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DEVELOPMENT AND EVALUATION OF THE OZONE/IMMOBILIZED FUNGAL PROCESS FOR PULP MILL EFFLUENT TREATMENT

BY
HUAZHONG MAO

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN

ENVIRONMENTAL ENGINEERING

DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA
SPRING, 1996

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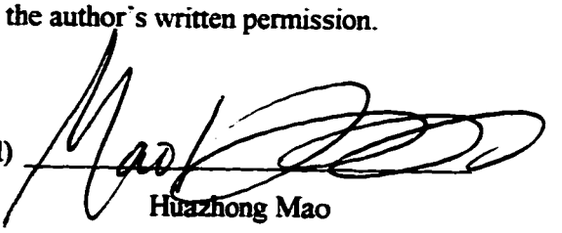
(signed) 

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled DEVELOPMENT AND EVALUATION OF THE OZONE/IMMOBILIZED FUNGAL PROCESS FOR PULP MILL EFFLUENT TREATMENT submitted by HUAZHONG MAO in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ENVIRONMENTAL ENGINEERING

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Dedicated to my parents, my wife and daughter

Shangfu Mao
Xu Fan
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ABSTRACT

A mechanistic model and dimensionless biodegradable potential index (BPI) were developed for systematically assessing biodegradable potential of pulp mill effluents. Three representative effluents, BTKPME, bleachery and combined effluents, were investigated.

Using ultrafiltration, raw and treated effluents were fragmented into molecular weight cutoff (MWCO)<1,000, 1,000<MWCO<5,000, 5,000<MWCO<10,000 and MWCO>10,000. HPSEC, TOX, TOC, COD, BOD, BPI were used in the evaluation. Two ozone reactor systems were developed for investigating impacts of ozonation.

The studies demonstrated that 1) the model and BPI were simple but reliable means for evaluation of biodegradable potentials; 2) color intensity (C.I.) was an intrinsic property of pulp mill effluents; it correlated well with MW and BPI; 3) there was an optimum ozone dose at which both BPI and decolorization were maximized; 4) BTKPME was the best target for ozone decolorization; 5) ozone application methods had insignificant effects on efficacy of ozone decolorization and dechlorination; 6) ozone decolorization was a dynamic process in removing and producing biodegradable components; in decolorization of BTKPME, BPI increased one unit with every 12 mg/L consumed ozone ranging from 0 to 200 mg/L; 7) kinetics of ozone decolorization follows 3/2 orders with respect to C.I.

The mechanisms of ozone/fungal decolorization and dechlorination were elucidated. Ozone preferentially destroyed the chromophoric structures in lignins and converted the high MW components into more biodegradable organics. *P. chrysosporium* had much less preference on the size and structures. Depolymerization seemed to be paralleled with dearomatization. Some low MW products were not detected at 280 nm. The ozonation enhanced the fungal treatment. The enhancement appeared to be through dual mechanisms: ozonation reduced MW and modified the lignin structures which became much more accessible to ligninolytic enzymes; and the newly formed organics may partially meet requirements for co-substrate and serve the inducers of the enzymes.

The ozone/immobilized fungal system were developed and evaluated under continuous operation. The bioreactor was reliable and effective in handling and sustaining the activity of *P. chrysosporium*. The system could reduce 90% of color and 80% of TOX in BTKPME simultaneously. The continuous study also verified the synergistic effects between ozone and immobilized fungal processes.

ACKNOWLEDGMENTS

The author sincerely appreciates the thesis supervisor, Professor Daniel W. Smith, who provided guidance, encouragement and technical support throughout the research program and the preparation of this manuscript. In particular, the encouragement to publish several papers in a variety of forums and providing opportunities to communicate with the leaders in the field of environmental studies are greatly appreciated.

The author would also like to thank Mr. Steve Cooper, Environmental Manager, and Dr. Henry Foster, engineering advisor, Weyhauser Co. for sample collection, their comments and providing necessary technical information related to the study. Important help was also obtained from several fellow graduate students in sample preparation and analysis and in some experiments.

A special thank you is extended to Professor Stephen J. Stanley and Dr. Shawn Xie for their critical comments in preparing some of the published papers.

The generous financial support by Grande Prairie Mill, Weyhauser Canada is also greatly appreciated. Some financial support by AQUATEX CORPORATION through internal research grants for supporting me to present the paper in 12th Ozone Congress were also appreciated.

Special thanks are extended to many colleagues in AQUATEX CORPORATION for their great encouragement and valuable helps whenever the author needed. In particular, Mr. Jose J. Lourenco and Mr. Bruce George are greatly appreciated.

My thanks also go to Mrs. Jun Ma, who painstakingly examined each page and every reference. A large measure of whatever success this research enjoys will be due in no small part to her unrelenting exactitude. Finally, I could not find proper words to express my deep appreciation to my wife, Jun Ma, and my dear daughter, Leslie Mao, for their sacrifice, considerate and encouragement for me to overcome the technical challenges and the incredible non-technical difficulty over the graduate studies.

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LIST OF SYMBOLS AND ABBREVIATIONS

$[C.I.]_t$	Color Intensity at time t=t
$[C.I.]_0$	Color Intensity at time t=0
C_{O3}	Concentration of Ozone in Liquid Phase
$D_{O3,G}$	Total Mass of Ozone Decomposed in Water-vapor-saturated (WVS) Gas Phase
k_1	First Order Kinetic Constant for Component A
k_2	First Order Kinetic Constant for Component B
K	Biodegradation Kinetics Constants in Simple First-order Model
k	Biodegradation Kinetic Constants Involved in Various Stages in Mechanistic Model
$k_{1G,}$	Stage I Kinetic Constant of 300 mg/L glucose and glutamic acid standard Solution
k_{3G}	Stage II Kinetic Constant of 300 mg/L glucose and glutamic acid standard Solution
k_G	Gas Phase Mass Transfer Coefficient
k_L	Liquid Phase Mass Transfer Coefficient
k_{TOC}	Pseudo-kinetic constant for ozone decolorization based on TOC
k_{COD}	Pseudo-kinetic constant for ozone decolorization based on COD
L_{C0}	Remaining Oxygen Uptake of Component C at Time $t = t_0$
L_A	Remaining Oxygen Uptake of Component A at Time t
L_B	Remaining Oxygen Uptake of Component B at Time t
L_C	Remaining Oxygen Uptake of Component C at Time t'
L	Remaining Oxygen Uptake of Sample at Time t
L_0	Ultimate Oxygen Uptake
$M_{O3,\ input}$	Total Mass of Ozone Input to the Reactor
$M_{O3,\ offgas}$	Total Mass of Ozone Leaving the Reactor in Offgas
$M_{O3,L}$	Total Mass of Ozone Residue in Liquid Phase
$P_{O3,}$	Partial Pressure of Ozone in Gas Mixture
Q_L	Total Volume of Treated Wastewater
t_0	Elapsed Time

t'	Time $t' = t-t_0$
t	Time for Biochemical Reaction
Y_A	Exerted Oxygen Uptake of Component A at Time t
Y_B	Exerted Oxygen Uptake of Component B at Time t
L	Dimensionless Biodegradable Potential
λ	Dimensionless Ultimate Biochemical Oxygen Demand
β	Dimensionless Kinetic Constant
α	Dimensionless Kinetic Constant
ε	Absorption Coefficient
λ	Wavelength (nm)
A	Component A
B	Component B
C	Component C
AOX	Absorbable Organic Holagens
BOD	Biochemical Oxygen Demand
BP	Biodegradable Potential
BPI	Biodegradable Potential Index
BTKPME	Biologically Treated Kraft Pulp Mill Effluent
C.I.	Color Intensity
COD	Chemical Oxygen Demand
CPPA	Canadian Pulp and Paper Association
C.U.	Color Unit
D	Chlorine Dioxide (ClO_2)
D_A	Molecular Diffusion Coefficient
$DE_{op}D_1ED_2$	Five Stages Bleaching Sequence
E_{op}	Extraction with oxygen and peroxide
H	Henry's Law Constant
H_A	Hatta Number

HPSEC	High Performance Size Exclusion Chromatography
I_o, I	Light Intensity
MW	Molecular Weight
MWD	Molecular Weight Distribution
MWCO	Molecular Weight Cut Off
OX	Organic Halogens
TOC	Total organic Carbon
TOX	Total Organic Halogens
TBOU	Total Biochemical Oxygen Uptake
UBOD	Ultimate Biochemical Oxygen Demand

CHAPTER 1. GENERAL INTRODUCTION

1.1 Background

The manufacture of paper from trees or other woody plants first requires a series of processes to modify naturally occurring fiber-to-fiber structures. These processes can be generally divided into four stages including preparation of fiber source, separation of fiber from other wood components, bleaching and paper making. The flowchart in Figure 1-1 schematically illustrates these four stages in the paper manufacturing processes.

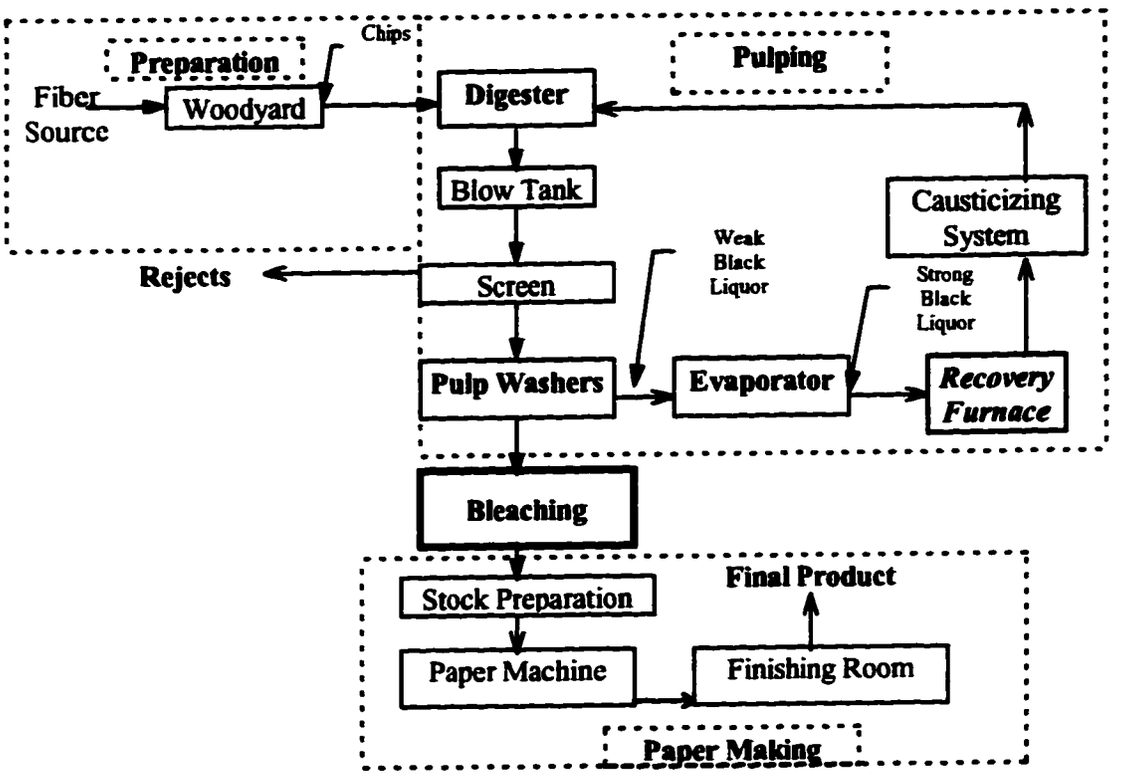


Figure 1-1. Schematic Illustration of Paper Manufacturing Processes.

1.1.1 Preparation of Fiber Source

The fibre source in Figure 1-1 refers to the logs of various sizes. The logs are sent to debarking processes (woodyard) which remove most of the bark from them. The debarked logs are then sent to the chippers where they are further reduced to chips which are usually less than

2.5 cm in length. Following the chipping process, the chips are screened to insure a uniform size, then the suitable chips are forwarded to the digesters in the pulping stage.

1.1.2 Separation of Fiber from Other Wood Components

Papermaking fibers in wood are held together in organized patterns by the materials called lignins. The lignins serve as the role of the cement which bonds the fibers together and imparts structural rigidity to the plant. Fiber separation is essential to the formation of a good paper sheet. This can be accomplished through the pulping process. In general, the pulping process can be categorized into physical, chemical processes and various combinations. The details of these processes have been covered comprehensively in the literature (Kringstad and Lindsrtöm, 1984; Sjöström, 1981; Sarkanen and Ludwig, 1971; Gierer, 1970; Pearl, 1967). Figure 1-2 summarizes the major pulping processes.

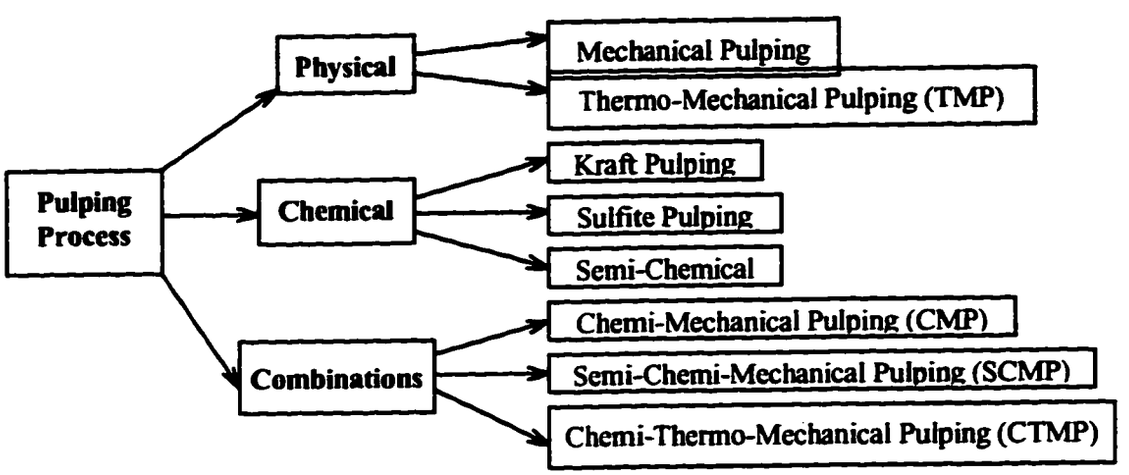


Figure 1-2. Comparative Summary of Major Pulping Processes

The kraft and sulphite pulping are two major chemical pulping processes. However, the most common pulping process in use today is the kraft, or sulfate, process accounting for more than 85% of total pulp production in Canada (Sinclair, 1990). This is probably because pulps made from the kraft process are characterized by their excellent strength properties. Other advantages of the kraft pulping process include the ability to maximize the recovery and recycle of pulping chemicals (see Figure 1-1) and the ability to pulp virtually any fiber source. In order to better understand the characteristics of kraft pulp mill effluents a brief overview of the kraft pulping process is presented.

In a kraft process, wood chips are charged into the digester and "cooked" under pressure with a mixture of the cooking chemicals, such as hot caustic soda and sodium sulphide. This solution is referred to as "white liquor". Lignins and wood extractives are solublized, leaving the insoluble cellulose fibres as pulp. The pulp is discharged from the digester after the appropriate cooking time has elapsed; this is referred to as "blowing the digester". The pulp is next screened to remove any oversize or undercooked constituents, such as knots. The accepted pulp, along with the black liquor from the cook, is then sent to the brown stock washers.

The brown stock washers are used to separate as much of the black liquor from the pulp as possible without causing too much dilution of the black liquor. The black liquor is composed of the spent and residual cooking chemicals and the dissolved solids including various fragments of lignin molecules. The recovered black liquor is usually sent to the recovery system while the washed pulp proceeds to either the bleach plant or directly to the paper mill. Most of the chemicals and dissolved solids are recovered from the spent (strong) black liquor and pulp washings (termed weak black liquor) through a series of steps involving concentration, combustion, clarification and causticizing. However, some of those residual chemicals and lignin derivatives are carried with the washed pulps to bleaching stages in bleached kraft mills.

1.1.3 Bleaching Processes

The bleaching process is used as an extension of the cooking stage to further brighten the pulp by removing residual lignin derivatives and other color-causing materials. The resulting product is a pulp of the desired brightness. Multiple stage bleaching is necessary to bleach kraft pulps to the desired brightness levels, and many different chemicals are used in the various bleaching stages.

In the past, the bleaching sequence C-E-H-CD-E-D (C=chlorine, E=alkali extraction, H=hypochlorite, D=chlorine dioxide) and various alternatives have been successfully used worldwide. In other bleaching processes, free chlorine or chlorine-containing chemicals serves as the major chemical. Due to the more stringent environmental regulations on the discharge of chlorinated organics and to reduce the total amount of wastewater discharged, it is now common that 100% chlorine dioxide (ClO_2) is substituted into all C-stages. For example, one of common bleaching processes, called $DE_{op}DED$ (E_{op} = alkali extraction with oxygen/peroxide), has been

practiced for years at mill scale. The effluent sampled from E_{op} stage of this bleaching process is usually called E_{op} filtrate.

The corrosiveness of the chlorides and other issues associated with bleaching effluents from various bleaching processes using chlorine containing chemicals present too many problems to be included in recovery systems. Thus, most of effluents from current bleaching plants have to be handled using external treatment processes for final disposal. For these reasons, the industry trend is toward developing bleaching processes with oxygen, hydrogen peroxide, ozone or the combination of these powerful oxidizing reagents. These alternative bleaching processes may allow the recycle of portions of the bleaching liquors in the future.

The final product from the bleach plant is sent to the paper mill for making paper sheets or other paper products.

1.2 Sources of Raw Wastewaters

Various effluents from a western Canadian kraft pulp mill were used as raw wastewaters in this study. The mill produces approximately 800 to 900 tonnes per day of the bleached kraft pulp. The raw material used in producing the pulp consisted of about 50 to 60% hardwood and 40 to 50% softwood depending on the season.

The bleaching process has undergone several modifications over the years to meet Alberta's increasingly stringent discharge standards. Since July, 1992, 100% chlorine dioxide substitution has been practiced and a five-stage bleaching sequence ($DE_{op}D_1ED_2$) is currently applied. The details of this bleaching process are illustrated in Appendix I.

The mill also developed primary and secondary treatment processes as illustrated in Appendix I. Figure I-1 in Appendix I shows that the alkaline and acidic effluents were separately collected and pretreated before they were mixed together for biological treatment. The biological treatment system consists of a two-celled aerated stabilization lagoon (ASB) with different functional zones. These processes remove the majority of biochemical oxygen demand (BOD) and total suspended solids (TSS). Currently, the treated effluent is directly discharged into the receiving water body through a mid-point channel diffuser. To further reduce impacts of pulp mill effluent discharge on the receiving water, the mill sponsored this study to develop an effective process to reduce color and chlorinated organics in bleached kraft pulp mill effluents.

The locations for sampling various raw effluents mentioned in this study are also shown in the figures in Appendix I.

1.3 Environmental Impacts of the Forestry Industry

The pulp and paper industry is one of the largest industrial consumers of water worldwide. It was estimated that trillions of tons of wastewater carrying more than $2.5x10^6$ tons of derivatives including about 250,000 tons of chlorinated organics were discharged annually by the industry throughout the world (Kringstad, *et al.*; 1984). Although effective pollution prevention measures reduced the water consumption and pollution load from the pulp and paper industry dramatically in past decades, the industry in Canada is still responsible for more than 20% of all the waste dumped into the nation's water bodies today (Sinclair, 1990; Environment Canada, 1991). These released effluents have been found to exert various adverse impacts on the receiving environment, notably depletion of dissolved oxygen, fish kills, and often dramatic alternations in various water quality parameters of the receiving waters. The major environmental impacts of discharging these effluents on the aquatic environment have been reviewed periodically with different emphasis (Poole *et al.* 1978; Gellman and Berger 1988; Anonymous, 1989; Crooks and Sikes, 1991). In brief, they can be outlined as, 1) discolorization of the receiving water and reduction of photosynthetic activities; 2) deteriorating water quality, both bacteriological and chemical; 3) acute and chronic toxicity; 4) taste and odor; 5) slime growth; 6) surface water foaming; and 7) damage to fisheries resources.

1.3.1 Pollutants in Pulp Mill Effluents

In the past, with the limited understanding of the nature of the organic compounds in the effluents wastewater, the principal pollutants, which were used by regulatory authorities and the industry to assess these adverse effects on receiving environments, were broadly classified into eight categories (USEPA, 1982; Springer, 1985): 1) oxygen-demanding substances such as biochemical oxygen demand (BOD); 2) disease-causing agents such as pathogenic microorganisms; 3) plant nutrients such as nitrogen and phosphorus; 4) inorganic chemicals and mineral substances such as pH and heavy metals; 5) sediments such as total suspended solids (TSS); 6) organic compounds such as color, various toxicants and other industrial chemicals; 7) radioactive substances; and 8) thermal discharges. The first five pollution parameters are usually defined by US Environmental Protection Agency (USEPA) and Canadian Environmental

Protection Act (CEPA, under Fisheries Act) as conventional pollutants. The pollution parameters associated with color, persistent or toxic organic compounds, odour, fish-flesh-tainting, slime growth, thermal effects, foam, and scum etc. are all classified as nonconventinal pollutants (USEPA, 1982; Environment Canada, 1983 and 1987). The complete definitions and characteristics of these pollution parameters have been discussed in detail by Springer (1985). The following section is intended to provide an overview as the background information for this study.

1.3.1.1 Conventional Pollutants

With several decades of effort by both regulatory authorities and industry, the problems associated with the conventional pollutants appeared to be largely reduced with the proper physical, chemical and biological treatments. The status and current situation have been extensively reported (USEPA, 1982; Environment Canada, 1981, 1983), and recently well summarized (Springer, 1985; Environment Canada; 1987). A detailed discussion is beyond the scope of this thesis. Some aspects will be briefly addressed as needed in later sections.

1.3.1.2 Nonconventional Pollutants

According to the above broad classification, chlorinated and non-chlorinated toxicants, color-causing substances and other associated organic matter are all classified as non-conventional pollutants, and currently represent the biggest problems to the industry and regulatory authorities. The primary objective of this research is to develop an advanced process for decolorization and dechlorination of kraft pulp mill effluents. Thus, more attention will be paid to the nonconventional pollutants associated with kraft pulp mill effluents.

1.3.2 Significance of Various Constituents in Kraft Pulp Mill Effluents

It was reported that the organic compounds contained in kraft pulp mill effluents not only varied in chemical types but also were distributed widely from low to very high molecular weight (MW) (Kringstad and Lindström, 1982 and 1984; Crooks and Sikes, 1991). In a view of low MW organic compounds associated with nonconventional pollutants, unbleached pulp mill effluents usually contained resin acids and soaps, fatty acids, diterpene alcohols, sugars, and aliphatic and aromatic hydrocarbons. Numerous volatile sulfur-containing compounds were also found in pulp mill effluents. By 1991, there were more than 47 Canadian mills employing

chlorine bleaching in their operation. It was estimated that 610,000 tonnes of chlorine are used annually to produce over 10 million tonnes of bleached pulp releasing over a million tonnes of chlorinated organic compounds to the aquatic environment (Environment Canada, 1991). After bleaching with chlorine, the effluents contained these chemicals as chlorinated phenols; chlorinated acids, alcohols, aldehydes, ketones, sugars, and aliphatic and aromatic hydrocarbons. It has also been reported that the low MW (<1000 g/mole) organic bound chlorine (OCl) only formed about 30% of the OCl in spent chlorination liquor and 5% of OCl in the caustic extraction liquor (Lindström *et al.*, 1981; Kringstad and Lindström, 1984), but this fraction has profound adverse effects on the aquatic ecosystem due to its acute toxicity (Leach and Thakore, 1975; McKague, 1981; Voss *et al.*, 1981a), genotoxicity and potential to accumulate (Walden, 1976; Walden *et al.*, 1977; Bjørseth *et al.*, 1981; Salkinoja-Salonen, 1981; Langi and Priha, 1988). This was also clearly reflected by the fact that more than half of all the priority pollutants, as designated by USEPA (1977), are halogenated organics.

A number of studies have been carried out to determine how levels of variety of toxicants such as chlorophenols and the toxicity of various effluents are related to the raw materials, pulping and bleaching techniques. Maximum production of chlorophenols, at 120% chlorine demand, occurred at a 1:1 ratio of chlorine and ClO_2 (Voss *et al.*, 1980, 1981a, 1981b; Gergov *et al.*, 1988). At higher levels of substitution, levels of chlorophenols dropped. The effects of ClO_2 substitution on toxicity have not been clearly established, although a reduction may be expected (Kutney *et al.*, 1984). In their study, Gergov *et al.* (1988) compared the mill with modern technology in both process and wastewater treatment with the conventional mill as a reference. The study (Gergov *et al.*, 1988) revealed several important facts. In bleaching of hardwood (angiosperms) kraft pulp, the formation of organically bound chlorine was lower than in the bleaching of softwood (gymnosperms) kraft pulp. The authors pointed out that this difference could be attributed to the differences between softwood and hardwood lignins in the pulp. The formation of chlorinated phenolics in hardwood pulp bleaching were only about a third of that for softwood pulp bleaching. Increase in ClO_2 from 14% to 66% reduced the formation of AOX by about 20%. About 10% of the total chlorine in the bleaching chemicals could be found as AOX in the bleach plant filtrates, the difference in the ratios is small between the modern and conventional mills. More importantly, the Daphnia toxicity determined for bleach plant filtrates did not correlate with the changes in the amounts of chlorinated organic compounds; at the

modern mill, the total effluent during the hardwood periods was nontoxic even in the streams before the activated sludge plant and; the acid filtrates proved to be mutagenic in all cases, with the softwood filtrates being twice as mutagenic as hardwood filtrates. The total untreated effluent was found to be Ames mutagenic only during the softwood pulping at the modern mill. During the hardwood pulping and in all cases at the conventional mill it was not mutagenic. Interestingly, extended pulping results in higher mutagenicity in untreated effluent which was suspected to be related to the higher content of chloroacetones.

The limited number of field surveys of bioaccumulated effluent constituents in aquatic biota (Voss and Yunker, 1983; Environment Canada, 1987; Crooks and Sikes, 1991) provided evidence that unchlorinated resin acids, chlorophenolic compounds and certain biodegradation products (chloroveratoles) could, in instances where effluent mixing and flushing were poor, accumulate in fish and shellfish. As with the laboratory findings, the results of these (freshwater, estuarine and marine) surveys indicated appreciably greater accumulations in fish livers than in muscle or whole-body tissues (where concentrations were frequently below the limits of detection).

Up to 80% of chlorinated organic compounds in bleachery effluents, which derived from lignin derivatives, are of high molecular weight (MW>1,000 g/mol.) (Kringstad and Lindström, 1984; Fandry *et al.*, 1989; Sågfors, 1988). The quantity of high molecular mass (HMM) substances in alkaline effluents was found to be 65 to 75%, and in acid stage effluents 20% of the UV-absorbing (A_{280} nm) components. Lignin derivatives were the major precursor of the HMM substances in the alkaline stage, and lignin derivatives and carbohydrates were the precursors of the acid stage HMM substances. The chlorine content of HMM substances was as high as 9.8% when ClO_2 charge had been low and less than 4% with a high ClO_2 charge; the low methoxyl group content and high oxygen content suggested that the substances were largely oxidized. The LC_{50} test with water fleas indicated no acute toxicity. IR spectra revealed that HMM substances from both the alkaline and acid stages in their neutral form had strong bands characteristics of carboxylic salts. There were little aromatic bands in the spectra of softwood HMM substances but there was a narrow aromatic band of low intensity in some of the alkaline HMM substances. There was little difference in molecular mass distribution among the hardwood and softwood HMM substances. More importantly, the high MW chlorolignins may bind or associate with the low MW toxic hydrophobic organics, and lead to wide distribution of

these toxicants by carrying them over and slowly releasing them into the aquatic system (Kukkonen, 1992; Carlberg *et al.*, 1988). Furthermore, these macromolecules may also be adsorbed by the sludge during biological treatment and the sediments of the receiving water, and appeared to cause no immediate adverse effects on aquatic ecosystem. However, the study (Lindström *et al.*, 1981; Kringstad *et al.*, 1984) revealed that the chlorinated aromatics of relatively low MW could be formed from high MW chlorolignins in the aquatic ecosystem through various physical, chemical and biological transformations. Several investigations (Neilson *et al.*, 1983, 1984 and 1990; Eriksson, 1985a and 1985b; Mao and Smith, 1995a) confirmed that the biodegradation/transformations were greatly enhanced and highly lipophilic chlorinated veratoles were formed as metabolites in the biological process with the presence of co-substrates under proper environmental conditions. It has also been demonstrated that even the most refractory fraction of the high MW chlorolignins may be converted to a variety of low MW organics under proper physical and chemical conditions (Archibald and Roy-Arcand, 1993). These and other unknown low MW byproducts in the receiving aquatic ecosystem can be expected to accumulate in the fatty tissues of higher organisms such as fish, and to cause profound adverse effects on the ecosystem over long-term since the organic compounds with MW<1000 g/mol. are able to pass through biological membranes while those of a higher MW are rarely able to do so (Kringstad and Lindström, 1984; Fandry *et al.*, 1989).

1.3.3 Chlorinated Organics and Their Surrogates (AOX/TOX/EOX)

A chlorinated organic substance is any organic compound that has one or more chlorine atoms attached to the molecule. The term organochlorine refers to the chlorine (only) that is attached to a chlorinated organic molecule. This distinction is quite important in the consideration of the mass of material involved and measurement of chlorine contents.

It has been estimated that only about 10 to 40% of low MW (MW<1000 g/mol.) chlorinated organic compounds in bleached pulp mill effluents have been characterized (Bjørseth, 1976; Kringstad and Lindstrom, 1984; Leach and Thakore, 1975; McKague, 1988), and it is unlikely that some of the chlorinated compounds in bleached pulp mill effluents will ever be identified. In addition, the compositions of these effluents varied greatly with the raw wood materials used and the operating conditions. Thus, it is impractical, if not impossible, to attempt to completely characterize effluents on the basis of individual substances. For instance, more

than 250 of the chemicals found in the pulp and paper mill effluents were chlorinated organic compounds and thousands of them remain unknown regarding their physical-chemical characteristics (Kringstad and Lindström, 1984; Suntio *et al.*, 1988). It is more reasonable as an interim step to measure the total organic chlorine concentration from bleached pulp mill effluents or in the aquatic environment.

Generic tests, such as Absorbable Organic Halogens (AOX) and Total Organic Halogens (TOX), have been found to provide reasonable indices of organochlorine concentrations in effluents and receiving waters since AOX or TOX measurement has good repeatability, comparative ease of use and low cost. There is no real difference between these two methods regarding the measured contents of the organic chlorine. But they differed in the methods and period for extraction of organic chlorine from the liquid phase (APHA, 1991; SCAN, 1989). Whether AOX or TOX should be regulated as one of the water and wastewater quality parameters is currently still under strong debate (Haskoning, 1991; Folke, 1991a and Folke *et al.*, 1991b and 1992) although the USEPA has recently issued regulations that treated effluent AOX level from non-TCF (Total Chlorine Free) bleached kraft facilities should be restricted to a monthly average of 0.156 kg/a.d. metric ton (Federal Register, 1993).

One side of the debate pointed out that an untolerable disadvantage of using TOX or AOX is the lack of specific information concerning the multitude of specific organic substances present. Also, the correlation of this parameter to other parameters, such as toxicity and mutagenicity, have been reported to be case specific (Bjørseth *et al.*, 1981; Salkinoja-Salonen *et al* ,1981; Kool *et al.*, 1984) since AOX or TOX comprises a plethora of individual substances with a vast variety of properties as discussed in the earlier sections. In other words, the major limitation of these surrogate tests is that they do not provide specific estimates of the potential toxicity, persistence, or bioaccumulation of specific chlorinated organic substances. For example, equal AOX values in bleached kraft mill effluents indicate neither identical composition of effluents nor equivalent toxicity to aquatic life (Haskoning, 1991). On the other side, as discussed earlier it is undeniable fact that the AOX or TOX parameter has been the most widely accepted measure for the "total" amount of organic chlorine compounds in water and wastewater, especially in a pulp mill effluent. In fact, the analysis of organic halides as a group parameter in water and wastewater recognizes that many such OX compounds are unaccounted for by methods designed to identify individual low MW compounds. These methods for individual

compound analysis usually identify and measure organic halides volatile enough to be purged from the water and trapped on an adsorbent (Bellar and Lichtenberg, 1974; Grob and Zurcher, 1976; Keith, 1981) or non-polar enough to be solvent extractable (USEPA, 1971; Dressman *et al.*, 1978; Keith, 1981) and then gas chromatographed. Such purgable/solvent extractable compounds have been found in many instances to represent no more than 25% of organic halides even in drinking water samples (Dressman *et al.*, 1979). Thus, the analysis for total organic halides, because it includes the measurement of potentially harmful organic halides for which no specific methods of analysis exist, is an unique indicator of chlorinated organics.

Moreover, TOX or AOX as a group parameter is especially useful in process control since they can be reliably determined within a short time, and with relatively simple equipment. More specifically, measuring TOX or AOX can serve to indicate the effects of various wastewater treatment unit processes on chlorinated organics. For example, the production of OX compounds by the use of various bleaching processes, and the efficiency of removal of OX products or their precursors by various treatment techniques can be reliably determined.

For further clarification, the Commission of the European Communities (EC) sponsored a comprehensive study on evaluation of usefulness of TOX or AOX as a regulatory parameter. In the report, Haskoning *et al.* (1991) thoroughly evaluated major features and shortcomings of TOX or AOX determination according to the compilation of a large amount of scientific evidence:

> "AOX includes the determination of volatile compounds and polar compounds, some of which do not bioaccumulate and/or which are not highly toxic. Thus it does not distinguish between less and more potentially hazardous constituents.... On the other hand AOX is the only parameter that assess the hazardous potential of the whole organo-chlorine pollution load. It is the only method that completely covers the high molecular mass organo-chlorine, a part which can be partially biotransformed into chlorophenols in the environment."

Further they concluded that "Based on the present application, standardization and environmental relevance, AOX is the most appropriate parameter to establish emission standards for chlorinated substances from the European industry at the moment". (Haskoning, 1991)

Consequently, this surrogate parameter has recently been proposed to be targeted as one of the most important parameters to regulate pulp mill effluent discharge based on the existing

scientific data. For instance, some European Economic Communities (EEC) countries have proposed AOX as a regulatory parameter for limitation of discharges of organochlorines in pulp and paper mill effluents. In Organization for Economic Collaboration and Development (OECD) countries (including Canada), the TOX or AOX is also coming into legal regulation of the pulp and paper mill effluents; and the USEPA has proposed the new standard for TOX regulation as discussed in the above section. Balancing all of these findings and considerations the parameter TOX was selected in this study to indicate the organochlorine content of kraft pulp mill effluents.

1.3.4 Distribution of Color Body

Color in pulp mill effluents mainly results from the operations of pulping and bleaching. The dominant color stream in the bleach plant is the caustic extraction (E) stage. Although the peeling and other various reactions involved in the pulping and bleaching processes may produce some chromophores, contributing the color to pulp mill effluents, it is certain that the lignin components contribute the majority of the color body in kraft bleachery effluents. For a representative bleached kraft mill, 135 kg of color per ton might be generated. The pulping process would contribute less than 30 kg/ton, and the bleaching plant more than 90 kg/ton, of which the caustic extraction stage would contribute more than 65 kg/ton. The remaining color, 15 kg/ton, originates in the wood yard, in recovery and causticizing. Thus, the bleach plant can be responsible for more than two-thirds of the total color load while contributing less than half of the effluent volume.

1.3.5 Problems Related to Color Discharge

With the advent of environmental legislation in forestry industry, wastewater treatment has referred primarily to removal of conventional pollutants, such as BOD and suspended solids. Color was not thought to be a major problem, and was classified as a nonconventional pollutant. There are still no federal regulations concerning color discharge in North America, but some states and Canadian provinces have established permissible limits of color discharge for some pulp mills. The reasons for color regulations at these locations are said to be for protection of fisheries or for aesthetic considerations, as the public associates color with pollution. In addition, Japan as well as some European and Scandanavian countries has proposed limitations on COD discharge; this is tantamount to a color discharge regulation since conventional biological treatment was ineffective in color reduction.

Even though there are no well-established color discharge regulations and there is little scientific data on color associated problems, the industry itself has attempted to address the color problem for many years. Previous reviews on the color problems have been prepared by Gillespie and Berger (1971), Tyler and Fitzgerald (1972), Gehm (1973), Timpe, *et al.* (1973), Gallay (1973), Vincent (1974), Gellman and Berger (1974), and Rush and Shannon (1976). More recently, Springer (1985) further compiled the possible effects of color body on the aquatic ecosystem:

1) Color retards sunlight transmission and may interfere with photosynthesis, thereby reducing the productivity of the aquatic community.
2) Natural stream color is altered, thus detracting from the visual appeal and recreational value of the receiving waters.
3) Color has impacts on downstream municipal and industrial water users, such as higher water treatment costs, difficulties with water treatment, and a multitude of industrial process operating problems.
4) Color bodies complex with metal ions, such as iron or copper, forming tar-like residues that remove the metals from stock available to stream organisms for normal metabolism.
5) Color may have direct inhibitory effects on some of the lower organisms in the food chain, thereby reduce the productivity of the receiving water.
6) Color in receiving waters may affect fish movements and fish productivity.
7) Color, derived from high MW effluent constituents such as chlorolignin, may be degraded to lower MW, potentially toxic chlorophenol derivatives (Environmental Canada, 1987; Neilson, *et al.*, 1983 and 1984) as outlined in earlier sections.
8) Color bodies exert long-term BODs (20 to 100 days) which is not measured by the BOD_5 tests (Mao and Smith, 1995a). The details on this topic will be addressed in Chapters 2 and 3.

1.4 Lignins – Chemical Source of Color and Chlorinated Organics

The earlier sections qualitatively described the color body and various organic compounds in the pulp mill effluents and their impacts on the aquatic environment at a

macroscale. It is essential to gain a better understanding of the nature, chemical structures, chemistry and physical characteristics of these compounds in order to develop most effective and practical strategies for dealing with them. Fortunately many pioneer researchers in this field have devoted great efforts to this topic for a century. The accumulated knowledge has allowed the development of quite a good understanding although some debate and uncertainty still exist regarding the complex nature of color.

1.4.1 Lignin: Definition, Distribution, Functions, and Structure

As defined by Sarkanen (1971), "lignins are polymeric natural products arising from an enzyme-initiated dehydrogenative polymerization of three primary precursors: trans-coniferyl, trans-sinapyl and trans-*p*-coumaryl alcohols". Figure 1-3 illustrates the chemical structures of these components.

Lignins are distributed widely throughout all higher member of the plant kingdom. Lignins form an essential component of the woody stems of arborescent gymnosperms (softwood) and angiosperms (hardwood) in which their amounts range from 10% to 36% (Sakakibara, 1991). Lignins are not, however, restricted to arborencent plants, but are found as integral cell wall constituents in all vascular plants including the herbaceous varieties. Their presence has been demonstrated in tissues associated with stems as well as in fruit, bark, bast, pith, cork cells, foliage and roots (Sarkanen and Ludwig, 1971, Pearl, 1967).

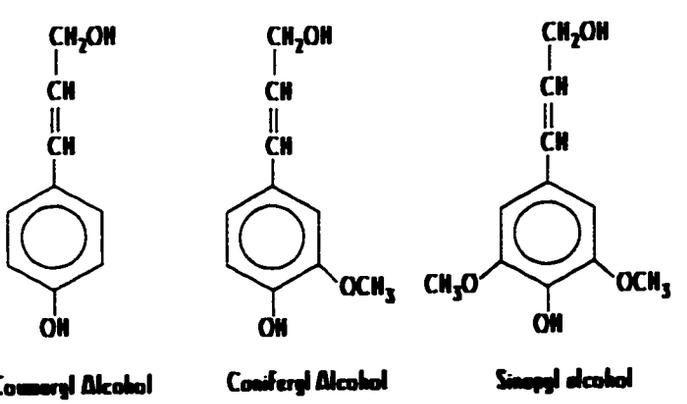


Figure 1-3. The Structures of Three Primary Monomeric Precursors of Lignins

As cell wall constituents, lignins do not merely act as "encrusting material" of some secondary nature. Rather, they perform multiple functions that are essential to the life of the

plant. By decreasing the permeation of water across the cell walls in the conducting xylem tissues, lignins play an important role in the intricate internal transport of water, nutrients and metabolites. Secondly, lignins impart rigidity to the cell walls and, in woody parts, act as permanent bonding agents between cells generating a composite structure outstandingly resistant towards impact, compression and bending. Finally, lignified tissues effectively resist attacks by microorganisms by impeding penetration of destructive enzymes into the cell wall (Sarkanen and Ludwig, 1971).

Obviously, complex structures are naturally required for the lignins to perform all these special functions. In fact, these multifunctional requirements indeed render lignins the most complex polymer among naturally occurring high MW materials, and investigations devoted to the elucidation of their structures have been underway for a long period of time. Accumulated evidence reveals that lignins can be divided into three broad classes according to their structural elements. These include softwood lignins, "guaiacyl lignins" or gymnosperm lignins; hardwood lignins, also called "guaiacyl-syringyl lignins" or dicotyledonous angiosperm lignins; and grass lignins or "syringyl lignins" or "*p*-hydroxyphenyl lignins" or monocotyledonus angiosperm lignins. Softwood lignins are mainly composed of guaiacyl units originating from the predominant precursor, trans-coniferyl alcohol, while hardwood lignins are copolymers of coniferyl and sinapyl alcohols, the ratio varying from 4:1 to 1:2 for the two monomeric units. Grass lignins contain *p*-hydroxyphenyl units derived from trans-*p*-coumary alcohol besides units originating from the foregoing two precursors. However, strictly speaking, almost all lignins consist more or less of all these three units, namely, guaiacyl, syringyl, and *p*-hydroxyphenyl moieties. Detailed descriptions have been compiled by Sarkanen (1971), Sjöström (1981) and Sakakibara (1991). Figures 1-4 and 1-5 show the recently revised lignin structural models for hardwood and softwood, respectively. It should be pointed out that they do not represent exact molecular structures, such as those for other natural polymers such as cellulose and proteins. This is simply because it has not been possible to isolate all the parts of the lignin completely from plant tissues without engendering structural changes.

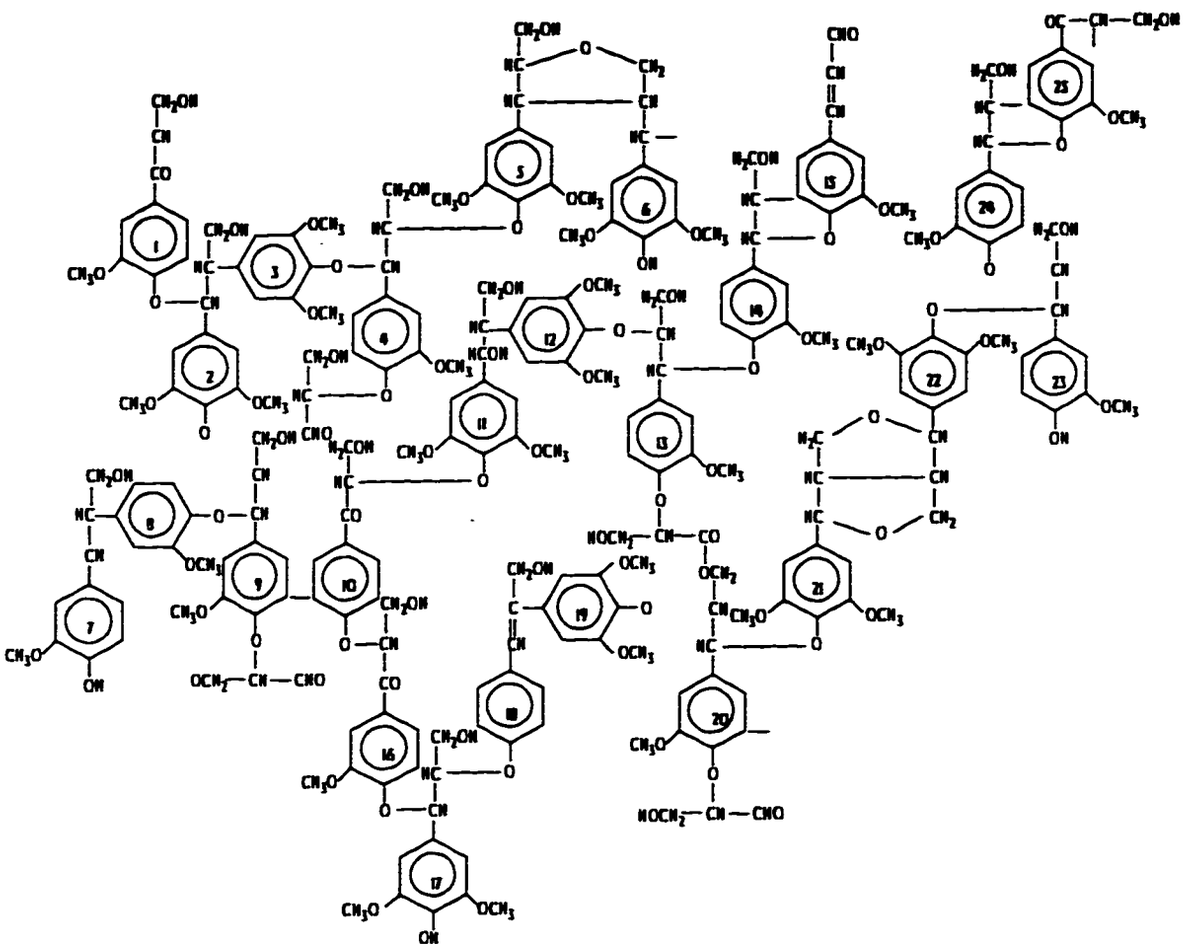


Figure 1-4. Structural Model of Hardwood Lignins (after Sjöstrom, 1981; Sakakibara, 1991)

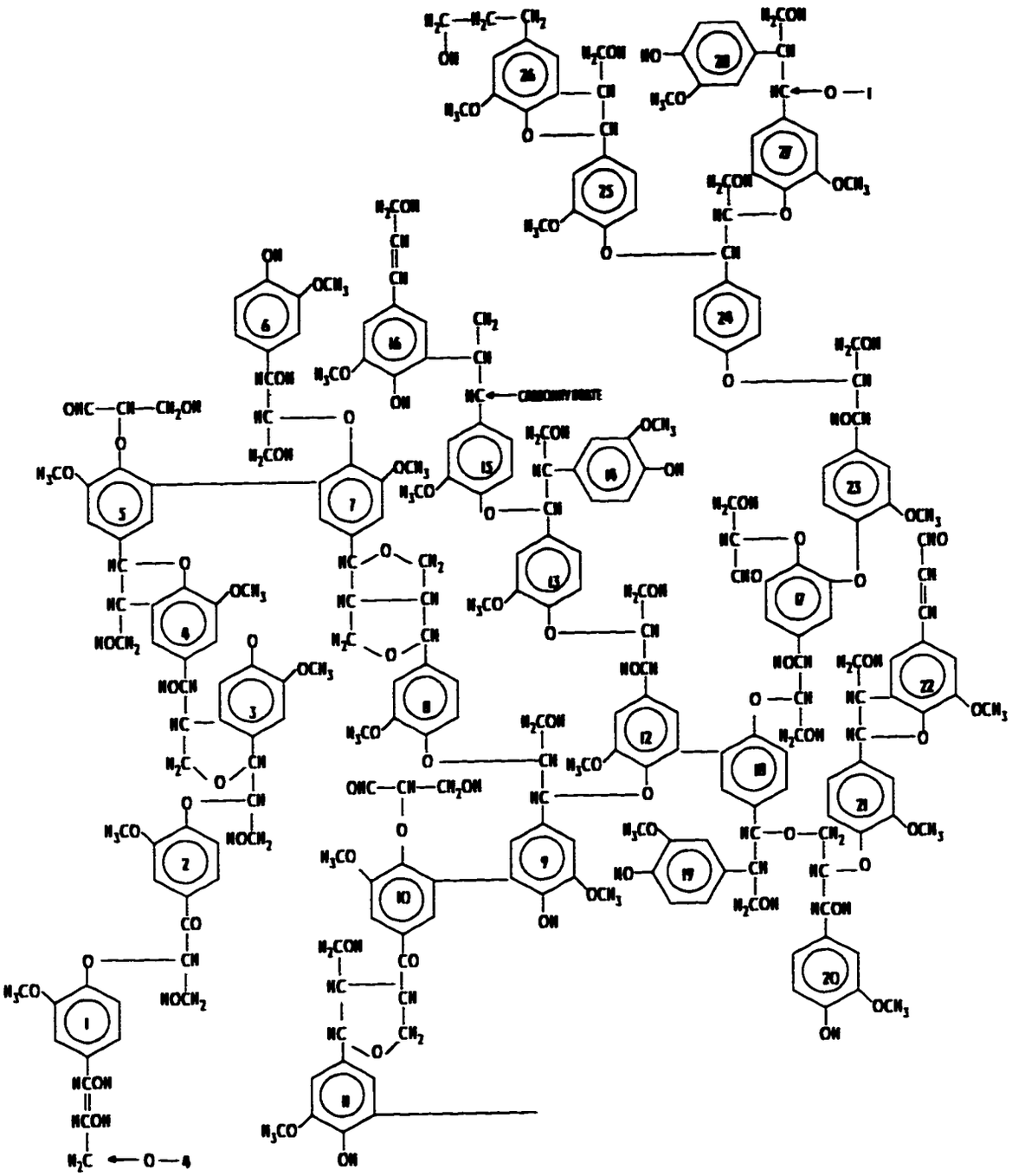


Figure 1-5. Structure Model of Softwood Lignin (after Sjostrom, 1981; Sakakibara, 1991)

1.4.2 Related Properties of Lignins

The physical and chemical properties of lignins and lignin derivatives have been reviewed with different emphasis (Brauns, 1952; Freudenberg, 1955; Brauns and Brauns, 1960; Sarkanen and Ludwig, 1971; Pearl, 1967; Sjöström, 1981; Sakakibara, 1991). The chemical characteristics of lignins summarized by Freudenberg (1955) may still serve a good representative:

> "A system of thermoplastic tri-dimensional polymers derived from coniferyl alcohol or other guaiacylpropane monomers. It is insoluble in water, in most organic solvents, and in strong sulfuric acid. It contains the major portion of methoxyl content of the wood. It has a characteristic ultraviolet absorption spectrum and gives characteristic color reactions with many phenols and aromatic amines. It has a variable elementary compositions and methoxyl content. It reacts readily with sodium bisulfite or thioglycolic acid to form soluble products. It is unhydrolyzable with acids, readily oxidizes, is soluble in hot alkali, and readily condenses with alcoholic and phenolic compounds. It yields up to 25% vanillin when oxidized with alkaline nitrobenzene and Hibbert's monomers when boiled under reflex with ethanol and little hydrogen chloride."

The following sections will briefly discuss some of these characteristics of lignins and lignin derivatives as background for this study. Some specific characteristics of lignin derivatives in kraft mill effluents related to this study are thoroughly addressed in Chapter 3 of this thesis.

1.4.2.1 Some Functional Structures of Lignins

The advance in chemistry, biochemistry, biochemical and analytical technologies have facilitated the progress in revealing the detailed physical and chemical structures of lignins. For example, the biosynthesis of lignins in-vitro provided excellent guidance in approaching this problem. Information on the degradation products from protolignin gave direct evidence about lignin structures. The quantitative analysis for various functional groups and linkage types in protolignins using UV, IR, NMR, particularly when used in conjunction with chemical modifications, have contributed essential information for understanding lignin structures. Many excellent reviews have treated these aspects in detail and comprehensive (Brauns, 1952; Brauns and Brauns, 1960; Sarkanen, 1971, Pearl, 1967; Sjöström, 1981; Sakakibara, 1991). The brief summary on the major aspects of these studies are presented here.

1.4.2.1.1 Typical Linkage Types and Functional Groups in Lignin Molecules

It has been observed that the composition of lignins estimated by various methods mentioned above changes not only with their sources but also with the methods used in their preparation from the same source, and even with the same method and the same source but under different conditions (Brauns, 1952; Brauns and Brauns, 1960; Sarkanen, 1971, Pearl, 1967; Sjöström, 1981; Sakakibara, 1991). However, the major types of linkage and functional groups in the lignin molecules have now been quite well identified, and relatively good agreement on their reactivity, functional and characteristics has been established although some debates on other minor functional groups still exist. Table 1-1 compares the composition of some lignin models with spruce lignins.

Table 1-1. Some Functional Groups and Structural Units – Comparisons of Spruce MWL and Lignin Models (per C6C3) (after Sakakibara, 1991)

Type of Functional Groups and Structural Units		Lignin Models			Spruce
		A	B	Mean Value	MWL
Aliphatic OH		1.00	1.00	1.00	1.09 (0.93)
Phenolic OH (free)		0.32	0.29	0.31	0.26 (0.33)
Total Carbonyl		0.21	0.21	0.21	0.20
	α C=O	0.07	0.04	0.06	0.06 to 0.07
	unconjugated carbonyl	0.11	0.11	0.11	0.10
	γ-Lactone	0	0.04	0.02	
	Ar-CH=CH-CHO	0.04	0.04	0.04	0.03 to 0.04
Ar-CH=CH-CH_2OH		0.04	0.04	0.04	0.03
Ar-CHOH-CHOH-CH_2OH		0	0.04	0.04	0.02
Ar-CH_2-CH_2-CH_2OH		0.04	0.04	0.04	
Ar-C-C-OCH_3		0.11	0.11	0.11	0.10 to 0.13 (0.06 to 0.08)

Figure 1-6 summarizes the main types of lignin structural units, which were obtained from the various collective studies on lignins (Sakakibara, 1991; Sjöström, 1981; Aldler, 1977). The linkage type β-O-4 in the lignin molecules was reported to be the most frequently detected and then phenylcoumaran, 5-5 biphenyl, diarylpropane β-1, benzyl-aryl ether α-O-4, and β-β linkage, 4-O-5 diphenyl ether and β-6 units have been confirmed by not only oxidation but also

hydrogenolysis. The linkages α-O-γ and α-O-4 have been detected but the frequencies of α-O-γ and α-O-4 have not yet been well-established. Other linkages are at least not frequent and correspond to minor ones (Sakakibara, 1991; Sjöström, 1981).

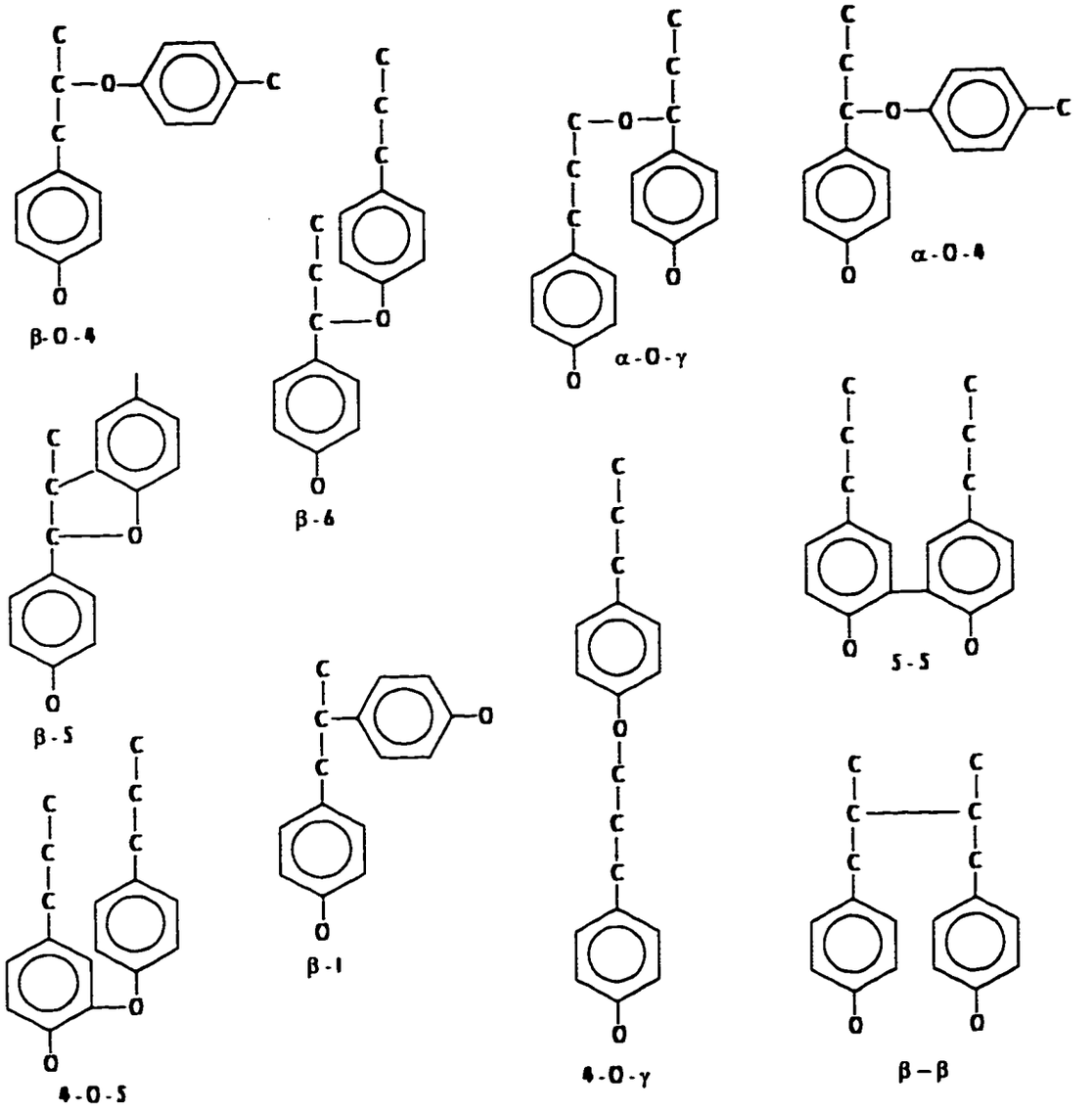


Figure 1-6. Common Linkages between Phenylpropane Units in the Lignin Molecules (after Sjöström, 1981; Sakakibara, 1991; Aldler, 1977)

In addition to these original functional groups, various residual functional groups derived from pulping and bleaching such as OH, O^-, COO^-, COOH, CHO, $COOCH_3$ etc. can be expected in the lignin components of pulp mill effluents. It was also reported that the chromophore-bearing components in the E1 effluent were predominantly polymeric, chlorine-containing oxidized lignin fragments with a low aromatic content (Eaton, 1981).

1.4.2.2 UV Spectra of Lignins and Lignin Derivatives

1.4.2.2.1 Chromophores and Auxochromes

The word 'chromophore' is often used to describe the chemical structure system containing the electrons responsible for the absorption in question. Specifically, an atomic group having a T electron, such as an unsaturated bond, is called a chromophore. An atomic group having isolated electron pairs, such as -OH, -COOH, and -OR are called an auxochrome. An auxochrome enables the light absorption of chromophores at a longer wavelength, thus intensifies the coloration of chromophoric structures.

The electron of an unsaturated bond in the chromophores (e.g., >C=C<, >C=O, >C=NH, -N=N-) can transfer relatively easily to an excited state with a relatively low level of energy. However, most of the simple unconjugated chromophores still give rise to such high-energy and, therefore, such short-wavelength absorption (<200 nm) that they are of little use in measuring UV spectra. The important chromophores are usually those in which conjugation is present. In principle, the molecular orbitals with the electrons in the molecules containing many such unsaturated groupings that all are conjugated will extend over these groups. The resulting high degree of delocalization of the electrons means the energy required for the transition decreases.

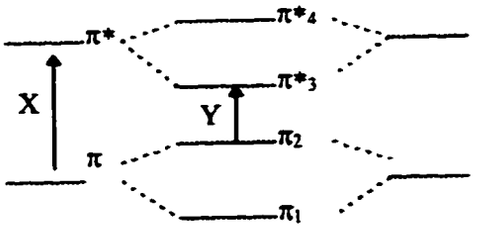


Figure 1-7. Schematic Illustration of Electron Transition Processes

For example, an isolated double bond (>C=C<) or lone-pair of electrons gives rise to a strong absorption maximum at about 190 nm, corresponding to the transition X in Figure 1-7 at too short a wavelength for convenient measurement in common solvents. When the molecular orbitals of two isolated double bonds are brought into conjugation, the energy level of the highest occupied orbitals is raised and that of the lowest unoccupied anti-bonding orbital lowered (see Figure 1-7). The $\pi \longrightarrow \pi^*$ transition, which occurs by absorption, is now associated with the smaller Y value. Moreover, when more than two orbitals overlap, that is, when the chromophore is a longer conjugated system, the separation of the energy levels is further reduced and absorption occurs at an even longer wavelength. For example, a long conjugated, β-carotene in which eleven units overlap absorbs the light of 520 nm to give red (William and Fleming, 1973).

1.4.2.2.2 Characteristics of UV Absorption of Chromophores

The visible and ultraviolet spectra of organic compounds are associated with transitions between electronic energy levels. The transitions are generally between a bonding or lone-pair orbital and an unfilled non-bonding or anti-bonding orbital. The wavelength of the absorption is then a measure of the separation of the energy levels of the orbitals concerned, which can be expressed as:

$$E = 1.38 \times 10^5 / \lambda \text{ (nm)} \qquad \text{[kJ/mol]} \qquad (1\text{-}1)$$

Two empirical laws have been formulated about the absorption intensity. Lamberts law states that the fraction of the incident light absorbed is independent of the intensity of the source. Beer's law states that the absorption is proportional to the number of absorbing molecules. From these laws, the following relationship can be formulated:

$$\text{Log } [I_0/I] = \varepsilon L C \qquad (1\text{-}2)$$

or

$$\text{Log } [I_0/I]/L = \varepsilon C \qquad (1\text{-}3)$$

I_0 and I are the intensities of the incident and transmitted light, respectively; L is the path length of the absorbing solution in cm; and C is the concentration in moles/liter. Log (I_0/I) is called the absorbance or optical density, ε is known as the molar extinction coefficient and has a unit of 1000 cm^2/mole but the unit is, by convention, rarely expressed.

If a one cm (10 mm) quartz cuvet is used for the full range of UV and visible spectra, the above equation can be re-written as

$$\text{Log}\,[I_0/I] = \varepsilon C \qquad (1\text{-}4)$$

thus, it has unit of 1/cm.

The irradiation of organic compounds may or may not give rise to excitation of electrons from one orbital (usually a lone-pair or bonding orbitals) to another orbital (usually a non-bonding or anti-bonding orbital). It is shown that:

$$\varepsilon = 8.7\text{x}10^{19}\, P\, a \qquad (1\text{-}5)$$

where P is called the transition probability (with values from 0 to 1) and a is the target area of the absorbing system which is usually called a chromophore which will be discussed further in later sections. With common chromophores of the order of 10 Å long, a transition of unit probability will have a value of 10^5. This is close to the highest observed values, though the values in excess of this have been measured (with unusually long chromophores). In practice, a chromophore giving rise to absorption by a fully allowed transition will have values greater than about 10,000, which are those with low transition probabilities.

1.4.2.2.3 Correlation of UV Absorption with Various Chromophoric Structures

It should be pointed out that there is no easy rule or set procedure for identifying or investigating a chromophore system. Too many factors affect the spectrum and the range of structures which can be found is too great, especially in a complex chromophore system. In general, the usual procedure, as summarized by William and Fleming (1973), is to compare the UV spectrum of an unknown substance in its general shape and in the intensity and position of its peaks, within the spectra of reasonable model compounds. These models are chosen to possess as nearly as possible the same chromophore system as that suspected for the unknown. However, for typical and relatively simple chromophoric systems, such as conjugated dienes, unsaturated ketones, and some substituted benzene ring compounds, some rules, with the exceptions, for prediction of the behaviour of the chromophore system have been established (William and Fleming, 1973). In general, these rules demonstrate the two most important points: the longer the conjugated system, the longer the wavelength of the absorption maximum; and the longer a particular kind of chromophore, the more intense the absorption. With more complicated chromophores, predictions become more difficult. A detailed treatment with full theoretical considerations can be found in a review by Murrell (1963).

1.4.2.2.4 UV Absorption Spectra of Aromatic Compounds

As demonstrated by William and Fleming (1973) simple benzenoid compounds, that is, those which contain one ring and do not contain additional unconjugated chromophores, absorbs at 184 nm (e=60,000), 203.5 (e=7400) and 254 (e=204 nm) nm in a hexane solution. The peak at 184 nm is usually called the K-band and the latter band, sometimes called the B-band, shows vibrational fine structure.

The absorption bands of benzene arise from the transition of an electron from the highest occupied to the lowest vacant orbital of a benzene π–π^* transition. In the highly symmetrical benzene molecules the transition corresponding to the 254 nm peak is symmetry 'forbidden'. The observation of this band in low intensity is made possible by small vibrational distortions of the ring (Jaffé and Orchin, 1962). When an aromatic ring is substituted by various chromophoric systems, the hexagonal electron symmetry of the aromatic ring was distorted. This causes changes in the transition energies as well as in the intensities. According to the general rules summarized by William and Fleming (1973), the wavelength and intensity of the absorption peaks increase with an increase in the extent of the chromophores. Also, as more and more conjugations were added to the benzene ring, the K-band (originally at 203.5 nm) effectively moves to longer wavelength, called red shifts, and moves "faster" than the B-band (originally at 254 nm) eventually overtaking it

William and Fleming (1973) and Goldschmid *et al.* (1971) compiled the experimental investigations on UV spectra shifts by various substituted groups with electron donating or withdrawing at different positions on the aromatic rings. In brief, it can be qualitatively described as:

ortho-para directing (electron-donating): $O^- > NH_2 > OCH_3 > OH^- > Cl^- > CH_3$

meta-directing (electron-withdrawing): $CHO > COCH_3 > COOH > COO^- > NH_3^+$

Both series of substituting groups increase the electron flow between the substituent group and aromatic ring irrespective of the direction of the flow. Both types of substituents lower the transition energies by stabilizing polar excited states and causing red shift on the spectra. Among these groups, O^- and CHO will cause the greatest red shift. Moreover, di-substitution of an aromatic ring with groups of opposite types (donating and withdrawing) causes greater red shift

than that with groups of the same type. The greatest red shift will occur in the case of para-disubstitution with groups of the opposite type.

1.4.2.2.5 Characteristic UV Spectra of Lignins

Since 1927, when Herzog and Hillmer (1927a and 1927b) first discovered the characteristic UV absorption of lignins in the solutions, spectra of a large number of lignin preparations and lignin model compounds have been investigated and critically reviewed (Brauns, 1952; Brauns and Brauns, 1960; Pearl, 1967; Goldschmid, 1971). Surprisingly, it was reported that UV spectra of different lignin preparations were usually quite similar in their general shape (Kleinert and Joyce, 1957). In principle, lignins and their derivatives usually show a strong absorption spectrum in the UV region, because of, more or less, their aromatic nature as discussed in earlier sections.

After carefully comparing the UV spectra of hardwood lignins with softwood lignins, Sakakibara (1991) pointed out that the typical lignin spectra decrease from a maximum near 205 nm to a shallow minimum near 260 nm, with a pronounced shoulder around 230 nm. The minimum is followed by a characteristic lower maximum near 280 nm and a gradual decrease towards the visible range of the spectrum. Clearly, this spectrum is a composite of the absorption bands of the different phenylpropane units that constitute the lignin polymer. Hess (1952) resolved the UV spectrum of spruce Brauns' lignin into six symmetrical absorption bands; their maxima were at 228, 262, 282, 312, 331, and 351 nm. Japanese authors (Tiyama *et al.*, 1967) using a computer program resolved the UV spectra of spruce milled wood lignin and of thiolignin into six bands with similar λ_{max} values. More recently Sakakibara (1991) reported that the major differences among the softwood and hardwood lignins are: softwood lignin shows a maximum at 280 to 285 nm and hardwood lignin at 274 to 276 nm. Interestingly, in contrast with lignins, polysaccharides derived from wood are transparent in the visible and near-ultraviolet region.

1.4.2.2.6 Contributions of Constituent Groups on UV Spectra of Lignins

As discussed in earlier sections, a lignin spectrum is a composite of the absorption bands of the different functional structures that constitute the lignin polymer. Kleinert and Joyce (1957) systematically investigated the UV spectra of guaiacyl-compounds found in lignins. The study demonstrated that there was a definite maxima throughout the wavelength range from 205 to 340

nm. However, there were variations in the numerical values of these maxima and also in the general shape of the UV spectra, indicating the strong influence of the substituents. Isoeugenol, which possesses a double bond conjugated to the benzene nucleus shows, when compared to eugenol, an increased short wavelength UV absorption. Eugenol, having the ethylene group at the end of propane chain, exhibits the shoulder seen between 25 and 235 nm, probably by an independent resonating of the double bond at higher wavelength. In vanillin the aldehyde carbonyl also produces a second maximum at about 230 nm. Also, a strong influence in the long wavelength UV absorption can be seen.

In a systematic study on the contribution of various functional units in lignins to the characteristic UV absorption, Aulin-Erdtman and Sandén (1968) examined the UV spectra of model compounds of the basic units of lignins with the non-ionized and ionized forms, *p*-hydroxyphenyl, guaiacyl, and syringyl derivatives with identical side-chains on the *p*-position. In addition, UV spectra (about the positions and intensities of the peaks) of various model structures with similar chemical bonds have also been extensively investigated in many other studies (Pew, 1962, 1963; Doub and Vandenbelt, 1955) and have been well summarized by Goldschmid (1971) and Sakakibara (1991). A few important points are taken here as a background to the discussions in following chapters in this study.

1. Phenolic Hydroxyl Groups

Free and etherified hydroxyl groups contribute significantly to the characteristic absorption maximum of the lignin UV spectrum near 280 nm. In neutral solutions, the spectra of free and etherified phenols are nearly identical. In alkaline solutions, on the other hand, ionization of the hydroxyl group causes red shifts.

2. Carbonyl Groups

a) Conjugated Carbonyl Groups

Carbonyl groups or double bonds conjugated with the aromatic rings are thought to contribute to the high absorption of the lignin UV spectrum in the region of 300 to 400 nm. Specifically, lignin units of Type 1 and Type 2 absorb near 350 nm; the Type 1 absorbs only in alkaline solutions when the hydroxyl group is ionized, whereas the latter absorbs near 350 nm at all pHs.

b) Non-conjugated carbonyl groups

In neutral solutions, as found in Figure 1-8, UV spectra of the model compounds such as G-CH_2CO-Me, S-CH_2CO-Me and G-CH_2-CO-CH_2OH were similar to those of simple guaiacylpropane compounds. They may have possible absorption maxima around 270 to 310 nm, but the intensity was lower than that of the maxima of phenolic conjugated α-ketones by a factor of 10. β-carbonyl groups, if present in lignins, have no effect on the neutral spectra. They may have a slight effect on the alkaline spectra around the 310 nm region where etherified aryl-α-carbonyl groups absorb.

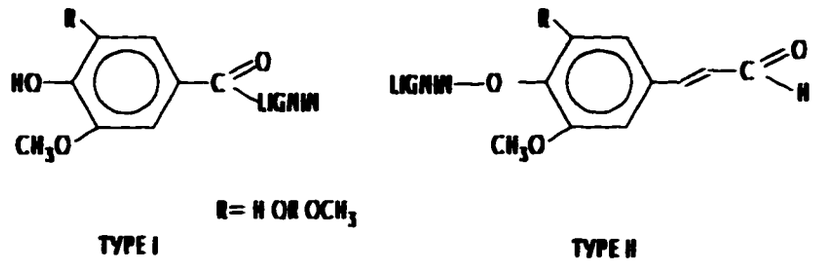


Figure 1-8. Typical Conjugation Structures in Molecules of Lignin Derivatives

3. Conjugated α–β Double Bonds

They usually have absorption maxima around 260 and 300 nm.

4. Biphenyl Groups

In principal, biphenyl structures usually have high absorption around 250 nm and 350 nm, and produce red shifts in characteristic UV spectra. The biphenyl structures are also the major structures contributing to the difference between characteristic UV spectra of lignins and the UV spectra of model structures.

1.4.2.3 Molecular Weight (MW) and Molecular Weight Distribution (MWD) of Lignins and Lignin Components

Knowledge of the MW and MWD of lignins and lignin derivatives is of importance for the elucidation of their macromolecular properties and better understanding of the behaviours of lignin derivatives during various physical, chemical and biological treatment processes. Consequently, many attempts have been made to determine the MW and MWD of lignin derivatives. As a result, a series of analytical methods based on the understanding of the physical

and chemical properties of lignins have been developed over past decades although none of them have given entirely satisfactory results. This was to be expected with such complex biopolymers as lignins. These methods include the cryoscopic, ebullioscopic, osmotic pressure, light scattering, ultracentrifuge, diffusion, conventional gel filtration, and various combinations of the above methods. The advantages, limitations and applications of these methods to the estimation of the MW and MWD of various lignin derivatives (up to 1971) have been well summarized by Brauns and Brauns (1952 and 1960), Pearl (1967) and Goring (1971). Since then, with the advance in analytical instrumentation, great progress has been made in developing more effective means of estimating the MW and MWD of lignin components in various non-aqueous and aqueous systems. In particular, the high performance size exclusion chromotography (HPSEC) has been greatly enriched recently by the advent of various advanced detector systems such as the combination of UV, RI, real time differential viscometer and the low angle laser light scattering photometer, etc. With these advanced detector systems, proper solvents and, effective calibration techniques the HPSEC has been repeatedly reported to be one of a reliable, simple, and effective means to estimate the MW and MWD of lignin derivatives under various conditions. For example, Himmel *et al.* (1989) has successfully determined the MWD of Aspen lignins using universal calibration; and Siochi *et al* (1989), Forss *et al.* (1989) and Rudatin *et al.* (1989) have estimated the MW and MWD of hydroxypropylated lignins and various kraft lignins, respectively, using various combinations of these techniques. The limitations and application of the HPSEC to characterization of MW and MWD of lignin derivatives in pulp mill effluents related to these study is discussed in some detail in Chapter 3.

Lignins, even as they exist in the original state in the living wood, are thought to be polydisperse with respect to the MW. This is because the enzymatic action in the biosynthetic process is limited to the formation of cinnamyl alcohol radicals and the subsequent coupling between these radicals to form lignins can not be expected to make any uniform structure, nor uniform molecular weight. In addition, as discussed in earlier sections, the multifunctions of lignin in the wood may also play a role in the evolution of lignin biosynthesis, thus contributing to the polydispersity. Moreover, the isolated lignins through various lignin preparation are in fact of lignin derivatives. Specifically, the isolated lignins are the various heterogeneous sizes of fragments of original lignin molecules, especially those from chemical preparation processes as discussed in numerous studies (Brauns and Brauns, 1960; Pearl, 1967; Goring, 1971;

Freudenberg 1955). As a result, the so-called lignins should perhaps be regarded as the mixtures of molecules, all possessing similar chemical structures, but with the possibility of certain structural and molecular weight difference.

As expected, the reported MW of various lignins and lignin derivatives are found to be distributed over an immense range, from less than 1000 (Rezanowich and Goring, 1960) to greater 10^6 g/mol for both lignosulphonates and alkali lignins (Gupta and Goring, 1960). However, more recent studies (Himmel *et al.*, 1989; Forss *et al.*, 1989; Froment and Pla, 1989), with the aid of advanced analytical systems, found that the MWD of various lignin preparations are quite similar with respect to the mode and shape of the distribution and close to the shape of normal distribution with some tailing peaks related to the various factors in preparation. Also, the majority of dissolved lignin molecules were found to be in the range of about 1000 to 100,000 g/mol. Some representative MW data of various lignin derivatives are listed in Table 1-2.

Table 1-2. Molecular Weight and Polydispersity of Some Lignins from Some Representative Preparations (Goring, 1971)

Wood	Method of Preparation	Range of MW	M_w/M_n
Spruce	Sulphonation	324 to 20,500	6.5
Spruce	Alkali Cook of periodate lignin	35,000 to $1.7x10^6$	5.7
Hemlock	Sulphonation	440 to 58,000	6.4
Spruce	Dioxane+HCl	4,300 to 85,000	3.1
Pine	Kraft	3,500 to 50,000	2.2
Hardwood	Kraft	2,900 to 100,000	2.8
Spruce	Kraft	1,800 to 51,000	>3

Mw=weight averaged molecular weight; Mn=number averaged molecular weight

The polydispersity of large polymer molecules is usually evaluated by the value of ratio of weight averaged MW (M_w) to the number averaged MW (M_n), M_w/M_n (Meister and Richards, 1989; Goring, 1971). These ratios varied widely with the methods for MW determination, the methods for lignin preparation, the source and form of lignins as well as the time of the MW data collected. Some examples of the ratios M_w/M_n for various lignin fractions and preparations are

also shown in Table 1-2. In addition, some recent studies (Sakakibara, 1991) found that the protolignin tends to consist of an immensely large polymer; hardwood lignins appear to have a tendency of lower MW than softwood lignins, which might also suggest that guaiacyl units are biopolymerized into higher MW structures than syringyl units.

1.4.2.4 Biodegradability and Biodegradation of Lignins and Lignin Components

As shown in earlier sections, unlike other natural polymers such as cellulose, proteins, and nucleic acids, lignins do not have a readily hydrolyzable bond recurring at periodic intervals along a linear backbone. Instead, the pronounced structural complexity of the lignin molecules is a three-dimensional, amorphous polymer with a seemingly random distribution of stable carbon-carbon and ether linkages between monomeric units (see Figures 1-4, 1-5 and 1-6). These types of structures are not amenable to normal modes of enzymatic degradation and naturally gives the biological recalcitrant property which in fact is one of the major functions that the lignins must perform to protect the living wood from infections or attacks by various microbial or pathogenic activities. However, it is certain that the lignins and lignin derivatives are biodegradable by some of microorganisms under proper environmental conditions although there existed some artifacts in those earlier studies due to the limitations on experimental design, and preparation and analysis on raw and degraded lignin products (Kirk, 1971; Crawford, 1981).

Over past decades, most of the studies (Crawford, 1981; Kirk *et al.*, 1980) have focused on screening, identifying, and evaluating the ability and effectiveness of fungi on degrading lignins in-situ and in-vitro. In these studies, a variety of fungi have been proved to be lignin degraders. These fungi can be classified into three major categories based on the type of wood decay caused by these organisms: white-rot, soft-rot, and brown-rot fungi.

Brown-rot fungi are usually defined as those wood-rotting fungi that decompose and remove wood carbohydrates, leaving a residue of modified lignins that is typically dark brown and almost equal in weight to the lignins in the original wood (Kirk, 1971; Ander and Eriksson, 1978). Brown-rot fungi appear initially to decay lignins in a manner similar to white-rot fungi. However, the brown-rotters apparently do not efficiently cleave lignin's hydroxyl-activated rings, or if they do open the rings, they are unable to significantly decompose resulting aliphatic moieties (Kirk, 1971). Thus, strictly speaking, the brown-rot fungi is the lignin modifier not lignin degrader or rotter.

Soft-rot fungi usually attack moist wood, producing a characteristic softening of surfaces of the woody tissues (Savory, 1954; Corbett, 1965; Ander and Eriksson 1978). As reported by Crawford (1981) and Shimada and Higuchi (1991), many species of soft-rot fungi are able to extensively degrade the lignins under various conditions. For example, two of fungi were reported to be able to degrade wood component of up to 92% of beech wood and lignin loss up to 45%, respectively. In addition, many poorly classified fungi, including those soil fungi, have also been reported to be able to degrade the lignins extensively.

Though the above evidence strongly implicates the important role for soft-rotters, brown-rot and other fungi as lignin degrades, inadequacies in methodologies and experimental design still left room for doubt as to the true lignin-degrading abilities of these fungi (Ander and Erriksson, 1978; Crawford, 1981; Shimada and Higuchi, 1991). Thus, before an understanding of the true importance of these fungi as lignin decomposers can be fully realized, additional work is required, preferably using ^{14}C-labelled DHP's and ^{14}C-lignin. In addition, there is little understanding concerning the enzymatic mechanisms whereby soft-rot fungi convert lignins to CO_2.

Of all the ligninolytic groups of fungi, the white-rot *Basidiomycetes* are probably the most efficient of all known lignin degrades. White-rot fungi are usually thought of as those fungi that are able to extensively decompose all the important structural components of wood, including both cellulose and lignins with ultimate formation of CO_2 and H_2O under the proper environmental conditions (Kalahatai, et al., 1974; Ander and Erriksson, 1978). In addition, producing extracellular lignase (enzymes which are probably involved in the processes of lignin degradation, see section 1.5.4) may be the fundamental features which distinguished white-rot fungi from nonligninolytic fungi (Kirk, 1971).

A large number of species of white-rot fungi belonging to a variety of fungal families were reported to be able to extensively degrade or alter various lignin preparations. For example, Lundquist *et al.* (1977) reported that the synthesized radiolabelled lignin was substantially degraded to nonvolatile products and some to CO_2. The authors concluded that industrial lignins could also be significantly bioalterable in the decolorization process and that, under favorable conditions industrial lignins were substantially biodegradable. However, different species degrade the various components of wood at different rates. Some deplete the lignins and carbohydrate components of wood at about the same proportional rates and others remove the

lignins from wood in preference to cellulose or hemicellulose (Ander and Erriksson, 1977; Lundquist *et al.*, 1977; Fukuzumi, 1980; Shimada and Higuchi, 1991). These studies also pointed out that among those lignin-degrading white-rot fungi, several species such as *Phanerochaete chrysosporium (ATCC 24725)*, *Coriolus versicolor* (ATCC34540), *Tinctoporia borbonica* have shown exceptional good lignin degradation ability under natural and simulated environments. A detailed summary on the characteristics of these representative fungi can be found in the reviews by Crawford (1981), Kirk, *et al.*, (1980), Boominathan and Reddy (1992), and Shimada and Higuchi (1991).

According to these findings and after preliminary screening studies, the white rot fungus *Phanerochaete chrysosporium* was selected as the microorganism for decolorization and dechlorination of kraft pulp mill effluents in this study. This is not only because this fungus is most commonly used model organism in previous lignin biodegradation studies; but also it has many unique features such as rapid growth and metabolisms of lignin, ability to grow optimally at relatively high temperature (up to 40°C), the ability to produce cornidia (asexual spores) and basidiospores (sexual spores, important advantage for easy culture of biomass), strong ability to grow on chemically defined media, and the species have been studied most extensively with respect to their taxonomy, ecology, physiology, biochemistry, molecular biology, genetics; and effectiveness on lignin degradation under various conditions.

The true range of microorganisms that are able to degrade lignins has been the subject of much debate in recent years. Of the three generally recognized groups of saprophytic microorganisms such as fungi, actinomycetes (filamentous bacteria), and eubacteria, only certain fungi previously had been thought to play a role in lignin degradation (Kirk, *et al.*, 1971; Kirk *et al.*, 1977). However, it is now generally agreed that lignin degradation is not an ability limited to the wood-rotting "white-rot" fungi. As summarized by Crawford (1981) and Kirk, *et al.* (1977), numerous bacteria have been reported to decompose lignins and lignin derivatives. These included *Pseudomonas spp.*, *Flavobacteria*, *Xanthomonas spp.*, *Bacillus spp.*, *Aeromonas spp.*, *Cellulomonas spp.*, *Chromobactria*, *etc.* Moreover, fundamental research into the biological processes for pulping and waste treatment has made some significant advances owing partly to the advent of gene-manipulation technology. It has been reported that some microbes have been engineered with the capability to produce enzymes that degrade lignins (Anon., 1984b; Anon., 1986; Srinivasan, 1987), at least under laboratory conditions. For example, a U.S. patent

(4,444,888) has even been granted for *Pseudomonas Aeruginosa*, "a novel microbe strain capable of decolorizing pulp and paper mill wastewater". However, as Kirk (1971 and 1977) and Crawford (1981) pointed out, in many of the earlier studies, weaknesses in experimental methods may have led to erroneous or at least questionable conclusions. For example, the publicized trial of the above patented bacterial mutants failed in the field for decolorization of kraft pulp mill effluents (Dogherty, 1982). Even some of the recent reports were equivocal because of problems with nonrepresentative or chemically modified lignins being used as growth substrates, and methods used to estimate residual lignins in culture media. Ander and Eriksson (1978) largely agreed with Kirk (1971 and 1977) and Crawford (1981), concluding that available evidence indicated that some bacteria may mediate a certain amount of lignins, but their true abilities and effectiveness in this regard were uncertain.

More recent experiments with better controlled designs appeared to confirm that although the bacterial lignin degraders are not distributed as widely as fungi, and do not decompose the lignin as extensively as fungi there is no question about the abilities of a pure bacteria culture to attack all lignin structural components such as methoxyl groups, aromatic rings and side chains. For example, with radio labelled lignin ^{14}C-DHP or ^{14}C-[LIGNIN] as substrates many studies (Trojanowski *et al.*, 1977; Haider, *et al.*, 1978; Gradziel *et al.*, 1978; Rabinson and Crawford, 1978; Phelan, *et al.*, 1979) have revealed that the species, such as *Nocardia, Bacillus, Actinomycete spp.* (as Streptomyces), *Pseudomonas,* are able to degrade the components and lignin derivatives. Among these species, some required the co-substrate, some of them even could use lignins or so-called lignin derivatives as a sole substrate. Among those requiring the co-substrate, it appeared that utilization of fungal-bacterial associations often speeds the degradation of lignincellulosic materials as compared to the degradation kinetics by either fungi or bacteria in pure culture. For example, Blanchette and Shaw (1978) observed significant increases in wood decay (weight loss) during 5 months decay treatments by combining bacteria (*Enterobacter sp.*) and yeasts (*Saccharomyces baillii* var. *bailii* and *Pichia pinus*) with wood-rotting basidiomycetes such as *Coriolus versicolor, Hirschioporus abietinus,* and *Poria placenta.* In this mutualistic relationship the bacteria were thought to increase fungal growth and catabolism by supplying vitamins or other growth promoting substances to the fungi. The bacteria in turn were able to utilize wood decay products released by fungal attack on the woody cell walls.

The further discussions about biodegradation, biodegradability, biodegradability tests, and mechanisms of biodegradation of lignin components, especially related to pulp mill effluents, are presented in Section 1.5.4 and Chapters 2, 3 and 5.

1.4.2.5 Correlation of Color with AOX, Toxicity, Biodegradability, and Molecular Weight

As discussed in earlier sections, the molecular chlorine or chlorine-containing compounds currently used as bleaching agents react with lignin components and other organic materials such as degraded cellulose, hemicellulose, and extractives released from wood during the pulping process, resulting in the formation of chlorinated organic compounds with various MWs. In comparison with their non-chlorinated analogues, chlorinated organics compounds may become more toxic; more lipophilic and therefore bioaccumulative; less biodegradable; and mutagenic. The type and concentration varied greatly with pulping and bleaching procedures employed by a mill. It is beyond the scope of this study to discuss the physical and chemical properties of all of the individual chlorinated organic substances found in bleached pulp mill effluents to date. Recently, Suntio, *et al.*, (1988) have described many environmentally relevant properties, such as specific octanol/water partition coefficients, bioconcentration potentials, of over 250 chemicals identified in pulp mill effluents. However, there is limited information concerning the correlation of AOX, MW, MWD, biodegradability of various pulp mill effluents in the existing literature. Obviously, these type of information are fundamental to the studies for developing advanced practical and economical processes for decolorization and dechlorination of pulp mill effluents. The comprehensive investigations on the compositions of various pulp mill effluents and the correlation of critical characteristics will provide fundamental knowledge to better the understanding of these processses.

1.4.2.6 Lignin Reactions

1.4.2.6.1 Possible Reactions in Kraft Pulping Processes

During the kraft pulping process, the majority of the lignins and some of the carbohydrates are dissolved simultaneously in an alkali solution, the former called a delignification process and the later called a peeling process. Sjostrom (1981) reported the competitive development of peeling and delignification processes in the kraft pulping. It was demonstrated that the carbohydrates were attacked already at a comparatively low temperature. It was also reported that the acetyl groups were completely removed and peeling process was

terminated long before the maximum cooking temperature had been attained. The reactivity of the polysaccharides varies depending on their accessibility as well as on their structures. Because of its crystalline nature and high degree of polymerization, cellulose suffers less losses than the hemicelluloses. The majority of the lignins are degraded or modified in the delignification processes, and the degraded lignins are usually dissolved in the cooking liquor as sodium phenolates. Hydrosulfide ions in the pulping liquor also react with the residual lignins in the pulp, but most of the sulfur-containing lignin products are decomposed during the later stages of the cook, with formation of elemental sulfur which combines with hydrosulfide ions to form polysulfide. However, the kraft lignins still contain 2-3% of sulfur corresponding to 20 to 30% of the charge. Some examples of the losses of various components from a few typical wood sources during two major pulping processes are presented in Table 1-3. These data also may indicate that some attacked carbohydrates in the pulping processes could be carried to the the bleaching processes which would further react with bleaching chemicals and represent a large portion of BODs and color body exerted by the bleaching effluents.

Table 1-3. Yields of Various Pulp Constituents after Sulfite and Kraft Pulping of Norway Spruce (Picea abies), Scots Pine (Pinus sylvestris), and Birch (Betula verrucosa) (after Sjöström, 1981)

	Spruce Sulfite		Birch Sulfite		Pine Kraft		Birch Kraft	
Constituent	in pulp	in wood	in pulp	in wood	in pulp	in wood	in pulp	in wood
CARBOHYDRATES								
Cellulose	41	41	40	40	35	39	34	40
Hemicellulose	5	18	1	3	4	17	1	3
Xylan	4	8	5	30	5	8	16	30
Others		4		4		5		4
Total Carbohydrates	50	69	46	74	44	67	51	74
LIGNINS	2	27	2	20	3	27	2	20
EXTRACTIVES	0.5	2	1	3	0.5	4	0.5	3
TOTAL	52	98	49	97	48	98	54	97

Most of the reactions in the alkaline delignification processes have been well clarified (Gierer, 1970; Maton, *et al.*, 1969; Maton, 1971). Table 1-4 lists some of the representative reactions and their characteristics in each category according to product analysis and model

compound studies. The details have been described in many excellent literatures (Pearl, 1967; Brauns and Brauns, 1960; Sarkanen and Ludwig, 1971; Sjöström, 1981). As shown in Table 1-4, the major reactions leading to the dissolution of the lignins under alkaline pulping conditions are the cleavage of α- and β-ether bonds in phenolic units of the lignins and of β-ether linkages in nonphenolic units of the lignins. Cleavage of ether linkages, promoted both by hydroxyl and hydrosulfide ions, results in increasing the hydrophilicity of the lignins because of the liberation of the phenolic hydroxyl groups. The major types of the reactions can be briefly summarized as follows.

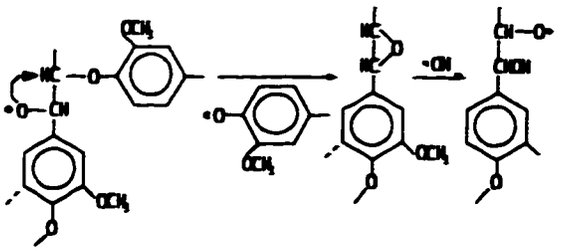
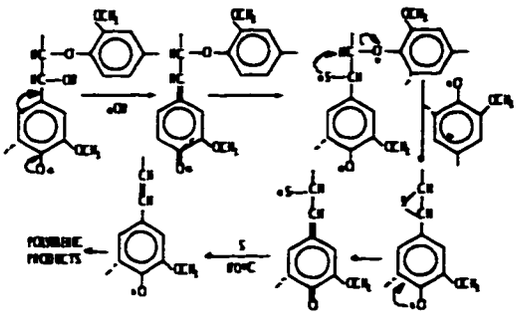
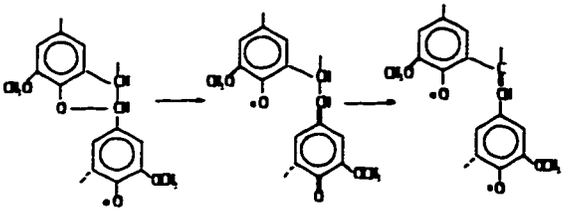
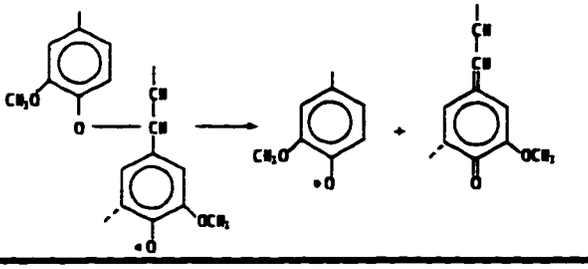
Alkaline cleavage of α-ether structures proceeds through the formation of quinonemethide intermediates, which require a phenolic hydroxyl group. Consequently, the α-ether linkage of nonphenolic units is essentially stable in alkali. The cleavage of β-Aryl ether bond and carbon-carbon bond and the condensation reactions also occurs simultaneously.

1.4.2.6.2 Formation of Chromophores in Kraft Pulping Processes

During kraft delignification, it has been reported that the specific light absorption coefficient of the residual lignins increases continuously, reaching ca. 500 m^2/kg at the end of pulping (Sjöström, 1981). For comparison, the corresponding value for wood lignins is about 20 to 40 m^2/kg. Thus, it is presumed that various chromophores may be generated from the various lignin reactions involved in the kraft pulping processes discussed above. In addition, some leucochromophores, which can be converted into chromophores by air or anti-oxidation, may also be present as intermediates in the pulp although some chromophoric groups can also be introduced into the polysaccharides. Figure 1-9 summarizes the major types of chromophoric groups which may derived from the lignin reactions involved in kraft pulping processes.

In the bleached kraft process, these chromophoric structures may be carried through pulps to the bleaching stages and dissolved in the bleaching spent liquors which may contribute to brownish color of pulp mill effluents.

Table 1-4. Representative Reactions of Lignins in Kraft Pulping Processes

Reactions	Illustration	Characteristics
cleavage of β-aryl ether bonds contained in etherified phenolic structures		This reaction promotes efficient delignification by fragmenting the lignins and by generating new free phenolic hydroxyl groups
cleavage of β-ary ether bonds contained in free phenolic structures		major mechanisms responsible for cleavage of β-ether bonds, e.g. delignification and reduce sulfur contents in pulping liquor
cleavage of α-ether bonds contained in various structures of lignin linkage	cleavage of α-ether bonds  cleavage of open α-aryl ether structure 	α-ether bonds are stable in all etherified structure but are readily cleaved in phenolic phenylcoumaran and pinoresinol structures; however, only in the case of open α-aryl ether structures the cleavage results in fragmentation of lignins.

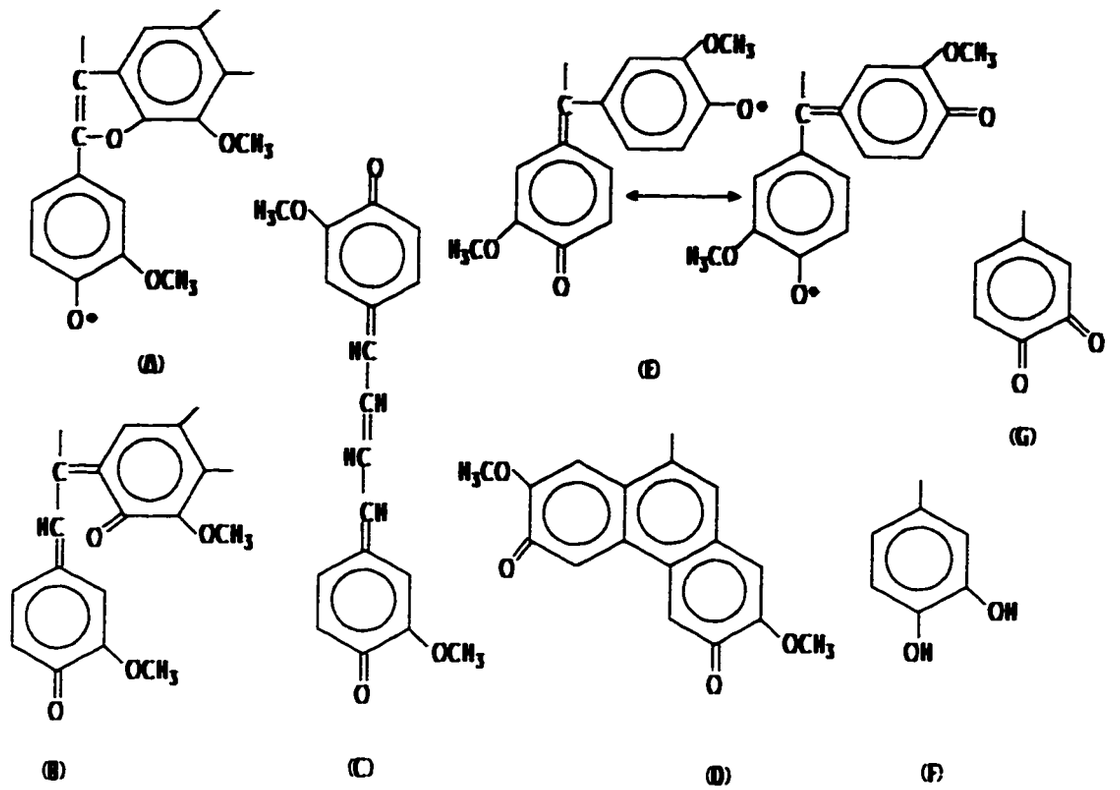


Figure 1-9. Examples of Proposed Leucochromophoric and Chromophoric Groups Derived from Lignins Involved in Pulping Processes (After Sjöström, 1981)

1.4.2.6.3 Possible Lignin Reactions in Bleaching Process Using ClO_2

Most of the bleaching processes using ClO_2 were usually carried out under acid conditions at elevated temperature since ClO_2 can be reduced to chlorite ions under slightly acidic conditions (pH 4 to 5):

$$ClO_2 + 4H^+ + 5e^- \longrightarrow Cl^- + 2H_2O$$

In this reaction five oxidation equivalents are released. On the other hand, in the alkaline media, ClO_2 is reduced to chlorite involving a change of only one oxidation equivalent:

$$ClO_2 + e^- \longrightarrow ClO_2^-$$

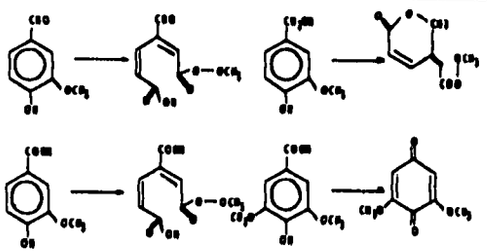
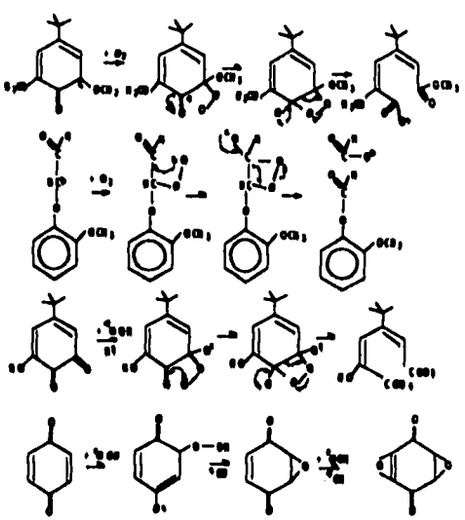
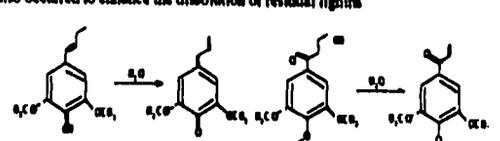
Also, two chlorine dioxide molecules disproportionate to form chlorate and chlorite ions in the presence of hydroxyl ions

$$2\,ClO_2 + 2\,OH^- \longrightarrow ClO_3 + ClO_2 + H_2O$$

The oxidation pathways of ClO_2 with lignin components under actual conditions are complex because a number of species including chlorine, chloric acid and chlorite are formed as intermediates. It is certain, however, that ClO_2 reacts rapidly with both free and etherified units at 60 to 70 °C but only sluggishly with etherified units at room temperature. Specifically, the free phenolic structures are oxidized faster, but chlorine dioxide also destroys the non-phenolic phenyl propane units and the double bonds present in the chromophores. After cleavage of the benzene ring various di-carboxylic acids may be formed, such as oxalic, muconic, maleic, and fumaric acids in addition to the products substituted with chlorine. As a result of depolymerization and formation of carboxyl groups the modified lignins are dissolved during chlorine dioxide treatment and in the sodium hydroxide extraction stage that usually follows. Studies on model compounds also showed that demethylation, ring cleavage, chlorination and *o*- and *p*-quinone formation all take place (Pearl, 1967; Sjostrom, 1981). Some of these representative lignin reactions and their characteristics in various bleaching stages are listed in Table 1-5.

As indicated in Table 1-5 the overall bleaching processes are dynamic processes in the generation and destruction of chromophores since in some stages, the reactions effectively destroy the chromophoric structures, but in others such as in E_{op} stage the reactions involved in oxygen/hydrogen peroxide may produce significant amount of chromophores. For example, it was reported that the lignin content of a softwood pulp after the chlorination and extraction stages decreased from about 2 to 3% to approximately 0.5 to 1%. The brightness of the pulp is 25 to 35% (ISO) and the light absorption coefficient (k) at 457 nm is 20 to 40 m^2/kg (Sjöström, 1981). The corresponding k value for the residual lignins is at least 2000 m^2/kg, indicating a substantial increase either in the number of chromophoric groups or in their absorptivity, or both.

Table 1-5. Representative Reactions in Various Bleaching Stages

Bleaching Stage	Reaction Conditions	General Characteristics	Possible Reactions with Lignins
Chlorine Dioxide (D) Stage		Although the free phenolic structures are oxidized faster, ClO_2 also destroys nonphenolic phenyl propane units and double bonds present in the pulp chromophores. After cleavage of aromatic rings various dicarboxylic acids are formed, such as oxalic, muconic, maleic, and fumaric acids in addition to products substituted with chlorine. As a result of partial depolymerization and formation of carboxyl groups the modified lignins are dissolved during ClO_2 stage and in E stage that usually follows.	
Alkaline Extraction with Oxygen/ Peroxide (Eop) Stage		Oxygen and peroxide bleaching have common features because in both cases the medium is alkaline, and the same reactiv species (oxygen and peroxides) are present, although in different proportions. The structures in lignin are converted in alkali to carbanions and conjugated carbonyl structures giving rise to electron attracting or repelling positions. As an electrophilic reagent, oxygen prefers negatively charged positions, whereas the nucleophilic peroxy anion (HOO-) reacts with positively charged positions. Both oxygen and hydrogen peroxide react with organic compounds to form hydroperoxides, although via different routes. By action of oxygen, lignin is degraded and chromophoric structures are formed, whereas hydrogen peroxide eliminates chromophores without any marked decomposition and dissolution of the lignin. However, the potentially oxidizable elements in the lignin system would probably not be available to react with the oxidants with the same ease as the corresponding structures in the model compounds, because in the mixture of lignin and pulp these structural elements are probably much less accessible.	
Alkaline Extraction (E) Stage		Conversion of phenolic hydroxyl and carboxyl groups to their more hydrophilic salts by alkali at high temperature, as a result, extensive lignin dissolution and simultaneous removal of about 70% of chlorine substituted in lignin took place. Absorption coefficient for the residual lignins increased significantly indicating the generation of chromophoric groups	In addition to those reactions discussed in pulping process the following reactions also occurred to enhance the dissolution of residual lignins 

1.5 Pollution Abatement Technologies

Today, a broad range of methods are being used to mitigate the impacts from pulp mill effluents on the aquatic environment. This variety of methods reflects the diverse approaches adopted by different regulatory agencies, differing management philosophies, and even geography. In particular, the pollution load from various bleaching processes has been reduced dramatically by various practices: One approach is to modify the pulping and bleaching processes to minimize the quantities of effluents and chlorinated organics produced within the plant, and thereby reduce discharge quantities. For example, oxygen (pre-)bleaching, enabling the effluents from this stage to be combined with cooking spent liquors and thus entering the chemical recovery system. This is usually a preferred approach in Europe to limit the impact of effluents on the aquatic environment. Another approach involves the development of the closed cycle bleached kraft pulp mill. The third approach is to use external pollution abatement systems. In reality, most of mills, more or less, now still rely on external treatment system to meet increasing regulatory requirements for discharge.The following sections provide a overview of existing pollution abatement technologies.

1.5.1 Existing External Treatment Technologies for Reduction of Conventional Pollutants

Most of the existing end pipe treatment systems use the primary and secondary treatment. Primary treatment followed by biological treatment such as aerated stabilization basins (ASB) and various activated sludge processes, are being widely practiced. These aspects have been comprehensively compiled by Environment Canada (1976), Joyce *et al.* (1986), and Mohammed (1990) over different periods. In brief, these technologies are able to reduce the concentration of many conventional pollutants such as BOD, TSS by greater than 90% but provide less than 30% reductions in color and chlorinated organics in bleached pulp mill effluents(Rush and Shannon, 1976; Obiaga and Ganczarczyk, 1974). This is largely due to the inability (or very slow biodegradation kinetics) of the microbial population to metabolize the lignin components.

1.5.2 Overview of Technologies for Removal of Color, AOX and Toxicity

There are two general strategies for the reduction of color and toxicants from pulp mill effluents. The first strategy attempts to modify the manufacturing process so that less color bodies and toxicants are produced or released. For example, recent developments in process

technology (such as oxygen/ozone bleaching and dynamic bleaching) have greatly reduced the effluent volume and load in the bleaching plant, and Rapson-Reeves concepts (Rapson, 1967; Reeve and Rowlandson, 1978) may eventually make the effluent free mill more widely feasible. However, implementation of these technologies is currently expensive. In addition, process modifications may not be technically feasible in all mills because of the lack of flexibility within the mill or a basic incompatibility between the proposed process modification and the desired end products. Furthermore, in most cases, the effluents, from the mills, even with most advanced in-plant technologies, still can not meet the regulatory standards. Therefore, alternative strategies are needed to satisfy the regulation requirements. The alternatives include advanced physical, chemical and biological processes such as ultrafiltration, carbon absorption, massive lime precipitation, ozonation, or fungi decolorization. These technologies appear to be technically feasible and usually have the least impact on the manufacturing facilities, but are typically the most expensive. The details and recent advances in these technologies have been comprehensively reviewed by Environment Canada (1976), Joyce and Petke (1983), and Springer (1985).

1.5.2.1 Physical/Chemical Processes

The available physical/chemical technologies can be categorized into two types: destruction and separation/concentration. The principle of physical processes is to separate or concentrate the large molecules, in particular, chromophoric organics, from the smaller molecules non-selectively. The efficiencies of the processes strongly depend on the molecular weight distribution, the nature of the organics, the characteristics of the process and the operating conditions. The major physical/chemical processes investigated for this purpose are irradiation, O_3/H_2O_2, $O_3/UV(\gamma)$, and various other advanced oxidation processes (AOPs) and heat destruction processes. Table 1-6 briefly summarizes the major characteristics and their current application states of these technologies. Tables 1-7 and 1-8 selectively compare the costs of some alternatives for reduction of color from E-stage and combined pulp mill effluents based on 1980 dollar values (Springer, 1985). As seen in Tables 1-6, 1-7 and 1-8, most of the technologies in this category merely concentrate the color or toxicants besides its expensive nature; a means for destruction of chromophore or toxicants must ultimately be exploited.

Consequently, it is imperative to develop alternative low-cost and technically-feasible technologies for ultimate removal of color and chlorinated organics as well as of other pollutants.

1.5.2.2 Biological and Various Combined Processes

As summarized in Table 1-6, the conventional biological treatment processes effectively reduce the concentration of individual resin and fatty acids in kraft mill effluents to levels that, in most instances, are below acutely lethal values. For example, biological treatment normally reduces the concentrations of non-chlorinated resin acids by up to 90% and the concentrations of fatty acids by over 70% (Dellinger, 1980; Rogers *et al.*, 1975; Leach *et al.*, 1977; Chung *et al.*, 1979; Willard, 1983). Usually chlorinated resin acids were found to be more resistant to biological treatment (Leach *et al.*, 1977; Chung *et al.*, 1979) and both chlorinated and non-chlorinated lignin components in pulp mill effluents have been repeatedly reported to be extremely biological recalcitrant in many situations.

As discussed in Section 1.4 and summarized in Table 1-6, up to now only some decolorization processes using fungi have been technically verified for removal of color and AOX derived from chlorinated and nonchlorinated lignins although some trials were made using various bacteria and other microorganisms. There is little information available concerning hybridizing the physical, chemical and biological processes for ultimate elimination of color body in pulp mill effluents although some concepts exist as recently pointed out by Springer (1985):

> "Further work should be done to discover the minimum amount of ozone that will allow subsequent biological treatment to decolorize pulp and paper mill effluents. Studies to date have dealt mainly with the ozonation process itself, and not the combined sequence of ozonation followed by biological treatment. It is possible that the right combination sequence might be economically viable."

Table 1-6 also lists some reported trials on biological and combined decolorization and dechlorination processes. The detailed discussion on fungi decolorization will be addressed in Sections 1.5.4.

Table 1-6. Summary of Characteristics and Application States of Various Color Reduction Technologies

Process	Agents Used	Effect on Color	Advantages	Disadvantages	Applied Scale	Reference
ozone decolorization	ozone	destroy	effective, 5 to 10 C.U./mg O_3; short retention time; can achieve any degree of decolorization; compatible with existing or new treatment process	ineffective in dechlorination expensive; has to be generate on site; carrier gas handling; high maintenance	pilot	Bauman and Lutz, 1974; Coburn, *et al.*, 1984; Melynk, et al, 1977; Rice and Browning; Roy-Arcan, et al, 1991.
biological decolorization (MyCoR)	various fungi	destroy	effective; can achieve greater than 80%; high level dechlorination simultaneously;	longer retention time; limited life span of fungi; contamination problems immature bioreactor design; relatively expensive;	small or pilot	Boman, et al, 1988; ANON, 1982; Anon., 1984b; Campbell, 1983; Gerrard, 1983.
other AOPs	radicals produced from H_2O_2/UV /ozone	destroy	widespread acceptance; effective; short retention time; can achieve any degree of decolorization; compatible with existing or new treatment process	relatively expensive; it using ozone, has to be generate on site; carrier gas handling; high maintenance; color effects on the effectiveness of UV	pilot	Anon., 1884a; Smith, 1990; Peyton, 1990.
photocatalysis /photo-oxidation	UV or γ-ray Titanium dioxide	destroy	emerging technology; compatible with ozone and other AOP processes potentially completely decolorization and dechlorination; simultaneous COD (50 to 80%)and BOD removal, lower pH;	may be expensive; only tested at bench scale; irradiation hazards; pretreatment for removing TSS and biodegradability is necessary	bench	Turchi, et al, 1989; Higashi, et al, 1991; Lenz, et al, 1971; Haberl et al,1991.
electrochemical processes	electrolysis	destroy	effective; short retention time; temperature has neglect effect on efficiency; simultaneous reduction in COD and toxicity; require relatively small space; color reduction up to 80% (entire bleach kraft effluent) and up to 90% with caustic extraction effluent;	high in operating cost; toxic by-products; inefficeincy in reactor design in developing stage	bench	Gupta, (1979); Sameshima, et al, 1975; Miguro, et al, 1976; Klaus, 1978; Barringer Research, 1976; Heren and Woodard, 1976; Nassar, et al, 1983.
thermal destruction	heat/oxygen	destroy	effective; short retention time	extreme pressure/temperature conditions; high cost; high maintenance	pilot	Deitrich and Randall, 1985; Barry, 1987; Mertz, 1984; Anon., 1985.

Table 1-6. Summary of Characteristics and Application States of Various Color Reduction Technologies (Continued)

Process	Agents Used	Effect on Color	Advantages	Disadvantages	Applied Scale	Reference
coagulation	polyamine	separation	some color removal; easy to handle low sludge production; can be applied in primary treatment; sludge disposal easy; does not affect the effluent conductivity	expensive for high level of decolorization; narrow optimum range; need careful control;	mill scale trial	Whiting and Hayes, 1985; Kisla, 1978; Smith and Malloy, 1990
stone process	alum/poly-amine/DAF	destroy	reported successful	not documented	full scale	Ackel, 1987; Ackel, 1988.
coagulation	lime	separate destroy	effective (>85% color removal) well established; most widely studied and enjoyed favor in 60's and 70's; compatible with mill recovery process relatively low cost	large amount of sludge (minimum lime); difficulty in sludge dewater; overload the clarifier; inability to treat the total mill effluents (massive lime); increase the load to recovery system; color reversion in treated effluent; foaming problems in primary clarifier or cooking liquor systems; all was shutdown	full scale	Anon, 1987; Hyaninen,1985; Lunar and Dence, 1970; Edde, 1964.
coagulation	alum	separate	achieve up to 89% color removal at relatively low dose	sludge is voluminous and difficult to handle and dewater; increase effluent conductivity and alum; alum toxicity	mill scale	USEPA, 1976; Smith and Berger, 1968; Davis, 1977; Spruill, 1973; Gould, 1970; Spruill, 1974; Gould, 1973; Oldham and Rush, 1978.
coagulation	iron salts	separate	achieve >80% color removal at relatively low dose; more economic compared to alum	precise pH control sludge handling problems dewater	mill scale trial	
membrane	membrane	separate	highly effective; compatible with existing and new treatment process; polysulfone membrane coated with polyethyleneimine shows promise; simultaneous COD (up to 70%), BOD (up to 30%) and toxicity (up to 50%) reduction; for ultrafiltration $0.33 to 0.88/ADT (EPA, pilot); water reuse potential;	extensive pretreatment; concentrates to handle; membrane fouling and clogging problems; expensive in operation and maintainence; complexity; limitations on reusing effluent from UF process	full scale trial	Smith and Christman, 1969; Willard, 1973; Middlebrooks, et al, 1969; Scott, 1974; Fuller, 1971; Zaidi, et al, 1991; Fremont, et al, 1973; Berger and Gillespie, 1970; Lundahl and Manasson, 1980; Fremont et al., 1973.

Table 1-6. Summary of Characteristics and Application States of Various Color Reduction Technologies (Continued)

Process	Agents Used	Effect on Color	Advantages	Disadvantages	Applied Scale	Reference
sorption	ion exchange resin	separation	highly effective (>90% color removal); simultaneous BOD and COD removal (up to 50%) compatible with existing and new treatment process; function better at low pH (2.0 to 2.5); suitable for post treatment	extensive pretreatment capacity vs. life; low efficiency after regeneration; resin fouling; high capital cost; need a additional color destroy equipment; potential chloride buildup	full scale	Fitch, 1985; Broddevall, 1976; Sanks, 1973; Chamberlin, et al, 1975; Rock, et al, 1974; Rock, et al, 1974; Anderson, et al, 1974; Lindberg and Lund, 1980; Borjeson and Lindberg, 1981.
ISEP process	activated carbon	separation	most popular carbon are GAC; well established; suitable for polishing; effective; continuous operation; relatively low sludge/regenerant some toxicity, BOD and COD reduction	high capital and O&M cost; regeneration problems	pilot trial	Ullrich, 1978; Rankin and Benddik, 1973; Timpe, et al, 1973; Timpe, et al, 1970; Timpe and Lang, 1973.
Land Treatment	soil/micro-organisms	separation/ destroy	well established very low cost;	accumulation of color and toxic pollutants in soil over time; clogging of treatment site; potential contamination of ground water; required large land	full scale trials	

Table 1-7. Costs of Decolorization -- Alternatives for the First Caustic Extraction-Stage Effluent (after Springer, 1985)

Type of Process	Scale of Mill	Costs (1980 $ Basis)			
		Color Removal (%)	Capital Cost ($/ton)	Operating Cost ($/ton)	Total Cost ($/ton)
Ion exchange	(300 tpd)	91	2.8	3.7	6.5
Ultrafiltration	(54.7 tpd)	87	1.7	3.8	5.5
Mini-lime	(625 tpd)	90	0.7	2.7	3.4
Massive-lime	Pilot plant	93	3.1	3.2	6.3
Polymeric Adsorbent	Pilot plant	85	0.6	1.3	1.9

Table 1-8. Costs of Decolorization --Alternatives for Combined Mill Effluent (after Springer, 1985)

Type of Process	Scale of Process	Costs (1980 $ Basis)			
		Color Removal (%)	Capital Cost ($/ton)	Operating Cost ($/ton)	Total Cost ($/ton)
Ozone	Pilot plant	85	1.0	4.0	5.0
Dissolved air flotation	Laboratory	90	NR*	6.9	NR
$Fe_2(SO_4)_3$	Laboratory	91	2.3	10.8	13.1
Alum	Laboratory	93	2.3	10.3	12.6
Activated carbon absorption	Pilot plant	80	2.2	7.8	10.0

1.5.3 Ozone Decolorization and Dechlorination

Ozone and ozone related AOP processes have been proven to be able to decolorize and dechlorinate pulp mill effluents, but the instability, high cost, and limited understanding of process chemistry in the past has prohibited the commercial applications of these technologies. The state of the art review of current studies on ozone decolorization and dechlorination are presented in Chapters 2, 4 and 5.

Preliminary studies have found that pretreatment with ozone or ozone-related AOP processes can greatly enhance the biodegradability of various kraft mill effluents (Smith and Mohammed, 1992; Mao and Smith, 1995b). However, as discussed in earlier sections, studies to date have dealt mainly with the ozonation or the AOP process itself and few are about the combined sequence of ozonation or AOP process followed by various biological treatments. Roy-

Arcand (1991) found the evidence of synergistic effects between ozone treatment and subsequent biological decolorization and dechlorination using the white rot fungi Tramete (*Coriolus versicolor*). Recently the preliminary study by Heinzle *et al.* (1992) on ozone/biotreatment of high strength sulfite pulp bleaching effluent using normal microbial consortia from sewage in aerobic/anaerobic fluidized bed bioreactor system shows great promising of this hybrid process. The results indicated that ozone can substantially increase biodegradability of high MW biologically-recalcitrant lignin components through partial degradation and dechlorination. However, the only global parameters COD and TOC were monitored in the study which greatly render the results much less convincing. The quantitative evaluation of influence of various process parameters on ozone decolorization and dechlorination are demonstrated in Chapters 4 and 5.

The general ozone chemistry is now well understood (Staehelin and Hoigné, 1985; Staehelin, *et al.*, 1984; Staehelin and Hoigné, 1982; Hoigné and Bader, 1976; Hoigné, 1982a and 1982 b), and has been applied to water and wastewater treatment successfully in many cases. Chapter 4 summarizes some available information of general ozone chemistry related to the ozone decolorization and dechlorination of pulp mill effluents. Some characteristic reactions of ozone with lignin model compounds and lignin components in pulp mill effluents were treated comprehensively in Chapters 6 and 7.

Until now, the existing knowledge provides very limited understanding of the chemistry and mechanisms of ozone decolorization and dechlorination of pulp mill effluents. This is probably because of the great complexity of ozone-lignin reaction system in addition to those difficulties mentioned in those studies (Smith and Mohammed, 1992; Mao and Smith, 1995b) and in Chapter 4. To facilitate the development of an advanced practical process involved in using ozone for decolorization and dechlorination of pulp mill effluents, obtaining the better understanding of these mechanisms and unique chemistry involved would be a critical step. Thus, some series of studies in Chapters 5, 6 and 7 were designed to further explore these aspects and the findings in these chapters appear to be very promising and helpful in understanding and developing an advanced hybrid system for decolorization and dechlorination of kraft pulp mill effluents.

1.5.4 Fungal Decolorization and Dechlorination

1.5.4.1 Biochemistry and Mechanisms of Fungal Decolorization and Dechlorination

The biochemistry and mechanisms of biodegradation of lignin components are of fundamental importance in developing and designing an effective biological process for decolorization and decolorization. Unfortunately, despite much effort during the past 20 years toward understanding the biochemistry and mechanisms of microorganisms decomposing lignin derivatives, little progress has been made on biodegradation of prototype, isolated, or industrial lignins. Most of what was learned in the past was achieved by studying the catabolic mechanisms of degrading aromatic or other simple model compounds by various bacteria, and much less work had been performed with the fungi, despite their well-known importance as degraders of lignin derivatives and benzenoid molecules within natural and controlled environments. As expected, the major difficulties contributing to these facts were 1) lack of reliable and reproducible isolation and purification techniques, 2) lack of simple, sensitive, and accurate assays for measuring lignin decomposition; 3) lack of means and knowledge to ascertain what specific enzymatic transformations occurring during microbial decay of lignins. Usually at best, in most of the earlier studies, gross chemical alternations (e.g., by quantification of various functional groups) in the lignin polymers before and after decay may be measured (Kirk and Chang, 1975). Although these difficulties greatly limited progress on the lignin biodegradation research, the previous studies did provide some important background for the later studies. For example, those findings on the unique structural features of lignin derivatives, together with their high MW led to many important conceptual discoveries, such as some of enzymes may be extracellular and relatively nonspecific, and the biodegradative mechanisms of lignins must be somewhat unusual and perhaps unique, and can be complex and difficult for elucidation.

During the past 10 to 15 years, after the rapid, specific, and sensitive assays for ligninolytic activity, utilizing ^{14}C-lignin preparations were successfully developed (Kirk, *et al.*, 1975; Crawford and Crawford, 1979), a great advance in the biochemistry and mechanisms of the lignin biodegradation by white-rot fungi and various microorganisms have been made through the analytical comparisons of nondecayed (sound) and residual lignins. The accumulative summaries with different emphasis on various aspects of lignin biodegradation can be found in a number of book and reviews (Crawford, 1981; Reddy, 1984; Eriksson and Kirk,

1985; Kirk, *et al.*, 1980; Buswell, 1991). The following sections primarily discuss the general findings through both the physiological and the new model compound studies with emphasis on the fungus *P. chrysosporium*. The necessary overview of the general findings with various microorganisms in the past will also be incorporated into the proper sections.

1.5.4.1.1 General Biochemistry and Microbial Physiology of Lignin Biodegradation

As discussed earlier, lignins are the high MW polymers of phenylpropanoid units; obviously the microbial decomposition involves microbial attack on aromatic rings and those chemical bond structures described in Figures 1-4, 1-5 and 1-6. Though there is as yet only fragmentary evidence to support the contention, it is almost a certainty that some enzymes and mechanisms of biodegradation of lignin fragments or benzene ring appear to be similar for both lignaceous and nonlignaceous natural benzenoid substances. Thus, the understanding of mechanisms and enzymes employed by microorganisms to break down the similar chemical bond structures and dearomatize benzenoid nuclei is essential to gain the knowledge of lignin biodegradation by either fungi or bacteria. In other words, the understanding of general mechanisms and enzyme systems employed by various microorganisms would greatly facilitate the understanding of the mechanisms and biochemistry employed by lignin-degrading microorganisms.

The type and nature of some known enzyme systems and lignin-degrading microorganisms have been comprehensively reviewed and summarized by Crawford (1980), Christman and Oylesby (1971), and Reddy (1983). Table 1-9 briefly outlines some representative microbial catabolism found in biodegradation of lignin-related aromatic compounds. The major enzymes and various microorganisms which were most frequently investigated and positively identified in lignin biodegradation processes were also included in Table 1-9. The earlier studies on these topics have also been comprehensively reviewed by Chapman (1972), Dagley (1971, 1977) and Sugumaran and Vaidyanathan (1978).

Several generalizations may be made concerning microbial catabolism of the lignin-related aromatic compounds compiled in Table 1-9. Aromatic compounds must be converted to ring-fission substrates, which generally contain a minimum of two hydroxyl substituents oriented in a ortho or para relationship to one another. These ring-fission substrates are then cleaved by a class of enzymes known as dioxygeases to give aliphatic compounds that are further degraded to

molecules that are readily funneled into the energy-yielding TCA cycle. The findings in Table 1-9 also revealed that the dioxygenases were the most common type of enzymes involved in almost all catabolic processes for metabolizing a variety of the model aromatic compounds. However, different dioxygenase had different structural specificity and functions. These variety of dioxygenases involved in these catabolic pathways have been described in detail by Crawford (1981).

It has also been noted that catabolism by these pathways of the ring-substituted aromatic compounds, particularly those substituted with halogen atoms, generally requires dissimilatory pathways with enzymes of low specificity. Enzymes of meta-fission pathways are frequently more tolerant of such ring substitution than are enzymes of ortho-fission pathways. Exceptions to these general rules exist which is usually called novel catabolic reaction pathways (Crawford, 1981).

It is also not at all surprising that lignin-model-compounds degrading microorganisms do not necessarily degrade lignins. As pointed out by Crawford (1981) the microorganisms to degrade various lignin model compounds is not necessarily able to degrade the lignin derivatives, at least not in significant kinetic rate (also see Section 1-4). Since the microorganisms usually catabolize these compounds by way of highly substrate-specific pathways such as the α-ketoadipate pathway (Stanier and Ornston, 1973). It is highly unlikely that such highly specific pathways also function in degradation of the lignin macromolecules. These pathways, however, might function in the catabolism of lignin model compounds or the mono-, dio-mers derived from lignin structures if these compounds or their precursors were released from lignin as degradative fragments following decay of the lignin polymer by other enzyme systems (Ishikawa *et al.*, 1963a and 1963b; Haars and Hüttermann, 1980). It is also probable that the same types of enzymes (mixed-function oxygenases and dioxygenases, Dagley, 1971) that mediate degradation of low MW aromatic compounds are involved in lignin biodegradation (Kirk and Chang, 1974), but this has not been unequivocally established.

Table 1-9. Summary of Major Mechanisms and Microorganisms Involved in Lignin Degradation

Microorganism	Substrate	Type of Bonds	Enzyme or Mechanism	Intermediates	Comments
Bacteria		ortho-fission of aromatic ring	β-ketoadipate or ortho-fission (dioxygenases)	catechol or protocatechuic acid	most commonly encountered pathways
Bacteria		meta-fission of substituted aromatic ring	meta-fission (dioxygenases)	catechol or protocatechuic acid or protocatechuate	less specific in variety of substitutions
Bacteria		meta-fission of substituted aromatic ring	homoprotocatechuate (dioxygenases)	homoprotocatechuic acid	less frequently encounted pathway few major species of bacteria were observed to possess these pathway
Bacteria/plants		meta-fission of substituted aromatic ring	gentisate (dioxygenases)	gentistic acid	frequently found in various genera of bacteria and plants
Bacteria		meta-fission of substituted aromatic ring	Homogentisate (dioxygenases/hydroxylases)	Homogentisic acid	found in few bacterial species
Bacteria		cleavage of aromatic ethers	mixed-function oxygenase or monooxygenases	free phenol and aliphatic aldehyde through an unstable hemiacetal	found in few species of Bacillus as novel catabolic reaction pathway
Bacteria		decarboxylation	unknown	guaiacol	found in few species of bacteria as novel catabolic reaction pathway
Bacillus		meta-fission like of 5-chloro-2-hydroxybenzoate	specific dioxygenase	a series of aliphatic acids	found in one strain of Bacillus as novel catabolic reaction pathway
strains of Bacillus and *Streptomyces sp.*	veratrate and vanillate	cleavage of C-C bond and aromatic ring-fission	specific decarboxylase, demethylation, then, through dioxygenase-catalyzed ring fission	protocatechute etc.	preliminary results

Table 1-9. Summary of Major Mechanisms and Microorganisms Involved in Lignin Degradation (Continued)

Microorganism	Substrate	Type of Bonds	Enzyme or Mechanism	Intermediates	Comments
Pseudomonas putida	dehydrodiconiferyl alcohol	cleavage of coumarin ring structure	unknown	coneferyl alcohol, ferulic acid, etc.	a model for coumarin ring structures of lignin, used as sole carbon source, preliminary results from product isolation studies
Pseudomonas putida	a-veratryl-b-guaiacylpropionic acidd and D, L-pinoresinol	cleavage of 1,2-diarylpropane structure of lignin	unknown	veratraldehyde, vanillin, veratric acid etc.	a model for 1,2-diarylpropane structures of lignin, preliminary results from product isolation studies
Pseudomonas putida and fungi (*Phanerochaete chrysosporium*)	guaiacylglycerol-β-coniferyl ether	cleavage of α,β ether bonds present in lignin molecules	unknown	β-hydroxypropiovanillone and coniferyl alcohol	preliminary results
Pseudomonas acidovorans and fungi (*Perennioporia subacida*)	veratrylglycerol-β-(o-methoxyphenyl) ether	cleavage of arylglycerol-β-aryl ether bonds	unknown	not confirmed	this linkage type represents 30-50% of the intermonomer bonds in spruce lignin
Fungi (*Coriolus versicolor*, *Aspergillus niger*, *Candida tropicalis* etc.)		meta-fission of substituted aromatic ring; ortho-fission of aromatic ring	various dioxygenase	variety of aliphatic compounds, catechol, protocatechate	more than 20 species of fungi have been reported to possess these metabolic pathways

Table 1-9. Summary of Major Mechanisms and Microorganisms Involved in Lignin Degradation (Continued)

Microorganism	Substrate	Type of Bonds	Enzyme or Mechanism	Intermediates	Comments
Fungi (*Glomerella cingulata*, *Aspergillus niger*, *Candida tropicalis etc.*)		decarboxylation of benzenoid compounds	unknown	not confirmed	more than 10 species of fungi have been reported to possess these enzymatic function
Fungi (*Penicillium patulum*, *Aspergillus niger*, *Candida tropicalis etc.*)		hydroxylations of benzenoid compounds	unknown	not confirmed	more than 100 species of fungi have been reported to possess these enzyme function
Fungi (*Phanerochaete chrysosporium*, *Sporotrichum pulverulentum*)	vanillate, protocatechuate, gallate, vanillic acid	cleavage of C-C bond and aromatic ring-fission	an oxidative vanillate decarboxylase/NADPH (NADH), decarboxylation	methoxyhydroquinone -> hydroxyquinol etc.	highly nonspecific, intracellular enzyme, may not directly involved in the early steps in lignin biodegradation, require presence of easily metabolized carbon source

1.5.4.1.2 Proposed Pathways for Biodegradation of Lignins and Lignin Components

Two general mechanisms whereby microorganisms might degrade lignin components have been proposed: a) depolymerization of lignin macromolecules with release of monomeric and dimeric lignin fragments which are transported into microbial cells where they are degraded, and b) dearomatization of the intact polymer by cleavage of rings while they are still bound in the macromolecule, followed by the erosion of the resulting polymeric, aliphatic network. The meager evidence appeared to be supportive of the latter alternative (Crawford, 1981). Kirk and Chang (1974) confirmed the important appearance of the vanillic acid group in the decayed lignins first observed by Hata (1966) in sprucewood lignin decayed by white-rot fungi. They proposed the possible occurrence of ring cleavage of aromatic rings while still in polymeric structures of lignin, and speculated that those ring cleavages might be catalyzed by extracellular oxygenases. After comparing decayed spruce lignins with that of sound lignins, Chua *et al.* (1982) found that the polymeric spruce decayed lignins contain relatively high MW aryl ether-linked vanillic acid, aryl ether-linked vanillyl alcohol etc., and saturated aliphatic moieties. Characterization of degraded lignin and ^{13}C-DHP with ^{13}C-NMR has provided further evidence of ring opening reactions (Chua *et al.*, 1982; Tai *et al.*, 1983).

It is also important to recognize that knowledge of the enzymology of lignin biodegradation is, rudimentary. Even the role of the well studied phenol oxidases in lignin degradation is still open to serious debate. Most available evidence concerning lignin degradation pathways is either circumstantial or indirect. Besides these limitations, only a few microorganisms have been examined in any detail as regards the pathways of lignin biodegradation. Because catabolic variations between superficially similar microorganisms are often immense, all organisms should not be expected to utilize similar degradative mechanisms. It is not unlikely that lignin degradation may proceed in nature by more than one mechanism. As a matter of fact, numerous low MW C_6-C_1 products such as 4-*O*-alkylated vanillic acid and vanillyl alcohol structures, 4-*O*-alkylated syringic acid and syringyl alcohol derivatives in spruce and birch lignins, respectively, have been detected following decay by *P. chrysosporium* (Chua, *et al.*, 1982; Tai, *et al.* 1983). These findings have firmly established C_α-C_β cleavage of the propyl side chain as a major depolymerization reactions. Cleavage of the C_α-C_β bond has also been demonstrated in ligninlytic cultures of *P. chrysosporium* using β-*O*-4 and β-1 lignin model

compounds and is catalyzed by extracellular lignin perioxidase isolated from the fungus (Tien and Kirk, 1983). Some other reactions have also been detected in lignin biodegradation include hydroxylation and reductive cleavage of the aryl ether bond.

1.5.4.1.3 Biochemistry and Enzymology of Lignin Degradation by White-rot Fungi

As discussed in Section 1.4, under appropriate conditions, white-rot fungi degrade all the major constituents of woody tissue, and there is little evidence so far to indicate that different white-rot fungi use fundamentally different mechanisms to degrade lignins and lignin derivatives. However, species vary considerably in the rates at which they attack the lignin and polysacchride components. In addition, fungal transformation of benzenoid compounds or lignin derivatives are often associated with processes that have been lumped under the term "secondary metabolism". In fact, lignin biodegradation by certain white-rot fungi is found to be a secondary metabolic process which followed the primary growth phase and triggered by nitrogen, carbon or sulfur limitation (Kirk, *et al.*, 1976; Kirk, *et al.*, 1978) as discussed in detail later.

Figure 1-10 schematically shows the interrelationships among fungal growth, consumption of nitrogen nutrients, ligninolytic activity, and a secondary metabolite veratryl alcohol. After inoculation of fungal spores, fungal mycelium increases at the primary phase (from day 0 to about day 3 to 4) along with the decrease in content of nitrogen nutrients, but the growth rate begins to level off around day 3 to 4, which is the time when the secondary or idiophasic phase begins under proper environmental conditions. At this point, the veratryl alcohol synthesis begins almost in parallel with the appearance of the ligninolytic activity. Since excess glucose as compared with the nitrogen source is present in the medium, glucose decreases very slowly. Figure 1-10 clearly indicates that nitrogen starvation triggers formation of the ligninolytic system and biosynthesis of the secondary metabolite veratryl alcohol.

Enzymology

Although the names of "LIPs" or "Ligninase" and MNPs or Manganese Peroxidase have not yet been rigorously defined, they may be properly used as general terms at this time to represent two critical enzyme systems, Lignin Peroxidase (LIPs) or Ligninase and Manganese involved in the lignin degradation processes by white-rot fungi.

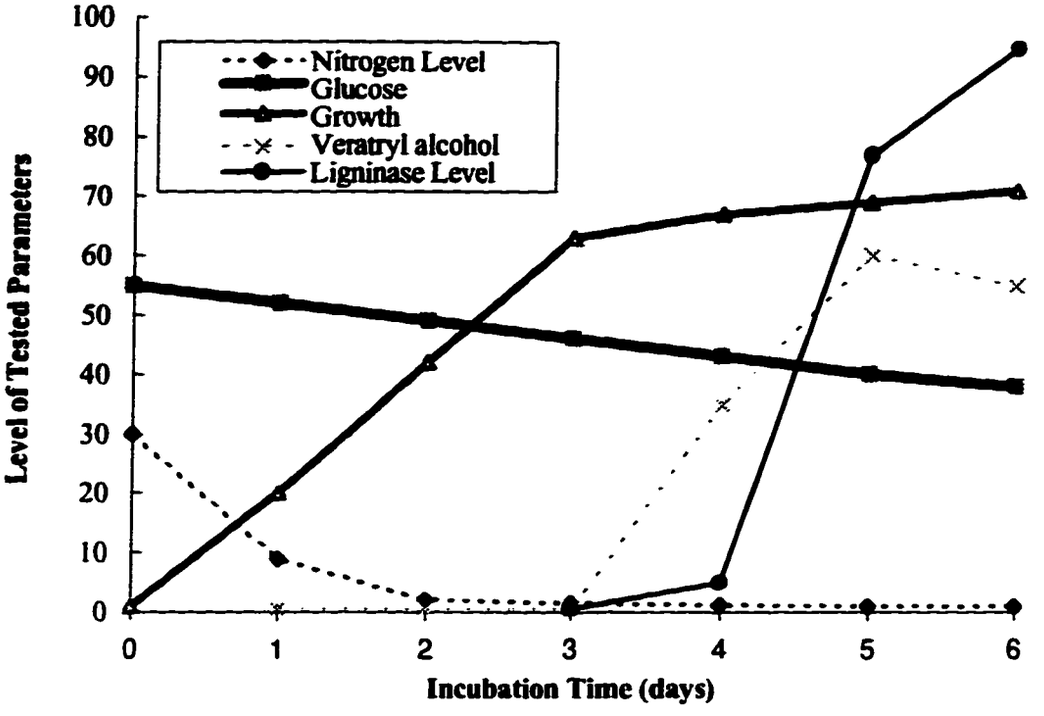


Figure 1-10. Schematic Illustration of Profiles of Secondary Metabolism of *P. chrysosporium* -- Correlation of Ligninolytic Activity and Veratryl Alcohol Biosynthesis Induced by Nitrogen Starvation (Developed from Shimada and Higuchi, 1991)

A) Lignin Peroxidase (Ligninase, LIPs)

Physiochemical Characteristics: an extracellular H_2O_2-dependent enzyme, a glycosylated hemoprotein with a MW of 38,000 to 43,000 g/mol. and contains one mole of protoporphyrin IX per mole of enzyme. It exhibits the structural and kinetic properties of a true perioxidase. The lignin peroxidase family contains multiple isozymes with pI values ranging from 3.3 to 4.7 which vary with strains, cultural conditions and isolation techniques.

Catalytic Properties: Details of various oxidation reactions have been described and reviewed elsewhere (Boominathan and Reddy, 1992; Buswell, 1991), and only the major types of reactions related to the earlier discussions will be briefly addressed here:

C_α-C_β cleavage: Since these C-C and C-O-C bonds account for about 55% of the total intermonomeric linkages in lignins (Sjöström, 1981; Shimada and Higuchi, 1991), C_α-C_β cleavage is likely to lead to extensive depolymerization.

C_α-Oxidation: β-*O*-4 and β-1 substructure are oxidized at C_α to form the corresponding ketone. Veratryl alcohol is also oxidized to veratrylaldehyde in an analogous reaction; Interestingly, C_α-oxidation strongly retards the metabolism of β-1 and β-O-4 models by ligninolytic culture of *P. chrysosporium* (Enoki and Gold, 1982; Kirk and Nakatsubo, 1983; Fenn and Kirk, 1984), but chemically induced C_α-oxidation in lignin greatly enhances depolymerization in direct proportion to the content of α-carbonyl groups (Fenn and Kirk, 1984).

Aromatic ring-cleavage: Cleavage of aromatic ring substructures in lignins to form α, β-cyclic carbonate, formate, and methyl oxalate esters of arylglycerol. Lignin peroxidase further catalyzes the ring cleavage of veratryl alcohol to yield γ- and δ-lactones.

Cleavage of the arylglycerol-β-aryl ether bond: Lignin peroxidase catalyzes the cleavage of the β-O-4 bond between the ethereal oxygen and 4-position carbon of B ring to form phenylglycerol products.

Other Types: They can also catalyze many other types of reactions, such as migration of phenoxy moieties from C_β to C_γ; hydroxylation of benzylic methylene groups; dihydroxylation at the C_α-C_β olefinic bond in styryl structures; phenol oxidation and polymerization of lignins.

B) Manganese Peroxidase (MWPs)

Manganese Peroxidase is an extracellular H_2O_2-dependent and M(II)-dependent, lactate-activated peroxidase with pI values ranging from 4.2 to 4.9; have a molecular weight between 45,000 to 47,000 g/mol and contains one mole of iron protoporphyrin IX with a high-spin, predominantly pentacoordinate, ferric iron and with histidine coordinated as the fifth ligand. Manganese peroxidase oxidizes Mn(II), which in turn can oxidize a variety of organic substrates.

1.5.4.1.4 Influence of Physiological Parameters

Lignin degradation by *P. chrysosporium* has been shown to be a secondary metabolic event and is triggered in response to N, C, or S starvation (Jeffries *et al.*, 1981; Kirk *et al.*, 1978; Reid and Seifert, 1980; Keyser *et al.*, 1978). Several nutritional and cultural parameters are now well known to be specific to the lignin degrading fungi and strongly influence the

manifestation and activity of the ligninolytic system and the appearance of other idiophasic features which are, or might be, directly associated with ligninolytic activities in fungi.

A). Oxygen Tension

The large oxidative nature of lignin biodegradation is reflected in the marked influence of oxygen level on the ligninolytic systems of white-rot fungi. Kirk *et al.* (1978) reported that little lignin degradation occurred in the culture of *P. chrysosporium* maintained at less than 5% oxygen level, but the rate and extent were improved dramatically with increasing oxygen partial pressure above the culture. Similar effects were found on *Coriolus versicolor* (Kirk, *et al.*, 1978). Increasing oxygen partial pressure in the cultural system was found not only to increase both the titer of the ligninolytic complex of *P. chrysosporium* and the rate of lignin oxidation by the system after its formation (Bar-Lee and Kirk, 1981; Faison and Kirk, 1985) but also to enhance the production of H_2O_2 (Faison and Kirk, 1983). These may be the partial explanation of this stimulatory effects of O_2. However, the enhancement by further increasing the oxygen partial pressure above 1.0 atm was reported to be marginal (Reid and Seifert, 1980).

B). Carbon Co-substrate Requirements

Theoretically, native lignin is potentially capable of serving as the sole carbon source for both energy and carbon requirements for sustaining biodegradation. A previous study reported that a white-rot fungus *Polyporus versicolor* could use lignin as a sole source of carbon and energy for its growth (Pelczar *et al.*, 1950). However, further studies suggested that ligninolytic activity was triggered irrespective of the presence of lignins in the medium, and the presence of lignins did not appear to enhance the lignin degradation. These observations are clear indications that lignin biodegradation is substantially different from that of other biopolymers and the energy in lignin is of little importance or insufficient to support the growth of fungi or lignin degradation. More recent studies confirmed that, in order for decomposition of lignins to proceed, white-rot fungi require a more easily-biodegradable carbon as co-substrate and whether or not presence of lignins has little impact on the development of the ligninolytic system (Kirk *et al.*, 1976; Ander and Erriksson, 1975). For example, *P. chrysosporium* failed to degrade ^{14}C-DPHs to $^{14}CO_2$ in the absence of suitable growth substrates (Kirk, *et al.*, 1976; Kirk, *et al.*, 1978). Drew and Kadam (1979) reported an obligate co-substrate requirement for ^{14}C-kraft lignin degradation for the white-rot fungi: *Phanerochaete chrysosporium, Coriolus versicolor,*

and *Sporotrichum pulverulentum*. Similar results were obtained with an imperfect fungus *Aspergillus fumigatus*.

Moreover, under nitrogen sufficient conditions, the nature and the concentration of the easily-biodegradable carbon source also has a great influence on the rate and extent of lignin degradation. Kirk *et al.* (1978) found that cellulose, glucose and xylose, among most of simple carbohydrates are good carbon sources for lignin degradation; and glycerol, succinate are poor although they could serve as carbon sources. Prasad and Joyce (1991) also observed that the glucose was the best substrate to enhance decolorization, and the optimal glucose concentration was about 500 mg/L to achieve 57% of color reluction. These authors also reported that other less favorable organics such as pulp and pith can be used as co-substrates to improve decolorization, and further speculated that the decolorization process can be hastened if the fungus was initially cultivated on pulp and pith and subsequently transferred to an extraction stage effluent for decolorization. Earlier Eaton *et al.* (1982) had also demonstrated that the residual fibre could serve as an co-substrate for lignin degradation. It has also been shown that in certain situations, the presence of an excess, easily-metabolizable carbon source could substantially inhibit the lignin biodegradation. In fact, Buswell *et al.* (1984) reported that lignin peroxidase activities were considerably higher in glycerol-supplemented cultures of *P. chrysosporium* INA-12 than in glucose-grown ones and related this elevated activity to the relatively poor rate of fungal growth on glycerol. It is also important to recognize that all of these parameters may interact with each other.

C). Efflects of Nutrients

The level of nutrient nitrogen provided in the growth and degradation medium has a profound influence on ligninolytic activity by *Phanerochaete chrysosporium*. Previous studies demonstrated that the addition of excess nutrient nitrogen in the carbon rich medium will restore primary growth and suppress both lignin perioxidase activity and lignin degradation (Keyser *et al.*, 1978; Faison and Kirk, 1985; Kirk *et al.*, 1978; Fenn and Kirk, 1981). Of the nitrogenous compounds examined in these studies, NH_4Cl, glutamine, glutamate, and histidine were the most effective, repressing ligninolytic activity by 50%, 76%, 83%, and 76%, respectively, compared with negative control cultures with no N added. Addition of NH_4^+ and L-glutamate also suppressed the synthesis of veratryl alcohol, a secondary metabolite (Fenn and Kirk, 1981; Keyser *et al.*, 1978; Lundquist and Kirk, 1978; Shimada *et al.*, 1981). These and other results

indicated that nitrogen metabolism affects ligninolytic activity as a part of secondary metabolism and that glutamate metabolism plays a key role in the regulation of lignin degradation by *Phanerochaete chrysosporium*. Reid and Seifert (1980) also showed that degradation by *Phanerochaete chrysosporium* of ^{14}C-lignin-labelled aspen wood is triggered by N starvation and inhibited by high levels of N. However, the repression of ligninolytic activity on the addition of NH_4^+ and L-glutamate does not appear to be mediated by suppression of central carbon metabolism since both stimulated the oxidation of succinate and glucose (Fenn and Kirk, 1981). Reid and Seifert (1980) also suggested that the C/N ratio is a better predictor of lignin degradation than the absolute C and N levels. Odier and Roch (1983) also reported that lignin biodegradation by *Phanerochaete chrysosporium and Phelebia radiata* is dependent on the C/N ratio in the medium. However, in the case of *Sporotrichum pulverulentum* and *Dichomitus squalens*, no relation between lignin degradation and the C/N ratio could be established, suggesting that the suppression of ligninolytic activity by high levels of nutrient nitrogen is variable among white-rot fungi.

Ligninolytic activity in nitrogen-rich carbohydrate-limited cultures of *Phanerochaete chrysosporium* is triggered in response to carbohydrate starvation and is accompanied by autolysis. Lignin degradation ceases when autolysis does, suggesting that autolysis provides the carbon and energy for lignin degradation and cell maintenance (Jefferies *et al.*, 1981).

Lignin degradation was reported to be depressed in 7 days on depletion of sulfur in cultures which initially contained limiting levels of SO_4^{2-} (20 mM) and non-limiting levels of carbohydrate and nitrogen (Jefferies *et al.*, 1981). Phosphorus starvation did not have a corresponding effect on lignin degradation. When the initial SO_4^{2-} concentration was 200 mM, no ligninolytic activity appeared. Dual limitations of SO_4^{2-} and N did not result in more rapid or more extensive lignin degradation. The basis for the effect of sulfur on lignin degradation by *Phanerochaete chrysosporium* is not known and some conflicting results exist in the literature.

D). Influence of Culture Agitation

Culture agitation, especially during the primary growth stage, was originally reported to have great impacts on the growth of fungal biomass and development of ligninolytic activities. Kirk *et al.* (1977) found that agitation strongly inhibited the conversion of ^{14}C-DHP to $^{14}CO_2$ by *P. chrysosporium*. Eaton *et al.* (1980) noted that both biomass growth and ligninolytic activities

were strongly suppressed by the agitation of the culture of *P. chrysosporium*. Prasad and Joyce (1991) also observed that the effluent turned turbid in the agitated culture, but he explained that this phenomenon was due to the fragmentation of cells caused by mechanical stress. In addition, biosynthesis of veratryl alcohol (Shimada, *et al.*, 1981) and production of lignin peroxidase (Faison and Kirk, 1985) were also dramatically inhibited in agitated cultures although comparable biomass growth was still observed in both agitated and controlled stationary cultures. Although the physiological basis is still unclear it was speculated that the agitation may result in severe damage to fungal hyphae leading to pellet formation which in turn may restrict oxygen and substrate availability (Kirk, *et al.*, 1978; Eaton, *et al.* 1980; Yang *et al.*, 1980).

In reality, for any bioreactor system to achieve good contact and mixing a certain level of agitation seems to be unavoidable. Recent investigations seemed to provide some remedies to this difficulty. Leisola *et al.* (1985) reported that the addition of veratryl alcohol and an increase in the oxygen partial pressure improved the lignin peroxidase activity in mildly agitated carbon-limited or nitrogen-limited cultures. Jager *et al.* (1985) showed that the addition of nonionic surfactants such as Tween 80 or Tween 20 to the culture medium may significantly reduce the adverse effects of agitation on the fungal biomass. Obviously, this may be debated for its applicability at a large scale. In fact, these were and probably still are the great limitations to their use in large-scale biotechnological processes. The manipulation of bioreactor design incorporated with the new immobilized living cell techniques may be another promising solution for this problem. Some of new developments in this area will be addressed in section 1.5.4.2 and the detailed treatment will be presented in Chapters 7 and 8.

E). Other Factors

An optimum pH ranging from 4 to 4.5 was found for lignin degradation, and the substantial pH decrease occurred in degradation media without pH control. Thus, control of pH or the choice of buffer was found to be critical to lignin degradation in biotechnological studies using fungi, especially, *Phanerochaete chrysosporium*. Prasad and Joyce (1991) demonstrated that a pH of 4 was optimum for decolorization. Sodium 2,2′-dimethylsuccinate was found to be a better buffer compared to others, whereas *o*-phthalate was found to be inhibitory in some cases (Fenn and Kirk, 1979).

Additional factors known to be associated with enhanced lignin degradation were other microelements. Casamino acids, or a mixture of an ammonium salt plus asparagine, are optimal for biomass growth, but high nitrogen concentration in the medium decreases ligninolytic activity. Thiamine is required for growth, and the balance of trace metals is also important. Kirk *et al.* (1976 and 1978) noted that supplement of Cu, Fe or Mn to the medium may improve the lignin degradation by *P. chrysosporium*. However, the improvement is limited and the application at a large-scale has to be justified.

1.5.4.2 Engineering Aspects of Immobilized Cell Bioreactor Systems

Immobilized cell bioreactor systems have been successfully applied in various industries, primarily in fermentation and pharmaceutical processes, for the past ten years. However, they are expanding into water and wastewater treatment and becoming more and more popular.

1.5.4.2.1 Characteristics and Advantages of Immobilized Cell Bioreactor Systems

The modern concept of living cell immobilization is a further refinement of the original concept of the fixed-film process. A fixed-film process utilizes a support media surface such as rocks, rotating disks, or some solid mass for the biomass to adhere to. In the modern immobilized cell bioreactor system, instead of the biomass restricting to adhering to the surface of the media, the living microorganisms are entrapped, covalently coupled, or space-contained in suitable immobilization materials. These concepts of immobilization attempt to keep the active biomass in contact with the substrate and nutrients at all times. In effect, the cell retention time is increased to the point where cell reproduction could balance endogenous decay and lysis. Therefore, net production of biomass could be greatly reduced, (i.e. unnecessary biomass production could be minimized absolutely). Another important feature of the immobilized living cell system is that cells becoming aged or rendered inactive by environmental conditions (i.e. nutrient deficiency or adverse effects) can be regenerated by 'reviving' the cells.

In addition, it has been repeatedly reported that the immobilized living cell systems, compared to suspended cultural systems (Tampion and Tampion, 1987; Atkinson, 1986):

- usually possess high and relatively long-term stability and biocatalytic activity;
- enable the three-dimensional interactions between the microbial biomass and the substrates;

- allow better control of biomass growth and biomass hold-up;
- permit manipulation of the growth rate in continuous systems, independent of the dilution rate (i.e. eliminate potential washing-out effects);
- provide an effective means to optimize cell physiology to maximize microbial activities and desired metabolization behaviours;
- are able to well protect the living biomass from various adverse environments, such as shear stress, pH, toxicants; and
- provide spatial location flexibility within bioreactors of different microbial populations.

1.5.4.2.2 Materials and Methods for Immobilization of Living Biomass

One of the most striking aspects of the studies on the immobilization of biocatalysts during the last twenty years has been the way in which the methods employed have been simplified with advances in microbiology and development of new immobilization materials. In general, early methods used fairly sophisticated chemical means adopted from immobilizing isolated enzymes. There has been a gradual realization that such covalent immobilization methods are unnecessary for success. Gentler means such as absorption on certain immobilized materials were shown to be perfectly satisfactory especially for the immobilization of various living cells for the biotreatment employed in wastewater treatment. It was then demonstrated that in many cases it was unnecessary to purify individual enzymes from the microbial cells that produced them. In fact, the immobilized living cell system has been proven to be extremely beneficial for multi-biochemical reaction systems, especially in the field of wastewater purification.

Useful immobilization methods and materials must satisfy mechanical, physical, and chemical criteria dictated by the conditions of their intended applications, in addition to achieving adequate levels of bioactivity at a reasonable cost. The influence of immobilization methods and materials on the performance and applicability of immobilized living cell systems can be viewed through:

- complexity;
- the toxicity of materials;
- the permeability of the carrier matrix to substrate and products;

- the mechanical strength and durability of the immobilized material;
- strength of immobilization (cell leakage)
- ease of scale-up; and
- cost and commercial availability of materials.

A simplified classification and comparison of technologies for immobilization of living cells is given in Table 1-10. As shown in Table 1-10, techniques such as flocculation and adsorption which were extensively used in a traditional attached growth bioreactor systems such as a conventional RBC and a trickling filter, are simple and cheap, but the major problem is considerable cell leakage, which results in excess type biomass production from wastewater treatment. On the other hand, entrapment with gel materials and covalent techniques are effective for better control of the biomass hold-up in immobilized cell bioreactors. However, the potential toxicity, and other adverse effects on the living biomass, including such restrictions as cell regrowth, mass transfer limitations, and the rate of metabolization rendered these methods less attractive in the wastewater field. In addition, these methods are usually expensive. Some effects of these techniques on the living biomass will be discussed further in later sections.

Table 1-10. A Simple Classification and Comparison of Cell immobilization Techniques

Technique	Effects on Cells	Advantage	Disadvantage
Flocculation	mild, close to natural for some species	simple, cheap	considerable cell leakage mass transfer limitation, species dependent
Adsorption (on surface)	mildest, close to natural for some species	simple, cheap, reusable, low maintenance	considerable cell leakage sludge production
Entrapment	some tolerable effects	better control of biomass hold-up	more complex, diffusional limitations, some toxicity, may be expensive
Covalent Coupling	harsh, some adverse effects	permanent on biomass	some toxicity, diffusional limitations, very expensive, less feasibility
Containment (in situ)	some effects but mild; close to natural for some species	simple, reusable, better control of biomass hold-up	may be expensive, possibly diffusional limitations

For a process in wastewater treatment to be commercially successful, the overall system should be simple, low cost, and easily scaleable due to the high volumes of wastewater that must be dealt with as inexpensively as possible. Additionally, the parameters determining the feasibility of such processes should be easily performed, controlled, stable, and reproducible in operation. Balancing all these considerations, it appeared that containment with initial adsorption for immobilization would be an advantageous choice for wastewater treatment, since new immobilization materials with suitable properties such as pore size and distribution are commercially available now (see Chapters 7 and 8).

1.5.4.2.3 Factors Influencing Activity of Immobilized Living Cells

Immobilized living cell technology is only now beginning to move out of the initial phase of demonstrating the bio-potential of new technology to full commercialization. To further advance into full-scale, commercial installations, which can effectively use the unique 'revival' capability of an immobilized living cell system requires the understanding of many underlying mechanisms and other unique features of the system.

The factors affecting the activity of immobilized living cell systems can be classified under three major categories:

- mass transfer and bioreactor configuration;
- cell physiology; and
- immobilization methods and materials.

It should be stated that progress in control and understanding of immobilized living cell systems must also go hand in hand with the development of appropriate immobilization methods and bioreactor systems. These factors and their inter-relationships are shown schematically in Figure 1-11. As discussed in a previous section, some factors related to immobilization methods and materials affect activity either directly or indirectly through their impact on the factors in the other two categories. The influence of the factors and their interactions in the other two categories on the activities of an immobilized living cell system are discussed in following sections.

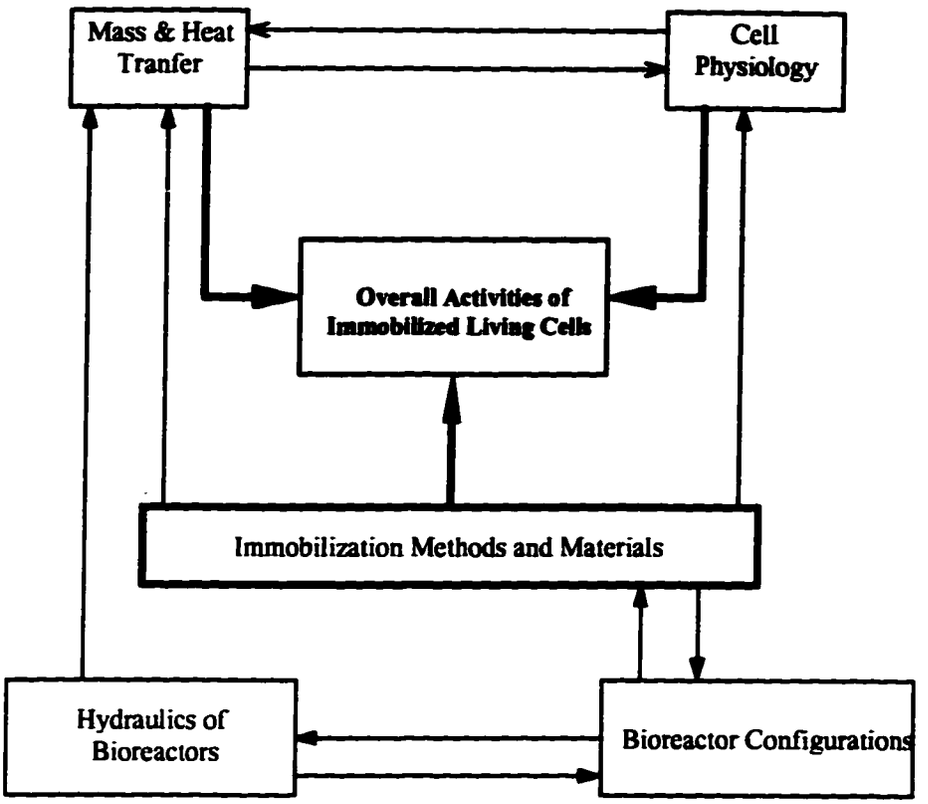


Figure 1-11. Components and Factors Affecting Overall Activities in Immobilized Living Cell Systems

1.5.4.2.4 Mechanisms of Biomass Control in Immobilized Cell Systems

Besides substrate concentration and hydrodynamics in the operating system, the immobilization methods and materials used are the primary determining factors affecting particle biomass hold-up. The influence of hydrodynamics on immobilized living cell systems will be further discussed in sections 1.5.4.2.5 and 1.5.4.2.7.

- Solid Support Particles:

The biomass hold-up is limited to the size of the particles and the thickness to which a film of biomass can accumulate. This may be controlled either by the concentration of limiting substrate or by the extent of attrition, resulting from interparticle or particle-wall contact. The rate of attrition will strongly depend on the balance between the shear forces imposed on the particle and the forces of adhesion binding the cells to the particle surface. These forces are micro-organism dependent and vary widely for different species. The nature of this immobilized

biomass has little difference from the biomass in the fixed film bioreactor mentioned earlier. thus, the regrowth and leakage of biomass in the bioreactor systems are less controllable.

- Biomass Support Particles (BSP):

As with solid support particles, the use of BSPs involves passive immobilization. The biomass hold-up is therefore dependent to some extent on the ability of the micro-organisms to colonize the particles. Nevertheless, a wide variety of micro-organisms have been successfully immobilized using both stainless steel and synthesized foam BSPs, including species which are not natural film formers, e.g., brewing yeasts (Webb, *et al.*, 1986).

Particle biomass hold-up is readily controlled in any type of bioreactor system employing BSPs. While cells are contained within the pores of the particles, any excess biomass occurring due to cell growth on the outside of the particles can be effectively removed or controlled by the forces of attrition arising from interparticle contact, hydrodynamic shear or regulation of the microenvironment through proper design of bioreactor systems, as discussed later.

For conditions under which particles are not physically "full" of biomass, particle biomass hold-up is a function of the substrate concentration within the bioreactor and is controlled by the concentration of the substrates, the rate of diffusion of nutrients into, and products out of, the particle (Atkinson, *et al.* 1984). In foam type BSPs, particle biomass hold up is also dependent on the pore size and the distribution of the particle (or specific surface area) (Black *et al.* 1984), but this dependence is organism specific and general rules concerning the choice of particle size cannot be made at this time.

- Gel Particles:

Since the use of gel particles involves active immobilization, particle biomass hold-up is selected prior to bioreactor operation. This initial particle biomass hold-up will, however, only remain constant if no further growth occurs. In cases where cells continue to grow during bioreactor operations, the biomass may become redistributed throughout the gel particles (Wada *et al*; 1979). The regrowth of biomass may lead to particle swelling and this overgrowth may result in breakage of the gel particles. The overall biomass hold-up may also change as a result of particle swelling.

Although particle biomass hold-up in gel particles may be controlled biologically by the concentration of limiting substrate in the bioreactor, physical control is very difficult. For example, if too few cells are immobilized, growth will lead to an increase in biomass hold-up and may damage the particles if proper control is not practiced, while too many cells will be wasteful in terms of the seed fermentation. In wastewater applications, the substrate concentration is very difficult to control, thus, this may lead to significant operational problems.

Of the three immobilized particle types discussed above, the BSP concept is utilized in this study as described in detail in Chapters 7 and 8.

1.5.4.2.5 Influence of Bioreactor Configurations

The extent of the impacts of bioreactor configurations is strongly dependent on the nature of the immobilized living cells, biochemical reactions and mass transfer in the reactors. Zeldowich (1939) is probably the first to define the effectiveness factor (λ) for numerical evaluation of these effects.

$$\lambda \equiv \frac{\text{observed rate of reaction with mass transfer resistance}}{\text{hypothetical rate of reaction if mass transfer resistance were absent}}$$

The magnitude of the effectiveness factor indicates what the rate controlling phenomenon is. If λ=1, there is no mass transfer limitation, and the rate controlling step is the reaction kinetics. If λ <1, there is mass transfer limitation and the rate controlling step may be mass transfer or both.

Aris (1975) developed a correlation of the effectiveness factors with various particle shapes including the slab, cylinder, and sphere based on a first order reaction and a normalized modulus ϕ ($\phi = \frac{V_p}{A_p}(k / D_e)^{1/2}$). He found that with the first order biochemical reaction, the effects of the shape on the effectiveness factor was relatively small. However, Baily and Ollis (1977) reported that the slab geometry was found to provide a considerably higher effectiveness factor λ for Monod-form kinetics with the observed dimensionless modulus ϕ between 0.05 and 1.0 which is defined as:

$$\phi = \frac{R_{obs}}{D_e S^*}\left[\frac{V_p}{A_p}\right]^2 \qquad (1\text{-}6)$$

where,

R_{obs} = observed reaction rate;

V_p = volume of immobilized material;

A_p = surface area of immobilized material;

D_e = effective diffusion coefficient for the diffusing species in the immobilized material;

S^* = substrate concentration.

According to above defined observed modulus ϕ, Bailey and Ollis (1977) developed the criteria for assessing the magnitude of mass transfer effects on overall kinetics for Monod type of biochemical reaction kinetics as shown in Table 1-11.

Table 1-11. Criteria for Assessing the Magnitude of Mass Transfer Effects on Overall Kinetics (after Bailey and Olis, 1977)

Criterion	Value of λ	Limiting Rate Process	Extent of Mass-transfer Limitation
$\phi < 0.3$	~ 1	Reaction	Negligible
$\phi > 3$	$\propto 1/\phi$	Diffusion	Large

The observed modulus and the criteria in Table 1-11 suggest that the particle size and geometry are the major factors determining the extent of impacts on the activity of the immobilized living cell system.

Mass transfer resistance increases with the increasing size of immobilized particles. The geometry of particle also influences the mass transfer resistance mainly through the ratio of available surface area (external and internal) to particle volume which alternatively affects the effectiveness factors in the bioreactor systems as discussed above.

Increasing the immobilized cell density in the immobilized carriers may lead to the higher overall rates of biochemical reactions, but may result in decreased effectiveness factors depending on biochemical reactions and bioreactor configuration. There is also a limit to the maximum biomass hold-up in each type of the immobilized particles. Alternation in hydrodynamics of the bioreactor can take place not only due to fouling from excess cell growth, but also due to break-up of the support matrix material itself. The matrix stability must also

have a constraining effect on the selection of the bioreactor configuration because the generation of both area and flow at the interface may require energy inputs incompatible with the mechanical integrity of the support matrix that may arise in scale-up. The characteristic mechanisms responsible for decrease of the activities in an immobilized living cell system are summarized in Table 1-12.

Table 1-12. Summary of Characteristic Mechanisms Affecting the Activities of Immobilized Living Cell Systems (Emery and Mitchell, 1986)

Type	Causes	Observations
Reversible	1. accumulation of cells, substrate, products (including gas, insoluble organics or inorganics); 2. reversible inhibition	1. blockage of channels and pores etc 2. accumulation of inhibitory materials
Irreversible	1. mechanical 2. physicochemical	1. loss of the support matrix (together with attached or entrapped cells) by solution or abrasion; 2. breakage of the cell matrix link and diffusion of cells out of the reaction system
Regulatory	1. cell matrix interaction on intracellular regulations 2. microenvironment effects	changes in cell physiology

There remains a large need for research and development of a considerable body of information with respect to configuration of immobilized cell systems. Mass and heat transfer processes, and the effects of geometry and power input on matrix stability and the manipulation of biological activity are key areas for future investigation.

1.5.4.2.6 Interactions of Immobilized Biomass with Micro-Environment

The development and application of the immobilized living cell technology poses many new and interesting, but potentially difficult problems concerning the control of the living biomass, and the understanding of their interactions with the microenvironment created by the immobilization events. Optimization of biocatalyst properties, particularly operating stability, catalytic activity, and minimization of undesired biomass growth and metabolic activities can

only be achieved if appropriate attention is paid to the physiological 'status' of cells during the precultivation stage, the immobilization stage, and during the subsequent process operation.

The first two aspects, which are very important in a pure culture, and non-viable cell immobilization or other applications, have been discussed in detail by Anderson (1986). In brief, prior to immobilization the selected strains should have the required metabolisation properties for the specified applications, high catalytic activity, low interfering enzyme activity, good stability during and after immobilization, absence of pathogenic and toxigenic properties, and adherence of flocculation properties if appropriate.

Following immobilization, surviving or viable cells will respond to, or be affected by, the new micro-environment in ways which can be beneficial or detrimental to the intended process. In many cases the changes observed, when the activities of immobilized cells have been compared with free cells, have been quite unexpected. Many such effects have been recorded but usually with little or no real understanding of the underlying biochemical or physiological changes which have been responsible for. For example, with a wide range of cell types the immobilization improves stability against environmental damage (Scherer *et al.*, 1981, Karube *et al.*, 1976, Kayano *et al.*, 1981), increases the productivity and reduces the undesired byproducts (Navarro and Durand, 1977; Anderson and Blain, 1980). In addition, in some cases, the reproduction and growth rate of immobilized cells differ dramatically from the free cell cultures (Jirku, *et al.*, 1980; Bandyopadhyay and Ghose, 1982; Brodelius *et al.*, 1980);

This is hardly surprising in view of the limited understanding of cell physiology and the complex and poorly-understood micro-environmental conditions to which the cells are exposed when on or within the immobilization support material; particularly, water activity, physico-chemical gradients to which cells are exposed within the support materials, such as dissolved oxygen and CO_2 etc. and many other subtler effects such as stereo-effects (Tampion and Tampion, 1987). By properly manipulating these mechanisms and combining them with the proper bioreactor design and operation, one could achieve unique performance with immobilized bioreactor systems such as resulting in low biomass production, long term stability, and high efficiency.

The great advantages of cell immobilization discussed in the above sections were obviously the underlying incentives responsible for many comprehensive studies. For instance,

the immobilized fungal decolorization system not only provided good protection of fungal biomass from damaging of severe agitation but also had other great advantages over their counterpart suspended biomass system such as improved the biodegradation and easy in biomass handling.

1.5.4.2.7 Bioreactor Systems for Fungal Decolorization and Dechlorination

Considerable research effort is now being directed towards the development of bioreactor systems for large-scale cultures of living fungi and production of ligninolytic enzymes for use in commercial processes, especially in biopulping, biobleaching and decolorization and dechlorination of pulp mill effluents.

The fungal decolorization and dechlorination of pulp mill effluents initially were carried out in 125 mL flasks with stationary shallow cultures (Marton, *et al.*, 1969; Fukuzumi, *et al.*, 1977; Eaton, *et al.*, 1980). However, this assay technique had many limitations, and microbial processes are more readily studied in the agitated submerged cell suspension. As discussed in previous sections, earlier studies (Eaton, *et al.*, 1980; Leatham and Kirk, 1983) revealed that the agitation of cultures had two different effects on decolorization and dechlorination. One is the detrimental effects as a result of reducing the production of key enzymes, ligninolytic enzymes, involved in decolorization and dechlorination; another is the enhancing effect due to improving oxygen supply and mass transfer. To minimize the adverse effects of agitation various immobilization techniques summarized in the previous sections for protection of biomass were extensively explored in addition to attempts on physiological improvement including the addition of veratryl alcohol and veratrylaldehyde (Leisola and Fiechter, 1985), and Tween 80 (Davies and Wilson, 1990) to the decolorization media.

The trials to immobilization of fungal biomass on different microcarriers are since being carried out worldwide for decolorization of pulp mill effluents and production of ligninolytic enzymes. As a result, the fungal biomass has been immobilized at a laboratory scale with various immobilization materials, such as fungal pellets, polyurethane foam (PUF), nylon web, porous glass fibre, and various gel types of materials. Linko and Zhong (1987) systematically compared different carriers for immobilization of fungus *P. chrysosporium*, such as agar, agarose, and k-carrageenan gel beads, nylon web, and polyurethane foam (PUF) in repeated batch shake cultures and in a 10 L continuous bioreactor. The results suggested that the nylon web was the

best carrier among all of them, and both nylon web and the PUF could support the bioactivity for at least 38 days. However, the SEM micrograph did not reveal any significant difference in the mycelial growth. In a separate study, PUF was identified as an excellent carrier for this fungus (Kirkpatrick and Palmer, 1987).

The advance in immobilization technology for better handling of fungal biomass led to further progress in the development of reliable bioreactor systems for decolorization and dechlorination. After evaluation of several traditional designs of the bioreactor system, Eaton *et al.* (1982) concluded the RBC had some unique operating features over other conventional bioreactor systems when trying to develop a bioreactor system for decolorization. These include large surface area for immobilization of the large amount of biomass (20 to 40 g VS/m^2 of disk), low substrate to microorganism (F/M) ratio (0.002 to 0.035), low maintenance costs, low energy requirements and simple construction and operation and the high ability to withstand large hydraulic and organic surges (Antonie, 1976; Tyagi *et al.* 1993) in addition to the common features identified in other applications. The preliminary results using RBC with an attached fungal thin-film (Eaton *et al.*, 1982) showed that about 80% color removal from E1 effluent could be achieved in one day. It was also reported that the performance of a modified RBC, which used PUF to attach to the disks as the porous support media to treat a petroleum refinery wastewater was greatly enhanced.

This concept was extended in many ways. Kirk *et al.* (1986) reported increased ligninase titers using a 3-L bench-scale fermenter with plastic disc immobilized with a mutant strain, *P. chrysosporium* SC26; the mutant, unlike wild-type strain, can firmly attach the plastic disk in the bioreactor. Recently, Hatakka *et al.* (1987) have described the production of ligninases by *P. radiata* in a laboratory bioreactor containing a plastic support matrix to which the fungal mycelium was loosely bound. Paszczynski *et al.* (1986) scaled-up a bioreactor system for the semi-continuous production lignin peroxidase and Mn (II) peroxidase in which the fungal mycelium was attached to the roughened interior surface of a slowly rotating 20 L glass carboy.

Historically, perhaps the most commonly used aerobic bioreactors in wastewater treatment are the trickling filter, packed or fixed bed bioreactors, although the RBC has recently gained more attention. Livernoche *et al.* (1981) developed a continuous decolorization process employing a packed column bioreactor with fungal mycelium immobilized in calcium alginate gel beads. About 80% of color removal could be obtained within three days when using a combined

pulp mill effluent. Linko *et al.* (1986) successfully produced lignin peroxidase in continuous and repeated batch modes in a horizontal column reactor by immobilizing spores of *P. chrysosporium* in the beads of agarose and agar gel. The biocatalysts was found to be more stable in agarose gel. Messner et al. (1987 and 1991) reported that the porous material immobilized with fungal biomass loaded in trickling filter type of bioreactor could achieve about 70% of color removal from E1 effluent within 12 hours.

A continuous stirred-tank reactor (CSTR) system for the production of LIPs using preformed mycelial pellets was also described recently (Michel *et al.*, 1990). Unfortunately attempts to produce LIP-producing mycelial pellets in the CSTR itself were not successful. However, in another study, it was reported that LIP was produced continuously in significantly high yield by immobilizing *P. chrysosporium* with nylon web or polyurethane in a modified Biostat E bioreactor with a working volume of 7.6 L, agitated with either air or oxygen alone, or in combination with a mechanical stirrer (Linko *et al.*, 1986). Under these conditions, LIP production started rapidly, and activities of about 600 U/L were obtained in about three days. A total activity yield of 10,000 U was obtained during one week of continuous production. A significantly high yield of ligninases by *P. chrysosporium*, was also achieved using stirred tank fermenters equipped with a spiral of silicon tubing on which the fungus grew in the form of mycelial mats (Willershausen *et al.*, 1987). Semicontinuous lignin peroxidase production has also been attained using *P. chrysosporium* immobilized in cubes of polyurethane foam (PUF) to minimize shear effects. More importantly, the PUF-immobilized fungus could be stored at 4°C for a period of at least two months and could still be reactivated to the former enzyme producing state within 48 hours. *P. chrysosporium* strain INA-12 immobilized with PUF produced high LIP levels in 24 hours in repeated batch cultures when biomass was partially regenerated on day five. The enzyme synthesis continued at a high level for eight batches, with only 7% activity lost every 24 hours.

These lab-scale or small-pilot bioreactor systems have been started to be further scaled-up in decolorization and dechlorination of pulp and paper mill effluents. Two patents have been granted in Europe and North American. One is called the MyCoR system, developed by Chang and Kirk (1987) in USA, and another called the MyCoPOR system (Messner, 1987), a process in which the fungus is immobilized on a porous carrier in a trickling filter type of bioreactor. Unfortunately there is no commercial application of either patent up to now. This implied that

some serious shortcomings still existed in those bioreactor systems. Therefore, a combination of selection for LIP-overproducing strains of fungi together with further optimization of culture parameters and development of immobilized living cell bioreactor systems would be very useful for a large scale production of these enzymes and their use in decolorization and dechlorization. More studies are obviously needed to bring these technology to the commercial application.

1.5.4.3 Fungal Decolorization and Dechlorination

Several investigators demonstrated that kraft mill effluents can be decolorized by 99% in 4 days by white-rot fungi, in particular, *P. chrysosporium*, at a batch scale. Decolorization of black liquor and E1 liquor with simultaneous degradation of various chlorinated organics have also been extensively studied under various situations. The details will be addressed in Chapters 7 and 8.

1.6 Objectives and Scopes

A limited literature survey revealed that ozone treatment could improve the biodegradability of varieties of recalcitrant organics by oxidative modification of those biological-resistant structures. However, most of the studies were either qualitative or on simulated situations. The data and methods are relatively insufficient in most cases to support the detail and quantitative assessment or comparison. In addition, the underlying mechanisms of ozone reactions were usually derived or speculated from studies on model compounds or general theory of ozone chemistry. More importantly, the synergistic effects of ozone and fungi on the structures of lignins and lignin derivatives were further complicated due to the many unique metabolic characteristics and enzyme systems of fungi. These phenomena have been recognized recently, some progress has been made in certain aspects, but a better understanding of the underlying mechanisms is absolutely necessary to apply these hybrid technologies to minimize the impact of pollution from the forestry industry.

Thus, this research program, which intended to integrate today's needs of forestry industry, planned to study how ozone treatment could improve the biodegradability of the lignin derivatives in a real situation; to investigate the underlying mechanisms of ozone and fungal decolorization and dechlorination; to quantitatively evaluate the synergistic effects of ozone treatment with fungal decolorization and dechlorination; and to integrate these technologies to develop an advanced practical process for decolorization and dechlorination of most problemic

wastewater, the bleached kraft pulp mill effluents. In particular, this research program was designed to achieve the following primary objectives.

1. Chapter 2 focused on developing a reliable and quantitative assay procedures for assessing and comparing the biodegradable potential of slowly biodegradable complex organics, specifically, lignin components in bleached pulp mill effluents.

2. In Chapter 3 the methods for characterization and differentiation of pulp mill effluents were first established; then, the critical characteristics of various pulp mill effluents were systematically analyzed to determine which portion of the pulp mill effluents would be most suitable as a target for this study.

3. Chapter 4 concentrated on investigating and establishing ozone application methods and systems for reliably studying ozone decolorization and dechlorination of pulp mill effluents.

4. The effectiveness of ozone decolorization and dechlorination on various kraft pulp mill effluents was also evaluated statistically in Chapter 4.

5. Chapter 5 quantitatively and comparatively evaluate the improvement of ozone decolorization and dechlorination on the biodegradable potentials of lignin components.

6. Chapters 6 and 7 intended to systematically investigate and get a better understanding of the underlying mechanisms of ozone, fungal and ozone/fungal decolorization and dechlorination.

7. Chapter 7 also quantitatively evaluated the synergistic effects of ozone decolorization and dechlorination on fungal decolorization and dechlorination of pulp mill effluents.

8. The detail design of a reliable bioreactor system for studying ozone/fungal decolorization and dechlorination under a continuous mode was described in Chapter 8.

9. Chapters 8 also evaluated the performence of the new bioreactor system, and collected and analyzed the results from the continuous operation for scaling up the bioreactor system for possible field applications.

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CHAPTER 2. A MECHANISTIC MODEL FOR ASSESSING BIODEGRADABILITY OF PULP MILL EFFLUENTS*

2.1 INTRODUCTION

Over the years the concept of biodegradability has come to have different meanings among different groups of researchers. In the field of water and wastewater treatment, biodegradability often implied biotreatability (Gledhill, 1975; Porter and Snider, 1976; Suzuki et al., 1978). In simulated ecosystem studies (Liu et al., 1981; Metcalf et al., 1971), bioavailability was substituted for biodegradability. In many other instances, the persistivity was used for the indication of biodegradability (Jones et al., 1974; NRCC, 1978).

The diversity of definitions of biodegradability led to the development of a diversity of methods for assessment. These included shaker flask, CO_2 evolution, simulated activated sludge, model ecosystem, oxygen uptake, BOD_5/COD or BOD_2/TOC ratio (Gledhill, 1975; Metcalf et al, 1971; OECD, 1971; Sturm, 1973; Federal Register, 1979; Gilbert, 1987, 1988) and the various combinations as reviewed by Howard (1975).

The significant differences among the methods were the test period, the test environment and the parameters used for evaluation. For complex wastewaters, global parameters, either TOC or COD, were usually used for evaluation under the assumption that the reduction was solely due to biodegradation. This assumption is obviously subject to question because in aerobic systems there are many physical and chemical processes, in addition to the biological processes, responsible for TOC removal. Moreover, the TOC remained in the system may not have resulted from the parent compounds. Even if the TOC reduction was due to biological activities, the amount and rate of oxygen uptake may be subject to different mechanisms, such as degradation vs transformation, and exposure period. The environments to which the test substances were exposed were usually simulated based on a specific interest or situation for the study. The field conditions and the test conditions, however, were usually not fully comparable in each situation, resulting in wide variability in results.

The test conditions and exposure periods, which varied greatly among the methods, may significantly affect biodegradability. Nyholm et al. (1992) found that the kinetics of biochemical

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* A version of this chapter has been published. Mao, H.-Z. and Smith, D. W. (1995) Wat. Res., 29, 1957-1975.

reactions involved in biodegradability tests varied significantly with the test environments, exposure periods and among the methods. A comparative study (Means and Anderson, 1981) found that CO_2 evolution, simulated activated sludge, and Gledhill's method always gave some positive artifacts of biodegradability. The possible reasons for these observations were: 1) the test seeds were larger in volume and density; the organics (either from organic chemicals or biomass) in the seeds, therefore, may have served as the energy source or co-substrate; 2) the large amount of biomass may have resulted in immediate organic removal by adsorption; 3) in the CO_2 evolution test, the endogenous respiration of a large amount of biomass may have significantly contributed to the CO_2 production. The authors pointed out that the reported biodegradability was strongly method-dependent, and was extremely difficult to compare among different methods.

The objectives of this study were 1) to develop a reliable mechanistic model for assessing and predicting the biodegradability or biodegradable potential of complex wastewaters through a better understanding of the mechanism involved in the biodegradation of complex industrial wastewaters; and 2) to test and verify its reliability and applicability to various representative wastewaters using commonly accepted long-term biochemical oxygen uptake tests.

2.2 THEORETICAL DEVELOPMENT OF THE MECHANISTIC MODEL

The oxygen uptake test is essentially a bioassay procedure in which living microbial consortia consume dissolved oxygen while utilizing organic matter for energy and carbon sources through complex biochemical reactions. Oxygen uptake is usually exerted through three categories of biochemical reactions: 1) the utilization of readily degradable organic substances for energy and synthesis; 2) endogenous respiration once the food supply has become severely limiting; and 3) the slow degradation or transformation of organic materials which are relatively resistant to the biological mineralization (Ettinger, 1956; Gaudy and Gaudy, 1980). Included in the resistant fraction are some enzymes produced by the living microorganisms (Ettinger, 1956), the resistant substances initially present in the test sample, and those transformed during biochemical processes (Gaudy and Gaudy, 1980). The biomass, as one of the intermediates in biochemical processes, decreases continuously during endogenous metabolism as the system advances toward complete stabilization of biodegradable materials. However, the determination of ultimate oxygen uptake can not be constructed as a definition of total biodegradable organic

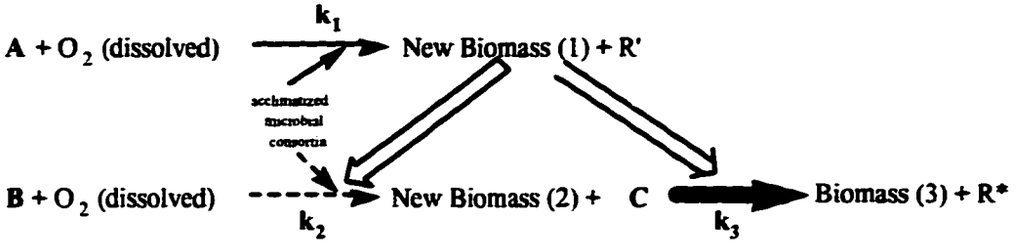
content since a small percentage (about 5 to 10%) of the assimilated carbon may remain as cellular components (Busch, 1966; Gaudy and Gaudy, 1980).

Theoretical models indicate that an infinite time must elapse if a biochemical reaction is to proceed to completion. For practical purposes, a 20-day period has frequently been selected as representing the time required for reaching greater than 90% completion of the reactions for relatively biodegradable materials. The adoption of the 5-day incubation period was based partly on the assumption that a reasonably large percentage of the ultimate oxygen uptake was exerted in this time. However, experience has shown that many natural and synthetic substances do not behave accordingly (Martone, 1976; Porter and Snider, 1976) since the biodegradation of these materials may advance slowly or erratically. In extreme cases, some of the more complex substances, such as lignins, may exert no measurable oxygen uptake in five (5) days.

The widespread use of one-stage first-order kinetics to describe oxygen uptake has contributed to the erroneous notion that ultimate oxygen uptake can be accurately predicted from short-term oxygen uptake studies. Indications were that one-stage first-order kinetics are generally not representative of the long-term oxygen uptake (Busch, 1958; NCASI, 1982; Skinner and van Roodselaar, 1992). An accurate interpretation of oxygen uptake required an understanding of toxicity, biochemical stability, the manner in which biochemical reactions progress, and the extent to which acclimation of microorganisms influences those factors. The short-term oxygen uptake test and one-stage first-order kinetics provided no information of this sort and, as a result, had naturally fallen under scrutiny with regard to the biodegradation of recalcitrant organics in complex wastewaters (Porter and Snider, 1976; NCASI, 1982; Skinner and van Roodselaar, 1992).

By incorporating these observations and the new findings in this study, it was assumed that in a batch bioreactor, 1) the individual biochemical reactions involved in the biodegradation followed the first-order kinetics; 2) the biochemical-oxygen-consuming materials could be divided into three components **A**, **B**, and biologically inert; 3) the component **A** was biodegraded with a kinetic constant k_1. This process provided carbons and energy for regenerating/maintaining biomass and energy for the essential bio-transformation of a less-biodegradable component **B**; 4) the biotransformation of the component **B** occurred in parallel with the biodegradation of the component **A** during the period of time t_0. The biotransformation

may require some dissolved oxygen, however, the amount would be relatively small, thus it was neglected in the model development; 5) after time t_0 elapsed, the major part of component A was metabolized; at the same time, the microbial consortia was induced to shift from one dominating group to another favorable one so that they were able to partially transform or enzymatically modify the component B to the intermediate form as a part of component C, or they changed their metabolism process such that they could use either the component C as a substrate more efficiently or component B directly and effectively (such as nitrification process). Figure 2-1 schematically illustrates these possible biochemical reactions and their relationships assuming fully acclimatized microbial consortia.



Where, A represents easily-biodegradable component, B represents the component which is less-biodegradable and may be biotranformed into component C in the presence of component A. C represents the intermediate products of various biotransformation processes, C is usually more biodegradable than component B. R' and R* represent end-product such as CO_2, H_2O, mineral matter and other residues. Solid arrows represent biodegradation processes; dash arrows represent biotransformation processes, and empty fat arrows stand for the possible flows of process energy and involved microbial consortia.

Figure 2-1. Pathways Involved in Biodegradation of Various Biological-oxygen-consuming Material in Complex Wastewaters During Oxygen Uptake Tests in a Batch Bioreactor

Thus, in a batch bioreactor, the disappearance rate of total oxygen-consuming material can be derived as,

$$\frac{dL}{dt} = -(k_1 + k_2)\,L$$

$$\therefore \quad L = L_0\, e^{-(k_1+k_2)t} \qquad (2\text{-}1)$$

where, L_0 represents the total amount of biochemical-oxygen-consuming material in the complex wastewater (equivalent to ultimate oxygen uptake), and L is the total remaining oxygen-

consuming material in the wastewater, respectively. Hereafter, the concentration of L is expressed as dissolved-oxygen-equivalent concentration, mg/L.

From Figure 2-1 and Equation (2-1), at time t, the exerted oxygen uptakes Y_A and Y_B can be expressed as Equations (2-2) and (2-3), respectively.

$$Y_A = L_0 - L - \int_0^t k_2 L dt$$

$$= L_0 - L_0 e^{-(k_1+k_2)t} - \int_0^t k_2 L_0 e^{-(k_1+k_2)t} dt \qquad (2\text{-}2)$$

$$Y_B = L_{C0} + \int_0^{t'} k_2 L' dt' - L_C \qquad (2\text{-}3)$$

where, Y_A and Y_B are the exerted oxygen uptakes of component A and B at time t and t', respectively; L_{C0} and L_C are the concentrations of component C at time $t=t_0$ and the remaining concentration of component C at time t' ($t'=t-t_0$), respectively. L' is the total remaining concentration at time t'.

By integrating and re-arranging Equation (2-2), it becomes

$$Y_A = \frac{k_1 L_0}{k_1 + k_2}(1 - e^{-(k_1+k_2)t}) \qquad (2\text{-}4)$$

$$\text{When } t \to \infty, Y_A \to L_{A0} = \frac{k_1 L_0}{k_1 + k_2}, \text{ and } t \to 0, Y_A \to 0 \qquad (2\text{-}5)$$

In a specific wastewater, the k_1, k_2 and L_0 were constant. Thus, the ultimate oxygen uptake exerted by component A could be estimated using Equation (2-5). However, the development pattern of component A not only depended on the characteristics and concentration of component A but also was greatly affected by both component B and C. The general development pattern of component A with the presence of B and C is schematically illustrated in Figure 2-2.

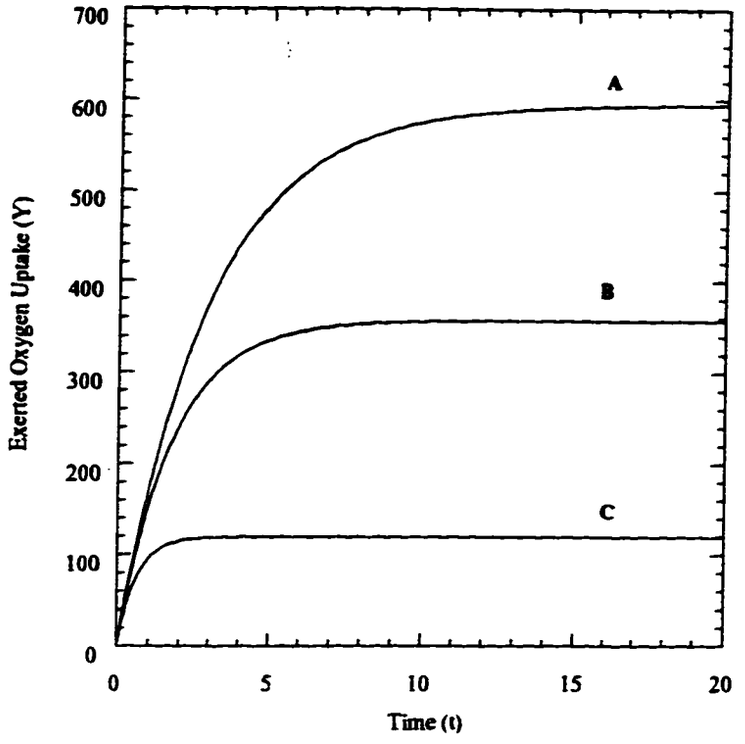


Figure 2-2. Schematic of Development Pattern of Biochemical Oxygen Uptake by Component **A** with the Presence of Component **B** and **C** (Curve A: $k_2/k_1=5$; B: $k_2/k_1=1$; C: $k_2/k_1=1/5$)

Similar to Y_A, the change of component **C** along time t can be formulated as Equation (2-6) according to Figure 2-1 and assumptions 4) and 5).

$$\frac{dL_C}{dt} = k_2 L \qquad (2\text{-}6)$$

Substituting Equation (2-1) into Equation (2-6) and integrating with $Y_C = 0$ at $t = 0$

$$\therefore \quad L_{C0} = \int_0^t k_2 L_0 e^{-(k_1+k_2)t} dt = \frac{k_2 L_0}{k_1 + k_2}(1 - e^{-(k_1+k_2)t_0}) \qquad (2\text{-}7)$$

and,

$$L' = L_0' e^{-(k_1+k_2)t'} \qquad (2\text{-}8)$$

where, $L_0' = L_0 e^{-(k_1+k_2)t_0}$

From Figure 2-1, the relationship of L_C with time t' ($t'=t-t_0$) can be elaborated as follows:

$$\frac{dL_C}{dt'} = k_2 L' - k_3 L_C$$

$$= k_2 L_0 e^{-(k_1+k_2)t_0} e^{-(k_1+k_2)t'} - k_3 L_C \qquad (2\text{-}9)$$

The boundary conditions for differential equation (2-9) are:

$t' = 0,\ L_C = L_{C0}$

Therefore, the solution for differential equation (2-9) is

$$Lc = \frac{k_2 L_0 e^{-(k_1+k_2)t_0}}{k_3 - k_1 - k_2}(e^{-(k_1+k_2)t'} - e^{-k_3 t'}) + \frac{k_2 L_0 (1 - e^{-(k_1+k_2)t_0})}{k_1 + k_2} e^{-k_3 t'} \qquad (2\text{-}10)$$

When $t' \to \infty,\ L_C \to 0$ (2-11)

The concentration profile of component C is schematically shown in Figure 2-3. It revealed that the concentration of C (express as L_c) will reach the maximum; then, gradually decrease to zero. As shown in Figure 2-3, L_c was strongly dependent on both elapsed time t_0 and biodegradation kinetics.

By substituting equations (2-6), (2-7), and (2-10) into equation (2-3), and re-arranging it as equation (2-12),

$$Y_B = \frac{k_2 L_0}{k_1 + k_2}[1 - e^{-k_3 t'} - \frac{k_3 e^{-(k_1+k_2)t_0}}{k_3 - k_1 - k_2}(e^{-(k_1+k_2)t'} - e^{-k_3 t'})] \qquad (2\text{-}12)$$

Since $t' = t-t_0$, Equation (2-12) can be re-written as:

$$Y_B = \frac{k_2 L_0}{k_1 + k_2}[1 - e^{-k_3(t-t_0)} - \frac{k_3 e^{-(k_1+k_2)t_0}}{k_3 - k_1 - k_2}(e^{-(k_1+k_2)(t-t_0)} - e^{-k_3(t-t_0)})] \quad (t > t_0)$$

(2-13)

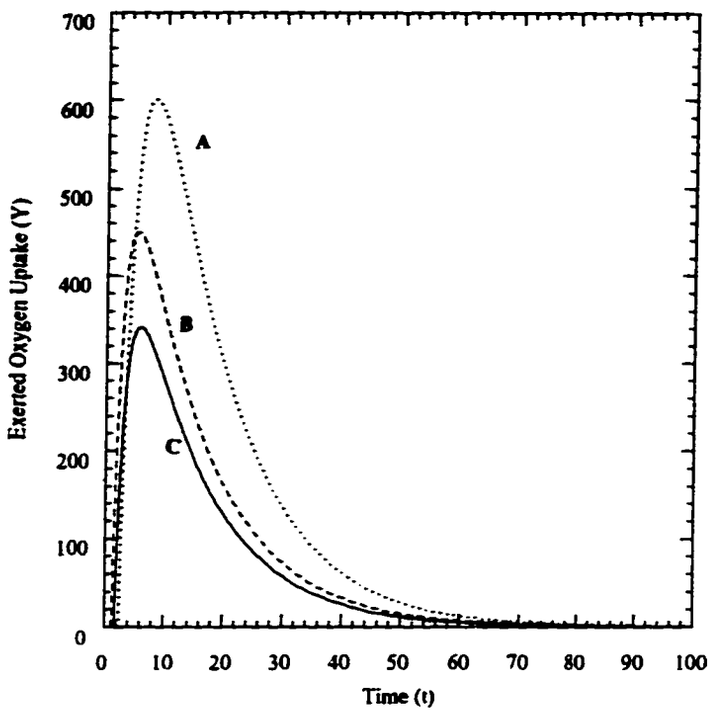


Figure 2-3. Schematic of Concentration Profile of Component C (Curve A: k_1/k_2=5, t_0=5; B: k_1/k_2=1, t_0=5; C: k_1/k_2=1, t_0=10)

$$\text{When } t' \to \infty, Y_B \to L_{B0} = \frac{k_2 L_0}{k_1 + k_2}, \text{ and } t' \to 0, Y_B \to 0 \qquad (2\text{-}14)$$

Equations (2-11), (2-13) and (2-14) and Figure 2-1 define the exerted oxygen uptake Y_B by the component **B** through intermediate form component **C** in any complex wastewater as the function of the kinetic constants k_1, k_2 and k_3. Figures 2-2, 2-3 and 2-4 schematically demonstrates the transition status of the three components during the complete metabolic processes assumed to be carried out in a closed batch bioreactor. As shown in these figures, Y_B not only directly related to these absolute values but was greatly influenced by the ratios of k_2/k_1 and k_3/k_1, since any intermediate would accumulate in the system without significant oxygen uptake, and subsequently resulted in the change of the microbial consortia involved in the biochemical reactions if these ratios were unbalanced. In a real system, these ratios should be unique to the system with specific substrates and microbial consortia. However, the amount of component **C** would vary with time, the characteristics of component **B** and the microbial consortia; the development of component **C** or development of new metabolic processes was

indicative of the status shifting of oxygen-consuming material, microbial consortia or both. As often seen in the nitrification process of domestic wastewater, for example, this phenomenon indicated that the total amount of nitrifying bacteria reached significant density after certain incubation time so they started to exert the significant amount of biochemical oxygen uptake.

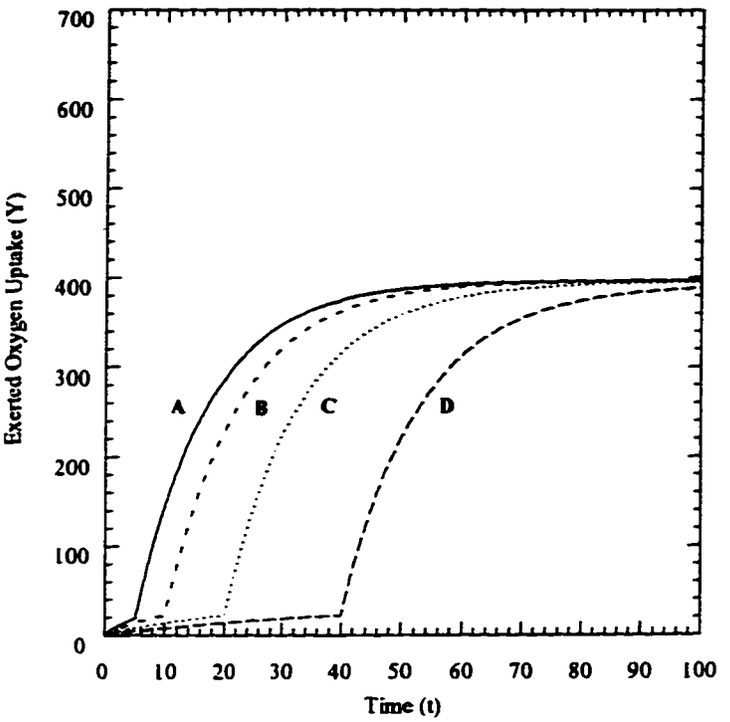


Figure 2-4. Schematic of Development Pattern of Biochemical Oxygen Uptake by Component B (k_1/k_2=1 and k_3=0.08 day^{-1}; Curve A: t_0=5, B: t_0=10; C: t_0=20; D: t_0=40)

Therefore, the total biochemical oxygen uptake (TBOU) of the complex wastewater after incubation time t using the fully acclimatized microbial consortia can be described as follows:

$$TBOU = Y_A + Y_B.$$

Thus,
$$TBOU = \frac{k_1 L_0}{k_1 + k_2}(1 - e^{-(k_1+k_2)t}) + \frac{k_2 L_0}{k_1 + k_2}[(1 - e^{-k_3(t-t_0)} - \frac{k_3 e^{-(k_1+k_2)t_0}}{k_3 - k_1 - k_2}(e^{-(k_1+k_2)(t-t_0)} - e^{-k_3(t-t_0)})] \qquad (t > t_0) \qquad (2\text{-}15)$$

$$\text{When } t \to \infty, TBOU \to \frac{k_1 L_0}{k_1 + k_2} + \frac{k_2 L_0}{k_1 + k_2} = L_0 \qquad (2\text{-}16)$$

Equation (2-16) reveals that the development pattern of the TBOU will be dependent on all three components and will reach the ultimate biological oxygen uptake when incubation time increases toward infinite.

In Equation (2-16),

$t > t_0$, $\quad \therefore \quad t - t_0 > 0$; and $t_0 \geq 0$;

also, $k_1 \geq 0$, $\quad k_2 \geq 0$, $\quad$ and $\quad k_3 \geq 0$;

Thus, $e^{(k_1+k_2)t_0} \geq 1$, $\quad e^{(k_1+k_2)(t-t_0)} \geq 1$, $\quad$ and $\quad e^{k_3(t-t_0)} \geq 1$;

Then, $e^{-(k_1+k_2)t_0}(e^{-(k_1+k_2)(t-t_0)} - e^{-k_3(t-t_0)}) \leq 1$;

Similarly, $\left|\frac{k_3}{k_3 - k_2 - k_1}\right| = \left|\frac{1}{1 - \frac{k_2}{k_3} - \frac{k_1}{k_3}}\right| < 1$ since $k_1 > k_3$ and $k_2 > k_3$ (see Figure 2-1)

Therefore, $[\frac{k_3 e^{-(k_1+k_2)t_0}}{k_3 - k_2 - k_1}(e^{-(k_1+k_2)(t-t_0)} - e^{-k_3(t-t_0)})] << 1$

In any complex industrial wastewater, it can be assumed that the TBOU would be greater than 1, that is, TBOU>1, so Equation (2-16) could be simplified into Equation (2-17).

$$\text{TBOU} = \frac{k_1 L_0}{k_1 + k_2}(1 - e^{-(k_1+k_2)t}) + \frac{k_2 L_0}{k_1 + k_2}(1 - e^{-k_3(t-t_0)}) \qquad (t > t_0) \quad (2\text{-}17)$$

2.3 MATERIALS AND METHODS

2.3.1 Samples for Model Evaluation

The samples used for the model evaluation were a solution of glucose/glutamic acid (Type A), grab samples of ASB influent (Type B) and effluent (Type C), and a grab sample of E_{op} filtrate (Type D). The sample Type A served as an easily-biodegradable organic mixture and standard called a GG solution. The ASB influent was sampled from the inlet of aerated stabilization basins (ASB), and represented the mixture of industrial and municipal wastewater.

The ASB effluent, which was from the discharge point of the ASB, represented the biologically-treated secondary effluent. The E_{op} filtrate was the bleachery effluent from E_{op} stage of $DE_{op}D_1ED_2$ (see note in Table 2-1) bleaching sequence (Mao and Smith, 1994a), and represented the industrial wastewater with a high concentration of both easily- and poorly-biodegradable organics. Unless stated otherwise, these samples were characterized according to Standard Methods (APHA, 1992).

2.3.2 Sample Preparation and Analysis

2.3.2.1 Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC)

Standard Methods was followed in COD analysis except that excess $Hg(SO)_4$ was added before the addition of $Ag_2(SO)_4$ to reduce the interference of the chloride in the wastewater samples.

The high temperature system (@ 850°C) of Total Organic Carbon Analyzer (Dohrman Xertex Carbon Analyzer) was employed to maximize the oxidation efficiency of the kraft lignins, and to minimize the known interference of the chloride and other unknown factors in wastewater samples (Alken, 1992). Total Organic Carbon (TOC) was obtained by subtracting TIC from TC.

The standards were always run along with the samples to ensure that the instrument was operating properly and the proper procedures were followed.

2.3.2.2 Sample Fractionation

E_{op} filtrate, ASB influent, raw and ozone treated ASB effluents were fractionated into four fragments using a Millitan Acrylic Ultrafiltration System (Millipore Co.) equipped with 1,000, 5,000, and 10,000 molecular weight cutoff (MWCO) membranes. The details have been described elsewhere (Mao and Smith, 1994a).

2.3.3 Biodegradability Improvement Studies Using Ozone Treatment

The procedure and reactor for the ozone treatment study were similar to those described by Smith *et al.* (1992) and Mohammed (1990) with minor modifications for more accurate determination of the consumed ozone dose. The details have been described elsewhere (Mao and Smith, 1994 b and 1995).

2.3.4 Oxygen Uptake Studies

2.3.4.1 Acclimatization of Microbial Consortia

The microbial consortia from ASB effluent was used as the initial seed in a batch activated sludge system for acclimatizing the microorganisms to either E_{op} filtrate or ASB influent with sufficient nutrients. The seeds were acclimatized for about two weeks before the first oxygen uptake study was conducted, and were maintained by replacing 10% of the total reactor volume with the fresh wastewater (twice a week) over the entire experimental period.

2.3.4.2 Oxygen Uptake Tests

Unless stated otherwise, the oxygen uptake tests performed in all the experiments are the triplicates at each dilution with a proper number of blanks and controls for statistical analysis of data. The graduated cylinders and membrane electrode techniques described in Standard Methods were adopted for the sample dilution and dissolved oxygen measurements, respectively, since the color in the mill effluents would interfere with the titration method for D.O. determination.

Before starting the test, 2 mL of acclimatized seed solution, which was settled for 30 minutes, was directly added to one litre of the diluted wastewater sample, and the contents were carefully mixed. In the long-term tests, the different dilution of the samples and the period of D.O. measurement were carefully designed such that 1) the dilution was sufficient to exert a measurable dissolved oxygen consumption between measurements; 2) the initial D.O. was sufficient to last throughout the desired duration of the experiments with D.O. greater than 1 mg/L at the end of experiments. This ensured that the reaeration of the samples could be avoided since this has been shown to give higher oxygen uptake (McKeown, 1981; Whittemore, 1982). In all the tests, Pyrex glass beads, which were thoroughly washed with a chromic acid solution, were added to the test bottles prior to filling the seeded samples to be incubated at 20±1°C. The bottles were mixed twice a week and mixed thoroughly immediately before D.O. measurements to reduce the wall effects.

2.3.5 Data Analysis

Non-linear parameter estimations were performed using either Simplex or Quasi-Newton methods pre-programmed in Systat® statistics package. In the regression analysis, the residuals

of regression were always plotted for assessing the distribution of residuals, and the goodness of fit of the model. In addition, Hessian Matrix was always calculated to check the possibility of intercorrelation of the parameters. In the regression analysis the corrected R^2, which is defined as $[1 - \frac{\text{residual sum - of - squares}}{\text{corrected sum - of - squares}}]$, were used for evaluation.

2.4 RESULTS AND DISCUSSION

Table 2-1 summarizes the characteristics of the three types of wastewaters used in this study, and Table 2-2 compares the levels of their biochemical oxygen uptake over different incubation periods. The results in Tables 2-1 and 2-2 demonstrated that these wastewaters could represent the desired categories of wastewaters for model evaluation with respect to COD, TOC, BOD_5, BOD_5/COD, BOD_{20} and NH_3-N.

Table 2-1. Summary of Characteristics of Eop Filtrate, ASB Influent and Effluent[7]

Parameter		E_{op} Filtrate	ASB Influent	ASB Effluent
pH		9.65±0.15	5.6±0.2	7.1±0.3
Temperature	(°C)	75	45 (15 to 55)	15 to 40
Dissolved Oxygen	(mg/L)	< 0.5	<0.8	>2
BOD_5	(mg/L)	540 ± 15	460±10	25.1
TSS[2]	(mg/L)	55±5	20±2	70±4
TDS[3]	(mg/L)	4720±70	1860±35	2557±20
TDVS[4]	(mg/L)	2563±17	1103±25	559±6
COD	(mg/L)	3058±44	1368±17	810
TDOC[5]	(mg/L)	1287±23	466±5	
TOC	(mg/L)	1296±14	493±4	276.3
BOD_5/COD		0.177	0.332	0.0309
COD/TDOC		2.38	2.94	
NH_3-N	(mg/L)	<0.1	5.89	< 1.5
TNOD[6]	(mg/L)	<5	<25	< 5

Note: 1. D= chlorine Dioxide, E_{OP} = alkaline Extraction of Oxygen/Peroxide, E= alkaline Extraction; 2. TSS = Total Suspended Solids;
3. TDS = Total Dissolved Solids; 4. TDVS = Total Dissolved Volatile Solids;
5. TDOC = Total Dissolved Organic Carbon; 6. TNOD = Theoretical Nitrogenous Oxygen Demand
7. see Table I-1 in Appendix I.

Table 2-3 summarizes the characteristics of each fragment derived from the ultrafiltration separation and the recovery rate in the separation process. The results in Table 2-3 indicated that the ultrafiltration process caused negligible loss or alteration of the processed wastewaters with regard to COD, TOC and BOD_5. Thus, the fragments from the ultrafiltration process were representative portions of these components in the raw wastewaters. Table 2-3 also reveals that the distribution pattern of ASB effluent mimiked that of ASB influent and E_{op} filtrate with regard to COD and TOC. It is important to recognize, however, that the distribution of BOD_5 among the fragments of ASB effluent differed considerably from that of ASB influent and E_{op} filtrate.

Table 2-2. Oxygen Uptake of E_{op} Filtrate and ASB Influent and Effluent over Different Periods

Sample	5-day		20-day		Ultimate BOD		BOD_5/COD
	BOD[1]	Level (%)	BOD	Level (%)	BOD	Level (%)	
E_{op} Filtrate	540±15[2]	56.4	790±14	82.5	958±30	100	0.177
ASB Influent	460±10	64.2	619±7	86.5	716±18	100	0.332
ASB Effluent (A)	25±4	18.7	56±3	41.8	134±10	100	0.0309
ASB Effluent (B)	11.7±2.5	9.2	29.4±3.7	23.1	127.7±6.1	100	0.0179
NR[3]	29.4±2.8	19.1	49.1±3.4	31.8	153.9±12	100	0.0494

1: BOD: mg/L; 2: ±: indicates the standard deviation; 3: ozone treated ASB effluent

Table 2-3. Characteristics of Raw and Four Fragments of E_{op} Filtrate, ASB Influent and Effluent

PARAMETER		FRAGMENTS						
		MWCO<1000	MWCO>1000 MWCO<5000	MWCO>5000 MWCO<10000	MWCO>10000	TOTAL[1]	RAW[2]	DIFF[3]
COD	ASB Effluent	132.2(20%)	83.9(13%)	111.4(17%)	306.2(47%)	633.7	654.2	20.5
(mg/L)	ASB Influent	595.6(47%)	127.1(10%)	184.7(15%)	359.8(29%)	1267.2	1260.8	-6.4
	Eop Filtrate	897.9(30%)	272.4(9%)	384.7(13%)	1185.9(40%)	2732.8	3000.3	267.5
TOC	ASB Effluent	52.1(21%)	35.8(15%)	46.3(19%)	113.4(46%)	247.6	246.7	-0.9
(mg/L)	ASB Influent	195.8(44%)	55.9(12%)	76.6(17%)	137.1(31%)	465.3	444.0	-21.3
	Eop Filtrate	376.3(30%)	106.7(9%)	148.5(12%)	444.7(36%)	1076.2	1239.8	163.6
BOD_5	ASB Effluent	12.2(95%)	3.8(29%)	4.2(33%)	6.6(51%)	26.8	12.9	-13.9
(mg/L)	ASB Influent	263.1(79%)	25.6(8%)	34.4(10%)	18.2(5%)	341.3	333.9	-7.4
	Eop Filtrate	421.8(80%)	25.5(5%)	20.3(4%)	37.3(7%)	504.9	524.0	19.1

Note: 1. TOTAL is the sum of parameter value from all fragments; 2. RAW is Raw effluent used in fractionation study;
3. DIFF is the difference between the Raw and TOTAL; 4. Number in bracket is the percentage of that fragment in raw effluent;
5. ASB influent and effluent were sampled at April 20, 1993.

Figure 2-5 illustrates the typical UV spectra of each fragment derived from the fractionation. It demonstrated that all but the fragment with MWCO<1,000 (F1) had a characteristic 205 nm UV absorption peak identified as the unique feature of kraft lignins (Brauns and Brauns, 1960; Pearl, 1967; Kleinert and Joyce, 1957). Figure 2-5 also demonstrates that the fragment with MWCO>10,000 (F4) contributed more than 50% of the absorbance around 205 nm, and the absorbance increased greatly with the increase of the molecular-weight. This observation was consistent with the true color measurement, and correlated very well with the results of TOC and COD shown in Table 2-3. These results also agreed with the previous findings reported in the literature (Sakakibara, 1991; Brauns and Brauns, 1960). In particular, the density of the characteristic-functional groups in the lignins and their derivatives increased with the size of the molecules; conversely, the biodegradability of lignins and their derivatives decreased greatly with the size of the molecules.

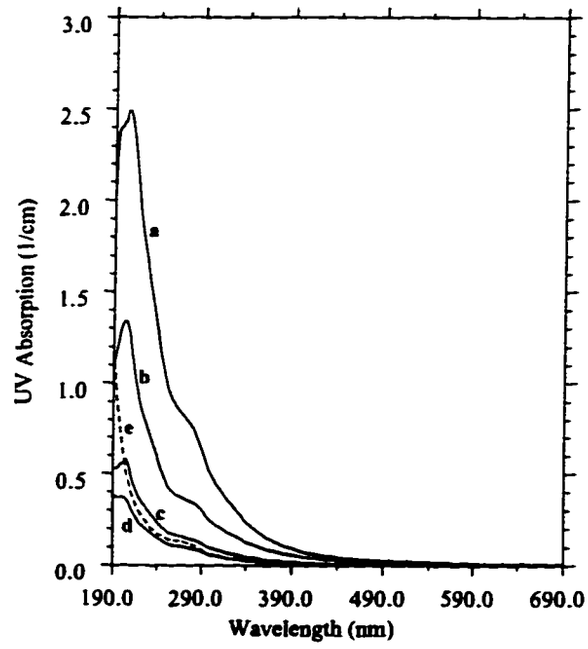


Figure 2-5. Typical UV Spectra of Raw and Four Fragments (ASB Influent at pH = 7.6) **a**: Raw, **b**: F4, MWCO>10,000, **c**: F3, 5,000<MWCO<10,000, **d**: F2, 1,000<MWCO<5,000, **e**: F1, MWCO<1,000

In short, these findings suggested that the wastewaters were well fractionated, and each could serve as a desired substrate for model evaluation in terms of the effects of various components on the biodegradability of complex industrial wastewaters.

2.4.1 Estimation of Kinetic Parameters in New Model

Two methods were developed to estimate the kinetic parameters in the new model based on the mechanism described in the model development.

2.4.1.1 Directly Using Long-term Oxygen Uptake Data (Method A)

Figures 2-6 through 2-9 illustrate the series of semi-log plots of the oxygen uptakes by the each fragment of E_{op} filtrate and ASB effluent (called Effluent (A)). In these figures, the original oxygen uptake data were transformed using Y=Ln (X). The kinetic constants were estimated using a simple first-order model according to the assumptions made in the model development. The regression equations based on these estimations were also illustrated in Figures 2-6 through 2-9, respectively.

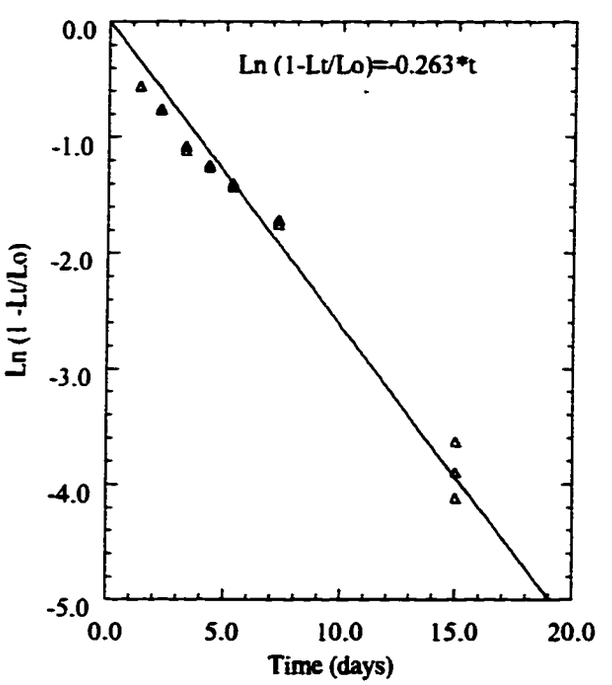


Figure 2-6. Estimation of Kinetic Parameter of Fragment with MWCO<1,000 (F1, Eop filtrate) Based on First-order Biochemical Reactions

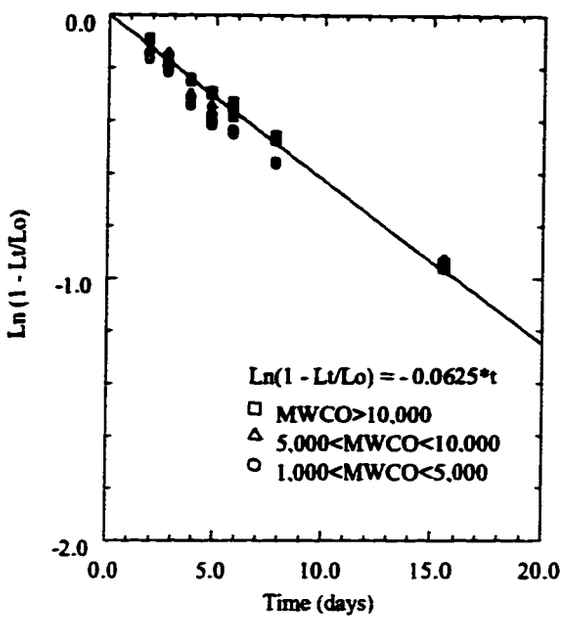


Figure 2-7. Estimation of Kinetic Parameter of Other Three Fragments (F2, F3, F4, E_{op} filtrate) Based on First-order Biochemical Reactions

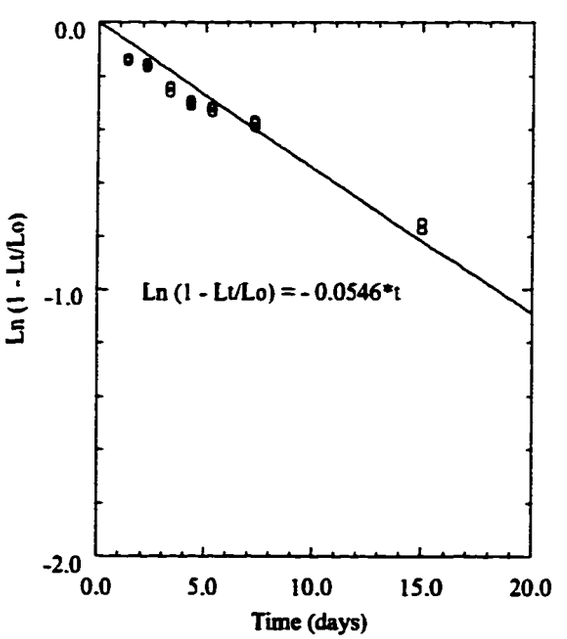


Figure 2-8. Estimation of Kinetic Parameter of Fragment with MWCO<1,000 (F1, ASB Effluent) Based on First-order Biochemical Reactions

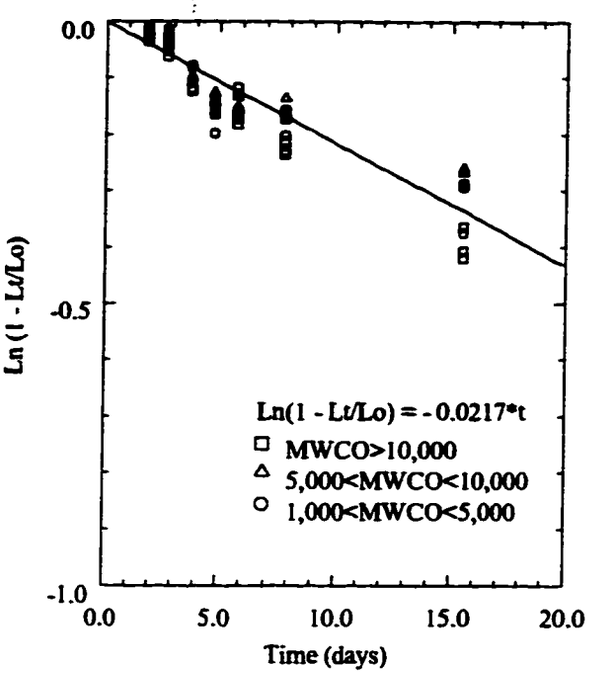


Figure 2-9. Estimation of Kinetic Parameter of Other Three Fragments (F2, F3, F4, ASB Effluent) Based on First-order Biochemical Reactions

The above estimated kinetic constant from F1 was used as k_1, and the kinetic constant estimated from F2 (1,000<MWCO<5,000), F3 (5,000<MWCO<10,000), and F4 was used as k_3 in the new model. Then, the first 20 days and first 50 days of biochemical oxygen uptake data from E_{op} and ASB effluent, respectively, were employed to estimate the other parameters in the new model. Table 2-4 compiles all the estimated kinetic parameters, their 95% confidence intervals, and corrected R^2s.

To examine the reliability of the above parameter estimation the complete sets of oxygen uptake data by the raw and ozone treated ASB effluent, raw ASB influent, raw E_{op}, and GG solution were directly used for the estimation of these kinetic parameters based on the new model. Figure 2-10 demonstrates the development patterns of the oxygen uptake by all four types of samples over 100 days, and Figure 2-11 compares the development pattern of the oxygen uptake by a raw ASB effluent (Effluent (B)) with that by the ozone-treated ASB effluent sample at a dose level of about 80 mg/L. The results from these parameter estimations were also summarized in Table 2-4. In addition, Figures 2-12 through 2-14 compare the series of long-term

oxygen uptake data from all four fragments of both raw and ozone treated ASB effluents. The kinetic parameters of each fragment in this series of tests were also estimated using a one-stage first-order kinetic model, the original data, and the non-linear parameter estimation. The ASB effluent in these series of tests was different from the Effluent (A), and subsequently called Effluent (B). These estimations were again listed in Table 2-4 for comparison.

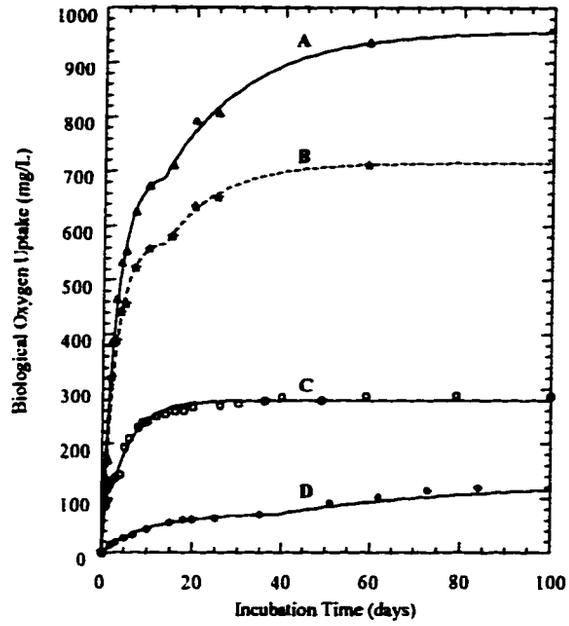


Figure 2-10. The Measured and Predicted Development Patterns of Oxygen Uptake among Different Samples Based on Raw Oxygen Uptake Data and Mechanistic Model (A: E_{op} Filtrate; B: ASB Influent; C: 300 mg/L Glucose/Glutamic Acid Solution; D: ASB Effluent)

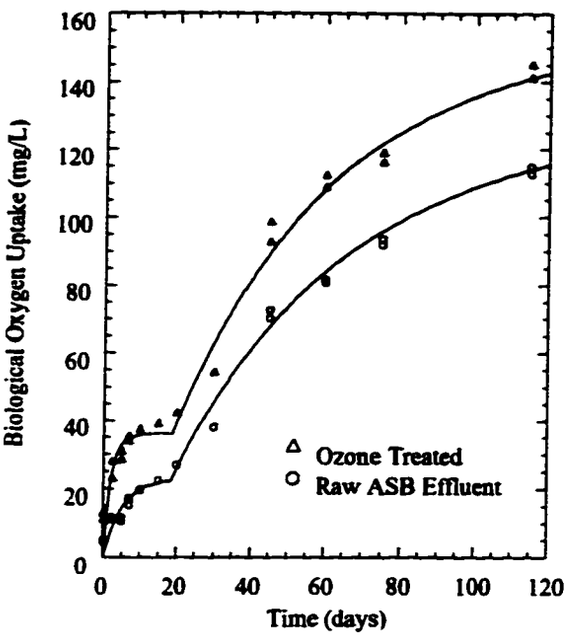


Figure 2-11. Comparison of the Predicted and Measured Development Patterns of Oxygen Uptake of Ozone Treated ASB Effluent with Those of Raw ASB Effluent Based on Mechanistic Model

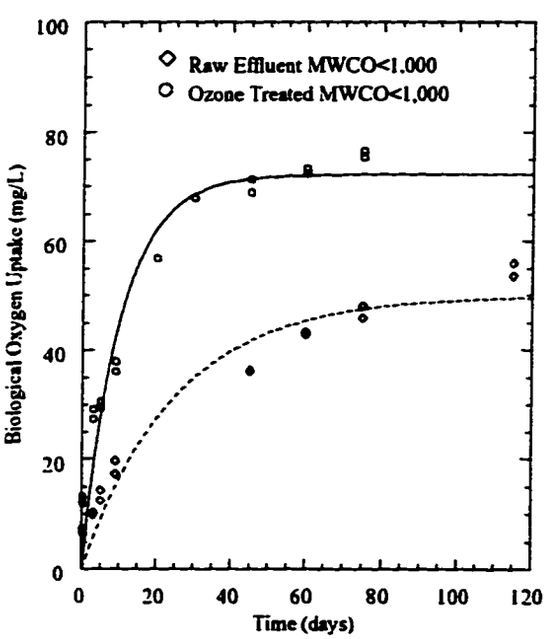


Figure 2-12. Comparison of the Measured and Predicted Long-term Oxygen Uptakes of F1 from Ozone Treated ASB Effluent with Those from Raw ASB Effluent Based on Simple First-order Model

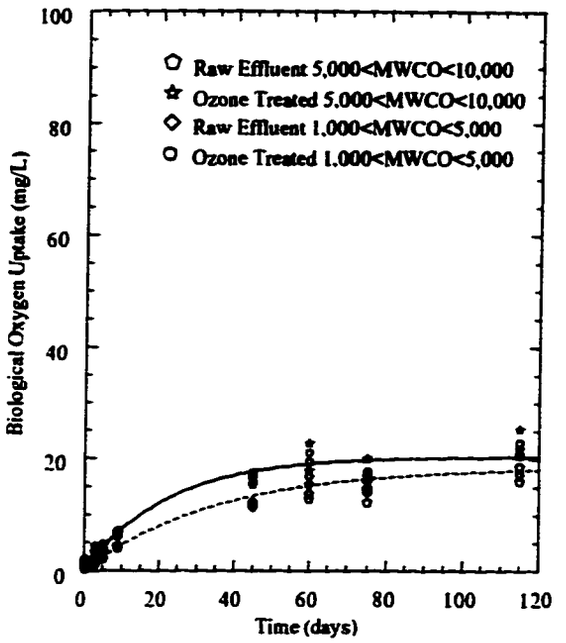


Figure 2-13. Comparison of the Measured and Predicted Long-term Oxygen Uptakes of F2 and F3 from Ozone Treated ASB Effluent with Those from Raw ASB Effluent Based on Simple First-order Model

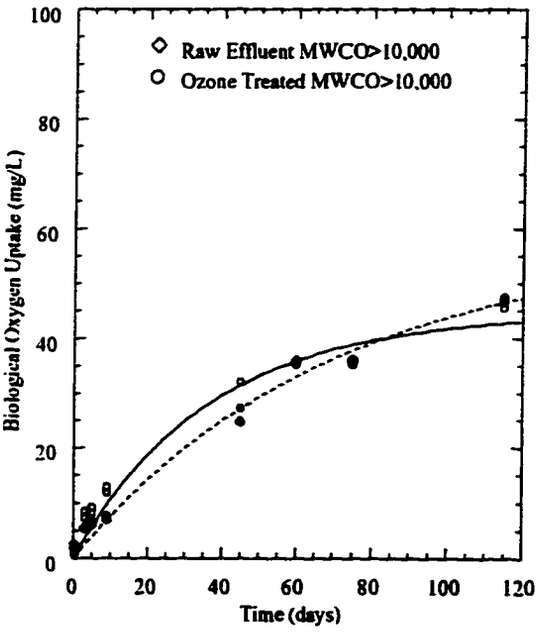


Figure 2-14. Comparison of the Measured and Predicted Long-term Oxygen Uptakes of F4 from Ozone Treated ASB Effluent with Those from Raw ASB Effluent Based on Simple First-order Model

Table 2-4. Parameters Estimated Using Either Mechanistic Model (for raw effluents) or One-stage First-order Model (for Fragments)

Wastewater	Fragment	Estimated Parameters					95% Confidence Interval				corrected
		L_o	k_1	k_2	k_3	K*	L_o	k_1	k_2	K or k_3	R^2
Eop Filtrate	Raw	957.9±29.7**	0.244±0.0123	0.0922±0.0131	0.0531±0.0181		897.7 - 1018.1	0.219 - 0.269	0.0656 - 0.119	0.0165 - 0.0897	0.986
	F1					0.263±0.00590					
	F2+F3+F4					0.0625±0.00109					
ASB Influent	Raw	715.4±18.4	0.282±0.0155	0.0703±0.0146	0.0812±0.0361		678.0 - 752.8	0.251 - 0.314	0.0407 - 0.0998	0.0079 - 0.155	0.974
Effluent (A)	Raw	134.2±10.2	0.0501±0.0041	0.0426±0.0048	0.0196±0.0060		113.7 - 154.8	0.0417 -0.0584	0.0329 - 0.0524	0.0073 -0.0318	0.994
	F1					0.0546±0.00188					
	F2+F3+F4					0.0217±0.00109					
Effluent (B)	Raw	127.7±6.1	0.0310±0.00981	0.146±0.0818	0.0206±0.00271		114.7 - 140.7	0.0101 - 0.0519	0.0287 - 0.320	0.0148 - 0.0263	0.994
	F1	49.9±3.82				0.0403±0.0130	41.7 - 58.1			0.0125 - 0.0682	0.935
	F2	18.7±1.65				0.0285±0.00742	15.2 - 22.3			0.0126 - 0.0444	0.949
	F3	17.3±1.29				0.0256±0.00538	14.5 - 20.1			0.0139 - 0.0372	0.973
	F4	58.1±5.00				0.0140±0.00216	47.4 - 68.8			0.00939 - 0.0187	0.983
O_3 Treated Effluent (B)	Raw	153.9±7.96	0.0996±.00494	0.326±0.0446	0.0234±0.00385		136.9 - 170.8	0.0510 - 0.174	0.182 - 0.635	0.0152 - 0.0316	0.989
	F1	72.5±2.66				0.0993±0.0142	66.8 - 78.2			0.0689 - 0.130	0.932
	F2	20.4±0.96				0.0452±0.00898	18.3 - 22.4			0.0258 - 0.0646	0.958
	F3	22.4±1.68				0.0338±0.00870	18.8 - 26.0			0.0152 - 0.0525	0.946
	F4	44.7±3.57				0.0274±0.00628	37.0 - 52.3			0.0139 - 0.0408	0.962

*: K=kinetic constant estimated using simple first-order model; **: standard deviation

2.4.1.2 Using Short-term Oxygen Uptake Data Incorporating TOC/COD Parameter (Method B)

This method employs 20 days biochemical oxygen uptake tests (20-day tests), TOC and COD for the estimation of all parameters in the model. However, in some cases, the elapsed time t_0 may be longer than 20 days. In this circumstance a proper series of tests should be conducted which must last 7 to 10 days longer than elapsed time t_0 to accurately determine the elapsed time t_0.

In this method, the 20 days biochemical oxygen uptake data from each fragment were used for the estimation of the kinetic constants k_1 and k_3. The estimation methods were similar to those described in Method (A). In addition, the 20 days biochemical oxygen uptake data from the original wastewaters were plotted against the incubation time to estimate the elapse time t_0. As demonstrated in Figures 2-10 and 2-11 the intersection between the two phases with different respiration rates determined the elapsed time t_0 and the ultimate oxygen uptake which was equivalent to L_{A0} by component A as described in the model development. Table 2-5 compares the results from Method (B) with those from Method (A).

Table 2-5. Comparison of Estimated Parameters Using Method (B) with Those Using Method (A)

Wastewater	t_0		L_{A0}	k_2		L_0	
	(A)	(B)		(A)	(B)	(A)	(B)
Eop	13.2	13.5	720	0.0922	0.0869	958	962
ASB Influent	13.7	12.5	580	0.0703	0.0656	715	715
ASB Effluent (A)	39	38	72	0.0426	0.0431	134.2	145.8
ASB Effluent (B)	18.2	17.5	27	0.146	0.150	127.7	122.2
O_3 Treated Effluent (B)	18.7	17.5	38	0.326	0.298	153.9	149.5

(A)=Method (A); (B)=Method (B)

In order to estimate the ultimate biochemical oxygen uptake without conducting long-term tests a regression analysis on the characteristics of various raw and ozone-treated pulp mill effluents used in this study was performed to establish the empirical relationships among the ultimate oxygen uptake, k_1, k_3, and TOC/COD ratio. The analysis results were formulated as Equation (2-18).

$$L_0 = 87.6\,[BOD_{20}(\frac{k_3}{k_1})]^{0.72}[\frac{TOC}{COD}]^{1.60} \qquad (2\text{-}18)$$

Where, L_0 and BOD_{20} are the ultimate and 20-day biochemical oxygen uptake of the original wastewater, respectively. k_1 and k_3 are the estimated kinetic constants. TOC and COD are total organic carbon and chemical oxygen demand of the original wastewater.

The ultimate oxygen uptake estimated from the actual measured long-term oxygen uptakes (over 100 days) with the predicted ultimate oxygen uptakes of various raw and ozone-treated effluents based on Equation (2-18) were also compared in Table 2-5. The results suggested that the ultimate oxygen uptake could be reliably predicted by Equation (2-18) using the intrinsic biological and chemical parameters of the raw wastewaters. At least, the results demonstrated that the ultimate biochemical oxygen uptake of a specific type of industrial wastewater such as pulp mill effluents could be satisfactorily predicted using these parameters indicated in Equation (2-18). However, the coefficients in Equation (2-18) may vary with the characteristics of wastewater.

After the determination of L_0, the kinetic constant k_2 was estimated by substitution L_0, L_{A0}, k_1 into Equation (2-5). Thus, the new model was properly calibrated for the tested wastewater and can be used for the prediction of biochemical oxygen uptake of the specified wastewater over any desired period. The calibrated model will be specially useful for modeling the dissolved oxygen profile in the surface water and evaluating the biodegradation kinetic constants.

In summary, the results revealed that 1) both methods reliably estimated the kinetic parameters in the new model and the estimations agreed with each other well with regard to the experimental errors; 2) the kinetic constants of all the F1s were much higher than kinetic constants of the other three fragments F2, F3, F4, and the fragment F2 was very similar to the other two large fragments F3 and F4 with regard to the kinetic constants of biochemical reactions; 3) the kinetic constant estimated from the oxygen uptake data by the fragment F1 was within the 95% confidence interval of k_1 estimated by the new model, and the kinetic constants from the other three fragments were within the 95% confidence interval of k_3; 4) the sum of the ultimate oxygen uptake from all fragments fell within the 95% confidence interval of the ultimate oxygen uptake of the respective raw wastewater.

2.4.2 Model Evaluation

For both statistical and visual evaluation, the predicted biochemical oxygen uptake of the wastewaters used in this study by the calibrated new model were compared in Figures 2-10 and 2-11. In this evaluation, the kinetic parameters were estimated using Method (A). Examining the corrected R^2s in Table 2-4 and the predicted curves in Figures 2-10 and 2-11 suggested that the new model could predict various types of biochemical oxygen uptakes by the various pulp mill effluents used in this study over the long-term very well.

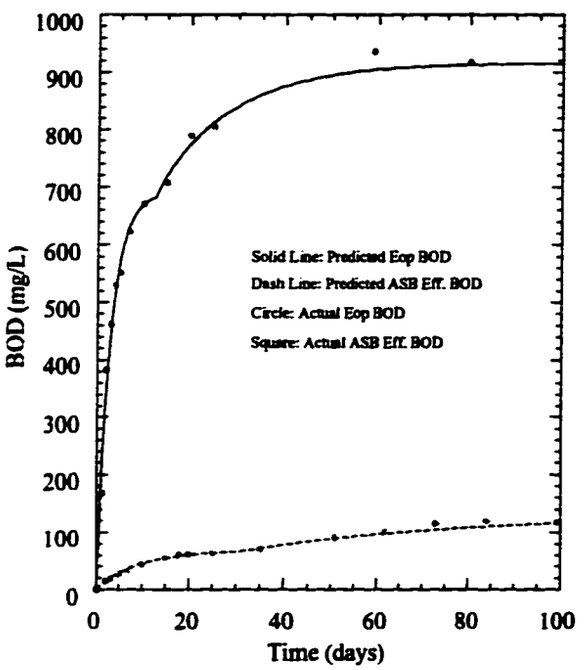


Figure 2-15. The Measured and Predicted Development Patterns of Oxygen Uptakes of E_{op} Filtrate and ASB Effluent Based on the Mechanistic Model

Moreover, to test the reliability of the new model predictions on the long-term oxygen uptake of pulp mill wastewaters, the kinetic parameters estimated from the short-term oxygen uptake data by each fragment (Method (B)) were substituted into the new model, and the calibrated models were used to predict the oxygen uptake of each wastewater over a long term. The measured and predicted results were compared in Figure 2-15, and some of the representative points of oxygen uptake were comparatively compiled in Table 2-6. Figure 2-15

and Table 2-6 demonstrate that the model derived from the estimated parameters using the short-term oxygen uptake data of the corresponding fragments satisfactorily predicted the long-term (over 100 days) oxygen uptake by the respective wastewater under the specified conditions.

Table 2-6. Comparison of Predicted with Measured Oxygen Uptakes by Various Wastewaters at Various Representative Incubation Time Points

Time (days)	Type of Data	Wastewater Samples			
		E_{op}	ASB Effluent	A1-42	A1-47
50	Measured	894			128
	Predicted	921			132.6
52	Measured		92.5	3180	
	Predicted		90.7	3073	
60	Measured	925	100.2	3270	138
	Predicted	936	102.4	3249	140.4
75	Measured		113.4	3370	187
	Predicted		106.3	3493	182.9
80	Measured	938	116.8		
	Predicted	950	108.8		
100	Measured			3735	207
	Predicted			3745	214.6
120	Measured			3860	220
	Predicted			3865	225.3
140	Measured			3960	235
	Predicted			3941	230.9
150	Measured			3990	239
	Predicted			3969	233.6

It may be further deduced that the model can be satisfactorily used to predict the long-term oxygen uptake provided that the model was properly calibrated under desired conditions. In fact, this deduction has been recently verified by the extensive studies on the oxygen uptakes by various effluents from all the pulp and paper mills operated in Alberta, Canada (MacDonald and Radermacher, 1993). Figures 2-16 and 2-17 illustrate the two sets of oxygen uptake data adopted from the above study. Figure 2-16 represents a pulp mill effluent with a high BOD, which usually corresponded to raw pulp mill effluent, and Figure 2-17 represents a effluent with

low BOD, which usually corresponded to the effluent treated by conventional biological processes, such as ASB. In both cases, the first 80 days of oxygen uptake data were used to estimate the kinetic parameters based on the new model. Then the derived models using the above estimated kinetic parameters were used to predict the oxygen uptake up to 160 days. The predicted oxygen uptake data were also plotted in Figures 2-16 and 2-17. Some of representative points were again compared in Table 2-6. These results suggested that the new model reliably predict the oxygen uptake by various pulp mill effluents over the long term after the proper calibrations.

By recognizing the problems in understanding and assessing the biodegradability of various wastewaters, scientific efforts (Skinner, 1992; NASCI, 1982; Swamee and Ojha, 1991; MacDolnad and Radermacher, 1993) have been made to analyze and interpret short- and long-term oxygen uptake by complex wastewaters. As a result, several semi-empirical oxygen uptake models, as illustrated in Equations (2-19), (2-20) and (2-21), have been extensively studied to fit various types of oxygen uptake data.

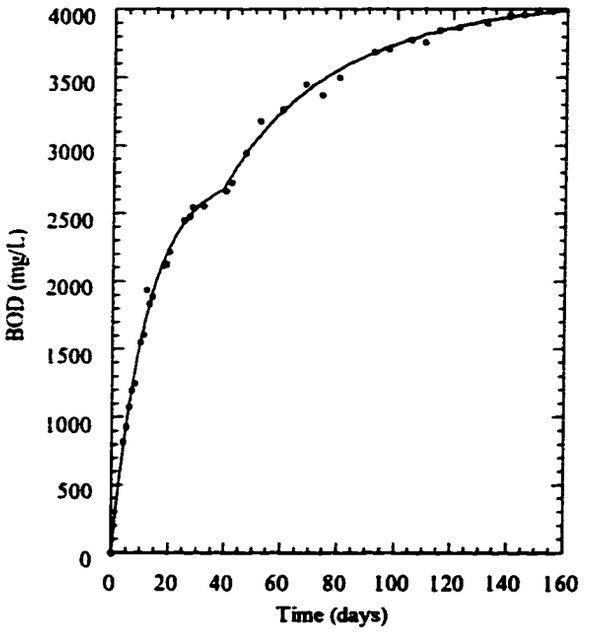


Figure 2-16. The Measured and Predicted Development Patterns of Oxygen Uptakes Adopted from Ref: Figure A1-47 Based on Mechanistic Model (MacDolnad and Radermacher, 1993)

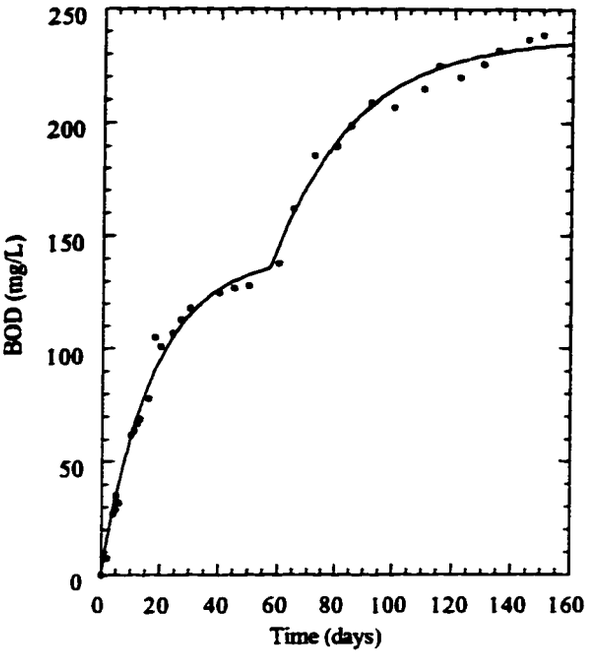


Figure 2-17. The Measured and Predicted Development Patterns of Oxygen Uptakes Adopted from Ref: Figure A1-42 Based on Mechanistic Model (MacDolnad and Radermacher, 1993)

$$BOD_t = BOD_u (1 - e^{-kt}) \quad (2\text{-}19)$$

$$BOD_t = BOD_{u1} (1 - e^{-k_1 t}) + BOD_{u2} (1 - e^{-k_2 t}) \quad (2\text{-}20)$$

$$BOD_t = [BOD_{u1F} (1 - e^{-k_{1F} t}) + BOD_{u1S} (1 - e^{-k_{1S} t})] + BOD_{u2} (1 - e^{-k_2 t}) \quad (2\text{-}21)$$

where, BOD_t is the BOD after incubation time t; BOD_u, BOD_{u1}, BOD_{u2} are ultimate BODs; and k, k_1, k_2 are kinetic rate constants; BOD_{u1F}, BOD_{u1S}, and k_{1F}, k_{1S} are ultimate BODs and kinetic rate constants for the first stage fast and slow components, respectively.

Table 2-7 comparatively shows the estimated parameters based on Equations (2-19), (2-20) or (2-21) using the data from this study along with those reported in the literature. The regression analysis using the oxygen uptake data from this study yielded non-convergence based on Equation (2-21). The possible reasons for lack of convergence may be parameter correlation and insufficient data collection for regression analysis, which was also found to cause problems with parameter estimation in another study (Skinner and van Roodselaar, 1992). The results in

Table 2-7 also showed that the estimations using Equation (2-21) based on the data provided in the literature (Hiidenheimo and Wilson,1974) were very poor, and can not be used for comparison. This is probably because Equation (2-21) is sensitive to experimental design at the critical stage of development of oxygen uptake, which may generate a long narrow confidence region of the estimated parameters (Berthouex and Hunter, 1971a and 1971b).

Comparing Table 2-7 with Table 2-4 revealed that 1) the parameters obtained from Equation (2-20) significantly deviated from the kinetic parameters estimated by the new model and in a recent study (MacDonald and Radermacher, 1993); 2) the kinetic constant k_1 from the new model was very close to the kinetic constants estimated by the first-order model (Equation (2-19)) and agreed well with those reported in literature based on short-term oxygen uptake and with similar wastewater characteristics.

Consequently, the fragment with MWCO<1,000 appeared to be responsible for the oxygen uptake in stage one (see Figure 2-1), and the parameter k_1 in the new model largely represented the kinetic constant of biodegradation of this fragment. The other three fragments were major contributors of the oxygen uptake in stage two, and the parameter k_3 largely represented the rate constant of the biochemical reactions at this stage (see Figure 2-1). In other words, the estimated kinetic constants k_1 and k_3 based on the new model developed in this study appeared to be the representative kinetic parameters involved in the biodegradation of these substrates. On the other hand, the study of oxygen uptake by each fragment of E_{op} and ASB effluent ascertained that the estimated parameters listed in Table 2-7 were not the true parameters involved in the biochemical reactions. To a large extent, these parameters were empirical, or semi-empirical, and had very limited value for interpreting the true meaning related to biochemical reactions and understanding the oxygen uptake in the aquatic system.

Table 2-7. The Estimated Oxygen Uptake Parameters for Various Pulp Mill Effluents

Sample		Estimated BOD Parameters						Reference
Bleaching Agent	Type	Dual First Order Model (Equation (20) or (21))				First Order Model (Equation. (19))		
		BOD_{u1} or BOD_{u1F}/BOD_{u1S}	k_1 or k_{1F}/k_{1S}	BOD_{u2}	k_2	BOD_u	k	
ClO_2/H_2O_2	E_{op} Filtrate	590	0.39	416	0.030	818	0.24	This study
ClO_2/H_2O_2	ASB Influent	511	0.40	267	0.024	635	0.28	This study
Cl_2	Treated mill effluent	103	0.142	287	0.014			Skinner, 1992
Cl_2	Treated mill effluent	23	0.312	287	0.026			Skinner, 1992
Cl_2	Bleachery Effluent					364	0.20	Mohammed, 1990
Cl_2	Primary Effluent					264	0.25	Mohammed, 1990
Cl_2	Secondary Effluent					63	0.15	Mohammed, 1990
	ASB Influent	18.9/272	0.97/0.20	63	0.046	336	0.13	Hiidenheimo, 1974
	Biologically Treated					143	0.083	Hiidenheimo, 1974
	ASB Influent*	204	0.71 to 2.4	141	0.046			Hiidenheimo, 1974
	Biologically Treated*	52.4	19.9	76.4	10671			Hiidenheimo, 1974
Cl_2	River Water**	33/105	1.05/0.17	103	0.19			Raabe, 1968

Note: k values are based on e.

* The parameters were estimated using Systat® nonlinear function based on data reported in the literature;

** River receiving pulp mill discharge.

These findings also demonstrated that the raw oxygen uptake data, even collected over a long term, did not provide an effective means of comparing the biodegradability among different wastewaters although they presented some aspects of biodegradability of a specific group of organic matter. This is because the biochemical reactions, just like any other chemical reaction, involved stoichiometry and kinetics which were markedly influenced by the nature of the substrate, the microorganisms, and the reaction conditions. The nature of substrate included such characteristics as the composition of wastewater, the complexity of the molecules of individual fractions, types of enzymes, and the free energy involved in the biochemical reactions. These were well demonstrated in Table 2-2 in which the ratio of BOD_5 to ultimate BOD or COD varied significantly with the source of wastewater from a pulp mill although they correlated well with the development patterns of oxygen uptake in Figure 2-10 and previous studies (Martone, 1976; Busch, 1958 and 1966; Porter and Snider 1976).

2.4.3 Pathways and Microbial Activities Involved in Biodegradation Processes

As shown in Figures 2-10 and 2-11, the development patterns of oxygen uptake by E_{op} filtrate, ASB influent, raw and ozone treated ASB effluent, and 300 mg/L glucose/glutamic acid all appearrf to occur in two stages although they significantly differed from each other with regard to the degree of oxygen uptake and the starting point of Stage Two. This phenomenon also occurred extensively in almost all the development patterns of oxygen uptake by the effluents from various pulp mills in Alberta (MacDonald and Radermacher, 1993). Moreover, the phenomenon was also observed in the development patterns of oxygen uptake of the effluents from various conventional pulping and bleaching processes (Martone, 1976; Raabe, 1968), other wastewaters (Busch, 1958 and 1966; Porter and Snider, 1976; Gaudy and Gaudy, 1980), and glucose/glutamic acid solution (Busch, 1958; Martone, 1976; Porter and Snider, 1976; Gaudy and Gaudy, 1980) although some studies have not clearly recognized these facts. However, as observed in all these studies, the degree and behavior of the bio-transformation process occurring in the biodegradation processes may differ greatly among the wastewaters and may be affected dramatically by the change of the composition of the wastewater.

Table 2-8 further compares the characteristics of raw and four fragments between ASB influent and effluent (operated at a steady state). The results in Table 2-8 revealed that all three large fragments had similar percentage TOC reduction after biological treatment. As also

discussed earlier. k_1 of biologically treated effluent was very close to k_3 of raw ASB influent and E_{op} filtrate (see Table 2-4). These results appeared to demonstrate that 1) two different categories of enzymes and substrates may be involved in two series of biochemical reactions. In other words, the portion of microbial consortia became more competitive when the available carbon and energy sources shifted through a series of biochemical reactions. Earlier Lawrance and Sakamoto (1959) also observed that the addition of the isolated lignins had an insignificant effect on the short-term oxygen uptake of mixtures of various wood sugars; 2) it was apparent that, after the concentration of the fragment with MWCO<1,000 became limited, a portion of the microbial population would be induced to excrete some kind of extra-membrane enzymes which may be able to attack those large molecules. Most probably these enzymatic processes were non-selective, and the process may be kinetically controlling in overall biochemical reactions involved in the metabolization of lignins and their derivatives. For example, some previous studies have shown that the functional groups on some of the lignin fragments were somewhat modified in conventional biological processes (Raabe,1968; Watkins 1970); moreover, as demonstrated earlier the portion of the fragment with MWCO<1,000 and all other three fragments are involved in biochemical reactions with a rate constant k_3 although it is certain that the substrate with MWCO>1000 will have difficulty penetrating into the cell membrane.

Table 2-8. Comparison of Composition of Raw ASB Influent with That of Biologically Treated Effluent

FRAGMENTS	TOC/TOCraw		COD/CODraw		BOD_5/BOD_{5raw}	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
Raw	100%	56%	100%	51.9%	100%	3.9%
MWCO<1,000	44%	11.7%	47%	10.5%	79%	3.7%
1,000<MWCO<5,000	12%	8.1%	10%	6.7%	8%	1.1%
5,000<MWCO<10,000	17%	10.4%	15%	8.8%	10%	1.3%
MWCO>10,000	31%	25.5%	29%	24.3%	5%	2%

These observations suggested that there existed a common mechanism governing the biodegradation processes of all kinds of biochemical-oxygen-consuming material; and the biodegradation processes elucidated in Figure 2-1 appeared to explain this mechanism satisfactorily. That is, under optimum conditions in a closed batch bioreactor with well-

acclimatized microbial consortia, the biodegradation appeared to follow two stages linked by a transformation process. Specifically, in a batch bioreactor, when the acclimatized microbial consortia were exposed to a complex organic mixture, the relatively easily-biodegradable organics would be consumed first in a relatively high rate since they could serve as the common substrate to the whole population. After a certain period of time, this substrate became limited, and only the competitive portion of the microbial consortia, which can efficiently use the less-biodegradable substrate can continue to survive. At the same time, some members of the microbial population may be able to use the metabolites from another population; thus the transformation processes emerge. However, the transformation processes strongly depended on the composition of both wastewater and microbial consortia. Finally, the smaller part of the surviving population has to continue to struggle to develop some more efficiently enzymatic systems in order to use the residues to keep surviving for an extended period of time. During this period the total population was getting smaller and smaller, and eventually all may died out at the end. At this point, the whole reactor reached the biological stable status.

In short, the assumptions made in the model development were sound, and Figure 2-1 was sufficient to represent the mechanism of biodegradation of complex industrial wastewaters.

2.4.4 Sensitivity Analysis

A wide range of stoichiometric and kinetic conditions were selected to verify how the model prediction would respond to the change of stoichiometric and kinetic conditions of biochemical reactions involved in oxygen uptake tests.

Figure 2-18 shows the effects of stoichiometric changes of complex wastewater samples on the predicted oxygen uptake over the long term. Figure 2-18 demonstrates that the new model predicted the stoichiometric changes well, and was very sensitive to the stoichiometric change of the biochemical reactions in each type of wastewater. Figures 2-19 and 2-20 illustrate the portions of the development pattern of oxygen uptake responding to the different kinetics of the biochemical reactions. In these cases, the change in kinetics reflected the effects of the activity of enzymes or microbial viability, density, or both of the inoculated microbial consortia and the quality or composition of substrate, co-substrate, or both in oxygen uptake tests.

Comparing Figure 2-18 with Figures 2-19 and 2-20 it was found that the stoichiometry changed the degree of oxygen uptake, but the kinetics only altered the development pattern of

oxygen uptake by the microbial consortia; the total oxygen uptake eventually reached the same level if the proper reaction time was provided. That is, the former reflected the bioavailability of organics and the later reflected the rate of biodegradation of organics in the wastewater.

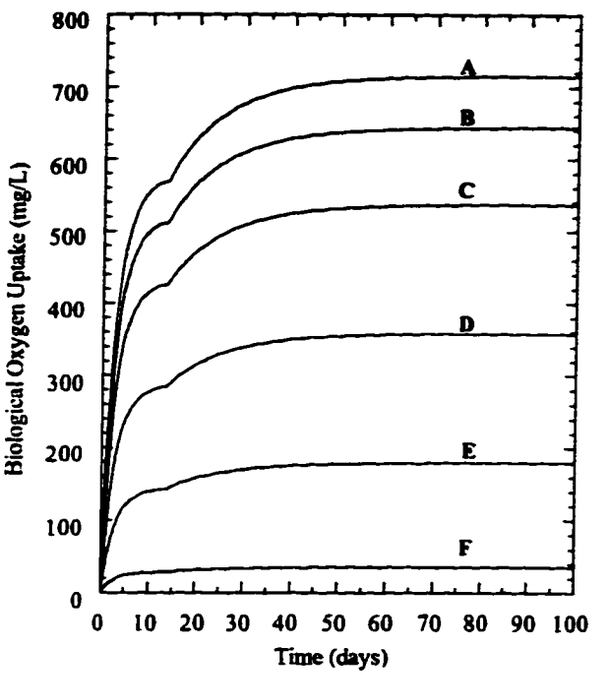


Figure 2-18. Effects of Stoichiometric Change of Biochemical Reactions Involved with Common Wastewater Containing Municipal and Industrial Wastewater Fractions. From G to A: assume UBOD=5, 10, 25, 50, 75, 90 and 100% UBOD

Figures 2-18 through 2-20 also show that the predicted oxygen uptake decreased more rapidly with decreasing stoichiometry of the overall biochemical reactions and the kinetic constant of the second biochemical reactions. These agreed well with the model and other studies. As discussed earlier, the first portion of organics in wastewater was partially degraded while partially transformed into other forms including biomass. Thus, the k_1 value varied within a relatively narrow range and had comparatively less effect on overall biodegradability. On the other hand, k_3 had comparatively greater influence on the biodegradable potential of wastewater since the relatively less biodegradable portion (usually a large portion in complex industrial wastewaters) and the transformed portions were combined together, and were eventually converted to CO_2 and non-biodegradable residues through the biochemical reactions in stage two, which usually represented the controlling kinetic process.

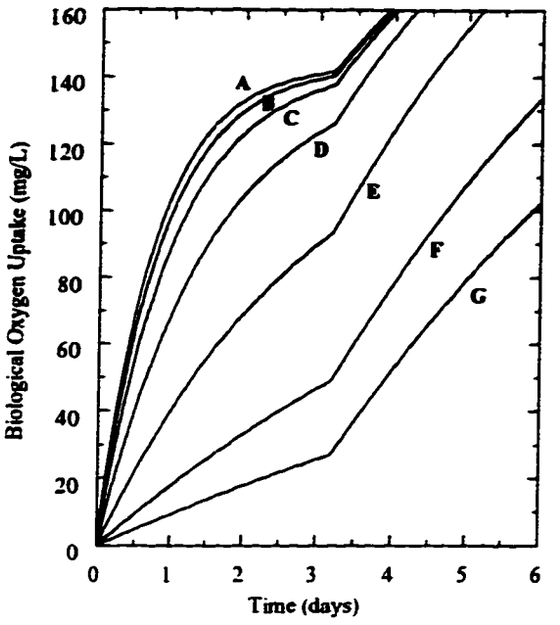


Figure 2-19. Effect of Kinetics of Biochemical Reactions on Development Patterns of Easily Biodegradable Organics (based on Glucose/Glutamic Acid Solution) (From G to A: assuming k_1=5, 10, 25, 50, 75, 90, and 100% k_1)

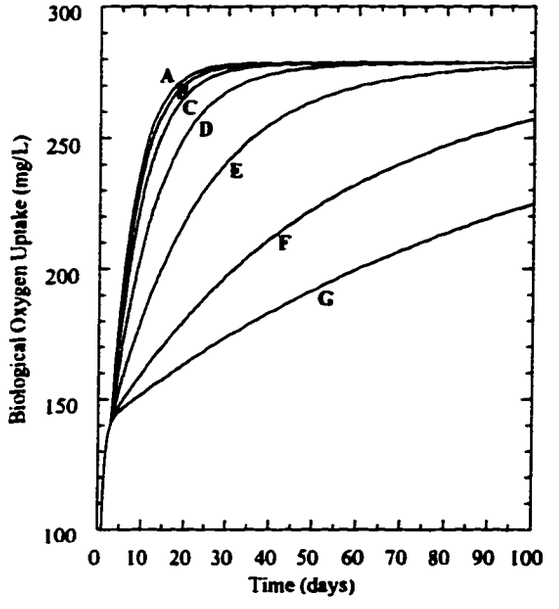


Figure 2-20. Effect of Kinetics in Stage Two on Development Patterns of Glucose/Glutamic Acid Solution (From G to A: assuming k_3=5, 10, 25, 50, 75, 90, 100% k_3)

2.5 CONCLUSIONS

The biodegradation of complex wastewaters in a closed batch reactor can be modelled well using the two stage biochemical reaction processes linked by a bio-transformation process. The sensitivity analysis of the new mechanistic model indicated that 1) the new model was sensitive to the change of various stoichiometric and kinetic conditions; and 2) the stoichiometry and kinetics of the biochemical reactions involved in Stage Two seemed to have comparatively greater influence on the overall biodegradation process.

All the parameters in the new model could be reliably estimated using either short- or long-term oxygen uptake data (Method (A) or (B)). Short-term tests seemed to be a effective means for calibrating the model for the wastewaters with high biochemical oxygen uptake (BOU) while the relatively long term tests appeared to be necessary for the wastewaters with low BOU.

Moreover, extensive model evaluation revealed that the new model not only fitted various oxygen uptake data but provided a effective means for 1) accurately estimating the mechanistic-kinetic constants involved in the biodegradation process; thus, assessing the quality of biodegradable materials in complex wastewaters; 2) effectively evaluating the quantity of the biodegradable materials in complex wastewaters; and 3) reliably predicting the oxygen uptake over the long term under specified conditions. As a result, the properly calibrated model can be very useful in water quality modeling. However, the kinetic data reported in the literature were largely empirical, and significantly deviated from the kinetic constants from the mechanistic model. Thus, they had very limited value regarding the understanding and comparison of biodegradability of complex wastewater among scientific works.

The results also suggested that the established approach for evaluation was simple, reliable and applicable to assessing the biodegradability of various raw and treated pulp mill effluents and their mixture with municipal wastewater. However, the reliability to assess biodegradable potential of low molecular weight and highly toxic chemicals (still toxic to microbial consortia at a very low concentration) remained to be confirmed.

2.6 ACKNOWLEDGEMENTS

This study was partially financed by Weyerhaeuser Canada Inc. The authors greatly appreciate the assistance of Mr. Steve Cooper, Environmental Manager, Grande Prairie

Operations' Mill, Weyerhaeuser Canada Inc. for providing representative effluent samples. The authors also thank Professor S. J. Stanley for his review comments and guidance. Thanks are also extended to Mr. Murray Carmichael for the help with the ozone study and some analyses.

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CHAPTER 3. COMPARATIVE ANALYSIS ON KRAFT PULP MILL EFFLUENTS FOR DEVELOPING AND OPTIMIZING ADVANCED TREATMENT PROCESSES

3.1 INTRODUCTION

Forestry industry has been developing the closed-cycle process for several decades (Rapson, 1967; Reeve, *et al.* 1979; NCASI, 1988). Unfortunately, the corrosion and associated recovery problems limited the possibility of completely recycling the chlorine-based bleaching plant effluents (Reeve, *et al.*, 1979; NCASI, 1990). As a result, many bleached kraft mills are actively searching for technologies which have minimum impacts on the process and are able to meet more strigent regulations on discharges of the color and chlorinated organics in the effluents.

To develop and optimize such an advanced treatment process for reduction of both color and chlorinated organics as TOX or AOX, it is imperative to fully understand the nature of these organics, properly determine the compositions, correlation and distribution of the various components in the wastewater.

The chemical and biological characteristics of the effluents from pulp mills employing conventional bleaching processes have been extensively studied (Hardell, 1977a; Howard and Walden, 1971; Collins *et al.*, 1969; Pfister and Sjöström, 1979a and 1979b) and reviewed by Krinstad and Lindström (1984). Further studies (Hardell, 1977b; Pfister and Sjöström, 1979c; Gergov *et al.*, 1988; Heimburger *et al.* 1988a and 1988b; Walsh *et al.*, 1992; NCASI, 1992) on the wastewaters from modified bleaching processes revealed that changes to conventional bleaching practices may cause significant effects on the characteristics of pulp mill effluents.

The spent liquors from O-C-E bleaching sequence were characterized with respect to molecular wight (MW), dry organic solids, TOC, COD, color and BOD_7 with emphasis on the composition of the dissolved materials (Pfister and Sjöström, 1979c). The study revealed that with oxygen prebleaching of pine pulp, over 90% of dissolved materials in spent chlorination liquor had MW less than 10,000 g/mol., while only about 70% of dissolved materials in alkali extraction liquor had the similar range. This agreed well with the color decrease observed by Hardell (1977b). However, the ionization difference spectra failed to reveal any significant

structural difference between the dissolved materials in the corresponding spent liquors from the oxygen prebleaching and the conventional bleaching sequences. In contrast, BOD_7 in the spent liquor from alkali extraction was two times as high as that in chlorination spent liquor, and this corresponded well to the low concentration of the organically bound chlorine and high level of low MW organics present.

Several earlier studies (Hardell 1977b, Pfister, 1979c; Gergov *et al.*, 1988) found that with substitution of chlorine dioxide (ClO_2, D) in bleaching processes, total organic material dissolved in D stage was increased while the total color was decreased by about 50% with the pine pulp and 20% with the birch pulp. In both cases, organically bound chlorine was reduced by more than 50%. The authors suggested that all of these changes were due to the stronger oxidation power of ClO_2. It was also observed that the absorptivity at 465 nm was independent with MW when MW of the component was greater than 10,000 g/mole, and dramatically decreased with decreasing the MW of the organics when the MW ranged from 1,000 to 10,000 g/mole. This might be one of the reasons responsible for the significant color decrease in the bleaching process using ClO_2.

Recently, NCASI (1992) conducted an extensive study to further investigate the effects of ClO_2 substitution on BOD and color. This study found that the effect on BOD varied with the process and pulp quality. In general, the color was significantly reduced, and BOD load decreased when ClO_2 substitutions were greater than 70%. After evaluating the influence of different levels of ClO_2 substitutions in various bleaching sequences at a laboratory and mini-pilot scale under well-controlled experimental conditions, Graves *et al.* (1993) confirmed that ClO_2 substitution had profound effects on color, AOX, BOD_5, but little effects on the C/Cl ratio.

Nevertheless, these studies were conducted using either simulated wastewater or at a laboratory or pilot scale under well-controlled conditions. The characteristics, composition and composition distributions of the effluents from these alternative bleaching processes at a full scale may vary with the specific process and operating conditions. After comprehensively surveying seven B.C. mills with a variety of bleaching processes employing elemental chlorine, Howard and Walden (1971) pointed out that the characteristics of the effluents varied dramatically, and there was little correlation among the general characteristics such as TOC, COD, BOD and toxicity. However, it was consistently observed that combination of various mill effluents tended to lower the toxicity. On the other hand, after surveying five mills with

representative modified bleaching processes Pryke (1989) concluded that, in general, the mill results supported the laboratory findings reported in the literature with less degree of improvement. Few studies at a mill scale have been found to systematically assess the impacts of the alternative bleaching sequences such as $DE_{op}DED$ on long-term biodegradability, composition distributions, color, molecular weight distribution (MWD) and their correlation with various effluents from kraft pulp mills. Consequently, better knowledge on these aspects would certainly facilitate to select the proper target wastewater, and develop and optimize the advanced treatment system.

Both activated sludge and aerated lagoon processes are widely used for treatment of pulp mill wastewaters. These methods have been reported to remove biodegradable organics and suspended solids effectively (Chen, et al., 1974; Hrutfiord, *et al.*, 1975; Haberl, *et al.*,1991). It was also reported that some reduction of color was achieved in laboratory-scale activated sludge. The polymerization and biomass bridging actions by some polymers of biological origin such as polysaccharides and polyamino acids were assumed to be the mechanisms responsible for the color removal. This color removal process was non-selective and had little effect on the MWD (Obiaga and Ganczarczyk, 1974). Several other studies also observed that some bacterial species could attack the color-causing lignin components (Watkins, 1970; Odier and Monties, 1977; Woodard, et al., 1964). However, it was repeatedly observed in the field that the color reduction in conventional biological treatment process was negligible probably because the color reduction was balanced by concentration through evaporation water loss in the aeration process.

Moreover, the accumulated evidence suggested that conventional biological treatment processes were not only ineffective in reducing color-causing lignin components but also inefficient in removing a variety of chlorinated organics (Hrutfiord *et al.*, 1975; Leuenberger, *et al.*, 1985; Voss, 1983; Kringstad, *et al.*, 1984; Gergov, *et al.*, 1988). A considerable concentration of the chlorinated organic compounds with low MW in spent liquors and biologically treated effluents have been detected (Leuenberger, *et al.*, 1985; Voss, 1983; Gergov, *et al.*, 1988; Jokela and Salkinoja-Salonen, 1992; Jokela *et al.*, 1993). Nevertheless, there is limited knowledge concerning the chlorinated organics with high MW in the spent liquors from alternative bleaching sequences, combined mill effluents and biologically treated kraft pulp mill effluents (BTKPME). Some of preliminary reports seemed to be contradictory (Sågfors and Starck, 1988; Jokela and Salkinoja-Salonen, 1992; Jokela *et al.*, 1993).

In this study, the state-of-the art ulltrafiltration separation and HPSEC techniques were employed to investigate the MW, MWDs and distributions of various components in kraft pulp mill effluents with respect to COD, color, TOX, TOC, BOD_5, UV absorbance and their correlations. In addition, the biodegradable potential tests developed in another study (Mao and Smith, 1995) was used to assess the biodegradable potential of various components and raw effluents. The major objectives of this study were to gain further information about the influence of alternative bleaching process on the characteristics of various mill effluents and their respective components under field operating conditions, to provide preliminary guide for identifying the target stream of the kraft pulp mill effluents for an advanced treatment system to apply to, and for selecting and optimizing the external advanced decolorization and dechlorination processes. A comparison will be made of the results obtained in this research and those reported by the earlier investigators.

3.2 MATERIALS AND METHODS

3.2.1 Mill Descriptions and Sampling

During the study period the mill produced bleached kraft pulp at approximately 885 ADT per day. The majority of the raw material was softwood, and consisted of about 50 to 60% pine and 40 to 50% spruce depending on the seasons.

Wastewaters for this study were grab samples of various effluents from a well-operated Alberta kraft pulp mill employing $DE_{op}DED$ bleaching sequence with 100% ClO_2 substitution. The details about bleaching process and wastewater treatment facilities have been decribed in Appendix I. The designated sampling locations for E_{op} filtrate, ASB influent and effluent were schematically shown in Appendix I. The E_{op} filtrate represented the second-stage bleaching effluent. ASB influent and effluent represented the combined mill effluent and biologically treated secondary effluent from a kraft pulp mill using $DE_{op}D_1ED_2$, respectively.

3.2.2 Sample Preparation and Analysis

3.2.2.1 Ultrafiltration Fractionation

E_{op} filtrate, ASB influent and effluent were fractionated into four components using Millitan Acrylic Ultrafiltration System (Millipore Co.) equipped with 1,000, 5,000, and 10,000 molecular weight cutoff (MWCO) membranes. The effluent was first filtered through Watman glass-fiber filters (934-AH) and 0.8μm filters (MSI Micron Separations Inc.) to remove the

suspended and colloidal solids. The filtrate was then concentrated with 10,000 MWCO membrane to about 10% (v/v, for E_{op} filtrate) or 5% (v/v, for ASB effluent) of the initial volume. The retentate (called F4 with MWCO>10,000) was consequently washed three times using about 250 mL Milli-Q water each. The washing water was collected separately and used for washing next component of the retentate. The filtrate which passed through the 10,000 MWCO membrane was processed similarly using 5,000 MWCO membrane to obtain the retentate as F3 component (5,000<MWCO<10,000). The filtrate from 5,000 MWCO membrane was further treated with 1,000 MWCO membrane. The retentate and filtrate were called F2 component (1,000<MWCO<5,000) and F1 component (MWCO<1,000), respectively. Each component was diluted to the original volume and analyzed for true color, TOX, COD, TOC, BOD_5, BOD_{20}, and UV absorbance.

3.2.2.2 Biodegradable Potential Tests

For quantitatively evaluating the long-term biodegradability of these pulp mill effluents and their components, the biochemical oxygen demand (BOD) development of the raw effluents and their respective components obtained from ultrafiltration separation were collected over about 120 days. The procedures and the mechnistic model described in another study (Mao and Smith, 1995) for evaluation of the biodegradability of complex industrial wastewater were adopted. In particular, the mechanistic model was used to estimate the biodegradable potentials and biodegradation kinetic parameters of raw effluent samples and the simple first-order BOD model was employed to estimate the kinetic parameters of the various components.

3.2.2.3 Sample Analysis

Unless stated otherwise, the procedures recommended in Standard Methods (APHA AWWA WPCF, 1992) were followed in sample analysis with special attention paid on the different characteristics of pulp mill effluents documented by TAPPI and CPPA. These include pH, temperature, dissolved oxygen, nitrogen, total suspended solids (TSS), total dissolved solids (TDS), total volatile solids (TVS), COD, and TOC.

3.2.2.3.1 Estimation of Total Organic Halogens

The total organic halogens (TOX) were extracted from the wastewater samples using microcolumn protocols recommended in Standard Methods (APHA AWWA WPCF, 1992) and analyzed using Euroglass AOX Analyzer. In each series of tests, the high or low concentrations

of *p*-chlorophenol standards and the blank check on the adsorbed halogen level of GAC were carried out. All the samples were diluted to the level within the range of the concentration in the standard curve. The low level of suspended or colloidal particles remained in the sample since the samples were not pretreated before GAC adsorption. Thus, the results from these tests will subsequently be called as TOX. Figure 3-1 shows the TOX standard curve using *p*-chlorophenol as an standard for the estimation of the TOX level in raw and treated pulp mill effluents.

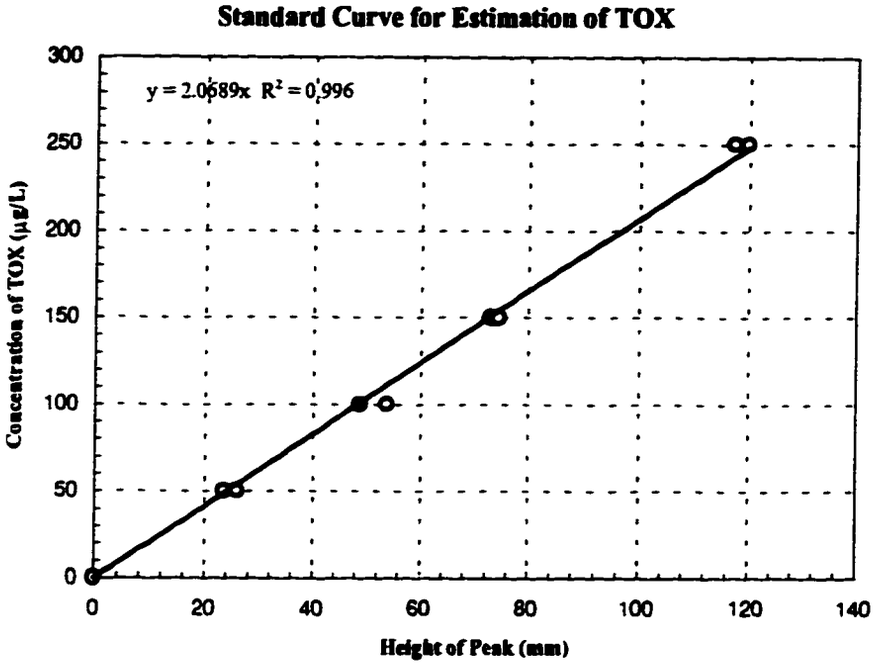

Figure 3-1. Standard Curve for Estimation of TOX in Pulp Mill Effluents

3.2.2.3.2 True Color Determination

The Canadian Pulp and Paper Association (CPPA) standard method H5.P (CPPA, 1974) was employed with minor modifications for determination of true color. Briefly, prior to filtration, the raw samples were properly diluted with Milli-Q water if necessary, and pH (Fisher Accumet® pH meter, Model 805 MP) was adjusted to pH=7.6 using either hydrochloric acid or NaOH solution with proper concentrations to assure the dilution less than 1%. Following pH adjustment, the samples were first filtered through glass-fibre filters (Watman 934-AH) and then through 0.8 µm filters (MSI Micron Separations Inc.). To minimize the effects of filtration on

the color removal the filtration time was carefully controlled to within 30 seconds. Finally the absorbance of the filtrate was measured at 465 nm using Spectronic-20 (Bausch & Lomb) and converted to CPPA color units.

3.2.2.3.3 Determination of UV Absorbance

The UV spectra (ranged from 190 to 690 nm) of raw effuents and various components were determined on UV-Vis spectrophotometer (Model HP8542A) using 10 mm quartz cuvette. All the samples for UV analysis were filtered through 0.8 μm non-absorbable filters (MSI micron separations Inc.) and pH were adjusted to 7.6 if desired.

3.2.2.3.4 Estimation of MW and MWD

High performance size exclusion chromotography (HPSEC) was employed to analyze the molecular weight (MW), molecular weight distribution (MWD), and polydispersity of the raw effluents and their respective components. The HPSEC column (TSK Progel G-3000xl) was calibrated with the protein standards (Sigma Co.) and lignin reference standards. It had been demonstrated that the effects caused by the electrolyte nature of bleached-kraft lignin derivatives could be significantly reduced by using 0.5M NaCl solution (Forss, *et al.*, 1989). In this study, the resolution and reliability of calibration were further improved by using LiCl-tris buffer as an eluent and eluting at a flow rate of 0.8 mL/min. The lignin reference standards were made using ultrafiltration techniques as described earlier for verifying the reliability of the calibration.

3.2.3 Data Analysis

Systat® statistic program was used in this study for statistical analysis of data and for estimation of kinetic parameters in the mechanistic model. In particular, non-linear parameter estimations were performed using either Simplex or Quasi-Newton methods pre-programmed in Systat® statistics package. In the regression analysis, the residuals of regression were always plotted for assessing the distribution of residuals, the goodness of fit of the model. In addition, Hessian Matrix was always calculated to check the possibility of intercorrelation of the parameters. In the regression analysis the corrected R^2 was defined as $\left[1 - \frac{residual\ sum\text{-}of\text{-}squares}{corrected\ sum\text{-}of\text{-}squares}\right]$.

3.3 RESULTS AND DISCUSSION

3.3.1 Calibration of HPSEC Column and Reliability of Ultrafiltration Separation

The reliability of HPSEC for estimation of MW of complex polymers is dependent on not only the performance of the SEC column but also on the calibration and the characteristics of the eluent. Currently there are many standard molecular markers available for SEC calibration. However, the behavior of synthetic polymer standards such as Polyethylen Glycoles (PEG) has been found to be significantly deviate from that of lignin components in pulp mill effluents (Forss, *et al.*, 1989; Rudatin *et al.*, 1989; Siochi *et al*; 1989). Study on the behaviour of natural protein standard using an conventional SEC indicated that the protein markers from natural source show much closer elution behaviour to these lignin components (Forss, *et al.*, 1989).

Three different molecular markers, natural protein (with narrow MW), commercially available PEG and the reference lignin standards (with wide MWD), were compared on the same column under the same conditions. The results shown in Figure 3-2 suggested that under the modified experimental conditions the behaviour of the protein markers were very similar to that of the lignin derivatives isolated by ultrafiltration process. In other words, the calibration of HPSEC column was reasonably reliable for estimation of MW and MWD of the lignin components in pulp mill effluents. However, it is necessary to keep in mind that for combined pulp mill effluents there were a variety of complex polymers, the reliability of this calibration can be deteriated at a certain level. In addition, the calibration was qualitative and relative since the concentration in the reference lignin standards was difficult to determine accurately. In other words, the change in MW was in relative sense.

Figure 3-3 shows the typical MW distribution (based on the UV absorbance monitored at 280 nm) of raw and respective components of E_{op} filtrate obtained from ultrafiltration separation. The MW distribution illustrated in Figure 3-3 confirmed that the ultrafiltration provided a reasonable and reproducible separation considering the polydispersity of the heavily-modified lignin components and the presence of various complex organics such as wood sugar, hemicellose in the E_{op}. However, the long tail of elution curves indicated that a certain amount of low MW organics was still associated with the large molecules during the ultrafiltration separation. In addition, the MW of the individual components from the ultrafiltration processes differed considerably from the MWCO of the membrane specified by the manufactures. These

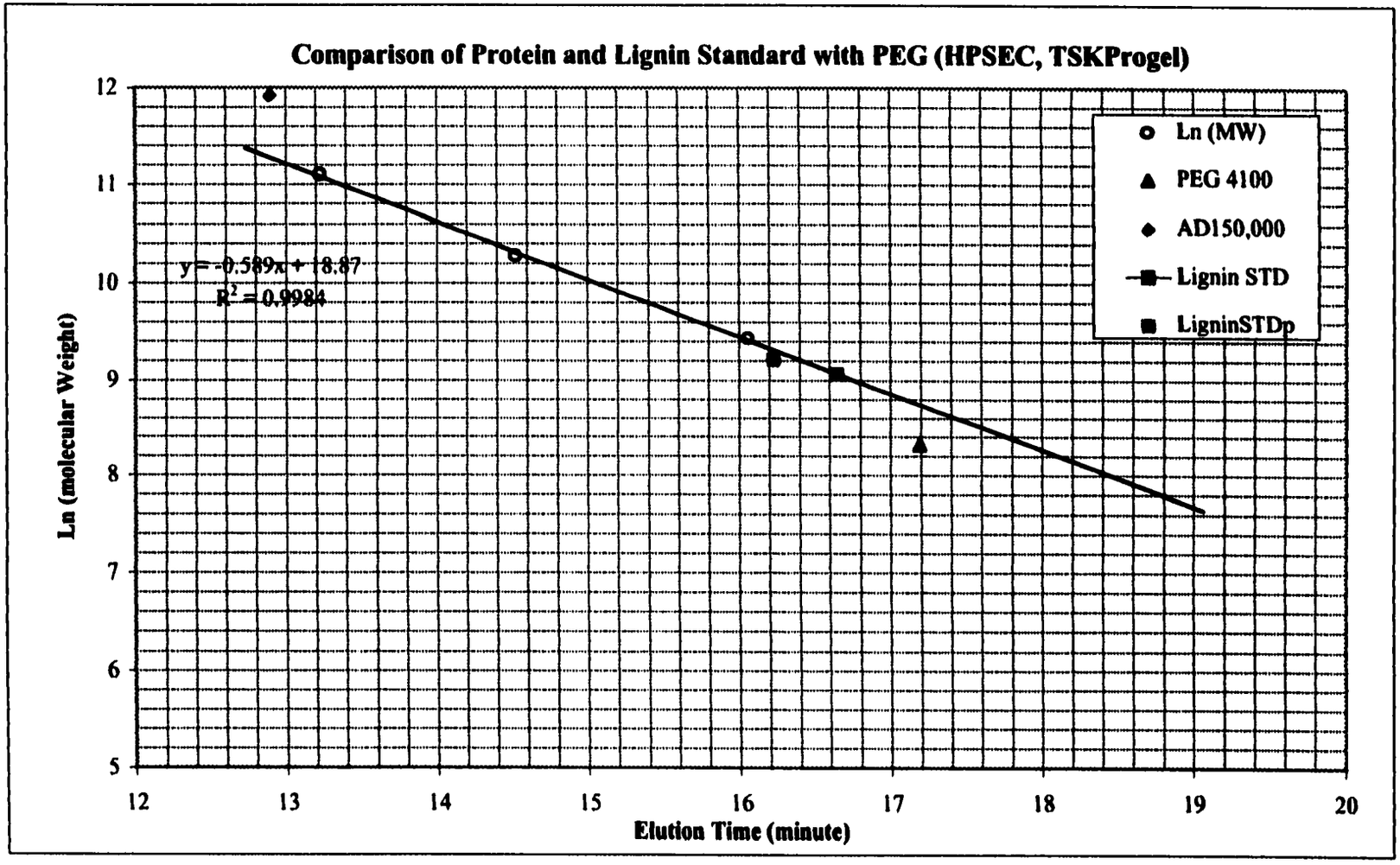

Figure 3-2. Standard Curve for Estimation of Molecular Weight Distribution of Pulp Mill Effluents

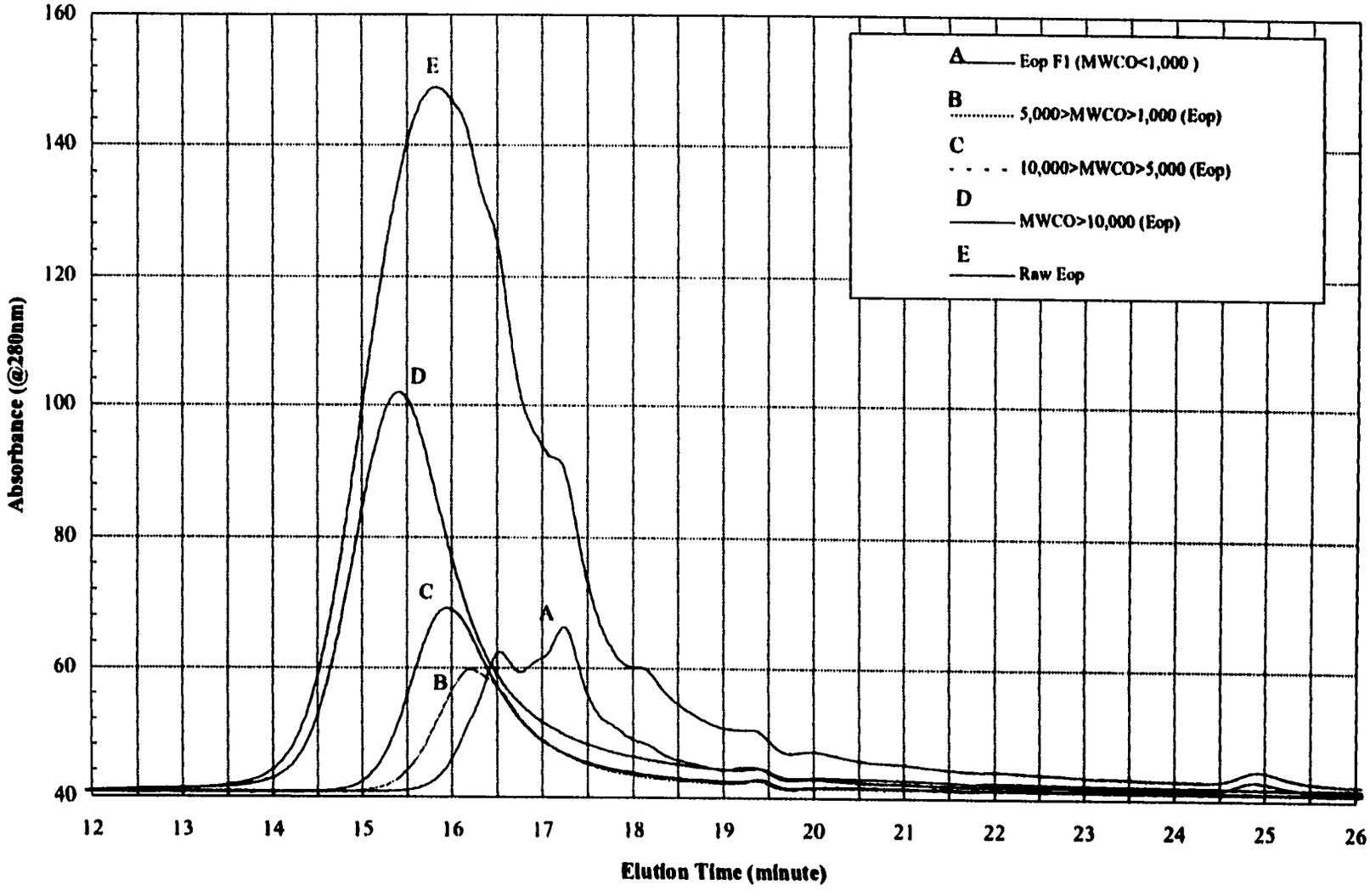

Figure 3-3. Typical Molecular Weight Distribution Patterns of Pulp Mill Effluents and Their Respective Components (E_{op} Filtrate)

observations suggested that the MWCO of the ultrafiltration membrane is the only approximate indication of the magnitude of MW range for separation purpose. The more reliable estimation of the MW of certain polymers can only be achieved by using either calibrated HPSEC or other methods.

Figure 3-4 further compares the MWDs of the washing components (MWCO<1,000) and F1 without combining washing water from influent. This observation confirmed that some low MW organics were retained or associated with the high MW components during ultrafiltration process. This is probably due to the concentration polarization and hydrogen bonds among various organic molecules, especially lignin derivatives. As a result, the separation was less efficient if the ratio of MW between two components was less than 10 (for example, 1,000<MWCO<5,000 vs. 5,000<MWCO <10,000). Nevertheless, even in this situation some separation did occur, and it seemed to be satisfactory for certain purposes such as biodegradability tests (see later section for details).

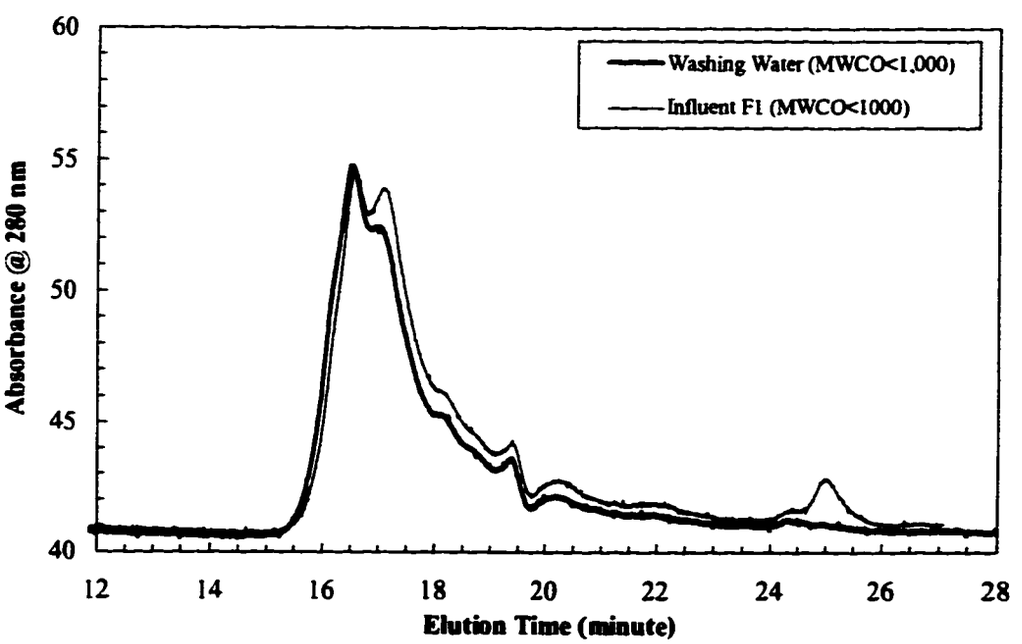

Figure 3-4. Comparison of MWD of F1 without Washing Component with MWD of Washing Water Component

3.3.2 General Characteristics of Various Kraft Pulp Mill Effluents

The general characteristics of three raw effluents were summarized in Table 3-1 (also see Table I-1 in Appendix I). Figures 3-5, 3-6 and 3-7 compare the UV spectra ranged from 190 to 690 nm, the differential MW distribution, and cummulative-weight-fraction MW distribution, respectively.

Table 3-1 shows that the three effluents had considerably different characteristics.

Table 3-1. Summary of Characteristics of E_{op} Filtrate, ASB Influent and Effluent

Parameter		E_{op} Filtrate	ASB Influent	ASB Effluent
pH		9.65±0.15	5.6±0.2	7.1±0.3
Temperature	(°C)	75	45 (15 to 55)	15 to 40 ?
BOD_5	(mg/L)	540 ± 15	460±10	25.1
True Color	(color unit)	3050±50	1650±20	1444±20
TSS	(mg/L)	55±5	70±4	20±2
TDS	(mg/L)	4720±70	1860±35	2557±20
TDVS	(mg/L)	2563±17	1103±25	559±6
TDVS/TDS		0.54	0.59	0.22
COD	(mg/L)	3058±44	1368±17	810
TDOC	(mg/L)	1287±23	466±5	
TOC	(mg/L)	1296±14	493±4	276.3
TDC	(mg/L)	1420±15	492±4	
TC	(mg/L)	1426±12	523±5	326.9
BOD_5/COD		0.177	0.332	0.03
C.I. (C.U./TOC)		2.35	3.33	5.23
COD/TDOC		2.38	2.94	
COD/TC		2.14	2.62	2.48
TDC/TC		0.996	0.941	
TDVS/TDOC		1.99	2.37	2.02
TOX	(mg/L)	22.0	9.9	6.57
TOC/TOX		58.9	49.8	45.3
NH_3-N	(mg/L)	<0.1	5.89	< 1.5
TNOD	(mg/L)	<5	<25	< 5

Note: TDS = Total Dissolved Solids; TDVS = Total Dissolved Volatile Solids;
TSS = Total Suspended Solids TDC=Total Dissolved Carbon;
TNOD = Theoretical Nitrogenous Oxygen Demand; C.I.=color intensity

1) E_{op} filtrate had the highest levels of true color, TOC, COD, BOD_5, TOX and C/Cl ratio and the ASB effluent had the lowest. These results confirmed that the existing ASB can remove greater than 95% of biodegradable organics as BOD_5, about 44% of TOC and 40% of

COD, but only about 38% of TOX. However, the true color level in ASB effluent was only about 12% lower than that in ASB influent and about 40% of the true color in E_{op} filtrate. Considering the possible effects of UV and the adsorption on the chromophoric structures of the lignin molecules in the ASB (Obiaga and Ganczarczyk, 1974; Archibald and Roy-Arcand, 1993) these findings confirmed that the existing microbial consortia in ASB had little effect on the true color derived from the lignin components when other carbon sources were available.

2) The BOD_5 in ASB influent was close to the BOD_5 in E_{op} filtrate, but ASB influent had about two and ten times BOD_5/COD as high as E_{op} filtrate and ASB effluent, respectively. This may be contributed by biodegradation, the addition of sanitary sewage and nutrients, and the evaporation due to the extended aeration in the ASB (more than 12 days total detention time).

3) In contrast, the ASB effluent had the highest color intensity (C.I.) and the E_{op} filtrate had the lowest C.I.

4) All the effluents had relatively high level of TDS. However, the content of TDS was considerably different among the effluents. ASB influent had the lowest TDS but the highest ratio of TDVS/TDS which was close to TDVS/TDS ratio of E_{op} filtrate. On the other hand, TDS in ASB effluent was about 35% higher than that in ASB influent, but the ratio of TDVS/TDS from ASB effluent was only about 40% of that from ASB influent.

5) ASB influent had 5.89 mg NH_3-N/L which is common but at the low range of that in sanitary sewage. As expected, the NH_3-N in ASB effluent was relatively low and that in E_{op} filtrate was very low (NH_3-N<0.1mg/L) due to limited nitrogen source, the high pH and temperature. Thus, the nitrogeous oxygen demand in biodegrable potential tests can be expected to be at insignificant level if nitrification inhibition was practiced.

Figure 3-5 shows that with the proper dilution of the raw effluents, all the UV spectra had the characteristic peaks reflecting the presence of lignin structures. In particular, they all had the absorbance shoulder around 280 nm and similar absorbance around 205 nm in the properly diluted samples. Nevertheless, the absorbance around 280 nm appeared to be much weaker than those reported in the literature (Brauns and Brauns, 1960; Sarkanen, *et al.* 1971). In addition, both E_{op} filtrate and ASB influent had a stronger peak around 214 nm than ASB effluent and E_{op} filtrate had the highest absorbance within the range from 214 to 250 nm. On the other hand, the corresponding peaks in ASB effluent almost completely disppeared. These bigger overlapped

peaks appeared to further render the characteristics of lignins in the above effluents to less obvious.

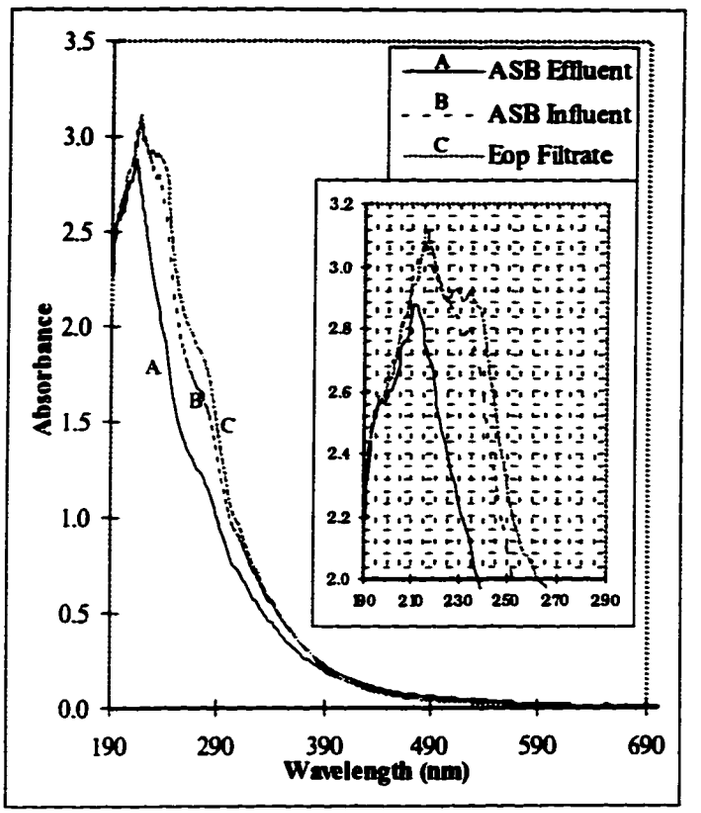

Figure 3-5. Comparison of UV Spectra of E_{op} Filtrate with ASB Influent and Effluent (E_{op} filtrate: 1:15 dilution; ASB influent and effluent: 1:5 dilution)

It has been reported that the absorbance around 205 nm represents lignin contents in the various pulp mill effluents with a high concentration of other organics (Kleinert and Joyce, 1957; Brauns and Brauns, 1960; Sarkanen, *et al.* 1971; Morohoshi, 1991). Lignins also have absorbance peak around 280 nm, but many other organics with aromatic structures, conjugated or non-conjugated double bonds in the wastewater such as wood extractives, hemicellose, carbohydrates and various organics from sewage (Collins, et al., 1969; Williams and Fleming, 1973) also contributed to the absorbance in this region. By comparing the UV spectra of E_{op} filtrate and ASB influent with ASB effluent it is reasonable to conclude that the content of non-

lignin components (or biodegrable components) was in the order of E_{op} filtrate> ASB influent>> ASB effluent. This agreed well with those results shown in Table 3-1.

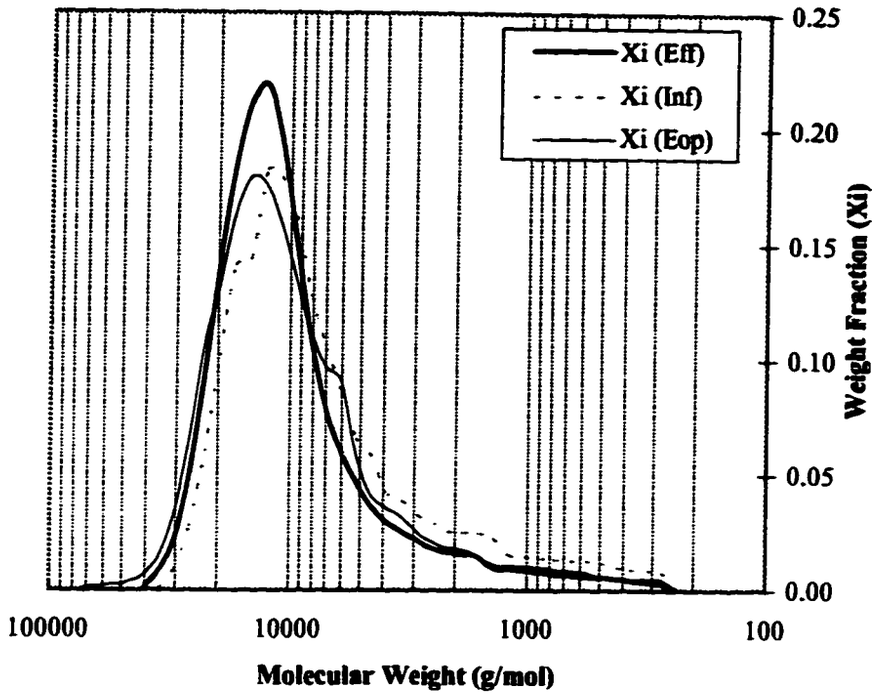

Figure 3-6. Comparison of Differential Molecular Weight Distribution (DMWD) of E_{op} Filtrate, ASB Influent and Effluent (0.8 mL/minute, UV detector @280 nm)

Figure 3-6 demonstrates that the DMWD of three effluents differed sunstantially. The DMWD of both E_{op} filtrate and ASB influent showed two major distribution peaks. In contrast, the DMWD in ASB effluent was much closer to the Guassian distribution.

Figures 3-6 and 3-7 show that the molecular weight distributions (MWD) of organics in all the wastewaters skewed to low MW range more or less. The maximum MW was about 80,000 g/mol., then MW continously distributed over less than 100 g/mol. with the majority of them distributed within 1000 to 15,000 g/mol. Among three effluents, MW of the E_{op} filtrate distributed over the widest range. That is, the E_{op} filtrate contained a portion of organics with MW>35,000 g/mol, and some of them were as high as 80,000 g/mol. On the other hand, ASB effluent had similar range (about 200 to 35,000 g/mol.) of MWD to that of ASB influent. More importantly, the MW distribution was significantly shifted to the higher MW range after

biological treatment. Combining these findings with the results in Table 3-1 suggested that 1) some of the organics with MW>20,000 g/mol. in bleaching effluent (E_{op}) disppeared before arriving at the biological treatment process (the details will be discussed later); 2) an appreciable amount of the relatively high MW organics with low true color, which had absorbance around 280 nm were removed in conventinal biological treatment along with low MW biodegradable organics.

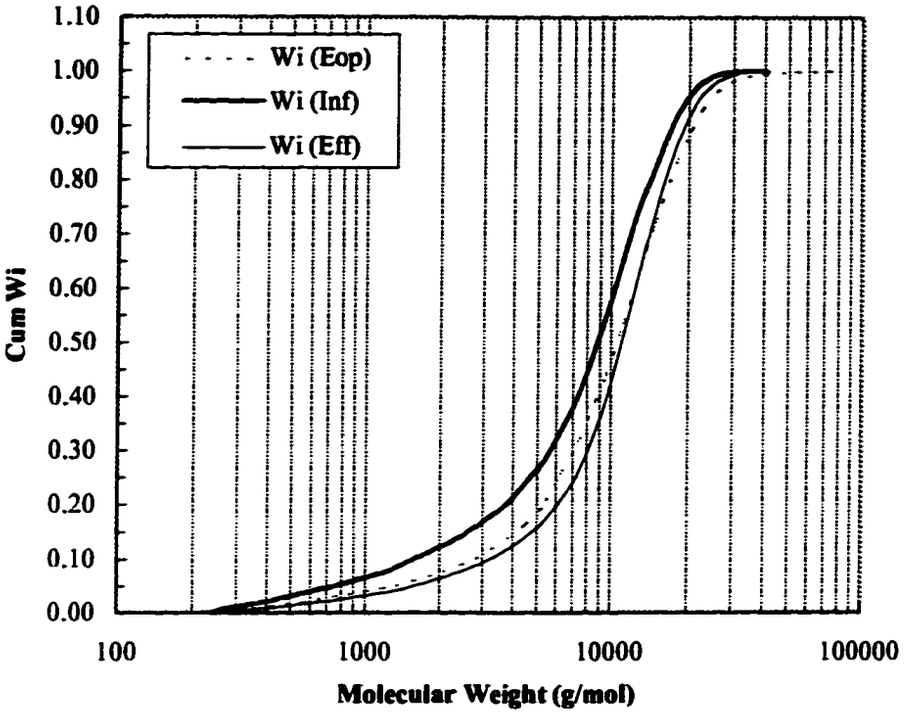

Figure 3-7. Comparison of Cumulative Weight Fraction (Wi) Molecular Weight Distribution of E_{op} Filtrate, ASB Influent and Effleunt

In summary, the observations in Table 3-1 and Figures 3-5, 3-6 and 3-7 suggested that 1) the E_{op} filtrate had considerable impacts on the MWD of the combined mill effluent (ASB influent), and the sanitary sewage signifiacntly improved the biodegradable content of pulp mill effluents; 2) both E_{op} filtrate and ASB influent contained high concentrations of biodegradable organics, and E_{op} filtrate also had high level of TOX. The biodegradable organics were largely removed after conventional biological treatment (ASB); some of the organics in E_{op} filtrate which had low C.I. were also partially removed; 3) the content of lignin components in ASB influent

was almost the same as that in ASB effluent, and about 30% of that in E_{op} filtrate; 4) the characteristics of lignin components appeared to be largely altered by stronger oxidation power of ClO_2 than Cl_2 in the $DE_{op}DED$ bleaching process. The above findings certainly helped to explain the low levels of color, TOX, C.I. and C/Cl ratio in the pulp mill effluents using 100% ClO_2 substitution.

3.3.3 Composition Distributions of Various Pulp Mill Effluents

The contribution of each component from E_{op} filtrate, ASB influent and effluent to true color, COD, TOC, BOD_5 and TOX of the respective raw effluents were compiled in Table 3-2. The distributions in percentages of each component in the respective raw effluents were presented in bracket in Table 3-2. Data from Table 3-2 indicated that the ultrafiltration separation had a recovery greater than 92% with the lowest on the true color regardless the type of effluent. Therefore, the characteristics of each component can represent the distributions of the organic mixture in the raw wastewaters with respect to the parameters used for characterization.

Table 3-2 reveals that the three effluents had similar distribution pattern with regard to the true color but substantial difference on other parameters. In gerneral, the component with MWCO>10,000 (F4) contributed greater than 57% of total true color; the rest of three components each contributed less than 15% of color, with the order of F3 (MWCO<10,000) > F2 (MWCO<5000) >F1 (MWCO<1000). The contribution of each component to BOD_5 (in percentage) in ASB influent closely followed that of E_{op} filtrate; however, the distribution pattern of BOD_5 in ASB effluent was extremely different from that of either ASB influent or E_{op} filtrate and the patterns of other parameters. The BOD levels in the components F2, F3, and F4 appeared to be proportional to their TOC levels although they were relatively low. Moreover, the distribution patterns of TOC and COD among the components of three effluents were somewhat different from each other with a great difference between ASB effluent and E_{op} filtrate (the details will be discussed in later sections).

3.3.3.1 Contribution of Components with MWCO<1,000 (F1)

The component F1 from all effluents contributed more than 79% of BOD_5 and 30% of TOX but less than 10% of true color. However, the contribution of F1 to TOC and COD differed dramatically among different effluents. The component F1 from E_{op} filtrate, which only

contributed about 7% of total true color, contributed 30% of total COD and TOC, respectively. The influent F1, which also contributed only 7% of total true color, contributed 47% of total COD and 44% of total TOC of ASB influent. In contrast, effluent F1, which had similar true color to influent F1, only contributed 20% of total COD and TOC, respectively. More importantly, E_{op} F1 had the lowest COD/TOC ratio but it was very close to that of ASB effluent. On the other hand, the influent F1 had very high COD/TOC ratio. This indicated that influent F1 contained a higher concentration of the organics with low oxidation status. This is consistent with the high BOD_5/COD ratio.

Table 3-2. Characteristics of Raw and Four Components of E_{op} Filtrate, ASB Influent and Effluent

PARAMETER		FRAGMENTS						
		MWCO<1000	MWCO>1000 MWCO<5000	MWCO>5000 MWCO<10000	MWCO>10000	TOTAL[1]	RAW[2]	DIFF[3]
Color	ASB Effluent[5]	94(7%)	104(8%)	187(14%)	885(64%)	1270	1390	120
(C.U.)	ASB Influent[5]	138(10%)	109(8%)	217(15%)	823(58%)	1287	1430	143
	Eop Filtrate	208(7%)	247(8%)	454(15%)	1704(57%)	2613	2989	376
COD	ASB Effluent	132.2(20%)	83.9(13%)	111.4(17%)	306.2(47%)	633.7	654.2	20.5
(mg/L)	ASB Influent	595.6(47%)	127.1(10%)	184.7(15%)	359.8(29%)	1267.2	1260.8	-6.4
	Eop Filtrate	897.9(30%)	272.4(9%)	384.7(13%)	1185.9(40%)	2732.8	3000.3	267.5
TOC	ASB Effluent	52.1(21%)	35.8(15%)	46.3(19%)	113.4(46%)	247.6	246.7	-0.9
(mg/L)	ASB Influent	195.8(44%)	55.9(12%)	76.6(17%)	137.1(31%)	465.3	444.0	-21.3
	Eop Filtrate	376.3(30%)	106.7(9%)	148.5(12%)	444.7(36%)	1076.2	1239.8	163.6
BOD_5	ASB Effluent	12.2(95%)	3.8(29%)	4.2(33%)	6.6(51%)	26.8	12.9	-13.9
(mg/L)	ASB Influent	263.1(79%)	25.6(8%)	34.4(10%)	18.2(5%)	341.3	333.9	-7.4
	Eop Filtrate	421.8(80%)	25.5(5%)	20.3(4%)	37.3(7%)	504.9	524.0	19.1
TOX	ASB Effluent	2.01(31%)	0.891(14%)	1.08(16%)	2.67(41%)	6.65	6.57	-0.08
(mg/L)	ASB Influent	3.2(32%)	5.7(58%)[6]	N/A	N/A	8.9	9.9	1.0
	Eop filtrate	6.9(31%)	16(73%)[6]	N/A	N/A	22.9	22.0	-0.9

Note:
1. TOTAL is the sum of parameter value from all fragments;
2. RAW is the analysis results of Raw effluent used in fractionation study;
3. DIFF is the difference between the Raw and TOTAL;
4. Number in parenthesis is the percentage of that fragment in raw effluent;
5. ASB influent and effluent were sampled at April 20, 1993;
6. All the components with molecular weight>1,000.

3.3.3.2 Contribution of Components with 10,000>MWCO>1,000 (F2 and F3)

The contribution of F2 and F3 to the true color was about 8% and 15% of total true color. The differences in COD and TOC distributions among effluents and between the components F2 and F3 were much less profound compared to component F1. They were in the order of Effluent>Influent>E_{op}. However, the contribution of BOD_5 differed dramatically. The BOD_5 of effluent F2 and F3 were relatively small although the contributions in percentage were high. On the other hand, the contributions of influent F2 and F3 to the BOD_5 in percentage were twice as high as the E_{op} filtrate. Again, this implied that there was an additional source contributing to this component in the pulp mill effluents and this portion contained considerable amount of biodegradable components.

3.3.3.3 Contributions of Components with MWCO>10,000 (F4)

All components with MWCO>10,000, regardless of the source of effluents, contributed more than 57% of the true color, 29% of COD, 31% of TOC of the respective raw effluent; This was in sharp contrast to the contributions of F1s. The effluent F4 had the highest contributions to true color (64%), COD (47%), and TOC (46%), and the influent F4 had the lowest but the true color was close to the E_{op} filtrate. Both influent F4 and E_{op} F4 contributed to less than 7% of total BOD_5; however, BOD_5 of effluent F4 was about 50% of BOD_5 of raw ASB effluent. The BOD_5 of the F4s from the respective effluents were in the order of E_{op} filtrate >ASB influent >ASB effluent.

Table 3-3. Comparison of Compositions of Raw ASB Influent with That of Biologically Treated Effluent

FRAGMENTS	TOC/TOC_{raw}		COD/COD_{raw}		BOD_5/BOD_{5raw}	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
Raw	100%	56%	100%	51.9%	100%	3.9%
MWCO<1,000	44%	11.7%	47%	10.5%	79%	3.7%
1,000<MWCO<5,000	12%	8.1%	10%	6.7%	8%	1.1%
5,000<MWCO<10,000	17%	10.4%	15%	8.8%	10%	1.3%
MWCO>10,000	31%	25.5%	29%	24.3%	5%	2%

Table 3-3 further examines the effects of conventional biological treatment on the compositions of pulp mill effluents. These results indicated that the ratios of TOC and COD of

the three components with MWCO>1,000 from ASB effluent consistently lower (about 4 to 7%) than those from ASB influent.

These obervations confiirmed the previous findings that there was a significant amount of high MW organics derived from E_{op} filtrate which had low color, could be biodegraded slowly. It is also important to recognize that there may be some low MW organics which were associated with the component F4s, and the retained low MW organics in F4s seemed to be in the order of ASB influent>Eop filtrate>>ASB effluent.

Figures 3-8 through 3-10 compile the characteristic UV spectra of the organic mixtures in various components under original wastewater conditions (without dilution or pH adjustment). The following observations were made through comparing the UV spectra in these figures.

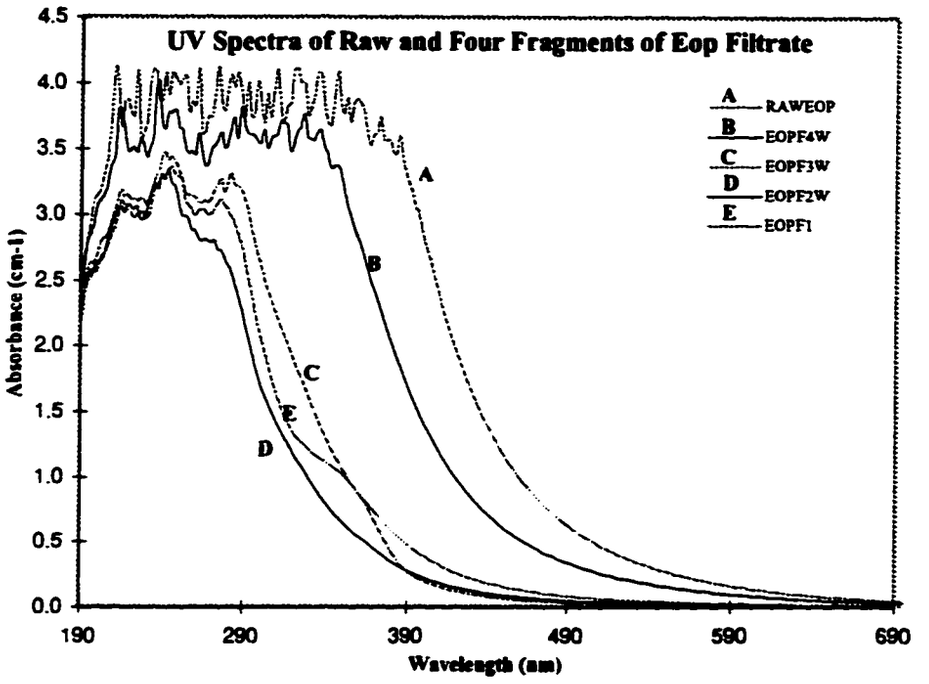

Figure 3-8. UV Spectra of Raw E_{op} Filtrate and Its Four Components

1) Considering the overall characteristics the UV spectra of ASB influent and their components from ultrafiltration separation resembled the UV spectra of the diluted E_{op} filtrate.

2) In contrast, the UV spectra of F2, F3 and F4 between ASB influent and effluent differed dramatically. Specifically, the absorbance shoulder from 214 to 310 nm on the UV

spectra of the components F2 and F3 from ASB effluent almost completely disappeared compared to the UV spectra of the respective components from ASB influent; the same shoulder on the spectra of effluent F4 was also considerably smaller compared to that of influent F4. These findings corresponded well with the results show in Table 3-2. Based on the characteristics of UV spectra of lignin components and the MWD of the effluents shown in Figures 3-5 and 3-6 it can be reasonably deduced that these peaks possibly represented some of high MW organics which were derived from either non-lignin sources such as wood sugar, hemicellose or carbohydrates or heavily modified lignin derivatives which caused very little true color but produced the absorbance at this region. As a result, the color intensity (C.I., C.V./mg TOC) of these components were improved after conventional biological treatment.

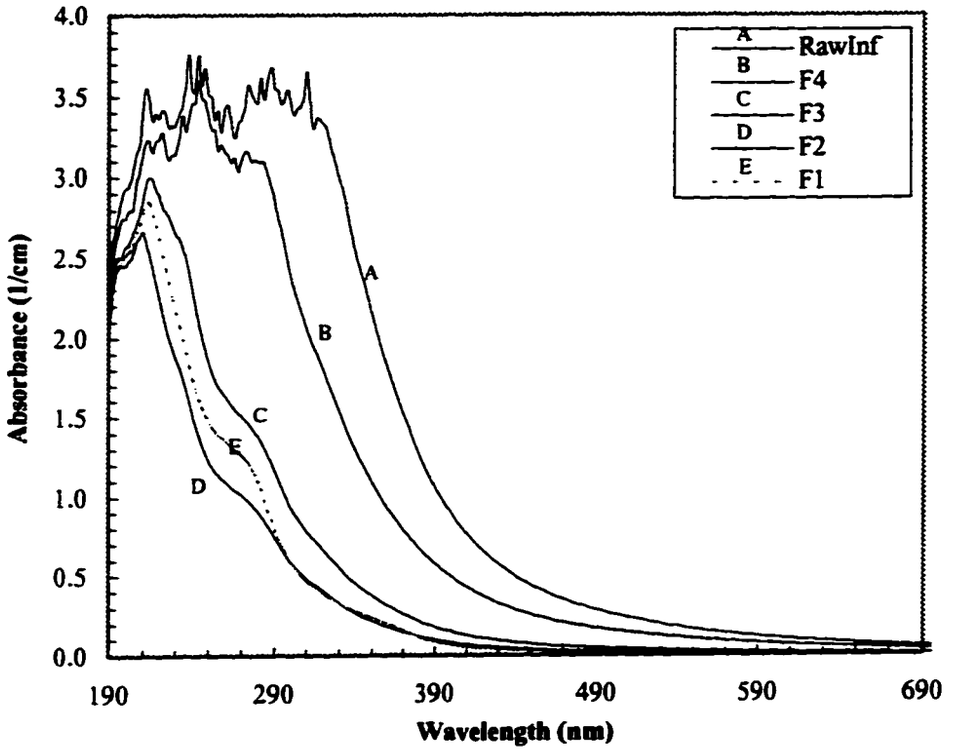

Figure 3-9. UV Spectra of Raw ASB Influent and Its Four Components

3) More importantly, the UV spectra of effluent F1 were similar to the UV spectra of influent F1. These observations suggested that some biodegradable organics in ASB influent, which may derived from non-lignin sources, were not UV detectable within the wavelength

ranges (from 190 to 690 nm); this also indirectly confirmed the earlier observations in HPSEC analysis. Thus, the MWD estimated by HPSEC based on UV absorbance appeared to have limited value to evaluate the content of the low MW biodegradable organics. Therefore, the MWD presented in Figures 3-6 and 3-7 have to be interpreted with the results listed in Table 3-2 to obtained the true picture. Nevertheless, the SEC/UV system could provide some comparative information on the effects of various physical, chemical and biological processes on the lignin derivatives. Even in this case, the simple UV absorbance measurement may be misleading since some organics may be transformed to certain structure which may have stronger or weaker absorbance in certain wavelength range.

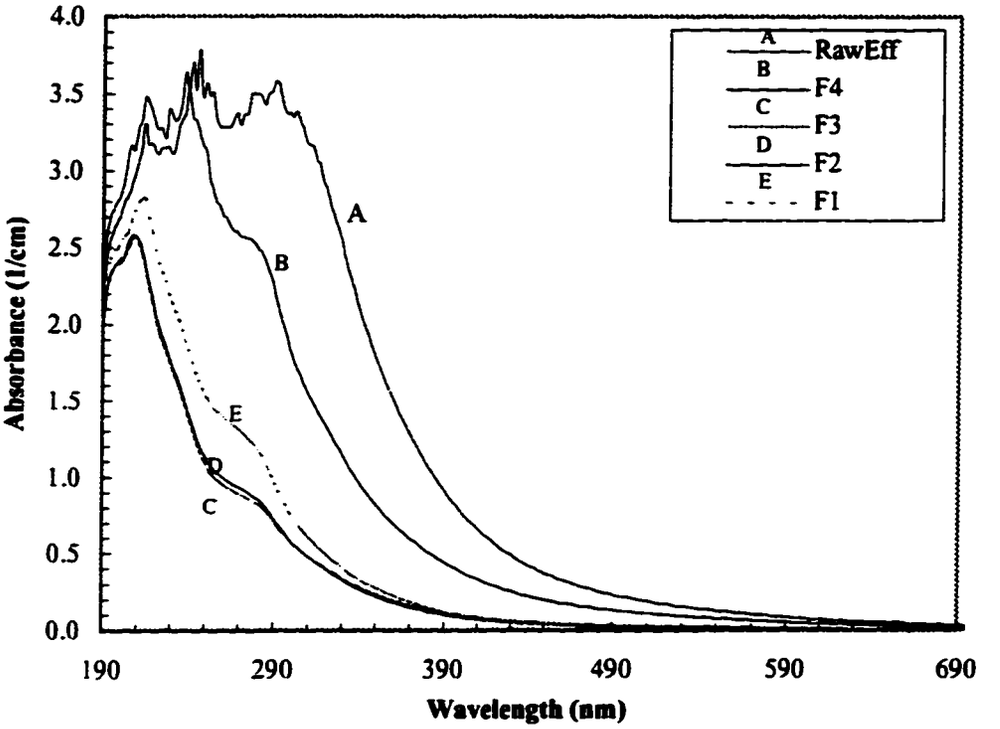

Figure 3-10. UV Spectra of Raw ASB Effluent and Its Four Components

3.3.4 Biodegradable Potentials (BP) of Effluents and Their Components

Table 3-4 compares the BOD developments of three raw effluents over several typical periods. Figures 3-11 through 3-14 further compare the BOD developments of the raw and respective components from above pulp mill effluents over 120 days. The estimated kinetic

constants of raw and their respective components of all three effluents were summarized in Table 3-5.

Table 3-4. BOD Development of E_{op} Filtrate, ASB Influent and Effluent over Different Period

Sample	5-day		20-day		60-day	
	BOD*	Level (%)	BOD	Level (%)	BOD	Level (%)
E_{OD} Filtrate	552±6	59	790±14	84	936±24	100
ASB Influent	457±6	64	619±7	87	712±13	100
ASB Effluent	25	28.4	57	64.8	88	100

*BOD: mg/L

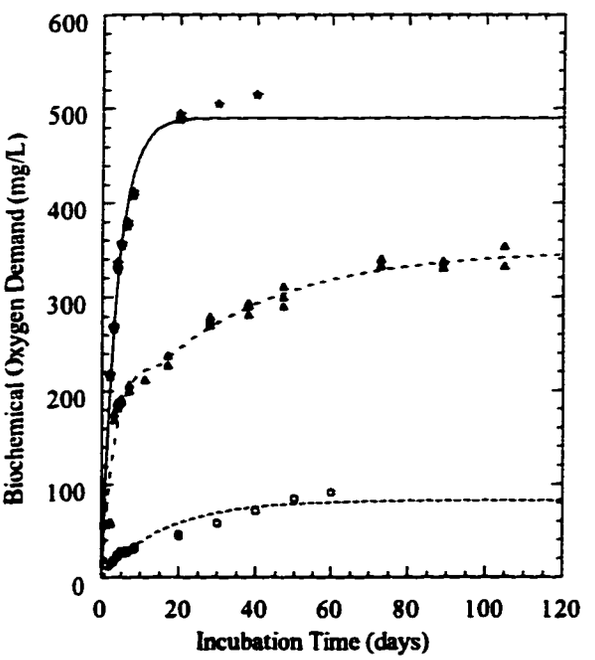

Figure 3-11. Comparison of BOD Development in Components F1 with MWCO<1,000 (star: Eop filtrate, triangle: ASB influent, circle, ASB effluent)

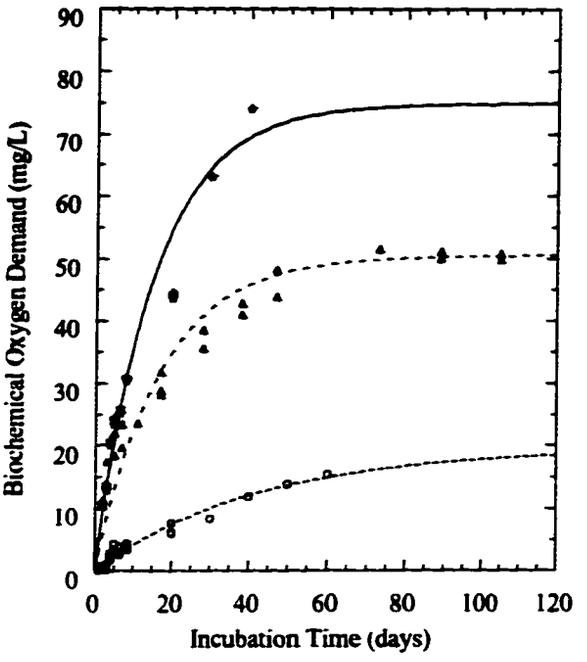

Figure 3-12. Comparison of BOD Development in Components F2 with 1,000<MWCO<5,000 (star: Eop filtrate, triangle: ASB influent, circle, ASB effluent)

The results suggested that 1) the absolute concentrations of the biodegradable content or biodegradable potential estimated over 100 days in the three raw effluents were in the order of E_{op}>Influent>>Effluent; however, the relative biodegradable content (BOD_5/TOC) was in the order of Influent >E_{op}>>Effluent; 2) BOD_5 in both E_{op} and influent only represented about 60% of 60 days BOD value, less than 30% in effluent. The similar observations were reported by Nemerow (1963); 3) the kinetics of BOD development in ASB effluent was considerably slower than that of either ASB influent or E_{op} filtrate.

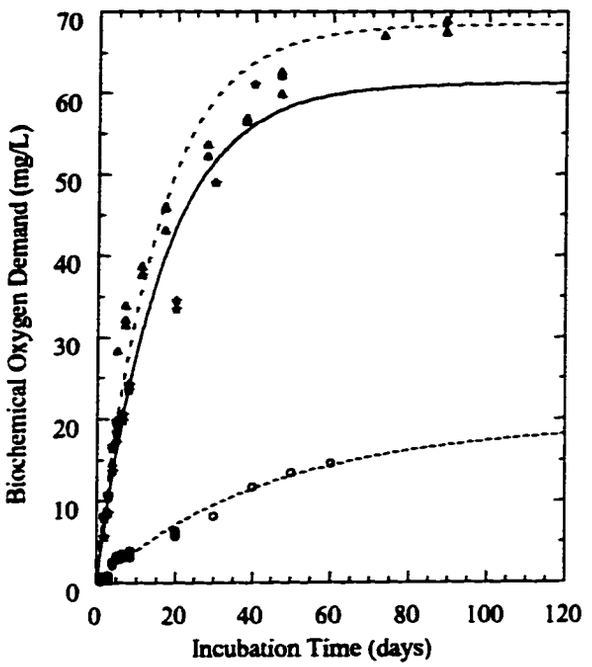

Figure 3-13. Comparison of BOD Development in Components with 5,000<MWCO<10,000 (star: Eop filtrate, triangle: ASB influent, circle, ASB effluent)

The results in Table 3-5 and Figures 3-11 through 3-14 revealed that 1) the ultimate BOD (UBOD) of each component was additive among the components from the same effluent; the BOD_5 of all three raw effluents was proportional to the concentrations of F1 which usually contributed more than 50% BP of any pulp mill effluent; 2) the components F2, F3 and F4 contributed an appreciable portion of BP; however, the percentage in ASB effluent was much higher than that in either ASB influent or E_{op} filtrate; 3) there was a relatively small difference in BP among the components with MW>1,000 from same pulp mill effluent but considerable difference among the components from different effluents; 4) influent F4 had much lower BP than E_{op} F4; in contrast, influent F3 had considerable higher BP than E_{op} F3; 5) the BPs and other kinetics constants shown in Table 3-5 and MWD in Figures 3-3 and 3-4 suggested that the amount of the retained low MW organics either biodegradable or biologically recalcitrant seemed to be relatively small and independent from the absolute concentration of the high MW organics if the retaining effects of ultrafiltration membrane on the lower MW organics were non-selective

between UV detectable and non-UV detectable organics and the washing procedures were carefully practiced.

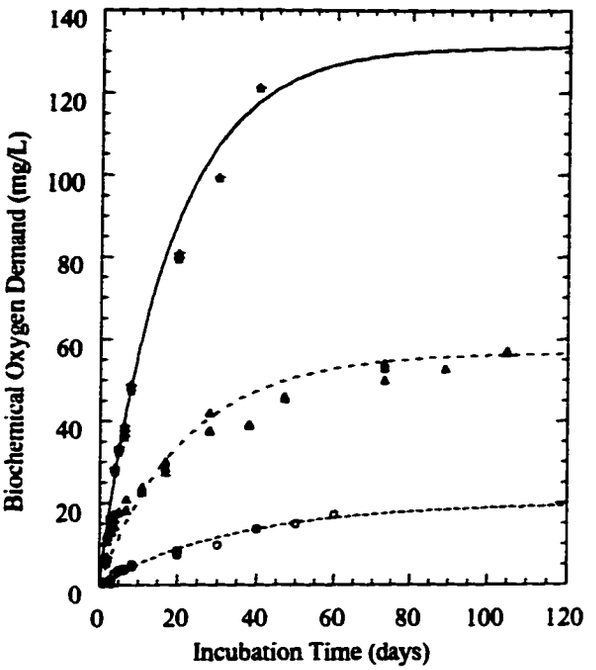

Figure 3-14. Comparison of BOD Development in Components F4 with MWCO>10,000 (star: Eop filtrate, triangle: ASB influent, circle, ASB effluent)

Statistic analysis shown in Table 3-5 demonstrated that at 5% significance level, k_1 of E_{op} filtrate was insignificantly different from k_1 of ASB influent and Ks values (estimated with simple first order model) of either E_{op} F1 or influent F1; k_3 of E_{op} filtrate was insignificantly different from k_3 of ASB influent and K values of F2, F3, F4 of either E_{op} filtrate or ASB influent; k_1 of ASB effluent was significantly different from k_1 of either E_{op} filtrate or ASB influent, but insignificantly from K of F1 of ASB effluent and k_3 of either E_{op} filtrate or ASB influent; k_3 of ASB effluent was significantly different from K values of F2, F3 and F4 from ASB effluent which were much smaller than the kinetic constants of other components from either E_{op} filtrate or ASB influent, but considerably larger than those reported from conventional pulp mill effluents (Mao and Smith, 1995; Mohammed and Smith, 1993; Hiildenheimo, 1974). The BOD of the component with MWCO<1000 from either E_{op} filtrate or ASB influent was

fitted to the simple first order model less satisfatorily than those from ASB effluent. These observations further confirmed that 1) some of the lignin derivatives from $DE_{op}DED$ bleaching process could be biodegraded at an increased rate (Mao and Smith, 1995) compared with less modified commercial lignin Indulin AT (Raabe, 1968) and lignin components from conventional bleachery effluent (Hiildenheimo, 1974; Mohammed, 1990); 2) some of the lignin derivatives may still have similar BP to those lignin components derived from the conventinal pulp mill effluents. In summary, the organics in pulp mill effluents can be divided into three general groups: a) biodegradable organics which were low in true color and MW; some of them were non-UV detectable; b) biodegradable organics which had medium color, some of them had low MW with low UV absorbance, others had MW>1,000 g/mol. with strong UV absorbance in the range of 214 to 280 nm; c) biologically resistant organics which were lignin-derived, high in true color; most of them seemed to have MW>1,000 g/mol; 2) in general, the organics with low color and MW<1,000 g/mol. usually had a higher BP value and the biodegradable potential decreased with increasing MW; when MW>1,000 g/mol. the difference in the BP value was getting smaller. However, there was a significant difference in BP among the high MW organics with low color and high color; 3) the biodegradable portion in the components F2s, F3s and F4s may be partially due to the high MW organics in group b) and partially due to the retained low MW organics in group a); 4) the amount of retained low MW biodegradable organics appeared to be proportional to the original concentration in the raw effluent and strongly depended on the nature of the organics; it also appeared that the concentration of high MW organics had little effects on the concentration of the retained low MW organics.

3.3.5 Correlations among MW, MWD, BP, C.I., TOX and C/Cl Ratio

Figures 3-15 through 3-18 systematically compare the MWDs of all the components from all three effluents separated by ultrafiltration process. For proper comparison of MWDs and MWD patterns, the components from E_{op} was diluted in 1:5 while the rest was diluted in 1:1 for analysis on HPSEC; however, only the peaks from F1s appeared to be comparable with respect to the absolute concentration; the height of the peaks from F2s, F3s and F4s appeared deviated considerably from the absolute concentration due to the relatively large error in the preparation of very small concentrated components for HPSEC analysis. These analysis showed that 1) the components F2 and F3 from all effluents, which had similar BP, also had very similar MWD; 2) among F4s, the MWD of E_{op} F4 extended to much higher MW range, and MWD of

Table 3-5. Parameters Estimated Using Mechanistic Model (raw effluent) or First Order Model (Fragments)

Sample	Fragment	Estimated Parameters					95% Confidence Interval				R^2
		UBOD	k_1	k_2	k_3	K	UBOD	k_1	k_2	K/k_3	
Eop Filtrate	Raw	957.9±29.7	0.244±0.0123	0.0922±0.0131	0.0531±0.0181		897.7 - 1018.1	0.219 - 0.269	0.0656 - 0.119	0.0165 - 0.0897	0.986
	F1	490.2±7.2				0.260±0.0102	475.3 to 505.1			0.239 to 0.281	0.972
	F2	74.9±3.4				0.0656±0.00523	67.7 to 82.1			0.0545 to 0.0766	0.972
	F3	61.2±3.6				0.0617±0.00625	53.6 to 68.8			0.0485 to 0.0749	0.960
	F4	131.2±4.8				0.0625±0.00109	121.1 to 141.3			0.0493 to 0.0639	0.987
ASB Influent	Raw	729.2±18.0	0.278±0.0208	0.146±0.0255	0.0674±0.0223		692.8 to 765.6	0.236 to 0.320	0.0946 to 0.198	0.0234 to 0.112	0.988
	washing	158.3±4.2				0.114±0.0086	149.5 to 167.1			0.0964 to 0.132	0.962
	F1*	303.4±7.7				0.166±0.0211	287.8 to 319.1			0.123 to 0.209	0.819
	F1	349.2±11.5	0.207±0.0215	0.108±0.0275	0.0308±0.00919		325.6 to 372.8	0.163 to 0.250	0.0518 to 0.164	0.0114 to 0.0490	0.951
	F2	50.6±1.7				0.0590±0.00739	47.0 to 54.1			0.0437 to 0.0744	0.913
	F3	68.4±1.8				0.0672±0.0067	64.6 to 72.3			0.0534 to 0.0889	0.922
	F4	56.6±2.1				0.0455±0.0057	52.3 to 60.9			0.0340 to 0.0570	0.906
ASB Effluent	Raw	134.2±10.2	0.0501±0.0041	0.0426±0.0048	0.0196±0.0060		113.7 to 154.8	0.0417 to 0.0584	0.0329 to 0.0524	0.0073 to 0.0318	0.994
	F1	82.5±6.1				0.0610±0.00991	70.0 to 95.1			0.0405 to 0.0816	0.892
	F2	19.6±2.5				0.0239±0.00468	14.5 to 24.7			0.0142 to 0.0336	0.957
	F3	19.5±2.5				0.0221±0.00428	14.4 to 24.7			0.0132 to 0.0310	0.960
	F4	20.0±1.6				0.0288±0.00388	16.7 to 23.3			0.0208 to 0.0369	0.974

* : 95% confidence interval; **; standard deviation

effluent F4 was slightly higher than influent F4 but considerably lower than E_{op} F4. This observation also correlated well with BP shown in earlier sections.

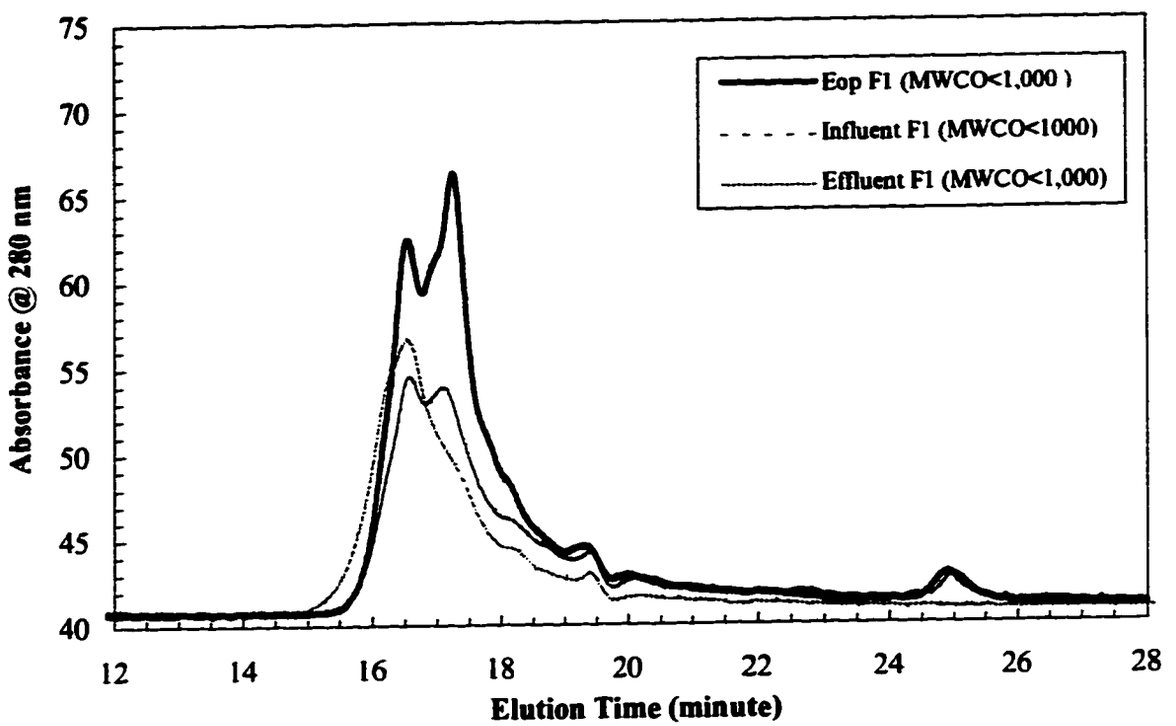

Figure 3-15. Comparison of MWD of Component F1s (with MWCO<1000) (F1 from E_{op} was diluted in 1:5 while the rest was diluted in 1:1)

Figure 3-15 shows that the MWD of influent F1 was very similar to that of E_{op} F1 behaving like the diluted E_{op} filtrate except the elution peak around 25 minutes on the MWD of ASB influent was same as that of E_{op} filtrate. But the corresponding peaks around 17 and 25 minutes disappeared on MWD of ASB effluent; thus, the part of the elution peaks seemed to be derived from E_{op} filtrate, i.e. wood source, and the part from municipal source. Again, the group of organics around 25 minutes seemed to have very low absorptivity at 280 nm. In fact, as discussed earlier, some of the low MW organics derived from municipal source in ASB influent were non-UV detectable at 280 nm.

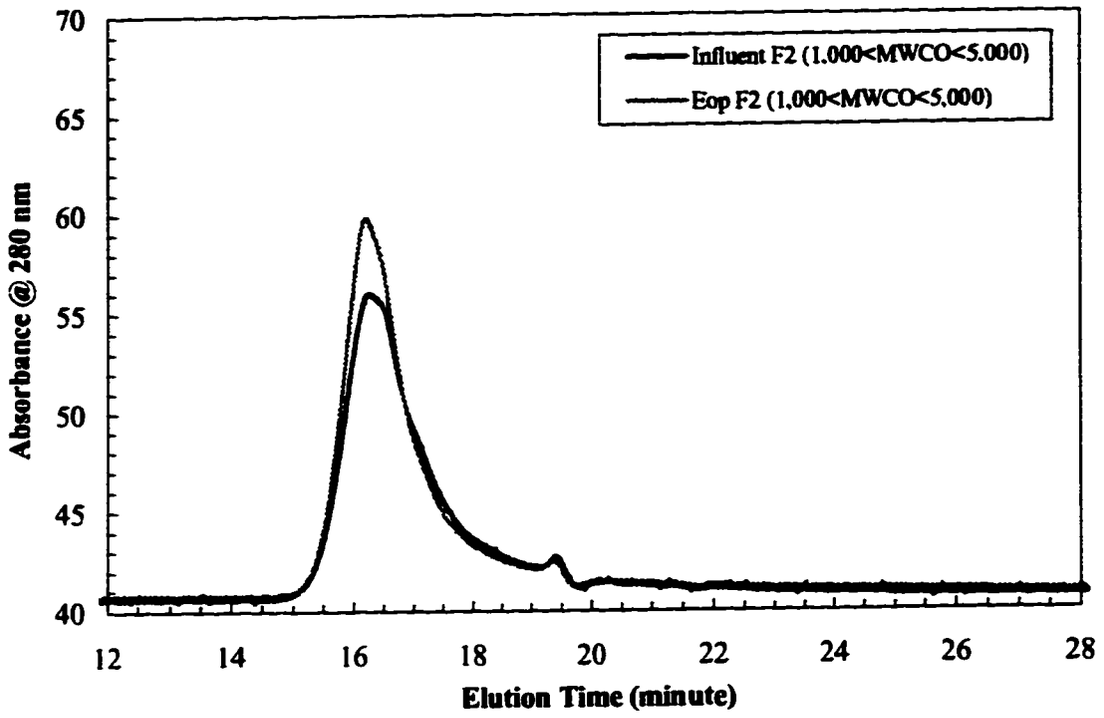

Figure 3-16. Comparison of MWD of Component F2 (with 1.000<MWCO<5000)

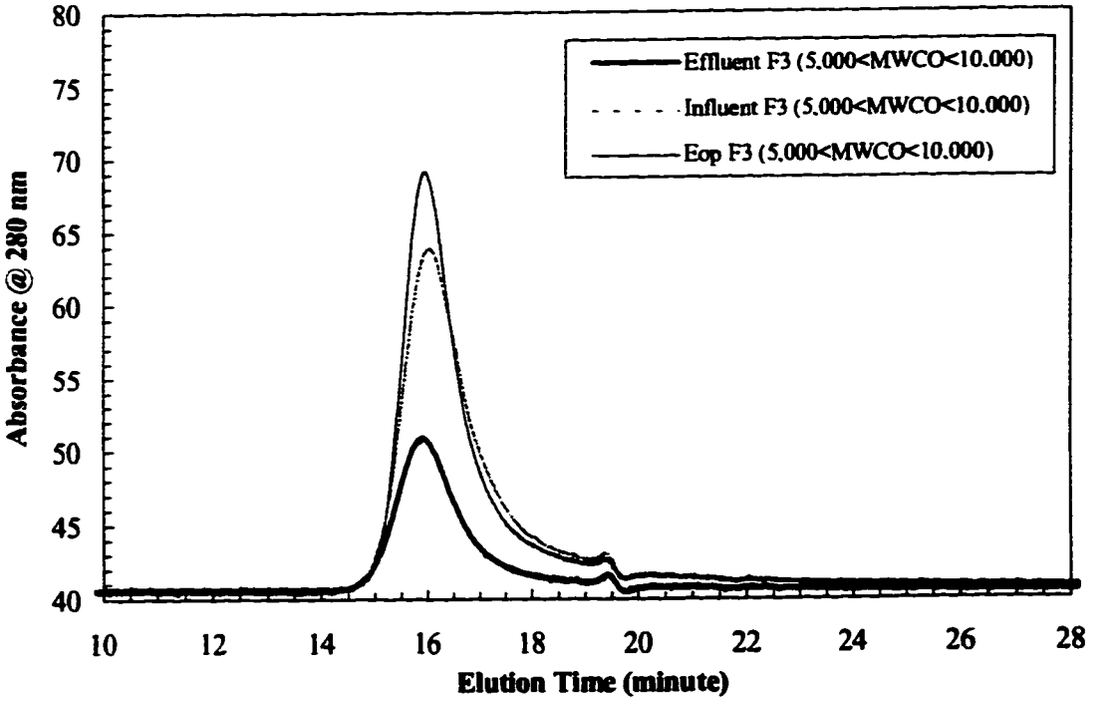

Figure 3-17. Comparison of MWD of Component F3s (with 5000<MWCO>10.000)

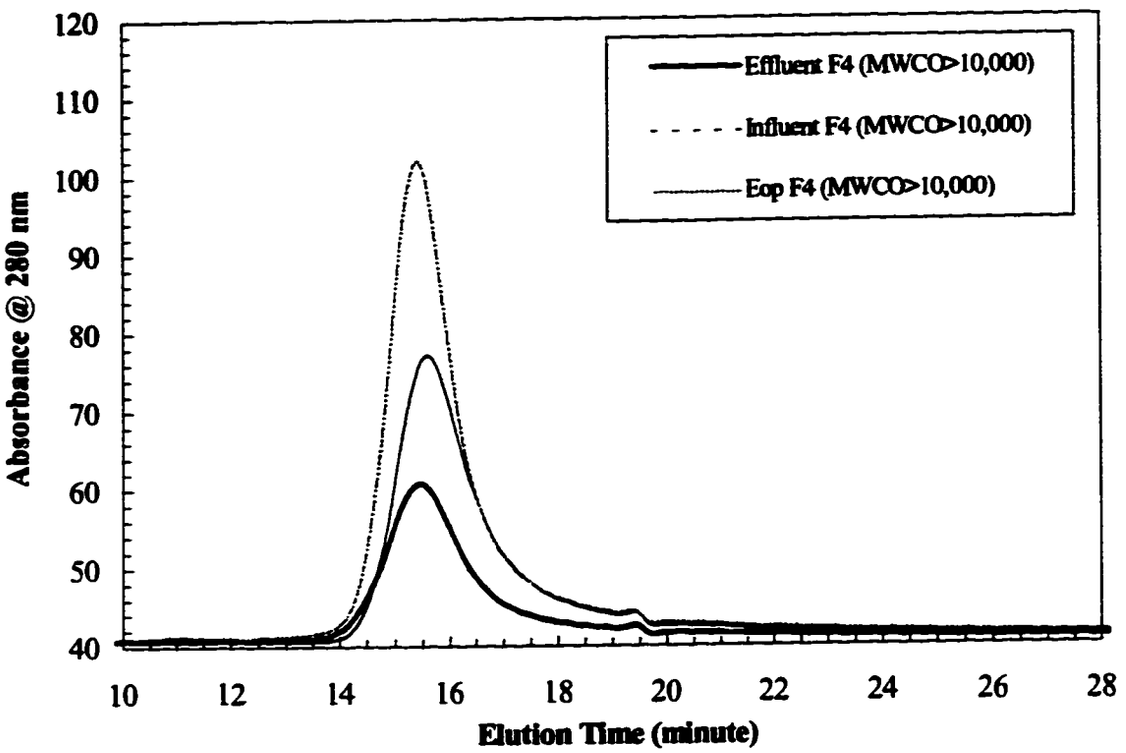

Figure 3-18. Comparison of MWD of Component F4 (with MWCO>10,000)

Moreover, with respect to the concentration and MW of the organics, the MWD of effluent F1 extended to higher range than MWD of either influent F1 or E_{op} F1. The higher MW portion of organics in influent F1 was more likely due to the change in molecular association among the organic molecules induced by the change of solvent environment and concentrations of organics between ultrafiltration and HPSEC (Johnson, *et al.*, 1989); less likely due to the different shapes of the molecules or the different organics contributed by other source of pulp mill effluents instead of E_{op} filtrate. This was seen more obviously by comparing the washing water and component F1. As shown in Figure 3-4, although the MWD pattern of washing water was similar to that of influent F1 the considerable difference was noticed 1) the MWD slightly shifted to high MW range; 2) the height of some peaks were slightly lower; and 3) an elution peak around 25 minutes disappeared.

These observations correlated well with the BP, C.I. and the UV spectra.

1) The organics eluted out around 17 and 25 minutes together with the other non-UV detectable organics with low MW derived from municipal source appeared to be responsible for

the first stage of BOD development shown in Figures 3-11 and another study (Mao and Smith, 1995), and the organics eluted out around 16 minutes and the other high MW organics were responsible for the second stage of BOD development. On the other hand, the two stage phenomenon was much less obvious in either E_{op} F1 or effluent F1 since in E_{op} filtrate the organics involved in second stage was in such a high concentration that the BOD development overwhelmed the first stage; in ASB effluent the concentration of the organics involved in the first stage was so low that it seemed only existed one group of biodegradable organics involved in stage two.

2) The peak around 16 minutes in F1s of the effluents most probably corresponded to group c) organics; that is, the biologically resistant organics with a relatively low MW which were derived from lignin source and responsible for the true color. The peaks around 17 and 25 minutes on the MWD of ASB influent and E_{op} filtrate may represent group b) and the majority of them was removed in the ASB; and the rest of the peaks having longer elution time on the MWD of either E_{op} filtrate and ASB influent disappearred on the MWD of ASB effluent; this indicated that the majority of the low MW biodegradable organics which was almost completely removed after conventional biological treatment may represented the group a).

3) All the above observations were reflected thoroughly in the UV spectra of raw and respective components shown in Figures 3-8 through 3-10.

Table 3-6 compares the changes in C.I. after ultrafiltration with that after ASB treatment. The results demonstrated that 1) there was only one C.I. distribution in a given pulp mill effluent, a physical separation process could only separate the designated component which has the specific C.I. from the whole effluent but it could not improve its C.I. In other words, C.I. represents one of the intrinsic properties of pulp mill effluents with respect to decolorization and dechlorination. For instance, the C.I. of a component with MWCO>5,000 (F4+F3 (b)) from effluent (b) had much lower C.I. than the same component from effluent (a) (since the effluent (b) was sampled from the modified operation); ultrafiltration could not increase the C.I. of F4+F3 (b) to the level of the C.I. of F4+F3 (a) from effluent (a); 2) conventional biological treatment appeared to be almost as efficient as the ultrafiltration process with 10,000 MWCO membranes. This is probably because the ASB effectively removed a variety of biodegradable components which had low C.I., and left the color-causing organics with little or no change. As shown in Table 3-6 the C.I. of effluent (a) was very close to the component obtained from the

ultrafiltration using MWCO>10,000 membrane in concentrating ASB influent to 5% of the original volume. On the other hand, ultrafiltration could rarely achieve this level of C.I. by concentrating E_{op} filtrate no matter which membrane is used.

Table 3-6. Comparison of Improvement on C.I. by Ultrafiltration with Conventional Biological Treatment

Sample	E_{op} Filtrate	Influent	Effluent (a)	Effluent (b)
RAW	2.41	3.22	5.63	4.08
F4 (MWCO>10,000)	3.83	6.01	7.8	
F4+F3 (MWCO>5,000)	3.64	4.87	6.71	5.05
F4+F3+F2 (MWCO>1,000)	3.43	4.26	6.02	

Note: Eop, Influent and Effluent (a) sampled in April 1993; Effluent (b) sampled in January, 1994

Table 3-7. Further Comparison of Major Characteristics of Raw Effluents and Their Components

FRAGMENT	C.I. (Color/TOC)			BOD_5/COD			C/Cl		
	E_{op}	Inf.	Eff.	E_{op}	Inf.	Eff.	E_{op}	Inf.	Eff.
Raw	2.41	3.22	5.63	0.175	0.269	0.0204	47.0	52.2	37.5
MWCO>10,000	3.83	6.00	7.80	0.0315	0.0506	0.0216			42.5
5,000<MWCO<10,000	3.06	2.83	4.12	0.0528	0.186	0.0377			42.9
1,000<MWCO<5,000	2.31	1.95	3.31	0.0936	0.201	0.0453	43.7	47.3	40.2(42.1)*
MWCO<1,000	0.55	0.71	1.96	0.470	0.442	0.0923	54.5	61.2	25.9

Eop = Eop filtrate; Inf.= ASB Influent; Eff. = ASB Effluent; * Number in parenthesis is the average of the fragments with molecular weight>1,000.

Table 3-7 further compares the C.I. (color/TOC) and C/Cl (TOC/TOX) ratios of various components and their respective raw samples along with BOD_5/COD. It can be seen that although the general trends among them were similar all the components from ASB effluent were considerably different from those from either E_{op} filtrate or ASB influent. In particular, 1) C.I.s of the raw pulp mill effluents were in the order of Effluent:Influent:E_{op}=2.34:1.34:1.00; 2) ASB effluent had 35% to over 250% higher C.I. in all components over a full range of MW; 3) C.I.s of component F4 was 4 to 7 times higher than those of component F1, but the differences were much less profound between F2 and F3. 4) C.I.s of F2 and F3 of ASB influent were considerably lower than E_{op} filtrate while influent F4 had 57% higher than E_{op} F4. Again this suggested that during the mixing and transporting of pulp mill effluents either some of the high MW organics with low C.I. in E_{op} filtrate were transformed to lower MW organics or other effluents instead of

E_{op} filtrate contributed a considerable amount of the organics with different C.I. In most cases, with increasing MW, the C.I. and C/Cl ratio increased rapidly while the BP and BOD_5/COD decreased. The C/Cl ratio of the components in ASB effluent seemed to increase with increasing MW up to MW>5,000 g/mol., then started to level off. These implied that 1) the degree of chlorine substitution was higher in the components with higher MW in either E_{op} filtrate or ASB influent; 2) after proper conventional biological treatment the degree of chlorine substitution in the residual organics became much higher in the component with low MW; in other words, a considerable portion of low MW chlorinated organics was resistant to conventional biological treatment; and the biodegradation of chlorinated vs non-chlorinated portion with MW>5,000 g/mol. appeared to be non-selective.

Moreover, comparing the component from ASB effluent with those from E_{op} filtrate and ASB influent it appeared that the biological process had little effects on the C/Cl ratio of the components with high MW, instead, the major effects was on the component with low MW. This observation was somewhat different from that reported by another study (Graves, *et al.*,1993);

Consequently, it is reasonable to conclude that the conventional biological treatment seemed to be a good concentrator in terms of increasing C.I. for improving the efficiency of ozone decolorization and dechlorination. The C.I. increased with MW in all pulp mill effluents but ASB effluent had the greatest increase while the E_{op} and ASB influent had similar magnitude; the C/Cl ratio decreased considerably with MW in the components from E_{op} filtrate and ASB influent, in sharp contrast, the trend of C/Cl ratio was reverted considerably after biological treatment. It also appeared that C/Cl ratio was proportional to C.I. in the components with high MW.

3.4 ENGINEERING IMPLICATIONS

The methods for decolorization and dechlorination of pulp mill effluents can be divided into three general categories according to the nature and operating principles of the processes: 1) physical separation (Phy.) such as ultrafiltration, RO, nanonfiltration, massive lime etc.; 2) advanced chemical destruction (ACD), such as ozone, various AOPs; and 3) advanced biochemical destruction (ABD) such as living fungi, immobilized enzymes etc. The effectiveness and applicability of these methods along with the critical parameters of the pulp mill effluents

discussed in the earlier sections are briefly summarized in Table 3-8. The detail comparison have been described in Chapter 1.

Table 3-8. Summary of Conceptual Selection of Methods for Decolorization and Dechlorination of Pulp Mill Effluents

Type of Effluent	True Color	Flow (%)	C.I.	C/Cl Ratio	Bio. Ratio	HMW/LMW Ratio	Applicability			
							Phy	ACD	ABD	ACD+ABD
Eop	50%	15 to ??	low	medium	medium	medium	E+RF+ RP+U	LE+ RP+U	E+RF+RP+Ex	EE+RF+ RP+Ex
ASB influent	100%	100%	medium	high	high	low	E+RF+ RP+U	LE+ RP+Ex	E + RP + Ex	E+RF+ RP+Ex
ASB effluent	100%	100%	high	low	low	high	EE+RF+RP+ E	EE+RP+EC	E+RP +EC	EE +EC

Note: EE= technically effective and efficient; E=technically effective; LE=technically less effective; RP=require post treatment; RF=require pretreatment; EC=economic; Ex=expensive; U=unaffordable

As summarized in Table 3-8, E_{op} filtrate contributed about 50% of pulp mill's total true color but only about 15% to 30% of the total flow depended on operation. This appeared to be a reasonable target effluent for treatment; however, more than 50% color problem would still remain even if the color of E_{op} filtrate was completely removed; in addition, the E_{op} filtrate had high pH (pH>12) and low NH_3-N which would affect most membranes used in the separation process and biological treatment. Moreover, both E_{op} filtrate and ASB influent contained a large amount of biodegadable organics which would affect the efficiency of the advanced decolorization processes, such as ozonation. ASB influent contributed 100% of true color, pH was suitable for biological treatment but the flow and TSS were much higher compared to E_{op} filtrate. In contrast, ASB effluent had very low BP, TSS, and C/Cl, but very high C.I., thus, it is most suitable for advanced chemical destruction, and it is also suitable for advanced biochemical decolorization with minimum pH adjustment.

The physical separation processes can be effective in separation of the organics with different MW but unable to diffrentiate the non-color or non-chlorinated organics from the colored or chlorinated ones if the MW was similar. Both E_{op} filtrate and ASB influent contained a appreciable portion of low color, highly chlorinated organics (group b) with the similar MW to the organics with high color; in addition, the true color was continuously distributed over whole MW range; thus, the physical separation process can only achieve partial decolorization and

dechlorination, and produce a considerable amount of concentrates with relatively high biodegradable content for further disposal. On the other hand, the majority of those organics were biodegradable which could be removed in biological treatment. More importantly, for all effluents, the extensive pre- and post-treatment have to be practiced for removal of TSS and biological particles which would foul the membrane. Together with the disposal of both concentrated and filtrate may render these methods as unaffortable even with the most advance technology.

The principle of advanced chemical destruction is to destroy chromophoric functional structures or alter the nature of the color absorbing properties of lignin components. By manipulating the operating conditions the process can be partially selective in attacking the chromophoric structures. In particular, for various AOP decolorization processes involving using ozone, the pH, temperature and radical scavenger compositions can be effectively used in controlling the selectivity. However, the degree of control is limited by the composition and characteristics of wastewater such as C.I., TDS, biodegradable contents and relative reactivity of various components; in general, the less complex in composition, the better the efficiency is, the higher the C.I. is, the better the efficiency is, the less concentration of the competing organics is, the better efficiency is. Both E_{op} filtrate and ASB influents contained high concentration of group a) organics which can compete ozone dramatically with the chromophoric structures, in addition, their high TDS or TSS content would also render the AOP decolorization and dechlorination efficiency very low. Moreover, the high MW organics in group b), although they had low density of chromophoric strucuture, they may have sufficeint density of the structures competing the oxidants such as ozone or the enzymes such as lignase for decolorization and dechlorination. There is also significant differences of C.I. among the fragments with MWCO>10,000, and relative small differences among the fragments with 1000<MWCO<10,000. Therefore, the removal of these components would benefit advanced chemical and biochemical decolorization processes. On the other hand, as discussed earlier, ASB behavored the good pretreatment for selectively removing these components; thus, it seemed that ASB effluent would be the first choice for this method to deal with, the E_{op} filtrate would be the second if the decolorization have to be practiced since the total flow of E_{op} filtrate was much lower compared to ASB influent. This method usually simultaneously improves the overall biodegradability of the pulp mill effluents. Thus, they cause additional requirement for removal of biodegradable components as

BOD. As a result, to achieve complete decolorization and dechlorination this method may still be rated as expensive.

Advanced biological decolorization processes employing living fungi were technically proved to be effective for decolorization and dechlorination of pulp mill effluents. Those color causing lignin components may be partially removed from the wastewater by these systems. However, as discussed in Chapter 1, currently they are not practical and economical. Consequently it seems that chemical oxidation processes such as ozone etc. hybridized with biological decolorization may provide a good potential for complete decolorization and dechlorination of biologically treated pulp mill effluents with a reasonable cost.

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CHAPTER 4. INFLUENCE OF OZONE APPLICATION METHODS ON EFFICACY OF OZONE DECOLORIZATION OF PULP MILL EFFLUENTS*

4.1 INTRODUCTION

Today, a much better understanding of ozone physicochemistry has been made possible by many pioneer researchers through extensive studies on ozone and ozonation of various model compounds in water and wastewater. A number of studies (Ruth, *et al.*, 1981; Masschelein, 1982; Caprio, 1982; Shevchenko, 1987; Langlais, 1991) have proposed that the absorption of ozone into water follows Henry's law satisfactorily within the temperature range of 0 to 30°C. The estimated apparent Henry's law constant (H) of ozone is about 1.95×10^3 atm/O_3 molar fraction at 0°C and 8.30×10^3 atm/O_3 molar fraction at 30°C, respectively. Ozone is generally considered as a slightly soluble gas in high quality water with neutral or acidic pH (Ruth, *et al.*, 1981; Kuo, 1982; Masschelein, 1982; Caprio, 1982; Munter, 1983; Langlais, 1991). Considering the solubility of oxygen in water under the partial pressure of oxygen in air and ozone in gas mixture the solubility of ozone is only about thirteen times that of oxygen over the temperature range of 0 to 30°C (Masschelein, 1982; Langlais, 1991). Obviously, in many cases for ozonation of wastewater, mass transfer may be a kinetically controlling process (Richards, *et al.* 1977; Kuo, *et al.* 1982). Under certain circumstances, such as a high concentration of organics with relatively high reactivity towards ozone, gas phase mass transfer could become the major contributor to overall resistance of mass transfer and potentially exert important effects on the ozonation process (Munter, 1983; Ovechkin, 1981; Westerterp, 1984; Singer and Gurol, 1983).

Ozone is relatively stable in dry O_2/N_2 gas phase. But upon dissolution in water, ozone, by reacting with hydroxyl ion ($[OH^-]$), is relatively unstable. Its half-life has been reported to range from 0.5 to 100 minutes depending on the quality, pH and temperature of the water (Hoigné, 1982a, Langlais, 1991). Comprehensive studies (Bühler, 1984; Forni, 1982; Glaze, 1987a and 1987b; Hoigné, 1976; Staehelin, 1982; Staehelin, 1984) have revealed that very low concentration of $[OH^-]$ ions in high quality water, by rapidly reacting with dissolved ozone

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* A version of this chapter has been published. Mao, H.-Z. and Smith, D. W. (1995) Ozone Sci. Eng., 17, 205-236.

molecules, can effectively initiate a radical chain reaction. In this complex chain reaction a series of single-electron and atom transfer processes are involved, and the intermediary of OH• radicals are produced. In turn, the OH• radicals rapidly react with ozone so that the chain propagating steps can repeat again and again as long as the supply of ozone continues. Therefore, hundreds of ozone molecules may be reacted by a single initiation step. In the presence of common water contaminates, such as bicarbonate, carbonate, and the organic constituents, however, the chain propagation can be terminated by the trapping of the hydroxyl radical and of course, the chain may also be terminated by radical-radical coupling processes (Staehelin et al., 1984; Staehelin and Hoigné, 1985).

It has been shown that the ozone decomposition could exert steering effects on both chemical reactions and mass transfer (Kuo *et al.*, 1977a; 1977b). These studies demonstrated that with a high ratio of mass transfer interface to bulk liquid volume the decomposition could change the mass transfer from liquid phase to gas phase controlling process when pH shifted from acidic to alkaline range. As a result, the majority of ozone may be depleted before entering chemical reactions.

The effects of total ozone decomposition in the water-saturated gas phase on the mass transfer and chemical reactions have received very limited investigations. However, the preliminary study by Thievent (1980) indicated that the half life of ozone in this gas mixture was close to that of dissolved ozone when the gas mixture is saturated with water vapor at certain pressures and temperatures.

When ozone is introduced into the water containing organics with various reactivities towards ozone, ozone may also react with them through one or more of following three possible pathways as illustrated in Figure 4-1 (Hoigné and Bader, 1976; Hoigné, 1982a; Hoigné, 1982b; Forni et al., 1982):

1. direct reactions of the solute with ozone molecules;

2. oxidation of the solute by OH• radicals formed in the reaction chain; and

3. solute induced oxidation reactions (chain reaction).

Many investigators (Hoigné , 1982a, 1982b; Hoigné and Staehelin, 1982; and 1985; Staehelin et al., 1984; Forni et al., 1982) have successfully demonstrated that 1) the kinetic rate

constants of radical reactions were usually several orders of magnitude higher than those of direct ozone reactions under the same conditions they tested; 2) the direct ozone reactions have much higher selectivity than radical type reactions; 3) the characteristics of wastewater can exert a steering influence on the selectivity, rate and extent of ozone reactions in a given application; and 4) the operating conditions can also have important effects on the completion and selectivity of the reactions (Hoigné, 1982a; Hoigné, 1982b).

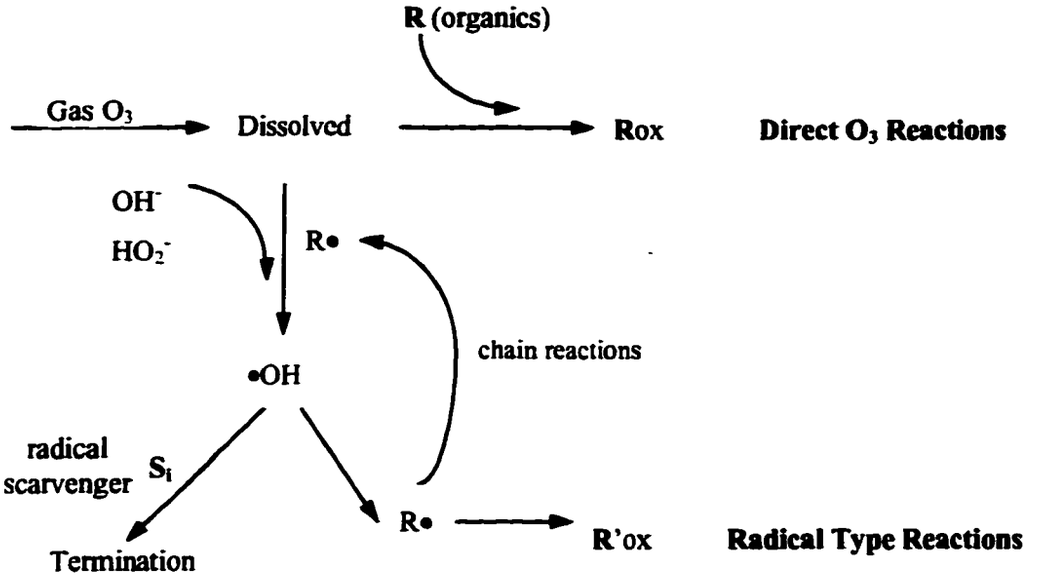

Figure 4-1. Schematic Illustration of Primary Ozone Reactions with Various Organics in Wastewater (Hoigné, 1976 and Bader, 1982a, 1982b; Forniet al., 1982)

Thus, the organic materials (R) in the wastewater may selectively become oxidized by ozone molecules through direct ozone reaction pathways under neutral or acidic pH conditions and at a low concentration of radical initiator. The direct ozone reaction usually yield some intermediate reaction products which can be oxidized further to form more stable reaction products if ozone addition is continued. However, through direct ozone reaction, only extended ozonation would lead, by a series of consecutive oxidation, to 'mineralization' of some of the organic materials. In practice, ozonation processes in wastewater treatment are terminated long

before such a mineralization is achieved. As a result, only the reactions with the most favorable kinetics can occur under the specific operating conditions. Conversely, the indirect, free radical reactions normally lead to a greater completion in practical lengths of time i.e. tend to produce more stable reaction products if sufficient ozone was supplied (Shevchenko et al., 1987; Hoigné, 1982a, 1982b). Thus, some organic substances that are known to be less reactive with ozone may be readily attacked by free radicals even with the presence of many other organics. In addition, R• or ROO• radicals formed in radical chain reaction (see Figure 4-1) may also lead to a series of indirect radical reactions. In wastewaters, however, such chain reactions may be quenched by many other types of dissolved materials which act as chain terminating reagents.

Polydisperse nature and colour are two major characteristics of degradative lignin fragments in pulp mill effluents. More than 200 organics have been identified, and hundreds of organics are known to be present in various concentrations in pulp mill effluents (Krinstad, et al. 1984; Suntio, et al. 1988). Moreover, a number of studies (Mao and Smith, 1994; Sågfors and Starck, 1988) have demonstrated that the molecular weight distribution of these organics ranged from less than 1000 to over 100,000 g/mol. The complexity of these organics raised great difficulties in completely elucidating their reaction scheme with ozone. However, a number of studies (Joyce, et al.; 1983 and Environment Canada, 1976) suggest that the lignin fragments in bleachery effluents contained a combination of chromophors derived from pulping process, and some additional, possibly different, chromophoric structures created by the bleaching process. These included C=C double bonds conjugated with the aromatic ring, quinonemethides and quinones which may also serve as oxidative species creating further chromophoric structures.

As reported by Hoigné (1982a) these functional groups are very reactive with ozone through either direct or radical reaction mechanisms (for example, a kinetic rate constant is in the order of greater than 106 $(mol/L)^{-1}$ s^{-1}). In addition, a variety of other functional groups and chemical bonds have also been reported to exist in various organics found in pulp mill effluents (Krinstad, et al. 1984; Suntio, et al. 1988; Sågfors Starck, 1988). Therefore, reactions with wide spectra of kinetics can naturally be expected when ozone is introduced into the pulp mill effluents. Nevertheless, the ozone reactions with the lignin fragments with higher density of chromophoric functional groups would be theoretically most competitive while other reactions could potentially occur along with them. The degree and variety of the reactions, however, would

strongly depend on the ratio of chromophoric functional groups to other less reactive functional groups and those factors discussed earlier.

Extensive studies on the ability of ozonation to destroy lignins and lignin derivatives in pulp mill effluents occurred long before the complex mechanisms were clearly understood (Bauman and Lutz, 1974; Katuscak et al., 1971a and 1971b; Majumdar and Sproul, 1974; Melnyk et al., 1977; Nebel et al., 1974), and have since been carried on without practical application (Heinzle, *et al.* 1992; Sozànska and Sozànski, 1991; Joyce and Petke, 1983; Rice, *et al.* 1981).

Historically most of ozone treatment studies (Bauman and Lutz, 1974; Majumdar and Sproul, 1974; Melnyk et al., 1977; Nebel et al., 1974; Patton et al., 1979; Stern and Gasner, 1974) on pulp mill effluents focused more on ozone treatability of either bleachery effluents or combined mill effluents than on that of secondary effluents. There existed many controversial results among those studies and those scattered results led to confusion as to the efficacy of ozone treatment and as to which waste stream would be most suitable for ozone treatment. By careful examination of those studies the estimation of ozone dose and the period of contact appeared to be two major sources of the confusion. In turn, the confusion was due to the limited understanding of the physicochemistry of dissolved ozone and the chain reactions in aqueous system.

For clarity, the ozone doses in this study were defined as the used ozone dose and the consumed ozone dose.

$$Used\ Ozone\ Dose = [M_{O_3,input} - M_{O_3,offgas}] / Q_L$$

$$Consumed\ Ozone\ Dose = [M_{O_3,input} - M_{O_3,offgas} - M_{O_3,L} - D_{O_3,G} - D_{O_3,L}] / Q_L$$

where,

$M_{O_3,input}$ = *total mass of input ozone to the reactor*;
$M_{O_3,offgas}$ = *mass of ozone leaving the reactor in offgas*;
$M_{O_3,L}$ = *mass of ozone residual in liquid phase*;
$D_{O_3,G}$ = *mass of ozone decomposed in water - vapor - saturated gas phase*;
$D_{O_3,L}$ = *mass of ozone decomposed in liquid phase*;
Q_L = *total volume of treated wasteweater.*

For fast reactions and at low ozone dose level the ozone decomposed in liquid phase and ozone residual in liquid phase are usually negligible.

Recently Sozánska and Sozánski (1991) re-examined the suitability of either ozone alone or ozone combined with coagulation for treatment of biologically treated pulp mill effluent (BTPME). They pointed out that there was a distinct point of inflexion on decolorization of BTPME, and that colour removal was not satisfactory until the used ozone dose exceeded 50 mg/L. The BOD_5 increase and odor reduction were also claimed to be additional benefits of ozonation of BTPME. Other accumulated evidence also indicated that the biodegradability improvement of ozone treated pulp mill effluents strongly depended on the ozone dose, reaction conditions (contact time, pH, temperature, etc.) and the characteristics of microorganisms used in evaluation of biodegradability (Stern and Gasner, 1974; Patton et al., 1979; Mohammed, 1990; Mohammed and Smith 1992). The results from these preliminary studies ascertained that ozonation can significantly improve the biodegradability of pulp mill effluents. However, it is hard to compare the degree of improvement since the many uncertainties in the experimental techniques and qualitative experimental design limited the data interpretation.

More recently, Roy-Arcand and Archibald (1991) demonstrated that the decolorization of bleachery effluents by ozone was nonlinear, mimicked the pattern observed by Stern and Gasner (1979), with rapid chromophore destruction in the first 3 minutes of ozonation, corresponding to the addition of 400 mg/L ozone.

The concept of ozone dose was again not well-defined in most of these studies. It seemed that it was equivalent to the used ozone dose since neither the residual nor decomposed ozone was monitored. However, data collected by Mohammed and Smith (1992), using a better controlled method for ozone dose estimation, qualitatively supported the three-phased characteristics of ozone treatment of BTPME but the details were somewhat different.

Most of the earlier studies paid little attention to the effects of the ozone application methods on the efficacy of ozone treatment. A study (Bauman, 1974) appeared to suggest that ozone addition rate (OAR), by varying ozone concentration in gas phase, did not affect the efficacy of ozone treatment. Conversely, a recent study (Amero *et al.*, 1993) demonstrated that OAR could change the performance of ozone treatment of bleachery effluents dramatically.

The major barrier to solve aforementioned practical problems in ozone treatment of pulp mill effluents could, in part, be attributed to difficulties in clearly understanding of properties of ozone, the mechanisms of its complex reactions as well as to difficulties in process control and ozone handling. Therefore, careful investigations on these aspects are not only important for properly interpreting the results of ozone treatment but are vital in successful application of ozone in industrial wastewater treatment, especially in decolorization of pulp mill effluents.

This study provides statistical evaluations of the effects of the ozone application methods on true colour, COD, TOC and BOD_5. This was done by assessing the efficacy of ozone treatment on two types of pulp mill effluents with the aid of a statistical experimental design. An additional objective was to identify the sources of confusion in the earlier studies and provide reasonable explanations.

4.2 MATERIALS AND METHODS

4.2.1 Raw Wastewater

Grab samples of E_{OP} filtrate and the effluent from aerated stabilization basin (ASB) were selected as raw wastewaters for evaluation. E_{OP} filtrate is the effluent from a bleaching process using $DE_{OP}D_1E_1D_2$ sequence (D: chlorine dioxide, E: alkaline extraction, OP: oxygen/peroxide). The details about the source and sampling of each wastewater were described elsewhere (Mao and Smith, 1994). All the effluents were stored in 20 L plastic containers at 4°C after arrival at the Environmental Engineering & Science Laboratory, University of Alberta. Before the experiments, the wastewater was thoroughly mixed, and the desired amount of wastewater samples were taken into a large acid-washed glass bottle. The wastewater samples in the glass bottle were settled and warmed to room temperature.

4.2.2 Sample Analysis

4.2.2.1 Determination of COD, TOC and BOD

Unless stated otherwise, the raw and treated wastewater samples in all experiments were analyzed using same procedures described by Mao and Smith (1994 and 1995). In the analysis of TOC, COD, and biochemical oxygen demand, the proper standards were always tested with the sample analysis. All tests were run in triplicates except COD analyses were replicates with proper control and blank in every series of analysis.

To minimize the possible variance caused by the possible change of characteristics of raw wastewater samples between each series of experiments, the raw and control wastewater samples were always analyzed along with the treated samples.

4.2.2.2 Measurement of True Colour

The Canadian Pulp and Paper Association (CPPA) standard method H5.P (CPPA, 1974) was employed for determination of true colour with minor modification. The detailed procedures have been described elsewhere (Mao and Smith, 1994 and 1995). Briefly, prior to filtration, the raw samples were properly diluted with Milli-Q water if necessary, and pH was adjusted to 7.6 using a proper concentration of either hydrochloric acid or NaOH solution to assure the dilution less than 1%. Following pH adjustment, the samples were first filtered through a glass-fibre filter (Whatman 934-AH), and then through a 0.8 μm filter (MSI Micron Separations Inc.) To minimize the effects of filtration on the colour measurement the filtration time was carefully controlled to within 30 seconds. Finally the absorbance of the filtrate was measured at 465 nm using Spectronic-20 (Bausch & Lomb) and converted to CPPA colour units.

4.2.2.3 Determination of UV Absorbance

The UV spectra (ranged from 190 to 810 nm) of raw and ozone treated effluents were determined on UV-Vis spectrophotometer (Model HP8542A) using 10 mm quartz cuvette. All the samples for UV analysis were filtered through 0.8 μm non-absorbable filter (MSI Micron Separations Inc.) and pH adjusted to 7.6 if necessary.

4.2.3 Ozone Treatment Study

4.2.3.1 Ozone Generation and Measurement

Ozone was produced by a PCI ozone generator (Model C2P-9C-4) using extra dry oxygen. Ozone output from ozone generator could be directly monitored using PCI UV ozone monitor (Model HC12) at ambient temperature and pressure. However, the ozone monitor was only used as the indication of approximate ozone concentration level in output gas since the operation conditions of the monitor is significantly different from those in the reactor systems.

Therefore, the ozone concentration in the input gas under the operating conditions of ozone reactor was determined using the semi-batch method (APHA AWWA WPCF, 1992). The

duplicate measurements were conducted before each run and after at least 1 hour stabilization of ozone output and another measurement was performed after each run. Any measurement which deviated greater than 5% was removed, and an additional run was conducted. The average of all valid measurements were used to estimate ozone dose. During the ozone determination, caution (Rice, 1986) was taken to avoid the limitations of KI method, and minimize the decomposition induced by water vapor in gas phase (see later section).

4.2.3.2 Ozone Treatment Studies

Two reactor systems were employed in this study. System I can add the desired ozone dose to the wastewater at once; and System II can apply ozone to wastewater with a variety of addition rates by varying the flow and concentration of ozone in gas mixture.

4.2.3.2.1 Ozone Reactor System I

The reactor System I and the operation procedure are similar to those described by Smith *et al.* (1992) and Mohammed (1990) with minor modifications for more accurate determination of the consumed ozone dose. The modified setting was schematically illustrated in Figure 4-2.

Before each experiment, the ozone generator was approximately set at the desired ozone concentration output and stabilized for at least 30 minutes; and the cylindrical part (B) of the reactor was filled with ozone-demand-free water. Then, the ozonated gas stream (with constant ozone concentration) was allowed to flow through the spherical part (A) of reactor for 10 minutes at the constant flow and desired pressure (from 20 to 35 kPa). At the 10-minute point, valves V1 and V2 were simultaneously closed, and the reactor was then switched to KI trap line and purged for another 10 minutes using high grade N_2. After 10 minutes purging with N_2, total amount of ozone was determined as described in earlier section. Usually replicates were conducted at one ozone dose and another was run if the difference is greater than 5%. At the end of each run, the ozone concentration was re-measured using the same procedures.

After the ozone dose determination, the ozone-demand-free water in the cylindrical part (B) was replaced with the known volume raw wastewater (about 474 mL). The same procedure described above was used but the wastewater and ozone gas mixture were continuously mixed for 10 minutes by tipping and shaking the reactor back and forth. This was followed by purging with N_2.

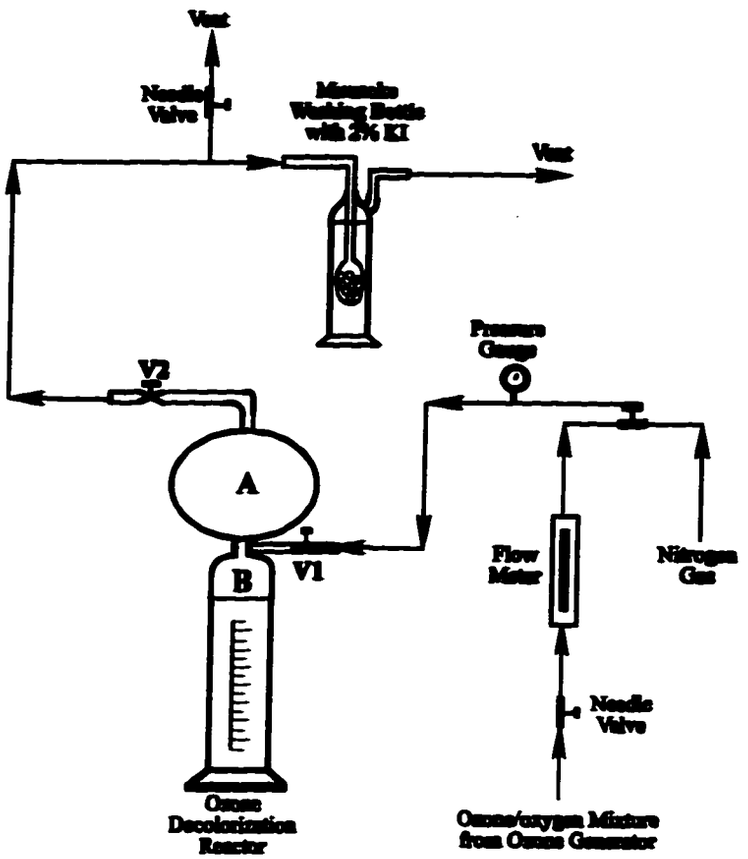

Figure 4-2. Schematic of Ozone Reactor System I for Ozone Treatment Study

4.2.3.2.2 Ozone Reactor System II

The ozone reactor system II (see Figure 4-3), which can provide a desired ozone addition rate and better controlled operations, was employed in the experiments required for varying ozone addition rates. The details of system design and operation procedures were elaborated elsewhere (Mao and Smith, 1994 and 1995). In this study, the same procedures and settings were precisely followed.

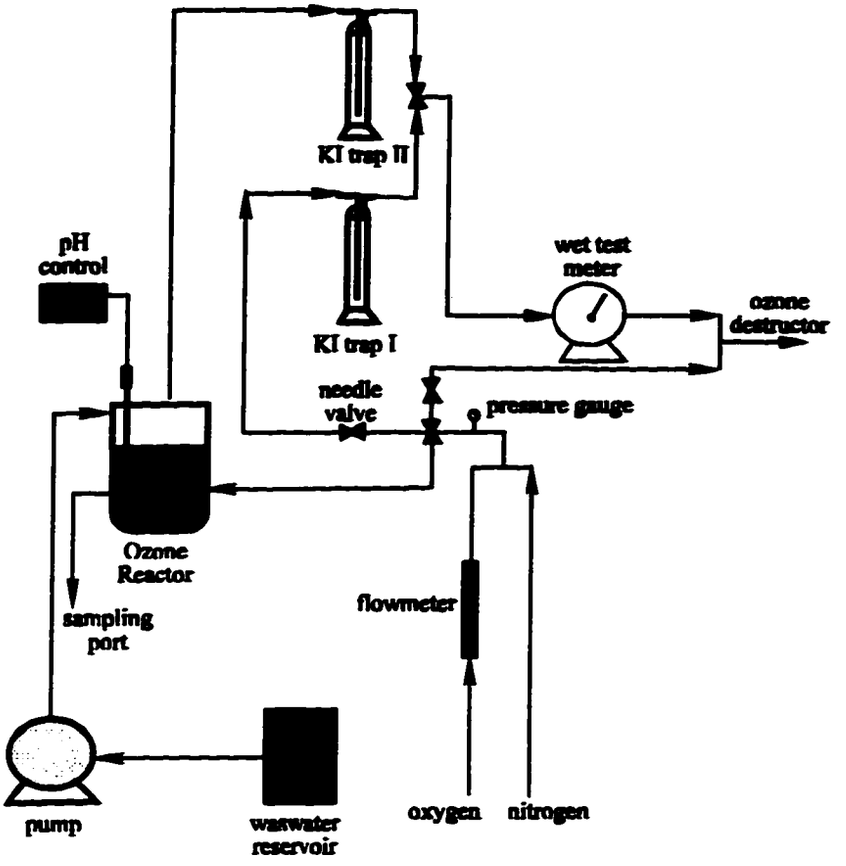

Figure 4-3. Schematic of Ozone Reactor System II for Ozone Treatment Study

4.2.3.2.3 Experimental Design and Data Analysis

To facilitate the statistical data analysis, the block paired experimental design (Hunter, *et al.*, 1979) was applied for evaluation of the effects of the ozone application methods. In each block, one type of effluent and reactor system were used, the pure oxygen run was conducted as a control for assessing the effects of oxygen on the wastewater; and triplicates were conducted under exactly the same operating conditions. The control and treated samples were analyzed in triplicates (except COD in replicates) immediately after the experiments. The experiments in the blocks with same wastewater but different reactor systems were conducted under same conditions except slight differences in used ozone dose (since it is very difficult to obtain the exact used ozone dose), thus, these experiments can be approximately considered as paired block in statistical data analysis.

Unless stated otherwise, Systat® Statistic Package was employed for statistical data and regression analysis. Specifically, the t-test and ANOVA were performed on the block paired

experiments using pooled standard deviations in each paired block; the least square estimation was employed in regression analyses of the combined results from both systems. The amount of ozone decomposition was the average of triplicates from each operating condition.

4.2.3.2.4 Ozone Decomposition in Water-Vapor-Saturated (WVS) Gas Phase

As discussed earlier ozone is very reactive with many substances including the OH^- in water, the ozone lost due to decomposition should be excluded from the consumed ozone dose.

In order to determine the amount of ozone decomposed in WVS gas phase, the cylindrical part (B) of reactor was filled with either 0.05% or 0.1% KI solution (which have similar ozone demand to raw ASB effluent and E_{op} filtrate, respectively) and the reactor system was pressurized at about 28 kPa. Unless stated otherwise, the procedure and other operating conditions were same as those described above. The paired experiments were designed as follows:

One used the same procedure as described in the ozone study but only mixed for 2 minutes to saturate the ozonated gas mixture with water vapor, then immediately following 10 minutes nitrogen purging through 2% KI series of traps to determine the remaining ozone.

Another was mixed for 2 minutes, allowed to stay for another 10 minutes, and then purged for 10 minutes using N_2 gas. All the experiments at each condition were conducted in triplicates.

4.3 RESULTS AND DISCUSSION

4.3.1 Characteristics of Wastewater

The effects of biological treatment and 100% chlorine dioxide substitution with the addition of oxygen/peroxide in the bleaching process on the characteristics of various pulp mill effluents have been thoroughly elaborated in the earlier study (Mao and Smith, 1994). The major characteristics of the wastewaters used in this study are summarized in Table 4-1, and the BOD development of raw ASB effluent over 100 days at 20°C is given in Figure 4-4 along with that of the control sample (pure oxygen treated ASB effluent).

The data in Table 4-1 demonstrate that the characteristics of E_{op} filtrate sharply differed from those of ASB effluent with regard to pH, BOD_5, true colour, colour intensity (C.U./mg

TOC or COD), COD, TOC. Thus, the two effluents can be representatives of pulp mill effluents for the purpose of this evaluation.

Table 4-1. Summary of Characteristics of E_{op} Filtrate and ASB Effluent

Parameter		E_{op} Filtrate	ASB Effluent
pH		9.65±0.15	7.1±0.3
BOD_5	(mg/L)	540 ± 15	25.1±1.2
True Colour	(colour unit)	3050±50	1440±20
TSS	(mg/L)	55±5	70±4
TDS	(mg/L)	4720±70	2560±20
TDVS	(mg/L)	2560±17	560±6
TDVS/TDS		0.54	0.22
COD	(mg/L)	3060±44	810±14
BOD_5/COD		0.177	0.03
TOC	(mg/L)	1300±14	276.0±5.8
TDVS/TDOC		1.99	2.02

Note: TDS = Total Dissolved Solids; TDVS = Total Dissolved Volatile Solids;
TSS = Total Suspended Solids.

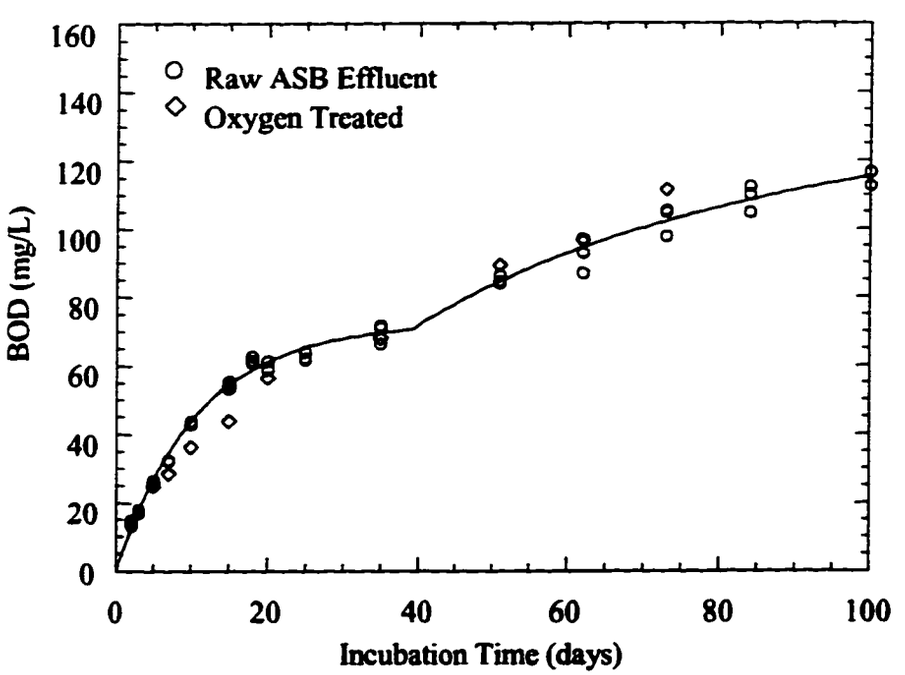

Figure 4-4. BOD Development of Raw and Oxygen Treated (as Control) ASB Effluent over 100 Days

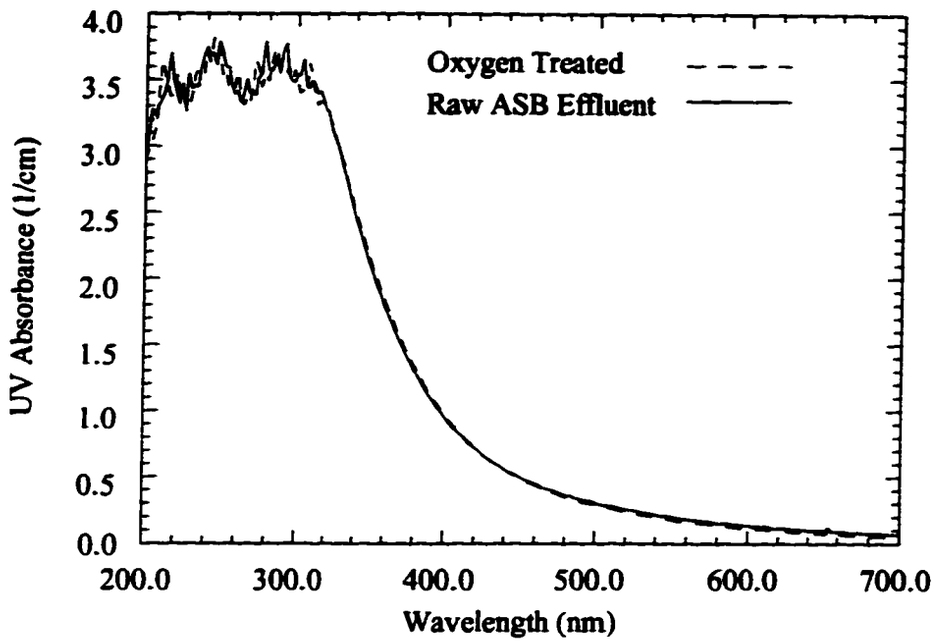

Figure 4-5. UV Spectra of Raw ASB Effluent and Oxygen Treated Control

4.3.2 Ozone Treatment Studies

4.3.2.1 Basis and Reliability of Experimentation and Evaluation

Figure 4-5 shows the UV spectra of raw and oxygen treated ASB effluent after pH adjusted to 7.6 (CPPA, 1974). Figure 4-5 appears to indicate that after oxygen treatment the UV absorbance pattern in the range of 204 to 350 nm slightly differ from those in raw ASB effluent. This is probably due to the partial removal of foaming/volatile material in ASB effluent during the mixing with oxygen. However, the pure oxygen treatment does not affect the UV absorbance at 465 nm which is reflective of the true colour measurement.

The results in Table 4-2 have further proved that oxygen treatment has negligible effects on the true colour of the wastewaters. Table 4-2 also indicates that TOC, COD and BOD_5 have negligible change in all pure oxygen runs compared with raw wastewater. Moreover, as shown in Figure 4-4, the long term BOD development of pure oxygen treated ASB effluent over 100 days also appears to closely follow that of raw ASB effluent.

Table 4-2. Effects of Ozone and Pure Oxygen on Various Process Parameters

Parameter	E_{op} Filtrate		ASB Effluent			
	Raw	O_2 Treated	Raw	O_2 Treated	NR1	NR2
True Colour (CPPA unit)	3050	3100	1440	1440	800	760
COD (mg/L)	3060	3010	810	750	610	600
TOC (mg/L)	1300	1280	276	273	248	238
BOD_5 (mg/L)	540	520	25.1	24.8	35	39

Note: NR1 and NR2 are ozone treated samples employing reactor system II; the ozone dose for NR1 and NR2 are about 84 and 85 mg/L, respectively.

Therefore, the effects of oxygen on the efficacy of ozone treatment was not important regarding true colour, TOC and COD. The biodegradability improvement after oxygen containing ozone treatment at various dose levels can be concluded as the sole effects of the ozone treatment.

Figure 4-6 compares the wide range of UV absorbance of raw ASB influent and effluent and the two replicates (NR1 and NR2) of ozone treated ASB effluent using System II. The ASB influent and effluent were sampled at same time under steady state operating conditions and analyzed on the same date at pH=7.6. Curves c and d in Figure 4-6 demonstrate that effects of ozone on UV absorbance over the range from 204 to 465 nm in NR1 are nearly duplicated in NR2. However, Figure 4-6 suggests that at this sampling time the ASB not only removed a major part of biodegradable organics (non-colored) but slightly altered some large lignin fragments (colored) (Pearl, 1967) through various physical and biological processes.

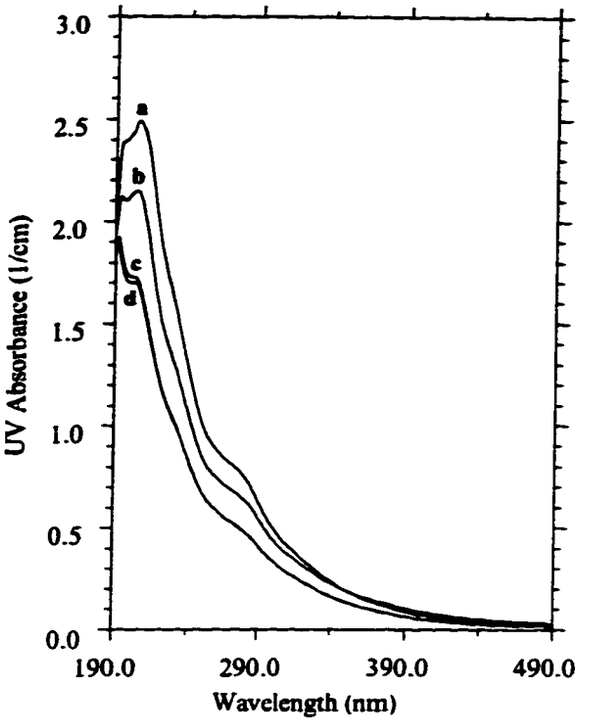

Figure 4-6. Wide Range of UV Spectra of Raw ASB Influent, ASB Effluent and Two Replicates of Ozone Treated ASB Effluent (a: Raw Influent; b: Raw Effluent; c: NR1 (Replicate No.1); d: NR2 (Replicate No.2). 1:10 dilution)

The wide range of UV absorbance in Figure 4-6, true colour, COD, TOC and BOD_5 in Table 4-2 reveal that the two replicates reproduce each other with regard to those monitored parameters and the reactions of ozone with the organic molecules in the wastewater samples. Consequently it is reasonable to state that the two systems are reliable for assessing the performance of ozone treatment, and the experimental results are reproducible and the accurate reflections of the ozone reactions with the organics in the wastewater.

4.3.2.2 Comparative Studies on Ozone Application Methods

Results in Table 4-3 demonstrate that decolorization efficiency using System I, expressed as C.U./mg used O_3, is statistically significantly lower at 1% significance level than that using System II both at two used ozone doses and with the two types of wastewaters. The degree of improvement on the short-term biodegradability of the wastewater tested in System I, expressed as mg BOD_5/mg used O_3, is not statistically significantly different at 1% significance level but is statistically significantly lower at 5% significance level than that tested in System II.

The statistical differences in COD and TOC removal between two systems, however, are not consistent for the two wastewater samples investigated. Removal efficiency of COD and TOC in System I, expressed as mg COD or TOC/mg used O_3, are statistically significantly lower at 5% significance level than those in System II when tested with ASB effluent at a medium used ozone dose but not significantly different at 5% significance level from those in System II when tested with E_{op} filtrate. Besides ozone application methods following three possible circumstances may also contribute to the inconsistency 1) significant errors in the estimation of the ozone dose; 2) different characteristics of the tested effluents (see Table 4-1); 3) inconsistency in the performance of ozone reactor systems. As discussed earlier, however, inconsistency in the performance of ozone reactor systems is less likely. Thus, the other two possibilities remain suspicious and should be further investigated.

Nevertheless, data in Table 4-3 appear to show that the ozone application methods (constant rate vs. one-time addition) have statistically significant effects on ozone treatment efficiency (at 5% significance level) at least with regard to the removal of true colour and the degree of improvement on BOD_5. A similar observation has recently also been reported by Amero *et al.* (1993) with respect to decolorization of bleachery effluents. The authors demonstrated that the OAR had substantially effects on the efficiency of ozone decolorization of the bleachery effluents. After examining the experiments in their study, however, it was found that both the lab and pilot reactors had very large head space, and obviously these large space would allow relatively high retention time of residual ozone gas; moreover, the residual ozone gas was obviously saturated with water vapor under the system pressure. Therefore, the ozone contactor system used in the study almost mimics the ozone reactor in System I used in this study. These observations and the earlier discussions on ozone chemistry raise the great doubts on the above conclusions since the ozone decomposition in WVS gas phase is theoretically significant, and could have important effects on the measurement of the consumed (but not used) ozone dose. Thus, it is absolutely necessary to determine these unseeable effects in many ozone treatment studies in order to reliably evaluate the real performance of ozone treatment on complex industrial wastewater.

Table 4-3. Comparison of Performance of Two Ozone Treatment Systems Based on Applied Ozone Dose

Wastewater	Used Ozone Dose	Colour		COD		TOC		BOD_5	
	(mg/L)	CPPA C.U.	C.U. per mg O_3	mg/L	COD per mg O_3	mg/L	TOC per mg O_3	mg/L	BOD_5 per mg O_3
Raw Eop (I)	0	3050		3000		1230		540	
Ozone Treated (I)	195	2370	3.49	2850	0.82	1210	0.092	588	0.246
Raw E_{nn}(II)	0	2900		2980		1240		518	
Ozone Treated (II)	152	2300	3.98	2850	0.86	1230	0.092	562	0.289
Raw Effluent (I)	0	1420		745		280		21	
Ozone Treated (I)	117	642	6.65	672	0.62	268	0.10	47	0.222
Raw Effluent (II)	0	1390		677		244		7	
Ozone Treated (II)	83	757	7.60	607	0.84	237	0.084	29	0.265

Table 4-4. Comparison of Performance of Two Ozone Treatment Systems Based on Consumed Ozone Dose

Wastewater	Consumed Ozone Dose	Colour		COD		TOC		BOD_5	
	(mg/L)	CPPA C.U.	C.U. per mg O_3	mg/L	COD per mg O_3	mg/L	TOC per mg O_3	mg/L	BOD_5 per mg O_3
Raw E_{nn} (I)	0	3050		3000		1230		540	
Ozone Treated (I)	170	2370	4.01	2850	0.94	1210	0.11	588	0.282
Raw E_{nn}(II)	0	2900		2980		1240		518	
Ozone Treated (II)	152	2300	3.98	2850	0.87	1230	0.093	562	0.289
Raw Effluent (I)	0	1420		745		280		21	
Ozone Treated (I)	103	642	7.55	672	0.72	268	0.12	47	0.252
Raw Effluent (II)	0	1390		677		244		7	
Ozone Treated (II)	83	757	7.60	607	0.84	237	0.084	29	0.265

Note: (I): Ozone Reactor System I; (II): Ozone Reactor System II; CPPA C.U. (or C.U.) = Colour Units based on CPPA method

4.3.2.3 Ozone Decomposition in WVS Gas Phase

Figure 4-7 illustrates the distribution of used ozone dose in the two-phased ozone reactor. Obviously the residual ozone in the gas phase was relatively small and found to be close to the residual ozone in treatment runs (less than 2% of total used ozone dose) in many cases.

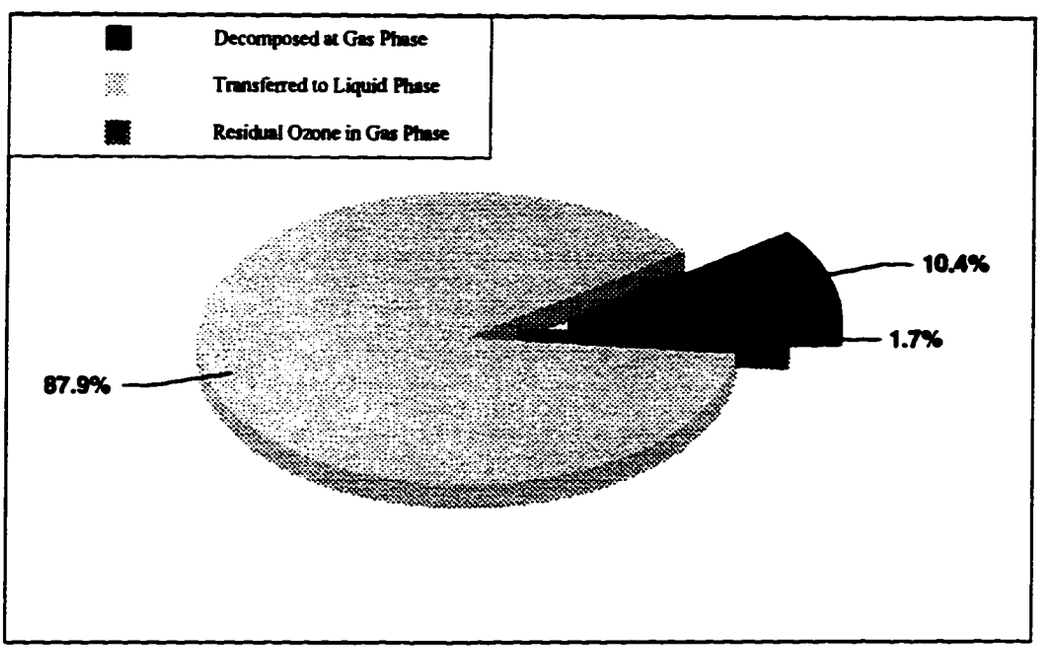

Figure 4-7. Approximate Distribution of Used Ozone Dose in Ozone Reactor System I

Figure 4-7 also demonstrates that there is more than 10% used ozone dose could be decomposed in WVS gas phase during ozone treatment and these decomposed ozone in gas phase can not be avoided in the ozone reactor like System I by the manipulation of operation procedures. In every series of experiments the ratio of decomposed ozone to used ozone dose at each experimental condition (with constant retention time) almost remains constant so the consumed ozone dose can be reliably estimated in each series of experiments, and the estimated consumed ozone dose in this study may better represent the ozone actually utilized by the wastewater treated with System I.

It is certain that the decomposed ozone, if included in the estimation of the consumed ozone dose (so the reported ozone dose became used ozone dose), could result in significant

errors in evaluation of the efficacy of ozone treatment. It is also important to recognize that the decomposed ozone will increase with increasing remaining ozone in a off-gas and increasing retention time of off-gas in a ozone contactor. The remaining ozone in turn positively correlated to OAR or ozone concentration in a gas mixture to the ozone contactor, decreasing mass transfer in the ozone contactor. Consequently these findings could be sufficient in explaining why decreasing ozone addition rate (by decreasing ozone concentration in feed gas) can apparently improve the ozone decolorization efficiency of bleachery effluents in the bubbling column contactor (Amero, *et al.* 1993). On the other hand, the reactor in System II has negligible headspace; the ozone decomposition in WVS gas phase, thus, could be negligible and the used ozone dose in this case is equivalent to the consumed ozone dose. This finding certainly helps explain why the performance of two reactor systems at same used ozone dose can be statistically significantly different (the details will be discussed in a later section).

4.3.2.4 Comparative Studies on Efficacy of Ozone Treatment Using Two Ozone Reactor Systems

Figures 4-8 and 4-9 compare the reduction of true colour, TOC and COD in System I with those in System II over the wide range of ozone dose levels using E_{op} filtrate and ASB effluent, respectively. The ozone dose in both Figure 4-8 and Figure 4-9 is the consumed ozone dose which is properly estimated according to the ratio of used ozone dose to decomposed ozone in the system.

These results reveal that the efficacy of ozone treatment in each experimental run using System I, based on reduction of true colour, COD, and TOC, agrees well with those made using System II at the similar consumed ozone dose level and with two different types of pulp mill effluents. In both cases, the effectiveness of ozone treatment on reduction of these parameters is in the order of colour>COD>TOC.

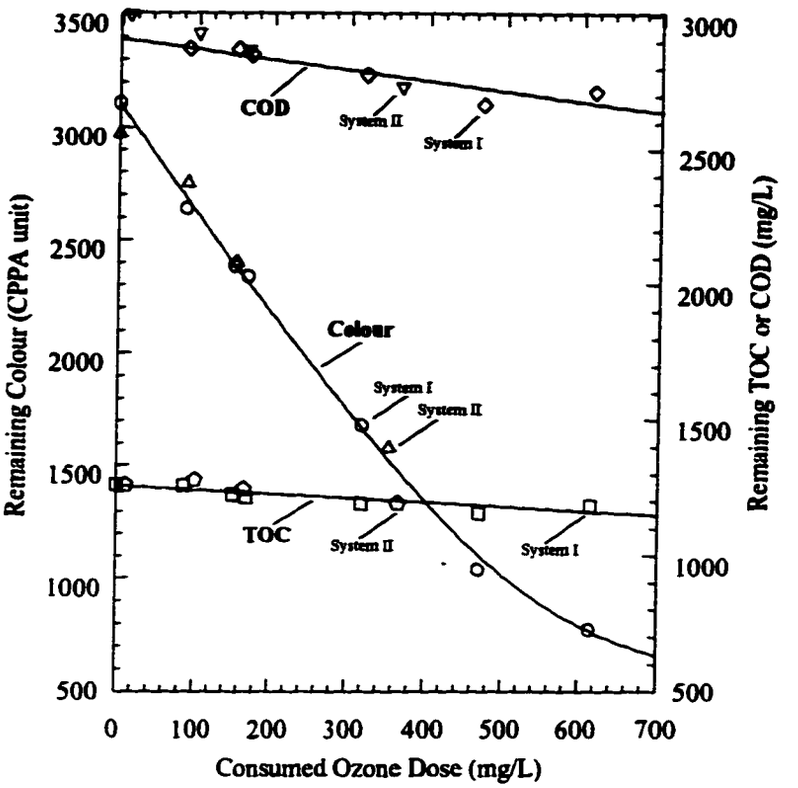

Figure 4-8. Efficacy of Ozone Treatment on True Colour, TOC and COD of E_{op} Filtrate over the Wide Range of Consumed Ozone Dose Levels

Nonetheless, the mode and performance of ozone decolorization appear to be wastewater dependent. As revealed in Figures 4-8 and 4-9, the decolorization mode of ASB effluent contrast sharply with that of E_{op} filtrate. For E_{op} filtrate (see Figure 4-8), up to 65% decolorization, the colour reduction appears to be linear with respect to the consumed ozone dose; after that, the decolorization efficiency only decreases slightly. By contrast, the decolorization of ASB effluent is obviously nonlinear, and almost mimics the three-phased mode reported in the literature (Mohammed and Smith, 1992; Mohammed, 1990; Patton, 1979; Stern, 1974). In addition, the decolorization of ASB effluent is obviously much more effective than that of E_{op} filtrate within the tested range of the consumed ozone doses. The statistical comparison of the decolorization mode between systems and two types of wastewaters will be detailed later in this study.

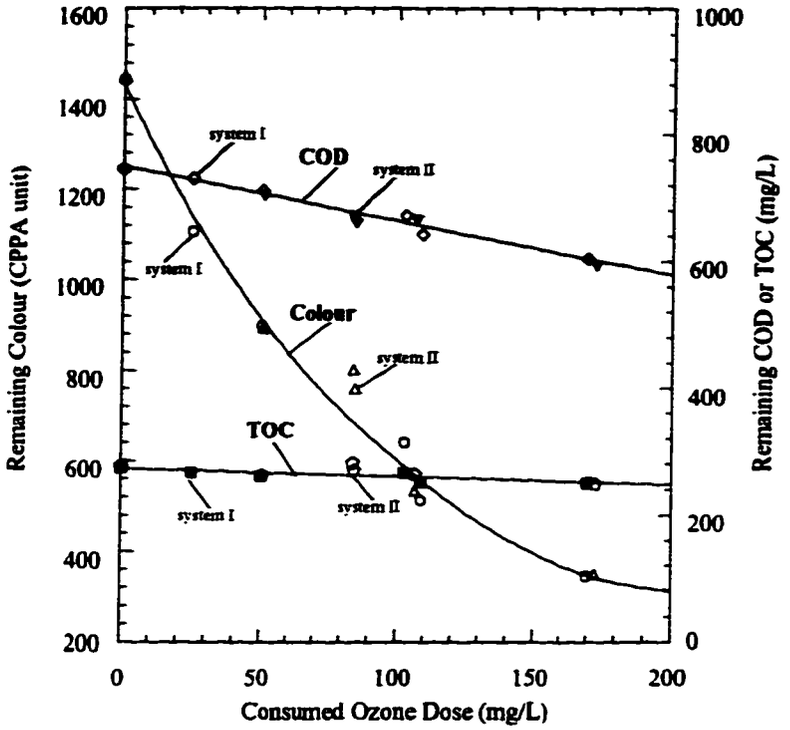

Figure 4-9. Efficacy of Ozone Treatment on True Colour, TOC and COD of ASB Effluent over a Wide Range of Consumed Ozone Dose Levels

Figure 4-10 further confirms that, with respect to decolorization efficiency in C.U./mg consumed O_3, the mode and performance of ozone decolorization of either E_{op} filtrate or ASB effluent in System II are closely followed by those in System I provided that the proper consumed ozone dose is employed in the interpretation of ozone treatment results. Figures 4-8, 4-9 and 4-10 also suggest that decolorization efficiency positively correlates with colour intensity (C.U./mg TOC or COD) and pH of the raw wastewater as indicated in Table 4-1 although it is independent of the ozone application methods. In this particular case, E_{op} filtrate has high pH (about 10) and low colour intensity (with high ratio of non-colored organics), but ASB effluent has neutral pH (about 7) and high colour intensity. As a result, the overall efficiency of decolorization of E_{op} filtrate is much lower than that of ASB effluent (see Figure 4-10). However, the colour intensity and pH have completely reversed effects on the degree of dependence of decolorization efficiency on the level of the consumed ozone dose. As also demonstrated in Figure 4-10, the decolorization efficiency of ASB effluent decreases sharply with increase in the consumed ozone dose level or the level of decolorization but ozone

decolorization efficiency of E_{op} filtrate is much less dependent on the level of decolorization (or the consumed ozone dose level).

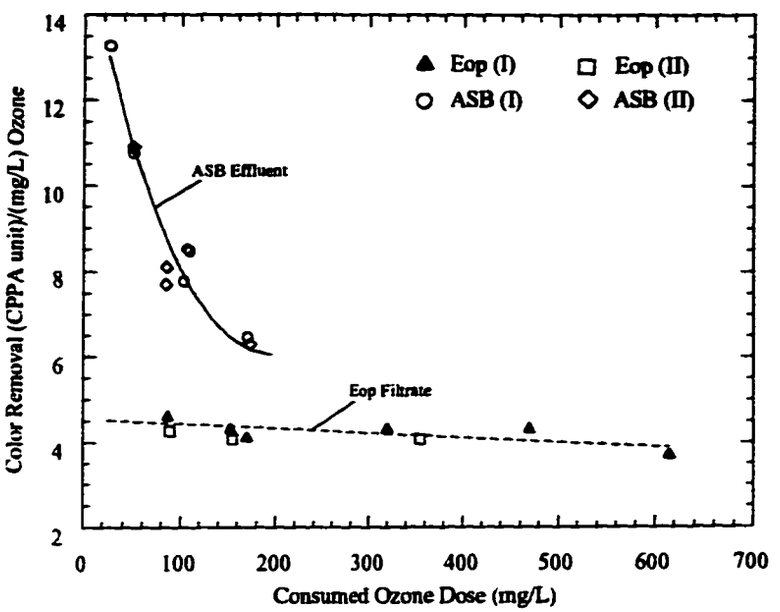

Figure 4-10. Comparison of Efficiency of Ozone Treatment in System I with that in System II Using Either E_{op} Filtrate or ASB Effluent over a Wide Range of Consumed Ozone Dose Levels

From these results it can be reasonably deduced that colour intensity and pH of raw wastewater are determinant factors in selecting decolorization mode and reaction scheme in ozone decolorization of pulp mill effluents; in particular, 1) in decolorization of E_{op} filtrate, indirect ozone reactions could be dominant and the competition for ozone by decomposition, non-colored organics and colored organics could be strong as soon as the ozone is introduced into the wastewater; 2) in decolorization of ASB effluent, direct reaction is obviously dominant and the competition for ozone by non-colored organics and decomposition is relatively weak.

It is also important to recognize that, unlike true colour removal, the mode and performance of ozone treatment on COD, as shown in Figures 4-8, 4-9 and 4-11, appears to be wastewater independent; moreover, the ratio of mg COD removal per mg consumed ozone is between 0.95 (at low ozone dose) and 1.15 (at high ozone dose). This implies that at this level of treatment almost all consumed ozone has been incorporated into the compounds for increasing

their oxidation status and that decomposed ozone in liquid phase is negligible. Even so, the treated effluents, up to 75% decolorization, still have relatively high chemical oxygen demand; as a result, the ozone residual in liquid phase was negligible. On the other hand, as observed in both this study and earlier studies (Amero, *et al.*,1993; Heinzle *et al.*, 1992; Prat, *et al.*, 1990) the ozone concentration in the residual ozone gas was gradually increased with increasing decolorization level.

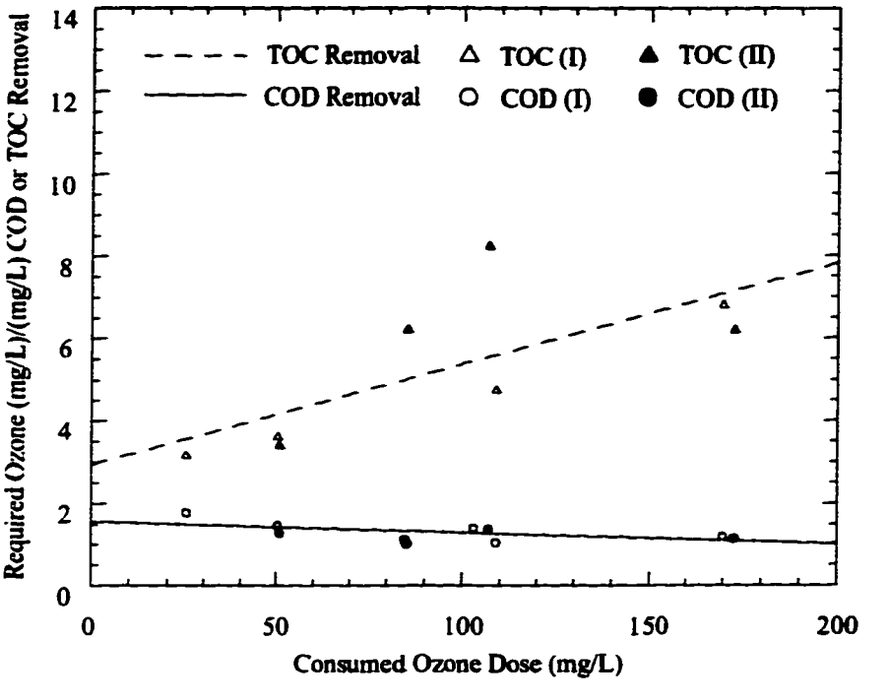

Figure 4-11. Efficiency of Ozone Treatment in Removal of TOC and COD from ASB Effluent

Contrast sharply with decolorization, ozone is less effective on COD removal and much less effective on TOC removal than true colour removal even at high ozone dose levels. As demonstrated in Figure 4-11, the results of TOC removal from ASB effluent at various ozone dose levels are relatively small and so scattered that the TOC reduction after ozone treatment was almost at the level of instrumental error. These results further prove that the oxidation in ozone treatment of pulp mill effluents is far from the completion.

These results also implied that in ozone treatment of pulp mill effluents the reactions of ozone with chromophoric structures are dominant and the reactions with both coloured and some competing non-coloured organic components, at the decolorization level less than 75%, are so fast that the mass transfer resistance in gas phase may be in the similar order of magnitude as that in liquid phase. A similar phenomenon have also been observed in a number of previous ozonation studies (Singer and Gurol, 1983; Ovechkin, *et al.* 1981; Munter, *et al.* 1983; Richards, *et al.* 1977; Matrozov, *et al.* 1976). In an instantaneous reaction, for example, between ozone and an aqueous solution of potassium iodide, the resistance of the liquid phase reduces to almost zero (Matrozov, *et al.* 1976). On a very fast reaction, for instance, for oxidation of phenol by ozone in alkaline medium (pH=11.5), the proportion of gas phase resistance is found to be in the range of 10 to 60% (Ovechkin et al., 1981; Munter, *et al.* 1983); even during the oxidation in acidic medium (pH below 7), the gas phase resistance is over 15% of total mass transfer resistance (Ovechkin et al., 1981; Singer and Gurol, 1983).

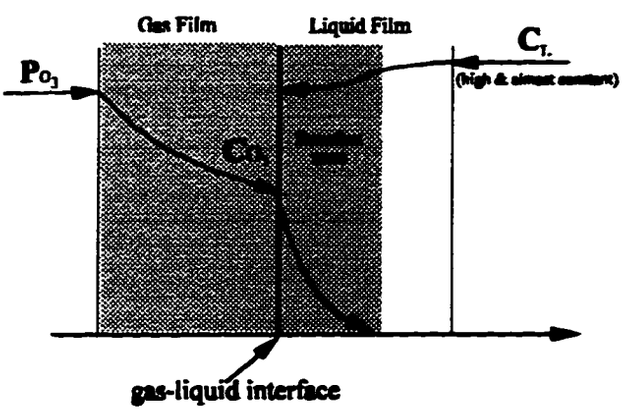

Figure 4-12. Possible Concentration Profile in the Boundary Region for Fast Reactions Between Ozone Molecules or Radicals through the Interface and Lignin Fragments Present in the Bulk of Wastewater

In fact, these observations are naturally in accordance with the results predicted by two-film theory under the circumstance of enhanced mass transfer by fast chemical reactions in liquid phase (Danckwerts, 1970; Levenspiel, 1972; Fahien, 1983; Westerterp, *et al.* 1984). As illustrated in Figure 4-12, the concentration of C_L and rate of chemical reaction has great influence on the thickness of reaction zone provided that the mixing in the system is well-controlled.

It has been demonstrated (Danckwerts, 1970; Westerterp, *et al.* 1984) that gas phase mass transfer would become dominant if the following criterion is satisfied;

$$\frac{k_G H}{k_L E_A} \ll 1 \qquad (1)$$

Where k_G and k_L are gas and liquid phase mass transfer coefficient, respectively; H is Henry's law constant, and E_A is mass transfer enhancement factor.

It was reported that k_G=1.0 mole/(m^2*sec*atm) (Charpentier, 1982), k_L=1x10^{-4} m/sec, and H=5.19x10^3 atm/O_3 mole fraction (Perry's Chemical Engineers Handbood). This Henry's law constant is equivalent to 0.0933 atm/(mole/m^3). Substituting these parameters into the equation $E_A >> \frac{k_G H}{k_L}$=933.

In case of fast irreversible first order reactions with high concentration of reactant in liquid phase (Danckwerts, 1970; Levenspiel, 1972; Fahien, 1983; Westerterp, *et al.* 1984),

$$E_A = H_a \qquad (H_a \geq 2) \qquad (2)$$

Where the Hatta number $H_a = \sqrt{\frac{k_1 D_A}{k_L^2}}$ (k_1 is the kinetic rate constant of irreversible first order reaction and D_A is the molecular diffusion coefficient);

Thus,

$$E_A = H_a = \sqrt{\frac{k_1 D_A}{k_L^2}} >> \frac{k_G H}{k_L} \qquad (3)$$

$$\therefore \qquad k_1 >> \frac{k_G^2 H^2}{D_A} \qquad (4)$$

It has been reported that at 20°C, k_G=2.1x10^{-3} m/s based on $N_A=k_G(C_{iG}-C_{iL})$(Munter et al., 1983); (which is equivalent to dimensionless Henry's law constant H=4.17 based on $H=C_{iG}/C_{iL}$) and D_A=1.74x10^{-9} m^2/s (Langlais et al., 1991). Substituting these parameters into Equation (4), it results k_1>>4.40x10^4 s^{-1}. It has also been reported that k_G values range from 10^{-4} to 10^{-1} m/s for the pure physical absorption of gas into liquid in common gas-liquid contacting

devices (Montgomery, 1985; Zarzycki and Chacuk, 1993). Thus, the range of k_l values to meet the above criteria would be in the order of magnitude 64 to 6.4×10^7 s^{-1}.

Hoignè (1982a) has actually shown that ozone reactions with many aromatic, quinonic or C=C structures have a kinetic rate constant in the order of greater than 10^5. For example, in the reaction of ozone with the phenolate structure, its kinetic rate constant is greater than 10^9 (Hoignè, 1982a) which is in the range of rate constant of the radical reactions. So it is obvious that above criterion can be easily satisfied in ozone decolorization of pulp mill effluents. Physically, this criterion can be visualized that the reactions are so fast that they may reduce the reaction zone to almost zero.

In summary, the results from above comparative studies suggest that 1) if the consumed ozone dose was properly determined in ozone treatment of pulp mill effluent and the mass transfer is consistently controlled, the ozone application methods (System I vs System II) appear to have negligible effects on the mode and performance of ozone treatment; the characteristics of wastewater, particularly colour intensity and pH, has steering influence on the mode and efficiency of decolorization, but negligible effects on reduction of COD and TOC and 2) the gas phase mass transfer may play a role in the process of ozone treatment of pulp mill effluents up to 75% decolorization level.

4.3.2.5 Statistical Significance of Effects of Ozone Application Methods on Mode and Performance of Ozone Treatment Over Wide Range of Ozone Dose Levels

Based on the ratio of used ozone to decomposed ozone established in above studies, the used ozone dose in Table 4-3 is properly converted into the consumed ozone dose and the efficiencies of treatment in System I are re-evaluated based on the estimated consumed ozone dose. The final results are summarized in Table 4-4.

The results in Table 4-4 demonstrate that the removal efficiencies of true colour, COD, TOC in System I, express as C.U., mg COD or mg TOC per mg consumed O_3, are not statistically significantly different at 1% significance level from those in System II; and the degree of improvement of short-term biodegradability in System I, expressed as mg BOD_5 per mg consumed O_3, is also not statistically significantly different at 5% significance level from that in System II.

Thus, it is reasonable to conclude from statistical point of view that 1) the tested ozone application methods do not have statistically significant effects on the mode and performance of ozone treatment regarding to true colour, COD, TOC and BOD_5 when tested with two types of pulp mill wastewaters and at two different ozone dose levels. Bauman and Lutz (1974) had observed similar phenomena although the result was very preliminary.

Figure 4-13 shows the 95% confidence intervals for decolorization of E_{op} filtrate. The confidence intervals are constructed using the data from System I based on the consumed ozone dose. For statistical comparison over a wide range of ozone dose levels, the results from decolorization of E_{op} filtrate using System II are also plotted in Figure 4-13. The results in Figure 4-13 demonstrate that all the decolorization runs from System II fall well within the 95% confidence intervals constructed using the results from System I. The t-test on the slope and intercept of two regression analyses suggests that, with regard to decolorization, the efficacy of ozone treatment using System I is not statistically distinguishable at 5% significance level from that in System II over the wide range of ozone dose investigated.

Consequently, the results from both System I and System II are combined together for regression analysis based on linear model $Y = aX + b$. The results from regression analysis are summarized in Table 4-5 and formulated in equation (5).

$$Y = 3007 - 3.891[O_3] \qquad (R^2 = 0.987) \qquad (5)$$

Where Y is the remaining true colour in CPPA unit; and $[O_3]$ is the consumed ozone dose in mg/L.

The regression line based on equation (5) is also plotted in Figure 4-13. The regression analysis further demonstrates that test results from both System I and System II belong to the same population and fit linear model satisfactorily (with R^2=0.987 and low standard error, see Table 4-5).

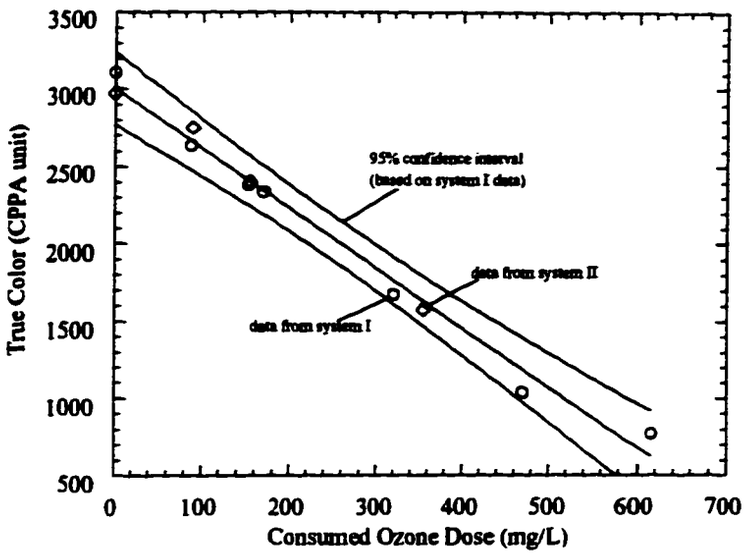

Figure 4-13. 95% Confidence Intervals (indicated by two band lines around the regression line) of Decolorization of E_{op} Filtrate and Its Regression Analysis

The semilog plot of the results from decolorization of ASB effluent in Figure 4-14 suggests its exponential characteristics and its striking difference from those of E_{op} filtrate. Thus, the decolorization results from both systems are transformed using Y=Ln(remaining color) and the transformed results from both systems are again combined together for regression analysis based on the log linear model Ln(Y) = aX + b.

Table 4-5. Results from Regression Analysis of Ozone Decolorization of Two Types of Pulp Mill Effluents Based on Poured Results from Both System I and System II

Wastewater	Coefficient a		Constant b		R^2
	estimate	standard error	estimate	standard error	
E_{op} filtrate	3006.5	47.1	-3.891	0.155	0.987
ASB effluent	7.250	0.0451	-0.00822	0.00044	0.974

The regression analysis results are also summarized in Table 4-5 and formulated in equation (6).

$$Y = 1444e^{-0.00822[O_3]} \qquad (R^2 = 0.974) \qquad (6)$$

Where Y is the remaining true colour in CPPA unit; and $[O_3]$ is the consumed ozone dose in mg/L.

The regression line based on equation (6) is illustrated in Figure 4-14. Similarly, the 95% confidence intervals based on data from System I are established. For direct comparison, the data from System II are directly overlaid on the confidence intervals as indicated in Figure 4-14. Again, the t-tests on the slope and intercept of two regression analysis suggest that no statistically significant difference between two systems can be stated at 5% significance level with regard to reduction of true colour in ASB effluent over a wide range of tested ozone doses.

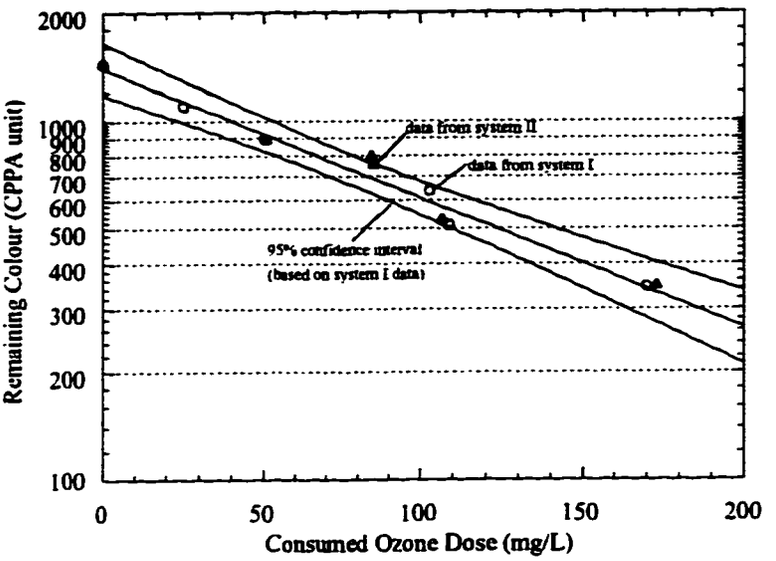

Figure 4-14. 95% Confidence Intervals of Decolorization of ASB Effluent and Its Regression Analysis

In this case, regression analysis also demonstrated that the log linear model fits the decolorization data better (R^2=0.974 with low standard error, see Table 4-5) and further confirms that ozone application methods do not have statistically significant effects on the mode and performance of ozone decolorization provided that the ozone dose was properly determined and the mass transfer was properly controlled and consistent in system but the characteristics of wastewater have important influence on the mode and efficiency of decolorization.

4.4 CONCLUSIONS

The careful investigations on ozone treatment of two major effluents from a pulp mill using two types of specially designed ozone reactor systems demonstrate that:

1) Over the wide range of the consumed ozone doses the ozone application methods (constant rate vs. one time addition) does not have statistically significant effects on the efficacy of ozone treatment at 5% significance level provided that the ozone is properly handled and the mass transfer is consistently controlled in the treatment systems.

2) In common ozone contactor systems including System I in this study, the ozone decomposition in WVS gas phase is significant and unavoidable but in most cases these phenomena are usually neglected and certainly contribute to significant confusion found in the previous studies.

3) The ozone application methods do not change the competitiveness of ozone reactions with various compounds in wastewater but affect the availability of ozone for reacting with the target organics.

4) The efficacy of ozone treatment of pulp mill effluents are wastewater and parameter specific. Particularly, the colour intensity and pH of wastewater can interfere severely with the ozone treatment; and in this study ozone treatment is more effective in decolorization of ASB effluent than decolorization of E_{op} filtrate; the effects of the colour intensity and pH on COD, TOC and BOD_5, however, are much less profound than on true colour.

5) At decolorization levels less than 75%, ozone decolorization of pulp mill effluents is greatly influenced by the rapid rate of reactions (mass transfer enhancement) which suggests that the gas phase mass transfer resistance may plat a role.

6) The kinetics of decolorization of ASB effluent is significantly different from that of decolorization E_{op} filtrate. A log-linear model fits the decolorization data of ASB effluent better while a linear model fits E_{op} data satisfactorily.

4.5 ACKNOWLEDGMENTS

This study was partially financed by Weyerhaeuser Canada Inc.. The authors greatly appreciate Steve Cooper, Environmental Manager of Grande Prairie Operations', Weyerhaeuser

Canada Inc. for providing representative effluent sample and some valuable discussions. The authors also like to thank Mr. Murray Carmichael for the great help in ozone treatment experiments and some TOC and COD analysis and Mr. Edwin Liem for the help in experiment preparation and some COD analysis.

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CHAPTER 5. COMPARATIVE EVALUATION OF IMPACTS OF OZONE DECOLORIZATION AND FOAM SEPARATION ON BIODEGRADABLE POTENTIALS OF PULP MILL EFFLUENTS

5.1 INTRODUCTION

Both activated sludge and aerated lagoon processes have been widely used for treatment of pulp mill wastewaters. These processes effectively reduce the biodegradable organics and suspended solids, but they are unable to attack the majority of the color-causing lignin components that some are usually chlorinated (Mao and Smith, 1994a; Graves, et al., 1993; Chen, et al., 1974; Davis, 1969). However, it was reported that under proper conditions the lignin components could exert a significant amount of biochemical oxygen demand (BOD) over a relatively long period (Mao and Smith, 1994a and 1995a; McDonald and Radermacher, 1993).

Ozone treatment has been studied as a method of improving the biodegradability and reducing the color of pulp mill effluents. The earlier studies found that ozone decolorization can increase BOD_5 of biologically treated pulp mill effluent by more than 100% (Bauman, 1974; Cauman and Lutz, 1974; Mohammed, 1990); but the effects of ozone decolorization on combined mill effluents varied with characteristics of wastewater. The accumulated evidence indicated that the improvement in biodegradability was also dependent on the ozone dose, reaction conditions (contact time, pH, temperature, etc.) and the microorganisms used in the evaluation process. When ozone treatment of combined mill effluents at low ozone dose, the BOD_5 increased slightly; however, it was found that BOD_5 was reduced considerably with further increasing the ozone dose (Ng, et al., 1978; Davis, 1969). Stern (1974) also found that when ozone dose exceeded a certain level, further increase of ozone dose had little improvement in the biodegradability of a kraft mill effluent. Patton (1979) observed that the BOD increase was significantly larger at low pH (pH<5) than that at high pH (pH>10). Patton (1979) also demonstrated that five times of the biomass was produced with ozonated desugared spent sulfite

lignin (SSL) than with raw desugared SSL using a rough "strain" of yeast. However, the increase in biomass was strain-dependent since only minor increase was observed if using a "smooth" variant of *Torula* yeast. Similar findings have been reported in other studies (Stern, 1974; Mohammed, 1990; Smith and Mohammed, 1992) employing 5-days BOD tests.

The results from these preliminary studies ascertained that ozonation altered the biodegradability of pulp mill effluents. However, it is difficult to compare the results among the studies due to many uncertainties in the experimental conditions, estimation of ozone dose (Mao and Smith, 1995b), and the different concepts of biodegradability as discussed by Mao and Smith (1995a). Recently a mechanistic model developed by Mao and Smith (1995a) seemed to provide a effective means to assess long-term biodegradability (or biodegradable potential as defined later) of complex industrial wastewatert especially pulp mill effluents.

In addition, there is insufficeint quantitative investigations on how and which molecular weight portion of the lignin components these processes have impacts on, and what are their contributions to the biodegradability of pulp mill effluents. This knowledge is of important concern to the research engineers, industries, public and regulatory authorities since 1) some of structure-modified chlorinated lignins could be toxic, mutagenic, or even carcinogenic (Kringstad and Lindström,, 1984); 2) some could be the procursors for these toxicants through the physical, chemical and microbial transformation (Nelson, et al, 1983 and 1990); 3) the distribution and removal of these lignin derivatives in either secondary treatment processes or the receiving environments are contingent on the biodegradability; and 4) it is fundamental information for developing and assessing a advanced biotreatment system to minimize the impacts of effluent discharges from pulp mills.

The objectives of this study were: (1) to establish a concept and quantitative procedures for systematic assessing and comparing the biodegradable potentials of various pulp mill effluents; (2) quantitatively evaluate the influence of ozone decolorization, foam separation, by comparing it to conventional biological treatment, on the biodegradable potentials of lignin

components in the pulp mill effluents, and (3) examine the contributions of different lignin components to the biodegradable potentials of pulp mill effluents and their competitiveness with biodegradable components defined as BOD_5 or BOD_7. Additional objective was to examine the correlation or interactions among the biodegradable potentials, color intensity (C.I.) and other important parameters. To achieve these objectives various pulp mill effluents were treated using ozonation and foam separation. The raw and treated effluents were further fragmented using ultrafiltration processes. The long-term biochemical oxygen uptake tests and the mechanistic biodegradable potential model (Mao and Smith, 1995a) were employed in the evaluation. The focus was on the effects of ozone decolorization.

5.2 MATERIALS AND METHODS

5.2.1 Sources of Wastewater

Four types of samples with representative characteristics (see Table 5-1) were chosen for evaluation. Type I was a 300 mg/L solution of glucose/glutamic acid (GG solution) (Fisher Scientific) which served as an biodegradable organic mixture and standard solution. The grab samples of combined pulp mill effluent called ASB influent (sampled at the inlet of aerated stabilization basin (ASB)), which represented the mixture of municipal and pulp mill wastewaters, was used as Type II. The grab samples of bleaching effluent called E_{op} filtrate (sampled from E_{op} stage of $DE_{op}D_1ED_2$ bleaching sequence, see Table 5-1) was used as Type III which represented the wastewater with high concentrations of both biologically resistant lignin components and other biodegradable wood components in pulp mill effluents. The grab samples of biologically treated effluent called ASB effluent (sampled at the discharge point of ASB) was used as Type IV which represented the effluent with low concentrations of biodegradable material and relatively high concentrations of lignin components from pulp mill effluents. Unless stated otherwise, these wastewaters were characterized according to Standard Methods (APHA, 1992).

Table 5-1. Summary of Characteristics of Bleaching Effluent (E_{op} Filtrate), Combined Pulp Mill Effluent (ASB Influent) and Biologically Treated Effluent (ASB Effluent)

Parameter		GG solution	ASB Influent	E_{op} Filtrate	ASB Effluent
pH			5.6±0.2	9.65±0.15	7.1±0.3
Temperature	(°C)		45 (15 to 55)	75	15 to 40
D.O.	(mg/L)		<0.8	< 0.5	>2
BOD_5	(mg/L)	205±25	460±10	540 ± 15	25.1
TSS[2]	(mg/L)		20±2	55±5	70±4
TDS[3]	(mg/L)		1860±35	4720±70	2557±20
TDVS[4]	(mg/L)		1103±25	2563±17	559±6
COD	(mg/L)	305±5	1368±17	3058±44	810
TDOC[5]	(mg/L)		466±5	1287±23	N/A
TOC	(mg/L)	142	493±4	1296±14	276.3
BOD_5/COD		**0.602**	**0.332**	**0.177**	**0.03**
COD/TDOC			2.94	2.38	N/A
NH_3-N	(mg/L)		5.89	<0.1	< 1.5
TNOD[6]	(mg/L)		<25	<5	< 5

Note: 1. D= chlorine Dioxide, E_{OP} = alkaline Extraction with Oxygen/Peroxide, E= alkaline Extraction;
2. TSS = Total Suspended Solids;
3. TDS = Total Dissolved Solids;
4. TDVS = Total Dissolved Volatile Solids;
5. TDOC = Total Dissolved Organic Carbon;
6. TNOD = Theoretical Nitrogenous Oxygen Demand

5.2.2 Experiments

5.2.2.1 Ozone Decolorization

For ozone decolorization and biodegradability studies, the raw wastewater samples were first treated with ozone at designed comsumed ozone dose levels. All the ozone experiments were performed using the ozone reactor system as shown in Figure 5-1 and according to the procedures described in other studies (Mao and Smith, 1994b and 1995b). In brief, the ozone gas with constant pressure, flowrate and concentration was introduced into the ozone contactor at the bottom. The pulp mill effluents were completely mixed with ozone gas in the contactor for a desired period. The residual ozone in offgas were trapped into KI and the consumed ozone dose was estimated based on the difference between total ozone input and ozone in offgas.

5.2.2.2 Foam Separation

Foaming is a common characteristic of the pulp mill effluents. The ASB influent was found to foam heavily during ozone treatment. It was observed that the newly formed foams in the reactor trapped the ozone/oxygen gas and rapidly carried them through the reactor. As a

result, the efficiencies of ozone decolorization were reduced due to great reduction in the contact time and mass transfer efficiency. These also led to high residual ozone concentration in offgas. To improve the efficiency of ozone decolorization, foam separation before ozone treatment appeared to be necessary.

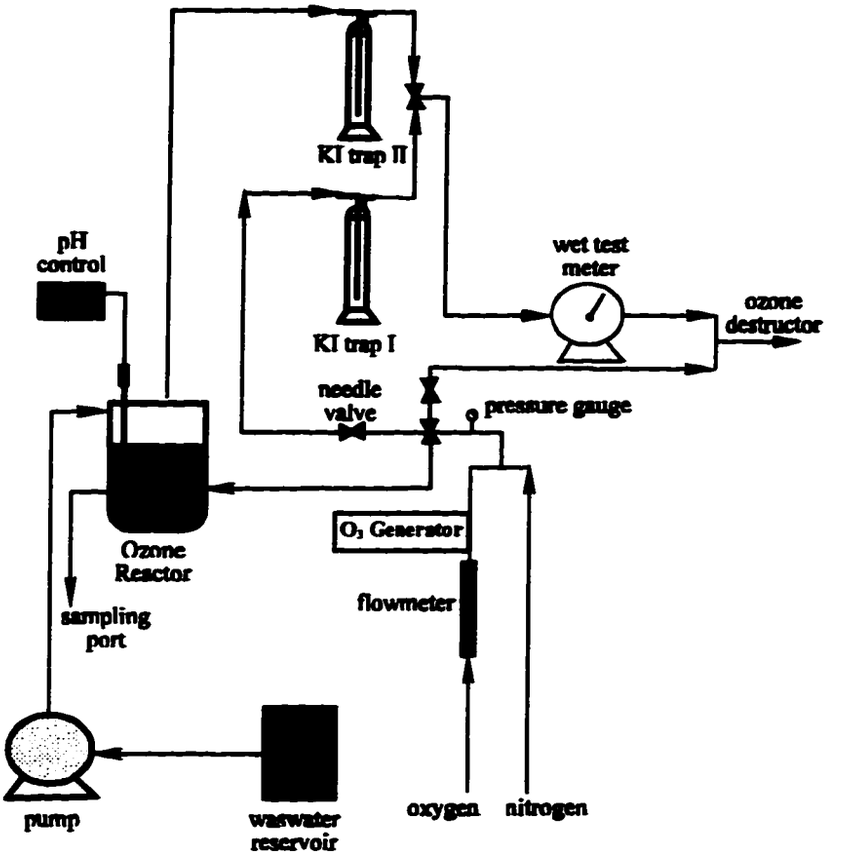

Figure 5-1. Schematic of Ozone Reactor System for Ozone Treatment Study

The foam separation was carried out in a 2-L reactor system at room temperature in a batch mode. This experimental system was similar to those shown in Figure 5-1 except for without operation of the ozone system. In each run, the reactor was filled with 1.5 L of raw ASB influent, then, the high grade pure nitrogen was continuously dispersed through a gas distributor located at the bottom of reactor at about 500 mL/minute for about 1.5 hours. The foam

component was collected through the top outlet of the reactor. The remaining liquid in the reactor after foam separation was collected as the defoamed component. The pH of raw ASB effluent was not adjusted prior to foam separation since it was within the range of 7 to 8 which was reported to be most effective for foam separation (Ng, *et al.*, 1976). The pure nitrogen was selected as foaming gas instead of air or pure oxygen in order to eliminate the potential effects of oxidation reactions, which had been reported in the literature (Ng, *et al.*, 1976; NCASI, 1964; Brunner and Stephern, 1965).

5.2.3 Sample Preparation and Analysis

5.2.3.1 Ultrafiltration Separation

The raw and treated samples were separated into four components using Millitan Acrylic Ultrafiltration System (Millipore Co.) equipped with 1,000, 5,000, and 10,000 molecular weight cutoff (MWCO) membranes. The procedures described by Mao and Smith (1995a) were followed in this study. These processes had been shown to be reliable in separation of pulp mill effluents.

5.2.3.2 Determination of True Color and Other Characteristics

The Canadian Pulp and Paper Association (CPPA) standard method H5.P (CPPA, 1974) and NCASI method (NCASI, 1971) were incorporated for the determination of true color with minor modifications (Mao and Smith, 1994b).

The procedures recommended in Standard Methods (APHA, 1992) were followed for sample analysis with considerations of the special characteristics of pulp mill effluents documented by TAPPI and CPPA. These include pH, temperature, dissolved oxygen, nitrogen, total suspended solids (TSS), total dissolved solids (TDS), total volatile solids (TVS), BOD_5, COD, and TOC.

5.2.4 Data Analysis

Systat® statistic program was used in this study for statistical analysis of data and for estimation of kinetic parameters in the model. In particular, non-linear parameter estimations

were performed using either Simplex or Quasi-Newton methods pre-programmed in Systat® statistics package. In the regression analysis, the residuals of regression were always plotted for assessing the distribution of residuals, the goodness of fit of the model. In addition, Hessian Matrix was always calculated to check the possibility of intercorrelation of the parameters. In the regression analysis the corrected R^2 was defined as $\left[1-\frac{residual\ sum-of-squares}{corrected\ sum-of-squares}\right]$.

5.2.5 Evaluation of Biodegradable Potential

A mechanistic model proposed by Mao and Smith (1995a) describes,

$$TBOU=\frac{k_1 L_0}{k_1+k_2}(1-e^{-(k_1+k_2)t})+\frac{k_2 L_0}{k_1+k_2}(1-e^{-k_3(t-t_0)}) \qquad (t>t_0) \quad (5\text{-}1)$$

where, TBOU = total biochemical oxygen uptake at time t; k_1, k_2, k_3 are kinetic constants involved in different stages of biodegradation; L_0 is the ultimate oxygen uptake; t_0 is the elapsed time before the stage two starts.

To unify the concept of biodegradability and establish systematic procedures for evaluation this study proposes the Biodegradable Potential (BP) as a uniform concept of biodegradability. Based on the experimental conditions and kinetics described in above mechanistic model, the biodegradable potential BP can be defined as the biochemical oxygen uptake with most favorable biodegradation kinetics by the acclimatized microbial consortia under ideal conditions over a specified long enough period, such as 100 days, from both practical and ecological points of view.

The above study also demonstrated that the development of TBOU showed a two-stage pattern which is the indication of the shift of microbial consortia. This development was effected not only by the absolute values of k_1, k_2 and k_3 but also dependent on the ratio of k_1/k_2 and k_1/k_3 (Mao and Smith, 1995a) since unbalanced ratios will result in the change of the microbial consortia involved in the biochemical reactions. In a real system, those ratios should be unique to the system with specific substrates and microbial consortia. It is also important to recognize that

it is absolutely necessary to compare the biodegradable potential at one uniform basis since TBOU varies widely with the compositions of wastewater. To meet these requirements for assessing and comparing the biodegradability, a series of dimensionless parameters is defined. from Eqation (5-1):

$$\overline{L} = \frac{k_1 L_0}{k_1 + k_2} \Big/ \frac{k_2 L_0}{k_1 + k_2} = \frac{k_1}{k_2} \tag{5-2}$$

$$\lambda = \frac{L_0}{COD} \tag{5-3}$$

$$\alpha = \frac{k_1}{k_{1G}} \tag{5-4}$$

$$\beta = \frac{k_3}{k_{3G}} \tag{5-5}$$

where COD (Chemical Oxygen Demand) is in mg/L, k_{1G} and k_{3G} are the kinetic constants of 300 mg/L glucose and glutamic acid standard solution, respectively.

As discussed in chapters 2 and 3, the biodegradable content of a wastewater depended on both the biodegradation kinetics (quality) and the total concentration (quantity) of the organics. Therefore, to uniformly assess BP of a wastewater the dimesionless index is necessary and can be established as a biodegradable potential index (BPI),

$$BPI = C\lambda(\alpha\beta\ \overline{L})^a \tag{5-6}$$

where C and a are constants to be determined from the experimental results.

To let BPI have 100 percent scale for practical purpose, however, assume BPI=100 represents maximum possible biodegradable potential, and define that the BPI of 150 mg/L glucose and 150 mg/L glutamic acid standard solution is 95 according to the findings in this study and previous study (Mao and Smith, 1995a; Gaudy and Gaudy , 1980). These studies found that the ultimate TBOU estimated using the mechanistic model was about 95% of COD. Moreover, by assuming biologically resistant organic mixtures such as the lignin components in

pulp mill effluents BPI≈5 as the basis for comparison and estimation of the constants in Equation (5-6).

5.3 RESULTS AND DISCUSSION

5.3.1 Basis of Evaluation and Reliability and Applicability of BPI

Table 5-2 compares the total biochemical oxygen uptake (TBOU) and the ratios of BOD_5 to TBOU and BOD_5/COD of the four representative samples shown in Figure 5-2 which was adopted from a previous study (Mao and Smith, 1994a). Table 5-3 further summarizes the characteristics of ASB effluent, ASB influent and E_{op} filtrate with respect to the distribution of true color, COD, TOC and BOD_5 among the components with different MW.

Table 5-2. Total Biochemical Oxgen Uptake (TBOU) of Bleaching Effluent (E_{op}), Combined Mill Effluent (ASB Influent) and Biologically Treated Effluent (ASB Effluent) over Different Periods

Sample	5-day		20-day		Ultimate BOD		BOD_5/COD
	BOD*	Level (%)	BOD	Level (%)	BOD	Level (%)	
E_{OD} Filtrate	540±15**	56.4	790±14	82.5	958±30	100	0.177 (0.314)
ASB Effluent	25±4	18.7	61±6	45.5	134±10	100	0.0309(0.165)
ASB Influent	460±10	64.2	619±7	86.5	716±18	100	0.332(0.336)
GG solution	174±25	59.2	278±14	94.6	294±17	100	0.602(0.913)

*BOD: mg/L; ** ±: indicates the standard deviation; Level (%) = BOD_5/Ultimate BOD

The results demonstrated that 1) the kinetic development of BOD mimicked each other between ASB influent and E_{op} filtrate, and the trend was similar to 300 mg/L glucose/glutamic acid but the ultimate BOD and ratios of BODs/COD differed considerably. The development pattern of ASB effluent differed substantially from all other patterns; 2) all components in ASB effluent had very low BOD_5, and the contributions to BOD_5 in among the components ASB effluent were in the order of F2<F3<F4 which was considerably different from that of either ASB influent or E_{op} filtrate; 3) the ASB process removed greater than 95% of BOD_5, about 44% of TOC, and 48% of COD, but had little effect on the color which usually represented lignin components. About 32% among the removed 44% of TOC was derived from the component with

MWCO<1000 (F1). The majority of the removed portion may be the non-lignin wood component, such as large molecules of hemicellose.

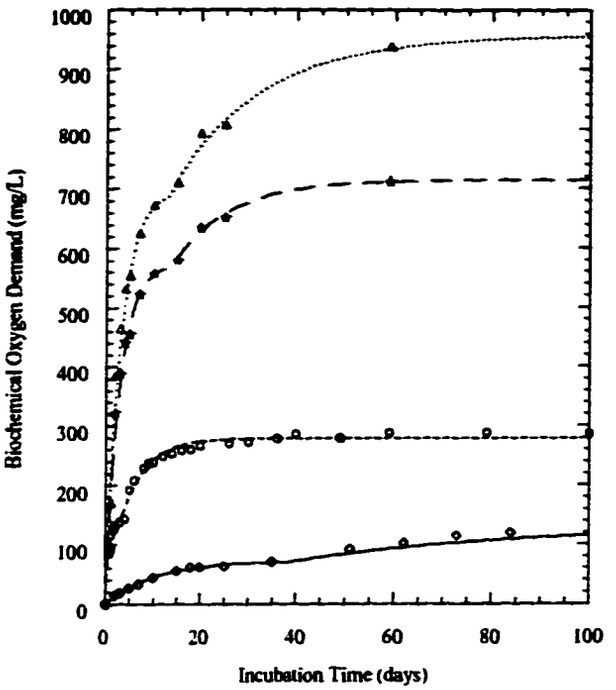

Figure 5-2. Comparison of Kinetic Development Patterns of BOD by ASB Effluent with Those by Other Pulp Mill Effluents and Standard Solution (square: ASB effluent, circle: glucose/glutamic acid; star: ASB influent, triangle: E_{op} filtrate)

Table 5-4 further compares the ratios of TOC, COD and BOD_5 of the four components from ASB effluent to the respective parameters from raw ASB influent. The results in Tables 5-2, 5-3 and 5-4 suggested that 1) the ratios of BODs to ultimate BOD or COD varied greatly with the source of wastewater and the period of the kinetic development. The similar observations have been reported in previous studies (Martone, 1976; Busch, 1958; Porter and Snider 1976). Obviously, the assessment of biodegradability would be different with different selections of parameters over different periods, but when the period was getting longer, the difference was getting smaller; 2) F1 from ASB effluent was entirely different from F1s of either ASB influent or E_{op} filtrate with regard to BOD_5, COD, BOD_5/COD, and COD/TOC; 3) a large portion of

color-causing lignin derivatives with MWCO<1,000 in pulp mill effluents was still biologically resistant; this indicated that the resistance of lignin derivatives to the biological degradation seemed to relate to both the structure and size of the molecules; 4) further comparison of the distribution of color in three effluents revealed the TOC and COD levels of the components with MWCO>1,000 had little correlation with the BOD_5 but the BOD_5 in F1 correlated well with either COD or TOC; 5) the ratio BOD_5/TOC or BOD_5/COD appeared to inversely correlate with the color intensity (C.I.), expressed as C.U./mg TOC or COD, which usually increases with the MW of organics in the pulp mill effluents (Mao and Smith, 1994a).

Table 5-3. Characteristics of Raw and Four Components of Bleaching Effluent (E_{op}), Combined Mill Effluent (ASB Influent) and Biologically Treated Effluent (ASB Effluent)

Parameter		Component					
		MWCO<1,000 (F1)	MWCO>1,000 MWCO<5,000 (F2)	MWCO>5,000 MWCO<10,000 (F3)	MWCO>10,000 (F4)	TOTAL[1]	RAW[2]
Color (C.U.)	ASB Effluent[4]	94(7%)	104(8%)	187(14%)	885(64%)	1270	1390
	ASB Influent[4]	138((10%)	109(8%)	217(15%)	823(58%)	1287	1430
	Eop Filtrate	208(7%)	247(8%)	454(15%)	1704(57%)	2613	2989
COD (mg/L)	ASB Effluent	132.2(20%)	83.9(13%)	111.4(17%)	306.2(47%)	633.7	654.2
	ASB Influent	595.6(47%)	127.1(10%)	184.7(15%)	359.8(29%)	1267.2	1260.8
	Eop Filtrate	897.9(30%)	272.4(9%)	384.7(13%)	1185.9(40%)	2732.8	3000.3
TOC (mg/L)	ASB Effluent	52.1(21%)	35.8(15%)	46.3(19%)	113.4(46%)	247.6	246.7
	ASB Influent	195.8(44%)	55.9(12%)	76.6(17%)	137.1(31%)	465.3	444.0
	Eop Filtrate	376.3(30%)	106.7(9%)	148.5(12%)	444.7(36%)	1076.2	1239.8
BOD_5 (mg/L)	ASB Effluent	12.2(95%)	3.8(29%)	4.2(33%)	6.6(51%)	26.8	12.9
	ASB Influent	263.1(79%)	25.6(8%)	34.4(10%)	18.2(5%)	341.3	333.9
	Eop Filtrate	421.8(80%)	25.5(5%)	20.3(4%)	37.3(7%)	504.9	524.0

Note: 1. TOTAL is the sum of parameter value from all components; 3. DIFF is the difference between the Raw and TOTAL;
2. RAW is the analysis results of Raw effluent used in fractionation study; 5. Number in bracket is the percentage of that component in raw effluent;
4. ASB influent and effluent were sampled at April 20, 1993.

Furthermore, these results also suggested that 1) there is a considerable difference in C.I. between F1 and F2, F3 or F4, and relatively small difference among the components with 1000<MWCO<10,000; 2) the component with MW>1000 may exert biochemical oxygen demand with a slower rate which was also demonstrated in a previous study (Mao and Smith, 1994a and 1995a).

Table 5-4. Comparison of Composition of Raw Combined Mill Effluent (ASB Influent) with That of Biologically Treated Effluent (ASB Effluent)

FRAGMENTS	TOC/TOC_{raw}		COD/COD_{raw}		BOD_5/BOD_{5raw}	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
Raw	100%	56%	100%	51.9%	100%	3.9%
MWCO<1,000	44%	11.7%	47%	10.5%	79%	3.7%
1,000<MWCO<5,000	12%	8.1%	10%	6.7%	8%	1.1%
5,000<MWCO<10,000	17%	10.4%	15%	8.8%	10%	1.3%
MWCO>10,000	31%	25.5%	29%	24.3%	5%	2.0%

Note: TOC_{raw} = TOC of Raw influent; TOC = TOC of various components; COD_{raw} = COD of Raw influent; COD = COD of various component; BOD_{5raw} = BOD_5 of raw influent; BOD_5 = B_{OD5} of various components.

In summary, these results suggested that 1) the stoichiometry and kinetics of the biochemical reactions involved in BOD development are remarkably influenced by both the nature and compositions of the wastewater even under the same conditions. Thus, the BOD_5, even BOD_{20} of pulp mill effluents containing lignin components only represented some stochiometric aspect of the parameter; certainly a better parameter including both stochiometry and knetics is desired for assessing and comparing the biodegradability in most cases; 2) the ASB effluent contained a very low concentration of biodegradable component and relatively high concentration of biologically resistant lignin components; E_{op} filtrate and ASB influent had substantially different characteristics with respect to those parameters listed in Tables 5-1, 5-2 and 5-3. Thus, ASB effluent could serve a basis for this evaluation; and E_{op} filtrate and ASB influent can be used to verify the reliability and applicability of BPI.

Table 5-5 shows the estimated values, standard errors (A.S.E.), and 95% confidence intervals of the constants in Equation (6). The parameters used for estimation were summarized in Table 5-6. The estimations were based on the results from series of oxygen uptake tests of the desired samples over 100 days, and the assumptions that BPI of ASB effluent is about 5 and BPI of GG solution is about 95. These results indicated that with the relatively limited test results both the estimated standard errors and 95% confidence intervals were reasonable and reliable. Thus, the BPI procedures and TBOU tests can be satisfactorily used to estimate the constants in Equation (6), and the estimated constants could be used for estimation of BPI of pulp mill

effluents. It is also important to recognize that the BPI considers not only the stoichiometry of biochemical reactions but also the kineties of biodegradation of complex organics in wastewater.

Table 5-5. Estimated Value and 95% Confidence Interval of Constants in Equation (5-6)

Parameter	Estimate	A.S.E.	95% Confidence Interval	
			Lower	Upper
C	99.5	2.65	91.1	107.9
a	0.155	0.0475	0.0041	0.306

Table 5-6. Parameters for Estimation of Constants in Equation (5-6) and Estimated BPIs

Sample	α	β	L	λ	BPI	Estimated BPI
GG solution -1	0.904	0.582	1.34	0.961	95	90.6
GG solution -2	1.094	1.410	1.06	0.915	95	98.3
GG solution -3	1.01	1.04	1.13	0.938	95	95.8
ASB effluent-1	0.0813	0.146	1.18	0.165	5	8.5
ASB effluent-2	0.0666	0.157	0.44	0.202	5	8.7
E_{op} Filtrate	0.438	0.681	2.646	0.313	N/A	30.0
ASB Influent-1	0.499	0.863	1.904	0.499	N/A	48.1
ASB Influent-2	0.506	1.041	4.011	0.497	N/A	55.5

Note: ASB influent and effluent were sampled at two different time.

Table 5-6 also compares the estimated BPIs of various raw pulp mill effluents based on equation (5-6). According to the numerical values of BPIs in Table 5-6 the biodegradable potential of each organic mixture was obvious in the order of GG solution >> ASB Influent >> E_{op} filtrate >> ASB effluent. This assessment is largely in agreement with those observations reported in various treatability studies (Stern, 1974; Smith and Mohammed, 1992; MacDonald and Radermacher, 1993; Mao and Smith, 1995a). Consequently, it is reasonable to state that the

parameter BPI is a simple but systematic engineering dimensionless index to reliably assess the biodegradable potential of pulp mill effluents which represented one of most complex industrial wastewaters.

5.3.2 Influence of Foam Separation on BP, Color and Other Characteristics of ASB Influent

Figure 5-3 shows the kinetic development of the BOD of the raw ASB influent, foam and defoamed components over 100 days. The major characteristics of the respective components were compared in Table 5-7. The results in Figure 5-3 and Table 5-7 revealed that the defoamed component had less than 5% lower color, COD and TOC than the raw ASB influent; but more than 19% and 11% lower BOD_5 and ultimate biochemical oxygen demand (UBOD) than the raw ASB influent, respectively. By sharp contrast, the foam component had about 10%, 6%, 17% higher color, COD and UBOD than the raw ASB influent, respectively. However, its BOD_5 and TOC were almost the same as those of the raw ASB influent. In addition, the kinetic development of the defoamed component differed considerably from the pattern of either the foam or raw ASB influent. Again the difference among either TBOU/UBOD or TBOU/COD ratio varied notably with the incubation periods. This also confirms that the biodegradability evaluation strongly depended on the period selected for TBOU.

On the other hand, the BPI appeared to be the solution to the problems. As shown in Table 5-7, the BPI of either foam fragment or defoamed fragment was more than 24% lower than that of raw ASB influent, but the BPI of the defoamed fragment was about 5% higher than that of the foam fragment. Thus, the BPI predicted that the foam separation substantially reduced the biodegradable potentials of the organic mixture and the defoamed component had slightly higher biodegradible potential than foam component. These predictions seemed to agree with the observations reported in previous treatability studies (NCASI, 1964; Ng, *et al.* 1973; Ng, *et al.* 1976). The reasons responsible for these effects were probably the combination of concentrating and partitioning. Since some toxicants usually had various interactions with each

other when they presented in the mixture, such as synergistic vs antagonistic effects, and the level of interactions was strongly dependent on the concentration and species.

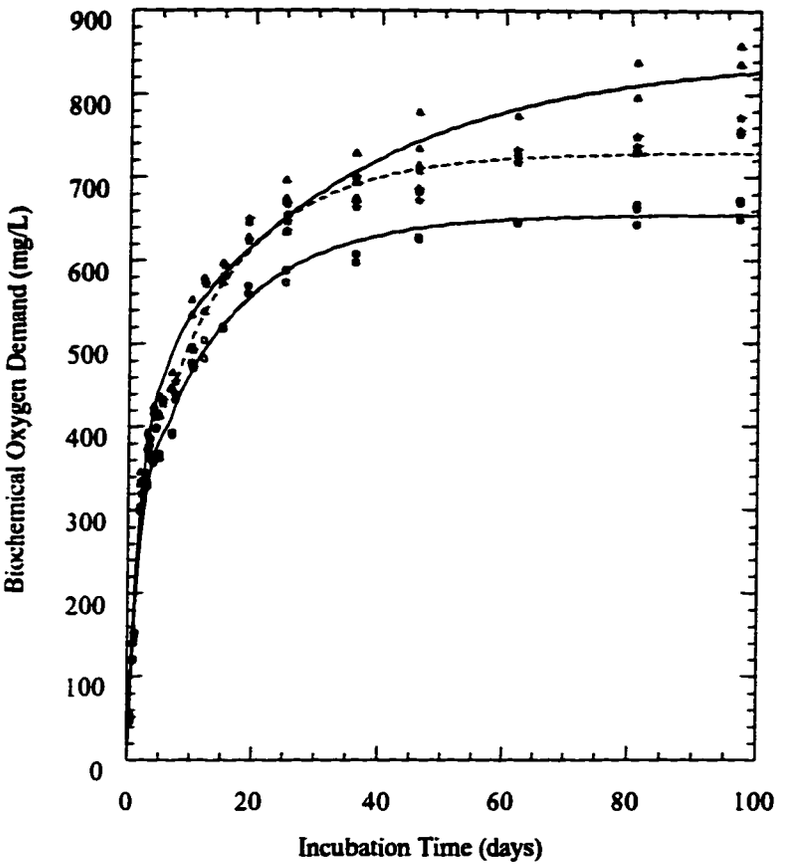

Figure 5-3. Comparison of Kinetic Development Patterns of BOD by Raw ASB Influent with Those by Foam and Defoamed Components over 100 Days
(Triangle: Foam Component; Star: Raw ASB Influent; Circle: Defoamed Component)

In conclusion, 1) due to the effects of surfactants on partitioning and concentrating the foam separation process partitioned the compounds in raw ASB influent into two general characteristic groups; the foam component may contain more hydrophobic organics with higher C.I. than the defoamed component; 2) the biodegradable potential of the organic mixture in the defoamed component was higher than that of the foam component, but much lower than that of raw ASB influent. Therefore, the results from ozone decolorization of the defoamed component would be more conservative for comparison with raw ASB influent.

Table 5-7. Characteristics of Raw, Foam and Defoamed Components of Combined Mill Effluent (ASB Influent)

Parameter	Raw ASB Influent	Foam Component	Defoamed Component
Color (C.U.)	1569	1720	1517
COD (mg/L)	1439	1516	1392
TOC (mg/L)	495	509	485
BOD_5 (mg/L)	432	456	363
Ultimate Oxygen Demand (mg/L)	729	847	655
BOD_5/UBOD	0.593	0.538	0.554
UBOD/COD	0.507	0.559	0.471
BOD_5/COD	0.300	0.301	0.261
BPI	56.5	43.3	45.3

5.3.3 Effects of Biodegradable Components on Efficacy of Ozone Decolorization

Table 5-8 summarizes different effects of ozone decolorization on three pulp mill effluents at two representative decolorization levels. Figures 5-4 and 5-5 compare the residual color, ozone doses, and efficacy of ozone decolorization of three pulp mill effluents.

These results demonstrated that both the decolorization level and biodegradable components expressed as BOD_5 had profound effects on the efficacy of ozone decolorization; the higher the concentration of the biodegradable components presented in the effluents the lower the ozone decolorization, efficiency was. As shown in Table 5-8 and Figures 5-4 and 5-5, the efficiency of decolorization (evaluated using color reduction in CPPA color unit per mg O_3 consumed) was decreased by 82%, 30% and 18% for ASB effluent, influent, and E_{op} filtrate, respectively, when the decolorization level increased from 35% to 75%. More importantly, the efficiency of decolorization of ASB effluent was about 98% higher than that of ASB influent at the 35% decolorization level, and about 146% higher than that of E_{op} filtrate at 35% decolorization level, respectively. On the other hand, the efficiency of decolorization of E_{op} filtrate at 35% level was more than 35% lower than that of ASB effluent at 75% decolorization, and very close to that for ASB influent at 75% decolorization, respectively.

Table 5-8. Comparison of Efficiency of Decolorization at 35% and 75% Decolorization Levels

Level of Colour Reduction	Related Parameters	Wastewater		
		ASB Effluent	ASB Influent	E_{op} Filtrate
Raw Wastewater	Color	1440 to 1480	1480 to 1650	3000 to 3100
	COD (mg/L)	810	1439	3058
	BOD_5 (mg/L)	26.5	366 (441)	540
	C.I. (C.U./BOD_5)	55.1	3.86	5.65
	C.I. (C.U./TOC)	5.29	3.15	2.35
	BPI	8.5	55.5	30.0
35% Color Reduction	Consumed Ozone Dose	50	95	250
	C.U./mg Ozone*	11.1	5.6	4.5
	$BOD_5/BOD_{5,ww}$	1.12	0.92	1.08
75% Color Reduction	Consumed Ozone Dose	180	270	615
	C.U./mg Ozone	6.1	4.3	3.8
	$BOD_5/BOD_{5,ww}$	2.78	0.97	1.26

* CPPA color units/mg consumed ozone.

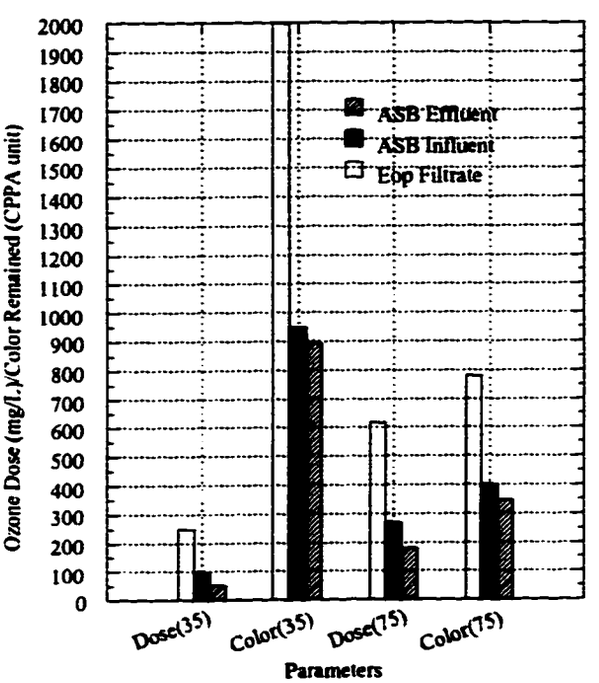

Figure 5-4. Comparison of Consumed Ozone Dose and Residual Color in Decolorization of E_{op} Filtrate, ASB Influent and Effluent at 35% and 75% Decolorization Levels (Dose(35)= ozone dose required for 35% decolorization; Color(35)= residual color after 35% decolorization; Dose(75)= ozone dose required for 75% decolorization; Color(75)= residual color after 75% decolorization)

These observations seemed to correlate well with BPI. As shown in Tables 5-1, 5-2 and 5-7, both the C.I., which is an intrinsic characteristic of color-causing lignin derivatives (Mao and Smith, 1994a), and the ratio of C.I. of the component with MWCO>10,000 to C.I. of the component with 1000<MWCO<10,000 inversely correlated with the biodegradable potential (BPI). Moreover, the efficiency of decolorization was also inversely correlated to BPI. Specifically, the higher the BPI of a pulp mill effluent had, the lower the C.I. is, and the lower the efficiency of ozone decolorization is.

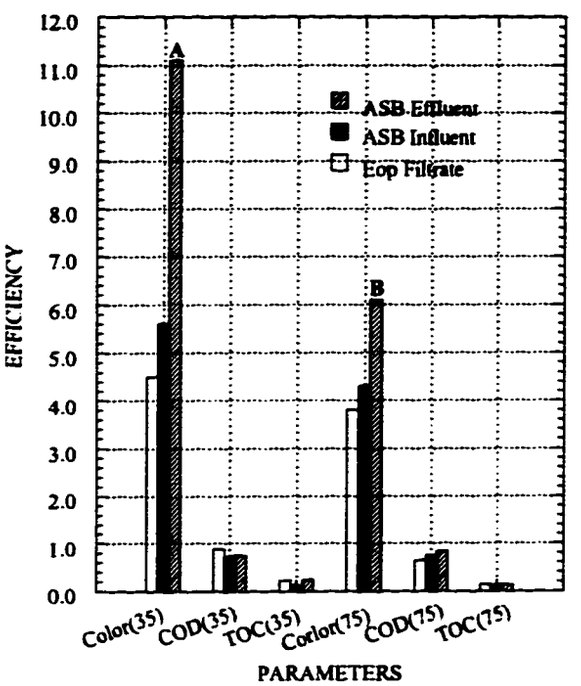

Figure 5-5. Comparison of Efficiency of Ozone Decolorization on Various Pulp Mill Effluents at 35% and 75% Decolorization Levels

5.3.4 Effects of Ozone Decolorization on Biodegradable Potential of Pulp Mill Effluents

Figure 5-6 compares the ratios of different BODs of treated to raw pulp mill effluents at the two levels of ozone decolorization. The results revealed that after 75% decolorization, 1) for ozone decolorization of ASB influent, there was more than 10% decrease in the total amount of biodegradable components (as BOD_7); 2) by contrast, after ozone treatment of both ASB effluent

and E_{op} filtrate, the amount of biodegradable components was increased; 3) at a similar ozone dose level, the increase in the ratio BOD_7/BOD_{7raw} was maximized, by contrast, the decrease in BOD_7/BOD_{7raw} of ASB influent was the highest. This suggested that the ozonation had different effects on different types and MW organics. However, as shown in Figure 5-6, the level of increase in biodegradable components differed dramatically between E_{op} filtrate and ASB effluent. For E_{op} filtrate, the increase was almost marginal; on the other hand, there was more than 150% increase in biodegradable components (as BOD_5) in ASB effluent at 75% ozone decolorization level. Moreover, as also shown in Figure 5-6, at 75% decolorization level, the increase of biodegradable organics in the treated E_{op} filtrate was substantially greater than that at 35% decolorization level.

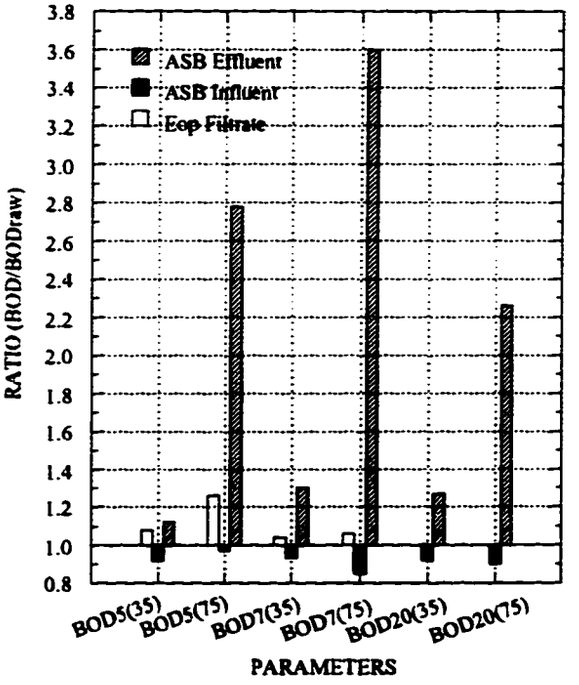

Figure 5-6. Effects of Ozone Decolorization on Biodegradable Organics in Various Pulp Mill Effluents (numbers in the bracket indicate the level of decolorization)

Figure 5-7 further compares the effects of ozone decolorization on the generation of biodegradable organics in the ASB effluent at different ozone dose levels. It revealed that the amount of ozone required to produce one mg/L of BOD was dramatically decreased when ozone dose increased from 50 mg/L to about 80 mg/L, reached minimum at the optimum dose, then, increased gradually after optimum dose up to 250 mg/L. The similar tendency was observed but less profound for ozone decolorization of E_{op} filtrate. It is also noted that at the same dose level, the required ozone dose for producing each mg/L of BOD was decreased with increasing the incubation time of BODs. That is, the ozone required for producing 1.0 mg/L of BOD_{20} was less than that for producing 1.0 mg/L of BOD_5. Again, these agreed well with the assessment using BPI. For instance, the ASB effluent, experienced a greater increase in the biodegradable organics at all tested ozone dose levels, which had very low BPI but high C.I. The E_{op} filtrate, which had relatively high BPI and low C.I., had a relatively smaller increase compared to ASB effluent. By contrast, ASB influent, which had very high BPI and low C.I., had a significant decrease in biodegradable organics at the tested ozone dose levels (at 5% level). By contrast, there was little correlation among BPI, TOC and COD removal. Also, as shown in Figure 5-5, ozone was ineffective in removal of TOC and COD in pulp mill effluents compared with other physical, chemical and conventional biological processes (Mao and Smith, 1994a and 1995a). In other words, ozonation should not be considered as an effective process for TOC and COD removal from pulp mill wastewaters.

In summary, the change of biodegradable organics (expressed as BOD_5, BOD_7 or BOD_{20}) during ozone decolorization was strongly related to both BPI and C.I. of the effluents as well as ozone dose levels, but much less effected by either the total quantity of biodegradable organics as BOD_5 or TOC. It also seemed that there existed optimum doses at which the ozone decolorization efficiency or the effects on the biodegradable potential were maximized.

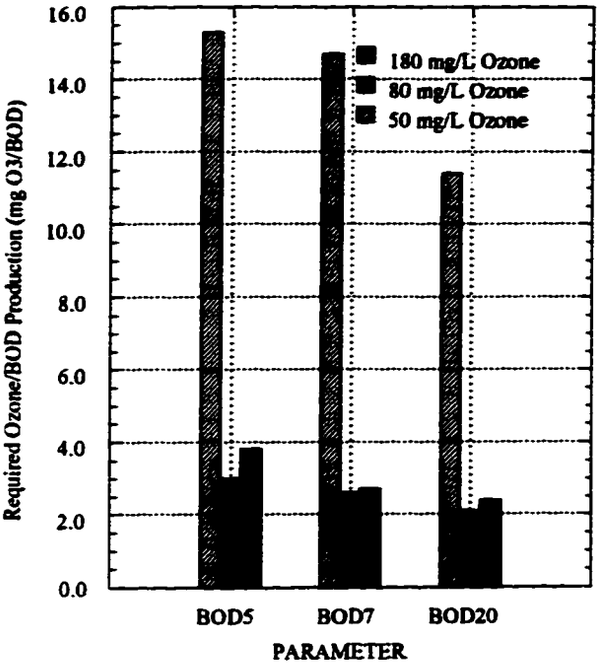

Figure 5-6. Comparison of Required Ozone for Producing One mg/L of BODs at Different Ozone Dose Levels (ASB Effluent)

5.3.5 Impacts of Ozone on Individual Lignin Components and Correlation of BP and BPI with Ozone Decolorization

Figure 5-7 qualitatively compares the kinetic development of BOD from the ozone treated samples at a dose level of about 250 mg/L with that of the raw defoamed component over 100 days. The fitted lines in this and following figures were based on the mechanistic model. Figure 5-7 further demonstrates that the organics in the raw ASB influent, which were biodegraded before 55 days, were reduced notably after ozone decolorization at the dose level of 250 mg/L. However, ozone decolorization increased the total quantity of the component which could be only degraded by the acclimatized microbial consortia over a longer period. These are consistent with the observations in above sections and a previous study (Mao and Smith, 1995a).

To quantitatively and comparatively evaluate these changes induced by ozone decolorization the biodegradation kinetic parameters were estimated using the results from the long-term biochemical oxygen uptake tests and the mechanistic model (Equation (5-1)). BPIs of all samples were calculated based on Equation (5-6). The results of these estimations were compiled in Table 5-8 along with the corrected R^2.

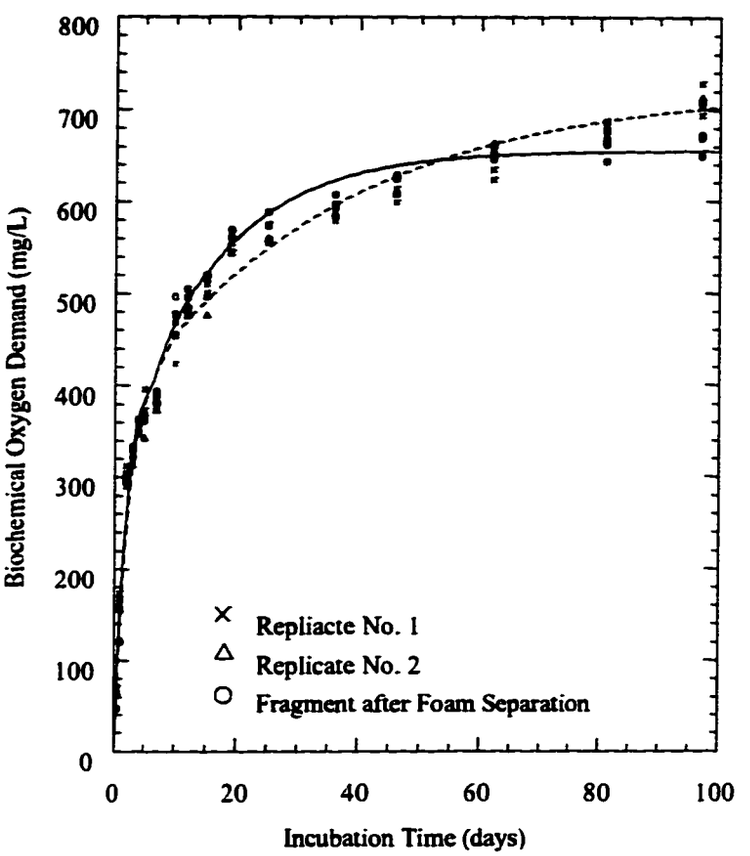

Figure 5-7. Comparison of Kinetic Development Patterns of BOD by Raw with that by Ozone Treated Defoamed Component (two replicates, ASB influent) over 100 Days

As shown in Table 5-8, after ozone decolorization of the defoamed components, the k_1 and k_3 decreased by more than 21%, 57%, respectively, although the total quantity of biodegradable components increased by more than 10%.

Table 5-8. Comparison of Biodegradation Kinetic Constants of Raw, Ozone Treated and Their Components of Pulp Mill Effluents

Sample	Estimated Parameters						
	UBOD	k_1 or K	k_2	k_3	T	corrected R^2	BPI
Defoamed Fragment	655	0.322	0.185	0.067	6.3		45.3
Ozone Treated (245 mg/L of O_3, NR-1)	723	0.260	0.181	0.0283	6.1		45.9
Ozone Treated (268 mg/L of O_3, NR-2)	718	0.252	0.183	0.0287	5.5		45.1
Raw Effluent	127.7	0.034	0.154	0.021	18.5	0.997	5.5
Ozone Treated (80 mg/L of O_3)	153.9	0.108	0.351	0.023	18.8	0.996	10.1
Raw F1	49.9	0.04				0.982	
Ozone Treated F1	72.2	0.099				0.987	
Raw F2	18.7	0.028				0.982	
Ozone Treated F2	20.4	0.045				0.988	
Raw F3	17.3	0.026				0.989	
Ozone Treated F3	22.4	0.034				0.983	
Raw F4	58.1	0.014		0.019*		0.993	
Ozone Treated F4	44.7	0.027		0.029*		0.988	
Ozone Treated (50 mg/L of O_3)	132	0.0996	0.335	0.027			8.2
Ozone Treated (180 mg/L of O_3)	272	0.0747	0.0639	0.0147			20.2

UBOD = Ultimate Biochemical Oxygen Demand; K= estimated kinetic constant from simple first order BOD kinetic model; F1= MWCO<1,000 dalton; F2=1,000<MWCO<5,000 dalton; F3=5,000<MWCO<10,000 dalton; F4 = MWCO>10,000 dalton. *: TOC-aveaged K values from F2, F3 and F4.

Obviously the above assessment by simply using all those estimated kinetic constants were semi-quantitative at most since it is still very difficulty to systematically compare or assess the degree of ozone effects on biodegradability. By contrast, the dimensionless BPI seemed to provide a reliable scale for this evaluation. As shown in Table 5-8, BPI of two replicates of ozone treated defoamed component of ASB influent were very close, and there was little difference between the BPIs of raw and treated ASB influent. Consequently, it can be quantitatively stated that there was insignificant difference in the biodegradable potential between raw and ozone treated defoamed fragments at 5% significance level. In other words, the biodegradable potential remained almost the same after ozone treatment at about 250 mg/L although the type and quantity of biodegradable components were altered substantially, and both samples had about 45% level of biodegradable potential compared to 300 mg/L GG solution.

Figure 5-8 compares the development patterns of the long-term biochemical oxygen demand of raw and ozone treated ASB effluents at the dose level of about 80 mg/L. Figures 5-9, 5-10, and 5-11 further compare the development patterns of the long-term biochemical oxygen demand of the individual components from these samples. The parameters of the individual components were estimated based on the simple first-order BOD model

($BOD = BOD_u(1 - e^{-kt})$) since the earlier study (Mao and Smith, 1995a) and Figures 5-9, 5-10, and 5-11 have demonstrated that they could fit the simple first-order model satisfactorily. The estimates were also summarized in Table 5-8.

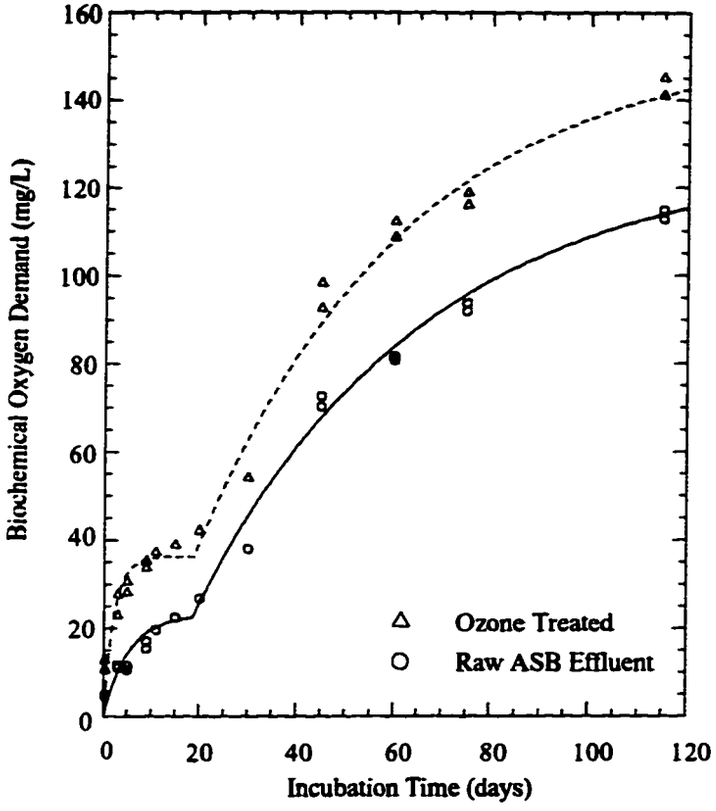

Figure 5-8. Comparison of Kinetic Development Pattern of Long-term BOD by Raw with That by Ozone Treated ASB Effluents (about 80 mg/L) over 120 days

The results in Figure 5-8 demonstrated, again qualitatively, that ozone decolorization appeared to greatly improve the biodegradability of ASB effluent over the entire incubation period. For example, 5-day BOD of ozone treated samples increased by 180% compared to 5-day BOD of raw ASB effluent. It is also evident, as shown in Figures 5-9 through 5-11, that the ozone decolorization had considerable impacts on a full spectra of organics in ASB effluent. Specifically, the high MW portion of lignin components, which initially was biologically

resistant, was largely converted into lower MW portions, and the majority of them belonged to component F1 which was much more biodegradable.

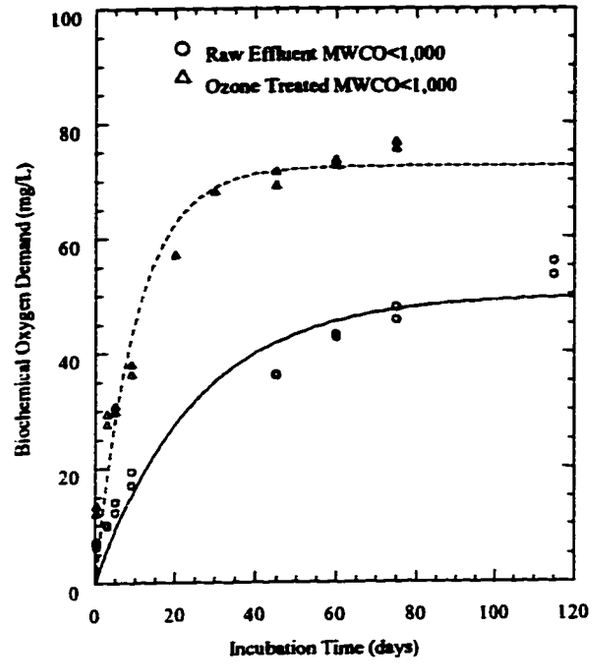

Figure 5-9. Comparison of Kinetic Development Pattern of Long-term BOD by Components with MWCO<1,000 from Raw with That from Ozone Treated ASB Effluents (about 80 mg/L)

The results in Table 5-8 demonstrated that after ozone decolorization, the UBOD in F4 was reduced by 23% whereas the biodegradation rate increased by about 100%; by contrast, both biodegradation rate and UBOD in F1 increased by about 150% and 45%, respectively. Similarly, both biodegradation rate and UBOD in F2 and F3 were improved after ozone decolorization. Nevertheless, the improvements were less profound.

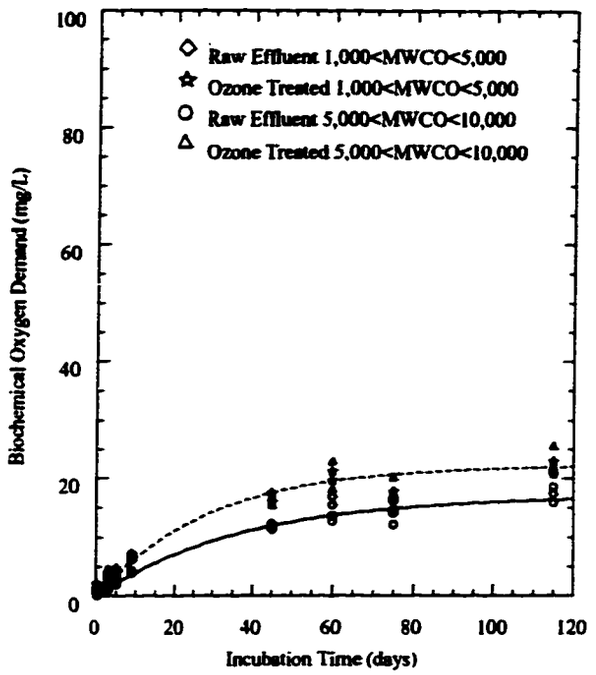

Figure 5-10. Comparison of Development Pattern of Long-term BOD by Components with 1,000<MWCO<5,000 and Components with 5,000<MWCO <10,000 from Raw with those from Ozone Treated ASB Effluents (about 80 mg/L) over 120 Days

As also seen in Table 5-8, kinetic constants of both F1s were very close to the respective k_1s estimated from the whole samples. The TOC-averaged kinetic constants of the components F2, F3, and F4 from raw ASB effluent were also very close to the k_3s of their counterparts. In addition, the kinetic constants of F2, F3, and F4 decreased in the order of F4<F3<F2 but the differences among them were close to the error level in the estimation. Furthermore, TOC-averaged kinetic constants of F2, F3, F4 from NR-1 and NR-2 were about 50% higher than the respective k_3 from raw ASB effluent; and k_2 of NR-1 and NR-2 were about twice as high as that of raw ASB effluent.

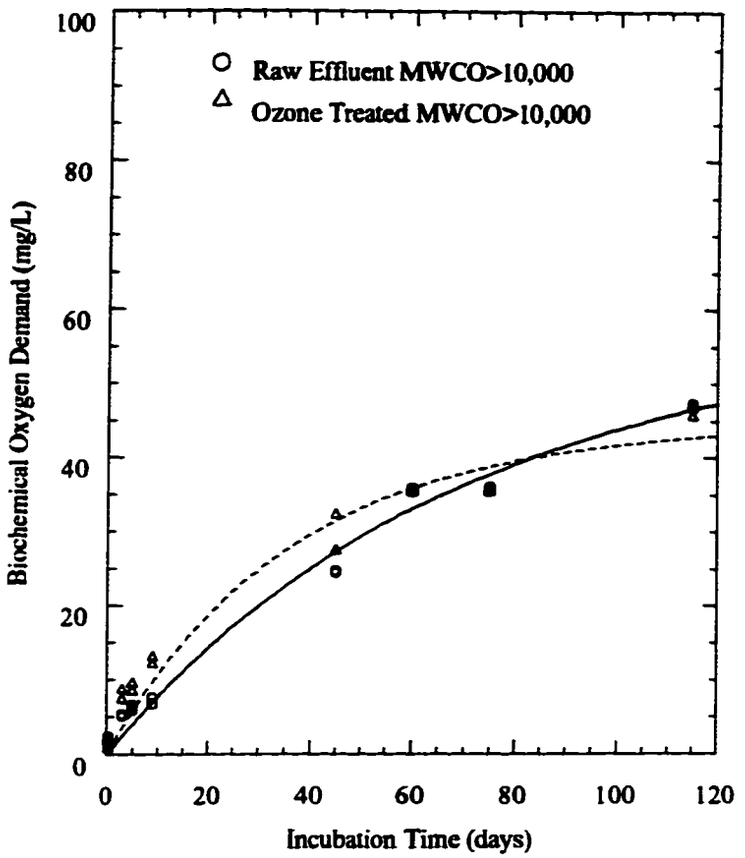

Figure 5-11. Comparison of Development Pattern of Long-term BOD by Components with MWCO>10,000 from Raw with That from Ozone Treated ASB Effluents Over 120 Days

It is also important to note that unlike COD, TOC, and true color, the distribution of BOD among the components in both raw and ozone treated pulp mill effluents was extremely skewed to the low MW components (F1). In particular, the component F1 contributed almost 50% BOD to the whole samples, and accounted for the major increase in the total BOD after ozone decolorization; in addition, the BOD levels in the components F2, F3, and F4 in treated samples appeared to be proportional to their TOC levels although they were relatively low. These results confirmed that the biodegradable potential of all components from ASB effluent was greatly improved after ozone decolorization at the dose level of about 80 mg/L.

Figure 5-12 systematically examines the effects of ozone decolorization with different dose levels on the kinetic development of the BOD of ASB effluent over 100 days. It is evident that biodegradable potential increased considerably with increasing ozone dose but the degree of improvement strongly depended on ozone dose and the development period; all the kinetic development patterns appeared to be two stage as described by Mao and Smith (1995a). The degree of kinetic development and length of each stage varied with the ozone dose levels.

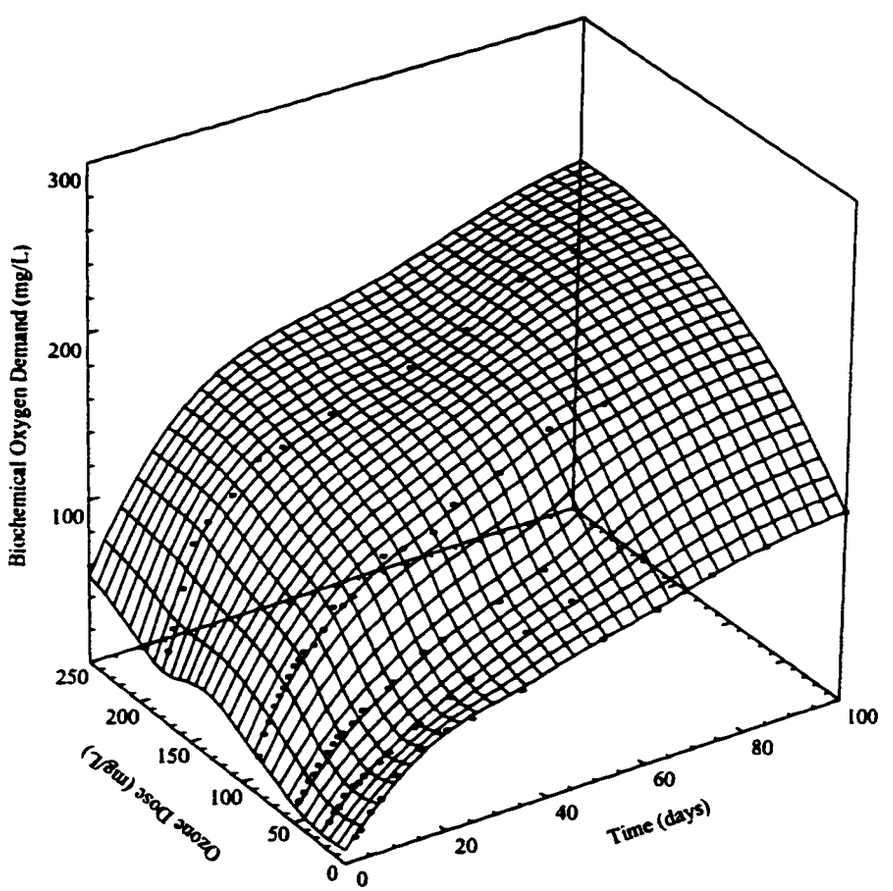

Figure 5-12. Effects of Ozone Decolorization at Various Ozone Dose Levels on Development Patterns of Long-term Biochemical Oxygen Demand of Biologically Treated Pulp Mill Effluents (ASB Effluent) over 100 Days

With respect to the biodegradation process, the microorganisms involved in stage I in the acclimatized microbial consortia became dominant with the support of the favorable substrates

including newly formed components. When these substrates were getting exhausted, the dominant microbial consortia was forced to shift to those organisms which could use the less favorable substrate involved in stage II. At low ozone dose levels, the concentration of the biodegradable substrate was relatively low and the difference between two categories of substrates was small, so the shifting time was relatively short, and the transition mode was relatively smooth. As the ozone dose increased the shifting time for these species became longer since the concentrations of the substrates involved in stage I was higher, and the difference between them was becoming larger.

In addition, because of the high oxidation power of ozone and the polydisperse nature of lignin derivatives would also allow the ozone reactions to produce heterogeneous reaction products with various functional structures during ozone decolorization. Thus, there was a great likelihood of producing some functional structures which were able to serve as inducers of certain enzymes in the microbial consortia under appropriate conditions for the metabolization of variety of substrates. This may also contributed to the increase of BPI.

Quantitatively, Figure 5-13 and Table 5-8 show that with respect to ozone decolorization of ASB effluent, the BPI increased linearly with increasing ozone dose with the slope to be 0.84 BPI/(10 mg/L) consumed ozone. However, it can be expected that this relationship would vary with the characteristics of wastewater.

In summary, with respect to the effects on biodegradable potential of the effluents, ozone decolorization was a dynamic process. Some of the biodegradable components originally present in the wastewater, if in high concentrations, may be significantly deteriorated in both quantity and quality at certain ozone dose levels. On the other hand, if in low concentrations ozone decolorization would convert some lignin components to more biodegradable components. The amount of newly formed biodegradable components was strongly dependent on characteristics of raw wastewater and the ozone dose level. In particular, the high MW lignin components such as F4, F3 and F2 appeared to be converted to the smaller ones such as F1 and F2 which were more

biodegradable. Thus, F4s, F3s, F2s in NR-1 and NR-2 (after ozone decolorization) did not follow the same distribution as found in raw ASB effluent. All examined characteristics were also significantly modified. Some of the original molecules of F2 and F3 components in raw ASB effluent may have also been transformed into F1 component, and at the same time some of the new F2 and F3 components may have been generated in the ozone reactions with the larger molecules in lignin components. However, some chromophoric structures located at the short branch of the lignin molecules in the original F4 and F3 may also be directly converted to F1s with similar characteristics of the original F1s.

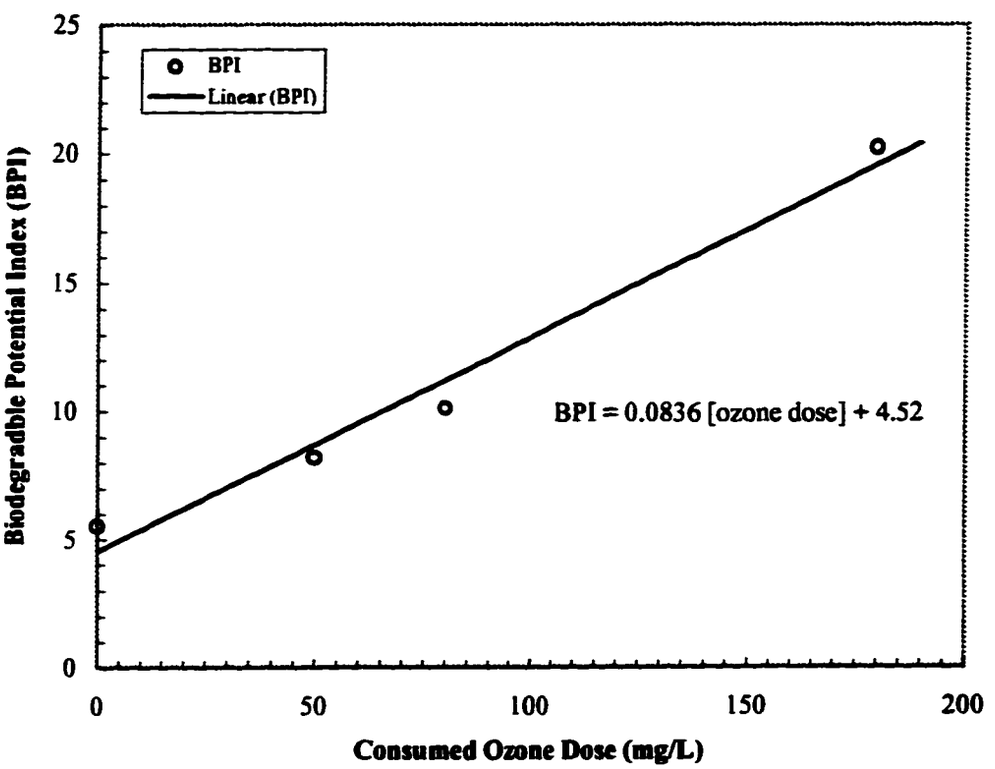

Figure 5-13. Correlation of BPI with Consumed Ozone Dose in Ozone Decolorization of ASB Effluent

5.4 CONCLUSIONS

Ozone decolorization was a dynamic process in removing and producing a wide spectrum of biodegradable components in pulp mill effluents. When the BPI was low and C.I. was high the amount of newly formed biodegradable components was much greater than those destroyed by ozone reactions. As a result, the biodegradable potential improved, which was indicated by BPI increase. On the other hand, the amount of newly formed biodegradable components was cancelled out as reflected with the unchanged or decreased BPI. With respect to ozone decolorization of ASB effluent, the BPI increased one unit with every 12 mg/L consumed ozone dose ranging from 0 to 200 mg/L.

The experimental evidence also suggested that 1) the biological process would be a proper pretreatment process for improving the efficacy of ozone decolorization of pulp mill effluents, 2) the dimensionless BPI could be a satisfactory engineering means for evaluation of the biodegradable potentials of the pulp mill effluents induced by ozone treatment and foam separation and, 3) in pulp mill effluents, F1 contributed the majority of BPI but little of C.I. By contrust, F4 was determinent factor in C.I. but little influence on BPI.

Foam separation substantially reduced the BP and tended to partition the organics by their hydrophilicity and toxicity. The defoamed component, which contained more hydrophilic components from pulp mill effluents, had slighly lower C.I. but higher BPI.

5.5 ACKNOWLEDGMENTS

This study was partially financed by Weyerhaeuser Canada Inc. The authors greatly appreciate the assistance of Mr. Steve Cooper, Environmental Manager, Grande Prairie Operations' Mill, Weyerhaeuser Canada Inc. for providing representative effluent samples. Thanks are also extended to Mr. Murray Carmichael for the help with the ozone study and some analyses. Authors also greatly appreaciate Dr. Y. Xie for his valuable comments and careful review of the manuscript.

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CHAPTER 6. TOWARD ELUCIDATING MECHANISM AND KINETICS OF OZONE DECOLORIZATION AND DECHLORINATION OF PULP MILL EFFLUENTS*

6.1 INTRODUCTION

The effluents from kraft pulp mills usually contain a large amount of poorly-biodegradable lignin derivatives which contribute a brownish color and chlorinated organics (Mao and Smith, 1994a).

The application of ozone to improve the biodegradability and the decolorization of pulp mill effluents has been studied for several decades. The previous studies (Stern, 1974; Patton, 1979; Roy-Arcand, 1991; Sazanska and Sazanska, 1989; Mao and Smith, 1995a) revealed that a ozone decolorization process is composed of three characteristic phases. In the first phase the rate of decolorization is high and the color reduction is linear with the ozone dose; in the second transit phase the relationship of ozone consumption and decolorization varies with the operating conditions; in the third phase the rate of decolorization is considerably slower and strongly depends on the ozonation conditions. The inflection point between the high and slow rate phases is at about 80% of decolorization. The recent studies (Sozañska and Sozañski, 1989; Mao and Smith, 1995a and 1994b) further demonstrated that the above observations are more applicable to the biologically treated pulp mill effluent than the raw bleachery effluent or combined pulp mill effluents. Moreover, it was found that at a low ozone addition rate (O.A.R.) or at a low ozone dose level the efficiencies of both decolorization and the overall utilization of the applied ozone can be improved (Amero, *et al.*, 1993; Mao and Smith, 1994b).

For optimizing the efficiency of ozone treatment, efforts have been made to understand the underlying mechanism and kinetics of these phenomena. Based on the color characteristics and relative reaction rates with ozone, the organic compounds in the pulp mill effluents are classified into three general characteristic groups: Group A reacts quickly with ozone, group B reacts relatively slowly and has to compete for ozone with group C which consists of non-colored low-molecular-weight biodegradable organics (Melnyk and Netzer 1975; Prat, *et al.*, 1989). The kinetic model based on this concept reasonably well predicts color reduction by ozone treatment.

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*A version of this chapter has been published. Mao, H.-Z. and Smith, D. W. (1995) Ozone Sci. Eng., 17, 419-448.

However, this approach cannot satisfactorily explain the mechanism and the different reaction kinetics in ozone decolorization and dechlorination processes.

Using a dialyses technique, Roy-Arcand and Archibald (1991) found that the low-molecular-weight organics are less colored, with 0.7 C.U./mg COD (15% of total color, 50% of total COD) as opposed to 4.0 C.U./mg COD (85% of total color, 50% of total COD). Gel permenation chromatography (GPC) analysis of these fragments revealed that 1) the distribution of the 280 nm absorbance in the raw effluent shows a multimode pattern with two peaks around 11,000 and 4,000 daltons; 2) ozonation seems uniformly to reduce the distribution of the 280 nm absorbance. Unfortunately, the UV detector at 280nm failed to detect the low-molecular-weight degradation products; also the authors did not monitor the absorbance shift within the whole range of wavelength after structural alternation. Based on a few data collected, it was concluded that ozone preferentially attacks the chromophoric structures and is more effective in destroying the chromophoric structure on smaller molecules at the same degree of decolorization (50% color removal).

Table 6-1. Comparison of Improvement of Color Intensity (C.U./mg TOC) of Pulp Mill Effluent by Ultrafiltration Separation with Conventional Biological Treatment

	Color Intensity (CPPA color unit/mg TOC)			
Sample	**E_{op} Filtrate**	**Influent**	**Effluent (a)**	**Effluent (b)**
RAW	2.41	3.22	5.63	4.08
F4 (MWCO>10,000)	3.83	6.01	7.8	
F3+F4(MWCO>5,000)	3.64	4.87	6.71	5.05
F2+F3+F4(MWCO>1,000)	3.43	4.26	6.02	

Note: E_{op}, Influent and Effluent (a) sampled in April 1993; Effluent (b) sampled in February, 1994; MWCO= Molecular Weight Cut Off

Color intensity (C.I.) appears to represent one of the intrinsic properties of pulp mill effluents with respect to decolorization and dechlorination. Here the C.I. is defined as the density of the chromophoric functional groups in the molecules of oxidized lignins and their derivatives in the pulp mill effluents, and is measured in either C.U./mg TOC (C.I./TOC) or C.U./mg COD (C.I./COD). Using a variety of physical, chemical and biological separation techniques Mao and Smith (1994a) found that 1) there is only one color intensity (C.I.) distribution in a given pulp

mill effluent; 2) the physical separation processes examined can not improve its C.I; instead, they could only separate the designated fragment which has the specific C.I. from the whole effluent. Table 6-1 is adopted from the above study. As demonstrated in Table 6-1, the C.I. of a fragment with MWCO>5,000 (F3+F4(b)) from effluent (b) had much lower C.I. than the same fragment from effluent (a) (since the effluent (b) was from the modified bleaching process); ultrafiltration can not increase the C.I. of F3+F4 (b) to the level of the C.I. of F3+F4(a) from effluent (a). Moreover, conventional biological treatment appeared to be almost as efficient as the ultrafiltration process with 10,000 MWCO membranes in the separation of low C.I. organics from the color-causing organics. For example, the C.I. of effluent (a) was very close to the fragment obtained from the ultrafiltration using MWCO>10,000 membrane in concentrating ASB influent to 5% of the original volume. On the other hand, ultrafiltration rarely achieved this level of C.I. by concentrating E_{op} filtrate regardless of what type of membrane was used.

In this study, we conducted a series of experiments to study the effects of ozone reactions on various portions of lignin derivatives in pulp mill effluents. The raw wastewater samples used in these experiments were obtained either directly from the pulp mill processes or the laboratory preparation using ultrafiltration and freeze drying techniques. The research objectives were to 1) develop a simple kinetic model; 2) determine the kinetic constants for modelling the color reduction using preozone treatment of pulp mill effluents; 3) elucidate the mechanism of ozone decolorization and dechlorination. The kinetic data obtained in this study further confirmed the concept of C.I., and the reported data in the literature seemed to support the new kinetic model. This study also found that ozone preferentially attacks chromophoric structure in oxidized lignin derivatives. These reactions greatly reduced the molecular size and halogen content and increased the biodegradability. The findings of this study could be helpful for optimizing the efficiency of ozone decolorization and dechlorination of pulp mill effluents.

6.2 MATERIALS AND METHODS

6.2.1 Sources of Wastewater

Two types of wastewater with different characteristics (see Table 6-2) were chosen to represent a variety of organics and their combinations in the pulp mill effluents. The grab samples of E_{op} filtrate from E_{op} stage of $DE_{op}D_1ED_2$ (see Table 6-2 footnote) bleaching sequence was used as Type I which represented high concentrations of poorly-biodegradable

organic material such as lignin fragments and high concentrations of relatively easily-biodegradable organics such as wood sugar and hemicellose. ASB effluent (sampled at the discharge point of ASB) was used as Type II which represented biologically treated secondary effluent.

Table 6-2. Summary of Characteristics of E_{op} Filtrate and ASB Effluent

Parameter		E_{op} Filtrate	ASB Effluent[5]
pH		9.7±0.5	7.1±0.5
BOD_5	(mg/L)	540 ± 15	12.2±1.5
TSS[1]	(mg/L)	55±5	70±4
TDS[2]	(mg/L)	4720±70	N/A
TDVS[3]	(mg/L)	2563±17	N/A
COD	(mg/L)	3058±44	585±25
TDOC[4]	(mg/L)	1287±23	
TOC	(mg/L)	1296±14	237.2
BOD_5/COD		0.177	0.021
COD/TDOC		2.38	2.47

Note: 1.TSS = Total Suspended Solids; 2. TDS = Total Dissolved Solids;
3. TDVS = Total Dissolved Volatile Solids; 4. TDOC = Total Dissolved Organic Carbon;
5. sampled at Ferurary. 1994.

6.2.2 Sample Preparation and Analysis

6.2.2.1 Sample Preparation

In this series of experiments the raw composite wastewater samples were prepared using the concentrated fragments from either E_{op} filtrate or ASB effluent (see Table 6-2). The concentrated fragments were produced using ultrafiltration fragmentation and freeze-drying techniques.

Sample Fractionation Using Ultrafiltration: The raw and treated E_{op} filtrate and ASB effluent were fractionated into four fragments using Millitan Acrylic Ultrafiltration System (Millipore Co.) equipped with 1,000, 5,000 and 10,000 molecular weight cutoff (MWCO) membranes. The details were described elsewhere (Mao and Smith, 1995b).

Concentrating Fragments for Composite Samples Using Freeze Drying: The desired effluent was first fragmented using 5,000 MWCO membrane according to the protocols described elsewhere (Mao and Smith, 1995b). After fragmentation, the filtrate was further

concentrated to the desired levels using a freeze drier. All the fragments were preserved at 4°C till experiment time.

Before the experiments, the raw composite samples were prepared such that the initial true color was close to the desired true color level while the initial color intensity was varied over a wide range using the proper fragments. The pH of the samples was adjusted to 7.5 ±0.2 (which is within the pH range of raw ASB effluent). These samples will be subsequently called as raw composite samples. Table 6-3 summarizes the major characteristics of these samples.

6.2.2.2 Sample Analysis

Unless stated otherwise, the procedures recommended in Standard Methods (APHA, 1992) were followed by considering the special characteristics of pulp mill effluents documented by TAPPI and CPPA. These included pH, total suspended solids (TSS), total dissolved solids (TDS), total volatile solids (TVS), COD, and TOC.

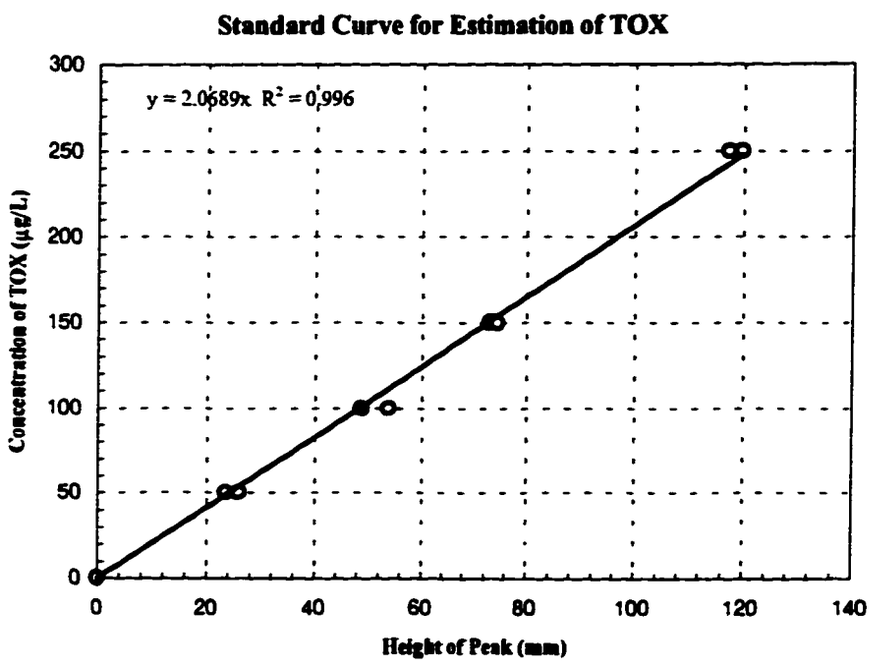

Figure 6-1. Standard Curve for Estimation of TOX in Pulp Mill Effluents

Total Organic Halogens: The total organic halogens (TOX) were extracted from the wastewater samples using microcolumn protocol recommended in Standard Methods and

analyzed using Euroglass AOX Analyzer. In each series of tests, the high or low concentrations of *p*-chlorophenol standards and the blank check on the adsorbed halogen level of GAC were carried out, and each sample was diluted with milli-Q water to the level within the range of the concentration in the standard curve. The low level suspended or colloidal particles remained in the sample since the samples were not pretreated before GAC adsorption. Thus, the results from these tests will subsequently be called as TOX. Figure 6-1 shows the TOX standard curve using *p*-chlorophenol as standard for the estimation of the TOX level in raw and treated pulp mill effluents.

UV Absorbance: The UV spectra of raw effluents were determined with a UV-Vis (Model HP8542A) using 10 mm quartz cuvette. All the samples for UV analysis were filtered through 0.8 μm non-absorbable filter (MSI micron separations Inc.) and pH was adjusted to 7.6 if desired.

True Color Determination: The Canadian Pulp and Paper Association (CPPA) standard method H5.P (CPPA, 1974) and NCASI method (NCASI, 1971) were incorporated for the determination of true colour with minor modifications (Mao and Smith, 1995a).

Molecular Weight Distribution: High performance size exclusion chromotograph (HPSEC) was employed to analyze the molecular weight distribution (MWD) of the raw effluents and their fragments. The HPSEC column (TSK Progel G-3000xl) was calibrated with the protein standards (Sigma Co.) and lignin reference standards. In this study, the resolution and reliability were further improved by using LiCl-tris buffer as eluent and eluting at a proper flow rate.

6.2.3 Ozone Treatment Studies

The protocols for ozone treatment studies were essentially identical to those described in previous studies (Mao and Smith, 1994b, 1995a, and 1995b).

6.3 RESULTS AND DISCUSSION

6.3.1 C.I. and MWD of Various Pulp Mill Effluents

The UV spectra and molecular weight distribution of the raw composite samples are compared in Figures 6-2 and 6-3, respectively. The other major characteristics of the raw composite samples are compiled in the first part of Table 6-3. In Figure 6-2 the UV spectra of all

raw samples (properly diluted) appeared to be quite similar to those spectra of kraft lignins reported earlier (Brauns, *et al.* 1960; Kleinert and Joyce, 1957a and 1957b; Saeknen and Ludwig, 1971). By examining the details of Figure 6-2, however, it was found that the UV spectrum of IIZ-05raw was the only UV spectrum which really resembles the characteristics of UV spectra of kraft lignins in the solution (Brauns, *et al.* 1960; Sarkanen, *et al.* 1971; Pearl, 1967). Even so, the absorbance around 280 nm was much weaker than those reported in the literature. These observations suggested that the characteristics of original lignins appeared to be largely changed in a bleaching process using 100% ClO_2 substitution. These findings agree well with those in the previous study (Mao and Smith, 1994a).

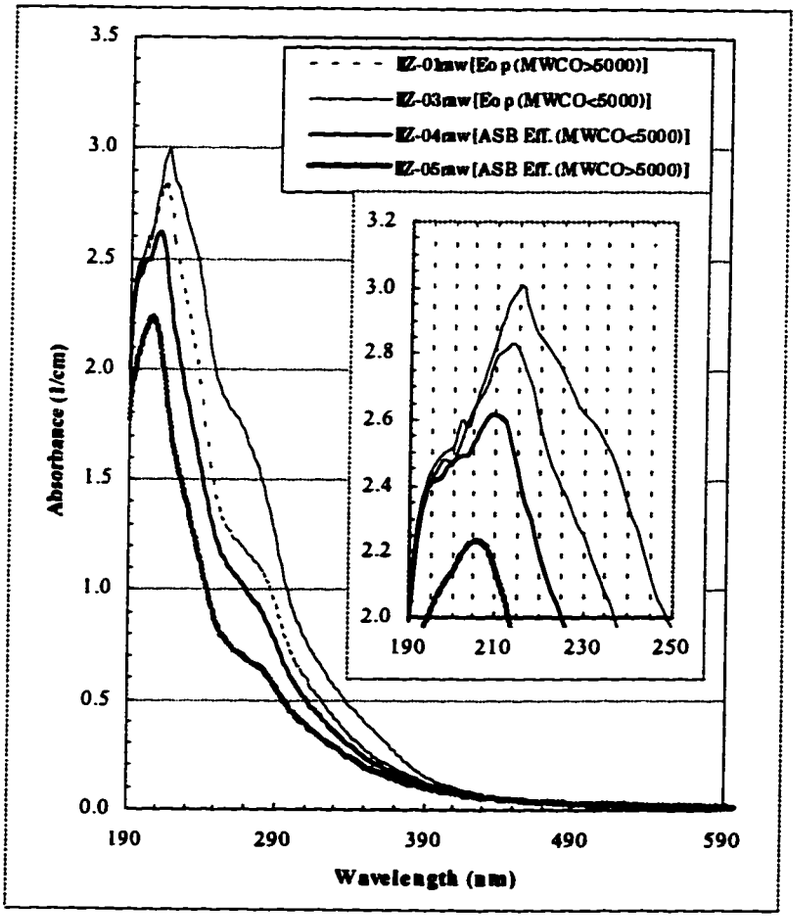

Figure 6-2. Comparison of UV Spectra of Raw Composite Samples Used in Ozone Decolorization and Dechlorination Studies (see Table 6-3 for description)

It has been reported that the Cl substitutions on the unsaturated bonds or aromatic rings usually lead to the red shift (Williams and Fleming, 1973). Moreover, low-molecular-weight organics with aromatic rings or conjugated, non-conjugated unsaturated bonds usually have absorbance maxima around 202 or 216 nm (Sarkanen, *et al.* 1971; Williams, *et al.* 1973). The UV spectra in Figure 6-2 revealed that, with the exception of the peak from IIZ-05raw, all other three peaks appeared to consist of either two or three overlaid peaks: peak A around 202nm, peak B around 205nm, and peak C around 216nm. It is also noted that the peaks in Figure 6-2 appeared constantly to have a red shift along with the decrease of the initial C.I. These observations correlated well with C.I. and TOX results compiled in Table 6-3. In particular, as demonstrated in Table 6-3, IIZ-03raw had the highest concentration of chlorinated and low-molecular-weight (lowest C.I.) organics, it had the largest shift. By similar reasoning, the absorbance maximum of IIZ-01raw shifted to around 214nm, and IIZ-04raw to only about 210nm since it had relatively low concentration of chlorinated and low-molecular-weight organics (with higher C.I.).

The difference in COD, TOC, and the absorbance around wavelengths of 202, 216 and 280 nm between IIZ-05raw and other raw composite samples seemed to correlate with the C.I. The difference may also represent the concentration of either low-molecular-weight compounds or high-molecular-weight biodegradable organics such as wood sugar and hemicellose in these composite samples. Table 6-3 shows that IIZ-05raw sample was derived from the fragment with MWCO>5,000 of ASB effluent which was well-treated with a conventional biological process; thus it contained a very low concentration of biodegradable and low-molecular-weight organics (MWCO<5,000) and very low concentration of biodegradable organics. As discussed in other studies (Mao and Smith, 1994a), these low-molecular-weight organics usually have lower color intensity (C.I.), higher biodegradability and a very weak absorbance peak at 205nm, but strong absorbances around 202 and 216 nm. Figure 6-2 and Table 6-3 also show that all raw composite samples had similar true color level and absorbance at 465nm initially. This suggested that the color-causing compounds in these composite samples may give similar absorbance at other wavelengths such as 202, 216 and 280 nm. However, the absorbance at 280nm differed greatly among the composite samples, and the difference increased with decreasing C. I.

Table 6-3. Major Characteristics of Raw and Ozone Treated Samples in Ozone Optimization Studies

Sample	Description	Characteristics							Ozone Dose		Efficiency			
		Color (C.U.)	COD (mg/L)	TOC (mg/L)	TOX (mg/L)	C.I.	BOD_5 (mg/L)	BOD_{10} (mg/L)	Used (mg/L)	Consumed (mg/L)	ΔC.I.	ΔTOX	P-TOX	C.U./mgO_3
IIZ-0315raw	Diluted Raw E_{ap}	1086	1547	660	18.21	1.65	N/A	N/A						
IIZ-01raw	MWCO>5000 from E_{ap}	1112	1234	540	15.00	2.06	102.2	209						
IIZ-03raw	MWCO<5000 from E_{ap}	1227	2010	922	23.52	1.33	566	858						
IIZ-04raw	MWCO<5000 from ASB Eff.	1183	919	463	17.81	2.56	24.2	77.6						
IIZ-05raw	MWCO>5000 from ASB Eff.	1219	608	280	9.52	4.35	5.1	21.5						
IIZ-031501	Ozone Treated	761	1415	645	15.72	1.18	N/A	N/A	95.3	79.5	0.47	2.49	1.59	4.09
IIZ-031502	Ozone Treated	771	1449	637	15.76	1.21	N/A	N/A	99.3	75.3	0.44	2.45	1.51	4.18
IIZ-01	Ozone Treated	784	1214	530	13.58	1.48	113.8	243	86.6	59.1	0.58	1.42	1.18	5.55
IIZ-02	Ozone Treated	777	1204	533	13.18	1.46	117.9	234	92.3	60.9	0.60	1.82	1.22	5.47
IIZ-03	Ozone Treated	929	1978	897	21.12	1.08	531	819	88.2	88.2	0.25	2.40	1.76	3.39
IIZ-04	Ozone Treated	694	852	433	14.61	1.64	44.8	115	89.4	88.5	0.92	3.20	1.77	5.93
IIZ-05	Ozone Treated	647	592	273	7.60	2.37	27.6	56.2	98.4	63.5	1.98	1.92	1.27	9.01

Note: C.I.=color intensity (C.U./mg TOC); P-TOX=predicted removal of TOX by ozone treatment.

Figure 6-3 further demonstrates that 1) with a similar concentration of color-causing lignin structures IIZ-01raw and IIZ-05raw had a similar molecular weight distribution pattern but IIZ-01raw had 50% higher absorbance at 280nm than IIZ-05raw; 2) IIZ-04raw had a higher low-molecular-weight proportion than the above two but lower than IIZ-03raw. IIZ-03raw, which was derived from the fragment with MWCO<5000 of E_{op} filtrate, had two larger peaks at low molecular weight range than IIZ-04raw. Consequently, it is resaonable to state that 1) IIZ-01raw had a much higher concentration of high-molecular-weight biodegradable organics such as wood sugar and hemicellose than IIZ-05raw; 2) by similar reasoning IIZ-03raw contained the highest concentration of biodegradable but relatively low-molecular-weight organics and IIZ-04raw also contained a higher concentration of low-molecular weight biodegradable organics than IIZ-05raw but a much lower concentration compared with IIZ-03raw.

6.3.2 Kinetics of Ozone Decolorization and Dechlorination

A series of experiments were designed to investigate the effects of the initial color intensity (C.I.) and different components of pulp mill effluents on the efficacy of ozone decolorization and dechlorination. All the ozone treatment experiments in this series were conducted under the same operating conditions. The experimental conditions were: pH=7.5 ±0.2; temperature 20 ±2°C, O.A.R.= 20 ±1 mg ozone/minute, the reaction time t = 8.9±0.25 minutes, and in Reactor System II without any headspace. The Reactor System II consisted of a CSTR ozone reactor and a control system for the ozone addition to the wastewater at the desired rate and concentration. The details were described in another study (Mao and Smith, 1995a). The results from these experiments are compiled in Table 6-3 and Figures 6-4 through 6-9.

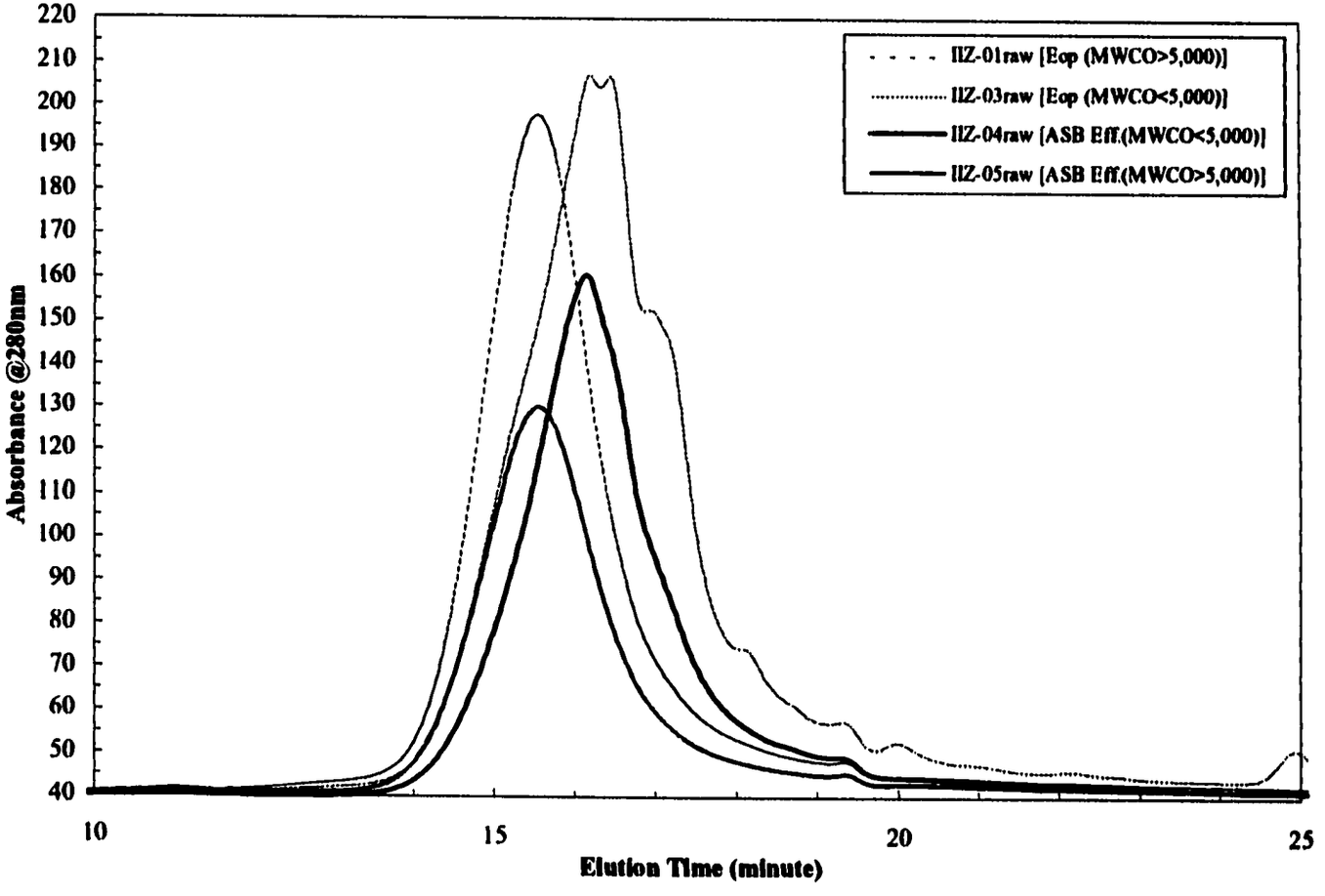

Figure 6-3. Comparison of Molecular Weight Distribution of Various Organics in Raw Composite Samples

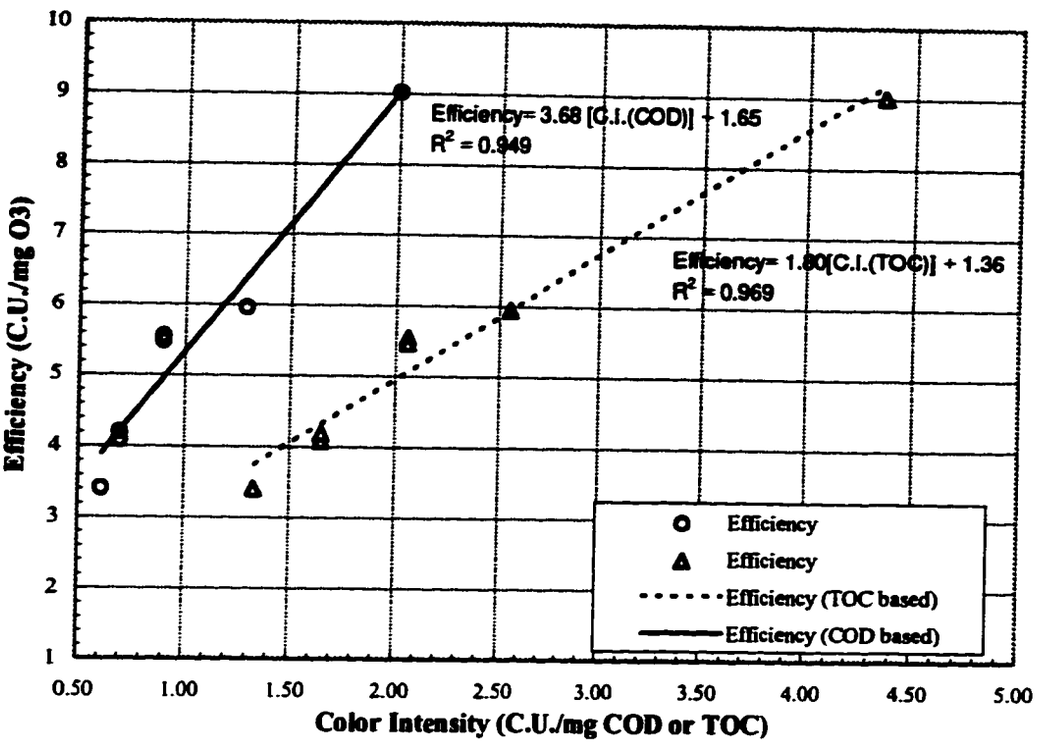

Figure 6-4. Effects of Color Intensity on Ozone Decolorization Efficiency of Pulp Mill Effluent

Figure 6-4 shows that the efficiency of ozone decolorization increased linearly with the increase of the color intensity (initial C.I.) of raw composite samples. The efficiency of ozone decolorization improved by about 1.8 C.U./mg consumed O_3 with every one unit increase in initial C.I. (TOC based) and by about 3.7 C.U./mg consumed O_3 with every one unit increase in initial C.I. (COD based). These results suggest that, under the same operating conditions, 1) the initial C.I., either TOC based or COD based, is a determining factor influencing the efficacy of ozone decolorization of pulp mill effluents; 2) TOC based C.I. seems to be a more sensitive parameter. In other words, the efficiency of ozone decolorization improved greatly by increasing the relative ratio of the concentration of chromophoric functional groups to that of either low- or high-molecular-weight (low C.I.) biodegradable compounds in the pulp mill effluents.

On the other hand, the C.I. appeared to have little effect on the efficiency of ozone dechlorination. As shown in Table 6-3, the TOX removal was almost independent of the C.I.s over the tested range. However, it appeared that, at this ozone dose level, the TOX reduction seemed to be proportional to the initial concentration of chlorinated organics in the composite

samples. More studies are needed to elucidate further the effects of the initial conditions on the TOX removal.

Under above operating conditions, the kinetics of the overall ozone decolorization reactions can be described in Equation (6-1) with respect to the density of chromophoric functional groups expressed as C.I.:

$$-r_{[C.I.]} = -\frac{dC}{dt} = -\frac{d[C.I.]}{dt} = k'[C_{O_3}]^a[C.I.]^b \qquad (6\text{-}1)$$

In this series of experiments, the O.A.R. was constant and the ozone demand was high, thus, $k'[C_{O_3}]^a$ can be considered as a pseudo-kinetic constant k; therefore, the initial reaction rate can be written as:

$$\left(-\frac{dC}{dt}\right)_{initial} = \left(-\frac{\Delta C}{\Delta t}\right)_{initial} = k\left([C.I.]\right)^b \qquad (6\text{-}2)$$

According to initial rate method (Levenspiel, 1972) and applying "ln" on both side of equation (6-2):

$$\ln\left(\frac{[C.I.]_0 - [C.I.]_t}{t-0}\right)_{initial} = \ln(k) + b\{\ln([C.I.]_0)\} \qquad (6\text{-}3)$$

where $[C.I.]_0$ and $[C.I.]_t$ represent the concentration of the chromophoric functional groups at time t=0 and t=t (expressed in C.I. units), t is the initial reaction time with ozone based on the concept of initial reaction rate method (Levenspiel, 1972), and k is the pseudo kinetic constant.

In this series of experiments the ozone addition time t was kept constant (t = 8.9±0.25 minutes) in a batch reactor. Thus, the Δt=t-0 was the same in each run. As also shown in Table 6-3, the initial true color of each composite sample was kept almost the same, and the decolorization remained at low levels (less than 35%); thus, the slopes of the plot of $\ln\left(\frac{[C.I.]_0 - [C.I.]_t}{t-0}\right)_{initial}$ vs $\{\ln([C.I.]_0)\}$ (according to differential method of analysis) should represent the reaction order of the overall ozone reactions and the intercept should be the kinetic constant ln(k):

$b_1 = 1.44$, $\ln(k_1) = -3.12$, or $k_1(TOC)) = 0.0442 \pm 0.003$ (minute^{-1})

$b_2 = 1.61$, $\ln(k_2) = -3.38$, or $k_2(COD) = 0.0340 \pm 0.002$ (minute^{-1})

The results of linear regression analysis were summarized in Eqs (6-4) and (6-5), and are plotted in Figure 6-5.

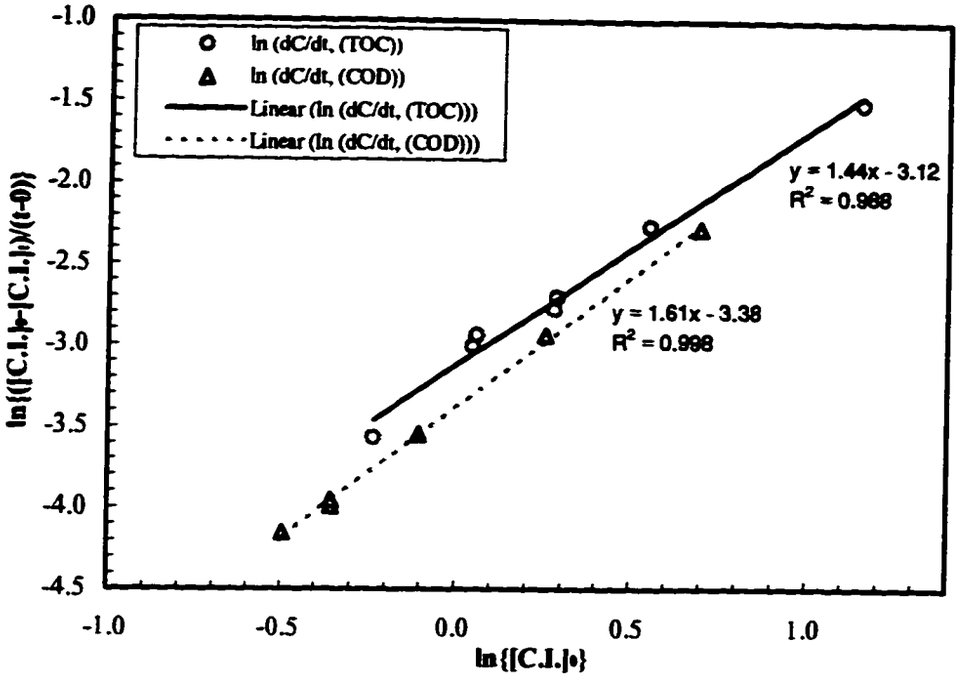

Figure 6-5. Kinetics of Ozone Decolorization of Pulp Mill Effluents (initial kinetic rate vs. initial C.I.s)

$$-r_{[C.I.]} = -\frac{dC}{dt} = -\frac{d[C.I.]}{dt} = 0.0442\,[C.I.]^{1.44} \qquad \text{(TOC based)} \qquad (6\text{-}4)$$

$$-r_{[C.I.]} = -\frac{dC}{dt} = -\frac{d[C.I.]}{dt} = 0.0340\,[C.I.]^{1.61} \qquad \text{(COD based)} \qquad (6\text{-}5)$$

The regression analysis suggests that the data fit Eq (6-3) well (R^2= 0.988 (TOC based) or 0.998 (COD based)). Further examining Eqs (6-4) and (6-5) reveals that the slopes of two regression lines are essentially the same and very close to 3/2 orders of reaction considering errors in the given experiments and in COD and TOC analysis.

Comparing Eq (6-2) with Eqs (6-4) and (6-5), the pseudo-kinetic constants for overall ozone decolorization reactions are:

$$k_{TOC} = 0.0442 \text{ (minute}^{-1}\text{)} \quad (6\text{-}6)$$

$$k_{COD} = 0.0340 \text{ (minute}^{-1}\text{)} \quad (6\text{-}7)$$

After substituting 3/2 orders into Eqs (6-4) and (6-5), respectively, integrating them gives the kinetic model in terms of true color and TOC or COD for ozone decolorization under conditions of t=0, [C.I.]=[C.I.]$_0$:

$$[color]_t = \frac{4\,[color]_0}{\left[\{0.0442\left(\frac{[color]_0}{[TOC]_0}\right)^{1/2} t + 2\}\right]^2} \frac{[TOC]_t}{[TOC]_0} \quad (6\text{-}8)$$

$$[color]_t = \frac{4\,[color]_0}{\left[\{0.0340\left(\frac{[color]_0}{[COD]_0}\right)^{1/2} t + 2\}\right]^2} \frac{[COD]_t}{[COD]_0} \quad (6\text{-}9)$$

The data reported in previous ozone decolorization study (Mao and Smith, 1995a) were adopted to verify the applicability of the above kinetic model to prediction of color reduction in ozone decolorization at low levels. The predicted results are compared in Table 6-4 with the reported results. Table 6-4 suggests that the kinetic model can be used satisfactorily to predict the residual true color levels after ozone decolorization at low levels with a proper constant O.A.D.

Therefore, it is reasonable to state that, at this level of ozone decolorization, the overall ozone decolorization reactions appeared to follow the 3/2 order kinetics with respect to the concentration of chromophoric functional groups expressed in C.I. These findings further confirmed that increasing initial C.I. (not necessarily initial true color) would definitely increase the ability of the chromophoric functional groups to compete for ozone with the low C.I. compounds, and the degree of competition is concentration-dependent (Mao and Smith, 1994a and 1995a).

Table 6-4. Comparison of Predicted C.I. and True Color by Kinetic Model with Those Reported in Previous Study (Mao and Smith, 1995a)

Source	Related Parameters	Wastewater			
		ASB Effluent	ASB Influent (A)	ASB Influent (B)	E_{up} Filtrate
Raw Wastewater	$[Color]_o$ (C.U.)	1460	1480	1480	3050
	$[COD]_o$ (mg/L)	810	1439	1439	3058
	C.I. (C.U./ mg COD)	1.80	1.09	1.09	1.02
Data from Previous Study (at 35% Color Reduction)	Required Ozone Dose	50±5	95±5	94±5	250±5
	$[Color]_t$ (C.U.)	885	931	965	2050
	$[COD]_t$ (mg/L)	775	1340	1343	2850
	C.U./mg Ozone*	1.14	0.695	0.719	0.719
	C.U./mg Ozone	11.1	5.6	5.5	4.5
	Reaction Time (minute)	9.0± 0.5	9.0± 0.5	9.0± 0.5	9.0± 0.5
Predicted Values by kinetic model	C.I. (C.U./mg COD)	1.24	0.773	0.773	0.751
	True Color (C.U.)	960 ± 15	1030 ± 15	1030 ± 15	2140 ± 35

Note: *: CPPA color units/mg consumed ozone.

6.3.3 Possible Mechanism of Ozone Decolorization and Dechlorination

Figures 6-6 through 6-9 compare the UV spectra of raw and ozone treated samples listed in Table 6-3. These figures demonstrate that after ozone treatment the UV absorbance was reduced most in IIZ-05, and least in IIZ-03. This finding correlates well with the observations discussed in the earlier sections and with the measurement of the true color and C.I. Moreover, the comparion of Figure 6-2 with Figures 6-6 through 6-9 reveals that the measurable blue shifts occurred in all but IIZ-05 at the absorbance maximum after ozone treatment, and the magnitude of the blue shift was inversely proportional to the initial C.I. as demonstrated in Table 6-5.

Table 6-5. Comparison of Ozone Effects on λ_{max} of UV Spectra of Composite Samples with Different C.I.

Sample	Initial C.I.	λ_{max} (nm)		
		Raw	Ozonated	Blue Shift
IIZ-031703	1.33	216	212	4
IIZ-031701	2.05	214	211	3
IIZ-031704	2.65	210	207	2
IIZ-031705	4.35	205	205	0

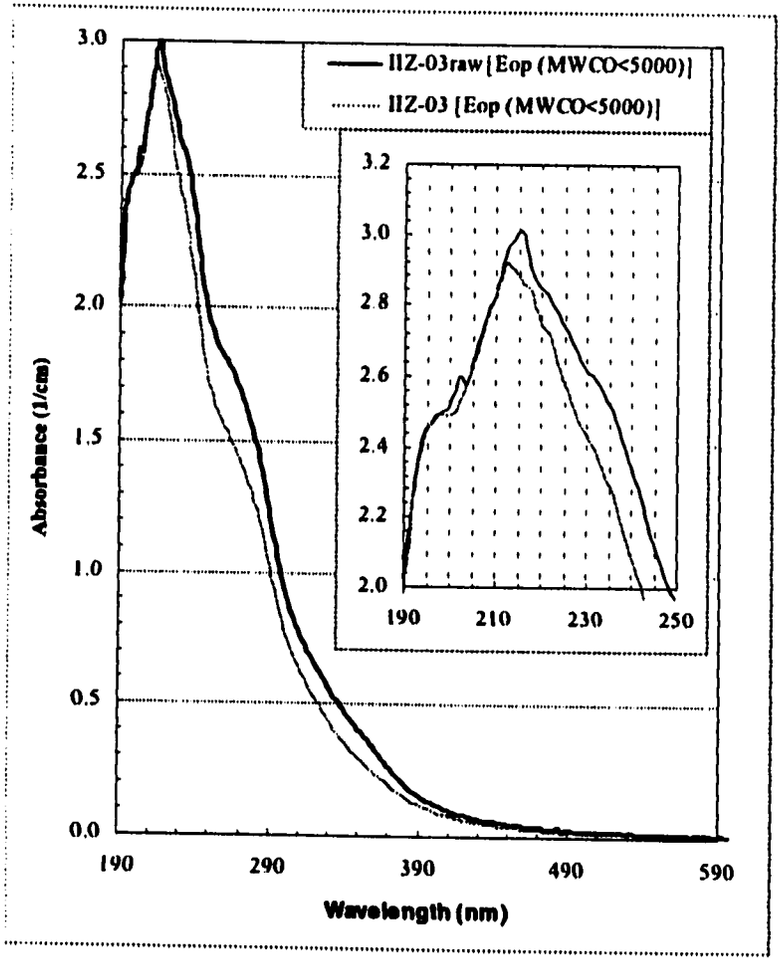

Figure 6-6. Comparison of UV Spectra of Raw and Ozone Treated Samples with C.I.=1.33 C.U./mgTOC

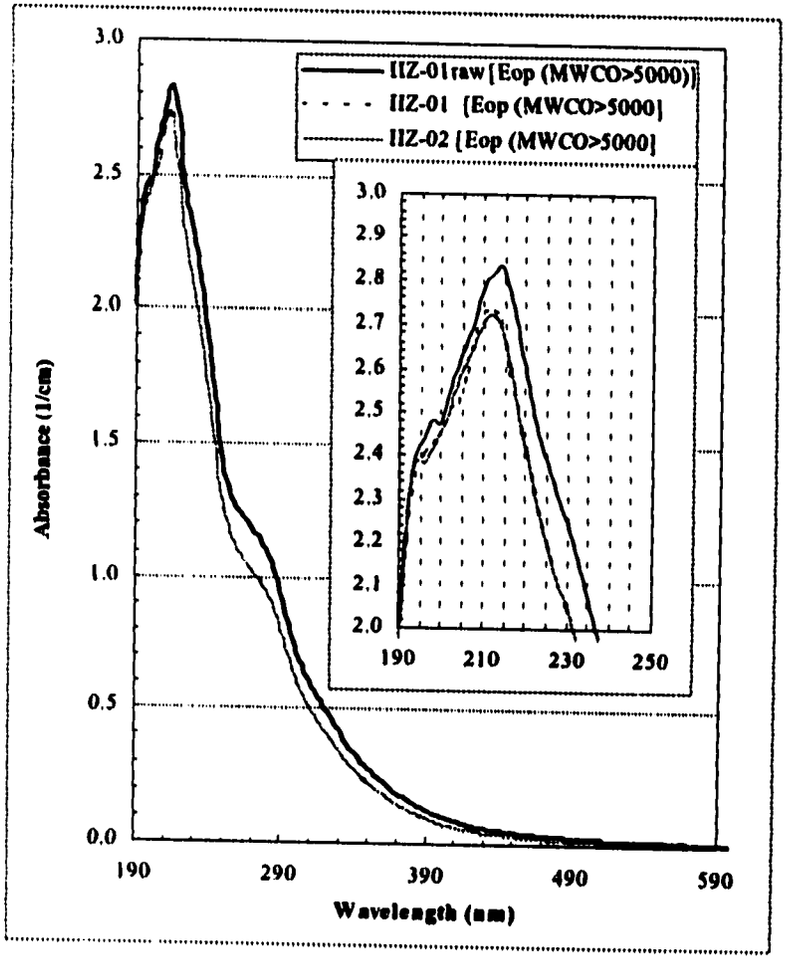

Figure 6-7. Comparison of UV Spectra of Raw and Ozone Treated Samples with C.I.=2.06 C.U./mgTOC

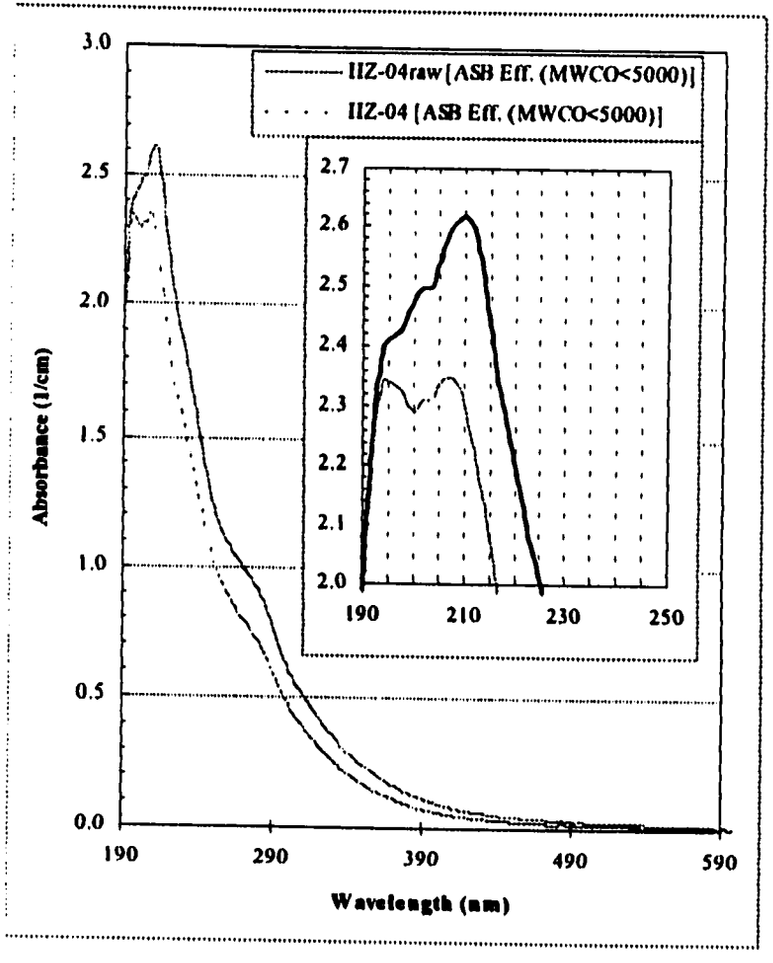

Figure 6-8. Comparison of UV Spectra of Raw and Ozone Treated Samples with C.I.=2.56 C.U./mgTOC

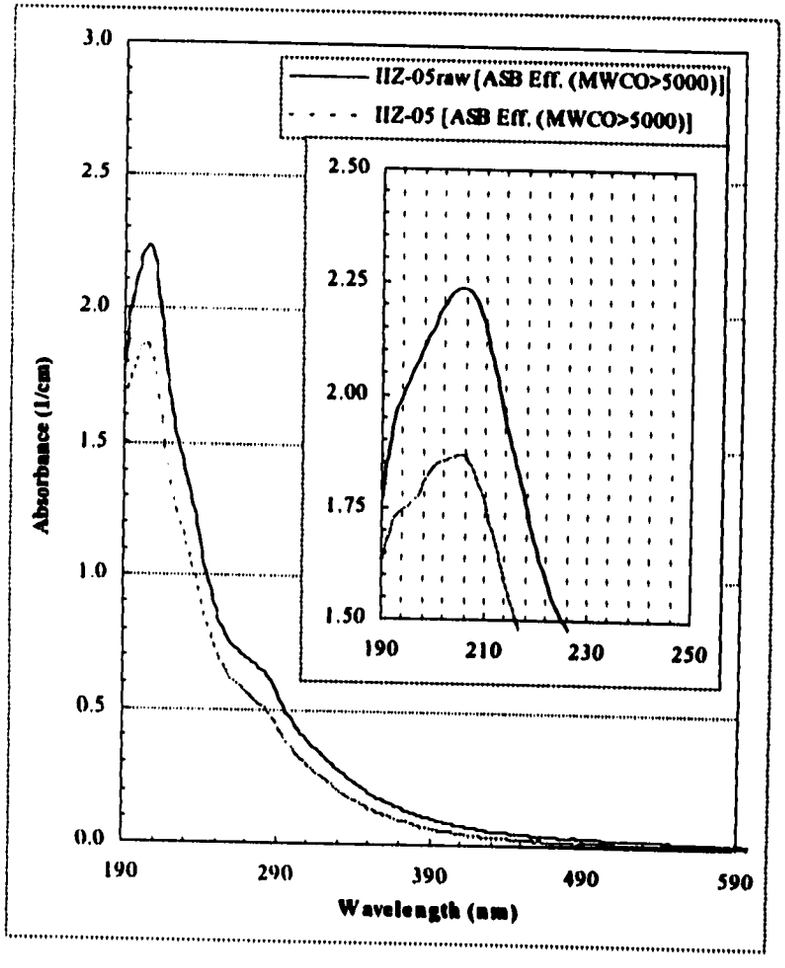

Figure 6-9. Comparison of UV Spectra of Raw and Ozone Treated Samples with C.I.=4.35 C.U./mgTOC

These observations indicate that more low-molecular-weight organics seemed to be destroyed by ozone when the ratio of fragment with MWCO<5,000 to fragment with MWCO>5,000 was high in the raw composite samples. Specifically, the absorbance of the peak around 216nm was reduced much more than that around 205nm. As a result, the peak would have more blue shift toward 205nm due to superimposing effects discussed in the earlier sections. On the other hand, the effect of fragments with MWCO<5,000 on the blue shift would be much less profound when the ratio was low. In an extreme case of IIZ-05raw it was observed that there appeared to have no shift after ozone decolorization since there was a negligible amount of fragment with MWCO<5,000 in the composite samples of IIZ-05raw.

Figure 6-10 further compares the changes in BOD_5 and BOD_{20} of the raw composite samples after ozone treatment. The effects demonstrated in Figure 6-10 well support the above observations. Specifically, at the same ozone dose level, when initial C.I. was low and initial BOD_5 was high, corresponding to earlier discussions, the ratio of fragment with MWCO<5,000 to fragment with MWCO>5,000 was high (as also shown in Table 6-3 and Figures 6-6 through 6-9), the BOD_5 and BOD_{20} were significantly reduced (at 5% level); whereas the ratio was low, i.e., the C.I. was high and BOD_5 was low, the BOD_5 and BOD_{20} were significantly increased after ozone treatment (at 5% level). These findings can also be deduced from the observations presented in the earlier sections. From biological point of view, in the former cases, there was more chance for the biodegradable-fragment (MWCO<5,000) to compete for ozone, and the majority of ozone introduced into the reactor was consumed by the organics in the biodegradable-fragment. As a result, a larger amount of biodegradable fragment was destroyed during ozone decolorization, and the amount of destroyed biodegradable-fragment overwhelmed the amount of the biodegradable-fragment produced in the ozone reactions with organics in the other fragments. When the ratio was low, the ability of the biodegradable-fragment to compete for ozone decreased, and more ozone would react with poorly-biodegradable fragments. Consequently, the BOD_5 and BOD_{20} were significantly increased (at 5% level). These conclusions also confirm that the change of biodegradability is strongly dependent on the composition of the raw samples.

In summary, the results from this series of experiments revealed the following:

1) The fragments with MWCO<5,000 had a low C.I. and strong UV absorbance maximum λ_{max} around 202 or λ_{max} around 216nm while the fragments with MWCO>5,000 had a high C.I. and strong UV absorbance maximum λ_{max} around 205nm or 280 nm;

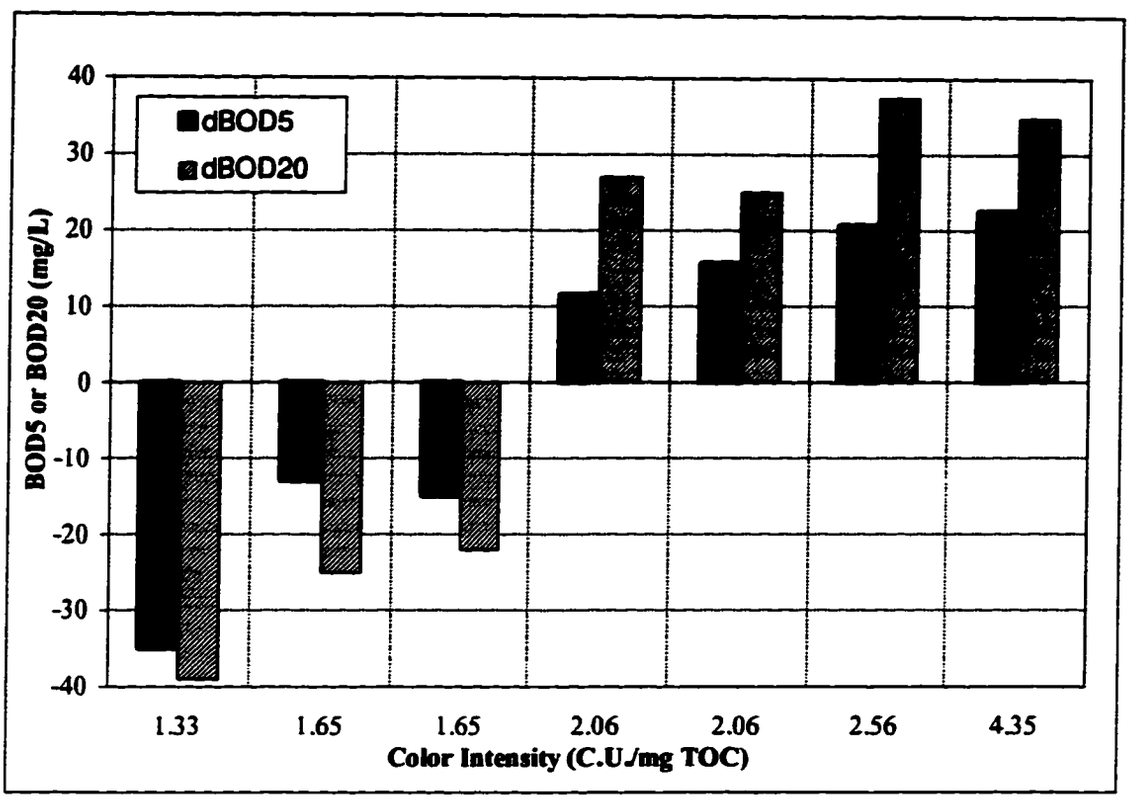

Figure 6-10. Effects of Ozone Reactions on BOD_5 and BOD_{20} during Decolorization Processes at Dose Level about 80 ppm (ΔBOD5=change of BOD5 after ozone treatment; ΔBOD20=change of BOD20 after ozone treatment)

2) the systematic red shifts occurred among the characteristic UV absorbance maximum of raw composite samples. These red shifts appeared to be due to the high concentration of low C.I. organics derived from the chlorination and the addition oxidation reactions in the pulping and bleaching process; ozone treatment induced the blue shifts among the composite samples;

3) the C.I. was proportional to the absorbance at 205nm or at 465nm, and inversely correlates with the UV absorbance around 216nm;

4) the absorbance at 205nm was greatly affected by the strength of the peak around 216 nm, and it was very difficult to isolate the absorbance of chromophoric structure in pulp mill effluents when the concentration of the fragment with MWCO<5,000 was high.

Figures 6-11 through 6-14 further demonstrate the effects of the ozone decolorization on the molecule weight (MW) and molecular weight distribution (MWD) of the composite samples. In Figure 6-11, the MW and MWD in the two replicates reproduced each other. This implied that the ozone decolorization study and HPSEC analysis were reliable considering the various errors in the experimentation.

Figures 6-11 and 6-14 demonstrate that 1) the MWD patterns of IIZ-01 (IIZ-02) and IIZ-031705 were close to normal distribution which agreed well with those reported earlier (Roy-Arcand and Archibald, 1991), but the MWD patterns of IIZ-03 and IIZ-04 deviated from the normal distribution; 2) the MWD of IIZ-01 and IIZ-05 shifted to the lower molecule weight range. This suggests that a large amount of high-molecular-weight lignin fragments was broken down to the lower-molecular-weight organics after ozone decolorization. Unfortunately, some low-molecule-weight products of ozone decolorization could not be detected at 280 nm on a UV detector attached to the HPSEC. However, as discussed in later section, it has been shown that the low-molecule-weight products were produced in the ozone decolorization process; 3) by contrast, Figure 6- 12 shows that the MWD of IIZ-03 shifted slightly to higher molecular weight range. Especially, the reduction of the two lower molecular weight peaks were greater than that of the relative high-molecular-weight peak. A similar phenomenon is observed in Figure 6-13 but to a lesser degree. These observations are consistent with the earlier observations. Specifically, IIZ-05 had a higher C.I., as a result, ozone decolorization caused the greatest MWD shift to a lower molecular weight range. When C.I. decreased, the degree of shift decreased as well. Further decreasing C.I. after a certain point the opposite shift occurred (as shown Figures 6-12 and 6-13). This appears to be the underlying mechanism for explanation of the three phases phenomena reported in the earlier studies (Mao and Smith, 1995a; Stern, 1974; Mohammed, 1990).

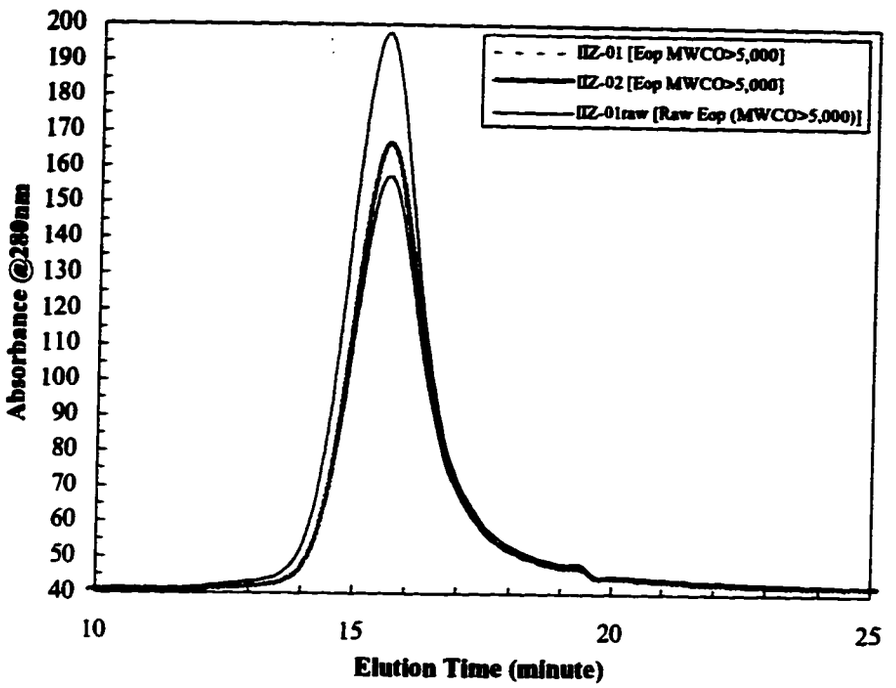

Figure 6-11. Effects of Ozone Decolorization on Molecular Weight and Molecular Weight Distribution of IIZ-031701Raw

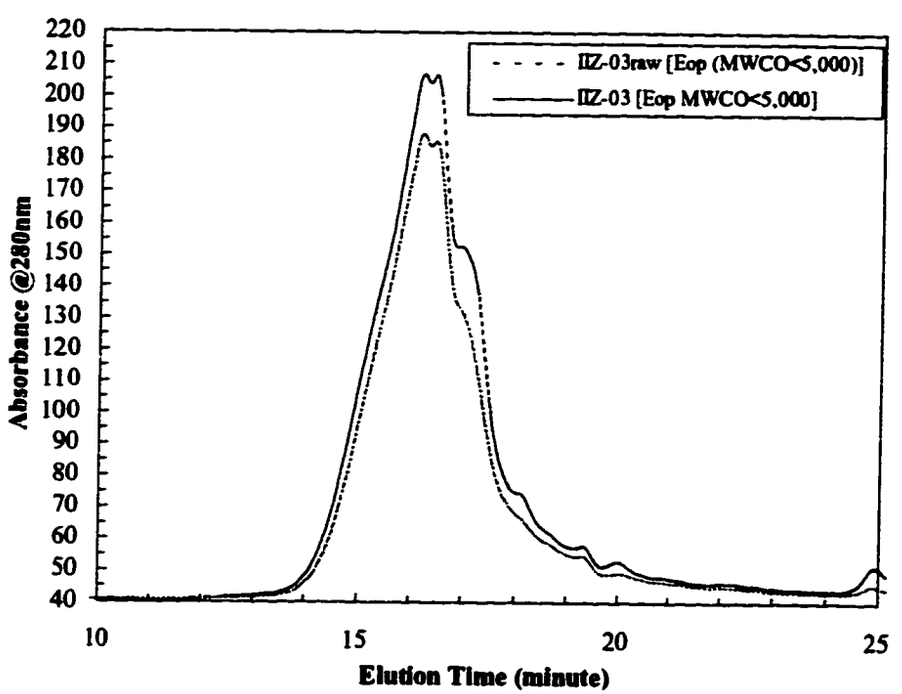

Figure 6-12. Effects of Ozone Decolorization on Molecular Weight and Molecular Weight Distribution of IIZ-031703Raw

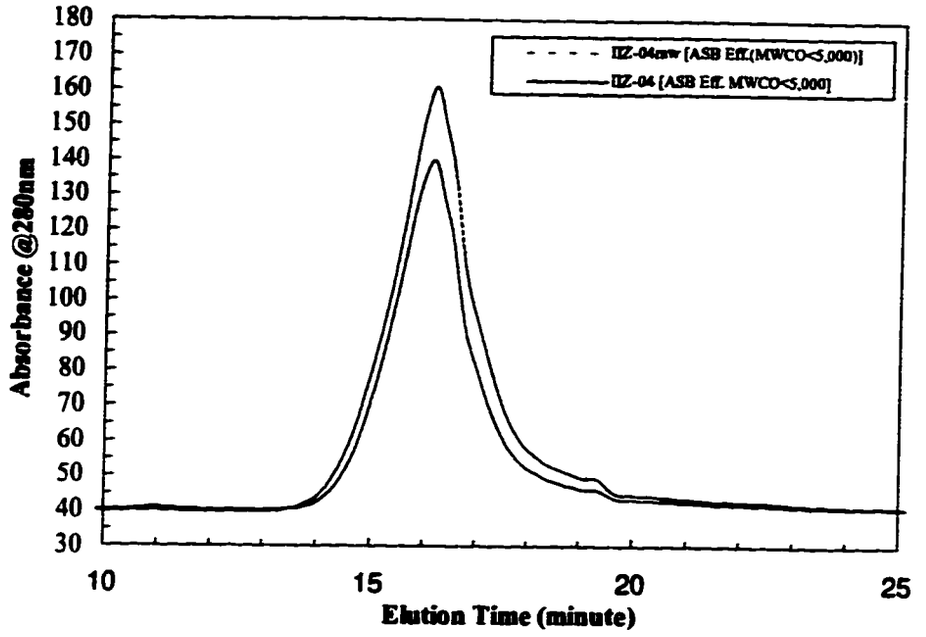

Figure 6-13. Effects of Ozone Decolorization on Molecular Weight and Molecular Weight Distribution of IIZ-031704Raw

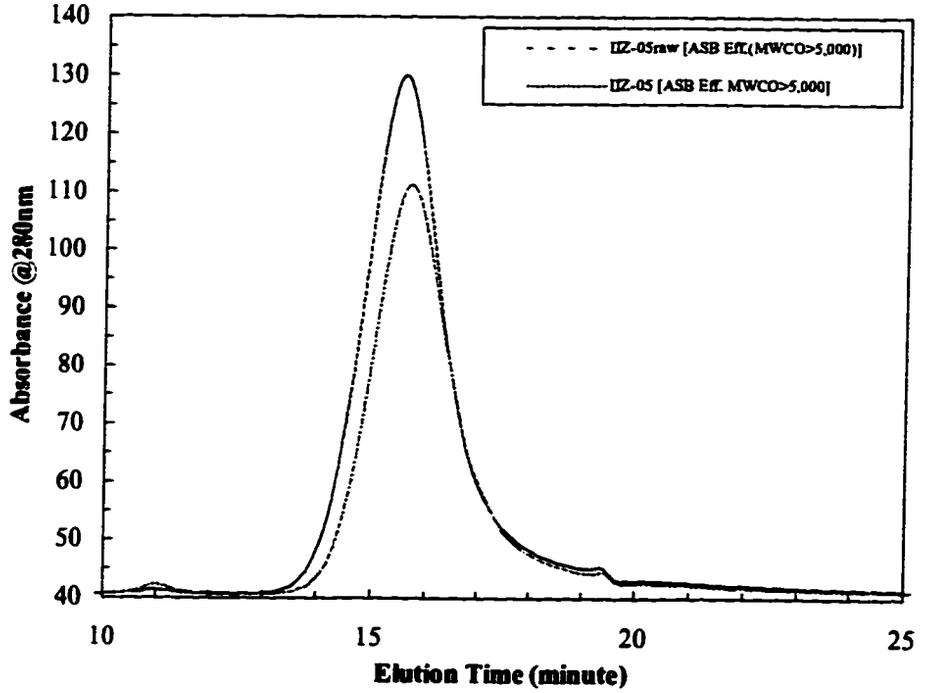

Figure 6-14. Effects of Ozone Decolorization on Molecular Weight and Molecular Weight Distribution of IIZ-031705Raw

However, Figures 6-11 through 6-14 also indicate that ozone decolorization did not appear to have a profound impact on MWD patterns of all composite samples. This result appears to agree well with another report (Roy-Arcand and Archibald, 1991).

Table 6-6 quantitatively compares the characteristics of raw and two replicates of ozone treated samples (NR-1 and NR-2). NR-1 and NR-2 were ozonated under the same conditions (pH, temperature, ozone addition rate (O.A.R.), and ozone dose level (about 80 mg/L)). Table 6-6 demonstrates that the NR-2 reproduced NR-1 very well within the wide range of the tested parameters. This is consistent with the measurement of the wide range of UV spectra presented in other studies (Mao and Smith, 1995a and 1994b) and molecular weight distribution in Figure 6-11 (IIZ-01 and IIZ-02).

Table 6-6. Characteristics and Distribution of Raw and Ozone Treated ASB Effluent

Parameter	Sample	FRAGMENTS						
		MWCO<1000	MWCO>1000 MWCO<5000	MWCO>5000 MWCO<10000	MWCO>10000	TOTAL[1]	RAW[2]	DIFF[3]
Color	Raw Effluent[5]	94(7%)[4]	104(8%)	187(14%)	885(64%)	1270	1390	120
(C.U.)	NR-1	62((8%)	74(9%)	132(17%)	438(55%)	706	798	92
	NR-2	69(9%)	78(10%)	119(15%)	439(57%)	705	756	51
COD	Raw Effluent	132.2(20%)	83.9(13%)	111.4(17%)	306.2(47%)	633.7	654.2	20.5
(mg/L)	NR-1	135.6(23%)	81.7(10%)	93.7(15%)	233.2(29%)	544.2	593.2	49.0
	NR-2	164.8(30%)	78.7(9%)	104.1(13%)	231.1(40%)	578.7	596.9	18.2
TOC	Raw Effluent	52.1(21%)	35.8(15%)	46.3(19%)	113.4(46%)	247.6	246.7	-0.9
(mg/L)	NR-1	75.4(31%)	34.7(14%)	38.1(16%)	89.4(37%)	237.6	243.2	5.6
	NR-2	71.4(29%)	31.6(13%)	41.3(17%)	87.1(36%)	231.4	235.9	4.5
BOD_5	Raw Effluent	12.2(95%)	3.8(29%)	4.2(33%)	6.6(51%)	26.8	12.9	-13.9
(mg/L)	NR-1	41.8(118%)	10.2(29%)	11.8(33%)	13.1(37%)	76.9	35.4	-41.5
	NR-2	41.6(118%)	12.1(34%)	9.6(27%)	12.1(34%)	75.4	37.1	-38.3
TOX	Raw Effluent[5]	2.01(27%)	1.43(19%)	1.37(18%)	3.38(45%)	8.19	7.51	-0.68
(mg/L)	NR-1[6]	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	NR-2	1.75(35%)	0.79(16%)	1.08(22%)	2.17 (43%)	5.79	5.01	-0.78

Note: 1. TOTAL is the sum of parameter value from all fragments;
2. RAW is the analysis results of Raw effluent used in fractionation study;
3. DIFF is the difference between the Raw and TOTAL;
4. Number in bracket is the percentage of that fragment in raw effluent;
5. large errors due to smaller samples; 6. samples missing for TOX analysis.

6.3.3.1 TOC and COD

Table 6-6 shows that about 25% of the TOC of the fragment with MWCO>10,000 (F4) was transformed into the smaller fragments with MWCO<10,000 (F3, F2 or F1) after ozone

treatment. Contrasting sharply with this were the negligible changes in total amount of TOC, and the TOCs of both fragments F2 (1,000<MWCO<5,000) and F3 (5,000<MWCO<10,000). It is also important to recognize that there were negligible changes in CODs of both fragments F2 and F3 after ozone treatment at a dose level of about 80 mg/L while there was more than 60 mg/L COD reduction in both NR-1 and NR-2. At the same time, the COD of F1 appeared to increase after ozone treatment. These results suggest that after ozone treatment some of the molecules of lignins and their derivatives in ASB effluents would be smaller in size and in higher oxidation status than the originals. However, the products resulting from ozonation reactions would be extremely heterogeneous since the lignins and their oxidative fragments are extremely polydisperse and irregular in their molecular structure (Pearl, 1967; Sarkanen, *et al.*,1971).

The chemistry of ozonation reactions with lignins and their derivatives suggests (Brauns, *et al.*, 1960; Sarkanen, *et al.* 1971) that F4 should have a higher ability to compete for ozone but it is impossible to have 100% selectivity. Therefore, it seems more reasonable to conclude, combining the findings presented in earlier sections, that during ozone treatment the fragment F4 was first broken down to smaller fragments such as F3, F2, and F1 fragments. In the meantime, F3 may have been partially oxidized to fragments F2 and F1 etc. The ratio of (F2+F3) to F4 seems to be another factor in governing the selectivity and efficiency of ozone decolorization reactions since both fragments had a relatively high density of reactive functional groups toward ozone molecules.

6.3.3.2 True Color

Table 6-7 further compares the true color and C.I. of each fragment. In contrast to the effects of ozone on the TOC and COD of each fragment, the results in Tables 6-6 and 6-7 show that the fragments F1, F2 and F3 had almost similar percentages of color reduction (about 30±4%) while the true color of fragment F4 was reduced by more than 50% after ozone treatment. In other words, the reductions expressed in the absolute CPPA color unit among F1, F2 and F3 were proportional to the initial color level. F4 did not follow this trend, however. It is also important to note that the effects of ozone treatment on the C.I. were significantly different among the fragments (at 5% level). The C.I. of fragments F4 and F1, after ozone treatment, were reduced by more than 35% and 50%, respectively. The TOC increase in the F1 appeared to be

the major factor responsible for the decrease of C.I., whereas the color reduction in the F4 was the major factor responsible for the decrease of C.I.

Table 6-7. Comparison of Changes of Color and Color Intensity of Raw and Each Fragment Samples

Parameter	Sample	F1	F2	F3	F4	Whole
True Color Reduction**	NR-1	-32 (34%)	-30 (29%)	-55 (29%)	-447 (51%)	-592 (43%)
	NR-2	-25 (27%)	-26 (25%)	-68 (36)%	-446 (51%)	-634 (46%)
	Raw Effluent	100%	100%	100%	100%	100%
Color Intensity (based on final TOC)	NR-1 C.I.*	0.8	2.1	3.5	4.9	3.3
	NR-2 C.I.	0.9	2.5	2.9	5.0	3.2
	Raw Effluent	1.8	2.9	4.1	7.8	5.6
Color Intensity (based on initial TOC)	NR-1	1.2	2.1	3.7	3.9	3.2
	NR-2	1.3	2.2	3.3	3.9	3.1
	Raw Effluent	1.8	2.9	4.1	7.8	5.6

Note: * C.I. = color intensity (CPPA C.U./mg TOC); ** in CPPA color unit, number in bracket are percentage reduction.

6.3.3.3 Total Organic Halogens (TOX)

The distributions of TOX among the fragments from raw and one of ozone treated ASB effluent are also listed in Table 6-6. The results in Table 6-6 demonstrate that 1) the F4 fragments in both raw and treated samples contributed about 45% of total TOX; 2) the TOX was abnormally distributed to low and high molecule ranges; 3) the ozone treatment did not appear to change the distributions of TOX among the high and low molecular weight fragments. These findings also imply that the substituted Cl in the lignin molecules were attacked non-selectively by ozone during decolorization process which appeared to destroy the majority of conjugated double bonds and some aromatic structures.

Figure 6-15 further demonstrates that the TOX removal from ASB effluent at different ozone dose levels. In Figure 6-15 the residual TOX in the samples vs. the consumed ozone doses fits a simple linear model at the dose levels up to 200 mg/L. The equation shown in Figure 6-15 suggests that the efficiency of ozone dechlorination was about 20 μgTOX/mg O_3. This finding implies that ozone treatment is much less effective on the removal of TOX compared with other processes. In short, ozone treatment non-selectively dechlorinates the pulp mill effluent but is relatively inefficient in removing chlorinated organics.

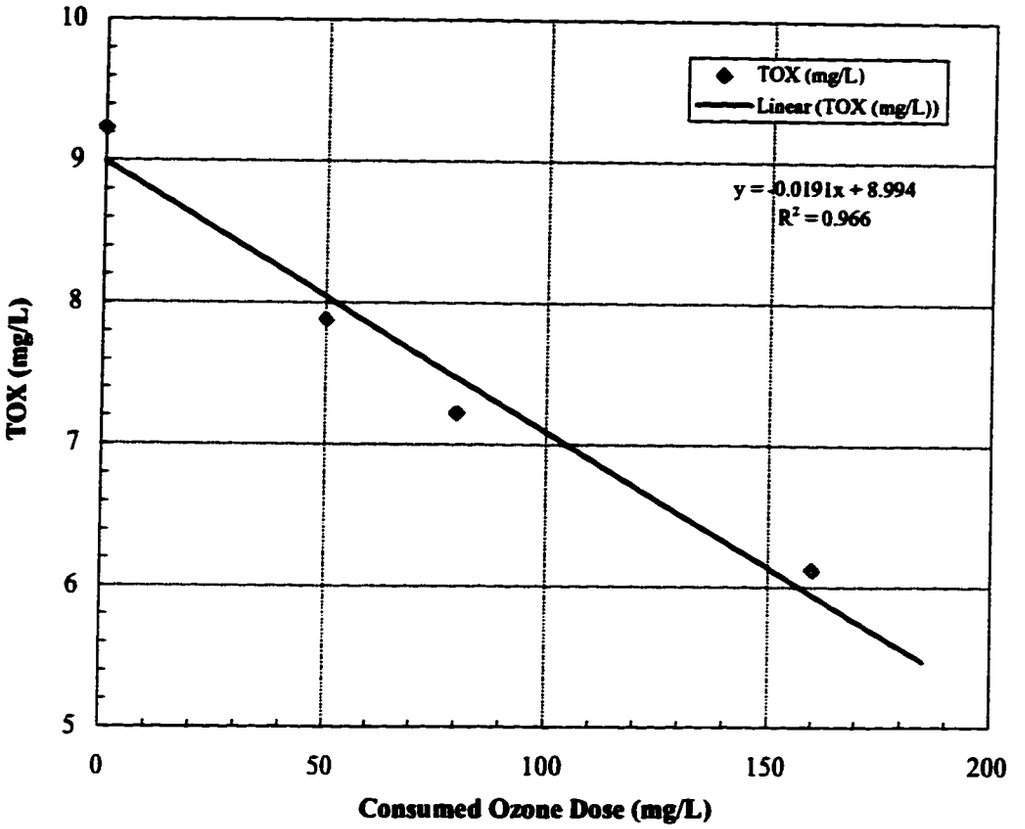

Figure 6-15. TOX Removal in Ozone Treatment of ASB Effluent at Different Dose Levels

6.3.3.4 Biodegradability

The biodegradability data in Table 6-6 quantitatively describe how ozone treatment improved the biodegradability of raw ASB effluent and their respective fragments. The comprehensive evaluation on the effects of ozone treatment on biodegradability have been reported in another study (Mao and Smith, 1995b). These findings demonstrate that the BODs in the fragments from ozone treated samples were 90 to 240% higher than those in the respective fragments from raw ASB effluent; by comparing with 5-day BOD of raw ASB effluent, 5-day BODs of ozone treated whole samples increased by 180%. Bauman and Lutz (1974) reported a similar observation. These findings support the earlier observations that ozone treatment could significantly increase the BODs in all fragments of ASB effluents with a high concentration of poorly-biodegradable fragments. It is also important to recognize that 1) unlike COD, TOC, and

true color, the distributions of BOD_5 among the fragments in both raw and ozone treated pulp mill effluents were extremely skewed to the low molecular weight fragments (F1). In particular, the fragment F1 contributed almost all BOD_5 to the whole samples, and accounts for the major increase in the total BOD_5 after ozone treatment; 2) by comparing the BOD_5 in a raw sample with those in the fragments, fractionation itself seemed to improve the 5-day BOD. This is likely due to the nature of microbial consortia used in biodegradability tests; in other words, the most favorable populations in the microbial consortia would always try to use the only available carbon source to survive. Less likely, this may be due to the reduced adverse or toxic effects of large chlorinated molecules on the microbial consortia when bioavailable carbon is very low; even less likely this may be due to the conversion of poorly-biodegradable molecules into biodegradable molecules in the fragmentation process; 3) the BOD levels in the fragments F2, F3, and F4 appeared to be proportional to their TOC levels although they were relatively low.

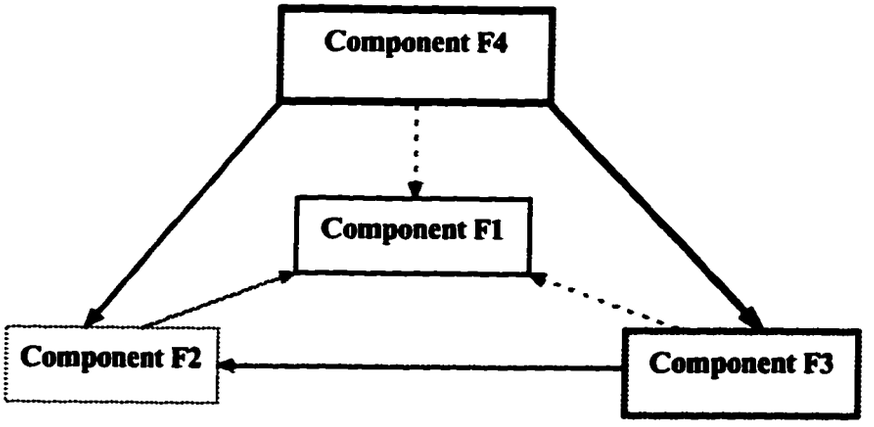

Figure 6-16. Pathways of Transformation of Lignins and Their Derivatives in Ozone Decolorization Process (width of lines indicates the degree of preference)

In summary, when ozone decolorization was performed in a batch reactor with complete mixing, the lignins and their derivatives from pulp mill effluents seemed to be converted to the smaller molecules through the pathways illustrated in Figure 6-16. As shown in Figure 6-16 the major chromophoric structures, conjugated double bonds were preferentially attacked by ozone. These reactions resulted in the production of greater numbers of smaller molecules which were

were not those F4s, F3s, and F2s from raw pulp mill effluents. Most of their characteristics were greatly altered. More specifically, the original portion of F2s and F3s may have been transformed into F1s, and at the same time the new portion of F2s and F3s may have been generated by the ozone reactions with larger lignin fragments. However, some chromophoric structures located at the short branch of the lignin molecules in the original F4s, F3s, and F2s may also have been directly converted to F1s with similar characteristics of the original F1s.

6.4 CONCLUSIONS

The following conclusions were drawn from above studies:

1) Color intensity, which is an intrinsic property of pulp mill effluents, is a determining factor influencing the efficacy of ozone decolorization.

2) The kinetics of ozone decolorization appears to follow 3/2 orders kinetic relationship with respect to C.I., and the new kinetic model reasonably well predicts the color reduction.

3) Ozone decolorization has little effect on the molecular weight distribution pattern of the organics with MWCO>1000 detected at 280nm; it converts a large amount of high-molecular-weight fragments (MWCO>1000) into low-molecular-weight fragment (MWCO<1000) which are more biodegradable.

4) The biodegradability is improved if both the initial C.I. and the ratio of high-molecular-weight to low molecular weight fragments are high. On the other hand, the biodegradability deteriorated if the C.I. and the ratio are low.

5) Ozone treatment has little effect on TOC of pulp mill effluents when the dose level is lower than 200 mg/L.

6) Ozone appears to preferentially attack the chromophoric functional groups of lignins and their derivatives but to remove chlorinated organics non-selectively.

7) Ozone treatment induces significant blue shifts on the UV spectra of pulp mill effluents.

6.5 ACKNOWLEDGMENTS

This study was partially financed by Weyerhaeuser Canada Inc. The authors greatly appreciate the assistance of Mr. Steve Cooper, Environmental Manager, Grande Prairie

Operations' Mill, Weyerhaeuser Canada Inc. for providing representative effluent samples. The thanks are also extended to Mr. Murray Carmichael for the help with the ozone study and some analyses.

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CHAPTER 7. EXPERIMENTAL EVIDENCE OF OZONE ENHANCEMENT ON FUNGAL DECOLORIZATION AND DECHLORINATION OF BIOLOGICALLY TREATED KRAFT PULP MILL EFFLUENT

7.1 INTRODUCTION

Ozonation has been shown to be one of most effective means for destroying chromophoric structures in lignin components from pulp mill effluents and converting some of them into biodegradable components (Mao and Smith, 1995a; Prat, 1989, Amero and Hilleke, 1995; Melnyk et al., 1976 and 1977; Baurman and Lutz, 1974). Prat (1989) modified the concept proposed by Melnyk et al. (1976 and 1977) and grouped organic compounds in pulp mill effluents into three groups: group I comsisted of a high molecular weight (MW) lignin components with high density of chromophores and reacted quickly with ozone; group II contained various relatively low color-causing compounds and reacted relatively slowly with ozone, and therefore, had to compete with group III which consisted of those biodegradable or low MW non-colored organic compounds. The kinetic model based on this reaction scheme fitted the data from ozonation of a Kraft bleaching effluent well. More recent studies (Mao and Smith, 1995a; Sozánska and Sozánski., 1991) revealed that the concept was also applicable to ozone decolorization of biologically treated kraft pulp mill effluents (BTKPME). These studies demonstrated that the increase in BOD and simultaneous TOX and odor reductions are additional benefits of ozone treatment. Many studies (Kaneko et al., 1981 and 1983; Nakano et al., 1982; Ferron et al., 1995; Kratzl, et al., 1976; Eriksson and Gierer, 1985; Katuscak, et al., 1971) on ozone reactions with isolated lignins and lignin model compounds also confirmed that ozone preferably attacked double bonds and aromatic structures in lignin components and converted some of them into biodegradable components which contribute to BOD5.

The white-rot fungi *Phanerochaete chrysosporium (ATCC24725) and Coriolus versicolor (ATCC34540)* have been considered as two of the most promising organisms for decolorization and dechlorination of effluents from alkaline extraction (E1) stage (Sundman, *et al.*, 1981; Eaton *et al.*, 1982; Prasad and Joyce, 1991) and combined bleaching effluents (Eaton *et al.* 1980; Livernoche *et al.*, 1983). However, there were few studies using these and other

fungi for decolorization and dechlorination of either combined kraft pulp mill effluents or BTKPME.

Despite the ability of ligninolytic enzyme system of these fungi to degrade lignin components it has been reported that a portion of the heterogeneous lignin derivatives in pulp mill effluents still resist fungal attack (Bergbauer, *et al*, 1992; Sunderman *et al.*, 1981). In other words, the degradation kinetics of this portion was quite slow. These observations naturally led to further hypothesize that some of the lignin derivatives which escaped from conventional bio-treatment may be even more resistant to fungal attack since they had been through a series of transformations and modifications exerted by the physical, chemical and biological processes.

Moreover, the fungal decolorization and dechlorination are secondary metabolizing events and require the presence of easily biodegradable organics as a co-substrate (Kirk, et al., 1978). To date only a few simple carbohydrates have been studied for this purpose. As discussed in above studies the ozone treatment of BTKPME could produce some biodegradable organics in addition to the benefit from structural modifications induced by series of oxidation reactions. However, there is little information concerning 1) if the lignin-derived biodegradable components generated in ozone treatment could serve some requirements for the co-substrate, and 2) if the ozone-modified lignin structures could be better accessible to ligninolytic enzyme system. In other words, whether the ozone pretreatment of BTKPME would produce significant synergistic, additive or negative effects on fungal decolorization and dechlorination.

Recently, the above concerns and the attempts to explore advantages and the synergistic effects of both processes have led to investigating various hybrid ozone/biological treatments. The preliminary study by Heinzle et al. (1992) showed promising results of hybrid ozone/biotreatment process for treatment of high strength sulfite pulp bleaching effluents using the microbial consortia from sewage in aerobic/anaerobic fluidized bed bioreactor system. The results indicated that ozone could significantly increase biodegradability of the high MW components. Unfortunately, the only effects on global parameters COD, TOC and AOX were studied. Roy-Arcan and Archibald (1991) demonstrated the measurable synergistic effects between ozone and fungal decolorization of E-stage effluent and concluded that ozone treatment produced a small improvement in the bioavailability of organics in the effluent. These organics

may partially satisfy the requirements for the co-substrate for fungal decolorization. However, very limited experimental evidence was presented.

The current study intended to quantify the synergistic effects of ozone applications on the following fungal decolorization and dechlorination of BTKPME. To achieve these objectives, a series of experiments were designed to 1) compare the effectiveness of ozone, fungus, and hybrid ozone/fungal processes under various physiological conditions, and 2) investigate the effects of structural alternations and biodegradable components produced by ozone treatment on the effectiveness of the following fungal treatment. The experimental evidence indicated that the ozone partial decolorization had greatly improved both kinetics and degree of fungal decolorization and dechlorination. Also, physiological parameters were found to play an important role in the enhancement.

7.2 MATERIALS AND METHODS

7.2.1 Wastewater Samples and Analysis

The previous ozone decolorization studies (Mao and Smith, 1995a and 1995c) demonstrated that ozone treatment was the most effective on biologically treated effluents with respect to the reduction of color and improvement on the biodegradability. Thus, an bleached kraft pulp mill effluent from an aerated stabilization basin (called ASB effluent) was selected as the target wastewater.

Unless stated otherwise, the raw and treated wastewater samples in all experiments were analyzed according to the procedures recommended in Standard Methods (APHA, AWWA WPCF, 1992). In determination of TOC and BOD, the procedural standards were always carried out with the sample analysis to ensure reliability of analysis and the seed solutions being effective. All tests were run in triplicates except COD analyses were run in duplicates.

To eliminate the possible variance caused by the change of raw wastewater samples between each series of experiments, the raw wastewater samples were always analyzed along with the treated samples.

7.2.2 Determination of True Color

The Canadian Pulp and Paper Association (CPPA) standard method H5.P (CPPA, 1974) was employed with minor modifications for determination of true color. Briefly, prior to filtration, the raw samples were diluted with Milli-Q water if necessary, and pH (Fisher Accumet® pH meter, Model 805 MP) was adjusted to pH=7.6±0.1 using either hydrochloric acid or NaOH solution with the concentration selected to assure the dilution less than 1%. Following pH adjustment, the samples were first filtered through glass-fibre filters (Watman 934-AH) and then through 0.8 μm filters (MSI Micron Separations Inc.). To minimize the effects of filtration on the color removal the filtration time was carefully controlled to within 30 seconds. Finally the absorbance of the filtrate was measured at 465 nm using Spectronic-20 (Bausch & Lomb) and converted to CPPA color units.

7.2.3 Measurement of UV Absorbance

The UV spectra of raw ASB effluents and various components were determined on UV-Vis spectrophotometer (Model HP8542A) using 10 mm quartz cuvette. All the samples for UV analysis were filtered through 0.8 μm non-absorbable filter (MSI Micron Separations Inc.) and pH was adjusted to 7.6 if desired.

7.2.4 Estimation of Total Organic Halogens (TOX)

The total organic halogens (TOX) were extracted from the wastewater samples using microcolumn protocols recommended in Standard Methods (APHA, AWWA WPCF, 1992) and analyzed using Euroglass AOX Analyzer.

In each series of tests, the high or low concentrations of *p*-chlorophenol standards and the blank check on the adsorbed halogen level of GAC were carried out, and each sample was diluted to the level within the range of the concentration in the standard curve. The low level of suspended or colloidal particles remained in the sample since the samples were not pretreated before GAC adsorption. Thus, the results from these tests would subsequently be called as TOX. Figure 7-1 shows the TOX standard curve using *p*-chlorophenol as standard for the estimation of the TOX level in raw and treated pulp mill effluents.

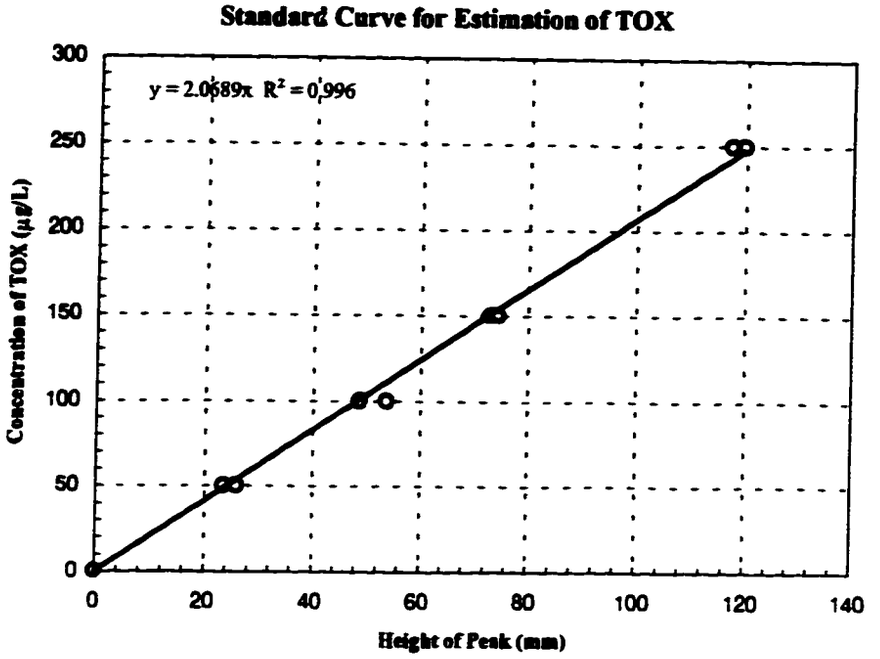

Figure 7-1. Standard Curve for Estimation of AOX/TOX in Pulp Mill Effluents

7.2.5 Estimation of Molecular Weight and Molecular Weight Distribution

High performance size exclusion chromotography (HPSEC) was employed to analyze the molecular weight (MW), molecular weight distribution (MWD), and polydispersity of the raw effluents and their respective components in this study. The HPSEC column (TSK Progel G-3000xl) was calibrated with protein standards (Sigma Co.) and lignin reference standards based on techniques described in previous studies (Mao and Smith, 1995d; Yau, et al., 1979). It had been demonstrated that the effects caused by the electrolyte nature of bleached-Kraft lignins and their derivatives could be significantly reduced by using 0.5M NaCl solution (Forss, *et al.*, 1989). In this study, the resolution and reliability of calibration were further improved by using LiCl-tris buffer as an eluent and eluting at a flow rate of 0.8 mL/min. The lignin reference standards were made using ultrafiltration techniques as described elsewhere for verifying the reliability of the calibration (Mao and Smith, 1995b). As shown in a previous study (Mao and Smith, 1995c and 1995d), the calibration of HPSEC column was reasonably reliable for comparison of MW and MWD of the lignin derivatives in pulp mill effluent. However, it is necessary to keep in mind that the concentration in the reference lignin standards were difficult to

precisely determine, the calibration was qualitative and relative. In other words, the changes in MW and MWD were considered to be relative to each other.

7.2.6 Experimental Design

The details of the experimental design are schematically illustrated in Figure 7-2.

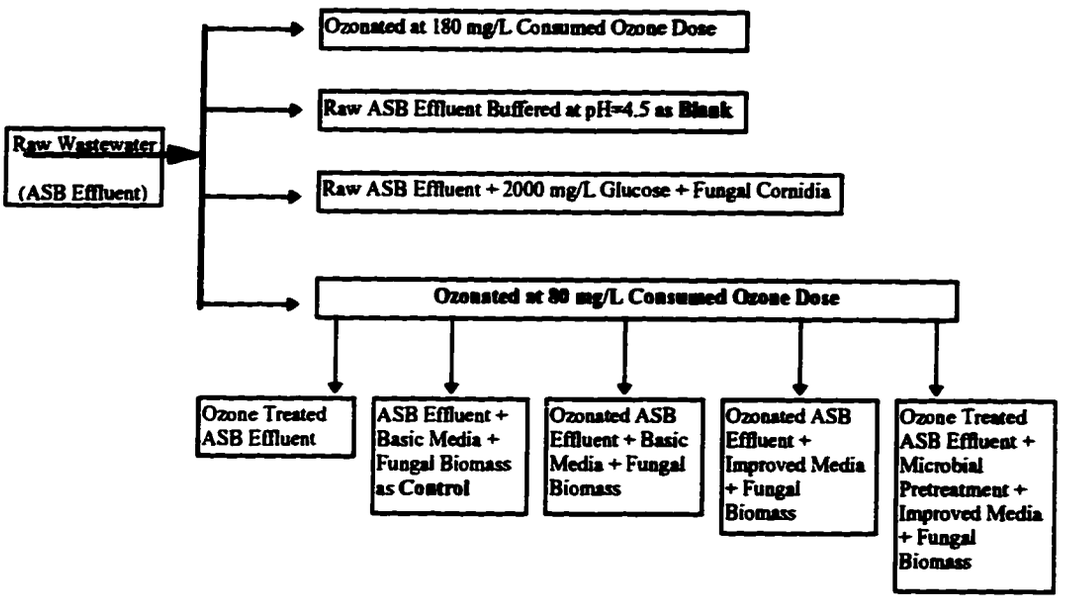

Figure 7-2. Schematic Illustration of Experimental Design for Decolorization and Dechlorination Studies

7.2.7 Organisms, Media and Culture Conditions

The fungus *Phanerochaete chrysosporium* (ATCC24725) was maintained through periodic transfer on 3% malt extract agar slants (pH=4.5±0.5) and preserved at 4°C. To start the culture, the conidial inoculum was prepared and inoculated into one of the media described below:

Basal Medium: 50 mM 2,2-dimethylsuccinate (DMS) buffer, 1.47 mM KH_2PO_4, 0.21 mM $MgSO_4$, 0.09 mM $CaCl_2$.

Medium A: basal medium, 0.22 mM NH_4Cl, 0.1 mg of thiamine*HCl and 1 mL/L of mineral solution as previously described (Kirk, *et al.*, 1978; Wolin *et al.*, 1963).

Improved Medium: Basal Medium, 0.05% Tween 80, 0.5 mM $MnSO_4$, 0.1 mg of thiamine*HCl and 1 mL/L of mineral solution as previously described (Kirk, *et al.*, 1978; Wolin *et al.*, 1963).

The concentration of all above media were made to be 50 times of the concentration used in the study and all the media were stored at 4°C as stock solutions.

7.2.8 Experiments

Ozone Decolorization and Dechlorination

One batch of grab ASB effluent samples with very low concentration of biodegradable organics (see Table 7-1) were used in all series of experiments as the raw wastewater. For ozone studies, the raw ASB effluent was first treated with ozone at about 80 mg/L dose level under the operating conditions as described in another study (Mao and Smith, 1995a). The separated ozone experiments were conducted at about 180 mg/L of ozone dose level under the same operating conditions as those with 80 mg/L. All the ozone experiments were performed using the ozone reactor system as shown in Figure 7-3 and the procedures described in previous studies (Mao and Smith, 1995a and 1995b).

The raw and treated ASB effluent samples were stored at air tight container at 4°C (not more than one month), and used as target wastewaters for further fungal treatment studies. Several series of parallel experiments were performed to investigate the various effects of ozone treatment on fungal decolorization and dechlorination as illustrated in experimental design section.

Fungal Decolorization and Dechlorination

In these studies, the fungal biomass was first grown in a chemically defined medium containing medium A and 1% glucose as a starting growth carbon source. Inoculated media in cotton-stopped 500 mL flasks were incubated for 4 days at 37°C without agitation. After the fungal mycelium was fully developed and the ligninolytic enzyme system started to be established, the fungal biomass was carefully harvested and washed three times with the sterilized 0.8% NaCl solution and then, dewatered by gravity draining for 10 minutes under aseptic conditions. This biomass was defined as wet biomass in the fungal treatment studies. The wet biomass was weighted by the desired amount and used for each series of experiments.

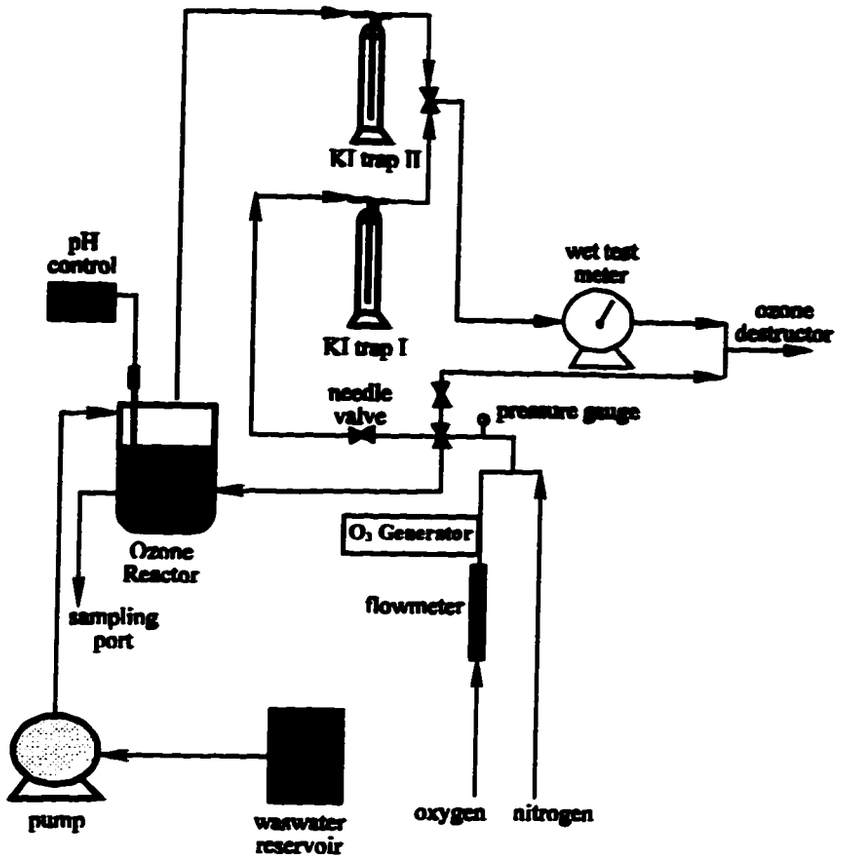

Figure 7-3. Schematic of Ozone Reactor System for Ozone Treatment Study

In fungal experiments, 150 mL raw wastewater samples containing desired carbon-free media were put in 500 mL cotton-stopped flasks. Each test, which was conducted in triplicate, was inoculated with about 10.0±0.5 g wet biomass. Control or blank samples were run in parallel for each experiment. Each set of experiments was repeated three times unless otherwise noted. Inoculations were routinely carried out in a laminar-flow workbench that was disinfected and irradiated with UV light. Inoculated wastewater samples were incubated at 35±2°C for desired period and the flasks were flashed with about 2 L pure oxygen gas every 24 hours at a rate of about 1000 mL/minutes. At the end of the desired decolorization period, the treated samples were first filtered through Watman AH-934 fiber filters to remove the biomass, then, adjusted the pH to 7.6 for determination of true color and UV spectra, TOX, MW and MWD.

7.3 RESULTS AND DISCUSSION

7.3.1 Effects of Ozone Treatment on Lignin Components

Table 7-1 summarizes the major characteristics of raw, ozone treated ASB effluents at about 80 mg/L (two replicates, NR-1 and NR-2) and 180 mg/L (NR-3) consumed ozone doses, respectively. Figure 7-4 shows the UV spectra at pH=7.6 of raw ASB influent (for comparison), ASB effluent and the two replicates (NR-1 and NR-2). These results indicated that 1) the ASB effluent contained a very low concentration of biodegradable organics, a relatively high concentration of chlorinated organics with high color intensity (C.I., C.U./mg TOC). It also appeared that the conventional biological treatment had some impacts on lignin components in addition to removal of biodegradable organics through various physical, chemical and biological processes; 2) ozone treatment was reproducible and reliable since the two replicates reproduced each other with regards the ozone effects on UV spectra of lignin components and other monitored parameters; 3) at an ozone dose of 80 mg/L, the ozone treatment reduced the color by about 45%, and converted less than 5% of the poorly biodegradable lignin derivatives ($BOD_5/COD<0.02$) into more biodegradable organics as BOD_5, but more than 10% of COD reduction was observed. These results also agreed well with previous studies (Mao and Smith, 1995a; Roy-Arcand and Archibald, 1991); 4) further ozone treatment (at about 180 mg/L) reduced color by more than 75% and converted about 20% of the poorly biodegradable lignin components into biodegradable organics (expressed as BOD_5); and 5) in both cases, ozone treatments cleaved some of the C-Cl bonds in lignin derivatives leading to partial dechlorination. It seemed that the majority of the C-Cl bonds in lignin components remained unattacked, and less than 10% of the lignin derivatives were completely mineralized as the TOC changed little after treatments.

Table 7-1. Summary of Major Characteristics of Raw and Ozone Treated ASB Effluent

Parameter	Raw ASB Effluent	Ozone Treated ASB Effluent		
		NR-1 (@80 mg/L)	NR-2 (@80mg/L)	NR-3 (@180mg/L)
True Color (CPPA C.U.)	1390	798	756	346
Color Reduction		43%	46%	75%
TOC (mg/L)	247	243	236	229
BOD_5 (mg/L)	12.9	35.4	37.1	99.2
COD (mg/L)	654	593	597	507
BOD_5/COD	0.0197	0.0597	0.0621	0.196
TOX (mg/L)	7.51	5.01	n/a	4.37

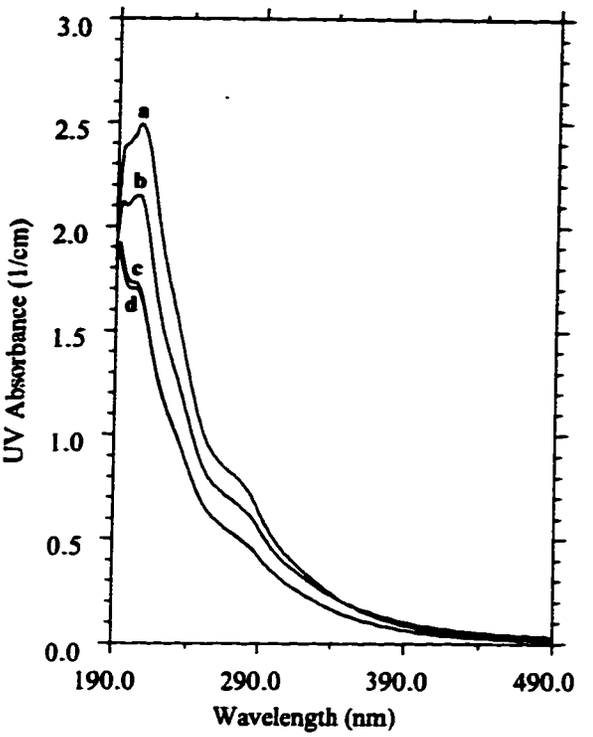

Figure 7-4. UV Spectra of Raw ASB Influent, ASB Effluent and Two Replicates of Ozone Treated ASB Effluent (a: Raw Influent; b: Raw Effluent; c: NR-1; d: NR-2)

7.3.2 Effectiveness of Ozone/Fungal Decolorization and Dechlorination

Figure 7-5 illustrates the effectiveness of different treatments on decolorization and dechlorination of ASB effluent. It is important to point out that, 1) in all cases, the fungal biomass was washed with sterilized 0.8% NaCl solution and there is no supplement of any organic carbon source as a co-substrate, ASB effluent served as sole carbon and energy source. The data in Figure 7-5 indicated that the color of the blanks (raw ASB effluent) increased slightly near the end of incubation. These blank samples were not inoculated with fungal biomass but contained all the components of basic media and the initial microbial consortia present in ASB effluent. The color of the controls, which contained raw ASB effluent without any pretreatment but inoculated with same amount of wet fungal biomass, were reduced by about 32% after 4 days treatment, and the color was also slightly increased after further incubation. In contrast, with the aid of ozone pretreatment at about 80 mg/L, a total about 58% and 68% color reductions could be reached within the first day incubation with the same amount of wet fungal biomass in basic and improved media, respectively. The final color of treated wastewater

samples was reduced to 270±20 with basic media and 180±20 with improved media after 4 days incubation time, respectively. Again, it was observed that further incubation appeared to provide no improvement, instead, the color levels increased slightly. This was probably due to composite effects of slow rate color removal, evaporation and newly released colored metabolites from fungal biomass.

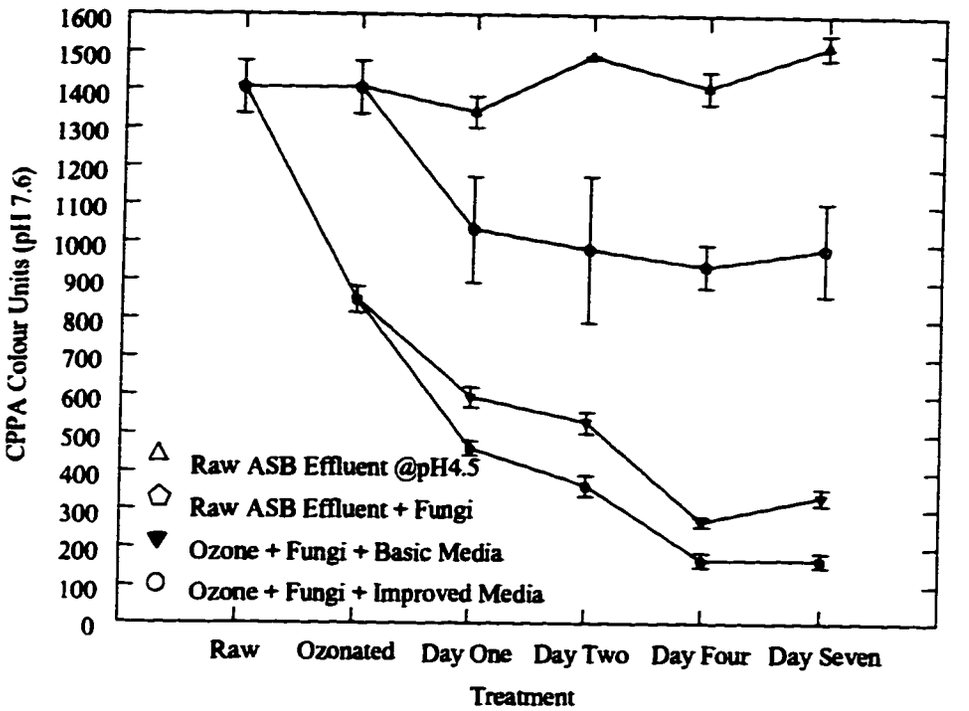

Figure 7-5. Comparison of Decolorization Using Different Treatment Scenarios over Different Periods in a Series of Batch Experiments

Figure 7-5 also demonstrates that 1) the color reduction during the first 24 hours were similar in the cases of control and basic media, but the ozone treatment at about 80 mg/L gave better color removal in following fungal decolorization especially in days 2 and 3; 2) the improved media seemed to be able to improve and sustain the catalytic capacity of ligninolytic enzyme system for decolorization of the ASB effluent. It was also observed that, although not extensively monitored, the annoying odor disappeared almost entirely while the effluent was undergoing this color reduction. These observations were general in a agreement with those reported in a recent study (Prasad and Joyce, 1991). In that study the pregrown biomass of

fungus *Trichoderma sp.* was used for decolorization of E1 effluent, and it was demonstrated that a maximum color reduction of 30% within 3 days at pH=10 was obtained without any substrate addition and further increases in incubation time did not improve the decolorization. However, it was also observed that there was no decolorization during the first 24 hours of exposing the biomass to the E1 effluent. These observations indirectly confirmed that the ozone treatment could more effectively improve the performance and kinetics of fungal decolorization of BTKPME.

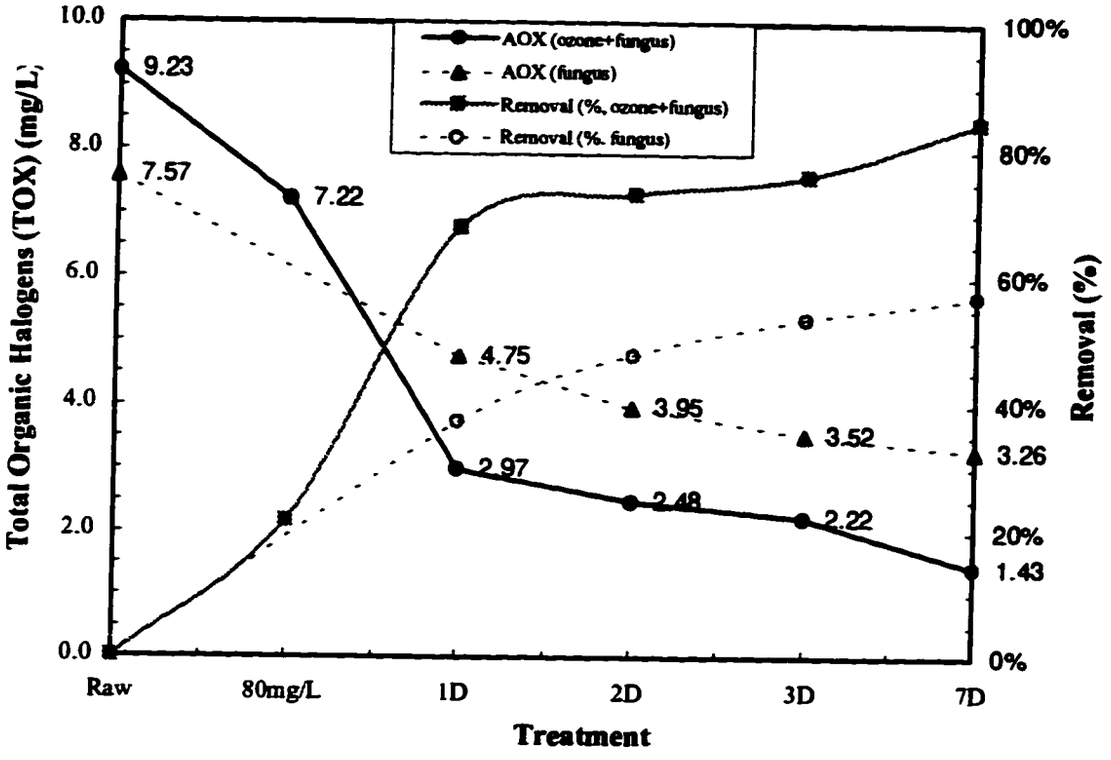

Figure 7-6. Comparison of Removal of TOX by Fungal Dechlorination alone with That by Hybrid Ozone/fungal Dechlorination with Improved Media (80mg/L=ozone treatment at 80 mg/L; 1D, 2D, 3D and 7D means incubation for 1, 2, 3, and 7 days, respectively).

Figure 7-6 compares the effectiveness of dechlorination by hybrid ozone/fungus system with that by fungal treatment alone. With fungal treatment alone in improved media, 55% of TOX was removed over 4 days incubation with 37% removal at first 24 hours. In a early study, Pellinen *et al.* (1988) also observed that about 48% of TOCl was transformed within the first 24

hours of dechlorination of raw E1 effluent, and total dechlorination was about 62%. By contrast, the TOX removal by ozone/fungal treatment was about 80% over same period of incubation and the 59% removal at the first 24 hours. Thus, these results suggested that ozone treatment at 80 mg/L also appeared to increase the TOX removal in the following fungal treatment.

However, the mode and pattern of ozone impacts on dechlorination seemed to be slightly different from those of decolorization. The ozone treatment at about 80 mg/L seemed to only improve the TOX removal during the first 24 hours, then the removal rate of TOX appeared to be comparable in both cases. This may indirectly suggest that the mechanisms for dechlorination was different from that for decolorization, ozone treatment at this level could only create a limited amount of suitable structures which were favorable to the dechlorination enzyme system as discussed in the previous studies (Kirk, 1987, Crawford, 1981). In addition, C-Cl bonds may not be necessary to concentrate on chromophoric structures and seemed to distribute widely among the lignin molecules. For example, in ozone treatment, C-Cl bonds were much more resistant and had less chance to be attacked by ozone molecules, especially at a high decolorization level.

Figure 7-7 shows the effects of the addition of the improved media and removal of biodegradable components from ozone treated ASB effluent (at about 80 mg/L) on the kinetics and degree of decolorization and dechlorination. In Groups A and B decolorization was conducted under the same operating conditions (incubated for 4 days at 37°C) and with almost the same amount of the wet biomass pregrown in the same media. But in Group A the improved non-carbon media was added to the ozone treated effluents while in Group B only the ozone treated effluents were used in the decolorization studies. In Groups C and D the ozone treated effluent was pretreated with acclimatized microbial consortia to partially remove biodegradable components which were assumed to be produced by ozone treatment. It was also observed that microbial pretreatment slightly reduced some of the color from the samples. The further fungal decolorization and dechlorination were performed with the improved non-carbon media.

Comparing Group A with Group B in Figure 7-7 suggested that the addition of the improved media resulted in improvement in both kinetics and degree of decolorization and dechlorination although the enhancement on dechlorination was less profound. As mentioned earlier this is probably because the media could improve the synthesis of the enzymes or the

kinetics of the rate-limiting steps in the biochemical reaction processes involved in the decolorization and dechlorination. However, the mechanism and physiological effects of the improved media was not further investigated in this study.

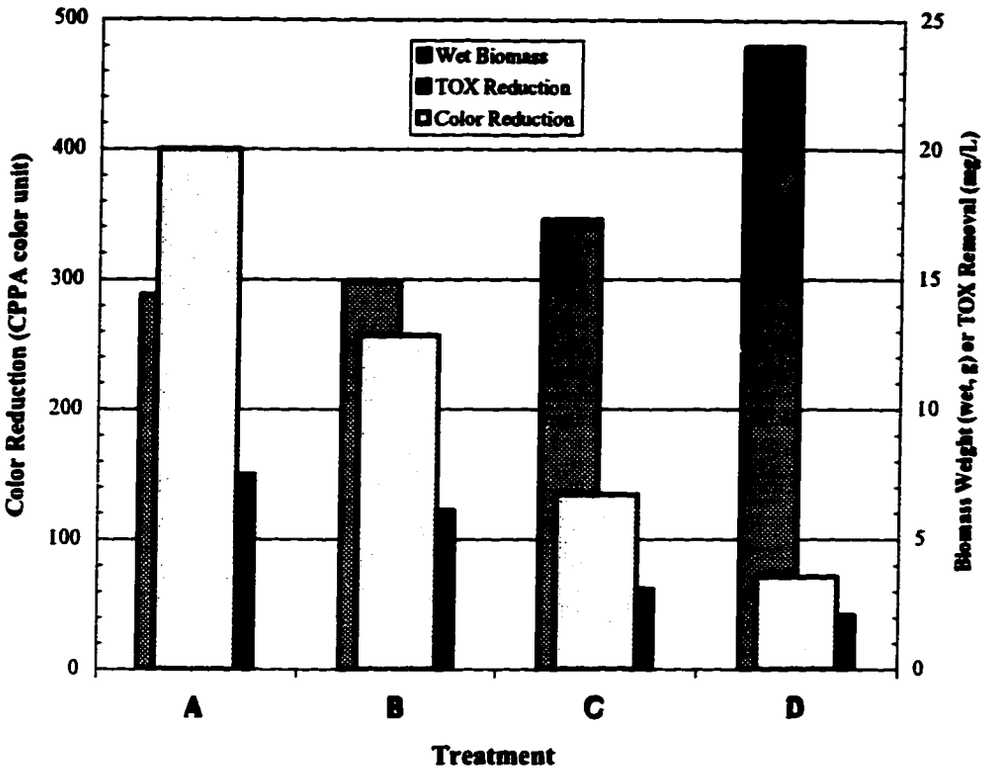

Figure 7-7. Comparison of Effects of Improved Media and Biodegradable Components on Fungal Decolorization and Dechlorination (A: with improved media, initial color level about 750 C.U. B: without improved media, initial color level about 750 C.U.; C: with improved media, initial color level about 600 color units; D: without improved media, initial color level about 500 color units)

Figure 7-7 also demonstrates that by pretreating ozonated samples in Groups C and D with acclimatized microbial consortia to the color levels of about 500 to 600 color units (C.U.) the efficiency of decolorization and dechlorination by the fungus deteriorated by about 50% and 70%, respectively. This was the case although the initially added wet biomass was higher by 15% and 60%, respectively. These results strongly suggested that the new components produced in the ozone treatment could improve the fungal decolorization and dechlorination.

Figure 7-8 illustrates TOX removal along with color reduction using hybrid ozone/fungal process. It demonstrated that the dechlorination occurred in parallel with the decolorization, and the two processes seemed to be compatible. That is, when decolorization reduced the color-causing compounds by about 90%, the reduction of TOX reached about 80%. Combining these results with those from earlier decolorization and dechlorination studies (Mao and Smith, 1995a; Faison and Kirk, 1985; Pellinen *et al.*, 1988) suggested that 1) the fungus may be able to simultaneously produce the two metabolically connected enzyme systems; one portion of these enzyme systems may serve some roles in another system, and were working cooperatively to perform the two biochemical processes in parallel; 2) the kinetics of dechlorination appeared to be slower than decolorization. Some preliminary similar evidence had also been observed in another study (Pellinen, et al., 1988).

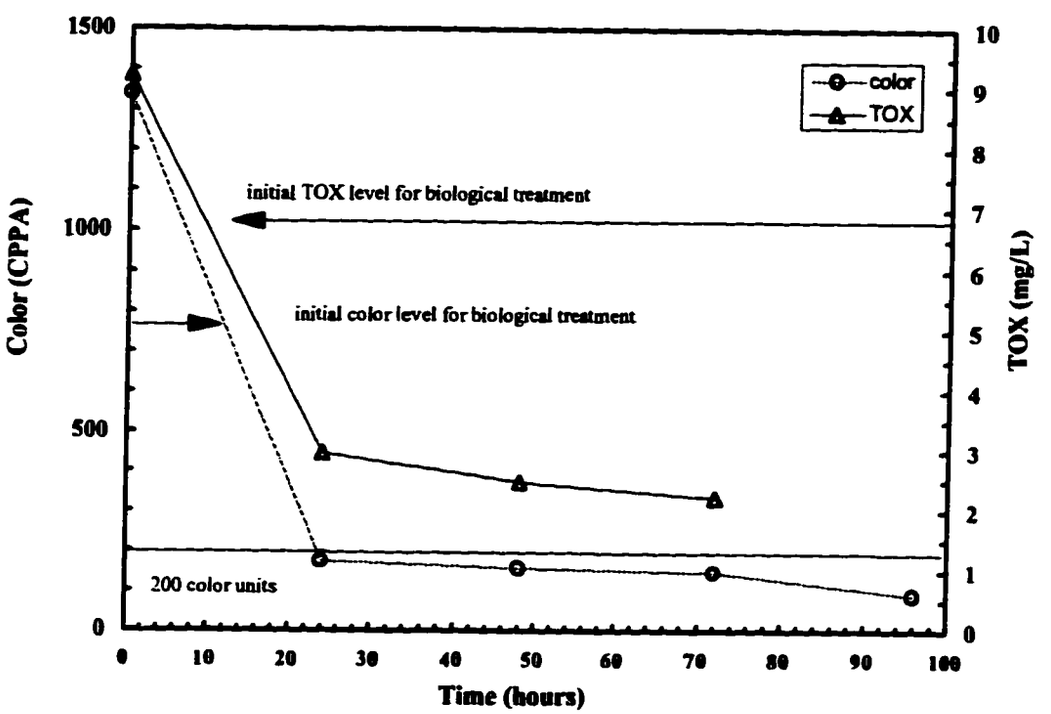

Figure 7-8. TOX Removal along with Color Reduction Using Ozone/fungal Decolorization and Dechlorination of BTKPME

In conclusion, ozone treatment at about 80 mg/L improved fungal decolorization and dechlorination of BTKPME. With the improved media the ozone/fungal process could achieve about 90% of the color reduction and 80% of TOX removal simultaneously. C-Cl bonds seemed to be spread throughout the molecules of lignin components. The evidence of improvement appeared to be more clear in days 2 and 3. It was also possible that 1) there existed a variety of dechlorination enzymes working in parallel with decolorization enzyme system, and both enzyme systems were induced near the end of primary growth phase; 2) the ligninolytic enzyme system may lead to some "accidental" partial dechlorination during breaking down the chromophoric structures.

7.3.3 Evidence of Ozone Impacts on UV Absorbance

Figure 7-9 compares the UV spectra of the control (see Figure 7-3), the samples treated with fungus alone and with hybrid ozone/fungus (the figure inserted in the up right corner was the enlarged peak portion in the original figure for comparison). Figure 7-10 further compares the UV spectra of the control with those of samples treated with fungus alone over 1, 2, and 4 days, respectively. These results suggested that 1) ozone treatment at about 80 mg/L facilitated fungal treatment in reducing the peaks in the region of 190 to 210 nm in addition to the reduction of the shoulder around 280 nm; 2) ozone/fungal treatment appeared to be able to reduce the UV absorbing structures in the molecules of lignin components, which had relatively strong UV absorbance over the range of 190 to 490 nm; 3) fungal treatment alone substantially reduce the intensity of the two major peaks which could also be effected by ozone treatment; however, as shown by Lines B, C, and D, after the 24 hours incubation, the reduction of peaks around 205 and 280 nm was getting less.

Figure 7-11 shows the different effects on the UV spectra between ozone treatment alone and ozone/fungal treatment (the figure inserted in the up right corner was the enlarged peak portion in the original figure for comparison). These observations illustrated that ozone treatment had some preference over reduction of the peaks around 205 nm; but after ozone treatment, the further fungal treatment was obviously in favor of reduction of the absorption from about 210 to 390 with the greatest reduction around 280 nm.

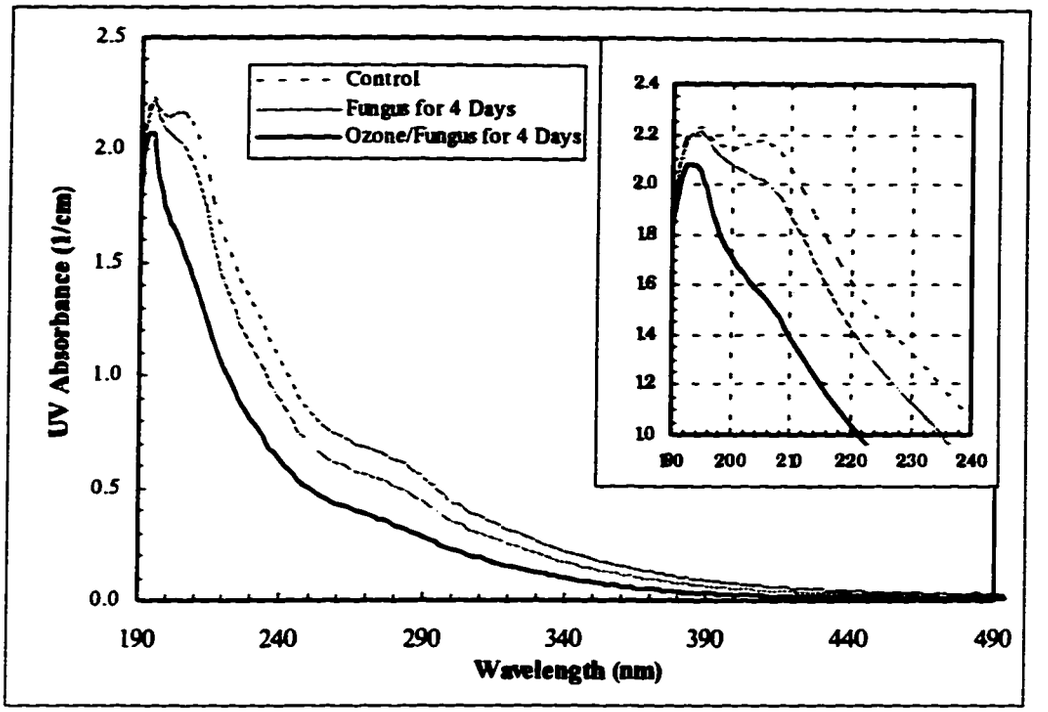

Figure 7-9. UV Spectra of Control and Samples Treated with Different Scenarios (All Samples were diluted as one part of sample in nine parts of Milli-Q water)

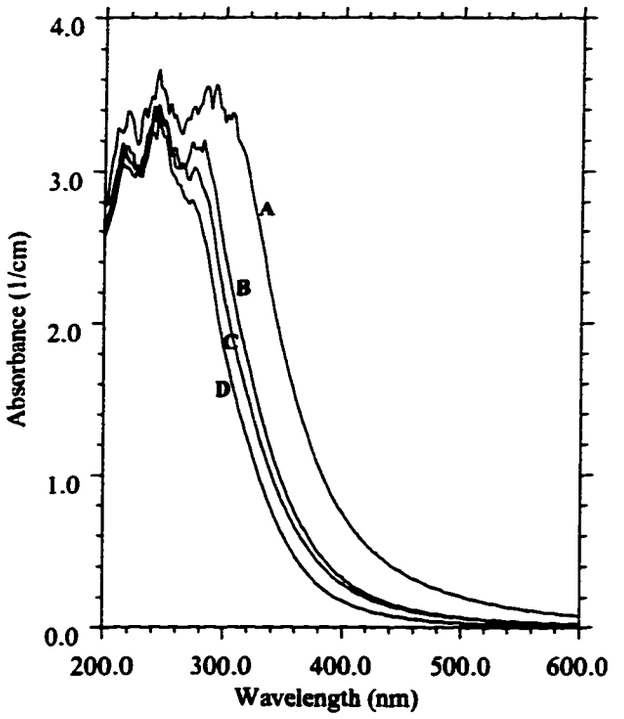

Figure 7-10. Comparison of UV Spectra of Samples Treated with Fungus alone over Different Periods (A: Control; B: Day One; C: Day Two; D: Day Four)

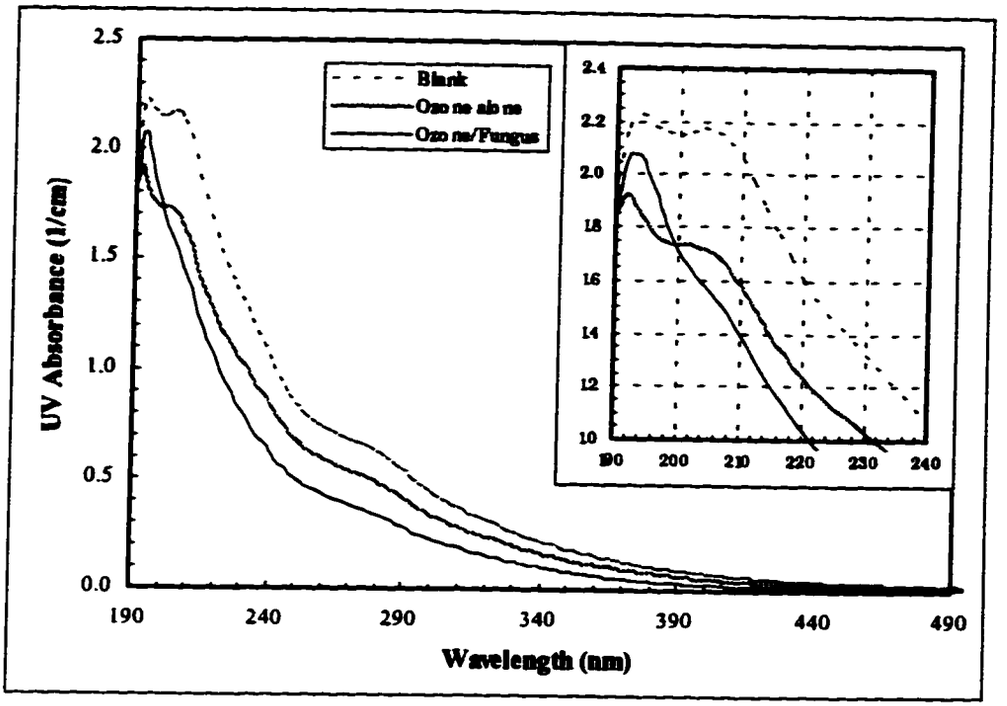

Figure 7-11. Comparison of Effectiveness of Ozone Treatment alone with That of Ozone/fungal Treatment on Effluent. (all samples were diluted as one part of sample in nine parts of Milli-Q water)

Figure 7-12 compares the UV spectra of the sample at pH=4.5 with that at pH=7.6 treated with ozone/fungal process, respectively. Previous studies (Mao and Smith, 1995a; Livernoche, *et al.*, 1983) demonstrated that pH has profound effects on the color of the raw pulp mill effluents and ozone treated samples. However, this is not the case in the samples treated by the ozone/fungal process in this study. As shown in Figure 7-12, pH had negligible effect on the UV spectra of the treated sample after it was adjusted from pH 4.5 to pH 7.6.

Figure 7-13 further compiles the changes (by comparing them to the UV absorbance of raw ASB effluent) of UV absorbance over the range of 200 to 590 nm after different treatment scenarios. In Figure 7-13 the UV spectra of 'Additive' (Line C) is the simple addition of the changes from the UV spectra of ozone (Line B) and fungal treatment alone (Line A) (just for comparison purposes). These results confirmed that 1) ozone treatment alone (Line B) preferentially reduced the UV absorbance around 205 nm although a large reduction of peaks around 280 nm was also observed; and 2) fungal treatment alone (Line A) had much less preference over the various molecules of the lignin components compared to ozone treatment, but

it did have some preference over the structures which gave UV absorbance around 240 nm; 3) ozone treatment at about 80 mg/L almost had the same impacts on the UV absorbance in the region of 340 to 590 nm as fungal treatment alone for four days.

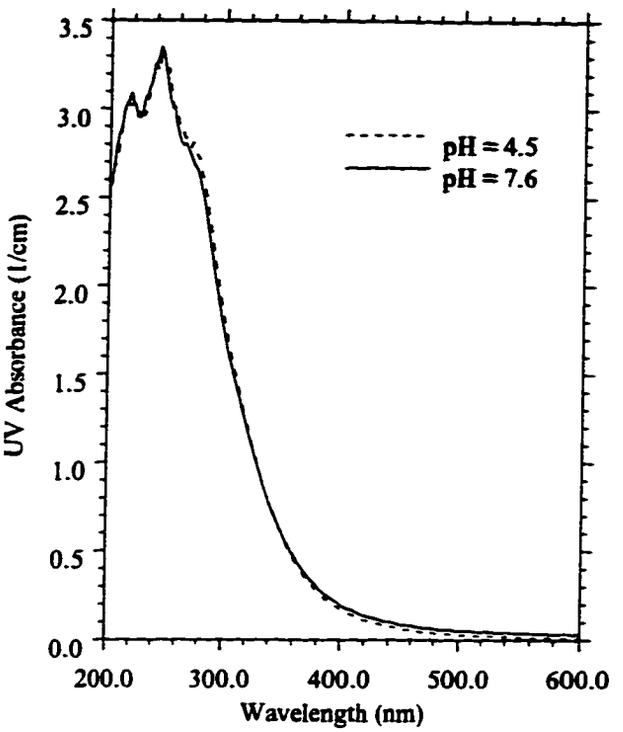

Figure 7-12. Effect of pH on UV Spectra of Samples Treated by Hybrid Ozone/Fungal Decolorization Process over Four Days

More importantly, comparing the change in UV absorption of samples treated with the ozone/fungal process (Line D) with the UV spectra shown by the Additive Line C confirmed that ozone treatment at about 80 mg/L followed by fungal decolorization increased the reduction of UV absorbance over the region of 190 to 340 nm. The greatest increase in reduction of the UV absorbance was more than 50% and was observed to be around 235 nm; the second was about 20% and around 280 nm, and the least was about 10% around 205 nm. The enhancement on reduction of the UV absorbance in the region of 340 to 590 seemed to be much less profound, but no adverse effects were observed.

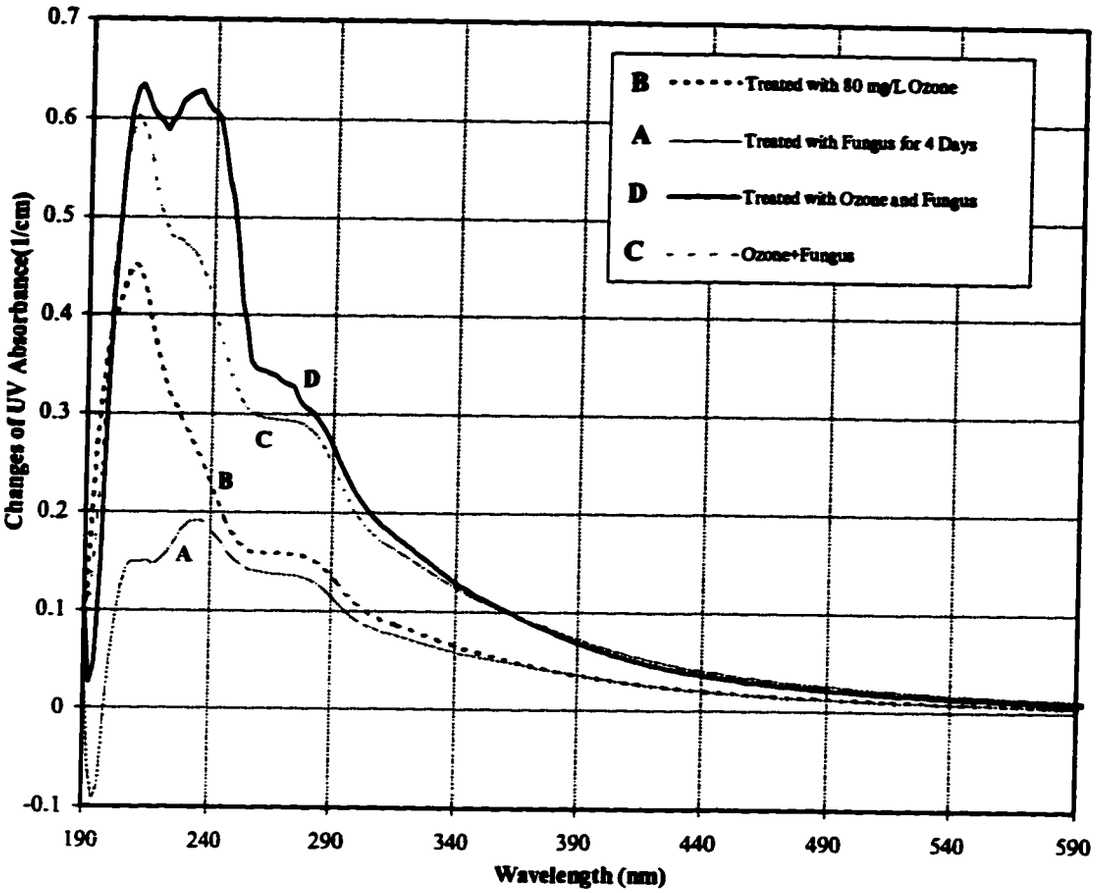

Figure 7-13. Changes in UV Absorbance at 280 nm after Different Treatments (by Comparing to UV Spectra of Raw ASB Effluent)

Figure 7-14 compares the UV absorbance of samples treated by the ozone/fungal process for 4 days (Line A), by ozone treatment alone at 75% decolorization (at about 180 mg/L ozone; Line B) and by fungal treatment alone with addition of 2000 mg/L glucose for pregrowth of fungal biomass (Line C). UV spectra in Figure 7-14 revealed that the addition of 2000 mg/L glucose for pregrowing biomass resulted in a measurable increase in the UV absorbance of the peaks in the range from 205 to 280 nm. This may be partially contributed by the residual glucose and partially contributed by the increased amount of metabolites. In addition, the level of decolorization was also the lowest in this case. This may be due to the high concentration of substrates suppressed the development of ligninolytic system which was also observed in another study (Forney, *et al.* 1982). Figure 7-14 again demonstrated that ozone treatment enhanced the

kinetics of the fungal process which can not be achieved by either of the two treatments. More importantly, the pregrown fungal biomass (in the chemically defined media) appeared to have better decolorization performance than the fungal biomass directly grown in the ASB effluent with the addition of 2000 mg/L glucose.

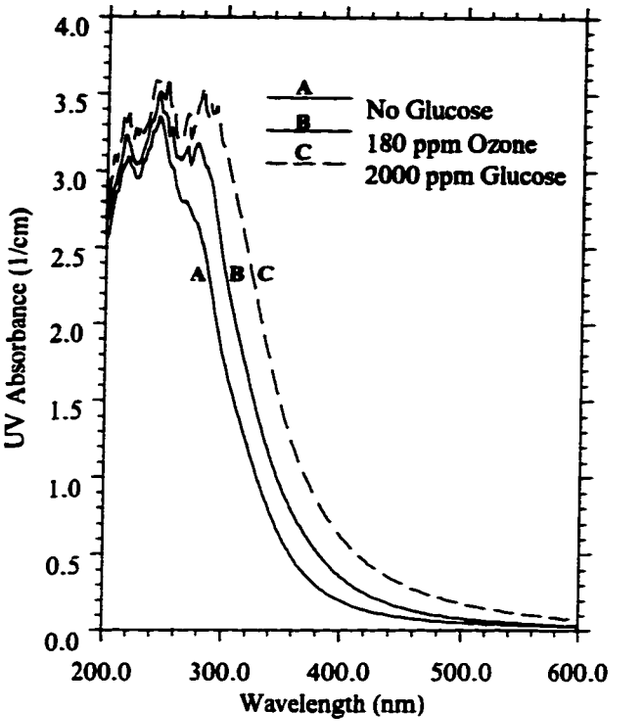

Figure 7-14. Comparison of Effectiveness of Ozone/Fungal Decolorization and Dechlorination with Improved Media with Either Ozone alone or Biological Treatment with Addition of 2000 mg/L Glucose as Pregrowth Substrate on Decolorization (A: preozonated with fungi treatment for 4 days B: 180 mg/L Ozone Treatment; C: Fungal treatment for 9 days (5 days after decolorization start) with addition of 2,000 mg/L glucose).

In summary, ozone treatment enhanced the reduction of UV absorbance in the region of 190 to 340 nm by the fungal process, but the improvement in the region of 340 to 590 nm was less profound. More specifically, ozone treatment preferentially attacked the chromophoric structures in the molecules of lignin components in ASB effluent, but was much less efficient in reducing the UV absorbance around 214 and 244 nm. The UV absorbance in these regions may represent more complex and relatively low MW organics with various functional groups such as C-Cl bonds. Fungal treatment had some preference over the chromophoric structures in lignin

derivatives, but much less profound than ozone treatment. Pregrown fungal biomass had better decolorization performance, and it is not absolutely necessary to supplement an easily metabolizable carbon source to ozone treated ASB effluent for decolorization and dechlorination.

7.3.4 Impacts on Molecular Weight (MW) and Molecular Weight Distribution (MWD)

Figure 7-15 shows the effects of ozone treatment at about 80 and 180 mg/L dose levels on the MW and MWD of ASB effluent. For comparison, the MWD of the lignin reference standard (1000<MWCO<5000 g/mol.) was also plotted in Figure 7-15. As shown in Figure 7-15, after ozone treatment at about 80 mg/L, the majority of lignin molecules were still greater than 1000 g/mol; and the MWD mimicked the pattern of the raw ASB effluent. These results suggested that at about 80 mg/L dose level, the ozone treatment reduced the MW of lignin components substantially, but had little impact on MWD. On the other hand, after ozone treatment at about 180 mg/L, the majority of molecules were broken down to less than 1000 g/mol. More importantly, the MWD was changed from very close to a Gaussian distribution to a bimodal pattern. This observation correlated well with the BOD_5 and COD in Table 7-1 as in both cases there was large increase in BOD_5 after ozone treatment, but the increase after 180 mg/L ozone treatment was much greater than that after 80 mg/L.

Figure 7-16 shows the MWDs before and after fungal treatment in a batch system with the incubation time of 24 hours, 72 hours and 96 hours, respectively. It can be seen that the MW and MWD of the organics before and after fungal treatment shifted toward low MW range considerably. This was also evident that there appeared a small shoulder around 17 minutes which is new compared to the raw ASB effluent. Thus, these suggested that a large amount of high MW organics were removed or converted into the low MW organics with very low UV absorbance at 280 nm. In other words, the fungal treatment appeared to preferably reduce the high MW chromophoric components.

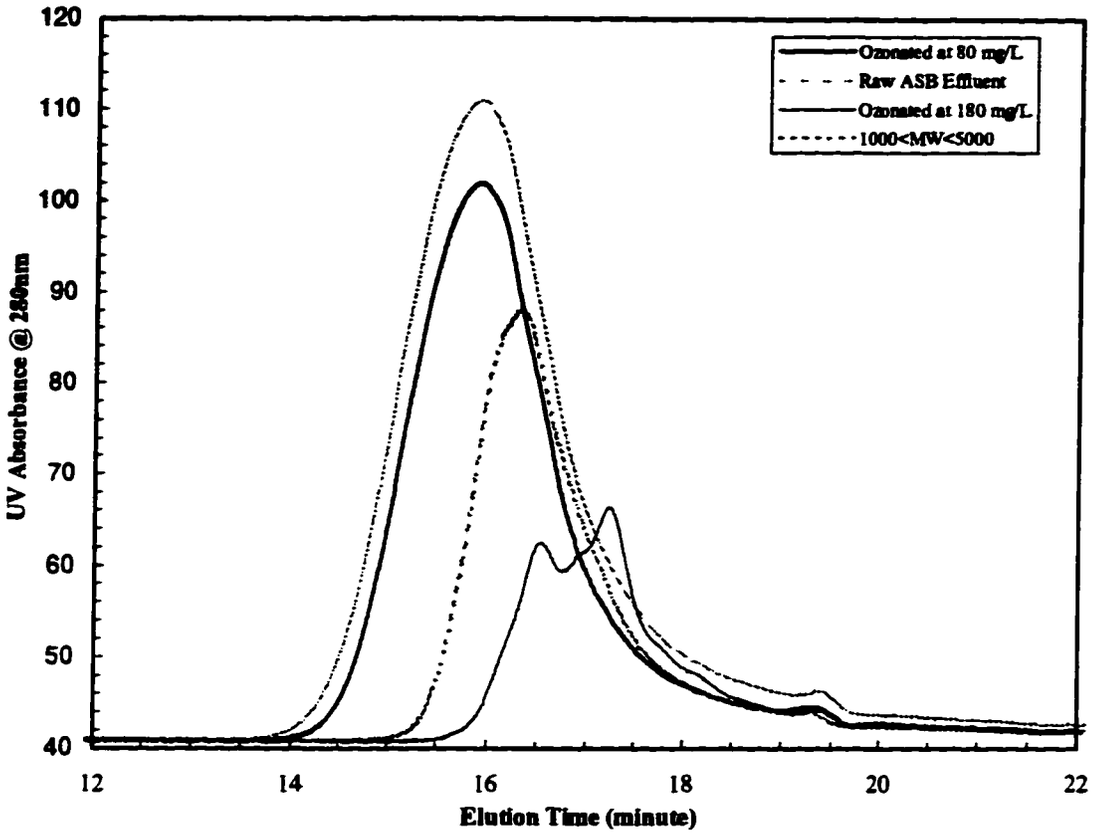

Figure 7-15. Changes in UV Absorbance @ 280 nm after Ozone Treatment at 80 mg/L and 180 mg/L Dose Levels, Respectively. (eluted @ 0.8mL/min with 0.1 M LiCl - Tris Buffer)

Figure 7-17 compares the MW and MWD of raw ASB effluent (Control, Line A), ozone treatment alone (Line B), fungal treatment alone for 4 days (Line C), and the ozone/fungal treatment for 4 days (Line D), respectively. Combining these results with those in Figures 7-15 and 7-16 further confirmed that after 4 days fungal treatment, most of high MW lignin derivatives had been destroyed, but very little low MW organics were detected at 280 nm, this is probably due to 1) a portion of the organics were completely mineralized to CO_2 end products; and 2) as also found in previous studies (Mao and Smith, 1995d; Pellinen *et al.*, 1988), some of newly formed low MW organics could not be detected at 280 nm. However, the shoulder around 17 minutes was presumably the peak representing some of the low MW organics produced in ozone/fungal processes. After the ozone/fungal treatment for 4 days, a small portion of organics

with MW>1000 g/mol. and with certain degree of color still survived the treatment process. Thus, it seemed that some of the high MW portion may be more resistant to either of the treatment, and the level of decolorization and dechlorination using the ozone/fungal process may be limited to less than 90%. The fungal treatment seemed to be more effective than ozone (at 80 mg/L) on destroying high MW lignin derivatives, but much less effective than ozone treatment at 180 mg/L. The ozone/fungal process was able to reduce the high MW portion of lignin derivatives more than either of the two process alone. In other words, the ozone treatment enhanced the accessibility of ligninolytic enzyme to the resistant structures, thus improved the kinetics and degree of fungal degradation.

The mechanism unlined these observations appeared to be well supported with the experimental evidence shown in other studies. As reported by Kaneko *et al.* (1981 and 1983) and Nakano *et al.* (1982), guaiacyl compounds were more reactive toward ozone than veratryl compounds. Moreover, the presence of an α-carbonyl group on an aromatic nuclei led to a reduction of its reactivity with ozone compared to a benzyl alcohol structure. The ozonation of cinnamic acid and the three acids which correspond to the three monomers of lignins (*para*-coumaric, ferulic, and sinapic acids) were made by Haluk and Metche (1986). It was found that, after ozone treatment, these acids were transformed into benzoic aldehydes. Among the other identified compounds, benzoic and *para*-hydroxybenzoic acid were present in large quantities while vanillic acid was observed at a trace level. Syringic acid was not identified. Aliphatic structures detected were produced from the oxidative cleavage of the side chain or from the opening of the aromatic rings at a high ozone dose level. Only glyoxylic acid was shown for carboxylic acids.

All these structures were less competitive when large amounts of chromophoric structures (conjugated double bonds) were present, and the eliminated amount of those organics may be compensated for by the production of those groups of organics from breaking down the high MW components.

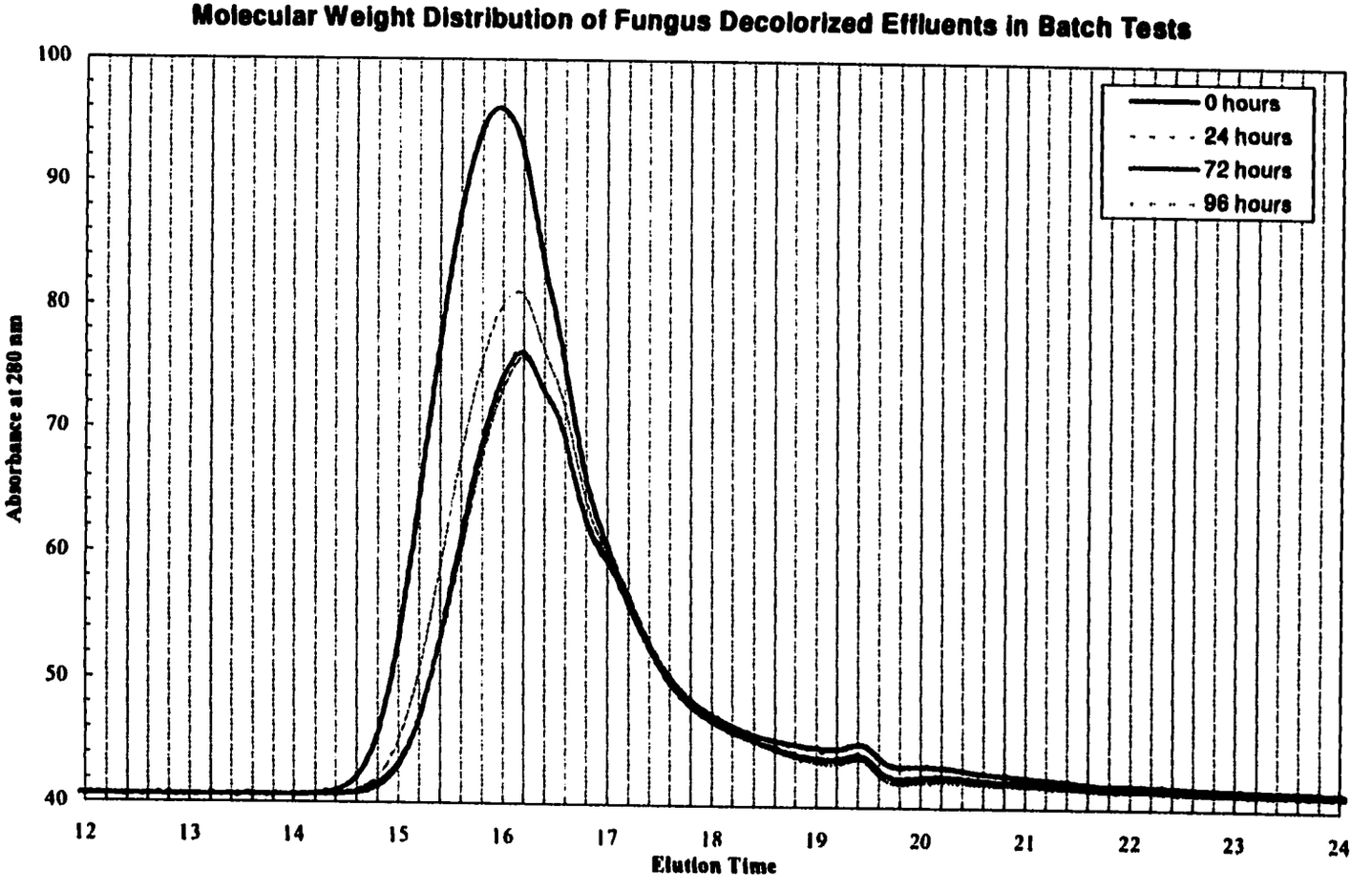

Figure 7-16. Changes in UV at 280 nm Absorbance Fungal after Treatment in Batch Tests

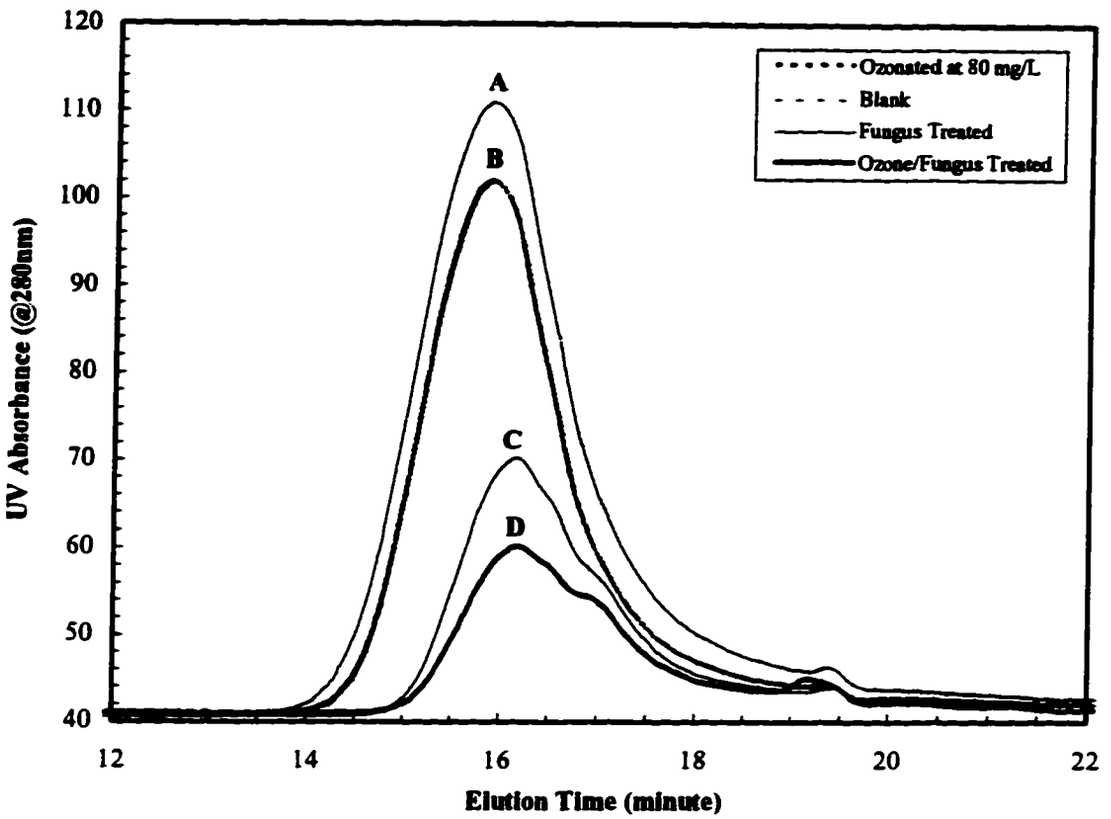

Figure 7-17. Changes in UV Absorbance @ 280 nm after Different Treatments

More recently, investigation of ozone reactions with two other lignin model compounds coniferyl alcohol and ferulic acid further confirmed that at a low ozone dose level (mole ratio O_3/C less than 1.5), both compounds were highly reactive toward ozone (Ferron *et al.*, 1995). It was found that the dissolved ozone molecules first attacked the side chain containing conjugated ethylenic bonds which was significantly delocalized in the lignin structures. Under acidic conditions, larger ozone doses led to attack of the aromatic ring structures. Based on these findings, Ferron *et al.* (1995) proposed the two-stage mechanism as ozone reactions with lignin components: the fragmentation of lignin molecules at the delocalized conjugated ethyenic bonds which may result in significantly smaller lignin molecules in some cases, and further reactions which may lead to opening various aromatic ring structures to form various aldehydes and acids.

In separate studies (Mao and Smith, 1995a and 1995c) it was demonstrated that the efficiency and kinetics of ozone decolorization were proportional to the color intensity (C.I.)

expressed as C.U./mgTOC and inversely correlated with the concentration of biodegradable organics expressed as BOD_5. Moreover, the ratio of C.I. of the fragment with MWCO>10,000 to C.I. of the fragment with 1000<MWCO<10,000 related well to the efficiency and kinetics of decolorization of various pulp mill effluents. For example, it was demonstrated in the above studies that the efficiency of decolorization was decreased by 82% when the decolorization level increased from 35% to 75%.

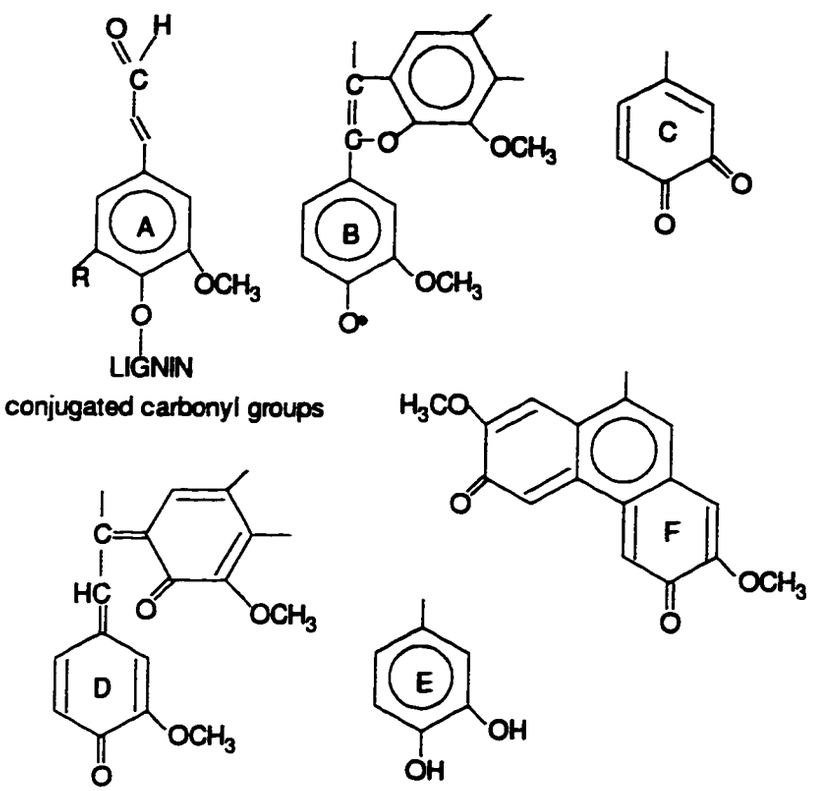

Figure 7-18. Proposed Dominant Chromophoric Structures in 1 Lignin components in ASB Effluent (Developed from Sjöström, 1981, Goldschmid, 1971)

In this study, it was observed that at about 80 mg/L consumed ozone dose level, the ozone treatment had a rapid initial color reduction (>40%), but had a limited BOD_5 increase, and most of the left-over organics still had MW greater than 1000 g/mole. According to the above findings, these observations could lead to the following explanations of the nature of residual lignin components in ASB effluent and the synergistic impacts of ozone treatment on fungal decolorization and dechlorination: The chromophoric structures shown in Figure 7-18 seemed to

be dominant in BTKPME according to the UV spectra determined in this study, the characteristics of BTKPME and the findings about these structures in the previous studies (Sjöström, 1981; Goldschmid, 1971). Most of these chromophoric structures contain the conjugated double bonds (Structures B, D) and some of them may locate on the relative short side chain of molecules as found in the structure model of lignins proposed by various researchers (Sjöström, 1981; Sakakibara, 1991). Thus, when those structures were destroyed at least two effects could be expected: 1) the color would be reduced; and 2) the UV spectra would have blue shifts which was also observed in another study (Mao and Smith, 1995c).

Moreover, some of these chromophores contained quenonic structures (Structure E, F, C) or conjugated carbonyl groups (Structure A). In addition, some of non-conjugated aromatic structures in the lignin derivatives may be somewhat modified in ASB effluent so the difference between the reactivity of aromatic ring structures and that of conjugated double bonds toward ozone may be dramatically narrowed.

Therefore, at a low ozone dose level (about 80 mg/L), a large portion of ozone molecules may be involved in cleavage of conjugated double bonds; at the same time, a significant amount of ozone molecules may be consumed in opening of aromatic ring structures through some reactions as shown in Figure 7-19. As a result, the MW after ozone treatment at 80 mg/L may not dramatically reduced as speculated (Katuscak, 1971), but the structures may be changed substantially.

These observations on ozone enhancement also appeared to be in a good agreement with the findings from physiological studies. Eaton *et al.* (1980) observed that the low MW organics in E1 effluent could stimulate the growth of *P. chrysosporium* in a medium containing cellulose. Ulmer *et al.* (1984) reported an apparent induction of the total ligninolytic system after incubation of *P. chrysosporium* cultures with a dioxane-HCl lignin from wheat straw. Similarly, Greene and Gould (1983) found an accelerated production of H_2O_2 by this organism when incubated with lignocellulosic materials in vitro. Faison and Kirk (1985) confirmed that both ligninase activity and production of H_2O_2 increased in lignin-induced cultures. It is most unlikely that lignins derivatives could act as an inducer because the large size and low solubility precluded them from crossing the cytoplasmic membrane to interact with enzyme system. Consequently, they inferred that the low MW compounds derived from or related to lignin

degradation intermediates would most probably be able to serve the actual inducers of ligninolytic enzyme system.

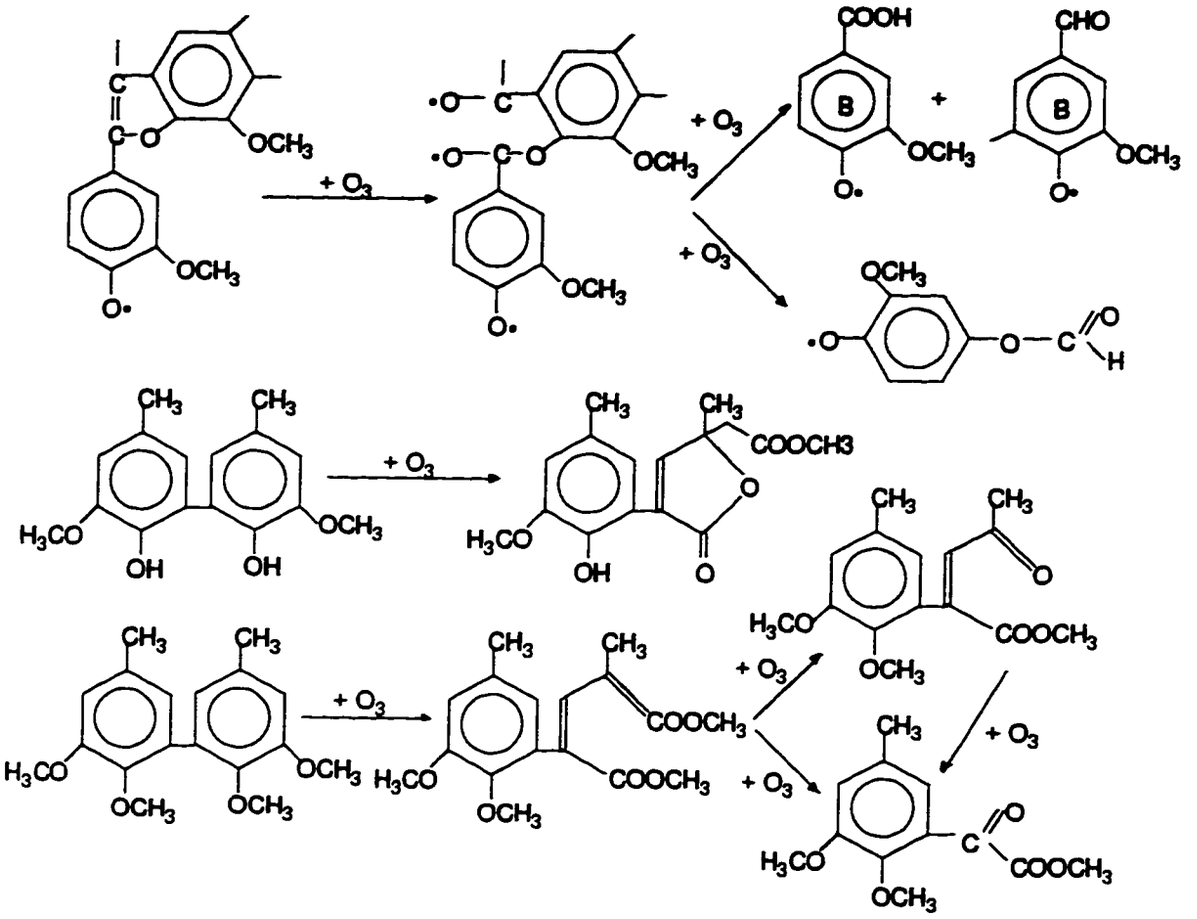

Figure 7-19. Examples of Ozone Reactions with Some Representative Chromophoric Structures in Residual Lignin Components in ASB Effluent (developed from Eriksson and Gierer, 1985; Ferron et al. 1995; Kaneko, et al., 1981 and 1983; Melnyk and Netzer, 1976)

Furthermore, it was reported (Lundquist, *et al.*, 1977) that the lignin derivatives with relatively low MW from the kraft pulping process, in which the side chain structures were largely modified, were degraded by *P. chrysosporium* at a greater rate than protolignin controls. The ozone treatment not only substantially reduced the MW of the lignin derivatives but also may have dramatically altered the structures of side chains in the molecules of lignin derivatives.

Earlier Sundman *et al.* (1981) also showed that in the fungal decolorization process, there was little preference between destruction of chromophores in the polymer and decomposition of the polymer to low MW, colorless, soluble/volatile products. More recently, many physiological studies (Kirk, 1987; Tai *et al.*, 1983) confirmed that the ligninolytic enzyme system had little structural specificity among the lignin structures such as etherified bonds, aromatic ring structures and conjugated double bonds.

In short, the fungal biodegradation of lignin components involved very complex enzyme systems which carried out oxidative fission of aromatic rings, conjugated double bonds, and breaking down part of the polymeric structures. Some of these processes could be the rate-limiting or act as bottleneck steps restricting the overall lignin degradation. Therefore, if these rate-limiting steps or structures could be destroyed or modified using ozone instead of enzymes in fungal decolorization process the overall kinetics of the processes may be greatly improved or accelerated. Ozone treatment, even at relatively low dose levels, could substantially modify the structures and lead to substantially increasing the accessibility of ligninolytic enzymes to the newly modified molecules. More importantly, some of the low MW organics derived from lignin components and lignin degradation intermediates could most probably serve as the inducers of ligninolytic enzyme systems. These compounds may be produced in a ozone partial decolorization process. Obviously, the degree of improvement by ozone would strongly depend on the consumed ozone dose and the reaction conditions.

7.4 CONCLUSIONS

In summary, ozone treatment can enhance the kinetics and overall performance of the fungal decolorization and dechlorination process. With the aid of improved media, the ozone/fungal decolorization and dechlorination process can simultaneously achieve about 90% of color and 80% of TOX removal within 24 hours. The experimental evidence in this study and the results from the previous studies (Chapters 4 and 6; Kirk, 1987; Kirk et al., 1978) suggested that the ozone enhancement of fungal decolorization and dechlorination was through dual mechanisms: 1) ozone partial decolorization may produce a variety of low MW organics which could serve the inducers of ligninolytic enzyme system; 2) ozone treatment may subsequently lead to the increased accessibility for ligninolytic enzymes to the molecules of lignin components by modifying the structures and reducing the molecular size.

More importantly, this study found that 1) the pregrown fungal biomass appeared to have better decolorization and dechlorination than the fungal biomass directly grown in the ASB effluent with the addition of 2000 mg/L glucose; 2) it is not absolutely necessary to supplement an easily biodegradable carbon source for decolorization and dechlorination after the fungal biomass were pregrown in a chemically defined media; 3) it seemed that the level of decolorization and dechlorination using the ozone/fungal process may be limited to less than 90%.

7.5 ACKNOWLEDGMENTS

The authors greatly appreciate Mr. Steve Cooper, Environmental Manager of Grande Prairie Operations' Mill, Weyerhaeuser Canada Inc. for providing invaluable information and help related to this project and representative effluent samples.

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CHAPTER 8. DEVELOPMENT AND EVALUATION OF OZONE/IMMOBILIZED FUNGAL PROCESS FOR DECOLORIZATION AND DECHLORINATION OF PULP MILL EFFLUENTS

8.1 INTRODUCTION

Previous studies have demonstrated that many species of fungi could effectively degrade the high MW lignin derivatives which were largely responsible for the color and TOX. After screening more than 100 species Davis and Wilson (1990) and other investigators (Mittar et al., 1992; Fukuzuimi, 1980) concluded that there were more than a dozen of fungi could effectively reduce the color and chlorinated organics in pulp mill effluents. Among them a few white-rot fungi including *P. chrysosporium* were exceptionally efficient. Using a stationary culture of *P. chrysosporium* Eaton *et al.* (1980) reported that a 60% color removal of E1 effluent within 2 to 4 days, and the fungal biomass could be reused for at least 60 days. Livernoche *et al.* (1982) obtained an 80% color removal from a bleach plant effluent within 3 days using *C. versicolor*. By immobilizing the same fungus in calcium alginate gel, about 80% removal of the color from a combined bleach kraft effluent was obtained continuously (Livernoche *et al.*, 1982). A 99% of color reduction from bleach plant effluents was also reported by Fukuzumi (1980). Later on Eaton *et al.* (1982) and Cambell *et al* (1982) developed a decolorization process using RBC type of bioreactor system. The process could decolorize more than 80% of E1 effluent within 24 hours, and the fungal biomass on the discs of RBC could be used continuously for over 35 days with no apparent loss in decolorization capacity when cellulose was amended to the E1 effluent as co-substrate. Royer *et al.* (1991) also successfully employed a system consisting of fluidized bed reactors with immobilized *C. versicolor* in calcium alginate beads to decolorize the E1 effluent. Using the high-MW components of pulp mill effluents for fungal decolorization and dechlorination study, Pellinen (1988) found that the rates of decolorization, dechlorination and decomposition of chlorolignins were different based on the COD parameter. In one day, color decreased by 65%, TOCl by 49%, and COD by only 14%. Thus, the author suggested that the three processes -- decolorization, dechlorination, and decomposition of chlorolignin -- are metabolically-connected with a higher rate of decolorization. The results from this study also suggested that there were no major differences in degradation rate of molecules of varying sizes,

and the absorption of the lignin derivatives by fungal biomass was insignificant due to its relatvely high solubility in water.

It has been repeatedly reported that the efficiency of decolorization and dechlorination by the fungal biomass was becoming lower after about 20 days. Prasad and Joyce (1991) noticed that in a RBC with sandwich type immobilized fungal biomass system the performance of decolorization of E1 effluent started to drop sharply from initial 57% level after 18 days continuous operation. Using ^{14}C label kraft lignins, Lundquist *et al.* (1977) found the CO_2 production could last for 75 days, but the majority would be collected within the first 20 days. These observations were largely confirmed recently in a kinetic study (Yin, *et al.* 1989) and in a study using another strain of white-rot fungus *Trichoderma sp.* (Prasad and Joyce, 1991) in a continuous flow mode of RBC. These studies revealed that the active life time of fungal biomass could be sustained around 20 days without significant loss of decolorization performance when biodegradable carbon sources were supplemented.

Moreover, it was reported that the half life of fungal pellets in fixed bed bioreactor was higher in batch than in continuous operations, and strongly depended on the initial color level of pulp mill effluents. Royer *et al.* (1991) demonstrated that about 93% decolorization could be obtained after 24 hours when initial color was 1380 C.U. and rapidly decreased to 28% when initial color was 31780 C.U. The author explained that lack of essential nutrient elements and potential toxicity of bleach effluent may be responsible for these observations. By contrast, Prasad and Joyce (1991) found that the performance and life span of fungal biomass in a continuous operation were better than those in batch tests. More importantly, recent studies (Mao and Smith, 1995; Roy-Arcand and Archibald, 1991) found that the ozone partial decolorization could substantially enhance the following fungal treatment in the batch tests under optimal conditions. However, little information is available on how ozone partial decolorization would improve the decolorization and dechlorination in a continuous bioreactor system, and what impacts of ozone dose on kinetics, degree of degradation and HRTs of bioreactor system can be expected. Further studies are obviously needed to clarify these questions.

It is now well established that the degradation of lignin components by white-rot fungi is by large an oxidative process and requires oxygen. Eaton *et al.* (1980) demonstrated that no difference in fungal growth was observed when stationary cultures were incubated under atmospheres of air or air enriched with oxygen. However, when this incubation was continued

into the decolorization period, substantial differences in the rate of decolorization was observed, with the rate being higher at higher oxygen concentration, indicating that oxygen diffusion was apparently the limiting factor for decolorization in the stationary culture. When the culture was agitated under proper environment, this oxygen limitation seemed to be reduced substantially. However, they and other investigators cautioned that the severe agitation should be avoided in the initial growth period for suspended submerged cultures since the mechanical stresses of agitation resulted in poorer performance of fungal treatment.

To minimize the adverse effects of agitation and the problems associated with recycling of biomass in a continuous process the fungal biomass has been immobilized in a laboratory scale with polyurethane foam (PUF), pellet, nylon web, calcium alginate gel, and many other types of immobilizing materials. Recently, Linko and Zhong (1987) systematically compared different carriers such as agar, agarose, k-carrageenan gel beads, nylon web, and PUF for immobilization of fungus *P. chrysosporium* under agitated cultural conditions. The results suggested that nylon web was the best carrier, and both nylon web and PUF can support the bioactivity for at least 38 days. However, SEM micrograph did not reveal significant difference in the mycelia growth. In another separate study, PUF was found to be an excellent carrier for this fungus (Kirkpatrick and Palmer, 1987). In addition, the immobilized living cell system not only provided the protection to the fungal biomass from various adverse effects but also had several advantages over the suspended biomass growth system: 1) the biodegradation kinetics and efficiency were significantly improved; 2) the immobilized biomass was much easier to handle; 3) the efficiency, reliability and reproducibility were better and sustainable. Besides these, PUF system has many other advantages: 1) available in quantity; 2) low cost of immobilization; 3) ease of scaling-up; 4) good mechanical strength; and 5) low toxicity.

The potentials of fungal decolorization and dechlorination have been realized since early 1980's, however, it has not yet been applied in a large scale due to various serious shortcomings which render the technology impractical (Bourbonnais, *et al.*, 1991). Obviously, the bioreactor system is a critical component for a continuous operation in addition to maintaining high efficiency and extending the fungal life span. Great efforts has been made during the past decade toward resolving these problems in the biodegradation of lignin components from pulp mill effluents at a large scale. Several types of bioreactor system have been examined and tested at various scales. These include trickling filters (Messner, *et al.*, 1991), RBCs (Chang, *et al.*,1987;

Yin, *et al.*, 1991) packed/fixed bed (Royer *et al.*, 1991), and expanded/fluidized bed bioreactor systems (Pallerla, *et al.*, 1995). Some of them have been patented in North America and Europe (Chang, *et al.* 1987; Messner, *et al.*, 1987). Prasad and Joyce (1991) recently further examined the decolorization and dechlorination performance of three common types of bioreactors: oxidative pond, packed bed with aeration, and RBC with sandwich type immobilized fungal biomass system. The results confirmed that RBC was most attractive type of bioreactor. It is also interesting to note that the plug flow mode of the continuous RBC process had slightly better decolorization kinetics than completely mixing mode. This may be due to the kinetics of decolorization which was reported to be approximately first order when the initial color level was less than 8000 C.U. (Yin, *et al.*, 1989).

Recently the USEPA launched a new regulation on reducing the discharge of chlorinated organics (0.15 kg TOX/ADT) after incorporating the new research findings and consulting industrial and scientific communities (Federal Register, 1993). Regulations on reduction of TOX and color in Canada will soon be subject to review. Conventional biological treatments such as aerated stabilization basins (ASB) and activated sludge are common practice in existing pulp mills. The effluents from ASB still contain a large amount of chlorinated, colored organics derived from lignin components. Previous studies (Mao and Smith, 1994 and 1995b) also revealed that more than 90% of the color causing components in the ASB effluent had molecular weights (MW)>1,000 g/mole, and were known to be recalcitrant to conventional microbial population in aerobic treatment processes.

The objectives of this work are to develop a bioreactor system with an immobilized fungal biomass to achieve a continuous decolorization and dechlorination of BTKPME, and to evaluate the feasibility of such a process under various conditions. Additional objectives are to use this bioreactor system to verify the effectiveness of the ozone-fungal decolorization and dechlorination under continuous operation conditions, to study the effects of ozone dose levels and other key process parameters on the efficiency of a continuous decolorization and dechlorination, and to study the correlations of color and TOX removal with TOC, molecular weight (MW), and molecular weight distribution (MWD) of lignin components in pulp mill effluents. To achieve these goals, an immobilized technique employing PUF microcarrier and a laboratory-scale bioreactor system with these immobilized fungal biomass were developed. The SEM techniques were employed to evaluate the physiology and dendy of the immobilized fungal

biomass. Series of parallel batch and continuous tests were carried out under various conditions using this new bioreactor system and ozone treated ASB effluents at about 50 and 80 mg/L ozone dose levels. The process parameters, true color, TOX, TOC, UV spectra, MW, and MWD were used to assess the performance of the process and the system. The results showed that the new system can achieve about 90% decolorization and 80% dechlorination of BTKPME under a continuous operation. The treated effluent could meet the proposed USEPA TOX regulations and had less than 200 C.U. (CPPA).

8.2 MATERIALS AND METHODS

8.2.1 Sample Preparation and Analysis

Unless stated otherwise, the procedures for characterization of raw and treated samples were essentially identical to those described in Chapter 7.

8.2.1.1 Ultrafiltration Separation of Treated Samples

The raw and treated samples were concentrated to about 50% of the original volume using Millitan System equipped with MWCO=1000 membrane. All fragments were analyzed for TOC using the established procedures. The detail procedures have been described in Chapter 2.

8.2.1.2 Dry Weight of Fungal Biomass

The fungal biomass was recovered from the bioreactor and dried overnight at 104°C , then, put into dessicator to a constant weight.

8.2.1.3 Estimation of Molecular Weight and Molecular Weight Distribution

High performance size exclusion chromotograph (HPSEC) was used to assess the effects on molecular weight (MW) and molecular weight distribution (MWD) of the raw and treated samples. The detail procedures have been described in Chapters 2 and 7.

8.2.2 Organism and Culture Conditions

The fungus *Phanerochaete chrysosporium* (ATCC24725) was maintained on 3% malt extract agar slants (pH=4.5±0.5) and preserved at 4°C as described in Chapter 7. The fungus biomass was cultured similarly in a chemically defined medium as described in Chapter 7.

8.2.3 Preparation of Specimens for Scanning Electron Microscopy (SEM) Examination

Different stages of fungal biomass were used for comparative investigation of changes during immobilization and decolorization. Small pieces of each sections were fixed by immersion in 2% glutaraldehyde solution in 0.1M phosphate buffer for 2 hours. The specimens were post-

fixed with 1% OsO_4 solution for 2 hours. After two washes in distilled water they were dehydrated by 15 minutes changes in graded series of ethanol until 10% and kept them there for 1 hour (4 changes of 15 minutes). Afterwards, the specimens were transferred to mixtures of amyl acetate-ethanol. The concentration of the first substance was gradually increased through six steps until 100%. The fixed specimens were critical point dried with liquid CO_2. Dried specimens were mounted onto aluminum stubs and coated with approximately 100 to 200 Å of gold-palladium. Micrographs were obtained with Hitachi S-2500 Scanning Electron Microscope.

8.2.4 Development of Immobilization Techniques

After a preliminary screening study and with the consideration of the findings reported in the literature, the issues discussed in Chapter 1, the material selected for immobilization was polyurethane foams (PUF), which are commercial products.

For comparative studies, the immobilization material PUF was cut into 10-mm cubes. For continuous studies, the PUF was shaped to fit the bioreactor design (see Appendix II). In both cases, the PUF were conditioned before the use for immobilization of fungal biomass. The conditioning process intended to improve the stability and catalytic capability of immobilized fungal biomass as shown in later experiments. During conditioning processes, all the immobilization materials were first soaked in a diluted buffer solution for 24 hours at 50°C, then, autoclaved at 121°C for 15 minutes. The diluted buffer solution contained 0.1M DMS buffer, 0.1% Tween 80, 0.25M NaCl, 0.1M $CaCl_2$, 0.1M $MgSO_4$, 0.05M $MnSO_4$ and 0.1M NH_4NO_3.

Following conditioning, the material was inoculated with the fungal conidia suspension under strict aseptic conditions. The initial inocula was the newly sub-cultured fungus *P. chrysosporium* (ATCC24725) conidia suspension with about 10^6 spores/mL. After initial inoculation, the inoculated PUFs was left for drying in the clean biological fumehood for about 30 minutes to allow the fungal spores to be effectively attached to the immobilization material. Following this, the inoculated PUFs were completely soaked in the chemically defined growth media as described in Chapter 7 for 24 to 36 hours and incubated at 37°C. During this period of time, the material was turned over about every 4 hours under strict aseptic conditions.

In the comparative study, the immobilization materials inoculated with the desired spores were first pregrown in a 1% glucose medium for 3 days, after the fungal biomass was fully developed and properly immobilized on the PUF particles, and the ligninolytic enzyme system was well established; this immobilized biomass was transferred to the target wastewater and

incubated at 37±2°C for desired periods with either buffer or pH control system under the moderate mixing conditions. The target wastewater was ASB effluent, which was treated at various desired ozone doses with and without the addition of the mineral media, was used as raw wastewater for decolorization and dechlorination studies, the details were described in Chapter 7.

In the continuous decolorization study, when the preliminary fungal biomass started to develop, the material was assembled into each immobilized system under the aseptic conditions (see photographs1 and 2). The bioreactor chambers, the immobilization system, pump tubings and various other components must be completely disinfected with a fungicide for 24 hours before use. After the initial startup, the bioreactor was maintained at pH=4.5±0.5 with a pH control system and proper concentration of NaOH. When the desired fungal biomass was fully developed in the bioreactor the ASB effluent treated with ozone at various dose levels was adjusted to pH=4.5±0.5 using 1N H_2SO_4 and applied continuously to the immobilized fungal bioreactor. The effluent from the bioreactor was sampled about every eight hours and stored at 4°C for analysis.

8.2.5 Design of Bioreactor System

8.2.5.1 Design Considerations

Considering of the findings from the bench-scale studies (see Chapters 1 and 7) and previous physiological studies (Kirk *et al*, 1978), the following criteria were incorporated into designing the bioreactor system to achieve the decolorization and dechlorination of BTKPME.

1. Biomass has to be pre-grown in the proper growth media other than raw pulp mill effluents.

2. The active biomass has to be properly immobilized in the bioreactor so that: a) the biomass can continuously access the lignin components in the raw wastewater; b) desired mixing, or other potential effects in the bioreactor would cause the minimum adverse effects on the living biomass.

3. During pre-growth and regeneration period, for proper immobilization and mass transfer, it is necessary to prevent the surface of the immobilization system from being densely covered by the biomass, thus, providing the proper agitation in the bioreactor during these period is absolutely necessary.

4. The fungus for decolorization is very sensitive to the pH change and has a very narrow optimum pH range; in addition, the decolorization will significantly lower the pH of the wastewater; thus it is critical to equip the bioreactor with a reliable pH control system.

5. Temperature of bioreactor should be controllable within the optimal range (35 to 39°C).

6. To enhance the decolorization and dechlorination it appears necessary to use oxygen enriched air or pure oxygen.

7. A plug flow pattern is favored to the kinetics of decolorization and dechlorination, but mixing along the cross section is required to reduce the possible limitations on mass transfer of dissolved oxygen and substrates.

8. The apparent hydraulic retention time (HRT) of bioreactor should be adjustable by changing either the level of liquid in the bioreactor or the feeding flowrate of the raw wastewater.

9. The reactor should be covered to reduce the evaporation and prevent any potential contamination; and

10. The bioreactor chamber should be completely drainable.

8.2.5.2 Components of Immobilized Bioreactor System

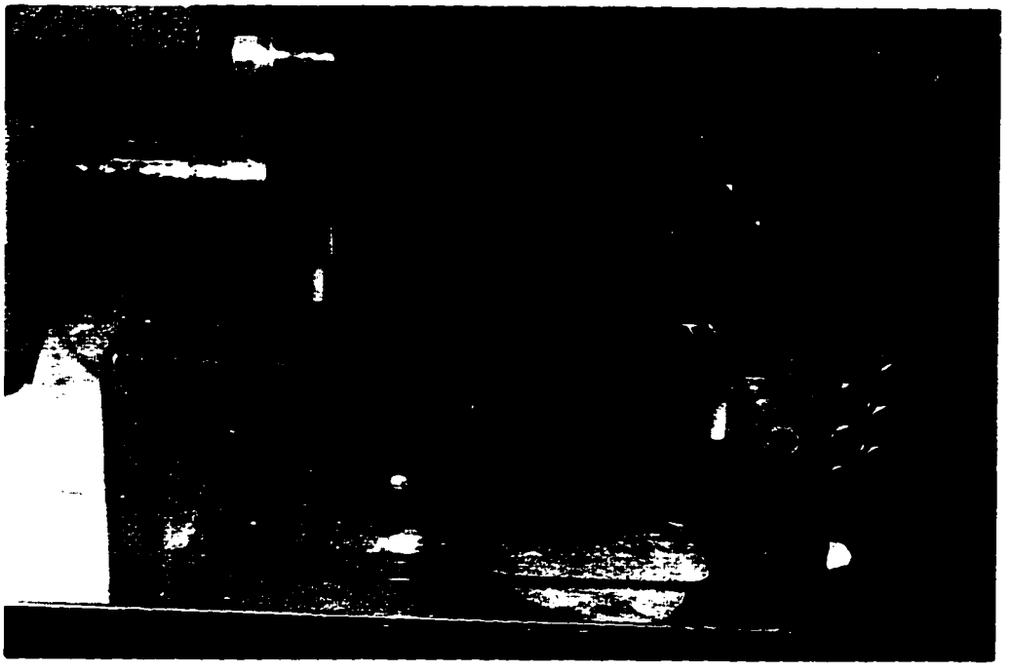
The new bioreactor system consists of six major components: 1) two-compartment bioreactor chamber with a cover for holding the wastewater and the immobilized biomass in a well-control environment; 2) the heating jack for temperature control; 3) an oxygen delivery system for providing sufficient dissolved oxygen for decolorization and dechlorination; 4) pH control system; 5) fungus immobilization systems to support active biomass for decolorization and dechlorination; and 6) a rotating system. Drawings No. 1 through No.5 illustrate the details of the structure and dimensions of the new bioreactor system. These drawing were collected in Appendix III. The description of the major components of the bioreactor system and their functions are summarized in Table 8-1.

Table 8-1. Summary of the Major Components of the Bioreactor

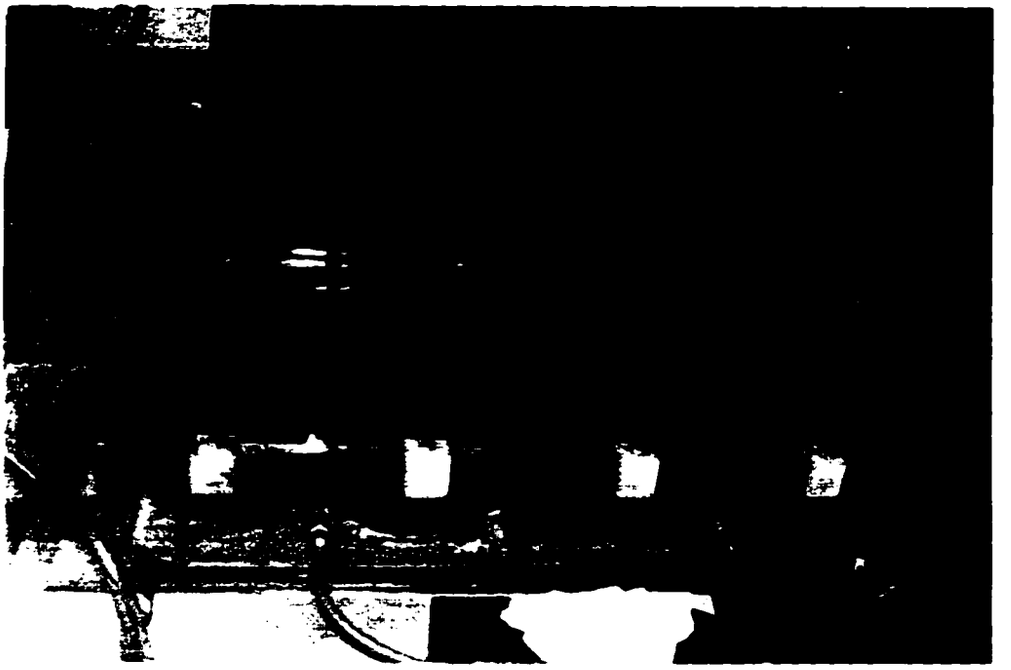
Drawing No.	Description	Function
No. 1	Front view of the bioreactor chamber without immobilization and rotation systems	provide a proper environment for decolorization and prevent contamination
No. 2	Top view of the bioreactor without the cover, immobilization and rotation systems	
No. 3	Structure view of immobilization system	support biomass and provide necessary environment for decolorization and dechlorination
No. 4	Detail view of distribution plates	distribute the nutrients and gas in the immobilization system
No. 5	Injection system	introduce the nutrients and oxygen gas

The top, left, and front view of the bioreactor system after the complete assembly are shown in Photographs 1, 2, and 3, respectively, and the individual components of the bioreactor system after the proper assembly are shown in Graphs A through J which were illustrated in Appendix II. The top view of the bioreactor system loaded with immobilized fungal biomass and after a period of operation is demonstrated in Photograph 4.

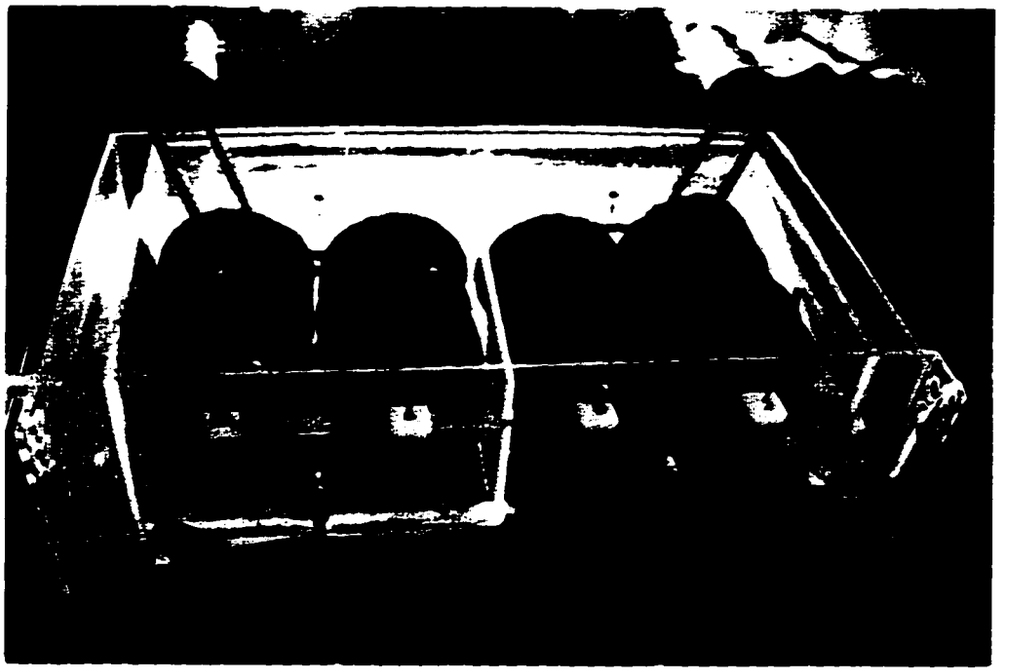
Photograph 1. Top View of Newly Assembled Bioreactor System (without Top Lid)



Photograph 2. Left View of Newly Assembled Bioreactor System (without Top Lid)



Photograph 3. Front View of Newly Assembled Bioreactor System (without Top Lid)



Photograph 4. Bioreactor System Loaded with Immobilized Fungal Biomass and after Several Series of Decolorization Experiments

8.2.6 Experiments

The ASB effluent was used as the raw wastewater for both comparative and continuous studies. The characteristics were similar to those summarized in Chapters 3 and 7.

8.2.6.1 Ozone Treatment Study

To enhance the decolorization and dechlorination the raw ASB effluent was treated with ozone at various ozone doses and under optimum operating conditions as described in previous studies (Mao and Smith, 1995a) and Chapter 7. The protocols for ozone treatment study and sample analysis were essentially identical to those described in Chapters 4 and 7.

8.2.6.2 Comparative Studies on Immobilized Biomass System

Series of parallel experiments were designed to optimize the process conditions for a continuous study. The protocols for optimization study were essentially the same as those described in Chapter 7 except for using immobilized biomass in some series of experiments. The detail design are schematically illustrated in Figure 8-1

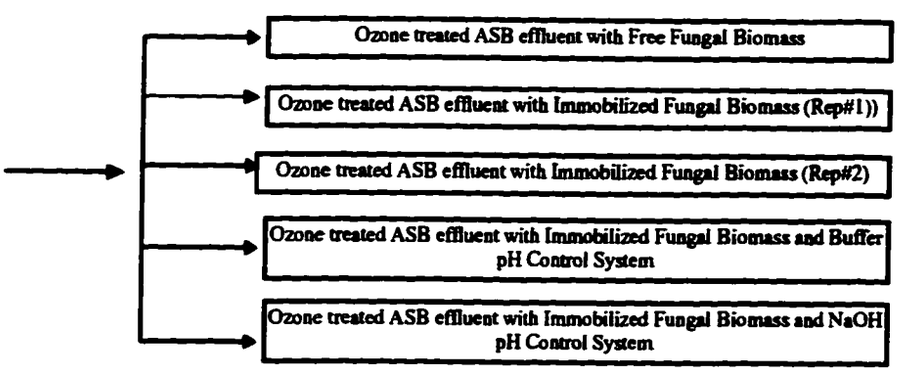


Figure 8-1. Schematic Illustration of Experimental Design for Comparative Studies

8.2.6.3 Continuous Decolorization and Dechlorination Study

The experimental system for continuous decolorization studies are schematically illustrated in Figure 8-2. In Figure 8-2, the feeding tank was preserved in the ice bath to reduce the potential biodegradation and the feeding pump was controlled at the precision of 0.01 mL/minute with a computer flow control system. The hot water (at about 50°C) in the hot water tank was circulated through the heating jack to maintain the bioreactor at 37±2°C by adjusting

the flowrate. The four identical cylinder type of immobilized fungus systems were installed uniformly in the bioreactor chamber, and the two systems in each side were linked by a plastic chain system and rotated with a variable speed DC motor. During initial startup, the rotating speed was increased from about 0.5 to 2 r.p.m. with every 12 hours interval. After the initial startup, the systems were continuously rotated at the desired speed depending on the loading and hydraulic retention time.

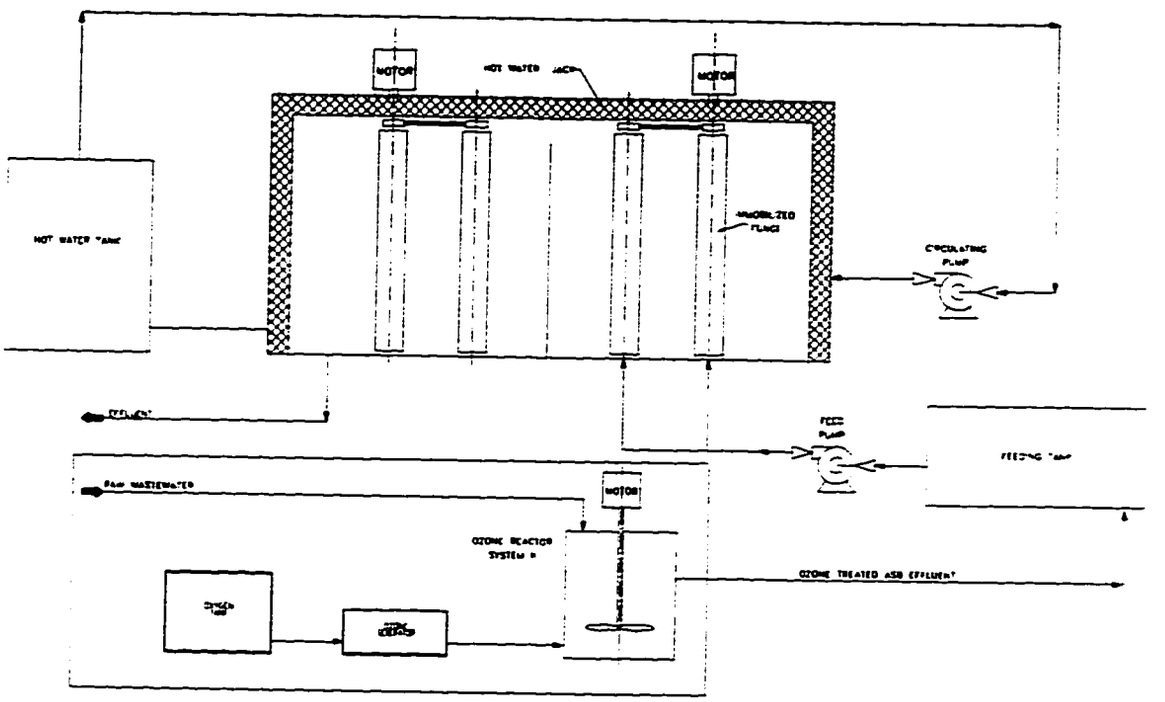


Figure 8-2. Schematic of Immobilized Ozone/Fungal Decolorization and Dechlorination System

8.2.6.4 Experimental Design for Continuous Decolorization and Dechlorination

Figure 8-3 illustrates the series of continuous decolorization and dechlorination tests. Due to the limited funding some of the tests were continuously run only about five (4 to 5) HRTs time which was relatively short but give acceptable results for comparison. All the fungal decolorization and dechlorination tests were conducted at the optimum conditions as summarized in Table 8-2. No effort was made to optimize of the bioreactor operation.

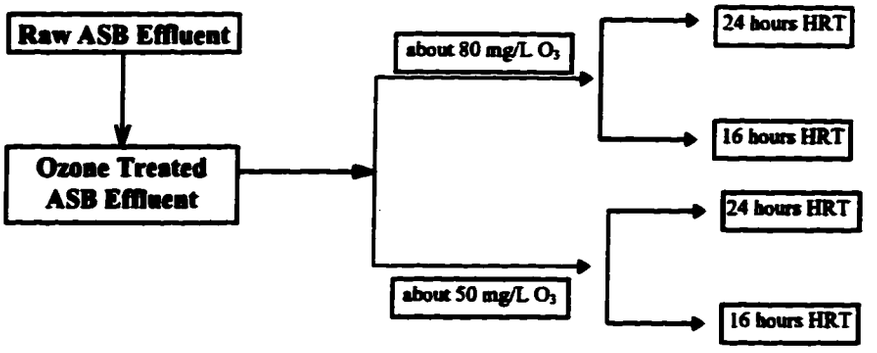


Figure 8-3. Schematic Illustration of Experimental Design for Continuous Decolorization and Dechlorination Studies

Table 8-2. Conditions for Continuous Decolorization and Dechlorination Tests

Parameter	Conditions	Comments
pH	4.5±0.5	critically controlled, optimum range
Temperature	37±2°C	degree of effect unknown, optimum range
Wastewater	ASB effluent	low biodegradability
Oxygen flow	0.5 L/min	affect D.O. in bioreactor
Rotation speed	0.5 to 2 rpm	affect the mass transfer in bioreactor

8.3 RESULTS AND DISCUSSION

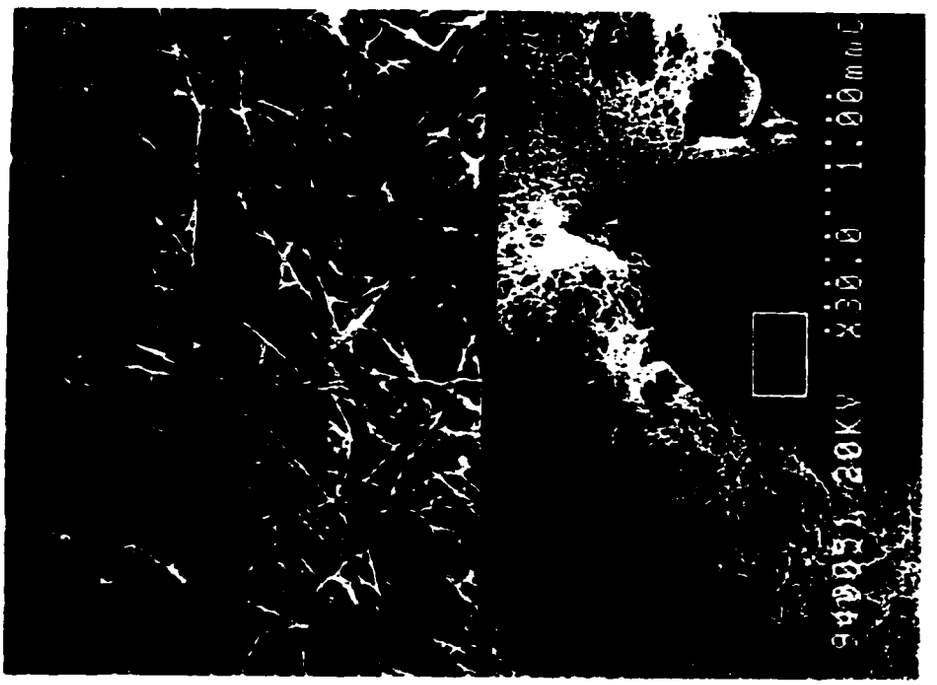
8.3.1 Characteristics and Effectiveness of Immobilization Techniques and Immobilized Biomass

Photographs 5 through 8 show the SEM micrographs at 30, 300, and 1,200 magnifications of the plains located on the surface (outside) and at a middle section (inside) of the PUF cube immobilized with metabolically active fungal biomass. Photographs 5 and 6 show that the fungus grew naturally within the micropores of PUF both inside the cubes and on the outside surface. However, some signs of the mixing effects on fungal growth near the surface could be seen from the partially empty pores near the outside surface. Some of the taxonomic features of this fungus can be observed more clearly in Photographs 7 and 8 with higher magnifications, i.e., the active hyphae grown in the micropores within the PUF cube laid uniformly and naturally, and the structural and morphological characteristics of the fungal hyphae, such as smooth, lateral branching and lacking clamp, appeared to be metabolically

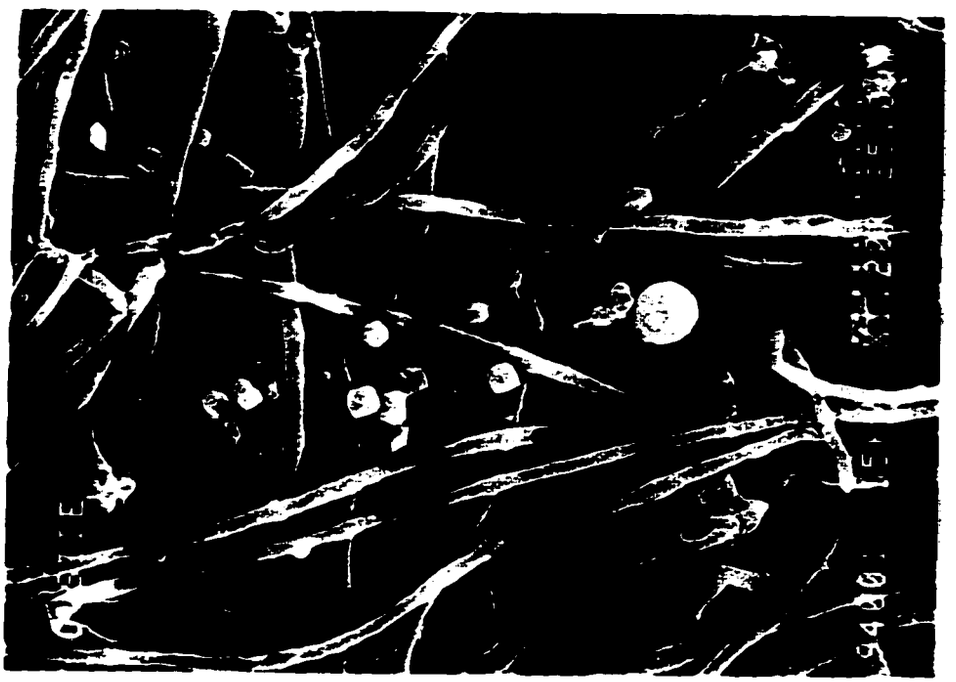
healthy and similar to those as reported in the natural culture (Moore-Landerker, 1990). It is also important to recognize that although these PUF cubes were incubated under agitated conditions, there is little suspended biomass in the bulk media and the density of the biomass in a PUF cube averaged 21±2 g per g PUF (wet weight) after 3 days. These observations strongly suggested that 1) there is little, if any, mass transfer limitation within the microcarriers; 2) this immobilization techniques not only immobilized the living fungal biomass properly in a well protected environment with respect to agitation or mixing effects but also provided a higher density of fungal biomass than the conventional suspended culture; 3) the agitation in the growth media did has the potential to cause the damage or adversely effects to the hyphae.

The experimental observations also suggested that the newly developed immobilized fungal system has many other advantages over the suspended growth system. The most important one was that it greatly eased biomass handling such as the separation and regeneration in a bioreactor; thus, it provided the effective means to reuse biomass or potentially to achieve a continuous operation, and it substantially increased the density of active biomass in a bioreactor (averaged 21±2g per g PUF) which resulted in significantly reducing the HRT of the bioreactor. Also it has the potential to increase the tolerance of fungal biomass to various shock loading and other adverse effects such as pH change. As can be seen in SEM graph the immobilized biomass was in some way protected by the micro-shell. Moreover, the immobilization material, PUF particles, were light in weight, flexible in shape, and they also can withstand high temperature (such as autoclave for 15 min at 121°C), wide pH range and other conditions in wastewater, so they were also easy to be handled on a large scale.

Photograph 5. SEM Micrograph of Outside Surface of PUF Cube Immobilized with Fungal Biomass (magnification = 30)



Photograph 6. SEM Micrograph of a Middle Section of PUF Cube Immobilized with Fungal Biomass (magnification = 30)



Photograph 7. SEM Micrograph of Outside Surface of PUF Cube Immobilized with Fungal Biomass (magnification = 1,200)

Photograph 8. SEM Micrograph of a Middle Section of PUF Cube Immobilized with Fungal Biomass (magnification = 1,200)

8.3.2 Effectiveness of Immobilized Living Biomass System

Figure 8-4 compares the kinetics and degree of decolorization by the immobilized biomass (replicates A and B) with that by the suspended biomass using the equivalent amount of the wet biomass. The weight of the wet biomass in each test was about 10±0.5 g and there is no mixing in the tests with suspended biomass. It demonstrated that using immobilized biomass both kinetics and degree of decolorization were superior to those suspended biomass. The final effluent was decolored by more than 90% after 24 hours compared to about 60% using suspended biomass. This is probably because some of the biomass is less effectively accessible to chromophoric organics in the suspended growth system due to limitations of mass transfer, unavoidable agitations and other factors such as the dissolved oxygen level. Moreover, Figure 8-4 also shows that the two tests (A and B) using immobilized biomass reproduced each other well with respect to the errors in determination of true color and wet biomass.

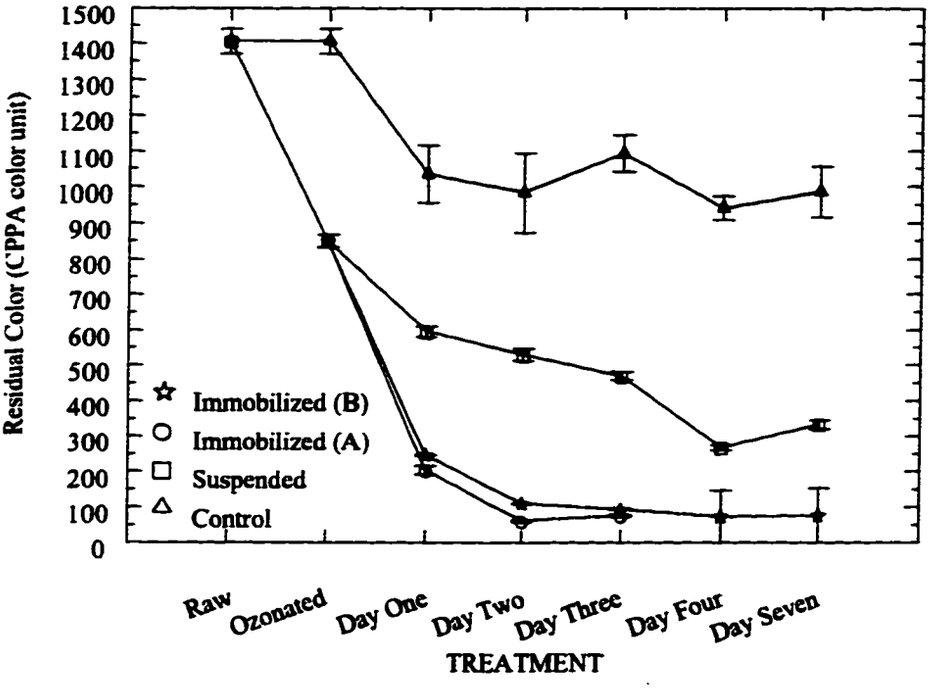


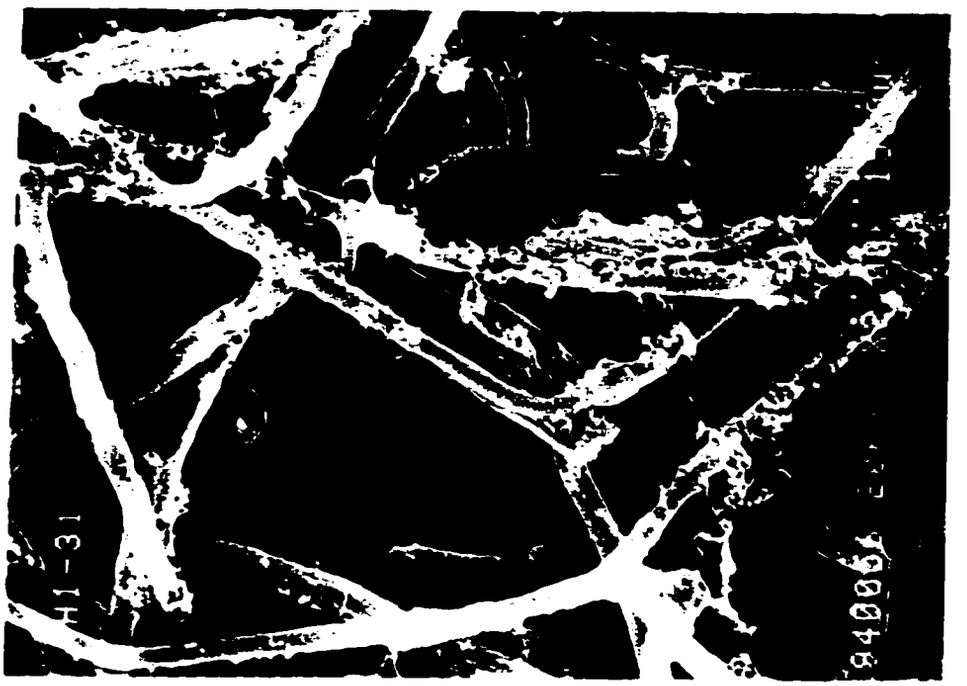
Figure 8-4. Comparison of Effectiveness of Immobilized with Free Biomass in Decolorization of Ozone Treated ASB Effluent under pH Controlled with DMS Buffer

Photographs 9 and 10 show the SEM micrographs of the immobilized biomass in the two replicates after 3 days decolorization. The morphological and structural characteristics of

the fungal hyphae in both cases had little change compared to those shown in Photographs 5 through 8. In addition, no damage to any hyphae were observed in both cases although the decolorization was carried in a continuously agitated environment. The density of fungal hyphae also appeared to be the same as that before decolorization. However, a substantial amount of sheath-like polymeric materials were formed around the fungal hyphae and some of them extended a quite distance from the fungal hyphae. Moreover, some micro-scale particles or clusters were observed around fungal hyphae. These particles were new and appeared to be high MW polymer colloids. Not until recently it was believed that contact between the fungal hyphae and lignin components was necessary for the lignin biodegradation. Studies on wood decayed by *Sporotrichum pulverulentum* (Ruel *et al.*, 1981), and on biodegradation of simulated kraft lignins (Gómez-Alarcón *et al.*, 1991) concluded that the ligninolytic enzymes can diffuse onto the wall of fungal hyphae, and the lignin degradation may not be considered as a true contact phenomenon. It was speculated that these sheath-like materials observed in this study may be a portion of the mixture of ligninolytic enzymes and large lignin molecules. Thus, the immobilized fungal biomass, which were well protected by the micropores in PUF, favored the expansion of fungal hyphae and its interaction with lignin derivatives; it offered the double-advantage of both the contact and near-contact between lignin and fungal hyphae which may represent the natural environment of the fungal growth on wood. However, more studies on biochemistry, physiology, and morphology would be necessary to clarify these preliminary observations.

These observations suggested that the immobilized biomass system did provide better efficiency, reliability, and reproducibility through the reliable control of active biomass and other factors. In addition, with the high density of immobilized biomass the process would enable the decolorization system to better deal with the potential washout and contamination, and potentially provided superior performance for decolorization and dechlorination of pulp mill effluents on a large continuous scale.

Photograph 9. SEM Micrograph of a Middle Section of PUF Cube Immobilized with Fungal Biomass after 3 Days Decolorization (Replicate#1) in pH Controlled Medium (magnification = 1,200)



Photograph 10. SEM Micrograph of a Middle Section of PUF Cube Immobilized with Fungal Biomass after 3 Days Decolorization (Replicate#2) in pH Controlled Medium (magnification = 1,200)

8.3.3 Effect of pH Control System

Figure 8-5 compares the effectiveness of the pH control system with that of the buffer system for pH control during the decolorization process using the immobilized biomass. It appeared that with the aid of proper mixing the pH control system using 0.5N NaOH could effectively maintain the pH around the optimal level for decolorization. In fact, it could slightly improve the performance of decolorization at a high level of decolorization. This may be partially due to better controlled pH (it was found that in some cases the buffer capacity was not sufficient) or elimination of the adverse effects of the buffer on the immobilized biomass at a very low level of bioavailable carbon source.

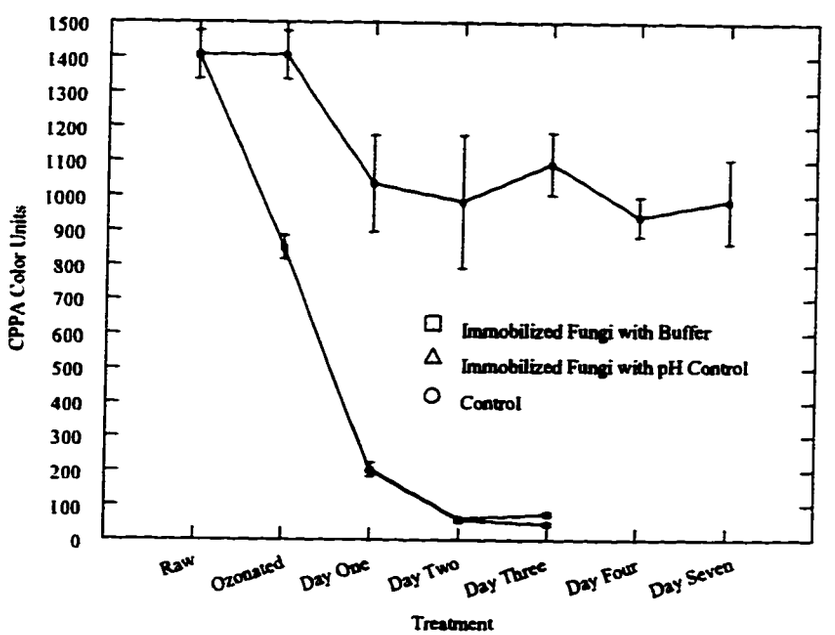
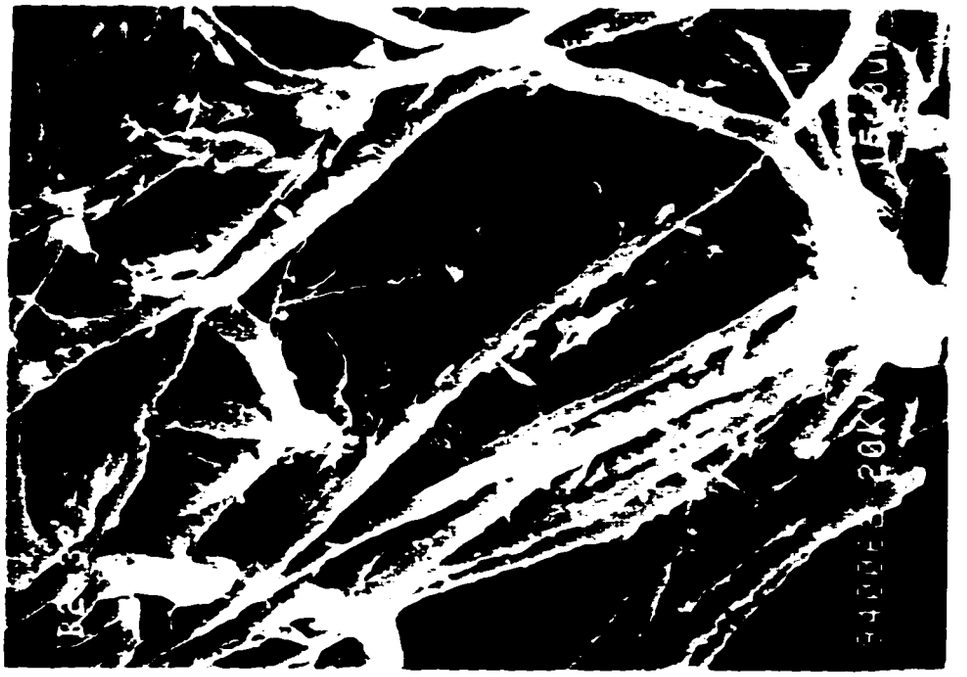


Figure 8-5. Comparison of Effectiveness of pH Control System with That of Buffer System on Decolorization Efficiency of Immobilized Biomass

Photograph 11 shows the SEM micrograph (1200X) of the immobilized biomass after 3 days decolorization in a buffered medium. Comparing the structural and morphological characteristics of fungal hyphae in Photograph 11 with those in Photographs 9 and 10 revealed that the hyphae after 3 days decolorization in a buffered medium appeared to be soften and less smooth, and start to collapse slightly; in addition, some of the hyphae seemed to have secreted

some polymeric materials and make some of the hyphae to stick together. The physiological mechanisms for these observation was not investigated further in this study, but it may be reasonable to assume that this may partially related to the improvement of decolorization performance under pH control system.

In conclusion, after 24 hours of decolorization the hybrid ozone/fungal process could achieve greater than 90% color reduction. The pH control system could effectively control the pH at the optimal level during decolorization and dechlorination. The process was also reliable and reproducible under the tested operating conditions.



Photograph 11. SEM Micrograph of a Middle Section of PUF Cube Immobilized with Fungal Biomass after 3 Days Decolorization in Buffered Medium (magnification = 1,200)

8.3.4 Continuous Decolorization and Dechlorination Study

Figures 8-6 and 8-7 show the operational performance of bioreactor using immobilized fungus ATCC24725 under continuous operation. The feed wastewater in these series of continuous tests was ASB effluent treated at about 50 and 80 mg/L ozone dose levels, respectively. Both HRT=16 and 24 hours were tested under each ozone dose level, and in all the tests the conditions, such as pH and temperature in the bioreactor, were controlled within the range of optimum conditions as established in Chapter 7 and summarized in Table 8-2. For comparison, the proposed USEPA TOX regulation (0.15 kg AOX/ADT) and the color level of 200 color unit (CPPA) were also illustrated in each figure. When interpreting these figures it is important to know that there was a shutdown of the bioreactor operation. The shutdown occurred between the series of 80 mg/L tests and a test of 50 mg/L with HRT=24 hours. The shutdown was caused by the immobilized rotating systems breaking down. However, during the series of the test at each ozone dose level, the ozone treated ASB effluents were fed to the bioreactor continuously and HRT was varied by only changing the flow rate of feed wastewater.

Over the period of the continuous operation it was observed that the newly developed immobilization techniques not only could reliably sustain the high density of fungal biomass in the bioreactor chamber but also well protected the immobilized biomass from the agitation and other potential adverse effects, and uniformly exposed to the wastewater samples in the bioreactor. This was evident in an accident caused by the malfuntion of pH controlling system. As shown in Figure 8-7, there was a period at which the pH of the system was higher than 6, under this condition the decolorization efficiency was decreased considerably. However, the system was able to recover quickly after the pH was controlled back to the optimum range. Moreover, the bioreactor system could effectively provide optimum environment for the fungal biomass as designed, such as temperature, dissolved oxygen and microelements. As a result the efficiency of fungal decolorization and dechlorination were maximized, and there was no apparent loss of capacity of fungal biomass in the continuous operation.

The results in Figures 8-6 and 8-7 demonstrated that the overall performance of the tests at 80 mg/L, and 24 hours HRT was the best among all; and in all series of tests, the residual color and TOX levels of the treated effluents were relatively constant over the operation period. In most cases, the residual color of treated effluents leveled off within the range of 180 to 220 C.U.; more importantly, in the two tests with new fungal biomass less than 100 C.U. and 2.5

mg/L TOX could be achieved in treated effluent. However, this usually lasted for about first 40 hours. In addition, the reduction of TOX seemed to follow the color removal but the kinetics and degree were substantially lower than those of decolorization. This was also observed in the previous studies (Roy-Arcand and Archibald, 1991; Pellinen et al, 1988). The rate and degree of dechlorination were also substantially lower compared to those obtained in the batch tests of this study and in Chapter 7.

Furthermore, Figures 8-6 and 8-7 show that when the HRT of the bioreactor was decreased from 24 to 16 hours, the residual color and TOX were increased slightly, then, started to level off after the transition period; and the effects of HRT on the decolorization and dechlorination seemed to be more profound at about 50 mg/L than those at about 80 mg/L ozone dose level. However, after reaching steady state (greater than 4 HRTs), the influence of HRT and ozone dose on the kinetics and degree of decolorization appeared to be minimum. In both cases, the treated effluents had about 200 CPPA units. Contradictory to decolorization, the dechlorination seemed to be slightly better at about 50 mg/L than that at about 80 mg/L ozone dose. This was probably due to the enzymatic capability of each batch of fungal biomass and interactions of other physiological factors. Unfortunately there is little information concerning this matter. Further study is warrented.

In short, the performance of bioreactor system was reliable and stable with respect to decolorization and dechlorination. With ozone dose greater than 50 mg/L and under optimum conditions the color and TOX of ASB effluents could be reduced to about 200 C.U. and 3.15 mg/L, respectively.

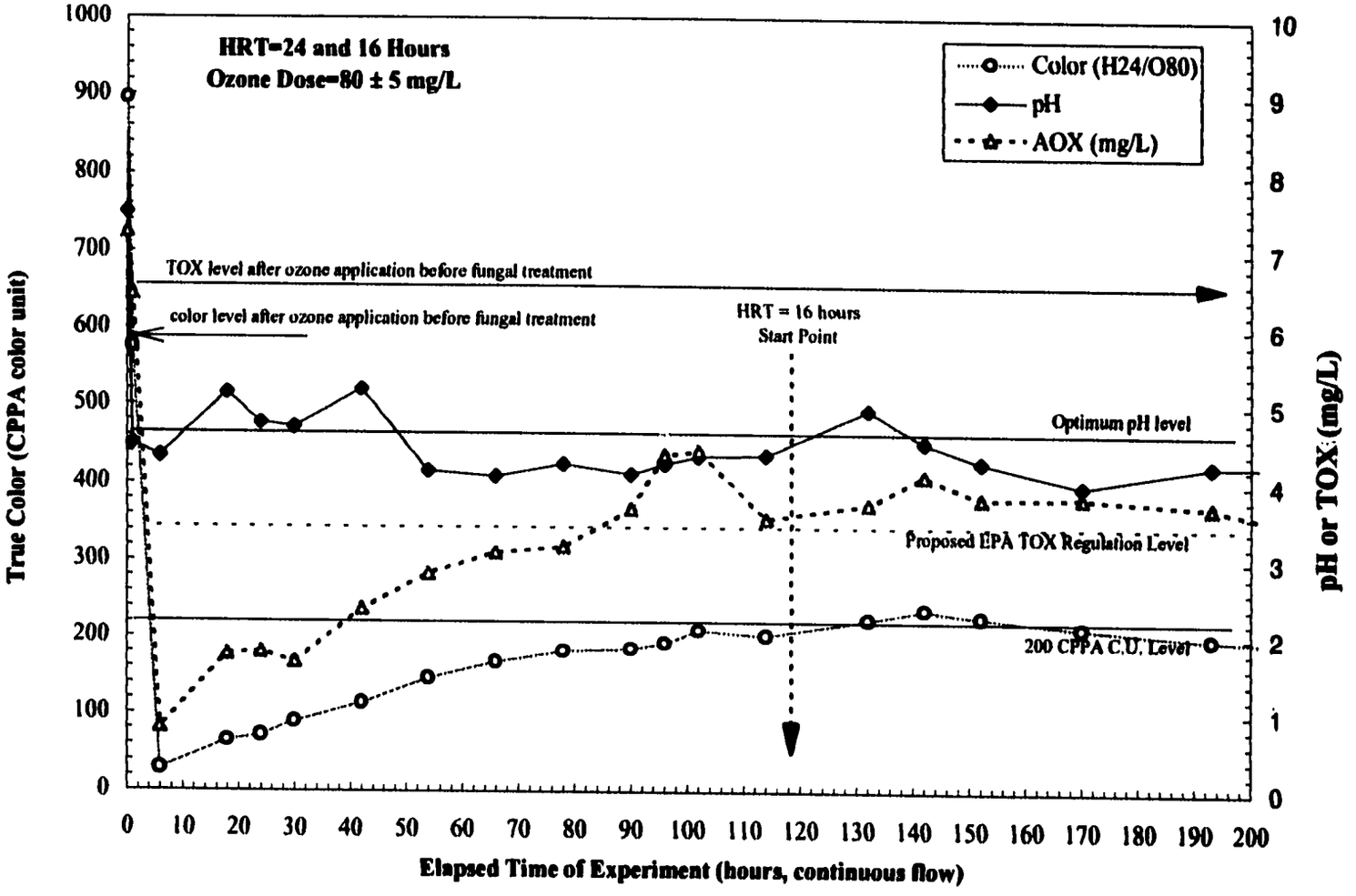


Figure 8-6. Reduction of Color and TOX with HRT=24 and 16 Hours and Ozone Dose = 80±5mg/L in the Hybrid Decolorization System Using Ozone and Immobilized Living Fungal Biomass Along with the pH Conditions

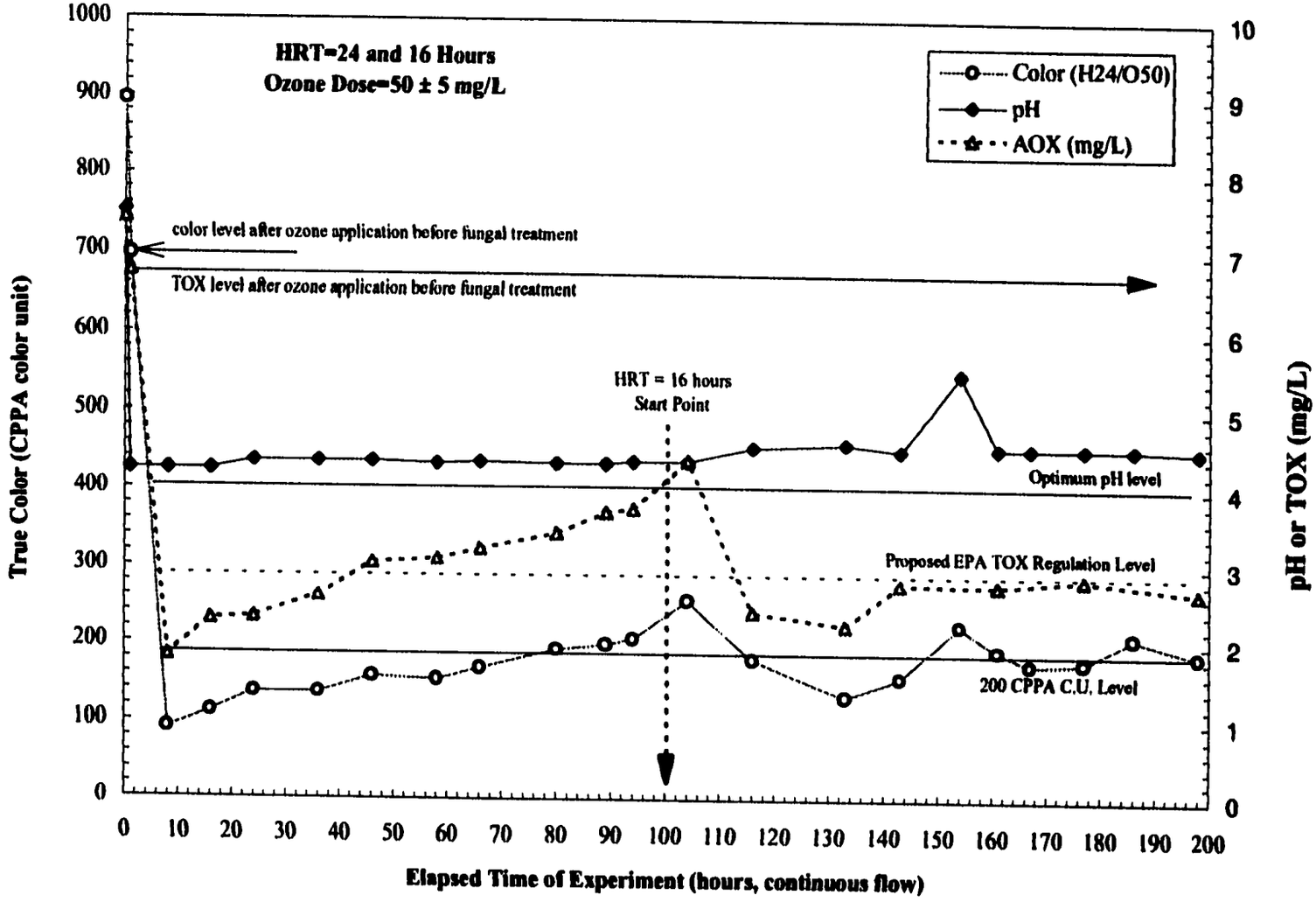


Figure 8-7. Reduction of Color and TOX with HRT=24 and 16 Hours and Ozone Dose=50±5mg/L in the Hybrid Decolorization System Using Ozone and Immobilized Living Fungal Biomass Along with the pH Conditions

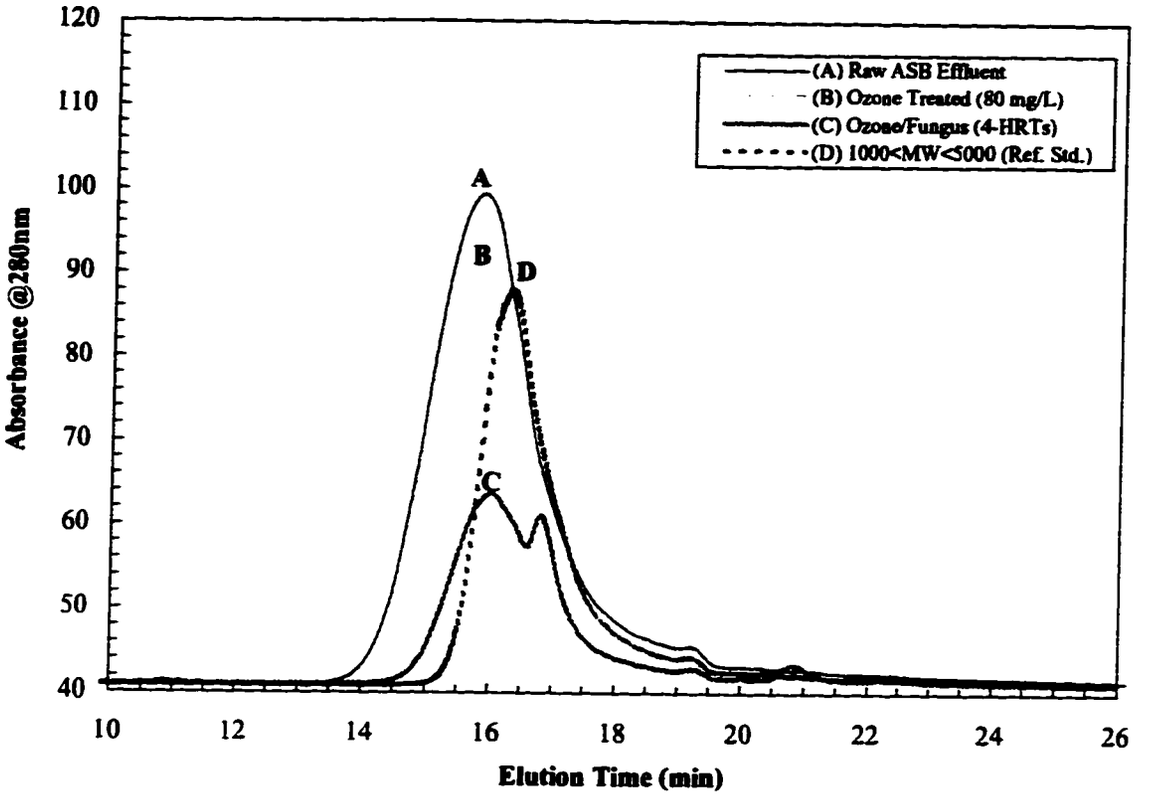


Figure 8-8. Molecular Weight Distribution of Raw ASB Effluent, Ozone Treated at Dose=80 mg/L and Ozone/fungal Treated Sample along with Lignin Reference Standard.

Figure 8-8 compares the MWD of raw ASB effluent (line A), ozone treated at 80 mg/L (line B), and ozone/fungal treated sample after about 4 HRTs (line C) continuous decolorization. Comparing the shape and elution time in Figure 8-8, it appeared that the MWD were changed substaintially after ozone/fungal treatment. The first peak on Line C seemed to correspond to the peak in the raw ASB effluent or ozone treated sample (i.e. control). However, the intensity of these peaks were reduced considerably compared to either raw and ozone treated ASB effluents. In addition, as indicated by lignin reference standard curve, the first peak was shifted to the lower MW range (1000 to 5000 g/mole). These observations corresponded well to color reduction and findings reported in previous studies, and also agreed well with the decolorization and dechlorination discussed above and some findings in previous studies (Yin, et al., 1991; Campbell, et al., 1982; Sundman, et al., 1981).

The second peak in Figure 8-8 appeared to be new compared to the MWD of the raw and ozone treated ASB effluent. There are three possible sources for the second peak:

1) it may represent a component which was much more biologically-resistant and remained in the treated effluents after the ozone/fungal treatment (Bergbauer, et al., 1992);

2) it may represent the ligninolytic enzymes which were excreted into the wastewater in the bioreactor by fungal biomass. Figure 8-9 compares the TOC of the component with MWCO>1000 from the samples of raw, ozone treated and ozone/fungal treated ASB effluent. It demonstrated that after ozone/fungal treatment, the TOC of the component with MVCO>1000 was dramatically increased compared to the TOC of the same component from either raw ASB effluent or ozone treated ASB effluent. It was also observed in the continuous study that the effluent after fungal decolorization has very high turbidity, and when the pH of the effluent was raised from about 4.5 to pH>7.6 there was a significant amount of white flocs precipitated out immediately. This was not observed with the ozone treated ASB effluent. Thus, this observation may indicate that the decolorized effluents contained an appreciable amount of high MW protein-type organics. This had been demonstrated even in pilot scale tests under continuous operations (Jager et al., 1985; Linko et al., 1986).

However, the preliminary studies indicated that the ligninolytic emzymes may be membrane bound and only be induced and released into the media under certain physiological conditions (Kirk, et al, 1987; Glenn and Gold, 1985; Srebotnik, et al., 1988). Moreover, the reported MW of the ligninolytic enzyme protein ranged from 38,000 to 46,000 which was far large than the molecules detected in either the first peak or second peak (Boominathan and Reddy, 1992). Thus, it appeared that these enzyme molecules were not detected under the conditions employed in HPSEC analysis. This was also the case in the batch study in Chapter 7 and other previous studies (Pellinen et al. 1988; Sundman, et al., 1981). The reasons that these enzyme molecules were not detected on HPSEC at 280nm presumably are 1) the concentration of these enzymes in the wastewater was relatively low and some of these enzymes had very low absorbance at 280 nm (Boominathan and Reddy, 1992); 2) partially due to their being entrapped by the precolumn as the pH of the elutant used in HPSEC was about 8.

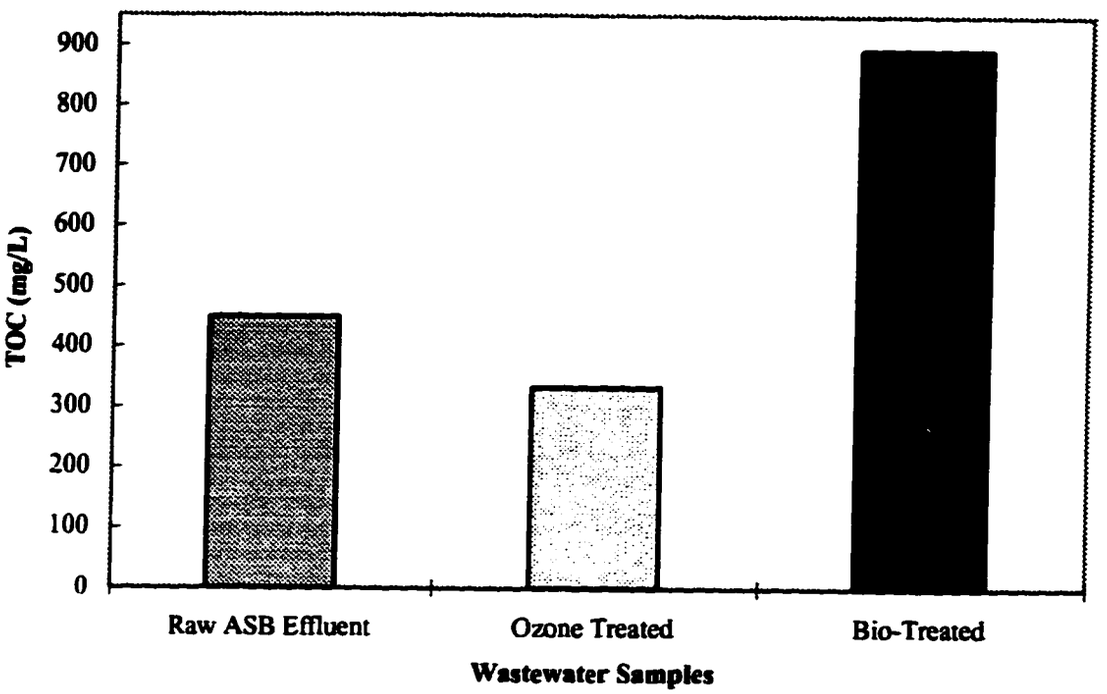


Figure 8-9. Comparison of TOC of Ozone/fungal Treated Sample (after about 96 Hours) with Those of Raw and Ozone Treated ASB Effluent (MWCO>1000 fragment in 50% concentrated)

3) The second peak may represent the low MW metabolites from the biodegradation of high MW lignin components other than enzymes. More importantly, it can be seen that besides the the second peak there were several small peaks on Line C located in very low MW range (less than 1000 g/mole). These peaks are all new compared to either raw ASB effluent or ozonne treated effluent. These phenomena had also been repeatedly observed in many other physiological studies. Using radioactive lignins Chua *et al.* (1983), Faix *e al.* (1985) and Lundquist (1977) all noticed the accumulation of low MW degradation products from fungal decolorization and dechlorination. Moreover, another study (Sundman et al., 1981) also revealed that in fungal decolorization of E1 effluent the high MW of lignin derivatives were largely decomposed to low MW, colorless, soluble/volatile products. However, Pellinen et al. (1988) failed to detect the low MW degradation products at 254 and only a low concentration of low MW products was found using UV detector at 280 nm in another study (Mao and Smith, 1995b).

Consequently, the second peak may partially compose of the variety of relatively low MW organic compounds with the fungal-resistant structures and may be partially contributed by

the low MW biodegradation products. These biological recalcitrant structures may partially derive from the ozone treatment process or partially derived from the enzymatic transformation process, and biologically resistant portion of lignin components. It is also important to pointed out that these structures were not non-biodegradable, but the kinetic rate was much slower compared to the rest of the lignin derivatives. The rest peaks on Line C would most probably represent groups of very low MW biodegradation products.

In brief, ozone/fungal system could effectively decolorize and dechlorinate BTKPME in continuous mode. In most cases, the residual color and TOX in treated effluents were less than 200 C.U. and 3.15 mg/L (0.15 kg TOX/ADT), respectively. HPSEC analysis confirmed that the high MW lignin derivatives, which were the sources of color and TOX, was largely destroyed, and some of them were possibly converted into several different groups of low MW organics, these organics seemed to have low absorbance at 280 nm.

8.4 CONCLUSIONS

This study demonstrated that under continuous operations, the ozone/fungal system could reduce the color and TOX of raw BTKPME to about 200 CPPA C.U. and 3.15 mg/L, respectively. However, the TOC increased substantially after treatment which may affect the ultimate disposal of treated effluent. More studies are needed to address this issue.

The immobilization techniques could successfully handle the fungal biomass in a bioreactor system for a continuous decolorization and dechlorination. The immobilized bioreactor system developed in this study provided a promising technique for decolorization and dechlorination in a continuous mode. It was found that HRT had little impact on decolorization under the tested conditions as far as the minimum 16 hours of HRT was provided. Further increasing HRT may not significantly improve the efficiency of the fungal treatment. The dechlorination may require the HRT greater than 24 hours to meet the proposed EPA regulations. However, increasing HRT may also affect the concentration of enzymes and low MW organic substrates in the bioreactor system.

This continuous study also confirmed the findings observed from the batch tests in Chapter 7. That is, ozone substantially enhanced the kinetics of decolorization and dechlorination by destroying or modifying the biologically resistant structures in the lignin components or by improving the access of fungal biomass to these structures. During the continuous operations

there was negligible loss in the activity of decolorization and dechlorination of fungal biomass. However, the difference of overall decolorization performance between 50 and 80 mg/L ozone treated samples was marginal; and the effects on dechlorination was inconsistent, it appeared the interactions among the process parameters may play important roles in both cases.

8.5 RECOMMENDATIONS

According to the findings in this study it was thought to be proper to further investigate the critical parameters within a wider range, to address some unsolved issues and collect more detail information for the pilot study. The transition phase proposal was developed to serve the recommendations for the further investigation. The detail proposal was presented in Appendix IV.

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CHAPTER 9. SUMMARY AND CONCLUSIONS

Today, the color and chlorinated organics are probably the two problems from the forestry industry which concern the public and regulatory authorities most. Thus, the new regulations have been proposed around the world, and in certain regions some preliminary actions have already been initiated based on the limited scientific investigations. Obviously the new regulations intend to encourage the industry to reduce the impacts of the color and chlorinated organics on the receiving environment. To respond to the new regulatory requirements and further minimize the impact of pulp mill effluents on the receiving environment, the forestry industry initiated the research program described in this thesis to develop a practicable technology for reduction of color and chlorinated organics. Further investigations revealed that the lignin derivatives in the bleached kraft pulp mill effluents are the major source of the color and chlorinated organics, and the existing treatment technologies are ineffective in removal of either nonchlorinated or chlorinated lignin components. Therefore, the ultimate objective of this study was to develop and evaluate a feasible process for simultaneous reduction of color and TOX in a continuous flow mode, and to provide a rationale and experimental basis for the process scale-up.

In particular, the following basic issues were considered to be critical and interrelated in the research, development and evaluation of an advance process for reduction of lignin components in the bleached kraft pulp mill effluents:

1) What technology is the most effective on simultaneous reduction of color and TOX in the effluents? Which stream of the pulp mill effluents is the best target to be treated with the new decolorization and dechlorination process?

2) If the biological treatment were going to be evaluated as a part of the treatment, what are the biodegradable potentials of chlorinated and non-chlorinated lignin components in the effluents? How are they correlated with other characteristics such as color, TOX, MWD, COD? In addition, how can the experiments be designed for quantitative evaluation?

3) Advanced treatment processes involving ozonation and various fungi have been reported to be a few of most effective means of destroying chlorinated and non-chlorinated chromophoric structures in kraft pulp mill effluents. How does ozone or fungi attack the lignin

components in the effluents; and how can the effectiveness of these treatments be evaluated quantitatively? Furthermore, what kind of reactor or bioreactor systems can accommodate these advanced processes for decolorization and dechlorination in a continuous mode, which is critical for industrial applications.

4) What synergistic effects could be expected between ozone and fungal decolorization and dechlorination? What are the underlying mechanisms of ozone, fungal and ozone/fungal processes? These are critical for better understanding, designing and scaling-up the new treatment processes.

The outcome of this research program and recommendations are summarized accordingly in this chapter.

9.1 Comparative Evaluation of Biodegradable Potential (BP), BPI and Their Correlation with Color, C.I., TOX, MWD

The diversity of definitions of biodegradability led to the development of a diversity of methods for assessment. These included oxygen uptake, BOD_5/COD or BOD_5/TOC ratio, shaking flask, CO_2 evolution, simulated activated sludge, model ecosystem and the various combinations. As a result, it is very difficult, if not impossible, to select a method for systematic evaluation of the biodegradability and its changes induced by various physicochemical and biological processes, and to compare and correlate the reported biodegradability data with the other characteristics and existing results. To narrow these tremendous differences in defining the biodegradability, the concept of biodegradable potential (BP) and a mechanistic model which quantitatively described the BP of pulp mill effluents was first developed and verified. This concept and the model seemed to be valid and the first step leading to establishment of a systematic approach for assessing the biodegradability of complex industrial wastewaters.

Specifically, on the basis of the BP concept, this study proposed a two-stage biodegradation process linked by a bio-transformation process as a mechanism involved in the biodegradation of complex industrial wastewaters, in particular, the lignin components in pulp mill effluents. A mechanistic oxygen uptake model was developed based on the proposed mechanism. The applicability and reliability of the new mechanistic model was evaluated using various representative organic mixtures including glucose/glutamic acid solutions, three types of wastewaters, ozone treated secondary effluents, and various components derived from these

effluents. The results revealed that through proper calibrations, the new mechanistic model could satisfactorily predict the oxygen uptake by various complex industrial wastewaters over the long term, and the new model could be used to reliably assess and to effectively compare the biodegradability of complex wastewaters. Moreover, the new model was also a practical and sensitive measure for evaluating the mechanistic-kinetic constants involved in the biodegradation processes since it considered both stoichiometry and kinetics of biochemical reactions. The reliability and applicability of the new model was also verified using the data from another independent study (MacDonald and Radermacher, 1993).

The model evaluation also suggested that the BP and the BPI provided a uniform, reliable but simple engineering means of assessing the alteration of biodegradability induced by various physical and chemical treatment processes. It was evident that the BP or BPI correlated well with the biodegradability data determined in this study, and those collected using other means established in the previous studies (Smith and Mohammed, 1992; MacDonald and Radermacher, 1993; Mao and Smith, 1995a). They also correlated well with other characteristics such as color, C.I. and MW.

To identify the target effluent and to select and optimize processes for decolorization and dechlorination, the various portions of the kraft bleached pulp mill effluents were systematically characterized with the newly developed evaluation method. The parameters such as the BPs of raw and individual components, true color, COD, TOC, TDS, BOD_5 and TOX were used in the characterization. The full range of UV spectra, BPI, C.I., MW, MWD and their correlation were also investigated. The results demonstrated that the organics in pulp mill effluents continuously distributed over a range from 100 to 80,000 g/mole with the majority within the range of 100 to 15,000 g/mole. With increasing MW of the organics the C.I. increased but the BP or BPI decreased, and the organics with MW>1,000 g/mole contributed greater than 80% of BP. In addition, with respect to the biodegradability and true color, the organics in the pulp mill effluents could be divided into three general categories: easily-biodegradable with low C.I. and low MW, biodegradable with medium C.I. and both high and low MW, and poorly-biodegradable with high C.I. and high MW. Also it was found that some of high MW organics in the pulp mill effluents were slowly biodegradable but had low C.I. More importantly, this series of study revealed that biologically treated pulp mill effluent (BTKPME) called ASB effluent would be a good choice for applying advanced decolorization and dechlorination processes. Also

the hybrid AOP with advanced fungal processes warranted the further study on its potential for complete decolorization and dechlorination of pulp mill effluents, and the selection and optimization of advanced decolorization and dechlorination system was wastewater and mill specific.

9.2 Impacts of Ozonation on BP, BPI, Color, C.I., TOX and MWD

The mechanisms and kinetics of ozone decolorization and dechlorination of pulp mill effluents remained largely unknown although the physicochemistry and reactions of ozone with various organics, especially the model compounds of lignin components, were well studied. Thus, the series of experiments were designed to quantitatively investigate the effectiveness and influence of ozonation on the color, TOX, BP and BPI of the pulp mill effluents. To facilitate statistical evaluation, the two representative types of pulp mill effluents, E_{op} filtrate and ASB effluent, were selected for tests and the experiments were statistically designed as paired block experimental series. The pooled experimental errors in each block were used for t-test and ANOVA analysis.

At first, two different ozone application modes were systematically investigated using two specially-designed ozone reactor systems. System I consisted of two-phased reactor which could introduce the total amount of ozone to the wastewater at one time with proper mixing while System II provided ozone to wastewater with a desired rate by accurately controlling the flow and concentration of the ozone/oxygen gas mixture in a once through flow mode. The investigations revealed that more than 10% of the used ozone dose was decomposed in water-vapor-saturated gas phase in System I. A negligible amount of ozone was decomposed in the gas phase in System II. Thus, the ozone decomposition initiated by the water vapor in gas phase contributed to the observed effects. According to the ratio of decomposed ozone to the used ozone the consumed ozone dose was established and the effectiveness of ozone treatment with each system was re-evaluated. The results suggested that over the wide range of ozone dose levels the ozone application methods did not have statistically significant effects on ozone treatment of both wastewaters with regard to true color removal (at 1% significance level), COD and TOC removal (at 5% significance level), and the improvement of short-term biodegradability as BOD_5 (at 5% significance level). Also, it was found that the ozone application methods did not change the competitiveness of ozone reactions with various organics in wastewater but

affected the availability of ozone for reacting with the target organics. The biodegradable (low C.I.) organics in the effluents had the greatest potential to reduce the efficacy of ozone decolorization of the pulp mill effluents. In addition, the consumed ozone dose determined the level of ozone decolorization and it is possible that the gas phase mass transfer resistance may play a role in the ozonation of pulp mill effluents.

Using the established dimensionless index BPI, a study was conducted to examine the impacts of ozone decolorization and foam separation on BP and BPI of bleachery effluent (E_{op} filtrate), combined pulp mill effluent and BTKPME as well as their respective components (with MWCO<1,000, 1,000<MWCO<5,000, 5,000<MWCO <10,000, and MWCO>10,000). The series of tests confirmed that the dimensionless BPI was a simple but reliable means for evaluation of biodegradable potential and its changes in pulp mill effluents after the treatment. It was also demonstrated that ozone decolorization was a dynamic process in removing and producing the biodegradable components in pulp mill effluents. The effects of ozone decolorization on BP depended on BPI, C.I. and ozone doses; there was an optimum ozone dose level at which both BP and decolorization efficiency were maximized. With respect to ozone decolorization of BTKPME, the BPI increased one unit with every 12 mg/L consumed ozone dose ranging from 0 to 200 mg/L. The foam separation process showed different impacts on the combined mill effluent. It partitioned the biodegradable components in combined pulp mill effluents into two groups. It appeared that the defoamed component containing hydrophilic organics had lower BPI but higher C.I.

9.3 Effectiveness of Ozone/Fungal Process on Simultaneous Reduction of Color and TOX

This study also investigated the enhancement of ozone pretreatment on fungal decolorization and dechlorination of BTKPME. Specifically, several series of parallel experiments with ozone and fungus *Phanechaete chrysosporium* (ATCC 24725) were performed to compare the effectiveness of ozone, fungus, and hybrid ozone/fungus decolorization and dechlorination under various physiological conditions; and assess the impacts of ozone-induced structural alternation and biodegradable components on the effectiveness of following fungal treatment. The analytical HPSEC was employed to examine the impacts of the processes on the MWD. The true color, TOX, UV absorbance, BOD_5 and COD were also used to quantify the interactions between ozone and fungal decolorization and dechlorination.

Experimental evidence suggested that the effectiveness of ozone treatment alone on simultaneous reduction of color and TOX in the effluents was in the order ASB effluent>> ASB influent> E_{op} filtrate. The efficiency of ozone decolorization and dechlorination was also strongly dependent on the level of reductions. The efficiency was in the ratio of ASB effluent: ASB influent: E_{op} filtrate = 2.5:1.2:1.0 at 35% decolorization level compared to the ratio of 1.6:1.1:1.0 at 75% decolorization level. The fungus *Phanechaete chrysosporium* (ATCC24725) alone could reduce the color and TOX simultaneously by about 65% and 55% over seven days, respectively.

Moreover, the ozone pretreatment, in addition to partial decolorization and dechlorination of BTKPME, greatly enhanced the kinetics and degree of decolorization and dechlorination using fungus *Phanechaete chrysosporium*. The physiological conditions was also found to play an important role in ozone-induced synergistic effects on fungal treatment. With the aid of ozone treatment and improved microbiological media, the hybrid ozone/fungal process could simultaneously achieve about 90% of true color removal and 80% of TOX reduction from BTKPME. More importantly, the pregrown fungal biomass performed better than the biomass grown in pulp mill effluents, and with the use of pregrown fungal biomass, it is not necessary to supplement easily biodegradable carbon source for fungal decolorization and dechlorination.

9.4 Mechanisms of Ozone and Fungal Decolorization and Dechlorination

To better understand the ozone and fungal decolorization and dechlorination processes, several series of parallel experiments were designed to elucidate the mechanism and kinetics of ozone, fungal and ozone/fungal decolorization and dechlorination processes. In these tests, both raw and ozone treated samples were separated into four components to assess the effects of ozonation on individual components. Analytical HPSEC was employed to determine the changes induced by ozonation in molecular weight distribution of lignins and their derivatives.

The investigations revealed that the color intensity, which is an intrinsic property of pulp mill effluents, was a determining factor on the efficiency of ozone decolorization; and the kinetics of ozone decolorization appeared to be 3/2 orders with respect to C.I. With C.I. as a kinetic parameter the new kinetic model satisfactorily predicted the ozone decolorization of pulp mill effluents. Moreover, it was found that the chromophoric components of lignins and their derivatives preferentially competed for ozone with other organics. As a result, ozone decolorization converted a large amount of high-MW components (MWCO>1000) into low MW

components (MWCO<1000) which was more biodegradable, and induced varied blue shifts in the UV spectra of various pulp mill effluents. On the other hand, ozone treatment seemed to dechlorinate the organics non-selectively and inefficiently compared with other processes.

The experimental evidence also suggested that the fungal biodegradation of lignin components involved very complex enzyme systems which carried out oxidative fission of aromatic rings, conjugated double bonds, and breaking down part of the polymeric structures. Some of these steps could be the rate-limiting processes or act as bottleneck steps restricting the overall kinetics of lignin degradation. Therefore, if these rate-limiting steps or structures could be destroyed using ozone instead of enzymes in fungal decolorization processes the overall kinetics of the process may be greatly improved or accelerated. In fact, it was found that the ozone pretreatment not only led to partial decolorization and dechlorination but also produced a substantial amount of structurally modified organics which could serve either as a co-substrate or as enzymatic inducers in fungal decolorization and dechlorination. Also, some modified structures in lignin components were much more easily accessible to the ligninolytic enzymes involved in decolorization and dechlorination.

More importantly, the experimental evidence appeared to support the speculation that the mechanism of dechlorination by immobilized fungi and ozone enhancement were somewhat different from those for decolorization. Ozone pretreatment at about 80 mg/L could only create a limited amount of suitable structures which were favorable to the dechlorination enzyme system. The results in this study and previous studies also revealed that the immobilized fungi may be able to simultaneously produce the two metabolically connected enzyme systems; one portion of these enzyme systems may serve some roles in the another system allowing the processes to occur in parallel. However, the kinetics of dechlorination appeared to be slower than decolorization.

9.5 Advanced Ozone/Immobilized Bioreactor System for Continuous Decolorization and Dechlorination of BTKPME

To achieve better efficiency and reliable continuous operation, a proper technology for immobilization of fungal biomass was developed and quantitatively evaluated. Both decolorization tests and SEM examination evidenced that the immobilization technique developed in this study was effective for handling living fungi which are sensitive to damage by agitation

and other adverse environmental effects. Also, the newly developed immobilized fungal system has many other advantages over the suspended growth system. The most important one was that it greatly eased biomass handling such as the separation and regeneration in a bioreactor. It provided the effective means to reuse biomass or potential to achieve a continuous operation, and it substantially increased the density of active biomass in a bioreactor (averaged 21±2g per g PUF) which resulted in significantly reducing the HRT of the bioreactor. Moreover, the immobilization material (PUF particles), was light in weight, flexible in shape, and can withstand high temperature, wide pH range and other conditions in wastewater, so they were also easy to be handled on a large scale.

By integrating ozone decolorization and the immobilized fungal biomass into the bioreactor design the new ozone/fungal bioreactor system was developed. The evaluation study suggested that the ozone/fungal bioreactor system was reliable and stable with respect to decolorization and dechlorination. Thus, it provided a promising means for decolorization and dechlorination in a continuous mode. With ozone dose greater than 50 mg/L and under optimum conditions the color and TOX of BTKPME could be reduced to about 200 C.U. and 3.15 mg/L, respectively. It was difficult to accurately define the impact of HRT on decolorization under the tested conditions as extended test runs at steady state conditions were not performed. Increasing HRT beyond 16 hours may not significantly improve the efficiency of the fungal treatment. The dechlorination may require the HRT to be greater (24 hours) to meet the proposed EPA regulations (0.15 kg TOX/ADT). However, increasing HRT may also affect the concentration of enzymes and low MW organics substrates in the bioreactor system.

9.6 Conclusions

Experimental investigations conducted in this research program led to successfully development of an advance ozone/fungal immobilized bioreactor system for decolorization and dechlorination of the pulp mill effluents. The study also revealed some fundamental mechanisms involved in ozone and fungal decolorization and dechlorination of pulp mill effluents. Specifically, the following conclusions can be drawn through the experimental evidence revealed in this study:

1) Ozone treatment alone is effective in destroying the chromophoric structures, but less effective in attacking C-Cl bonds in the lignin components. Ozone pretreatment could

substantially enhance the immobilized fungal decolorization and dechlorination of the pulp mill effluents. The underlining mechanisms responsible for the synergistic effects are: the ozone treatment provided partial decolorization and dechlorination, the ozone treatment produced a considerable amount of modified structures which were much more accessible to the fungal enzyme systems, and the ozone treatment converted the high MW lignin components to lower MW organics, some of which may have been able to serve the inducers or co-substrates in biodegradation of lignin derivatives.

2) The newly developed ozone/immobilized fungal bioreactor system provided a promising means for the continuous decolorization and dechlorination of the pulp mill effluents. With the optimum test conditions, the ozone-immobilized bioreactor system achieved about 90% of color reduction and 80% of TOX removal simultaneously. With an ozone dose greater than 50 mg/L, HRT greater than 24 hours and under optimum conditions, the color and TOX of BTKPME was reduced to about 200 C.U. and 0.15 kg TOX/ADT, respectively, using this bioreactor system.

3) The lignin components in the bleached kraft pulp mill effluents can be degraded biologically, but the biodegradation kinetics are usually slow, even using the efficient lignin degraders such as white rot fungi. The biodegradation of the pulp mill effluents involves two stages linked by a biotransformation process. The mechanistic model developed in this study can reliably predict the oxygen uptake by the acclimatized microbial consortia involved in the lignin biodegradation. The findings were also supported by independent studies of biodegradation of pulp mill effluents in Alberta, Canada.

4) BPI is a reliable and simple dimensionless index for systematically assessing the impacts of various physicochemical and biological processes on the biodegradable components of complex industrial wastewaters, especially pulp mill effluents. During ozone treatment of BTKPME, the BPI increased one unit with every 12 mg/L ozone dose increase.

5) The color intensity, C.I. (C.U./mg TOC), is an intrinsic property of pulp mill effluents. The higher C.I. value a pulp mill effluent has, the lower the BPI value, the higher the MW of the organic components.

6) Conventional biological treatment appeared to be as effective as the ultrafiltration (with a MWCO=10,000 membrane) in concentrating color causing lignin components in pulp

mill effluents. The BTKPME is the choice for hybrid ozone/immobilized fungal process for decolorization and dechlorination.

7) The mechanism of decolorization by ozone alone is through destroying UV absorbing structures, i.e., chromophores in the effluents. The ozone reactions with the lignin components break down the conjugated double bonds and aromatic rings, and usually lead to converting high MW lignin derivatives into more biodegradable organics with low MW. The degree of conversion is strongly dependent on the ozone dose and organics present in the wastewater. Fungi seem to destroy the lignin derivatives non-selectively, and the kinetics are usually slow. It appeared that two different enzyme systems are involved in decolorization and dechlorination. It seems that the two enzymes were produced simultaneously and worked cooperatively.

9.7 Recommendations

According to the findings in this study it was thought to be proper to further investigate the critical parameters within a wider range, to address some unsolved issues and collect more information for the pilot study. The transition phase proposal was developed to serve the recommendations for the further investigation. The proposal was presented in Appendix IV.

9.8 References

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APPENDIX I

BLEACHING PROCESS,
SCHEMATICS OF WASTEWATER TREATMENT FACILITIES,
SAMPLING LOCATIONS
AND
RAW EFFLUENTS HANDLING RECORDS

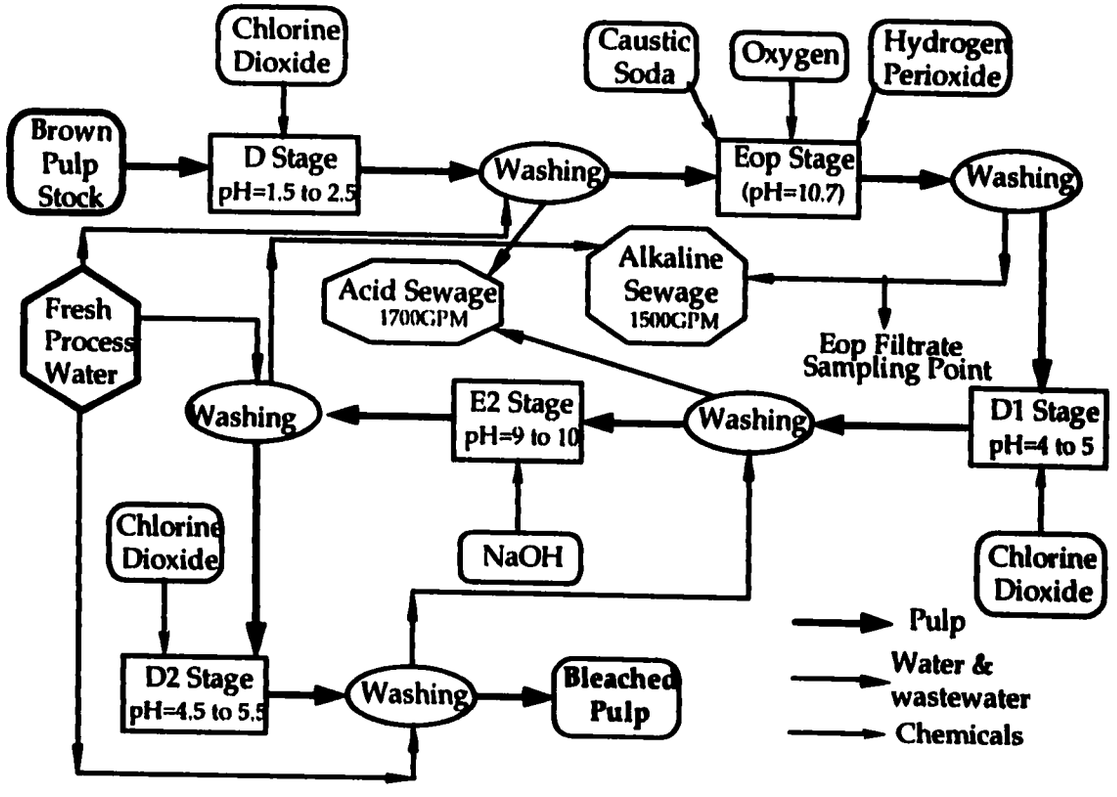


Figure I-1. Schematic Illustration of Five Stage $DE_{op}DED$ Bleaching Process and Sampling Point for E_{op} Filtrate

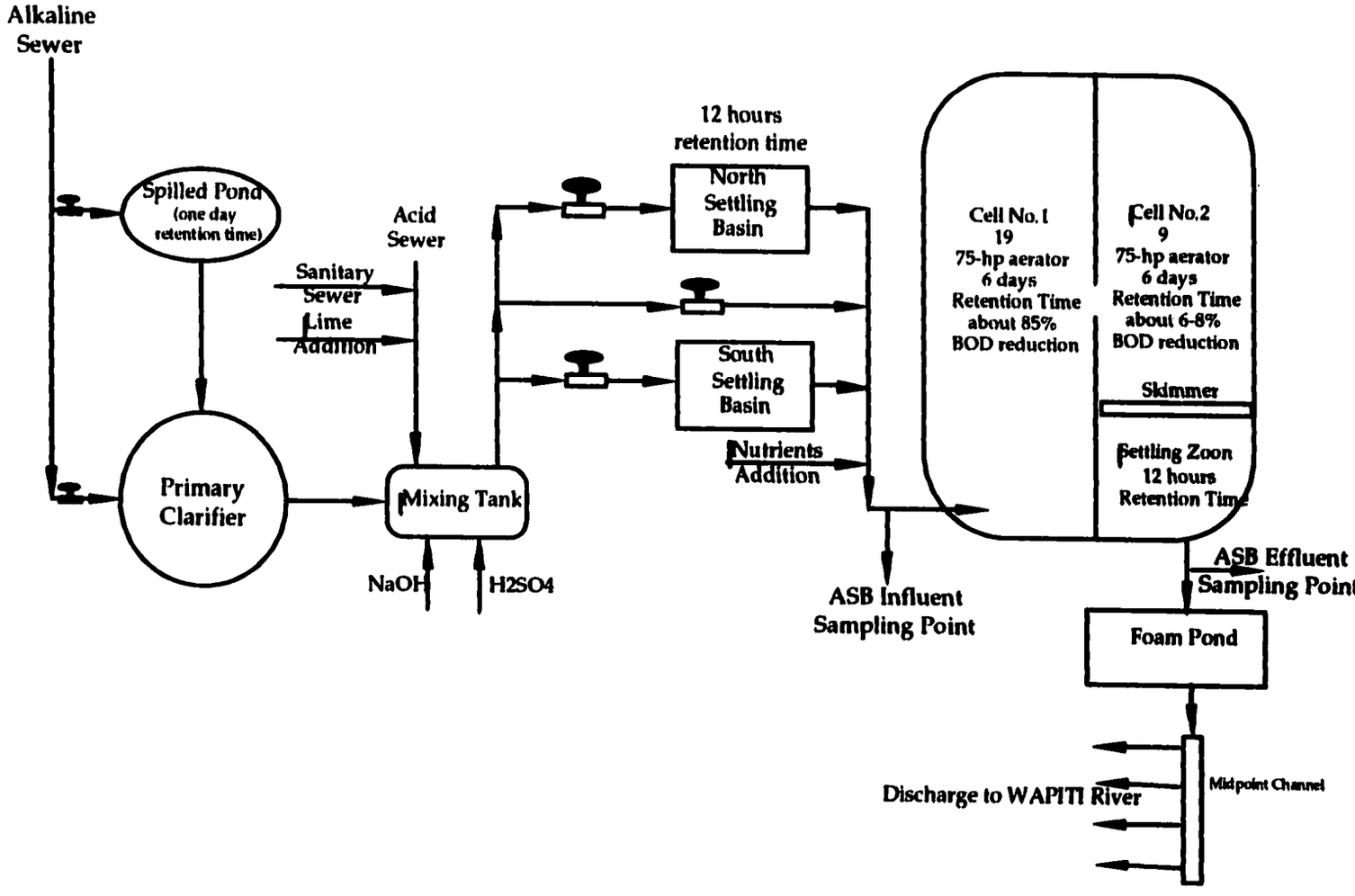


Figure I-2. Schematic Illustration of Wastewater Treatment Facility and Sampling Points of ASB Influent and Effluent

Table I-1. Summary of Some of COD Analysis on Three Raw Pulp Mill Effluents Sampled during Various Periods of This Study

Run	Sample	Sampling Date	COD (mg/L)	Analysis Date
1	E_{op} Filtrate	August 4, 1992	3022	August 18, 1992
2	E_{op} Filtrate	August 4, 1992	3049	August 18, 1992
3	ASB Influent	August 4, 1992	1304	August 18, 1992
4	ASB Influent	August 4, 1992	1298	August 18, 1992
5	E_{op} Filtrate	August 4, 1992	3098	October 9, 1992
6	E_{op} Filtrate	August 4, 1992	2887	October 9, 1992
7	ASB Effluent	November 4, 1992	778	November 13, 1992
8	ASB Effluent	November 4, 1992	799	November 13, 1992
9	ASB Effluent	November 4, 1992	830	November 13, 1992
10	E_{op} Filtrate	August 4, 1992	2996	February 13, 1993
11	E_{op} Filtrate	August 4, 1992	3004	February 13, 1993
12	ASB Effluent	December 19, 1992	677	December 30, 1992
13	ASB Effluent	December 19, 1992	694	December 19, 1992
14	ASB Effluent	December 19, 1992	660	February 23, 1993
15	ASB Effluent	December 19, 1992	656	February 23, 1993
16	E_{op} Filtrate	August 4, 1992	2976	March 6, 1993
17	E_{op} Filtrate	August 4, 1992	2978	March 6, 1993

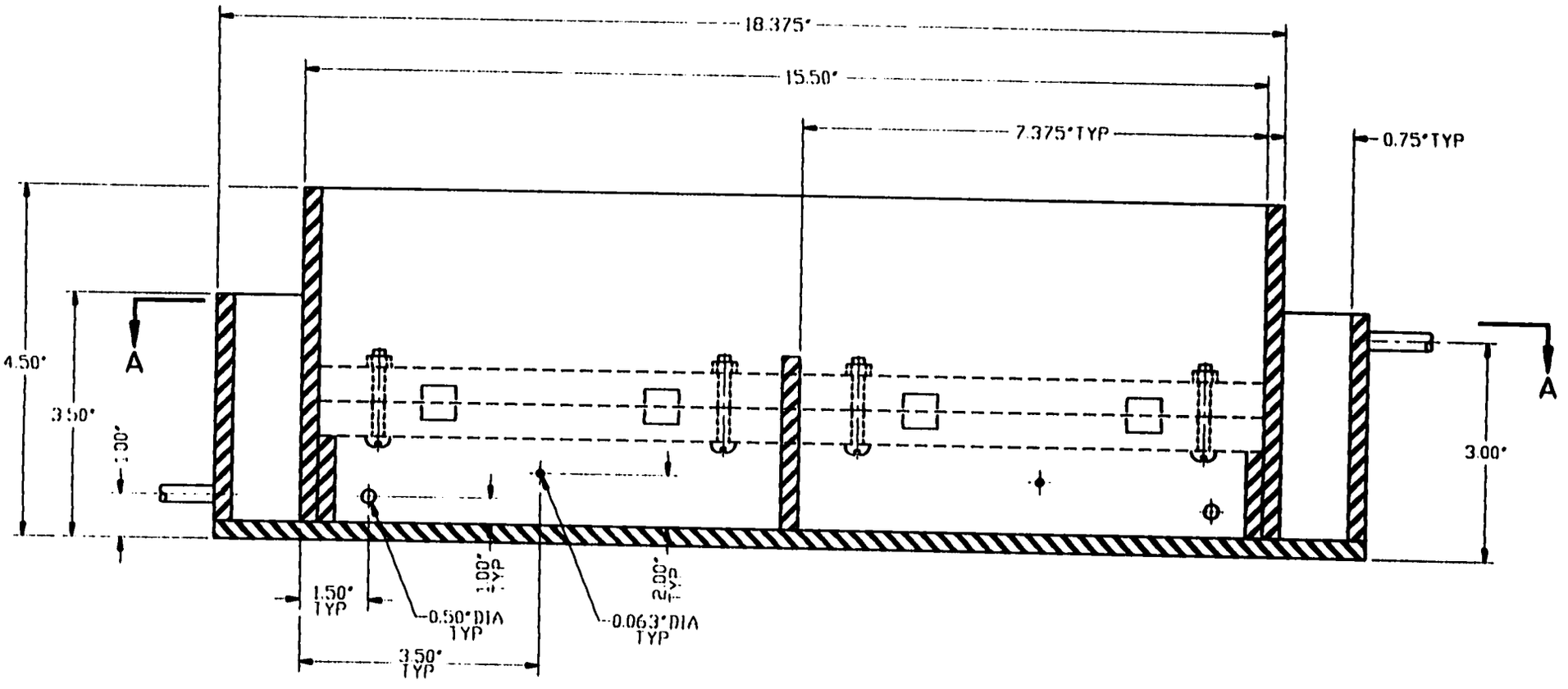
Table I-1. (continued) Summary of Some of COD Analysis on Three Raw Pulp Mill Effluents Sampled during Various Periods of This Study

Run	Sample	Sampling Date	COD (mg/L)	Analysis Date
18	ASB Influent	April 20, 1993	1439	April 22, 1993
19	ASB Influent	April 20, 1993	1439	April 22, 1993
20	ASB Effluent	April 20, 1993	677	April 22, 1993
21	ASB Effluent	April 20, 1993	676	April 22, 1993
22	ASB Influent	April 20, 1993	1449	June 10, 1993
23	ASB Influent	April 20, 1993	1447	June 10, 1993
24	ASB Effluent	April 20, 1993	634	July 8, 1993
25	ASB Effluent	April 20, 1993	634	July 8, 1993
26	ASB Influent	April 20, 1993	1410	July 23, 1993
27	ASB Influent	April 20, 1993	1424	July 23, 1993

Note: All the Effluents were sampled by the qualified operator in the mill and carried to Environmental Engineering and Science (EE&S) lab; the samples arrived at EE&S Lab were immediately well preserved in air tight containers in 4°C cold room; and the preserved samples were well mixed before taking for each series of tests; in addition to COD check, the desired characteristics of raw samples were always analyzed along the treated samples in each series of tests.

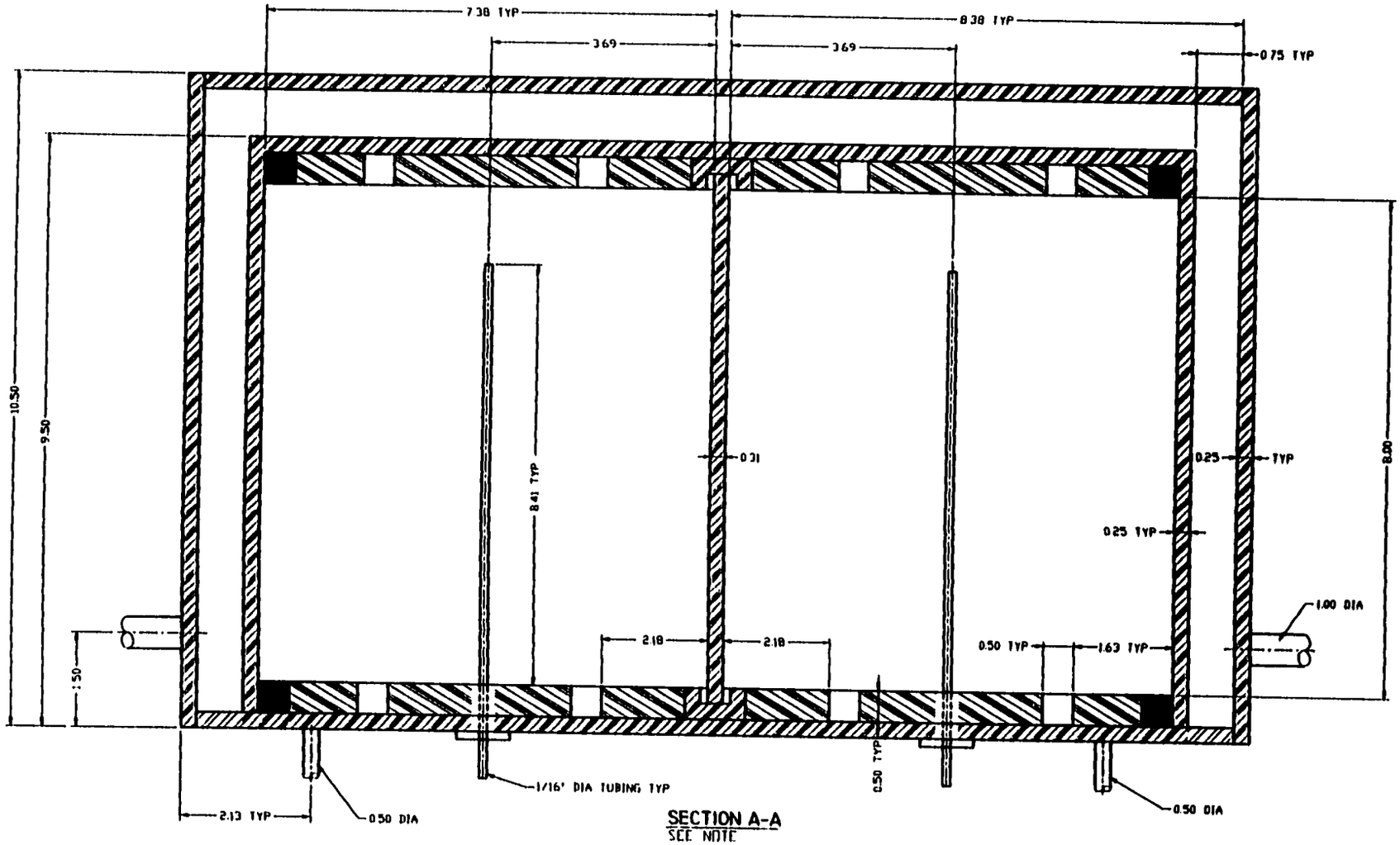
APPENDIX II

STRUCTURES AND DIMENSIONS OF BIOREACTOR SYSTEM



NOTE: FOR SECTION A-A SEE DWG NO 02.

DRAWN BY: HUAZHONG MAO	DATE: 4/17/94	TITLE: BIOREACTOR SYSTEM FOR DECOLORIZATION OF PULP MILL EFFLUENTS ENVIRONMENTAL ENGINEERING DEPARTMENT OF CIVIL ENGINEERING UNIVERSITY OF ALBERTA	DWG NO: 01
		SCALE: AS NOTED	TITLE: FRONT VIEW BIOREACTOR CHAMBER



SECTION A-A
SEE NOTE

NOTE: FOR REFERENCE SEE DRAWING NO 01.

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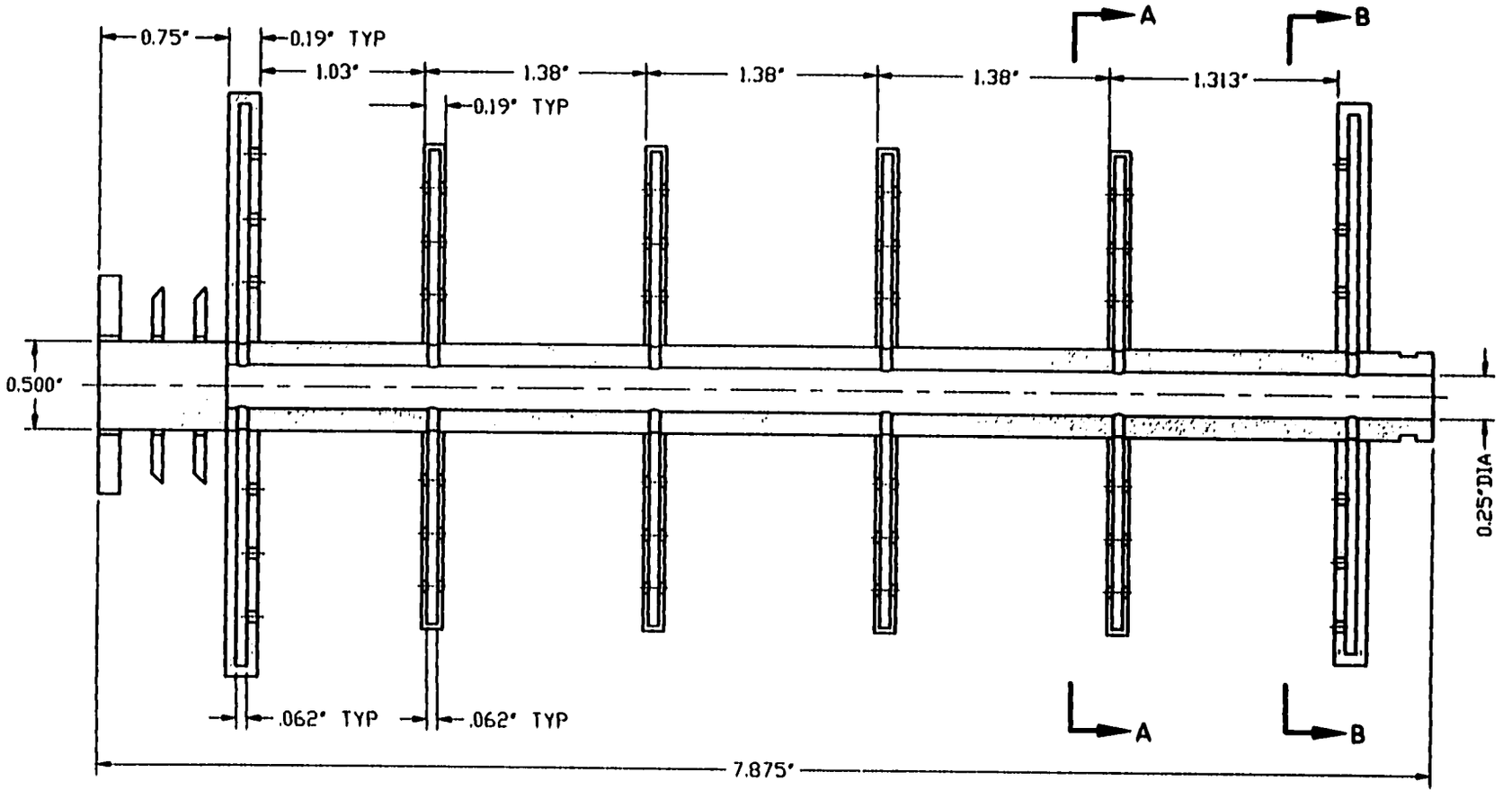

NOTE: FOR SECTIONS A-A AND B-B SEE DRAWING NO 04.

DRAWN BY: HUAZHONG MAO	DATE: 4/17/94	TITLE: BIOREACTOR SYSTEM FOR DECOLORIZATION OF PULP MILL EFFLUENTS ENVIRONMENTAL ENGINEERING DEPARTMENT OF CIVIL ENGINEERING UNIVERSITY OF ALBERTA	DWG NO: 03
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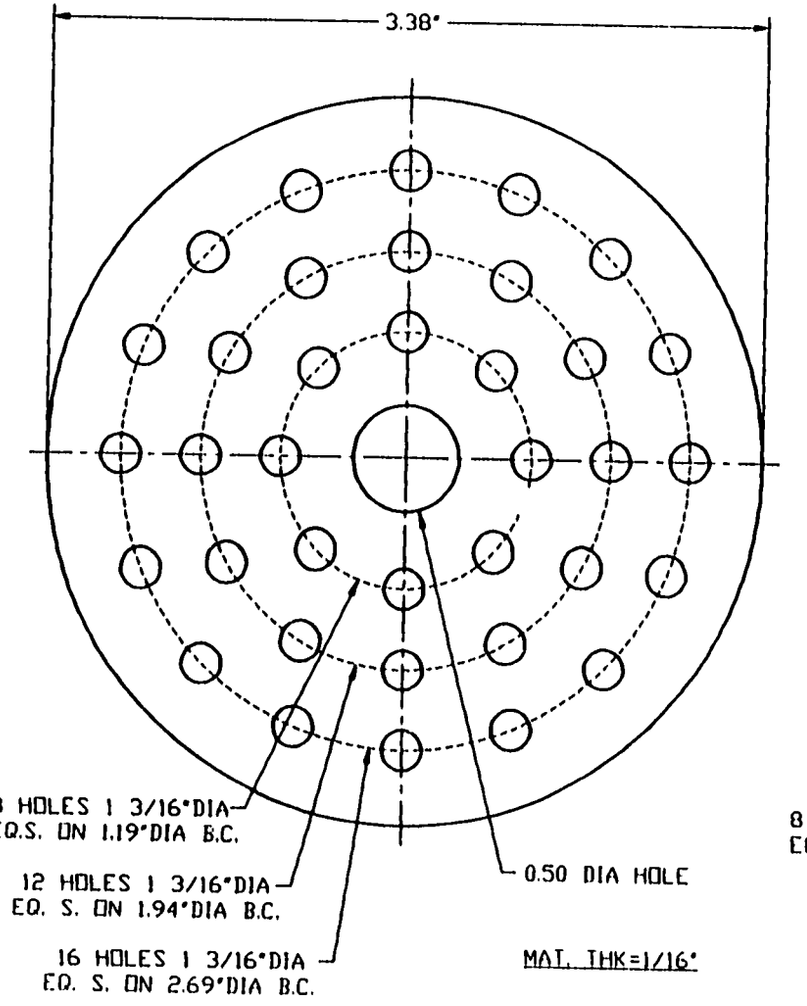
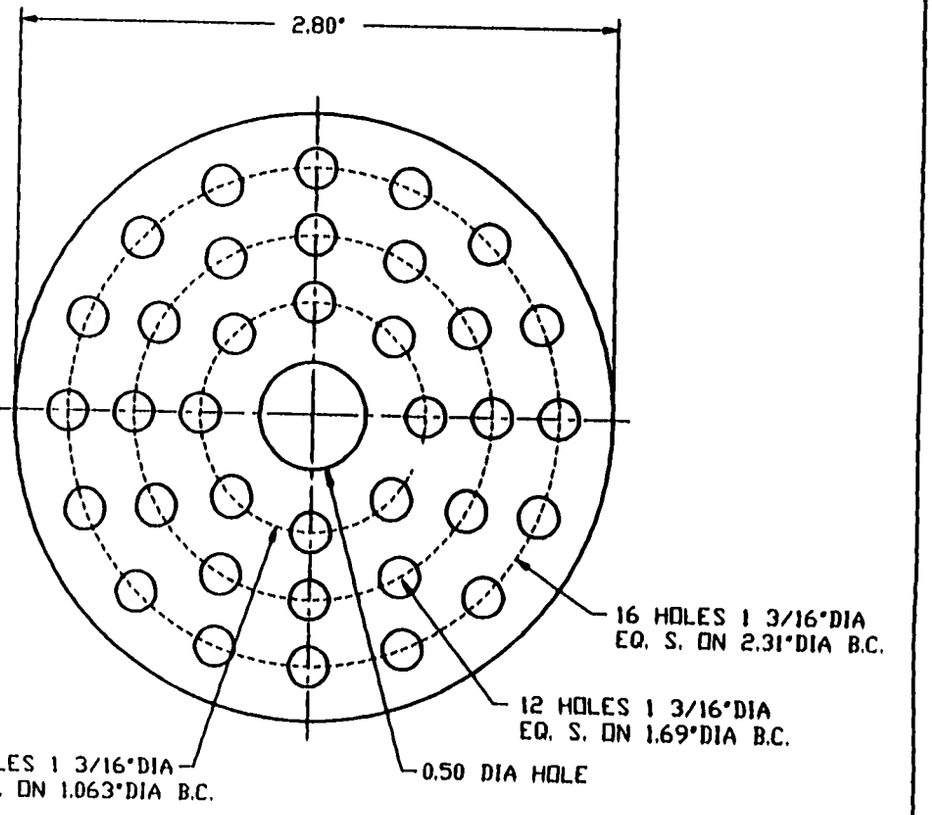


SECTION B-B
SEE NOTE



SECTION A-A
SEE NOTE

NOTE: FOR REFERENCE SEE DRAWING NO 03.

DRAWN BY: HUAZHONG MAO	DATE: 4/17/94	TITLE: BIOREACTOR SYSTEM FOR DECOLORIZATION OF PULP MILL EFFLUENTS ENVIRONMENTAL ENGINEERING DEPARTMENT OF CIVIL ENGINEERING UNIVERSITY OF ALBERTA	DWG NO: 04
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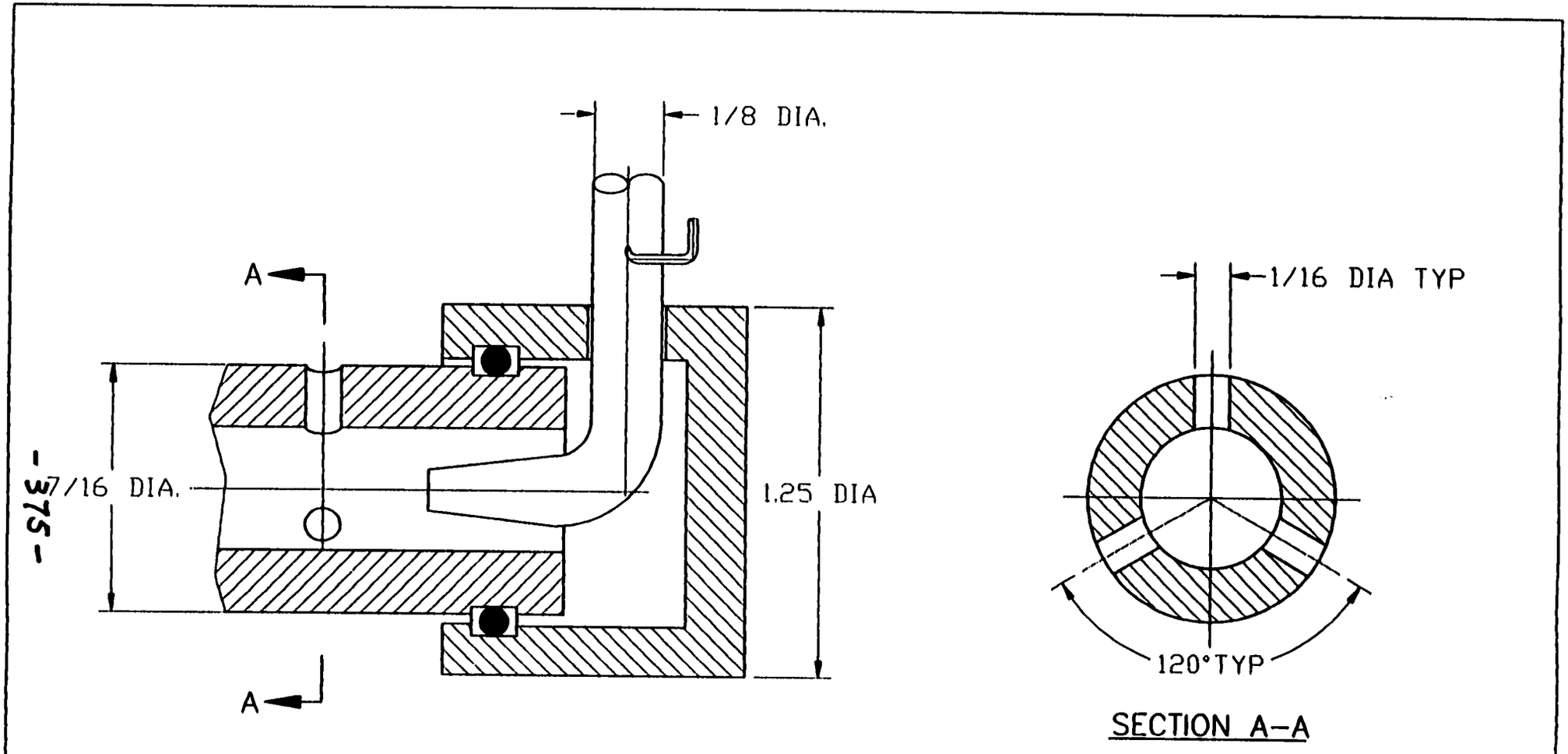
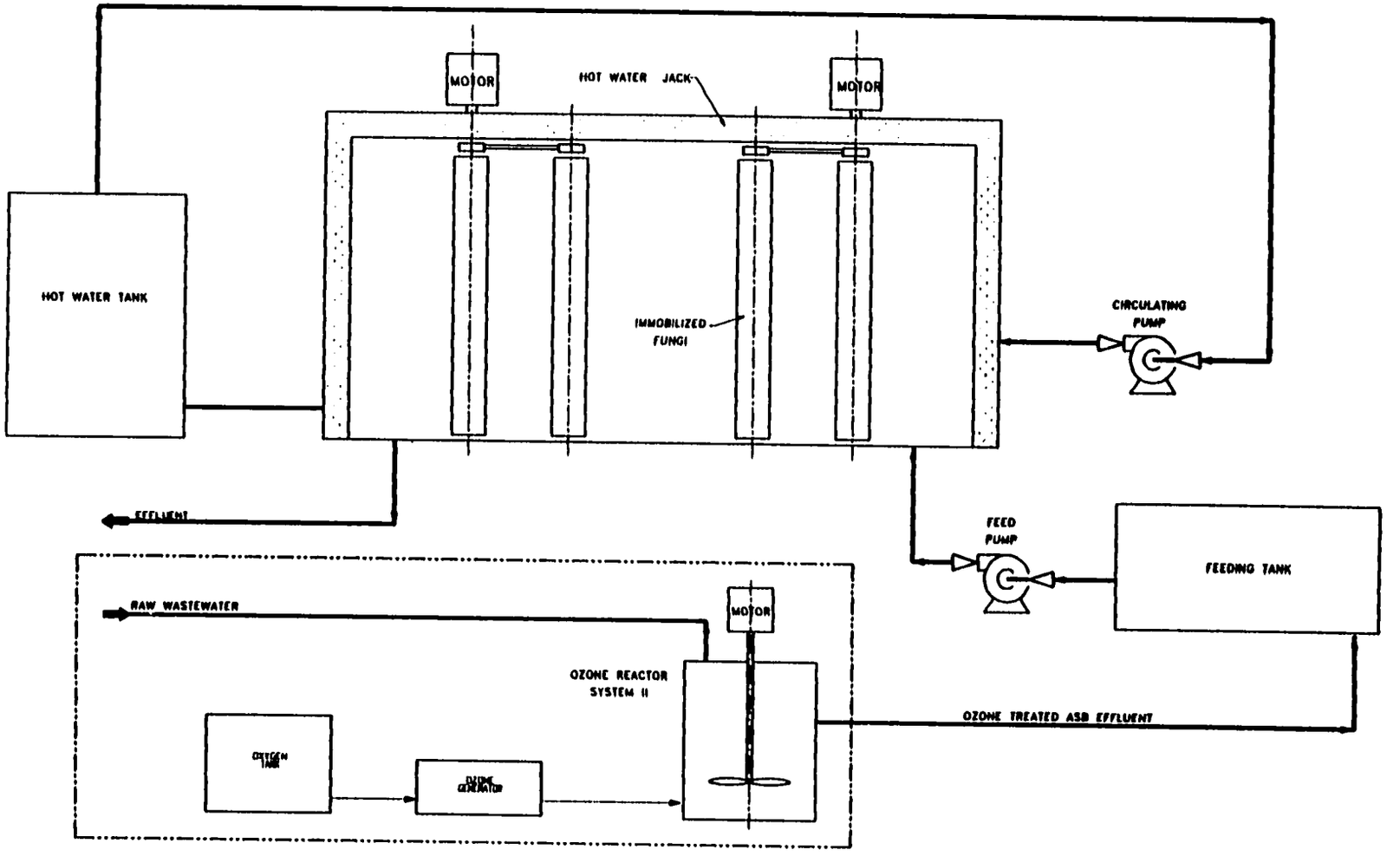


SECTION A–A

DRAWN BY: HUAZHONG MAO	DATE: 4/17/94	TITLE: BIOREACTOR SYSTEM FOR DECOLORIZATION OF PULP MILL EFFLUENTS ENVIRONMENTAL ENGINEERING DEPARTMENT OF CIVIL ENGINEERING UNIVERSITY OF ALBERTA SCALE: AS NOTED	DWG NO: 05 TITLE: INJECTOR BIOREACTOR CHAMBER

APPENDIX III

INDIVIDUAL COMPONENTS OF BIOREACTOR SYSTEM



DRAWN BY: HUAZHONG MAO	DATE: 4/17/94	TITLE: BIOREACTOR SYSTEM FOR DECOLORIZATION OF PULP MILL EFFLUENTS ENVIRONMENTAL ENGINEERING DEPARTMENT OF CIVIL ENGINEERING UNIVERSITY OF ALBERTA	DWG NO: 06
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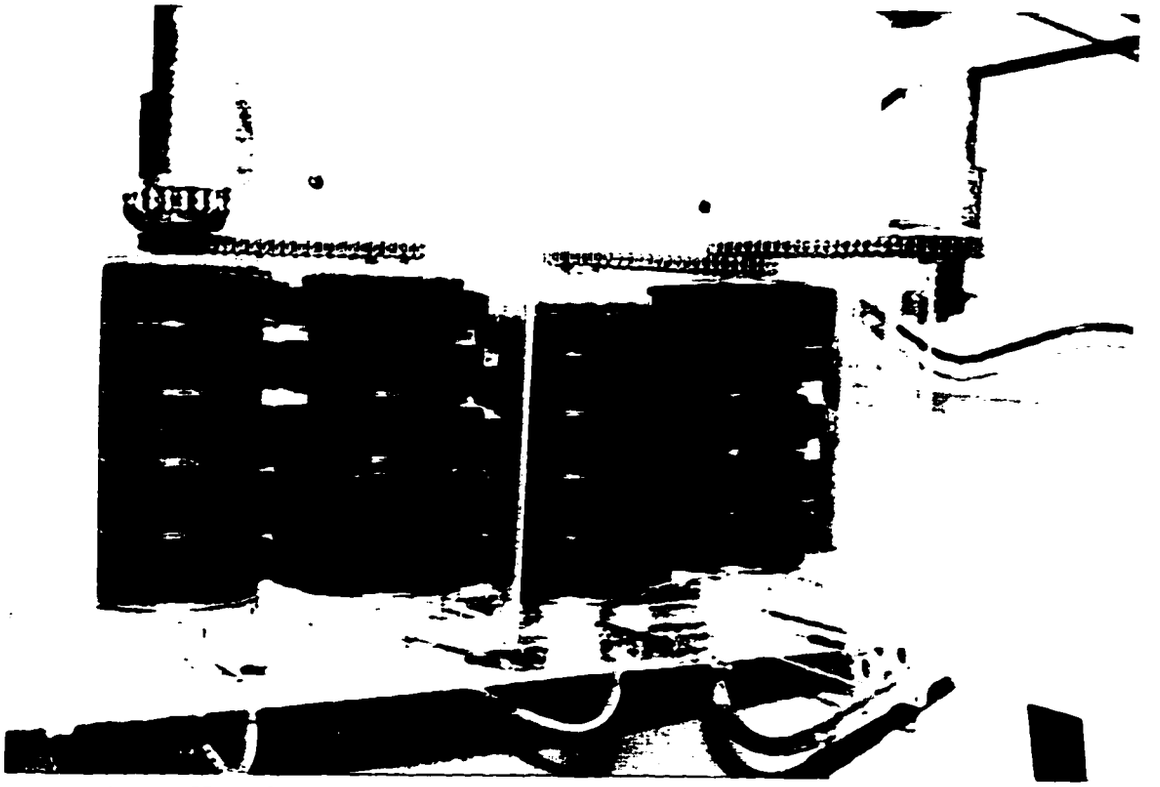
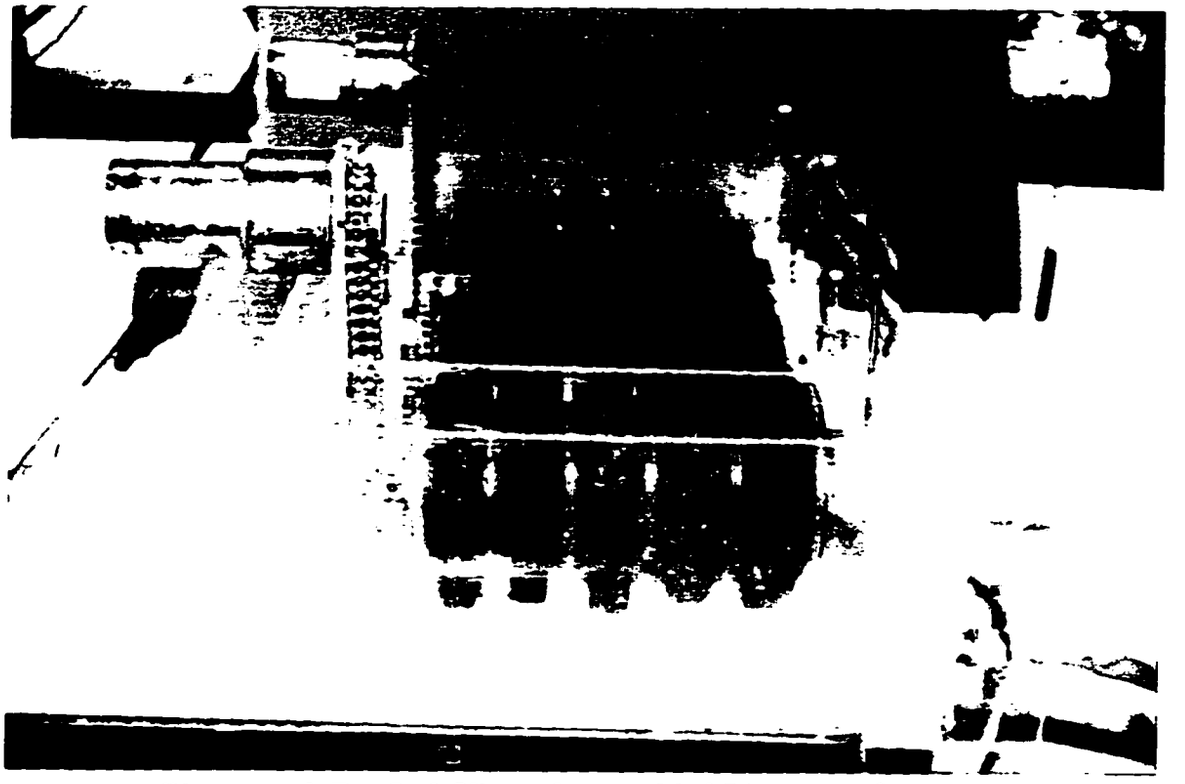
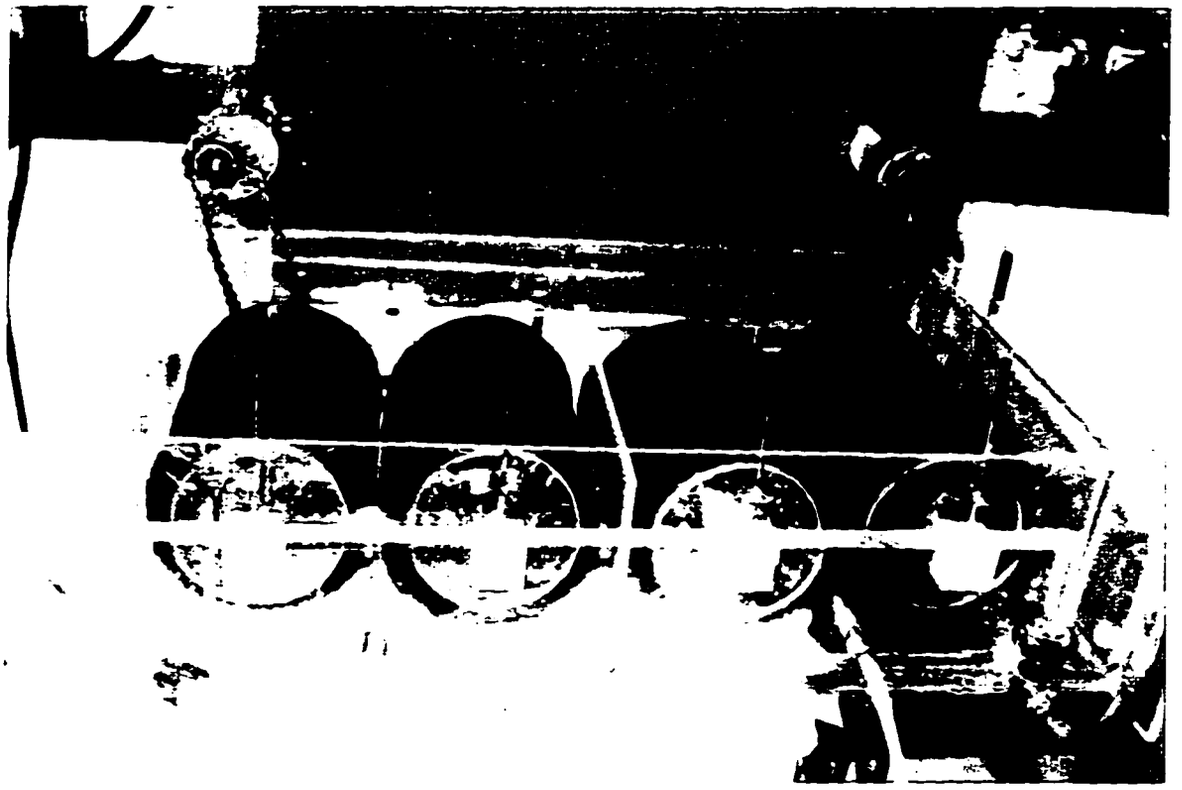
-376-(a)



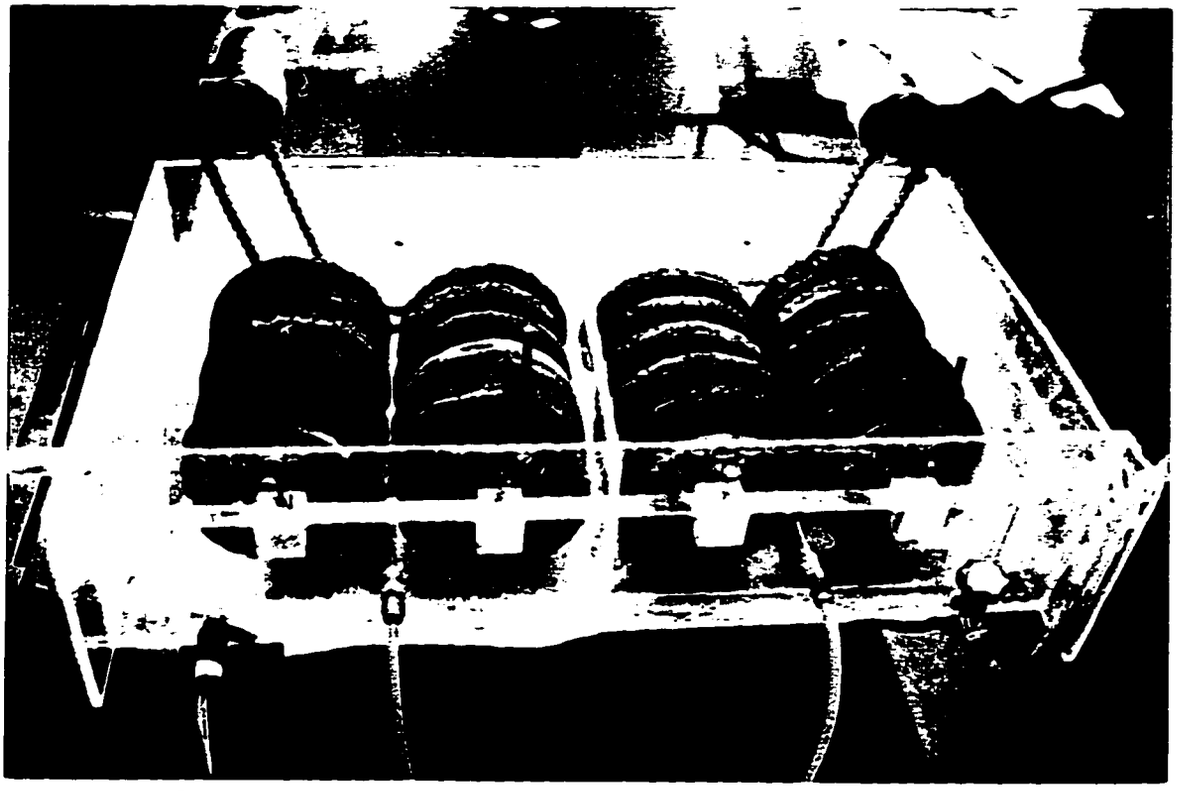
Photograph A. Top View of Assembled Bioreactor System (Portion of Bioreactor Chamber)



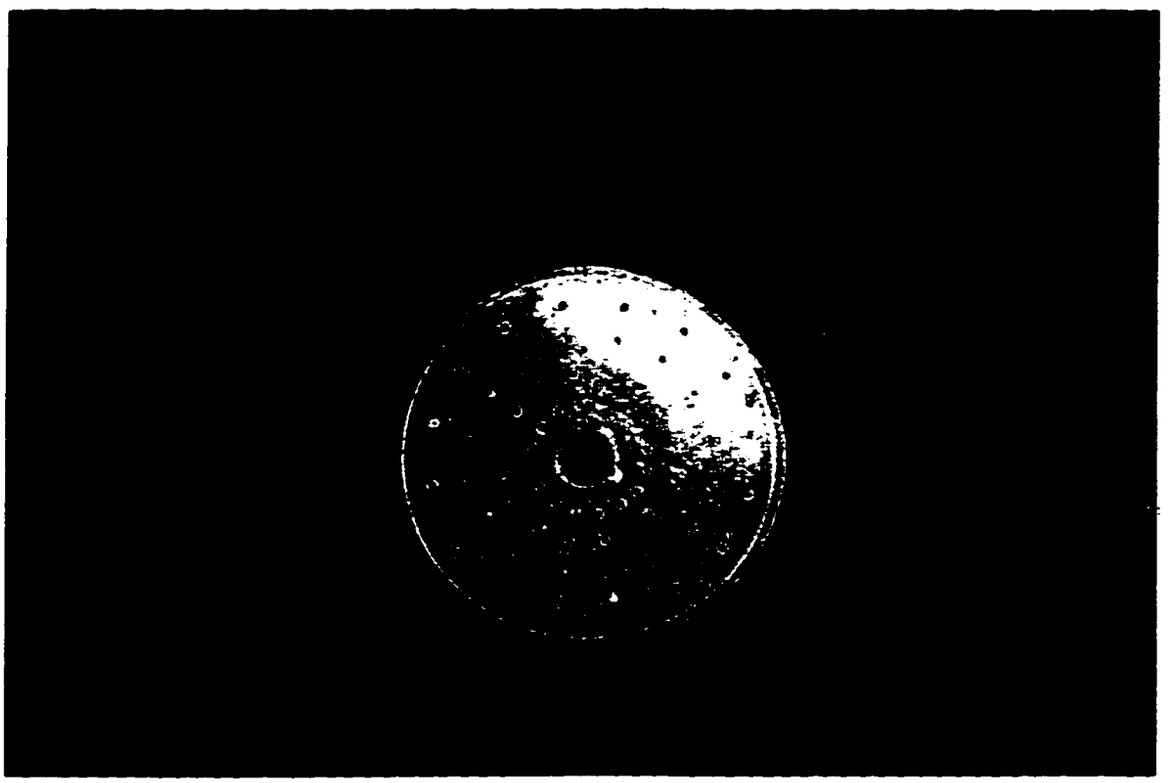
Photograph B. Left View of Assembled Bioreactor System



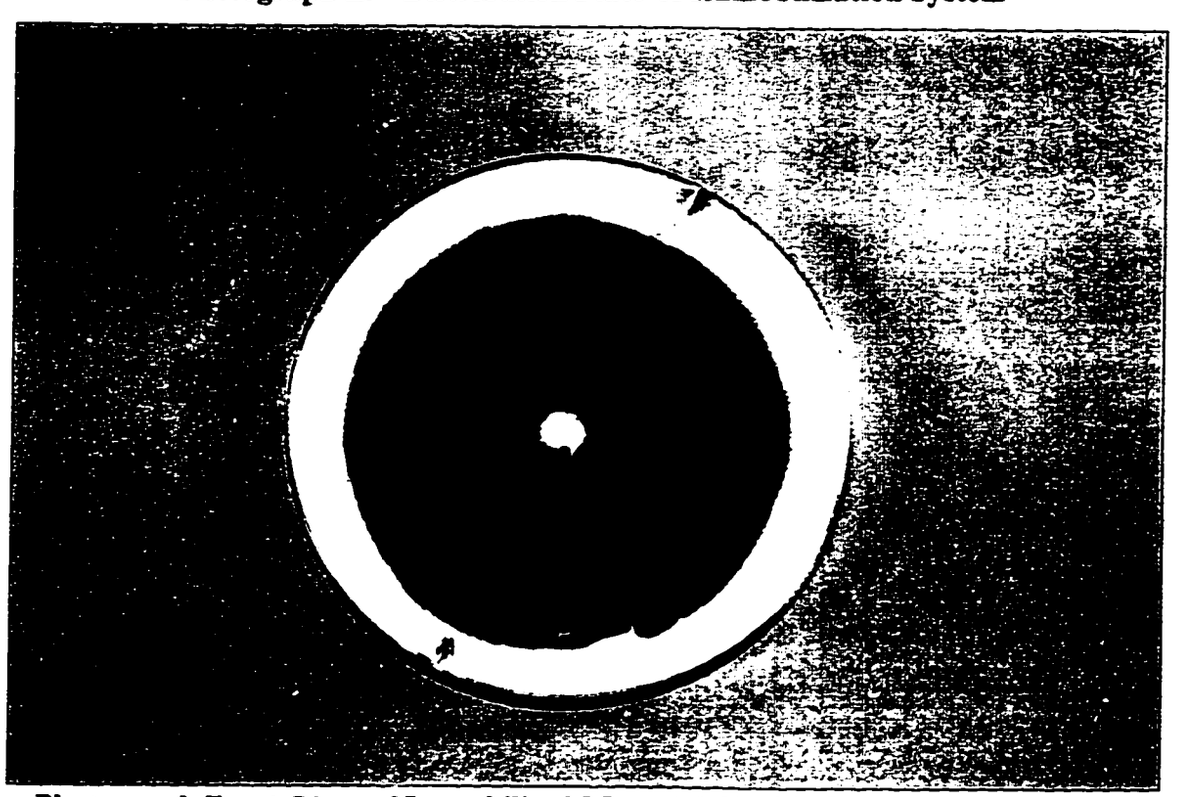
Photograph C. Top View of Newly Assembled Bioreactor System



Photograph D. The Bioreactor System After Several Series of Decolorization Tests

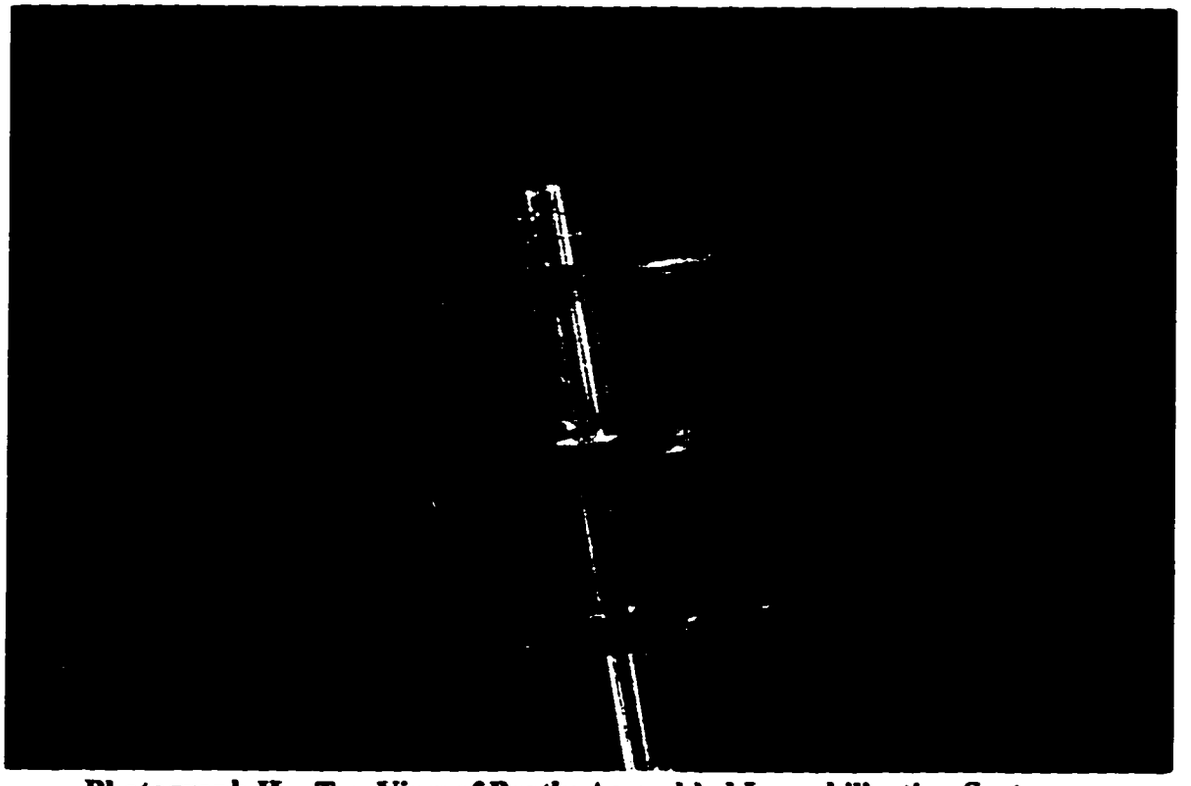


Photograph E. Distribution Plate of Immobilization System

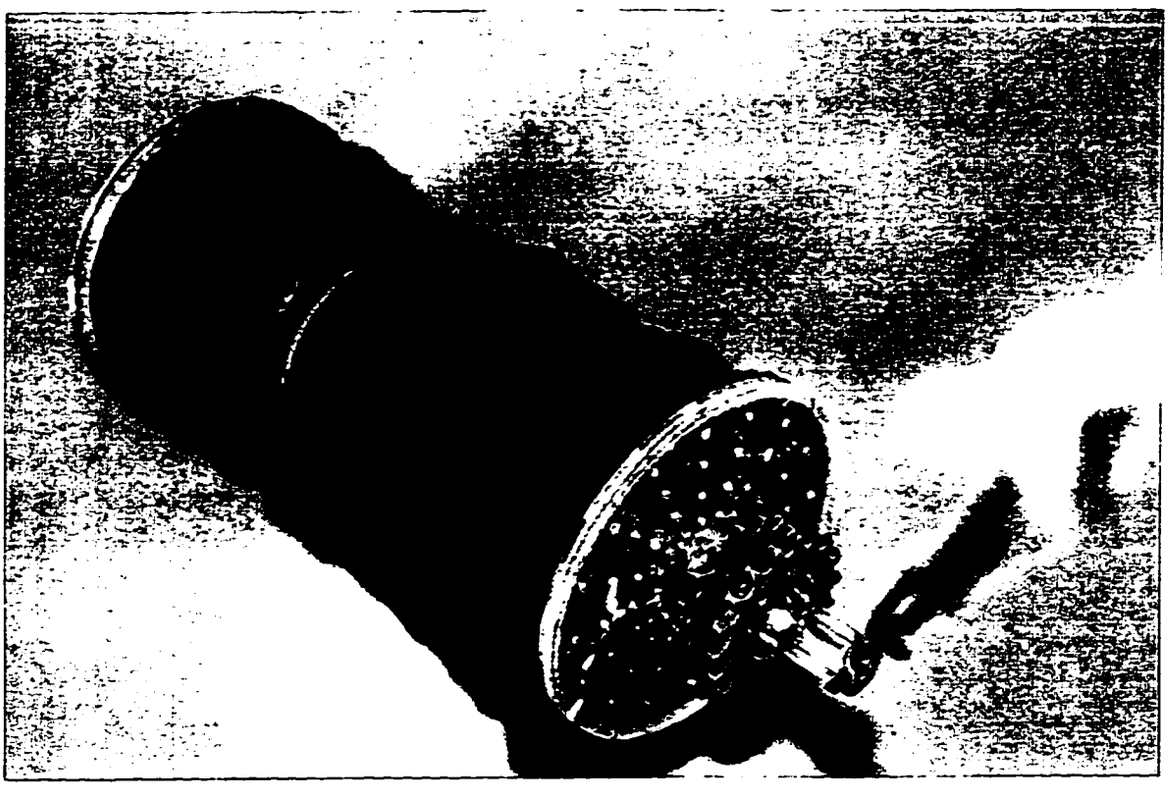


Photograph F. A Piece of Immobilized Material Used in Immobilization System

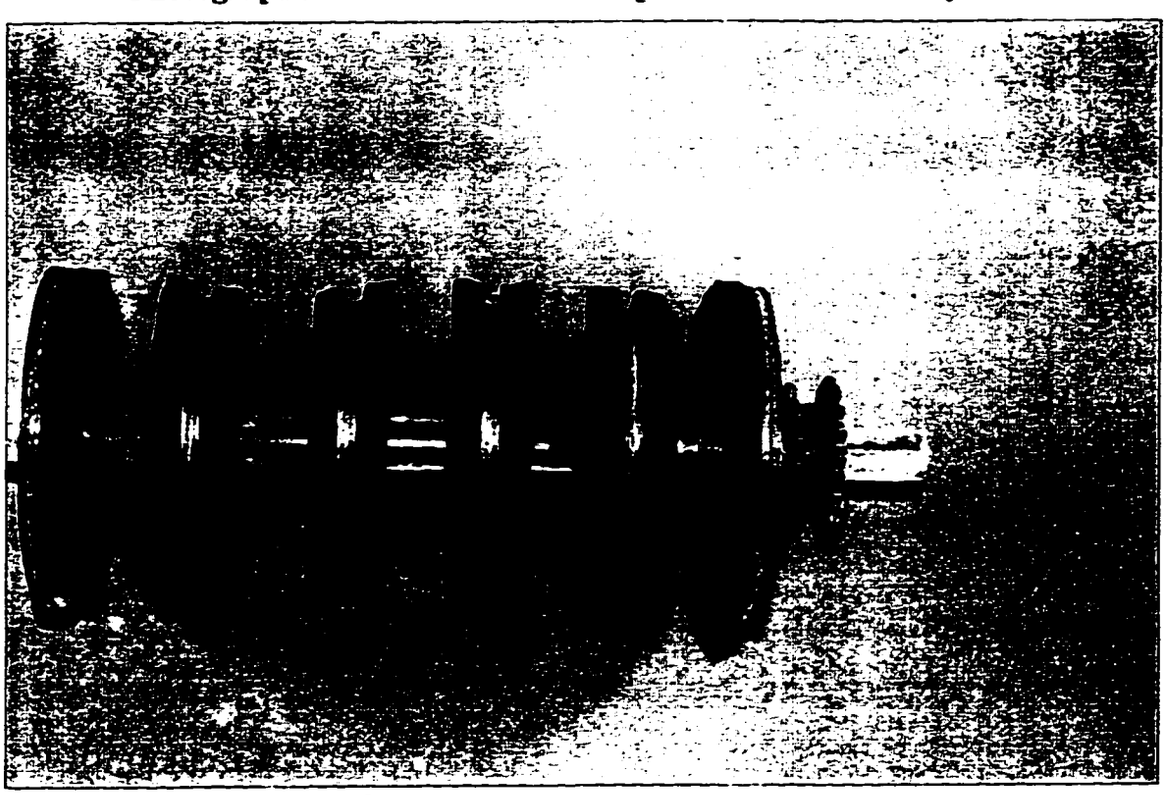
Photograph G. Sideview of Partly Assembled Immobilization System



Photograph H Top View of Partly Assembled Immobilization System



Photograph I Side View of a Complete Immobilization System



Photograph J Front View of a Complete Immobilization System

APPENDIX IV

RECOMMENDATIONS

TRANSIT PHASE PROPOSAL

Environmental Engineering & Science
Department of Civil Engineering
University of Alberta

PROPOSAL

(Transition Phase)

Advanced Engineering System for Decolorization and Dechlorination of Pulp Mill Effluents

Prepared by

Daniel W. Smith

Huazhong Mao

Prepared for

Grande Prairie Operation's Mill

Weyerhaeuser Canada Inc.

AUGUST, 1994

BACKGROUND

In Phases I and II an advanced hybrid process for decolorization and dechlorination of biologically-treated pulp mill effluent was successfully developed at a lab scale. The results suggested that the new process can achieve greater than 85% decolorization and greater than 70% removal of TOX. However, the experiments performed using newly-developed bioreactor system were very limited, and many process parameters need to be further investigated to provide engineering data base for designing and operating the pilot tests.

OBJECTIVES

The objectives of the proposed Transition Phase are to

1) further modify and finalize the design of the new bioreactor system and establish the complete operating procedures;

2) further study the key process parameters, such as temperature, critical concentrations of various critical nutrients, detention time, ozone dose, initial color levels, etc.,

3) investigate the effective range of the identified process parameters and their effects on the efficiencies of decolorization and dechlorination using a statistical design approach;

4) further study on the modes of operation and regeneration of the biomass in order to extend the life span of the biomass;

5) determine the effective (maximum) life span of the immobilized biomass and optimize the overall process;

6) establish the enzyme assay procedures for characterizing lignin-related enzymes in the treated pulp mill effluents and quantitatively evaluating their enzymatic activities; and

7) preliminary evaluation/comparison of the economic feasibility of the new process.

SCHEDULE AND BUDGETARY INFORMATION

A) SCHEDULE

According to the experience in operating the new bioreactor system and the time-consuming characteristics of continuous biological treatment studies this Transition Phase will require at least six full months without any interruption if the proposed activities are thoroughly studied. Considering the unavoidable unexpected events in experimental process development it is suggested that 7 to 8 months would be a proper period to secure the satisfactory completion of the proposed study including writing the final report. The preliminary schedule and budget are summarized in Tables 1 and 2, respectively.

TABLE IV-1. LIST OF RESEARCH ACTIVITIES

Timing	Activities
by Oct. 1, 94	Contract approval
by Oct. 31, 94	Maintain the operation of existing bioreactor, establish enzyme assay, first progress report
by Dec. 31, 94	Study on process parameters; second progress report
by Feb. 28, 95	Optimize the overall process using statistical design approach; third progree report
by Apr. 30, 95	Continuous study at established optimal conditions to collect the data for designing pilot tests
by May 30, 95	Data analysis and final report for transit phase

B) BUDGETARY INFORMATION

It is important to recognize that the following budget is only for the Transition Phase. It does not include any tests or preparation for Phase III (pilot study). The decision to proceed to Phase III is suggested to be discussed toward the end of the Transition Phase after reviewing the results from Phases I, II and the Transition Phase.

TABLE IV-2. LIST OF TENTATIVE BUDGET

SALARIES

Personnel	Task/Material	Timing	Charge
Dr. D. W. Smith	direction and review of report		no charge
Huazhong Mao	provide the consulting service		$21,000.00*
Technician	sample analysis. assistance in 24-hour continuous studies	7 person-month	$15,400.00
Graduate Student	performing required tests	9 person-month	$13,500.00
Total Salaries			$49,900.00

EXPENSES

Supplies	expendable. chemicals. others		$10,000.00
Additional Bioreactor			$5,000.00
Emzyme Assay kit			$7,500.00
Report Preparation			$3,000.00
Total Expenses			$25,500.00
Subtotal			$75,400.00
University of Alberta	**Overhead**		
Student (15%)			$2025.00
Other (40%)			$16,360.00
Subtotal			$18,385.00
PROJECT TOTAL			$93,285.00

PLEASE NOTE

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UMI

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B) BUDGETARY INFORMATION

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Report Preparation		$3,000.00
Total Expenses		$25,500.00
Subtotal		$75,400.00

University of Alberta	**Overhead**	
Student (15%)		$2025.00
Other (40%)		$16,360.00
Subtotal		$18,385.00
PROJECT TOTAL		$93,285.00