NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

UNIVERSITY OF ALBERTA

THE ROLE OF GRANULAR ACTIVATED CARBON AS A SUPPORT MEDIUM IN THE ANAEROBIC DEGRADATION OF PHENOL

By

© STEPHEN A. CRAIK

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF CHEMICAL ENGINEERING

EDMONTON, ALBERTA

SPRING 1990

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

ISBN 0-315-60283-X

micro.

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Stephen A. Craik

TITLE OF THESIS: The Role of Activated Carbon as Support Medium in the Anaerobic Degradation of Phenol

DEGREE: Master of Science

YEAR THIS DEGREE GRANTED: 1990

SIGNED: Steve Craik

PERMANENT ADDRESS:

Apt. 8, 10636–85 Avenue
Edmonton, Alberta
T6E 2K7

Date: March 9, 1990

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE ROLE OF GRANULAR ACTIVATED CARBON AS A SUPPORT MEDIUM IN THE ANAEROBIC DEGRADATION OF PHENOL submitted by STEPHEN A. CRAIK in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

M.R. Gray (Supervisor)

R.E. Hayes

P.M. Fedorak

S.E. Hrudey

Date: March 9, 1990

ABSTRACT

An experimental program was undertaken with the objective of determining the role that granular activated carbon (GAC) plays in enhancing the anaerobic bio–treatment of a phenolic wastewater, apart from serving as a physical adsorbent. GAC was employed as a biological support medium in a laboratory–scale, experimental bioreactor, treating a model wastewater containing inorganic nutrients and phenol as the major carbon source. The bioreactor was operated at pseudo–steady state in order to evaluate the phenol–degrading kinetics of a culture attached to GAC particles maintained in an expanded–bed column. The attached culture did not exhibit superior phenol–degrading activity over non–attached culture forms. The specific phenol–degrading activities of the attached, flocculated and suspended cultures were all in the range of 0.14–0.19 mg phenol/mg VSS/d.

Serum bottle cultures containing GAC–supported and flocculated biomass from the reactor were subject to nominal shock loads of phenol concentration in the supernatant of 1000, 1500 and 2000 mg/L. Above 1500 mg/L both types of culture experienced essentially total inhibition of phenol–degrading activity. Below 1200 mg/L, the activity of the GAC–supported culture was not inhibited whereas that of the flocculated culture was reduced significantly. Prevention of feedback inhibition of phenol degradation by adsorption of the metabolic intermediate, acetic acid, was proposed as the mechanism which accounted for the superior performance of the GAC culture.

Of particular significance was the rapid development and retention of a layer of flocculated biomass above the expanded–bed of GAC in the experimental reactor. S.E.M. observations indicated the formation of biomass granules up to 0.3 mm in size. Growth of the biomass layer and absence of a complete biofilm confirmed that the GAC was transferring biological activity into the supernatant. Methanogenic

activity within the GAC bed was vital in preventing wash–out of the flocculated/granular biomass. Re–seeding of the supernatant with active culture and hydraulic stabilization of a bed of flocculated biomass are two important mechanisms by which GAC was observed to enhance the treatment of phenolic wastewaters. Both have significant implications on the design of industrial wastewater treatment processes.

ACKNOWLEDGEMENTS

I would like to thank my graduate supervisor, Dr. Murray Gray as well as Dr. Phil Fedorak and Dr. Steve Hrudey, who acted as co–supervisors, for the help, advice and encouragement they provided during the course of this project. I would also like to express thanks to the staff and graduate students of the Department of Microbiology at the University of Alberta for their cooperation and assistance which allowed me to carry out my experimental work. Neil Fairbairn and Debbie Roberts, in particular, were extremely helpful to me in my endeavors with anaerobic bacteria. I would finally like to thank all the other staff members at the University of Alberta who provided me with assistance with my work.

Financial support for this project was provided by a strategic grant from the Natural Sciences and Engineering Council of Canada. NSERC also provided the bulk of my personal funding, in the form of a graduate scholarship, during my stay at the University of Alberta .

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

auth.	=	authentic
BS	=	Berl saddles
C	=	effluent substrate concentration in chemostat
C_e	=	adsorption equilibrium concentration of solute
C_a	=	concentration of solute in liquid addition in adsorption isotherm assay.
C_o	=	feed substrate concentration in chemostat
CCWW	=	coal conversion wastewater
CF	=	correction factor for thermogravimetric analysis
CFRB	=	continuous–flow recylce bioreactor
COD	=	chemical oxygen demand
CONC.	=	concentration
D	=	dilution rate
dd	=	double distilled
df	=	degrees of freedom
dil.	=	dilution
δx	=	absolute uncertainty on estimate of quantity x
EBR	=	expanded–bed reactor
F	=	volumetric flowrate in chemostat reactor
FBR	=	fluidized–bed reactor
FLOC	=	floccculated biomass
GAC	=	granular activated carbon
GC	=	gas chromatography
HAc	=	acetic acid
HP	=	Hewlett–Packard

HPLC	=	high performance liquid chromatography
k	=	pre–exponential constant in Freundlich iostherm equation
K_i	=	substrate inhibition constant
K_s	=	Monod kinetic half–rate constant
m	=	sample mass in thermogravimetric analysis
m_b	=	mass of total biomass in chemostat
m_c	=	mass of activated carbon
m_f	=	mass of flocculated biomass in chemostat
m_p	=	total mass of phenol in serum bottle cultures
$\Delta \overline{m}$	=	percent change in sample mass in thermogravimetric analysis
Δ m	=	absolute change in sample mass in thermogravimetric analysis
μ	=	specific growth rate of bacteria
n	=	order of sustrate inhibition
1/n	=	exponential constant in Freundlich isotherm equation
n_1, n_2	=	number of samples of random variables x_1 and x_2
NAC	=	non–activated carbon
PBR	=	packed(fixed)– bed reactor
q	=	mass adsorbate per mass adsorbent at equilibrium
r	=	correlation coefficient of linear regression analysis
R	=	reaction rate
rpm	=	revolutions per minute
RR	=	Raschig rings
s	=	standard deviation
S	=	substrate concentration
S.E.M.	=	scanning electron microscopy

synth.	=	synthetic
S_p	=	calculated parameter for confidence interval determination
t	=	time
$t_{\alpha/2}$	=	Students t–distribution parameter
T_o	=	initial temperature in thermogravimetric analysis
T_1	=	drying temperature in thermogravimetric analysis
T_2	=	volatilization temperature in thermogravimetric analysis
TGA	=	thermogravimetric analysis
UASB	=	upflow anaerobic sludge blanket
V	=	substrate biodegradation activity (rate)
V_a	=	volume of concentrated phenol solution added in adsorption isotherm assay
V_c	=	biodegrading activity of GAC supported culture
$\bar{V}_c$	=	specific biodegrading activity of GAC immobilized culture
V_f	=	biodegrading activity of flocculated culture
$\bar{V}_f$	=	specific biodegrading activity of flocculated culture
V_l	=	liquid volume hold–up in chemostat
V_l	=	supernatant liquid volume in serum–bottles
V_{max}	=	maximum substrate biodegrading activity
V_o	=	overall biodegrading activity of chemostat
$\bar{V}_o$	=	specific overall biodegrading activity of chemostat
V_s	=	biodegrading activity of suspended culture

∇_s	=	specific biodegrading activity of suspended culture
VOA	=	volatile organic acid
VS	=	volatile solids
VSS	=	volatile suspended solids
X	=	suspended biomass concentration in a chemostat
$x_{b/c}$	=	biomass concentration on GAC (mass VS/mass dry GAC)
$X_{b/c}$	=	biomass concentration on GAC [mass VS/(mass VS + mass dry GAC)]
$\bar{x}_1, \bar{x}_2$	=	means of random variables x_1 and x_2
$Y_{x/s}$	=	biomass yield (mass biomass/mass substrate)
$1-\alpha$	=	confidence interval

1 INTRODUCTION

1.1 BACKGROUND

Application of the anaerobic digestion process to the treatment of industrial wastewaters has attracted significant attention in recent years. Anaerobic digestion is a biological process in which organic compounds are reduced, mainly to methane and carbon dioxide gases, by a complex group of bacteria in an environment devoid of oxygen. The anaerobic process has been used since the last century for the reduction of domestic sewage sludge and has also been successfully adopted for the treatment of wastewaters arising from the food and beverage industries. Effort in developing the process for production of biogas via the anaerobic degradation of agricultural waste and municipal refuse was inspired by the energy crisis of the early 1970's and the subsequent search for cheap fuel alternatives. It is only in the last decade or so that the anaerobic process has been considered for the treatment of aqueous effluents from chemical, petrochemical, petroleum and coal industries.

Phenol and phenolic compounds are prevalent in the effluents of the following industrial operations:

1. coal conversion, *i.e.* liquefaction and gasification,
2. petrochemical processes, especially those involving phenolics as raw materials, intermediates or end products, *i.e.* phenolic resin manufacturing,
3. petroleum refining,

as well as others. The Environmental Protection Agency in the U.S. lists phenol and several phenolic derivatives, including isomers of cresol, dimethylphenol, chlorophenol and nitrophenol, as priority pollutants. As well as increasing the biochemical oxygen demand of receiving waters, even very small concentrations (ppm) of phenolics are toxic to aquatic life and can cause taste and odor problems in drinking water.

Appropriate methodologies for treatment of industrial, phenolic wastewaters vary widely, being a function of wastewater composition, strength, flowrate and required effluent quality. Options for treatment include physical and chemical methods as well as the aerobic and anaerobic biological processes. Complete removal of phenolics from wastewater using physical or chemical methods such as solvent extraction, oxidation, air stripping and adsorption is often uneconomical. Biological processes represent attractive alternatives from an economic standpoint. Phenolic wastewaters, like most industrial wastewaters, often contain a complex mixture of organic and inorganic compounds, many of which inhibit or are toxic to bacteria above certain concentration levels, and are difficult to treat biologically. In fact, phenolic compounds themselves have often been considered to be difficult to degrade. Combined treatment strategies are often most effective for the treatment of high strength wastewaters. Toxic and inhibitory compounds are reduced to levels acceptable for biological treatment by a physical or chemical method and are subsequently degraded to even lower levels in a biological step. Final polishing of the effluent in a physical or chemical step, such as activated carbon adsorption or ozone oxidation, may be required to meet effluent standards. Traditionally, the aerobic biological process, specifically the extended aeration activated sludge process, has been employed for treatment of phenolic wastewaters.

In the biological treatment of industrial wastewaters, including phenolic wastewaters, the anaerobic process offers certain potential advantages over its more established aerobic counterpart. Most importantly, excess sludge production is lower and energy efficiency is greater. Difficulties in application have arisen primarily as a result of a poor understanding of the complex microbial interactions involved and a lack of a rational basis for optimizing the design and operation of treatment reactors. Research has resulted in significant improvements in these areas and has fostered a renewed interest in the anaerobic process. Advanced

reactor design concepts have been developed with the emphasis on increasing bacterial retention times to offset the characteristic slow growth of anaerobes. The slow growth rate is often considered to be the major drawback of the process, resulting in protracted reactor start–up times and process instabilities. Immobilization of bacteria by attachment to an inert support is a common method of increasing retention times in biological reactors. Agglomeration of dispersed bacteria into flocs and the formation of granules of biomass with good settling properties is another method.

1.2 PROJECT OBJECTIVES

Granular activated carbon (GAC) has proven to be an effective bacterial support in attached–growth treatment reactor systems. The inherent adsorption capacity of activated carbon will protect a biological system against the harmful concentrations of toxic and/or inhibitory compounds in the wastewater feed which may be present during normal operation or as a result of process upsets. *In situ* use of GAC can, therefore, improve the stability of the process. The degree to which the use of GAC contributes to the improvement of the anaerobic bio–treatment of phenolics has not been fully appreciated. It has been proposed that GAC plays an additional role in process enhancement aside from functioning as a simple support medium and adsorbent buffer. Immobilization of bacteria has often been suggested as a reason for the stimulation of substrate degradation kinetics. It is possible that this effect is present in anaerobic systems incorporating GAC as a support medium.

The present study attempted to elucidate further the mechanisms by which GAC serves to enhance the anaerobic degradation of phenolics apart from its role as a physical adsorbent. Specifically, the project objectives were:

1. to establish the effect of immobilization of an anaerobic, phenol–degrading enrichment culture on GAC on the kinetics of phenol degradation,

2. to establish the characteristics of the immobilized culture which are important in the design of treatment reactors,
3. to determine the relationship between the use of GAC as a support medium and the development and retention of unsupported biomass in treatment reactors.

In order to achieve these objectives, an experimental program, involving the operation of laboratory–scale anaerobic bioreactors, was undertaken. GAC was incorporated into the reactor design as a bacterial support.

1.3 OUTLINE OF EXPERIMENTAL PROGRAM

A steady–state experiment was designed to overcome the difficulties inherent in using batch reactors to determine bio–degradation kinetics of systems incorporating GAC as a microbial support. A continuous–flow recycle bioreactor (CFRB) was employed in which a phenol–degrading anaerobic culture was immobilized by attachment to GAC. The GAC particles were maintained in the reactor as an expanded bed. To eliminate wastewater composition as an uncontrolled variable, a well–defined model wastewater containing a synthetically prepared mixture of inorganic nutrients and phenol, as the major carbon source, served as the reactor feed. Phenol concentrations experienced by the culture within the reactor were maintained below inhibitory levels.

Originally, the experimental objectives were restricted to a study of the properties of the attached culture. Shortly after start–up of the experimental bioreactor, however, a significant quantity of biomass was observed to be accumulating in the reactor as flocculated and settled material. Visual observations suggested the onset of granulation. Retention of this active, flocculated biomass could have implications on the design of treatment reactors. It was, therefore, decided that the phenomenon merited further investigation. The objectives were

expanded to include a study of the properties of the flocculated biomass and the factors involved in its retention.

The resulting research program consisted of three related experimental studies which are briefly described here.

1. Bioreactor Studies

The specific phenol degradation kinetics of the attached and flocculated cultures were determined in the experimental bioreactor. The objective was to compare the kinetics of the attached, flocculated and suspended bacteria and to determine if attachment to GAC or formation of flocs enhanced the specific phenol degrading capability of the bacteria.

2. Shock Loading Experiments

GAC–attached and flocculated biomass from the bioreactor were subject to spikes of inhibitory phenol concentration in batch culture experiments in order to determine the relative abilities of the two culture types to respond to these stresses.

3. Additional Studies of the GAC–Attached and Flocculated Cultures

Qualitative information on the nature and extent of the attached growth on GAC removed from the bioreactor was provided by scanning electron microscopy. A newly developed technique based on thermogravimetric analysis enabled a direct measurement of the quantity of attached biomass. *In situ* measurements of phenol adsorption kinetics were carried out with the purpose of providing an indirect assessment of the effect of different hydraulic regimes within the reactor on the attached culture.

Scanning electron microscopy was used to characterize qualitatively the biomass flocs which developed in the bioreactor and to determine if granulation had

occurred. A reactor containing only flocculated biomass and no GAC was operated with the objective of elucidating the mechanisms that accounted for the excellent retention of the flocculated biomass that was experienced in the GAC reactor. Of specific interest was the role of GAC in helping to establish and/or retain this biomass.

1.4 THESIS STRUCTURE

This thesis report is comprised of two main parts. The first part provides background material relevant to the project. Chapter 2 is a brief overview of the anaerobic microbial process and the current technology in anaerobic treatment of wastewaters. Chapter 3 is a review of the literature dealing with the anaerobic treatment of phenolic wastewaters with an emphasis on the role of GAC as a biological support. Literature on microbial granulation in anaerobic systems is also reviewed. The second part of the report describes the experimental program in detail, including procedures, presentation and analysis of results, and discussion. A separate chapter has been dedicated to each of the three experimental studies described previously (Chapters 5, 6 and 7). Key results and observations are combined and summarized and the implications on process technology are discussed in Chapter 8. Conclusions and recommendations for future work are then presented in Chapters 9 and 10, respectively.

2 OVERVIEW OF THE ANAEROBIC DIGESTION PROCESS

The objective of this chapter is to provide a brief overview of the anaerobic digestion process in industrial wastewater treatment. The discussion will be limited to the methanogenic anaerobic process which is responsible for the degradation of organic matter to methane and carbon dioxide gases although non–methanogenic processes, such as microbial sulfate reduction, are also important in anaerobic waste treatment (Stronach *et al.*, 1986). The anaerobic conversion of organic substrates is carried out in a sequence of tightly coupled hydrolysis, oxidation and reduction reactions that are catalyzed by a complex consortium of closely interacting bacteria. In nature, methanogenic processes play an important role in the carbon cycle, contributing to the degradation of dead organic matter. Methanogenic bacteria are responsible for the decay of vegetation in swamps and bogs and the production of swamp gas containing methane. Methane production in the rumen of cows also occurs via the methanogenic process. Engineered application of methanogenic processes has been almost entirely restricted to the degradation of both solid and aqueous wastes resulting from domestic, agricultural and industrial operations and the associated production of biogas. The following sections will cover the advantages and limitations of the anaerobic process in industrial wastewater treatment, the basic microbial interactions involved and concepts in advanced treatment reactor design.

2.1 ADVANTAGES AND DISADVANTAGES

The benefits of the anaerobic process must be assessed in comparison to aerobic biological treatment. Although both processes utilize the metabolic abilities of microorganisms, primarily bacteria, to degrade undesired organic constituents, fundamental differences between the two exist. The biochemical degradation

pathways and the nature of the bacteria involved are remarkably different. The anaerobic processes are characterized by reductive pathways and oxidative pathways that employ terminal electron acceptors other than molecular oxygen. The anaerobic consortium responsible for the degradation of a particular organic compound is far more complex than the single aerobic organism which is often capable of degrading the same organic. In general, growth rates and substrate utilization rates of the anaerobic consortia are much lower than those of aerobic bacteria (Sahm, 1984). This is a result of the low efficiency of energy utilization in anaerobic metabolic pathways. For instance, anaerobic bacteria are only able make use of 5% – 7% of the chemical energy of organic substrates in the production of new cell material (Sahm 1984). Most of the remaining chemical energy is converted into end products (*i.e.* methane and carbon dioxide).

The principle advantages of the anaerobic process which make it an attractive alternative to aerobic treatment are:

1. lower biomass production
2. lower nutrient requirements
3. potential for greater energy efficiency.

According to Sahm (1984) aerobic bacteria typically convert 50% of the organic substrate in the wastewater into more cells or biomass. The production of this biomass, termed excess sludge, usually represents a significant solids waste disposal problem in itself. In anaerobic processes, on the other hand, only about 5% of the substrate is converted to biomass while the remainder is catabolized to product gas. In addition, anaerobic sludge has better characteristics for waste disposal (Obayashi and Gordan, 1985).

The lower nutrient requirement per quantity of substrate degraded is also a result of the lower cell yield. In addition to a carbon source, nutrients such as nitrogen, phosphorus, sulphur, certain vitamins and trace amounts of metal ions are

generally required for growth of bacteria. Most industrial wastewaters require nutrient supplementation and this can be a considerable expense to the treatment process. The amount of supplementation is proportional to the cell yield of the bacteria.

Industrial–scale aerobic–treatment reactors require aeration for efficient operation. Providing large quantities of air to ensure adequate aeration and mixing represents a significant operating cost in aerobic–treatment processes. Anaerobic processes have no such requirement. In addition, the product of anaerobic degradation is a gas typically containing between 50% and 90% methane. The energy content of this gas can be recovered and used to provide the energy requirements, *i.e.* heating, mixing and pumping, for the treatment system. Treatment processes employing anaerobic digestion have the potential for energy self–sufficiency (*i.e* Van den Berg, 1984).

The principle limitations of the anaerobic process are:

1. start–up and recovery times are long,
2. the microbial process is complex and poorly understood,
3. it is a pretreatment process because unconverted organics remain in the wastewater.

The low energy efficiency of anaerobic metabolism means that the growth rates are low. Minimum doubling times of the anaerobic bacteria in treatment processes are typically on the order of days (Henze and Harremoes, 1983) whereas those of aerobes are generally much lower, on the order of minutes or hours. This low growth rate means that the time required to develop an active culture in a large treatment reactor from a small inoculum is long. For the same reason, the recovery from process upsets which result in a loss of viable biomass is slow.

The microbiology of anaerobic degradation and the nature of the interactions between the individual bacterial species involved have not been fully elucidated. As

a consequence, the causes of process instabilities and failures are frequently misunderstood. This makes it difficult to establish a rational basis for monitoring and controlling the process.

As a result of these limitations, research emphasis has been on improving the understanding of the microbiology of anaerobes and elucidating the specific degradation pathways, as well as on the development of reactor designs which increase bacterial retention. In the treatment of industrial wastewaters, the anaerobic process has been recognized as essentially a pretreatment process. Although it has been demonstrated that bulk organic removal at high rates is possible, further treatment of effluent to remove nitrogen compounds, such as ammonia and nitrate, or residual organics is usually required. A final aerobic or chemical/physical polishing step can achieve this.

2.2 MICROBIOLOGY

The stages in the anaerobic degradation of organic matter have been described by numerous reviewers (*i.e.* Sahm, 1984, Stronach *et al.*, 1986, Wilkie and Colleran, 1988). Sahm (1984) describes three major groups of bacteria performing three major steps in the overall degradation of complex organics to methane and carbon dioxide as in Figure 2.1.

STAGE 1: Hydrolysis and Acetogenesis

Large organic polymers *i.e.* proteins, polysaccharides, fats, etc. are first broken down into smaller subunits and solubilized by hydrolytic enzymes secreted by the bacteria. Soluble organics are fermented and anaerobically oxidized to acetate, hydrogen, carbon dioxide or higher organic acids and alcohols. A complex group of both facultative and strictly anaerobic bacteria are involved in this stage of the process, the composition of which is dependent on the type of substrate or substrates in the waste. Very little is known about the individual species involved

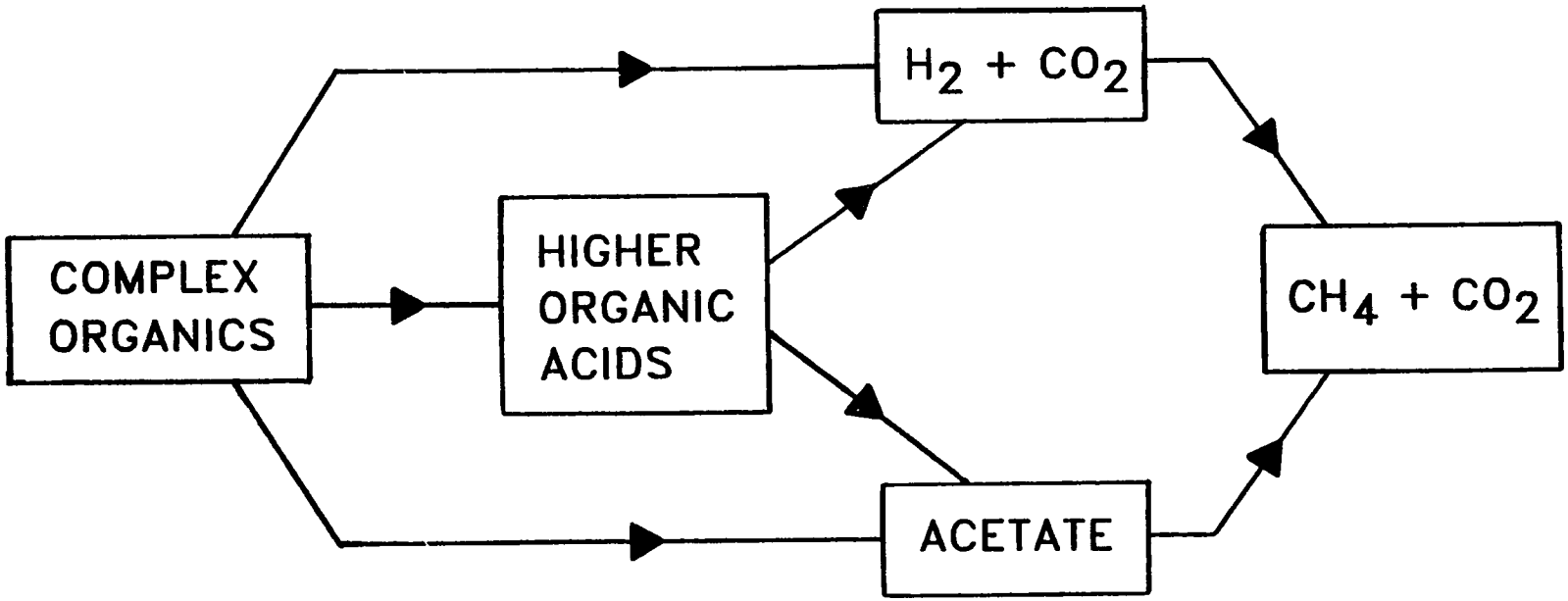

FIGURE 2.1: **Major Stages in Methanogenic Anaerobic Degradation**

and in most cases they have not been isolated or characterized. They are often referred to as the hydrolytic, fermentative bacteria and acetogenic bacteria.

STAGE 2: Acetogenesis

The higher organic acids *i.e.* propionate, butyrate, and longer chain acids, and alcohols, *i.e.* ethanol, are oxidized to acetate, carbon dioxide and hydrogen by a group of strictly anaerobic bacteria called the obligate hydrogen–producing acetogens. This is a newly discovered group of bacteria, a few species of which have been recently identified. The hydrogen producing reactions catalyzed by these bacteria are thermodynamically unfavorable unless the concentration of molecular hydrogen is kept extremely low. For instance, the degradation of propionate to hydrogen, carbon dioxide and acetate will only proceed if the partial pressure of the hydrogen product in the digester is maintained between 10^{-4} and 10^{-6} atm (McCarty and Smith, 1986). The metabolic activities of these bacteria must therefore be coupled to the consumption of hydrogen by the methanogenic bacteria. Efficient inter–species transfer of hydrogen between the two different bacteria is crucial to the success of the degradation process.

STAGE 3. Methanogenesis

The final stage of the degradation process is the production of methane and carbon dioxide by the methanogens. Many species of methanogens have now been isolated from wastewater treatment reactors and natural environments and characterized. Taylor (1982) lists 17 species as being isolated in pure culture and others have been identified since. They are members of a unique family of bacteria, the archaebacteria, which are quite distinct from typical procaryotes, and are very strict anaerobes, requiring redox potentials below – 330 mV for growth and metabolism (Hungate, 1969). Maintenance of such an oxygen–free environment in a treatment reactor depends on the oxygen scavenging action of facultative anaerobes present in the consortium. The range of substrates which the methanogens are able

to utilize is quite limited. The two major classes of these bacteria are the acetoclastic, or acetate–utilizing, and hydrogenotrophic, or hydrogen–utilizing, methanogens. About 70% of the methane produced in anaerobic processes is from the breakdown of acetate. Other substrates reported to have been utilized by methanogens are formate and methanol. Higher organic acids and alcohols are not degraded directly by the methanogens but must first be broken down by the acetogenic bacteria.

Continued successful degradation of organic substrates to gaseous products requires a careful balance of active populations of the bacteria performing the various reactions. The failure or partial failure of any single species to perform its individual task results in the accumulation of inhibitory intermediates. The organic acids are considered to be strong inhibitors of methanogenesis and rising concentration of these compounds in treatment reactors is an indication of potential process failure. The tight coupling of the metabolic activities of the acetogenic and methanogenic bacteria illustrates the degree of symbiosis displayed by the anaerobic consortium. The acetogens provide the necessary substrates for the methanogens and in turn depend on the activity of methanogens to maintain an environment of low hydrogen concentration. The degradation of organic acids to methane and carbon dioxide is generally considered to be the rate–limiting step in the degradation of soluble organics. This is a reflection of the low metabolic rates of the acetogenic and methanogenic species.

As with all biological processes, the anaerobic process is sensitive to environmental conditions. Important environmental conditions in wastewater treatment processes are:

1. temperature,
2. pH,
3. wastewater composition.

Most of the bacteria in anaerobic treatment are mesophilic, preferring an intermediate temperature range (*i.e.* 25°– 45°C). Lin *et al.* (1987) reported an optimum temperature of 35°C for the methanogenic degradation of volatile organic acids. Adjustment of wastewater temperature may be required for efficient operation. The pH for optimum growth of the methanogenic consortium is in the neutral range, between 6 and 8 (Sahm, 1984). A healthy culture creates a buffering capacity by the production of bicarbonate which enables the system to counteract a reduction in pH caused by a build–up of organic acids produced by the acetogenic bacteria. This buffering capacity is, however, limited. For many industrial wastewaters pH adjustment may be required.

The treatability of an industrial wastewater by biological means is a strong function of the wastewater composition. As was mentioned earlier, the wastewater may lack an appropriate balance of nutrients. As well as the macronutrients required for growth and energy of the anaerobic bacteria, such as carbon, nitrogen, phosphorus and sulphur, the requirement of certain micronutrients has been identified. Examples are the trace metals such as nickel, molybdenum, selenium and tungsten as well as iron (Stronach *et al.*, 1986). Industrial wastewaters often contain compounds which are toxic or inhibitory to anaerobic bacteria above certain concentration levels. These include sulphide, cyanide, thiocyanate, ammonia and heavy metals. Most aromatic compounds, including phenolics, are inhibitory substrates. Given an appropriate acclimation time and procedure, many such compounds are eventually degraded by anaerobic cultures.

2.3 ADVANCED TREATMENT REACTOR DESIGN

The greatest need in anaerobic treatment of industrial wastewaters is for reactor designs that:

1. reduce start–up times,

2. increase process stability.

The slow growth rate and the time required to acclimate a starter culture to a particular industrial wastewater combine to increase the length of time for reactor start–up. Process stability requires that a high concentration of active, acclimated bacteria be maintained in the reactor during process upsets. Wastewater treatment systems routinely experience wide fluctuations in both hydraulic and organic loadings. In particular, these systems are prone to shock loadings. To be effective, a treatment system must be able respond to and recover quickly from these upsets.

In response to these problems, the strategy employed in advanced treatment reactor design is to increase the retention time of the bacteria. The importance of bacterial retention is illustrated by considering the simplest of reactor designs, the well–mixed digester. This is a continuous, stirred–tank reactor in which the bacteria are uniformly dispersed in the liquid medium. Suspended bacteria are continuously washed out with the reactor effluent. The mass balance equation for biomass is:

$$\frac{dX}{dt} = (\mu - D) \cdot X \qquad (2.1)$$

where μ is the specific growth rate of the bacteria, D is the dilution rate (inverse of hydraulic residence time) and X is the reactor biomass concentration (Bailey and Ollis, 1986). Hence, the only non–trivial steady–state has a dilution rate fixed by the growth rate. Low growth rates result in large residence times and, for a fixed volumetric flowrate of wastewater, large reactor volumes. The constant wash–out of biomass means that it is also an inherently unstable design. A process upset, such as a nutrient imbalance, a high concentration of inhibitory compounds or a temperature shock, that causes a transient reduction in the growth rate will result

in a decline of the bacterial content of the reactor if the volumetric flowrate remains fixed. If this loss of active culture persists the entire population will eventually be washed out of the reactor.

Decoupling of the dilution rate from the growth rate is achieved by cell immobilization. This technique is commonly used in bioreactor design to increase the retention time of cells by physically separating them from the liquid. Two methods are employed in establishing this separation in anaerobic treatment reactors. The first is the agglomeration of biomass into particles with good settling characteristics, often in combination with recycle of biomass. The second method is by attachment of the biomass to an support medium. Fortunately, the anaerobic bacteria have a strong tendency to adhere both to themselves, forming flocs and granules, and to solid surfaces. In doing so, the bacteria secrete a polymer material which may aid in the adhesion process by acting as a binding agent (Stronach *et al.*, 1986). The secretion of polymer in attached growth biological systems is common and the exo–polymer is referred to as a glycocalyx (Costerton *et al.*, 1978). Coverage of a surface by a uniform layer of bacterial growth is a process known as biofilm formation. Flocculation and biofilm formation occur in many cell systems and are particularly useful in anaerobic reactor designs.

Three basic attached growth reactor designs are shown in Figure 2.2(a). Packed–bed reactors are often called anaerobic filters because the bacteria tend to become trapped in the interstices of the packing. The anaerobic filter process was first developed by Young and McCarty (1969). A variety of support media have been employed in anaerobic filters, including sand, crushed gravel, plastic or ceramic rings and granular activated carbon (*i.e.* Van den Berg, 1984). A good support medium is one to which the bacteria adhere readily and which provides a large surface area for attachment and growth (Young and Dahab, 1983). The fluidized and expanded bed designs overcome the plugging and channeling problems that are

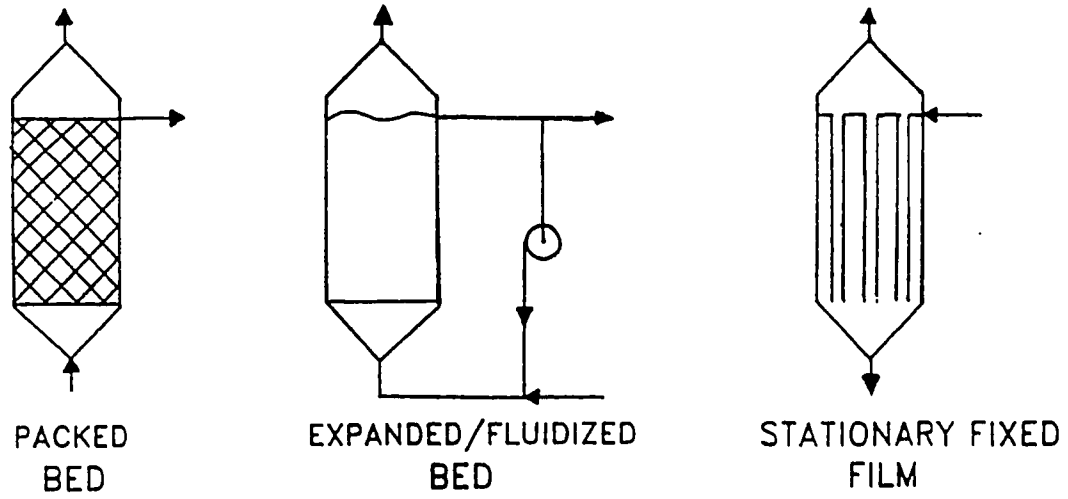

FIGURE 2.2(a): Attached Growth Reactor Designs

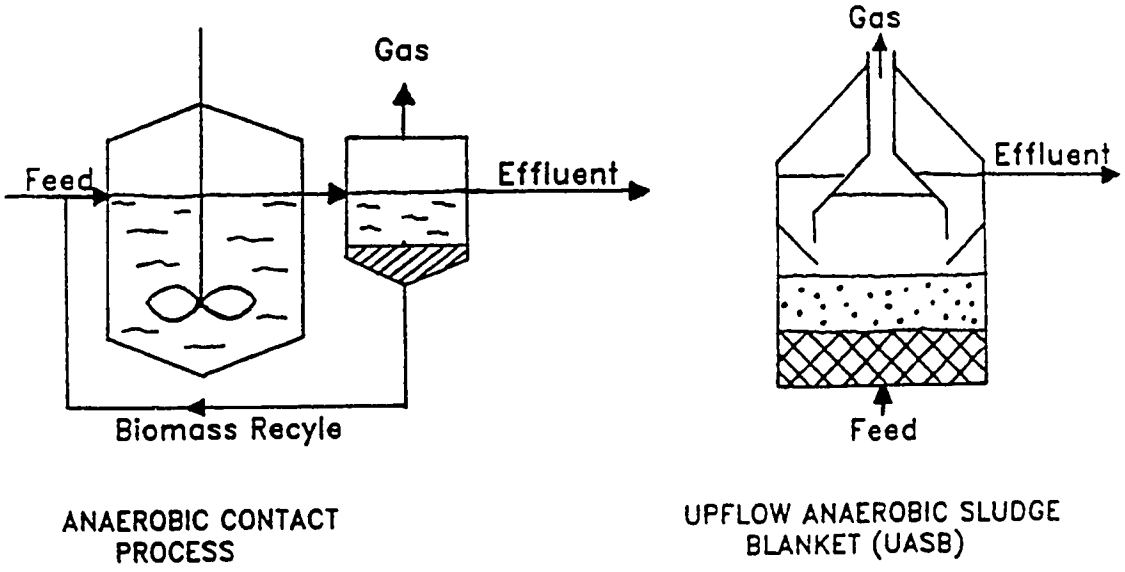

FIGURE 2.2(b): Agglomerated Growth Reactor Designs

often encountered in anaerobic filters and are generally more efficient (Switzenbaum, 1983). Stationary fixed–film reactors employ vertical tubes as the biological support. A continuous biofilm forms on the walls of these tubes.

Reactor designs based on the formation of biomass agglomerates are the anaerobic contact process and the upflow anaerobic sludge blanket (UASB) process, both illustrated in Figure 2.2(b). In the contact process, biomass is separated from the effluent of a well–mixed digester by flocculation and settling. The biomass is then returned to the digester. The contact reactor is effective in treating wastewater with a high content of settlable solids, particularly biodegradable solids, as this tends to promote settling of the bacteria. Originally developed in the Netherlands for the treatment of sugar refinery wastewater (Lettinga *et al.*, 1980), the UASB is an interesting design that has attracted attention for full–scale applications in Europe. The concept is dependent on the formation of a fast–settling, granular biomass. A dense granular layer is established at the bottom of the reactor vessel. Above this layer, a blanket of lighter material forms. Fine, suspended material is quickly washed out of the reactor so that when steady operation is established, the effluent contains very little biomass. An arrangement of baffles in the upper portion of the reactor aids in gas separation and prevents wash–out of biomass particles by flotation. There is no mechanical mixing. Gas production and release in the settled layers provides the required mixing. The granulation phenomenon, upon which this design is based, is discussed in Chapter 3.

Other interesting designs are the hybrid reactors and the two–phase process. Hybrid reactors combine the basic features of the above designs. An example is a UASB reactor that incorporates a packed bed above the sludge bed to increase the efficiency of biomass retention (Lettinga *et al*,, 1983). The principle goal of increasing biomass retention remains the same. In the two–phase process the hydrolytic and acidogenic stage of the degradation pathway is separated from the

acetogenic and methanogenic phases by using two reactor vessels, based on the realization that the metabolic activities of the two major groups of bacteria defined by this separation are more effectively optimized independently (Cohen *et al.*, 1979).

3 LITERATURE REVIEW

The objective of the research project described in this thesis was to study the role of granular activated carbon (GAC) in the enhancement of anaerobic phenol degradation. Literature pertaining to the anaerobic degradation of phenol is reviewed in this chapter. Emphasis is placed on studies in which GAC was employed to improve the treatability of phenolic wastewaters. The formation of granular biomass became important during the course of the experimental program, therefore, a section is dedicated to the subject of granulation in anaerobic treatment systems.

3.1 DEGRADATION OF PHENOL

Application of coal conversion technologies, such as the liquefaction and gasification processes, for the production of synthetic fuels has resulted in an increase in the generation of phenolic wastewaters. The data of Table 3.1, from Fedorak and Hrudey (1984a), describe the phenolic content of a full strength wastewater from a coal liquefaction process. Phenol and the alkyl phenolics (isomers of cresol and dimethylphenol) were the major phenolics present. The dominant organic in the wastewater was phenol, accounting for 62% of the total phenolics and 49% of the organic content in the H –coal effluent. Ammonia and cyanide, potential inhibitors of biological processes, were also present at low levels (6.4 mg/L and 0.21 mg/L respectively).

Phenol and phenolic compounds have long been considered to be highly refractory to biological treatment. Improved culture techniques have enabled the successful anaerobic degradation of some phenolics. Phenol was shown to be converted to methane and carbon dioxide in anaerobic cultures (Healy and Young, 1978). The batch, serum bottle cultures were inoculated with domestic sewage

TABLE 3.1: CONCENTRATION OF VARIOUS PHENOLICS AND VOA'S IN AN H–COAL LIQUEFACTION WASTEWATER[1]

Compound	Concentration(mg/L)[2]
Phenol	4900
o–Cresol	586
m–Cresol	1230
p–Cresol	420
2,4/2,5–Dimethylphenol	63
3,5–Dimethylphenol	213
3,4–Dimethyphenol	44
Total Phenolics	7456
Acetic acid	280
Propionic acid	160
n–Butyric acid	50
n–Valeric acid	35

Notes:

1. information from Fedorak and Hrudey (1984a)
2. determined by gas chromatography

sludge and required a two week acclimation period prior to the onset of phenol degradation and gas production. The stoichiometry for the conversion of phenol to methane and carbon dioxide is given by the general equation for methanogenic degradation of organic compounds developed by Symons and Buswell (Stronach *et al.*, 1986):

$$C_6H_6O + 4\ H_2O \longrightarrow 2.5\ CO_2 + 3.5\ CH_4 \qquad (3.1)$$

Healy and Young (1978) reported methane production at 89% of the theoretical predicted by equation 3.1.

An important consideration in the optimization of phenolic treatment processes is that substrate inhibition kinetics are exhibited at certain concentration levels. Neufeld *et al.* (1980) applied a substrate inhibition model to determine the kinetic parameters of phenol degradation. The maximum specific degradation rate was 0.08 mg phenol/mg VSS/d which is, as the authors pointed out, about 0.5 – 0.8% of the corresponding aerobic degradation rate. VSS refers to volatile suspended solids and is a measure of biomass commonly employed in the biological wastewater treatment field. Inhibition of phenol degradation occurred at phenol concentrations of greater than 686 mg/L. However, the reported growth yield was 0.82 g VSS/g phenol, which is very high compared to typical growth yields for anaerobes (*i.e.* 5%, Sahm, 1984), and no methane production was observed. Because of these drawbacks, the results of the Neufeld *et al.* (1980) study must be interpreted with skepticism. Fedorak and Hrudey (1984b) reported inhibition of methane production at phenol concentrations of greater than 1200 mg/L in batch cultures and presented evidence that the methanogenic bacteria in the phenol–degrading consortium were less susceptible to phenol inhibition than were the phenol–degrading bacteria. They also showed that the presence of other

phenolics can inhibit the degradation of phenol. The effect of some alkyl phenolics on methane production was tested. The only other phenolic, other then phenol, observed to be degraded to methane was *p*–cresol. Dimethyl phenols at concentrations above 500 mg/L were inhibitory to methane production from phenol. Cresols produced the same effect at concentrations above 1000 mg/L. Wang *et al.* (1989) observed complete degradation of phenol at initial concentrations as high as 1400 mg/L in batch cultures, although a reduction in degradation rate was seen at initial concentrations above 600 mg/L. In agreement with the conclusions of Fedorak and Hrudey (1984b), phenol inhibited the action of the phenol–degrading organisms and not that of the methanogens. It should be pointed out that the results of kinetic studies will depend largely on the properties of the inoculum used, *i.e.* type, source and age, as well as culturing procedures and medium composition.

The biochemical pathway catalyzed by the phenol–degrading anaerobic consortium has not been clearly elucidated. Originally it was shown that phenol was reduced to cyclohexanol and then cyclohexanone prior to cleavage of the aromatic ring and further degradation to organic acids (Evans, 1977). Some recent work by Knoll and Winter (1987 and 1989) and Kobayashi *et al.* (1989) has suggested an alternate pathway involving the formation of benzoate in an initial step prior to ring cleavage. Benzoate was detected as an intermediate in phenol degradation (Knoll and Winter, 1987). It is conceivable that both pathways exist and that the one selected in a given situation depends on the nature of the consortia present and the environmental conditions imposed. Figure 3.1, from Knoll and Winter (1987), describes both of these hypothetical pathways.

Even though the complete degradation pathway is uncertain, the consensus in the literature is that acetate is the major intermediate. Knoll and Winter (1987) measured a concentration of acetate as high as 590 mg/L during batch degradation of phenol. This build–up was transient and all of the acetate was eventually

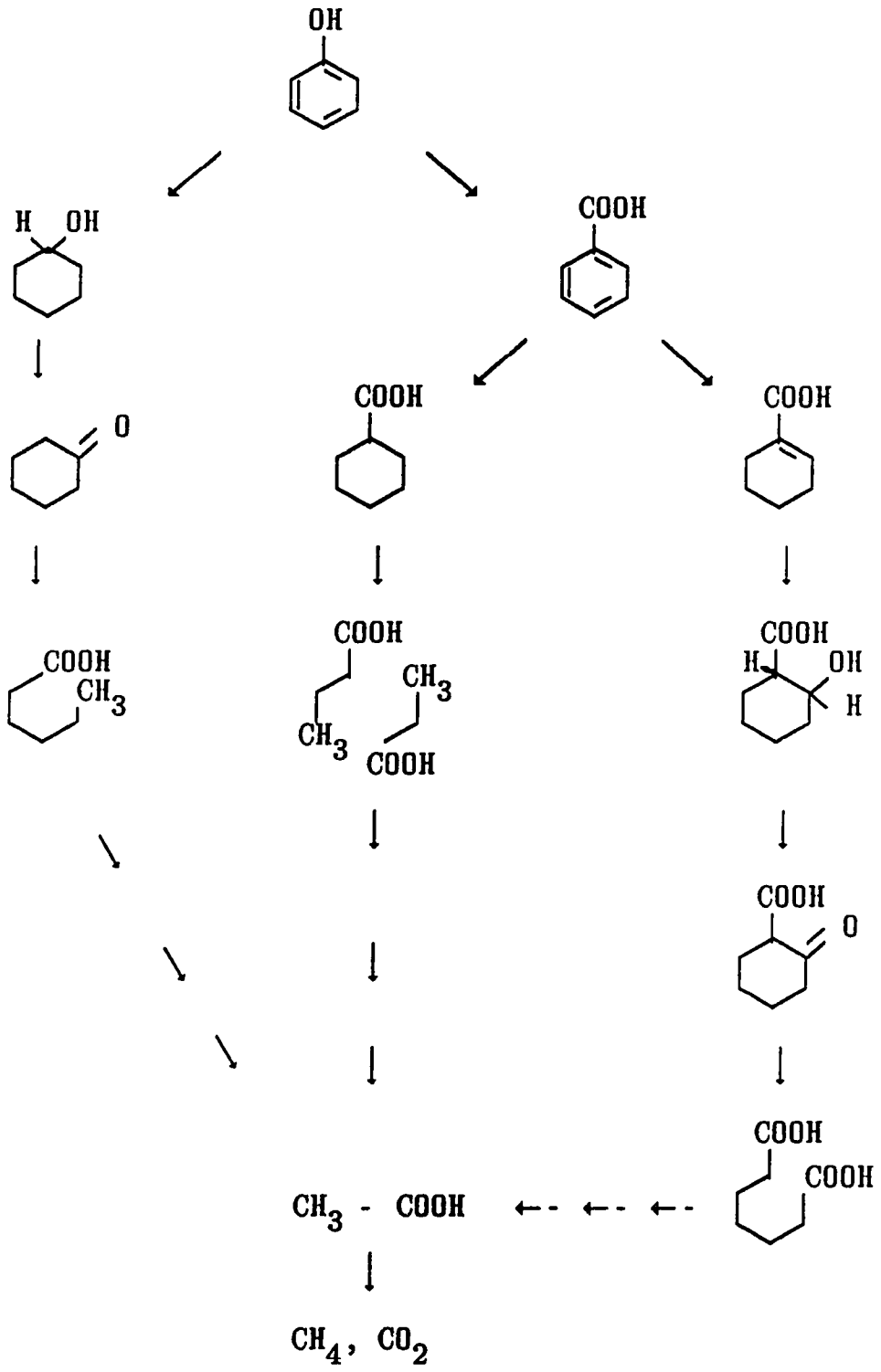

FIGURE 3.1: **Proposed pathways for the anaerobic degradation of phenol (Adapted from Knoll and Winter, 1987)**

converted to product gases. Wang *et al.* (1989) reported that acetate was the only intermediate which they detected in their experiments, although the levels were much lower at between 10 and 20 mg/L.

A full characterization of the phenol–degrading consortium is also lacking, however, some recent work has produced a partial description of the bacterial types involved. Using scanning electron microscopy, Dwyer *et al.* (1986) distinguished three different morphological types in a methanogenic, phenol–degrading enrichment immobilized in agar. An acetoclastic methanogen was recognized by the filamentous morphology characteristic of the acetate–utilizing *Methanothrix* species. A hydrogen–utilizing methanogen was also identified. A small, oval bacterium was hae proposed phenol–oxidizing organism. Based on this three member consortium and the recognition of acetate as the major intermediate in the pathway, the set of reactions catalyzed by the individual bacterial species is most likely:

$$C_6H_6O_6 + 5\ H_2O \longrightarrow 3\ CH_3COOH + 2\ H_2 \qquad (3.2)$$

$$3\ CH_3COOH \longrightarrow 3\ CH_4 + 3\ CO_2 \qquad (3.3)$$

$$2\ H_2 + 0.5\ CO_2 \longrightarrow 0.5\ CH_4 + H_2O \qquad (3.4)$$

Equation 3.2 is anaerobic oxidation of phenol to acetic acid and hydrogen and equations 3.3 and 3.4 are acetoclastic and hydrogenotrophic methanogenesis, respectively. The sum of these equations yields the stoichiometry for the overall conversion of phenol to product gases predicted by Symons and Buswell (equation 3.1).

Conversion of phenol present in wastewater from a coal conversion process to methane and carbon dioxide has been shown. Methane production was observed in batch anaerobic treatment of volumetric dilutions containing up to 6% of the wastewater in Table 3.1 (Fedorak and Hrudey, 1984a). Unidentified inhibitory

compounds in the wastewater retarded the production of methane from volatile organic acids, indicating that the methanogens were affected. Ether extraction of the wastewater reduced the degree of inhibition substantially. Inhibition by ether–extractable components was not attributed to the major phenolics.

3.2 ROLE OF GAC IN TREATMENT OF PHENOLIC WASTEWATERS

Granular activated carbon has been widely used in both biological and non–biological wastewater treatment processes. The purpose of this section is to review the state of knowledge of the role of GAC in the anaerobic treatment of industrial, phenolic wastewaters. The focus will be on the research work that has aided in establishing that role.

When employed *in situ* in a biological treatment reactor, such as a packed, expanded or fluidized–bed bioreactor, the two main recognized functions of GAC are as follows. Firstly, it serves to increase the microbial retention time by acting as a support medium to which the culture readily attaches. The surface character of GAC, particularly the predominance of surface irregularities, such as macropores and crevices, make it an ideal support medium by providing a large surface area for attachment and growth. These features also provide a protective environment, sheltering the bacteria from the effects of hydraulic shear and inter–particle abrasion. Secondly, by virtue of the adsorption capacity of GAC, it is able to reduce the concentration of toxic and/or inhibitory compounds, which are often present in industrial wastewaters, to levels low enough to permit biological activity. Non–biodegradable constituents may also be removed, thereby making the treatment process more complete. By the same mode, GAC can function as an excellent system buffer by absorbing the effect of sudden surges of inhibitory substrates in the process influent. As discussed in the previous section, above certain levels, phenolic substrates inhibit anaerobic bacteria. At very high levels

they are toxic. Shock loads of these substrates are quickly adsorbed by the GAC preventing the exposure of the bacteria to high aqueous concentrations. Upon returning to normal loading conditions these substrates are slowly desorbed and biodegraded, thus renewing the adsorption capacity of the carbon. This degradation of previously adsorbed material has been given the term bioregeneration.

Several recent studies have investigated these roles in anaerobic bio–treatment of synthetic and authentic, or dilutions of authentic phenolic wastewaters. Both continuous and batch reactor systems have been employed. Table 3.2 gives a chronological listing of these studies.

In anaerobic batch serum bottle experiments, Fedorak *et al.* (1985) showed that the addition of GAC allowed treatment of higher concentrations of wastewater from an H–coal liquefaction process (see Table 3.1). In addition, the acclimation time for the production of methane from the phenolic compounds in the wastewater was shortened. Combinations of H–coal effluent concentrations between 0 and 20% (0 – 1500 mg/L phenolics) and GAC doses from 0 to 2500 mg/L were tested. In the cultures containing low concentrations of both GAC and H–coal wastewater, reduction of the concentration of the major phenolics by adsorption was negligible; however, acclimation times were noticeably reduced due to adsorption of inhibitory materials. At the concentrations experienced in these cultures the major phenolics would not be considered inhibitory. In the cultures containing higher GAC and wastewater concentrations the removal of phenolics by adsorption accounted for further reductions in acclimation times. Adsorbed phenolics were later available for bioregeneration. Batch experiments on the treatability of coal–coking wastewater (Fedorak and Hrudey, 1987) indicated severe inhibition of methanogenesis. Addition of GAC only partially relieved the inhibitory effects of the wastewater. The identity of the inhibitory substance was not confirmed, however, cyanide, which was present in the wastewater and which is not strongly adsorbed, was

TABLE 3.2: STUDIES EMPLOYING GRANULAR ACTIVATED CARBON

INVESTIGATOR	SYSTEM	WASTEWATER
Suidan *et al.* (1981)	GAC PBR	synth. catechol, o–cresol
Khan *et al.* (1981)	GAC PBR	synth. phenol, glucose
Khan *et al.* (1982)	GAC PBR, coal PBR	synth. phenol, glucose, auth. aircraft paint stripping–bath
Cross *et al.* (1982)	RR PBR + GAC PBR	dil. auth. CCWW
Suidan *et al.* (1983a)	BS PBR + GAC EBR + aerobic nitrification	synth. CCWW
Suidan *et al.* (1983b)	same as 1983a	dil. auth. CCWW
Harper *et al.* (1984)	GAC FBR	dil. auth. CCWW
Fedorak and Hrudey (1985)	Batch serum bottles	dil. auth. CCWW
Kim *et al.* (1986)	RR PBR + GAC FBR	synth. phenol
Wang *et al.* (1986)	GAC EBR	synth. phenol
Suidan *et al.* (1987)	same as 1983a	synth. CCWW
Fedorak and Hrudey (1987)	Batch serum bottles	dil. coal–coking wastewater
Fox *et al.* (1988)	BS PBR + GAC EBR	synth. CCWW
Gardner *et al.* (1988)	GAC FBR, NAC FBR	Refinery sour–water stripper bottoms
Pfeffer and Suidan (1989)	GAC FBR	dil auth. CCWW
Kindzierski (1989)	Sequential–batch serum bottles	synth. phenol

DEFINITIONS OF SYMBOLS FOR TABLE 3.2

PBR	.....	Packed bed reactor
EBR	.....	Expanded bed reactor
FBR	.....	Fluidized bed reactor
BS	.....	Berl saddles
RR	.....	Rashig rings
GAC	.....	Granular activated carbon
NAC	.....	Non–activated carbon
CCWW	.....	Coal conversion wastewater
synth.	.....	synthetic
auth.	.....	authentic
dil.	.....	volume dilution

suspected.

Suidan *et al.* (1981) successfully treated a synthetic, catechol–containing wastewater using two GAC packed–bed reactors in series. Catechol removals of greater then 99% were achieved at steady–state on a feed of 1000 mg/L catechol. Based on volumetric loading, biological conversion rates were comparable to those achieved in aerobic systems. In the same study, no degradation of *o*–cresol was observed. Using a similar system, Khan *et al.* (1981) achieved high removals of phenol and glucose. These and similar studies from Table 3.2 exhibited the same reactor start–up characteristics. In an initial phase, no biodegradation of phenolics occurred and removal was attributed almost exclusively to adsorption. With the onset of breakthrough, phenolic substrate was made available to the bacteria and methanogenic activity commenced. Bioregeneration was then observed when methane production exceeded the theoretical maximum attributable to the biodegradation of the organics in the feed. Methane production increased until a steady–state was achieved. At this point of operation, biological breakdown was the main means of phenolic removal. Wang *et al.* (1986) used partially saturated GAC to eliminate the initial adsorption phase in treatment of a synthetic, phenol–containing wastewater. They also detected a loss of attached biomass at higher loading rates which they attributed to increased gas shearing.

In attempts to determine the relative importance of the mechanisms which account for the effectiveness of GAC in anaerobic bio–treatment, several investigators carried out studies in which the performance of GAC was compared to that of other media. Khan *et al.* (1982) demonstrated the superior performance of GAC over non–porous anthracite coal in terms of organic removal, methane production and biomass retention for a system treating both a synthetic, phenol bearing wastewater and a diluted aircraft paint stripping–bath wastewater. The authors suggested substrate polarization and the ability of the GAC to shelter dense

bacterial populations as mechanisms for improved performance. The GAC system readily absorbed a surge in feed phenol concentration after 275 days of continuous operation, demonstrating the buffering capacity of the system. Suidan *et al.* (1983a) employed a system consisting of a reactor packed with Berl saddles followed by a reactor containing an expanded bed of GAC to treat a synthetic analog of a coal gasification wastewater containing a complex mixture of phenolics and known inhibitors (*i.e.* cyanide, ammonia, thiocyanate). This treatment was followed by an aerobic nitrification step. There was very little removal of organics in the Berl saddle reactor although removal efficiencies in the GAC reactor were high. An identical system was used to treat a solution containing 10% authentic coal gasification wastewater (Suidan *et al.*, 1983b), with similar results. The low biological activity in the Berl saddle reactor is suggestive of the special role that GAC plays in adsorption of toxic and inhibitory material. Cross *et al.* (1982) observed the same low bio–activity in a Rashig ring packed reactor treating a dilution of coal conversion wastewater. In a later study, Suidan *et al.* (1987) noticed methane production from glucose in a Berl saddle reactor even in the presence of a synthetic coal conversion wastewater, suggesting that the inhibitive constituents of the wastewater affect the bacteria that perform the initial breakdown of the aromatic ring, rather then the methanogens. This observation is contrary to observations made by Fedorak and Hrudey (1987) in treating coal coking wastewater. Gardiner *et al.* (1988) compared the performance of non–activated carbon to GAC in treating refinery sour–water stripper bottoms in a fluidized–bed. They also investigated the effect of varying GAC particle size. The poor performance of the non–activated carbon system relative to the GAC system reflects the necessity of toxicity removal by adsorption. The superior performance of the smaller GAC particles observed at higher wastewater loading rates was attributed to increased surface area for microbial growth and decreased mass

transfer resistance during adsorption.

These studies were able to demonstrate quite clearly that the adsorption capacity of activated carbon was crucial in reducing the level of toxic compounds in order to permit biological treatment. It was realized, however, that the adsorption capacity of the GAC for toxic and/or inhibitory constituents of coal conversion wastewaters was limited and would eventually become exhausted. This need was first identified by Harper *et al.* (1984) in a study in which a solution containing $10-12\%$ coal conversion wastewater was treated in a fluidized–bed reactor. Shortly after saturation of the GAC by cresols, reactor performance declined sharply indicated by a decrease in COD removal efficiency and cessation of gas production. An accumulation of volatile acids was observed, suggesting that the methanogens were inhibited first. Performance improved immediately after replacement of 10% of the carbon with fresh material. To maintain performance levels for the longer term, subsequent carbon replacements were required. At the end of the study the authors suggested an optimum steady–state carbon replacement schedule of $0.6-0.8$ kg GAC per kg of organic carbon fed to the system, noting that removal of too large a portion of the GAC results in excessive loss of biomass. Suidan *et al.* (1987), Fox *et al.* (1988) and Pfeffer and Suidan (1989) also reported the requirement of GAC replacement in the systems they were studying.

Kindzierski (1989) carried out a study with the objective of identifying the features of a biological support that account for enhanced removal and eventual conversion of phenol to methane. Properties of three different potential supports (Filtrasorb F300 activated carbon, Dowex MSC–1 cation exchange resin and Ambergard XE–352 anion exchange resin) which determined their abilities to function as microbial supports were characterized and compared. These included surface chemistry, pore volume and surface area available for microbial attachment and proliferation, and phenol adsorptive capacity. Initial bacterial affinity for the

supports was determined by adsorption studies with radiolabelled cells. The actual performance of the supports was determined in sequential–batch serum bottle experiments which employed a model wastewater containing phenol as the carbon source and the necessary nutrients for growth. Scanning electron microscopy enabled a qualitative description of the attached culture characteristics. Since the model wastewater did not contain the toxic and inhibitory compounds prevalent in an authentic wastewater, the study was able to provide further insight into those characteristics, aside from adsorption capacity, that enable a material to function as an effective microbial support.

Pore capacity and associated surface area were determined to be the most important features that encourage attachment and growth of biomass on the supports. Based on pore–size distribution and a knowledge of bacterial sizes, the internal surface area available for attachment and growth was estimated to be 0.0173 m^2/g for the F300 activated carbon, 0.227 m^2/g for the XE–352 resin and close to zero for the MSC–1 resin. Cell adsorption studies showed that the XE–352 resin possessed a higher initial affinity to *Pseudomonas aeruginosa* cells in suspension than did the F300 activated carbon. The MSC–1 resin displayed negligible adsorption affinity. Results of the phenol bio–activity assays showed that there was no increase in surface attached bio–activity of the F300 activated carbon after 75 days of culturing. The supernatant, however, possessed a significant amount of activity suggesting that the activated carbon had become overloaded biological growth. Continued growth on the surface was being offset by removal mechanisms such as hydraulic shear and inter–particle abrasion. Even after 88 days the bio–activity of the XE–352 supported culture continued to increase and the activity in the supernatant was far less then that which was observed in the activated carbon cultures. There was negligible bio–activity associated with the MSC–1 resin. Scanning electron microscopy revealed extensive attachment and

growth of the bacteria inside the surface pores and crevices of the activated carbon and the pores and internal areas of the XE–352 resin. Very little attachment was observed on the exposed outer surface of any of the supports. Because the MSC–1 resin did not possess a porous structure or an irregular surface no growth was fostered on this support. The large surface pores of the activated carbon and the XE–352 resin offer an environment where the bacteria are protected from the effects of hydraulic shear and inter–particle abrasion. Response to pulses of high phenol concentration showed that the suspended bacteria from the XE–352 resin culture were more susceptible to substrate inhibition then were the attached bacteria. The phenol adsorption capacity of the resin was negligible suggesting that the protection of the attached bacteria from the inhibitory concentration of phenol in the bulk liquid could be attributed to mass transfer limitations within the immobilized culture.

Of particular interest in the Kindzierski study was the observation that the biomass–loaded activated carbon was capable of rapidly transferring bio–activity into the liquid medium. Some results of sequential–batch degradation experiments which illustrate the re–seeding of the supernatant are given in Figures 3.2(a) and 3.2(b). After 75 days of growth on the GAC a sequence of supernatant withdrawals and replacements was commenced. In this procedure, 88% of the supernatant liquid was withdrawn from the culture and replaced with cell–free medium. This quantity was the largest aliquot of liquid which could be withdrawn without removing a portion of the carbon particles. The removed supernatant was divided into two fractions. A 12% fraction represented the portion which remained behind with the activated carbon. The second fraction was the remaining 76%. Each of the three cultures, the activated carbon culture and the 12% and 76% supernatant cultures, was fed the same amount of phenol. Figure 3.2(a) shows the decrease in phenol concentration in the supernatant of each of three cultures with time until there is no

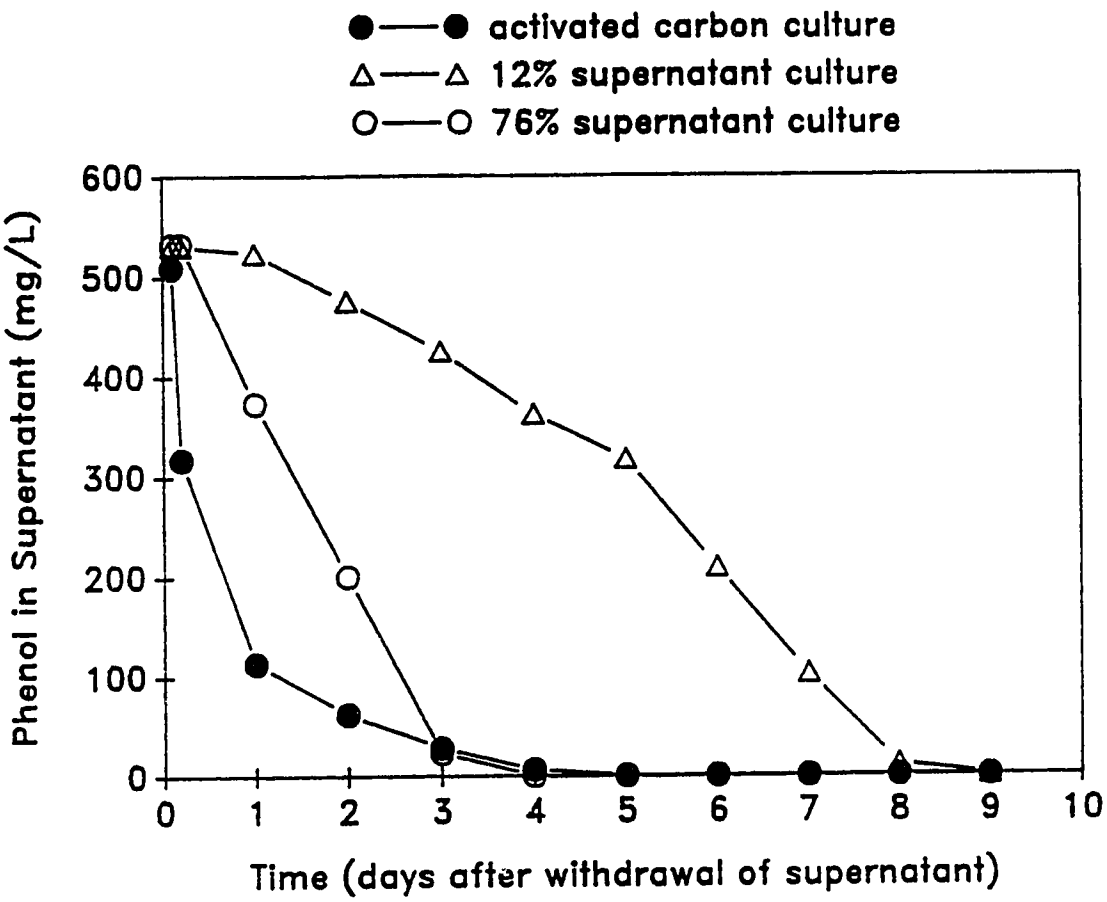

FIGURE 3.2(a): Phenol biodegradation in GAC–containing sequential–batch cultures after the first of a series of supernatant withdrawals and replacements (data from Kindzierski, 1989)

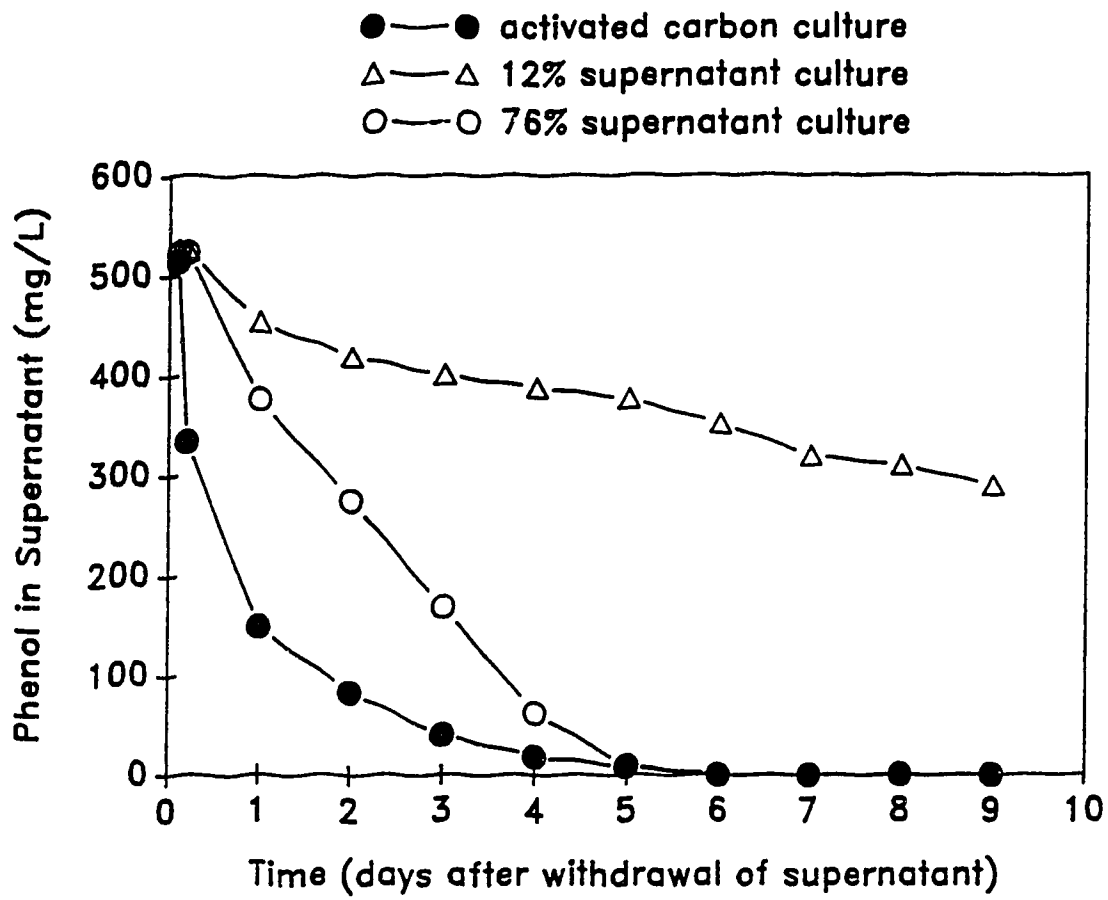

FIGURE 3.2(b): Phenol biodegradation in GAC–containing sequential–batch cultures after the fourth of a series of supernatant withdrawals and replacements (data from Kindzierski, 1989)

activated carbon culture which was due to adsorption. This phenol was subsequently desorbed from the activated carbon and biodegraded. The 76% supernatant culture possessed a significant amount of phenol–degrading activity relative to the activated carbon–containing culture. The procedure was repeated sequentially and the results of the fourth supernatant withdrawal and replacement are presented in Figure 3.2(b). As the figure shows, the 76% supernatant still possessed significant phenol–degrading activity although to a lesser degree than in the first supernatant withdrawal. The activity of the activated carbon–containing culture was approximately the same. This suggests that the activated carbon–containing culture was rapidly transferring bio–activity to the supernatant liquid in the form of new growth. If it was not, the activity of the supernatant would have decreased by 98% by the fourth withdrawal because of the sequential dilution procedure.

The previous work has, therefore, been successful in partially characterizing the role GAC in enhancing anaerobic treatment of phenolic wastewaters. The importance of the adsorption capacity of GAC in treating authentic wastes has been unequivocally established. Kindzierski's work provided new information on the nature of GAC as a microbial support. Under the hydraulic conditions experienced in constantly shaken serum bottles, the colonization of the GAC eventually reached a maximum after which any further growth was transferred into the supernatant. This re–seeding of the liquid medium could have significant implications in treatment reactor design. None of the literature mentioned in this review has reported the specific phenol degradation rate of bacteria immobilized on GAC. The difficulties in determining the phenol degradation kinetics in GAC systems are as follows:

1. The effects of adsorption and desorption of phenol on the apparent

degradation rates cannot be easily assessed. Kindzierski (1989) demonstrated the effect of bacterial growth on the phenol adsorption kinetics. The rate of uptake and adsorption capacity were both noticeably reduced, presumably as a result of blockage of the macropores with biomass as illustrated by Figure 3.3.

2. The determination of specific degradation rates requires an estimate of the quantity of active biomass. No satisfactory method for directly measuring the quantity of biomass attached to the GAC in anaerobic system is available.

3. Biological activity is a function of many variables and wastewater treatment systems are extremely complex. The selection of an appropriate set of control conditions is difficult.

Serum bottle studies suffer from an additional disadvantage in evaluating the performance of attached growth systems in that the environment of hydraulic shear and inter–particle abrasion is not representative of the environment that exists in actual treatment reactor configurations such as the packed, expanded or fluidized–bed reactors.

3.3 GRANULATION IN ANAEROBIC TREATMENT REACTORS

Development of biomass granules with good settling properties and high specific activities is an excellent method of increasing bacterial retention times in treatment reactors. No inert support medium is required, therefore the inactive fraction of the reactor volume is reduced. This phenomenon of biomass granulation has the potential to influence greatly the design of advanced treatment reactors. The mechanisms involved in the initiation and promotion of bacterial granulation, however, appear to be quite complex and are far from being fully understood. The aim of this section is to provide a brief review of the current understanding of the

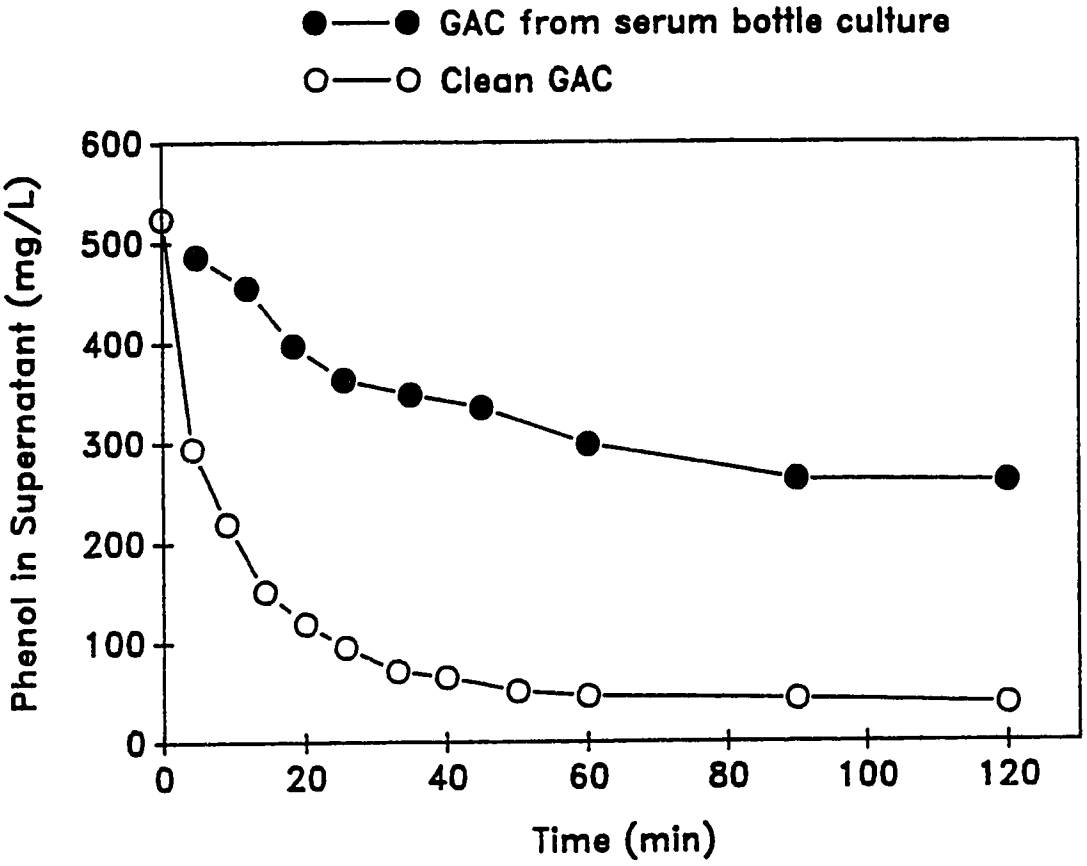

FIGURE 3.3: Phenol adsorption onto clean GAC (without bacteria) and GAC from serum bottle cultures (in the presence of attached growth) (data from Kindzierski, 1989)

granulation phenomena in anaerobic treatment systems.

Interest in biomass granulation in anaerobic treatment was initiated by the development of the upflow anaerobic sludge blanket (UASB) reactor. As reported by Lettinga *et al.* (1980 and 1983), the formation of dense layers of flocculated and granular biomass is integral to the UASB design concept. The distinction between granules and the bacterial flocs which are generally observed in anaerobic treatment processes can be considered as follows. Flocculation occurs when cells in suspension form conglomerations having relatively loose structures, or flocs. Upon settling together, the flocs form a uniform layer of biomass and are no longer individually distinguishable. Granules are more dense and particulate in appearance and remain visible as separate entities after settling. They also have significantly higher settling velocities.

Many factors appear to be involved in the granulation process. Hulsoff Pol *et al.* (1983) summarize factors that are considered to be important in the development of granular biomass in UASB reactors as follows:

1. environmental conditions such as like wastewater composition, the availability of nutrients, the concentration of univalent and divalent cations, the presence of inhibitory compounds and finely dispersed non–biodegradable matter, temperature, pH, etc.,
2. the type of inoculum (*i.e.* source, composition, specific activity),
3. the process conditions applied during start–up such as like organic loading rate and quantity of inoculum used.

Hulshoff Pol *et al.* (1983) carried out an experimental study with the objective of evaluating the impact of several of these factors on the development of granules. They discovered that wastewater composition and start–up procedures play a dominant role. The addition of a small amount of granular biomass with the inoculum did not have a significant effect on granule formation. A selection process

was observed to take place during the initial reactor start–up phase in which the smaller, suspended biomass particles were washed out with the effluent. The larger, granular particles were retained and growth was subsequently concentrated on this granular biomass. When returned to the reactor, the suspended material was not able to form granules and was soon washed out again. Flotation of flocculated and granular biomass, resulting in wash–out, occurred due to the increased gas production and retention of gas bubbles within the particles which accompanied higher organic loadings. An optimal concentration of Ca^{2+} for granule promotion was suggested by their findings. Divalent cations are known to exert a positive influence on the flocculation of anaerobic biomass. Using mixtures of acetate, propionate and butyrate as substrates, two distinct types of granules were observed to develop in separate reactors. The filamentous granules were composed mainly of long, multicellular filaments of rod–shaped organisms resembling the acetate–utilizing methanogen, *Methanothrix soehngeii*. Rod–type granules also consisted of *Methanothrix* species but as short multicellular fragments. The filamentous granules all contained some kind of inert material which originated with the inoculum. Filamentous and rod–shaped granules of up to 2 mm and 5 mm in size, respectively, were observed and the average settling velocity of both types of granules was
0.5 m/min.

Granulation has been observed to occur readily in methanogenic thermophilic systems as well as in mesophilic systems. Wiegant and Man (1986) observed similar *Methanothrix* granule types in a study of thermophilic granulation of acetate–degrading anaerobes. They were also able to cultivate *Methanosarcina* granules by applying a selection pressure, but it was discovered that these granules were more readily washed out of the system. Addition of inert support media and the type of inoculum did not effect the formation of *Methanothrix* granules. The

authors noted, however, that in another study, on mesophilic digestion, addition of hydroanthracite particles did significantly reduce the time required for granulation to be observed.

The chemical composition of various bacterial granules grown on sugar refinery wastewater and synthetic volatile–acid mixtures was reported by Dolfing *et al.* (1985). The protein, carbohydrate, total organic carbon and nitrogen contents were found to be comparable to that of bacteria in general. The ash content was somewhat higher, suggesting that inert organic material was incorporated into the particle matrix. Scanning and transmission electron microscopy indicated the presence of significant extracellular polymer material in the matrix. Secretion of a polysaccharide glycocalyx has been suggested as a mechanism by which bacteria bind to each other to form flocs or granules. Extracellular sugars accounted for $1-2\%$ of the dry weight of the granules. According to Stokes Law, the settling velocity of a particle is directly proportional to the particle size and the effective particle density. The effective density of the granules was determined to be $1.00-1.05\ g/cm^3$ which is approximately equal to that of dispersed cells. This suggests that the observed settling properties of the granules were mainly a result of an increase in particle size reulstinf from simple agglomeration of the bacteria.

In a densely packed granule, significant resistance to the diffusion of substrate into the particle interior might be encountered. In particles of sufficient size, mass transfer limitations can be expected to reduce the efficiency of substrate conversion. Dolfing *et al.* (1985) showed that the effect of mass transfer limitations on the kinetic ability of methanogenic granules is dependant on the specific activity of the bacteria within the granule and the thickness of the bio–layers of which the granules are composed. Mass–transfer limitations were found to be significant at low substrate concentrations and in large granules with high methanogenic activity. No mass transfer limitations were detected for the degradation of volatile organic

acids in experiments with granules from an industrial reactor. The granules were less than or equal to 2 mm in size. The methanogenic activity of bacteria degrading a synthetically prepared medium containing a simple carbon source, such as organic acids, and all the appropriate nutrients for growth and metabolism will be greater than what would be achieved in a treatment reactor treating a complex, real wastewater. The author of the study concluded, therefore, that mass transfer limitations will not be of much influence in industrial reactors.

Although experimental studies, such as those mentioned above, have provided some useful information on the granulation process, there are still many unanswered questions. For instance, mechanisms responsible for for the initiation of granule formation are not known. The granulation process is most likely influenced by several different factors acting simultaneously. Characterization of granules grown under certain specific conditions is possible in these types of studies, however, the results are specific to the system. It is difficult, if not impossible, to elucidate general mechanisms and draw fundamental conclusions. A generalized procedure for optimization of granule growth is not available. Studies so far have involved a limited range of wastewater substrates (*i.e.* organic acids, sugar refinery wastewaters), even though the granulation process is a strong function of wastewater composition.

4 EXPERIMENTAL METHODS

4.1 ANAEROBIC CULTURE TECHNIQUES

Maintenance of strict anaerobic conditions was required during all operations involving the phenol–degrading anaerobes. Procedures employed for growing the phenol–degraders were based on the principles of anaerobic technique developed by Hungate (1969). All vessels, containers, fittings, tubing and attachments were thoroughly flushed with a mixture of 30% CO_2 in N_2, prior to the addition of medium or bacterial culture. Oxygen was removed from the gas mixture by passing it through a glass column containing a copper heating coil. Before each use, the heated copper coil was reduced by passing a mixture of 5% H_2 in N_2 through the column for 15 min. Syringes and needles employed for the transfer of gases or liquids into or out of the culture systems were rinsed several times with the 30% CO_2 in N_2 gas mixture before use.

4.2 CULTURE MEDIUM

The complete culture medium used by Fedorak and Hrudey (1986) was used in the present study. The medium was composed of stock solutions containing components necessary for growth *i.e.* phosphate, cation salts, trace metals and B vitamins. Buffering of the pH was provided by the addition of bicarbonate. Sodium sulphide was added as a reducing agent and resazurin served as a redox indicator. The compositions of the stock solutions are given in Table 4.1. The final medium composition, without phenol, is given in Table 4.2.

Medium was prepared by combining the stock solutions with double–distilled water (dd H_2O) in the proportions listed in Table 4.2 in an appropriate size Erlenmeyer flask. 3.5 L of medium were prepared in a 4–L flask for reactor feed and 800 mL were prepared in a 1–L flask for other purposes. Boiling chips were added

TABLE 4.1: COMPOSITION OF STOCK SOLUTIONS USED IN CULTURE MEDIUM

SOLUTION	COMPOUND	CONCENTRATION IN dd H_2O (g/L)
Mineral Solution I[1]	NaCl	50
	NH_4Cl	50
	$CaCl_2 \cdot 2H_2O$	10
	$MgCl_2 \cdot 6H_2O$	10
Mineral Solution II	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	10
	$ZnSO_4 \cdot 7H_2O$	0.1
	H_3BO_3	0.3
	$FeCl_2 \cdot 4H_2O$	1.5
	$CoCl_2 \cdot 6H_2O$	10.0
	$MnCl_2 \cdot 4H_2O$	0.03
	$NiCl_2 \cdot 6H_2O$	0.03
	$AlK(SO_4)_2 \cdot 12H_2O$	0.1
Vitamin B Solution	Nicotinic acid	0.1
	Cyanobalamine	0.1
	Thiamine	0.05
	p–Aminobenzoic acid	0.05
	Pyridoxine	0.25
	Pantothenic Acid	0.025
Phosphate Solution	KH_2PO_4	50
Resazurin Solution		0.1
Sodium Sulphide[2]	$Na_2S \cdot 9H_2O$	25

Notes:

1. dissolved in 0.01 M HCl rather than dd H_2O

2. prepared with boiled dd H_2O and sealed in gassed serum bottles

TABLE 4.2: COMPOSITION OF COMPLETE CULTURE MEDIUM[1]

COMPONENT	AMOUNT
Mineral Solution I	1.0 mL
Mineral Solution II	0.1 mL
Vitamin B Solution	0.1 mL
Phosphate Solution	1.0 mL
Sodium Bicarbonate	0.57 g
Double Distilled Water	97.0 mL
Resazurin Solution	1.0 mL
Sodium Sulphide Solution	1.0 mL

Notes:

1. no phenol in this medium

and the contents of the flask were brought to a full boil which was maintained for two minutes in order to remove dissolved O_2. The medium was then cooled under a stream of O_2–free 30% CO_2 in N_2 by immersing the flask in an ice–water bath. After cooling to room temperature, the sodium bicarbonate was added to the flask. Bubbling of gas through the medium was continued until the pH was between 6.9 and 7.1. Final reduction of the medium was accomplished by addition of 10 mL of 2.5% Na_2S solution per 1 L of complete medium.

4.3 SERUM BOTTLE PROCEDURES

Before addition of culture and/or medium, the serum bottles were flushed with 30% CO_2 in N_2 gas. After the addition, the headspace of the bottles were flushed for a few more minutes. The bottles were then sealed with butyl rubber stoppers which were secured in place with crimped aluminum caps. The serum bottle contents were then reduced by addition of 2.5% Na_2S solution. Additions of concentrated phenol feed solution or Na_2S solution were made using plastic syringes fitted with 22–gauge needles.

Stock solutions of concentrated phenol feed were prepared for use in batch feedings of the bioreactor and the serum bottle cultures. The required quantity of phenol was accurately weighed into 158–mL serum bottles. The bottles were flushed with O_2–free 30% CO_2 in N_2 gas for 20 min. While flushing of the headspace continued, exactly 100 mL of boiled and pH–adjusted medium was transferred anaerobically from the preparation flask into the serum bottles in pre–flushed 25–mL pipettes. The headspace of the bottles was flushed for a few more minutes and then the bottles were stoppered and capped. The contents were then reduced by addition of 1 mL of 2.5% Na_2S solution.

4.4 PHENOL ANALYSIS

Phenol concentrations of liquid samples were determined on a Hewlett–Packard model 5700A gas chromatograph. The GC was equipped with a flame ionization detector and a 2 m x 2 mm I.D. stainless steel column packed with 5% polyphenylether (Chromatographic Specialties) coated 60/80 mesh Tenax GC packing (Alltech Assoc.). Helium (30 mL/min) was the carrier gas. Hydrogen (40 mL/min) and air (300 mL/min) were supplied to the flame ionization detector. Oven, injector and detector temperatures were 200°C, 250°C and 250°C respectively. Peak areas were obtained from a Hewlett–Packard model 3390A integrator.

Suspended solids were allowed to settle in the liquid samples withdrawn from the bioreactor. Using a 10–μL Hamilton syringe, 3 μL of the supernatant was were injected into the GC and the peak area was determined. Sampling of the serum bottle cultures was accomplished by first inverting the bottles and allowing the bulk of the suspended solids to settle and then inserting the needle of a 10–μL Hamilton syringe through the stopper and withdrawing a portion the supernatant. In the case of samples with high phenol concentrations, (*e.g.* 500 mg/L – 2000 mg/L), the sample was first volumetrically diluted with dd H_2O, using an Eppendorf micropipette and/or a 10–μL Hamilton syringe, to reduce the concentration to between 100 and 500 mg/L. Three microlitres of the resulting dilution were injected into the GC and the peak area determined.

Peak areas were compared to calibration curves to determine the phenol concentration. Calibration curves were determined by linear regression analysis of the peak areas resulting from injections of a series of standard solutions with concentrations spanning the range of the sample concentrations. The standards were carefully prepared in volumetric glassware and the concentrations were accurately known. A new calibration curve was determined following any

disturbances in the carrier or detector gas flowrates or after re–lighting of the detector flame. Injections of standards were carried out in a manner identical to injection of samples. All injections of samples and standards were performed at least 3 times and the average peak area was determined.

4.5 ACETIC ACID ANALYSIS

Acetic acid concentration was determined on a HP 5790 GC equipped with a 2 m x 2 mm I.D. glass column packed with GP 10% SP1000/1%H_3PO_4 on 100/200 Chromosorb WAW (Supelco) and a flame ionization detector. Helium (20 mL/min) was the carrier gas and air (300 mL/min) and hydrogen (30 mL/min) were supplied to the flame ionization detector. Oven, injector and detector temperatures were 130°C, 200°C and 250°C respectively. Peak areas were obtained using a HP model 3390A integrator.

Supernatant of liquid samples from the bioreactor was acidified by combining 100 μL of the supernatant with 20 μL of 4 N phosphoric acid solution. Prior to injection of sample, the GC column was also conditioned by injecting 1.0 μL of 0.1% formic acid solution. One microlitre of sample was injected into the GC using a 5–μL Hamilton syringe. A calibration curve was determined each day samples were run. Quantitatively prepared standard solutions, spanning the range of the sample data, were acidified and injected in the same manner as the samples. All injections of samples or standards were performed at least three times and the average peak area was determined.

4.6 METHANE ANALYSIS

The concentration of methane in the headspace of the bioreactor was determined on a Microtek GC equipped with a flame ionization detector and a 2 m x 2 m I.D. glass column packed with Chromosorb WAW (Supelco). Nitrogen

(20 mL/min) was the carrier gas. Air (300 mL/min) and hydrogen (30 mL/min) were supplied to the detector. The oven, injector and detector were maintained at ambient temperature. Samples (0.1–mL) were injected into the GC with Lo–dose gas tight syringes (Becton Dickinson). Peak areas were recorded on an HP3390A integrator.

Peak areas were converted into concentrations by comparing the data to a calibration curve consisting of 3 points; 0, 29.9 and 100% methane. Standards were prepared by filling 158–mL serum bottles with 30% CO_2 in N_2, 29.9% methane and 100% methane gases from prepared gas cylinders and then sealing the bottles with rubber septa. A calibration curve was prepared each day samples were run. The average peak areas were determined for three injections of standard and five injections of headspace gas. The methane concentration of the headspace gas determined from the GC analysis was corrected for moisture content by assuming saturation at the reactor temperature (*i.e.* 35 °C). This determination of methane concentration was not intended to be highly precise and was only used as a check on methanogenic activity and in the approximate of the reactor phenol balance.

4.7 SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy was used to view samples of the the colonized GAC and flocculated biomass from the bioreactor. Edith Schwalt in the Faculty of Dentistry at the University of Alberta, Edmonton, Alberta performed the entire S.E.M. procedure, including sample preparation. Kindzierski (1989) described the sample preparation procedure:

1. Support materials were fixed overnight in 2.5% glutaraldehyde in Milloning's Buffer.
2. Samples were rinsed 3 times (15 min each) with fresh Milloning's buffer.

3. Samples were dehydrated in a series of ethanol solutions for 15 min each (50, 70, 80, 90% ethanol solutions)
4. Samples were dehydrated 3 times (10 min each) in fresh 100% ethanol.
5. Samples were critical–point dried in a See Vac Inc. Critical Point Dryer (Florida, U.S.) with liquid CO_2.
6. Samples were mounted on aluminum stubs with silver conductive glue and dried overnight in a desiccator.
7. Samples were sputtered with gold (75–150 nm thickness) in an Edwards Sputter Coater (Model S150B)
8. Samples were examined and photographed in a Philips SEM (Model 505).

4.8 VOLATILE SUSPENDED SOLIDS

Volatile suspended solids (VSS) concentration was determined by a standard method (APHA, 1985, Section 208). A glass microfibre filter (Whatman GF/C, 4.25 cm) was placed in an aluminum dish, dried for 15 min in a 550°C air oven, and cooled to room temperature in a desiccator. The weight of the filter and dish were determined to the nearest 0.1 mg. Samples containing settlable biomass, *i.e.* biomass flocs, were well shaken to suspend the biomass immediately before filtering. A precise volume of a liquid sample containing biomass solids was filtered through the pre–wetted filter on a suction flask apparatus. The filtered solids were washed with 50 mL of dd H_2O. The filter was then carefully returned to the aluminum dish and dried at 105°C for 1 h. The dish and filter were cooled in a desiccator, weighed and then fired in a 550°C oven for 15 min. The cooling and weighing was repeated and the weight loss was determined. Total suspended solids (TSS) concentration was determined along with the VSS. Triplicate determinations were performed and the average was reported.

4.9 THERMOGRAVIMETRIC ANALYSIS

Thermogravimetric analysis was used to estimate the quantity of biomass immobilized on the GAC particles in the bioreactor. In thermogravimetric analysis, the weight of a small (mg size) sample of material is continuously and accurately monitored while the sample is subjected to a desired temperature environment. The weight gain or loss of the sample as a function of temperature is determined.

A Perkin Elmer TGS–2 Thermogravimetric System (model no. 319–0272) combined with a Perkin Elmer System 4 Microprocessor Controller (model no. 419–0018) was employed for the analysis. The TGS–2 consists consists of:

- TGS–2 Analyzer Unit
- Heater Control Unit
- Balance Control Unit
- Chart Recorder

The analyzer unit is a sensitive micro–balance that suspends the sample in a small furnace. A precisely controlled temperature profile can be programmed on the microprocessor controller. Temperatures up to 1000°C can be obtained in the furnace. During a run, the sample weight and furnace temperature are continuously displayed on the chart recorder. The absolute sample weight or the percent weight change can be recorded, as desired. The gaseous atmosphere in the sample furnace can be selected by the system operator.

The procedures followed for calibrating the system and analyzing the GAC samples are described in the equipment manuals provided by Perkin Elmer. No modifications to equipment or procedures were required.

5 BIOREACTOR STUDY

5.1 EXPERIMENTAL BIOREACTOR DESIGN

The design, construction and operation of a continuous–flow recyle bioreactor (CFRB) accounted for the greatest portion of the experimental effort of this research program. The objective of the experimental design was to concentrate the active biomass on particles of granular activated carbon rather than as suspended cells. In fact, it was desired that the concentration of suspended biomass be kept to a minimum so that the biological activity of the reactor system would be largely a result of immobilized biomass activity. Figure 5.1 is a schematic of the CFRB design. The reactor consisted of a well–mixed tank to which the model wastewater was continuously fed. Liquid holdup in the tank was maintained constant, at approximately 460 mL, by an effluent overflow. The most important feature was a recirculation loop which incorporated a 3.2 cm I.D. column containing a bed of activated carbon. Medium was continuously pumped from the tank, through the GAC bed, and back to the tank.

The following operating criteria were critical to the design of the CFRB experiments:

1. The rate of medium recirculation was maintained very high relative to the the reactor feed rate and the phenol degradation rate of the immobilized culture. This was to ensure that the percent conversion of phenol per pass in the column was small and, therefore, the system could be considered to be well–mixed. This assumption simplifies the kinetic analysis.
2. The overall dilution rate used was several times greater than the specific growth rate of a suspended methanogenic culture to ensure the wash–out of suspended cells.
3. A steady–state with respect to the phenol concentration within the reactor

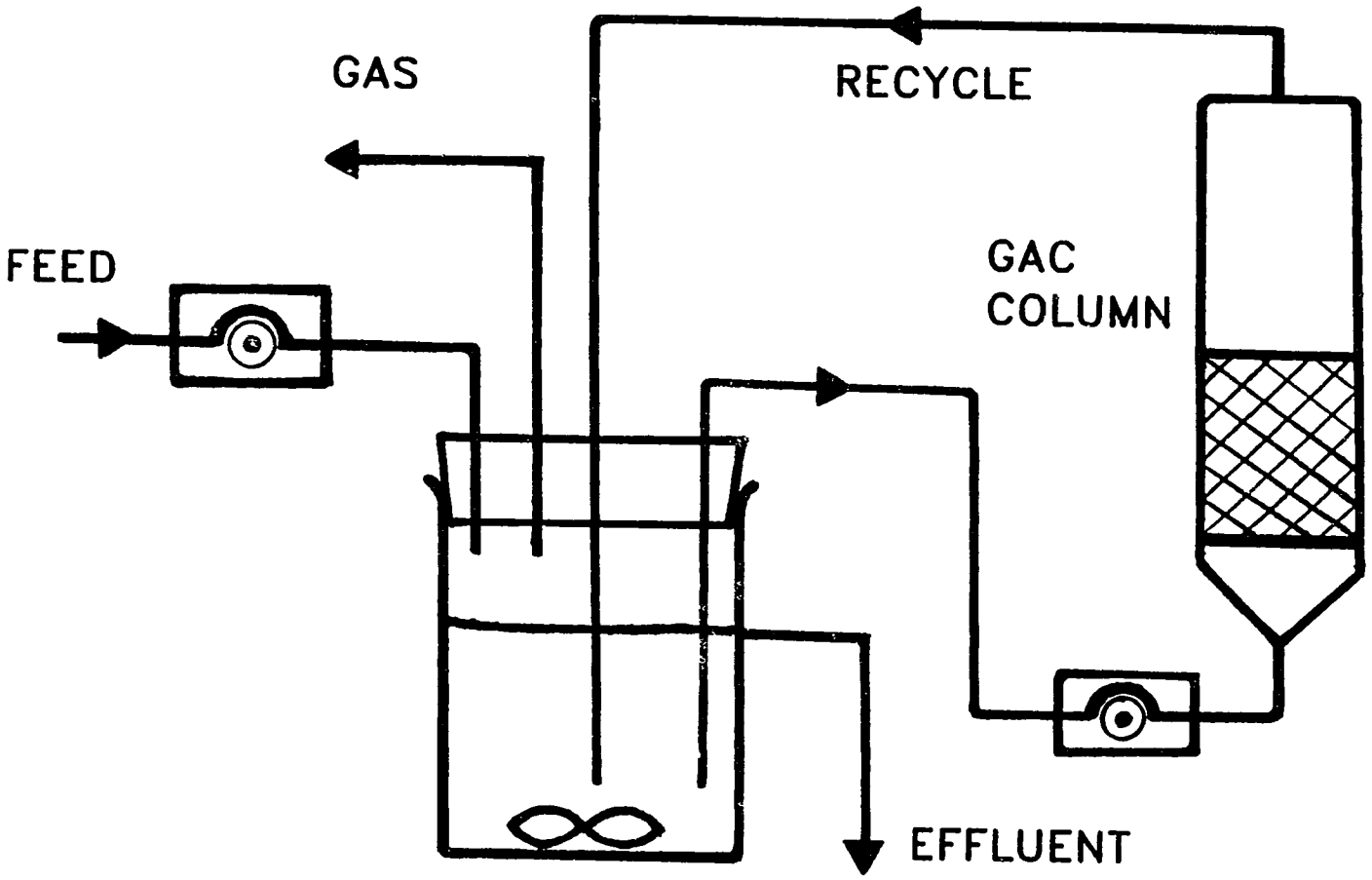

FIGURE 5.1: Schematic of Experimental Reactor (Feed System, Gas and Effluent Collection Systems not Shown)

was achieved so that the net adsorption and desorption of phenol was zero. Adsorption of phenol onto GAC is an equilibrium process which can be described by an adsorption isotherm. It was, therefore, also important that the phenol concentration was measurable to ensure that an adsorption equilibrium did in fact exist (*i.e.* that breakthrough of phenol had occurred). Doran and Bailey (1986) used a similar experimental bioreactor design to study the fermentation properties of the yeast *Saccharomyces cerevisiae* immobilized on gelatin.

The source of inoculum for the bioreactor was a 2–L phenol–degrading enrichment culture. This culture had originally been inoculated with anaerobic sludge from a domestic sewage treatment operation and had been maintained on a synthetic feed containing phenol as the major carbon source for a period of over three years. The same synthetic feed was employed as the model wastewater in the bioreactor. System temperature was maintained at the same temperature as the inoculum source *i.e.* 35°C. The support medium selected was F300 granular active carbon, manufactured by Calgon Corporation, sieved to 20 – 40 U.S. standard mesh size. Kindzierski (1989) employed the same GAC support and medium in his experiments, therefore, some basic knowledge of the performance of this material as a biological support in the anaerobic phenol–degrading system already existed.

Anaerobic conditions were achieved inside the reactor by ensuring that the medium employed was prepared and reduced as described in Section 4.2., that the oxygen was purged from the system prior to start–up and that the entire system was sealed from the atmosphere during operation. Materials were selected and procedures designed carefully so that oxygen transfer into the system would be minimized. Other features of the system were:

– transparent glass construction to permit qualitative observation of the system,

– feed system with variable speed peristaltic pump to allow variation of dilution rate,

– reversible, variable speed, peristaltic pump for medium recirculation to allow various modes of GAC bed operation (*i.e.* packed, expanded, fluidized),

– effluent collection and measurement system,

– liquid displacement gas collection system,

– reactor liquid and headspace gas sampling systems,

– total liquid hold–up of approximately 600 mL,

– GAC hold–up of 5.1 g.

A detailed description of the entire reactor system is provided in Appendix I.

5.2 PSEUDO STEADY–STATE KINETIC ANALYSIS

The amount of wash–out resistant flocculated biomass which had accumulated in the bioreactor was observed to be slowly increasing with time as a consequence of natural biological growth. As a result, a true steady–state, in which the biomass content of the reactor would be constant, could not be achieved. A pseudo steady–state assumption was made which exploited the slow growth of the anaerobic culture. If the phenol loading and degradation rates are sufficiently large relative to the biological growth rate, then the concentration of phenol in the reactor will change very slowly. Over short time periods, the change in biomass and phenol concentration would be small. A pseudo steady–state would prevail from which a reasonable estimate of phenol–degrading activity could be obtained. A second important assumption concerning biomass growth is that, after a sufficient duration of operation, the GAC would become fully–loaded with attached biomass and any further growth on the GAC would be balanced by removal mechanisms. This condition was observed by Kindzierski (1989) in serum bottle cultures after 75 d of growth. Negligible change in the quantity of flocculated biomass and

constant biomass concentration on the GAC were the major assumptions made in the kinetic analysis.

In a completely mixed, continuous system, at steady–state with respect to substrate concentration, the mass balance on substrate is:

$$V_0 = F\,(C_o - C) \tag{5.1}$$

where:

F is volumetric flowrate,

C_0 is feed substrate concentration,

C is reactor substrate concentration,

V_0 is the overall biodegrading activity (rate) of the reactor.

In a system containing GAC and an adsorbable substrate, the amount of adsorbed substrate is also constant at constant concentration in the medium, therefore, the term for substrate adsorption is not required in the steady–state equation.

Three forms of biomass were considered to exist in the reactor; the attached, flocculated and suspended forms. The reactor biodegrading activity (or rate) is the sum of the corresponding contributions from each of these forms as described by the following equation:

$$V_0 = V_c + V_f + V_s \tag{5.2}$$

where:

V_c is the biodegrading activity of the attached culture,

V_f is the biodegrading activity of the flocculated culture,

V_s is the biodegrading activity of the suspended culture.

The specific biological activity, or degradation rate, is defined here as the phenol–degrading activity per unit of biomass. If all forms of biomass in the system

are assumed to exhibit the same specific activity, then the overall activity of the reactor can be represented by:

$$V_o = \nabla_o \cdot m_b \tag{5.3}$$

where:

∇_o is the overall specific biodegrading activity of the reactor,

m_b is the amount of biomass in the system.

In this study the specific activities of the attached, flocculated and suspended biomass were not assumed to be the same. Expressions for the individual activities of these biomass forms, in terms of specific activities are:

$$V_c = \nabla_c \cdot m_c \cdot x_{b/c} \tag{5.4}$$

$$V_f = \nabla_f \cdot m_f \tag{5.5}$$

$$V_s = \nabla_s \cdot X \cdot V_l \tag{5.6}$$

where:

∇_c is the specific degradation rate of the GAC attached culture,

∇_f is the specific degradation rate of the flocculated biomass,

∇_s is the specific degradation rate of the suspended biomass,

m_c is the mass of GAC,

$x_{b/c}$ is the mass concentration of biomass on the carbon,

X is the concentration of suspended biomass,

V_l is the liquid volume of the system,

m_f is the mass of flocculated biomass.

The overall biological degradation rate, V_o, is easily determined from pseudo steady–state data using equation 5.1. Further information is required to determine the activities of the individual culture forms. At very high dilution rates, the contribution of suspended cells will be negligible because they are essentially washed

out of the system. A novel technique of separating the flocculated biomass from the GAC particles inside the reactor while maintaining an active anaerobic culture was developed. This technique, which will be discussed in more detail in a later section, involved a hydraulic separation based on the difference in density between the two materials. Flocculated biomass was completely removed from the column and collected in the tank portion of the reactor while the GAC remained in the column.

The tank contained no GAC and the biodegrading activity of the flocculated biomass, V_f, was determined in a batch test with the GAC column isolated. The difference between this batch activity and the overall reactor activity, V_o, determined from the pseudo steady–state data yielded the activity of the attached culture, V_c, as described by this equation:

$$V_c = V_o - V_f \tag{5.7}$$

V_s was assumed to be zero because the concentration of suspended biomass is very low.

Determination of the specific phenol–degrading activities of the attached and flocculated cultures required measurement of the parameters m_c, $x_{b/c}$, and m_f in equations 5.4 and 5.5. Ideally, a quantitative measure of active biomass would be used. This measurement would include live, phenol–degrading cells and would not include dead or inactive cells and extracellular organic material that is associated with the cells. Unfortunately, such a measure is not available for mixed–population systems, including anaerobic systems anaerobic systems, principally because of the complexity of the consortia involved. The quantification of biomass used in this study was the volatile suspended solids measurement or VSS, described in Section 4.8. It measures the quantity of volatile organic material in a known volume of liquid by a simple gravimetric technique. Measurement of the quantity of

flocculated biomass, m_f, was by direct application of the VSS method. Measurement of $x_{b/c}$, the mass of attached biomass per mass of GAC support, was less straightforward and required a modification of the VSS technique using thermogravimetric analysis equipment (see Section 4.9). The activity of the suspended biomass was determined by performing a batch–degradation experiment on the reactor inoculum.

The above method allows determination of the biological degradation rates at a single phenol concentration. The specific substrate degradation rate, however, is generally a function of the substrate concentration in most biological systems. Neufeld *et al.* (1980) applied a substrate inhibition equation of the following form to describe anaerobic phenol biokinetics:

$$V = \frac{V_{max}}{\left[\ 1 + (K_s/S) + (S/K_i)^n\ \right]} \qquad (5.8)$$

where:

- V is the substrate biodegradation rate,
- V_{max} is the maximum substrate biodegradation rate,
- S is the substrate concentration,
- K_s is the Monod kinetic half–rate,
- K_i is the inhibition constant,
- n is order of inhibition.

Complete evaluation of the equation parameters V_{max}, K_s, K_i and n requires determination of the substrate utilization rate at several different substrate concentrations. The above equation predicts that a substrate concentration range may exist within which the effects of substrate limitation and substrate inhibition are both very small. This occurs when S is much greater than K_s and much smaller

than K_i simultaneously. This condition does not necessarily exist for a given biological system. If it does exist the kinetic equation simplifies to a zero–order form:

$$V = V_{max} \tag{5.9}$$

Kindzierski (1989) observed zero–order kinetic behaviour at phenol concentrations between 500 and 50 mg/L. The main purpose of the kinetic study undertaken for this research was to compare the kinetic performance of the three culture forms in the reactor. The most convenient way of doing so was to determine the maximum degradation rates by maintaining the phenol concentration within the zero–order range. In principle, a single psuedo steady–state run was required to measure V_0, V_f and V_c.

5.3 PROCEDURES

5.3.1 BIOREACTOR START–UP

The tank lid was secured in place and the feed, effluent, recirculation, gas and sample lines were attached. Filtrasorb 300 activated carbon that had been crushed, washed , and sieved to 20 – 40 U.S. standard mesh size (420 μm to 840 μm particle size) was obtained. Prior to use, the activated carbon was washed several times with dd H_2O, to remove fines, and then dried in a 105°C air oven for 48 h. 10.1 g of of dry activated carbon were added to the reactor column and the column was sealed. The butyl rubber line from the top of the column was connected to the tank but the recirculation pump tubing was left disconnected at the pump inlet. The entire reactor, including the column and all the lines, was flushed with O_2–free 30% CO_2 in N_2 for 16 h. Approximately 700 mL of boiled and gassed medium,

prepared as described in Section 4.2, were added to the reactor through the lower end of the column. As the column and the tank of the reactor were filling with medium, the tank headspace was continuously flushed with the O_2–free gas. After filling, the recirculation loop was completed by connecting the free end of the pump tubing to the butyl rubber recirculation line and the reactor was sealed by clamping the ends of the feed, effluent gas and sample lines.

The reactor was secured on the stirring plate and the column secured to a clamp stand in a 35°C walk–in incubator. The recirculation pump tubing was attached to the recirculation pump head and medium circulation and stirring were commenced. The reactor contents were reduced by adding 7 mL of 2.5% Na_2S solution. A volume of concentrated phenol stock solution was added to bring the aqueous concentration phenol to 250 mg/L after adsorption on the GAC was complete. Medium was circulated through the column for 4 d prior to inoculation on the fourth day.

Before inoculating the tank, 5 mL of a reduced 0.05 g/L resazurin solution were added to the tank to determine if the medium was reduced. (Resazurin indicator in the initial medium charged to the reactor would have been adsorbed onto the GAC during circulation.) A colour change to pink indicated that the medium was oxidized. Addition of 3 mL of 2.5% Na_2S solution was sufficient to reduce the medium in the tank. 100 mL of inoculum from the 2–L digester were transferred directly to the tank via the sample line. The transfer was made in two 50–mL stages using degassed 60 mL plastic syringes.

The gas collection system was flushed with O_2–free 30% CO_2 in N_2 and connected to the gas line from the reactor. Gas production and methane content of the headspace were monitored to ensure that a healthy, methanogenic culture was established. The culture in the tank was maintained on batch feedings of reduced concentrated phenol stock solution for a period of 8 d. On the eighth day,

circulation through the column in upflow mode was commenced. Batch feedings and monitoring of methane production continued. Occasionally, the circulation of medium was stopped and the column contents were thoroughly mixed by inverting the column several times. This was done to alleviate bed plugging problems and to ensure that the GAC particles were adequately exposed to bacteria.

5.3.2 TRIAL PERIOD

During a trial period of 286 d duration, various modifications to the reactor system and operating procedures were made. The reactor was operated in both batch and continuous modes during this period. Phenol was available to the culture at most times, so biomass growth was continuous, and methane production continued, although at times the measured production was erratic. Problems overcome in the final design included bed plugging and channeling, plugging of the feed and recirculation lines and associated fittings, and leakage of gas from the system. The final design is as described in Appendix I.

After approximately 2 months of operation, the flocculated biomass had established itself in the column of the reactor. Figure 5.2 is a sketch indicating the locations where this biomass had concentrated. The most significant accumulation was in a layer above the GAC bed. The settled height of this top layer was approximately 1 cm. An accurate measurement was not possible because it was difficult to distinguish the boundary between the biomass layer and the GAC bed. A smaller amount of flocculated biomass had accumulated at the entrance to the column, beneath the support screen. The observation of small particles of biomass, less then a mm in size, was an indication that granules were beginning to form.

A wash–out procedure was performed 168 d following inoculation in which the flocculated biomass was removed from the column by hydraulic means. This wash–out procedure involved first washing the biomass in the bottom of the column

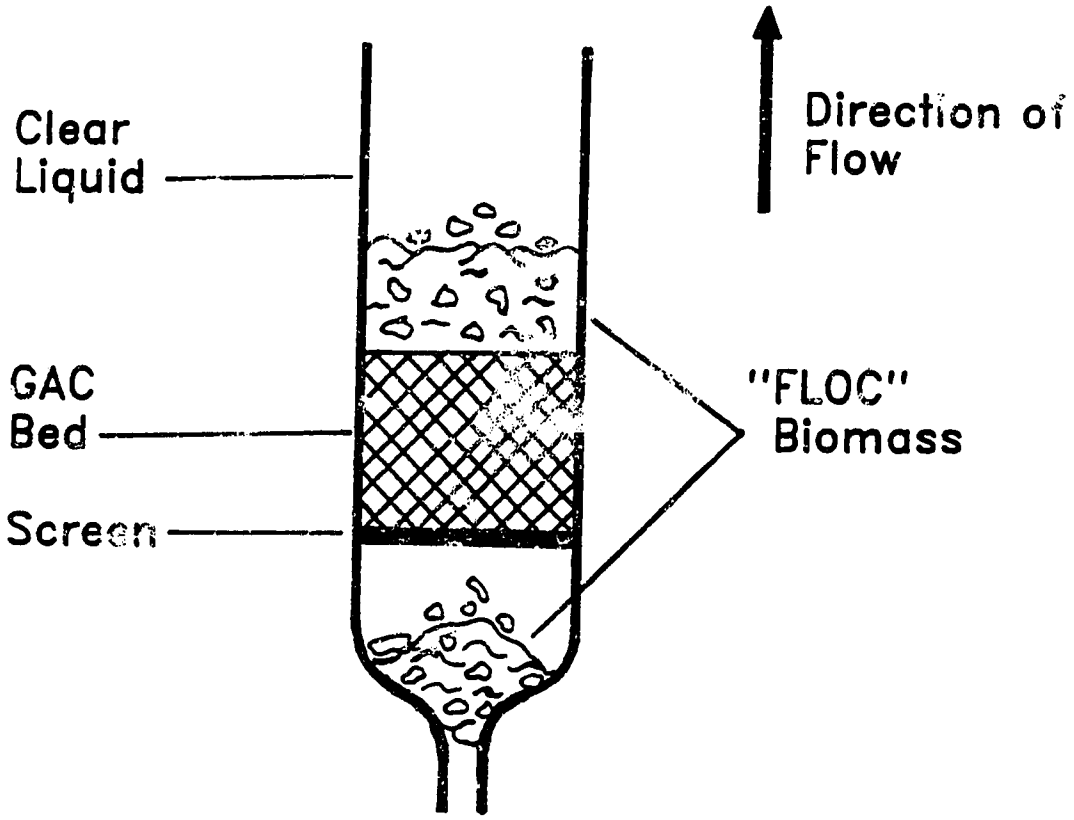

FIGURE 5.2: Locations of Flocculated Biomass in Bioreactor Column

into the tank by reversing the recirculation flow. It was necessary to turn the entire column on its side so that the bed would not plug from compression of biomass and GAC particles. The biomass in the tank was allowed to settle without stirring. After complete settling, the column was restored to a vertical position. The flocculated biomass above the GAC bed was then washed into the tank from the top of the column by increasing the upward flowrate until the GAC bed was fully fluidized (recirculation pump setting of 8). At this upward flowrate the flocculated particles were quickly washed–out of the column. The bed was left in fluidized mode for 15 min. Although the GAC bed expanded significantly during fluidization, their was sufficient freeboard above the bed to prevent entrainment of GAC particles. The biomass in the tank was again allowed to settle and the entire procedure was repeated until no flocculated biomass was observed to remain in the column. This wash–out procedure, which took about 4 h to complete, was also used at the end of the the CFRB runs.

After wash–out of the flocculated biomass, the column was isolated using screw–type pinch clamps on the butyl rubber tubing recirculation lines and separated from the rest of the reactor. The tank was dismantled and thoroughly cleaned. It was then re–assembled, gassed and filled with fresh medium using the same procedures as in the original start–up. The column, still sealed and containing the active culture attached to the GAC, was then carefully re–attached to the tank and continuous operation was commenced.

After a further two months of continuous operation the flocculated biomass was re–established at approximately the former level. This result suggests that the GAC in the reactor was saturated with biomass growth and a re–seeding of the medium was taking place. Collection of the data for the CFBR runs was commenced 286 d after inoculation.

5.3.3 CONTINUOUS OPERATION

The commencement of a CFRB run involved feed flask preparation and hook–up, preparation of the effluent collection system, zeroing of the gas collection system and setting of the recirculation flowrate. The objective during a run was to minimize disturbances to the system so that a pseudo–steady state could be achieved rapidly. During the run, the feed rate, the feed concentration and the recirculation rate were not changed. It was necessary to change effluent containers and to re–level the gas collection system.

3.5 L of medium were prepared in a 4–L Erlenmeyer flask as described in Section 4.2. After bubbling with O_2–free 30% $C0_2$ in N_2 until the pH was between 6.9 and 7.1, a pre–weighed quantity of reagent grade phenol was added to the medium. Bubbling continued for an additional 10 min and the pH was checked to ensure that it was still within the desired range. The butyl rubber feed line, with a new piece of pump tubing attached, was flushed with O_2–free 30% CO_2 in N_2 for 10 min. The rubber stopper, to which the feed line was attached, was inserted into the flask and the secured in place with cable ties. The end of the pump tubing and the outer section of Tygon tubing at the top of the flask were clamped. 35 mL of a 2.5% Na_2S solution were added through the rubber septum at the top of the flask. The inner section of Tygon tubing was then clamped. The sealed flask was inverted several times to thoroughly mix the contents. The addition of the sodium sulphide solution resulted in the instantaneous formation of a very fine, black precipitate of iron sulphide. Before hook–up of the feed, the iron sulphide precipitate was allowed to settle to the bottom of the flask for 5 d. Otherwise, the feed pump fittings tended to become clogged with iron sulphide solids during the run.

After 5 d, the feed line was filled with feed medium by removing the clamp from the pump tubing and pressurizing the flask by connecting the O_2–free gas line to the Tygon tubing. A small amount of medium was allowed to drain from the

feed line before re–clamping. A portion of this liquid was retained for determination of feed phenol concentration by GC analysis. The feed flask was then connected to the reactor at the feed pump outlet fitting and allowed to equilibrate to the incubator temperature for 1 d before feeding was commenced.

The effluent collection cylinder was flushed with O_2–free 30% CO_2 in N_2 for 30 min. A solution composed of 100 mL dd H_2O, 5 mL of resazurin and 30 g of NaCl that had been boiled for 2 min was added to the cylinder while flushing continued. One millilitre of 2.5% Na_2S solution was then added to reduce this solution. The cylinder was then sealed with a rubber stopper and transported to the incubator where it was rapidly connected to the rest of the effluent collection system. The initial liquid level in the cylinder was measured. The purpose of the salt and resazurin solution was to stop biological activity and gas production and to provide an indication of whether the effluent was reduced. (Resazurin indicator in the reactor feed was adsorbed onto the GAC and therefore did not provide any indication of the oxidation state of the reactor contents).

Before commencement of the run, the level in the gas collection system was raised to near the top of the collection cylinder by the method described in Appendix I. The recirculation pump was set at 1.0. This setting provided an upward flowrate through the column of 45 mL/min and a superficial velocity of 7.5 cm/min. This resulted in a GAC bed expansion of approximately 12% and was sufficient to alleviate problems of bed plugging and channeling. A significant amount of the GAC originally added to the column was lost during the start–up and trial periods. The height of the settled GAC bed in the column at the start of the CFRB run was 19 mm. The original height, before inoculation, was 37 mm. The mass of GAC remaining in the column was, therefore, estimated to be 5.1 g.

A feed pump setting of 0.8 was employed during the CFRB runs. A nominal feed flowrate of 350 mL/d was supplied by the pump at this setting. The ratio of

the recirculation rate to the feed rate was 188 to 1 and the system was, therefore, assumed to be well–mixed. Based on a total liquid hold–up of 608 mL, the hydraulic residence time of the reactor was 1.8 d. A single feed flask was capable of delivering over 5 times the liquid hold–up of the column. This was deemed sufficient to ensure that the reactor had adjusted to the feed concentration by the time the contents of the feed flask were depleted. The transient disturbance in reactor concentration in an ideal continuous stirred–tank reactor (CSTR) can be shown to be greater then 99% complete at 5 residence times following a step change in the feed concentration (see Appendix II.E).

The following operations were performed daily during the course of the CFRB runs:

1. A 1–mL liquid sample was withdrawm from the tank (See Appendix I for method) for determination of phenol and acetic acid concentrations by GC analyses by the methods of Sections 4.4 and 4.5.
2. The volume of gas in the gas collection system was measured and the cumulative mmoles of dry gas produced was calculated.
3. The liquid level in the effluent container was measured and the feed flowrate was checked.
4. To replace the volume of medium removed by continuous feeding, 350 mL of O_2–free 30% CO_2 in N_2 were added to the headspace of the feed flask, through the rubber septum at the top of the flask, using several 60–mL pre–flushed plastic syringes fitted with 22 gauge needles.

The samples withdrawn from the reactor were either analyzed immediately or stored in sealed test tubes at –4°C until analysis. The daily withdrawal of 1 mL was considered negligible compared to a feed flowrate of 350 mL/d. An average feed flowrate for the run was determined by dividing the total volume collected by the duration of the run. When the effluent container reached capacity it was replaced

with an identical unit prepared in the same manner as the first and a VSS determination was done on the effluent collected using the method of Section 4.8. It was necessary to filter 250 mL of effluent for each determination because of the very low biomass concentration. The headspace of the tank was sampled on the final day of the run for determination of methane concentration. Five 0.1 mL gas samples were withdrawn for GC analysis as described in Section 4.6. The run was considered complete when the feed flask was almost empty.

As soon as the run was completed the flocculated biomass was washed–out from the column, as previously described in Section 5.3.2. After collecting the flocculated biomass in the tank, the column was isolated by clamping the recirculation lines. Mixing of the tank contents was commenced. The concentration of phenol in the tank was raised to between 200 and 300 mg/L by addition of a small volume of concentrated phenol stock solution. The batch degradation of phenol in the tank was monitored over a 8 to 10 h period. Samples (0.5–mL) were withdrawn from the tank every 1.5 to 3 h and the phenol concentration of the samples was determined by GC. At the end of the sampling period, a 20 mL sample was withdrawn for determination of the VSS concentration of the tank. Combined with a knowledge of the tank volume, the VSS measurement gave an estimate of the quantity of flocculated biomass that was washed–out from the column. The flocculated biomass was washed back into the top of the column in preparation for the next run.

5.3.4 DEGRADATION KINETICS OF BIOREACTOR INOCULUM

The 2–L digester which served as an inoculum source for the CFRB experiment had been enriched on a phenol containing feed for approximately 3.5 years prior to the start–up of the bioreactor. The digester was maintained on a draw and feed basis. Before each feeding, digester stirring was stopped to allow

suspended biomass to settle. A portion of the supernatant was withdrawn and replaced by an equal volume of feed solution composed of the synthetic medium, described in Section 4.2, and phenol. Stirring was then resumed. The digester was fed 3 times per week. The liquid phenol concentration was approximately 250 mg/L after each feeding and decreased to below detectable levels within 24 h.

The specific degradation of the inoculum was determined by monitoring the rate of phenol degradation in the 2–L digester after a regularly scheduled feeding. Liquid samples (0.5 mL) were withdrawn for determination of phenol concentration by GC every 2 h using a sampling procedure similar to that employed with the CFRB. Stirring in the digester was maintained during sampling. The 2–L digester was equipped with a gas collection system to allow measurement of volumetric gas production as well. Samples were collected and gas production was measured for a period of 24 h after which time a 20 mL sample was withdrawn for determination of the VSS concentration of the digester.

5.4 RESULTS

5.4.1 CFRB RUNS

Data and results of two successful CFRB runs are presented in this section. Detailed calculations involved in the development of the results are presented in Appendix II. A complete CFRB run consisted of two distinct phases; the continuous (pseudo steady–state) phase and the batch degradation phase. The two runs were carried our consecutively using the same reactor and cultures established within the reactor. The first run, Run 1, was commenced 286 d after the initial inoculation and both phases were completed within 11 days. Run 2 was initiated 10 d after the conclusion of Run 1 and was similarly completed within 11 d. During the 10–d period between runs, the reactor was maintained in batch mode.

The important variables measured during the continuous phase are presented in Table 5.1 and Figures 5.3 and 5.4. Phenol loading rate was the major adjustable parameter in the system. The loading rate could be varied by manipulating the feed phenol concentration and the volumetric flowrate of the feed. It was more convenient to maintain a fixed flowrate and to vary the feed concentration. The phenol loading rates selected resulted in reactor phenol concentrations that were easily measured by the GC method described in Section 4.4 and were within a range in which the kinetics of substrate degradation were known to be zero–order (*i.e.* 50 – 500 mg/L, Kindzierski, 1989).

The concentration of biomass, measured as VSS, in the effluent was low in both runs (28 mg/L and 14 mg/L in Runs 1 and 2, respectively). Biomass content in the effluent was largely a result of the occasional wash–out of biomass flocs from above the GAC bed and the shearing of bacterial growth, that had accumulated on the outer walls of the glass tubing in the tank, rather then genuine suspended growth. The supernatant liquid in the reactor was observed to be clear during the CFRB runs. Two important points were suggested from these observations. First, the high dilution rate proved effective in maintaining the concentration of suspended cells at a very low level and in providing a selection pressure for immobilized growth forms in the reactor. Second, the new biomass produced by re–seeding of the supernatant by the GAC attached culture was concentrating as flocculated biomass.

The profiles of phenol and acetic acid concentration with time during the continuous phases of Run 1 and Run 2 are shown in Figure 5.3. and 5.4, respectively. The day when feeding was commenced from a newly prepared feed flask was designated as day 0. Similar trends were observed with respect to reactor phenol and acetic acid concentrations in both runs. Phenol concentration rapidly increased from a low level, initially, to a maximum after 1 or 2 d operation and

TABLE 5.1: PSEUDO STEADY-STATE DATA FROM CONTINUOUS PHASE OF CFRB RUNS

	RUN 1	RUN 1
AVERAGE FEED FLOWRATE (mL/d)	332	343
FEED PHENOL CONC. (mg/L)	639	803
FINAL REACTOR PHENOL CONC. (mg/L)	51	208
FINAL REACTOR HAc CONC. (mg/L)	330	293
AVERAGE EFFLUENT VSS (mg/L)	28	14
FINAL % CH_4 IN PRODUCT GAS[1]	75	75

Notes:

1. on a dry gas basis

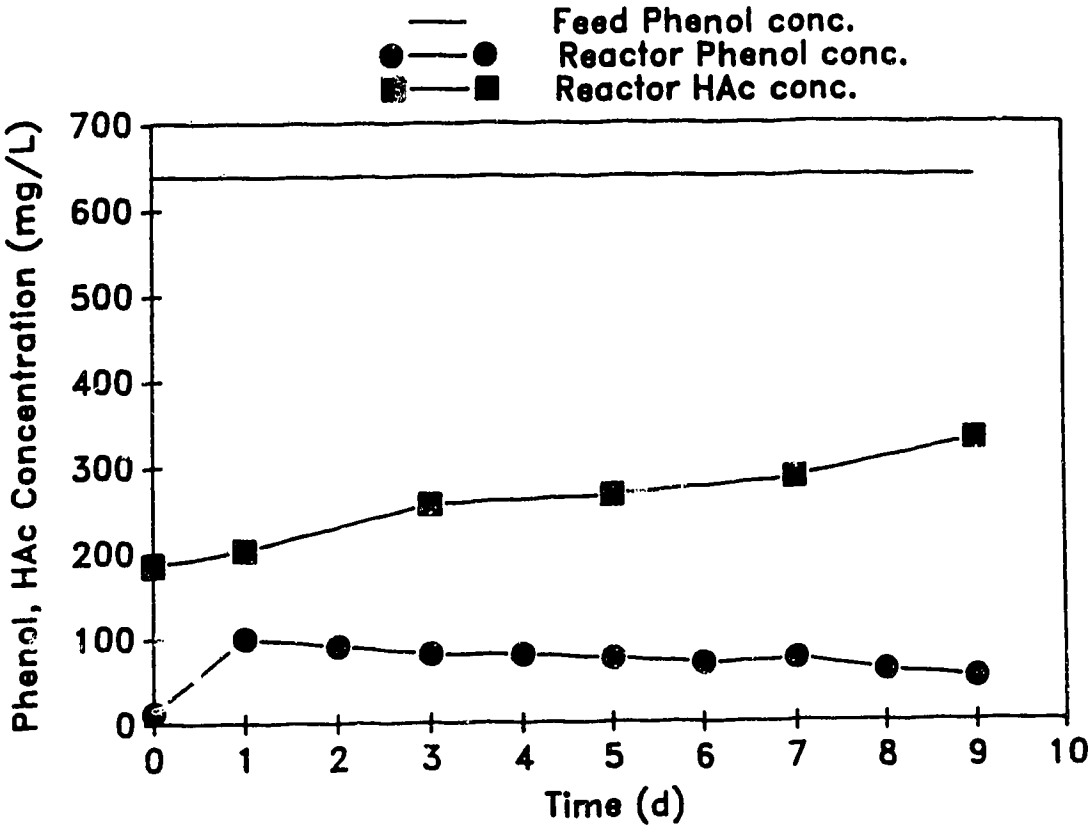

FIGURE 5.3: Pseudo steady–state phenol and acetic acid concentration profiles with time in CFRB RUN 1

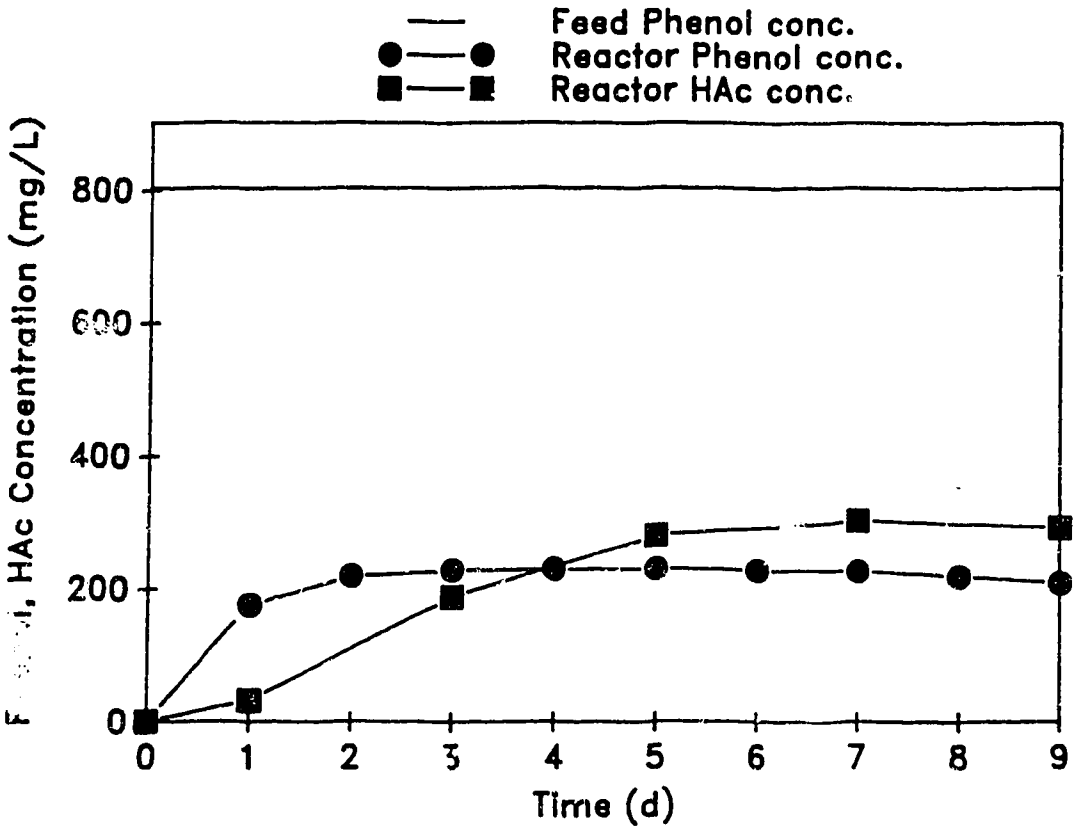

FIGURE 5.4: Pseudo steady–state phenol and acetic acid concentration profiles with time in CFRB RUN 2

then gradually declined until termination of the continuous phase. The initial increases were caused by positive step changes in phenol feed concentration. The gradual decline which followed the adjustment of the reactor to the higher feed concentration reflects the slow accumulation of active biomass and illustrates the difficulty of achieving a true steady–state in the reactor.

The concentration of the intermediate product of degradation, acetic acid, increased to significant levels in both runs. As Figure 5.4 shows, the acetic acid was completely degraded during the batch phase between runs but the concentration rapidly returned to previous levels after continuous feeding was commenced for the second run. Figures 5.3 and 5.4 tend to suggest that the acetic acid level was approaching a similar steady–state concentration in both runs of approximately 300 mg/L. This level of acetic acid did not appear to inhibit phenol degradation or gas production.

The accumulation of product gas in the gas collection system during the continuous phases of Run 1 and Run 2 is shown in Figure 5.5 and Figure 5.6 respectively. Continuous accumulation of gas and a high concentration of methane in the reactor headspace at the conclusion of the runs (Table 5.1) confirms that a healthy methanogenic population was established in the reactor. Linear regression fits of the data of Figures 5.5 and 5.6 yielded gas production rates of 4.3 mmole/d and 3.5 mmole/d (total dry gas) respectively. Correlation coefficients of the linear fit were 0.998 and 0.993, respectively. Because the amount of active biomass retained in the reactor was expected to be greater in the second run, due to growth, gas production in the second run was expected to be slightly greater. The gas observed gas gas production is not consistent with this expectation. The data of Figure 5.6 exhibits a discontinuity in the trend of gas production around the sixth day which tends to lower the calculated production rate. In addition, during the trial period, measured gas production was often erratic, even though phenol