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PULP MILL EFFLUENT INDUCED COAGULATION AND FLOCCULATION IN RECEIVING WATERS

by

STEPHANIE YOUNG 

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

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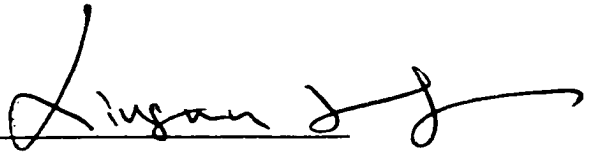
(signed) 

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Pulp Mill Effluent Induced Coagulation and Flocculation in Receiving Waters** submitted by **Stephanie Young** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Environmental Engineering**.

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DEDICATION

This work is dedicated to my M.Sc. and Ph.D. supervisor Dr. Daniel. W. Smith who made my dream come true, and memory of Dr. Daniel. W. Smith for his encouragement for me to overcome the technical challenges and incredible non-technical difficulties over eight-year graduate studies. Without his encouragement, it would have been impossible for me to complete the Ph.D. degree, which has been my dream, and was the only reason I came to Canada from a half way around the world (P.R. China).

ABSTRACT

Field surveys have found floc formation and deposition of an organic matrix, and suspended sediments, downstream of pulp mills in some rivers. This phenomenon is referred to as pulp mill effluent induced coagulation and flocculation (PMEICF).

PMEICF has created much concern about dissolved oxygen (DO) reduction in rivers and at river bottoms, and about the reliability of the existing models for predicting transport and fate of sediments and contaminants in rivers. If PMEICF causes DO reduction, it may have negative impacts on aquatic habitats, biodiversity and reproductivity of bottom dwellers. If transport models do not include PMEICF effect, they may not be adequate for rivers receiving pulp mill effluent. However, most of the existing transport models could not include this effect, due to a lack of understanding of the mechanisms for its formation. This situation prompted further study. This study includes confirmation of PMEICF occurrence, determination of substances causing and factors affecting PMEICF formation, postulation of mechanisms for PMEICF formation, and evaluation of the impact of PMEICF on DO in rivers or at river bottoms.

In conclusion, PMEICF occurred in the Athabasca River and the Wapiti River. It was more prominent in removal of particles with sizes less than 10 μm. Substances attributed to PMEICF formation were Na^+, Ca^{2+}, fibers, hydrolytic lignin, protein, sucrose, cellulose, starch, and extracellular polymeric substances. The major mechanisms for PMEICF formation were sorption, coagulation, flocculation and bioflocculation.

The critical finding from 140-day carbonaceous biochemical oxygen demand (CBOD) tests was that deposited flocs exerted approximately 9 times higher CBOD than

a suspension with the same type and concentration of substrate. This may cause DO reduction at river bottoms if the river assimilative ability is low, especially in the winter when the ice cover prevents atmospheric reaeration.

The significance of the study is to develop better understanding of particle formation due to PMEICF. Through this new knowledge it is hoped that improved transport models and effluent treatment technologies or regulations will be developed. This will facilitate efforts for effective management of river water (RW) quality.

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adt	air-dried tonne
AES	atomic emission spectrometry
AOX	adsorbable organic halogens, μg/L
Avg.	average
BAP	blood agar plate
BKME	bleached Kraft mill effluent
BOD	biochemical oxygen demand, mg/L
CBOD	carbonaceous BOD, mg/L
$CBOD_u$	ultimate CBOD, mg/L
$CBOD_t$	CBOD at time t, mg/L
COD	chemical oxygen demand, mg/L
COM	conventional optical microscope
CTMP	chemical thermo-mechanical pulp
DKN	dissolved Kjeldahl nitrogen
DO	dissolved oxygen, mg/L
DOC	dissolved organic carbons, mgC/L
EG	ethylene glycol
EOX	extractable organic halogens, μg/L
EPS	extracellular polymeric substances

F_1	fractionated PME (MWCO < 1,000 dalton)
F_2	fractionated PME (1,000 dalton < MWCO < 5,000 dalton)
F_3	fractionated PME (5,000 dalton < MWCO < 10,000 dalton)
F_4	fractionated PME (MWCO > 10,000 dalton)
$\overline{G_c}$	mean velocity gradient used for rapid mixing, s^{-1}
$\overline{G_f}$	mean velocity gradient used for flocculation, s^{-1}
GN-ENT	gram negative enteric
GN-FAS	gram negative fastidious
GN-NENT	gram negative non-enteric
GP	gram positive
HPSEC	high performance size exclusion chromatograph
ICP	inductively coupled plasma
RI	refractive index
k	rate constant, t^{-1}
L	TRE (or PRE) at time t, %
L_o	ultimate TRE (or PRE), %
MAC	MacConkey solid agar
MS	mass spectrometry
M_W	molecular weight, daltons
$\overline{M_z}$ / $\overline{M_w}$	measure of the breadth of MWD
$\overline{M_{z+1}}$	Z + 1 average molecular weight, dalton
$\overline{M_n}$	number-average molecular weight, dalton

$$\overline{M}_n = \frac{\sum N_i M_i}{\sum N_i} = \frac{\sum W_i}{\sum (W_i / M_i)}$$

$\overline{M}_w$ weight-average molecular weight, dalton

$$\overline{M}_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} = \frac{\sum W_i M_i}{\sum W_i}$$

$\overline{M}_z$ Z average molecular weight, dalton

$$\overline{M}_z = \frac{\sum N_i M_i^3}{\sum N_i M_i^2}$$

MWCO molecular weight cut-off, dalton

MWD molecular weight distribution, dalton

NBOD nitrogenous BOD, mg/L

NH_3-N ammonia nitrogen, mg/L as nitrogen

NO_2-N nitrite, mg/L as nitrogen

NO_3-N nitrate, mg/L as nitrogen

Ø lag time, day

P Index polydispersity index

Peak $\overline{M}_w$ peak molecular weight, dalton

PEG polyethylene glycol

PME pulp mill effluent

PMEICF pulp mill effluent induced coagulation and flocculation

PRE particle removal efficiency, %

RFA resin and fatty acids

RI refractive index

RW	river water
SCLM	scanning confocal laser microscope
SEM	scanning electron microscope
SIM	simulation index
stdev.	standard deviation
TC	total carbon, mgC/L
TDP	total dissolved phosphorus, mg/L
TDS	total dissolved solids, mg/L
TEM	transmission electron microscope
TIC	total inorganic carbon, mgC/L
TKN	total Kjeldahl nitrogen, mg/L as nitrogen
TMP	thermo-mechanical pulp
TOC	total organic carbon, mgC/L
TON	threshold odour number, unitless
TOX	total organic halogens, μg/L
TP	total phosphorus, mg/L as P or PO_4^{3-}
TRE	turbidity removal efficiency, %
TSA	tryptic soy agar
TSI	triple sugar iron agar
TS	total solids, mg/L
TSS	total suspended solids, mg/L
UV	ultraviolet

1 INTRODUCTION

Floc formation and deposition of an organic matrix and suspended sediments downstream of pulp mills in rivers is referred to in this study as pulp mill effluent induced coagulation and flocculation (PMEICF). Discharge of pulp mill effluent (PME) into aquatic ecosystems causes PMEICF. This may deteriorate the water quality.

Thousands of chemicals and a large amount and variety of bacteria from PME are discharged into rivers. Some of them tend to have an affinity for ambient suspended particles, which can lead to various chemicals becoming attached and transported downstream. This may induce the flocculation of suspended sediments and formation of settleable flocs that may, in turn, deposit at river bottoms. The deposited flocs may release biodegradable chemicals, refractory compounds and even toxic materials into rivers. This may increase the amount of contaminants in benthic sediments, fish or other biota (Carlberg, 1986; Carlberg, 1988; Judd *et al.*, 1995; Martinsen, 1994; Tavendale, 1996), and cause lower dissolved oxygen (DO) concentrations and higher toxicity at the river bottoms. Low DO concentrations may adversely affect development of fish eggs and aquatic organisms (Adams, 1994; Carlberg, 1986; Carlberg, 1988; Judd *et al.*, 1995; Martinsen, 1994; Tavendale, 1996). Therefore, PMEICF may have negative impacts on aquatic habitats, biodiversity and reproductive ability of bottom dwellers (Gifford, 1994; Maldiney and Mouchel, 1995). It may also affect food chains and threaten human health (Adams, 1994).

1.1 Problem Statement

Knowledge of PMEICF is an important component for understanding the transport and fate of sediments and contaminants in the aquatic ecosystem (Lau and Krishnappan, 1992). Its occurrence is of concern as it may cause DO reduction at river bottoms, and may influence the accuracy of the existing models for predicting the transport and fate of sediments and contaminants in rivers. If PMEICF causes DO reduction, it may have negative impacts on aquatic habitats, biodiversity and reproductive ability of bottom dwellers. If the transport models do not include the effect of PMEICF, they may not be adequate for rivers receiving PME. However, most of the existing transport models do not include this effect, due to a lack of understanding of the mechanisms for its formation, and uncertainties related to the occurrence of PMEICF and substances causing and factors affecting PMEICF formation (Irvine *et al.*, 1995; Ng *et al.*, 1996; Ziegler and Nisbet, 1994). In fact, the majority of transport models were developed under the assumption that all particles behave as individual particles and flocculation does not exist (Ongley *et al.*, 1992). These models may not be adequate for rivers receiving PME. This is particularly true when a large percentage of sediments and contaminants are settled out instead of being carried downstream. The situation prompted this research.

1.2 Objectives

The objectives of this study were to confirm the existence of PMEICF in two Alberta rivers, to identify substances causing and factors affecting PMEICF formation, to postulate the mechanisms for PMEICF formation, and to evaluate the effect of PMEICF

on DO reduction at river bottoms. The significance of the study is to provide knowledge, which will improve the transport models through the inclusion of PMEICF, and lead to the adjustment of effluent treatment technologies or regulations to minimize the impacts of these effluents on aquatic ecosystems. This will facilitate efforts to effectively monitor long-term BOD, DO, toxicity and sediment impact, which are the most significant long-term issues related to RW management.

2 LITERATURE REVIEW

The literature review presents the basic information relevant to Alberta pulp mills, overview of PME and its impacts, mechanisms and models for coagulation and flocculation and information related to PMEICF.

2.1 Overview of Pulp Mills in Alberta

There are currently seven pulp or pulp and paper mills operating in Alberta: four are bleached Kraft pulp mills (in Kraft pulping process lignin is solubilized in a solution of sodium sulphide and caustic soda), two are Chemi-Thermo-Mechanical Pulp (CTMP) mills, and one is a Thermo-Mechanical Pulp (TMP) mill. The bleached Kraft pulp mills are: Alberta Pacific Forest Industries Inc. at Boyle, Daishowa-Marubeni International Ltd. at Peace River, Weldwood of Canada Ltd. at Hinton, and Weyerhaeuser Canada Ltd. at Grande Prairie (formerly Procter & Gamble). The CTMP pulp mills are Millar Western Pulp Ltd. at Whitecourt, and Slave Lake Pulp Corporation at Slave Lake. The TMP pulp mill is Alberta Newsprint Company Ltd. at Whitecourt. A comparative summary of the major pulping processes is presented in Figure 1. Locations of these pulp mills were detailed elsewhere (Chambers, 1996).

Secondary treatment methods of PME are used in all the pulp mills in Alberta. Aerated lagoons are used in the Kraft mills (except for Alberta Pacific Forest Industries Inc. at Boyle, which uses activated sludge), and activated sludge with secondary clarifiers is used in the CTMP mills. As the high molecular weight organic compounds are resistant to biological treatment, the treated PME has a dark brown color and contains

various compounds. The color of PME in Alberta ranges from 200 to 1200 CU (Alberta Environmental Protection, 2000).

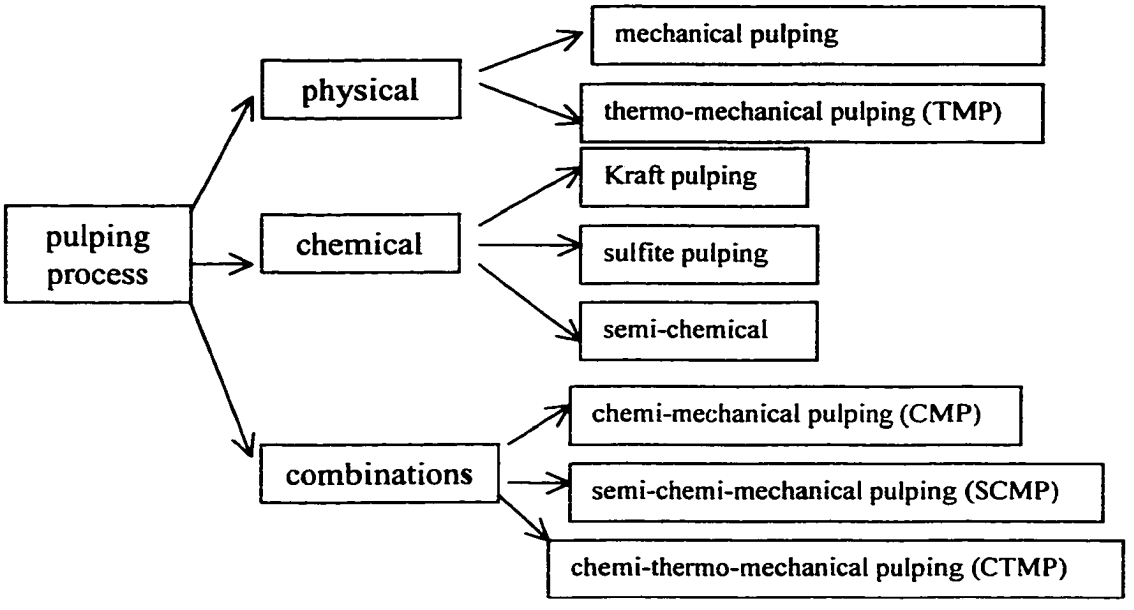


Figure 1. Comparative summary of major pulping processes (after Mao, 1996)

Study sites selected were the Weldwood of Canada Ltd. and the Weyerhaeuser Canada Ltd. mills. Therefore, these two pulp mills will be discussed in further detail.

Weldwood of Canada Ltd. runs the first pulp mill in Alberta (it became operational in 1950's). It is a large bleached Kraft mill located in Hinton, and has implemented oxygen delignification since 1990 and 100% chlorine dioxide substitution

since 1993. The mill produces an average of 1,150 metric tonnes of pulp per day from the softwood furnish blend of 65 to 70% lodgepole pine, 20 to 25% black and white spruce and 10% fir. Average daily water usage by the mill is 110,000 m^3/day, and approximately 95 m^3 of effluent is produced for every air-dried tonne of pulp produced. The total discharge of effluent is higher because the mill also treats the municipal wastewater from Hinton.

The effluent treatment processes used by Weldwood of Canada Ltd. are reviewed below. First, the effluent passes into a primary mechanical clarifier (13,753 m^3, 61 m in diameter) that reduces settleable solids. Then the primary treated effluent flows into an aerated stabilization basin that has a 4.5-day retention time, and has 34 mechanical aerators (2500 HP total or 1864 KW total). If needed, granular 12-51-0 (P-N-K) fertilizer (which enhances secondary treatment) is added to the basin, which generally achieves a 91% reduction in $CBOD_5$. Treated effluent passes through a quiescent zone to reduce suspended solids, then it is mixed with non-contact cooling water from the mill and discharged into the Athabasca River.

Weyerhaeuser Canada Ltd. runs the pulp mill at Grande Prairie. In 1973 the mill began operations, and discharged effluent that was treated in a primary clarifier and aerated lagoons. The mill gradually increased the level of substitution of chlorine dioxide for chlorine to bleach pulp, from 7% to 25% in 1987, from 25% to 70% in 1990 and from 70% to 100% in 1992. At present (from a softwood supply) approximately 900 air dried tonnes of pulp is produced per day. The process involves wood digestion under heat and pressure in alkaline liquor, containing caustic soda (NaOH) and sodium sulphide (Na_2S), to produce unbleached pulp. The pulp is then bleached using chlorine dioxide, hydrogen

peroxide and oxygen. Water from the Wapiti River is drawn in and treated for the process, and PME is treated using a primary clarifier, 14 day (nominal) aerated biological lagoons, quiescent zone, reaeration zone and a foam-removal pond. The treated PME is then discharged into the Wapiti River through a diffuser.

2.2 Overview of PME and Its Impacts

Presented in this section are the characteristics of PME, and its environmental impact and fate.

2.2.1 Characteristics of PME

The pulp and paper industry is one of the largest consumers and contaminators of water in the world. In pulp mills, water is used for transporting wood in the mill, for the cooking and grinding processes, for carrying the separated fibers through bleaching, for refining and for the sheet forming phases of manufacturing. The PME composition is determined by pulping, bleaching and effluent treatment technology. Generally pulping processes produce alkaline and acidic effluents, which are mixed together before biological treatment.

Thousands of chemicals exist in PME, and more than 300 have been identified (Gifford, 1994; Holmbom *et al.*, 1992; McKague *et al.*, 1989; Suntio *et al.*, 1988). The identified PME chemicals include unsaturated fatty acids, chlorinated phenols, diterpene alcohols, juvabiones and resin acids (Walden and Howard, 1977). A large number of trace organic compounds have been identified in PME (McKague *et al.*, 1989). Some of them may be carcinogenic and mutagenic, such as dioxins, furans, and polycyclic

aromatic hydrocarbons (PAHs). In addition, PME also contains inorganic ions, organic polymers and biopolymers. Both Kraft and CTMP mill effluents are rich in Na^+ and SO_4^{2-} and Kraft PME contains a high concentration of Cl^-. The predominant polymers in PME are high molecular weight lignin derivatives that are color causing compounds and resist biological treatment. Other organic polymers are also found, including fibers, wood sugars, lignins, cellulose, hemicellulose, starch and proteins.

Concentrations of PME chemicals were affected largely by dilution and downstream transport. The chemical composition of PME depends on a variety of factors that are mill specific such as the pulping and bleaching technology, the type (i.e. softwood or hardwood) and age of wood chips used, and the effluent treatment technology (Sentar, 1997). The unbleached PME usually contains resin and fatty acids, soap, diterpene alcohols, sugars, aliphatic and aromatic hydrocarbons, and numerous volatile sulfur-containing compounds (Mao, 1996). The chlorine bleached Kraft PME contains chlorinated phenols, chlorinated acids, alcohols, aldehydes, ketones, sugars, and aliphatic and aromatic hydrocarbons.

Although the substitution of chlorine dioxide for elemental chlorine has significantly decreased the amount of chlorinated by-products, the biologically treated PME still contains persistent and highly bioaccumulative substances and lignin derivatives. These compounds along with other toxic and persistent chemicals from agricultural, industrial and municipal sources are released into receiving waters, which may have a negative impact on aquatic ecosystems.

2.2.2 Environmental Impact of PME

Although effective pollution prevention measures decrease the water consumption and the pollution load from pulp mills, PME discharge still accounts for a significant portion of the total waste discharged into receiving waters. The discharge loading of PME contaminants is determined by the pulping and bleaching technology, effluent treatment methods and effluent flow.

The effect of PME on aquatic organisms and food webs can be stimulatory or inhibitory (Bothwell, 1992; McLeay, 1987; Owens, 1991). Recent regulations have emphasized toxicity effects (Owens, 1991), as PME may deteriorate water quality both bacteriologically and chemically and damage fisheries (Anonymous, 1989; Crooks and Sikes, 1991; Gellman, 1988; Mao, 1996; Robinson *et al.*, 1994). Some of the organic compounds present in PME are toxic to fish. Most of the acute toxicity may be due to resin and fatty acids as well as chlorinated resin and fatty acids and low molecular weight organochlorines such as chlorinated phenols (McLeay, 1987; Oanh *et al.*, 1999).

The discharge of PME significantly increases the concentration of dissolved organic compounds, which may have impacts on flora and fauna in rivers. This may cause depletion of DO, fish mortality and dramatic changes to RW parameters (color, odor, taste, and acute and chronic toxicity). The discharge of PME may cause the formation of surface water foam, enhances slime growth, decrease diversity and richness of algae species, increase algae production due to nutrient-enhancement effects (algal nutrients, nitrogen and phosphorus in PME) (Bothwell, 1992). This may cause change in the taxonomic structure of the algal community composition and affect their growth (Amblard *et al.*, 1990; Hall *et al.*, 1991; Lowell *et al.*, 1995). The discharge of PME may

also cause the formation and deposition of flocs near the pulp mill outfall. This may adversely affect benthic dwellers.

2.2.3 Environmental Fate of Pulp Mill Chemicals

The fate of pulp mill chemicals in receiving waters has been studied extensively (Gifford, 1994; Grimvall *et al.*, 1991; Lindström-Seppä and Oikari, 1990; Neilson, 1991; Owens *et al.*, 1994; St. Lucie Press, 1994; Robinson *et al.*, 1994, Millar, 1994). It has been found that the fate of PME chemicals is determined by their physical and chemical properties and environmental conditions. Once PME chemicals have become associated with particulate matter, their fate during transport is determined by advection, diffusion, sorption, desorption, coagulation, flocculation, sedimentation, resuspension, volatilization, photolysis, hydrolysis, biodegradation, biotransformation, diffusive exchange at the sediment and water interface, and short-term events (floods and high wind stress). Moreover, the fate of chemicals sorbed to particles is directly linked to the fate of the particles, which is dependent on the size distribution, morphology, zeta potential and composition of the particles, and the chemistry, hydrological and physical conditions of the receiving waters.

The partition of PME chemicals between air, water and river sediments is often evaluated by vapor pressure, water solubility, bioconcentration factor and octanol-water partition coefficients (estimates partitioning between organic and aqueous phases). Most of the chemicals released into the aquatic environment are hydrophobic (high octanol-water partition coefficients and low water solubility). They have a high affinity for suspended sediments, organic materials and biological tissues. They tend not to exist in

the dissolved form (as hydrophilic compounds do), but rather adsorb onto organic substances such as biological tissue or attach to the suspended sediments, forming settleable flocs. It has been found that the organic halogens such as chloroguaiacols and chlorocatechols accumulated in the bethic sediments (Kukkonen *et al.*, 1996). The hydrophobic compounds may be taken up by organisms in the sediment, which initiates accumulation in the food chain (Gifford, 1994).

2.3 Coagulation and Flocculation

Reviewed are characteristics of river sediments, basic mechanisms for coagulation and flocculation, polymer-enhanced flocculation and bioflocculation.

2.3.1 Characteristics of River Sediments

River sediments consist of colloids, dissolved and suspended solids, microorganisms, aquatic organisms and plants (such as algae and diatoms). The size ranges of some of them can be found elsewhere (Stumm, 1977). Suspended sediments are major carriers of contaminants in rivers. They are classified as cohesive (<63 μm) and cohesionless sediments (Krishnappan, 1991). Cohesive sediments are most closely associated with contaminants (Droppo and Ongley, 1989; Horowitz and Elrick, 1987; Karickhoff, 1981; Krishnappan, 1991; Stone and Mudroch, 1989; Wang, 1978). The transport process of cohesive sediments is significantly different from that of cohesionless sediments (Krishnappan, 1996). Cohesionless sediments tend to behave as individual particles and are less dependent on flow pattern. However, cohesive suspended particles, especially submicron colloidal particles, tend to sorb significant quantities of both inorganic and organic substances due to their large specific surface

areas and potentially high surface free energies. These may be the principal transport vectors for toxic compounds, such as heavy metals, hydrophobic materials and radioisotopes.

Flocculation of sediments depends on particle mineralogy, the chemical matrixes of PME and RW, and river hydraulic conditions. Sediments consist largely of clay particles. Clay particles tend to be negatively charged in natural waters (Hunter and Liss, 1979; Niehof and Loeb, 1972) and to be surrounded by a diffusive layer where an excess of positive charges accumulates in the interfacial region. Opposite charge ions accumulate in the interfacial region together with the primary charge to form an electrical double layer. Since same charge particles tend to repel, they cannot be effectively agglomerated to form larger and more settleable flocs. Therefore, they are normally transported as discrete particles downstream in rivers (Brinke and Dronkers, 1993). However, cations in PME can destabilize the particles by reducing the repulsive forces. Once destabilized, particles tend to agglomerate and form flocs.

2.3.2 Basic Mechanism for Coagulation and Flocculation

Coagulation refers to the chemical process of particle destabilization, and flocculation refers to the physical process of producing contacts (Amirtharajah and O'Melia, 1990). Coagulation and flocculation are essential processes in rivers as they result in the formation of sediment flocs with much higher settling velocities. The process of coagulation and flocculation can be induced by turbulence, differential settling, Brownian motion or microorganisms (Eisma *et al.*, 1991).

The general mechanisms for coagulation and flocculation include four types:

compression of the double layer, adsorption to produce charge neutralization, enmeshment in a precipitate or sweep coagulation, and adsorption to permit inter-particle bridging (Amirtharajah and O'Melia, 1990).

Compression of the double layer is described by the electrostatic model of Derjaguin, Landau, Verwey, and Overbeek (DLVO theory), which states that the addition of indifferent electrolytes, ions of opposite electrical charge to the colloids can compress the electrical double layer to the point that attractive van der Waals' forces prevail and precipitation occurs. The amount of electrolyte required to achieve coagulation by this mechanism is practically independent of the concentration of the indifferent electrolyte, but is dependent upon the valence of the indifferent electrolyte. Based on the empirical Schulze-Hardy rule and the theoretical DLVO model, Amirtharajah & O'Melia (1990) concluded that the higher ion charges, the less the ion dosages required. However, when alum is added to the suspension, it does not function solely as indifferent electrolyte, it also undergoes hydrolysis reactions in addition to electrostatic ones.

Adsorption and charge neutralization occur when a chemical species of opposite charge to that of the colloid adsorbs onto the surface of particle. This will reduce the particle charge; the particles will coagulate by virtue of attractive forces. Destabilization by this mechanism is stoichiometric, and the required dosage of coagulant is proportional to the charge of the coagulant.

Enmeshment in a precipitate or sweep coagulation is explained below. When metal salts such as alum or iron salts are used in a certain pH range, they will precipitate as metal hydroxides [e.g. $Al(OH)_3$ (s) or $Fe(OH)_3$ (s)]; colloidal particles can be enmeshed in these precipitates as they are formed by colliding with them in the process.

This has been termed "sweep-floc" coagulation by Packham (1965). An inverse relationship exists between optimum coagulant dosage and colloid concentration. At a high colloid concentration a lower dosage of coagulant is needed. At lower colloid concentration more coagulant is required. The conditions for optimum coagulation do not correspond to a minimum zeta potential (Sheludko, 1966).

Adsorption to permit inter-particle bridging is discussed below. Destabilization by bridging occurs when segments of a polymer chain absorb on more than one particle, thereby linking the particles together. Polymer bridging can occur between dissimilarly and similarly charged materials. Bridging of negatively charged colloids by high molecular weight cationic and anionic polymers are example of the first and second kind of bridging, respectively.

For floc formation, the particles must undergo two processes: collision and adhesion. Collisions caused by Brownian motion are called perikinetic flocculation, and collisions caused by velocity gradients are referred to as orthokinetic flocculation (van Leussen, 1988). Perikinetic flocculation tends to form weak flocs, and orthokinetic flocculation tends to form strong, more spherical flocs.

There are four dominant mechanisms for adhesion (Krishnappan, 1996; van Leussen, 1988), which are salt flocculation, bioflocculation, pelletization and interaction between chemical coating of particles. Adhesion forms flocs that grow from zero order to first order to second order flocs and so on. Three or four level floc structures are commonly observed (van Leussen, 1988). As the order increases, the size and porosity of flocs increase. This decreases the floc density and strength (Droppo *et al.*, 1997; van Leussen, 1988). These flocs may break up in more turbulent conditions.

2.3.3 Polymer-Enhanced Flocculation

The major mechanism for polymer-enhanced flocculation includes both adsorption and inter-particle bridging (Amirtharajah and O'Melia, 1990; O'Melia, 1969). The adsorption process is accomplished by 1) transport of particles close to the polymer chain, 2) collision between particles and polymer and 3) adhesion. As low bonding energy per segment suffices to render the affinity of the particles on the segment, most of the collisions will result in adhesion. Collision and adhesion are fast reaction processes, and the transport process governs the rate of polymer adsorption. The primary transport mechanisms are: 1) perikinetic flocculation driven by thermal forces, 2) orthokinetic flocculation due to bulk fluid and turbulent motions, and 3) differential settling due to larger particles overtaking smaller particles. Orthokinetic flocculation is the dominant mechanism in polymer flocculation with mixing input. The rate of particle transport is dependent on the mixing intensity, as mixing has an impact on both the length of the segment of the polymer chain and the conformation of the long chain polymer.

Polymers can enhance flocculation, but can also inhibit flocculation. This depends on the relative quantities of polymer and solid particles, the affinities of the polymer for solids and water, and the electrolyte type and concentration. Polymer concentration and ionic strength are critical. Polymer can destabilize the particles, but can also re-stabilize them when polymer concentration is high. Therefore, an optimum polymer concentration exists for polymer flocculation.

Polymers enhance flocculation through bridging mechanisms. Inter-particle bridging occurs when a particle-polymer-particle aggregate is formed. When a polymer molecule comes into contact with a clay particle, some of the reactive groups on the

polymer chain adsorb onto the particle surface, leaving other portions of the molecule extending into the solution. If a second particle with some vacant adsorption sites contacts these extended loops and tails, attachment can occur. A particle-polymer-particle aggregate is formed when the polymer serves as a bridge (Gregory, 1978). More polymer molecules continue to adsorb onto these and other particles, until thousands or even millions are tied together in large masses called flocs. Depending on the rate that polymer molecules are introduced into the suspension, a more or less tightly knit or dense network of flocs can be found that will settle readily when liquid turbulence is minimized (Amirtharajah and O'Melia, 1990).

2.3.4 General Review of Mathematical Models

As previously mentioned, the final purpose of this study is to modify the existing transport models with the inclusion of the effect of PMEICF. Therefore, the related mathematical models are reviewed. These include the models for predicting the kinetics of flocculation and floc growth, and the transport and fate of sediments and contaminants in rivers.

2.3.4.1 Models for Kinetics of Flocculation

Existing models for the kinetics of flocculation were extensively reviewed by Yang (1996). These models were proposed and analyzed based on particle size distribution data. The models evaluated the rate of aggregation either with or without inclusion of the rate of disaggregation. Some models were developed exclusively for either perikinetic or orthokinetic flocculation. The models for perikinetic and orthokinetic flocculation were also reviewed by Yang (1996). The reviewed models were

developed based on laboratory studies that included the use of coagulants. No model was found to predict the kinetics of PMEICF in rivers. Further research needs to be conducted in this area.

2.3.4.2 Models for Kinetics of Floc Growth

Models for the kinetics of floc growth were reviewed to aid in developing a model for predicting the growth of sediment floc in rivers. The models for floc growth generally are classified into conceptual models and mathematical models. Conceptual models were reviewed by Stanley (1995), and the mathematical models were reviewed by Yang (1996). In addition, a floc growth kinetic model for orthokinetic flocculation was developed. By assuming various upper particle-size limits and the existence of floc break-up, it was found that floc growth approached a steady value if floc break-up was insignificant, and floc growth fluctuated if floc break-up and reformation were significant (Yang, 1996). A model for the kinetics of alum floc growth was developed by Francois (1988), and Yang (1996) confirmed later that the model adequately fitted the kinetics of polymeric floc growth. However, no model was found to predict the kinetics of sediment-floc growth in rivers. This area needs to be studied in further detail.

2.3.4.3 Models for Transport and Fate of Sediments and Contaminants in Rivers

Numerical models for predicting the transport and fate of sediments and contaminants in rivers have been extensively studied. Putz and Smith (1998) and Putz (1996) verified a two-dimensional river mixing and transport model that can handle unsteady input conditions. The model was used to predict the transport of PME.

A review of the existing models found that few models included a parameter for

flocculation effects, and few models took into consideration the deposition and resuspension processes (Gailani *et al.*, 1991; Ziegler and Nisbet, 1994). Therefore, most of the models need to be improved to include the above effects, especially when the model is used to predict the transport and fate of the sediments and contaminants in rivers that receive PME.

2.4 PMEICF in Receiving Waters

Presented are field observations for the occurrence of PMEICF and factors that may affect PMEICF formation.

2.4.1 Evidence for the Occurrence of PMEICF

Few field studies have been conducted on flocculation in RW. These studies measured in-situ particle size distributions of suspended sediments in rivers. Krishnappan *et al.* (1994) was the first to find evidence that can likely be linked to PMEICF. This includes rapid aggregation and deposition of suspended sediments downstream of the pulp mill outfall in rivers, and an increased amount of benthic sediments near the outfall. Other researchers also found elevated concentrations of contaminants in sediments downstream of the outfall (Judd *et al.*, 1995; Martinsen, 1994), and build-up of fiberbanks and detectable concentrations of pulp mill chemicals in fish or biota (Carlberg, 1986; Tavendale, 1996). This provides supportive evidence for the occurrence of PMEICF in receiving waters.

Detailed field observations were provided by Krishnappan *et al.* (1995). They conducted field tests in the Athabasca River below the Weldwood Pulp Mill/Municipal outfall at Hinton, Alberta, Canada in 1993. The study measured the size distributions of

suspended sediments using a submersible laser particle size analyzer developed at the National Water Research Institute (NWRI), Burlington, Ontario, Canada. They performed fall and winter sediment surveys. The fall survey results showed that 50% of the total sediment load of the river settled out after travelling 175 km downstream of the effluent discharge. The winter survey results indicated that the majority of incoming sediment deposited within 20 km of the outfall. The deposition of the sediments was assumed to be due to PMEICF, which formed settleable flocs. To confirm these results from the field tests, Krishnappan *et al.* (1995) conducted laboratory experiments using a rotating flume developed at NWRI. It was found that PME enhanced the flocculation of river sediments and increased the sediment deposition rate. Thus, the conclusion was drawn that PMEICF has a significant impact on transport characteristics of sediments and contaminants in rivers. Judd *et al.* (1996) reported that much higher concentrations of pulp mill chemicals were found in sediments downstream of the effluent discharge point than upstream. The analysis of sediments found downstream showed that chlorophenolic compounds, resin acids and resin acid-derived neutral compounds had concentrations as high as 1 ng/g, 6.2 mg/g and 0.6 mg/g, respectively. Carlberg and Stuthridge (1994) reviewed studies of selected chemicals in PME that were found downstream in sediments. As shown in Table 1, researchers found that the concentrations of the chemicals decreased rapidly after they were discharged into receiving waters. This may be attributed to dilution, hydrodynamic transport, sorption, flocculation, sedimentation, hydrolysis, photolysis and biological processes. Therefore, knowledge of the interactions of sediments and contaminants from PME and the mechanism for PMEICF formation better enables researchers to accurately simulate transport and fate of sediments and

contaminants within the aquatic environment (Evans, 1996; Ongley *et al.*, 1992; Tye *et al.*, 1995).

2.4.2 Arguments Against the Occurrence of PMEICF

As previously discussed, the supportive evidence for PMEICF was observed in the laboratory and field, but the occurrence of PMEICF is still under debate because the phenomenon has not yet been observed in some rivers. Evans (1996) reported that Krishnappan and Engel conducted field studies on the Fraser River in the fall of 1994 and spring freshet of 1995. Most of the measurements were made in the immediate vicinity of a Kraft pulp mill. The tested results indicated that a low degree of flocculation of sediments occurred within the effluent plume, which was not likely to affect the overall transport of suspended sediments within the Fraser River. Based on laboratory test results (Evans, 1996), Evans indicated that flocculation was not enhanced by the addition of PME to a suspension of trapped Fraser River sediments. The author suggested that PMEICF was only relevant for smaller discrete particles with diameters less than 10 μm. However, most of the field researchers failed to measure the sizes in this range due to instrument limitations and many problems involved in their experimental determinations (Krishnappan and Engel, 1994). As a result, only limited data are available on particle size distributions of natural colloid particles (Filella *et al.*, 1993) and fine particles in the size range less than 10 μm. Therefore, the occurrence of PMEICF was under debate and further confirmatory experiments need to be conducted.

Although the evidence was not observed in all rivers, the phenomenon of PMEICF is still thought to exist. This is because the formation of PMEICF is a complex

combination of physical, chemical and biological processes, and the degree of PMEICF varies spatially and temporally. Low degrees of PMEICF provided slight or unobserved evidence, but this can not be used to deny the existence of PMEICF in receiving waters.

Table 1. Concentration of PME chemicals in downstream sediments (Carlberg and Stuthridge, 1994)

Compound Class	Effluent	Treatment	Recipient	Country	Distance (km)	Concentration (ug. g^{-1} d.w)	Reference
EOX	BKME	secondary	river	New Zealand	1.5	6	(Stuthridge *et al.*, 1992)
					20	2.8	
					80	0.4	
	BKME	secondary	marine	Sweden	5	5270	(Hakanson *et al.*, 1988)
					15	70	
					36	250	
	BKME	secondary	marine	Sweden	1	1100	(Martinsen, 1994)
					4	720	
					12	70	
					29	30	
TOX	BKME	secondary	lake	Finland	1	9600	(Maatela *et al.*, 1990)
					1.5	1260	
	BKME	secondary	lake	Finland	3	4870	(Paasivirta *et al.*, 1988)]
					15	980	
					40	790	
					55	460	
					85	340	
	BKME	secondary	river	New Zealand	1.5	433	(Stuthridge *et al.*, 1992)
					20	467	
					80	217	
	BKME	secondary	marine	Sweden	1	3600	(Martinsen, 1994)
					9	700	
					150	300	
Chloro-	BKME	secondary	lake	Finland	3	0 8 (149 0)*	(Paasivirta *et al.*, 1990)
Phenolics					15	0 1 (44 8)	
					40	0 1 (5 3)	
					55	0 1 (3 5)	
					85	nd (0 2)	
	BKME/TMP	secondary	lake	Finland	2 5	1 9 (54 4)	(Holmbom *et al.*, 1992)
					5 5	1 0 (32 3)	
					10	1 2 (31 7)	
					14	1 0 (27 5)	
	BKME	secondary	river 1	New Zealand	1.5	0 159	(Judd *et al.*, 1995)
					11	0 043	
					20	0 001	
					80	0 002	
	BKME	secondary	river 2	New Zealand	2 5	0 017	(Judd *et al.*, 1996)
					14	0 016	
					16 5	0.007	
	BKME	secondary	marine	Sweden	1	1 0 (8 4)	(Kvernheim *et al.*, 1993)
					2	(7.2)	
					6	(1.3)	
					11	(0.1)	
Resin acids	News-print	unspecified	estuarine	Australia	0	87	(Volkman *et al.*, 1993)
					1.5	34	
					6	5	
					12	2	
	BKME	secondary	river	Canada	2	211	(Lee and Peart, 1991)
					5	88	
	BKME/TMP	secondary	lake	Finland	2.5	1600	(Holmbom *et al.*, 1992)
					5.5	600	
					10	350	
					14	200	
	BKME	secondary	river 1	New Zealand	1.5	85	(Judd *et al.*, 1995)
					11	56	
					20	37	
					80	1	
	BKME	secondary	river 2	New Zealand	2.5	23	(Judd *et al.*, 1996)
					14	7	
					16.5	10	

* Free (bound)

2.4.3 Factors that May Affect PMEICF Formation

Chemical matrices of PME and RW and environmental conditions may affect PMEICF formation.

2.4.3.1 Chemical Matrix of PME

The chemical matrix of PME may have an effect on PMEICF formation. The matrix is often characterized by the following factors: 1) composition and concentrations of solids, biosolids, metal ions, polymers and biopolymers, 2) properties of the chemicals in the effluent such as solubility, lipophilic tendency, chemical stability, susceptibility to degradation, charge density, molecular structures, molecular weight distributions, and 3) the effluent conditions such as ionic groups, alkalinity, salinity, pH and temperature. These characteristics are determined by the pulping process and effluent treatment technique.

2.4.3.2 Chemical Matrix of RW

Chemical matrix of RW may have effect on PMEICF formation. The matrix primarily includes: 1) chemical and biological compositions of RW, 2) concentrations of colloids, suspended sediments, humic acids, algae, nutrients and other organic carbon contents, 3) particle size distributions of suspended sediments, and 4) characteristics of RW such as ionic strength, pH, alkalinity, salinity, temperature and DO. It is necessary to point out that the characteristics of RW undergo significant temporal and spatial variation. This is largely due to the variation of river hydraulic conditions and different plumes discharged from point and non-point sources (snowmelt, spring runoff and sewer overflow). Therefore, the phenomenon of PMEICF may not be observed at all times or

locations. It has been found that the maximum deposited flocs were evident in the winter when the flow was minimum (Krishnappan *et al.*, 1995).

2.4.3.3 Environmental Factors

Environmental factors may affect PMEICF formation. River hydraulic conditions determine turbulence regime and mixing intensity. Temperature has effect on density, viscosity, DO in RW, and biological activities. Increasing the water temperature may promote microbial activities and enhance flocculation (Warren and Zimmermann, 1994). Sunlight may promote photosynthesis or photolysis. Wind causes turbulence, shear, erosion (resuspension) and aeration. Major storms increase concentrations of primary clay particles and suspended sediments in rivers.

2.5 Bioflocculation

The microbial involvement in biofloc formation is well documented (Biddanda, 1985; Mueller, 1996; Muschenheim *et al.*, 1989; Paerl, 1974; Pavoni *et al.*, 1972; Rao *et al.*, 1991; Riley, 1963). However, there has been little consideration of microbial effects on the transport behavior of clays (Dade and Nowell, 1991; Dade *et al.*, 1996; Parchure and Mehta, 1985) and on PMEICF formation. Research needs to be conducted on these issues.

2.5.1 Bacteria Species

Bacteria are categorized as either floc-forming (Rao *et al.*, 1991) or non-floc-forming bacteria (Friedman and Dugan, 1968). Floc-forming bacteria often have sizes less than 0.5 μm in diameter (Paerl, 1975). They tend to clump together, or cling onto

particle surfaces. As found, floc-forming bacteria include *Klebsiella* spp., *E. Coli Streptococcus* spp., *Zoogloea* spp., *Zooglea ramigera, Bacillus cereus, Escherichia intermedium, Paracolobactrum aerogenoides,* and *Nocardia actinomorpha* (Al-Shahwani *et al.*, 1986; Dudley *et al.*, 1980; Friedman and Dugan, 1968; McKinney and Horwood, 1952; Roth *et al.*, 1989). However, limited work has been conducted to identify floc-forming bacteria from PME (Fulthorpe *et al.*, 1993).

2.5.2 Bacteria from PME

A wide variety of bacteria species are present in PME. Research has been done to identify them, and it was found that 80% of PME-bacteria were gram-negative organisms (Liss and Allen, 1992). Numerous culturable bacteria in PME were identified and characterized by Fulthorpe *et al.* (1993). A review of literature found some PME bacteria, such as *Ancyclobacter aquaticus* (phenetic clusters), *Klebsiella* spp. (phenetic clusters), *Sphaerotilus natans*, *Sphingomonas yanoikuyae*, *Zoogloea ramigera, Microbulbifer hydrolyticus* and *Marinobacterium georgiense*(Geesey, 1982; González *et al.*, 1997; Tsernoglou and Anthony, 1971). The *Pseudomonas* group, which was the largest of the clusters, including *P. stutzeri.* and *A. aquaticus* were present in both RW and PME (Fulthorpe *et al.*, 1993). The bacteria identified from Weyerhaeuser PME include *Haliscomenobacter hydrossis*, type 0914 and type 0411, *Thiothrix I, Sphaerotilus natans*, nitrifying bacteria (*Nitrosomonas*), *Beggiatoa* spp., and *Nostocoida limicola II.* Some of them may be floc-forming bacteria or secrete biopolymers that may induce flocculation in RW.

2.5.3 Extracellular Polymeric Substances

Bacteria secrete extracellular polymeric substances (EPS), which are also referred to as exopolymers. They appear as fibrils (ribbon-like or thread-like), and have a diameter ranging from 2 to 20 nm (Dade *et al.*, 1996; Droppo and Ongley, 1994; Geesey, 1982; Heissenberger *et al.*, 1996; Leppard, 1996). EPS are colloidal in nature and glue-like (Dade *et al.*, 1996). They contain acid, largely polysaccharide in composition (Bache *et al.*, 1996; Leppard, 1996; Sicre *et al.*, 1994), and some lipopolysaccharides (Liss *et al.*, 1996), protein (Liss *et al.*, 1996; White, 1995), or strands of RNA or DNA (Pavoni *et al.*, 1972; van Leussen, 1988). The morphology or structure of EPS was visualized by Sanin and Vesilind (1996), and their chemical compositions, which depend on bacteria species and environmental conditions (Geesey, 1982), were described by Leppard (1996).

EPS have an anchoring or bridging ability (Biddanda, 1985; Dade *et al.*, 1996; Droppo and Ongley, 1992; Eisma and Kalf, 1987; Geesey, 1982; Paerl, 1974; van Leussen, 1988). Using correlative microscopy to observe the river floc, Liss *et al.* (1996) and Droppo *et al.* (1997) found EPS bind organic and inorganic components together through bridging mechanism. EPS can also interact with, and transport contaminants and enzymes (Leppard, 1986), and can complex and detoxify metal ions (Geesey, 1982; Leppard, 1986).

Reviewed literature also indicated that EPS assist in the formation of biofilms. The colonization and growth of a biofilm at a solid-liquid interface were found by Mueller (1996) and reviewed by Joyce (1999). The process includes substratum conditioning by organic molecules, transport of cells to the surface, adsorption of cells to

the substratum, transformation of reversibly adsorbed cells to irreversibly adsorbed cells, growth and erosion of cells. Adsorption and growth processes were further studied by Allison and Sutherland (1987) and Muschenheim *et al.* (1989). It was found that the bacteria tend to colonize on the surface in a planar manner, until a critical size is reached. Further growth occurs away from the surface, which forms a complex three-dimensional biofilm.

2.6 Techniques for Morphology Study

The techniques for floc morphology studies that are generally used are scanning electron microscope (SEM), X-ray SEM, the conventional optical microscope (COM), the transmission electron microscope (TEM) and the scanning confocal laser microscope (SCLM). COM can observe the structure of large flocs (maximum magnification being 900). SEM and X-ray SEM can determine the size, shape and structure of smaller sized flocs, and the ultrastructure of flocs when the magnification is high. TEM can observe the ultrastructure of flocs. For example, EPS in the floc pores and the bacterial coating may be detected with TEM (Leppard, 1992). SCLM is a possible bridge between COM and TEM; and can produce a 3-D image.

Correlative microscopy is a new technique. It is used to examine specimens with more than one microscope to obtain a 3-D image of the structure. The microscopes used in combination are generally COM, TEM and SCLM. Because flocs are fragile and unstable, stabilization techniques developed by Droppo *et al.* (1996a; 1996b) can be used to permit observation of the same samples under all three microscopes. Liss *et al.* (1996) and Droppo *et al.* (1997) used correlative microscopy to study the freshwater floc. It was

found that a mixture of organic and inorganic compounds were held together by a fibrillar matrix. The presence of pores within the floc structure was also observed. At higher magnification, it was evident that the pores were filled with a 3-D matrix consisting of EPS. Liss *et al.* (1996) also studied the engineered PME floc using TEM. Results suggested that bacteria were more abundant in the engineered floc particles than the fresh water ones, and both EPS and bacteria-bacteria associations were present.

3 EXPERIMENTAL METHODS

Major experimental apparatus and methodology are presented in this section.

3.1 Experimental Apparatus

Extensive experimentation has been conducted in this study using many different apparatuses. Special focus was given to the followings: pH meter, turbidity meter, particle size analyzer, mixing devices, respirometer system, ultrafiltration system, high performance size exclusion chromatograph, video microphotography system and Biolog® identification system.

3.1.1 pH Meter

The pH of samples was measured with a Fisher Scientific Accumet® pH meter 25. The pH meter was calibrated using pH= 4, pH=7 and pH=10 standard buffer solutions provided by Fisher Scientific. Calibration was performed on a daily basis.

3.1.2 Turbidity Meter

The turbidity of the suspension was determined using a Hach Turbidimeter (Model 2100 A). The turbidity meter was calibrated using manufacturer supplied formazin suspension standards prior to the measurement and between each measurement, as suggested by the supplier.

3.1.3 Particle Size Analyzer

Particle sizes and size distributions were analyzed with a light-blockage particle size analyzer (HIAC/ROYCO MODEL 8000A). This employs a laser-light source along with advanced data acquisition and processing systems. The HRLD-150 light-obstruction sensor was used in the particle size analyzer. The sensor ranges from 1 to 150 μm with the resolution of 1 μm. The sensor was calibrated using latex particles in water with flow rate of 25 mL/min. The optimum concentration range was 0 to 18,000 count/mL. A 10 mL sample was analyzed in each run, but a minimum of 35 ml of sample was required for each measurement because of the large tare (dead volume) held in the tubes (about 25 mL).

The particle size analyzer sizes particles into eight user-specified channels. The number of particles counted in each channel and the particle size distribution are strongly dependent on the channel setting. The lowest size channel was set at the smallest size limit for the sensor in use to verify that the maximum recommended particle concentration was not exceeded. The fixed channel settings were utilized in order to obtain consistent results. The channel settings employed throughout our study are presented in Table 2.

Table 2. Channel Setting for the Particle Size Analyzer

Channel Number	Channel Setting (μm)
1	2
2	4
3	6
4	8
5	10
6	15
7	20
8	25

Table 2 demonstrates that successive and non-uniform class-size intervals were selected. The narrow channel widths were set for the sizes less than 10 μm, but wide widths were chosen for the higher channels. Using this set of channel settings, the particle size analyzer's ability to define the particle size distribution of a sample was limited by its resolution capabilities rather than the channel settings chosen by the analyst. If necessary, particle counts were normalized for channel width to eliminate the impact of channel width on the particle size distribution.

The particle size analyzer was calibrated and standardized before being used. Internal and external calibrations were performed. The internal calibration curve of latex particles in water was selected for these flocculation experiments. The particle size analyzer was calibrated from time to time during the process of measurement. The external calibration standards (d = 2.08, 9.70, 19.81 μm, distributed by Coulter Source INC., and manufactured by EPICS Division of Coulter Corporation) were used to standardize the analyzer.

The standard solutions used to standardize the particle size analyzer were prepared by gently mixing the standard particles with Milli-Q® water and surfacant for 30 minutes to ensure complete dispersion, where the addition of surfacant aids to disperse the standard particles. The concentration of the standard suspension was controlled at approximately 4500 counts/mL, which is 25% of the manufacturer's recommended maximum concentration. The use of this concentration limited the coincidence errors to less than 3 %.

After the standard suspensions were well mixed, samples were taken and exposed

for at least one minute to remove the bubbles. The samples were analyzed in triplicate using the particle size analyzer. The channel settings of particle size analyzer were that the lowest size channel was set at the smallest size limit for the sensor in use to verify that the maximum recommended particle concentration was not exceeded, and remaining channels were set up in 1-μm increments around the mean calibration sphere diameter. The data were taken following the procedures described below:

(1) Perform instrument warm-up procedure for 30 minutes. Then execute the field standardization using the "auto-adjustment" prior to the initiation measurement in order to ensure low noise in the data.

(2) Flush the sampling line and the sensor volume five times using Milli-Q® water prior to the initial measurement, record the particle counts from the past 2 or 3 flushes. If the total background counts were greater than that of the clean system, more flushing was required until the counts dropped to the level of a clean system. This was necessary to eliminate the possibility of contamination of samples by residuals in the sampling line and the sensor volume of the particle size analyzer.

(3) Adjust the sample flow rate to 25 mL/min using Milli-Q® water or a test sample. The flow rate was checked and adjusted periodically throughout the experiment to maintain a constant flow rate. Adjustments were made very slowly via a potentiometer on the pump controller.

(4) Take an aliquot of approximately 40 mL of the sample from the side sampler in the reactor every three minutes from the beginning of flocculation. Expose the sample for 1 minute to remove bubbles, then measure immediately with very gentle mixing while

the big flocs were present, and without mixing when the flocs were very fine.

(5) Flush the sampling line several times with Milli-Q water between each test, and also prior to instrument shutdown for the removal of all residual particles.

3.1.4 Mixing Devices

Two types of mixing devices were used for flocculation studies. They were a standard jar test apparatus (Hudson and Wagner, 1981) and a vertically oscillating grid mixing system as shown in Figure 2. A standard jar test apparatus includes a 2L square jar (115 mm × 115 mm × 115 mm) with a 76-mm diameter flat blade impeller. The calibration curve between mean velocity gradient and paddle mixing rate can be found elsewhere (Yang, 1996). A vertically oscillating grid mixing system includes a grid mixer, which was made of 10 rods with diameter of 6.4 mm (grid mesh was 11 mm). The calibration curve between the mean velocity gradient and the vertical grid speed was determined by Liem (1998).

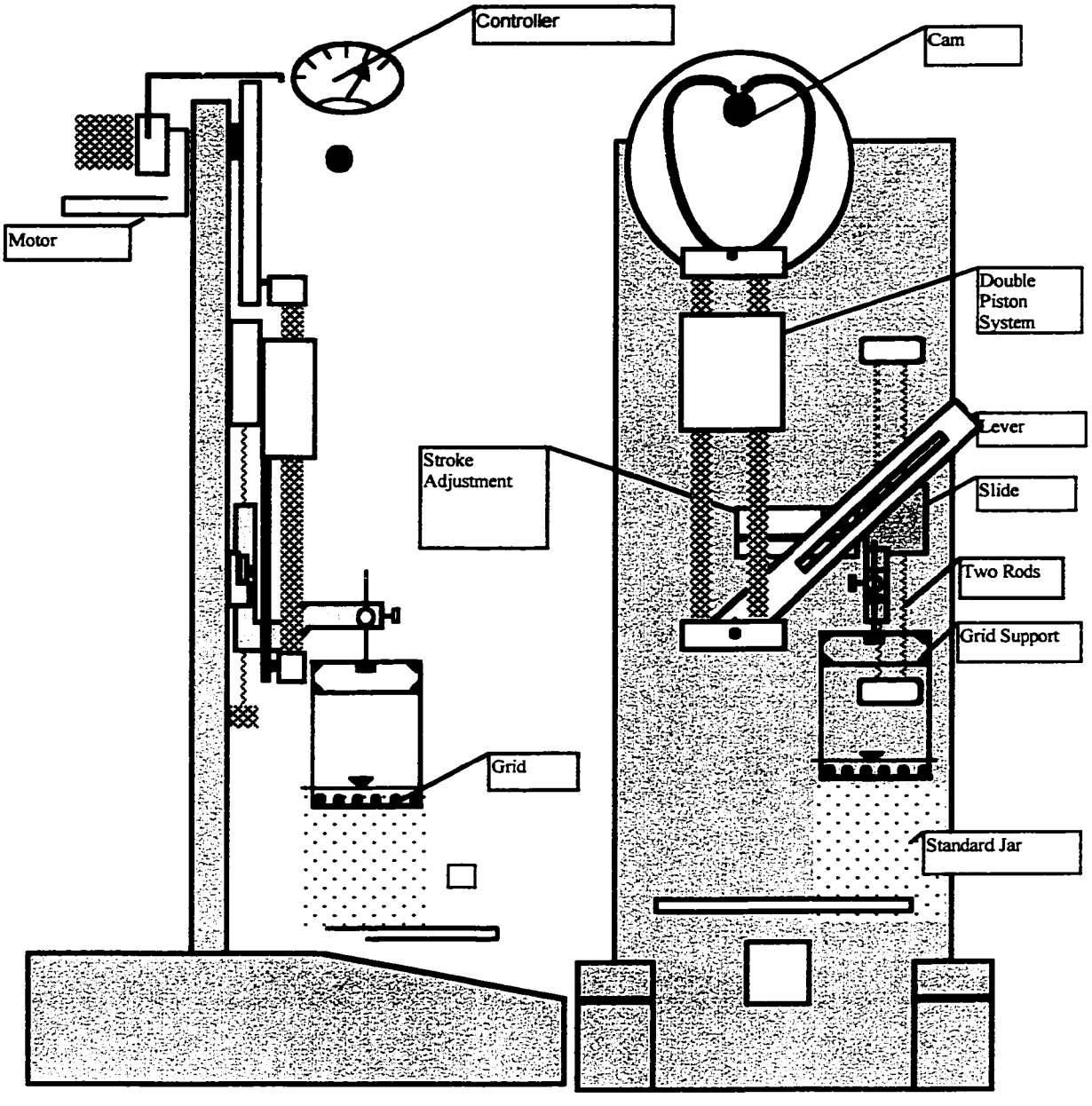


Figure 2. A vertically oscillating grid mixing system (after Liem, 1998)

3.1.5 Respirometer

A Micro Oxymax V 5.3 respirometer (Columbus Instruments International Corporation, Columbus, OH) was used for long-term BOD tests. The instrument uses a highly sensitive gas selective membrane technology to continuously measure O_2 and CO_2 concentrations in the overhead space of each sample. The concentrations are recorded using a microprocessor data acquisition system and are converted to accumulated BOD over a specific time period.

3.1.6 Ultrafiltration System

The Minitan™ acrylic ultrafiltration system (Millipore Co.) was used for PME separation. It consisted of a reservoir, a pump and a polyvinylidene fluoride (PVDF) membrane filters (glycerine was used as a wetting agent and formaldehyde was used to prevent microbial growth). Molecular weight cutoffs (MWCO) for the membranes were 1,000 dalton, 5,000 dalton and 10,000 dalton. The membrane fractionated pre-filtered PME (0.45 μm Watman glass-fiber filters to remove the suspended solids) into four fractions: MWCO < 1,000 dalton (F_1), 1,000 dalton < MWCO < 5,000 dalton (F_2), 5,000 dalton < MWCO < 10,000 dalton (F_3) and MWCO > 10,000 dalton (F_4). It should be noted that the cut-off for certain molecules was a function of molecular size and shape, rather than weight. Those molecules were smaller than the cut-off values appear in permeate; larger molecules are rejected and remain in retentate.

Ultrafiltration process is summarized below. PME was pumped from the reservoir into the membrane. Permeate flow was diverted to a collection line, and retentate (the flow that does not permeate the membrane) was recycled back to the

reservoir. Pressure drop across the membrane was controlled by a valve on the retentate line and by pump speed (flow rate). A higher flow rate was used to minimize concentration gradients at the membrane surface. The ultrafiltration process was operated in concentration mode. The system volume was not held constant, and the concentration of all molecules had permeation factors less than unity increase over time, in both the reservoir and the permeate. In concentration mode, the initial volume was concentrated to a final retentate volume, which was 20 % of the initial volume.

3.1.7 High Performance Size Exclusion Chromatograph

The Shimadzu high performance size exclusion chromatograph (HPSEC) manufactured by Shimadzu Co. was used to determine molecular weight distributions. It was equipped with two columns in sequence. A gel filtration guard column (TSKgel PW_{XL}, 40 mm × 6.0 mm, Sigma Co.) was followed by a gel filtration column (TSK gel $G3000PW_{XL}$, 300mm × 7.8 mm, Supelco Co.). The HPSEC retention and resolution were determined by the column performance, the characteristics of the substrate and mobile phase, the operating variables and the calibration.

For the calibration, it was important to know that the separation using HPSEC, which is a reverse phase liquid chromatography, was based on molecular size, not weight. As the sample passes through columns, the small compounds permeate the matrix pores to a greater degree than the larger components, and are retained longer. Therefore, the largest materials elute first and the smallest last. Ionic and hydrophobic interactions may disturb this order. This can be overcome by choosing suitable mobile phases for sample solubility, and eliminating unwanted solute or substrate effects. Prior studies determined

that the adverse effects caused by the electrolyte nature of bleached-Kraft lignin derivatives could be significantly reduced by using LiCl-tris buffer as an eluent (Mao, 1996). Thus, 0.5 M LiCl-tris buffer was chosen as an eluent, and eluted at a flow rate of 0.8 mL/min. The column eluent in HPSEC was continuously monitored by either an UV detector or a refractive index (RI) detector.

Molecular weight standards used for construction of calibration curves should be matched with the actual samples, not only with respect to charge and hydrophobicity, but also with molecular size and shape. HPSEC relies on transformation of elution time or volume into a molecular weight scale, by observing the elution of polymer standards of known M_W or MWD. This implies that the relationship between the size and M_W of the standards and fractionated PME should be at least similar.

Many standard molecular markers are commercially available for column calibration. For M_W ranging from 66,000 to 6,500 Dalton, the HPLC column was calibrated using an UV-Vis detector at λ = 280 nm (SPD-10A VP, Shimadzu Co.) and a protein molecular weight marker kit (MW-GF-70 Kit). For M_W ranging from 6000 to 62 dalton, the column was calibrated using a RI detector (RID-10A, Shimadzu Co.) and polyethylene glycol/ethylene glycol standards. The mobile phase used LiCl-tris buffer, eluting at a flow rate of 0.8 mL/min.

The protein standards were used for the column calibration. The standards were measured with an UV detector, which is a solute-property or selective detector. As natural protein and lignin compounds from PME have comparative structure, the elution behaviour of protein standards was similar to that of lignin components (Forss *et al.*, 1989; Mao, 1996). The protein molecular weight marker kit used was MW-GF-70

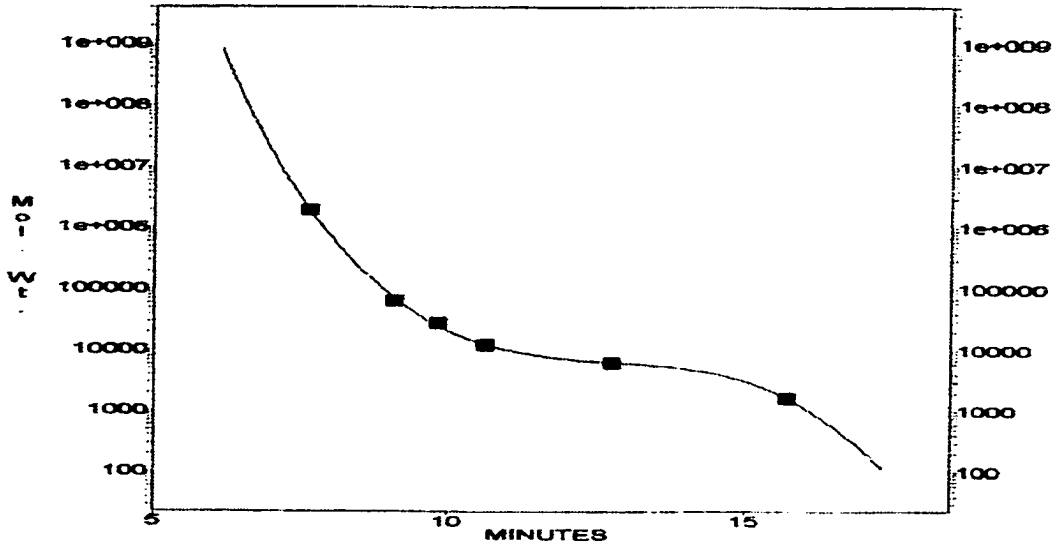
(Sigma Co.). This kit included Albumin (M_W = 66,000), Carbonic Anhydrase (M_W = 29,000), Cytochrome c (M_W = 12,400) and Aprotinin (M_W = 6,500). The calibration curve was constructed as plots of known molecular weights versus elution volume and presented in Figure 3-a. It was found that the calibration of the HPSEC column was reasonably reliable when $M_W > 5,000$ (linear relationship). Thus, it was effective only for measuring components with $M_W > 5,000$.

PEG/EG standards (Superco Co.) with a RI detector were used to construct the calibration curve for $M_W < 6,000$. The standards included 6,000, 4,600, 2,000, 1,000 and 62. It was reported that polyethylene glycol is nonionic hydrophilic compound, comparable with soluble starch, cellulose and sugars, although the behaviour of the PEG/EG standards significantly deviate from that of lignin components in PME (Forss *et al.*, 1989; Rudatin *et al.*, 1989; Siochi *et al.*, 1989). As the PEG/EG standards could not be detected using an UV detector, a RI detector was used. The RI detector is a bulk-property or general detector. It measures a change in an overall physical property of the solvent or mobile phase due to the presence of the solute. It is more sensitive to a compound with a smaller M_W. The calibration curve constructed from the PEG/EG standards is shown in Figure 3-b. A perfect linear curve was obtained when $M_W < 6,000$.

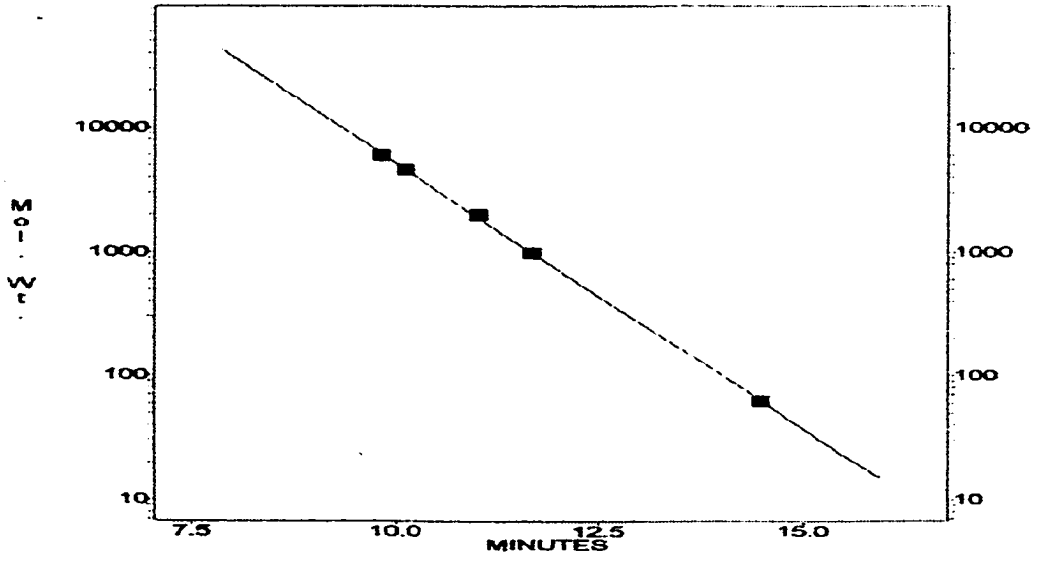
3.1.8 Video Microphotography System

The video microphotography system developed by Shawwa (1998) was used for on-line monitoring of the floc formation. The system was made of a CCD video camera (768 h × 494 v pixels) and a fiber optic source. A micro len (zoom 6000, D.O. Industries, New York) was mounted on the camera using 63.5 × magnification and a 508 mm

monitor. The system was capable of capturing 30 frames per second. The video images were captured using Media Studio VE software (version 2, Ulead Systems). The captured floc images were recorded using a high quality VCR that was connected to a video capture board via a PC computer. The captured floc images were selected and saved in separate files using the same software. The floc size distributions were analyzed using the IMAQ^{TM} version software.



a: (Protein standards with a UV detector)



b: (PEG/EG standards with a RI detector)

Figure 3. Calibration curves for the HPSEC column

3.1.9 Biolog® Identification System

The Biolog® identification system was used to identify bacteria species. Before the Biolog® procedure was initiated, bacterial isolates had to be classified as Gram negative, non-enteric (GN-NENT), Gram negative, enteric (GN-ENT), Gram negative, fastidious (GN-FAS) or Gram positive (GP). The tests required for classification into the one of the above categories were the Gram stain, oxidase and catalase, reaction in a triple sugar iron (TSI) slant and growth on a MacConkey (MAC) plate.

The bacteria samples for the Biolog® identification system were detailed elsewhere (Joyce, 1999). It consisted of nutrients, biochemicals, and tetrazolium violet (a redox dye), a special inoculating fluid (GN/GP-IF, provided by Biolog Inc.) and an anti-capsule agent. Samples (150 μL) were injected into 96 wells in the MicroPlate then incubated for a specified amount of time. If the bacterial isolate was able to utilize the carbon source, the carbon source was oxidized, as such the redox dye changed from colourless to violet. The pattern of purple and clear wells was recorded at the end of the incubation period; this pattern is referred to as the fingerprint. If adequate, the identification was made after the fingerprint was entered into Biolog's Microlog computer program. The computer program lists the top ten potential IDs, with the associated probability and simulation index (SIM), whether or not identification is made. Therefore, if no identification is made, possible IDs are listed and the top one may still be the correct name of the microorganism present.

3.2 Experimental Methods

Methdologies used for field survey and experimental methods that are not used

widely are reviewed briefly. In addition, all the analytical techniques used in the study are summarized in Table 4.

3.2.1 Methodology for Confirmatory Study

Inconsistent results from different rivers regarding the occurrence of PMEICF have created debate. Uncertainty of its occurrence led to few studies on PMEICF formation. This encouraged us to conduct further research to verify its existence. Confirmatory studies were conducted in the field and laboratory.

3.2.1.1 Field Confirmation

Field surveys were conducted on two rivers in Alberta, which receive biologically treated PME. The Athabasca River near Hinton receives PME from Weldwood of Canada Ltd., and the Wapiti River near Grande Prairie receives PME from Weyerhaeuser Canada Ltd.

The survey sites selected were 100, 300 and 1000 m upstream of the mill outfall, and 0, 50, 100, 500, 1000 and 1500 m downstream of the outfall. The survey focused on collecting evidence to reinforce the argument that PMEICF does exist. Sediment flocs were the major focal point. Suspended sediments were collected from RW and deposited sediments were collected from the bottom of the river. Methodology of sampling, transport and analytical methods were crucial to retain the in-situ floc structures. As fragile sediment flocs may be broken-up during sampling, a pump was avoided. As shown in Figure 4, the depth of surveyed rivers made hand dipping possible when collecting the samples.

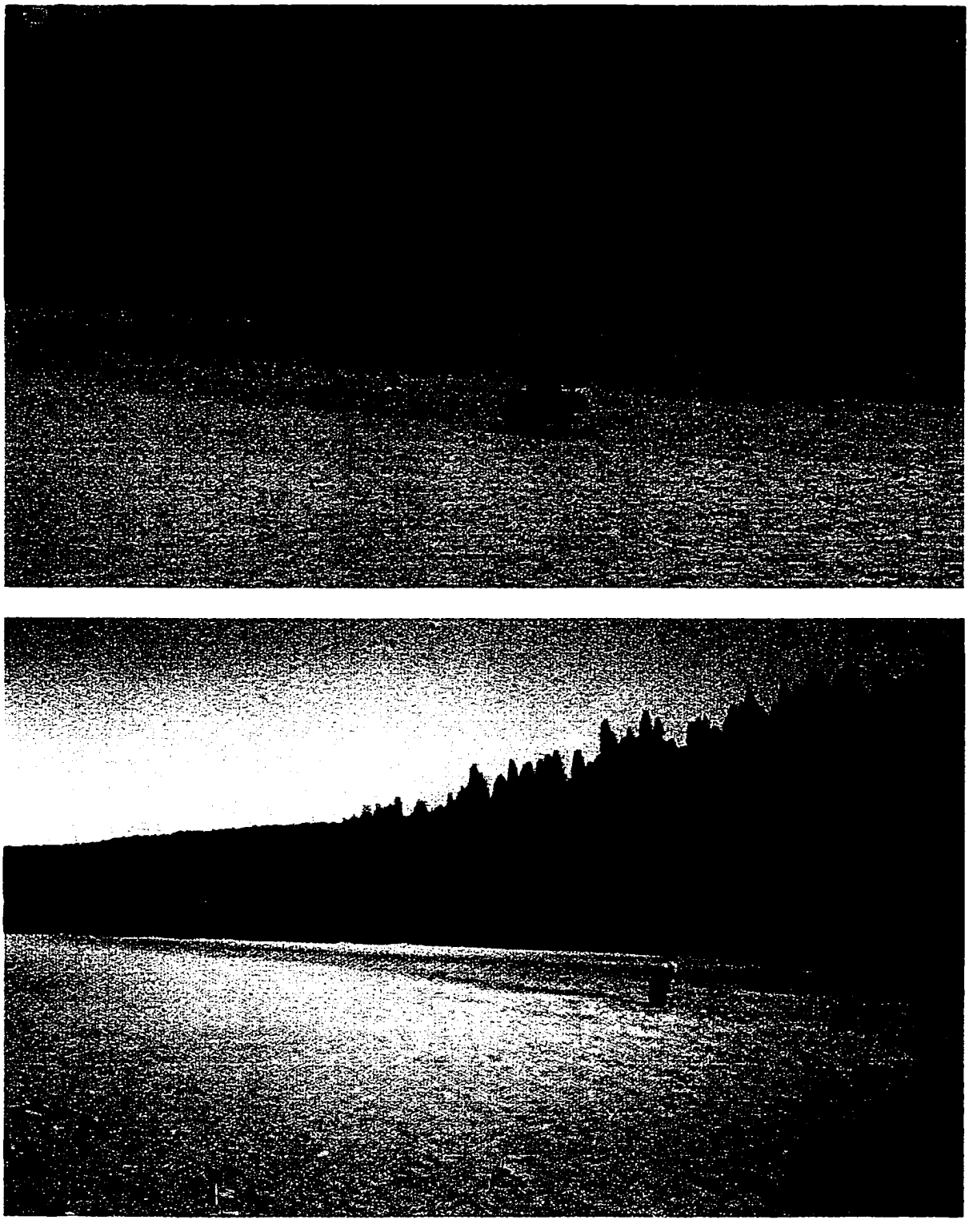


Figure 4. Sampling benthic sediment flocs in Athabasca River near Hinton (August 1998)

3.2.1.2 Laboratory Confirmation

Laboratory confirmatory tests were conducted using flocculation tests in different scenarios to include the effects of temporal and spatial variations, and to closely simulate river conditions. For example, long-term flocculation tests (100 hours) were carried out at a low mixing intensity to simulate winter ice-cover conditions in rivers, and short-term flocculation tests were conducted to simulate conditions immediately downstream of the outfall. Both fresh (daily) and preserved summer samples were tested using both grid and jar mixing apparatus for up to 11 consecutive days to include the effect of sample age and microbial activities.

3.2.2 Samples

Samples used for flocculation tests were either a mixture of PME and RW (1:1 by volume) or RW. Sampling sites for PME were the Weyerhaeuser Canada Pulp Mill at Grande Prairie, Alberta, Canada, and the Weldwood of Canada Ltd. pulp mill in Hinton, Alberta, Canada. Sampling sites for RW were the Wapiti River near Grande Prairie and the Athabasca River near Hinton. PME grab samples were collected from lagoon outfalls, and RW grab samples were collected from upstream of the outfall. Fresh samples of PME and RW (<20 h) were sent daily by the mills, and the preserved samples were from one batch that were collected at one time and kept in the cold room (T = 2°C). Most of samples for PME chemicals such as hydrolytic lignin, protein, sucrose, cellulose and starch were purchased (Sigma Co.), and used directly for flocculation tests. The only exception was the fibers that were separated from the PME. The fibers were washed to remove solids and soaked for 24 hours in water solutions with pH 2, 7.6 or 12 (adjusted

with HCl or NaOH), and dried overnight at 103°C. The pH values 2, 7.6 and 12 were chosen, as they are the approximate pH values for effluents from chlorination stage (pH is around 2), combined effluents from chlorination stage and alkaline extraction stage (pH is around 7.6), and from alkaline extraction stage (pH is around 12).

3.2.3 Mixing Conditions for Flocculation

Two mixing systems were used for flocculation tests, as described in the section 3.1.4. The flocculation tests began with different mixing rates, and the optimum mixing rate was chosen which produced the maximum number of flocs at the lowest energy input. When a standard jar test apparatus was used, samples were mixed for 5 minutes at $\overline{G_c} = 200\ s^{-1}$, then for up to 3 hours at $\overline{G_f} = 13\ s^{-1}$, and finally settled for 0.5 hours before the samples were analyzed. When a grid mixing apparatus was used, the samples were mixed for 1 minute at a vertical grid speed of 10 mm/s ($\overline{Gc} = 15\ s^{-1}$), and for up to 100 hours at 2 mm/s ($\overline{G_f} = 4\ s^{-1}$), and settled for 0.5 hours before the samples were analyzed.

3.2.4 Measurement of Particle Size Distributions

Samples used for particle size distribution were diluted to 1:100 using Milli-Q water, so that the maximum recommended particle concentration (18,000 count/mL) would not be exceeded. Particle size distributions were measured immediately for diluted samples after they were taken, and calculated for the original samples with inclusion of the effect of the particles in the dilution water.

3.2.5 Bacterial Isolation and Identification

The bacteria were isolated from PME and RW. All samples were filtered through

a 0.45 μm filter, and incubated in three different media for up to 5 days, to allow for growth of slow-growing organisms. The media used were 1) R2A (Difco), 2) a mixture of standard plate count agar (Difco) with 30% PME, and 3) a medium composed of 50% by volume PME and agar. The nutrients present in the prepared media can be found elsewhere (Joyce, 1999). The bacteria samples were diluted numerous times to obtain isolated colonies. The individual colonies were isolated by streak plating onto the same media that they were accustomed to. Isolates were streaked onto solid medium a minimum of 5 times to ensure purity. They were then grown in a similar liquid broth and kept frozen in a glycerol solution at -70°C until needed.

To resume growth, bacteria were thawed and a small amount of freezing solution was transferred to a test tube containing the corresponding broth. The test tubes were incubated for 4 days until growth was visible when the bacteria suspension became turbid. The bacteria suspensions were reintroduced into RW samples to determine if they could induce flocculation. Short-term (0.5 to 3.0 hours) and long-term (4 days) bioflocculation tests were conducted at different mixing conditions to form the maximum number of flocs.

Bacteria identification was conducted following the standard procedure outlined by Biolog Inc. The isolated bacteria were sub-cultured onto MacConkey (MAC) plates, blood agar plates (BAP) and the corresponding media plates, and incubated overnight or longer. Catalase, Gram stain and oxidase tests were conducted, and a triple sugar iron (TSI) slant was inoculated. This enabled bacteria to be classified into the Gram negative non-enteric (GN-NENT), Gram negative enteric (GN-ENT), and Gram negative

fastidious (GN-FAS) or Gram positive (GP) categories. Testing conditions specified for the Biolog® are listed in Table 3. The MicroPlates were inoculated following the standard procedure outlined by Biolog® Inc, then incubated for a specified amount of time. The fingerprint (the pattern of purple and clear wells) was recorded and entered into the computer program. A top ten list of possible IDs was identified for each isolate.

Table 3. Testing conditions specified by Biolog® Inc. (after Joyce, 1999)

Parameter	GN-ENT	GN-NENT	GN-FAS
Subculture time	16 to 24 hours	16 to 24 hours	24 to 48 hours
Subculture media	TSA with blood	TSA with blood	Chocolate
Subculture temperature	35°C	30°C	35°C /CO_2
Inoculum concentration	63%	52%	20%
Supplement	Thioglycolate	NA	Thioglycolate
Incubation temperature	35°C /humidity	30°C	35°C /CO_2
Incubation time	4 to 6 hours	16 to 24 hours	16 to 24 hours
SIM for ID	0.75	0.5	0.5

Note: TSA stands for tryptic soy agar

3.2.6 Long-term BOD

BOD is the most significant parameter to measure the required oxygen for the biological degradation of organic matter, and to measure waste loading. It can also be used to assess the persistence and fate of organic matter in rivers. BOD is divided into carbonaceous BOD (CBOD) that is attributed to organic carbon, and nitrogenous BOD (NBOD) that is attributed to inorganic nitrogen. The CBOD is determined when nitrification inhibitor is used. Normally BOD_5 is conducted, but long-term BOD tests are preferable for biologically treated PME that contains refractory substances that are resistant to biodegradation.

In this study, long-term CBOD was determined using respirometric techniques for samples of PME, RW, a mixture of PME and RW (1:1 by volume) and the bottom sludge formed from a mixture of PME and RW (1:1 by volume). The CBOD data were collected every 12 hours for a total of 140 days. Results for duplicate samples were averaged and used for plots of exerted CBOD versus incubation time.

With the addition of nitrification inhibitors carbonaceous BOD (CBOD) was determined in replicate at 12-hour intervals for 140 days. A 200-mL CBOD sample consisted of 1 mL of seeds that were concentrated from PME and grew for a month in PME with aeration, 3 mL of nutrients (APHA *et al.*, 1995) and 3 mL of minerals (APHA *et al.*, 1995) and nitrification inhibitors [2 mL of 2-chloro-6- (trichloro methyl pyridine) at 10 mg/L]. The pH of samples was adjusted to 7 ± 0.1 using HCl and NaOH. Samples of bottom sludge were blended and diluted to 1:2 and 1:5 using seed blank. The seed blank consisted of seeds (10 mL), nutrients (3 mL), nitrification inhibitor (2 mL) and distilled water (191 mL).

3.2.7 Analytical Techniques

Turbidity removal efficiency (TRE) and particle removal efficiencies (< 10 μm) (PRE) were the major parameters used to characterize flocculation performance. Other parameters were also determined. The analytic methods used in this study are summarized in Table 4.

Table 4. Summarization of analytical methods

Analytical Parameter	Instrument	Method
pH	Accumet® - pH meter 50 (digital) (Fisher Scientific)	Standard Methods (APHA *et al.*, 1995)
Conductivity	Accumet® - pH meter 50 (digital) (Fisher Scientific)	Standard Methods (APHA *et al.*, 1995)
Color	Pharmacia Biotech Ultrospec® 3000 UV / visible spectrophotometer	Standard Methods (APHA *et al.*, 1995)
Absorbance (ranged from 190 to 690 nm)	Pharmacia Biotech Ultrospec® 3000 UV / visible spectrophotometer	Standard Methods (APHA *et al.*, 1995) (samples were filtered and pH were adjusted to 7.6)
Total Suspended Solids (TSS)		Standard Methods (APHA *et al.*, 1995)
Total Solids (TS)		Standard Methods (APHA *et al.*, 1995)
Turbidity	Orbeco-Hellige – Digital Direct – Reading Turbidimeter (Orbeco Analytical Systems Inc.)	Standard Methods (APHA *et al.*, 1995)
Particle Size Analyzer	A light-blockage particle size analyzer (HIAC/ROYCO MODEL 8000A)	PSA Manual from manufacturer
Chemical Oxygen Demand (COD)	COD Reactor (HACH Company)	Standard Methods (APHA *et al.*, 1995)
Total Organic Carbon (TOC)	2100 Lab TOC Total Organic Carbon Analyzer - Astro Model 2100 (Zellweger Analytics Inc.)	Standard Methods (APHA *et al.*, 1995)
Adsorbable Organic Halide (AOX)	Euroglas (Delft, Holland) Total Organic Halide Analyzer	Euroglas BV Manual for Determination of AOX, POX and EOX
Biochemical Oxygen Demand (BOD)	Micro Oxymax V5.3 Respirometer (Columbus Instruments International Corporation)	Standard Methods (APHA *et al.*, 1995) and instrument manual
Alkalinity	Mettler DL25 Titrator (Mettler Instruments)	Standard Methods (APHA *et al.*, 1995)
Metal Ions	Inductively Coupled Plasma (ICP), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)	EPA 6010 Analyzed by Maxxam Analytics Inc.
Molecular Weight and Molecular Weight Distribution	Shimadzu High Performance Liquid Chromatograph (HPLC), Mass Spectrometry (MS) and Electrospray Mass Spectrometry (EMS)	*EMS was analyzed by the Department of Chemistry at the University of Alberta
Morphology Study	Scanning Electron Microscope (SEM). HITACHI SEM-S2500 (HITACHI Ltd. Toyko, Japan)	Standard SEM Methods analyzed by Department of Dentistry and Pharmaceutical Sciences, Electromicroscope Unit, Surgical and Medical Research Institute.
	x-ray Scanning Electron Microscope (x-ray SEM). The JEOL-JSM-630 1FXV Scanning Electron Microscope (FACEY, 1999)	Secondary SEM Methods, analyzed by Department of Geology
	Conventional Optical Microscope	Department of Biological Science, University of Alberta
	Transmission Electron Microscope	
	Scanning Confocal Laser Microscope	
On-line Floc Size Distribution	Video microphotography system	IMAQ™ version software for sizing flocs

4 RESULTS AND DISCUSSION

Experimental results include experimental reliability, confirmatory studies, evaluation of effect of physical, chemical and biological factors on PMEICF formation, investigation of the effect of PMEICF on DO, and postulation of mechanisms for PMEICF formation.

4.1 Experimental Reliability

The experimental reliability was evaluated to ensure that the results were accurate. The evaluation was focused on analytic instruments, flocculation systems, results and mathematical models.

4.1.1 Instrument Reliability

The precision of the particle size analyzer was evaluated, as PRE is one of the major parameters used to characterize flocculation performance. Figure 5 shows that the average of particle counts for RW samples from the same batch (with continuous mixing) was 35,847 ± 1,171 count/mL (samples were diluted to 1:100 in order not to exceed the maximum recommended particle concentration of 18,000 count/mL). The low standard deviation suggested that the precision of analyzer was reasonable high until it broke down. The results remained unstable after it was repaired and recalibrated by the manufacturer. Replication results were measured and calculated, but are not promising. Therefore, this portion of PRE results was not presentable.

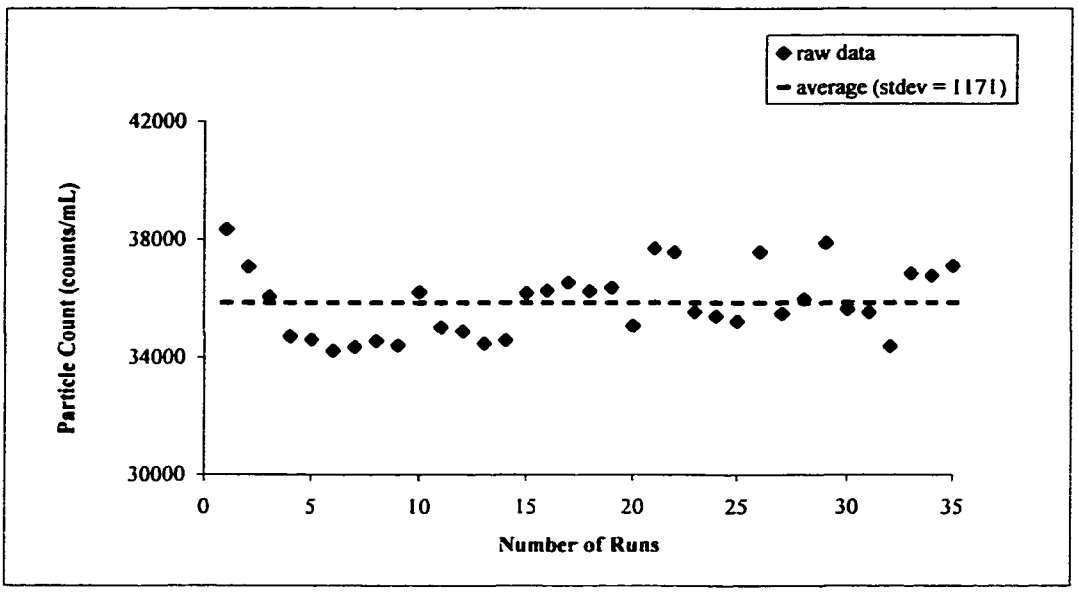


Figure 5. Variation of particle counts for RW samples between runs (samples were well mixed before they were taken)

4.1.2 Model Adequacy

Unlike the semi-logarithmic relationship shown in Equation 1 that can only be used for flocculation curves with exponential growth (Figure 6-a to Figure 6-c, but not Figure 6-d to Figure 6-f).

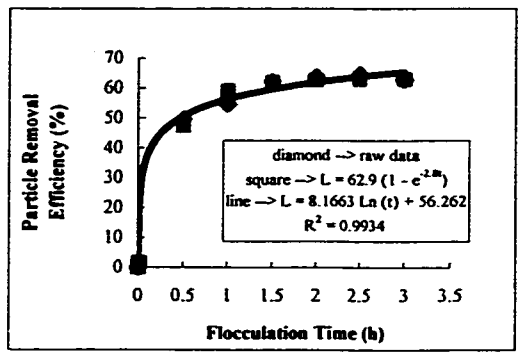
$$L = a \, Ln\,(t) + b \qquad (1)$$

where L stands for TRE (or PRE) at flocculation time t.

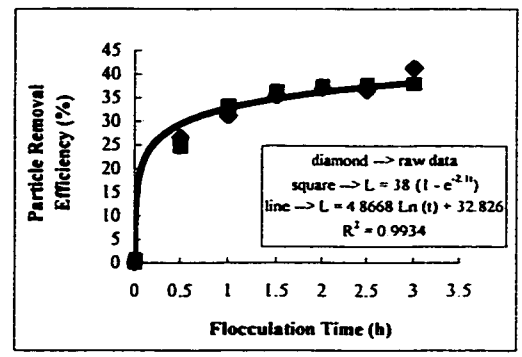
Figure 6 revealed that the model in Equation 2 was the most adequate for simulating flocculation curves with either linear or exponential growth, and was chosen to use in this study.

$$L = L_o (1 - e^{-kt}) \qquad (2)$$

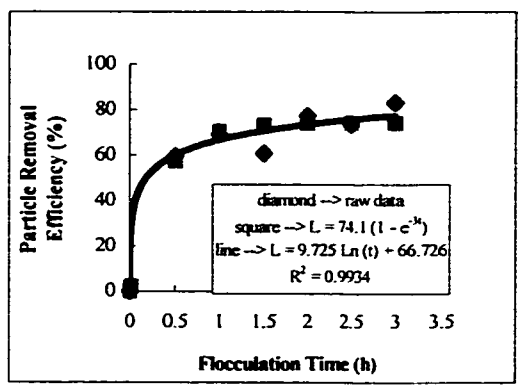
where L stands for TRE (or PRE) at flocculation time t, L_o is its ultimate value, and k is the rate constant. The values for k and L_o were used to quantitatively evaluate the rate and degree of flocculation.



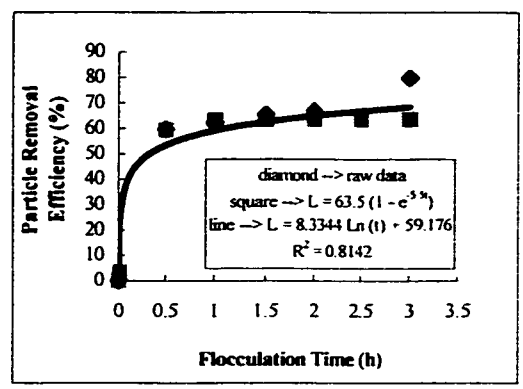
a (April 22, 1999)



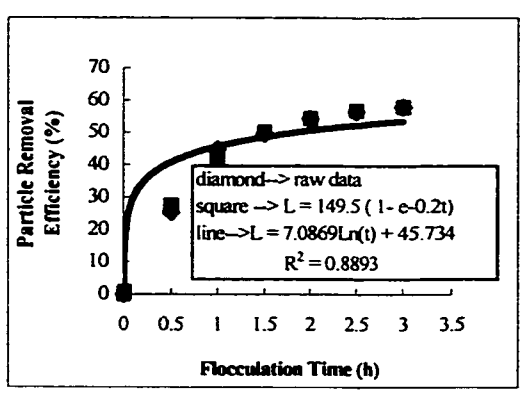
b (May 15, 1999)



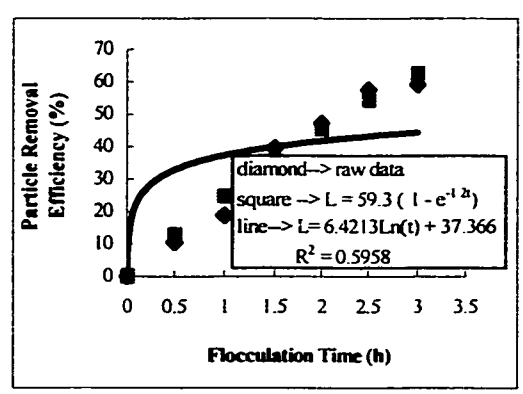
c (May 12, 1999)



d (May 5, 1999)



e (May 22, 1999)



f (April 13, 1999)

Figure 6. Model simulation for flocculation curves (samples were a mixture of PME and RW with volume ratio of 1:1)

4.1.3 Flocculation System Reliability

Flocculation system reliability was evaluated by determining the repeatability of flocculation results. Flocculation tests were replicated 24 times (from May 27 to July 9, 1999) using the same experimental set-up and samples from the same batch that was kept in a cold room (2°C). Detailed experimental dates and sample ages are summarized in Table 5. The experiments were conducted at close intervals at the beginning of the evaluation and longer intervals towards the end of the study. TRE and PRE were measured, L_0 (maximum degree of flocculation) and k (rate constant) were determined from model simulation based on Equation 2.

Replication of flocculation results obtained from the same experimental set-up is presented in Figure 7 (TRE) and Figure 8 (PRE). Averages and standard deviations of L_0 k, TRE and PRE are summarized in Table 6. Scattered results of TRE were evident from Figure 7. This may be attributed to random errors caused by the mixing apparatus, sampling, aging of samples and instrument instability. Sample age was the major factor attributed to high standard deviation. Figure 8 shows upward trends for PRE, L_0 and k, but this was not seen for TRE, L_0 and k (Figure 7). This indicates that the older the samples, the higher degree the bioflocculation due to the higher EPS concentrations. It should be emphesized that the replicate experiments were begun with preserved samples. Therefore, the sample age in Table 5 did not represent the real sample age, it was only used to measure the interval between runs.

Inconsistent trends for TRE and PRE were largely attributed to bioflocculation, which tended to transfer more colloidal particles into suspended particles or small sized bioflocs that was removed during flocculation. This phenomenon was readily detected

by PRE, but not by TRE.

Table 5. Experimental dates and sample ages for replicate experiments

Number of Runs	Experiment Date (1999)	Sample Age (day)	Number of Runs	Experiment Date (1999)	Sample Age (day)
1	May 27	1	13	May 31	5
2	May 27	1	14	June 2	7
3	May 27	1	15	June 3	8
4	May 27	1	16	June 7	12
5	May 27	1	17	June 11	16
6	May 28	2	18	June 14	19
7	May 28	2	19	June 17	22
8	May 28	2	20	June 19	24
9	May 28	2	21	June 28	33
10	May 29	3	22	June 30	35
11	May 30	4	23	July 5	40
12	May 30	4	24	July 9	44

Table 6. Statistical values for PRE, L_0 and k (t = flocculation time, stdev = standard deviation)

	Particle Removal Efficiency (%)						L_o (%)	k (h^{-1})
	t = 0.5 h	t = 1.0 h	t = 1.5 h	t = 2.0 h	t = 2.5 h	t = 3.0 h		
average	11.38	20.86	27.61	33.00	37.89	41.40	24.33	12.17
stdev	11.44	12.18	12.21	13.53	14.40	14.34	10.58	4.15
	Turbidity Removal Efficiency (%)						L_o (%)	k (h^{-1})
	t = 0.5 h	t = 1.0 h	t = 1.5 h	t = 2.0 h	t = 2.5 h	t = 3.0 h		
average	16.54	27.68	34.99	40.47	45.42	47.83	30.18	14.32
stdev	9.18	11.42	10.95	10.91	11.00	9.78	8.64	3.07

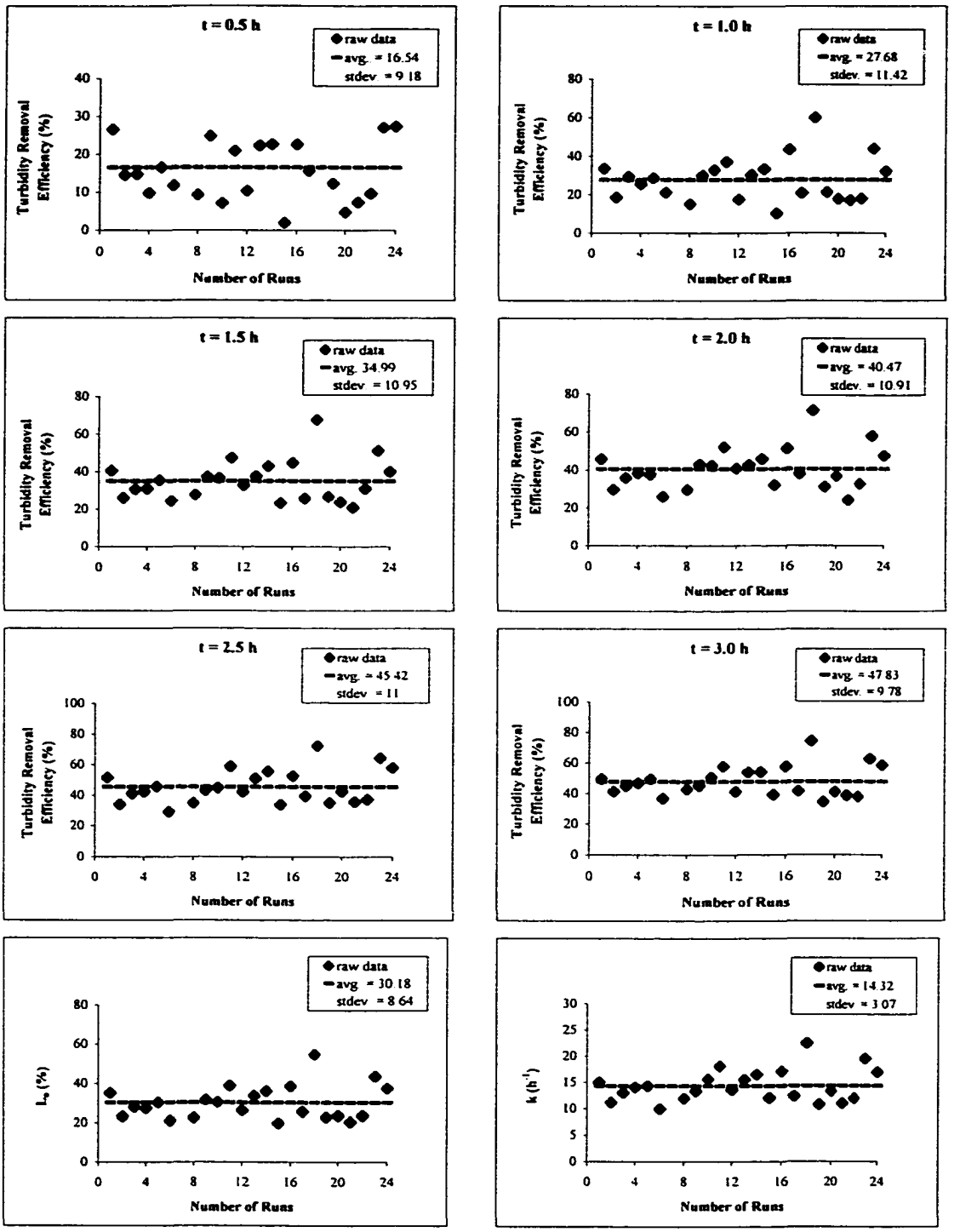


Figure 7. Variation of TRE, Lo and k (determined based on TRE) between runs over 43 days (samples were a mixture of PME and RW with volume ratio of 1:1)

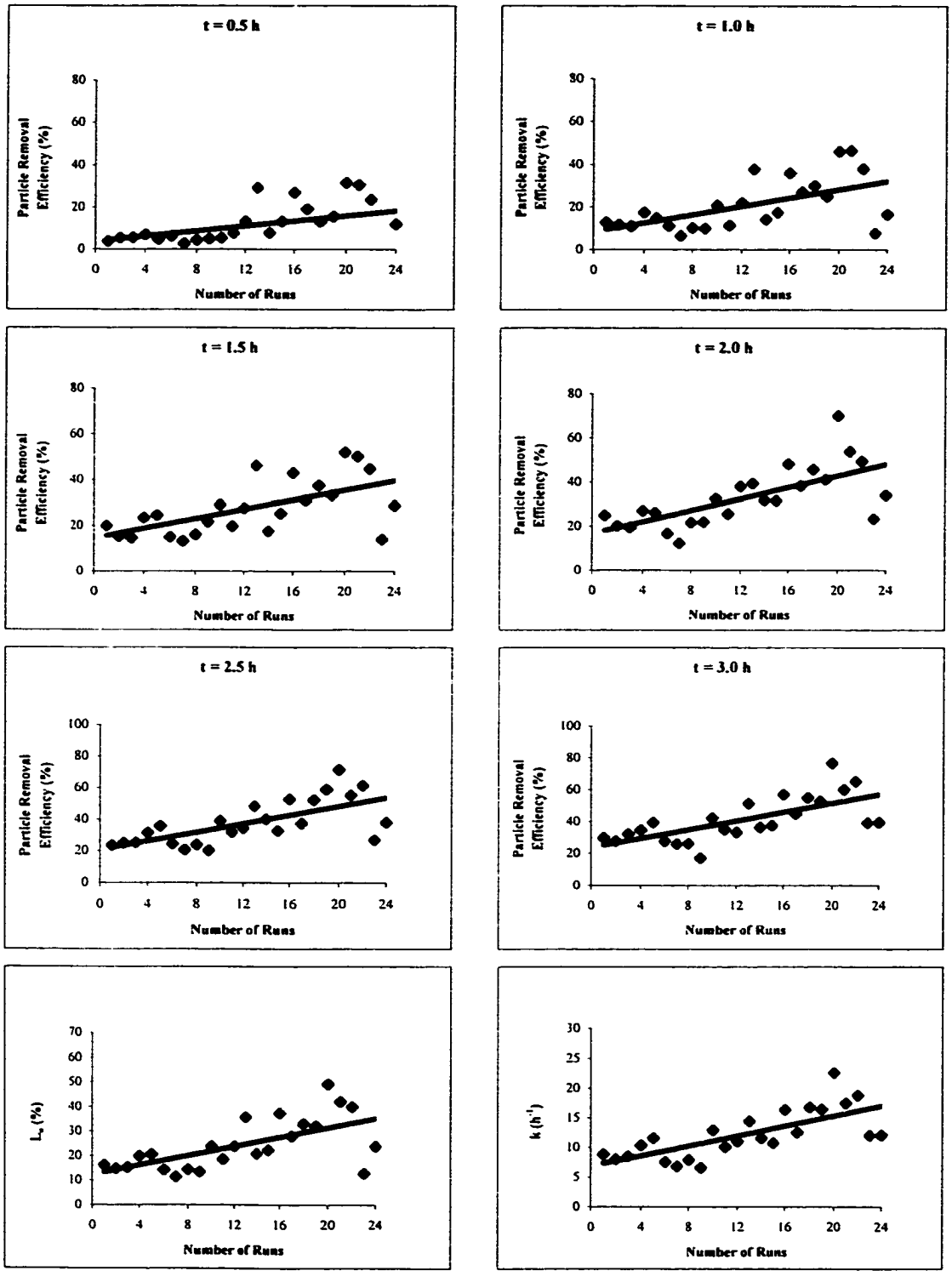


Figure 8. Variation of PRE, L_o and k (determined based on PRE) between runs over 43 days (samples were a mixture of PME and RW with volume ratio of 1:1)

4.1.4 Experimental Results Reliability

Evaluation of accuracy and reproducibility of experimental results suggested that the results were reliable. The majority of experimental results were the means of triplicates or duplicates, although a few AOX results were from single analyses. Both fresh (< 20 h) and preserved samples were used, but most results presented were replicated using daily fresh samples. Some experiments were also replicated using samples from different seasons in different years. Where appropriate, single-factor ANOVA analyses (analysis of variance) were conducted to test the null hypothesis that there was no difference between the means for two sets of experiments. If differences were detected, the statistical significance of the data was tested using a two-tailed t-test under equal variance assumptions. All statistical tests were carried out at the 5% significance level.

Parameters used to evaluate flocculation performance were reliable. Instead of using turbidity and particle size distributions, TRE and PRE were used to characterize flocculation performance. Using TRE and PRE accounts for the variation of initial particle concentrations attributed to seasonal and spatial variations or sample preparation, so that the results from different experimental scenarios are comparable. In addition, the experimental results were used with the consideration of effect from other factors. Most of the results were obtained from fixed experimental conditions at room temperature between 20 to 22 °C (the temperature of samples used for flocculation was also between 20 to 22°C). However, the effect on the results from temperature, initial particle concentration, dilution, pH, alkalinity and mixing were evaluated under the same

experimental set-up to understand how these factors influence the results obtained.

4.2 Confirmatory Studies

Confirmatory studies were conducted in the field and laboratory.

4.2.1 Field Survey

Field survey was conducted on the Athabasca River and the Wapiti River.

4.2.1.1 Survey of the Athabasca River

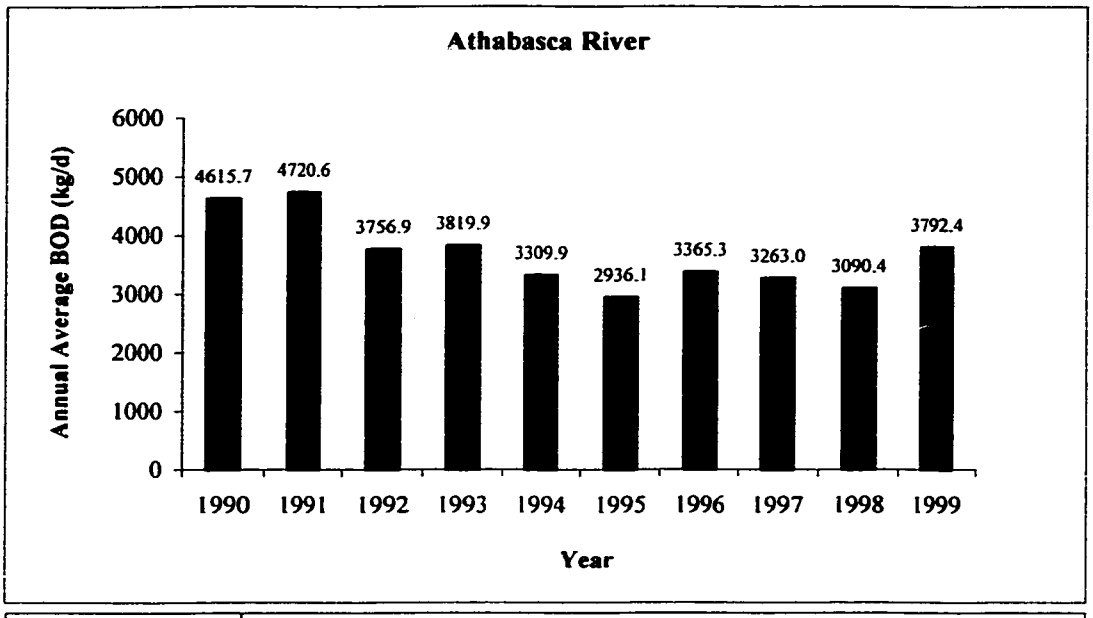
The Athabasca River starts in the Rocky Mountains of west central Alberta in Jasper National Park. It then flows Northeast in Alberta across the boreal mixed wood ecoregions and boreal foothills to Lake Athabasca, where it first joins the Peace River and eventually forms the Slave River. Within the Athabasca River Basin potential sources of ecotoxicity come from major point-sources on the mainstream, such as continuous municipal discharge, PME, oil extraction effluent, period discharge and non-point (diffuse) sources. Survey sites were chosen near Hinton, upstream and downstream of the Weldwood of Canada pulp mill outfall. The major point source from upstream of the outfall is the municipal sewage from the Town of Jasper. Weldwood pulp mill discharges the combined effluent of Weldwood effluent and sewage from the Town of Hinton. The top photo in Figure 9 shows the vicinity of the pulp mill outfall where PME (yellow color) was visualized (the pink color was the result of Rhodomine WT dye tests conducted during the same time period). The bottom photo in Figure 9 shows the background of downstream of the Athabasca River where no PME was evident.

Figure 9. The Athabasca River downstream of the pulp mill outfall near Hinton (left: Dr. Daniel W. Smith, right: Stephanie Young)

The Athabasca River currently receives PME from five pulp mills; near Hinton it receives the effluent from the Weldwood mill. The Weldwood mill discharges combined PME and municipal effluent, as such the loading for total phosphorus is greater than Weyerhaeuser Canada Ltd. at Grande Prairie that only discharges bleached Kraft mill effluent. BOD_5 loading from all mills to the river is presented in Figure 10 where a general downward trend for average BOD loading (kg/d) from 1990 to 1998 is evident, but a slight increase in 1999.

The Athabasca River underwent significant diurnal and seasonal variations in river flow rate. Figure 11 reveals flow fluctuates from year to year. It averaged 335 m^3/s for August 1998 and 460 m^3/s for August 1999 (the diurnal variation in August is presented as the surveys were conducted during that time). Figure 11 also shows that monthly flow undergoes significant variation during the year. A low flow was evident between November to April. The minimal flow occurred during January and February, and peak flows occurred in July after the mountain snow-packs melted (433 m^3/s for July of 1998 and 637 m^3/s for July of 1999).

Floc formation and the amount of deposited sediment flocs depend on river flow velocity that controls shear. High flow velocity promotes the floc formation, but it may also cause them to break up. Bottom sediments might be flushed away or resuspended at high flow rate, but most sediment flocs would remain at the bottom of the river in winter at minimum flow.



	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
Slave Lake (CTMP), (kg/d)	1107.2	887.4	621.9	455.8	235.0	295.3	269.8	388.8	341.7	548.8
Millar Western (CTMP), (kg/d)	1016.1	1121.1	576.5	330.8	763.7	593.5	538.0	364.9	207.5	351.1
ANC (TMP newsprint), (kg/d)	110.5	162.8	229.9	116.6	140.2	157.8	136.0	93.9	141.6	88.8
Weldwood (BKM), (kg/d)	2381.9	2549.3	2328.6	1922.8	1829.8	1664.5	2165.0	2128.7	2222.0	2581.5
Al-Pac (BKM), (kg/d)				993.9	341.2	225.0	256.5	286.7	177.6	222.2
Total Avg. BOD (kg/d)	4615.7	4720.6	3756.9	3819.9	3309.9	2936.1	3365.3	3263	3090.4	3792.4

Figure 10. Discharges of annual average BOD to the Athabasca River (data from Alberta Environmental Protection, 2000)

From the field survey conducted in August 1998, it was found that a significant quantity of larger sized flocs formed from the outfall to approximately 1000 m downstream of the outfall. The size and amount of flocs decreased further downstream in the river (some loss was due to settling). Figure 12 further reveals that the bottom sediment flocs collected upstream of the outfall were discrete (Figure 12-a), but larger, denser flocs were found at different locations downstream of the outfall. Examples of the suspended flocs (from RW) are presented in Figure 12-b, and examples of the deposited flocs (from the river bottom) are presented in Figure 12-c and Figure 12-d. Previous research also found that downstream of the outfalls pulp mill chemicals decreased in RW (Carlberg and Stuthridge, 1994), but increased in the sediments (Judd *et al.*, 1996; Judd *et al.*, 1995; Martinsen, 1994). Therefore, it can be concluded that the phenomenon of PMEICF existed in the Athabasca River.

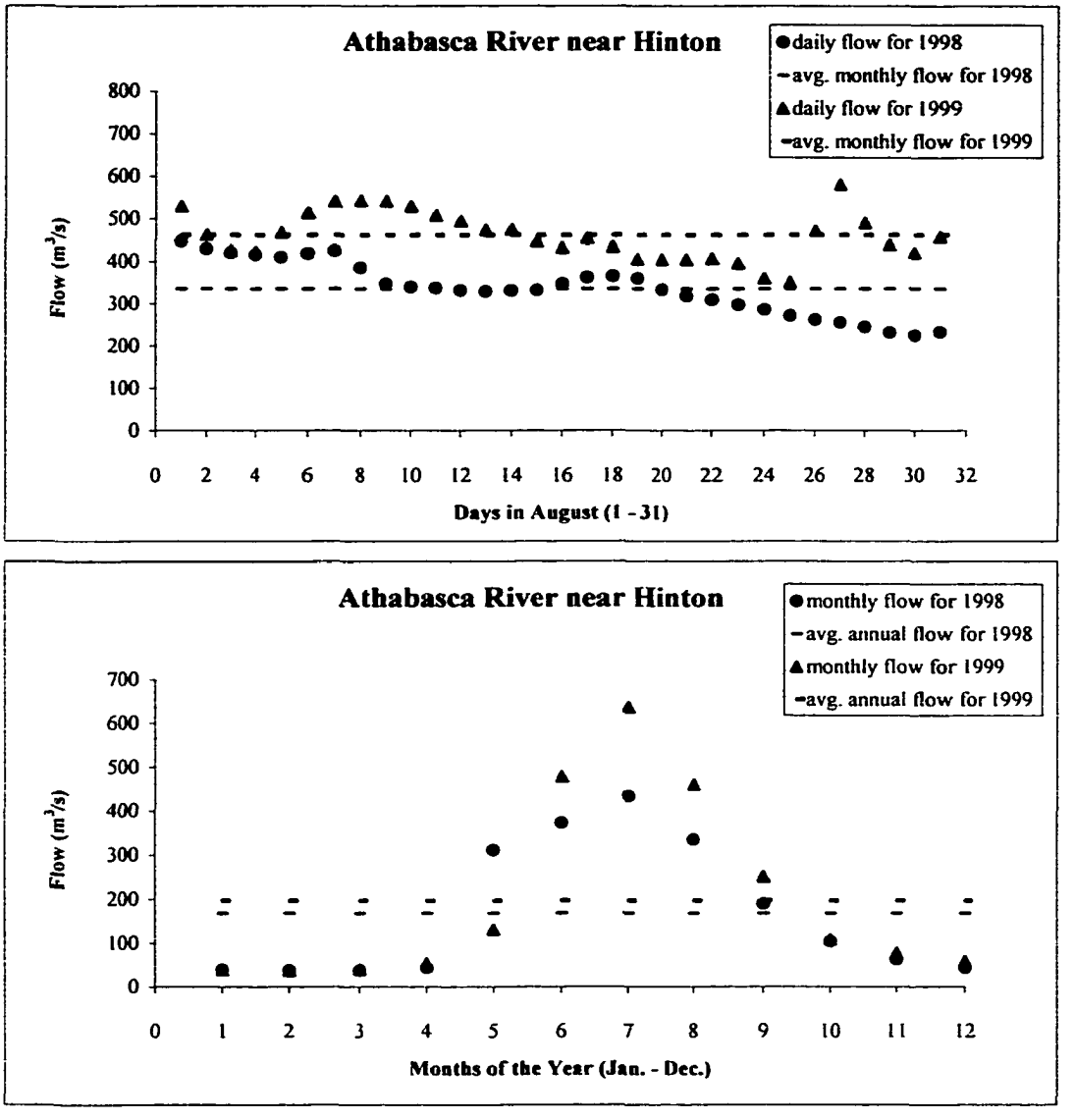
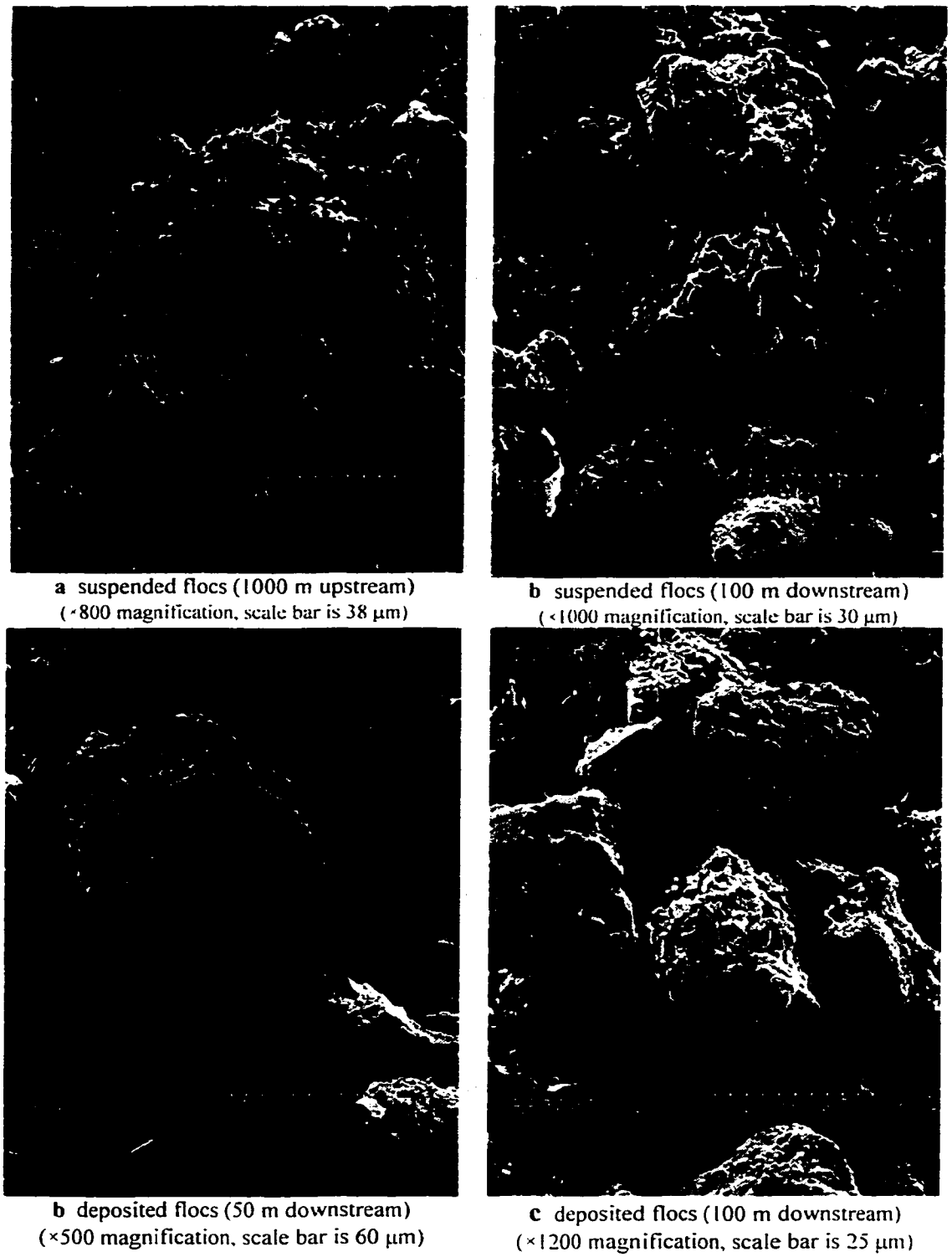


Figure 11. Daily and monthly flows for the Athabasca River (data was provided by Alberta Environmental Protection, 2000)



a suspended flocs (1000 m upstream)
(×800 magnification, scale bar is 38 μm)

b suspended flocs (100 m downstream)
(×1000 magnification, scale bar is 30 μm)

b deposited flocs (50 m downstream)
(×500 magnification, scale bar is 60 μm)

c deposited flocs (100 m downstream)
(×1200 magnification, scale bar is 25 μm)

Figure 12. Sediment flocs collected from the Athabasca River near Hinton

4.2.1.2 Survey of the Wapiti River

The Wapiti River originates south of Dawson Creek, which is located in east-central British Columbia in the Rocky Mountains. The Wapiti River flows east across the boreal foothills, boreal uplands and boreal mixed wood ecoregions of Alberta. It joins the Smoky River about 42 km downstream of Grande Prairie. Currently, the Wapiti River receives continuous industrial effluent discharge from Weyerhaeuser Canada Ltd. at Grande Prairie. The upstream of Weyerhaeuser pulp mill outfall in Wapiti River also receives sewage discharge from the Grande Prairie municipality. The Wapiti River also receives the discharge from sewage lagoons near the Wapiti-Smoky drainage basin. In addition, there are 20 natural gas processing plants, which discharge wastewater to the Wapiti-Smoky drainage. Alberta Environmental Protection has given the mill stringent limits for BOD, TSS, AOX and color. For example, Weyerhaeuser's limit for color is approximately 90 kg/adt (adt stands for air-dried tonne) currently, 61 kg/adt in 2002, and 45 kg/adt in 2007, assuming no increase in production.

Like the Athabasca River, the Wapiti river flow also underwent significant diurnal and seasonal variations. Figure 13 shows that daily flow fluctuates during the month. The flow in August averaged 35 m^3/s for 1998 and 69 m^3/s for 1999. Peak flows occurred in July 1998 and June 1999 after the mountain snow-packs melted (289 m^3/s for July of 1998 and 247 m^3/s for June of 1999). The low flow occurred during December to March, and the lowest flows occurred in February of 1998 (11.4 m^3/s) and December of 1999 (9.9 m^3/s).

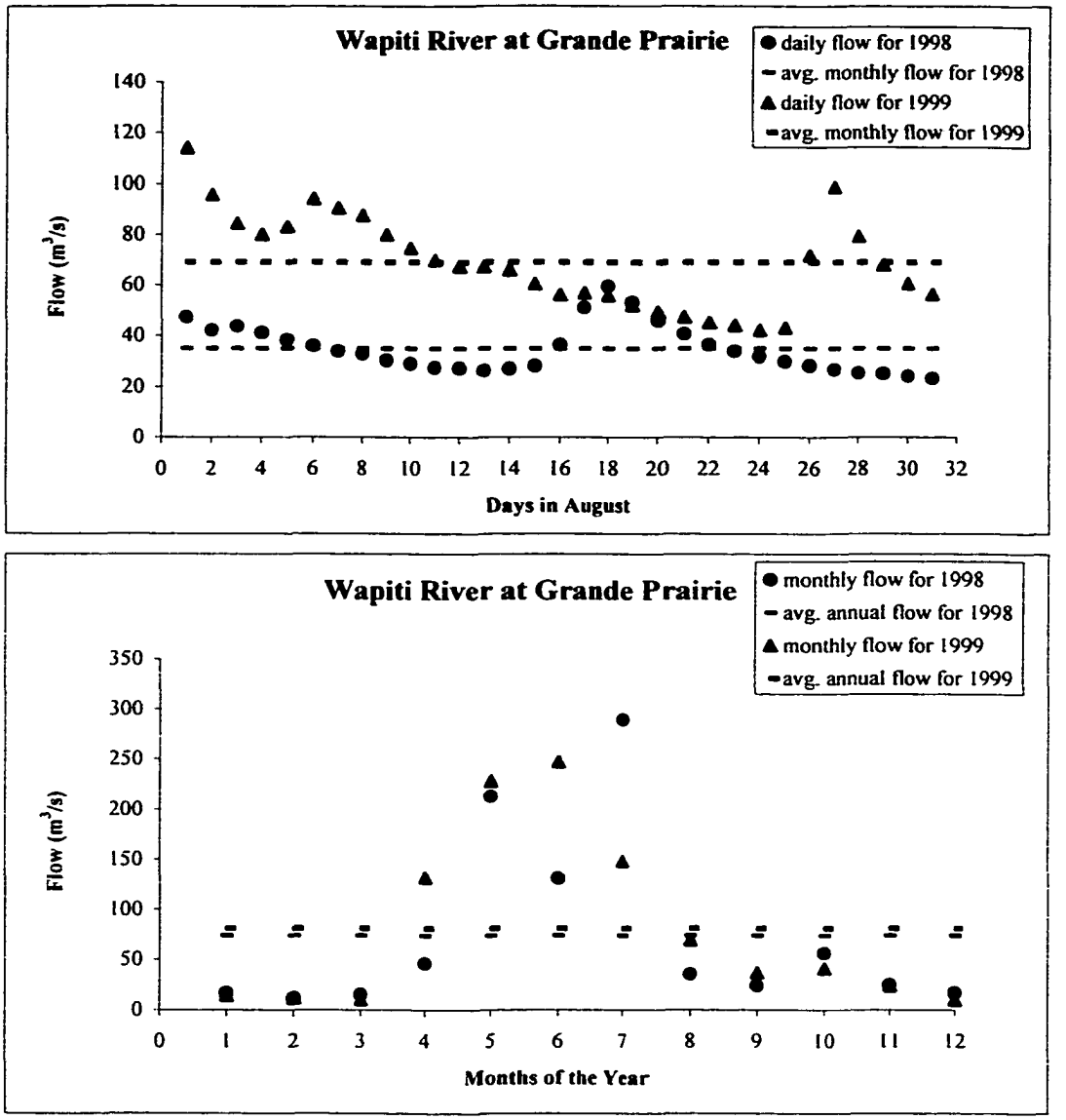


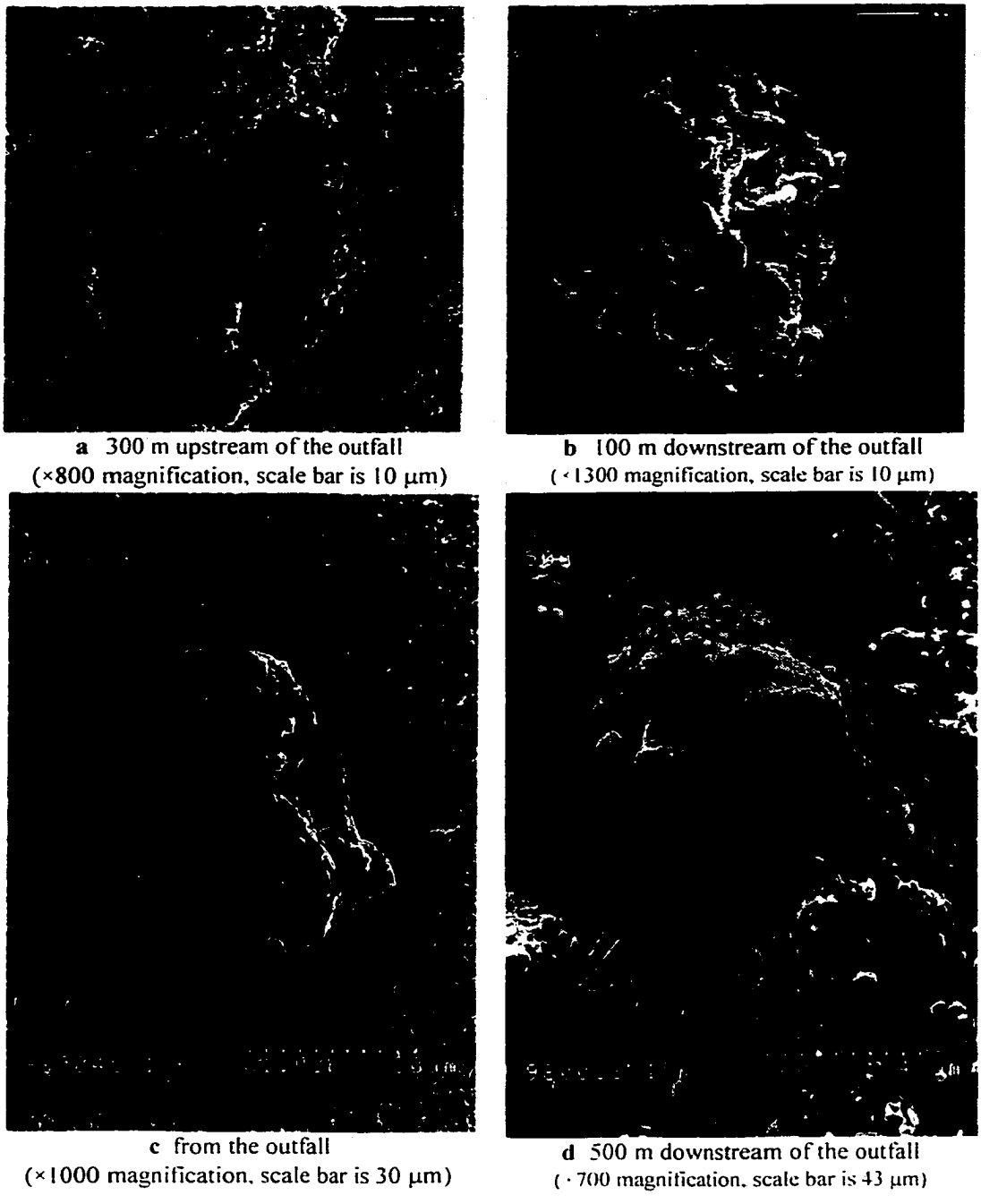
Figure 13. Daily and monthly flow for the Wapiti River (data was provided by Alberta Environmental Protection, 2000)

The same phenomenon observed in the Athabasca River was also evident in the Wapiti River. Figure 14 shows that the bottom sediments collected upstream of the outfall were discrete, but larger, denser flocs formed at different locations downstream of the outfall. This indicates that PMEICF occurred in the Wapiti River.

In summary, field observations on the Athabasca and Wapiti rivers consistently showed that larger flocs formed downstream of the outfalls, although the conditions for the rivers are quite different. The Athabasca River has higher river assimilative ability than the Wapiti River, as it is deeper and generally has high flows and high turbulence. Scouring caused by high turbulence results in high concentrations of suspended solids and fewer aquatic plants during the summer months. On the other hand, the Wapiti River is shallow and has lower flow and higher density of algae and aquatic plants.

4.2.2 Laboratory Confirmation

Confirmatory experiments were also conducted in the laboratory to reinforce the field findings. Tested samples were a mixture of Weldwood PME and Athabasca RW, and a mixture of Weyerhaeuser PME and Wapiti RW. The confirmatory experiments were replicated using samples from different seasons of the years (from 1997 to 2000). Physicochemical characteristics of RW and PME for most of the samples are summarized in Table 7. It was noted that there was no significant variation for characteristics of PME during the study period. However, the turbidity of RW samples significantly varied in different seasons of the year. Higher turbidity was found in the spring samples.



a 300 m upstream of the outfall
(×800 magnification, scale bar is 10 μm)

b 100 m downstream of the outfall
(×1300 magnification, scale bar is 10 μm)

c from the outfall
(×1000 magnification, scale bar is 30 μm)

d 500 m downstream of the outfall
(×700 magnification, scale bar is 43 μm)

Figure 14. Sediment flocs from the Wapiti River near Grande Prairie

Table 7. Characteristics of Samples of Weyerhaeuser PME (after biological treatment prior to discharge, data were provided by Weyerhaeuser pulp mill at Grande Prairie.)

Parameter	Result	Units	Sample Date	Sample Type
AOX	6.8	mg/L	Dec. 1/97	Composite sample
	5.3		June 1/98	
	6		June 8/98	
BOD_5	21	mg/L	Dec. 3/97	Composite sample
	18		May 27/98	
	16		June 4/98	
TSS	20	mg/L	Dec. 3/97	Composite sample
	14		May 27/98	
	12		June 4/98	
Color	1000	CU	Dec. 3/97	Composite sample
	980		May 27/98	
	1100		June 4/98	
COD	603	mg/L	Dec. 3/97	Composite sample
	470		May 27/98	
	562		June 4/98	
Ammonia	1.55	mg/L	Dec. 3/97	Composite sample
	1.18		May 27/98	
	1.95		June 4/98	
TOC	162	mg/L	Dec 3/97	Grab Sample
	136		June 4/98	
	148		July 8/98	
	132		Aug 6/98	
	160		July 6/99	
	181		Aug 3/99	
	179		Aug 19/99	
DOC	124	mg/L	Dec 3/97	Grab Sample
	111		June 4/98	
	116		July 8/98	
	103		Aug 6/98	
	133		July 6/99	
	140		Aug 3/99	
	143		Aug 19/99	
TON	500	mg/L	June 4/98	Grab Sample
	500		July 8/98	
	241		Aug 6/98	
	500		July 6/99	
	500		Aug 3/99	
Chlorophenol	1.1		Aug 6/98	
	0.3		Aug 3/99	
	0.1		Aug 19/99	
Chloroform	<0.5	µg/L (ppb)	Dec 3/97	Grab Sample
	0.5		Aug 6/98	
	0.5		Aug 3/99	
PCB	0.05		July 8/98	
Aluminum (extractable)	0.67	mg/L	Dec 3/97	Grab Sample

Table 7 continued . . .

Parameter	Result	Units	Sample Date	Sample Type
Chlorate	2	mg/L	Dec 3/97	Grab Sample
	2.1		June 4/98	
	19.8		July 8/98	
	18.2		Aug 6/98	
	26.4		July 6/99	
	12.8		Aug 3/99	
	24.1		Aug 19/99	
	9		Aug 23/99	
	8.4		Aug 30/99	
Chlorite	<0.5	mg/L	Dec 3/97	Grab Sample
	2.4		June 4/98	
	0.5		July 8/98	
	0.5		Aug 6/98	
	0.5		July 6/99	
	0.5		Aug 3/99	
	0.5		Aug 19/99	
Resin & Fatty Acids	<0.01	mg/L	Dec 3/97	Grab Sample
	0.035		June 4/98	
	0.02		July 8/98	
	0.02		Aug 6/98	
	0.01		July 6/99	
	0.01		Aug 3/99	
	0.02		Aug 19/99	
Sulfide	0.078	mg/L	Dec 3/97	Grab Sample
	0.112		June 4/98	
	0.079		July 8/98	
	0.083		Aug 6/98	
	0.074		July 6/99	
	0.156		Aug 3/99	
	0.128		Aug 19/99	
Threshold Odor Number	50	mg/L	Dec 3/97	Grab Sample
DKN	2.5	mg/L	Dec. 3/97	Grab Sample
	4.3		May 27/98	
	4		June 4/98	
	3.3		July 2/98	
	3.4		July 8/98	
	3.4		July 15/98	
	5		July 22/98	
	4.1		July 29/98	
	2.4		Aug 6/98	
	2.8		Aug 12/98	
	2.8		Aug 18/98	
	3.1		Aug 25/98	
	1.8		July 6/99	
	2.6		Aug 3/99	
	5		Aug 19/99	

Table 7 continued . . .

Parameter	Result	Units	Sample Date	Sample Type
Total Dissolved	0.71	mg/L	Dec. 3/97	Grab Sample
Phosphorus	0.98		May 27/98	
	1.11		June 4/98	
	1.18		July 2/98	
	1.45		July 8/98	
	1.23		July 15/98	
	1.22		July 22/98	
	1.18		July 29/98	
	1.01		Aug 6/98	
	1.06		Aug 12/98	
	0.93		Aug 18/98	
	1		Aug 25/98	
	0.74		July 6/99	
	1.08		Aug 3/99	
	0.89		Aug 19/99	
Nitrate	0.09	mg/L	Dec. 3/97	Grab Sample
	0.05		May 27/98	
	0.05		June 4/98	
	0.05		July 2/98	
	0.05		July 8/98	
	0.05		July 15/98	
	0.05		July 22/98	
	0.05		July 29/98	
	0.05		Aug 6/98	
	0.05		Aug 12/98	
	0.05		Aug 18/98	
	0.05		Aug 25/98	
	0.05		July 6/99	
	0.05		Aug 3/99	
	0.05		Aug 19/99	
Nitrite	<0.2	mg/L	Dec. 3/97	Grab Sample
	0.02		May 27/98	
	0.02		June 4/98	
	0.2		July 2/98	
	0.2		July 8/98	
	0.2		July 15/98	
	0.2		July 22/98	
	0.2		July 29/98	
	0.2		Aug 6/98	
	0.2		Aug 12/98	
	0.2		Aug 18/98	
	0.2		Aug 25/98	
	0.2		July 6/99	
	0.2		Aug 3/99	
	0.2		Aug 19/99	

Table 7 continued . . .

Parameter	Result	Units	Sample Date	Sample Type
Ammonia	1.34	mg/L	Dec. 3/97	Grab Sample
	2.2		May 27/98	
	2.65		June 4/98	
	1.2		July 2/98	
	2.08		July 8/98	
	1.8		July 15/98	
	1.55		July 22/98	
	1.95		July 29/98	
	1.82		Aug 6/98	
	1.62		Aug 12/98	
	1.62		Aug 18/98	
	1.83		Aug 25/98	
	0.98		July 6/99	
	1.89		Aug 3/99	
	1.59		Aug 19/99	
Total Phosphorus	0.93	mg/L	Dec. 3/97	Grab Sample
	1.17		May 27/98	
	1.33		June 4/98	
	1.39		July 2/98	
	1.54		July 8/98	
	1.4		July 15/98	
	1.46		July 22/98	
	1.36		July 29/98	
	1.22		Aug 6/98	
	1.17		Aug 12/98	
	1.13		Aug 18/98	
	0.68		Aug 25/98	
	0.95		July 6/99	
	1.32		Aug 3/99	
	1.21		Aug 19/99	
TKN	4.1	mg/L	Dec. 3/97	Grab Sample
	6.5		May 27/98	
	5.2		June 4/98	
	5.8		July 2/98	
	6.2		July 8/98	
	4.6		July 15/98	
	7.2		July 22/98	
	5.2		July 29/98	
	3.7		Aug 6/98	
	2.8		Aug 12/98	
	3.6		Aug 18/98	
	4		Aug 25/98	
	3.5		July 6/99	
	4.2		Aug 3/99	
	5		Aug 19/99	

Table 7 continued . . .

Abbreviations:

AOX	Adsorbable organic halogens	RFA	Resin & fatty acids
BOD_5	Biochemical oxygen demand	TDP	Total dissolved phosphorus
COD	Chemical oxygen demand	TDS	Total dissolved solids
DKN	Dissolved Kjeldahl nitrogen	TKN	Total Kjeldahl nitrogen
DOC	Dissolved organic carbons	TOC	Total organic carbons
Mn	Manganese	TON	Threshold odour number
NH_3-N	Ammonia nitrogen	TP	Total phosphorus
NO_2-N	Nitrite	TSS	Total suspended solids
NO_3-N	Nitrate		

Note:
1. Samples were analyzed immediately after collection.
2. The samples were analyzed in the mill laboratory.

Confirmatory studies in the laboratory included sedimentation (without mixing) and flocculation tests. Long-term sedimentation tests (100 h) were conducted on PME, RW and a corresponding mixture of both with volume ratio of 1:1, where PME and RW were used as references.

Figure 15 shows that, in comparison to RW, no increase in TRE was observed when PME was mixed with RW. In fact, the TRE of RW was slightly higher than that of the mixture after 100 hours sedimentation. This is because RW had a higher initial turbidity and larger sediments than samples of PME or a mixture. However, laboratory observations found the immediate appearance of pinpoint flocs as soon as PME was mixed with RW. This indicates that coagulation occurred. Therefore, without mixing, PME induce mainly coagulation, so that large flocs were not evident during sedimentation test (100 h).

The sedimentation results show that mixing is the critical factor for floc formation, which indicates that PMEICF was dominated by orthokinetic flocculation where a collision of particles was caused by velocity gradients, which depends on river hydraulic conditions. The peak flow in Alberta rivers often occurs between June to July, when the mountain snow-packs melt. This increases mixing intensity. The higher mixing intensity promotes flocculation, but it may also break-up sediment flocs. In addition, benthic sediment flocs may be resuspended and flushed away at high river flows. Therefore, the phenomena of PMEICF may not be observed at all times. The minimum flow in Alberta rivers often occurs between December to February. The higher degree of PMEICF is expected when flocculation time is long enough, and the greater number of benthic flocs is evident at river bottoms. These concepts were confirmed in the laboratory using flocculation tests.

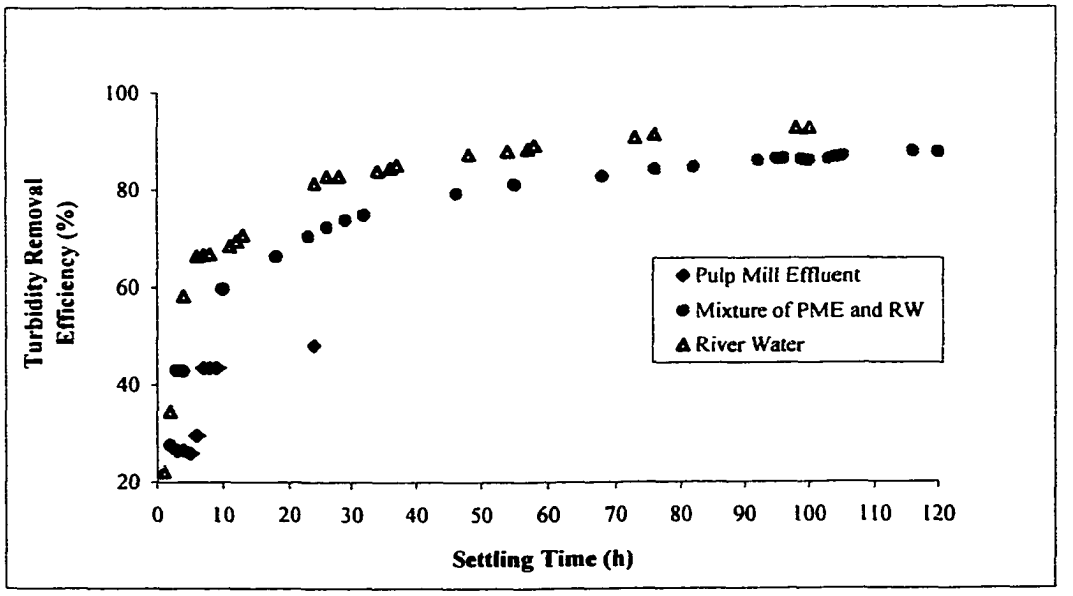


Figure 15. Sedimentation curves without mixing (Weyerhaeuser PME and Wapiti RW, June 1998)

Figure 16 shows that PRE for the sample of a mixture is 38.61%, but 21.95% for RW and 25.96% for PME. Figure 19 also shows much higher PRE for samples of a mixture of PME and RW than that of RW samples. In addition, Table 8 shows that PRE for samples of a mixture was higher than that of RW samples. Higher PRE for small particles (<10 μm) from the samples of mixture indicates that PMEICF was more prominent in removal of particles with sizes less than 10 μm.

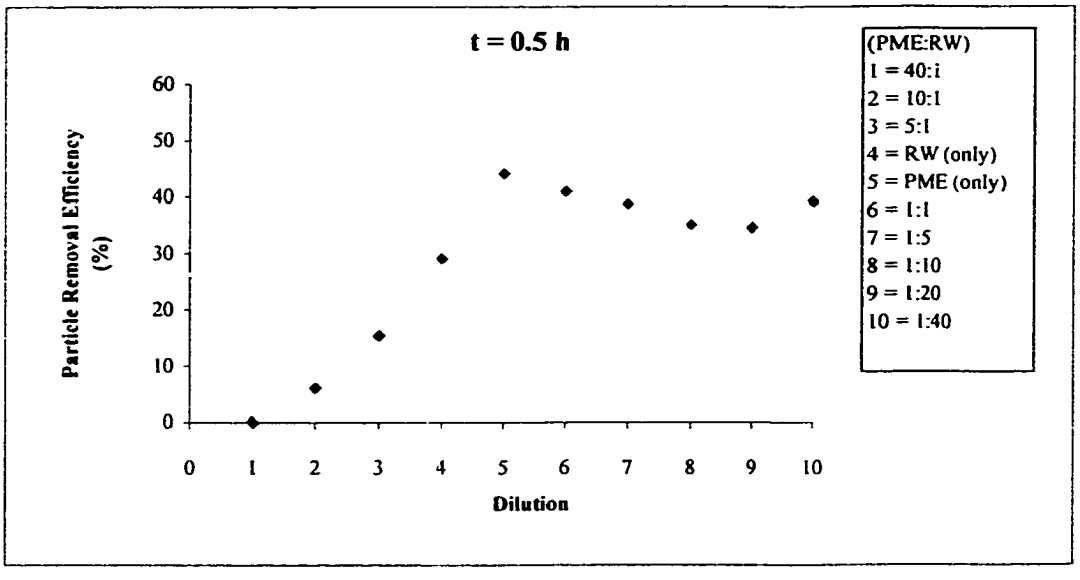


Figure 16. Effect of mixing on particle removal efficiency for summer samples [samples consisted of a mixture of GPME and WRW (all were 1 day old) with different volume ratios, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{Gf} = 13\ s^{-1}$]

Occurrence of PMEICF was also confirmed from Figure 17 and Figure 18, as flocs were evident either on-line in a consecutive time period using the video

microphotography system, or observed using SEM. Figure 17 provides on-line microphotography images for floc settling, which were recorded every 3 seconds during flocculation process. From Figure 17 floc settling velocity can be determined when a scale bar is provided, as such the density of floc can also be determined if the floc settling velocity is known. However, it is not the focus of this study. Figure 17 shows the images of larger, denser flocs formed from samples of a mixture of PME and RW. However, similar types of flocs were not evident from flocculation of RW samples.

Figure 18 shows the images of suspended and deposited flocs formed in the laboratory, which further reinforced that orthokinetic flocculation dominates flocculation mechanisms, because it has been found that orthokinetic flocculation forms larger, denser, spherical flocs (van Leussen, 1988).

In addition, Figure 20 further demonstrates that flocculated samples of a mixture of PME and RW (1:1 by volume) contained fewer residual particles with small sizes (<10 μm) when compared with flocculated RW samples. Higher PRE (<10 μm) suggested that PMEICF was more effective at removing small particles with sizes less than 10 μm. Moreover, Table 9 shows that more than 90% of total suspended solids formed settable flocs after flocculation.

As the laboratory finding is consistent with field observations, it can be concluded that PMEICF does occur in the Athabasca River and the Wapiti River, and orthokinetic flocculation is the dominant flocculation mechanisms. Further research is suggested in the future to determine floc settling velocity and floc density.

Table 8. PRE for samples of RW and a mixture of PME and RW (1:1 by volume)

	PRE (%)		
	d = 2 μm	d = 5 μm	d = 10 μm
RW	74	66	44
PME+RW(1:1)	92	93	92

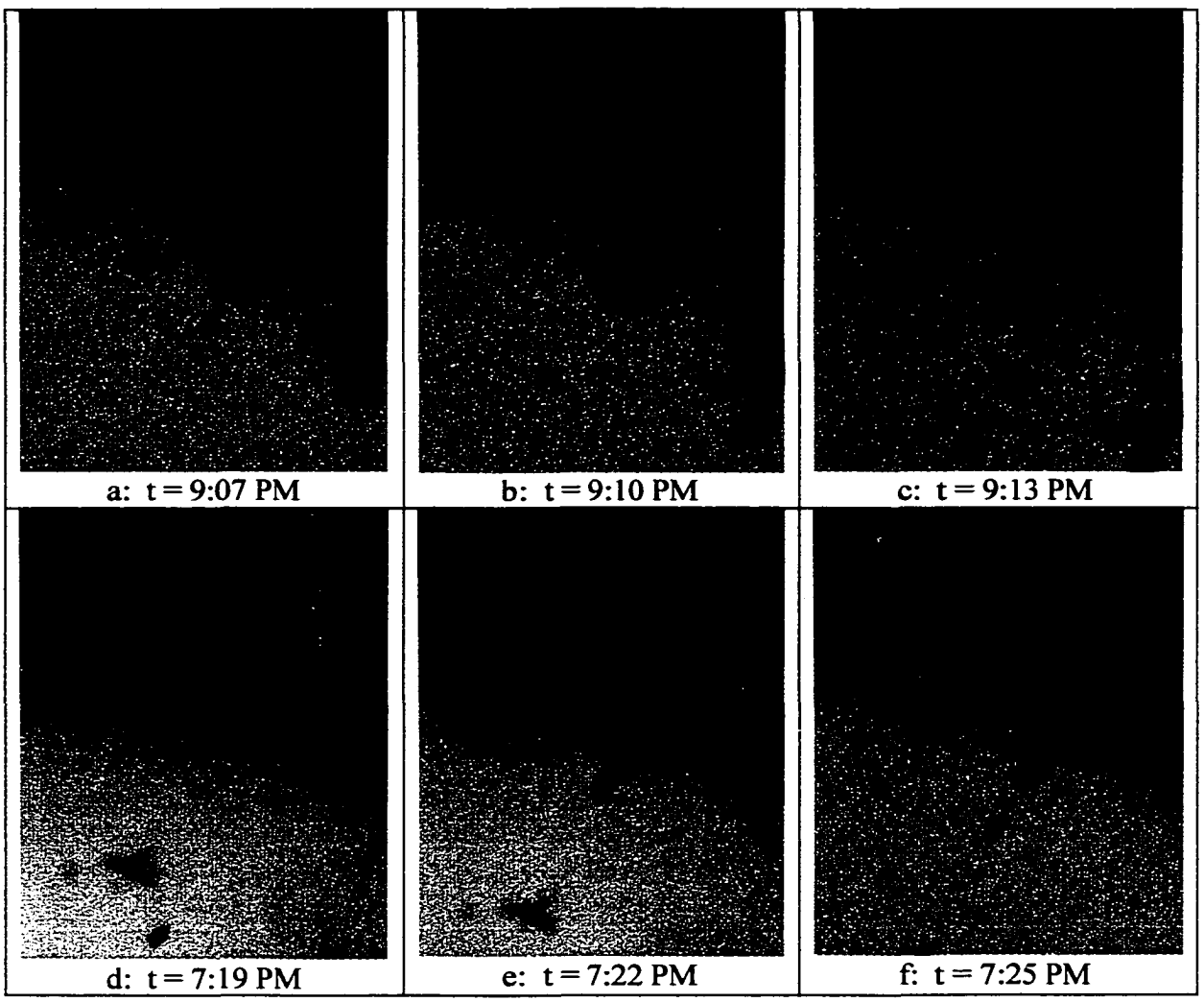


Figure 17. On-line microphotography images for floc settling (t stands for the time when flocs were taped). Flocs were formed from flocculation of a mixture of Weyerhaeuser PME and Wapiti RW (1:1 by volume) for 100 hours at impeller speed of 2 rpm

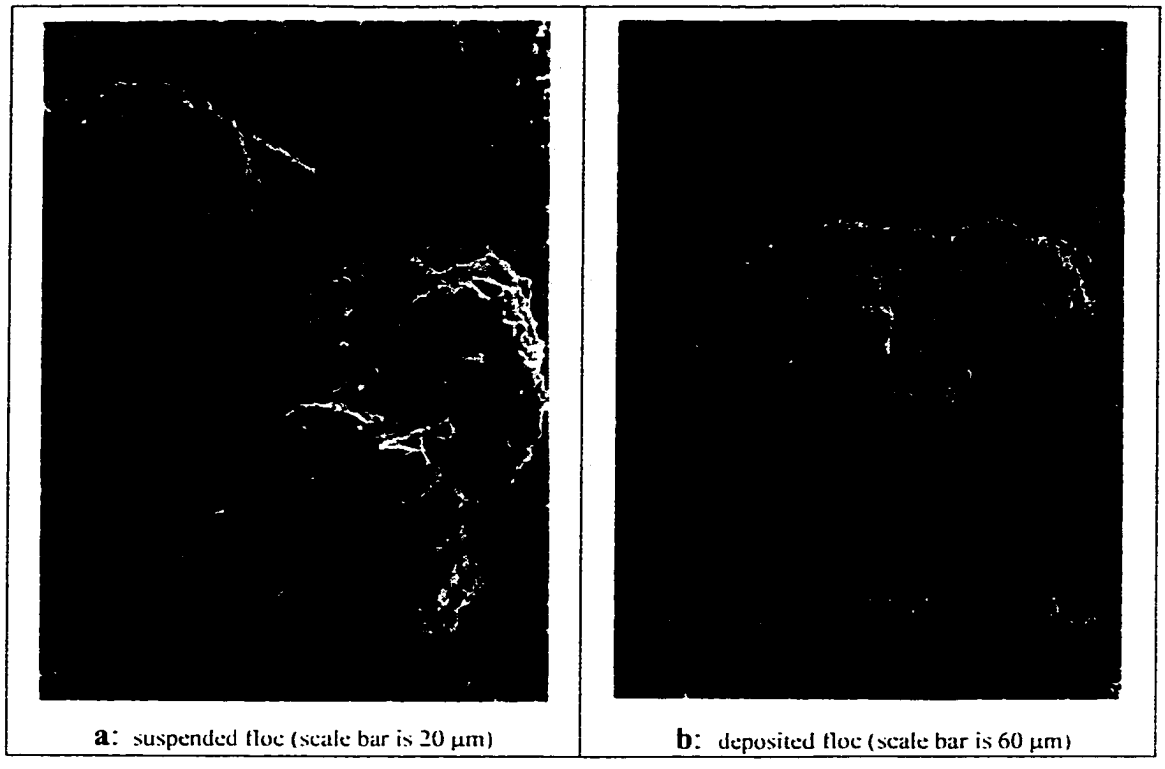


Figure 18. SEM images of suspended and deposited flocs formed in the laboratory from flocculation of samples of a mixture of Weyerhaeuser PME and Wapiti RW (1:1 by volume) for 100 hours at vertical grid speed of 2 mm/s

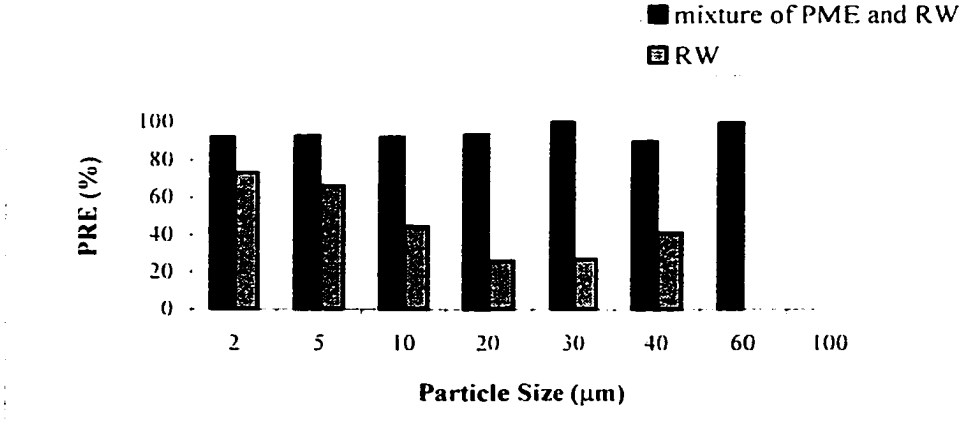


Figure 19. Comparison of values of PRE obtained from RW samples and samples of a mixture of Weldwood PME and Athabasca RW (1:1 by volume). Flocculation tests were conducted at vertical grid speed of 2 mm/s for 100 hours and 20-hour sedimentation (August 19, 1998)

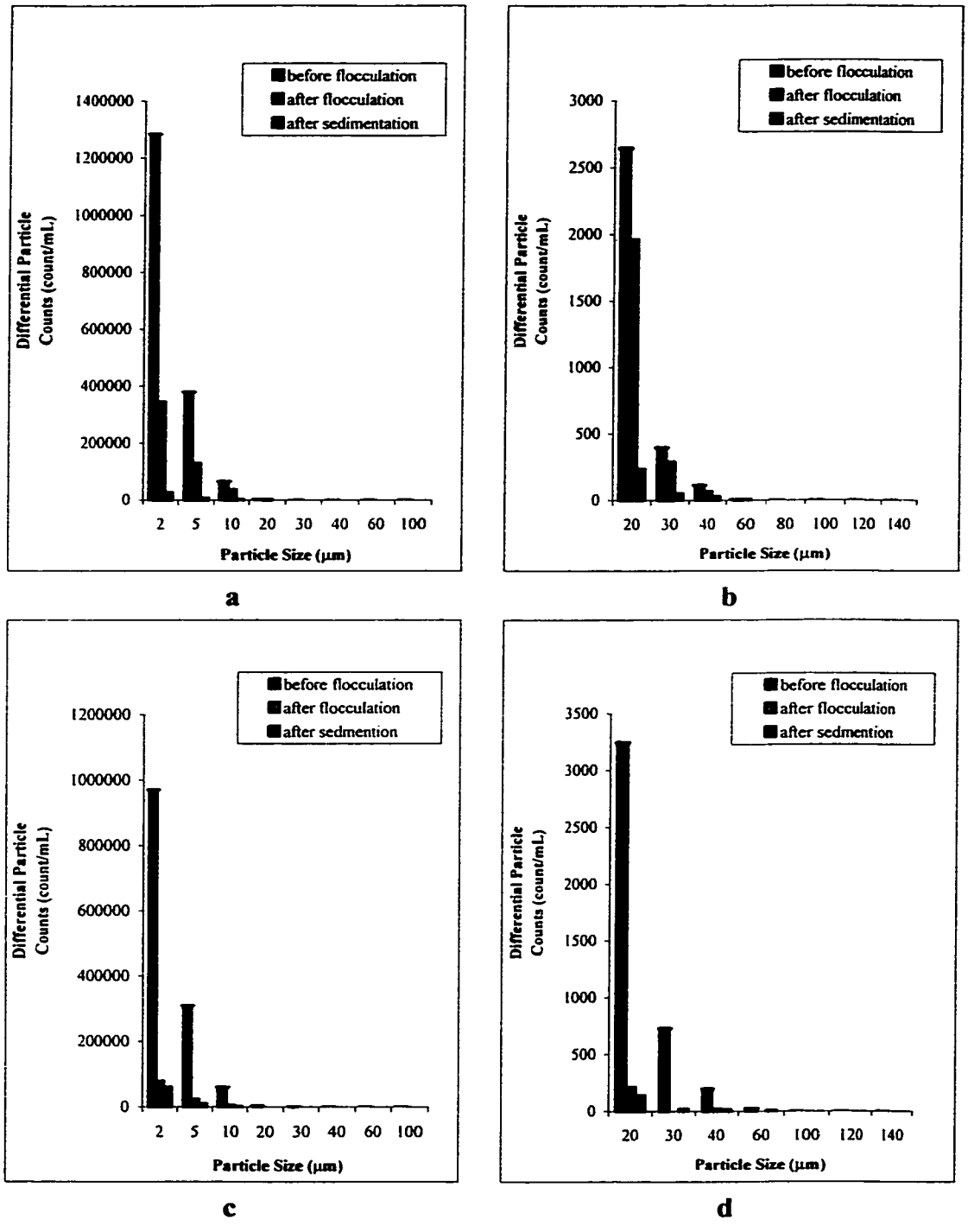


Figure 20. Particle size distributions of Wapiti RW (a: 0 μm < size < 20 μm and b: 20 μm < size < 60 μm) and a mixture of Weyerhaeuser PME and Wapiti RW with a volume ratio of 1:1 (c: 0 μm < size < 20 μm and d: 20 μm < size < 60 μm). Flocculation tests were conducted at vertical grid speed of 2 mm/s for 100 hours and 20-hour sedimentation (August 19 1998)

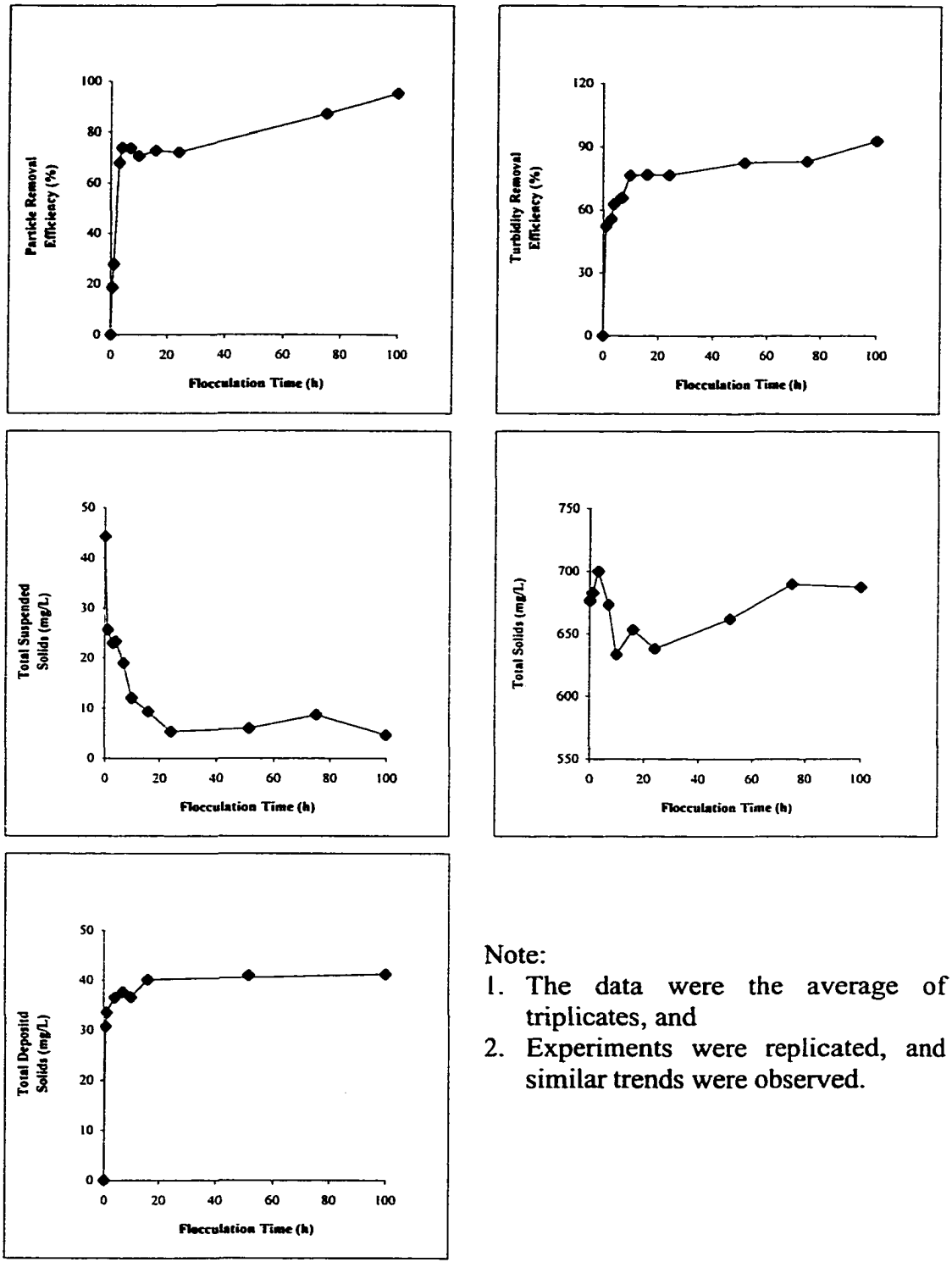
Table 9. Variation in characteristics for samples of a mixture of Weyerhaeuser PME and Wapiti RW with a volume ratio of 1:1 (sample date: May to June of 1998; the results are averages of duplicates or triplicates)

	Parameter		Results
Before Mixing	TS	(mg/L)	1145
	TSS	(mg/L)	208
	Turbidity	(NTU)	115.8
	Color	(CU)	586
	pH		7.3
	TC (measured)	(mg/L)	144
	TIC (measured)	(mg/L)	61
	TOC (calculated)	(mg/L)	83
After 100 h Mixing At 2 mm/s	TS (top)	(mg/L)	1117
	TS (bottom)	(mg/L)	1113
	TSS (top)	(mg/L)	15
	TSS (bottom)	(mg/L)	19
	Turbidity	(NTU)	5.37
After 20 h settling	Turbidity	(NTU)	6.08
	Color	(CU)	573
	pH		7.3
	TC (measured)	(mg/L)	156
	TIC (measured)	(mg/L)	63.5
	TOC (calculated)	(mg/L)	92.5

Notes:

1. TS = Total Solids, TSS = Total Suspended Solids and TOC = Total Organic Carbon
2. The increase of the turbidity after sedimentation was due to the disturbance of the suspension when the grid mixer was taken out
3. Bottom = samples taken from ¼ of the suspension height
   Top = samples taken from ¾ of the suspension height

The above results confirm that PMEICF occurred in the Wapiti River. To prove its existence in the Athabasca River, long-term flocculation tests (up to 100 h) were replicated using a mixture of Athabasca RW and Weldwood PME. Figure 21 demonstrates that TRE, PRE and total deposited solids increased, and total suspended solids decreased when flocculation time increased, but the degree of flocculation tends to be asymptotic to a limiting value after a certain time, called the critical time. Only a slight increase after the critical time was attributed to the limited availability of small sized discrete particles. Total solids decreased up to approximately 20 h, and then slightly increased up to 100 h. The increase was due to the increase in total dissolved solids attributed to sorption of dissolved species onto colloidal particles. Sorption can be classified into physical sorption and chemical sorption. Physical sorption was attributed to van der Waals forces, ion-dipole interactions and dipole-dipole interactions. Chemical sorption was attributed to the bonds between ions in the solution and the surface of fine particles, condensation reactions with OH^- groups on the surface of chemicals.



Note:

1. The data were the average of triplicates, and
2. Experiments were replicated, and similar trends were observed.

Figure 21. Flocculation of samples of a mixture of PME and RW (1:1 by volume) at vertical speed of 2 mm/s (sampling date: August 19, 1998)

In conclusion, a large number of flocs were formed in the laboratory and observed in the field; significant particle removal (especially with sizes less than 10 μm) was achieved when PME was mixed with RW (1:1 by volume). Existing evidence supports the conclusion that PMEICF occurred in both the Athabasca and Wapiti Rivers. It was also found that mixing is the critical factor for the formation of PMEICF, orthokinetic flocculation was the primary mechanism for flocculation, and PMEICF was more prominent in removal of particles with sizes less than 10 μm.

4.3 Effect of physical factors on PMEICF Formation

Physical factors evaluated included sample age, temperature, mixing and dilution.

4.3.1 Sample Age

The methodology of sampling is very critical for obtaining meaningful, representative results and allowing valid comparison, as insufficient sampling leads to erroneous conclusions. To determine a cost-effective sampling methodology, effect of sample age on flocculation in RW was evaluated for 11 consecutive days (July 19-31, 1999). Samples used for evaluation were daily fresh (< 20 h old) and preserved (< 11 days) samples collected from the Weyerhaeuser pulp mill and the Wapiti River. The fresh samples were shipped daily from the sites, and preserved samples came from one batch collected on July 19, 1999 that was kept in a cold room (2 °C). The study included the variation of characteristics of samples for 11 consecutive days and replication of flocculation experiments for the same period of time.

Results for variation of characteristics of samples of PME or RW could not be

used to determine whether to use fresh or preserved samples. The characteristics evaluated for samples of PME, RW and a mixture of both (1:1) included pH, TS, TSS, TOC, COD, AOX, turbidity, particle size distributions, color, conductivity and absorbance at 280 nm and 340 nm. From Appendix A it was found fresh samples are preferred based on the results of TC, TOC and color; preserved samples are preferred based on the results of COD and AOX results; but no differences in terms of using fresh and preserved samples based on the results of TS, TSS, turbidity, conductivity, absorbance and pH. Therefore, it is difficult to choose the better sampling method based on the above analysis.

However, results for variation of characteristics of samples of a mixture of PME and RW (1:1 by volume) shown in Appendix B, and results from flocculation of samples of a mixture of PME and RW (1:1 by volume) shown in Appendix C and Table 10 suggest that preserved samples ($\leq$ 11 days) were as valid as daily fresh samples when the maximum age was 11 days. The conclusion may still be effective for sample age greater than 11 days, but it was not evaluated in this study.

Based on the above analysis, the most cost-effective sampling methodology was used in this study. Preserved samples were used for the preliminary study without significant biological reactions, but all the important experiments were replicated using daily fresh samples. Using fresh samples was essential for any experiments that included biological reactions, but it was more expensive considering the distance from the mill site. Using preserved samples from one batch eliminated the daily variation of sample from sites, but it was not appropriate for studies that involved significant biological reactions. The most cost-effective and reliable approach was to use preserved samples

for preliminary tests, and to use fresh samples for replicates and final data collection.

Table 10. The average and standard deviation of TRE and PRE for fresh and preserved samples using grid and jar mixing apparatuses

	characteristics	sample status	time[1] (h)	average (%)	stdev[2]
grid mixing	TRE	fresh	0.5	27.62	7.77
			1.0	34.34	9.58
		preserved	0.5	32.05	10.80
			1.0	40.90	10.20
	PRE	fresh	0.5	20.28	10.04
			1.0	30.94	10.52
		preserved	0.5	23.64	14.64
			1.0	24.82	5.46
paddle mixing	TRE	fresh	0.5	27.50	6.44
			1.0	35.78	7.64
		preserved	0.5	30.47	6.15
			1.0	40.18	8.05
	PRE	fresh	0.5	18.18	9.12
			1.0	24.92	8.04
		preserved	0.5	20.62	10.12
			1.0	24.30	8.80

1. time = flocculation time. 2. stdev = standard deviation

4.3.2 Temperature

Effect of temperature on flocculation was evaluated, as temperature is one of the significant water pollutant indicators. Flocculation experiments were conducted at 2 to 30°C using samples of a mixture of PME and RW (1:1 by volume). Samples were collected from different sites at different seasons of the year to eliminate the effect of temporal and spatial variations. The same experiments were replicated several times to ensure that the results were accurate. The temperature was controlled using either a cold room or a hot water bath. After reviewing the experimental results, similar trends were observed from flocculation curves using samples from different mills or seasons. An example of the curves is provided in Figure 22, which shows that temperature has an effect on flocculation; the lowest flocculation efficiency occurred at 4 to 5°C, and the highest occurred at approximately 15°C (from 12 to 18°C).

Figure 23 shows that temperature has no effect on the pH of flocculated samples. Figure 24 shows slight increases in absorbance at 280 nm (not at 340 nm) when temperature was increased (absorbance values at different wavelengths for samples of a mixture of PME and RW with volume ratio of 1:1 are presented in Figure 25). Figure 26 reveals slight increases in conductivity when temperature was increased. However, it was found from Figure 24 and Figure 26 that absorbance and conductivity were not affected significantly by flocculation time.

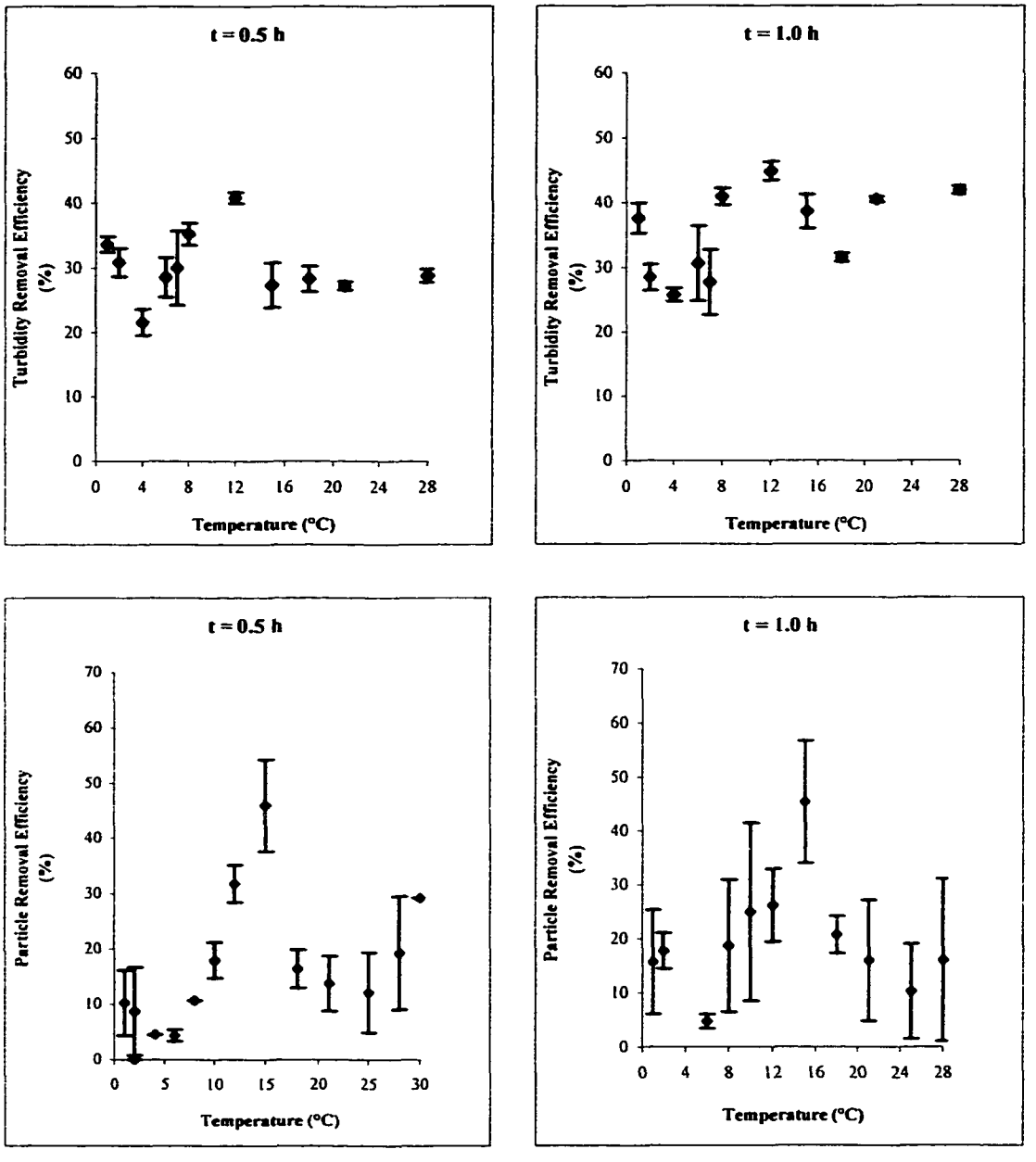


Figure 22. Effect of temperature on PMEICF (samples were a mixture of Weyerhaeuser PME and Wapiti RW at 1:1 by volume, August 1999)

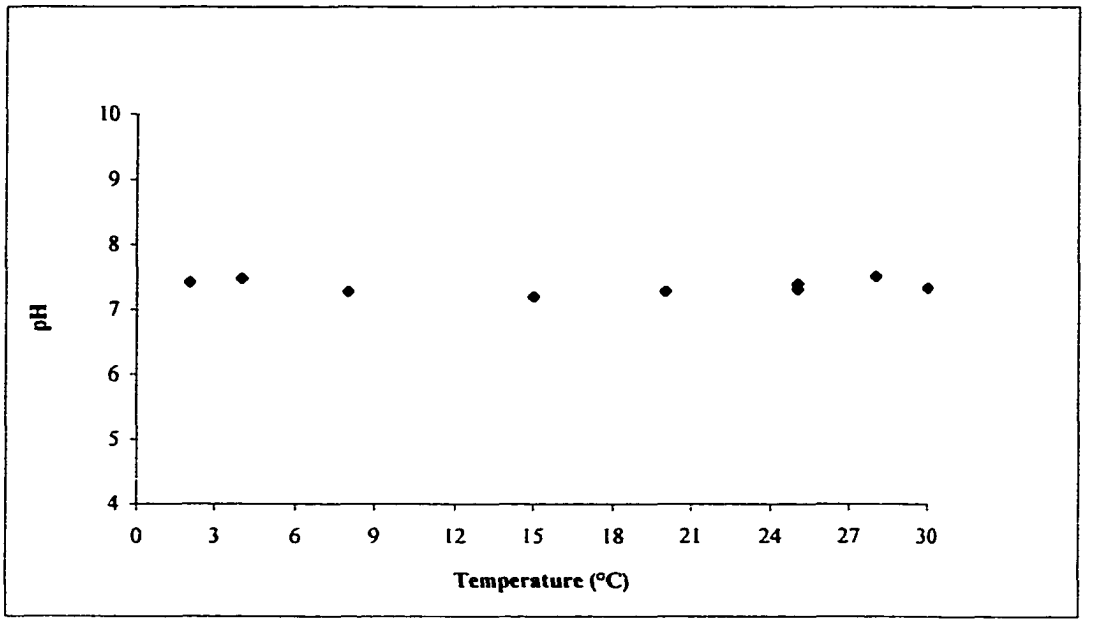


Figure 23. Effect of temperature on pH for summer samples [samples consisted of a mixture of PME and RW (all were 1 day old) with a volume ratio of 1:1, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{Gf} = 13\ s^{-1}$]

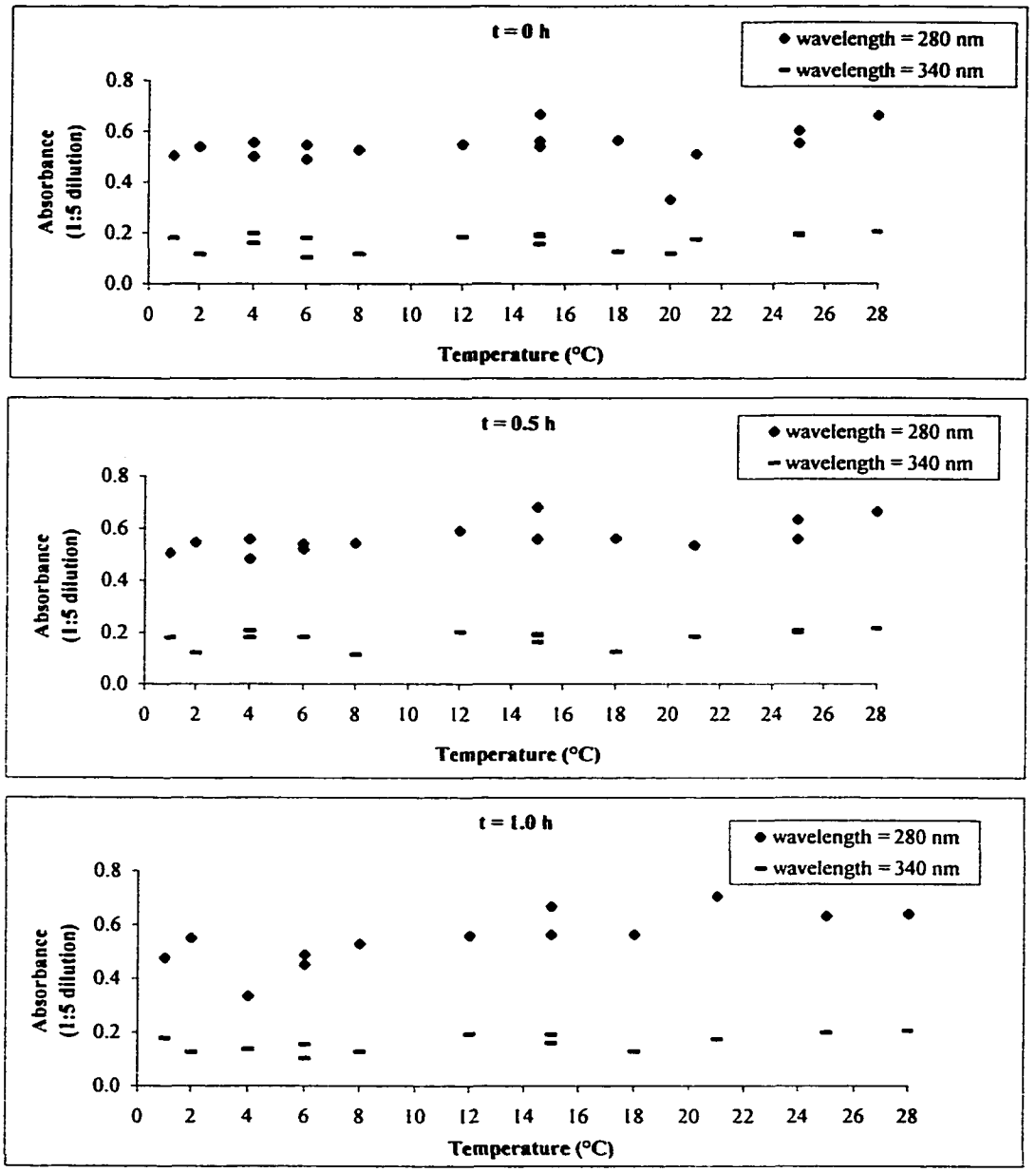


Figure 24. Effect of temperature on absorbance (samples were a mixture of Weyerhaeuser PME and Wapiti RW at 1:1 by volume, August 1999)

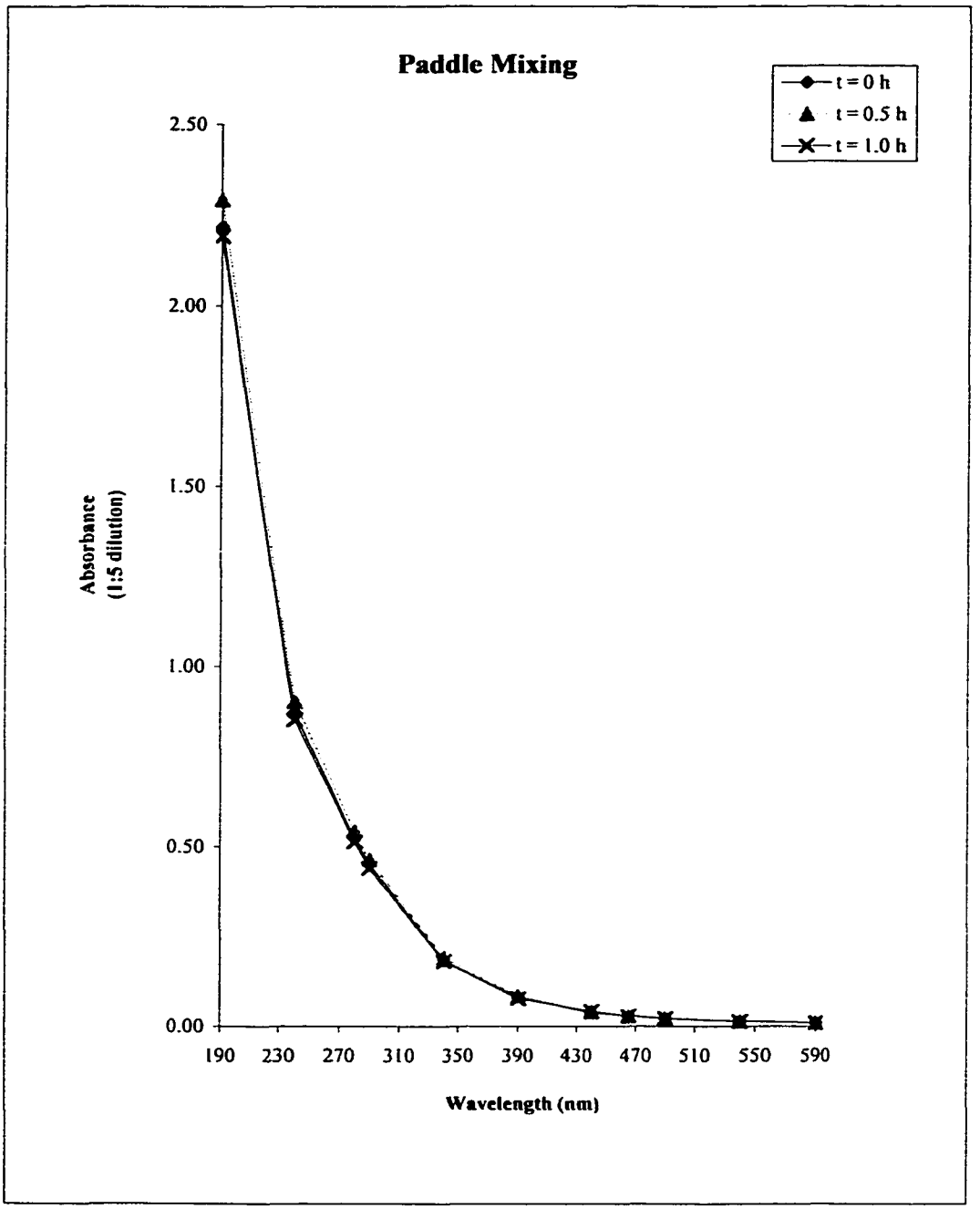


Figure 25. Absorbance at different wavelengths for mixture of PME and RW (1:1 volume ratio), mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{Gf} = 13\ s^{-1}$

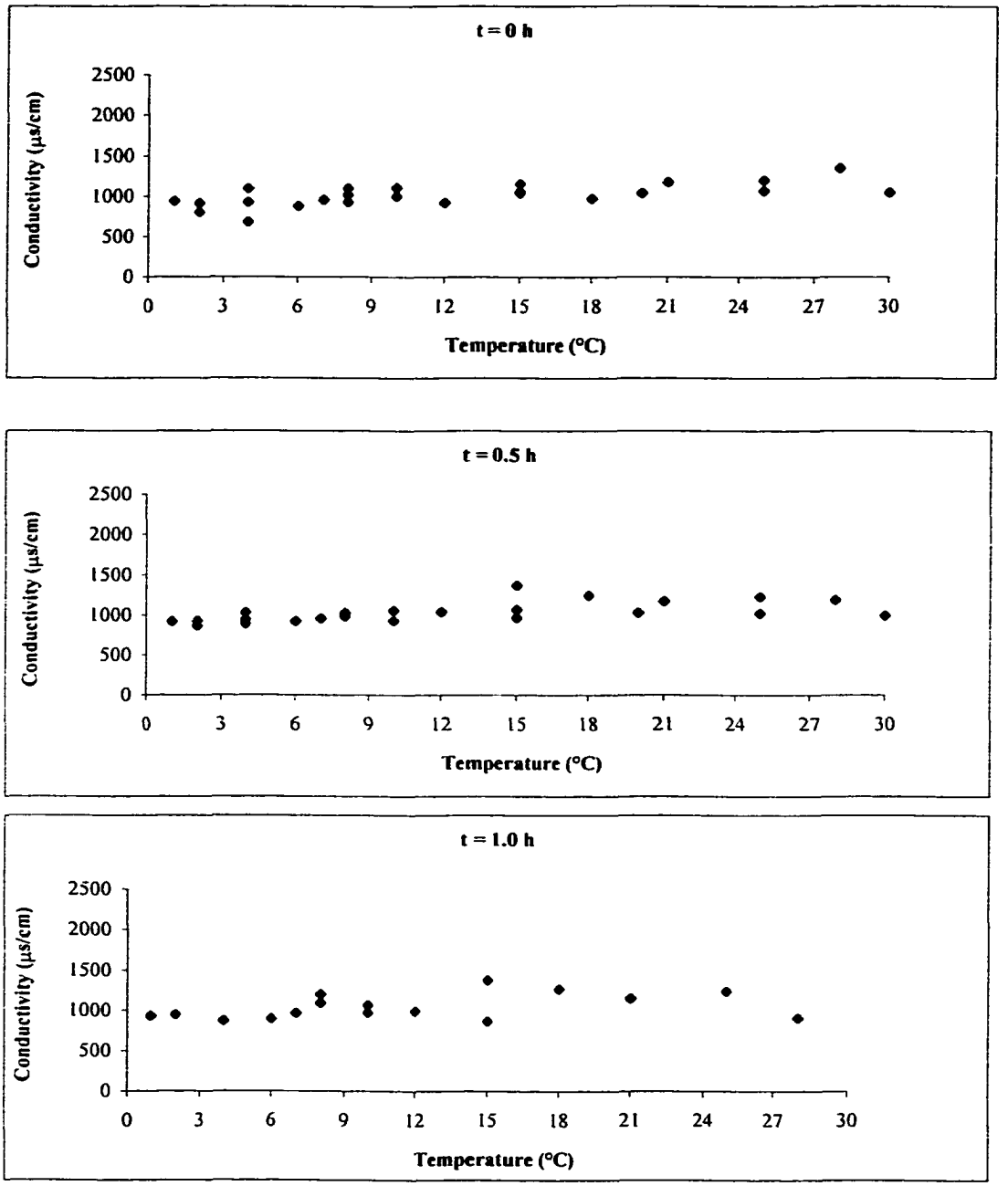


Figure 26. Effect of temperature on conductivity (samples were a mixture of Weyerhaeuser PME and Wapiti RW at 1:1 by volume, August 1999)

Figure 27 shows that temperature affects the flocculation rate (k) and the ultimate flocculation efficiency (L_o), but trends are not clear (where L_o and k are determined from Equation 2). Inconsistent k values make it difficult to reach conclusions. The values for L_o, obtained using samples from Weldwood PME and Athabasca RW, suggested that the minimum L_o values reached at 4 to 5 °C, and the highest values occurred at approximately 15°C. This is consistent with the previous findings based on flocculation curve analyses. When samples of a mixture of Weyerhaeuser PME and Wapiti RW were used, L_o consistently increases from 2 to 30 °C. The inconsistent effects of temperature on L_o due to samples from different locations were not clear. However, most of experimental results suggest that the lowest degree of flocculation occurred at 4 to 5 °C and the highest degree of flocculation occurred at approximately 15°C (ranged from 12 to 18°C). At 4 to 5°C, the solubility of chemicals was low, and the density and viscosity of samples reached the higher value (which reduced mobility of ions and particles), causing low degree and rate of flocculation and small sized flocs. At higher temperature, the rate of flocculation is high, but DO may have been low. This is because the elevated water temperatures reduce the oxygen saturation capacity of water, and microorganisms, higher level of aquatic organisms and plants were actively respiring. Low DO may affect microbial activities and EPS production, and affect flocculation. Therefore, the combined effects show that there existed the optimal temperature for flocculation of RW. However, the optimal value was determined by many factors. Most of the experimental results in this study pointed out that it occurred at approximately 15°C (ranged from 12 to 18°C).

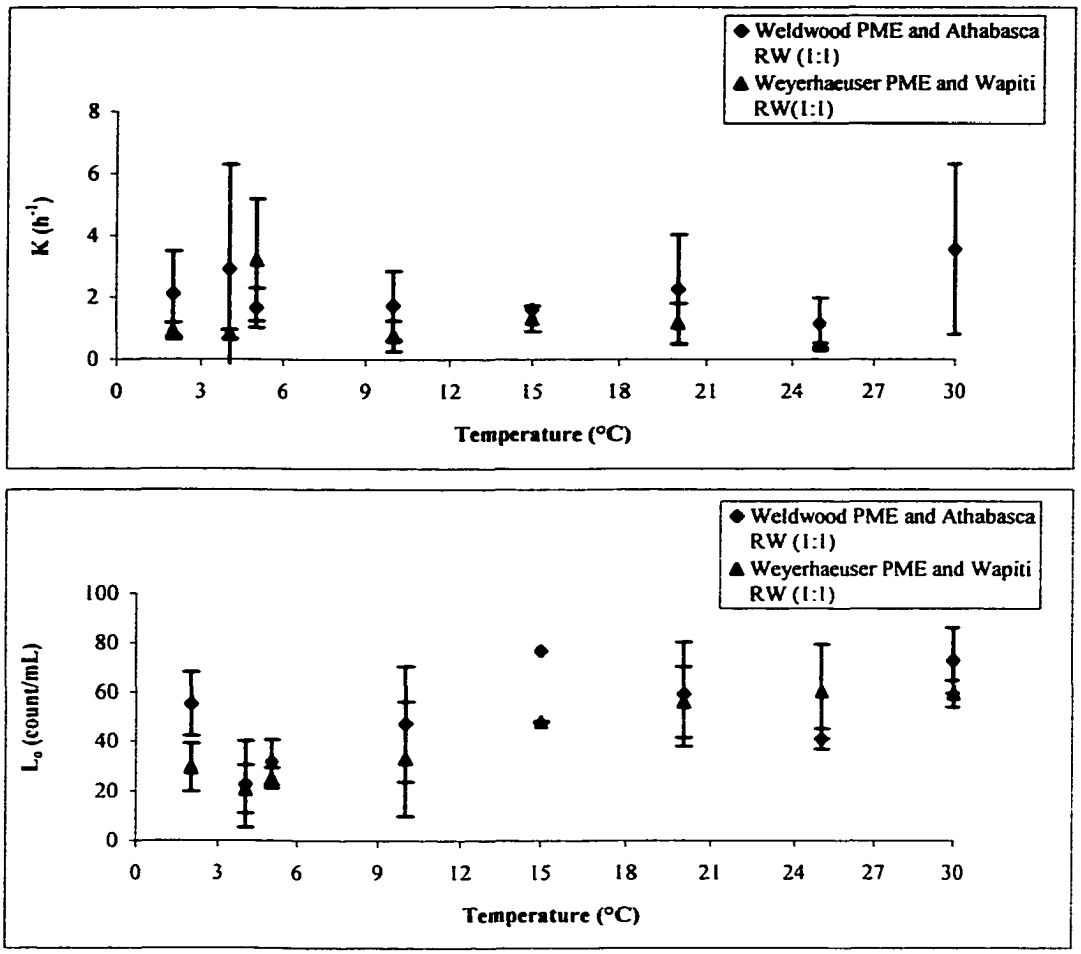


Figure 27. Effect of temperature on the rate and ultimate degree of flocculation (Samples were a mixture of Weyerhaeuser PME and Wapiti RW at 1:1 by volume, August 1999)

4.3.3 Mixing

Mixing determines chemical dispersion rate, the kinetics and degree of flocculation, floc size distributions and the number of deposited flocs. Floc formation depends on the eddy size that is responsible for inter-particle contacts. As found in the laboratory, critical mixing intensity existed, beyond which significant floc break-up occurred (Young *et al.*, 2000). As found in the field, mixing is determined by river hydraulic conditions, which undergo significant seasonal variations. Higher flow when the ice melts in the early spring provides more mixing. Higher mixing promotes floc formation, but it may also break up the floc. In addition, higher mixing may flush away or resuspend the deposited sediment flocs, which remain at the river bottoms during winter under low flow conditions. Therefore, the effect of mixing on flocculation in RW is a complicated issue. Extensive investigation of its effect is beyond the scope of this study.

Provided is the effect of mixing on PMEICF in the laboratory set-up using samples of a mixture of PME and RW (1:1 by volume). The samples were daily fresh summer samples collected from the Wapiti River and Weyerhaeuser pulp mill at Grande Prairie. The purpose was to provide perspective on the effect of mixing on experimental results obtained at the same laboratory set-up. The fast mixing rate of $\overline{G}_c$ was used to simulate the hydraulic condition near the pulp mill outfall, and slow mixing rate $\overline{G_f}$ was used to simulate the hydraulic condition downstream of the pulp mill outfall. However, it should be pointed out that the values of $\overline{G}_c$ and $\overline{G_f}$ used in most of flocculation tests conducted in this study were not determined based on the above hydraulic conditions. It

was determined in the laboratory, based on the maximum formation of flocs at the minimum energy input. In the future study, it is suggested to relate $\overline{G_c}$ to the hydraulic condition near the pulp mill outfall and $\overline{G_f}$ to the hydraulic condition downstream of the pulp mill outfall.

Figure 28 shows that TRE increased when the mixing rate was increased. However, the same trend was not clearly evident from PRE curves shown in Figure 29 primarily because of the instability of the particle size analyzer after instrumentation breakdown and re-calibration. As shown in Figure 28, the TRE at 13 s^{-1} used throughout the flocculation tests falls into the middle value for the TRE range. However, it may be more useful to relate the mixing rates used in flocculation tests to hydraulic conditions of pulp mill outfall and downstream of the outfall in rivers.

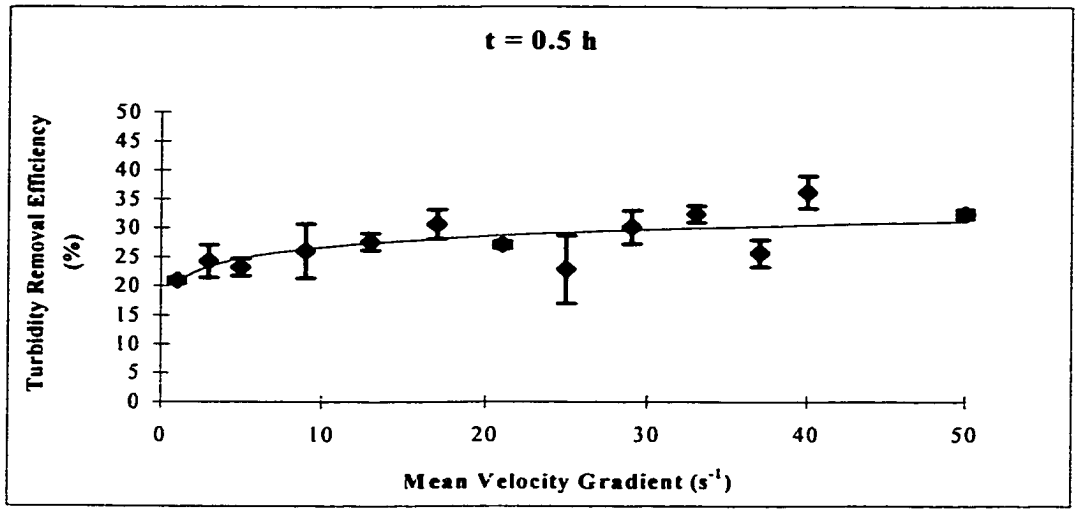


Figure 28. Effect of mixing on PMEICF (samples were a mixture of PME and RW (1:1 by volume, August 1999)

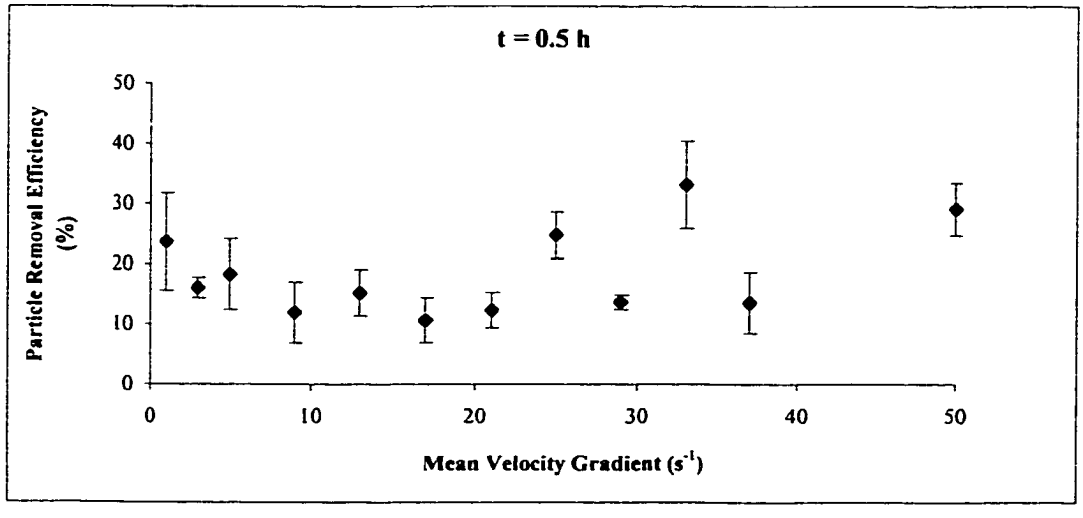


Figure 29. Effect of mixing on particle removal efficiency for summer samples [samples consisted of a mixture of PME and RW (all were 1 day old) with a volume ratio of 1:1, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{Gf}$ up to $50\ s^{-1}$]

4.3.4 Dilution

Dilution affects PMEICF by affecting the concentration of chemicals and particles. The purpose of the study was to determine whether the volume ratio of 1:1 (PME:RW) used for preparing samples for flocculation tests was representative. Figure 30 shows upward trends for TRE when the dilution increased (more RW), and the curve tend to reach a plateau at 1:5 (PME:RW). It was found from Figure 30 that the higher the dilution (but less than 1:5), the greater the TRE. This is attributed to elutriation. Figure 30 also presents that TRE at 1:1 (by volume) falls into the middle value of the range. Therefore, samples of a mixture of PME and RW with volume ratio of 1:1 were considered to be representative, and used throughout the study. Previous research found that more flocs were evident at 1:1 by volume (Evans, 1996).

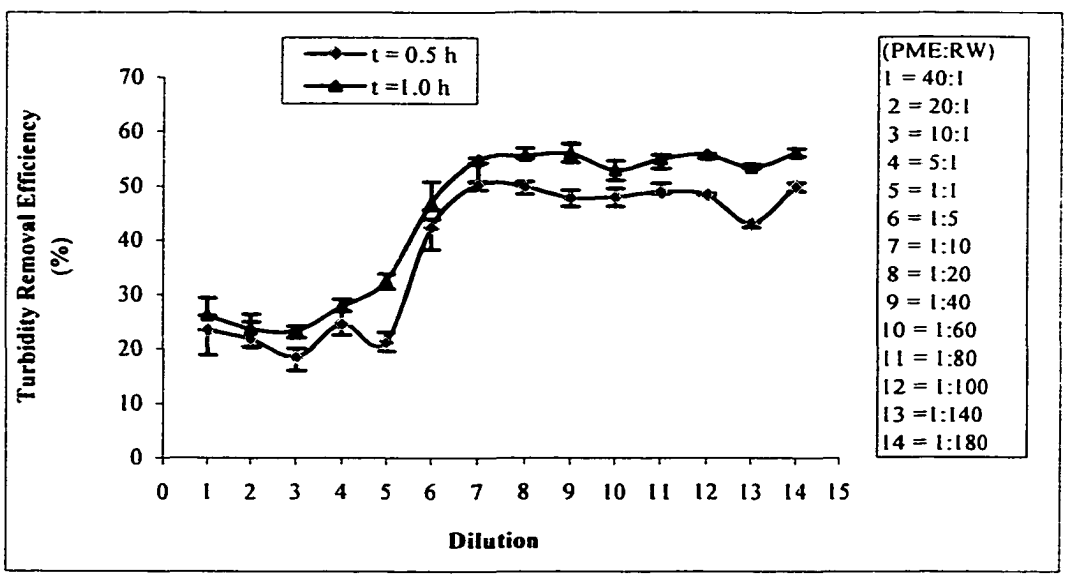


Figure 30. Effect of dilution on PMEICF (Weyerhaeuser PME and Wapiti RW, August 1999)

4.4 Effect of Chemical Factors on PMEICF Formation

Chemical factors evaluated included pH, alkalinity, isolated PME chemicals and fractionated PME.

4.4.1 pH

The pH depends on the concentration of OH^-, H^+ and carbonate species. Changes in pH can affect the nature of ionic species themselves, sorption sites, polymer configuration, sweep-floc domain, the occurrence of precipitation and complexation reactions. All of these are ecosystem-specific.

The effect was evaluated using both samples of PME and samples of a mixture of PME and RW at 1:1 by volume. Using samples of PME, Figure 31 reveals that total solids in flocculated PME (the top was supernatant and the bottom was sludge) increase when pH increases, but the total suspended solids only increase slightly. This indicates that the increase of total solids was attributed to the increase in total dissolved solids. This may be due to more dissolved compounds were adsorbed onto the surface of collodial particles when pH increased. Using samples of a mixture of PME and RW at 1:1 by volume, TRE and PRE increased when pH increased. Figure 32 shows that TRE obtained from different flocculation times slightly increased when pH increased, but significant increases in PRE were evident after samples were flocculated for 1 h.

Therefore, it was concluded that total dissolved solids, TRE and PRE increased when pH increased from 6 to 10. It is necessary to point out that pH in RW undergoes diurnal and seasonal variations. In the summer, the organisms produce higher pH values during the day, but lower values at night and when photosynthetic activity was low.

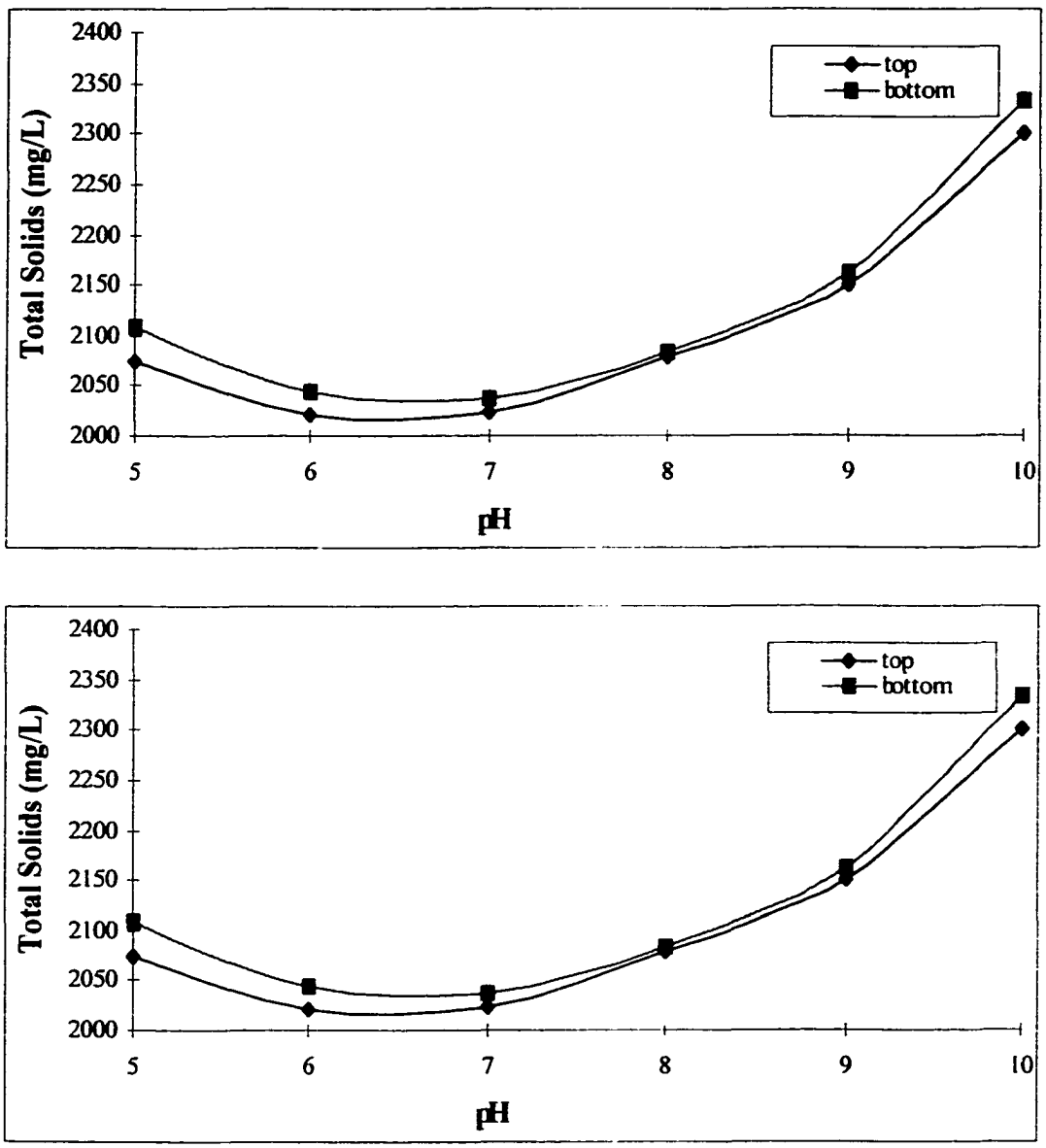


Figure 31. Effect of pH on solid concentrations of flocculated PME samples (Dec. 1998), which were flocculated for 73 hours at $\overline{G_f} = 0.54\ s^{-1}$ (2 rpm)

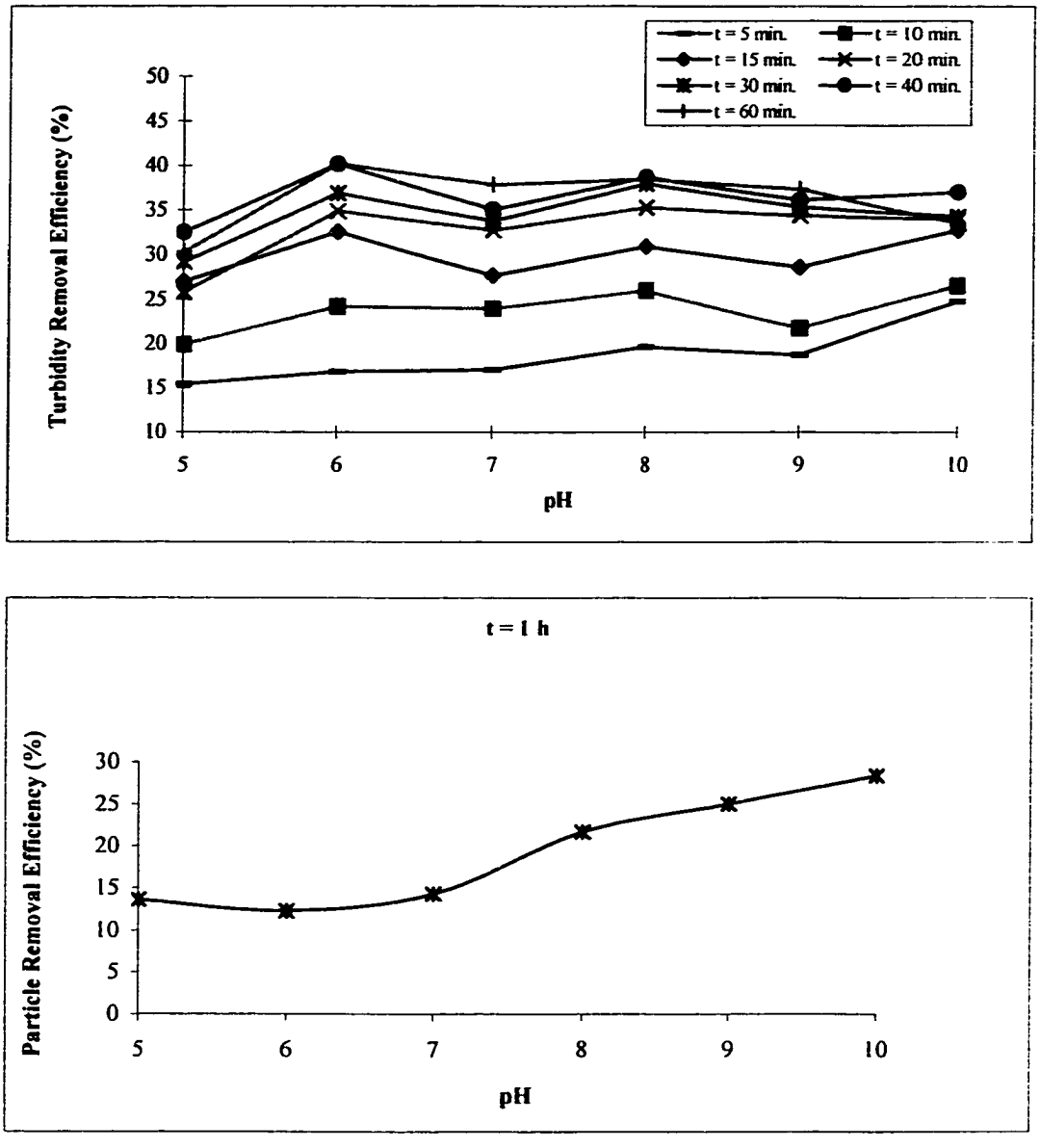


Figure 32. Effect of pH on PMEICF. Samples of a mixture of PME and RW (1:1 by volume) were flocculated for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 1 hour at $\overline{Gf} = 13\ s^{-1}$, August 1999)

4.4.2 Alkalinity

Alkalinity is defined as the capacity for solutes to react with hydrogen ions (Hem, 1989). It is determined by aqueous HCO_3^- and CO_3^{2-}. Figure 33 show that alkalinity is directly proportional to pH. The effect of alkalinity on flocculation was evaluated using both samples of PME and samples of a mixture of PME and RW at 1:1 by volume.

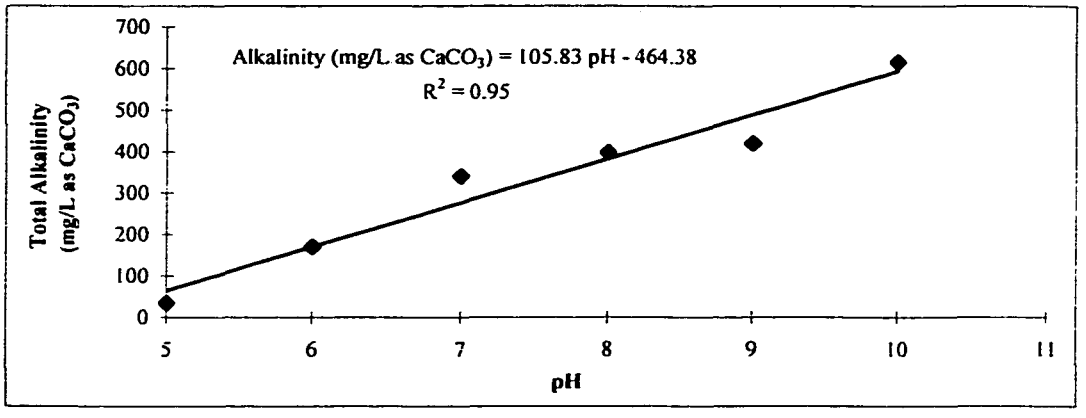
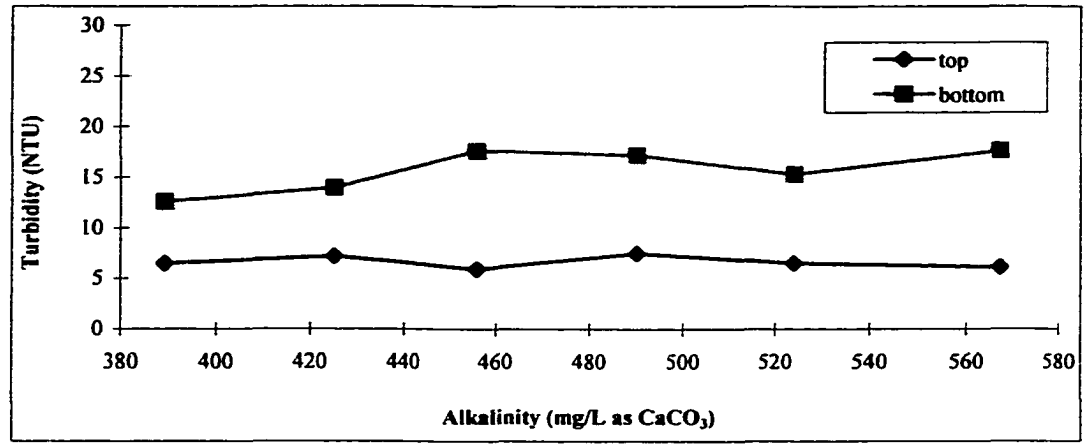


Figure 33. Correlation of pH and alkalinity for PME

Figure 34 reveals that total solids for PME increased when alkalinity increased in the range from 389 to 569 mg/L as $CaCO_3$, but not turbidity. Experimentation also found that alkalinity has insignificant effect on total suspended solids.



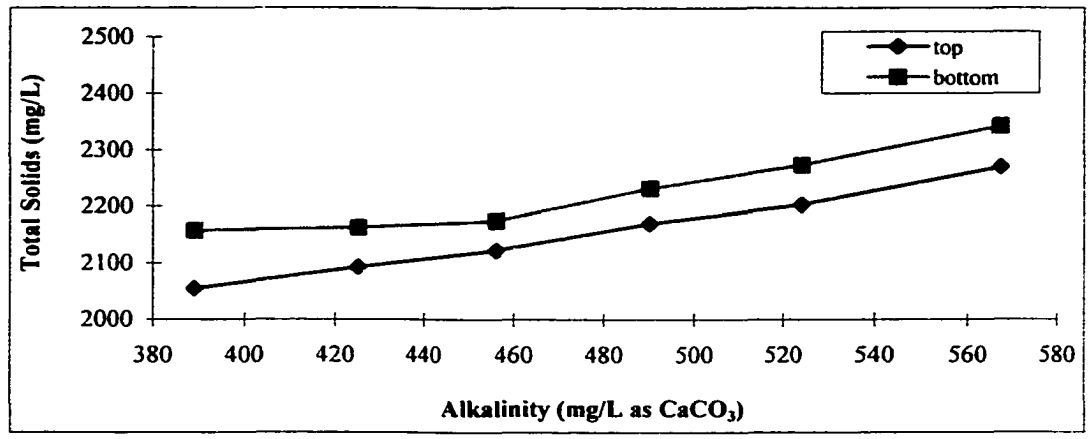
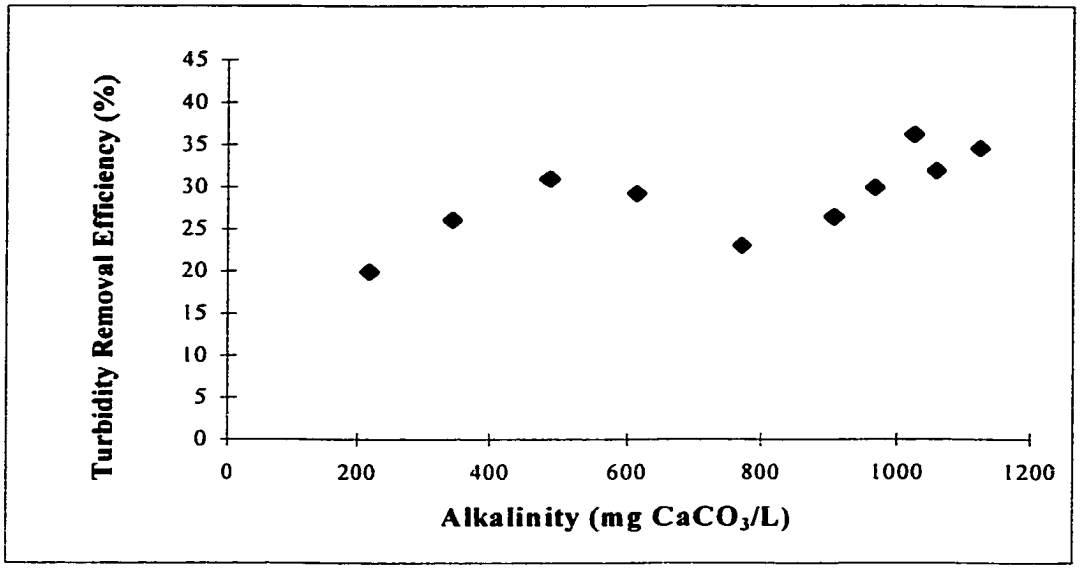


Figure 34. Effect of alkalinity on the total solids of Weyerhaeuser PME winter samples (mixed for 96 hours at $\overline{G_f} = 0.54\ s^{-1}$, or 2 rpm).

Note: 1). bottom stands for samples taken from ¼ of the suspension height.
2). top stands for samples taken from ¾ of the suspension height.

Figure 35 shows the upward trend of TRE for samples of a mixture of PME and RW at 1:1 (by volume), when alkalinity increased from 200 to 1200 mg $CaCO_3$/L. The maximum PRE was reached at 1023 mg $CaCO_3$/L. It should be noted that PRE results at alkalinity less than 600 mg $CaCO_3$/L were not available due to the instrument instability. The conclusion can be drawn, based on the above analysis, that alkalinity has an effect on turbidity, but its effect on PRE below 600 mg $CaCO_3$/L was not clear due to the inconsistent results from the particle counter.



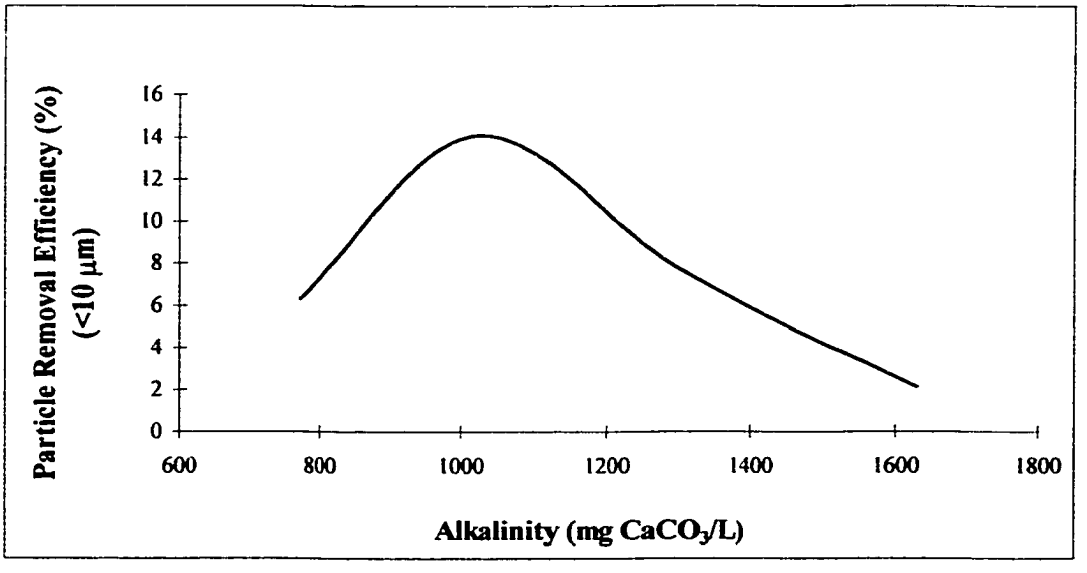


Figure 35 Effect of alkalinity on PMEICF (samples consisted of a mixture of PME and RW at 1:1 by volume, which were mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{Gf} = 13\ s^{-1}$, August, 1999)

4.4.3 PME Chemicals

Cations and organic polymers were the major PME chemicals evaluated.

4.4.3.1 Cations

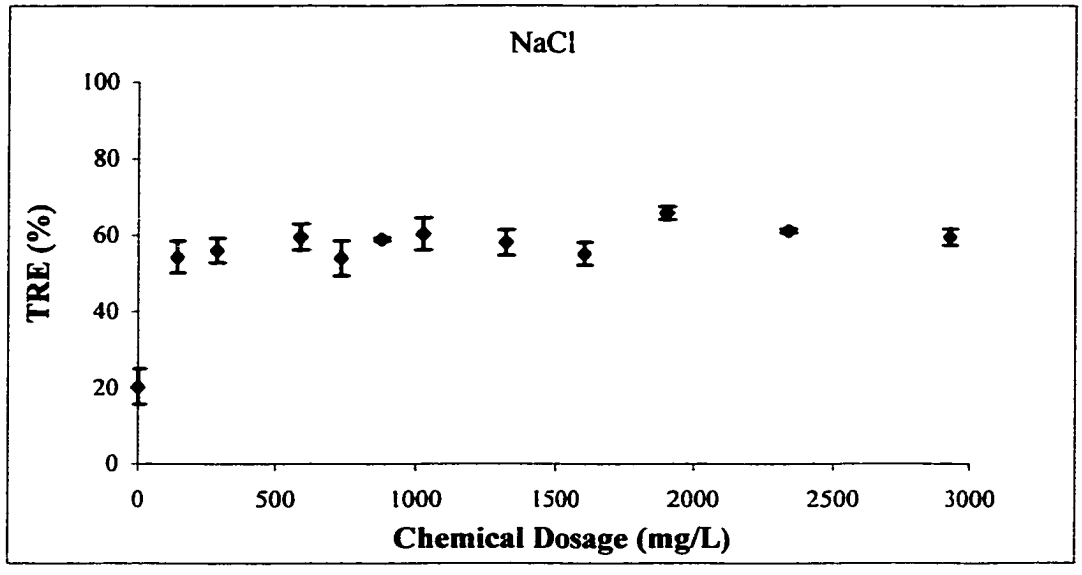
The major cations present in PME were identified by ICP-AES and ICP-MS. Table 11 demonstrates that Na^+, Mg^{2+}, Ca^{2+} and K^+ were present in Weyerhaeuser PME, but the other ions were found in extremely low concentrations. Therefore, only the effects on PMEICF of Na^+, Mg^{2+}, Ca^{2+} and K^+ were evaluated. The evaluation was conducted for a wide chemical dosage range to thoroughly understand their effect. However, the primary focus was on the lower dose range, especially the dosage closest to what was present in PME.

Table 11. Element composition of PME and RW (from ICP-AES and ICP-MS analyses, EPA 6010 analyzed by Maxxam Analytic Inc.)

Elemental Concentrations (mg/L), grab summer samples							
Unfiltered Samples (total concentrations)						Filtered Samples (dissolved concentrations)	
Name of Element	Wapiti RW (1998)	Weyerhaeuser PME (1998)	**Name of Element**	Wapiti RW (1998)	Weyerhaeuser PME (1998)	**Name of Element**	Wapiti RW (1999)
Aluminum (Al)	0.16	0.54	Zinc (Zn)	0.01	0.08	Calcium (Ca)	38.10
Chromium (Cr)	0.00	0.00	Phosphorus (P)	0.20	0.90	Magnesium (Mg)	9.70
Copper (Cu)	0.01	0.01	Barium (Ba)	0.08	0.33	Potassium (K)	1.10
Iron (Fe)	0.22	0.27	Silicon (Si)	1.51	2.87	Sodium (Na)	6.80
Magnesium (Mg)	7.20	11.70	Beryllium (Be)	0.00	0.00	Iron (Fe)	<0.01
Manganese (Mn)	0.00	0.58	Boron (B)	0.02	0.02	Manganese (Mn)	<0.001
Molybdenum (Mo)	0.00	0.00	Cadmium (Cd)	0.00	0.00	**Name of Element**	Weyerhaeuser Effluent (1999)
Potassium (K)	0.70	12.20	Calcium (Ca)	30.70	73.20	Calcium (Ca)	65.80
Sodium (Na)	3.70	346.00	Cobalt (Co)	0.00	0.00	Magnesium (Mg)	10.60
Strontium (Sr)	0.11	0.25	Lead (Pb)	0.01	0.00	Potassium (K)	18.30
Sulphur (S)	3.30	124.00	Nickel (Ni)	0.02	0.00	Sodium (Na)	440.00
Titanium (Ti)	0.00	0.00	Silver (Ag)	0.00	0.00	Iron (Fe)	0.36
Vanadium (V)	0.01	0.00	Uranium (U)	0.00	0.00	Manganese (Mn)	0.71

Flocculation curves were plotted for the effect of Na^+, Mg^{2+}, Ca^{2+} and K^+ at different dosages (Appendix D). After reviewing them it was found TRE curves reach asymptotic values when more than 2.5 meq/L (milli-equivalence/Litre) of cations were added. Thus, the discussion of chemical effect will be focussed on chemical dosages that are equal to or less than 2.5 meq/L. Figure 36 shows that all evaluated cations can induce flocculation at 2.5 meq/L, Na^+ has the strongest inducing ability and K^+ has a minimal effect. Na^+ from PME is the major contributor to flocculation, as 34% of increase in TRE was obtained when 146 mg/L of Na^+ was added, but the dosage effect did not increase beyond that when the Na^+ was added. In addition, the results from both the maximum increase in TRE and the F-value from ANOVA analysis showed that the ability to increase TRE ranks $Na^+ > Ca^{2+} > Mg^{2+} > K^+$, when the same equivalence of ions were added into the RW samples.

It was found that when the cation concentration in PME is higher than its critical concentration, PMEICF occurs (the critical concentration is defined as the minimum concentration beyond which the increase in TRE is statistically significant). Table 12 shows that Na^+ and Ca^{2+} concentrations in Weyerhaeuser PME were high enough to induce flocculation in RW, but K^+ present in PME was unable to induce flocculation due to its low concentration. The lowest Mg^{2+} dosage evaluated was 60 mg/L, TRE increases were statistically significant when $Mg^{2+} \geq 60$ mg/L. However, the Weyerhaeuser PME contained 10.6 mg/L of Mg^{2+}, the inducing ability at 10.6 mg/L was not evaluated, as this concentration was not available when the study was conducted.



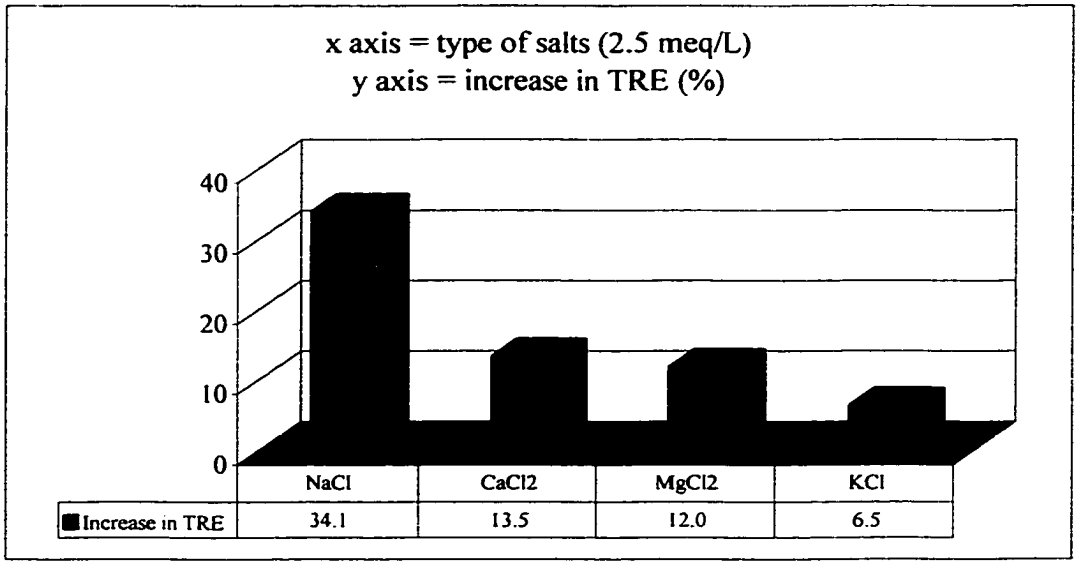


Figure 36. Effect of cations on flocculation in RW (samples were the Wapiti RW, August 1999)

Table 12. Cation concentrations in Weyerhaeuser PME and critical concentration for PMEICF formation

Cations	Concentration in PME (mg/L)	Critical concentration (mg/L)
Na^+	440	146
Ca^{2+}	65.8	40
Mg^{2+}	10.6	≤ 60
K^+	18.3	1560

In conclusion, all ions evaluated have the ability to induce turbidity reduction. However, at the concentrations found in raw PME, Na^+ and Ca^{2+} can induce flocculation, but K^+ can not (Mg^{2+} may, but this is uncertain). It is necessary to point out that all the evaluated cations were simultaneously present in PME, but the combined effect of the ions may not be additive. Moreover, when PME was discharged into the river, pulp mill chemicals were diluted due to mixing. The chemicals started to spread in longitudinal, transverse and vertical directions. Therefore, their concentrations changed temporally and spatially. In addition, seasonal variation of river flow significantly affected the concentration of cations in rivers. Higher ion concentrations were found in the winter when the river flow was low. This might assist in the formation of sediment flocs. Field survey has been found more deposited sediment flocs in the winter (Krishnappan *et al.*, 1995). This could be due to either higher degree of PMEICF or less flushing and resuspension of bottom sediments at a low flow observed in the winter. After careful consideration of the above impacts, the experimental findings are still meaningful. This is because the cations caused physio-chemical reactions, which occurred immediately (at this time frame PME was not significantly diluted). This was demonstrated by the field observations in which more sediment flocs were found in the vicinity of the pulp mill outfall.

The possible mechanisms for cation-induced coagulation and flocculation are discussed below. Addition of Na^+ (or NaCl) increases ionic strength, compresses the diffuse layer surrounding the colloidal particles, and decreases zeta potential. This is a benefit for the adsorption of Ca^{2+} and Mg^{2+} onto the surface of colloidal particles, leading to charge netralization. Cations can also affect the configuration of polymer chains and the segment length of a polymer, which influence its bridging ability.

The above postulation is reinforced by laboratory and field observations. Immediate formation of pinpoint flocs in the laboratory as soon as the ions were added into RW samples indicated that coagulation occurred. The laboratory findings are consistent with field observations from surveys conducted in 1998 on the Wapiti River at Grande Prairie and the Athabasca River near Hinton, Alberta, Canada, which received the discharge of PME treated by aerated stabilisation basins. More sediment flocs were evident in the vicinity of pulp mill outfalls, but diminished further downstream.

4.4.3.2 Organic Polymers

The possibility of inducing flocculation in RW by organic polymers was evaluated. Polymers were chosen based on the nature and properties of pulp mill chemicals. Polymers evaluated were fibers, hydrolytic lignin, protein, sucrose, cellulose and starch. Polymer was added into samples either alone or in conjunction with inorganic salt. As only a limited amount of protein was available at a very high cost, only the effect of protein in conjunction with inorganic salt was evaluated under the assumption that protein induces flocculation through the bridging mechanism. Flocculation curves are presented in Appendix D.

Fibers can induce flocculation in RW. PME contains a large quantity of fibers. The effect of fibers on PMEICF was evaluated for a wide dose range. The fiber surface was treated under different pH levels to simulate fibers isolated from the chlorine dioxide oxidation stage of the bleaching process (pH = 2), the final combined effluent (pH = 7.6) and the alkali extraction stage of the bleaching process (pH = 12). Figure 37 demonstrates that fibers can enhance flocculation at all tested dosages. Without NaCl addition, fibers treated at different pH levels enhanced flocculation to different extents. A maximum increase in TRE of 22 to 23% was obtained for fibers treated at pH = 7.6 or pH = 2, but only 12% was obtained for fibers treated at pH = 12. When 585 mg/L NaCl was added in conjunction with fibers, the fibers treated at different pH levels enhanced flocculation to similar extent at most chemical dosages. The highest maximum increase in TRE (up to 40%) occurred for fibers treated at pH = 2, but the increase in TRE was only 25 to 26% for fibers treated at pH = 7.6 or pH = 12. This shows that salt addition was more beneficial for flocculation when fibers were treated at pH = 2.

A hypothesis to explain the mechanism for fiber enhanced flocculation is that fibers provide surface area for particle attachment. They function as bridges, bind particles together and form flocs. Figure 38 shows the ultrastructure of flocs formed downstream of the Weyerhaeuser pulp mill outfall in the Wapiti River. It confirms that fibers were involved in the formation of flocs by showing particles clinging to the surface of the fiber. This finding reinforced observations made by other researchers (Carson and Hudson, 1997; Kankaanpaa *et al.*, 1997; Krishnappan *et al.*, 1998).

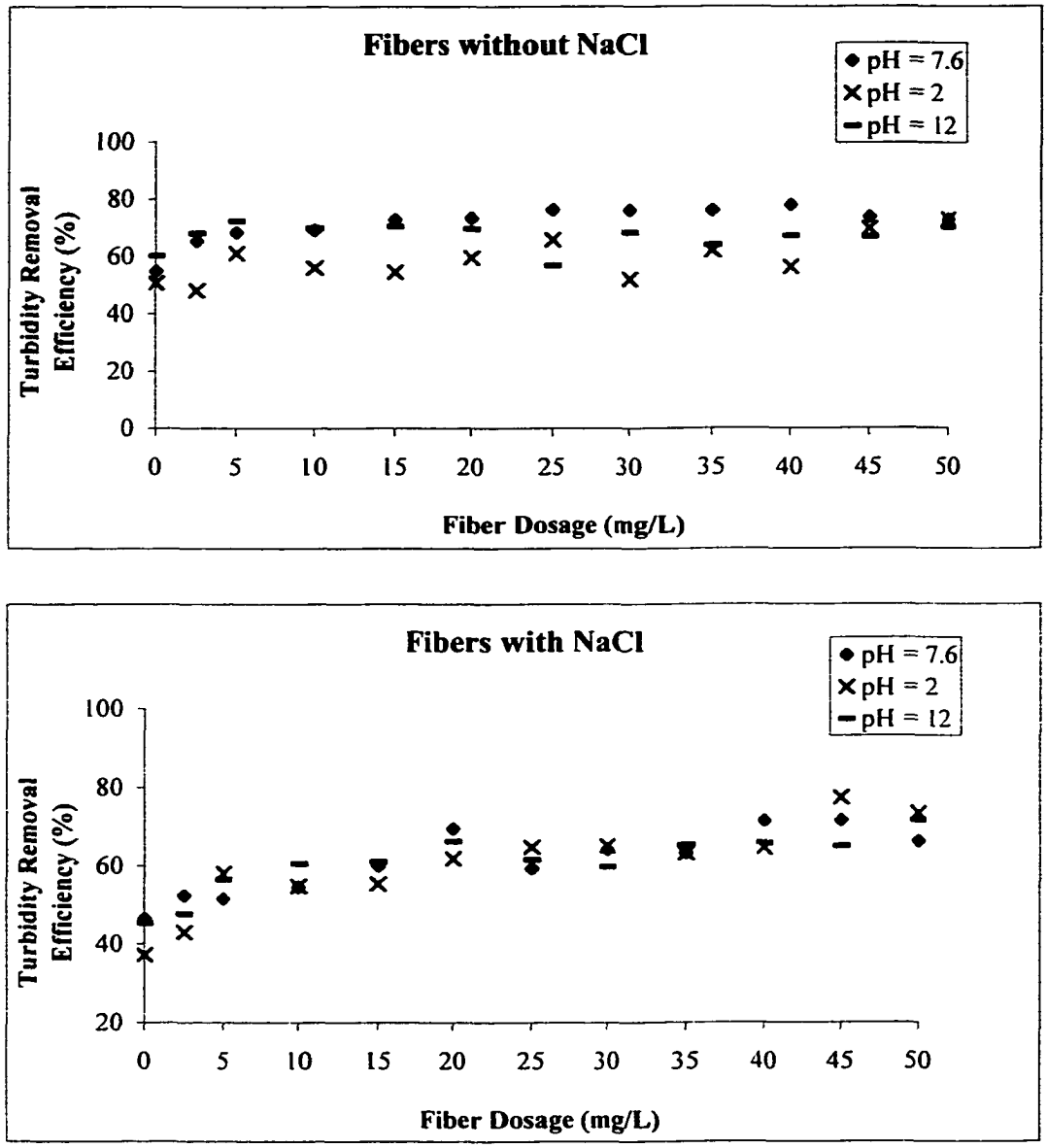
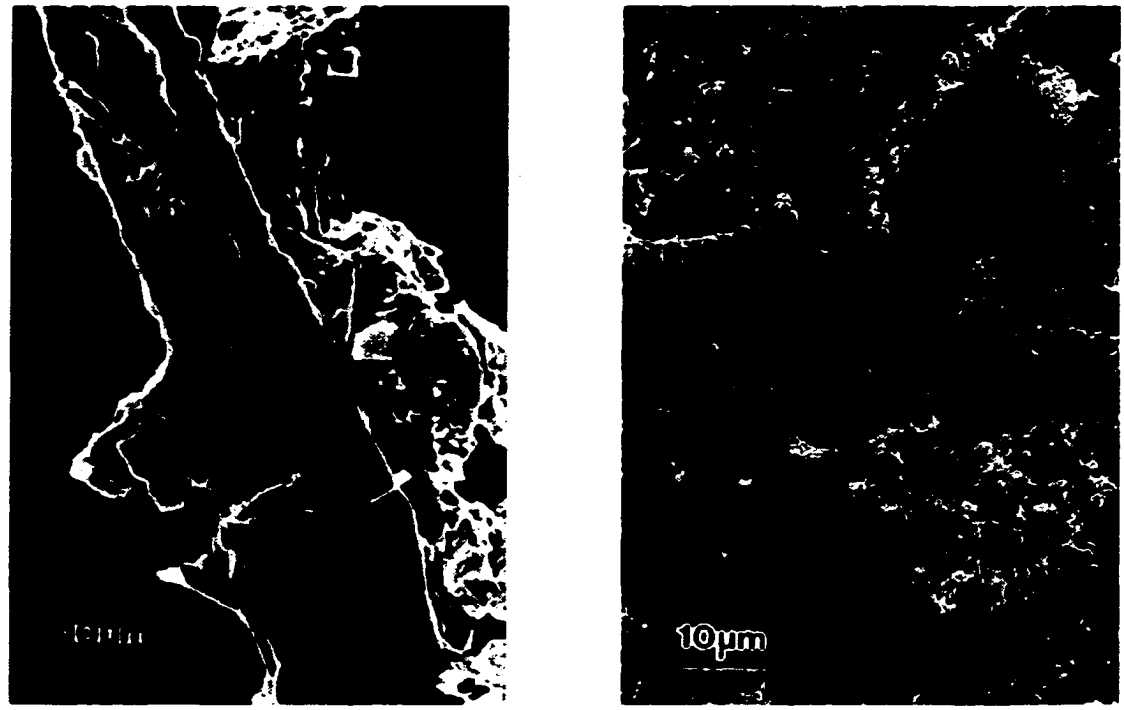


Figure 37. Effect of fibers on flocculation in RW (NaCl addition was 585 mg/L or 20 mL 1N NaCl was added into a 1.98L RW sample)



(a) (b)

Figure 38. SEM images of the ultrastructure of deposited sediment flocs collected from 500 m downstream of the Weyerhaeuser pulp mill outfall in the Wapiti River at Grande Prairie.

Hydrolytic lignin can induce flocculation, but to a lesser extent. Lignin derived polymers are the major pollutants in PME. They are among the most resistant to microbial degradation (Kringstad and Lindström, 1984; Santos and Duarte, 1998), and are abundant biopolymers in vascular plants (25 to 30 wt % of wood) (Louchouarn *et al.*, 1997). The effect of hydrolytic lignin on flocculation was examined. Figure 39 shows that hydrolytic lignin only slightly (though ANOVA analysis and t-tests proved statistically significant) enhanced flocculation at certain dosages, but to a slightly greater extent when inorganic salts such as $CaCl_2$ were added. The t-test results suggest that the

increase in TRE was statistically significant when 20 mg/L lignin was used (with or without salt). Lignin induced flocculation, as TRE increased after the addition of lignin (in comparison to that of no lignin addition). The maximum increases in TRE were 5.2% with the addition of 20 mg/L of lignin suspension, and 6.5% with the addition of 30 mg/L of lignin suspension in conjunction with 555 mg/L of $CaCl_2$ solution. It was found that absorbance at λ = 280 nm decreased after flocculation. This reinforces that lignin induced flocculation [absorbance of lignin compounds peaks around 280 nm, although many other organic compounds with aromatic structures, conjugated or non-conjugated double bonds in compounds such as wood extractives, hemi-cellulose, and carbohydrates also contributed to the absorbance at λ = 280 nm (Williams and Fleming, 1973). In conclusion, hydrolytic lignin can induce flocculation in RW, but to a lesser extent than other organic polymer evaluated.

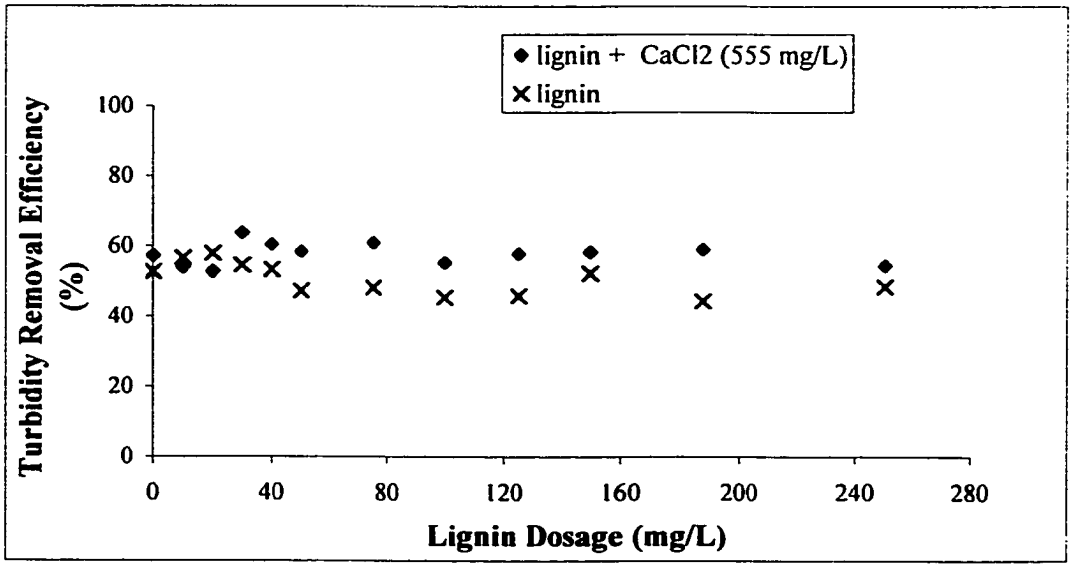


Figure 39. Effect of hydrolytic lignin on flocculation in RW ($CaCl_2$ addition was 585 mg/L or 20 mL 1N $CaCl_2$ was added into a 1.98L sample)

The mechanism for lignin related flocculation has been explained (Sjöström, 1981; Springer, 1993). Lignin is formed by removing water from sugars to create aromatic structures. The OH^- groups in lignin (either the alcohol OH's on the chains or the phenolic OH's on the aromatic rings) can react with each other or with the aldehyde or ketone groups, and form heavy cross-linked, large, three-dimensional lignin molecules. Hydrophobic lignin provides more surface area for suspended sediments to adsorb onto (McCarthy, 1994; McCarthy *et al.*, 1997). Lignin exploits the strength of cellulose and gives flexibility, it may also function as a bridge binding particles together to form flocs. In addition, it may form hydrogen bonds or react with cations (Lindstrom *et al.*, 1974; Louchouarn *et al.*, 1997; Orsa and Holmbom, 1994), which facilitates the lignin bridging mechanism and enhances the floc formation. However, it should be kept in mind that lignins also undergo degradation by fungi (especially white-rot fungi) under aerobic or anaerobic conditions, although they are more refractory than polysaccharides or amino-acids (Louchouarn *et al.*, 1997).

Figure 40 shows that protein, sucrose, cellulose and starch also have the ability to induce flocculation in RW. However, it should be pointed out that protein (with $CaCl_2$) induced flocculation only at 14 mg/L (the increase in TRE is statistically significant), but not at other dosages. The protein-induced flocculation (without salt) was not tested due to the high price of the protein sample.

Figure 39 and Figure 40 show that all tested polymers have ability to increase TRE (as proven by ANOVA analysis), but to different extents. When polymer was used alone the maximum increase in TRE ranks: 28.3% (cellulose) > 22 to 23% (fibers treated at pH = 7.6 or pH = 2) > 12.5% (starch) > 12.1% (fibers surface treated at pH = 12) >

11.9% (sucrose) > 5.2% (hydrolytic lignin). When polymer was used with inorganic salt, the maximum increase in TRE (statistically significant) ranks: 25 to 26% (fibers were surface treated at pH = 7.6 and pH = 12) > 17.7% (starch + NaCl) > 11.2% (cellulose + NaCl) > 6.5% (hydrolytic lignin + $CaCl_2$) > 6% (sucrose + NaCl) > 3.2% (protein + $CaCl_2$). The results show no clear relationship in terms of the effect of salt on the maximum increase in TRE. This is because salt has antagonistic effects on PMEICF when it was used with polymer. It can enhance coagulation, but can also hinder flocculation by affecting the steric structure of the polymer, which may reduce the polymers bridging ability. The net effect was determined by the concentration of salt and polymer, and polymer structure and property.

In conclusion, pulp mill chemicals were evaluated separately for the possibility of inducing flocculation in RW. The evaluation studies were conducted using standard jar tests, and TRE was used to characterize flocculation performance. t-tests and ANOVA analyses were used to ensure the reliability of the results. The experimental results show that Na^+, Ca^{2+}, fibers, hydrolytic lignin, protein, sucrose, cellulose and starch, which are associated with bleached Kraft PME, can induce flocculation in RW to different extents. This finding will assist in postulating the mechanisms for PMEICF formation, so that the transport models can be modified to include flocculation effect.

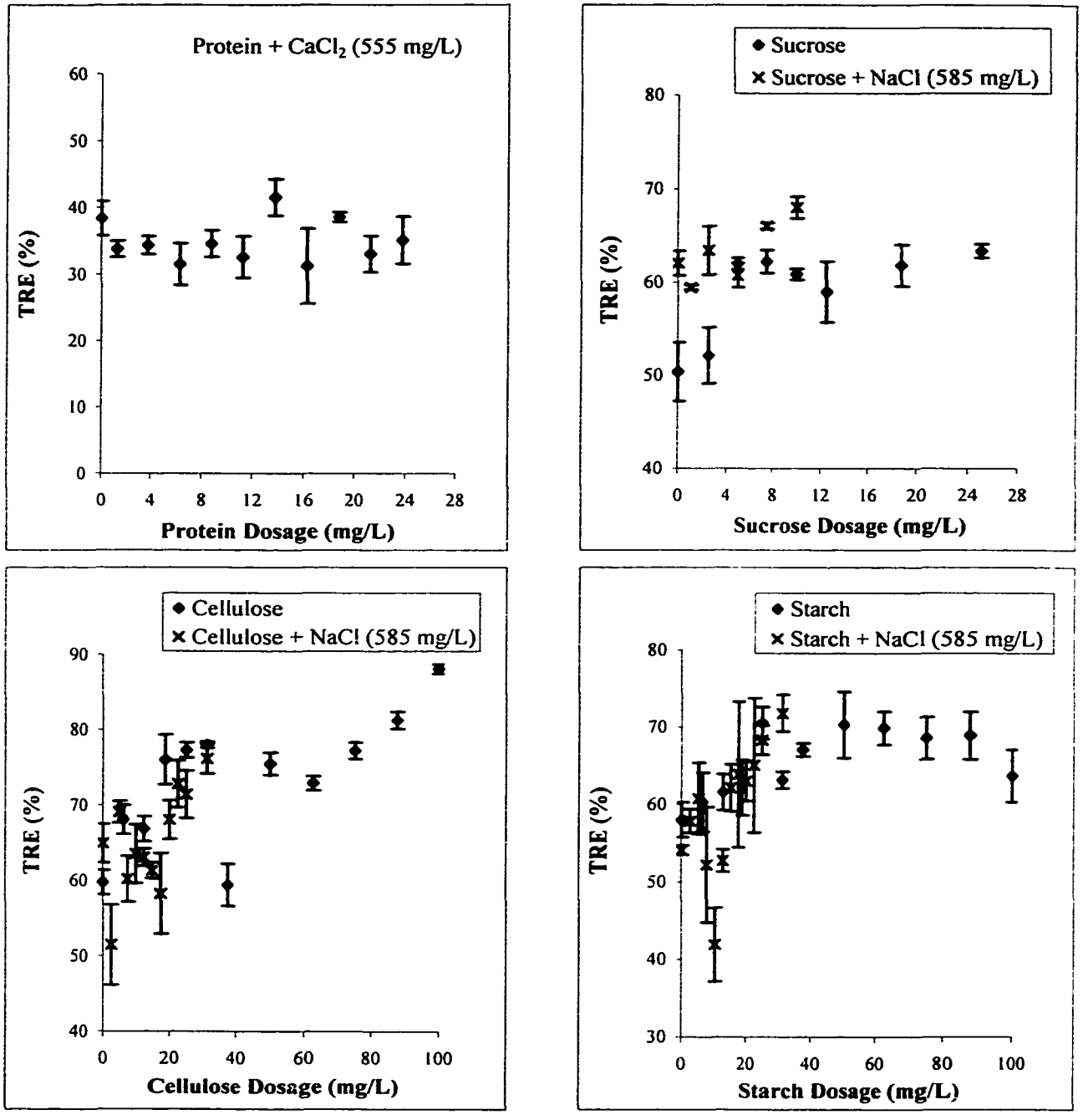


Figure 40. Effect of PME related polymers on flocculation in RW ($CaCl_2$ addition was 555 mg/L or 20 mL 1N $CaCl_2$ was added into a 1.98L RW sample; NaCl addition was 585 mg/L or 20 mL 1N NaCl was added into a 1.98L of sample)

4.4.4 Fractionated PME

As previous proven, PME can induce flocculation in RW. However, it is important to identify which portion of PME plays a more important role in PMEICF formation. This will assist in understanding mechanisms for PMEICF formation. The studies were carried out using flocculation tests by re-introducing fractionated PME into RW samples. Therefore, separation of PME and flocculation in RW by fractionated PME are discussed.

4.4.4.1 Size Fractionation by Ultrafiltration

Ultrafiltration was used to fractionate PME. Separation techniques that are often used are ultrafiltration, centrifugation and size exclusion chromatography (SEC). There have been several attempts to separate and size the organic compounds in PME using chromatographic methods (Connors *et al.*, 1980; Dixon *et al.*, 1992; Faix *et al.*, 1981; Forss and Stenlund, 1969; Jokela and Salkinoja-Salonen, 1992; Lewis *et al.*, 1983; Marchessault *et al.*, 1982; Saski *et al.*, 1997). However, fractionation of PME has mostly been conducted using ultrafiltration. Therefore, this method was used in this study.

The ultrafiltration system provided visible separation, because different colors of fractionated PME were evident from Figure 41. The higher the MWD, the darker the color (the dark color was attributed to high M_W lignin compounds). The order for color decrease is F_4 > raw effluent > F_3 > F_2 > F_1.

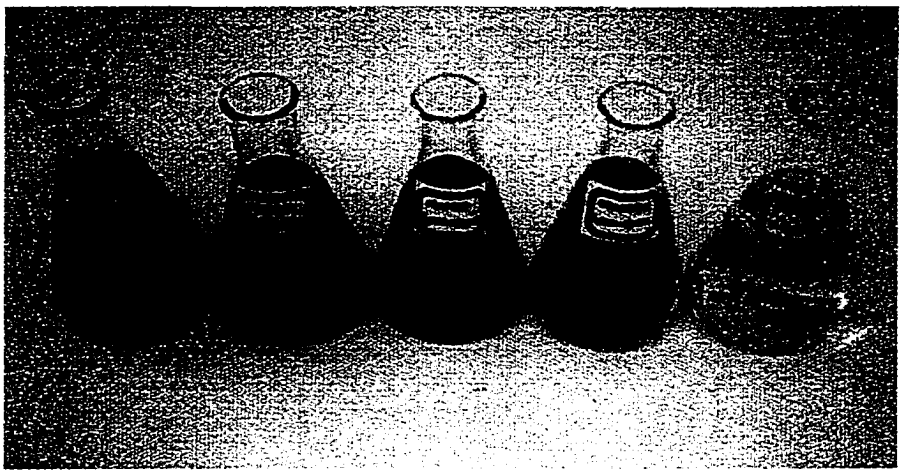


Figure 41. Color differences between fractionated Weyerhaeuser PME (from left to right: raw PME, F_4, F_3, F_2, and F_1)

The rough ranges of MWD for F_1 to F_4 were known from MWCO, but concentrations were difficult to determine precisely. Separation efficiency was qualitatively evaluated by measuring molecular weight distributions (MWD), TOC, color, AOX for fractionated PME. It can also be evaluated by measuring absorbance, using HPLC with a diode array detector (Mao, 1996). Table 13 shows significant differences in TOC, color and AOX from F_1 to F_4. This reveals that separation occurred using the ultrafiltration system. Table 13 also shows that F_4 has the highest color, but F_3 has the highest TOC. However, it is necessary to keep in mind that the concentration of fractionated PME was difficult to precisely determine; thus, the values of the above parameters were considered qualitative and relative.

Table 13. Characteristics of fractionated Weyerhaeuser PME

PARAMETER	ULTRAFILTRATION COMPONENTS			
	F_1	F_2	F_3	F_4
	MWCO < 1,000	1,000 < MWCO < 5,000	5,000 < MWCO < 10,000	MWCO > 10,000
COLOR (CU) (Nov. 1999)	48	184	898	3905
AOX (mg/L) (Nov. 1999)	1.69	3.27	8.95	21.68
TOC (mg/L) (March, 1998)	9.65	29.29	79.26	38.08
TOC (mg/L) (May, 1998)	17.40	55.58	90.77	57.19
TOC (mg/L) (Dec, 1998)	11.68	54.69	92.53	48.52

4.4.4.2 Sizing of Fractionated PME

The reliability of the ultrafiltration system was verified by measuring MWD for fractionated PME. High performance size exclusion chromatograph (HPSEC) was used to measure MW and MWD of the raw and treated samples. The results were shown in Table 14.

Table 14. Summary of HPSEC results for MWD of fractionated PME

	Protein Standard with UV Detector - 1st peak				Protein Standard with UV Detector - 2nd peak	
	MWCO < 1000 (F_1)	1000 < MW < 5000 (F_2)	5000 < MW < 10000 (F_3)	MWCO > 10000 (F_4)		MWCO > 10000
Mw	6903	7424	7811	8976	Mw	4057248
Mn	4421	6829	6202	5604	Mn	Cal. Error
Mz	7322	7704	8240	9980	Mz	13001851
Mz + 1	7529	7905	8545	11025	Mz + 1	25916095
P Index	1.531	1.087	1.259	1.602	P Index	Cal. Error
Peak Mw	7966	8151	8651	9503	Peak Mw	2749893
Mz / Mw	1.061	1.028	1.055	1.112	Mz / Mw	3.205
Mz + 1 / Mw	1.091	1.065	1.094	1.228	Mz + 1 / Mw	6.388
	Polyethylene Glycol / Ethylene Standard with RI Detector - 1st peak		Polyethylene Glycol / Ethylene Standard with RI Detector - 2nd peak		Polyethylene Glycol / Ethylene Standard with RI Detector - 3rd peak	
	MWCO < 1000	1000 < MW < 5000	MWCO < 1000	1000 < MW < 5000	MWCO < 1000	1000 < MW < 5000
Mw	739	771	317	336	36	37
Mn	711	729	314	333	35	36
Mz	772	834	320	340	36	37
Mz + 1	814	940	323	343	37	38
P Index	1.039	1.058	1.01	1.009	1.029	1.028
Peak Mw	788	788	319	321	32	32
Mz / Mw	1.044	1.081	1.009	1.01	1.015	1.014
Mz + 1 / Mw	1.101	1.219	1.019	1.021	1.031	1.028

Table 14 shows expected results were obtained for F_3 and F_4 using protein standards with an UV detector, and for F_1 using PEG/EG standards with a RI detector. However, the results for F_2 were not promising ($1{,}000 < M_W < 5{,}000$). It was assumed that F_2 contained a variety of complex polymers, the reliability of the calibration deteriorated due to the lack of suitable M_W standards for F_2. Great efforts were made to determine MWD for F_2, but the results were not promising. These included using different mobile phases, M_W standards and detectors for HPSEC, and using Mass Spectrometry (MS) and Electronic Spray/MS. Thus, MWD for F_2 is still unknown. Future work needs to be done in this area. Exploring suitable standards and using simultaneous multiple in-line detectors are suggested.

4.4.4.3 Role of Fractionated PME in Flocculation

Effect on flocculation in RW by F_1, F_2, F_3, F_4, $F_1 + F_2 + F_3$, $F_1 + F_2$, $F_1 + F_4$ and $F_1 + F_2 + F_4$ were evaluated using flocculation tests. Samples of a mixture of the Wapiti RW (August 1999) and fractionated PME were mixed for 5 minutes at $\overline{G}_c = 200\ s^{-1}$ and flocculated up to 60 minutes at $\overline{G_f} = 13\ s^{-1}$. TRE and PRE were used to characterize flocculation performance. Flocculation curves presented in Figure 42 to Figure 44 and Appendix E. The maximum increases in TRE and PRE (in comparison to the RW control) were used to evaluate the ability to induce flocculation by fractionated PME. However, it is necessary to keep in mind that the maximum values occurred at different dosages. The maximum increases in TRE and PRE were determined from flocculation curves shown in Figure 42 to Figure 44 and Appendix E, and summarized in Table 14.

Table 15 reveals that all fractionated PME can induce flocculation, but only at

certain dosages, and to different extents. F_1 and F_3 had similar effects on flocculation in RW. Their effects were much greater than F_2 and F_4, which also had comparable effects. Figure 44 indicates that the best flocculation efficiency was obtained with the addition of $F_1 + F_2$ (MW < 5,000). This can be reinforced by the fact that the highest value of maximum increases in TRE and PRE occurred with the addition of $F_1 + F_2$ in comparison with other results listed in Table 15. It was postulated that fractionated PME contained both coagulants and flocculants. The coagulants were the cations from F_1, where Na^+ played an important role as previously proven. The flocculants were the organic polymers. Polymers with 1,000 < MW < 5,000 consist largely of the polar breakdown products of lignin (with lesser amounts of lignin at various degradation stages), as well as polysaccharides, proteins, soluble starch, cellulose and sugar. These polymers were found to have ability to induce PMEICF. Of importance, the combined effects from PME chemicals with different M_W are provided by evaluating the effect of fractionated PME on flocculation in RW. It was found that the polymer flocculation inducing ability improved when the polymer and cations co-existed. This can be reinforced by the fact that the flocculation inducing ability by the fractionated PME with MWCO < 5,000 is much stronger than that with MWCO < 1,000 (contained more cations). In addition, it was found that cations greatly enhanced the F_4 flocculation inducing ability. This indicates that some polymers in F_4 may function as flocculants, inducing flocculation by bridging mechanism. This further reinforces the results obtained from the previous section.

More small sized particles were removed and more flocs were broken up by prolonged flocculation time. Table 15 shows that PRE (<10 μm) increased when

flocculation time increased due to better particle absorption, but TRE decreases due to break-up of fragile flocs. Inconsistent results from PRE and TRE suggested to determine the absorbance for raw and treated samples at λ = 280 nm and λ = 340 nm for all flocculation tests. A large quantity of absorbance results revealed that flocculation had almost no influence on absorbance. Therefore, the results can not be used for the evaluation study.

In conclusion, all fractionated PME molecular size groups can induce flocculation, but to different extents at different dosages. The highest induced ability was attributed to the fractionated PME with M_W < 5,000. This further reinforced that cations and organic polymers of polysaccharides, proteins, soluble starch, cellulose, sugar and the polar breakdown products of lignin are the major chemicals inducing flocculation in RW. This also reveals that the co-existence of cations and organic polymers provided better inducing ability.

Table 15. Maximum increases in removal efficiencies attributed to fractionated PME

Fractionated PME	Maximum increase in TRE (%)		Maximum increase in PRE (%)	
	t_r = 0.5 h	t_r = 1.0 h	t_r = 0.5 h	t_r = 1.0 h
F_4 (MWCO > 10, 000)	1.83	4.33	14.82	11.85
F_4 + 555 mg/L $CaCl_2$ (Blank = RW)	4.38	7.08	failure	failure
F_4 + 555 mg/L (Blank = RW+ $CaCl_2$)	2.69	4.06	failure	failure
F_3 (10, 000 > MWCO > 5,000)	11.12	10.45	25.38	26.36
F_3 + 555 mg/L	11.53	10.43	16.18	failure
F_2 (5, 000 > MWCO > 1,000)	2.26	1.98	25.41	0.31
F_1 (MWCO < 1,000)	14.47	4.6	17.28	39.08
F_1 + F_2 (MWCO < 5,000)	35.21	15.69	45.68	61.2
F_1 + F_2 + F_3 (MWCO < 10, 000)	13.39	10.85	21.5	6.52
F_1 + F_2 + F_4 (MWCO < 5,000 + > 10,000)	failure	failure	12.64	12.18
F_1 + F_4 (MWCO < 1,000 + > 10,000)	8.85	9.56	4.61	31.71

Note: 1). $CaCl_2$ = 555 mg/L, adding 20 mL 1N $CaCl_2$ into a 1.98L sample
2). failure = tests did not work due to equipment failure

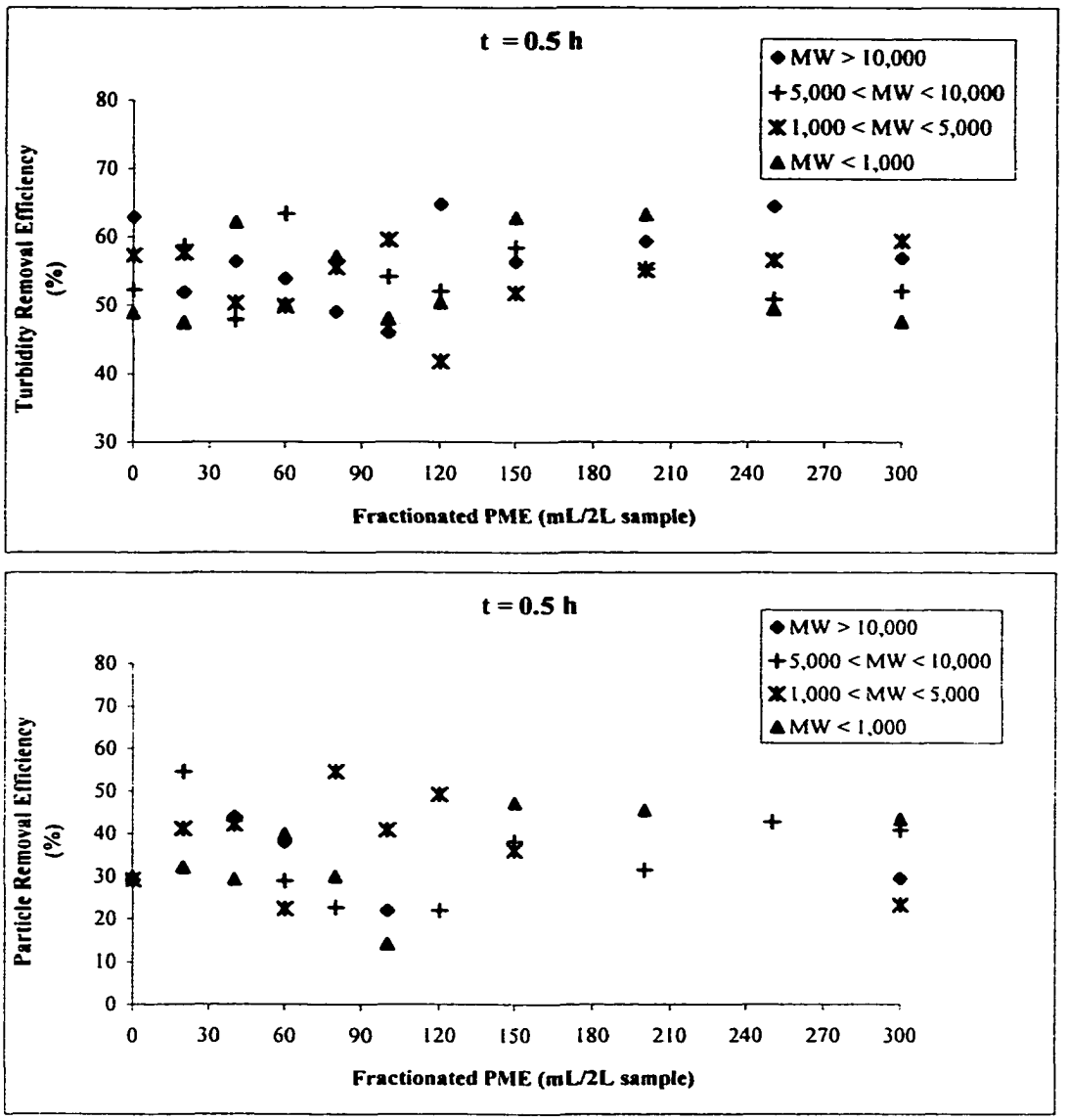


Figure 42. Effect of fractionated PME on flocculation in Wapiti RW (August 1999)

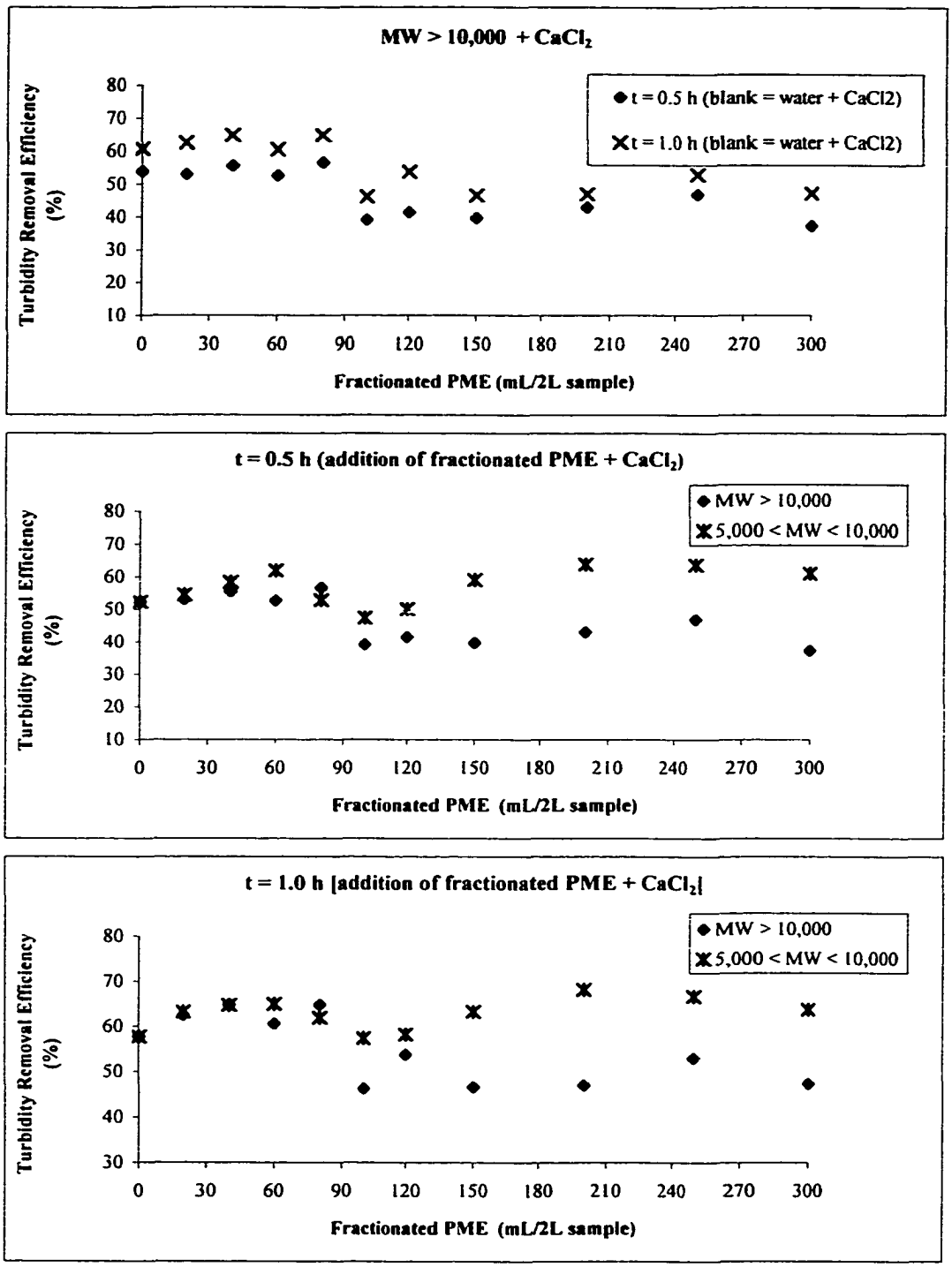


Figure 43. Combined effect of salt and fractionated PME on flocculation in Wapiti RW samples (August 1999). $CaCl_2$ = 555 mg/L, by adding 20 mL 1N $CaCl_2$ into a 1.98L sample

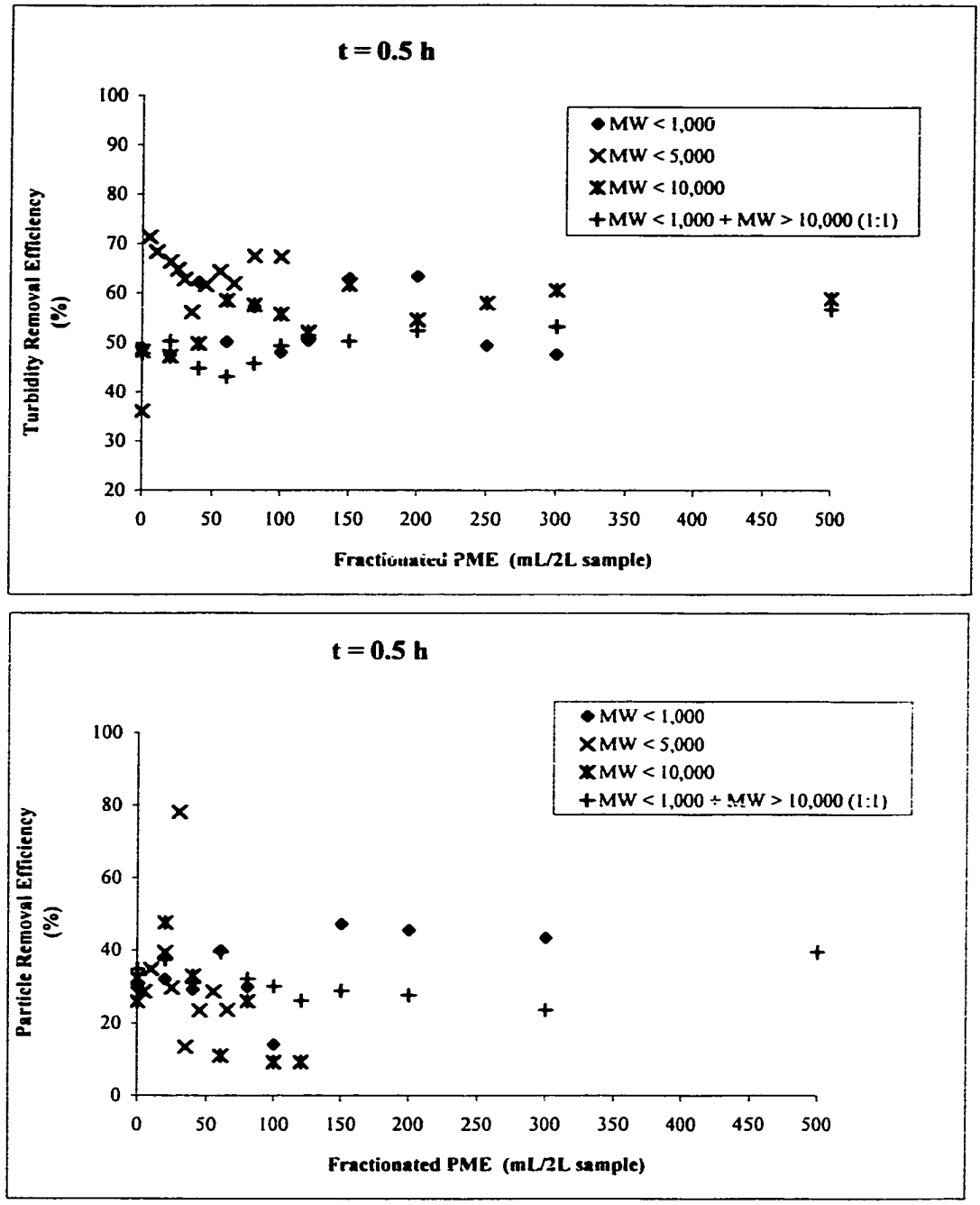


Figure 44. Effect of fractionated PME on flocculation in Wapiti RW

4.5 Effect of Biological Factors on PMEICF Formation

Evaluation of the effect of biological factors on flocculation in RW was focused on microbial activity, with some consideration of aquatic organisms and plants. The evaluation included morphology studies, isolation and identification of PME-related bacteria, determination of floc-forming bacteria, and postulation of the mechanisms for bioflocculation.

4.5.1 Morphology Study

The microbial involvement of induced floc formation was examined using a Conventional Optical Microscope (COM), Scanning Electron Microscope (SEM), x-ray SEM, Transmission Electron Microscope (TEM) and Scanning Confocal Laser Microscopy (SCLM). The morphology of bacteria, biopolymers, biofilm, bioflocs, field flocs, and aquatic organisms and plants were observed from the gross to fine scale. SEM and TEM were used to view two dimensional floc structures, and COM and SCLM were used to view three dimensional floc structures. SCLM results are not presented as problems occurred during the observation. For example, the dye bound to the agar and the specimens became fluoresced even though the optimum staining time of 5 to 10 minutes was used with concentrated dye (FITC, 2 mg/L). As a result, with the fluoresced specimens, the image was difficult for the human eye to examine. Moreover, the dye would occasionally not bind to the bacteria and only the agar fluoresced (Joyce, 1999). Therefore, images presented were from SEM and x-ray SEM, unless TEM was noted.

Morphology observation results reveal that PME contains bacteria species, some of the species possess varied means of attachment and secrete EPS, and the secreted EPS

contributes to adsorption and flocculation. Aquatic organisms and plants are also evident in the examined flocs.

PME contains a large quantity and variety of bacteria species (Figure 45). Some of them have been identified (Fulthorpe *et al.*, 1993; Geesey, 1982; González *et al.*, 1997; Liss and Allen, 1992; Mohn, 1995; Tsernoglou and Anthony, 1971; Weyerhaeuser, 2000), but most of them are still unknown (Joyce, 1999). Attempts have been made in this study to further identify PME-related bacteria. Figure 46-a and Figure 46-b reveal that majority of PME-bacteria appear to be cocci and bacilli in shape. Some spirilla or fiber-like bacteria were also observed under COM. Observed bacteria ranged from approximately 0.9 to 1.5 μm in length, and 0.27 to 0.36 μm in width (Joyce, 1999).

Some bacteria possess varied means of attachment that may assist in adsorption and floc formation. The means included: 1) adhesive stalk formation (Figure 46-b and Figure 46-c), 2) star formation (a centre sphere with star-like appendages as shown in Figure 46-d), 3) capsular secretions (fuzzy edges in the polymeric matrix as shown in Figure 47-c), and 4) fibrillar appendages (Figure 46-c, Figure 47-c, Figure 47-d and Figure 48-c). This causes bacteria-bacteria association, bacteria-colloids association, leading to the formation of biofilm, bioflocs and field flocs.

Bacteria from PME secrete EPS that are visible in flocs. Morphology observations have shown the existence of EPS. Figure 47-d reveals fuzzy edges around bacteria, and Figure 48-b shows bacteria that appear coated. This could be due to the presence of EPS in the form of a capsule, as a nucleus is visible (Joyce, 1999). Moreover, single, long strands of EPS can be seen in Figure 47-d, Figure 52-a and Figure 52-d. Semi-transparent EPS bundles are also evident in Figure 46-c. A network of EPS is shown in

Figure 47-c and Figure 48-c. In Figure 46-c EPS bundles appear as long strands suspended between bacteria clusters and fibers.

EPS contributed to adsorption and floc formation. Bacteria-bacteria association is viewed in Figure 47-a and Figure 48-d (floc-forming bacteria tend to clump together). Individual bacteria latching onto clay particles is evident in Figure 47-b. EPS clump bacteria together to form clusters as shown in Figure 46-c. A network of EPS, bacteria and clay particles is observed in Figure 47-c and Figure 48-c. Bacteria clump together and form microbial biofilms, a gelatinous matrix, which is revealed in Figure 48-a and Figure 48-b. Besides bacteria, biofilms may also contain algae, fungi and other microscopic organisms (Sentar, 1997).

The formation of denser bioflocs confirms that PME bacteria have a strong ability to secrete EPS that helps bond the colloidal sub-micron particles together. Figure 49 also illustrates that the bioflocs latch onto the fiber surface, forming larger flocs through the fibers bridging ability. Large field flocs were observed in Figure 51, and the ultrastructure of field flocs is evident in Figure 51-b and Figure 52. Under higher magnification Figure 50-d shows that bioflocs consist largely of colloidal sub-micron particles. This indicates that bioflocculation is the primary factor in transferring dissolved colloids into suspended particles or flocs. The ability of biofloc to transport and settle sediment and contaminants has been reported in the literature (Liss *et al.*, 1996).

From the observation of a large quantity of floc images it was found that bacteria, colloids, suspended particles or bioflocs clasped onto the surface of diatoms and other organic materials forming settleable flocs. This can be further reinforced by observation

of the composition of field flocs (Figure 51 and Figure 52).

Morphology observations suggest that the field flocs have a varying degree of porosity, and consist mainly of inorganic material, such as clay particles and fine flocs. They also contain microbial communities, biopolymers, such as EPS and cellular debris, and organic polymers such as fibers. Different kinds of diatoms from the Wapiti River (Figure 53 and Figure 54) and some aquatic organisms from Weyerhaeuser PME (Figure 56) were also detected in the field flocs.

In addition, different types of diatoms are visualized from field flocs as shown in Figure 51. Higher life forms of aquatic organisms exist in PME and are observed in field flocs. These include rotifers, stalks, flagellates, suctorians, testate amoebae, daphne and copepods (Cole, 1994). Examples of some of the aquatic organisms visualized from PME are presented in Figure 56. These organisms have the ability to assist in the formation of flocs by providing sites for particulate matter to lodge onto, or bridge particles together by using fiber-like appendages on the organisms. For example, copepods have been found to transfer suspended matter into pellets, which increases settling velocities (van Leussen, 1988).

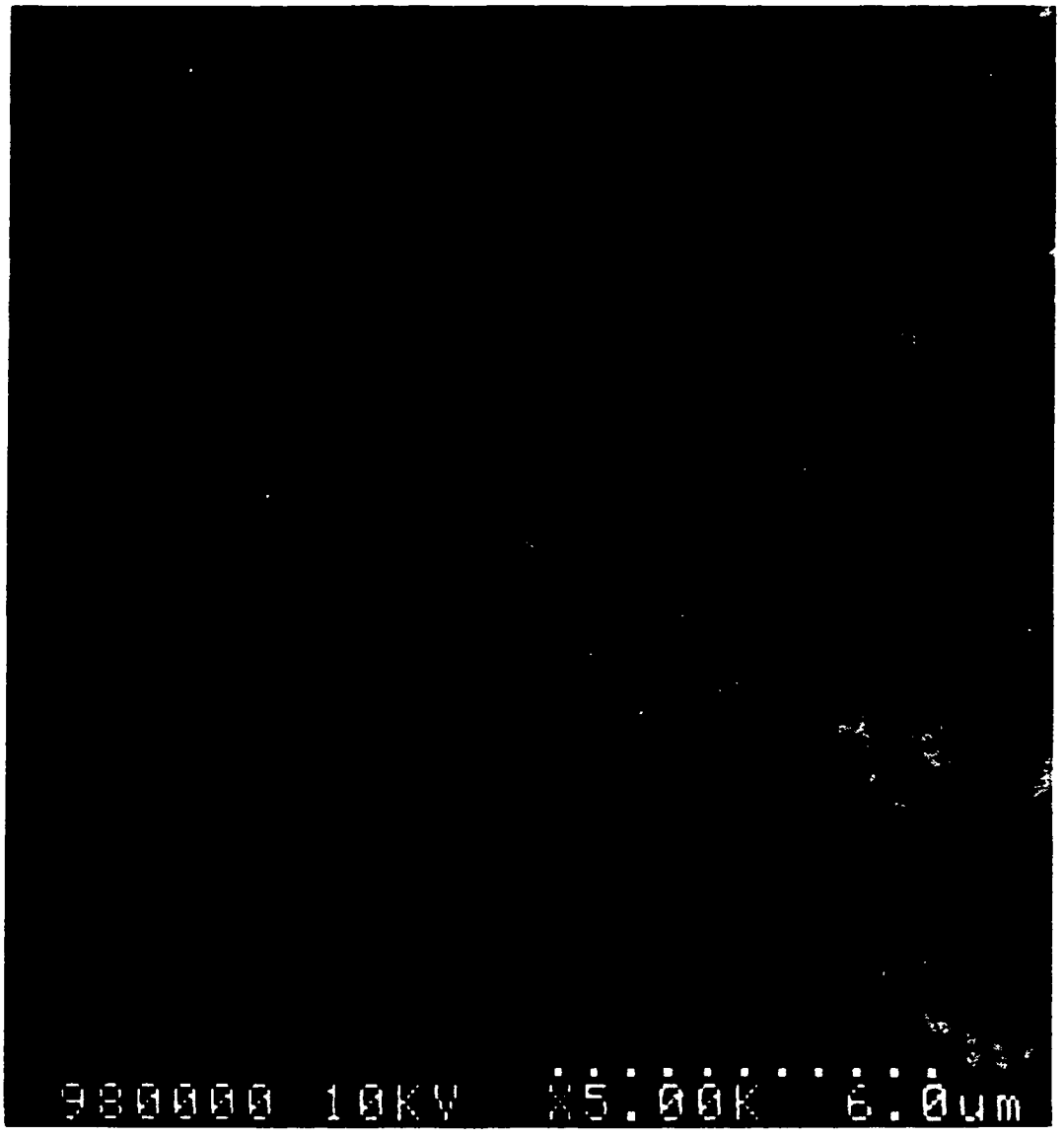


Figure 45. Bacteria from Weyerhaeuser PME (August 1998)

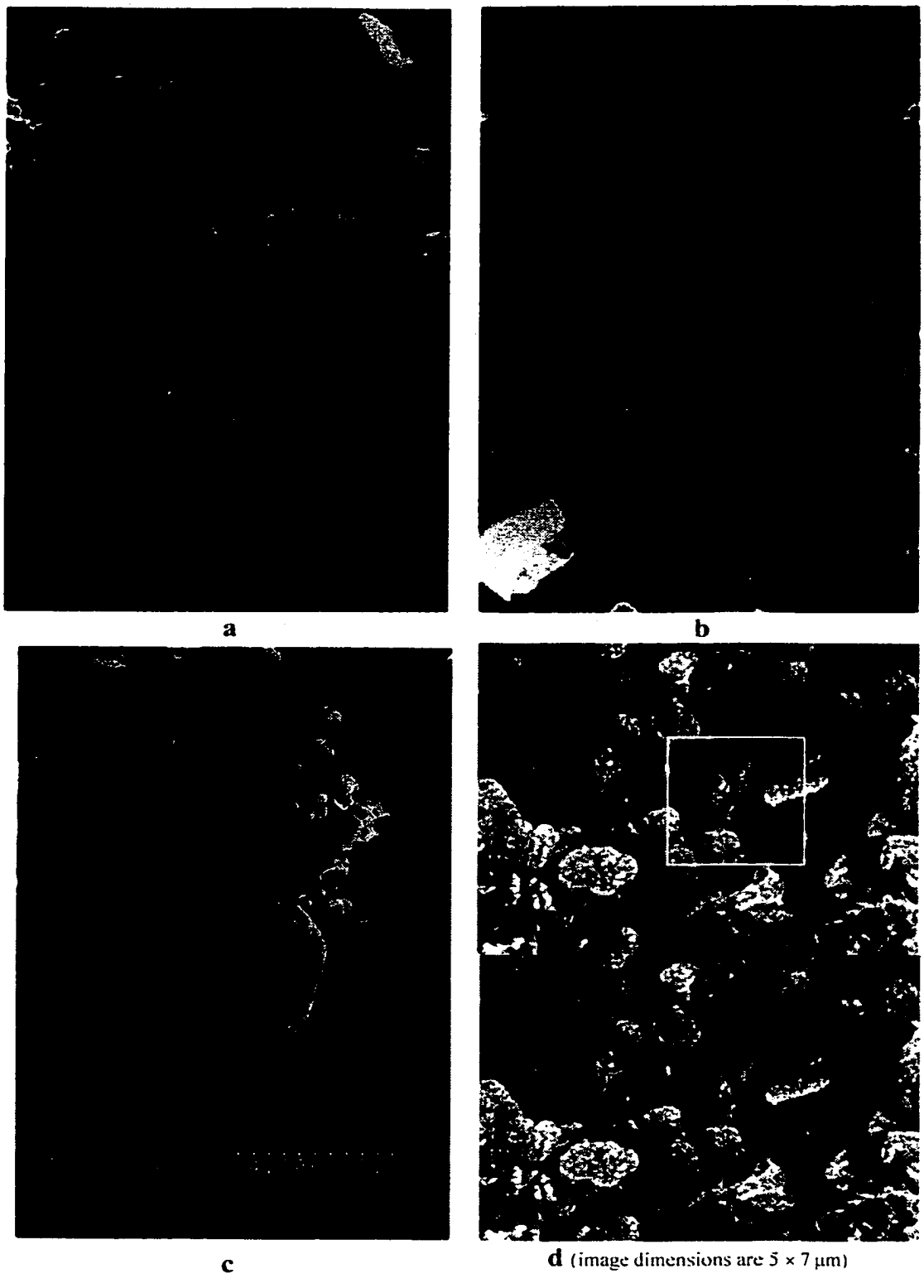


Figure 46. Individual and clusters of PME bacteria

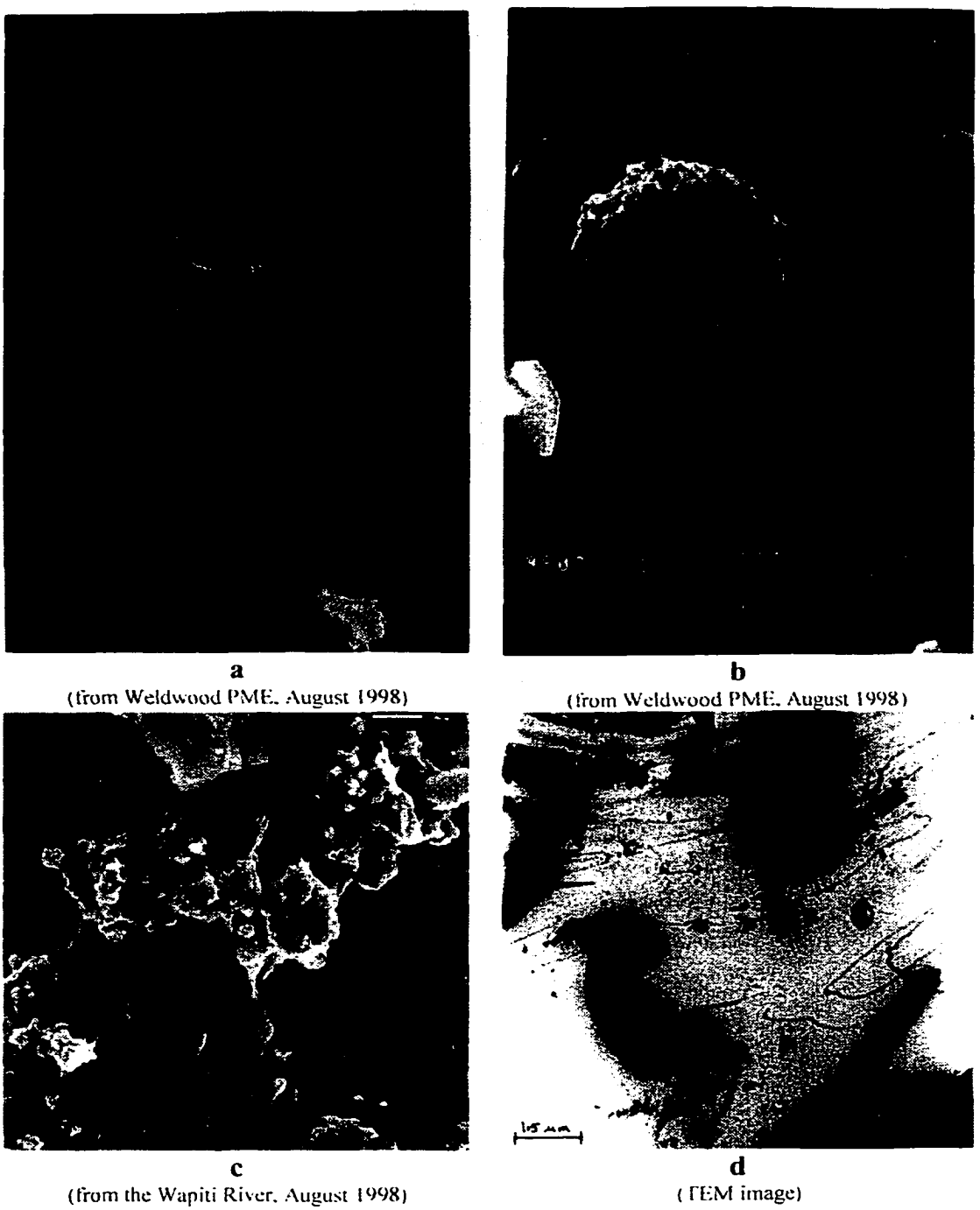
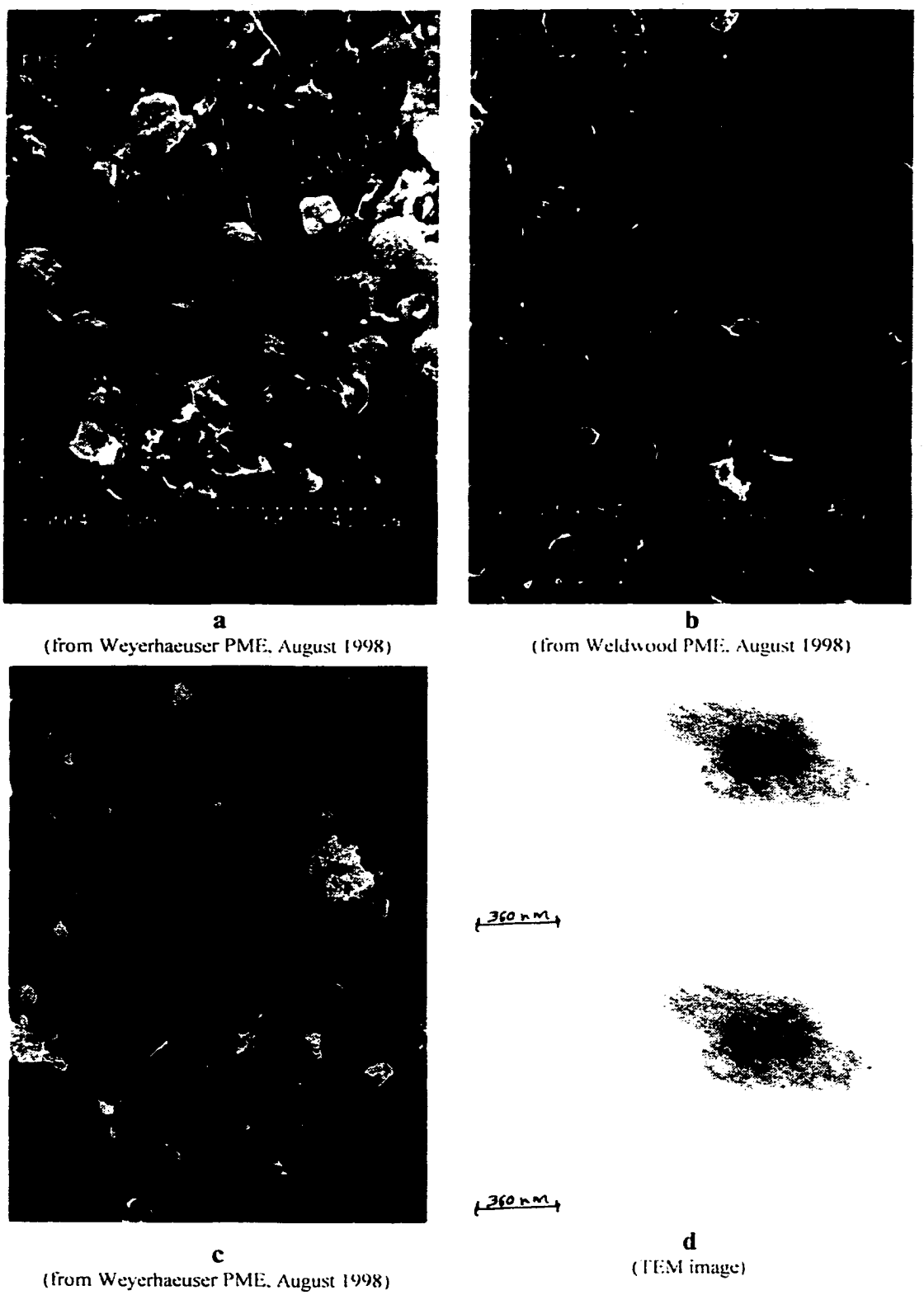


Figure 47. PME bacteria and fibrils (EPS)



a
(from Weyerhaeuser PME, August 1998)

b
(from Weldwood PME, August 1998)

c
(from Weyerhaeuser PME, August 1998)

d
(TEM image)

Figure 48. Bacteria-bacteria association and biofilms

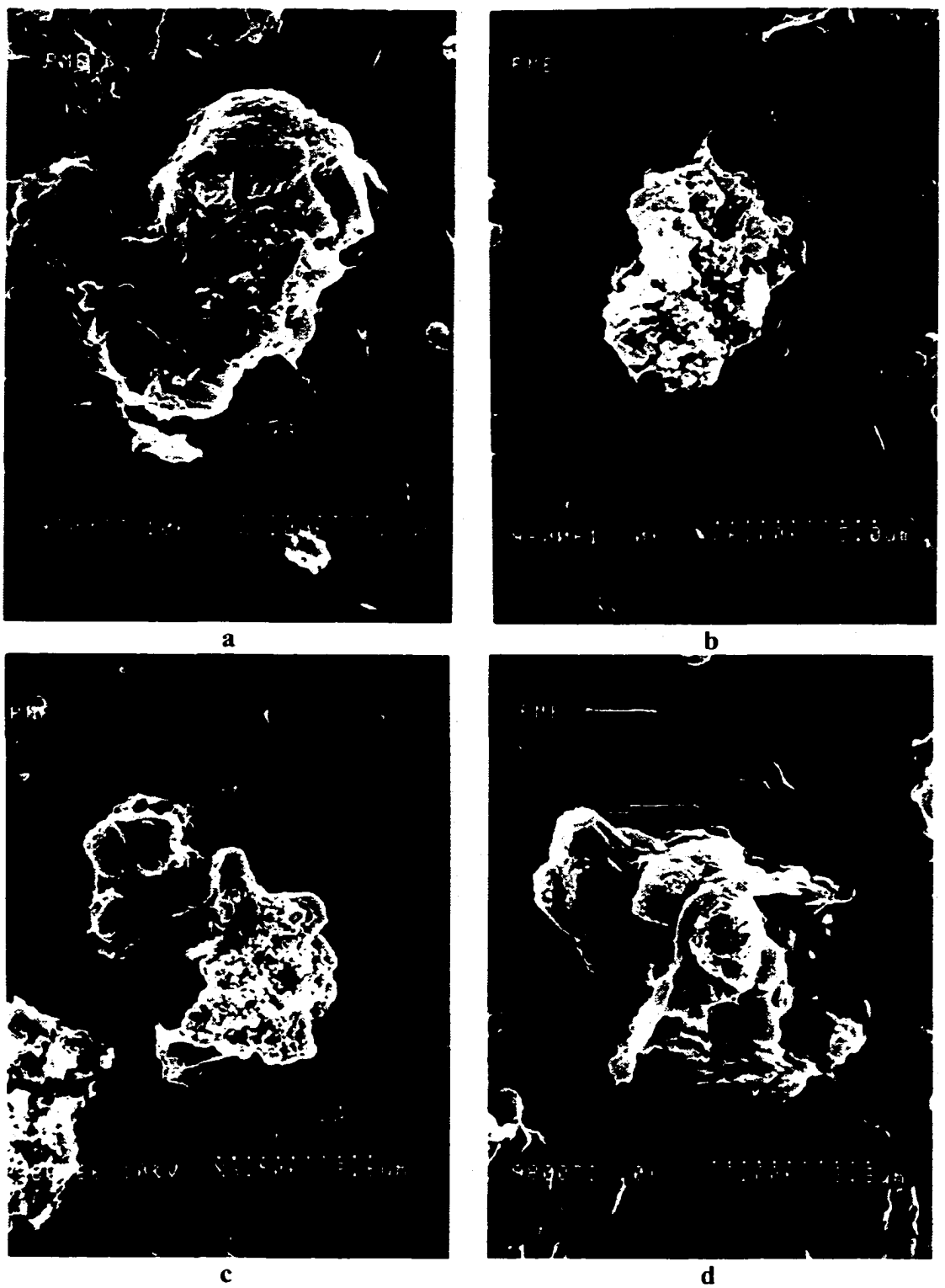
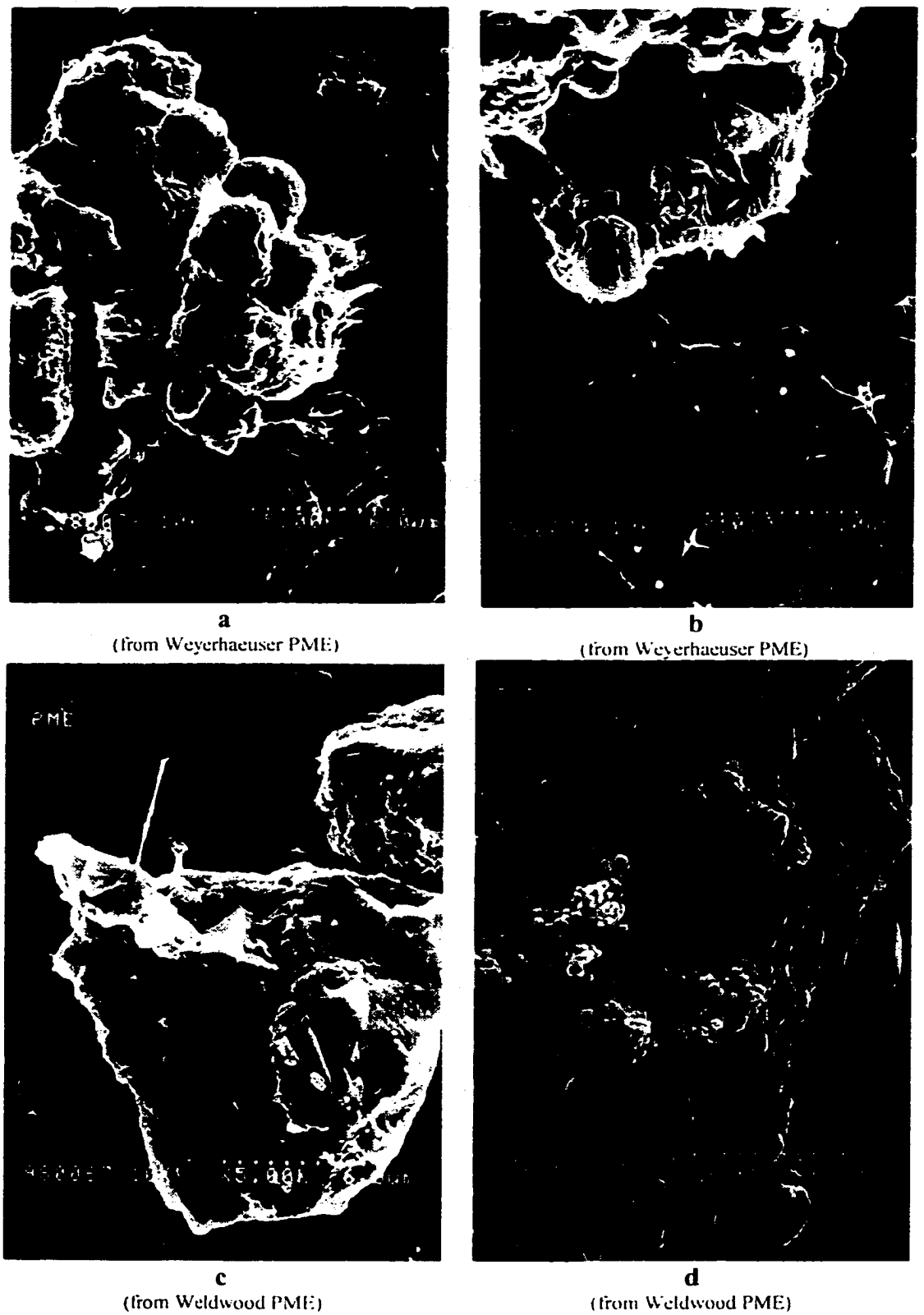


Figure 49. Bioflocs from Weyerhaeuser PME collected in August 1998



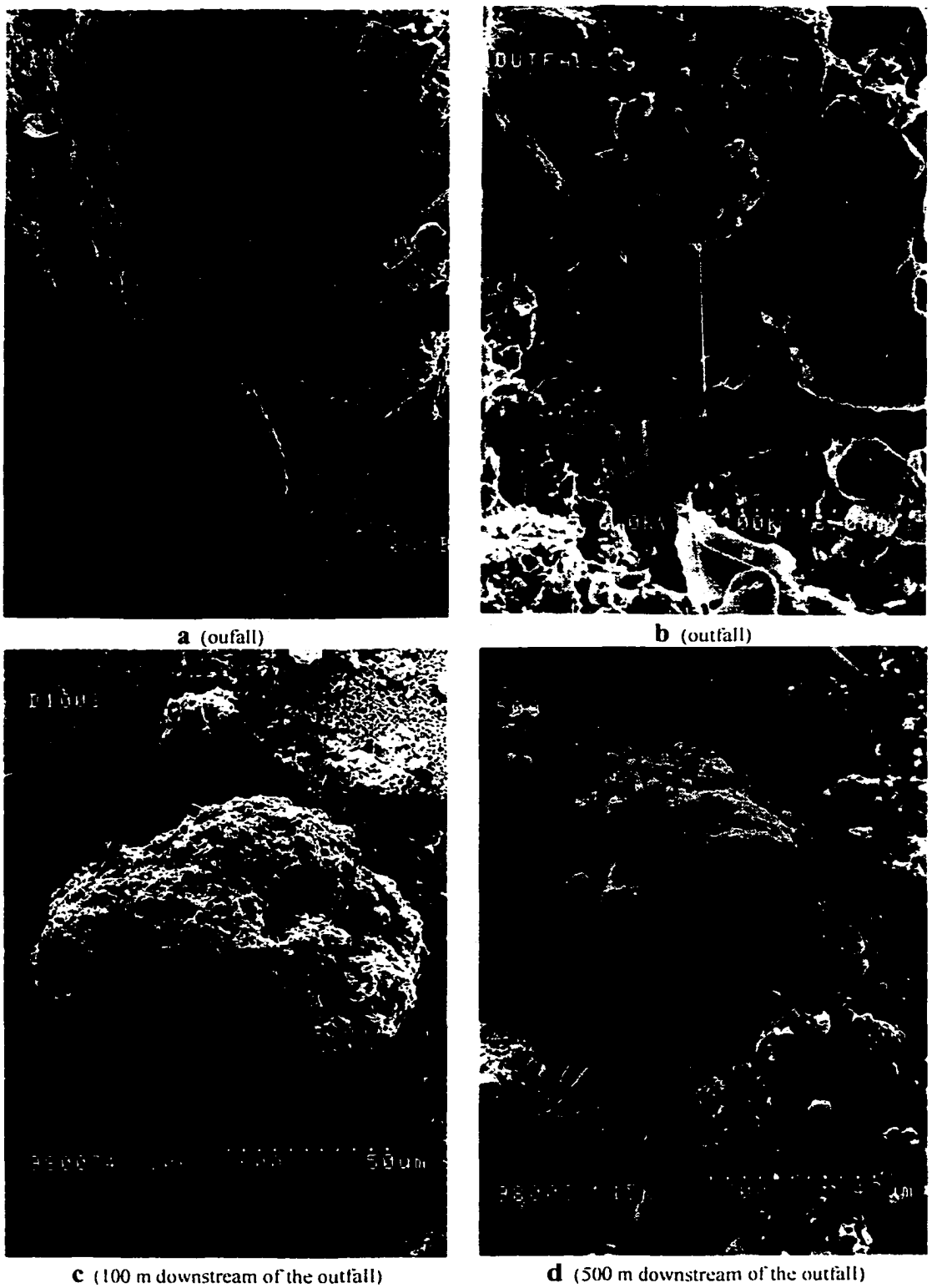
a (from Weyerhaeuser PME)

b (from Weyerhaeuser PME)

c (from Weldwood PME)

d (from Weldwood PME)

Figure 50. Bioflocs from PME (August 1998)



a (oufall) **b** (outfall)

c (100 m downstream of the outfall) **d** (500 m downstream of the outfall)

Figure 51. Field flocs from the Wapiti River (August 1998)

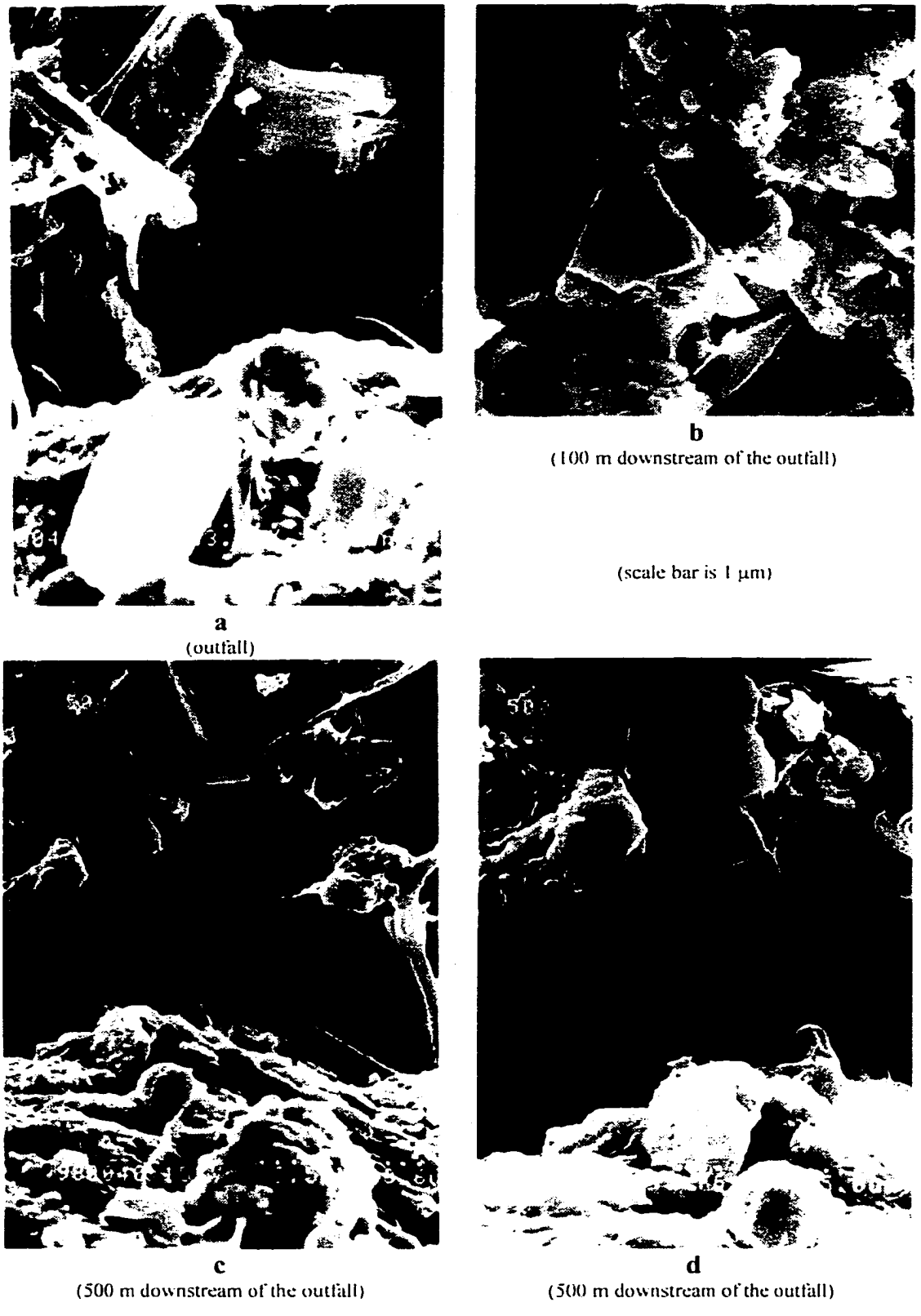


Figure 52. Ultrastructure of field flocs from the Wapiti River (August 1998)

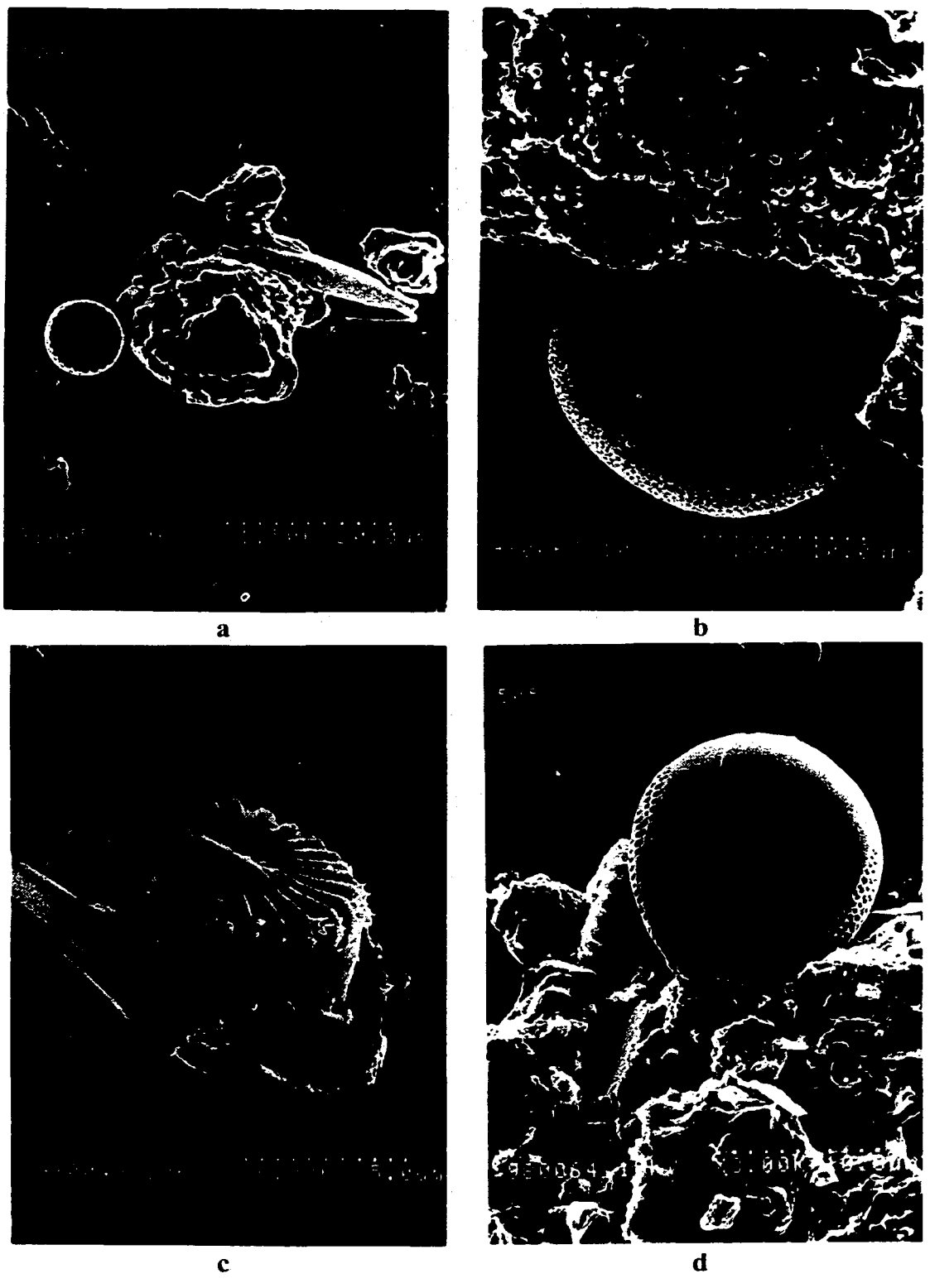


Figure 53. Aquatic plants from 500 m downstream of the outfall in the Wapiti River (August 1998)

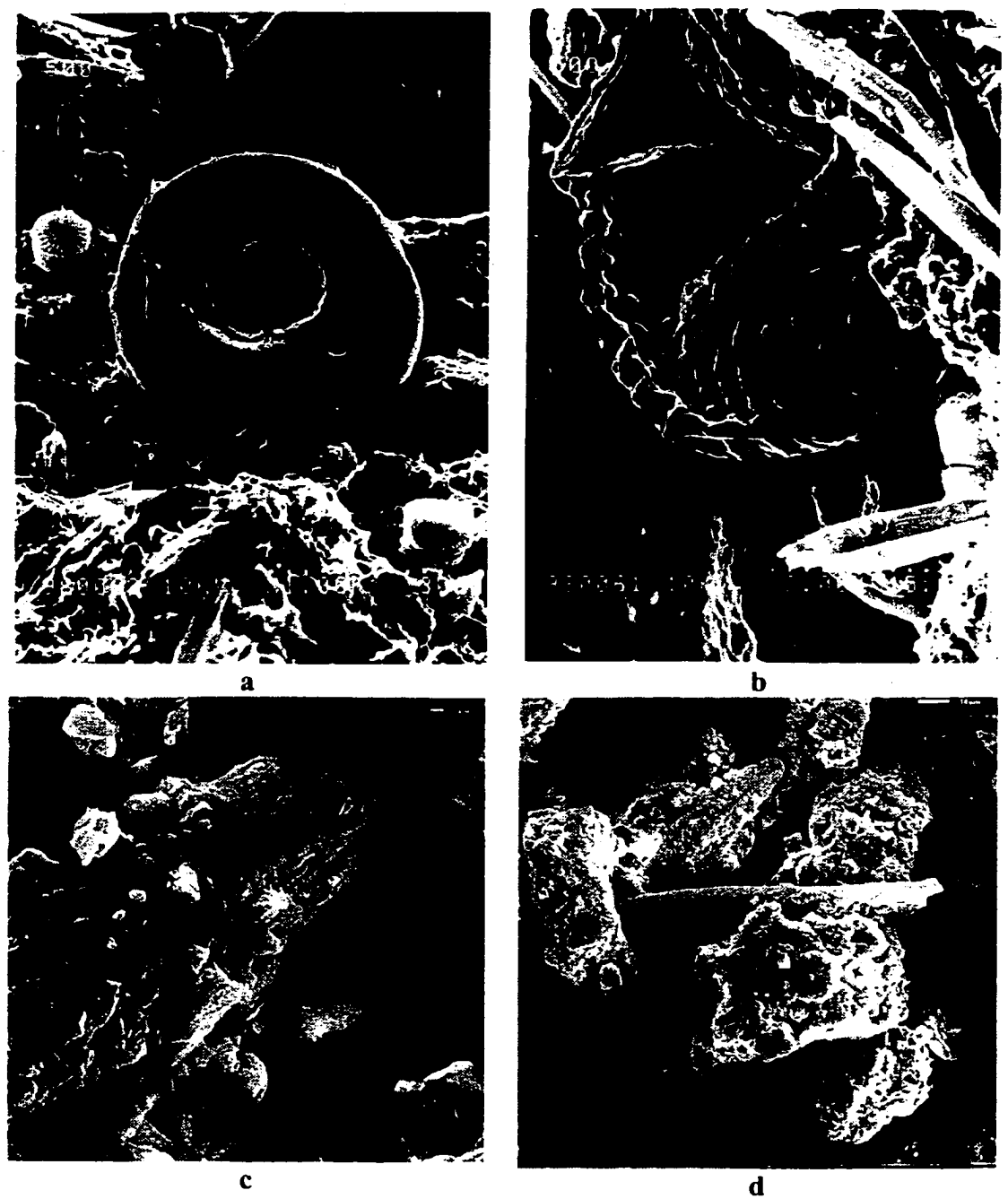
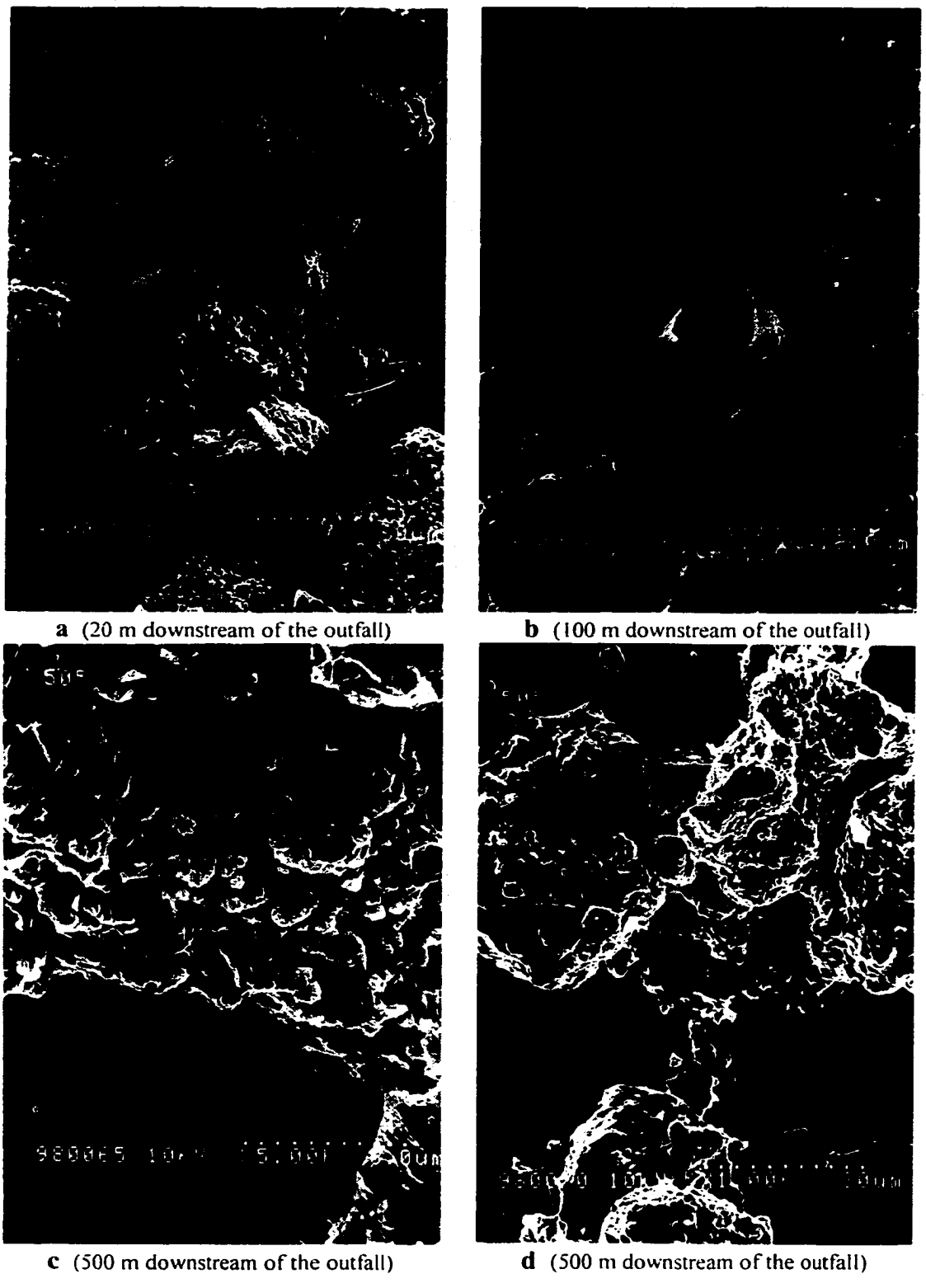


Figure 54. Aquatic plants from the Wapiti River (August 1998)



a (20 m downstream of the outfall)

b (100 m downstream of the outfall)

c (500 m downstream of the outfall)

d (500 m downstream of the outfall)

Figure 55. Diatom involvement in the formation of field flocs (August, 1998)

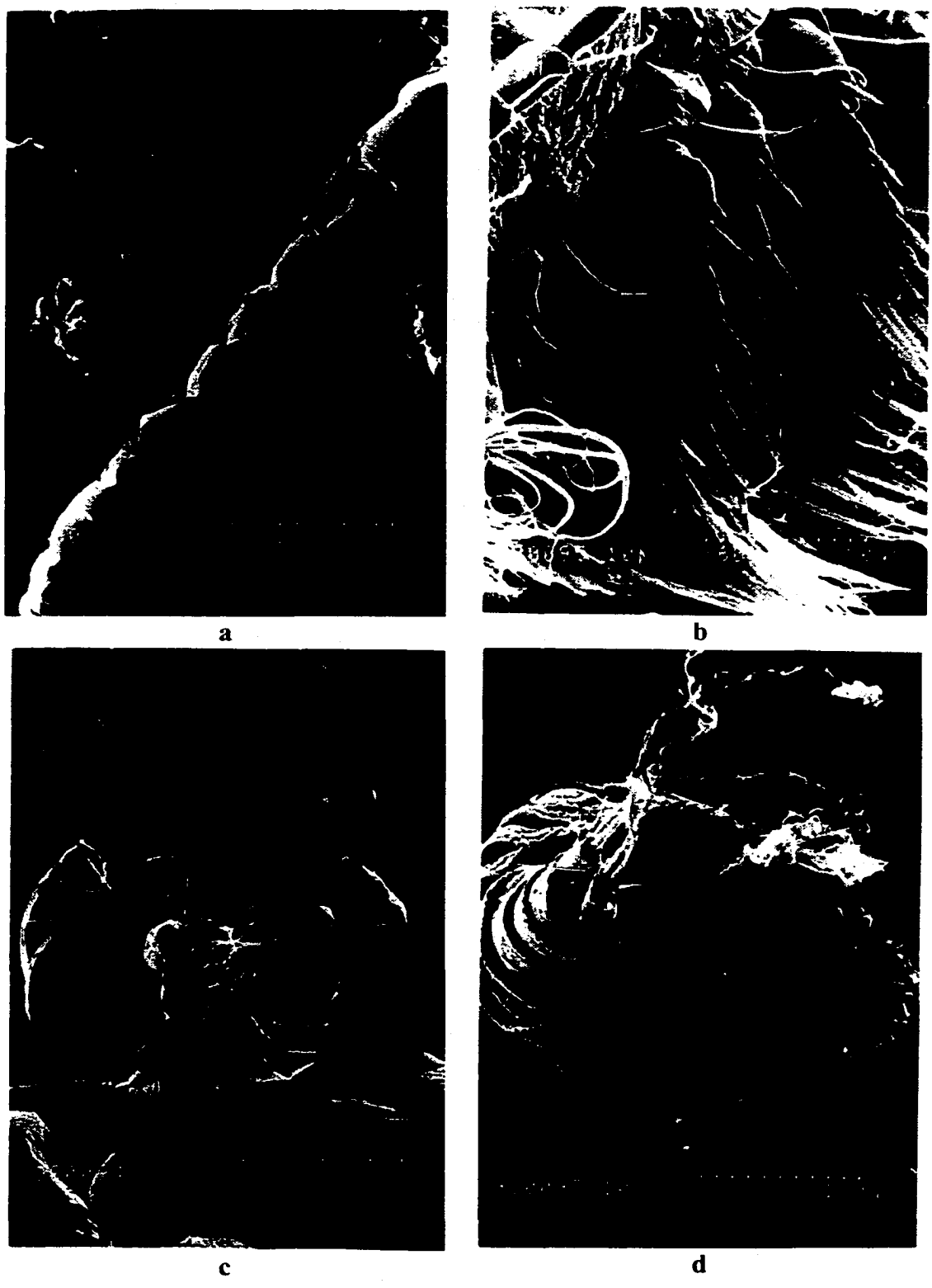


Figure 56. Aquatic organisms in Weyerhaeuser PME (August 1998)

4.5.2 Bacteria Isolation and Identification

Techniques currently used for identifying bacteria species are the water membrane filter technique, standard plate count technique, most probable number, and the Biolog® identification system. Biolog® identification system was used in this study. Before identification, bacteria needed to be isolated and characterized. Heterotrophic plate counts were used for isolation.

Figure 57 shows that colonies from different sources had different colors. This indicates that each source of bacteria has its own characteristic fauna. The 36 colonies were isolated from the heterotrophic plates; their characteristics are summarized in Table 16. The bacteria cultures were characterized using gram staining, the oxidase test, the catalase test, a triple sugar iron (TSI) test and growth on MAC plates. It was found from Table 17 that most of the bacteria were Gram negative, which is consistent with previous findings (Fulthorpe *et al.*, 1993; Liss and Allen, 1992; Liss *et al.*, 1996). Most of the Gram negative bacteria were classified as GN-NENT, except for SF and HC. They were classified as GN-ENT due to their acid/acid reactions with a TSI slant. The classified isolates were then identified using the Biolog® identification system. Table 18 reveals that 13 of the 27 isolates were positively identified.

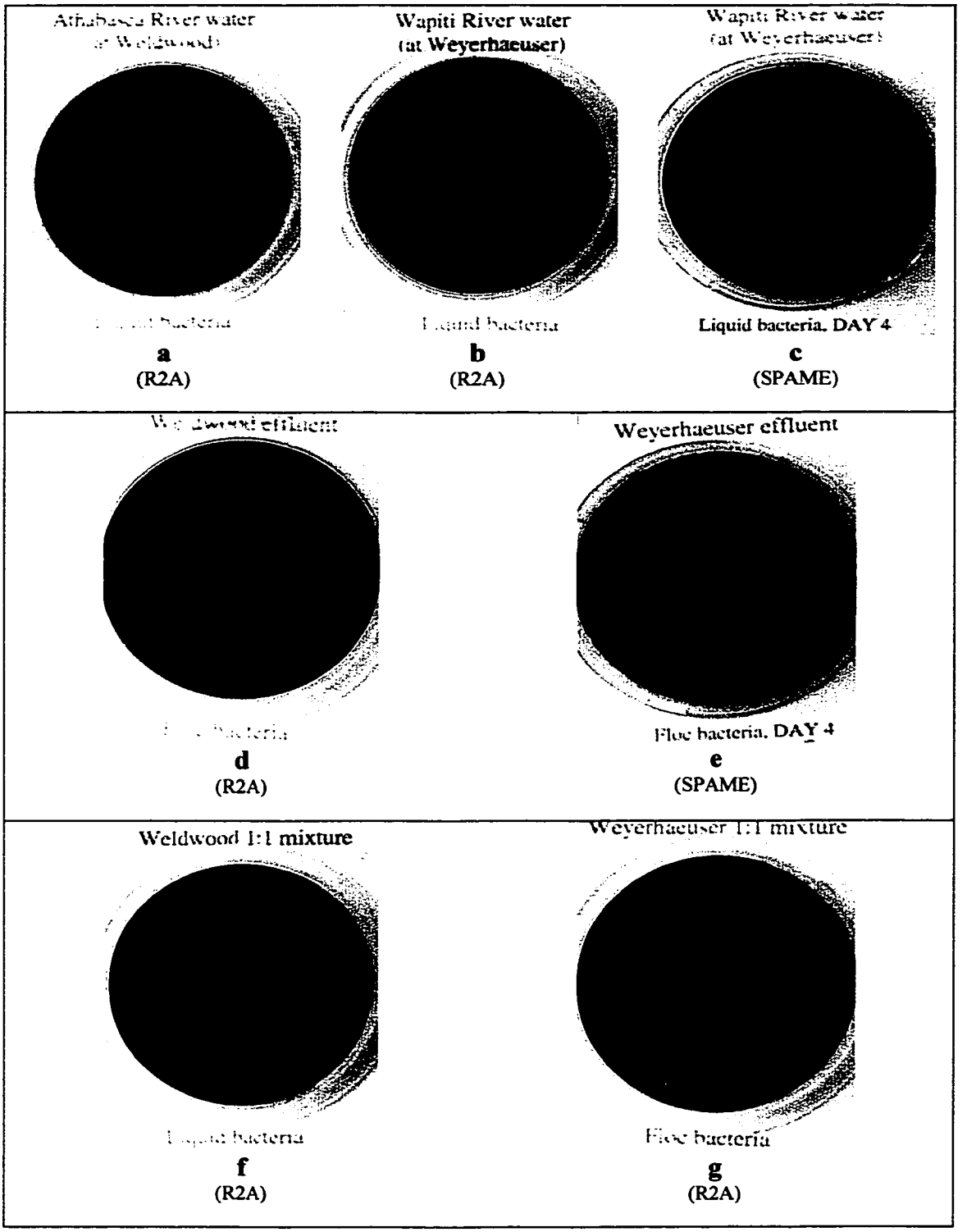


Figure 57. Bacteria isolates from different sources (Joyce, 1999)

Table 16. Characteristics of isolates (Joyce, 1999)

Isolate	Source	Site	Physical Description (on corresponding media)
A	mixture (1:1)	Weldwood and Athabasca River	white, round, slow-growing, ~2 mm diameter after 3d growth
B	floc (1:1)	Weldwood and Athabasca River	bright, yellow colonies, very slow-growing, well-defined, ~1 mm diameter after 2d growth
C	mixture (1:1)	Weldwood and Athabasca River	white, round, slow-growing, ~2 mm diameter after 3d growth
D	mixture (1:1)	Weldwood and Athabasca River	white, round, slow-growing, ~2 mm diameter after 3d growth
E	mixture (1:1)	Weyerhaeuser and Wapiti River	white
F	PME	Weldwood	white
G	PME	Weldwood	pale yellow
H	PME	Weyerhaeuser	bright yellow
I	RW	Athabasca River	pink colonies, ~5 mm diameter after 2d growth, flat colonies
HA	mixture (1:1)	Weyerhaeuser and Wapiti River	bright yellow
HB	mixture (1:1)	Weyerhaeuser and Wapiti River	yellow, ~2 mm diameter after 2d growth
HC	mixture (1:1)	Weyerhaeuser and Wapiti River	off-white, ~3 mm diameter after 1d growth, variable size
HD	mixture (1:1)	Weyerhaeuser and Wapiti River	off-white-clear, ~1 cm after 2d growth, flat but raised in middle,
HE	mixture (1:1)	Weyerhaeuser and Wapiti River	creamy white, various sizes, <1 mm-2 mm diameter after 2d growth
HF	mixture (1:1)	Weyerhaeuser and Wapiti River	white, tiny colonies, <1 mm diameter after 2d growth
HG	mixture (1:1)	Weyerhaeuser and Wapiti River	white, ~2 mm after 2d growth
HH	RW	Wapiti River	white, ~ 1mm diameter after 1d growth
HI	PME	Weyerhaeuser	tiny, yellow colonies, ~1 mm diameter after 2d growth, slow-growing
HJ	floc (1:1)	Weyerhaeuser and Wapiti River	white/clear colonies, ~1 diameter after 2d growth, slow-growing
HK	floc (1:1)	Weyerhaeuser and Wapiti River	tiny, white-clear colonies, <1 mm diameter after 2d growth
HL	floc (1:1)	Weyerhaeuser and Wapiti River	clear colonies, ~1 mm diameter after 3d growth, irregular edges, flat
HM	floc (1:1)	Weyerhaeuser and Wapiti River	white-clear colonies
SA	RW	Wapiti River	pale yellow colonies, ~2 mm diameter after 2d growth
SB	RW	Wapiti River	yellow, 2-3 mm diameter 2d growth, round with peak in middle
SC	RW	Wapiti River	white-clear, oval-shaped, ~3 x ~2 mm after 1d growth, lots of mucous
SD	RW	Wapiti River	yellow, tiny, <1 mm diameter after 1d growth, irregular size, shape
SE	mixture (1:1)	Weyerhaeuser and Wapiti River	yellowish colonies, ~1 mm diameter after 1d growth, peak in middle
SF	floc (1:1)	Weyerhaeuser and Wapiti River	yellow-beige, peaked in middle, ~5 mm diameter after 1d growth, lot of mucous
SG	floc (1:1)	Weyerhaeuser and Wapiti River	yellowish, ~1 mm diameter after 1d growth, not perfectly round
SH	floc (1:1)	Weyerhaeuser and Wapiti River	creamy-yellow, v. spread out, jagged edges, ~ 5 mm after 1d growth, lots of mucous
SI	mixture (1:1)	Weyerhaeuser and Wapiti River	pale yellow, 1-2 mm diameter after 3d growth, smooth edges, peaked in middle
SJ	mixture (1:1)	Weyerhaeuser and Wapiti River	yellow-orange, very slow-growing, ~1 diameter after 3d growth, convex
SK	mixture (1:1)	Weyerhaeuser and Wapiti River	white
SL	mixture (1:1)	Weyerhaeuser and Wapiti River	orange
SM	mixture (1:1)	Weyerhaeuser and Wapiti River	off-white
SN	mixture (1:1)	Weyerhaeuser and Wapiti River	yellow-orange, very slow-growing, ~1 diameter after 3d growth, convex

Table 17. Summary of characterization studies (Joyce, 1999)

ID	BAP	MAC	Gram stain	oxidase	catalase	TSI	Comments
A	growth (48), tiny, grey	no growth (72)	Gram negative bacilli, curly	+	+	no change / no change	
B	no growth (96 hrs)	no growth	Gram negative bacilli, curly	-	-	no growth (48 hrs)	grows well on R2A
C	grey, opaque (fine indifferent, 48 hrs)	no growth	Gram negative bacilli, curved, pleomorphic (variable length), var. thickness	+	+	no change / no change	
D	grey, opaque (fine indifferent, 48 hrs)	no growth	same as above	+	+	no change / no change (72 hrs)	
E	no growth	no growth					
F	no growth	no growth					
G	no growth	no growth					
I	small, opaque	no growth (72)	Gram negative bacilli, in chains, mostly 2's	-	+	no growth (72)	grows well on R2A
HB	growth (48), tiny	no growth (72)	Gram negative bacilli, curly, chains	+	-	no growth (48)	grows well on R2A
HC	coliform, indifferent	pink (48)		-	+	Acid / Acid	late lactose fermenter
HD	small, grey	no growth (72)	Gram negative bacilli, mostly single	+	+	Alkaline / no change	
HE	no growth (72)	no growth (72)	Gram negative bacilli, tiny, single rods	+	+	no growth (48)	grows on R2A
HF	tiny, grey	white (48)	Gram negative bacilli, single, some chains	+	+	no change / no change	
HG	tiny (48)	no growth (48)	Gram negative bacilli, mostly single	+	+	Alkaline / no change (48)	
HH	tiny, grey	no growth (24)	Gram negative bacilli, thin rods	+	+	Alkaline / Acid	
HI	no growth (48)	no growth (48)	Gram negative bacilli, curly	+	-	no growth (48)	grows on R2A
HJ	small grey (48)	no growth (48)	Gram negative bacilli, tiny, single rods	+	-	Alkaline / Alkaline	
HK	tiny (24)	no growth (24)	Gram negative bacilli, thin rods	-	+	Alkaline / no change (48)	
HL	tiny (24)	no growth (24)	Gram negative bacilli, single rods	+	-	Alkaline / no change (48)	
SA	tiny, opaque (48)	white (48)	Gram negative bacilli, single	+	+	Alkaline / no change	
SB	tiny, opaque (24)	no growth (24)	Gram negative bacilli, single, short chains	-	+	no change / no change (48)	
SC	coliform, indifferent	white		+	+	Alkaline / no change	
SD	no growth (72)	no growth (72)	Gram negative cocci	-	+	no growth (48)	grows on R2A
SE	coliform, hemolytic	white		+	+	Alkaline / no change	
SF	coliform, hemolytic	white		+	+	Acid / Acid	late lactose fermenter
SG	coliform, indifferent	white		+	+	Alkaline / no change	
SH	coliform, hemolytic (48)	white	Gram negative bacilli, single rods	+	+	Alkaline / no change	
SI	tiny (24)	no growth (24)	Gram negative bacilli, tiny rods	+	+	no change / no change (48)	
SJ	no growth (48)	no growth (48)	Gram negative bacilli, in clumps	-	+	no growth (96)	grows on R2A
SK	no growth	no growth					
SL	no growth	no growth					
SN	no growth (48)	no growth (48)	Gram negative cocci, single	-	+	no growth (72)	grows on R2A

Table 18. Summary of bacteria identified using Biolog® identification system (Joyce, 1999)

ID	Biolog® ID	ID	Biolog® ID
A	no ID, resembles D in appearance and biochemical tests	HK	Aquaspirillum metamorphum
B	*Roseomonas* genomospecies 6	HL*	no ID, *possibly Lampropedia hylina*
C	no ID, resembles D in appearance and biochemical tests	SA	*Brevundimonas vesicularis*
D	*Agrobacterium* like-cystic fibrosis	SB	*Flavobacterium marinotypicum*
I*	*Pasteurella pneumotropica*	SC	*Comamonas testosteroni*
HB*	*Achromobacter cholinophagum*	SD**	
HC	no ID, likely *Enterobacter* spp.	SE	no ID, likely *Pseudomonas* spp.
HD	*Acidovorax delafieldii*	SF	no ID, possibly *Aeromonas* spp.
HE	*Acidovorax delafieldii*	SG	Bordetella bronchiseptica
HF	Aquaspirillum metamorphum	SH	no ID, likely *Pseudomonas* spp.
HG*	no ID	SI*	no ID, possibly *Pasteurella trehalosi*
HH	*Pseudomonas* spp.	SJ	no ID, likely *Pseudomonas aurantiaca*, resembles SN
HI*	no ID, possibly *Aquaspirillum putridiconchylium*	SN	no ID, likely *Pseudomonas aurantiaca*, resembles SJ
HJ*	*Pseudomonas echinoides*		

4.5.3 Identification of floc-forming Bacteria

The identification of floc-forming bacteria was conducted by a series of long-term and short-term bioflocculation tests.

Long-term bioflocculation tests were conducted for up to 96 hours to allow enough time for bioflocculation to occur. This was based on the finding that it took at least 14 hours to visualize the formation of bioflocs (Biddanda, 1985) and significant formation of bioflocs occurred after 48 hours of flocculation (Muschenheim *et al.*, 1989). The samples used for the long-term bioflocculation tests were sterile mixtures of PME and RW with a volume ratio of 1:1. The sterile samples were obtained by either successive autoclaving or adding chloroform. In comparison with adding chloroform, successive autoclaving was the chosen method for long-term bioflocculation tests, as there was no chemical addition. However, it was found that autoclaving increased the

number of tiny particles, and changed particle size distributions of RW samples (Joyce, 1999). Thus, the sterile samples provided ideal environmental conditions, but did not realistically simulate the river environment. The efficiency of bioflocculation was evaluated by decreasing particles with sizes 2 to 4 μm in diameter and decreasing turbidity. Long-term bioflocculation results are shown in Figure 58, the bacteria were categorized and floc-forming bacteria were identified. The findings were summarized in Table 18.

Short-term bioflocculation tests were performed for 0.5 hours to simulate the conditions downstream of the pulp mill outfall in rivers. As it was found that the sterile samples did not realistically simulate the river environment, non-sterile RW samples were used for short-term bioflocculation tests. This was more representative of the actual physical and chemical composition of RW. The suspensions of bacteria isolate were re-introduced into the RW samples (bacteria suspensions were incubated for 4 days at 30 °C to allow for growth of slow-growing bacteria and enough secretion of EPS from bacteria). The samples were flocculated for 0.5 h at different mixing intensities in order to maximize floc formation. TRE, PRE, absorbance at 280 nm and 340 nm, pH and conductivity were used to characterize flocculation performance. t-tests were used to determine if bacteria significantly contributed to flocculation. Bacteria were considered to be floc-forming bacteria (+) if enhanced flocculation abilities were noted in all runs, possibly floc-forming bacteria (+/-) if mild flocculation abilities were noted in at least one run, and not floc-forming bacteria (-) if no flocculation abilities were noted or an increased number of small particles were noted (such as negative PRE attributed to SE).

The results, determined from bioflocculation curves (Appendix F), are summarized in Table 19. It was found from short-term flocculation test results that HL, HJ, SD, HK, SH, SJ, HI and HE were floc-forming bacteria, SE, HB, HH, I, C, HC, A_2, HG, D, B, HF were possibly floc-forming bacteria, and HD and SF were not floc-forming bacteria. However, long-term flocculation test results suggest that HC, HL, HJ, HH and SF were floc-forming bacteria, SE, SH, HB, I, A_2, HG, B, HF and D were possibly floc-forming bacteria, and SD, HK, HD and C were not floc-forming bacteria. Both short-term and long-term tests identified HJ and HL as floc-forming bacteria. Results were inconsistent for the remaining. HH and HC from short-term tests were possibly floc-forming bacteria, but were floc-forming when long-term tests were done. HD and SF from short-term tests were not floc-forming bacteria (HD and SF may not be able to secrete EPS). Results from all the tests show that HD is not floc-forming bacteria. When flocculation proceeded, it was observed that many flocs formed due to the addition of HJ, HL, HI, HE, or SJ suspension; therefore, these bacteria were floc-forming bacteria.

Inconsistent results from short-term and long-term flocculation tests may be attributed to different experimental settlings. It has been found that EPS production and biofloc formation are environment specific (Flemming, 1993; White, 1995). In this study, chemical and biological compositions of sterile and non-sterile samples are different, so bacteria function differently. Unlike the sterile samples used for long-term tests, the non-sterile samples were used for the short-term experiments to closely simulate river conditions. Non-sterile samples were used because autoclaving can change the physical and chemical properties and particle size distributions of samples. Bacteria that usually thrive in non-sterile PME and RW may not do so in the autoclaved ones,

conversely, some species may prefer the autoclaved PME and RW. Bacteria that usually produce large amounts of EPS may not do so in the autoclaved solution and the reverse may be true. In addition, long-term tests evaluated the effect of isolated bacteria on the mixture of PME and RW, but short-term tests evaluated the effect of isolated bacteria on raw RW. Although the extent of bioflocculation was species and environment specific, the experimental results demonstrated that some PME bacteria have the ability to induce flocculation in RW, but to varying extents. A major contributor to bioflocculation was EPS secreted from bacteria.

Table 19. Identification of floc-forming bacteria

			$\overline{G_f}$ = 2 s^{-1}		$\overline{G_f}$ = 5 s^{-1}										$\overline{G_f}$ = 13 s^{-1}		Short-term Bioflocculation	Long-term Bioflocculation
			TRE	PRE	TRE			PRE			Absorbance				TRE	PRE	(up to 3 h)	(up to 96 h)
			with $CaCl_2$	with $CaCl_2$	with NaCl	with $CaCl_2$	without salt	with NaCl	with $CaCl_2$	without salt	without salt		with $CaCl_2$		with $CaCl_2$	with $CaCl_2$		
ID	**Source**	**Biolog® ID**									280 nm	340 nm	280 nm	340 nm				
HL	floc	no ID, *possibly Lampropedia hylina*	+	+					+	+			-	-	+	+	floc-forming	floc-forming
SE	1:1 mixture	no ID, likely *Pseudomonas* spp.	-	-					-	-			-	+	-	-	possibly floc-forming	possibly floc-forming
HJ	floc	*Pseudomonas echinoides*	+	+/-					+	+/-			-	-	+	+/-	floc-forming	floc-forming
SD	RW		+	+					+	+			-	-	+	+	floc-forming	not floc-forming
HK	floc	Aquaspirillum metamorphum	+	+					+	-			+	+	+	-	floc-forming	not floc-forming
SH	floc	no ID, likely *Pseudomonas* spp.	+	+/-					+	+			+	+	+	+	floc-forming	possibly floc-forming
HD	1:1 mixture	*Acidovorax delafieldii*			-	-	-	-	-	-	-	-					not floc-forming	not floc-forming
HB	1:1 mixture	*Achromobacter cholinophagum*			-	-	+	-	-	-	-	-					possibly floc-forming	possibly floc-forming
HH	RW	*Pseudomonas* spp.			-	-	-	-	+	-	-	-					possibly floc-forming	floc-forming
SF	floc	no ID, possibly *Aeromonas* spp.			-	-	-	-	-	-	-	-					not floc-forming	floc-forming
I	RW	*Pasteurella pneumotropica*			-	-	+	-	+	+	-	-					possibly floc-forming	possibly floc-forming
C	1:1 mixture	no ID, ressembles D in appearance and biochemical tests			-	-	-	-	+	-	-	-					possibly floc-forming	not floc-forming
HC	1:1 mixture	no ID, likely *Enterobacter* spp.			-	-	+	-	+	+/-	-	-					possibly floc-forming	floc-forming
A_2	1:1 mixture	no ID, ressembles D in appearance and biochemical tests			-	-	-	-	-	+	+	+					possibly floc-forming	possibly floc-forming
HG	1:1 mixture	no ID			-	-	+	-	-	-	-	-					possibly floc-forming	possibly floc-forming
D	1:1 mixture	*Agrobacterium* like-cystic fibrosis			-	-	-	+	+	+	-	-					possibly floc-forming	not floc-forming
B	floc	*Roseomonas* genomospecies 6			-	-	+	-	-	-	-	-					possibly floc-forming	possibly floc-forming
HF	1:1 mixture	Aquaspirillum metamorphum			-	-	+	-	+/-	-	-	-					possibly floc-forming	possibly floc-forming
SJ	1:1 mixture	no ID, likely *Pseudomonas aurantiaca*							+	+/-					+	+	floc-forming	possibly floc-forming
HI	PME	no ID, possibly *Aquaspirillum putridiconchylium*							+	+/-			-	-	+	+	floc-forming	possibly floc-forming
HE	1:1 mixture	*Acidovorax delafieldii*							+	+			-	-	+	+	formed good flocs	possibly floc-forming

Note: + stands for floc-forming bacteria
+/- stands for weak floc-forming bacteria
- stands for non-floc-forming bacteria

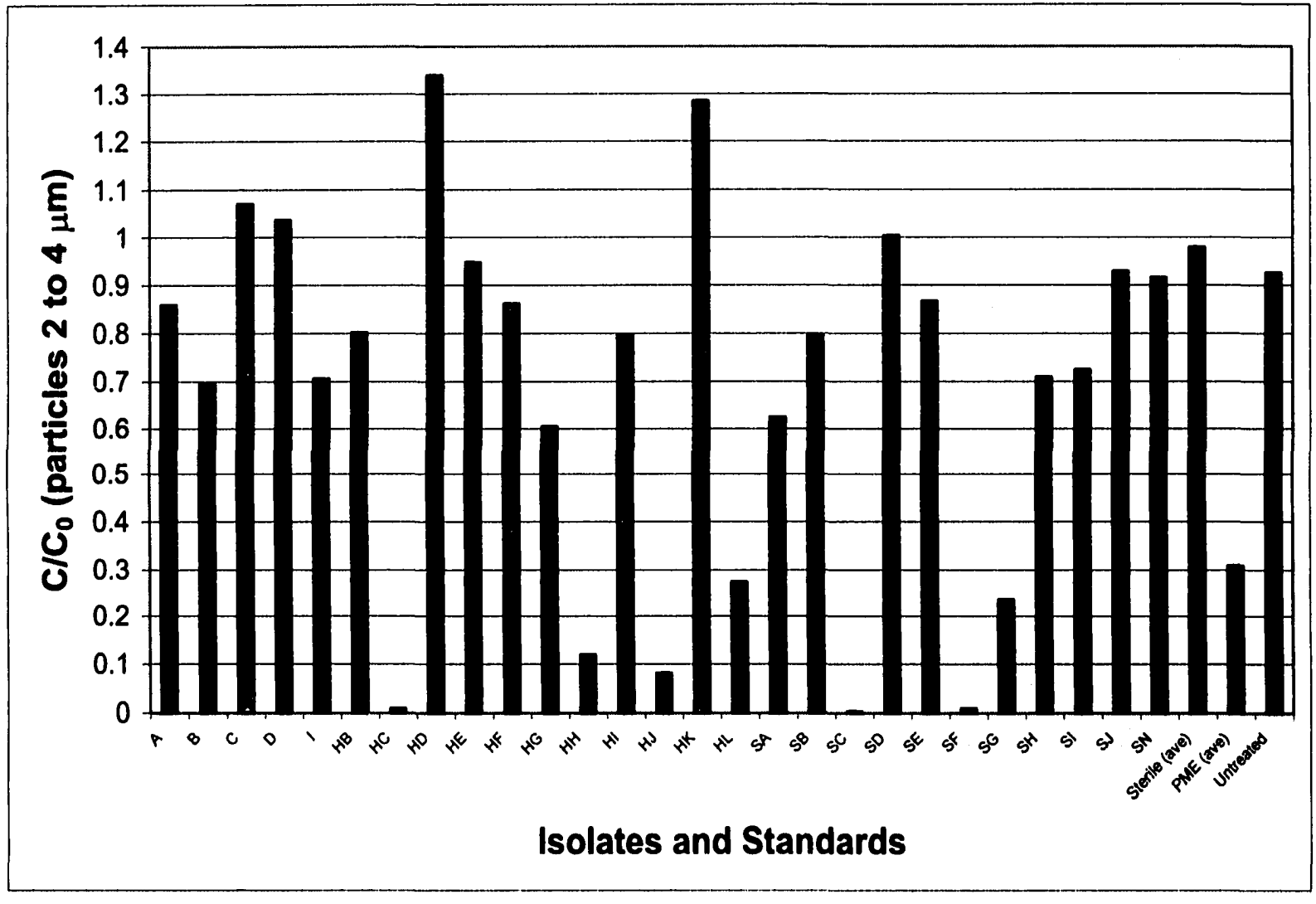


Figure 58. Effect of bacteria on flocculation of fine particles (2 to 4 μm). C_0 is the initial concentration of particles. C is the concentration after 4 days bioflocculation (Joyce, 1999)

4.6 Effect of PMEICF on Dissolved Oxygen

The most significant concern facing the river management is whether PMEICF causes low DO in RW or benthic sediments. This issue was addressed using long-term biochemical oxygen demand (BOD) tests. In this study, CBOD was measured for samples of PME, RW, a mixture of PME and RW (1:1 by volume) and a bottom sludge formed from the mixture. The 25-day CBOD plots reveal the detailed variation of CBOD versus incubation time, and the 140-day CBOD profiles provide overall perspective of the rate and degree of biodegradation of organic matter in the samples. Without a lag period, CBOD exertion curves were adequately fitted by the first order model:

$$CBOD_t = CBOD_u (1 - e^{-kt}) \quad (3)$$

where $CBOD_t$ is the load at time t, $CBOD_u$ is the ultimate CBOD, and k is the rate constant. With a lag period, CBOD exertion curves were adequately fitted by

$$CBOD_t = CBOD_u (1 - e^{-k(t-\emptyset)}) \quad (4)$$

where $\emptyset$ is the lag time in days. Both the least square method and the maximum likelihood method can be used for estimating model parameters. 95% joint confidence regions can be plotted to determine the accuracy of parameter estimates.

Figure 59 to Figure 62 show that CBOD was not affected by flocculation time, but rather by incubation time. For RW samples Figure 59 shows a one-day lag period followed by a period of high rate of DO use and a period of lower rate of DO use. The lag time is due largely to the low seed concentration, or the time required for the seed

culture to become acclimated to the RW samples. Once the acclimation is accomplished, substrate degradation proceeds in a logarithmic fashion. CBOD exertion reaches a plateau after 7 days, and the ultimate CBOD is about 1.4 mg/L.

For PME samples Figure 60 shows a continuous increase in CBOD up to the termination of the experiment (140^{th} day). The ultimate CBOD of about 2.1 mg/L occurred at the 140^{th} day. The plots are relatively straight for the first 17 days revealing that bacteria culture was using more DO in the early part of the test. It is obvious that the continuous increase in CBOD for PME is attributed to high molecular weight lignin derivatives. Although lignin derivatives are more refractory than polysaccharides or amino acids, they are still not inert and undergo degradation under aerobic conditions. No lag period is evident from Figure 60. It can be explained by the fact that the seed cultures were concentrated and grown for a month in PME with aeration. Thus, they had already become acclimated to the environment. In addition, PME contains bacteria, which compensated for a low seed concentration or poor seed quality.

For samples of a mixture of PME and RW with volume ratio of 1:1, Figure 61 shows a one-day lag period followed by a period of high rate DO uptake and a slower DO uptake phase. CBOD exertion gradually tapers off, but at a lower rate when compared to pure PME. It reached a plateau after 40 days. An ultimate CBOD of about 1.6 mg/L was evident.

For samples of bottom sludge formed from a mixture of PME and RW with volume ratio of 1:1, Figure 62 shows the mixture of a two-day lag period. The CBOD reaches a plateau after 7 days, and an ultimate CBOD is about 3 mg/L for 1:5 dilution and approximately 7.5 mg/L for 1:2 dilution. This consistently points out that an ultimate

CBOD for undiluted bottom sludge is about 15 mg/L. This indicates that there was no toxicity effect in BOD samples. Therefore, no microtoxicity test was conducted. The CBOD results determined from Figure 59 to Figure 62 were summarized in Table 20.

Table 20 Long-term CBOD results (a lag time was taken out)

Sample ID	$CBOD_5$ (mg/L)	$CBOD_{21}$ (mg/L)	$CBOD_{100}$ (mg/L)	Ultimate CBOD (mg/L)
RW (t = 0 h)	1.20	1.35	1.35	1.35
RW (t = 0.5 h)	1.20	1.35	1.35	1.35
PME (t = 0 h)	0.44	1.38	1.88	2.00
PME (t = 0.5 h)	0.44	1.50	2.00	2.12
Mixture[1] (t = 0 h)	1.13	1.30	1.50	1.65
Mixture (t = 0.5 h)	1.13	1.30	1.50	1.65
Bottom Sludge[2]	12.40	14.80	15.00	15.00

1. Mixture consists of PME and RW (1:1 by volume)
2. Bottom sludge were formed from samples of a mixture of PME and RW (1:1 by volume)

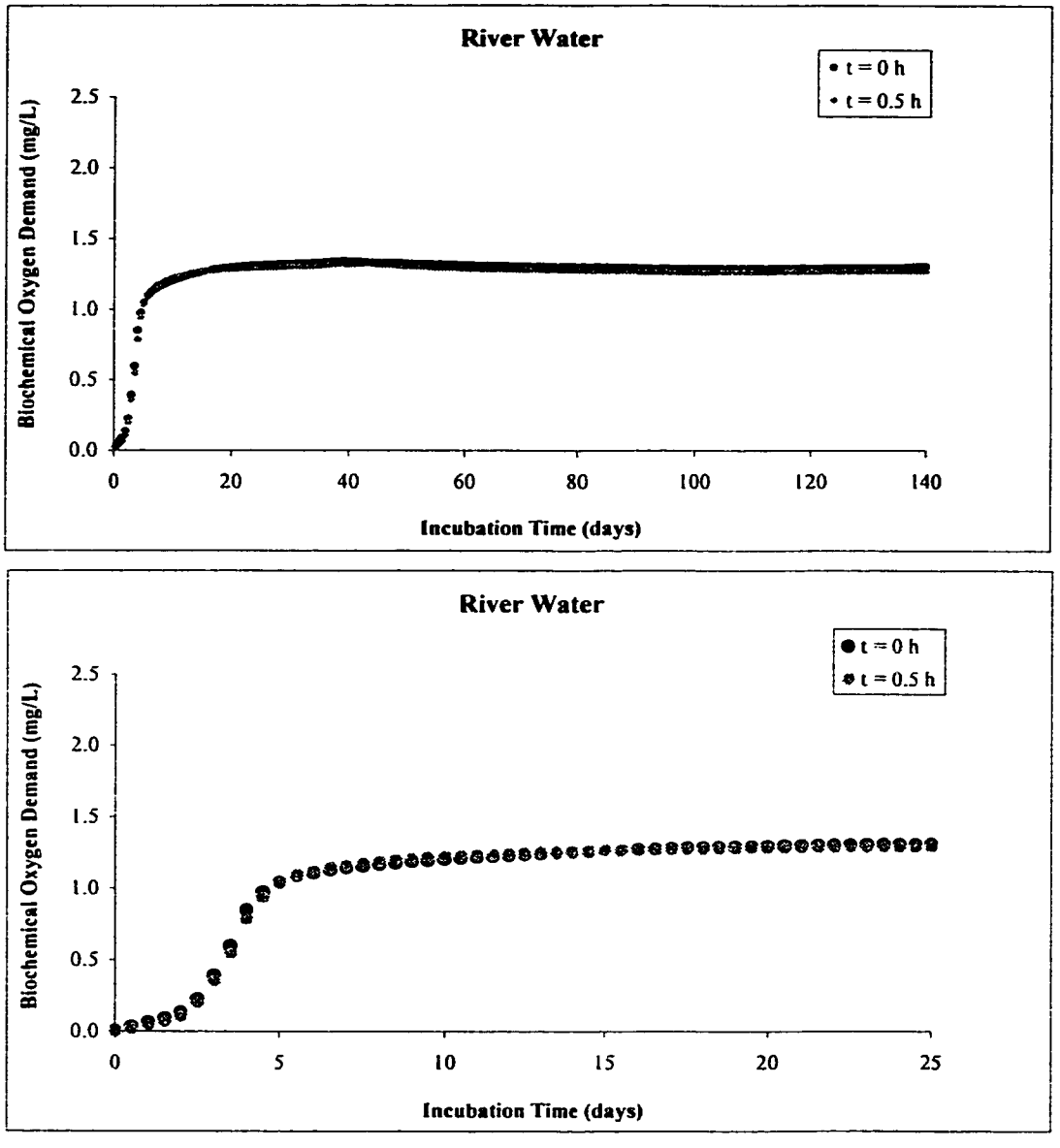


Figure 59. CBOD for RW samples before flocculation (t = 0 h) and after flocculation (t = 0.5 h)

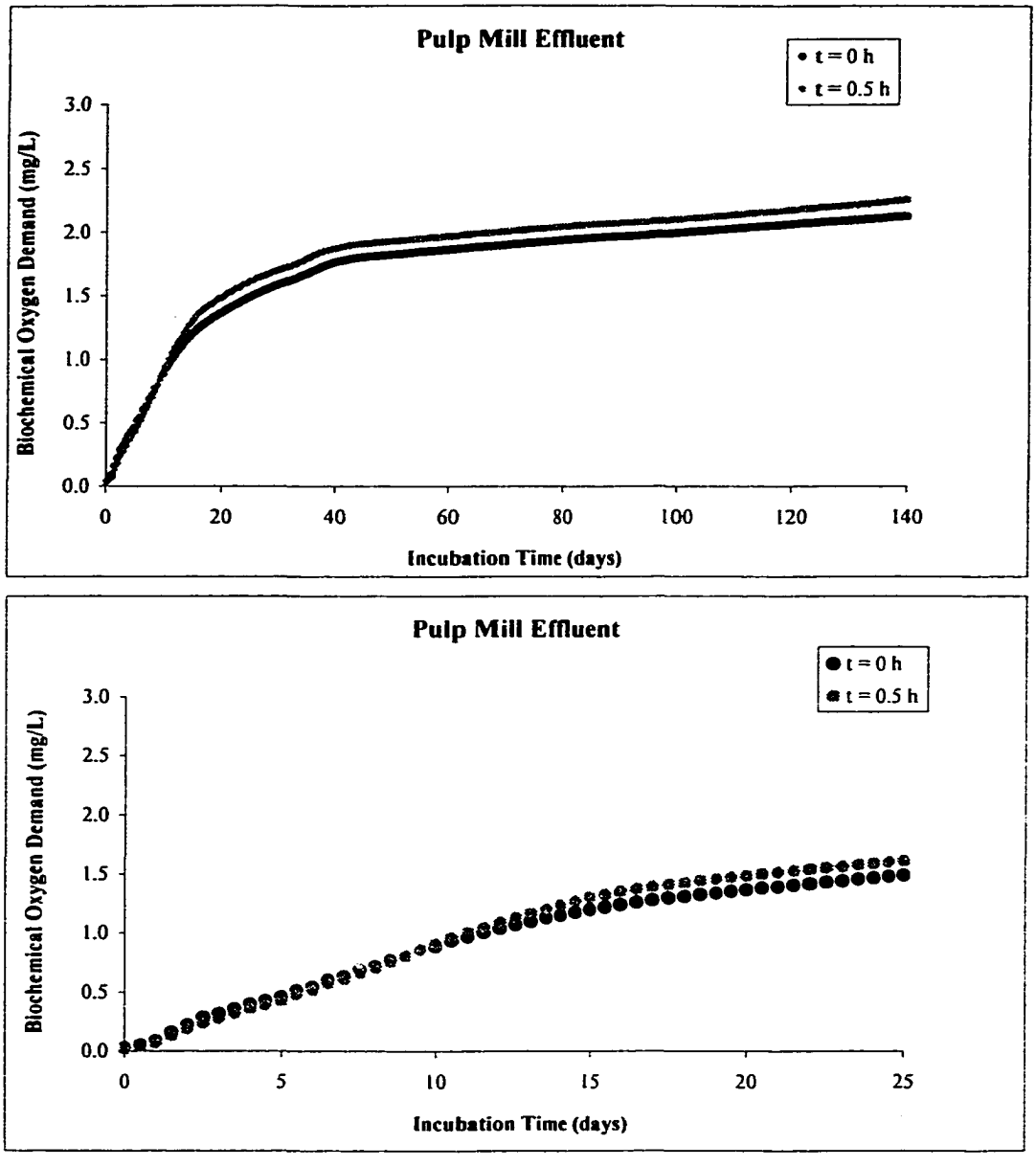


Figure 60. CBOD for PME samples before flocculation (t = 0 h) and after flocculation (t = 0.5 h) (top: 140-day CBOD profile, bottom: 25-day CBOD profile)

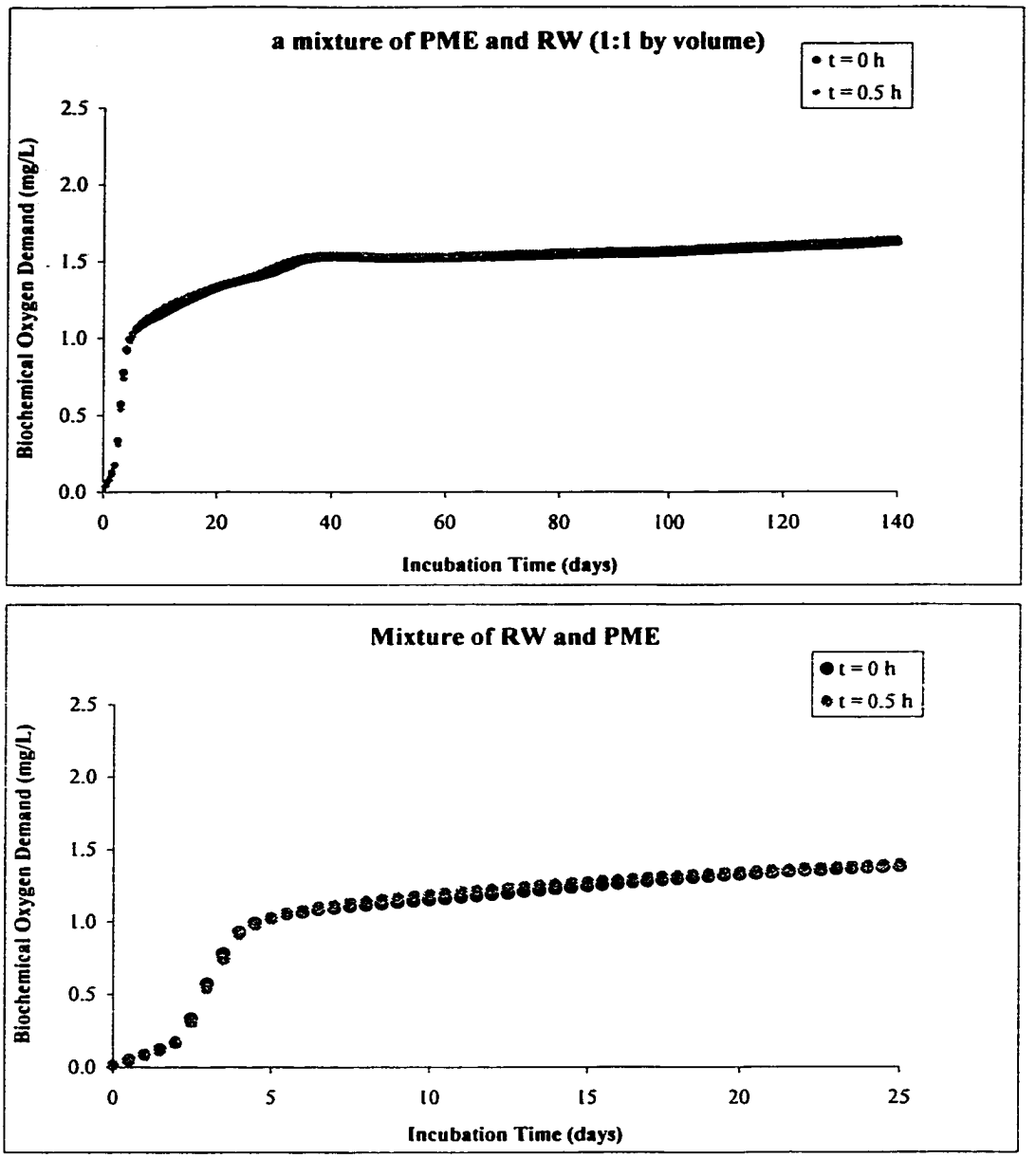


Figure 61. CBOD for samples of a mixture of PME and RW (1:1) before flocculation (t = 0 h) and after flocculation (t = 0.5 h) (top: 140-day CBOD profile, bottom: 25-day CBOD profile)

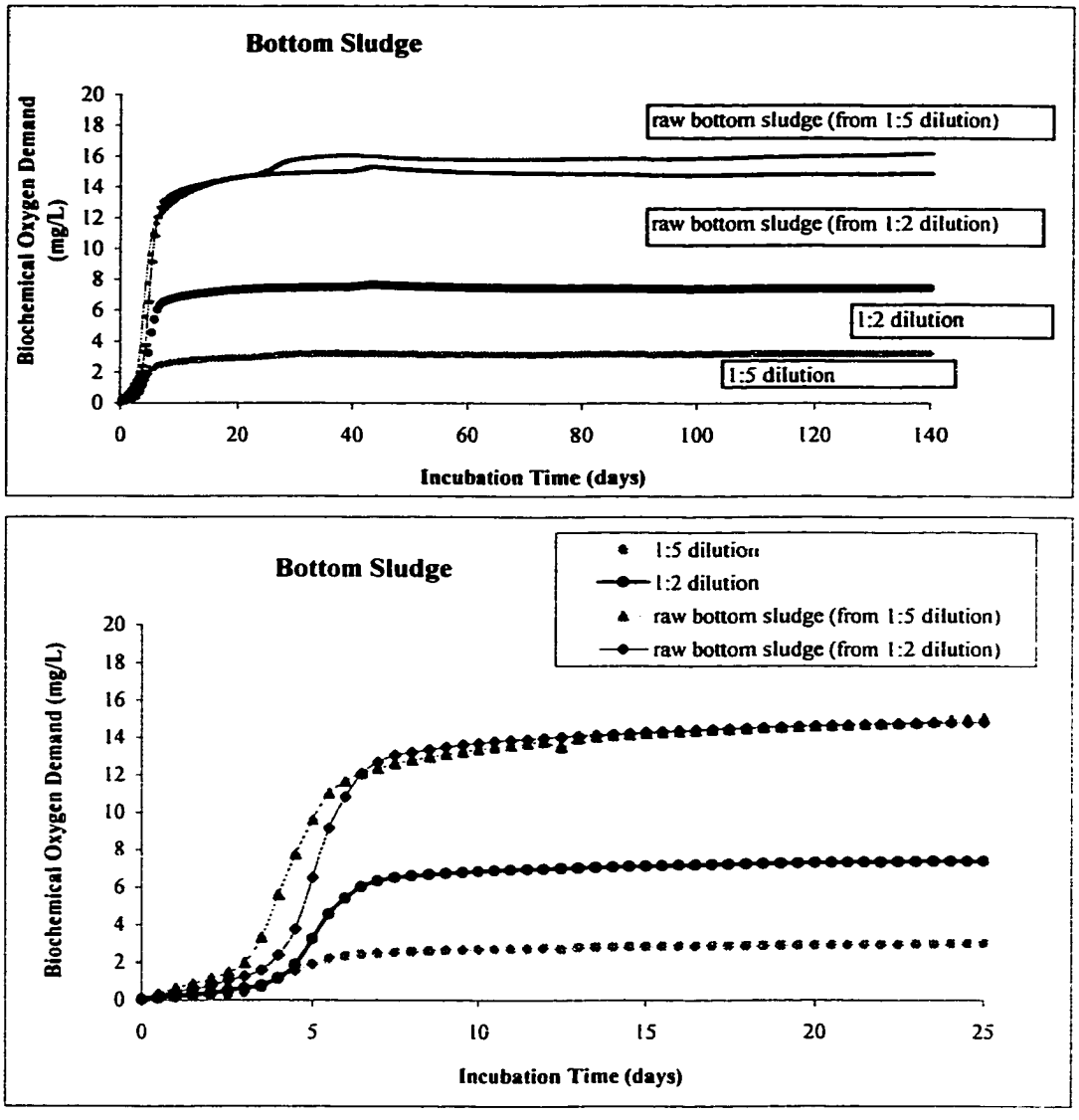


Figure 62. CBOD for the samples of bottom sludge before flocculation (t = 0 h) and after flocculation (t = 0.5 h) (top: 140-day CBOD profile, bottom: 25-day CBOD profile)

It should be emphasized that CBOD is affected by temperature, pH, salinity, the type and concentration of substrate and viable microorganisms, and the quantity and quality of nutrients, trace metals and vitamins. Care should be taken when CBOD results are compared. The CBOD results obtained from this study were from the same experimental conditions, except for different type and concentration of substrate. As found in Table 21, CBOD results for a mixture of suspension and sludge are comparable. This is because the substrate was the same and total solids of samples were fairly close, although the sludge had much higher total suspended solids. After comparing CBOD results it was found that when TS of the samples are similar, CBOD for the sludge was 9 times higher than the suspension. This indicates that bottom sludge had higher concentrations of biodegradable contaminants that caused a high rate of DO depletion during biodegradation, forming either innocuous molecules or relatively persistent and toxic intermediates.

In aquatic ecosystems this indicates that bottom sediments are the primary contaminant sinks, which may cause DO reduction at river bottoms. Bottom sediments may undergo mixing, mineralization, accumulation, biodegradation and resuspension. Biodegration and resuspension of bottom sediments may exert high BOD that may cause DO reduction. The resuspension of bottom sediments and other processes such as direct uptake of contaminants in bottom sediment by biota, bioturbation (biological disturbance), and re-establishment of new equilibria with overlying water can prolong the time period during which contaminants in bottom sediments remain bioavailable and thus bioaccumulate (Allan, 1986).

This study found that CBOD exertion for the bottom sludge is much higher than a suspension with the same chemical composition and similar total solids concentration. This may affect DO in rivers or river bottoms if the river assimilative ability is low, and may negatively affect biological health.

Table 21. Characteristics of samples for CBOD tests (March 29, 2000)

Sample ID	Color (cu) (duplicates)	TS[3] (mg/L) (triplicate)	TSS[4] (mg/L) (triplicate)	COD (mg/L) (triplicate)	AOX (mg/L) (triplicate)
RW (t = 0 h)	4.11	240.0	41.33	7.30	0.0247
RW (t = 0.5 h)	4.11	234.3	26.67	5.21	0.0213
PME (t = 0 h)	1111.90	2013.3	26.67	718.60	8.9568
PME (t = 0.5 h)	1106.30	2016.0	25.33	730.00	9.3165
Mixture[1] (t = 0 h)	541.80	1106.0	38.67	360.90	4.3028
Mixture (t = 0.5 h)	556.70	1123.3	17.33	357.70	4.6011
Bottom Sludge[2]		1172.0	60.00		

1) Mixture consists of PME and RW (1:1 by volume)
2) Bottom sludge was formed from samples of a mixture of PME and RW (1:1 by volume)
3) TS = total solids
4) TSS = total suspended solids

DO is essential for all aerobic aquatic organisms. It determines microbial activities, the rate of chemical reactions and biological degradation. DO is generally determined by temperature, streams oxygen demand and the river assimilative ability. A streams oxygen demand is determined by decomposition of organic and inorganic matter. Other factors that may affect the streams oxygen demand are photosynthesis and respiration of river biota (bacteria, fungi, algae, higher aquatic plants and invertebrates), resuspension of benthic sediments, inputs of groundwater or tributary water, and inputs of oxygen-consuming effluent from industrial and municipal discharges. The river

assimilative ability largely depends on river hydraulic conditions. The river hydraulic conditions undergo significant seasonal variations. A higher river flow provides more mixing and better dispersion and reaeration. This may prevent DO reduction in the water column. However, higher river flow may also cause DO reduction due to resuspension (erosion) of bottom sediment flocs that release biodegradable chemicals, refractory compounds and toxic materials.

DO in rivers may vary substantially through the year. In a warm climate, biodegradable organics can lead to lower DO concentrations due to lower saturation capacity of water and higher numbers of aquatic organisms and plants. However, in a cold climate, low DO is particularly evident during the winter when ice cover restricts reaeration of oxygen-depleted waters, and a large number of deposited flocs are retained on river bottoms. It has been reported that in the Athabasca River the lowest DO concentration occurred during the winter when ice cover restricts reaeration (Lowell and Culp, 1996). It has also been reported that PME had adverse effects on DO concentration (and phosphorus) in the Athabasca and Wapiti-Smoky Rivers during the winter at the low-flow conditions (Culp and Chambers, 1994).

In conclusion, benthic sediment flocs exerted approximately 9 times higher CBOD than that of the suspension with same chemical composition and total solids concentration. This may affect DO concentrations in RW or at river bottoms if the river assimilative ability is low.

The accumulation of organic matter is great in the benthic sediment layer. The dissolved oxygen may become exhausted. This may lead to anoxic conditions at river bottoms near pulp mill outfalls. A sequence of various redox reactions with organic

matter may occur in the benthic sediment layer. For example, cellulose can undergo β-bond transformation in anoxic conditions forming glucose. Glucose undergoes biodegradation by bacteria using oxygen. This may cause significant DO reduction at river bottoms, especially in the winter during ice cover.

Low DO may negatively affect fish eggs, aquatic organisms and habitats. It may also affect biodiversity and reproductivity of bottom dwellers such as benthic invertebrates that are a major source of food for northern river fish (Gifford, 1994; Lowell and Culp, 1996; Maldiney and Mouchel, 1995). Further research needs to be conducted to evaluate the DO concentrations, oxidation reduction potential, pH, conductivity in RW and at river bottoms near pulp mill outfalls. If DO reduction is evident, measures need to be taken to minimize the degree of PMEICF. Removal of cations from PME may be one of the options. Reverse osmosis, ion exchange or electrodialysis is suggested.

4.7 Mechanisms for PMEICF Formation

Based on the experimental results it was postulated that sorption, coagulation, flocculation and bioflocculation are the major mechanisms that contributed to PMEICF formation.

Sorption is an essential process for PMEICF formation. It depends on the hydrophobicity of PME chemicals and concentration of PME chemicals and particulate matter. When PME is discharged into rivers, particulate matter in RW offers a site for sorption. The hydrophobic organic PME chemicals tend to be adsorbed onto the surface of suspended particulate matter, or become associated with or incorporated into other organic particulate phases both biotic and abiotic, including organic colloids.

Sorption in the field is usually much more complex. It occurs on several substrates in a variety of ways, involving physical sorption, chemical sorption, biological sorption, ion exchange, polymerization and complexation. Physical, chemical and biological sorptions are the most important factors controlling the partitioning of substances between dissolved and solid phases. Physical sorption is attributed to van der Waals forces, ion-dipole interactions, dipole-dipole interactions or by hydrogen bonding. Cations in PME tend to associate with particulate matter by hydrogen bonding or ion-dipole interactions. For nonpolar or slightly polar PME organic chemicals, hydrophobic bonding is more important (hydrophobic bonding refers to an affinity for positioning at a water phase boundary). Chemical sorption is attributed to bonds between ions in solution and the surface of particulate, and condensation reactions with OH^- groups in lignin and some other organic compounds. Biological sorption is attributed to EPS through bridging mechanism and is related to algae, bacteria and higher level aquatic organisms. Ion exchange is attributed to negative or positive charged lattice compensation by negative or positive ions from solution. Polymerization may be attributed to photolysis. Complexation may be attributed to cations from PME (Ca^{2+} and Mg^{2+}) and natural organic compounds such as humic substances, lignin and fibrilic materials (Santos and Duarte, 1998). Complexation can cause greater release of cations from sediments. It is necessary to keep in mind that formation of metal/organic complexes in solution may enhance adsorption if the organic ligands are adsorbed onto mineral surfaces. On the other hand, it may prevent adsorption if the complexing ligands form strong metal/ligand complexes that are not adsorbed onto mineral surfaces.

PME caused coagulation in RW, as many pin point flocs formed as soon as PME was added into RW samples. Coagulation induced by Na^+ and Ca^{2+} compressed the double layer and destabilized the negatively charged particles. Coagulation is significantly affected by ionic strength (pH and alkalinity have slightly effects). The ionic strength depends on the salt concentration, which is affected by the river flow and dilution. Higher salt concentrations occur in the winter when the river flow is low.

Flocculation (dominated by orthokinetic flocculation) was induced by PME-related organic polymers through bridging mechanisms, including fibers, hydrolytic lignin, protein, sucrose, cellulose and starch. Degree of flocculation is determined significantly by the concentration of cation and polymer, and mixing intensity.

Bioflocculation is induced by EPS secreted by bacteria. Degree of bioflocculation is determined significantly by EPS concentration, nutrient availability, and environmental conditions (pH, alkalinity, ionic strength and hydraulic conditions).

EPS concentration is one of the most critical factors (Droppo *et al.*, 1997; Droppo and Ongley, 1994). Floc formation upstream of the outfall was insignificant due to the lower EPS concentrations, although large quantities of bacteria were present in RW (Burns, 1982; Chróst, 1991; Confer and Logan, 1997). However, floc formation downstream of the outfall was significant due to higher concentrations of EPS and cations discharged from PME. It should be emphesized that the EPS concentration in rivers was not possibly high enough to limit free sites available for EPS and hinder bioflocculation. This was true even when rivers were in oligotrophic environments (nutrient deficient) where more EPS were secreted by copiotrophs (need more nutrients) that enter into a starvation-survival phase (Humphrey *et al.*, 1983).

Nutrient availability was essential for bioflocculation. The ability of some bacteria to secrete EPS is also controlled by the nutrient regime. Nutrient deficiency was found to promote bioflocculation. Lower nutrient concentrations occurred when bacteria were discharged into rivers from pulp mill lagoons. Bacterial strains respond to low nutrient conditions differently (Kjelleberg and Hermansson, 1984). Copiotrophs (micro-organisms requiring large amounts of nutrients) may enter into a starvation-survival phase, particularly in oligotrophic environments where nutrients are deficient (Humphrey *et al.*, 1983; Kjelleberg and Hermansson, 1984). When bacteria enter a starvation-survival phase, they undergo dwarfing, fragmentation and adsorption to each other or other surfaces. Dwarfing reduces the size of bacteria, and fragmentation increases the individual cell number, but without growth (Mueller, 1996). Bacteria tend to attach to organic matter or sediments, especially when they undergo starvation (Goulder, 1977; Rao *et al.*, 1991). Starved bacteria appear to have a greater affinity for adhesion (Mueller, 1996), as they swim faster and go shorter lengths in random free runs. It is crucial that bacteria are considered stickier and will attach to organic matter more readily in the starvation phase (this attachment could be used as a bacteria survival mechanism, (Mueller, 1996). This is supported by the statement that bioflocculation occurred most readily in an endogenous growth phase (Pavoni *et al.*, 1972).

Environment conditions play important role in bioflocculation (Flemming, 1993; White, 1995). The hydrophobicity and binding affinity were increased under low nutrient and high mixing intensity (Lazarova *et al.*, 1994), which were the predominate conditions when bacteria were discharged into river.

A mechanism for EPS-induced bioflocculation is postulated below. EPS are anionic (Geesey, 1982), containing the functional groups -COO^- and OH^- (Sanin and Vesilind, 1996), which mediate the interaction between cations, such as Ca^{2+} and Na^+ (Biddanda, 1985; Leppard, 1985). EPS have anchoring abilities (Biddanda, 1985; Geesey, 1982; Muschenheim *et al.*, 1989), and provide cohesion and structural integrity to microorganisms (Flemming, 1993). This provides their ability to transfer colloidal particles into suspended solids, and small sized bioflocs, and ability to agglomerate them into large sized flocs or aggregates through bridging mechanism. EPS can also complex and detoxify metal ions (Geesey, 1982; Leppard, 1986), and interact with contaminants, transport pollutants and enzymes downstream of rivers (Leppard, 1986).

The morphology study revealed the existence of individual EPS, which bundled in bacteria-bacteria association, bacteria-clay particle association (especially colloidal particles), and assisted in forming bioflocs and field flocs. Although EPS is the major contributor for bioflocculation, it is necessary to emphesize that they are not necessary for bioflocculation to occur (Friedman and Dugan, 1968). Many bacteria species are known to form flocs, but not produce EPS. These bacteria may induce bioflocculation if the flocculation time is long enough. As proven in this study, SF were not floc-forming bacteria based on short-term flocculation tests, but were floc-forming based on long-term flocculation tests. This demonstrates that bioflocculation was not solely due to the presence of EPS, but also due to the bacteria type.

As mentioned earlier, EPS are anionic. The existence of cations is essential for bioflocculation with EPS involvement or for some bacteria having a net negative charge (Busch and Stumm, 1968; Levy *et al.*, 1992; Rao *et al.*, 1991; Sanin and Vesilind, 1996;

van Leussen, 1988). However, it was found in this study that cations have antagonistic effects on biofloccualtion. It may enhance or inhibit biofloccualtion, depending on the bacteria type.

In conclusion, PMEICF formation was attributed to PME-related cations, organic polymers, biopolymers and microorganisms. EPS has ability to transfer colloidal particles into suspended solids. Cations (Na^+, Ca^{2+}) destabilize particles by compressing the diffuse layer surrounding the colloidal particles. Organic polymers (fibers, hydrolytic lignin, protein, sucrose, cellulose and starch) and biopolymers (EPS) induce flocculation by binding destabilized particles together through bridging mechanism, forming large sized settleable flocs or aggregates. It was also found that bioflocculation was responsible for the removal of sub-micron particles, and PMEICF was more relevant to the removal of small sized particles, especially with sizes less than 10 μm.

5 CONCLUSIONS

This study includes: 1) confirmation of PMEICF occurrence in laboratory and field, 2) evaluation of sampling methodology extensively within 11 consecutive days, 3) isolation and identification of PME-related or RW-related bacteria using Biolog® identification system, 4) morphology studies for observation of ultrastructures of bacteria, EPS, varied means of attachment, bacteria-bacteria association, bacteria-colloids association, biofilms, bioflocs and field flocs, 5) identification of floc-forming bacteria using both short-term (0.5 hours) and long-term (96 hours) bioflocculation tests, 6) identification of PME-related cations and organic polymers attributed to PMEICF formation, 7) fractionation of PME using the Minitan^{TM} ultrafiltration system, 8) determination of MWD of fractionated PME components using HPSEC, 9) determination of the major fractionated PME component attributed to PMEICF formation, 10) determination of affecting factors on PMEICF formation, including physical factors (temperature, mixing, dilution, sample age), chemical factors (pH, alkalinity, isolated PME chemicals, fractionated PME), and biological factors (microbial activities, with some inclusion of high level of aquatic organisms and plants), 11) evaluation of impact of PMEICF on DO in rivers or at river bottoms, and 12) postulation of mechanisms for PMEICF formation.

A confirmatory study was conducted in the laboratory and on two rivers in Alberta that receive the discharge of biologically treated PME. It was proven that PMEICF does occur in the Athabasca River and the Wapiti River, orthokinetic

flocculation dominates flocculation mechanisms, and PMEICF was more prominent in removal of particles with sizes less than 10 μm.

Evaluation of sampling methodology was conducted within eleven consecutive days using both fresh samples (< 20 h) and preserved samples (≤ 11days). It was found that preserved samples (≤ 11 days) were as valid as daily fresh samples for flocculation tests without significant biological reactions (the conclusion may still be effective for sample age greater than 11 days, but it was not evaluated in this study). It was suggested that the most cost-effective and reliable approach was to use preserved samples (≤ 11 days) for the preliminary studies without significant biological effects, and fresh samples for the studies that included biological reactions, and for replicates and final data collection.

The bacteria in either PME or a mixture of PME and RW were found to be *Aquaspirillum* spp., *Acidovorax* spp., *Pseudomonas* spp., *Enterobacter* spp., *Comamonas* spp., *Brevundimonas* spp., *Flavobacterium* spp., *Achromobacter* spp., *Pastuerella* spp., and *Aeromonas*.

Morphology observations revealed varied means of attachment of some bacteria that may assist in adsorption and floc formation. The attachment mechanisms included adhesive stalk formation, star formation, capsular secretions and fibrillar appendages. These cause bacteria-bacteria association and bacteria-colloids association, leading to the formation of biofilm, bioflocs and field flocs. The fact that bioflocs consisted largely of colloidal sub-micron particles suggested that bioflocculation was the primary factor in transferring dissolved colloids into suspended particles or flocs. The ultrastructure of field flocs revealed that they consisted largely of clay particles, fine flocs, bacteria, EPS,

fibers, diatoms, aquatic organisms (such as rotifers, stalks, flagellates, suctorians, testate amoebae, daphne and copepods), and aquatic plants (such as diatoms).

Floc-forming bacteria were identified using both short-term (0.5 h) and long-term (96 hours) flocculation tests. The isolated bacteria suspensions (with or without ID) were incubated at 30°C for 4 days, and then added into either RW samples or samples of a mixture of PME and RW (1:1 by volume). Short-term flocculation test results suggested that microorganisms identified as HL, HJ, SD, HK, SH, SJ, HI and HE were floc-forming bacteria. Microorganisms identified as SE, HB, HH, I, C, HC, A_2, HG, D, B, HF were possibly floc-forming bacteria, and microorganisms identified as HD and SF were not floc-forming bacteria. Results from long-term flocculation tests suggested that microorganisms identified as HC, HL, HJ, HH and SF were floc-forming bacteria (*Pseudomonas* spp., *Enterobacter* spp. and *Comamonas testosteroni.* were possibly involved in floc formation), SE, SH, HB, I, A_2, HG, B, HF and D were possibly floc-forming bacteria, and SD, HK, HD and C were not floc-forming bacteria. Both short-term and long-term tests identified HJ and HL as floc-forming bacteria, and HD was not floc-forming bacteria. Laboratory observation suggested that HJ, HL, HI, HE, or SJ were floc-forming bacteria, as significant number of flocs were evident during bioflocculation tests.

Mechanism studies were conducted using flocculation tests on samples of a mixture of PME and RW (1:1 by volume) to identify substances inducing and factors affecting PMEICF formation, and to postulate the mechanisms for PMEICF formation.

Predominant cations in PME were identified to be Na^+, K^+, Ca^{2+} and Mg^{2+}, using ICP, ICP-AES and ICP-MS. Chemicals attributed to PMEICF formation were found

cations, organic polymers and biopolymers. The cations included Na^{+}, Ca^{2+}. Organic polymers included fibers (with or without acidic or alkaline surface treatment), hydrolytic lignin, protein, sucrose, cellulose and starch. Biopolymers were found to be EPS. Morphology observation confirmed that EPS were significantly involved in the floc formation, and EPS have ability to transfer colloidal particles into dissolve solids. Therefore, PMEICF was more relevant to the removal of small sized particles, especially with sizes less than 10 μm.

PME was fractionated using the Minitan™ ultrafiltration system. Occurrence of separation was confirmed by measurement of MWD using HPSEC for fractionated PME, observation of the significant color differences for fractionated PME, and determination of color, AOX, TOC for fractionated PME. It was found that separation was occurring.

The fractionated PME was added into RW samples for flocculation studies. It was found that all fractionated PME can induce flocculation, but to different extents at different dosages. The fractionated PME with MWCO < 5,000 was the major contributor to PMEICF formation. This further reinforced that cations and organic polymer of polysaccharides, proteins, soluble starch, cellulose and sugar and the polar breakdown products of lignin are the major chemicals inducing flocculation in RW (the MW of these chemicals are MW < 5,000). It was also found that chemicals in the F_4 function as mainly flocculants, salt addition enhanced flocculation efficiency.

Factors affecting PMEICF were confirmed to be pH, alkalinity, ionic strength, temperature, mixing and dilution. Total solids from PME and PRE for the mixture of PME and RW (1:1 by volume) increased with an increase in pH from 5 to 10. Optimum alkalinity existed at which flocculation efficiency reached its maximum value. Ionic

strength changes the chemistry of the samples and affects the configuration of polymer chains. It had antagonistic effects on bioflocculation and PMEICF. The lowest degree of flocculation occurred at 4 to 5 °C and the highest degree of flocculation occurred at approximately 15°C (ranged from 12 to 18°C). Mixing is critical for the PMEICF formation, and orthokinetic flocculation dominates flocculation mechanisms. Higher flocculation efficiency reached at higher dilution. TRE at dilution of 1:1 by volume was in the middle of the TRE range tested; thus, 1:1 was representative.

Mechanisms for PMEICF formation include sorption, coagulation, flocculation and bioflocculation. Sorption is an essential process for PMEICF formation. It was attributed to physical sorption, chemical sorption, biological sorption, ion exchange, polymerization and complexation reactions. Immediate formation of pinpoint flocs as soon as PME was added into RW samples suggested that PME enhanced coagulation in RW. Coagulation was attributed to Na^+ and Ca^{2+}. Na^+ compressed the double layer, and Ca^{2+} or Mg^{2+} brought about charge nutralization. Flocculation, dominated by orthokinetic flocculation, was attributed to fibers, hydrolytic lignin, protein, sucrose, cellulose and starch, which agglomerated the destabilized particles into flocs through bridging mechanism. Salt has antagonistic effect on PMEICF, and its dosages played an important role. Bioflocculation was contributed to EPS secreted from bacteria. EPS contributed to adsorption and floc formation. It also has ability to transform colloidal particles into suspended particles, and bind them together, forming small sized bioflocs or large sized flocs.

The critical finding from this study was that deposited flocs exert approximately 9 times higher CBOD than a suspension with the same chemical composition and similar

solid concentration. This may cause DO reduction at river bottoms if the river assimilative ability is low. This may cause anoxic conditions at river bottoms in the winter during ice cover. To prevent from low DO in rivers, it is necessary to minimize the degree of PMEICF. Desalinating PME (remove cations) may be one of options. Reverse osmosis, ion exchange or electrodialysis is suggested.

6 SUGGESTIONS FOR FUTURE RESEARCH

In the future, the following research is suggested:

1) to determine MWD for F_2 by exploring suitable standards and using simultaneous multiple in-line detectors.
2) to determine the relationship between the mean velocity gradient ($\overline{G_c}$ and $\overline{G_f}$) used in the laboratory and hydraulic conditions near pulp mill outfall and in the mixing zone downstream of the outfall in rivers.
3) to determine DO concentrations, oxygen reduction potential, pH, conductivity at river bottoms near pulp mill outfall at different seasons of the year. Especially attention should be paid in the winter when ice cover restricts reaeration of oxygen-depleted waters.
4) to develop measure to control PMEICF formation. Removal of cations from PME may be one of the options. Reverse osmosis, ion exchange or electrodialysis is suggested.
5) to modify the existing transport models to take account of PMEICF.
6) to adjust effluent treatment technology and discharge regulation to minimize its negative effects. This will facilitate efforts for effective management of river water quality.

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APPENDICES

APPENDIX A

Variation of Characteristics of Samples of Raw PME, RW and a Mixture of Both

Fresh samples were taken from July 19th to 29th, and tested from July 20th to 30th. Most fresh samples were tested less than one day after they were taken from outfall; however, the samples taken on July 23rd were tested on the 26th; the samples for July 24th and 25th were tested on the 27th; and the samples for July 26th were tested on the 28th. Preserved samples were taken on July 19th and tested each day from July 20th to July 30th.

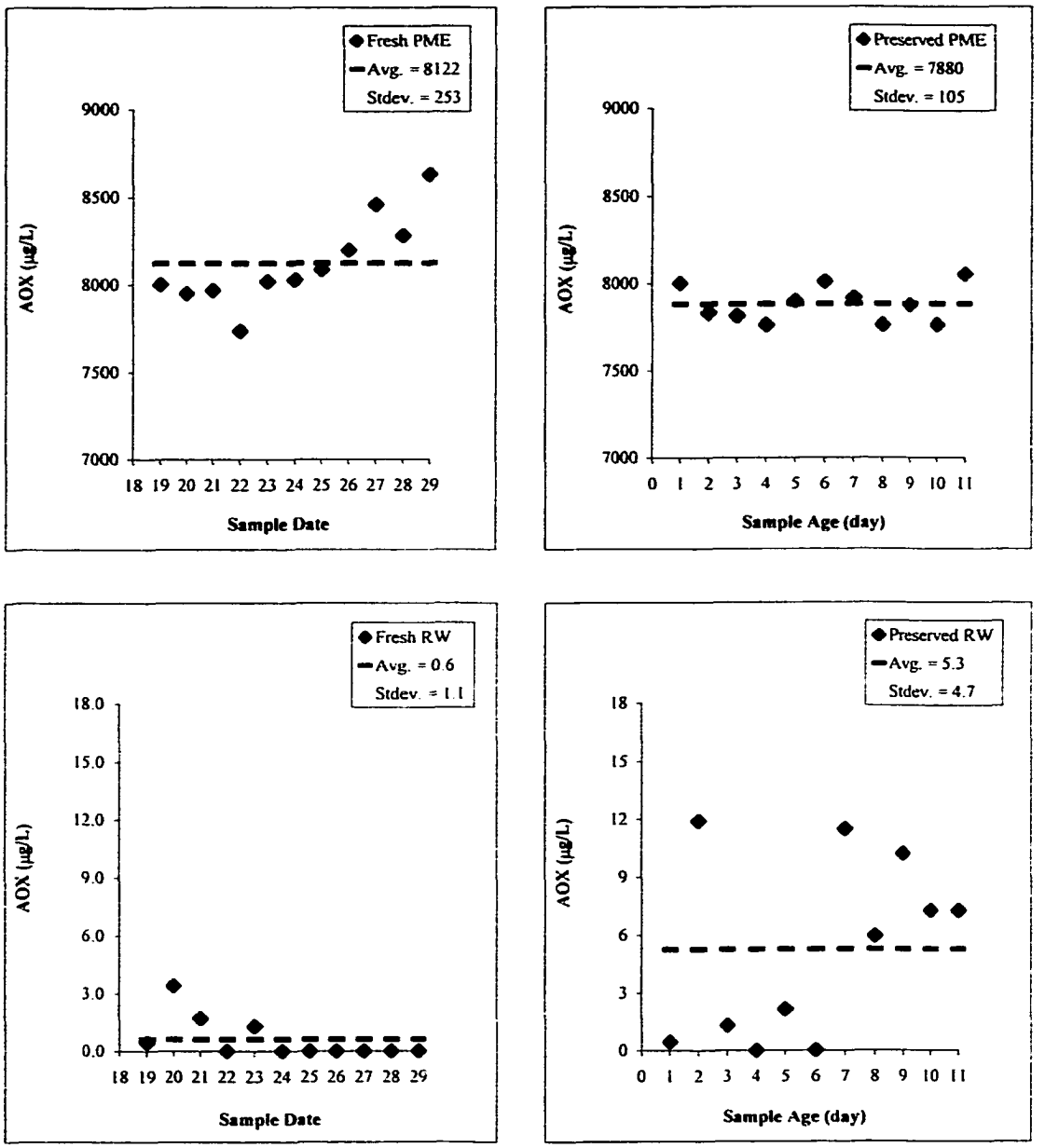


Figure A1. Effect of sample age on AOX for fresh and preserved PME or RW samples

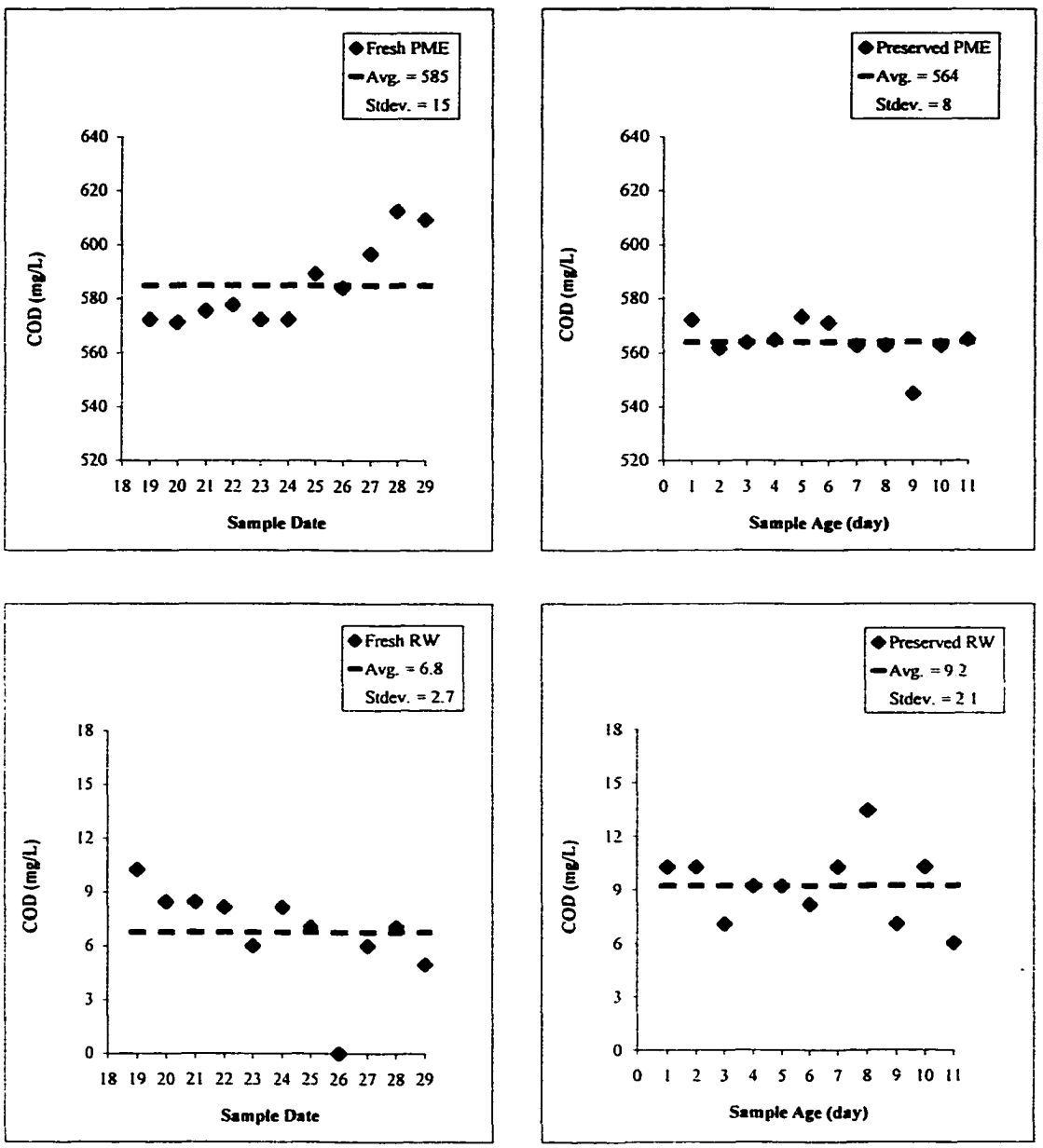


Figure A2. Effect of sample age on COD for fresh and preserved PME or RW samples

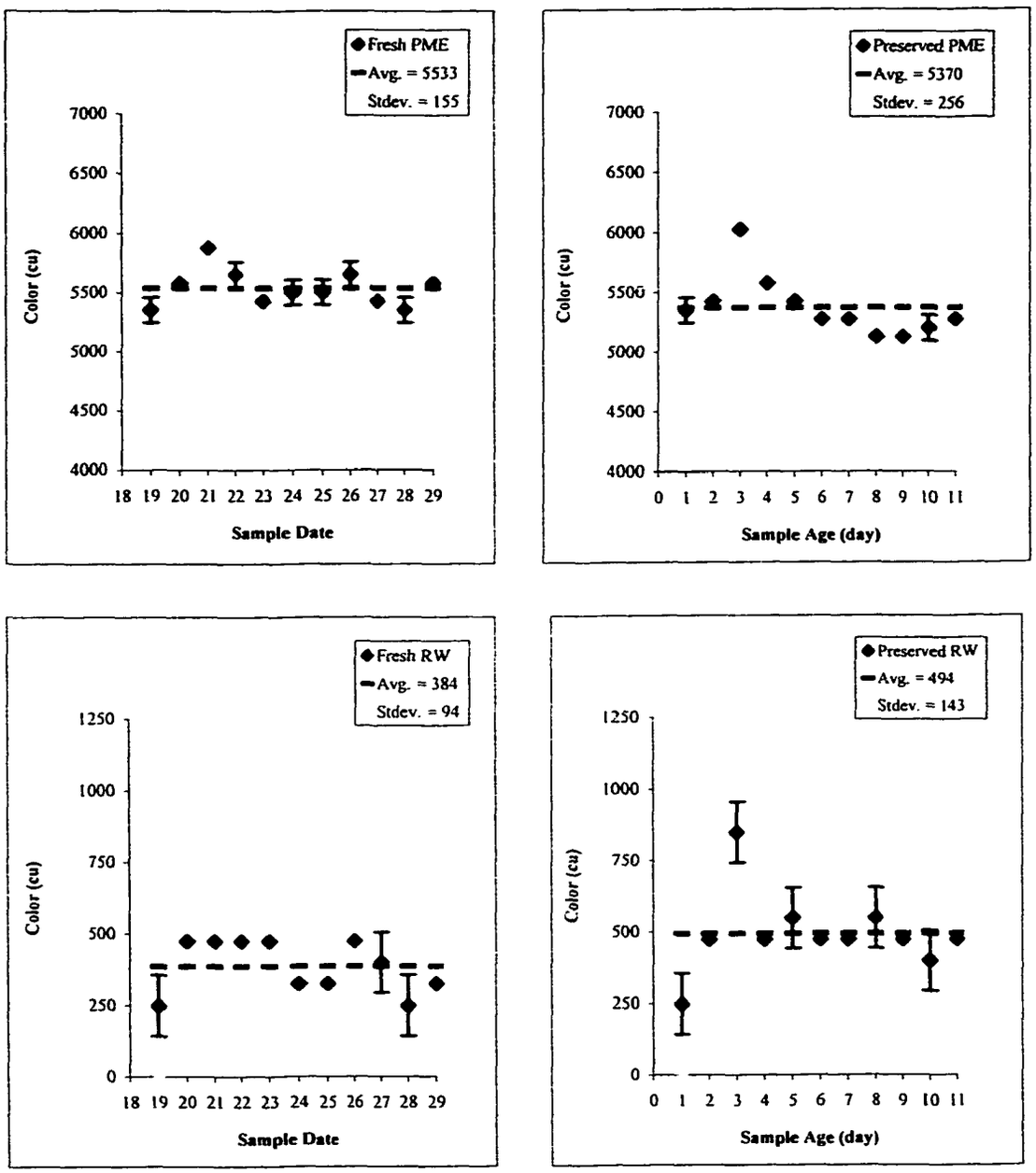


Figure A3. Effect of sample age on color for fresh and preserved PME or RW samples

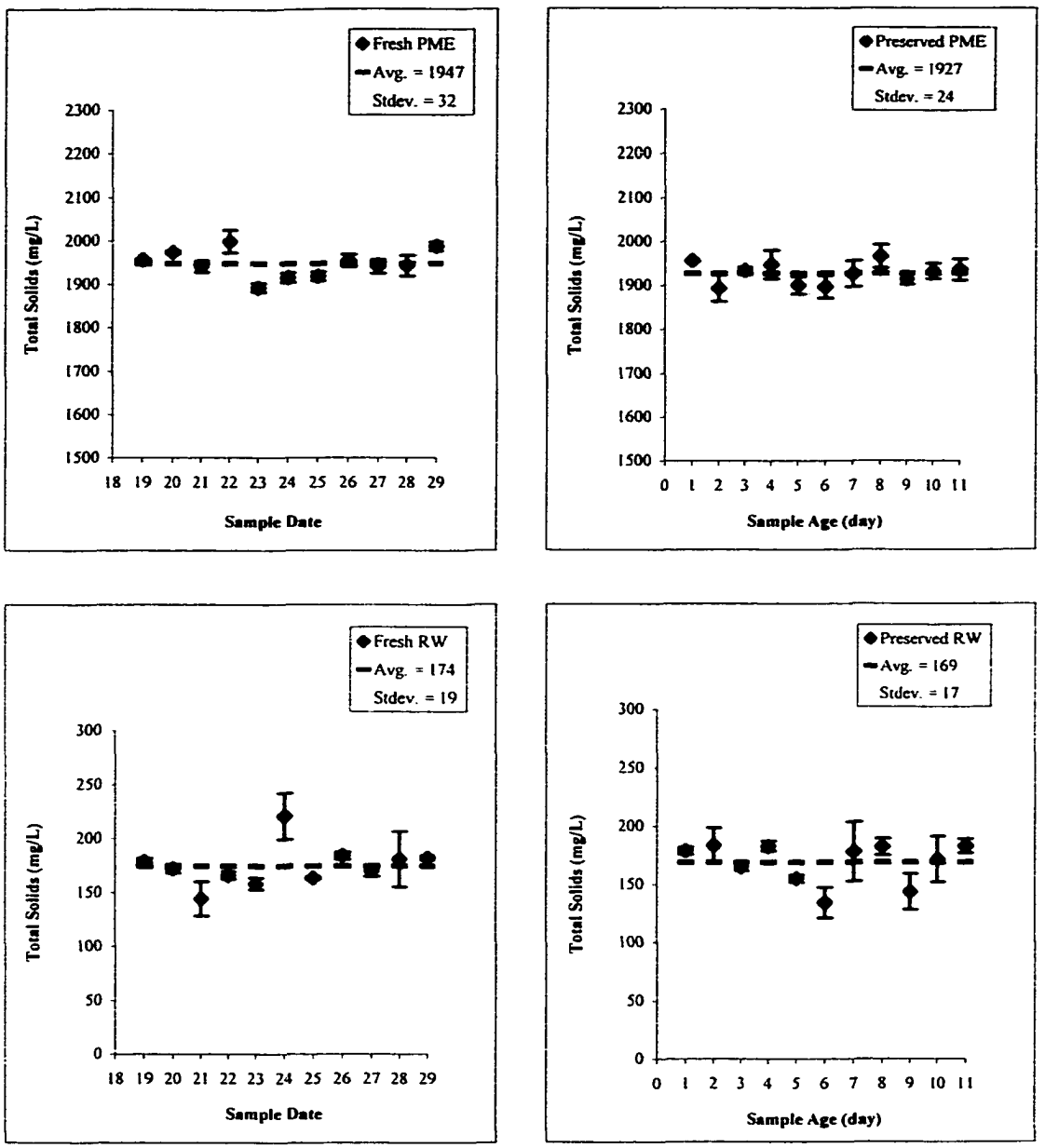


Figure A4. Effect of sample age on total solids for fresh and preserved PME or RW samples

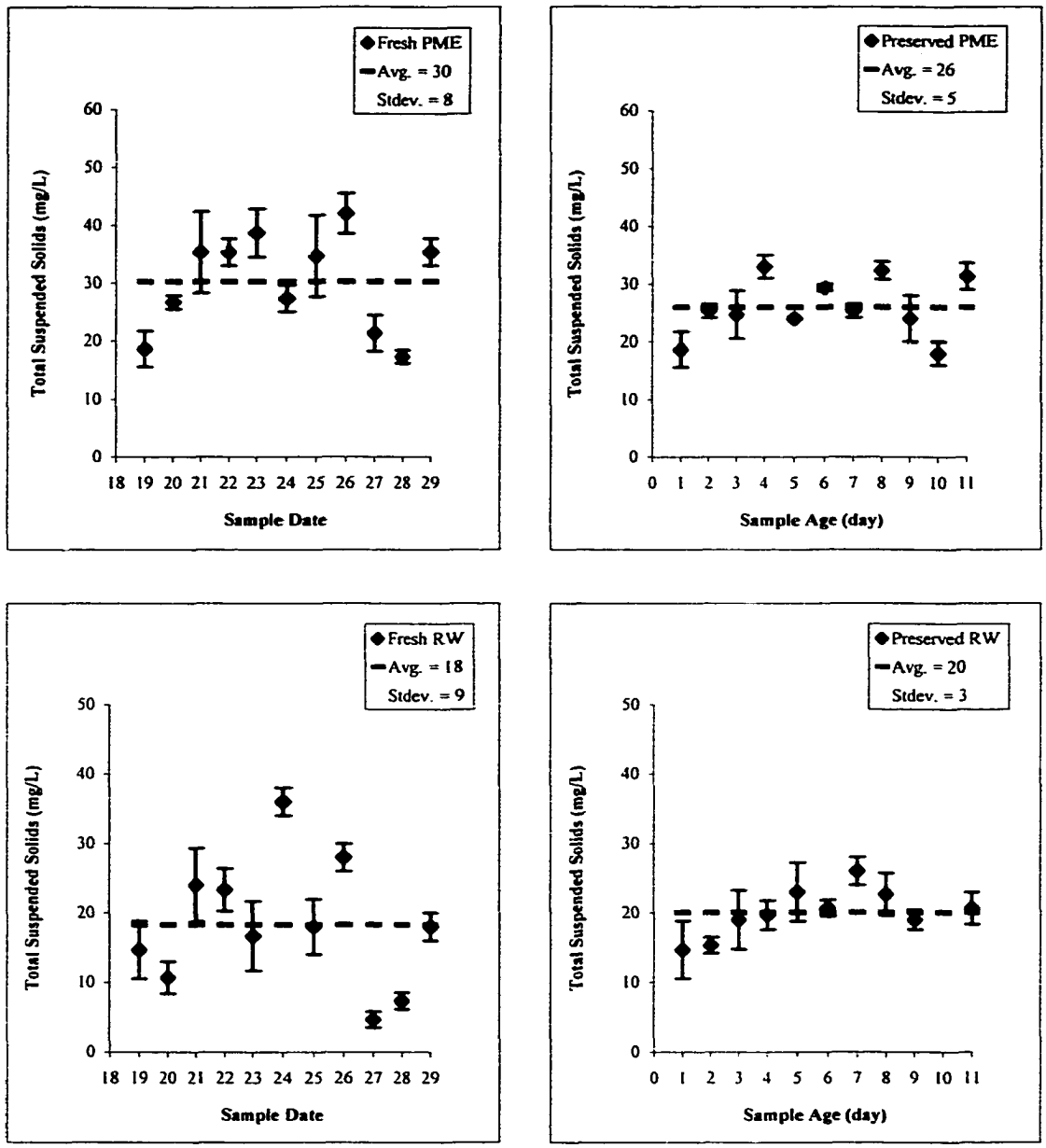
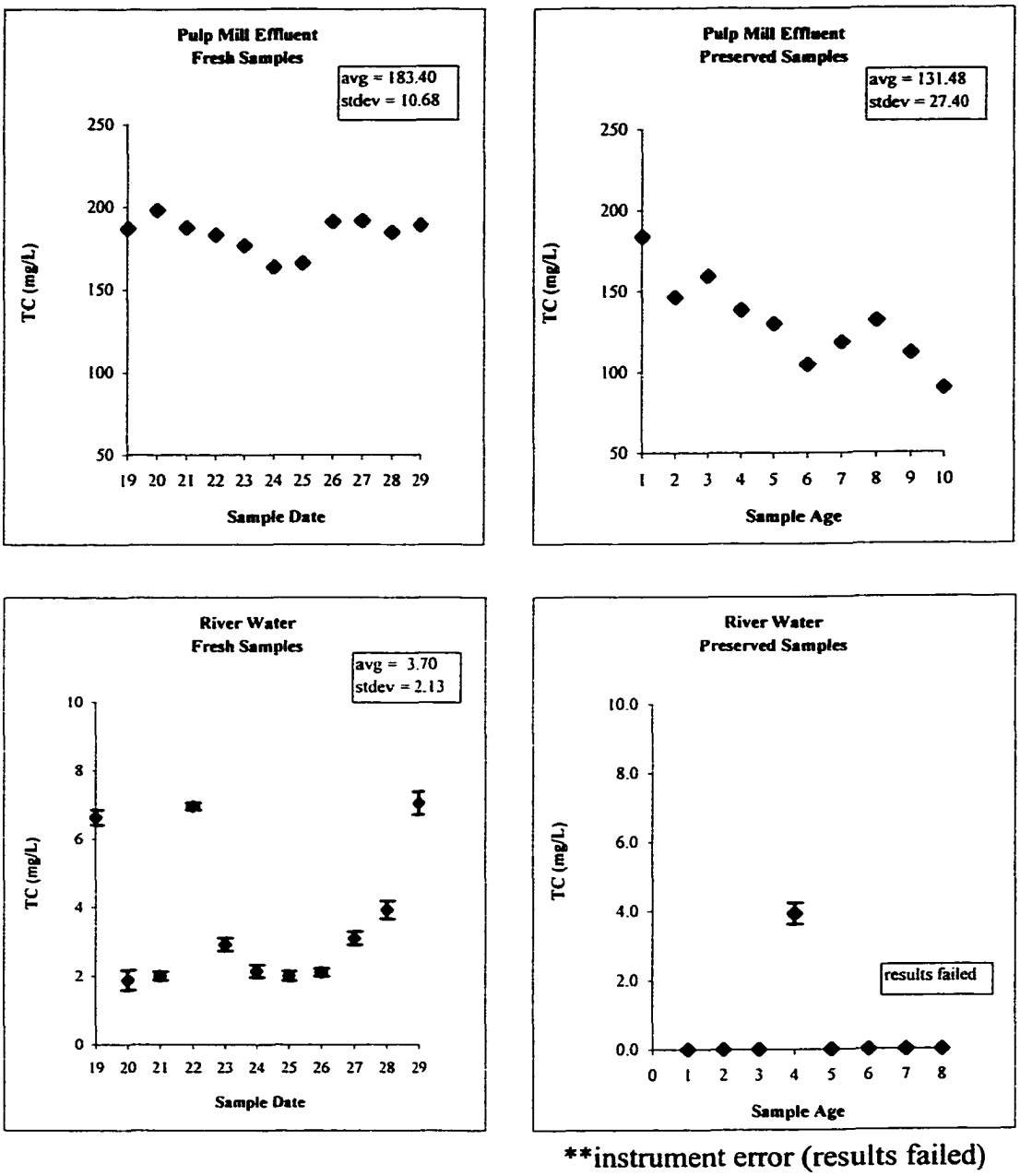
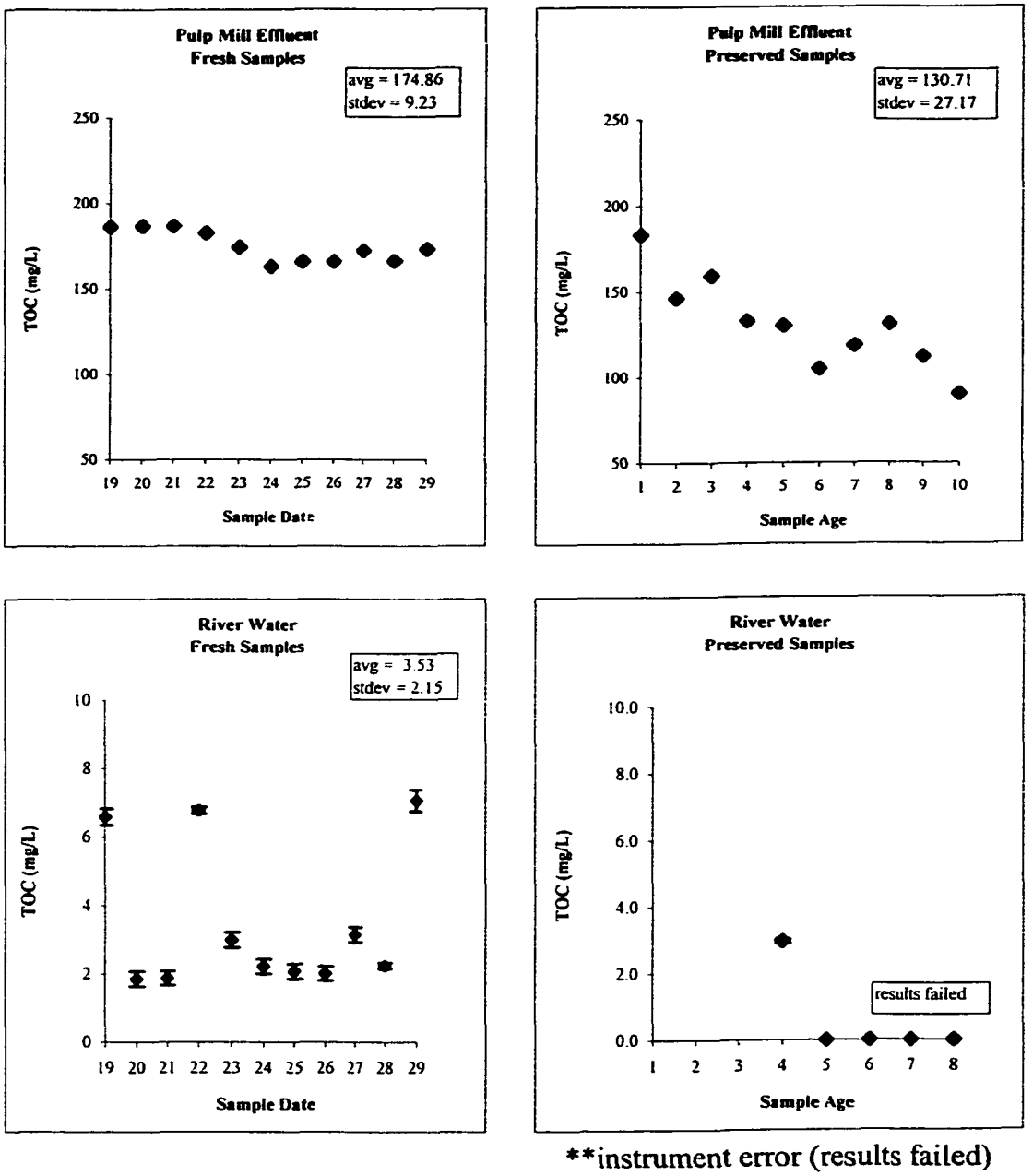


Figure A5. Effect of sample age on total suspended solids for fresh and preserved PME or RW samples



**instrument error (results failed)

Figure A6. Effect of sample age on TC for fresh and preserved PME or RW samples



**instrument error (results failed)

Figure A7. Effect of sample age on TOC for fresh and preserved PME or RW samples

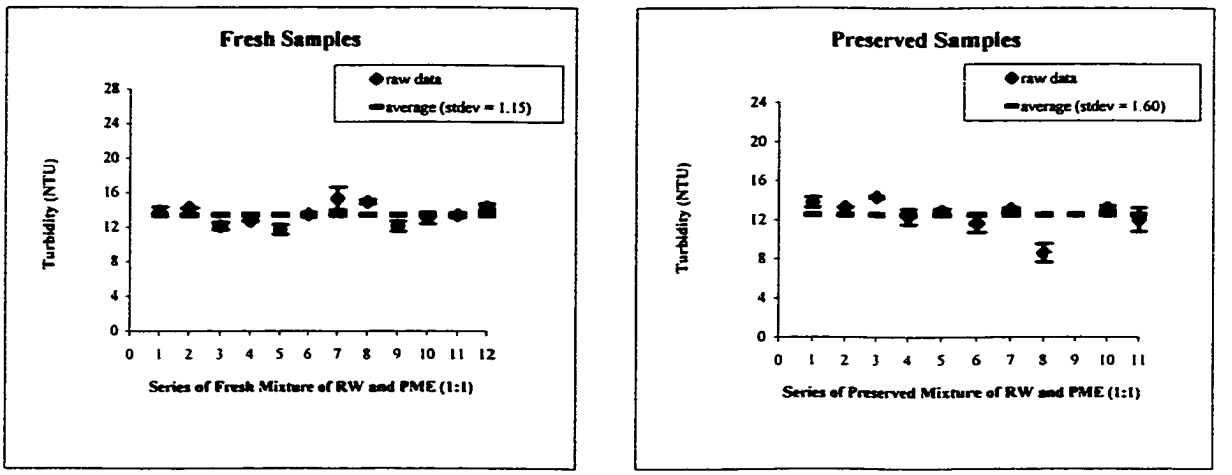


Figure A8. Consecutive daily measurements of turbidity for summer samples (1:1 volume mixtures of PME and RW samples)

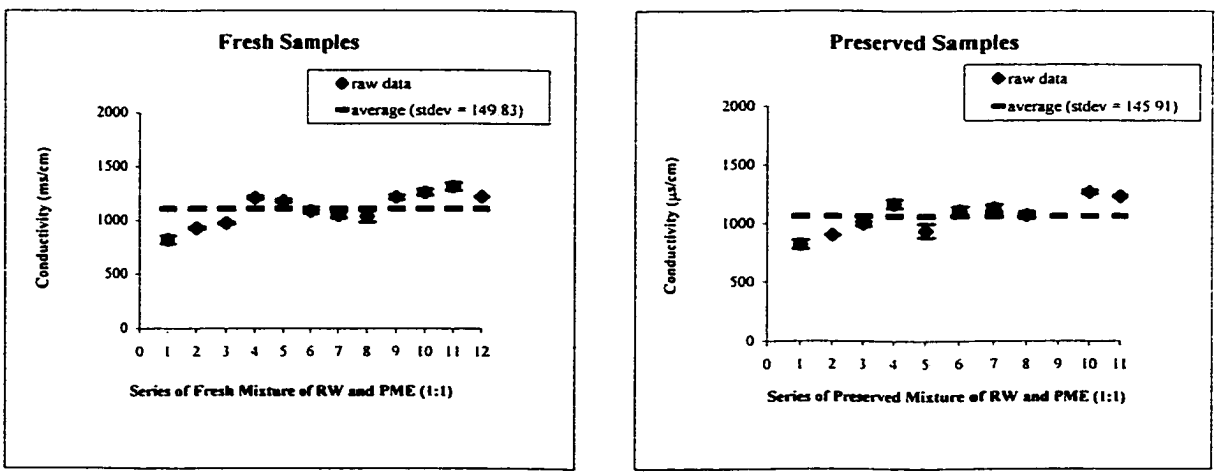


Figure A9 Consecutive daily measurements of conductivity for summer samples (1:1 volume mixtures of PME and RW samples)

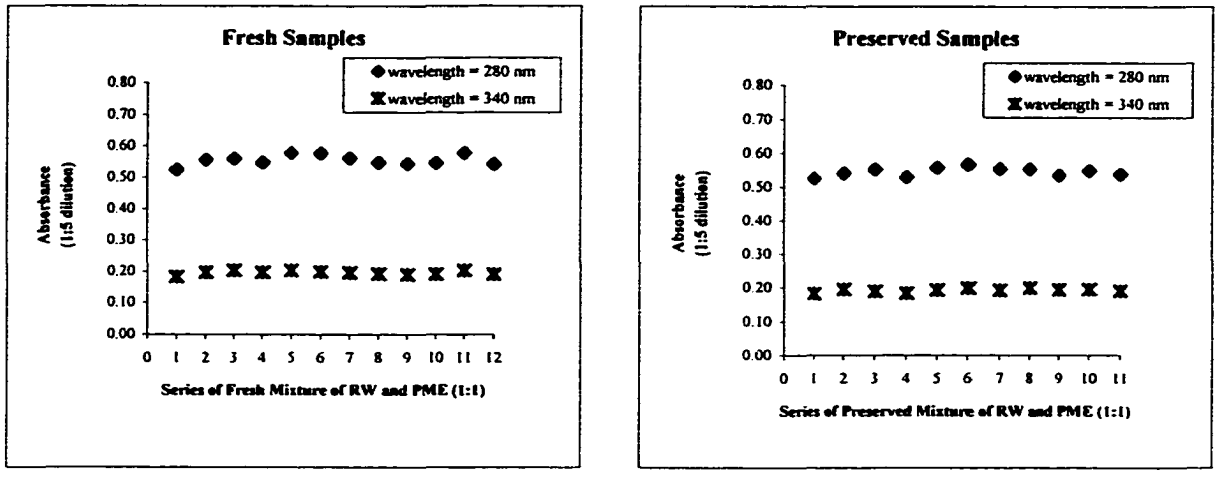


Figure A10. Consecutive daily measurements of absorbance for summer samples (1:1 volume mixtures of PME and RW samples)

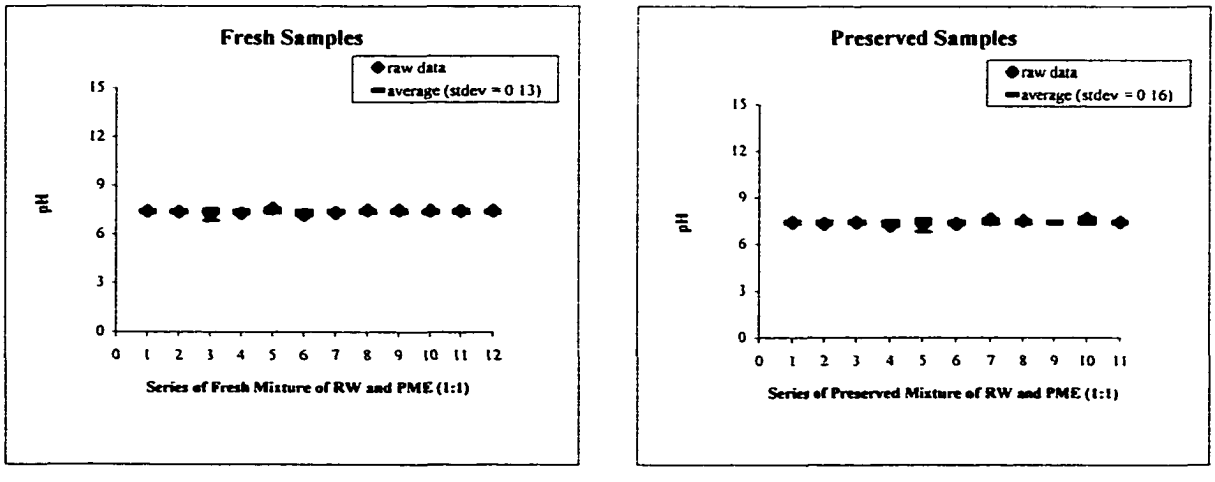


Figure A11. Consecutive daily measurements of pH for summer samples (1:1 volume mixtures of PME and RW samples)

APPENDIX B

Variation of Characteristics of Samples for Flocculation Tests

Samples, used for flocculation tests in both grid and standard jar test apparatuses, were a mixture of PME and RW (1:1 by volume). Fresh samples were taken from July 19th to 29th, and tested from July 20th to 30th. Most samples were tested less than one day after they were taken; however, the samples taken on July 23rd were tested on the 26th; the samples for July 24th and 25th were tested on the 27th; and the samples for July 26th were tested on the 28th. Preserved samples were taken on July 19th and tested each day from July 20th to July 30th.

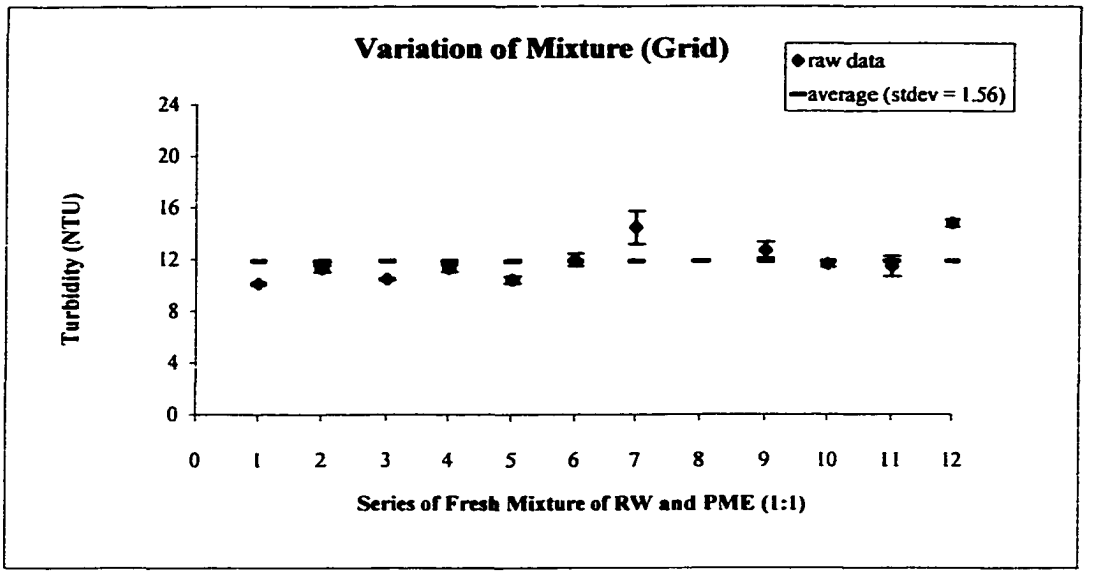


Figure B1. Consecutive daily measurements of turbidity for summer samples (1:1 volume mixtures of fresh PME and RW samples)

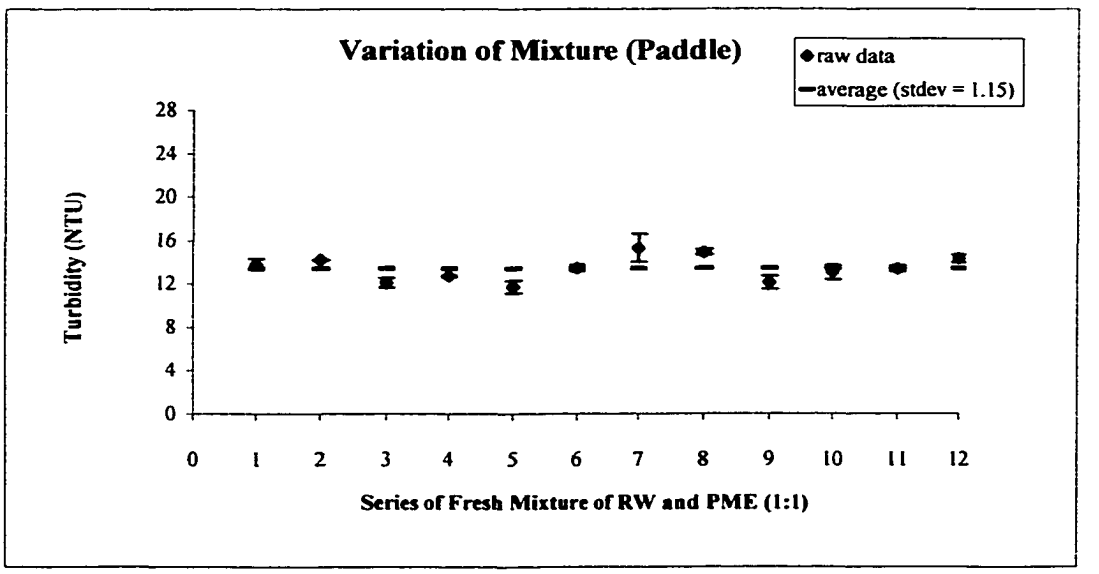


Figure B2. Consecutive daily measurements of turbidity for summer samples (1:1 volume mixtures of fresh PME and RW samples)

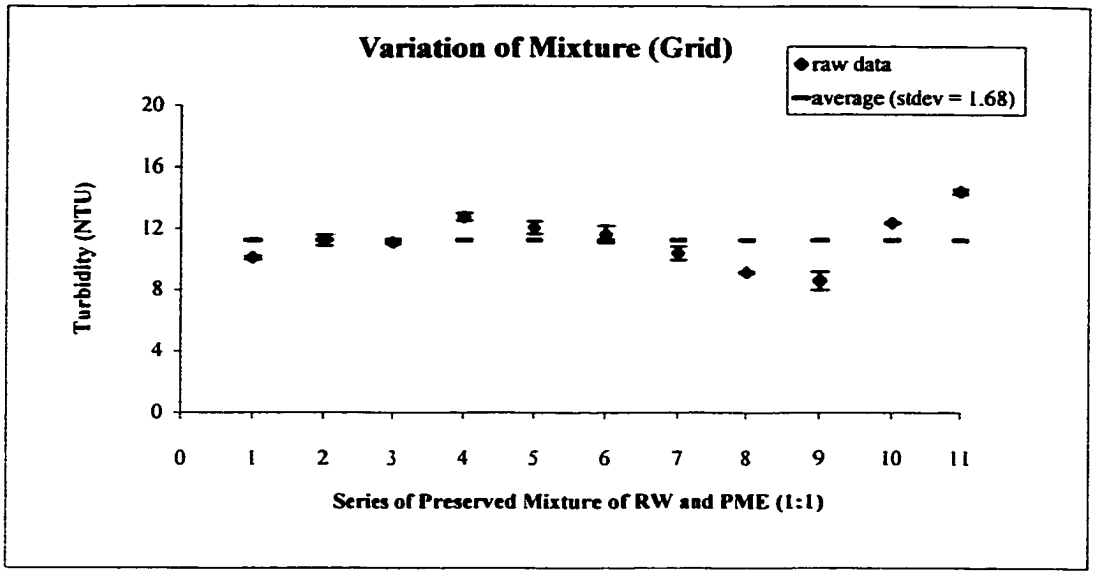


Figure B3. Consecutive daily measurements of turbidity for summer samples (1:1 volume mixtures of preserved PME and RW samples)

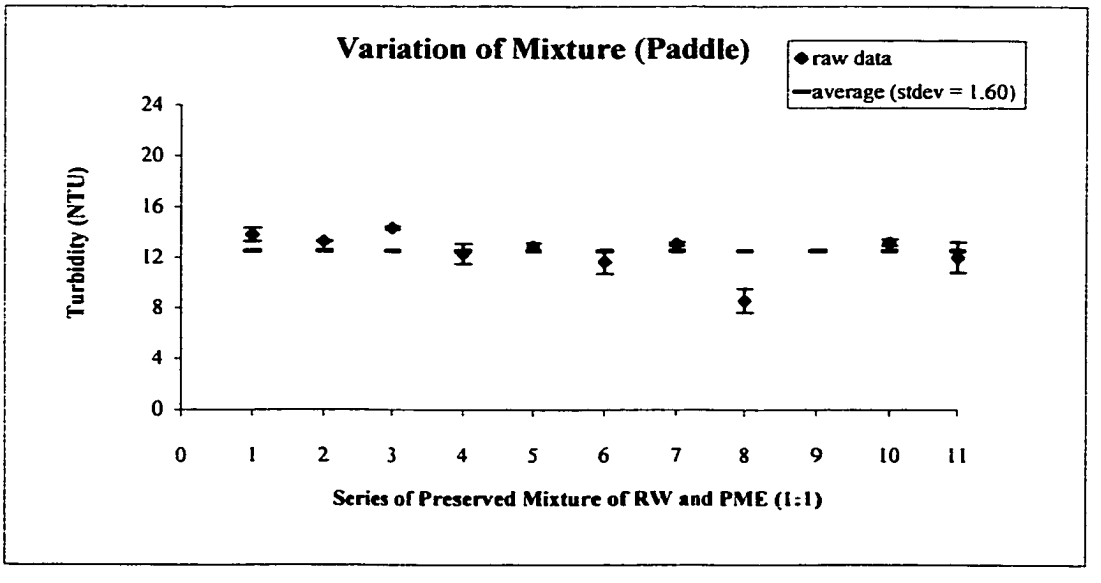


Figure B4. Consecutive daily measurements of turbidity for summer samples (1:1 volume mixtures of preserved PME and RW samples)

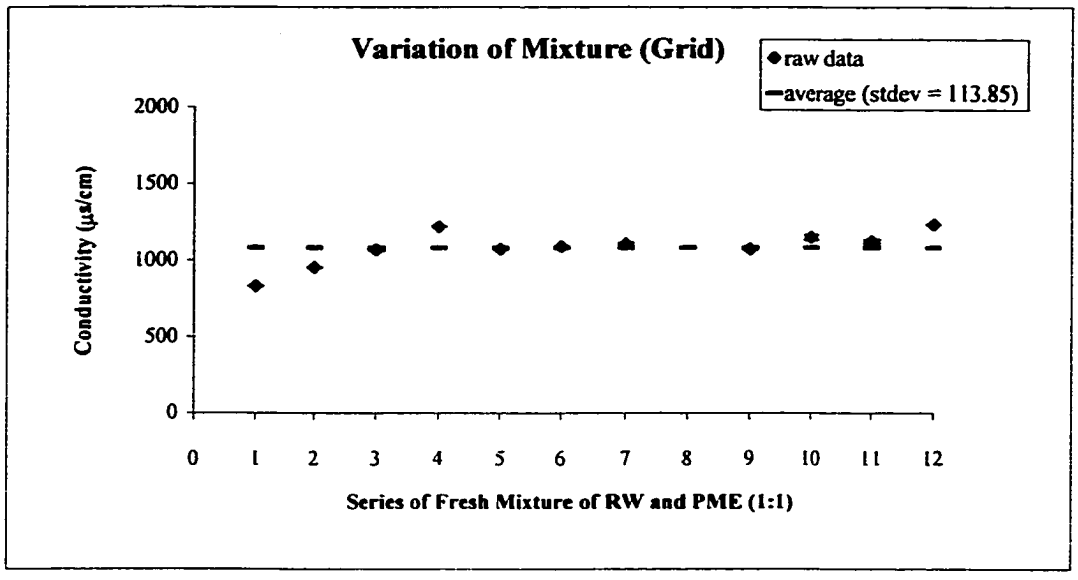


Figure B5. Consecutive daily measurements of conductivity for summer samples (1:1 volume mixtures of fresh PME and RW samples)

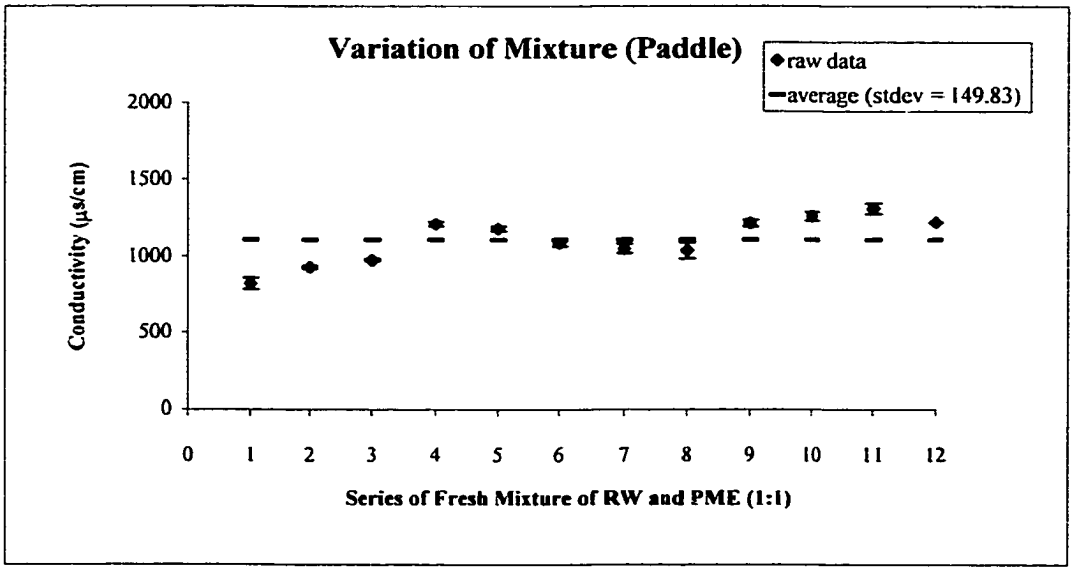


Figure B6. Consecutive daily measurements of conductivity for summer samples (1:1 volume mixtures of fresh PME and RW samples)

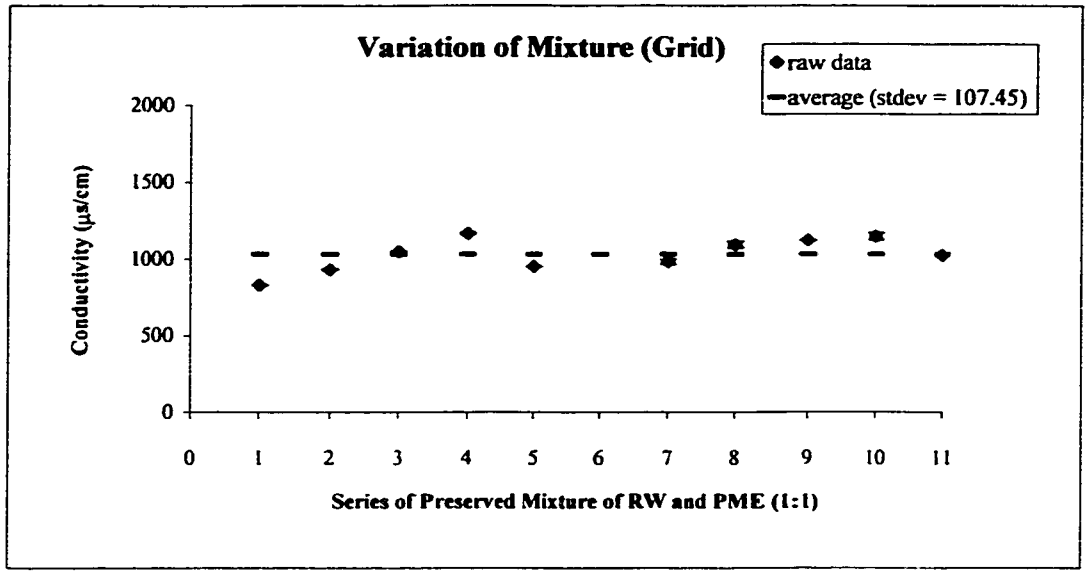


Figure B7. Consecutive daily measurements of conductivity for summer samples (1:1 volume mixtures of preserved PME and RW samples)

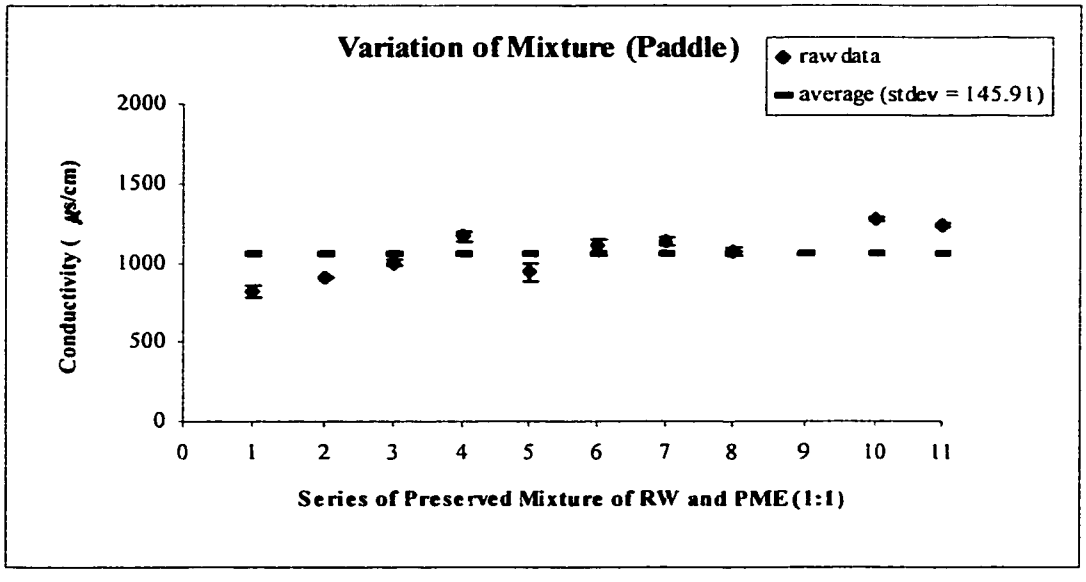


Figure B8. Consecutive daily measurements of conductivity for summer samples (1:1 volume mixtures of preserved PME and RW samples)

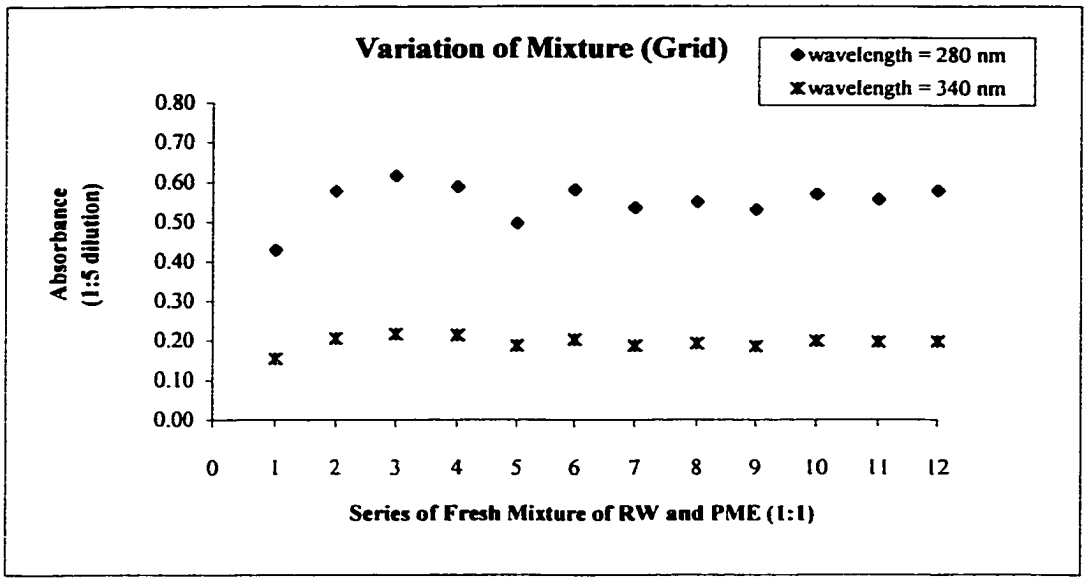


Figure B9. Consecutive daily measurements of absorbance for summer samples (1:1 volume mixtures of fresh PME and RW samples)

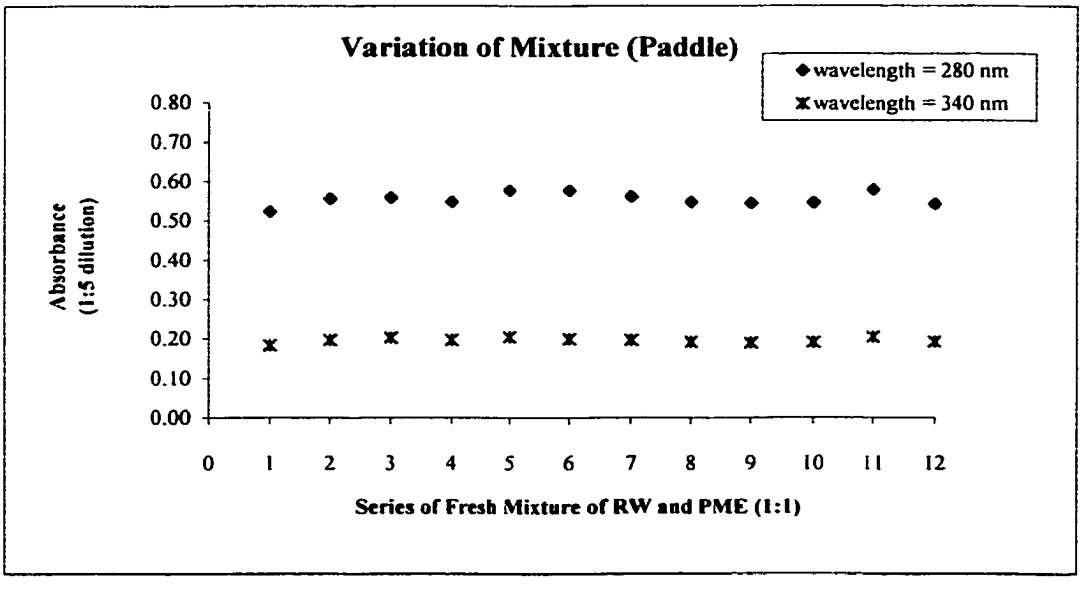


Figure B10. Consecutive daily measuremen.ts of absorbance for summer samples (1:1 volume mixtures of fresh PME and RW samples)

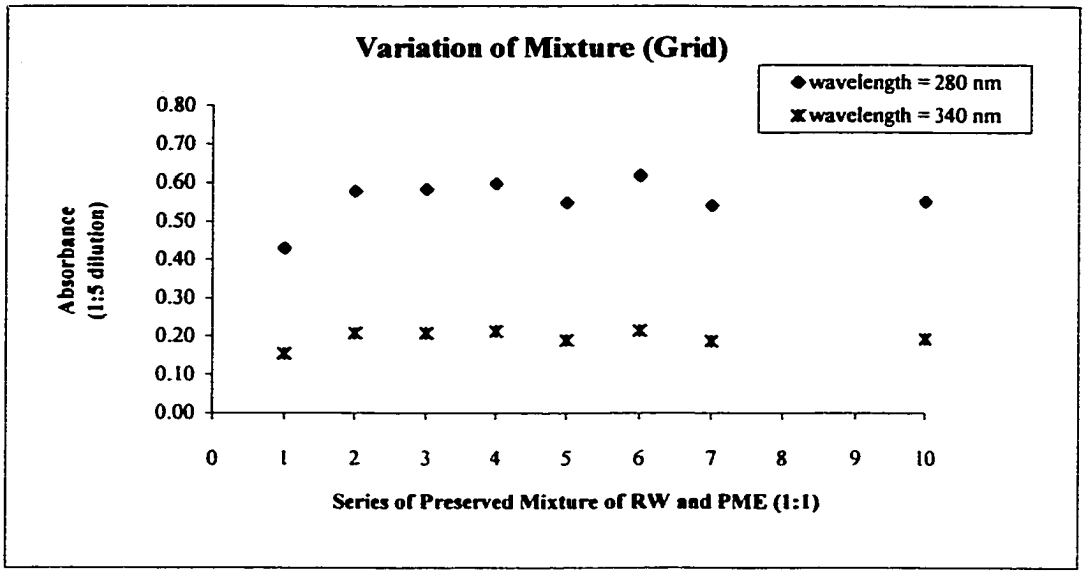


Figure B11. Consecutive daily measurements of absorbance for summer samples (1:1 volume mixtures of preserved PME and RW samples)

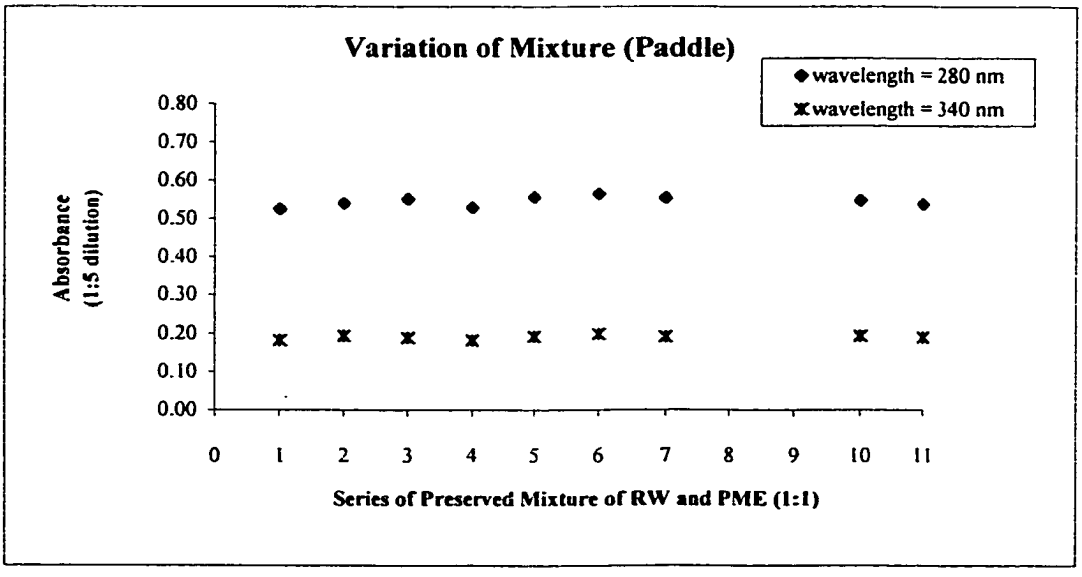


Figure B12. Consecutive daily measurements of absorbance for summer samples (1:1 volume mixtures of preserved PME and RW samples)

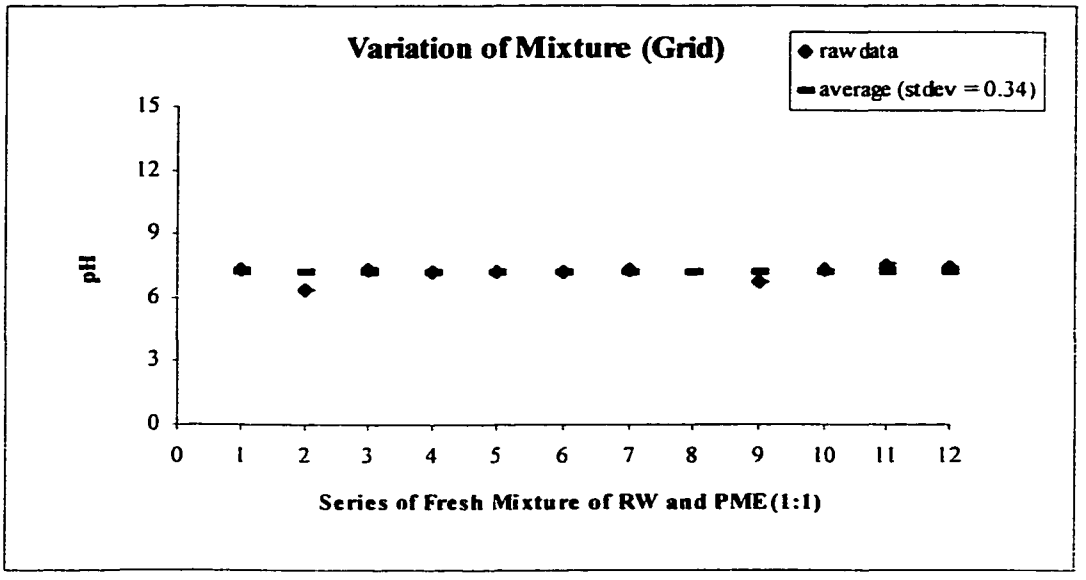


Figure B13. Consecutive daily measurements of pH for summer samples (1:1 volume mixtures of fresh PME and RW samples)

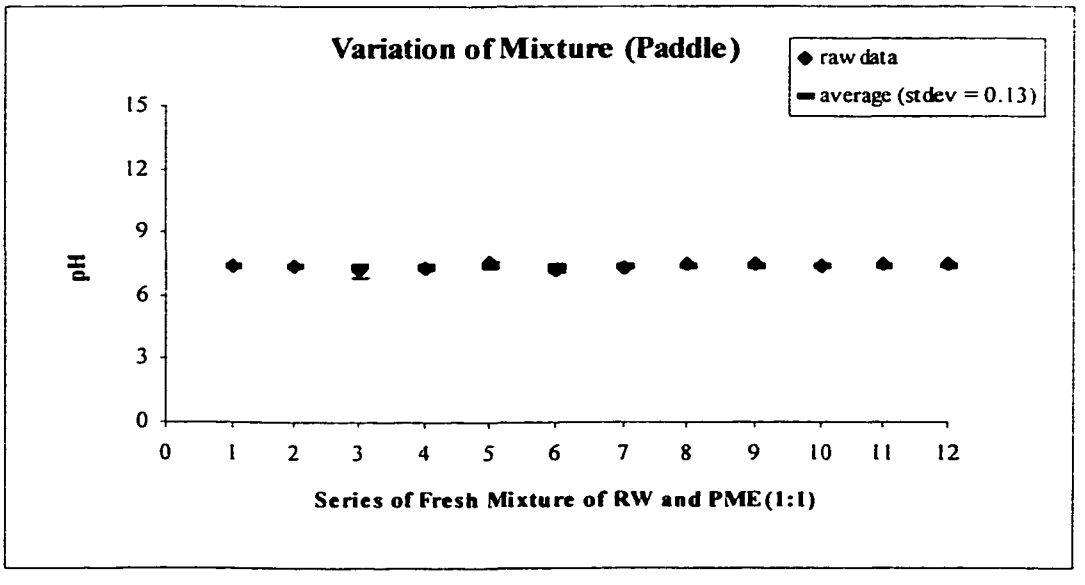


Figure B14. Consecutive daily measurements of pH for summer samples (1:1 volume mixtures of fresh PME and RW samples)

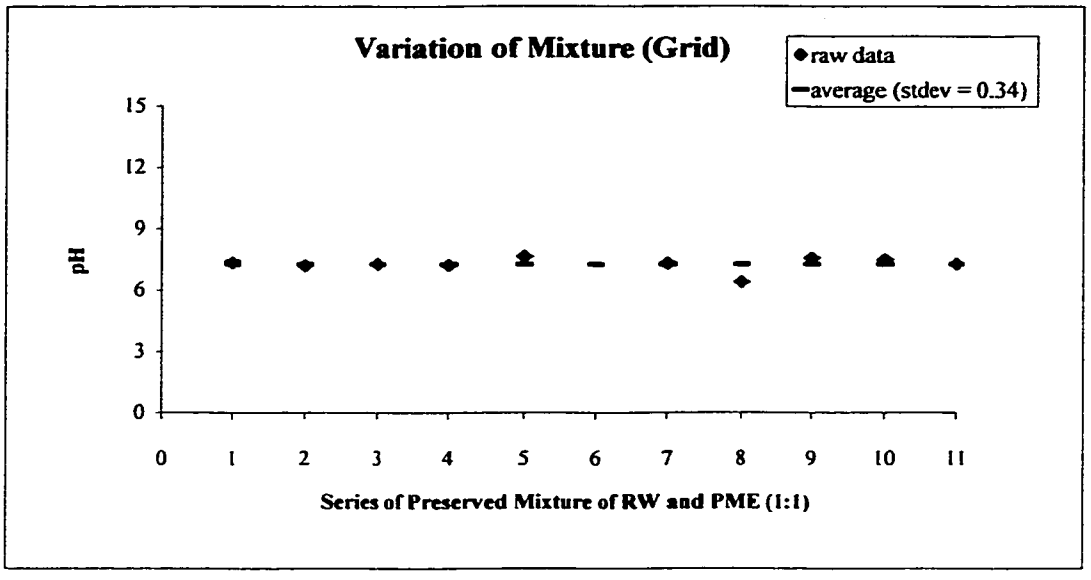


Figure B15. Consecutive daily measurements of pH for summer samples (1:1 volume mixtures of preserved PME and RW samples)

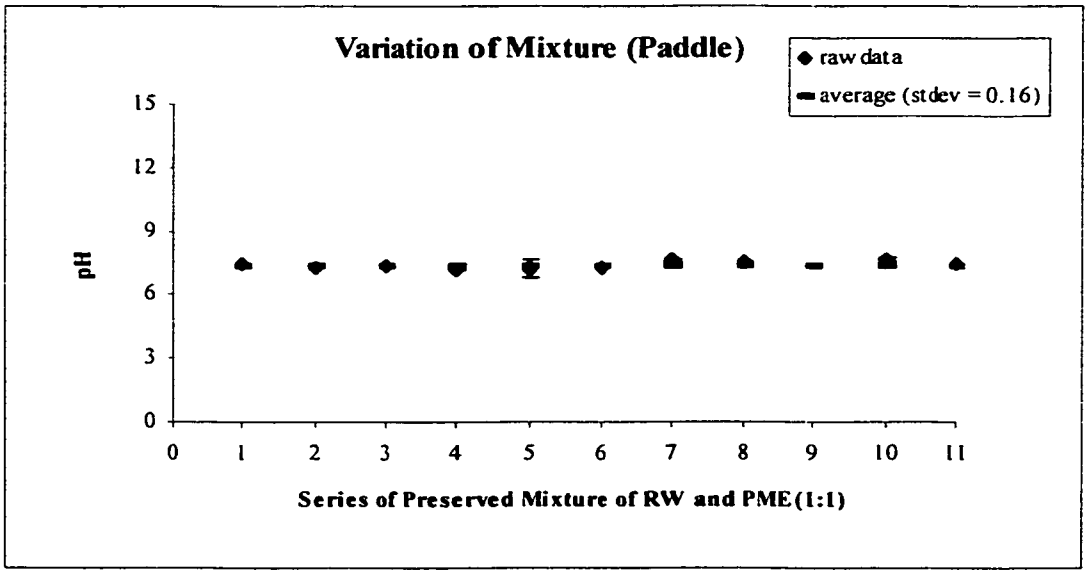
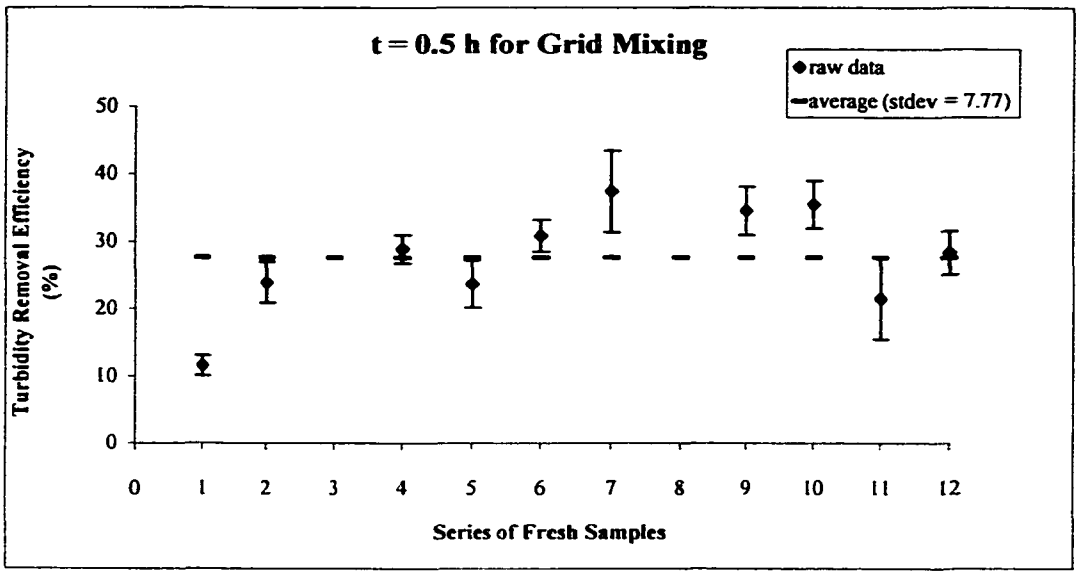


Figure B16. Consecutive daily measurements of pH for summer samples (1:1 volume mixtures of preserved PME and RW samples)

APPENDIX C

Variation of Characteristics of Flocculated Samples of a Mixture of PME and RW (1:1 by Volume)

Fresh samples were taken from July 19th to 29th, and tested from July 20th to 30th. Most samples were tested less than one day after they were taken; however, the samples taken on July 23rd were tested on the 26th; the samples for July 24th and 25th were tested on the 27th; and the samples for July 26th were tested on the 28th. Preserved samples were taken on July 19th and tested each day from July 20th to July 30th.



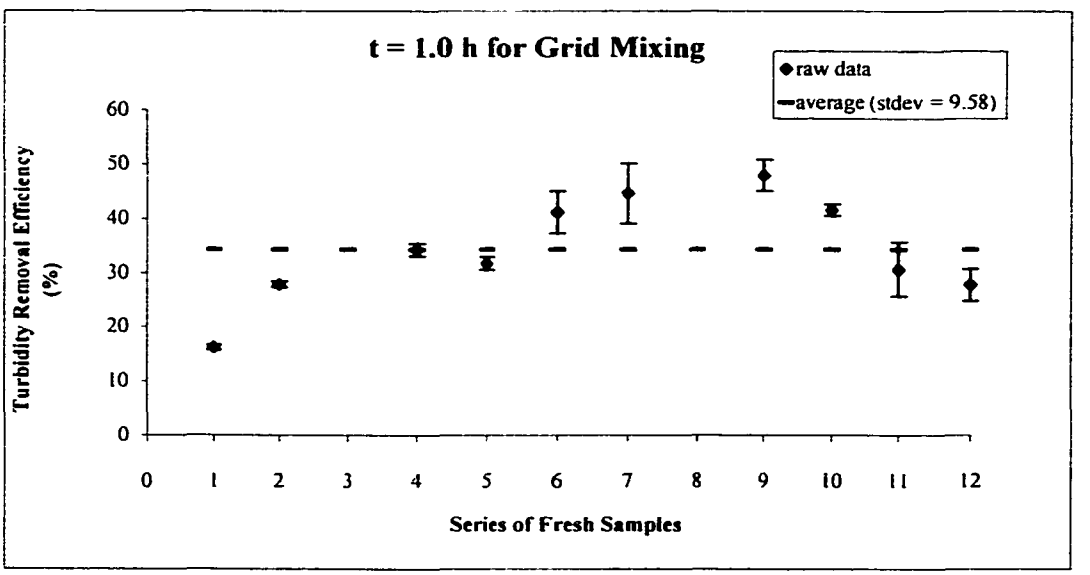
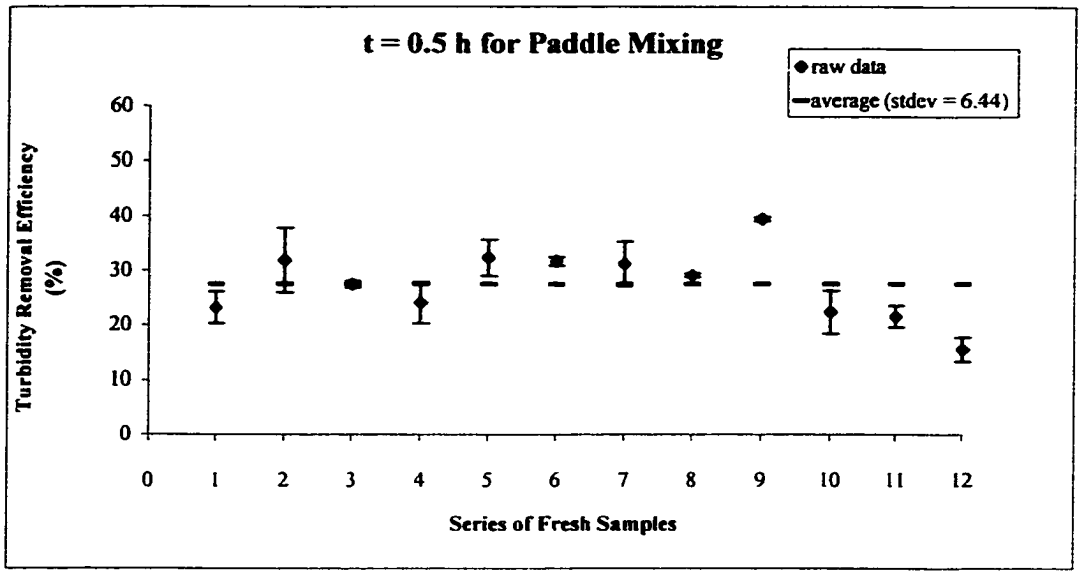


Figure C1. Consecutive daily testing for turbidity removal efficiency for fresh mixtures of PME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.



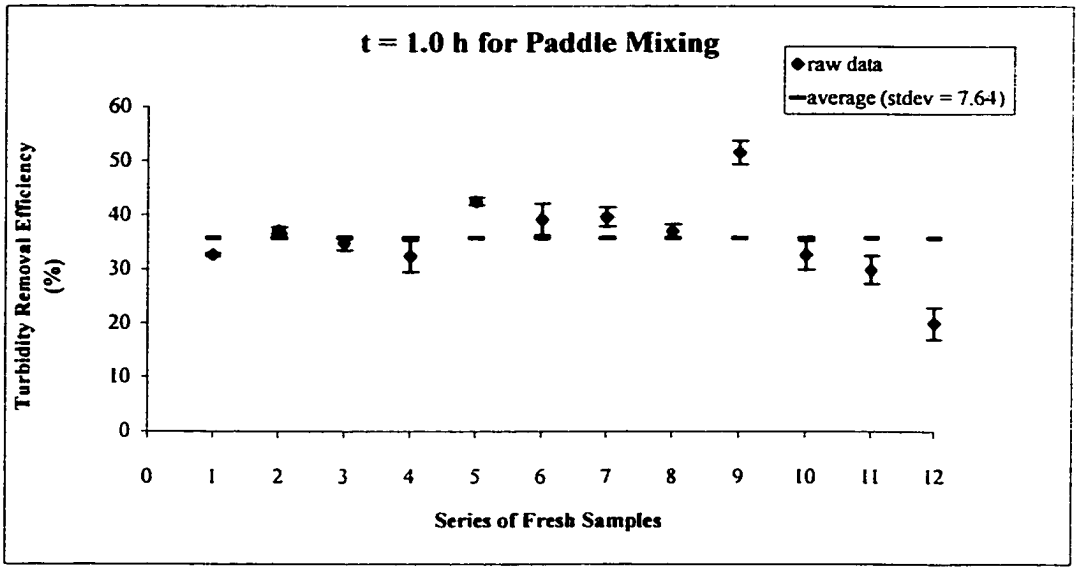
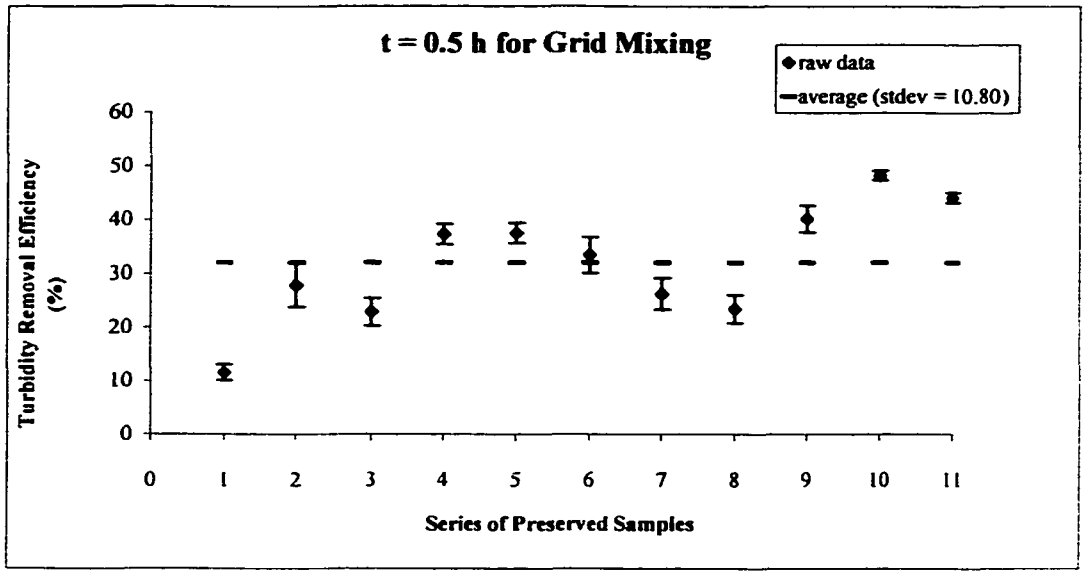


Figure C2. Consecutive daily testing for turbidity removal efficiency for fresh mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 1h at $\overline{Gf} = 13\ s^{-1}$).



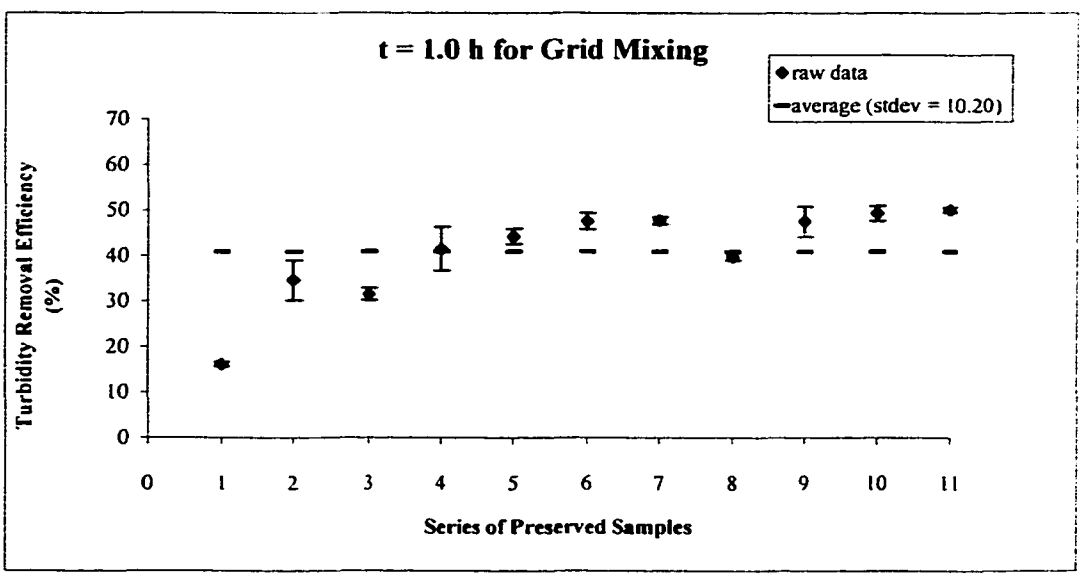


Figure C3. Consecutive daily testing for turbidity removal efficiency for preserved mixtures of PME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.

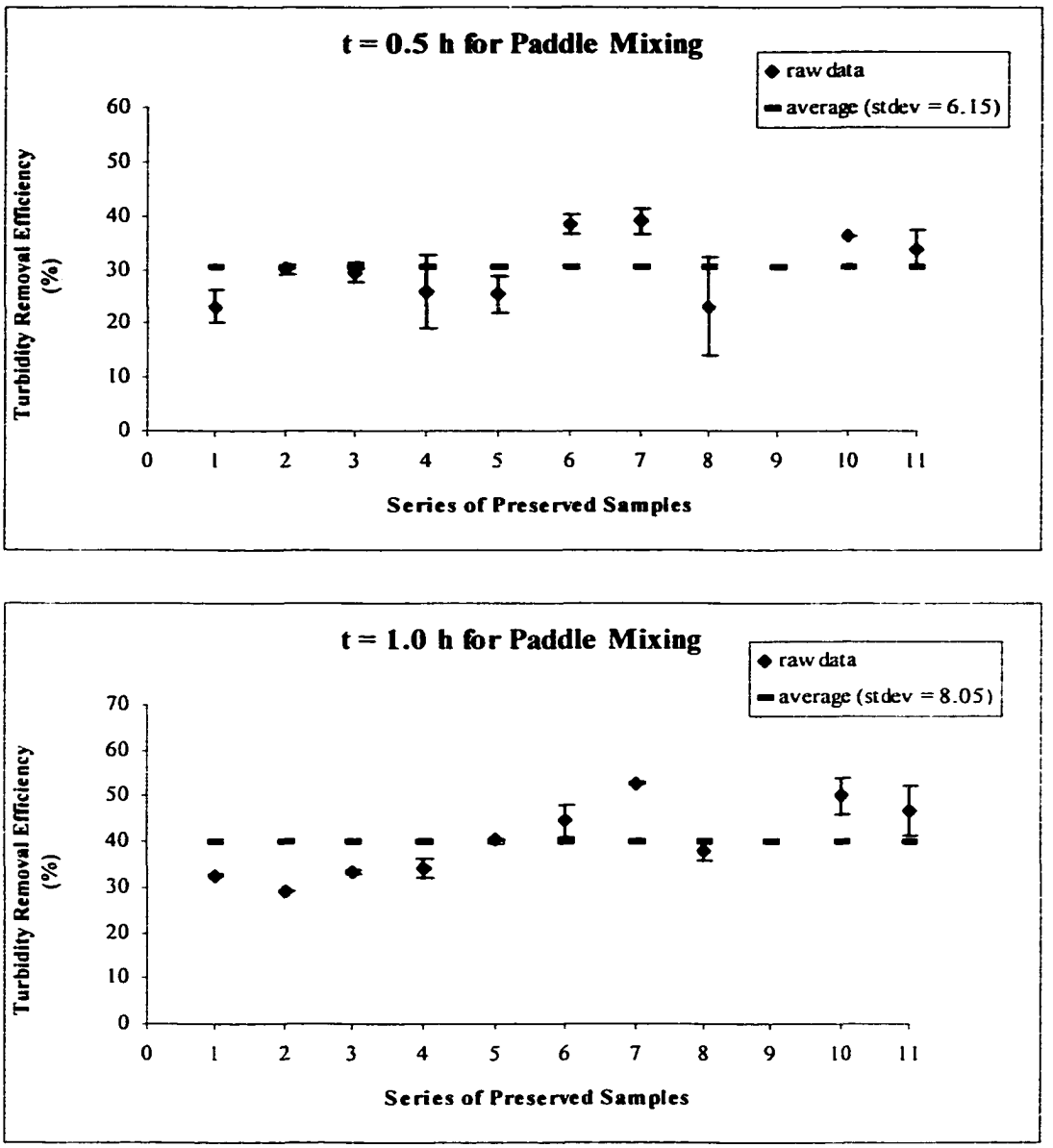
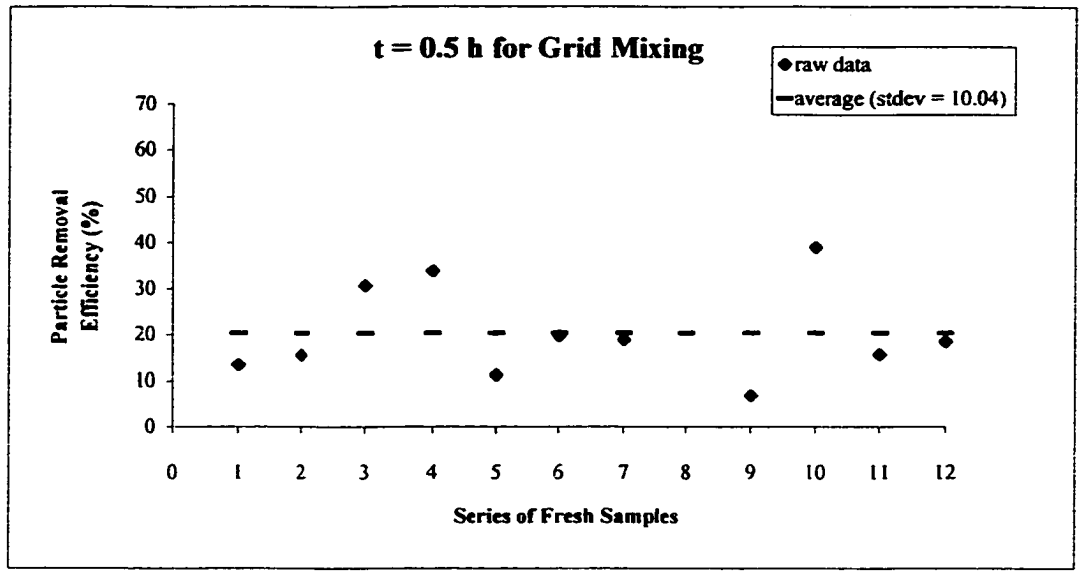


Figure C4. Consecutive daily testing for turbidity removal efficiency for preserved mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 1h at $\overline{Gf} = 13\ s^{-1}$).



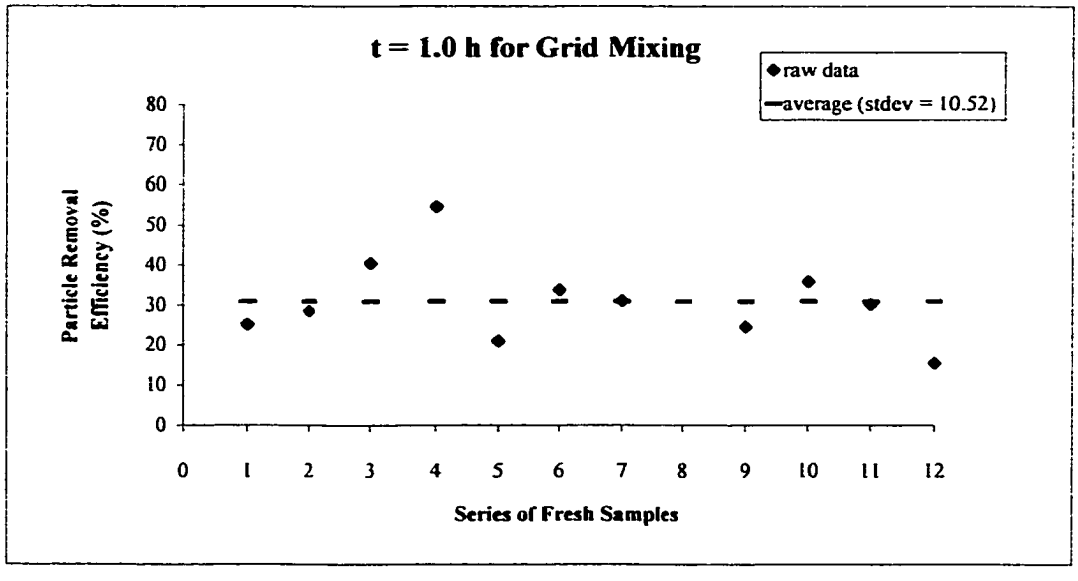


Figure C5. Consecutive daily testing for particle removal efficiency for fresh mixtures of PME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.

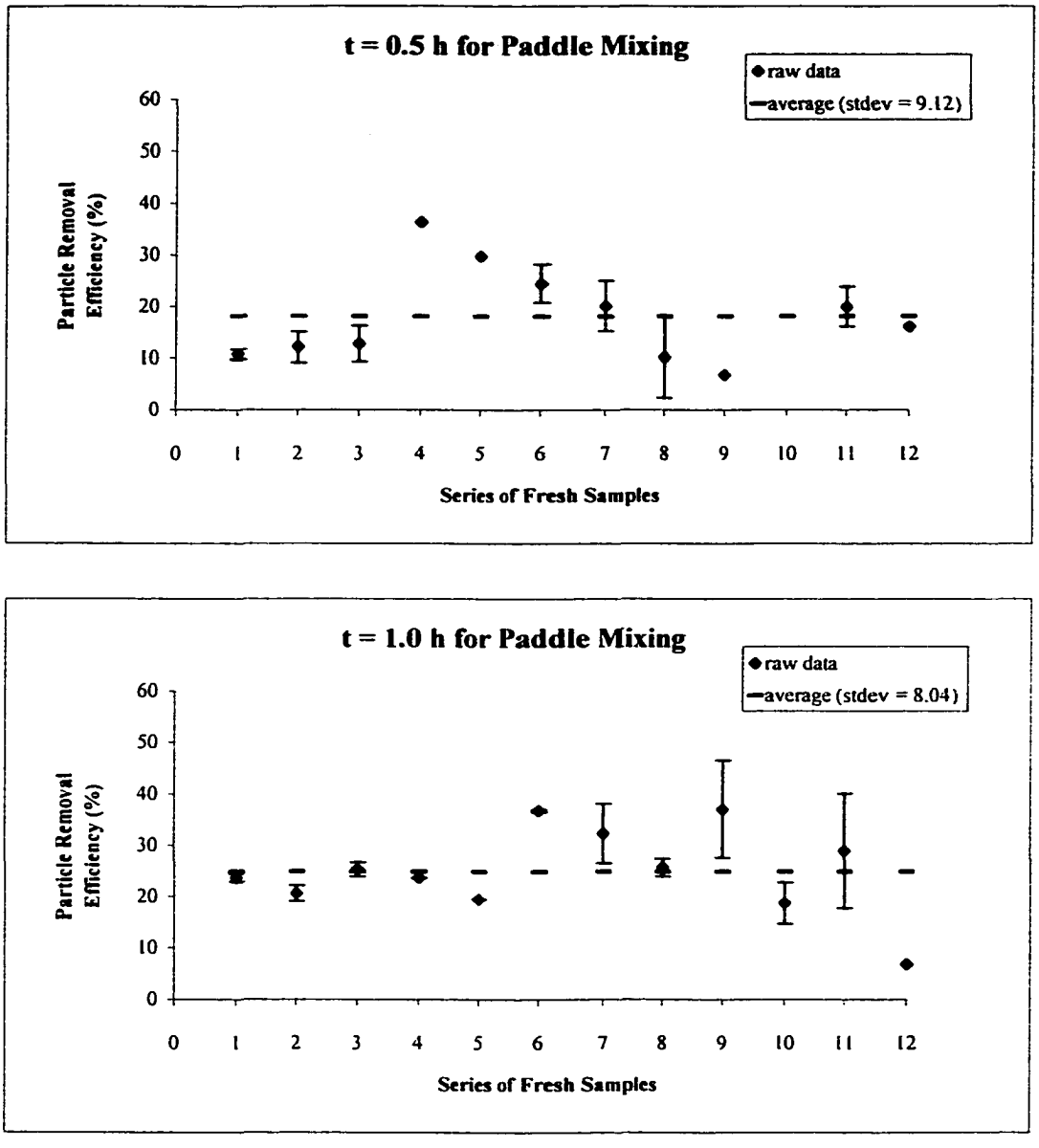
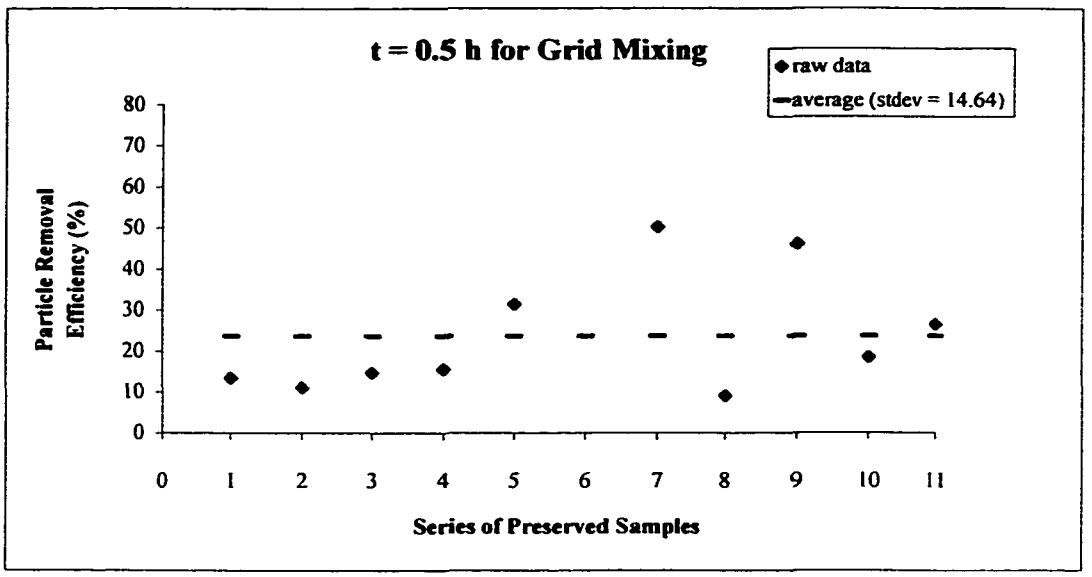


Figure C6. Consecutive daily testing for particle removal efficiency for fresh mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 1h at $\overline{Gf} = 13\ s^{-1}$).



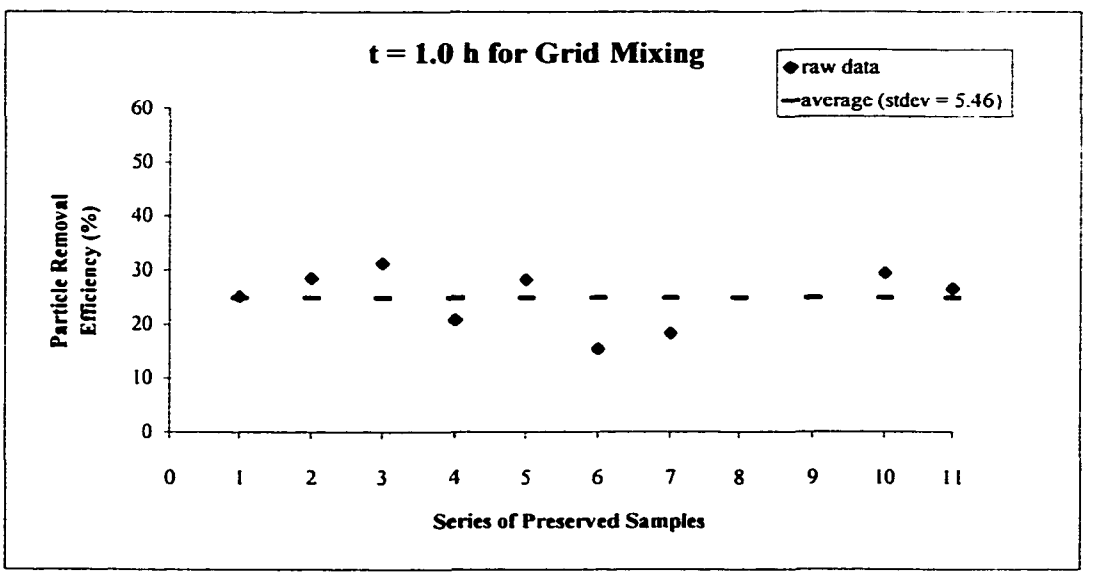
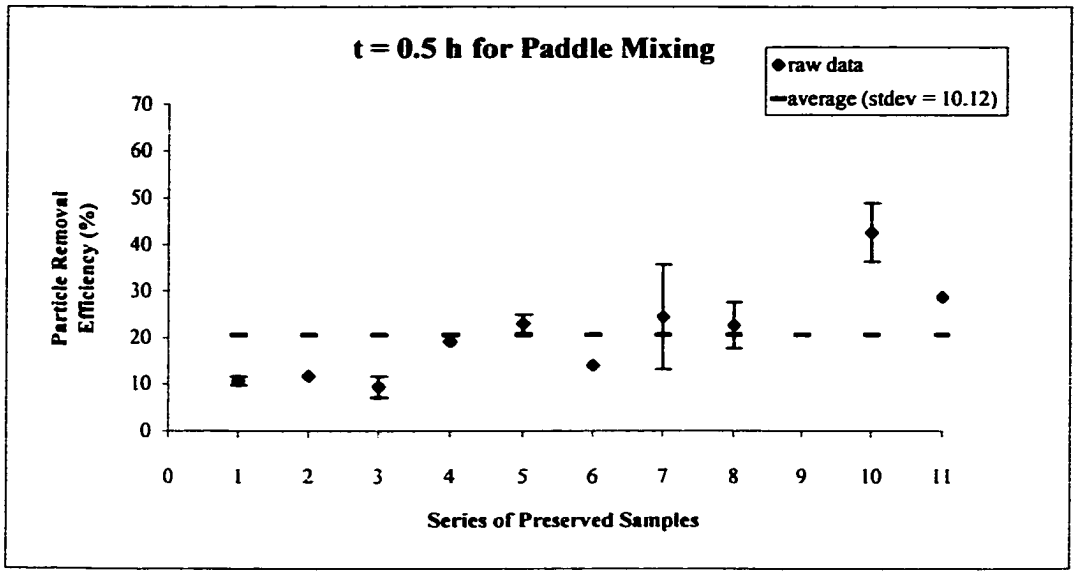


Figure C7. Consecutive daily testing for particle removal efficiency for preserved mixtures of PME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.



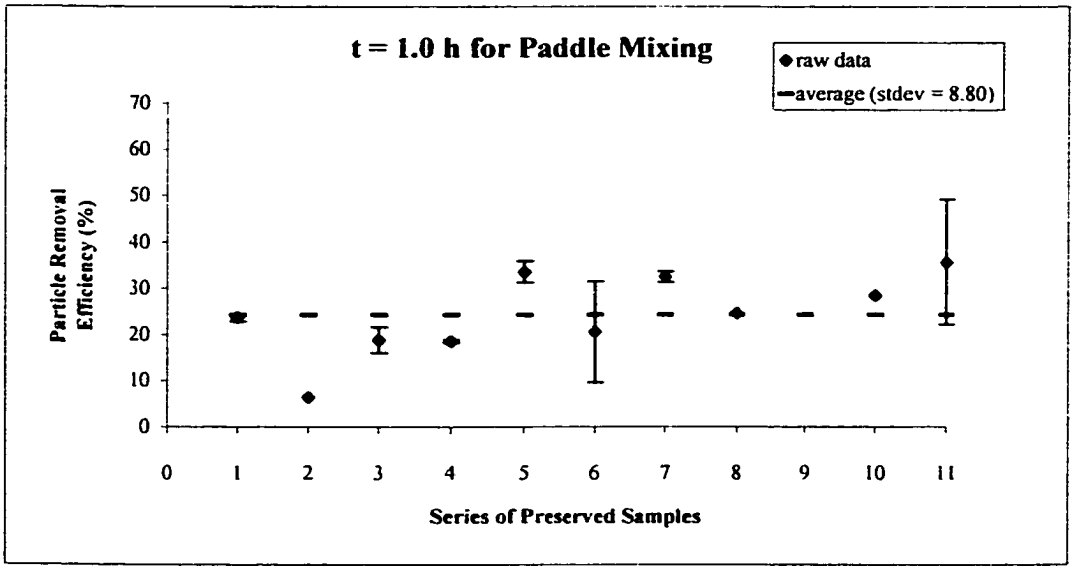


Figure C8. Consecutive daily testing for particle removal efficiency for preserved mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 1h at $\overline{Gf} = 13\ s^{-1}$).

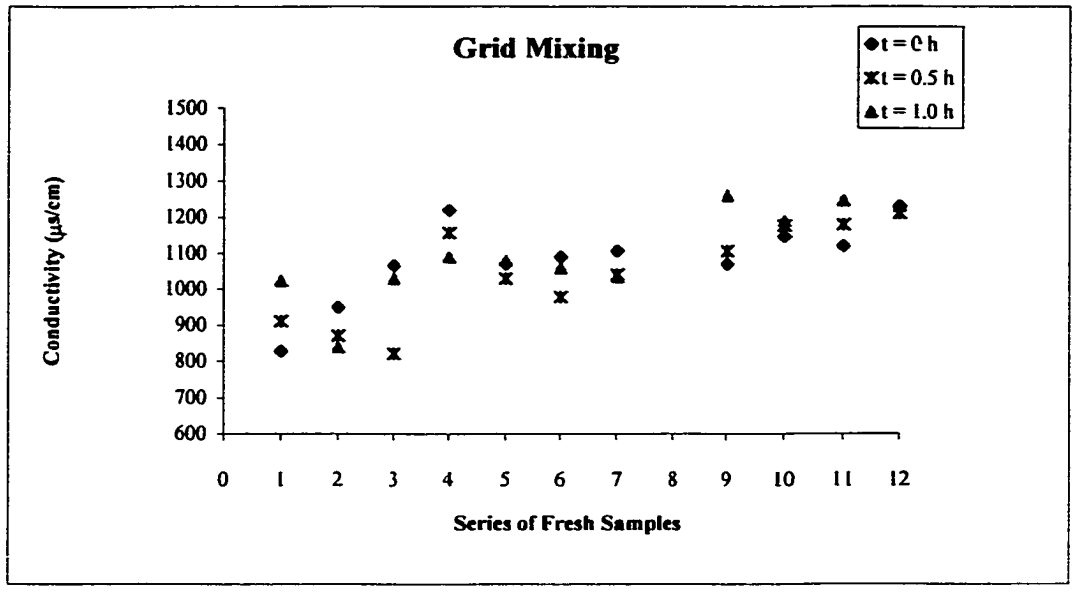


Figure C9. Consecutive daily testing for conductivity for fresh mixtures of PME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.

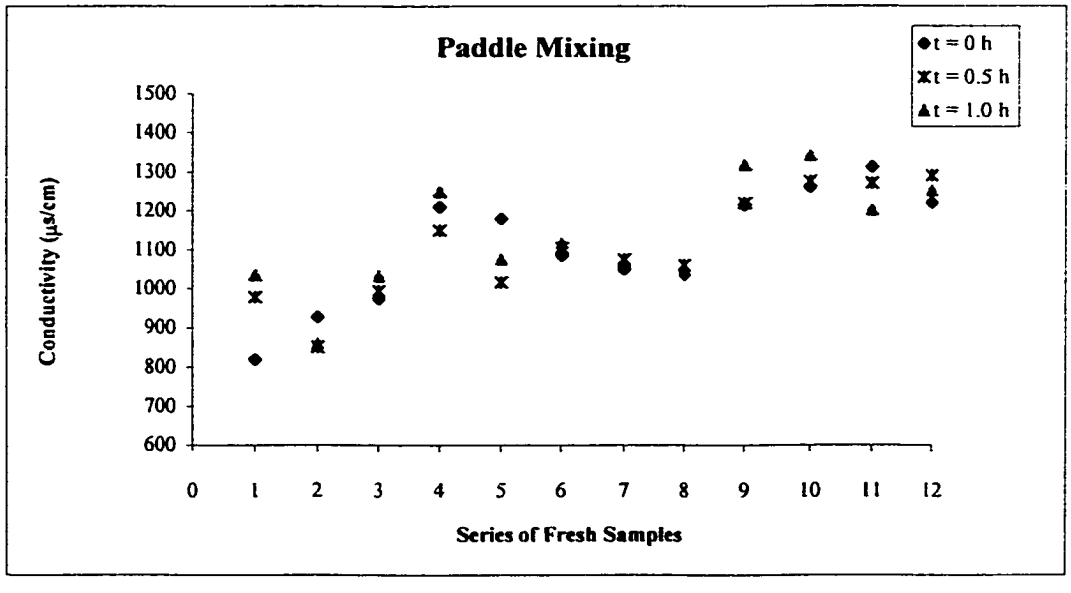


Figure C10. Consecutive daily testing for conductivity for fresh mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc}$ = 200 s^{-1} and up to 1h at $\overline{Gf}$ = 13 s^{-1}).

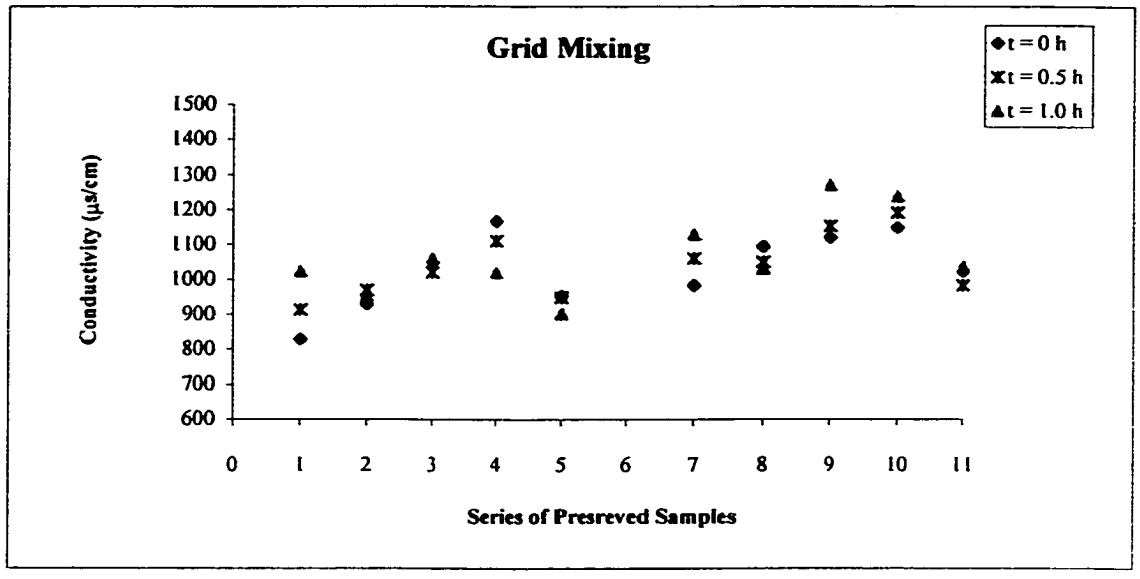


Figure C11. Consecutive daily testing for conductivity for preserved mixtures of GME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.

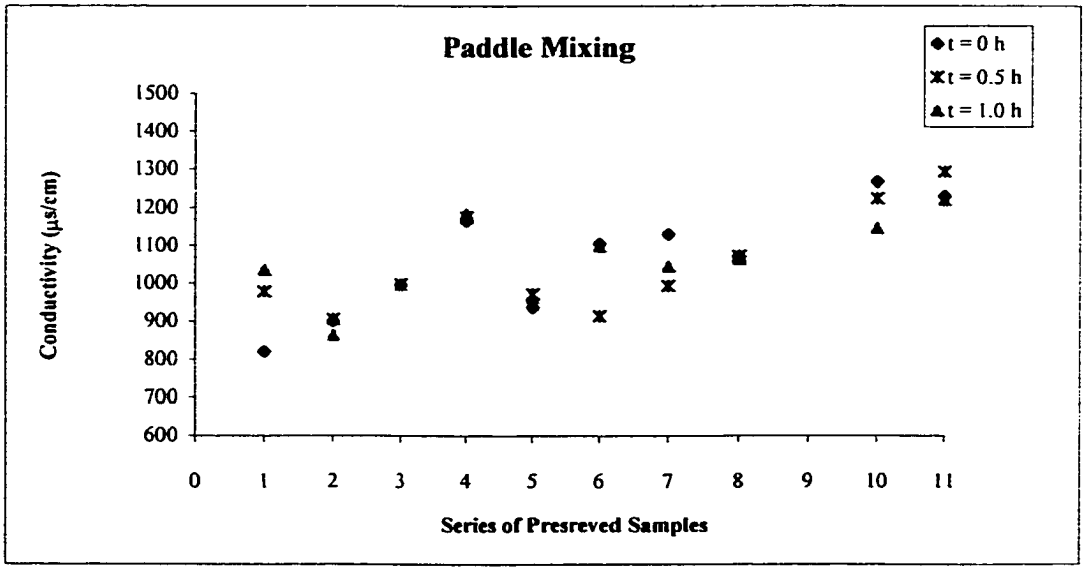


Figure C12. Consecutive daily testing for conductivity for preserved mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 1h at $\overline{Gf} = 13\ s^{-1}$).

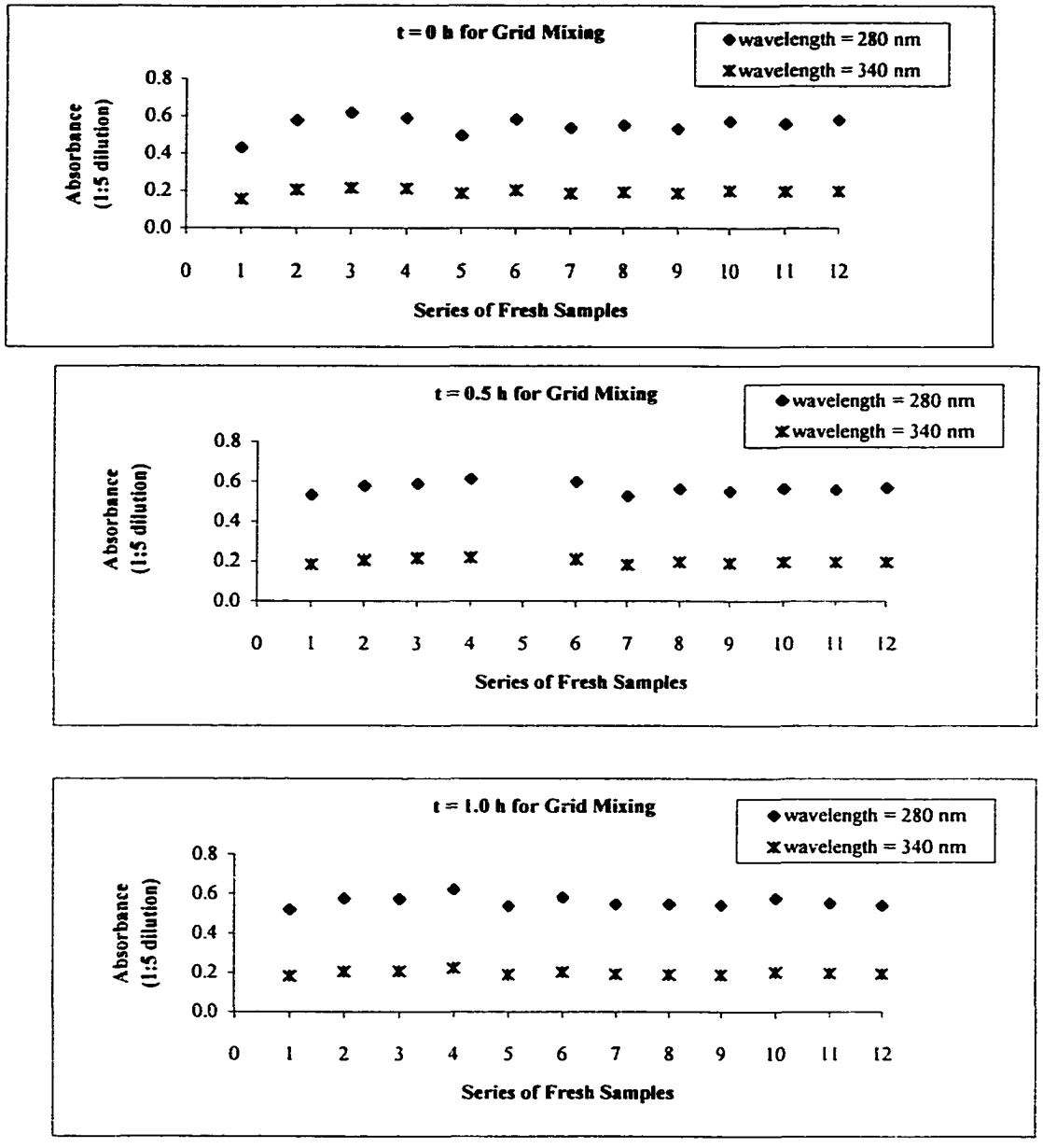
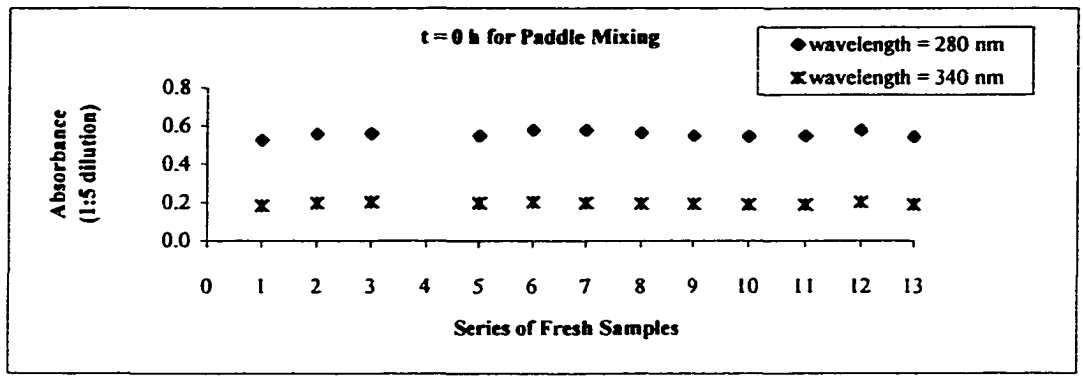
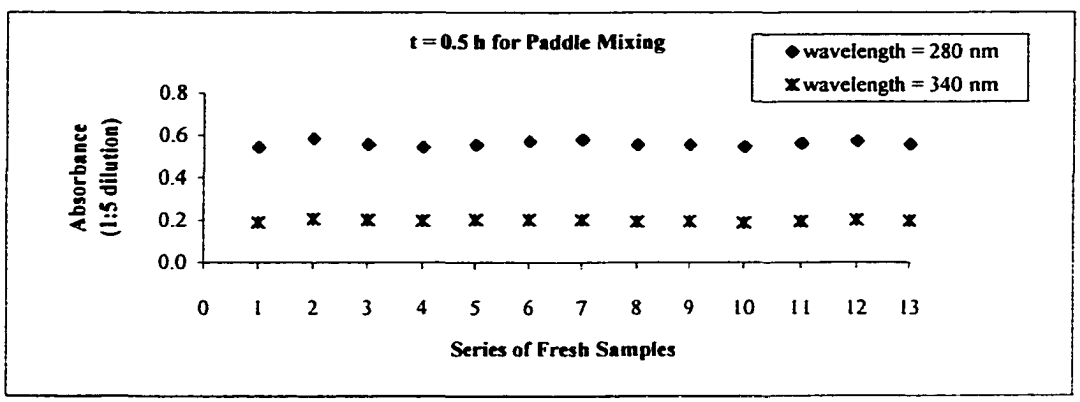


Figure C13. Consecutive daily testing for absorbance for fresh mixtures of PME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.





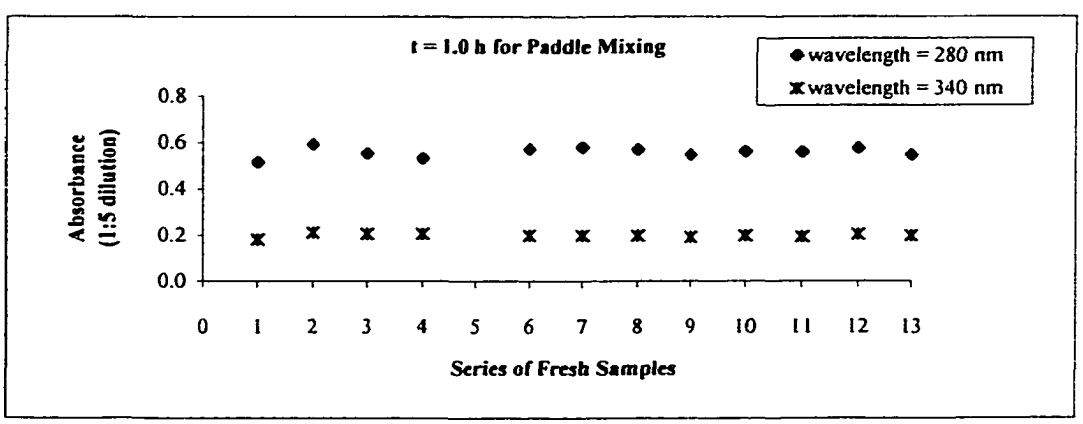


Figure C14. Consecutive daily testing for absorbance for fresh mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc} = 200$ s^{-1} and up to 1h at $\overline{G_f} = 13$ s^{-1}).

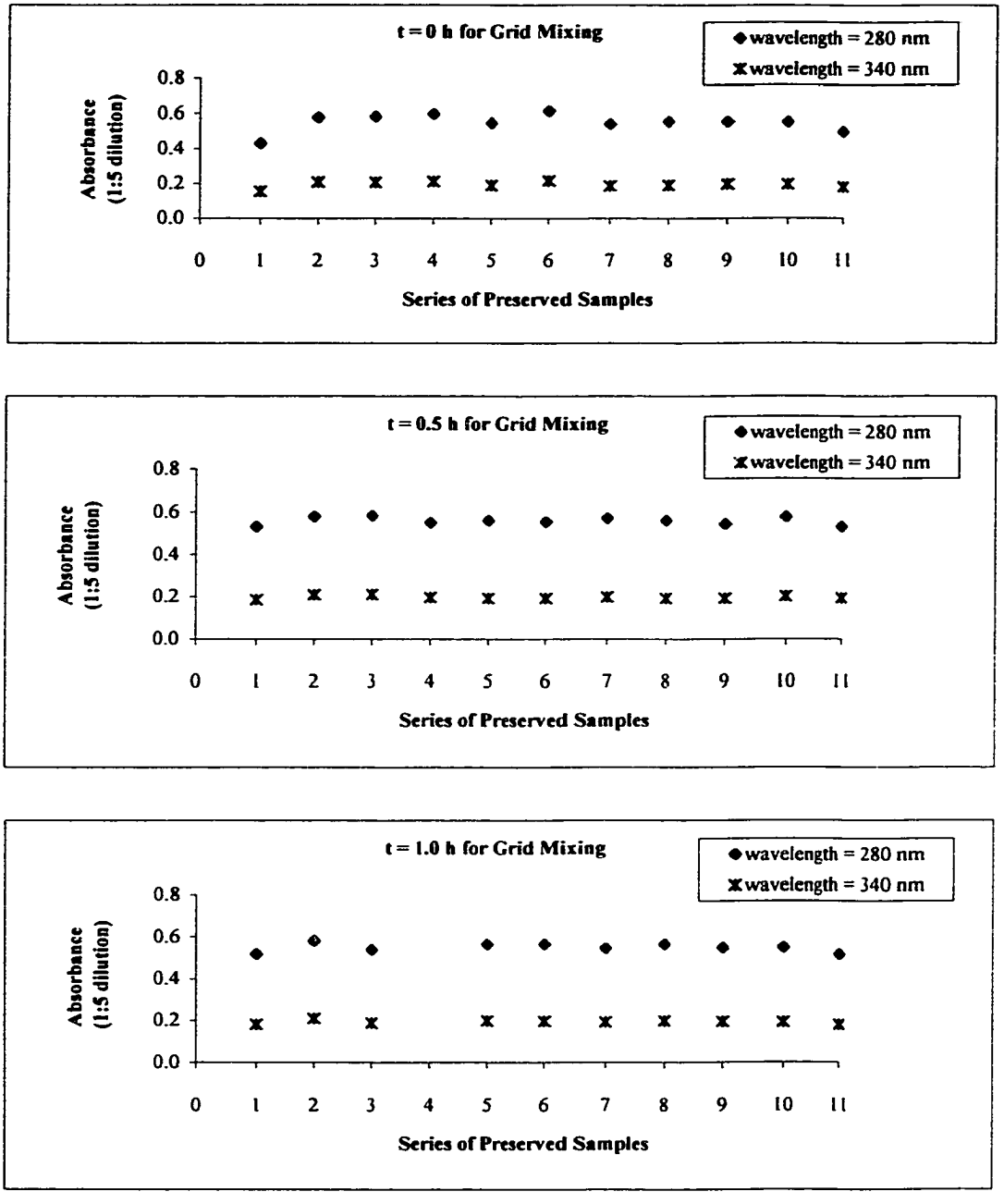
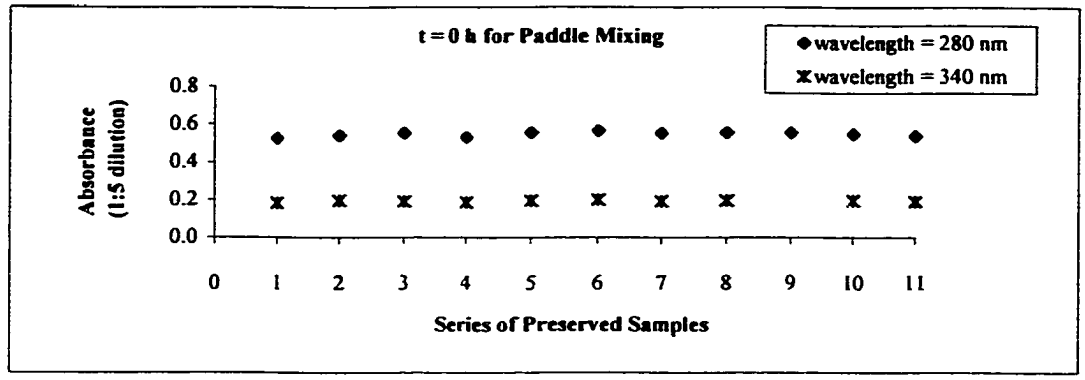
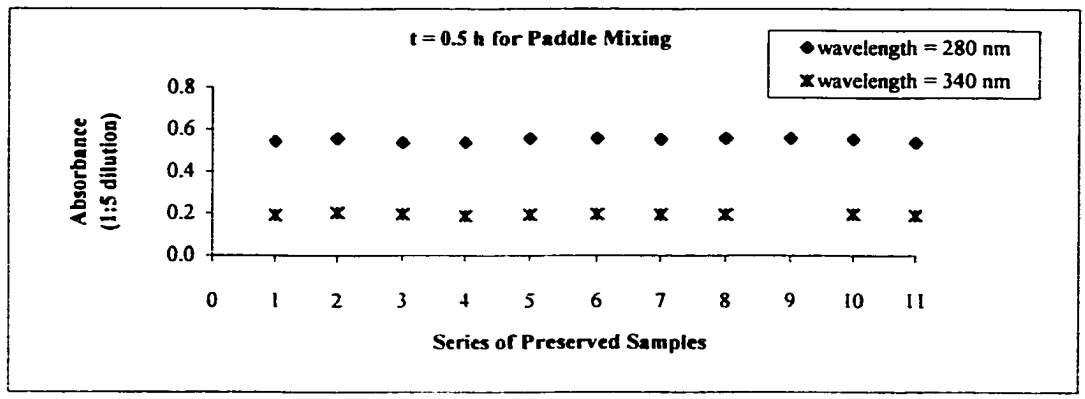


Figure C15. Consecutive daily testing for absorbance for preserved mixtures of PME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.





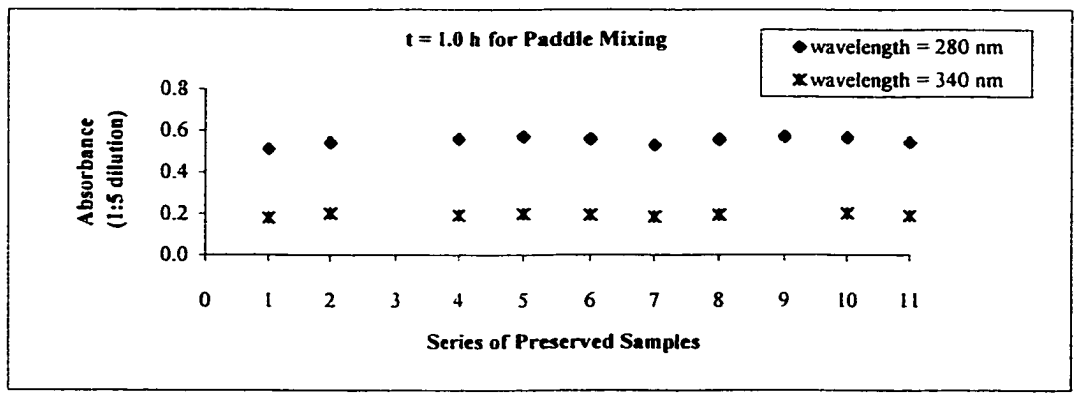


Figure C16. Consecutive daily testing for absorbance for preserved mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc}$ = 200 s^{-1} and up to 1h at $\overline{G_f}$ = 13 s^{-1}).

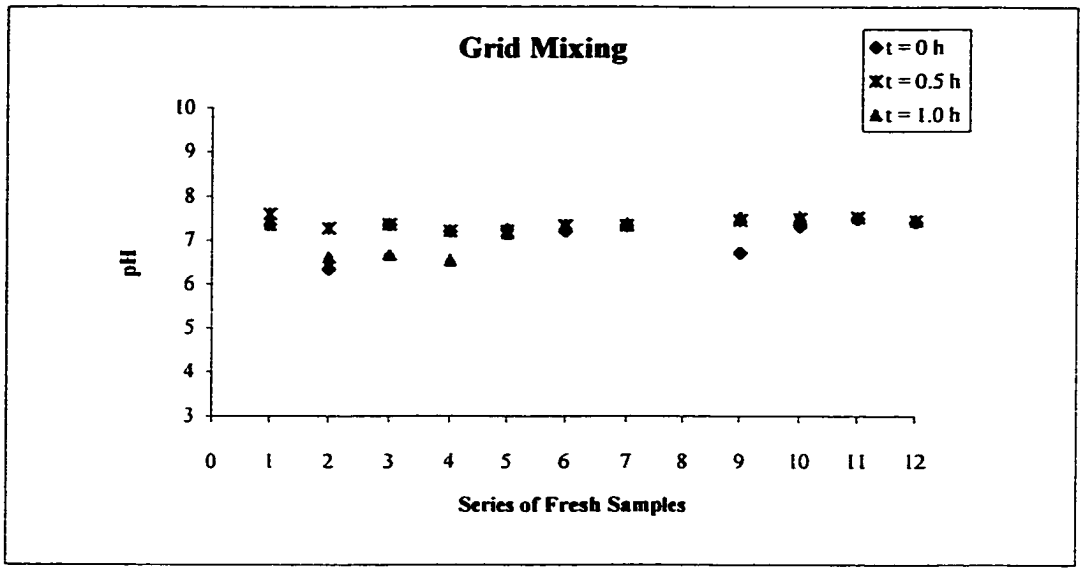


Figure C17. Consecutive daily testing for pH for fresh mixtures of PME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.

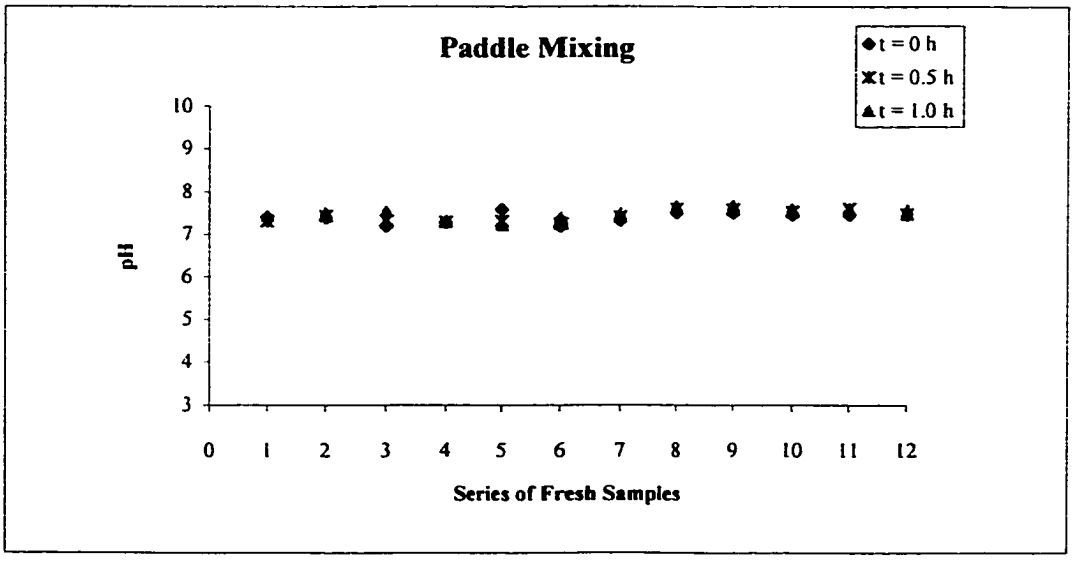


Figure C18. Consecutive daily testing for pH for fresh mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 1h at $\overline{Gf} = 13\ s^{-1}$).

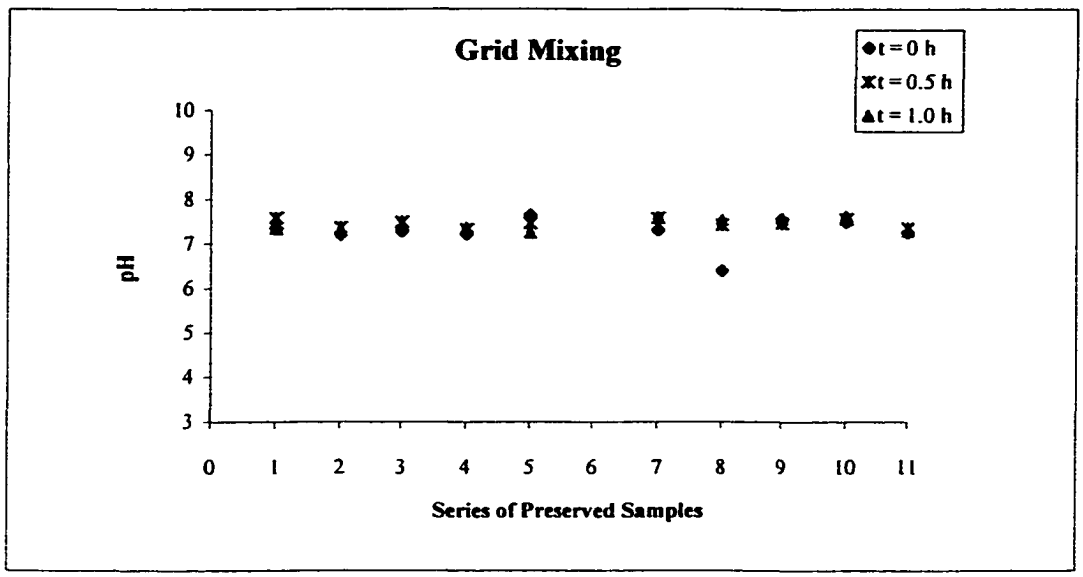


Figure C19. Consecutive daily testing for pH for preserved mixtures of PME and RW (1:1 ratio volume) using the grid mixing system at a vertical grid speed of 2 mm/s.

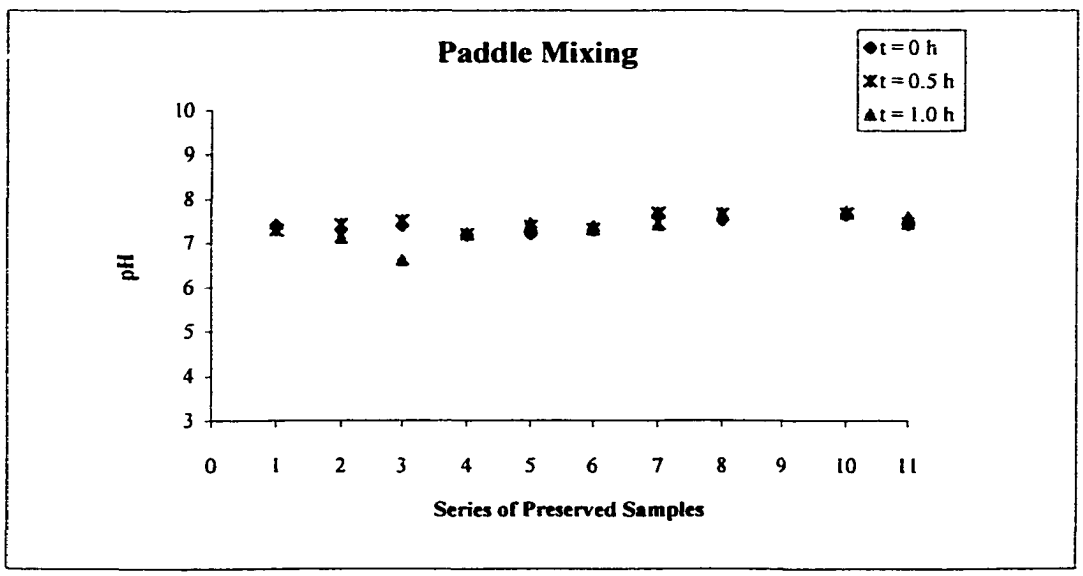


Figure C20. Consecutive daily testing for pH for preserved mixtures of PME and RW (1:1 ratio volume) using the paddle mixing system (5 minutes at $\overline{Gc} = 200$ s^{-1} and up to 1h at $\overline{Gf} = 13$ s^{-1}).

APPENDIX D

Effect of isolated pulp mill chemicals on PMEICF

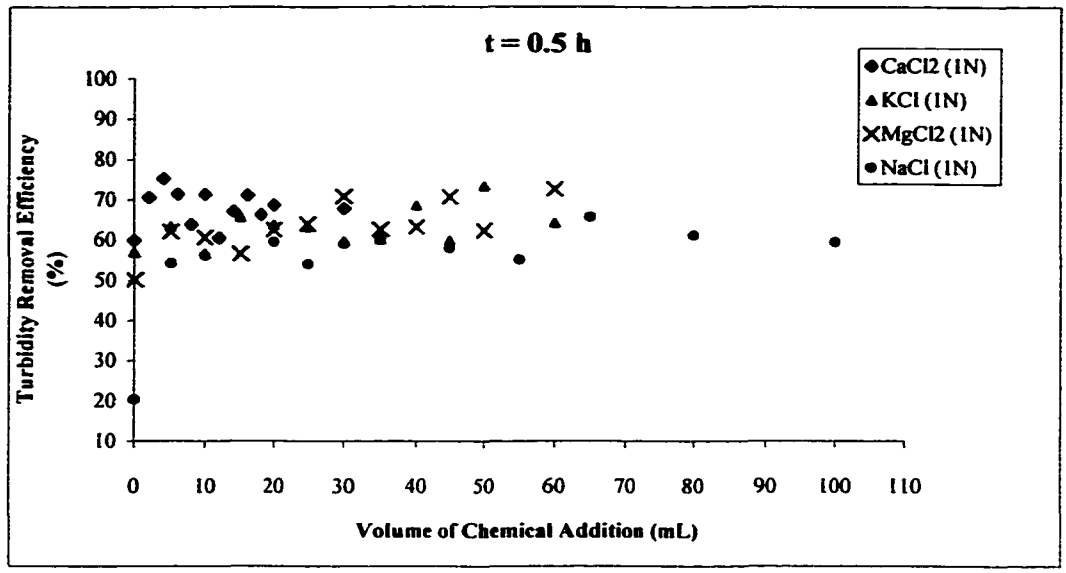


Figure D1. Effect of salts on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{G_f} = 13\ s^{-1}$)

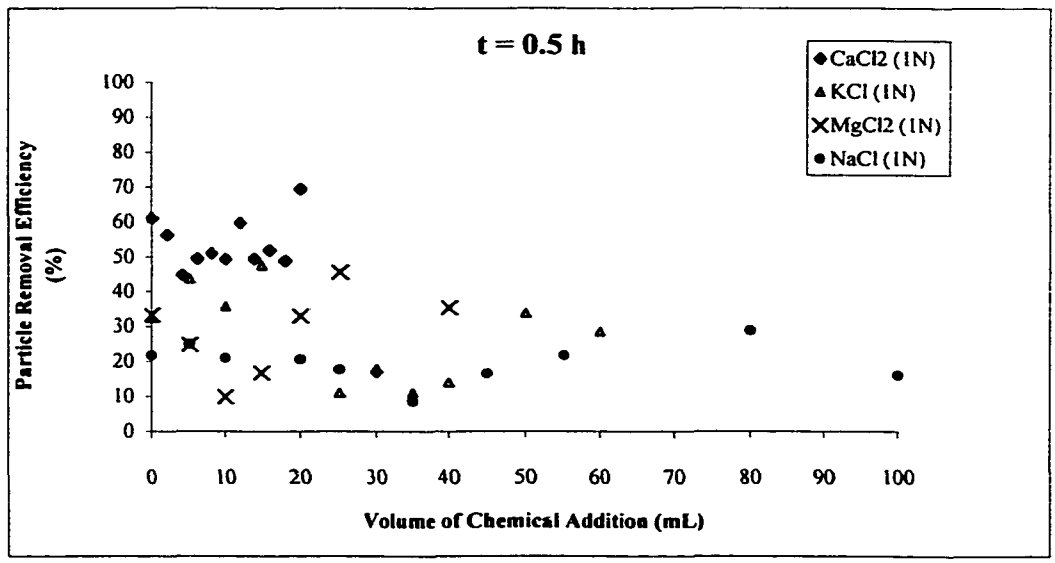


Figure D2. Effect of salts on particle removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{G_f} = 13\ s^{-1}$)

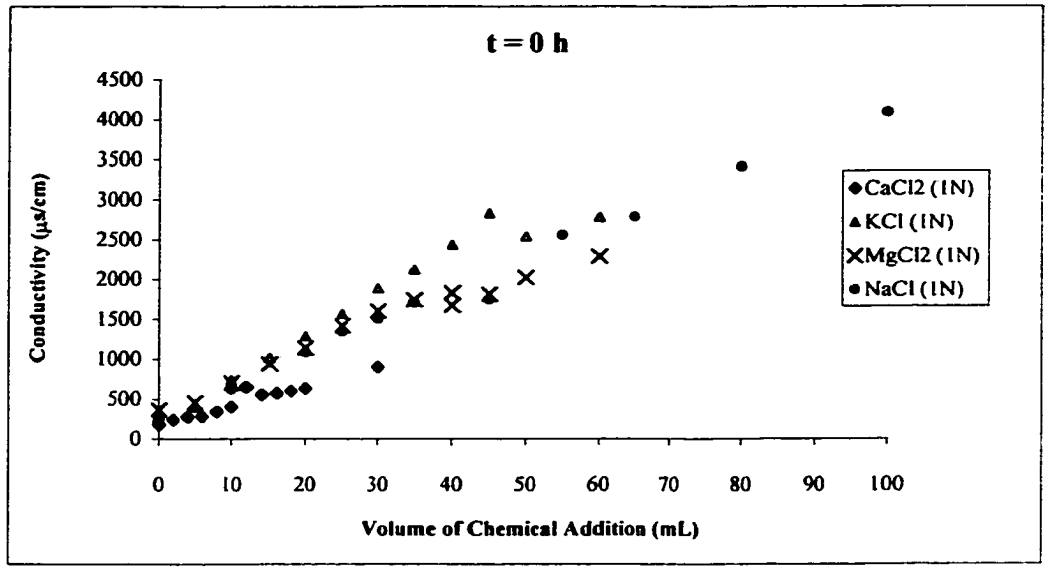


Figure D3. Effect of salts on conductivity for summer samples (samples were one day old Wapiti River Water)

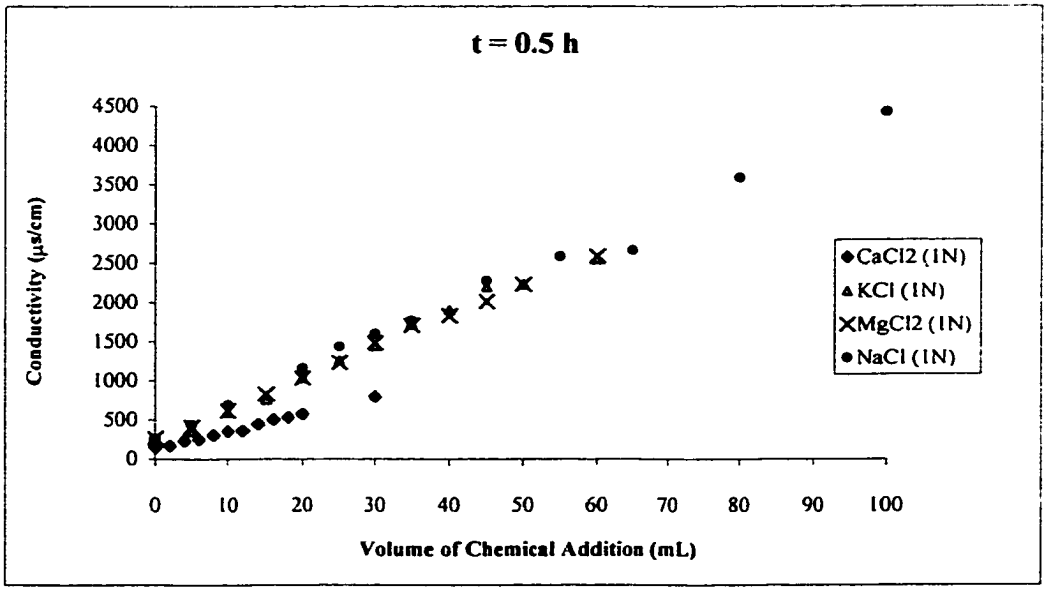


Figure D4. Effect of salts on conductivity for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$= 200 s^{-1} and 30 minutes at $\overline{G_f}$ = 13 s^{-1})

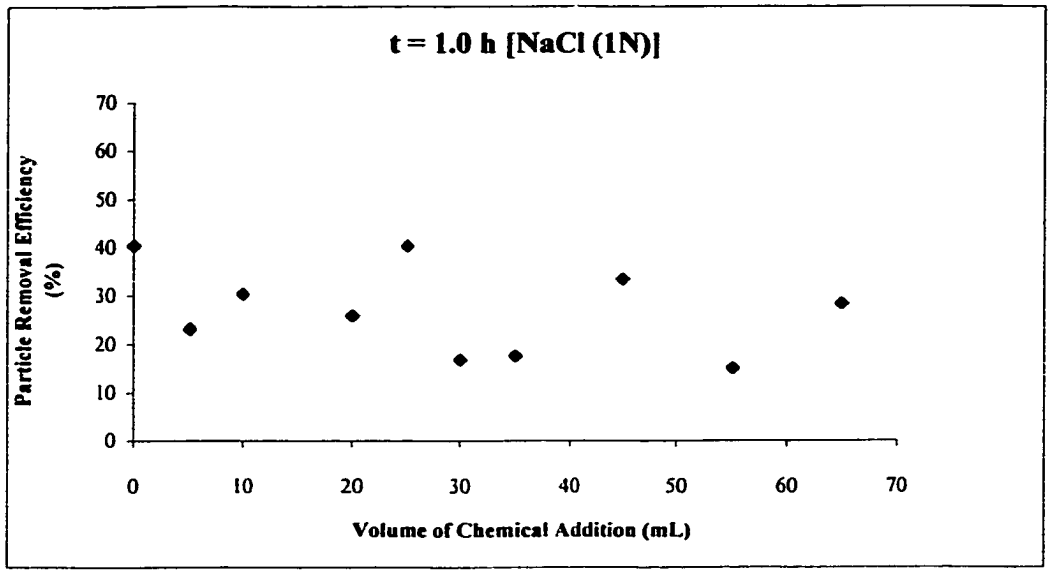


Figure D5. Effect of NaCl on particle removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 60 minutes at $\overline{Gf} = 13\ s^{-1}$)

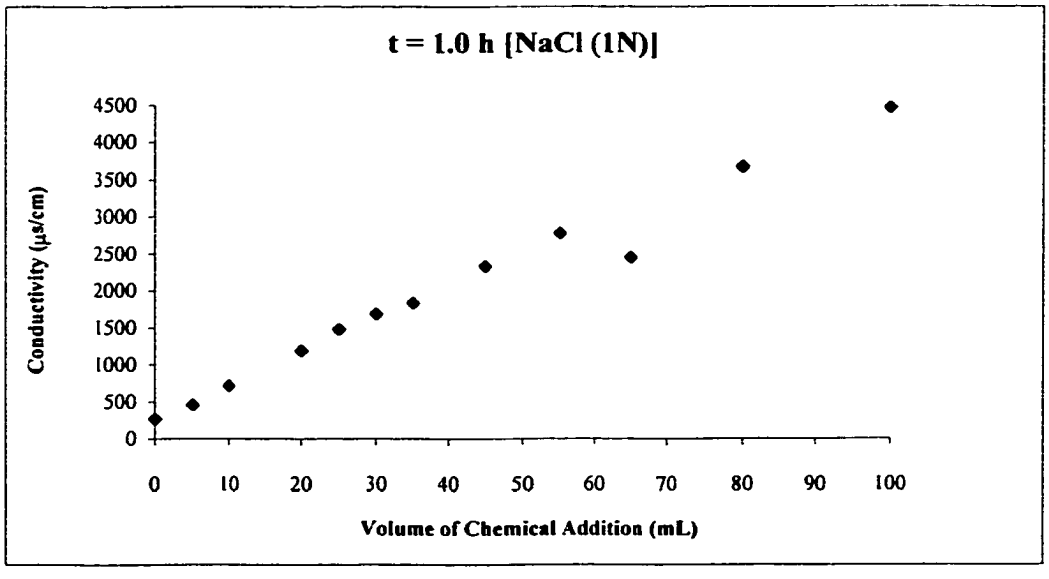


Figure D6. Effect of NaCl on conductivity for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 60 minutes at $\overline{Gf} = 13\ s^{-1}$)

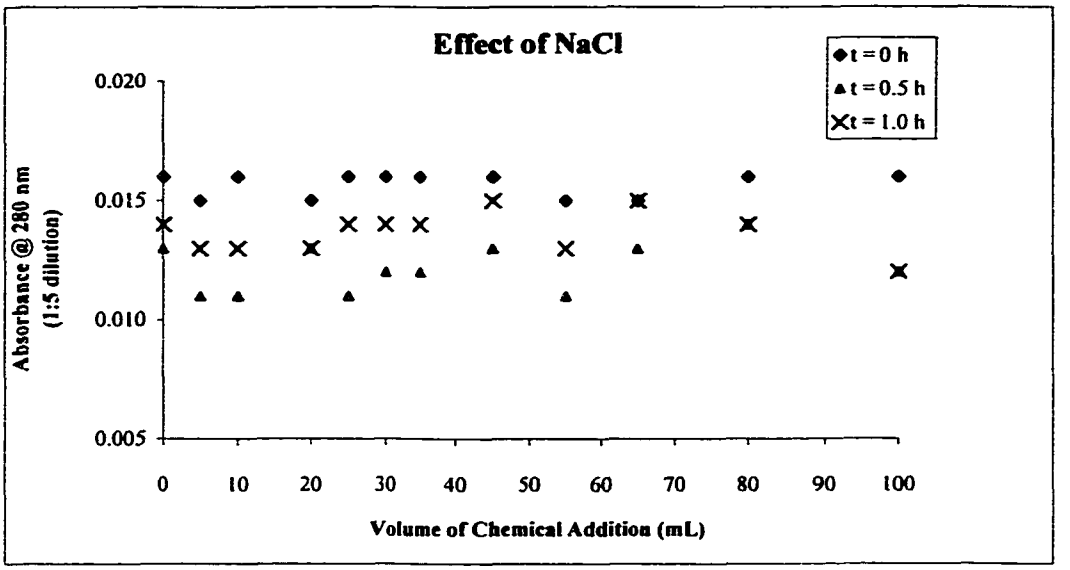


Figure D7. Effect of NaCl on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$= 200 s^{-1} and up to 60 minutes at $\overline{Gf}$ = 13 s^{-1})

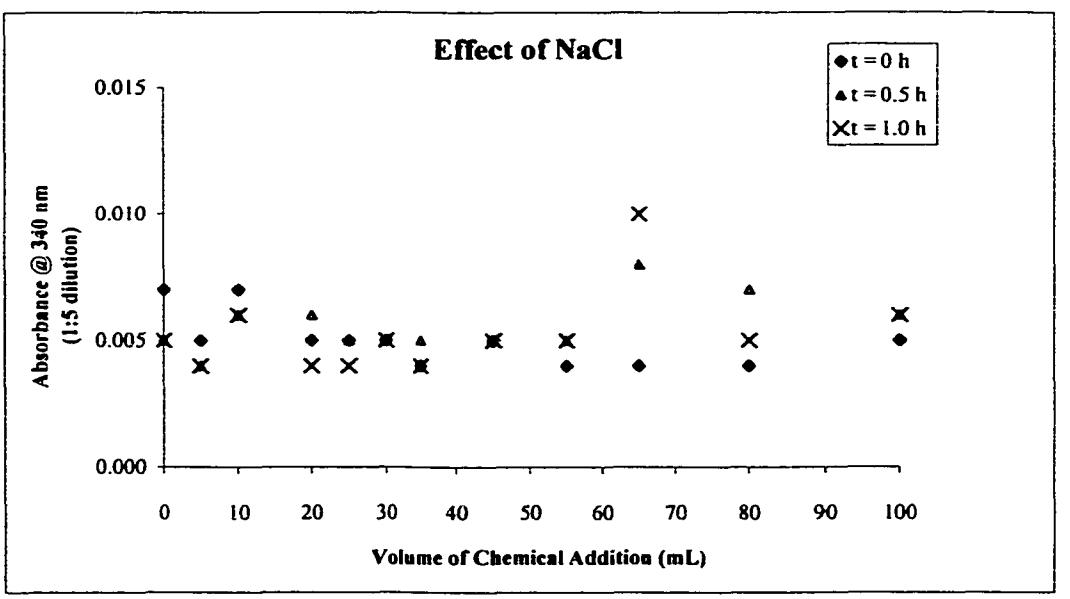


Figure D8. Effect of NaCl on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$= 200 s^{-1} and up to 60 minutes at $\overline{Gf}$ = 13 s^{-1})

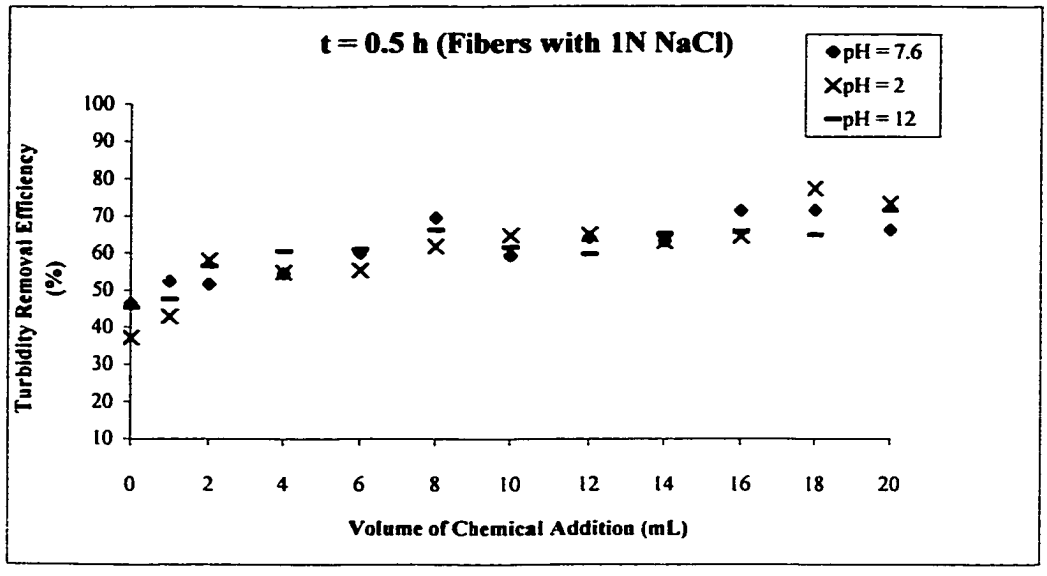


Figure D9. Effect of fibers (with NaCl) on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and 30 minutes at $\overline{G_f}$ = 13 s^{-1})

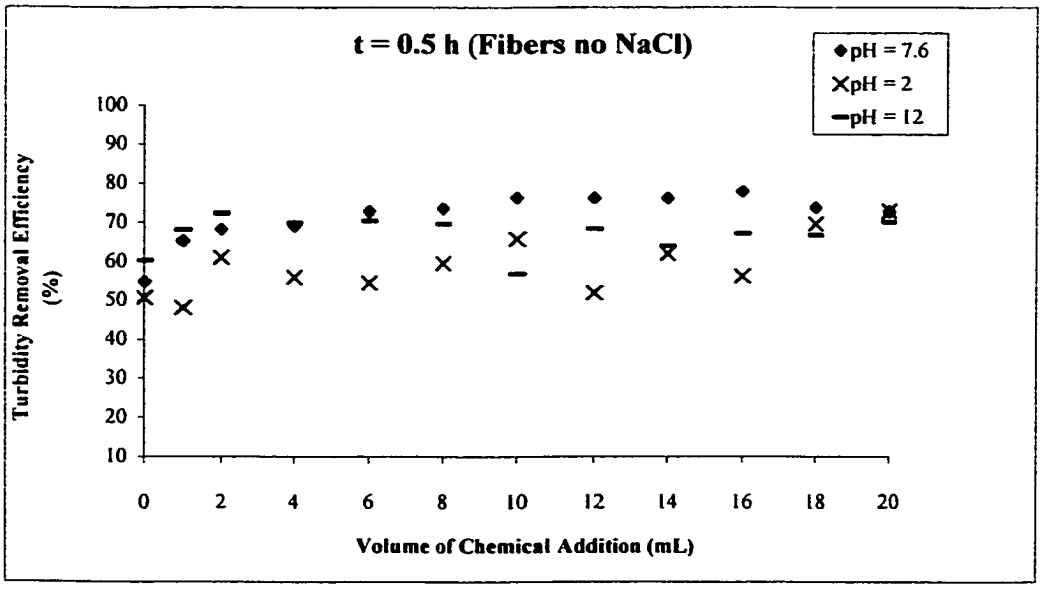


Figure D10. Effect of fibers (no NaCl) on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and up to 30 minutes at $\overline{G_f}$ = 13 s^{-1})

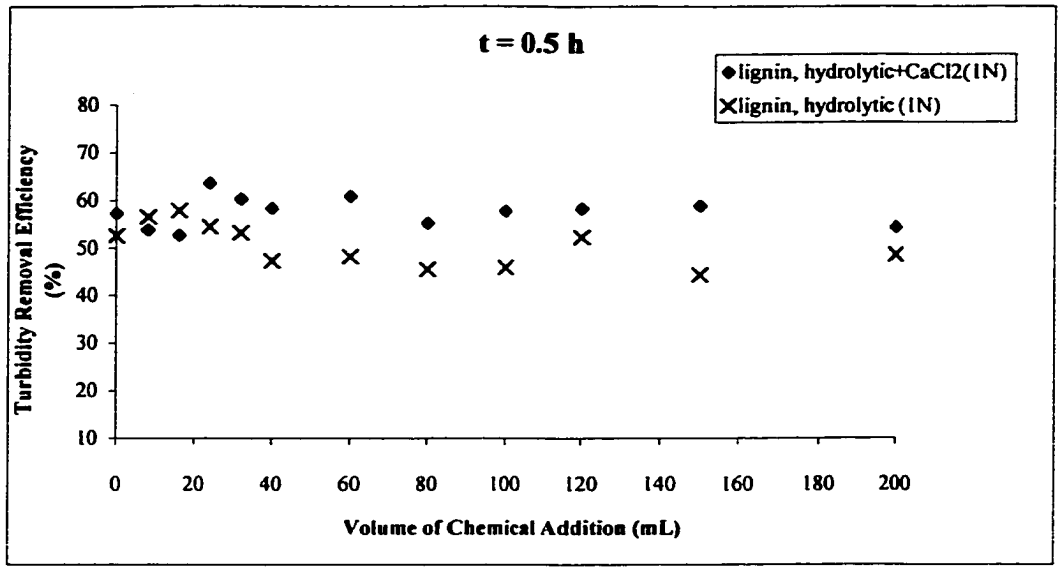


Figure D11. Effect of lignin on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{Gf} = 13\ s^{-1}$)

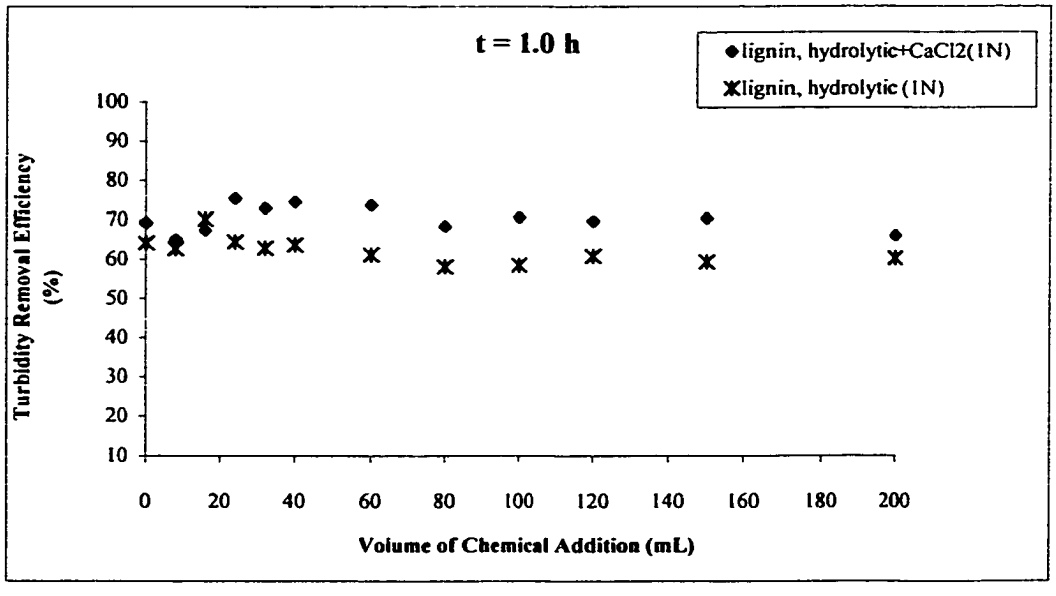


Figure D12. Effect of lignin on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 60 minutes at $\overline{Gf} = 13\ s^{-1}$)

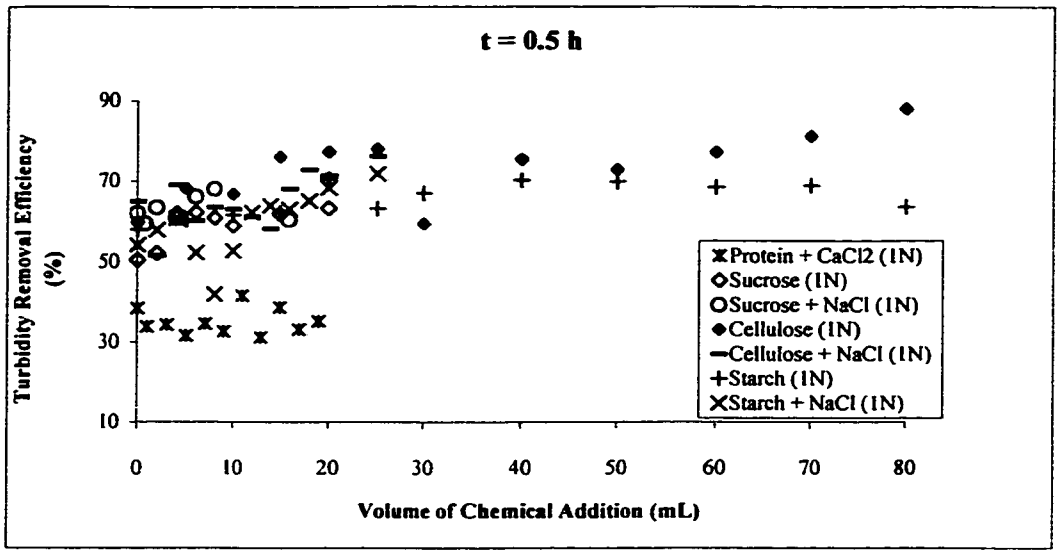


Figure D13. Effect of polymers on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{G_f} = 13\ s^{-1}$)

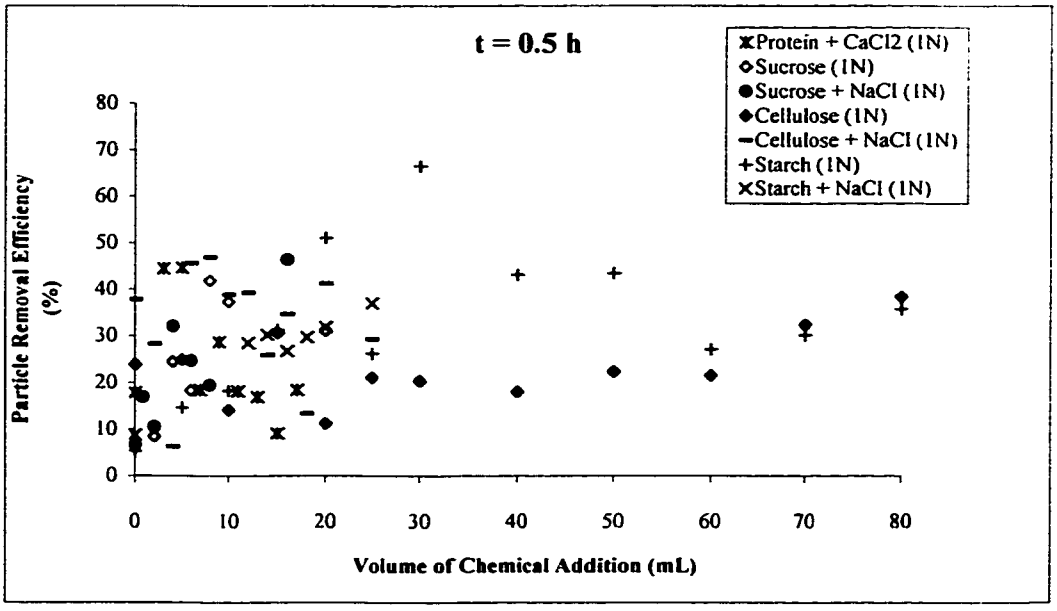


Figure D14. Effect of polymers on particle removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{G_f} = 13\ s^{-1}$)

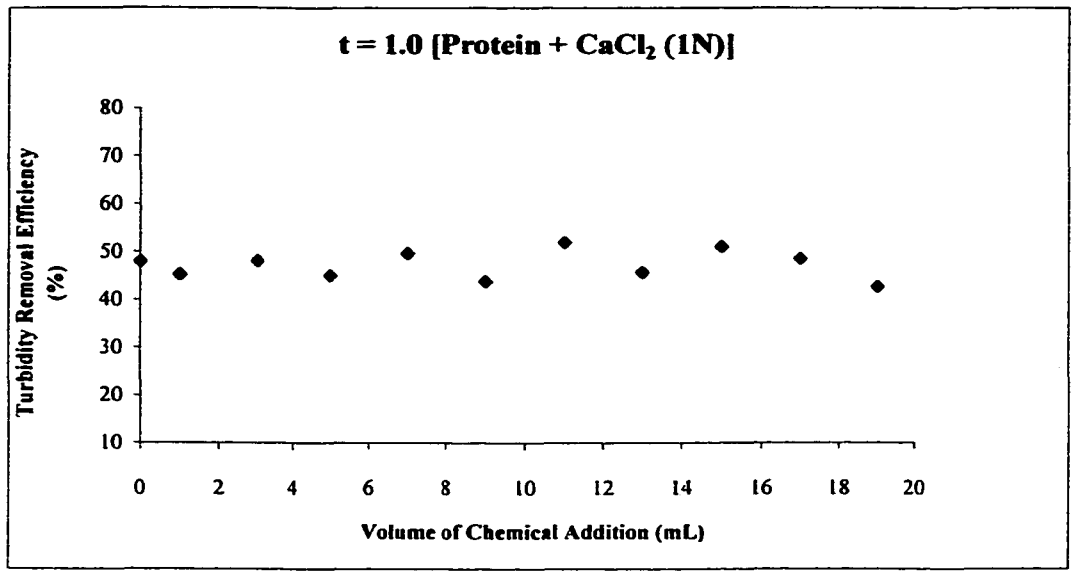


Figure D15. Effect of protein (with $CaCl_2$) on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and 60 minutes at $\overline{Gf}$ = 13 s^{-1})

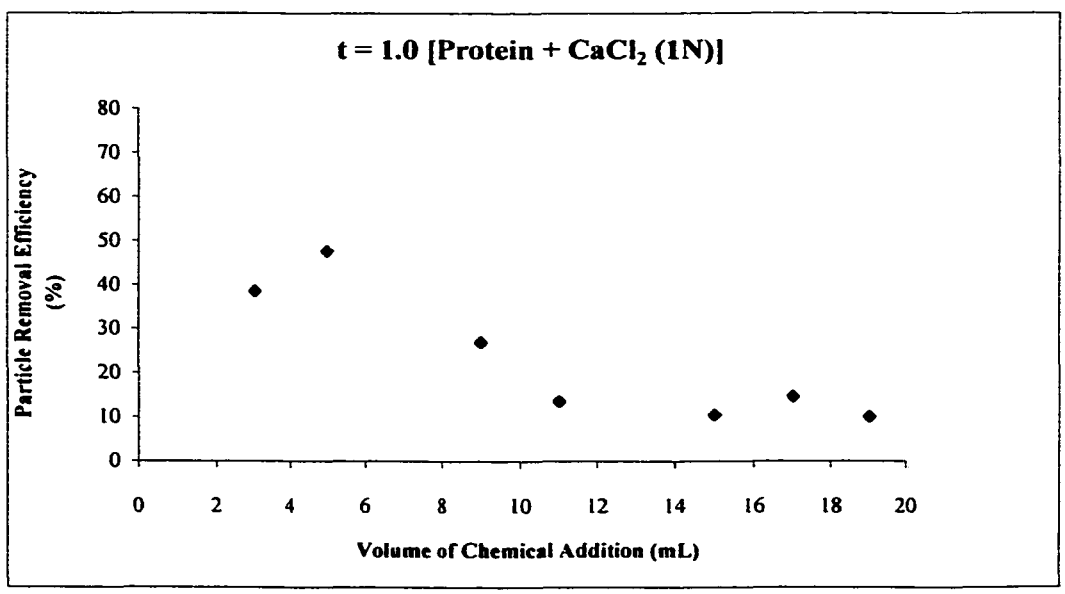


Figure D16. Effect of protein (with $CaCl_2$) on particle removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and up to 60 minutes at $\overline{Gf}$ = 13 s^{-1})

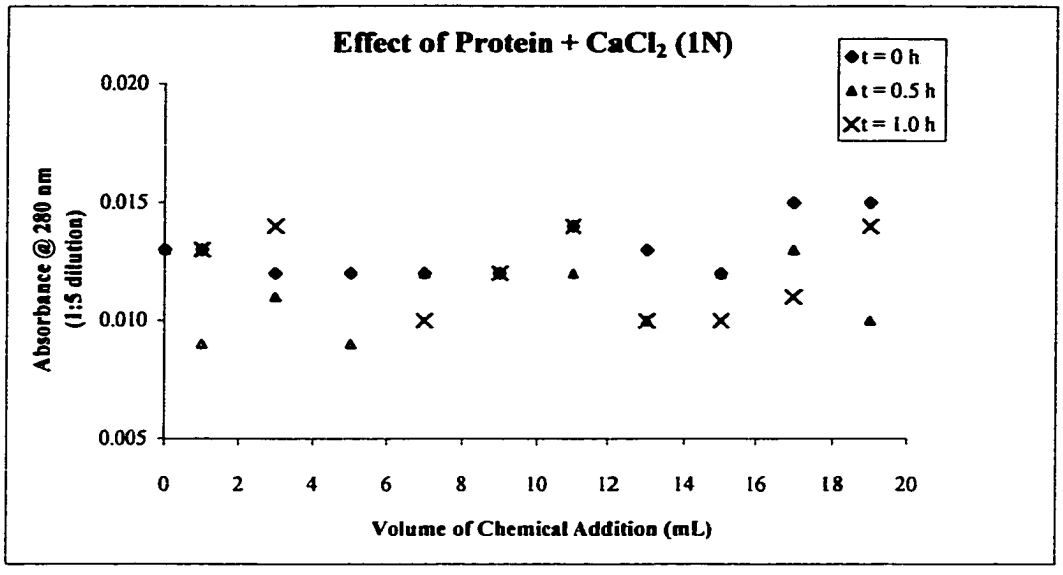


Figure D17. Effect of protein (with $CaCl_2$) on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{Gf} = 13\ s^{-1}$)

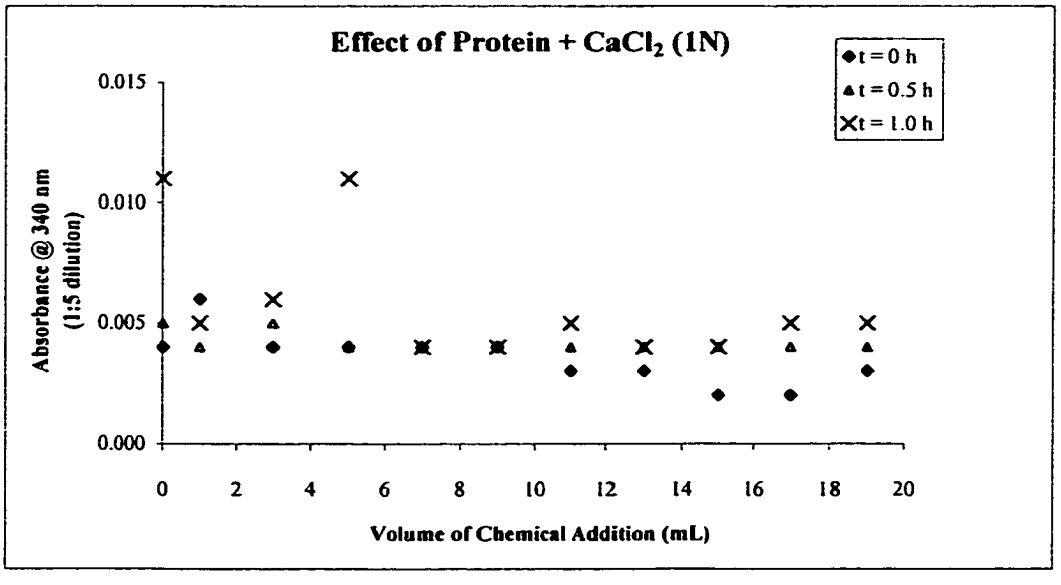


Figure D18. Effect of protein (with $CaCl_2$) on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{Gf} = 13\ s^{-1}$)

APPENDIX E

Effect of Fractionated PME on PMEICF

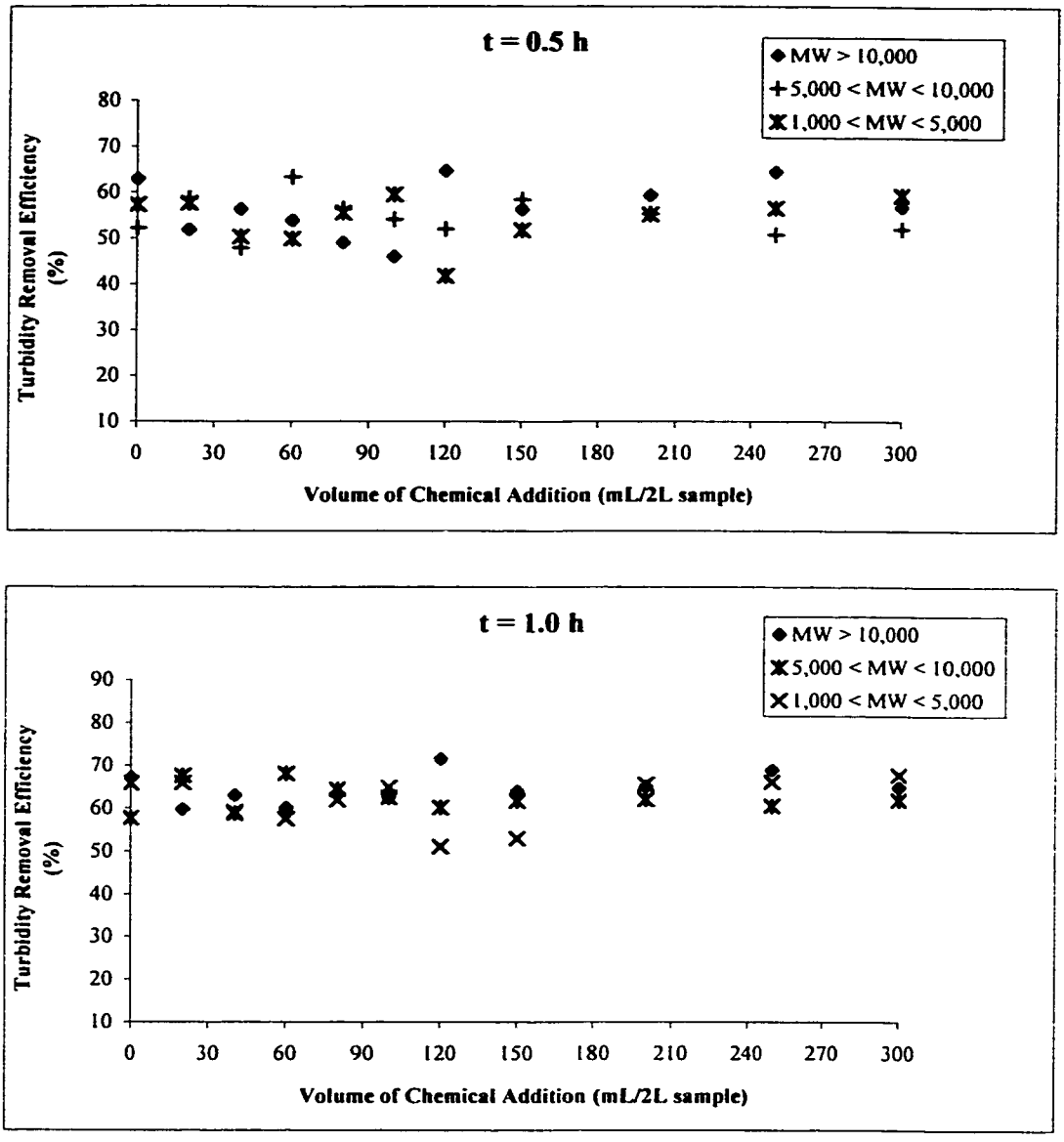


Figure E1. Effect of pulp mill chemicals from ultrafiltration fractionation on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{G_f} = 13\ s^{-1}$)

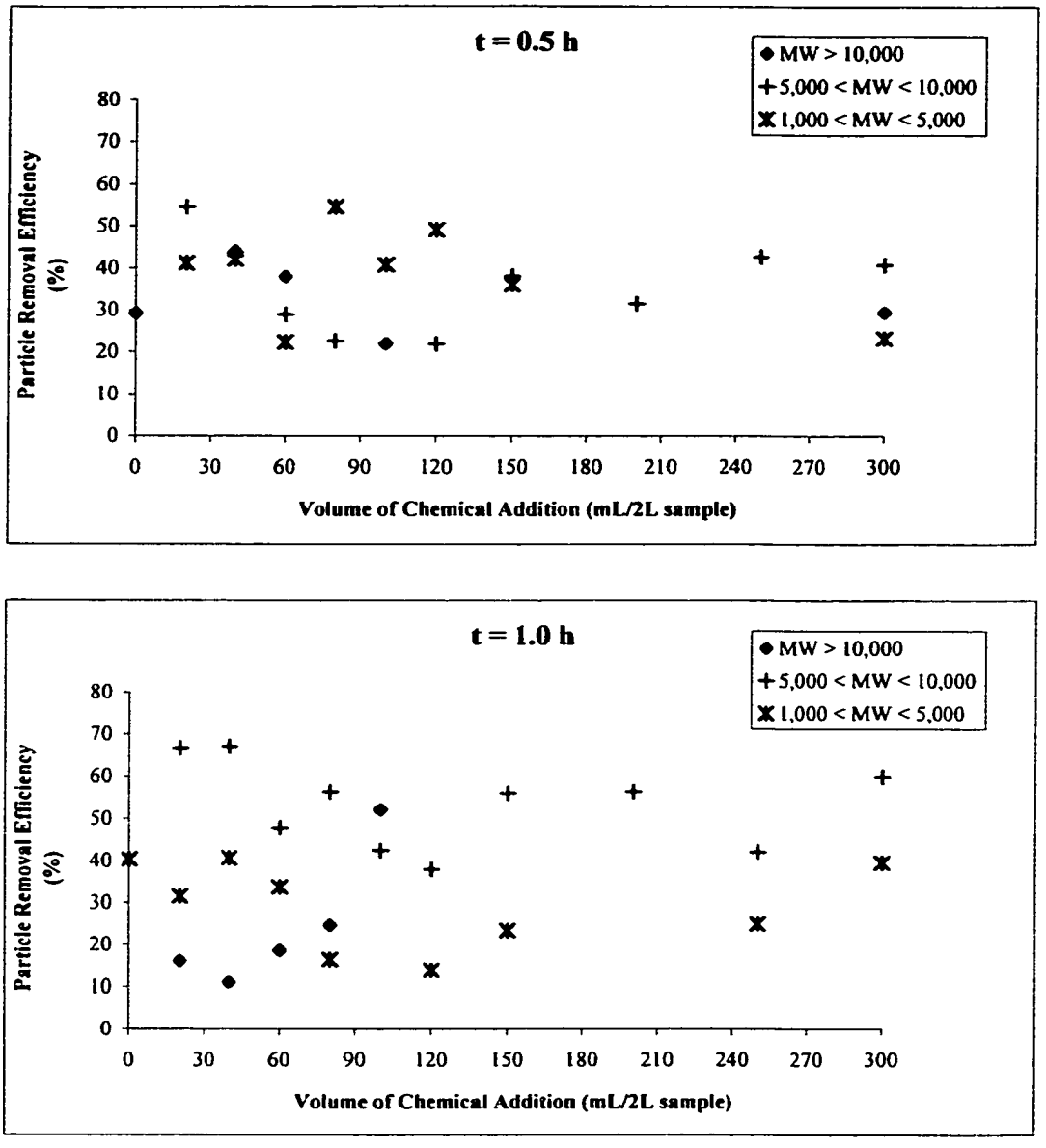
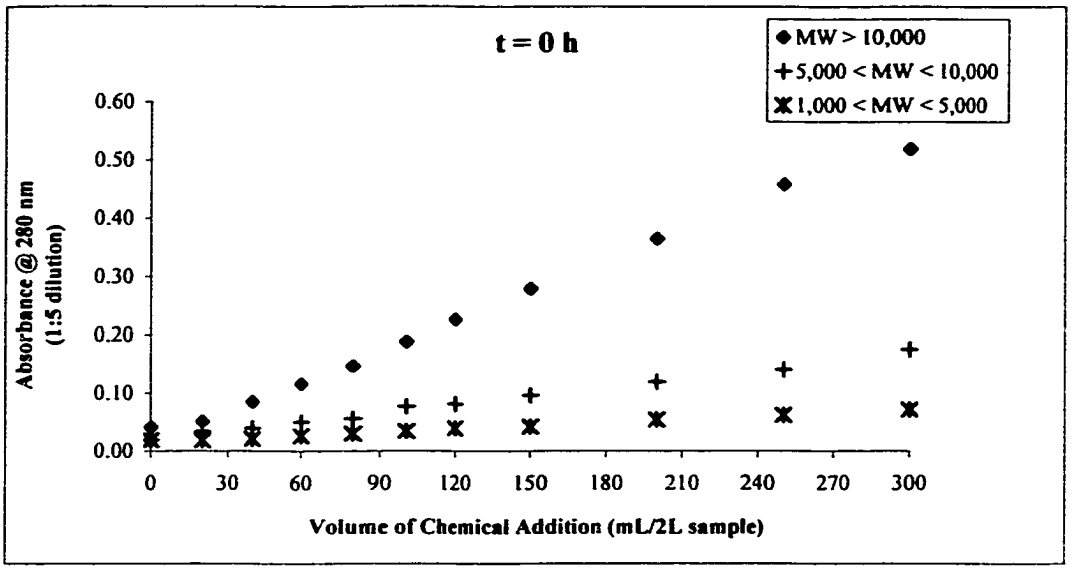


Figure E2. Effect of pulp mill chemicals from ultrafiltration fractionation on particle removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and up to 60 minutes at $\overline{G_f}$ = 13 s^{-1})



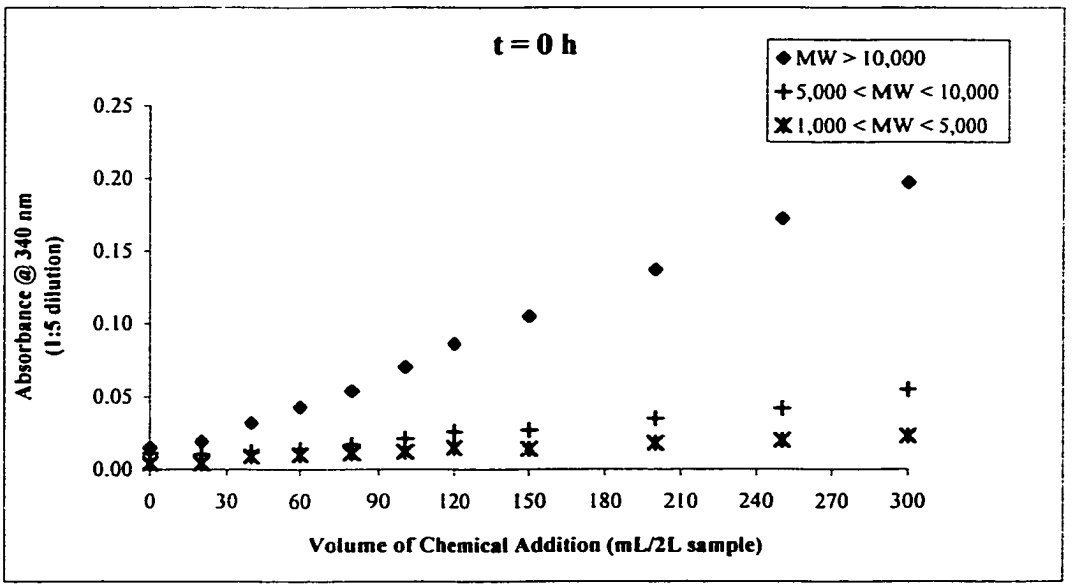
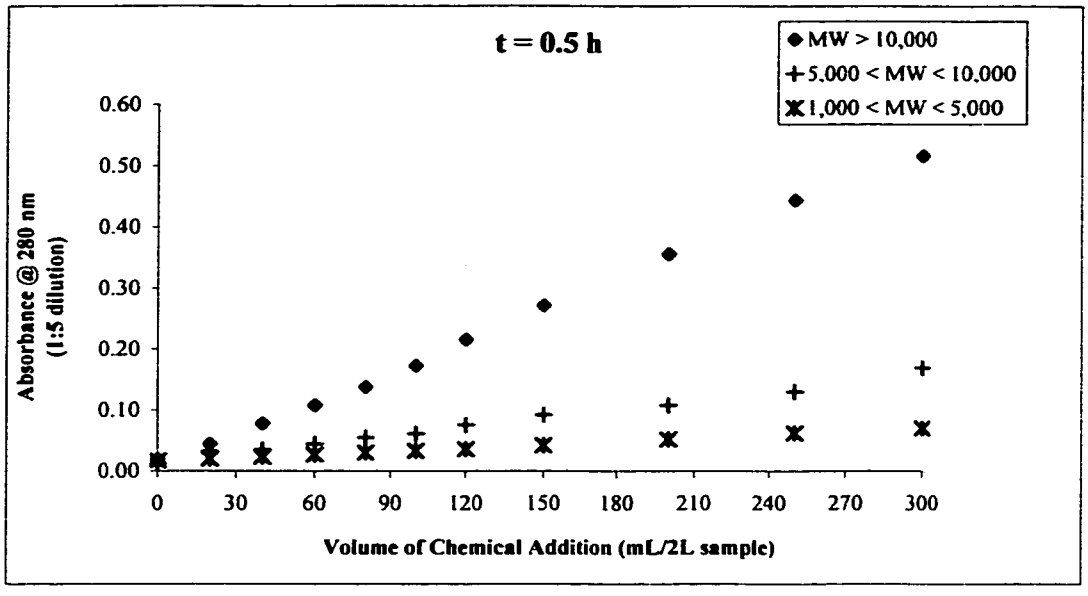


Figure E3. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 0 minutes at $\overline{Gf} = 13\ s^{-1}$)



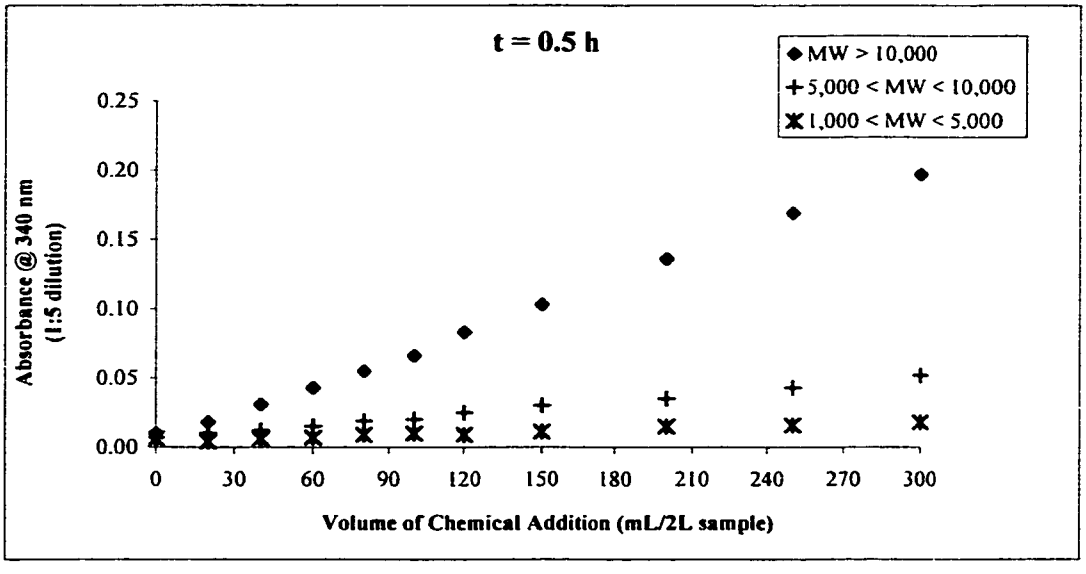
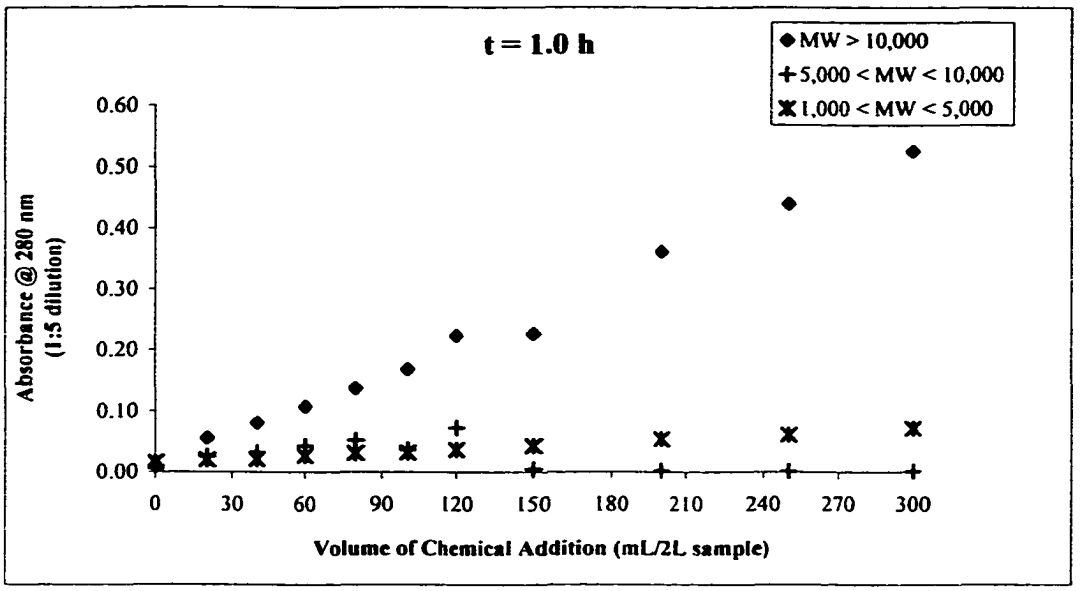


Figure E4. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{Gf} = 13\ s^{-1}$)



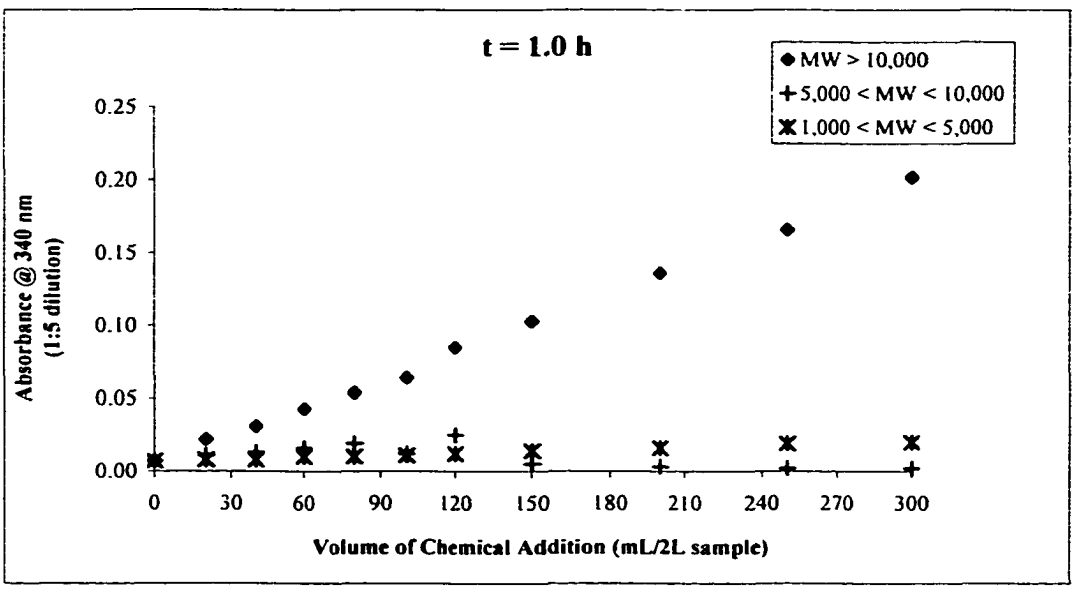
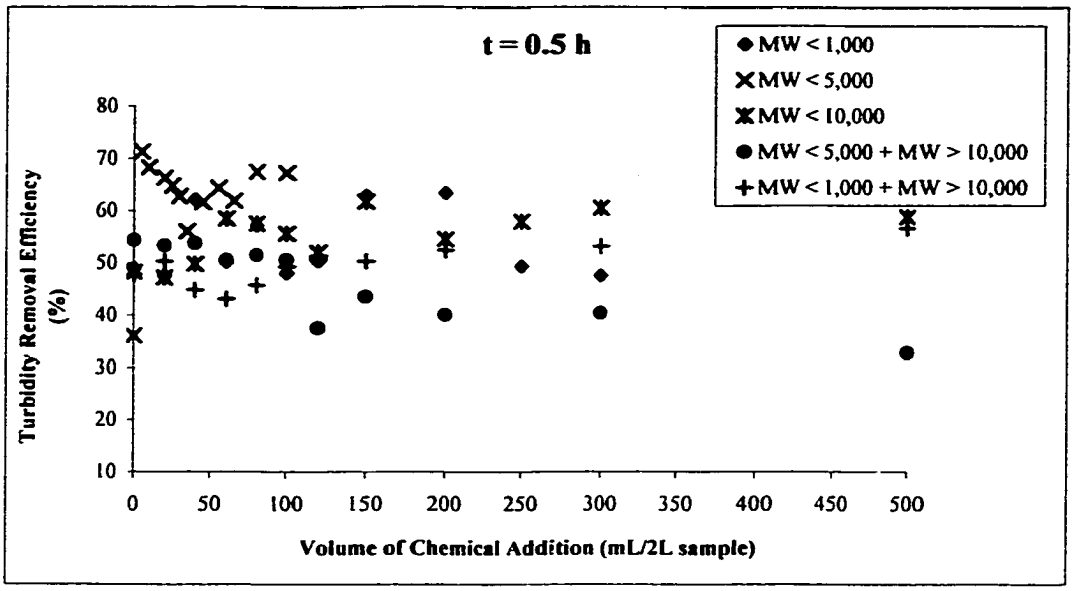


Figure E5. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 60 minutes at $\overline{Gf} = 13\ s^{-1}$)



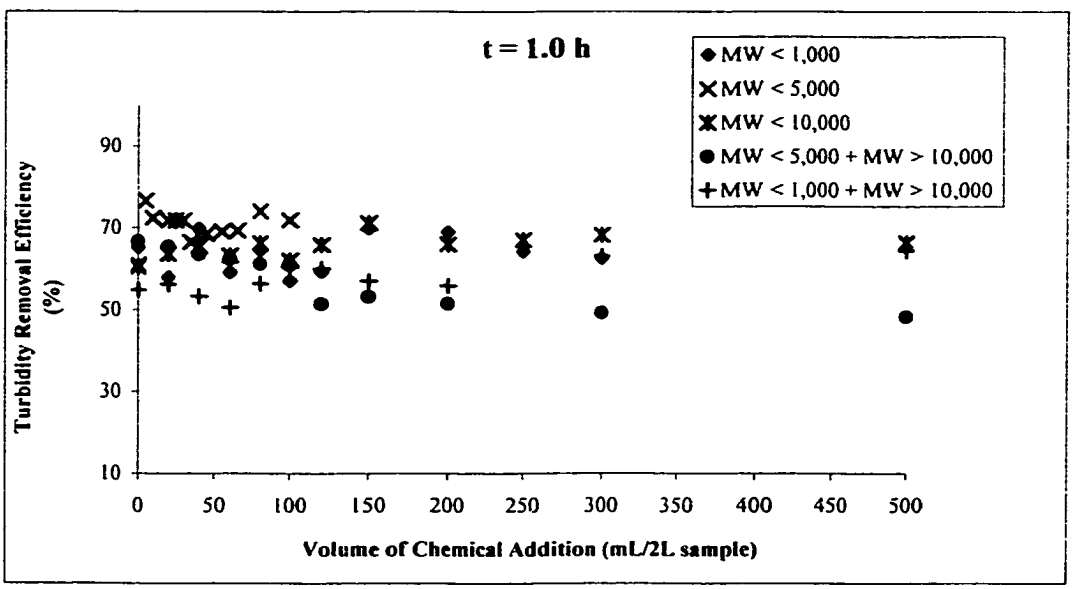
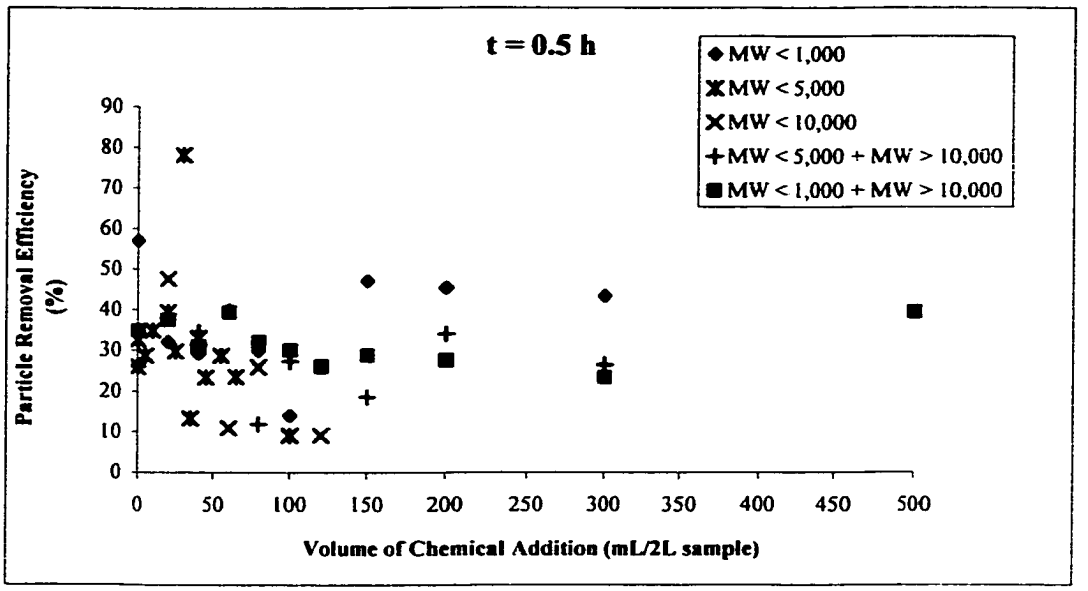


Figure E6. Effect of pulp mill chemicals from ultrafiltration fractionation on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{Gf} = 13\ s^{-1}$)



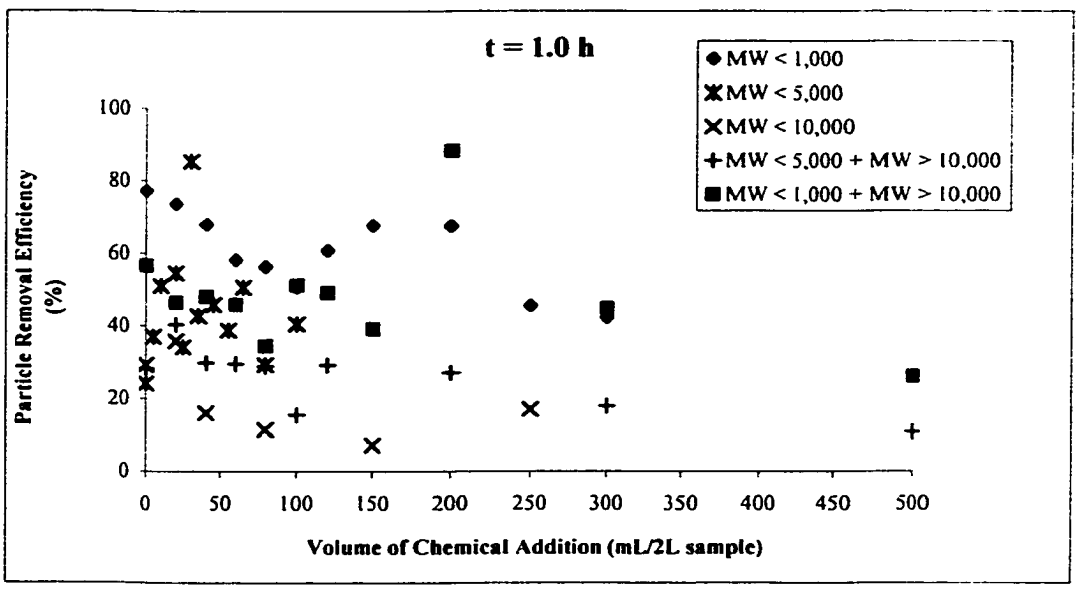
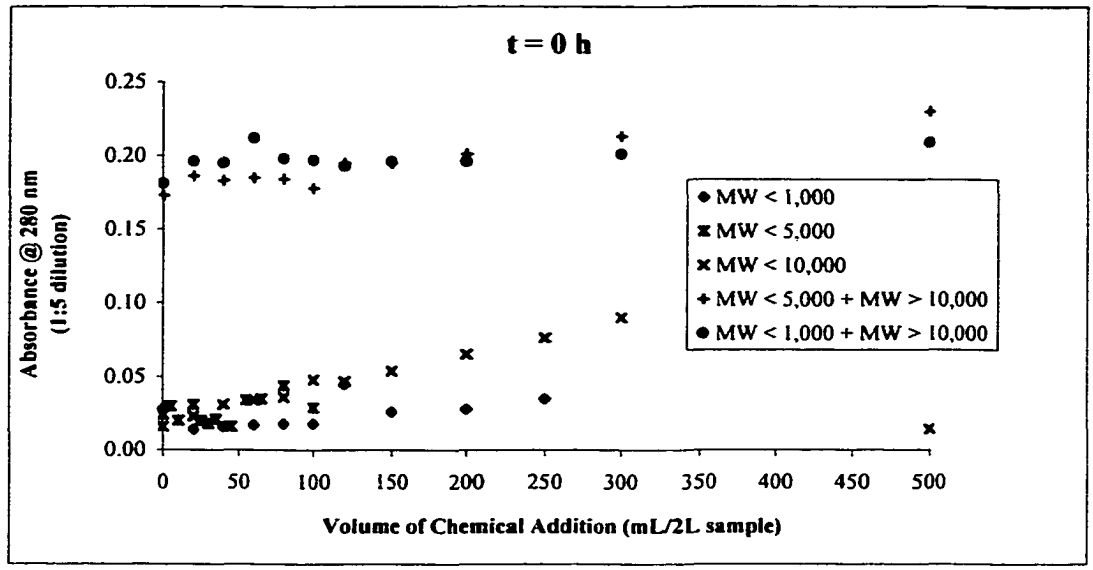


Figure E7. Effect of pulp mill chemicals from ultrafiltration fractionation on particle removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and up to 60 minutes at $\overline{Gf}$ = 13 s^{-1})



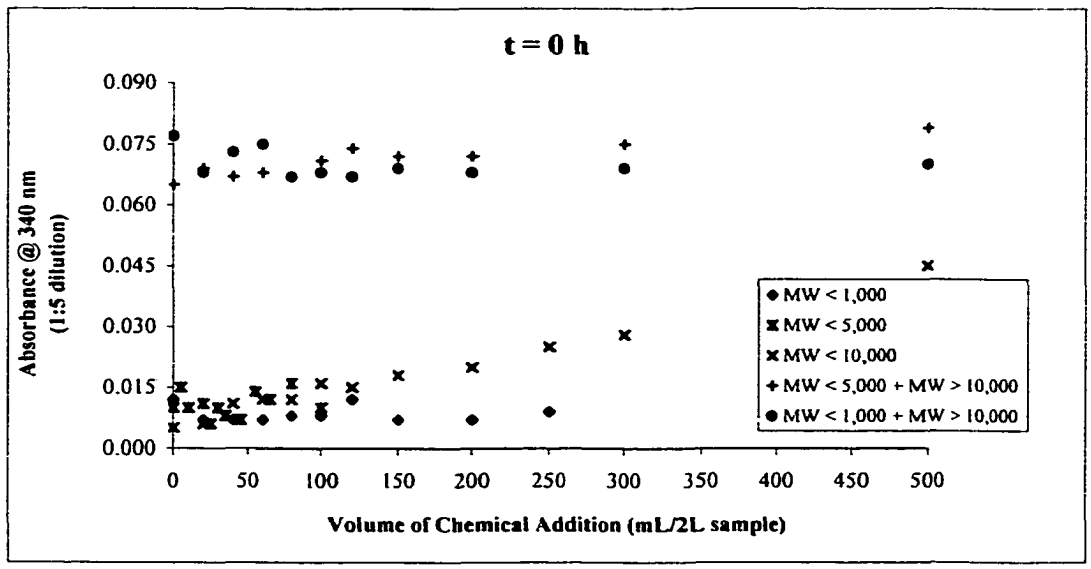
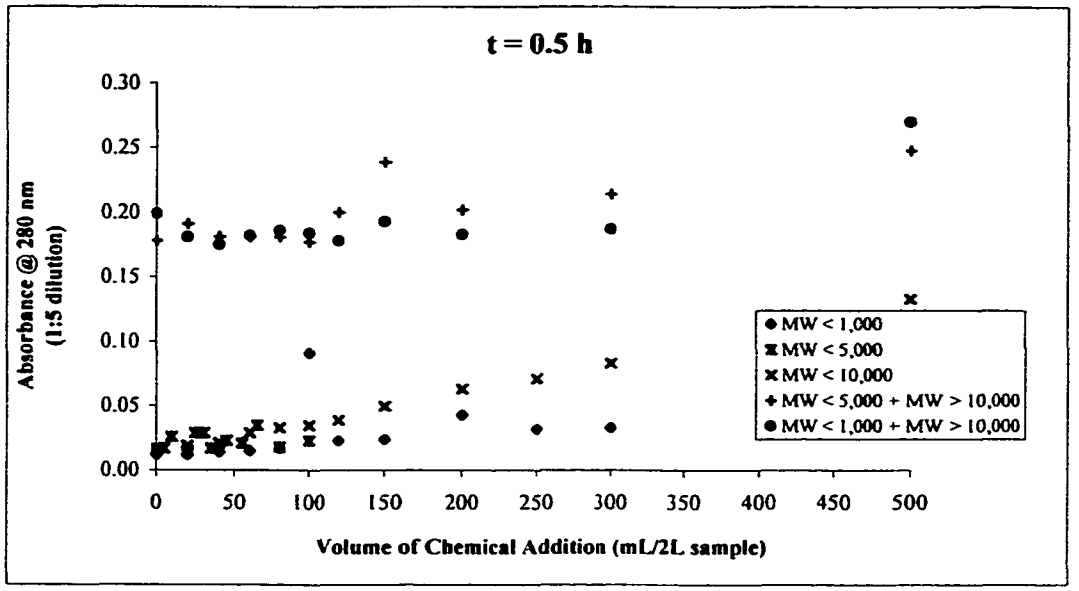


Figure E8. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and 0 minutes at $\overline{Gf}$ = 13 s^{-1})



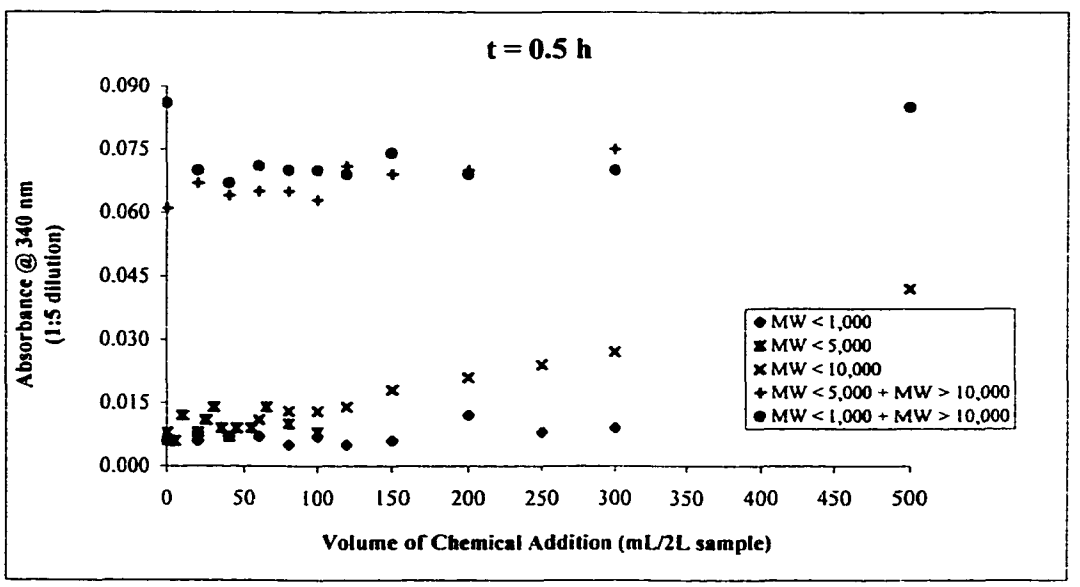
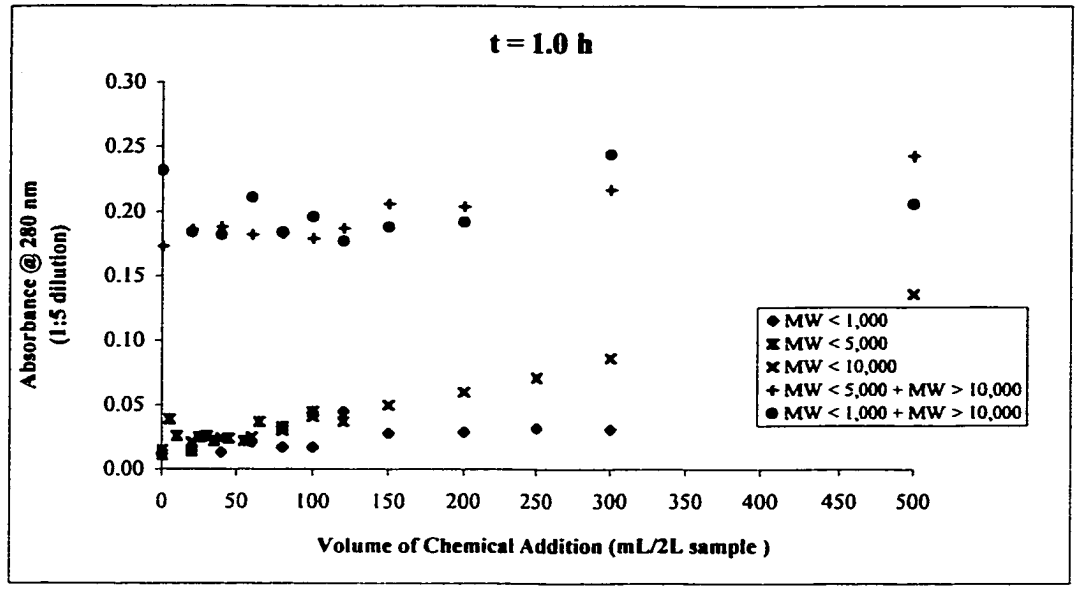


Figure E9. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and 30 minutes at $\overline{Gf} = 13\ s^{-1}$)



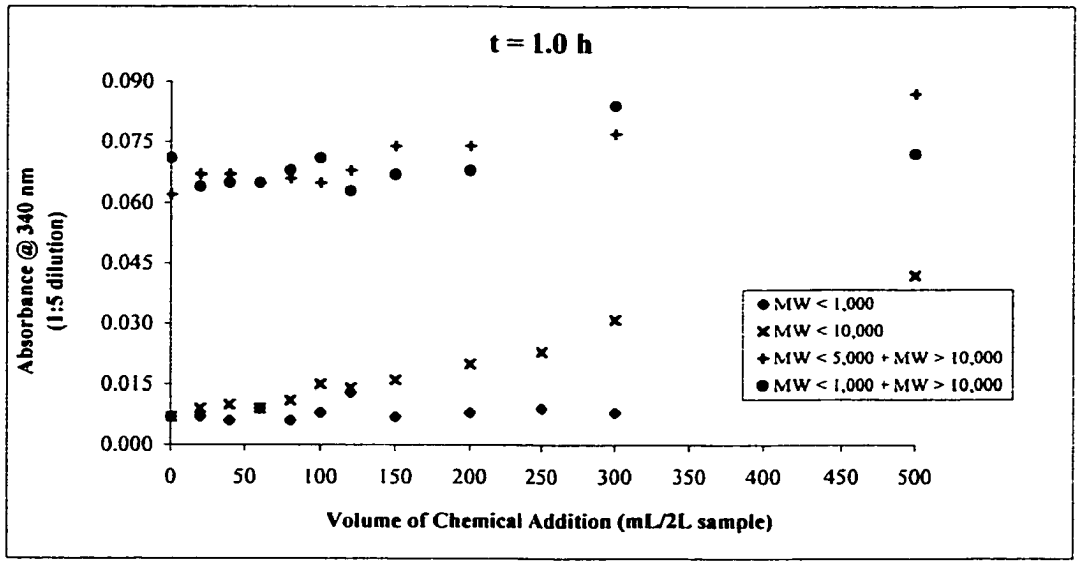
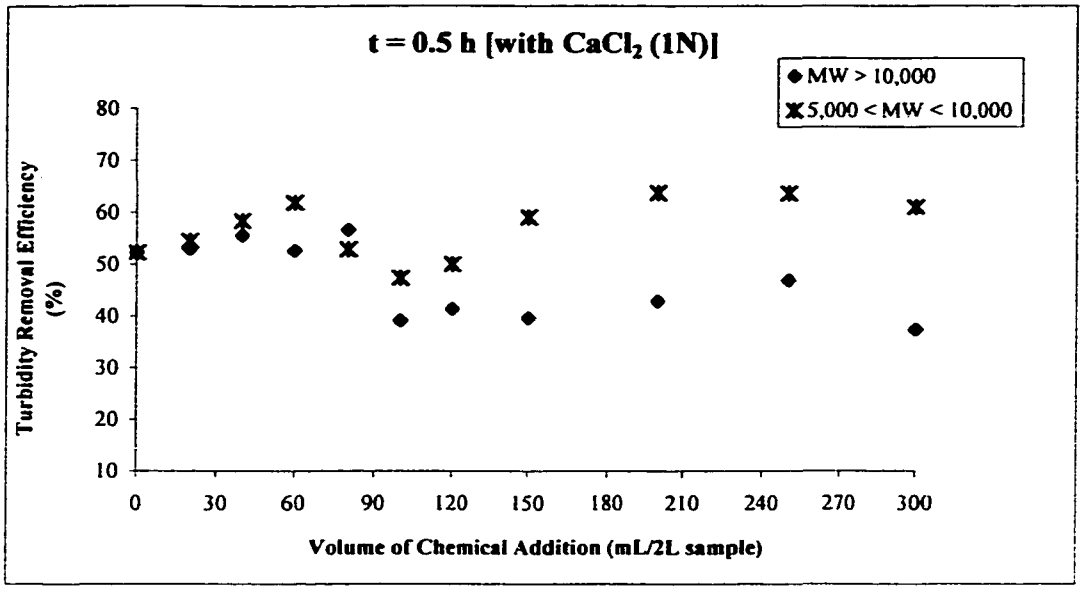


Figure E10. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and 60 minutes at $\overline{G_f}$ = 13 s^{-1})



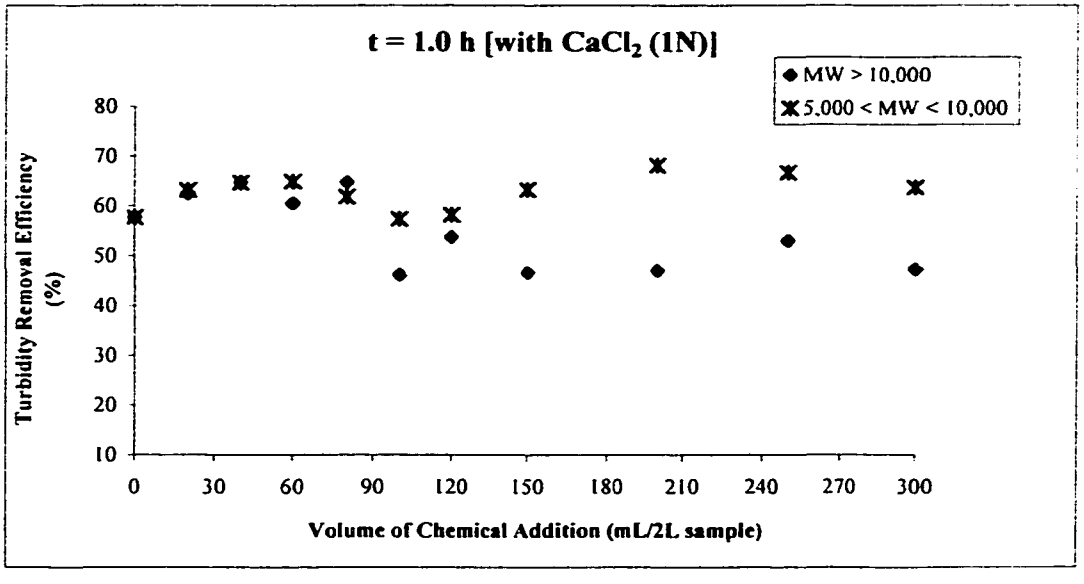


Figure E11. Effect of pulp mill chemicals from ultrafiltration fractionation on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and up to 60 minutes at $\overline{Gf}$ = 13 s^{-1})

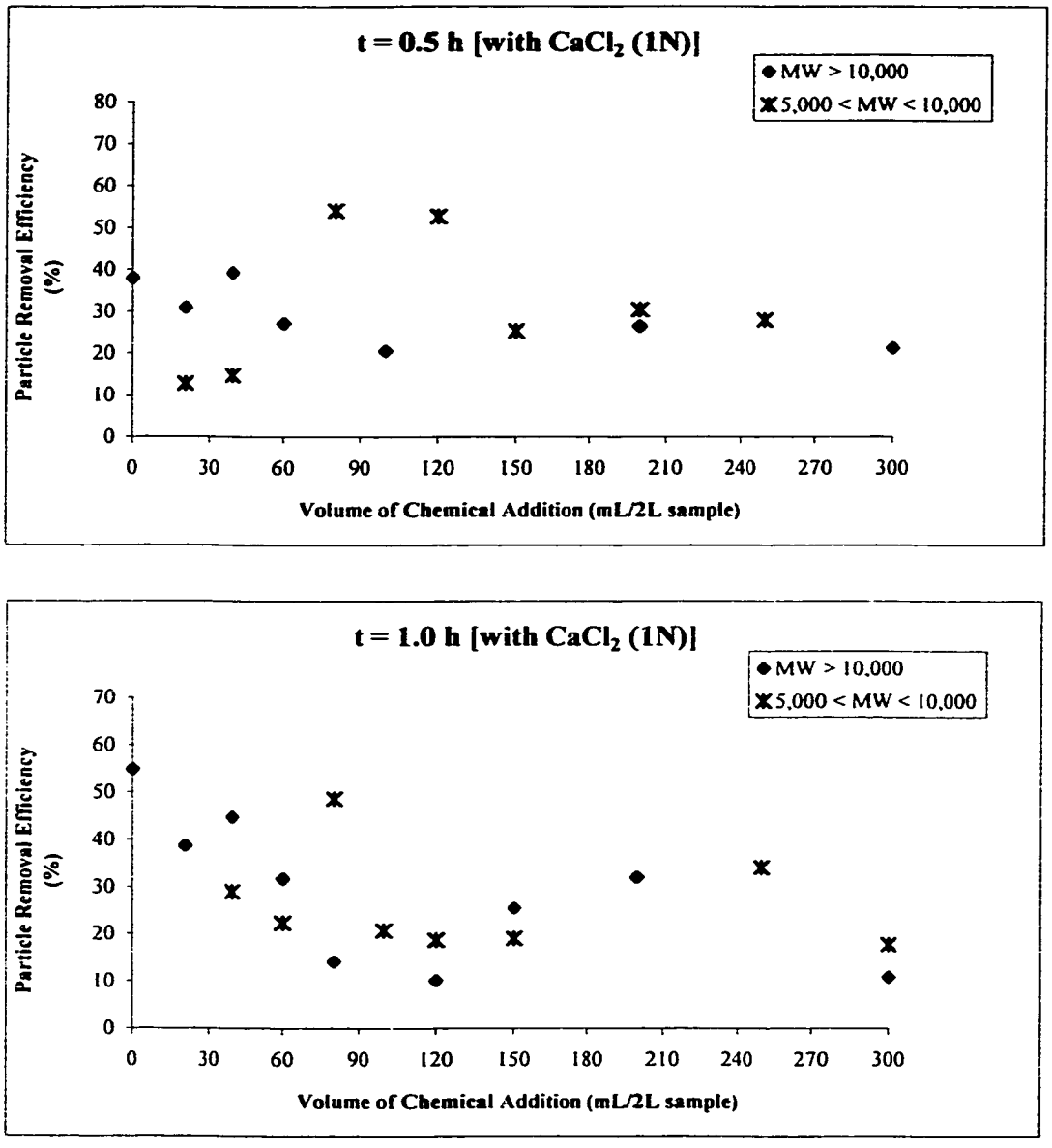
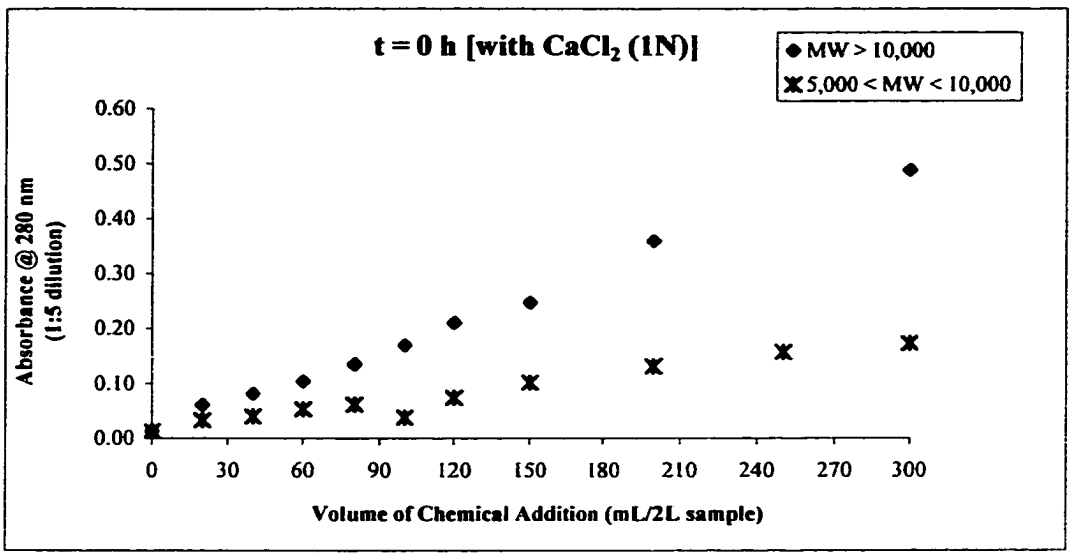


Figure E12. Effect of pulp mill chemicals from ultrafiltration fractionation on particle removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{Gf} = 13\ s^{-1}$)



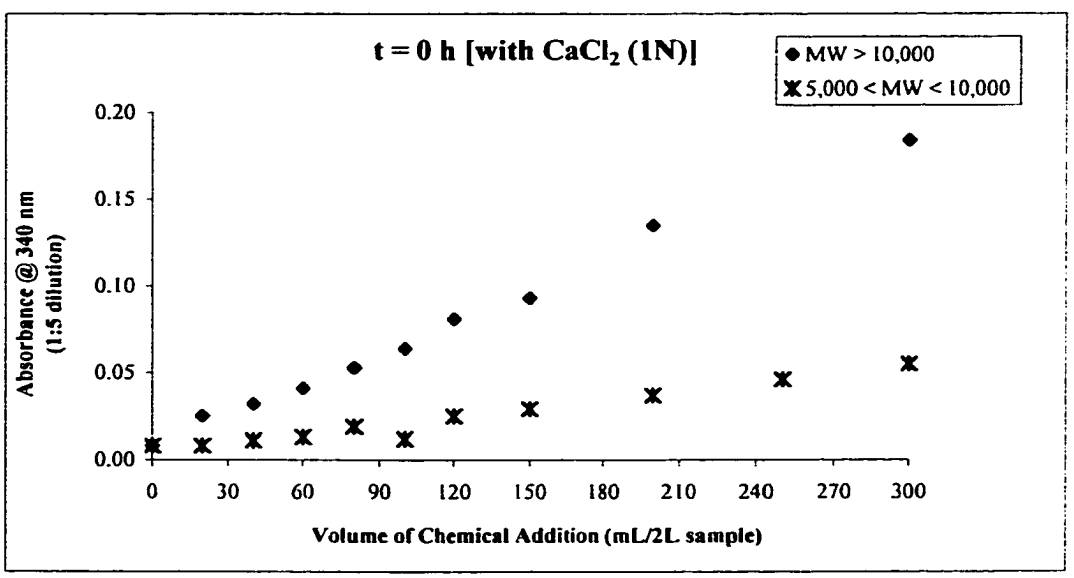


Figure E13. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and 0 minutes at $\overline{Gf}$ = 13 s^{-1})

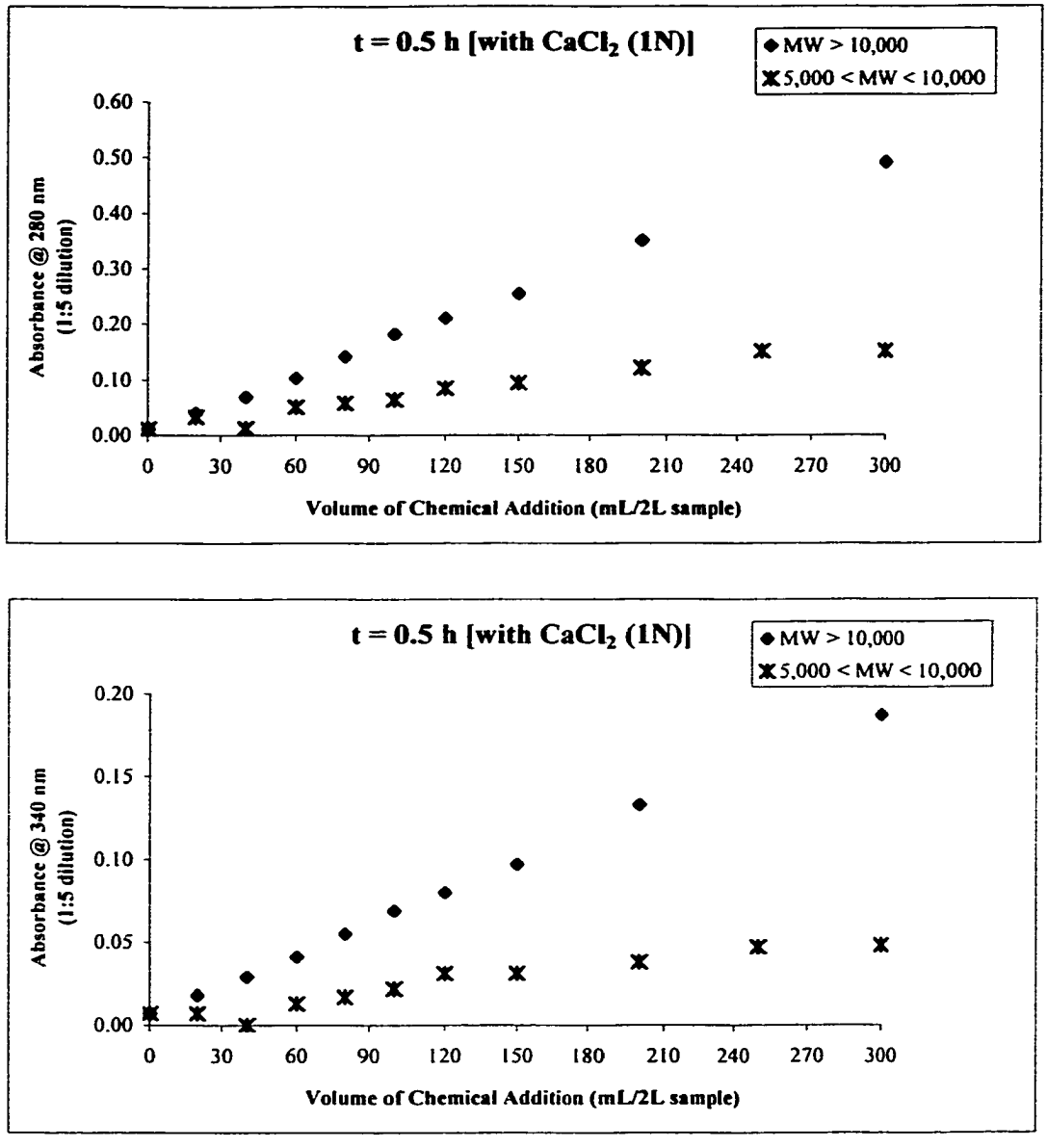


Figure E14. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and 30 minutes at $\overline{Gf}$ = 13 s^{-1})

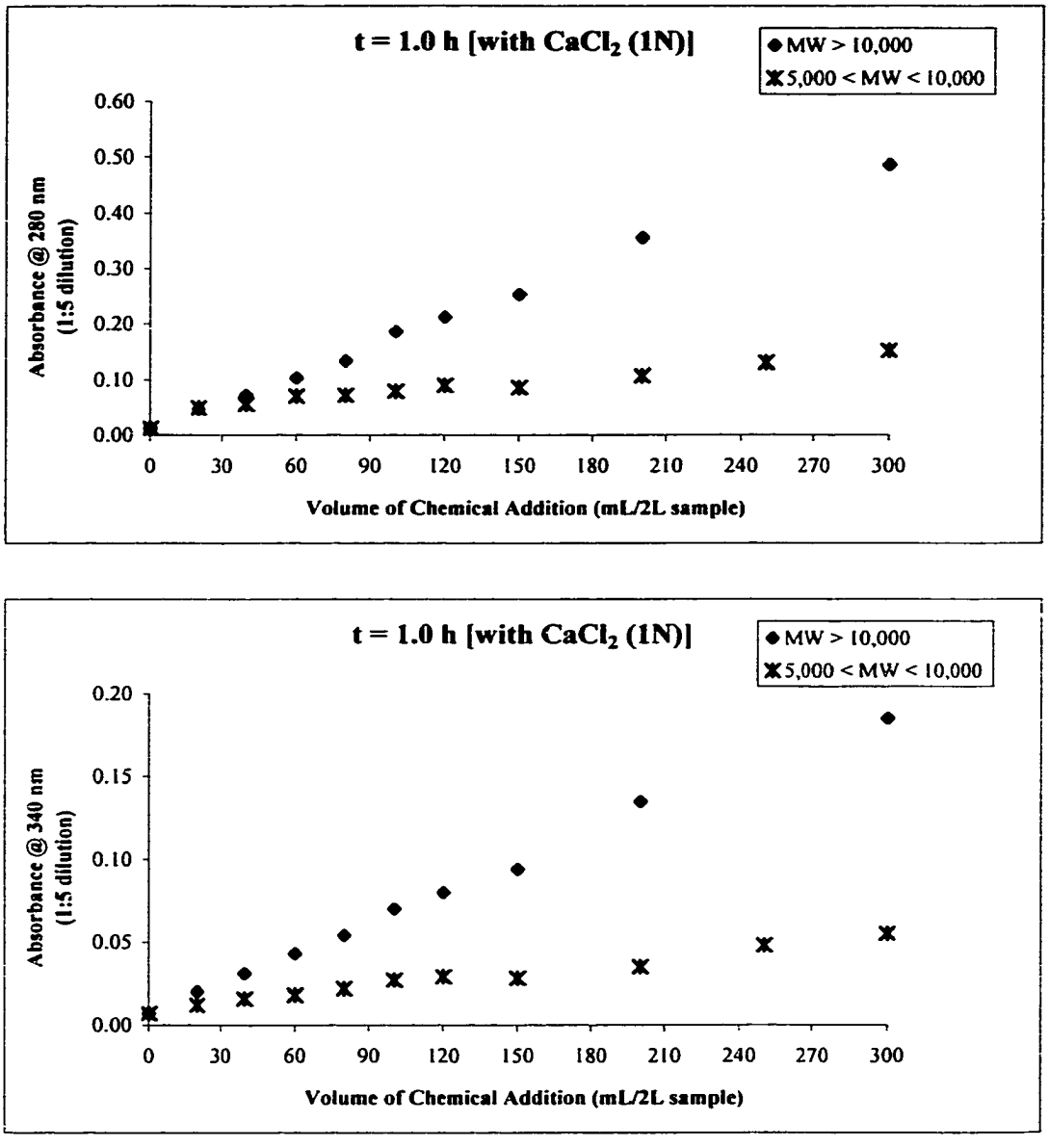


Figure E15. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and 60 minutes at $\overline{G_f}$ = 13 s^{-1})

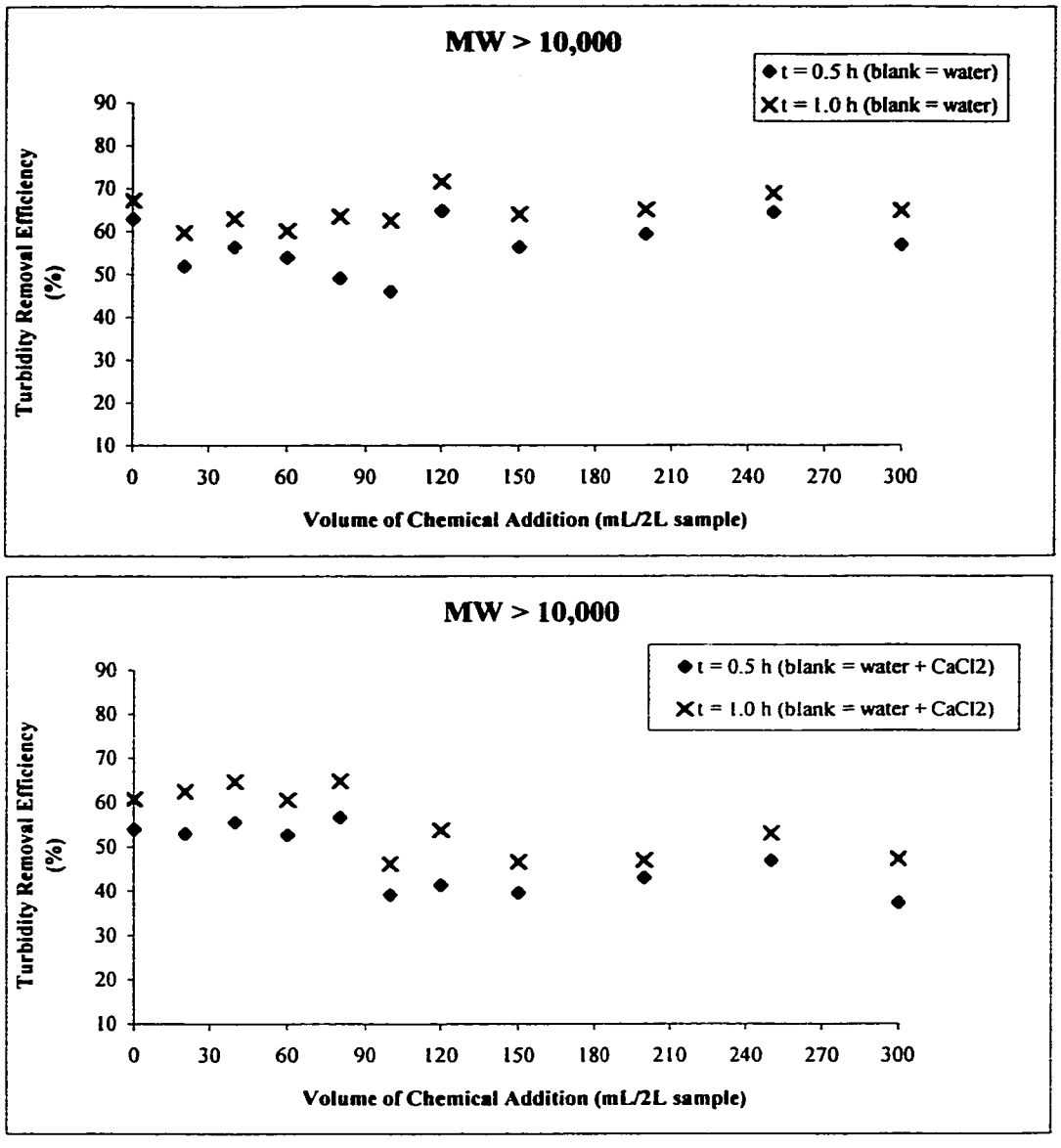
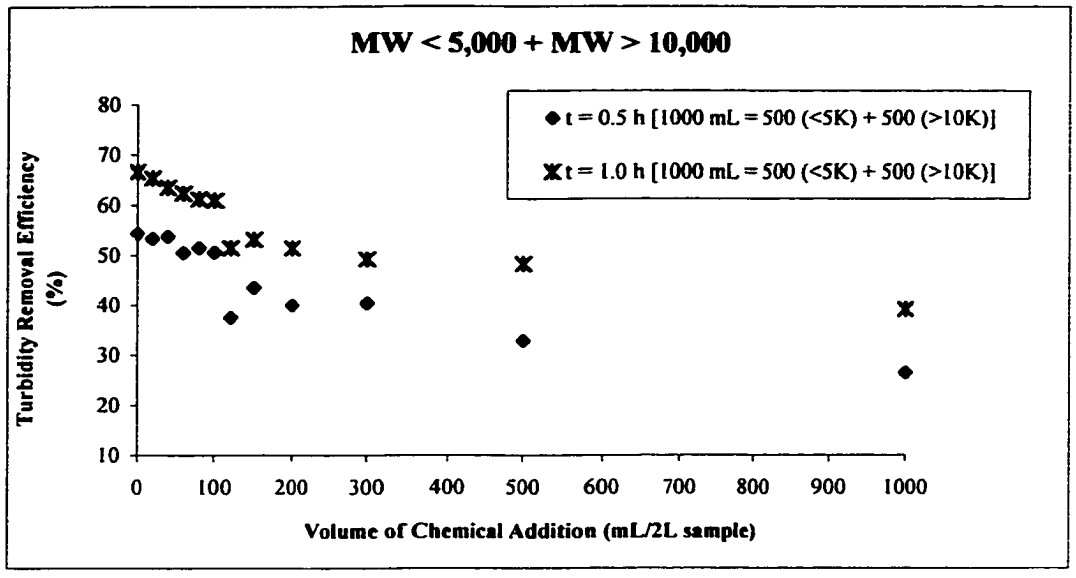


Figure E16. Effect of pulp mill chemicals from ultrafiltration fractionation on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{G_f} = 13\ s^{-1}$)



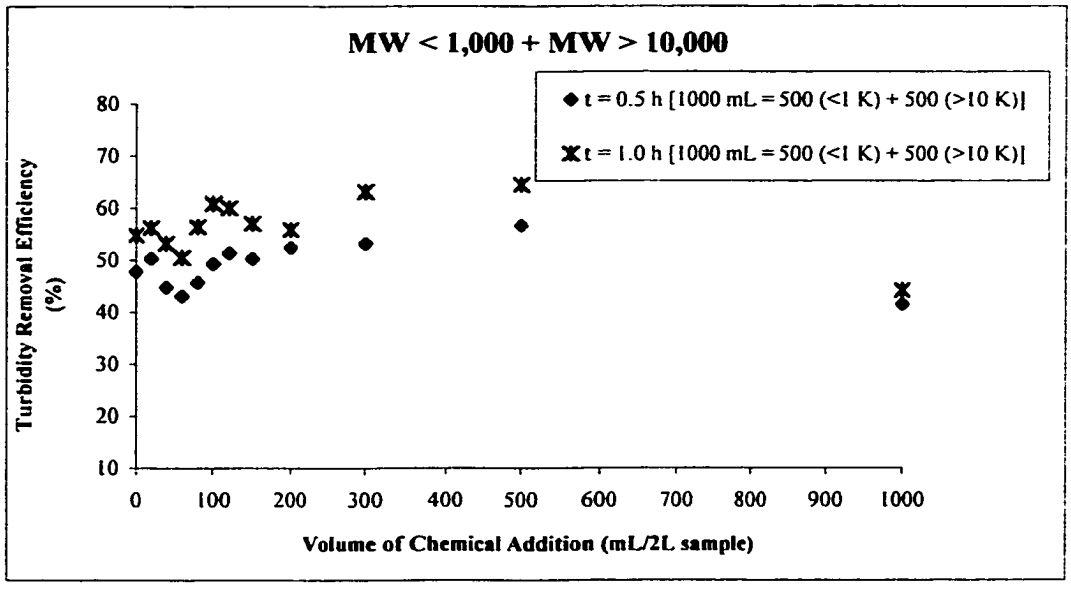


Figure E17. Effect of pulp mill chemicals from ultrafiltration fractionation on turbidity removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and up to 60 minutes at $\overline{G_f}$ = 13 s^{-1})

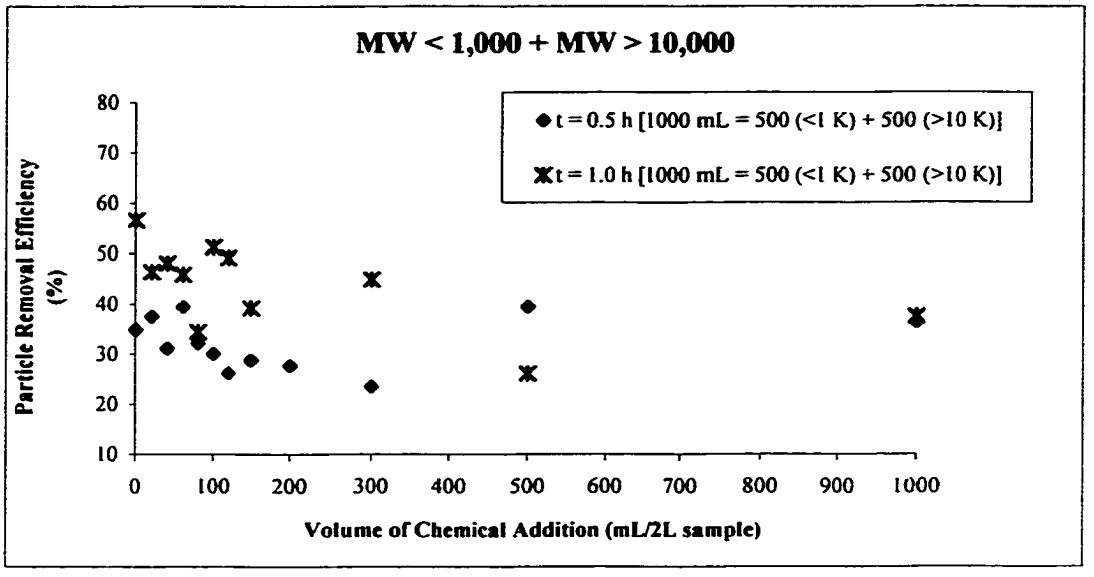
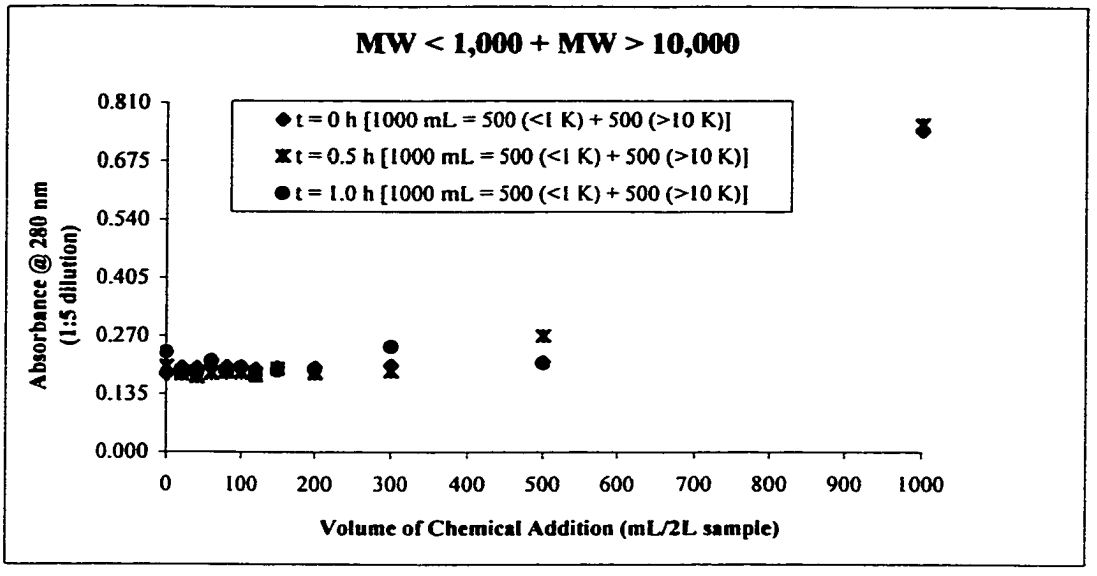


Figure E18. Effect of pulp mill chemicals from ultrafiltration fractionation on particle removal efficiency for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{G_f} = 13\ s^{-1}$)



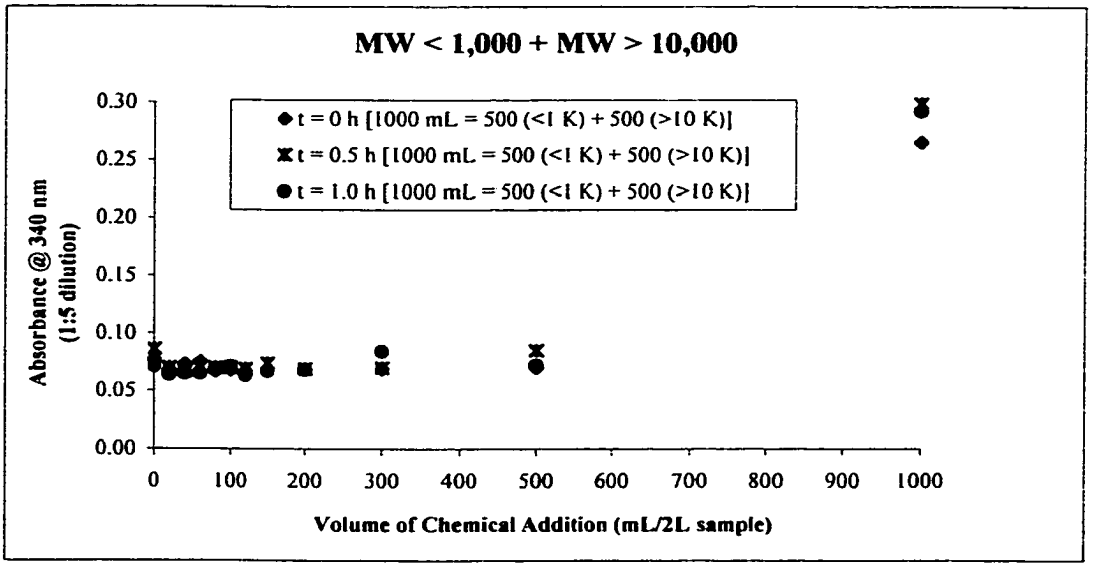


Figure E19. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc} = 200\ s^{-1}$ and up to 60 minutes at $\overline{Gf} = 13\ s^{-1}$)

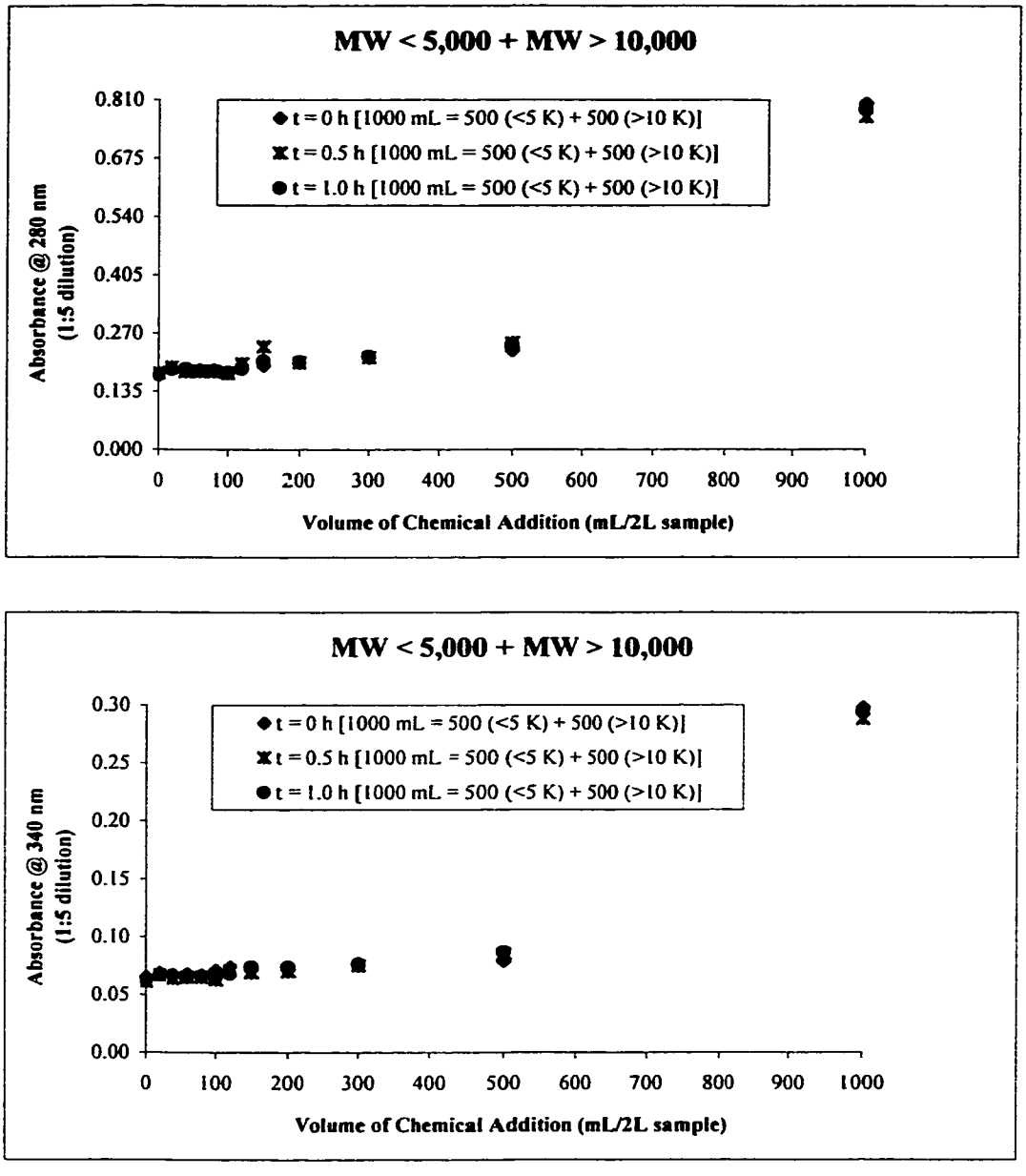


Figure E20. Effect of pulp mill chemicals from ultrafiltration fractionation on absorbance for summer samples (samples were one day old Wapiti River Water, mixed for 5 minutes at $\overline{Gc}$ = 200 s^{-1} and up to 60 minutes at $\overline{Gf}$ = 13 s^{-1})

APPENDIX F

Bioflocculation Results

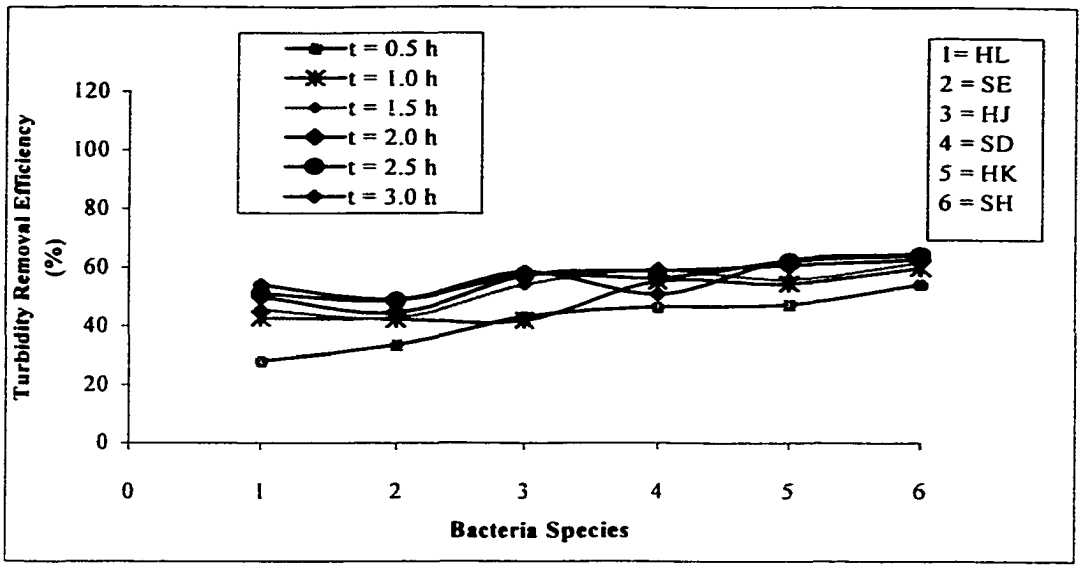


Figure F1. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 3 hours at $\overline{G_f} = 2\ s^{-1}$]

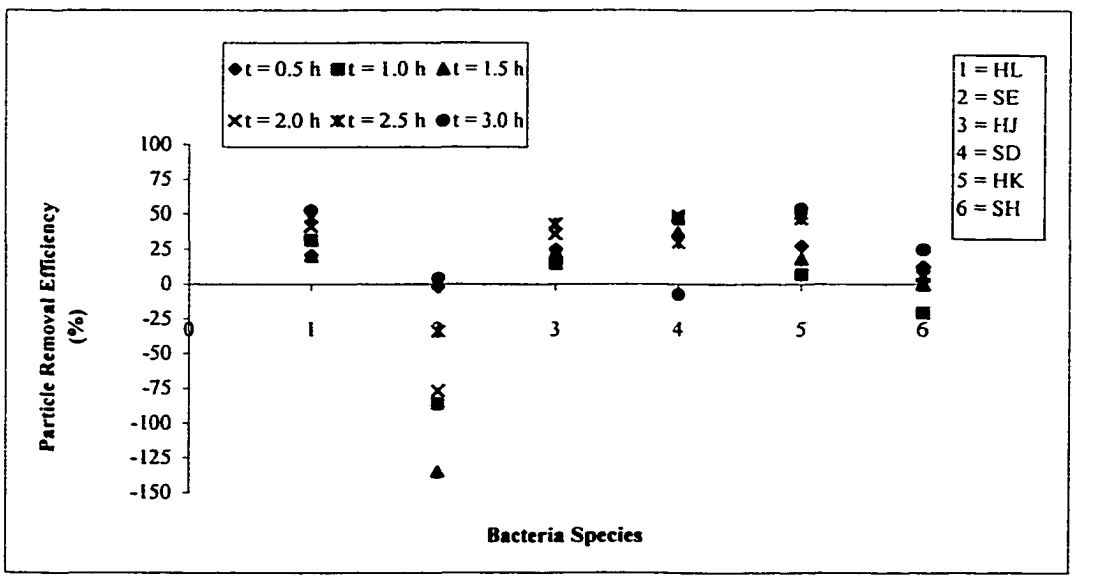


Figure F2. Effect of bacteria species on particle removal efficiency for fresh summer RW samples [20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 3 hours at $\overline{G_f} = 2\ s^{-1}$]

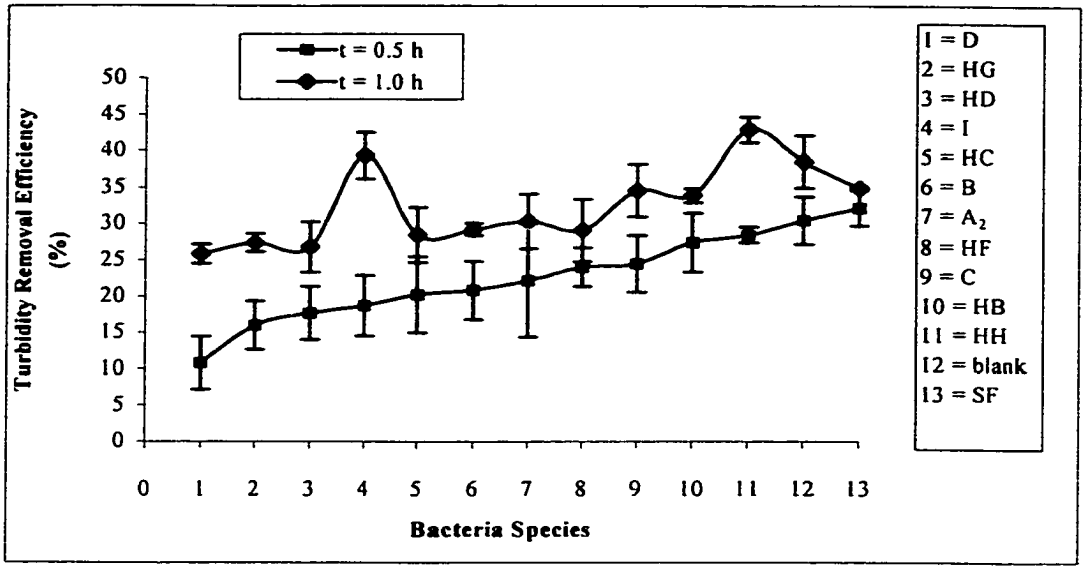


Figure F3. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [20 mL of 1N NaCl and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 1 hour at $\overline{G_f} = 5\ s^{-1}$]

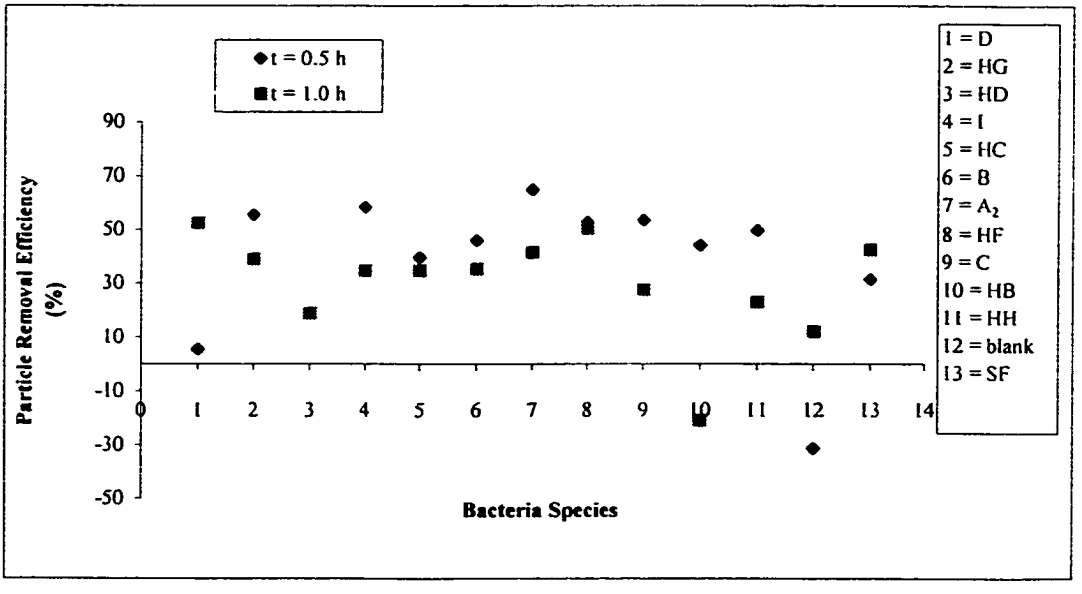


Figure F4. Effect of bacteria species on particle removal efficiency for fresh summer RW samples [20 mL of 1N NaCl and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 1 hour at $\overline{G_f} = 5\ s^{-1}$]

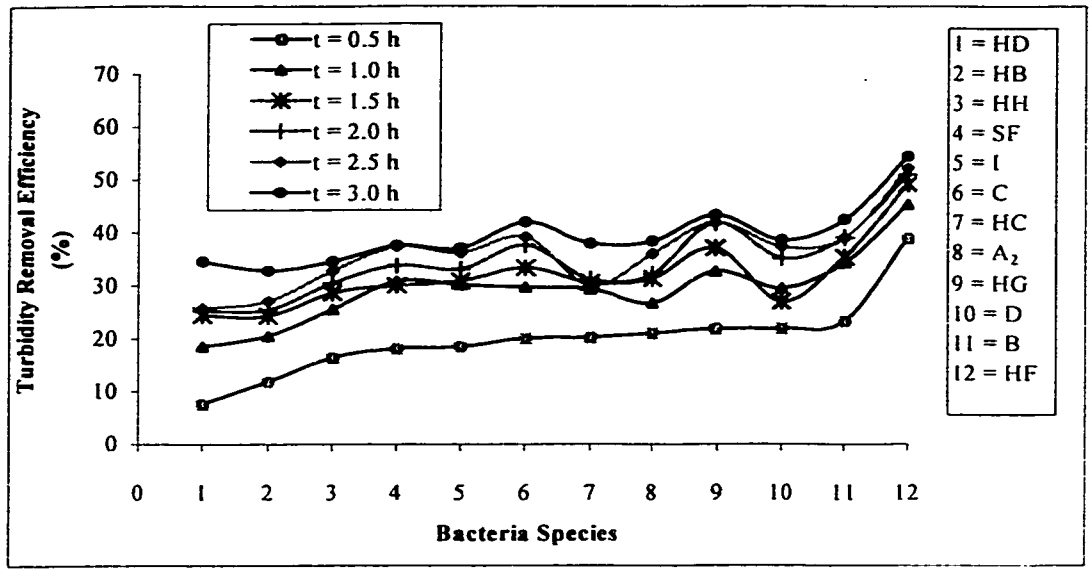


Figure F5. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 3 hours at $\overline{G_f} = 5\ s^{-1}$]

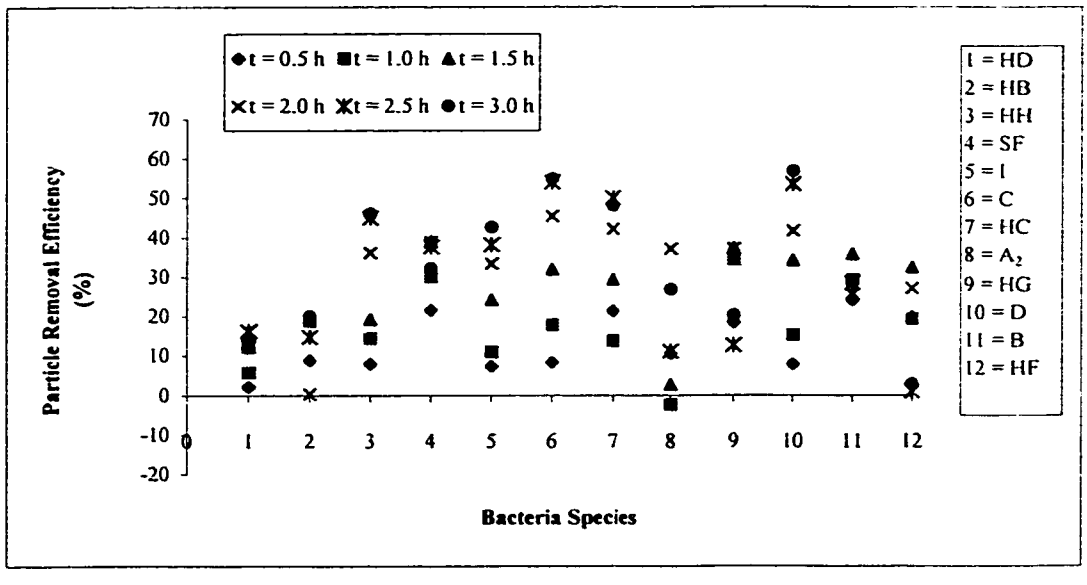


Figure F6. Effect of bacteria species on particle removal efficiency for fresh summer RW samples [20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. samples were mixed up to 3 hours at $\overline{G_f} = 5\ s^{-1}$]

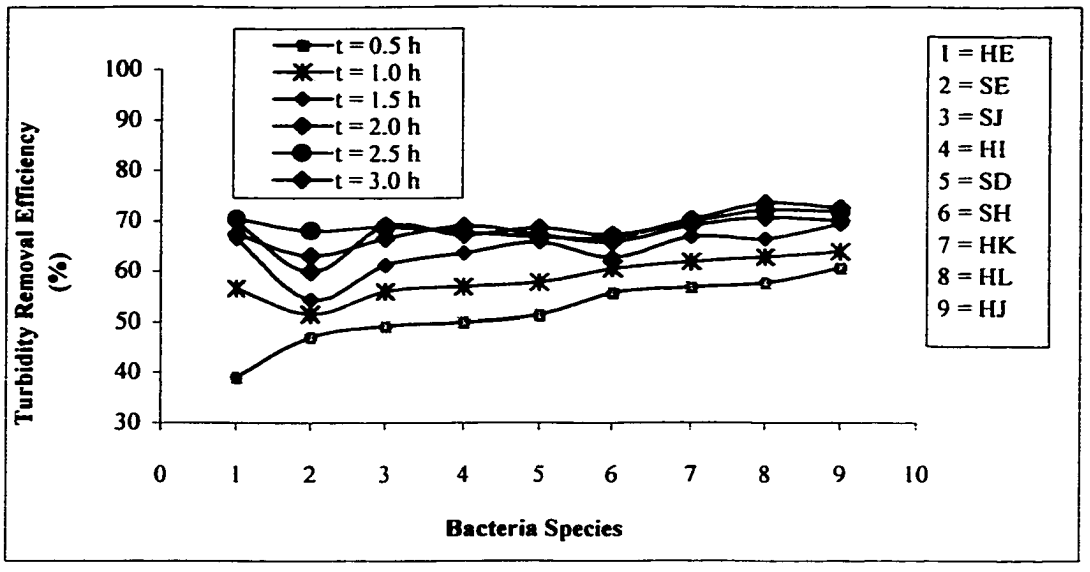


Figure F7. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 3 hours at $\overline{Gf} = 13\ s^{-1}$]

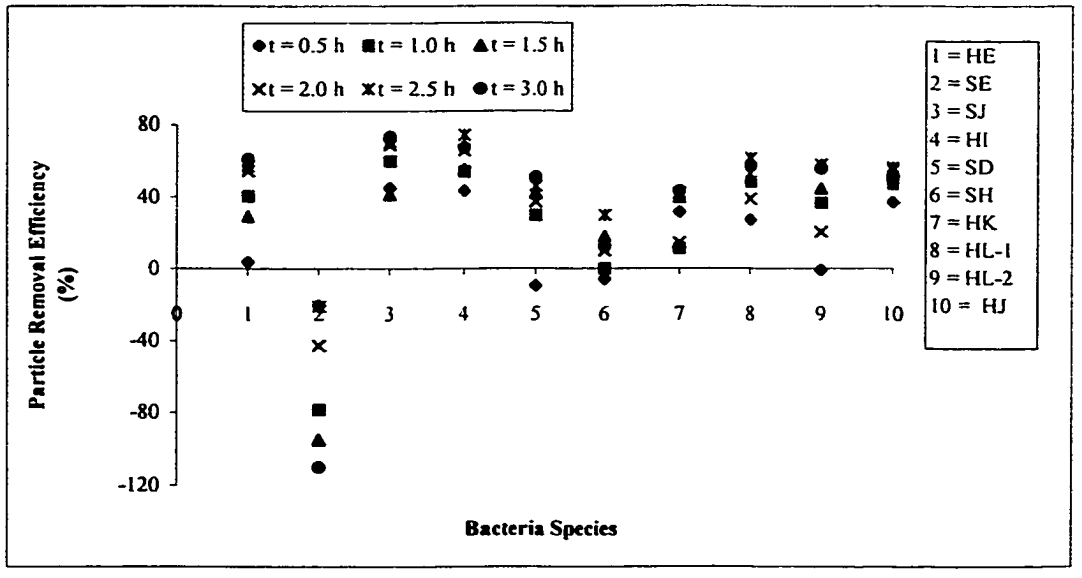


Figure F8. Effect of bacteria species on particle removal efficiency for fresh summer RW samples [20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 3 hours at $\overline{Gf} = 13\ s^{-1}$]

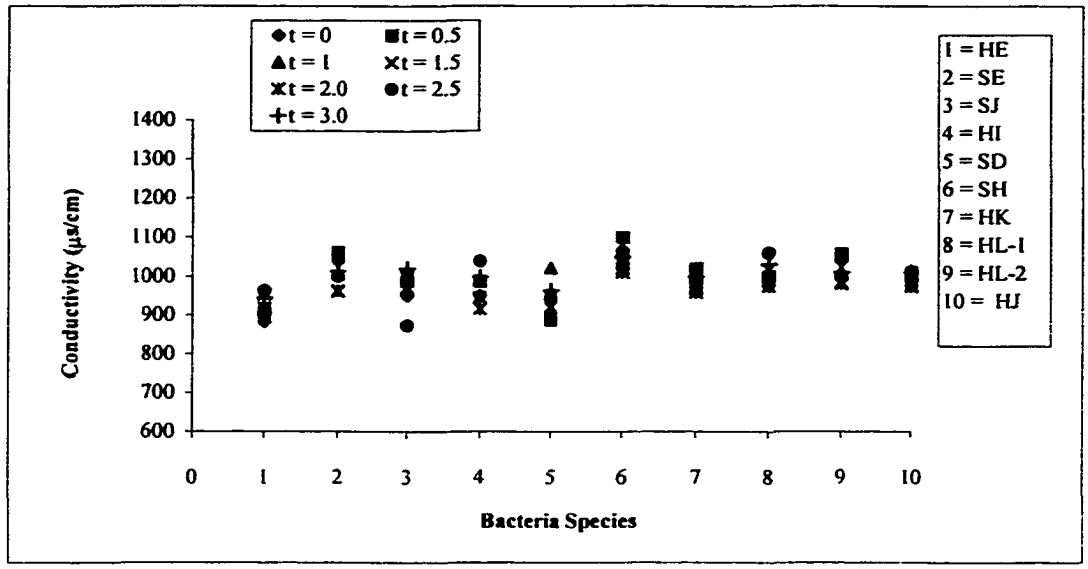


Figure F9. Effect of bacteria species on conductivity for fresh summer RW samples [20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 3 hours at $\overline{G_f} = 13\ s^{-1}$]

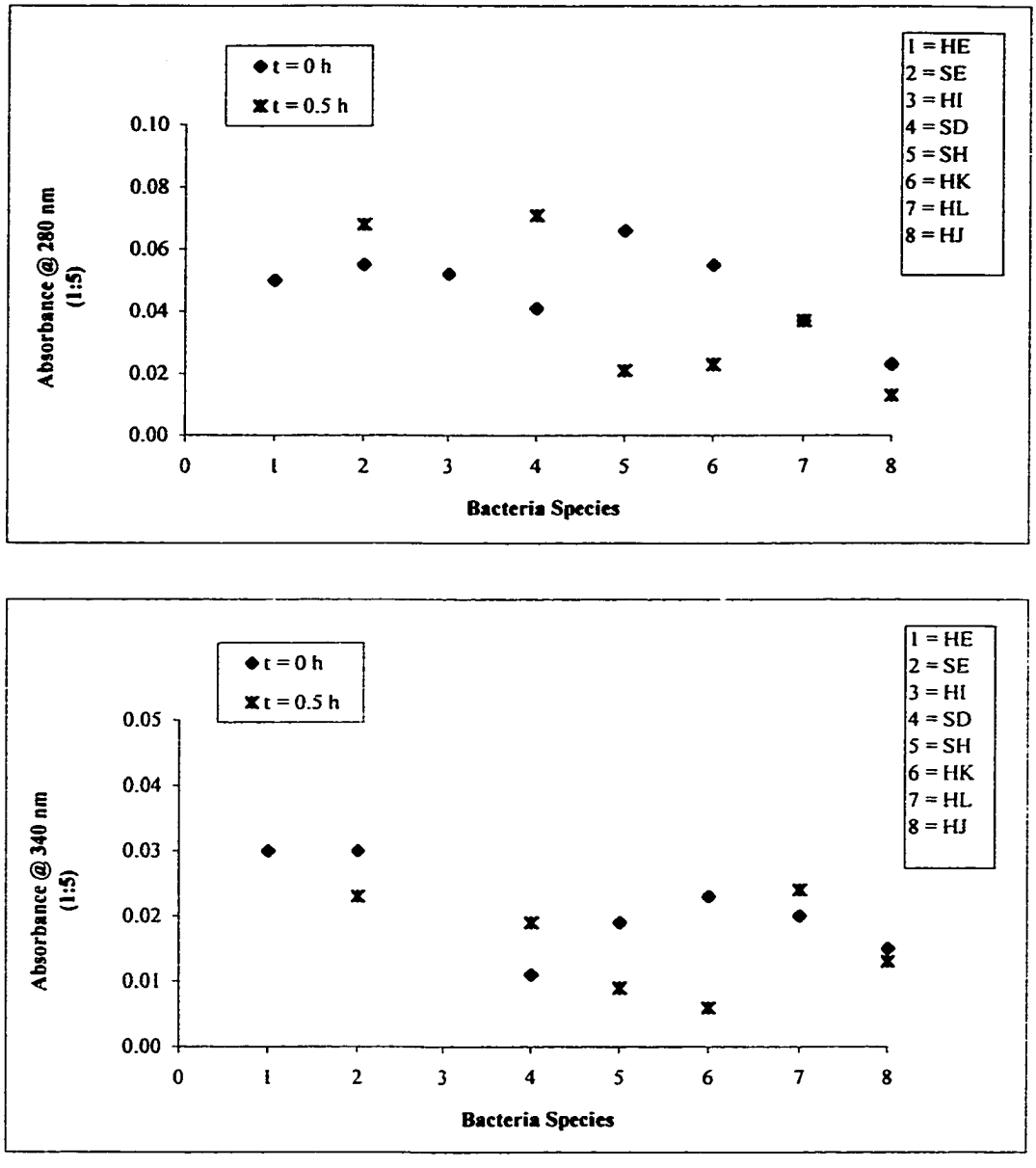


Figure F10. Effect of bacteria species on absorbance for fresh summer RW samples [20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hours at $\overline{G_f} = 13\ s^{-1}$]

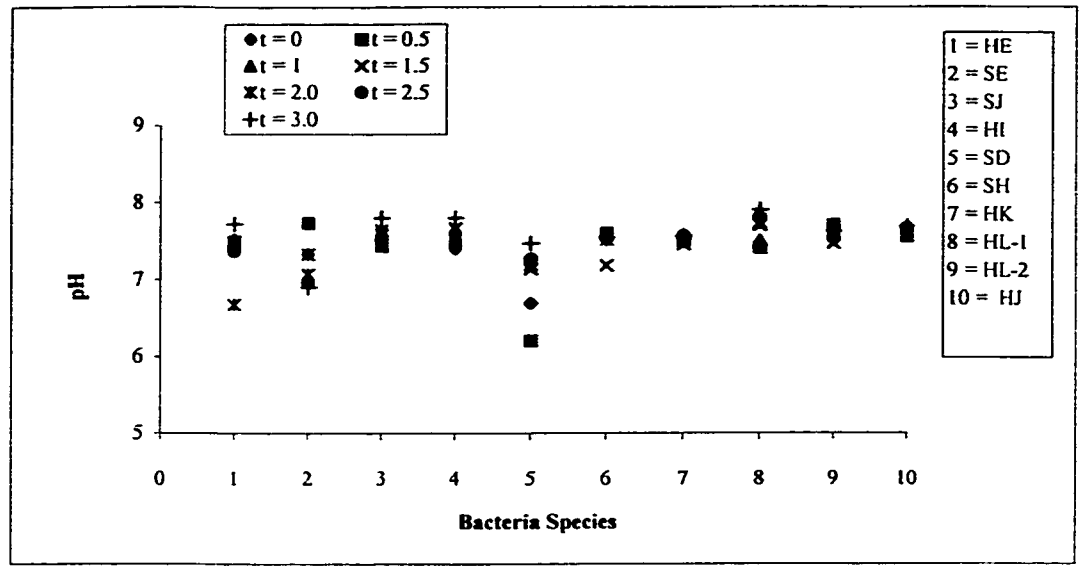


Figure F11. Effect of bacteria species on pH for fresh summer RW samples [20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 3 hours at $\overline{G_f}$ = 13 s^{-1}]

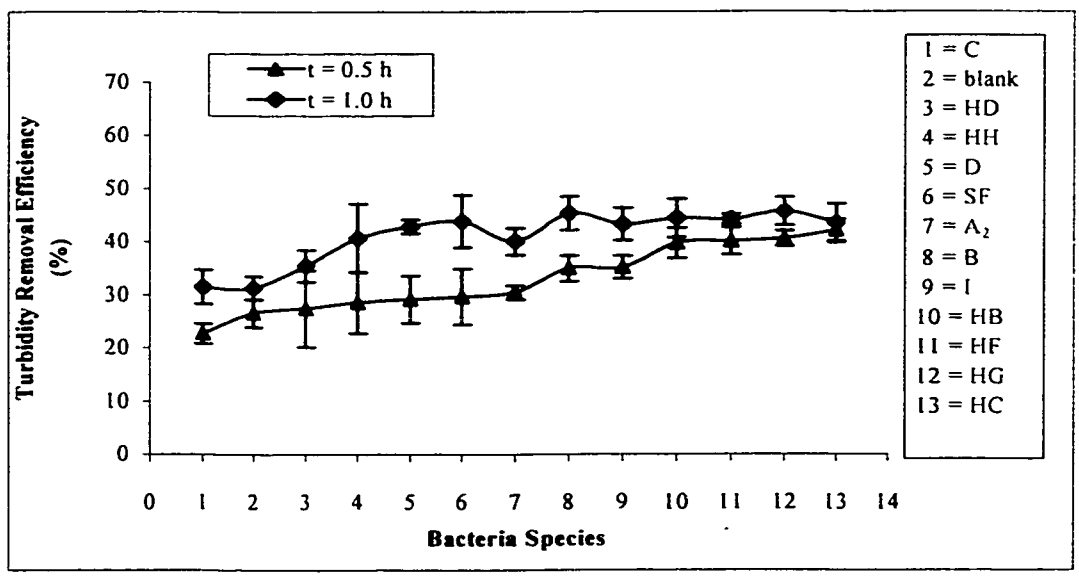


Figure F12. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [5 mL of bacteria suspension (no salt addition), bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 1 hour at $\overline{G_f}$ = 5 s^{-1}]

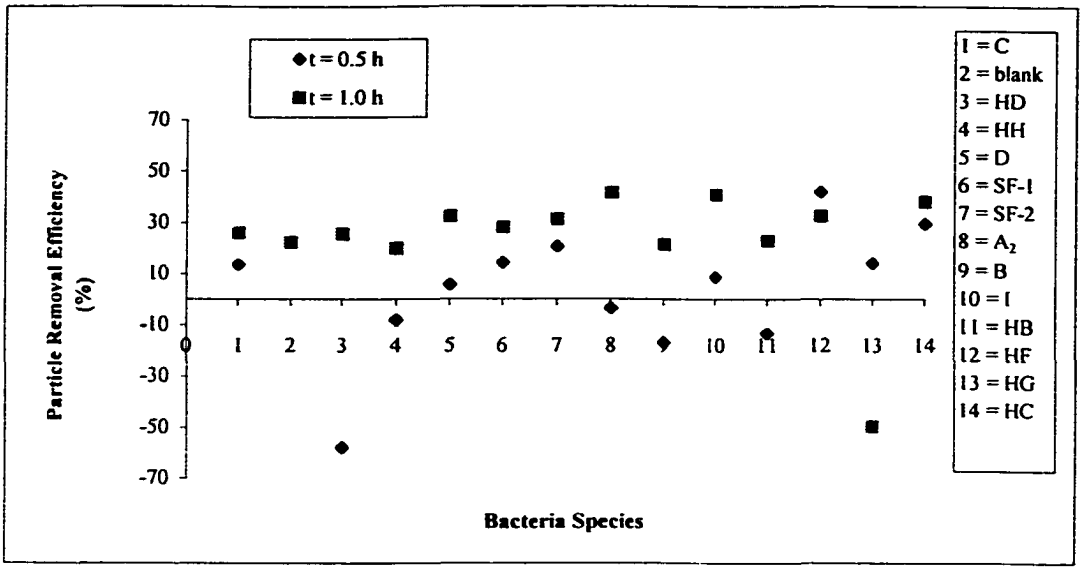
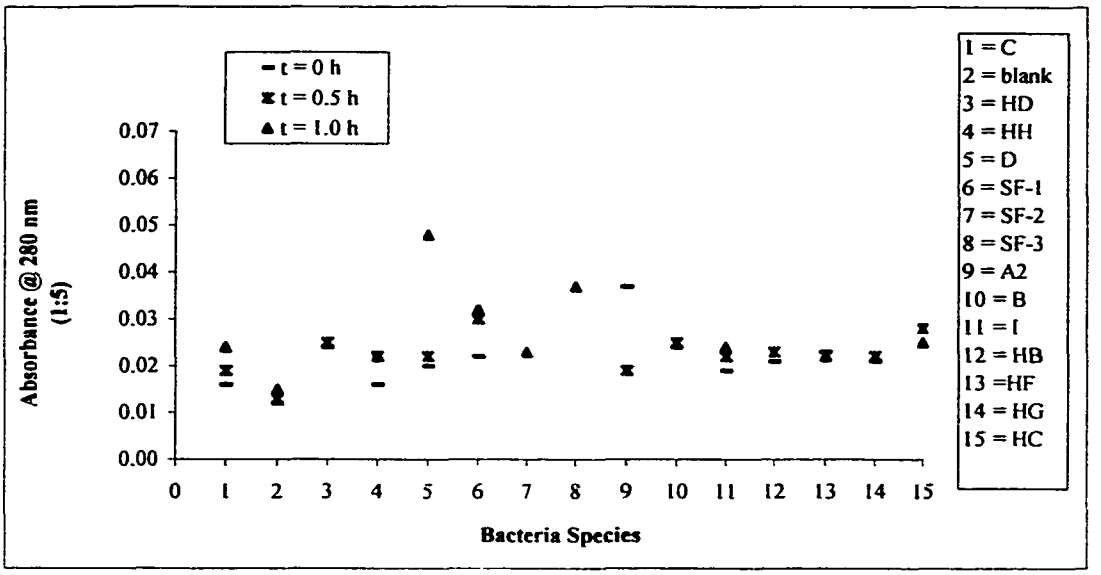


Figure F13. Effect of bacteria species on particle removal efficiency for fresh summer RW samples [5 mL of bacteria suspension (no salt addition), bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 1 hour at $\overline{G_f} = 5\ s^{-1}$]



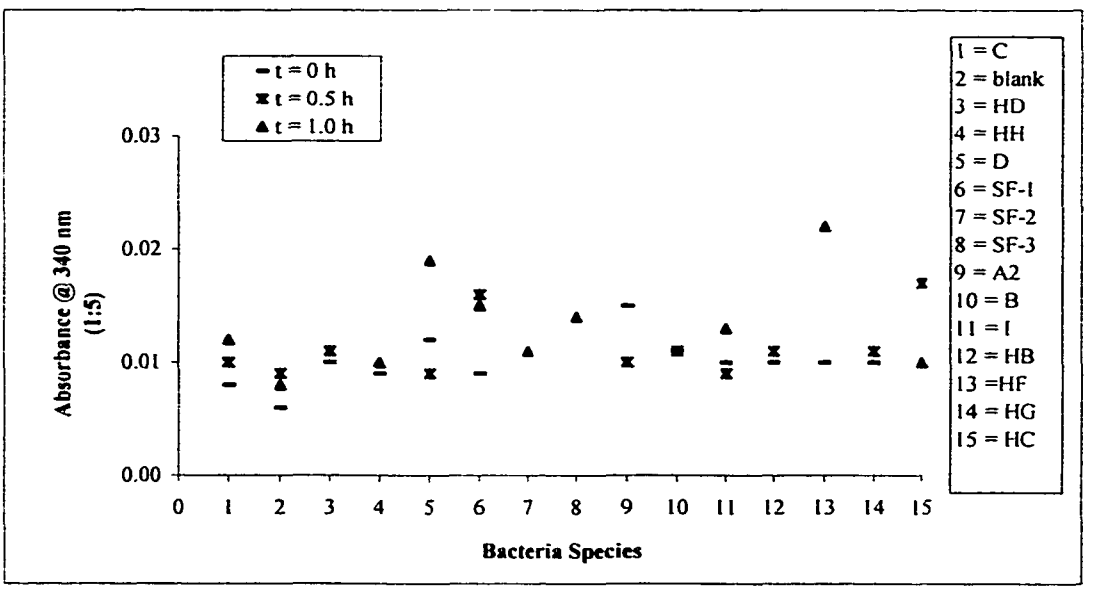


Figure F14. Effect of bacteria species on absorbance for fresh summer RW samples [5 mL of bacteria suspension (no salt addition), bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 1 hour at $\overline{G_f} = 5\ s^{-1}$]

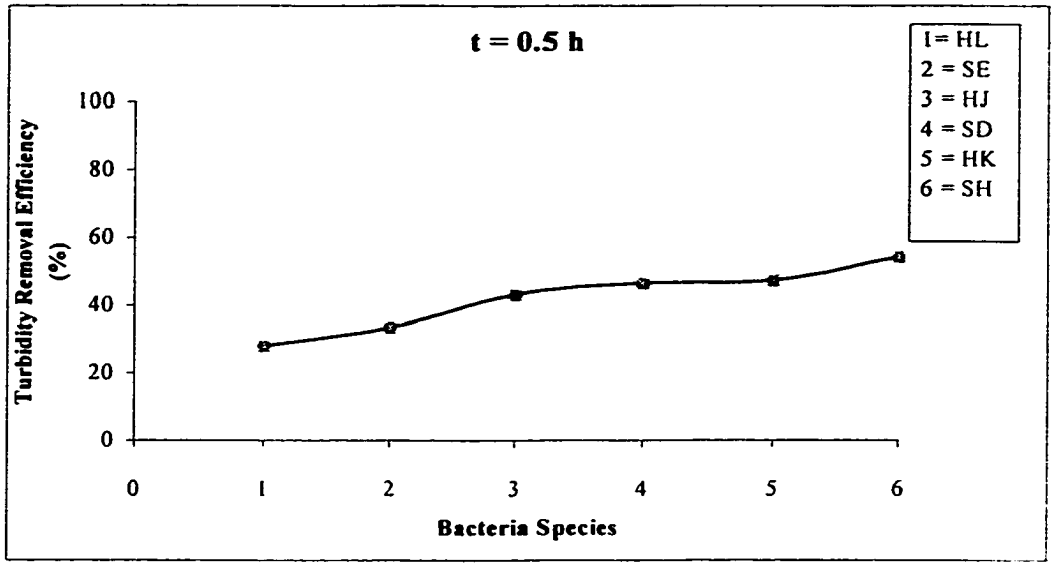


Figure F15. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [with 20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f} = 2\ s^{-1}$]

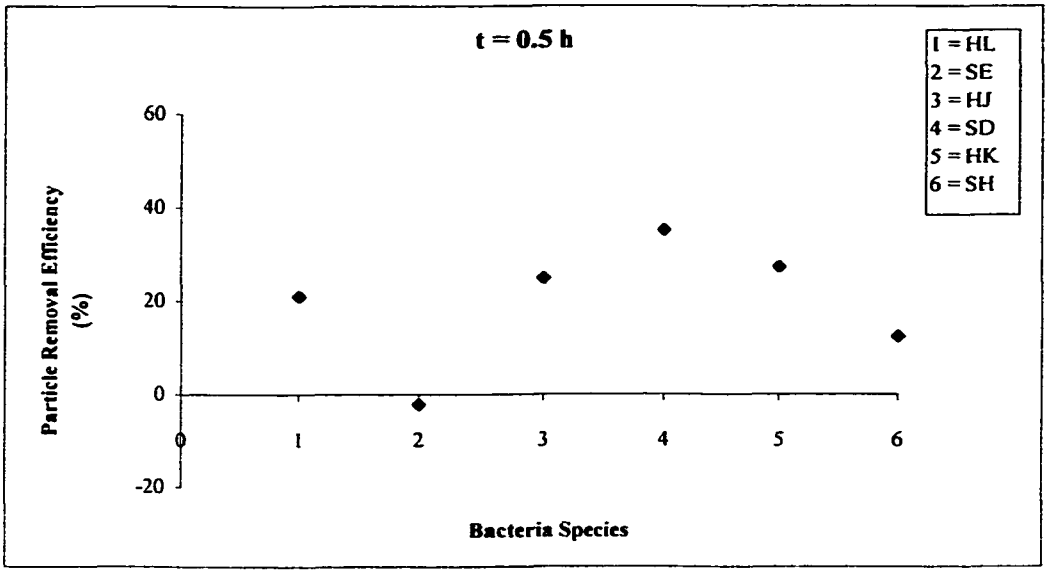


Figure F16. Effect of bacteria species on particle removal efficiency for fresh summer RW samples [with 20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f} = 2\ s^{-1}$]

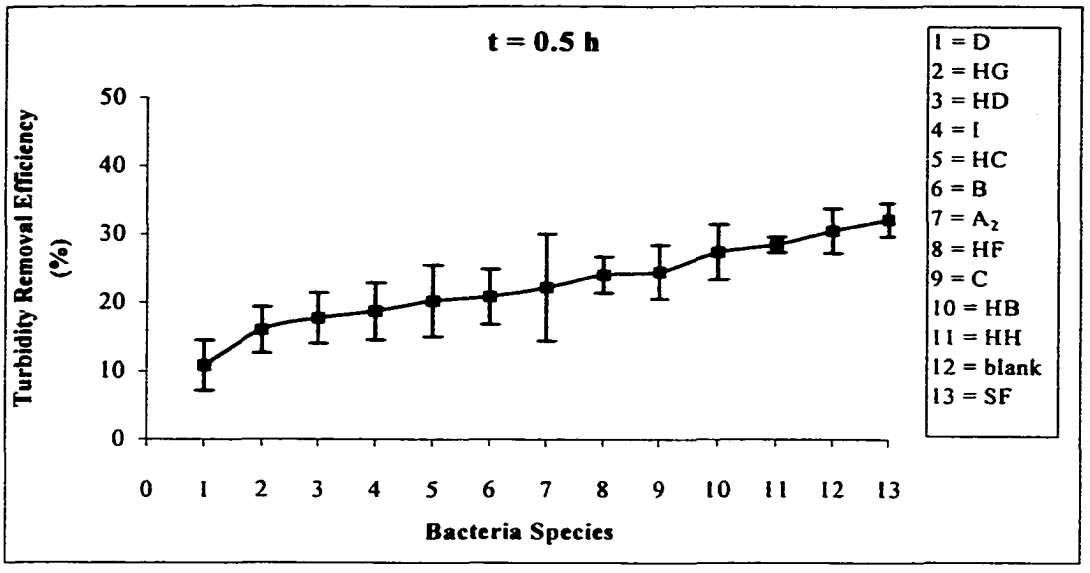


Figure F17. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [with 20 mL of 1N NaCl and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f} = 5\ s^{-1}$]

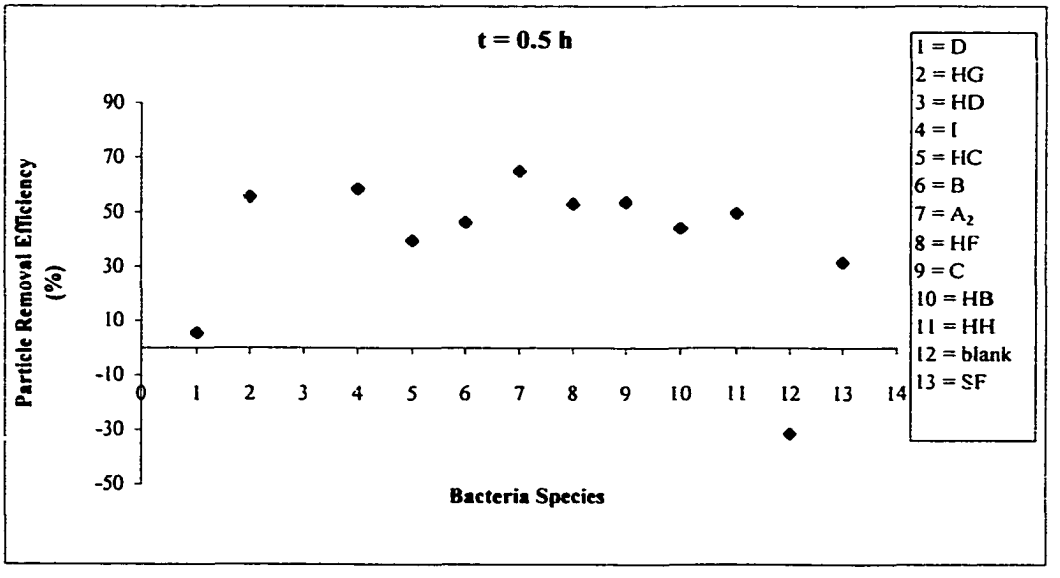


Figure F18. Effect of bacteria species on particle removal efficiency for fresh summer RW samples [with 20 mL of 1N NaCl and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f} = 5\ s^{-1}$]

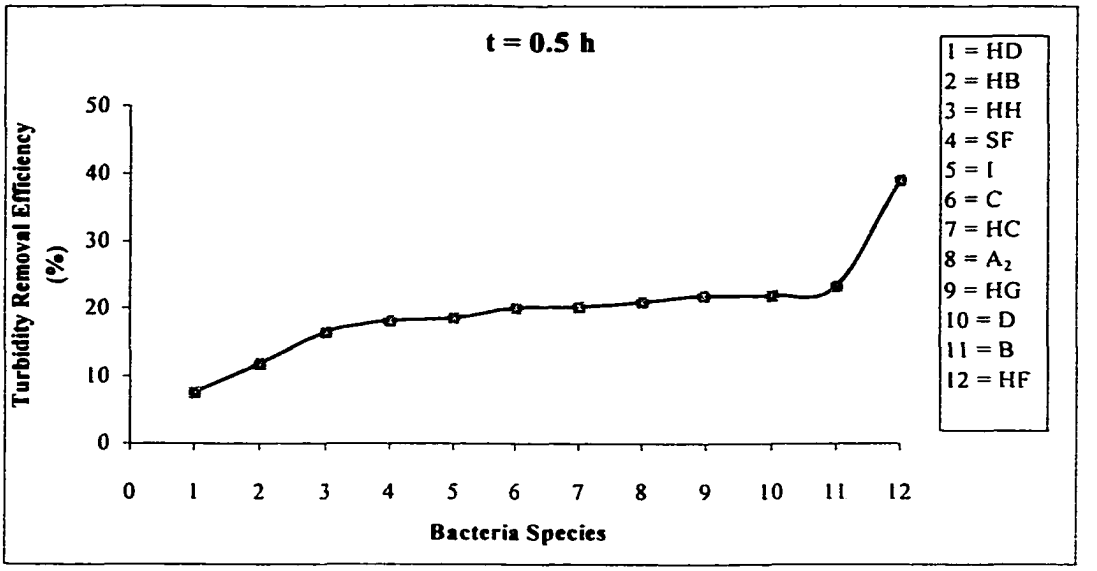


Figure F19. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [with 20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f} = 5\ s^{-1}$]

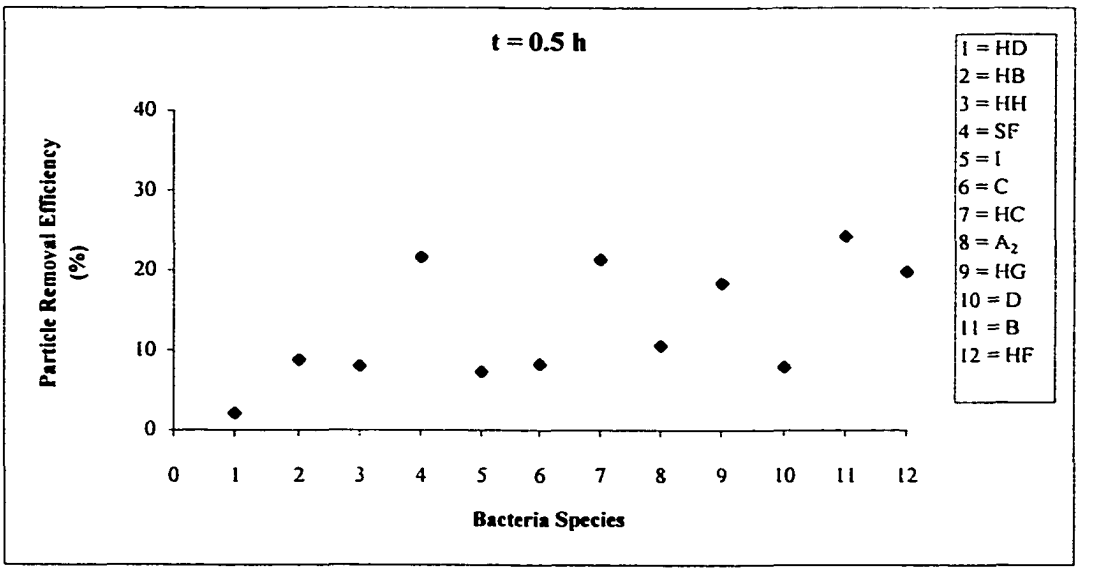


Figure F20. Effect of bacteria species on particle removal efficiency for fresh summer RW samples [with 20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f} = 5\ s^{-1}$]

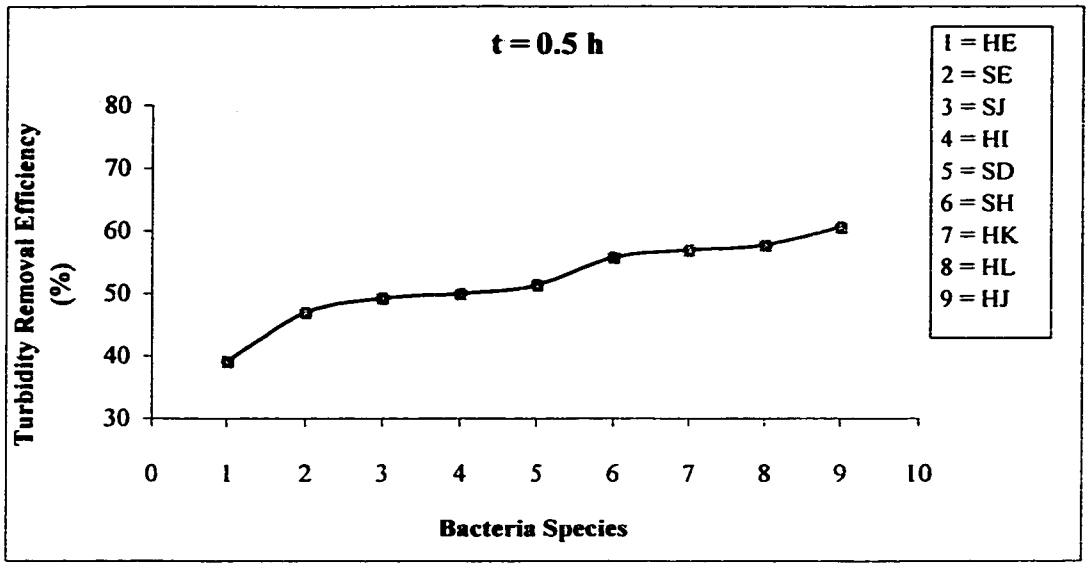


Figure F21. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [with 20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f}$ = 13 s^{-1}]

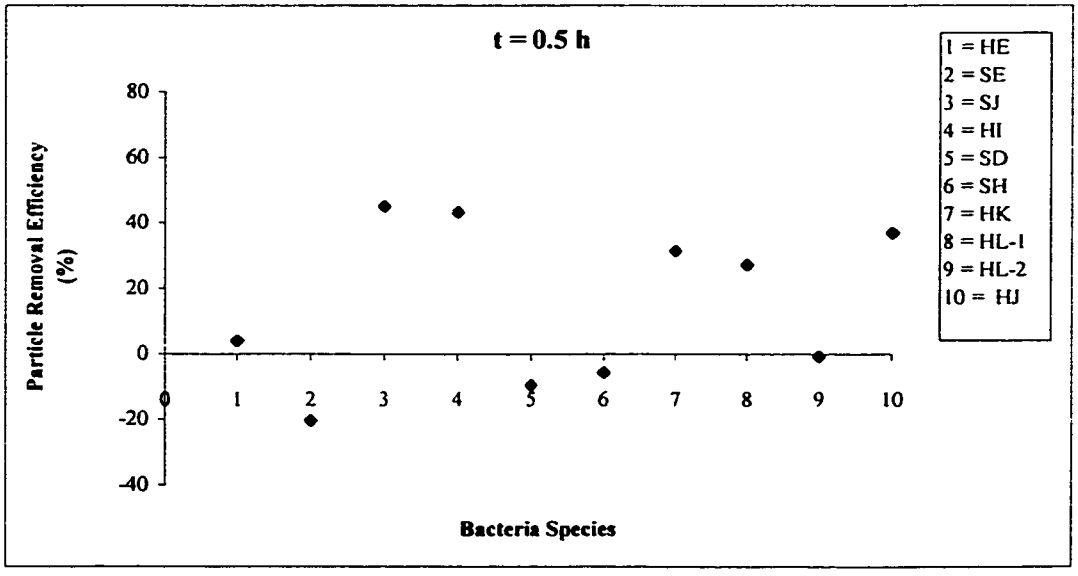


Figure F22. Effect of bacteria species on particle removal efficiency for fresh summer RW samples [with 20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f}$ = 13 s^{-1}]

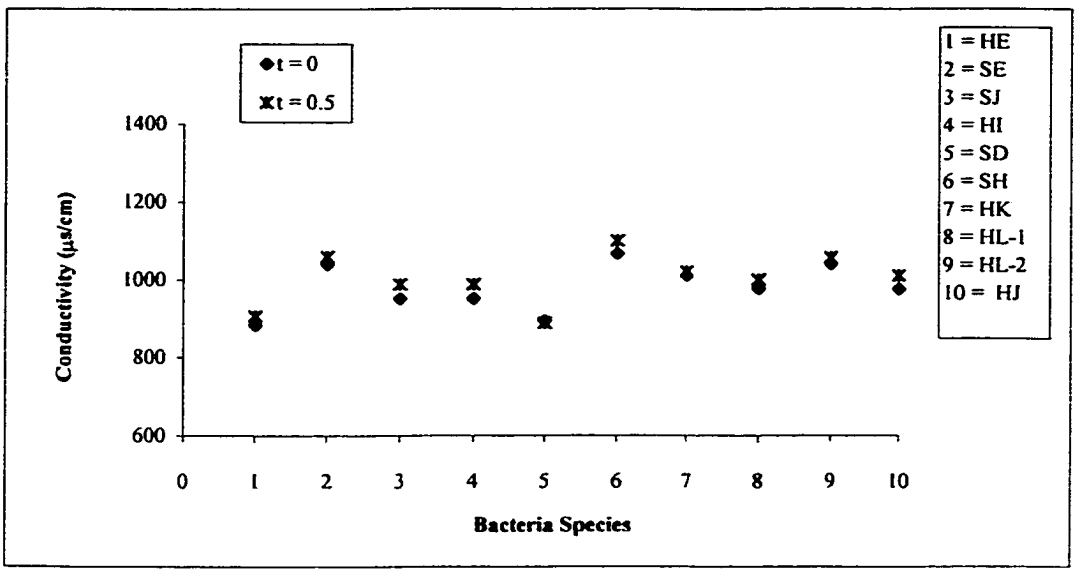


Figure F23. Effect of bacteria species on conductivity for fresh summer RW samples [with 20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f} = 13\ s^{-1}$]

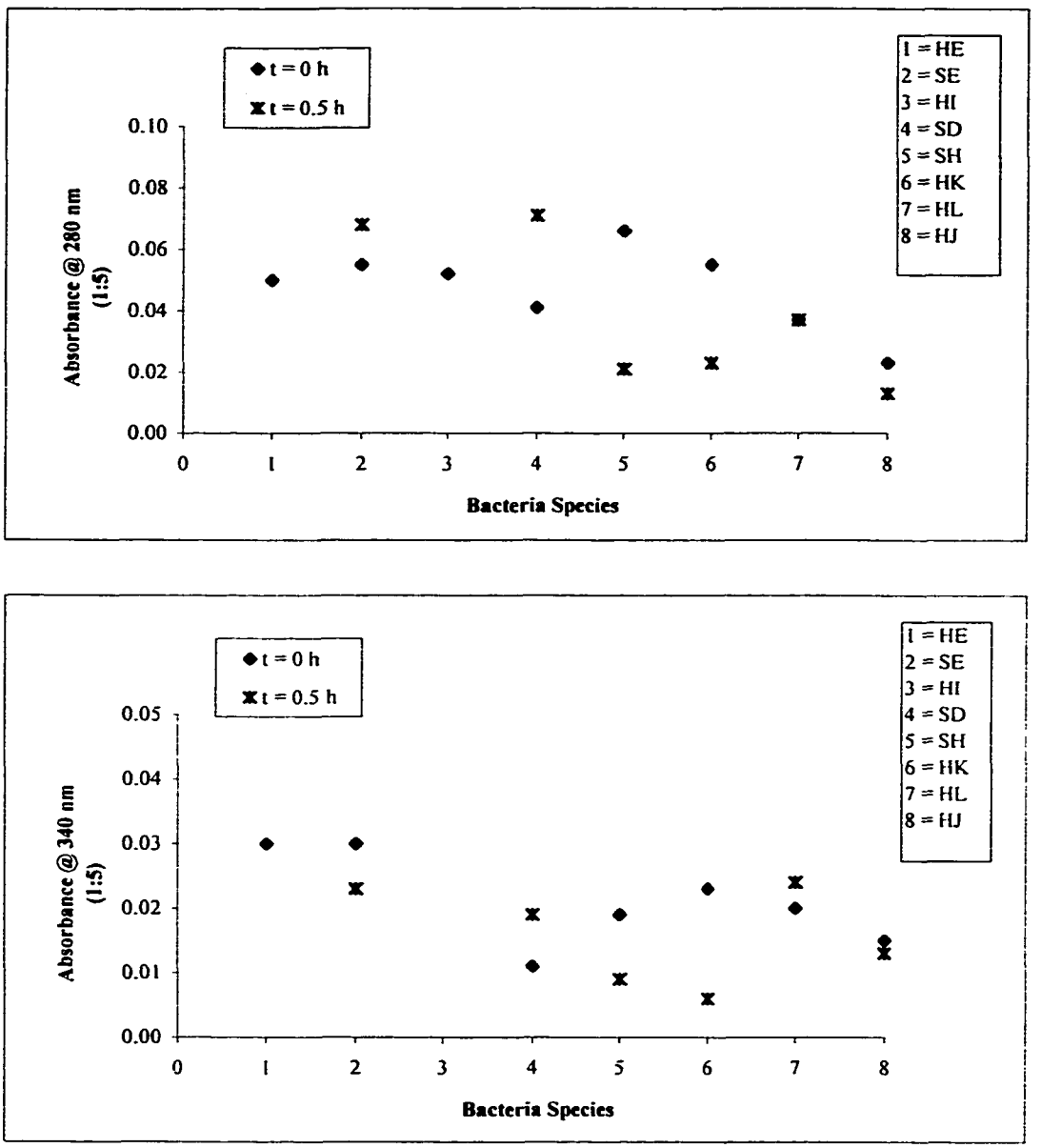


Figure F24. Effect of bacteria species on absorbance for fresh summer RW samples [with 20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f} = 13\ s^{-1}$]

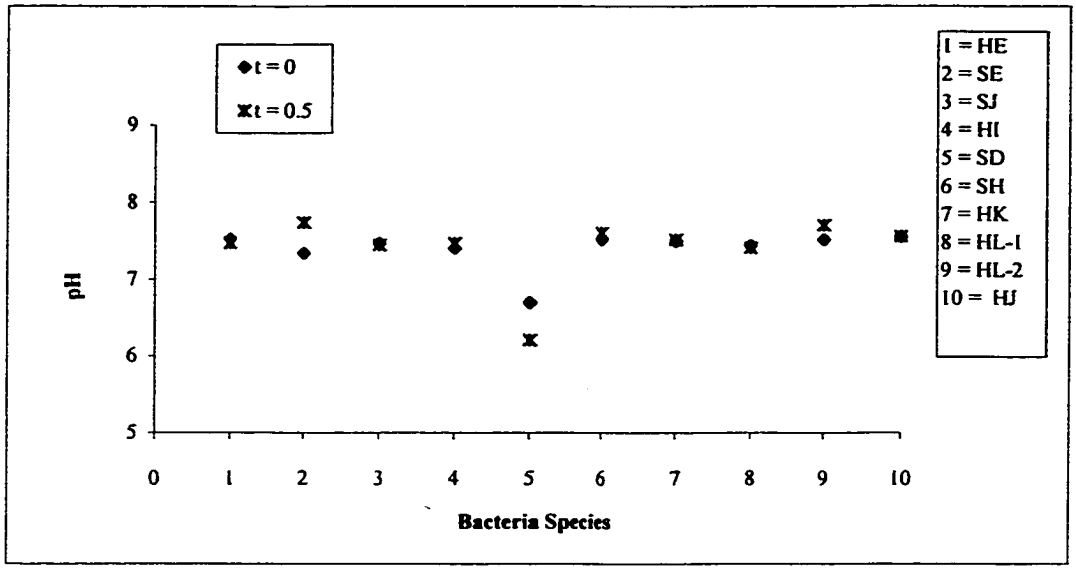


Figure F25. Effect of bacteria species on pH for fresh summer RW samples [with 20 mL of 1N $CaCl_2$ and 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 0.5 hour at $\overline{G_f} = 13\ s^{-1}$]

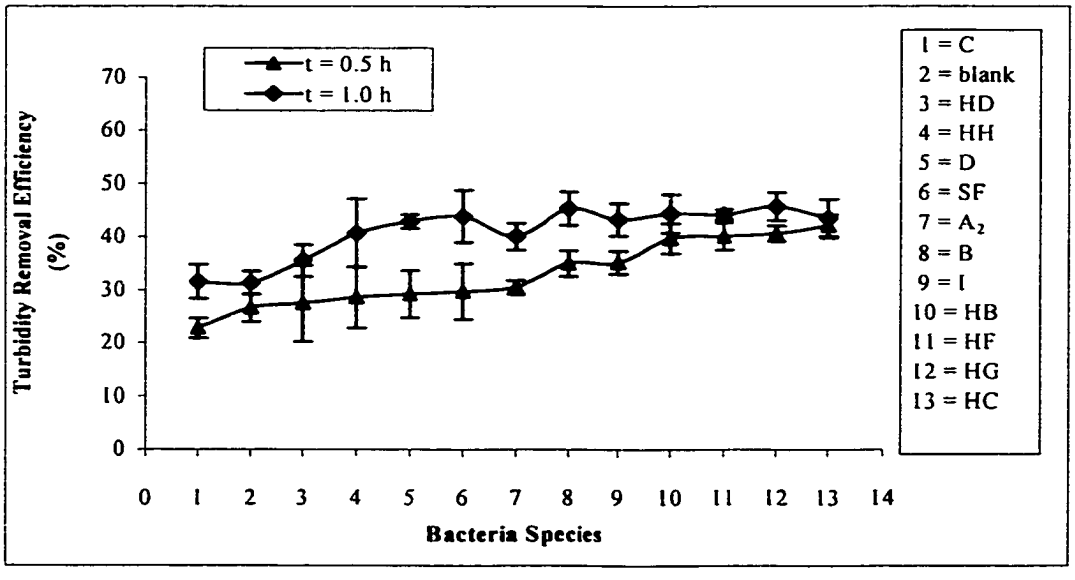


Figure F26. Effect of bacteria species on turbidity removal efficiency for fresh summer RW samples [no salt addition, 5 mL of bacteria suspension; bacteria were separated from PME and incubated for 4 days at 30°C. Samples were mixed up to 1 hour at $\overline{G_f} = 5\ s^{-1}$]