The Impact of Aircraft Deicing Wastes on the Biological Wastewater Treatment Process

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To my parents and to the newest
member in our family, Amruthanjali

ABSTRACT

In order to ensure aircraft safety during winter conditions, glycol-based deicing and anti-icing fluids are employed prior to takeoff. These products can exert a severe impact on the environment if allowed to go untreated. The present study is related to the treatment of glycol-contaminated wastewaters by the activated sludge process.

The specific objectives of the research were to : 1) determine the effects of process parameters such as biomass concentration, deicing fluid concentration and temperature on the biodegradation kinetics. 2) determine the mechanism of deicing fluid removal and model the reaction rates. 3) determine the effects of microbial changes on the treatment process. 4) evaluate the advantages of a sequencing batch reactor for the treatment of deicing wastes. 5) corroborate our laboratory results with field data from an operating wastewater facility treating deicing wastes.

The results from the field show that despite the increases in influent organic matter during the deicing season, there were very little changes in effluent values of organic matter. Furthermore, influent concentrations of deicing fluid between 10 and 30 mg/L were reduced to trace amounts (i.e. below 5 mg/L) throughout the deicing season. However, as witnessed by the high sludge volume index during the deicing season, the presence of deicing fluid creates settling problems in the clarifier.

The laboratory batch experiments indicate optimal substrate removal rates at biomass concentrations of 1000 mg/L and 2000 mg/L. Very low biomass levels lead to inhibition whereas a high biomass level of 3000 mg/L is unnecessary since the food to microorganism ratio is such that only a fraction of the biomass participates in the degradation reaction. With regards to deicing fluid concentration, organic matter removal rates tend to increase as the deicing fluid is increased. However, at the highest level of deicing fluid, certain inhibitory effects are present. As expected, higher temperatures produce much higher removal rates with the ethylene glycol substrate showing less variation with temperature than the other organic compounds present in the wastewater.

With regards to the mechanism of removal, the results showed very little adsorption of organic matter onto the biomass within the first hour of contact. In addition, the total organic matter removal (TOC and COD) followed first order kinetics with respect to substrate concentration.

Lastly, sequencing batch reactor operation allowed for much higher removal rates as the microbial population is acclimatized to the substrate with increasing cycles. With regards to the microbial population, the Biolog results showed that there was a decrease in the variety of compounds that could be degraded as the biomass was exposed to the deicing fluid. Furthermore, most population changes occurred at the very beginning of the deicing season and in the first half of the SBR experiments.

RÉSUMÉ

Afin d'assurer la sécurité pendant les conditions d'hiver, les dégivrants et anti-givrants à base de glycols sont employés avant le décollage. Cependant, ces produits peuvent exercer un impact sévère sur l'environnement s'ils ne sont pas traités. La présente étude implique le traitement d'eaux usées contaminées de glycols par le procédé de boues activées.

Les objectifs spécifiques de la recherche étaient de : 1) déterminer les effets de paramètres tels que la concentration de biomasse, concentration de dégivrant et la température fluide sur la cinétique de biodégradation. 2) déterminer le mécanisme d'enlèvement du fluide dégivrant et modéliser les taux de réaction. 3) déterminer les effets des changements microbiens sur le procédé de traitement. 4) évaluer les avantages d'un réacteur de cuvée séquentielle pour le traitement des dégivrants. 5) corroborer nos résultats de laboratoire avec les données d'une station d'eaux usées traitant les produits dégivrants.

Les résultats sur le champ indiquent que les augmentations en matière organique pendant la saison de dégivrage produisent très peu de changements dans les concentrations de matière organique dans les effluents. De plus, les concentrations en amont de dégivrant d'avion entre 10 et 30 mg/L sont réduites en dessous de 5 mg/L durant la saison de dégivrage. Cependant, en observant les résultats de l'indice de volume des boues, la présence des dégivrants d'avion crée des problèmes dans le clarificateur.

Les expériences en cuvée indiquent des taux d'enlèvement de matière organique optimales avec des concentrations de biomasse de 1000 mg/L et 2000 mg/L. Un niveau de biomasse très bas peut mener à l'inhibition et un niveau élevé de 3000 mg/L n'est pas exigé car seulement une fraction de la biomasse participe dans la réaction de dégradation avec une quantité limitée de substrat. Les taux d'enlèvement ont tendance à augmenter lorsque la concentration du produit dégivrant est haussée mais il y a certains effets inhibiteurs au plus haut niveau de dégivrant. Une température élevée produit un plus haut taux d'enlèvement avec le substrat d'éthylène glycol démontrant moins de variation avec la température que les autres composés.

En ce qui concerne le mécanisme, nos résultats ont démontré très peu d'adsorption sur la biomasse dans la première heure de contact. De plus, l'enlèvement de la matière organique totale (COT et DCO) suit une cinétique de premier ordre.

Dernièrement, l'opération de réacteur de cuvée séquentielle permet de plus hauts taux d'enlèvement car la population microbienne est acclimaté au substrat sur divers cycles. Au niveau de la population microbienne, les résultats Biolog indiquent une diminution dans la variété de composés qui peuvent être dégradés quand la biomasse a été exposée au liquide dégivrant. De plus, la plupart des changements de la population surviennent au début de la saison de dégivrage et dans la première moitié des expériences de cuvée séquentielle.

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NOMENCLATURE

BOD	Biochemical Oxygen Demand
C	Concentration of Organic Matter
CAC	Chemical Oxygen Demand Adsorption Capacity
COD	Chemical Oxygen Demand
$\frac{dC}{dt} = r$	Rate of Change of the Reactant per Amount Biomass
DIF	Deicing Fluid
DIS	Deicing Season
DGGE	Denaturing Gradient Gel Electrophoresis
DO	Dissolved Oxygen
EG	Ethylene Glycol
FAS	Ferrous Ammonium Sulphate
GC	Gas Chromatograph
k	Reaction Rate Constant
k'	Reaction Rate Constant per Amount Biomass
k_e	Biomass Decay Coefficient
K_S	Substrate Concentration with $\mu = 0.5 * \mu_{max}$
k_{T1}	Reaction Rate Constant at Temperature T1
k_{T2}	Reaction Rate Constant at Temperature T2
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
OLR	Organic Loading Rate
OUR	Oxygen Uptake Rate
PCA	Principal Component Analysis
PEG	Polyethylene Glycol

PHB	Poly-(R)-3-Hydroxybutyric Acid
r_D	Rate of Biomass Decay
r_S	Rate of Substrate Utilization
r_X	Rate of Biomass Growth
RPM	Rotations per Minute
S	Substrate Concentration
SBBR	Sequencing Bio-Film Batch Reactor
SBR	Sequencing Batch Reactor
SOS	Soluble Organic Substrate
SOUR	Specific Oxygen Uptake Rate
SVI	Sludge Volume Index
TOC	Total Organic Carbon
TSS	Total Suspended Solids
T1	Temperature 1
T2	Temperature 2
μ	Biomass Growth Coefficient
μ_{max}	Maximum Value of the Biomass Growth Coefficient
UASB	Upflow Anaerobic Sludge Blanket
VSS	Volatile Suspended Solids
X	Biomass Concentration
Y	Biomass Yield Coefficient
θ	Temperature correction coefficient

CHAPTER 1

INTRODUCTION

Deicing and anti-icing fluids (primarily composed of glycols) play a crucial role in preventing the degradation of the aircraft's aerodynamic profile by removing and inhibiting the formation of ice and snow on aircrafts prior to takeoff. Therefore, since many fatal accidents have been attributed to the inadequate deicing of aircrafts during winter months, glycol-based deicing fluids ensure safe and efficient winter aircraft operations.

While these chemicals help ensure aircraft safety, glycol-contaminated runoff from the airport has a serious negative impact on the environment. During deicing and anti-icing operations, important quantities of these fluids flow into the water streams adjacent to the airport and the sewer system. In fact, deicing one commercial aircraft typically results in a pollution load approximately equivalent to the daily wastewater discharge of more than 5000 inhabitants [Backer et al., 1994]. Along with such a high biochemical oxygen demand ($BOD_5 \approx 600\ 000$ mg/L), deicing fluids may also exert toxic effects on the receiving aquatic ecosystems.

Recognizing the environmental impact caused by the deicing and anti-icing operations of airports, many countries have adopted guidelines to limit the quantity of glycols found in water streams. For example, Environment Canada has fixed the limit of glycol in water bodies at 100 mg/L [Canada Gazette, 1994].

At international airports such as Denver International Airport (DIA) and Mirabel airport (Montreal, PQ), deicing fluids are collected and sent to a municipal wastewater treatment plant. For example, Mirabel airport collects the glycol-containing wastes from the deicing apron and stores them in large reservoirs. Thereafter, they are pumped to the St-Canut (PQ) municipal wastewater treatment plant. Thus, during deicing season, the operations at Mirabel Airport cause a supplementary loading on the wastewater treatment plant. From the aforesaid, it is relevant to investigate the impact of deicing and anti-icing fluids on the activated sludge process that is in place at the treatment plant of St-Canut.

1.1 Background

1.1.1 Activated Sludge Process – Continuous Operation

The activated sludge process is widely used for the treatment of municipal and industrial wastewaters [Viessman and Hammer, 1998]. The sludge consists of a mixed microbial population that includes bacteria, protozoa, fungi, rotifers and nematodes [McKinney, 1962]. This biomass is put into contact with the organic matter and degrades the latter aerobically. The process requires a biological reactor in which there is a ready supply of oxygen (aeration tank), a separator in which the biological solids are separated from the effluent (secondary clarifier) and a system of sludge recycling pumps (Figure 1.1.1). In the traditional system, the wastewater is mixed with the active biological solids

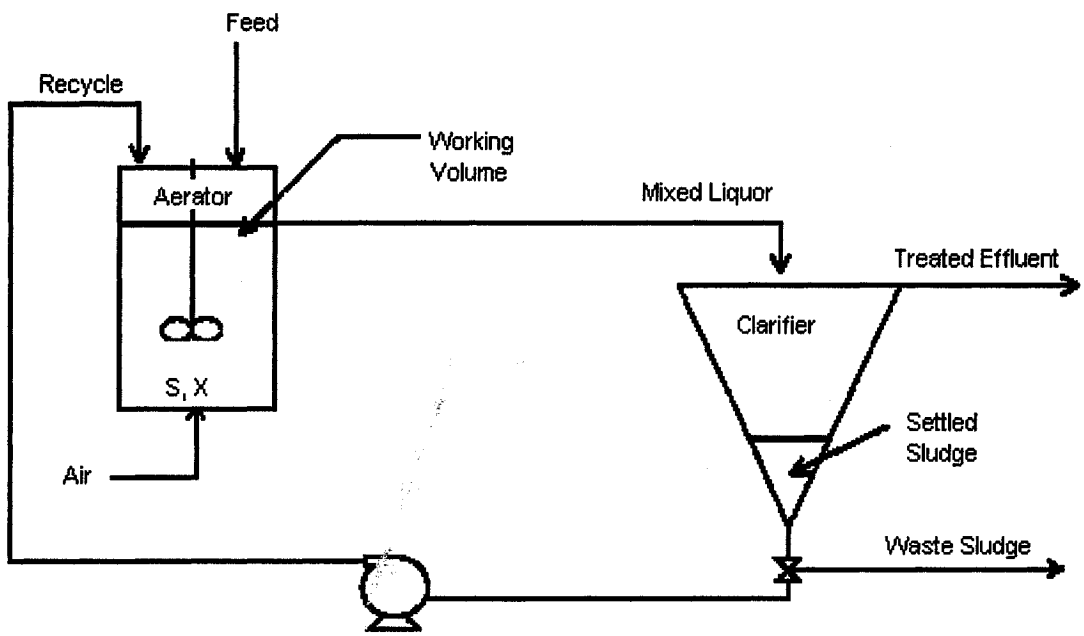
immediately before or immediately after entering the biological reactor. The two streams are mixed in such proportions so that in the aeration tank, the sludge-wastewater mixture termed mixed liquor usually contains between 1500 and 3500 mg/L of mixed liquor suspended solids (MLSS) by dry weight [Tchobanoglous and Schroeder, 1987]. Thereafter, the biodegradation of the organic material has been described as essentially a two-step process. First, the activated sludge rapidly adsorbs the suspended organic solids contained in the wastewater [Reynolds and Richards, 1996]. This phase is generally quite rapid and ranges from 20 to 45 minutes in most cases. Following adsorption, the organic solids are oxidized by the microorganisms throughout the reactor.

Once the organic matter has been biologically oxidized in the reactor, the mixed liquor flows to the clarifier where the biological solids are separated from the mixed liquor by decantation. Thereafter, the sludge settles to the bottom and is recycled (a part of it is also sent to wastage). The treated supernatant wastewater flows over the weirs and into the effluent channels. In the case of municipal wastewaters, the effluent is usually disinfected before it is discharged into the receiving body of water. Industrial effluents are normally not disinfected since they may not contain pathogens [Reynolds and Richards, 1996].

In order to control the desired level of mixed liquor suspended solids (MLSS) in the biological reactor, a fixed amount of sludge is recycled from the solid-liquid separator as defined by the recycle ratio. This ratio is dependent on

the desired MLSS concentration in the aeration tank and the concentration of the settled activated sludge. In addition, a part of the settled sludge is disposed of as daily wastage from the clarifier.

Figure 1.1.1 : Schematic diagram of the activated sludge process



Many factors, such as wastewater strength, temperature, pH, hydraulic retention time and biomass retention time affect the performance of this system. Since the most important component of the process is the biomass, a great deal of emphasis must be placed on the maintaining adequate sludge characteristics. An important property of the sludge is its settling ability and literature has suggested that the sequencing batch reactor offers an advantage in this area as compared to traditional activated sludge systems.

1.1.2 Activated Sludge Process – Sequencing Batch Reactor (SBR)

The SBR is basically an activated sludge wastewater treatment system in which different treatment operations are carried out in one vessel. The SBR accomplishes both aeration and clarification in one vessel (following a timed sequence) whereas the continuous flow process requires multiple tanks. In general, an SBR cycle consists of the following 5 phases : fill, react, settle, decant and idle/waste sludge. In the "fill" phase, the reactor is filled with wastewater (onto the sludge blanket) and biodegradation is initiated. The aeration continues during the "react" phase until the desired level of biodegradation is achieved. During the "settle" phase, the aerators are turned off and quiescent conditions allow the biomass to settle, leaving the treated supernatant above. The treated effluent is then decanted during the "decant" phase. Finally, in the "idle/waste sludge" phase, part of settled sludge is wasted and the reactor is once again ready to start another cycle. The SBR technology offers a number of advantages over other activated sludge systems such as :

- Lower capital and operation costs (only one vessel is used for all process operations)
- Greater ability to meet effluent standards (due to the use of "batch" kinetics)
- Better settling characteristics (cyclic feast-famine conditions produce better settling)
- Greater system flexibility and control (cycle sequence be easily modified)

1.2 Scope and Objectives

1.2.1 Scope of the Research

Existing literature suggests that deicing fluids are effectively biodegraded using a mixed culture of microorganisms as in the activated sludge process [Sabeh, 1996. Nitschke et al., 1996]. However, the presence of these chemicals in the bioreactor may entail the growth of unwanted filamentous microorganisms and create additional sludge production [Aéroports de Montréal, 1999]. Thus, there is a need for further research in this area since the specific kinetics of biodegradation as well as the changes in microbial population provoked by this additional loading has been poorly covered.

In biological systems, when a new chemical compound is introduced to the environment, certain types of microorganisms will thrive while others that are not able to assimilate this compound will perish. Therefore, the type of activated sludge population present in a wastewater treatment plant depends, to a certain degree, on the type of wastewater treated. As changes in wastewater characteristics occur, changes in types of microbial population follow. These changes, in turn, affect the capacity of the resulting biomass to degrade a certain type of wastewater. This process of adaptation is called microbial acclimatization and is crucial for effective wastewater treatment.

Regarding the mechanism of degradation, literature suggests that organic matter is utilised following a 2-step process : adsorption onto the biomass

followed by biochemical oxidation. Existing literature proposes that the adsorption process occurs at a much faster rate than the actual degradation reaction. Hence, a soluble substrate concentration versus time profile is expected to consist of two distinct plateaux. The first describing the fast initial decrease due to adsorption and the second, the slower degradation process.

The present thesis is concerned with the study of the degradation of deicing fluid in three different, but related systems : 1) Conventional activated sludge process as studied by the field data, 2) Batch experiments conducted under various experimental conditions and, 3) Sequencing batch reactor experiments

First, samples from an actual full-scale treatment plant treating airport de-icing fluids were collected. These field data were employed to set a basis for the results obtained from the various laboratory experiments and to study the practical aspects of treating airport deicing wastes by the activated sludge process. The wastewater treatment plant of St-Canut (PQ) was selected as it receives large amounts of glycol-containing wastewaters.

Next, the effects of biomass concentration, deicing fluid concentration and operating temperature on the treatment of deicing wastewaters were determined. In order to do so, laboratory batch experiments were conducted with different deicing fluid concentrations as well as various biomass concentrations.

Parameters such as Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), Ethylene Glycol (EG), Specific Oxygen Uptake Rate (SOUR), Sludge Volume Index (SVI), pH, etc. were periodically monitored to evaluate the degradation process and the state of the microbial population. Further batch experiments were carried out using both glycol-acclimatized and unacclimatized biomass in order to compare the removal rate of the ethylene glycol with different biomass populations. In addition to the batch experiments, adsorption experiments (shake flasks) were conducted to gain insight into the mechanism of ethylene glycol removal. Once this was tested, the results obtained from these adsorption experiments could be incorporated into the kinetic model.

Lastly, as the advantages of operating the sequencing batch reactor are numerous, a full study on the treatment of deicing wastes using this type of setup was conducted. The improvements in sludge settling characteristics and kinetic rates with continuous cycling were examined. In addition, the effect of the operating temperature and a sudden change in temperature on the removal rates and biomass properties was also investigated.

Throughout the study, the Biolog technique was employed as a tool in characterizing the changes in microbial population during the treatment process. This allowed to assess the differences in microbial population for the various laboratory experiments as well as at the St-Canut wastewater treatment plant.

It should be noted that in addition to the Biolog technique, the different parameters calculated from the data are also used to characterize the microbial population. More specifically, the degradation rates (COD, TOC and EG) indicate the ability of the population to assimilate the organic matter. The specific oxygen uptake rates (SOUR) indicate the general state of the population and the sludge volume index (SVI) measures the settleability of the biomass. By examining all these indicators, an overall picture of the microbial population is obtained.

1.2.2 Objectives of the Research

The following specific objectives can be derived from the scope of the research :

- Determination of the degradation kinetics of wastewaters containing deicing fluid. This includes the effect of process parameters (such as deicing fluid concentration, biomass concentration and temperature) as well as the specific mechanism for organic matter removal.
- Monitoring the microbial changes during the treatment of deicing wastes with regards to phenotypic (Biolog) changes.
- Implementation of a sequencing batch reactor (SBR) to treat deicing wastes and an evaluation of its performance based on kinetics and biomass characteristics.
- Corroborate the laboratory results with field data obtained from samples collected at municipal treatment plant (St-Canut).

Structure of the Thesis

This thesis consists of 7 Chapters. Following the Introduction Chapter (Chapter 1), Chapter 2 presents the background and the literature for the properties and treatment methods of glycol-based deicing/antiicing fluids. Chapter 2 also includes a literature review on the different wastewater treatment systems as well as the techniques employed for monitoring microbial populations. The various reactor setups as well as the analytical procedures used to follow the degradation process are discussed in Chapter 3.

Chapters 4 to 6 present the results and discussion of the field data analysis as well as the laboratory experiments. In Chapter 4, the results of the field data analysis (Section 4.1) and the preliminary batch experiments (Section 4.2) are shown.

Chapter 5 presents the batch reactor experiments conducted under various operating conditions and the shake flask experiments conducted to verify the adsorption mechanism. More specifically, the experiments using unacclimatized sludge (at various deicing fluid and biomass concentrations) are discussed in Section 5.1. The repeatability of biological experiments is discussed in Section 5.2. In Section 5.3, the role of adsorption in the removal of organic matter by biomass is presented. Subsequently, the acclimatized biomass experiments are presented and comparisons between unacclimatized and acclimatized biomass are made in Section 5.4. The effect of the operating

temperature on reaction rates is discussed in Section 5.5. The kinetic modelling of organic matter removal for the different batch experiments is presented in Section 5.6

Chapter 6 presents the experimental results obtained from applying the sequencing batch reactor setup (SBR) to the treatment of deicing wastes. The effect of cycle length (Section 6.1), biomass acclimatization (Section 6.2), temperature (Section 6.3) and the overall trends in SBR operation (Section 6.4) are presented. Section 6.5 presents the results for the kinetic modelling of organic matter removal in the SBR.

Lastly, Chapter 7 presents the conclusions derived from the experimental results, suggestions for future work and the original contributions to knowledge obtained from this study.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

In order to obtain an in-depth understanding of the literature on which the current research project is based, this chapter was divided into four main categories. First, the properties, environmental impacts and treatment methods of glycols and deicing/antiicing fluids are discussed. Following this section, a deeper look into the activated sludge process, including the mechanism and kinetics of degradation, is undertaken as this process is commonly applied to the treatment of deicing wastes. Thirdly, various studies on sequencing batch reactors (SBRs) are presented and provide a basis to develop the treatment of deicing wastes using this type of reactor. Lastly, literature on the monitoring of microbial population with the Biolog technique is shown in order to understand the scope of this method in providing data on the biomass changes.

2.1 Glycols and Deicing/Antiicing Fluids

2.1.1 Properties of glycols and deicing/antiicing fluids

Glycols or dialcohols (refer to Figures 2.1.1 and 2.1.2) are compounds having two hydroxyl groups attached to carbon atoms by an aliphatic chain [Miller, 1979]. Ethylene glycol (EG) was discovered in 1856 by Wurtz and owes its name to its intermediary position between glycerine (trialcohol) and alcohol. Glycols remain miscible in water as long as they contain less than six carbon

atoms and remain particularly soluble up to 10 carbon atoms [Coffey, 1965]. The properties of ethylene and diethylene glycol are presented in Table 2.1.1.

Table 2.1.1 : Miscellaneous properties of ethylene glycol and diethylene glycol

Property	**Ethylene Glycol**	**Diethylene Glycol**
Molecular formula	$C_2H_6O_2$	$C_4H_{10}O_3$
Molar mass	62.07	106.12
Characteristics	Visquous liquid, colorless, odorless	Visquous liquid, colorless, odorless
Boiling point (°C)	197.6	245.0
Density (at 20°C, g/ml)	1.1135	1.118
Flash point (°C) (open/closed)	116 / 111	139/124
Flammability limits (volume)	3.2% / 15.3%	2.0% / not available
Fusion point (°C)	- 13.0	- 6.5
Solubility in water (at 20°C)	miscible	miscible
Vapor density (air =1)	2.14	3.66
Vapor pressure (at 20°C, mm Hg)	0.055	less than 0.01

[CSST, 1989 and 1991] and [Verschueren, 1985]

Figure 2.1.1 : Ethylene Glycol

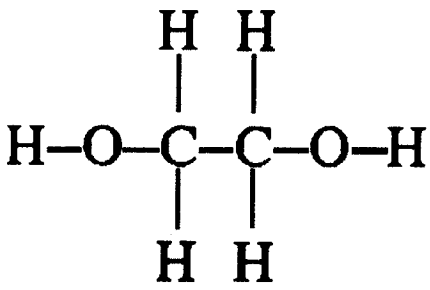
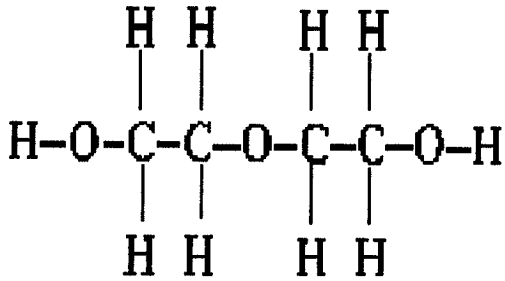


Figure 2.1.2 : Diethylene Glycol



In general, deicing and antiicing liquids are made up of glycols, water and chemicals such as wetting agents, corrosion inhibitors and colouring agents. The chemical compositions of a few of the most common deicing and antiicing fluids are presented in Table 2.1.2. The composition of these chemicals differs for each manufacturer and in fact, many european and american deicing chemicals use different glycols than those used in Canada.

Table 2.1.2 : Chemical composition of deicing and antiicing fluids

Chemical (basis of weight)	UCAR-ADF D (Deicing)	UCAR-XL (Deicing)	UC-5.1 (Antiicing)	UCAR-ULTRA (Antiicing)
Ethylene Glycol	49%	54%	46%	59%
Diethylene Glycol	5%	0%	21%	0%
Water	44.5%	45.5%	27.1%	39%
Additives	1.5%	0.5%	5.9%	2%

[Union Carbide, 1989 and 1994]

2.1.2 Fate of Glycols in the Environment

In order to evaluate the environmental impact of deicing fluids, their effect upon the zones affected by the dispersion of these chemicals can be examined. Firstly, part of the glycol is present as vapour which can be inhaled directly by the workers who handle them prior to and during spraying. A study conducted to evaluate the extent of exposition to the chemicals in the deicing area concluded that the use of protective masks greatly reduces health risks [Gérin and Viau, 1993]. It is also well known that ethylene glycol and diethylene glycol have low vapor tensions. Thus, since airport deicing operations are conducted at low temperatures under winter conditions, it is unlikely that any significant amounts of glycol will volatilise and persist in the atmosphere [Sabeh, 1996].

An important fraction of the deicing fluids remains in the deicing area and eventually finds its way into the drainage system. A certain quantity is released into the environment during the aircraft's takeoff and another part is mixed with the precipitations contained within the deicing zone. A laboratory study evaluating of the amount of glycol that remains on the aircraft (using a wind speed of 12 km/hr) found that 16% of the deicing fluid remained on the aircraft, 35% of the chemical was swept away by the wind and 49% was scattered on the ground near the aircraft [LGL-Love, 1979].

Table 2.1.3 presents a few environmental characteristics of ethylene glycol and diethylene glycol :

Table 2.1.3 : Environmental characteristics of ethylene glycol and diethylene glycol

Parameter	Ethylene glycol	Diethylene glycol
Biochemical Oxygen Demand (BOD_5, mg/L)	750 000	890 000
Chemical Oxygen Demand (COD, mg/L)	1 550 000	1 750 000
Total Organic Carbon (TOC, mg/L)	430 000	500 000

[Sabeh, 1996]

2.1.3 Treatment of Glycols and Deicing/Antiicing Fluids

Biochemical oxidation is one of the most commonly used techniques for the removal of organics contained in wastewaters. Other methods that have been used for the removal of glycols include adsorption, wet oxidation and ozonation. A study on the adsorption of petrochemicals by activated carbon revealed that ethylene glycol has a very low affinity for activated carbon. Their results showed that only approximately 70 mg out of 1 000 mg of ethylene glycol (in 1 litre of water) was adsorbed by 5 g of activated carbon [Giusti *et al.*, 1974].

Wet oxidation relies on the oxidation of organics in the presence of oxygen or air at very high pressures and temperatures. Imamura *et al.* (1986) studied the wet oxidation of polyethylene glycol (PEG) and found that the presence of a catalyst greatly enhances the degradation of PEG. Another study relating to the degradation of PEG was conducted using ozone (O_3) since it is a very powerful oxidant that can react rapidly with organics present in water. Hence, Suzuki *et al.* (1978) observed an increase in the biodegradability of polyethylene glycol when pre-treated by this oxidizing agent.

Due to the widespread use of biochemical oxidation, considerably more literature is available on the biodegradation of glycols than on the aforementioned treatment methods. In a laboratory scale study, the biodegradation of ethylene glycol and diethylene glycol was evaluated using water from different rivers [Evans and David, 1974]. Their results tend to indicate that the biodegradation of these chemicals is often slow in natural environments. Therefore, in case of low temperatures during winter, their breakdown will usually occur downstream from their point of their origin. Other experimental studies indicate that the biodegradation of diethylene glycol tends to be slower than that of ethylene glycol. This is probably due to the existence of an ether link in the structure of diethylene glycol (see Figure 2.1.2) [Gerhold and Maloney, 1966].

Grabinska-Loniewska (1974) identified the types of bacterial species able to degrade ethylene glycol. The study classified 44 different species depending

upon the extent of their growth on the ethylene glycol (EG) substrate. Another study isolated the bacterial populations capable of degrading ethylene glycol in a rotating biological contactor [Gould *et al.*, 1989]. The study concluded that the optimal pH for this process is 5.0 and results show a biodegradation of 70% to 80% after 5 hours at a temperature between 20°C and 25°C and an initial ethylene glycol concentration of 3.5 g/L.

With regards to diethylene glycol, Koganovski *et al.* (1987) identified many bacterial species that were able to utilize this carbon source. Their research revealed that all the cultures that were isolated belonged to the *Pseudomonas* and *Bacillus* species. They further conclude that the optimal conditions for the biodegradation of ethylene glycol occur when using a mixed microbial culture.

Jank *et al.* (1973) undertook the first study on the biodegradation of deicing fluids. The first part of their work was a bench-scale activated sludge study to determine the optimum loading conditions and design parameters for the treatment of deicing fluids. The experimental results showed that an activated sludge system treating a combination of deicing fluid and domestic sewage at less than 10°C produced an effluent having BOD_5 and suspended solids concentrations not exceeding 20 mg/L and 25 mg/L, respectively at a loading of 0.15 kg BOD_5/ kg MLSS-day. Growth of filamentous microorganisms and the resulting sludge bulking condition were responsible for the low loading condition. The second half of their study dealt with the toxicity of aircraft deicing fluids and

process effluents to rainbow trout. These bioassays showed that, at an acceptable organic loading, the concentration of deicer in the feed solution would be such that the effluent from the activated sludge process would not be toxic to rainbow trout.

More recent studies [Sabeh, 1996 and Aéroports de Montréal, 1999] evaluated the impact of a controlled spill of deicing fluid on the operation of an effluent treatment plant. This research was conducted at Mirabel airport in order to determine the feasibility of treating glycol-contaminated wastewater at a local wastewater treatment station. The results obtained indicate that it is feasible to treat deicing wastes in a municipal wastewater plant using the activated sludge process. However, the addition of deicing fluid provoked a marked increase in the growth of filamentous microorganisms in the bioreactor and additional sludge production.

Nitschke *et al.* (1996) also provide valuable information pertaining to the effects of deicing fluids on the biological treatment process. Their experiments simulate the effect of glycol "shock loadings" on their laboratory constructed activated sludge treatment plants. They conclude that glycol-containing wastewaters can cause severe disturbances in sewage treatment. Thus, special emphasis should be placed on avoiding "shock loads" and maintaining an acclimatised activated sludge in the treatment plant.

Lastly, anaerobic treatment of dilute aircraft deicing fluid wastewaters has been reported to be successful using an Upflow Anaerobic Sludge Blanket (UASB) reactor [Darlington and Kennedy, 1998]. However, as can be expected, COD specific removal rates are much lower than those reported in studies conducted under aerobic conditions.

2.2 Activated Sludge Mechanism

2.2.1 Removal of Organic Matter by Adsorption

Adsorption involves the transfer of a constituent from the liquid phase to the solid phase. The adsorbate is the substance (usually the pollutant) that is removed from the liquid phase at the interface. The adsorbent is the solid liquid or gas phase onto which the adsorbate accumulates. Principal adsorbates used in water and wastewater industry are activated carbon and a few synthetic polymer based products, the latter being used to as a last resort due to their high cost [Metcalf &Eddy, 2003]. While the aeration tank provides a proper environment for flocculated microorganisms to grow, this biomass removes the organics from the wastewater by adsorption and subsequent aerobic biological oxidation [Eckenfelder 1986]. Hence, it is of interest to review the adsorption process as this allows the evaluation of the experimental data based on this mechanism.

Tan and Chua (1997) have studied the COD adsorption capacity of activated sludge known often as CAC. They recommend this to be a key

parameter for controlling the whole process. In this case, CAC was determined by mixing activated sludge with the settled sewage and measuring the COD reduction per unit mass of activated sludge over a one minute period immediately thereafter. The article presents a method of assessment of the physical adsorption capacity of activated sludge at an operational level.

Several researchers including Selvakumar and Hsieh (1989) have observed removal of organic compounds by microbial biomass. In their study, measured quantities of biomass were placed in test tubes into which different concentrations (from 50 up to 200 mg/L) of liquid organic compound were added. They report that the adsorption of the organic matter can be adequately expressed by the Freundlich Isotherm.

Bell and Tsezos (1987) focused on the adsorption of several pesticides by microbial biomass. According to their studies, refractory organics can be removed by physical adsorption. Specific examples of this adsorption are lindane penta-chlorophenol, diazinon and 2-chloro-bi-phenyl. However, they caution against the possibility of desorption of the pollutants as the process of adsorption is reversible.

Susumu *et al.* (1970) demonstrated that the adsorption mechanism of biomass in the activated sludge treatment of wastewater is identical to the same phenomenon exhibited by charcoal. In explaining the mechanisms of elimination

of organic matter in wastewater by the activated sludge process, Schulz *et al.* (1978) observed that all substances were eliminated at a rate greater than that of biodegradation. The removal rate was found to be dependant on the concentration of activated sludge but nearly independent of the substrate concentration suggesting thereby that adsorption was the key mechanism.

2.2.2 Substrate and Biomass Kinetics

In order to be able to design a suitable biological wastewater treatment facility for domestic or industrial effluents, one needs to properly understand the kinetics of biological growth. These include a) utilization rate of soluble substrate b) rate of soluble substrate generation from organic matter c) rate of biomass growth with soluble substrate d) kinetic coefficients for substrate utilization and biomass growth e) rate of oxygen uptake f) temperature effects g) observed and net yield and h) volatile suspended solids and active biomass [Metcalf and Eddy, 2003]. Standard textbooks on wastewater treatment offer ample information on the above topics. First, the traditional equations that govern substrate utilization and biomass growth are presented. Thereafter, the equations that are required for the specific experiments of the current research project are derived. Lastly, a few examples of kinetic studies are given.

The mechanism for organic matter removal is quite complex and there are many kinetic models that describe biomass growth and substrate utilisation. One of the most often used is the Monod relationship [Droste, 1997 and Eckenfelder,

1999] which is a well-known expression that models the removal of single substrates (i.e. substrates that are directly transportable into the cell). It is described by the following expressions :

$$r_X = \mu X = Y r_S \qquad \text{Eq. 2.1}$$

where

r_X = Rate of biomass growth, mass/(volume-time)

μ = Growth coefficient, time^{-1}

X = Biomass concentration, mass/volume

Y = Yield coefficient, mass biomass produced/mass substrate utilised

r_S = Rate of substrate utilisation, mass/(volume-time)

The growth coefficient is given by :

$$\mu = \mu_{max} \left(\frac{S}{K_S + S} \right) \qquad \text{Eq. 2.2}$$

where

μ_{max} = Maximum value of the growth coefficient, time^{-1}

S = Substrate concentration, mass/volume

K_S = Saturation constant (substrate concentration with $\mu = 0.5 * \mu_{max}$)

In addition to biomass growth during substrate utilisation, there is a term to represent biomass death. Thus, the expression for biomass decay (often referred to as endogenous decay) is given by :

$$r_D = k_e X \qquad \text{Eq. 2.3}$$

where

r_D = Rate of biomass decay, mass/(volume-time)

k_e = Endogenous decay coefficient, $time^{-1}$

X = Biomass concentration, mass/volume

Therefore, the expressions for biomass growth and decay can be combined to describe net biomass growth and substrate utilisation. The other kinetic models are similar to the Monod model, however they include terms that may take into account substrate inhibition and the presence of multiple substrates.

As was shown in the above section, the Monod model describes the biomass and substrate utilization kinetics under microbial growth conditions. However, the specific kinetics of organic matter removal can be described by kinetic models that are independent of biomass growth. The models are basically empirical models wherein the rate of substrate utilization is described by a zero order or first order rate law [adapted from Fogler, 1999]:

$r_s = - k'$ (Zero Order) Eq. 2.4

$r_s = - k' C_s$ (First Order) Eq. 2.5

where

r_s = rate of substrate utilization per amount biomass, mg/ (g TSS-hr) or equivalent

k' = reaction rate constant per amount biomass, mg/(g TSS-hr) or equivalent for zero order and L/(g TSS-hr) or equivalent for first order

C_s = reactant concentration , mg/L or equivalent

These constants can be obtained from the integration of the design equation (using a constant volume batch reactor) :

$$-\frac{dC_s}{dt} = \frac{k}{X} = k' \quad \text{(Zero Order)} \quad \text{Eq. 2.6}$$

$$-\frac{dC_s}{dt} = \frac{k\ C_s}{X} = k'\ C_s \quad \text{(First Order)} \quad \text{Eq. 2.7}$$

where

$\frac{dC_s}{dt}$ = rate of change of the reactant per amount biomass, mg/(g TSS-hr) or equivalent

C_s = reactant concentration , mg/L or equivalent

k = reaction rate constant, mg/(L-hr) or equivalent for zero order and hr^{-1} or equivalent for first order

X = biomass concentration, g/L or equivalent

k' = reaction rate constant per amount biomass, mg/(g TSS-hr) or equivalent for zero order and L/(g TSS-hr) or equivalent for first order

Therefore, the reaction follows zero-order kinetics if the plot of the reactant concentration vs time is linear (slope is equal to –k). The reaction is found to follow first-order kinetics if the plot of $\ln(C_s)$ vs time is linear (the slope is equal to –k).

An important variable that has a significant effect on the reaction rate of chemical and biological reactions is temperature. Since microbial cells and their cell contents are at the same temperature as their environment, and since metabolic reactions are biochemical-enzyme-catalyzed reactions, an increase in temperature generally increases the rate of reaction [Schuler and Kargi, 2002]. The dependence of the reaction rate on temperature normally follows an Arrhenius type relationship. In activated sludge modeling, however, this dependence is simplified to an equation of the form shown below :

$$\frac{k_{T2}}{k_{T1}} = \theta^{T2-T1} \qquad \text{Eq. 2.8}$$

where

k_{T1} = Reaction rate constant at temperature T1, °C

k_{T2} = Reaction rate constant at temperature T2, °C

θ = Temperature correction coefficient

T1 = Temperature 1, °C

T2 = Temperature 2, °C

The value of θ varies from 1.01 to 1.10 depending upon the type of wastewater and the geographical location of the wastewater treatment plant. It may be added that from practical point of view, the variation of the value of the rate constant with temperature has a tremendous impact on the design of treatment plant units under the cold climatic conditions of Canada. As can be seen from Equation 2.8, if the wastewater temperature decreases from summer to winter months, the value of k decreases. This means, that in order to have the same degree of organic matter removal in winter, the retention time of wastewater in the treatment unit should be longer translating into bigger reactor volumes and ensuing costs.

Over the past 20 years, various researchers around the world have studied the kinetics of microbial growth and proposed kinetic models based on empirical calculations or experimental results. A few such examples are presented below.

By using samples taken from a municipal wastewater treatment plant in Japan, Fujie *et al.* (1988) empirically determined parameters such as instantaneous bio-sorption and rate of biological oxidation of all the soluble organic substrate (SOS) as a function of soluble COD concentration. They proposed a simplified kinetic model for the removal of soluble organic substances

(SOS) in the activated sludge aeration tank. The authors also express empirically the bio-sorption of SOS per unit dry mass of activated sludge as a function of SOS concentration in the influent wastewater.

A simple structured kinetic model is applied to the activated sludge system by Padukone and Andrews (1989) which illustrates some of the possibilities and difficulties in producing a comprehensive model for all the variations of the activated sludge process. The rate equations are chosen so as to reduce to the Monod equation during balanced growth. The authors mention that the mathematics can be simplified by lumping adsorbed colloidal matter, intracellular storage products and extra cellular polysaccharides into a single "stored substrate" term. However, they admit that this would obscure some important, time-dependent differences between these categories.

Lastly, to examine the range of kinetic constants, Nyholm *et al.* (1996) estimated the rate constants for four chemicals with widely different biodegradability characteristics and using sludges of different origins. They found that their values agree well with standard values of biodegradation of wastewater in European municipalities.

2.3 Sequencing Batch Reactors

All the variations of the conventional activated sludge process involve spatial movements of biomass from tank to tank in a continuous fashion. As the tank in each process has a definite volume, the process time spent by a waste in the tank is fixed. For a given flow rate, varying the retention times of a tank means changing the volume of tanks which is impractical and uneconomical. However, the same results can be achieved in a batch reactor by altering the environment temporally. By employing a batch reactor or a series of batch reactors in this fashion, the so-called SBR system is obtained [Metcalf and Eddy, 2003].

In the Sequential Batch Reactor System, aeration and clarification take place sequentially but in the same tank as opposed to the conventional system. The operational cycle of SBR consists of five steps 1) Fill 2) React 3) Settle 4) Decant and 5) Idle [Henry and Heinke, 1996]. Most full-scale SBR systems provide at least two reactors which alternatively receive influent and hence, can provide continuous treatment. Sludge is wasted from the reactor during decant or idle phases when the settled sludge volume reaches a certain level. Construction and operation of SBRs are said to be 20% less than for conventional treatment due to absence of sludge pumping equipment and secondary settling tank [US EPA, 1986].

There are about 200 installations of SBR in the US and a few dozen in Canada. Over the last 10-15 years, various studies all over the world have developed kinetic models of SBRs treating municipal and industrial wastes. Nakazawa and Tanuca (1991) conducted experiments on a pilot plant treating municipal waste In Japan. They developed a mathematical model, based on steady-state conditions, which has the following characteristics : a) An increase in aeration time per cycle decreases the sludge production b) The fill period with aeration (the highest aeration time ratio) gives the highest oxygen consumption per cycle but the lowest average oxygen consumption rate c) The fill period without aeration (i.e. lowest aeration time ratio) gives the lowest average oxygen consumption per cycle but the highest average oxygen consumption rate d) The fill period with aeration gives lowest soluble BOD_L (BOD ultimate) in a reactor throughout a cycle, the reverse being true also e) The highest aeration time ratio gives a lower level of BOD_L throughout the cycle, and, finally; f) Filamentous bacteria are controlled by having lower aeration time for the anoxic period during fill period.

A comparative study was conducted using SBRs and continuous up flow anaerobic sludge blanket (UASB) reactors operating at organic loading rates (OLR) between 0,60-19,7 g COD/L [Kennedy and Lentz, 2000]. The results show that the performance of both types was very similar at low to intermediate OLR. The UASB reactors performed better at a higher OLR than the anaerobic SBR. The sequencing batch reactor showed a soluble COD removal of 71 to 92%

whereas for the continuous UASB, the soluble COD removal rate was between 77-91% for same HRT and feed conditions. Thus, there is not much difference between the two systems. In case of an anaerobic SBR treatment of municipal landfill leachate, if the specific organic loading rate during the fill cycle is less than 3000 mg/L of COD per g VSS per day, there is a risk of system failure. Besides COD removal, both systems successfully reduced toxicity (as measured by the Microtox test). Neither operating system was able to consistently lower sulfides, chlorides and BOD concentrations to meet sewer discharge norms.

Nakhla *et al.* (1997) developed a mathematical model for SBR that takes into account the fill and reaction periods and compared against experimental data from the literature. Using the Monod equation, their model predicts the time dependent microbial and substrate concentrations during SBR treatment. According to them, wastes containing low concentrations of inhibitory agents permit an instantaneous fill for optimum operation compared to high strength inhibitory wastes which need longer fill terms. Their models compare very favourably with experimental data like MLVSS versus time up to 40 days obtained by Misbahuddin and Farooq (1991). In addition, the modelling results obtained by Hsu (1986) compare very well to the effluent concentrations versus time for up to 100 days. However, little or no information is available as to type of inhibitory or non-inhibitory wastes used in the present study.

A wastewater treatment plant treating 2 Megagallons a day (approximately 7600 m^3/day) of mainly domestic wastewater in Lenoir N.C. completed an expansion of the plant including conversion of 4 existing treatment tanks to a four SBR basins. In doing so, they report achieving influent equalization within the tanks, nutrient removal, improved solids settleability as well as controlling filamentous growth of organisms. The above features allowed the municipality to increase plant capacity without constructing new tanks and also achieve better nitrification of effluent. The subsequent computer based automation resulted in fewer hours for operating personnel thereby reducing the overall cost of operation and maintenance [Waresak,1997].

Irvine *et al* (1997) offer an overview of controlled unsteady state processes and technologies. They discussed the SBR process cycle with aerobic and anoxic phases to achieve nitrification and de-nitrification quasi simultaneously. In practice, in most SBRs, there are two or more identically operated tanks that provide for the time sequencing of operations such as equalization and sedimentation. Operated systems maximize overall contaminant reduction by allowing for a wide range of aerobic, anoxic and anaerobic reactions to take place. This will render the microbiological, bio-chemical and mathematical modelling of such controlled unsteady-state system quite complex and impressively large.

A mathematical model which describes the volume changes of the reactor and the biodegradation kinetics of a SBR has been developed and calibrated by using the experimental data from a full-scale plant operating at 4-hour cycle (Novak *et al*, 1997). The model describes the behaviour of selected parameters such as volume, suspended solids concentration, oxygen uptake rate (OUR), ammonia and nitrate/nitrogen in the selector compartment and the main aeration tank in ideally mixed and filled reactors. However, one must apply the model with caution by collecting enough information on the hydraulic regime of the system in question. For example, the hydraulic information such as residence time distribution in each volume of the reactor can be employed for better model precision.

Morgenroth and Wilderer (1998) reviewed the historical evolution of the SBRs. Mass balance equations are presented which enable a quantitative comparison of the SBR system with continuous flow systems used in the activated sludge process. In a sequencing bio-film batch reactor (SBBR), wastewater is treated using microorganisms that grow on a support media similar to a trickling filter in a continuous system. Arnz *et al.* (2000) conducted experiments on lab-scale as well as semi-full-scale reactors using modelling and tracer studies. They investigated hydraulic retention time distribution function and flow patterns under varying hydraulic loading rates. They found that introduction of a new batch of wastewater and withdrawal of treated water can be executed simultaneously without risk of contaminating the effluent of the reactor with

untreated influent. Like many other studies, they found that the full displacement strategy enables a reduction of cycle time and enhanced exploitation of reactor volume.

Under dynamic operating conditions in wastewater treatment when biomass grows, the substrate removal is not by simple growth and oxidation. For example, storage in the form of PHB is the main mechanism of acetate removal. This has been confirmed by several years of studies by Dionisi *et al.* (2001) on the influence of periodic operation on the performance of the activated sludge process. On the basis of the results obtained, an empirical kinetic model was developed and applied to typical tests. The authors, of course, caution against application of their specific results to SBR design and operation as they were based on tests conducted using a synthetic medium with a single carbon source, which is very different from the actual wastewater conditions.

There has been a new approach in SBR process for treating municipal wastewater destined for agriculture. Lin and Cheng (2001) modified the SBR to accommodate a continuous flow of wastewater while retaining the sequencing operations of the other steps. The modified SBR system was found to reduce a total of 70% of COD and the water quality was found to be excellent and suitable for agricultural irrigation. It should be mentioned that the above results are those

of a lab-scale experimental setup and therefore, it is not known whether same results can be anticipated in a full scale SBR.

Recently, SBR were employed to treat brewery wastewaters in order to produce acceptable effluent for discharging into streams. The influent used in the SBR was, in fact, the effluent discharged by an up-flow anaerobic sludge blanket (UASB) operating in a brewery in Portugal. Rodrigues *et al.* (2001) employed a lab scale cylindrical SBR to run their experiments. They report that a nitrification efficiency of 97% and a satisfactory removal of NH_4^-. De-nitrification was suppressed when the bulk liquid oxygen level increased to about 7 mg/L. The creation of a primary toxic phase in the reaction cycle was considered to be the best treatment option.

2.4 Monitoring Changes in Microbial Population

Changes in microbial population have an all pervading impact on the activated sludge process in terms of settling properties, the types of compounds that are degraded by the consortium and the rates at which they are removed. Amongst many characterization methods, the Biolog technique is employed to appraise the microbial community. Below are a few examples of studies that employ this technique to characterize various microbial populations.

The Biolog method is a phenotypic technique (relating to carbon source utilization) that provides a community analysis of the metabolical changes that

occur in the activated sludge microorganisms. This technique is based on the utilization of substrates (96 total) contained in a microplate to which the sludge sample is added. A statistical analysis is performed on the resulting carbon source utilization data to reveal trends in metabolical patterns.

Garland and Mills (1991) employed this technique to characterize and classify heterotrophic microbial communities. Their research involved the testing of aquatic, soil and rhizosphere samples and produced community-dependent patterns of sole-carbon-source utilization. Their results showed that intensive spatial and temporal analysis of microbial communities (through statistical analysis) could produce ecologically relevant classifications of heterotrophic microbial communities.

A subsequent study [Victorio *et al.*, 1996] used a similar approach to classify the microbial communities involved in various wastewater treatments systems. They employed principal component analysis (PCA) to determine the changes in carbon source utilization and developed a complete methodology to prepare raw sludge samples for inoculation in the microplate. The main results showed that distinct microbial populations were created at the different wastewater systems. In addition, they determined that the Biolog procedure could be employed to detect population changes due to changes operating conditions or treatment plant upsets.

Lastly, Schneider *et al.* (1998) added evidence that phenotypic fingerprinting with the Biolog technique was sensitive enough to detect microbial changes that can affect treatment system performance. In particular, they found observable changes between treatment plant samples taken during a toxic upset and samples collected during normal operation. Hence, they concluded that phenotypic fingerprinting successfully followed metabolical pattern changes during plants upsets and could be more sensitive in revealing changes than other techniques such as microbial plate counts and microscopic analysis.

CHAPTER 3

MATERIALS AND METHODS

To implement the objectives cited in Section 1.2.2, a study of the biodegradation of glycols was undertaken involving shake flasks, batch reactor and sequencing batch reactor experiments. In addition, an existing wastewater treatment plant was studied in order to evaluate its capacity to treat glycol-contaminated wastewaters during winter months.

3.1 Shake Flask Setup

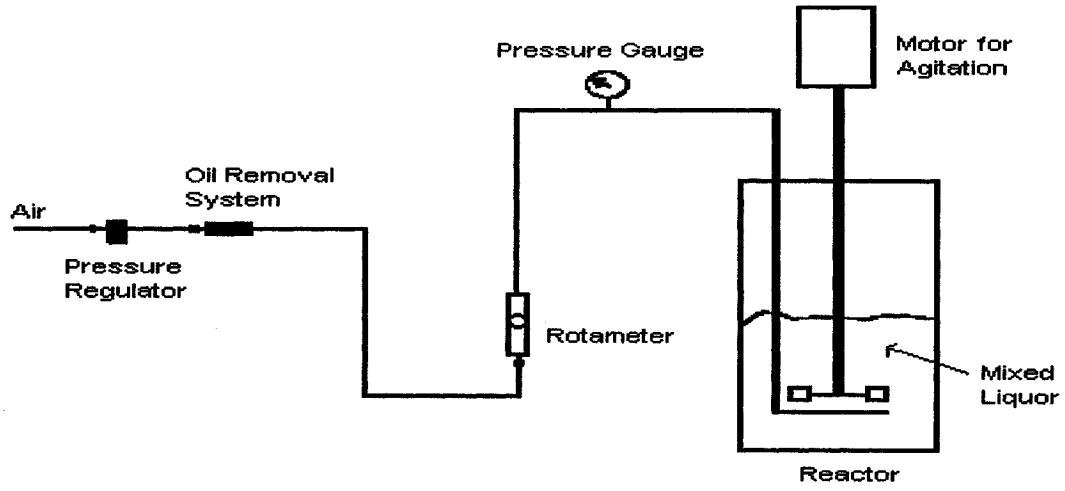
To gain insight into the mechanism of deicing fluid adsorption, shake flask experiments were conducted under various conditions. Calculated proportions of wastewater and biomass were placed into 250 mL Erlenmeyer flasks. The flasks were tightly plugged in order to minimize oxygen transfer into the bulk liquid. The flasks were then placed on a mixer (200 RPM) and samples were taken periodically to verify the extent of removal by adsorption onto the biomass.

3.2 Batch Reactor Setup

Figure 3.2.1 shows the schematic diagram of the experimental set-up that was used to the conduct the batch experiments. A Microferm© Fermentor unit provides online mixing with speed control for the 15-litre closed glass vessel. For the batch experiments conducted at the lower temperature, a 2-litre plastic vessel was employed. The compressor supplies the air and a line pressure regulator

and an online rotameter regulate its flow rate. In order to prevent any oil residues from entering the reactor, a cotton filter is installed between the line regulator and the rotameter. The TSS of the activated sludge must be determined in order to mix the correct proportions of biomass and wastewater. The wastewater is prepared in two parts : the first part consists of a synthetic base of carbon sources and nutrients and the second part is comprised of deicing fluid (refer to Section 3.4). Once the biomass is mixed with the correct amount of wastewater, tap water is added to complete to a working volume of 6 L (or 1.5 L in the case of the lower temperature experiments). The reactor is then clamped to the unit and the aeration and mixing are started. In order to achieve dissolved oxygen levels above 2 mg/L (for biomass activity and growth), the air flow is set to about 0.5 L/min. The mixing speed is maintained at approximately 300 RPM for adequate mixing without creating stagnant zones or vortex conditions.

Figure 3.2.1 : Batch reactor set-up



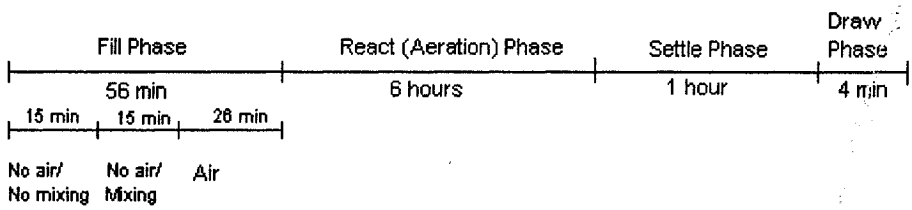
For most of these experiments, samples were taken at time = 0, 1, 2, 4, 6 and 8 hours. However, the samples for the experiments carried out at low biomass concentrations were taken every 4.5 hours (total run time of 45 hours).

3.3 Sequencing Batch Reactor Setup

In order to conduct the sequencing batch reactor experiments, the same basic units as the batch reactor were employed. However, in addition, 2 peristaltic pumps (Masterflex, model 7553) as well as a programmable logic controller (ChronTrol, model XT) were required to pump the raw and treated wastewaters as well as control the various phases (fill, react, settle, draw and idle) of operation.

An activated sludge volume of 375 mL is initially placed in the reactor to which the wastewater is added for a total operating volume of 1.5 L. Based on the literature, an 8 hour cycle included a "fill" phase of 56 minutes, a "react" phase of 6 hours (or 10 hours for the 12 hour cycle), a "settle" phase of 1 hour and a "draw" phase of 4 minutes. In the "fill" phase, the first 15 minutes are without aeration and mixing and thereafter, a further 15 minutes are without aeration (see Figure 3.3.1). Samples were taken at the beginning of the fill phase (labelled as t = 0 hours), during the aeration ("react") phase (labelled as t = 1, 2, 3, 5 and 7 hours) and after the "draw" phase (treated effluent, labelled as t = 8 hours).

Figure 3.3.1 : Timeline of an 8-hour SBR cycle



3.4 Wastewater

In all laboratory experiments, two types of synthetic wastewaters were used : a nutrient solution and a solution containing deicing fluid. These synthetic solutions were preferred over a domestic wastewater since they are amenable to more reproducible experimental conditions. The first synthetic wastewater provides basic nutrients and carbon sources to the microorganisms. It includes adequate proportions of carbon, nitrogen and phosphorus as well as other trace

elements required for microbial growth. This particular formulation was selected as it was employed by Nitschke et al. (1996) in their study of the influence of glycol deicing agents on the sewage treatment process. The composition of this solution is given in Table 3.4.1. The glycol wastewater is simply a mixture of tap water added with a controlled amount of deicing fluid. The four levels of deicing fluid employed are presented in Table 3.4.2 and Section 2.1 of the literature review presents the composition of the deicing fluid (UCAR-XL was employed for all experiments). Both solutions are prepared less than an hour before start-up in order to prevent any degradation of the synthetic mediums. Appropriate quantities of wastewaters, activated sludge and tap water are then added to the reactor and the experiment is started.

Typically, the initial TOC and COD values of the synthetic wastewater (without the deicing fluid) varied between 40 mg/L and 55 mg/L for the TOC and 120 mg/L and 160 mg/L for the COD. This variation is probably due to the different batches of ingredients such as peptone, beef extract, etc. used during the experimentation period.

Table 3.4.1 : Composition of the synthetic base (without deicing fluid)

Ingredient	**COD ≈ 140 mg / L (data in mg/L)**
Peptone	73
Beef Extract	51
Urea	14
NaCl	3.2
K_2HPO_4	12.9
$NaHCO_3$	90
Tap Water	add to working volume of reactor

[Nitschke et al., 1996]

Table 3.4.2 : Deicing fluid concentrations

Type of Experiment	**Deicing fluid concentration (in mg/L)**
Normal operation	0
Glycol acclimatization	35
Deicing season	65
Glycol shock load	130

3.5 Analytical Procedures

In the batch set-up, activated sludge and mixed liquor samples were taken in order to conduct the following measurements : Chemical Oxygen Demand

(COD), Total Organic Carbon (TOC), Ethylene glycol concentration, Total and Volatile Suspended Solids (TSS/VSS), Sludge Volume Index (SVI), pH and temperature. In addition, periodic measurements of Dissolved Oxygen (DO) were taken in order to calculate the Specific Oxygen Uptake Rate (SOUR). In order to prevent residual biodegradation, all samples were acidified to pH < 2 with concentrated sulfuric acid (method prescribed by Standard Methods for preserving samples) and were stored in a fridge maintained between 2-6 °C. Unless otherwise mentioned, the analytical methods described above were performed using the techniques prescribed in Standard Methods [APHA, 1989].

COD and TOC measurements characterise the environmental impact of the wastewater and the total organic matter present. TSS and VSS provide an indication of the amount of biomass (microorganisms) in the system and SVI describes the settleability of the sludge (important for sludge settling in the clarifier). The monitoring of parameters such as pH, DO and temperature help ensure a stable environment for the respiration and growth of microorganisms in the reactor. In addition to these standard variables, the Biolog© technique was also employed. This method offers information on the type of microbial culture present in the activated sludge system. A microplate containing 95 different substrates is injected with the activated sludge sample. If the cells utilize the particular substrate, the well turns purple due to the reduction of a dye. The degree of utilization is then determined by the intensity of colour in the particular

well. Thus, the microbial culture can be characterised by observing the types of substrates it is able to degrade.

It should be noted that most samples were analyzed in triplicate and yielded reproducible results. Thus, all the comparisons presented in the results and discussion represent significant variations in the various rates.

3.5.1 Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. The dichromate reflux method is preferred over other methods due to its superior oxidizing ability and applicability to a wide variety of samples. The specific procedure used was the closed reflux titrimetric methods. This technique involves refluxing the samples in a strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). Thus, the organic matter is oxidized by a boiling mixture of chromic and sulfuric acids (2 hour reflux time). After digestion, the remaining dichromate is titrated using ferrous ammonium sulphate (FAS) to determine the amount of potassium dichromate consumed and the organic matter is quantified in terms of oxygen equivalent.

For this procedure, several reagents must be prepared at least one day in advance in order to permit proper dissolution and cooling of the solutions. A

standard potassium dichromate solution of 0.0167 M is prepared by adding 4.913 of dried $K_2Cr_2O_7$ crystals with 167 mL of concentrated sulfuric acid and completing to 1000 mL with distilled water. The sulfuric acid reagent consists of reagent grade Ag_2SO_4 added to concentrated H_2SO_4 at a rate of 5.5 g Ag_2SO_4 / kg H_2SO_4 (silver sulphate is added to improve the oxidation of straight-chain aliphatic compounds). A standard ferrous ammonium sulphate (FAS) titrant of approximately 0.01 M is prepared by dissolving 3.92 g of Iron (II) sulphate hexahydrate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) in distilled water and adding 20 mL of concentrated sulfuric acid. The solution is then completed with distilled water upto a total volume of 1000 mL.

Once the solutions are ready, standard 10 mL culture tubes and caps are washed (twice) with a 20 % v/v solution of H_2SO_4 in order to prevent contamination. Each ampoule is then prepared by combining 2.5 mL of the wastewater, 1.5 mL of the dichromate solution and 3.5 mL of the sulfuric acid reagent (samples were prepared in either triplicate or quadruplicate in order to obtain reliable values). The ampoules are capped and inverted several times to mix completely (caution was employed to avoid overheating of the ampoule while mixing). In addition to the wastewater samples, four blanks were prepared containing the reagents and a volume of distilled water equal to that of the wastewater. Out of these four blanks, two are digested in order to provide a blank reading and two are set aside in order to determine the molarity of the FAS solution. Once all the ampoules are prepared, they are placed in a block digester

preheated to 150 °C and refluxed for 2 hours. They are then cooled to room temperature and are titrated using the FAS solution and 2-3 drops of ferroin indicator. The end point is observed when a sharp colour change from blue-green to reddish brown occurs.

The equation employed to calculate the COD is presented in Appendix A. The above procedure can be employed for COD values ranging between 50-300 mg O_2/L. For values higher then 300 mg O_2/L, the samples must be diluted. For values less than 50 mg O_2/L, an alternate procedure using lower concentrations of reagents was employed.

Some possible interferences for this test include halides and nitrite. However, these were judged to be insignificant for the wastewater samples in the present study.

3.5.2 Total Organic Carbon (TOC)

Total Organic Carbon represents the total organic (carbon) content of the wastewater whereas COD is the total oxidizable material in the sample. Unlike COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganics that can contribute to the oxygen demand measured by COD. This technique involves the oxidation of organic compounds to carbon dioxide by persulphate in the presence of a UV light source (UV lamp). The CO_2 generated

as a result of this reaction is measured by a nondispersive infrared analyser. Both units are comprised in the TOC Analyser (Dohrmann® Division, Rousemount® Analytical Inc). First, the apparatus is calibrated using a 400 ppm standard of Potassium Hydrogen Phthalate (commonly known as KPH). The filtered samples are then injected using a 200 μL syringe. The detection range at this particular setting is 0.05 to 400 ppm. In all cases, samples must be run with both the lamp on and the lamp off. This is due to the fact that both organic carbon and inorganic carbon (such as carbonates and dissolved CO_2) are measured with the lamp on (total carbon) whereas only inorganic carbon is measured with the lamp off. Thus, the numerical value with the lamp off is simply subtracted from the value with the lamp on in order to quantify the total organic content of the wastewater (i.e. TOC = total carbon - inorganic carbon).

For the TOC test, excessive acidification (below pH = 1) can hinder the oxidation of organic carbon. Furthermore, the intensity of the ultraviolet light reaching the sample may be reduced by highly turbid samples. In this case, both these interferences were considered to be negligible.

3.5.3 Ethylene Glycol Analysis

The analysis for ethylene glycol was performed using a Hewlett-Packard (model 5890) Gas Chromatograph (GC). After testing many types of columns, the DB wax column was chosen for its higher resolution and separation of the ethylene glycol peak and other peaks of the glycol family of compounds. As an

internal standard, propylene glycol was selected and peak calibration was conducted with a 100 ppm Ethylene Glycol and Propylene Glycol standard. The program consisted of an initial temperature of 120 °C with a ramp of 2 °C /min upto 129 °C. Thereafter, an increase of 25 °C /min until a final temperature of 200 °C (held for 5 minutes).

3.5.4 Total and Volatile Suspended Solids (TSS and VSS)

Total suspended solids (TSS) is the term applied to the amount of solids retained by a filter whereas Volatile suspended solids (VSS) is the portion of TSS that is volatilised (i.e. combusted) at a high temperature. Thus, TSS represents the organic and inorganic matter contained in the sludge and VSS quantifies the organic matter (i.e. micro-organisms) present in the sludge. These quantities were determined using a vacuum filtration system and Millipore type AP40 filter disks having a pore size of 45 μm (the standard size for the retention of biomass). For TSS, a known volume of sample is filtered and the retained solids are collected and dried in an oven between 103-105 °C (the filtrate is collected and stored for analysis of COD, TOC, etc.). Thus, the difference in dry weight before and after filtration is used to calculate the TSS. Once the TSS is known, the sample dish containing the filter paper is placed in an oven at a temperature of 550 ± 50 °C for approximately 15 minutes. The sample is weighed and the difference in dry weight before and after ignition is used to calculate the VSS.

3.5.5 Sludge Volume Index (SVI)

The sludge volume index (SVI) is a measure of the settling characteristics of activated sludge and other biological suspensions. Although SVI is not supported theoretically, it has been shown to provide useful information in routine process control. Physically, this parameter represents the volume in millilitres occupied by 1 g of suspension after 30 minutes of settling. In general, the threshold value separating adequate sludge settling characteristics from poor settling characteristics is considered to be approximately 250 mL/g. The procedure to determine SVI involves filling a 1L graduated cylinder with a well mixed sludge suspension (or mixed liquor) and measuring the level (i.e. volume) of solids after 30 minutes. Prior to this, the determination of suspended solids of the suspension must be carried out. Thus, the SVI can be calculated using the following expression :

$$SVI_{30} = \frac{\left(\frac{V_{30}}{V_{cyl}}\right) x\, 1000\ mg/g}{TSS} \qquad \text{Eq. 3.1}$$

where : SVI_{30} = SVI after 30 minutes (in ml/g)

V_{30} = settled sludge volume after 30 minutes (in mL)

V_{cyl} = volume of the cylinder (1000 mL)

TSS = TSS of the suspension (in mg/mL)

3.5.6 Oxygen and Specific Oxygen Uptake Rate (OUR and SOUR)

Dissolved oxygen is essential for biological metabolism and growth. Therefore, periodic measurements of dissolved oxygen (DO) are crucial in monitoring microbial activity. In the present study, dissolved oxygen was measured using a model 810 Orion probe and DO/°C meter. The meter is first calibrated in water-saturated air before insertion of the probe into the reactor. The specific oxygen uptake rate (SOUR) represents the amount of O_2 consumed by the mass of microorganisms per unit time. It is usually expressed in mg O_2/(min*g biomass) [Tchobanoglous and Schroeder, 1987]. In order to calculate the SOUR, several DO measurements are taken in a short time interval. First, the oxygen supplied to the reactor is shut off and a first measurement is taken once the reading is stable. Subsequent measurements are taken every 30 seconds to a total of 4 minutes (note that the response time of the DO probe is less than several seconds). The DO concentration is then plotted versus time in order to determine the slope of the curve (see sample curve in Appendix B). In all cases, the decreases are found to be linear. The SOUR is calculated by dividing the slope (mg O_2/min*L) by the value of TSS (g/L) in the reactor. It is often simpler to calculate the Oxygen Uptake Rate (OUR) by dividing the decrease in oxygen concentration by the time period over which the change was measured. Hence, the OUR is expressed in mg/(L*min) or mg/(L*s).

3.5.7 pH and Temperature

Periodic pH measurements were taken to verify proper biological activity as most activated sludge microorganisms are pH-sensitive and thrive at pH's between 7 and 8. An Accumet AR50 pH/Ion/Conductivity meter was employed for this purpose and was calibrated using 5 points at pH = 2, 4, 7, 10 and 12. Temperature was measured using the Orion 810 DO/°C meter at the same time as dissolved oxygen.

3.5.8 Biolog Microplate Technique

As previously mentioned, the Biolog technique was employed to characterize the microbial population of the activated sludge and mixed liquor. This analytical method involves 3 major steps : microplate preparation, image analysis and statistical analysis [adapted from Peters, 1998 and Vadodaria, 1999].

1) Microplate preparation

Depending on the type of microorganism to be tested, several types of Biolog microplates are available. However, since most activated sludge microorganisms are Gram-negative species (i.e. do not retain the dye when stained due to their smaller cell wall) [Gray, 1990], GN microplates were employed for this analysis. Each microplate consists of 96 individual wells out of which 95 contain a carbon source and 1 serves as a control well (contains no carbon source). The 96 wells in the microplate are presented in Appendix C.

In addition to the individual substrates, each well contains a dye as well as nutrients in a dried-film form. Upon inoculation with the cell suspension, these chemical are reconstituted. As an indicator of substrate utilization, the tetrazolium dye is irreversibly reduced producing a bright purple colouring. The Biolog microplates are stored at 2-8 °C and are brought to room temperature approximately 2 hours prior to use. The technique employed for microplate inoculation is described by Victorio *et al.* (1996).

First, fresh samples of activated sludge or mixed liquor are collected (10 mL for each microplate to be inoculated). To these samples, deflocculating agents (Tween 80 and sodium pyrophosphate) are added in order to obtain a concentration of 0.01 % v/v of each (0.1 mL of 1 % solutions each). The samples are homogenized by vigorous shaking (Fisher Vortex) and then centrifuged for 5 min at 5000 RPM. The supernatant is then collected and disposed off while the recovered solids are washed with a 0.1 M phosphate buffer and centrifuged at 10000 RPM for 10 min. This process is repeated twice more (total of 3 washings with the phosphate buffer) and biological solids are re-suspended in 2 mL of a 0.85% saline solution.

In order to obtain the desired cell density for the inoculum, a turbidimeter is calibrated using turbidity standards and a blank saline solution set to 100 % transmittance. The turbidity standards establish the upper (58%) and lower (53%) transmittance limits for the desired cell density of 3×10^8 cells/mL. Thus,

the cell suspension is slowly added to the vial containing the saline solution until the desired turbidity range is achieved. All micro-wells are then inoculated with precisely 150μL of the suspension. The microplates are incubated in a closed box for 48 hours at room temperature to allow for carbon source utilisation. Once the incubation period is complete, the microplates are ready for image analysis.

2) Image analysis

In order to quantify carbon source utilisation, a picture of the microplate is taken using a Sony Hi-Resolution CCD-IRIS monochrome digital camera. The microplate is placed in a closed box with holes to accommodate a fiber optic light source and the camera lens in order to provide consistent lighting. The camera is mounted on a stand and maintained at a constant distance from the microplate. Once the picture is taken using Visilog 5.1 software, the colour intensity was determined using a macro that calculates the average pixel value of a 5X5 matrix of pixels taken from the center of each well. These values are taken to compute the raw difference data (obtained by subtracting the colour intensity of the control well from each of the 96 wells). Each value is then divided by the average colour intensity in order to obtain the normalised data. The resulting data is analysed using principal component analysis (PCA).

3) Statistical Analysis

For the statistical analysis, SIMCA-P 3.01 software was employed. Principal Component Analysis (PCA) is a multivariate statistical tool that basically

rotates a swarm of data about their centroid in order to reveal intrinsic patterns. The method consists of rewriting a matrix as a sum of linearly independent matrices. These matrices are then expressed as a product of two vectors : a score (column) vector and a loading (row) vector. Once these vectors are determined, they are plotted in order to reveal differences between microplates. The score vectors are used to describe the greatest variation among the data. They reveal trends, groupings and outliers as well as highlight the difference between microplates. On the other hand, the loading vectors describe which of the variables (carbon sources) are important in the data matrix. Thus, it is simpler to list the important carbon sources based on well colour intensity than to plot the loading vectors. The score vectors are plotted in decreasing order of variability. Hence, the first principal component axis accounts for the largest portion of variability among the data. The second axis of the score vector accounts for the second largest portion of variance and so on. Therefore, after the first 2 principal components, the remaining components account for very little of the data variability and are usually discarded from further analysis.

A 1 x 96 matrix represents each microplate and at least four matrices (i.e. four rows) are required by SIMCA-P software for statistical analysis. Once the normalised data was entered, the numerical values of the first two PC's of the loading vector were calculated. The PC1 of the loading vector was used to identify the chemical compounds that cause the differences between microplates. In addition, the average variability and standard deviation of the variability of the

replicate microplates were calculated. From this, a threshold value of the average variability plus 3 standard deviations (represents approximately 99.9% of the data) was established to identify the compounds whose consumption significantly changed between populations. Along with this information, normalised colour intensity values are used to explain the metabolic changes occurring between microbial populations. A cut-off value of 1.10 for the normalised colour intensity was selected to identify the most utilised carbon sources. The chemical species having colour intensities above this limit represent roughly a third of the total number of substrates.

In addition to the numerical outputs, a plot of the first two principal components (PC's) of the score vector provides information on the similarities or differences between microbial populations (or microplates). In this type of plot, each microplate is represented by a single point and the two axes (x and y) represent the first two PC's of the score vector. Appendix D shows the numerical outputs used for analysis.

CHAPTER 4

RESULTS AND DISCUSSION : FIELD DATA AND PRELIMINARY BATCH EXPERIMENTS

In general, within each Section, the results are divided into six parts. First, data with regards to the variations of TSS (and VSS) are presented. Thereafter, the results for the profiles and rates of COD and TOC removal are shown. This is followed by the results on the removal of ethylene glycol. It should be noted that all ethylene glycol present in the system is introduced as deicing fluid (refer to Section 2.1 of the Literature Review for the composition of the deicing fluid). The data on the oxygen uptake rates is then presented. Lastly, the results relating to sludge volume index (SVI) and the Biolog results are given.

4.1 Field Data Analysis

As a complement to laboratory experiments, field samples provided data on the actual wastewater treatment system. The municipal treatment station of St-Canut has been receiving large quantities of these deicing fluids on a regular basis since 1998. The purpose of these experiments was to examine the evolution of the treatment of deicing wastes as the deicing season progressed. Specifically, the removal of COD, TOC and Ethylene Glycol as well as the changes in microbial population were monitored. The field samples were collected in the period ranging from December 2000 to April 2001 and corresponds to approximately 120 days. During the deicing season (DIS), the

frequency of sampling was increased in order to closely follow the impact of these wastes on the activated sludge process. Table 4.1.1 indicates the various dates samples were collected. In general, three types of samples were collected: influent wastewater, activated sludge (from the recycle stream), and treated effluent. For the activated sludge, TSS and VSS were determined in addition to the Biolog microplate analysis. Influent and effluent samples were analysed for their COD, TOC and EG content.

Table 4.1.1 : Dates of field sample collection

	Dates
December 2000	8th, 13th, 19th and 26th
January 2001	3rd, 8th, 12th and 19th
February 2001	5th and 26th
April 2001	9th

a) TSS and VSS

The TSS of the settled sludge in the clarifier was measured since perturbations in the microbial population (such as the excessive growth of filamentous bacteria) negatively affect the settling characteristics of the sludge and thus, modify the concentration of the recycled biomass. Therefore, significant decreases in the TSS may indicate poor process performance since the sludge is not settling properly in the clarifier. Figure 4.1.1 shows that the TSS of the settled sludge decreases as the deicing season progresses and reaches

values around 3500 mg/L which are relatively low. Moreover Figure 4.1.2 also shows a decrease in the TSS levels of the aeration tank during the deicing period. Recycled sludge levels at the St-Canut plant are approximately 5000 to 6000 mg/L during the rest of the year and hence, the sustained treatment of deicing wastes seems to affect negatively the biomass levels of the recycled sludge and correspondingly produces lower concentrations TSS in the aerator.

Figure 4.1.1 : TSS and VSS of the recycled sludge during the deicing season

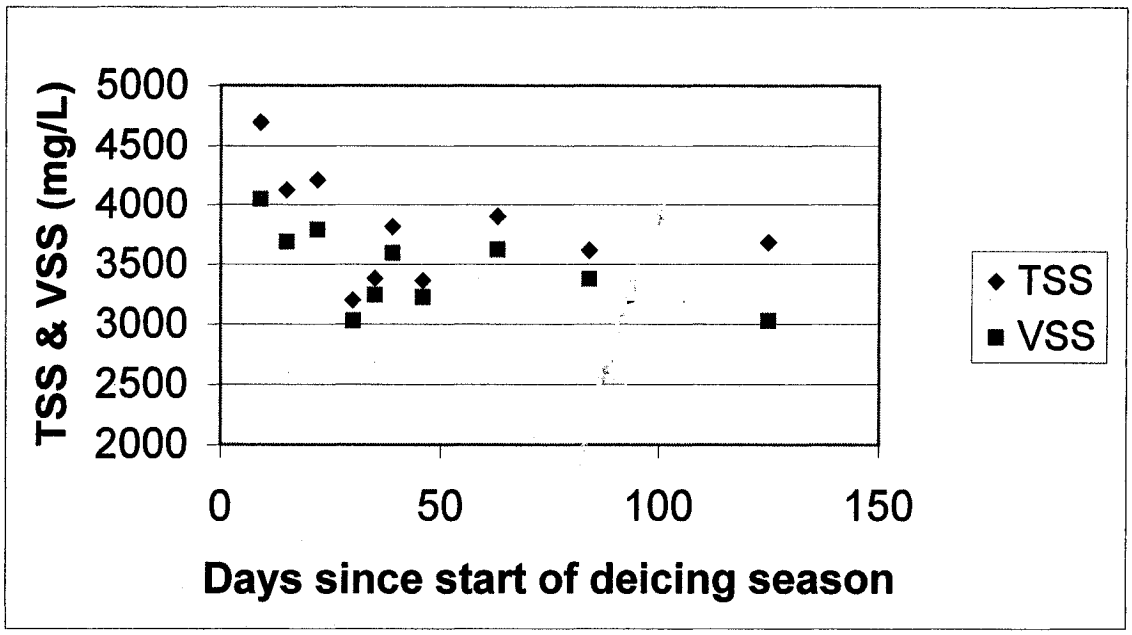
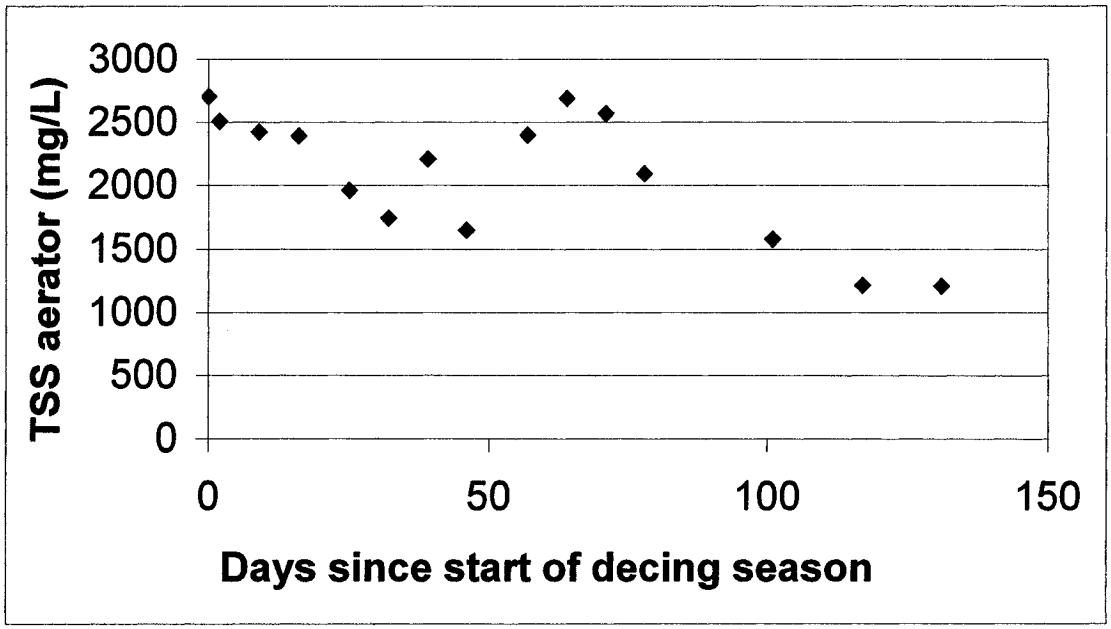


Figure 4.1.2 : TSS in the aerator during the deicing season



b) Variation of COD and TOC

Figures 4.1.3 and 4.1.4 show that until the onset of winter conditions the values of the COD and TOC parameters are rather low. However, as the deicing season progresses, there is a steady increase of the COD and TOC to approximately 250 mg/L and 85 mg/L, respectively. As shown in these Figures, this increase of organic loading does not affect in a significant way the effluent COD and TOC values. Over this period, influent COD varied up to as much as 170 mg/L (*i.e.* from the highest of 310 mg/L to the lowest of 140 mg/L) while the effluent values varied only up to 30 mg/L (*i.e.* from the highest of 50 mg/L to the lowest of 20 mg/L). It is important to note that, even though the percent variation is higher for the effluent compared to the influent, the absolute differences offer greater insight into the ability of the system to react to the increase in organic

loading during the deicing season. The same trend prevails with the TOC where the influent variations of up to 60 mg/L cause little change in the effluent. Therefore, with regards to overall organic matter removal, the field results suggest that the microorganisms in the activated sludge process can tolerate the additional organic loading by the deicing wastes.

Figure 4.1.3 : Influent and effluent COD during the deicing season

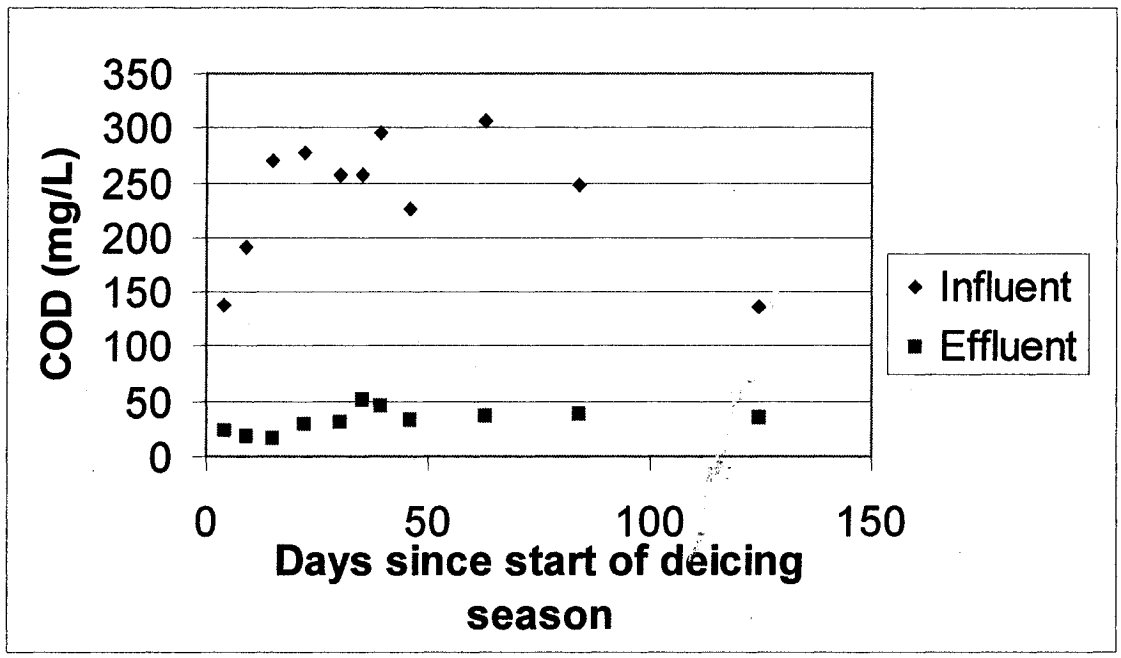
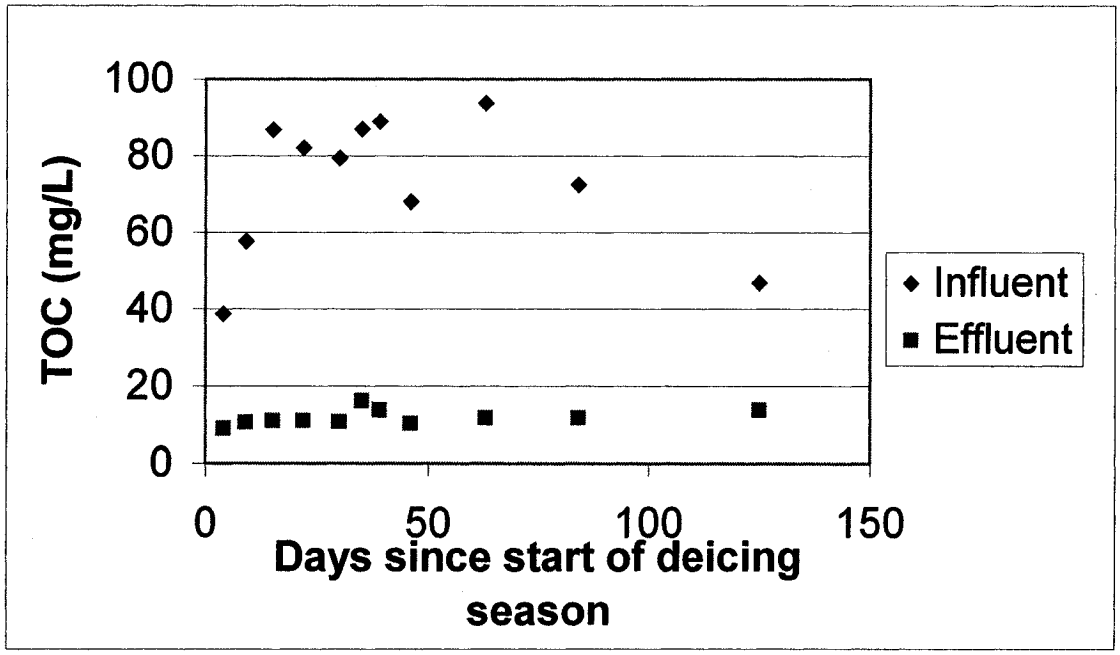


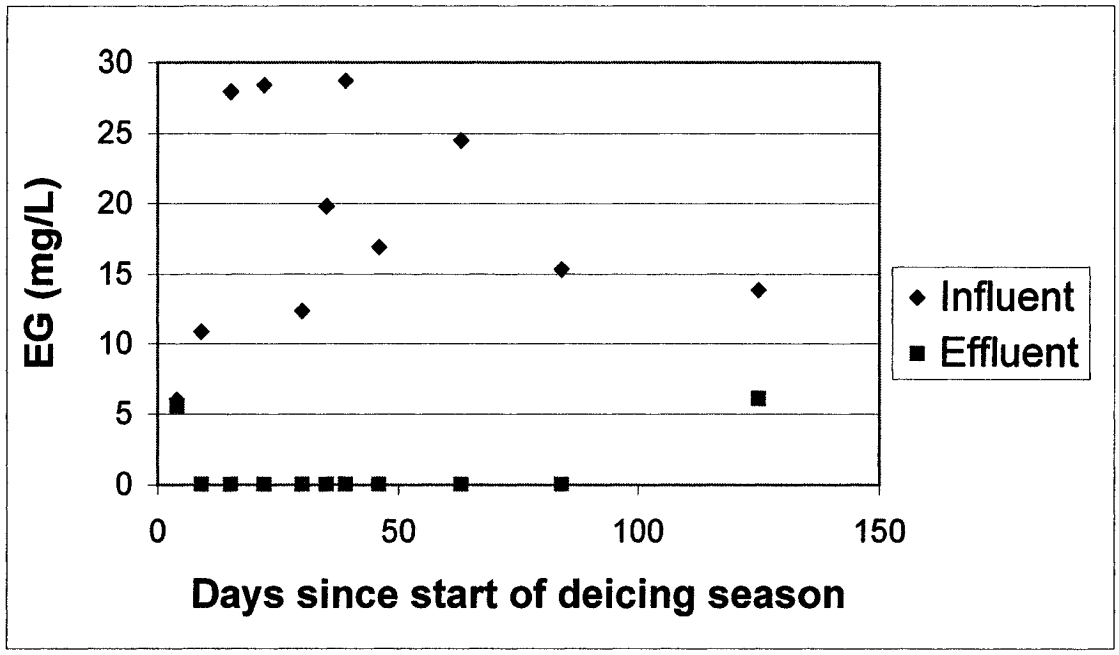
Figure 4.1.4 : Influent and effluent TOC during the deicing season



c) EG

As for ethylene glycol, Figure 4.1.5 presents the concentration in both the influent and effluent. Once again, similar patterns are observed for the degradation of ethylene glycol. In most cases, influent concentrations of approximately 10 to 30 mg/L are reduced to trace levels (i.e. less than 5 mg/L) in the effluent. The only two instances where this is not observed are at the very beginning and the end of deicing season. However, microbial acclimatization is a key process in establishing the efficiency of removal of a particular substrate. Thus, these two effluent values higher than 5 mg/L can be explained by the lower affinity of the biomass for ethylene glycol as smaller concentrations were fed to the reactor at that time.

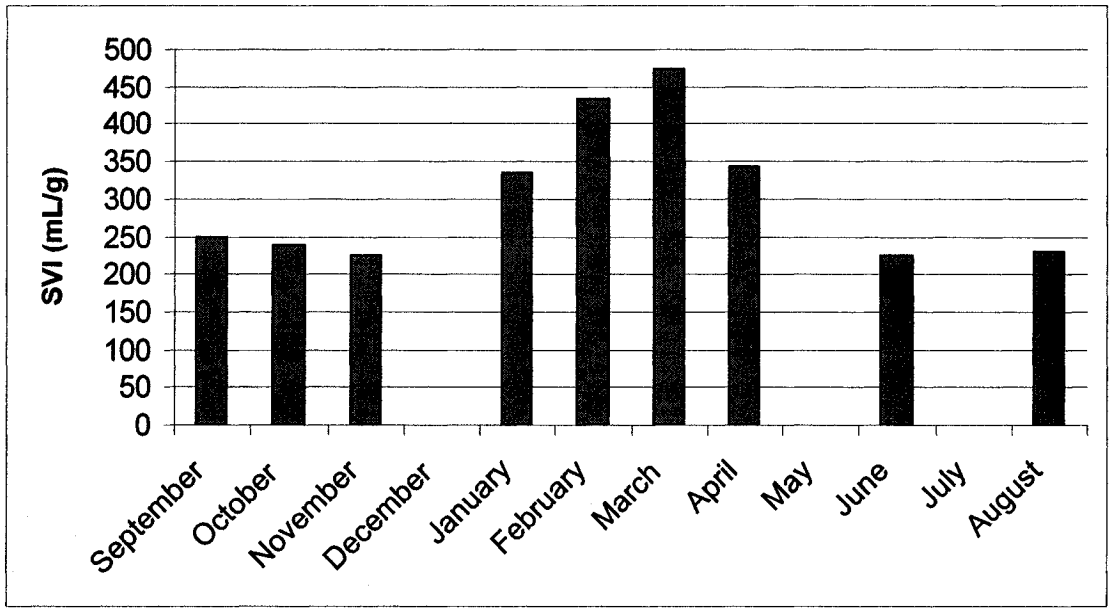
Figure 4.1.5 : Influent and effluent EG during the deicing season



d) Sludge Volume Index (SVI)

In general, sludge is considered to have poor settling characteristics if it has an SVI above 250 mL/g (Standard Methods, 1989). Previous literature has shown that the presence of deicing wastes decreases the settleability of activated sludge by promoting the growth of filamentous organisms. The results (Figure 4.1.6) from the St-Canut treatment plant follow this trend as they clearly indicate that deicing wastes negatively affect the settling characteristics of the biomass.

Figure 4.1.6 : SVI values during the year



BIOLOG RESULTS

The changes in carbon-source utilisation for the field samples are presented using 9 sets of microplates that were analyzed in order to follow the microbial population from the beginning of the deicing season (sample 1) till the end (sample 9). Figure 1 (in Appendix D) shows a plot of the first two principal components (PC's) of the score vector. As can be seen from the figure, there is a clear evolution of the microbial population as the deicing season progresses. In fact, the population at the beginning of the deicing season (sample 1) is the furthest apart from those at the end of the deicing period (samples 8 and 9). These changes can be caused by the presence of the deicing fluid as well as the decrease in the temperature during the winter months. The effect of temperature on the changes in the microbial population will be further covered by the SBR experiments in Chapter 6.

The substrates causing the greatest variability among microplates are the carbohydrates (see Tables 1 to 5 in Appendix D). In general, the utilisation of this group of compounds decreases as the deicing season progresses. More specifically, the consumption of carbohydrates such as maltose, galactose, lactose, etc. decreases during the deicing season.

Furthermore, most of the changes in carbon source utilisation occur in the first few samples. These samples approximately correspond to the period during which biomass acclimatisation took place (i.e. during the first month). The number of highly metabolized compounds (i.e. compounds whose normalized consumption is above 1.10) stays relatively the same from the beginning to the end of the deicing season. This parameter is important as it indicates the ability of a specific microbial population to highly metabolize a variety of compounds. In this case, the majority of highly metabolised compounds consist of carboxylic acids, amino acids and carbohydrates (refer to Appendix C and Tables 1 to 9 in Appendix D for the specific names of the compounds).

4.2 Preliminary Batch Experiments

The purpose of the preliminary experiments was to evaluate different wastewater compositions and select experimental conditions for further batch experiments. The experiments were carried out using the batch reactor set-up as described in Section 3.2 of the Materials and Methods Chapter. Three

different wastewater samples were tested (refer to Section 3.4 for the composition of the synthetic wastewater) and parameters such as TSS, DO, COD and TOC were monitored through time. The three types of wastewater were : a) Municipal wastewater from St-Canut + No deicing fluid, b) Synthetic wastewater + No deicing fluid, c) Synthetic wastewater + Deicing fluid. The results are presented in Figures 4.2.1 to 4.2.6 (for each Figure, the type of wastewater tested is indicated between brackets).

4.2.1 Effect of Wastewater Composition

Initially, an experiment was performed using wastewater from the St-Canut treatment plant. This wastewater was combined with an appropriate amount of activated sludge (also obtained from St-Canut, PQ) in order to obtain a biomass concentration of about 2000 mg/L in the mixed liquor. This level of biomass was selected as it falls into the range employed for normal process operation [Tchobanoglous and Schroeder, 1987]. For this experiment, no deicing fluid (DIF) was used. These results are presented in Figures 4.2.1 and 4.2.2. Biomass levels seem to change very little from the beginning of the experiment to the end indicating that the amount of substrate is adequate to maintain the micro-organisms but not enough to promote growth of biomass. From the COD and TOC curves, it is observed that most of the organic content is degraded within the first 5 hours of operation with very little change after that time. Finally, the dissolved oxygen (data not shown) was maintained at levels above 2 mg/L indicating favourable environmental conditions [Reynolds and Richards, 1996].

Figure 4.2.1 : Variation of the TSS concentration (Municipal wastewater, no DIF)

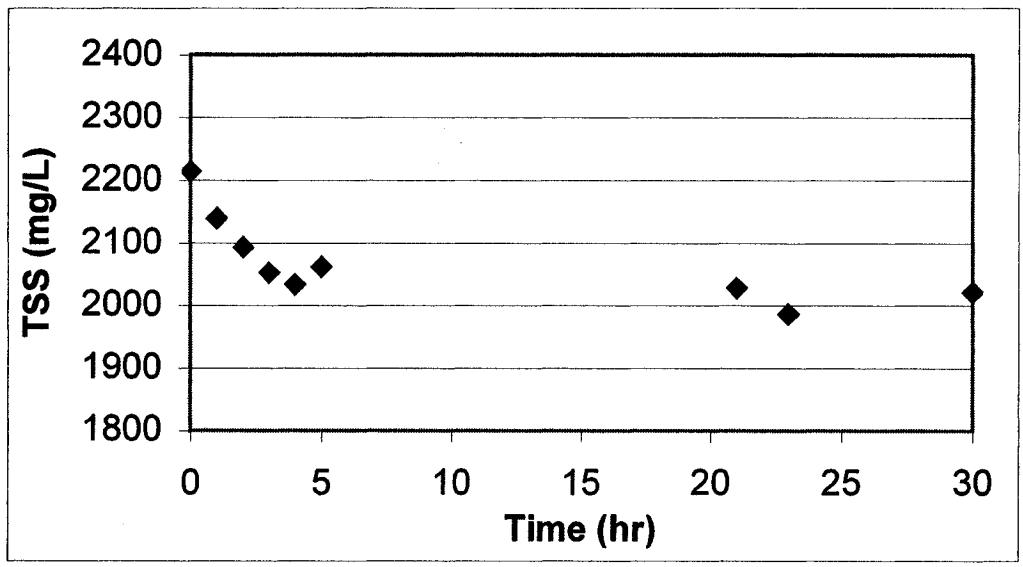
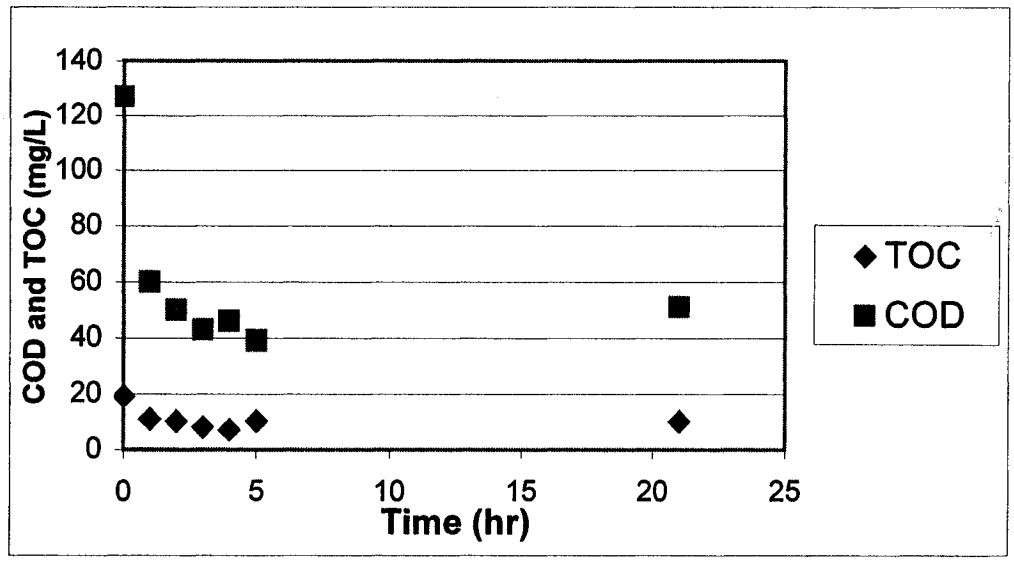


Figure 4.2.2 : Variation of COD and TOC (Municipal wastewater, no DIF)



Figures 4.2.3 and 4.2.4 show the results when the synthetic wastewater is employed. Once again, TSS readings do not indicate growth and most biological removal activity occurs in the first few hours of reactor operation. In fact, there is

practically no change in COD and TOC concentration after 6 hours. The DO levels are also above the 2 mg/L threshold (data not shown).

Figure 4.2.3 : Variation of the TSS concentration (Synthetic wastewater, no DIF)

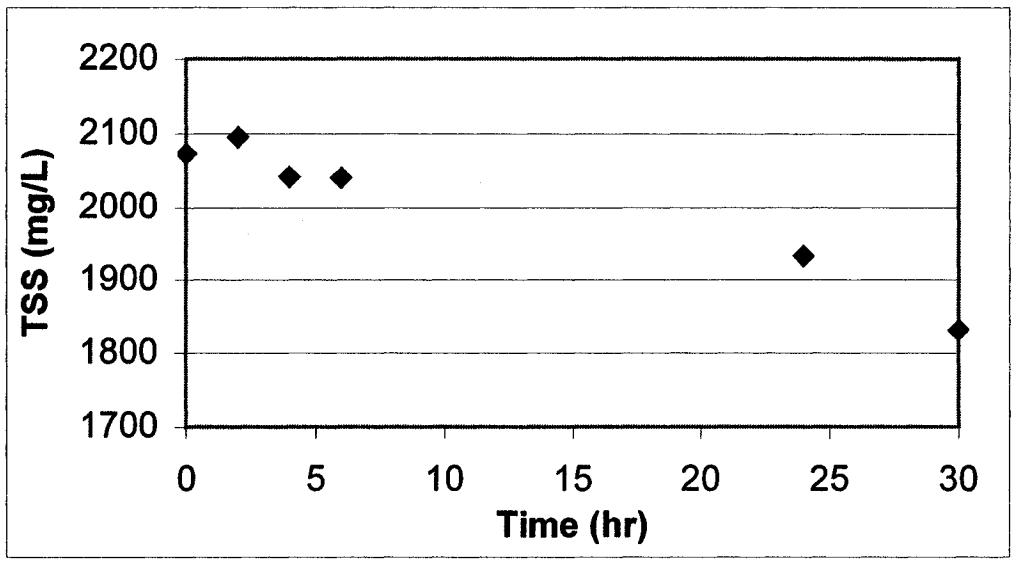
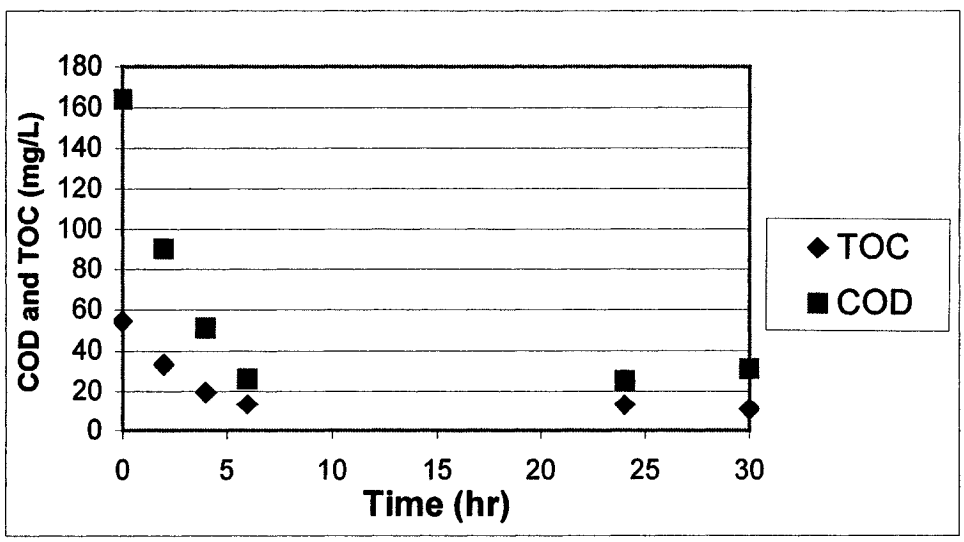


Figure 4.2.4 : Variation of COD and TOC (Synthetic wastewater, no DIF)



The final preliminary experiment was carried out using the synthetic base to which 40 mg/L of deicing fluid was added. Figures 4.2.5 and 4.2.6 show the results of this test. First, very little change in the TSS and DO levels (data not shown) is observed. Even though the initial COD and TOC are higher than the previous experiments, the degradation rates follow the same trends.

Figure 4.2.5 : Variation of the TSS concentration (Synthetic wastewater, DIF)

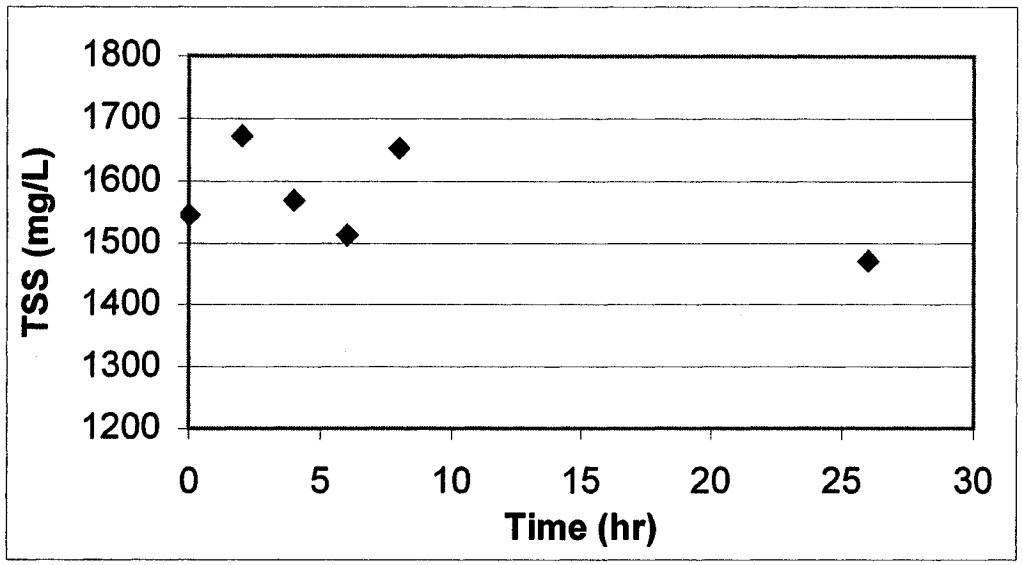
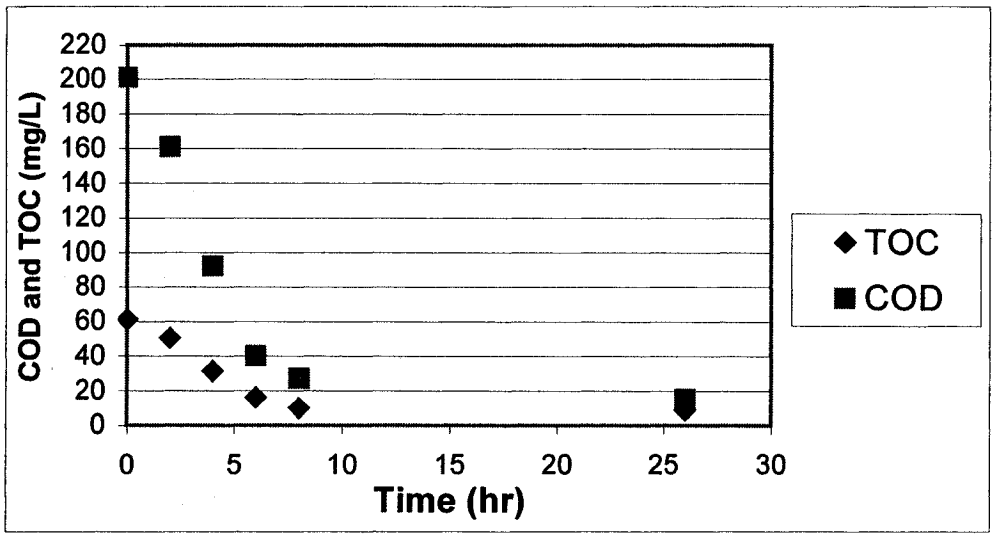


Figure 4.2.6 : Variation of COD and TOC (Synthetic wastewater, DIF)



4.2.2 Choice of Experimental Conditions

Due to the inherent variability of the municipal wastewater composition from day to day, it may be difficult to achieve reproducible conditions using this type of waste in the laboratory. Thus, it is preferable to employ a synthetic wastewater as its composition can be better defined. Based on the experimental data presented in this section, all three media provided easily degradable carbon sources for the microorganisms present in activated sludge. In general, most of the COD and TOC removal occurs within the first few hours of the experiment. As there is little change in biomass concentration, the organic matter present contributes solely to the maintenance of the microbial population.

The experimental results demonstrate efficient degradation of the synthetic wastewater (84 % decrease in COD and 76 % decrease in TOC within the first 6 hours). Furthermore, the addition of de-icing fluid to the synthetic base

does not hinder biodegradation as similar trends in COD and TOC removal are observed (80 % decrease in COD and 74 % decrease in TOC within the first 6 hours). Therefore, based on the data obtained from the preliminary experiments, wastewater streams containing a synthetic wastewater with or without de-icing fluid were chosen for all subsequent experiments.

As seen from the preliminary data, most of the organic matter (as reflected by the COD and TOC) is removed within the first 5 to 8 hours of the experiment. Therefore, experiments lasting longer than 20 hours are unnecessary in studying the biodegradation of the synthetic wastewater and deicing fluid. Biomass concentrations of approximately 1500 to 2200 mg/L provide a sufficient amount of microorganisms to degrade the organic matter in the wastewater within the first 8 hours. In addition, the TSS results show that there is adequate substrate for the maintenance of the microbial population but not for growth. With regards to dissolved oxygen, the results obtained with an air flow rate of 500 mL/min (at room temperature and standard pressure) and 300 RPM of mixing indicate adequate conditions for microbial metabolism. Based on the above data, a reaction time of 8 hours was chosen for further experiments. Furthermore, initial biomass and air flow rate levels of approximately 2000 mg/L and 500 mL/min, respectively, provide adequate conditions for the biodegradation of the artificial waste and deicing fluid.

CHAPTER 5

RESULTS AND DISCUSSION : BATCH EXPERIMENTS

5.1 Effect of Biomass and Deicing Fluid Concentration

The objective of the experiments described in this section was to investigate the effect of various biomass and deicing fluid concentrations on the removal rates of organic matter (COD, TOC and EG) and on the microbial population. This was achieved by conducting batch experiments at four different sludge concentrations and four different deicing fluid concentrations.

In general, the St-Canut wastewater treatment plant maintains TSS levels in the aeration tank between 1500 and 3000 mg/L throughout the year. Hence, biomass concentrations of 2000 and 3000 mg/L were selected as they represent the levels encountered in many municipal activated sludge treatment units. In addition batch experiments at a very low TSS concentration of 300 mg/L were conducted in order to verify whether or not organic matter utilization would lead to biomass growth and the kinetics of removal under a higher food to microorganism ratio. Since results between the 300 mg/L and 2000 mg/L TSS level were different, batch experiments at a fourth biomass level of 1000 mg/L were conducted in order to have a broad experimental region and verify the rates of removal at an intermediary biomass concentration.

Deicing fluid concentrations of 0, 35, 65 and 130 mg/L were employed. The case of zero concentration corresponds to when no deicing activities are conducted at the airport (i.e. non-winter operation). In order to acclimatize activated sludge micro-organisms to a new carbon source, low concentrations of this compound are first introduced and subsequently raised to normal treatment levels. These two situations were represented by the 35 and 65 mg/L concentrations respectively. On certain occasions, additional amounts of deicing fluid are treated during peak air traffic operations. This final case is represented by the 130 mg/L concentration of deicing fluid. In all four cases, the synthetic base described in Section 3.4 of the Materials and Methods is provided.

Therefore, as there are four different biomass concentrations coupled with four different deicing fluid concentrations, this leads to a total of 16 batch experiments. It should be mentioned that these 16 experiments represent the basis to which all other batch experiments (glycol-acclimatized, lower temperature, replicates) were compared. Moreover, these 16 runs were conducted with an unacclimatized population (with respect to the deicing fluid) and at room temperature.

5.1.1 Effect of Biomass Concentration

a) TSS and VSS

Tables 5.1.1 to 5.1.4 show the initial and final values for TSS and VSS for the 16 batch experiments. The values remain relatively stable for all the

experiments conducted at 1000 mg/L, 2000 mg/L and 3000 mg/L of TSS. For the experiments at low biomass concentrations, a more noticeable increase in the biomass level of 300 mg/L (compared to the other three TSS levels) was expected as there was a much greater amount of substrate per gram of biomass (hence less competition for substrate). Moreover, these experiments lasted longer (45 hours instead of 8 hours for all the other batch experiments) and allowed for a longer window for microbial growth. However, the total and volatile suspended solids (TSS and VSS) analysis shows that there is not a significant change in the amount of biomass during the experiment. Therefore, the comparison of biomass levels shows that there is very little microbial growth under the specific TSS concentrations of 300 mg/L, 1000 mg/L, 2000 mg/L and 3000 mg/L. It must be noted that, since VSS values closely follow the same trend as the TSS values, only the TSS results will be shown in further sections.

Table 5.1.1 : TSS and VSS values for initial sludge concentration of 300 mg/L

Deicing conc. (mg/L)	Initial TSS (mg/L)	Final TSS (mg/L)	Initial VSS (mg/L)	Final VSS (mg/L)
0	280	270	250	240
35	270	250	200	230
65	270	240	190	190
130	300	390	290	300

Table 5.1.2 : TSS and VSS values for initial sludge concentration of 1000 mg/L

Deicing conc. (mg/L)	Initial TSS (mg/L)	Final TSS (mg/L)	Initial VSS (mg/L)	Final VSS (mg/L)
0	990	1030	870	920
35	960	1050	820	890
65	980	1040	880	900
130	990	1080	830	980

Table 5.1.3 : TSS and VSS values for initial sludge concentration of 2000 mg/L

Deicing conc. (mg/L)	Initial TSS (mg/L)	Final TSS (mg/L)	Initial VSS (mg/L)	Final VSS (mg/L)
0	2140	1930	1700	1540
35	2150	1970	1720	1580
65	1950	2100	1580	1710
130	1960	1960	1580	1470

Table 5.1.4 : TSS and VSS values for initial sludge concentration of 3000 mg/L

Deicing conc. (mg/L)	Initial TSS (mg/L)	Final TSS (mg/L)	Initial VSS (mg/L)	Final VSS (mg/L)
0	2940	2930	2340	2340
35	2920	2910	2300	2330
65	2570	2740	1970	2130
130	3100	3080	2380	2410

b) COD and TOC

For the 16 different batch experiments, the COD and TOC concentration profiles are presented in Figures 5.1.1 to 5.1.16. The specific removal rates of COD and TOC are shown in Figures 5.1.17 to 5.1.32. For the case of 0 mg/L deicing fluid, the concentrations profiles of COD and TOC (Figures 5.1.3 and

5.1.4) are found to be similar for biomass levels of 2000 mg/L and 3000 mg/L of TSS. Thus, in this case, the ratio of specific rates (i.e. rate per gram TSS) corresponds approximately to the ratio of total suspended solids. For example, the initial specific COD removal rate in the absence of deicing fluid is approximately 18 mg COD/hr-g TSS with a biomass concentration of 2000 mg/L (Figure 5.1.19). Using a TSS concentration of 3000 mg/L, the initial COD removal rate is around 12 mg COD/hr-g TSS for 0 mg/L deicing fluid (Figure 5.1.20). This trend in specific removal rate for 2000 mg/L and 3000 mg/L of TSS was also noted for all other deicing fluid concentrations. This suggests that COD and TOC removal rates (expressed as mg/L-hr) do not depend on the biomass concentration present in the reactor at these two concentrations. This is contrary to what is expected since a higher amount of microorganisms should be able to degrade a fixed amount of organic matter in a shorter time. However, a possible explanation can be that not all the biomass participates in the degradation reaction. Thus, at high concentrations of biomass, because of the limited supply of nutrients, the biomass appears to use the substrates less efficiently. Under such a low food-to-microorganism ratio, all of the substrate utilised is directed towards the maintenance of the biomass population.

With regards to the experiments performed at the lower biomass concentrations of 300 mg/L and 1000 mg/L of TSS (Figures 5.1.17 to 5.1.32), the specific removal rates of TOC and COD are significantly higher than at the two higher biomass concentrations. More specifically, for the intermediate deicing

fluid concentrations of 35 mg/L and 65 mg/L, there is very little difference in the specific rates of removal between 300 mg/L and 1000 mg/L. However, at the highest concentration of deicing fluid, there is a tapering off of the specific rate for 300 mg/L of TSS but an increase in the specific rate at 1000 mg/L of TSS. These observations suggest that there are two opposing effects which balance the specific removal rate. On one hand, it may be expected that a decrease in biomass concentration would always lead to an increase in specific rate since a fixed quantity of organic matter is being degraded by a smaller amount of microorganisms. This is true to a certain extent as there is a trend of increasing specific rate of removal (for all deicing fluid concentrations) as the biomass level is lowered. However, at a biomass level of 300 mg/L of TSS, there is an inhibition of the biomass activity caused by a very high food-to-microorganism ratio. Hence, this leads to a decrease in the specific rates of organic matter removal. These opposing effects may explain the attainment of similar removal rates for 300 mg/L and 1000 mg/L of TSS at intermediate deicing fluid concentrations and the higher removal rates for 1000 mg/L when compared to 300 mg/L of TSS at the highest deicing fluid concentration where inhibitory effects may reduce microbial activity.

Figure 5.1.1 : COD and TOC concentration (0 mg/L DIF, 300 mg/L TSS)

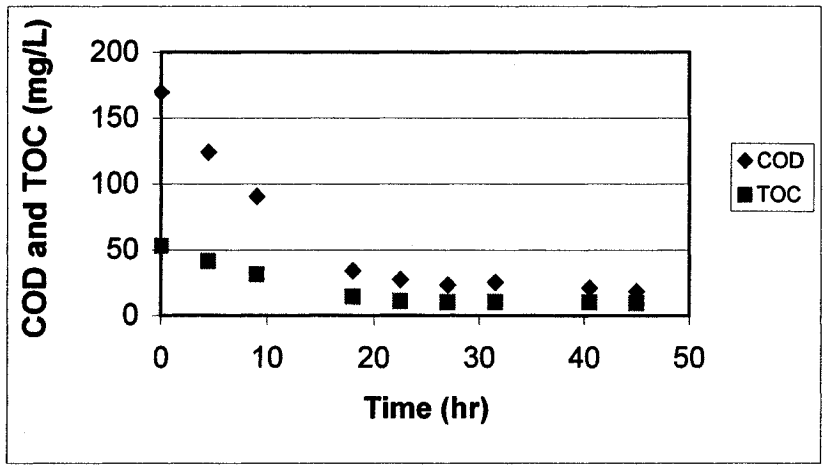


Figure 5.1.2 : COD and TOC concentration (0 mg/L DIF, 1000 mg/L TSS)

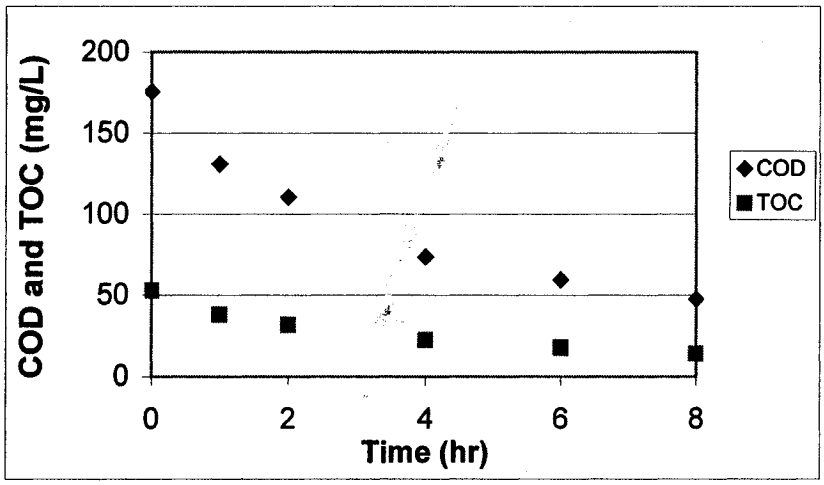


Figure 5.1.3 : COD and TOC concentration (0 mg/L DIF, 2000 mg/L TSS)

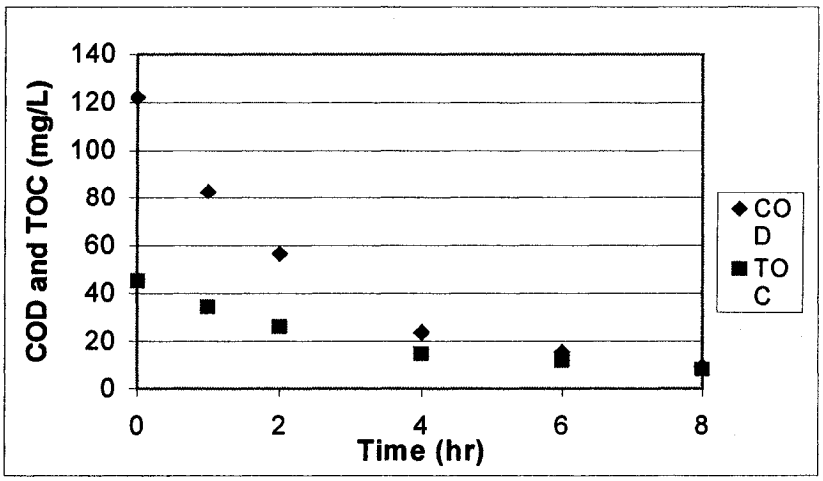


Figure 5.1.4 : COD and TOC concentration (0 mg/L DIF, 3000 mg/L TSS)

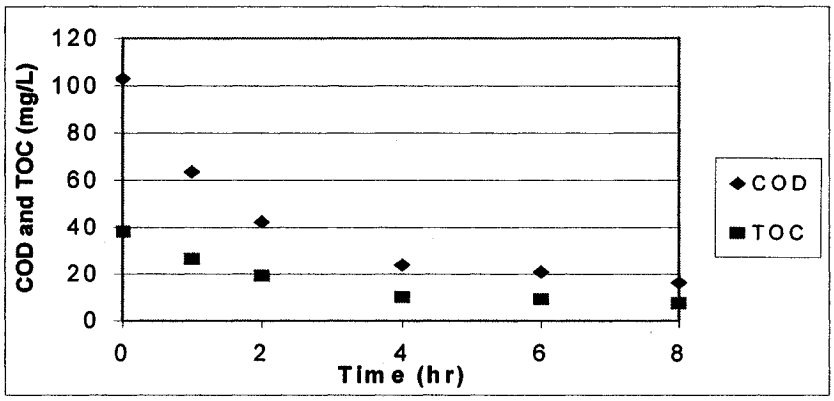


Figure 5.1.5 : COD and TOC concentration (35 mg/L DIF, 300 mg/L TSS)

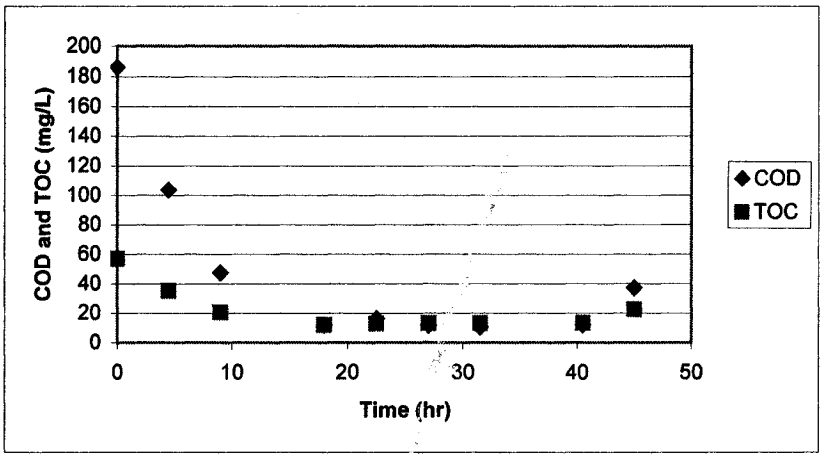


Figure 5.1.6 : COD and TOC concentration (35 mg/L DIF, 1000 mg/L TSS)

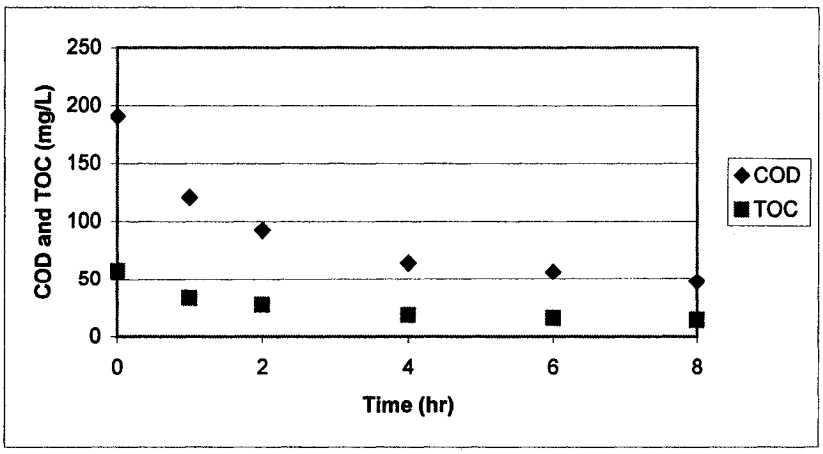


Figure 5.1.7 : COD and TOC concentration (35 mg/L DIF, 2000 mg/L TSS)

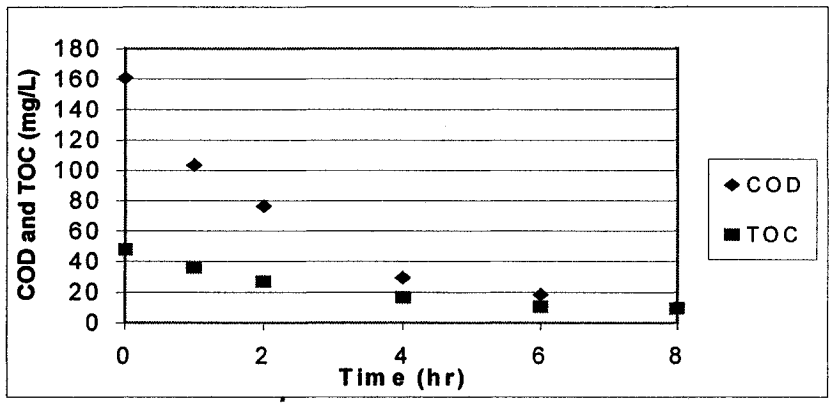


Figure 5.1.8 : COD and TOC concentration (35 mg/L DIF, 3000 mg/L TSS)

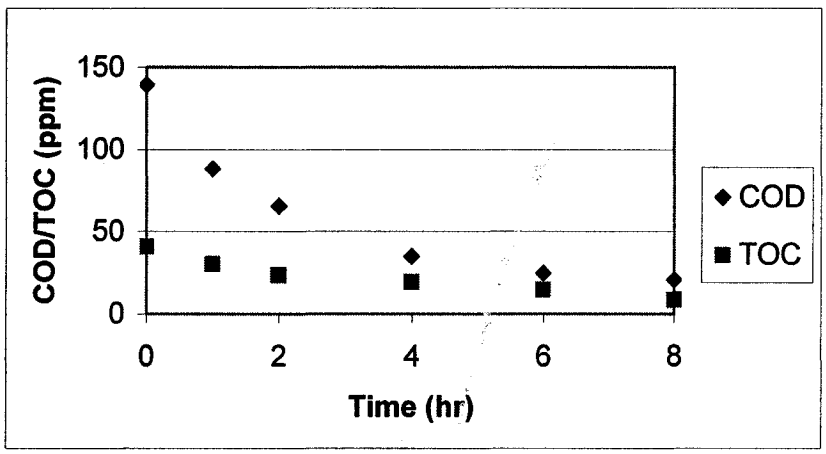


Figure 5.1.9 : COD and TOC concentration (65 mg/L DIF, 300 mg/L TSS)

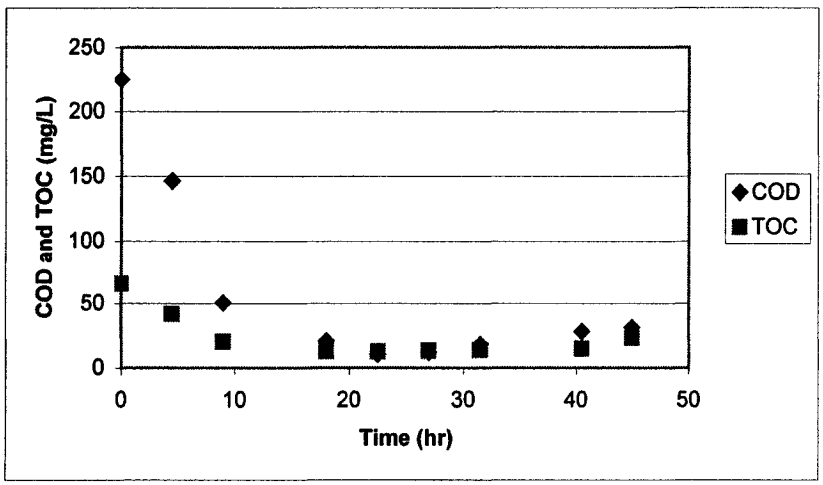


Figure 5.1.10 : COD and TOC concentration (65 mg/L DIF, 1000 mg/L TSS)

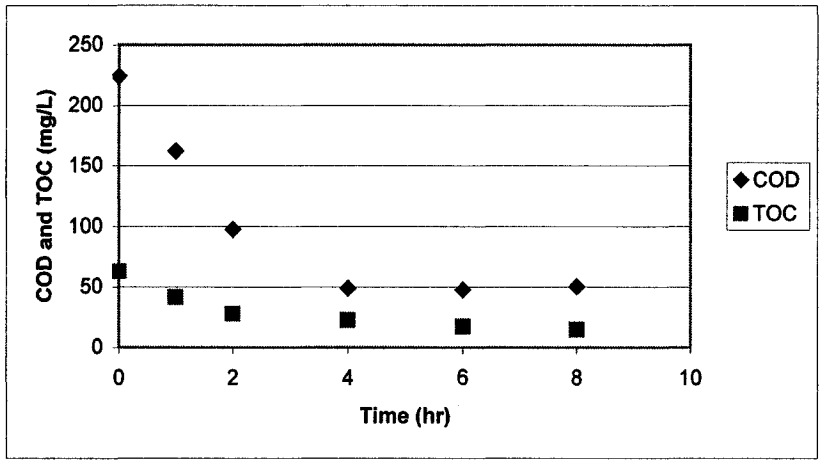


Figure 5.1.11 : COD and TOC concentration (65 mg/L DIF, 2000 mg/L TSS)

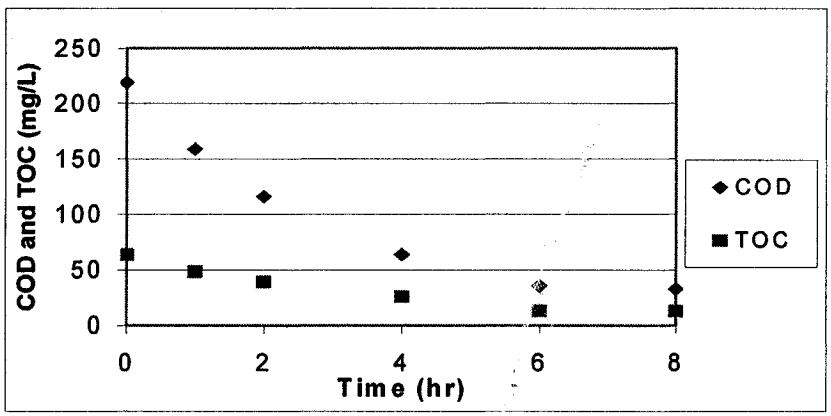


Figure 5.1.12 : COD and TOC concentration (65 mg/L DIF, 3000 mg/L TSS)

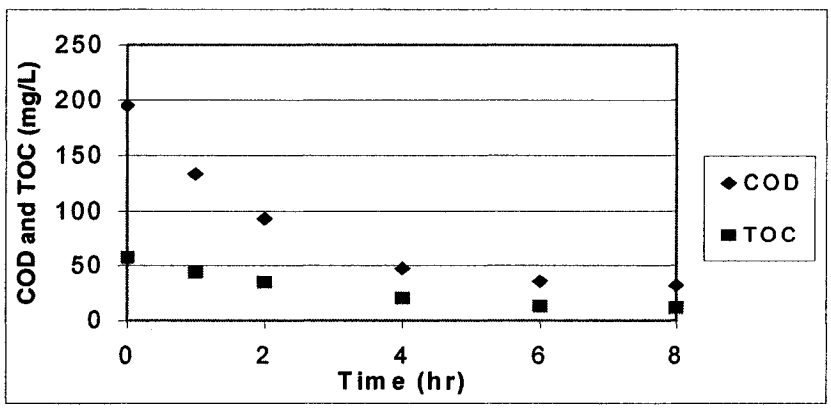


Figure 5.1.13 : COD and TOC concentration (130 mg/L DIF, 300 mg/L TSS)

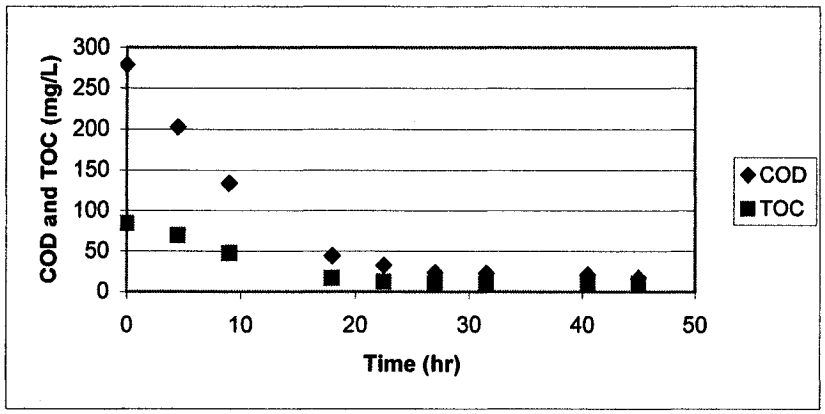


Figure 5.1.14 : COD and TOC concentration (130 mg/L DIF, 1000 mg/L TSS)

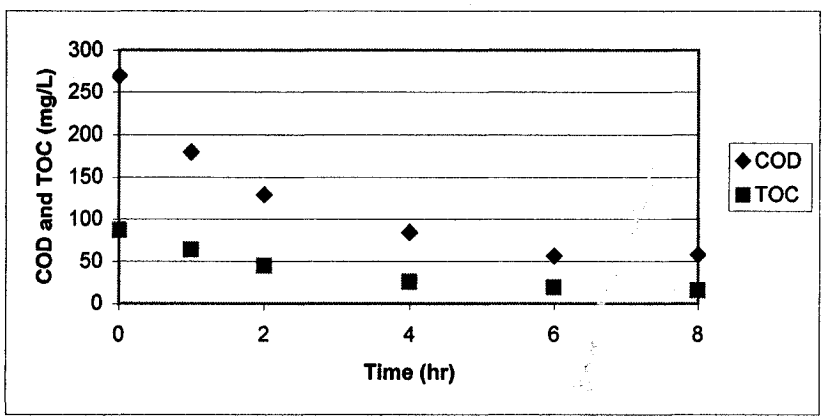


Figure 5.1.15 : COD and TOC concentration (130 mg/L DIF, 2000 mg/L TSS)

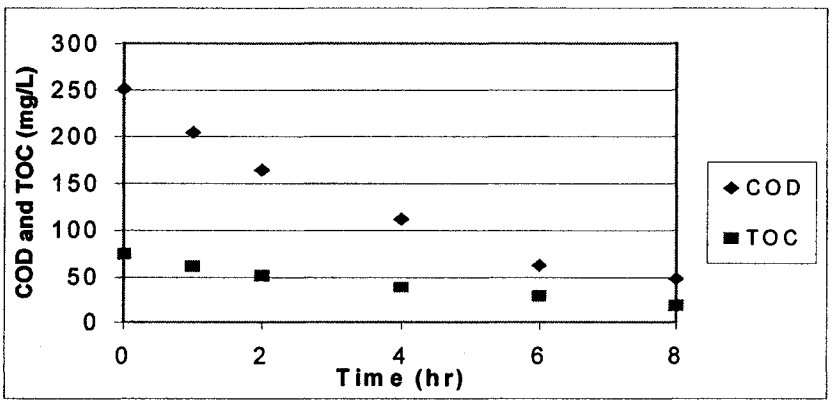


Figure 5.1.16 : COD and TOC concentration (130 mg/L DIF, 3000 mg/L TSS)

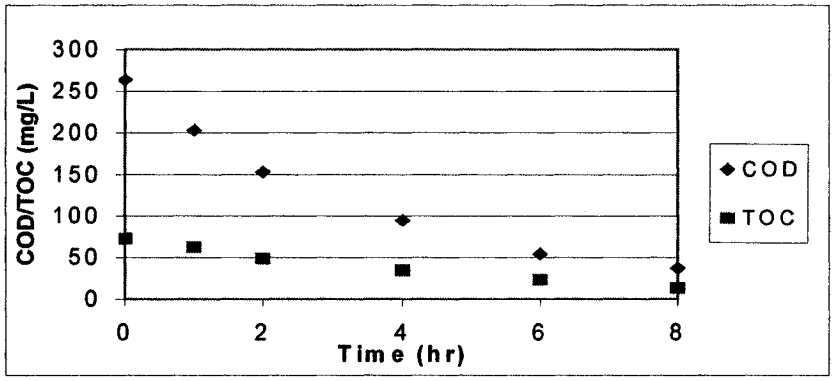


Figure 5.1.17 : COD and TOC specific rate (0 mg/L DIF, 300 mg/L TSS)

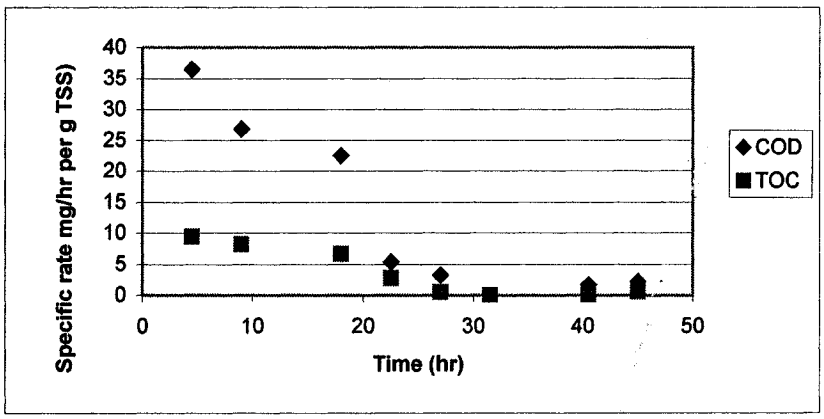


Figure 5.1.18 : COD and TOC specific rate (0 mg/L DIF, 1000 mg/L TSS)

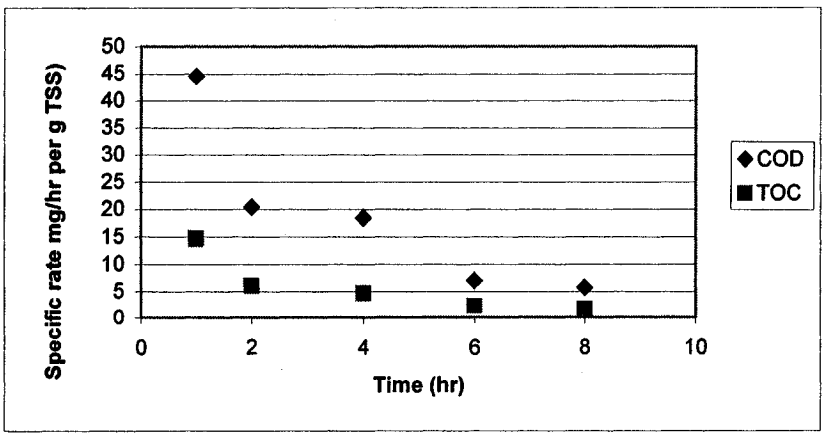


Figure 5.1.19 : COD and TOC specific rate (0 mg/L DIF, 2000 mg/L TSS)

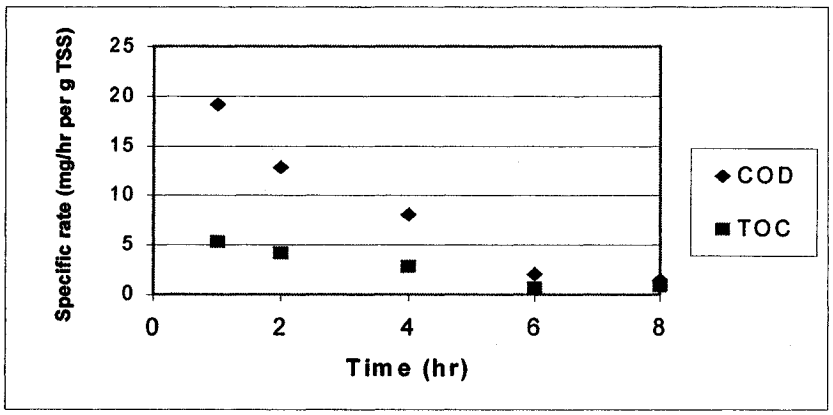


Figure 5.1.20 : COD and TOC specific rate (0 mg/L DIF, 3000 mg/L TSS)

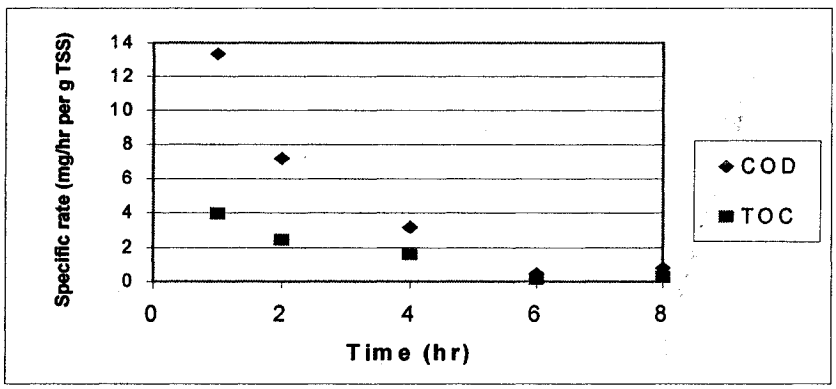


Figure 5.1.21 : COD and TOC specific rate (35 mg/L DIF, 300 mg/L TSS)

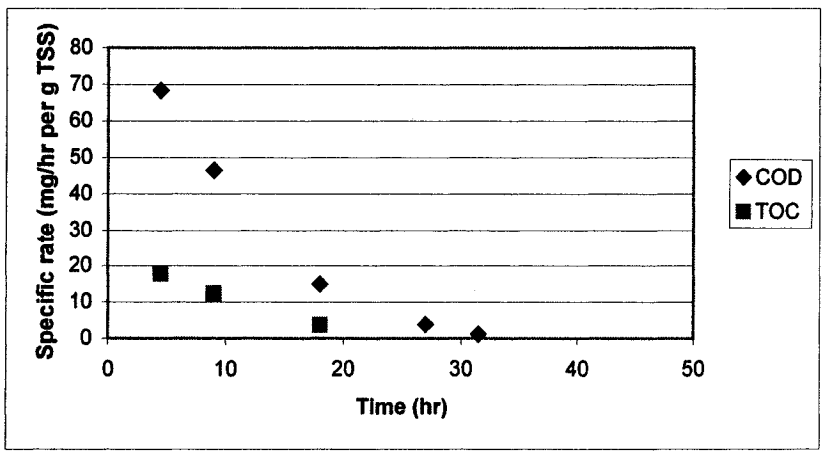


Figure 5.1.22 : COD and TOC specific rate (35 mg/L DIF, 1000 mg/L TSS)

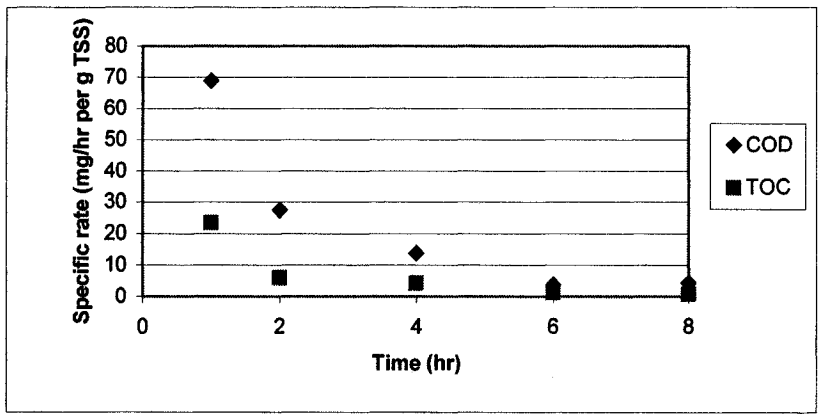


Figure 5.1.23 : COD and TOC specific rate (35 mg/L DIF, 2000 mg/L TSS)

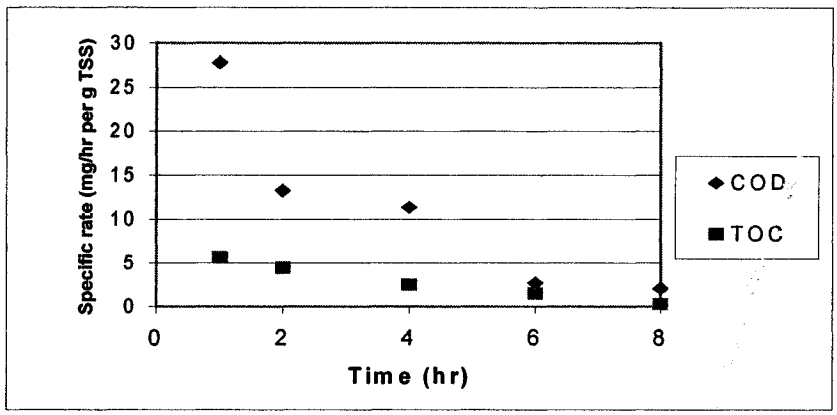


Figure 5.1.24 : COD and TOC specific rate (35 mg/L DIF, 3000 mg/L TSS)

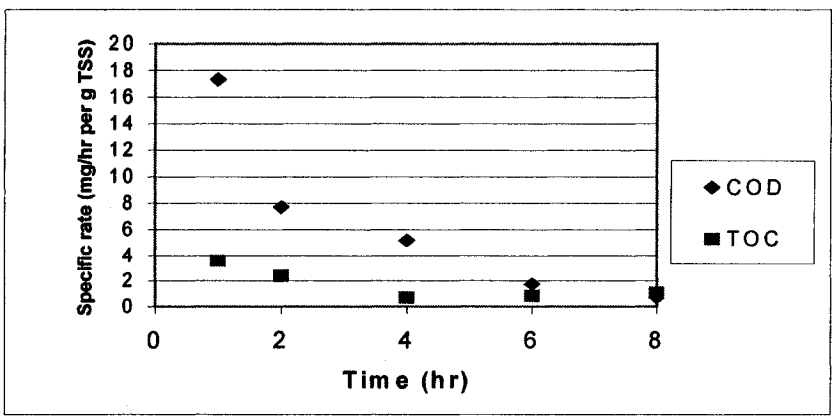


Figure 5.1.25 : COD and TOC specific rate (65 mg/L DIF, 300 mg/L TSS)

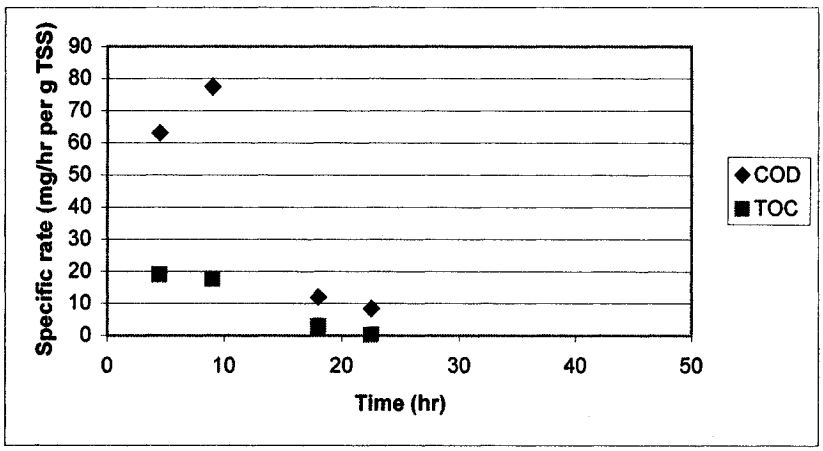


Figure 5.1.26 : COD and TOC specific rate (65 mg/L DIF, 1000 mg/L TSS)

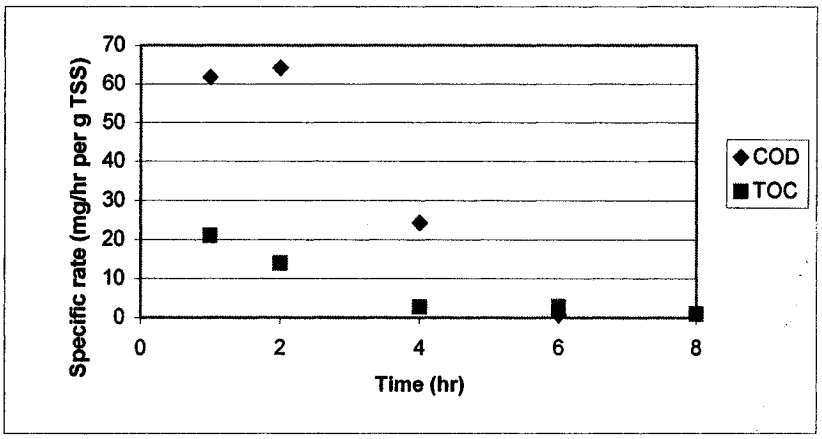


Figure 5.1.27 : COD and TOC specific rate (65 mg/L DIF, 2000 mg/L TSS)

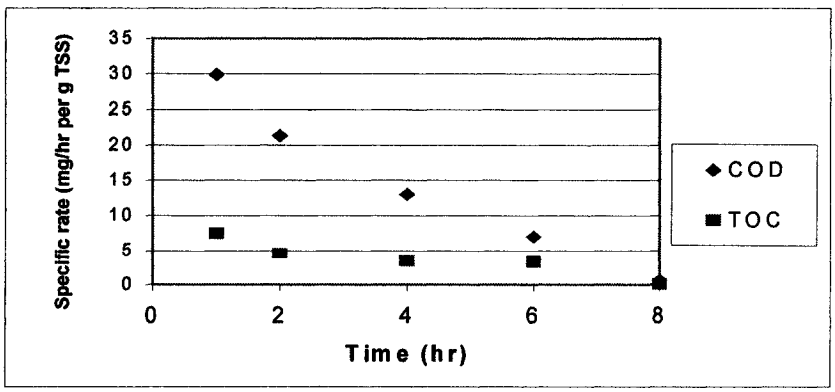


Figure 5.1.28 : COD and TOC specific rate (65 mg/L DIF, 3000 mg/L TSS)

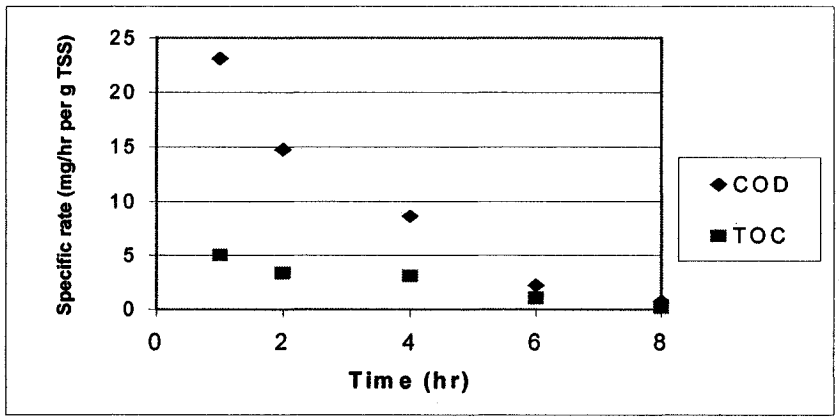


Figure 5.1.29 : COD and TOC specific rate (130 mg/L DIF, 300 mg/L TSS)

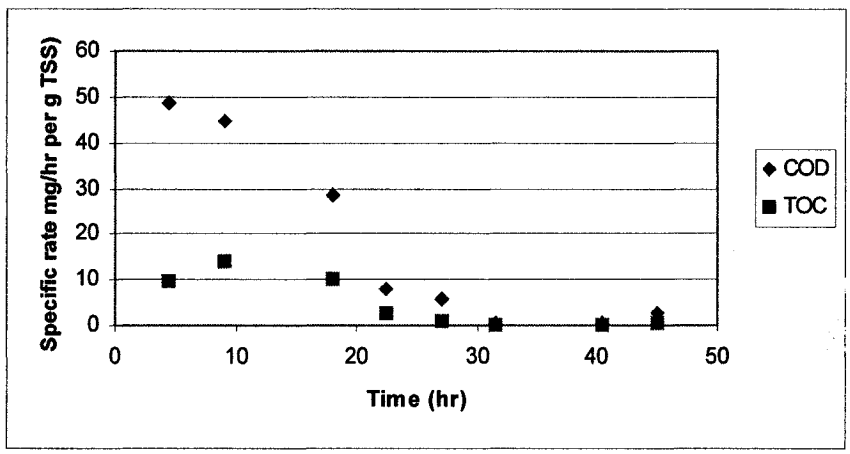


Figure 5.1.30 : COD and TOC specific rate (130 mg/L DIF, 1000 mg/L TSS)

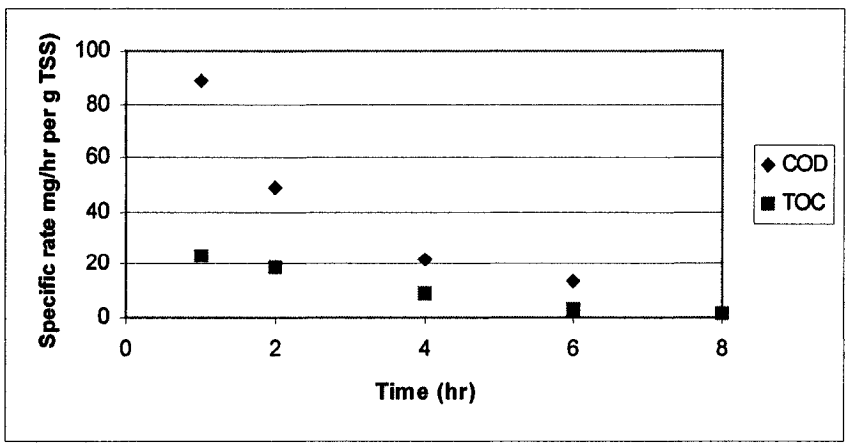


Figure 5.1.31 : COD and TOC specific rate (130 mg/L DIF, 2000 mg/L TSS)

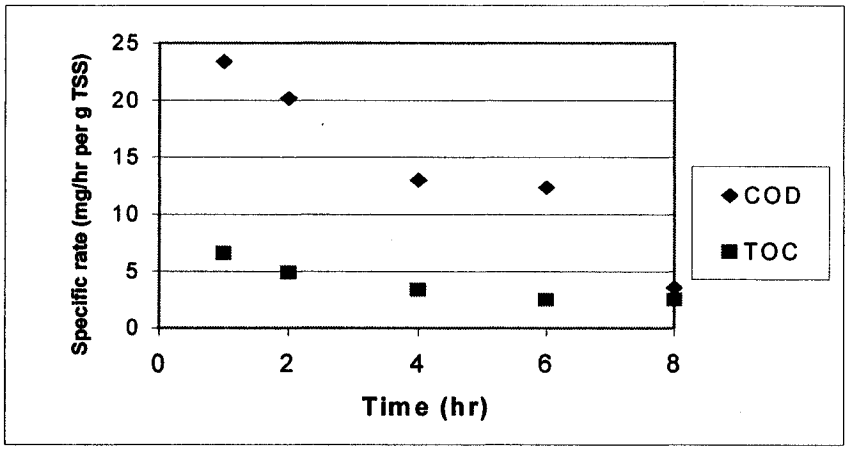
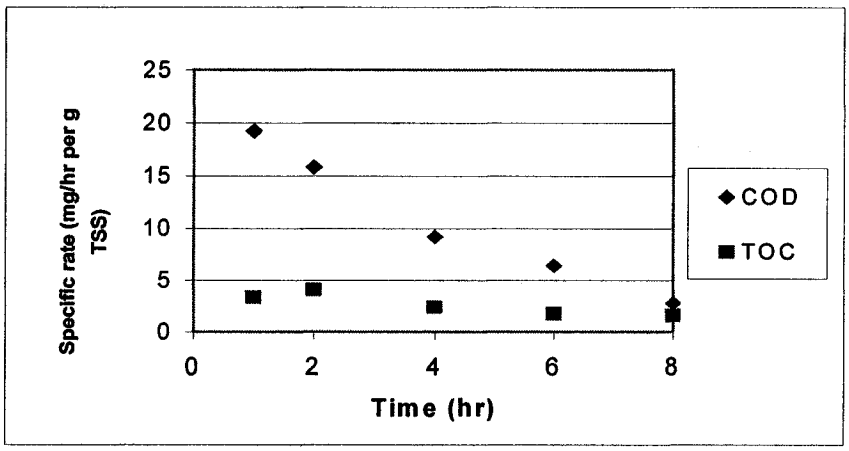


Figure 5.1.32 : COD and TOC specific rate (130 mg/L DIF, 3000 mg/L TSS)



c) EG

The decrease in the concentration of Ethylene Glycol (EG) is presented in Figures 5.1.33 to 5.1.35. At the lowest deicing concentration of 35 mg/L (Figure 5.1.33), ethylene glycol is removed within the first hour for the three biomass levels of 1000 mg/L, 2000 mg/L and 3000 mg/L of TSS. Furthermore, at the lowest TSS concentration of 300 mg/L, there is complete removal in less than 4.5 hours of operation. The amount of biomass has very little impact on the profile of

ethylene glycol removal at the lowest concentration of deicing fluid. This can be explained by the fact that under substrate limiting conditions, there is more than enough biomass to degrade the organic matter. For the intermediate deicing fluid concentration of 65 mg/L (Figure 5.1.34), there are differences between ethylene glycol degradation profiles as a function of biomass concentration. More specifically, ethylene glycol is reduced to trace amounts (i.e. less than the detectable limit of 5 mg/L) within the first four hours for 1000 mg/L, 2000 mg/L and 3000 mg/L of TSS. However, the lowest biomass level requires between 4.5 and 9 hours to completely remove the same concentration of ethylene glycol. Similar degradation profiles are observed at the highest concentration of deicing fluid wherein the lowest concentration of biomass does not completely degrade the ethylene glycol within the same timeframe as the three higher TSS levels. Hence, the effect of biomass concentration becomes more pronounced as the amount of substrate increases.

Figures 5.1.36 and 5.1.37 show the initial removal rate and the initial specific removal rate of EG as a function of initial DIF concentration. At the lowest DIF level, the initial removal rates (Figure 5.1.36) show very little variation between 1000 mg/L, 2000 mg/L and 3000 mg/L of TSS biomass concentrations. In addition, at the two higher deicing fluids, there is once again very little change in the initial removal rate between 2000 mg/L and 3000 mg/L of TSS but an increase in the initial rate for 1000 mg/L of TSS. As can be expected, in all cases, the initial rates are the lowest for the biomass concentration of 300 mg/L.

With regards to initial specific removal rate (Figure 5.1.37), the values are higher for the TSS level of 300 mg/L than those at 2000 mg/L and 3000 mg/L for all three deicing fluid concentrations. In fact, the highest initial specific rates are obtained at the intermediate biomass concentration of 1000 mg/L of TSS. This suggests, once again, the possibility of substrate inhibition at the lowest biomass level and substrate limiting conditions at the two highest biomass concentrations. Thus, at the biomass levels of 2000 mg/L and 3000 mg/L, all microorganisms may not be participating in the degradation reaction of ethylene glycol (as was similarly found with the degradation reactions of COD and TOC).

Figure 5.1.33 : Removal of Ethylene Glycol for initial deicing fluid of 35 mg/L

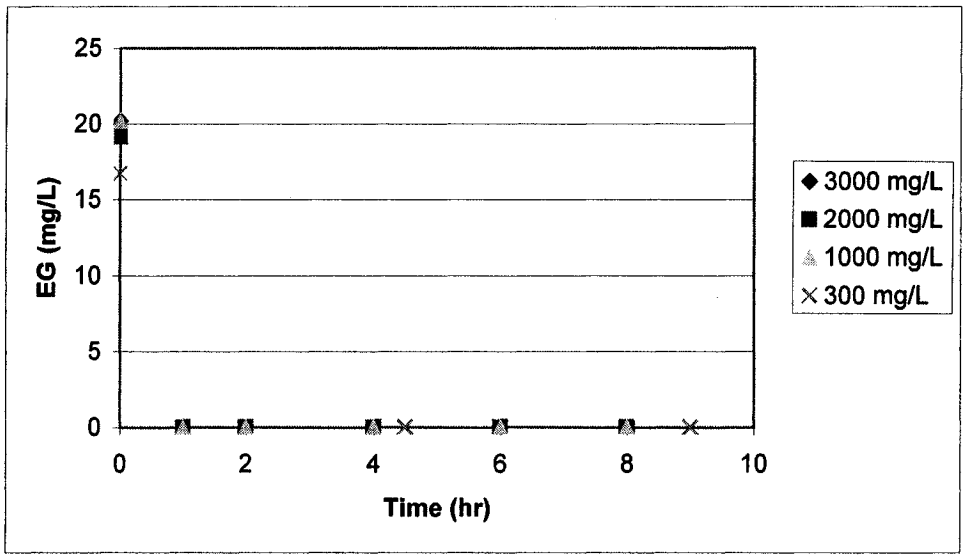


Figure 5.1.34 : Removal of Ethylene Glycol for initial deicing fluid of 65 mg/L

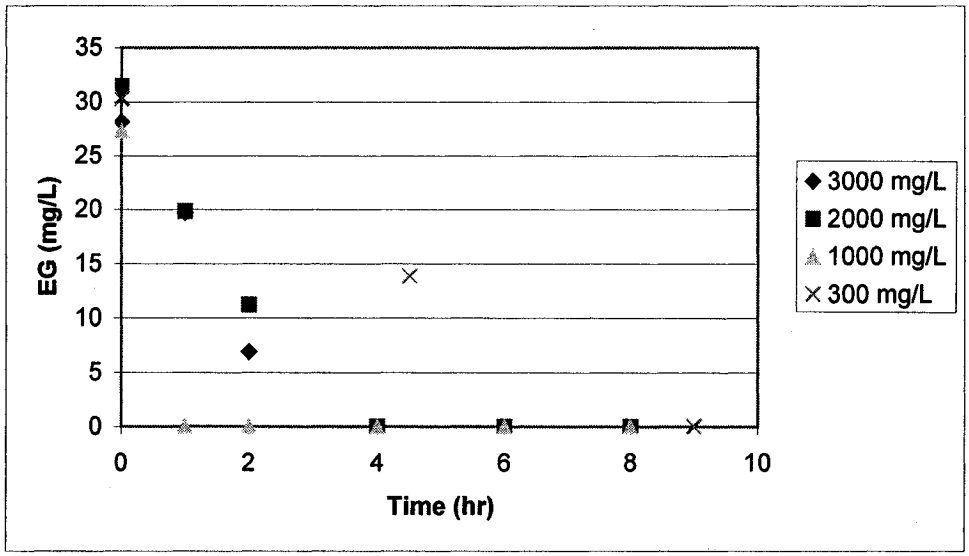


Figure 5.1.35 : Removal of Ethylene Glycol for initial deicing fluid of 130 mg/L

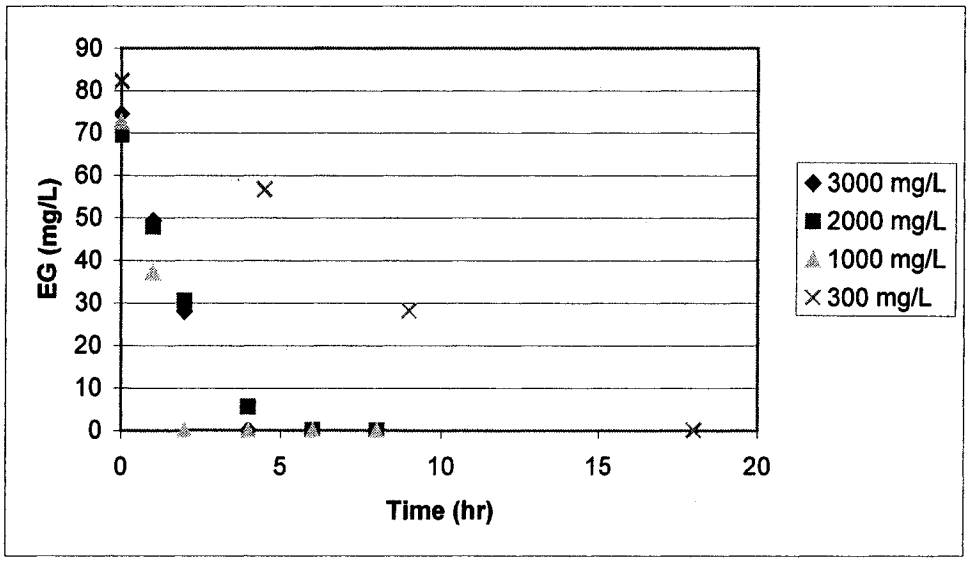


Figure 5.1.36 : Initial rate of Ethylene Glycol removal vs. initial DIF

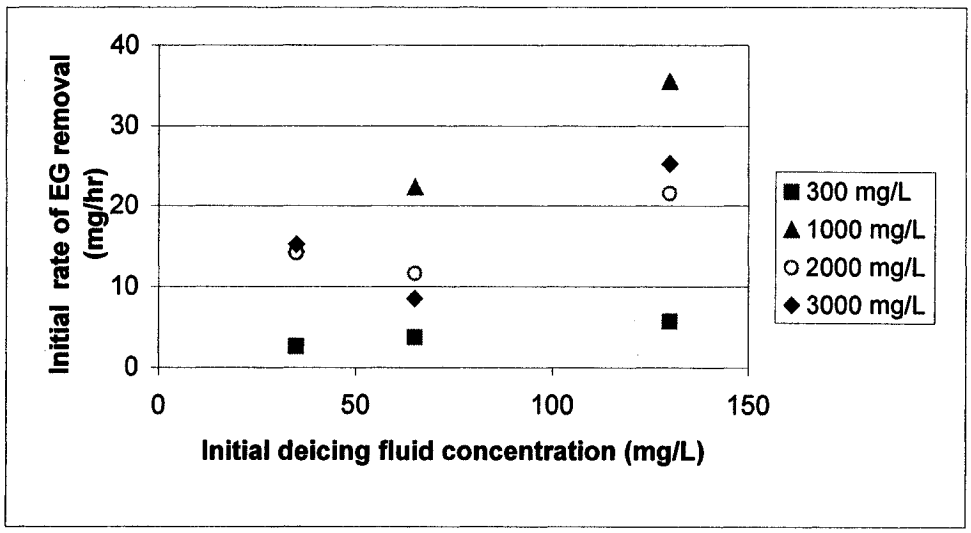
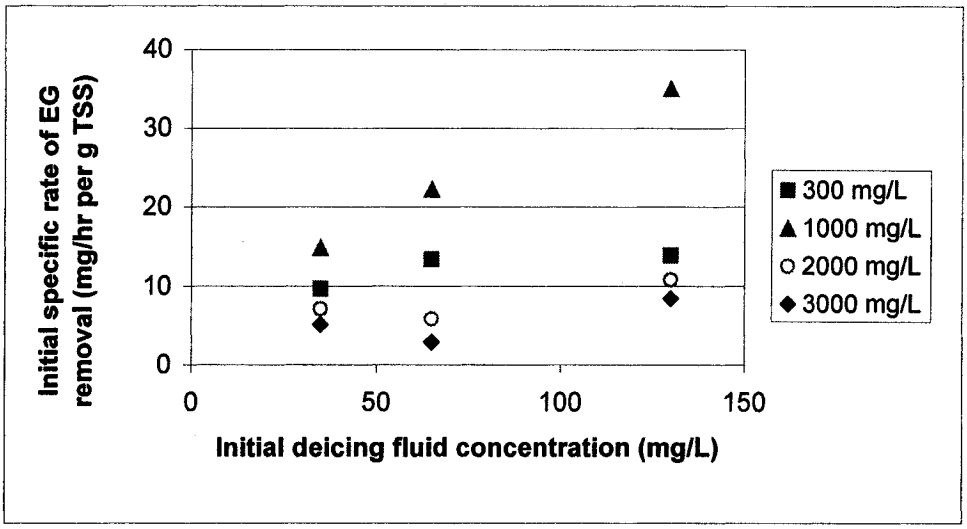


Figure 5.1.37 : Initial specific rate of Ethylene Glycol removal vs. initial DIF



d) OUR and SOUR

The results for the oxygen uptake rates and specific oxygen uptake rates are shown in Tables 5.1.5 to 5.1.12 and Figures 5.1.38 and 5.1.39. It must be noted that there is always a decreasing trend in the OUR and SOUR with time

since microbial respiration is tied to substrate utilization. Hence, as substrate utilization rates decreases with time, there is a corresponding decrease in microbial respiration.

In the absence of deicing fluid, the oxygen uptake rate is much higher for the biomass concentration of 3000 mg/L compared to the biomass concentrations of 1000 mg/L and 2000 mg/L. As expected, the lowest uptake rates in the absence of deicing fluid occur at the biomass level of 300 mg/L of TSS. This also occurs at the deicing fluid concentration of 35 mg/L, where the initial rate of oxygen consumption is approximately four to five times higher for the 3000 mg/L biomass level than the three other biomass concentrations. Smaller differences in the oxygen uptake rates are observed between the biomass concentrations of 1000 mg/L, 2000 mg/L and 3000 mg/L of TSS for the two higher deicing fluid concentrations. As in the previous cases, the oxygen uptake rates are lower at the 300 mg/L biomass level for both these deicing fluid concentrations.

As expected, taking into account the respiration rate per gram of biomass, the results are quite different. In most cases, the highest specific oxygen uptake rates occur at the lowest biomass concentration of 300 mg/L. Moreover, the general trend is that the specific oxygen uptake rate tends to increase as the biomass level decreases. Thus, the amount of biomass influences the

respiration rates indicating that the biomass is more active under higher food to micro-organism ratios.

Table 5.1.5 : OUR for different concentrations of DIF at 300 mg/L TSS

DIF conc.	0 mg/L	35 mg/L	65 mg/L	130 mg/L
Time (hr)	OUR (mg/L*min)	OUR (mg/L*min)	OUR (mg/L*min)	OUR (mg/L*min)
0	0.08	0.11	0.09	0.28
4.5	0.11	0.10	0.09	0.19
9	0.10	0.09	0.03	0.17
18	0.06	0.02	0.03	0.12
22.5	0.03	0.01	0.02	0.10

Table 5.1.6 : OUR for different concentrations of DIF at 1000 mg/L TSS

DIF conc.	0 mg/L	35 mg/L	65 mg/L	130 mg/L
Time (hr)	OUR (mg/L*min)	OUR (mg/L*min)	OUR (mg/L*min)	OUR (mg/L*min)
1	0.23	0.17	0.41	0.40
2,5	0.24	0.10	0.26	0.37
5	0.20	0.06	0.20	0.22
7	0.10	0.05	0.17	0.19

Table 5.1.7 : OUR for different concentrations of DIF at 2000 mg/L TSS

DIF conc.	0 mg/L	35 mg/L	65 mg/L	130 mg/L
Time (hr)	OUR (mg/L*min)	OUR (mg/L*min)	OUR (mg/L*min)	OUR (mg/L*min)
1	0,09	0,12	0,60	0,56
2,5	0,04	0,06	0,30	0,28
5	0,11	0,03	0,16	0,27
7	0,07	0,08	0,06	0,16

Table 5.1.8 : OUR for different concentrations of DIF at 3000 mg/L TSS

DIF conc.	0 mg/L	35 mg/L	65 mg/L	130 mg/L
Time (hr)	OUR (mg/L*min)	OUR (mg/L*min)	OUR (mg/L*min)	OUR (mg/L*min)
1	0,53	0,66	0,64	0,53
2,5	0,16	0,24	0,32	0,32
5	0,14	0,14	0,19	0,30
7	0,11	0,13	0,11	0,23

Table 5.1.9 : SOUR for different concentrations of DIF at 300 mg/L TSS

DIF conc.	0 mg/L	35 mg/L	65 mg/L	130 mg/L
Time (hr)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)
0	0.30	0.41	0.34	0.69
4.5	0.39	0.36	0.33	0.46
9	0.36	0.32	0.12	0.41
18	0.21	0.09	0.12	0.29
22.5	0.09	0.04	0.09	0.23

Table 5.1.10 : SOUR for different concentrations of DIF at 1000 mg/L TSS

DIF conc.	0 mg/L	35 mg/L	65 mg/L	130 mg/L
Time (hr)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)
1	0.23	0.17	0.40	0.39
2,5	0.24	0.10	0.26	0.37
5	0.20	0.06	0.19	0.22
7	0.10	0.05	0.17	0.19

Table 5.1.11 : SOUR for different concentrations of DIF at 2000 mg/L TSS

DIF conc.	0 mg/L	35 mg/L	65 mg/L	130 mg/L
Time (hr)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)
1	0.04	0.06	0.30	0.28
2,5	0.02	0.03	0.15	0.14
5	0.05	0.01	0.08	0.13
7	0.03	0.04	0.03	0.08

Table 5.1.12 : SOUR for different concentrations of DIF at 3000 mg/L TSS

DIF conc.	0 mg/L	35 mg/L	65 mg/L	130 mg/L
Time (hr)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)
1	0.18	0.23	0.24	0.17
2,5	0.05	0.08	0.12	0.10
5	0.05	0.05	0.07	0.09
7	0.04	0.04	0.04	0.07

Figure 5.1.38 : Initial OUR for different TSS levels

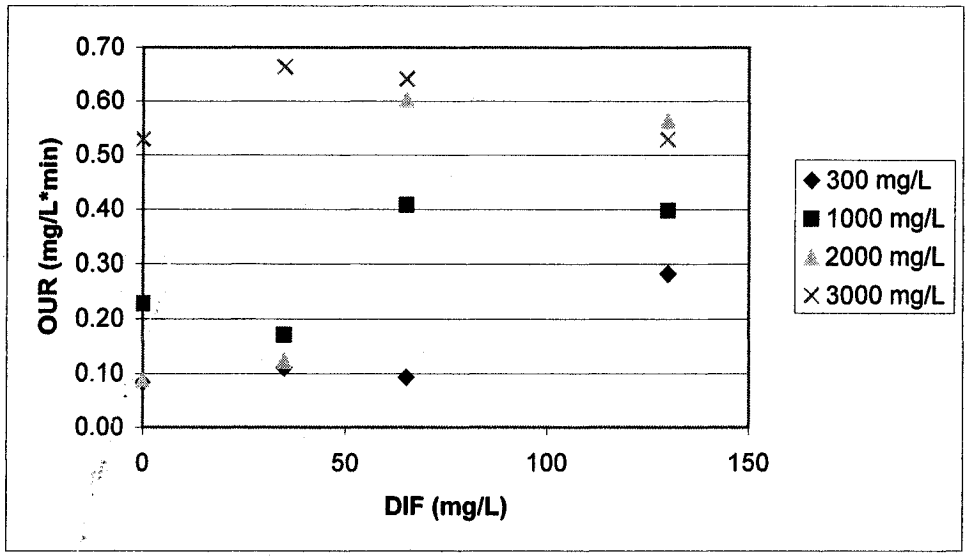
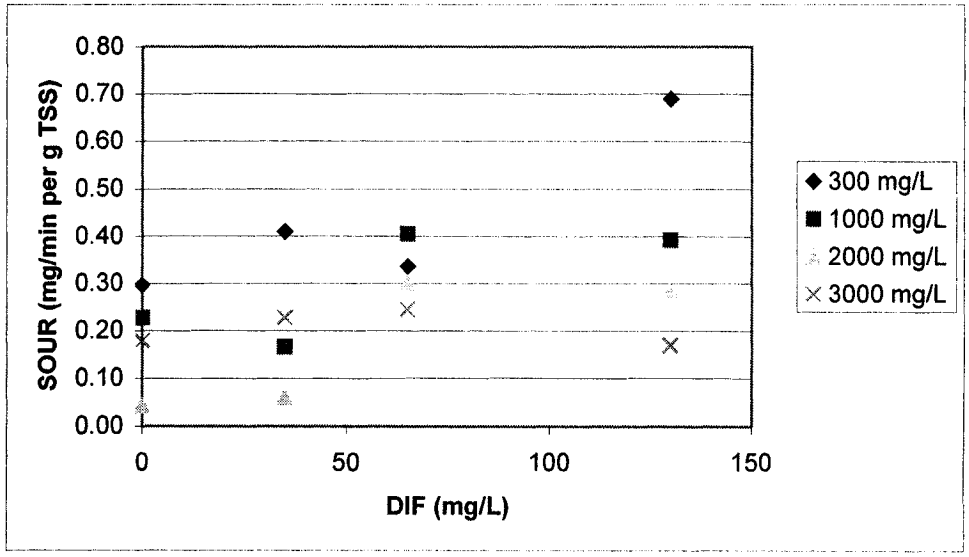


Figure 5.1.39 : Initial SOUR for different TSS levels



e) Sludge Volume Index (SVI)

With regards to sludge volume index, higher TSS values yield higher initial SVI in most cases (see Tables 5.1.13 and 5.1.16). However, final SVI values do not seem to be affected by the amount of biomass present in the reactor. This suggests that the settleability of sludge is not very much influenced by the biomass concentration.

Table 5.1.13 : Initial and Final SVI values at 300 mg/L of TSS

Deicing conc. (mg/L)	0	35	65	130
Initial SVI	140	80	90	140
Final SVI	220	240	250	250

Table 5.1.14 : Initial and Final SVI values at 1000 mg/L of TSS

Deicing conc. (mg/L)	0	35	65	130
Initial SVI	70	60	80	80
Final SVI	80	70	60	70

Table 5.1.15 : Initial and Final SVI values at 2000 mg/L of TSS

Deicing conc. (mg/L)	0	35	65	130
Initial SVI	380	360	280	250
Final SVI	250	230	240	220

Table 5.1.16 : Initial and Final SVI values at 3000 mg/L of TSS

Deicing conc. (mg/L)	0	35	65	130
Initial SVI	350	550	380	430
Final SVI	200	440	250	210

5.1.2 Effect of Deicing Fluid Concentration

The results for the 16 basic batch experiments have been presented in Section 5.1.1. These results will now be analyzed based on the effect of deicing fluid concentration. Thus, the results are discussed for each of the four initial biomass concentrations (300 mg/L, 1000 mg/L, 2000 mg/L and 3000 mg/L of TSS).

a) TSS and VSS

As Tables 5.1.1 to 5.1.4 show, there is no significant change in the biomass levels with an increase in the concentration of deicing fluid for each of the four initial biomass concentrations. Even at the highest deicing fluid concentration, there is an insufficient amount of organic matter to promote significant microbial growth (for all four cases). Therefore, an increase in the amount of organic matter is not sufficient to induce significant microbial growth under these experimental conditions where the amount of biomass is too large and the timeframe (8 hr and 45 hr) is relatively short.

b) COD and TOC

In general, for all four initial biomass concentrations, there is an increase in initial specific removal rates of TOC and COD as the deicing fluid concentration is augmented (see Figures 5.1.40 to 5.1.43 below). However, for the lowest initial biomass level of 300 mg/L of TSS, there is a decrease in the removal rates at the highest deicing fluid level. This decrease is more marked than at the three other levels of biomass concentrations and can be explained by the possibility of substrate inhibition. Thus, at a reduced level of biomass, the microbial population is more sensitive to an increase in deicing fluid level than at the higher biomass levels.

c) EG

The specific rates of ethylene glycol removal are expected to follow a similar trend as the COD and TOC rates but they do not (Figure 5.1.37). Hence, for the lowest initial TSS level of 300 mg/L, an increase in the initial specific removal rate of ethylene glycol is observed even at the highest concentration of deicing fluid. This suggests that, even though there seems to be an overall inhibitory effect caused by a large amount of total substrate (as measured by the COD and TOC), ethylene glycol is preferentially degraded by the microorganisms present in the activated sludge. For the three higher initial biomass concentrations, increases in the deicing fluid concentration lead to slight increases in its specific removal rate.

These results suggest that the ethylene glycol is a preferred substrate and is degraded more rapidly than the other organic products present in the synthetic wastewater base. Moreover, at low biomass levels, increased amounts of deicing fluid affect the overall rates of organic matter removal but do not negatively hinder ethylene glycol removal rates.

d) OUR and SOUR

For all four initial biomass levels, the oxygen and specific oxygen uptake rates follow a trend similar to the rates of ethylene glycol removal as the concentration of deicing fluid is increased (see Tables 5.1.5 to 5.1.12). Thus, there is a general increase in the respiration rates as more organic matter is

present in the reactor. At this point it would be premature to suggest inferences describing the relative contribution in microbial activity (and respiration rates) from the ethylene glycol substrate versus the overall organic matter (described by TOC and COD values). However, it should be kept in mind that respiration rates can be affected by the type of substrate to be biodegraded.

e) Sludge Volume Index (SVI)

For all four initial biomass concentrations, no specific trends are apparent as the amount of deicing fluid in the reactor is increased (Tables 5.1.13 to 5.1.16). These results could have been anticipated since the settling characteristics of the biomass are not expected to change within the relatively short time frame of these experiments (8 hr and 45 hr).

Figure 5.1.40 : Initial specific rate of COD and TOC removal (300 mg/L of TSS)

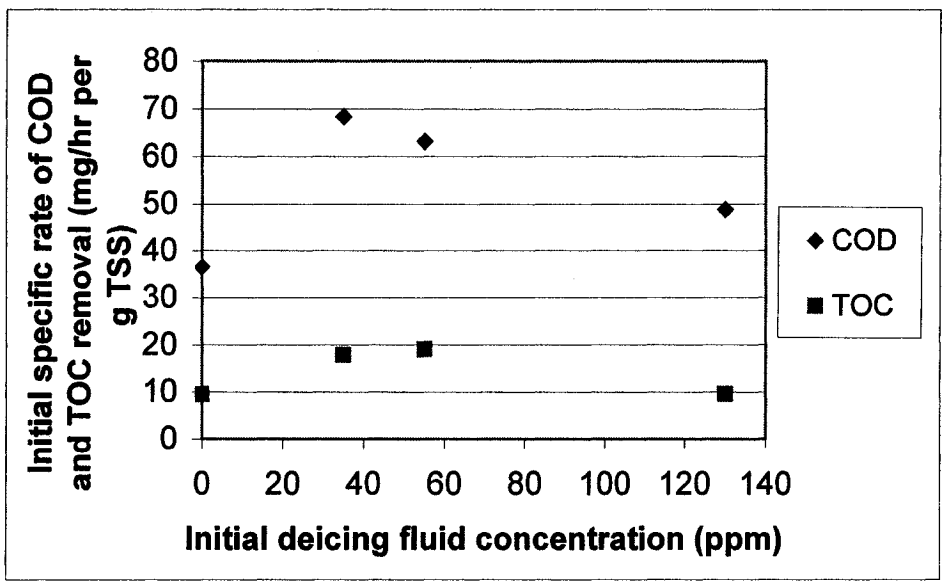


Figure 5.1.41 : Initial specific rate of COD and TOC removal (1000 mg/L of TSS)

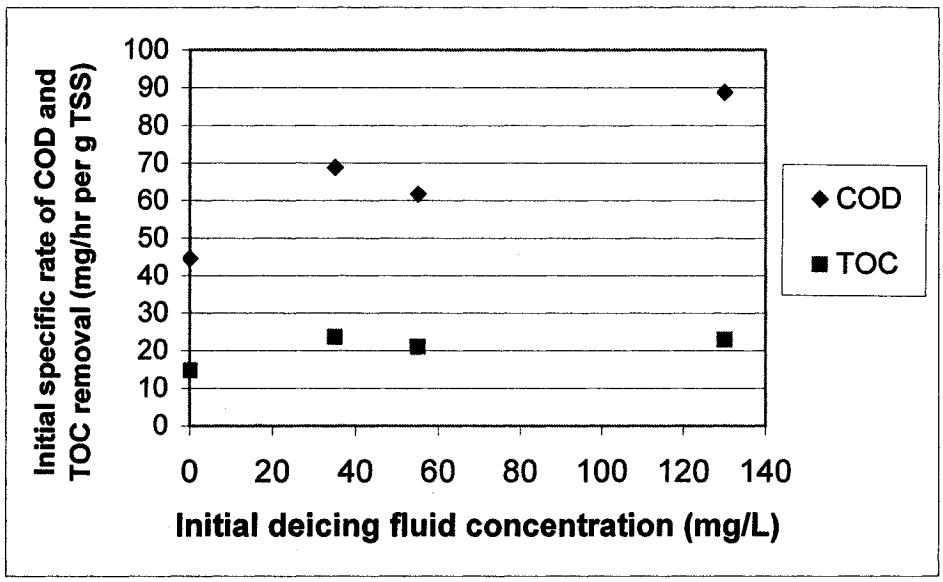


Figure 5.1.42 : Initial specific rate of COD and TOC removal (2000 mg/L of TSS)

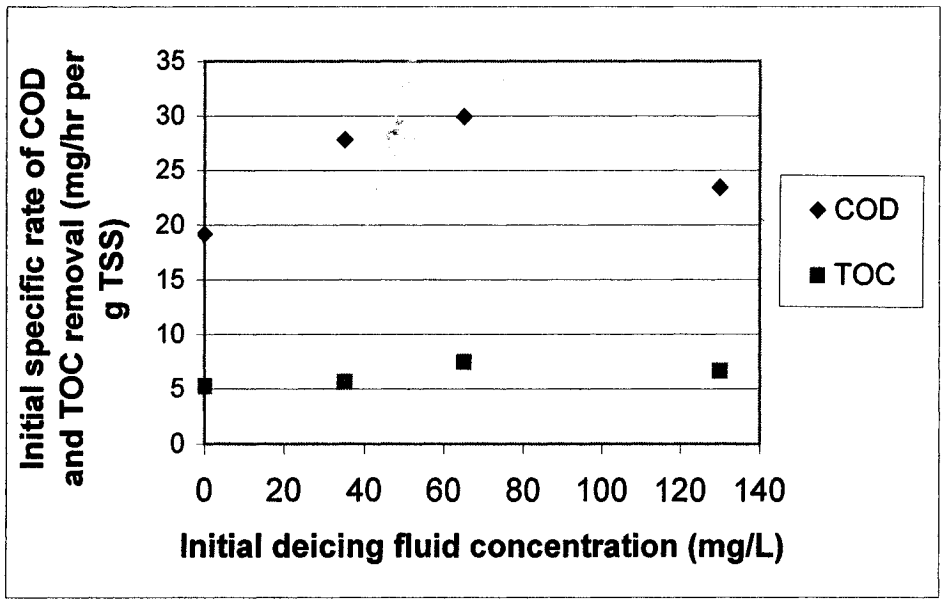
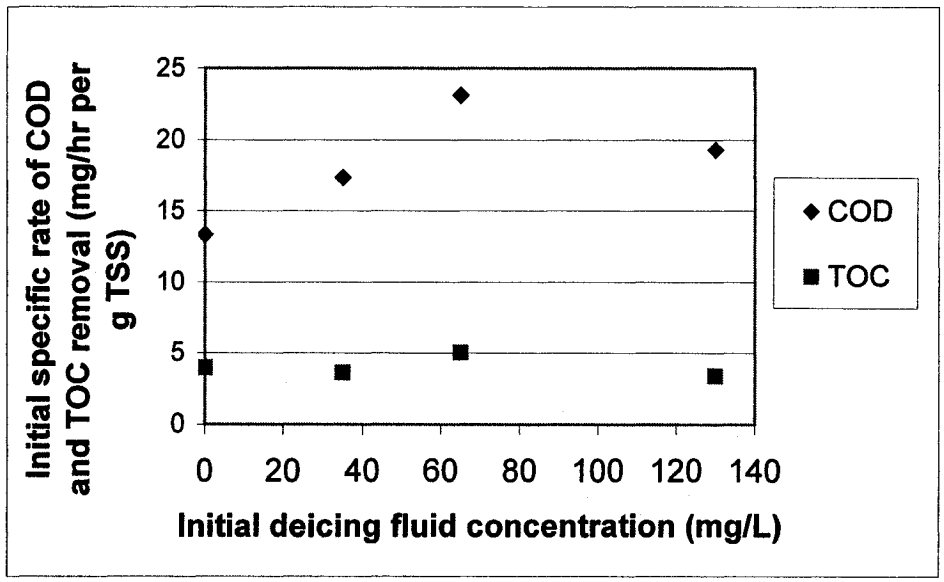


Figure 5.1.43 : Initial specific rate of COD and TOC removal (3000 mg/L of TSS)



BIOLOG RESULTS

As described in Section 3.5, along with the PCA analysis, the variability in the microplate replicates was employed to evaluate the changes in the utilization of a particular substrate between the initial and final populations. Hence, with this method, there are very little differences occurring in most of the batch experiments. In these cases, at most only one or two substrates varied significantly from start to finish. These experiments were conducted under the conditions of 1000 mg/L, 2000mg/L and 3000 mg/L of TSS with the four different deicing fluid concentrations. The statistical analysis for these experiments is presented In Appendix D. However, the experiments conducted at 300 mg/L of TSS show more differences between the initial and final populations. Other than the time frame being longer (45 hr run instead of 8 hr run), the high food-to-microorganism ratio might have also played in role in creating more significant

changes in the substrate utilization pattern resulting from a change in the microbial population. These results are presented below.

The Biolog results for the four base experiments conducted at the lowest biomass concentration of 300 mg/L are presented in Tables 6 to 10 of Appendix D. There is no effect of the deicing fluid concentration on the number of compounds that experience a change above the calculated threshold (see Table 6 in Appendix D). In most cases, there are approximately 10 compounds that show significant changes between initial and final populations. The makeup of these compounds is mainly carbohydrates and carboxylic acids.

With respect to the changes in the number of compounds that exhibit high consumption (normalized consumption above 1.10), the largest difference occurs at the highest deicing fluid concentration (see Tables 7 to 10 in Appendix D). More specifically, there is a decrease in the number of compounds between the initial and final microbial populations. This result is also confirmed by the replicate experiment performed at the highest deicing fluid concentration (see Table 12 in Appendix D). In all experiments, the types of compounds that are highly consumed remain relatively the same.

5.2 Repeatability in the Activated Sludge Experiments

One of the main challenges associated with biological systems is the repeatability of experiments as microorganisms and microbial populations undergo significant variations with time. Replicate experiments were conducted to determine to which extent the data obtained from the batch experiments described in Section 5.1 change under the same experimental conditions but with two different biomass samples (both unacclimatized) obtained from St-Canut at different time periods during the summer months. The experimental conditions were chosen at random without repeating the same biomass level and deicing fluid concentration twice. Thus, the following replicate experiments were conducted ; Experiment 1 : 2000 mg/L TSS and 35 mg/L DIF, Experiment 2 : 1000 mg/L TSS and 65 mg/L DIF, Experiment 3 : 300 mg/L TSS and 130 mg/L DIF). In order to abbreviate this Section, only the main observations will be discussed with all the adjoining Figures and Tables presented in Appendix E.

a) TSS

As can be seen from Tables 5.2.1 and 5.2.2 (Appendix E), the TSS values show similar results for replicate experiments with no significant microbial growth observed. It is natural that the values between replicates are similar since the amount of biomass that is placed in the reactor is controlled and due to the fact that there is insignificant biomass growth, the final values also display the same trend.

b) COD and TOC

Figures 5.2.1 to 5.2.12 (Appendix E) present the COD and TOC profiles as well as the specific rates for the replicate experiments. From these figures, similar trends between replicate experiments for the most of the 3 experimental conditions are observed. However, certain differences in values are observed due to a few factors. First of all, in all cases, the initial values of COD and TOC between replicates are not exactly the same. Hence, in spite of preparing a synthetic wastewater, there is still variability in the initial values (refer to Section 3.4) and this translates into differences in the trend of COD and TOC decrease. In addition, different microbial populations were used for replicate experiments and this can have an impact on the degradation rates of organic matter.

The difference between initial values also has a significant effect on the initial specific rates of removal. However, there is a tendency for the specific rates to follow each other more closely after the initial value. This is an interesting result, as one could have expected the difference in specific rates to be maintained when starting with different initial COD and TOC values.

For the lowest biomass concentration of 300 mg/L (Figures 5.2.5, 5.2.6, 5.2.11 and 5.2.12) there are more important differences between replicate experiments compared to the two higher biomass levels. More specifically, the differences between specific rates are maintained throughout a longer period of the experiment at this biomass concentration. This can be explained by the high

food-to-microorganism ratio under these conditions and thus, the micro-organisms require a longer time period to handle the disparity in initial TOC and COD compared to the two higher TSS concentrations.

c) EG

In the case of ethylene glycol removal, similar trends of removal for the 3 experimental conditions are observed (Figures 5.2.13 to 5.2.15 of Appendix E). Moreover, the specific rates of removal are also similar between replicates for the 3 conditions (Figures 5.2.16 to 5.2.18). Thus, neither the disparity caused by differences in initial values of TOC and COD nor the differences between replicates at the lowest TSS value are observed. The initial concentration of ethylene glycol in the medium is much easier to control than the synthetic wastewater base. In addition, ethylene glycol is degraded at a much faster rate than the other components of the wastewater and its rate does not require a longer time period to stabilize even at the lowest biomass concentration.

d) OUR and SOUR

As can be seen from Tables 5.2.3 to 5.2.6 (Appendix E), there are quite large differences between OUR and SOUR replicate values for the cases of 300 mg/L and 2000 mg/L of TSS. For the case of 1000 mg/L, very similar values between the replicate experiments for the OUR and SOUR are observed. These observations can be explained by many factors. First of all, amongst the experiments, the replicates performed at 1000 mg/L have the most similar initial

organic matter content, (COD, TOC and EG) and biomass concentration. In the other two cases, the differences between either the initial organic matter content or the biomass concentrations are more pronounced. By examining the replicates at 2000 mg/L of TSS (Tables 5.2.3 and 5.2.5), higher values for the second replicate are expected since the experiment was started with higher initial values of COD and TOC in this case. The replicates at 300 mg/L of TSS (Tables 5.2.4 and 5.2.6) show larger disparities in the initial values and OUR and SOUR and then follow each other for subsequent values. Hence, the oxygen uptake rates and specific oxygen uptake rates will tend to follow the same trends as the COD and TOC values rather than the ethylene glycol values. This can be explained by the fact that ethylene glycol represents only a fraction of the total amount of organic matter given by COD or TOC.

e) Sludge Volume Index (SVI)

The SVI values for replicate experiments are shown in Table 5.2.7 of Appendix E. Since all sets of replicates experiments were conducted at different periods of the year and with different sludge populations, it is very difficult to determine any trends between replicates. This parameter lends itself to a more significant interpretation when many measurements are taken over an extended period of time (as in the case of the field data study).

BIOLOG RESULTS

As stated in Section 5.1, only the batch experiments conducted at the lowest biomass concentration of 300 mg/L show differences between the initial and final populations. A similar result was found for the replicate runs whereby only the experiment conducted at 300 mg/L of TSS shows significant changes in the microbial population. The Biolog results for this experiment were presented along with the other batch experiments in Section 5.1 (see p.104).

Although the Biolog technique was not used to characterize the differences between the starting populations of the batch experiments, the SBR Biolog results (see p. 171) show a relatively stable microbial population at the St-Canut treatment plant (during the unacclimatized period).

5.3 Adsorption of Organic Matter by Biomass

Adsorption experiments were performed to gain insight into the mechanism of ethylene glycol removal by the biomass. To achieve this objective, shake flask experiments were conducted at 3 different biomass concentrations (300 mg/L, 1000 mg/L and 2000 mg/L of TSS) and 2 different deicing fluid concentrations (65 mg/L and 130 mg/L). In each case, the synthetic wastewater base was employed and samples were taken at t = 0, 15, 30, 50, 70, and 90 minutes. In addition, a last sample was taken at 10 days to quantify the amount of residual organic matter. The results are presented in Figures 5.3.1 to 5.3.5.

Figure 5.3.1 : Adsorption experiments at 300 mg/L of TSS

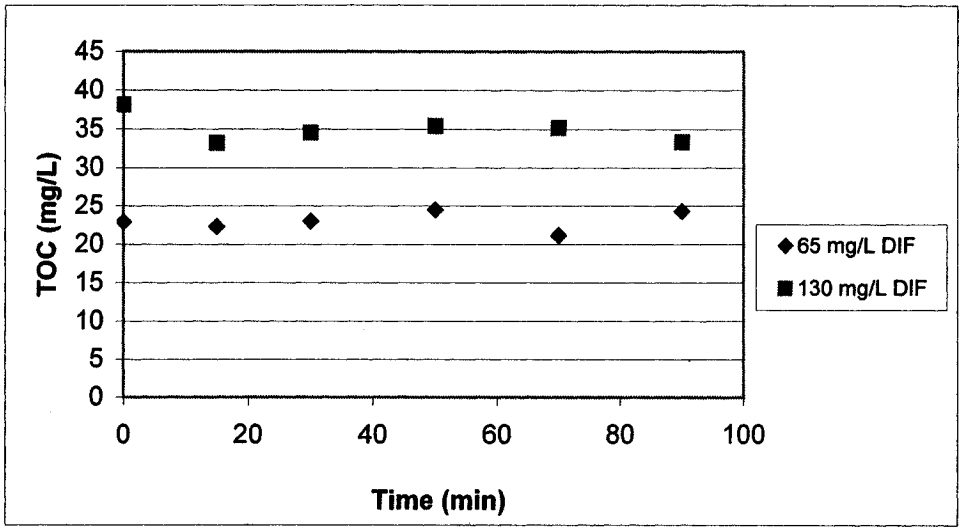


Figure 5.3.2 : Adsorption experiments at 1000 mg/L of TSS

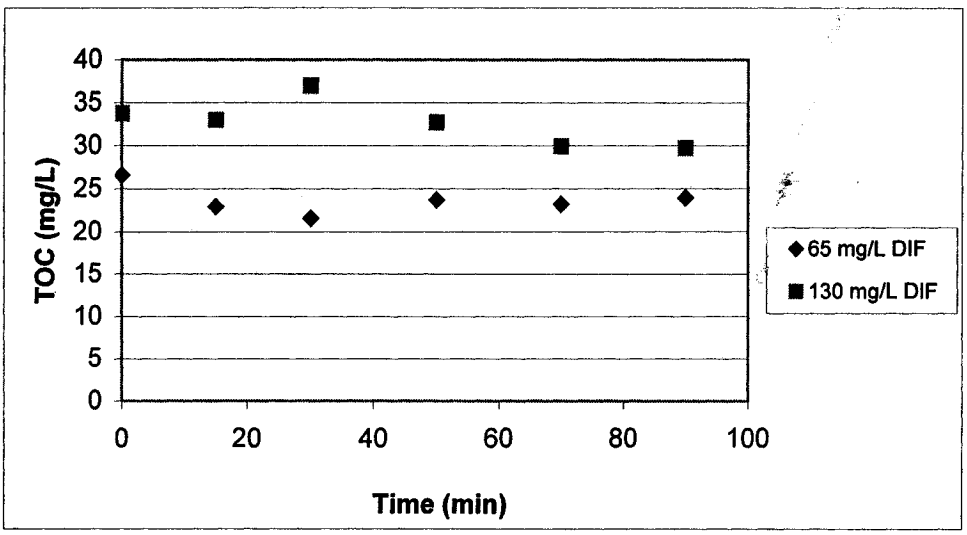


Figure 5.3.3 : Adsorption experiments at 2000 mg/L of TSS

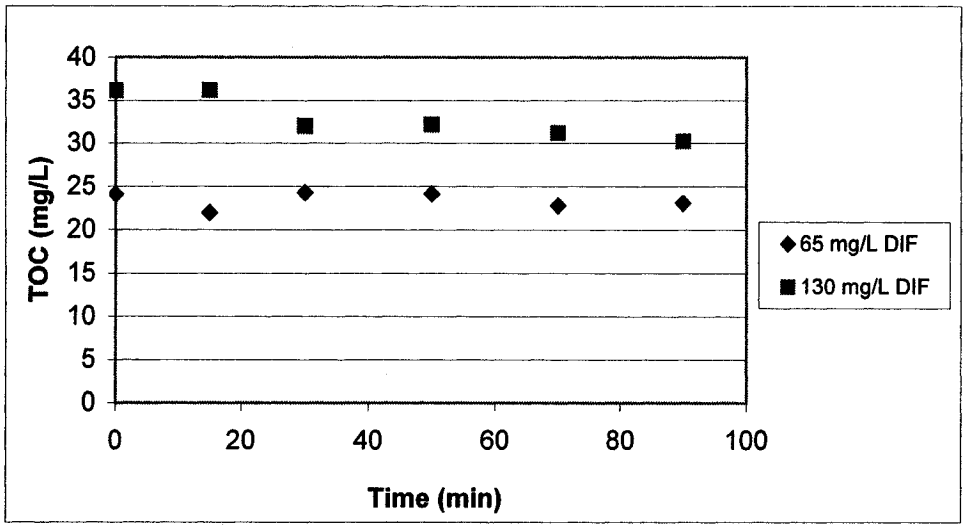


Figure 5.3.4 : Adsorption experiments at 2000 mg/L of TSS (Ethylene Glycol)

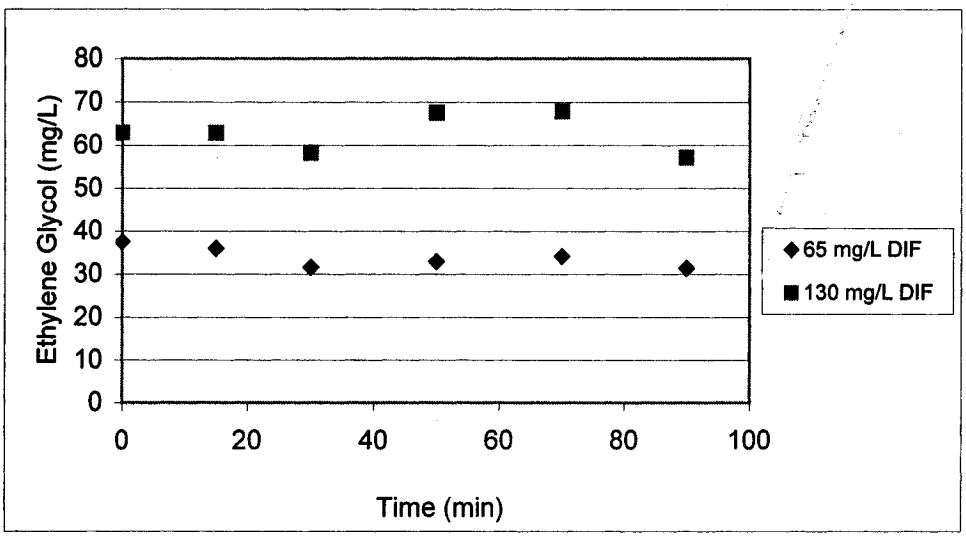
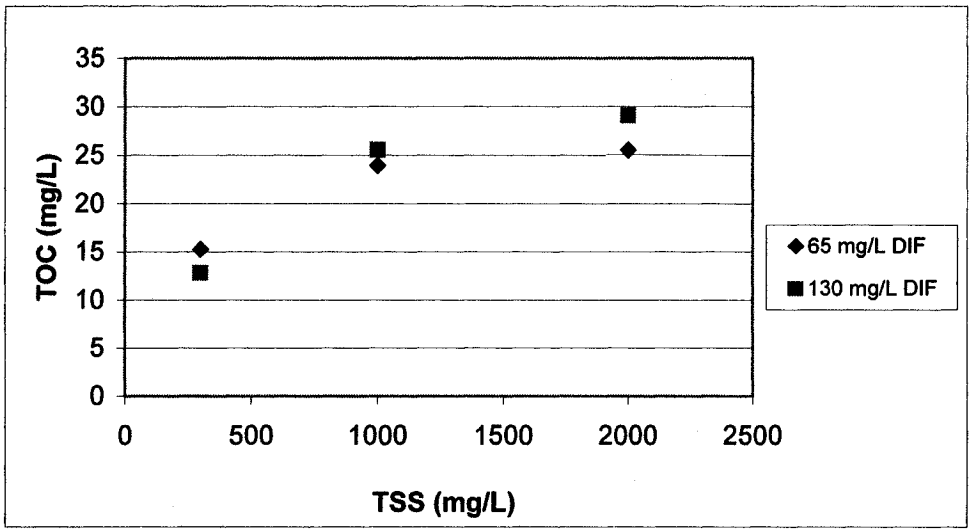


Figure 5.3.5 : Residual TOC at 10 days for various TSS



The most striking result is that under the various conditions, very little organic matter seems to be removed by adsorption (as shown by the TOC results). In addition, the ethylene glycol concentration remains relatively stable throughout the runs. At most, there is only a slight reduction in TOC values with time (Figures 5.3.1 to 5.3.3) but this is nowhere as important as the decreases observed in the multiple batch experiments performed under similar biomass and organic matter concentrations. In fact, there is up to approximately 16% reduction in TOC within 90 minutes of contact between the biomass and substrate. Moreover, even samples taken after 10 days of contact show non-negligible TOC residual levels in most cases (Figure 5.3.5).

The repeatability of the results leads to believe that adsorption may in fact, not be a necessary step for biodegradation to occur. As ethylene glycol is a readily degraded substrate, the microbial population can directly assimilate the

carbon source from solution. Although a review of cellular biology is beyond the scope of this thesis, scientific literature suggests various mechanisms (other than adsorption) for the transport of substrate to the cell wall in order to be assimilated.

Therefore, based on the results obtained, the adsorption process (including isotherms, etc.) will not be incorporated into the kinetic model describing the biodegradation of deicing wastes.

5.4 Effect of Biomass Acclimatization

At the St-Canut (PQ) wastewater plant, treatment of deicing wastes takes place from December to April. During the first two weeks, the activated sludge microorganisms are gradually acclimatized to the deicing fluid. The concentrations of deicing fluid are progressively increased in the following two weeks. Thus, after approximately one month, the acclimatization process is judged to be complete and the treatment plant is operated at full capacity. When the deicing wastes are depleted, the treatment plant returns to its normal operation.

In order to gain insight into glycol-acclimatized microorganisms, three laboratory batch experiments were performed using activated sludge collected during peak deicing season. An activated sludge concentration of 2000 mg/L was employed for all three tests as results from the previous section show little

differences between biomass concentrations of 2000 and 3000 mg/L. The first experiment was performed using only the synthetic base while the two others used the synthetic base and deicing fluid concentrations of 65 and 130 mg/L respectively.

5.4.1 Effect of Deicing Fluid Concentration : Acclimatized Biomass

a) TSS

As with the unacclimatized biomass, there are very few changes in TSS for the different experiments (Table 5.4.1). Therefore, no significant microbial growth is observed for the acclimatized biomass in both the presence and absence of deicing. This result is expected given the relatively short time frame (8 hr) of the batch experiments conducted at 2000 mg/L.

Table 5.4.1 : TSS values for initial sludge concentration of 2000 mg/L

Deicing conc. (mg/L)	Initial TSS (mg/L)	Final TSS (mg/L)
0	2010	1840
65	1970	1850
130	1740	1820

b) COD and TOC

The decrease of COD and TOC concentration are presented in Figures 5.4.1 to 5.4.3 and their specific removal rates are given in Figures 5.4.4 to 5.4.6. First, as the deicing fluid concentration increases, the specific removal rates also exhibit a clear increase (Figures 5.4.4 to 5.4.6). In fact, the initial specific removal rate of COD is approximately 4 times greater at the deicing level of 130

mg/L than in the case where only the synthetic base is present. These results show that the acclimated microorganisms degrade the deicing fluid quite easily.

Figure 5.4.1 : COD and TOC concentration (0 mg/L DIF, 2000 mg/L TSS)

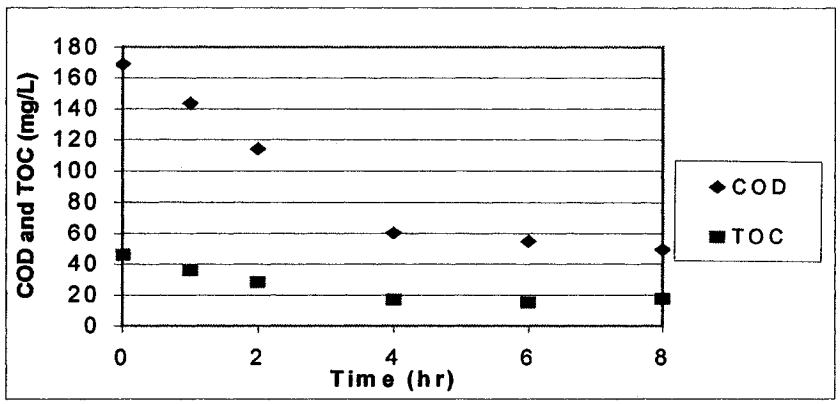


Figure 5.4.2 : COD and TOC concentration (65 mg/L DIF, 2000 mg/L TSS)

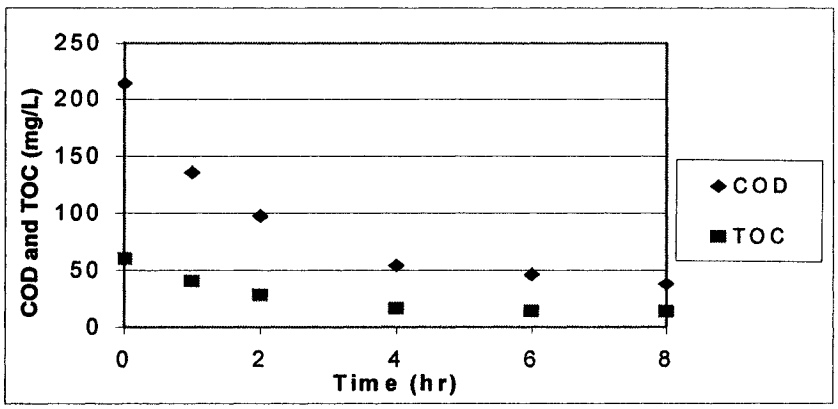


Figure 5.4.3 : COD and TOC concentration (130 mg/L DIF, 2000 mg/L TSS)

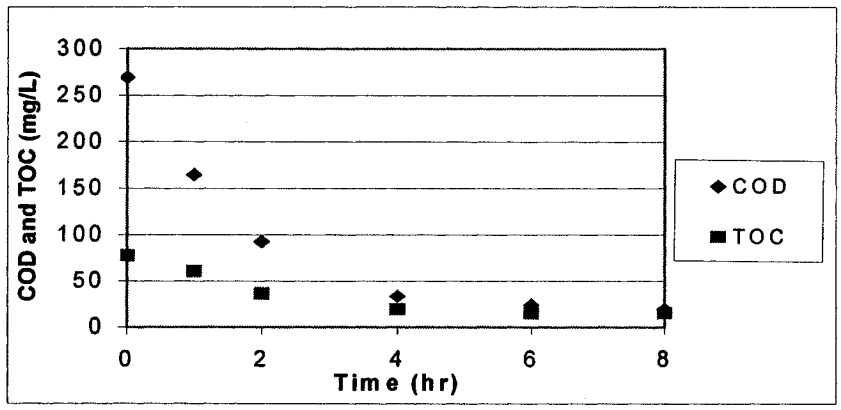


Figure 5.4.4 : COD and TOC specific removal (0 mg/L DIF, 2000 mg/L TSS)

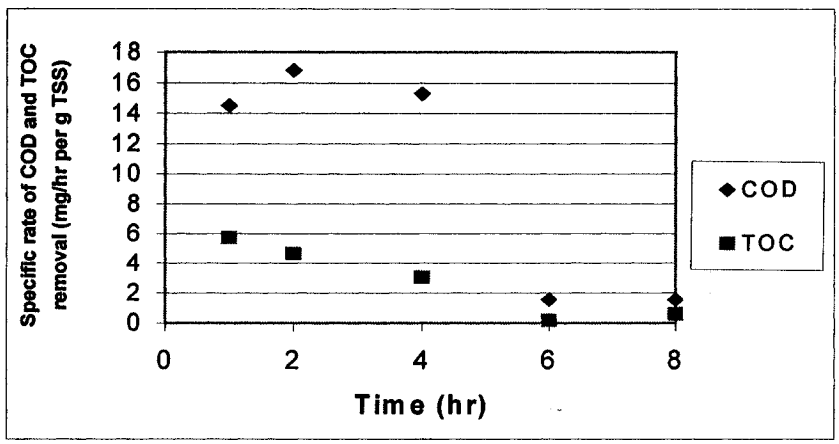


Figure 5.4.5 : COD and TOC specific removal (65 mg/L DIF, 2000 mg/L TSS)

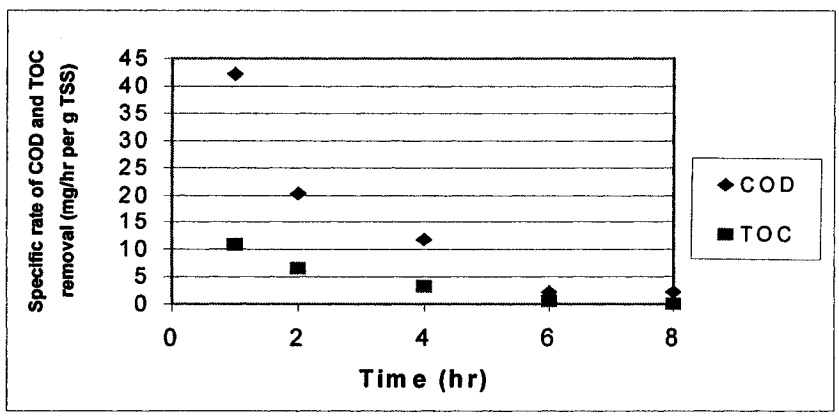
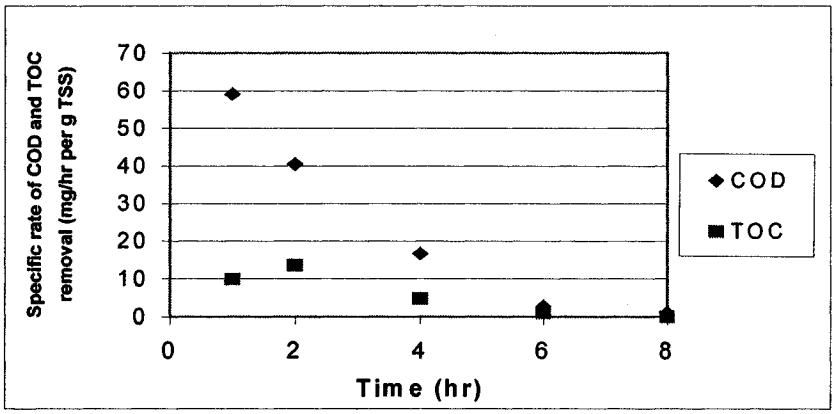


Figure 5.4.6 : COD and TOC specific removal (130 mg/L DIF, 2000 mg/L TSS)



c) EG

In addition, ethylene glycol is almost completely removed within the first hour at both deicing fluid concentrations (Figures 5.4.7 and 5.4.8). As can be seen in Figure 5.4.9, the rate of ethylene glycol removal increases as its concentration is increased.

d) SOUR

The specific oxygen uptake rates are relatively similar for all three deicing fluid concentrations (see Table 5.4.2).

e) Sludge Volume Index (SVI)

Table 5.4.3 shows very poor settling characteristics of the final sludge for all three experimental conditions.

Figure 5.4.7: Removal of Ethylene Glycol with Time for initial DIF of 65 mg/L

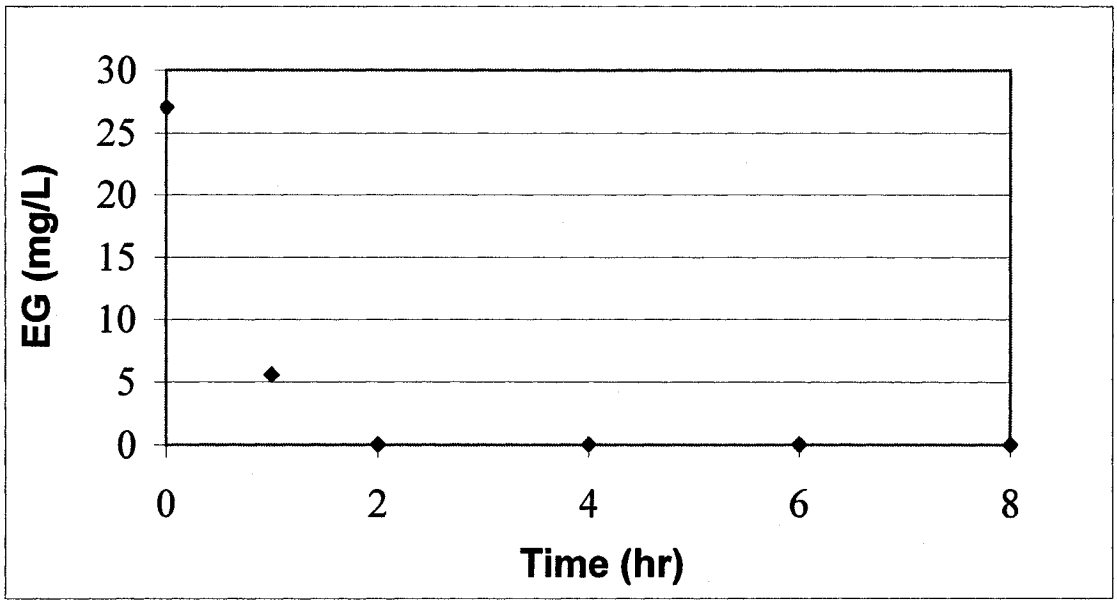


Figure 5.4.8 : Removal of Ethylene Glycol with Time for initial DIF of 130 mg/L

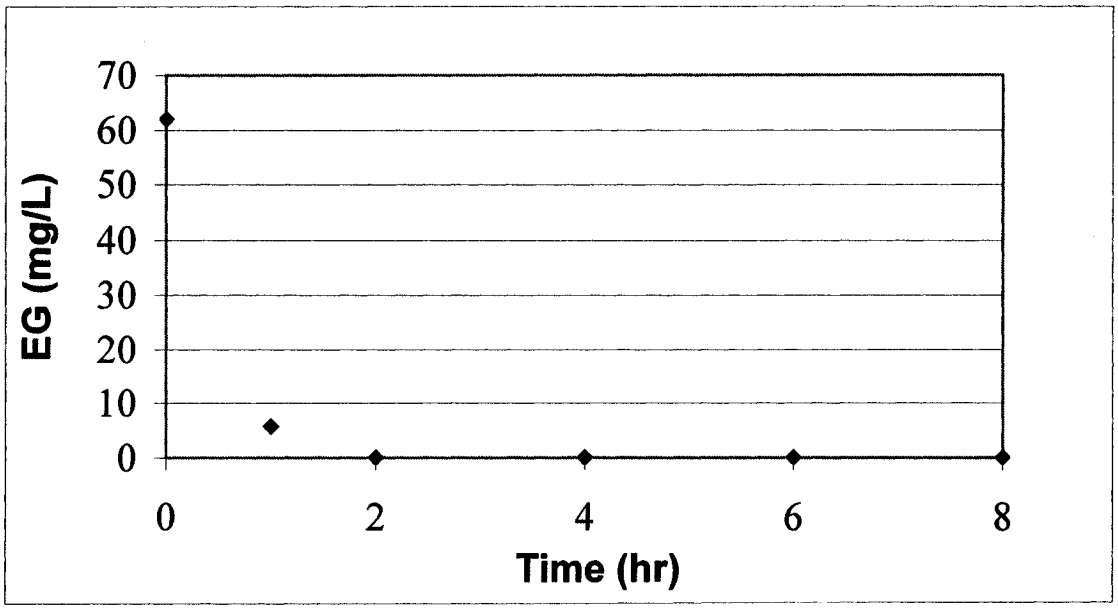


Figure 5.4.9 : Initial rate of Ethylene Glycol removal vs. initial DIF

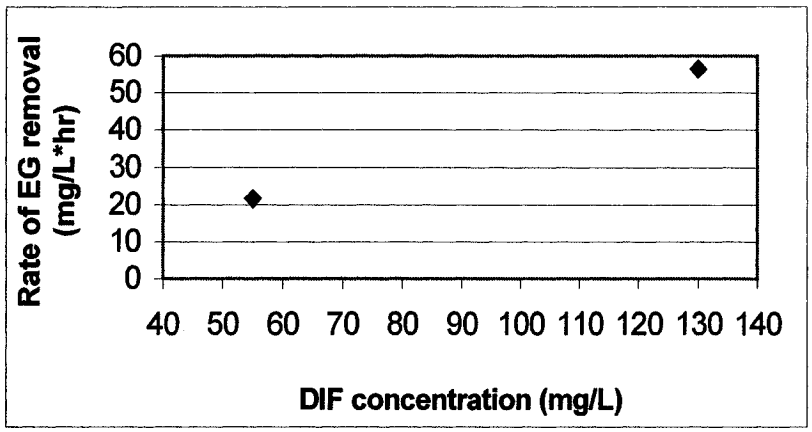


Table 5.4.2 : SOUR for different concentrations of DIF

DIF conc.	0 mg/L	65 mg/L	130 mg/L
Time (hr)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)	SOUR (mg/min per g TSS)
1	0.21	0.28	0.30
2,5	0.20	0.24	0.28
5	0.09	0.12	0.13
7	0.07	0.11	0.09

Table 5.4.3 : Initial and Final SVI values at 2000 mg/L of TSS

Deicing conc. (mg/L)	0	65	130
Initial SVI	230	240	160
Final SVI	330	430	470

Overall, the results indicate that an increase in the deicing fluid concentration is met with a corresponding increase in microbial activity for the acclimatized biomass.

5.4.2 Comparison of Non-acclimatized vs. Acclimatized Biomass

As previously stated, microbial acclimatization plays an important role in determining the effectiveness of a mixed culture to degrade a particular chemical compound. In this section, the differences between unacclimatized and glycol-acclimatized activated sludge are examined. Hence, the results from the three experiments using acclimatized sludge shall be compared to those obtained with unacclimatized sludge under the same experimental conditions (TSS = 2000 mg/L).

a) TSS

With regards to TSS, the values are relatively constant for both types of sludges (Table 5.1.3 in Section 5.1 and Table 5.4.1 in Section 5.4). Since the experiments were conducted in a relatively short time frame (i.e. 8 hours), further experiments with a longer time frame would be required to determine the differences between the unacclimatized and acclimatized populations with regards to biomass growth.

b) COD and TOC

The comparative COD and TOC specific rates with and without acclimatization are presented in Tables 5.4.4 to 5.4.9. When no deicing fluid is present, the specific removal rates of COD and TOC are slightly higher with the unacclimatized biomass. However, these trends seem to be reversed at both ethylene glycol concentrations where the specific removal rates are much higher

for the acclimatized sludge. These results indicate a very high affinity of the acclimatized sludge for the glycol substrate.

Table 5.4.4 : COD specific removal rates for 0 mg/L deicing fluid

	Unacclimatized	Acclimatized
Time (hr)	COD rate (mg/h per g TSS)	COD rate (mg/h per g TSS)
0	-	-
1	20	13
2	13	15
4	8	13
6	2	1
8	2	1

Table 5.4.5 : TOC specific removal rates for 0 mg/L deicing fluid

	Unacclimatized	Acclimatized
Time (hr)	TOC rate (mg/h per g TSS)	TOC rate (mg/h per g TSS)
0	-	-
1	5	5
2	4	4
4	3	3
6	1	0
8	1	1

Table 5.4.6 : COD specific removal rates for 65 mg/L deicing fluid

	Unacclimatized	Acclimatized
Time (hr)	COD rate (mg/h per g TSS)	COD rate (mg/h per g TSS)
0	-	-
1	30	39
2	21	19
4	13	11
6	7	2
8	1	2

Table 5.4.7 : TOC specific removal rates for 65 mg/L deicing fluid

	Unacclimatized	Acclimatized
Time (hr)	TOC rate (mg/h per g TSS)	TOC rate (mg/h per g TSS)
0	-	-
1	8	10
2	5	6
4	3	3
6	3	1
8	0	0

Table 5.4.8 : COD specific removal rates for 130 mg/L deicing fluid

	Unacclimatized	Acclimatized
Time (hr)	COD rate (mg/h per g TSS)	COD rate (mg/h per g TSS)
0	-	-
1	23	52
2	20	36
4	13	15
6	12	2
8	4	1

Table 5.4.9 : TOC specific removal rates for 130 mg/L deicing fluid

	Unacclimatized	Acclimatized
Time (hr)	TOC rate (mg/h per g TSS)	TOC rate (mg/h per g TSS)
0	-	-
1	7	9
2	5	12
4	3	4
6	3	1
8	3	0

c) EG

The decrease in EG is presented in Figures 5.4.10 and 5.4.11. For the acclimatized sludge, almost complete removal is achieved within the first hour at both DIF concentrations while it takes approximately 4 hours for the unacclimatized biomass to achieve similar removal. Thus, ethylene glycol removal rates are much higher for the acclimatized biomass (Figure 5.4.12). In fact, the difference between the activities of the two populations for the removal of EG is much greater at the higher DIF concentration.

Figure 5.4.10 : Removal of Ethylene Glycol with Time for initial DIF of 65 mg/L

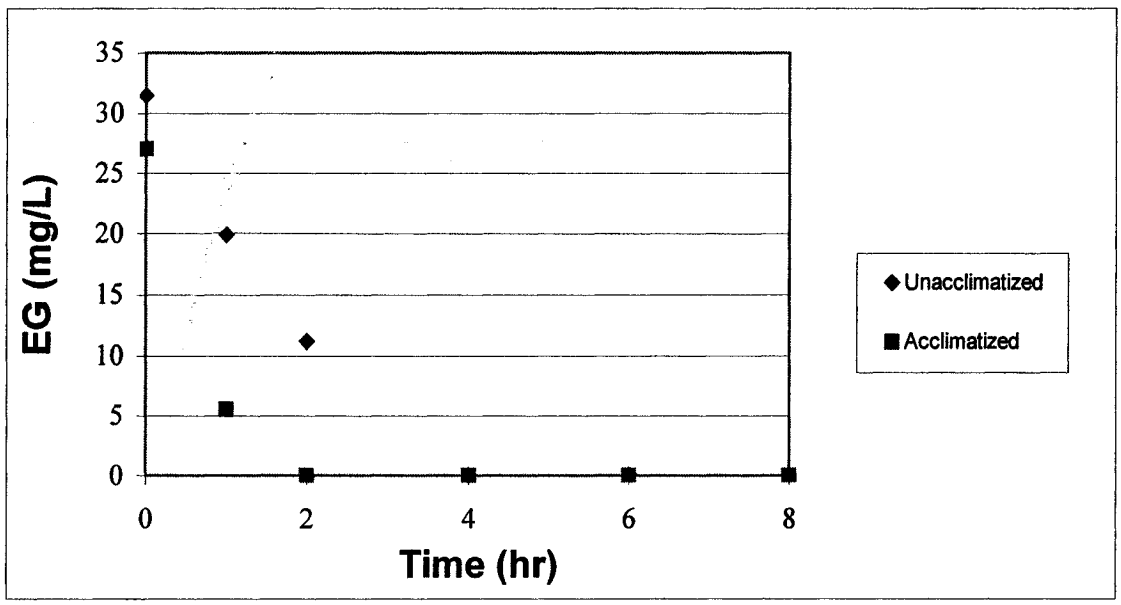


Figure 5.4.11 : Removal of Ethylene Glycol with Time for initial DIF of 130 mg/L

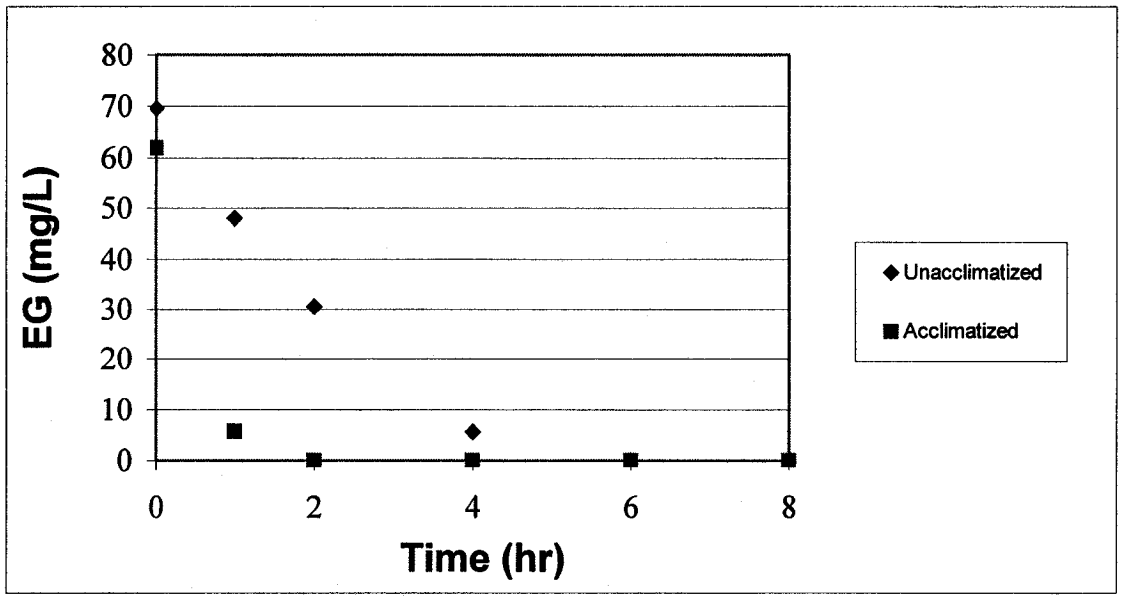
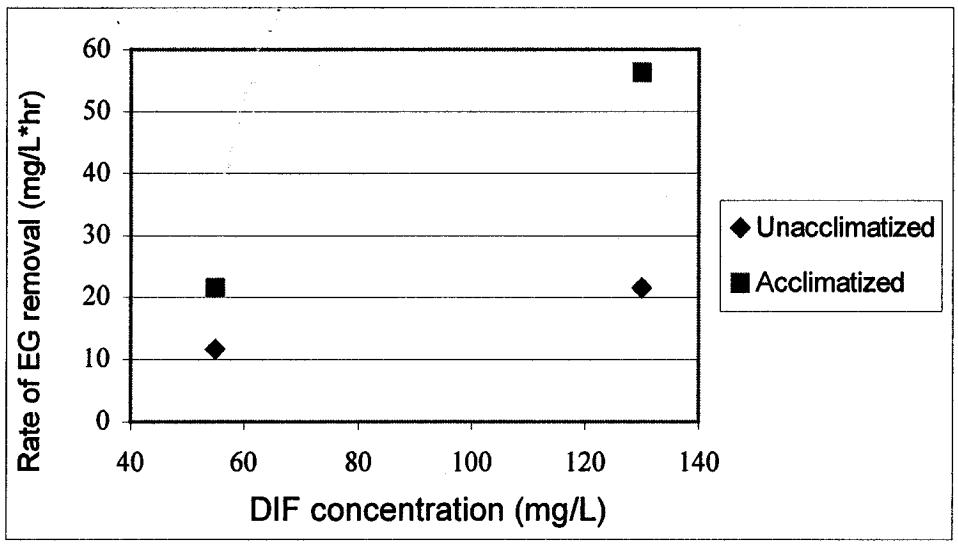


Figure 5.4.12 : Initial rate of EG removal vs. initial DIF



d) SOUR

From Table 5.4.10, specific oxygen uptake rates are higher for the acclimatized biomass treating the synthetic base. However, at both deicing fluid levels, the initial SOUR does not differ much between both types of sludges. Hence, overall, the acclimatized populations show higher activities than the unacclimatized populations.

e) Sludge Volume Index (SVI)

SVI values tend to indicate poorer settling characteristics of the acclimatized sludge for all three wastewater conditions but especially when deicing fluid is present (see Table 5.4.3).

Table 5.4.10 : Comparison of Specific Oxygen Uptake Rates

	Unacclimatized	Acclimatized	Unacclimatized	Acclimatized	Unacclimatized	Acclimatized
DIF conc.	0 mg/L		65 mg/L		130 mg/L	
Time (hr)	SOUR (mg/min per g TSS)		SOUR (mg/min per g TSS)		SOUR (mg/min per g TSS)	
1	0.04	0.21	0.30	0.28	0.28	0.30
2,5	0.02	0.20	0.15	0.24	0.14	0.28
5	0.06	0.09	0.08	0.12	0.13	0.13
7	0.03	0.07	0.03	0.11	0.08	0.09

Table 5.4.11 : Comparison of initial and final SVI

	Unacclimatized	Acclimatized	Unacclimatized	Acclimatized	Unacclimatized	Acclimatized
Deicing conc. (mg/L)	0		65		130	
Initial SVI	380	230	280	240	250	160
Final SVI	250	330	240	430	220	470

The results indicate that acclimatized micro-organisms are much more efficient than unacclimatized biomass in degrading glycol-contaminated wastewaters. In contrast, this sludge seems to fair poorly when only the synthetic base is present. Even though its sludge settling characteristics are slightly inferior, the high removal rates show the advantages of acclimatized biomass.

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Comparison of Unacclimatized and Acclimatized Populations

Table 15 (Appendix D) shows the compounds whose consumption changes (above the calculated threshold limit) between the final populations of the three sets of comparative experiments. The number of compounds whose consumption varies between the unacclimatized and acclimatized populations ranges from 6 to 11 and they mainly consist of amino acids, carboxylic acids and carbohydrates. Although the number of highly consumed compounds is relatively similar, there are higher consumption values for the unacclimatized populations (Tables 16 to 18 in Appendix D). Thus, the acclimatized populations

exhibit a poorer ability than the unacclimatized population to metabolize the variety of substrates that are contained in the microplate. A similar trend is found and further discussed in the Biolog results for the sequencing batch reactor (SBR) experiments (refer to Section 6.4)

5.5 Effect of Temperature

In order to gain insight into the effect of operating at a low temperature, batch experiments were conducted at 5°C. The operating conditions at which the low temperature batch experiments were conducted are presented in Table 5.5.1.

Table 5.5.1 : Experimental conditions of low temperature batch experiments

Temperature = 5°C	TSS (mg/L)	DIF concentration (mg/L)
Experiment 1	300	0
Experiment 2	2000	0
Experiment 3	300	130

A low temperature experiment with a higher deicing fluid concentration (2000 mg/L of TSS and 190 mg/L DIF) was studied by a sequencing batch reactor experiment (refer to Section 6.3). These four experiments allowed the comparison of summer (20°C) and winter (5°C) conditions in the presence and absence of deicing fluid. In addition, the kinetics were compared at normal operating biomass levels of 2000 mg/L and at a low biomass concentration of 300 mg/L.

a) TSS

Tables 5.5.2 and 5.5.3 show the initial and final TSS values for the experiments conducted at room temperature (approximately 20°C) and those conducted at low temperature (approximately 5°C). From these results, one can see that there is very little change between the initial and final values of biomass concentrations for both the high and the low temperatures. In particular, the decrease in temperature and ensuing decrease in kinetics may play a role in hindering the growth of biomass under these conditions. Moreover, biomass growth did not occur at room temperature so no increase in TSS values at the lower temperature of 5 °C was expected.

Table 5.5.2 : TSS values for experiments run at 20°C

Deicing conc. (mg/L)	Initial TSS (mg/L)	Final TSS (mg/L)
0	2140	1930
0	280	270
130	300	390

Table 5.5.3 : TSS values for experiments run at 5°C

Deicing conc. (mg/L)	Initial TSS (mg/L)	Final TSS (mg/L)
0	1970	1900
0	300	340
130	400	340

b) COD and TOC

Figures 5.5.1 to 5.5.6 present a comparison of the COD and TOC profiles (with respect to time) obtained for the three sets of experiments performed at 5 °C and 20 °C. From these figures, it is observed that the removal kinetics are significantly lowered when the experiments are performed at 5 °C. In certain cases, final COD values are an order of magnitude higher between these two temperatures. In order to examine these trends more closely, the initial specific rates for COD and TOC removal were calculated and are presented in Figures 5.5.7 and 5.5.8. The initial specific rates of TOC and COD removal are almost the same for both temperatures at the biomass concentration of 2000 mg/L. Thus, the difference in rates between temperatures is the least pronounced for the experiment conducted at the higher biomass level.

When comparing the rates for the experiments conducted at 300 mg/L of biomass, the absence of deicing fluid results in a greater difference in kinetics between the high and low temperatures. Therefore, an increase in the TSS and/or the presence of a higher amount of substrate (through the addition of the deicing fluid) can diminish the impact of a decrease in biodegradation kinetics associated with a lower operating temperature.

Figure 5.5.1 : COD profile for 20°C vs. 5°C (300 mg/L of TSS and 0 mg/L DIF)

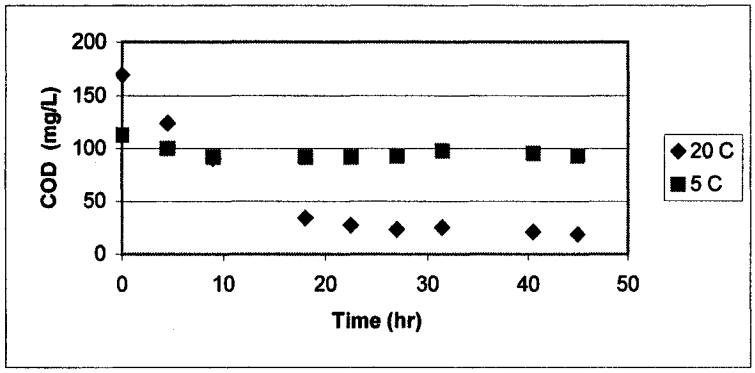


Figure 5.5.2 : TOC profile for 20°C vs. 5°C (300 mg/L of TSS and 0 mg/L DIF)

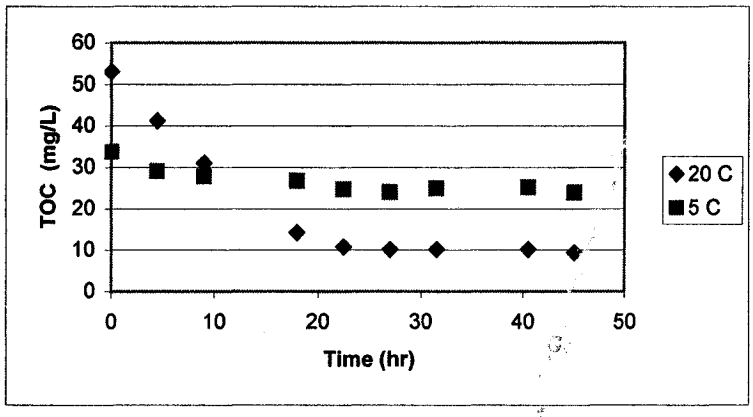


Figure 5.5.3 : COD profile for 20°C vs. 5°C (2000 mg/L of TSS and 0 mg/L DIF)

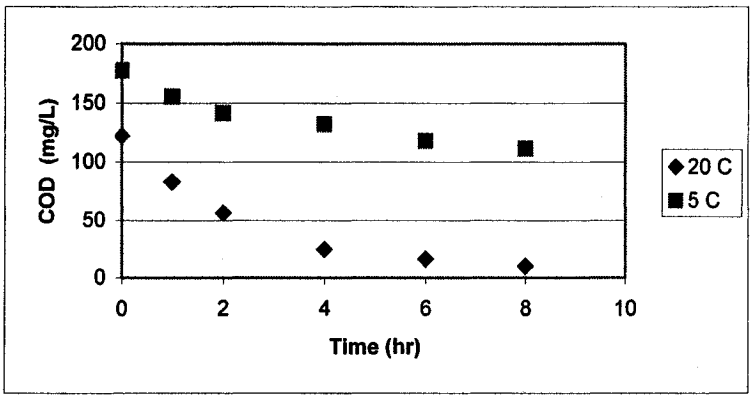


Figure 5.5.4 : TOC profile for 20°C vs. 5°C (2000 mg/L of TSS and 0 mg/L DIF)

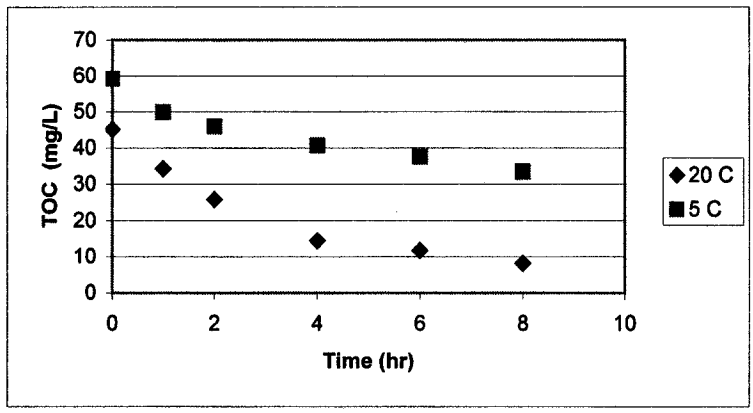


Figure 5.5.5 : COD profile for 20°C vs. 5°C (300 mg/L of TSS and 130 mg/L DIF)

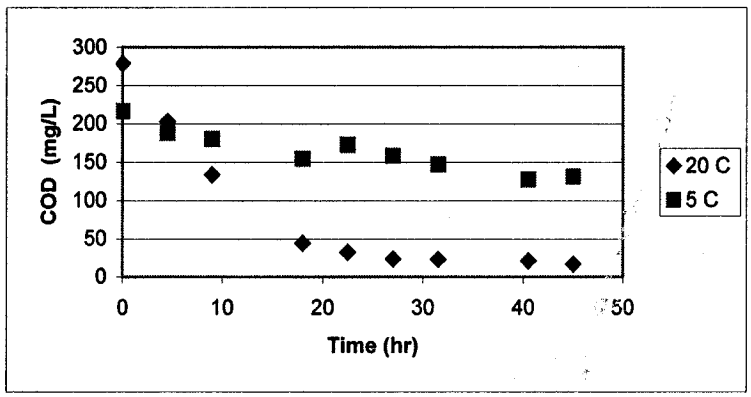


Figure 5.5.6 : TOC profile for 20°C vs. 5°C (300 mg/L of TSS and 130 mg/L DIF)

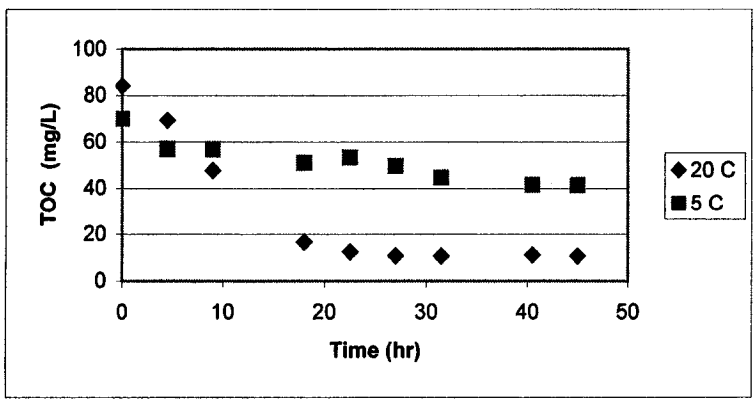


Figure 5.5.7 : Initial specific rate of COD removal for 20°C vs. 5°C (see Table 5.5.1 for the experimental conditions)

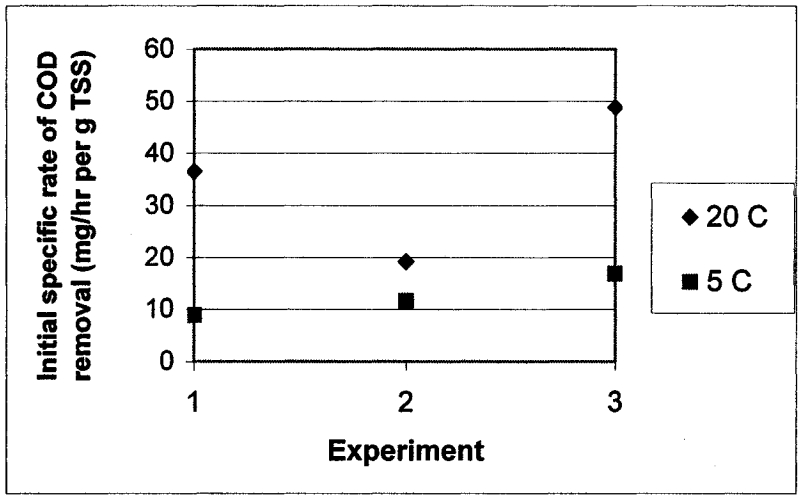
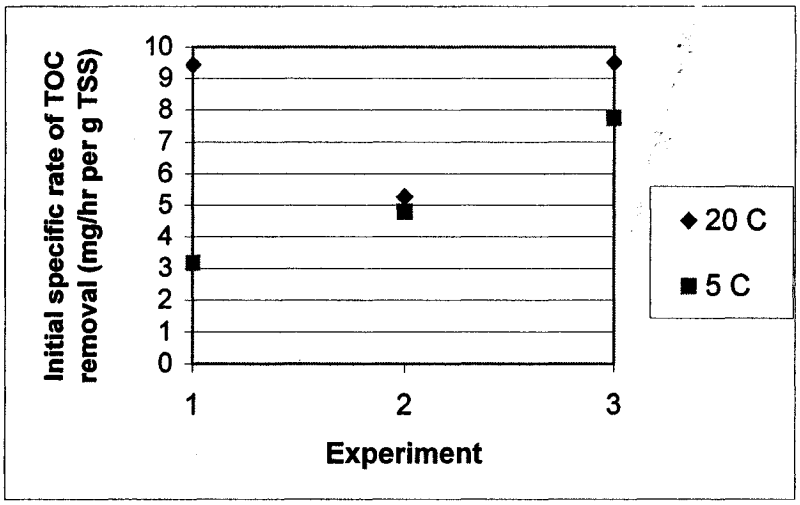


Figure 5.5.8 : Initial specific rate of TOC removal for 20°C vs. 5°C (see Table 5.5.1 for the experimental conditions)



c) EG

Figures 5.5.9 and 5.5.10 show the comparison of the ethylene glycol profile (with respect to time) and the specific removal rate for the high and low temperatures. As expected, there is a noticeable decrease in removal kinetics

when the experiment is conducted at the lower temperature. However, for both temperatures, ethylene glycol is degraded within the first 18 hours of operation and high residual concentrations are not observed as in the case of COD and TOC. In fact, the experiment performed at 5 °C shows a very little change in the COD and TOC values following the complete removal of the deicing fluid. Hence, these results further support the experimental evidence that ethylene glycol is a preferred substrate and entails an increase in microbial activity when it is present.

Figure 5.5.9 : EG profile for 20°C vs. 5°C (300 mg/L of TSS and 130 mg/L DIF)

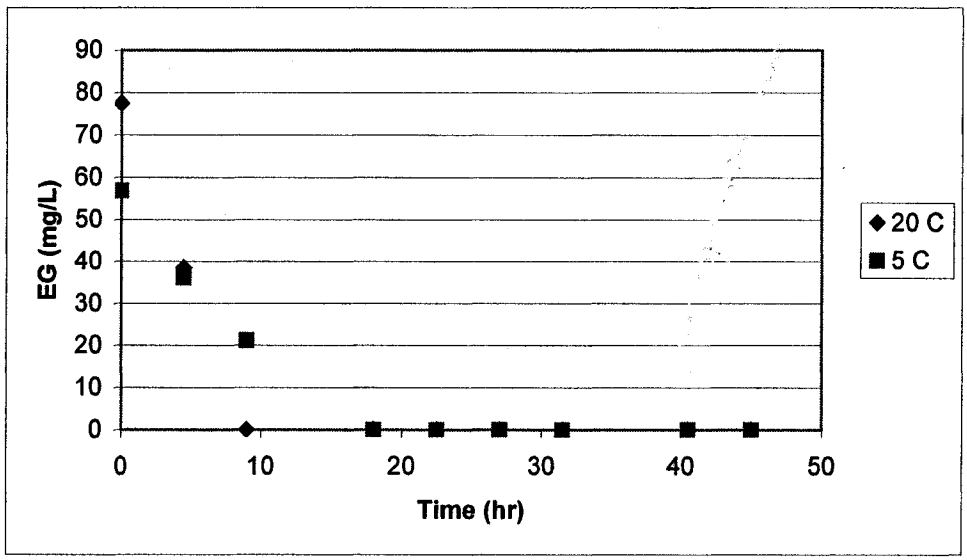
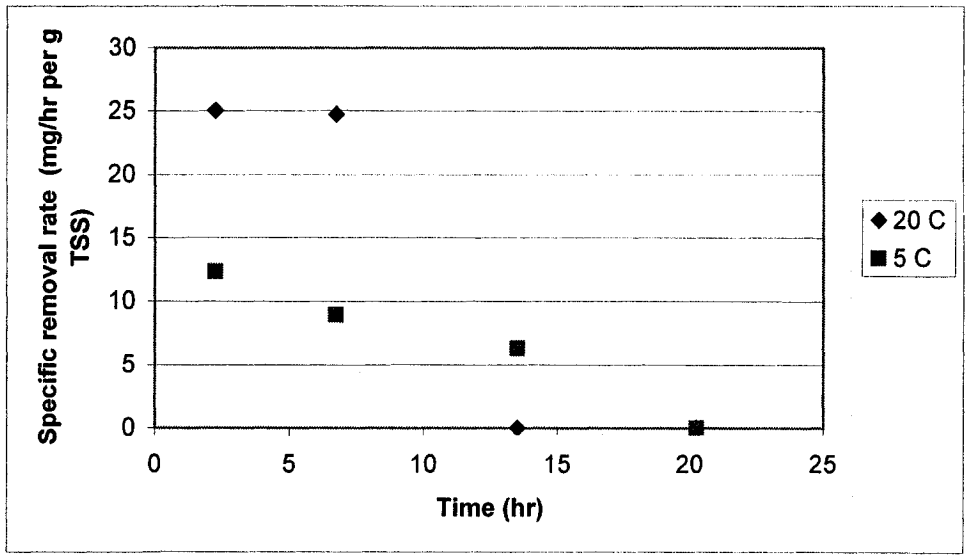


Figure 5.5.10 : Specific rate of Ethylene Glycol removal for 20°C vs. 5°C



d) OUR and SOUR

Tables 5.5.4 and 5.5.5 show the specific oxygen uptakes rates for the three sets of experiments. The first startling result is that the oxygen consumption tends to be higher at the lower temperature for all experimental conditions. This is not expected since one would expect that faster kinetics would require a greater consumption of oxygen in order to degrade the organic material. However, the stress on the microorganisms caused by the decrease in temperature may lead to oxygen consumption that is not associated with the substrate degradation.

As expected, the presence of deicing fluid increases the respiration rate and there is a greater increase in the specific oxygen uptake rates at 5 °C than at 20 °C. Hence, the effect on microbial activity caused by the deicing fluid seems

to be greater at the lower temperature where the microorganisms are under more stress.

Table 5.5.4 : SOUR for 20°C vs. 5°C (experiments done at 300 mg/L of TSS)

	Experiment 1 (300 mg/L TSS, 0 mg/L DIF)		Experiment 3 (300 mg/L TSS, 130 mg/L DIF)	
Temp.	20°C	5°C	20°C	5°C
Time (hr)	SOUR (mg/min per g TSS)		SOUR (mg/min per g TSS)	
0	0.30	0.48	0.32	0.78
4.5	0.39	0.41	0.64	0.34
9	0.36	0.31	0.56	0.27
18	0.21	0.29	0.31	0.34
22.5	0.09	0.28	0.06	0.36
27	0.08	0.28	0.06	0.24
31.5	0.08	0.28	0.06	0.21
40.5	0.10	0.22	0.04	0.17
45	0.07	0.27	0.05	0.16

Table 5.5.5 : SOUR for 20°C vs. 5°C (experiment done at 2000 mg/L of TSS)

	Experiment 2 (2000 mg/L TSS, 0 mg/L DIF)	
Temp.	20°C	5°C
Time (hr)	SOUR (mg/min per g TSS)	
1	0.04	0.11
2.5	0.02	0.09
5	0.05	0.09
7	0.03	0.08

e) Sludge Volume Index (SVI)

Table 5.5.6 shows the initial and final values for the sludge volume index (SVI) between the experiments run at 5 °C and 20 °C. However, since the initial values vary extensively between the experiments done at these two temperatures, there are very little conclusions that can be drawn with regards to

the effect of temperature on the settleability of the biomass. Please refer to Section 4.8 (SBR experiments) for more conclusive results on this topic.

Table 5.5.6 : Initial and Final SVI values for 20°C vs. 5°C

	Experiment 1		Experiment 2		Experiment 3	
Temp.	20°C	5°C	20°C	5°C	20°C	5°C
Initial SVI	140	40	380	40	140	40
Final SVI	220	20	250	60	250	20

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There are less significant changes between the initial and final populations for the experiments performed at the lower temperature than those conducted at room temperature. In fact, there are very few (0 and 2) compounds that exhibit a change above the threshold value (see Table 11 in Appendix D). Moreover, the number of high consumption compounds remains practically the same for the initial and final populations for the low temperature experiments (see Tables 13 and 14 in Appendix D). Hence, the evolution of the biomass depends on the operating temperature as the low temperature leads to a smaller change in the microbial population. The effect of temperature on the microbial population will be further discussed in Chapter 6.

5.6 Kinetic Modelling : Batch Experiments

As was shown in the previous sections, there are noticeable differences in rates of organic matter removal based on parameters such as biomass

concentration, temperature and other variables. Traditionally, in order to quantify these results, a mathematical model is established to describe two phenomena : substrate utilization and biomass growth. However, the experimental conditions were such that no significant microbial growth was observed in all cases. Hence, traditional models based mainly on Monod kinetics that link biomass growth to substrate utilization are not applicable to the current analysis. The shortcomings of the Monod model became apparent as there are significant rates of organic matter removal with very little observable biomass growth. According to Monod model, no biomass growth translates into no substrate removal and vice versa. This approach neglects the possibility of substrate utilization to merely maintain the microbial population without biomass growth.

Hence, the approach that was employed is to model the rate of substrate utilization using the equations presented in Section 2.2. For each experiment, the order of the substrate utilization reaction was determined using a regression analysis (refer to Section 2.2) and thereafter, kinetic constants for the removal of TOC and COD (see Appendix F for details of regression). It must be noted that ethylene glycol kinetic constants were also determined but due to the limited number of data, conclusive values could not be obtained. Therefore, ethylene glycol kinetic modeling was excluded from this Section.

The order was determined based on the linearity of the graphs obtained using the 0th and 1st order kinetic model (based on the closeness of the value of

R^2 to 1). . Based on the regressions (see Appendix F), the results indicate that TOC and COD follow 1st order kinetics. This result was consistent for almost all batch experiments.

In addition, the effect of temperature was determined using a model based on the Arrhenius equation. The following sections present the numerical values of these constants and the impact of different variables on their magnitude. In most cases, a limited number of data points were used in the calculation of the rate constants. Thus, these constants should be used with caution and only for comparing the experimental conditions. It must also be noted that normally for a fixed microbial population, the rate constants (at a given temperature) should be the same no matter what the experimental conditions are. Hence, large variations in the kinetic constants can be attributed to differences in the microbial population.

Effect of Biomass Concentration

Tables 5.6.1 and 5.6.2 present the kinetic constant values (k') for the COD and TOC of the 16 basic batch experiments. The results show a consistent decrease in the kinetic constant k' as the biomass concentration increases. More specifically, the values at the two lower biomass concentrations (300 mg/L and 1000 mg/L of TSS) are similar to each other as are the values at the two higher concentrations (2000 mg/L and 3000 mg/L of TSS). Based on these results, the microbial activity decreases at the two highest biomass concentrations where the

limited amount of food may not allow for all the biomass to participate in the degradation reaction. The linearity of the graphs employed to determine the values of the kinetic constant are generally very high. In fact, for the 32 kinetic constants found for the COD and TOC, only 1 case demonstrates a regression coefficient (R^2) less than 0.97.

Table 5.6.1 : TOC kinetic constant k' for batch experiments

k' values (L/(g TSS-hr))	TSS = 300 mg/L	TSS = 1000 mg/L	TSS = 2000 mg/L	TSS = 3000 mg/L
DIF = 0 mg/L	0.27	0.21	0.14	0.11
DIF= 35 mg/L	0.32	0.36*	0.13	0.10
DIF = 65 mg/L	0.48	0.40	0.11	0.11
DIF = 130 mg/L	0.27	0.30	0.08	0.06

* = R^2 less than 0.97 (0.93 in this case)

Table 5.6.2 : COD kinetic constant k' for batch experiments

k' values (L/(g TSS-hr))	TSS = 300 mg/L	TSS = 1000 mg/L	TSS = 2000 mg/L	TSS = 3000 mg/L
DIF = 0 mg/L	0.32	0.21	0.20	0.12
DIF= 35 mg/L	0.58	0.35	0.20	0.12
DIF = 65 mg/L	0.50	0.39	0.15	0.13
DIF = 130 mg/L	0.30	0.28	0.10	0.08

Effect of Deicing Fluid Concentration

From Tables 5.6.1 and 5.6.2, there is a general increase in the kinetic constant value as the deicing fluid concentration is raised. This result is not surprising as it has been established that an increase in the concentration of

deicing fluid entails an increase in the rates of TOC and COD removal. Similarly to the removal rates, there is a slight dip in the reaction constants at the highest value of the deicing fluid. This result is found for all four biomass concentrations but the data does not enable to discern at which biomass concentration this effect is more pronounced.

Effect of Biomass Acclimatization

When comparing the results from the previous section (Tables 5.6.1 and 5.6.2) to Tables 5.6.3 and 5.6.4, in the presence of deicing fluid, higher values of the TOC and COD kinetic constants (k') are obtained for the acclimatized population. These results mimic the trend observed in the removal rates as the acclimatized population seems to thrive when the deicing fluid is present. However, the acclimatized population demonstrates similar kinetic constants when the wastewater contains only the synthetic base. Once again, the R^2 coefficients from the determination of kinetics are above 0.98 for all three acclimatized experiments.

Table 5.6.3 : TOC kinetic constants for the glycol-acclimatized population

DIF (mg/L)	0	65	130
TSS (mg/L)	2000	2000	2000
k' (L/(g TSS-hr))	0.14	0.18	0.20

Table 5.6.4 : COD kinetic constants for the glycol-acclimatized population

DIF (mg/L)	0	65	130
TSS (mg/L)	2000	2000	2000
k' (L/(g TSS-hr))	0.15	0.18	0.30

Effect of Temperature

As can be expected (based on the removal rate results) the kinetic constants for TOC and COD removal show a significant decrease at the lower temperature (Tables 5.6.5 and 5.6.6 compared to Tables 5.6.1 and 5.6.2). Using a model based on the Arrhenius equation, the theta values (see Section 2.2) obtained are towards the higher end of the spectrum as typical values for the activated sludge range between 1.01 and 1.09 (Table 5.6.7). This indicates more important differences between the values obtained at the high and low temperature.

Table 5.6.5 : TOC kinetic constants for batch experiments done at 5°C

DIF (mg/L)	0	0	130
TSS (mg/L)	2000	300	300
k' (L/(g TSS-hr))	0.07	0.07	0.04

Table 5.6.6 : COD kinetic constants for batch experiments done at 5°C

DIF (mg/L)	0	0	130
TSS (mg/L)	2000	300	300
k' (L/(g TSS-hr))	0.06	0.07	0.05

Table 5.6.7 : Values of θ for TOC and COD kinetic constants

DIF (mg/L)	0	0	130
TSS (mg/L)	2000	300	300
θ for TOC	1.06	1.09	1.13
θ for COD	1.09	1.10	1.13

Comparative Results for Replicates

The replicates for the 3 experiments show varying degrees of repeatability in the values for the kinetic constants (Tables 5.6.8 and 5.6.9 compared to Tables 5.6.1 and 5.6.2). This is directly linked to the repeatability of the experiments and depends on a similarity of initial conditions for each replicate experiment. As was previously discussed (refer to section 5.2), the experiments conducted at 2000 mg/L of TSS show the biggest difference in initial organic matter concentration between replicates. In addition, the replicate trials at the lowest biomass level were carried out with very similar initial conditions. Therefore, since the TOC and COD follow first order kinetics, the constants are expectedly higher for the replicate experiment at 2000 mg/L as the initial concentration of organic matter was approximately 25% higher. The results for the COD and TOC constants are more similar for the two other sets of experiments. Once again, regression coefficients describing the fit of the 1st order model show high values for the replicate experiments.

Table 5.6.8 : TOC kinetic constants for replicate batch experiments

DIF (mg/L)	35	65	130
TSS (mg/L)	2000	1000	300
k' (L/(g TSS-hr))	0.31	0.44	0.21

Table 5.6.9 : COD kinetic constants for replicate batch experiments

DIF (mg/L)	35	65	130
TSS (mg/L)	2000	1000	300
k' (L/(g TSS-hr))	0.33	0.49	0.26

Summary of Results and Discussion (Chapter 5)

The first important result is that degradation rates (TOC, COD and EG) vary significantly with operating conditions such as initial biomass levels, deicing fluid concentration and temperature. Experiments conducted at the same conditions but with a different biomass sample also show differences in the degradation kinetics. Moreover, the changes in kinetics are clearly shown in the comparison of unacclimatized and acclimatized populations where the two populations respond very differently to the presence of deicing fluid. Therefore, although the rate constants obtained for these runs should be independent of the experimental conditions (at a fixed temperature), significant differences are observed. These results suggest that there are significant differences in the microbial population between each experiment. These variations are further examined with the Biolog technique which shows the specific changes in the microbial population for the batch experiments.

CHAPTER 6

RESULTS AND DISCUSSION : SEQUENCING BATCH REACTOR EXPERIMENTS

Sequencing batch reactor (SBR) experiments were conducted in order to gain insight into the treatment of deicing wastes with this type of reactor setup. More specifically, as literature suggests that the treatment of deicing fluid has a negative impact on sludge settling characteristics, SBR operation was selected as it is reported to improve sludge settling. In addition, SBR has many advantages (refer to Section 1.1) when compared to conventional wastewater treatment systems and thus, the kinetic rates as well as the microbial population dynamics observed under various conditions can be employed to help design SBR systems treating municipal and deicing wastes.

It must be noted for each individual day, only the data from one 8 hour (or 12 hour) cycle is presented. The TSS and sludge blanket results present the corresponding day directly on the time axis. On the other hand, the results for the COD, TOC, EG and SOUR present the day at which the samples were taken in the legend of each Figure. Moreover, vertical dashed lines separate the individual 8 hour (or 12 hour) cycles. For example, Figure 6.1.7 presents the TOC data for a total of 9 separate 12 hour cycles obtained between day 1 and day 12 of the SBR experiment.

6.1 Effect of Cycle Length

First of all, the total suspended solids for the 12 hour cycles and the 8 hour cycles remain relatively constant with respect to time (Figures 6.1.1 and 6.1.2). The initial TSS values between the two experiments are slightly different but the cycle time does not seem to have an effect on the change in biomass with time. Thus, as one might have expected, there is insufficient amounts of substrate to promote microbial growth for either type of cycle. However, there seems to be enough substrate for biomass maintenance even for the cycle time of 12 hours where the fixed amount of substrate does not produce any significant biomass decay in spite of residual organic matter levels being attained within the first 5 to 6 hours of operation.

Regarding, biomass concentration in the treated effluent, there are significant decreases in the 12 hour cycle and the 8 hour cycle (Figures 6.1.3 and 6.1.4). A similar result is obtained for the blanket height (Figures 6.1.5 and 6.1.6). These two parameters (effluent TSS and sludge blanket height) are related as a better settling sludge produces a more compact sludge blanket with fewer floating microorganisms ; thus, less sludge is removed out with the effluent. The sequencing batch reactor is said to operate under starve-feed conditions since at the beginning of each cycle, there is a large amount of substrate fed into the reactor whereas at the end of each cycle, there is very little substrate left in the reactor. As the starve-feed cycling of sequencing batch reactor operation is known to have a positive effect on sludge settling, it can seem that a longer

starvation period may have a more beneficial impact on the sludge settling characteristics.

Figure 6.1.1 : Reactor TSS for 12 hour cycles

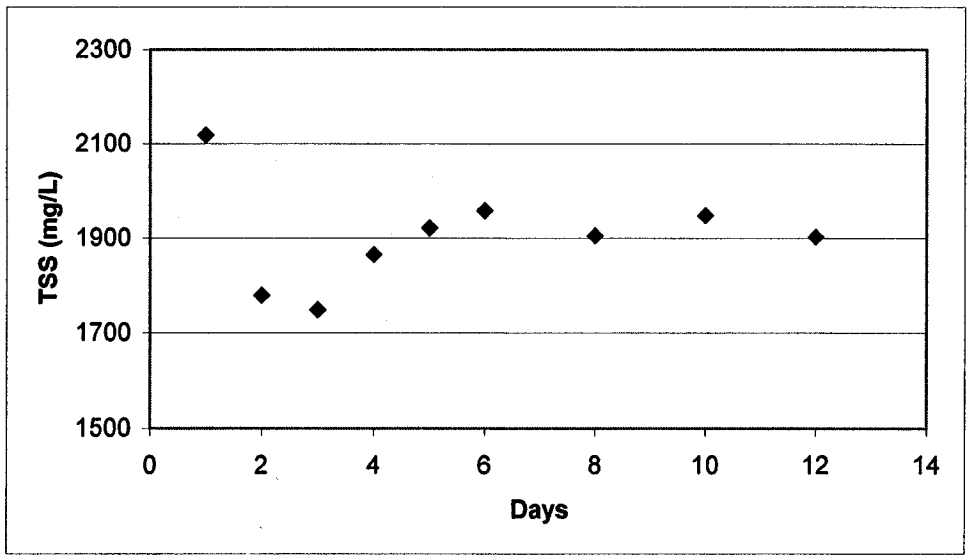


Figure 6.1.2 : Reactor TSS for 8 hour cycles

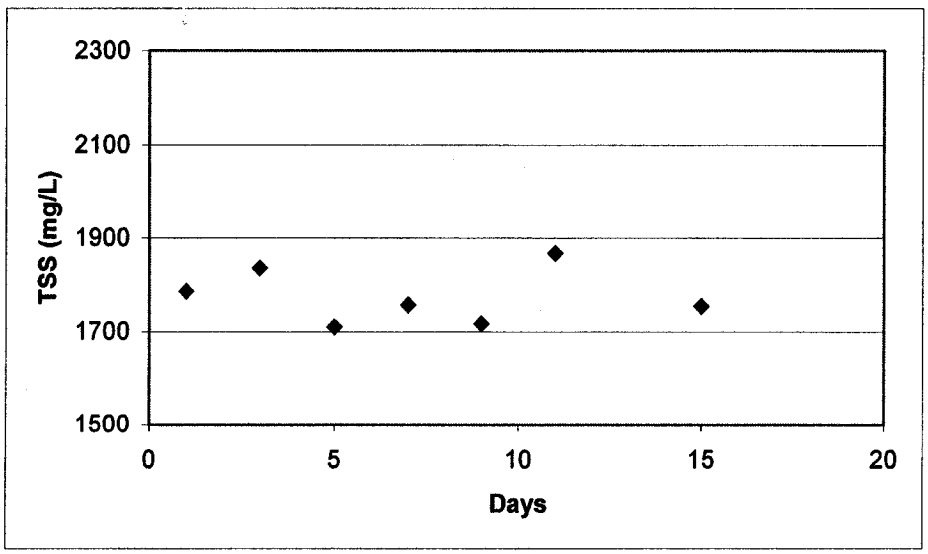


Figure 6.1.3 : Effluent TSS for 12 hour cycles

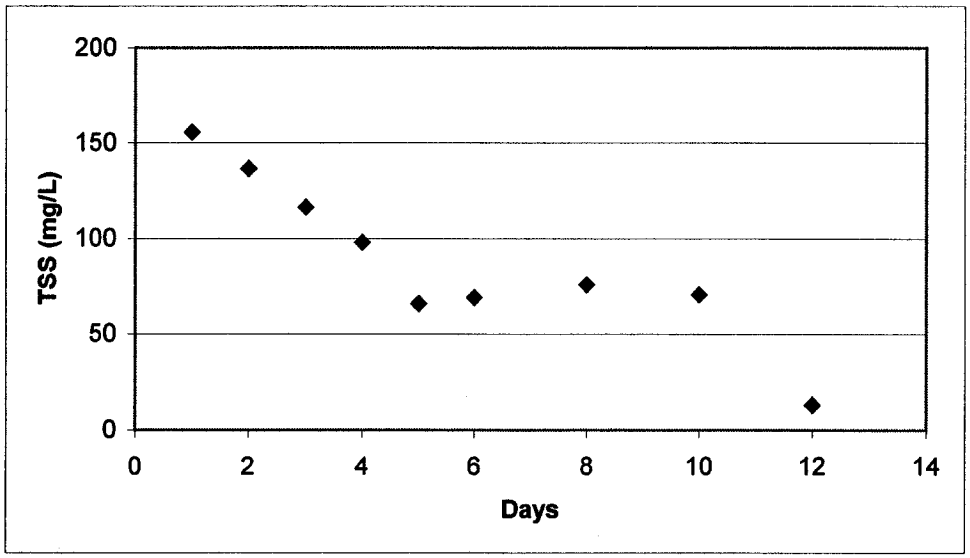


Figure 6.1.4 : Effluent TSS for 8 hour cycles

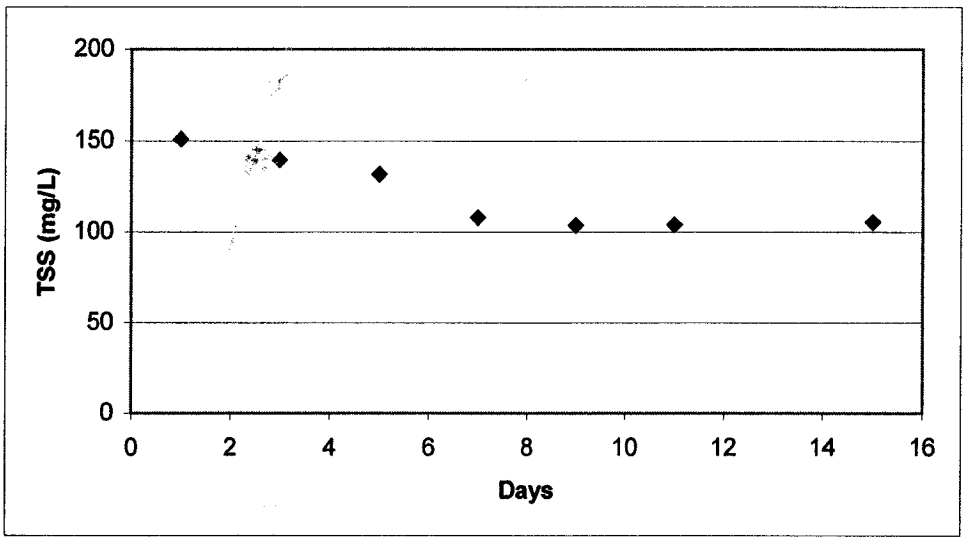


Figure 6.1.5 : Sludge blanket height for 12 hour cycles

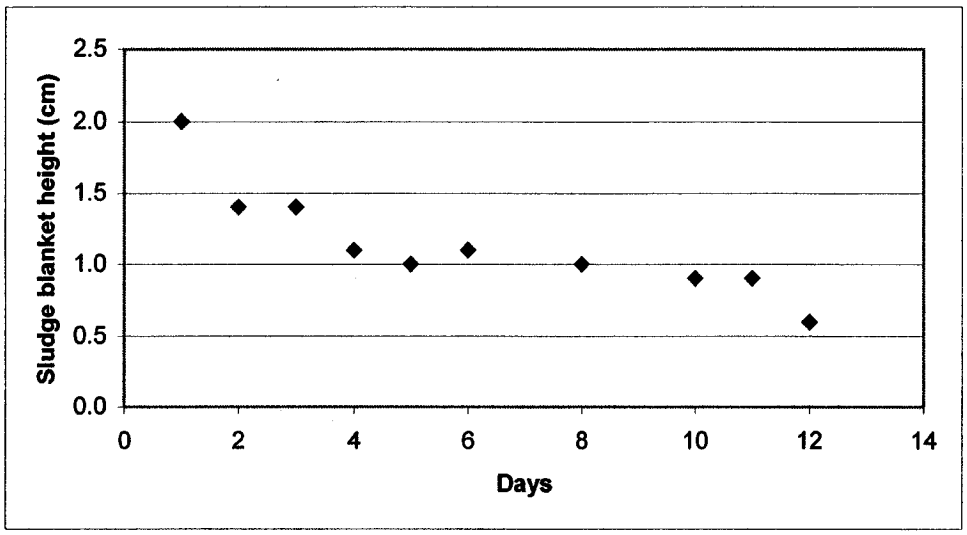
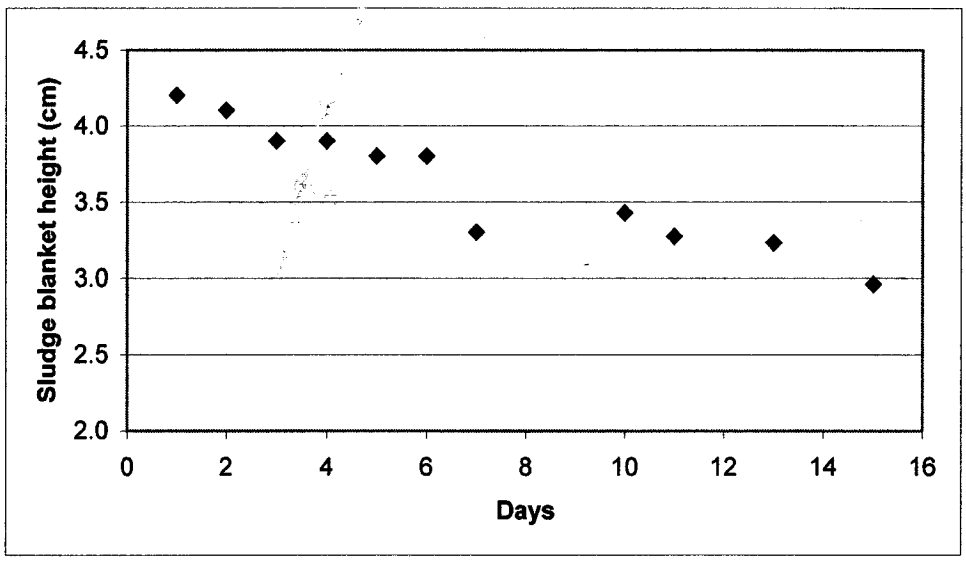


Figure 6.1.6 : Sludge blanket height for 8 hour cycles



The graphs for COD and TOC removal are given in Figure 6.1.7 (12 hour cycles) and Figures 6.1.8 and 6.1.9 (8 hour cycles). As can be seen from these graphs, both cycle times allow for almost complete removal of organic matter. In

fact, the TOC does not vary very much after 4-6 hours of aeration. Hence, residual values are obtained for both cycle times well before the end of the aeration period. Therefore, the main observation is that both cycle times allow for almost complete removal of organic matter with residual TOC and COD values approximately equal for both time cycles.

Figure 6.1.7 : TOC profile for 12 hour cycles (2000 mg/L TSS, 190 mg/L DIF)

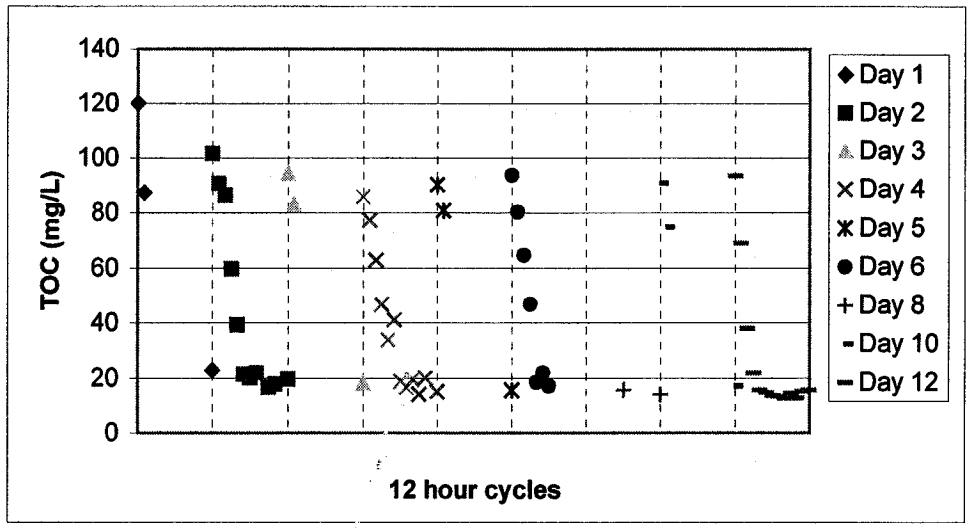


Figure 6.1.8 : TOC profile for 8 hour cycles (2000 mg/L TSS, 190 mg/L DIF)

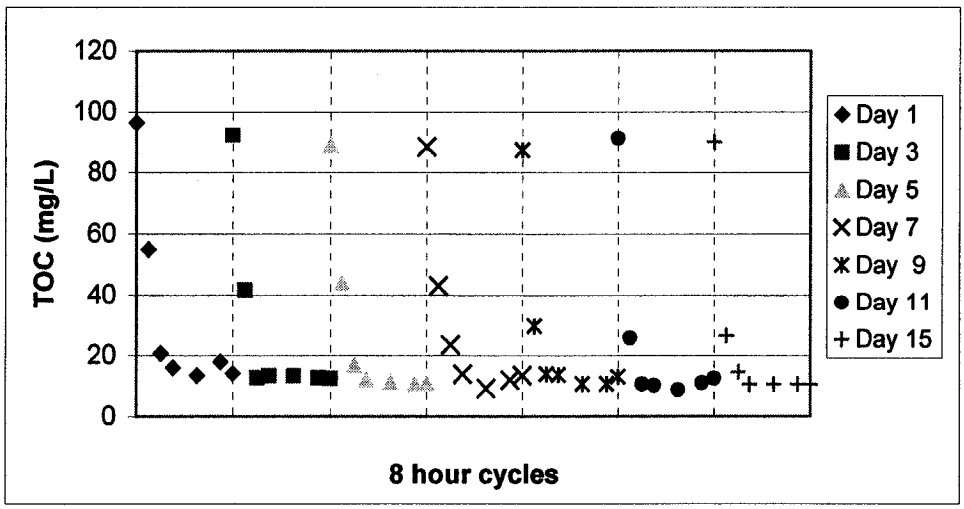
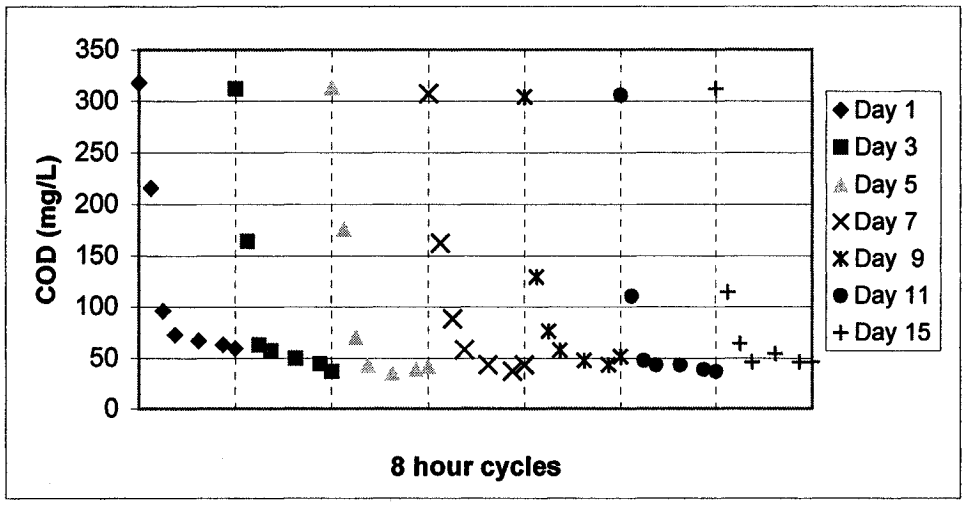


Figure 6.1.9 : COD profile for 8 hour cycles (2000 mg/L TSS, 190 mg/L DIF)



Figures 6.1.10 (12 hour cycles) and 6.1.11 (8 hour cycles) show the removal of ethylene glycol with time. Similarly to the TOC and COD, ethylene glycol is completely removed within the first few hours (approximately 3 to 4 hours) of aeration for both cycle times. Therefore, both cycle times allow for complete removal of ethylene glycol within the first few hours of operation and in relation to the TOC and COD values, it is removed at a quicker rate (it is a preferred substrate).

Figure 6.1.10 : EG profile for 12 hour cycles (2000 mg/L TSS, 190 mg/L DIF)

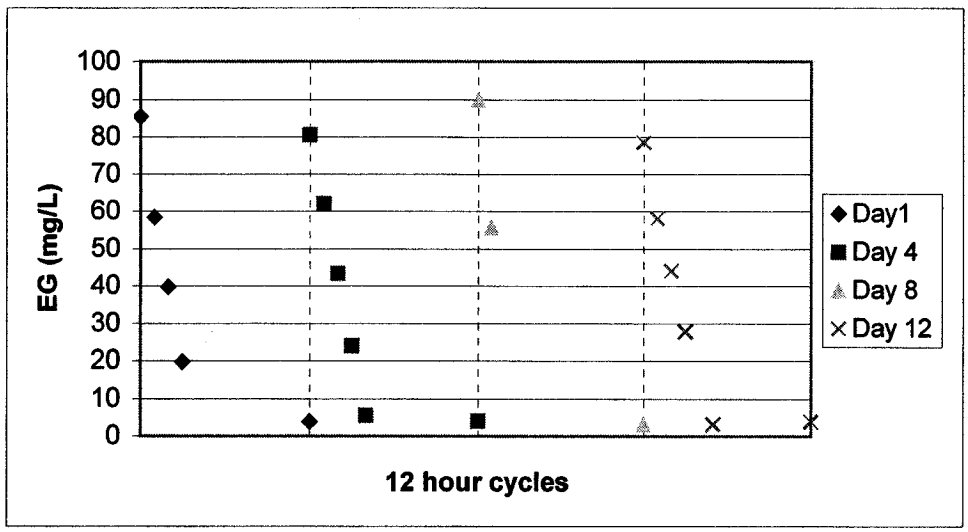
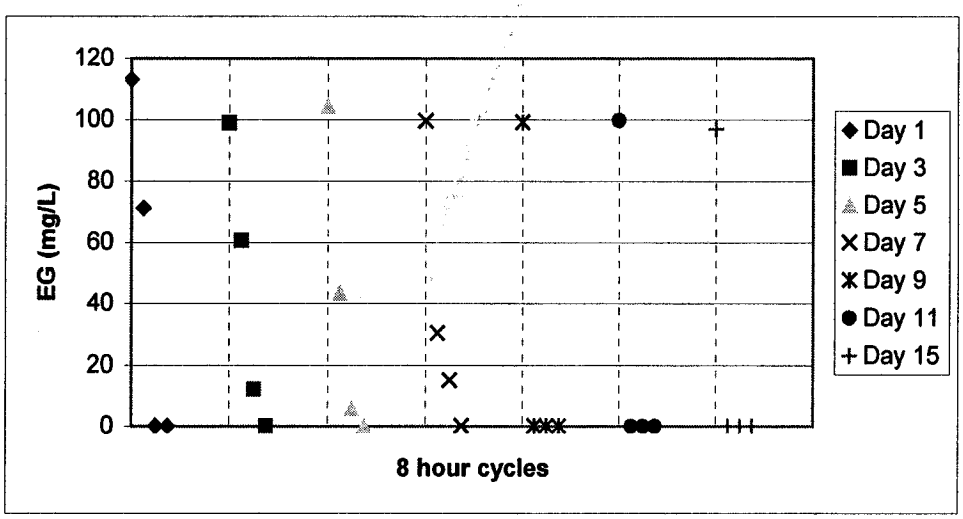


Figure 6.1.11 : EG profile for 8 hour cycles (2000 mg/L TSS, 190 mg/L DIF)



From Figures 6.1.12 and 6.1.13, it is seen that the specific oxygen uptake rates show a significant decrease with time that can be associated with the decrease in available substrate for both cycle times. With respect to difference between cycle times, the 8 hour cycles show relatively higher SOUR values

during the initial cycles than the 12 hour cycles. This result can be correlated to the amount of organic matter since, as seen by the comparative TOC and COD graphs, there is a higher amount of degradation in the first few hours for the 8 hour cycle. In addition, for both cycle lengths, there is a general decreasing trend in the set of SOUR as the experiment progresses from day to day.

Figure 6.1.12 : SOUR for 12 hour cycles

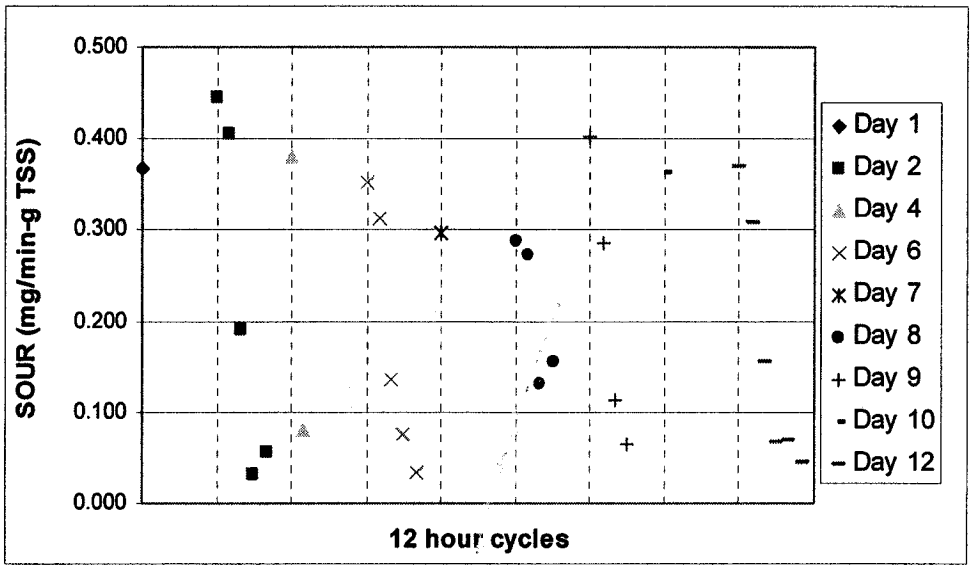
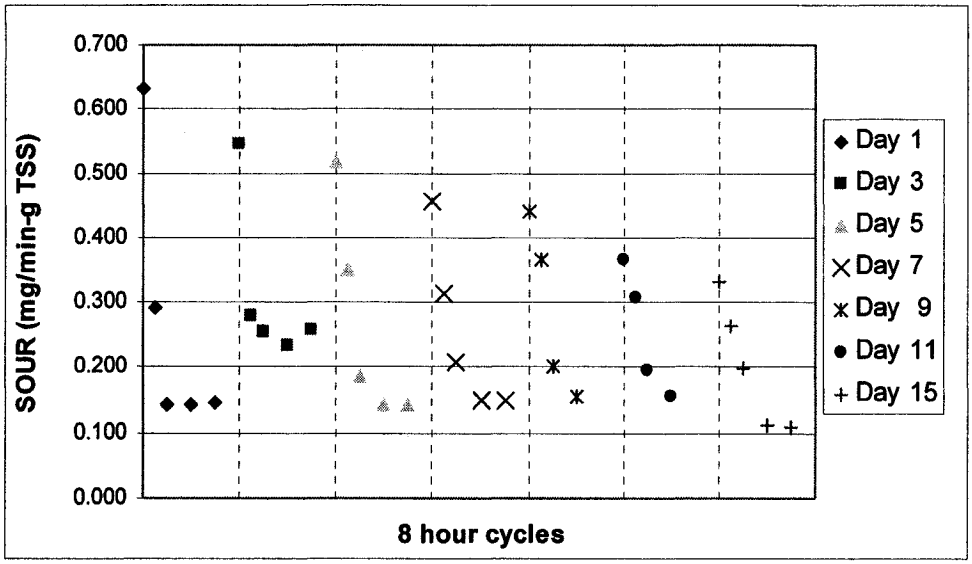


Figure 6.1.13 : SOUR for 8 hour cycles



Therefore, based on these results, it became apparent that a cycle time of 12 hours was not necessary for complete removal of all organic matter to residual levels. Moreover, an 8 hour cycle provided adequate improvements in sludge settling characteristics. Hence, further experiments were conducted with this cycle time and the results are shown in the following Sections.

6.2 Acclimatization

Past literature has shown that the treatment of wastes by sequencing batch reactors provides an interesting alternative to traditional wastewater treatment systems. More specifically, as the biomass is subjected to the same type of wastewater over many cycles, one would expect an acclimatization phenomenon to take place. This would entail positive effects such as the development of a specialized population to treat the specific wastewater with

resulting improvements in the rates of organic matter removal and sludge settling characteristics. These parameters will be examined more closely in the next paragraphs.

As was already seen (Figures 6.1.1 and 6.1.2), there seems to be very little or no growth with the experimental conditions. Thus, the biomass acclimatization with time does not engender any change in growth conditions as there is still a high amount of biomass for a fixed amount of organic matter. With regards to the sludge blanket height, there is a gradual decrease with time. Therefore, the operation of the sequencing batch reactor does have a positive impact on sludge settling as the resulting sludge blanket is more compact. In addition, the amount of biomass present in the treated effluent also shows a decrease with time. As presented in the literature, the presence of deicing fluids has been shown to have a negative impact on sludge settling. Therefore, overall, the positive effects of SBR operation seem to outweigh the negative effects of prolonged exposure of the biomass to the deicing fluid. Thus, over a period of 45 cycles (or 15 days), the net effect is a gradual improvement of sludge settling characteristics.

The results for TOC for the various cycles are presented in the previous section (Figure 6.1.8). There is a clear decrease in the TOC values of the samples taken at the beginning of each aeration period (i.e. the second point in each individual cycle) from day 1 to day 15. This clearly points to an acclimatization phenomenon as the wastewater input stays relatively constant at

approximately 90 mg/L of TOC (i.e. the first point in each individual cycle). There is approximately a 50% reduction as the TOC content of the sample taken at the beginning of the aeration period goes from 55 mg/L at day 1 to 25 mg/L at day 15. By examining the results more closely, it is observed that after approximately 27 cycles (9 days), the values remain relatively constant for the rest of the experiment. Therefore, the acclimatization occurs mostly at the beginning of the cycles and tapers off in the later stages of the experiment. With regards to residual values, there is little change with time as the residual values (i.e. the last point in each individual cycle) go from approximately 15 mg/L of TOC at day 1 to 10 mg/L of TOC at day 15. Thus, the acclimatization affects the initial aeration period to a larger extent but does not have as great an impact on the amount of organic matter that remains refractory to the microorganisms.

Similar results are found with the COD values with an approximate reduction of 50% in the values at the beginning of the aeration period from day 1 to day 15 (Figure 6.1.9). Moreover, the acclimatization period seems to occur between day 1 and day 9 with a less marked difference in the COD profile thereafter. Lastly, the residual values do not decrease in a very significant fashion from cycle 1 to cycle 45.

These results clearly indicate the importance of acclimatization as removal rates are significantly higher as the biomass is exposed to many cycles of operation. Considering that the sludge settling characteristics are also

enhanced, there is a clear advantage to operating with a sequencing batch reactor.

The results for ethylene glycol (Figure 6.1.11) present additional information on the removal rates of the different substrates during acclimatization. The relative effect of acclimatization on the ethylene glycol seems to be even greater than on the other components of the wastewater. Hence, the ethylene glycol concentration at the beginning of the aeration period (i.e. the second point in each individual cycle) is reduced by more than 50% by cycle 21 (day 7) and goes to undetectable levels (i.e. below 5 mg/L) thereafter. Hence, to a great extent, the changes in TOC and COD profiles through acclimatization can be attributed to the ethylene glycol carbon source. In this case, this substrate is completely removed.

With an increase in the organic matter removal rates through acclimatization, the specific oxygen uptake rates seem to follow a decreasing trend from cycle 1 to cycle 45 (Figure 6.1.13). There are a few interesting tendencies in this curve that should be pointed out. Firstly, as the initial rates of removal increase with acclimatization, one would have expected the specific oxygen uptake rate to also show higher values at the beginning of each aeration period as SOUR is an indication of microbial activity. However, this is not the case, as the SOUR values at the beginning of each aeration period tend to decrease from day 1 to day 15. This can be explained by the fact that as the acclimatization process takes place, the amount of organic matter degraded

during the filling of the reactor (which takes 56 minutes) shows a significant increase. Therefore, by the time that the SOUR at the beginning of the aeration period is measured, the microbial activity has decreased since there is less organic matter to be degraded. Another interesting result is that the SOUR reaches a similar plateau for the different cycles of operation. Thus, the acclimatization process does not affect the microbial activity when the substrate has been exhausted.

6.3 Effect of Temperature

Sequencing batch reactor (SBR) experiments were conducted at a high (20°C) and low (5°C) temperature since deicing fluid can be present in the wastewater treatment plant during winter as well as summer climates. In addition, since the rates of removal are expected to be higher under a warmer temperature, an additional experiment was carried out to verify the effect of a temperature disturbance on the treatment of deicing wastes. This experiment was operated at 20°C for seven days then decreased to 5°C between day 8 to day 17 (10 days total).

The results for the SBR experiment performed at room temperature (20°C) have been presented in the earlier sections (refer to Figures 6.1.2, 6.1.4, 6.1.6, 6.1.8, 6.1.9, 6.1.11 and 6.1.13). The results for the SBR experiment performed at 5°C and the SBR experiment conducted at two different temperatures (two-

temperature experiment) are shown below and a comparison between the 3 experiments is then presented.

As can be seen from Figures 6.1.2, 6.3.1 and 6.3.2, there is no particular effect of temperature on the values of biomass present in the reactor for the different cycles. Although kinetics are found to be much faster for the higher temperature (see following sections), there is no significant growth in all of the 3 types of experiments conducted because of the high amount of biomass in the reactor (around 2000 mg/L) in relation to the amount of substrate present. Thus, even though a much longer time frame than the batch experiments is employed, the organic matter only contributes to cell maintenance but not growth. This result seems unaffected by the changes in operating temperature.

Similar results are found for the amount of biomass contained in the treated effluent (Figures 6.1.4, 6.3.3 and 6.3.4). For the experiments conducted at 5°C and at 20°C to 5°C, there is a decrease in the amount of TSS in the effluent from the beginning to the end of the experiment as was the case with the first experiment conducted at 20°C. However, there is no significant trend in the magnitude of this decrease in effluent biomass with respect to the operating temperature. Lastly, it is found that the blanket becomes more compact with time for both sets of experiments (Figures 6.3.5 and 6.3.6). As with the other cases, it is difficult to establish the relative importance of this improvement with respect to temperature.

Figure 6.3.1 : Reactor TSS for 8 hour cycles (T = 5°C)

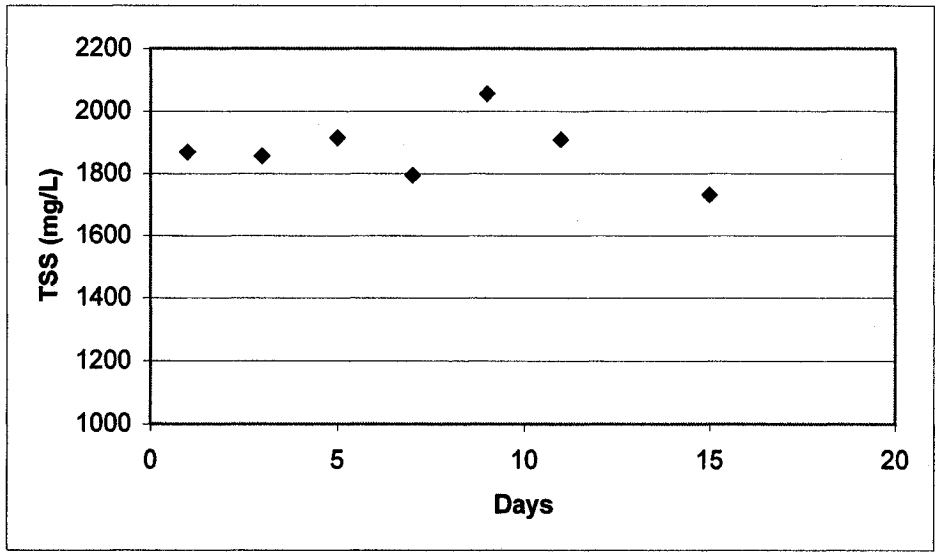


Figure 6.3.2 : Reactor TSS for 8 hour cycles (T = 20°C to 5°C)

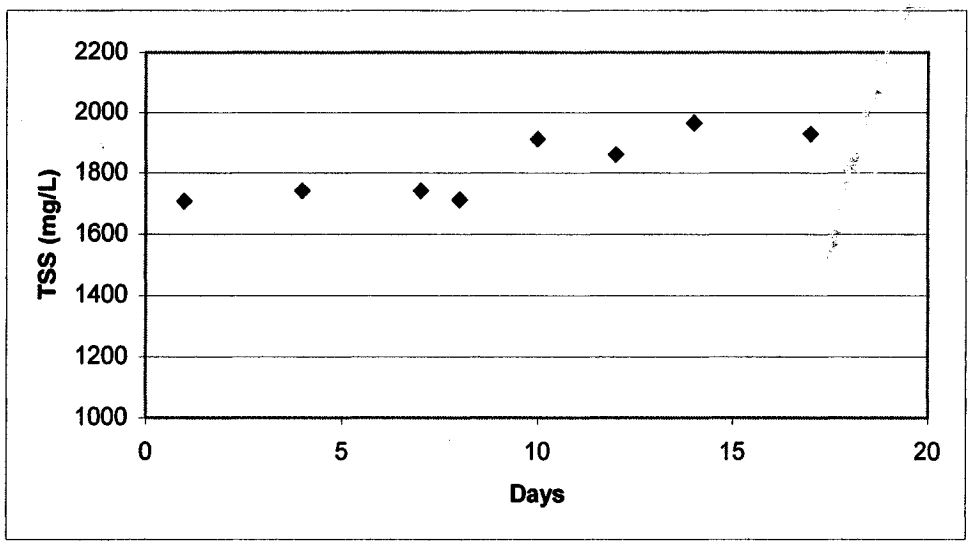


Figure 6.3.3 : Effluent TSS for 8 hour cycles (T = 5°C)

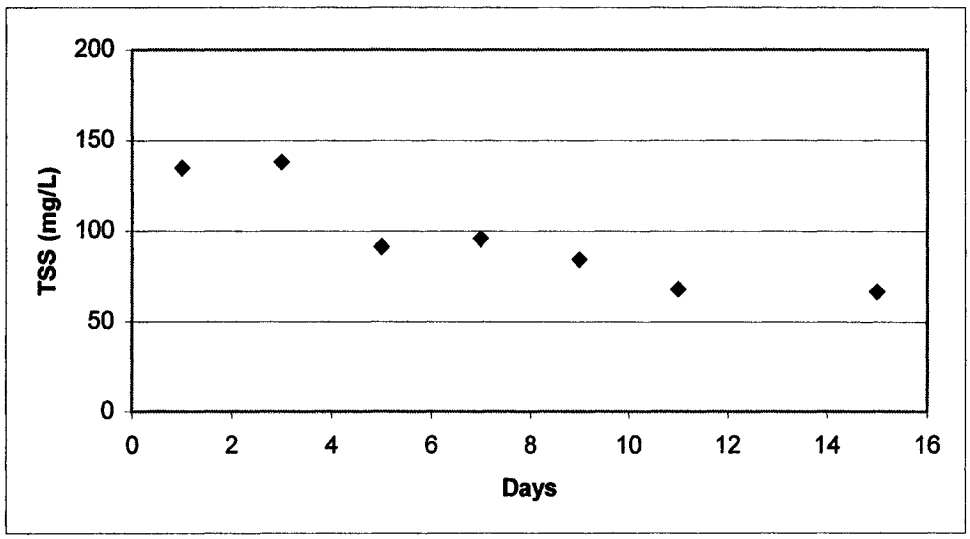


Figure 6.3.4 : Effluent TSS for 8 hour cycles (T =20°C to 5°C)

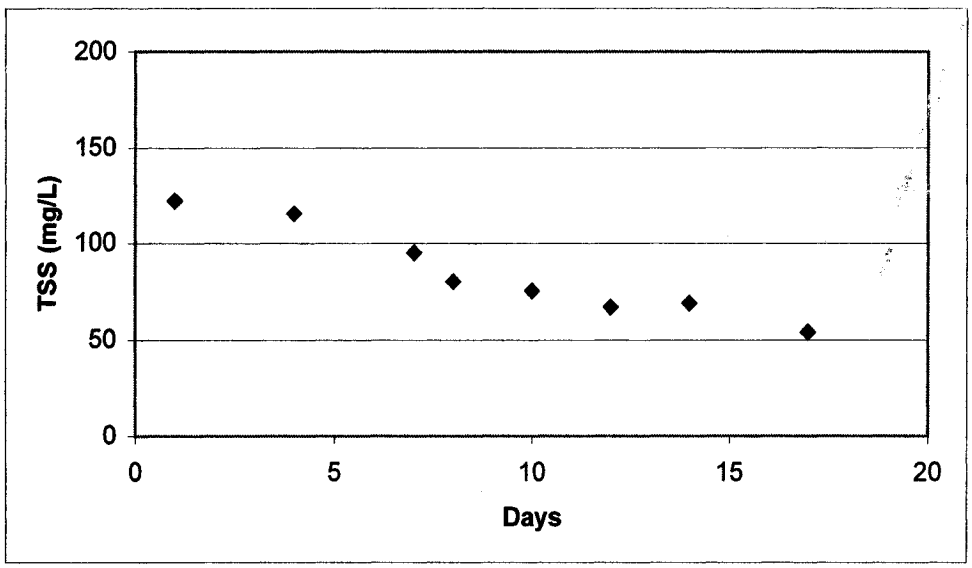


Figure 6.3.5 : Sludge blanket height for 8 hour cycles (T = 5°C)

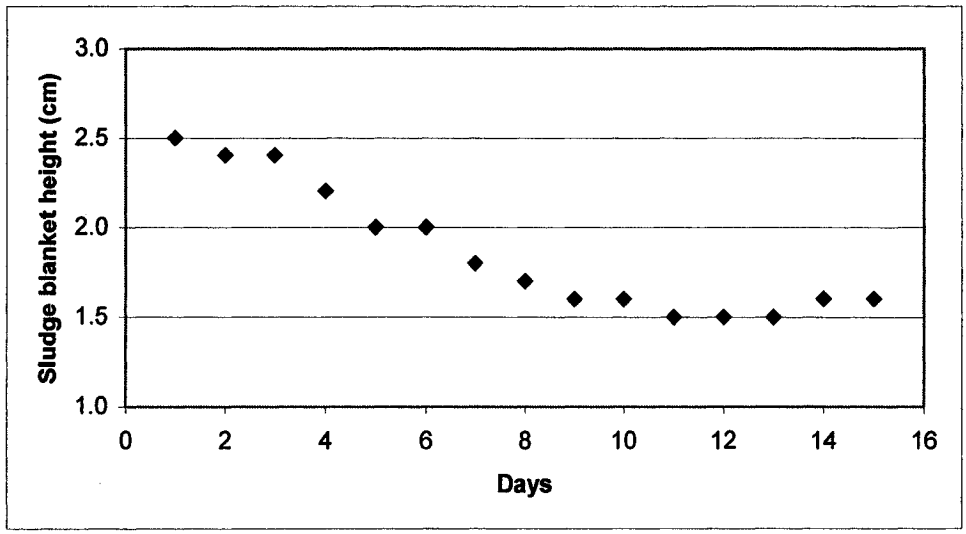
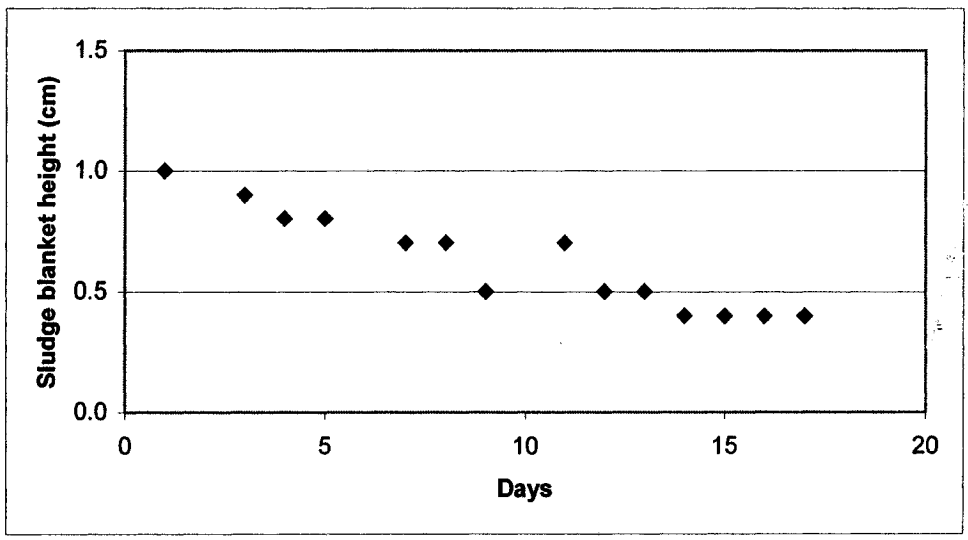


Figure 6.3.6 : Sludge blanket height for 8 hour cycles (T = 20°C to 5°C)



From Figures 6.3.7 to 6.3.10, the decrease in operating temperature has a dramatic effect on the rates of organic matter removal. The values at the beginning of each aeration period (i.e. the second point in each individual cycle) are not only much higher for the experiment at 5°C, but the differences between

the two temperatures become more important from day 1 to day 15. In fact, at day 1, the TOC value at the beginning of the aeration period is approximately 50% higher for 5°C than for 20°C whereas, at day 15, the value for 5°C is almost double that of 20°C. In addition, it is noticed that when operating at the lower temperature, there are continuing changes in the TOC and COD profiles with time even up to day 15. Thus, the acclimatization process seems to occur during a longer time frame than at 20°C where there is a tapering off phenomenon after approximately 9 days. This result is also seen in the case of the two-temperature experiment where the values are evolving even at day 17.

Another interesting effect of the change in temperature is on the amount of residual organic matter (i.e. the last point in each individual cycle) present in the reactor after each 8 hr cycle. In the case of 20°C, these values are lower than the values obtained when operating at 5°C. This can be attributed to the acclimatization period being longer when operating at the lower temperature and thus, the amount of residual organics stays higher for a prolonged period of time. A second factor is the temperature can affect the microorganism's metabolism and render a larger fraction of the organic matter unavailable for degradation (bioavailability of the substrate).

With regards to the two-temperature experiment, one would have expected the reaction rates after the change to 5°C to be different from those when operating the whole experiment at 5°C. The reason being that this

biomass would have prior contact with the substrate (at 20°C) and would demonstrate improved rates of removal. However, when comparing the two-temperature experiment on the first day at 5°C (day 8) to day 1of the experiment at 5°C, it is seen that the removal rates are quite similar. This trend continues throughout with values for the two-temperature experiment being approximately equivalent to those for the experiment at 5°C on equivalent days of operation. Therefore, this suggests that the populations that are favoured by operating the reactor at 20°C versus 5°C are relatively different. In terms of practical applications, this means the biomass population should be acclimatized at the temperature at which the reactor is to be operated.

Figure 6.3.7 : TOC profile for 8 hour cycles (T = 5°C)

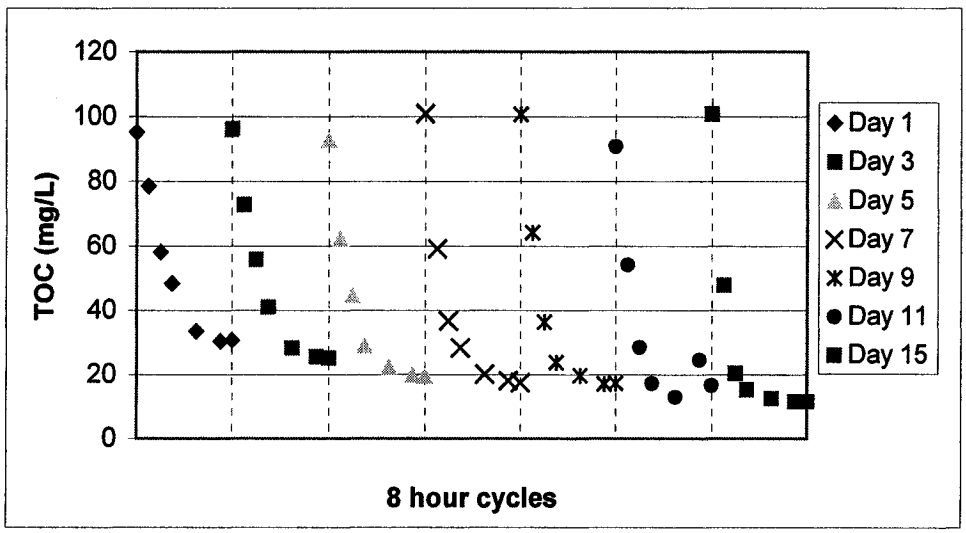


Figure 6.3.8 : TOC profile for 8 hour cycles (T = 20°C to 5°C)

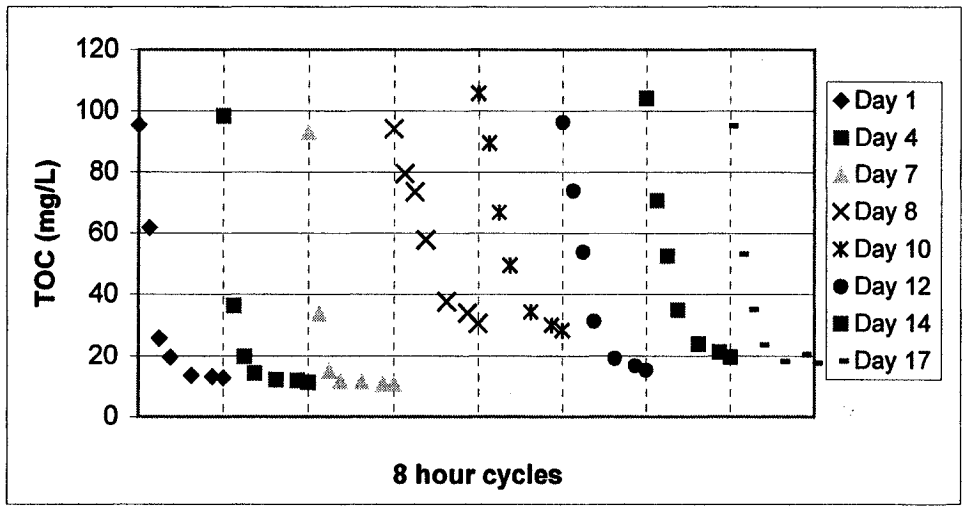


Figure 6.3.9 : COD profile for 8 hour cycles (T = 5°C)

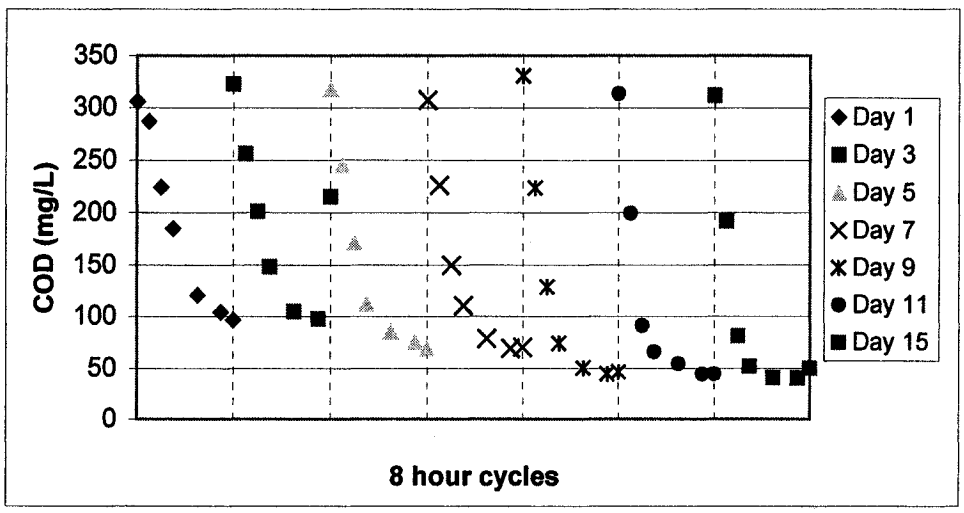
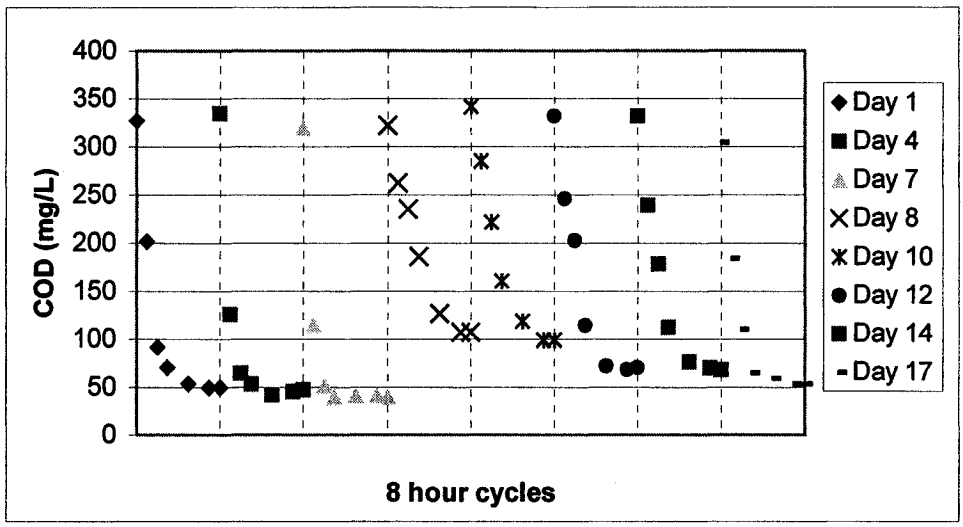


Figure 6.3.10 : COD profile for 8 hour cycles (T = 20°C to 5°C)



With regards to the ethylene glycol substrate, there are significantly higher removal rates when operating at the higher temperature of 20°C (Figures 6.1.11, 6.3.11 and 6.3.12). The value of ethylene glycol at the beginning of the each aeration period (i.e. the second point in each individual cycle) is reduced to trace amounts by day 9 for the experiment at 20°C whereas the corresponding value for the experiment at the lower temperature is gradually decreasing even at day 15. Therefore, there is a rapid attainment of stable conditions for the experiment at the higher temperature. As expected, a sudden change to a lower temperature results in reappearance of ethylene glycol in the samples taken at the beginning of the each aeration period. Similarly to the experiment conducted ay 5°C, there values of ethylene glycol do not stabilize even after day 17 of the two-temperature experiment.

Figure 6.3.11 : EG profile for 8 hour cycles (T = 5°C)

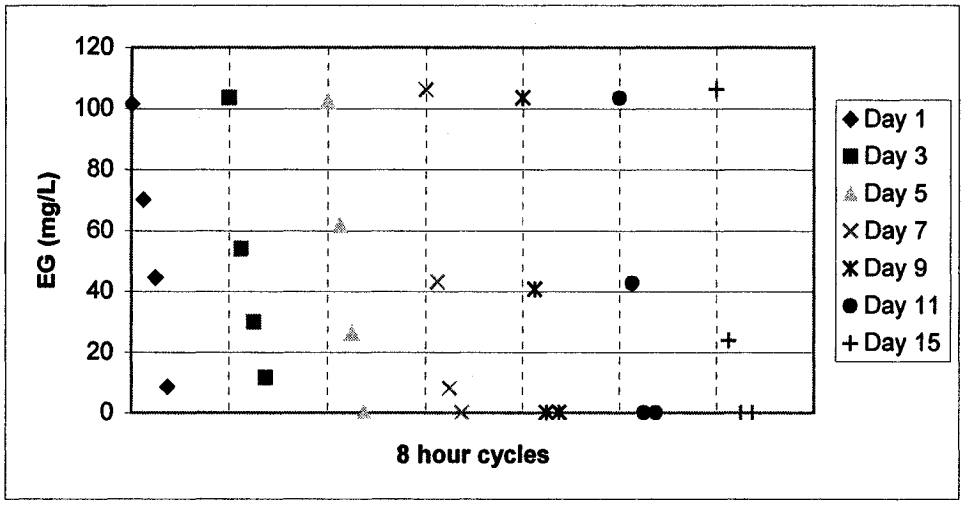
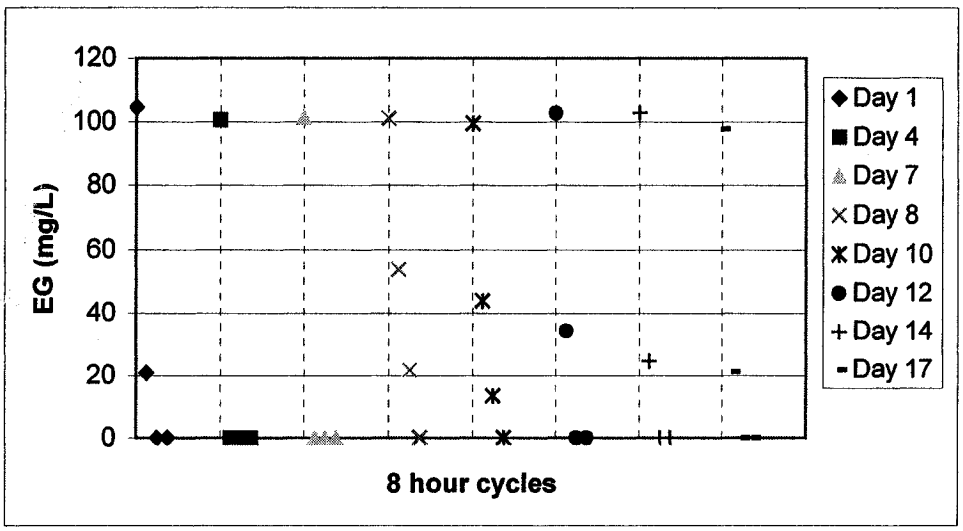


Figure 6.3.12 : EG profile for 8 hour cycles (T = 20°C to 5°C)



Figures 6.1.13, 6.3.13 and 6.3.14 present the specific oxygen uptake rates for the various sequencing batch reactor cycles. As previously seen, the experiment conducted at the higher temperature shows a gradual decrease in the SOUR from cycle 1 to 45. The first trend that is striking is the increasing trend of the specific oxygen uptake rate with time when operating at the lower

temperature. This result may seem surprising as there is a gradual increase in initial rates occurring from the beginning to the end of the experiment. Hence, one would have expected a decrease in the SOUR with time but to a lesser magnitude than at 20°C as the organic matter removal rates take longer to increase. These initial results are further reinforced by the two-temperature experiment where the trend in specific oxygen uptake rates reverses itself when the temperature is decreased to 5°C. However, as the lower temperature slows down the reaction rates, most organic matter removal occurs after the fill period of the reactor. Hence, as the microorganisms are acclimatized at the lower temperature, the removal rates show a corresponding increase. Furthermore, as stated previously, there are other factors that can affect the specific oxygen uptake rate outside of the trends in organic matter removal rates such as the changes in microbial metabolism under the stress of a lower temperature.

Figure 6.3.13 : SOUR for 8 hour cycles (T = 5°C)

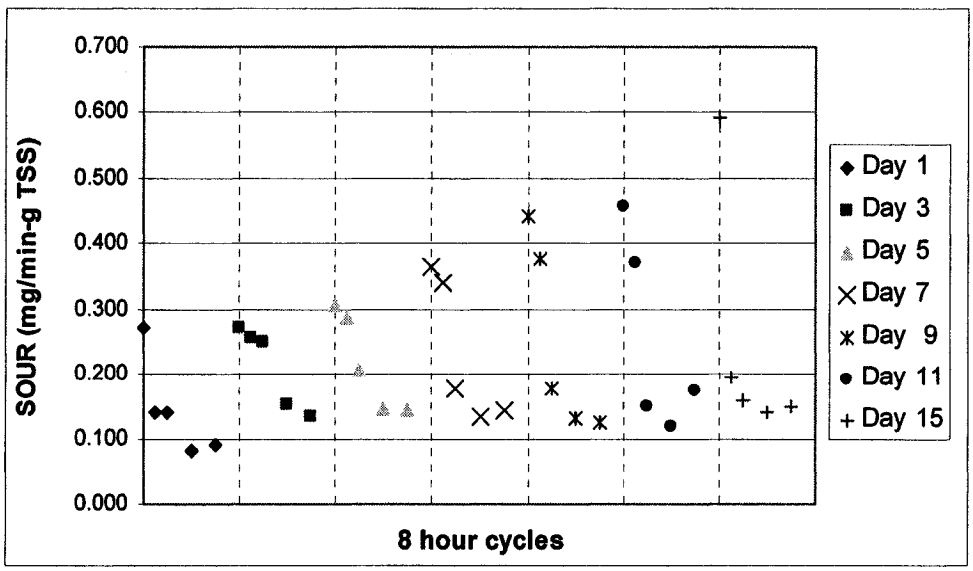
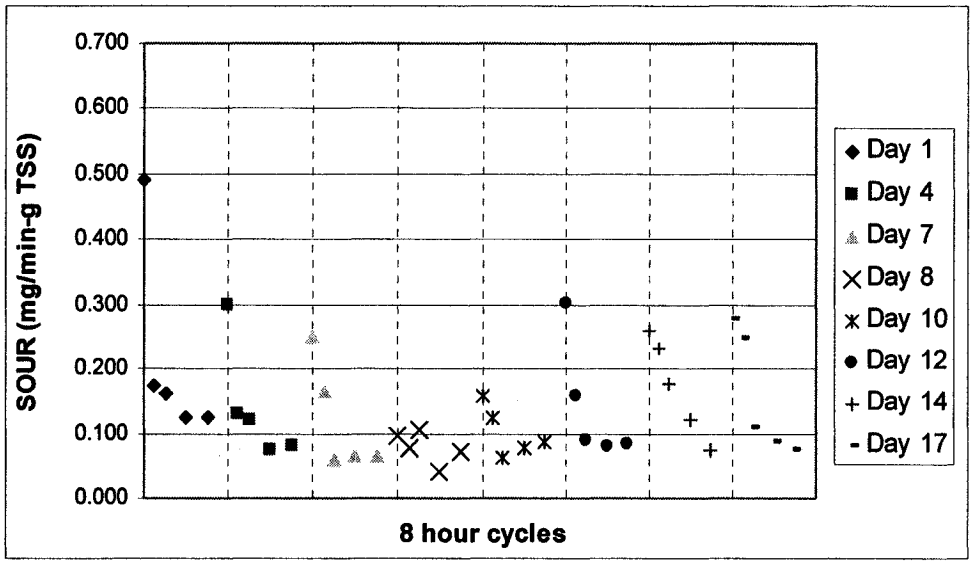


Figure 6.3.14 : SOUR for 8 hour cycles (T = 20°C to 5°C)



6.4 Overall Trends in Sequencing Batch Operation

As has been seen with the experimental results, there are many advantages to operating a sequencing batch reactor. Firstly, the data has revealed that there is an improvement in sludge settling characteristics over many cycles (as measured per the effluent TSS and the sludge blanket height). Since literature has shown that the prolonged exposure to deicing fluid has negative effects on the sludge settling characteristics, this aspect of sequencing batch reactor operation is particularly attractive. Furthermore, dramatic improvements in the rates or organic matter removal are also found as the cycles are increased. More specifically, the rates of ethylene glycol removal show a very fast increase with cycles, greater even than the increase in TOC and COD rates. With regards to temperature effects, it was determined that acclimatization

takes place differently at both sets of temperatures with stabilization occurring more rapidly at the higher operating temperature.

BIOLOG RESULTS

The Biolog results for the three sequencing batch reactor experiments are presented in Tables 19 to 27 of Appendix D. Out of the three experiments, the experiment conducted at 20°C shows the highest number of compounds that undergo significant change between the initial and final populations (see Table 19 of Appendix D). Moreover, the magnitude of these changes is relatively higher with the normalized consumption going down as much as 1 unit in a few cases (see Table 20 of Appendix D). The categories of compounds that vary significantly from day 1 to day 15 are mainly carbohydrates, carboxylic acids and amino acids.

From the field data analysis, most changes occurred at the beginning of the deicing season. Thus, the sequencing batch reactor experiment conducted at 20°C can be compared to the one at 5°C to determine if there are similar patterns in carbon source utilization. From Tables 23 and 24 (Appendix D), the number of compounds that demonstrate significant change is much higher in the first 7 days of each experiment as compared to the last 7 days. In quantitative terms, for the run at 20°C, there are 21 compounds whose consumption change to a significant extent from day 1 to day 7 but only 3 thereafter (from day 7 to day 15). Similar results are obtained for the lower temperature experiment with a

value of 21 (from day 1 to day 7) as compared to only 2 carbon sources significantly changing from day 7 to day 15. With regards to the number of highly metabolized compounds, there is a decrease between the initial (day 1) and final (day 15) populations for both experiments. However, the change in the experiment operated at the higher temperature is more significant as the number of highly utilized compounds goes from 44 to 35 (see Tables 20 and 21 of Appendix D).

Next, the results obtained for the 20°C and 5°C experiments can be compared to the two-temperature experiment conducted initially at 20°C then changed at day 8 to 5°C. In this case, the numbers of compounds that change, based on the threshold value, is more similar to the experiment performed at 5°C than the one at 20°C (see Table 19 of Appendix D). By examining the results carefully, there is a noticeable separation between the sections conducted at the different temperatures (see Table 25 of Appendix D). Hence, there are 24 compounds that undergo significant changes between day 1 and day 7 but only 5 substrates that vary to an important degree between day 8 and day 17 (end of experiment). This reinforces the previous findings that lower temperature conditions entail less significant changes in the carbon source utilization patterns. Moreover, most changes in substrate utilization patterns seem to occur at the beginning of each experiment.

As for the number of highly metabolized substrates, there are no significant changes between the beginning and the end of the two-temperature experiment (see Table 22 of Appendix D). Once again, the types of highly metabolized compounds are mainly carbohydrates, carboxylic acids and amino acids.

When comparing the initial and final populations of the three SBR experiments between themselves, some very interesting results can be extracted (Table 26 and 27). First, based on the consumption of compounds, there are limited differences between the initial populations employed for the sequencing batch reactor. This provides evidence that the treatment plant at St-Canut maintains a relatively stable population based on the carbon source utilization patterns. However, there are much more important differences between the final populations of the three experiments. As Table 27 shows, the most significant changes in utilization patterns occur between the final populations of the experiments run at the two different temperatures. Moreover, the smallest change occurs between the final populations that were exposed to the low temperature for a prolonged period of time (10 to 15 days). Therefore, there is once again a trend such that the operating temperature has an effect on the type of population obtained with the lower temperature producing fewer changes in metabolical patterns.

It should be noted that an attempt was made to detect the evolution of the microbial population for the SBR experiments using the Denaturing Gradient Gel Electrophoresis (DGGE) technique. This method characterizes the microbial population on a genetic level as it analyzes the total community DNA of a given population. Although the initial DGGE results point to the same conclusions as the Biolog (i.e. that the microbial population changes), this technique has many shortcomings. Further details are given in Appendix G.

6.5 Kinetic Modelling : Sequencing Batch Experiments

Similarly to the batch experiments, the order of reaction as well as the kinetic constants were calculated for each 8 hour cycle of the different SBR runs. More specifically, quantitative results were obtained for TOC and COD removal. As acclimatization is an important phenomenon in the activated sludge process, the sequencing batch reactor experiments would allow to quantify the magnitude of the change in removal rates with respect to cycling time and when operating at various temperatures. The following sections present the results for the kinetic evaluation.

Effect of Cycling

The data with regards to the kinetic constants of TOC and COD removal are presented in Tables 6.5.1 to 6.5.3. For the experiment performed at the higher temperature, there is a slight increasing trend in the kinetic constant for TOC and COD removal but there is scatter in the data (Table 6.5.1). By examining the order of reaction, there is a good fit of the data to a 1^{st} order model

(data not shown). The trials performed with 0^{th} order reveal that although the first few cycles can be fitted to this model, there is a rapid loss of linearity of the fit after day 7 (data not shown). The results shown for the operating temperature of 5°C clearly indicate a strong increase in reaction rates from day 1 to day 15. In this case, the rate constant approximately triples between the beginning and the end of the experiment. Lastly, the two-temperature experiment shows some interesting results at the transition period between the 7^{th} and 8^{th} day. These results will further be discussed in the following section.

Table 6.5.1 : TOC and COD kinetic constants for SBR operated at 20°C

TOC	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 15
k' (L/(g TSS-hr))	0.43	0.54	0.49	0.38	0.54	0.58	0.52
COD	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 15
k' (L/(g TSS-hr))	0.34	0.44	0.44	0.36	0.41	0.50	0.45

Table 6.5.2 : TOC and COD kinetic constants for SBR operated at 5°C

TOC	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 15
k' (L/(g TSS-hr))	0.13	0.15	0.20	0.24	0.24	0.30	0.38
COD	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 15
k' (L/(g TSS-hr))	0.09	0.14	0.18	0.20	0.25	0.29	0.36

Table 6.5.3 : TOC and COD kinetic constants for SBR operated at 20°C to 5°C

TOC	Day 1 20°C	Day 4 20°C	Day 7 20°C	Day 8 5°C	Day 10 5°C	Day 12 5°C	Day 14 5°C	Day 17 5°C
k' (L/(g TSS-hr))	0.39	0.46	0.52	0.09	0.14	0.20	0.18	0.24
COD	Day 1 20°C	Day 4 20°C	Day 7 20°C	Day 8 5°C	Day 10 5°C	Day 12 5°C	Day 14 5°C	Day 17 5°C
k' (L/(g TSS-hr))	0.37	0.47	0.53	0.10	0.13	0.18	0.18	0.27

Effect of Temperature

The removal of deicing wastes occurs during both winter and warmer conditions at the St-Canut treatment plant. Hence, it is important to know how the operating temperature can influence kinetics in order to meet the established effluent standards. Tables 6.5.1 to 6.5.3 (see above) show the comparative data for the three runs and Tables 6.5.4 and 6.5.5 show the tabulated theta values for the comparison between the experiments run at 20°C and 5°C. As can be expected, in all cases the kinetic constants are higher for the experiment performed at 20°C but are of the same order of magnitude as the run at 5°C. In addition, there is a smaller proportional change with cycling time in the kinetic constants at the higher temperature. Therefore, acclimatization has a lesser impact at the higher temperature where removal rates are already reasonably fast.

With regards to the theta values, that the range is between 1.02 and 1.09 which approximately falls within the limits for the activated sludge process. There is also a steady decrease in the value of theta from day 1 to day 15. This indicates that operating temperature has a lesser impact on the removal rates of two acclimatized populations. Although acclimatization seems to take longer at 5°C, the removal rates of the two final populations become more similar.

The TOC and COD kinetic constants for the 20°C experiment are comparable to those of the corresponding section of the two-temperature

experiment (i.e. days 1 to 7 of the two-temperature experiment). Moreover, a similar result is found when comparing the kinetic constants for the 5°C experiment to the corresponding section of the two-temperature experiment (i.e days 8 to 17 of the two-temperature experiment). This is surprising, as one could have expected that cycling at 20°C for 7 days would have had a positive impact on the kinetic rates obtained after the change to 5°C. As mentioned previously, these results can indicate that acclimatization is a temperature dependant phenomenon and a given population should be acclimatized at the specific operating temperature for optimal organic matter removal. Hence, acclimatization seems to have little impact when sudden changes in the operating temperature occur.

Table 6.5.4 : Values of θ for TOC kinetic constants

TOC	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 15
θ	1.08	1.09	1.05	1.03	1.04	1.04	1.02

Table 6.5.5 : Values of θ for COD kinetic constants

COD	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 15
θ	1.09	1.08	1.05	1.04	1.02	1.04	1.02

Summary of Results and Discussion (Chapter 6)

As demonstrated by the sequencing batch reactor (SBR) experiments, changes in degradation kinetics occur when operating the reactor during an extended period of time. In addition, the operating temperature has an impact on the evolution of the kinetics during the SBR runs. In this case, the rate constants are not only different between individual experiments (as with the batch experiments) but also vary within the same experiment. More specifically, as the biomass is exposed to the wastewater over many cycles, the rate constants change from the beginning to the end of the experiment. These changes in the microbial population are captured by the Biolog results which show a clear evolution in the types of compounds consumed from the beginning to the end of each experiment. In addition, Biolog data show that the evolution in microbial population is linked to the operating temperature of the SBR.

CHAPTER 7

CONCLUSIONS, FUTURE WORK AND ORIGINAL CONTRIBUTIONS

7.1 Conclusions

The field data indicate that activated sludge micro-organisms were able to tolerate the additional organic loading caused by the deicing wastes since ethylene glycol was reduced to trace levels (i.e. lower than 5 mg/L) in the effluent. However, sludge settling characteristics are negatively affected by the deicing fluid as SVI values were well above threshold of 250 mL/g during the deicing season.

The rates of TOC and COD removal increase only slightly as the concentration of deicing fluid is increased. With respect to ethylene glycol, its removal rate slightly increases as its initial concentration is increased. In most cases, over 90% removal is achieved within the first four hours of the experiment indicating its rapid assimilation.

The advantage of acclimatization is apparent as the rates of COD and TOC removal show significant increases as the concentration of initial deicing fluid is increased. In addition, for the acclimatized population, there is a more notable increase in ethylene glycol removal as the deicing fluid concentration is increased.

Under virtually all conditions, very little or no significant microbial growth was observed. This is due to the relative amounts of organic matter and biomass in the reactor that only seem to provide enough substrate for maintenance. In addition, the relative time frame of the batch experiments (8 or 45 hr) may not be enough for adequate growth to be observed.

Experimental results show very little removal of the organic matter in the first hour attributable to adsorption. Thus, the kinetic model describing the process can be simplified to the determination of kinetic rate constants under various operating conditions. With respect to TOC and COD, it was found that specific removal rates followed 1^{st} order kinetics. Hence, rate constants were calculated to quantify the effects of biomass concentration, deicing fluid concentration, microbial acclimatization and operating temperature.

The results from our sequencing batch reactor experiments show important microbial and kinetic changes over a 15 to 17 day period. First, we see that continuous cycling produces important increases in the organic matter removal rates. The relative importance of cycling is more pronounced under the higher operating temperature as the residual levels are attained more quickly and substrate concentrations stabilize faster. With regards to settling characteristics, there are definite improvements with time as sludge blanket heights and effluent biomass concentration shows significant decreases.

Finally, the Biolog technique has shown that, for the field samples, most changes in metabolic patterns occurred during the period which biomass acclimatization (to deicing fluid) took place at the wastewater treatment plant. In addition, the field samples and low biomass experiments show that the presence of deicing fluid slightly decreases the variety of the compounds that are degraded by the activated sludge. A similar result was found with the sequencing batch reactor experiment as there is a decrease in the variety of compounds that are degraded from the beginning to the end of the run. However, the changes are much less pronounced under a lower operating temperature where the initial and final populations are more similar. Lastly, most changes in the carbon source utilization patterns occur at the beginning of the experiment (first 7 days) and stabilize thereafter (in the last 8 to 10 days).

7.2 Future Work

Based on the results obtained, the following suggestions for future work are made :

- Conduct experiments with higher food-to-microorganism ratios (i.e. higher concentrations of deicing fluid) in order to determine conditions at which biomass growth occurs. This can serve as the basis for a more comprehensive kinetic model that incorporates biomass growth and describes deicing fluid degradation.

- Pursue sequencing batch reactor (SBR) experiments with extended run times in order to monitor microbial population changes and removal kinetics on a long-term basis. This will provide an in-depth link between removal kinetics and the type of microbial population present in the system.
- Extend the monitoring of the microbial population to substrates that, unlike ethylene glycol, are difficult to degrade with activated sludge. An understanding of the impact of refractory compounds on population dynamics is essential for wastewater treatment plant operation.

7.3 Original Contributions

From the current study, the following original contributions to knowledge can be derived :

- A complete study of the biodegradation of ethylene glycol based deicing fluids including the determination of kinetics with respect to various operating parameters (biomass concentration, deicing fluid concentration and temperature) and the removal mechanism.
- The evaluation of microbial changes, including substrate acclimatization, based on removal kinetics and biomass indicators such as the phenotypic (Biolog) analysis.
- Investigation of the sequencing batch reactor (SBR) applied to the treatment of deicing wastes.

- Illustration of the acclimatization process with the sequencing batch reactor (SBR) relating to changes in sludge characteristics and degradation kinetics.
- Corroboration of experimental data with field data results obtained from an operating plant treating municipal as well as deicing wastes.

REFERENCES

1. Aéroports de Montréal (1999) *Déversement Contrôlé de Glycol à la Station d'Épuration de St-Canut : Phase II.* Aéroports de Montréal, pp. 1-49.
2. APHA (1989) Standard Methods for the Examination of Water and Wastewater, 17th Edition. APHA publication.
3. Arnz, P. Esterl, S. Nerger, C. Delgado, A. and Wilderer, P.A. (2000) Simultaneous Loading and Draining as a Means to Enhance Efficacy of SBRs, Water Research. v 34, n 5, pp. 1763-1766.
4. Backer, D.S., Smith, D., Habben, C.E. (1994) *Deicing Dilemma.* Journal of Civil Engineering, July 1994, pp. 56-59.
5. Bell, John, P. and Tsezos, M. (1987) *Removal of Hazardous Organic Pollutants by Biomass Adsorption.* Journal WPCF, v 59, n 4, pp. 191-198.
6. Canada Gazette (1994) *Glycol Guidelines Order of Council.* Department of the Environment, Canada Gazette, Part I, pp. 840-842.
7. Coffey, S. (1965) Rodd's Chemistry of Carbon Compounds. Vol. 1, 2nd Edition. Elsevier Publishing Company, London.
8. Crittenden, J.C., Vaitheeswaran, K., Hand, D.W., Howe, E.W., Aieta, E.M., Tate, C.H., McGuire, M.J., Davis, M.K. (1993) *Removal of Dissolved Organic Carbon Using Granular Activated Carbon.* Water Research, v 27, n 4, pp. 715-721.
9. CSST (1991) *Répertoire Toxicologique pour l'Éthylène Glycol.* Commission de la Santé et de la Sécurité du Travail au Québec, Gouvernement du Québec.
10. CSST (1989) *Répertoire Toxicologique pour le Diéthylène Glycol.* Commission de la Santé et de la Sécurité du Travail au Québec, Gouvernement du Québec.
11. Curtis, T.P. and Craine, N.G. (1998) *The Comparison of the Diversity of Activated Sludge Plants.* Water Science and Technology, v 37, pp. 71-78.

12. Darlington, C. and Kennedy, K.J. (1998) *Biodegradation of Aircraft Deicing Fluid in an Upflow Anaerobic Sludge Blanket (UASB) reactor.* J. Envir. Sci. Health. Pt. A, v 33, n 3, pp.339-351.
13. Dionisi, D., Majone, M., Tandoi, V. and Beccari, M. (2001) *Sequencing Batch Reactor : Influence of Periodic Operation on Performance of Activated Sludges in Biological Wastewater Treatment.* Ind. Eng. Chem. Res., v 40, pp. 5110-5119.
14. Droste,R.L (1997) *Theory and Practice of Water and Wastewater Treatment.* John Wiley, Toronto.
15. Eckenfelder, W.W. (1986) The Activated Sludge Process : State of the Art. CRC Critical Reviews in Environmental Control CRC Press, pp. 111-178.
16. Eckenfelder, W.W. (1999) Industrial Water Pollution Control. McGraw-Hill, New York.
17. Evans, W.H., David, E.J. (1974) *Biodegradation of Mono-, Di-, and Triethylene Glycols in River Waters under Controlled Laboratory Conditions.* Water Research, v 8, n 2, pp. 97-100.
18. Fogler, S.H. (1999) Elements of Chemical Reaction Engineering, 3rd Edition. Prentice Hall PTR, New Jersey.
19. Fujie, K., Tsubone, T., Monya, H. and Kubota, H. (1988). *A Simplified Kinetic Model to Simulate Soluble Organic Substances Removal in an Activated Sludge Aeration Tank.* Water Research, v 22, n 1, pp. 29-36.
20. Garland, J.L. and Mills, A.L. (1991) *Classification and Characterisation of Heterotrophic Microbial Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilisation.* Appl. Environ. Microbiol., v 57, n 8, pp.2351-2359.
21. Gaulin, J.P. (2003) Selective Caffeine Removal by Microbial Consortia. Master's Thesis. McGill University.
22. Gerhold, R.M., Maloney, G.M. (1966) *Structural Determinants in the Oxidation of Aliphatic Compounds by Activated Sludge.* Journal of WPCF, v 38, n 4, pp. 562-579.

23. Gérin, M., Viau, C. (1993) *Research Project on Aviation Workers Exposure to Ethylene Glycol : Final Report.* Département de Médecine du Travail et d'Hygiène du Milieu, Faculté de Médecine, Université de Montréal.
24. Giusti, D.M., Conway, R.A., Lawson, C.T. (1974) *Activated Carbon Adsorption of Petrochemicals.* Journal of WPCF, v 46, n 5, pp. 947-965.
25. Gould, W.D., Chalykoff, C., McCready, R.G.L., Salley, J. (1989) *Microbial Degradation of Ethylene Glycol using a Rotating Biological Contactor.* Extractive Metallurgy Laboratory, CANMET.
26. Grabinska-Loniewska, A. (1974) *Activated Sludge Bacteria participating in the Biodegradation of Methanol, Formaldehyde, and Ethylene Glycol.* Acta Microbiol. Pol. Ser. B, v 6, n 2, pp.75-81.
27. Henry, J.G., Henike, G.W. (1996) Environmental Science and Engineering, 3rd Edition. Prentice Hall, New Jersey, pp. 460-462.
28. Hsu, E.H. (1986) *Treatment of a Petrochemical Wastewater in Sequencing Batch Reactors.* Environmental Progress., v 5, n 2, pp.71-81.
29. Imamura, S., Nakamura, M., Kawabata, N., Yoshida, J.I., Ishida, S. (1986) *Wet Oxidation of Polyethylene Glycol Catalysed by Manganes-cerium Composite Oxide.* Ind. Eng. Chem. Prod. Res. Dev., v 25, n 1, pp.34-37.
30. Irvine, R.L., Wilderer, P.A. and Flemming, H.C. (1997) *Controlled Unsteady-State Processes and Technology - An Overview.* Water Science and Technology, v 35, v 1, pp. 1-10.
31. Iwamoto, T., Tani, K., Nakamura, K., Suzuki, Y., Kitagawa, M., Eguchi, M. and Nasu, M. (2000) *Monitoring Impact of In Situ Biostimulation Treatment on Groundwater Bacterial Community by DGGE.* FEMS Microbiology Ecology, v 32, pp. 129-141.
32. Jank, B.E., Guo, H.M., Cairns, V.W. (1973) *Biological Treatment of Airport Wastewater Containing Aircraft De-icing Fluids.* Environmental Protection Service, Environment Canada.
33. Kennedy, K. J. and Lentz, E.M. (2000) *Treatment of Landfill Leachate Using Sequencing Batch and Continuous Flow Upflow Anaerobic Sludge Blanket (UASB) Reactors.* Water Research, v 34, n 14, pp. 3640-3656.

34. Koganovski, A.M., Udod, V.M., Nesynova, L.I., Nevinnaya, L.V. (1987) *Degradation of Diethylene Glycol by Microorganisms.* Soviet Journal of Water Chemistry and Technology, v 9, n 2, pp.169-171.
35. Lallai, A. and Mura, G. (1989) *Kinetics of Growth for Mixed Cultures of Micro-organisms Growing on Phenol.* The Chemical Engineering Journal, v 41, pp. 55-60.
36. LGL-Love (1979 *Analysis of Shrinkage in Volume of Glycol De-icing Fluid Recovery Systems.* Transport Canada.
37. Lin, S.H. and Cheng, K. W. (2001) *A New SBR for Treatment of Municipal Sewage Waste Water for Agricultural Use.* J. Desalination, v 133, pp. 41-51.
38. McKinney, R.E. (1962) Microbiology for Sanitary Engineers. McGraw Hill, New York.
39. MacNaughton, S.J., Stephen, J.R., Venosa, A.D., Davis, G.A., Chang, Y.J. and White, D.C. (1999) *Microbial Population Changes During Bioremediation of an Experimental Oil Spill.* Appl. Env. Microbiol., v 65, n 8, pp. 3566-3574.
40. Metcalf and Eddy (2003) Wastewater Engineering : Collection, Treatment and Disposal. McGraw-Hill, New York.
41. Miller, L.M. (1979) *Investigation of Selected Potential Environmental Contaminants : Ethylene Glycol, Propylene Glycol , and Butylene Glycol.* U.S. Environmental Protection Agency.
42. Misbahuddin, M. and Farooq, S. (1991) *Biological Treatment of a Petrochemical Wastewater Using Sequencing Batch Reactors.* Environmental Technology, v 12, n 2, pp. 131-145
43. Morgenroth, E. Wilderer, P.A. (1998) *Sequencing Batch Reactor Technology : Concepts, Design and Experiences.* Journal CIWEM, v 12, pp. 314-321.
44. Muyzer, G. (1999) *DGGE/TGGE a Method for Identifying Genes from Natural Ecosystems.* Current Opinion in Microbiology, v 2, pp. 317-322.
45. Nakazawa, H., Tanaka, K. (1991) *Kinetic Model of SBIAS Process for Municipal Wastewater Treatment.* Water Science and Technology, v 23, Kyoto, pp. 1097-1106.

46. Nakhla, A., Ahmed, A.M. and Farooq, S. (1997) *Modeling of SBRs Treating Inhibitory and Non Inhibitory Waste Waters.* Water Env. Research, v 69, n 1, pp. 6-13.

47. Nitschke, L., Wagner, H., Metzner, G., Wilk, A., Huber, L. (1996) *Biological Treatment of Waste Water Containing Glycols from De-icing Agents.* Water Research, v 30, n 3, pp. 644-648.

48. Novak, L., Goronezy, M.c. and Wanner, J. (1997) *Dynamic Math. Modelling of SBRs With Aerated and Mixed Filling Period.* Water Science and Technology, v 35, n 1, pp. 105-112.

49. Nyholm, F. Ingersleu, U.T. Berg, J. P. Petersen and Frimer – Larsen, H. (1996). *Estimation of Kinetic Rate Constants for Biodegradation of Concentration.* J. Chemosphere, v 33, n 5, pp. 851-864.

50. Padukone, N. and Andrews, G.F. (1989) *A Simple, Conceptual, Mathematical Model for the Activated Sludge Process and its Variants.* Water Res., v 23, n 12, pp. 1535-1543.

51. Peters, J.J. (1998) The Activated Sludge Treatment of Pulp and Paper Wastes. Master's Thesis. McGill University.

52. Reynolds and Richards (1996) Unit Operations and Processes in Environmental Engineering, 2nd Edition. PWS Publishing, Boston.

53. Rodrigues, A.C., Brito, A.G., Melo, L.F. (2001) *Post-treatment of a Brewery Waste Water Using SBR*, Water Env. Research, v 73, n1, pp. 45-51.

54. Sabeh, Y. (1996) Impacts Environnementaux, Toxicité et Biodégradation des Dégivrants pour Avions. Ph.D. Thesis, Université de Sherbrooke.

55. Selvaknmar, A. and Hsieh, H.N. (1989) *Removal of Organic Compounds by Microbial Biomass.* Purdue Industrial Waste Conference Proceedings, Lewis Publishers, Michigan, pp. 275-281.

56. Schneider, C.A., Mo, K. and Liss, S.N. (1998) *Applying Phenotypic Fingerprinting in the Management of Wastewater Treatment Systems.* Water Science and Technology, v 37, n 4, pp. 461-464.

57. Schuler, M.L. and Kargi, F. (2002) Bioprocess Engineering : Basic Concepts, 2nd Edition. Prentice Hall PTR, New Jersey.

58. Schulz-Rettmer, Reiner, Yawari and Touradj (1978). *Mechanisms of the Elimination of Organic Substances from Wastewater by Activated Sludge*, Zeitschrift für wasser und abwasser forschung, v 11, n 6, pp. 205-209.
59. Susumu, H. and Masanori, F. (1970) *Purification Theories and Mechanism of Activated Sludge.* Hakko Kogaku Zasshi, v 48, n 5, pp. 263-269.
60. Suzuki, J., Hukushima, K., Suzuki, S. (1978) *Effect of Ozone Treatment upon Biodegradability of Water-soluble Polymers.* Environmental Science and Technology, v 12, n 10, pp.1180-1183.
61. Tan, K.N. and Chua, H. (1997) *COD Adsorption of AS-Its Determination and Application in the Activated Sludge Process.* Environmental Monitoring and Assessment, Kluwer Academic Publishers, Netherlands, v 44, pp. 211-217.
62. Tchobanoglous, G., Schroeder, E.D. (1987) Water Quality. Addison Wesley, New York.
63. Union Carbide (1989) *Liquide Dégivrant UCAR-ADF D et UCAR XL-54.* Fiche Technique Santé-Sécurité.
64. Union Carbide (1994) *Liquide Anti-givrant UCAR UC-5.1 et UCAR Ultra.* Fiche Technique Santé-Sécurité.
65. U.S. EPA (1986) *US Environmental Protection Agency Summary Report Sequencing Batch Reactors.* Cincinnati, Ohio Tech. Transfer Report EPA, pp. 625-886.
66. Vadodaria, S. (1999) Effect of Black Liquor on the Activated Sludge Process. Master's Thesis. McGill University.
67. Verschueren, K. (1985) Handbook of Environmental Data on Organic Chemicals, 2nd Edition. Van Nostrand Reinhold Company, New York.
68. Victorio, L., Gilbride, K.A., Allen, D.G. and Liss, S.N. (1996) *Phenotypic Fingerprinting of Microbial Communities in Wastewater Treatment Systems.* Water Research, v 30, n 5, pp. 1077-1086.
69. Viessman, W., Hammer, M.J. (1998) Water Supply and Pollution Control, 6th Edition. Addison Wesley, New York.
70. Waresak, M.J. (1997) *City Converts Waste Water Treatment Facility to SBRs,* Water Engineering and Management, Jan. 97, pp. 22-39.

APPENDIX A

$$COD(as\ mg\ O_2/L) = \frac{(A-B)\,x\,M\,x\,8000}{V_{sample}} \qquad \text{Eq. 3.1A}$$

where : A = mL of FAS used for digested blank (average of 2)

B = mL of FAS used for wastewater sample

M = molarity of FAS

V_{sample} = volume of sample in ampoule (2.5 mL)

The value of 8000 is a conversion factor

The molarity of FAS is calculated using the following relationship :

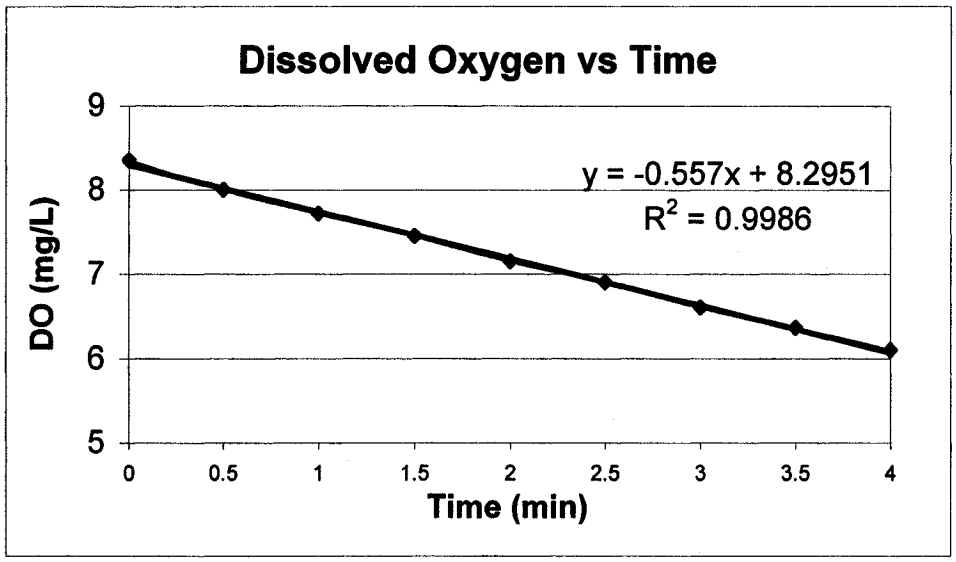
$$M = \frac{V_{dic}}{V_{FAS}}\ x\ 0.10 \qquad \text{Eq. 3.2A}$$

where : V_{dic} = volume of dichromate solution in ampoule (1.5 mL)

V_{FAS} = volume of FAS used for undigested blank (average of 2)

The value of 0.10 is a conversion factor.

APPENDIX B

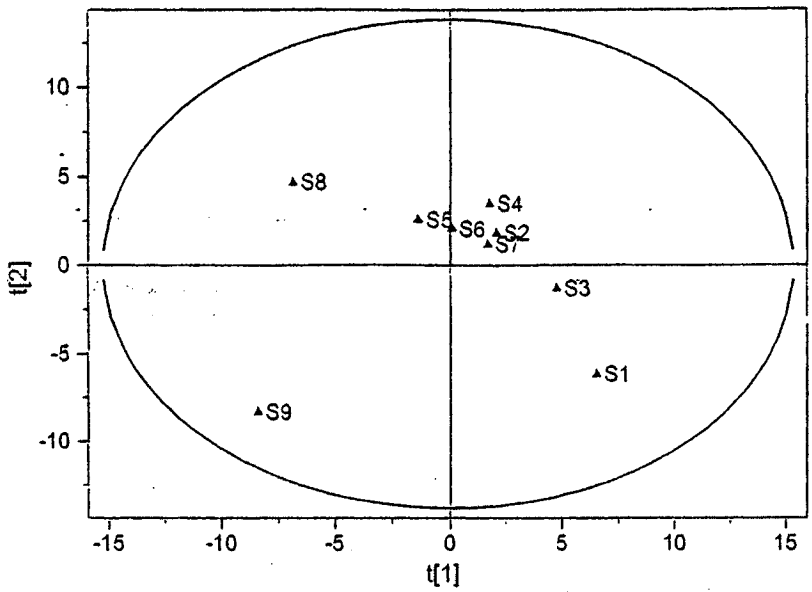

Dissolved Oxygen vs Time
y = -0.557x + 8.2951
R2 = 0.9986
DO (mg/L)
9
8
7
6
5
0
0.5
1
1.5
2
2.5
3
3.5
4
Time (min)

A1 water	A2 α- cyclodextrin	A3 dextrin	A4 glycogen	A5 tween 40	A6 Tween 80	A7 N-acetyl-D-galactos-amine	A8 N-acetyl-D-Glucosamine	A9 adonitol	A10 L- arabinose	A11 D- arabitol	A12 cellobiose
B1 i- erythritol	B2 D- Fructose	B3 L- fucose	B4 D- galactose	B5 gentiobiose	B6 α-D- glucose	B7 m- inositol	B8 α-D- lactose	B9 lactulose	B10 maltose	B11 D- mannitol	B12 D- mannose
C1 D- melibiose	C2 β-methyl D-glucoside	C3 D- psicose	C4 D- raffinose	C5 L- raffinose	C6 D- Sorbitol	C7 sucrose	C8 D- Trehalose	C9 turanose	C10 xylitol	C11 methyl pyruvate	C12 mono-methyl succinate
D1 acetic acid	D2 cis- aconitic acid	D3 citric acid	D4 formic acid	D5 D- galactonic acid lactone	D6 D- Galacturonic Acid	D7 D- gluconic acid	D8 D- Glucosamini c acid	D9 D- glucuronic acid	D10 α-hydroxy butyric acid	D11 β-hydroxy butyric acid	D12 γ-hyroxy butyric acid
E1 p-hydroxy phenylacetic acid	E2 Itaconic acid	E3 α-keto butyric acid	E4 α-keto glutaric acid	E5 α-keto valeric acid	E6 D, L- Lactic acid	E7 malonic acid	E8 Propionic acid	E9 quinic acid	E10 D- saccharic acid	E11 sebacic acid	E12 succinic acid
F1 bromo succinic acid	F2 Succinamic Acid	F3 glucuron- amide	F4 alaninamide	F5 D- alanine	F6 L- Alanine	F7 L-alanyl- glycine	F8 L- Asparagine	F9 L- aspartic acid	F10 L- glutamic acid	F11 glycyl-L- aspartic acid	F12 glycyl-L- glutamic acid
G1 L- histidine	G2 Hyroxy L- Proline	G3 L- leucine	G4 L- ornithine	G5 L- phenyl- alanine	G6 L- Proline	G7 L- pyroglutamic acid	G8 D- Serine	G9 L- serine	G10 L- threonine	G11 D, L- carnitine	G12 γ-amino butyric acid
H1 urocanic acid	H2 Inosine	H3 uridine	H4 thymidine	H5 phenyl ethylamine	H6 Putrescine	H7 2-amino ethanol	H8 2,3- butanediol	H9 glycerol	H10 D, L-α- glycerol phosphate	H11 glucose-1- phosphate	H12 glucose-6- phosphate

Biolog GN MicroPlate™ Panel

APPENDIX D

Figure 1 : Field Data Analysis



Si denotes the samples in chronological order (December to April)

APPENDIX D

Table 1 : Field Samples (PC's and Sample 1)

		PC1	PC2
A3	Polymers	0.198	0.015
B10	Carbohydrates	0.181	-0.022
A4	Polymers	0.176	-0.081
B7	Carbohydrates	0.161	-0.093
B4	Carbohydrates	0.158	-0.111
B11	Carbohydrates	0.156	0.012
A5	Polymers	0.156	0.110
B12	Carbohydrates	0.148	0.108
A10	Carbohydrates	0.146	0.057
B8	Carbohydrates	0.142	-0.114
A8	Carbohydrates	0.141	0.061
B3	Carbohydrates	0.135	0.002
C2	Carbohydrates	0.128	-0.105
C8	Carbohydrates	0.126	-0.096
A12	Carbohydrates	0.125	0.134
B1	Carbohydrates	-0.122	0.050
F6	Amino acids	-0.124	-0.067
F12	Amino acids	-0.127	0.010
D12	Carboxylic acids	-0.130	0.120
F3	Amides	-0.131	0.047
H3	Aromatics	-0.136	0.009
E3	Carboxylic acids	-0.136	-0.070
F7	Amino acids	-0.138	-0.031
E5	Carboxylic acids	-0.148	0.037
G3	Amino acids	-0.148	-0.082
E4	Carboxylic acids	-0.153	-0.061
H1	Aromatics	-0.162	-0.089
G4	Amino acids	-0.172	-0.071
E2	Carboxylic acids	-0.175	-0.025

Sample 1

		Norm. Col. Intensity
H9	Alcohols	1.357
D7	Carboxylic acids	1.327
C3	Carbohydrates	1.318
G1	Amino acids	1.318
F11	Amino acids	1.310
H2	Aromatics	1.307
D8	Carboxylic acids	1.302
D2	Carboxylic acids	1.296
G9	Amino acids	1.293
E6	Carboxylic acids	1.285
D4	Carboxylic acids	1.285
H11	Phosphorylated HC	1.285
H4	Aromatics	1.277
F9	Amino acids	1.277
B7	Carbohydrates	1.274
H3	Aromatics	1.260
F8	Amino acids	1.252
A7	Carbohydrates	1.252
E10	Carboxylic acids	1.246
D3	Carboxylic acids	1.235
B3	Carbohydrates	1.235
C4	Carbohydrates	1.224
C2	Carbohydrates	1.224
C1	Carbohydrates	1.218
A4	Polymers	1.210
B9	Carbohydrates	1.207
D6	Carboxylic acids	1.199
B4	Carbohydrates	1.199
A3	Polymers	1.191
C9	Carbohydrates	1.188
C6	Carbohydrates	1.185
C8	Carbohydrates	1.185
D5	Carboxylic acids	1.179
C7	Carbohydrates	1.168
B5	Carbohydrates	1.163
B8	Carbohydrates	1.160
C5	Carbohydrates	1.149
D9	Carboxylic acids	1.138
G8	Amino acids	1.129
E1	Carboxylic acids	1.127

Table 2 : Field Samples 2 and 3

Sample 2

		Norm. Col. Intensity
H9	Alcohols	1.334
H3	Aromatics	1.306
G1	Amino acids	1.304
D7	Carboxylic acids	1.286
E6	Carboxylic acids	1.281
D8	Carboxylic acids	1.273
C3	Carbohydrates	1.27
C7	Carbohydrates	1.263
C4	Carbohydrates	1.247
C2	Carbohydrates	1.247
G9	Amino acids	1.24
F11	Amino acids	1.237
H2	Aromatics	1.237
D2	Carboxylic acids	1.23
D3	Carboxylic acids	1.227
B6	Carbohydrates	1.227
D4	Carboxylic acids	1.224
C1	Carbohydrates	1.219
E10	Carboxylic acids	1.217
B3	Carbohydrates	1.217
H4	Aromatics	1.214
C9	Carbohydrates	1.212
D6	Carboxylic acids	1.196
C6	Carbohydrates	1.196
G8	Amino acids	1.196
B7	Carbohydrates	1.186
F10	Amino acids	1.181
F9	Amino acids	1.176
C8	Carbohydrates	1.176
H11	Phosphorylated HC	1.168
B4	Carbohydrates	1.168
F8	Amino acids	1.166
D9	Carboxylic acids	1.163
B5	Carbohydrates	1.161
A4	Polymers	1.155
B9	Carbohydrates	1.155
B8	Carbohydrates	1.153
C5	Carbohydrates	1.153
H10	Phosphorylated HC	1.153
A3	Polymers	1.148
D5	Carboxylic acids	1.127
A7	Carbohydrates	1.104

Sample 3

		Norm. Col. Intensity
C3	Carbohydrates	1.373
H3	Aromatics	1.346
H4	Aromatics	1.336
H9	Alcohols	1.325
D7	Carboxylic acids	1.312
E6	Carboxylic acids	1.312
D3	Carboxylic acids	1.293
C7	Carbohydrates	1.290
D8	Carboxylic acids	1.288
D4	Carboxylic acids	1.282
G1	Amino acids	1.269
B7	Carbohydrates	1.269
D2	Carboxylic acids	1.261
F8	Amino acids	1.256
G9	Amino acids	1.251
F11	Amino acids	1.251
C1	Carbohydrates	1.245
C4	Carbohydrates	1.235
B9	Carbohydrates	1.224
C2	Carbohydrates	1.221
C6	Carbohydrates	1.221
B3	Carbohydrates	1.219
H2	Aromatics	1.213
D6	Carboxylic acids	1.203
B5	Carbohydrates	1.195
D9	Carboxylic acids	1.190
E10	Carboxylic acids	1.182
C5	Carbohydrates	1.182
C8	Carbohydrates	1.176
A3	Polymers	1.176
D5	Carboxylic acids	1.174
B11	Carbohydrates	1.171
B4	Carbohydrates	1.166
F9	Amino acids	1.163
A4	Polymers	1.163
H11	Phosphorylated HC	1.160
B6	Carbohydrates	1.150
B8	Carbohydrates	1.144
G8	Amino acids	1.118
B10	Carbohydrates	1.113

Table 3 : Field Samples 4 and 5

Sample 4

		Norm. Col. Intensity
H3	Aromatics	1.337
D8	Carboxylic acids	1.321
C3	Carbohydrates	1.319
H4	Aromatics	1.308
D4	Carboxylic acids	1.305
F11	Amino acids	1.303
H9	Alcohols	1.292
E6	Carboxylic acids	1.271
D3	Carboxylic acids	1.268
E10	Carboxylic acids	1.268
B3	Carbohydrates	1.266
D2	Carboxylic acids	1.255
G1	Amino acids	1.250
G9	Amino acids	1.247
D7	Carboxylic acids	1.242
D9	Carboxylic acids	1.242
D6	Carboxylic acids	1.234
C5	Carbohydrates	1.232
H2	Aromatics	1.210
B7	Carbohydrates	1.205
B9	Carbohydrates	1.203
F8	Amino acids	1.197
C4	Carbohydrates	1.189
D5	Carboxylic acids	1.189
F9	Amino acids	1.189
A9	Carbohydrates	1.189
C2	Carbohydrates	1.187
C6	Carbohydrates	1.176
C8	Carbohydrates	1.176
A3	Polymers	1.152
B4	Carbohydrates	1.145
A10	Carbohydrates	1.139
B5	Carbohydrates	1.137
A4	Polymers	1.134
H11	Phosphorylated HC	1.131
C1	Carbohydrates	1.123
A7	Carbohydrates	1.123
C7	Carbohydrates	1.121
B8	Carbohydrates	1.118
C9	Carbohydrates	1.118
H10	Phosphorylated HC	1.108
F10	Amino acids	1.102

Sample 5

		Norm. Col. Intensity
G1	Amino acids	1.341
H3	Aromatics	1.336
C3	Carbohydrates	1.336
H9	Alcohols	1.333
D8	Carboxylic acids	1.293
E6	Carboxylic acids	1.288
H4	Aromatics	1.285
C7	Carbohydrates	1.269
D2	Carboxylic acids	1.258
H2	Aromatics	1.258
D7	Carboxylic acids	1.253
F11	Amino acids	1.250
D4	Carboxylic acids	1.245
D3	Carboxylic acids	1.228
E10	Carboxylic acids	1.223
D6	Carboxylic acids	1.220
G9	Amino acids	1.215
C4	Carbohydrates	1.212
C1	Carbohydrates	1.207
B3	Carbohydrates	1.202
B9	Carbohydrates	1.188
C9	Carbohydrates	1.188
C5	Carbohydrates	1.183
F8	Amino acids	1.183
D5	Carboxylic acids	1.177
H11	Phosphorylated HC	1.177
C6	Carbohydrates	1.175
D9	Carboxylic acids	1.172
F9	Amino acids	1.172
C8	Carbohydrates	1.172
B5	Carbohydrates	1.167
C2	Carbohydrates	1.161
B7	Carbohydrates	1.159
B6	Carbohydrates	1.150
G8	Amino acids	1.140
H10	Phosphorylated HC	1.132
B4	Carbohydrates	1.124
F10	Amino acids	1.115
A9	Carbohydrates	1.107
A3	Polymers	1.107
A4	Polymers	1.102

Table 4 : Field Samples 6 and 7

Sample 6

		Norm. Col. Intensity
H9	Alcohols	1.344
H3	Aromatics	1.339
C3	Carbohydrates	1.337
E10	Carboxylic acids	1.297
E6	Carboxylic acids	1.275
D2	Carboxylic acids	1.275
F11	Amino acids	1.275
D4	Carboxylic acids	1.262
H4	Aromatics	1.260
D3	Carboxylic acids	1.245
D7	Carboxylic acids	1.237
G1	Amino acids	1.232
B9	Carbohydrates	1.232
G9	Amino acids	1.230
C4	Carbohydrates	1.230
D8	Carboxylic acids	1.227
C7	Carbohydrates	1.227
D6	Carboxylic acids	1.227
B3	Carbohydrates	1.222
H11	Phosphorylated HC	1.222
C5	Carbohydrates	1.217
H2	Aromatics	1.202
C6	Carbohydrates	1.202
B7	Carbohydrates	1.202
C2	Carbohydrates	1.200
H10	Phosphorylated HC	1.200
D5	Carboxylic acids	1.197
F8	Amino acids	1.185
C1	Carbohydrates	1.175
B5	Carbohydrates	1.162
C9	Carbohydrates	1.157
A7	Carbohydrates	1.147
D9	Carboxylic acids	1.145
B4	Carbohydrates	1.142
A3	Polymers	1.132
A4	Polymers	1.132
C8	Carbohydrates	1.127
A9	Carbohydrates	1.125
F9	Amino acids	1.107
B8	Carbohydrates	1.102
A11	Carbohydrates	1.102
G8	Amino acids	1.100

Sample 7

		Norm. Col. Intensity
H9	Alcohols	1.367
H3	Aromatics	1.362
H2	Aromatics	1.329
F11	Amino acids	1.303
C3	Carbohydrates	1.301
D4	Carboxylic acids	1.272
E10	Carboxylic acids	1.262
G9	Amino acids	1.262
C4	Carbohydrates	1.262
E6	Carboxylic acids	1.257
G1	Amino acids	1.252
C5	Carbohydrates	1.247
H4	Aromatics	1.244
H11	Phosphorylated HC	1.244
D7	Carboxylic acids	1.242
B9	Carbohydrates	1.237
D8	Carboxylic acids	1.232
B3	Carbohydrates	1.229
D2	Carboxylic acids	1.226
C6	Carbohydrates	1.221
D3	Carboxylic acids	1.216
F8	Amino acids	1.214
C1	Carbohydrates	1.209
D6	Carboxylic acids	1.206
C2	Carbohydrates	1.201
F9	Amino acids	1.201
H10	Phosphorylated HC	1.188
B4	Carbohydrates	1.180
D9	Carboxylic acids	1.178
D5	Carboxylic acids	1.173
B5	Carbohydrates	1.170
C7	Carbohydrates	1.165
B7	Carbohydrates	1.165
A3	Polymers	1.165
C8	Carbohydrates	1.163
C9	Carbohydrates	1.160
A7	Carbohydrates	1.152
B8	Carbohydrates	1.150
A4	Polymers	1.140
A9	Carbohydrates	1.140
C11	Esters	1.119
A11	Carbohydrates	1.104

Table 5 : Field Samples 8 and 9

Sample 8

		Norm. Col. Intensity
H3	Aromatics	1.343
G1	Amino acids	1.333
H9	Alcohols	1.293
H2	Aromatics	1.286
H4	Aromatics	1.276
D8	Carboxylic acids	1.258
D4	Carboxylic acids	1.256
C3	Carbohydrates	1.248
E6	Carboxylic acids	1.243
G9	Amino acids	1.241
F11	Amino acids	1.221
D2	Carboxylic acids	1.221
E10	Carboxylic acids	1.218
D7	Carboxylic acids	1.211
D3	Carboxylic acids	1.198
D6	Carboxylic acids	1.191
C5	Carbohydrates	1.188
B3	Carbohydrates	1.181
F9	Amino acids	1.181
D9	Carboxylic acids	1.178
C9	Carbohydrates	1.178
B5	Carbohydrates	1.176
B9	Carbohydrates	1.168
C4	Carbohydrates	1.163
H10	Phosphorylated HC	1.158
H11	Phosphorylated HC	1.156
C6	Carbohydrates	1.156
D5	Carboxylic acids	1.156
F8	Amino acids	1.143
B7	Carbohydrates	1.139
A7	Carbohydrates	1.139
C1	Carbohydrates	1.134
G8	Amino acids	1.131
A9	Carbohydrates	1.124
H1	Aromatics	1.119
C2	Carbohydrates	1.104
A4	Polymers	1.101

Sample 9

		Norm. Col. Intensity
H9	Alcohols	1.392
H3	Aromatics	1.387
G1	Amino acids	1.371
H4	Aromatics	1.346
H2	Aromatics	1.336
G9	Amino acids	1.325
D4	Carboxylic acids	1.322
E6	Carboxylic acids	1.322
D7	Carboxylic acids	1.295
C3	Carbohydrates	1.285
D3	Carboxylic acids	1.279
E10	Carboxylic acids	1.268
F8	Amino acids	1.263
D6	Carboxylic acids	1.260
F11	Amino acids	1.255
D2	Carboxylic acids	1.250
D8	Carboxylic acids	1.244
D5	Carboxylic acids	1.231
C4	Carbohydrates	1.225
H11	Phosphorylated HC	1.214
C5	Carbohydrates	1.212
C9	Carbohydrates	1.212
C1	Carbohydrates	1.209
B3	Carbohydrates	1.201
D9	Carboxylic acids	1.201
B5	Carbohydrates	1.201
C2	Carbohydrates	1.201
F9	Amino acids	1.190
B9	Carbohydrates	1.185
C6	Carbohydrates	1.185
G8	Amino acids	1.179
B7	Carbohydrates	1.166
F10	Amino acids	1.163
C8	Carbohydrates	1.161
H10	Phosphorylated HC	1.155
B4	Carbohydrates	1.142
H1	Aromatics	1.136
A2	Polymers	1.126
B8	Carbohydrates	1.123
C7	Carbohydrates	1.120
A4	Polymers	1.104

Table 6 : Compounds that undergo change (T = 20° batch experiments)

Microplate Code	Compound Type	Difference between Initial and Final
Experimental conditions : 300 mg/L TSS and 0 mg/L DIF		
C3	Carbohydrates	0.377
D4	Carboxylic acids	0.376
E1	Carboxylic acids	0.368
H6	Amines	0.338
A9	Carbohydrates	0.326
H7	Alcohols	0.293
E4	Carboxylic acids	0.255
D8	Carboxylic acids	0.242
G2	Amino acids	0.239
A10	Carbohydrates	0.218
Experimental conditions : 300 mg/L TSS and 35 mg/L DIF		
A9	Carbohydrates	0.435
B12	Carbohydrates	0.348
G1	Amino acids	0.310
F2	Amides	0.293
F9	Amino acids	0.276
A12	Carbohydrates	0.266
A11	Carbohydrates	0.259
H12	Phosphorylated HC	0.242
C12	Esters	0.230
F8	Amino acids	0.229
H2	Aromatics	0.223
Experimental conditions : 300 mg/L TSS and 65 mg/L DIF		
H10	Phosphorylated HC	0.306
A9	Carbohydrates	0.303
G1	Amino acids	0.243
H4	Aromatics	0.243
Experimental conditions : 300 mg/L TSS and 130 mg/L DIF		
D4	Carboxylic acids	0.487
E1	Carboxylic acids	0.467
C3	Carbohydrates	0.390
A9	Carbohydrates	0.389
C5	Carbohydrates	0.335
G2	Amino acids	0.308
H7	Alcohols	0.298
F8	Amino acids	0.295
D8	Carboxylic acids	0.289

Table 7 : 300 mg/L TSS with 0 mg/L DIF

Principal Components

		PC1	PC2
C3	Carbohydrates	0.130	0.008
D4	Carboxylic acids	0.130	-0.015
C4	Carbohydrates	0.129	-0.008
B4	Carbohydrates	0.129	0.022
A9	Carbohydrates	0.128	0.034
A7	Carbohydrates	0.127	-0.024
B10	Carbohydrates	0.126	0.043
D9	Carboxylic acids	0.126	0.049
D8	Carboxylic acids	0.126	0.053
B3	Carbohydrates	0.126	0.038
C3	Carbohydrates	-0.125	0.040
F3	Amides	-0.125	-0.017
A7	Carbohydrates	-0.126	-0.008
C4	Carbohydrates	-0.126	-0.026
C1	Carbohydrates	-0.126	0.023
D5	Carboxylic acids	-0.127	-0.014
B3	Carbohydrates	-0.127	0.014
C5	Carbohydrates	-0.127	0.013
D3	Carboxylic acids	-0.127	0.002

Initial Consumption

E10	Carboxylic acids	1.34
H9	Alcohols	1.33
G9	Amino acids	1.30
D3	Carboxylic acids	1.29
F11	Amino acids	1.29
H3	Aromatics	1.28
D8	Carboxylic acids	1.28
D9	Carboxylic acids	1.27
D7	Carboxylic acids	1.27
F8	Amino acids	1.27
C3	Carbohydrates	1.27
G1	Amino acids	1.26
F9	Amino acids	1.26
D2	Carboxylic acids	1.26
H2	Aromatics	1.25
E6	Carboxylic acids	1.24
H4	Aromatics	1.24
D4	Carboxylic acids	1.24
C9	Carbohydrates	1.23
C5	Carbohydrates	1.23
H11	Phosphoryl. HC	1.22
H10	Phosphoryl. HC	1.21
D6	Carboxylic acids	1.20
C4	Carbohydrates	1.20
C6	Carbohydrates	1.19
D5	Carboxylic acids	1.19
G8	Amino acids	1.18
B3	Carbohydrates	1.17
C8	Carbohydrates	1.15
B7	Carbohydrates	1.15
B5	Carbohydrates	1.15
C2	Carbohydrates	1.14
F10	Amino acids	1.13
C7	Carbohydrates	1.13
B4	Carbohydrates	1.13
F3	Amides	1.12
B9	Carbohydrates	1.12
B8	Carbohydrates	1.11
A9	Carbohydrates	1.11
E9	Carboxylic acids	1.10

Final Consumption

F9	Amino acids	1.46
F8	Amino acids	1.45
F11	Amino acids	1.40
D2	Carboxylic acids	1.35
H3	Aromatics	1.33
E10	Carboxylic acids	1.33
D7	Carboxylic acids	1.31
H9	Alcohols	1.29
G1	Amino acids	1.29
G9	Amino acids	1.27
D6	Carboxylic acids	1.27
H11	Phosphoryl. HC	1.26
H2	Aromatics	1.25
G8	Amino acids	1.23
E6	Carboxylic acids	1.22
E4	Carboxylic acids	1.22
D9	Carboxylic acids	1.21
H10	Phosphoryl. HC	1.19
D5	Carboxylic acids	1.19
B9	Carbohydrates	1.17
H4	Aromatics	1.17
D3	Carboxylic acids	1.16
F10	Amino acids	1.16
C2	Carbohydrates	1.16
C6	Carbohydrates	1.15
B7	Carbohydrates	1.13
C5	Carbohydrates	1.12
E9	Carboxylic acids	1.12
B3	Carbohydrates	1.11
C8	Carbohydrates	1.11
C7	Carbohydrates	1.10
A4	Polymers	1.10
H12	Phosphoryl. HC	1.10
A3	Polymers	1.10

Table 8 : 300 mg/L TSS with 35 mg/L DIF

Principal Components

		PC1	PC2
H4	Aromatics	0.137	0.006
D4	Carboxylic acids	0.135	0.016
B11	Carbohydrates	0.135	0.035
D2	Carboxylic acids	0.135	0.004
A12	Carbohydrates	0.134	0.026
A10	Carbohydrates	0.133	-0.045
D8	Carboxylic acids	0.131	-0.019
C10	Carbohydrates	0.129	0.032
G3	Amino acids	0.129	0.016
C1	Carbohydrates	0.127	0.065
D5	Carboxylic acids	0.127	-0.058
G7	Amino acids	-0.126	0.049
F9	Amino acids	-0.126	-0.070
F4	Amides	-0.127	-0.059
H7	Alcohols	-0.127	0.033
F2	Amides	-0.128	-0.043
F5	Amino acids	-0.129	-0.057
E11	Carboxylic acids	-0.132	0.050
G2	Amino acids	-0.132	0.017
H2	Aromatics	-0.132	-0.046
H1	Aromatics	-0.133	-0.021
G9	Amino acids	-0.133	-0.043
A8	Carbohydrates	-0.134	-0.027
F10	Amino acids	-0.134	-0.039
F8	Amino acids	-0.134	-0.037
E9	Carboxylic acids	-0.134	-0.005
F7	Amino acids	-0.135	0.007
G12	Amino acids	-0.135	0.021
F1	Carboxylic acids	-0.136	0.023
E1	Carboxylic acids	-0.137	-0.010

Initial Consumption

F9	Amino acids	1.44
F8	Amino acids	1.43
G9	Amino acids	1.37
F10	Amino acids	1.36
G8	Amino acids	1.36
H2	Aromatics	1.36
F6	Amino acids	1.35
F5	Amino acids	1.32
F11	Amino acids	1.30
F7	Amino acids	1.30
G6	Amino acids	1.26
H6	Amines	1.24
G12	Amino acids	1.23
G7	Amino acids	1.22
C3	Carbohydrates	1.21
E10	Carboxylic acids	1.20
A8	Carbohydrates	1.19
H10	Phosphoryl. HC	1.17
H1	Aromatics	1.16
H9	Alcohols	1.16
E6	Carboxylic acids	1.15
D7	Carboxylic acids	1.15
D2	Carboxylic acids	1.12
C4	Carbohydrates	1.11
C8	Carbohydrates	1.11
D9	Carboxylic acids	1.11
C9	Carbohydrates	1.11
G2	Amino acids	1.10

Final Consumption

F9	Amino acids	1.71
F8	Amino acids	1.66
H2	Aromatics	1.58
G8	Amino acids	1.55
F10	Amino acids	1.54
F6	Amino acids	1.51
G9	Amino acids	1.50
F5	Amino acids	1.50
F7	Amino acids	1.42
F11	Amino acids	1.40
G1	Amino acids	1.37
F2	Amides	1.36
G12	Amino acids	1.34
E6	Carboxylic acids	1.33
H1	Aromatics	1.31
G7	Amino acids	1.29
H6	Amines	1.29
E10	Carboxylic acids	1.26
A8	Carbohydrates	1.26
G2	Amino acids	1.25
G6	Amino acids	1.22
C9	Carbohydrates	1.17
C3	Carbohydrates	1.17
C7	Carbohydrates	1.16
E7	Carboxylic acids	1.15
C8	Carbohydrates	1.15
C6	Carbohydrates	1.10
D6	Carboxylic acids	1.10
C4	Carbohydrates	1.10

Table 9 : 300 mg/L TSS with 65 mg/L DIF

Principal Components

		PC1	PC2
E8	Carboxylic acids	0.144	-0.002
B2	Carbohydrates	0.141	-0.028
F8	Amino acids	0.138	-0.048
D5	Carboxylic acids	0.138	-0.007
E1	Carboxylic acids	0.138	-0.036
G8	Amino acids	0.138	-0.051
F9	Amino acids	0.137	-0.055
G7	Amino acids	0.136	-0.026
D9	Carboxylic acids	0.135	0.010
B6	Carbohydrates	0.135	0.044
B4	Carbohydrates	0.134	0.067
F2	Amides	0.133	-0.005
E7	Carboxylic acids	0.132	-0.010
H7	Alcohols	0.132	0.012
C7	Carbohydrates	0.131	0.039
H1	Aromatics	0.130	-0.056
G2	Amino acids	0.129	0.037
E9	Carboxylic acids	0.129	-0.077
E6	Carboxylic acids	0.128	-0.033
G11	Amino acids	0.125	-0.091
D2	Carboxylic acids	-0.128	-0.054
H12	Phosphorylated H	-0.129	-0.058
A12	Carbohydrates	-0.131	-0.035
H3	Aromatics	-0.133	0.069
C5	Carbohydrates	-0.135	0.060
C1	Carbohydrates	-0.139	-0.047
C4	Carbohydrates	-0.139	0.029
A10	Carbohydrates	-0.140	-0.025
A9	Carbohydrates	-0.140	-0.040
H11	Phosphorylated H	-0.141	-0.021
B12	Carbohydrates	-0.141	0.036
D3	Carboxylic acids	-0.141	-0.002
H4	Aromatics	-0.142	-0.004
D4	Carboxylic acids	-0.144	0.005

Initial Consumption

F9	Amino acids	1.44
F8	Amino acids	1.43
G9	Amino acids	1.37
F10	Amino acids	1.36
G8	Amino acids	1.36
H2	Aromatics	1.36
F6	Amino acids	1.35
F5	Amino acids	1.32
F11	Amino acids	1.30
F7	Amino acids	1.30
G6	Amino acids	1.26
H6	Amines	1.24
G12	Amino acids	1.23
G7	Amino acids	1.22
C3	Carbohydrates	1.21
E10	Carboxylic acids	1.20
A8	Carbohydrates	1.19
H10	Phosphoryl. HC	1.17
H1	Aromatics	1.16
H9	Alcohols	1.16
E6	Carboxylic acids	1.15
D7	Carboxylic acids	1.15
D2	Carboxylic acids	1.12
C4	Carbohydrates	1.11
C8	Carbohydrates	1.11
D9	Carboxylic acids	1.11
C9	Carbohydrates	1.11
G2	Amino acids	1.10

Final Consumption

F8	Amino acids	1.60
F9	Amino acids	1.58
G8	Amino acids	1.53
H2	Aromatics	1.45
G9	Amino acids	1.42
F6	Amino acids	1.40
F10	Amino acids	1.39
F7	Amino acids	1.39
F5	Amino acids	1.34
G12	Amino acids	1.31
G7	Amino acids	1.31
G1	Amino acids	1.30
F11	Amino acids	1.26
G6	Amino acids	1.26
A8	Carbohydrates	1.23
E6	Carboxylic acids	1.23
F2	Amides	1.22
G2	Amino acids	1.21
H6	Amines	1.21
H1	Aromatics	1.21
D7	Carboxylic acids	1.20
E7	Carboxylic acids	1.19
C7	Carbohydrates	1.19
C8	Carbohydrates	1.15
E10	Carboxylic acids	1.15
D9	Carboxylic acids	1.14
D6	Carboxylic acids	1.13
G11	Amino acids	1.13
H5	Amines	1.12
E8	Carboxylic acids	1.11
C6	Carbohydrates	1.10

Table 10 : 300 mg/L TSS with 130 mg/L DIF

Principal Components

		PC1	PC2
C2	Carbohydrates	0.127	0.010
F7	Amino acids	0.126	0.005
G6	Amino acids	0.126	0.028
E1	Carboxylic acids	0.125	0.031
G7	Amino acids	0.125	-0.023
D2	Carboxylic acids	0.125	-0.035
E9	Carboxylic acids	0.125	0.035
H5	Amines	0.125	-0.027
C3	Carbohydrates	-0.125	0.040
F3	Amides	-0.125	-0.017
A7	Carbohydrates	-0.126	-0.008
C4	Carbohydrates	-0.126	-0.026
C1	Carbohydrates	-0.126	0.023
D5	Carboxylic acids	-0.127	-0.014
B3	Carbohydrates	-0.127	0.014
C5	Carbohydrates	-0.127	0.013
D3	Carboxylic acids	-0.127	0.002

Initial Consumption

E10	Carboxylic acids	1.34
H9	Alcohols	1.33
G9	Amino acids	1.30
D3	Carboxylic acids	1.29
F11	Amino acids	1.29
H3	Aromatics	1.28
D8	Carboxylic acids	1.28
D9	Carboxylic acids	1.27
D7	Carboxylic acids	1.27
F8	Amino acids	1.27
C3	Carbohydrates	1.27
G1	Amino acids	1.26
F9	Amino acids	1.26
D2	Carboxylic acids	1.26
H2	Aromatics	1.25
E6	Carboxylic acids	1.24
H4	Aromatics	1.24
D4	Carboxylic acids	1.24
C9	Carbohydrates	1.23
C5	Carbohydrates	1.23
H11	Phosphoryl. HC	1.22
H10	Phosphoryl. HC	1.21
D6	Carboxylic acids	1.20
C4	Carbohydrates	1.20
C6	Carbohydrates	1.19
D5	Carboxylic acids	1.19
G8	Amino acids	1.18
B3	Carbohydrates	1.17
C8	Carbohydrates	1.15
B7	Carbohydrates	1.15
B5	Carbohydrates	1.15
C2	Carbohydrates	1.14
F10	Amino acids	1.13
C7	Carbohydrates	1.13
B4	Carbohydrates	1.13
F3	Amides	1.12
B9	Carbohydrates	1.12
B8	Carbohydrates	1.11
A9	Carbohydrates	1.11
E9	Carboxylic acids	1.10

Final Consumption

F8	Amino acids	1.56
F9	Amino acids	1.45
F11	Amino acids	1.37
D2	Carboxylic acids	1.37
H9	Alcohols	1.35
D7	Carboxylic acids	1.34
G9	Amino acids	1.34
H11	Phosphoryl. HC	1.32
H2	Aromatics	1.30
G8	Amino acids	1.29
C2	Carbohydrates	1.28
H3	Aromatics	1.27
E10	Carboxylic acids	1.26
G1	Amino acids	1.25
E9	Carboxylic acids	1.25
E6	Carboxylic acids	1.22
F10	Amino acids	1.22
D6	Carboxylic acids	1.21
H12	Phosphoryl. HC	1.20
C8	Carbohydrates	1.20
H10	Phosphoryl. HC	1.19
A4	Polymers	1.17
C6	Carbohydrates	1.17
E4	Carboxylic acids	1.16
C7	Carbohydrates	1.16
E1	Carboxylic acids	1.15
G2	Amino acids	1.14
F7	Amino acids	1.10

Table 11 : Compounds that undergo change (Replicate and T = 5° experiments)

Microplate Code	Compound Type	Difference between Initial and Final

Experimental conditions : Replicate of 300 mg/L TSS and 130 mg/L DIF

A9	Carbohydrates	0.606
D8	Carboxylic acids	0.420
H7	Alcohols	0.385
B7	Carbohydrates	0.384
A12	Carbohydrates	0.347
F11	Amino acids	0.330

Experimental conditions : 300 mg/L TSS and 0 mg/L DIF run at 5°C

A6	Polymers	0.386
H10	Phosphorylated HC	0.345

Experimental conditions : 300 mg/L TSS and 130 mg/L DIF run at 5°C

None

Table 12 :Replicate of 300 mg/L TSS with 130 mg/L DIF

Principal Components

		PC1	PC2
E10	Carboxylic acids	0.140	0.001
B7	Carbohydrates	0.139	-0.009
E8	Carboxylic acids	0.138	0.028
F11	Amino acids	0.138	0.032
A8	Carbohydrates	0.138	-0.031
B6	Carbohydrates	0.136	-0.026
B5	Carbohydrates	0.135	0.028
A9	Carbohydrates	0.135	0.032
E12	Carboxylic acids	0.133	0.050
D9	Carboxylic acids	0.133	0.059
D8	Carboxylic acids	0.132	-0.061
B11	Carbohydrates	0.131	-0.009
C5	Carbohydrates	0.130	0.062
C11	Esters	0.129	-0.043
D5	Carboxylic acids	0.128	0.057
B4	Carbohydrates	0.128	0.039
A12	Carbohydrates	0.127	0.071
F1	Carboxylic acids	-0.129	-0.067
F8	Amino acids	-0.130	-0.040
H2	Aromatics	-0.131	-0.063
F3	Amides	-0.131	-0.061
F6	Amino acids	-0.133	0.037
G7	Amino acids	-0.134	0.031
H11	Phosphoryl. HC	-0.136	-0.042
F4	Amides	-0.136	-0.016
H7	Alcohols	-0.136	-0.036
G6	Amino acids	-0.138	0.016
F7	Amino acids	-0.140	0.007
D1	Carboxylic acids	-0.140	-0.016
C3	Carbohydrates	-0.140	-0.014

Initial Consumption

H3	Aromatics	1.56
H2	Aromatics	1.50
H4	Aromatics	1.46
G1	Amino acids	1.46
H1	Aromatics	1.45
C5	Carbohydrates	1.41
D6	Carboxylic acids	1.41
D9	Carboxylic acids	1.41
G9	Amino acids	1.40
C3	Carbohydrates	1.40
E10	Carboxylic acids	1.39
D3	Carboxylic acids	1.39
D5	Carboxylic acids	1.36
C9	Carbohydrates	1.36
H9	Alcohols	1.35
D2	Carboxylic acids	1.34
H11	Phosphoryl. HC	1.32
F9	Amino acids	1.30
C2	Carbohydrates	1.30
C6	Carbohydrates	1.30
E6	Carboxylic acids	1.29
C1	Carbohydrates	1.28
D7	Carboxylic acids	1.27
B3	Carbohydrates	1.27
F8	Amino acids	1.26
B9	Carbohydrates	1.26
F11	Amino acids	1.24
C4	Carbohydrates	1.24
H12	Phosphorylated H	1.23
G8	Amino acids	1.23
B5	Carbohydrates	1.23
F1	Carboxylic acids	1.22
C8	Carbohydrates	1.22
B4	Carbohydrates	1.20
A2	Polymers	1.18
B8	Carbohydrates	1.16
C11	Esters	1.13
C7	Carbohydrates	1.12
H10	Phosphorylated H	1.12
D4	Carboxylic acids	1.11
A7	Carbohydrates	1.10

Final Consumption

H3	Aromatics	1.56
H2	Aromatics	1.50
H9	Alcohols	1.46
H11	Phosphoryl. HC	1.46
C3	Carbohydrates	1.45
H1	Aromatics	1.41
H4	Aromatics	1.41
G9	Amino acids	1.41
F1	Carboxylic acids	1.40
G1	Amino acids	1.40
H10	Phosphoryl. HC	1.39
D7	Carboxylic acids	1.39
C1	Carbohydrates	1.36
D3	Carboxylic acids	1.36
D6	Carboxylic acids	1.35
F8	Amino acids	1.34
H12	Phosphoryl. HC	1.32
C9	Carbohydrates	1.30
F9	Amino acids	1.30
C4	Carbohydrates	1.30
F3	Amides	1.29
C6	Carbohydrates	1.28
C2	Carbohydrates	1.27
B3	Carbohydrates	1.27
D9	Carboxylic acids	1.26
D5	Carboxylic acids	1.26
D2	Carboxylic acids	1.24
C8	Carbohydrates	1.24
G8	Amino acids	1.23
D4	Carboxylic acids	1.23
F10	Amino acids	1.23
E6	Carboxylic acids	1.22

Table 13 : 300 mg/L TSS with 0 mg/L DIF at 5°C

Principal Components

		PC1	PC2
C1	Carbohydrates	0.151	-0.002
D7	Carboxylic acids	0.150	0.027
G2	Amino acids	0.148	-0.043
G3	Amino acids	0.147	0.047
C8	Carbohydrates	0.142	-0.056
G10	Amino acids	0.142	-0.068
E1	Carboxylic acids	0.142	-0.065
B1	Carbohydrates	0.141	0.055
C2	Carbohydrates	0.134	-0.082
G1	Amino acids	0.133	0.062
H10	Phosphoryl. HC	0.133	0.082
E10	Carboxylic acids	0.132	0.095
D2	Carboxylic acids	0.131	-0.061
C7	Carbohydrates	0.130	-0.055
H11	Phosphoryl. HC	0.126	0.083
D4	Carboxylic acids	-0.126	-0.083
B10	Carbohydrates	-0.128	-0.091
B7	Carbohydrates	-0.131	0.097
D11	Carboxylic acids	-0.132	0.079
G5	Amino acids	-0.132	0.000
A6	Polymers	-0.133	-0.002
B9	Carbohydrates	-0.135	-0.087
A12	Carbohydrates	-0.142	-0.044
H5	Amines	-0.146	0.040
A9	Carbohydrates	-0.146	0.044
G7	Amino acids	-0.146	0.045
A11	Carbohydrates	-0.148	-0.042

Initial Consumption

H2	Aromatics	1.81
H11	Phosphoryl. HC	1.62
G9	Amino acids	1.62
C8	Carbohydrates	1.60
D7	Carboxylic acids	1.56
G8	Amino acids	1.54
C7	Carbohydrates	1.52
D6	Carboxylic acids	1.50
H9	Alcohols	1.48
C2	Carbohydrates	1.46
C4	Carbohydrates	1.41
H12	Phosphoryl. HC	1.38
H10	Phosphoryl. HC	1.37
D3	Carboxylic acids	1.34
C6	Carbohydrates	1.33
E6	Carboxylic acids	1.32
C3	Carbohydrates	1.32
E10	Carboxylic acids	1.30
D9	Carboxylic acids	1.29
F8	Amino acids	1.26
H3	Aromatics	1.26
F9	Amino acids	1.22
D5	Carboxylic acids	1.22
A7	Carbohydrates	1.22
F11	Amino acids	1.21
C1	Carbohydrates	1.20
B4	Carbohydrates	1.20
H4	Aromatics	1.20
B8	Carbohydrates	1.19
G1	Amino acids	1.14
F10	Amino acids	1.13
G6	Amino acids	1.13
C5	Carbohydrates	1.13
A3	Polymers	1.11
D2	Carboxylic acids	1.11
E7	Carboxylic acids	1.11

Final Consumption

H2	Aromatics	1.69
H9	Alcohols	1.50
G9	Amino acids	1.49
D7	Carboxylic acids	1.49
F9	Amino acids	1.44
C7	Carbohydrates	1.43
D6	Carboxylic acids	1.40
C8	Carbohydrates	1.38
G8	Amino acids	1.37
C4	Carbohydrates	1.35
H11	Phosphoryl. HC	1.34
C2	Carbohydrates	1.34
C6	Carbohydrates	1.33
E6	Carboxylic acids	1.28
H3	Aromatics	1.25
H12	Phosphoryl. HC	1.25
F8	Amino acids	1.24
B8	Carbohydrates	1.23
D9	Carboxylic acids	1.20
F6	Amino acids	1.19
C3	Carbohydrates	1.19
E7	Carboxylic acids	1.19
B4	Carbohydrates	1.17
F10	Amino acids	1.17
F7	Amino acids	1.17
D5	Carboxylic acids	1.16
D3	Carboxylic acids	1.16
B10	Carbohydrates	1.15
H4	Aromatics	1.13
F11	Amino acids	1.13
D4	Carboxylic acids	1.11
G7	Amino acids	1.11
G1	Amino acids	1.11

Table 14 : 300 mg/L TSS with 130 mg/L DIF at 5°C

Principal Components

		PC1	PC2
H2	Aromatics	0.152	0.023
C1	Carbohydrates	0.150	0.039
E3	Carboxylic acids	0.149	-0.022
H11	Phosphoryl. HC	0.148	-0.015
H10	Phosphoryl. HC	0.147	-0.022
C2	Carbohydrates	0.146	0.060
E11	Carboxylic acids	0.145	-0.023
A2	Polymers	0.145	-0.001
C4	Carbohydrates	0.144	0.029
G4	Amino acids	0.142	-0.037
D2	Carboxylic acids	0.139	-0.080
E10	Carboxylic acids	0.137	0.040
H3	Aromatics	0.132	0.086
C3	Carbohydrates	0.131	-0.093
C8	Carbohydrates	0.130	0.090
B3	Carbohydrates	-0.126	0.009
F4	Amides	-0.128	0.023
C11	Esters	-0.129	0.086
A8	Carbohydrates	-0.130	0.063
D4	Carboxylic acids	-0.131	0.092
A6	Polymers	-0.134	-0.023
A5	Polymers	-0.136	0.053
D11	Carboxylic acids	-0.139	-0.077
A11	Carbohydrates	-0.141	-0.043
H6	Amines	-0.146	-0.060
A12	Carbohydrates	-0.146	0.043
G7	Amino acids	-0.149	0.023
A10	Carbohydrates	-0.150	0.010
B9	Carbohydrates	-0.151	-0.033
B7	Carbohydrates	-0.153	-0.017

Initial Consumption

H2	Aromatics	1.81
H11	Phosphoryl. HC	1.62
G9	Amino acids	1.62
C8	Carbohydrates	1.60
D7	Carboxylic acids	1.56
G8	Amino acids	1.54
C7	Carbohydrates	1.52
D6	Carboxylic acids	1.50
H9	Alcohols	1.48
C2	Carbohydrates	1.46
C4	Carbohydrates	1.41
H12	Phosphoryl. HC	1.38
H10	Phosphoryl. HC	1.37
D3	Carboxylic acids	1.34
C6	Carbohydrates	1.33
E6	Carboxylic acids	1.32
C3	Carbohydrates	1.32
E10	Carboxylic acids	1.30
D9	Carboxylic acids	1.29
F8	Amino acids	1.26
H3	Aromatics	1.26
F9	Amino acids	1.22
D5	Carboxylic acids	1.22
A7	Carbohydrates	1.22
F11	Amino acids	1.21
C1	Carbohydrates	1.20
H4	Aromatics	1.20
B4	Carbohydrates	1.20
B8	Carbohydrates	1.19
G1	Amino acids	1.14
F10	Amino acids	1.13
G6	Amino acids	1.13
C5	Carbohydrates	1.13
A3	Polymers	1.11
D2	Carboxylic acids	1.11
E7	Carboxylic acids	1.11

Final Consumption

H2	Aromatics	1.56
D7	Carboxylic acids	1.53
G9	Amino acids	1.48
H9	Alcohols	1.47
D6	Carboxylic acids	1.46
C8	Carbohydrates	1.44
C7	Carbohydrates	1.41
H12	Phosphoryl. HC	1.37
H11	Phosphoryl. HC	1.33
C2	Carbohydrates	1.33
G8	Amino acids	1.30
C6	Carbohydrates	1.28
D9	Carboxylic acids	1.26
E6	Carboxylic acids	1.24
F9	Amino acids	1.24
F8	Amino acids	1.23
D3	Carboxylic acids	1.22
B8	Carbohydrates	1.22
C4	Carbohydrates	1.21
H4	Aromatics	1.21
D5	Carboxylic acids	1.20
E10	Carboxylic acids	1.19
B4	Carbohydrates	1.19
H3	Aromatics	1.19
E7	Carboxylic acids	1.19
F10	Amino acids	1.18
D4	Carboxylic acids	1.18
C3	Carbohydrates	1.18
A3	Polymers	1.17
G1	Amino acids	1.15
B2	Carbohydrates	1.13
B10	Carbohydrates	1.12
A8	Carbohydrates	1.12
B6	Carbohydrates	1.11
A4	Polymers	1.11

Table 15 : Compounds that undergo change (unacclimatized vs acclimatized)

Microplate Code	Compound Type	Difference between Initial and Final

Experimental conditions : 2000 mg/L TSS and 0 mg/L DIF

C10	Carbohydrates	0.437
H7	Alcohols	0.399
B1	Carbohydrates	0.337
H4	Aromatics	0.289
C12	Esters	0.288
C9	Carbohydrates	0.284
F11	Amino acids	0.259
E9	Carboxylic acids	0.243
D2	Carboxylic acids	0.227
B3	Carbohydrates	0.223
F8	Amino acids	0.218

Experimental conditions : 2000 mg/L TSS and 65 mg/L DIF

G1	Amino acids	0.331
D4	Carboxylic acids	0.295
H6	Amines	0.286
E1	Carboxylic acids	0.286
F11	Amino acids	0.273
H5	Amines	0.232
H10	Phosphorylated HC	0.230

Experimental conditions : 2000 mg/L TSS and 130 mg/L DIF

E1	Carboxylic acids	0.359
G1	Amino acids	0.312
C9	Carbohydrates	0.307
F12	Amino acids	0.284
H6	Amines	0.246
G10	Amino acids	0.245

Table 16 : 2000 mg/L TSS with 0 mg/L DIF (unacclimatized vs acclimatized)

Principal Components

		PC1	PC2
A12	Carbohydrates	0.142	0.001
F12	Amino acids	0.142	-0.023
D4	Carboxylic acids	0.142	0.009
A10	Carbohydrates	0.142	0.031
C9	Carbohydrates	0.142	-0.022
B1	Carbohydrates	0.141	-0.027
G6	Amino acids	0.141	-0.037
B5	Carbohydrates	0.134	-0.056
D11	Carboxylic acids	0.132	0.049
C1	Carbohydrates	0.131	-0.076
F11	Amino acids	0.130	0.076
C10	Carbohydrates	0.127	-0.083
F2	Amides	0.126	-0.085
G9	Amino acids	0.126	-0.046
F7	Amino acids	0.126	0.043
F9	Amino acids	-0.126	0.085
H3	Aromatics	-0.130	-0.068
C2	Carbohydrates	-0.131	-0.059
A11	Carbohydrates	-0.132	-0.042
E4	Carboxylic acids	-0.132	0.069
H5	Amines	-0.133	-0.045
F1	Carboxylic acids	-0.134	-0.068
A7	Carbohydrates	-0.135	0.061
F8	Amino acids	-0.139	0.030
H4	Aromatics	-0.142	-0.029
B3	Carbohydrates	-0.142	0.023
B7	Carbohydrates	-0.143	-0.017
B9	Carbohydrates	-0.144	0.013
H7	Alcohols	-0.144	-0.008
D2	Carboxylic acids	-0.144	-0.002

Unacclimatized Consumption

H4	Aromatics	1.55
F8	Amino acids	1.53
D2	Carboxylic acids	1.43
H3	Aromatics	1.40
F9	Amino acids	1.36
B3	Carbohydrates	1.36
H11	Phosphoryl. HC	1.33
B9	Carbohydrates	1.33
B7	Carbohydrates	1.32
G1	Amino acids	1.29
D8	Carboxylic acids	1.28
H9	Alcohols	1.28
H12	Phosphoryl. HC	1.26
H2	Aromatics	1.25
E9	Carboxylic acids	1.21
A7	Carbohydrates	1.21
G9	Amino acids	1.20
F10	Amino acids	1.20
E10	Carboxylic acids	1.20
D6	Carboxylic acids	1.20
D7	Carboxylic acids	1.19
C6	Carbohydrates	1.17
E6	Carboxylic acids	1.17
C2	Carbohydrates	1.16
A2	Polymers	1.16
G8	Amino acids	1.13
C3	Carbohydrates	1.13
H1	Aromatics	1.11
H10	Phosphoryl. HC	1.11
D9	Carboxylic acids	1.10
A4	Polymers	1.10

Acclimatized Consumption

F11	Amino acids	1.35
G1	Amino acids	1.35
H3	Aromatics	1.32
D8	Carboxylic acids	1.31
F8	Amino acids	1.31
H9	Alcohols	1.27
F9	Amino acids	1.27
G8	Amino acids	1.27
H4	Aromatics	1.26
H11	Phosphoryl. HC	1.25
C9	Carbohydrates	1.25
G9	Amino acids	1.24
H2	Aromatics	1.23
D6	Carboxylic acids	1.22
D9	Carboxylic acids	1.21
E10	Carboxylic acids	1.21
D2	Carboxylic acids	1.20
E6	Carboxylic acids	1.20
D7	Carboxylic acids	1.20
C3	Carbohydrates	1.19
F10	Amino acids	1.18
H10	Phosphoryl. HC	1.17
B9	Carbohydrates	1.16
C8	Carbohydrates	1.16
D5	Carboxylic acids	1.15
H12	Phosphoryl. HC	1.14
C1	Carbohydrates	1.14
B7	Carbohydrates	1.14
D3	Carboxylic acids	1.14
B3	Carbohydrates	1.13
D4	Carboxylic acids	1.12
C6	Carbohydrates	1.12
H1	Aromatics	1.10

Table 17 : 2000 mg/L TSS with 65 mg/L DIF (unacclimatized vs acclimatized)

Principal Components

		PC1	PC2
A6	Polymers	0.133	-0.007
F8	Amino acids	0.131	-0.034
B7	Carbohydrates	0.131	-0.023
A7	Carbohydrates	0.130	-0.006
H6	Amines	0.128	-0.052
E5	Carboxylic acids	0.128	0.020
G7	Amino acids	0.128	0.026
A5	Polymers	0.127	-0.030
E1	Carboxylic acids	0.125	0.068
A4	Polymers	0.125	0.053
E4	Carboxylic acids	0.125	0.069
C2	Carbohydrates	0.125	-0.019
F6	Amino acids	0.125	-0.062
B8	Carbohydrates	-0.125	0.045
C11	Esters	-0.127	-0.060
C3	Carbohydrates	-0.127	-0.023
F12	Amino acids	-0.128	-0.026
B11	Carbohydrates	-0.129	-0.052
C12	Esters	-0.130	-0.036
D4	Carboxylic acids	-0.130	-0.043
D10	Carboxylic acids	-0.130	0.006
E12	Carboxylic acids	-0.131	-0.037
H11	Phosphorylated H	-0.131	-0.034
G1	Amino acids	-0.133	0.009

Unacclimatized Consumption

F8	Amino acids	1.48
F9	Amino acids	1.34
B7	Carbohydrates	1.33
D2	Carboxylic acids	1.33
H3	Aromatics	1.29
E6	Carboxylic acids	1.28
E10	Carboxylic acids	1.28
H2	Aromatics	1.27
G9	Amino acids	1.27
G8	Amino acids	1.26
D7	Carboxylic acids	1.25
F10	Amino acids	1.23
H4	Aromatics	1.23
A4	Polymers	1.22
B3	Carbohydrates	1.21
H9	Alcohols	1.20
D6	Carboxylic acids	1.20
C7	Carbohydrates	1.20
H11	Phosphoryl. HC	1.19
D8	Carboxylic acids	1.19
C2	Carbohydrates	1.18
D9	Carboxylic acids	1.18
D5	Carboxylic acids	1.15
C9	Carbohydrates	1.14
E9	Carboxylic acids	1.12
H12	Phosphoryl. HC	1.12
E4	Carboxylic acids	1.11
C5	Carbohydrates	1.11
F7	Amino acids	1.11
A3	Polymers	1.10
C8	Carbohydrates	1.10
F11	Amino acids	1.10

Acclimatized Consumption

F11	Amino acids	1.37
G1	Amino acids	1.36
H9	Alcohols	1.32
H3	Aromatics	1.31
D8	Carboxylic acids	1.30
F9	Amino acids	1.30
G9	Amino acids	1.29
E10	Carboxylic acids	1.28
H11	Phosphoryl. HC	1.28
H4	Aromatics	1.28
F8	Amino acids	1.27
C9	Carbohydrates	1.26
G8	Amino acids	1.24
D9	Carboxylic acids	1.24
H10	Phosphoryl. HC	1.22
E6	Carboxylic acids	1.21
D6	Carboxylic acids	1.21
H2	Aromatics	1.21
D2	Carboxylic acids	1.19
H12	Phosphoryl. HC	1.19
D4	Carboxylic acids	1.19
D7	Carboxylic acids	1.18
D5	Carboxylic acids	1.16
F10	Amino acids	1.16
B9	Carbohydrates	1.16
B7	Carbohydrates	1.15
B3	Carbohydrates	1.14
C8	Carbohydrates	1.13
C4	Carbohydrates	1.13
F3	Amides	1.13
C3	Carbohydrates	1.12
C5	Carbohydrates	1.12
D3	Carboxylic acids	1.12
H1	Aromatics	1.12
C7	Carbohydrates	1.11
C6	Carbohydrates	1.11
C1	Carbohydrates	1.10
C2	Carbohydrates	1.10

Table 18 : 2000 mg/L TSS with 130 mg/L DIF (unacclimatized vs acclimatized)

Principal Components

		PC1	PC2
C6	Carbohydrates	0.141	-0.016
F5	Amino acids	0.141	0.021
B7	Carbohydrates	0.140	0.026
C7	Carbohydrates	0.140	-0.021
H6	Amines	0.139	-0.032
D12	Carboxylic acids	0.139	-0.038
A2	Polymers	0.136	-0.003
B2	Carbohydrates	0.136	-0.050
G2	Amino acids	0.136	0.052
F9	Amino acids	0.135	-0.022
C2	Carbohydrates	0.133	0.063
E4	Carboxylic acids	0.132	0.025
E1	Carboxylic acids	0.131	0.044
D2	Carboxylic acids	0.129	-0.075
A12	Carbohydrates	0.127	0.057
B3	Carbohydrates	0.127	-0.065
D7	Carboxylic acids	0.127	-0.078
A5	Polymers	0.126	0.059
H4	Aromatics	-0.125	0.077
D11	Carboxylic acids	-0.125	0.077
E6	Carboxylic acids	-0.126	-0.076
G1	Amino acids	-0.130	0.074
D3	Carboxylic acids	-0.131	-0.055
F11	Amino acids	-0.132	0.067
G10	Amino acids	-0.134	-0.049
D4	Carboxylic acids	-0.134	0.057
F3	Amides	-0.135	0.009
C11	Esters	-0.139	0.036
G12	Amino acids	-0.140	0.028
C12	Esters	-0.142	0.010

Unacclimatized Consumption

F9	Amino acids	1.45
F8	Amino acids	1.37
D2	Carboxylic acids	1.33
B7	Carbohydrates	1.32
B9	Carbohydrates	1.30
G8	Amino acids	1.29
D8	Carboxylic acids	1.27
D7	Carboxylic acids	1.24
G9	Amino acids	1.24
H11	Phosphoryl. HC	1.24
E10	Carboxylic acids	1.24
H2	Aromatics	1.23
H3	Aromatics	1.23
C6	Carbohydrates	1.22
D6	Carboxylic acids	1.22
B3	Carbohydrates	1.22
H10	Phosphoryl. HC	1.20
A4	Polymers	1.19
H9	Alcohols	1.18
C7	Carbohydrates	1.18
H12	Phosphoryl. HC	1.18
E1	Carboxylic acids	1.18
C2	Carbohydrates	1.16
E6	Carboxylic acids	1.16
D5	Carboxylic acids	1.16
D9	Carboxylic acids	1.16
E4	Carboxylic acids	1.14
C8	Carbohydrates	1.14
B4	Carbohydrates	1.14
H4	Aromatics	1.14
F10	Amino acids	1.13
E9	Carboxylic acids	1.13
F11	Amino acids	1.12

Acclimatized Consumption

F11	Amino acids	1.35
H9	Alcohols	1.34
D8	Carboxylic acids	1.33
G1	Amino acids	1.33
H3	Aromatics	1.30
F9	Amino acids	1.28
G9	Amino acids	1.27
D9	Carboxylic acids	1.26
H11	Phosphoryl. HC	1.25
F8	Amino acids	1.25
E10	Carboxylic acids	1.25
H4	Aromatics	1.25
E6	Carboxylic acids	1.24
H10	Phosphoryl. HC	1.23
D2	Carboxylic acids	1.23
D6	Carboxylic acids	1.23
C9	Carbohydrates	1.23
G8	Amino acids	1.22
D4	Carboxylic acids	1.22
D7	Carboxylic acids	1.21
F10	Amino acids	1.20
H2	Aromatics	1.20
B9	Carbohydrates	1.17
C3	Carbohydrates	1.16
B7	Carbohydrates	1.15
C8	Carbohydrates	1.13
D5	Carboxylic acids	1.13
H1	Aromatics	1.13
H12	Phosphoryl. HC	1.13
F12	Amino acids	1.12
B3	Carbohydrates	1.11
C6	Carbohydrates	1.11
D3	Carboxylic acids	1.10
C4	Carbohydrates	1.10

Table 19 : Compounds that undergo change (SBR experiments)

Microplate Code	Compound Type	Difference between Initial and Final
Experimental conditions : 2000 mg/L TSS and 190 mg/L DIF at 20°C		
A7	Carbohydrates	1.049
A9	Carbohydrates	1.040
A2	Polymers	0.799
H3	Aromatics	0.763
H4	Aromatics	0.751
A11	Carbohydrates	0.738
E11	Carboxylic acids	0.690
A6	Polymers	0.687
G7	Amino acids	0.681
F11	Amino acids	0.679
B7	Carbohydrates	0.649
G12	Amino acids	0.623
D8	Carboxylic acids	0.598
G6	Amino acids	0.588
D4	Carboxylic acids	0.558
D11	Carboxylic acids	0.557
G2	Amino acids	0.552
A5	Polymers	0.540
H6	Amines	0.522
E5	Carboxylic acids	0.498
F5	Amino acids	0.482
F6	Amino acids	0.472
C3	Carbohydrates	0.460
C9	Carbohydrates	0.439
E9	Carboxylic acids	0.428
Experimental conditions : 2000 mg/L TSS and 190 mg/L DIF at 5°C		
A12	Carbohydrates	0.661
H4	Aromatics	0.601
D4	Carboxylic acids	0.596
H7	Alcohols	0.567
B7	Carbohydrates	0.459
B12	Carbohydrates	0.432
F12	Amino acids	0.430
F1	Carboxylic acids	0.430
G3	Amino acids	0.402
E5	Carboxylic acids	0.397
A3	Polymers	0.394
B11	Carbohydrates	0.389
H5	Amines	0.377
E3	Carboxylic acids	0.374
A9	Carbohydrates	0.368

Table 19 (continued) : Compounds that undergo change (SBR experiments)

Microplate Code	Compound Type	Difference between Initial and Final
Experimental conditions : 2000 mg/L TSS and 190 mg/L DIF at 20°C to 5°C (switch)		
C11	Esters	0.564
B3	Carbohydrates	0.546
A10	Carbohydrates	0.535
F3	Amides	0.508
H7	Alcohols	0.483
E5	Carboxylic acids	0.483
B8	Carbohydrates	0.481
C3	Carbohydrates	0.467
A12	Carbohydrates	0.467
B11	Carbohydrates	0.455
G10	Amino acids	0.455
G6	Amino acids	0.432
G7	Amino acids	0.422

Table 20 : SBR1 (2000 mg/L TSS with 190 mg/L DIF at 20°C)

Principal Components

		PC1	PC2
A7	Carbohydrates	0.151	0.001
A11	Carbohydrates	0.145	0.021
A9	Carbohydrates	0.144	-0.038
B7	Carbohydrates	0.142	-0.056
H3	Aromatics	0.142	-0.043
D5	Carboxylic acids	0.138	-0.043
D4	Carboxylic acids	0.138	-0.072
D6	Carboxylic acids	0.137	-0.006
C9	Carbohydrates	0.137	-0.022
D8	Carboxylic acids	0.136	-0.074
F11	Amino acids	0.135	0.065
G9	Amino acids	0.133	0.031
F3	Amides	0.125	0.020
F8	Amino acids	-0.127	0.015
H5	Amines	-0.128	0.091
E4	Carboxylic acids	-0.129	-0.070
H12	Phosphorylated H	-0.129	0.078
H8	Alcohols	-0.129	0.054
G2	Amino acids	-0.135	-0.006
E11	Carboxylic acids	-0.137	-0.064
G7	Amino acids	-0.138	-0.056
E5	Carboxylic acids	-0.141	0.021
E6	Carboxylic acids	-0.142	0.027
F7	Amino acids	-0.142	0.035
F5	Amino acids	-0.143	0.017
E8	Carboxylic acids	-0.144	0.017
A6	Polymers	-0.144	0.049
D11	Carboxylic acids	-0.144	0.033
G6	Amino acids	-0.145	-0.034
A5	Polymers	-0.148	0.029
F6	Amino acids	-0.150	0.000
E9	Carboxylic acids	-0.150	0.006
G12	Amino acids	-0.151	-0.011

Initial Consumption

H3	Aromatics	1.47
F8	Amino acids	1.37
H2	Aromatics	1.37
F11	Amino acids	1.36
H9	Alcohols	1.36
G9	Amino acids	1.35
E10	Carboxylic acids	1.35
H4	Aromatics	1.34
D8	Carboxylic acids	1.34
D7	Carboxylic acids	1.32
H12	Phosphoryl. HC	1.30
F9	Amino acids	1.28
C3	Carbohydrates	1.27
D2	Carboxylic acids	1.27
C9	Carbohydrates	1.26
C1	Carbohydrates	1.26
D6	Carboxylic acids	1.26
A4	Polymers	1.25
E6	Carboxylic acids	1.25
D9	Carboxylic acids	1.23
G1	Amino acids	1.22
C4	Carbohydrates	1.20
A3	Polymers	1.20
H11	Phosphoryl. HC	1.20
B7	Carbohydrates	1.19
C8	Carbohydrates	1.19
C7	Carbohydrates	1.18
E9	Carboxylic acids	1.18
G8	Amino acids	1.17
A2	Polymers	1.16
F10	Amino acids	1.15
D5	Carboxylic acids	1.15
F3	Amides	1.15
B4	Carbohydrates	1.15
A9	Carbohydrates	1.15
C2	Carbohydrates	1.14
D3	Carboxylic acids	1.14
B8	Carbohydrates	1.12
E4	Carboxylic acids	1.12
A7	Carbohydrates	1.12
C6	Carbohydrates	1.12
B5	Carbohydrates	1.11
B9	Carbohydrates	1.11
H1	Aromatics	1.10

Final Consumption

F9	Amino acids	1.62
E9	Carboxylic acids	1.60
F8	Amino acids	1.58
E6	Carboxylic acids	1.50
G7	Amino acids	1.50
G2	Amino acids	1.47
G8	Amino acids	1.46
D2	Carboxylic acids	1.46
F10	Amino acids	1.46
E10	Carboxylic acids	1.44
G12	Amino acids	1.41
F6	Amino acids	1.39
G6	Amino acids	1.39
G1	Amino acids	1.37
E4	Carboxylic acids	1.36
H12	Phosphoryl. HC	1.36
F5	Amino acids	1.35
D3	Carboxylic acids	1.35
A6	Polymers	1.35
H11	Phosphoryl. HC	1.31
D11	Carboxylic acids	1.30
A5	Polymers	1.28
H2	Aromatics	1.27
H9	Alcohols	1.26
F7	Amino acids	1.23
D7	Carboxylic acids	1.23
E11	Carboxylic acids	1.22
H6	Amines	1.21
A4	Polymers	1.20
C4	Carbohydrates	1.18
A3	Polymers	1.18
C7	Carbohydrates	1.15
G9	Amino acids	1.13
E12	Carboxylic acids	1.11

Table 21 : SBR2 (2000 mg/L TSS with 190 mg/L DIF at 5°C)

Principal Components

		PC1	PC2
F1	Carboxylic acids	0.169	0.049
H1	Aromatics	0.157	0.047
H3	Aromatics	0.154	0.086
H7	Alcohols	0.146	-0.051
E3	Carboxylic acids	0.145	-0.048
F7	Amino acids	0.144	0.056
H10	Phosphorylated H	0.142	0.053
F11	Amino acids	0.141	0.061
F8	Amino acids	0.141	0.083
E5	Carboxylic acids	0.136	-0.060
F9	Amino acids	0.134	0.094
E7	Carboxylic acids	0.132	0.048
G3	Amino acids	0.131	-0.143
H4	Aromatics	0.127	-0.040
A10	Carbohydrates	-0.131	-0.009
A4	Polymers	-0.140	0.089
C7	Carbohydrates	-0.142	0.139
D11	Carboxylic acids	-0.151	-0.112
B4	Carbohydrates	-0.151	0.091
A8	Carbohydrates	-0.151	0.101
B7	Carbohydrates	-0.152	0.002
B2	Carbohydrates	-0.160	0.034
A12	Carbohydrates	-0.162	0.038
B6	Carbohydrates	-0.163	0.039
A6	Polymers	-0.163	-0.075
B10	Carbohydrates	-0.170	0.050
A3	Polymers	-0.171	0.048
B12	Carbohydrates	-0.171	-0.002
B11	Carbohydrates	-0.171	0.040
A5	Polymers	-0.173	-0.023

Initial Consumption

H9	Alcohols	1.60
G9	Amino acids	1.57
H2	Aromatics	1.55
C8	Carbohydrates	1.52
C2	Carbohydrates	1.51
D7	Carboxylic acids	1.50
H11	Phosphoryl. HC	1.50
C7	Carbohydrates	1.49
G8	Amino acids	1.45
H12	Phosphoryl. HC	1.42
C3	Carbohydrates	1.41
C6	Carbohydrates	1.41
D6	Carboxylic acids	1.36
B4	Carbohydrates	1.36
C1	Carbohydrates	1.32
B8	Carbohydrates	1.32
A3	Polymers	1.31
C4	Carbohydrates	1.30
B10	Carbohydrates	1.30
B6	Carbohydrates	1.30
B2	Carbohydrates	1.29
A8	Carbohydrates	1.27
E10	Carboxylic acids	1.25
H3	Aromatics	1.25
A4	Polymers	1.25
D3	Carboxylic acids	1.22
F9	Amino acids	1.22
C9	Carbohydrates	1.22
B11	Carbohydrates	1.18
F8	Amino acids	1.16
F10	Amino acids	1.16
B12	Carbohydrates	1.15
D9	Carboxylic acids	1.15
B9	Carbohydrates	1.14
E6	Carboxylic acids	1.13
A2	Polymers	1.13
H1	Aromatics	1.12
H10	Phosphoryl. HC	1.12
A12	Carbohydrates	1.11

Final Consumption

H3	Aromatics	1.59
H4	Aromatics	1.51
H2	Aromatics	1.49
F1	Carboxylic acids	1.39
H11	Phosphoryl. HC	1.39
G9	Amino acids	1.38
C3	Carbohydrates	1.37
F8	Amino acids	1.37
F9	Amino acids	1.31
H9	Alcohols	1.30
D9	Carboxylic acids	1.27
C4	Carbohydrates	1.27
D7	Carboxylic acids	1.27
C2	Carbohydrates	1.26
D6	Carboxylic acids	1.26
H12	Phosphoryl. HC	1.25
G8	Amino acids	1.24
C5	Carbohydrates	1.24
H7	Alcohols	1.24
C6	Carbohydrates	1.23
H10	Phosphoryl. HC	1.23
D5	Carboxylic acids	1.23
G1	Amino acids	1.23
H1	Aromatics	1.22
E6	Carboxylic acids	1.22
C8	Carbohydrates	1.21
F11	Amino acids	1.21
E7	Carboxylic acids	1.21
D4	Carboxylic acids	1.21
C9	Carbohydrates	1.18
C1	Carbohydrates	1.16
C7	Carbohydrates	1.14
D8	Carboxylic acids	1.13
E10	Carboxylic acids	1.12
F10	Amino acids	1.11
F7	Amino acids	1.10

Table 22 : SBR3 (2000 mg/L TSS with 190 mg/L DIF at 20°C to 5°C)

Principal Components

		PC1	PC2
B8	Carbohydrates	0.162	-0.053
B12	Carbohydrates	0.155	-0.038
A12	Carbohydrates	0.150	0.031
B11	Carbohydrates	0.146	0.083
C9	Carbohydrates	0.145	-0.095
A10	Carbohydrates	0.143	0.084
B10	Carbohydrates	0.137	0.028
A8	Carbohydrates	0.137	0.119
C8	Carbohydrates	0.133	0.013
C3	Carbohydrates	0.125	0.114
E3	Carboxylic acids	-0.127	-0.033
G6	Amino acids	-0.130	-0.020
E6	Carboxylic acids	-0.132	-0.091
F5	Amino acids	-0.133	-0.107
F9	Amino acids	-0.138	0.089
F4	Amides	-0.139	-0.092
H7	Alcohols	-0.144	-0.022
G4	Amino acids	-0.146	0.049
F6	Amino acids	-0.149	-0.068
F2	Amides	-0.152	0.066
G3	Amino acids	-0.152	0.037
F8	Amino acids	-0.153	0.104
G5	Amino acids	-0.155	0.016
F3	Amides	-0.158	-0.024
E4	Carboxylic acids	-0.159	0.088
E7	Carboxylic acids	-0.163	0.009
F7	Amino acids	-0.164	-0.030
F10	Amino acids	-0.168	0.036
G7	Amino acids	-0.168	-0.025
E5	Carboxylic acids	-0.170	-0.035

Initial Consumption

H2	Aromatics	1.59
H9	Alcohols	1.53
H11	Phosphoryl. HC	1.48
G9	Amino acids	1.48
H3	Aromatics	1.47
C3	Carbohydrates	1.44
D7	Carboxylic acids	1.39
D9	Carboxylic acids	1.37
F9	Amino acids	1.36
C9	Carbohydrates	1.35
D6	Carboxylic acids	1.35
C5	Carbohydrates	1.35
C2	Carbohydrates	1.34
C6	Carbohydrates	1.34
H12	Phosphoryl. HC	1.33
F8	Amino acids	1.32
C4	Carbohydrates	1.32
C8	Carbohydrates	1.32
A2	Polymers	1.29
G1	Amino acids	1.28
C1	Carbohydrates	1.26
B5	Carbohydrates	1.26
D3	Carboxylic acids	1.26
B3	Carbohydrates	1.24
D2	Carboxylic acids	1.22
H10	Phosphoryl. HC	1.21
H4	Aromatics	1.20
C7	Carbohydrates	1.20
H1	Aromatics	1.20
F1	Carboxylic acids	1.18
E10	Carboxylic acids	1.15
B8	Carbohydrates	1.14
B4	Carbohydrates	1.13
D5	Carboxylic acids	1.12
B2	Carbohydrates	1.12
A4	Polymers	1.11
F11	Amino acids	1.10
G8	Amino acids	1.10

Final Consumption

F9	Amino acids	1.74
F8	Amino acids	1.68
D2	Carboxylic acids	1.59
H2	Aromatics	1.58
H11	Phosphoryl. HC	1.48
G8	Amino acids	1.43
G9	Amino acids	1.43
H1	Aromatics	1.41
F10	Amino acids	1.40
F7	Amino acids	1.38
H3	Aromatics	1.38
D8	Carboxylic acids	1.34
F1	Carboxylic acids	1.34
H4	Aromatics	1.34
G7	Amino acids	1.33
G6	Amino acids	1.33
H9	Alcohols	1.32
E7	Carboxylic acids	1.31
D7	Carboxylic acids	1.31
F6	Amino acids	1.29
D6	Carboxylic acids	1.28
E9	Carboxylic acids	1.28
H7	Alcohols	1.27
H12	Phosphoryl. HC	1.24
D9	Carboxylic acids	1.23
G3	Amino acids	1.19
D3	Carboxylic acids	1.19
E10	Carboxylic acids	1.19
G10	Amino acids	1.18
H6	Amines	1.18
C4	Carbohydrates	1.16
H10	Phosphoryl. HC	1.16
E6	Carboxylic acids	1.15
E8	Carboxylic acids	1.15
F2	Amides	1.15
C2	Carbohydrates	1.14
C9	Carbohydrates	1.14
H5	Amines	1.14
F5	Amino acids	1.14
E5	Carboxylic acids	1.13
C8	Carbohydrates	1.12
E4	Carboxylic acids	1.12
D4	Carboxylic acids	1.12

Table 23 : Comparison of compounds that undergo change (SBR1)

Microplate Code	Compound Type	Difference between Initial and Final
Day 1 to Day 7		
A9	Carbohydrates	1.031
A7	Carbohydrates	0.887
H3	Aromatics	0.867
A6	Polymers	0.804
B7	Carbohydrates	0.674
D8	Carboxylic acids	0.659
D4	Carboxylic acids	0.641
H4	Aromatics	0.638
F12	Amino acids	0.616
D11	Carboxylic acids	0.604
A11	Carbohydrates	0.594
F5	Amino acids	0.574
E1	Carboxylic acids	0.541
G6	Amino acids	0.540
E5	Carboxylic acids	0.531
A5	Polymers	0.516
G2	Amino acids	0.500
F6	Amino acids	0.480
D1	Carboxylic acids	0.465
G7	Amino acids	0.431
G12	Amino acids	0.422
Day 7 to Day 15		
E1	Carboxylic acids	0.719
A2	Polymers	0.684
F11	Amino acids	0.417

Table 24 : Comparison of compounds that undergo change (SBR2)

Microplate Code	Compound Type	Difference between Initial and Final
Day 1 to Day 7		
D4	Carboxylic acids	0.622
F12	Amino acids	0.618
H4	Aromatics	0.616
A9	Carbohydrates	0.552
F3	Amides	0.550
A8	Carbohydrates	0.519
A12	Carbohydrates	0.503
B7	Carbohydrates	0.502
B11	Carbohydrates	0.496
B6	Carbohydrates	0.485
C7	Carbohydrates	0.482
A3	Polymers	0.468
C8	Carbohydrates	0.444
B4	Carbohydrates	0.439
C2	Carbohydrates	0.438
B12	Carbohydrates	0.430
B10	Carbohydrates	0.413
G3	Amino acids	0.410
F11	Amino acids	0.388
G10	Amino acids	0.370
B2	Carbohydrates	0.361
Day 7 to Day 15		
H7	Alcohols	0.396
G10	Amino acids	0.375

Table 25 : Comparison of compounds that undergo change (SBR3)

Microplate Code	Compound Type	Difference between Initial and Final

Day 1 to Day 7

A11	Carbohydrates	0.799
A10	Carbohydrates	0.773
B11	Carbohydrates	0.715
E5	Carboxylic acids	0.678
E3	Carboxylic acids	0.650
F7	Amino acids	0.634
F6	Amino acids	0.581
F5	Amino acids	0.561
F4	Amides	0.560
F3	Amides	0.539
H5	Amines	0.519
B3	Carbohydrates	0.517
G5	Amino acids	0.507
C5	Carbohydrates	0.506
G7	Amino acids	0.504
H10	Phosphorylated HC	0.497
A8	Carbohydrates	0.495
A12	Carbohydrates	0.489
H7	Alcohols	0.474
A2	Polymers	0.469
C3	Carbohydrates	0.468
D9	Carboxylic acids	0.462
D4	Carboxylic acids	0.460
G3	Amino acids	0.443

Day 8 to Day 17

A6	Polymers	0.654
C11	Esters	0.576
D2	Carboxylic acids	0.575
F5	Amino acids	0.500
B9	Carbohydrates	0.453

Table 26 : Comparison of Initial Populations

Microplate Code	Compound Type	Difference between populations

SBR1 to SBR2

A9	Carbohydrates	0.704
D8	Carboxylic acids	0.534
F12	Amino acids	0.497
D4	Carboxylic acids	0.445
H4	Aromatics	0.432
F3	Amides	0.425

SBR1 to SBR3

A9	Carbohydrates	0.782
F3	Amides	0.641
F12	Amino acids	0.465
B7	Carbohydrates	0.458

SBR2 to SBR3

None

Table 27 : Comparison of Final Populations

Microplate Code	Compound Type	Difference between populations
SBR1 to SBR2		
G12	Amino acids	0.951
A7	Carbohydrates	0.949
H4	Aromatics	0.919
H3	Aromatics	0.882
E9	Carboxylic acids	0.805
E11	Carboxylic acids	0.789
A5	Polymers	0.759
A6	Polymers	0.732
D4	Carboxylic acids	0.709
A9	Carbohydrates	0.705
A2	Polymers	0.671
D11	Carboxylic acids	0.654
G2	Amino acids	0.632
A12	Carbohydrates	0.607
H7	Alcohols	0.597
C3	Carbohydrates	0.562
A11	Carbohydrates	0.528
F11	Amino acids	0.527
D2	Carboxylic acids	0.518
G6	Amino acids	0.497
E4	Carboxylic acids	0.479
G7	Amino acids	0.478
E1	Carboxylic acids	0.450
C5	Carbohydrates	0.440
H1	Aromatics	0.434
F6	Amino acids	0.414
F5	Amino acids	0.412
E7	Carboxylic acids	0.409

Table 27 : Comparison of Final Populations

Microplate Code	Compound Type	Difference between populations
SBR1 to SBR3		
A6	Polymers	1.001
A5	Polymers	0.926
A12	Carbohydrates	0.779
H4	Aromatics	0.746
G12	Amino acids	0.693
H3	Aromatics	0.667
E11	Carboxylic acids	0.626
H7	Alcohols	0.626
D4	Carboxylic acids	0.621
H1	Aromatics	0.621
D8	Carboxylic acids	0.604
A7	Carbohydrates	0.600
G2	Amino acids	0.562
A2	Polymers	0.554
C11	Esters	0.553
B12	Carbohydrates	0.538
E7	Carboxylic acids	0.512
G10	Amino acids	0.503
A8	Carbohydrates	0.445
E12	Carboxylic acids	0.434
SBR2 to SBR3		
A9	Carbohydrates	0.725
D2	Carboxylic acids	0.647
G10	Amino acids	0.535
E9	Carboxylic acids	0.485
C11	Esters	0.475
G6	Amino acids	0.437
F9	Amino acids	0.430

APPENDIX E

Table 5.2.1 : Initial and Final TSS for Replicate 1 (i.e. original experiments)

Deicing conc. (ppm)	Initial TSS (mg/L)	Final TSS (mg/L)
35	2150	1970
65	980	1040
130	300	390

Table 5.2.2 : Initial anf Final TSS for Replicate 2 (i.e. replicate experiments)

Deicing conc. (ppm)	Initial TSS (mg/L)	Final TSS (mg/L)
35	1900	1880
65	990	1070
130	390	420

Figure 5.2.1 : COD profile for replicates (2000 mg/L TSS, 35 mg/L DIF)

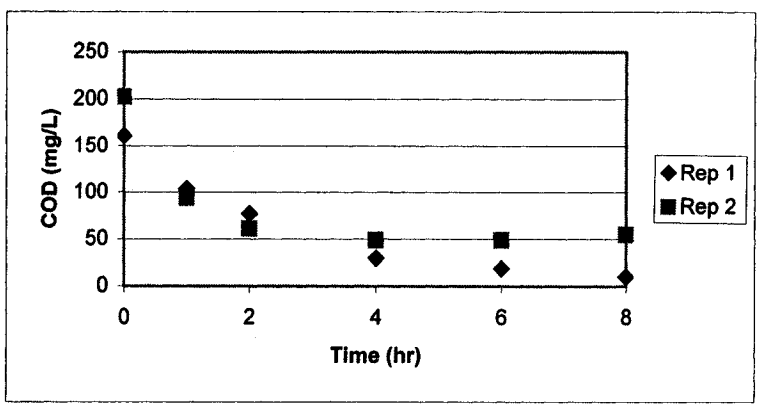


Figure 5.2.2 : TOC profile for replicates (2000 mg/L TSS, 35 mg/L DIF)

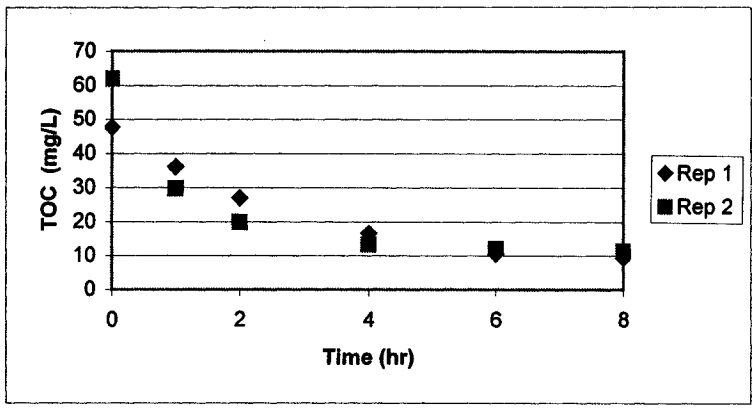


Figure 5.2.3 : COD profile for replicates (1000 mg/L TSS, 65 mg/L DIF)

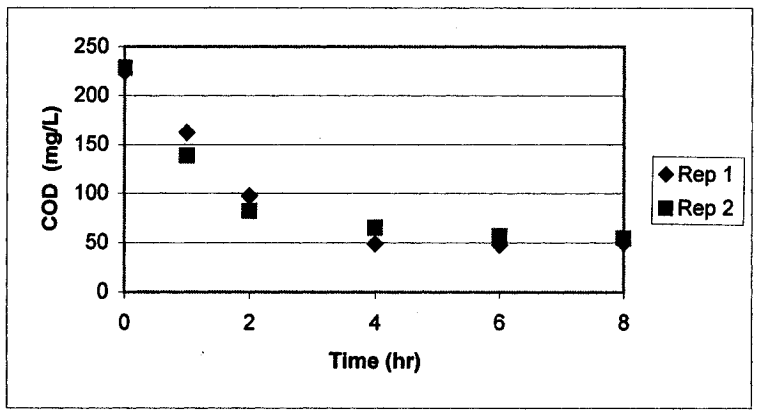


Figure 5.2.4 : TOC profile for replicates (1000 mg/L TSS, 65 mg/L DIF)

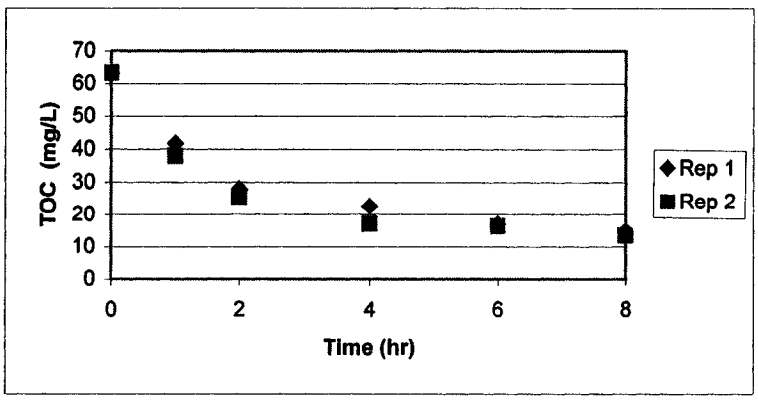


Figure 5.2.5 : COD profile for replicates (300 mg/L TSS, 130 mg/L DIF)

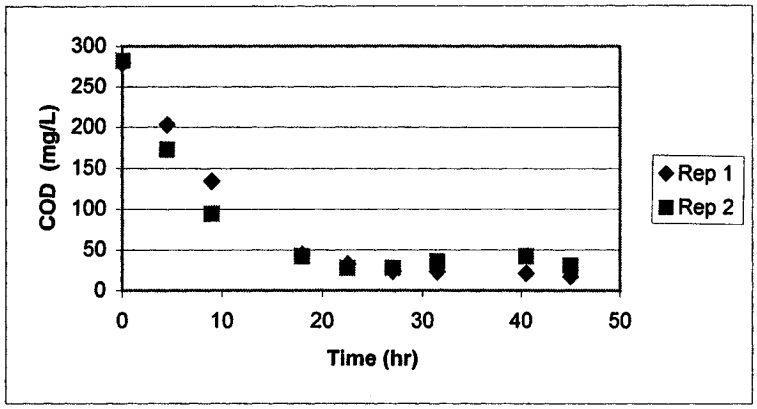


Figure 5.2.6 : TOC profile for replicates (300 mg/L TSS, 130 mg/L DIF)

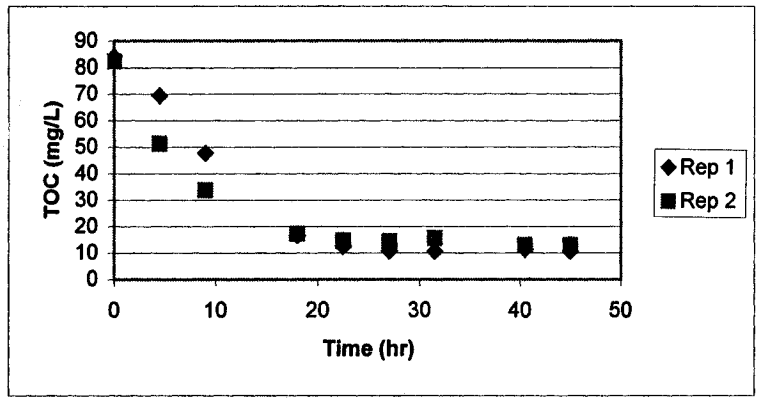


Figure 5.2.7 : Specific COD rate for replicates (2000 mg/L TSS, 35 mg/L DIF)

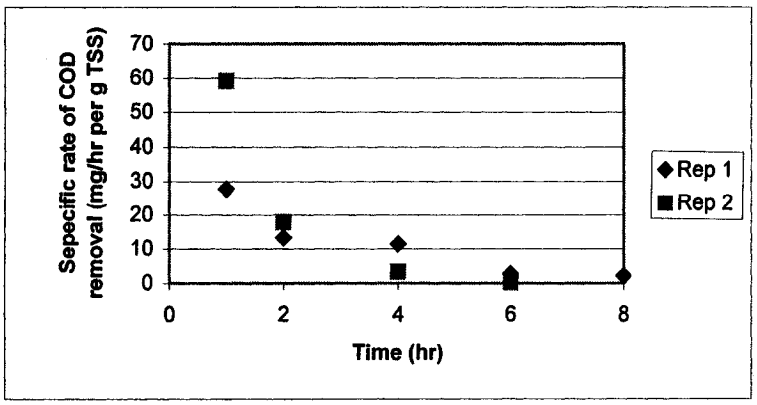


Figure 5.2.8 : Specific TOC rate for replicates (2000 mg/L TSS, 35 mg/L DIF)

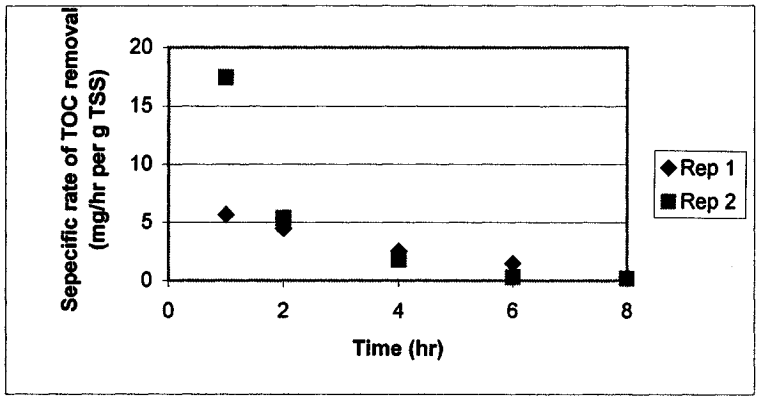


Figure 5.2.9 : Specific COD rate for replicates (1000 mg/L TSS, 65 mg/L DIF)

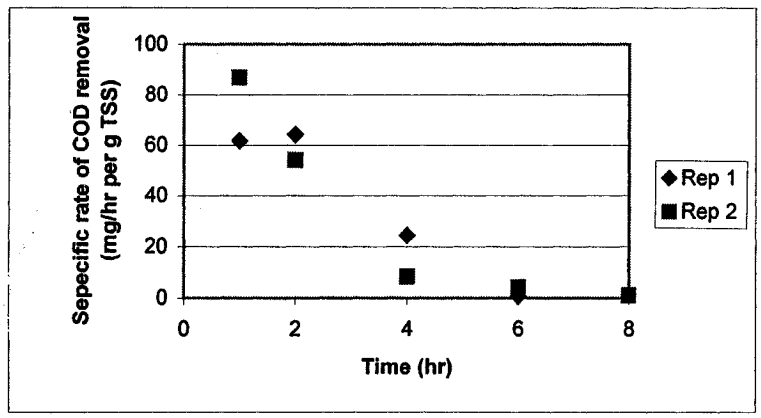


Figure 5.2.10 : Specific TOC rate for replicates (1000 mg/L TSS, 65 mg/L DIF)

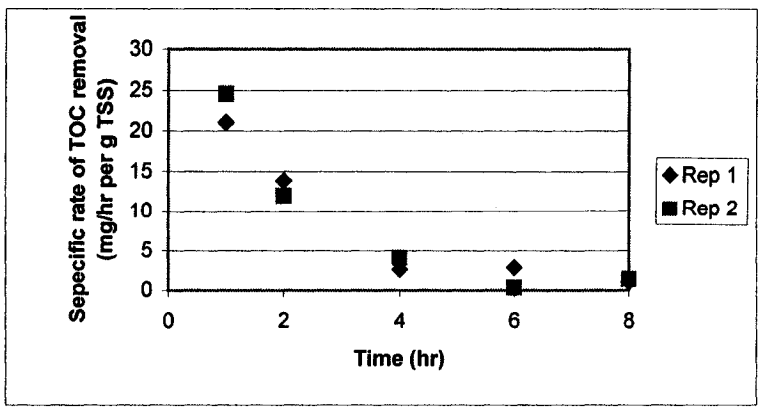


Figure 5.2.11 : Specific COD rate for replicates (300 mg/L TSS, 130 mg/L DIF)

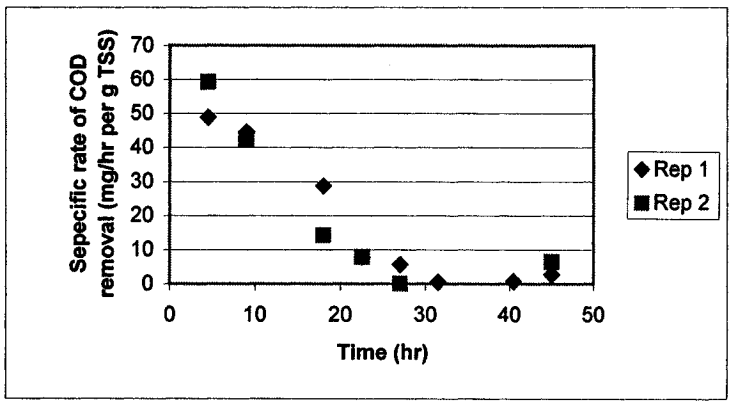


Figure 5.2.12 : Specific TOC rate for replicates (300 mg/L TSS, 130 mg/L DIF)

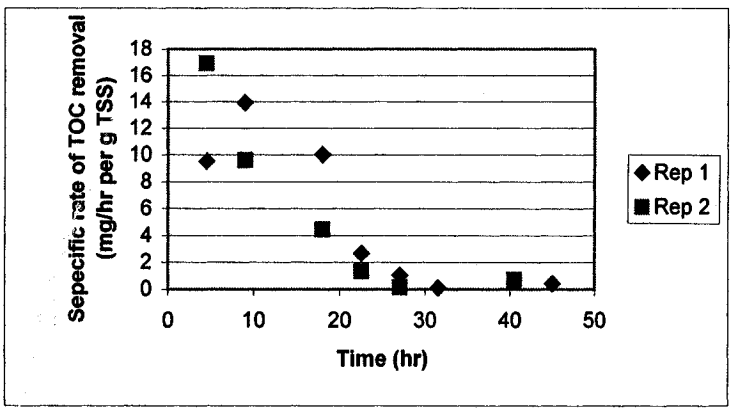


Figure 5.2.13 : EG profile for replicates (2000 mg/L TSS, 35 mg/L DIF)

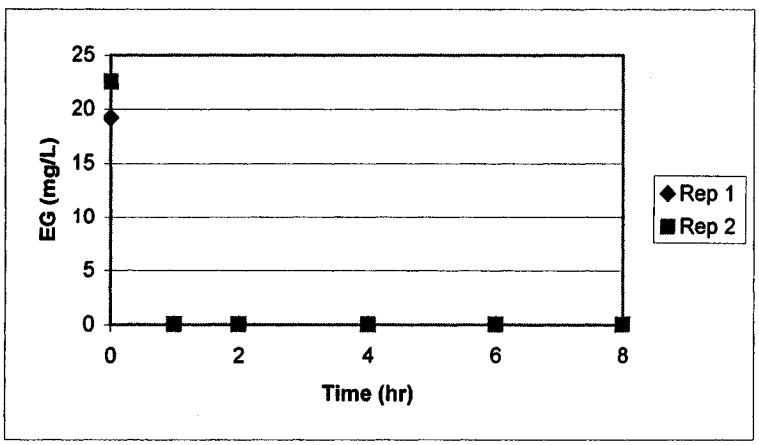


Figure 5.2.14 : EG profile for replicates (1000 mg/L TSS, 65 mg/L DIF)

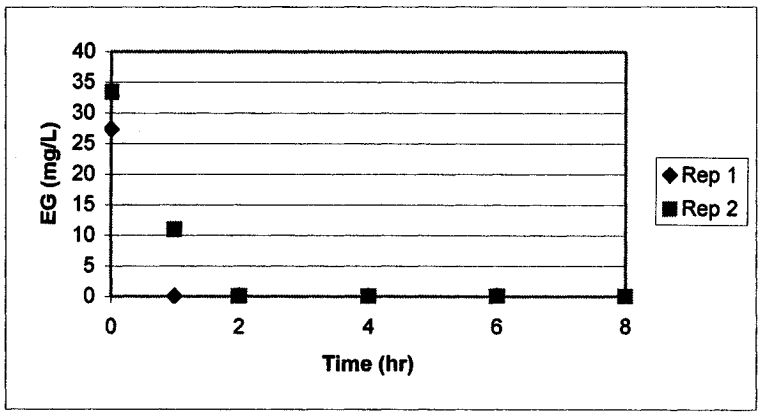


Figure 5.2.15 : EG profile for replicates (300 mg/L TSS, 130 mg/L DIF)

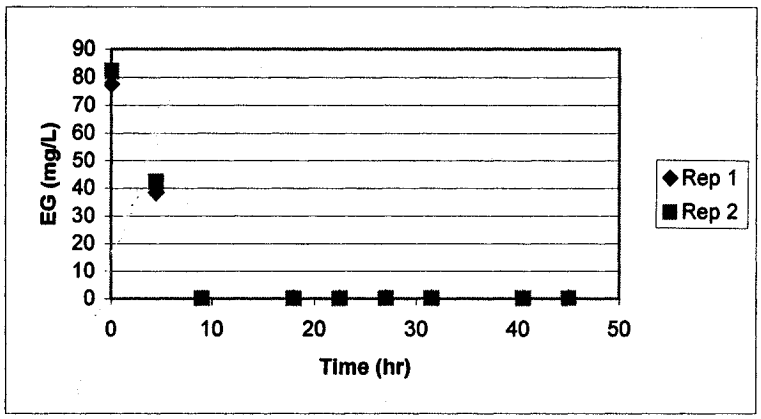


Figure 5.2.16 : Specific EG rate for replicates (2000 mg/L TSS, 35 mg/L DIF)

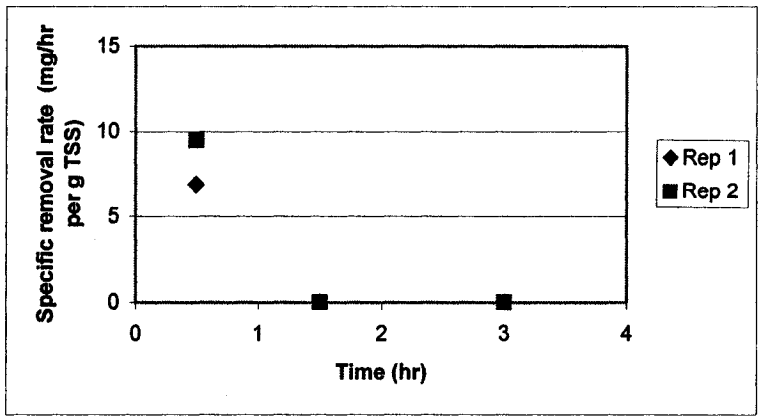


Figure 5.2.17 : Specific EG rate for replicates (1000 mg/L TSS, 65 mg/L DIF)

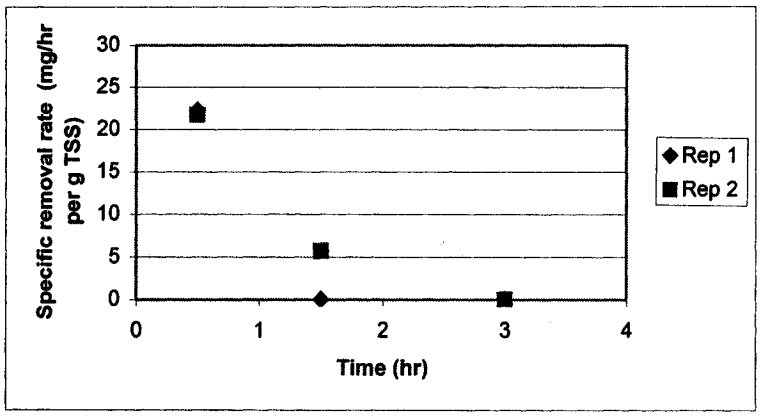


Figure 5.2.18 : Specific EG rate for replicates (300 mg/L TSS, 130 mg/L DIF)

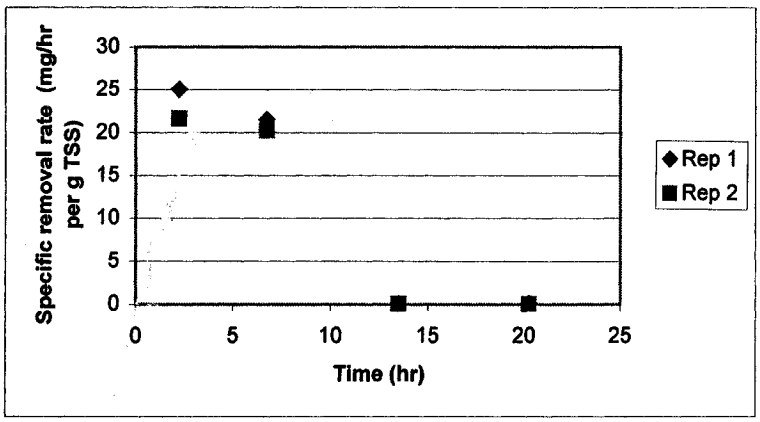


Table 5.2.3 : OUR for replicate experiments (Experiments 1 and 2)

	Experiment 1*		Experiment 2*	
Temp.	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Time (hr)	OUR (mg/L-min)		OUR (mg/L-min)	
1	0.12	0.63	0.41	0.42
2.5	0.06	0.57	0.26	0.32
5	0.03	0.30	0.20	0.20
7	0.08	0.24	0.17	0.17

Table 5.2.4 : OUR for replicate experiments (Experiment 3)

	Experiment 3*	
Temp.	Replicate 1	Replicate 2
Time (hr)	OUR (mg/L-min)	
0	0.11	0.28
4.5	0.22	0.19
9	0.19	0.17
18	0.11	0.12
22.5	0.02	0.10
27	0.02	0.08
31.5	0.02	0.06
40.5	0.01	0.06
45	0.02	0.05

Table 5.2.5 : SOUR for replicate experiments (Experiments 1 and 2)

	Experiment 1*		Experiment 2*	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Time (hr)	SOUR (mg/min per g TSS)		SOUR (mg/min per g TSS)	
1	0.06	0.34	0.40	0.40
2.5	0.03	0.31	0.26	0.31
5	0.01	0.16	0.19	0.19
7	0.04	0.13	0.17	0.16

Table 5.2.6 : SOUR for replicate experiments (Experiment 3)

	Experiment 3*	
Temp.	Replicate 1	Replicate 2
Time (hr)	SOUR (mg/min per g TSS)	
0	0.32	0.69
4.5	0.64	0.46
9	0.56	0.41
18	0.31	0.29
22.5	0.06	0.23
27	0.06	0.19
31.5	0.06	0.16
40.5	0.04	0.15
45	0.05	0.13

Table 5.2.7 : Initial and Final SVI values for replicate experiments

	Experiment 1		Experiment 2		Experiment 3	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
Initial SVI	360	60	80	50	140	40
Final SVI	230	50	60	50	250	20

*Experimental conditions of replicate batch experiments :

Experiment 1 : 2000 mg/L TSS and 35 mg/L DIF

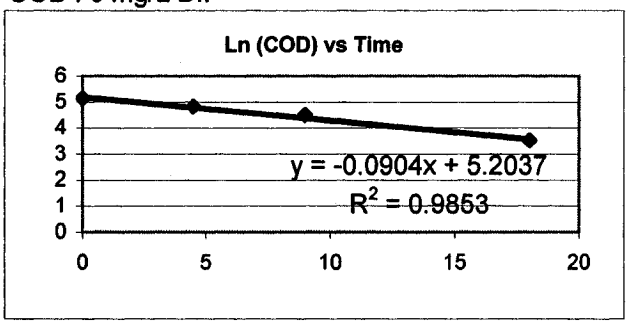
Experiment 2 : 1000 mg/L TSS and 65 mg/L DIF

Experiment 3 : 300 mg/L TSS and 130 mg/L DIF

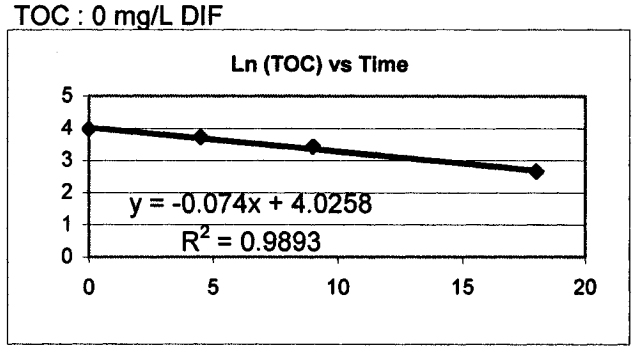
APPENDIX F

1st Order regression for batch experiments conducted at 300 mg/L of TSS

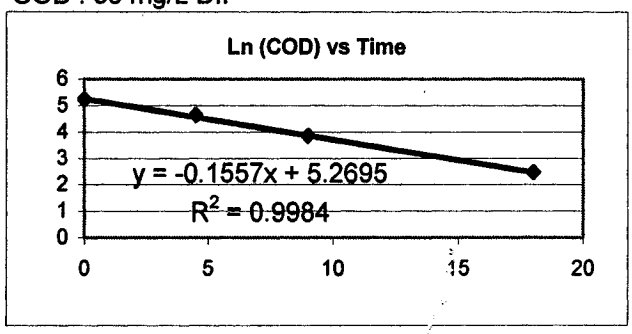
COD : 0 mg/L DIF



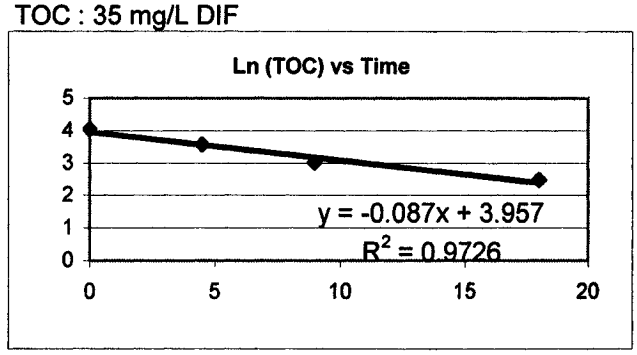
TOC : 0 mg/L DIF



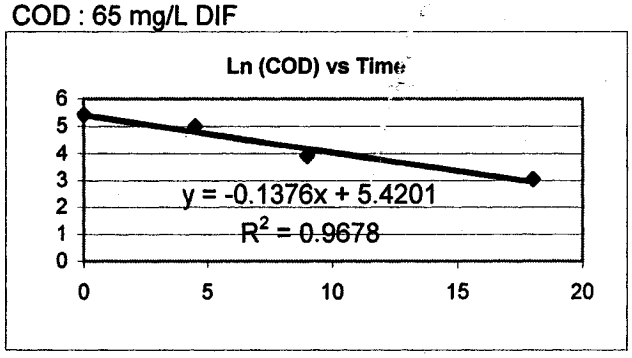
COD : 35 mg/L DIF



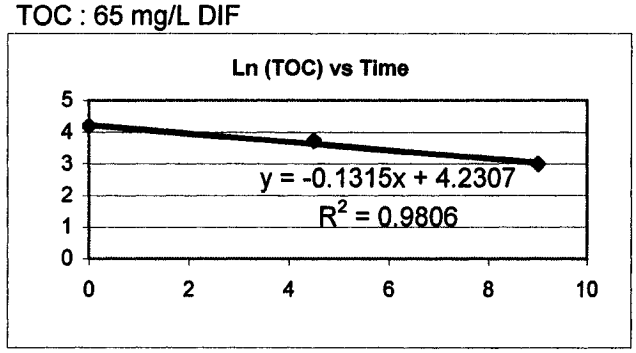
TOC : 35 mg/L DIF



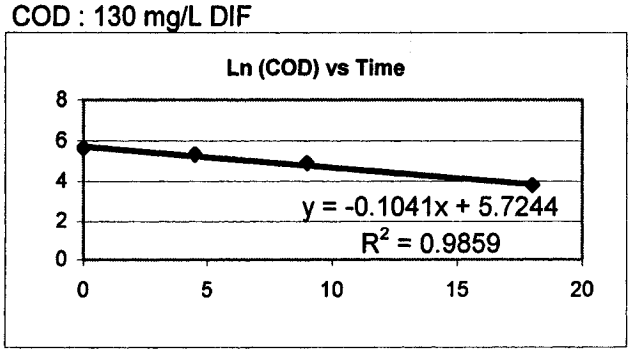
COD : 65 mg/L DIF



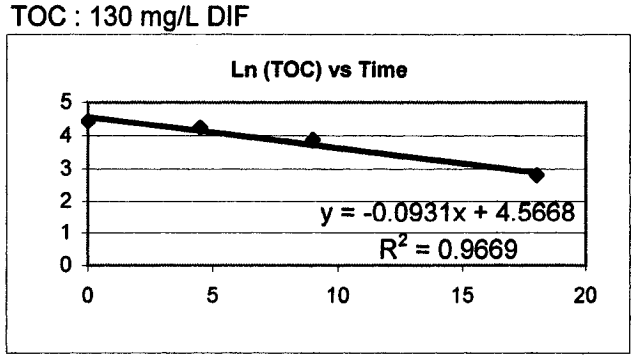
TOC : 65 mg/L DIF



COD : 130 mg/L DIF

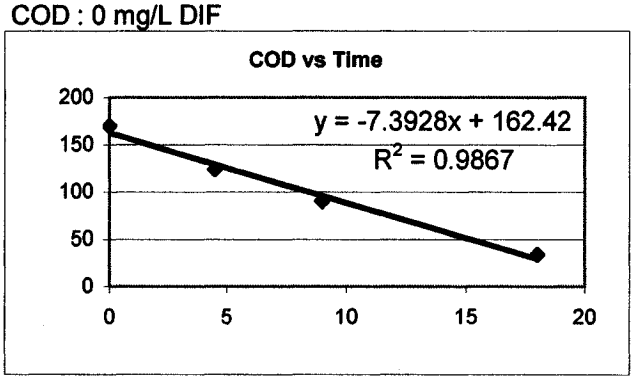


TOC : 130 mg/L DIF

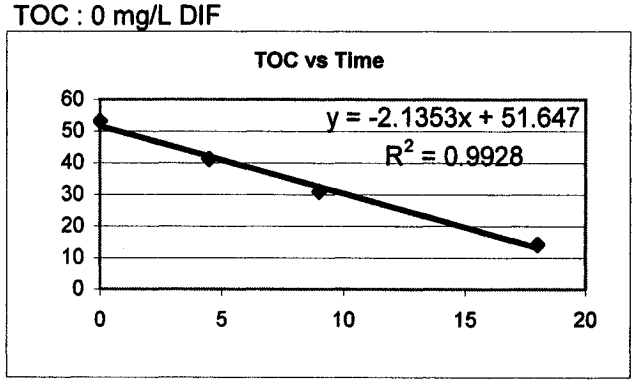


0th Order regression for batch experiments conducted at 300 mg/L of TSS

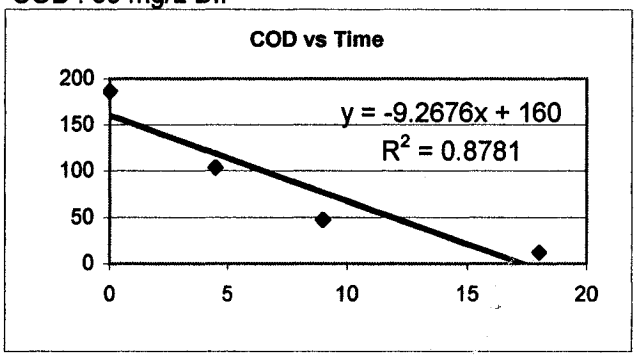
COD : 0 mg/L DIF



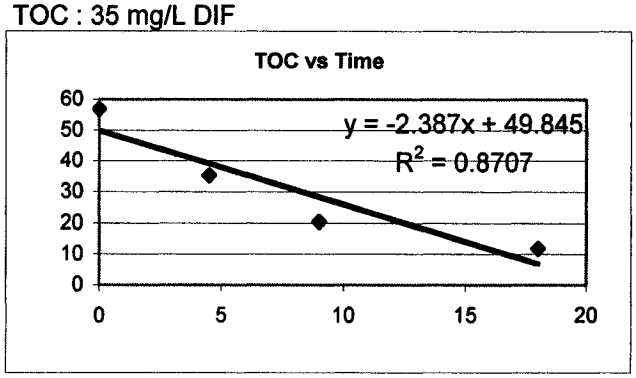
TOC : 0 mg/L DIF



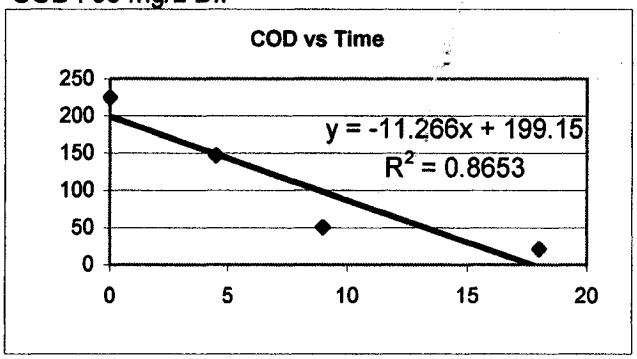
COD : 35 mg/L DIF



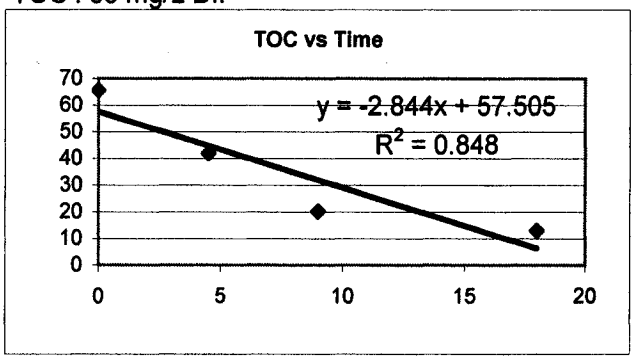
TOC : 35 mg/L DIF



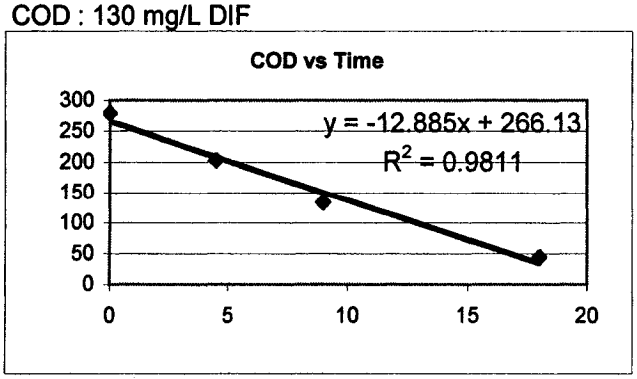
COD : 65 mg/L DIF



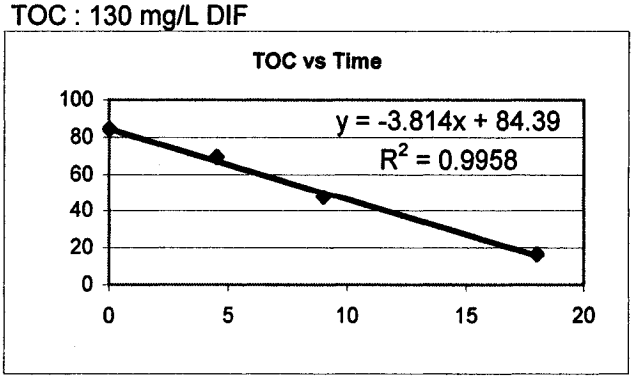
TOC : 65 mg/L DIF



COD : 130 mg/L DIF

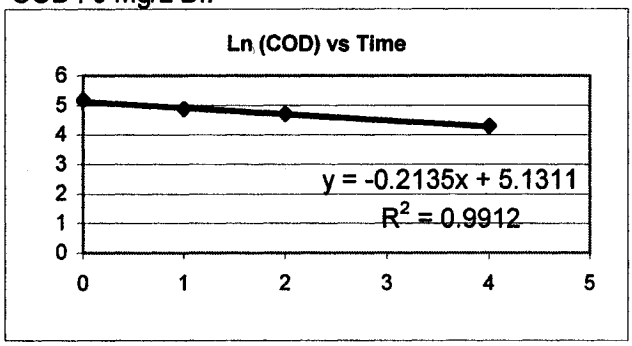


TOC : 130 mg/L DIF

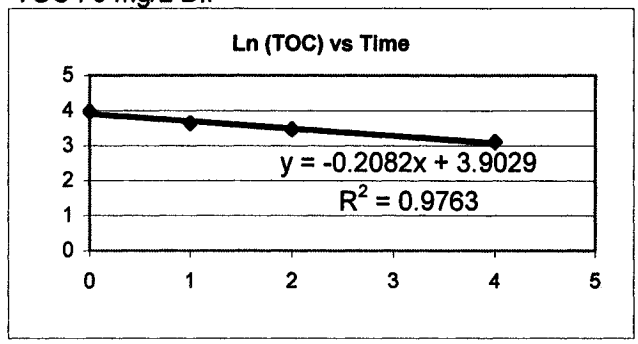


1st Order regression for batch experiments conducted at 1000 mg/L of TSS

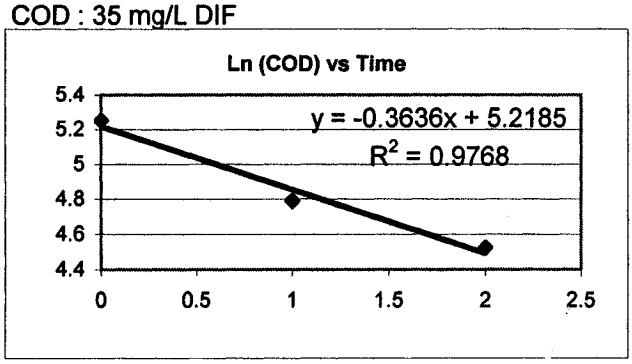
COD : 0 mg/L DIF



TOC : 0 mg/L DIF



COD : 35 mg/L DIF



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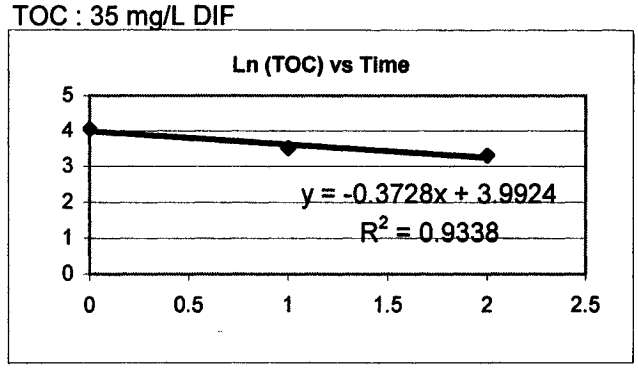
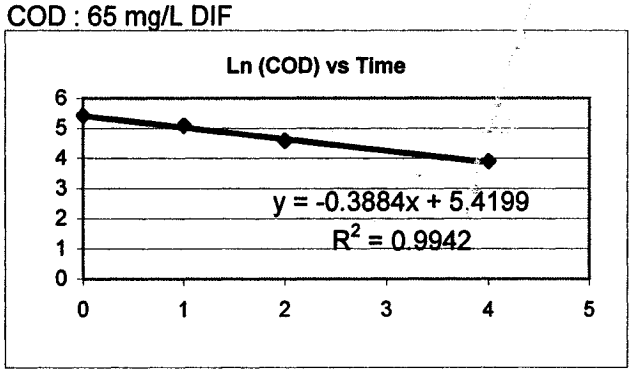


COD : 65 mg/L DIF



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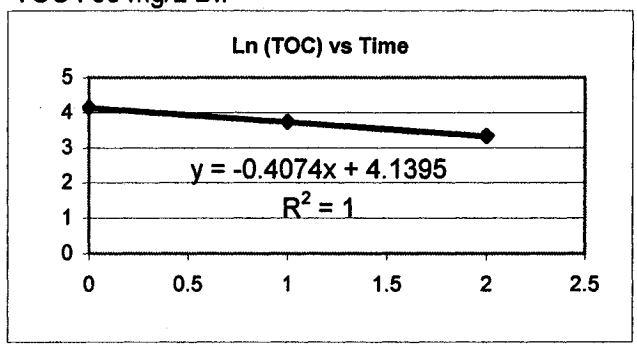
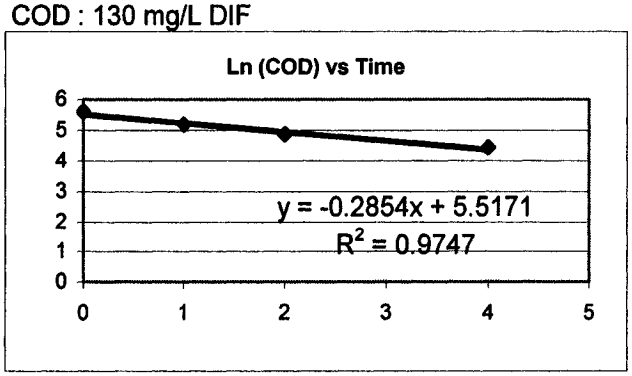


COD : 130 mg/L DIF



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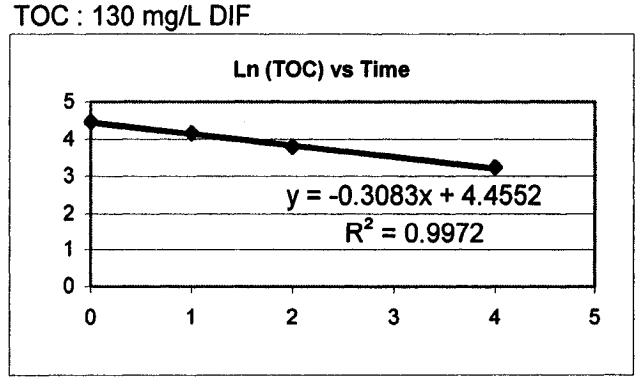
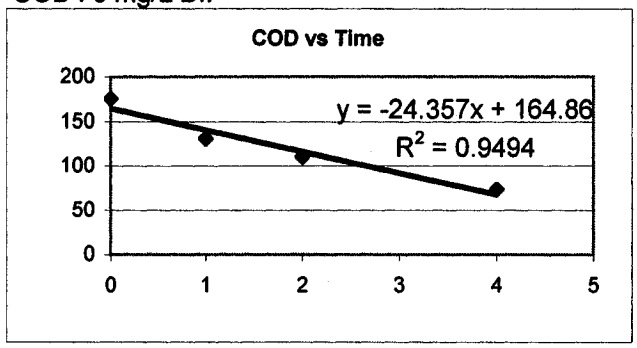


0th Order regression for batch experiments conducted at 1000 mg/L of TSS

COD : 0 mg/L DIF



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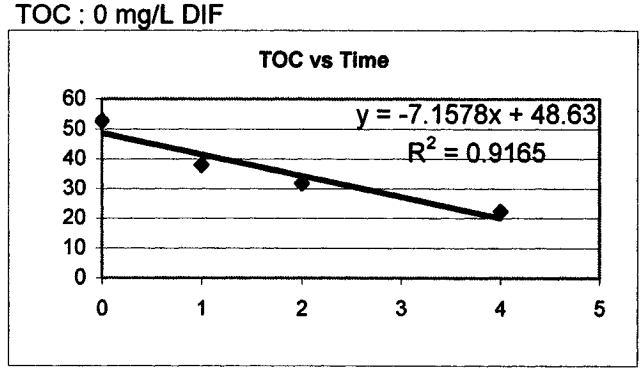
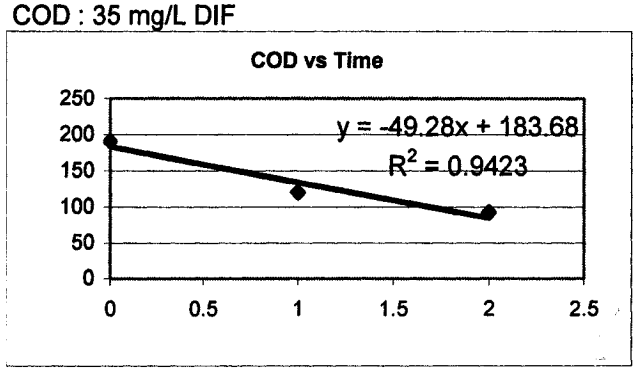


COD : 35 mg/L DIF



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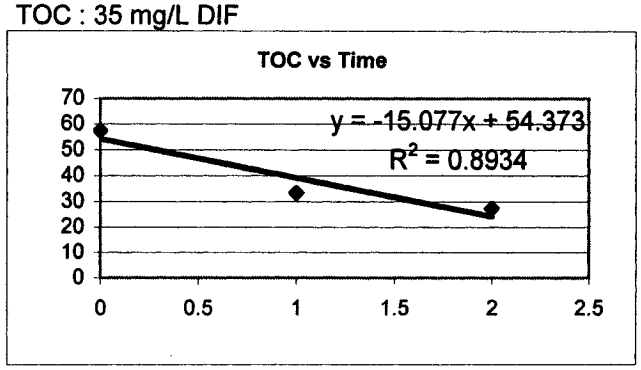
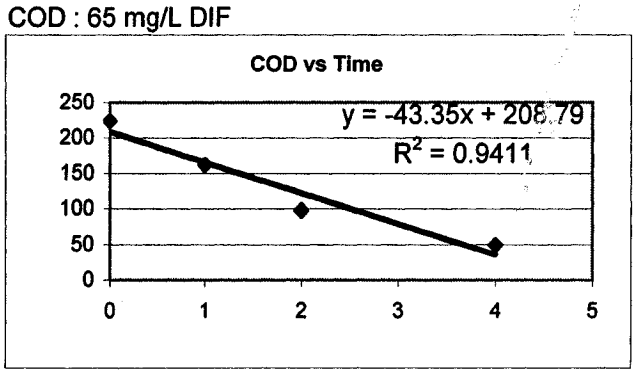
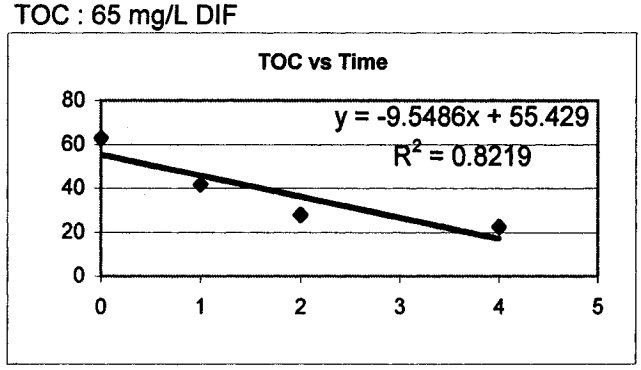


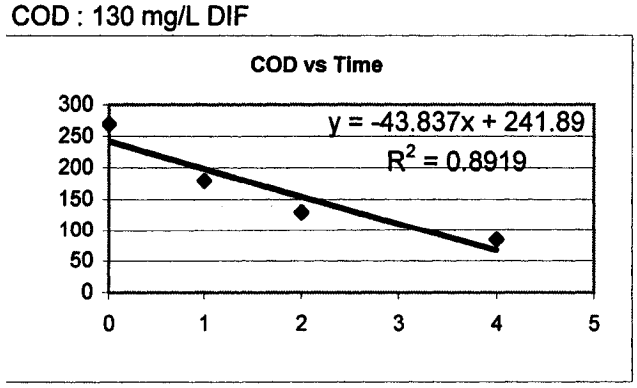
COD : 65 mg/L DIF



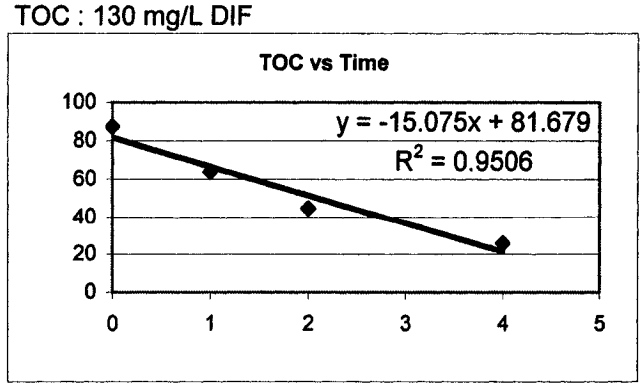
TOC : 65 mg/L DIF



COD : 130 mg/L DIF

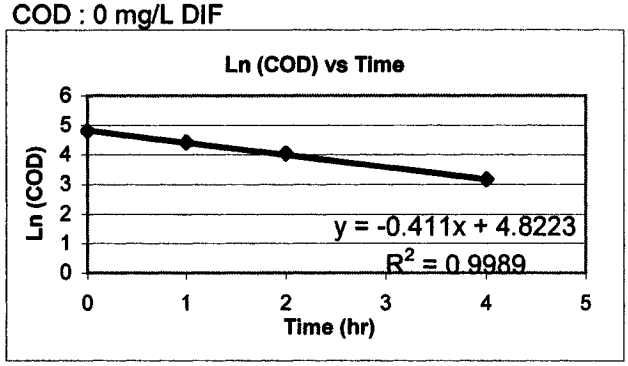


TOC : 130 mg/L DIF

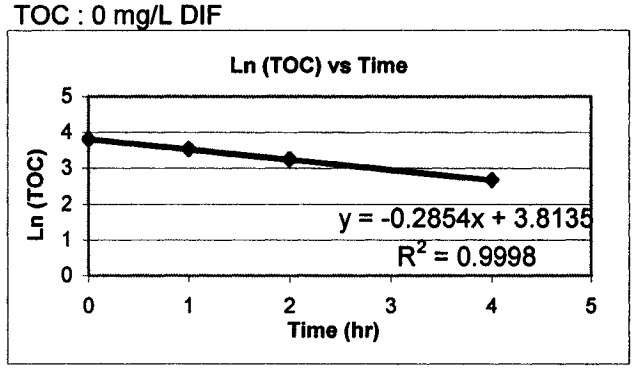


1st Order regression for batch experiments conducted at 2000 mg/L of TSS

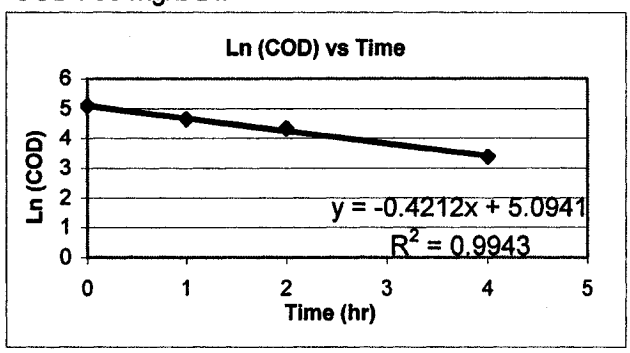
COD : 0 mg/L DIF



TOC : 0 mg/L DIF



COD : 35 mg/L DIF



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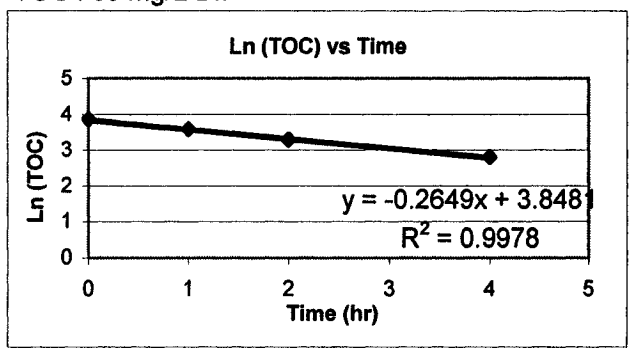
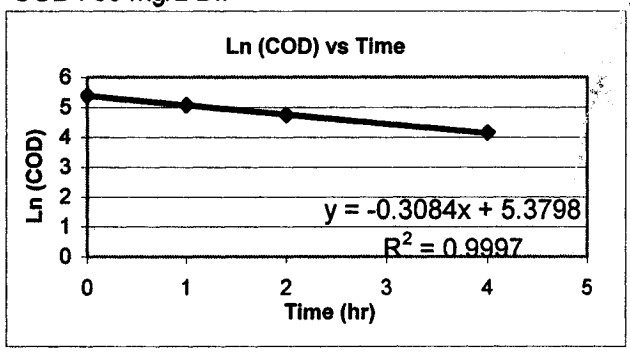
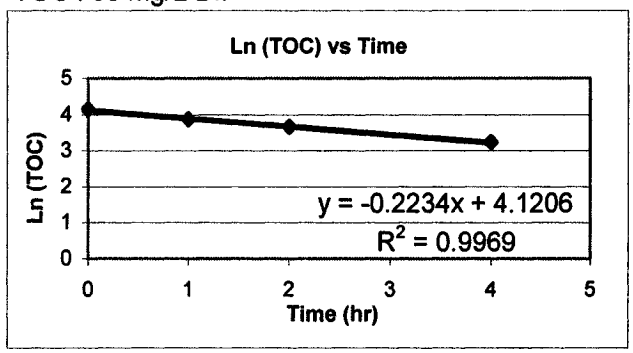


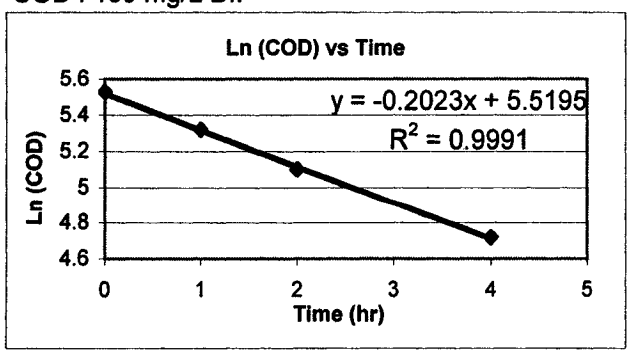
COD : 65 mg/L DIF



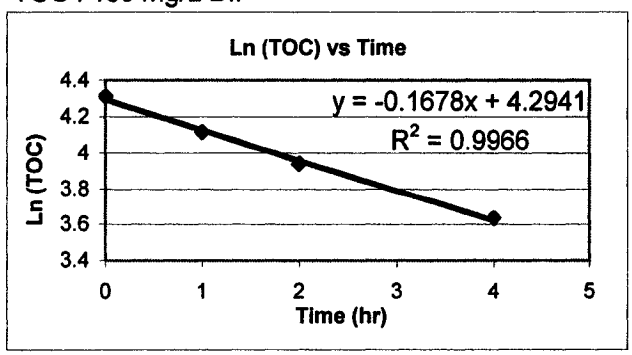
TOC : 65 mg/L DIF



COD : 130 mg/L DIF

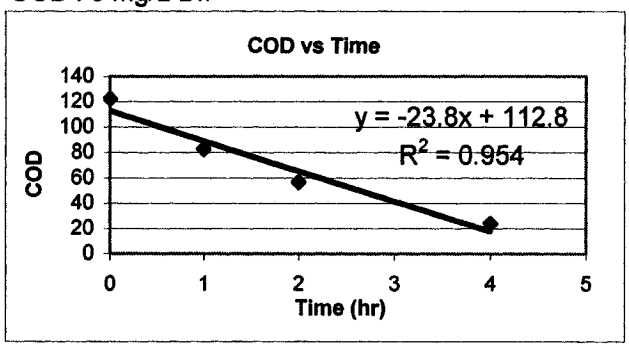


TOC : 130 mg/L DIF

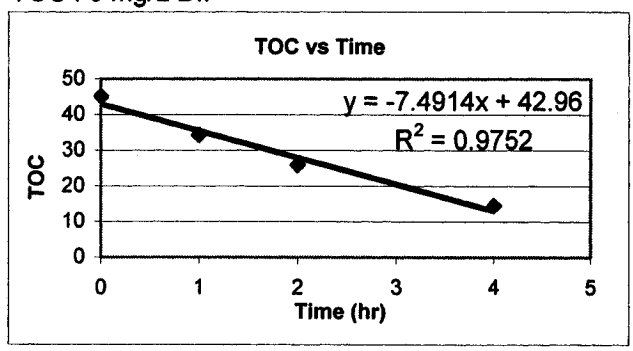


0th Order regression for batch experiments conducted at 2000 mg/L of TSS

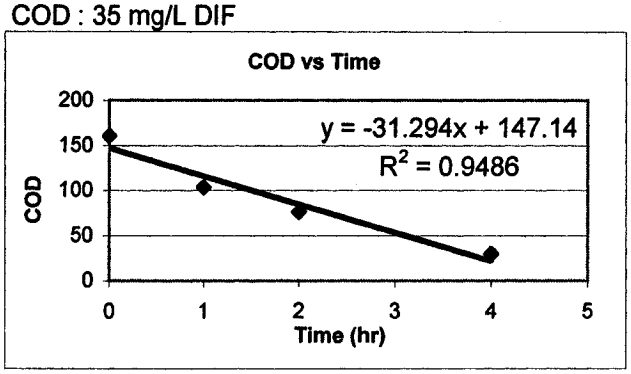
COD : 0 mg/L DIF



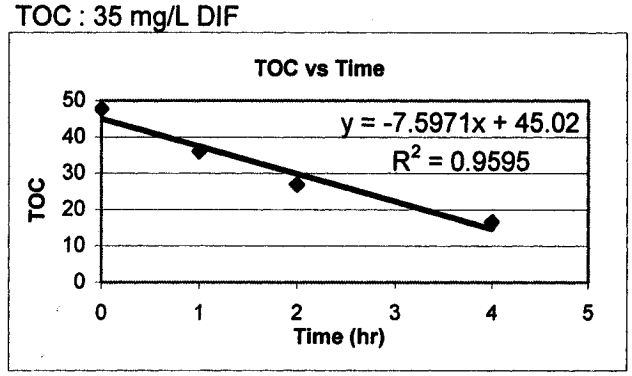
TOC : 0 mg/L DIF



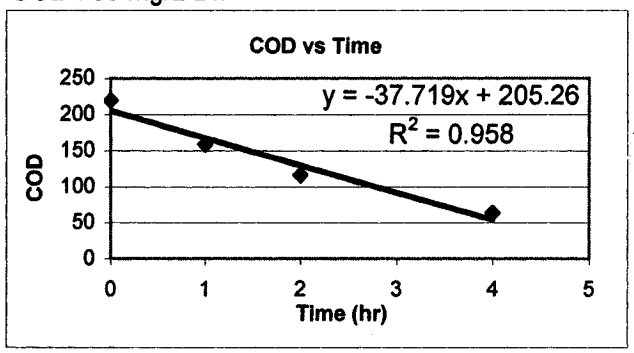
COD : 35 mg/L DIF



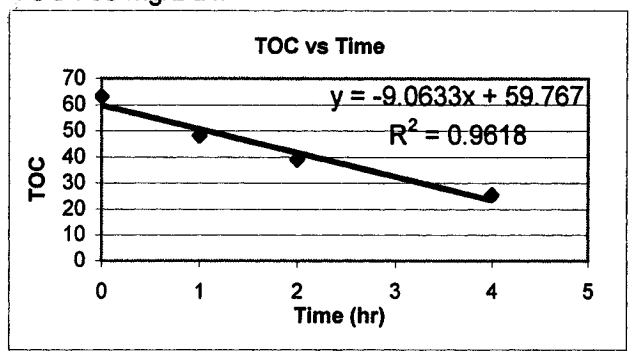
TOC : 35 mg/L DIF



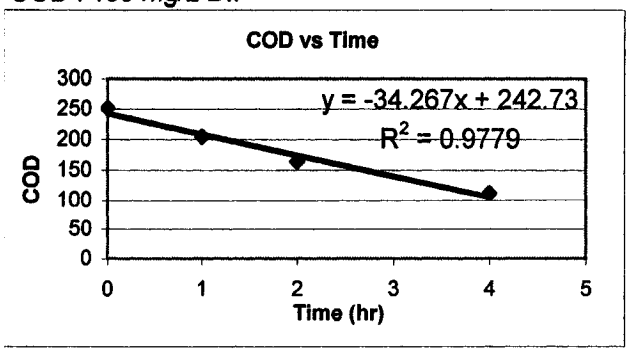
COD : 65 mg/L DIF



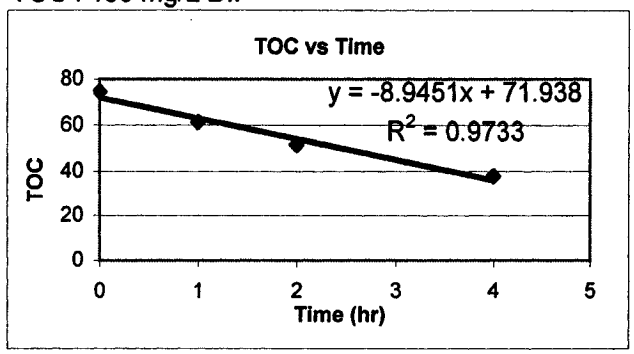
TOC : 65 mg/L DIF



COD : 130 mg/L DIF

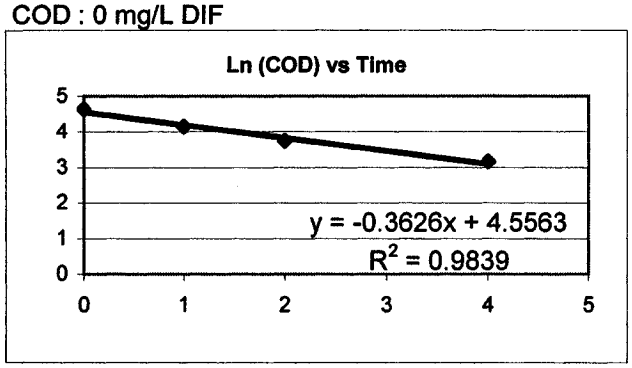


TOC : 130 mg/L DIF

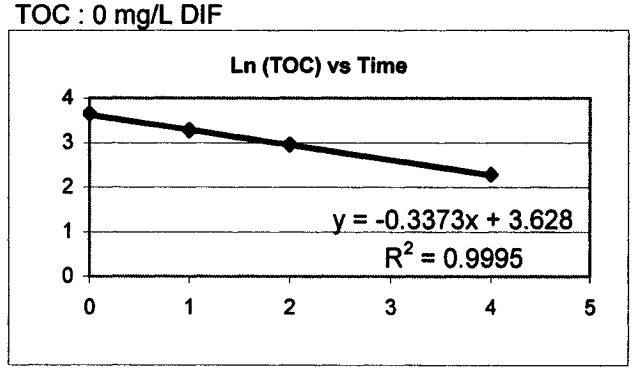


1st Order regression for batch experiments conducted at 3000 mg/L of TSS

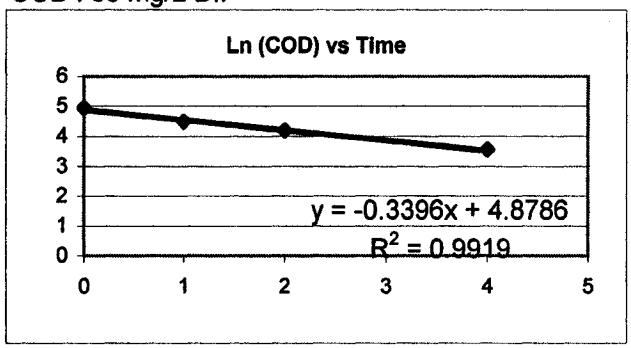
COD : 0 mg/L DIF



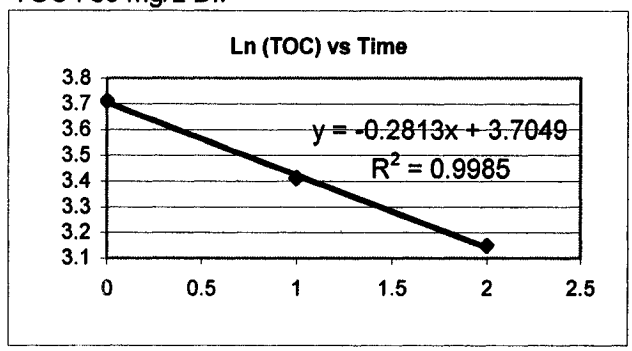
TOC : 0 mg/L DIF



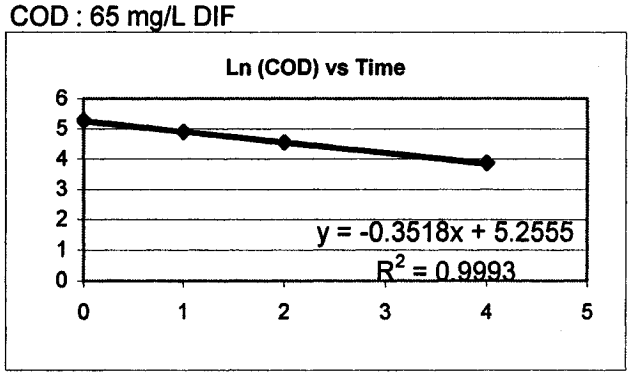
COD : 35 mg/L DIF



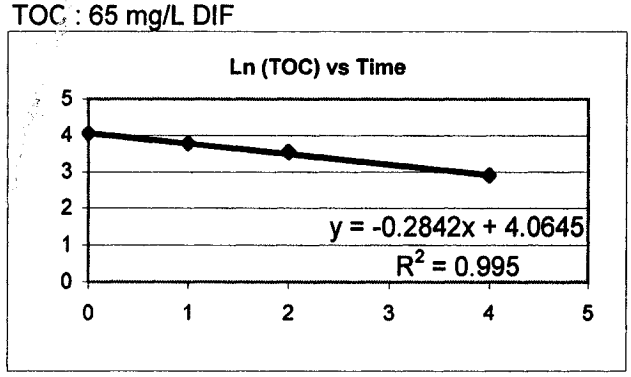
TOC : 35 mg/L DIF



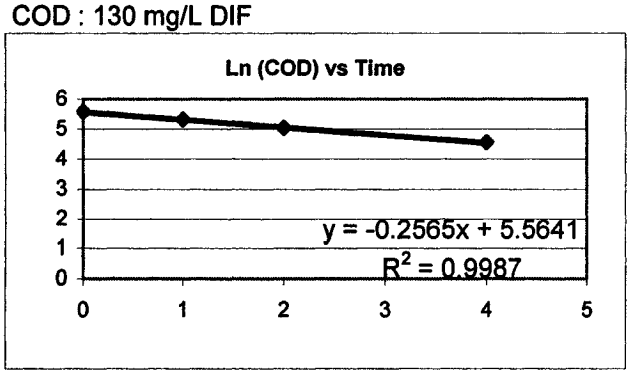
COD : 65 mg/L DIF



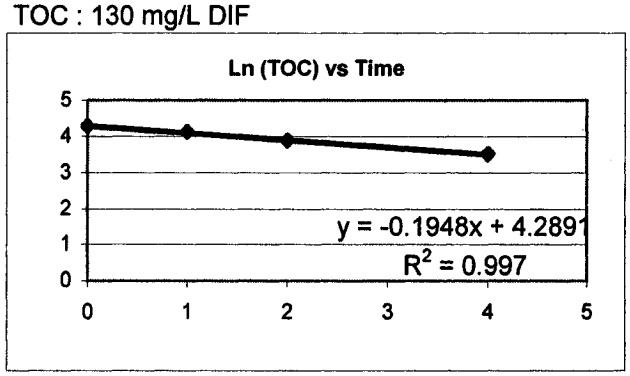
TOC : 65 mg/L DIF



COD : 130 mg/L DIF

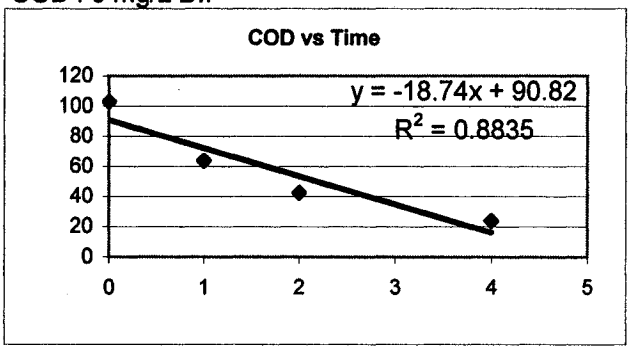


TOC : 130 mg/L DIF

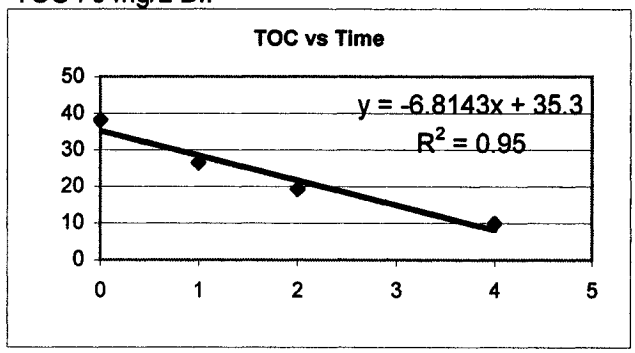


0th Order regression for batch experiments conducted at 3000 mg/L of TSS

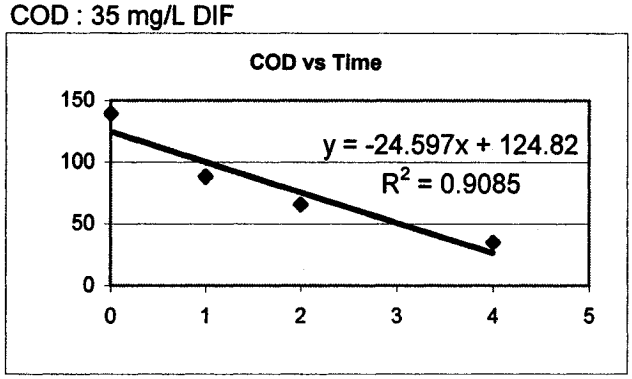
COD : 0 mg/L DIF



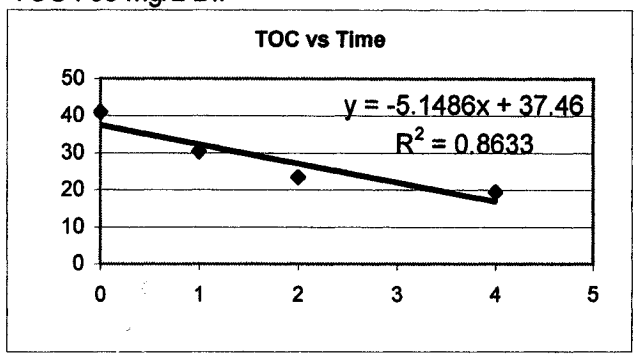
TOC : 0 mg/L DIF



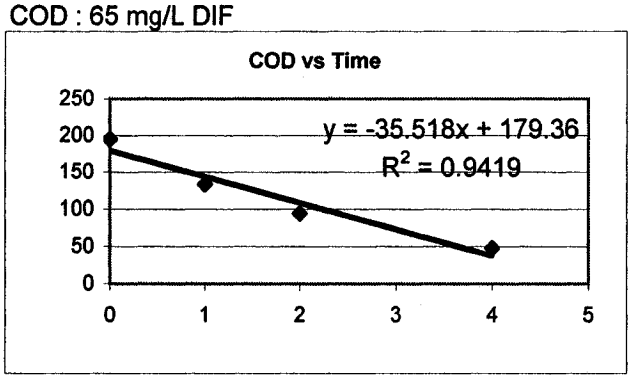
COD : 35 mg/L DIF



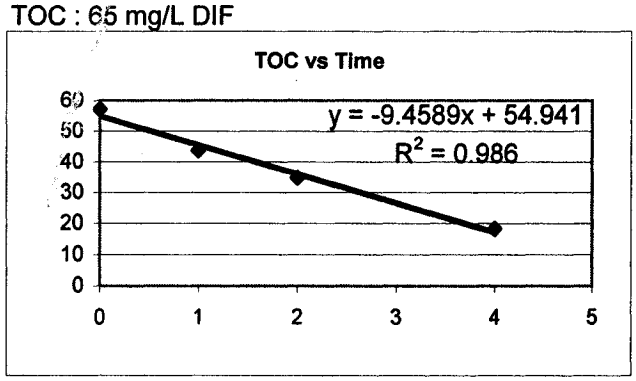
TOC : 35 mg/L DIF



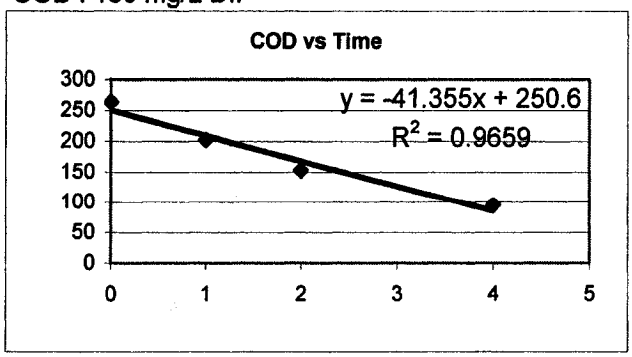
COD : 65 mg/L DIF



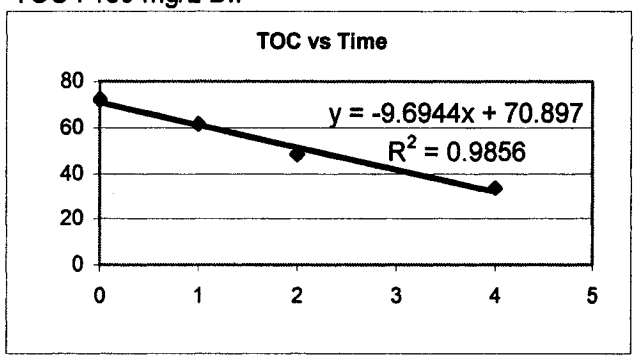
TOC : 65 mg/L DIF



COD : 130 mg/L DIF

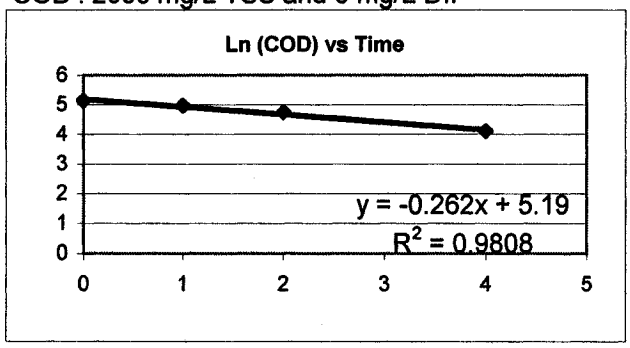


TOC : 130 mg/L DIF

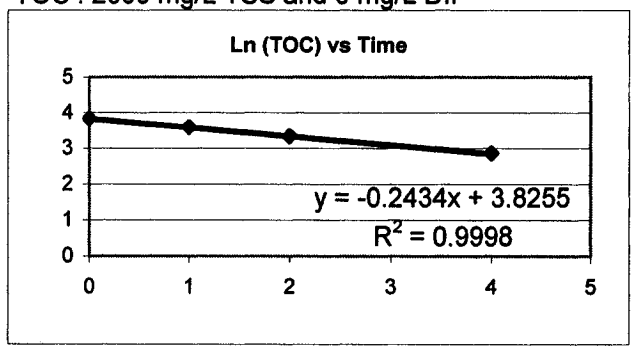


1st Order regression for acclimatized batch experiments

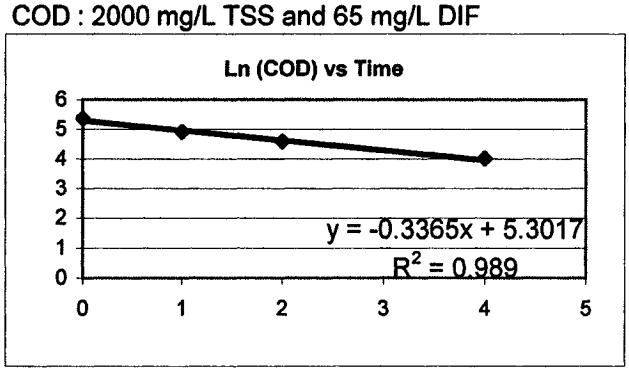
COD : 2000 mg/L TSS and 0 mg/L DIF



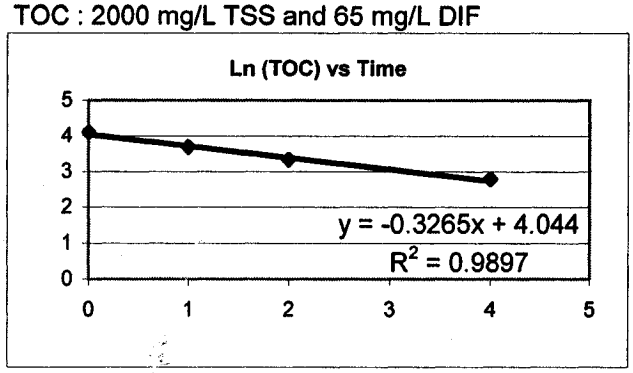
TOC : 2000 mg/L TSS and 0 mg/L DIF



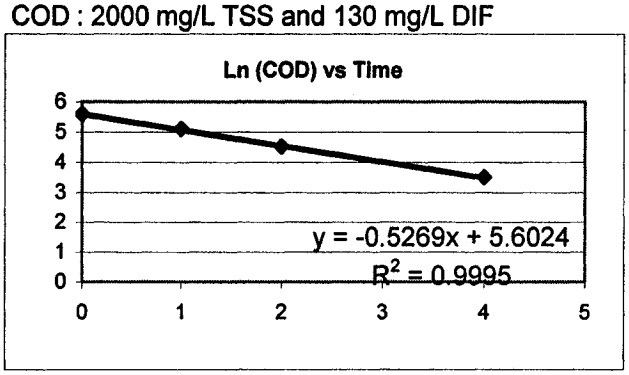
COD : 2000 mg/L TSS and 65 mg/L DIF



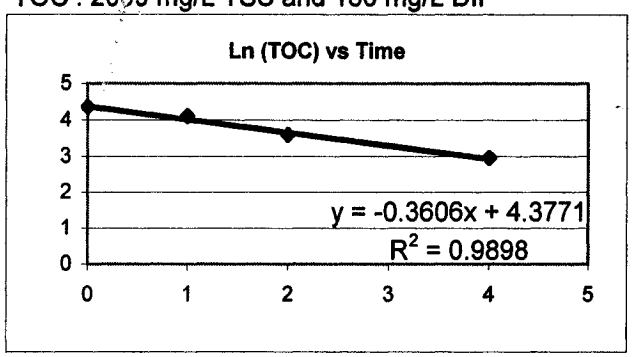
TOC : 2000 mg/L TSS and 65 mg/L DIF



COD : 2000 mg/L TSS and 130 mg/L DIF

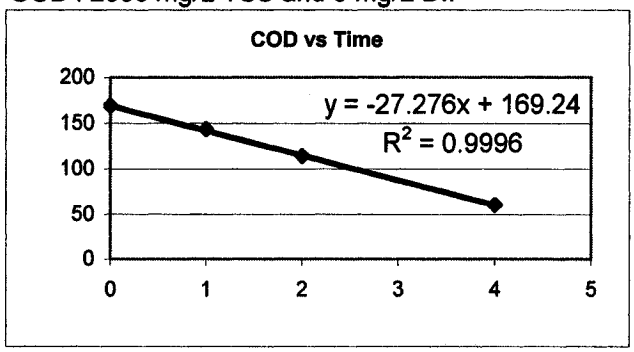


TOC : 2000 mg/L TSS and 130 mg/L DIF

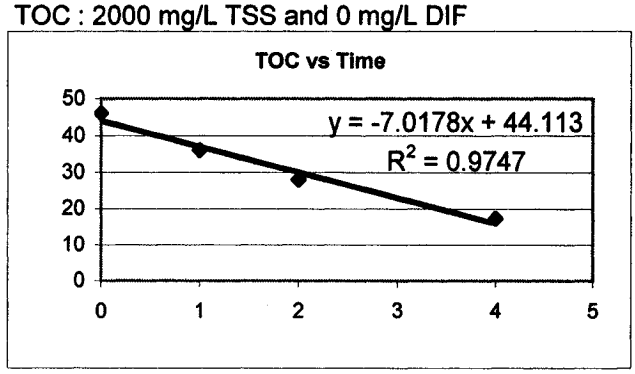


0th Order regression for acclimatized batch experiments

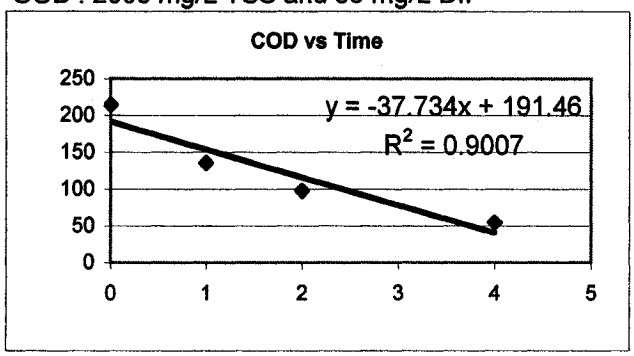
COD : 2000 mg/L TSS and 0 mg/L DIF



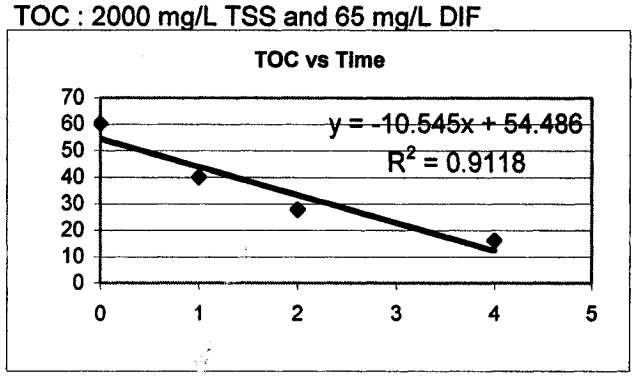
TOC : 2000 mg/L TSS and 0 mg/L DIF



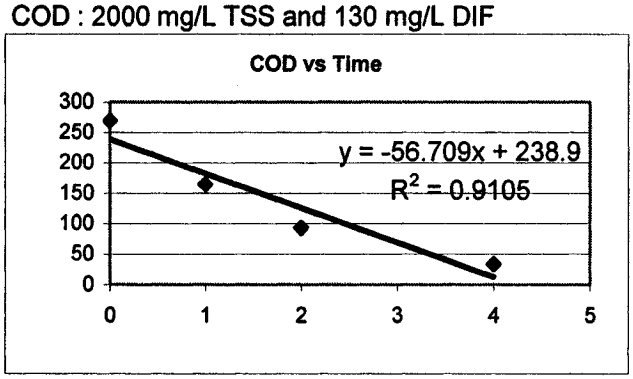
COD : 2000 mg/L TSS and 65 mg/L DIF



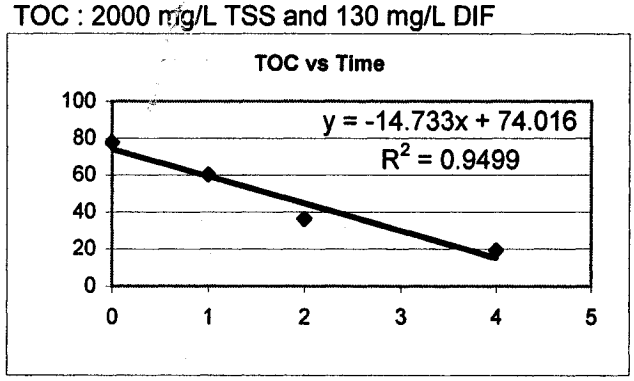
TOC : 2000 mg/L TSS and 65 mg/L DIF



COD : 2000 mg/L TSS and 130 mg/L DIF

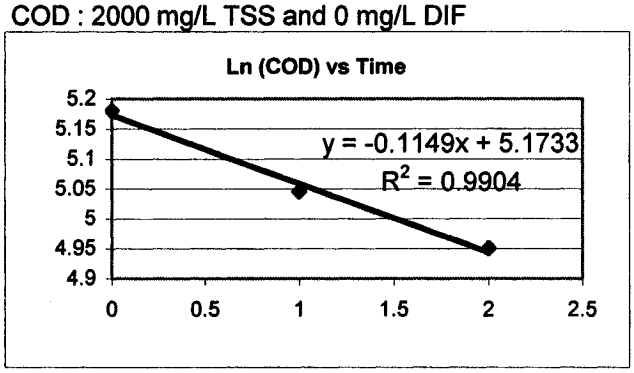


TOC : 2000 mg/L TSS and 130 mg/L DIF

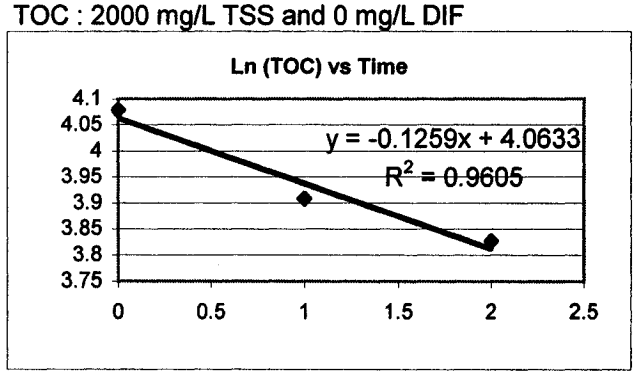


1st Order regression for batch experiments conducted at 5°C

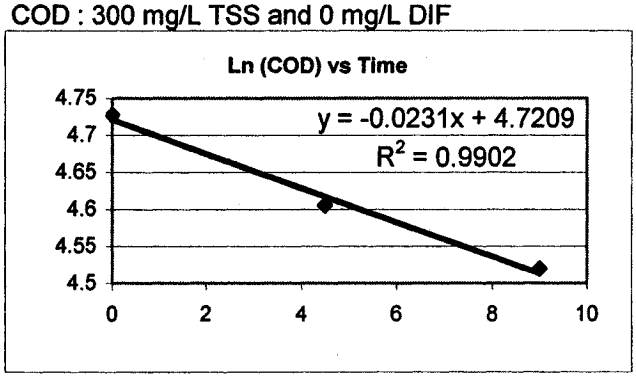
COD : 2000 mg/L TSS and 0 mg/L DIF



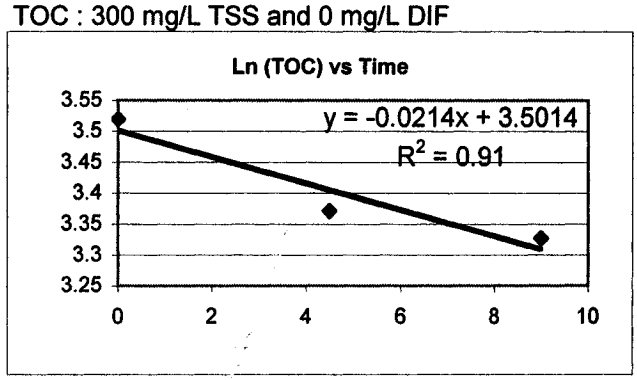
TOC : 2000 mg/L TSS and 0 mg/L DIF



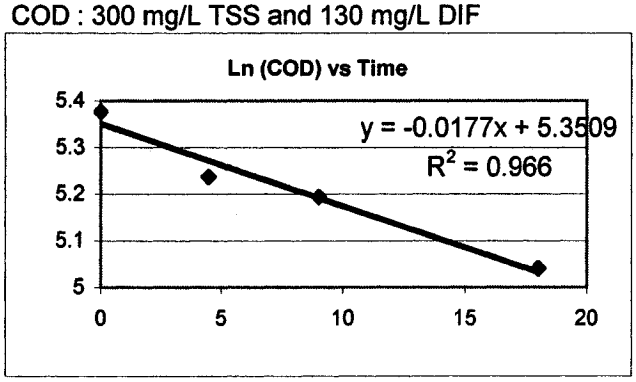
COD : 300 mg/L TSS and 0 mg/L DIF



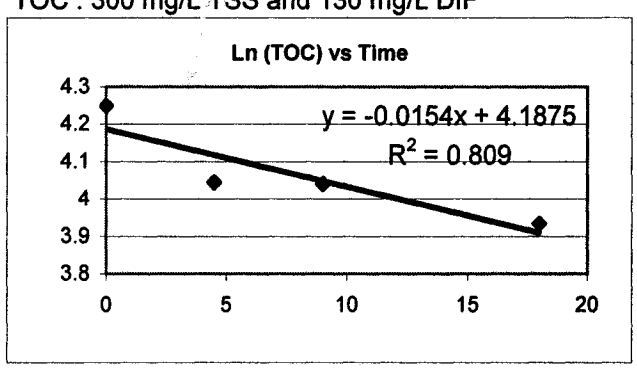
TOC : 300 mg/L TSS and 0 mg/L DIF



COD : 300 mg/L TSS and 130 mg/L DIF

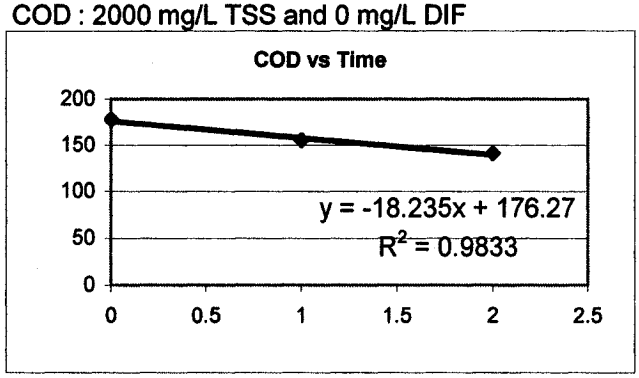


TOC : 300 mg/L TSS and 130 mg/L DIF

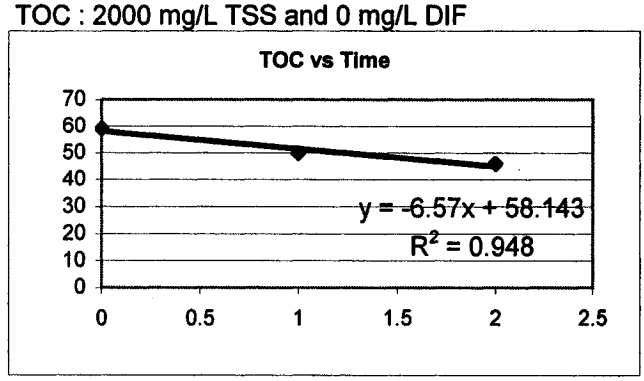


0th Order regression for batch experiments conducted at 5°C

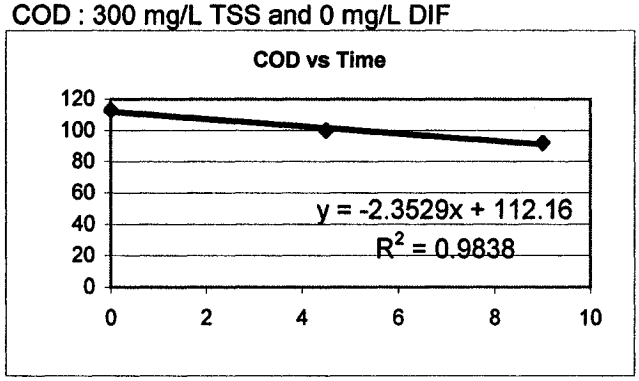
COD : 2000 mg/L TSS and 0 mg/L DIF



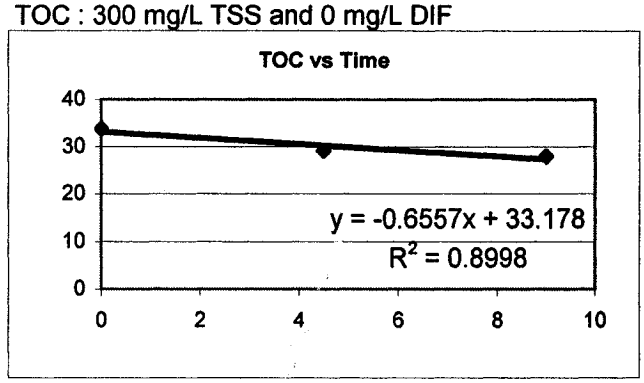
TOC : 2000 mg/L TSS and 0 mg/L DIF



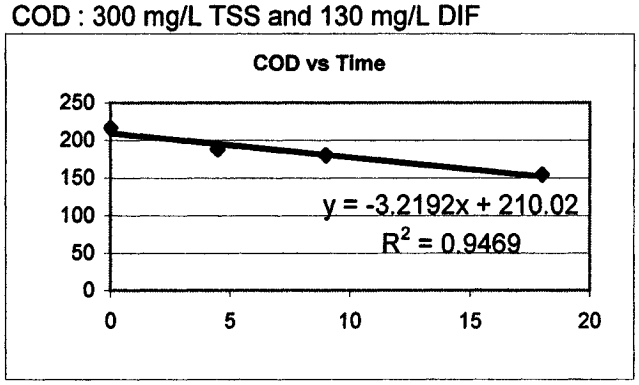
COD : 300 mg/L TSS and 0 mg/L DIF



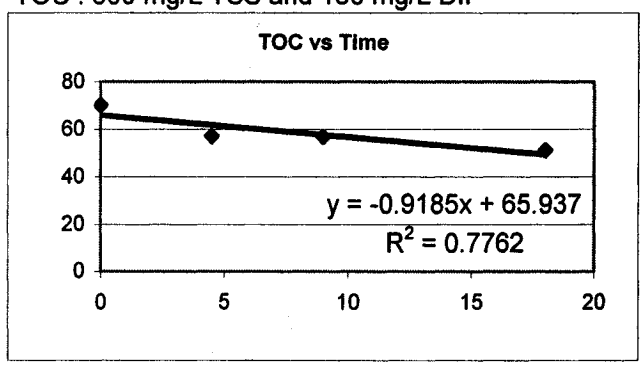
TOC : 300 mg/L TSS and 0 mg/L DIF



COD : 300 mg/L TSS and 130 mg/L DIF

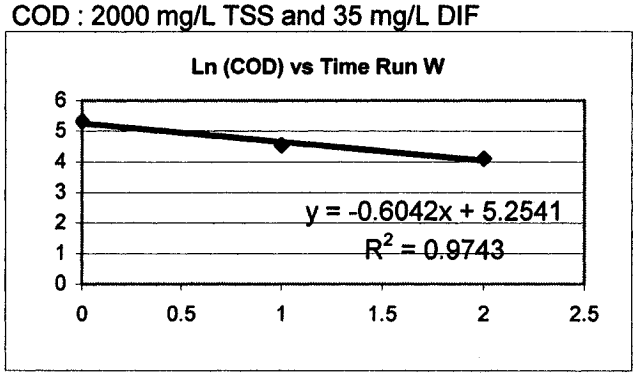


TOC : 300 mg/L TSS and 130 mg/L DIF

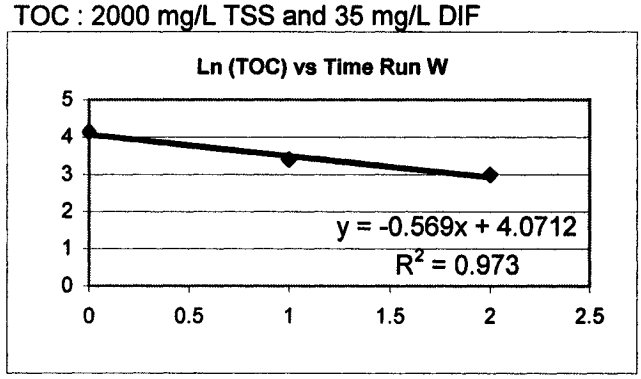


1st Order regression for replicate batch experiments

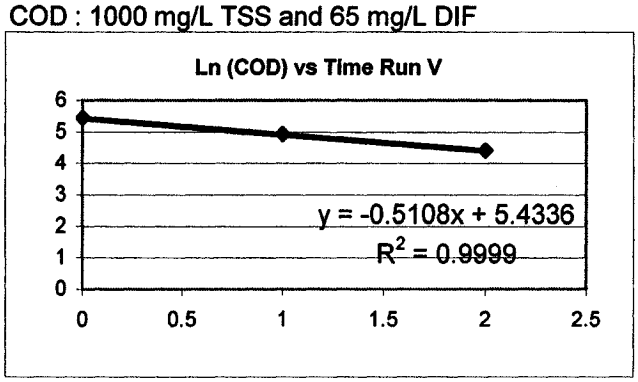
COD : 2000 mg/L TSS and 35 mg/L DIF



TOC : 2000 mg/L TSS and 35 mg/L DIF



COD : 1000 mg/L TSS and 65 mg/L DIF



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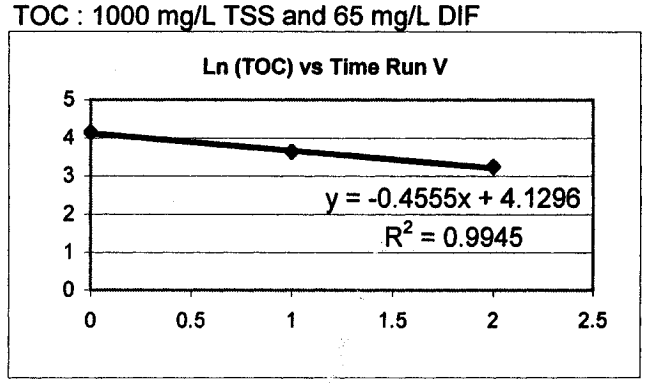

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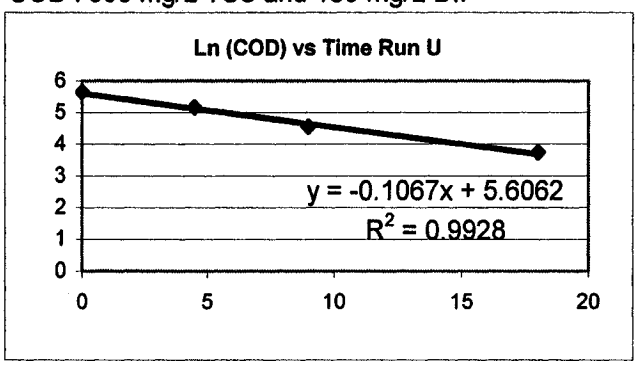

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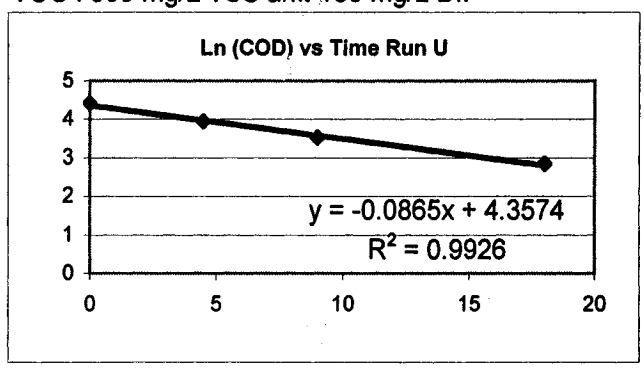
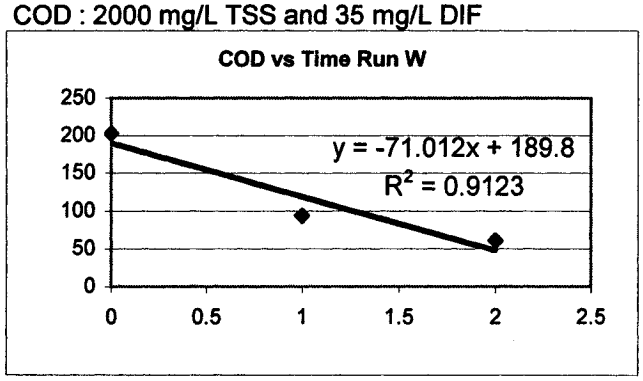
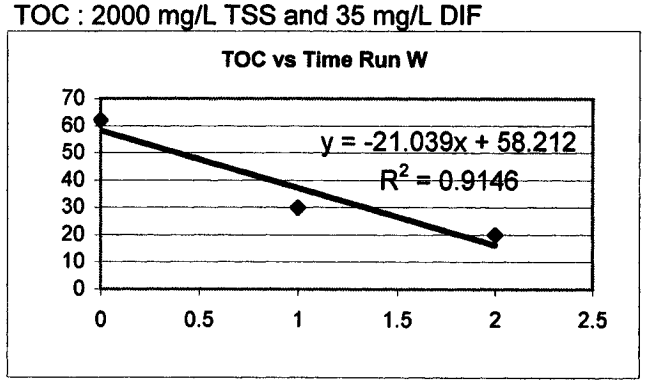


0th Order regression for replicate batch experiments

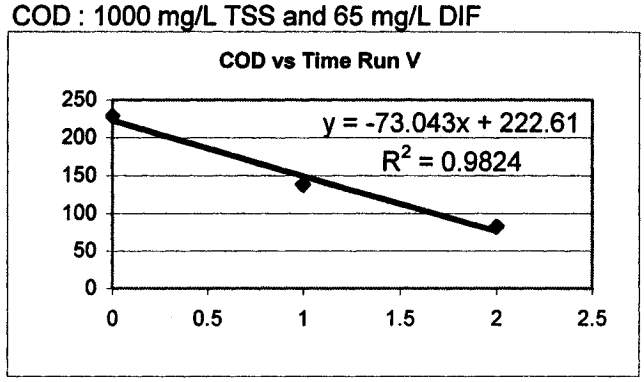
COD : 2000 mg/L TSS and 35 mg/L DIF



TOC : 2000 mg/L TSS and 35 mg/L DIF



COD : 1000 mg/L TSS and 65 mg/L DIF



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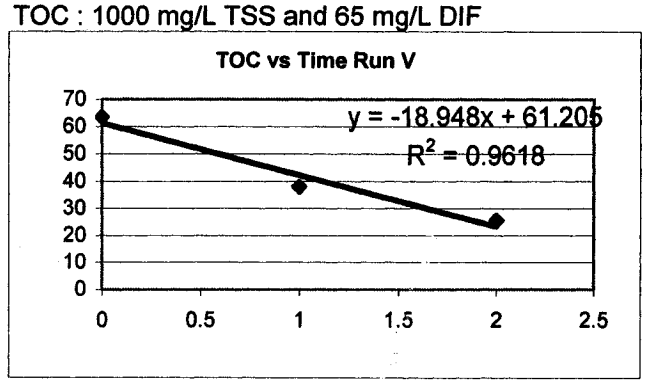
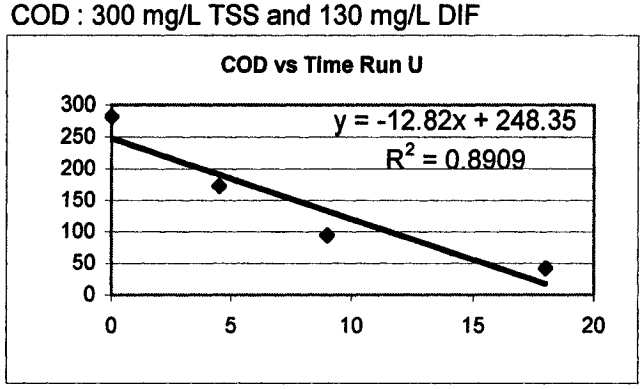
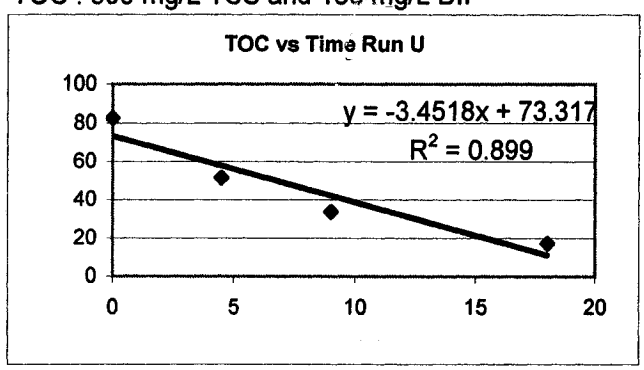


COD : 300 mg/L TSS and 130 mg/L DIF



TOC : 300 mg/L TSS and 130 mg/L DIF



APPENDIX G

Denaturing Gradient Gel Electrophoresis (DGGE) Technique

1. Literature Review

In addition to phenotypic changes, there is increasing interest in detecting the fundamental changes at the genetic level that are responsible for the ensuing differences in carbon source utilization patterns. Denaturing Gradient Gel Electrophoresis (DGGE) has been widely employed to characterize the total community DNA of a given population. The general strategy for genetic fingerprinting of microbial communities is first, the extraction of SNA and RNA from the sample, second, the amplification of the genes encoding for the 16S rRNA (important marker of microbial diversity) and third, the analysis of amplification products by a genetic fingerprinting technique such as DGGE [Muyzer, 1999]. The DGGE technique is based on the electrophoretic mobility of partially melted double-stranded DNA molecules in polyacrylamide gels. Thus, the differences in electrophoretic mobility create banding patterns in the gels and characterize a specific microbial population.

Macnaughton *et al.* (1999), used this technique to monitor the in-situ microbial community structures in an experiment simulating a coastal oil spill. DGGE analysis of 4 types of treatment plots (no oil control, oil alone, oil plus nutrients, oil plus nutrients plus an indigenous inoculum) revealed significant differences in the structure and the diversity of dominant bacterial community.

Prominent bands were subsequently excised for sequence analysis and revealed that oil treatments encouraged the growth of gram negative microorganisms.

Iwamoto *et al.* (2000) studied the impact of in-situ biostimulation treatment on a groundwater bacterial community by DGGE. The results revealed that the bacterial community was disturbed after the start of the treatment, continued to change for approximately 45 to 60 days, then formed a relatively stable community different from the original one. DGGE analysis was employed to monitor the shifts in the dominant methanotrophs during the field experiment. Sequence analysis from the gene fragments of the DGGE bands implied that biostimulation treatment caused a shift in the type of methanotrophs.

Finally, Curtis and Craine (1998) used DGGE to compare the diversity of total microbial communities present in different activated sludge plants. They found no variation within the plants studied and concluded that a single sample of an activated sludge plant was sufficient for a plant to plant comparison. The results showed an ability to distinguish between plants although further work is required to find the most appropriate basis for such comparisons. In addition, organisms from raw sewage were found in the mixed liquor samples and since these organisms have no functional significance in the treatment process, they complicate plant to plant comparisons. Nevertheless, the authors conclude that despite certain drawbacks, there are numerous advantages in taking and comparing a relatively large number of samples.

2. Materials and Methods

The DGGE technique is performed in 3 major steps : DNA extraction, DNA amplification and separation of DNA fragments [adapted from Gaulin, 2003].

1) DNA extraction

The first step in DNA extraction is to collect the mixed liquor or activated sludge sample and lyse the cells. The lysis of cells is conducted using an apparatus (nebulizer) that propels the sample at high pressure against a ball where the cell wall is broken open without any damage to the contents within. Following this step, various extractions are performed in order to separate and purify the DNA from the rest of the cell contents and unwanted compounds. The resulting DNA sample is quantified by absorbance to verify its purity with respect to the amount of proteins and contaminants. Once the purity is ascertained, the sample is ready for amplification with the PCR technique.

2) DNA amplification

In order to isolate and amplify the 16S rDNA fragments from the total genomic DNA of the microorganisms, the Polymerase Chain Reaction (PCR) is employed. This technique uses specific primers that contain complementary sequences to the fragment that need to be amplified. Thereafter, the fragments are amplified through 25-30 PCR cycles resulting in the synthesis of over a million copies of the double stranded DNA sample. The amplified fragments of interest can then be separated using the denaturing gel.

3) Separation of DNA fragments

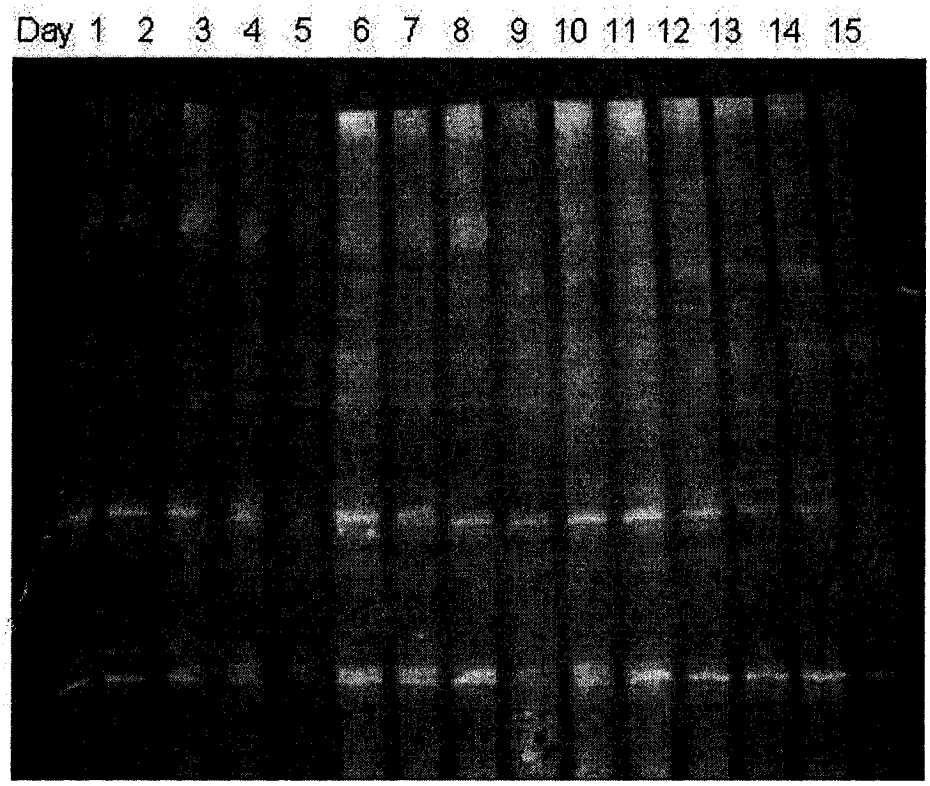
Denaturing gradient gel electrophoresis separates the amplified DNA fragments based on their electrophoretic mobility. The fragments are subjected to a linear increasing gradient gel and an electrical potential which induces the fragments to migrate. When the fragment reaches a certain gradient concentration in the gel, the DNA will partially melt from a helical to a partially open structure. This melting decreases the electrophoretic mobility of the fragment and therefore, fragments with different sequences will migrate to separate areas in the gel. These differences in electrophoretic mobility create banding patterns in the gel that can be used to characterize the specific microbial population. It should be noted, that the bands from the DGGE gels can be further excised in order to identify the actual species present in the original sample. However, this technique was deemed beyond the scope of the current project for various reasons.

3. Results and Discussion

Denaturing Gradient Gel Electrophoresis was utilized to characterize the evolution of the microbial populations based on their banding patterns with respect to time. As was seen from the Biolog results, the 8 hour batch experiments do not provide a sufficient timeframe in which significant changes of the microbial population can be determined. Therefore, the DGGE test was employed to monitor the microbial population in the sequencing batch reactor (SBR) experiments. The results of this test applied to the SBR experiment

conducted at room temperature and with an 8 hour cycle are shown in Figure 6.5.1.

Figure 6.5.1 : DGGE banding patterns for Day 1 to Day 15



As can be seen, there are noticeable changes in the banding patterns with respect time for this particular experiment. However, no additional information can be drawn for the DGGE analysis since it is difficult to identify or quantify the different microorganisms that undergo change. First of all, due to the available resources, it was not possible to excise the bands from the gel in order to determine which species were present in the sample. In addition, there is sufficient evidence that shows that band intensity cannot be directly correlated to

the amount of the microorganisms present in the sample. Furthermore, a specific microorganism can exhibit more than one band. Therefore, due to all these constraints, this technique was not pursued beyond the first SBR experiment and the significant results for the changes in the microbial population were carried out with the Biolog technique.