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The Activated Sludge Treatment of Pulp and Paper Wastewater

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ABSTRACT

Biological treatment of pulp and paper wastes by activated sludge is widely practiced in industry to reduce the organic content and toxicity of the wastewater. Most industrial applications require the treatment of a combination of streams from various processes. The composition of the combined stream varies since shock loadings and spills may occur. The objective of this research was to determine the effects of these variations on the microbial community and, ultimately, on the kinetics of the reduction of biological oxygen demand (BOD) and chemical oxygen demand (COD). This will improve the understanding of control requirements for the process.

Effluents from two mills in Quebec were examined. Initially, effluents from a chemithermomechanical pulp (CTMP) mill were used in this research. The objective was to study the effect of six different waste streams mixed with total mill effluent (0-100%) on the reduction of COD and on the microbial population. The effluents from the ultra-high yield pulping process, wood washing process, acid plant, and paper machine were found to have adverse effects on the biomass and its reduction of COD. The remainder of this research focused on treating effluents from a Kraft mill and investigated the effect of hydraulic residence time (HRT) and step inputs of 1%, 2.5% and 5% black liquor on the reduction of COD and BOD_5 and on the microbial population. Generally, the system reduced the COD by 63-72% and the BOD_5 by 90-99%, regardless of HRT or black liquor concentrations. Changes in HRT had no effect on the substrate uptake rate; it ranged from 0.31 mgCOD/min·gVSS to 0.38 mgCOD/min·gVSS. However, the substrate uptake rate did increase from 1.0 mgCOD/min·gVSS to 1.6 mgCOD/min·gVSS with an increase in black liquor concentration. Furthermore, the SOUR increased from about 10 mgO_2/gVSS·h to 30 mgO_2/gVSS·h, indicating that the black liquor caused an increase in microbial activity. The microbial population changed over time for different step inputs of black liquor and the sludge quickly adapted to the new substrate loading. The Biolog assay was an effective tool for indicating these changes.

RÉSUMÉ

Les déchets de l'industrie des pâtes et papiers sont couramment traités par des boues activées afin de réduire le contenu organique et la toxicité des eaux usées. Dans la plupart des applications industrielles, on doit traiter des effluents combinés provenant de divers procédés. La composition de l'effluent combiné peut varier à cause de déversements accidentels. L'objectif de cette étude est de déterminer les effets de ces variations sur la communauté microbienne et sur la cinétique de la réduction des demandes biologique et chimique d'oxygène (DBO et DCO). Les résultats de cette étude serviront à améliorer la compréhension des besoins de contrôle du procédé.

Des effluents provenant de deux usines du Québec ont été examinés. La recherche a porté d'abord sur des effluents d'une usine de pâte chemi-thermomécanique. On a étudié l'effet de six effluents différents mélangés à l'effluent total de l'usine (0% à 100%) sur la population microbienne et la réduction de DCO. On a ensuite étudié le traitement des effluents d'une usine Kraft. On a analysé l'effet du temps de résidence hydraulique (TRH) et d'échelons de liqueur noire de 1%, 2.5% et 5% sur la réduction de DCO et DBO_5 ainsi que sur la population microbienne. En général le système a réduit la DCO de 63% à 72% et la DBO_5 de 90% à 99% indépendamment du TRH et de la concentration de liqueur noire. Les changemets de TRH n'ont eu aucun effet sur le taux d'utilisation du substrat qui a varié de 0.31 mgDCO/min·gSVS à 0.38 mgDCO/min·gSVS. Cependant le taux d'utilisation du substrat a augmenté de 1.0 mgDCO/min·gSVS à 1.6 mgDCO/min·gSVS suite à une augmentation de concentration de liqueur noire. De plus, la liqueur noire a causé une augmentation d'activité microbienne tel qu'indiqué par le taux d'utilisation spécifique d'oxygène qui a augmenté de 10 mgO_2/h·gSVS à 30 mgO_2/h·gSVS. La population microbienne a changé avec le temps pour différents échelons de liqueur noire et les boues se sont rapidement adaptées à la nouvelle charge de substrat. Le test Biolog s'est avéré un outil efficace pour indiquer ces changements.

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NOMENCLATURE

AOX	adsorbable organic halides
AWCD	average well colour development
BKME	bleached Kraft mill effluent
BOD	biological oxygen demand
BOD_5	biological oxygen demand (mg/l)
C	control well in the Biolog microplates
COD	chemical oxygen demand (mg/l)
CFSR	continuous flow stirred reactor
CSTR	continuous stirred tank reactor
CTMP	chemithermomechanical pulp
DCA	detrended correspondence analysis
DO	dissolved oxygen (mg/l)
DOC	dissolved organic carbon (mg/l)
GN	gram negative microplates produced by Biolog
HMW	high molecular weight fraction
HRT	hydraulic residence time (h)
k_d	decay rate ($time^{-1}$)
K_s	saturation constant (mass/volume)
LMW	low molecular weight fraction
MLSS	mixed liquor solids concentration (mg/l)
MLVSS	mixed liquor suspended solids concentration (mg/l)
N	nitrogen
P	phosphorus
PC	principal component
PCA	principal component analysis
Q	flow rate (volume/time)
r	reaction rate (mass/volume·time)
R	response well in the Biolog microplates
RFA	resin and fatty acids
S	limiting substrate concentration (mass/volume)
SBR	sequencing batch reactor
SMP	soluble microbial products
SOUR	specific oxygen uptake rate (mg O_2/g VSS·h)
SRT	solids retention time (days)
SVI	sludge volume index (ml/g)
t	time
TKN	total Kjeldahl nitrogen
TME	total mill effluent
TMP	thermomechanical pulp
TOC	total organic carbon
TSS	total suspended solids (mg/l)
V	aerator volume (ml or l)
VSS	volatile suspended solids (mg/l)
WSF	waste sludge flow rate (ml/day)

X	biomass concentration (mass/volume)
Y	yield coefficient (mass of biomass/mass of substrate)

subscripts:

a	aerator
c	clarifier
e	effluent
f	feed
r	recycle
s	substrate
w	waste
x	biomass

Greek letters:

α	recycle ratio
μ	specific growth rate ($time^{-1}$)
μ_m	maximum specific growth rate ($time^{-1}$)
θ	sludge age (days)

1. INTRODUCTION

1.1 BACKGROUND

Biological treatment of wastewater is widely used in industry. One successful process used extensively is the activated sludge process since it can effectively reduce the organic content and toxicity of various wastewaters. The activated sludge itself is a complex microbial population that includes bacteria, protozoa, and many other types of organisms. These microorganisms are mixed in suspension with the wastewater under aerobic conditions and metabolise the soluble organic compounds present. The bacteria form aggregates or flocs that can easily be separated from the treated wastewater in a settling tank. Protozoa and other grazers feed on the edges of the flocs. Because of its complex nature, the activated sludge process is difficult to control and extensive research is being carried out to improve its control.

Several factors, such as the reactor temperature, pH, hydraulic residence time (HRT), and the nature and composition of the microbial population, may affect the performance of any biological treatment system. Organisms typically thrive at a narrow range of temperature and pH. Changes in these parameters may lead to the growth of unwanted organisms, such as filamentous bacteria, which cause bulking. Furthermore, most activated sludge populations consist of a mixed culture, and the more diverse the culture is the more adaptable the sludge is to different effluent streams. Therefore, the nature of the sludge is an important consideration. Treatability of the effluent is further complicated by such factors as biodegradability, solubility of organic material, and the presence of toxins. Toxins may include various bleaching chemicals, trace metals, and non-biodegradable compounds that could inhibit treatment.

Another factor that significantly affects the performance of the biological wastewater treatment system is the composition of the wastewater itself. Most industrial applications require the treatment of a combination of streams from various treatment processes. It is impossible to keep the composition of the combined stream constant over time since the

raw materials to the process may change, or variations such as shock loadings and spills may occur. It is desirable to determine the effects of these variations on the microbial community and ultimately on the kinetics of the reduction of biological oxygen demand (BOD) and chemical oxygen demand (COD), thus leading to more accurate modeling of the process.

The activated sludge process is commonly used in the treatment of pulp and paper wastes. In the present research, effluents from two mills in Quebec were used. Mill A is a chemithermomechanical pulp (CTMP) mill while Mill B uses the Kraft process. These processes are briefly outlined below. The intention of this project was to investigate several operating parameters that describe the performance of the activated sludge process in order to determine the bioreaction kinetics. This will allow for more accurate modeling of the activated sludge system and improve the understanding of control requirements for the process. A review of the treatability of the effluents by activated sludge has been provided followed by the results and discussion for experiments done using effluents from the two different mills.

1.1.1 Description of Pulp and Paper Processes

a) The Kraft Process

The Kraft process uses sodium hydroxide (NaOH) and sodium sulphide (Na_2S) (collectively referred to as white liquor) at a high temperature and under pressure to reduce the wood chips to pulp. The non-fibrous wood not turned into pulp is recycled along with most of the cooking chemicals (Gray, 1990). This mixture is referred to as black liquor. Black liquor consists of about 55% alkali lignin, 3% resin and fatty acids (RFA), 4% formic acid, 5% acetic acid and 30% laconic acids. These acids form sodium salts and sulphonation products. The black liquor is concentrated and burned in a recovery furnace yielding a sodium carbonate (Na_2CO_3) and Na_2S smelt. This is dissolved to form green liquor and then causticised by adding quick lime (CaO) to convert the Na_2CO_3 to NaOH thereby regenerating the white liquor (Smook, 1992; Sutermeister, 1941). The pulp is washed and concentrated in multiple effect evaporators.

The off-gases are cleaned in wet scrubbers. The water used for washing and scrubbing is treated in the biological wastewater treatment plant (Betz, 1980). The Kraft process may require chlorine bleaching to produce a paper of acceptable brightness.

The Kraft mill (Mill A) uses hardwood as its raw material. The main component is maple (which constitutes about 60% of the total); however, birch and poplar are also used. Hardwoods generally do not yield any extractives such as resin and fatty acids, which contribute significantly to toxicity. However, the effluent may contain chlorinated aromatics derived from the bleaching process. The Kraft mill uses a $C_DE_{O.P.}DED$ bleaching scheme. C_D refers to the use of ClO_2 (about 70%) and Cl_2 (g) in the first stage of bleaching. $E_{O.P.}$ refers to extraction using O_2 and peroxide. DED refers to a second bleaching stage using only ClO_2, followed by extraction (without O_2 or peroxide) followed by a final bleaching stage with ClO_2. Chlorine derivatives from this bleaching process may be present in the mill effluent. Also, plant upsets may result in a black liquor spill that ends up in the final mill effluent and affects the treatment performance. The effluent is characteristically dark in colour and tends to foam.

b) The Chemithermomechanical Process (CTMP)

The chemithermomechanical (CTMP) process involves mechanical grinding of the wood chips under pressure and heat in the presence of sodium sulphite (Na_2SO_3). Sodium sulphite is added to soften the wood chips and improve the yield of the pulp. This initial stage is referred to as the ultra-high yield process and is responsible for the greatest portion of organic matter in the total mill effluent that must be treated by the activated sludge. The pulp is then refined by washing and screening before being sent to the paper machines. The refining stages and the paper machines use a great deal of water and this water must also be treated. Other sources of wastewater include an acid plant and a ground wood process and the wastewater generated by these processes is also combined with the total mill effluent for treatment.

The CTMP mill (Mill B) uses softwood for its raw material. Softwoods typically contain resin and fatty acids (RFA) as well as neutral wood extractives which give the final mill

effluent a high BOD and COD. Sodium sulphite further increases COD since more RFA are released. Sulphite is toxic to the biomass and high concentrations must be reduced prior to biological treatment. Different chemicals such as peroxide (H_2O_2) and sodium thiosulphate ($Na_2S_2O_4$) are also added to brighten the pulp depending on what type of paper is produced and the desired brightness. This affects the wastewater treatment system further downstream. (Smook, 1992; Betz, 1980).

1.1.2 Activated Sludge Process Description

The basic activated sludge process consists of an aeration basin and a settling basin (clarifier). A typical setup is illustrated in Figure 1.1. The aeration basin is designed as a continuous stirred tank reactor (CSTR) of volume, V and is completely mixed. The aerator contains the microbial organisms and receives a continuous flow of mill effluent, Q_f. Air is necessary for respiration and is added to the system. Air also promotes mixing, improves mass transfer and allows the maximum contact between the floc and the wastewater ensuring that the organisms are adequately exposed to the food supply. The activated sludge process is usually designed to operate at food limiting conditions to maintain cell growth in the endogenous phase. This reduces the quantity of biomass produced (Klopping et al, 1995; Gray, 1990).

From the aerator the mixed liquor (mixture of wastewater and biomass) is sent to the clarifier at a rate of $(Q_f + Q_r)$ as shown in Figure 1.1. The flocculated biomass settles out leaving a final effluent virtually free of solids. Some of the sludge is recycled to the aerator at the rate, Q_r to provide continuous seeding and the remainder of the sludge is wasted at a fixed flow rate, Q_w. Recycle keeps the concentration of the cells higher than the normal steady-state level and increases the conversion. It may also improve the stability of the system by minimising the effects of process fluctuations (Curds, 1973).

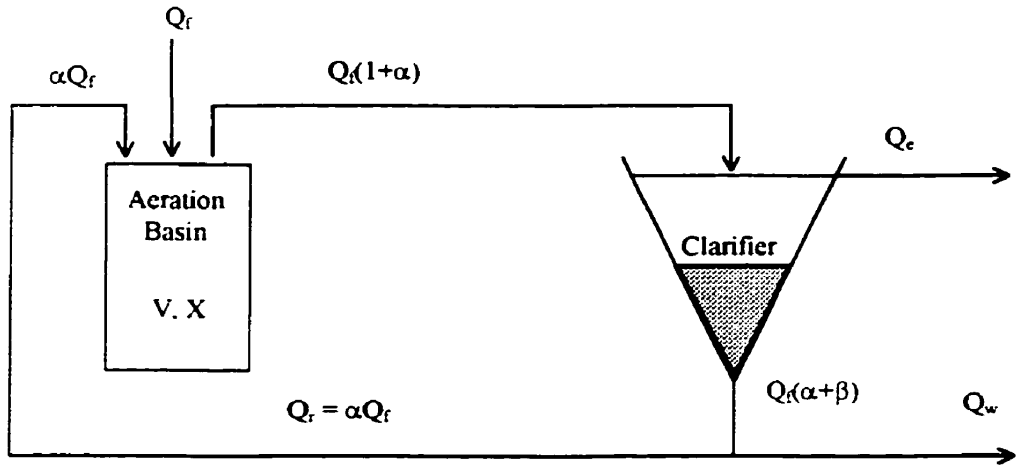

Figure 1.1: Activated Sludge Plant Flow Diagram with a Single CSTR.

The overall substrate balance for the system is as follows:

$$V\frac{dS}{dt} = Q_f S_f - Q_e S_e - Q_w S_w - r_s V \tag{1.1}$$

where: V = the volume in the aerator

S = the substrate concentration (mass/volume)

Q = flow rate (volume/time)

r = substrate utilisation rate (mass/volume·time)

subscript f = feed

subscript e = effluent

subscript w = waste

subscript s = substrate

Similarly, the overall biomass balance for the system is as follows:

$$V\frac{dX}{dt} = Q_f X_f - Q_e X_e - Q_w X_w + r_x V \tag{1.2}$$

where: X = the biomass concentration (mass/volume)

subscript x = biomass

The rate of biomass growth, r_x (mass/volume·time) is usually represented by

$$r_x = \mu X \tag{1.3}$$

where: μ = specific growth rate (time^{-1})

Models for the activated sludge process are typically based on the assumption that the specific growth rates of the organisms obey Monod kinetics and are related to their specific substrate concentrations by the Monod Equation for endogenous metabolism,

$$\mu = \frac{\mu_m S}{K_s + S} - k_d \tag{1.4}$$

where: μ_m = maximum specific growth rate (time^{-1})

K_s = saturation constant, substrate concentration at one-half the maximum growth rate (mass/volume)

S = limiting substrate concentration (mass/volume)

k_d = decay rate (time^{-1})

As an organism grows, it is assumed that the rate at which the organism uses substrate is described by the following relation:

$$r_x = -Y r_s \tag{1.5}$$

where: Y = yield coefficient

The substrate balance over the aerator is as follows:

$$V\frac{dS}{dt} = Q_f S_f + \alpha Q_f S_r - (1+\alpha)Q_f S - r_s V$$

(1.6)

where: α = sludge recycle ratio

subscript r = recycle

The biomass balance over the aerator is as follows:

$$V\frac{dX}{dt} = (1+\alpha)Q_f X_r - (1+\alpha)Q_f X + r_x V$$

(1.7)

The sludge age can be defined as the average length of time that a microorganism or sludge particle spends in the aeration tank. If the proportion of microbial cells is assumed constant than sludge age may be considered equivalent to solids retention time (SRT). SRT is usually controlled through sludge wasting by the following equation:

$$SRT = \frac{VX}{Q_e TSS_e - Q_w X_w}$$

(1.8)

where: SRT = solids retention time or sludge age (days)

1.2 SCOPE AND OBJECTIVES

Since the composition of the effluent varies for each pulping process due to such factors as different raw materials and process conditions, the response of the activated sludge used to treat that particular effluent will also vary. Different groups of microorganisms will thrive under different conditions. Therefore, in this project a study was performed that took into account the effect of variations in effluent composition on the microbial population. To do this, the sludge and effluent were characterised.

Through shake flask experiments, the relevant effluents were characterised analytically to determine such descriptors as chemical oxygen demand (COD), total organic carbon (TOC), and five-day biological oxygen demand (BOD_5). To characterise the sludge, the total and volatile suspended solids (TSS and VSS) and sludge volume index (SVI) were measured and phenotypic fingerprinting using the Biolog technique was employed. In a continuous reactor, the reduction of BOD_5, COD, and TOC was examined in order to determine the rate constants of the biological reactions taking place in the treatment system.

Effluents from two different pulping mills were examined in this study. Initially, the intention was to use only effluent from a CTMP mill (Mill A) for this research. However, since effluent from Mill A became unavailable due to changes in the mill's status within the company, the scope had to be altered to include the study of effluent from a second mill (Mill B) employing the Kraft process. The original plan of the research was to characterise the CTMP mill effluent using shake flask experiments and to ascertain which of its constituents had the strongest effect on the overall reduction of COD. Once the waste streams with the most significant effect were identified, testing could be limited to these streams and experiments in a continuous reactor could be performed.

However, after only two preliminary experiments were performed in the continuous reactor, effluent was no longer available as stated above. Thus, experiments were undertaken with effluent samples from Mill B employing the Kraft process. Several different hydraulic residence times were investigated using a continuous reactor with sludge recycle to determine the effect of HRT on the reduction of COD and BOD_5. Once these were completed, a series of step experiments adding black liquor at various concentrations were performed to determine the effect of black liquor on the microbial population and its ability to reduce the COD and BOD_5.

1.2.1 Statement of Objectives

Mill A

1. To identify which of the effluent streams have the greatest effect on the biomass and its ability to reduce the COD.
2. To characterise the effluent and sludge using shake flask experiments and the following analytical techniques: COD, TOC, BOD_5, TSS, VSS, SVI, and phenotypic fingerprinting using the Biolog technique.

Mill B

3. To determine the kinetics of the reduction of COD and BOD_5 by the activated sludge.
4. To study the effect of black liquor on the sludge population and the reduction of COD and BOD_5 to improve the understanding of what occurs during a system upset.

2. LITERATURE REVIEW

2.1 MICROBIOLOGY OF ACTIVATED SLUDGE

Activated sludge consists of a diverse collection of microorganisms. Organisms require energy and carbon sources for cell production and proper functionality. The carbon may be derived from organic matter or carbon dioxide. Cells using CO_2 are classified as autotrophic while cells that utilise organic carbon are heterotrophic. Microorganisms can be metabolically classified by the energy source they require. Photosynthetic autotrophs use light as their energy source while chemosynthetic autotrophs derive their energy from inorganic oxidation-reduction reactions. Heterotrophs derive their energy from organic oxidation-reduction reactions. All microbes can be further classified by their oxygen requirements. Aerobic species need oxygen for growth, anaerobic species need an environment free from oxygen, and facultative species can thrive with or without oxygen present (Metcalf and Eddy, 1979).

2.1.1 Composition of Activated Sludge and Growth Factors

Microorganisms are classified as protista, plants or animals. Protista include fungi, algae, bacteria, and protozoa. Bacteria are the main group present in activated sludge followed by protozoa. Bacteria are single-celled organisms that use soluble food and generally reproduce by binary fission. They can be sub-divided by temperature requirements. Cryophilic bacteria thrive in the temperature range of -2-30°C, mesophilic bacteria prefer temperatures of 20-45°C, while thermophilic bacteria can live at temperatures between 45-75°C. The optimal pH for bacterial growth is between 6.5 and 7.5. Heterotrophic bacteria are the most important type present in activated sludge.

Protozoa are single-celled, motile organisms that include amoebas, flagellates, free-swimming and stalked ciliates. These feed on the bacteria and help maintain a balance within the population. Most protozoa are aerobic heterotrophs. Another group sometimes present in healthy sludge are rotifers. Rotifers are aerobic, heterotrophic,

multicellular animals that consume dispersed and flocculated bacteria as well as some organic matter. They help improve the efficiency of the treatment process (Metcalf and Eddy, 1979). Aerobic organisms grow best in non-limiting dissolved oxygen concentrations greater than 6.0 mg/l, but a dissolved oxygen concentration between 1-2 mg/l is sufficient for most active aerobic heterotrophic microbial activity (Gray, 1990).

2.1.2 The Growth Curve

Typically, most organisms follow a similar growth pattern when they are cultivated in a batch reactor. This is described by the growth curve and is illustrated in Figure 2.1. It can be divided into several phases as follows: lag phase, acceleration phase, exponential (log) growth phase, deceleration growth phase, stationary phase, and finally the endogenous or death phase. The lag phase represents the acclimation period for the cells and is characterised by long generation times. The acceleration phase occurs when the generation time decreases and the growth rate begins to increase. This is followed by the exponential growth phase, which involves a constant and maximal specific growth rate. The rate of metabolism is only limited by microbial generation and its ability to process the substrate. Next, the microbes enter the decelerating growth phase where the growth rate slows down as the substrate concentration becomes limiting and generation time increases once again. When the cells have almost exhausted the nutrients and substrate, the microbes enter the stationary phase. At this stage, growth is offset by death and the concentration of toxic metabolites begins to rise. Once the concentration of toxic metabolites becomes unfavourable to the population and the cell death rate begins to exceed the growth rate, the microbes enter the endogenous (log-death) phase. During this phase the microbes metabolise their own protoplasm and lysis occurs. (Gray, 1990; Metcalf and Eddy, 1979).

It is best to operate activated sludge reactors in the endogenous growth phase. During this phase, the total mass of microorganisms begins to decrease as cells use up stored reserves and begin to die. This leads to good BOD removal because the microbes are oxidising all available substrate. Also, floc-forming bacteria are only flocculant in a

stationary growth phase such as the endogenous (or declining) phase (Klopping et al, 1995; Gray, 1990). Furthermore, the log growth phase promotes poor settling conditions for the sludge, which leads to a higher concentration of solids in the effluent as well as inefficient BOD removal (Barr et al, 1996).

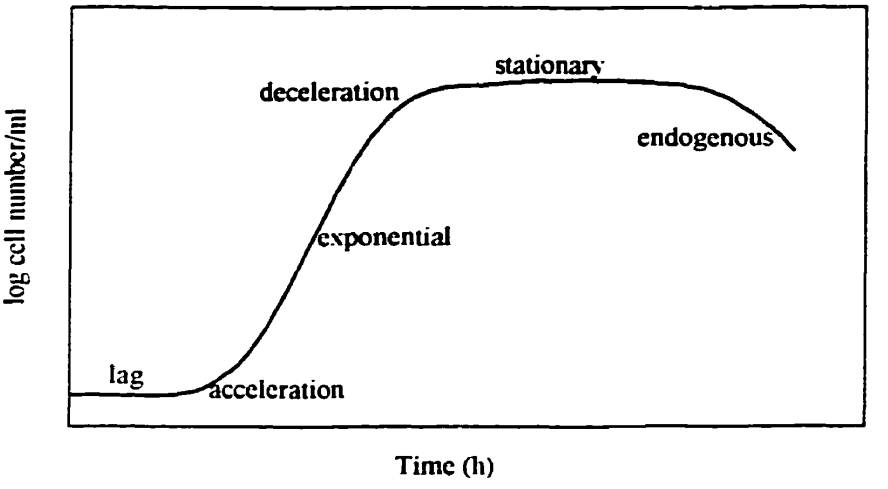

Figure 2.1: Typical Growth Curve for a Single Bacterial Population Grown in a Batch Reactor

2.2 NUTRIENT ADDITION

All organisms not only require food and an energy source for proper growth, but they also require nutrients, such as nitrogen, phosphorus, and trace amounts of sulphur, iron, potassium, calcium, magnesium, manganese, copper, zinc, and molybdenum. Nutrient deficiencies may cause bulking or the production of troublesome scums, foam or slime (Klopping et al, 1995). Therefore, in industrial practice nutrients, namely nitrogen and phosphorus, are added to the wastewater as a necessary step prior to treatment by activated sludge. The most readily utilised form of nitrogen is ammonia, but in pulp and paper waste treatment systems it is difficult to denitrify excessive amounts of ammonia so other nitrogen sources are also added (urea, amino acids, and peptides). The most readily utilisable form of phosphorus is ortho-phosphate so phosphorus is usually added as ortho-phosphoric acid (Saunamaki, 1994).

Usually, the addition of nutrients is determined by the ratio of BOD:N:P or COD:N:P. Commonly used ratios in literature are 100:5:1 for BOD:N:P and 100:3:1 for COD:N:P (Franta et al, 1994; Klopping et al, 1995; Gray, 1990; Grau, 1991). These 'rules of thumb' occasionally lead to excess nitrogen and phosphorus in the system. Excess nutrients cause eutrophication in the receiving bodies of water, while nutrient deficiency can reduce the treatment efficiency and cause sludge bulking. Several studies have been done to optimise nutrient addition. Saunamaki (1994) concentrated on the effects of adding phosphorus to pulp and paper mill effluent. He determined that less phosphorus can be added than the 'rule of thumb' states. He deemed that the addition of phosphorus was generally unnecessary at pulp mills since they can manage without, while paper mills need supplemental phosphorus. He found that the best ratio for a newsprint mill was a BOD:P of 100:0.3-0.5. Mobius (1991) stated that optimisation of nutrient addition generally leads to a ratio of 100:3.5:0.6 for BOD:N:P.

2.3 TREATMENT OF PULP AND PAPER MILL EFFLUENT BY THE ACTIVATED SLUDGE

It is common practice to study the performance of laboratory or industrial reactors in an activated sludge treatment system by analysis of COD and BOD_5 removal. Many studies have been done on pulp mill wastewater treatment systems using activated sludge. This section contains a review of the treatment of CTMP and Kraft mill effluents as well as a review on the treatment of other types of pulp and paper wastewaters.

2.3.1 Treatment of CTMP Wastewater

Few papers have been written on the treatment of CTMP wastewater. Liu et al (1993) studied the effects of HRT, pH, temperature, and DO on the performance of continuous aeration tanks used to treat effluent from a CTMP washing operation. The criteria they used to determine the performance were the removal of COD, TOC, BOD_5, RFA (resin and fatty acids), and total carbohydrates measured as apparent glucose. They determined that, for their particular effluent, the optimal conditions for treatment were as follows: pH between 6.5 and 7.5, HRT of 48 h, temperature of 20°C, and a DO greater than 2 mg/l.

The authors established that about 88% of BOD_5 and 94% of RFA could be removed from the effluent studied using an HRT of 0.5 days. To study the kinetics and determine the parameters they used the Monod and first order reaction models to represent the rate of substrate removal and concluded that both models successfully described the biodegradation.

2.3.2 Treatment of Kraft Mill Wastewater

Barr et al (1996) considered how HRT, SRT (solids retention time) and temperature affect the performance of activated sludge reactors treating bleached Kraft mill effluent (BKME). The authors studied high operating temperatures (45-47°C) inherent in BKME treatment. In a series of steady state experiments operated at 35°C, where they held the HRT at 12 h and the SRT at 15 d, the authors noted removal efficiencies of 87.9% for BOD while only 32.4% for COD and the food to mass ratio (F/M) ranged between 0.37 and 0.45. The specific oxygen uptake rate (SOUR) was 16.5 $mgO_2/gMLVSS \cdot h$ (where MLVSS is the mixed liquor volatile suspended solids) and the acute toxicity removal was 97.7%. By altering the HRT and SRT they found that HRT influenced BOD removal more strongly than SRT. As HRT decreased BOD, COD, adsorbable organic halides (AOX) and toxicity removal all decreased while SOUR increased.

Kemeny and Banerjee (1997) investigated some relationships among effluent constituents, namely AOX and COD, in an aerated lagoon of a bleached Kraft pulp mill. They determined that residual COD and AOX are due to lignin residuals not degraded during treatment. After treatment, there was a strong correlation between AOX and COD. Colour increases across the lagoon and exceeds inlet colour by 22%. Colour and COD correlated well at the exit of the lagoon indicating colour is derived from carbon that is not removed during treatment. The authors also concluded that conductivity is a good indicator of the presence of black liquor in the effluent.

In a study involving molecular weight fractions, Bullock et al (1996) fractionated BKME into high and low molecular weight fractions (HMW and LMW) and found that HMW

was the main contributor to AOX, colour and COD in treated effluent. In a series of batch experiments operated at room temperature with a 48 h HRT and 10 d SRT, the system generally achieved BOD and COD removals of greater than 90% and 60% respectively, and the system volatile suspended solids concentration was generally between 1000 mg/l and 1500 mg/l. The authors found that HMW accounted for 75-85% of the AOX, 65% of the NO_x, and contained almost three times as much total Kjeldahl nitrogen (TKN) than the LMW (TKN as derived from the wood furnishing or pulping process) and very little ammonia or phosphorus. They observed good microbial growth in the unfractionated effluent, but the LMW and HMW fractions both had poor growth. This suggests there was a synergistic effect between the two fractions. The authors found that nitrogen was the limiting factor in treatment of the LMW since most of it was contained in the HMW, but that the LMW was readily degradable (70% removal of soluble COD) with the provision of nutrients. The HMW was found to be biologically recalcitrant.

2.3.3 Treatment of Other Pulp Mill Wastewaters

Lo et al (1994) characterised the effluents of two thermomechanical pulp (TMP) newsprint mills to determine the variation and contribution of process elements on BOD_5 and toxicity to trout. The authors collected total mill effluent (TME) samples and samples from the TMP and paper machine operations to determine which streams contributed the most to BOD_5, RFA and toxicity. They examined the effects of HRT and sludge age on BOD_5 reduction and toxicity to trout for those particular streams and found that treating TME achieved good BOD_5 removal (93%) and toxicity removal (treated 96 h LC_{50} value over 100%) for HRT's between 8 and 24 h. Varying the sludge age from 10-20 days had no effect. Treating the more concentrated TMP effluent did not achieve satisfactory toxicity removals at short sludge ages (<10 d) and HRT's (<18 h), but provided high removal efficiencies of BOD_5 (96%). In a second paper the same authors (1994) derived the biokinetic parameters using their results. They used a first order reaction model to determine the BOD_5 removal rate, O_2 usage and biomass yield

parameters and concluded that the TMP processes were the greatest sources of BOD_5 and toxicity to trout, but a TME treatment process was more adaptable for design purposes.

Using sequencing batch reactors, Franta et al (1994) studied factors affecting the composition and concentration of residual organics in the effluent of biological wastewater treatment plants. They tested mechanical pulp mill wastewater and a synthetic wastewater to estimate the fraction of residual organics in the effluent originating from activated sludge metabolism. They determined that only 10% of effluent (residual) COD had microbial origin. The rest of the residual organics could be assumed to come from higher molecular weight compounds derived from lignins. The authors concluded that the best COD removal and sludge settling for papermill wastewater were achieved with the highest sludge age (20 d) and longest react period (22 h).

Franta and Wilderer (1997) studied the reduction of residual organics using sequencing batch reactors by modifying the sludge age, react period (batch phase) and fill period. In terms of biodegradability, they generally achieved a reduction in COD between 85-93% and a 98% reduction of BOD_5, while the SVI (sludge volume index) ranged from 90-170 ml/g. Low molecular weight compounds such as alkenes, terpenes, diterpenes, resin acids, aromatics and aliphatics were significantly reduced in the effluent indicating that they are readily biodegradable. Higher molecular weight fractions, such as 2-methoxyphenol derivatives, required high sludge ages to achieve significant degradation ratios. They found that the system achieved the minimum residual COD at sludge ages of 15 and 25 days. Overall, they achieved the best residual COD removal with a sludge age of 15-25 days, a react period of 12 h and a fill phase of 0.5 h.

2.4 CHARACTERISATION TECHNIQUES

Pulp and paper mill effluent has been thoroughly characterised using various techniques. Some commonly used analytical techniques include: COD, BOD and TOC analysis, toxicity tests such as MICROTOX, Daphnia Magna, and trout tests, spectroscopic tests using infrared (IR) and ultraviolet (UV) radiation, and other tests including high

performance liquid chromatography (HPLC) and gas chromatography/mass spectroscopy (GC/MS). The characterisation of activated sludge is more difficult than for effluent because it is a complex mixture of microorganisms. Sludge may be characterised on the basis of settling properties, microscopy and viability. Common techniques include: sludge volume index (SVI), total and volatile suspended solids (TSS and VSS or MLVSS - mixed liquor volatile suspended solids). Phenotypic fingerprinting is another technique that has been shown to be successful in characterising sludge (Victorio et al, 1996).

2.4.1 Characterisation of Effluent

Nyholm (1996) suggested a scheme for assessment of biodegradability of industrial effluents in terms of degradable and persistent toxicity. He suggests combining lumped parameters such as COD and DOC (dissolved organic carbon) with the measurement of specific compounds through the use of HPLC/MS or GC/MS as well as toxicity tests to get a better understanding. He recommended a tiered strategy, which begins by compiling all available data in order to get clues on which substances may be present in the effluent. Then techniques including simple chemical analyses such BOD_5, COD, VSS, and the determination of ammonia and ad hoc group specific parameters should be applied. Finally, for aerobic stabilisation he suggested that investigators should assess the degradable and persistent toxicity as well as the overall 'ready' biodegradability.

2.4.2 Characterisation of Sludge

A technique recently developed by Biolog Inc. in Hayward, California has been reported by several research groups (Fulthorpe et al, 1992; Fulthorpe and Allen, 1994) to be effective in the characterisation of bacterial communities in the activated sludge when coupled with statistical analysis. The Biolog technique uses microplates comprised of 95 wells each containing different sole-carbon sources and tetrazolium violet, a redox dye that turns purple upon respiration. An additional well containing no nutrients acts as a control well. The pattern of purple wells may be analysed to determine which species was inoculated on the plate (a more detailed description of the Biolog technique is given in Materials and Methods, section 3.2.8).

Garland and Mills (1991) used the Biolog technique to study the rate of colour development of various microbial communities. They applied principal component analysis (PCA) to determine intrinsic relationships. PCA is an ordination technique that essentially rotates a swarm of data about its centre and projects the data onto new axes or principal components. This analysis extracts the greatest variance in the first two principal components and these may be plotted in two dimensions to reflect any intrinsic patterns within the data (see Appendix B for a complete description on PCA).

Prior to the use of PCA, Garland and Mills (1991) manipulated the data. First, they determined the raw difference values for each response well within a plate by subtracting the colour response of that well from the colour response of the control well (C-R). Then, the data was transformed by dividing the raw difference values by the average well colour development (AWCD) : $(C-R)/\{[\Sigma(C-R)]/95\}$. This step is necessary to eliminate discrepancies among plates due to variations in inoculum density and the rate of colour formation (Garland, 1996). They incubated whole communities in Biolog GN plates (a type of plate that contains various substrates typically utilised by the gram-negative bacteria inherent in the activated sludge) and read the colour response with a digitising video scanner. They determined that inoculum cell density affected the colour development rates and suggested that colour change in the Biolog community level assay is caused by growth of bacteria, rather than respiration.

Victorio et al (1996) also took a community-level approach in the application of the Biolog system. They characterised the microbial community involved in the biodegradation of wastewater treatment systems including two bleached Kraft mill effluent (BKME) treatment systems. The paper outlines the experimental procedure and sample processing (see Materials and Methods). The authors chose homogenisation with deflocculating agents (Tween 80 and sodium pyrophosphate) as the best method for microbial recovery (releasing the highest viable cell count) before inoculation of the Biolog plates. They determined differences among the sole-carbon source utilisation pattern of the microbial samples using PCA of the phenotypic fingerprints following the same procedure as Garland and Mills (1991) to manipulate the data and to plot the new

principal components. In comparing three different wastewater treatment systems, the authors concluded that each system had a unique microbial population. They also concluded that the Biolog system should be able to detect changes in the microbial community due to changes in various parameters or system upsets.

Schneider et al (1998) determined that fingerprinting can indicate a shift in the microbial pattern during system upsets such as a toxic breakthrough and was, in fact, more sensitive than conventional plate counts and microscopy. Thus, the authors confirmed that phenotypic fingerprinting was sensitive enough to detect changes that affect the system performance. They also used PCA. The authors measured the colour response of each well and obtained the colour development for a particular well by subtracting the colour response of that well at inoculation from its colour response after a 24 h incubation (C-R). This data was considered the raw difference data. The overall colour development of the plate was expressed as AWCD : [Σ(C-R)]/95]. The data was transformed by dividing the raw difference value for each well by the AWCD of the plate [(C-R)/AWCD] to express the pattern of sole-carbon source utilisation of that plate. The authors then performed PCA on the transformed data to determine the relationships among different samples (plates) and found distinct differences between samples taken during a toxic upset and samples taken during normal operation.

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Effluents

Pulp mill effluents were received approximately once per week in shipments of about 16-32 litres. These shipments were transported in coolers with ice packs to keep the effluents cool and minimise changes in effluent properties. Sludge was shipped in the same manner (about 2-4 litres per week or as needed). Sludge and effluent were refrigerated immediately upon receipt and stored at 2-4°C until needed (sludge was used as soon as possible to ensure that it was always fresh and that the cells were still viable). Prior to use, the sludge was allowed to settle and then the supernatant was decanted to concentrate the sludge. Total mill effluent (TME), which is the combination of all the waste streams at the mill, was taken after primary settling that removes most of the large fibres and particles while sludge was taken from the return activated sludge line. Constituent effluents were taken from their respective points prior to joining the total mill effluent.

3.1.2 Nutrient Addition

Nitrogen and phosphorus were supplemented based on COD using an experimentally determined ratio of COD:N:P of 100:3.0:1.0-2.0. The experiments are outlined in Chapter 4. Nitrogen was added as urea (H_2NCONH_2) and phosphorus was added as ortho-phosphoric acid (H_3PO_4). Equivalent amounts of these chemicals were calculated using stoichiometry as shown in Appendix A. Once the nutrients were added to the wastewater it was ready for use in subsequent experiments.

3.2 ANALYTICAL PROCEDURES

Unless otherwise specified, all analytical assays were performed following the protocols outlined in Standard Methods (APHA 1986).

3.2.1 Total Suspended and Volatile Suspended Solids Concentration (TSS and VSS)

Total suspended solids (TSS) refers to the portion of total solids retained by a filter after drying while volatile suspended solids (VSS) is the portion of TSS that can be oxidised and represents the organic matter in the sludge. TSS and VSS were determined via vacuum filtration of a known volume of sample using the Millipore® filtration apparatus and type AP40 glass-fibre filter disks or equivalent. Prior to use, the filter disks were washed, dried and, if VSS were to be measured, ignited in a muffle furnace. The filtrate was collected and stored at 2-4°C for later analysis while the filter was dried in the oven at 103-105°C to constant weight. The increase in weight was the TSS. To measure VSS, the filter was ignited in a muffle furnace preheated to 550 ± 50°C to combust all volatile matter. The difference in weight before and after ignition was the VSS.

3.2.2 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is the oxygen equivalent of the organic and inorganic matter in a sample that may be oxidised by a strong chemical oxidant. The closed reflux, titrimetric method from Standard Methods (1986) was used for this procedure. It involves refluxing the samples in a strong acid environment at a high temperature with a known excess of potassium dichromate. The remaining dichromate is measured by titration with ferrous ammonium sulphate (FAS). Samples were filtered and stored at 2-4°C for not more than two weeks before analysis.

It was necessary to prepare several reagents a day or two before beginning the assay to allow time for the solutions to dissolve and cool. Standard potassium dichromate digestion solution was prepared to a concentration of 0.0167 M by adding 4.913 g of previously dried $K_2Cr_2O_7$ and 167 ml of concentrated H_2SO_4 to about 500 ml of distilled water, cooling and bringing the mixture to 1000 ml. Sulphuric acid reagent was prepared by adding silver sulphate (Ag_2SO_4) to concentrated H_2SO_4 at the rate of 5.5 g Ag_2SO_4/ kg H_2SO_4 (silver sulphate is added as catalyst to improve the oxidation of straight-chain aliphatic compounds). Standard FAS titrant was prepared to a concentration of about

0.01 M by dissolving 3.92 g of iron (II) ammonium sulphate hexahydrate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) in distilled water and adding 20 ml of concentrated H_2SO_4, cooling and diluting to 1000 ml.

Standard 10 ml ampules and caps were pre-washed with 20% H_2SO_4 to prevent contamination. The ampules were prepared by combining 2.5 ml of sample, 1.5 ml of digestion solution and 3.5 ml of sulphuric acid reagent. Appropriate caution was taken when working with the strong oxidising and acid reagents. It was necessary to dilute the samples (the filtrate collected during the tests for TSS and VSS) so that the resulting COD would be within the range of 100-300 mg/l. This range was acceptable for this test and it avoided the necessity of using very small volumes of the titrant. Four blank ampules containing distilled water instead of sample were also prepared. Two of these were digested to provide a blank reading and two were set aside to determine the molarity of the FAS. The ampules were digested on a heating block preheated to 150°C for 2 h then cooled to room temperature prior to titration. 1-2 drops of ferroin indicator were added and the samples were titrated with FAS while stirring. The observed end point was a change from blue-green to reddish brown. The COD was determined by the following equation:

$$COD\left(\frac{mgO_2}{l}\right) = \frac{\left(FAS_{blank} - FAS_{sample}\right) * Molarity_{FAS} * 8000}{V_{sample}} \qquad \textbf{(3.1)}$$

where: FAS_{blank} = volume of FAS titrated for blank (ml)

FAS_{sample} = volume of FAS titrated for sample (ml)

V_{sample} = volume of sample in ampule (ml)

The constant 8000 is a conversion factor

The molarity of FAS was obtained from the following:

$$Molarity_{FAS} = \frac{V_{dig}}{V_{FAS}} * 0.10 \qquad \textbf{(3.2)}$$

where: V_{dig} = volume of 0.0167M $K_2Cr_2O_7$ solution titrated (ml)

V_{FAS} = volume of FAS used in titration (ml)

The constant 0.10 is a conversion factor

Standard Methods (1986) lists some possible interferences in the COD test. Silver sulphate, added as catalyst, reacts with halides to produce precipitates that are only partially oxidised. Mercuric sulphate may be added to overcome complications due to the presence of halides, but in this case it was assumed that the presence of halides in the samples was insignificant. Also, interference due to nitrite (NO_2^-) was insignificant.

3.2.3 Total Organic Carbon (TOC)

Total organic carbon (TOC) is the total organic content of the wastewater. The method involves the oxidation of organic molecules to carbon dioxide (CO_2) by persulphate in the presence of ultraviolet light. The CO_2 is measured by a nondispersive infrared analyser producing quantitative results. The samples were pre-filtered and then 40 or 200 µl, depending on the expected TOC concentration, were injected into a DC-80 Total Organic Carbon Analyser (Dohrmann® Division, Rousemount® Analytical Inc.). The detection range for 200 µl is between 10 and 400 mg/l, while for 40 µl it is between 200 and 2000 mg/l. The instrument was calibrated using either a 400 ppm standard (potassium hydrogen phthalate) in the case of 200 µl samples or 2000 ppm standard in the case of the 40 µl samples.

3.2.4 5-Day Biological Oxygen Demand (BOD_5)

The 5-day biological oxygen demand (BOD_5) measures the oxygen utilised via biological degradation during five days of incubation at room temperature. The sample was diluted with dilution water, which was prepared by adding phosphate buffer (pH of 7.2),

magnesium sulphate (22.5g/l), calcium chloride (27.5g/l), and ferric chloride (0.25g/l) reagents at the rate of 1 ml/l of dilution water. These reagents were bought already prepared to the correct molarities for the BOD_5 assay. A nitrification inhibitor (2-chloro-6-(trichloro-methyl) pyridine) was added at the rate of 1 mg/l of dilution water to ensure that only carbonaceous demand was measured. The dilution water was aerated for several hours to allow time for the nitrification inhibitor to dissolve and to ensure that the dilution water was saturated with air. Standard glucose-glutamic acid solution (150 mg of each dried and dissolved in 1000 ml of distilled water) was prepared and stored for up to two months at 2-4°C and warmed to room temperature before use. Seed was prepared by emptying one capsule of Polyseed® (Polybac®) into 500 ml of dilution water and stirring for 1 h prior to use. Polyseed® is a uniform preparation of a range of bacteria responsible for the degradation of municipal and industrial wastes, and it allows for more reproducible measurements of BOD_5 following the procedure outlined in Standard Methods.

Standard Methods (1986) suggests doing several controls to ensure the quality of the dilution water and seed. One such control is the seed control. A seed control was performed by plotting the DO depletion versus volume of seed. This plot should produce a straight line of which the slope is DO depletion per ml of seed and the y-intercept is less than 0.1 mg/l. To do this the DO depletion over five days was measured for six dilutions of 3, 5, 10, 15, 20 and 25 ml of seed. Two blank bottles containing only dilution water were also incubated and the DO depletion was measured. It was usually less than 0.2 mg/l, ensuring high quality dilution water. Another check involved incubating two bottles containing 3 ml of seed and 6 ml of standard glucose-glutamic acid solution. According to Standard Methods (1986), the BOD_5 should be about 204 mg/l.

All samples from the aerator were filtered as described by the TSS method (section 3.2.1) and stored in a freezer until the BOD_5 assay could be performed. Then the samples were thawed and diluted by estimating the BOD_5 and adding the required volume of sample to a standard 300 ml BOD bottle. 3 ml of seed were added and the bottle was filled with dilution water. The initial DO was measured by inserting the DO probe and stirring

mechanism into the BOD bottle and waiting until the reading stabilised before recording the value. Once the initial DO was determined, the bottles were topped off with more dilution water, stoppered and covered with parafilm to create a water seal and prevent evaporation. The samples were incubated in darkness at room temperature for five days before the final DO was measured as before. The BOD_5 was calculated by the following equation:

$$BOD_5 = \frac{(D_1 - D_2) - (B_1 - B_2) * f}{P} \qquad \textbf{(3.3)}$$

where: BOD_5 is expressed in mg/l

D_1 = DO of diluted sample immediately after preparation (mg/l)

D_2 = DO of diluted sample after 5 d incubation at 20°C (mg/l)

P = decimal volumetric fraction of sample used

B_1 = DO of seed control before incubation (mg/l)

B_2 = DO of seed control after incubation (mg/l)

f = ratio of seed in diluted sample to seed in seed control

3.2.5 Specific Oxygen Uptake Rate (SOUR)

The specific oxygen uptake rate (SOUR) is the amount of O_2 used per unit time per unit mass of activated sludge ($mgO_2/min \cdot g$). An estimate of SOUR was obtained by turning off the air in the reactor while maintaining agitation and recording the dissolved oxygen concentration at time zero and every 30 seconds for a total of 4 min. The DO concentration was plotted over time to determine the slope of the best fit line and the SOUR was calculated as the slope divided by the VSS at that time (see Figure 3.1).

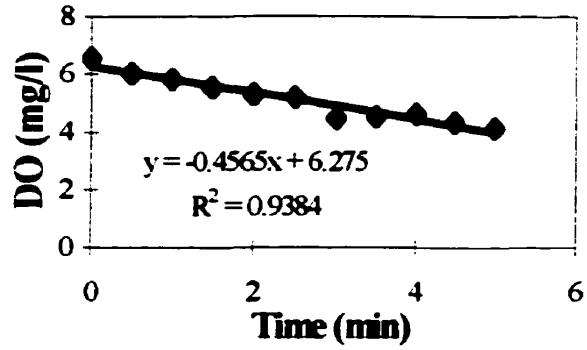

Figure 3.1: Typical Variation of DO with time as used for the Determination of SOUR

3.2.6 Sludge Volume Index (SVI)

Sludge volume index (SVI) is a measure of sludge settleability and is the volume occupied by 1g of sludge after 30 minutes of settling. According to the procedure outlined in Standard Methods (1986), 1 litre of mixed liquor should be used to measure the SVI. However, due to the small size of the aerator, it was impossible to use one full litre of mixed liquor. Since it was necessary to get an idea of settling characteristics of the flock, the test was modified and only 50 ml of mixed liquor from the aerator (or flasks) were used. The level of sludge at various intervals (usually 30 and 60 min.) was recorded and the respective modified SVI was calculated by the following equation:

$$SVI_{30} = \frac{\left(V_{30} / V_{bot}\right) * 1000 mg/g}{TSS_a} \qquad (3.4)$$

where: SVI_{30} = SVI after 30 min. (ml/g)

V_{30} = sludge level after 30 min. (ml)

V_{bot} = volume of the sample bottle (i.e. 0.05 L)

TSS_a = TSS in the aerator (mg/l)

3.2.7 pH, Temperature, Dissolved Oxygen (DO), and Conductivity

pH was measured using a Fisher Accumet® pH meter calibrated using a 2-point or 3-point calibration. Temperature was measured by the DO probe at the same time as DO. DO was measured using an Orion DO probe and meter calibrated in air saturated with water at room temperature with a correction for atmospheric pressure. Conductivity was measured using a Cole-Parmer conductivity meter.

3.2.8 Biolog Microplate Technique and Analysis

A. Microplate Preparation

The Biolog technology relies on the irreversible reduction of a tetrazolium dye as an indicator of carbon-source utilisation. There are several types of Biolog microplates that may be used depending on the type of organism to be tested. Since activated sludge consists mainly of gram-negative species (Gray, 1990), GN microplates that are specific to gram-negative species were used. A GN microplate panel is comprised of 95 substrate containing wells and a control well without a carbon source. A graphical representation of a GN microplate is given in Figure 3.2. Each well is also supplied with dye and nutrients in a dried-film form, which is reconstituted upon inoculation of the cell suspension. If the cells utilise the substrate, the tetrazolium redox dye turns purple. Patterns of metabolic response for a community are produced, and these patterns can be read using imaging software and then analysed statistically to determine the community response to the treatment. This is described below.

The microplates were stored at 2-4°C and warmed to room temperature prior to use. Fresh samples of mixed liquor were collected from either shake flasks or the reactor and deflocculating agents, sodium pyrophosphate and Tween 80, were each added to the sample at a concentration of 0.01% v/v. The samples were homogenised by vigorous shaking and any remaining solids were removed by centrifugation at 5000 rpm for five minutes. The recovered suspensions were washed three times with 0.1 M phosphate buffer by centrifugation at 10 000 rpm for ten minutes and then resuspended in about 2 ml of 0.85% saline. This method was adapted from that described by Victorio et al (1996).

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

Figure 3.2: Biolog GN MicroPlate™ Panel

The turbidity range was established by using turbidity standards provided by Biolog and an uninoculated saline tube to set the 100% transmittance. The turbidimeter was re-set to 100% transmittance with the same uninoculated saline tube (this should be done for each tube) and the cell suspension was slowly added until the inoculum density fell within the acceptable turbidity range. The approximate cell density was adjusted to $3x10^8$ cells/ml and this gave a transmittance level of about 53% to 58%. All microwells were filled with precisely 150 µl of suspension, taking caution not to splash or carry over any chemicals. The plates were incubated in a closed box at room temperature for 48 h. After 48 h, pictures of the microplates were taken using either a low-resolution Kodac DC40 'point-and-shoot' digital camera or, in later experiments, a Sony Hi-Resolution CCD-IRIS monochrome digital camera interfaced to a computer.

B. Image Processing

Initial images were obtained using a hand held, low-resolution camera. An attempt was made to hold the camera at the same height each time; however, the distance between the camera lens and the microplate may have varied from one image to the next. Also, the lighting was not as consistent since a fibre optic light source was not yet available. The pictures were all taken in the same room with the blinds drawn in an attempt to minimise inconsistencies in the lighting; however, there were some problems with glare and reflection. In order to avoid this lack of precision, a more standardised procedure using the Hi-Resolution camera was developed. The camera was mounted on a stand at a constant distance from the microplate. The microplate itself was placed in a box with white walls and a lid to prevent outside light from entering and creating glare and reflection. A hole was cut into the lid to accommodate the camera lens and two fibre optic light sources were directed through the hole and aimed at the walls inside the box to produce consistent lighting.

The data were analysed with the aid of the Visilog 5.1 image analysis software. A typical image is represented in Figure 3.3. Colour intensity in each well was determined using a macro that computed the average pixel value of a 5x5 matrix of pixels taken from the centre of each well (shown as small squares in the figure). These values were pretreated

following the procedure outlined in a paper by Garland and Mills (1991). This involved computing the raw difference data by subtracting the colour intensity for the response well from the colour intensity for the control well (C-R; where C is A1 in Figure 3.2 and R is each response well). Then each value was divided by the average well colour development (AWCD) to normalise the data: AWCD = Σ(C-R)/95. This data, referred to as the transformed data, was then statistically analysed using principal component analysis (PCA).

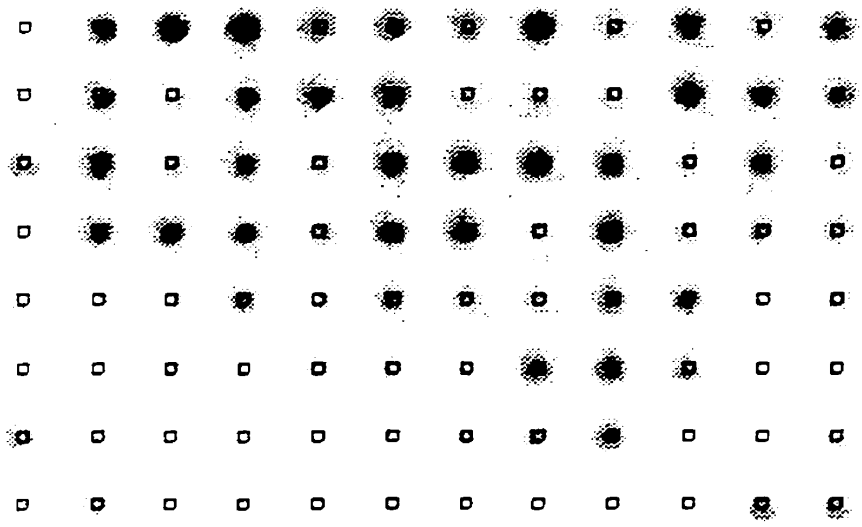

Figure 3.3: Typical Biolog Microplate Image Taken using the Hi-Resolution Digital Camera with an Overlay Showing the 5x5 Pixel Matrices at the Centre of Each Well.

C. Statistical Analysis

PCA is a multivariate method that essentially rotates a swarm of data points about their centroid to reveal any intrinsic patterns. SIMCA-P statistical software was used to develop the PCA models. It follows the nonlinear iterative partial least squares (NIPALS) method of calculating the principal components. The method consists of rewriting a data matrix as a sum of linearly independent matrices. These can in turn be expressed as products of two vectors, a score (column) vector, t and a loading (row) vector, p^T. Once the vectors, t and p^T, are found they can be plotted two dimensionally to

reveal any intrinsic patterns present in the data. A plot of the score vectors, t_1 and t_2, is usually all that is required to demonstrate differences among plates. Scores are vectors that contain the greatest variation that can be explained among the data. Score plots reveal trends, groups, and outliers (observations that lie beyond the region within the Hotelling t^2 confidence ellipse). Loading vectors indicate which of the variables are important in the approximation of the data matrix. Loading vectors may also be plotted; however, these figures are difficult to read and it is more effective to simply list the important variables.

A typical plot of the scores of the first two principal components (PC's) is given in Figure 3.4. This figure should be read in the following manner. The x-axis labelled t[1] is the scores vector for the first PC while the y-axis labelled t[2] is the scores vector for the second PC. The points in the figure are labelled according to experiment (by capital letters) and time the sample for the microplate was taken. In this particular figure, the letter 'G' happens to represent a 5% black liquor step experiment. The times are with respect to the addition of black liquor. Therefore, '0h bef' signifies a microplate done at steady state just before the addition of black liquor, while '0h aft' signifies a microplate done immediately after the black liquor step was initiated. The points denoted by '14 h' and '65 h' represent plates done at 14 and 65 h after the step. These times may vary slightly for different experiments. Finally, the point denoted by 'SS new' represents a plate done when the system reached a new steady state in the presence of black liquor. In this particular figure, the points are all slightly apart from one another indicating that the plates were changing over time. It should be noted that each figure represents the scores for each set of plates in a particular model and separate models were developed for individual experiments as well as for certain plates from several experiments in order to compare them.

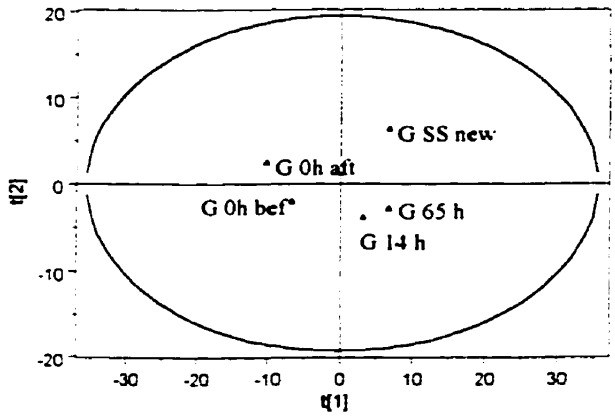

Figure 3.4: Typical Scores Plot for the First Two Principal Components

For the hydraulic residence time experiments, slightly different notation was used to represent points for individual plates. The experiments are still distinguished by using different capital letters; however, in these experiments the samples for the microplates were taken on different days and from different locations. A typical point will be denoted by say 'B6s', where the 'B' represents that particular experiment, the number '6' represents the date the plate was done, and the letter 's' represents the location from which the sample was taken, in this case, the refrigerated sludge. The letters 'a' and 'c' represent samples taken from the aerator and clarifier respectively.

For the analysis, the data matrix was set up by placing the intensity values for each well into a single row. Therefore, rows represent individual plates while columns represent the colour intensity in each microwell. In this way, differences among the plates could be determined. However, PCA cannot test for specific differences. More details on the setup of the data matrix and the pretreatment of the data, as well as a mathematical description of the PCA model, are given in Appendix B.

In order to determine the variation of the PCA scores, a variability study was performed by two different researchers who each prepared several microplates inoculated with the

same sample of sludge. The variance of the readings for each of the 95 wells in the variability studies was very small indicating that the Biolog assay is very reproducible. The variance for selected wells in one of the variability studies is much smaller than the variance for the same wells in a series of plates done during an experiment (see Table 3.1) and the ratio of experimental variances to replicate variances was greater than two for 97% of the microwells.

Table 3.1: Comparison of Experimental and Replicate Variances for Selected Wells from the Microplate

Well	Variance		Ratio
#	Experiment	replicates	exp/rep
A2	0.0812	0.0067	12.14
A3	0.3183	0.0025	126.96
A4	0.3267	0.0013	255.77
A5	0.2323	0.0300	7.73
A6	0.1721	0.0005	326.07
A7	0.2762	0.0461	6.00
A8	0.2408	0.0003	796.20
A9	1.0180	0.0696	14.63
A10	0.3705	0.0048	77.56
A11	1.3690	0.0361	37.92
A12	0.0466	0.1496	0.31

3.3 EXPERIMENTAL PROCEDURES

3.3.1 Shake Flask Experiments

Shake flask experiments were carried out using 500 ml Erlenmeyer flasks on a rotary shaker operating at about 180-200 rpm. The flasks were incubated for 48 h at room temperature (about 21-24°C) and samples were taken at various time intervals (usually 0, 24 and 48 h) to measure the TSS, COD and TOC. In preparation of the mixed liquor for the flasks, it was necessary to first determine the TSS in the activated sludge. Once the TSS of the sludge was known, the sludge to wastewater ratio was computed to get the desired initial TSS concentration for the experiment. The wastewater consisted of either 100% TME or any combination of effluents in known ratios required for that particular

experiment. Before adding the sludge, nutrients were added to the wastewater as described above (see Appendix A for sample calculations).

Once the nutrients and sludge were mixed with the wastewater (referred to as a batch), about 100 ml of each batch were poured into one of several flasks and placed on the rotary shaker; duplicate flasks were prepared for each batch. Initial samples of about 10 ml each were filtered in duplicate immediately upon mixing the batches for determination of actual initial TSS, COD and TOC. TSS, COD and TOC were determined at prescribed time intervals.

3.3.2 Reactor Experiments

A 2 litre Multi-Gen lab top continuous reactor with recycle (via a clarifier) was used in the experiments (see Figure 3.5). The working volume of the reactor was 1.4 litres and aeration and agitation were employed. The working volume of the clarifier was 1 litre.

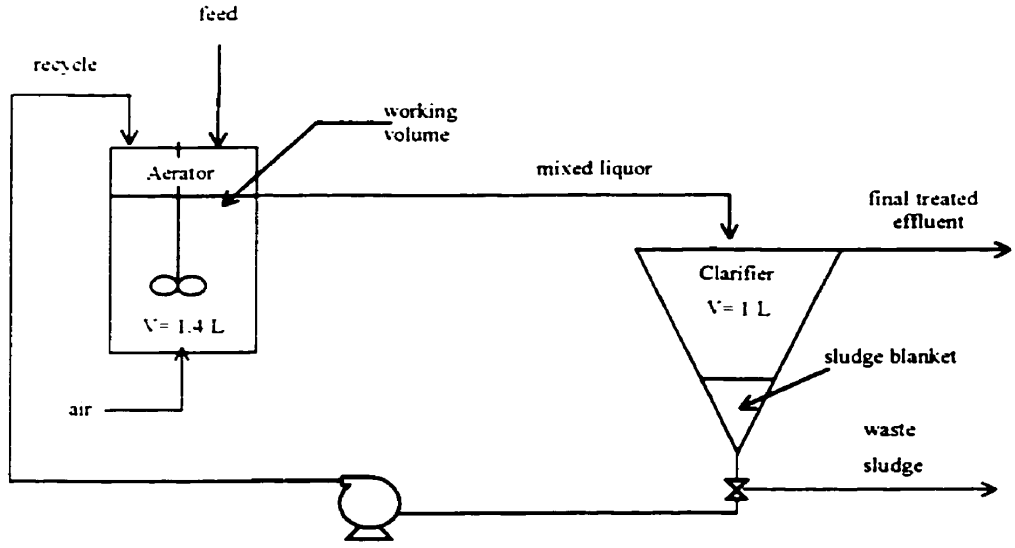

Figure 3.5: Schematic of the Lab Top Continuous Reactor

a) Batch Period (or Acclimation Period)

To allow for the acclimation of the sludge, the reactor was started as a batch reactor and run for about 16 h. This time was determined by Milin (1996) as the time required for the

cells to reach the endogenous phase. At the same time, six shake flasks of 200 ml each, containing the same desired initial solids concentration as in the reactor, were prepared and placed on a rotary shaker at about 200 rpm for about 16 h (overnight). These flasks were used to start up the clarifier.

As in the flask experiments, it was necessary to determine the TSS of the sludge in order to determine the amounts of sludge and effluent to combine to get the desired sludge inoculum concentration. A batch of effluent was prepared, as described for the shake flask experiments, by first adding nutrients to the wastewater and then adding Antifoam A® (Dow Corning, Inc.) at the rate of 100-200 µl per 2000 ml of effluent (more antifoam had to be added when black liquor was to be used). The effluent was poured into the reactor and the required amount of sludge was added to bring the final working volume to 1.4 litres. Agitation and air were applied and a few minutes were allowed to pass for complete mixing before a sample was taken from the aerator and filtered in duplicate as a time zero measurement. The filtrate was collected for measurement of COD and TOC and the filter paper holding the solids was used to determine TSS and VSS.

b) Continuous Phase

The continuous phase started just as the sludge entered the endogenous phase. The pump speed was adjusted until the desired flow rate was achieved. The continuous phase began after approximately 16 h of acclimation (batch phase). After about 14-16 h on the shaker, the flasks were removed and poured into the clarifier and enough time was allowed for the sludge to settle (about 1 h). Another sample was taken just after the continuous phase began.

Sampling frequency sometimes varied due to experimental requirements, but generally sampling was done as described here. The aerator was sampled 2-3 times per day for TSS, COD, and TOC and once per day for VSS. The clarifier was sampled once per day for TSS, COD, and TOC. The influent and effluent were sampled once every other day for TSS, COD, TOC and BOD_5. pH, temperature, DO, SOUR, and SVI were usually measured once a day and later, with the addition of black liquor, conductivity was also

measured once a day. The feed flow rate was checked once per day to make sure it was still at the desired value as the influent tube gradually got coated by fine fibres in the feed thus reducing the feed flow rate. It was necessary to frequently change the feed tube.

Sludge age was adjusted to the desired value by wasting from the clarifier once per day based on the previous day's TSS in the aerator and clarifier with the following equation:

$$WSF = \frac{V_a * TSS_a}{\theta * TSS_c} \qquad (3.5)$$

where: WSF = waste sludge flow rate in ml/day

V_a = the aerator working volume in ml

θ = sludge age in days

subscript a = aerator

subscript c = clarifier

The Biolog microplate analysis was performed when the reactor reached steady state and whenever a significant change in the population may have occurred (i.e. when adding black liquor).

c) Black Liquor Step Procedure

To determine the effect of black liquor on the activated sludge system a series of black liquor step experiments were performed with concentrations of 1, 2.5 and 5% black liquor in TME. The reactor was run as above with TME until the system reached steady state (usually 2-4 weeks depending upon initial sludge concentration). Then black liquor, containing the appropriate amount of nutrients, was added to the aerator and clarifier by replacing the mixed liquor in the aerator, or sludge in the clarifier, with an equal volume of black liquor. At the same time, the feed was brought to the desired black liquor concentration.

Just prior to and just after the step input the system was sampled as usual (to determine COD, TOC, BOD_5, TSS and VSS at various locations) and samples were also collected from the aerator for the Biolog technique and put aside. Antifoam A® was added to the aerator as necessary to control foaming. Microplates were also done at about one HRT after the step, two or three days after, and at the new steady state. The sampling frequency was the same as in the HRT experiments.

4. RESULTS AND DISCUSSION FOR EXPERIMENTS USING CTMP MILL EFFLUENT

In this chapter, the experimental results and discussion for the treatment of effluents from a CTMP mill in Quebec will be presented. Initially, preliminary experiments were executed using shake flasks with total mill effluent. The results and discussion for these experiments are given in section 4.1. Then shake flask experiments with constituent effluents from the CTMP mill were performed. These results are given in section 4.2 and the experiments are compared in section 4.3. Finally, preliminary experiments were performed using a CSTR. These results are provided in section 4.4 and the discussion follows in section 4.5. Further continuous reactor experiments were not carried out because the effluent was no longer available.

4.1 PRELIMINARY EXPERIMENTS

The objective of the preliminary experiments was to determine the effects of initial sludge concentration, nutrient addition, and changes in the quality of the untreated wastewater. These experiments were carried out using shake flasks. Also, experiments were performed to determine the variability of the total suspended solids (TSS) and chemical oxygen demand (COD).

4.1.1 Sludge Inoculum Experiments

Initially, two experiments were performed in shake flasks using different nominal sludge inoculum concentrations. In these experiments, total mill effluent (TME) from a CTMP mill was examined to test the effect of various sludge concentrations on the reduction of chemical oxygen demand (COD). The COD:N:P was 100:3.0:1.0, as described in literature (Franta et al, 1994; Klopping et al 1995; Gray, 1990; Grau, 1991). The flasks were placed on a rotary shaker for 48 h at 21-24°C with an approximate speed of 185 rpm. The results for different sludge concentrations are shown in Table 4.1.

Relatively high percent reduction in COD was achieved, regardless of the sludge inoculum concentration. The COD was generally reduced from initial concentrations of 750-1000 mg/l to final concentrations of 250-300 mg/l. Based on this observation, a nominal sludge inoculum concentration of 2000 mg/l was chosen for subsequent experiments to reflect industrial conditions. The growth of the sludge was less than 35%. A higher growth rate was not expected since initial inoculum of sludge was high and the effluent, limited by the substrate concentration, could not support further growth. The effect of time on COD reduction was also examined and it was noted that most of the reduction in COD occurred within the first 24 h, after which there was no decrease. Similarly, the sludge concentration did not change significantly between 24 and 48 h.

Table 4.1: Various Sludge Inoculum Concentrations in Total Mill Effluent from the CTMP Mill (time = 48 h)

Inoculum Conc. (mg/l)	% COD Reduction
500	73
1000	69
2000	71
3000	66

4.1.2 Nutrient Experiments

In order to determine the appropriate amount of nutrients to add to the wastewater from the CTMP mill, an experiment was executed where nutrients were added on the basis of effluent COD by the ratio COD:N:P. In the first part of the experiment, the nitrogen concentration was varied while the phosphorus concentration was held constant and in the second part, the nitrogen was held constant while the phosphorus concentration was varied. The experiments were performed using shake flasks inoculated with 500 and 2000 mg/l of sludge using TME from the CTMP mill and incubated on a rotary shaker at room temperature for 48 h. The objective of this test was to determine the best COD:N:P. Results are given in Table 4.2 and Table 4.3.

The best COD:N ratio appeared to be 100:3 for both inoculum concentrations because it achieved the greatest percent growth and reduction in COD. The best COD:P ratio with respect to the reduction in COD appeared to be only 100:0.2 for an inoculum concentration of 500 mg/l while it was 100:2 for an inoculum concentration of 2000 mg/l. This ratio was higher than expected which suggests that for the system in question, the industrial ratio is too low with respect to the amount of phosphorus. Also, greater growth occurs with a ratio of 100:2 and an inoculum concentration of 2000 mg/l. In general, since most subsequent experiments used the inoculum concentration of 2000 mg/l, the best COD:N:P ratio for CTMP mill effluent was deemed to be 100:3:2.

Table 4.2: Nutrient Experimental Results for CTMP Total Mill Effluent (time = 48 h) - Varying the Nitrogen Concentration

Nutrient	Inoculum Conc. (mg/l)		Inoculum Conc. (mg/l)		Inoculum Conc. (mg/l)	
Ratio	500	2000	500	2000	500	2000
COD:N (P=1)	% Growth		% Reduction in COD		ΔCOD/TSS	
100:2	28	32	77	59	0.46	0.07
100:3	51	35	88	78	0.50	0.08
100:4	41	29	81		0.33	0.04

Table 4.3: Nutrient Experimental Results for CTMP Total Mill Effluent (time = 48 h) - Varying the Phosphorus Concentration

Nutrient	Inoculum Conc. (mg/l)		Inoculum Conc. (mg/l)		Inoculum Conc. (mg/l)	
Ratio	500	2000	500	2000	500	2000
COD:P (N=3)	% Growth		% Reduction in COD		ΔCOD/TSS	
100:0.2	40	25	58	51	1.4	0.34
100:0.5		14	52		1.0	
100:1	44	13	57	57	1.1	0.39
100:2	45	30	55	60	1.1	0.40

4.1.3 Variability Experiments

To determine the variability in the shake flask procedure, an experiment was completed using ten flasks. The nominal inoculum concentration was 2000 mg/l and a COD:N:P ratio of 100:3.0:1.0 was used as suggested in literature. The exercise proved that the values were within a reasonable range of each other. The average reduction in COD was 67% with a standard deviation of 1.63. The average growth was 10% with a standard deviation of 3.20. Not much growth was expected since the nominal sludge concentration was high. Results are shown in Table 4.4.

Table 4.4: Shake Flask Variability Results for CTMP Total Mill Effluent - 10 Flasks

Flask #		TSS (mg/l)	% Growth	COD (mg/l)	% Reduction	ΔCOD TSS
(t=0 h)	1	1990	0	1526	0	0.00
	2	1930	0	1491	0	0.00
(t=48 h)	3	2060	5	523	65	0.48
	4	2180	11	506	66	0.46
	5	2100	7	489	68	0.49
	6	2220	13	489	68	0.46
	7	2170	11	454	70	0.49
	8	2200	12	489	68	0.46
	9	2170	11	506	66	0.46
	10	2210	13	471	69	0.47
	11	2200	12	506	66	0.46
	12	2050	5	540	64	0.47
Mean		**2156**	**10**	**497**	**67**	**0.47**
Std. Dev.		**63**	**3**	**25**	**2**	**0.01**

4.1.4 Discussion For Preliminary Experiments

Preliminary experiments indicate that most of the substrate removal occurred within 24 h of treatment. Leaving the flasks for 48 h only improved the COD reduction by a small fraction and did not lead to further growth of the sludge; in fact, it often led to a reduction in the biomass concentration. It appears that the sludge entered the endogenous phase

after 24 h and may have begun to lyse once the substrate was depleted. Moreover, in these experiments the initial concentration of sludge was quite high thus there was not much opportunity for additional growth due to the lack of sufficient carbon source.

The nutrient experiments indicated that the optimal ratio should be COD:N:P of 100:3:2. Nutrient addition displayed a dependence upon the initial biomass concentration in the system. More sludge required more phosphorus and nitrogen.

4.2 SHAKE FLASK EXPERIMENTS FOR COMBINATIONS OF INDIVIDUAL STREAMS WITH TOTAL MILL EFFLUENT

The total mill effluent from the CTMP mill is mainly composed of six separate effluent streams derived from the following processes: paper machine (PM), ultra high yield (UHY), wood washing (WW), screen room (SR), acid plant (AP), and ground wood (GW). The streams are combined into one total mill effluent stream (TME) which is sent on to the treatment plant. The objective of these experiments was to determine which of the effluents in question had the greatest effect on the reduction of COD by the activated sludge.

The individual streams were combined with TME in concentrations of 0, 5, 10, 20, 40, 60, 80, and 100% by volume and placed in shake flasks on a rotary shaker at about 180-200 rpm for 48 h at room temperature. Concentrations greater than 60% for each individual stream are seldom or never realised on an industrial scale; however, these concentrations were examined in the experiments to investigate the effect of each undiluted stream. In each of the following experiments, a high nominal inoculum concentration of 2000 mg/l was used since the growth rate of the organisms was not of primary interest in these experiments. The COD:N:P was 100:3.0:2.0 as determined in the nutrient experiments. The results presented in the subsequent figures include the COD, percent reduction in COD, TSS, percent growth in TSS and the COD utilised by TSS at the experimental time of 24 or 48 h.

4.2.1 Paper Machine Effluent (PM)

The paper machine effluent (PM) had a COD of 690 mg/l, which was lower than the TME at 2300 mg/l; therefore, when the TME was diluted with the PM, the COD decreased as shown in Figure 4.1a. The percent reduction in COD varied between 60% and 80% for both 24 and 48 h as shown in Figure 4.1b with no significant improvement in the reduction of COD over time. Figure 4.1c and Figure 4.1d indicate that as the percentage of PM increased the total suspended solids (TSS) decreased from 2200 mg/l to 1900 mg/l and percent growth decreased from 22% to about 8%. This suggests that the paper machine effluent had some toxic effects on the sludge. From Figure 4.1e, it is evident that even low concentrations of PM had a detrimental effect on the specific reduction of COD (ΔCOD/TSS). The specific reduction of COD dropped from 0.74 to 0.40 with a concentration of only 5% PM. This may be due to toxic effects of the PM on the biomass.

4.2.2 Ultra High Yield Effluent (UHY)

The ultra high yield effluent (UHY) had a higher COD (about 2850 mg/l) than the TME (about 1630 mg/l); therefore, when the UHY was added to the TME, the COD increased as shown in Figure 4.2a. As the concentration of UHY increased the percent reduction in COD, TSS and percent growth all generally decreased as illustrated in Figure 4.2b, c and d. Most notably there was no growth within the first 24 hours when UHY was greater then 20%. After 48 h, however, only concentrations of 80% and 100% prevented growth. This suggests that the ultra high yield effluent may have had initial adverse effects on the sludge, but that the sludge adapted over time. The results indicate that a longer reaction time of 48 h allowed the system to remove slightly more COD. Also, the specific reduction in COD (ΔCOD/TSS) decreased from about 0.65 to 0.30 at concentrations of UHY greater than 40% as shown in Figure 4.2e. This may further suggest that the UHY adversely affected the biomass.

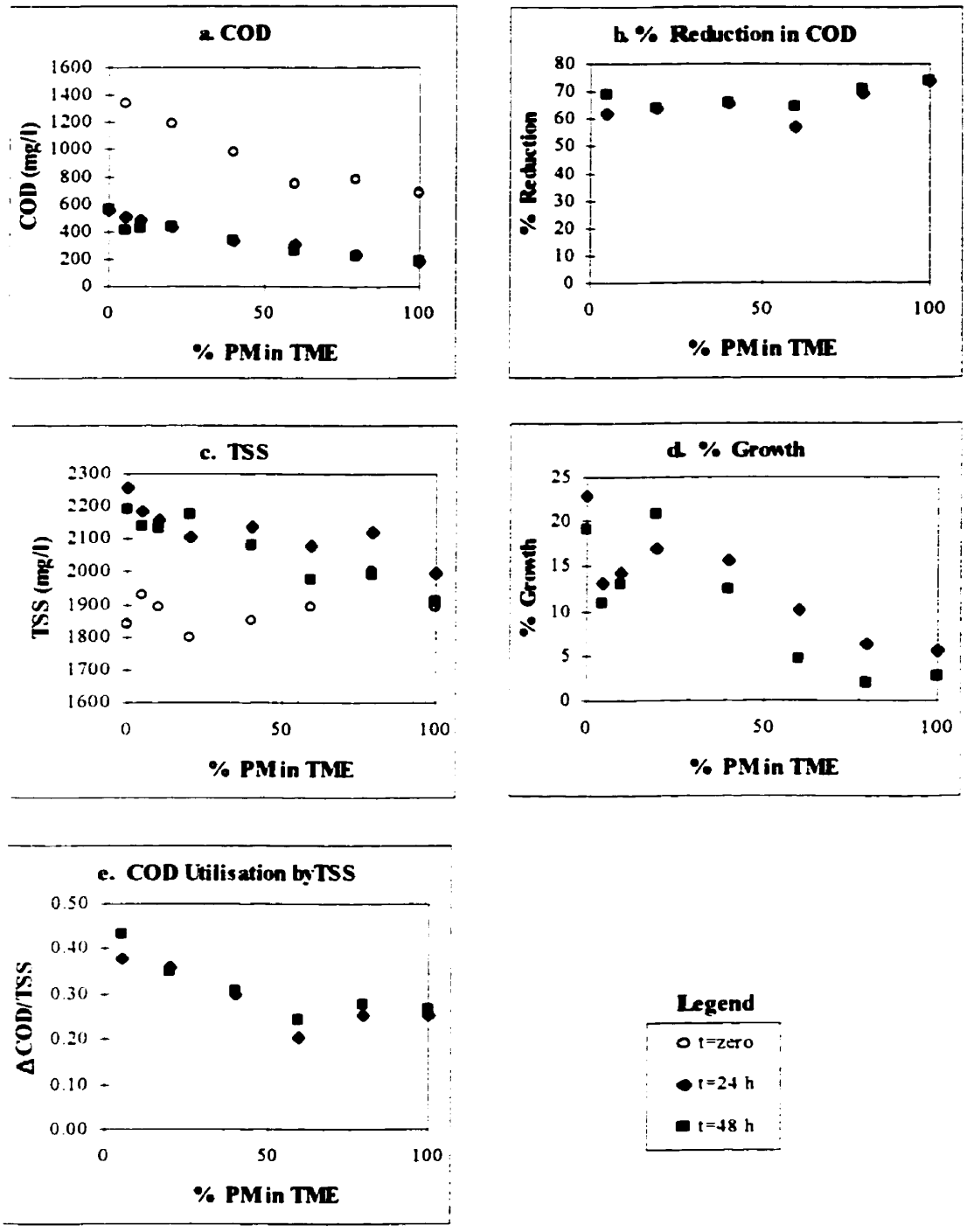

Figure 4.1a, b, c, d and e: Effect of Varying Concentrations of Paper Machine Effluent in Total Mill Effluent from a CTMP mill on Various System Descriptors

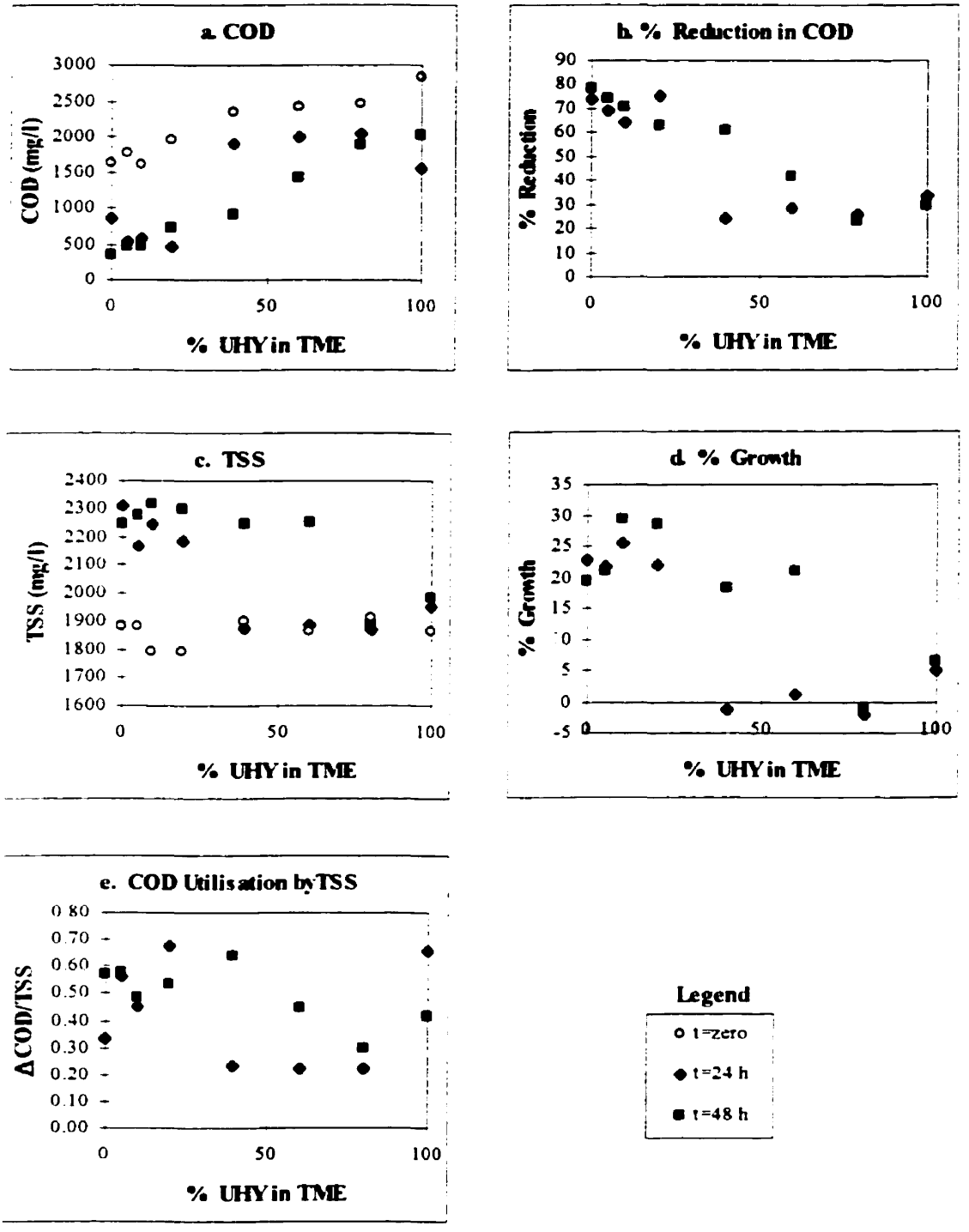

Figure 4.2 a, b, c, d and e: Effect of Varying Concentrations of Ultra High Yield Effluent in Total Mill Effluent from a CTMP mill on Various System Descriptors

4.2.3 Wood Washing Effluent (WW)

The wood washing effluent (WW) had a lower COD (250 mg/l) than the TME (950 mg/l); therefore, when the TME was diluted with the WW, the COD decreased as shown in Figure 4.3a. This experiment was run for 72 h due to an emergency shutdown of McGill University for one day. This was beneficial in that it proved that longer reaction times still did not improve greater reduction in COD. As demonstrated in Figure 4.3b, c, and d, the percent reduction in COD decreased from 65 to 25% with increasing amounts of wood washing effluent as did the TSS and the percent growth. Figure 4.3d indicates that the percent growth of the sludge actually decreased after 72 h. This could be due to cell lysis as the microbes ran out of substrate. Looking at Figure 4.3e, which shows the specific reduction in COD (ΔCOD/TSS), it seems that greater concentrations of WW were detrimental to the specific reduction of COD causing it to decrease from 0.3 to 0.03. Furthermore, no improvement was achieved with the longer reaction time of 72 h as demonstrated in the figures.

4.2.4 Screen Room Effluent (SR)

The COD for the TME and the SR were both in the range of 1100-1200 mg/l. Therefore, as can be seen in Figure 4.4a, the COD did not vary significantly with increasing amounts of screen room effluent. Likewise, the reductions for the mixtures were in the same range of 70 to 80% as shown in Figure 4.4b, and the TSS were generally in the same range of 2200 to 2350 mg/l as shown in Figure 4.4c. The percent growth in each mixture was in the same range of 15 to 20% at concentrations of 5% SR or greater. The specific reduction in COD (ΔCOD/TSS) is illustrated in Figure 4.4e and the data remain fairly constant with increasing concentrations of SR. Therefore, the SR had little effect on the reduction of COD or on the biomass. In all the figures, the 48 h trends were generally slightly less in magnitude than the 24 h trends.

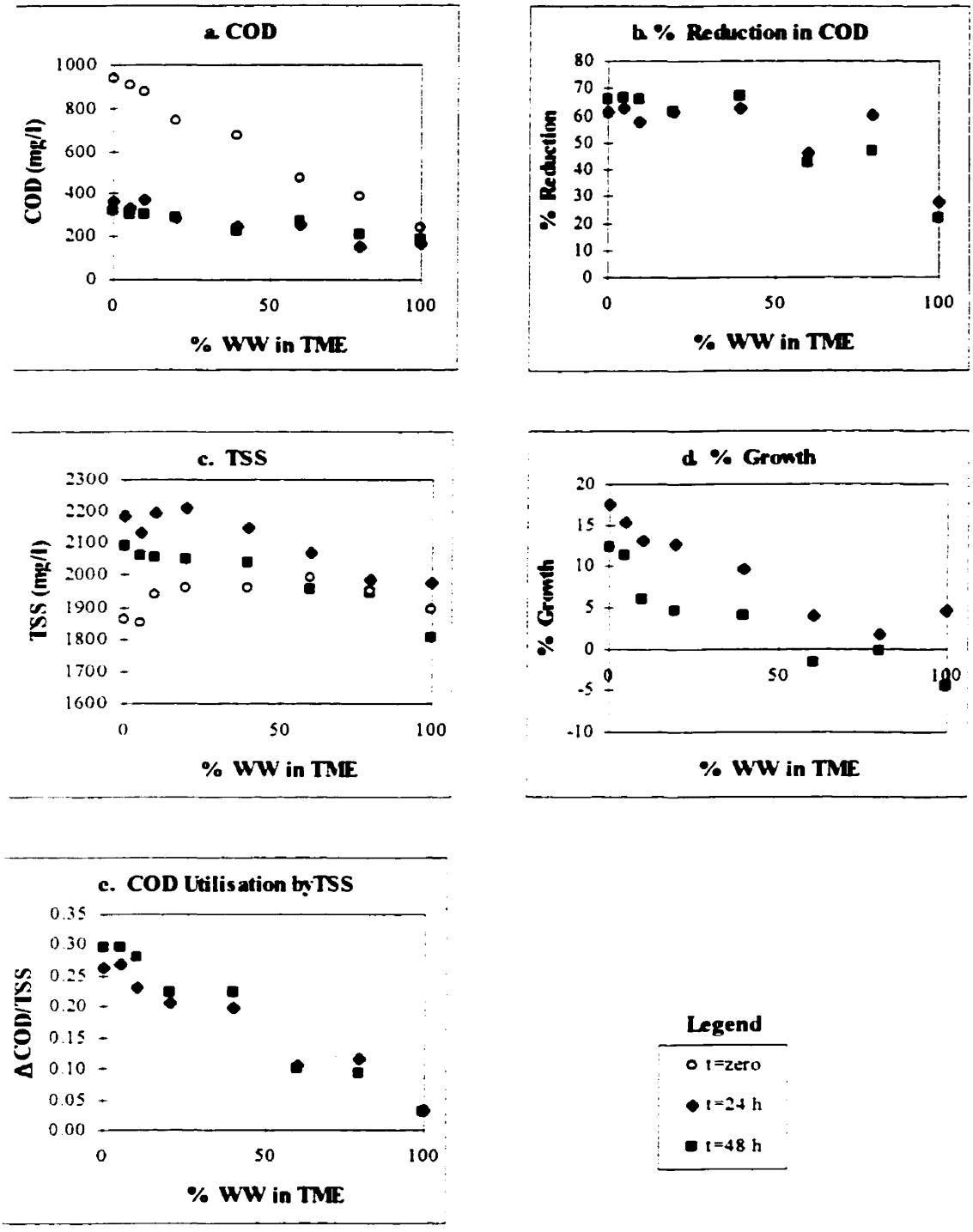

Figure 4.3a, b, c, d and e: Effect of Varying Concentrations of Wood Washing Effluent in Total Mill Effluent from a CTMP mill on Various System Descriptors

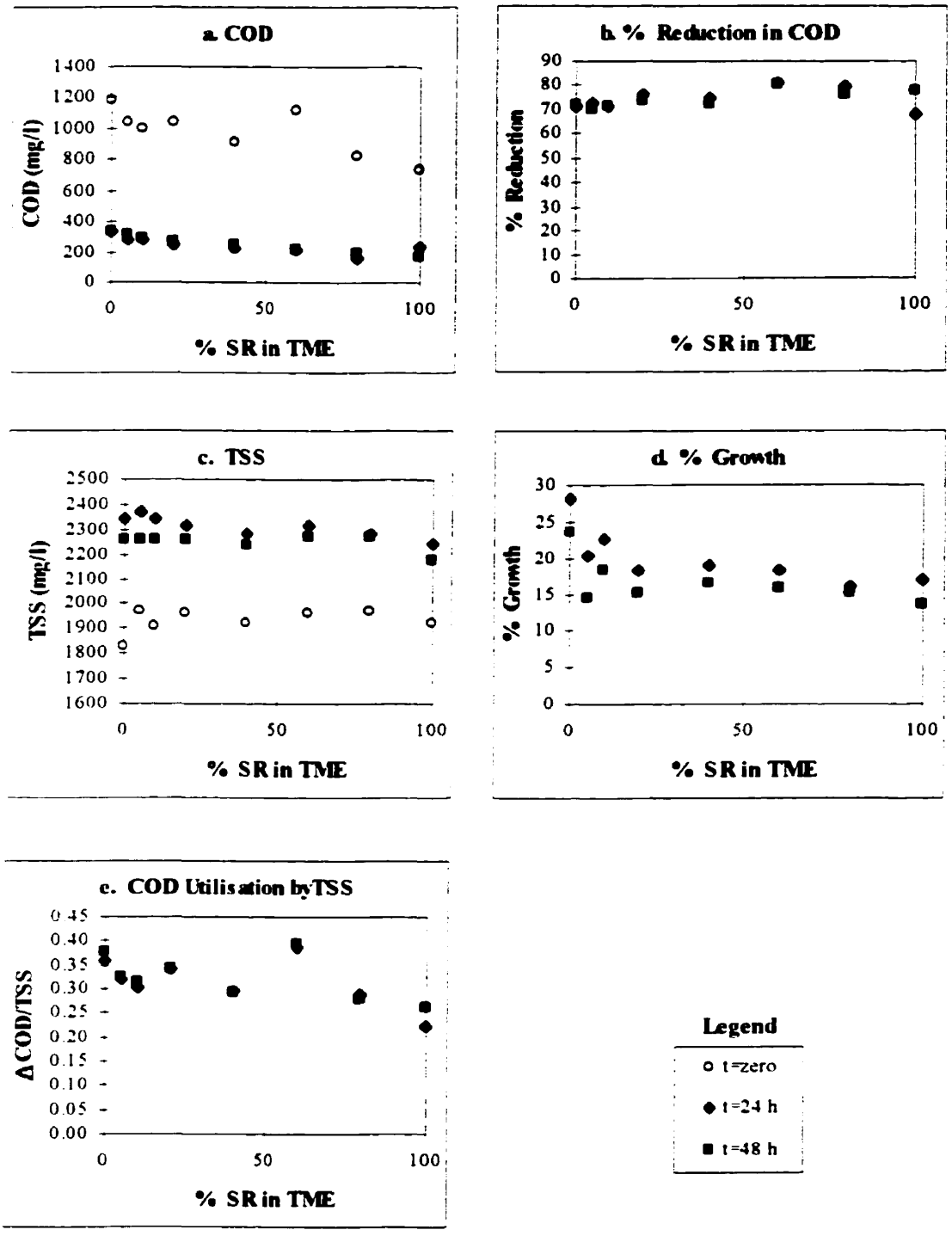

Figure 4.4 a, b, c, d and e: Effect of Varying Concentrations of Screen Room Effluent in Total Mill Effluent from a CTMP mill on Various System Descriptors

4.2.5 Acid Plant Effluent (AP)

The acid plant effluent (AP) had a very low COD of approximately 50-60 mg/l thus the COD decreased with increasing amounts of AP as shown in Figure 4.5a. The 24 and 48 h COD are in the same range, regardless of AP concentration. This can be explained by Figure 4.5b where the reduction in COD decreases from 75% to zero for higher concentrations of AP. This is due to the fact that at high concentrations of AP there was very little organic matter for the sludge to consume. Figure 4.5c it can be seen that the TSS decreased from 2150-1850 mg/l with increasing amounts of AP. This could be due to toxicity of the AP on the cells, but may also be explained by the very low COD of the AP. This effect is further evident in Figure 4.5d where the percent growth drops from 20% to zero with increasing amounts of AP. Figure 4.5e shows that the specific reduction in COD (ΔCOD/TSS) decreased from 0.42 to zero with increasing concentrations of AP. These observations indicate that the acid plant effluent provided very little organic matter for sludge consumption and may also have been toxic to the sludge.

4.3 COMPARISON OF THE VARIOUS EFFLUENTS

Several of the constituent effluents from the CTMP mill exhibited adverse effects on the biomass. These include the paper machine effluent (PM), the ultra high yield effluent (UHY), the wood washing liquid (WW), and the acid plant effluent (AP). The screen room effluent did not exhibit any adverse effects.

In most cases, higher concentrations of the effluent (usually greater than 40%) inhibited growth (Figures 4.1, 4.3 and 4.5c and d). With increasing concentrations of the ultra high yield effluent, however, the growth rate simply slowed down (Figure 4.2c and d). In the mill, the UHY accounts for almost half the TME; thus the sludge was likely acclimated to it at lower concentrations. In the case of the wood washing and acid plant effluents, the COD concentrations were quite low (Figure 4.3a and Figure 4.5a). Therefore, the sludge may simply have run out of substrate rather than experiencing adverse conditions. The screen room effluent had no effect on the biomass (Figure 4.4c and d).

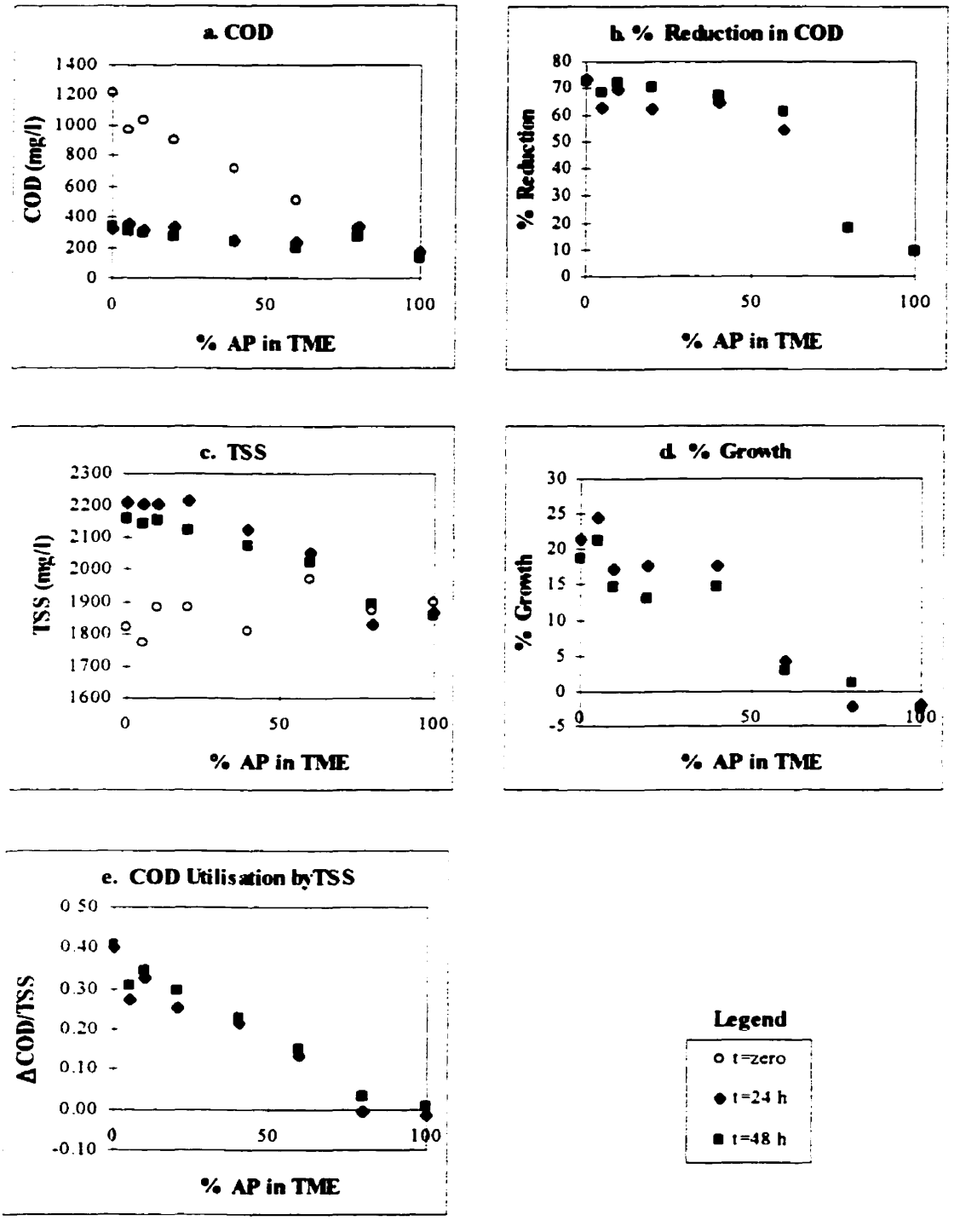

Figure 4.5 a, b, c, d and e: Effect of Varying Concentrations of Acid Plant Effluent in Total Mill Effluent from a CTMP mill on Various System Descriptors

Increasing concentrations of ultra high yield effluent caused a delay in the reduction of COD (Figure 4.2b) and had a similar effect on the specific COD utilisation (Figure 4.2e). This delay is likely due to the fact that the UHY accounts for such a large portion of the TME as stated above. Higher concentrations of wood washing and acid plant effluent led to lower COD reductions (Figure 4.3b and Figure 4.5b). This is due to a lack of substrate since both effluents had very low COD's. However, in both cases, the specific utilisation of COD decreased with an increase in effluent concentration (Figure 4.3e and Figure 4.5e) suggesting that the reduction of COD was effected by growth. The paper machine effluent and the screen room effluent had no effect on the COD (Figures 4.1 and 4.4a and b). Also, the screen room effluent had no effect on the specific COD utilisation (Figure 4.4e) while for the paper machine effluent it decreased slightly with increasing amounts of effluent (Figure 4.1e).

4.4 PRELIMINARY REACTOR EXPERIMENTS

Having determined which streams had the greatest effect on the reduction of COD, continuous reactor experiments were planned using only the most significant streams. However, before we got to this point, effluents from the CTMP mill in Quebec were no longer available. Therefore, only two preliminary reactor experiments were completed and the results for these are also given below. In these, TME was used alone. The COD:N:P ratio was 100:3.0:1.0. In the first experiment the reactor was operated continuously without sludge recycle and in the second experiment, recycle was added to prevent washout of the sludge.

4.4.1 CSTR using Total Mill Effluent without Recycle

This experiment used a hydraulic residence time (HRT) of 28 h and a high nominal sludge inoculum concentration of 6000 mg/l. The HRT and initial TSS were much higher than any studied by Milin (1996). However, as shown in Figure 4.6a, the system tended towards washout since the solids concentration showed a continuous decline. Also, the system only achieved a 50% reduction in the COD (from 1300 mg/l to 600 mg/l) as shown in Figure 4.6b. The temperature remained relatively constant at about 20-22°C,

but the pH in the aerator decreased from about 8.0 to 5.8. The dissolved oxygen concentration (DO) generally remained above 6 mg/l while the specific oxygen uptake rate (SOUR) based on TSS rose from 4.3 to 14.5 $mgO_2/gTSS \cdot h$. This increase can be explained by the reduction in biomass.

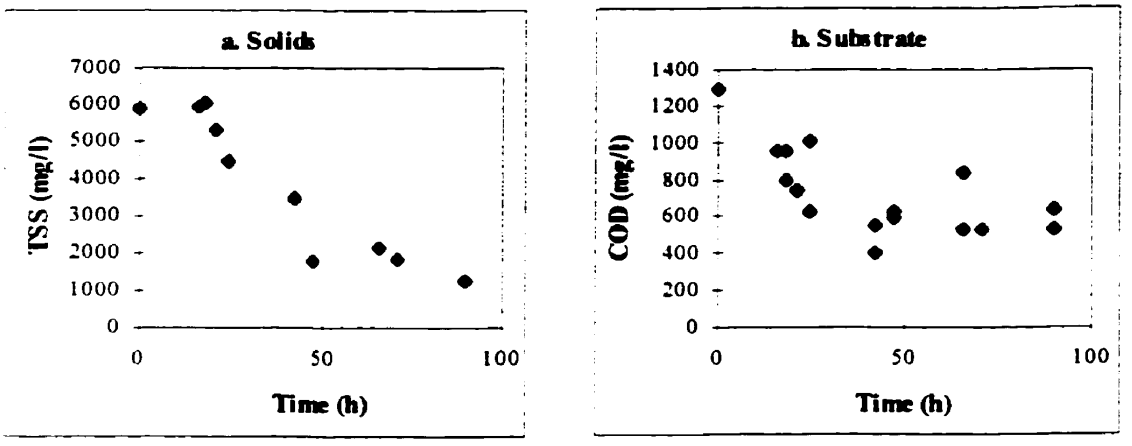

Figure 4.6 a and b: TSS and COD for the CSTR without Sludge Recycle Using TME from the CTMP Mill (HRT = 28 h)

4.4.2 CSTR using Total Mill Effluent with Recycle

In order to prevent washout, sludge recycle was initiated with a flow rate equal to that of the feed stream (i.e. the sludge recycle ratio was 1:1). This ratio was chosen to ensure that plenty of sludge would be present in the system, but in industry, the sludge is typically recycled at a rate of 40% of the feed flow rate. The HRT was only 9.6 h due to tube size limitations and the nominal sludge inoculum concentration was 4000 mg/l. From Figure 4.7a it can be seen that the TSS in the aerator generally remained constant. Initially, there was a decrease in the TSS because the recycle rate was not fast enough. To counteract this, periodic recycling as well as continuous recycling was started, bringing the concentration of the solids under control. The COD in the aerator and clarifier was generally about the same at any given time (see Figure 4.7b). After about 150 h, the COD dropped and then stabilised at about 400 mg/l resulting in a COD

reduction of 75%. That is roughly the time that periodic recycling was initiated. This suggests that the improved recycling led to improvements in the reduction of COD.

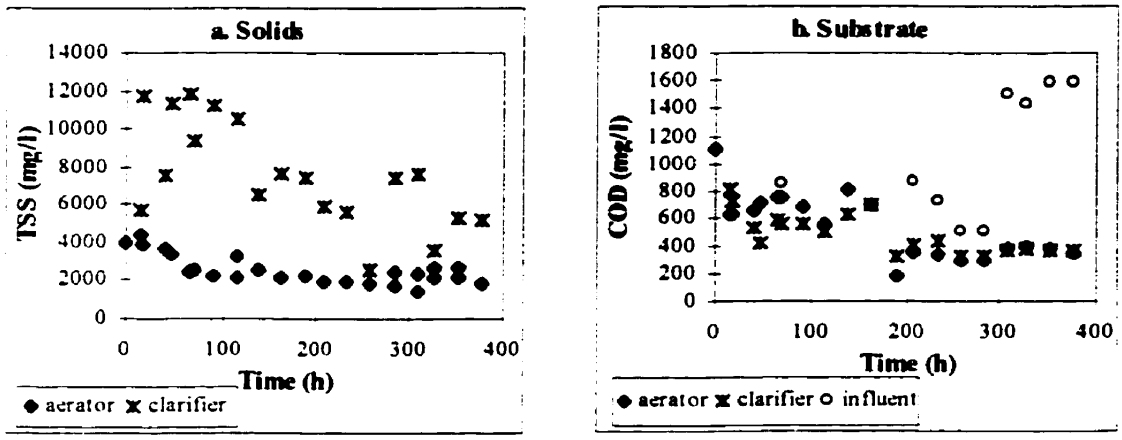

Figure 4.7 a and b: TSS and COD for the CSTR with Sludge Recycle Using TME from the CTMP Mill (HRT = 9.6 h)

Table 4.5: SVI for the CSTR with Sludge Recycle using TME from the CTMP Mill (HRT = 9.6 h)

Time (h)	SVI_{30} (ml/g)	SVI_{60} (ml/g)
63	234	202
138	330	271
210	440	366
281	140	112
326	333	238
374	189	162

During the experiment the temperature, pH, DO and SOUR were monitored. The temperature remained relatively constant at 24°C, the pH was in the range of 5.5-7.5 and the DO was about 6 to 8 mg/l. The SVI (sludge volume index) was also determined for both 30 and 60 minutes of settling and these results are shown in Table 4.5.

Several microplates using the Biolog technique were done during this experiment as described in section 3.2.8. Three microplates using samples taken from the aerator on three different days were incubated to determine if the sludge population was changing on a day-to-day basis. Three more microplates were done on samples taken from the aerator, clarifier and a new shipment of sludge to compare the populations in each. The low-resolution camera was used to take pictures of the microplates and the colour intensities were analysed using PCA (principal component analysis) as outlined in Materials and Methods. The scores for the first two principal components are displayed in Figure 4.8.

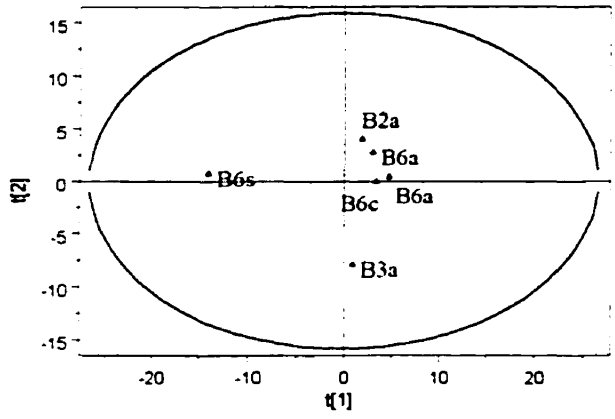

Figure 4.8: Scores for the First Two Principal Components for Microplates Inoculated with Samples Taken from Different Locations and at Different Times for CTMP TME. (a = aerator, c = clarifier, s = sludge, numbers represent different dates - t[1] is the scores vector for the first PC and t[2] is the second).

The patterns for the three plates (and one replicate), prepared using samples taken from the aerator over time, were visually very similar suggesting that the sludge population did not vary. In Figure 4.8a, the microplates done on two different days (denoted by B2 and B6) are very similar while the plate done on another day (denoted by B3) is slightly different. This could be due to differences in lighting or focus when the image was obtained since at this time, a constant setup for taking the picture had not yet been developed. As for the plates taken from the aerator, clarifier and refrigerated sludge on the same day (denoted by B6), the patterns for the aerator and clarifier were similar while

that for the sludge appeared different. This difference is demonstrated in Figure 4.8a (see point B6s). This suggests that the new sludge requires an acclimation period.

4.5 COMPARISON OF REACTOR EXPERIMENTS

The continuous reactor without recycle tended towards washout as indicated in Figure 4.6a. The solids concentration decreased from 6000 to 1000 mg/l within 96 h. The COD level was constant because even the remaining biomass was effective in its reduction from 1300 to 600 mg/l (a reduction of 54%). Once recycle was added, the concentration of solids stabilised at 2000 mg/l (see Figure 4.7a) and substrate removal (from 1600 to 400 mg/l) improved to 75% (compare Figure 4.6b to Figure 4.7b). Thus, improved recycling led to improvements in the reduction of COD. Biolog microplates done on samples taken from the aerator, clarifier and refrigerated sludge suggest that the refrigerated sludge was less viable than the sludge in the aerator, which is why an acclimation period is necessary to allow the sludge time to adapt to the effluent and operating conditions and to revitalise the biomass. Also, a comparison between microplates inoculated with aerator samples taken on a day-to-day basis indicates that the sludge population was constant over time.

5. RESULTS AND DISCUSSION FOR EXPERIMENTS USING KRAFT MILL EFFLUENT

In this chapter, the results and discussion for the treatment of effluents from a Kraft mill in Quebec will be presented. Initially, a series of experiments were executed at various hydraulic residence times (HRT's) in a CSTR with sludge recycle to determine the effect of HRT on the reduction of chemical and biological oxygen demand (COD and BOD_5). These results are given in section 5.1 and the experiments are compared in section 5.2. Once these experiments were completed, the effect of a step input of black liquor at various concentrations was determined. These results are given in section 5.3 and are compared in section 5.4.

5.1 EXPERIMENTS WITH THE TOTAL MILL EFFLUENT

Five experiments were performed at HRT's of 8, 12, 16, and 20 h in the 2 litre CSTR with a sludge recycle ratio of 1:1 (i.e. the flow rates of the feed and recycle streams were equal). The sludge age was adjusted to 5 days for an HRT of 8 and 12 h and 10 days for an HRT of 16 and 20 h by wasting sludge from the clarifier daily (see section 3.3.2 for the equation used to adjust sludge age). In each experiment, total mill effluent (TME) was mixed with activated sludge at a nominal inoculum concentration of 4000 mg/l and the COD:N:P was 100:3.0:1.0. The influent values for the COD, TOC and BOD_5 were found to vary during the experiments since the influent consisted of grab samples received from the mill on a weekly basis.

The reactor was operated at room temperature with aeration and agitation and the system was allowed to run until steady state was reached. Steady state was considered to occur when the concentration of total suspended solids and volatile suspended solids (TSS and VSS) remained constant in the aerator for at least 3 HRT's. In all the experiments, the biomass was the last parameter to reach steady state. The TSS and VSS in the aerator followed the same trend in all experiments and correlations were developed between them. The same was true for chemical oxygen demand and total organic carbon (COD

and TOC) and correlations were derived for these two parameters as well. The five-day biological oxygen demand (BOD_5) was determined in all but the first experiment (a 12 h HRT) and correlations between COD and BOD_5 for the influent and effluent were found.

5.1.1 Hydraulic Residence Time of 12 h

The system ran for about 135 h with a sludge age adjusted to 5 days and it took about 40 h to reach steady state based on biomass concentration, as can be seen from Figure 5.1a. The COD and TOC in the aerator remained roughly constant after 40 h, as shown in Figure 5.1b and c, supporting the idea that the system reached steady state. The TOC followed the same trend as the COD.

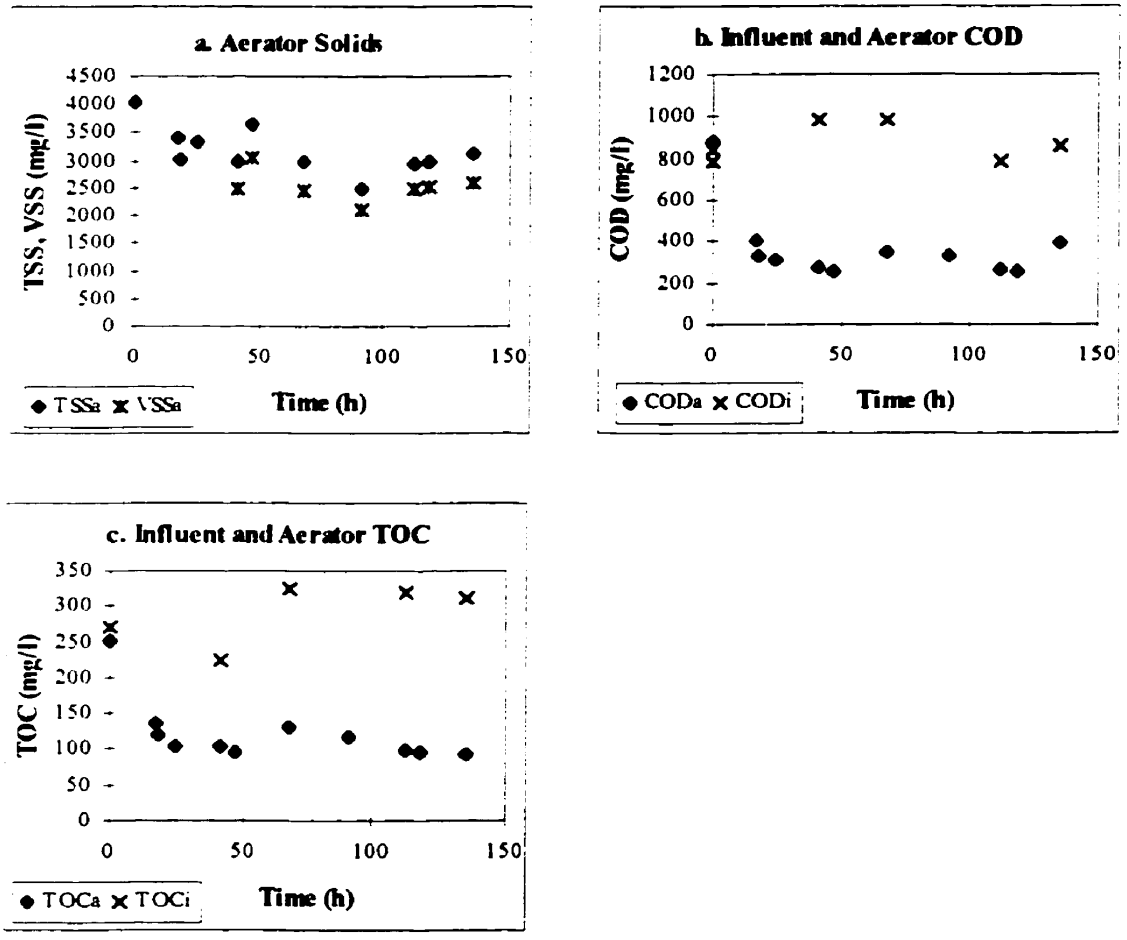

Figure 5.1a, b and c: System Parameters for an HRT of 12 h using Kraft Total Mill Effluent

From Table 5.1 it can be seen that the COD and TOC exhibited low standard deviations during the steady state operation. The average COD in the aerator at steady state was about 302 mg/l and the average influent COD was about 919 mg/l resulting in a COD reduction of about 67%. The COD uptake rate was 0.337 mgCOD/min·gVSS.

The average steady state values for the aerator and clarifier as well as the ratios of VSS/TSS and COD/TOC are given in Table 5.1. The VSS/TSS was very similar between the aerator and clarifier at about 0.84 and had a low standard deviation over a period of time at about 0.01. This is a useful result since it is easier to measure TSS and the VSS/TSS ratio can be used to quickly estimate the VSS at any desired time and location. Therefore, the measurement of VSS was limited only to the aerator in future experiments. The ratio of TOC/COD was also calculated and from Table 5.1 it is clear that they are similar in the aerator and clarifier at about 0.35-0.36 with a standard deviation of 0.05 in the aerator and 0.03 in the clarifier. This ratio can be used to estimate the TOC if the COD is known thus limiting the number of analyses that must be performed.

Table 5.1: Steady State Values and Ratios for an HRT of 12 h with Kraft Total Mill Effluent

	Aerator		Clarifier	
	Average	Std Dev	Average	Std Dev
TSS (mg/l)	3031	328	5427	2534
VSS (mg/l)	2538	263	3843	1679
VSS/TSS	0.84	0.01	0.84	0.01
COD (mg/l)	302	65	274	18
TOC (mg/l)	105	19	99	4.4
TOC/COD	0.35	0.05	0.36	0.03

From Table 5.1 it can also be seen that the coefficient of variation (i.e. the ratio of the mean to the standard deviation) for the TSS and VSS in the aerator was only about 10% while it was much higher in the clarifier at about 50%. This can be explained by the fact that the solids concentration in the clarifier fluctuated considerably during the run. This is a result of a number of factors that are discussed in section 5.2.

The pH, temperature, and DO generally remained constant throughout the experiment (pH was about 7.0, T was 24°C, DO was 6.5 mg/l). The specific oxygen uptake rate (SOUR), which is indicative of biomass activity, increased slightly after 80 h due to a slight drop in the concentration of solids, but it generally remained in the range of around 10 $mgO_2/gVSS \cdot h$. The SVI was initially quite high as can be seen in Table 5.2. One explanation for the poor settling at the beginning was due to inadequate wasting of the sludge. Once wasting was improved, settling also improved. The significance of SVI is discussed in greater detail later in this chapter in section 5.2.

Table 5.2: SVI for an HRT of 12 h with Kraft Total Mill Effluent

Time (h)	SVI_{30} (ml/g)	SVI_{60} (ml/g)
47	343	343
93	373	362
114	332	319
136	318	315

Three microplates using the Biolog technique were inoculated with samples taken from the aerator, clarifier, and refrigerated sludge. These were done during the steady state period and the plates were incubated at room temperature for 48 h. The patterns for all three plates were visually very similar suggesting that the sludge population was constant. PCA can only be performed on a data set of at least four or more observations; therefore, it was not possible to model these results within this experiment. However, it is possible to compare these results to the next experiment using PCA and this is done in section 5.2.

5.1.2 Hydraulic Residence Time of 20 h

The sludge age in this experiment was increased from 5 days to 10 days to prevent depletion of the sludge in the clarifier because, with a 5 day sludge age, the calculated waste sludge flow rate (WSF) was too high (see Materials and Methods for the calculation of WSF). The system ran for over 340 h and reached steady state after 220 h as demonstrated in Figure 5.2a. Figure 5.2illustrates that the COD in the aerator generally remained constant and the average steady state value was 219 mg/l with a

standard deviation of 25 as shown in Table 5.3. The average influent COD was about 774 mg/l with a standard deviation of about 30 and the system achieved a reduction of approximately 72%. TOC followed a similar trend as shown in Figure 5.2c. The COD uptake rate was 0.376 mgCOD/min·gVSS.

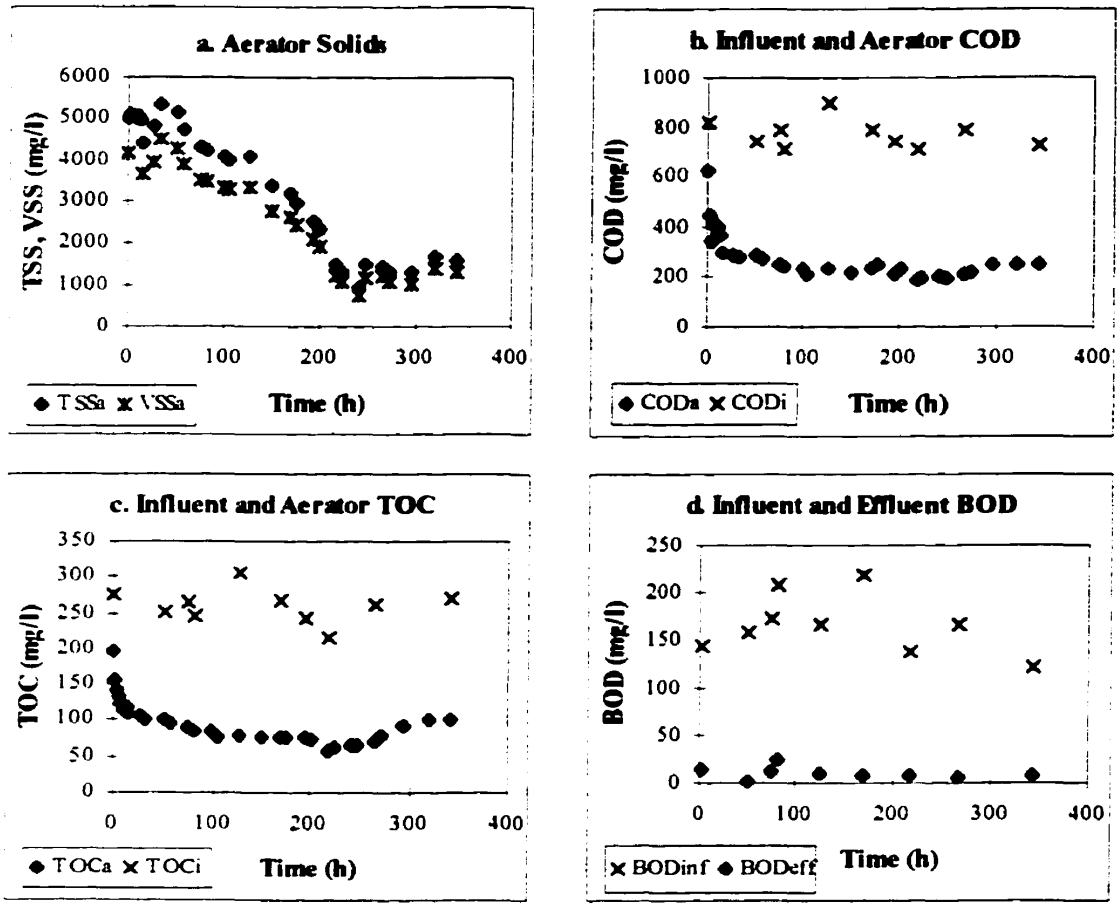

Figure 5.2 a, b, c and d: System Parameters for an HRT of 20 h with Kraft Total Mill Effluent

Figure 5.2d represents the BOD_5 for the system. Samples were taken from the influent and the final effluent and both the BOD_5 and COD were determined. The ratio, given in Table 5.3, was about 0.2 for the influent and 0.03 for the effluent; they differed by a factor of slightly more than 10. These ratios are useful in estimating the BOD_5 since the BOD assay takes five days to produce results while COD is a relatively quick and easy

measure of substrate concentration. The average influent BOD_5 was about 143 mg/l with a standard deviation of about 23 which is reasonably low while the average effluent BOD_5 was about 7 mg/l with standard deviation of 1.4. This gives a reduction in BOD_5 of 95% and the BOD uptake rate was 0.123 mgBOD/min·gVSS.

Table 5.3: Steady State Values and Ratios for an HRT of 20 h with Kraft Total Mill Effluent

	Aerator		Clarifier		Influent		Effluent	
	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
TSS (mg/l)	1396	222	11530	3621				
VSS (mg/l)	1143	185	9431	2942				
VSS/TSS	0.82	0.01	0.82	0.007				
COD (mg/l)	219	25	203	30	741	41	259	34
TOC (mg/l)	77	17	80	13	250	29	102	24
TOC/COD	0.34	0.03	0.37	0.04	0.34	0.04	0.36	0.06
BOD_5 (mg/l)					143	23	7	1
BOD_5/COD					0.20	0.02	0.03	0.004

The TSS and VSS clearly followed the same trend. Average steady state values for these and other parameters are given in Table 5.3. The TSS and VSS in the aerator each had a coefficient of variation of 16% while that for the clarifier was 31% which is lower than the previous experiment due to improvements in settling. Various ratios are also provided in Table 5.3. Once again, the ratios were very similar in both the aerator and clarifier. The VSS/TSS was 0.82 with a standard deviation of only 0.01 for both the aerator and clarifier while the TOC/COD was about 0.35 at all locations with a standard deviation of about 0.03.

The trends of pH, temperature, DO, and SOUR were monitored for the system and these generally remained constant (pH was 7.1, T was 24°C, DO was 6.8 mg/l, SOUR was 8.4 mg/g·h). The 30 and 60 minute SVI tended to increase during the experiment as can be seen in Table 5.4 (see Materials and Methods for the calculation of SVI). This is due, in part, to the fact that SVI is inversely proportional to TSS in the aerator. These results are discussed in section 5.2.

Table 5.4: SVI for an HRT of 20 h with Kraft Total Mill Effluent

Time (h)	SVI_{30} (ml/g)	SVI_{60} (ml/g)
51	186	178
75	221	210
170	297	282
195	377	254
295	336	229
341	423	308

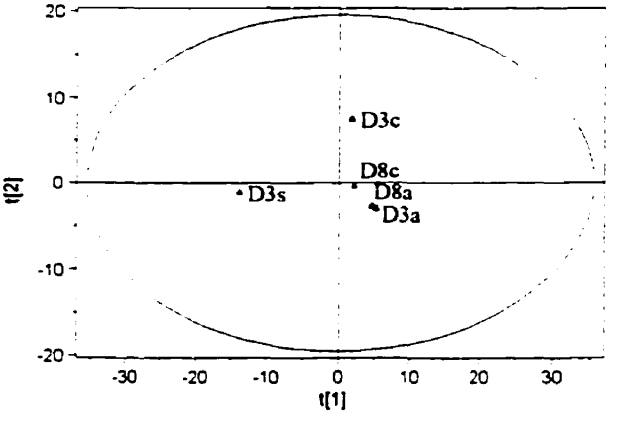

Figure 5.3: Scores for the First Two Principal Components for Microplates Inoculated with Samples Taken from Different Locations at Steady and Non-Steady States for the Kraft Mill Effluent at an HRT of 20 h (a = aerator, c = clarifier, s = sludge, numbers represent dates -- t[1] is the first principal component and t[2] is the second).

For this experiment Biolog microplates were prepared on two different days. The low-resolution camera was used to take pictures of the microplates, the colour intensities were analysed using PCA, and a plot of the scores of the first two PC's is given in Figure 5.3. The first set of plates (represented by the number 3 in the figure) were done at around 170 h, during the declining phase of the solids, using samples taken from the aerator (a), clarifier (c), and refrigerated sludge (s) used as inoculum. The second set was done

during the steady state period at about 300 h (represented by the number 8) using samples taken from the aerator and clarifier. For the first set, the patterns for each plate were different for each location: the plate inoculated with refrigerated sludge showed the greatest variation. The microplates taken during the steady state period exhibited similar patterns in both the aerator and clarifier. Comparing the patterns from day to day, the microbial populations were slightly different in the clarifier, but still very similar in the aerator.

5.1.3 Hydraulic Residence Time of 8h

The system ran for almost 200 h with a sludge age of 5 days and steady state was assumed after 185 h. However, as illustrated in Figure 5.4a, the biomass concentration in the aerator varied considerably during the run and never fully stabilised. A reason for this may be that the HRT was too short to allow the cells time to regenerate. The COD also fluctuated considerably as shown in Figure 5.4b, but appeared to be stabilising after about 180 h. Figure 5.4c shows similar fluctuations for the TOC. The average COD in the aerator at the approximate steady state was about 294 mg/l and the average influent COD was about 789 mg/l which gives a COD reduction of about 63%. The COD uptake rate was 0.820 mgCOD/min·gVSS, which is considerably higher than any of the experiments with a higher HRT.

Figure 5.4d shows the influent and effluent BOD_5. The BOD_5 of the effluent was constant throughout the experiment despite the fluctuation of the solids and COD suggesting that the system still achieved sufficient BOD_5 reduction even at non-steady state conditions. Influent BOD_5 was 227 mg/l and effluent BOD_5 was 9 mg/l, which gives a reduction of 96%. The ratios for BOD/COD were 0.29 and 0.03 for the influent and effluent respectively (shown in Table 5.5). These values are analogous to the other HRT experiments. The BOD uptake rate was 0.175 mgBOD/min·gVSS.

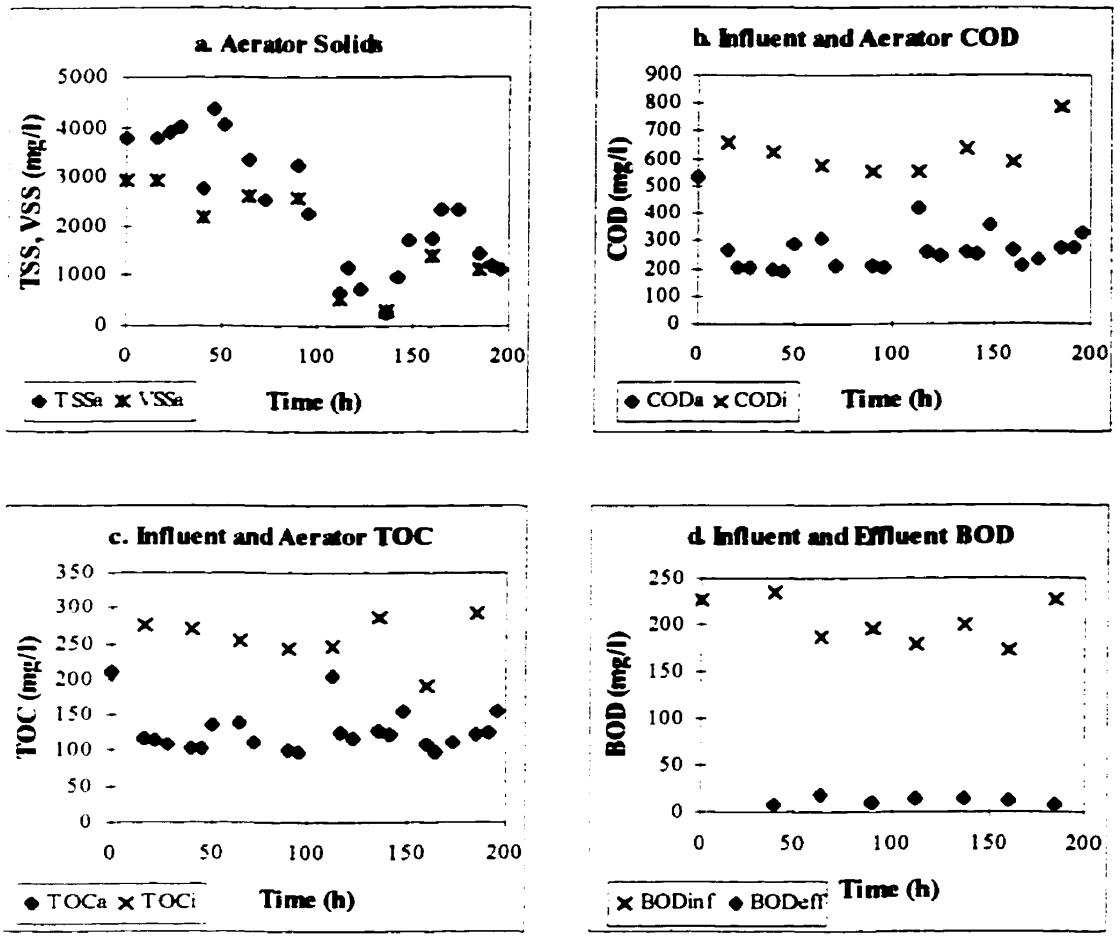

Figure 5.4 a, b, c and d: System Parameters for an HRT of 8 h with Kraft Total Mill Effluent

The ratio of VSS/TSS is about 0.79 which is slightly lower than other HRT experiments. Also, the TOC/COD ratio is about 0.46 for the aerator which is considerably higher than the other HRT experiments. This could be due to the fact that a final steady state had not been reached. The standard deviation is not presented here, as there were not enough points at the assumed steady state to accurately determine standard deviation.

The pH, temperature and DO generally remained constant throughout the experiment (pH was 7.5, temperature was 24°C, DO was 6.5 mg/l). The SOUR increased significantly (from 10 to 25 $mgO_2/gVSS \cdot h$) after 100 h due to the large drop in the concentration of

solids. SVI was determined and these results are given in Table 5.6. The highest SVI occurred during the period of lowest solids concentration.

Table 5.5: Steady State Values and Ratios for an HRT of 8 h with Kraft Total Mill Effluent

	Aerator	Clarifier	Influent	Effluent
	Average	Average	Average	Average
TSS (mg/l)	1265	7240		
VSS (mg/l)	1135			
VSS/TSS	0.79			
COD (mg/l)	294	237	789	250
TOC (mg/l)	137	112	292	117
TOC/COD	0.46	0.47	0.37	0.47
BOD_5 (mg/l)			227	9
BOD_5/COD			0.29	0.03
VSS/ΔCOD	2.21			

Table 5.6: SVI for an HRT of 8 h with Kraft Total Mill Effluent

Time (h)	SVI_{30} (ml/g)	SVI_{60} (ml/g)
64	274	244
136	414	
160	350	237

5.1.4 Hydraulic Residence Time of 16 h

The sludge age was adjusted to10 days and the system was allowed to run for over 475 h and reached steady state at about 350 h as shown in Figure 5.5a for the concentration of solids (TSS and VSS). Figure 5.5 (b, c and d) shows that the COD, TOC and BOD_5 reached steady state within the first 50 h. The standard deviation for the COD in the aerator was only 8.7 (shown in Table 5.7). At steady state the average COD for the influent was 958 mg/l and for the aerator it was 276 mg/l corresponding to a reduction of 71%. The COD uptake rate was 0.311 mgCOD/min·gVSS. The steady state values for BOD_5 were about 199 mg/l for the influent and 2 mg/l for the effluent (shown in

Table 5.7), corresponding to a very high a reduction of 99%. The ratios for the influent and effluent BOD/COD were 0.21 and 0.008 respectively. The latter value is considerably lower than ratios determined for other experiments suggesting that the system was running at full efficiency in terms of BOD reduction. The BOD uptake rate was 0.091 mgBOD/min·gVSS.

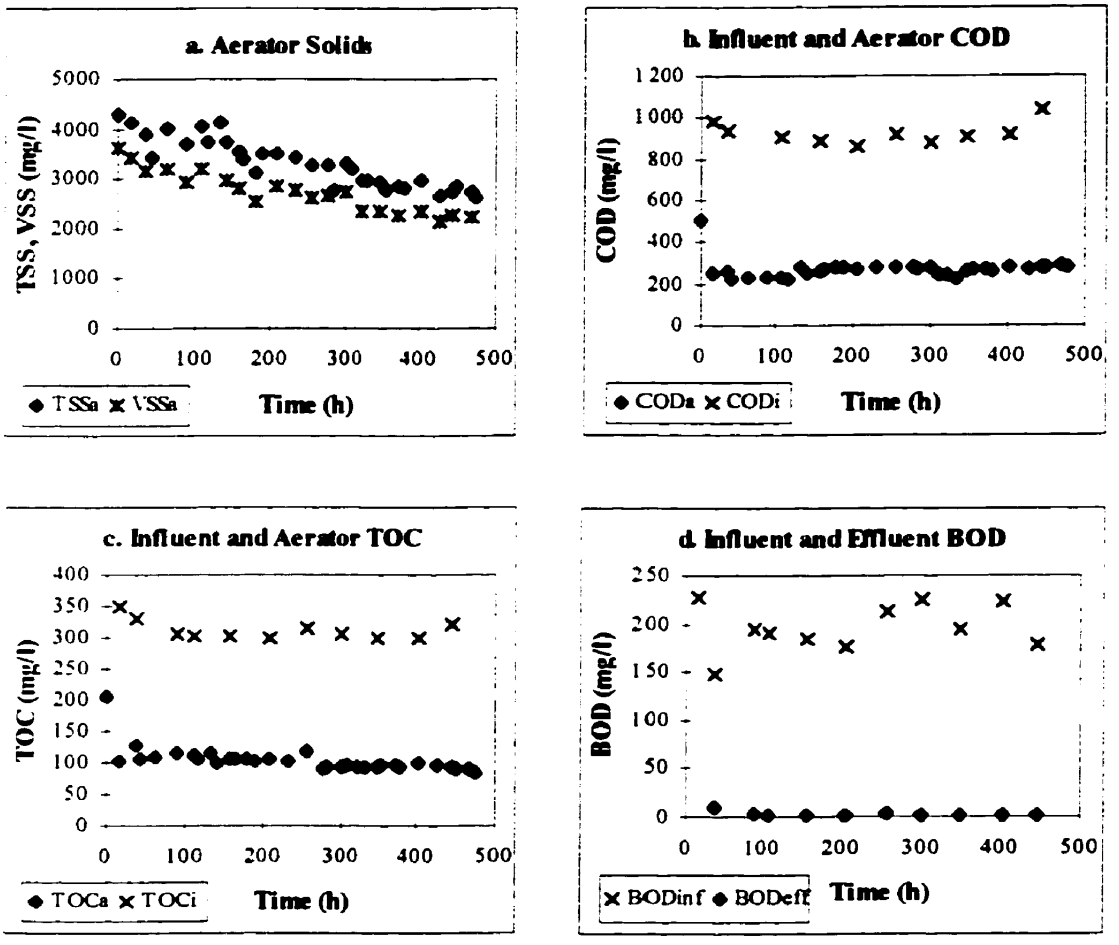

Figure 5.5 a, b, c and d: System Parameters for an HRT of 16 h using Kraft Total Mill Effluent

The TSS and VSS had very low standard deviations of only 113 and 78 respectively while the standard deviation for the TSS in the clarifier was also reasonably low compared to other experiments at 1284 (see Table 5.7). The VSS/TSS was 0.8 with a

standard deviation of 0.02 and the TOC/COD was 0.34 with a standard deviation of 0.02. These ratios are comparable to those obtained for the 12 and 20 h HRT experiments

Table 5.7: Steady State Values and Ratios for an HRT of 16 h using Kraft Total Mill Effluent

	Aerator		Clarifier		Influent		Effluent	
	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
TSS (mg/l)	2795	113	5096	1284				
VSS (mg/l)	2263	78						
VSS/TSS	0.80	0.02						
COD (mg/l)	276	9	259	16	958	76	3034	45
TOC (mg/l)	94	4	88	0.5	307	13	102	1.0
TOC/COD	0.34	0.02	0.34	0.02	0.32	0.01	0.34	0.05
BOD_5 (mg/l)					199	23	2	0.2
BOD_5/COD					0.21	0.04	0.008	0.002
VSS/ΔCOD	3.34	0.33						

The average temperature was 22°C, pH was 6.7, DO was 6.6 mg/l, and SOUR was 9.6 $mgO_2/gVSS \cdot h$. The SVI increased slightly during the experiment as the TSS decreased for the same reasons as before. These results are shown in Table 5.8.

Table 5.8: SVI for an HRT of 16 h with Kraft Total Mill Effluent

SVI	Time (h)															
(mg/l)	39	110	134	158	179	207	233	257	278	301	349	374	403	428	447	470
SVI_{30}	227	237	239		317	238	283		297	299	330	346	323	358	352	349
SVI_{60}	222	227	230	266	304	268	274	288	282	287	313	332	306	339	330	327

5.2 COMPARISON OF EXPERIMENTS WITH TOTAL MILL EFFLUENT

The following is a discussion on the results obtained during the experiments using total mill effluent from the Kraft mill. The purpose of these experiments was to determine the effect of HRT on substrate reduction and the microbial population.

5.2.1 Effect of HRT on Substrate Reduction and Rate of Utilisation

The system achieved similar reductions in COD and BOD_5 at each HRT investigated as shown in Table 5.9. The reduction in COD averaged between 63-72% and the reduction in BOD_5 was between 94-99%. The HRT had no effect on the COD or BOD uptake rates. The COD uptake rate generally ranged from 0.311 to 0.376 mgCOD/min·gVSS and the BOD uptake rate was around 0.091 to 0.175 mgCOD/min·gVSS (the BOD uptake rate shows greater variation since it was measured using samples taken from the waste collection vessel rather than the aerator – see section 3.2.4 in Materials and Methods). The only experiment that showed any significant difference was the 8 h HRT experiment in which the uptake rate for COD was 0.820 mgCOD/min·gVSS, but the BOD uptake rate for this experiment was not much higher than the other experiments. This suggests that even though the system never truly reached steady state it still managed to effectively reduce the BOD. Excluding the 8 h HRT value the overall average uptake rate for COD was 0.341 mgCOD/min·gVSS and for BOD it was 0.107 mgBOD/min·gVSS. The average BOD uptake rate including the 8 h value was 0.130 mgBOD/min·gVSS.

5.2.2 Comparison of TSS/VSS, TOC/COD and BOD/COD

Table 5.9 gives the values of the various ratios obtained in this research. The ratio of VSS/TSS was uniform at about 0.80 showing that the biomass had the same carbon content in all the experiments. The ratio for TOC/COD was relatively constant at about 0.34 for HRT's of 12, 16 and 20 h. This indicates that approximately 34% of the total COD was organic material. A higher value of 0.46 was obtained for the 8 h HRT experiment that did not reach a final steady state. Ratios for BOD/COD were derived separately for the influent and treated effluent as they typically differed by a factor of almost ten. The BOD/COD ratio for the influent as received from the pulp mill was fairly constant averaging 0.22 (the ratio of the 8 h HRT was higher due to differences in samples delivered to the lab on a weekly basis). The ratio of the COD to BOD of the treated effluent varied between 0.03 to 0.01.

Table 5.9: Comparison of Ratios and Kinetic Parameters at Steady State for Various HRT's

HRT (h)	8*	12	16	20
Sludge Age (days)	5	5	10	10
COD uptake (mg/min)/g	0.820	0.337	0.311	0.376
BOD uptake (mg/min)/g	0.175		0.091	0.123
% Reduction in COD	63	67	71	72
% Reduction in BOD	96		99	95
TOC/COD	0.46	0.35	0.34	0.34
BOD/COD influent	0.29		0.21	0.20
BOD/COD effluent	0.034		0.008	0.028
VSS/TSS	0.79	0.84	0.80	0.82
SOUR ($mgO_2/gVSS \cdot h$)	26	11	10	8
SVI_{30} (ml/g)	346	341	300	307
SVI_{60} (ml/g)	241	335	287	260

*indicates that the experiment did not reach a final steady state

5.2.3 Sludge Age

The experiments with lower HRT (12 and 8 h) used a sludge age of 5 days while the experiments with a higher HRT (16 and 20h) required longer sludge ages of 10 days to prevent depletion of the sludge in the clarifier. This can be explained by considering the calculation for the waste sludge flow rate (WSF) which is the mechanism used to control sludge age. The WSF is calculated by dividing by the sludge age (see Materials and Methods for the equation for WSF). Thus greater wasting causes shorter sludge ages. Low sludge ages are desirable in that they select faster growing, non-filamentous species, which are less likely to cause sludge bulking. However, with a higher HRT, the flow rate is slower and less sludge is entering the clarifier. Therefore, wasting must be low enough to permit the accumulation of a reasonable amount of sludge in the clarifier, but not so low that it leads to poor settling (i.e. high sludge ages lead to the proliferation of slower growing filamentous bacteria). Initially, in a few experiments, problems were encountered with sludge bulking due to inadequate wasting of the sludge. Once the WSF was increased, settling improved.

5.2.4 Settling of the Sludge

There were a number of other problems concerning the clarifier including plugging of the recycle line and problems in the design of the clarifier itself. The sludge tended to stick to the sides of the clarifier or tunnels would form through the sludge blanket so that only treated effluent would pass on to the recycle line. Periodic manual raking was applied by gently scraping the sludge from the sides of the clarifier and slightly stirring it to remove the tunnels, but this was not frequent enough to eliminate the above mentioned problems. Therefore, the sludge concentration in the under flow of the clarifier never stabilised. This was the case in all the CSTR experiments that involved sludge recycle.

As a measure of sludge settling properties, the sludge volume index (SVI) was determined at time intervals of 30 and 60 minutes. In most cases, the values did not change significantly from 30 to 60 minutes of settling (for the equation for SVI see Materials and Methods). The steady state values for SVI are given in Table 5.9. Excluding the values for the 8 h experiment that failed to reach a final steady state, the values for SVI decrease linearly with an increase in HRT. This trend is shown in Figure 5.6. With long HRT's, nearly all the fed substrate is consumed by the cells resulting in low BOD values. Hence, the cells exist in the endogenous growth phase characterised by slow growth rates that promote good settling. Furthermore, floc-forming bacteria are only flocculant in a stationary growth phase such as the endogenous phase.

5.2.5 Specific Oxygen Uptake Rate

The SOUR is indicative of biomass activity. As the HRT increases the SOUR decreases (see Figure 5.6), because the biomass is receiving substrate at a reduced rate causing the growth rate to slow down. With fewer cells being produced, less oxygen is utilised.

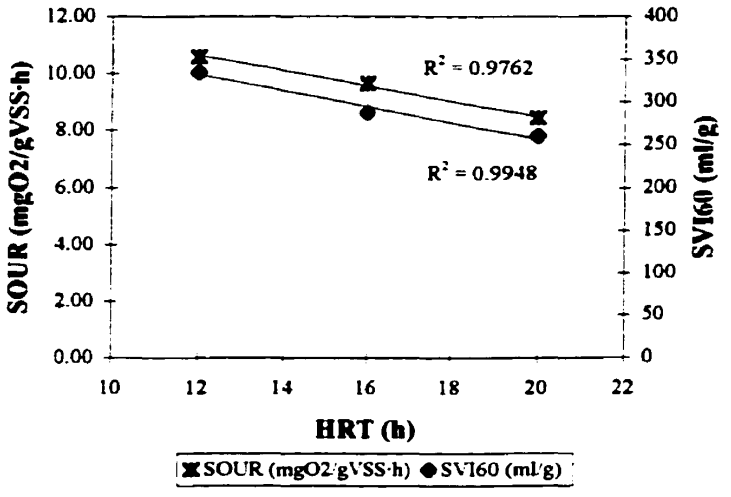

Figure 5.6: Comparison of SOUR and SVI for Various HRT

5.2.6 Microbial Response to HRT as Indicated by the Biolog Microplate Results

Principal component analysis (PCA) was used to compare the microplate results for samples taken at different times and from different locations during the 12 and 20 h HRT experiments. Microplate results for HRT's of 12 and 20 h are compared below. The PCA scores are shown in Figure 5.7 for the first two principal components (PC's). In the figure, each experiment is denoted by the letters 'C', and 'D' for 12 and 20 h HRT's respectively.

Comparing these scores to those of the variability study (section 3.2.8C), it appears that there were differences among microbial samples taken at each HRT on various days and from various locations. The point that demonstrates the greatest difference is the point for the sludge in the 20 h HRT experiment (point D3s in Figure 5.7). An explanation for this is that in the 20 h HRT experiment, the sludge had been refrigerated for longer than usual. This is another indication that the sludge may have to go through a period of acclimatisation. Comparing between experiments, there is a difference for each HRT investigated; the points for each experiment are in distinct regions.

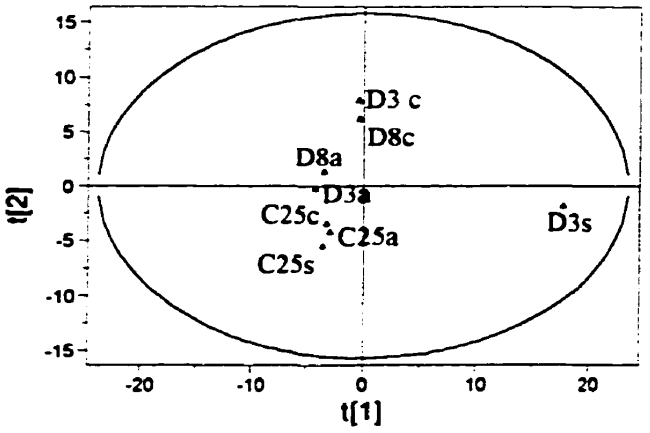

Figure 5.7: Scores for the First Two Principal Components (t[1] and t[2]) for Microplates Inoculated with Samples Taken from the Aerator, Clarifier and Sludge at Various Times for HRT's of 12 and 20 h using Kraft Mill Effluent.

Within the 12 h HRT experiment, the scores for the microplates inoculated with samples taken on the same day (denoted by C25) from the aerator, clarifier, and refrigerated sludge indicate that the microbial population was the same in each location. For an HRT of 20 h, the scores indicate that very slight differences exist between the aerator and clarifier. This is likely due to the longer HRT that would favour the development of slower growing organisms and give the sludge more time to shift.

5.3 STEP INPUT OF BLACK LIQUOR

Black liquor spills are an occasional problem at the mill. Black liquor consists mainly of lignin, hydrolysis salts and sulphonation products and has a very high COD of about 160 000 mg/l so even small concentrations of black liquor in the TME can be a shock to the treatment system. To study this effect, step inputs of black liquor were added to the aerator. For these experiments steps of 5, 2.5 and 1% black liquor in TME by volume were examined. The system was operated at room temperature at an HRT of 16 h and a sludge age of 10 days with a sludge recycle to feed ratio of 1:1. The COD:N:P ratio was 100:3.0:1.0. Aeration and agitation were provided and in each case the system was

allowed to reach steady state with only TME as feed before applying the step. The purpose of the experiments was to establish the effect of black liquor on the activated sludge and the reduction in COD and BOD_5.

5.3.1 Step Input of a 5% Solution of Black Liquor in TME

The reactor was kept running from the experiment with an HRT of 16 h so that the system was already at steady state when the black liquor step was added. To initiate the step, black liquor was added to the aerator, clarifier and feed stream simultaneously (see Materials and Methods on the experimental procedure). The initial TSS prior to the step was about 1700 mg/l. Within 15 minutes of feeding the 5% solution the TSS increased to 1950 mg/l due to the greater concentration of solids in the black liquor (see Figure 5.8a). The TSS in black liquor was about 600 mg/l causing a rise in the feed TSS from 20 mg/l to 220 mg/l.

After 16 h, the TSS dropped dramatically to 1100 mg/l indicating that the microbial population had declined. However, after this initial decline, the biomass population began to grow at a rapid rate. In just over 60 h it increased to almost 4000 mg/l and finally reached a steady state concentration of almost 5000 mg/l with standard deviation of about 340. This trend can be seen in Figure 5.8a and the new steady state values are shown in Table 5.10. From the Table it is obvious that at steady state the TSS and VSS showed very little variation with coefficients of variation of less than 10%. The ratio for VSS/TSS was still in the same range as past experiments even in the presence of black liquor. It also exhibited a low standard deviation.

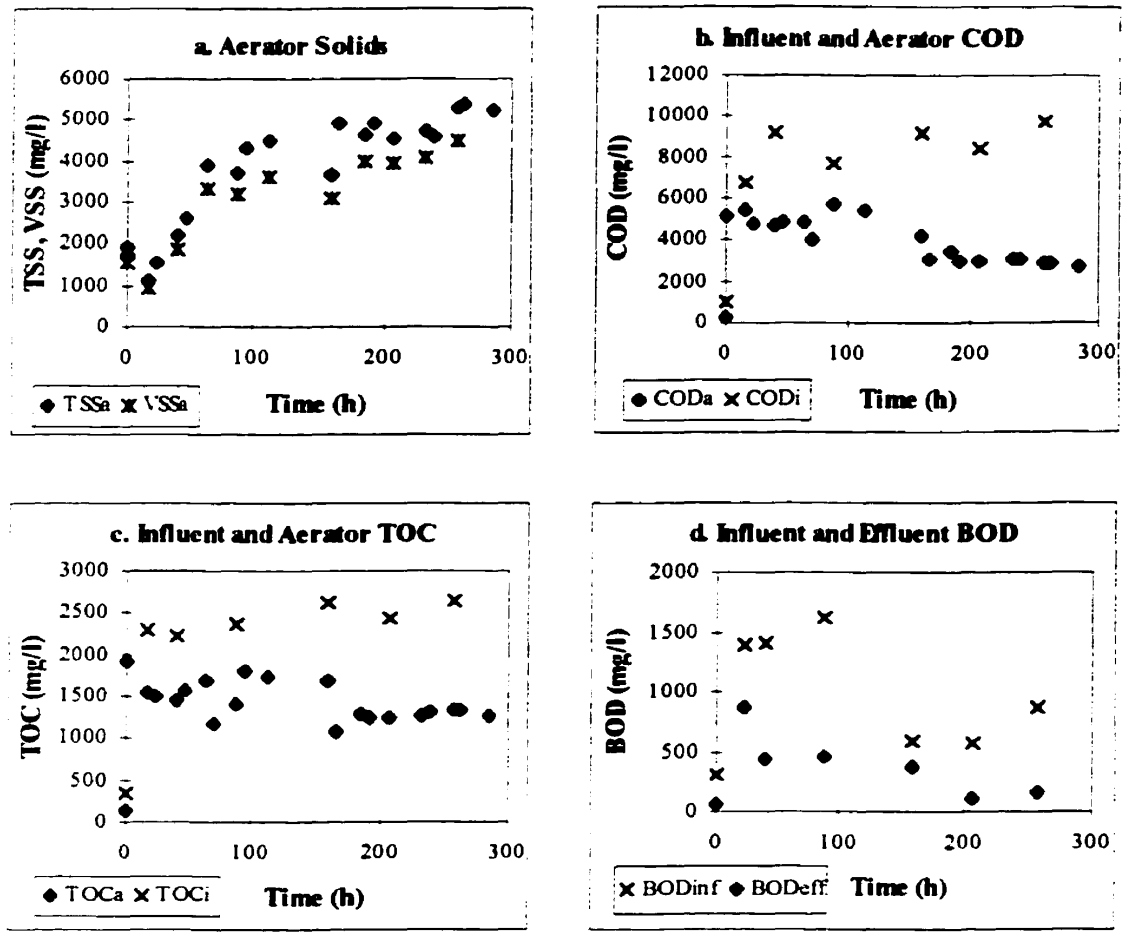

Figure 5.8 a, b, c and d: System Parameters for a Black Liquor Step of 5% in Kraft Total Mill Effluent (HRT = 16 h)

Just prior to the step, the COD concentration was 320 mg/l in the aerator and immediately after the black liquor step the concentration increased to 5100 mg/l (the COD of the black liquor was about 160 000 mg/l). This value gradually decreased to a steady state value of about 3022 mg/l. Since the inlet COD was about 9085 mg/l this is a reduction of 67%, which is comparable to past experiments without black liquor. A plot of COD is given in Figure 5.8b. Figure 5.8c is a similar plot showing TOC for the aerator and influent and it follows the same pattern as the COD. The ratio TOC/COD (see Table 5.10) is in the same range as the other experiments at 0.43 with a standard deviation of 0.03. The COD uptake rate was quite high at 1.63 mgCOD/min·gVSS. This is much higher than any of the previous experiments with just total mill effluent suggesting that the activity of the

sludge is greater. The BOD_5 (Figure 5.8d) for the influent was 720 mg/l and for the effluent at steady state it was 146 mg/l for a reduction of 80% (Table 5.10). The ratio of BOD to COD is also given in Table 5.10. The BOD uptake rate was 0.145 mgBOD/min·gVSS.

Table 5.10: Steady State Values for a Step Input of 5% Black Liquor in Kraft Total Mill Effluent (HRT = 16 h)

	Aerator		Clarifier		Influent		Effluent	
	Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev
TSS (mg/l)	4927	343	7615	6919				
VSS (mg/l)	4118	250						
VSS/TSS	0.86	0.01						
COD (mg/l)	3022	216	3290	859	9085	947	6350	541
TOC (mg/l)	1284	38	1213	182	2552	153	1681	235
TOC/COD	0.43	0.03	0.39	0.10	0.28	0.01	0.26	0.01
BOD_5 (mg/l)					720	205	146	34
BOD_5/COD					0.08	0.01	0.02	0.01
Cond (mS)	7.0	0.11						
Cond/COD (S/mg/l)	2.3	0.21						

The temperature, pH, DO and conductivity were monitored throughout the experiment. The temperature remained between 22 and 23°C. After the black liquor step, the pH rose from 7.3 to 8.6 and then stabilised at 8.2. Influent pH was adjusted to about 6.5. Conductivity was around 5.4-7.0 mS and the ratio of conductivity(S)/COD(mg/l) was 2.3 with a standard deviation of 0.2. Most significantly though, the DO dropped from 5.8 to 0.3 mg/l with the addition of black liquor. It was impossible to increase the dissolved oxygen concentration. This indicates that the cells were utilising the oxygen as fast as it was being replenished and for this experiment, the oxygen became the limiting factor. Because the DO was so low it was impossible to measure the SOUR. It was also observed that the SVI decreased after the addition of black liquor, which suggests that the biomass settled better. SVI results are shown in Table 5.11. The SVI_{30} just prior to the addition of black liquor was about 350 ml/g (SVI_{60} was 330 ml/g).

Table 5.11: SVI for a Step Input of 5% Black Liquor in Kraft Total Mill Effluent (HRT = 16 h)

Time (h)	SVI_{30} (ml/g)	SVI_{60} (ml/g)
160	151	105
185	160	119
207	136	109
232	158	116
257	93	74
285	137	107

Biolog microplates were done on samples taken just prior to, just after, 14 h after and 65 h after the step of 5% black liquor as well as at the new steady state to see the response of the population to the addition of black liquor. Quantitative results were obtained using PCA as outlined in Materials and Methods and the scores are presented in Figure 5.9.

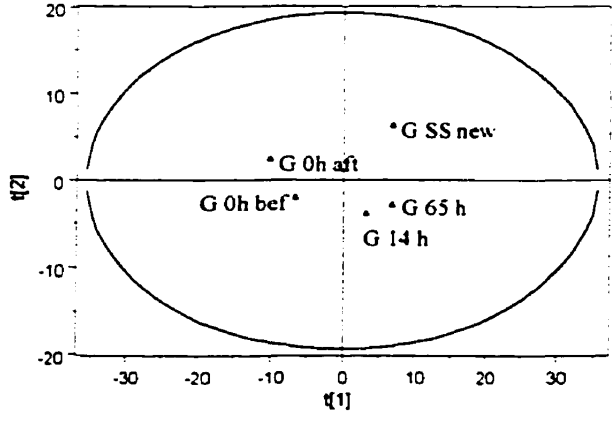

Figure 5.9: Scores for the First Two Principal Components for Microplates Taken at Various Times Before and After a Black Liquor Step of 5% in Kraft TME with an HRT of 16 h (t[1] is the first principal component, t[2] is the second).

Prior to the step, the population pattern visually appeared normal (as compared to past steady state patterns). Then, just after the step, the pattern contained significantly fewer wells showing colour development suggesting that many of the species had been reduced

to such an extent that they no longer demonstrated any affect on the colour development. After only 14 h, the pattern became stronger (i.e. it contained more purple wells) and after 65 h this new pattern was more pronounced. A final microplate was done when the system reached steady state and this pattern differed from previous steady state patterns without black liquor. These differences, demonstrated in Figure 5.9, suggest that there was a shift in the microbial population.

5.3.2 Step Input of a 2.5% Solution of Black Liquor in TME

In this experiment, the reactor was started up using only TME with a nominal TSS of about 2500 mg/l and was allowed to run until steady state was reached, at which time a step of 2.5% black liquor was added. The initial TSS, just prior to the step, was about 2600 mg/l. Results are given for both steady states before and after the addition of black liquor in Table 5.12. The concentration of solids did not change with the addition of black liquor. However, one day after the step input, the TSS went up from 2600 to 3930 mg/l. It then proceeded to drop slightly until climbing back up to a steady state value of about 3900 mg/l; this can be seen in Figure 5.10a. The ratios for VSS/TSS were very similar before and after the step (0.81 and 0.83) and also compare well to other experiments.

The steady state COD in the aerator prior to the step was about 311 mg/l and after the step the COD increased to 3150 mg/l (see Figure 5.10b). It then dropped gradually to a new steady state of 1644 mg/l in the aerator. The percent reduction of the COD in aerator was about 63% both before and after the step. Figure 5.10c shows a similar trend in the TOC. Ratios and steady state values are given in Table 5.12 for before and after the black liquor step. The ratio of TOC/COD compares reasonably well to other experiments. Also, the ratio of conductivity/COD was 5.6 before the addition of black liquor and 2.7 after black liquor. This indicates that the presence of black liquor causes a reduction in the conductivity/COD ratio. The addition of black liquor also improved the COD uptake rate, causing it to increase from 0.255 mgCOD/min·gVSS before the step, to 0.997 mgCOD/min·gVSS after the step.

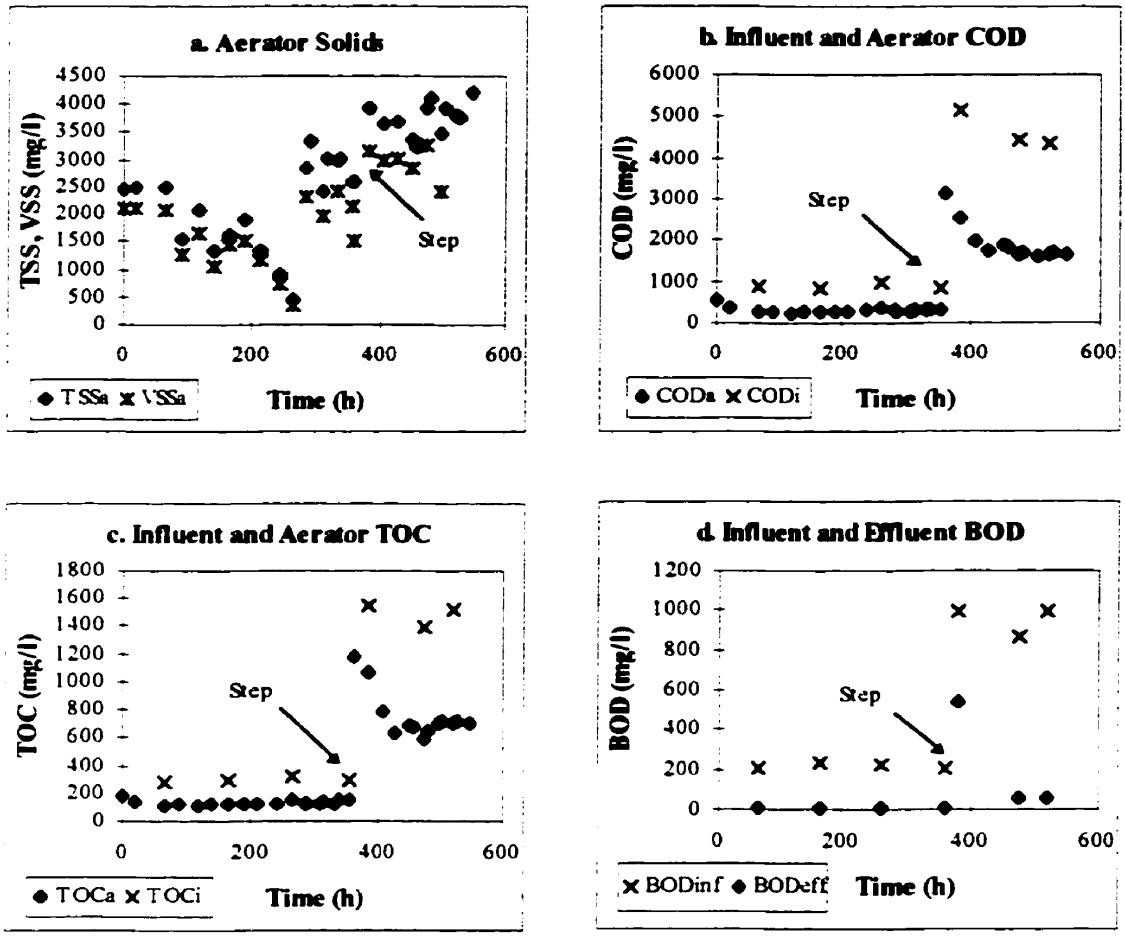

Figure 5.10 a, b, c and d: System Parameters for a Black Liquor Step of 2.5% in Kraft Total Mill Effluent (HRT = 16 h)

The influent and effluent BOD_5 was determined and the results are shown in Figure 5.10d. Before the step, the steady state BOD_5 was 207 mg/l for the influent and 6 mg/l for the effluent as shown in Table 5.12. After the black liquor step, the BOD_5 reached a new steady state of 931 mg/l for the influent and 58 mg/l for the effluent. The reduction in BOD_5 was initially 97% and dropped slightly to 94% after the step. The BOD uptake rate was 0.095 mgBOD/min·gVSS before the step and 0.319 mgBOD/min·gVSS after the step.

Table 5.12: Comparison of Steady State Averages for a Black Liquor Step of 2.5% in Kraft Total Mill Effluent (HRT = 16 h)

	Aerator		Clarifier		Influent		Effluent	
	0% BL	2.5% BL	0% BL	2.5% BL	0% BL	2.5% BL	0% BL	2.5% BL
TSS (mg/l)	2899	3894	8241	12080				
VSS (mg/l)	2214	2850						
VSS/TSS	0.81	0.83						
COD (mg/l)	311	1644	314	1565	839	4409	428	2696
TOC (mg/l)	141	684	136	549	294	1458	165	971
TOC/COD	0.46	0.41	0.41	0.34	0.35	0.33	0.39	0.36
BOD_5 (mg/l)					207	931	6	58
BOD_5/COD					0.25	0.21	0.014	0.022
Cond. (mS)	1.7	4.4						
Cond./COD (S/mg/l)	5.6	2.7						

Table 5.13: SVI for a Black Liquor Step of 2.5% in Kraft Total Mill Effluent (HRT = 16 h)

SVI	Time (h)											
(ml/g)	166	190	212	283	307	330	382	451	474	497	521	546
SVI_{30}	542	450	448	309	332	247	178	163	182	201	211	190
SVI_{60}	449	349	261	253		181	115	107	142	158	171	152

With the addition of black liquor, the temperature remained the same at 24°C while the pH, conductivity, and SOUR increased and the DO decreased. The average values before and after the step respectively are as follows: pH increased from 6.8 to 8.3, conductivity increased from 1.74 to 4.37 mS, DO decreased from 7.6 to 4.9 mg/l and SOUR increased from 9.5 to 31.1 $mgO_2/gVSS \cdot h$. The notable increase in the SOUR after the step indicates enhanced biomass activity. The SVI decreased with the addition of black liquor (at 382h) as in the previous experiment, confirming that the addition of black liquor does improve settling (see Table 5.13).

As in the previous experiment, Biolog microplates were done on samples taken just prior to, just after, 14 h after and 48 h after the step with 2.5% black liquor as well as at the new steady state. This was demonstrated quantitatively using PCA and the results are shown below in Figure 5.11 as well as in section 5.4, where they are compared to other experimental results.

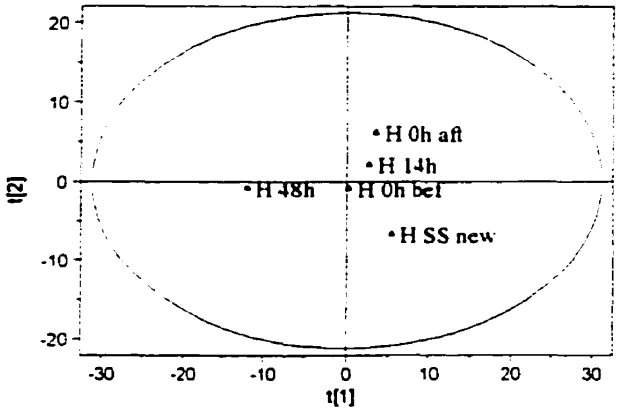

Figure 5.11: Scores for the First Two Principal Components for Microplates Taken at Various Times Before and After a Black Liquor Step of 2.5% in Kraft TME with an HRT of 16 h (t[1] is the first principal component, t[2] is the second).

Prior to the step, the population pattern appeared normal (as compared to past steady state patterns). The plate done just after the step was similar to the one prior, but contained several wells that were lighter than corresponding wells in the previous plate. This suggests that a concentration of 2.5% black liquor did not cause as severe a stress to the microbial population as did the 5% step. After only 14 h, the pattern became darker; however, the 48 h pattern contained some newly purple wells that had not exhibited colour development before. Finally, a microplate done at the new steady state produced a pattern that was more similar to that for the regular steady state (with no black liquor). The difference between the pattern obtained for the 48 h plate and the others seems clear in Figure 5.11, where the 48 h plate is set apart from the other plates. The results indicate that the microbial population changed during the experiment.

5.3.3 Step Input of a 1% Solution of Black Liquor in TME

The reactor was run without black liquor (initial TSS was 2800 mg/l) until steady state was reached at which time a step of 1% black liquor was added. Steady state results for before and after the addition of black liquor are given in Table 5.14. The TSS fluctuated substantially during the initial phase of the experiment due to problems with bulking and plugging in the return sludge line, as can be seen in Figure 5.12a. Once the TSS stabilised to some degree the step was initiated. This caused a slight increase in the TSS, which then proceeded to increase further to about 3100 mg/l within one day of the step input. The TSS then dropped, due to a plug in the return sludge line, before increasing again to 2800 mg/l. Then the TSS slowly decreased until a steady state value of about 1700 mg/l was reached. The steady state TSS value before the black liquor step was about 1600 mg/l so it was only slightly higher with the 1% black liquor. Ratios and steady state averages are given in Table 5.14.

The COD for the aerator, clarifier and influent are shown in Figure 5.12b. The steady state COD in the aerator prior to the step was about 300 mg/l. Immediately after the black liquor step, the COD in the aerator increased and then decreased within one HRT to 940 mg/l. From there, it reached the steady state value of about 797 mg/l within two HRT's. The percent reduction for the COD in the aerator was 65% both prior to and after the step input. This compares very well to previous experiments. Figure 5.12c shows a similar trend for the TOC. The ratios for TOC/COD were 0.54 and 0.42 before and after the step respectively. The COD uptake rate increased from 0.442 to 1.014 mgCOD/min·gVSS.

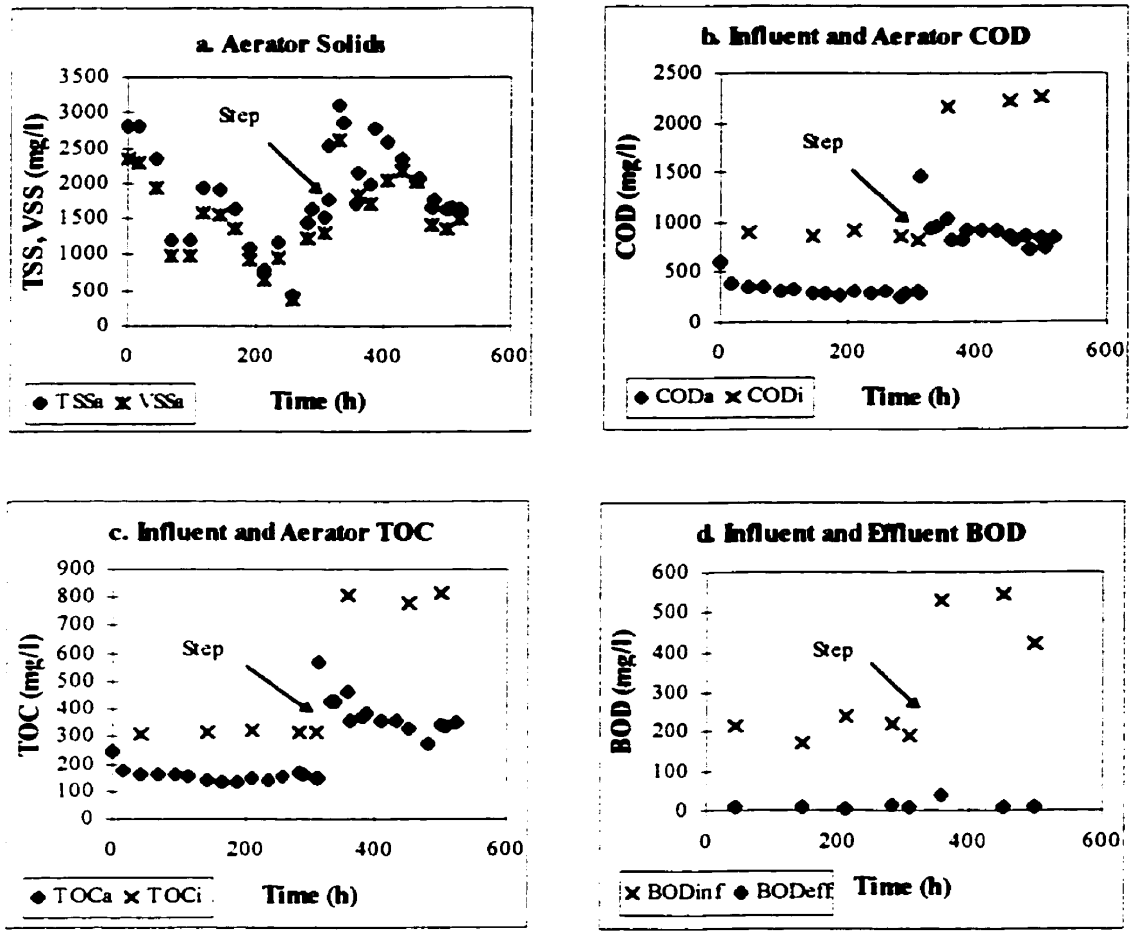

Figure 5.12 a, b, c and d: System Parameters for a Black Liquor Step of 1% in Kraft TME (HRT = 16 h)

The influent and effluent BOD_5 was determined and the results are shown in Figure 5.12d. At steady state before the step, the BOD_5 was 207 mg/l for the influent and 10 mg/l for the effluent. At the new steady state after the black liquor step, the BOD_5 was 425 mg/l for the influent and 12 mg/l for the effluent. The reduction in BOD was initially 95% and increased slightly to 97% in the presence of black liquor. The BOD uptake rate increased from 0.161 to 0.300 mgBOD/min·gVSS.

Table 5.14: Comparison of Steady State Averages for a Black Liquor Step of 1% in Kraft Total Mill Effluent (HRT = 16 h)

	Aerator		Clarifier		Influent		Effluent	
	0% BL	1% BL	0% BL	1% BL	0% BL	1% BL	0% BL	1% BL
TSS (mg/l)	1593	1674	8113	9357				
VSS (mg/l)	1275	14367						
VSS/TSS	0.86	0.85						
COD (mg/l)	293	797	287	696	832	2274	421	1019
TOC (mg/l)	156	326	145	298	316	818	180	404
TOC/COD	0.54	0.42	0.50	0.43	0.38	0.36	0.43	0.40
BOD_5 (mg/l)					207	425	10	12
BOD_5/COD					0.25	0.19	0.03	0.01
Cond. (mS)	1.5	2.4						
Cond./COD (S/mg/l)	5.3	2.9						

The average values for the system trends before and after the step respectively were as follows: temperature was 22°C and 24°C, pH was 6.96 and 6.87, conductivity was 1.49 and 2.42 mS, DO was 8.11 and 6.94 mg/l, and SOUR was 15.76 and 34.77 $mgO_2/gVSS \cdot h$. Once again the SOUR demonstrated a significant increase with the addition of black liquor. The SVI decreased slightly with the addition of black liquor (at 330 h) as previously, but not to as great an extent (see Table 5.15).

Table 5.15: SVI for a Black Liquor Step of 1% in Kraft TME (HRT = 16 h)

SVI	Time (h)														
(ml/g)	18	43	115	144	165	211	283	308	329	356	378	451	473	496	518
SVI_{30}	427	807	495	504	576	600	440	444	322	333	325	345	300	120	135
SVI_{60}	410	774	474	472	545	409	318	370	315	213	225	259	203	90	123

Biolog microplates were prepared for samples taken just prior to, just after, 16 h after and 46 h after the step with 1% black liquor to see the response of the population to the addition of black liquor. PCA was used to quantify the results and a plot of the scores of

the first two principal components is given in Figure 5.13. These results are compared to those of previous experiments in section 5.4.

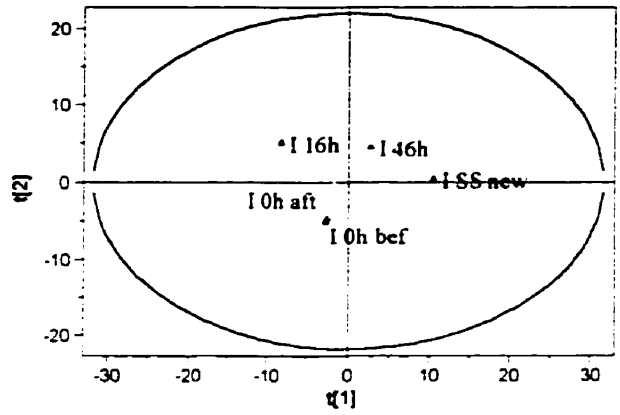

Figure 5.13: Scores for the First Two Principal Components for Microplates Taken at Various Times Before and After a Black Liquor Step of 1% in Kraft TME with an HRT of 16 h (t[1] is the first principal component, t[2] is the second).

Prior to the step, the population pattern was slightly different from previous steady state patterns. There were a few wells demonstrating colour development that normally do not, implying that the microbial population in this experiment was different from those in past experiments. Just after the step, the pattern was very similar to the one just prior to it suggesting that a concentration of 1% black liquor did not cause much of a shock to the microbial population. This similarity is clearly demonstrated in Figure 5.13, where the points I 0h bef and I 0h aft are very close. After only 16 h, the pattern became darker and both it and the 46 h pattern contained some newly purple wells that had not exhibited colour development before. Finally, a microplate done at the new steady state showed a pattern very similar to that of the 46 h plate. Overall, there was a difference between corresponding microwells in the plates taken just prior to the step and at the new steady state. The results indicate that there was a shift in the microbial population.

5.4 COMPARISON OF BLACK LIQUOR STEP EXPERIMENTS

The following is a discussion of the results obtained during the black liquor step experiments using total mill effluent and black liquor from the Kraft mill. The purpose of these experiments was to determine the effect of black liquor on the microbial population and its ability to reduce the substrate concentration. In general, higher black liquor concentrations resulted in higher COD uptake rates while the system still managed to reduce the organic content of the wastewater to the same degree as in the HRT experiments. The black liquor also induced a shift in the microbial population.

5.4.1 Effect of Black Liquor on Substrate Reduction and Rate of Utilisation

In these experiments, the substrate concentration tended to increase from the clarifier to the final treated effluent in the waste collection beaker. For each step input of black liquor, the values for COD in the clarifier and final effluent respectively were as follows: for a step of 5% they were 3290 mg/l and 6350 mg/l, for 2.5% they were 1565 mg/l and 2700 mg/l and for 1% they were 670 mg/l and 1020 mg/l. This increase was noticed in the hydraulic residence time experiments with total mill effluent; however, it was not as significant. In this case, with the high substrate concentrations brought on by the addition of black liquor, the increase was greater. This may be a consequence of the composition of the black liquor since it contains dissolved organic salts and other chemicals. However, it is more likely that due to higher biomass concentrations in the aerator, more cells remained in the final effluent after settling and they proceeded to lyse, releasing their cell contents and contributing to the rise in substrate concentration.

In most cases of the black liquor step inputs, the system still achieved satisfactory reductions in COD and BOD_5 as shown in Table 5.16. All experiments obtained at least 63-67% reduction in COD and these values did not vary significantly within an experiment, before and after the addition of black liquor. Two of the experiments also attained high reductions in BOD_5 in the presence of black liquor at 94% and 97% and these values did not differ significantly from those of the steady state before the addition of black liquor. The only experiment that did not achieve adequate BOD_5 reduction was

that with the step of 5% black liquor, which only reduced the BOD_5 by about 80%. This may indicate that the system cannot handle such a large increase in fed substrate concentration. This may be discussed by inspecting the COD uptake rate.

Table 5.16: Comparison of Ratios and Kinetic Parameters at Steady State for Various Black Liquor Steps (HRT = 16 h, Sludge Age = 10 days)

Black Liquor Conc. (%)	5.0%		2.5%		1.0%	
	Bef	Aft	Bef	Aft	Bef	Aft
COD uptake (mg/min)/g	0.31	1.6	0.26	1.0	0.44	1.0
COD uptake factor (aft/bef)		5.2		3.9		2.3
BOD uptake (mg/min)/g	0.09	0.15	0.10	0.32	0.16	0.30
BOD uptake factor (aft/bef)		1.6		3.4		1.9
% Reduction in COD	71	67	63	63	65	65
% Reduction in BOD	99	80	97	94	95	97
TOC/COD	0.34	0.43	0.45	0.41	0.54	0.42
BOD/COD influent	0.21	0.08	0.25	0.21	0.25	0.19
BOD/COD effluent	0.008	0.02	0.01	0.02	0.03	0.01
Cond./COD (S/(mg/l))		2.3	5.7	2.7	5.3	2.9
VSS/TSS	0.80	0.86	0.81	0.83	0.86	0.85
SOUR (mgO_2/gVSS·h)	10	Fast	10	31	16	35
SVI_{30} (ml/g)	300	140	296	196	442	185
SVI_{60} (ml/g)	287	104	217	156	344	139

With the addition of black liquor, the COD uptake rate increased significantly as shown in Table 5.16. A step input of 5% black liquor resulted in an uptake rate of 1.6 mgCOD/min·gVSS, which was 5.2 times greater than that for the same system before the addition of black liquor. For the step of 2.5%, the uptake rate was 1.0 mgCOD/min·gVSS, which was 3.9 times greater then prior to black liquor. Finally, the 1% step gave an uptake rate of 1.0 mgCOD/min·gVSS, which was 2.3 times greater than before black liquor was added. The initial uptake rate for the 2.5% step was higher than usual at 0.44 mgCOD/min·gVSS compared to a value of 0.26 mgCOD/min·gVSS for the 2.5% step. To reduce the effects of this disparity, a plot of the COD uptake rate factor (COD uptake rate at steady state for the step concentration of black liquor divided by the

steady state uptake rate prior to the addition of black liquor) for increasing black liquor concentrations is given in Figure 5.14. The figure illustrates that the COD uptake rate factor increases with an increase in black liquor concentration.

The BOD uptake rate also increased with the black liquor steps as shown in Table 5.16. For the step of 5%, the rate increased 1.6 times (from 0.09 to 0.15 mgBOD/min·gVSS). The 2.5% step caused the rate to increase 3.4 times (from 0.10 to 0.32 mgBOD/min·gVSS) and the 1% step caused the rate to 1.9 times increase (from 0.16 to 0.30 mgBOD/min·gVSS). These values show a large degree of variation since the BOD_5 was measured on samples taken from the final effluent after settling, where, as mentioned above, the substrate concentration was much higher. Thus, these results are not as reliable as those for the COD. Therefore, the BOD uptake factor is not plotted in Figure 5.14.

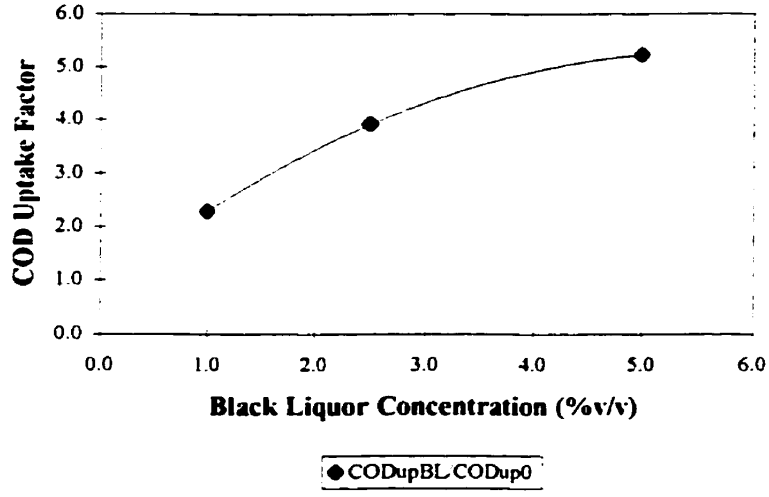

Figure 5.14: Effect of Black Liquor on the COD Uptake Rate for Kraft Mill Effluent

The black liquor causes a large increase in the feed substrate concentration and results in a situation where the substrate is in excess. Therefore, the system is no longer operating in the endogenous phase, but in the logarithmic growth phase where the growth rate of the microbial population increases in order to utilise the available substrate. The rate of

metabolism is only limited by microbial generation and the population's ability to process the substrate. In the case of the 5% step, where the BOD_5 reduction was only 80%, the biomass may have reached its maximum capacity for substrate reduction. One further explanation for the removal of only 80% of the BOD could be that since the DO dropped to levels below 0.5 mg/l and became the limiting factor in BOD reduction, the biomass may have been prevented from reducing the BOD to the extent that it normally does.

5.4.2 Comparison of VSS/TSS, TOC/COD, Conductivity/COD and BOD/COD

Table 5.16 shows the various ratios obtained for different black liquor concentrations. The ratios did not change with the addition of black liquor. This implies that these ratios may be used to estimate certain parameters in the system regardless of present conditions at the treatment plant (i.e. system upsets). However, one ratio that is present in the table that had not been used in the previous experiments is that of conductivity/COD in siemens (S) per mg of COD per litre. As demonstrated in the conductivity experiment as well as in literature (Kemeny and Banerjee, 1997), conductivity is a good indicator of black liquor content in the wastewater. The ratio for conductivity/COD tends to decrease linearly with an increase in black liquor concentration, as illustrated in Figure 5.14. This is only true for treated effluent, as the conductivity/COD ratio appeared mainly constant in the conductivity test using untreated effluent. Another thing to note is that the ratio of conductivity to COD was lower for the system containing black liquor at about 2.62 S/(mg/l) than it was in the experiments with just TME where it was about 5.46 S/(mg/l).

5.4.3 Effect of Black Liquor on Sludge Settling Properties and Activity

The same problems with the clarifier as discussed in the section for the HRT experiments were also encountered here. In terms of settling, the SVI was lower in the presence of black liquor suggesting that the black liquor improves the settling properties of the sludge. A reason for this may be that by adding black liquor, we are essentially increasing the food to mass ratio (F/M) and high F/M loading selects for faster growing, non-filamentous cells. The SVI did not, however, vary significantly with black liquor concentration. Thus the improvement in settling may have been a result of the different

type of substrate present and not the concentration of substrate. The composition of black liquor may have been the factor that selected for non-bulking bacteria. Furthermore, in the parallel discussion on settling properties as a function of HRT, it was noted that high HRT's lead to improvements in SVI because the system is operating in the endogenous phase with slow growth rates. Therefore, with the increase in substrate concentration, the system would enter the logarithmic growth phase characterised by high growth rates that should, logically, lead to poor settling. Since this was not the case, the composition of the black liquor must be the factor that caused improvements in settling.

As the fed substrate concentration increased (with the addition of black liquor), the SOUR increased, causing the DO to decrease. This is a result of rapid biomass production as it utilises the excessive amounts of substrate fed into the reactor, thus metabolising oxygen at a higher rate. Steady state values for SOUR are given in Table 5.16.

5.4.4 Microbial Response to Black Liquor as Indicated by the Microplate Results

For each black liquor step experiment, Biolog microplates were prepared for samples taken from the aerator at steady state prior to the addition of black liquor to determine whether the microbial population was consistent throughout the experiments. PCA was applied to demonstrate any variations among the plates. The scores of the first two PC's are given in Figure 5.15. In the figure, the letters 'G', 'H', and 'I' represent the experiments with steps of 5%, 2.5% and 1% respectively. The samples for the microplates were taken just prior to the step input. A plate was also prepared for the 16 h HRT experiment at steady state and is represented by 'F 0%' in the figure.

Replicate plates done during a variability study (mentioned in section 3.2.8) are also shown in Figure 5.15. The points for the scores of these plates are clustered together indicating that the plates were very similar to one another. Concomitantly, the steady state patterns just before the addition of black liquor show some degree of scatter. Thus, each experiment started with a different microbial population. This was unavoidable since the sludge was collected and delivered from the mill on a weekly basis and

conditions, such as effluent composition, vary at the mill. In order to determine the extent of this difference, it would be necessary to run the PCA on a set of plates inoculated with several completely different microbial populations from separate systems. This would show whether these plates (in Figure 5.15) were actually quite similar in comparison to completely different populations.

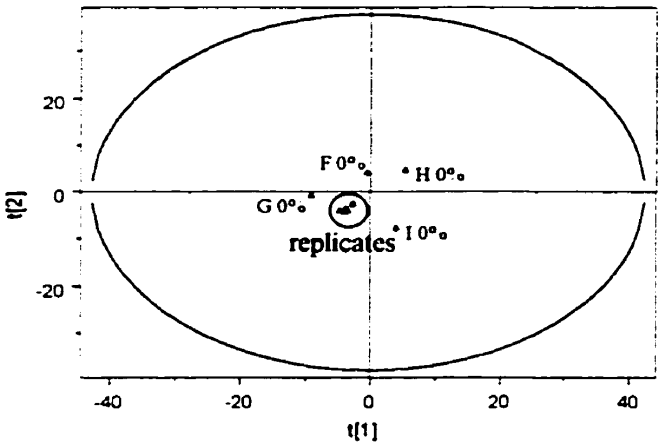

Figure 5.15: Scores for the First Two Principal Components (t[1] and t[2]) for Images of Microplates Inoculated with Samples from the Aerator Treating Kraft Mill Effluent at Steady State before a Black Liquor Step (Replicates are also shown).

After each step input, the system reached a new steady state. Scores for the microplates done at each new steady state are shown in Figure 5.16 as well scores for the replicate microplates. Once again, the points for the replicates are clustered together while the points for the microplates done at the new steady states are scattered, indicating that different black liquor step inputs resulted in different microbial populations at the new steady state. Thus, substrate concentration affects the microbial population. As mentioned above, the extent to which these plates differ must be determined through a comparison with several completely different microbial populations.

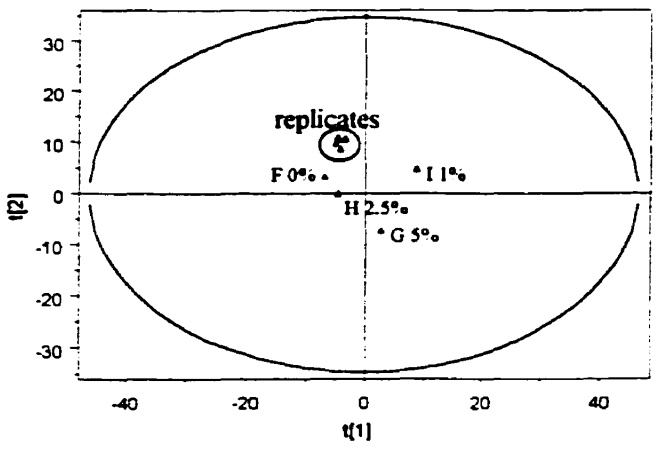

Figure 5.16: Scores for the First Two Principal Components (t[1] and t[2]) for Images of Microplates Inoculated with Samples from the Aerator Treating Kraft Mill Effluent at Steady State after a Black Liquor Step (Replicates are also shown).

For each black liquor step experiment, the replicate scores were compared to the experimental scores in the same manner as above and in each case, the points for the scores for each microplate were considerably more scattered in the figures than the points for the replicate plates. Thus, the microbial population underwent a shift with each concentration of black liquor.

6. CONCLUSIONS AND RECOMMENDATIONS

In the experiments using various effluents from the CTMP mill, it was found that the ultra high yield, paper machine, acid plant and wood washing effluents all exhibited adverse effects on the microbial population. In all cases, the growth of the biomass decreased with increasing concentrations of these effluents in the total mill effluent as did the specific COD reduction. Increasing concentrations of each effluent also caused a decrease in the reduction of COD in all cases, but the experiment using paper machine effluent where the activated sludge still achieved sufficient reduction in COD.

For the Kraft mill effluent, the activated sludge system sufficiently reduced the substrate in all cases investigated, regardless of variations in hydraulic residence time (HRT) or black liquor concentration. The system achieved reductions of 63-72% in the COD and 90-99% in the BOD_5. The only exception occurred with a 5% black liquor step input where only 80% of the BOD_5 was reduced. However, typical upsets at the mill rarely, if ever, lead to black liquor levels as high as 5% of the total mill effluent. Therefore, it may be concluded that the sludge can handle most black liquor shocks typically experienced at the mill.

The hydraulic residence time had no effect on the substrate uptake rate. The COD uptake ranged from 0.31 to 0.38 mgCOD/min·gVSS at steady state for each HRT studied. However, longer HRT's led to decreases in the specific oxygen uptake rate (SOUR) and sludge volume index (SVI). Thus longer HRT's reduced the activity of the biomass and produced a sludge with inferior settling properties. The step input of black liquor, on the other hand, caused a significant rise in the substrate uptake rate and biomass activity. The values for the substrate uptake rate and SOUR were much higher in the experiments with black liquor than in the experiments with only total mill effluent (TME). Furthermore, the COD uptake rate increased (from 1.0 to 1.6 mgCOD/min·gVSS) with respect to rising black liquor concentrations as did the SOUR, indicating an increase in biomass activity. The SVI decreased with a step input of black liquor, but did not change for different black liquor concentrations.

The Biolog microplate technique has been shown to be a useful tool for demonstrating general changes in the microbial population. Although it is somewhat labour intensive and costly, it is a valuable technique in that it gives an idea about the changes in the population that cannot otherwise be determined. The results from the Biolog microplates confirmed that the microbial population experiences a shift during a system upset such as a black liquor spill. However, to determine the extent to which the population actually changed, it is recommended that principal component analysis be used to compare the microbial populations in these experiments to several completely different populations from separate systems. The results obtained from these experiments do show that step inputs of black liquor caused the microbial population to vary over time, resulting in different steady state populations. However, even though the microbial population varied, the system still achieved sufficient substrate reduction as stated above. In conclusion, even though the black liquor induced a change on the microbial population, the biomass proved to be very robust and capable of effectively treating the pulp mill effluent under many non-ideal conditions.

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APPENDIX A. SAMPLE CALCULATIONS

MILL	Mill B	**EXP:**	
COD (mg/l)	1000	**DATE:**	
COD:N:P	100:3.0:1.0		

Batch volume required	Vt (ml) =	1380
Inoculum conc. wanted	TSSi (mg/l) =	4000
Actual sludge conc.	TSSo (mg/l) =	8935

$$\text{TSS (mg/ml)} = \frac{\text{(dry sample \& filter weight (g) - filter weight (g))*1000 (mg)}}{\text{sample volume filtered (ml)}}$$

$$\text{Vs (ml)} = \frac{\text{TSSi*Vt}}{\text{TSSo}} = \frac{4000 * 1380}{8935} = 618$$

$$\text{Ve (ml)} = \text{Vt - Vs} = 1380 - 618 = 762$$

NUTRIENTS

PROPERTIES:	**%**	**sp.**	**MW**		
Phosphoric Acid:	85	1.685	98	**P MW**	31
Urea:	99	1.335	60.06	**N MW**	14

Conc. of urea (determined by solution) = 30.514 mg/ml

conc. N (mg/l) =	3/100*COD	=	30
conc. P (mg/l) =	1/100*COD	=	10

Vb = volume of effluent batch = 2000 ml (usually)

$$\text{Vacid (ml)} = \frac{\text{conc.P (mg/l)*98*Vb(ml)}}{\text{31*1000ml/l*density of acid (mg/ml)*0.85}} = \frac{10*98*2000}{31*1000*1685*0.85} = 0.044$$

$$\text{Vurea (ml)} = \frac{\text{conc.N(mg/l)*60.06*Vt(ml)}}{\text{28*1000ml/l*conc. of urea (mg/ml)}} = \frac{30*60.06*2000}{28*1000*30.514} = 4.21$$

APPENDIX B. PRINCIPAL COMPONENT ANALYSIS (PCA)

Principal component analysis is an ordination method. Ordination is the projection of a multi-dimensional swarm or matrix of data points onto a two-dimensional plot that will allow any intrinsic pattern to become apparent. In the case of PCA, the data swarm is projected onto a differentially oriented space without any differential weighting of the species (Pielou, 1984). Several strategies for PCA exist. The classical method performs an eigenanalysis on the data matrix while another method, commonly used by computers, follows an iterative procedure. The SIMCA-P software, used in this research to determine the principal components, applies the iterative technique. Thus it will be described in greater detail in this section followed by a brief description of the classical eigenanalysis approach. Before getting into that however, a description of the data collection and manipulation process is given below as well the set up of the data matrix.

Data was collected using digital cameras and Visilog 5.1 image analysis software. The software applied a macro to determine the average value in a 5x5 matrix of pixels at the centre of each well. This value represents the colour intensity of that well. Before PCA could be performed, it was necessary to manipulate the data to improve the outcome of the results. The method followed was one described by Garland and Mills, 1991. Initially, the raw difference data was calculated by subtracting the value of the response well from that of the control well (C-R), reducing the control well to zero. Then, the data was normalised by dividing by the average well colour development (AWCD) of that plate: AWCD = Σ(C-R)/95. This step was necessary to account for differences in the degree of colour development for each plate (Garland, 1996). This is referred to as the transformed data. Once the transformed data is obtained for each plate the plates may be compared to one another using PCA.

PCA is applied on a single s x n data matrix X where s is the number of observations and n is the number of variables. These data are represented by a diffuse and irregular swarm of n points in s-space (Pielou, 1984). In this case, s is the number of microplates to be compared and n is the number of microwells within a plate. Thus there are 95 variables.

The matrix X is set up by placing the intensity values for all the wells into a single row. Therefore, rows represent individual plates (observations) while columns represent the colour intensity in each microwell (variables). In this way, differences among the plates may be determined. PCA cannot test for specific differences.

SIMCA-P statistical software follows the nonlinear iterative partial least squares (NIPALS) method of calculating the principal components. This approach (as describe by Geladi and Kowalski, 1986) consists of rewriting a data matrix X as a sum of linearly independent matrices. These can in turn be expressed as products of two vectors, a score (column) vector t and a loading (row) vector p^T. Thus the PCA decomposition of the data matrix X can be written as follows:

$$X = t_1 p_1^T + t_2 p_2^T + \ldots + t_a p_a^T$$

where a is the number of components or tp^T products required to exactly describe the X matrix. In other words, each principal component (PC) is described by a score vector and a loading vector. The first score vector is the one that passes through the data swarm in the direction that describes the greatest portion of the variation among the data. The second score vector is orthogonal to the first and passes through the data swarm in the direction that describes the second greatest portion of the variation and so on so that the first few components explain the greatest portion of the variation. A loading vector shows how the variables are combined to form the scores (i.e. it shows the responsible variables). Alternately, X can be written as:

$$X = TP^T$$

where T is a matrix whose columns are the score vectors t, while P^T is a matrix whose rows are the loading vectors p^T. To illustrate the significance of the PCA decomposition, consider a matrix X with two columns. This would correspond to a data matrix where colour intensities for only two corresponding wells are recorded.

$$X = \begin{bmatrix} x_{11} & x_{12} \\ x_{21} & x_{22} \\ x_{31} & x_{32} \\ x_{41} & x_{42} \\ x_{51} & x_{52} \\ x_{61} & x_{62} \end{bmatrix}$$

Each column of the matrix X defined above contains colour intensity values for two microwells in six different microplates. Each row of the matrix X consists of two entries. If one thinks of these as plane coordinates, then each row of the matrix X describes a point in a plane whose axes X_1 and X_2 correspond to the colour intensities. Thus matrix X consists of six points as illustrated in Figure B.1b.

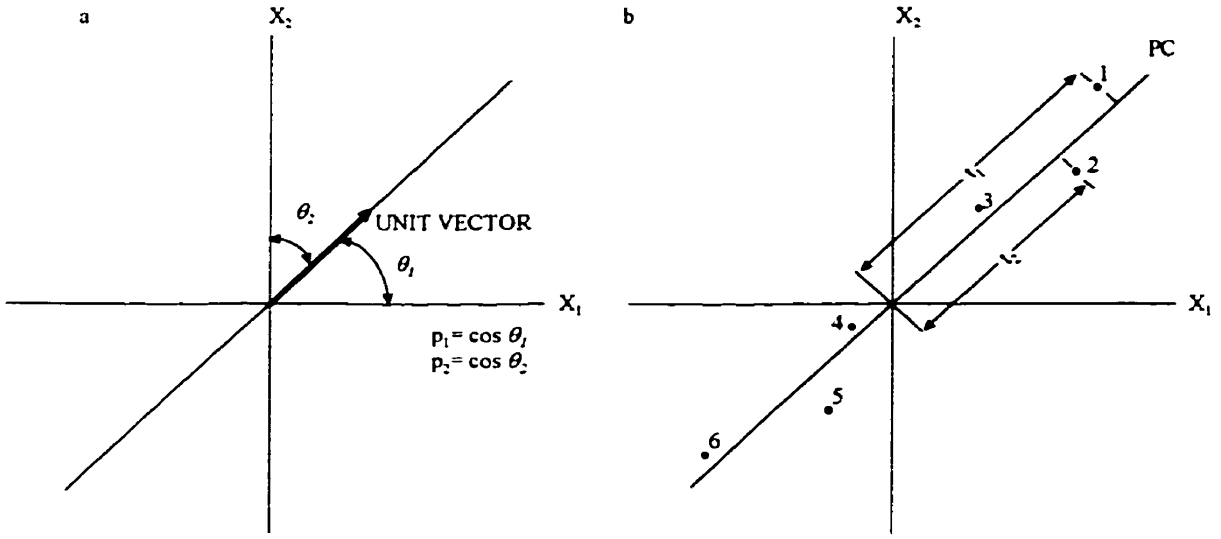

Figure B.1a and b: Geometrical Interpretation of PCA Scores t and Loadings p^T (Geladi and Kowalski, 1986)

Next, assume that matrix X is to be described by a single principal component. This means that we would like to write X as:

$$X = tp^T - E$$

where the matrix E contains the residuals. In this case the principal component is the line that best fits the six points illustrated in Figure B.1b (i.e. the line that minimises the sum of the squares of the distances from the points to the line). The elements of the loading vector p^T are the direction cosines of the principal component line, while the elements of the score vector t are the coordinates of the six points along the principal component line. Thus the principal component decomposition

$$X = \begin{bmatrix} x_{11} & x_{12} \\ x_{21} & x_{22} \\ x_{31} & x_{32} \\ x_{41} & x_{42} \\ x_{51} & x_{52} \\ x_{61} & x_{62} \end{bmatrix} = \begin{bmatrix} t_1 \\ t_2 \\ t_3 \\ t_4 \\ t_5 \\ t_6 \end{bmatrix} \begin{bmatrix} p_1 & p_2 \end{bmatrix} + E = tp^T + E$$

is nothing but a change of coordinate systems which allows one to describe the information contained in the X matrix in a new coordinate system of reduced dimensions. Once the direction cosines p_1 and p_2 of the principal component line are defined, only one coordinate, t is needed to define each of the six points. This is illustrated in Figure B.1a and b.

The conclusions drawn from this two-dimensional example can be easily applied to cases with more than two dimensions such as a full data set which contains 95 variables and as many plates as desired. In addition, one can also easily see that the residual E is reduced if X is approximated by more than one principal component (recall that a was defined above as the number of components required to describe X without any error).
The NIPALS method calculates the principal components one at a time, while making sure that each component is orthogonal to the previous one. The first component, given by $t_1 p_1^T$, is calculated from the X matrix so that:

$$X = t_1 p_1^T + E_1$$

Then, the residual E_1 is obtained by subtracting the first component from X:

$$E_1 = X - t_1 p_1^T$$

The second component, given by $t_2 p_2^T$, is calculated from the residual E_1:

$$E_1 = t_2 p_2^T + E_2$$

after which the residual E_2 is obtained and used to calculate the third component and so on, until the ath component for which $E_a = 0$.

The NIPALS algorithm is given below:

Step	Interpretation
1. Let t_i be any vector x_j from X : $t_i = x_j$	Any known vector can be used to start off.
2. Calculate p_i^T : $p_i^T = t_i^T X \, t_i^T t_i$	Find vector p_i^T which projects X onto t_i.
3. Normalize p_i^T : $p_i^T{}_{new} = p_i^T{}_{old} / p_i^T{}_{old}$	Make p_i^T a unit vector (of direction cosines).
4. Recalculate t_i : $t_i = X p_i \, p_i^T p_i$	Readjust scores in direction p_i^T (X onto p_i^T)
5. If $t_i(4.) = t_i(2.)$ stop, else goto 2.	If t_i is the same, the iteration has converged and $t_i p_i^T$ is the ith component; replace X by its residual and start again at 1.

(Geladi and Kowalski, 1986)

SIMCA extracts as many components as it considers significant. The criterion for selecting the number of PC's is cross-validation. In this approach, parts of the data are kept out of the model development and then predicted by the model and compared with the actual values. Cross-validation computes the PRESS (predicted error sum of squares) residuals which are the squared differences between observed and predicted values for the data kept out of the model fitting. This procedure is repeated until each data element has been kept out once, so that the final PRESS contains contributions from all data. Then,

for each dimension, the PRESS/SS (where SS is the residual sum of squares of the previous dimension) is computed. With this, a component's significance is determined. The method also computes the $(PRESS/SS)_k$ for each X variable x_k. A component (model dimension) is significant if it obeys one of the following cross-validation rules:

Rule 1: Q^2>limit

where Q^2 is the fraction of the total variation of X's that can be predicted by a component: $Q^2 = 1.0 - PRESS/SS$

Rule 2: at least $\sqrt{K}$ has Q^2V>limit

where K is the number of X-variables and Q^2V is the fraction of variation of a variable, x_k : $Q^2V = (1.0 - PRESS/SS)_k$ for each variable k

Another criterion for significance is if the component's eigenvalue is greater than 2.0. It should be noted here that SIMCA also computes the R^2 or adjusted R^2 depending on which is chosen. In this case the adjusted R^2 was chosen and this is simply the fraction of variance of all the X's explained by the current component.

Once the vectors t and p^T are found they can be plotted two dimensionally to reveal any intrinsic patterns present in the data. Plots of the score vectors, t_1, t_2 and occasionally t_3 are usually all that is required to demonstrate differences among plates. Score plots display the observations in the new coordinate system defined by the PCA model where each axis is mutually perpendicular. Loading plots of p_1, p_2 and if necessary, p_3 should also be included to fully describe the data. A plot of the loading vectors indicates which of the variables are important in the approximation of the X matrix and corresponds to the directions in the score plot.

Upon conversion, the NIPALS solution is equivalent to that determined by eigenanalysis (Geladi and Kowalski, 1986). For the sake of completion, this method is described below.

The first step of the eigenanalysis method is to determine the sum of the squares of the cross products matrix R by multiplying the matrix X by its transpose as follows:

$$R_{xxx} = X_{xxn} X^T_{nxx}$$

This produces a matrix with an equal number of rows and columns. Then the eigenvalues λ_i and eigenvectors u_i are computed for R. One can combine these n eigenvectors, which are column vectors each with n elements, by letting them be columns in an $n \times n$ matrix and obtain the orthogonal matrix of eigenvectors U. At this point the eigenvectors may be normalised such that their inner product or sum of squares equals 1; that is,

$$u_i^T u_i = 1$$

which is done by calculating a scaling factor k for each eigenvector by the following equation:

$$k_i = \frac{1}{\sqrt{\sum_{q=1}^{n} u_{qi}^2}}$$

Then, for each eigenvector

$$u_i = k_i \begin{bmatrix} u_{1i} \\ u_{2i} \\ \mathrm{M} \\ u_{ni} \end{bmatrix}$$

The new coordinates can be computed by multiplying the orthogonal matrix U by the data matrix X:

$$Y_{xxn} = X_{xxn} U_{nxn}$$

This results in a matrix of the original $s \times n$ dimensions only now each row s corresponds to one principal component axis and the coordinates of each data point are no longer the values used to describe colour development (i.e. optical densities), but weighted sums of all the values in the microwell (Ludwig and Reynolds, 1988, Pielou, 1984). Plotting the first two principal components will give a two dimensional plot with n points (this is equivalent to plotting the loading vectors p_1 and p_2 computed by the NIPALS method). If all the points are plotted one would see that the pattern of the points relative to one another is unchanged. Essentially, the only change that has occurred is that the entire swarm as a single entity has been rotated about its centre of gravity, which is the origin of the new coordinate frame. In the same sense, plotting the eigenvectors u_1 and u_2 is equivalent to plotting the scores t_1 and t_2 determined by the NIPALS method.