THERMOPHILIC AND MESOPHILIC AEROBIC BIOLOGICAL TREAMENT OF WASTEWATER

By

SHAWN LANGEVIN

A thesis submitted in conformity with the requirements for the Degree of Masters of Science in Engineering, Faculty of Engineering of Lakehead University

ABSTRACT

To achieve closed cycle operation and subsequent reuse of treated effluent, individual streams of pulp and paper mill effluents are being evaluated for their suitability for in-mill treatment. This thesis includes two individual studies. The first part focused on the feasibility of thermomechanical pulping (TMP) condensate treatment using thermophilic and mesophilic sequencing batch reactors (SBR). The second part of this thesis, based on the results of the first part and literature, developed an improved fundamental understanding of how environmental (temperature) and operating (dissolved oxygen (DO)) conditions affecting sludge properties and their role in bioflocculation and settling using a synthetic wastewater of glucose. The main results and conclusions are summarized below.

1.) The treatment of TMP condensate was feasible in terms of chemical oxygen demand (COD) removal for both thermophilic and mesophilic aerobic biological treatment. Thermophilic treatment showed COD removals of 74-84% with 13% being removed due to the aeration effects, while mesophilic demonstrated COD removals of 79-91% with 9% being removed due to aeration effects. The flocculation of the thermophilic sludge was found to be poorer than that of mesophilic sludge as evident by the higher amount of suspended particles in the treated effluent. Also, the settling of the thermophilic sludge was worse than that of mesophilic sludge as demonstrated by the amount of sludge bulking that occurred. The reason for the poorer sludge bulking is believed to be due to the higher level of filamentous microorganisms found in thermophilic sludge and the reason for poorer flocculation is unknown. Treatment of TMP condensate by thermophilic aerobic treatment is feasible in terms of COD removal; however sludge separation needs to be improved.

2.) Effects of temperature (mesophilic (35°C) vs. thermophilic (55°C)) and DO concentration (under thermophilic conditions) on sludge properties were studied using well-controlled sequencing batch reactors fed with a synthetic wastewater of glucose. Under similar dissolved oxygen level, the thermophilic sludge had a poorer flocculating and settleability than that of the analogous mesophilic sludge. Under thermophilic condition, an increase in DO level led to a slightly improved flocculating ability and a poorer settleability. A poorer settleability was related to a higher level of filaments. Analysis of extracellular polymeric substances (EPS) using chemical analysis indicates that thermophilic sludge had a higher level of total EPS than that of mesophilic sludge under similar DO level, and an increase in DO resulted in an increase in total EPS in thermophilic sludge. In the thermophilic system, an increase in sludge volume index correlated to an increase in total EPS, and a decrease in effluent suspended solids correlated to an increase in total EPS. But these correlations disappeared when the results of the mesophilic system are included, this is understandable due to the difference in microbial community between thermophilic and mesophilic systems. The results suggest that either the electrostatic interactions or the quantity of total EPS can not explain the differences in flocculating behavior between thermophilic and mesophilic sludge, pointing in the direction of the specific roles of individual EPS molecule governing flocculation in different biological systems. The strategy of increasing DO level can not solve the biomass separation problems associated with thermophilic sludge.

ACKNOWLEDGEMENTS

I am grateful to my supervisor Dr. B. Q. Liao, for introducing me into the challenging field of biological wastewater treatment and for his inspiration and direction throughout the course of this study and preparation of this thesis. This study is supported by Natural Sciences and Engineering Research Council of Canada (NSREC). I would like to thank Mr. Devin Kelly for all his help with lab work and collection of data. I would also like to thank Mr. Gary Rathje (lab technician), Allan Mackenzie and Ain Raitsakas (LU instrumentation lab) for all their help with equipment and analytical tools needs during my study. I would also like to thank Dr. Hongjun Lin (post doctorate) and Weijue Gao (PhD student) for their help with lab work and analysis. Finally, I would like to thank Abitibi Bowater Canada Inc, Thunder Bay for their support and for supplying us with the TMP condensate used during the study

Table of Contents

LIST OF TABLES

LIST OF FIGURES

Figures **Page**

1.0 Introduction

1.1 Current problems associated with pulp and paper wastewater treatment

The pulp and paper manufacturing process utilizes large volumes of water and chemicals, thus produces large volumes of wastewater. It has been found the amount of wastewater produced can be as high as 60 m^3 per tonne of paper produced, depending on the type of pulping processes used in the plant (Thompson *et al.*, 2001). The pulp and paper manufacturing processes utilize large amounts of energy and is a significant source of environmental pollution, with organic pollutant concentrations (COD) being as high as 11000 mg/L (Thompson *et al.*, 2001). Pulp and paper effluents contain many pollutants that have a negative impact on the natural environment (air and water) that include: organics, inorganics, volatile organic pollutants, color and toxicity causing chemicals (Environment Canada, 1999).

With growing concern over the health of the environment, regulations on effluent discharge limits have grown stricter with large penalties for violations of these regulations (Environment Canada, 1999). These regulations have forced pulp and paper mills to pay more attention to effluent quality prior to discharge. This attention has lead to a re-evaluation of existing effluent treatment technologies that exist in the mill. An option that most pulp and paper mills have adopted, in order to meet government regulations, is to install biological treatment processes as a secondary treatment system.

The reduction of the use of fresh water is an attractive idea and thus pulp and paper mills have recently been exploring options that can be adopted in order to achieve the reduction of amount of water that is utilized during the pulping process. This can be done by improving both the pulp and paper manufacturing process or improving the waste water treatment process to improve the effluent quality such that the water can be reused within the paper manufacturing process as opposed to being discharged to the environment. A novel idea that has been conceived for certain types of pulp and paper mills is the idea of a zero liquid effluent, where no waste water effluent is discharged to the environment, it is all reused within the mill, it has also been referred to as complete closed cycle operation (Wisemen and Ogden, 1996; Vogelaar *et al.*, 2002). In order to achieve zero liquid effluent, the treatment of each individual waste water stream needs to be studied. The treatment of individual Kraft and thermomechanical pulping (TMP) waste water streams have been studied (Rintala and Lepistö, 1992; Barr *et. al.*, 1996; Berube and Hall, 2001; Jarhen *et al.*, 2001).

Due to the implementation of treatment technologies that allow for water system closure, an overall decrease of the volume of water utilized in Canadian pulp and paper mills has been found (Elliot and Voss, 2002).

1.2 TMP wastewater characteristics and treatment options

The TMP process accounts for 40% of the pulp and paper mills in Canada (Bosker, 2007). The TMP process generates a considerable amount of wastewater, which is highly polluted and requires treatment before discharging into the receiving water. Among the various TMP wastewater streams, TMP condensate is produced when wood chips are mechanically ground under pressure and at temperatures in excess of 100°C (Artamo, 2000). Due to the pressure and heat being applied, the moisture content contained in the wood is evaporated and when this takes place volatile organic compounds (VOCs) are released into the treatment water. TMP condensate mainly consists of volatile organic oils, methanol, acetic acid, formic acid, and pulp fibres (Conrad and Huck, 1996; Artamo, 2000). In striving closed cycle operation of pulp and paper mills, individual wastewater streams have to be evaluated for their suitability to in-mill treatment and subsequent reuse.

Currently steam stripping and mesophilic biological treatment are the most common methods used to treat pulp and paper wastewaters. To date, there are many options for treating TMP wastewaters:

- Activated Sludge Process (ASP)
- Anaerobic Biological Treatment
- Aerated Lagoons
- Aerobic Biofilters
- Ozonation and Oxidation
- Aerobic High Rate Compact Reactors
- Aerobic Moving Bed Biofilm Reactors
- Aerobic Suspended Carrier Biofilm Reactors

Details of pulp and paper wastewater treatment will be presented in the second Chapter: literature review.

1.3 Motivation for current study

In order to achieve pulp and paper mills operating in a completely closed cycle, there is increased interest in investigating the feasibility of treating individual waste streams for subsequent reuse throughout the mill. If process waters were reused throughout the plant, the amount fresh water required would decrease. Also, process water from a thermophilic treatment system would eliminate the pre-cooling and post-heating processes and thus save energy.

Also, Treatment technologies that are currently in place (steam stripping and aerobic mesophilic biological treatment) are efficient but suffer from limitations that could be remedied by using newer treatment technology such as thermophilic biological treatment.

In theory, operation of a wastewater treatment system under thermophilic (50-55°C) conditions would have advantages over mesophilic (30-35°C), such as: higher reaction rate, lower sludge yield, excellent process stability, and energy savings. Studies have been conducted on the feasibility of using thermophilic biological treatment as opposed to traditional mesophilic treatment (Tripathi and Allen, 1999; Vogelaar *et al.*, 2002; Vogelaar *et. al.*, 2005). Generally, the research shows that thermophilic treatment is comparable to mesophilic treatment in terms of COD removal but suffers from poorer sludge settling and higher turbidity in treated effluent. It is therefore desirable to understand what factors affect the settleability and flocculating ability of thermophilic sludge and develop strategies to improve settleability and flocculating ability of thermophilic sludge. Well-controlled studies are needed to explain the relationship between temperature, DO and flocculation and settling in order to understand the observed difference between thermophilic and mesophilic treatment systems.

1.4 Research objectives

The overall objective of this study was to develop better treatment technologies for pulp and paper wastewater treatment to achieve closed cycle operation. Specific objectives include:

1. To investigate the feasibility of using sequencing batch reactors for TMP condensate treatment under thermophilic (55°C) and mesophilic (35°C) temperatures. COD removal efficiency, sludge settleability and flocculating ability under thermophilic and mesophilic temperatures were evaluated under various hydraulic retention times (HRTs). Floc characteristics such as floc size, filaments, and zeta potential, were studied.

2. To study the effects of temperature (thermophilic vs. mesophilic) and dissolved oxygen (under thermophilic conditions) on sludge properties and the role of sludge properties in flocculation and sludge settling using synthetic wastewater of glucose. Sludge properties such as extracellular polymeric substances (EPS), filament content, floc size and zeta potential were analyzed in order to explain the reasons for poor flocculation and settling under thermophilic conditions. The effect of dissolved oxygen levels on the sludge properties was also studied in an attempt to surpress the growth of filamentous bacteria which hinder settling.

2.0 Treatment Technologies for Pulp and Paper Wastewater

There are two categories of treatment technologies when it comes to the treatment of wastewater, these technologies are physiochemical treatment and biological treatment. The focus of this literature study was biological treatment of wastewater and more specifically pulp and paper wastewater (TMP wastewater). The topics of importance for this study include biological treatment technologies, treatment of TMP wastewater, advantages and disadvantages of thermophilic biological treatment compared to mesophilic biological treatment, and effect of environmental and operating (temperature and dissolved oxygen) conditions on sludge properties and performance.

2.1 Biological Treatment

Biological treatment involves the treatment of water using microorganisms that consume the organic material that is contained in the wastewater. Using the organic contaminants and nutrients (nitrogen, phosphorus and micronutrients) the bacteria produce new cells and gases (methane and carbon dioxide) that can be utilized for further applications. There are two classes of bacteria that can be used for biological treatment: bacteria that require oxygen (aerobic) and bacteria that do not require oxygen (anaerobic). Both types of bacteria have been studied for potential application and improvement of biological treatment. Laboratory-scale studies have been conducted in an attempt to combine aerobic and anaerobic treatment technologies to take use the advantages of both types of treatment and minimizing their disadvantages.

2.2 Anaerobic Biological Treatment

Anaerobic digestion is frequently used for secondary treatment of industrial wastewater (Pearson, 1990). Generally, in terms of biological treatment processes, anaerobic treatment is considered to be the most suitable method for the treatment of high strength wastewater. Before the 1980s, anaerobic treatment was limited due because at the time most pulp and paper waste waters were low strength but with the introduction of new processes a need to treat high strength wastewater became apparent (Bajpal, 2000; Pokhrel and Viraraghavan, 2004). Anaerobic treatment has some attractive advantages over aerobic treatment such as (Thompson et. al, 2001) lower sludge production, lower chemical usage and consumption, smaller land requirements due to smaller reactor volumes, and methane production from anaerobic digestion can be used as a fuel source.

Anaerobic technologies that are currently used to treat pulp and paper waste streams include: anaerobic filters, up-flow sludge blanket, fluidized bed, anaerobic lagoons and anerobic contact reactors. Anaerobic treatment has been studied for a variety of pulp and paper wastewater types such as: debarking, TMP, CTMP, Kraft Condensate, Chlorine bleaching and Sulfite spent liquor with all showing promising results of 50-90% COD removal (Rintala and Puhakka, 1994).

2.3 Treatment of Thermomechanical Pulping (TMP) wastewater

The treatment of TMP wastewater can be very difficult because TMP wastewater tends to contain contaminants that are very difficult to degrade such as resin acid, fatty acids and wood extractives. A characteristic of TMP wastewaters is that the solubilisation of resin acids can be increased due to the intense heat and mechanical action required in the pulping process. This causes increased contaminant concentrations in TMP effluents (McLeavy, 1987). The sources of contaminants of the TMP process include: components of wood that are dissolved in the process water – hemicelluloses, pectins, lignin, extractives and inorganic salts, process chemicals from mechanical pulping and bleaching, possible lubrication and sealant leaking into process water, papermaking chemicals that have been recirculated back to pulping process and organic or inorganic chemicals that came in with fresh water intake and contamination from the wood debarking process (Artamo, 2000).

A component of importance to remove is resin, which refers to a mixture of components which are present in wood chips, such as steroids, waxes, glycerides, resin acids, terpenes and fatty acids (Backlund *et al.*, 1990).

The removal process of resin from the pulp is a complicated process including: the pulp has to be diluted to 3-5% consistency, the pulp is dried for 15-30 minutes at a temperature of 50^0C - 80^0C, then the pulp is pressed to a dryness of about 30%, the pressed water is then treated for the resin before reuse (Grant *et al.*, 2001).

At present, a number of technologies have been tested for TMP wastewater treatment (Pokhrel and Viraraghavan, 2004). The activated sludge process (ASP) has been extensively studied (Shere and Daly, 1982) with varying results and is a well developed technology. Aerated lagoons are a technology that has been proposed, but to date has not been well studied into the feasibility to treat TMP wastewater specifically (Welander *et al.*, 1997). Aerobic biofilters for treating TMP wastewater has also been studied with promising results (Kantarddjieff, 1997). Ozonation and oxidation have been tested as means to treat TMP waste streams (Laari *et al.*, 1999; Verenich *et. al.*, 1999; Korhonen and Tuhkanen, 2000; and Ledakowicz *et. al.*, 2006). Anaerobic treatment has been studied specifically for application to the treatment of TMP wastewater with all showing promising results in terms of COD removals. Semicontinous batch digesters and upflow anaerobic sludge blanket (UASB) reactors were used for TMP whitewater with COD removals of 65-75% (Rintala and Lepisto, 1992). Jahren *et al.* (1999) investigated the treatability of TMP effluent for both anaerobic and aerobic biological treatment and found COD removals of 84% and 86% respectively. An anaerobic-aerobic biofilm reactor operating under thermophilic conditions has been studied for the treatment of TMP whitewater showing results of 60% COD removal (Jahren and Odegaard, 1999). Nitrogen fixating bacteria was used to treat TMP wastewater showing 82-87% COD removal (Slade, 2001).

In recent years, novel aerobic processes for treating TMP wastewater have been well studied. These include aerobic high rate compact reactors (Magnus *et al.*, 2000), moving bed biofilm reactors (Jahren *et al.*, 2001), suspended carrier biofilm reactor (Assenlin *et al.*, 2000) and biological high-efficiency compact reactors (Magnus *et al.*, 2000), which have all shown promising results. A combination of anaerobic and aerobic technologies has been attempted in order to take advantage of the benefits of both types of systems. Anaerobic pre-treatment before aerobic bioreactors (Jahren and Oedegaard, 1999) and aerobic pretreatment prior to anaerobic bioreactor treatment (Shaw *et al.*, 2002) have been studied with promising results, however more work is needed in this area as well before implementation. Although a number of studies have been conducted to treat TMP wastewater - a combination of various streams generated in the TMP process (Jahren *et. al.*, 1999). To the best of our knowledge, no reports are available for the treatment of TMP condensate specifically.

A combination of anaerobic pre-treatment with aerobic post-treatment of TMP wastewater has been studied under thermophilic conditions (55°C). Anaerobically pre-treated then aerobically treated TMP wastewater showed an overall removal of COD of 65-75% (Rintala and Lepisto, 1992). A study using anaerobic pre-treatment followed by an aerobic moving bed biofilm reactor found overall COD removals of 60% (Jahren and Oedegaard, 1999).

Generally TMP mill effluents are difficult to treat and thus require very large and expensive biological treatment systems to achieve acceptable effluent quality. Usually TMP wastewater treatment includes biological secondary treatment for TMP effluents. It has been reported that resin and fatty acids removal has been successfully achieved (79-99%) (Liver and Hall, 1996a; Liver and Hall, 1996b). Resin and fatty acids are removed as pollutants and are not useful for any industrial application.

TMP waste streams have been shown to be efficiently treated by the utilization of biological process, however most studies have been conducted using TMP whitewater, which refers to all waters of a paper mill that have been separated from the stock or pulp suspension, either on the paper machine or accessory equipment, such as thickeners, washers, save-alls, and from pulp grinders. It carries a certain amount of fiber and may contain varying amounts of fillers and dyestuffs and it would be attractive to investigate the treatability of individual streams such as TMP condensate and pressate for closed cycle operation and subsequent reuse.

2.4 Thermophilic and Mesophilic biological treatment

In recent years, thermophilic aerobic biological treatment of pulp and paper effluents has received much attention (Berube and Hall, 2001; Dias *et al.*, 2005). The advantages of thermophilic treatment include low sludge yield, high reaction rate, and excellent process stability (Lapara and Alleman, 1999; Tripathi and Allen, 1999; Morgan-Sagastume and Allen, 2003). The lower sludge yield is a result of a higher rate of decays at thermophilic temperatures, due to this the cost of handling the sludge for storage and treatment is decreased (Berube and Hall, 2001; Rozich and Bordacs, 2002). The high reaction rate allows for a decrease in hydraulic retention times and an increase in organic loading rates (OLRs) (Lim *et al.*, 2001; Rozich and

Bordacs, 2002). It has also been found that thermophilic treatment is more efficient than mesophilic treatment at removing specifically targeted compounds which allows for the production of a better quality effluent (Tripathi and Allen, 1999; Berube and Hall, 2001). Studies have found thermophilic systems are comparable to mesophilic systems in terms of BOD and COD removal in pulp and paper mill effluents and have been found to tolerate varying operational conditions such as temperature, pH and flow rates more effectively than mesophilic systems. However, mesophilic sludge has been found to have much better settling properties and effluent quality than that of thermophilic sludge (Lapara and Alleman, 1999; Thompson *et. al.*, 2000; Vogelaar *et. al.*, 2002).

Research has found that overall thermophilic effluent demonstrates more turbidity than mesophilic (Tripathi and Allen, 1999). A higher fraction of small particles such as free bacteria as a result of increased rate of cell lysis and small colodial particles, have been found in thermophilic effluents, which tends to cause a higher residual COD. Suvilampi and Rintala (2003) reported that a possible reason for the higher effluent turbidity and higher residual COD in thermophilic treatment systems is due to the absence of higher organisms in thermophilic sludge, which have been credited with the removal of free bacteria in mesophilic conditions. However, research is underway to develope heat resistant microorganisms that could be used in thermophilic treatment systems. Biomass retention has proven to be more difficult under thermophilic conditions, some possible reasons could be that under thermophilic conditions flocculation is poorer than that of mesophilic and that sludge settling has been found to be poorer as well. Poor flocculation leads to a sludge being retained in the effluent and when the effluent is discharged, a loss of biomass occurs. Also, due to the higher levels of filamentous bacteria, the settling ability of thermophilic sludge tends to be poorer than that of mesophilic sludge and poor settling could lead to sludge bulking, which could also lead to loss of biomass. More research is needed in the area of factors effecting flocculation and sludge settling to gain a better understanding of the phenomenon at work.

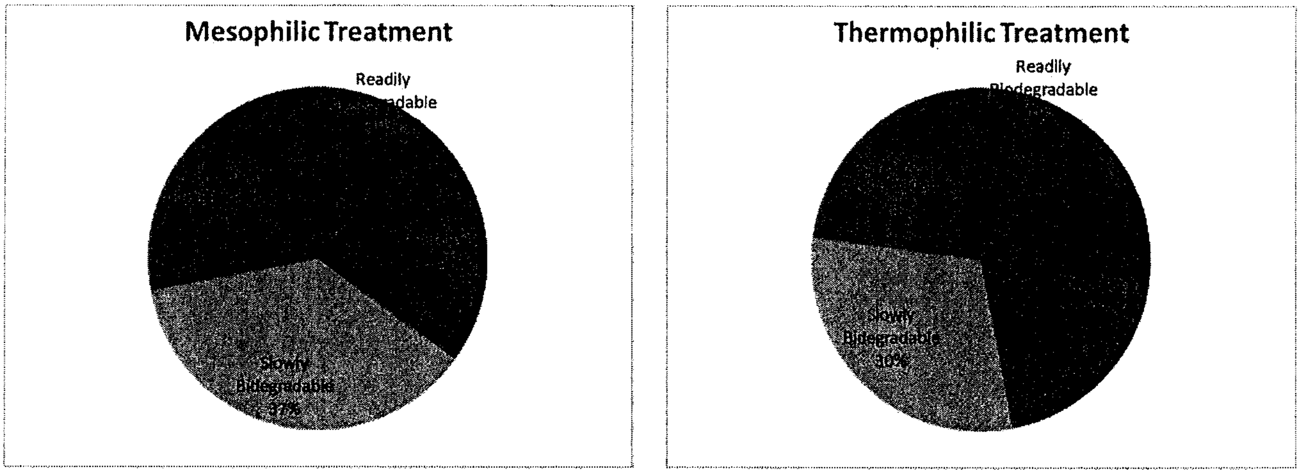


Figure 2.4.1 – Comparison of Mesophilic (35^{0}C) and Thermophiic (55^{0}C) biodegradation of COD (Vogelaar *et al.*, 2002).

A study was conducted investigating the feasibility of utilizing an activated sludge treatment process to treat anaerobic effluent (Vogelaar *et al.*, 2002). Figure 2.4.1 shows that thermophilic conditions allow for a higher inert COD fraction but lower slowly and very slow biodegradable COD fractions than that of mesophilic conditions. This comparison suggests that the degradation of COD is less efficient under thermophilic conditions than it is under mesophilic conditions due to the higher amount of inert COD that is produced under thermophilic conditions.

In order to understand why the observed differences between mesophilic and thermophilic performance, a study was conducted assessing the performance of thermophilic and mesophilc activated sludge (Vogelaar *et al.*, 2005).

Factors that are believed to effect the amount of filament bacteria growth in wastewater treatment systems include temperature dependence of the hydrophobic interactions, effect of temperature, biomass type and dissolved oxygen, zeta potential, hydrophobicity and particle size distribution (Vogelaar *et. al.*, 2005).

The type of wastewater that was studied was anaerobically pre-treated paper process water from a completely closed water system. The study confirmed as expected that mesophilc and thermophilic sludge demonstrate a significant difference in bioflucculation ability. It was found that under mesophilic conditions bioflocculation occurs when aerobic biodegradation of the effluent COD has been shown to have occurred. Under thermophilic conditions it was found that very little bioflocculation occurred at all no matter if biodegradation had occurred. In terms of temperature dependence on the hydrophobic reactions, no correlation was found to be able to conclude it is a major factor. It was found that thermophilic sludge contained a larger portion of small particles and were less resistant to shear forces than that of mesophilic sludge which has been found by other researchers as well (Vogelaar *et al.*, 2005). The study concluded that it is most likely the difference in sludge flocculation was mainly due to changes in extracellular polymeric substances (EPS), with temperature, that are found on the outside of the cell wall (Vogelaar *et. al.*, 2005). But the EPS of thermophilic sludge has not been well studied.

Thermophilic aerobic wastewater treatment has been studied and tested for a variety of types of wastewater to replace traditional mesophilic treatment. The studies that have been conducted range in scale from many laboratory studied to a few pilot scale studies to even a few full scale studies (Suvilampi and Rintala, 2003). The types of treatment processes that have been tested for thermophilic treatment include biofilm reactor, activated sludge process and batch and continuous stirred tank reactors (Suvilampi and Rintala, 2003). Many different types of wastewaters have been studied mainly on a lab-scale with COD removals being reported as 15-95% depending on the type of wastewater in question. This leads to the observation that not all types of wastewater are equally feasible to be treated thermophilically. Types of wastewater that have shown favourable results with thermophilic aerobic treatment include are shown below in table 2.4.1.

Table 2.4.1 Thermophilic Aerobic Wastewater Treatment

Type of Wastewater	Source
Slaughterhouse Effluent	Gariepy *et al.*, 1989
Distillation Residue from Potato Slop	Cibis *et al.*, 2002
Fish Food Waste	Rozich and Bordacs, 2002
TMP Whitewater	Rintala and Lepisto, 1992
Kraft Mill Effluent	Barr *et al.*, 1996

A number of studies have been conducted with the objective of directly comparing operation of thermophilic and mesophilic treatment in terms of COD removal and sludge settling, these can be seen below in table 2.4.2.

Table 2.4.2 Comparison of Mesophilic and Thermophilic Systems

Type of Wastewater	Source
Slaughterhouse Effluents	Couillard and Zhu, 1993
Bleached Kraft Mill Effluent	Barr *et.al,* 1996
News Print Whitewater	Tardif and Hall, 1997
Pulp and Paper Effluent	Malmqvist *et.al,* 1999
Bleached Kraft Mill Effluent	Tripathi and Allen, 1999
Pharmaceutical Waste	Lapara *et.al,* 2001
Kraft Mill Effluent	Suvilampi and Rintala, 2001
Anaerobic Pretreated Paper Process Water	Vogelaar *et.al,* 2002a
Recycled Paper Mill Effluent	Vogelaar *et.al,* 2002b
Synthetic Acetic Acid Feed	Vogelaar *et.al,* 2003
Anaerobic Pretreated Paper Process Water	Vogelaar *et.al,* 2005

The most commonly used treatment systems are sequencing batch reactors (SBR) and activated sludge process (ASP) (Suvilampi and Rintala, 2003). All of the studies came to the conclusion that mesophilic treatment demonstrates a higher rate of COD removal which is consistent with well known theory. It has been found although thermophilic treatment systems tend to demonstrate a lower COD removal, some specific compounds such as long-chain fatty acids, are much more efficiently removed under thermophilic conditions (Tripathi and Allen, 1999).

The characteristics of thermophilic aerobic biological treatment make it an attractive treatment alternative when compared to mesophilic aerobic biological treatment. However, there are still some problems in terms of sludge flocculation and settling that reduce the overall efficiency of the thermophilic treatment process and further work is needed to fully understand why this occurs.

2.5 Flocculation and Settling in Thermophilic and Mesophilic Biological Treatment

2.5.1 – Flocculation of Thermophilic and Mesophilic Sludge

Many studies have been conducted on the feasibility of using thermophilic biological treatment as an alternative to traditional mesophilic biological treatment. The results usually show that in terms of COD removal, thermophilic biological treatment is comparable to mesophilic biological treatment, however in terms of effluent quality; mesophilic treatment is much better than thermophilic treatment (Lapara and Alleman, 1999; Thompson *et. al.*, 2000; Vogelaar *et. al.*, 2002).

The causes of the observed effluent turbidity under thermophilic conditions are still unknown, however there have been theories developed to explain the higher turbidity. Vogelaar *et al.*

(2002) found that it is possible for a thermophilic treatment system to produce a clear effluent, if the effluent contains very little colloid material, suggesting that effluent turbidity could be caused by colloidal material that is contained in the raw wastewater and is not retained in the sludge during treatment.

Another suggested reason for poor flocculation is the absence of protozoa bacteria and other higher life forms of bacteria in the thermophilic sludge when compared to mesophilic sludge (Lapara and Alleman, 1999). This is because protozoa are known to consume activated sludge flocs and target free bacteria as a food source, which would remove the free bacteria that was not able to flocculate in the sludge (Ratsak *et al.*, 1996).

Vogelaar *et al.* (2002) suggest that the hydrophobic interaction of sludge particles is decreased by the change in temperature and therefore could result in a decrease in flocculation and settling performance of the sludge.

Vogelaar *et al.* (2002) conducted a study to study the effect of both the effluent colloidal material and the role of protozoa in the both mesophilic and thermophic treatment systems. The results of the study showed that the effluent turbidity in the thermophilic treatment system was caused by both the influent colloidal material that were not retained in the sludge and due to erosion (cell lysis) of the thermophilic sludge which causes small free bacteria to be released into the effluent. The DGGE analysis showed that the microbial communities of the thermophilic and mesophilic reactors were vastly different; however, the effect of protozoa on effluent turbidity was negligible for both the thermophilic and mesophilic system leading to the conclusion that it is not a determining factor in the observed difference in effluent quality.

2.5.2 – Settling of Thermophilic and Mesophilic Sludge

A major problem that has been found with thermophilic treatment systems is that sludge bulking has been found to occur. Sludge bulking is an indication that sludge settling is poor and has been frequently found to occur under thermophilic conditions only (Lapara and Alleman, 1999; Thompson *et. al.*, 2000; Vogelaar *et. al.*, 2002). Sludge bulking causes undesirable amounts of sludge to be discharged with the treated effluent which can lower the designed solids retention time for the treatment system which can affect treatment efficiency (Thompson *et al.*, 2001). Sludge bulking has caused operational problems since 1920s in activated sludge plants (Morgan and Beck, 1928). Bulking is said to have occurred when the sludge volume index is higher than 120 mL/g MLSS (Thompson *et al.*, 2001). The mechanisms that govern floc formation are still widely unknown, however it is believed that filamentous bacteria form a matrix that allow for floc forming bacteria to attach and start to settle, settling problems occur when filamentous bacteria start to grow out from the floc (Sezgin *et al.*, 1978).

Although it is still unknown what cause or suppresses the growth rate of filamentous bacteria, there have been some studies conducted in order to try to quantify the effect of operating conditions on the growth of certain known filamentous bacteria. Some conditions believed to promote the growth of filamentous bacteria include: low dissolved oxygen (< 2 mg/L), low organic loading, nitrogen or phosphorus deficiency and increase sulphide concentrations in wastewaters (Jenkins *et al.*, 1986; Wanner, 1994).

Many theories have been developed to try to explain the causes of filamentous bacteria growth in wastewater treatment sludge, these include: kinetics, accumulation-regeneration, starvation and nutrient deficit, sludge age, dissolved oxygen and temperature have all be theorized to effect filament growth (Dalentoft and Thulin, 1997; Thompson *et al.*, 2001).

2.5.3 – Extracellular Polymeric Substances (EPS) and their role in Settling and Flocculation

Recently, a factor that has been considered to possibly be very important to explain the flocculation process is the extracellular polymer substance (EPS) production and composition. EPS was originally defined as any polysaccharide or peptidoglycan structure of bacteria origin lying outside the cell membrane (Schmidt and Ahring, 1994). It is now widely accepted that EPS now include proteins and other macromolecules that exist in a wide variety of environments (Shin *et al.*, 2001). It has been found that EPS is essential for floc formation in both aerobic and anaerobic sludge; however aerobic sludge tends to have higher protein content (Morgan *et al.*, 1990; Schmidt and Ahring, 1994; Jia *et al.*, 1996). It is believed that the EPS acts as glue in order to aid in the flocculation process, whereby it holds the cells together (Liao *et al.*, 2001; Li and Yuan, 2002). The EPS are considered to be a significant contributor to the overall sludge mass and allow for linkages in biofilms, flocs and granules (Jia *et al.*, 1996; Hakkarainen and Sillanpaa, 2007). It has also been found that the quantity of EPS is directly related to the zeta potential of the sludge flocs (Mikkelsen and Keiding, 2002) and the charge value is dependent on the composition of EPS in the sludge flocs (Thompson and Forester, 2003). The EPS is believed to be the most important factor with respect to floc structure and shear sensitivity due to the floc stabilizing ability of the EPS to form stable gel networks surrounding the sludge formation (Mikkelsen and Keiding, 2002; Neyens and Baeyens, 2003). EPS has two origins in a biological wastewater treatment system: EPS can be released from the cells by metabolic exertion or by cell lysis or the raw wastewater itself could contain EPS to begin with (Hakkarainen and Sillanpaa, 2007).

EPS is able to either hinder or aid in cell flocculation, the internal hydrophobicity of the cells is higher if the EPS concentration is lower and thus the flocculation is improved (Hakkarainen and Sillanpaa, 2007). However if the EPS concentration is too high, the steric effect and gel formations may hinder flocculation (Neyens and Baeyens, 2003). High quantities of EPS also cause the weakening of the bond between cells in the sludge flocs which can cause greater cell erosion in turbulent environments. Also, loosely bounded EPS tend to contain large amounts of bound water, therefore with increased amounts of loosely bounded EPS there is an increase in the amount of bounded water in the floc structure. An increase in bounded water in the floc structure can cause a weakening of floc structure and also cause poor settling (Hakkarainen and Sillanpaa, 2007; Li and Yang, 2007). EPS can also affect the hydrophobicity of the cells because the EPS are negatively charged, EPS could make the cells surface more negatively charged and therefore appear to be more hydrophobic due to interaction with inorganic cations. EPS is also known to be a problem when using a membrane based reactor for the treatment of wastewater due to the negative charge on the EPS, it will attach onto the membrane surface causing a cake layer that lowers the overall efficiency of the membrane (Shin *et al.*, 1999). It is believed that by altering the process or environmental conditions, it may be possible to alter the EPS concentration and composition to a more favourable state (Neyens and Baeyens, 2003).

Recently studies have started to focus on the relationship between EPS, operation conditions (temperature and dissolved oxygen) and overall performance of the biological treatment system. The focus has been looking at the effect of dissolved oxygen (controlled by increasing the aeration intensity) on EPS production, flocculation and settling of the sludge. Studies have found that the dissolved oxygen has a drastic effect on the both the sludge volume index and carbohydrate concentration of the sludge (Shin *et al.*, 2000). In a short-term study, it was found that increasing the dissolved oxygen causes the carbohydrate concentration to increase and protein concentration stays relatively constant thus increasing the C/P ratio (Shin *et. al.*, 2000; Shin *et. al.*, 2001; Kim *et. al.*, 2004). The dissolved oxygen tends to cause more carbohydrate to form and thus allows for more bounded water to be held in the floc (Hakkarainen and Sillanpaa, 2007; LI and Yang, 2007) which then causes the settling to decease and thus the SVI increases. Shin et. al, (2001) also suggest that the type of EPS is more important than the quantity of the EPS, thus meaning further work should be done looking at the specific polymers that exist in the EPS.

The effect of temperature on the production and quantity of the EPS is an important phenomenon to explore in order to fully understand the observed differences between thermophilic and mesophilic aerobic biological treatment systems. The effect of temperature on the EPS production and quantity has only been studied in a limited capacity for all types of wastewater treatment systems including pulp and paper wastewater treatment. That is why it was desirable to conduct a study to correlate the relationship between system temperature and EPS formation. Gorret et al. (2001) looked the effect of temperature, pH and yeast extract on the growth of EPS production. The results showed that the optimal pH range for maximum EPS growth was in the range of 6-6.5. The results show that temperature did have an effect on the amount of EPS that was produced; the temperature was tested in the range of 19 – 40 °C. It was found that the growth yield of EPS was increased when the temperature was lower which would suggest under thermophilic temperatures the amount of EPS produced would be lower than at mesophilic conditions although this has not been researched.

2.6 Summary of Literature Review

Based on the literature review of publications in this field, conclusions about the present state of anaerobic biological treatment, treatment of pulp and paper wastewater with aerobic biological treatment, treatment technologies for TMP wastewater specifically, thermophilic aerobic biological treatment and effect of environmental conditions on sludge performance can be summarized as follows:

Anaerobic biological treatment has traditionally been used for industrial wastewater but the application for pulp and paper wastewater did not become prominent until 1980. Anaerobic biological treatment is considered the best option for treatment of high strength wastewater and has advantages over aerobic biological treatment such as: low sludge production, low chemical consumption, low space requirements and methane production for energy. Anaerobic biological treatment is an attractive technology option that requires further research.

TMP whitewater has been studied extensively with both anaerobic biological treatment, aerobic biological treatment and a combination of both types of treatment with varying but some promising results being found. During the course of the literary review, no studies involving TMP condensate specifically was found. TMP wastewater is a significant effluent to be treated due to the properties of TMP wastewater: high strength, high temperature and contains chemicals that are highly toxic to marine life. Many different types of reactors have been tested for TMP wastewater treatment; however the potential of using sequencing batch reactors to treat TMP wastewater is so far unknown.

Thermophilic biological treatment has become a widely researched topic in the field of biological wastewater treatment due to its advantages over traditional mesophilic treatment: low sludge yield, higher reaction rate, higher process stability and the ability to remove chemicals mesophilic cannot due to higher reaction and degradation rates under thermophilic conditions. Generally thermophilic biological treatment is comparable to mesophilic biological treatment in terms of COD removal, however thermophilic biological treatment usually suffers from poor sludge flocculation and settling leading to turbulent effluents. Biomass retention is also proven to be more difficult under thermophilic conditions due to high effluent turbidity and sludge bulking and washout. Technologies that allow for the treatment under thermophilic conditions but help nullify biomass separation problems such as membrane technologies have been studied with some promising results.

The major disadvantage of thermophilic biological treatment is that sludge flocculation and settling are much poorer than mesophilic biological treatment. Research into the mechanisms and sludge properties that govern sludge flocculation and settling is needed to understand options to improve the flocculation and settling of thermophilic sludge. It is believe that dissolved oxygen, aeration intensity, hydrophbicity, EPS properties and temperature play a vital role in the sludge flocculation and settling of sludge. But little studies have been conducted on thermophilic sludge properties, such as EPS production and composition, and the effect of environmental (such as temperature) and operating (such as DO) conditions on sludge properties and their role in bioflocculation and settling.

2.7 Significance of this Study

The thermophilic and mesophilic biological treatment of TMP condensate using a sequencing batch reactor has not yet been studied and it would be ideal to study the treatability of individual TMP water streams to allow for full system closure and subsequent reuse of treated effluent as process water.

The second part of the study focuses on the effect of environmental conditions such as temperature and dissolved oxygen on sludge properties such as hydrophobicity, surface charge (zeta potential), particle size, sludge morphology and EPS. This study was done to develop an improved fundamental understanding of the observed difference in settling and effluent quality between thermophilic and mesophilic biological treatment. The settling was evaluated using the sludge volume index (SVI) and flocculating ability was evaluated using effluent suspended solids (ESS). If more is known about the factors that govern the flocculation of sludge, perhaps an engineering solution can derived to allow for thermophilic biological treatment to be more efficient. Strategies, such as elevated DO level, that have been widely used in the mesophilic biological wastewater treatment for improving sludge flocculation and settling, was tested in the thermophilic sludge to improving settling and flocculation in this study.

3.0 Experimental Materials and Methods

This chapter introduces the thermophilic and mesophilic sequencing batch reactors (SBR) that were used throughout both parts of experimental work, the properties of the TMP condensate used for part 1 of the study and the synthetic feed used for part 2 of the study. The methods for data collection and reactor system analysis are also described here.

3.1 Laboratory scale sequencing batch reactor system

The laboratory experimental system consists of two parallel sequencing batch reactors (SBR) (effective volume: 1.8L/each) operated at either 55°C or 35°C with on-line pH controller (pH = 7.0) over a period of 143 days for the treatment of TMP condensate using thermophilic and mesophilic SBRS. The same experimental system was used in the second part of the research, effect of dissolved oxygen and temperature on sludge properties, as well until the aeration on the thermophilic reactor was changed to a tank containing 32% oxygen and 68% nitrogen for period of 209 days. The experimental setup is shown below in Figure 3.1.1. A refrigerator was used for storing the TMP condensate and synthetic feed that was used in both parts of the study, at 4°C. Two preheating tanks were used to increase the temperature of the synthetic feed from 4°C to 35°C and 55°C, respectively, before it entered the SBRs. Two water baths that circulated water at different constant temperatures through the jacket of SBRs, result in each SBR operated at a certain constant temperature (35 ± 1°C, 55 ± 1°C, respectively). 1L of the TMP condensate or synthetic glucose based feed was added to each SBR in each cycle. The time of filling, reaction, settling and discharging was 10, 660 (or 540, 300), 40 and 10 min, respectively. The mixing intensity in each SBR was similar by setting the same rotating speed of the magnetic stirring bar on the bottom of each SBR and the similar air flow rate (1.5L/min). For the second part of the study, the aeration in the thermophilic reactor was controlled by the flow meter. Seed of activated sludge was from the laboratory-scale SBRs treating TMP wastewater at 55 and 35°C, respectively, in a previous study. The MLSS was maintained at 2350 ± 92 and 2670 ± 123 mg/L for the thermophilic and mesophilic SBR, respectively during part 1 of the study and maintained at 2880 ± 480 mg/L, 2330 ± 170 mg/L and 3550 ± 527 mg/L for the thermophilic low DO, thermophilic high DO and mesophilic SBRs for part 2.

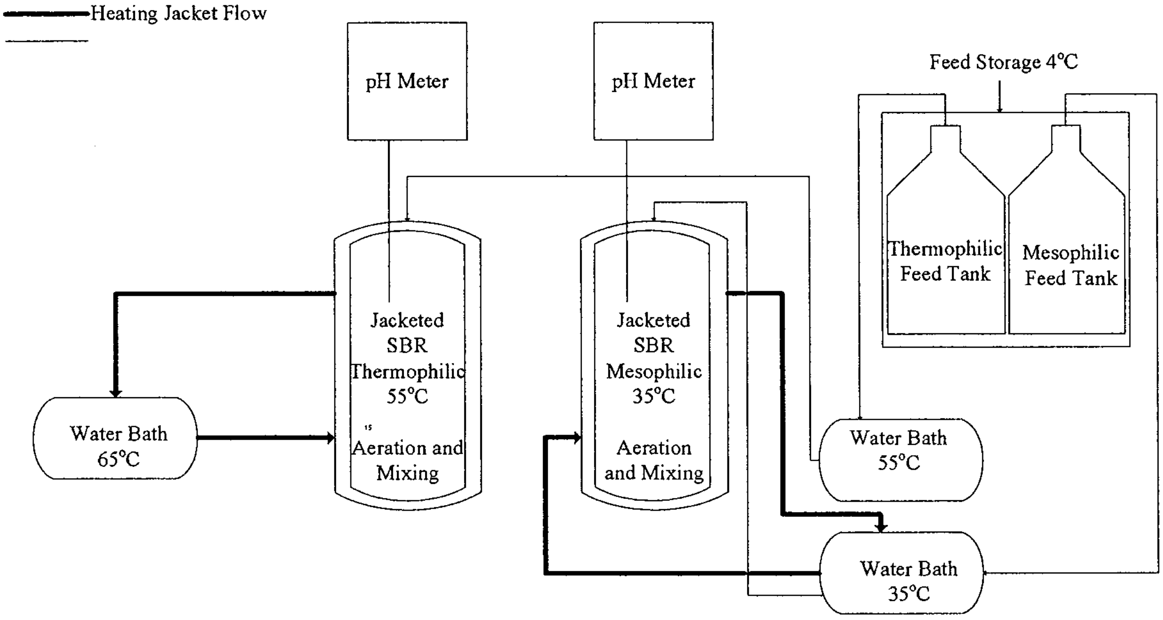


Figure 3.1.1 - Schematic of Experimental System

3.2 Experimental feed characteristics

During the course of the study, there were two wastewater feeds that were studied: TMP condensate and a synthetic glucose-based wastewater.

3.2.1 TMP condensate feed

The TMP condensate was obtained from Abitibi-Bowater Inc. in Thunder Bay, Ontario. The characteristics of the TMP condensate are shown in Table 3.2.1. Chemical analyses of the feed indicate that the TMP condensate did not contain enough nutrients for bacteria growth. Therefore, additional nutrients, including N, P and inorganic salts were added to the influent (Table 3.1.2) before biological treatment, according to a COD: N:P ratio of 100:5:1

Table 3.2.1. Characteristics of raw TMP condensate

Total COD (TCOD)	700-1150 mg/L
Soluble COD (SCOD)	550 - 1000 mg/L
Phosphorus	6.4mg/L
Nitrogen	1.2 mg/L
Ca^{+2}	11.7 mg/L
K^{+1}	3.5 mg/L
Mg^{+2}	2.56 mg/L
Na^{+1}	14.10 mg/L

Table 3.2.2. Chemical Composition of Experimental Feed

NH_4Cl	120 – 185 mg/L
K_2PO_4	25 - 45 mg/L
$NiCl_2$	0.1 μM
$CaCl_2.7H_2O$	5.0 μM
$CuCl_2 \cdot 2H_2O$	0.01 μM
$FeCl_3 \cdot 6H_2O$	5.0 μM
$MnCl_2 \cdot 4H_2O$	0.1 mM
$ZnCl_2$	0.01 μM
$CoCl_2 \cdot 6H_2O$	0.1 μM
Na_2SeO_3	0.01 μM

3.2.2 Synthetic glucose based feed

The synthetic feed that was used in the second part of the study utilized glucose as the substrate because the glucose is very readily biodegradable. The feed was made from a mixture of nutrient salts, distilled water and a glucose solution. The nutrients were used in the same quantities as part 1 that is outlined in the above Tables 3.2.2. The glucose was added in a manner that would produce a total COD of 1000 mg/L.

3.3 Experimental operating conditions

During the course of the study, the reactors systems from the two parts of the study varied slightly and the following sections outline the operating conditions that were used in each part.

3.3.1 Experimental conditions for the treatment of TMP condensate

The experimental operation conditions for the treatment of TMP condensate is shown below in Table 3.3.1.

Table 3.3.1. Operating conditions of the SBR system for the treatment of TMP condensate

	Thermophilic SBR	Mesophilic SBR
Cyclic Time (hr)	12,8,6	12,8,6
Solids Retention Time (day)	20 ± 2	14 ± 1
	14 ± 1	11 ± 1
	10 ± 1	10 ± 1
Organic Loading (kg/m^{3}d)	0.7 – 1.3	0.7 – 1.3
Operating Temp (°C)	55 ± 1	35 ± 1
Operating pH	7.2 ± 0.2	7.2 ± 0.2
Dissolved Oxygen (mg/L)	1.1– 2.6	4.0 – 6.5

3.3.2 Experimental conditions for the treatment of synthetic glucose based feed

The experimental operation conditions for the treatment of synthetic glucose based feed are shown below in table 3.3.2 and table 3.3.3.

Table 3.3.2. Operating parameters of the SBR system for the treatment of synthetic feed.

	Thermophilic Low DO	Mesophilic	Thermophilic High DO
Cyclic Time (hr)	12	12	12
Solids Retention Time (day)	10 ± 1	10± 1	10± 1
Organic Loading (kg/m^{3}d)	1.1	1.1	1.1
Operating Temp (°C)	55± 1	35± 1	55± 1
Operating pH	7.2 ± 0.2	7.2 ± 0.2	7.2± 0.2
Dissolved oxygen (mg/L)	1.0-2.5	4.0-6.0	3.5-5.5

Table 3.3.3. Experimental operating conditions for treatment of synthetic feed

	Phase 1	Phase 2	Phase 3
Thermophilic	Day 1-82 Air Aeration 1.5 L/min DO = 1.0 – 2.5 mg/L	83-176 32% O_2 + 68% N_2 Aeration 1.5 L/min DO = 3.5 – 5.5 mg/L	177-209 Air Aeration 1.5 L/min DO = 1.0 – 2.5 mg/L
Mesophilic	Day 1-209 Air Aeration 1.5 L/min DO = 4.0 – 5.8 mg/L		

3.4 Experimental Methods

3.4.1 Analytical Methods for COD, MLSS, and ESS

Mixed liquor suspended solids (MLSS), soluble chemical oxygen demand (COD), and effluent suspended solids (ESS) were determined according to Standard Methods (APHA, 2005). The MLSS and ESS were determined by drying a sample and comparing the weights of a dry filter paper with the dried sample and filter paper. The COD was determined using the closed reflux calorimetric method. The samples for these measurements were taken from the SBRs at the end of the reaction (MLSS and COD) and at the end of settling (ESS) just before discharging.

3.4.2 Sludge Volume Index (SVI)

The sludge volume index was measured using a 500 mL graduated cylinder filled with sludge and being allowed to settle for 30 mins. The volume of sludge after 30 mins is then used to calculate the SVI from the following equation:

$$SVI = \frac{V}{(MLSS * 0.5L)}(1000 mg)$$

Where:
SVI – Sludge Volume Index (mL/g Biomass)
V – Settling Volume (mL)
MLSS – Concentration of Biomass in Sludge (mg/L)

3.4.3 Sludge Morphology and Filamentous Microorganism Levels

The general sludge morphology and the abundance of filamentous microorganisms was extensively examined (3 times/week) with a light microscope (Olympus IX51 inverted microscope, Tokyo, Japan) at a magnification of the objective lens 10x. The number of filaments was classified into levels 0 to 6 according to Jenkins et al. (2003). A smaller score corresponds to a lower level of filaments.

3.4.4 Extracellular Polymeric Substance (EPS) Extraction and Chemical Analysis of EPS

The extraction of the bound EPS was carried out by the cation ion exchange method (Frølund, et al. 1996), using cation exchange resin (CER) (Dowex Marathon C, Na+ form, Sigma-Aldrich, Bellefonte, PA). A known quantity of sludge (0.25 – 0.40 g) of sludge was centrifuged at 5000 rpm for 15 mins at 4 °C, the supernatant was then collected for further analysis of the free EPS. The sludge pellets were re-suspended to their original volume using a buffer consisting of 2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9mM NaCl and 1 mM KCl at pH 7. The buffer-sludge mixture was then centrifuged at 5000 rpm for 15 mins and the supernatant was then discarded and the sludge pellets were again re-suspended in buffer solution ready for extraction. Then, the sludge was transferred to an extraction beaker with a magnetic stirrer and the CER (80 g/g-MLSS) added. The suspension was stirred (Corning 171 Scholar Stirrer, Corning, USA) for the selected stirring intensity and extraction time of 2 hrs in an ice bath. The CER/sludge mixture was then centrifuged at 13000 rpm and 4 °C for 20 mins to separate the extracted EPS. The extracted EPS was then collected and stored at -23 °C until chemical analysis could be performed. The polysaccharide (carbohydrate) and protein concentrations were measured by the use of colorimetric methods Gaudy (1962) and Lowry et al. (1951), respectively.

3.4.6 Analytical Methods for Zeta Potential

Zeta potential of the non-settleable fraction of sludge flocs in the treated effluent as ESS was measured by Zetacompact Z8000 model (CAD Instrumentation, Les Essarts Le Roi, France). The electric field added on the solution containing non-settleable flocs was controlled by a cell voltage of Zetacompact Z8000 and was fixed at 80 V. The pH of the treated effluent was in the range of 7.2±0.3

3.4.7 Floc Particle Size Distribution

Floc size distribution, of mixed liquor suspended solids (MLSS) and effluent suspended solids (ESS) in treated effluent (after 40 minutes settling), was determined by using a Mastersize 2000E (measuring range 0.04-2000µm) made by Marvin Instrument (Worcestershire, UK). The Malvern instrument uses light scattering and the data is given as percentage distribution by volume. Three measurements were taken for each SBR in each week of the experimental time.

3.4.8 Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) 16.0 software. One-way analysis of variance (ANOVA) was used to determine the statistical significant differences in values for the EPS production (soluble and bound) and the zeta potential data, the less significance difference (LSD) test at $p < 0.05$ was performed.

4.0 Results and Discussion

This chapter contains two parts. The first part reports the results and discussion of the TMP condensate treatment. Based on the observed differences in flocculation and settling between thermophilic and mesophilic sludge in the first part, a fundamental study on the effect of temperature (mesophilic vs. thermophilic) and dissolved oxygen (DO) (under thermophilic condition) on sludge properties and their role in bioflocculation and settling was conducted using a synthetic wastewater of glucose. The second part of this chapter reports the results and discussion of the fundamental study using synthetic wastewater.

4.1 Treatment of TMP Condensate Using Thermophilic and Mesophilic Sequencing Batch Reactors

4.1.1 Soluble COD removal

Figure 4.1.1 shows the variation of influent and effluent soluble COD (SCOD) with time. Effluent SCOD varied from 50 to 200 mg/L. The SCOD removal efficiency varied slightly under different cyclic times for both the thermophilic and mesophilic SBRs. During the study, 4 drums of TMP wastewater sample (180L/each) were used with varying influent SCOD values; this is the reason for the spike in influent SCOD at day 44 until day 68. At day 87, the SCOD removal efficiency dropped to 60% for the mesophilic SBR and 39% for the thermophilic SBR. This could be attributed to system instability due the change in cyclic time from 12 hr to 6hr. The smaller cyclic time (6hr) might result in toxic shocking due to a significantly increased organic loading rate. Thus, the cyclic time was changed from 6hr to 8hr at day 88. After that, the SCOD removal efficiency recovered. Over the course of the study, the mesophilic SBR demonstrated a higher SCOD removal efficiency than the thermophilic SBR, the average removal efficiency of the mesophilic SBR was 3-5% higher than that of the thermophilic SBR (Table 4.1.1).

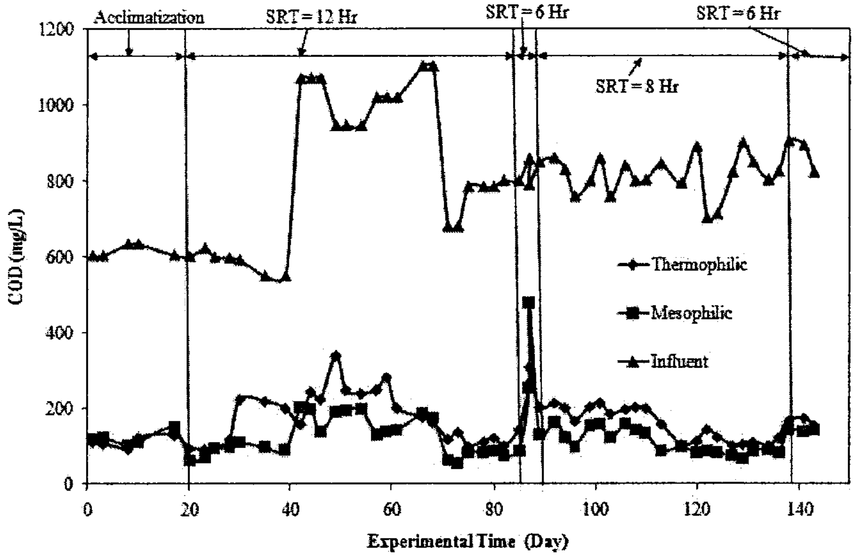


Figure 4.1.1 Performance of soluble COD removal of thermophilic and mesophilic SBRs over the study period.

Table 4.1.1 Average SCOD removal efficiency at different HRTs

HRT (hr)	Run Time (days)	Mesophilic SCOD Removal (%)	Thermophilic SCOD Removal (%)
12	87	83 ± 3	80 ± 4
8	47	88 ± 3	83 ± 3
6	7	81 ± 2	77 ± 3

It was expected that the longer the reaction time would lead to a higher SCOD removal efficiency. Therefore it would be expected that the 12 hr cyclic time would have better SCOD removal than the 8 and 6 hrs cyclic time. However, the SCOD removal efficiency did not change significantly at different cyclic times. This phenomenon can be explained by referring to Figure 4.1.2. Figure 4.1.2 shows the decrease in the concentration of effluent SCOD with reaction time in one operational cycle. Most of the consumable COD was removed within either a 5 hr period for mesophilic condition or a 7 hr period for thermophilic condition. This could explain the similar SCOD removal efficiency at different cyclic times. The presence of about 100 mg/L SCOD in treated effluent strongly suggests that non-biodegradable compounds exits in treated effluent. The non-biodegradable compounds remaining in treated effluent could also include biopolymers from cell lysis or soluble microbial products (SMPs). Analysis of treated effluent showed that carbohydrate and proteins, which are compounds of SMPs, were present in treated effluent (data not shown).

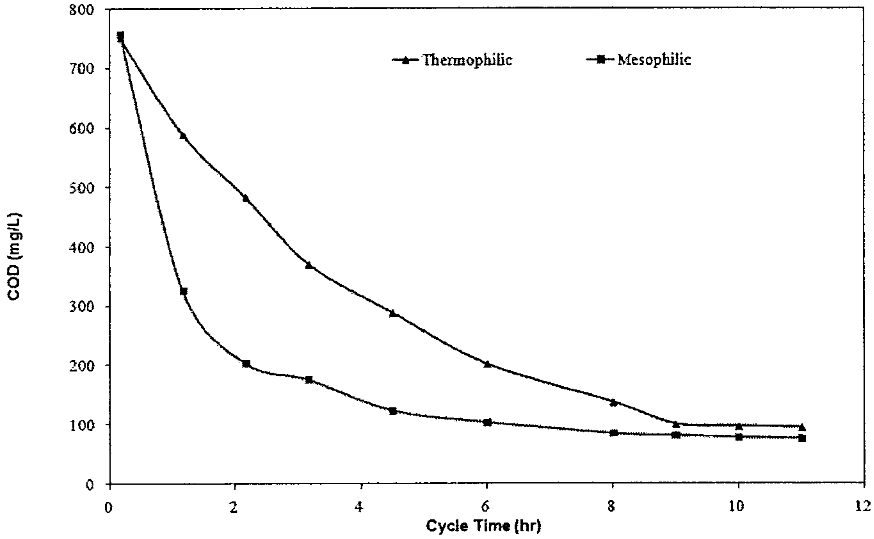


Figure 4.1.2 COD concentration over the course of a 12 hr reaction cycle for themophilic and mesophilic SBRs

Due to the problem of poor settling of the thermophilic SBR, occasionally sludge bulking would occur; which results in the loss of biomass and therefore a lower biomass concentration in the SBR (Suvilampi and Rintala, 2003; Vogelaar *et. al.*, 2002). There have been other studies done to compare biomass production and COD removal for thermophilic and mesophilic systems with varying results reported. Some studies have concluded that thermophilic sludge has a higher reaction rate than mesophilic sludge (Barr *et. al*, 1996; Lim *et al.*, 2001), while others have agreed with the findings out this study and have stated thermophilic sludge has lower reaction rate than mesophilic sludge (Lapara *et. al.*, 2001; Vogelaar *et. al.*, 2002). The COD removal rate based on a 12 hr cycle was calculated for the treatment of TMP condensate with thermophilic having a higher reaction rate (0.29 mg COD removed/mg

MLSS.d) than that for mesophilic (0.24 mg COD removed/ mg MLSS.d). COD reaction rates were determined to be Studies have shown that thermophilic and mesophilic bacterial communities are completely different and it should not be expected that they would have the same reaction rates (Lapara and Alleman, 1999) and that thermophlic conditions cause a more specific and less diverse community (Lapara et. al, 2001). Any sudden change microbial community could cause a change in SCOD removal in the thermophilic community due to the lower diversity of the community. It has been found that thermophilic systems tend to form more free bacteria, these are bacteria that do not form floc but stay free in the effluent causing a higher effluent COD (Suvilampi and Rintala, 2003). Cell lyses of bacteria causes effluent COD to increase due to the organics that are released when a cell is broken apart, cell lyses is more likely to happen at higher temperatures and therefore would have a greater effect under thermophilic conditions. That might explain the slight higher SCOD in the effluent from the thermophlic SBR.

The SCOD removal can be contributed from two sources: biodegradation and stripping during aeration. In order to understand the contribution of stripping on the SCOD removal, the change in SCOD with time in one cycle under stripping conditions was measured without the presence of activated sludge. The results (Figure 4.1.3) showed that there was slightly decrease in SCOD from the beginning to the end of the cycle under stripping conditions, which accounted for 9% and 13% of SCOD removal in the mesophilic and thermophilic SBR, respectively. The results suggest the majority of SCOD removal was attributed to biodegradation.

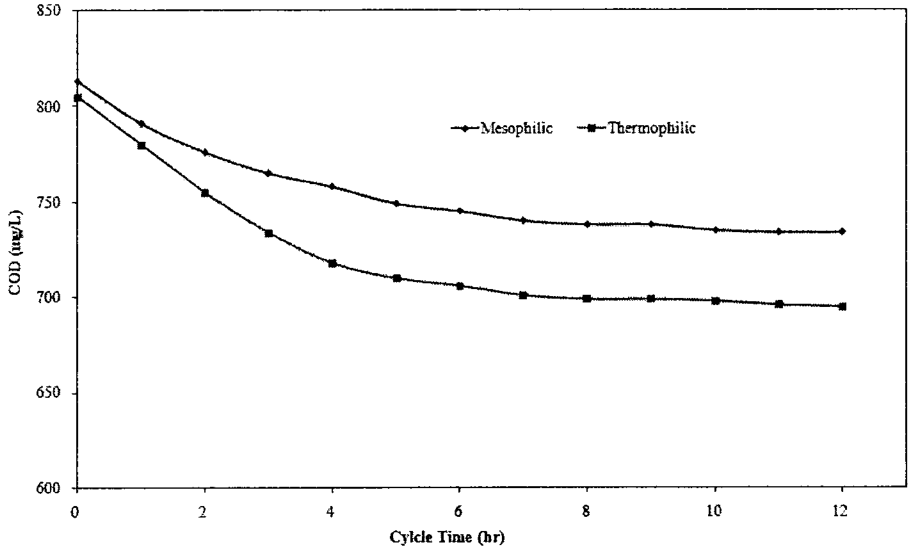


Figure 4.1.3. COD cycle with just stripping by aeration

4.1.2 Sludge settleability and flocculating ability

One of the major tasks for the activated sludge processes is biomass separation. The efficiency of biomass separation can be evaluated in terms of the settleability (sludge volume index (SVI)) and flocculating ability (effluent suspended solids (ESS)).

Changes in settleability of the thermophilic and mesophilic sludge, during the course of this study, were shown in Figure 4.1.4. The mesophilic sludge had a good settleability (SVI<100 mL/g MLSS) (except for days 85-95), while the thermophilic sludge had a modest sludge bulking (100<SVI<200 mL/g MLSS) for most of the time. The results suggest that thermophilic sludge usually had a poorer settleability than mesophilic sludge. This observation is consistent with the findings of a number studies in that an increase in temperature, particularly mesophilic vs. thermophilic temperature, results in poorer sludge settleability (Suvilampi and Rintala, 2003). The poorer settleability of the thermophilic sludge can be explained by the presence of a higher level of filaments in the thermophilic sludge (Figure 4.5(A) and 4.5 (B)) and the improved settleability was related to a decreased level of filaments

(Figure 4.1.5(C) and 4.1.5(D)). Figure 4.1.6 shows a comparison of normal mesophilic sludge compared to a day where a higher SVI was measured, day 87. The increase in SVI of the mesophilic sludge, during days 85-95, was also related to an increase in filaments level during these days. These results suggest that the higher SVI strongly correlated to a higher level of filaments.

The presence of different levels of filaments between thermophilic and mesophilic sludge might be related to the different dissolved oxygen (DO) level in the SBRs. From Figure 4.1.7, it is clear that the thermophilic sludge had a much lower DO level than that of the mesophilic sludge. A number of studies have shown that the DO in the water has a great impact on the type of bacteria that exists within activated sludge, particularly filamentous bacteria growth is suppressed when a DO of 2 mg/L is achieved (Jenkins *et. al.*, 2003). This suggests that it maybe possible to improve biomass settleability of thermophilic sludge by using pure oxygen aeration. This should be further studied.

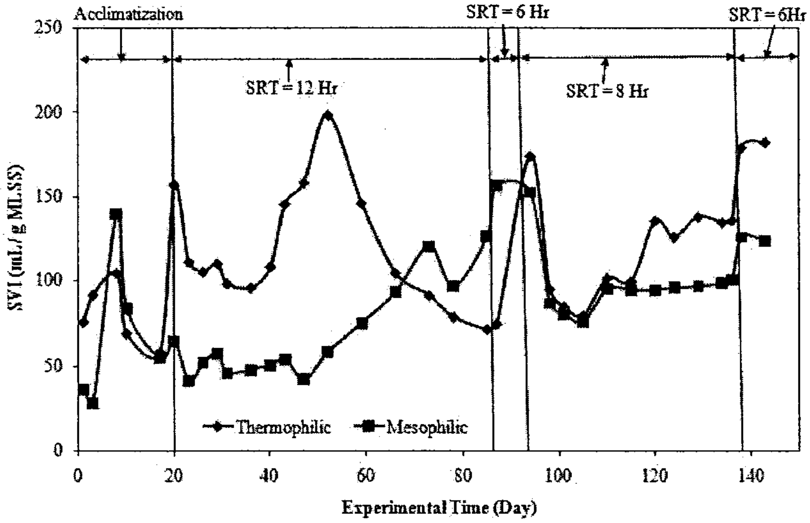


Figure 4.1.4. Sludge volume index (SVI) of the thermophilic and mesophilic SBRs over the study period

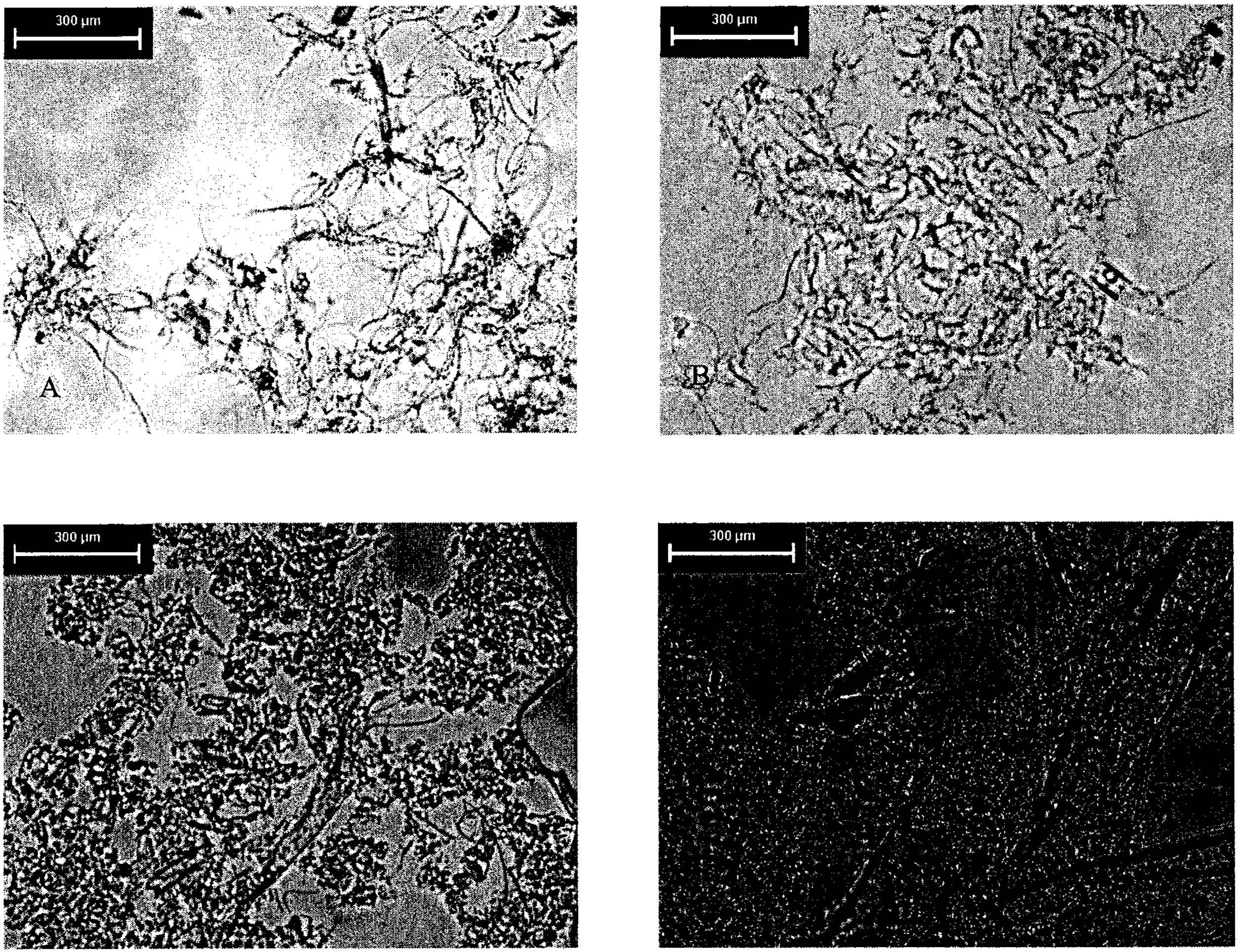


Figure 4.1.5. Microscopic images of thermophilic sludge at A) day 55, B) day 143, C) day 38, D) day 87

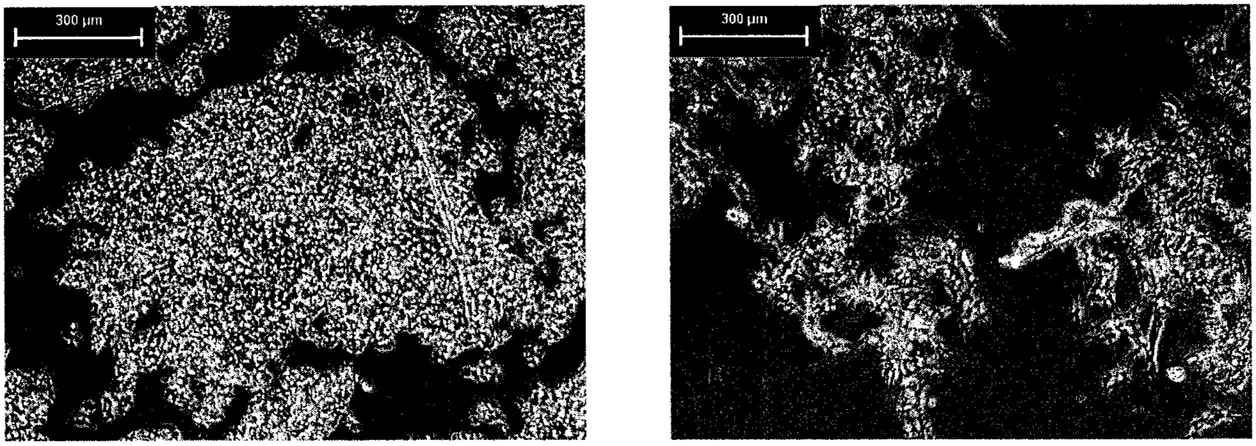


Figure 4.1.6. Microscopic images of mesophilic sludge A) Day 22, B) Day 87

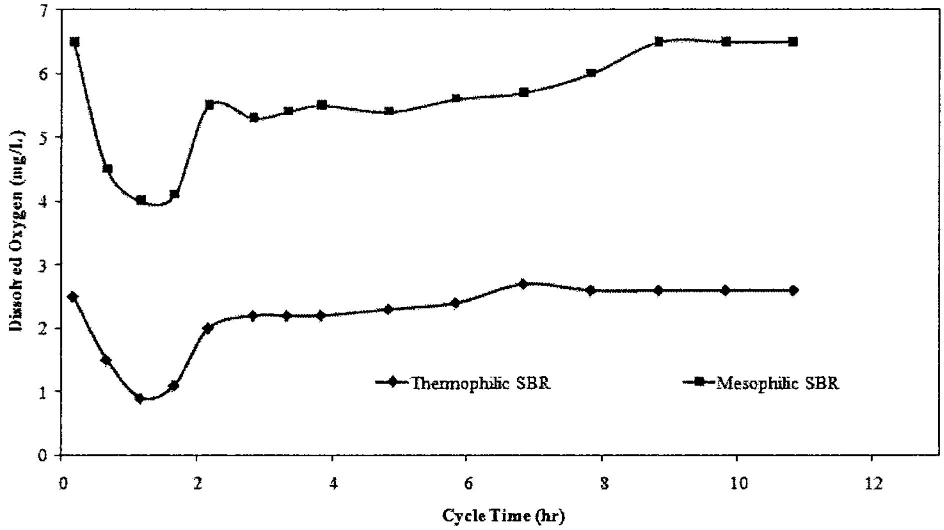


Figure 4.1.7 Changes in dissolved oxygen level in one operational cycle (HRT=12 hr).

Flocculating ability of sludge is different from the settleability of sludge. Sludge settleability is reflected by the SVI or zone settling velocity, which is a property of the flocculated sludge. Flocculating ability of sludge is the ability of fine particles to form large flocs and is reflected by the residual fine particles (i.e. effluent suspended solids (ESS)) in supernatant after flocculation. Figure 4.1.8 shows the variations of ESS in treated effluent with experimental time. In the first 50 days of operation, the ESS of the thermophilic SBR was slightly lower than that of the mesophilic SBR. This was the acclimation period of time of the thermophilic SBR. After the thermophilic SBR reached at a more stable operation, the ESS of the thermophilic SBR was higher than that of the mesophilic SBR. At day 87, a large jump in the ESS for both the thermophilic and mesophilic SBRs was observed, this was because the system underwent a period of instability following the change in cyclic time of 12 to 6 hrs. The large jump in ESS level was recovered after the cyclic time was switched from 6 to 8 hrs. The finding that the ESS levels of thermophilic SBR was higher than that of mesophilic SBR is in agreement with previous studies (Tripathi and Allen, 1999; Vogelaar *et. al.*, 2002). The result suggests that thermophilic sludge has a poorer flocculating ability than that of the mesophilic sludge. The real reasons that cause the poorer flocculating ability are still unknown. Vogelaar *et al.* (2005) suggest this maybe related to the extracellular polymeric substances production and properties after the DLVO theory and hydrophobic interaction fail to explain the poorer flocculating ability of thermophilic sludge. Further studies on sludge properties of thermophilic sludge is needed to improve our understanding of the mechanisms involved in flocculation of thermophilic sludge.

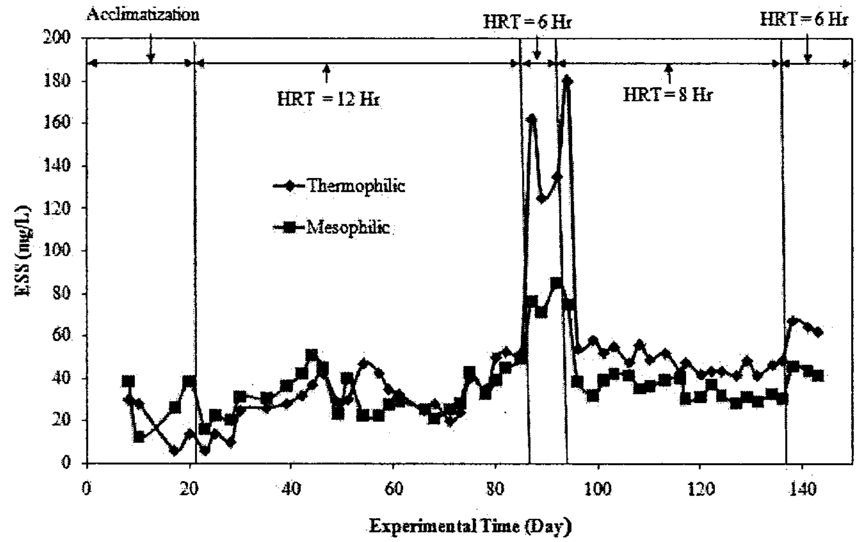


Figure 4.1.8. Effluent suspended solids of the thermophilic and mesophilic SBRs over the study period

4.1.3 Floc characteristics

Floc properties, including particle size, EPS production and composition and surface charge, play an important role in governing sludge flocculation and settling. The average zeta potential of the thermophilic and mesophilic sludge was -10.8 ± 0.5 and -11.3 ± 0.7 mv, respectively. In contrast to the significant difference in ESS concentration and SVI level between thermophilic and mesophilic sludge, there was no significant difference in zeta potential between the thermophilic and mesophilic sludge.

This result is consistent with the findings of (Vogelaar *et al.* 2005) in that the DLVO theory is not valid in explaining the difference in bioflocculation and settleability between thermophilic and mesophilic sludge. It is likely the biopolymer bridging mechanism by EPS plays a more important role in controlling bioflocculation (Vogelaar et al., 2005). Further studies should focus on the quantity and composition of EPS from thermophilic and mesophilic sludge, and also on the distribution of a given EPS with regard to floc surfaces.

Floc size distribution of the MLSS and non-settleable fraction of sludge flocs in treated effluent is shown in Figures 4.1.9. Figure 4.1.9 (A) shows that on average the particle size of the mesophilic sludge is larger than that of the thermophilic sludge. Mesophilic has a larger floc size than thermophilic which supports the pervious observation that mesophilic sludge flocculates more efficiently than thermophilic sludge. Figure 4.1.9(B) shows that a larger portion of fine colloidal particles in the size range of 0.1 to 10um existed in the treated effluent of the thermophilic SBR. However, there was a small fraction of large particles (100 ~ 1000um) in the thermophilic MLSS (Figure 4.1.9). This might not be surprising, as the presence of filaments in the thermophilic sludge provides the backbones of formation of large flocs.

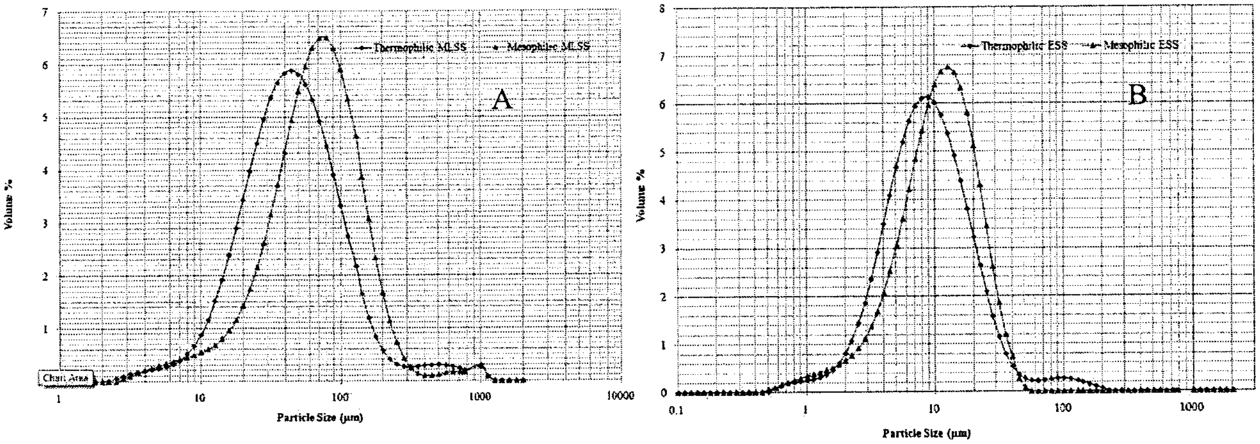


Figure 4.1.9 Particle size distribution for A) mixed liquor suspended solids, B) effluent suspended solids.

4.1.4. Summary

Treatment of TMP condensate at thermophilic (55°C) and at mesophilic (35°C) temperature was feasible in terms of COD removal. The majority of the SCOD was removed by biodegradation with a small fraction (9-13%) stripped by aeration. The settleability and flocculating ability of thermophilic sludge were comparable to or slightly poorer than that of the mesophilic sludge. The filaments level in the thermophilic sludge was usually higher than that of the mesophilic sludge. A higher SVI strongly correlated to a higher level of filaments. The poorer flocculating ability of thermophilic sludge could not be explained by the DLVO theory. Further studies should focus on EPS production and composition in order to understand the difference in flocculating ability between thermophilic and mesophilic sludge. The results suggest that both thermophilic and mesophilic SBR are promising technology for in-mill treatment of TMP condensate and subsequent reuse of treated effluent.

4.2 Effect of Temperature and Dissolved Oxygen on Sludge Properties and Their Role in Biofloculation and Settling

4.2.1. Overall Performance of the thermophilic and mesophilic SBRs

Figures 4.2.1, 4.2.2 and 4.2.3 show the changes in sludge volume index (SVI), effluent suspended solids (ESS) and residual soluble COD in treated effluent with time. The average values of SVI, ESS and residual soluble COD under different conditions are presented in Table 4.2.1.

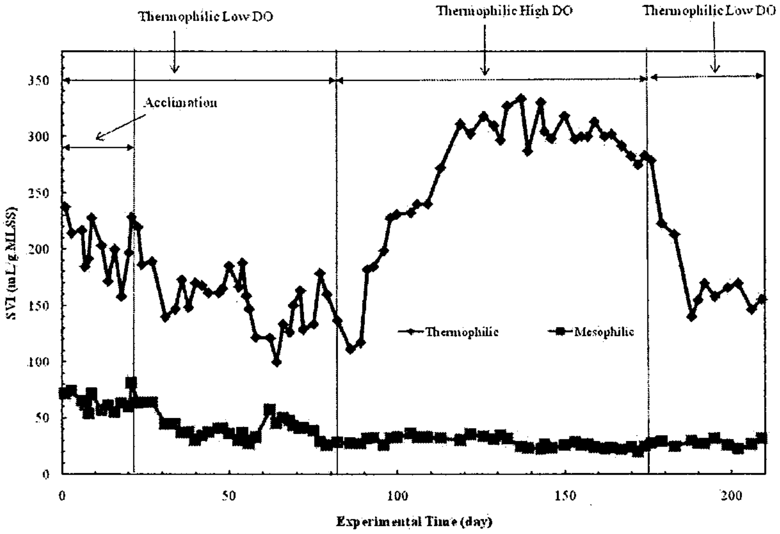


Figure 4.2.1. Variation of sludge volume index with experimental time

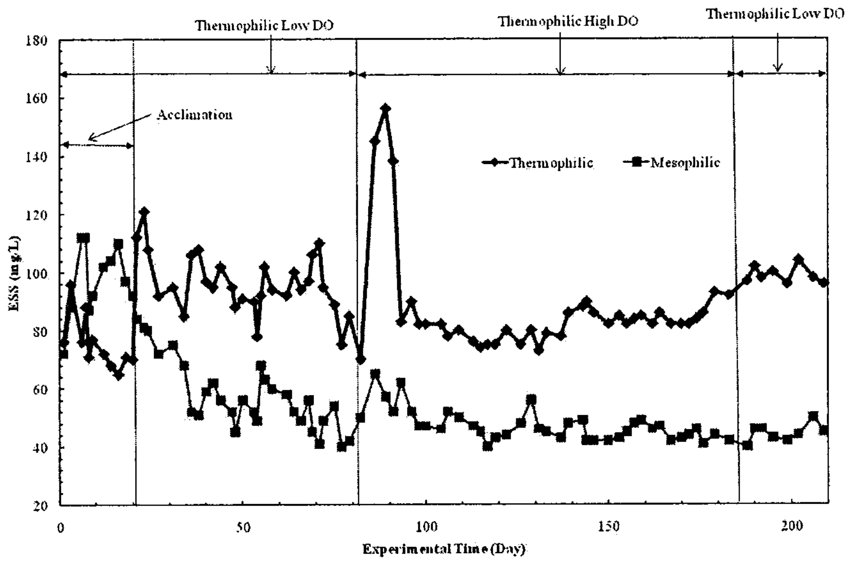


Figure 4.2.2 Variation of effluent suspended solids with experimental time

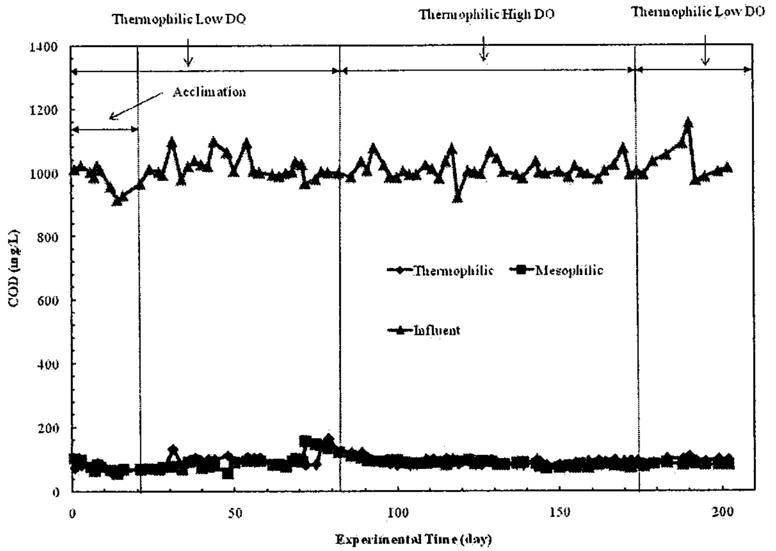


Figure 4.2.3. Variation of soluble COD in treated effluent and feed with experimental time

Table 4.2.1 Average sludge properties under different tested conditions

	Mesophilic	Thermophilic	
		Low DO	High DO
SVI (mL/g MLSS)	32 ± 8	157 ± 26	270 ± 60
COD (% Removal)	92 ± 2	91± 2	90 ± 1
ESS (mg/L)	49 ± 7	96 ± 10	81 ± 5
MLSS (g/L)	3.5 ± 0.5	2.8 ± 0.4	2.3 ± 0.2

As shown in Figure 4.2.1, the settleability of thermophilic sludge was poorer (higher SVIs) than that of the mesophilic sludge. The thermophilic sludge had filamentious bulking problem while the mesophilic sludge had excellent settleability. An increase in the DO level resulted in an increase in the SVI under the thermophilic temperature (55°C). A subsequent decrease in the DO level, after the period of time of higher DO level, led to an improvement in settleability. This result confirmed the findings of this study that the lower DO level favored to a better settleability under thermophilic conditions at the beginning of this study (202 days). Overall, the results of the poorer settleability of the thermophilic sludge, as compared to the analogous mesophilic sludge, is consistent with the findings of a number of previous studies (Berube and Hall, 2000; Cenens *et. al.*, 2000; Rozich A. and Bodacs, 2002; Vogelaar *et al.*, 2002a; Vogelaar *et al.*, 2002b). However, some other studies, although the cases are rare, found that the settleability of thermophilic sludge was better than or comparable to that of mesophilic sludge (Lapara and Alleman, 1999).

Effect of DO on the settleability of mesophilic sludge has been extensively studied (Jenkins *et al.*,2003). Literature data are contradictory and the relationship between DO and settleability of mesophilic sludge is still unclear (Martins *et al.*, 2003). But it is generally believed that an increase in DO would improve the settleability of mesophilic sludge (Jenkins *et al.*, 2003). A critical DO level of 2 mg/L is required to suppress the overgrowth of filaments in biomass separation (Jenkins *et al.*, 2003; Martins *et al.*, 2003). However, the results from this study suggest that an increase in the DO level resulted in a deterioration of the settleability of thermophilic sludge. This result is consistent with the findings of previous studies (Benefield *et al.*, 1975; Houtmeyers *et al.*, 1978; Palm *et al.*, 1980) in that sludge bulking could occur at higher DO levels. The contradictory results suggest that the role of DO level in controlling the settleability of sludge is complex and may depends on the relative importance of other factors other than DO. The results from this study suggest that the widely used strategy of raising DO level to minimize filamentous sludge bulking in mesophilic sludge may not be applicable to thermophilic sludge.

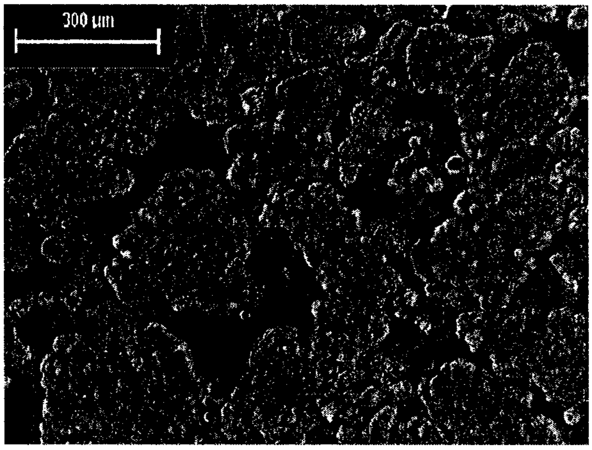
As shown in Figure 4.2.2, the flocculating ability of the thermophilic sludge was poorer (higher ESS level) than that of the analogous mesophilic sludge. An increase in the DO level led to a slightly improved flocculating ability under the thermophilic temperature (55°C). The results of poorer flocculating ability of thermophilic sludge are in good agreement with the findings of previous studies (Suvilampi and Rintala, 2003). Literature data suggests that more colloidal particles or free bacteria are present in the treated effluent of thermophilic processes (Suvilampi and Rintala, 2003). The slightly improved flocculating ability of thermophilic sludge at a higher DO level could be due to an increased level of filaments at the higher DO level, which providing more binding sites from the filaments for free bacteria or colloidal particles attachments.

Figure 4.2.3 shows that the residual soluble COD in the treated effluent of the thermophilic SBR was slightly higher than that of the mesophilic sludge. Considering the fact of that glucose is easily biodegradable compound, the residual soluble COD might be soluble microbial products (SMPs) rather than glucose. Analysis of the treated effluent showed that proteins and carbohydrates were present in treated effluent, implying the presence of SMPs in treated effluent. A calculation of the COD removal rate, based on the data of COD measured at different reaction time in one operational cycle, under both thermophilic and mesophilic conditions suggest the COD removal rate of the thermophilic sludge was higher than that of the mesophilic sludge under similar DO level. An increase in the DO level resulted in a higher COD removal rate under thermophilic conditions.

4.2.2 Properties of themophilic and mesophilic sludge

Filaments level: Significant difference in the level of filaments was observed under different tested conditions. Figure 4.2.4 shows the image analysis of typical sludge samples of mesophilic and thermophilic sludge. The mesophilic sludge contained no or a few filaments (level: 0-1), while the thermophilic sludge had a significant higher level of filaments (4-5). An increase in the DO level from 1-2.5 ppm to 3.5 – 5.0 ppm by using 32% oxygen gas led to an increase in the filaments level. The increase in the filaments level with an increase in the DO level is not consistent with the findings of previous studies (Jenkins *et. al.*, 2003; Martins et. al, 2003) with mesophilic sludge. It is generally agreed that a minimum of DO level at 2 mg/L should be maintained to suppress the overgrowth of filaments in mesophilic sludge (Jenkins *et. al.*, 2003; Martins et. al, 2003).

A DO level of smaller than 2 mg/L will promote the growth of filaments (Wanner 1994; Jenkins *et. al.*, 2003; Martins et. al, 2003). If this conclusion can be applied to thermophilic sludge, then it is not surprising to see the presence of a significant amount of filaments in thermophilic sludge at the lower DO levels (Lapara and Allemen, 1999; Rozich and Bordacs, 2002; Suvilampi and Rintala, 2003) aerated with air.



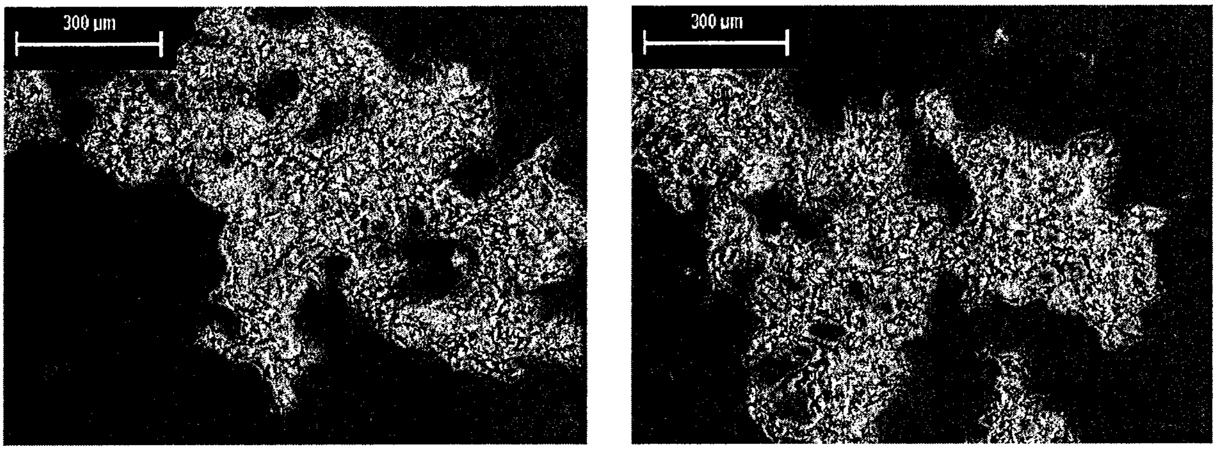


Figure 4.2.4 Morphology of sludge under A) Mesophilic B) Thermophilic Low DO C) Thermophilic High DO

EPS production and composition:

Extensive studies on EPS production and composition were conducted over a period of 6 months. Under similar DO level, the average total bound EPS of the mesophilic sludge (17.70 mg/g MLSS) was significantly lower than that (32.04 mg/g MLSS) of the thermophilic sludge ($p<0.05$) (Fig. 4.2.5). The different in the total bound EPS between thermophilic and mesophilic sludge might be related to the different microbial communities and cell lysis rates. It is known that thermophilic sludge contained different microbial community, as compared to the mesophilic sludge (Lapara *et al.*, 2000). An increase in temperature would result in an increase in the cell lysis rates (Lapara *et al.*, 2000). Under thermophilic condition, an increase in the DO level resulted in an increase in the total EPS (Fig. 4.2.5). The increased total amount of bound EPS with an increase in the DO level in thermophilic sludge is consistent with the findings of previous studies with activated sludge (Shin *et al.*, 2001). Shin *et al.* (2001) found higher airflow rates increased the amount of carbohydrate in the EPS but the protein level was almost constant. However, there was no significant difference in the ratio of protein to carbohydrate in bound EPS under different tested conditions (Fig.4.2.5).

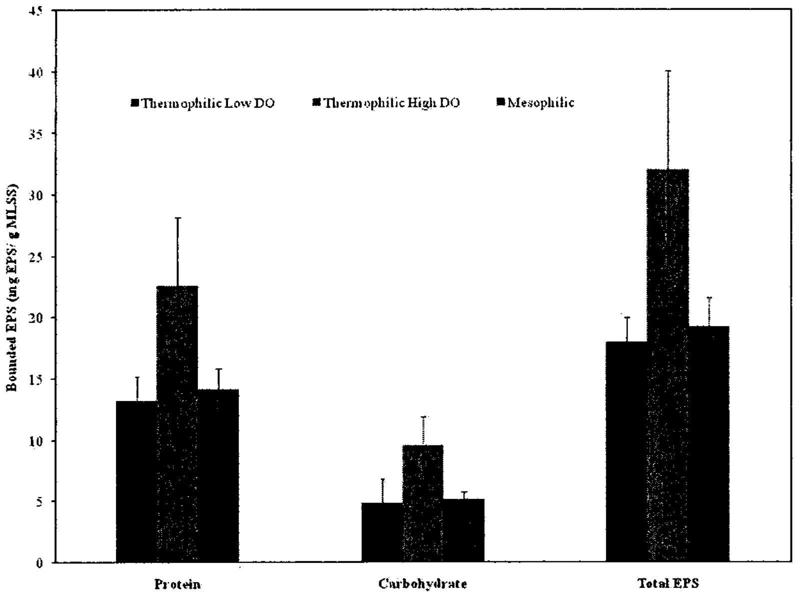


Figure 4.2.5 Comparison of bound EPS among different tested conditions (measurement number, n=12-15 under each condition)

Fig. 4.2.6 shows the soluble EPS in treated effluent under different tested conditions. There are no statistically significant differences among the tested conditions. But there was an increasing trend in protein and total EPS content with the thermophilic high DO condition, although not statistically significant. Under similar DO level, the quantity of soluble EPS in the thermophilic effluent was usually similar to that of the mesophilic effluent. Under thermophilic conditions, an increase in the DO level in thermophilic sludge led to an increase in the total amount of soluble EPS. The results are consistent with the findings of previous studies with pure culture (Lee *et al.*, 2001; Kim *et al.*, 2005). Lee *et al.* (2001) and Kim *et al.* (2005) found that the amount of soluble extracellular polysaccharides increased with an increase in the DO level. The increased soluble EPS may change the surface tension of the solution and thus affect bioflocculation.

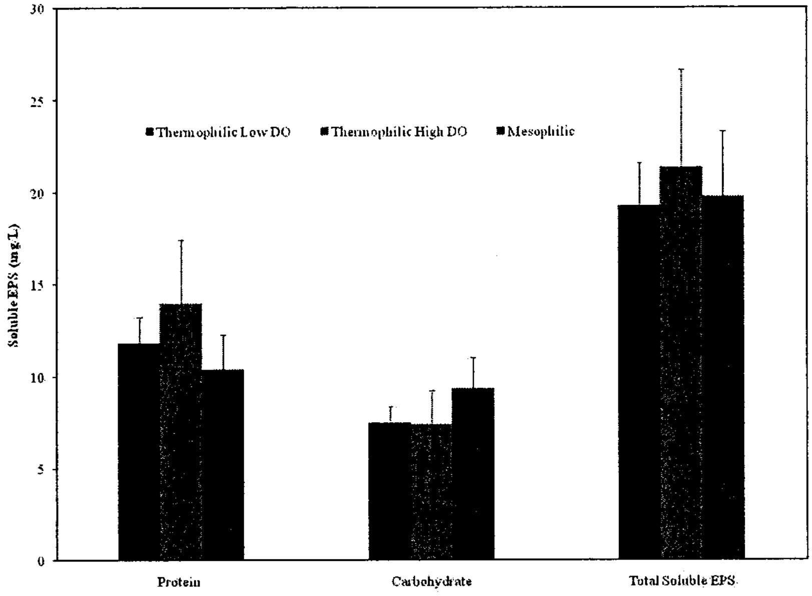


Figure 4.2.6. Comparison of soluble EPS among different tested condition ($p>0.05$)

Zeta potential: Under similar DO level, the average zeta potential of the thermophilic sludge (-12.6 ± 0.8) was similar to that (-13.2 ± 0.9) of the mesophilic sludge. Under thermophilic condition, the DO level had limited effect on the zeta potential of the thermophilic sludge. The results are consistent with the findings of Vogelaar *et al.* (2005) in that there was no significant difference in zeta potential between thermophilic and mesophilic sludge.

4.2.3 Correlations between sludge properties and settleability as well as flocculating ability

A strong correlation between the level of filaments and SVI was observed for all the measured results. A higher level of filaments is correlated to a higher SVI. This is consistent with the findings of previous studies (Jenkins *et al.*, 2003; Martins *et al.*, 2003). The results suggest that sludge bulking in the thermophilic system was caused by the overgrowth of filaments. An increase in the DO level was not effective to minimize the growth of filaments and even further promoted the growth of filaments. Therefore, the strategy of using elevated DO level to control filamentous bulking problems widely used in mesophilic systems might not be applicable to the thermophilic system. However, the improved bioflocculation ability of thermophilic sludge (less EPS) at higher DO could be attributed to the slightly increased filaments level. Previous study found that the increased filaments level provide more binding sites on the backbone of filaments for free cells or smaller aggregates to attach and thus the level of free cells or colloids in the suspension is decreased with an increase in the level of filaments (Thompson *et al.,* 2001; Suvilampi *et al.,* 2006).

In addition to the importance of filaments in sludge bulking, the EPS has been reported to be an important factor in controlling SVI and ESS (Liao *et al.*, 2001; Li and Yang, 2007). From Table 4.2.1 and Figure 4.2.5, it is clear that there were no significant correlations between SVI and total EPS or between ESS and Total EPS, if all the results, including mesophilic and thermophilic results, are considered. However, an increase in the total EPS corresponded to an increase in the SVI and a decrease in the ESS, for the thermophilic results. This might not be surprising, as EPS could be very different in terms of molecular weight, composition, especially when they are produced by very different biological systems (thermophilic vs. mesophilic). The results suggest that, with potentially similar EPS composition and molecular weight produced in the thermophilic system, an increase in total EPS resulted in an increase in SVI. The results are consistent with the findings of Liao *et al.* (2001) and Li and Wang (2007). The improved bioflocculation with an in crease in the total EPS could be due to the fact that more EPS provided more chances for cells embedded in EPS so that free cells or small aggregates are less. However, for different biological systems, the roles of specific EPS molecules (composition, molecular weight, surface charge, hydrophobicity etc.) are more important than the total EPS in controlling bioflocculation and settling. Further studies should focus on the roles of specific EPS molecules (composition, molecular weight, surface charge and hydrophibicity) produced in different biological systems.

4.2.4 Summary

This study investigated the effects of temperature and dissolved oxygen concentration on sludge properties and their role in bioflocculation and settleability using well-controlled sequencing batch reactors. The main conclusions are summarized below.

Under similar DO level, the thermophilic sludge had a poorer flocculation ability and settleability than mesophilic sludge. The poorer settleability of thermophilic sludge was related to a higher filaments level.

Under the same thermophilic temperature (55°C), an increase in the DO level from 1.0 - 2.5 ppm to 3.5 – 5.5 ppm resulted in a slightly improved flocculating ability and even poorer settleability. This result suggests that an increase in DO was not effective in improving the biomass separation efficiency in thermophilic sludge.

Temperature had a significant impact on EPS production and composition ratio. Under similar DO level, the total EPS of the thermophilic sludge was significantly higher than that of the mesophilic sludge. Under thermophilic conditions, an increase in DO resulted in an increase in the quantity of total EPS.

Under the same thermophilic temperature (55°C) but different DO levels, an increase in the level of filaments under higher DO was the dominate factor that caused the deterioration of settling ability and improvement of bioflocculation ability. In addition, the total EPS played some roles in settling and bioflocculation in the thermophilic system. An increase in the quantity of total EPS was related to an increase in SVI and a decrease in ESS. But no correlations between SVI and total EPS and between ESS and total EPS were observed, if the results from both mesophilic and thermophilic systems are considered. The results suggest that the roles of specific EPS molecules (composition, molecular weight, surface charge, and hydrophobicity ets.) are more important than the quantity of total EPS in controlling bioflocculation and settling.

Temperature has a greater effect on the sludge properties than DO and therefore, the temperature has a greater effect on the flocculation and settling performance. This leads to the conclusion in terms of biomass separation, temperature has the greater effect.

5.0 Conclusions and Recommendations

5.1 Conclusions

The current studies were conducted to investigate the feasibility of treating TMP Condensate wastewater streams individually using thermophilic aerobic biological wastewater treatment as opposed to traditional mesophilic treatment. Also, the effect of temperature and dissolved oxygen on sludge properties was studied under well-controlled conditions to assess the effect each parameter has separately from the other. The focus of the study was the effect of temperature and dissolved oxygen on the EPS composition and production to explain poor flocculation of thermophilic sludge. The following conclusions were made at the conclusion of the experimental study.

- The treatment of TMP condensate using thermophilic and mesophilic sequencing batch reactors demonstrates good results in terms of COD removal, with both systems showing COD removals of 77-91%, with air stripping only responsible for 9-13% of the COD removal. There was only a minor difference between in removal rates between thermophilic and mesophilic sludge (3-5%). In terms of sludge settling and biomass separation, thermophilic sludge demonstrated poorer settling (SVI) and a more turbid effluent (ESS) than that of mesophilic sludge. Thermophilic sludge also demonstrates a higher level of filamentous bacteria which leads to poorer settling as observed. The poorer flocculating ability of thermophilic sludge could not be explained by the DLVO theory. Other factors needed to be investigated into the cause of the poor sludge flocculation and settling under thermophilic conditions.

- In terms of bounded EPS, both temperature and dissolved oxygen had significant effects on EPS production and composition. Under similar DO level, the total EPS of the thermophilic sludge was significantly higher than that of the mesophilic sludge. Similarly, an increase in DO level under thermophilic conditions resulted in an increase in the total EPS production. In the thermophilic system, sludge volume index (SVI) positively correlated to the quantity of total EPS and the effluent suspended solids (ESS) negatively correlated to the quantity of total EPS. But these correlations disappeared when results of both the thermopphilic and mesophilic systems are included. The results suggest that the quantity of total EPS had significant impact on flocculation and settling in similar biological system. However, the roles of specific individual EPS molecules (composition, molecular weight, surface charge, hydrophobicity etc.) and types of microorganisms are more important than the quantity of total EPS in governing bioflocculation and settling for different biological systems (thermophilic vs. mesophilic). The use of elevated DO level did not improve the biomass separation in terms of settleability.

5.2 Recommendations

Although this thesis explored the effects of temperature and dissolved oxygen on the sludge properties, particularly EPS production and composition and filaments level, to explain the observed difference in flocculation and settling between mesophilic and thermophilic treatment, still many questions remain that require further investigation.

An in-depth knowledge of EPS composition and molecular weight distribution is needed to understand the specific roles of individual EPS molecules in bioflocculation. To achieve this, gel permeation chromatography (GPC) can be used to characterize the molecular weight distribution of extracted EPS solutions (currently stored at -20°C). surface analysis using X-ray photoelectron spectroscopy (XPS) technique can be carried out to determine elemental composition, empirical formula, chemical state and electronic state of the elements that exist within a material up to ppm level. In addition, transmission electron microscope (TEM) technique can be used to obtained the information of EPS at nanometer on sludge surfaces. It is anticipated that the use of these correlative analytical techniques would enable us to develop an improved fundamental understanding of EPS on bioflocculation and the cause of differences in bioflocculation between thermophilic and mesophilic systems. Further studies on EPS are being carried out in these areas mentioned above.

5.0 References

American Public Health Association - American Water Works Association - Water Environment Federation,, *Standard Methods for examination of water and wastewater*, (2005). 21st ed., Washington, DC, USA.

Artamo A. (2000). *Reprint from Book 5 Mechanical Pulping, Chapter 7 – Thermomechanical pulping.* WILEY-VCH Verlag GmbH & Co. KGaA, Helsinki.

Assenlin C., Colin D. and Graff S. (2000). Effluent treatment for chronic toxicity removal with the suspended carrier biofilm reactor. *Tappi International Environmental Conference and Exhibit,* Technical Association for Pulp and Paper Industry, Norcross, GA, USA. *2,* 805-811.

Backlund A, Ferritsius O and Tistad G (1990) Treatment of mechanical pulp to remove resin. *United States Patent* (19), 4992.989.

Bajpal P. (2000) Treatment of pulp and paper mill effluents with anaerobic technology. Pira international, Randall Road, Leatherhead, UK.

Barr T., Taylor J. and Duff S. (1996). Effect of HRT, SRT and temperature on the performance of activated sludge reactors treating bleach kraft mill effluent. *Water Research,* **30**, 391-400.

Benefield L.D, Randall C.W and King P.H. (1975) The simulation of filamentous microorganisms in activated sludge by high dissolved oxygen concentration. *Water, Air and Soil Pollution,* **5,** 113-123.

Berube P and Hall E. (2000) Fate and removal kinetics of contaminants contained in the evaporator condensate during treatment for reuse using a high temperature membrane bioreactor. *Pulp and paper technical association of Canada.*

Berube P. and Hall E. (2001) Effects of elevated operating temperature on methanol removal kinetics from synthetic kraft pulp mill condensate using a membrane bioreactor. *Water Research*, **34**(18), 4359-4366.

Bosker T. (2007). *Thermomechanical pulp mill effluent effects on fish reproduction: an investigation of cause.* PhD thesis of university of New Brunswick, Canada.

Cibis E, Kent C.A, Krzywonos M,Garncarek Z, Garncarek B and Miskiewicz T (2002) Biodegradation of potato slops from a rural distillery by thermophilic aerobic bacteria. *Bioresouce Technology.* **85,** 57-61.

Cenens C, Smets I, Ryckaert V and van Impe I. (2000) Modelling the competition between floc-forming and filamentous bacteria in activated sludge wastewater treatment systems – I Evaluation of mathematical models based on kinetic selection theory. *Water Reseource,* **34,** 2525-2534.

Conrad D.J. and Huck P.M. (1996). Chlorination by-products from ferulic acid (a lignin model compound) and biologically treated themomechanical and chemithemomechanica pulp mill effluents, *Wat. Res.* **30**(11), 2776-2784.

Couillard D and Zhu S, (2003) Thermophilic aerobic process for the treatment of slaughterhouse effluents with protein recovery. *Environmental Pollution,* **79**, 121-126.

Dalentoft E and Thulin P. (1997) The use of aerobic selectors in activated sludge systems for the treatment of from the pulp and paper industry. *Water Science and Technology.* **35,** 181-188.

Dias, J. C. T., Rezende, R. P., Silva, C. M. and Linardi, V. R. (2005). Biological treatment of Kraft pulp mill foul condensates at high temperatures using a membrane bioreactor. *Process Biochemistry,* **40**, 1125-1129.

Environment Canada (1999), *National Pollutant Release Inventory*

Elliot A. and Voss R. (2002). Consequences of increasing effluent concentration on secondary treatment performance . *Pulp and Paper Canada,* **105**(7), 159-169.

Frolund B., Palmgren R., Keiding K. and Nielson P.H. (1996). Extraction of extracelluar polymers from activated sludge using a cation exchange resin. *Water Research,* **30**(8), 1749-1758.

Gariepy S, Tyagi R.D, Couillard D and Tran F (1989) Thermophilic process for protein recovery as an alternative to slaughterhouse waste treatment. *Biological Wastes.* **29,** 93-105.

Gaudy A.F. (1962). Colorimetric determination of protein and carbohydrate. *Industrial Water Wastes,* 7(1), 17-21.

Gorret N, Maubois J.L, Engasser J.M and Ghoul M.(2001) Study of the effects of temperature, pH and yeast extract on growth and exopolysaccharides production by Propionibacterium acidi-propionici on milk microfltrate using a response surface methodology. *Journal of applied microbiology.* **90,** 788-796.

Grant J, Li Z, Court G, Crowell M, Mobarak N, Cole S and Sain M. (2001) Analysis and optimization of used water networks in a TMP mill. Annual meeting Canadian pulp and paper association, C105-C108.

Hakkaranien K and Sillanpaa M. (2007) Flocculation in pulp and paper mill sludge process, *Research Journal of Chemistry and Environment.* **11**(3), 96-103.

Houtemeyers J, Verachtert H, Van den Eynden, Poffe R. (1980) Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge process. *Applied Microbiology and Biotechnology.* **9,** 137-149.

Jahren S.J and Oedegaard H. (1999). Treatment of thermomechanical pulping (TMP) whitewater in thermophilic (55 °C) anaerobic– aerobic moving bed biofilm reactors. *Water Science and Technology,* **40**(8), 81– 90.

Jahren S.J., Rintala J. and Ødegaard H., (1999). Anaerobic Thermophilic (55°C) treatment of TMP wastewater in reactors based on biomass attachment and entrapment. *Water Science and Technology,* **40**(11-12), 67-75.

Jahren S.J., Rintala J. and Ødegaard H. (2001). Aerobic moving bed biofilm reactor treating thermomechanical pulping whitewater under thermophilic conditions. *Water Research,* **36** (4), 1067–1075.

Jenkins D., Richard M., and Daigger GT. (2003). *Manual on the Causes and Control of Activated Sludge Bulking, Foaming, and Other Solids Separations Problems, 3rd Edition,* Lewis Publishers, Boca Ranton, Florida.

Jia X.S., Furumai H. and Fang H.H.P. (1996) Extracellular polymers of hydrogen-utilising methanogenic and sulphate reducing sludges. *Water Research,* **30**(6), 1439-1444.

Kantardjieff A and Jones JP. (1997). Practical experiences with aerobic biofilters in TMP sulfite and fine paper mills in Canada. *Water Science and Technoogy,* **35**(2 – 3), 227– 234.

Kim H.H, Na J.G, Chang Y.K and Lee S.J. (2005) Effects of dissolved oxygen control on cell growth and exopolysaccaride production in batch culture of Agaricus blazei. *Korean Journal of Chemical Engineering.* **22**(1), 80-84.

Korhonen S and Tuhkanen T. (2000). Effects of ozone on resin acids in thermomechanical pulp and paper mill circulation waters. *Ozone: Science and Engineering,* **22**(6), 575–584.

Laari A, Korhonen S, Tuhkanen T, Verenich S and Kallas J. (1999). Ozonation and wet oxidation in the treatment of thermomechanical pulp (TMP) circulation waters. *Water Science and Technology,* **40**(11– 12), 51– 58.

Lapara T.M. and Alleman J.E, (1999). Thermophilic aerobic biological wastewater treatment, *Water Research.* **33**(4), 895-908.

LaPara T.M., Nakatsu C.H., Pantea L.M. and Alleman J.E. (2000) Phylogenetic analysis of bacterial communities in mesophilic and thermophilic bioreactors treating pharmaceutical wastewater. *Applied Environmental Microbiology,* **66,** 3951-3959.

LaPara T.M., Nakatsu C.H., Pantea L.M. and Alleman J.E. (2001) Aerobic Biological Treatment of a Pharmaceutical Wastewater: Effect of Temperature on COD Removal and Bacterial Community Development. *Water Research,* **35**(18), 4417-4425.

Ledakowicz S., Michniewicz M., Jagiella A., Stufka-Olczyk A. and Martynelis M. (2006). Elimination of resin acids by advanced oxidation processes and their impact on subsequent biodegradation. *Water Reseach,* **40**(18), 3439 – 3446.

Lee H.S, Park S.H, Lee J.H and Lee H.K. (2001) Effect of aeration rates on production of extracellular polysaccharide, EPS-R by marine bacterium Hahella chejuensis. *Biotechnology Bioprocess Engineering,* **6**, 359-362.

Li X.Y. and Yuan Y. (2002) Settling Velocities and permeabilities of microbial aggregates. *Water Research.* **36,** 3110-3120.

Li X.Y. and Yang S.F. (2007) Influence of loosely bounded extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research,* **41,** 1022-1030.

Liao B.Q., Allen D.G., Droppo I.G., Leppard G.G. and Liss S.N. (2001) Surface properties of sludge and their role in bioflocculation and settleability. *Water Research.* **35**, 339-350.

Lim B., Huang X., Hu H-Y., Goto N. and Fujie K. (2001) . Effects of temperature on biodegradation characteristics of organic pollutants and microbial community in a solid phase aerobic bioreactor treating high strength organic wastewater. *Water Science and Technology,* **43**(1), 131-137.

Liver S.W and Hall E.R. (1996a) Interactions of resin acids with aerobic and anaerobic biomass – I degredation by non-acclimated inocula, *Water Research,* **30**(3) 663-671.

Liver S.W and Hall E.R. (1996b) Interactions of resin acids with aerobic and anaerobic biomass – II partitioning of biosolids, *Water Research,* **30**(3) 672-681.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randell R.J. (1951) Protein measurement with folin phenol reagent. *Journal of Biological Chemistry,* **193**(1), 265.

McLeavy D. and Associates Ltd. (1987) Aquatic toxicity of pulp and paper mill effluent: A review. Environment Canada, Beauregard Press Ltd.

Magnus E, Carlberg GE and Norske HH. (2000) TMP wastewater treatment including a biological high-efficiency compact reactor. *Nordic Pulp and Paper Research Journal.* **5**(1), 37 – 45.

Malmqvist A, Ternstrom A and Welander T, (1999) In-mill biological treatment for paper mill closure. *Water Science and Technology,* **40**(11-12), 43-50.

Martins A.M.P, Heijen J.J, van Loosdrecht M.C.M (2003) Effect of dissolved oxygen concentration on sludge settleability. *Applied Microbial Biotechnology.* **62,** 586-593.

Mikkelsen L.H. and Kieding K. (2002). Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. *Water Research,* **36,** 2451-2462.

Morgan J.W., Forster C.F. and Evison L. (1990). A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. *Water Research* **24**(6), 743-750.

Morgan-Sagastume F. and Allen D.G. (2003). Effects of temperature transient conditions on aerobic biological treatment of wastewater, *Water Research* , **37**(15), 3590–3601.

Neyens E and Baeyens J. (2003) A review of thermal sludge pretreatment processes to improve dewaterability. *Journal of Hazardous Material.* **B98,** 51-67.

Palm J.C, Jenkins D and Parker D.S. (1980) Relationship between organic loading, dissolved oxygen, concentration and sludge settleability in the completely mixed activated sludge process. *Journal of Water Pollution Control Federation.* **52,** 2484-2506.

Pearson J. (1990) Mills opt for anaerobic treatment. *Pulp and Paper International,* **32**, 65-68.

Pokhrel D. and Viraraghavan T. (2004). Treatment of pulp and paper mill wastewater - a review, *Science of the Total Environment,* **333**(1-3) , 37– 58.

Ratsak C.H, Maarsen K.A and Kooijman S (1996). Effect of protozoa on carbon mineralization in activated sludge. *Water Research.* **30,** 1-12.

Rintala J and Lepistö S. (1992). Anaerobic treatment of thermomechanical pulping whitewater at 35–70°C. *Water Research,* **26**(10), 1297-1305.

Rintala J. and Puhakka J.A. (1994). Anaerobic treatment in pulp and paper mill waste management - review. *Bioresource Technology,* **47** (1), 1-18.

Rozich A and Bordacs K (2002). Use of thermophilic biological aerobic technology for industrial wastewater treatment. *Water Science and Technology.* **46**(4-5), 83-89.

Schmidt J and Ahring B. (1994) Extracellular polymers in granular sludge from different upflow anaerobic sludge blanket (USAB) reactors. *Applied Microbiological Technology.* **42,** 457-462.

Sezgin M, Jenkins D, Parker D, (1978) A unified theory of filamentous activated sludge bulking. *Journal of Water Pollution Control Federation.* **50**, 362–381.

Shaw C.B., Carliell C.M. and Wheatley A.D. (2002) Anaerobic/aerobic treatment of coloured textile effluents using sequencing batch reactors. *Water Research,* **36**(8), 1993-2001.

Shere S.M. and Daly P.G. (1982). High rate biological treatment of TMP effluent. *Pulp and Paper Canada.* **83**(1), 61-66.

Shin H.S, An H.H, Kang S.T, Choi K.H. and Jun K.S. (1999) Fouling characterstics in pilot scale submerged membrane bioreactor.

Shin H.S., Kang S.T. and Nam S.Y. (2000) Effect of Carbohydrates to protein ratio in EPS on sludge settling characteristics. *Biotechnological Bioprocess Engineering.* **5,** 460-464.

Shin H.S., Kang S.T. and Nam S.Y. (2001) Effect of carbohydrates and proteins in the EPS on sludge settling characteristics. *Water Science and Technology.* **43**(6), 193-196.

Slade A.H, Andersen S.M., Blackwell B.G., Dare P.H and Pearson H. (2001) Nitrogen fixation in the activated sludge treatment of themromechanical pulping wastewater. *TAPPI Envinromental Health and Safety Conference.* Charlotte, NC, USA.

Standard Methods for examination of water and wastewater, (1998). 20th ed. American Public Health Association - American Water Works Association - Water Environment Federation, Washington, DC, USA.

Suvilampi J. and Rintala J. (2003). Thermophilic aerobic wastewater treatment, process performance, biomass characteristics, and effluent quality, *Reviews in Environmental Science & Bio-Technology,* **2**(1), 35–51

Suvilampi J, Lehtomaki A and Rintala J, (2006). Biomass characterization of laboratory-scale thermophilic-mesophilic wastewater treatment processes. *Environmental Technology.* **27**(1), 41-51.

Thompson G., Swain J., Kay M. and Forster C.M. (2001). The treatment of pulp and paper mill effluent: a review. *Bioresource Technology,* **77** (3), 275 – 288.

Thompson G and Forester C.F. (2003) Bulking in activated sludge plants treating paper mill wastewaters. *Water Research.* **37,** 2636-2644.

Tripathi C. and Allen D. (1999). Comparison of mesophilic and thermophilic aerobic biological treatment in sequencing batch reactors treating bleached kraft pulp mill effluent. *Water Research,* **33**(3), 836-846.

Verenich S., Laari A., Kallas J. (1999). Wet oxidation of concentrated wastewaters of paper mills for water cycle closing. *Waste Management,* **20** (4), 283-293.

Vogelaar, J.C.T., van Lier, J.B., Klapwijk, B., de Vries, M.C. and Lettinga, G. (2002a). Assessment of effluent turbidity in mesophilic and thermophilic activated sludge reactors: origin of effluent colloidal material. *Applied Microbiol Biotechnology,* **59**(1), 105–111.

Vogelaar, J.C.T., Bouwhuis B., Klapwijk A., Spanjers H. and van Lier, J.B. (2002b). Mesophilic and thermophilic activated sludge post-treatment of paper mill process water. *Water Research,* **36**(7), 1869-1979.

Vogelaar J.C.T., Klapwijk, B., Temmink H. and van Lier, J.B. (2003) Kinetic comparisons of mesophilic and thermophilic aerobic biomass. *Journal of Industrial Microbial Biotechnology,* **30**(1), 81-88.

Vogelaar J.C.T., De Keizer A., Spijker S. and Lettinga G. (2005). Bioflocculation of mesophilic and thermophilic activated sludge. *Water Research,* **39**(1), 37-46.

Wanner J. (1994) The implementation of bulking control in the design of activated sludge systems. *Water Science and Technology*. **29,** 193-202.

Welander, T., Lofqvist, A. and Selmer, A. (1997). Upgrading aerated lagoons at pulp and paper mills. *Water Science and Technology*, **35** (2-3), 117-122.

Wiseman N. and Ogden G. (1996). Zero liquid effluent technologies for the paper industries. *Paper Technology,* **37**(1). 31-38.

Appendix A –COD Removal Data

Part 1 - Treatment of TMP Condensate

Experimental Time (Day)	Influent COD (mg/L)	Thermophilic SBR (mg/L)	Thermophilic Removal (%)	Mesophilic SBR (mg/L)	Mesophilic Removal (%)
1	604	113	81	118	80
3	604	107	82	123	80
8	634	94	85	103	84
10	634	125	80	112	82
17	605	130	79	150	75
20	602	96	84	65	89
23	623	93	85	73	88
25	600	95	84	95	84
28	598	120	80	99	83
30	592	225	62	110	81
35	550	220	60	98	82
39	550	201	63	90	84
42	1070	160	85	203	81
44	1070	245	77	200	81
46	1070	225	79	140	87
49	946	340	64	192	80
51	946	250	74	195	79
54	946	240	75	198	79
57	1020	250	75	130	87
59	1020	283	72	140	86
61	1020	202	80	142	86
66	1102	178	84	187	83
68	1102	165	85	176	84
71	680	120	82	65	90
73	680	138	80	55	92
75	785	100	87	85	89
78	785	112	86	83	89
80	785	123	84	86	89
82	801	102	87	75	91
85	801	146	82	89	89
87	858	267	69	256	70
87	790	310	61	480	39
89	850	204	76	130	85
92	860	215	75	165	81
94	830	202	76	125	85

96	760	167	78	100	87
99	800	206	74	156	81
101	860	215	75	159	82
103	760	186	76	123	84
106	840	198	76	158	81
108	800	204	75	143	82
110	803	201	75	134	83
113	845	160	81	88	90
117	794	103	87	98	88
120	890	115	87	84	91
122	703	145	79	89	87
124	713	125	82	84	88
127	823	103	87	74	91
129	901	106	88	69	92
131	850	112	87	89	90
134	803	102	87	93	88
136	825	123	85	85	90
138	905	167	82	142	84
141	894	175	80	139	84
143	823	156	81	143	83

Part 2 – Effect of Temperature and Dissolved Oxygen on EPS Production

Study Day	Influent COD (mg/L)	Thermophilic SBR (mg/L)	Thermophilic Removal (%)	Mesophilic SBR (mg/L)	Mesophilic Removal (%)
1	1012	72	93	103	90
3	1024	83	92	97	91
6	1003	73	93	76	92
7	986	64	94	63	94
8	1023	89	91	82	92
9	1009	82	92	76	92
12	957	62	94	65	93
14	916	65	93	55	94
16	930	65	93	68	93
21	965	68	93	68	93
24	1012	72	93	70	93
27	1003	78	92	69	93
28	994	71	93	75	92
31	1100	133	88	77	93
34	980	83	92	69	93
36	1020	96	91	92	91
38	1039	105	90	97	91
40	1025	96	91	73	93
42	1020	101	90	78	92
44	1100	100	91	79	93
48	1065	112	89	56	95
50	1005	96	90	89	91
54	750	38	95	69	91
56	1095	107	90	94	91
58	1004	105	90	94	91
62	1001	104	90	93	91
64	994	86	91	81	92
66	989	84	92	82	92
68	998	81	92	75	92
69	1003	103	90	94	91
71	1034	105	90	100	90
72	1165	116	90	104	91
75	1025	97	91	91	91
77	965	83	91	156	84
79	980	85	91	146	85
82	1002	145	86	142	86
86	1000	165	84	132	87
89	998	123	88	120	88

91	987	120	88	110	89
93	1034	120	88	103	90
96	1008	104	90	95	91
98	1078	94	91	93	91
100	1023	92	91	91	91
102	987	84	91	97	90
104	984	83	92	96	90
106	1006	89	91	90	91
109	994	81	92	87	91
111	994	84	92	86	91
113	1023	100	90	87	91
115	1012	100	90	88	91
117	983	94	90	89	91
119	1034	103	90	82	92
122	1076	101	91	93	91
124	923	85	91	91	90
126	1006	91	91	95	91
129	1000	83	92	85	92
131	996	82	92	93	91
133	1065	99	91	92	91
137	1045	90	91	83	92
139	1003	87	91	82	92
143	994	84	92	87	91
144	982	82	92	89	91
146	1034	98	91	85	92
150	1000	93	91	75	93
153	995	82	92	71	93
155	1003	83	92	74	93
157	987	81	92	78	92
159	1021	89	91	72	93
162	1001	90	91	84	92
164	995	93	91	73	93
167	980	96	90	81	92
170	1004	92	91	85	92
172	1023	98	90	80	92
174	1076	93	91	78	93
176	994	96	90	72	93
179	1005	97	90	84	92
183	993	86	91	78	92
188	1034	90	91	84	92
190	1056	103	90	88	92
192	1092	100	91	81	93

195	1156	110	90	88	92
199	976	96	90	84	91
202	987	93	91	82	92
206	1003	100	90	81	92
209	1013	98	90	82	92

Appendix B – Effluent Suspended Solids Data

Part 1 - Treatment of TMP Condensate

Experimental Time (Day)	Thermophilic ESS (mg/L)	Mesophilic ESS(mg/L)
8	30	38
10	28	12
17	6	26
20	14	38
23	6	16
25	14	22
28	10	20
30	25	31
35	26	30
39	28	36
42	32	42
44	37	51
46	43	45
49	29	23
51	30	40
54	47	22
57	43	23
59	35	28
61	33	29
66	25	25
68	28	21
71	20	25
73	24	28
75	40	43
78	35	33
80	50	39
82	53	45
85	52	49
87	162	76
89	125	71
92	135	85
94	180	75
96	54	38
99	58	32
101	52	39
103	55	42
106	48	41
108	56	35
110	49	36
113	52	39

116	43	40
117	48	30
120	42	31
122	44	37
124	44	32
127	42	28
129	49	31
131	42	29
134	47	33
136	49	30
138	67	46
141	64	43
143	62	41

Part 2 - Effect of Temperature and Dissolved Oxygen on EPS Production

Experimental Time (Day)	Thermophilic ESS (mg/L)	Mesophilic ESS (mg/L)
1	76	72
3	96	88
6	76	112
7	88	112
8	71	87
9	77	92
12	72	102
14	68	104
16	65	110
18	71	97
20	70	92
21	112	84
23	121	81
24	108	80
27	92	72
31	95	75
34	85	68
36	106	52
38	108	51
40	97	59
42	95	62
44	102	56
47	95	52
48	88	45

50	91	56
53	90	52
54	78	49
55	92	68
56	102	63
58	94	60
62	92	58
64	100	52
66	94	49
68	97	56
69	106	45
71	110	41
72	95	49
75	89	54
77	75	40
79	85	42
82	70	50
86	145	65
89	156	57
91	138	52
93	83	62
96	90	52
98	82	47
100		
104	82	46
106	78	52
109	80	50
113	76	47
115	74	45
117	75	40
119	75	43
122	80	44
126	75	48
129	80	56
131	73	46
133	79	45
137	78	43
139	86	48
143	88	49
144	90	42
146	86	42
150	82	42

153	85	43
155	82	45
157	84	48
159	85	49
162	82	46
164	86	47
167	82	42
170	82	43
172	82	44
174	84	46
176	86	41
179	93	44
183	92	42
188	97	40
190	102	46
192	98	46
195	100	43
199	96	42
202	104	44
206	98	50
209	96	45

Appendix C – Sludge Volume Index Data

Part 1 – Treatment of TMP Condensate

Experimental Time (Day)	Thermophilic SVI (mL/g MLSS)	Mesophilic SVI (mL/g MLSS)
1	75	35
3	91	28
8	104	140
10	68	83
17	57	54
20	157	64
23	111	41
26	105	51
29	110	57
31	98	45
36	96	47
40	108	50
43	145	53
47	158	41
52	198	58
59	146	75
66	104	93
73	91	120
78	78	96
85	71	126
87	74	156
94	174	152
98	95	86
101	84	80
105	79	75
110	101	95
115	99	94
120	135	94
124	126	95
129	138	96
134	135	98
136	136	100
138	179	125
143	182	124

Part 2 – Effect of Temperature and Dissolved Oxygen on Sludge Properties

Experimental Time (Day)	Thermophilic SVI (mL/ g MLSS)	Mesophilic SVI (mL/g MLSS)
1	237	71
3	214	74
6	217	65
7	184	61
8	191	53
9	228	71
12	203	56
14	171	61
16	200	55
18	158	63
20	197	60
21	228	81
23	219	63
24	186	64
27	189	63
31	140	44
34	147	44
36	172	37
38	148	37
40	170	30
42	167	34
44	161	37
47	161	40
48	165	40
50	185	36
53	166	30
54	187	37
55	158	29
56	147	26
58	122	32
62	121	57
64	100	45
66	133	50
68	126	47
69	150	43
71	163	40

72	129	41
75	133	38
77	179	29
79	160	26
82	136	28
86	112	27
89	117	27
91	182	31
93	184	32
96	198	25
98	227	32
100	231	32
104	232	36
106	240	32
109	240	33
113	272	32
119	311	30
122	303	35
126	318	33
129	310	30
131	297	34
133	327	31
137	333	24
139	287	23
143	330	23
144	304	26
146	298	23
150	318	25
153	297	28
155	300	26
157	300	26
159	313	23
162	300	22
164	302	23
167	292	21
170	283	24
172	274	20
174	283	24
176	278	27
179	222	29
183	213	24
188	140	30

190	155	26
192	169	26
195	158	31
199	166	26
202	169	22
206	146	26
209	155	32

Appendix D – Mixed Liquor Suspended Solids Data

Part 1 – Treatment of TMP Condensate

Experimental Time (Day)	Thermophilic MLSS (g/L)	Mesophilic MLSS (g/L)
1	0.00	2.26
3	0.00	1.14
8	0.48	0.43
10	0.73	0.23
17	0.87	1.30
20	0.89	0.78
23	1.08	1.23
26	1.10	1.20
29	2.35	2.57
31	2.25	2.52
36	2.30	2.53
40	2.50	2.56
43	2.34	2.26
47	2.53	2.42
52	2.52	2.60
59	2.40	2.68
66	2.30	2.50
73	2.20	2.50
78	2.30	2.60
85	2.30	2.65
87	2.30	2.78
94	2.35	2.80
98	2.32	2.85
101	2.37	2.79
105	2.35	2.70
110	2.41	2.86
115	2.45	2.80
120	2.35	2.85
124	2.43	2.75
129	2.40	2.80
134	2.46	2.85
136	2.35	2.90
138	2.16	2.67
143	2.25	2.71

Part 2 – Effect of Temperature and DO on Sludge Properties

Experimental Time (Day)	Thermophilic MLSS (g/L)	Mesophilic MLSS (g/L)
1	2.95	3.50
3	2.80	4.05
6	3.00	4.30
7	3.20	4.10
8	3.45	4.90
9	2.99	3.80
12	3.20	3.90
14	3.50	3.95
16	3.50	4.20
18	3.80	4.30
20	3.00	4.70
21	2.50	3.70
23	2.65	4.30
24	2.95	4.56
27	2.65	3.95
31	3.50	4.50
34	3.40	3.85
36	2.90	4.10
38	3.10	3.50
40	2.65	4.00
42	2.75	3.25
44	2.80	3.15
47	2.80	2.80
48	2.85	2.50
50	2.70	2.50
53	3.01	2.67
54	2.67	3.00
55	2.84	3.45
56	3.20	3.78
58	3.70	4.01
62	2.90	3.50
64	3.00	3.37
66	3.00	3.00
68	2.85	2.75
69	2.67	2.56
71	2.76	2.50

72	2.80	2.45
75	3.00	2.60
77	2.80	3.50
79	2.50	3.50
82	2.20	3.55
86	2.60	3.55
89	2.56	3.35
91	2.20	3.20
93	2.44	3.20
96	2.52	3.17
98	2.64	3.15
100	2.60	3.10
104	2.55	3.07
106	2.50	3.10
109	2.50	3.06
113	2.35	3.00
115	2.30	3.70
117	2.35	3.30
119	2.25	3.35
122	2.18	3.15
126	2.20	3.65
129	2.26	3.28
131	2.19	3.21
133	2.20	3.17
137	1.95	3.85
139	2.30	4.10
143	2.00	4.00
144	2.30	3.85
146	2.15	3.90
150	2.20	3.95
153	2.20	3.75
155	2.20	3.90
157	2.30	3.85
159	2.15	4.05
162	2.30	4.15
164	2.20	3.95
167	2.25	3.95
170	2.30	3.80
172	2.15	4.10
174	2.05	3.50
176	2.30	3.80
179	2.70	3.50

183	2.35	3.70
188	2.50	3.05
190	2.20	3.40
192	1.95	3.40
195	1.65	3.20
199	1.75	3.50
202	1.95	3.60
206	2.05	3.65
209	2.00	3.30

Appendix E – Zeta Potential Data

Part 1 - Treatment of TMP Condensate

Experimental Time (Day)	Thermophilic Zeta Potential (mV)	Mesophilic Zeta Potential (mV)
10	-13.5	-11.5
17	-11.3	-12.5
20	-11.9	-11.4
23	-10.6	-10.5
26	-11.4	-10.9
29	-11.7	-11.8
31	-9.5	-10.3
36	-10.9	-11.7
40	-9.7	-11.6
43	-10.6	-10.5
47	-10.9	-12.4
52	-10.6	-10.8
59	-11.4	-11.7
66	-9.8	-10.8
73	-9.5	-12
78	-10.6	-11.5
85	-10.4	-11.7
87	-9.8	-11.4
94	-10.5	-11.3
98	-10.7	-10.5
101	-9.8	-11.7
105	-10.8	-11.7
110	-9.9	-11.9
115	-10.8	-12
120	-11.2	-10.5
124	-11.4	-10.8
129	-10.5	-11
134	-10.1	-10.5
136	-10.3	-11.2
138	-10.9	-11.3
143	-10.8	-10.7

Part 2 – Effect of Temperature and Dissolved Oxygen on EPS Production

Experimental Time (Day)	Thermophilic Zeta Potential (mV)	Mesophilic Zeta Potential (mV)
37	-12.3	-11.4
39	-12.7	-13.4
41	-14.4	-12.5
45	-13.7	-15.3
49	-14.8	-11.9
59	-13.6	-11.4
63	-14.3	-12.6
65	-12.6	-13.2
114	-13.7	-14.3
116	-14.2	-14.2
118	-12.8	-11.5
124	-12.9	-12.9
127	-13.6	-11.9
130	-13.9	-13.2
137	-14.3	-13.6
139	-13.7	-13.1
143	13.2	-12.7
146	-12.8	-14.5
151	-14.5	-14.2
153	-13.5	-15.1
156	-12.7	-13.7
159	-13.8	-12.6
163	-14.3	-14.2
165	-13.5	-14.7
189	-12.7	-13.2
191	-13.7	-12.7
197	-14.6	-13.8
201	-13.6	-14.2

Appendix F – Bounded EPS Extraction and Analysis Data

Part 2 – Effect of Temperature and DO on Sludge Properties

Thermophilic Low DO:

Experimental Time (Day)	Protein Concentration (mg P/ g MLSS)	Carbohydrate Concentration (mg C/ g MLSS)	Total EPS (mg/ g MLSS)	P/C
36	13.63	6.54	20.17	2.08
38	15.48	5.36	20.84	2.89
40	8.65	6.34	14.99	1.36
44	11.80	4.24	16.04	2.78
48	9.58	4.50	14.08	2.13
54	12.50	5.80	18.30	2.16
56	10.36	6.60	16.96	1.57
58	14.69	5.17	19.86	2.84
62	12.16	4.27	16.43	2.85
64	23.13	10.20	33.32	2.27
68	8.28	4.08	12.36	2.03

Thermophilic High DO:

Experimental Time (Day)	Protein Concentration (mg P/ g MLSS)	Carbohydrate Concentration (mg C/ g MLSS)	Total EPS (mg/ g MLSS)	P/C
113	20.82	14.28	35.10	1.46
115	22.47	15.67	38.14	1.43
117	25.02	14.25	39.27	1.76
123	22.47	2.67	25.14	8.41
126	29.23	3.77	33.00	7.74
129	18.28	3.49	21.77	5.24
136	25.22	8.09	33.30	3.12
138	16.07	6.80	22.87	2.36
142	22.62	8.28	30.91	2.73
145	26.30	9.34	35.64	2.81
152	20.03	15.96	35.99	1.26
155	18.13	15.02	33.15	1.21
158	26.10	8.86	34.96	2.95
162	18.62	6.70	25.32	2.78
164	26.82	9.25	36.07	2.90

Thermophilic Low DO:

Experimental Time (Day)	Protein Concentration (mg P/ g MLSS)	Carbohydrate Concentration (mg C/ g MLSS)	Total EPS (mg/ g MLSS)	P/C
188	9.20	2.49	11.69	3.70
190	20.80	2.80	23.60	7.44
196	20.00	3.60	23.60	5.55
200	10.40	2.42	12.82	4.31
202	10.00	2.56	12.56	3.90

Mesophilic:

Experimental Time (Day)	Protein Concentration (mg P/ g MLSS)	Carbohydrate Concentration (mg C/ g MLSS)	Total EPS (mg/ g MLSS)	P/C
36	6.32	6.89	13.21	0.92
40	14.56	7.34	21.90	1.98
44	6.92	2.56	9.48	2.70
48	18.17	2.34	20.51	7.76
58	12.19	3.33	15.52	3.66
62	14.53	3.62	18.15	4.01
113	14.85	7.34	22.19	2.02
115	13.54	7.40	20.94	1.83
117	12.88	6.81	19.69	1.89
123	9.67	4.15	13.82	2.33
126	13.70	3.35	17.05	4.09
129	16.60	4.11	20.71	4.04
136	12.46	5.73	18.18	2.18
138	10.15	5.12	15.27	1.98
142	9.64	3.48	13.12	2.77
145	15.54	7.57	23.11	2.05
150	17.67	3.70	21.36	4.78
152	12.00	6.94	18.94	1.73
155	11.54	5.94	17.48	1.94
158	9.61	3.92	13.53	2.45
162	19.00	3.77	22.77	5.03
164	9.85	4.62	14.47	2.14
188	8.87	4.86	13.74	1.82
200	17.67	2.10	19.77	8.42

Appendix G – Soluble EPS Extraction and Analysis Data

Part 2 – Effect of Temperature and DO on Sludge Properties

Thermophilic

Experimental Time (Day)	Soluble Protein (mg/L)	Soluble Carbohydrate (mg/L)
36	14.24	8.65
44	11.54	8.65
48	16.98	6.78
56	7.81	5.34
58	9.65	7.76
117	15.65	9.49
126	14.28	8.12
136	12.56	5.34
152	16.83	6.87
162	10.45	7.07
190	13.92	9.43
200	8.43	5.71

Mesophilic:

Experimental Time (Day)	Soluble Protein (mg/L)	Soluble Carbohydrate (mg/L)
36	12.11	9.24
44	5.76	9.17
48	12.8	8.98
62	7.86	11.35
117	10.76	12.65
126	13.89	10.68
136	9.56	7.94
155	12.87	8.56
162	8.56	4.31
190	11.03	12.80
200	9.12	7.13

Appendix H – COD and Dissolved Oxygen Cycle

Part 1 – Treatment of TMP Condensate

COD cycle over 12 hour period:

Time (hr)	Thermophilic COD (mg/L)	Mesophilic COD (mg/L)
0.17	750.342	756.345
1.17	588.5597	325
2.17	482.9113	203
3.17	370.0596	175
4.50	288.4222	123
6.00	201.9826	103
8.00	137.1529	84.3287
9.00	100.23	80.34
10.00	96.476	77.34
11.00	94.345	75.34

Air Stripping COD cycle:

Time (hr)	Thermophilic COD (mg/L)	Mesophilic COD (mg/L)
0	805	813
1	780	791
2	755	776
3	734	765
4	718	758
5	710	749
6	706	745
7	701	740
8	699	738
9	699	738
10	698	735
11	696	734
12	695	734

DO cycle:

Time (hr)	Thermophilic DO (mg/L)	Mesophilic DO (mg/L)
0.17	2.5	6.5
0.67	1.5	4.5
1.17	0.9	4
1.67	1.1	4.1
2.17	2	5.5
2.83	2.2	5.3
3.33	2.2	5.4
3.83	2.2	5.5
4.83	2.3	5.4
5.83	2.4	5.6
6.83	2.7	5.7
7.83	2.6	6
8.83	2.6	6.5
9.83	2.6	6.5
10.83	2.6	6.5

Part 2 –Effect of Temperature and Dissolved Oxygen on Sludge Properties

Thermophilic Low DO COD Removal Cycle:

Time (hr)	COD (mg/L)	COD (mg/L)
0	1002	987
0.5	756	819
1	650	686
1.5	600	534
2	523	425
3	400	319
4	336	206
5	235	175
6	167	124
7	145	95
8	123	86
9	107	73
10	104	73
11	97	72

Thermophilic High DO COD cycle:

Time (hr)	COD (mg/L)	COD (mg/L)
0	976	1035
0.5	746	823
1	634	702
1.5	570	587
2	523	476
3	400	321
4	314	206
5	212	165
6	153	104
7	135	95
8	107	86
9	104	77
10	104	75
11	91	75

Thermophilic Low DO cycle:

Time (hr)	Thermophilic DO (mg/L)	Mesophilic DO (mg/L)
0.2	2.4	5.8
0.7	1.4	4.3
1.2	1	4
1.7	1.2	4.2
2.2	2.1	5.1
2.8	2.3	5.3
3.3	2.3	5.4
3.8	2.2	5.5
4.8	2.3	5.5
5.8	2.4	5.5
6.8	2.5	5.6
7.8	2.5	5.6
8.8	2.5	5.7
9.8	2.5	5.7
10.8	2.5	5.7

Time (hr)	Thermophilic DO (mg/L)	Mesophilic DO (mg/L)
0.1	2.5	5.7
0.6	1.5	4.3
1.2	1	4
1.8	1.2	4.2
2.5	2	4.9
3	2.3	5.2
3.5	2.3	5.2
4	2.4	5.4
5	2.4	5.4
6	2.4	5.6
7	2.5	5.6
8	2.5	5.6
9	2.5	5.6
10	2.6	5.7
11	2.6	5.7

Thermophilic High DO cycle:

Time (hrs)	Thermophilic DO (mg/L)	Mesophilic DO (mg/L)
0.33	5.5	5.6
0.83	4.7	4.8
1.33	4	4.5
1.83	3.5	4.1
2.50	4	4.2
3.00	4.1	4.4
3.50	4.1	4.6
4.00	4.3	4.7
5.00	4.4	4.9
6.00	4.6	4.9
7.00	4.8	5.1
8.00	5.1	5.4
9.00	5.2	5.3
10.00	5.3	5.4
11.00	5.5	5.4

Time (hrs)	Thermophilic DO (mg/L)	Mesophilic DO (mg/L)
0.10	5.5	5.8
0.50	4.8	4.8
1.50	3.8	4.5
2.00	3.5	4
2.50	4	4.1
3.00	4	4.3
3.50	4	4.5
4.00	4.2	4.5
5.00	4.5	4.7
6.00	4.6	5
7.00	4.8	5.1
8.00	5.1	5.2
9.00	5.2	5.3
10.00	5.3	5.5
11.00	5.3	5.5