

Enhanced Biological Phosphorus Removal
for Liquid Dairy Manure

Yanjuan Hong

Thesis submitted to the faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Biological Systems Engineering

Dr. Jactone Arogo Ogejo
Dr. Katharine F. Knowlton
Dr. Amy J. Pruden

November 13, 2009
Blacksburg, VA

Keywords: Enhanced biological phosphorus removal, polyphosphate accumulating organisms, volatile fatty acids, phosphorus recovery, dairy manure, manure management, manure treatment

Copyright 2009, Yanjuan Hong

Enhanced Biological Phosphorus Removal for Liquid Dairy Manure

Yanjuan Hong

Abstract

. Enhanced biological phosphorus removal (EBPR) has been widely used in municipal wastewater treatment, but no previous studies have examined the application of EBPR to treat dairy manure. This study was conducted to evaluate the (i) performance of pilot-scale EBPR systems treating liquid dairy manure, to balance the ratio of nitrogen to phosphorus in manure to meet crop nutrient requirements, (ii) effects of dissolved oxygen and solids retention time on the efficiency of EBPR, and (iii) effectiveness of gravity thickening for reducing the volume of harvested EBPR aerated mixed liquor.

Two pilot-scale EBPR systems were used in this study. The ratio of the manure chemical oxygen demand expressed as volatile fatty acids to phosphorus used ranged from 18:1 to 45:1. The phosphorus removal efficiencies of the EBPR system were investigated at three solids retention times (4, 6 and 10 d), and three dissolved oxygen levels (3, 4 and 6 mg O₂/L). The total phosphorus removal was highest (84%) at 10 d solids retention time and lowest (63%) at 4 d solids retention time. The sludge from the 6 d solids retention time tests had better sludge settling characteristics with a sludge volume index of 62 mL/g compared to 80 mL/g for the 4 d solids retention time. The EBPR system achieved 90% dissolved reactive phosphorus removal when the system

was operated at 4 mg O₂/L, and the ratio of nitrogen to phosphorus in effluent increased to about 5:1, which was higher than the normal ratio in dairy manure. On the other hand, phosphorus removal performance deteriorated when dissolved oxygen level was 3 mg O₂/L. In the gravity thickening tests, 93-95 % total suspended solids (TSS) was removed from the settled supernatant, with 1.2 to 1.54 % total solids (TS) in the settled solids after 90 min gravity-induced thickening. The extent of phosphorus release during gravity thickening process needs to be further investigated.

Acknowledgements

I would like to express my appreciation to my advisor, Dr. Jactone Arogo, for his time and guidance throughout this research. I would also like to thank my committee members, Dr. Katharine F. Knowlton and Dr. Amy J. Pruden, for their invaluable comments and assistance in my study. I am very grateful to Dr. Clifford W. Randall and Dr. Chao Shang for their time and patience to answer my questions.

I am very appreciative of Hope White, Jody Smiley and Julie Jordan for being so supportive and helpful, when I was using IC, GC and other facilities in their labs. I want to thank Karen Hall for her hard work and continuous support for my sample analysis. Also, I want to thank Monika Corbett and Jo DeBusk for kindly teaching me many laboratory skills. Many thanks go to Lifeng Li for his tremendous support, help and encouragement during my graduate studies.

Finally, I would like to extend thanks to Sonja Galley, Chris Brown, Abigail Schmidt, Kareka Aradi, Brittany Willing, Brittany Thompson, Yanwen Shen, Leslie Sarmiento, Porter Knight, Alex and Henry for their great help with manure separation and other lab work.

TABLE OF CONTENTS

1	Introduction.....	1
1.1	Background.....	1
1.2	Treatment Technologies	2
1.2.1	Natural Treatment System.....	2
1.2.2	Physical Treatment.....	3
1.2.3	Chemical Treatment	3
1.2.4	Biological Treatment.....	4
1.2.4.1	Anaerobic Treatment.....	4
1.2.4.2	Aerobic Treatment.....	5
1.3	Enhanced Biological Phosphorus Removal	6
1.3.1	Microbiology of Enhanced Biological Phosphorus Removal	9
1.3.2	Factors Affecting EBPR	12
1.3.2.1	Temperature.....	12
1.3.2.2	pH.....	13
1.3.2.3	Feed.....	14
1.3.2.4	Dissolved Oxygen	15
1.3.2.5	Solids Retention Time	16
1.3.3	Sludge Thickening	17
1.4	Summary	20
2	Objectives.....	22
3	Materials and Methods	23

3.1	Manure Collection and Preparation.....	23
3.2	Reactor Design and Operation.....	23
3.2.1	Reactor Design	23
3.2.2	Sequencing Batch Reactor	26
3.2.3	Reactor Operation	29
3.2.3.1	Feeding	29
3.2.3.2	Evaluation of Pilot Scale Enhanced Biological Phosphorus Removal Systems for Liquid Dairy Manure	29
3.2.3.3	Calculating Phosphorus Content	30
3.2.3.4	Effects of SRT on Phosphorus Removal	30
3.2.3.5	Calculating Solids Retention Time.....	31
3.2.3.6	Effects of DO on Phosphorus Removal.....	31
3.2.3.7	Sludge Volume Index	32
3.2.4	Reactor Maintenance.....	32
3.3	Gravity Thickening Tests	33
3.4	Sampling and Analysis.....	35
3.5	Statistical Analysis	38
3.5.1	Hypothesis Testing	38
3.5.2	Power Analysis	39
4	Results and Discussion	42
4.1	Evaluation of Pilot Scale Enhanced Biological Phosphorus Removal Systems Treating Liquid Dairy Manure	42
4.1.1	Manure Characteristics	42
4.1.2	COD:P	44

4.1.3	DRP	47
4.1.4	TP	52
4.1.5	VFA	56
4.1.5.1	TSS and VSS	61
4.2	Effects of Different Parameters on Phosphorus Removal	66
4.2.1	Solids Retention Time	66
4.2.1.1	Power Analysis	77
4.2.2	Dissolved Oxygen	80
4.3	Gravity Thickening Tests	95
5	Conclusions	100
6	Future Work	103
7	References	105

List of Figures

Figure 1. Schematic of the EBPR system.....	25
Figure 2. Schematic of an 8-h cycle in the SBR (Arogo et al., 2008). At the end of the cycle, aerated mixed liquor was pumped out for 3 min followed by 30 min settling, and then effluent (supernatant) was pumped out for 7 min.	28
Figure 3. Schematic of an Imhoff settling cone as a thickener	34
Figure 4. DRP concentrations in influent to SBR, effluent and aerated mixed liquor in SBR 1.	50
Figure 5. DRP concentrations in influent to SBR, effluent and aerated mixed liquor in SBR 2.	51
Figure 6. TP concentrations in influent to SBR, effluent and aerated mixed liquor in SBR 1.	54
Figure 7. TP concentrations in influent to SBR, effluent and aerated mixed liquor in SBR 2.	55
Figure 8. TSS in influent to SBR, effluent and aerated mixed liquor of SBR 1 at different COD:P ratios.	62
Figure 9. TSS in influent to SBR, effluent and aerated mixed liquor of SBR 2 at different COD:P ratios.	63
Figure 10. VSS in influent to SBR, effluent and aerated mixed liquor of SBR 1 at different COD:P ratios.	64
Figure 11. VSS in influent to SBR, effluent and aerated mixed liquor of SBR 2 at different COD:P ratios	65
Figure 12. DRP concentrations in influent to SBR, effluent and aerated mixed liquor of SBR 1 at different SRTs, 4 d from day 516 to 705, 6 d from day 712 to 774 and 10 d from day 782 to 839.	68
Figure 13. DRP concentrations in influent to SBR, effluent and aerated mixed liquor of SBR 2 at 4 d SRT from day 516 to 839.	69

Figure 14. TP concentrations in influent to SBR, effluent and aerated mixed liquor of SBR 1 at different SRTs, 4 days from day 516 to 705, 6 days from day 712 to 774 and 10 days from day 782 to 839.....	70
Figure 15. TP concentrations in influent to SBR, effluent and aerated mixed liquor of SBR 2 at 4 d SRT from day 516 to 839.....	71
Figure 16. Sludge volume index test using 1 L graduated cylinder.....	76
Figure 17. Plot of TP removal effect size versus standard deviation for fixed power of 80% and total sample size of 4.....	78
Figure 18. DRP concentrations in influent to SBR, effluent and aerated mixed liquor at different DO levels in treatment reactor (SBR 1).	82
Figure 19. DRP concentrations in influent to SBR, effluent and aerated mixed liquor at 6 mg O ₂ /L in control reactor (SBR 2).	83
Figure 20. TP concentrations in influent to SBR, effluent and aerated mixed liquor at different DO levels in treatment reactor (SBR 1).	84
Figure 21. TP concentrations in influent to SBR, effluent and aerated mixed liquor at 6 mg O ₂ /L in control reactor (SBR 2).....	85
Figure 22. Typical DO and temperature profile for SBR 1 in 3 mg O ₂ /L phase...	93
Figure 23. Typical DO and pH profile for SBR 2.....	94
Figure 24. Variation of TSS in supernatant versus thickening period within Trial 1.	97
Figure 25. Variation of TSS in supernatant versus thickening period within Trial 2.	97
Figure 26. Gravity thickening tests using Imhoff cones	99

List of Tables

Table 1. Characteristics of the liquid manure collected after solid-liquid separation.	43
Table 2. Ratio of COD equivalents of VFA to DRP in influent to two SBR systems.	46
Table 3. VFA concentrations in feed and fermenter of SBR 1.	59
Table 4. VFA residues in effluent and aerated mixed liquor of SBR 1.	60
Table 5. Mean DRP removal, TP removal, P content, TSS, VSS and SVI in treatment reactor and control reactor at different SRTs.	75
Table 6. TP removal data from a previous research.	79
Table 7. Mean DRP removal, TP removal, P content, TSS, VSS and SVI in treatment reactor and control reactor at different DO levels.	91
Table 8. Concentrations of TP, sCOD and TKN in effluent and aerated mixed liquor in treatment reactor (SBR 1) at different DO levels.	92
Table 9. Comparison of TSS in the supernatant of two thickening trials.	98

1 Introduction

1.1 Background

Dairy manure is a valuable source of elements that are essential for plant growth, including nitrogen (N), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), calcium (Ca), zinc (Zn), copper (Cu), manganese (Mn), sulfur (S) and aluminum (Al). It is usually land-applied as fertilizer to improve soil fertility and promote crop growth. But when manure is applied on N-based crop needs, usually P in manure is in excess. After a long time of manure over-application, P gradually accumulates in soil (Maguire et al., 2001), and is likely transported in runoff as soil erodes. Agriculture is a main cause of water quality impairment after inappropriate application of P to field, and when P enters natural water bodies through runoff, it causes eutrophication, oxygen depletion and biodiversity reduction (Danalewich et al., 1998).

Typically, the ratio of N to P (N:P) in manure is lower than the ratio of crop growth requirement. Manure that is used as a fertilizer to meet crop N demand tends to add much more P to the cropland than the crops need, resulting in P buildup in soils. Agronomic soil P test report showed that more than 20 states in the U.S. had 50% soil samples categorized as “high” in P (Sharpley et al., 2003), which raised the awareness of the importance of P management. Some states have been working with USDA and state cooperative extensions to provide guidance for producers on how to manage manure application on their farms to maximize nutrient utilization and in turn conserve soil and water quality. Producers are advised to tailor manure application to specific soil

conditions. If the soil is low in P, manure can be applied to the field to meet crop growth requirements. If P level in field is rather high, manure application is not recommended in order to reduce the risks of P runoff, and manure can be transported to the land in high demand of P. On the other hand, the importance of managing P in manure to balance N:P ratio is reflected. Dietary P reduction and animal feed grouping strategies can be employed to reduce manure P excretion and the potential of P overapplication (Van Horn et al., 1996; Dunlap et al., 1997; Powell et al., 2002). Proper manure treatment can also be used to balance the ratio of N:P in manure to meet the crop agronomic requirements.

1.2 Treatment Technologies

Numerous types of treatment technologies can be used to manage P, including physical, chemical and biological methods, and in some cases a combination of these methods.

1.2.1 Natural Treatment System

Natural treatment system such as wetland is a treatment method that incorporates a variety of physical, chemical and biological transformations. Adsorption, biochemical reaction, chemical precipitation, ion exchange, sedimentation and many other processes take place in the complex system. Constructed wetlands have drawn increasing attention for their potential on treating animal wastewater since 1990s, while they have already been utilized to treat municipal and domestic wastewater for decades (Knight et al., 2000). Compared to natural wetlands, constructed wetlands are more

flexible and controllable by selecting appropriate landform, substrates and plants on the purpose of removing organic residues, nutrients, fecal coliform and solids (Kadlec and Knight, 1996; Higgins et al., 2000). Few data were reported on P removal efficiencies of constructed wetlands for treating concentrated animal wastewater. Tyson (1996) reported that 58-74% P was removed from dairy wastewater after constructed wetland treatment.

1.2.2 Physical Treatment

Gravity sedimentation and mechanical solid-liquid separation can be used to remove solids from wet manure. In general, 35% of P in manure solid fraction can be removed by mechanical separation (Kintzer and Moffitt, 2005). To enhance P removal efficiency using physical treatment methods, chemicals are usually added to the manure. Other manure treatment methods including drying, incineration and pyrolysis, which aim to reduce manure volume, generate heat and produce oil, may require substantial energy input (Hatfield and Stewart, 1998).

1.2.3 Chemical Treatment

Chemical P removal process is a widely used technology to reduce P concentration in wastewater by adding metal (such as Fe, Al, Ca, Mg) salts or polymers to cause chemical coagulation, flocculation and precipitation. Properly operated chemical P removal process enabled full-scale wastewater treatment plants (WWTP) to meet stringent regulations by reducing P to a very low concentration (Takacs et al., 2006). Some Fe-based flocculants were applied to remove P from liquid pig manure and

the effluent P concentration could meet the 2 mg/L discharge standard (Meers et al., 2006). Ferric chloride and alum were used to treat separated flush dairy manure, achieving 80% and 90% P removal respectively (DeBusk et al., 2008). While removing P from manure, chemical process produces substantial sludge due to the addition of chemicals. The costs for purchasing chemicals and treating such large amount of sludge are considerable, and the availability of chemically-bounded P to the crops need to be evaluated (Maguire et al., 2001).

1.2.4 Biological Treatment

Biological manure treatment is a process that takes advantage of microbial metabolic mechanisms to remove pollutants and nutrients by subjecting microorganisms to conditions with or without aeration. Compared to chemical treatment, biological treatment process without chemical addition is more cost effective, produces less sludge and effluent with lower salinity (Janssen et al., 2002). More detailed information will be provided in the following subsections.

1.2.4.1 Anaerobic Treatment

Anaerobic treatment is a process where microorganisms reduce biodegradable organic matter and possibly pathogens in the absence of air. Examples of anaerobic treatment include anaerobic lagoon and anaerobic digestion. Anaerobic lagoon is an open-air pond with adequate depth to keep good anaerobic condition and prevent odor emissions (Hatfield and Stewart, 1998). It is operated mainly to reduce organic and N concentrations from high strength animal waste. Complex organic compounds are

degraded into simple compounds associated with emission of odorous ammonia (NH_3) gas and hydrogen sulfide (H_2S). Anaerobic digester is an enclosed tank that is strictly controlled in the absence of oxygen, at mesophilic (30-40°C) or thermophilic temperature (55-65°C). The main purposes of anaerobic digestion are to degrade organic compounds and generate biogas which basically consists of 60% of methane (CH_4) and 40% of carbon dioxide (CO_2) (Hatfield and Stewart, 1998). During the process, organic N is converted to NH_3 and P is converted to soluble inorganic P.

1.2.4.2 Aerobic Treatment

Aerobic treatment is a biological process taking place in the presence of oxygen. Aerobic treatment is advantageous to organic compounds removal, odor control and pathogen reduction. During the process, N can be removed via release of nitrogen gas (N_2) after undergoing nitrification and denitrification, but P is not reduced. Aerobic treatment is not as popular as anaerobic treatment for treating animal waste on site, due to the fact that it is relative costly for installation and maintenance of aeration and mixing equipment in aerobic process. Composting is a process usually operated at thermophilic temperature in an aerobic environment, such as aerated static pile (Van Horn et al., 2003). With appropriate moisture content, ratio of C:N, air supply, mixing and turning, microorganisms selected by the process will decompose biodegradable organics, reduce solid volume and pathogens (Surampalli et al., 2004). P content is not reduced. Composting produces a stabilized final product that could be a good soil amendment.

Sequencing batch reactor (SBR) is a system that employs different processes (anaerobic, anoxic and aerobic) in one reactor at different stages, by which organic matter and nutrients can be removed. Differing from tanks-in-series configuration, an SBR is a single sludge bioreactor that treats wastewater in batches with a typical time sequence of fill, react, settle, draw and idle (Grady et al., 1999), and the length of these sequential periods can be altered to achieve specific design objectives. In contrast to conventional activated sludge process, SBR is known for its operation flexibility, easy control over microbial growth and good nutrient removal performance, and it doesn't require return activated sludge pumping (Surampalli et al., 2004). Given the effectiveness of nutrient removal, SBR has been increasingly implemented to remove P from industrial and municipal wastewater, and could be a good alternative for P removal from animal waste other than chemical treatment.

1.3 Enhanced Biological Phosphorus Removal

Enhanced biological P removal (EBPR) has been widely implemented for municipal and industrial wastewater treatment in past four decades (Blackall et al., 2002; de-Bashan and Bashan, 2004; Oehmen et al., 2007).

It should be noted that P is an essential element for microbial growth and metabolism. It is an important component of cell membrane structure, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and adenosine triphosphate (ATP) in microbial cells. Normally, microorganisms will take up P for their cellular biosynthesis in biological treatment process, and certain amount of P removal is expected to occur. McCarty

(1970) (quoted by Pokethitiyook (1990)) used $C_{60}H_{87}O_{23}N_{12}P$ to represent bacteria biomass constituents in activated sludge treatment process, which indicated that normally microorganisms could take up about 2% of P from substrate for their growth in an activated sludge process. However, a much higher P removal than the normal bacteria P uptake for growth in an activated sludge process was observed and first reported by Srinath et al. (1959) (quoted by Pattarkine (1991)), after whom extensive research has been done to examine the phenomenon of enhanced P removal in activated sludge system. It was later found that larger amounts of P could be stored as polyphosphate in microbial cells other than the normal P requirement for maintenance.

Barnard (1976) suggested that it is necessary to put an anaerobic zone ahead of other zones (aerobic or anoxic/aerobic) to achieve luxury P uptake, in that it is important to keep the redox potential low before bacteria could take up P in aerobic zones. This helped to develop a basic configuration of EBPR process. The simplest configuration of EBPR process is an anaerobic zone followed by an aerobic zone or keeping cyclic anaerobic and aerobic in an SBR, which result in the selection of polyphosphate accumulating organisms (PAOs) (Lan et al., 1983; Wentzel et al., 1990; Comeau et al., 1996).

In the upstream anaerobic phase, in the absence of dissolved oxygen (DO) and nitrate, organic compounds are broken down into readily biodegradable organics, mostly volatile fatty acids (VFAs). Meanwhile, PAOs will hydrolyze intracellular polyphosphate (Pereira et al., 1996; Mino, 2000) and release orthophosphate into liquid

phase to generate energy that can be utilized to store VFAs as poly- β -hydroxyalkanoates (PHAs) (Mino, 2000). In the subsequent aerobic phase, oxygen is the dominant electron acceptor and PHA serves as energy source, PAOs will take up more than two times the amount of P they need for cell growth and store it as polyphosphate (Liu et al., 1996). After undergoing anaerobic P release and aerobic P uptake in the EBPR process, P would be highly accumulated in the biomass. Thus, P content in the liquid phase is very low, while P content in the sludge is much higher. By wasting the P-enriched sludge, excess P is removed from the treated wastewater as part of biomass. In municipal wastewater treatment, the low P content effluent undergoes tertiary treatment before being discharged to natural water bodies. In animal waste treatment process, the low P effluent which is more balanced in N:P ratio will be used for irrigation to croplands or pasturelands. The P-enriched sludge will be further treated, which will be discussed later.

While no previous research was found that investigated the application of EBPR on treating dairy manure, some researchers have reported the applications of EBPR on dairy milking and processing wastewater. Mason and Mulcahy (2003) compared the characteristics of domestic wastewater with farm dairy wastewater which was flushed wastewater from milking parlor and dairy shed. Dairy wastewater contained 4.4 to 5 times as much nutrient as domestic wastewater in terms of total kjeldahl nitrogen (TKN) and total P (TP). Danalewich et al. (1998) evaluated the potential of biological nutrient removal by analyzing ratios of five-day biochemical oxygen demand (BOD_5):TP,

BOD₅:TKN, chemical oxygen demand (COD):TP and COD:TKN. They concluded that successful nitrification, denitrification and P removal from milk processing wastewater could be achieved simultaneously. Bickers et al. (2003) reported appropriate EBPR technology can be applied to treat fermented dairy processing wastewater.

1.3.1 Microbiology of Enhanced Biological Phosphorus Removal

After decades of development and implementation, EBPR has been widely used in municipal WWTPs (Janssen et al., 2002; Stephens et al., 2003). However, since the biochemical reactions involved in EBPR process are highly complex and no pure PAO cultures are available (Mino et al., 1998), the progress of understanding the mechanisms in EBPR process is relatively slow. Several researchers proposed possible biochemical transformation mechanisms to explain the cyclic P-release and P-uptake phenomenon (Comeau et al., 1986; Wentzel et al., 1991; Mino et al., 1995; Smolders et al., 1995).

Substantial work has been done to study the microbial community involved in EBPR. Fuhs and Chen (1975) first isolated *Actinobacteria* from P-enriched sludge and suggested it was a group of microorganisms that were responsible for P removal. This was supported by other researchers who also thought *Actinobacteria* were capable of storing P in their cells in addition to the metabolic requirement (Fuhs and Chen, 1975; Malnou et al., 1984; Kong et al., 2005). However, the isolated *Actinobacteria* by Fuhs and Chen (1975) didn't show acetate uptake and PHA synthesis in spite of the observed P uptake under aerobic condition. Brodisch and Joyner (1983) suggested there might

be other microorganisms that were able to remove P other than *Actinobacteria*, because they found that *Aeromonas* and *Pseudomonas* accounted for major part of the microbial population in P removal process. Gradually, it was recognized that the microbial community structure in EBPR system was not dominated by a specific group of microorganisms but rather quite diverse and has a broad spectrum of microorganisms (Mino et al., 1998; Mino, 2000). Many studies found beta *Proteobacteria* was dominant in P-enriched EBPR sludge and presumed to be responsible for P removal (Hesselmann et al., 1999; Crocetti et al., 2000; Jeon et al., 2003). After more specific analysis of the beta *Proteobacteria*, Hesselmann et al. (1999) found a type of bacterium possessing the characteristics of polyphosphate storage and PHA synthesis was very similar to *Rhodocyclus* of beta *Proteobacteria* subset, but was taxonomically different. They named it a new genus called *Candidatus Accumulibacter phosphatis*. Recently, He et al. (2007) used quantitative real-time polymerase chain reaction (qPCR) and polyphosphate kinase 1 gene primer to investigate the microbial community structure of lab-scale and full-scale EBPRs, and found that *Candidatus Accumulibacter* were varied and might be ecologically distinct.

The detection of PAOs that were responsible for excess P removal was mainly relied on culture-dependent methods in the early stage since 1970s. Bacterial colonies were isolated from other microbial population by serial dilutions, spread plate or other techniques, and then cultivated for pure culture enrichment in a medium under suitable conditions (Wagner et al., 1993; Stante et al., 1997). However, some bacteria are

unculturable on artificial media and pure PAO cultures that have all the necessary EBPR characteristics including VFA uptake, PHA synthesis, P release, PHA degradation and P uptake are difficult to isolate. Mino et al. (1998) suggested that it is important to keep anaerobic and aerobic alternation and avoid coexisting of carbon source and electron acceptors during isolation process. The development of molecular techniques independent of cultivation is of great importance for understanding the microbiology of EBPR. Nowadays, the methods commonly used to detect and identify PAOs can be classified into two major groups, microscopic based and PCR based, respectively. In microscopic based methods, microscopy analysis is usually combined with staining or fluorescent in situ hybridization (FISH). Methylene blue and Neisser stain are usually used for staining polyphosphate granules and sudan black stain can be used for staining PHA granules (Crocetti et al., 2000; Griffiths et al., 2002). In FISH analysis, an oligonucleotide probe which is complementary to the 16s rRNA of specific bacteria will be hybridized to the fixed sample and the target gene with fluorescence can be detected (Crocetti et al., 2000; Beer et al., 2006). In PCR based methods, after DNA extraction, nucleic acid primers with complementary sequence to the target gene can be hybridized to target specific gene, and by repeating the DNA polymerization, the target genes are amplified (Madigan et al., 2006). Cloning and denaturing gradient gel electrophoresis (DGGE) can be used to separate the PCR-amplified genes and sequence for phylogenetic affiliation analysis (Onuki et al., 2002; Jeon et al., 2003; Madigan et al., 2006). Meanwhile, microbial population can be quantified using qPCR

analysis. To minimize the bias and increase accuracy, more than two of the identification methods can be used.

1.3.2 Factors Affecting EBPR

Factors that affect the enrichment of PAOs and the performance of P removal include solids retention time (SRT), temperature, pH, organic loading, influent P concentration, and DO. By optimizing these factors and creating suitable conditions for PAOs proliferation, high P removal efficiency and low P content in effluent, can be achieved.

1.3.2.1 Temperature

Temperature is a fundamental factor that affects the vitality of living organisms and consequently the P removal efficiency of EBPR systems. Erdal et al. (2003) investigated the effects of temperature on the EBPR, and found P removal decreased as temperature decreased. But after a longer period of steady-state, P removal efficiency increased twice more than that at 20°C. Temperatures less than 10°C were more favorable for PAO growth compared with non-PAOs. On the contrary, P removal was found to be much higher at 20°C and 25°C, compared to 5-15°C in swine manure treatment (Ndegwa et al., 2003). Whang and Park (2006) examined the effects of temperature and SRT on the competition between PAOs and non-PAOs. They found that at 20°C and 10 d SRT, PAOs were dominant in the system and complete P removal was achieved with 30 mg P/L in influent; while at 30°C and 10 d SRT, non-PAOs dominated in the system. Erdal et al. (2006) studied the relationship of washout SRT

and temperature in EBPR system, and showed that higher temperature lead to a shorter washout SRT, and the P removal efficiency was lower than that at lower temperature.

1.3.2.2 pH

As previously stated, robust EBPR system can be obtained by alternating anaerobic and aerobic conditions to the reactors. In the anaerobic phase, VFAs are taken up and stored as PHA by microorganisms in the mixed liquor, and P is released to the mixed liquor by degradation of intracellular polyphosphate. Without pH control, increase of pH in anaerobic phase can be observed due to removal of VFAs. Jeon et al. (2001) found P removal efficiency increased when the pH of anaerobic phase increased to 8.4 without pH control, while a controlled pH value of 7.0 had a detrimental effect on the EBPR performance. This is in accordance with the observations by Serafim et al. (2002) who reported P release during the anaerobic phase and the P content in biomass without pH control in anaerobic phase (between 7.8 and 8.5) was almost twice as much as that with pH control at 7.0 ± 0.2 . A study by Liu et al. (1996) showed that P release rate increased when pH was increased from 5.0 to 8.0, and decreased when the pH was above 8.0. Oehmen et al. (2005) found a low level of P release and uptake occurred when pH was controlled at 7, and suggested that it would be more favorable for the growth of PAOs when pH level was close to 8. Overall, these studies suggested that pH can be used as an operational variable to affect the performance of EBPR system, and pH level around 8.0 might have a positive effect on P removal efficiency and system stability.

1.3.2.3 Feed

The characteristics of feed play an important role in the performance of EBPR system, especially the P and influent organic substrate concentrations. Dissolved reactive P (DRP), also known as soluble reactive P (SRP), is the soluble biologically available P. Since insoluble P and most of the soluble organic P are considered to be biological unavailable, DRP is actually used to indicate the fraction of soluble orthophosphate (Pierzynski, 2000), and is a critical factor in nutrient management and water quality monitoring (Neal et al., 2003).

VFAs were most favorable for PAOs as external carbon source (Randall et al., 1997; Puig et al., 2008) compared to alcohols, starch and other organic compounds. Branched VFAs (especially isovaleric acid) were found to be more beneficial to the P removal efficiency in EBPR processes (Abughararah and Randall, 1991). To enhance P release and uptake in EBPR, prefermentation was introduced to obtain the necessary VFAs for more efficient anaerobic PHA synthesis (Randall et al., 1994; Skalsky and Daigger, 1995; Güngör et al., 2009).

Several optimal influent ratios of COD:P calculated using various forms of P (TP, Ortho-P) and COD (sCOD, total COD, COD_{VFA}) have been reported in literature. Some studies have suggested that at least 20 mg COD equivalent of acetic acid is needed to remove 1 mg soluble P (Abughararah and Randall, 1991; Lie et al., 1997). Carrera et al. (2001) examined six levels of influent COD:DRP ranging from 16:1 to 87:1 in a lab scale

A²/O EBPR system and determined that the optimum COD:DRP ranged between 41:1 and 48:1. Panswad et al. (2007) investigated effects of three sCOD:DRP ratios (50:1, 25:1 and 6.25:1) on the performance of P removal, and concluded higher P content in the feed would favor the growth of PAOs. Studies by Carucci et al. (1994) and Yagci et al. (2003) showed that P removal efficiency decreased when the influent COD/TKN decreased, and nitrate had a negative effect on P removal. In a full scale EBPR WWTP, Carucci et al. (1999) observed a trend that low COD loading caused higher P concentration in the effluent. The EBPR system can maintain good performance under slight fluctuations of organic loading, but abrupt changes in the feed COD can cause wash out of microorganisms (Ahn et al., 2006). Ahn et al. (2006) observed that when influent COD decreased abruptly from 150 to 50 mg/L, washout of microorganisms occurred.

1.3.2.4 Dissolved Oxygen

DO is a key operational parameter that affects the performance of EBPR system, and would be a major concern for farmers in terms of energy cost if the system was to be implemented on farms. Sufficient DO should be supplied to the aerobic stage of an EBPR system. Luxury P uptake takes place if there is enough PHA as energy source and oxygen as electron acceptor. However, excessive aeration will adversely affect the P removal efficiency. When excessive aeration occurs in the aerobic phase, PHA stored in the cells would be depleted, and thus P uptake decreases due to lack of energy

(Brdjanovic et al., 1998). Also, excessive aeration may induce the occurrence of nitrification.

Nitrate has a detrimental effect on the performance of P removal when it enters anaerobic zone (Brdjanovic et al., 1998; Yagci et al., 2003; Zou et al., 2006). In the presence of nitrate, the redox potential of anaerobic phase is increased (Ekama et al., 1983), which make denitrification process more favorable than PAOs' anaerobic VFA storage. Denitrifiers would compete with PAOs for the organic substrate available in the anaerobic phase by utilizing nitrate as electron acceptor. Thus, less organic substrate would be available for PAOs to synthesize PHA, resulting in less energy for P uptake under aerobic condition and a decline of P removal efficiency. On the other hand, if DO supply is limited so that the energy required for P uptake is limited, P removal performance would also decrease. Because of the critical role of oxygen, it is necessary to maintain an optimal DO concentration for the best performance of P removal.

1.3.2.5 Solids Retention Time

Solids retention time, also referred to sludge age or mean cell residence time (MCRT), is the average time that microorganisms stay in the reactors. It is one of the important factors that affect the efficiency of P removal as well as the sludge settleability in EBPR systems. Generally, SRT is related to the growth of microorganisms in reactors. When SRT is shorter than the minimum SRT required by PAOs, the formation of new biomass will be slower than the wastage of the present biomass, resulting in microorganisms washout (Grady et al., 1999).

At SRT of 1.2 and 2.1 d, PAOs washout was observed at 10 and 5°C, respectively, and the optimal removal efficiency happened at SRT of 16 to 24 d at 5°C, and 12 to 17 d at 10°C (Erdal et al., 2006). In a study conducted by Kargi and Uygur (2002), the maximum P removal rate (70%) was obtained at 10 d sludge age. Henze et al. (2002) found that low SRT (4 d) lead to poor settling properties in that low SRT might have caused changes in P release rate and capacity. Similar observations were made by Rodrigo et al. (1996). Rodrigo et al. (1996) found sludge volume index (SVI) decreased from 145 to 80 mL/g as the sludge age increased from 11 d to 65 d, which indicated sludge settleability was better when the SRT was longer. However, poorer sludge settling was observed at SRT of 20 d compared to 5 d (Ju et al., 2007). Higher removal rates were achieved when sludge age decreased, and P release versus VFA removal in the anaerobic reactor was fitted to a straight line (Rodrigo et al., 1996). Henze et al. (2002) pointed out that when raw wastewater had a high ratio of VFA:TP, performance was not affected by the SRT. Generally, an SRT of 8 to 10 d at a temperature of 20°C is an optimal combination for EBPR system, but the correlation of sludge settling properties and sludge age needs further study.

1.3.3 Sludge Thickening

Sludge thickening, also referred to sludge concentration, is a process used to reduce the volume of liquid portion in sludge and thus increase the solid content of sludge. Sludge thickening is required before any further waste activated sludge treatment, such as digestion and composting. Waste activated sludge usually contains

99.0-99.5% water (Cizinska et al., 1992; Randall et al., 1992). The cost of transporting and further treating such large volume of wet sludge is a big part of the whole process expenses. In EBPR process, although P has been accumulated as polyphosphate in microbial cells, there's still a chance that DRP would be released into the liquid phase, if sludge is exposed to an anaerobic condition for a long time (Eikum et al., 1975; Pitman et al., 1991; Ju et al., 2005). Thus, proper sludge thickening and treatment technologies are required to minimize P release and maximize nutrient recovery.

Gravity thickening, dissolved air flotation (DAF), gravity belt thickening, centrifugal thickening and rotary drum thickening are the typical technologies used for sludge thickening (Tchobanoglous et al., 1991). A sludge solid concentration varying from 2% to 7% can be achieved after a thickening treatment (Tchobanoglous et al., 1991; Randall et al., 1992).

Gravity thickening is a gravity driven settling process taking place in a circular tank with a mechanical scraper in the slope-shape bottom. Sludge is supplied into the center of the tank through pipe. Supernate overflows out of the tank and concentrated sludge is scraped from the bottom of the tank for further treatment. Gravity thickening is more often used to treat primary sludge than waste activated sludge, and more favorable for application in small plants (Tchobanoglous et al., 1991). This is because the settlement of waste activated sludge is relatively slow and gas emitted from activated sludge during thickening process may adversely affect sludge settling (Lang, 2005). On the other hand, comparing to other thickening methods, gravity thickening is

more economical and easy to operate, although usually the effluent suspended solid is relatively high (Park et al., 2004).

DAF is commonly used for activated sludge thickening. Air is pumped into a pressurized sludge and forms a mixture of air and sludge before it enters DAF tank. The dissolved air is released to form fine air bubbles as soon as the mixture of air and sludge is pressure-released. Fine air bubbles will attach to suspended solids and float to the top of the tank, where clear effluent passes through effluent weir and float sludge is discharged through float trough (Tchobanoglous et al., 1991). It was reported that DAF showed good thickening performance, but it may require substantial input for aeration and operation (Arora et al., 1995).

In other instances, Park et al. (2004) utilized mesh filtration with pore sizes of 100 to 500 μm for thickening and achieved a 85-95% of sludge volume reduction, despite that thickening was very fast in the beginning, and slowed down as solids clogged the surface of mesh. In order to minimize P release during sludge thickening, pre-aeration and a combination of linear screen and belt press were introduced by Pitman et al. (1991). Also, there were methods that combined chemical conditioning with sludge thickening to effectively reduce water content and concentrate the sludge. Chen et al. (2006) used metal salts and polyampholytes for conditioning and increased the dewaterability of P-rich sludge. However, this method is costly due to chemical dosage and produces chemical sludge that's difficult to dispose of.

When considering an appropriate thickening method for EBPR sludge, it is important to consider cost effectiveness, ease of operation and maintenance as well as risks of releasing P from biomass back to liquid phase. In this regard, it seems DAF process would have minimum P release because of the aerobic environment in the tank, but the cost of equipment installation and operation needs to be considered. Gravity thickening has advantages in cost and operation, if the sludge volume needs to be treated is not significant. The efficiency of sludge concentration and potential of P release need to be evaluated for consideration. It should be noted that sludge properties (settleability, compaction, flotation and filtration) were demonstrated to have a relationship with sludge thickening and dewatering performance in wastewater treatment processes (Merlo et al., 2007).

1.4 Summary

A review of the literature shows that P removal can be accomplished by biological and chemical processes and constructed wetland treatment. Chemical treatment is effective for P removal, but the cost of treatment due to chemical addition and sludge handling is high. Constructed wetland treatment requires low energy input and is environmental sound by carrying out the transformations among soil, water, air, plants and microorganisms. However, the P removal efficiency by wetland treatment varies and is slightly lower than the other two processes. In contrast, biological treatment is more attractive and feasible, because of its cost-effectiveness, reliability, easy operation and maintenance as well as good performance. Moreover, the low P

effluent could be a resource of more balanced N:P suitable for crop growths, and the P-rich sludge can be used as a fertilizer after suitable sludge treatment and processing. Previous studies have been conducted to investigate the application of EBPR on dairy milking center wastewater and dairy processing wastewater, whereas no previous research was seen to investigate the application of EBPR on dairy manure treatment.

2 Objectives

The objectives of this study were to:

- (1) Evaluate the performance of pilot scale EBPR systems treating liquid dairy manure;
- (2) Determine the effects of SRT and DO on the efficiency of EBPR process;
- (3) Evaluate the feasibility of using gravity thickening as a method to concentrate the P-rich sludge generated by EBPR.

3 Materials and Methods

3.1 Manure Collection and Preparation

The manure used in this study was collected from the free stall barn at the Virginia Tech Dairy Complex (Blacksburg, VA). The barn uses a flush system to remove manure from the barn floor. Manure is flushed four times a day, but the manure used in this study was collected when manure accumulated on the barn floor between two consecutive flushing times. The dairy farm was described in detail by DeBusk et al. (2008). Manure was manually scraped off the barn floor into a barrel, weighed and diluted with an equal amount of tap water to provide a ratio of 1:1 (w/w) of manure to water. The diluted manure was mixed thoroughly using hand-held power mixer for about 3 min and pumped into a reception tank for a mechanical manure separator. The diluted manure was then released into the mechanical separator with 3.18 mm screen openings (Knowlton et al., 2005) for solid-liquid separation. The liquid portion of the separated manure was used in this study. Liquid manure was collected in 20 L plastic buckets and stored at -20°C.

3.2 Reactor Design and Operation

3.2.1 Reactor Design

The pilot-scale EBPR system used in this study was designed by Güngör et al. (2009). It is located in a shed where room temperature is controlled at 20°C. The main components of the EBPR system were a fermenter and an SBR. A semi-continuous flow fermenter was used in combination with the SBR reactor to enhance VFA

production for PHA synthesis in the anaerobic stage of SBR (Güngör et al., 2009). The system comprised of two parallel combinations of fermenter and SBR sharing the same feed tank (120 L) and waste tank (120 L). The schematic of the EBPR reactors is shown in Figure 1. The working volume of fermenters and SBRs were approximately 29.4 L and 14.7 L, respectively. The hydraulic retention time (HRT) and SRT of the fermenters were 2 d, while the HRT and SRT of SBRs were initially designed to be 1 d and 4 d, respectively.

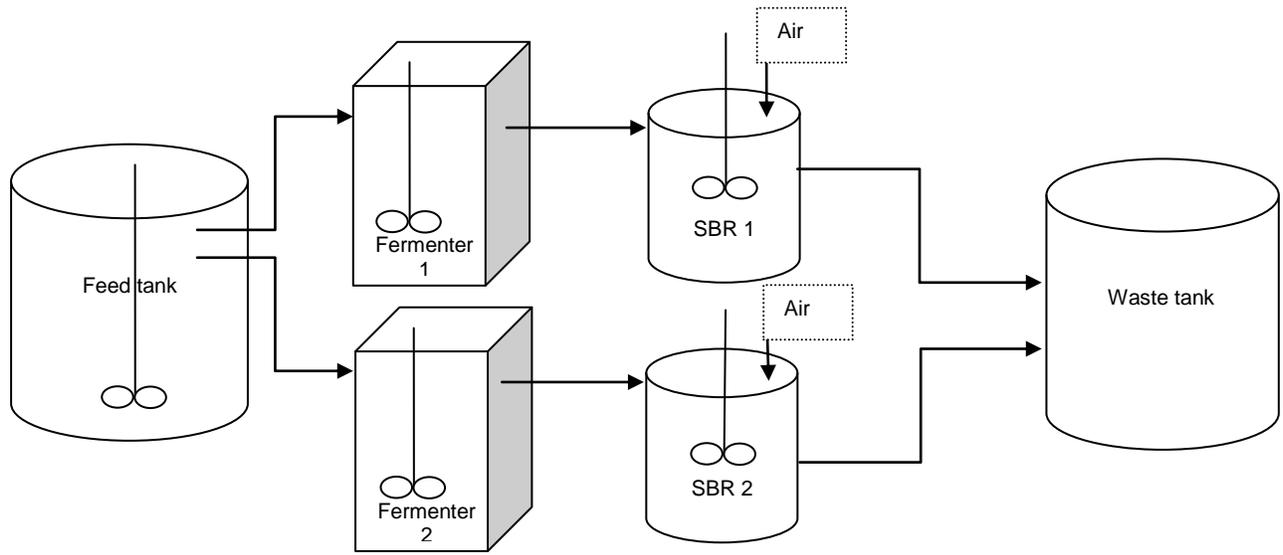


Figure 1. Schematic of the EBPR system

3.2.2 Sequencing Batch Reactor

The SBRs were operated by alternating anaerobic and aerobic conditions (Figure 2) to select for PAOs. Step feed was applied to introduce fermented manure (denoted as influent to SBR) into SBR reactor at the beginning of each anaerobic phase, with four steps feeding per cycle. As discussed earlier, nitrate had a detrimental effect on P removal when entering an anaerobic zone (Brdjanovic et al., 1998; Yagci et al., 2003; Zou et al., 2006), because the redox potential in anaerobic phase was increased when nitrate was available as an electron acceptor and P release would be inhibited (Ekama et al., 1983). Moreover, it was found that high organic loading could inhibit nitrification in the aerobic phase (Guo et al., 2008). In this regard, step-feed influent added in each anaerobic phase allows substantial VFA uptake and PHA storage. In subsequent aerobic phase, the high concentration of PHA compounds would inhibit nitrification and enable luxury P uptake.

The SBRs were operated three cycles per day, 8 h for each cycle. The 8-h cycle consisted of four sub-cycles, each including 1 min 50 s feeding, 40 min anaerobic conditions and 70 min aerobic conditions. In the fourth sub-cycle, there was a 40 min draining process. At the beginning of each sub-cycle, about 1.2 L of feed was pumped into each of the fermenters, and simultaneously the same amount of fermented manure flowed into corresponding SBRs. Immediately after feeding, SBR underwent 40 min anaerobic conditions. Then the mixers were turned on and air was pumped into the SBRs for 70 min when the anaerobic phase was over. Before the fourth aerobic phase

ended, about 1.2 L of aerated mixed liquor was discharged into waste tank, by which excessive P was removed. Then the mixed liquor settled for 30 min to achieve solid-liquid separation. Aeration and mixing were off in this stage to avoid turbulence. After settling, about 3.6 L of clear effluent (supernatant) was discharged into the same waste tank.

The mixer used consisted of a parallel shaft AC gearmotor (Dayton, Grainger, Lake Forest, IL) and a motor run capacitor (Dayton, Grainger, Lake Forest, IL). Air was supplied using air pumps (Gast, Benton Harbor, MI). Influent and effluent was pumped using peristaltic pumps (Masterflex L/S, Cole-Parmer, Vernon Hills, IL). Feeding, mixing, aeration and effluent pumping programs were controlled by three timers (Chrontrol, Cole-Parmer, Vernon Hills, IL).

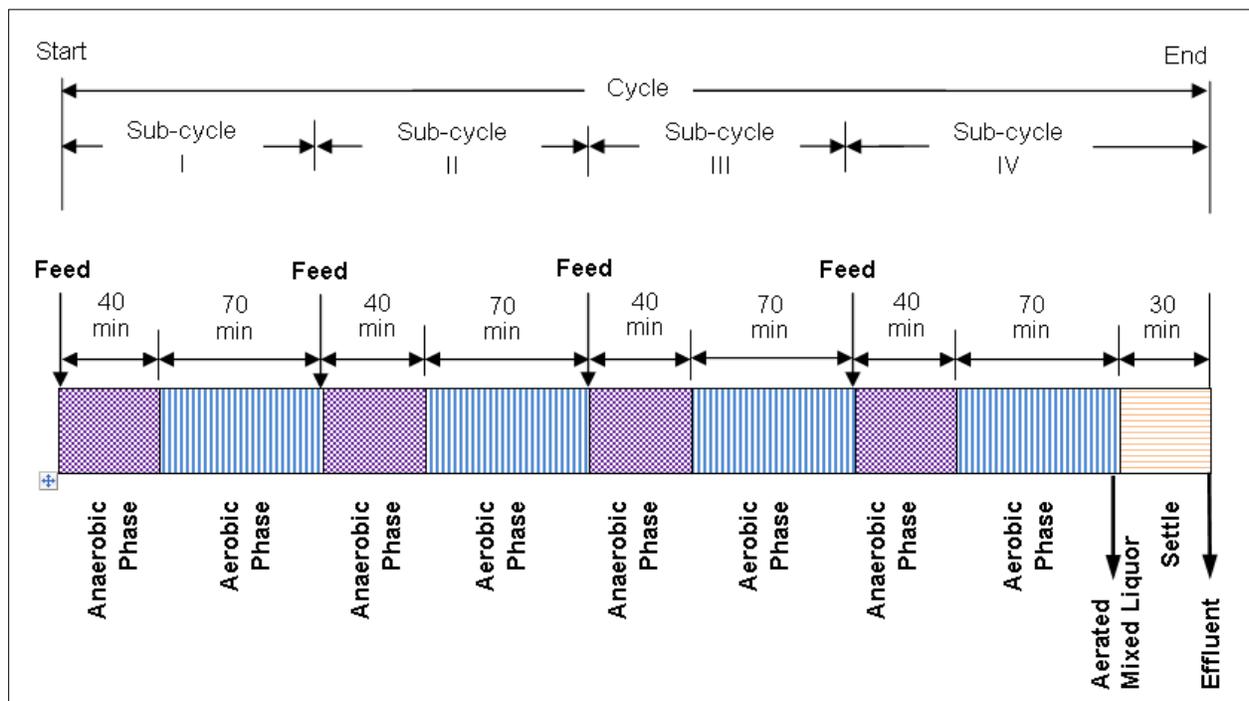


Figure 2. Schematic of an 8-h cycle in the SBR (Arogo et al., 2008). At the end of the cycle, aerated mixed liquor was pumped out for 3 min followed by 30 min settling, and then effluent (supernatant) was pumped out for 7 min.

3.2.3 Reactor Operation

3.2.3.1 Feeding

This study started 313 days after the EBPR system was constructed, and the experimental part was completed on day 999. The reactors were fed every Monday, Wednesday and Friday. Two days prior to use, the separated liquid manure was taken out of the freezer and stored in the shed at 20°C to thaw. The thawed manure was manually mixed and diluted with tap water to serve as feed to the fermenters. The ratio of manure to water used in different experimental stages will be described in detail in the following sections.

3.2.3.2 Evaluation of Pilot Scale Enhanced Biological Phosphorus Removal Systems for Liquid Dairy Manure

The two reactors, SBR 1 and SBR 2, were operated at 4 d SRT, with 6 mg O₂/L in aerobic phase and supplied with the same feed (sharing feed tank) for a period of 393 days.

For the first 181 days, both fermenters were fed with manure (mix A) with COD_{VFA}:DRP ratio of 45 (±30). Residual VFA in the effluent was observed and DRP removal was between 66% and 76%. To reduce the quantity of VFA residue and enhance P removal performance, we lowered the ratio of COD_{VFA}:DRP to 18 (±7) for 192 days using mix B, and both SBRs were reseeded with P-enriched activated sludge from East Burlington WWTP (Burlington, NC, USA) on day 493.

3.2.3.3 Calculating Phosphorus Content

The P content of biomass (%P) in aerated mixed liquor was estimated using the TP, DRP and VSS concentrations of the aerated mixed liquor according to Eq. 1:

$$\%P = \frac{(TP_{AML} - DRP_{AML})}{VSS_{AML}} \times 100 \quad \text{Eq. 1}$$

Where, AML denotes aerated mixed liquor.

3.2.3.4 Effects of SRT on Phosphorus Removal

SRT was introduced as a treatment factor to investigate whether it had an effect on EBPR performance. SBR 1 was subjected to the treatment by changing sludge wastage to obtain 4, 6 and 10 d SRT, while SBR 2 was operated at 4 d SRT as a control. Since VFA residue was reduced and the DRP concentration in the effluent decreased slightly when SBRs were fed with mix B compared to mix A, mix B was used as feed for this experimental stage. Both SBR 1 and SBR 2 were operated under 6 mg O₂/L in the aerobic phase and sharing the waste tank as usual.

A 57 L cylindrical polyethylene tank of 38 cm diameter and 56 cm length was added so that the two parallel combination systems would be fed from separate tanks. SBR 1 reactor was replaced by a 57 L cylindrical polypropylene tank of 38 cm diameter and 56 cm length to accommodate the changes of volume associated with increasing the SRTs.

3.2.3.5 Calculating Solids Retention Time

The SRT is defined as the mass of biomass in the SBR divided by the mass discharged from the reactor through AML and effluent (denoted by the symbol EFF) per day:

$$SRT = \frac{V_{reactor} \times TSS_{AML}}{(V_{AML} \times TSS_{AML} + V_{EFF} \times TSS_{EFF})/d} \quad \text{Eq. 2}$$

Where, SRT = solids retention time, day

$V_{reactor}$ = working volume of SBR reactor, L

TSS_{AML} = total suspended solids of aerated mixed liquor, mg/L

TSS_{EFF} = total suspended solids of effluent, mg/L

V_{AML} = volume of aerated mixed liquor drained per day, L

V_{EFF} = volume of effluent drained per day, L

d = day

3.2.3.6 Effects of DO on Phosphorus Removal

In this experimental stage, DO was introduced as a treatment factor to investigate whether it had an effect on EBPR performance. SBR 1 was subjected to the treatment by controlling DO level in the aerobic phase to 3, 4 and 6 mg O₂/L, while SBR 2 was operated at 6 mg O₂/L as a control. Since at 4 d SRT, SBR 1 achieved relatively

low DRP and TP in effluent, both SBR 1 and SBR 2 were operated at 4 d SRT and fed with mix B (one part of separated manure to four parts of tap water). Both SBRs shared the waste tank as usual.

A Hach LDO® process dissolved oxygen probe (Hach Company, Loveland, CO) was installed in SBR 1 to continually monitor the DO levels in SBR 1. HACH sc 100 universal controller (Hach Company, Loveland, CO) was used to receive data from the DO sensor. For the accuracy of DO measurement in SBR 1, HACH LDO sensor cap was cleaned three times a week to clear away sticky sludge. One 24 h DO profile was conducted every week to monitor DO levels in SBR 1 tank.

3.2.3.7 Sludge Volume Index

The volume of aerated mixed liquor and settled effluent were measured every week. The SVI test was performed by placing 1000 mL of mixed liquor into a 1000-mL graduated cylinder for 30-min settling (Grady et al., 1999). The volume of the settled solid after 30 min was recorded. The SVI (mL/g) was calculated by dividing the settled volume (SV) by mixed liquor suspended solids (MLSS).

3.2.4 Reactor Maintenance

Pump tubings were replaced and air diffusers in both SBR tanks were cleaned monthly. Sludge would clog pores of air diffusers, so it was necessary to clear away sticky sludge. Air diffusers were cleaned by being put in 1 L of 3 mol/L hydrochloric acid for about 15 min. Pins were used to unclog the pores when necessary. And finally,

diffusers were rinsed with tap water thoroughly. Usually, the air diffuser was cleaned during 40 min anaerobic phase. If it took longer, air pump was turned off to delay the onset of aerobic phase.

3.3 Gravity Thickening Tests

A 1000-mL Imhoff settling cone was used as a gravity thickener (Figure 3) to separate solid and liquid from aerated mixed liquor of EBPR. After settling, supernatant was drained from the top of the cone for sample analysis, and the concentrated solid was withdrawn from the screw closure at the bottom of the cone.

Aerated mixed liquor from SBR 2 was collected in a container. At the same time, about 100 mL mixed liquor was transferred into a bottle for TS and TSS analysis. The mixed liquor was kept aerated for 30 min. Then 1000 mL aerated mixed liquor was poured into an Imhoff cone for gravity thickening. Every 10 min, the volume of settled solid was recorded. Meanwhile, a 3.5-mL disposable transfer pipette was dipped into the clear supernate with the tip in the middle level of supernate (6-7 mm above solid-liquid interface), and about 10 mL of the supernate was collected in a 20-mL plastic dilution vial for TSS analysis. After two hours, 100~150 mL concentrated solid was collected from the bottom of the cone for TSS and TS analysis.

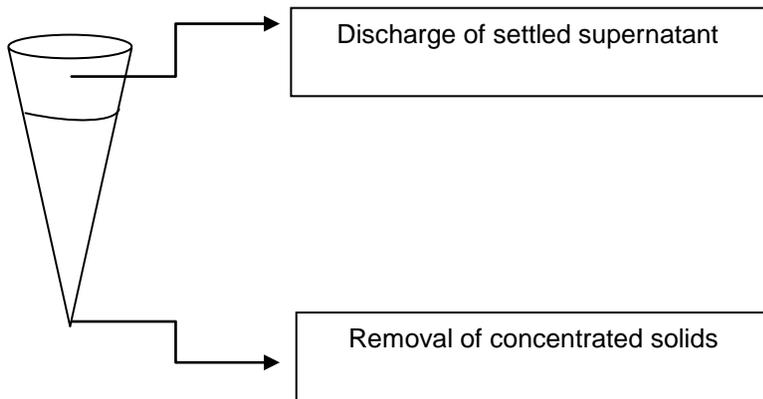


Figure 3. Schematic of an Imhoff settling cone as a thickener

3.4 Sampling and Analysis

Aerated mixed liquor and settled effluent from the reactors were collected in 250 mL bottles once every week for TP, DRP, VFA, nitrate, nitrite, and solids analysis. To prevent DRP release and VFA storage after sampling, about 60 mL of each sample was transferred into two 30-mL centrifuge tubes (Nalge Nunc International, Rochester, NY, USA) for immediate field centrifugation at 12600 x g for 20 minutes using F0630 rotor and Allegra X-22 Centrifuge (Beckman Coulter Inc., Fullerton, CA, USA). The supernatant was transferred to 50-mL round centrifuge tubes (Nalge Nunc International, Rochester, NY, USA). The supernatant was filtered through 0.7 µm microfibre filters (Whatman Inc., Florham Park, NJ, USA) and 0.45 µm glass fiber filters (Millipore, Billerica, MA, USA) for DRP, VFAs, nitrite and nitrate analysis. The untreated samples for TP analysis were stored at 4°C. The DRP was analyzed within 48 hours after sampling. The filtrate for VFA, nitrate and nitrite analysis was stored frozen to prevent biological activity, and thawed before analysis.

Before this study started, weekly samples from the feed tanks were collected for 7 weeks. The average DRP concentration in feed was 24 ($\pm 17\%$) mg/L and the average TP concentration in feed was 70 ($\pm 12\%$) mg/L. Feed tanks and fermenters were then collected once a month to characterize feed in this study. TP, DRP, VFA, soluble COD (sCOD) and solids were analyzed. The samples for sCOD analysis were filtered through 2.7 µm and 1.5 µm glass microfiber filters (Whatman Inc., Florham Park, NJ, USA), and acidified to the pH of less than 2.0 with 96.1% (w/w) sulfuric acid.

Total (TS) and volatile (VS) solids, total (TSS) and volatile (VSS) suspended solids, DRP, TP and sCOD were analyzed using standard methods (APHA, 1998). Nitrate and nitrite concentration were determined by Dionex ICS-3000 Reagent-free ion chromatography (Dionex Corp., Sunnyvale, CA, USA). VFA concentration were analyzed using a Hewlett Packard 5890 gas chromatograph with flame ionization detector (FID) and a Nukol fused silica capillary column for acetic, propionic, butyric, isobutyric, valeric, isovaleric, hexanoic, isocaproic, and heptanoic acids. Seven standards were prepared for calibration. Before analysis, 990 μL of standards and samples were added to 1 mL vials respectively and each was acidified by 10 μL of 85% phosphoric acid, capped tightly. Because of the high concentration of VFAs, samples from feed tanks and fermenters were diluted 1:3 (v/v) with nanopure water after acidification. A blank sample was prepared by adding 990 μL nanopure water to 10 μL phosphoric acid. The temperature of injector and FID detector were 200°C and 250°C, respectively. The GC oven temperature reached 80°C in first 3 min, rose to 140°C in 10 min at the speed of 6°C/min, and was held at 140°C for 1 min. The injection volume was 1.0 μL and each sample was analyzed in duplicate. COD concentration was calculated by multiplying VFA concentrations by different conversion factors of 1.07, 1.51, 1.82, 1.82, 2.04, 2.04, 2.13, 2.13 and 2.34 mg COD/mg VFA for acetic, propionic, butyric, isobutyric, valeric, isovaleric, hexanoic, isocaproic and heptanoic acids, respectively.

Manure separation was performed about once a month to ensure enough feed for the reactors. DO, pH and temperature for SBR were measured by WTW multi 340i

pocket meter (Wissenschaftlich-Technische Werkstätten GmbH, Germany). DO profiles in both SBR 1 and 2 were plotted to monitor the DO, pH and temperature in the shed.

3.5 Statistical Analysis

3.5.1 Hypothesis Testing

The reduction efficiencies of various parameters (DRP, TP, TSS and VSS) of reactors at different treatment factors (SRT and DO) were compared (across-reactor) using Excel spreadsheet according to the following statistical model:

$$y_{ij} = \mu + T_i + e_{ij} \quad \text{Eq. 3}$$

Where,

y_{ij} is response for experimental unit j of treatment i ,

μ is overall mean,

T_i is effect due to treatment i (SRT or DO), $i=1, 2, 3$,

e_{ij} is random error ($j=1, 2$).

Two-sample t-test in Microsoft Excel was performed to test whether SRT and DO had any effects on DRP and TP removal, TSS and VSS in effluent, P content of the biomass in the aerated mixed liquor, and the SVI of the aerated mixed liquor produced by the EBPR system.

For example, when evaluating the effects of DO on the EBPR performance, DO was the treatment factor and was introduced into SBR 1. SBR 1 was the experimental reactor and SBR 2 was the control reactor without treatment manipulation. Samples

collected from the two reactors were independent, and it is assumed that the sampled measures within each reactor were normally distributed with equal variances. So a two-sample t-test assuming equal variance was used in this case to test the hypothesis. The following steps summarize the procedure for the two-tailed hypothesis tests:

- Null hypothesis (H_0): The means of DRP removal efficiency between the control reactor and the experimental reactor are the same.
- Alternative hypothesis (H_a): The means of DRP removal efficiency between the control reactor and the experimental reactor are different.
- A p value of 0.05 was chosen as level of significance ($\alpha=0.05$), corresponding to a level of confidence of 95%. In Microsoft Excel, the default is $\alpha = 0.05$.
- If the t-test returns a probability less than or equal to 0.05, we reject the null hypothesis, and conclude that the means of DRP removal efficiency between the control and experimental reactors were different. If the t-test returns a probability greater than 0.05, we fail to reject the null hypothesis, and conclude that the means of DRP removal efficiency between the control and experimental reactors were not different.

3.5.2 Power Analysis

A statistical power analysis using JMP 8 statistical analysis software (SAS 2008) was conducted to determine the minimum detectable difference between treatments

with current sampling scheme (for the comparisons that return a probability greater than 0.05). The power analysis equation used was:

$$D = \left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta} \right) \sqrt{\frac{2\sigma^2}{n}} \quad \text{Eq. 4}$$

Where,

$D = \mu_1 - \mu_2$, is the minimum detectable difference between two treatment means that can be declared statistically different,

Z is z-value from standard normal distribution table,

α is significance level ($\alpha=0.05$ by default),

β is type II error that fails to reject a null hypothesis when it is not true,

σ is error standard deviation that can be estimated using root mean square error (RMSE) from a previous model fit,

n is sample size per group.

Power, $1 - \beta$, is the probability of getting a p-value equal or below a given significance level. To do a statistical power analysis in JMP, the minimum detectable difference, significance level, error standard deviation, sample size and power are needed for calculation in JMP. If four of the five parameters are known or indicated, the fifth parameter can be calculated accordingly.

There is a higher chance to find statistically significant differences with higher power, but the cost is higher due to increasing sample size. For calculation, a desired power of 80% was chosen to declare a significant difference between two means with significance level of 0.05. Total sample size is known with the current sampling scheme. However, error standard deviation is not available due to lack of information in previous research. So instead of obtaining a specific effect size, a plot of effect size versus standard deviation for fixed power and sample size was drawn.

4 Results and Discussion

4.1 Evaluation of Pilot Scale Enhanced Biological Phosphorus Removal Systems Treating Liquid Dairy Manure

4.1.1 Manure Characteristics

The characteristics of the liquid dairy manure collected after solid-liquid separation are listed in Table 1. The TP and DRP concentrations when utilizing mix B were 390 mg/L and 210 mg/L, 1.2 and 1.5 times as much as those when using feed mix A. The average TS and VS when using mix B were 3.9% and 3.1% respectively, slightly less than those when using feed mix A. The sCOD concentrations were close in period A and B, 16740 mg/L and 14620 mg/L, respectively. Both COD and P in liquid dairy manure were much higher than those in domestic wastewater. Of the nine volatile fatty acids measured, acetic acid accounted for 55% and 48% of total VFAs in period with mix A and mix B, respectively. Propionic acid was the second largest fraction of VFAs in the liquid manure, approximately 22%. Acetic acid, propionic acid and butyric acid together accounted for 92% and 89% of total VFAs respectively.

Table 1. Characteristics of the liquid manure collected after solid-liquid separation^{a,b}.

Period	TP (mg/L)	DRP (mg/L)	TS (%)	VS (%)	sCOD (mg/L)	VFA as COD (mg/L)		
						Total	Acetic	Propionic
Day 313-493	320±42 (8)	140±46 (8)	4.3±0.4 (8)	3.4±0.4 (8)	16740±2350 (8)	5090±1200 (7)	2810±600 (7)	1090±350 (7)
Day 514-705	390±42 (6)	210±21 (6)	3.9±0.3 (6)	3.1±0.3 (6)	14620±4280 (6)	5630±1250 (6)	2730±460 (6)	1330±300 (6)

^aValues presented are arithmetic mean±SD.

^bThe number of samples is indicated in parenthesis.

4.1.2 COD:P

The ratio of COD:P in the influent supplied to the anaerobic phase of EBPR is a critical factor in that it is closely correlated with P removal performance and effluent P concentration in EBPR system. All wastewater is either P limited or COD limited in biological P removal processes, and under such a limiting factor, the anaerobic phase will reach an equilibrium state with the growth of PAOs (Randall et al., 1992). If P is the limiting factor, readily biodegradable organics in anaerobic phase will not be fully utilized so that organic residue will be transferred to the subsequent aerobic phase of SBR. This might favor the growth of heterotrophic non-PAOs, resulting in the competition between PAOs and non-PAOs. On the other hand, if COD is the limiting factor, insufficient organic matter will limit P uptake due to insufficient energy supplied by PHA storage, and excess P will remain in effluent.

In this regard, the characteristics of fermented manure (influent to SBR) that is introduced to the anaerobic phase of SBR are very important. It is necessary to ensure suitable ratio of COD:P supplied to SBR to select for PAOs and achieve good P removal from dairy manure. Table 2 presents the ratios of COD:P in influent supplied to two SBR

reactors during the experiments of demonstrating EBPR. When fed with mix A, certain amount of organic residue (VFA) was observed in the effluent, which indicated that the influent supplied to SBR was P limited. The ratio of COD_{VFA} to DRP was 42 and 49 in SBR 1 and 2, respectively. To eliminate P-limiting factor, mix B was used to increase P fraction in the influent of SBR. The ratios of COD_{VFA} to DRP were decreased to 20:1 and 15:1 in SBR 1 and 2 respectively, in order to reduce VFA quantity in the effluent and enhance P removal efficiency.

Table 2. Ratio of COD equivalents of VFA to DRP in influent to two SBR systems^{a,b}.

Period	COD _{VFA} :DRP in influent to SBR	
	system 1	system 2
Day 313-495 Feed mix A	42±10 (7)	49±42 (7)
Day 514-705 Feed mix B	20±6 (6)	15±7 (6)

^aValues presented are arithmetic mean±SD.

^bThe number of samples is indicated in parenthesis.

4.1.3 DRP

The DRP concentrations of influent to SBR, effluent and aerated mixed liquor in SBR 1 are presented in Figure 4 and DRP profile for SBR 2 in Figure 5. In general, DRP concentration was high in feed tanks, but low in aerated mixed liquor and effluent. This is because after undergoing cyclic anaerobic and aerobic conditions, DRP was taken up and stored in the form of polyphosphate in the cells of microorganisms.

Average DRP removal efficiencies in SBR 2 were not significantly different when the ratio of $COD_{VFA}:DRP$ decreased from 49:1 to 15:1, but numerically, DRP removal efficiency was higher (78%) when the ratio of COD:P was 15:1, compared to 66% with the ratio of COD:P at 49:1. Similar observations were made in SBR 1, where DRP removal efficiencies were not significantly different between COD:P of 42 and 20, but DRP removal slightly increased from 76% to 79% after COD:P decreased. The lowest effluent DRP concentration in SBR 2 was 4.7 mg/L at average COD:P of 49, and reached 1.5 mg/L at lower COD:P of 15. A total of 31 mg/L of DRP was removed by SBR 2 during the most efficient P removal week. The highest TP accumulation calculated by dividing TP in mixed liquor by TP in influent to SBR were achieved in the

same week in two systems, with 200% and 213% in SBR 1 and 2 respectively at higher COD:P, and 317% and 288% respectively at lower COD:P.

A ratio of COD equivalent of VFAs to DRP between 15:1 and 20:1 might be good for PAO enrichment. The observation was in agreement with previous study conducted by Broughton et al. (2008), who obtained 100% P removal while using a $COD_{VFA}:DRP$ ratio of 15:1, and indicated that 13:1 of $COD_{VFA}:DRP$ ratio could be used to treat high strength dairy processing wastewater. Abughararah and Randall (1991) and Lie et al. (1997) suggested that a minimum of 20 mg COD equivalent of acetic acid should be added to remove 1 mg soluble P. On the other hand, Carrera et al. (2001) emphasized that influent COD:P played an important part in biological P removal operation, and a ratio of COD:DRP between 41:1 and 48:1 was recommended. It should be noted that glucose, sucrose and starch were used as organic substrate in their study, and these substrates must be fermented to readily biodegradable compounds before subjected to anaerobic conditions for VFA uptake and P release.

There was one outlier in Figure 4 that should be pointed out. On day 558, the aeration tubing of SBR 2 was broken. SBR 2 ran without aeration for about 1 day, which

may have caused the high DRP concentration in both effluent and mixed liquor, as well as a high TP concentration in settled effluent during that week (Figure 7). Thus, data points for SBR 2 of that week were not included for P removal calculation.

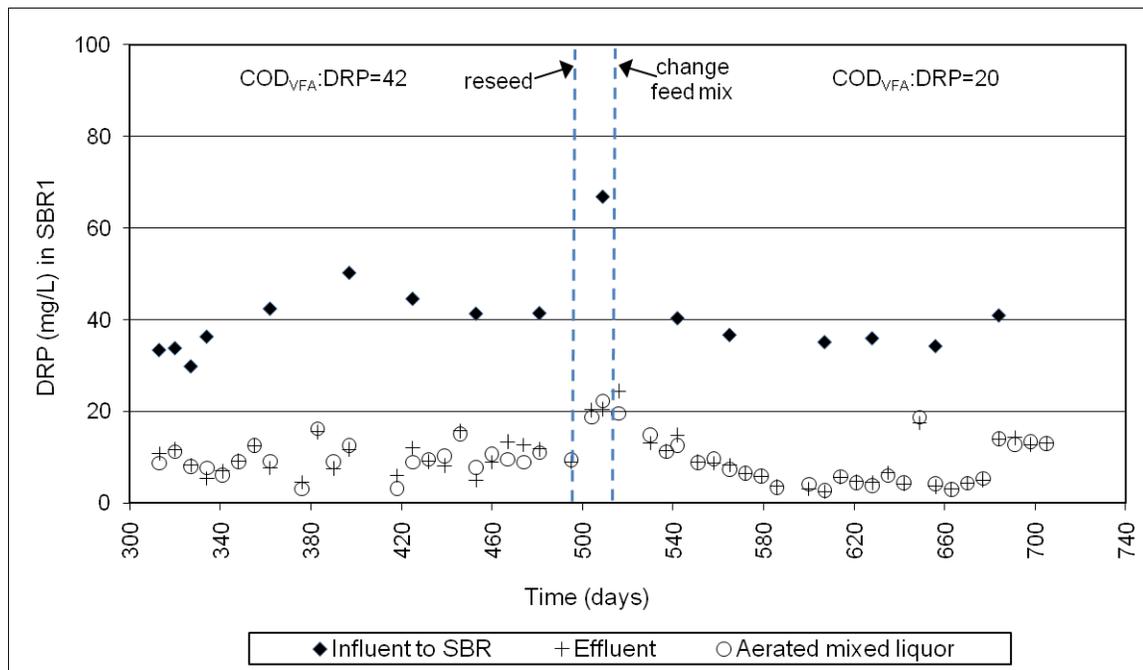


Figure 4. DRP concentrations in influent to SBR, effluent and aerated mixed liquor in SBR 1.

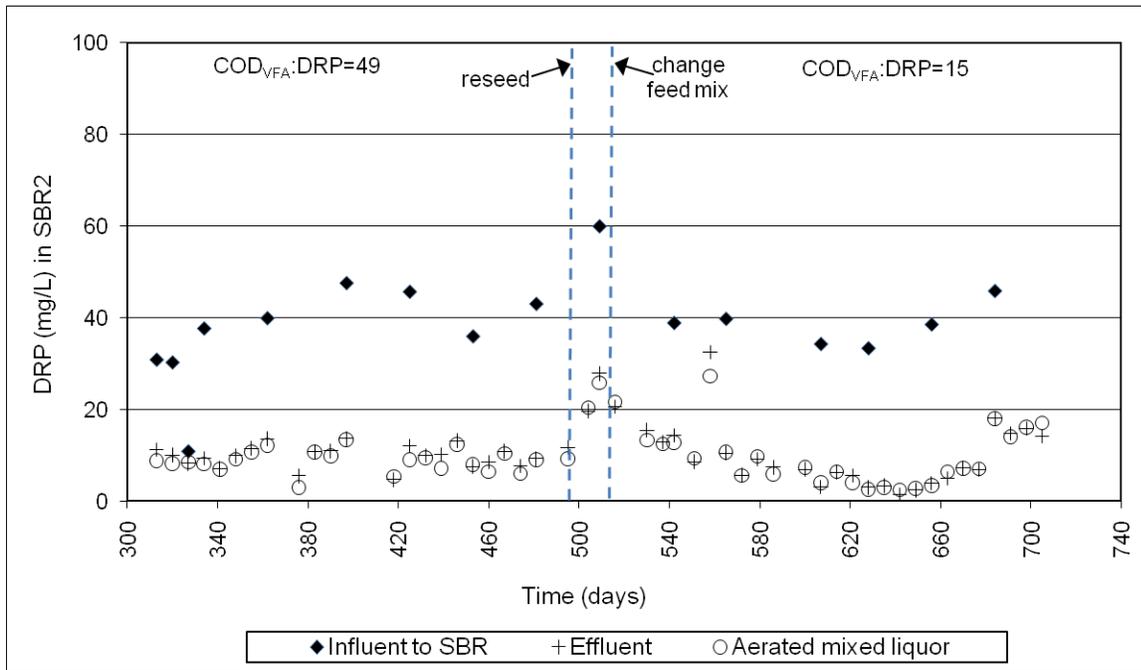


Figure 5. DRP concentrations in influent to SBR, effluent and aerated mixed liquor in SBR 2.

4.1.4 TP

The variation of TP concentrations in influent to SBR, effluent and aerated mixed liquor of SBR1 are presented in Figure 6 and SBR 2 in Figure 7. The average TP concentrations in influent and aerated mixed liquor of SBR 2 were 78 and 124 mg/L respectively at COD:P ratio of 49:1, and 66 and 156 mg/L, respectively at COD:P of 15:1. The concentrations of effluent TP in SBR2 were statistically different ($P < 0.05$) at COD:P of 49:1 and 15:1, with the lowest effluent TP of 25 and 6 mg/L, respectively. The TP removal in SBR 2 were significantly different between COD:P of 49:1 and 15:1, 34% and 71%, respectively. Mean TP accumulation in mixed liquor of SBR 2 was higher at COD:P of 15:1 than 49:1, 234% and 158%, respectively. A similar TP accumulation phenomenon was observed in SBR 1 that mean TP accumulation were significantly different between COD:P of 42:1 and 20:1, 231% and 154%, respectively. However, mean TP removal in SBR 1 between two COD:P ratios were not statistically different, with 29% and 56% at COD:P of 42 and 20, respectively. The P content in mixed liquor of SBR 1 and 2 were 1.7% and 1.6% respectively at COD:P of 20:1 and 15:1.

The ratio of VSS/TSS in SBR 1 decreased from 89% to 86% when COD:P in influent decreased from 42:1 to 20:1, but the P content in aerated mixed liquor of SBR 1 increased 50% at COD:P of 20:1. Similarly, the ratios of VSS/TSS in SBR 2 decreased from 92% to 85% at COD:P of 49:1 and 15:1, while the P content increased by 45% at COD:P of 15:1. These results were in agreement with the findings of McClintock (1990), who found a correlation between %P and the ratio of VSS to TSS. It has been observed that an increase in the ratio of VSS/TSS can be associated with a decrease in %P of mixed liquor. Punrattanasin (1997) also reported that VSS/TSS increased from 64% to 82%, and the %P in sludge decreased from 15.6% to 5.7%, when the ratios of COD/TP increased from 20 to 60.

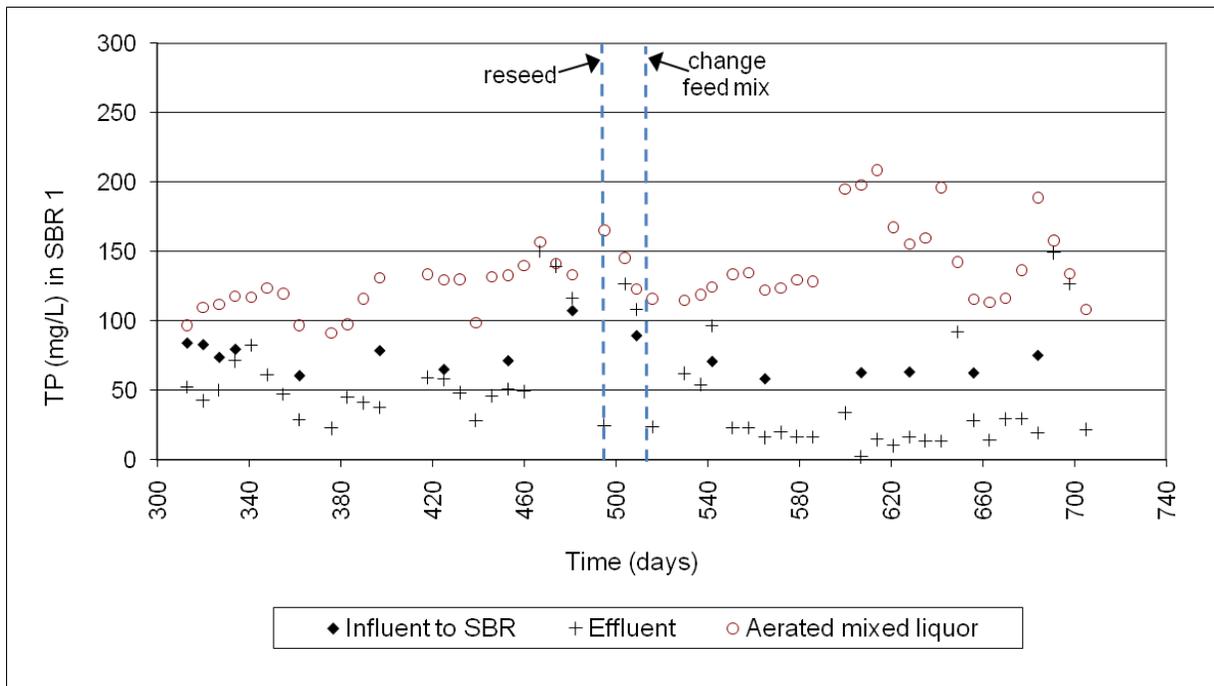


Figure 6. TP concentrations in influent to SBR, effluent and aerated mixed liquor in SBR

1.

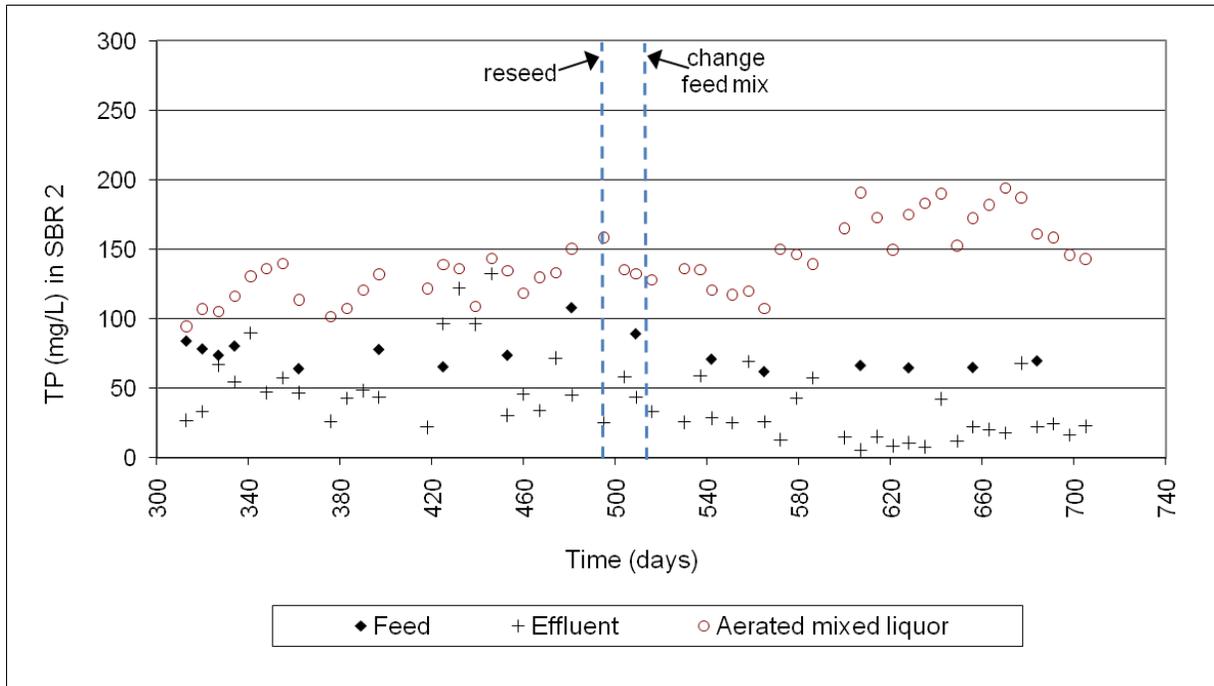


Figure 7. TP concentrations in influent to SBR, effluent and aerated mixed liquor in SBR

2.

4.1.5 VFA

. In the anaerobic phase where redox potential is low, VFAs are converted into PHA by PAOs and stimulate P release from intracellular polyphosphate hydrolysis. The hydrolysis, acidogenesis, acetogenesis and methanogenesis processes that occur under anaerobic condition break down organic matter into CO_2 and CH_4 . However, for EBPR proper process design and control that promotes only hydrolysis and acidogenesis to produce readily biodegradable organics (mostly VFAs), is desirable. The beneficial effects of prefermentation on the break-down of biodegradable COD, production of VFAs, and enhancement of P removal performance have been demonstrated by Randall et al. (1994) and Güngör et al. (2009).

When the ratio of COD:P in system 1 was 42:1, the COD_{VFA} in fermenter increased by 31% compared with the feed. Acetic acid, propionic acid and butyric acid increased by 18%, 64% and 16%, respectively. The average COD_{VFA} concentration in feed before entering fermenter was 1272 mg/L (Table 3). However, when the COD:P in system 1 was 20:1, the total COD_{VFA} in fermenter decreased by 33% compared to feed, with decreases in acetic, propionic and butyric acid concentrations respectively.

The average COD_{VFA} concentration in feed was 1126 mg/L (Table 3). Barajas et al. (2002) indicated that low temperature and an extreme redox potential that is low enough for methanogenesis or too high would lead to poor fermentation. This may explain the observations made here that VFA concentration decreased after fermentation. The redox potential in fermenter at COD:P of 20:1 might be decreased to methanogenesis level, so that VFA production reduced. The loss of VFA might also be partly due to VFA volatilization in fermenter.

The aerated mixed liquor in SBR 1 had 51 mg COD_{VFA} /L of acetic acid under COD:P of 42:1, and 12 mg COD_{VFA} /L of acetic acid under COD:P of 20:1 (Table 4). This suggests that acetic acid was not fully utilized for PHA synthesis, perhaps, causing a deficiency of energy for P uptake and the decrease of P removal efficiency. Chapin (1993) observed that when the acetate concentration in anaerobic phase exceeded 800 mg/L, the PAOs were unable to fully utilize it, creating a P limiting condition. The excess acetate that was available in the aerobic zone afterwards favored the dominance of non-PAOs, and induced the failure of EBPR. Acetic acid inhibition may have occurred in this study as well. The average acetic acid concentrations in fermenter of system 1

were 826 and 330 mg COD_{VFA}/L, at COD:P of 42 and 20 respectively (Table 3). The high concentration of acetic acid at COD:P of 42:1 might have affected the P removal performance, and resulted in numerically lower P removal efficiencies than COD:P of 20:1. It should be pointed out that almost 100% of propionic, butyric and valeric acid produced in fermenter were depleted in SBR for both COD:P ratios, whereas there were some residual acetic and isobutyric acid in effluent. Yagci et al. (2007) and Broughton et al. (2008) indicated propionic acid might be more favorable than acetic acid to the performance of P removal.

Table 3. VFA concentrations in feed and fermenter of SBR 1^{a,b}.

Period	n	VFA (mg COD/L)	Feed 1	Fermenter 1
COD:P=42 Day 313-493	7	Total	1272±300	1662±132
		Acetic acid	702±150	826±62
		Propionic acid	272±89	446±55
		Butyric acid	191±69	221±43
		Isobutyric acid	33±11	52±7
		Valeric acid	25±6	34±5
COD:P=20 Day 514-705	6	Total	1126±251	757±282
		Acetic acid	547±92	330±100
		Propionic acid	266±61	239±101
		Butyric acid	182±83	75±58
		Isobutyric acid	40±16	34±7
		Valeric acid	30±14	22±12

^aValues presented are arithmetic mean±SD.

^bn indicates the number of samples.

Table 4. VFA residues in effluent and aerated mixed liquor of SBR 1^{a,b}.

Period	n	VFA (mg COD/L)	Aerated Mixed Liquor 1	Effluent 1
COD:P=42 Day 313-493	8	Total	61±50	59±58
		Acetic acid	51±51	50±56
		Propionic acid	1±2	1±2
		Butyric acid	1±2	0±0
		Isobutyric acid	8±10	7±7
		Valeric acid	0±0	0±0
COD:P=20 Day 514-705	25	Total	20±15	21±15
		Acetic acid	12±9	12±9
		Propionic acid	0±0	0±0
		Butyric acid	0±0	0±0
		Isobutyric acid	7±5	8±6
		Valeric acid	0±0	0±0

^aValues presented are arithmetic mean±SD.

^bn indicates the number of samples.

4.1.5.1 TSS and VSS

TSS and VSS (Figure 8 and Figure 10) show that lower TSS in effluent was achieved when SBR 1 was operated under lower COD:P of 20:1 compared to 42:1. The same observations were made in Figure 9 and Figure 11, which illustrate that lower TSS in effluent was also achieved when SBR 2 was operated under COD:P of 15:1 compared to 49:1. There was much more VSS in the mixed liquor than influent to SBR and effluent during the experiment, which suggested microorganisms were growing and proliferating during the EBPR process with the organic substrate in the feed.

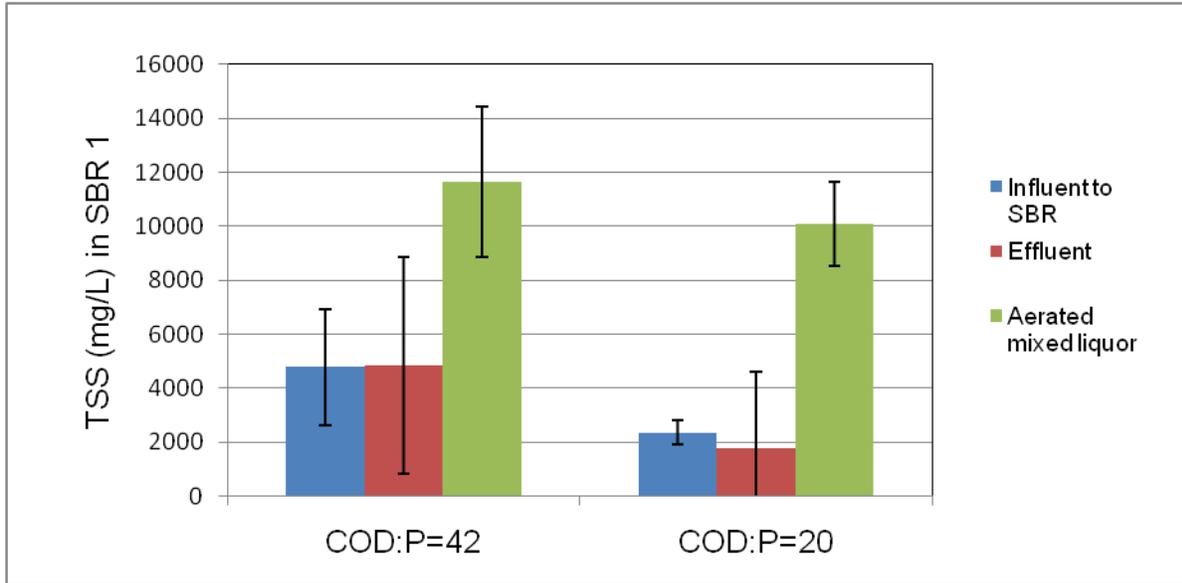


Figure 8. TSS in influent to SBR, effluent and aerated mixed liquor of SBR 1 at different COD:P ratios.

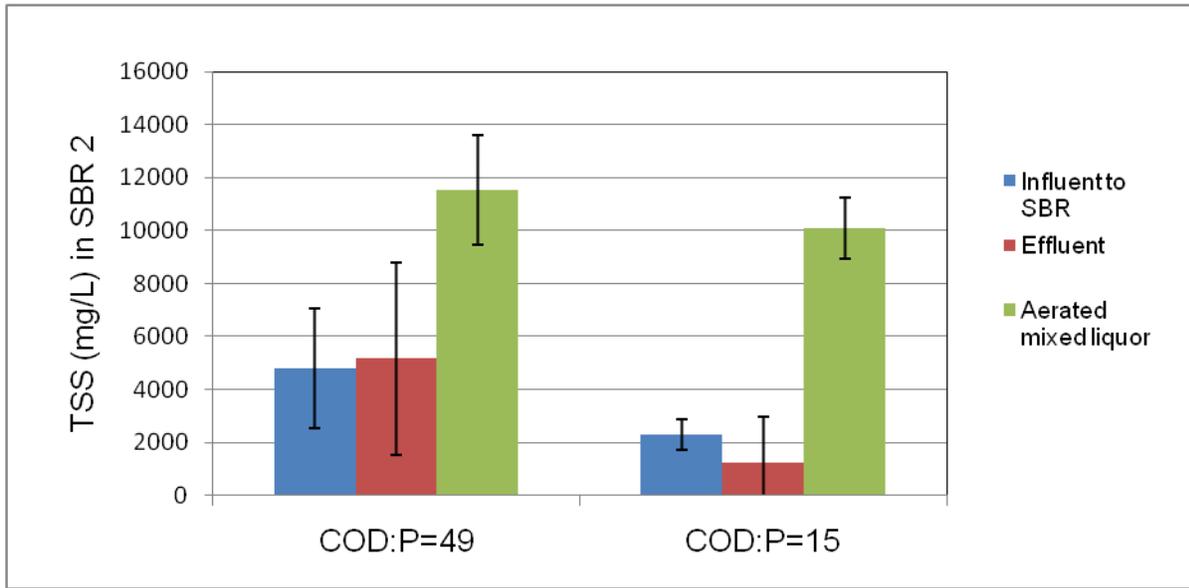


Figure 9. TSS in influent to SBR, effluent and aerated mixed liquor of SBR 2 at different COD:P ratios.

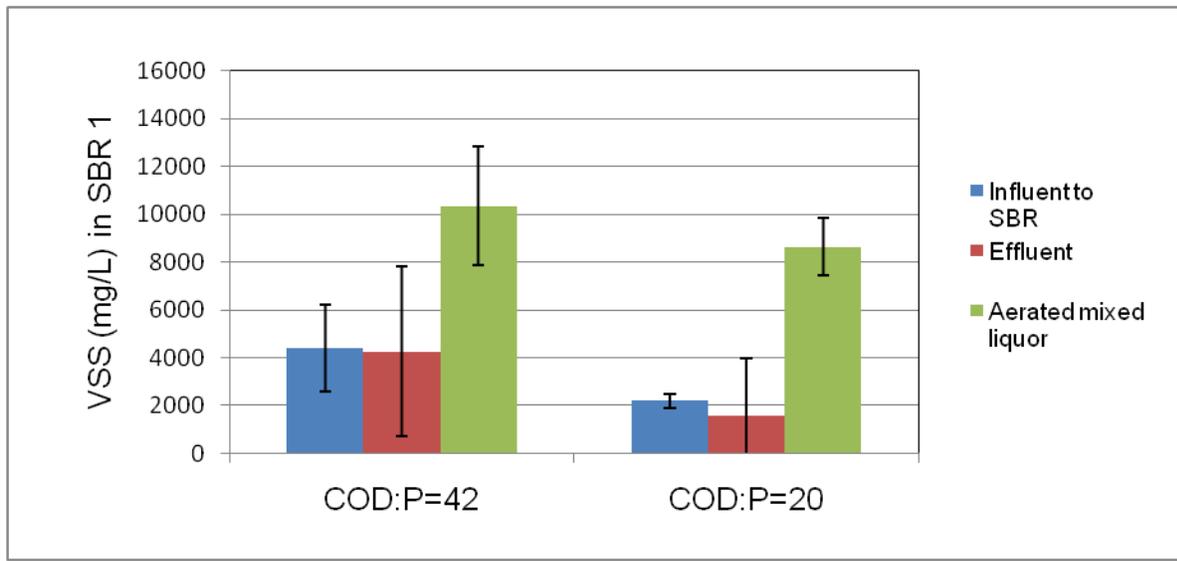


Figure 10. VSS in influent to SBR, effluent and aerated mixed liquor of SBR 1 at different COD:P ratios.

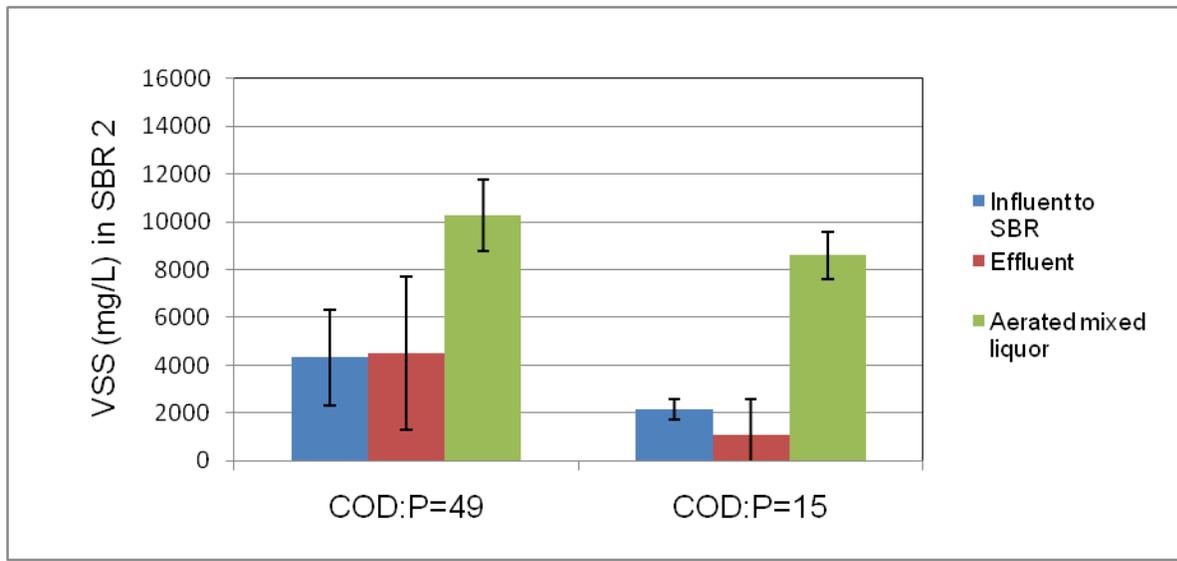


Figure 11. VSS in influent to SBR, effluent and aerated mixed liquor of SBR 2 at different COD:P ratios

4.2 Effects of Different Parameters on Phosphorus Removal

4.2.1 Solids Retention Time

Variations of DRP in influent, effluent and aerated mixed liquor of treatment reactor (SBR 1) are presented in Figure 12 and TP profile of SBR 1 is shown in Figure 14. The DRP concentration in effluent at 4 d SRT decreased gradually and stabilized on day 586. The average effluent DRP between day 586 and 677 was 4.4 mg/L. The lowest DRP and TP concentrations were 2.5 and 2 mg/L, respectively, occurring on day 607. The treatment reactor (SBR 1) achieved a maximum of 93% DRP removal at 4 d SRT. On day 649, the aeration tubing in treatment reactor dropped off accidentally, which consequently disturbed the performance of treatment reactor. The DRP and TP levels in the effluent went up to 17.5 and 92 mg/L, respectively. In 6-d SRT test, the average DRP levels in treatment reactor (SBR 1) effluent and mixed liquor were both 12 mg/L, with the lowest concentration of 8.3 and 7.8 mg/L respectively on day 754 (Figure 12). Kargi and Uygur (2002) suggested that a 10 d SRT would be an optimum condition for N, P and COD removal, as well as sludge settleability. In the 10-d SRT test, the DRP concentration in treatment reactor effluent increased compared to the concentration at

the 4 d SRT. Average DRP concentrations were 11 and 12 mg/L in the effluent and mixed liquor of treatment reactor. As the control reactor, SBR 2 was operated at 4 d SRT and 6 mg O₂/L. The DRP concentrations in influent, effluent and aerated mixed liquor is presented in Figure 13 and TP profile shown in Figure 15. The average TP concentrations in influent, effluent and mixed liquor were 79, 29 and 167 mg/L.

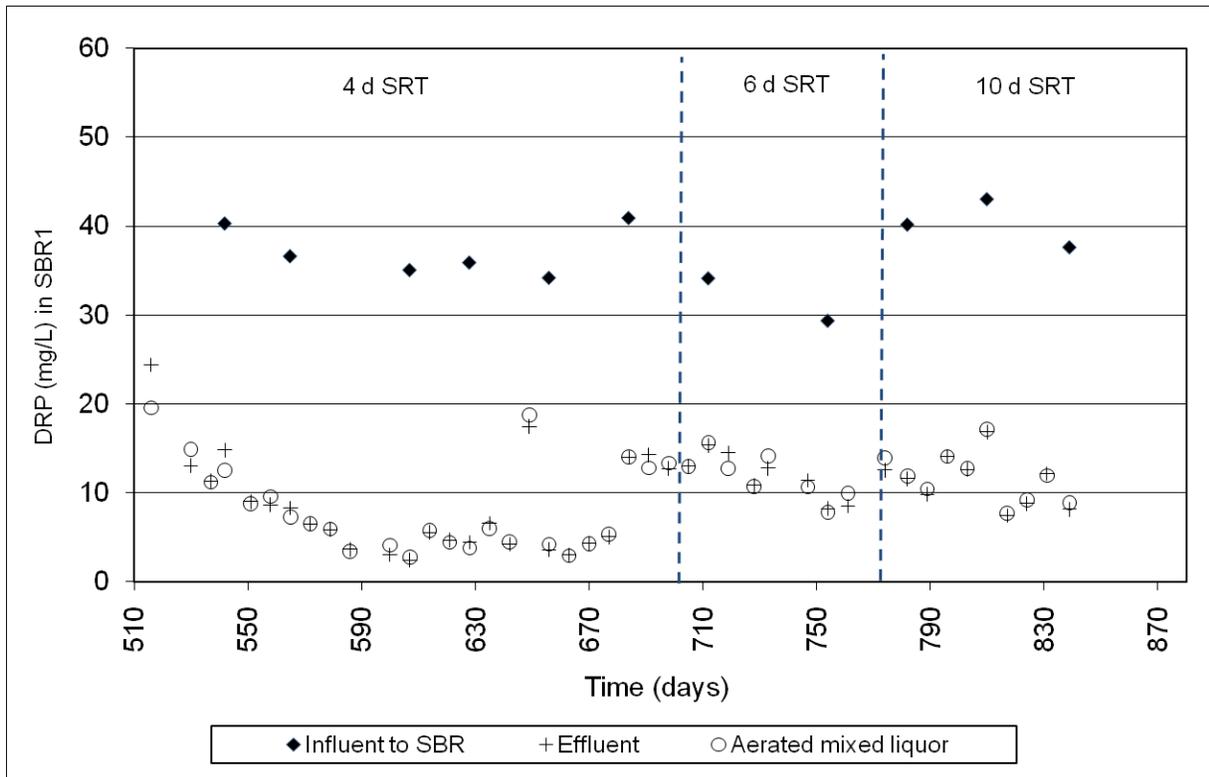


Figure 12. DRP concentrations in influent to SBR, effluent and aerated mixed liquor of SBR 1 at different SRTs, 4 d from day 516 to 705, 6 d from day 712 to 774 and 10 d from day 782 to 839.

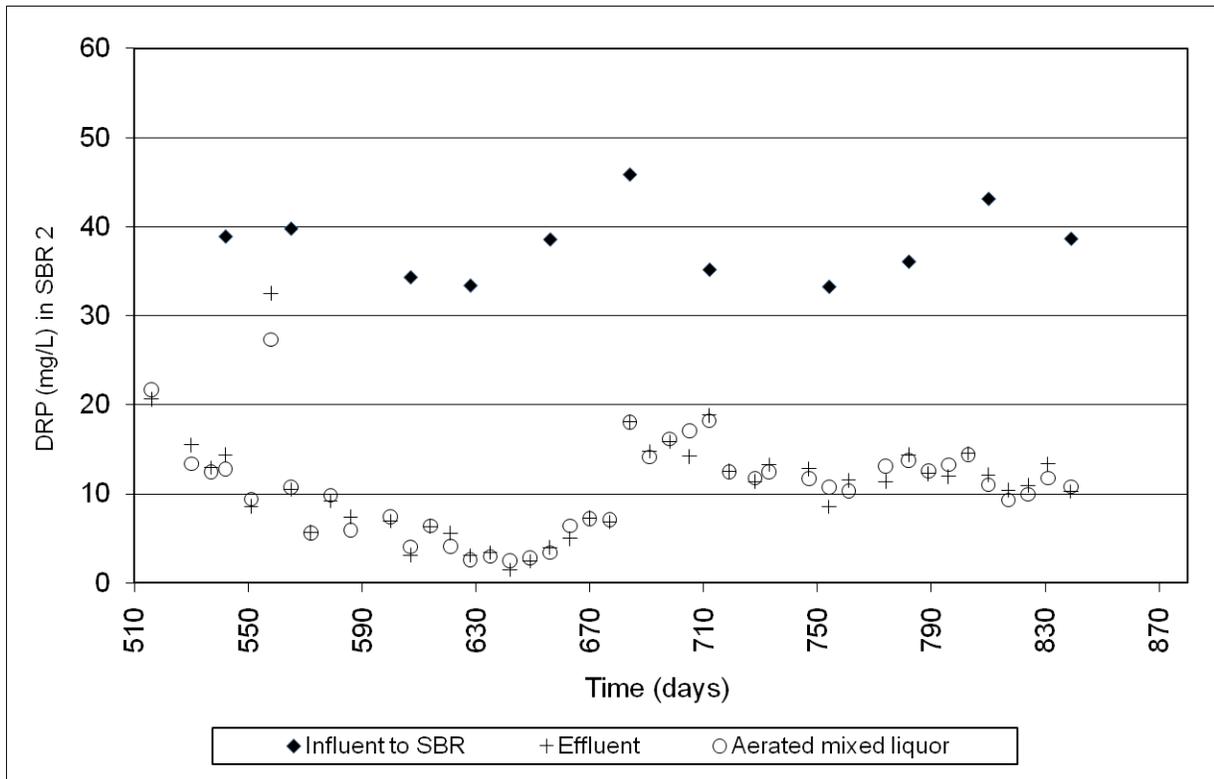


Figure 13. DRP concentrations in influent to SBR, effluent and aerated mixed liquor of SBR 2 at 4 d SRT from day 516 to 839.

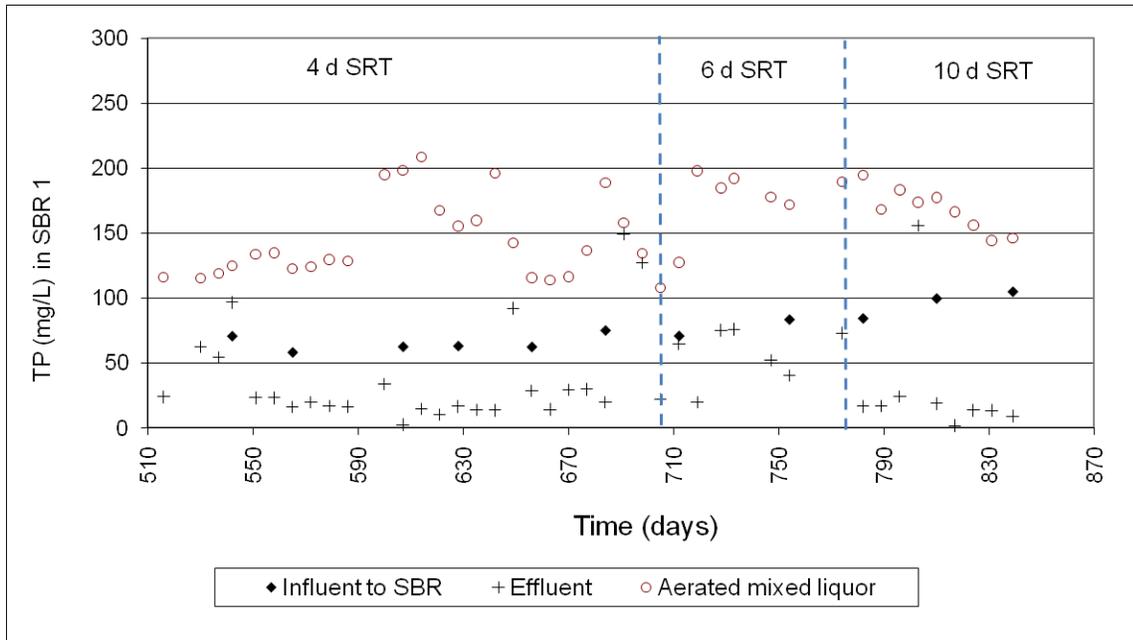


Figure 14. TP concentrations in influent to SBR, effluent and aerated mixed liquor of SBR 1 at different SRTs, 4 days from day 516 to 705, 6 days from day 712 to 774 and 10 days from day 782 to 839.

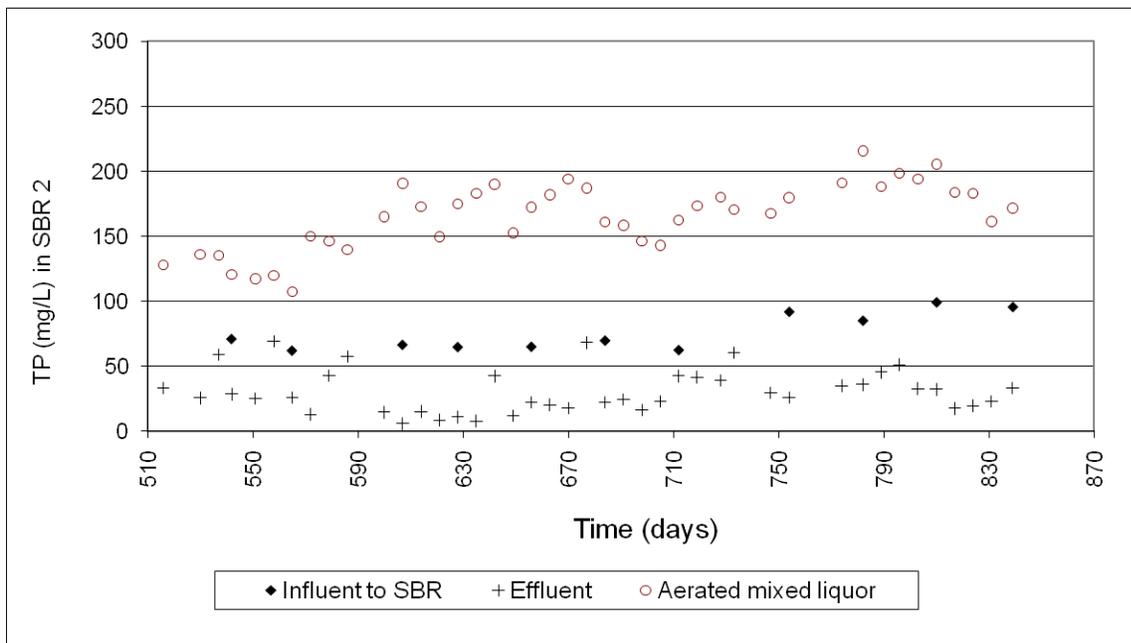


Figure 15. TP concentrations in influent to SBR, effluent and aerated mixed liquor of SBR 2 at 4 d SRT from day 516 to 839.

The SRT (Mamais and Jenkins, 1992; Rodrigo et al., 1996) and the composition of wastewater (Henze et al., 2002) can impact on P removal. Moreover, SVI is an indicator of sludge settleability (Merlo et al., 2007), and good sludge settleability would enable the thickening to occur faster, especially by gravity. An SVI of larger than 150 mL/g indicates poor sludge settling characteristics, and an SVI of lower than 80 mL/g suggests excellent settling characteristics (Grady et al., 1999). Figure 16 shows how SVI tests were performed.

The mean concentrations of different constituents in treatment and control reactors at different SRTs are presented in Table 5. When both treatment and control reactors were operated at 4 d SRT, there were no differences between two reactors in terms of DRP removal, TP removal, P content, TSS in effluent, VSS in effluent and SVI. This was expected since the two reactors were operated similarly in this phase.

When treatment and control reactors were operated at 6 d and 4 d SRT respectively, the mean DRP and TP removal efficiencies and P content were not significantly different. However, the treatment reactor statistically differed from the control reactor with regard to TSS and VSS concentrations in effluent and SVI. The

effluent TSS and VSS at 6 d SRT were 1662 and 1444 mg/L respectively, much higher than the effluent TSS and VSS at 4 d SRT, 678 and 595 mg/L, respectively. The mean SVI between treatment and control reactors were significantly different; whereas both reactors demonstrated excellent sludge settling properties with SVI of 80 mL/g and 62 mL/g at 4d and 6 d SRTs, respectively. This might be because at 6 d SRT, pin point flocs without the backbone support by certain filaments formed and loose floc particles would cause a low SVI but might also be readily brought into the effluent (Grady et al., 1999).

There were no significant differences between 4 d and 10 d SRTs in terms of DRP removal, P content, TSS and VSS in effluent and SVI. The TP removal efficiency at 10 d SRT statistically differed from the efficiency at 4 d SRT ($P < 0.05$). At the 10 d SRT, an average of 84% TP removal was achieved compared to 63% at the 4 d SRT in control reactor. Meanwhile, the SVI of 134 mL/g in treatment reactor indicated a poor sludge compaction property, which was probably caused by the growth of filamentous bacteria. The causative factors that will favor the growth of filamentous bacteria include low organic substrate in aerobic condition, low DO, long SRT and nutrient deficiency (Randall et al., 1992). A study conducted by Brodisch and Joyner (1983) showed that

filamentous bacteria *Microthrix* and *Nocardia* were also capable of removing P in addition to the major P removing bacteria named *Aeromonas* and *Pseudomonas* in the system. The growth of *Microthrix Parvicella* in the activated sludge process will result in high SVI and bulking. In this case, high SRT and low organic substrate might have stimulated the growth of filamentous bacteria such as *Microthrix* and *Nocardia*, which were able to take up P in aerobic phase, and consequently resulted in high TP removal but poor sludge settling characteristics.

Table 5. Mean DRP removal, TP removal, P content, TSS, VSS and SVI in treatment reactor and control reactor at different SRTs.

	Phase 1 ^a	Phase 2 ^b	Phase 3 ^c
DRP removal, %			
Treatment (n)	79 (6)	63 (2)	70 (3)
Control ^d (n)	78 (6)	60 (2)	68 (3)
P	0.87	0.87	0.82
TP removal, %			
Treatment (n)	56 (6)	30 (2)	84 (3)
Control ^d (n)	71 (6)	52 (2)	63 (3)
P	0.46	0.54	0.01
P content, %			
Treatment (n)	1.7 (26)	1.6 (6)	2.8 (9)
Control ^d (n)	1.6 (26)	1.8 (7)	2.5 (9)
P	0.55	0.25	0.54
TSS in Effluent, mg/L			
Treatment (n)	1746 (26)	1662 (8)	668 (9)
Control ^d (n)	1097 (26)	678 (8)	447 (9)
P	0.27	0.02	0.59
VSS in Effluent, mg/L			
Treatment (n)	1531 (26)	1444 (8)	581 (9)
Control ^d (n)	986 (26)	595 (8)	372 (9)
P	0.27	0.02	0.55
SVI, mL/g			
Treatment (n)	96 (17)	62 (3)	134 (9)
Control ^d (n)	84 (17)	80 (7)	109 (11)
P	0.07	0.01	0.29

^aIn phase 1, treatment reactor operated at 4 d SRT.

^bIn phase 2, treatment reactor operated at 6 d SRT.

^cIn phase 3, treatment reactor operated at 10 d SRT.

^dIn 3 phases, control reactor operated at 4 d SRT.

The number of samples is indicated in parenthesis.

P indicates the p value obtained by performing two sample t-test.



Figure 16. Sludge volume index test using 1 L graduated cylinder

4.2.1.1 Power Analysis

As can be seen in Table 5, TP removal efficiencies between the treatment and control reactors at 6 d and 4 d SRTs were not statistically different with a p value of 0.54. Power analysis was performed to find out the minimum significant difference between means of TP removal of treatment and control reactors. When the current sampling scheme with a total sample size of 4 is used and a power of 80% is desired, the minimum significant difference (effect size) between means of TP removal is a function of error standard deviation, as presented in Figure 17.

Since error standard deviation can be estimated using RMSE from a previous model fit, data was adapted from Chapin (1993) for specific minimum significant difference calculation. The RMSE was 5% in the previous research (Table 6) and total sample size is 4, consequently a minimum difference of 28% between TP removal efficiencies of treatment and control reactors can be declared as statistically different with an 80% power. Alternatively, with the actual variations I had in SBR reactors, if a minimum significant TP removal difference of 10% between treatment and control is desired, at least 12 total observations (6 per each group) would be needed.

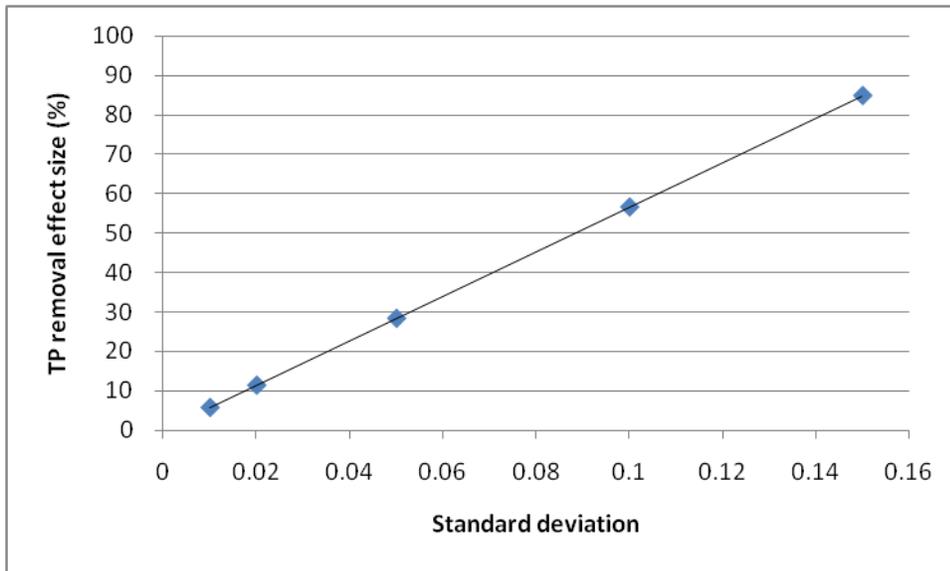


Figure 17. Plot of TP removal effect size versus standard deviation for fixed power of 80% and total sample size of 4.

Table 6. TP removal data from a previous research^{a,b,c}.

Date	Influent TP ^a (mg/L)	Effluent TP ^a (mg/L)	TP removal ^b (%)
July 24, 1991	16.3	4.3	74
July 26, 1991	20.4	11.1	46
July 29, 1991	22.2	16.7	25
August 5, 1991	21.9	14.9	32
August 7, 1991	20.3	7.2	65
August 10, 1991	20.3	5.5	73
August 19, 1991	22.9	19.1	17
August 28, 1991	25.7	22.6	12
August 30, 1991	34.5	28.1	19
September 30, 1991	20.4	17.1	16
October 3, 1991	21.2	17.7	17
October 9, 1991	26.3	22	16
October 11, 1991	26.3	22.7	14
November 7, 1991	23.5	16.2	31
November 11, 1991	23	8.2	64
November 13, 1991	24	16.1	33
November 15, 1991	21.8	12.4	43
November 21, 1991	24.3	6.7	72
November 25, 1991	19.1	16.5	14
November 29, 1991	22.3	18.6	17
Mean			35±23 ^c
RMSE			5

^aAdapted from Chapin (1993).

^bTP removal=((Influent TP-Effluent TP)/Influent TP)*100%.

^cValue presented is arithmetic mean±SD.

4.2.2 Dissolved Oxygen

Figure 18 presents the DRP profile of treatment reactor (SBR 1) in feed, effluent and aerated mixed liquor at different DO levels. SBR 1 was disturbed when DO level was set to be 3 mg O₂/L. The DRP concentration in effluent rose from 16.9 mg/L to 36.8 mg/L, which was very close to the DRP concentration in influent (Figure 18). So the DO level in SBR 1 was brought up to 6 mg O₂/L, in the hope of bringing the system back to normal function. After the period of adaptation, the DRP concentration in the effluent dropped to 16.3 mg/L on day 901, but then slightly increased. The fermenter in system 1 was cleaned and refilled on day 894, which might be one of the reasons causing the slight fluctuation. The observations were also reflected in Figure 20, which shows the fluctuation of TP concentration in effluent and mixed liquor. The P removal performance at 4 mg O₂/L improved a lot, with the lowest effluent DRP concentration of 2.1 mg/L on day 992. The treatment reactor (SBR 1) achieved highest DRP removal efficiency of 97%, compared to 73% removal efficiency in control reactor (SBR 2). DRP concentrations in influent to SBR, effluent and mixed liquor of control reactor were shown in Figure 19. The control reactor was operated at 6 mg O₂/L from day 866 to 999.

The average DRP in influent and effluent were 48 mg/L and 17 mg/L, with the lowest DRP concentration 9.7 mg/L. Figure 21 shows the variation of TP concentration in SBR 2 during DO test. The average TP concentrations in influent, effluent and mixed liquor were 92, 39 and 203 mg/L.

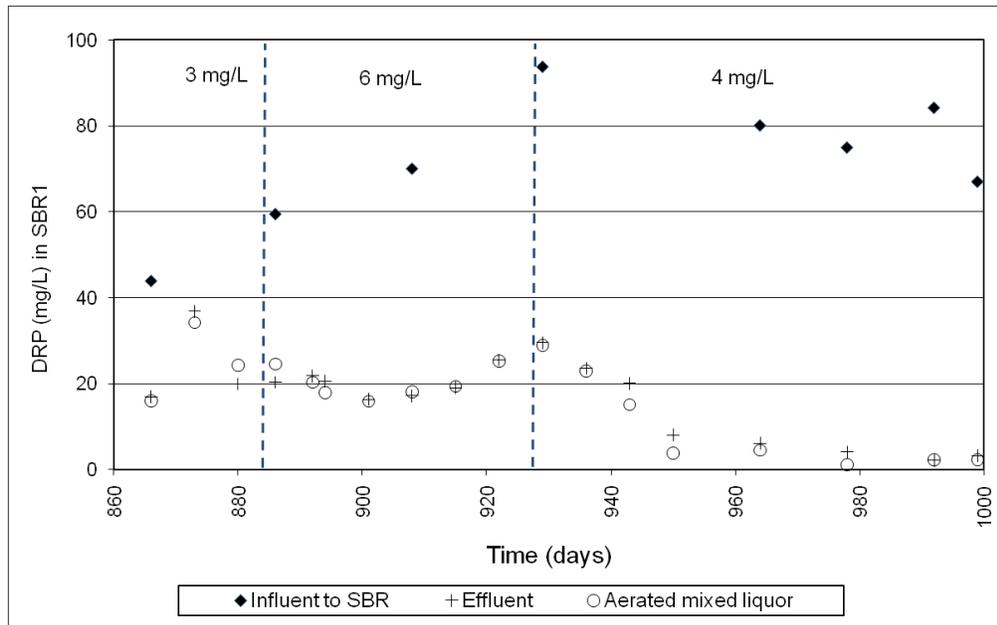


Figure 18. DRP concentrations in influent to SBR, effluent and aerated mixed liquor at different DO levels in treatment reactor (SBR 1).

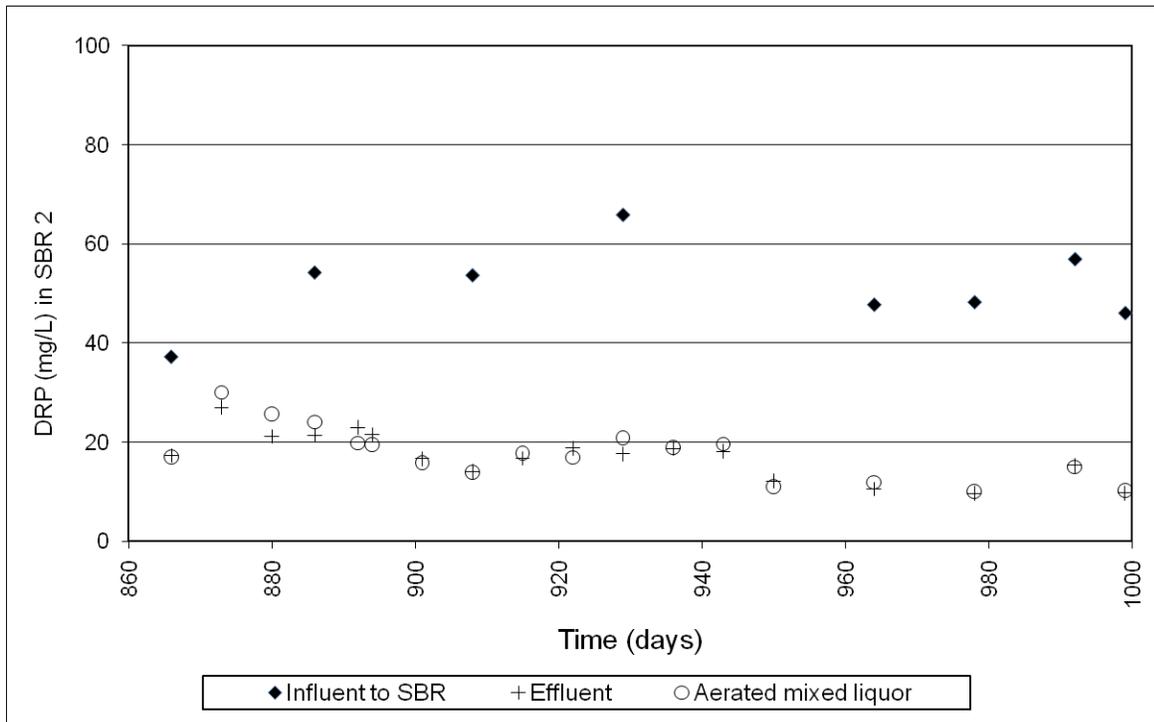


Figure 19. DRP concentrations in influent to SBR, effluent and aerated mixed liquor at 6 mg O₂/L in control reactor (SBR 2).

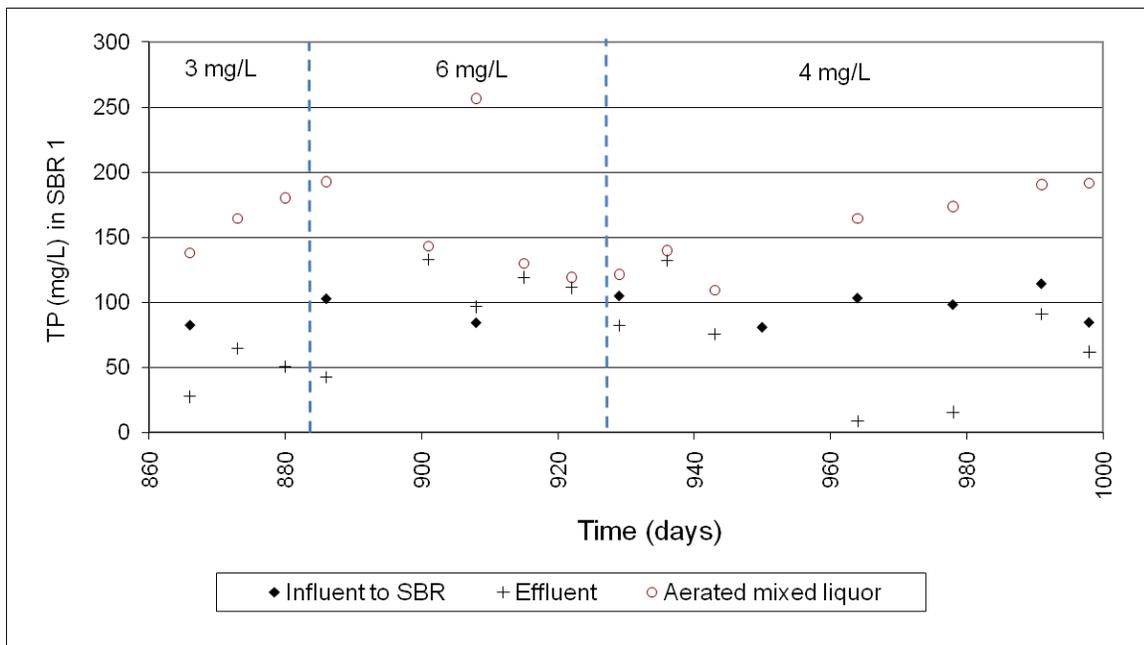


Figure 20. TP concentrations in influent to SBR, effluent and aerated mixed liquor at different DO levels in treatment reactor (SBR 1).

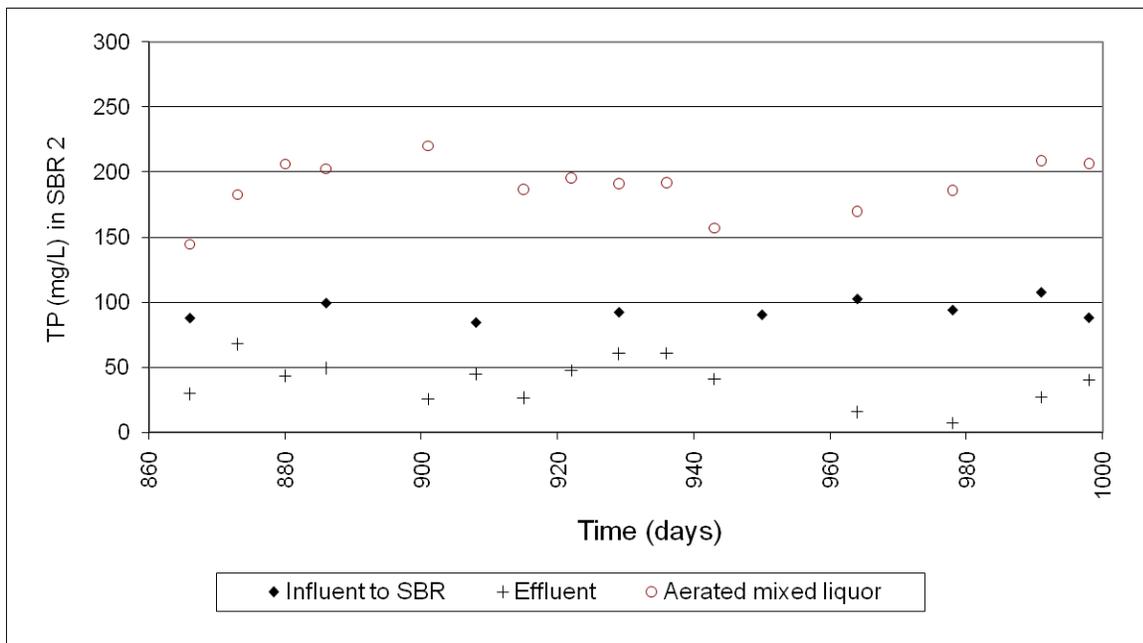


Figure 21. TP concentrations in influent to SBR, effluent and aerated mixed liquor at 6 mg O₂/L in control reactor (SBR 2).

Table 7 presents the mean concentrations of different constituents in treatment and control reactors at different DO levels. As stated earlier, the treatment reactor was disturbed when DO level was set at 3 mg O₂/L, and few data were collected for P removal comparison between treatment and control reactors. There were no significant differences between treatment and control reactors in terms of P content, TSS and VSS in effluent and SVI at 3 mg O₂/L.

There was no effect of 6 mg O₂/L on P removal, but interpretation of these results is difficult. This is because the treatment and control reactors were exposed to the same conditions in phase 2, but solids data indicated very different performance. There was one possible explanation in regard to this observation. The DO sensor installed in the treatment reactor was found blocked by sticky sludge for several times during the test period at 6 mg O₂/L, which prevented the sensor from detecting the actual DO level in the treatment tank. While the actual DO level in aerobic phase should be 6 mg O₂/L, the sensor controller detected a DO level (varying from 3 to 5 mg O₂/L) way below the set point of 6 mg/L, so that DO sensor kept the air pump running to introduce more air into the aerobic phase to meet the set point. This problem was solved in the midway of the 6

mg O₂/L test by wiping the DO sensor every other day to avoid the accumulation of sticky sludge.

The average DRP removal in treatment reactor at 4 mg O₂/L was statistically different from the removal in control reactor operated at 6 mg O₂/L, 90% and 77%, respectively. In regard to TP removal, P content, TSS, VSS and SVI, there were no significant differences between treatment and control reactors. However, the comparison may be questionable since the treatment reactor did not behave like control reactor when they were operated under the same conditions.

It should be noted that the average concentration of nitrate plus nitrite in the aerated mixed liquor of control reactor was 5.0 mg/L, while in the treatment reactor it was 2.1, 0.2 and 2.8 mg/L at 3, 4 and 6 mg O₂/L respectively. In an SBR, return activated sludge is not required because sludge settling and decanting are carried out within the reactor. Sludge remaining in the reactor will be subjected to anaerobic phase again at the end of one anaerobic/aerobic cycle. The nitrate concentration at the end of aerobic phase is critical, since nitrate has a detrimental effect on the performance of P removal when it enters anaerobic zone (Brdjanovic et al., 1998; Yagci et al., 2003; Zou

et al., 2006). In the presence of nitrate in anaerobic condition, denitrifiers will compete with PAOs for carbon source. When readily biodegradable organics are consumed through denitrification process in anaerobic phase, fewer organic matters would be available for PHA storage, resulting in less energy for P uptake in the following aerobic phase. Osborn and Nicholls (1978) suggested that the maximum nitrate concentration at the end of aerobic phase should be 2 mg/L in order to accomplish good P removal. The total concentration of nitrate and nitrite in the control reactor was higher than 2 mg/L and nitrate might have had an adverse effect on P removal efficiency. The adverse effect of nitrate might also have happened to the treatment reactor operated at 6 mg O₂/L, so that the TP removal was low. The total concentration of nitrate and nitrite of 0.2 mg/L in the treatment reactor indicated that nitrification didn't occur at the aerobic oxygen level of 4 mg/L. Factors that will affect nitrification process include toxic chemicals, temperature, DO, pH and organic loading (Randall et al., 1992). Generally, a low pH less than 7.0 and low DO concentration might be inhibitory for nitrifying bacteria, but this may vary depending on specific circumstances.

After biological manure treatment, the liquid portion (effluent) of the treatment process can be utilized for cropland irrigation. The ratio of N:P in the effluent was expected to be more balanced, in other words, N:P in the effluent should have been increased to meet the agronomic nutrient requirements. Table 8 lists the concentration of different constituents at different DO levels in treatment reactor. The ratios of TKN:TP in the effluent were 3.5, 4.7 and 2.6 at 3, 4 and 6 mg O₂/L, respectively. It can be seen that the ratio of TKN:TP was the highest when the system was operated at DO level of 4 mg O₂/L. The effluent TKN:TP around 5 is much higher than the normal N:P in manure, which is around 2:1 to 3:1. The balanced effluent would be more favorable to crop nutrient demand, and the P-enriched sludge can be further treated with thickening, dewatering, composting or digestion before its application on fields.

Typical DO and temperature profile is presented in Figure 22, which shows the room temperature was constant around 19.5°C on the measurement day, and the aerobic oxygen level in treatment reactor was controlled at a set point of 3 mg O₂/L. The oxygen concentration in anaerobic phase was zero. Typical DO and pH profile is shown in Figure 23. The pH in SBR was not controlled and varied from 7.7 to 7.9, which was in

a suitable range for P removal (Jeon et al., 2001; Oehmen et al., 2005). In the 40-min anaerobic phase, as the VFA in solution was taken up and stored as PHA in bacteria cells, the pH increased from 7.7 to 7.8.

Table 7. Mean DRP removal, TP removal, P content, TSS, VSS and SVI in treatment reactor and control reactor at different DO levels.

	Phase 1 ^a	Phase 2 ^b	Phase 3 ^c
DRP removal, %			
Treatment (n)	61 (1)	71 (2)	90 (5)
Control ^d (n)	53 (1)	67 (2)	77 (5)
P	-	0.71	0.05
TP removal, %			
Treatment (n)	67 (1)	22 (2)	49 (5)
Control ^d (n)	66 (1)	49 (2)	68 (5)
P	-	0.54	0.34
P content, %			
Treatment (n)	2.0 (3)	2.8 (5)	2.2 (7)
Control ^d (n)	2.0 (3)	2.9 (5)	2.1 (7)
P	0.84	0.89	0.74
TSS in Effluent, mg/L			
Treatment (n)	1115 (3)	3490 (6)	3287 (8)
Control ^d (n)	560 (3)	458 (6)	1015 (8)
P	0.37	0.01	0.06
VSS in Effluent, mg/L			
Treatment (n)	1014 (3)	3120 (6)	3028 (8)
Control ^d (n)	501 (3)	429 (6)	938 (8)
P	0.36	0.01	0.06
SVI, mL/g			
Treatment (n)	108 (3)	135 (7)	91 (11)
Control ^d (n)	107 (3)	98 (7)	92 (12)
P	0.86	0.04	0.87

^aIn phase 1, treatment reactor operated at 3 mg O₂/L.

^bIn phase 2, treatment reactor operated at 6 mg O₂/L.

^cIn phase 3, treatment reactor operated at 4 mg O₂/L.

^dIn 3 phases, control reactor operated at 6 mg O₂/L.

The number of samples (n) is indicated in parenthesis.

P indicates the p value obtained by performing two sample t-test.

Table 8. Concentrations of TP, sCOD and TKN in effluent and aerated mixed liquor in treatment reactor (SBR 1) at different DO levels^{a,b}.

DO Level (mg/L)	TP in aerated mixed liquor (mg/L)	sCOD in Effluent (mg/L)	TKN in Effluent (mg/L)	TP in Effluent (mg/L)	TKN:TP in Effluent
3	161±21 (3)	1072 (1)	148±53 (3)	48±19 (3)	3.5±1.6
4	156±33 (7)	1118±163 (6)	180±120 (8)	67±43 (7)	4.7±4.4
6	168±57 (5)	1320±45 (2)	244±110 (6)	100±35 (5)	2.6±1.0

^aValues presented are arithmetic means±SD.

^bThe number of samples (n) is indicated in parenthesis.

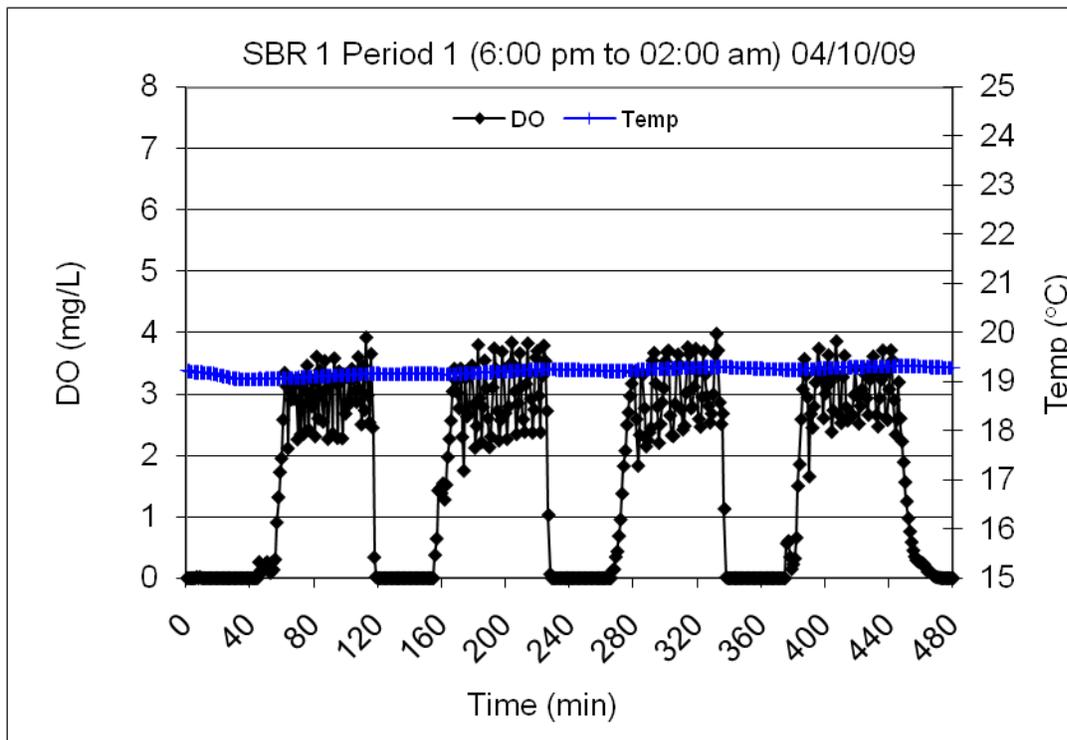


Figure 22. Typical DO and temperature profile for SBR 1 in 3 mg O₂/L phase

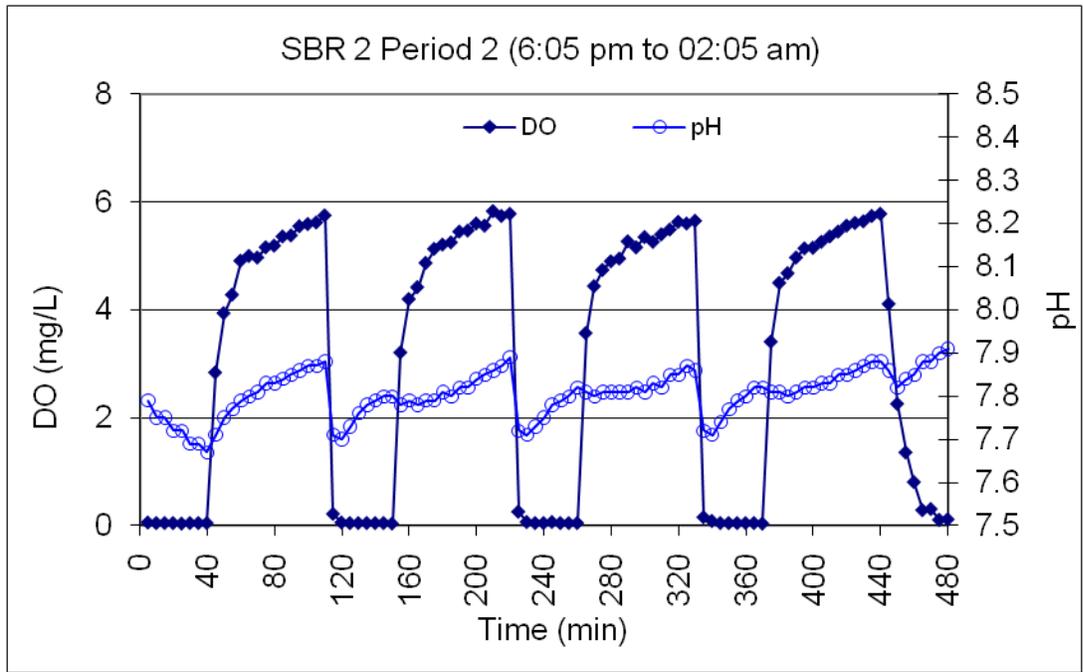


Figure 23. Typical DO and pH profile for SBR 2

4.3 Gravity Thickening Tests

Two gravity thickening preliminary tests were conducted on aerated mixed liquor drawn from SBR 2. In trial 1, the initial and final TS before and after 1.5 h thickening were 1% and 1.2 % respectively. In trial 2, the TS concentration before and after 2 h thickening were 1.06% and 1.54% respectively. Arora et al. (1995) reported a 2-7 % solids concentration after gravity thickening in full scale WTPs, and a 2-3 % solids concentration using bench scale DAF testing preconditioned with some polymers. The solids concentration after gravity thickening in this study were lower than the one reported by Arora et al. (1995), which might be because the fed sludge in this study was aerated mixed liquor drawn directly from the SBR tank, while the fed sludge used in their study was withdrawn from clarifiers. The changes in TSS in the supernatant with time in two trials are presented in Figure 24 and 25. Both figures show a sharp decline in TSS of the settled supernatant in the first 30 min and a slight decrease in the following 60 min. This suggests that it might be favorable to settle the sludge for about 30 min and then remove the thickened underflow.

The results of TSS removal rate in Trial 1 and 2 are summarized in Table 9. It can be seen that the two bench-scale thickening had 91% and 79% TSS removal from the settled supernatant in the first 30 min, and removed 95% and 93% TSS from the settled supernatant after 90 min gravity thickening. The gravity thickening kit used in this study is shown in Figure 26. It should be noted the sludge water content was still high after thickening, and it may be further treated by dewatering process to remove more water. In the mean time, it is desirable to evaluate the potential of P release with time from the P-rich sludge to the liquid phase during thickening process.

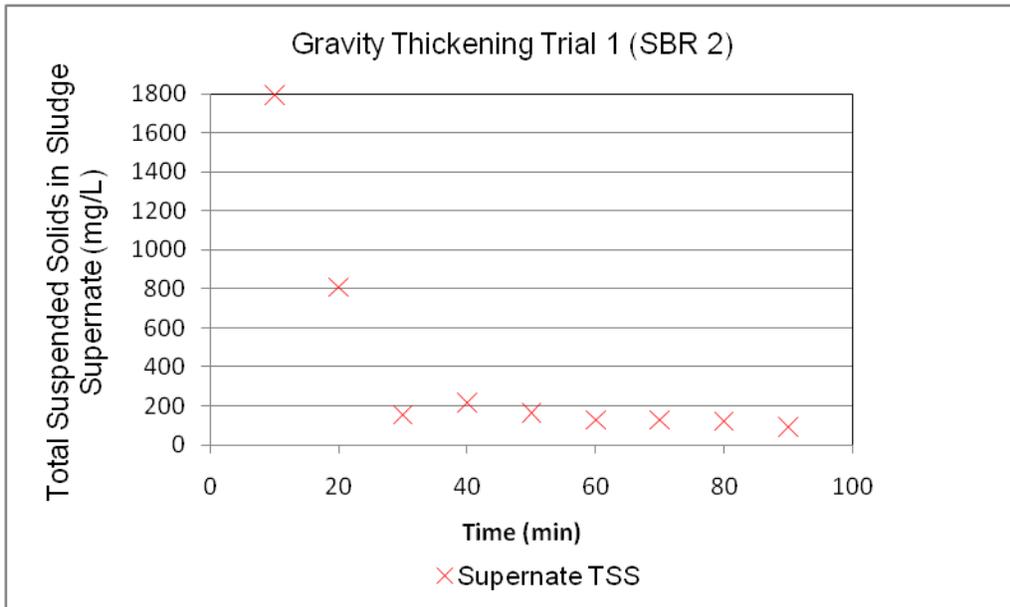


Figure 24. Variation of TSS in supernatant versus thickening period within Trial 1.

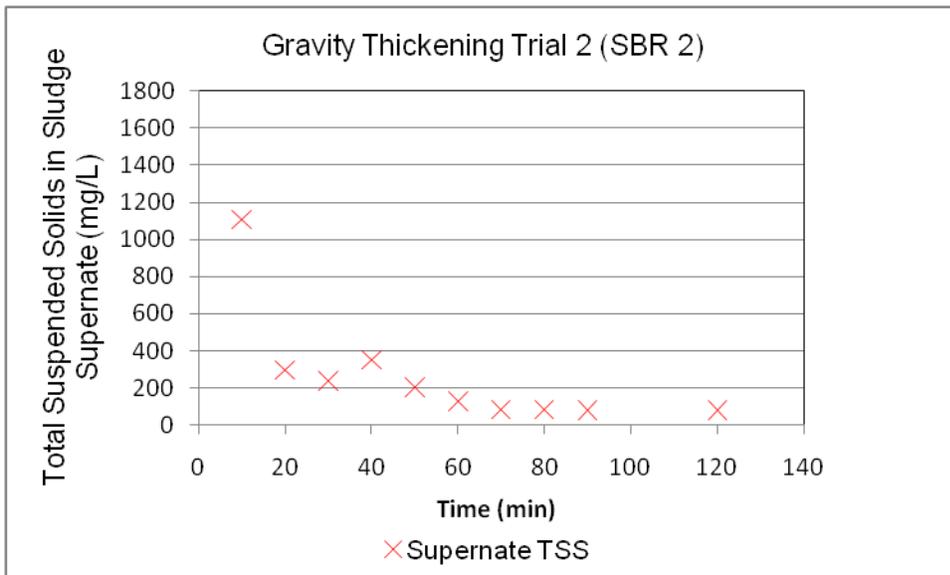


Figure 25. Variation of TSS in supernatant versus thickening period within Trial 2.

Table 9. Comparison of TSS in the supernatant of two thickening trials

Elapsed Time (min)	Trial 1		Trial 2	
	TSS in supernatant (mg/L)	TSS removal (%)	TSS in supernatant (mg/L)	TSS removal (%)
10	1793	-	1109	-
20	810	55	297	73
30	154	91	237	79
40	217	88	352	68
50	164	91	202	82
60	129	93	128	88
70	129	93	83	92
80	122	93	82	93
90	93	95	78	93
120	-	-	81	93



Figure 26. Gravity thickening tests using Imhoff cones

5 Conclusions

- With proper design and operation, EBPR could be a good alternative treatment to reduce P concentration in dairy manure. The influent COD:P ratio is a critical factor in EBPR design. It has a close correlation with P removal performance and effluent P concentration. Numerically, DRP removal efficiency increased when the ratio of COD_{VFA}:DRP decreased from 49:1 to 15:1. A ratio of COD equivalent of VFAs to DRP between 15:1 and 20:1 seems favorable for PAO enrichment.
- If the concentration of VFA as COD in anaerobic phase was high, P removal in EBPR system may be inhibited. We observed that acetic acid concentration of 826 mg COD_{VFA}/L in influent to SBR 1 at COD_{VFA}:P of 42 might have inhibited P removal efficiency and resulted in 51 mg COD_{VFA}/L of acetic acid residue in aerated mixed liquor. Almost 100% of propionic, butyric and valeric acid entering the anaerobic zone were depleted, whereas there were some residual acetic and isobutyric acid in the effluent.
- Sludge from the 6 d SRT had better settling characteristics compared to 4 d SRT. At 6 d SRT, the system generated an effluent with an average P concentration

of 57 mg/L, which was relatively high but suitable for applications to P deficient fields that require more P input to meet crop nutrient demand. It should be noted that the high P effluent also contained high concentration of solid (~1660 mg TSS/L), which might clog irrigation nozzles. Some solid pretreatment might be needed before land application.

- A 10 d SRT resulted in higher TP removal efficiency (84%), compared to 63% TP removal at 4 d SRT but the 10 d SRT might favor the growth of some filamentous bacteria, resulting in relatively poor sludge settleability.
- If a 10% minimum TP removal differences and an 80% power are desired, 12 total observations (6 per each group) are needed per treatment test to detect the treatment effects.
- It is important to maintain sufficient DO concentration in aerobic phase for luxury P uptake. A DO level of 3 mg/L did not sustain EBPR. When the system was operated at 4 mg O₂/L, effluent DRP concentration of 2.1 mg/L was achieved. The system achieved relatively higher average DRP removal at 4 mg O₂/L than 6 mg O₂/L, 90% and 77%, respectively.

- The effluent TKN:TP around 5 was achieved when the EBPR system was operated at 4 mg O₂/L. Such balanced N:P ratio would be good for irrigation and better match agronomic nutrient requirements. The low volume P-enriched treated manure sludge could be applied or transported to sites that are in P deficient.
- After gravity thickening, 93-95 % TSS was removed from the settled supernatant, and 1.2-1.54 % TS in the settled solids was achieved. The TSS in the settled supernatant was as low as 78-93 mg/L after first 120 min thickening. A sharp decline in TSS of the settled supernatant (91% and 79% reduction) in the first 30 min was observed in both trials, followed by a slight decrease in the following 60 min. It would be favorable to settle the sludge for about 30 min and then remove the thickened underflow.

6 Future Work

Extensive work needs to be done before scaling up the EBPR system to full scale application on dairy manure treatment. Based upon experimental observations and maintenance record, a more stable and reliable EBPR system is desired. Pump tubings and feed tanks need to be checked and cleaned frequently, so as to avoid the growth and attachment of microorganisms. Aeration pump, tubings and sensor need to be checked and properly maintained for consistent operation. From the perspective of farmers who are interested in manure treatment to comply with manure application regulations, the cost of implementing such treatment technology would be their top concern. Thus, it is necessary to further investigate the possibilities of water saving and energy input reduction, by examining the effects of feed preparation and oxygen on EBPR. By taking advantage of molecular biology techniques, it would also be important to study the microbial populations that are responsible for luxury P removal in EBPR process and provide more comprehensive understanding of biochemical mechanisms involved in the process. When evaluating various thickening methods, the extent of

DRP release from sludge into the liquid solution during different thickening processes should be taken into account.

7 References

- Abughararah, Z. H. and C. W. Randall (1991). "The Effect of Organic-Compounds on Biological Phosphorus Removal." *Water Science and Technology* 23(4-6): 585-594.
- Ahn, C. H., J. K. Park and K. S. Kim (2006). "Microbial adaptability to organic loading changes in an enhanced biological phosphorus removal process." *Journal of Environmental Engineering-Asce* 132(8): 909-917.
- APHA (1998). *Standard Methods for the Examination of Water and Wastewater*. Washington D.C., American Public Health Association.
- Arogo, J., K. F. Knowlton, N. G. Love, Y. Hong, K. Gilmore and K. Gungor (2008). *Enhanced Biological Phosphorus Removal for Liquid Dairy Manure*. ASABE Annual International Meeting. Providence, Rhode Island. June 29-July 2, 084477.
- Arora, H., J. R. Dewolfe, R. G. Lee and T. P. Grubb (1995). "Evaluation of Dissolved Air Flotation Process for Water Clarification and Sludge Thickening." *Water Science and Technology* 31(3-4): 137-147.
- Barajas, M. G., A. Escalas and R. Mujeriego (2002). "Fermentation of a low VFA wastewater in an activated primary tank." *Water Sa* 28(1): 89-98.
- Barnard, J. L. (1976). "A review of biological phosphorus removal in the activated sludge process." *Water Sa* 2(3): 136-144.
- Beer, M., H. M. Stratton, P. C. Griffiths and R. J. Seviour (2006). "Which are the polyphosphate accumulating organisms in full-scale activated sludge enhanced biological phosphate removal systems in Australia?" *Journal of Applied Microbiology* 100(2): 233-243.
- Bickers, P. O., R. Bhamidimarri, J. Shepherd and J. Russell (2003). "Biological phosphorus removal from a phosphorus-rich dairy processing wastewater." *Water Science and Technology* 48(8): 43-51.

Blackall, L. L., G. Crocetti, A. M. Saunders and P. L. Bond (2002). "A review and update of the microbiology of enhanced biological phosphorus removal in wastewater treatment plants." *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 81(1-4): 681-691.

Brdjanovic, D., A. Slamet, M. C. M. van Loosdrecht, C. M. Hooijmans, G. J. Alaerts and J. J. Heijnen (1998). "Impact of excessive aeration on biological phosphorus removal from wastewater." *Water Research* 32(1): 200-208.

Brodisch, K. E. U. and S. J. Joyner (1983). "The Role of Microorganisms Other Than *Acinetobacter* in Biological Phosphate Removal in Activated-Sludge Processes." *Water Science and Technology* 15(3-4): 117-125.

Broughton, A., S. Pratt and A. Shilton (2008). "Enhanced biological phosphorus removal for high-strength wastewater with a low rbCOD : P ratio." *Bioresource Technology* 99(5): 1236-1241.

Carrera, J., M. Sarra, F. J. Lafuente and T. Vicent (2001). "Effect of different operational parameters in the enhanced biological phosphorus removal process. Experimental design and results." *Environmental Technology* 22(12): 1439-1446.

Carucci, A., M. Kuhni, R. Brun, G. Carucci, G. Koch, M. Majone and H. Siegrist (1999). "Microbial competition for the organic substrates and its impact on EBPR systems under conditions of changing carbon feed." *Water Science and Technology* 39(1): 75-85.

Carucci, A., M. Majone, R. Ramadori and S. Rossetti (1994). "Dynamics of phosphorus and organic substrates in anaerobic and aerobic phases of a sequencing batch reactor." *Water Science and Technology* 30(6): 237-246.

Chapin, R. W. (1993). *Substrate induced failure of biological phosphorus removal*, Virginia Polytechnic Institute and State University, 1993.: ix, 126 leaves.

Chen, S. H., J. C. Liu, G. H. Cheng and W. C. Chang (2006). "Conditioning and dewatering of phosphorus-rich biological sludge." *Drying Technology* 24(10): 1217-1223.

Cizinska, S., V. Matejo, C. Wase, Y. Klasson, J. Krejci and G. Dalhammar (1992). "Thickening of Waste Activated-Sludge by Biological Flotation." *Water Research* 26(2): 139-144.

Comeau, Y., K. J. Hall, R. E. W. Hancock and W. K. Oldham (1986). "Biochemical Models for Enhanced Biological Phosphorus Removal." *Water Research* 20: 1511-1521.

Comeau, Y., D. Lamarre, F. Roberge, M. Perrier, G. Desjardins, C. Hade and R. Mayer (1996). "Biological nutrient removal from a phosphorus-rich pre-fermented industrial wastewater." *Water Science and Technology* 34(1-2): 169-177.

Crocetti, G. R., P. Hugenholtz, P. L. Bond, A. Schuler, J. Keller, D. Jenkins and L. L. Blackall (2000). "Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation." *Applied and Environmental Microbiology* 66(3): 1175-1182.

Danalewich, J. R., T. G. Papagiannis, R. L. Belyea, M. E. Tumbleson and L. Raskin (1998). "Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal." *Water Research* 32(12): 3555-3568.

de-Bashan, L. E. and Y. Bashan (2004). "Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997-2003)." *Water Research* 38(19): 4222-4246.

DeBusk, J. A., J. A. Ogejo, K. F. Knowlton and N. G. Love (2008). "Chemical phosphorus removal for separated flushed dairy manure." *Applied Engineering in Agriculture* 24(4): 499-506.

Dunlap, T. F., R. A. Kohn and K. F. Kalscheur (1997). "Effect of animal grouping strategies on nutrient losses from the dairy farm." *Journal of Dairy Science* 80(Suppl 1): 246.

Eikum, A. S., D. A. Carlson and A. Lundar (1975). "Phosphorus Release during Storage of Aerobically Digested Sludge." *Journal Water Pollution Control Federation* 47(2): 330-337.

Ekama, G. A., I. P. Siebritz and G. V. Marais (1983). "Considerations in the Process Design of Nutrient Removal Activated-Sludge Processes." *Water Science and Technology* 15(3-4): 283-318.

Erdal, U. G., Z. K. Erdal and C. W. Randall (2003). "The competition between PAOs (phosphorus accumulating organisms) and GAOs (glycogen accumulating organisms) in EBPR (enhanced biological phosphorus removal) systems at different temperatures and the effects on system performance." *Water Science and Technology* 47(11): 1-8.

Erdal, U. G., Z. K. Erdal and C. W. Randall (2006). "The mechanism of enhanced biological phosphorus removal washout and temperature relationships." *Water Environment Research* 78(7): 710-715.

Fuhs, G. W. and M. Chen (1975). "Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater." *Microbial Ecology* 2: 119-138.

Güngör, K., M. B. Müftügil, J. Arogo, K. F. Knowlton and N. G. Love (2009). "Prefermentation of liquid dairy manure to support biological nutrient removal." *Bioresource Technology* 100(7): 2124-2129.

Güngör, K., M. B. Muftugil, J. A. Ogejo, K. F. Knowlton and N. G. Love (2009). "Prefermentation of liquid dairy manure to support biological nutrient removal." *Bioresource Technology* 100(7): 2124-2129.

Grady, C. P. L., G. T. Daigger and H. C. Lim (1999). *Biological wastewater treatment : Second Edition, Revised and Expanded*. New York, M. Dekker.

Grady, C. P. L., G. T. Daigger and H. C. Lim (1999). "Biological Wastewater Treatment, Second Edition, Revised and Expanded." Marcel Dekker, Inc.

Griffiths, P. C., H. M. Stratton and R. J. Seviour (2002). "Environmental factors contributing to the "G bacteria" population in full-scale EBPR plants." *Water Science and Technology* 46(4-5): 185-192.

- Guo, J. H., Y. Z. Peng, Q. Yang, S. Y. Wang, Y. Chen and C. H. Zhao (2008). "Theoretical analysis and enhanced nitrogen removal performance of step-feed SBR." *Water Science and Technology* 58(4): 795-802.
- Hatfield, J. L. and B. A. Stewart (1998). *Animal waste utilization : effective use of manure as a soil resource*. Chelsea, Mich., Ann Arbor Press.
- He, S., D. L. Gall and K. D. McMahon (2007). "'Candidatus accumulibacter" population structure in enhanced biological phosphorus removal Sludges as revealed by polyphosphate kinase genes." *Applied and Environmental Microbiology* 73(18): 5865-5874.
- Henze, M., H. Aspegren, J. L. Jansen, P. H. Nielsen and N. Lee (2002). "Effect of solids retention time and wastewater characteristics on biological phosphorus removal." *Water Science and Technology* 45(6): 137-144.
- Hesselmann, R. P. X., C. Werlen, D. Hahn, J. R. van der Meer and A. J. B. Zehnder (1999). "Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge." *Systematic and Applied Microbiology* 22(3): 454-465.
- Higgins, J. P., S. Hurd and C. Weil (2000). "The use of engineered wetlands to treat recalcitrant wastewaters." *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* 35(8): 1309-1334.
- Janssen, P. M. J., K. Meinema and H. F. van der Roest (2002). *Biological Phosphorus Removal: Manual for Design and Operation*. London, IWA Publishing.
- Jeon, C. O., D. S. Lee, M. W. Lee and J. M. Park (2001). "Enhanced biological phosphorus removal in an anaerobic-aerobic sequencing batch reactor: Effect of pH." *Water Environment Research* 73(3): 301-306.
- Jeon, C. O., D. S. Lee and J. M. Park (2003). "Microbial communities in activated sludge performing enhanced biological phosphorus removal in a sequencing batch reactor." *Water Research* 37(9): 2195-2205.

Ju, L.-K., L. Huang and H. Trivedi (2007). "Simultaneous Nitrification, Denitrification, and Phosphorus Removal in Single-Tank Low-Dissolved-Oxygen Systems Under Cyclic Aeration " *Water Environment Research* 79(8): 912-920.

Ju, L. K., H. K. Shah and J. Porteous (2005). "Phosphorus release in aerobic sludge digestion." *Water Environment Research* 77(5): 553-559.

Kadlec, R. H. and R. L. Knight (1996). *Treatment wetlands*. Boca Raton, Lewis Publishers.

Kargi, F. and A. Uygur (2002). "Nutrient removal performance of a sequencing batch reactor as a function of the sludge age." *Enzyme and Microbial Technology* 31(6): 842-847.

Kintzer, B. and D. Moffitt (2005). *Physical Manure Treatment (Solid Separation)*. , Virginia Polytechnic Institute and State University, SERA-17.

Knight, R. L., V. W. E. Payne, R. E. Borer, R. A. Clarke and J. H. Pries (2000). "Constructed wetlands for livestock wastewater management." *Ecological Engineering* 15(1-2): 41-55.

Knowlton, K. F., N. G. Love and C. A. Parsons (2005). "Dietary phosphorus effects on characteristics of mechanically separated dairy manure." *Transactions of the Asae* 48(3): 1253-1258.

Kong, Y. H., J. L. Nielsen and P. H. Nielsen (2005). "Identity and ecophysiology of uncultured actinobacterial polyphosphate-accumulating organisms in full-scale enhanced biological phosphorus removal plants." *Applied and Environmental Microbiology* 71(7): 4076-4085.

Lan, J. C., L. Benefield and C. W. Randall (1983). "Phosphorus Removal in the Activated-Sludge Process." *Water Research* 17(9): 1193-1200.

Lang, M. (2005). *National Manual of Good Practice for Biosolids*, National Biosolids Partnership.

Lie, E., M. Christensson, K. Jonsson, K. Ostgaard, P. Johansson and T. Welander (1997). "Carbon and phosphorus transformations in a full-scale enhanced biological phosphorus removal process." *Water Research* 31(11): 2693-2698.

Liu, W. T., T. Mino, T. Matsuo and K. Nakamura (1996). "Biological phosphorus removal processes - Effect of pH on anaerobic substrate metabolism." *Water Science and Technology* 34(1-2): 25-32.

Madigan, M. T., J. M. Martinko and T. D. Brock (2006). *Brock biology of microorganisms*. Upper Saddle River, NJ, Pearson Prentice Hall.

Maguire, R. O., J. T. Sims, S. K. Dentel, F. J. Coale and J. T. Mah (2001). "Relationships between biosolids treatment process and soil phosphorus availability." *Journal of Environmental Quality* 30(3): 1023-1033.

Malnou, D., M. Meganck, G. M. Faup and M. Durostu (1984). "Biological Phosphorus Removal - Study of the Main Parameters." *Water Science and Technology* 16(10-1): 173-185.

Mamais, D. and D. Jenkins (1992). "The Effects of M_{cr}t and Temperature on Enhanced Biological Phosphorus Removal." *Water Science and Technology* 26(5-6): 955-965.

Mason, I. G. and J. Mulcahy (2003). "Volatile fatty acid production from farm dairy wastewater." *Transactions of the Asae* 46(3): 819-824.

McCarty, P. L. (1970). *Phosphorus and Nitrogen Removal by Biological Systems*. Wastewater Reclamation and Reuse Workshop, Lake Tahoe, California.

Mcclintock, S. A. (1990). "Effects of temperature and mean cell residence time on the performance of high-rate biological nutrient removal processes." Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Meers, E., D. P. L. Rousseau, E. Lesage, E. Demeersseman and F. M. G. Tack (2006). "Physico-chemical P removal from the liquid fraction of pig manure as an intermediary step in manure processing." *Water Air and Soil Pollution* 169(1-4): 317-330.

Merlo, R. P., R. S. Trussell, S. W. Hermanowicz and D. Jenkins (2007). "Effects of sludge properties on the thickening and dewatering of waste activated sludge." *Water Environment Research* 79(12): 2412-2419.

Mino, T. (2000). "Microbial selection of polyphosphate-accumulating bacteria in activated sludge wastewater treatment processes for enhanced biological phosphate removal." *Biochemistry-Moscow* 65(3): 341-348.

Mino, T., W. T. Liu, F. Kurisu and T. Matsuo (1995). "Modeling Glycogen-Storage and Denitrification Capability of Microorganisms in Enhanced Biological Phosphate Removal Processes." *Water Science and Technology* 31(2): 25-34.

Mino, T., M. C. M. Van Loosdrecht and J. J. Heijnen (1998). "Microbiology and biochemistry of the enhanced biological phosphate removal process." *Water Research* 32(11): 3193-3207.

Ndegwa, P. M., J. Zhu and A. C. Luo (2003). "Influence of temperature and time on phosphorus removal in swine manure during batch aeration." *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes* 38(1): 73-87.

Neal, C., B. Reynolds, M. Neal, S. Hughes, H. Wickham, L. Hill, P. Rowland and B. Pugh (2003). "Soluble reactive phosphorus levels in rainfall, cloud water, throughfall, stemflow, soil waters, stream waters and groundwaters for the Upper River Severn area, Plynlimon, mid Wales." *Science of the Total Environment* 314: 99-120.

Oehmen, A., P. C. Lemos, G. Carvalho, Z. G. Yuan, J. Keller, L. L. Blackall and M. A. M. Reis (2007). "Advances in enhanced biological phosphorus removal: From micro to macro scale." *Water Research* 41(11): 2271-2300.

Oehmen, A., M. T. Vives, H. B. Lu, Z. G. Yuan and J. Keller (2005). "The effect of pH on the competition between polyphosphate-accumulating organisms and glycogen-accumulating organisms." *Water Research* 39(15): 3727-3737.

Onuki, M., H. Satoh and T. Mino (2002). "Analysis of microbial community that performs enhanced biological phosphorus removal in activated sludge fed with acetate." *Water Science and Technology* 46(1-2): 145-153.

Osborn, D. W. and H. A. Nicholls (1978). "Optimization of Activated-Sludge Process for Biological Removal of Phosphorus." *Progress in Water Technology* 10(1-2): 261-277.

Panswad, T., N. Tongkhammak and J. Anotai (2007). "Estimation of intracellular phosphorus content of phosphorus-accumulating organisms at different P : COD feeding ratios." *Journal of Environmental Management* 84(2): 141-145.

Park, M. S., Y. Kiso, Y. J. Jung, M. Simase, W. H. Wang, T. Kitao and K. S. Min (2004). "Sludge thickening performance of mesh filtration process." *Water Science and Technology* 50(8): 125-133.

Pattarkine, V. M. (1991). *The role of metals in enhanced biological phosphorus removal from wastewater*, Virginia Polytechnic Institute and State University, 1991.: xx, 328 leaves.

Pereira, H., P. C. Lemos, M. A. M. Reis, J. P. S. G. Crespo, M. J. T. Carrondo and H. Santos (1996). "Model for carbon metabolism in biological phosphorus removal processes based on in vivo C-13-NMR labelling experiments." *Water Research* 30(9): 2128-2138.

Pierzynski, G. M. (2000). "Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters." *Southern Cooperative Series Bulletin No. # 396*.

Pitman, A. R., S. L. Deacon and W. V. Alexander (1991). "The Thickening and Treatment of Sewage Sludges to Minimize Phosphorus Release." *Water Research* 25(10): 1285-1294.

Pokethitiyook, P. (1990). *Nitrate utilization as the final electron acceptor in a biological phosphorus removal system*, Virginia Polytechnic Institute and State University, 1990.: x, 108 leaves.

Powell, J. M., D. B. Jackson-Smith and L. D. Satter (2002). "Phosphorus feeding and manure nutrient recycling on Wisconsin dairy farms." *Nutrient Cycling in Agroecosystems* 62(3): 277-286.

Puig, S., M. Coma, H. Monclus, M. C. M. van Loosdrecht, J. Colprim and M. D. Balaguer (2008). "Selection between alcohols and volatile fatty acids as external carbon sources for EBPR." *Water Research* 42(3): 557-566.

Punrattanasin, W. (1997). "Investigation of the effects of COD/TP ratio on the performance of a biological nutrient removal system." MS Thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.

Randall, A. A., L. D. Benefield and W. E. Hill (1994). "The Effect of Fermentation Products on Enhanced Biological Phosphorus Removal, Polyphosphate Storage, and Microbial-Population Dynamics." *Water Science and Technology* 30(6): 213-219.

Randall, A. A., L. D. Benefield, W. E. Hill, J. P. Nicol, G. K. Boman and S. R. Jing (1997). "The effect of volatile fatty acids on enhanced biological phosphorus removal and population structure in anaerobic/aerobic sequencing batch reactors." *Water Science and Technology* 35(1): 153-160.

Randall, C. W., J. L. Barnard and H. D. Stensel (1992). Design and retrofit of wastewater treatment plants for biological nutrient removal. Lancaster, Pa., Technomic Pub. Co., p 381.

Rodrigo, M. A., A. Seco, J. M. PenyaRoja and J. Ferrer (1996). "Influence of sludge age on enhanced phosphorus removal in biological systems." *Water Science and Technology* 34(1-2): 41-48.

Serafim, L. S., P. C. Lemos and M. A. M. Reis (2002). "Effect of pH control on EBPR stability and efficiency." *Water Science and Technology* 46(4-5): 179-184.

Sharpley, A. N., T. Daniel, T. Sims, J. Lemunyon, R. Stevens and R. Parry (2003). *Agricultural Phosphorus and Eutrophication. Second Edition. ARS-149.*, USDA Agricultural Research Service.

Skalsky, D. S. and G. T. Daigger (1995). "Waste-Water Solids Fermentation for Volatile Acid Production and Enhanced Biological Phosphorus Removal." *Water Environment Research* 67(2): 230-237.

Smolders, G. J. F., J. Vandermeij, M. C. M. Vanloosdrecht and J. J. Heijnen (1995). "A Structured Metabolic Model for Anaerobic and Aerobic Stoichiometry and Kinetics of the Biological Phosphorus Removal Process." *Biotechnology and Bioengineering* 47(3): 277-287.

Stante, L., C. M. Cellamare, F. Malaspina, G. Bortone and A. Tilche (1997). "Biological phosphorus removal by pure culture of *Lamproedia* spp." *Water Research* 31(6): 1317-1324.

Stephens, H. M., J. B. Neethling, M. Benisch, B. Baake, H. D. Stensel and R. Moore (2003). Reliability of Biological Phosphorus Removal. Proceedings of the Water Environment Federation, WEFTEC 2003: Session 31 through Session 40, Water Environment Federation.

Surampalli, R. Y., R. D. Tyagi and Environmental and Water Resources Institute (U.S.). Environmental and Multi-Media Council. (2004). *Advances in water and wastewater treatment*. Reston, Va., American Society of Civil Engineers.

Takacs, I., S. Murthy, S. Smith and M. McGrath (2006). "Chemical phosphorus removal to extremely low levels: experience of two plants in the Washington, DC area." *Water Science and Technology* 53(12): 21-28.

Tchobanoglous, G., F. L. Burton and Metcalf & Eddy. (1991). *Wastewater engineering : treatment, disposal, and reuse*. New York, Irwin/McGraw-Hill.

Tyson, T. W. (1996). *Constructed Wetlands for Animal Waste Treatment*. ANR-965., Alabama Cooperative Extension System.

Van Horn, H. H., G. L. Newton and W. E. Kunkle (1996). "Ruminant nutrition from an environmental perspective: factors affecting whole-farm nutrient balance." *Journal of Animal Science* 174(12): 3082-3102.

Van Horn, H. H., G. L. Newton, R. A. Nordstedt, E. C. French, G. Kidder, D. A. Graetz and C. F. Chambliss (2003). "Dairy Manure Management: Strategies for Recycling Nutrients to Recover Fertilizer Value and Avoid Environmental Pollution." Florida Coop. Ext. Serv. Circ CIR1016.

Wagner, M., R. Amann, H. Lemmer and K. H. Schleifer (1993). "Probing Activated-Sludge with Oligonucleotides Specific for Proteobacteria - Inadequacy of Culture-Dependent Methods for Describing Microbial Community Structure." *Applied and Environmental Microbiology* 59(5): 1520-1525.

Wentzel, M. C., G. A. Ekama, P. L. Dold and G. V. Marais (1990). "Biological Excess Phosphorus Removal - Steady-State Process Design." *Water Sa* 16(1): 29-48.

Wentzel, M. C., L. H. Lotter, G. A. Ekama, R. E. Loewenthal and G. V. R. Marais (1991). "Evaluation of Biochemical-Models for Biological Excess Phosphorus Removal." *Water Science and Technology* 23(4-6): 567-576.

Whang, L. M. and J. K. Park (2006). "Competition between polyphosphate- and glycogen-accumulating organisms in enhanced-biological-phosphorus-removal systems: Effect of temperature and sludge age." *Water Environment Research* 78(1): 4-11.

Yagci, N., E. U. Cokgor, N. Artan, C. Randall and D. Orhon (2007). "The effect of substrate on the composition of polyhydroxyalkanoates in enhanced biological phosphorus removal." *Journal of Chemical Technology and Biotechnology* 82(3): 295-303.

Yagci, N. O., R. Tasli, N. Artan and D. Orhon (2003). "The effect of nitrate and different substrates on enhanced biological phosphorus removal in sequencing batch reactors." *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* 38(8): 1489-1497.

Zou, H., G. C. Du, W. Q. Ruan and J. Chen (2006). "Role of nitrate in biological phosphorus removal in a sequencing batch reactor." *World Journal of Microbiology & Biotechnology* 22(7): 701-706.