2013

Impact of additional carbon on poly-hydroxybutyrates (PHB) accumulation and nutrient removal in a sustainable anaerobic/anoxic/oxic (A2O) membrane bioreactor

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Impact of additional carbon on poly-hydroxybutyrates (PHB) accumulation and nutrient removal in a sustainable anaerobic/anoxic/oxic (A_2O) membrane bioreactor

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Civil Engineering (Environmental Engineering)

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Iowa State University

Ames, Iowa

2013

DEDICATION

To my dearest family:

Thank you for the encouragement and support you've given me.

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ACKNOWLEDGEMENT

I thank Dr. Say Kee Ong for holding weekly meetings to discuss my thesis process, challenging my thinking and improving my visions in research. I really appreciate his patience and everything he has done.

I thank Dr. Thomas Loynachan for providing us the biomass freeze drier and help with bacterial observation.

I thank Mr. David Mozzena for his help in collecting activated sludge at Boone Water Pollution Control Center.

ABSTRACT

Recovering nutrient, energy, useful byproducts and reusing the treated wastewater may make a municipal wastewater treatment more sustainable. The approach employed in this study to increase the sustainability of wastewater treatment was to accumulate and harvest poly-hydroxybutyrates (PHB) in wastewater treatment plants. Additional carbon (acetate, supernatant of fermented sludge or thin corn stillage) was continuously fed along with synthetic municipal wastewater to a bench-scale anaerobic/anoxic/oxic (A_2O) membrane bioreactor to promote PHB accumulation in the biomass. The impact of addition of carbon to the anaerobic tank or anoxic tank was also studied. PHB content in the range of 10% of dry biomass weight was achieved by adding 1000 mg-C/L acetate to either the anaerobic tank or anoxic tank. In addition, removal of total nitrogen and total phosphorus by the A_2O MBR increased when acetate was added. Percent of nitrogen removal increased from 82.4% to 98%, and total phosphorus in effluent was reduced to as low as 0.4 mg/L. When supernatant of fermented sludge was added as additional carbon source, the PHB accumulation was about 4.2% of dry biomass weight. Adding supernatant of fermented sludge did not affect the effluent quality, and the total nitrogen and total phosphorus in the effluent were still within typical discharge limits. With thin corn stillage as an additional carbon source, a PHB content of 7.2% of dry biomass weight was obtained. However, use of corn stillage resulted in high TN, TP and COD in the effluent of the A_2O MBR.

CHAPTER 1. INTRODUCTION AND OBJECTIVES

1.1 Introduction

Since its first implementation in the modern world, wastewater treatment plants have been viewed as a disposal facility for the removal of pollutants before the treated wastewaters are discharged into bodies of water with minimal environmental damage. Recently, the question of the sustainability of planet earth and its limited resources have spurred a rethinking of our view of various urban infrastructure systems including that of the municipal wastewater disposal system. Urban infrastructure are built based on cost, technology available, convenience, and, to a certain extent, driven and governed by regulatory measures in place. In the case of wastewater disposal system, wastewater collection and treatments systems are designed to meet discharge limits for the treated wastewater and/or protecting the water quality of the watersheds. However, recent thinking is to reuse the various constituents in the wastewater and reuse the treated wastewater. This paradigm shift in viewing the wastewater treatment plant as a "factory" for the production of renewable products and as a water source has been heavily promoted in recent years.

Over the past 30 years, wastewater treatment technologies have improved mainly due to the more stringent nutrient discharge limits. A new technology called membrane bioreactor (MBR) which combines activated sludge process with membrane filtration has been successfully implemented. When combined with various anoxic and anaerobic tanks, MBRs have shown excellent nutrient removal, good flexibility and low sludge production in comparison to conventional treatment system. Brown et al. (2011) obtained 89%

removal of total nitrogen and 82% removal of total phosphorus. Ersu et al. (2008) reported total nitrogen and phosphorus removal at 91% and 88% respectively by a bench-scale MBR.

The excellent treatment potential of MBRs can be used to make the wastewater treatment system more sustainable. However, the capital and operating costs of MBRs are typically higher than conventional systems for the same throughput. Energy consumption is higher for MBR as the system uses a higher volume of air to scour the membranes and requires energy for the membrane filtration as opposed to gravity settling of the sludge. High energy consumption and high operating costs provide an opportunity to improve the sustainability of the MBR system as other aspects of the MBR such as low sludge production and recovery of phosphorus would offset the energy costs.

One possible way of increasing the sustainability of MBR or wastewater treatment plant in general is to recover PHB from the phosphorus removal process. PHB is an intracellular carbon and energy source synthesized by a wide range of microorganisms under nutrient-limiting conditions (Aderson et al., 1990). Because of its biodegradability and promising applications, PHB as an organic polymer has attracted interest in the medical, pharmaceutical and chemical industries. Studies have been conducted on PHB accumulation by activated sludge in sequencing batch reactors (SBR). Many of these studies are conducted with pure culture and with a specific clean substrate such as acetate. For example, Liu et al. (2011) were able to attain a PHB content of 67% of sludge dry weight by adding 6.0 g/L sodium acetate to a SBR with activated sludge from a municipal wastewater treatment plant. The sludge was acclimatized to acetate before it was placed in the SBR. If the same principle of PHB accumulation in a pure

culture can be applied to an MBR and at the same time recover phosphorus, nitrogen and energy, and treat the municipal wastewater for reuse, the wastewater treatment system can be made more sustainable.. Since the price of PHB may be as high as $10/kg (Gurieff and Lant, 2007), recovery and sale of PHB may help offset the operating and energy costs of the wastewater treatment system.

There are very few studies on PHB accumulation and recovery in continuous flow systems such as in activated sludge plants or MBRs which may make these wastewater treatment systems more sustainable. Even though PHB accumulation potential of activated sludge is known, there remain issues to be overcomed in order to optimize PHB accumulation, and, at the same time, treat the wastewater to regulatory limits, and if required, reuse the treated wastewater. There is a need to understand the treatment plant operating conditions and the responses of the plant to PHB accumulation for low carbon content characteristics of municipal wastewater (about 100 – 200 mg/L as C) and the need for the addition of a suitable carbon source. For example, acetate addition can improve PHB growth. This would mean additional operating costs since acetate needs to be purchased. In addition, the feeding point and the optimal concentration of acetate needed are still unresolved. Since there are many industrial wastewaters with high carbon that need treatment, these industrial wastewaters can be used as a source of additional carbon. A disadvantage of using industrial wastewater is the presence of nitrogen, phosphorus and toxic compounds in the industrial wastewaters which may impact the overall treatment efficiency of the municipal wastewater treatment plants.

1.2 Objectives

The goal of this study is to determine the accumulation of PHBs in MBRs through operating condition adjustments and by adding external carbon source to make the treatment system more sustainable. The specific objectives of the study are:

1. Determine the impact of three different additional carbon sources (acetate, fermented supernatant of activated sludge or corn stillage) and their carbon concentrations on PHB accumulation
2. Determine the influence of feeding location of additional carbon on PHB accumulation (comparison of feeding to anoxic or anaerobic tank)

The results of this study, combined with future work, can be utilized to further improve the sustainability of wastewater treatment plants through PHB accumulation in MBR systems.

1.3 Thesis organization

The thesis is organized into 4 chapters with 2 appendices. Chapter 1 provides the introduction and objectives of the study. Chapter 2 is a literature review comprising of information on PHB accumulation principles and work conducted by others. Chapter 3 presents the method and results of this study and is in a paper format to be submitted for publication, and Chapter 4 is the conclusion chapter.

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CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Rapid growth in population has threatened water sustainability of many watersheds especially in water-stressed regions. Efforts are made in water conservation, reuse of treated wastewaters, protect of watersheds through removal of pollutants in wastewaters and implementation of discharge limits. Discharge of nutrients to water bodies results in eutrophication which in turn affects the water quality and reduces the available water supply. The main causes of eutrophication to the receiving water bodies are nitrogen and phosphorus, and they are removed by a commonly used wastewater treatment process, biological nutrient removal. Nutrient removal processes can be accomplished in SBRs or in continuous flow systems. Conventional treatment process such as activated sludge system by itself may not be able to achieve nutrient discharge limitations in the future, but would require modification or additional unit processes.

In recent years, membrane bioreactors (MBR) in combination with anaerobic and anoxic tanks have been employed to maximize nitrogen and phosphorus removal. This A_2O MBR system offers excellent effluent quality, flexible operation time and high treatment efficiency and low sludge production. MBRs can be operated under long solids residence times (SRT) (> 25 days) and short HRTs (< 4 hours) without sacrificing treatment efficiency and, at the same time, have lower sludge production. A_2O MBR produces excellent treated effluent which can be reused or recycled with minimal further treatment. To increase the sustainability of wastewater treatment plants, efforts are being made to recover energy, nutrients and produce useful products such as PHB from

processes of wastewater treatment. Subsequent sections of this chapter will discuss nutrient removal and production of PHBs in wastewater treatment processes.

2.2 A_2O System

An A_2O activated sludge system consists of three stages/tanks which are maintained under anaerobic, anoxic and aerobic conditions. It is commonly used for removal of both nitrogen and phosphorus. Nitrogen is removed by nitrification (ammonia to nitrate) followed by denitrification (nitrate to nitrogen gas). Phosphorus is removed biologically by phosphorus accumulating organisms (PAOs). With a membrane in the aerobic reactor, this system works even better since the membrane provides excellent retention of the sludge resulting in reduced sludge production at longer SRTs and, at the same time, producing treated effluent with low suspended solids (< 1 mg/L).

2.2.1 Nitrification

Nitrification is a biological process that converts ammonium to nitrate nitrogen. It is a two-step process and each step is performed by two distinct groups of bacteria. Bacteria commonly involved in nitrification in wastewater treatment are the autotrophic bacteria *Nitrosomonas* and *Nitrobacter* (Metcalf and Eddy, 2003). Ammonia or ammonium is first converted to nitrite by *Nitrosomonas*, and then nitrite is converted to nitrate by *Nitrobacter*. The energy-yielding two–step oxidation of ammonia to nitrate is as follows (Metcalf and Eddy 2003):

Reaction by Nitroso-bacteria:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (2.1)$$

Reaction by Nitro-bacteria:

$$2NO_2^- + 2O_2 \rightarrow 2NO_3^- \quad (2.2)$$

Total oxidation reaction:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (2.3)$$

Overall reaction including cell synthesis (Crites and Tchobanoglous, 1998):

$$NH_4^+ + 1.863O_2 + 0.098CO_2 \rightarrow$$

$$0.0196C_5H_7NO_2 + 0.98NO_3^- + 1.98H^+ + 0.941H_2O \quad (2.4)$$

In A_2O system, nitrification happens in the aerobic stage. This is because bacteria in this process are strict “aerobes”, and the amount of oxygen in the water plays an important role in the process. At low dissolved oxygen (DO) level (< 0.5 mg/L), nitrification rates are greatly inhibited (Metcalf and Eddy, 2003). Usually a dissolved oxygen level of 2 mg/L or above is maintained. Besides dissolved oxygen in the wastewater, several other parameters such as pH, carbon: nitrogen: phosphorus ratios (C: N: P ratios) and the amount of ammonia in the influent may also affect the performance of nitrification. Optimum pH for nitrification is in the range 7.5 to 8.0, and pH values below 6.8 will cause significant decrease in nitrification. One mg of ammonia oxidized will consume about 7.14 mg of alkalinity. This will result in a drop in pH due to the consumption of alkalinity (Metcalf and Eddy, 2003).

2.2.2 Denitirification

Denitrification is the biological process of reducing nitrate to nitric oxide, nitrous oxide, and nitrogen gas in absence of dissolved oxygen. In the A_2O system, denitrification occurs under the anoxic stage. The nitrate produced in the aerobic stage is recycled back to the anoxic tank and used as the electron acceptor. This process is termed as substrate denitrification because the organic substrate from the influent provides the electron donor for the reduction of nitrate. It's also commonly known as preanoxic denitrification because the anoxic process precedes the aerobic process (Metcalf and Eddy, 2003) (Figure 2.1).

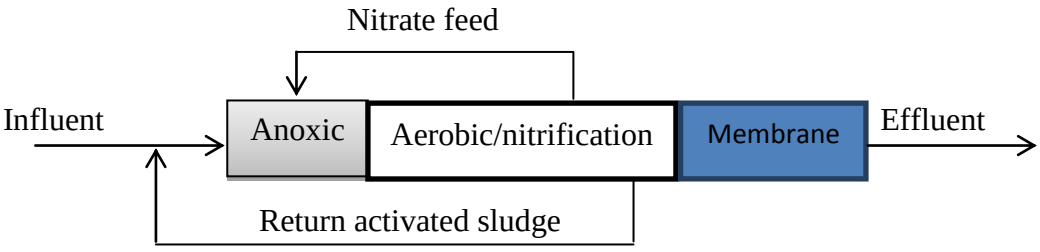

Figure 2.1 Preanoxic denitrification in A_2O system (adapted from Brown et al., 2011)

The reduction of nitrate to nitrogen gas involves several reduction steps:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad 2.5$$

Low molecular weight carbon such as volatile fatty acids (such as acetate), methanol and organics produced by endogenous decay are known to be favored as electron donors in the denitrification process. The process can be described as:

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \quad 2.6$$

In the heterotrophic denitrification reaction above, 3.57 g of alkalinity (as $CaCO_3$) is produced when 1 g of nitrate nitrogen is reduced. By denitrification about one-half of the alkalinity consumed by nitrification (7.14 g alkalinity as $CaCO_3$ per g of NH_4-N oxidized) can be recovered (Metcalf and Eddy, 2003).

2.2.3 Biological phosphorus removal

Biological phosphorus removal is based on the characteristics of PAOs. The following observations are the basis for phosphorus removal (Sedlak, 1991):

1. Under anaerobic condition, PAOs will assimilate easily biodegradable carbons such as volatile fatty acids and stored as PHB within the cells. At the same time, phosphorus will be released from the stored polyphosphates.

2. Under aerobic condition, the reactions will be reversed with the oxidation of the stored PHB and the energy released used for the uptake and storage of phosphorus in the cells.

Soluble orthophosphate in wastewater are stored as polyphosphate in the bacterial cells. By wasting portion of the biomass, stored phosphorus is removed from the solution and disposed with the wasted sludge (Metcalf and Eddy, 2003). Biological phosphorus removal systems will perform better when biodegradable soluble COD (bsCOD) or acetate is available at a steady rate. Changes in the intracellular storage reserves of glycogen, PHB and polyphosphates caused by periods of starvation or low bsCOD will rapidly lead to decreased phosphorus removal efficiency (Stephens and Stensel, 1998).

2.3 Factors Impacting Nutrient Removal

Hydraulic retention time (HRT), solids retention time (SRT), recirculation ratios and concentration of carbon have an impact on nitrogen and phosphorus removal. In an A_2O membrane bioreactor, Brown (2007) found that phosphorus removal increased with an increase in SRT from 10 to 25 days and then declined when the SRT was greater than 50 days. Nitrification and denitrification performances were improved with an increase of SRT until 70 days, and phosphorus removal decreased at prolonged SRT due to a reduction in excess sludge (Sung-Soo et al., 2004). Figure 2.2 drawn with the data from Brown et al. (2011) showed that phosphorus removal decreased when anoxic HRT increased, but increased when anaerobic HRT increased. On the other hand, longer anoxic HRT provided better nitrogen removal. By using the figure below, an optimal HRT combination of anaerobic and anoxic tanks can be selected.

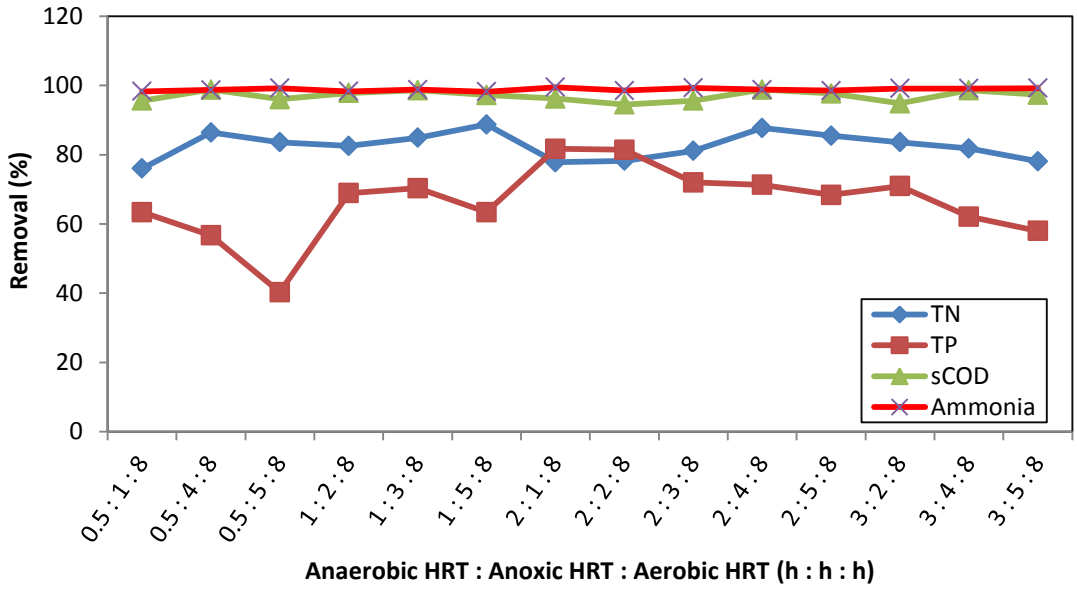

Figure 2.2 Steady – state removal results for various HRT combinations (adapted from Brown et al., 2011)

Work done by Ersu et al. (2006) showed that nitrogen removal increased from 76% to 85% and then 88% as permeate recirculation (PR) increased from 100% to 200% and 300% (Figure 2.3). Phosphorus removal increased from 65% to 88% as mixed liquor recirculation (MLR) increased from 100% to 300% (Figure 2.3). In an A_2O – biological aerated filter system, recirculation of nitrate is also an important factor for nitrogen and phosphorus removal. Nitrogen removal efficiencies increased from 65% to 87% as the recirculation rate of nitrate increased from 100% to 400%, and phosphorus removal efficiency also increased as recirculation of nitrate increased (Chen et al., 2011).

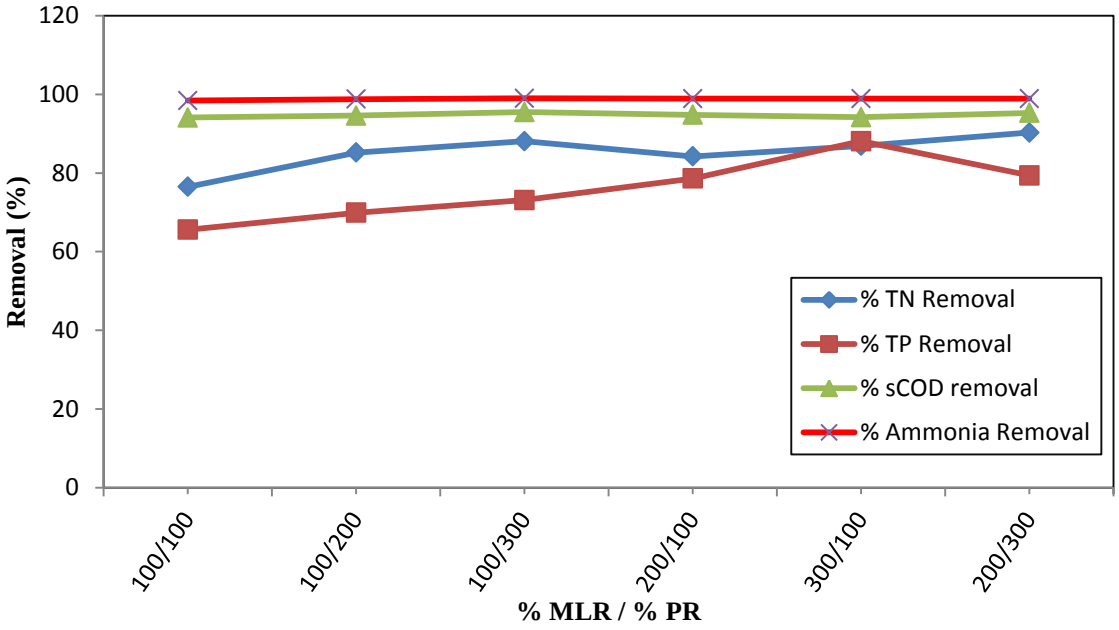

Figure 2.3 Steady – state removal results for various recirculation rates (adapted from Ersu et al., 2008)

Brown et al. (2011) reported that HRTs of 2 hours anaerobic, 4 hours anoxic and 8 hours aerobic gave the optimal nitrogen and phosphorus removal. The recirculation ratio used in their research was 100% MLR to the inlet feed, and 100% PR to the anoxic compartment. Ersu et al. (2008) reported that 300% MLR and 100% PR gave optimal nitrogen and phosphorus removal with HRTs of 2 hours anaerobic, 2 hours anoxic and 8 hours aerobic. By merging these two researches together, it is reasonable to hypothesize that at HRTs of 2 hours anaerobic, 4 hours anoxic and 8 hours aerobic and a recirculation ratio larger than 100% MLR/100% PR may improve removal efficiency.

Besides HRTs and recirculation ratios, SRT is an important factor for A_2O system. However, there is a conflict between SRT needed for nitrifiers which favor long SRT and PAOs which prefer short SRT (Van Loosdrecht et al., 1998). In order to obtain satisfactory removal for both nitrogen and phosphorus, a balanced but optimal SRT is necessary. Due to the increase of hydrolysis factors in anoxic and anaerobic compartments, nitrogen and phosphorus removal increased with SRT up to 50 days, but phosphorus removal decreased with SRT beyond due to an increase of endogenous decay in the aerobic tank resulting in release of phosphorus (Ersu et al., 2010). By comparing the performance of membrane bioreactor with different SRTs (20 days and 60 days) at low dissolved oxygen (0.1 - 0.2 mg/L), Hocaoglu et al. (2011) found that nitrification was reduced from 68% to 40% while denitrification was almost complete.

Concentration of substrates (carbon source) plays an important role in the entire treatment process, especially for denitrification and phosphorus removal. Sludge recycled back to anaerobic zone will introduce nitrate which is considered as inhibitor to biological phosphorus removal (BPR) activity. Nitrate recycled back can be denitrified which in turn will reduce the amount of organic substrate available for uptake by the PAOs. Introducing additional acetate to the anoxic zone of a BPR system is beneficial to nitrogen removal since it increases denitrification which results in a net phosphorus release and a net PHB accumulation. Although there is a net phosphorus release with high addition of acetate, it is hard to tell whether the release is detrimental to phosphorus removal since the overall phosphorus removal is dependent on phosphorus uptake rate in the aerobic zone and thus dependent on the PHB level and the aerobic retention time (Meinhold et al., 1998). However, it is possible that phosphorus uptake due to extra PHB

accumulation can be more than the net phosphorus release, therefore, resulting in an increased phosphorus removal. As shown in Figure 2.4, phosphorus uptake increased as more PHB were accumulated.

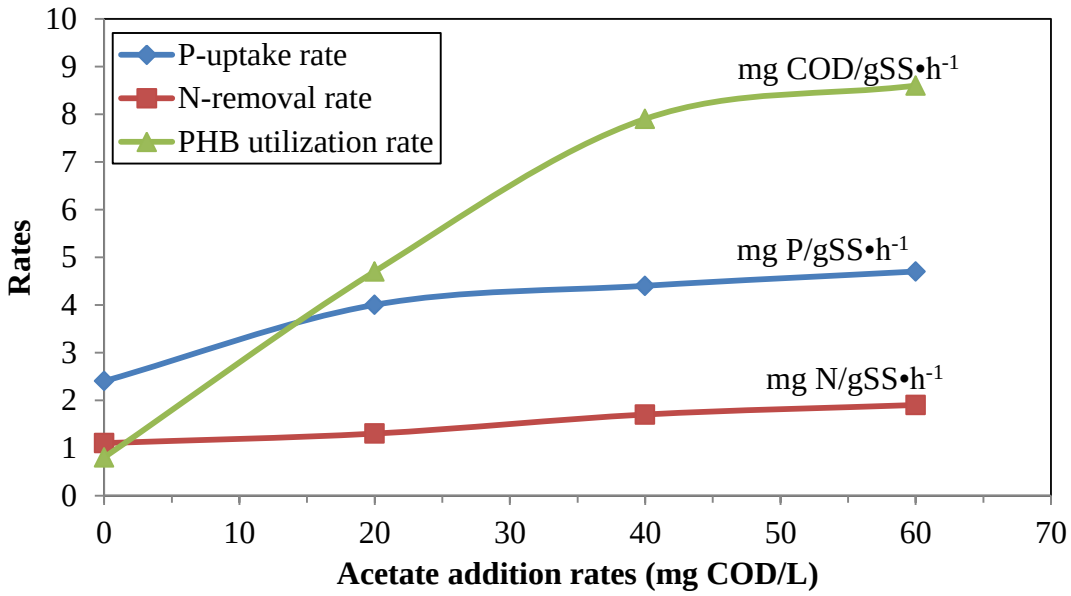

Figure 2.4 Phosphorus uptake, nitrogen removal and PHB utilization rates with various acetate addition rates, SS = 3.71 g/L (adapted from Meinhold et al., 1998)

2.4 PHB Accumulation

2.4.1 PHB accumulation

PHB are polymers accumulated by PAOs. PAOs accumulate polyphosphate as an energy reserve in their intracellular granules. These organisms utilize the energy and release orthophosphate to accumulate simple organics which are stored as PHB under anaerobic condition. Commercial PHB are mainly produced using pure microbial culture. The cost of maintaining a pure culture and the recovery process is high and this makes the costs of PHB as a raw material higher than conventional plastics such as

polypropylene. It is probable that PHB production based on mixed cultures and use of wastewater can greatly reduce the price of the biopolymer (Salehizadeh and Van Loosdrecht, 2004).

In recent years, there were many studies investigating PHB accumulation by activated sludge in SBRs with a focus on the operational process modeling and control, bacterial storage mechanisms and polymer characterization (Liu et al., 2011). Chua et al. (2003) found that sludge can accumulate PHB up to approximately 20% of dry biomass weight using municipal wastewater only, and with additional acetate supplement, the poly-hydroxyalkonates (PHA) content increased to 30% of biomass dry weight. At a COD: N ratio of 140, PHA in the biomass was found to be up to 39% of biomass dry weight (Chua et al., 1999). Satoh et al. (1998) reported that activated sludge can accumulate PHA to around 20% under anaerobic condition and 33% under aerobic condition in a lab-scale anaerobic-aerobic reactor. Using a "microaerophilic-aerobic" process which operated with limited oxygen demand in the aerobic zone, Satoh et al. (1998) was able to increase the PHA accumulation to as high as 62%. Sludge with low polyphosphate content (8%) can accumulate PHA of 51% at pH of 8 (Kasemsap et al., 2007). Wen et al. (2010) reported that the maximum PHA accumulation was 59% of cell dry weight when the C to N ratio was 125. The maximum PHB content of 67% sludge dry weight was attained when 6.0 g/L sodium acetate was added to a SBR (Liu et al., 2011).

SBRs were commonly used when investigating PHB accumulation by activated sludge. However, there are very few research investigating PHB accumulation in a continuous flow system such as A_2O system. Moreover, the carbon source used was clean compound such as acetate which needs to be purchased and is an expense to the wastewater treatment facilities. To reduce the cost of PHB production, industrial waste streams which must be treated before they can be disposed of can be used instead of clean compounds (Braunegg et al., 2004). Other sources include fermented sludge and corn stillage which contain volatile fatty acid that can be used for PHB accumulation as well.

2.4.2 Factors impacting PHB accumulation

Substrates in many waste streams may be suitable for producing PHB, but use of the waste stream may result in nutrient limited conditions and additional carbon may need to be supplemented for sufficient enrichment of PHB accumulating bacteria. Carbon to nitrogen ratio (C/N ratio) is an important measure for PHB accumulation. Johnson et al. (2010) found that with a C/N ratio of 13.2, PHB can be accumulated to as high as 39.8% with short SRT of 0.5 days in a SBR. Biomass with higher PHB accumulating capacities usually grow in carbon-limited SBRs while biomass with higher initial PHB content usually grow in nitrogen-limited SBRs (Johnson et al., 2010). Lemos et al. (1998) showed that the types of carbon sources (acetate, propionate and butyrate) had an impact on biopolymer production with acetate giving the best polymer production of the three carbon used. In addition, the components of the polymer were also different when different types of carbon sources were used. Biopolymer accumulated by using acetate

consisted 75.25% of PHB and 24.75% of poly-hydroxyvalerate (PHV). When using propionate, PHV was 71.95% of the total amount produced (Lemos et al., 1998). Pijuan et al. (2009) found that use of butyrate as a carbon source resulted in a relative even amount PHB and PHV, and the amount of PHV was much greater when using glucose.

SRT plays an important role in PHB accumulation. Using the same C/N ratio, Hohnson et al. (2010) showed that lower SRT contributes to higher PHB percent in the biomass (see Table 2.1).

Table 2.1 PHB production under different SRT (adapted from Johnson et al., 2010)

SRT	C/N Ratio	PHB Accumulated (PHB per active biomass)
4	13.2	5.3%
1	13.2	27%
0.5	13.2	39.8%

Chang et al. (2011) report that SRT of an enhanced biological phosphorus removal system is the core factor in determining whether anaerobic or anoxic sludge should be employed for PHA production. The anoxic sludge exhibited better PHA production compared to anaerobic sludge at 5 days SRT, while at 15 days SRT, anaerobic sludge performed better in accumulation of PHA as compared to anoxic sludge (Chang et al., 2011).

PHB accumulation was traditionally assumed to be related to carbon for growth and limitation of a nutrient such as nitrogen or phosphorus (Braunegg et al., 2004). The limitation of a nutrient (nitrogen or phosphorus) is an important parameter since carbon sources are diverted for direct growth instead of storage when there were excess nutrient.

Ciggin et al. (2009) found that storage yield based on PHB changed from 0.59 gCOD/gCOD to 0.4 and then to 0.33 gCOD/gCOD when 114 mg-N/L and 226 mg-N/L of nitrate, respectively, were injected into the SBR. For influent wastewater with low COD/N ratio, nitrate accumulation was found to be responsible for inhibition of PHB accumulation. Different nutrient limitation also led to different PHB accumulation. With phosphorus limitation, the PHB accumulated was significantly lower than the amount accumulated with nitrogen limitation (Wen et al., 2010). Common operating parameters such as pH and temperature are also important to PHB accumulation. Kasemsap et al. (2007) found that when pH was increased from 6 to 8, PHA accumulation increased significantly which may be due to the lower energy required for acetate uptake.

The parameters discussed above have significant impact on PHB production when using SBR for accumulation. When continuous flow systems are used to accumulate PHB, these parameters could also be important, especially the nitrate concentrations and SRT. With nitrogen removal, denitrifiers will compete with PAOs for carbon source and reduce PHB accumulation.

2.4.3 Possible carbon source for PHB accumulation

Although different types of carbon sources were investigated by various researchers, there are other options that have not been tested yet. Supernatant from fermented sludge was used to improve phosphorus removal, but few researchers have used it for PHB accumulation. Short chain volatile fatty acids such as acetic and propionic acids are formed when organic materials in sludge are anaerobically broken

down. With initial volatile suspended solids of 18,000 mg/L, the amount of volatile fatty acids can reach as high as 2920 mg/L after 4 days (Cokgor et al., 2006). Even though, fermented sludge may have a high concentration of volatile fatty acids and is an excellent carbon source for PHB accumulation, fermented sludge also has high nitrogen and phosphorus which may affect PHB accumulation. But Coats et al. (2011) obtained PHB content of 12% to 27% of dry biomass weight by using supernatant of fermented sludge as external carbon source in a SBR using sludge from a municipal wastewater treatment plant. Corn stillage is another possible option for providing carbon for PHB accumulation. Eskicioglu et al. (2011) obtained 5874 mg/L of volatile fatty acids when using thermophilic digestion of whole corn stillage. The large amount of volatile fatty acids from corn stillage can provide enough carbon sources for PHB accumulation. However, the amount of nitrogen and phosphorus in corn stillage are very large (initial TKN and TP of whole corn stillage was 5300 mg/L and 3506 mg/L) (Eskicioglu et al., 2011), and could negatively affect PHB accumulation.

2.5 Summary and Further Study

Previous investigations of PHB accumulation were mainly conducted by using SBR. There are very few studies using continuous flow systems such as A_2O system. Recent literature showed that the carbon source used in PHB accumulation were mostly "clean" such as acetate which may not be cost effective for practical application. Although different types of carbon sources were investigated, few studies used waste streams or products derived from waste streams as carbon sources. Use of waste streams

as a suitable carbon source for PHB accumulation which may make the wastewater treatment plant more sustainable remains unresolved.

There are many studies about optimizing wastewater treatment plants for the removal efficiency of nitrogen and phosphorus, but there are very few studies that combine nutrient removal and improve sustainability of the treatment systems accordingly. The possibilities of further recovering energy or useful products from membrane biological nutrient removal system remain unknown. Recent researches have shown excellent PHB accumulation by activated sludge in SBRs with a reported maximum PHB accumulation of 67% of dry sludge weight using acetate (Liu et al., 2011) and a PHA content of 27% of biomass using fermenter liquor (Coats et al., 2007) Although a continuous flow system is different from a SBR, the basic principles governing the accumulation of PHB in SBR may be applied to a continuous flow system. There is a need to determine the possibility of accumulating PHB for harvest in a continuous flow system to improve the sustainability of wastewater treatment system along with high removal efficiency.

The main cost of PHB accumulation comes from the use of carbon source. Use of substrate carbons such as industrial wastewater which may reduce the cost of PHB production have not been well investigated. Use of different waste streams to accumulate PHB in both SBR and MBR is an area of potential research. Shorter SRT gives better PHB accumulation, so shortening the SRT in an MBR system may increase PHB accumulation. But shorter SRT could negatively affect nutrient and carbonaceous removal efficiency. The optimal SRT for PHB accumulations in MBR system remains

unknown. HRTs of anaerobic, anoxic and aerobic could impact PHB accumulation in MBR too. The optimal HRTs for PHB accumulation remain unresolved.

There is a need for a comprehensive investigation to determine optimal conditions for PHB accumulation in MBR. Under such condition, PHB can be harvested along with satisfactory nutrient removal efficiency.

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CHAPTER 3. IMPACT OF ADDITIONAL CARBON ON POLY-HYDROXYBUTYRATES (PHB) ACCUMULATION AND NUTRIENT REMOVAL IN A SUSTAINABLE ANAEROBIC/ANOXIC/OXIC MEMBRANE BIOREACTOR

Abstract

Efforts are being employed to make municipal wastewater treatment plants more sustainable by recovering nutrients, energy, useful byproducts and reusing the treated wastewater. One approach to achieve this objective of sustainability is to accumulate and harvest poly-hydroxybutyrates (PHB) in wastewater treatment plants. A bench-scale anaerobic/anoxic/oxic (A_2O) membrane bioreactor was fed with additional carbon (acetate, supernatant of fermented sludge and thin corn stillage) to promote PHB accumulation in the biomass. The impact of carbon addition to the anaerobic tank or anoxic tank was also studied. Adding 1000 mg-C/L of acetate gave PHB content in the range of 10% of dry biomass weight for both addition of the carbon to the anaerobic tank or anoxic tank. Addition of acetate to the A_2O MBR also increased the removal of total nitrogen (from 82.4% to as high as 98%) and total phosphorus (0.4 mg/L of total phosphorus in effluent) in the synthetic municipal wastewater. PHB (4.2% of dry biomass) was found to accumulate when supernatant of fermented sludge and thin corn stillage (7.2% of dry biomass) were used as additional carbon sources. However, using corn stillage as an additional carbon source resulted in high TN, TP and COD in effluent.

3.1 Introduction

Since the first day of its introduction, wastewater treatment plants have been regarded as disposal facilities for municipal wastewaters. Pollutants are removed before the treated wastewaters are discharged into bodies of water to minimize damage to the environment. Recent thinking, however, shows a shift from viewing the treatment plant as a disposal facility to a "factory" for the recovery of essential nutrients, possible production of renewable products and energy, and as a reliable water source.

The overall goal of this recent thinking is to make the wastewater treatment plants more sustainable with significant contributions towards a sustainable wastewater utilization system. Besides recovering nutrients and energy, another approach in increasing the sustainability of wastewater treatment plants is to recover poly-hydroxybutyrates (PHB). PHB is an organic polymer synthesized by a wide range of microorganisms under nutrient-limiting conditions (Anderson et al., 1990). Unlike synthetic polymers, PHBs can be used as a base material in the medical, pharmaceutical and chemical industries where biodegradable organic materials are needed. PHB accumulations in pure microbial culture and with a specific clean substrate such as acetate have been studied extensively. For example, Liu et al. (2011) was able to attain 67% PHB content of dry biomass by adding 6.0 g/L sodium acetate in a sequencing batch reactor (SBR). Wendlandt et al. (2005) reported an accumulation of 51% PHB in the biomass by the methanotrophic strain, *Methylocystis* sp. GB 25 DSM 7674, when using methane as a carbon source. Fang et al. (2009) obtained 44% PHB content of the aerobic granules grown in a SBR using sodium acetate, ammonium and phosphates as the carbon, nitrogen and phosphorus sources.

The large amount of municipal wastewater may be a good source of organic carbon for PHB production. However, there are few studies on PHB accumulation and harvesting using municipal wastewater. Some limitations in the use of municipal wastewaters include the low carbon concentration of municipal wastewaters, the heterogeneous mixed culture of municipal wastewaters, and treatment systems currently in place are not designed for PHB accumulation. Over the last 30 years, nutrient discharge limits have become more stringent causing many wastewater treatment plants to incorporate biological nutrient removal (BNR) with anoxic and anaerobic tanks for nutrient removal. BNR plants have the potential to accumulate PHBs and, at the same time, treat municipal wastewater. The price of PHB may be as high as $10/kg (Gurieff and Lant, 2007), and recovery and sale of PHB may help offset the operating and energy costs of the municipal wastewater treatment plants and therefore making the treatment plants more sustainable. For example, Chua et al. (2003) obtained PHB content up to 30% of sludge dry weight using municipal wastewater supplemented with acetate in a SBR. Venkateswar Reddy et al. (2012) obtained PHA accumulation of 39.6% using aerobic mixed culture and fermented food waste as a substrate in a SBR. Coats et al. (2011) used fermented municipal wastewater solids in an SBR to accumulate approximately 28% of poly-hydroxyalkonates (PHA) in the biomass. Surprisingly, the majority of the studies conducted this far are in SBRs rather than in a continuous flow wastewater treatment system.

A recent treatment technology, membrane bioreactor (MBR) which operates with high biomass concentration (10,000 – 12,000 mg/L) as compared to conventional activated sludge system (3,000 – 4,000 mg/L), has the potential for accumulation and

harvesting of PHBs. Even though PHB accumulation potential in activated sludge systems is known, there remain issues to be overcomed in order to optimize PHB accumulation and at the same time treat the wastewater to regulatory limits and, if required, reuse the treated wastewater. There is a need to understand the treatment plant operating conditions and the response of the plant to PHB accumulation for low carbon content conditions such as municipal wastewater (about 100 – 200 mg/L as C) and the need for additional carbon source. For example, acetate addition can improve PHB growth, but the feeding path and optimal concentration of acetate is still unresolved for MBRs. Since there are many industrial wastewaters with high carbon concentrations that need treatment, these industrial wastewaters can be used as a source of additional carbon. However, the presence of nitrogen, phosphorus and toxic compounds in the industrial wastewater may affect the overall treatment efficiency of the municipal wastewater treatment plant.

The goal of this study is to determine the accumulation of PHBs in MBRs by adjusting the operating conditions and by adding external carbon source to make the treatment system more sustainable. The specific objectives of the study are:

1. Determine the impact of three different carbon sources (acetate, fermented supernatant of activated sludge and corn stillage) and their carbon concentrations on PHB accumulation,
2. Determine the influence of feeding location of additional carbon on PHB accumulation (comparison of feeding to anoxic or anaerobic tank).

The results of this study, combined with future work, can be utilized to improve the sustainability of wastewater treatment plants through the production and harvesting of PHB.

3.2 Methods and Materials

3.2.1 Membrane bioreactor

A bench-scale membrane bioreactor consisting of three separate tanks: anaerobic, anoxic and aerobic was set up as shown in Figure 3.1. The anaerobic tank is cylindrical shaped with a diameter of 6 inches and a total volume of 12 L. The actual working volume of the tank was 2 L when additional carbon was added to anoxic tank and 4 L when additional carbon was added to anaerobic tank. The cover of the anaerobic tank was greased to help seal the tank and maintain anaerobic condition. The anoxic tank is also cylindrical shaped with a diameter of 8 inches and a total volume of 12 L. The actual working volume for anoxic tank was 4 L. Magnetic stirrers were employed for both anaerobic and anoxic tank to keep the solids completely mixed. A 12 L rectangular reactor (20 cm length x 12 cm width x 50 cm depth) with a working volume of 8 L was used as aerobic tank with an HRT of 8 hours for a flowrate of 1 L/hr. An air diffuser was installed at the bottom of the tank to provide air and mixing (dissolved oxygen concentration was maintained > 2 mg/L). A flat plate framed membrane manufactured by Kubota Co., Japan was installed in the aerobic tank. Specifications of the flat plate membrane are provided in Table 3.1. The reactors were operated at a room temperature of $23.8 \pm 1.2\ ^{o}$ C.

Synthetic wastewater was pumped into the anaerobic tank from a 20 L container kept in a refrigerator at 4 to 5 ^{o}C. The composition of the synthetic wastewater is given in Table 3.2 where the COD was about 500 mg/L. The synthetic wastewater was pumped at a feeding rate of 1 L/hr. Wastewater flowed by gravity from the anaerobic tank to the anoxic tank and to the aerobic tank. Polypropylene tubes (6.4 mm diameter) were used to connect between the anaerobic, anoxic and aerobic tanks. The treated effluent was extracted through the membrane by a pump which was operated in a cycle of 10 minutes of pumping and 2 minutes of idle.

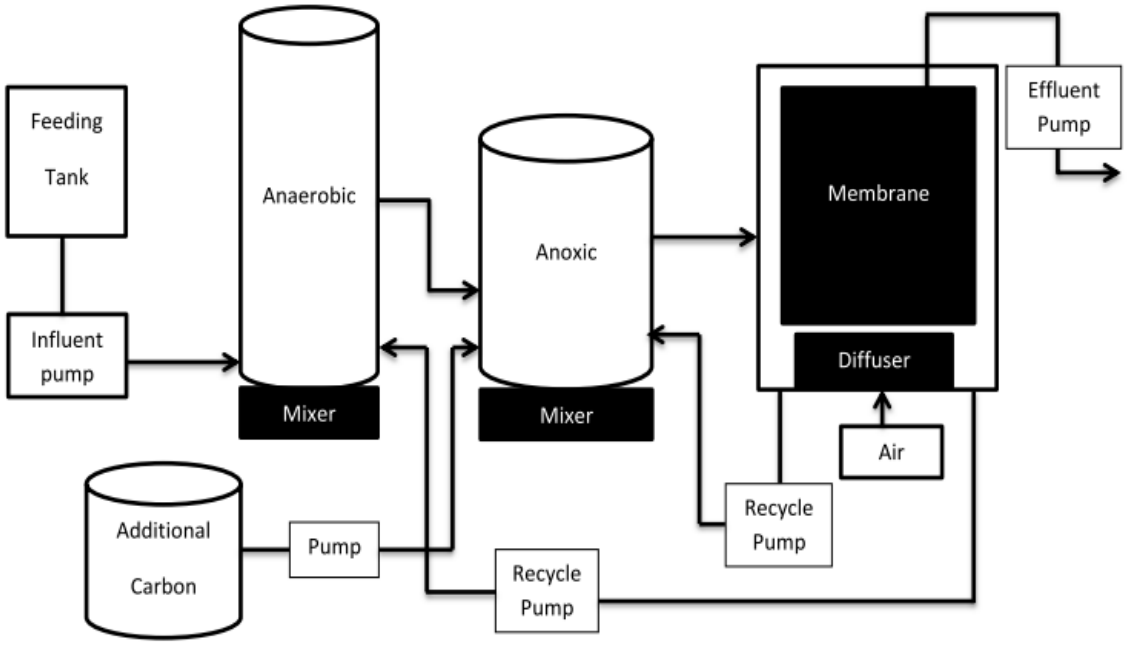

Fig. 3.1 A_2O MBR system process diagram (modified from Brown et al., 2011)

Table 3.1 Membrane filter specifications (Brown, 2007)

Parameter	Specification
Module Configuration	Plate-frame
Membrane Material	Cellulose
Pore Size	0.2 μm
Membrane Porosity	60% volume
Dimensions (width × thickness × height)	23 cm × 1 cm × 31 cm
Total Filtration Area	0.15 m^2
pH Range	5.5 - 10
Maximum Temperature	80 ^{o} C
Maximum Pressure	25 kPa

Table 3.2 Synthetic wastewater composition (adapted from Brown et al., 2011)

	Chemicals/Parameters	Concentration (mg/L)
Ingredients	Calcium sulfate	40
	Ferric chloride	3
	Isomil (Simulac^{TM})	20 mL (1% by volume)
	Magnesium sulfate	4
	Nutrient broth*	250
	Potassium chloride	5
	Sodium bicarbonate	63
	Sodium biphosphate monobasic	60
	Sodium citrate	500
Final Composition	Soluble COD (mg/L)	485.8
	Suspended solids (mg/L)	22.8
	Total nitrogen (mg/L-N)	48.2
	Nitrate (mg/L-N)	0.36
	Ammonia (mg/L-N)	24.2
	Total phosphorus (mg/L – P)	16.2
	pH	7.1

*Difco^{TM} nutrient broth (REF: 234000), Becton, Dickinson and Company, Sparks, MD

3.2.2 Operation of the treatment plant

The activated sludge used to seed the aerobic reactor was obtained from the aeration tank of the water pollution control center in Boone, Iowa. The average total suspended solids of the sludge added were 2,600 mg/L. The aerobic reactor was initially operated as a batch reactor for 8 hours. The sludge was allowed to settle and 3 L of supernatant was removed and a similar volume of synthetic wastewater was added. This was repeated for 6 cycles to allow the sludge to acclimatize to the synthetic wastewater. After that the membrane bioreactor was operated in a continuous feeding mode with wastewater fed into the anaerobic tank along with recirculation of the mixed liquor from the aerobic tank to the anaerobic and anoxic tanks. The HRT for the anaerobic and anoxic tanks were fixed at 2 and 4 hours and the HRT of the aerobic tank was fixed at 8 hours as per Brown et al. (2011) who found that these HTRs were optimal for this bench-scale MBR. The SRT in the aerobic tank was maintained at 25 days by manually wasting mixed liquor from aerobic tank each day. Recirculation ratio was varied to maximize the nutrient removal efficiency. The recirculation from the aerobic tank to the anoxic tank varied from 100 – 300% of the influent flowrate while the recirculation from the aerobic to the anaerobic tank was kept at 100% of influent flowrate. After the best recirculation rate was found, the HRT for anoxic tank and aerobic tank were reduced to half of the previous HRT by doubling the influent flowrate to 2 L/hr to see the change in COD and nutrient removal efficiency. Wastewater samples were collected from the feed tank, anaerobic tank, anoxic tank, aerobic tank and the final effluent after membrane separation. Based on the nutrient removal efficiency, HRTs and the recirculation ratios for the A_2O

MBR system were chosen for subsequent PHB accumulation studies. The percent of PHB in the biomass of each tank of the MBR was measured.

3.2.3 PHB growth experiments

To grow and harvest PHB in the MBR, experiments were conducted by adding three types of carbon sources along with the synthetic wastewater. The added carbon sources were: sodium acetate, supernatant from fermented sludge, and thin corn stillage liquid. The compositions of the three carbon sources are given in Table 3.3. For the acetate, experiments were conducted by pumping continuously the acetate solution (concentrations of 100, 500, 700, 900, 1000 mg/L-C) to the anoxic tank at a feeding rate of 1 L/hr. The reactor was operated for 3 days or longer to steady state conditions which was indicated by fairly constant COD removals for three measurements. At steady state conditions, samples from each tank and permeate were taken and measured for PHB, TN, nitrate, TP, and suspended solids. The injection point was then changed to the anaerobic tank with the same acetate feeding rate of 1 L/h and the same concentrations (see Table 3.4). At steady state, samples from each tank and permeate were taken and measured for the water quality parameters mentioned earlier. The impact of continuous addition of acetate at these two injection points was compared. For the supernatant from the fermented sludge, the supernatant was added to the anoxic or the anaerobic tank with a carbon concentration of 100 and 500 mg/L-C (based on the volatile fatty acids (VFAs) concentration which was assumed to be mainly made up of acetate) (Table 3.4). The flowrates used were 1 L/hr. In the case of corn stillage, the stillage was also added to the

anoxic or anaerobic tank with a carbon concentration of 100 and 500 mg/L-C (based on VFAs concentration which was assumed to be mainly made up of acetate) at a flow rate of 1 L/hr. All experiments were operated to steady state before samples from each tank and the permeate effluent were collected and analyzed.

Table 3.3 Compositions of added carbon sources

	Concentration		
Parameter	Acetate [a]	Supernatant (Fermented sludge)	Corn [b] Stillage
COD (mg/L)	Vary with concentration	2,186	44,500
Soluble COD (mg/L)	Vary with concentration	1,862	24,800
Volatile fatty acid (VFA) (mg/L)	Vary with concentration	1,120 ± 83	600
Suspended solids (mg/L)	1.3	14 ± 2	27,500
Total Nitrogen (mg/L)	0	69	3,600
Nitrate (mg/L as N)	0	15 ± 3	300
Ammonia (mg/L as N)	0	22 ± 3	40
Total Phosphorus (mg/L as P)	0	52	2,190
pH	N/A	5.3-6.6	4.5 – 6.7

[a] Carbon to COD ratio (C: COD) = 1: 1.6, N/A – not applicable
[b] Thin stillage from an ethanol plant named Lincolnway Energy, LLC. Nevada, IA

Table 3.4 Summary of feeding conditions to anoxic or anaerobic tank

Carbon Source	Concentration (mg/L-C)	Flowrates (L/hr)
Acetate	100, 300, 500, 700, 900, 1000	1
Supernatant from fermented sludge	100, 500	1
Corn Stillage	100, 500	1

3.2.4 Laboratory analysis

COD and soluble COD tests were conducted according to Method 5220 of Standard Methods (APHA, AWWA and WEF, 2005). TN, TP, NO_3^- - N, and NH_3-N were tested using test kits from Hach Company (Loveland, CO). The Hach methods are presented in Table 3.5. The concentrations of VFA in the supernatant of centrifuged samples of fermented sludge and corn stillage were analyzed using the distillation method (Method 5560) of Standard Methods (APHA, AWWA and WEF, 2005). The fermented sludge and corn stillage were centrifuged at 2000 × g and a sample volume of 100 mL was used in the VFA analysis.

The percent of PHB in the biomass was measured using a gas chromatography (GC) method proposed by Comeau et al. (1988). In this method, 20 mL of activated sludge or biomass samples were collected from each tank. The samples were first centrifuged at 1500 × g and the thickened biomass was transferred to 5 mL vials. The biomass in the vials was frozen in the freezer and then lyophilized by a 12 vial freeze-dry machine (Model FD-3-54, SP Scientific, Stone Ridge, NY). The rated vacuum pressure of the freeze-dry machine was 20 millitorr with a low temperature of - 40 ^{o} C. Ten mg of dried biomass was weighted and transferred to a 10 mL test tube, and 2 mL of acidified methanol (3% H_2SO_4) and 2 mL of chloroform were added. Poly[R-3-hydroxybutyric acid] combined with 2 mL of acidified acid and 2 mL of chloroform were used as the standard. The test tubes were capped and heated at 105 ^{o} C in a digestion reactor for 2 hours. After digestion, 5 mL of deionized water was added to the test tubes to extract the acids and particulate debris. The tubes were then shaken for 1 minute and centrifuged for 5 minutes at 2000 rpm. The dense chloroform phase containing the PHB was transferred

to a GC vial for split injection of a 1 µL sample into an Agilent Technologies Model 6890 Gas Chromatography/Mass Spectrometer (Agilent Technologies, Santa Clara, CA) equipped with a programmable autosampler and an Agilent 190915-433 column. The injection temperature was 220 ^{o}C. The temperature program of the GC was 45 ^{o}C for 2 minutes, followed by a ramp of 5 ^{o}C/min to 65 ^{o}C for 4 minutes, and a final ramp of 50 ^{o}C/min to 320 ^{o}C for 3 minutes. The total time of the GC run was 18.1 minutes.

Table 3.5 Hach methods used

Parameters	Hach Method Number
Total Nitrogen (TN)	10072
Total Phosphorus (TP)	10127
Ammonia Nitrogen (NH_3-N)	10031
Nitrate Nitrogen (NO_3^--N)	10020

3.3 Results and Discussion

3.3.1 Nutrient removal and PHB accumulation for various operating conditions

Results of the treatment plant for various recirculation ratios are shown in Table 3.6. For HRTs of 2 hours, 4 hours and 8 hours for anaerobic, anoxic and aerobic tank, the percent removal of phosphorus increased and then decreased with the percent of mixed liquor recycle to the anaerobic tank. sCOD appeared to be similar for all recycle conditions while the percent nitrogen removal were similar for 300% and 500% recycle of the mixed liquor to the anaerobic tank. Based on the conditions tested, recycle of mixed liquor at 300% to the anaerobic tank and 100% to the anoxic tank appeared to

provide the highest nitrogen percent removal and phosphorus percent removal. The nitrogen and phosphorus concentration in the effluent for this operating condition were 5.5 mg/L and 3.3 mg/L. PHB concentrations in the biomass in anaerobic, anoxic and aerobic tank were all less than 1%. The hydraulic retention times were then changed to 2 hours, 2 hours and 4 hours but the recirculation ratios were kept as 300% to the anaerobic tank and 100% to the anoxic tank. Under this condition, nitrogen percent removal and phosphorus percent removal were comparable to the previous conditions. The average soluble COD of the effluent for this condition was 28 mg/L and the lowest nitrogen and phosphorus concentration in effluent was 7.9 mg/L and 3.8 mg/L. The initial PHB accumulated in anaerobic, anoxic and aerobic tank were still less than 1%. Based on the operating conditions above, subsequent experiments were conducted using hydraulic retention times of 2 hours, 2hours and 4 hours, and recirculation ratios of 300% of the influent to the anaerobic tank and 100% of the influent flow to the anoxic tank.

Table 3.6 Nutrient removal for various recirculation ratios and HRT conditions

Anaerobic tank recycle (equivalent to influent flowrate)	Anoxic tank recycle (equivalent to influent flowrate)	Anaerobic, anoxic, aerobic HRTs (hrs)	sCOD removal (%)	Total nitrogen removal (%)	Total phosphorus removal (%)
100%	100%	2, 4, 8	96.6	77.6	60.7
300%	100%	2, 4, 8	96.2	88.2	79.8
500%	100%	2, 4, 8	95.7	89.6	72.3
300%	100%	2, 2, 4	94.5	82.4	76.2

3.3.2 Addition of acetate on proportionally PHB accumulation and nutrient removal

Figure 3.2 shows the steady state concentration of PHB in the biomass of the three tanks of the MBR for the addition of acetate to the anaerobic or anoxic tank while Figure 3.3, 3.4 and 3.5 provide the effluent concentrations of sCOD, TN and TP, respectively, for the anaerobic, anoxic and aerobic tanks. PHB accumulation in the anaerobic tank was found to increase from 0.8% to as high as 10.7% of dry biomass weight when the concentration of acetate added to anaerobic tank changed from 0 to 1,000 mg-C/L at a flowrate of 1 L/hr (Figure 3.2a). PHBs in the anoxic and aerobic tanks were about 2% lower than the PHB concentrations in the anaerobic tank. An average PHB of 8.3% dry biomass weight was detected in aerobic biomass when 1000 mg-C/L acetate was added. This may be due to the excessive carbon available in the aerobic tank that the phosphorus accumulating organisms (PAOs) need not use the stored PHBs.

Adding acetate to the anoxic tank increased the PHB presented in the biomass (Figure 3.2b). The PHB concentration was 10.9% of dry biomass for the addition of 1000 mg-C/L acetate to the anoxic tank. Using the PHB concentrations in the anaerobic and anoxic tanks for various acetate concentration, a student's t-test was conducted yielding a Prob < t of 0.1487 which is greater than 0.05. This means that there was no significant difference between addition of acetate in the anaerobic tank or in the anoxic tank. However, when the acetate added to the anoxic tank was at 500 mg-C/L, the PHB in the biomass of the aerobic tank was close to zero percent. This shows that addition of acetate into the anoxic tank needs to be 500 mg-C/L or more in order to have excess carbon for the PAOs not to use their stored PHBs. As expected, the effluent sCOD increased with an increase in the acetate concentration added (Figure 3.3). Chua et al. (2003) showed that

PHA accumulation as high as 20% of biomass dry weight was possible by using only municipal wastewater in a anaerobic/aerobic SBR. Addition of 30 mg-C/L acetate increased the PHB content to about 30% in a separate batch reactor. Coats et al. (2011) found that the PHA concentration varied from 12.2% to 28% in an SBR by combing sludge from a bench-scale activated sludge system and fermenter supernatant. In our experiments, the lower amount of PHB accumulated may be due to the continuous flow system of the MBR as opposed to a SBR where a feast-and-famine condition can be better maintained and controlled.

Nitrogen removal was as high as 98% when 1000 mg-C/L acetate was added to anoxic tank (Figure 3.4). Similarly phosphorus removal was up to 92% for 1000 mg-C/L of acetate added (Figure 3.5). Adding acetate to the anaerobic tank resulted in better phosphorus removal than adding to the anoxic tank (Figure 3.5c). Phosphorus in the effluent was reduced to as low as 0.3 mg/L with a 98% removal. Higher TN removal was obtained when the acetate was added to the anoxic tank while higher TP removal was obtained when the acetate was added to the anaerobic tank. In general, addition of carbon improved nutrient removal.

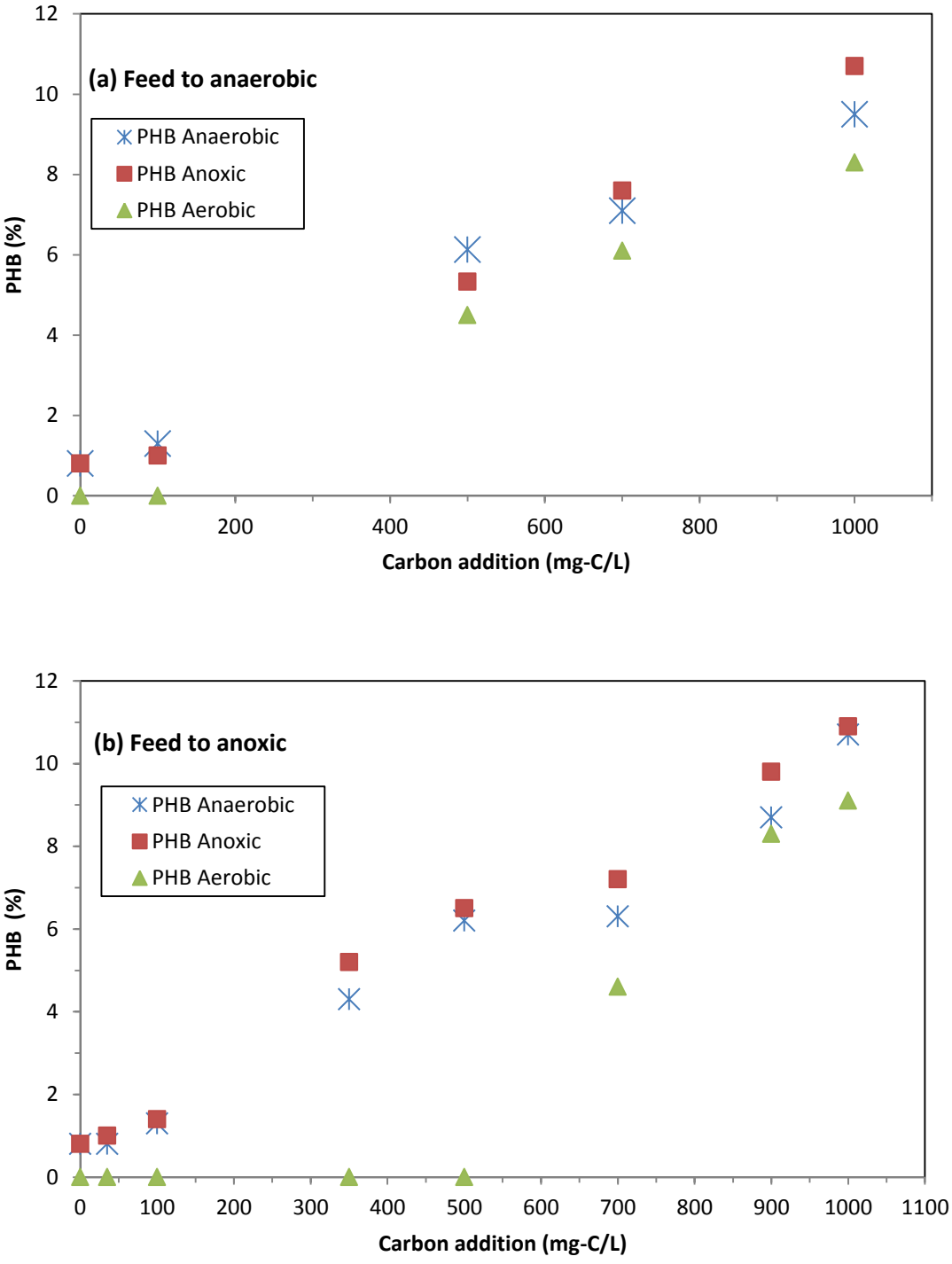

Figure 3.2 Impact of acetate addition on PHB accumulation, (a) feed to anaerobic tank and (b) feed to anoxic tank

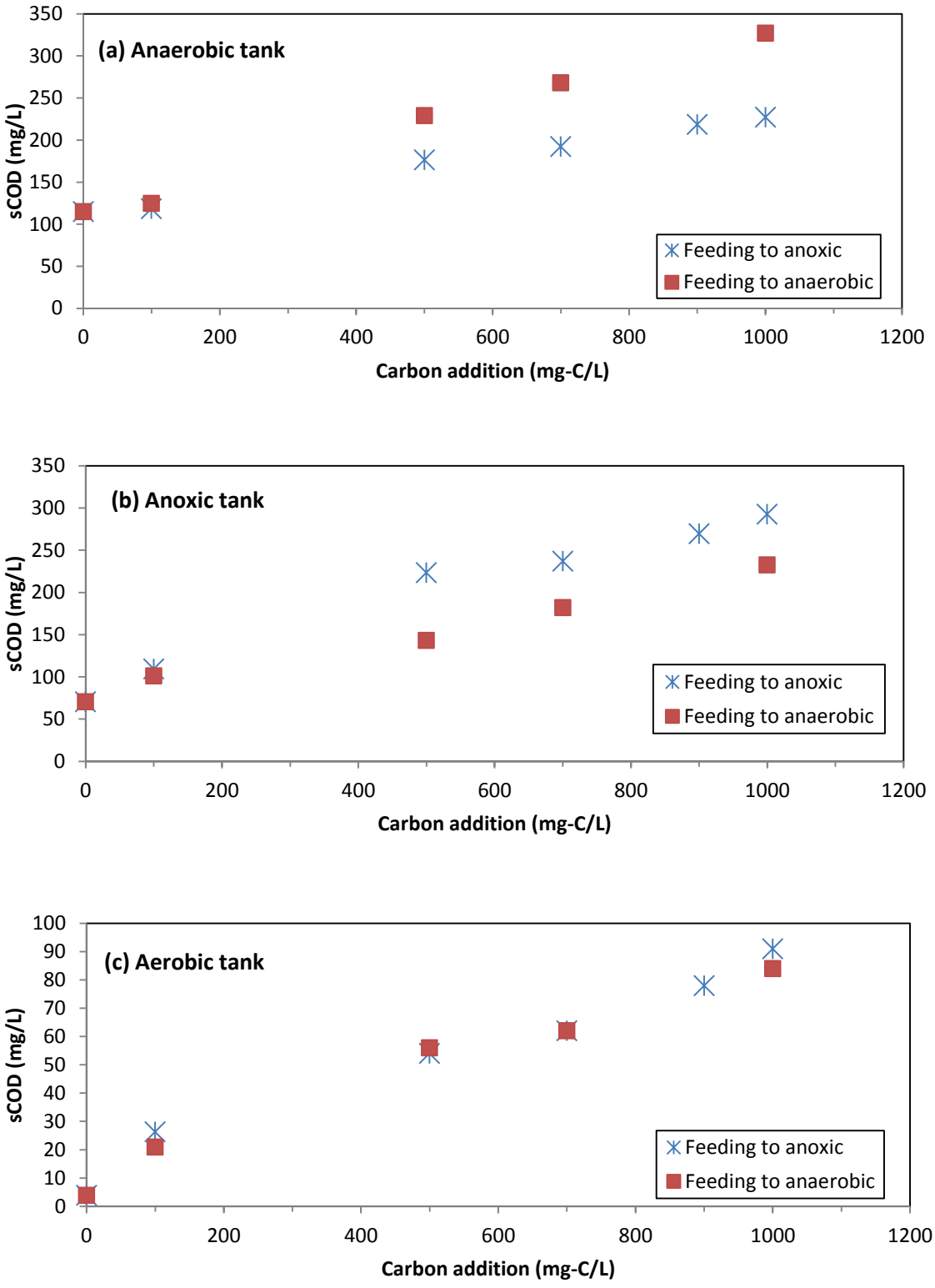

Figure 3.3 Impact of acetate addition on effluent soluble COD, (a) anaerobic tank, (b) anoxic tank, and (c) aerobic tank

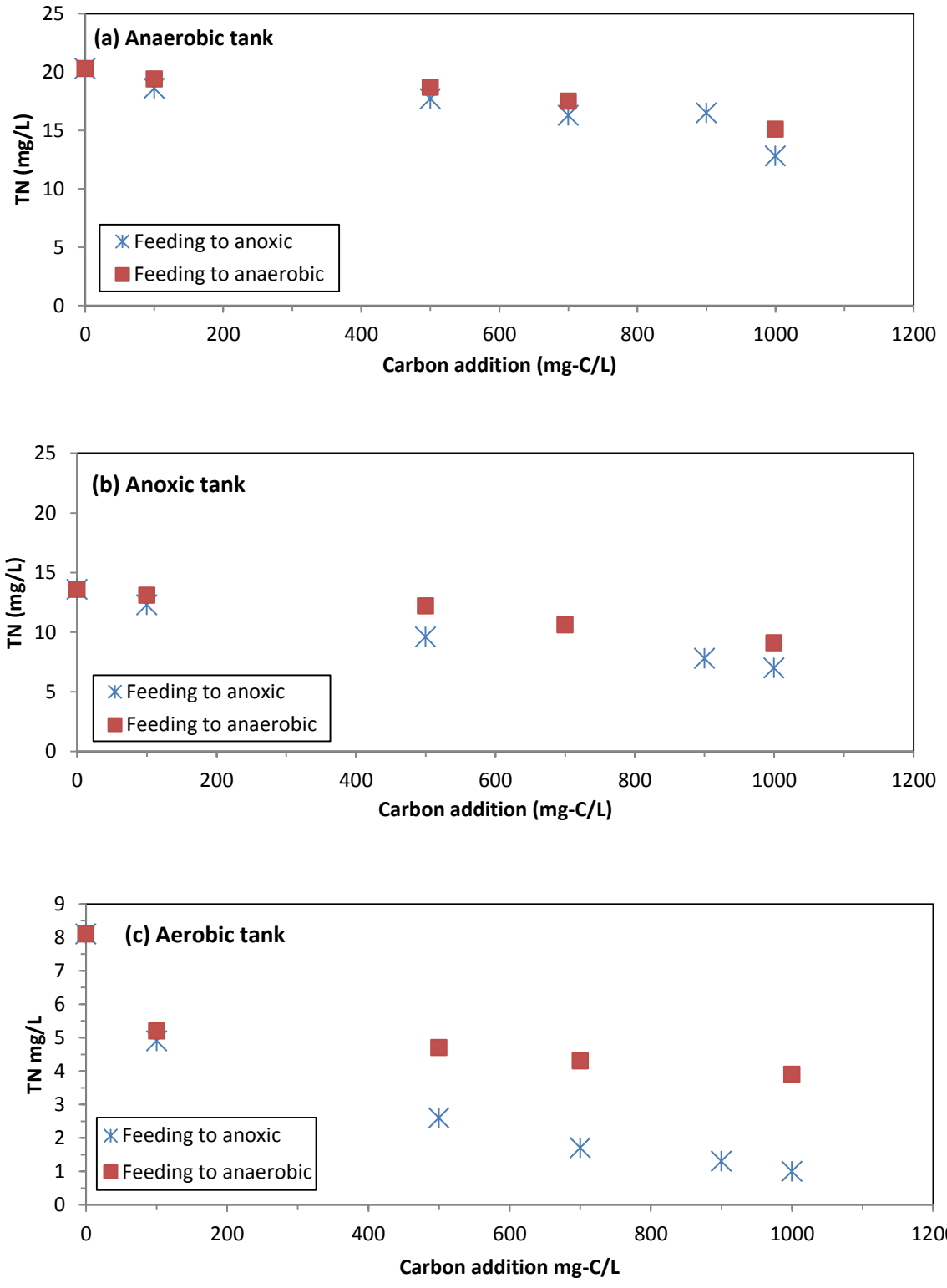

Figure 3.4 Impact of acetate addition on effluent TN, (a) anaerobic tank, (b) anoxic tank and (c) aerobic tank

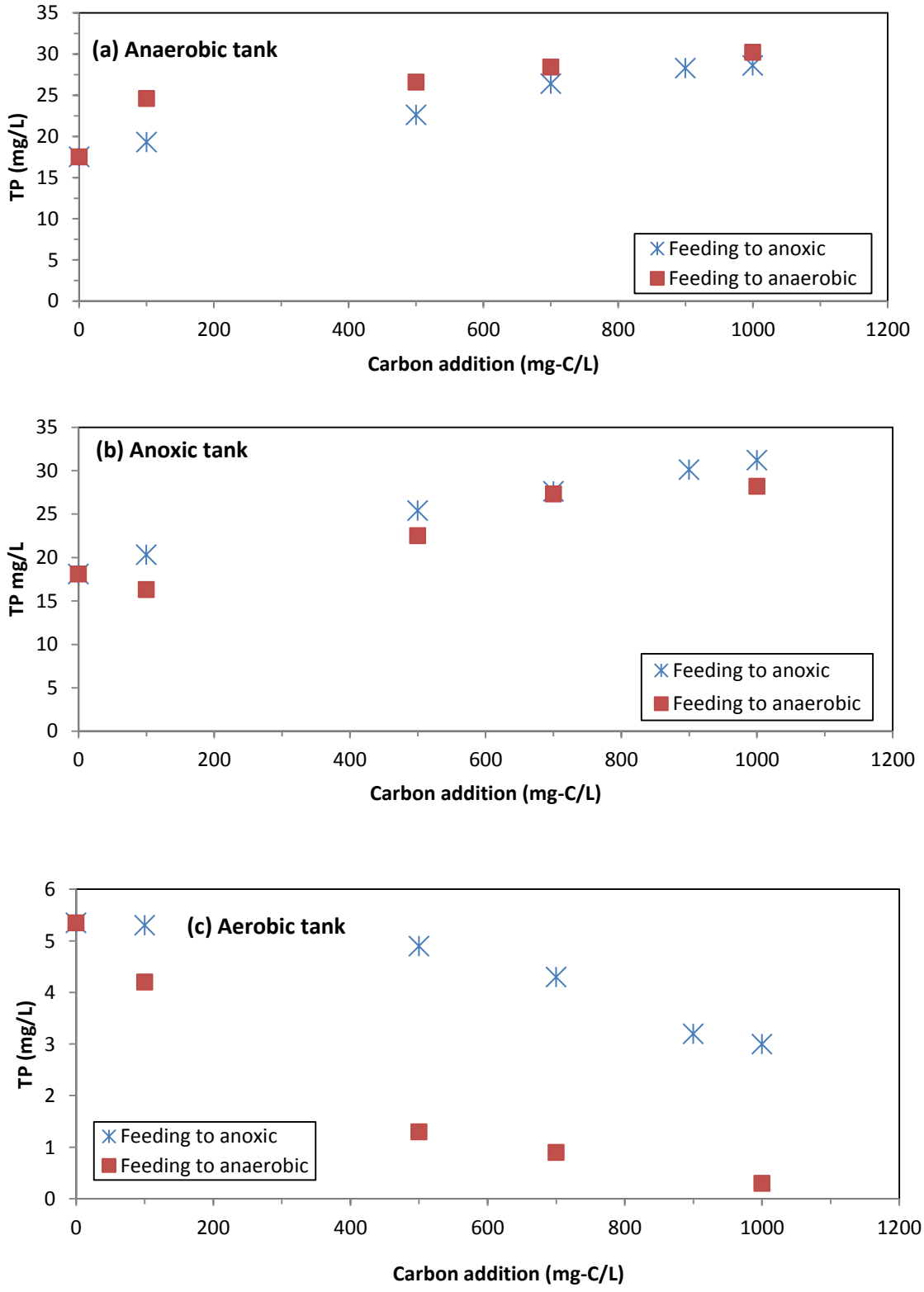

Figure 3.5 Impact of acetate addition on effluent TP, (a) anaerobic tank, (b) anoxic tank, and (c) aerobic tank

3.3.3 Addition of supernatant of fermented sludge on PHB accumulation and nutrient removal

Figure 3.6 showed that PHB accumulation increased from 0.8% to 4.2% of dry biomass weight when supernatant of fermented sludge with 500mg-C/L was added to the anaerobic tank at a flowrate of 1 L/hr. PHB in the biomass of aerobic tank was barely detectable. Adding the supernatant to anoxic tank gave PHB content of about 2.2% of biomass dry weight in the anaerobic tank which was about half of the PHB concentration when the supernatant was fed to anaerobic tank (Figure 3.6). This may be due to the nitrate in the supernatant which may have resulted in the carbon being used for denitrification and simultaneous growth of bacteria. Ciggin et al. (2009) showed that PHB formation and storage yield were reduced with increasing influent nitrate concentration. Figure 3.7 presents the sCOD concentrations in the effluent of each tank.

Figure 3.8 and 3.9 showed that the concentrations of TN and TP, respectively, in the effluent increased with an increase in the carbon concentration in the supernatant. The increase in the nitrogen and phosphorus concentrations in the effluent is probably due to the higher TN and TP concentrations in the supernatant. Using student's t-test, the percent PHB in the anaerobic and anoxic tanks were found to be significantly different ($Prob < t = 0.0001$) for the addition of the supernatant to the anaerobic tank and to the anoxic tank. There were no differences in the effluent TN and TP except for the TN concentration in the effluent of aerobic tank (Figure 3.8). One possible reason is the higher denitrification rate in the anoxic tank when the supernatant was added to the anoxic tank.

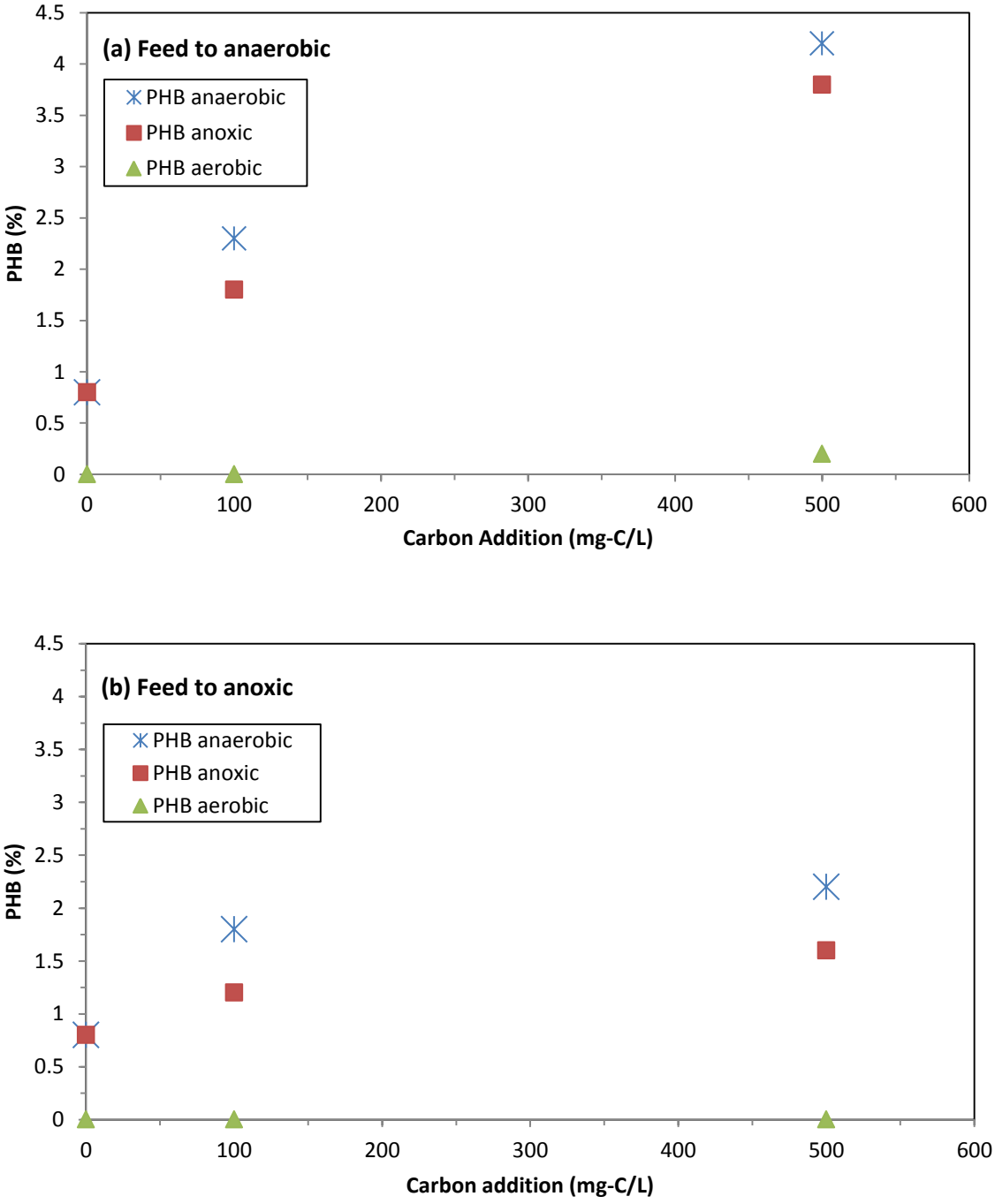

Figure 3.6 Impact of adding fermented sludge on PHB accumulation, (a) feed to anaerobic tank and (b) feed to anoxic tank. (Note: carbon added were based on VFA concentration in the supernatant, the total carbon in the supernatant was higher)

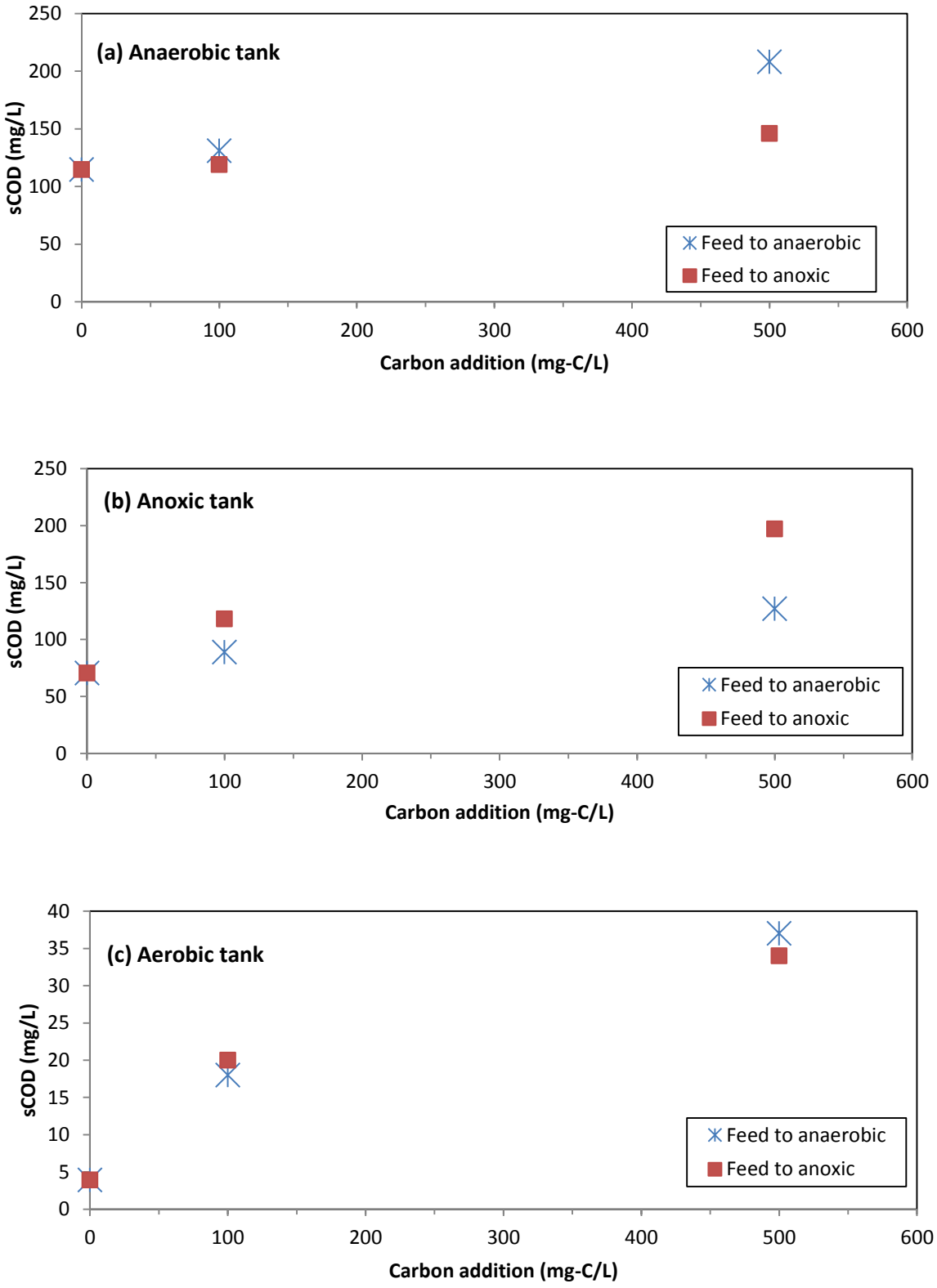

Figure 3.7 Impact of adding supernatant of fermented sludge on effluent sCOD, (a) anaerobic tank, (b) anoxic tank and (c) aerobic tank

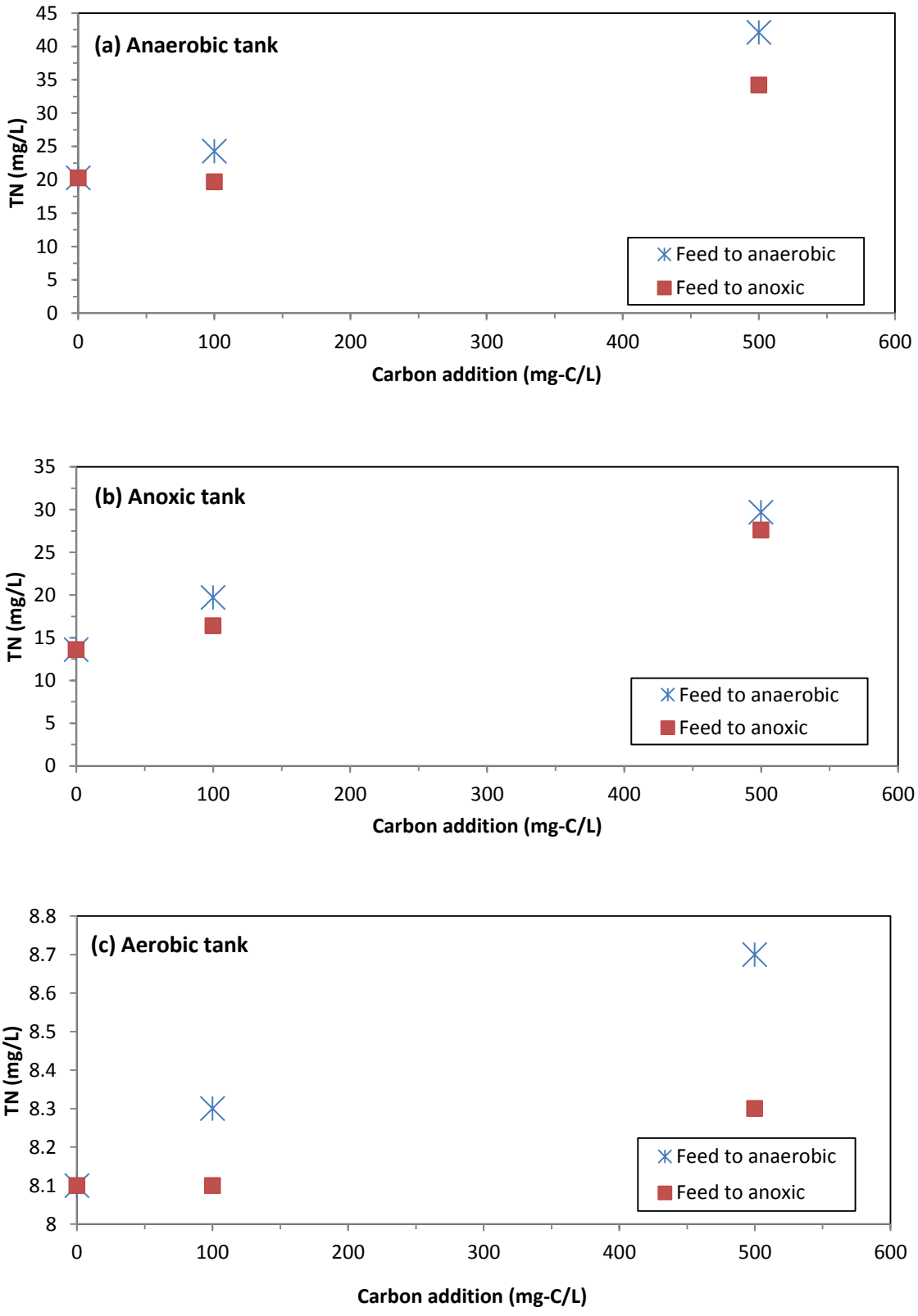

Figure 3.8 Influence of adding supernatant of fermented sludge on effluent TN, (a) anaerobic tank, (b) anoxic tank and (c) aerobic tank

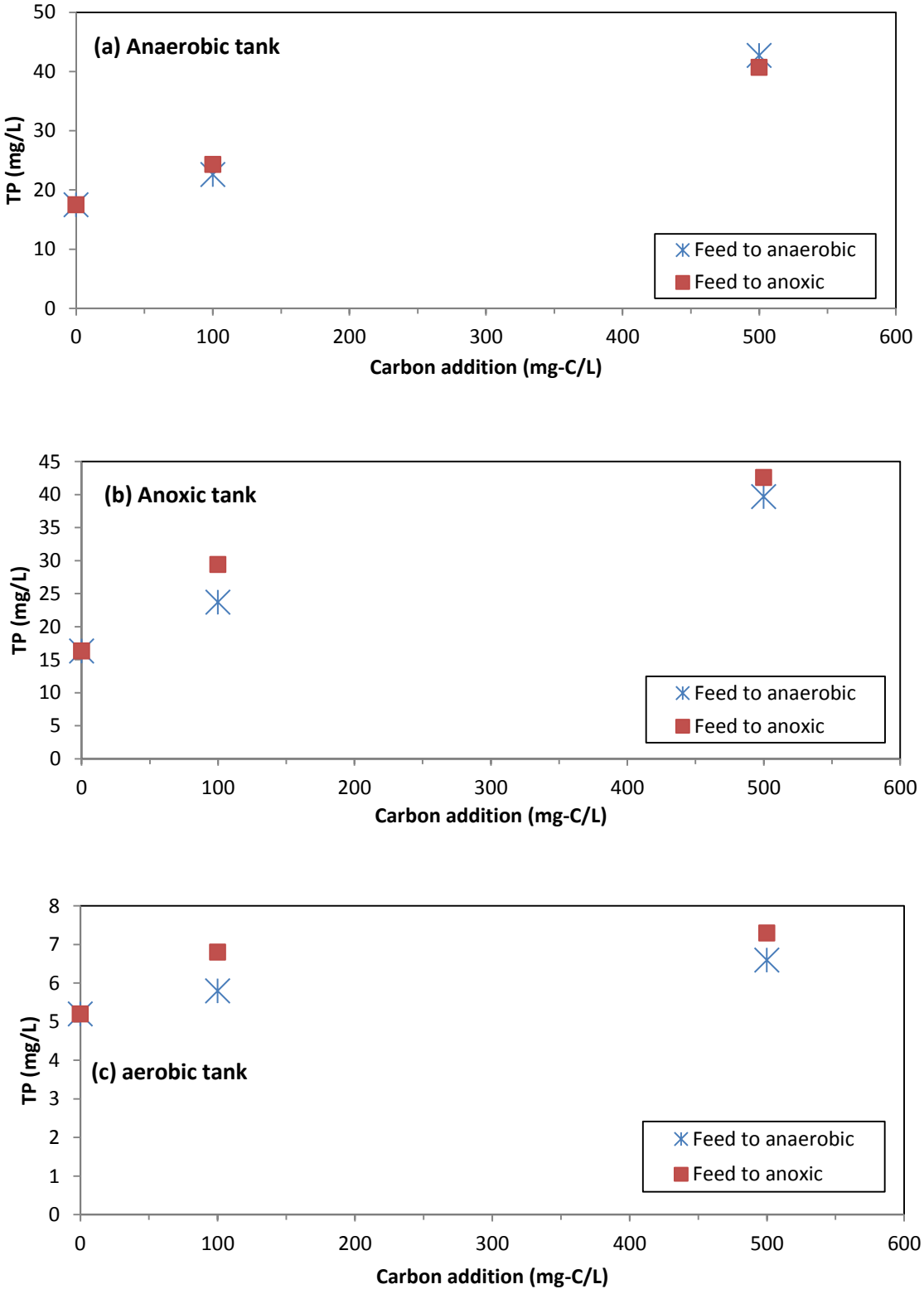

Figure 3.9 Impact of adding supernatant of fermented sludge on effluent TP, (a) anaerobic tank, (b) anoxic tank and (c) aerobic tank

3.3.4 Addition of thin corn stillage on PHB accumulation and nutrient removal

Figure 3.10 shows the percent PHB contents for the addition of corn stillage to the anaerobic tank or the anoxic tank. Despite the high concentration of nitrogen and phosphorus in thin corn stillage, the percent of PHB in the biomass was in the range of 7% for addition of 500 mg-C/L (based on VFA concentration) to anaerobic tank (Figure 3.10). PHB was also detected at about 5% in aerobic biomass. A lower percent of PHB (between 2 to 3%) was obtained for addition of the corn stillage to the anoxic tank (Figure 3.10b). The percent PHB in aerobic biomass was close to zero for the addition of thin corn stillage to the anoxic tank. Based on Figure 3.10, the two feeding locations resulted in different percent of PHB accumulation. The difference was probably caused by the high concentration of organic carbon other than VFAs, nitrogen and phosphorus in the corn stillage that was fed to the system. Because of the high concentration of sCOD, TN and TP, the effluent concentration of these parameters increased accordingly for an increase in the concentration of the thin corn stillage added. The effluent sCOD (Figure 3.11c), TN (Figure 3.12c) and TP (Figure 3.13c) were as high as 400 mg/L, 150 mg-N/L and 500 mg-P/L for an addition of 500 mg-C/L (based on VFA concentration) of the thin corn stillage. These effluent concentrations were far above the typical effluent discharge limits of a municipal wastewater treatment plant.

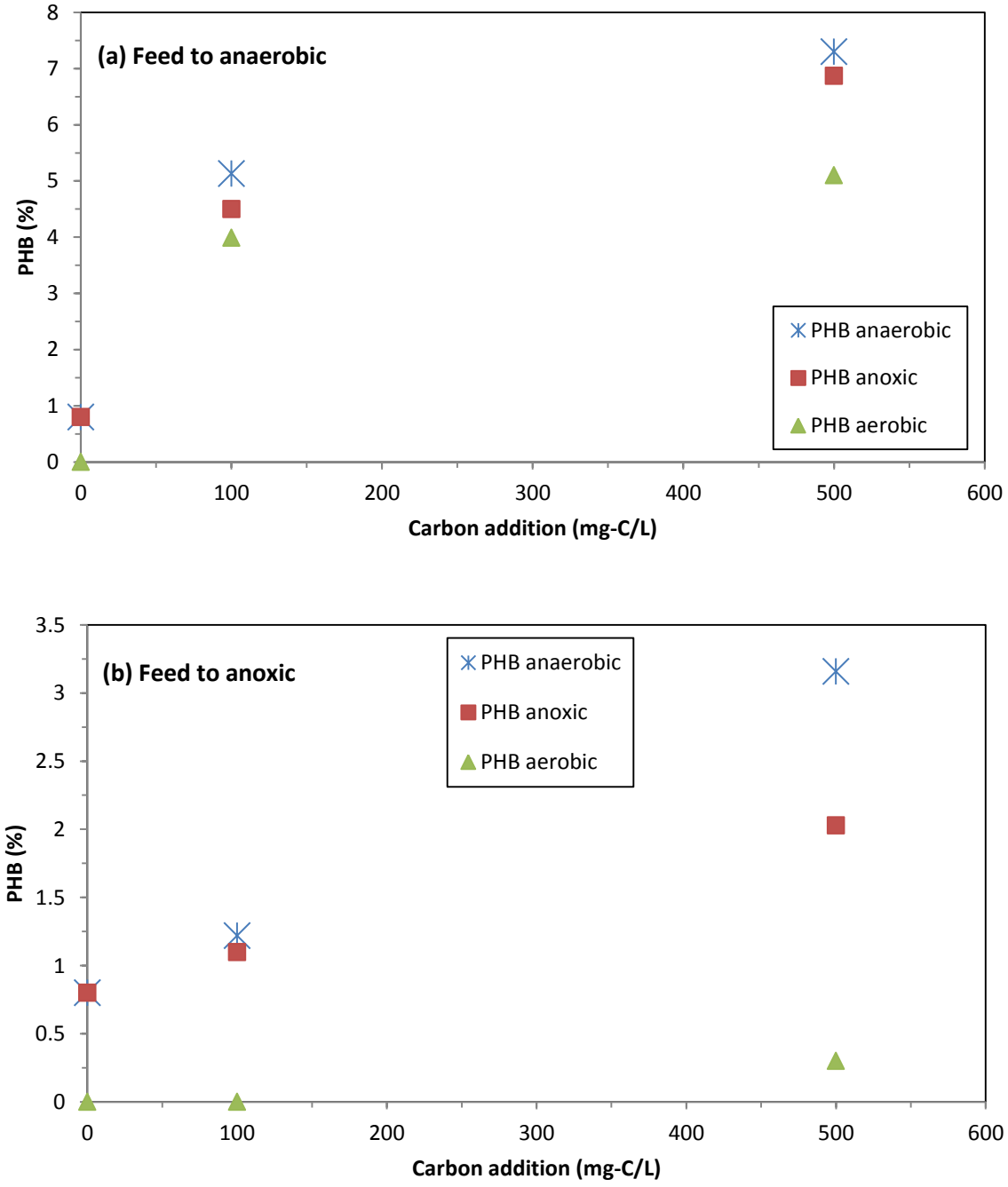

Figure 3.10 Impact of adding thin corn stillage on PHB accumulation, (a) feed to anaerobic tank and (b) feed to anoxic tank (Note: carbon added were based on the VFA concentration in the corn stillage, the total carbon in the corn stillage was higher).

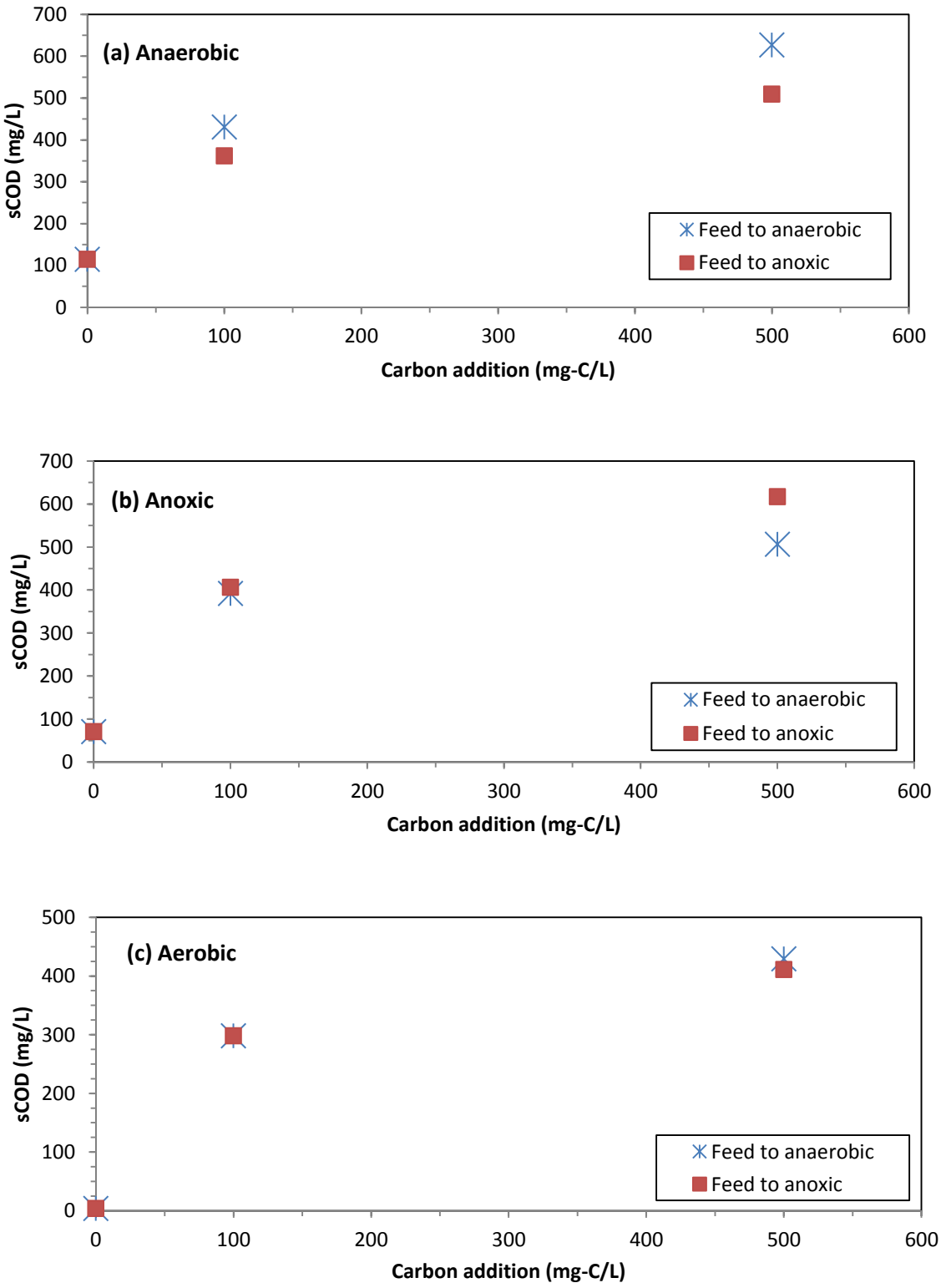

Figure 3.11 Impact of adding thin corn stillage on effluent sCOD, (a) anaerobic tank, (b) anoxic tank and (c) aerobic tank (Note: for sCOD > 500 mg/L the concentrations were out of range of the sCOD test).

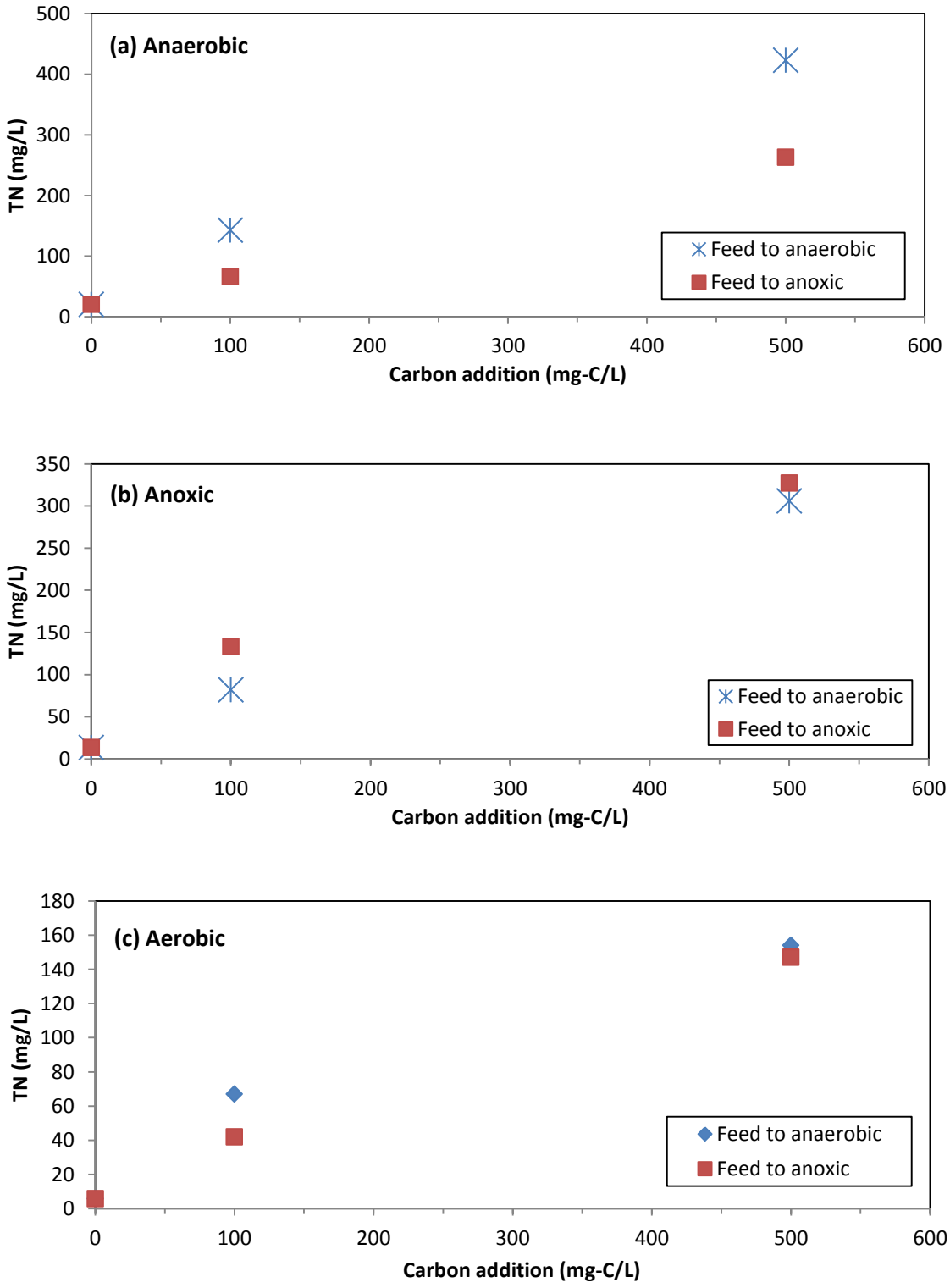

Figure 3.12 Impact of adding thin corn stillage on effluent TN, (a) anaerobic tank, (b) anoxic tank and (c) aerobic tank (Note: for TN > 150 mg/L as N, the concentrations measured were out of range of TN test).

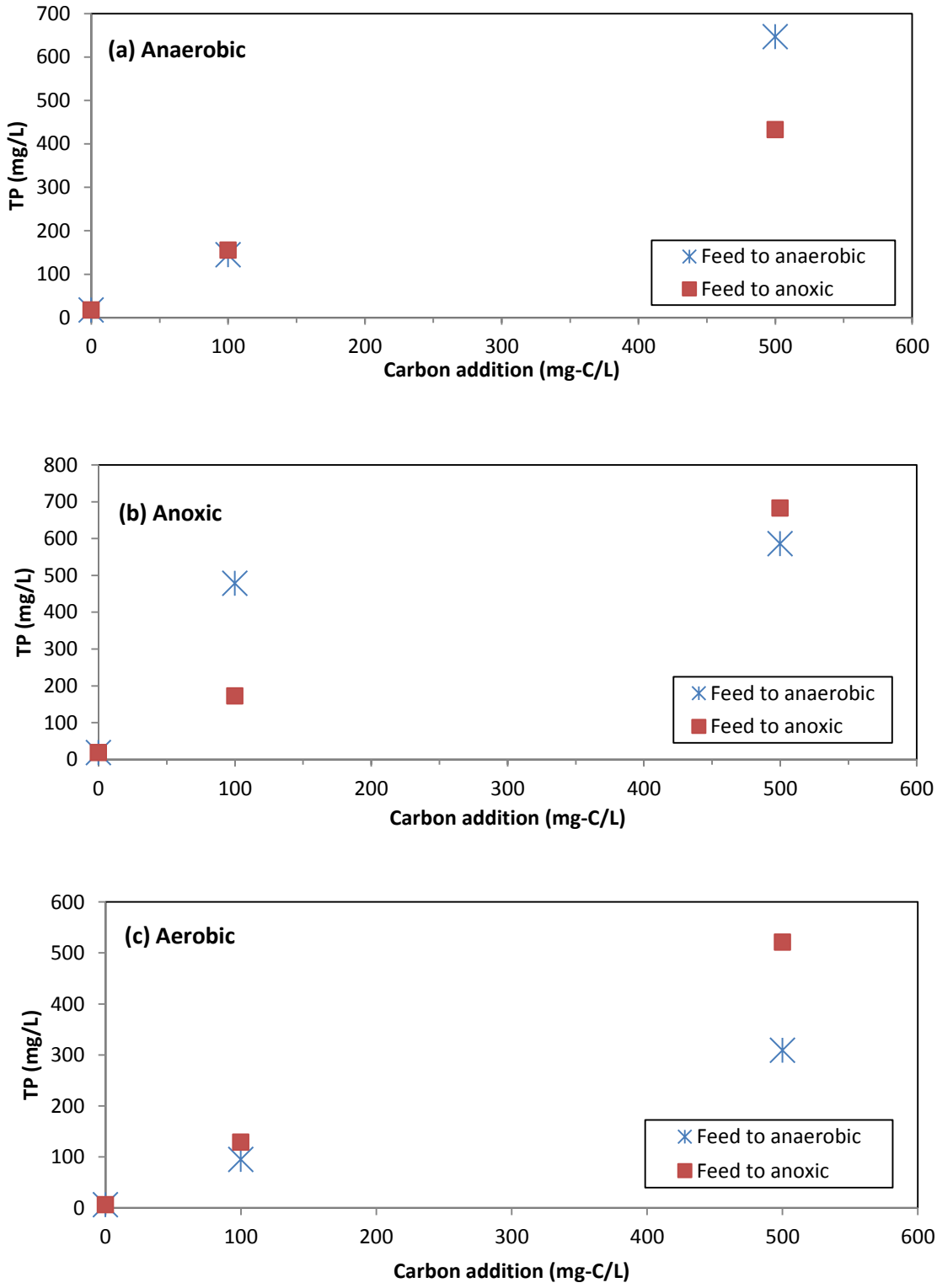

Figure 3.13 Impact of adding thin corn stillage on effluent TP, (a) anaerobic tank, (b) anoxic tank and (c) aerobic tank (Note: for TP > 100 mg/L, the concentrations measured were out of range of the TP test)

3.3.5 Engineering analysis of PHB production for a full-scale system

The location to harvest PHB is the aerobic tank of the system. In a full-scale A_2O membrane bioreactor treating 1 million gallon wastewater per day, to keep an SRT of 25 days, approximately 600 kg of biomass will be wasted every day. According to this study, the highest PHB content (when 1000 mg-C/L sodium acetate was added) in the aerobic biomass was about 9%. Thus, in the wasted 600 kg biomass, there are about 54 kg PHB. As reported by Gurieff and Lant (2007), the current market price for PHB exceeds $10 per kg. Therefore, this sustainable A_2O membrane bioreactor could generate gross annual revenue of approximately $197,100. However the annual cost for purchasing sodium acetate would be approximately $416,000. As a result, the revenue generated by recovery and sale of PHB cannot offset the cost unless PHB content of more than 25% in the aerobic biomass is achieved. Changing the SRT and HRT to improve the operation can increase PHB accumulation. The optimal condition to accumulate PHB in A2O membrane bioreactor is still unresolved, and it is still unknown whether PHB can accumulate more than 25% in the aerobic biomass or not. Future investigation focus on finding the optimal conditions is needed.

3.4 Conclusion

Addition of a clean carbon source such as acetate resulted in an increase in PHB accumulation in the biomass in all three tanks: anaerobic, anoxic and aerobic. The percent PHB (by dry biomass weight) obtained was 10.9% for an acetate addition of 1000 mg-C/L. Addition of acetate to the anaerobic tank or anoxic tank did not make any

difference in PHB accumulation if the carbon concentration added was greater than 500 mg-C/L. For acetate less than 500 mg-C/L, the PHB in the aerobic biomass decreased to about 4.5% and was close to zero for acetate concentrations of 300 mg-C/L..

Addition of supernatant of fermented sludge and thin corn stillage resulted in lower PHB accumulation as compared to addition of acetate. Inferences of high nitrogen (nitrate) and phosphorus were probably some of the reasons for the lower PHB accumulation.

It appeared that municipal wastewater treatment can be made more sustainable by producing PHBs. Although the percent of PHB in the biomass for this study were in the range of 10% (less than other studies), changing the HRT and SRT to improve the operation of the reactors can increase the PHB accumulation.

3.5 Reference

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CHAPTER 4 CONCLUSION

4.1 Conclusion

Conventional wastewater treatment plants such as activated sludge and MBRs consume large amounts of energy to treat the wastewater and at the same time remove nutrients and carbon with minimal recovery. In addition, the sludge produced requires further disposal. To make the wastewater treatment plants more sustainable, efforts are being made to minimize energy used, recover energy and nutrients or produce and harvest organic by-products such as PHBs. Because of their consistent and continuous source of carbon, municipal wastewater and industrial wastewater may be used for the PH production. However, operations of municipal wastewater treatment plants are not optimized to produce PHBs. In Chapter 3, it was determined that clean carbon source such as acetate resulted in an increase in PHB in the biomass in all three tanks of an A_2O membrane bioreactor. PHB content of 10.9% of dry biomass weight was obtained for an acetate addition of 1000 mg-C/L. It was found that high carbon concentration (> 500 mg-C/L) did not result in PHB consumption in the aerobic biomass. There was no significant difference in the PHB content of the biomasses in the three tanks. This means that PHB may be harvested from all three tanks.

Addition of supernatant of sludge and thin corn stillage resulted in lower PHB accumulation as compared to addition of acetate. Inferences of high nitrogen (nitrate) and phosphorus were probably some of the reasons for the lower PHB accumulation. With the supernatant as the carbon source, TN and TP removal were at levels similar to that without the addition of the acetate. Both TN and TP concentrations in the aerobic effluent

were less than 10 mg/L. This result indicated that PHB can be accumulated without affecting nutrient removal efficiency in MBRs using supernatant of fermented sludge as carbon source. With thin corn stillage as the carbon source, the results showed a very high TN and TP concentration in the effluent which is probably due to the high TN and TP concentrations in the corn stillage.

It appeared that producing PHB in municipal wastewater treatment process can make the treatment plants more sustainable. Although the percent of PHB in the biomass (in the range of 10%) for this study were much less than other studies, PHB accumulation can be further increased by changing the HRT and SRT to improve the operation of the reactors.

APPENDIX A. EXPERIMENT DATA FOR VARIOUS RECIRCULATION RATIOS AND HRT CONDITIONS

Table A. 1 Influent Characteristic

Run	Influent characteristics (mg/L where applicable)								
	Day	COD	TN	NO_3^- - N	NO_2^- - N	NH_3 - N	TP (Soluble)	TSS	pH
Base line (Aerobic MBR)	1	495							
	2	486	47.9	0.6	0.2	24.3	13.8		6.9
	5		46.7	0.7	0.3	21.2	15.2		6.8
	7	502							
	9	509	51.6	0.4	0.1		14.2		7.1
	14	496							
	16	468	47.2				16.1		7
	20	472	44.8	0.2	0.1		16.8		6.9
Run 1: 2,4,8 Recirculation: 100%/100%	22		46.3	0.1	0				
	25	503							
	26	476						21.2	
	27	458	46.2	0.3	0.1	27.2	16.6		7.1
	34						16.2	22.1	6.8
	52		45.8				15.1	23.1	
	70	502	52.1	0.8	0	28.6	17.1		
	77	511	51.4				17.1	25.1	
	83	492							
Run 2: 2,4,8 Recirculation: 300%/100%	90	501	49.6	0.2	0.1		17.3		
	97								
	104	447	46.2	0.5	0.2		15.2	23.6	7.4
	110	513							
	116	467	48.6	0.2	0.2	27.8		21.5	7.2
	123								
	128	516	51.3				16.9	23.7	7.2
	133	462	50.1	0.3	0.1	23.6			
	138							22.4	
	146	487	48.3				17.1		7.1

Table A.1 (continued) Influent Characteristics

Run	Influent Characteristics (mg/L where applicable)								
	Day	COD	TN	NO_3^- - N	NO_2^- - N	NH_3 - N	TP (Soluble)	TSS	pH
Run 3: 2,4,8 Recirculation: 500%/100%	150	495	50.1	0.2	0.1	26.2	16.2		7.1
	156	486						22.4	
	163		46.7	0.3	0.1	21.4	15.2		6.8
	172	502							
	180	509						20.6	7.1
	184								
	190						16.1		7
	197	472	44.8	0.6	0.2	20.7	16.8	23.1	6.9
	206	489					14.6		
	210								7.2
Run 4: 2,2,4 Recirculation: 300%/100%	216	476						21.2	
	220	458	46.2				16.6		7.1
	226	431					16.2	22.1	6.8
	232	437	45.8	0.1	0.1	21.2	15.1		
	236	479						23.4	
	241						16.3		7.3
	247	502	52.1	0.4	0.2	25.5	17.1		
	254	511					17.1	25.1	7.1
	268	492							
	276	501	49.6	0.2	0.2	23.1	17.3	24.3	7.2

Table A.2 Anaerobic Characteristics

Run	Anaerobic Characteristics (mg/L where applicable)									
	Day	sCOD	TN	NO_3^- - N	NO_2^- - N	NH_3 - N	TP (Soluble)	TSS	pH	DO
Run 1: 2,4,8 Recirculation: 100%/100%	22							4670	7.4	0.02
	25	131						4720	7.2	
	26	123	23.4	0.6	0.4	14.3	17.2			0.03
	27	117					16.8			
	34	134	23.1	0.6	0.3	15.1	16.2	4850	7	0.02
	52	109						4530		
	70	119	22.6	0.4	0.3	14.4	17.1	4620	7.2	0.03
	77	128	26.3	0.8	0.2	15.6	18.2	4590		
	83	133	24.7	0.5	0.4	15.2	17.9	4710	7.1	0.04
Run 2: 2,4,8 Recirculation: 300%/100%	90	118						4880		
	97	132	20.6	1.1	0.3	13.6	18.2	4920	6.9	
	104	121						4950		
	110	118	19.8	1.2	0.4	12.9	17.2		7.1	0.04
	116	122								
	123	115	22.1	0.9	0.5	11.8		5020	7.3	
	128	114						5010		
	133	120	19.3	1.3	0.6	12.3	18.1		7.1	0.03
	138	118						4980		
	146	123	18.7	1.3	0.5	13.2	18.2	4980	7.2	0.02
Run 3: 2,4,8 Recirculation: 500%/100%	150	97	16.2	0.5	0.1	14.6	16.2			
	156	106	20.8	0.9		15.1				0.05
	163									
	172	109	22.3	0.8	0	14.3	16.4	5630		0.03
	180									
	184	112	23.8	0.6	0	13.9	15.2	5480		
	190									
	197	99								
	206	107	24.6	0.3	0.1	15.2	15.3	5010		0.04
	210	98	23.4	0.6	0.2	14.4	16.2			

Table A.2 (continued) Anaerobic Characteristics

Run	Anaerobic Characteristics (mg/L where applicable)									
	Day	sCOD	TN	NO_3^- - N	NO_2^- - N	NH_3 - N	TP (Soluble)	TSS	pH	DO
Run 4: 2,2,4 Recirculation: 300%/100%	216	97						5080	7.4	0.02
	220							5320	7.2	
	226	92	15.2	0.9	0.1	10.3	16.3			0.03
	232	103					16.8			
	236	96	13.6	1.3	0	9.8	16.2	5440	7	0.02
	241	89						5360		
	247	102	14.3	0.9	0.1	11.1	17.1	5530	7.2	0.03
	254	97	16.1	0.8	0.2	11.7	18.2			
	268	95	13.9	1.2	0	10.6	17.9	5610	7.1	0.04

Table A.3 Anoxic Characteristics

Run	Anoxic Characteristics (mg/L where applicable)									
	Day	sCOD	TN	NO_3^- - N	NO_2^- - N	NH_3 - N	TP (Soluble)	TSS	pH	DO
Run 1: 2,4,8 Recirculation: 100%/100%	22							4970	7.4	0.2
	25	53						5220	7.2	
	26	61	11.8	0.2	0.1	6.2	16.2			0.2
	27	49					16.6			
	34	52	10.6	0.1	0.1	5.8	14.7	5050	7	0.3
	52	38						4730		
	70	50	12.1	0.1	0	4.7	14.3	4420	7.2	0.5
	77	47	12.3	0	0.2	4.9	13.8	4690		
	83	52	12.7	0.3	0	5.2	14.2	4410	7.1	0.1
Run 2: 2,4,8 Recirculation: 300%/100%	90	46						5680		
	97	39	7.3	0.2	0.1	5.3	18.6	5420	6.9	
	104	42						5550		
	110	44	6.9	0.1	0	4.9	17.6		7.1	0.2
	116	37								
	123	43	6.7	0.1	0.2	5.1		5420	7.3	
	128	41						5710		
	133	39	7.2	0.2	0	4.8	19.1		7.1	0.1
	138	46						5680		
	146	47	6.8	1.3	0.1	5.1	18.9	5980	7.2	0.08
Run 3: 2,4,8 Recirculation: 500%/100%	150	36	10.2	0.5	0.1	7.6	14.3			
	156	40	9.6	0.9		8.1				0.08
	163									
	172	37	8.9	0.8	0	7.4	15.6	5730	7.1	0.1
	180									
	184	41	10.1	0.6	0	7.2	12.8	5860	6.9	
	190									
	197	33								
	206	38	11.1	0.3	0.1	6.9	14.3	5620	7.0	0.2
	210	37	10.3	0.6	0.2	6.7	13.7			

Table A.3 (continued) Anoxic Characteristics

Run	Anoxic Characteristics (mg/L where applicable)									
	Day	sCOD	TN	NO_3^- - N	NO_2^- - N	NH_3 - N	TP (Soluble)	TSS	pH	DO
Run 4: 2,2,4 Recirculation: 300%/100%	216	39						5580	7.4	0.06
	220							5320	7.2	
	226	41	9.6	0.4	0.1	4.7	17.1			0.1
	232	41								
	236	38	8.9	0.7	0	5.1	16.9	5440	7	0.2
	241	43						5670		
	247	42	10.1	0.9	0.1	4.9	16.5	5830	7.2	0.09
	254	46	9.2	0.8	0.1	5.2	16.8			
	268	44	8.8	1.0	0	5.3	17.3	5610	7.1	0.1

Table A.4 Aerobic Characteristics

Run	Aerobic Characteristics (mg/L where applicable)									
	Day	sCOD	TN	NO_3^- - N	NO_2^- - N	NH_3 - N	TP (Soluble)	TSS	pH	DO
Run 1: 2,4,8 Recirculation: 100%/100%	22							7970	7.4	3.2
	25	10.8						8220	7.2	
	26		9.6	6.7	0.6	0.9	6.6			2.6
	27	10.6								
	34		10.6	7.2	0.8	0.6	6.1	8050	7	2.3
	52	9.8						8430		
	70	9.0	10.2	7.1	0.3	0.7	6.3	8420	7.2	3.5
	77	11.3	10.6				6.9	8690		
	83	11.6	11.1	7.6	0.4	0.5	6.8	8410	7.1	2.4
Run 2: 2,4,8 Recirculation: 300%/100%	90	5						8680		
	97	2	5.8	4.9	0.6	0.2	3.6	8420	6.9	
	104	5						8550		
	110	3	5.9	5.1	0.4	0.2	3.4		7.1	2.8
	116	3								
	123	5	5.4	4.7	0.5	0.1		8460	7.3	
	128	7						8760		
	133	8	4.9	3.8	0.5	0.3	3.1		7.1	3.1
	138	3						8690		
	146	5	6.0	3.7	0.8	0.6	3.3	8940	7.2	
Run 3: 2,4,8 Recirculation: 500%/100%	150	10	8.1	4.8	0.6	0.5	4.2			
	156	9	7.1	6.2						3.2
	163									
	172	8	6.9	5.8	0.5	0.3	4.6	8720	7.1	2.7
	180									
	184	11	7.1	6.1	0.6	0.2	4.5	8840	6.9	
	190									
	197	10								
	206	12	7.1	5.9	0.7	0.3	4.8	8690	7.0	3.1
	210	9	6.9	6.3	0.8	0.1	4.2			

Table A.4 (continued) Aerobic Characteristics

Run	Aerobic Characteristics (mg/L where applicable)									
	Day	sCOD	TN	NO_3^- - N	NO_2^- N	NH_3 - N	TP (Soluble)	TSS	pH	DO
Run 4: 2,2,4 Recirculation: 300%/100%	216	3						8580	7.4	2.5
	220							8370	7.2	
	226	4	8.8	6.9	0.8	0.6	5.1			2.9
	232	3								
	236	5	7.9	6.6	0.6	0.3	4.4	8640	7	2.8
	241	3						8850		
	247	7	8.6	7.1	0.7	0.6	5.3	8820	7.2	3.1
	254	6	8.3							
	268	5	8.8	6.8	0.6	0.8	5.0	8680	7.1	3.1

APPENDIX B. EXPERIMENT DATA FOR PHB ACCUMULATION

Table B.1 Anaerobic, anoxic and oxic characteristics with acetate addition to anaerobic tank

Carbon addition (mg-c/L)	0	100	500	700	1000
Anaerobic Tank					
PHB (%)	0.8	1.3	6.13	7.1	9.5
TN (mg/L)	20.3	19.4	18.7	17.5	15.1
TP (mg/L)	17.5	24.6	26.6	28.4	30.2
sCOD (mg/L)	114.7	134.6	229	268	327
Anoxic Tank					
PHB (%)	0.8	1.0	5.33	7.6	10.7
TN (mg/L)	13.6	13.1	12.2	10.6	9.1
TP (mg/L)	18.1	16.3	22.5	27.3	28.2
sCOD (mg/L)	70.5	101.3	143.1	182	232.6
Aerobic Tank					
PHB (%)	0	0	4.5	6.1	8.3
TN (mg/L)	8.1	5.2	4.7	4.3	3.9
TP (mg/L)	5.2	4.2	1.3	0.9	0.3
sCOD (mg/L)	3.9	21	56	62	84

Table B.2 Anaerobic, anoxic and oxic characteristics with acetate addition to anoxic tank

Carbon addition (mg-c/L)	0	100	500	700	900	1000
Anaerobic Tank						
PHB (%)	0.8	1.3	6.2	6.8	8.7	10.7
TN (mg/L)	20.3	18.6	17.7	16.3	16.5	12.8
TP (mg/L)	17.5	19.3	22.6	26.4	28.3	28.6
sCOD (mg/L)	114.7	118.4	176.3	192.3	218.4	227
Anoxic Tank						
PHB (%)	0.8	1.4	6.5	7.2	9.2	10.9
TN (mg/L)	13.6	12.3	9.6	8.9	7.8	7
TP (mg/L)	18.1	20.3	25.4	27.6	30.1	31.2
sCOD (mg/L)	70.5	109.3	223.4	237.1	269.4	292.6
Aerobic Tank						
PHB (%)	0	0	0	4.6	8.3	8.9
TN (mg/L)	8.1	4.9	2.6	1.7	1.3	1
TP (mg/L)	5.2	5.3	4.9	4.3	3.2	3
sCOD (mg/L)	3.9	26.3	54	62	78	91

Table B.3 Anaerobic, anoxic and oxic characteristics with supernatant added to anaerobic tank

Carbon addition (mg-c/L)	0	100	500
Anaerobic Tank			
PHB (%)	0.8	2.3	4.2
TN (mg/L)	20.3	24.3	42.1
TP (mg/L)	17.5	22.6	42.7
sCOD (mg/L)	114.7	131	208
Anoxic Tank			
PHB (%)	0.8	1.4	6.5
TN (mg/L)	13.6	19.7	29.7
TP (mg/L)	18.1	23.7	39.6
sCOD (mg/L)	70.5	89	127
Aerobic Tank			
PHB (%)	0	0	0.2
TN (mg/L)	8.1	8.3	8.7
TP (mg/L)	5.2	5.8	6.6
sCOD (mg/L)	3.9	18	37

Table B.4 Anaerobic, anoxic and oxic characteristics with supernatant added to anoxic tank

Carbon addition (mg-c/L)	0	100	500
Anaerobic Tank			
PHB (%)	0.8	1.8	2.2
TN (mg/L)	20.3	19.7	34.2
TP (mg/L)	17.5	24.3	40.7
sCOD (mg/L)	114.7	119	146
Anoxic Tank			
PHB (%)	0.8	1.2	1.6
TN (mg/L)	13.6	16.4	27.6
TP (mg/L)	18.1	29.4	42.6
sCOD (mg/L)	70.5	118	197
Aerobic Tank			
PHB (%)	0	0	0
TN (mg/L)	8.1	8.1	8.3
TP (mg/L)	5.2	6.8	7.3
sCOD (mg/L)	3.9	20	34

Table B.5 Anaerobic, anoxic and oxic characteristics with thin corn stillage added to anaerobic tank

Carbon addition (mg-c/L)	0	100	500
Anaerobic Tank			
PHB (%)	0.8	5.13	7.3
TN (mg/L)	20.3	143	423
TP (mg/L)	17.5	145	647
sCOD (mg/L)	114.7	431	627
Anoxic Tank			
PHB (%)	0.8	4.5	6.87
TN (mg/L)	13.6	82	306
TP (mg/L)	18.1	478	586
sCOD (mg/L)	70.5	392	506
Aerobic Tank			
PHB (%)	0	3.99	5.1
TN (mg/L)	8.1	67	154
TP (mg/L)	5.2	95	309
sCOD (mg/L)	3.9	298	429

Table B.6 Anaerobic, anoxic and oxic characteristics with thin corn stillage added to anoxic tank

Carbon addition (mg-c/L)	0	100	500
Anaerobic Tank			
PHB (%)	0.8	1.22	3.16
TN (mg/L)	20.3	66	263
TP (mg/L)	17.5	155	433
sCOD (mg/L)	114.7	362	509
Anoxic Tank			
PHB (%)	0.8	1.1	2.03
TN (mg/L)	13.6	133	327
TP (mg/L)	18.1	172	683
sCOD (mg/L)	70.5	406	617
Aerobic Tank			
PHB (%)	0	0	0.3
TN (mg/L)	8.1	42	147
TP (mg/L)	5.2	129	521
sCOD (mg/L)	3.9	298	411