

2014

Sustainable wastewater systems: Impact of operational strategies and carbon sources on poly-hydroxybutyrate (PHB) accumulation and nutrient removal in sequencing batch reactor

Miao Yu

Iowa State University

Follow this and additional works at: <https://lib.dr.iastate.edu/etd>

 Part of the [Environmental Engineering Commons](#)

Recommended Citation

Yu, Miao, "Sustainable wastewater systems: Impact of operational strategies and carbon sources on poly-hydroxybutyrate (PHB) accumulation and nutrient removal in sequencing batch reactor" (2014). *Graduate Theses and Dissertations*. 13767.
<https://lib.dr.iastate.edu/etd/13767>

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

**Sustainable wastewater systems: Impact of operational strategies and carbon sources
on poly-hydroxybutyrate (PHB) accumulation and nutrient removal in sequencing
batch reactor**

by

Miao Yu

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)

Program of Study Committee:
Say Kee Ong, Major Professor
Shiwu Sung
Thomas Loynachan

Iowa State University

Ames, Iowa

2014

Copyright © Miao Yu, 2014. All rights reserved.

DEDICATION

I would like to dedicate my thesis to my beloved parents.

TABLE OF CONTENTS

	Page
LIST OF FIGURES	v
LIST OF TABLES	vi
ACKNOWLEDGEMENTS	viii
ABSTRACT	ix
CHAPTER 1 INTRODUCTION AND OBJECTIVES	1
1.1 Introduction	1
1.2 Objectives	2
1.3 Thesis Organization	3
1.4 References	3
CHAPTER 2 LITERATURE REVIEW	5
2.1 Introduction	5
2.2 Nutrients Recovery from Municipal Wastewater as Struvite	6
2.3 Source Separated Urine	9
2.4 Carbon Recovery as Methane	9
2.5 Carbon Recovery as Poly-hydroxyalkanoates	11
2.5.1 PHB production in sequencing batch reactor	12
2.5.2 Influence of additional carbon source on PHB production	15
2.5.3 PHB extraction method	15
2.6 Conclusion and Further Study	17
2.7 References	18
CHAPTER 3 IMPACT OF OPERATIONAL STRATEGIES AND CARBON SOURCES ON POLY-HYDROXYBUTYRATE (PHB) ACCUMULATION AND NUTRIENT – REMOVAL IN SEQUENCING BATCH REACTOR	24
Abstract	24
3.1 Introduction	25
3.2 Methods and Materials	27
3.2.1 Source of biomass and wastewater composition	27
3.2.2 Sequencing batch reactor and operational conditions	27
3.2.3 PHB production batch experiments	28
3.2.4 Analysis methods	32
3.3 Results and Discussions	34
3.3.1 Performance of wastewater treatment and PHB accumulation under different operational conditions	34

	Page
3.3.2 Impact of acetate addition on PHB storage.....	35
3.3.3 Impact of acetate addition on wastewater effluent quality	37
3.3.4 Concentration profiles for Condition B	39
3.3.5 Impact of fermented corn stillage addition on PHB storage and effluent quality	42
3.4 Conclusion	46
3.5 References	46
 CHAPTER 4 GENERAL CONCLUSION	 48
4.1 Conclusion	48
4.2 Future Studies	49
 APPENDIX A. EXPERIMENTAL DATA FOR ALL TREATMENT CONDITIONS WITHOUT ADDITIONAL CARBON SOURCE	 50
 APPENDIX B. EXPERIMENTAL DATA FOR ALL TREATMENT CONDITIONS WITH ACETATE ADDITION	 53
 APPENDIX C. EXPERIMENTAL DATA FOR ALL TREATMENT CONDITIONS WITH FERMENTED CORN STILLAGE ADDITION	 56

LIST OF FIGURES

	Page
Figure 3.1 Operational conditions for SBR	29
Figure 3.2 Impact of addition of acetate on PHB production for three operational conditions	37
Figure 3.3 Impact of addition of acetate on wastewater treatment	38
Figure 3.4 Concentration profiles for Condition B with the addition of 1200 mg-C/cycle acetate.....	41
Figure 3.5 Impact of addition of fermented corn stillage on PHB production for Condition B.....	44
Figure 3.6 Impact of addition of fermented corn stillage on COD removal for Condition B.....	44
Figure 3.7 Impact of addition of fermented corn stillage on TN and TP removal for Condition B	45

LIST OF TABLES

	Page
Table 2.1 PHB recovery method and results	16
Table 3.1 Synthetic wastewater composition.....	29
Table 3.2 Composition of corn stillage and fermented corn stillage	31
Table 3.3 Composition of corn stillage, Mg(OH) ₂ -precipitated corn stillage and fermented Mg(OH) ₂ -precipitated corn stillage	31
Table 3.4 Hach methods used	33
Table 3.5 Nutrients removed and PHB accumulation under different operational conditions with synthetic wastewater only	34
Table A.1 Concentrations for Condition A.....	50
Table A.2 Concentrations for Condition B	51
Table A.3 Concentrations for Condition C	52
Table A.4 PHB concentrations for Condition A, Condition B and Condition C.....	52
Table B.1 PHB and nutrients concentration in effluent with acetate addition for Condition A.....	53
Table B.2 PHB and nutrients concentration in effluent with acetate addition for Condition B	53
Table B.3 PHB and nutrients concentration in effluent with acetate addition for Condition C	54
Table B.4 Volumes and concentrations of addition of acetate solution	54
Table B.5 Concentrations for Condition B with addition 1200 mg-C/cycle acetate.....	55
Table C.1 PHB and nutrients concentration in effluent with fermented corn stillage addition for Condition B	56
Table C.2 PHB and nutrients concentration in effluent with fermented Mg(OH) ₂ -precipitated corn stillage addition for Condition B ..	56

Table C.3 Volumes and concentrations of addition of fermented corn stillage.....	57
--	----

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my advisor, Dr. Say Kee Ong, for the continuous support of my master study and research, for his patience and his support. His guidance helped me throughout my research and writing of this thesis.

Besides my advisor, I would like to thank the rest of my committee members: Dr. Shihwu Sung and Dr. Thomas E Loynachan for their help and guidance during the research.

In addition, I would like to thank Mr. Dave Mozzena for his help in collecting activated sludge at Boone Wastewater Treatment Plant.

Last but not the least, I would like to thank my family for their encouragement and love.

ABSTRACT

In a sustainable wastewater treatment system, wastewater is viewed as a resource rather than a waste. Carbon recovery as poly-hydroxybutyrate (PHB) has attracted attention in the last two decades. In this study, the possibility of combining PHB accumulation in the biomass along with wastewater treatment was investigated. Three operational conditions and addition of acetate and fermented corn stillage as carbon sources in a sequencing batch reactor (SBR) were evaluated. The react phase of a 6 hour cycle of a SBR was divided into anaerobic and aerobic sequences to investigate the best operational condition to maximize PHB production and wastewater treatment. Condition B, where the react cycle was divided into two rounds of 45 minutes of anaerobic sequence and 2 hours of aerobic sequence each with the addition of acetate in the anaerobic sequence, gave a PHB content of $37.4 \pm 4.1\%$ (wt./wt.) in the biomass. The acetate added was 1200 mg-C/cycle. Using fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage, the PHB content in the biomass achieved was 24.3% (wt./wt.) and a TP content of 8.6% (wt./wt.) for the addition of 800 mg-C/cycle fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage. The results of this study showed that it is possible to recover both PHB and phosphorus by using fermented corn stillage as a carbon source and at the same time treat municipal wastewater.

CHAPTER 1. INTRODUCTION AND OBJECTIVES

1.1 Introduction

To solve the scarcity of global resources and to reduce energy consumption, there is a new thinking of making municipal wastewater treatment a sustainable system and infrastructure. In recent decades, wastewater is seen more as a source of valuable resources and a raw material carrier. Domestic wastewater contains organic matters and three main nutrients: phosphorus, nitrogen and potassium (Jessen et al., 2007). The goal of sustainable wastewater treatment systems is to provide the public with clean and safe water and at the same time ensure the social, environmental, and economic sustainability of communities that the water utilities serve (USEPA, 2012). Nitrogen, phosphorus and carbon sources present in wastewaters are all seen as recyclable sources. Combining wastewater treatment with resource recovery has been recognized as a promising method to realize the goal of a sustainable wastewater treatment system.

Among the various materials recovered from municipal wastewater, carbon in wastewaters can be recovered as poly-hydroxyalkonate (PHA), a biopolymer. This approach has attracted a lot of attention in recent years. Due to the increased concern of possible depletion of fossil fuels and high crude oil prices, biopolymers has been thought to be an alternative material to replace traditional polymer made from fossil fuels. PHAs are naturally produced by many bacteria. Industrial production of PHAs is usually based on pure microbial cultures and the operational cost can be high. The cost of developing pure culture fermentation and commercialization of PHAs is about 4-9 times higher than that of conventional plastics (Moita and Lemos, 2012). About 30% of the total PHA production cost is due to the cost of the feedstocks, such as glucose and sucrose (Lee, 1996). If PHAs are produced using mixed cultures and a carbon source of low cost, the production cost of PHAs can be significantly reduced. Synthesizing poly-hydroxybutyrate, a PHA

compound in the municipal wastewater treatment system, using municipal wastewater is one option to reduce the cost of PHAs production. Harvesting PHB from a municipal wastewater treatment system can be an economic strategy for a sustainable wastewater treatment system.

Researchers have shown that sequencing batch reactor (SBR), among all wastewater treatment systems, is very effective in accumulating PHBs within the biomass. Since the treatment sequences of SBRs can be easily manipulated, SBR is highly efficient in treating the BOD₅ in the wastewater and, at the same time, allow for nutrients removal. For example, SBRs have been shown to remove 89%-98% of BOD₅ with good total nitrogen removal greater than 75% (USEPA, 1992). By adding an anaerobic and anoxic sequence, SBR can accumulate PHBs and total phosphorus in the biomass which can then be recovered.

This is accomplished through a feast and famine approach to enhance PHB accumulation (Salehizadeh et al., 2004). The SBR process can also allow addition of carbon substrate at different times of the cycle sequences to manipulate feast/famine sequences and to maximize PHB synthesis (Coats et al., 2011). Research on PHB accumulation by using SBR typically uses pure cultures with some studies using mixed cultures. For instance, Serafilm et al. (2004) used acetate and propionate as carbon sources to grow PHB in a SBR system and obtained PHB content of 67.2% of cell dry weight. To reduce the cost of PHB production, researchers are working on manipulating the treatment operations and using low cost, waste carbon source to maximize production of PHB.

1.2 Objectives

This research will investigate the influence of different carbon sources and different operational strategies on PHB production in a SBR system. The specific objectives of this study are:

1. Determine the impact of treatment operation strategies (specially, the SBR cycle sequences) on PHB accumulation.
2. Determine the impact of adding carbon waste streams from other sources to supplement the carbon in the municipal wastewater for PHB accumulation.

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 nutrient recovery from wastewater, and PHB production with wastewater and work conducted by others. Chapter 3 presents the method and results of this study. Chapter 4 is the conclusion chapter.

1.4 References

- Coats, E.R., VandeVort, K.E., Darby, J.L., and Loge, F.J., (2011) Toward polyhydroxyalkanoate production concurrent with municipal wastewater treatment in a sequencing batch reactor system. *Journal of Environmental Engineering*. 137 (1), 46-54.
- Jessen, P.D., Vrale L., and Lindholm, O. (2007) Sustainable wastewater treatment. International conference on Natural Resources and Environmental Management and Environmental Safety and Health. November 27-29, Kuching, Malaysia.
- Lee, S.Y. (1996) Bacterial polyhydroxyalkanoates. *Biotechnology Bioengineering*. 49(1), 1-14.
- Moita, R., and Lemos, P.C. (2012) Biopolymers production from mixed cultures and pyrolysis by-products. *Journal of Biotechnology*. 157(4), 578-583.
- Salehizadeh, H., and Van Loosdrecht, M.C. (2004) Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance. *Biotechnology Advances*. 22 (3), 261-179.
- Serafilm, F.S., Lemo, P.C., Oliveira, R., and Reis, M.A. (2004) Optimization of polyhydroxybutyrate production by mixed cultures submitted to aerobic dynamic feeding conditions. *Biotechnology Bioengineering*. 87(2), 145-160.
- USEPA. (1992) Sequencing batch reactors for nitrifications and nutrient removal, EPA 832 R-92-003, U.S.Environmental Protection Agency, Washington, D.C.

USEPA, (2012). Planning for sustainability. EPA 832 R-12-001, U.S.Environmental Protection Agency, Washington, D.C.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Water, food and energy are three of the major resource concerns facing the world today. To live more sustainably, every aspect of our infrastructure is being examined to see whether usage of these resources can be minimized and at the same time recovered or reused. Useful materials can be recovered from waste materials and energy used can be reduced or recovered by better managing the resources available. One of the new approaches is making the urban infrastructure, sustainable by closing the urban water cycle loop. A component of the urban water cycle is the municipal wastewater treatment systems. The goal of sustainable wastewater treatment is to provide the public with clean and safe water and at the same time reduce resource used such as energy and enhance recovery of useful products (USEPA, 2012).

Conventional wastewater treatment process is capital-intensive and energy-intensive. Of all the energy used in the United States (US), drinking water and wastewater systems account for approximately 3-4 percent of energy use in the United States. In addition, wastewater treatment plants, result in emissions of more than 45 million tons of greenhouse gases annually (USEPA, 2013). Water pumping and energy consumption for the treatment process at a treatment plant can comprise about 56% of total energy use at the treatment plant (Means et al., 2004). Biodegradation of carbonaceous wastes in conventional wastewater treatment process results in carbon dioxide emission which contributes towards global climate change. Sewage sludge from the treatment plants are typically land applied or landfilled which may contribute towards methane emission. Currently, there are over 15000 municipal wastewater plants in service in the US (USEPA, 2007). Emission from wastewater treatment plants and high use of energy are contrary to the sustainable development of a responsible society.

To address some of these issues, there is a paradigm shift in viewing wastewater as a resource and not as a waste. Municipal wastewater contains organic matter and three main nutrients: nitrogen, phosphorus and potassium (Jessen et al., 2007). These materials can be recovered for use, if the treatment systems are designed or operated to harvest these materials. This review mainly focuses on current research and technology of resource recovery from municipal wastewaters.

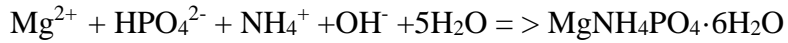
2.2 Nutrients Recovery From Municipal Wastewater as Struvite

Domestic wastewater contains three main nutrients: phosphorus, nitrogen and potassium. Large quantities of nitrogen and phosphorus present in the treated wastewater that are discharged into bodies of water are the main cause of eutrophication. Conventionally, nitrogen in the wastewater treatment plants is removed by biological methods. After nitrification, nitrate is converted into nitrogen gas by denitrification. A common approach for removing phosphorus from wastewater is by metal salts precipitation, but this approach makes the precipitate unrecoverable for possible reuse such as fertilizer (Donnert and Salecker, 1999).

In recent decades, there are many research on the recovery of nitrogen and phosphorus for use as fertilizer and other purposes. Phosphorus has been viewed by many scientists as a limited resource. Recovery of phosphorus from municipal wastewaters will be one of the methods in meeting the world's demand for phosphorus.

One of the approaches in recovering nutrients from wastewaters is the precipitation of magnesium ammonium phosphate hexahydrate ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$), a white crystalline compound, also known as struvite (Booker et al., 1999). Recovery of nutrients in wastewater treatment plants before they are formed or precipitated can protect treatment equipment as well as

provide a renewable nutrient source for agriculture. Struvite is formed according to the general reaction shown below, although the chemistry involved in struvite precipitation can be complicated (Doyle, 2002).



Struvite composition (i.e., nitrogen, phosphorus, and magnesium ions in equal molar concentrations) makes it a potentially marketable product for the fertilizer industry. (Booker et al., 1999). Research found that the nutrient existing in wastewater can supply 15% - 20% of Norway's fertilizer market if the nutrients in the wastewater can be completely recycled (Jenssen and Vatn, 1991). More than 40% of the fertilizer used in developing countries may be supplied by recovering nutrients from their municipal wastewaters (Etnier and Jessen, 1997). Struvite displays certain excellent fertilizer qualities under specific conditions such as low solubility as compared with standard fertilizers (Munch, 2001). Furthermore, struvite recovered from wastewater treatment plants has low heavy metal contents as compared to phosphate bearing rocks that are mined and supplied to the fertilizer industry (Driver et al., 1999).

The efficiency in recovering these nutrients is one of the more important factors in determining whether this method is practical and economical. Phosphorus removal efficiencies can range from 70% to 97% and nitrogen removal efficiency can range from 65% to 90% depending on treatment methods and types of wastewaters (Jaffer et al., 2002; Doyle et al., 2002; Liu et al., 2011). One challenge for the recovery of struvite in wastewater is the low magnesium concentration in municipal wastewater compared to nitrogen and phosphorus. Addition of magnesium may raise the cost of recovery (El Diwani et al., 2007).

The economic feasibility of recovering nutrients from wastewaters by precipitation as struvite was estimated by several researchers. Munch and Barr (2001) achieved an ortho-P removal

of 94% by adding magnesium hydroxide slurry as a magnesium source and as an agent for adjusting the pH. They estimated that, for this method, the cost for struvite recovery from Oxley Creek wastewater treatment plant would range from A\$13,000 to A\$149,000 per year (Munch and Barr, 2001). This equals to about \$276 per ton of struvite. The suggested market value of struvite in Australia was \$198-\$330. Jaffer et al. (2002) used MgCl_2 as magnesium source and sodium hydroxide to adjust the pH. Ninety seven percent phosphorus removal was achieved. The cost of chemicals used in a full-scale struvite crystallization plant could be at least \$65,755 per annum (Jaffer et al., 2002). Part of this cost can be recovered by selling the struvite product. The market value of struvite should be at \$283 per ton to outweigh the chemical costs used (Jaffer et al., 2001). Production of struvite can reduce the sludge production, which will result in operational savings such as decrease in landfill space needed for sludge disposal and lower maintenance problems due to struvite deposition (Shu et al., 2006; Le Corre et al., 2009). All these benefits can compensate for the cost of struvite production and bring benefits to the wastewater companies. Even though struvite is more expensive than phosphate fertilizers, struvite recovery can be a source which can alleviate the depletion of scarce phosphate bearing rocks.

Nutrient recovery technologies have not been widely applied due to the cost of nutrient recovery being higher than producing fertilizer from phosphorus bearing ore. To save money and to make wastewater treatment plant more efficient and sustainable, the challenge is to combine the nutrient recovery technologies with the wastewater treatment process more effectively that maximizes treatment and product recovery. Further research is still needed to achieve both nutrient recovery technologies and treatment of wastewater in practice.

2.3 Source-Separated Urine

In 1990s, separating urine and faeces attracted considerable attention in Europe as a sustainable wastewater treatment system (Kirchmann and Pettensson, 1995). Urine contains a high concentration of nutrients, especially nitrogen and phosphorus, i.e. about 80% of the nitrogen, 50% of the phosphorus in household wastewaters come from urine (Jonsson et al., 1997). Thus, separating urine from domestic faeces may enable the reuse of this nutrient more effectively (Maurer et al., 2006). Because of the high ammonia and phosphorus concentration in urine, struvite precipitation is one of the promising ways in extracting these nutrients from urine. Approximately, 90% nitrogen removal and around 98% phosphorus removal from urine can be achieved (Antonini et al., 2011; Ganrot et al., 2008; Kabdasli et al., 2006; Ban and Dave, 2004).

However, effective and convenient facilities for separating urine are not available and are being researched. For example, the No-Mix is a promising technology for collecting urine. No-Mix toilets have been installed in seven European countries and have received high acceptance (Lienert et al., 2010). For large scale installations, the efficiency of No-Mix technology need to be further enhanced and the benefits need to be demonstrated (Rossi et al., 2009).

2.4 Carbon Recovery as Methane

Methane and carbon dioxide production occurs through acetate cleavage. It mostly occurs during the anaerobic treatment process. Methane emission from wastewater treatment plants were estimated to account for approximately 5% of the total global greenhouse gas emissions every year (Czepllel et al., 1993). Methane is second to CO₂ in its global contribution to radiative forcing (El-Fadel et al., 2001). To control global warming, reduction of emission of CO₂ and CH₄ is important.

Methane is the main component of natural gas. If methane is recovered, it can be a source of energy. Recovery of methane from wastewater treatment plants is one of the strategies for controlling methane emission as well as harvesting energy for the sustainable operation of wastewater treatment plants.

Anaerobic fermentation is a typical approach in producing methane from wastewater sludge. The efficiency of hydrolysis and fermentation is one of the factors impacting the production of methane. In recent decades, various approaches have been used to improve the production of methane. In wastewater treatment plants, sludge digestibility is highly dependent on the sludge quality (primary, secondary or mixed), especially the contents of proteins, polysaccharides, lipids and humic acid like substances (Dumas et al., 2010). Besides the types and makeup of the sludge, treatment conditions such as temperature and digestion time are key factors which have considerable influence on methane production. Gao et al. (2011) combined up-flow anaerobic sludge fixed bed reactor, an anoxic tank and an aerobic membrane reactor to recover methane from domestic wastewater at different HRTs. Their results showed that by changing the HRT from 8 hr to 1 hr, the methane production increased from 115.0 mL CH₄/gCOD removed to 187.8 mL CH₄/gCOD removed. Dumas et al. (2010) compared the methane recovery efficiencies of hyper-thermophilic (65°C) aerobic reactor (TAR) coupled with a mesophilic (35°C) anaerobic digester (MAD) and that of a conventional mesophilic anaerobic digester (MAD). In their research using an SRT of 21 days and 42 days, the total methane yield was 30% and 41.3%, respectively for MAD-TAR and 26% and 41.1%, respectively for MAD. Therefore, based on different operating conditions and different reactors, methane production efficiency will change accordingly. Selection of the most suitable treatment process to recover methane for different sludge or organic material is very important with cost and efficiency in consideration.

Certain anions in wastewater influence methane recovery efficiency. Gimenez et al. (2012) tested the methane recovery efficiency from urban wastewater in a submerged anaerobic membrane bioreactor. They found that sulfate in the wastewater can impact and reduce methane producing efficiency. The methane recovery efficiency obtained at 33 °C was 57.4% but increased to 83% when there was no sulfate present in the wastewater. Wu et al. (2011) showed that Cu (II) present in water had an influence on methane production. They found that Cu(II) concentrations at 5 mg/L had a slightly stimulating effect on the activity of methanogenic bacteria, but the production rate was completely inhibited at a Cu(II) concentration of 300 mg/L.

Combined hydrogen (H₂) production with CH₄ production is another promising approach in building sustainability in sustainable wastewater treatment plants. H₂ is considered as a clean energy, if it can be produced by fermenting wastewater. However, production of H₂ by fermentation resulted in around 80%-90% of the initial COD remaining in the wastewater in the form of various volatile organic acids (Logan et al., 2004). Thus, the remaining COD can be used to produce methane, which can make the whole process more sustainable. Some research showed that this approach is feasible. Producing methane after hydrogen, is a promising way to enhance COD removal and recover energy efficiently (Mohan et al., 2008; Ting et al., 2004). However, though these two processes can be operated simultaneously, further economic evaluation is needed to ensure this approach is financially feasible.

2.5 Carbon Recovery as Poly-hydroxyalkanoates

Poly-hydroxyalkanoates (PHAs) are the polymers of hydroxyalkanoates which are produced by bacteria to store carbon and energy. In recent years, PHAs have attracted attention as a raw material for the production of biodegradable plastics. Conventional plastics produced from

nonrenewable resources such as petrochemicals are non-degradable unless modified with a biodegradable co-substrate. Non-biodegradable plastics are hard to dispose of and cause various environmental problems. Some countries have enacted a series of policies to reduce the use of plastics. However, to completely solve the environmental problems associated with plastics, a substitute material needs to be provided. PHAs are biosynthesized from renewable resources, allowing for a sustainable and closed-cycle process for the production and use of such polymers (Braunegg et al., 1998).

PHA has been industrially produced by pure cultures. However, wider production of PHA is limited by the cost of PHA production as compared to oil-derived plastics (Choi and Lee, 1997). About 30% of the total PHA production cost is due to the cost of the feedstocks, which are typically sugars such as glucose and sucrose (Lee, 1996). Thus, to decrease the cost of PHA production, lower cost carbon sources in mixed cultures instead of pure cultures need to be used. Industrial wastewaters have been considered as carbon sources for PHA production. Besides lowering the cost of PHA production, combining PHA production with wastewater treatment may result in making the municipal wastewater treatment process more sustainable and may enhance the economics of wastewater treatment plants. Additionally, if this combined approach is feasible, this approach can be applied universally, since wastewater treatment plant is found in all cities and municipal waste streams are continuously generated and abundant (Coats et al., 2011).

2.5.1 PHA Production in Sequencing Batch Reactor

Feast and famine approach is the most promising for high PHA accumulation among the treatment systems for industrial production of PHAs. (Salehizadeh et al., 2004). Feast and famine cycles can be divided into two parts, successive periods of substrate availability (feast period) and

no external substrate availability (famine period) (Salehizadeh et al., 2004). In municipal wastewater treatment plants, the SBR process configuration can allow addition of carbon substrate (all at the beginning of a cycle or at different times) and the operations can be manipulated to induce feast/famine (Coats et al., 2011).

Typically, in the operation of a SBR, there are five sequences in a cycle: fill, react, settle, decant and idle. Depending on the desired nutrients removal needed, the steps in one cycle can be adjusted to provide aerobic, anoxic and anaerobic periods (USEPA, 1992). Sequencing batch reactor is widely used for municipal and industrial wastewater treatment in recent decades, because of its simple configuration and high removal efficiency of COD and nutrients. In a study covering the performance of SBR for 19 plants. BOD removal in SBR ranged from 89-98% and total nitrogen percent removals were larger than 75% (USEPA, 1992). The main factors affecting COD and nutrients removal efficiency in SBR are hydraulic retention time (HRT), sludge residence time (SRT), dissolved oxygen, and C/N ratio. Operation of the SBR can be modified in various ways depending on different treatment requirements. Some modifications of the SBR in recent years, include continuous flow SBR, and anaerobic-aerobic sequencing batch reactor (Mahvi, 2008).

As an effective facility for producing PHB, the amount of PHB accumulation in SBR has been investigated using various carbon sources. Many studies used pure cultures with organic substrates such as glucose, methanol, ethanol and acetate. These pure cultures have been found to produce PHB at a concentration of over 80/% in the biomass (Lee, 1996). Hollender et al. (2002) evaluated the effect of acetate, glucose and a combination of acetate and glucose on PHA production under anaerobic-aerobic conditions in a 2-liter SBR. The treatment cycle for all experiments consisted of a 19.5-hour aerobic phase, followed by a 4-hour anaerobic phase, and a 0.5-hour period for settling. The organic substrate was added 10 min after the anaerobic process

began. The highest phosphate release (27 mg-P/L) and PHA storage (20 mg-C/g dry matter (DM)) during the anaerobic phase as well as the highest polyphosphate (8 mg-P/g DM) and glycogen storage (17 mg-C/g DM) during the aerobic phase were observed with acetate on the carbon source.

Levantesi et al. (2002) investigated PHA production in SBR by feeding with a mixture of volatile fatty acids (VFAs) consisting of acetate, propionate, butyrate and glycogen. The PHA accumulation reached 23% (wt./wt.) for a 2 hour anaerobic and 4 hour aerobic sequence over a 6-hour cycle.

Lemos et al. (1998) investigated the PHA accumulation using VFA as a carbon source in an SBR for with an 8 hour cycle. Each cycle comprised of an anaerobic (2 hour), aerobic (4 hour), settling (1 hour) and fill (1hour). PHA production in SBR was as high as 12% (wt./wt.).

Industrial and municipal wastewaters are relatively low cost carbon sources for PHA production. Coats et al. (2011) found that 0.23-0.31 mg PHA per mg of biomass per mg COD was obtained using primary solids fermenter liquor in an SBR. The SBR used has a volume of 15 L and was operated over a 24-hour cycle. Based on this study, they estimated that an SBR treating a flowrate of 1 million gallon per day could generate 11-36 metric tons of PHA annually. The proposed SBR system could generate a gross annual revenue of approximately \$110,000-\$360,000.

Chua et al. (2003) investigated the effect of pH, SRT, and acetate concentration on PHB storage in the biomass using municipal wastewater as the base wastewater. The SBRs were operated with a cycle of 4-hour consisting: supernatant decanting (15 min), influent feeding (5 min), anaerobic (1 hour), aerobic (2 hour) and settling period (40 min). Municipal wastewater supplemented with acetate could accumulate PHA up to 30% of sludge dry weight. Additionally,

pH in influent kept at 8-9 can favor PHA production. Coats et al. (2007) showed that PHA storage can be as high as 53% (wt./wt.) in the aerobic tank with fermenter liquor as the carbon source.

2.5.2 Influence of Additional Carbon Source on PHB Production

Using low-cost carbon sources is a logical approach to reduce the cost of PHB production. Many researchers have focused on PHA storage using acetate, VFA and glucose as additional carbon sources. Work done has indicated that these materials, especially acetate, are suitable for producing PHB. Other researchers have used high carbon content waste streams, such as cane molasses, cooking oil and corn stillage, to produce PHA. PHA production using these materials is promising, which can reach up to 43% dry weight and 36% dry weight using sugar cane molasses and waste cooking oil respectively (Wu et al., 2001; Haba et al., 2007;). Eskicioglu et al. (2011) found that VFA concentration can be over 5000 mg/L in the liquor of thermophilic digestion of corn stillage. With a high source of VFA content, fermented corn stillage can be a valuable source for PHB production. However, the high content of total nitrogen and phosphorus may have a negative effect and may lower PHB storage. Research on using fermented corn stillage for PHB production has not been conducted.

2.5.3 PHB Extraction

The method for PHB extraction is equally important in obtaining high PHB yields. Some recovery methods may result in the degradation of PHB reducing the yield of PHB. Over half of the production cost of PHB is associated with the recovery and purification of PHB. Typically, PHB is extracted in three steps. Firstly, cells need to be disrupted by using chemicals such as sodium hypochlorite, or by using a physical method such as passing cell suspensions through a two-stage homogenizer. PHB is then extracted with organic solvents to separate from the non-

PHB materials. The third step is separation of PHB from the solvent by solvent evaporation or precipitation in a non-solvent. To enhance PHB recovery, scientists have investigated the most appropriate method for each step of the extraction procedure.

Table 2.1 PHB recovery method and results

	Method	Temperature	Time	Yield	Purity	References
Disruption	Wash with deionized water and stored at - 20°C	--	--	--	--	Fiorese et al., 2009
Extraction	Chloroform	60 °C	2 hr (stirring)	--	--	
Separation	Solvent evaporation	--	--	94%	98%	
Disruption	Wash with deionized water and stored at - 20°C	--	--	--	--	Fiorese et al., 2009
Extraction	1,2 propylene carbonate	130 °C	30 min (condenser and stirring)	--	--	
Separation	acetone	20 °C	24 hr	95%	84%	
Disruption	Sodium hypochlorite	37 °C	1 hr	--	--	Sayyed et al., 2009
Extraction	Hot chloroform	100 °C – 145 °C	--	--	--	
Separation	Ethanol and acetone (1:1 v/v)	--	--	5.6g/l (100%)	--	
Extraction	Sodium hypochlorite (30% v/v) and chloroform (1:1)	40 °C	90 min (stirring)	--	--	Ghatnekar et al., 2002
Separation	hexane	--	--	95%	97%	

Table 2.1 shows the details and results of these PHB recovery methods. Fiorese et al. (2009) extracted PHB with chloroform and followed by solvent evaporation to recover the PHB. The results showed a PHB yield of 94% and a purity of 98%. The molecular weight of the extracted PHB was around 1.0×10^6 . In their research, they also extracted PHB using 1,2 propylene carbonate followed by separation using acetone. The advantages of using 1,2-propylene carbonate are its higher boiling point, its reuse over several times without purification, and its low toxicity (Fiorese

et al., 2009). Using this method, a yield of 95% and a purity of 84% were obtained. The polymer obtained was characterized to have a molecular weight of 7.4×10^5 and a polydispersity of 3.1. Sayyed et al. (2009) used chloroform to do the extraction, but used ethanol and acetone (1:1 v/v) to do the separation. With this method, a yield of almost 100% was achieved. Chloroform extracted PHB can also be separated by hexane. Ghatnekar et al. (2002) used this method to recover PHB from *Methylobacterium Sp V49* and obtained a yield of 95% and purity of 97%.

Most of the researchers showed that PHA production with municipal wastewater is industrially feasible. However to make this technology more economical, further research is needed with a focus on increasing the production of PHA and reducing the cost of raw material.

2.6 Conclusion and Further Study

This chapter reviewed the resource recovery in municipal wastewater treatment plants. Based on the work of others, some approaches have proven to be effective and feasible to recover useful products. But some approaches still need to be improved before they can be put into practice.

Nutrient and carbon recovery are important parts of a sustainable wastewater treatment system. Resource recovery from wastewater can contribute to be improved treated wastewater quality as well as alleviate the pressure of resource shortage. Full-scale plants have shown promising results of phosphorus and nitrogen recycling from wastewaters. Carbon recovery from wastewater helps to decrease the emission of carbon dioxide and methane into the atmosphere. Meanwhile, PHB production from wastewater will bring further benefits in providing raw materials for the production of biopolymers.

Thus far, nutrient and carbon recovery technologies other than methane are not widely used, since they are not economically feasible. As the price of crude oil increases further and the

total amount available depletes rapidly, industrial production of biopolymer needs to be realized as quickly as possible. Sequencing batch reactor shows that it is effective for PHBs production, since SBR can induce feast and famine conditions. Although there are many results showing nutrients removal and PHB production in sequencing batch reactor, there are not much research on optimizing both wastewater treatment and PHB production by modifying operational strategies in an SBR.

Carbon source is the main cost of PHB production. Using low cost carbon sources have not been fully investigated. Municipal wastewater as a common and low cost carbon sources may be suitable for PHB production. However, since the carbon content in municipal wastewater is low, to produce biomass with high PHB content, additional carbon source may be needed. Low cost carbon sources, such as industrial wastewaters, corn stillage and waste cooking oil, can be used as additional carbon source. But these sources may have negative impact on the final effluent, further investigations are needed.

Moreover, recovering phosphorus and producing PHB simultaneously in biological nutrient removal wastewater treatment plants is theoretically feasible. PHB production is part of a biological phosphorus removal process. Thus, if these two resources can be extracted at the same time, achieving a sustainable wastewater treatment system may be possible.

2.7 References

- Antonini, S., Paris, S., Eichert, T., and Clemens, J. (2011) Nitrogen and phosphorus recovery from human urine by struvite precipitation and air stripping in Vietnam. *Clean-Soil, Air, Water*. 39(12), 1099-1104.
- Ban, Z.S., and Dave, G. (2004) Laboratory studies on recovery of N and P from human urine through struvite crystallization and zeolite adsorption. *Environmental technology*. 25(1), 111-121.

- Booker, N.A., Priestley, A.J., and Fraser, I.H. (1999) Struvite formation in wastewater treatment plants: Opportunities for nutrient recover. *Environmental Technology*. 20(7) 777-782.
- Booker, N.A., Priestley, A.J., and Fraser, I.H., (1999) Struvite formation in wastewater treatment plants: opportunities for nutrient recovery. *Environ. Technol.* 20 (7), 777–782.
- Braunegg, G., Lefebvre, G., and Genser, K.F. (1998) Polyhydroxyalkanoates, biopolyesters from renewable resources: physiological and engineering aspects. *Journal of Biotechnology*. 65(2-3), 127-161.
- Byrom, D. (1987) Polymer synthesis by microorganisms: technology and economics. *Trends Biotechnology*. 5(9), 246-250.
- Choi, J., and Lee, S.Y. (1997) Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation. *Bioprocess Eng.* 17(6), 335–342.
- Choi, J., and Lee, S.Y. (1997) Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation. *Bioprocess Engineering*. 17(6), 335–342.
- Chua, A.S.M, Takabatake, H., Satoh, H., and Mino, T. (2003) Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of PH, sludge retention time, and acetate concentration in influent. *Water Research*. 37(15), 3602-3611.
- Coats, E.R., Loge, F.J., Wolcott, M.P., Englund, K., and McDonald, A.G. (2007) Synthesis of Polyhydroxyalkanoates in Municipal Wastewater Treatment. *Water Environment Research*. 79(12), 2396-2403.
- Coats, E.R., VandeVort, K.E., Darby J.L., and Loge F.J. (2011) Toward Polyhydroxyalkanoate production concurrent with municipal wastewater treatment in a sequencing batch reactor system. *Journal of Environmental Engineering*. 137(1), 46-54.
- Czeple, P.M., Crill, P.M., and Harriss, R.C. (1993) Methane emission from municipal wastewater treatment processes. *Environmental Science and Technology*. 27(12), 2472-2477.
- Donnert, D., and Salecker, M. (1999a) Elimination of phosphorus from waste water by crystallization. *Environmental Technology*. 20(7), 735–742.
- Doyle, J.D., Porsons, and S.A. (2002) Struvite control, formation and recovery. *Water research*. 36 (16), 3925-3940.
- Driver, J., Lijmbach, and D., Steen, I. (1999) Why recover phosphorus for recycling, and how? *Environmental Technology*. 20(7), 651–662.

- Dumas, C., Perez, S., Paul, E., and Lefebvre, X. (2019) Combined thermophilic aerobic process and conventional anaerobic digestion: Effect on sludge biodegradation and methane production. *Bioresource Technology*. 101(8), 2629-263.
- El-Fadel, M., and Massoud, M. (2001) Methane emissions from wastewater management. *Environmental Pollution*. 114(2), 177-185.
- El Diwani G., El Rafie, Sh., El Ibiari, N.N., and El-Aila, H.I. (2007) Recovery of ammonia nitrogen from industrial wastewater treatment as struvite slow releasing fertilizer. *Desalination*. 214(1-3), 200-214.
- Eskicioglu, C., Kennedy, K.J., Marin, J., and Strehler, B. (2011) Anaerobic digestion of whole stillage from dry-grind corn ethanol plant under mesophilic and thermophilic conditions. *Bioresource Technology*. 102(2), 1079-1086.
- Etnier, C., and Jessen, P.D (1997) The human waste resource in developing countries: Examples of options for reuse of nutrients in agriculture and aquaculture. Centre for International Environment and Development Studies, Noragric, Norway.
- Fiorese, M.L., Freitas, F., Pais, J., Ramos, A.M., de Aragao, G.M.F., and Reis, M.A.M. (2009) Recovery of polyhydroxybutyrate (PHB) from *Cupriavidus necator* biomass by solvent extraction with 1,2-propylene carbonate. *Life Science*. 9(6), 454–461.
- Ganrot, Z., Sivka, A., and Dave, G. (2008) Nutrient recovery from human urine using pretreated zeolite and struvite precipitation in combination with freezing-thawing and plant availability tests on common wheat. *Clean*. 36(1), 45-52.
- Gao D., An R., Tao Y., Li J., Li X., and Ren N. (2011) Simultaneous methane production and wastewater reuse by a membrane-based process: Evaluation with raw domestic wastewater. *Journal of Hazardous Materials*. 186(1), 383-389.
- Ghatnekar, M.S., Pai, J.S., and Ganesh, M. (2002) Production and recovery of poly-3-hydroxybutyrate from *Methylobacterium* sp V49. *Journal of Chemical Technology and Biotechnology*. 77 (4), 444-448.
- Gimenez, J.B., Marti, N., Ferrer, J., and Seco, A. (2012) Methane recovery efficiency in a submerged anaerobic membrane bioreactor (SAnMBR) treating sulphate-rich urban wastewater: Evaluation of methane losses with the effluent. *Bioresource Technology*. 118, 67-72.
- Haba, E., Vidal-Mas, J., Bassas, M., Espuny, M.J., Llorens, J., and Manresa, A. (2007) Poly3-(hydroxyalkanoates) produced from oily substrates by *Pseudomonas aeruginosa* 47T2 (NCBIM 40044): Effect of nutrients and incubation temperature on polymer composition. *Biochemistry Engineering Journal*. 35(2), 99-106

- Hollender, J., van der Krol, D., Kornberger, L., Gierden, E., and Dott, W. (2002) Effect of different carbon sources on the enhanced biological phosphorus removal in a sequencing batch reactor. *World Journal of Microbiology & Biotechnology*. 18(4), 355–360.
- Jaffer, Y., Clark, T.A, Pearce, P., and Parsons, S.A. (2001) Assessing the potential of full-scale phosphorus recovery by struvite formation. Second International Conference on Recovery of Phosphates from Sewage and Animal Wastes, March 12-14, Noordwijkerhout, Holland.
- Jaffer, Y., Clark, T.A., Pearce, P., and Parsons, S.A. (2002) Potential phosphorus recovery by struvite formation. *Water Research*. 36(7), 1834-1842.
- Jenssen, P.D., and Vatn, A. (1991) Ecologically sound wastewater treatment: Concepts and implementation. In: C. Etnier and B. Guterstam (eds.) *Ecological engineering for wastewater treatment*. Proc. International Conference on Natural Resources and Environmental Management and Environmental Safety and Health. Stensund, Bokskog, Sweden. pp. 148-160.
- Jenssen, P.D., Vrale, L., and Lindholm, O. (2007) Sustainable wastewater treatment. International conference on natural resources and environmental management and environmental safety and health. Kuching, Malaysia. November 27-29, 2007.
- Jonsson, H., Stentrom, T.A., Svensson, J. and Sundin, A. (1997) Source separated urine Nutrient and heavy metal content, water saving and faecal contamination. *Water Science and Technology*. 35(9), 145-152.
- Kabdasli, L., Tunay, O., Islek, C., Erdinc, E., Huskalar, S., and Tatli, M.B. (2006) Nitrogen recovery by urea hydrolysis and struvite precipitation from anthropogenic urine. *Water science and technology*. 53(12), 305-312.
- Kirchmann, H., and Pettersson, S. (1995) Human urine—chemical composition and fertilizer use efficiency. *Fertilizer Research*. 40(2), 149–154.
- Le Corre, K.S., Valsami-Jones, E., Hobbs, P., and Parsons, S.A. (2009) Phosphorus recovery from wastewater by struvite crystallization: A review. *Environmental Science and Technology*. 39(6), 433-477.
- Lee, B., Pometto, A.L., Fratzke, A., and Bailey, T.B. (1991) Biodegradation of degradable plastic polyethelene by phanerochaete and stretomyces species. *Applied Environmental Microbiology*. 57(3), 678-685.
- Lee, S.Y. (1996) Bacterial polyhydroxyalkanoates. *Biotechnology Bioengineering*. 49(1), 1-14.
- Lemos, P.C., Viana, C., Salgueiro, E.N., Ramos, A.M., Crespo, J.P.S.G., and Reis, M.A.M. (1998) Effect of carbon source on the formation of polyhyroxyalkanoates (PHA) by a

- phosphate-accumulation mixed culture. *Enzyme and Microbial Technology*. 22(8), 662-671.
- Levantesi, C., Serafim, L.S., Crocetti, G.R., Lemos, P.C., Rossetti, Blackall, L.L., Reis M.A., and Tandoi, V. (2002) Analysis of the microbial community structure and function of a laboratory scale enhance biological phosphorous removal reactor. *Environmental Microbiology*. 4(10), 559-569.
- Lienert, J., and Larsen, T.A. (2010) High acceptance of urine source separation in seven European countries: A review. *Environmental Science Technology*. 44(2), 556-566.
- Liu, Y., Kwag, J., Kim, J., and Ra, C. (2011) Recovery of nitrogen and phosphorus by struvite crystallization from swine wastewater. *Desalination*. 277(1-3), 364-369.
- Logan, B.E. (2004) Biologically extracting energy from wastewater: biohydrogen production and microbial fuel cells. *Environmental Science and Technology*. 38(9), 160A–167A.
- Mahvi, A.H. (2008) Sequencing batch reactor: A promising technology in wastewater treatment. *Iran. J. Environ. Health. Sci. Eng.* 5(2), 79-90.
- Maurer, M., Pronk, W., and Larsen, T.A. (2006) Review: treatment processes for source-separated urine. *Water Research*. 40(17), 3151–3166.
- Means, E. (2004) Water and wastewater industrial energy efficiency: a research roadmap. Awwa Research foundation. CEC-500-2004-901.
- Mohan, S.V., Mohanakrishna, G., and Sarma, P.N. (2008) Integration of acidogenic and methanogenic processes for simultaneous production of biohydrogen and methane from wastewater treatment. *International Journal of Hydrogen Energy*. 33(9), 2156 – 2166.
- Munch, E., Barr, K. (2001) Controlled struvite crystallisation for removing phosphorus from anaerobic digester sidestreams. *Water Research*. 35(1), 151–159.
- Münch, E.V., and Barr, K. (2001). Controlled struvite crystallisation for removing phosphorus from anaerobic digester sidestreams. *Water Resource*. 35(1), 151-159.
- Rossi, L., Lienert, J., and Larsen, T.A. (2009) Real-life efficiency of urine source separation. *Journal of environmental management*. 90(5), 1909-1917.
- Salehizadeh, H., and Van Loosdrecht, M.C.M. (2004) Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance. *Biotechnology Advances*. 22(3), 261-179.
- Sayyed, R.Z., Gangurde, N.S., and Chincholkar, S.B. (2009) Hypochlorite digestion method for efficient recovery of PHB from *Alcaligenes faecalis*. *Indian J Microbiology*. 49(3), 230–232.

- Shu, L., Schneider, P., Jegatheesan, V., and Johnson, J. (2006) An economic evaluation of phosphorus recovery as struvite from digester supernatant. *Bioresource Technology*. 97(17), 2211-2216.
- Ting, C.H., Lin, K.R., Lee, D.J., and Tay, J.H. (2004) Production of hydrogen and methane from wastewater sludge using anaerobic fermentation. *Water Science and Technology*. 50(9), 223–228.
- US EPA, (1992). Sequencing batch reactors for nitrifications and nutrient removal, U.S.Environmental Protection Agency, EPA 832 R-92-003.
- US EPA, (2004). Guidelines for water reuse, U.S.Environmental Protection Agency, EPA 625 R-04-108.
- US EPA, (2007) Water treatment plant operator on-site technical assistance training program - 104(g)(1). End of fiscal year 2007 accomplishment report. United States Environmental Protection Agency Office of Water, Office of Wastewater Management, Municipal Assistance Branch.
- US EPA, (2012). Planning for sustainability. U.S.Environmental Protection Agency, EPA 832 R-12-001.
- US EPA, (2013). Energy efficiency in water and wastewater facilities. U.S.Environmental Protection Agency.
- USGS, 2009. Estimated use of water in the United States in 2005. US Geological Survey, Reston, VA.
- Wu, D., Yang Z., and Tian, G. (2011) Inhibitory effects of Cu(II) on fermentative methane production using bamboo wastewater as substrate. *J Hazard Mater*. 195, 170-174.
- Wu, Q., Huang H., Hu, G., Chen, J., Ho, K.P., and Chen, G.Q. (2001) Production of poly-3-hydroxybutrate by *Bacillus* sp. JMa5 cultivated in molasses media. *Antonie van Leeuwenhoek, Int J Gen Mol Microbiol*. 80, 111-118.

CHAPTER 3. IMPACT OF OPERATIONAL STRATEGIES AND CARBON SOURCES ON POLY-HYDROXYBUTYRATE (PHB) ACCUMULATION AND NUTRIENT REMOVAL IN SEQUENCING BATCH REACTOR

Abstract

To make wastewater treatment plants more sustainable, one of the approaches is to recover nutrients and carbon from the wastewater. Since many treatment facilities are implementing biological nutrient removal system, poly-hydroxybutyrate (PHB) a biopolymer, which is synthesized in phosphorus accumulating organisms, can be recovered and harvested as an useful product. However the carbon in municipal wastewater is low in the range of 100 - 200 mg-C/L, which may be insufficient for significant PHB accumulation in the biomass. The objectives of the study were to evaluate the feasibility of combining wastewater treatment and PHB production in a sequencing batch reactor by adding various carbon sources. Three operational conditions, where the react phase of a 6-hour cycle was divided into anaerobic and aerobic sequences, were investigated to maximize PHB accumulation and wastewater treatment. Results showed that dividing the react cycle into two rounds of 45 minutes anaerobic period followed by a 2 hour aerobic period each and adding 1200 mg-C/cycle acetate gave PHB content of $37.4 \pm 4.1\%$. Acceptable wastewater treatment effluent quality was achieved in all three operational conditions. Addition of 800 mg-C/cycle (as in volatile fatty acids (VFA)) of fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage gave a % PHB concentration of 24.3% (wt./wt.) in the dry biomass and a %TP concentration of 8.6% (wt./wt.) in the dry biomass. The results showed that that it is possible to recover PHB and phosphorus by using corn stillage as a carbon source and at the same time treat municipal wastewater.

3.1 Introduction

Recycle of limited material resources and use of renewable energy are some of the strategies in realizing the sustainability of the planet earth. In last two decades, there is a paradigm shift in viewing municipal wastewater as a resource rather than a waste product. Municipal wastewater contains organic matter and nutrients, such as nitrogen phosphorus and potassium (Jenssen et al., 2007). Recovery of nutrients and energy has been practiced for a long time, but recovery of useful organic products such as poly-hydroxyalkanoate (PHA) in recent years have attracted interest.

Conventional plastics are mainly derived from petroleum oil. As a raw material for numerous products, plastics play an important role in our daily life. The annual world production of plastics was over 250 million tons/year in 2011 (PlasticsEurope, 2012). Since petrochemical plastics are not biodegradable, disposal of plastic products is a huge problem. Some countries have enacted various policies to reduce the use of plastics. A substitute material that is biodegradable is needed to replace the conventional plastic polymers made from petroleum oils.

PHA is a polymer material made naturally in microbial cells. PHAs can be produced by renewable organic sources making the manufacturing process a sustainable process. However, the cost of producing PHAs is higher than synthesizing plastic polymers from petroleum oil. Research indicates that almost 30% of the total PHA production cost is due to the cost of the carbon source (Salehizadeh et al., 2004). Municipal wastewater is a possible low-cost source of carbon for the synthesis of PHAs. With more stringent regulation for nitrogen and phosphorus discharge, many municipal wastewater treatment plants are implementing biological nutrient removal systems to treat their wastewater. These treatment plants can be used to produce PHAs and at the same time treat the wastewater. However, the carbon content in municipal wastewater is rather dilute (≤ 100

mg/L as carbon) and supplemental carbon may need to be added.

Sequencing batch reactor (SBR) is a widely applied facility for wastewater treatment and has proven to be effective in the accumulation of PHAs. The SBR process sequences can be manipulated to induce feast/famine for PHA synthesis and allow addition of separate carbon sources at different times (Coats et al., 2011). However, there is not much research that focuses on combining municipal wastewater treatment with PHA production. If both goals can be achieved, it will advance the sustainability of wastewater treatment plants.

The operational strategy of the SBR is one of the key factors which can affect PHA production. To optimize feast and famine process, the SBR sequences can be changed from anaerobic to aerobic condition accordingly. However, optimizing the feast/famine sequence may have a negative effect on the original purpose of removing COD and nutrients in the wastewater.

Several researchers have shown that using municipal wastewater as a carbon source cannot accumulate sufficient PHA in the biomass. Additional carbon source is needed. Acetate is widely used for PHA production. For instance, Chua et al. (2003) found that municipal wastewater supplemented with acetate could accumulate PHA up to 30% of sludge dry weight. Liquor from the fermented activated sludge has been used with an accumulation of 0.23-0.31 mg PHA per mg in biomass per mg COD (Coats et al., 2011). Corn stillage is another material which can be used as a carbon source. But not much work has been done with corn stillage. Eskicioglu et al. (2011) found that the VFA concentration can be over 5000 mg/L in the thermophilic digestion process of corn stillage. Since corn stillage is a waste product that needs to be disposed of, it can be an inexpensive source of carbon. However, the high phosphorus and nitrogen concentration in corn stillage may be a big challenge for effective treatment of the municipal wastewater and corn stillage. Presence of high total nitrogen may inhibit PHA growth.

The goal of this study is to investigate the accumulation of PHB in the biomass in SBR treating a synthetic municipal wastewater. To optimize production of poly-hydroxybutyrate (PHB), a PHA compound, the operational conditions of the SBR was varied. In addition, different external carbon sources were added. The specific objectives of the study are:

1. Determine the impact of operation strategies on PHB accumulation and wastewater treatment by changing the anaerobic/anoxic/aerobic sequences of an SBR.
2. Determine the impact of adding additional carbon sources with different concentrations on PHB accumulation and wastewater treatment.

3.2 Methods and Materials

3.2.1 Source of biomass and wastewater composition

Sludge from the aeration tank of a municipal wastewater treatment plant in Boone, IA was used as the seed for the SBR. The composition of the synthetic municipal is presented in Table 3.1 (Brown et al., 2011). The COD of the synthetic wastewater was about 500 mg/L.

3.2.2 Sequencing batch reactor and operational conditions

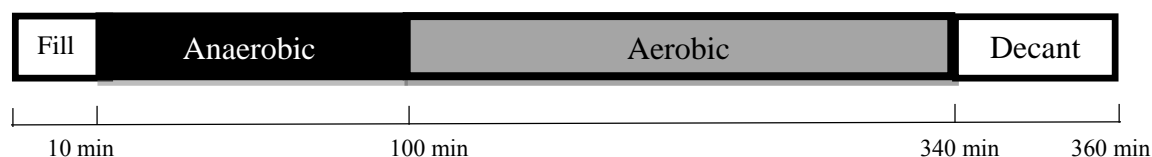
The SBR was made of plexi-glass with a working volume of 4 L. The SBR has a diameter of 3.5 inches and a water depth of 35 inches. The SBR was operated with a 6 hour cycle and 5 days SRT at a room temperature of $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The TSS in the reactor was maintained at approximately $5,000 \pm 500$ mg/L throughout the entire experiment. The SBR cycle consisted of 10 min of fill, 330 min of react and 20 min of decant. To investigate the optimal operational strategies for PHB production, three different react phases were investigated by turning the air in and off in the SBR. The three different react phases are given in Figure 3.1. In Condition A, the air off

(anaerobic) phase was 1.5 hour followed by a 4 hours air on (aerobic) process. In Condition B, the anaerobic phase was 45 minutes followed by a 2 hour aerobic period and these two phases were repeated. For Condition C, there were three repetition of a 30 minutes anaerobic phase followed by an 80 minutes aerobic phase. For all three conditions, the total anaerobic time was 90 minutes and the total aerobic time was 4 hour. The purpose of having different anaerobic and aerobic react time was to create a feast/famine condition to promote PHA accumulation. The phases of the SBR were controlled by ChronTrol controllers (XT4, ChronTrol Corp., San Diego, CA). Under anaerobic condition, the wastewater was recycled from the top to bottom to keep the wastewater and sludge mixed. The aerobic condition was maintained by supplying air at a rate of 2.5 L/min. For each cycle, 2.5 L supernatant was decanted at the end of the cycle by a peristaltic pump (Model No. 7553-20, Cole-Parmer, IL) and approximately 2.5 L of synthetic wastewater and additional carbon source were added at the beginning of each cycle and additional carbon source at the start of the anaerobic phases as described later.

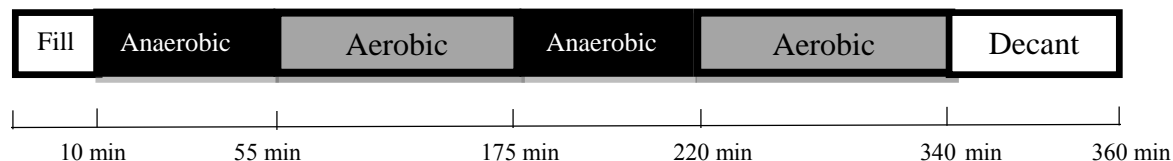
3.2.3 PHB production batch experiments

Two additional carbon sources, acetate and fermented corn stillage, were added to supplement the carbon of the synthetic municipal wastewater. The additional carbon sources were added at the beginning of each anaerobic phase.

(a) Condition A



(b) Condition B



(c) Condition C

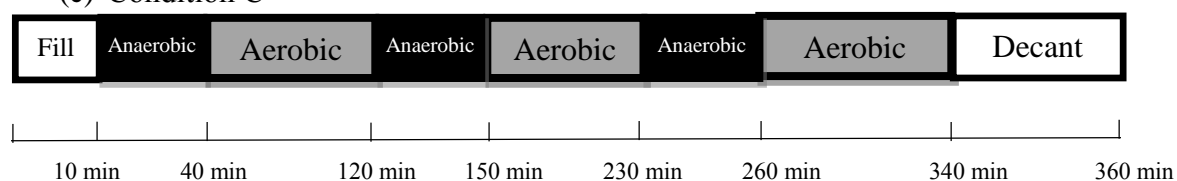


Figure 3.1 Operational conditions for SBR; (a) Condition A; (b) Condition B; (c) Condition C

Table 3.1 Synthetic wastewater composition

	Chemicals/Parameters	Concentration (mg/L)
Ingredients	Calcium sulfate	40
	Ferric chloride	3
	Isomil (Simulac™)	20 mL (1% by volume)
	Magnesium sulfate	4
	Nutrient broth	250
	Potassium chloride	5
	Sodium bicarbonate	63
	Sodium biophosphate monobasic	60
	Sodium citrate	500
Final composition	COD (mg/L)	489
	TOC (mg/L)	158
	Suspended solids (mg/L)	23.4
	Total nitrogen (mg/L-N)	47.6
	Nitrate (mg/L-N)	0.3
	Ammonia (mg/L-N)	24.5
	Total phosphorus (mg/L – P)	15.8
	pH	7.25

Four different amounts of acetate, 400 mg-C/cycle, 800 mg-C/cycle, 1200 mg-C/cycle and 1600 mg-C/cycle, were added into the SBR. The acetate solution was made by dissolving different amount of sodium acetate trihydrate (MW =136.08) in deionized water. In Condition A, the total amount of acetate was added once at the beginning of anaerobic phase. In Condition B and C, the total amount of acetate was added as two and three equal parts, respectively, at the beginning of each anaerobic phase.

Corn stillage was obtained from Lincolnway Energy, LLC (Nevada, IA). The corn stillage was fermented in a digester operating at $35\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$. Two liters of corn stillage and 6 liters of activated sludge from a wastewater treatment plant (Boone, IA) were added to the digester. The activated sludge used had a total solids concentration of 17,380 mg/L. The contents was fermented for 6 days to maximize VFA production. The composition of the corn stillage and the fermented corn stillage are presented in Table 3.2. Four different amounts of carbon masses of fermented corn stillage (based on VFA concentration which was assumed to be mainly made up of acetate), were added to the SBR. The amounts added were 200 mg-C/cycle, 300 mg-C/cycle, 400 mg-C/cycle and 500 mg-C/cycle (based on VFA).

Since the fermented corn stillage contained high concentrations of nitrogen and phosphorus which may affect PHB production and the final effluent quality, part of the TN and TP were reduced first by precipitation before fermentation. TN and TP were precipitated using magnesium hydroxide. Magnesium chloride (112.34g) was dissolved in 200 mL of deionized water and added to 4 L of thin corn stillage. The Mg : P ratio was kept at 1.6 : 1 and the pH was maintained at 9 by adding 2 N sodium hydroxide solution. After precipitation, the corn stillage was stored for one day at $4\text{ }^{\circ}\text{C}$ to allow the solids to settle. Two L of precipitated supernatant were added to digester along with 2.5 L of activated sludge to obtain a F/M ratio of 2. The pH of the digester was adjust to 7 by

Table 3.2 Composition of corn stillage and fermented corn stillage

Parameter	Corn stillage	Fermented corn stillage
COD (mg/L)	105,000	22,000
TOC (mg/L)	3,5600	7,600
Volatile Fatty acids (VFA) (mg/L)	849	1,680
Suspended Solids (mg/L)	28,900	90
Total Nitrogen (mg-N/L)	4,100	520
Nitrate (mg-N/L)	200	320
Ammonia (mg-N/L)	300	140
Total phosphorus (mg/L)	2,520	2,140
pH	5.2	4.8

Table 3.3 Composition of corn stillage, Mg(OH)₂-precipitated corn stillage and fermented Mg(OH)₂-precipitated corn stillage

Parameter	Corn stillage	Mg(OH) ₂ -precipitated corn stillage	Fermented corn stillage
COD (mg/L)	89000	52,000	19,000
TOC (mg/L)	32,300	20,000	10,700
Volatile Fatty acids (VFA) (mg/L)	758	732	2,785
Suspended Solids (mg/L)	26,300	100	90
Total Nitrogen (mg-N/L)	4,100	1,100	420
Nitrate (mg-N/L)	200	5	5.4
Ammonia (mg-N/L)	300	190	265
Total phosphorus (mg-P/L)	6,100	1680	900
pH	5.6	7.2	5.9

adding 2 N sodium bicarbonate solution. The digester was operated at $35\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and was maintained for 6 days before supernatant was withdrawn. Table 3.3 shows the composition of corn stillage after precipitation and after digestion. Three different amounts of carbon mass of fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage (based on VFA concentration which was assumed to be mainly made up of acetate), 200 mg-C/cycle, 400 mg-C/cycle and 800 mg-C/cycle, were added to the SBR.

3.2.4 Analysis methods

COD of samples were measured according to Method 5220 of the Standard Methods (APHA, 2005). Total nitrogen (TN), total phosphorus (TP), ammonia and nitrate concentrations were measured using HACH test kits (Loveland, CO). The HACH test method numbers are given in Table 3.4. The total phosphorus in the biomass of the SBR was measured using the sulfuric acid-nitric acid digestion method combined with vanadomolybdophosphoric acid colorimetric method (Method 4500-P, C) of the Standard Methods (APHA, 2005). The VFA concentrations of corn stillage and fermented corn stillage were determined by the distillation method (Method 5560, C) of the Standard Methods (APHA, 2005). pH of the wastewater was measured using an Orion 9207BN pH/ATC Triode (Thermo Scientific, Waltham, MA). Total organic carbon was measured by a Shimadzu TOC-Vws TOC Analyzer (Shimadzu Scientific Instrument, Columbia, MD).

PHB content was measured by a gas chromatograph mass spectrometer (GC). Approximately 30 mL of biomass was collected from the SBR at a specific time and centrifuged. Approximately 5 mL of the centrifuged biomass was transferred and frozen in a 5 mL vial. The frozen samples were freeze-dried by a freeze-dry machine (Model FD-3-54, SP Scientific, Stone Ridge, NY) at 2000 millitorr and $-40\text{ }^{\circ}\text{C}$ for at least 12 hours. Ten mg of each dried samples was

measured and transferred to a 10 mL tube. Two mL of 3% acidified methanol and two mL of chloroform were added and the vials sealed and heated at 105 °C for 2 hours to digest and extract the PHB in the dried biomass. Poly [R-3-hydroxybutyric acid] was used as a standard by using the same preparation method. After digestion and extraction, 5 mL deionized water was added to each tube. The tube were mixed vigorously and centrifuged for 10 minutes at 2000 rpm. After centrifuging, PHB was dissolved in 2 mL of chloroform. Approximately 1 mL of the dense chloroform was transferred to 1.5 mL GC vials and further diluted with chloroform if necessary. PHB were measured using an Agilent 7890A gas chromatograph mass spectrometer (GC-MS) with a 5975C MSD detector (Aglient Technologies, Santa Clara, CA) by injecting 1 µL samples into the GC. The gas chromatograph was equipped with a HP-5ms column, 30 m (length) × 0.25 mm (diameter) × 0.25 µm (film), (Aglient Technologies, Santa Clara, CA). The GC analytical run took 18.1 minutes. The start temperature was 45 °C for 2 min, then raised at 5 °C/min to 65 °C, holding for 4 min, followed by a ramp of 50 °C/min to 320 °C, and then holding for 3 min.

Table 3.4 Hach methods used

Regent	Hach Method Number
Total Phosphorus (TP)	10127
Total Nitrogen (TN)	10072
Ammonia Nitrogen (NH ₃ -N)	10031
Nitrate Nitrogen (NO ₃ -N)	10020

3.3 Results and Discussions

3.3.1 Impact of different operational conditions on PHB accumulation

Using synthetic municipal wastewater only, the COD and nutrient removal and PHB accumulation in the biomass for three operational conditions are provided in Table 3.5. The results showed that for all three operational conditions, the average COD removal efficiencies were greater than 94% and were statistically similar. The effluent TN, ammonia and TP concentration were less than 3 mg/L, 1.3 mg/L as N and 2 mg/L as P, respectively, for all three operational conditions. Conditional B appeared to show the highest percent of ammonia and TP removal. In addition, PHB in the biomass was the highest for Condition B. All three conditions showed that the SBR can achieve TN concentration of less than 10 mg/L as N but not a TP concentration of 1 mg/L as P as typically required of a wastewater treatment plant with a permit limit of 10 mg/as N and 1 mg/L as P.

Table 3.5 Nutrient removal and PHB accumulation under different operational conditions with synthetic municipal wastewater only

	Condition A	Condition B	Condition C
PHB in biomass (% d.w)	0.43%	1.16%	0.81%
Average effluent COD (mg/L)	13±2	19±2	20±5.5
Average COD removal efficiency (%)	96.1±0.6	94.3±0.6	94.1±1.5
Average effluent TN (mg/L as N)	2.73±0.15	2.21±0.12	3±0.29
Average TN removal efficiency (%)	80.2±2.6	84.1±2.9	78.4±3.1
Average effluent ammonia (mg/L as N)	1.23±0.15	0.6±0.2	1.1±0.21
Average ammonia removal efficiency (%)	73.9±1.9	87.6±4.3	76.8±5.2
Average effluent TP (mg/L as P)	1.63±0.12	1.33±0.15	1.78±0.1
Average TP removal efficiency (%)	78.2±1.1	82.2±2.1	76.3±1.5

The results also showed that using municipal wastewater only, the SBR cannot accumulate a high percent of PHB in the biomass because of the low carbon content of the wastewater.

3.3.2 Impact of acetate addition on PHB storage

The percent of PHB in the biomass for the synthetic wastewater and acetate addition for three operational conditions are shown in Figure 3.2. All three operational conditions showed that increasing the concentration of acetate resulted in an increase in the percent of PHB in the biomass. Condition A and Condition C showed a linear increase in the percent of PHB for an increase in acetate concentration. The highest PHB percent achieved was for 1600 mg-C/cycle of acetate at $28.2\% \pm 0.2\%$ and $31.9\% \pm 1.1\%$ for Condition A and Condition C, respectively. Condition B was the best operational condition of three conditions tested. For an acetate concentration of 400 mg-C/cycle, the % PHB in the biomass was $14.6\% \pm 1.3\%$ which was significantly different and higher than the % PHB for Condition A and Condition C. The highest % PHB were $37.4\% \pm 4.1\%$ and $32.9\% \pm 2.1\%$ for 1200 mg-C/cycle acetate and 1600 mg-C/cycle acetate, respectively. An acetate concentration of 1600 mg-C/cycle did not further enhance the % PHB in the biomass. This may indicate that the PHB accumulation in the biomass may have reached its limits. Repeat of two sets of experimental runs (as shown in Figure 3.2) after about 2 months later showed that the results of PHB accumulation were reproducible.

Chua et al. (2003) obtained PHB content of 30% in the biomass (dry weight) with 30 mg/L acetate addition and up to 20% of biomass (dry weight) with municipal wastewater only in an anaerobic/aerobic SBR. Coats et al. (2011) obtained 12.5% to 28.7% PHB using primary solids fermenter liquor in an SBR. These experiments obtained similar PHB content as in this experiment. Coats et al. (2007) also showed that PHA storage can be as high as 53% (wt./wt.) in the aerobic

tank with fermenter liquor as the carbon source. The higher PHA percent in the biomass in the Coats et al. experiments may be due to the higher F/M ratio (approximately 0.75) in their experiments. In our experiment, the highest F/M ratio was approximately 0.13 for the 1200 mg-C/cycle acetate addition for Condition B. Condition B most probably provided the better anaerobic and aerobic sequence times for the amount of carbon added to maximize the feast and famine process to accumulate PHBs. Feast and famine cycling can be divided into two parts, successive periods of substrate availability (feast period and no external substrate availability (famine period) (Salehizadeh et al., 2004). In Condition B, the anaerobic sequence of 45 minutes provided adequate time for PAOs to uptake the carbon and convert to PHB. The aerobic sequence of two hours was sufficient to avoid extra PHB consumption allowing the PHB to build up within the biomass. Additionally, dividing the feeding of additional carbon into two times may optimize the feast and famine process.

Condition A may not be ideal due to the long aerobic time which consumed PHB stored to accumulate phosphorus. Moreover, adding all the carbon source at one time at the start of the cycle may not allow the biomass to uptake all the carbon during the 1.5 hour anaerobic period.

For 1600 mg-C/cycle acetate addition, the % PHB in the biomass were statistically similar for all three operational conditions. Without occurring extra costs of providing acetate, Condition B with 800 mg-C/cycle of acetate appeared to be appropriate for optimizing PHB accumulation in SBR treating municipal wastewater.

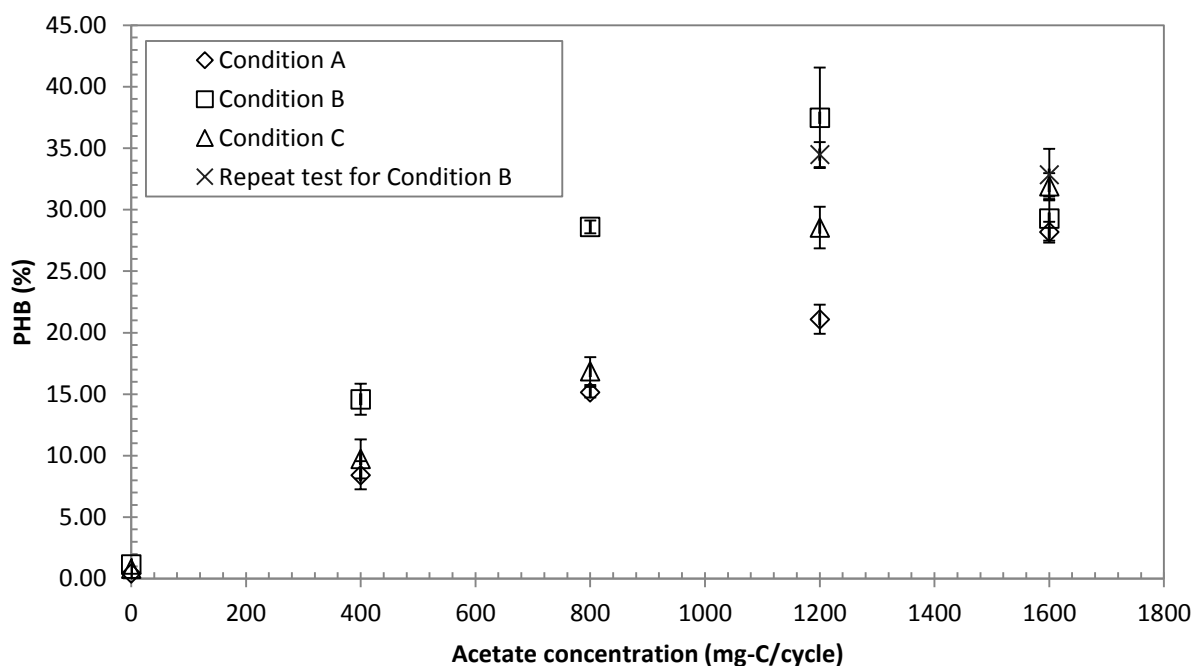


Figure 3.2 Impact of addition of acetate on PHB production for three operational conditions (TOC for municipal wastewater added per cycle was 378 ± 33 mg-C)

3.3.3 Impact of acetate addition on wastewater effluent quality

Figure 3.3 provides the COD, TN and TP concentrations after treatment for all three conditions with different amounts of acetate added. For COD removal, Condition A shows the best effluent concentration. Average COD percent removal ranged from 95.9% to 97.9%, 96.2% to 97.4% and 96.3% to 97.0% for Condition A, Condition B and Condition C respectively for different amounts of acetate added. A probable reason for the better performance of COD removal for Condition A was the longer aerobic sequence time.

All the conditions showed good performance for TP removal with TP concentration less than 1.5 mg/L as P. In fact addition of acetate, gave better TP removal than the removal for synthetic municipal wastewater alone. For all conditions, the % TP removals ranged from 85.3% to 96%, which were higher than TP removals without additional carbon added (ranged from 76.3%

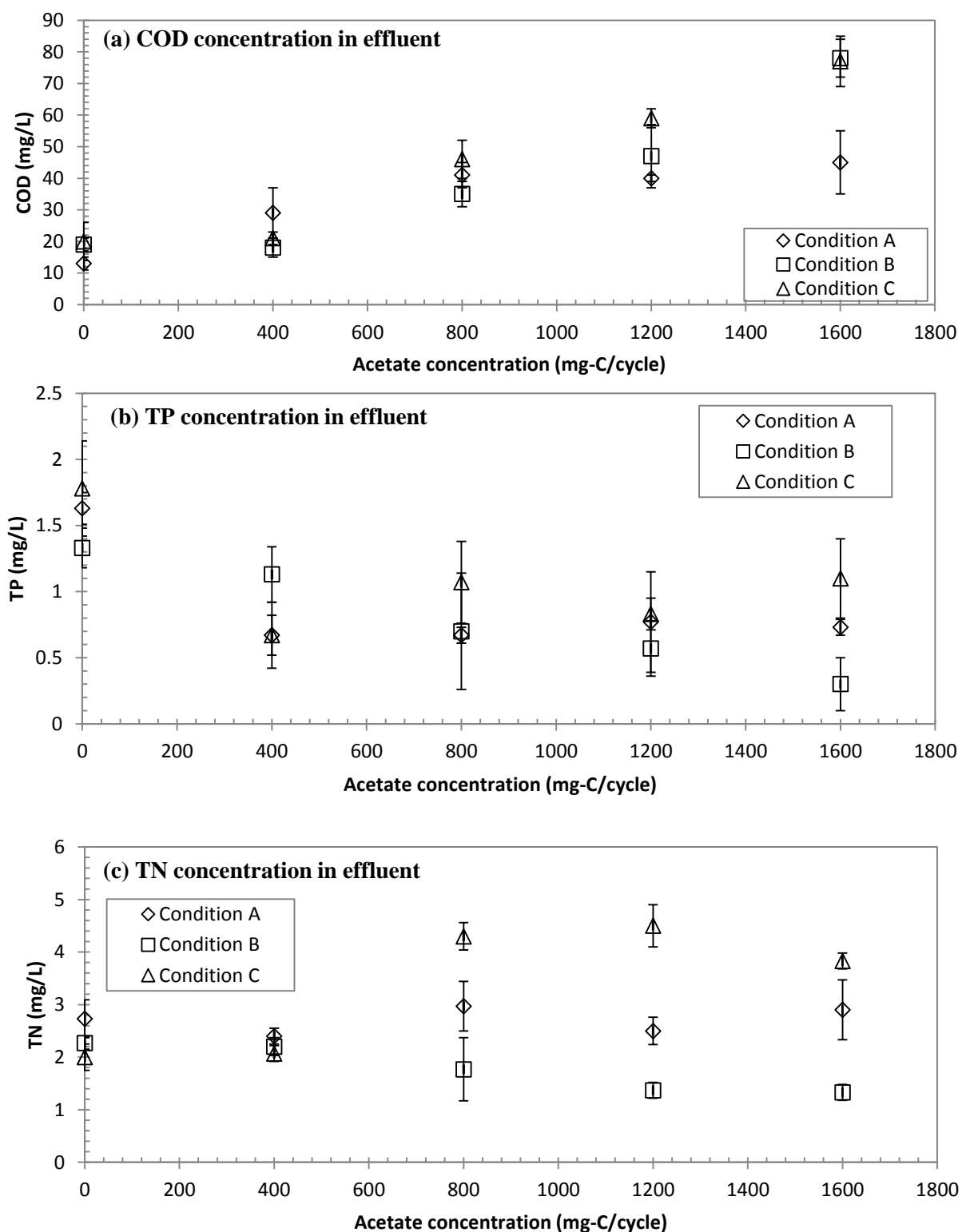


Figure 3.3 Impact of addition of acetate on wastewater treatment, (a) COD concentration in effluent, (b) TP concentration in effluent, (c) TN concentration in effluent (TOC for municipal wastewater added per cycle was 378 ± 33 mg-C)

to 82.2%). The highest TP percent removal (96%) was for Condition B with 1600 mg-C/cycle acetate added, which was in line with optimizing PHB accumulation.

Figure 3.3 (c) shows the total nitrogen concentration in the effluent. Condition B gave the highest TN percent removal at 90.4% with 1600 mg-C/cycle of acetate added to the reactor. TN percent removal for Condition C changed from 85.6% to 72.4% when the amount of acetate added increased. Overall, addition of acetate did not affect the effluent concentration of TN and TP except for COD. This shows that addition of acetate will not affect the treatment of municipal wastewater except for an increase in COD concentration, which was acceptable if 800 mg-C/cycle of acetate was used for operational Condition B.

The total phosphorus in the biomass was found to be $4.4\% \pm 1.2\%$ (dry weight) for 1200 mg-C acetate addition in Condition B. The results confirmed phosphorus uptake in the biomass which can be recovered along with PHB.

In general, with the right amount of acetate added the final COD concentration in the effluent can be within acceptable effluent discharge limits. Additional acetate also enhanced TP removal. Considering TN and TP removal and PHB accumulation, Condition B appeared to show the best results.

3.3.4 Concentration Profiles for Condition B

Figure 3.4 shows the concentration profiles of PHB, TP, COD, TN, ammonia and nitrate of Condition B with 1200 mg-C/cycle acetate added. For the first anaerobic condition just after the fill sequence, COD concentration decreased with time and % PHB in the biomass increased to as high as 37% with release of phosphorus as indicated by an increase in TP in solution (as high as 20 mg/L). For the first aerobic sequence after the anaerobic sequence, TP in solution decreased to

about 5 mg/L indicating uptake of phosphorus by the biomass. At the same time, the % PHB in the biomass decreased accordingly along with the COD in the wastewater solution. When more acetate was added in the second anaerobic sequence, the COD increased accordingly and during the anaerobic sequence, % PHB increased. While the TP was released as shown by the increase in TP in solution for the second aerobic sequence, the change in % PHB in the biomass was not as steep as in the first aerobic sequence. This may be due to the TP concentration in solution which was about one third of the concentration at the start of the cycle. TN and ammonia showed a large change in the aerobic sequence as compared to the anaerobic sequence. Nitrate was shown to increase in the aerobic sequences which was due to nitrification.

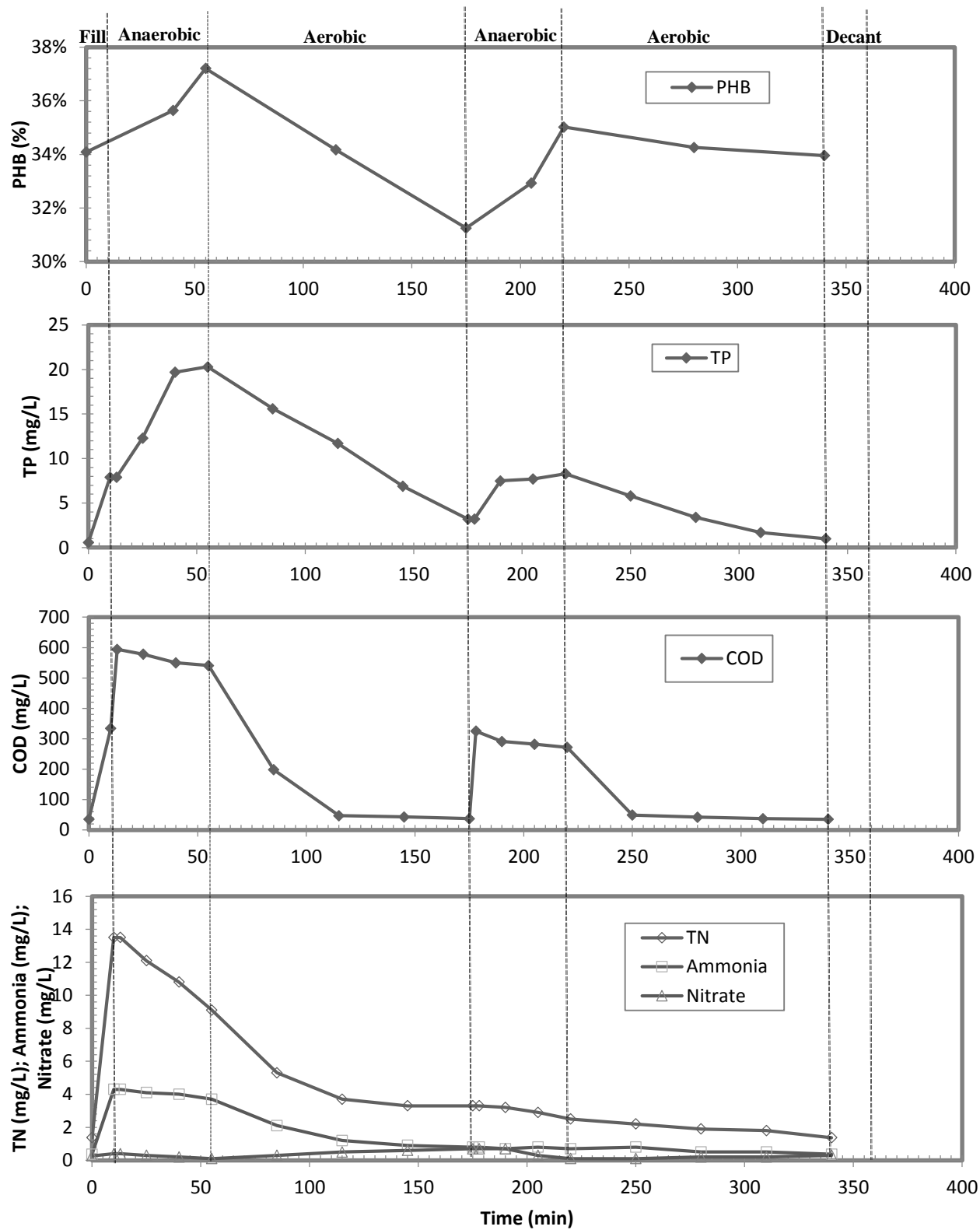


Figure 3.4 Concentration profiles for Condition B with the addition 1200 mg-C/cycle acetate (2.5 L of synthetic wastewater added during fill; 120 mL of acetate added at the start of each of the anaerobic sequence)

3.3.5 Impact of fermented corn stillage added on PHB storage and effluent quality

Figure 3.5 shows the % PHB in the biomass by adding fermented corn stillage and fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage. Operational condition for the tests was Condition B. The average PHBs in the biomass were 7.5%, 12.7% and 24.3% of dry weight for the addition of 200 mg-C/cycle, 400 mg-C/cycle and 800 mg-C/cycle (based on VFA concentration) respectively of fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage. For fermented corn stillage, the % PHBs were 7.3% and 9.7% for the addition of 200 mg-C/cycle and 400 mg-C/cycle (based on VFA concentration), respectively. The results showed use of fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage performed slightly better than fermented corn stillage. One possible reason is that fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage had higher VFA concentration and lower TN and TP concentration. Precipitation of corn stillage removed part of large molecular weight carbon compounds, making it easier to ferment the stillage. In comparison with the same amount of acetate-C (Figure 3.2), the % PHB in the biomass using fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage was lower by about 5%. This may be due to the higher nitrogen concentration in the fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage which may inhibited PHB storage.

COD concentration in the effluent (Figure 3.6) was as high as 96 mg/L and 145 mg/L for an addition of 200 mg-C/cycle fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage and 200 mg-C/cycle fermented corn stillage, respectively. The COD concentration increased with the amount of fermented corn stillage added. When 800 mg-C/L fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage was added, COD concentration in the effluent reached 310 mg/L.

TP concentrations in the effluent were 91.7 mg/L and 10.4 mg/L for the addition of 400 mg-C/cycle for fermented corn stillage and fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage, respectively (Figure 3.7a). Precipitation of phosphorus in corn stillage before fermentation

effectively reduced the additional phosphorus concentration supplied into reactor, which resulted in a lower TP concentration in the effluent.

The higher total phosphorus in the fermented corn stillage resulted in higher phosphorus percent concentrations in the biomass, i.e., 6.5%, 7.0% and 8.6% for 200 mg-C/cycle, 400 mg-C/cycle and 800 mg-C/cycle fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage, respectively. Since the TP concentration in fermented corn stillage was higher, TP concentration in the biomass was as high as 8.9% for 400 mg-C/cycle of fermented corn stillage added. The phosphorus in biomass can be recovered, making the wastewater treatment plants more sustainable.

Total nitrogen concentrations in the effluent were high at concentrations of 9.3 mg/L and 9.5 mg/L for the addition of 400 mg-C/cycle of fermented corn stillage and fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage, respectively (Figure 3.7b). For an addition of 800 mg-C/cycle of fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage, the TN concentration was as high as 19.2 mg/L.

Since corn stillage need to be disposed of, fermented corn stillage can be a good carbon resource for PHB production in municipal wastewater treatment plants. The relatively low cost of corn stillage may be feasible as an additional carbon source. However, the final effluent quality may be impacted by the addition of corn stillage and a secondary treatment unit may be needed to further treat the wastewater.

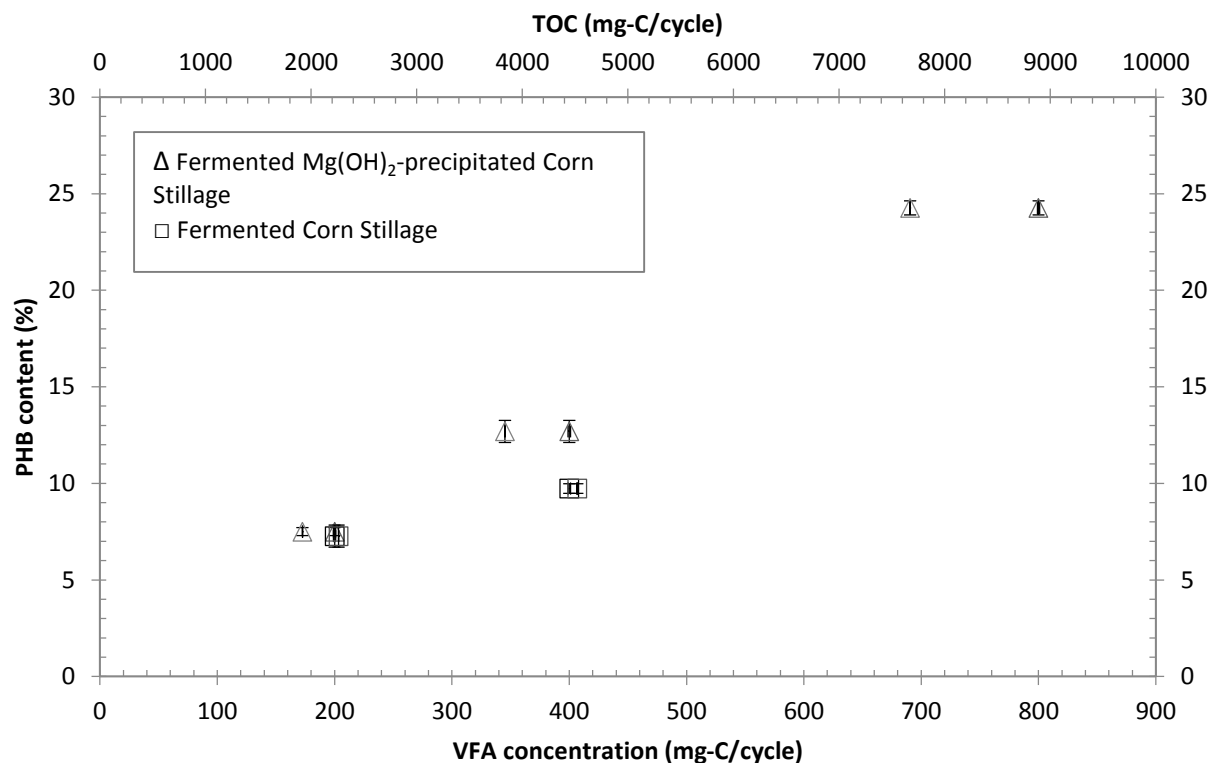


Figure 3.5 Impact of addition of fermented corn stillage on PHB production in Condition B

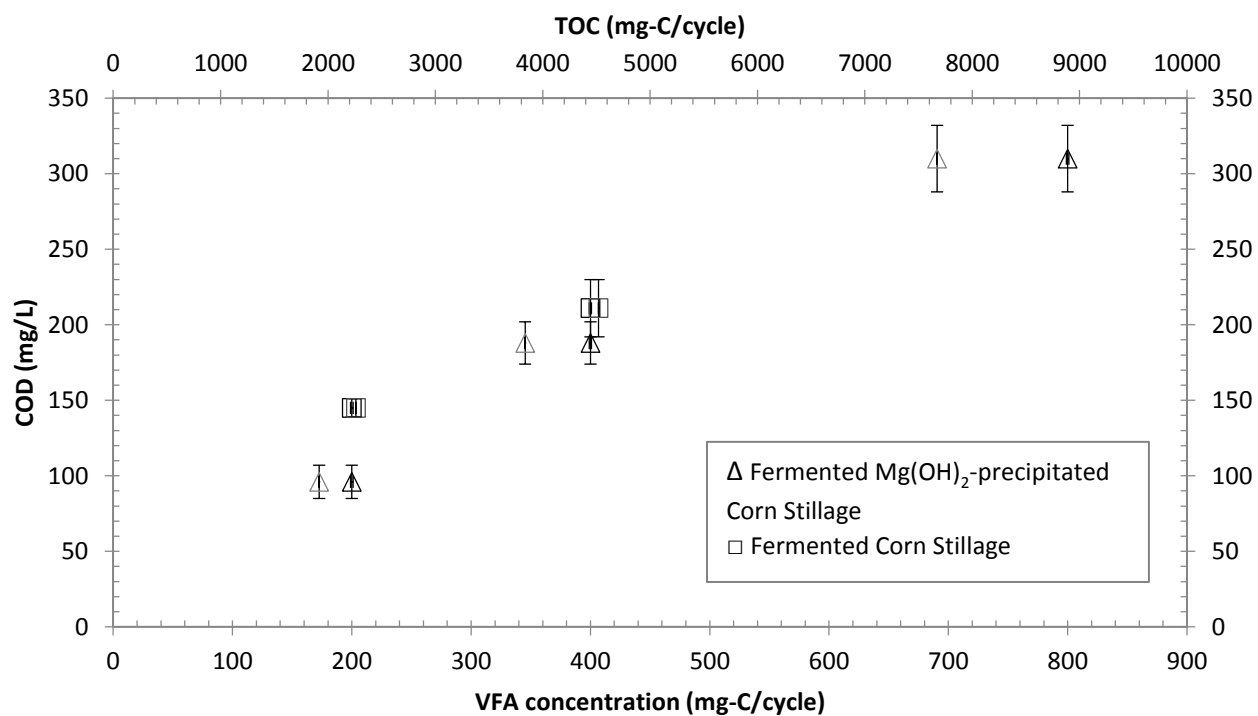


Figure 3.6 Impact of addition of fermented corn stillage on COD removal in Condition B

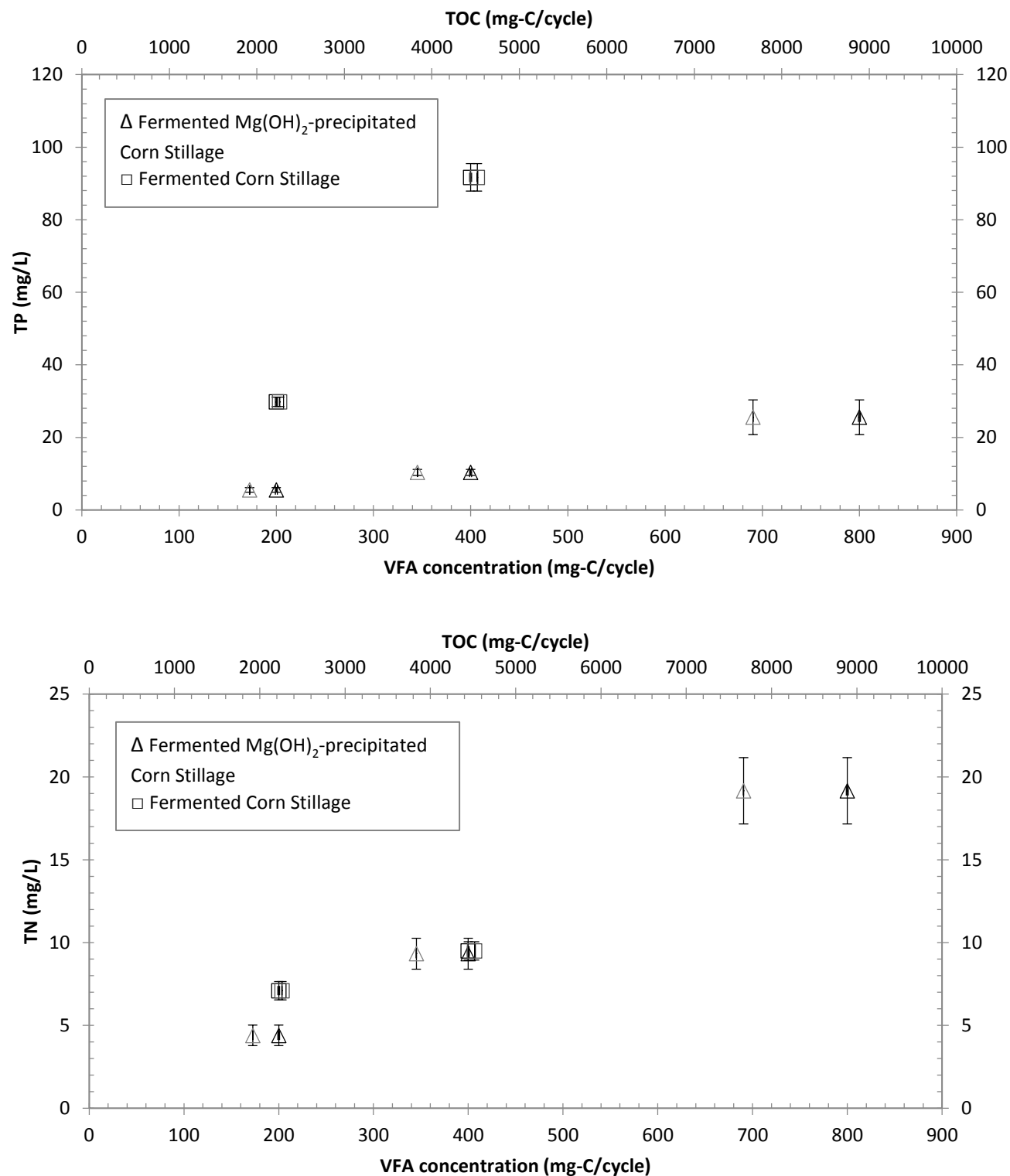


Figure 3.7 Impact of addition of fermented corn stillage on TP and TN removal in Condition B, (a) TP concentration in effluent, (b) TN concentration in effluent

3.4 Conclusion

Operational Condition B, where the react phase of 330 minutes was divided into two rounds of 45 minutes anaerobic sequence and 2 hour aerobic sequence each, gave the better % PHB content in the biomass. Percent PHB achieved within this condition was $37.4\% \pm 4.1\%$ with the addition of 1,200 mg-C of acetate per cycle. With the addition of acetate, nutrient removal improved for the treatment of municipal wastewater for all three operational conditions.

Fermented corn stillage with a high VFA concentration was used as an additional carbon source. The highest % PHB in the biomass obtained was 24.3% of biomass (dry weight) with the addition of 800 mg-C/cycle of fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage. At the same time the %TP content in the biomass was 8.2% for the addition of 800 mg-C/cycle fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage and 8.9% for the addition of 400 mg-C/cycle fermented corn stillage. These results showed the possibility of recovering both PHB and phosphorus together from the wastewater treatment process. However, using corn stillage resulted in high TN, TP and COD concentration in the effluent. Removing part of phosphorus and COD before fermentation by precipitation with magnesium hydroxide was an effective way in reducing the TP and TN concentrations in the corn stillage.

In summary, PHB production combined with municipal wastewater treatment in a sequencing batch reactor is possible with the addition of a carbon source such as industrial wastewaters. This approach can make the wastewater treatment process more sustainable.

3.5 References:

Chua, A.S.M, Takabatake, H., Satoh, H., and Mino, T. (2003) Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of PH, sludge retention time, and acetate concentration in influent. *Water Research*. 37(15), 3602-3611.

- APHA, AWWA and WEF, (2005) Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.
- Coats, E.R., Loge, F.J., Wolcott, M.P., Englund, K., and McDonald, A.G. (2007) Synthesis of polyhydroxyalkanoates in municipal wastewater treatment. *Water Environment Research*. 79(12), 2396-2403.
- Coats, E.R., VandeVort K.E., Darby J.L., and Loge F.J., (2011) Toward polyhydroxyalkanoate production concurrent with municipal wastewater treatment in a sequencing batch reactor system. *Journal of Environmental Engineering*. 137 (1), 46-54.
- Eskicioglu, C., Kennedy, K.J., Marin, J., and Strehler, B. (2011) Anaerobic digestion of whole stillage from dry-grind corn ethanol plant under mesophilic and thermophilic conditions. *Bioresource Technology*. 102(2), 1079-1086.
- Jenssen, P.D., Vrale, L., and Lindholm, O. (2007) Sustainable wastewater treatment. International conference on natural resources and environmental management and environmental safety and health. Kuching, Malaysia. November 27-29, 2007.
- PlasticsEurope. (2012) *Plastics-the Facts 2012*. An analysis of European plastics production, demand and waste data for 2011. Frankfurt, Germany.
- Rudolph, B. (1994) Treasure from trash. *Time International*. 144, 66–67.
- Salehizadeh, H., Van Loosdrecht, M.C.M. (2004) Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance. *Biotechnology Advances*. 22(3), 261-179.

CHAPTER 4. GENERAL CONCLUSION

4.1 Conclusion

Recovery of nutrients and carbon from wastewater has been regarded as a promising approach to realize the sustainability of municipal wastewater treatment plants. PHB is a biopolymer that can be produced by phosphorus accumulating organisms. Municipal wastewater, as a cheap carbon source can be used to produce PHB. However, due to the limited carbon concentration in municipal wastewater, addition of other carbon sources may be needed to achieve sufficient % PHB in the biomass. To maximize the % PHB accumulation, wastewater treatment operational condition must be optimized. In this research, the possibility of combining municipal wastewater treatment and PHB production with various operational conditions and the addition of carbon sources in sequencing batch reactor (SBR) was investigated. In Chapter 3, the results showed that Condition B, where the react phase was divided into two rounds of 45 minutes anaerobic period and 2 hours aerobic period each gave % PHB concentration of $37.4\% \pm 4.1\%$, with the addition of 1200 mg-C/cycle of acetate.

Using fermented corn stillage as additional carbon source, the % PHB content in the biomass was found to be lower than that with the same carbon amount of acetate. The % PHB content achieved was 24.3% of biomass (dry weight) with the addition of 800 mg-C/cycle fermented $\text{Mg}(\text{OH})_2$ -precipitated corn stillage. Additionally, the high COD, TN and TP concentration in fermented corn stillage resulted in high TP, TN and COD concentration in final effluent. This can be partially solved by precipitating TP and TN before fermenting the corn stillage. The results showed that after precipitation with $\text{Mg}(\text{OH})_2$, 72.2% total phosphorus and 73.12% nitrogen were removed from corn stillage. The fermented corn stillage also resulted in % TP concentration of 8.2% for the addition of 800 mg-C/cycle fermented $\text{Mg}(\text{OH})_2$ -precipitated corn

stillage and 8.9% TP for addition of 400 mg-C/cycle fermented corn stillage. Fermented corn stillage appeared to be a suitable carbon source for production of PHB and recovery of phosphorus.

In conclusion, it is possible to realize sustainable wastewater treatment by recovering PHB for municipal wastewater treatment process in sequencing batch reactor when an additional carbon source such as corn stillage is added.

4.2 Future Studies

This study shows it is possible to combine PHB recovery and municipal wastewater treatment together. To make the whole process more efficient and feasible, some further studies are needed. They are:

1. The PHB content in the biomass achieved in this study may not be high enough to reduce the cost of PHB production. Some further studies can focus on increasing the PHB in municipal wastewater treatment plants with less carbon source added but further fine-tuning the operational conditions
2. Use of corn stillage showed the possibility of recovering phosphorus and PHB together in municipal wastewater treatment systems. Future studies can investigate use of corn stillage more efficiently for PHB production and to avoid high TP, TN and COD concentration in effluent.
3. Further explore and find a carbon source to reduce the cost of PHB.

**APPENDIX A. EXPERIMENTAL DATA FOR ALL TREATMENT CONDITIONS
WITHOUT ADDITIONAL CARBON SOURCE**

Table A.1 Concentrations for Condition A (for Table 3.5)

	10 min	55 min	100 min	160 min	220 min	280 min	340 min
pH	7.1±0.2						7.3±0.2
TSS (mg/L)	4661±200						
COD (mg/L)	318						11.0
	350	188	164	36	26	12	13.0
	326						15.0
Average COD (mg/L)	331						13.0
TP (mg/L)	7.9						1.7
	7.2	8.9	14.3	22.1	4.2	2.2	1.5
	7.4						1.7
Average TP (mg/L)	7.5						1.6
TN (mg-N/L)	15.2						2.6
	12.1	7.1	7.5	3.6	2.3	2.8	2.7
	14.3						2.9
Average TN (mg-N/L)	13.9						2.7
Ammonia (mg-N/L)	4.5						1.2
	5.1	5.7	5.3	3.1	1.7	1.4	1.4
	4.6						1.1
Average Ammonia (mg-N/L)	4.73						1.2
Nitrate (mg-N/L)	0.40						0.2
	0.50						0.3
	0.30						0.3
Average Nitrate (mg-N/L)	0.40						0.27
PHB (w/w %)		0.90%	1.23%		0.73%		0.43%

Table A.2 Concentrations for Condition B (for Table 3.5)

	10 min	55 min	115 min	175 min	220 min	280 min	340 min
pH	7.2±0.1						7.3±0.1
TSS (mg/L)	4624±130						
COD (mg/L)	318	243	47	36	25	22	17.0
	350						19.0
	326						21.0
Average COD (mg/L)	331						19.0
TP (mg/L)	7.9	11.7	2.7	2.8	3.2	1.8	1.3
	7.2						1.2
	7.4						1.5
Average TP (mg/L)	7.5						1.3
TN (mg-N/L)	15.2	6.5	2.9	2.5	2.2	2.3	2.2
	12.1						2.4
	14.3						2.2
Average TN (mg-N/L)	13.9						2.3
Ammonia (mg-N/L)	4.5	6.1	2.4	1.9	1	0.4	0.2
	5.1						0.4
	4.6						0.6
Average Ammonia (mg-N/L)	3.23						0.4
Nitrate (mg-N/L)	0.40						0.1
	0.50						0.2
	0.30						0.1
Average Nitrate (mg-N/L)	0.40						0.13
PHB (w/w %)		0.97%		1.03%	1.62%		1.16%

Table A.3 Concentrations for Condition C (for Table 3.5)

	10 min	40 min	120 min	150 min	230 min	260 min	340 min
pH	7.2±0.1						7.3±0.1
TSS (mg/L)	4681±150						
COD (mg/L)	318	260	52	56	36	49	20.0
	350						25.0
	326						14.0
Average COD (mg/L)	331						20.0
TP (mg/L)	7.9	10.1	4.2	2.2	1.2	2.5	1.8
	7.2						1.7
	7.4						1.9
Average TP (mg/L)	7.5						1.8
TN (mg-N/L)	15.2	6.7	4	3.1	2.5	3.3	2.2
	12.1						2.4
	14.3						1.7
Average TN (mg-N/L)	13.9						2.0
Ammonia (mg-N/L)	4.5	4.7	3	2.5	0.8	1.7	0.9
	5.1						0.5
	4.6						0.6
Average Ammonia (mg-N/L)	4.73	00					1.1
Nitrate (mg-N/L)	0.40						0.2
	0.50						0.3
	0.30						0.2
Average Nitrate (mg-N/L)	0.40						0.23
PHB (w/w %)		1.24%	0.99%	1.37%	1.14%	1.21%	0.81%

Table A.4 PHB concentrations for Condition A, Condition B and Condition C

Condition A							
Time	10 min	55 min	100 min	160 min	220 min	280 min	340 min
PHB (w/w%)		0.90%	1.23%		0.73%		0.43%
Condition B							
Time	10 min	55 min	115 min	175 min	220 min	280 min	340 min
PHB (w/w%)		0.97%		1.03%	1.62%		1.16%
Condition C							
Time	10 min	40 min	120 min	150 min	230 min	260 min	340 min
PHB (w/w%)		1.24%	0.99%	1.37%	1.14%	1.21%	0.81%

APPENDIX B. EXPERIMENTAL DATA FOR ALL TREATMENT CONDITIONS WITH ACETATE ADDITION

Table B.1 PHB and nutrients concentration in effluent with acetate addition for Condition A (for Figures 3.2 and 3.3)

Acetate (mg-C/ cycle)	0	400	800	1200	1600
TSS (mg/L)		4939±110	5110±90	5310±100	5520±150
PHB content (%)	0.43%	8.41%±1.14%	15.16%±0.43%	21.09%±1.18%	28.18%±0.85%
TN (mg/L)	2.73±0.15	2.40±0.15	2.97±0.47	2.50±0.26	2.90±0.57
TP (mg/L)	1.63±0.12	0.67±0.15	0.67±0.06	0.77±0.38	0.73±0.06
Ammonia (mg-N/L)	1.23±0.15	0.87±0.06	0.77±0.15	0.73±0.06	0.67±0.15
Nitrate (mg-N/L)	0.27±0.06	0.33±0.06	0.70±0.17	0.77±0.25	0.87±0.06
COD (mg/L)	13±2	29±8	41±4	40±1	45±10

Table B.2 PHB and nutrients concentration in effluent with acetate addition for Condition B (for Figures 3.2 and 3.3)

Acetate (mg-C per time)	0	200	400	600	800
Acetate (mg-C/cycle)	0	400	800	1200	1600
TSS (mg/L)		4810±80	5042±100	5213±110	5589±80
PHB content (%)	1.16%	14.59%±1.27%	28.60%±0.53%	37.36%±4.09% 34.48%±1.01%	29.28%±1.79% 32.85%±2.09%
TN (mg/L)	2.27±0.12	2.20±0.17	1.77±0.6	1.37±0.15	1.33±0.15
TP (mg/L)	1.33±0.15	1.13±0.21	0.70±0.44	0.57±0.21	0.30±0.2
Ammonia (mg-N/L)	1.0±0.2	0.87±0.15	0.5±0.26	0.37±0.12	0.33±0.15
Nitrate (mg-N/L)	0.13±0.06	0.23±0.06	0.23±0.06	0.27±0.06	0.33±0.06
COD (mg/L)	19±2	18±3	35±4	47±10	78±6

Table B.3 PHB and nutrients concentration in effluent with acetate addition for Condition C (for Figures 3.2 and 3.3)

Acetate (mg-C per time)	0	135	270	400	530
Acetate (mg-C/cycle)	0	400	800	1200	1600
TSS (mg/L)		4798±100	5050±90	5227±110	5436±90
PHB content (%)	0.81%	9.74%±1.58%	16.88%±1.14%	28.55%±1.69%	31.92%±1.05%
TN (mg/L)	2±0.29	2.07±0.15	4.30±0.26	4.50±0.4	3.83±0.15
TP (mg/L)	1.78±0.1	0.67±0.25	1.07±0.31	0.83±0.12	1.10±0.3
Ammonia (mg-N/L)	1.1±0.21	0.63±0.21	0.9±0.1	0.87±0.06	0.73±0.06
Nitrate (mg-N/L)	0.23±0.06	0.33±0.06	0.83±0.06	1.00±0.1	0.97±0.26
COD (mg/L)	20±5.5	21±2	46±6	59±3	77±8

Table B.4 Volume and concentration of addition of acetate solution (for Table B.1, Table B.2 and Table B.3)

Acetate addition (mg-C/cycle)	Added acetate concentration (mg-C/L)	Added volume (mL/cycle)
400	1690 mg-C/L	240
800	3340 mg-C/L	240
1200	5000 mg-C/L	240
1600	6700 mg-C/L	240

Table B.5 Concentrations for Condition B with the addition 1200 mg-C/cycle acetate (for Figure 3.4)

Time (min)	PHB (w/w)	COD (mg/L)	TP (mg/L)	TN (mg/L)	Ammonia (mg/L)	Nitrate (mg/L)
0	34.09%	32	0.57	1.42	0.3	0.27
10	34.09%	334	7.9	13.5	4.3	0.4
13		594	7.9	13.5	4.3	0.4
25		578	12.3	12.1	4.1	0.3
40	35.64%	550	19.7	10.8	4	0.2
55	37.21%	541	20.3	9.1	3.7	0.1
85		198	15.6	5.3	2.1	0.3
115	34.17%	47	11.7	3.7	1.2	0.5
145		43	6.9	3.3	0.9	0.6
175	31.25%	37	3.2	3.3	0.8	0.7
178		325	3.2	3.3	0.8	0.7
190		291	7.5	3.2	0.7	0.7
205	32.93%	282	7.7	2.9	0.8	0.3
220	35.02%	272	8.3	2.5	0.7	0.1
250		49	5.8	2.2	0.8	0.1
280	34.26%	42	3.4	1.9	0.5	0.2
310		37	1.7	1.8	0.5	0.2
340	33.96%	35	1	1.37	0.37	0.3

APPENDIX C. EXPERIMENTAL DATA FOR ALL TREATMENT CONDITIONS WITH FERMENTED CORN STILLAGE ADDITION

Table C.1 PHB and nutrients concentration in effluent with fermented corn stillage addition for Condition B (for Figure 3.5, 3.6 and 3.7)

VFA (mg-C per time)	0	100	150	200	250
VFA (mg-C/cycle)	0	200	300	400	500
PHB content (%)	1.16%	7.27%±0.57%	8.47%±0.51%	9.73%±0.25%	10.83%±0.31%
TN (mg/L)	1.27±0.12	7.1±0.55	8.00±0.62	9.50±0.55	11.60±1.12
TP (mg/L)	1.33±0.15	29.80±1.3	63.50±12.3	91.67±3.79	116.67±6.1
TP in biomass (%)		6.70%	7.8%	8.9%	8.20%
Ammonia (mg-N/L)	0.4±0.2	1.37±0.35	2.3±0.46	2.87±0.32	3.40±0.4
Nitrate (mg-N/L)	0.13±0.06	0.87±0.15	1.00±0.26	1.67±0.15	2.33±0.32
COD (mg/L)	19±2	145±6	182±20	211±19	256±8

Table C.2 PHB and nutrients concentration in effluent with fermented Mg(OH)₂-precipitated corn stillage addition for Condition B (for Figure 3.5, 3.6 and 3.7)

VFA (mg-C per time)	100	200	400
VFA (mg-C/cycle)	200	400	800
PHB (%)	7.5%±0.21%	12.69%±0.57%	24.26%±0.36%
TP (mg/L)	5.53±0.6	10.36±0.8	25.56±4.79
TN (mg/L)	4.4±0.62	9.33±0.93	19.16±2
Ammonia (mg-N/L)	2.17±0.31	3.5±0.89	3.93±0.25
Nitrate (mg-N/L)	0.5±0.1	0.5±0.1	0.7±0.3
COD (mg/L)	96±11	188±14	310±22
TP in sludge (d.w %)	6.50%	7.00%	8.60%

Table C.3 Volume and concentration of addition of fermented corn stillage (for Table C.1 and Table C.2)

	VFA Concentration (mg-C/L)	Added volume (mL/cycle)	Added VFA (mg-C/cycle)
Fermented corn stillage	670	300	200
		600	400
Fermented $\text{Mg}(\text{OH})_2$ - precipitated corn stillage	1,115	180	200
		360	400
		720	800