Fermentation – Enhanced Sustainable Biological Phosphorus Removal

by

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EXECUTIVE SUMMARY

The success of enhanced biological phosphorus removal depends on the constant availability of volatile fatty acids (VFAs). To reduce costs of purchasing external carbon, waste streams would be a preferred source for nutrient removal. VFAs were shown to vary in the incoming sewage and fermentate from primary sludge (PS). Another available source of organic to generate VFAs is waste activated sludge (WAS).

The effect of solids retention time and biomass concentration, as well as the effect of temperature and requirement for mixing on generation of VFA from the fermentation of WAS were investigated. It was found that VFA yields from sludge fermentation increased with SRT. At the longest SRT of 10 days improved biomass degradation resulted in the highest soluble to total COD ratio and the highest VFA yield. WAS fermentation was found highly temperature-dependent. The overall VFA–COD concentration in the non-mixed reactors was much lower than the mixed reactors.

The study of fermentation of PS, WAS and a mixture of WAS and PS demonstrated that PS fermentation predictably generated a significantly higher amount of soluble COD than WAS. Co-fermentation of WAS with PS enhanced soluble COD production and increased the release of phosphate and ammonium. Fermentation of combined PS and WAS sludge generated a concentration of phosphate high enough to allow phosphorus recovery as struvite

The effect of using glycerol as an external carbon source in biological phosphorus removal was investigated. Using glycerol directly resulted in the failure of the process which maintained enhanced biological phosphorus removal. When glycerol was co-fermented with waste activated sludge, significant VFA production was observed. By

supplying the system with the VFA-enriched supernatant of the fermentate, biological phosphorus removal was enhanced. It was concluded that, if glycerol was to be used as external carbon source for biological phosphorous removal, the effective approach was to ferment glycerol with waste activated sludge.

According to the cost analysis, the economic benefit of WAS fermentation can be demonstrated in three ways: 1) cost saving in external carbon addition; 2) cost saving in sludge handling; 3) revenue from phosphorus. At current condition, the value of the recovered P product is insignificant relative to the cost of chemicals that required for recovery and capital cost of the facilities. However, P recovery becomes important when the sustainability take into account.

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In the course of the many years of study that have led to this thesis, there may be some individuals that I have inadvertently omitted. My humblest apologies go to those who nevertheless remain in my heart.

Contents

List of Table

List of Figure

ABBREVIATION

BNR	Biological nutrient removal
BOD	Biological oxygen demand
COD	Chemical oxygen demand
DNPAOs	Denitrifying Phosphorus accumulating organisms
DO	Dissolved Oxygen
DOC	Dissolved organic carbon
EBPR	Enhanced biological phosphorus removal
GAOs	Glycogen accumulating organisms
HRT	Hydraulic retention time
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
NEWPCC	NorthEnd Wastewater Pollution Control Centre
PAOs	Phosphorus accumulation organisms
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PRR	Phosphorus release rate
PS	Primary sludge
PUR	Phosphorus uptake rate
SBR	Sequencing batch reactor
sCOD	Soluble COD
SEWPCC	SouthEnd Wastewater Pollution Control Centre
SRT	Solids retention time
SS	Suspended solids
SVI	Sludge Volume Index
TCOD	Total COD
TN	Total nitrogen
TP	Total phosphorous
TSS	Total suspended solids
VFA	Volatile fatty acid

VSS	Volatile suspended solids
WAS	Waste activated sludge
WEWPCC	WestEnd Wastewater Pollution Control Centre
WWTP	Wastewater treatment plant

Chapter 1 **INTRODUCTION**

1.1 SIGNIFICANCE OF PHOSPHORUS REMOVAL FROM WASTEWATER

Eutrophication, the enrichment of water bodies with nutrient, is the phenomenon which leads to harmful algal blooms, hypoxia, and loss of submerged aquatic vegetation. This has caused increasing environmental concern. From a public health perspective, eutrophication may also cause risks to human health. Consumption of shellfish, contaminated with algal toxins, or direct exposure to waterborne toxins have serious negative consequences. Eutrophication, in particular, can create problems if the water is used as a source of drinking water. Since phosphorus has been identified as the most important limiting nutrient to the growth of algae and aquatic plants in water bodies (Schindler et al. 2008), eutrophication, is therefore, mainly a function of phosphorus concentration. Base on UNEP report (1994), there are 28-54% of lakes and reservoirs have eutrophication problems in different region around the world. This demonstrates clearly that the phenomenon is both widespread and significant. Municipal and industrial wastewaters contain significant quantities of phosphorus. Therefore, reducing nutrient input through advanced treatment and recovery of phosphorus to prevent eutrophication has become the main focus of environmental protection of receiving water bodies. Lake Winnipeg is the tenth largest freshwater lake in the world and the sixth largest in Canada. In recent years, due to excessive amounts of nutrients, primarily phosphorus and nitrogen, water quality has deteriorated in the lake. Lake Winnipeg is now experiencing the huge area of the blue-green algae blooming which initiate the Canada's Action Plan for Clean Water to clean up

Lake Winnipeg (Manitoba Clean Environment Commission, 2011). In Canada's Great Lakes region, provincial effluent permits require total phosphorus (TP) to be less than 0.25 to 0.5 mg/L, (Oleszkiewicz & Barnard, 2006). Three wastewater treatment plants in Winnipeg (NEWPCC, SEWPCC and WEWPCC) are undergoing expansions and technical upgrades to meet the discharge limits defined by the Manitoba Clean Environment Act. The Act mandates that TP effluent quality should be less than 1 mg/L (City of Winnipeg, 2007).

1.2 CHEMICAL PHOSPHORUS REMOVAL

Chemical precipitation has been used for phosphorus removal since 1893 (Rybicki, 1998). In chemical phosphorus removal, a metal salt (which is usually aluminum or iron salt) is used to convert the dissolved ortho-phosphorus in wastewater into a particulate form- metal phosphate-- which has low solubility. Then the resulting metal phosphate is usually removed by a solid separation process, such as settling, floatation or filtration (Tchobanoglous and Burton, 1991).

There are many parameters which highly influence the achievable efficiency of phosphorus' removal. They include the metal salt dose, the wastewater characteristics, the method of chemical addition, the location at which the chemical addition is introduced, the mixing condition, the reaction pH, the flocculation method employed and the process configuration (Parsons and Stephenson, 2004). It is well recognized that the chemical precipitation process at the WWTP is more complex than predicted by clinical laboratory experiments. Several reactions such as precipitation, co-precipitation, diffusion, adsorption, coagulation and flocculation are involved (Jenkins, 2007) in the precipitation process. Moreover, the

formation and adsorption of carbonates or hydroxides play an important role (Hermanowicz, 2006). In practice a chemical precipitation is a promising method for P removal from wastewater. Indeed, Neethling and Gu (2006) demonstrated that a chemical phosphorus removal process can achieve an effluent phosphorus limit of 0.05 mg/L.

A major and continuing concern of a chemical phosphorus removal from wastewater is the greater production of sludge and its additional handling. For example, the addition of metal salt to the primary clarifier, secondary treatment process and tertiary application will typically increase the total overall plant sludge production by 60-70, 35-45 and 10-40%, respectively (USEPA, 2010). Furthermore, other issues arising from the use of a chemical precipitation are: the negative effect on nitrification and sludge dewaterability which is caused by the residual coagulant, corrosion caused by the metal chemical and the larger amount of inorganic salts produced in the sludge and effluent (USEPA, 2010).

In chemical P removal process, depending on the physical configuration of the plant, chemical cost factors and the required effluent quality, the introduction of chemical application points can be varied. Figure 1-1 shows the alternative chemical addition points for P removal at the wastewater treatment plant. Generally, the metal salts maybe added at the primary clarifier (pre-precipitation), direct to the mixed liquor- either in the bioreactor or upstream of the final clarifier (co-precipitation), or feeding to the tertiary solid-liquid separation process (post-precipitation) (Parsons and Stephenson, 2004). Recently, attention has been paid to the chemical P removal from the sludge stream since 90% of P compounds are incorporated in the sludge. The metal salt is usually added after an anaerobic process,

such as sludge digestion or fermentation, where phosphorus release occurs. P removal and recovery can be achieved simultaneously if the metal salt is added to the supernatant obtained after solid-liquid separation.

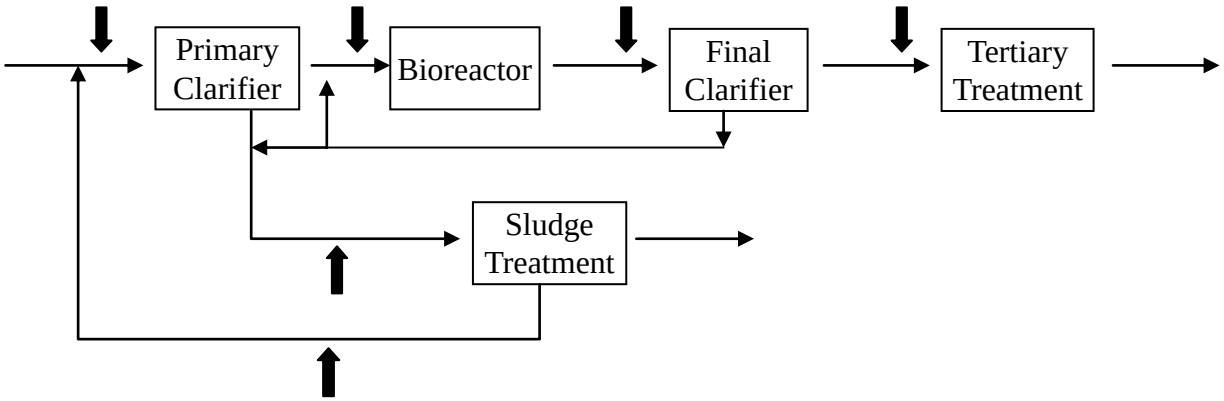


Figure 1-1 Alternative points of chemical addition for phosphorus removal

A BNR plant lacking adequate VFA in the influent requires supplementary chemical phosphorus. In this case, the necessary chemical dosing facilities need to be installed and they can also be used as a back-up system. Typically, simultaneous dosing of chemical in the activated sludge tank is applied. Dosing into the sludge line is another option practiced.

1.3 BIOLOGICAL PHOSPHORUS REMOVAL

The most recent process that has produced great interest is enhanced biological phosphorus removal (EBPR). This process has the potential to achieve very low phosphorus in an effluent at a relatively low cost and with minimum additional sludge production. Biological phosphorus removal was discovered accidentally around 1959 in full-scale wastewater treatment plants. The first full-scale processes were designed and introduced at

the end of 1970, see, for example the review of Van Loosdrecht, 1997. Historically, the BPR was developed empirically without a strong understanding of their microbiological bases (Seviour, 2003). However, research field became progressively more interdisciplinary until the 1980s, so that the cooperation between microbiologists and process-engineers gave a better understanding of the basic phenomena (Van Loosdrecht, 1997). With the rapid development of molecular tools, subsequently, the microorganisms, which are involved in the BPR process, continue to be studied more closely with an even better understanding of the basic phenomena.

1.3.1 Mechanisms of BPR

The basic concept of EBPR microbiology is that phosphate accumulating organisms (PAOs) accumulate polyphosphate as an energy reserve in intercellular granules (Figure 1.1). Under anaerobic conditions, with fermentation products, such as acetate and other volatile fatty acids (VFAs) present, PAOs uptake VFA and store them in the form of carbon deposits, i.e. polyhydroxyalkanoates– PHA. The energy to store this polymer is obtained from breakdown of glycogen and hydrolysis of internal cellular polyphosphate which is an energy-rich internal phosphorus chain. Since polyphosphate is broken down to ortho-phosphate for energy supply and is released into the liquid phase, the phosphate concentration in the anaerobic phase increases. Under aerobic conditions, the PHA is oxidized to produce energy for cell growth and glycogen replenishment. The energy is also used to take up orthophosphate which is stored as polyphosphate (Metcalf & Eddy, 2003). As a result, 80-90% of the influent phosphorus is removed with the wasted activated sludge which has higher phosphorus content. In the BPR process, the anaerobic phase is important

since it provides an unique and positive environment for PAOs. The anaerobic phase allows PAOs having the advantage of utilizing carbon without competing with other microorganisms (Matsuo et al. 1992). However, recent research showed that another group of microorganisms called Glycogen Accumulating Organisms (GAOs) can also store carbon sources anaerobically. They can compete with PAOs in the system for carbon source which lead to the deterioration of the bio-P process. Like PAOs, GAOs can uptake VFA and store them as PHA, however, unlike PAOs which use stored polyphosphate as energy source, GAOs use stored glycogen as the energy source in the anaerobic zone. The glycogen pool of GAOs is replenished in the aerobic zone by oxidizing the stored PHA. In other words, GAOs do not accumulate internal polyphosphate; therefore, there is no phosphorus release and uptake involved in both anaerobic and aerobic cycle. Due to the two different energy pathways, the form of PHA accumulated in the cell of PAOs and GAOs are also different, as indicated by Erdal et. al (2004). The main storage product of PAOs is PHB, while in GAO's case it is PHV.

Liu et al. (1997) suggested that the dominance of either PAOs or GAOs is dependent on the P:C (P:COD) ratio. They was found that PAOs outcompeted GAOs when excessive phosphorus was provided (P:C feeding ratio = 20:100). When the P:C ratio decreased to 2:100, GAOs became dominant. The coexistence of both groups of organisms occurred under a median P:C ratio (10:100). The study conducted by Ahn et al (2007) showed that higher organic loadings favoured glycogen-accumulating metabolism over phosphorus-accumulating metabolism. They suggested that the ratio of anaerobic phosphorus released to acetate uptake could be a good indicator of PAO population. When this ratio was lower than 0.4 mM/mM, GAOs were dominant over PAOs.

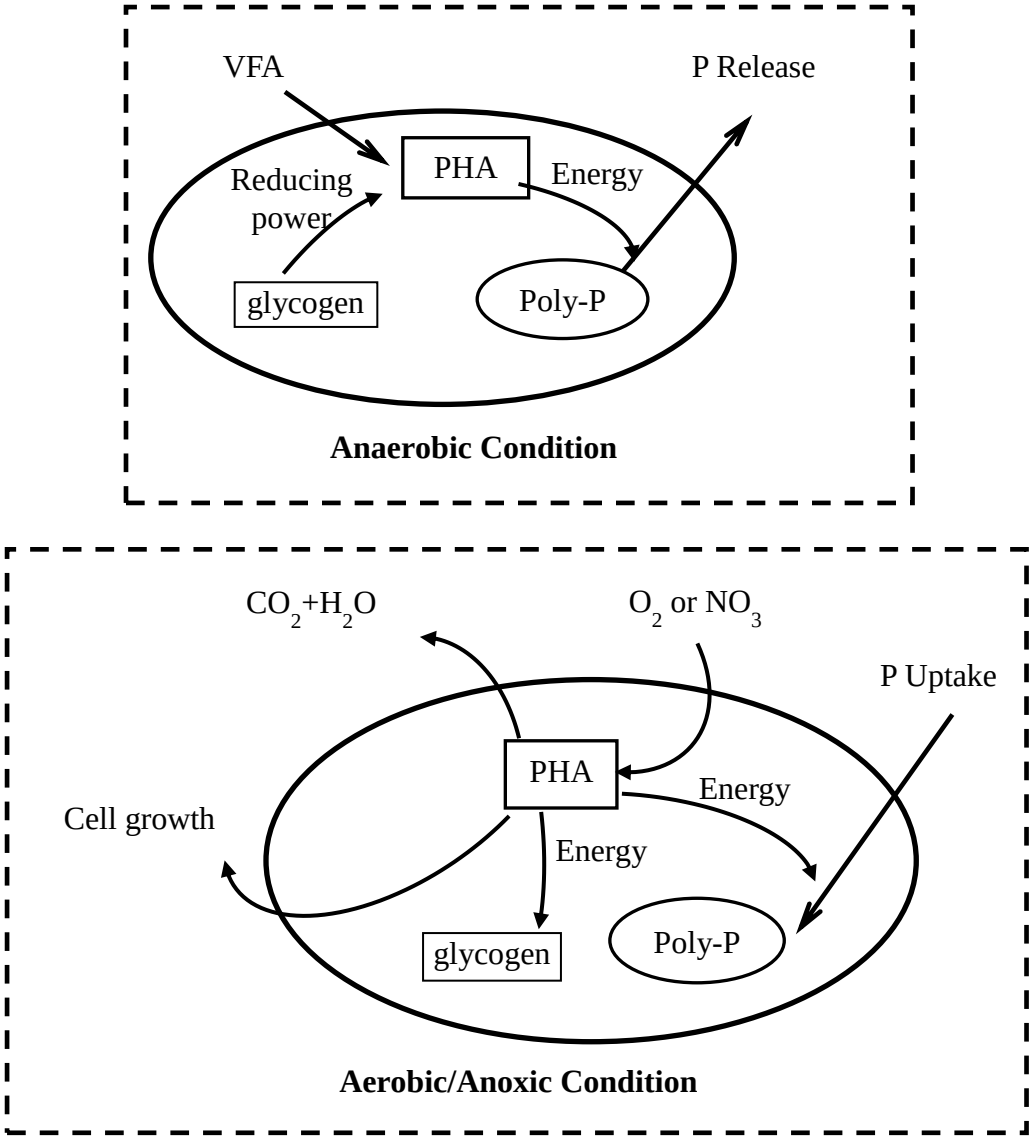


Figure 1-2 Pathway of PAO in Biological phosphorus removal

1.3.2 Microorganisms that involved in the BPR

Since the discovery of the biological phosphorus removal activities, it has been reported that it is linked with *Acinetobacter spp.* Activity (Fuhs and Chen, 1975). *Acinetobacter spp.* which is a group of gram-negative bacteria, belong to the gamma-subclass of

Proteobacteria (Kawaharasaki *et al.*, 1999). Bosch (1992) indicated that *Acinetobacters* are relatively small and can only remove approximately 10^{-10} mg phosphate/cell. Bosch (1992) also stated that the phosphorus content of *Acinetobacter* spp. can increase from 4 to 10% of its dry weight. This was agreed with the result that Deinema *et al.* (1985) and Pauli (1994) obtained. However, numerous researches (Meganck *et al.*, 1985, Lötter and Murphy, 1985, Cloete and Steyn, 1988, Zafiri *et al.*,1999) that aimed to identify the PAOs in activated sludge systems showed that there is no link between the number of *Acinetobacter spp.* present and the phosphorus removal capacity of the plant. Therefore, though *Acinetobacter spp.* is able to accumulate high levels of poly-P and thus might play a role in EBPR, its role in EBPR is still to be defined.

Recent research have demonstrated that phosphorus removing sludges are not dominated by a single bacteria but are composed of a few dominant bacterial species (Bond *et al.*, 1997; Mino *et al.*, 1998). Many candidates have been proposed as potential PAOs, such as 1) *Pseudomonas* (Kavanaugh, 1991; Okada *et al.*, 1992), 2) *Microlunatus phosphovorus* (Nakamura *et al.*, 1995), 3) *Nocardia* (Chuang and Ouyang, 2000) and 4) Gram-positive *Actinobacteria* (Kong et al., 2005) etc. However, those organisms do not exhibit all the characteristics of the EBPR biochemistry model.

With the help of the advanced molecular tools, such as fluorescence in situ hybridization (FISH), quantitative real-time polymerase chain reaction (qPCR) and micro-autoradiogrphy (MAR), the understanding of PAO phylogeny and ecophysiology has been greatly increased. Recent research have highlighted an uncultured *Rhodocyclus*- related bacterium, named *Candidatus Accumulibater phosphatis* (i.e. *Accumulibacter*) as one of the most

important PAO candidates. They have been found generally abundant in full-scale EBPR systems (5-20% of the total bacteria) and also in lab-scale EBPR reactors (Oehmen et al., 2007). The Lab-scale enrichments of *Accumulibacter* have demonstrated the characteristic of PAO biochemistry model, e.g. the anaerobic VFA uptake and storage of PHA, coupled with P release and glycogen degradation and followed by aerobic PHA degradation, P uptake and storage as Poly-P and glycogen replenishment. According to He et al. (2007), Peterson et al. (2008) and Kim et al (2010) the *Accumulibacter* strains consists of two distinct types. Each type includes multiple sub-clades among which at least 5 clades have been detected in EBPR plants. Further research on establishing the link between identity and biochemistry is required in order to better modeling the overall activities of PAO sludge.

1.3.3 Parameters which affect BPR process

The following parameters are important for the BPR process.

a. The influent composition of the wastewater that needs to be treated defines the operation and the efficiency of the BPR process. This composition includes the readily biodegradable COD, VFA, BOD:P and BOD:N ratios, and other characteristics such as the content of potassium, calcium and magnesium ion, etc. Generally speaking, removing 1 mg P requires 20 mg COD which is readily and slowly biodegradable (2002, STOWA). VFAs are essential for an effective BPR process. For instance, Barnard (1993) reported that 7-9 mg of VFA is needed to remove 1 mg of phosphorus. Clearly an insufficient supply of VFA can reduce the performance efficiency of the phosphorus removal.

b. A change of temperature will have a corresponding effect on the activity of PAOs and the biomass population, i.e. the fraction of PAOs in the sludge (2002, STOWA). Generally, P release and uptake rates of PAOs increase with a higher process temperature.

c. pH is a crucial factor for the anaerobic P release as well as aerobic P uptake. A low pH results in a slow P release/acetate uptake rate (Schuler and Jenkins, 2002). However, when pH value greater than 7.5, a higher P reduction will be observed in two ways: 1) through an increased polyphosphate uptake; 2) through an increased chemical precipitation with the metal ions presented in the wastewater such as calcium and magnesium. (2004, IWA).

d. Nitrate and oxygen are essential for P uptake; on the P-release side, nitrate and oxygen in the RAS stream or influent will interfere while being preferentially utilized in the anaerobic tank. This will result in the consumption of readily biodegradable COD (rbCOD) which decreases the amount of available rbCOD for PAOs.

e. A short sludge age/SRT is beneficial for bio-P process. Short SRT leads to decreased nitrification, which minimizes the negative impact of nitrate on the bio-P process. In addition, short SRT will decrease the chance of over aeration. Over aeration can result in the oxidation of the glycogen. The oxidation of glycogen in the aerobic phase leads to insufficient supply of reducing power for VFA uptake from glycogen in the anaerobic phase, which ultimately inhibits the bio-P process. In practice, the minimal SRT for bio-P process is less than 3 days.

1.3.4 Configurations of Bio-P process

Many configurations for application of bio-P process have been invented, developed and implemented in practice. Some of the BPR processes are developed from the configuration that focused on the biological nitrogen removal with the aim of protecting the BPR from nitrates and O_2 in the anaerobic zone. The most common processes are presented as following:

a. Phorodex (A/O)

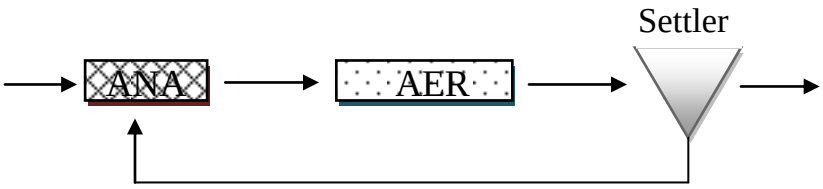


Figure 1-3 Flow diagram of the Phorodex (A/O) process

The Phorodex process is the basic process which was primarily developed for biological phosphorus removal with no requirement of nitrification. It consists of anaerobic tank followed by aerobic tank. To prevent nitrification, the sludge age is controlled short and aerobic tank is designed for high COD removal rate.

b. 3-stage Modified Bardenpho (A2/O)

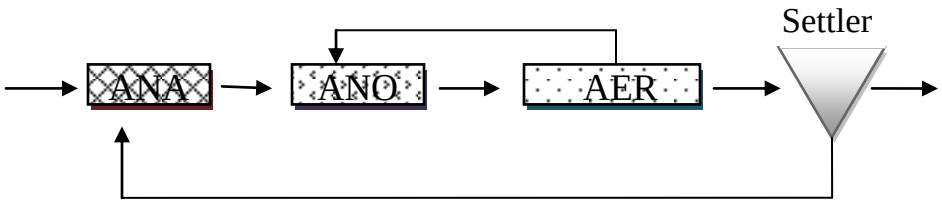


Figure 1-4 Flow diagram of 3-stage Modified Bardenpho (A2/O) process

The 3-stage Modified Bardenpho process is derived from the A/O process with addition of anoxic tank for denitrification. Nitrogen removal is introduced by the anoxic tank. In addition, the anoxic phosphorus removal will be enhanced via the anoxic tank. However, the P removal capacity was affected by the nitrate that carried to the anaerobic zone in the RAS stream.

c. 5- stage Modified Bardenpho

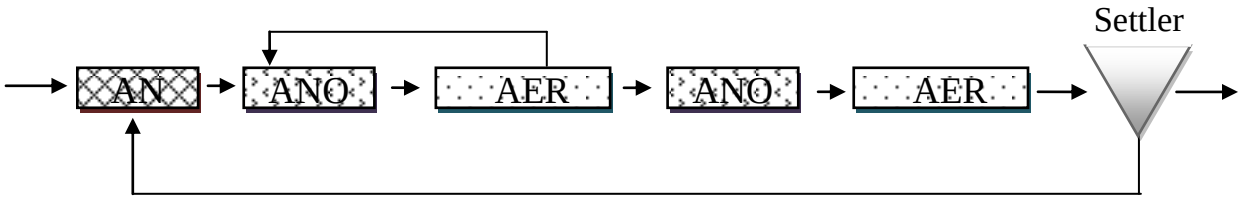


Figure 1-5 Flow diagram of the Modified Bardenpho process

The 5- stage Modified Bardenpho process improves both P and N removal. It consists one anaerobic tank followed by two series of anoxic and aerated tank which placed one after the other. The P release take place in the anaerobic tank and uptake take place in the following anoxic and aerobic tank. The second anoxic tank is provided for additional denitrification using the endogenous organic carbon as the carbon source. The final aerobic tank is used to strip the nitrogen gas and minimize the release of phosphorus. The nitrate in the RAS stream is minimized by the additional series of anoxic and aerate tank. However, larger tank volume is required.

d. UCT

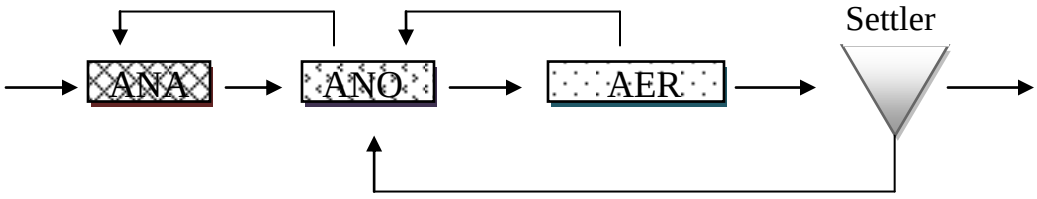


Figure 1-6 Flow diagram of UCT process

The UCT process was developed to minimize nitrate that enters the anaerobic tank by returning the activated sludge to the anoxic tank. The internal recycle from anoxic to anaerobic tank maximize the utilization of organic carbon for denitrification as well as for anaerobic phosphorus release. Also, anoxic phosphorus removal is enhanced. Larger anaerobic tank volume is needed to obtain the same phosphorus release or fermentation activity because the activated sludge is not recycled to the anaerobic tank.

e. Modified UCT

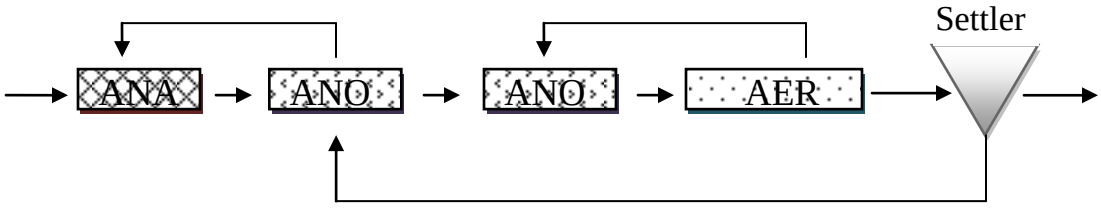


Figure 1-7 Flow diagram of Modified UCT process

By providing an additional anoxic tank, the nitrate recycled to the anaerobic tank is further reduced. The major portion of nitrate removal is taking place in the second anoxic tank which receives the internal nitrate recycle from the aerobic tank. Because of the introduction of an additional internal recycle, it is more complex operation and more complicated to control the process.

f. Johannesburg

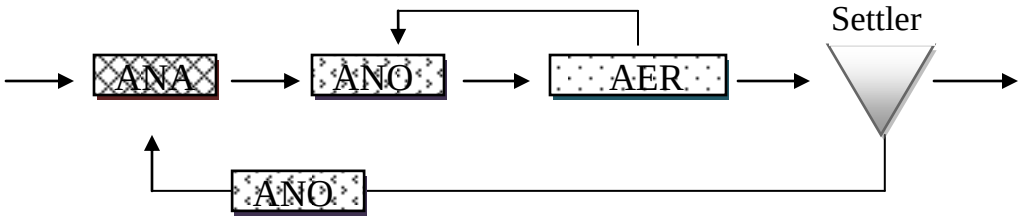


Figure 1-8 Flow diagram of Johannesburg process

Johannesburg process was developed for weak wastewaters to maximize the biological phosphorus removal. By adding an anoxic tank on the RAS stream, nitrates that enter anaerobic tank are minimized. With comparison to the UTC process, a higher MLSS concentration in the anaerobic tank requires a shorter anaerobic detention time.

g. BCFS (Biological-Chemical Phosphorus and Nitrogen removal)

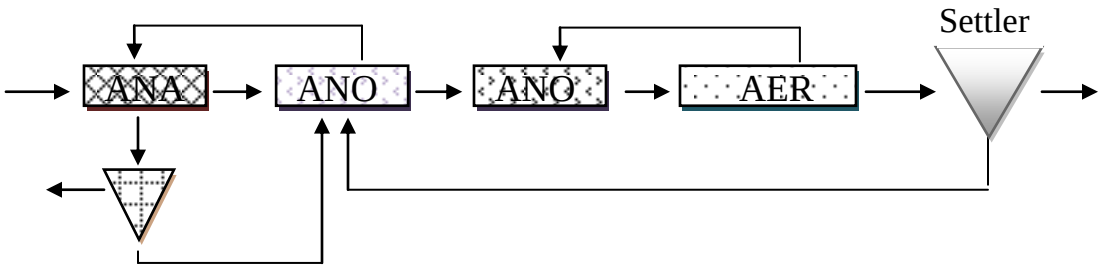


Figure 1-9 Flow diagram of BCFS process

BCFS process is the extension of the MUCT process, in which the advanced phosphorus removal is obtained by the addition of a stripping tank. This process is the combination of the biological and chemical P removal. The phosphorus rich wastewater is withdrawn from the anaerobic tank to the stripping tank where metal salt is added to precipitate phosphorus in the sludge line. The metal salts are used effectively since P concentration is high in the stripping tank. However, careful control of the dose of chemical is important since too much precipitation will make P unavailable for PAOs and thus deteriorate the EBPR process.

h. Phostrip

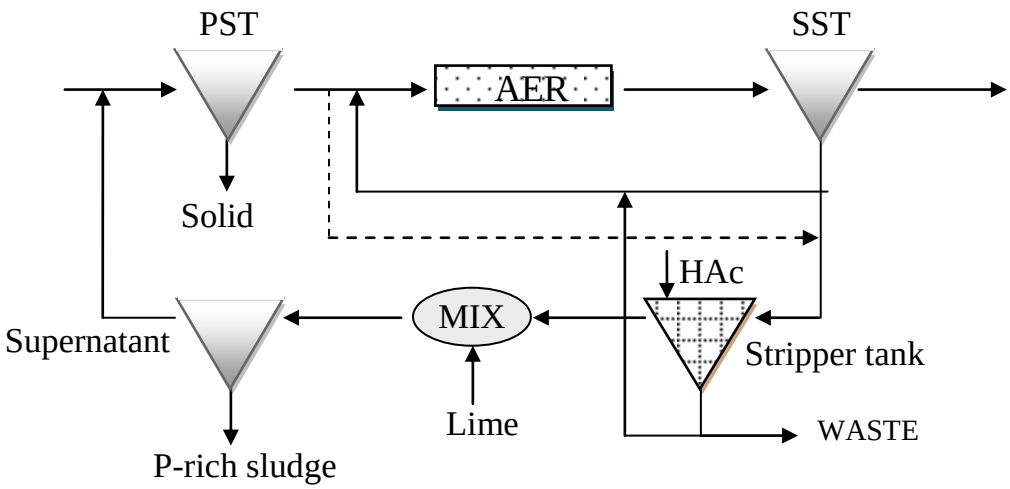


Figure 1-10 Flow diagram of Phostrip process

Phostrip is a side stream P removal process that combines biological and chemical phosphorus removal. A proportion of the return sludge is thickened with a long retention time at the stripper tank where undergoes an anaerobic condition. Phosphorus is released in the stripper tank and the supernatant stream is treated with lime. The precipitated phosphorus is removed through settling and the sludge from the stripper tank is recycled to the aerobic tank where P uptake occurs. Organic substrate (VFA) is added to the stripper tank to enhance phosphorus release.

1.4 CARBON ISSUE IN BIOLOGICAL PHOSPHORUS REMOVAL

The initial studies of EBPR as well as continuous research revealed that the carbon source that lead to good EBPR performance belong to a group of short chain, low molecular

monocaboxylic acid, e.g. volatile fatty acids (VFA). Researchers such as Fuhs and Chen (1975), Barnard (1984), Ekama et al. (1984) and Comeau et al. (1987) etc. all reported that substantial phosphorus release was observed when acetate or propionate were used instead of other substrate. They all concluded that the presence of VFA is an important factor for the biological P removal process. According to Ekama and Marais (1984) the VFA-COD concentration must be greater than 25 mg/L in the initial anaerobic zone in order to achieve significant biological P removal performance.

In practice, usually the feasibility of applying EBPR to a given wastewater has been evaluated using rule of thumb readily biodegradable COD/P, COD/P or BOD/P ratio, since early research has shown that the influent carbon to P ratio correlated very well with the TP content in the biomass and phosphorus removal functions (Liu et al., 1997, Schuler and Jenkins, 2003).

Since the amount of VFA required is a function of the influent TP, the VFA to TP ratio can be an indication of the EBPR capability of the system. rbCOD now is considered as a better measure since it represent the influent VFA and other organic compounds that can be fermented to VFA during anaerobic process. Research by Janssen (1996) demonstrated that 10 g of readily biodegradable carbon is required to remove 1 g phosphorus.

BOD especially $cBOD_5$ to TP ratio is also often used as an indicator of the adequacy of carbon substrate in a EBPR process. According to SCOPE (1998), the BOD to phosphorus ratio should be at least 20 to achieve good P removal. Randall et al (1992) showed that to achieve a reliable effluent TP concentration less than 1.0 mg/L, the ratio of BOD to TP should be 20:1 or greater.

COD to TP ratio has been widely used to assess the availability of substrate for EBPR process, due to the fact that COD is a more consistent and convenient measurement. Randall et al (1992) pointed out that TCOD to TP ratio of 45 or greater is essential for EBPR. According to Reddy (1998) the TCOD to P ratio of 50 is a conservative number for North American municipal wastewater, and is recommended for design purposes. A significant fraction of the organics in wastewater may not be available for EBPR since only valeric acid has a COD to P removed ratio higher than 45. This results in a higher COD requirement for phosphorus removal. Table 1-3 (WEF Manual of practice NO.34, 2010) is the summary of the typical ratio used to quantify minimum substrate to phosphorus ratio.

Table 1-1 Minimum substrate to phosphorus requirements for enhanced biological phosphorus removal (WEF Manual of practice NO.34, 2010)

Substrate Measure	Substrate to Phosphorus ratio*	Remarks
$cBOD_5$	25:1	Provides a rough/initial estimate. Based on typically available plant data
$sBOD_5$	15:1	Better indicator than $cBOD_5$
COD	45:1	More accurate than $cBOD_5$. Not measured by all plants
VFA	7:1 to 10:1	More accurate than COD. Involves specialized lab analysis
rbCOD	15:1	Most accurate. Measures VFA formation potential. Accounts for VFA formation in the anaerobic zone. Specialized laboratory analysis

* Minimum requirements

$cBOD_5$ = five-day carbonaceous biochemical oxygen demand

sBOD5 = five-day soluble BOD

Stumm & Morgan (1970) pointed out that in terms of the relative stoichiometric relationships between carbon, nitrogen and phosphorus when the biological oxidation of carbon is conducted; both nitrogen and P are present in excess. Conversely, wastewater is normally carbon-deficient in terms of nutrient removal. Many existing BNR processes do not meet constantly the strict permit requirements since wastewater does not always contain carbon substrate in amounts sufficient for nutrient removal. Carbon-deficient wastewater hinders denitrification which in turn affects EBPR. Morling (2001) observed when the availability of organic carbon was at a low level, return activated sludge (RAS) contained over 8 mg/L NO_3 and denitrification occurred in the anaerobic zone, which resulted in the deterioration of the EBPR process. The competition for organic carbon, particularly VFA, between PAOs and denitrifying bacteria has become an important factor that affects the BNR performance.

To tackle the problem, several external carbon sources such as ethanol, methanol and acetic acid have been added to compensate for this deficiency (Cho & Molof, 2004). As the cost of external carbon addition become more expensive, along with the concerns of safety, supply volatility and the long-term sustainability, many utilities have considered alternative sources for carbon augmentation, such as waste by-products from industrial applications. The process optimization including primary sludge fermentation is another sustainable solution to reduce supplemental carbon requirements. Generating VFA from waste activated sludge fermentation to augment both phosphorus and nitrogen removal is the primary focus of this study.

1.5 PHOSPHORUS RECOVERY

Phosphorus recovery is another important dimension in phosphorus removal. Phosphorus is a non-renewable resource and an essential element on which life depends and for which there is no substitute. The world's phosphorus supply is limited (Rittmann, et al. 2011). Therefore implementation of principles of sustainable development requires that phosphorus be removed or recovered from wastewater. Over the last few years, interest and development in phosphorus recycling has accelerated and several recovery techniques have been studied and developed.

P recovery from wastewater can be achieved mainly from two streams: P enriched side stream and/or sludge stream. The concept of side stream P recovery is that, side stream taken from the anaerobic part of the active sludge process where phosphorus has been released to the solution, contains a high concentration of phosphorus. Phosphate-rich streams from EBPR processes also have a high content of magnesium since, in phosphate release, magnesium and potassium are also released to maintain the ionic balance. By combining P-enriched side stream with an ammonium-rich stream (such as centrate), P can be recovered in the form of struvite (Magnesium Ammonium Phosphate, MAP) which has a good fertilizer market potential. Examples of this application are Slough WWTP (UK) and Shinji WWTP (Japan) (Levlin and Hultman, 2003). Another well know application of the side stream P recovery is the Phostrip process in which phosphorus is recovered from a return sludge stream by dosing with lime. Calcium phosphate is the product of this process which can be used by the phosphate industry. Phostrip process has been practiced widely in the USA, Germany, and the Netherlands. Investigations in recent years showed that phosphorus recovery is particularly successful in combination with biological phosphorus

removal in a side stream. The phosphorus recovery rate from the side stream can reach 40-50%.

An important alternative is to recover P from sludge stream since, in the wastewater treatment process, 90% of phosphorus compounds are incorporated in the sludge. This includes phosphorus recovery from anaerobic sludge treatment (anaerobic sludge digestion/fermentation) and sludge ash. The supernatant generated from an anaerobic sludge treatment contains a high concentration of ammonium and phosphorus and, with slight adjustment in the pH-value, struvite crystallization can be induced spontaneously. Successful applications of this technology are the Phosnix process (Japan), the Ostara process (Canada) and the Prisa process (Germany) (Berg and Schaum, 2005). To date the attention has also been given to testing the possibility of extracting phosphorus and heavy metals from sewage sludge ash. In contrast to wet sludge, P recovery by solid-liquid separation after alkaline or acidic treatment is significantly easier to realize due to the exclusive inorganic formation of the sludge ash. To recover P from sludge ash, the Sephos process (Germany) has been developed and SUSAN- an EU research project - has started a pilot plant in Leoben (Austria) (Cornel and Schaum, 2009). The phosphorus recovery rate from the sludge stream can reach up to 90%.

Even though the cost of recovering phosphorus from wastewater exceeds, by several times, that of taking phosphate from rock phosphate, limited phosphorus resources call for the continuous development of phosphorus recovery technologies in order to lower the cost. On the other hand, the future importance of P recovery will depend on the market price of raw phosphate rock, the recovery cost and general political conditions.

1.6 THE OBJECTIVE OF THIS RESEARCH

The primary goal is to develop the technology of enhanced biological phosphorus removal by focusing on internal fermentation of activated sludge, e.g. to minimize the external carbon required for biological phosphorus removal by increasing internal carbon generation through fermentation of the sludge in the process.

The detail objectives of this research study are:

- Study the impact of different condition such as temperature, mixing, SRT, and biomass concentration on WAS fermentation to generate VFA.
- Study the effect of sludge type e.g. primary sludge, WAS and mixture of PS and WAS on sludge fermentation.
- Study the performance of co-fermentation of WAS with glycerol
- Sludge reduction and investigating the potential of phosphorus recovery.

Detailed research objectives, results and discussions during the entire experiments period will be provided in the following chapters.

1.7 THE SCOPE OF THIS RESEARCH

The main goal of this research was to study WAS fermentation to generate VFAs for biological nutrient removal. The structure of the research is shown as Figure 1-11. This research was consisted of three sections: 1) to study the impact of different factors on WAS fermentation; 2) to study the in-line biomass fermentation to generate VFA for EBPR; 3) to study the co-fermentation of glycerol with WAS to generate VFA for EBPR.

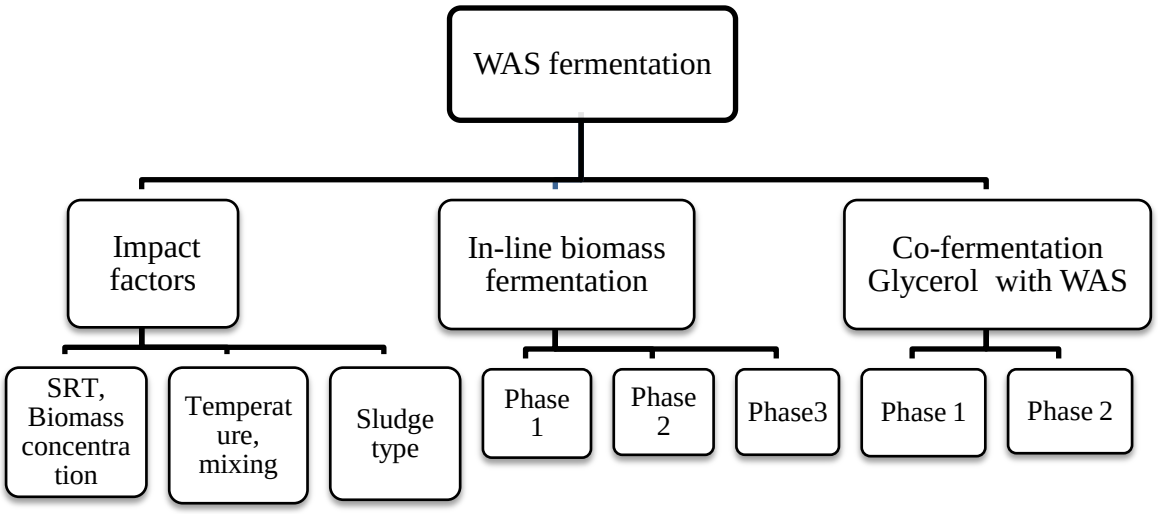
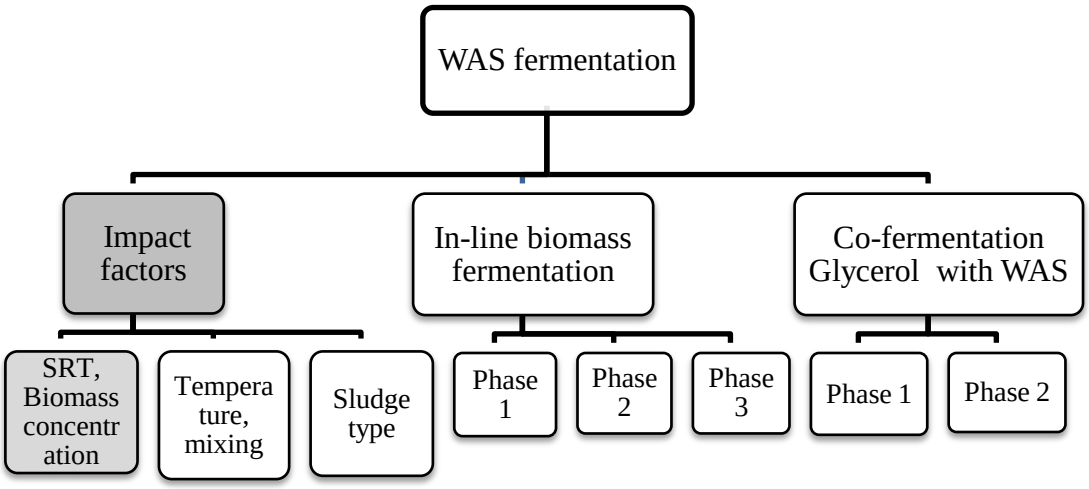


Figure 1-11 Research Scope

Chapter 2 **WASTE ACTIVATED SLUDGE FERMENTATION: EFFECT OF SOLIDS RETENTION TIME AND BIOMASS CONCENTRATION**[*]



[*] published in ***Water Research (2009)***, 43, 5180-5186.

2.1 INTRODUCTION AND SPECIFIC OBJECTIVES

Enhanced biological phosphorus removal (EBPR) processes are potentially the least costly methods to remove phosphorus from wastewater. The key to efficient EBPR performance is the presence of adequate volatile fatty acids (VFA) in the influent wastewater (Chu *et al*., 1994, Oleszkiewicz & Barnard, 2006). Influent wastewater often has low chemical oxygen demand (COD) and lacks adequate VFA to permit suitable P removal (Barajas *et al*., 2002) which forces the utility to seek carbon sources within the wastewater treatment plant (WWTP) itself. The addition of organic carbon from outside of the plant, unless it is a waste material, increases not only the plant's carbon footprint but also the operational cost of liquid treatment and sludge processing, since sludge production will increase as well.

The practice of primary sludge fermentation for on-site production of VFA is well established worldwide (Munch and Koch, 1999; Chanona, *et al*., 2006; Bouzas *et al*., 2007), however the reliability of VFA generation is often not adequate, particularly in flat terrain large sewer systems such as in St Paul-Minneapolis USA, Winnipeg South CA or Gdansk PL. In a number of treatment plants the mass of VFA produced from primary sludge fermentation is frequently below that required to ensure efficient removal of both phosphorus and nitrate, for example in the Noosa AUS biological nutrient removal (BNR) WWTP (Thomas *et al*., 2003). The factors that affect VFA production from primary sludge fermentation include operational parameters such as solids retention time (SRT), hydraulic retention time (HRT), pH, temperature etc. Perot *et al* (1998) conducted an experiment where the effects of pH, temperature and agitation speed were investigated with respect to VFA production. They concluded that optimal hydrolysis conditions appeared to be a pH of 6.8 at a temperature of 50 ℃ and an agitation speed of 545 rpm. Bouzas *et al.*

(2002) reported that higher total volatile solids concentrations resulted in higher VFA production, but that SRT above 6 d did not significantly improve the VFA yields, however, an important decrease of VFA was observed with an SRT of 4 d.

The only other substrate available for VFA production within a wastewater treatment plant is waste activated sludge (WAS) generated from soluble organic matter removal. Waste activated sludge differs from primary sludge which contains higher concentrations of easily biodegradable organic polymers (proteins, lipids carbohydrates) than WAS, permitting shorter hydrolysis and fermentation times. WAS contains mostly bacterial mass, and cell lysis is therefore the rate-limiting step (Turovskiy and Mathai, 2006). Fermentation of WAS is expected to release phosphorus and nitrogen into the liquid phase, as demonstrated during fermentation of primary sludge co-thickened with WAS by Danesh *et al* (1997) and McIntosh & Oleszkiewicz (1997). Because of the differences between primary sludge and WAS, operational conditions will have to vary accordingly. Few studies have been conducted on the operational parameters necessary for optimal WAS fermentation for VFA production. The objective of this study was to investigate the effect of SRT and biomass concentration on VFA production in terms of quantity and composition from WAS (biomass) fermentation. The potential for nutrient recovery using the end-products of WAS fermentation, nutrient release (N and P) from cell lysis during the fermentation was also studied.

2.2 MATERIALS AND METHODS

2.2.1 Experiment set-up

Bench-scale experiments were performed with two semi-continuous reactors. The reactors were initially seeded by adding 50% of the volume with the sludge (approximate TSS of 16

g/L) from a mother WAS fermenter. One reactor was operated at an SRT of 10 days, and the other at an SRT=7 d. When the tests in the latter reactor were finished, the SRT of this reactor was changed to 5 days. The reactors were operated under a complete mixing condition; therefore, hydraulic retention time (HRT) was same as SRT. For each biomass concentration test at the designated SRT, the reactor was operated with three SRT to reach steady state followed by a one SRT sampling period. The results from this sampling period are reported here. Nevertheless, sampling was regularly performed during each of the three SRT periods to monitor the reactor performance and to assure that the reactor reached steady state before the final reported sampling period.

Each reactor was fed daily with WAS obtained from two lab-scale sequencing batch reactors (SBR) which were maintained for biological phosphorus removal. Synthetic wastewater (Table 2-1) was used as the feed for the SBRs which were operated with 3 cycles per day. For each cycle, it went through a 20 minute filling period, a 1.5 h anaerobic period, a 3 h aerobic period, a 1.5 h anoxic period followed by 1 h of settling and finally a 40 min. decant and idle period. The waste activated sludge was withdrawn from the SBR at the end of the anoxic period and then settled in a cylinder. The supernatant was discarded through a siphon and the concentrated WAS was used in this experiment. In order to provide the reactor with constant and desired mass feed, the biomass concentration of the sludge was measured (TSS=18.89 g/L, VSS=15.48 g/L and alkalinity = 190 mg/L) and adjusted to the desired value prior to feeding, by diluting with de-ionized water. Each reactor had a working volume of 0.5 L. Magnetic stirrers were used to maintain solids in suspension. The pH was monitored throughout the experiment. The experiments were conducted at room temperature (20 – 22 ℃).

Table 2-1 Synthetic wastewater composition

Synthetic wastewater		**Mineral solution**	
Ingredients	Concentration (mg/L)	Ingredients	Concentration (g/L)
NaAc	255	$FeCl_3 \cdot 6H_2O$	1.5
Beef extract	65	H_3BO_3	0.15
Yeast extract	65	$CuSO_4 \cdot 5H_2O$	0.03
$MgSO_4 \cdot 7H_2O$	170	KI	0.03
$CaCl_2 \cdot 2H_2O$	14	$MnCl_2 \cdot 4H_2O$	0.12
P (K_2HPO_4)	9	$Na_2MoO_4 \cdot 2H_2O$	0.06
TN (organic)	14-15	$ZnSO_4 \cdot 7H_2O$	0.12
		$CoCl_2 \cdot 2H_2O$	0.15
Mineral solution	0.3 mL	EDTA	10

2.2.2 Analytical methods

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) measurements were performed according to Standard Methods (APHA, 1998). Hach COD digestion vials were used to measure COD. Dissolved phosphate and ammonium were measured using a Lachat Instrument Quik Chem 8500, following the Quik Chem orthophosphate method 10-115-01-1-O, and Quik Chem ammonia method 10-107-06-1-I. The analysis of VFA composition was conducted by means of a Varian CP-3800 Gas Chromatograph using a flame ionization detector (FID) and HP-FFAP capillary column (inner diameter of 0.25mm and length of 25m). VFA concentration was converted

to COD concentration by using the following conversion factors: 1.07 g COD/g acetic acid, 1.51 g COD/g propionic acid, 1.82 g COD/g butyric and isobutyric acid, 2.04 g COD/g valeric and isovaleric acid, 2.21 g COD/g caproic and iso-caproic acid and 2.34 g COD/g heptanoic acid.

VFA yield was expressed as the mass of effluent VFA-COD per unit mass of total COD in the feed (g VFA-COD /g TCOD). Solubilization was expressed as the mass ratio of the soluble COD to the total COD in the feed (SCOD / TCOD). P and N release was expressed as the mass of effluent soluble phosphate and ammonium per unit mass of total COD in the feed (mg PO_4-P/g TCOD, mg NH_4-N/g TCOD).

2.3 RESULTS AND DISCUSSION

2.3.1 Effect of SRT on VFA production

VFA yields in the fermenter with SRT of 5, 7 and 10 d, are presented in Figure 2-1. The general trend was that VFA yield increased with increased SRT; the longest SRT (10 d) resulted in the highest VFA yield. The maximum VFA yield from the influent COD was 0.14 g VFA/g TCOD at an SRT of 10 d with the feed biomass (VSS) concentration of 4.8 g/L, whereas with the similar feed biomass concentration at an SRT of 7 and 5 d the VFA yields were 0.09 and 0.07 g VFA/g COD, respectively. Much of the research on primary sludge fermentation also showed that VFA yield increased with increased SRT, with a suggested optimal SRT of 5-6 d (Skalsky and Daigger, 1995; Jiang et al. 2007; Bouzas et al, 2002). The indication from the present experiment was that, at room temperature (20-22 ℃), a longer SRT is required for WAS fermentation. This may be due to the difference in characteristics of these two types of sludge - with WAS being harder to ferment due to the content of cell biomass.

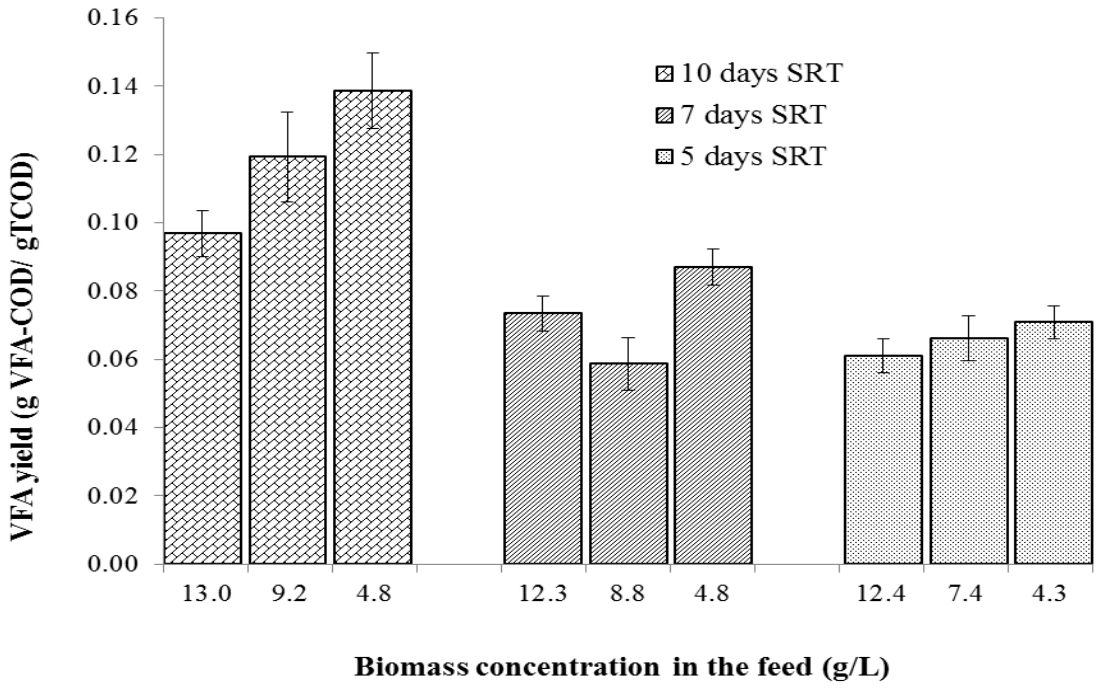


Figure 2-1 Effect of SRT and biomass concentration on VFA yield (the numbers are an average of 5-10 replicates)

Regardless of the SRT and the WAS concentration in the feed, the VFA yields of this experiment were in the range of 0.058-0.14 g VFA-COD/g TCOD, which is slightly lower than the value reported by Ubay-Cokgor *et al* (2005) 0.095-0.19 g VFA-COD/g TCOD, but slightly higher than Bannister and Pretorius (1998), 0.05-0.11g VFA-COD/g TCOD. Both cited yields were from primary sludge fermentation at an SRT of 6 days. Although it is difficult to compare the effectiveness of the WAS fermentation to primary sludge fermentation due to the variation of the primary sludge itself, WAS fermentation was shown to be as effective as primary sludge fermentation; however, longer SRT was required.

2.3.2 Effect of biomass concentration on VFA yield

The general trend was that, regardless of the SRT, VFA yields increased with a decrease in biomass concentration, when the biomass concentration of the feed was in the range of 4.3-13 g/L. The only apparent exception was at the SRT of 7 days, when the average biomass concentration was 8.8 g/L. The reason for this discrepancy may have been caused by the lack of biomass feed 2 days before the sampling period when effluent of a lab-scale anaerobic manure digester for gas production was used for the fermenter as feed. Therefore, methanogens were mistakenly introduced into the system. It was not realized until the end of the sample period. To inhibit these Archaea, 10 mM of 2-Bromoethanesulfonic acid (BESA) was injected to the system and methanogens were effectively inhibited within 2 days, after which an increase in VFA concentration was observed.

At an SRT of 10 d, biomass concentration had a larger impact on VFA yields than with an SRT of 7 and 5 d (Figure 2-1). Indeed, when the biomass concentration decreased from 13 g/L to 4.8 g/L, the VFA yield increased very significantly, by about 46%. For an SRT of 7 d, the VFA yield increased by 19% when biomass concentration decreased from 12.3 to 4.8 g/L. At a lower SRT of 5 d, there was a 16% increase in VFA yield as the biomass concentration decreased from 12.4 to 4.3 g/L. The observed VFA yield increased with decreased biomass concentration which was consistent with the primary sludge fermentation study by Skalsky and Daigger (1995). In their study, at an SRT of 2 d, there was a 50% increase in VFA yield when the solids concentration decreased from 2.6% to 0.43%. Banister and Pretorius (1998) also reported similar observation. In full scale practice, however, the decreased sludge concentration may require an increase of the reactor size - which would be considered a disadvantage.

One explanation for this behaviour could be that low biomass concentration created better mixing conditions in the reactor, which resulted in the improvement of the cell availability to the hydrolysis (Skalsky and Daigger, 1995).

Another reason could be that less of the inhibiting compounds, such as zinc or copper, were released into the system during fermentation when a lower biomass concentration was in the feed (Utgikar et al., 2004).

2.3.3 Effect of SRT on the degree of solubilization and the ratio between VFA to SCOD

Solubilization of the biomass, which could be expressed as the ratio of soluble COD to total COD, is the main objective of biomass fermentation. As can be seen from Table 2-2, the degree of biomass solubilization increased with increased SRT. The longest SRT resulted in the highest solubilization, approximate 18%, followed by 11% and 8% at SRT of 7 and 5 d respectively. This result, along with the VFA yield results, suggested that longer solids retention time enhanced the degradation of biomass and subsequently, its conversion to VFA. The solubilization results obtained from this experiment at an SRT of 10 d were comparable with primary sludge fermentation results reported by Ucisik and Henze (2008) where values of 15.9-17.9% were reported when using an SRT of 6 d.

Interestingly, the ratio of VFA-COD to soluble COD showed a different trend, that is, an increased SRT resulted in a lower VFA-COD/SCOD ratio. The average VFA-COD to SCOD ratio at an SRT of 10 days was 73%, followed by 80% and 86% for SRT of 7 and 5 d respectively. Longer SRT led to enhanced hydrolysis and fermentation.

During biomass fermentation, much of the biomass was solubilised resulting in 23-40% reduction of VSS. However, the results did not point to a clear correlation between SRT or biomass concentration and the VSS reduction (Table 2-2).

Table 2-2 Effect of SRT and biomass concentration on VFA production and nutrient release

SRT (d)	Feed TSS (g/L)	Feed VSS (g/L)	Effluent TSS (g/L)	Effluent VSS (g/L)	VSS reduction	*VFA (mg/L)	*VFA-COD (mg/L)	*Soluble COD (mg/L)	*VFA-COD/ SCOD	*SCOD /TCOD	*P-PO_4 /TCOD (mg/g)	*N-NH_4 /TCOD (mg/g)
10	16.03 (1.6)	13.04 (1.25)	11.62 (0.53)	8.97 (0.36)	31.3%	1330 (105)	1846 (130)	2600 (170)	71%	14%	17.2 (1.0)	26.4 (1.3)
10	11.30 (1.37)	9.21 (1.20)	7.31 (0.35)	5.56 (0.31)	39.6%	1172 (135)	1604 (179)	2300 (240)	70%	17%	19.7 (1.2)	31.1 (1.9)
10	5.97 (0.78)	4.85 (0.61)	4.06 (0.20)	3.07 (0.13)	36.7%	690 (61)	985 (81)	1290 (107)	76%	18%	19.6 (1.4)	30.2 (2.2)
7	15.14 (0.94)	12.30 (0.65)	10.59 (0.31)	8.17 (0.27)	33.6%	976 (42)	1321 (53)	1740 (90)	76%	10%	17.9 (1.1)	25.4 (1.6)
7	10.72 (0.88)	8.76 (0.65)	6.91 (0.44)	5.23 (0.33)	40.3%	581 (57)	749 (97)	940 (84)	80%	7%	20.3 (1.2)	29.4 (1.4)
7	5.98 (0.90)	4.80 (0.71)	4.34 (0.32)	3.33 (0.25)	30.7%	460 (29)	619 (34)	755 (40)	82%	11%	16.0 (1.3)	22.7 (1.4)
5	15.42 (0.78)	12.39 (0.65)	11.32 (0.49)	8.76 (0.56)	29.3%	861 (68)	1118 (89)	1330 (80)	84%	7%	15.1 (0.8)	22.9 (1.1)
5	9.20 (0.50)	7.41 (0.47)	6.33 (0.21)	4.94 (0.18)	33.3%	586 (57)	724 (68)	820 (54)	88%	7%	16.9 (1.2)	24.1 (1.0)
5	5.34 (0.54)	4.33 (0.52)	4.32 (0.23)	3.30 (0.16)	23.8%	350 (25)	451 (31)	520 (42)	87%	8%	13.1 (1.2)	19.9 (1.7)

Standard deviations are shown in brackets, number of samples 5-10.

*measured in the fermenter effluent

2.3.4 VFA Composition and production

The produced VFA composition is important as it can provide useful information on the degree of hydrolysis and fermentation. In this study acetic acid was the dominant product of biomass fermentation regardless of SRT – Figure 2-2. It was also observed that the percentage of acetic acid in the total VFA production decreased significantly (two- tailed, t-test, $\alpha < 0.05$) with the increased SRT. At an SRT = 5 d, acetic acid was approximately 66% of the total VFA produced. When the SRT increased to 7 and 10 d, acetic acid decreased to 59% and 49%, respectively. The fraction of propionic acid was fairly constant, regardless of SRT (16-18%). The fraction of long chain VFA (C4-C8) of the total VFA increased from 18%, 24% to 34% with the increased SRT from 5, 7 and 10 d, respectively.

Hydrogen is typically a by-product of non-methanogenic fermentation hence acetate production is linked to hydrogen production. As the rate of total fermentation increases so does the hydrogen partial pressure. The increased hydrogen partial pressure would redirect fermentation pathways towards the production of longer chained VFA (Han and Shin, 2004). This may have been the reason for the higher long-chain VFA production at higher SRT. Compared to SRT, biomass concentration showed much less impact on the VFA composition.

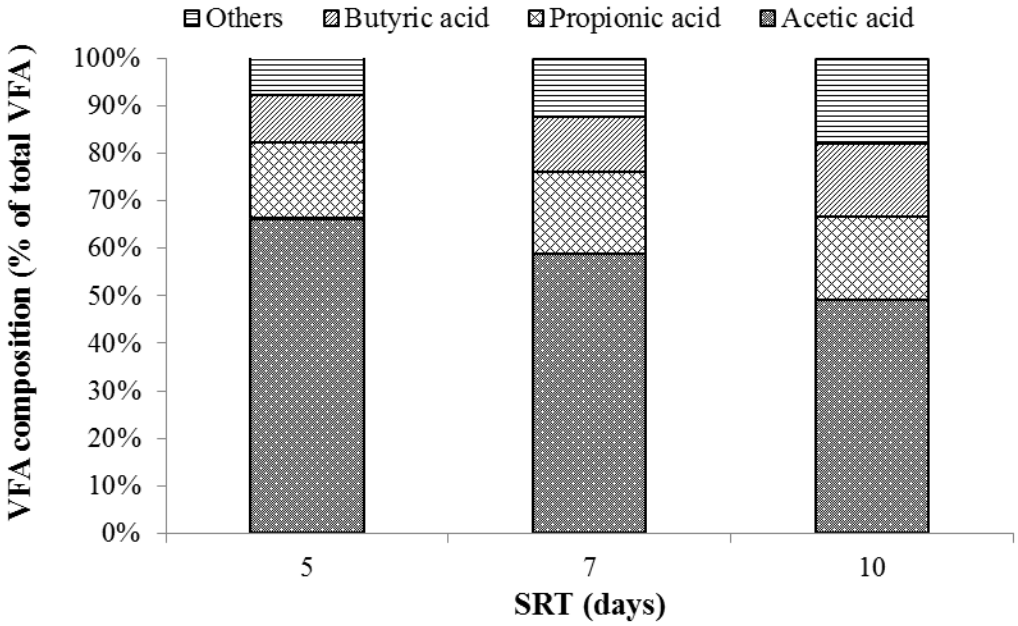


Figure 2-2 The effect of SRT on VFA composition

(At SRT=5 d, MLVSS = 4.33 g/L, t VFA = 350 mg/L; For SRT = 7 d, MLVSS = 4.80 g/L, tVFA = 460 mg/L; At SRT =10 d, MLVSS= 4.85 g/L, tVFA = 690 mg/L. The numbers are an average of 5-10 replicates)

Acetic acid and propionic acid are the primary substrates for biological phosphorus removal. Therefore it is important to look into their production during fermentation. Although it was shown in Figure 2-2 that the percentage of acetic acid in the VFA production decreased with the increased SRT, the overall concentration of acetic acid and propionic acid actually increased with the increased SRT as shown in Figure 2-3. This is due to the fact that the longest SRT of 10 d resulted in the highest total VFA concentration, 690 mg/L, whereas the SRT of 5 d resulted in the lowest total VFA concentration, 350 mg/L. Therefore, a longer SRT will result in an increased overall VFA concentrations, including those of acetic and propionic acid.

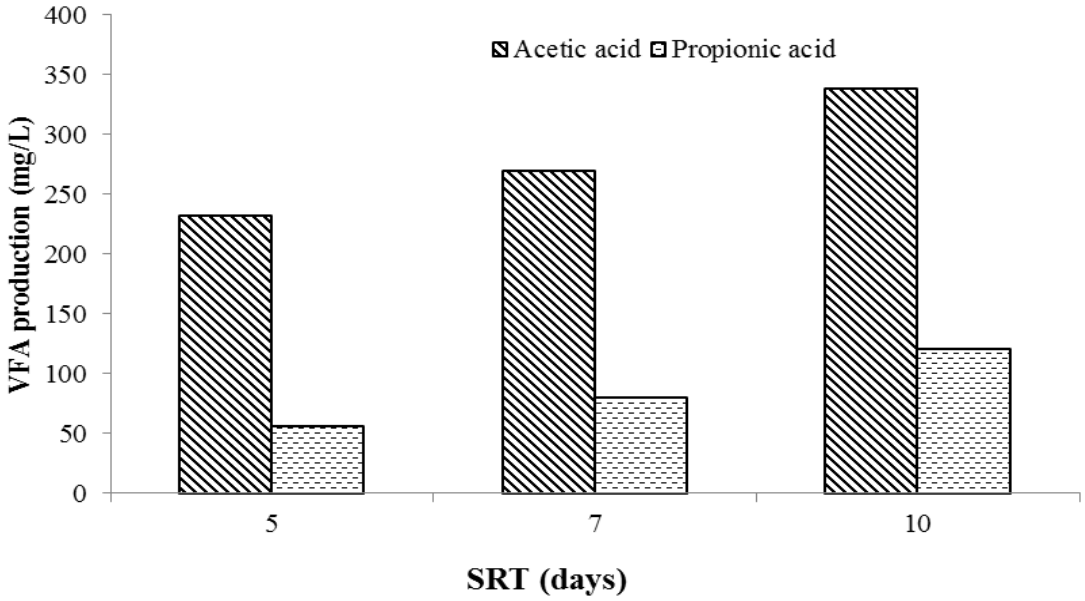


Figure 2-3 Acetic acid and propionic acid concentration in the fermenter effluent
(At SRT = 5 d, MLVSS = 4.33 g/L; SRT = 7 d, MLVSS = 4.80 g/L; SRT =10 d, MLVSS = 4.85 g/L. The numbers are an average of 5 to 10 replications)

2.3.5 The pH and alkalinity in the fermenter

Throughout the experiment, pH of the fermenter was between 6.1 and 6.8, without adding any acid. After start-up the pH of the fermenter dropped from 7.3 (feed) to 6.8 in 1 day and gradually dropped to 6.4 after 5 days. The pH then remained between 6.1 and 6.5 regardless of SRT and solids concentration. Although pH decreased due to the VFA production in the fermenter, the alkalinity of the fermenter effluent was higher than the influent. In addition, significant alkalinity variation (400 and 1220 mg/L as $CaCO_3$) was observed even though the pH in the fermenter remained stable. The highest alkalinity was measured in the fermenter with the highest VFA concentration. Fermentation caused lysis of cells and the release of cell content such as phosphate, ammonia, and metals as well as volatile fatty acids. The presence of VFA was in the form of various salts utilizing

alkali metals as their counter ions. Notably, as the pK values of these VFA are in the approximate range of 3.5-5 and while the low pH is conducive to their dissociation, high concentrations of the dissociated VFA contribute to alkalinity increase. This indicates that alkalinity is possibly a more effective parameter for indicating the degree of fermentation of waste activated sludge and VFA production rather than pH. The release of the ammonium and phosphate from the lysed cells (see section 3.6) increased the buffering capacity such that the VFA contributed less than expected to the pH of the system. Although methane was not monitored in this test, methane production was not expected due to the short SRT and low temperature. The optimal temperature for methane production is 35℃ with an SRT of 15 days. Switzenbaum et al. (1990) suggested that VFA accumulation in an anaerobic digester would result in a high VFA/Total Alkalinity ratio. Therefore in order to maintain a healthy methane production in an anaerobic digester, the ratio of VFA/TA should be in the range of 0.1-0.35. In this fermentation experiment an average VFA/TA ratio of 1.1 was observed. This much higher ratio suggested that methanogenesis was not occurring and only VFA were produced in the fermenter.

2.3.6 Phosphorus and nitrogen release

As nitrogen and phosphorus are present in the bacterial cells, the process of biomass fermentation will result in the release of these nutrients as ammonium and phosphate. A high level of nutrient release is a characteristic that distinguishes biomass fermentation from primary sludge fermentation. It was observed that SRT did not have a significant impact on the nutrient released in the feed per unit influent TCOD. Table 2 shows that the

average value of phosphorus release from this experiment was 17.3 mg PO_4-P/g TCOD and ammonium release was 25.8 mg NH_4-N/g TCOD. Both nutrient release values were much higher than the value from primary sludge reported by Ubay-Cokgo *et. al.* (2005). The value obtained from their study was 0.55 mg PO_4-P/g TCOD and 2.1 mg NH_4-N/g TCOD respectively.

With the variation of SRT and biomass concentration, the phosphate and ammonium concentrations in the WAS fermenter effluent were in the range of 83-327 mg P/L, and 126-514 mg N/L respectively. The high nitrogen and phosphorus release observed during WAS fermentation can be beneficial for sustainable nutrient recovery. Indeed, previous studies (Yuan *et al*, 2009) have shown that the released nitrogen and phosphorus could be effectively recovered through struvite formation by magnesium addition. With pH increase to 9.5, approximately 92% phosphorus and 72% of ammonium were removed respectively. Nutrient release and subsequent recovery from this biomass fermentation could be one unique advantage of waste activated sludge fermentation over the primary sludge fermentation as a source of VFA in an EBPR process.

Mavinic et al. (2000) showed that in order to achieve full EBPR it was necessary to provide a ratio of 8 g VFA (HAc)/ g $P_{removed}$. The mass balance of the fermenter from this experiment (Figure 2-4) showed that the effluent from the fermenter provided only 1.5 HAc/P, which was much lower than the recommended ratio. This suggested that during biomass fermentation the VFA were not produced in amounts sufficient to remove the released phosphorus. If the fermenter effluent was recirculated back to the main-stream to be used as a source of VFA, this would have increased the load of phosphorus to the main-stream. This increased load of phosphorus would overcome the benefit of the VFA

addition, exceeding process capacity leading to the deterioration of EBPR (Wild and Siegrist, 1999).

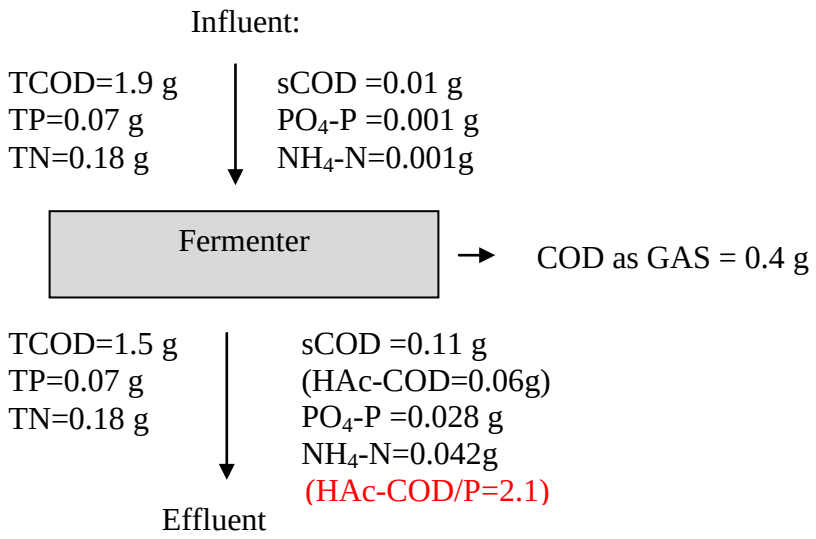


Figure 2-4 Daily mass balance for fermenter at SRT=5 d with influent solids concentration of 15.4 g/L

2.3.7 Effect of sludge type on VFA production and nutrient release

The type of sludge has significant impact on VFA generation and nutrient release during fermentation as shown in Table 3 In general, primary sludge contains a fairly high portion of easily biodegradable organic matter which can be fermented to VFA in a short time, with high VFA yields. The activated sludge contains mainly bacterial mass which requires complex enzymes for cell wall lysis and therefore a longer fermentation time is needed. Under similar SRT or HRT conditions, VFA yields from activated sludge are much lower than from primary sludge. However, because nitrogen and phosphorus are

present in substantial quantities within the bacterial biomass, fermentation of activated sludge results in their release. This does not happen during fermentation of primary sludge. The VFA yields at an SRT of 5 d from this study were quite comparable with those reported by Ucisik & Henze (2008). Fermentation of mixed primary and activated sludge is a compromise solution which enhances VFA production while the release of nutrients offers an opportunity for their recovery.

Table 2-3 Comparison of VFA production and nutrient release from fermentation of various types of sludge

Sludge type	Temperature (℃)	SRT /HRT (d)	Solids concentration (g/L)	VFA or COD production from substrate feed	N-released	P-released	Reference
Primary	35	5	8.5 g/L (VSS)	270 mg VFA-COD /g VSS	4.08 mg/g VSS	0.77 mg/g VSS	Ucisik & Henze [16]
Primary	18-28	6	TS=0.48-5.5%	50-110 mg VFA-COD/g TCOD	0.7-3.6 mg/g COD	0.3-0.8 mg/g COD	Banister & Pretorius [21]
Activated	35	5	8.5 g/L (VSS)	62 mg VFA-COD /g VSS	12.75 mg/g VSS	0.59 mg/g VSS	Ucisik & Henze [16]
Mixed primary & activated	35	5	8.5 g/L (VSS)	114 mg VFA-COD /g VSS	10.12 mg/g VSS	1.09 mg/g VSS	Ucisik & Henze [16]
Mixed primary & activated	18	2.5	3.3%	80 mg VFA/g VS	-	-	Skalsky & Daigger [18]
Activated sludge	22	5	12.4 g/L (VSS)	61 mg VFA-COD/g TCOD	22.9 mg/g TCOD	15.1mg/g TCOD	This study

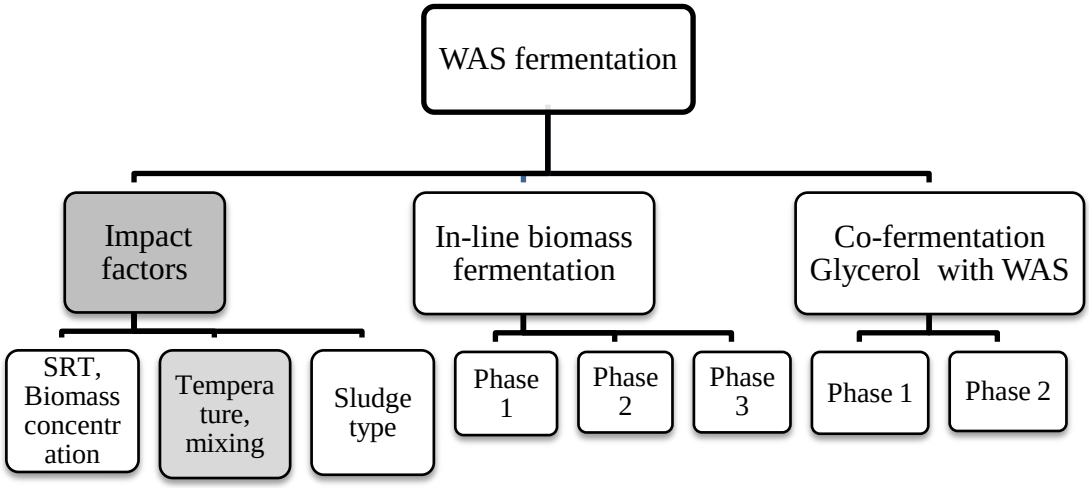
It should be pointed out that there is a great difficulty in comparing the VFA yields from waste activated sludge and mixtures due to the fact that researchers use different units for expression the VFA yield, such as: gVA/gVS, gVFA/g COD in the feed, g VFA-COD/g

VSS, g VFA-COD /g VSS-COD and g VFA(HAc)/ g TVS, etc. It was suggested that g VFA-COD/g TCOD in the feed maybe a proper unit to express to VFA yield due to the fact that COD is the consistent wastewater characterization unit adopted by the IWA ASM

2.4 CONCLUSION

The effect of SRT and biomass concentration on VFA production during waste activated sludge fermentation was studied in a set of semi-continuous experiments. The results indicated that VFA yields increased with the SRT; the longest SRT of 10 d resulted in the highest total VFA yield of 690 mg/L. The highest degree of solubilization, expressed as SCOD/TCOD, was also achieved at the longest SRT – a sign of enhanced degradation of the biomass. It was also found that VFA composition significantly changed with the SRT. Longer SRTs resulted in lower acetic acid fraction and a larger long-chain VFA fraction. Biomass concentration also played an important role in the VFA production. Under the same SRT condition, improved VFA yields were observed when biomass concentration decreased. High nutrient release was observed during Bio-P biomass fermentation. The average phosphorus release was 17.3 mg PO_4-P/g TCOD and ammonium release was 25.8 mg NH_4-N/g TCOD. Compared to the primary sludge fermentation of VFA generation, a longer SRT is required for WAS fermentation. On the other hand, WAS is more consistent in composition than primary sludge, therefore, WAS fermentation should yield a more stable product composition. Nutrient release during WAS fermentation offers an opportunity for their recovery.

Chapter 3 **VFA GENERATION FROM WASTE ACTIVATED SLUDGE: EFFECT OF TEMPERATURE AND MIXING**[*]



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3.1 INTRODUCTION

Enhanced biological phosphorus removal (EBPR) processes have been considered as one of the most effective and potentially least expensive ways to remove phosphorus from wastewater. The key to efficient EBPR performance lies in the presence of adequate volatile fatty acids (VFAs) in the influent wastewater (Chu et al., 1994; Danesh and Oleszkiewicz, 1997; Oleszkiewicz and Barnard, 2006). For low organic content (weak) wastewater, such as municipal wastewater, external VFA addition is becoming necessary to sufficiently stimulate phosphorous removal to meet the increasingly stringent effluent phosphorus limits. As the cost of purchasing external carbon continues to increase along with the overall greenhouse gas footprint of the treatment plant, research is focusing on on-site VFA production. One widely used method is to rely upon primary sludge for on-site production of VFA. Although the practice of using primary sludge fermentation is well established worldwide (Munch and Koch, 1999; Chanona et al., 2006; Bouzas et al., 2007), the actual process is difficult to control and the reliability of VFA generation is often not adequate for both EBPR and denitrification, particularly in flat terrain large sewer systems such as in St Paul-Minneapolis, MN, Winnipeg West, MB, Gdansk, PL or Noosa, AUS (Tomas et al., 2003). As the result of these complications, recent research has focused on generation of VFA from fermentation of biomass or waste activated sludge (WAS).

Fermenting WAS to produce VFA offers many benefits such as cost-effective reduction of mass of sludge for disposal or carbon footprint decrease. Fermentation is considered to be a two-step process occurring sequentially. The first step combines of disintegration and hydrolysis of complex organic material to soluble substrate: both are extracellular

biological as well as abiotic processes. During this step, the complex organic materials are broken-down and solubilized to substrate. The second step is acidification of soluble organics where the acid-forming bacteria use these soluble substrates as energy source for their growth, resulting in fermentation products such as VFA, lactate, ethanol, etc. The rate of hydrolysis and acidification is a function of factors such as pH, temperature, substrate loading, hydraulic and solids residence times (HRT and SRT) (Veeken and Hamelers, 1999). Among these factors, temperature is one of the most important factors when operating in a climate subjected to significant seasonal changes in temperature. Temperature can affect biochemical reactions in many ways, such as reaction rate, the reaction pathway, microorganism yields, death rate, etc (ADM1, 2002). The most common used kinetic relationship to describe hydrolysis process is a first order reaction proposed by Eastman and Ferguson (1981). Veeken and Hamelers (1999) studied the hydrolysis rates of selected biowaste components at 20, 30 and 40 ℃. They observed that for all the studied biowaste components the hydrolysis rate constants increased at higher temperatures.

Several studies reported on the effect of temperature on VFA production from primary sludge fermentation. Perot et al. (1998) concluded that optimal hydrolysis conditions of primary sludge appeared to be a pH of 6.8 at a temperature of 50 ℃ and an agitation speed of 545 rpm. Skalsky and Daigger (1995) showed that VFA production from primary sludge at 14 ℃ was approximately 42% less than that observed at 21 ℃. Zhang et al. (2009) also observed that the VFA production from primary sludge was improved with the enhancement of temperature from 10 to 35 ℃. However, so far no study has reported on the effect of temperature on WAS hydrolysis rate and VFA generation.

Besides temperature, mixing is another factor in biomass fermentation. Mixing is expected to keep the organic material in suspension in order to increase the contact between the microorganisms and substrate (Pfeffer, 1974). Stafford (1982) studied the effect of mixing on anaerobic sludge digestion and reported that for impeller speed of between 140 and 1000 rpm, no improvement in gas yields was observed. Gomez et al. (2009) compared mixing and static condition for bio-hydrogen production from waste fermentation and concluded that the performance of fermentation system could be improved by means of agitation. In sludge fermentation gravity thickener was the most common used facility. Under the incompletely mixed conditions in such a thickener-fermenter, the biomass settles while fermenting. The mechanical stirrer rotated at 1-2 rpm. In a bench-scale study by Danesh and Oleszkiewicz (1997) infrequent, short-time mixing of primary sludge in a clarifier-fermenter was found adequate as diffusion was thought to be responsible for most of the VFA release to the over-laying liquid.

The objectives of this experiment were 1) to study the WAS hydrolysis rate to offer guidance in process design; 2) to examine the effect of temperature and mixing on the rate and quality of VFA generated from biomass fermentation. Three temperatures applicable to the wastewater treatment plants in northern climates were employed (4, 14 and 24.6 ℃) and since the intent of WAS fermentation would be to provide VFA for EBPR, and 3) to monitor the release of phosphorus and ammonium during the fermentation process.

3.2 METHODOLOGY

WAS used in this study was obtained from the local wastewater treatment plant (Winnipeg South, Manitoba) which operates high purity oxygen COD removal process

with SRT maintained under 3 d. Bench-scale batch experiments were performed in nine 1.3 L reactors which operated under three different temperature conditions (4, 14 and 24.6 ℃). At each temperature, two conditions were tested: no mixing (one reactor) and complete mixing (2 reactors) at moderate speed of approximately 50 rpm. This speed was the slowest that still kept the particles in suspension in order to achieve complete mixing. Each reactor was closed/capped with a stopper. There was approximately 300 mL of headspace in each reactor, and the generated gases were exchanged with air during daily sampling. 10 mL of samples were taken daily for the following tests: COD, VFA, phosphate and ammonium. Samples were first centrifuged, and then were filtered with 0.45 μm syringe filters. At last the samples were diluted to 2-4 times for the composition analysis. For each reactor, solids were measured on the first and the last day. The test lasted for 20 d. Characteristics of the WAS used in the experiments are presented in Table 3-1.

Table 3-1 Sludge characterization

TSS (g/L)	9.73	pH	7.02
VSS (g/L)	7.15	VFA (mg/L)	2.1
SCOD (g/L)	0.025	NH_4-N (mg/L)	10.0
TCOD (g/L)	10.95	PO_4-P (mg/L)	3.5
TCOD/VSS (g/g)	1.53	Alkalinity (mg/L)	925

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) measurements were performed according to Standard Methods (APHA, 1998). Hach COD digestion vials (High range 20-1500 mg/L) were used to measure COD. Dissolved phosphate and ammonium were measured using a Lachat Instrument Quik Chem 8500, following the Quik Chem orthophosphate method 10-115-01-1-O, and Quik Chem ammonia method 10-107-06-1-I. The analysis of VFA composition was conducted by means of a Varian CP-3800 Gas Chromatograph using a flame ionization detector and HP-FFAP capillary column (inner diameter of 0.25 mm and length of 25 m). VFA concentration was converted to COD concentration by using the following conversion factors: 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric and isobutyric acid, 2.04 for valeric and isovaleric acid, 2.21 for caproic and iso-caproic acid and 2.34 for heptanoic acid.

VFA yield was expressed as the mass VFA-COD per unit mass of the initial VSS (VSS_0) in the reactor (g VFA-COD /g VSS_0). Solubilization was expressed as the mass ratio of the soluble COD to the total COD in the reactor (SCOD/TCOD). P and N release was expressed as the mass of soluble phosphate and ammonium per unit mass of VSS in the reactor (mg/g VSS).

3.3 RESULT AND DISCUSSION

3.3.1 Fermentation under completely mixed conditions

1.1 Determination of first-order hydrolysis rate constant and temperature coefficient

The primary products of hydrolysis process are soluble monomers which can be measured as soluble COD. According to Eastman and Ferguson (1981) the hydrolysis

can be expressed as a first order process with respect to degradable particulate components. The hydrolysis production in a completely mixed batch is given as:

$$X_a = X_{a0} + X_s (1- \exp [-K_{hT0} \theta^{(T0-T)} t]) \quad (1)$$

Where: X_a = hydrolysis production (COD mg/L)

Xs = degradable particulate components (COD mg/L)

T = temperature (℃)

t = time (d)

K_h = hydrolysis rate constant (d^{-1})

θ = temperature coefficient of K_h

Soluble COD productions at different temperatures in the reactors with mixing are shown in Fig. 3-1. It was observed that the hydrolysis in the 24.6 ℃ reactor was completed in 5 d which was indicated by the peak concentration of soluble COD at day 5. For 14 and 4 ℃ reactors, it took 7 and 9 d respectively to complete hydrolysis. Based on the soluble COD measurements during the hydrolysis process from the completely mixed reactors in this experiment, K_h values of 0.17, 0.08 and 0.04 d^{-1} at 24.6, 14, and 4 ℃, respectively, were calculated from the straight line portion of the curves.

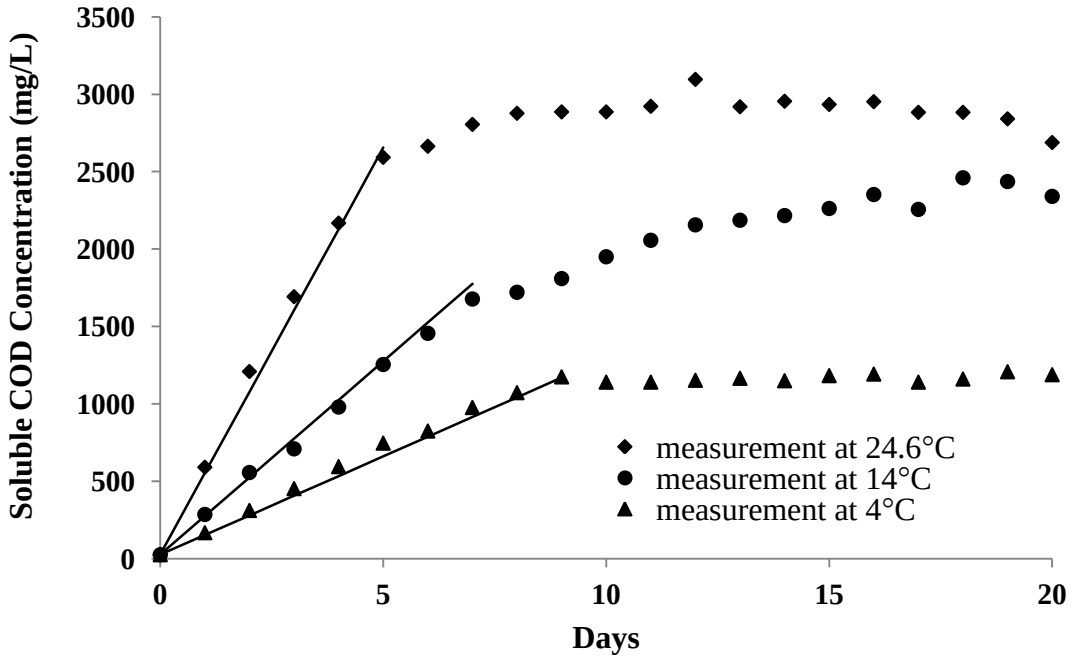


Figure 3-1 Measurement of soluble COD concentration at each temperature vs. the model (solid line)

Using Eq. 1 and K_h values obtained at each temperature, the K_h value at 20 ℃ and Arrhenius temperature coefficient (θ) was calculated as 0.12 d^{-1} and 1.07 respectively. As can be seen in Fig. 1, at each test temperature the theoretical data calculated from K_h 20 and θ showed excellent fit with the measurement data from this experiment. This indicated that using first order reaction to express hydrolysis process was valid in this experiment. Moser-Engeler et al. (1999) reported the kh value of 0.11 d^{-1} at 20 ℃ for the raw sludge (primary sludge, primary and WAS mixed sludge) fermentation, which was very close to the value obtained from this experiment. However, θ value obtained from this experiment is slightly lower than what they found, which is 1.09. This difference in the temperature coefficient could be the result of differences in the source of sludge used in the two experiments.

3.3.2 Hydrolysis and acidification

VFA-COD concentration (Fig. 3-2) at each temperature showed similar trend to the soluble COD concentration in the mixed reactors (Fig. 3-1). This suggested that although fermentation is a two-step process of hydrolysis followed by acidification, the two reactions are unlikely to be occurring sequentially. It was hypothesised that acidification, carried out by acidogenic bacteria occurred immediately once the soluble substrate from the hydrolysis was made available. Banister and Pretorius' research (1997) showed that as much as 40% of the total VFA production was generated in the first day of fermentation of primary sludge. Moser-Engeler et al. (1998) also reported that acid production rate during primary sludge fermentation can be characterised by the hydrolysis constant. The observation from this experiment, conducted on WAS was consistent with their observation.

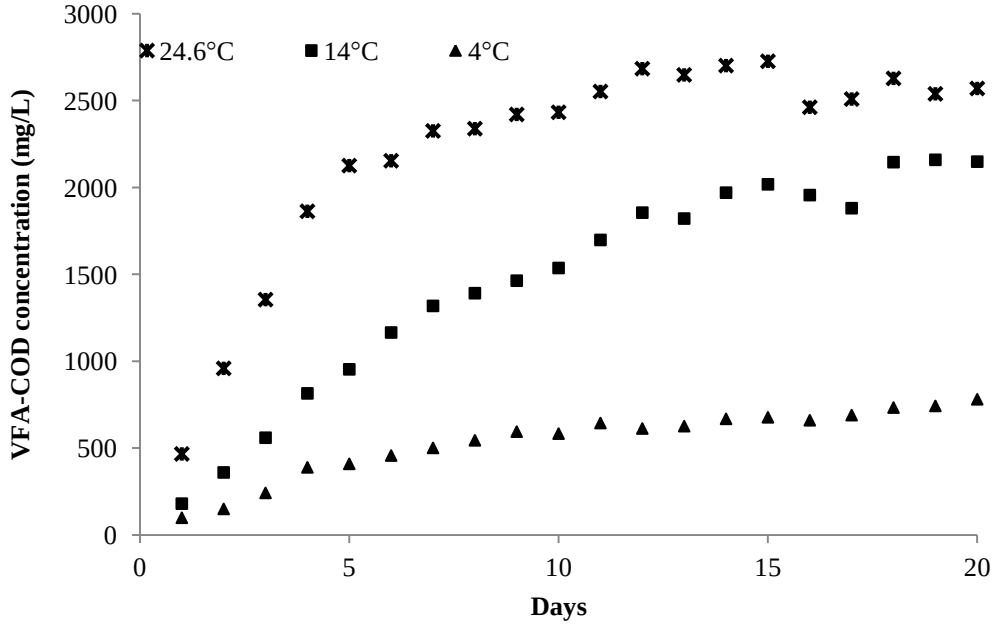


Figure 3-2 VFA production at different temperatures in reactors with mixing

(One set of data was presented from two reactors at each temperature)

3.3.3 VFA production at different temperatures

WAS fermentation at 24.6 ℃ (Fig. 3-2) was mainly completed after 5 d. The concentrations of acetic acid, propionic acid, butyric acid and valeric acids at day 6 were approximately 624, 395, 260 and 189 mg COD/L, respectively. The overall VFA-COD concentration curve showed a significant slowing down after 6 d of fermentation suggesting that the optimal number of days needed for VFA production was approximately 6 d for the digestion of this WAS at 24.6 ℃.

As expected, the time needed for complete fermentation increased with decreasing temperature. For the reactor operated at 14 ℃, it took about 14 d of fermentation for the reactor to near completion and generate a similar amount of VFA to that operated at 24.6 ℃ for 5 d (Fig. 3-2). As opposed to the 24.6 ℃ reactor, in the reactor of 14 ℃, there was no observed decrease of acetic acid occurring during 20 d fermentation. The overall VFA-COD concentration at 14℃ was comparable to the VFA-COD concentration produced at 24.6 ℃. This suggested that when temperature was lowered from 24.6 ℃ to 14 ℃, the fermentation rate decreased but was not inhibited.

Significant decrease of individual VFA concentrations and production rates were observed in the 4 ℃ reactor (Fig. 3-2). The VFA-COD reached a plateau of 595 mg/L after 9 d and no significant increase was observed after that. The VFA-COD concentration was approximately 28% of VFA-COD obtained at 24.6 ℃ after 6 d.

Feng et al. (2009) studied the effect of temperature on the VFA production from WAS under alkaline condition (pH = 10). They observed significant VFA increase when temperature was raised from 10 to 30 ℃. The study of temperature effect on acid phase anaerobic digestion of municipal and industrial wastewater conducted by Maharaj and

Elefsiniotis (2001) demonstrated that VFA production rate decreased significantly when temperature decreased from 25 ℃ to 8 ℃ at HRT of 30 h.

3.3.4 VFA composition at different fermentation temperatures

VFA composition is detailed for day 16 in Fig. 3-3. The VFA had reached a plateau in most of the reactors. No significant composition difference was observed between 24.6 and 14 ℃ mixing reactors. This again suggested that the decrease temperature from 24.6 to 14 ℃, slowed down but not inhibited the activity of microorganisms resulting in an overall decrease of the fermentation rate. For both reactors acetic acid and propionic acid were the major products accounting for approximately 40 and 30% of the total VFA production, respectively.

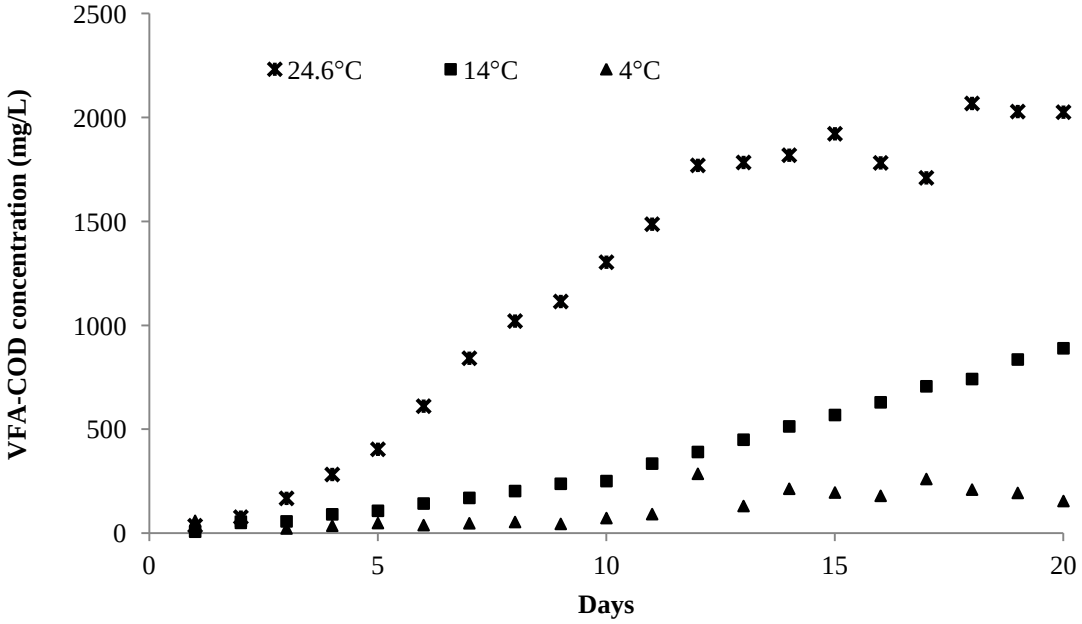


Figure 3-3 VFA productions under different temperatures in the reactors without mixing

In the mixed reactor operated under 4 ℃, acetate was the dominant product (55%). This observed change in the distribution of VFA at the lowest temperature can perhaps be explained by the operational condition affecting the types of particulates being hydrolyzed and consequently the types of VFA produced (Eastman and Ferguson, 1981). Another possible reason for this VFA distribution change could be that low temperature caused the change in microbial population and a shift in yield and the reaction pathway (ADM1, 2002).

3.3.5 The effect of mixing

The purpose of mixing is to provide an even distribution of the substrate for the growth of each group of microorganisms during fermentation. However, in practice the gravity thickeners commonly used as fermenters do not provide even and adequate mixing. Sludge concentrations increase with depth and VFA mass transfer is significant by diffusion to the supernatant. In order to compare the impact of mixing on the efficiency of fermentation the static, un-mixed reactors were also operated at different temperatures. It was observed that under the same temperature, both VFA production rates and mass of VFA were significantly reduced in the absence of mixing (Fig. 3-3). For the reactor operated under 24.6 ℃, it took 15 d for VFA-COD concentration to reach a peak of 1784 mg/L, which was about 83% of the concentration in the reactor with mixing at day 6.

Similar observation was made in the 14 ℃ reactors. The highest VFA-COD concentration in the un-mixed, static reactor was 890 mg/L, which was approximately

42% of that in the mixed reactor. The VFA composition was also different at 14 ℃. In the static reactor, acetic acid was the dominant VFA component and accounted for 69% of the total VFA concentration (Fig 3-4). In the mixed reactor at the same temperature acetic acid and propionic acid were the dominant VFAs produced.

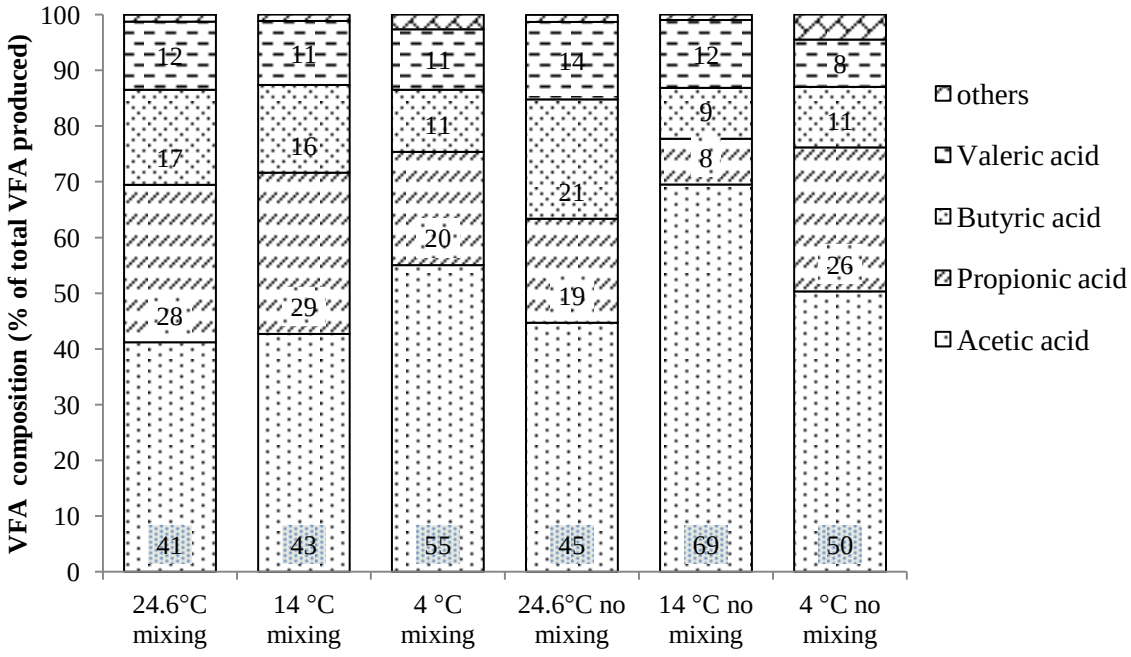


Figure 3-4VFA composition on day 16

The static reactor operated at 4 ℃ exhibited an increase in VFA-COD concentration was observed, however the plateau reached after 9 d, rather inconsistently, was low at under 200 mg/L of VFA-COD. The VFA-COD concentration in the un-mixed reactor was only 33% of that in the mixed reactor 4 ℃. The low VFA-COD concentration in the static reactor can be the result of two mechanisms: 1. Lack of mixing prevented the substrate supply from being uniformly distributed and available to microbial population thus decreasing the fermentation efficiency – as suggested by Stafford (1982); and 2. Slower

diffusion led to local increases in inhibitory biological intermediates and end-products (Stafford, 1982).

The result from this experiment demonstrated that mixing of sludge is an important factor for efficient acid fermentation of WAS. In all the static reactors, regardless of temperature, there is an apparent lag period at the beginning of the fermentation. The lower the temperature, the longer the lag period required. In this experiment 24.6 ℃ had a 3 d lag period, whereas 14 ℃ had a lag period of 9 d. It is suggested that time was required for the fermentation products to diffuse into the bulk solution and may not have reached equilibrium since the sample was taken from the top of supernatant,. Locally high concentrations of fermentation products and associated reduction in pH, in the sediments may also have been inhibitory.

The ratios of total VFA produced at day 5 and day 10 to the total VFA produced at day 20 at each temperature (Fig. 3-5), were much lower than the respective ratios in the un-mixed reactors. Among the static reactors, the ratios decreased with decreasing temperature. These observations confirmed the hypothesis that higher temperature and more intense mixing (or increased contact opportunity) are necessary for completion of the acid fermentation process. The low rates and mass of VFA-COD release in the static reactor put the concept of economical generation of VFA from WAS at the lowest temperature – here 4 ℃ – into question.

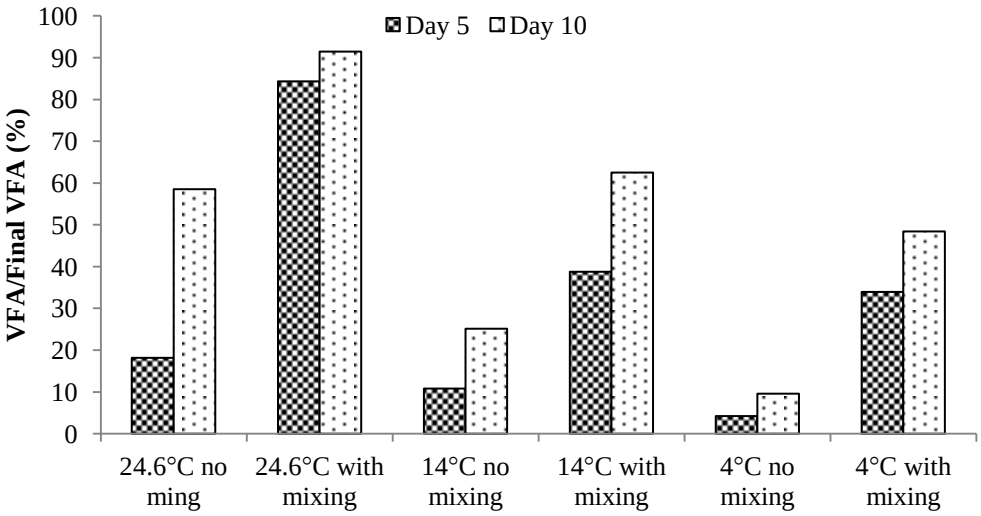


Figure 3-5 The percent VFA production on day 5 and day 10 to the final VFA production considered completed after 20 days.

3.3.6 Solubilisation and decrease in volatile solids produced

Solubilization of the biomass increased with temperature and with mixing. Mixed reactor operated at 24.6 ℃ provided the highest solubilisation of 25%, whereas the static reactor at 4 ℃ yielded only 2% solubilisation. VFAs were the primary products of fermentation process in the reactors operated at 24.6℃ and 14℃ and accounted up to 80-90% of soluble COD (Table 3-2). However, when the fermentation occurred at 4 ℃, the VFA-COD to soluble COD ratio decreased significantly to approximately 51-57%. This was most likely caused by the change in thermodynamic yields and microbial population (ADM1, 2002) at the lowest temperature. The decreased VFA-COD/sCOD ratio at low temperature suggested that if VFA generation is the main goal of fermentation, higher

fermentation temperature is required and that low-temperature fermentation (4 ℃) will be impractical.

Table 3-2 Summary of N, P released and VFA formation at the end of fermentation

Conditions		NH_4-N release ($mg/gVSS_o$)	PO_4-P release ($mg/gVSS_o$)	VFA yield (gVFA-COD /g VSS_o)	VSS destruction (%)	SCOD/ TCOD (%)	VFA-COD /SCOD (%)
24.6 ℃	Static	50	13	285	33	19	90
	Mixed	**64**	**14**	**355**	**40**	**25**	**87**
14 ℃	Static	20	8	123	23	9	81
	Mixed	**50**	**12**	**296**	**40**	**24**	**83**
4 ℃	Static	4	4	37	17	2	51
	Mixed	**23**	**8**	**109**	**22**	**11**	**57**

Along with VFA production, another important benefit of biomass fermentation is solids reduction. During biomass fermentation, much of the biomass was solubilised resulting in 22-40% VSS reduction. The highest VSS reduction of 40% was achieved in both mixed reactor operated under 24.6 ℃ and 14 ℃. The observation of similar VFA-COD/SCOD ratio, VFA yields and VFA compositions suggested that when the temperature dropped from 24.6 ℃ to 14 ℃, the fermentation reactions slowed down but did not changed significantly. When temperature dropped to 4 ℃ significant differences in all the parameters were observed, suggesting that at 4 ℃ the reactions not only slowed down but also changed.

3.3.7 Phosphorus and ammonium release

Fermentation of activated sludge results in the release of nitrogen and phosphorus from the decomposing biomass. The WAS used in this study originated from a non-EBPR plant and thus had typical low mass phosphorus concentrations of 1.2-1.5% phosphorus in TSS and typical nitrogen content of 8% TN, by weight. The release of nitrogen and phosphorus, expressed per g of VSS fed in (mg/g VSS_0) is presented in Table 2. The PO_4 and NH_4 amounts released are directly proportional to the degree of fermentation

The highest P and N release was achieved in the mixed reactor at 24.6 ℃ was 14 and 64 mg/g VSS_0, respectively. The release of both P and N from the reactors without mixing was significantly lower than that from the mixed reactors. The decrease in nutrient release was further hindered by the lower temperature.

In order to facilitate recovery of phosphorus as struvite from the fermentate the concentration of phosphorus must be as high as possible. The concentrations of 50-75 mg/L are accepted as the lower limit of economic feasibility of struvite recovery through the fluidized bed crystallization process (Benisch et al. 2009). Thus PO_4 concentration is the limiting factor. In the present experiment at day 10, PO_4 concentrations were 100, 74 and 50 mg/L in the mixed reactor operated at 24.6, 14 and 4 ℃, respectively. The concentrations in the static reactors were all below the 50 mg/L threshold. This suggested that the fermenter supernatant could be used for phosphorus recovery when mixing of the fermenting sludge is implemented.

3.4 CONCLUSIONS

Fermentation of waste activated sludge from a full scale non-EBPR facility was tested in three temperatures: 24.6, 14 and 4 ℃ in mixed and un-mixed, static conditions. The

objective was to test the rate and magnitude of solubilization of the total COD; volatile fatty acids (VFA) composition of the fermentate supernatant COD; technical feasibility of achieving appreciable VFA yields at lower temperatures; with and without mixing. On the basis of the results discussed above we can conclude:

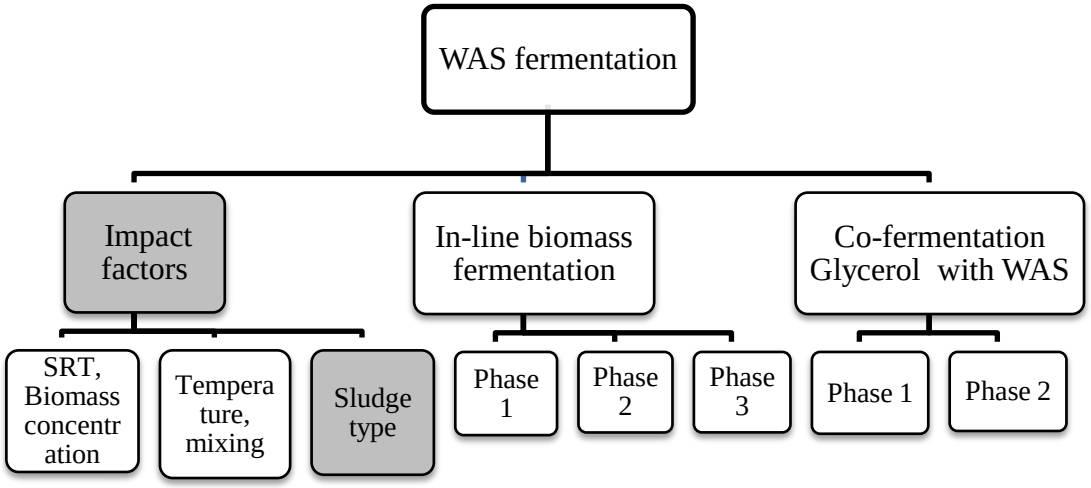
WAS fermentation was found to be a first order reaction with hydrolysis rate constant (k_h) values of 0.17, 0.08 and 0.04 d^{-1} at 24.6, 14 and 4 ℃, respectively. k_h at 20 ℃ and the Arrhenius temperature coefficient θ were 0.11 d^{-1} and 1.07, respectively.

Mixed reactor operated at 24.6 ℃ achieved the highest VFA-COD production of 2154 mg/L in the shortest time of 6 d, followed by the mixed reactor operated at 14 and 4 ℃, which obtained 2149 and 782 mg/L respectively at the end of fermentation. Lack of mixing drastically reduced the amount of VFA-COD generated, particularly at low temperatures. It was concluded that the process would not be feasible technically at the low temperature of 4 ℃, with or without mixing.

Concomitant release of NH_4 and PO_4 was directly proportional to the rate and magnitude of fermentation and was the highest in the mixed reactor at 24.6 ℃ which was 64 and 14 mg/g VSSo respectively. The high PO_4-P concentration in all the mixing reactors along with the high VFA concentration suggested that the fermentation effluent can be used as the substance for phosphorus recovery in form of struvite.

Solubilization of particulate solids led to decrease in the final amount of solids to be removed from the system. Volatile solids destruction of 40% was obtained for the reactor operated under 24.6 and 14 ℃ in this experiment after 10 d of fermentation.

Chapter 4 **EFFECT OF SLUDGE TYPE ON THE FERMENTATION PRODUCTS***



* published in ***Chemosphere,*** (2010), 80, 445–449

4.1 INTRODUCTION

Sustainable development challenges the wastewater industry to both remove inorganic nutrients as well as to recover them from the waste stream. Limited availability of readily biodegradable COD (rbCOD) in the raw wastewater often hinders complete biological removal of phosphorus and nitrates. Adequate supply of short-chain volatile fatty acids (VFA) is necessary for successful performance of the phosphorus accumulating organisms. (Chu et. al., 1994; Danesh and Oleszkiewicz, 1997; Oleszkiewicz and Barnard, 2006). When the influent wastewater contains low concentrations of organic carbon and VFA, external substrate and VFA addition is necessary to satisfy the demand for rbCOD and to fulfill the effluent demands for nutrient removal. This additional external substrate can be also produced in the wastewater treatment plant itself by fermenting the primary sludge. The fermentation of primary sludge to generate VFA is well established and has been practiced worldwide (Munch and Koch, 1999; Chanona et al., 2006; Bouzas et al., 2007). However, there are some issues in primary sludge fermentation need to be addressed, such as the difficulty in controlling the process and the reliability of VFA generation.

Waste activated sludge (WAS) fermentation to produce rbCOD as a supplement to PS fermentation is an untapped opportunity (Yuan et al., 2009). PS differs from WAS as it contains high concentrations of easily biodegradable organic polymers (proteins, lipids carbohydrates), permitting short hydrolysis and fermentation times. WAS, on the other hand, contains mostly bacterial mass; cell lysis is therefore the rate-limiting step. The fermentation of WAS is expected to release phosphorus and nitrogen into the liquid phase. Recent research (Yuan and Oleszkiewicz, 2010) conducted on artificial substrate-derived

solids has demonstrated that fermentation of WAS had the following advantages: (1) it provided internal carbon for biological nutrient removal (BNR) and (2) the nutrients released during the fermentation could be recovered in a useful form such as struvite. In addition, WAS fermentation decreases the mass of generated excess sludge which reduces the operating costs and increases the value of WAS. Andreasen et al. (1997) and Ucisik and Henze (2008) demonstrated that compared to PS, WAS has much slower biodegradability which was indicated by a lower resulting sCOD to total COD ratio. Skalsky and Daigger (1995) observed that blending fermented PS and WAS, resulted in a significant amount of phosphorus (50% of the WAS total phosphorus) release. The released phosphorus could be beneficially recovered with addition of magnesium salt to form struvite if the concentration and amounts are large enough.

The objective of this experiment was to study the feasibility of attaining sCOD through fermentation of PS, WAS and a mixture of PS and WAS from a full scale BNR plant and from a full scale plant removing only COD.

4.2 MATERIAL AND METHODS

The PS and WAS used in this study were obtained from two wastewater treatment plants in Winnipeg: South End Water Pollution Control Center (SEWPCC) and the West End Water Pollution Control Center (WEWPCC). The SEWPCC is a COD removal only facility with a solids retention time (SRT) of 2.5 d. The WEWPCC is a BNR facility with an SRT of 10 d. The PS from the WEWPCC undergoes fermentation in the plant's fermenter-thickeners. The PS was collected from the fermenter-thickener, i.e. after fermentation. The sludge blanket in the fermenter-thickener is approximately 1.5-2 m deep and the PS was collected from 0.1-05 m from the sludge blanket surface. During the

experiment period, one of the BNR reactors was out of service at WEWPCC. Therefore, ferric chloride was added to the primary clarifier to decrease the P load to the other reactor. The average total phosphorus in the primary effluent decreased by 1-1.5 mg/L due to the addition of ferric chloride.

4.2.1 Batch test fermenter

Fermentation batch tests of each type of sludge (PS, WAS and mixture of PS with WAS) were conducted by using 1 L reactors. The WEWPCC sludge was mixed in a 1:3 (v/v) ratio of PS to WAS, while the ratio of PS to WAS in the SEWPCC sludge mixture was 1:1 (v/v). In the preliminary experiment, mixed sludge of WEWPCC PS and WAS in the ratios of 1:1, 1:2, 1:3 and 1:4 were tested. The results showed that the 1:3 ratio resulted in the highest P and N release and further increases in the WAS portion did not have significant impact on P and N release; therefore, PS to WAS ratio of 1:3 was used in this experiment. In order to obtain a comparable solids load to the WEWPCC, PS and WAS mixing ratio of 1:1 was used for the SEWPCC. Table 4-1 shows the characteristics of each sludge. The reactor was sealed with a rubber stopper and it was opened daily for approximately 1 min for sampling. NH_4^+, PO_4^{3-} and sCOD were measured for each sample. All reactors were operated at 22 ℃ (±1 ℃) with complete mixing and a 4 d hydraulic residence time (HRT). The pH of the reactor was not controlled.

4.2.2 Semi-continuous flow fermenter

Two semi-continuous flow reactors co-fermenting PS with WAS from each wastewater treatment plant were setup. The SRT and HRT of each reactor were identical

and equal to 4 d. The PS/WAS ratio in the feed was 1:3 and 1:1 (v/v) for the WEWPCC and SEWPCC reactors, respectively. The influent column in Table 4-2 lists the characteristics of the feed. Each reactor was operated under anaerobic conditions with no headspace. The reactors were sealed with rubber stoppers. Any gas produced during the acidogenic fermentation process, such as carbon dioxide and hydrogen, dissolved inside the reactor and escaped from the system during the feeding and wasting. Feed was pumped to the bottom of the reactor and effluent exited the system from the top of the reactor. Samples were taken regularly from both feed and effluent for solids, NH_4^+, PO_4^{3-}, sCOD, total COD (tCOD), alkalinity and VFA analysis. The pH was not controlled since the sludge had fairly good buffering capacity. The reactors were operated at 22 ℃ (±1 ℃) for 3 months.

Table 4-1 Characteristics of sludge used in the batch tests

	WEWPCC Sludge			SEWPCC Sludge		
	PS	WAS	PS+WAS	PS	WAS	PS+WAS
TS (g/L)	42.5 (±4.5)	5.0 (±0.3)	14.3 (±0.4)	22.4 (±2.5)	3.9 (±0.9)	13.2 (±0.4)
VS (g/L)	40.2 (±2.8)	4.7 (±0.1)	12.2 (±1.8)	17.9 (±1.0)	3.2 (±1.1)	9.6 (±0.5)
sCOD (g/L)*	2980 (±660)	63 (±39)	810 (±138)	2970 (±450)	63 (±9)	1700 (±240)
tCOD (g/L)	84900 (±23600)	7230 (±580)	25700 (±3360)	58700 (±12700)	5760 (±1540)	22300 (±1520)
pH	4.80 (±0.3)	6.71 (±0.2)	5.79 (±0.3)	5.66 (±0.5)	6.29 (±0.2)	6.01 (±0.3)
NH_4^+-N (mg/L)*	36 (±19)	6 (±6)	19 (±3)	320 (±36)	20 (±0.5)	128 (±47)
PO_4^{3-}-P (mg/L)*	112 (±55)	13 (±17)	38 (±5)	110 (±29)	17 (±4)	47 (±31)
TN (mg/L)	1493 (±314)	497 (±194)	667 (±246)	1312 (±276)	509 (±197)	603 (±358)
TP (mg/L)	556	198	276	363	94	227

*Sample was measured after filtration.

Table 4-2 Volatile solids, COD and nutrient changes in each semi-continuous flow bench-scale fermenter

	VS			TCOD	SCOD			NH_4^+-N			PO_4^{3-}-P		
	Influent (g/L)	Effluent (g/L)	Net Loss (g/L)	Influent (mg/L)	Influent (mg/L)	Effluent (mg/L)	Net increase (mg/g VSS)	Influent (mg/L)	Effluent (mg/L)	Net increase (mg/g VSS)	Influent (mg/L)	Effluent (mg/L)	Net increase (mg/g VSS)
WEWPCC	14.9 (±2.5)	11.8 (±1.6)	3.1 (±2.0)	25500 (±5290)	850 (±340)	3530 (±830)	180 (±54)	29 (±17)	121 (±48)	6.2 (±2.6)	51 (±31)	149 (±30)	6.6 (±1.8)
SEWPCC	14.2 (±1.3)	11.1 (±1.4)	3.1 (±1.3)	24700 (±3270)	1800 (±500)	4240 (±930)	172 (±12)	181 (±62)	345 (±92)	11.4 (±4.7)	78 (±33)	127 (±40)	3.6 (±1.5)

4.2.3 Analytical methods

Total solids (TS) and volatile solids (VS) measurements were performed according to Standard Methods (APHA, 2005). Hach COD digestion vials were used to measure COD. Dissolved PO_4^{3-} and NH_4^+ were measured using a Lachat Instrument Quik Chem 8500, following the Quik Chem orthophosphate method 10-115-01-1-O, and Quik Chem ammonia method 10-107-06-1-I. The analysis of VFA composition was conducted by means of a Varian CP-3800 Gas Chromatograph using a flame ionization detector and HP-FFAP capillary column (inner diameter of 0.25 mm and length of 25 m). VFA concentration was converted to COD concentration using the following conversion factors: 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric and isobutyric acid, 2.04 for valeric and isovaleric acid, 2.21 for caproic and iso-caproic acid and 2.34 for heptanoic acid.

4.3 RESULTS AND DISCUSSION

4.3.1 Batch test

It was found that, predictably, PS generated significantly higher sCOD than WAS (Table 4-3) at both treatment plants. The differences between PS and WAS in sCOD production were 173 and 95 mg/g VSS in the SEWPCC and WEWPCC, respectively. The conversion to sCOD from the fermentation of the WEWPCC PS solids was only 61% of that from the SEWPCC PS. This was due to the fact that WEWPCC PS has already undergone fermentation in the plant's PS static fermenter-thickener. The WAS from SEWPCC also generated higher sCOD (approximately 20%) than the WEWPCC WAS sludge. This is most likely due to the shorter SRT maintained in the high-purity oxygen

reactors at the SEWPCC than at the WEWPCC: 2.5 versus 10 d, respectively. Sludge with a short SRT has a higher ratio of active biomass and of hydrolysable organic solids (which are biodegradable) to the non-biodegradable organic solids than the sludge with a longer SRT.

Table 4-3 Nutrients and sCOD released in each reactor from the batch test

	NH_4^+-N (mg/g VSS)		PO_4^{3-}-P (mg/g VSS)		sCOD (mg/g VSS)	
	SEWPCC	WEWPCC	SEWPCC	WEWPCC	SEWPCC	WEWPCC
PS	6.3	4.6	2.5	3.3	221.5	135.7
WAS	15.1	8.3	5.7	7.5	49.0	41.1
Mixed Sludge WAS+PS	15.5	7.8	5.4	8.8	231.0	150.5
Theoretical [a] Σ WAS+PS	7.6	5.6	2.9	4.4	195.6	111.1

[a] Theoretical calculation is based on the solids mass ratio (converted from v/v). For SouthEnd mixed sludge, the PS: WAS = 85%:15%, and WestEnd mixed sludge PS: WAS=74%:26%. Example calculation of SEWPCC NH_4^+-N: 6.3*85% +15.1*15% = 7.6 (mg/g VSS)

As expected, the released PO_4^{3-} and NH_4^+ from WAS during fermentation was much higher than from the PS (Table 4-3). PS has a lower nitrogen and phosphorus content than WAS (Table 4-1). WAS mainly consists of biomass, where nitrogen and phosphorus are major components of bacterial cells. Fermentation caused the cells to lyse, which results in the release of both nitrogen and phosphorus from the cell into the liquid phase. Differing from WAS, PS contains mainly organic polymers, such as protein, carbohydrate and lipids in which phosphorus content is fairly low. During PS

fermentation, enzymatic hydrolysis and ammonification occur, resulting in the formation of NH_4^+. Compared to WEWPCC sludge, both PS and WAS from SEWPCC released higher NH_4^+. The difference in PS can be explained in that partial NH_4^+ was already released from WEWPCC sludge during fermentation on site. The difference of NH_4^+ release in WAS might be due to the difference in the SRT. SEWPCC sludge has an SRT of 2-3 d which has a higher ratio of active biomass than WEWPCC sludge with an SRT of 10 d. In contrast to NH_4^+, both the PS and WAS from the WEWPCC released higher levels of PO_4^{3-} than the SEWPCC. Even though the WEWPCC PS had partial PO_4^{3-} release during fermentation at the plant, it still exhibited higher PO_4^{3-} release than the SEWPCC PS. One possible reason is that in addition to the hydrolysis, the WEWPCC PS contains precipitated P due to the addition of ferric chloride to the primary clarifier. This precipitated P (in the form of mixed complex polynuclear species) was re-solubilised at lower pH during fermentation. The BNR process results in a higher phosphorus release from WAS from the WEWPCC, because this process results in higher bio-P sludge content.

When PS and WAS from WEWPCC were co-fermented, increased sCOD production coupled with high P and N release were observed. The sCOD production was approximately 10% higher than that of fermentation from the PS alone. The theoretical sCOD production, calculated based on the solids mass ratio of PS and WAS, was approximately 111 mg/g VSS. The experiment yielded 151 mg/g VSS, which is 35% higher than the theoretically calculated value. In addition, the released P from co-fermentation was 8.8 mg/g VSS. This was also higher than fermenting WAS alone, although the WAS fraction in the mixed sludge was fairly low (26% of the total mass).

This result was twice the theoretically calculated value. A slight increase of NH_4^+ release was observed during co-fermentation. Increased sCOD production and P and N release were also found in the mixed sludge from the SEWPCC.

Regardless of the sludge source all mixed sludge had a higher sCOD, NH_4^+, and PO_4^{3+} release than the theoretically calculated values. This suggested that when PS is co-fermented with WAS, the reactions occurring during fermentation are different than when the two fermentation processes are occurring separately. This result can be explained by the following reasons: 1) the microorganisms in the PS are mainly anaerobic, while the microorganisms in the WAS are mainly aerobic. During the co-fermentation the anaerobic microorganisms in the PS will facility the hydrolysis process of WAS, which leads to the improvement of the WAS fermentation. 2) co-fermentation of the WAS and PS improve the balance and varieties of nutrients, provide synergistic effect of the microorganisms.

4.3.2 SCOD production and solids destruction in semi-continuous flow fermenter

In order to simulate potential large scale application, co-fermentation of WAS and PS was performed in the semi-continuous flow reactors. The objective was to compare the fermentation performance of sludge originating from wastewater treatment plants with and without biological nutrient removal.

The solubilization can be expressed as the ratio of increased sCOD during fermentation to total COD in the feed. Both fermenters showed similar degrees of solubilization of approximately 10%, as well as similar sCOD production (Table 4-2). The sCOD production from WEWPCC sludge was 180 mg/g VSS, which was slightly

higher than the results from the batch test. The sCOD production from SEWPCC sludge was 172 mg/g VSS, approximately 75% of the result from the batch test. This was probably due to the fact that the batch test was conducted using fresh sludge taken from the WWTP on the same day.

The sludge used for semi-continuous flow fermenter was taken from the WWTP weekly and was stored in the refrigerator at 4^{o}C. Significant sCOD increase (approx 719 to 1293 mg/L) was observed during one week time of storage of the SEWPCC sludge. Less sCOD was released from the WEWPCC sludge during storage which was approximately 150 to 574 mg/L. This was most likely due the supplementary addition of ferric chloride to the primary clarifier at WEWPCC to enhance COD removal. Higher sCOD released from the SEWPCC sludge (approximately 719 to 1293 mg/L) during storage also indicated that it was easier to hydrolyze at lower temperatures than the WEWPCC sludge (approx 150 to 574 mg/L). Along with the increased sCOD, the increased NH_4^+ and PO_4^{3-} concentration were also observed during storage of both sludges.

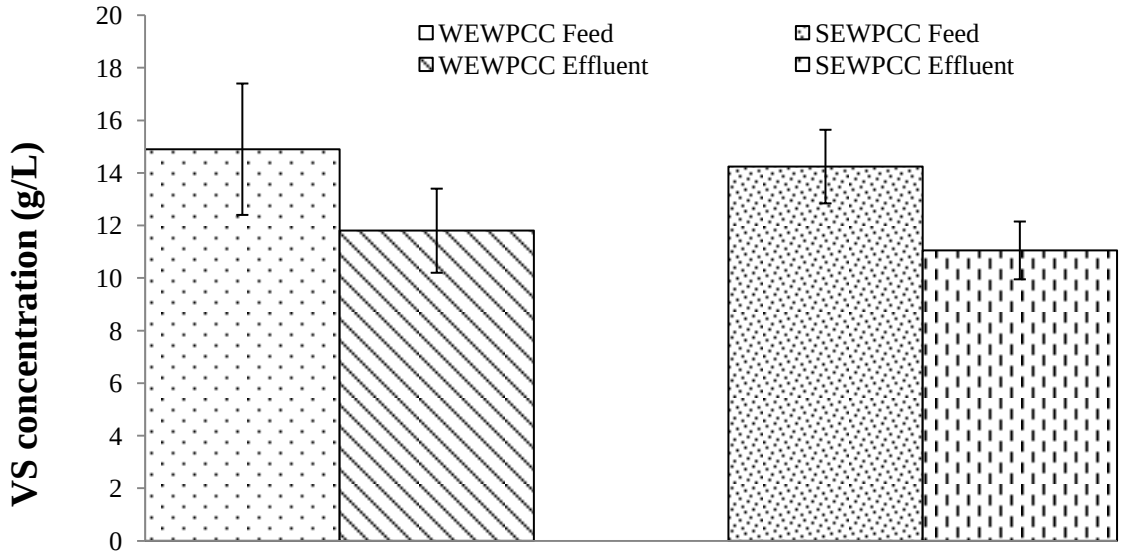


Figure 4-1Volatile solids concentration of the feed and effluent blended sludges from the two plants

Along with sCOD production, another important benefit of biomass fermentation is the reduction of excess biomass production. From this experiment, an average volatile solids destruction (VSD) of 21-22% was obtained during fermentation (Fig. 4-1). There was no significant difference of VSD observed in either sludge.

4.3.3 VFA to sCOD ratio and VFA composition

The main goal of sludge fermentation is to solubilise the solid organic particles and further convert to VFA which are the most important substrates for the enhanced biological phosphorus removal (EBPR) process. Therefore, it is important to know the VFA/sCOD ratio. The VFA-COD concentrations in both fermenters were measured as high as 3380 and 2860 mg L^{-1} from the WEWPCC sludge and the SEWPCC sludge,

respectively (Table 4-4). VFA were the major component of sCOD as indicated by the high ratio of VFA-COD to sCOD ratio of 93% for the WEWPCC and 82% from the SEWPCC sludge. Looking at the VFA-COD concentration as well as VFA-COD/sCOD ratio in the mixed WEWPCC sludge, one can conclude that in terms of VFA production, mixed sludge from the WEWPCC performed better than the mixed sludge from the SEWPCC. This was somewhat unexpected as the WEWPCC PS has undergone fermentation prior to the tests and WAS had almost a 5 times larger SRT.

Table 4-4 pH, Alkalinity and VFA in each reactor

	pH		Alkalinity (mg/L)		VFA-COD (mg/L)	VFA-COD/ sCOD
	Feed	Effluent	Feed	Effluent		
WEWPCC	5.7 (±0.2)	4.8 (±0.2)	479 (±134)	475 (±257)	3380 (±709)	92.8%
SEWPCC	6.0 (±0.2)	5.3 (±0.2)	905 (±145)	950 (±220)	2860 (±720)	81.9%

The VFA composition (Figure 4-2) provided information on hydrolysis and fermentation in addition to the suitability of supernatant as the carbon source. With respect to the EBPR process, acetic acid and propionic acid are the preferred substrates. The VFA composition produced from the two plants did not show significant differences.

Acetic acid, at 40-42% of the total VFA concentration, was the major VFA component, followed by butyric acid at 35-39%, 13-14% propionic acid and 9% valeric acid.

Due to the VFA production during fermentation, there was an observed decrease in pH by 0.7-0.9 units from the initial pH of 6.5-6.7 during fermentation observed in both reactors. Despite the decreasing pH, there was no significant change in the alkalinity of the WEWPCC fermenter effluent. The alkalinity of the SEWPCC fermenter effluent was slighter higher than that of the influent. This was probably due to the hydrolysis of organic matter resulting in the dissociation of anions and the lysis of cells releasing phosphate and ammonia, and producing acetic acid and dissolved CO_2, all contributing to alkalinity.

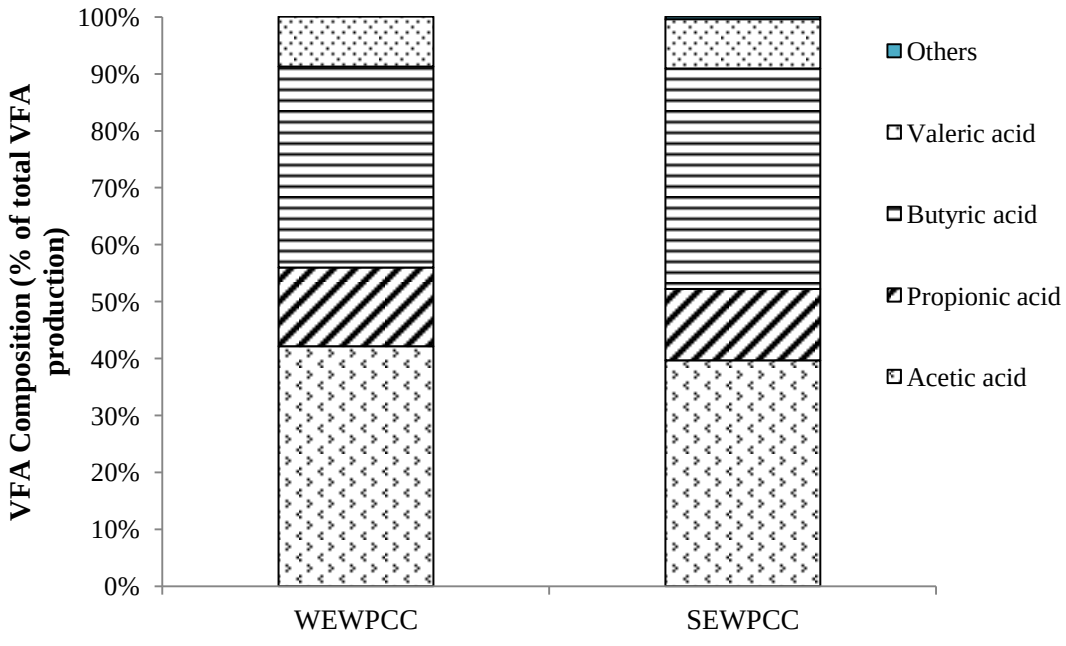


Figure 4-2 VFA composition in each reactor

4.3.4 Soluble phosphate and ammonium release

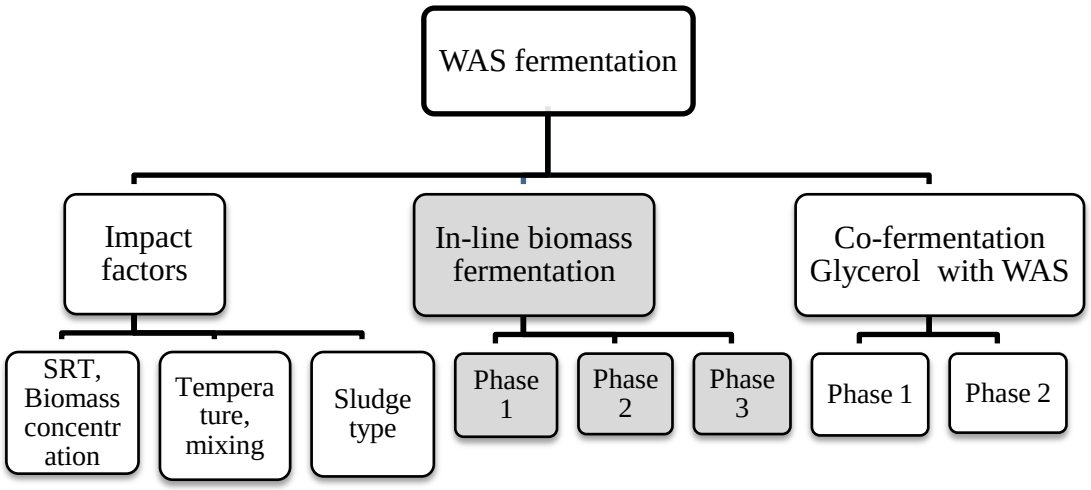
During the fermentation, the average NH_4^+ released from SEWPCC sludge was 11.4 mg/g VSS, higher than the WEWPCC of 6.2 mg/g VSS. The average PO_4^{3-} released from the SEWPCC sludge was 3.6 mg/g VSS, lower than the WEWPCC sludge of 6.6 mg/g VSS. This was consistent with the batch tests. The concentrations of released NH_4^+ and PO_4^{3-} from the semi-continuous flow were slightly lower than those of the batch test; however, they were higher than the theoretically calculated values from the batch test. In order to recover PO_4^{3-} and NH_4^+ as struvite, magnissium salt addition was necessary in at least an equal molar ratio to the limiting compound. Therefore, it is important to have the highest possible soluble PO_4^{3-} concentration, since PO_4^{3-} is the limiting element. The results from this experiment showed that although the average PO_4^{3-} released from the SEWPCC sludge was lower than from WEWPCC, the PO_4^{3-} concentration in the effluent from the SEWPCC sludge is comparable with the WEWPCC sludge (127 vs. 149 mg/L). It was reported that the minimal PO_4^{3-} concentration for struvite recovery via crystallizer such as the one reported by Benisch et al. (2009) was 60 mg/L; therefore, both sludge supernatants would be good candidates for PO_4^{3-} recovery.

4.4 CONCLUSIONS

PS and WAS from two plants which operated a BNR process and a COD removal process respectively, were fermented. Batch tests demonstrated that fermentation of PS from both plants generated significant amounts of sCOD, while WAS fermentation released considerable amounts of PO_4^{3-} and NH_4^+. Co-fermentation of WAS and PS enhanced both sCOD production and the PO_4^{3-} and NH_4^+ release. The semi-continuous flow co-fermentation study showed that, regardless of the sludge source, with a similar

total COD load, there was no significant difference in soluble COD production. The sCOD generated during fermentation consisted mainly of VFA. Solids from both plants have shown good potential for phosphorus recovery as struvite.

Chapter 5 **BIOMASS FERMENTATION TO AUGMENT BIOLOGICAL PHOSPHORUS REMOVAL**[*]



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5.1 INTRODUCTION

Since phosphorus, rather than nitrogen, is considered the leading factor contributing to the freshwater eutrophication (Schindler et al., 2008) its removal has become an essential part of wastewater treatment. Enhanced biological phosphorus removal (EBPR) is one of the most cost-effective ways to remove phosphorus from the liquid stream. The key to this process is the presence of adequate volatile fatty acid (VFA) (Chu et al., 1994; Oleszkiewicz and Barnard, 2006). For low organic content (weak) wastewater, external volatile fatty acid addition is becoming necessary to sufficiently stimulate phosphorous removal and meet the increasingly stringent effluent phosphorus limits. The purchase of VFA would increase the cost and carbon footprint to the point where it would become unsustainable. The practice of on-site VFA production by fermentation of primary sludge appears well established worldwide. However it was found that in flat terrain sewer systems, particularly e.g. in the Winnipeg West Manitoba, Canada (Zaleski, 2009), the VFA generation from the primary sludge fermentation is not adequate. In large flat sewers long residence times coupled with elevated temperatures lead to rapid depletion of the VFA production potential in the primary sludge arriving at the treatment plant. In some cases, beside the inconsistency of the VFA production, the total mass of VFA produced from primary sludge may be lower than required for both phosphorus and nitrate removal - as was the case in the Noosa plant in Australia (Thomas et al., 2003). To offset these problems research involving new methods of VFA generation is needed. Production of VFA from fermentation of in-process biomass, such as return activated sludge (RAS) or waste activated sludge (WAS) are some options.

Biomass fermentation benefits the plant by generating VFA and by reducing the overall production of WAS, which lowers sludge handling costs and plant's overall carbon footprint. The drawback is that the VFA are generated in conjunction with re-release of ammonia and particularly phosphates. The supernatant created during fermentation often contains high concentration of nutrients and therefore its recycle may actually offset the benefits of VFA produced. This phenomenon was observed by McIntosh and Oleszkiewicz (1997) who investigated micro-aerophilic fermentation of co-thickened primary sludge and WAS.

The phosphorus and ammonia need to be removed from supernatant before the stream is used as the additional source of VFA. One of the possible methods of ammonia and phosphorus removal is struvite precipitation. Struvite ($MgNH_4PO_4$, magnesium-amono-phosphate or MAP) is composed of equimolar amounts of magnesium, ammonium and phosphate. Removing ammonium and phosphate through struvite precipitation would allow the returned biomass fermentation supernatant to enhance the EBPR process while generating a value-added phosphorus fertilizer product.

Removing phosphorus through precipitation with lime has been practiced as a PhoStrip process (Daigger and Polson, 1991) to mitigate the problem of the ubiquitous presence of nuisance struvite deposits in various areas of biological phosphorus removal plants (Stratful et al., 2004). The objective of this study was to investigate VFA generation in the acid-phase fermentation of biomass and in-process struvite precipitation as a method of improved phosphorus removal and recovery.

5.2 MATERIAL AND METHODS

5.2.1 Experiment approach.

The experiment consisted of 3 phases.

Phase 1 Experimental set-up for Phase 1 fermentation of in-line biomass is shown in Fig. 5-1a. The biomass fermentation set-up consisted of a bench scale mother reactor and fermenter combination. The mother reactor was seeded with activated sludge from a lab sequencing batch rector with stable phosphorus removal and was operated in an anaerobic/aerobic configuration and went through three, 8-h cycles per day. In each cycle the reactor was fed with synthetic wastewater (COD of 300 mg/L, acetic acid of 30 mg/L, NH_4-N of 30 mg/L, PO_4-P of 10 mg/L). At the end of the 90 min anaerobic cycle a portion (6%) of the biomass was pumped to the fermenter. Subsequently, air was bubbled through the reactor to create aerobic conditions for 4.5 h. The dissolve oxygen (DO) was regular checked to make sure DO was over 2.0 mg/L. At the end of the aeration period, WAS was removed from the reactor to maintain the solids residence time (SRT) at 10 d. After 60 min of settling time the supernatant was decanted. Overall, the mother reactor had a hydraulic residence time (HRT) of 12 h. The reactor pH was controlled between 7.0-7.5.

The fermenter received biomass from the mother reactor three times a day. It was at the beginning of each cycle of the mother reactor that the effluent from the fermenter was added. The fermenter was well sealed and was operated under completely mixed conditions, with an SRT equal to HRT of 5 d. Phase 1 lasted 60 d. A control reactor, without connection to a fermenter, was also setup and operated under the same conditions as the mother reactor.

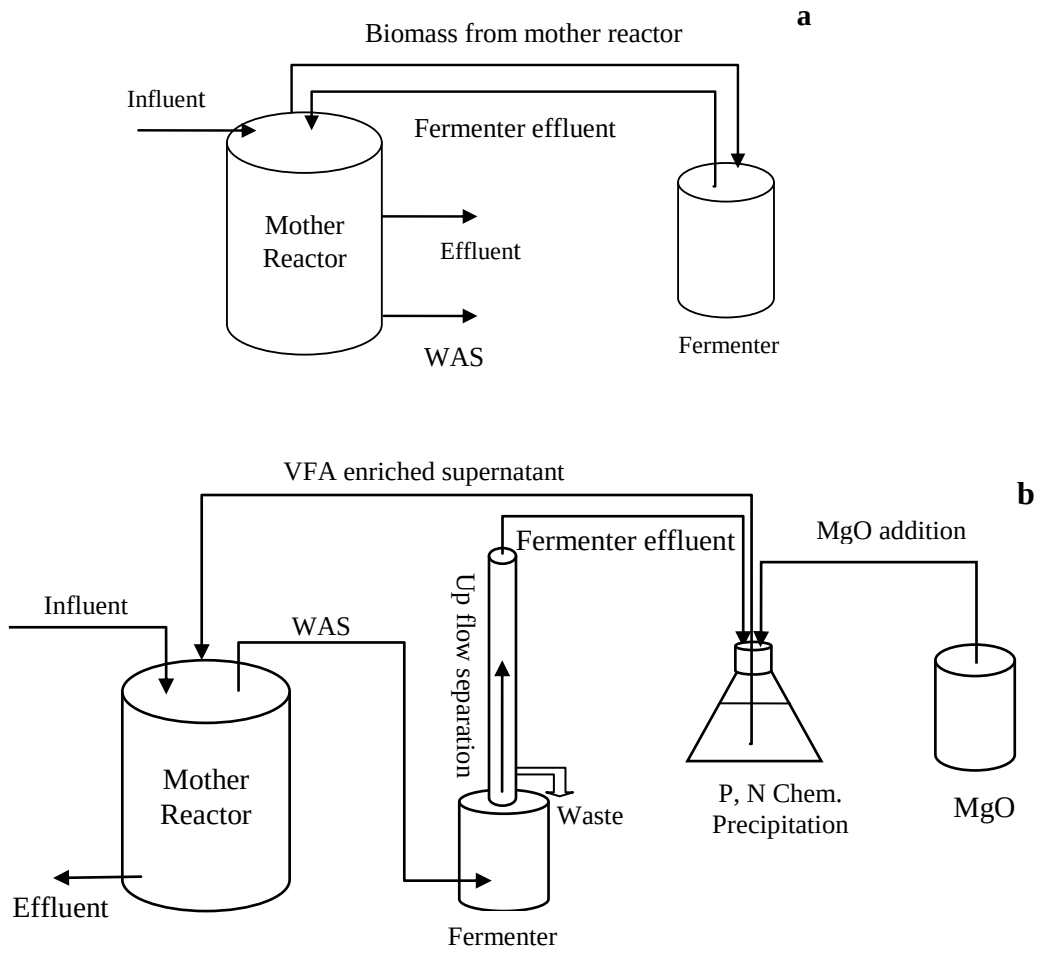


Figure 5-1Schematic graph of experiment setup (a: phase 1- Fermentation of biomass extracted from and returned to the mother reactor; b: phase 2, 3- Fermentation of wasted activated sludge and return of the fermentate supernatant)

5.2.2 Phase 2 and 3

The experimental set up of Phases 2 and 3 is shown in Fig. 5-1b. The setup and operation of mother reactor during Phase 2 and 3 was similar as Phase 1. The mother reactor was fed with the same substrates as in Phase 1 and went through 3 cycles per day.

The WAS was pumped out from the mother reactor at the end of the anaerobic period to the fermenter to maintain the SRT of the mother reactor at 10 d. The anaerobic phase lasted for 90 min, followed by an aeration phase of 4.5 h. The biomass was then settled and the supernatant decanted.

During Phases 2 and 3, the fermenter was connected to a vertical column to separate the biomass from the fermenter effluent (Fig. 5-1b). After biomass separation, the fermenter effluent was collected in a flask where 15 mg MgO in Phase 2 and 17 mg MgO in Phase 3 were added to precipitate phosphate and ammonium. Following chemical precipitation, the supernatant was pumped into the mother reactor as the VFA-rich liquor for phosphorus removal. In Phase 2, the supernatant was added to the mother reactor at the beginning of the cycle, whereas during Phase 3, the supernatant was added 30 min after the beginning of the cycle. Sample was also taken from the chemical precipitation flask to characterise the supernatant.

The final WAS biomass from the fermenter was manually wasted once per day from the bottom of the column. This final wasted biomass as well as the supernatant were then analysed to determine the characteristics of the fermenter effluent. Overall, the fermenter had an SRT equal to HRT of 5 d. The control reactor from Phase 1 also served as control reactor in Phase 2 and 3. Phase 2 and Phase 3 were operated for over two months. Throughout the experiment, the reactor and fermenter were operated under room temperature.

5.2.3 Analytical procedures

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were analyzed according to Standard Methods (APHA, 1998). Hach COD

digestion vials were used to measure the COD. Dissolved phosphate and ammonium were measured using a Lachat Instrument Quik Chem 8500, following the Quik Chem orthophosphate method 10-115-01-1-O, and Quik Chem ammonia method 10-107-06-1-I. Struvite crystals were examined under a phase contrast microscope (Nikon). Powder X-ray diffraction (XRD) analysis was carried out on a PANalytical X'Pert Pro diffractometer equipped with an X'Celerator detector using a Cu$K\alpha_{1,2}$ (λ = 1.540598, 1.544426 Å) radiation source.

The VFA composition was determined by means of a Varian CP-3800 Gas Chromatograph using a flame ionization detector and HP-FFAP capillary column (inner diameter of 0.25 mm and length of 25 m). VFA concentration was converted to COD concentration by using the following conversion factors: 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric and isobutyric acid, 2.04 for valeric and isovaleric acid, 2.21 for caproic and iso-caproic acid and 2.34 for heptanoic acid.

5.3 RESULTS AND DISCUSSION

5.3.1 Performance of the control reference reactor and full nitrification

The kinetic study and mass balance of the control reactor (Figs. 5-2 and 5-3) showed that low VFA (acetate) content in the feed resulted in poor phosphate removal. Influent 30 mg/L of acetate was completely removed in 15 min. There are approximately 8 mg NO_3-N /L remaining in the reactor from the preceding nitrification period and this was fully removed in the first 30 min. Since acetate is the preferred substrate (Ekama, et al., 1984 and Peng, et al., 2007) for both denitrifiers and phosphorus accumulating organisms (PAOs) there is a competition for acetate which is typically won by the denitrifiers and

results in lower acetate availability for the PAOs. This led to the low anaerobic phosphorus release and to the subsequent low aerobic phosphate uptake: 6 and 8.7 mg PO_4-P /L, respectively, which resulted in 2.7 mg/L phosphate net removal from the effluent - approximately 32% total phosphate removal.

In terms of ammonia removal, full nitrification was observed in the control reactor. The conversion of 26.4 mg/L of ammonia to nitrate occurred during 3 h of aeration which corresponds to approximately 8.8 mg/L of ammonia nitrified in 60 min. During the anaerobic phase, ammonia concentration has slightly increased due to the ammonification of the substrate. Beef extract and yeast extract, which have both relatively high nitrogen content, are the primary ingredients in the influent substrate. They were broken down and hydrolysed by the microorganisms during the anaerobic period with the release of small amounts of ammonia.

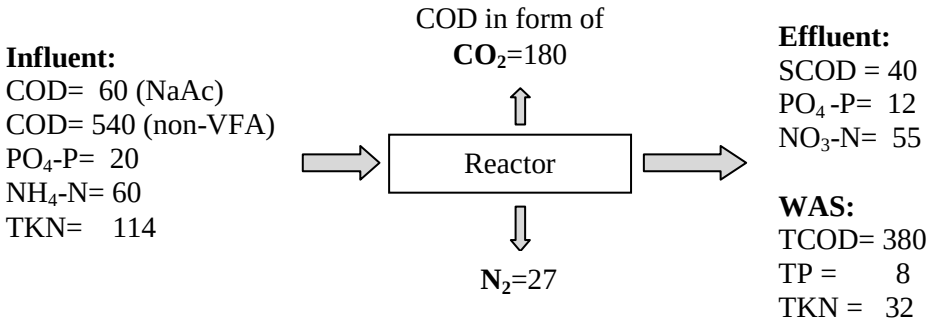


Figure 5-2 Mass balance in the control reactor. (Values are in mg/cycle. There were 3 cycles/day)

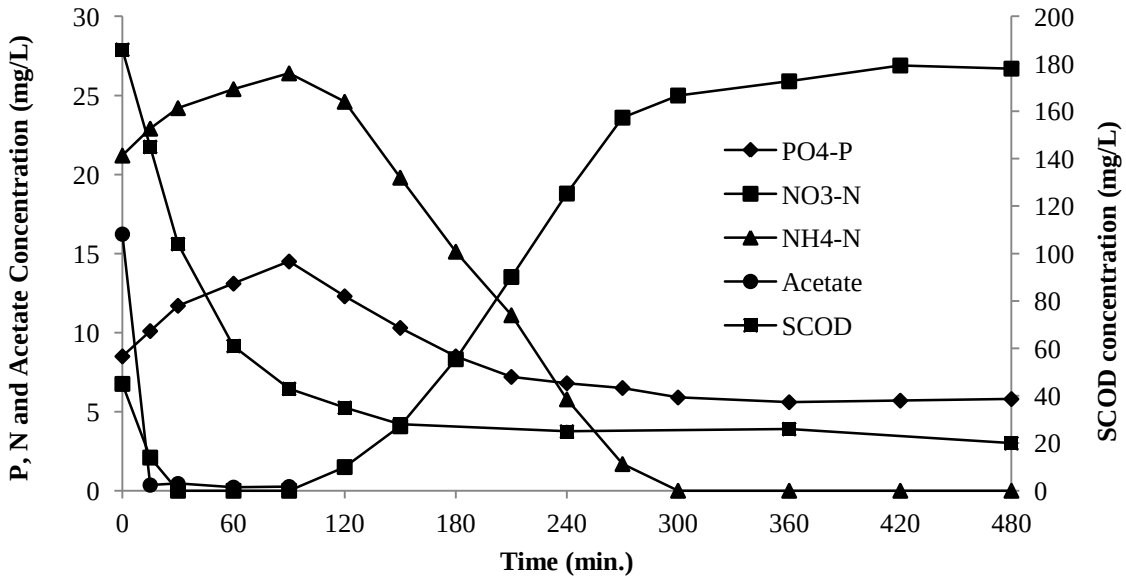


Figure 5-3 Nitrogen, phosphate, Acetate and SCOD profile of the control reactor

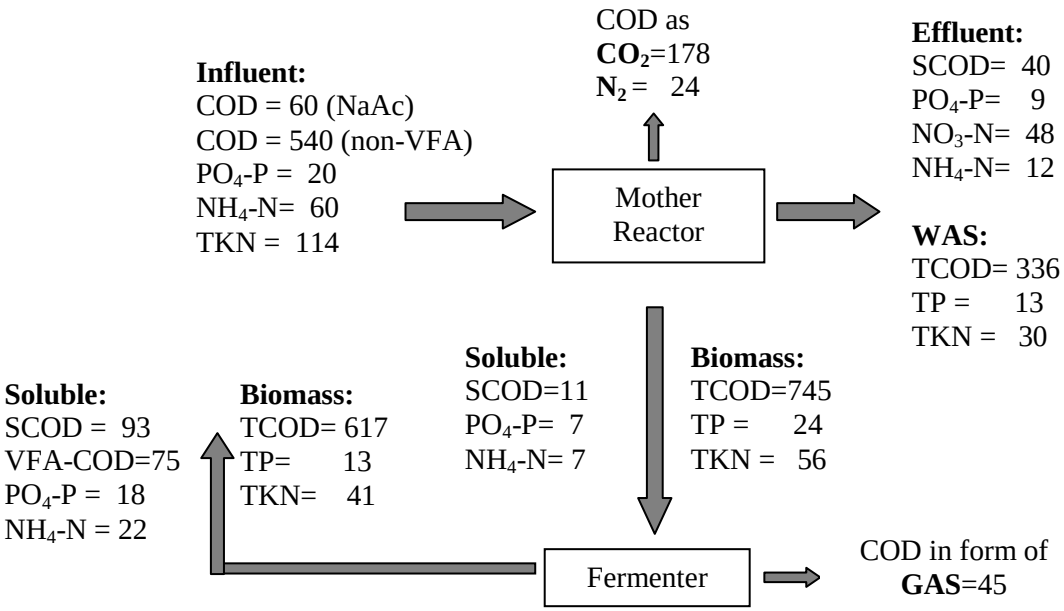


Figure 5-4Mass balance in the mother reactor and the fermenter (Phase 1). (Values are in mg/cycle. There were 3 cycles/day)

5.3.1 Performance of the mother reactor in Phase 1

5.3.1.1 Improvement of P removal with inline biomass fermentation

Compared to the control reactor, improvement of phosphorus removal was achieved in the mother reactor with VFA addition from in-line biomass fermentation. Biomass fermentation resulted in high VFA production (Fig. 5-4). The average VFA-COD concentration was approximately 305 mg/L in the fermenter. With the overall addition to the mother reactor of approximately 75 mg VFA-COD in each feed cycle, relatively high anaerobic phase phosphorus release of 27 mg/L was observed (Fig. 5-5) which is 21 mg/L larger than in the control reactor. Although aerobic phosphate uptake was 37 mg/L, and the net phosphorus removal was around 10 mg/L, there were still close to 4 mg PO_4-

P /L in the effluent. This remaining phosphorus is caused by increased phosphorus loading to the mother reactor. During fermentation the intracellular phosphorus was released into the fermentate which was then recirculated back to the mother reactor causing increased phosphorus loads. The mass of VFA in the fermentation products sent to the mother reactor was not sufficient to fully remove all the phosphorus from the system. This result implies that phosphorus must be removed from the fermenter effluent before the overall phosphorus removal capacity can be increased.

5.3.1.1 Deterioration of nitrification

In contrast to the improved phosphorus removal, ammonia removal rate deteriorated. Some 26.4 mg/L ammonia was removed in the control reactor in 3 h whereas in the mother reactor approximately 17.5 mg/L of ammonia was nitrified in the same period. Two hypotheses for this decline in nitrification can be offered: 1. lysis of nitrifying bacteria cells during fermentation resulted in their population depletion; or 2. there is an inhibition of nitrifying activity due to the fermentation products e.g. metals such as zinc or copper, (Utgikar et al., 2004) - which leads to an increase of the minimum SRT required for the nitrifiers to avoid washout.

Although similar amounts of ammonia were removed from both the mother reactor and the control reactor, the additional load of ammonia coming in with the fermentate was not nitrified and approximately 8 mg/L of NH_4-N were found in the effluent. This again implies that both P and N have to be removed from the fermentate before returning it to the mother reactor. The observation from Phase 1 led to modification of the process in Phase 2.

It was also observed that the VSS/TSS ratio in the mother reactor was 71%, i.e. lower than that of the control reactor at 78%. Fermentation results in the lysis of bacteria cells which causes both organic and inorganic compounds to be released into the liquid phase. The fermentation of the biodegradable fraction of the VSS led to the lowering of the VSS/TSS ratio in the mother reactor mixed liquor.

It should be pointed out that the advantage of biomass fermentation at the end of the anaerobic phase is that the anaerobic conditions are directly transferred to the fermenter. Less energy is wasted to achieve anaerobic conditions in the fermenter. In addition, such procedure will benefit phosphorus recovery since phosphorus is released anaerobically, and therefore a maximum concentration of phosphorus will be in the liquid phase and may be available for subsequent chemical precipitation.

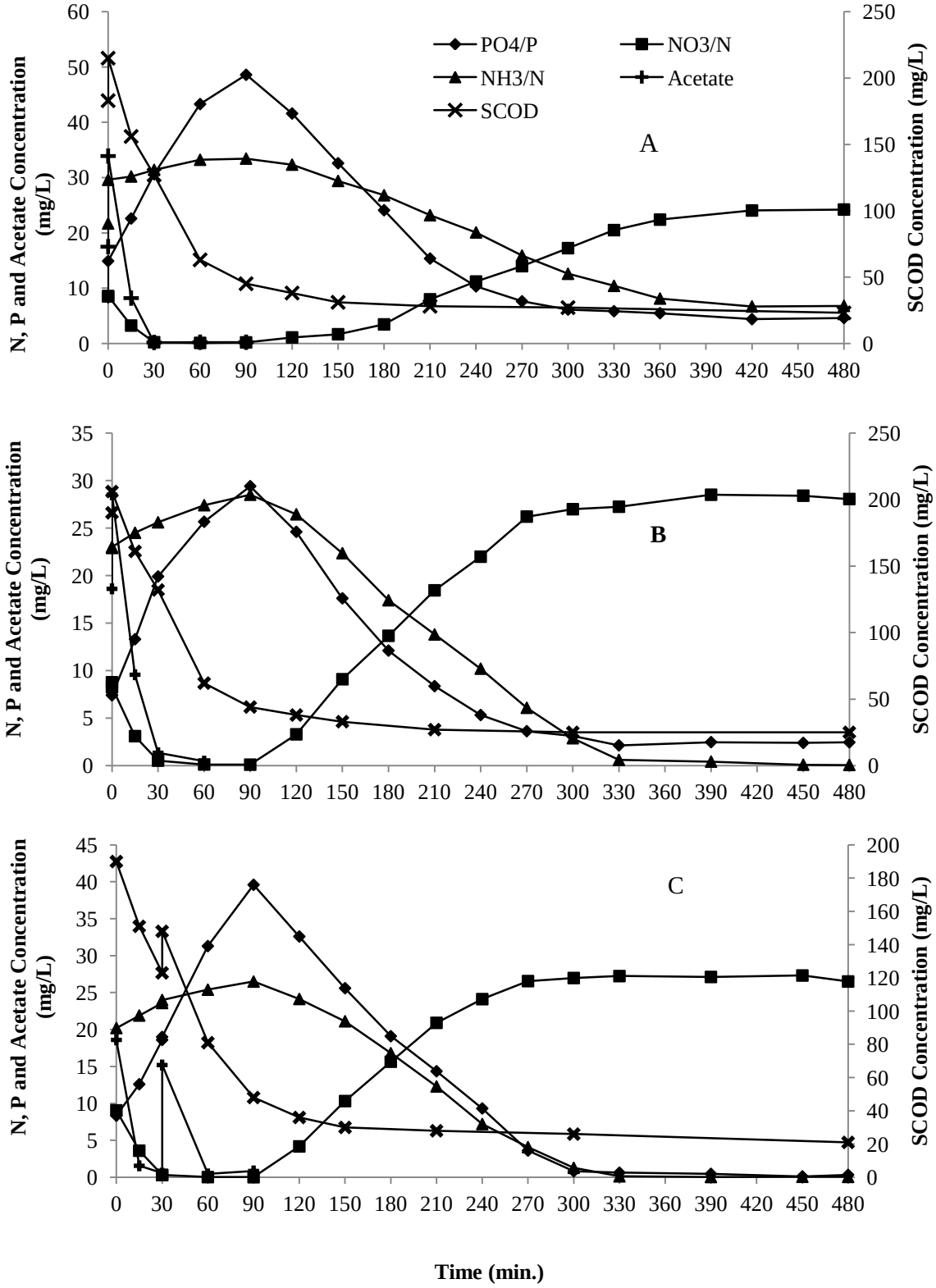


Figure 5-5 Nitrogen, phosphate, acetate and SCOD profiles in the mother reactor. A: Phase 1; B: Phase 2; C: Phase 3

5.3.2 Performance of the mother reactor in Phase 2 and 3

Based on the conclusions from Phase 1, the reactor setup was modified: 1. the fermenter was fed with biomass wasted from the mother reactor at the end of the anaerobic cycle instead of during the aerobic cycle; 2. phosphate, ammonium and biomass were removed from the fermentate before returning it to the mother reactor. The latter was achieved by applying an up-flow system to separate the biomass from supernatant, followed by adding MgO to the supernatant to precipitate phosphorus with ammonium as struvite – Fig. 5-1b. This allowed very little phosphate (< 0.5 mg) and ammonium (6 mg) to return back to the mother reactor.

5.3.2.1 P removal in the mother reactor

Phosphorus concentrations in the effluent decreased from 3.6 (phase 1) to 2.0 mg/L (phase 2) when P-loading to the mother reactor decreased from 18 mg to nearly 0 mg per cycle – Fig. 5-5B and Fig. 5-6. In Phase 2, only 3% of the biomass from the mother reactor was sent to the fermenter, compared to 6% of biomass in Phase 1. The decrease in biomass loading resulted in the lower overall amount of VFA-COD produced (Fig. 5-3, 5-6) and therefore a lower VFA addition to the mother reactor. Decreased VFA addition resulted in decreased anaerobic phosphorus release and aerobic phosphorus uptake. It was observed that the anaerobic phosphate release has decreased from 27 (phase 1) to 14 mg/L (phase 2), when VFA-COD addition was decreased from 75 (phase 1) to 42 mg (phase 2), and the subsequent aerobic uptake was 37 and 19 mg/L, respectively. These results confirm yet again how critical to the optimum PAOs performance is the presence of VFA.

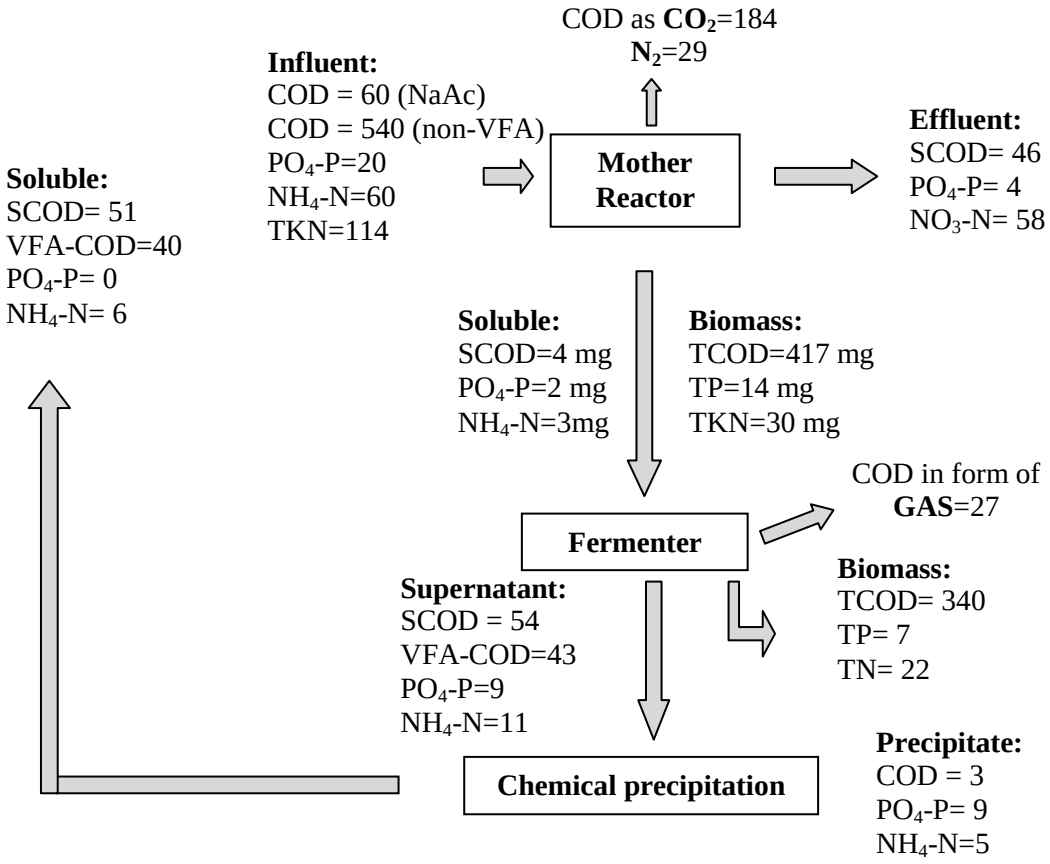


Figure 5-6Mass balance for the mother reactor and the fermenter (Phase 3). (All values are in mg/cycle. There were 3 cycles/day)

Although improvement in phosphorus removal was obtained compared to the control reactor, the presence of phosphorus in the effluent suggested that the amount of VFA added in phase 2 was not enough for the PAOs to fully perform phosphorus removal under the current process configuration. Nitrates block phosphorus release by successfully competing for VFA (Peng et al., 2007). This was confirmed in Phase 3, where the addition the VFA were added 30 min after the beginning of each cycle, i.e. once the denitrification had ceased. Fig. 5-5 C shows that up to 99% of phosphorus

removal was achieved in the mother reactor by simply delaying the time of VFA addition. The anaerobic phosphorus release increased to 22 mg/L, followed by the increased aerobic phosphate uptake of 30 mg/L. This experiment demonstrated that pre-denitrification is essential to achieve EBPR.

The amount of anaerobic phosphorus release during 1h in the reactors operated under different conditions are summarized in Table 5-1. It can be concluded that anaerobic phosphorus release rate is closely related to the availability of VFA to the PAOs. The more VFA that is available to the PAOs, the higher the PRR. This again demonstrated that VFA is the key substrate for PAOs performing phosphorus removal.

5.3.2.2 Nitrification in the mother reactor

Compared to Phase 1, the nitrification rate increased by approximately 34% (Table 5-1) in Phases 2 and 3, and there was no ammonia left in the effluent. Since WAS was used for fermentation as opposed to the inline biomass fermentation in Phase 1, there was no additional loss of the nitrifying bacteria and the nitrification activity in the system showed significant improvement. The nitrification rates during phase 2 and 3 were however slightly lower than the control reactor – Table 5-1. The reasons require further studies. It can be concluded that the modification of the reactor setup in Phases 2 and 3 significantly improved the nitrification rate and allowed the system to achieve full nitrification.

Table 5-1Comparison of parameters during individual phases

	Mother Reactor				Fermenter		
	VFA-COD addition (mg)	Phosphorus Release in 60' (mg P/L)	Nitrification in 60' (mg N/L)	VSS (g/L)	VFA-COD (mg/ $g_{biomass}$)	PO_4-P (mg/$g_{biomass}$)	NH_4-N (mg/ $g_{biomass}$)
Control	0	4.6	9.5	2.6			
Phase 1	75	21	6.7	2.5	151	24.2	30.2
Phase 2	40(addition at 0')	12.1	9.0	2.8	161	25.5	31.8
Phase 3	40(addition at 30')	18.1	8.9	2.8	160	25.4	31.2

5.3.3 Performance of the fermenter

Acetic and propionic acids are the primary substrates for biological phosphorus removal. Generation of these VFA from biomass fermentation was consistent during this experiment as shown in Table 5-1. Fermentation of 1 g of biomass produced approximately 157 mg of VFA-COD. The VFA were the primary products of fermentation as the average VFA-COD to SCOD ratio was 80%. Acetate is the component, accounting for 78% of the VFA composition followed by propionate at 10% of the total VFA produced.

Significant advantage of biomass fermentation is the decrease of the total overall mass of produced sludge (WAS). Compared to the control reactor, the decrease in WAS production was close to 8% during the in-line fermentation (Phase 1) and 20% during waste biomass fermentation (Phase 3). The lower decrease in Phase 1 is possibly due to

the increased concentration of solids fed to the mother reactor with the fermentate. This may have led to high inert composition in the sludge.

No significant change was observed in either ammonium or phosphate released from the biomass during all three phases of biomass fermentation – Table 5-1. The average phosphorus and ammonium released was about 25 mg PO_4-P /g VSS and 31 mg NH_4-N /g VSS biomass. This suggested that under the same SRT conditions, operation of the mother reactor does not have significant impact on the degree of biomass fermentation (degradation) and nutrient release.

5.3.4 Chemical phosphorus and ammonium precipitation

In Phases 2 and 3, MgO was used to precipitate N and P in the supernatant after fermentation, with the goal to form struvite. The composition of struvite is 1:1:1 molar ratio of magnesium, phosphate and ammonium. Theoretically approximately 12 and 14 mg MgO was required to react with phosphate in Phase 2 and 3 respectively. In order to assure a complete reaction, a slight overdose of MgO (15 and 16 mg in Phase 2 and 3 respectively) was used. Due to the low solubility of MgO, the residual MgO would precipitate out. MgO was used as Mg addition, because it increased the pH, which is favourable for struvite formation. The pH of MgO reagent is 10.2-10.5. Analysis of the supernatant showed that a nearly 1:1 molar ratio of P and N was removed from the supernatant. This suggested that ammonium and phosphate were precipitated out as struvite. The conclusion was further confirmed by XRD analysis (data not shown here). It was also noticed that close to 90-95% of P was removed from the supernatant while N removal was approx 70%. Struvite crystallization was found to be an effective method of removing (and recovering) both P and N.

5.3.5 Anaerobic carbon uptake in the reactors

Most of the carbon (90%) in the feed is from beef and yeast extracts, which primarily consist of proteins and carbohydrates, respectively. It was observed that the major fraction of COD was removed during the anaerobic period in both the control reactor and the mother reactor. This pattern followed a similar trend regardless of the amount of phosphates released (shown in Fig. 5-5b, 5-5c). This suggested that there is another group of facultative anaerobic bacteria - other than PAOs - that are present in the reactors. This group of microorganisms can utilize carbon anaerobically without engaging in phosphorus removal. Further investigation and characterization of this group of microorganisms is needed to understand their role in biological nutrient removal as it is important to optimise the utilization of the limited carbon source in the influent substrate.

5.4 CONCLUSIONS

- Biological phosphorus removal was closely linked to the availability of VFA in the anaerobic phase. Pre-denitrification was required to provide PAOs access to the limited VFA source.
- Biomass fermentation was found to be an effective method of generating VFA. At an SRT = 5 d, some 157 mg VFA-COD was produced by fermenting 1 g of biomass. Acetate and propionic acid were the primary products, and accounted for 75 and 10% of the VFA composition, respectively.
- The VFA produced by in-line biomass fermentation was not sufficient to remove the extra load of phosphorus released during the fermentation. In addition, the returned fermented biomass led to deterioration of nitrification.

- Fermentation of WAS followed by precipitation of re-released nitrogen and phosphorus with magnesium led to achieving lower concentration of effluent phosphorus and complete nitrification.
- Phosphorus could be recovered through struvite precipitation by addition of MgO to the WAS fermentate supernatant.

Chapter 6 **VOLATILE FATTY ACID AND NUTRIENT RECOVERY FROM BIOMASS FERMENTATION**[*]

6.1 INTRODUCTION

Since phosphorus, rather than nitrogen, is considered the leading factor contributing to the freshwater eutrophication (Schindler, et al., 2008) its removal from wastewater has become an essential part of wastewater treatment. Enhanced biological phosphorus removal (EBPR) is one of the most cost-effective ways to remove phosphorus from the liquid stream. The key to this process is the presence of adequate volatile fatty acids (Chu et. al., 1994; Oleszkiewicz and Barnard, 2006). For low organic content (weak) wastewater, external volatile fatty acids (VFA) addition is becoming necessary to sufficiently stimulate phosphorous removal to meet the increasingly stringent effluent phosphorus limits. The purchase of VFA would increase the cost and carbon footprint removal to the point where it would become unsustainable. The designers are turning to the practice of on-site VFA production fermentation of primary sludge. In some cases, beside the VFA production variability problems, the total mass of VFA produced from primary sludge may be lower than required for both phosphorus and nitrate removal - e.g. the Noosa plant in Australia (Tomas et al., 2003). To offset these problems research is needed on generating VFA from fermentation of in-process biomass, such as return activated sludge (RAS) or waste activated sludge (WAS).

[*] published in ***International Conference on Nutrient Recovery from Wastewater Streams,IWA, ISBN: 1843392321***, pp. 633-642.

Biomass fermentation benefits the plant by generating VFA and by reducing the overall production of WAS, which lowers sludge handling costs and plant's overall carbon footprint. The drawback is that the VFA are generated in conjunction with re-release of ammonia and particularly phosphates. Nutrients contained in such a fermented biomass supernatant may offset the benefits of VFA produced, as was shown by McIntosh & Oleszkiewicz (1997) during micro-aerophilic fermentation of co-thickened primary sludge and WAS.

The ubiquitous presence of nuisance struvite (magnesium-amono-phosphate or MAP) deposits in various areas of biological phosphorus removal plants (Stratful, et al., 2004) points requires phosphorus removal before separate treatment of sludge. Removing phosphorus through precipitation with lime has been practiced as PhoStrip before (Digger and Polson, 1991); however the value of the precipitate was too low for beneficial recovery. Controlled precipitation of struvite within the liquid wastewater treatment process would prevent clogging and scaling problems in downstream operations; would allow the return of biomass fermentation supernatant to enhance the EBPR process and could generate a useful phosphorus fertilizer MAP.

The objective of this study was to investigate VFA generation in acid-phase fermentation as an internal carbon source for biological nutrient removal. Additionally, struvite formation was investigated as an alternative to recovering nutrients from wastewater treatment to achieve sustainable sludge management.

6.2 MATERIAL AND METHODS

6.2.1 Experiment approach

The biomass fermentation set up consisted of a bench scale mother reactor and fermenter combination. The mother reactor was seeded with the activated sludge from a lab sequencing batch rector (SBR) with stable phosphorus removal and was operated in an anaerobic\aerobic configuration. The mother reactor is a SBR which goes through three, eight hour cycles per day. In each cycle the reactor is fed with synthetic wastewater (COD of 300 mg/L, acetic acid of 150 mg/L) after which an anaerobic environment is created by bubbling nitrogen gas through the reactor. It is at the end of the anaerobic cycle that a portion of the biomass is pumped into the fermenter. Following the anaerobic cycle which lasts for 90 minutes, air is bubbled through the reactor to create aerobic conditions for 4.5 hours. Finally, the reactor is allowed to settle and the supernatant is decanted. Overall, an SRT of 10 days is achieved in the mother reactor with an HRT of 12 hours.

The fermenter receives biomass from the mother three times each day and is manually wasted once per day. The wasted liquor is then analysed, filtered, and the supernatant is used for the struvite precipitation tests. The fermenter is anaerobic, well mixed, and kept at a temperature of 35℃. Overall, the fermenter has an SRT and an HRT of 7 days. Although the fermenter is designed to model an in-line fermenter, the wasted liquor is not fed back into the mother reactor as the goal of this experiment is to determine the characteristics of the liquor (i.e. VFA, P, N concentrations) and its suitability for the precipitation of struvite.

6.2.2 Phosphorus recovery via struvite formation

The struvite precipitation tests are performed on the supernatant from the fermenter. Biomass is filtered from the liquor and the resulting supernatant is refrigerated until sufficient quantities are available for testing. Five tests were performed at pH of 7.5, 8.0, 8.5, 9.0, and 9.5 respectively. For each test, approximately 2 litres of supernatant is gently mixed together. The pH is raised to the desired level with a 40% w/w sodium hydroxide solution and the supernatant is divided into four beakers. Varying doses of magnesium (0.6M magnesium chloride hydrate [$MgCl_2$ 6(H_2O)]) solution are added to each of the four beakers and are thoroughly mixed. Following a 24 hour period for struvite precipitation, the crystals are removed by filtration. The resulting supernatant is reserved for analysis and the crystals are dried and analysed.

6.2.3 Analytical procedure

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) measurements were performed according to Standard Methods (APHA, 1998). Dissolved phosphate and ammonium was measured by Lachat Instrument Quik Chem 8500, followed Quik Chem Method orthophosphate 10-115-01-1-O and Quik Chem Method ammonia 10-107-06-1-I. Varian CP-3800 gas chromatography was used for VFA measurement. Magnesium and calcium were analyzed by a Varian VISTA-MPX. Struvite crystals were examined under a phase contrast microscope (Nikon). Powder X-ray diffraction analysis was carried out on a PANalytical X'Pert Pro diffractometer equipped with an X'Celerator detector using a CuKα1,2 (λ=1.540598, 1.544426 Å) radiation source.

6.3 RESULTS AND DISCUSSION

6.3.1 VFA Production and Solids Reduction

Figure 6-1 illustrates concentrations of VFA as well as phosphate and ammonium in the fermenter over time. Although the fermenter was operated using a low solids concentration (MLVSS of 0.9-1.3 g/L), an increase in VFA concentration is observed with an average concentration of 354 mg/L. VFA as high as 658 mg/L was measured, and the average specific VFA production rate was measured as 0.12 g VFA/g VSS per day. Acetic acid was the dominant VFA produced with an average range of 66.5%, followed by propionic acid (14.6%). The ratio of VFA to soluble COD was observed as 58%. Since approximately 8mg of VFA is required to remove 1 mg P (Abu-ghararah and Randal, 1991), the fermentation of 1g wasted biomass will yield sufficient VFA for the removal of 15 mg P. This correlation effectively translates to a significant reduction in the demand for an external carbon source.

The large fluctuations of VFA concentrations were observed at approximately day 10, whereas phosphate and ammonium were fairly stable in this time frame (Figure 6-1). These fluctuations of VFA were most likely due to instrument malfunction. It was found at day 12 that the gas chromatograph flow rate regulator had malfunctioned, consequently the data recorded slightly before and after that day did not fit well with the rest of the recordings. The drop in VFA production around day 18 was probably a result of an accidental temperature increase of the fermenter to nearly 100℃ (day 15). This increase of temperature definitely disrupted the fermentation process and caused a decrease in the VFA production. Nevertheless, it is apparent that the general trend of the graph describes

an increase of VFA with time until a concentration of about 425 mg/L of VFA was reached, at which point the VFA production stabilized.

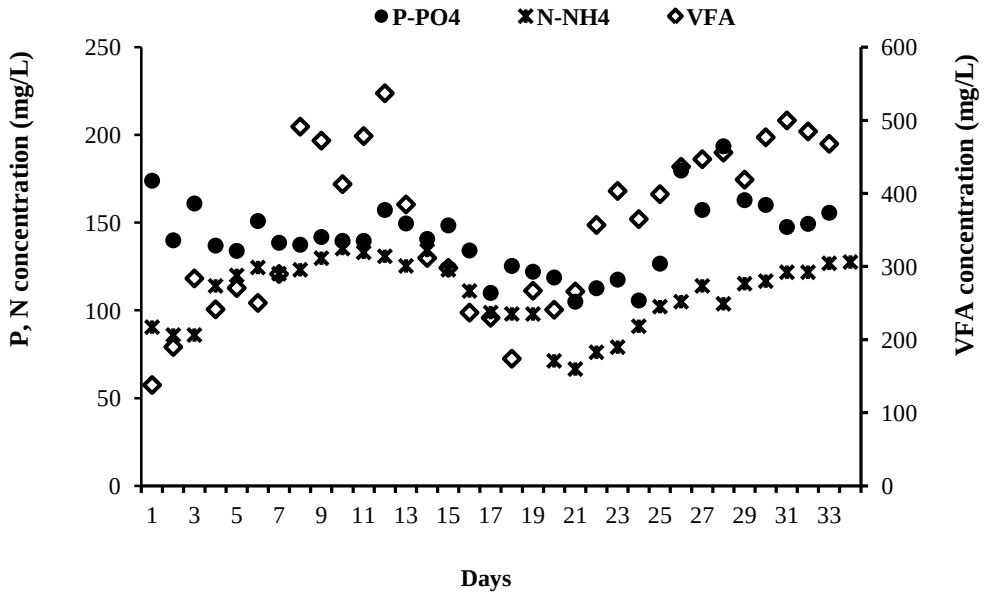


Figure 6-1 VFA, phosphate and ammonia concentration in the fermenter

Another goal of acid fermentation is solids reduction. Figure 6-2 exemplifies that 40% solids destruction was achieved via biomass fermentation. This solids destruction will provide significant reduction for the cost of sludge handling, as the entire sludge treatment process accounts for nearly 60% of the total operating cost for the wastewater treatment plants (Horan, 1990).

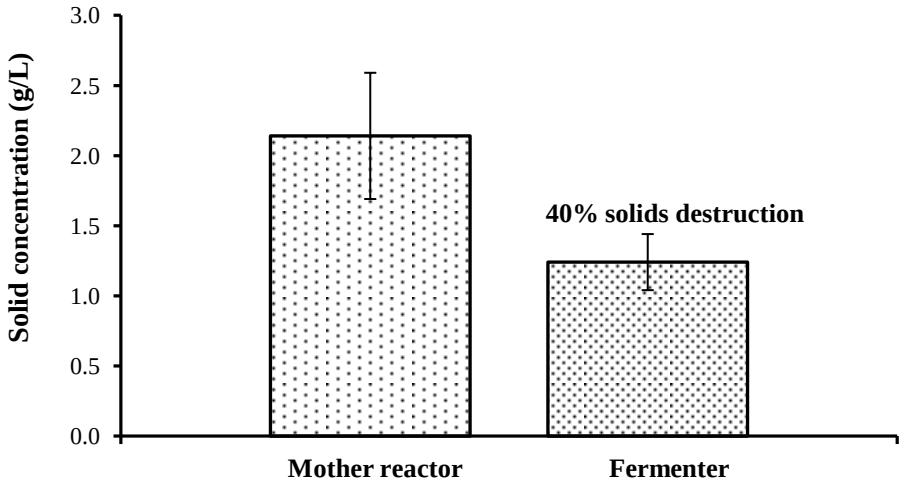


Figure 6-2 Solids concentration of mother reactor and fermenter

6.3.2 Nutrient recovery

6.3.2.1 Solubilisation of P and N

The concentrations of phosphorus as phosphate and nitrogen (N) as ammonium were measured in the fermenter. Figure 6-1 illustrates the high concentrations of both elements in the fermented liquor with the average concentration of nitrogen measured to be 110mg/L and the average concentration of phosphorus to be 142mg/L.

The elevated concentrations of phosphate and ammonium in the fermenter, due to cell lysis, reinforce the suitability of a struvite precipitation process at this stage. If a fermented liquor rich in VFA, P and N would be returned to the mother reactor, the newly produced VFA would be completely depleted in the process of taking up the P that has been added by the liquor. If on average it takes at least 8mg VFA for the removal of 1mg P (Abu-ghararah and Randal, 1991), the observed 120 mg/L of P in the fermented

liquor would require 960 mg/L of VFA, exceeding the total quality produced in the fermenter altogether. By combining the phosphorus and nitrogen together into struvite and precipitating it prior to adding the liquor back into the BNR system, establishes the benefit of added VFA for biological phosphorus removal, aiding the necessary reduction of nitrogen required further downstream.

The average released and solubilised ammonium and phosphate from the biomass fermentation was found to be 0.06 g NH_4-N/g VSS and 0.07 g PO_4-P/g VSS. One may argue that the amount of phosphorus released cannot be higher than that of ammonium. However, it should be noted that the biomass in the mother reactor was an enhanced culture containing significantly high population of PAOs in the MLSS instead of the conventional activated sludge. This did not have an effect on the amount of VFA produced since the fermentation of other bacteria will produce the same results. However, lower P concentration does have an effect on the potential for struvite formation since high concentrations of the constituent components are required for precipitation. It is important to determine if there are sufficient amounts of P-PO_4 and N-NH_4 available in the fermenter prior to an attempt to precipitate struvite. It is hypothesized that a reason for this heightened release of phosphorus rather than that of ammonium can be that the biomass was withdrawn from the mother reactor at the end of its anaerobic cycle, at which time the phosphorus release has already happened within the mother reactor.

Figure 6-1 further indicates that during the fermentation stage, the ammonium and phosphorus production followed the same trend as VFA generation. It can be concluded that high ammonium and phosphorus levels may be associated with high VFA production;

therefore, ammonia and phosphorus levels in the fermenter may be potentially used as an indicator in terms of the degree of biomass destruction.

6.3.2.2 Struvite formation test

Results suggest that the pH exhibits a stronger influence with phosphorus precipitation, rather than magnesium precipitation; this is illustrated in Figure 6-3. The rate of phosphorus removal increased while an increase in pH was experienced, as did the removal of ammonium and magnesium (Figure 6-4). It should be noted that the magnesium doses for a pH of 9 were different than those for the remaining trials. It was further hypothesized that the effects of pH could further influence the magnesium dosages for phosphorus removal. Initial studies began at a pH of 9 and appeared promising, further investigations were conducted to study if a wider pH range and magnesium dose still were able to provide beneficial results. However, results from the rest of the trials with different pH levels were in the agreement with the result from a pH of 9.0 using the magnesium dosage. This may be due to the over dosing of magnesium. It is suggested that lower magnesium dosages should be the focus of testing in future experimentation.

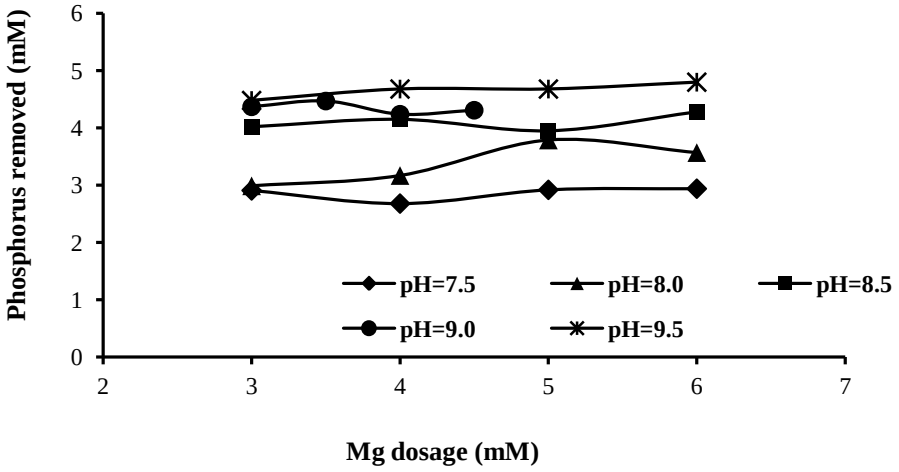


Figure 6-3 pH and Mg dosage on phosphorus removal

At a pH of 7.5, a small amount of crystallization was observed. It was found that much less magnesium was removed compared to ammonium or phosphate (data not show), and therefore not all the phosphate or ammonium was removed as struvite. Instead, it was thought that other reactions, such as calcium phosphate precipitation, were simultaneously competing for struvite formation, consequently forming other crystals containing phosphate and ammonia.

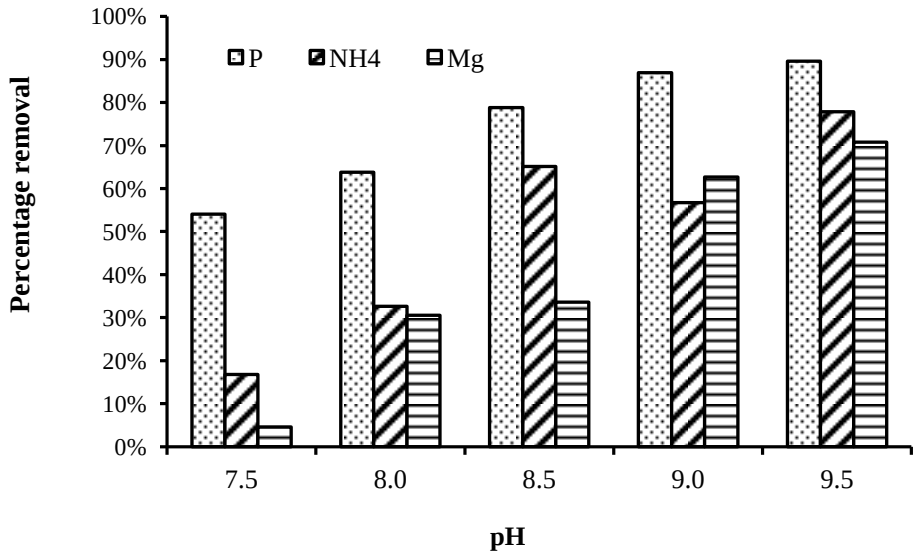


Figure 6-4 Phosphate, ammonia and magnesium removal with pH

With an increase in pH levels of 8.0 to 8.5, both ammonia and magnesium removal were greatly improved compared to pH 7.5 (Figure 6-4); however, it still does not exhibit the 1:1:1 ratio characteristic of struvite precipitation. When the pH is increased to 9.0, a nearly perfect 1:1:1 ratio of removed ions was exhibited indicating struvite formation. The obtained precipitated crystals were examined via microscope; an identified orthorhombic shape was observed (Figure 6-5). Further analysis via powder x-ray de-fraction (XRD) techniques verified that the obtained de-fraction pattern was consistent with struvite, suggesting a minimum purity of 99%. This pH condition of 9.0 is considered the optimum condition for phosphorus removal through struvite precipitation. The results from this test suggest that the optimal dose for magnesium salt at pH 9 was 0.38g/L, resulting in 84% of P-PO_4 and 60% N-NH_4 removal. At pH 9.5 significant ammonium removal was observed in the form of ammonia gas due to the high pH condition. The ammonium-ammonia equilibrium point occurs at pH 9.2 and so at pH

levels higher than 9.2, ammonium converts to ammonia gas. Compared to the pH 9.0 trial, there were very similar quantities of struvite formed; however, the added production of ammonia gas at this higher pH leads to the conclusion that a pH 9.5 is less favorable.

VFA concentration of the supernatant post struvite precipitation was analyzed. It was observed that only 5% VFA was lost in the bulk solution during the precipitation process. This suggests that the recovery of VFA and removal of phosphorus and ammonia can occur simultaneously without a negative synergistic impact.

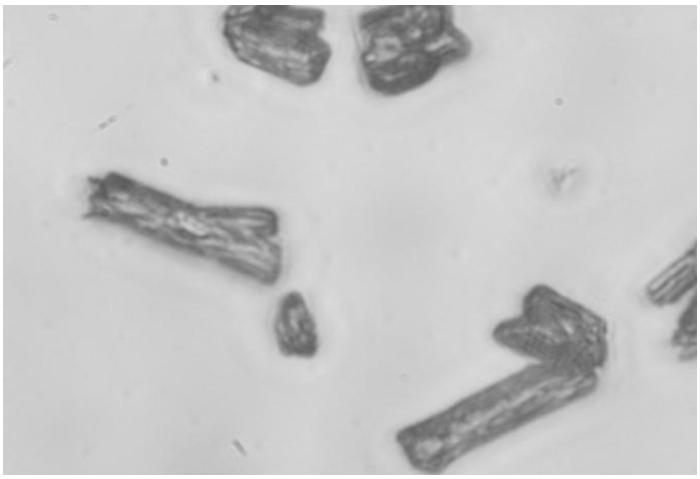


Figure 6-5 Picture of crystals from the fermentation liquor with $MgCl_2$ addition

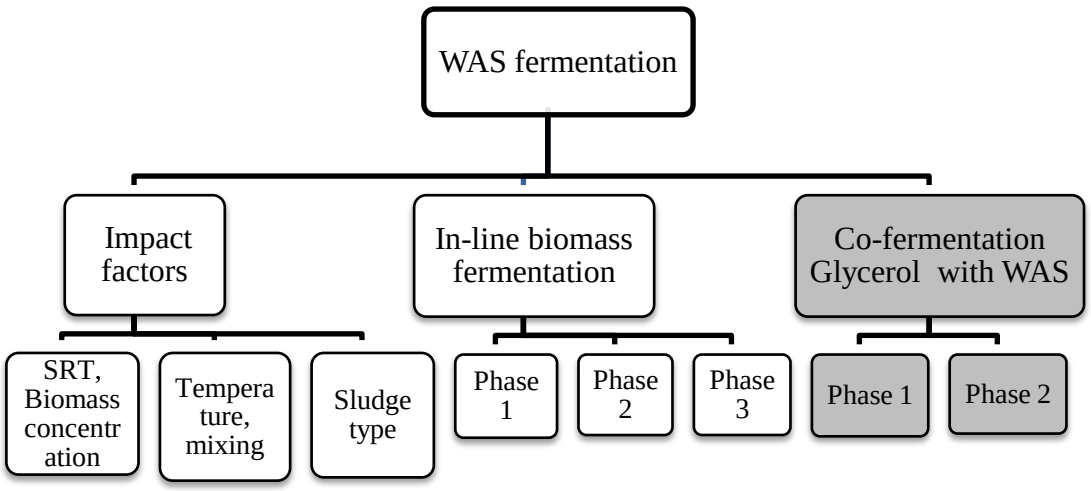
6.4 CONCLUSION

In light of this preliminary study, the following conclusions can be made:

- Fermentation of waste activated sludge is an effective approach for VFA production, while reducing the sludge generation.

- Significant release of phosphate and ammonia occurs in the fermentation process.
- Struvite formation is an effective method by which phosphorus can be recovered from wastewater. Struvite precipitation can remove high concentrations of phosphate and ammonia from wastewater through increasing the pH and magnesium dosing. The best pH for struvite formation from this study is 9, with the optimal dose of magnesium chloride being 0.38g/L.
- Nutrient recovery through struvite formation may be achieved without negative impacts on VFA generation.

Chapter 7 **ENHANCING BIOLOGICAL PHOSPHORUS REMOVAL WITH GLYCEROL**[*]



[*] published in ***Water Science & Technology***, (2010), 61(7), 1837-1843.

7.1 INTRODUCTION

Glycerol is a significant waste from the production of bio-diesel (Wandijken and Scheffers, 1986). About one kilogram of glycerol is created for every 10 kilograms of biodiesel produced. Due to its relative impurity, glycerol from bio-diesel production is considered a waste stream possessing low commercial value. According to the National Biodiesel Board, U.S. companies produced about 1.7 million m^3 of biodiesel in 2007, and about 60 new plants with a production capacity of 4.5M m^3 are slated to open by 2010. This generates an urgent need to develop suitable application and markets for waste glycerol these plants will produce. In the wastewater treatment field, it has been shown that glycerol can be an excellent carbon source for denitrification (Grabinska-Loniewska, 1985, Akunna, 1993, and Chen, 2008); as well as a co-substrate in boosting methane production in anaerobic digestion (Amon et al., 2006, Holm-Nielsen et al., 2007, Wohlgemut, 2008). At this moment there were no studies on the use of glycerol as a potential external carbon source for biological phosphorus removal.

The purpose of this study is to investigate the effect of glycerol on enhanced biological phosphorus removal in two configurations 1) direct application of glycerol as an additional carbon source in the anaerobic zone of the EBPR process; 2) supplementing the influent wastewater stream with VFA-enriched supernatant from the co-fermentation of glycerol and waste activated sludge (WAS).

7.2 METHODOLOGY

7.2.1 Experiment setup

7.2.1.1 Phase 1 - Glycerol as direct external carbon

A lab scale sequencing batch reactor (SBR) was set up as a glycerol testing reactor. It was seeded with activated sludge from a parent lab scale SBR with stable biological phosphorus removal activity. This parent SBR was also used as a control reactor. The control reactor was fed with synthetic wastewater (composition shown in Table 7-1) with a COD of 350 mg/L (50% of COD from acetate). The glycerol testing reactor was fed with the same synthetic wastewater for the first 10 days to ensure continued stable phosphorus removal. After 10 days acetate was replaced with glycerol which provided the same amount of COD. Both reactors had a working volume of 3 litres and were operated with a cyclical anaerobic/aerobic configuration. The reactors were operated with 3 cycles per day to achieve a hydraulic retention time (HRT) of 12 h and the solid retention time (SRT) was controlled at 10 d. Each operational cycle consisted of a 15 min. filling period, a 90 min. anaerobic period, a 300 min. aerobic period followed by 60 min. settling and 15 min. decanting. Throughout the two month testing period, the temperature was maintained between 20-22 ℃ and the pH was controlled between 7.0-7.5.

Table 7-1 Synthetic wastewater composition

Synthetic wastewater		Mineral solution	
Ingredients	Concentration (mg/L)	Ingredients	Concentration (g/L)

NaAc	215	$FeCl_3 \cdot 6H_2O$	1.5
Beef extract	80	H_3BO_3	0.15
Yeast extract	80	$CuSO_4 \cdot 5H_2O$	0.03
$MgSO_4 \cdot 7H_2O$	170	KI	0.03
$CaCl_2 \cdot 2H_2O$	14	$MnCl_2 \cdot 4H_2O$	0.12
P (K_2HPO_4)	9	$Na_2MoO_4 \cdot 2H_2O$	0.06
N (NH_4Cl)	15	$ZnSO_4 \cdot 7H_2O$	0.12
		$CoCl_2 \cdot 2H_2O$	0.15
Mineral solution	0.3 ml	EDTA	10

7.2.1.2 Phase 2 - Co-fermentation of glycerol with waste activated sludge (WAS)

A pair of lab scale SBRs was set up, consisting of a mother reactor and a fermenter (Figure 7-1). The mother reactor was seeded with the activated sludge from the control reactor, from Phase 1, and was operated as a phosphorous removal reactor under the same operational conditions as in Phase 1, with the exception of feed composition (COD of 270 mg/L from equal amounts of yeast and beef extract, COD of 30 mg/L from acetate). Less COD in the form of acetate was provided in the feed in order to test the effect of VFA on the EBPR. Excess biomass from this mother reactor was withdrawn at the end of the 90 minutes anaerobic period to a fermenter, where 140 mg glycerol (as COD) was added. To start the fermentation, this fermenter was seeded with active biomass from a lab scale anaerobic digester maintained on hog manure and glycerol. The fermenter was operated with 3 cycles per day, and with SRT and HRT of 3 d. The effluent from the fermenter was settled and added to the mother reactor at the beginning of each cycle as supplemental carbon source.

A control reactor, seeded with the activated sludge from the control reactor from Phase 1, was operated under the same condition as the mother reactor without connection to the fermenter. Phase 2 lasted for over two months.

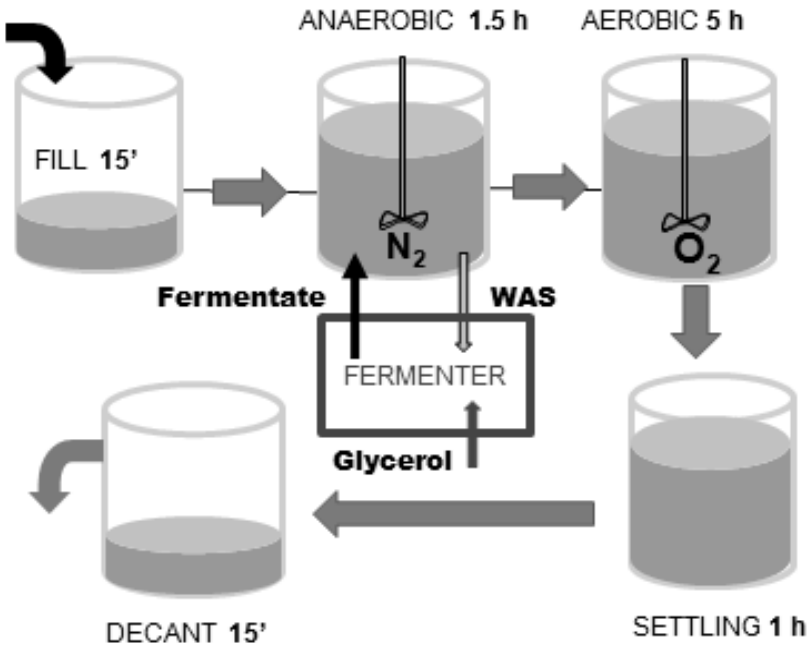


Figure 7-1 Experimental set-up in Phase 2

7.2.2 Analytical methods

Mixed liquor suspended and volatile solids (MLSS and MLVSS) measurements were performed according to Standard Methods (APHA, 1998). Hach COD digestion vials were used to measure COD. Dissolved phosphate and ammonium were measured using a Lachat Instrument Quik Chem 8500, following the Quik Chem orthophosphate method 10-115-01-1-O, Quik Chem ammonia method 10-107-06-1-I. The analysis of volatile

fatty acid (VFA) composition was conducted in a Varian CP-3800 Gas Chromatograph using flame ionization detector (FID) and HP-FFAP capillary column (inner diameter of 0.25mm and length of 25m). VFA concentration was converted to COD concentration by using the following conversion factors: 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric and isobutyric acid, 2.04 for valeric and isovaleric acid, 2.21 for caproic and iso-caproic acid and 2.34 for heptanoic acid. Glycerol COD concentration was calculated as 1.54 g/L.

7.3 RESULT AND DISCUSSION

7.3.1 Phase 1: Glycerol as direct external carbon source

Excellent phosphorus removal (95-99% removal) was achieved from the control reactor (shown in Fig. 7-2) where acetate was used as 50% of the carbon source. In contrast, poor phosphorus removal was observed from the glycerol testing reactor, the average phosphorus removal was only about 30%. For the first 8 days, the effluent of the glycerol testing reactor contained even higher phosphate (as high as 27 mg/L) than that in the influent. This indicated that a significant amount of phosphorus was released from the biomass into the effluent. Although phosphorus concentration in the effluent started to decrease from day 9, phosphorus removal was unstable and very low. This observation suggested that the PAOs, enriched when acetate was the primary carbon source, were not able to adapt to growth on glycerol when it was introduced to the system. It was hypothesized that the acetate-utilising PAOs washed out with time when acetate was replaced with glycerol, and were not replaced by glycerol-utilising PAOs but rather, by non-PAO glycerol utilising bacteria.

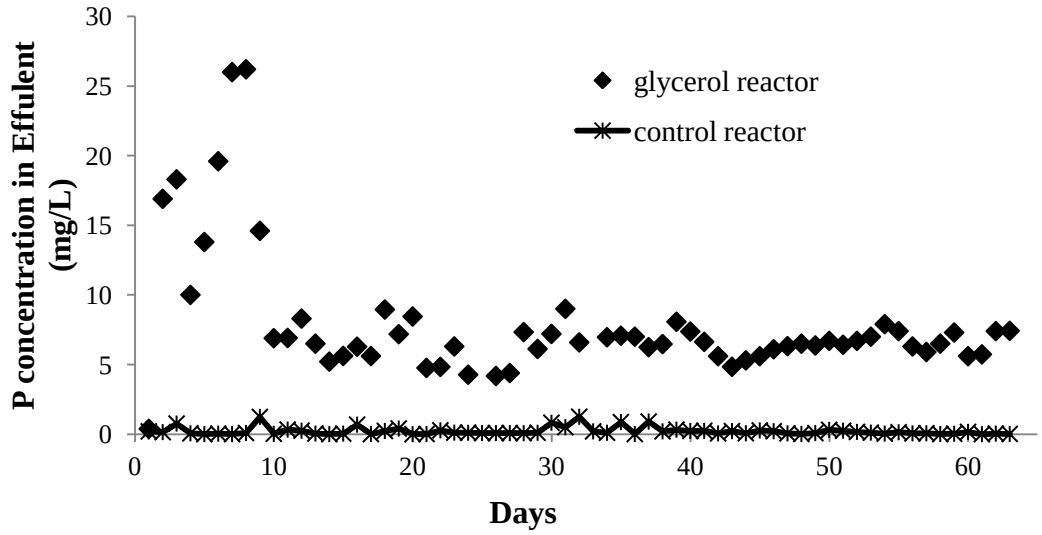


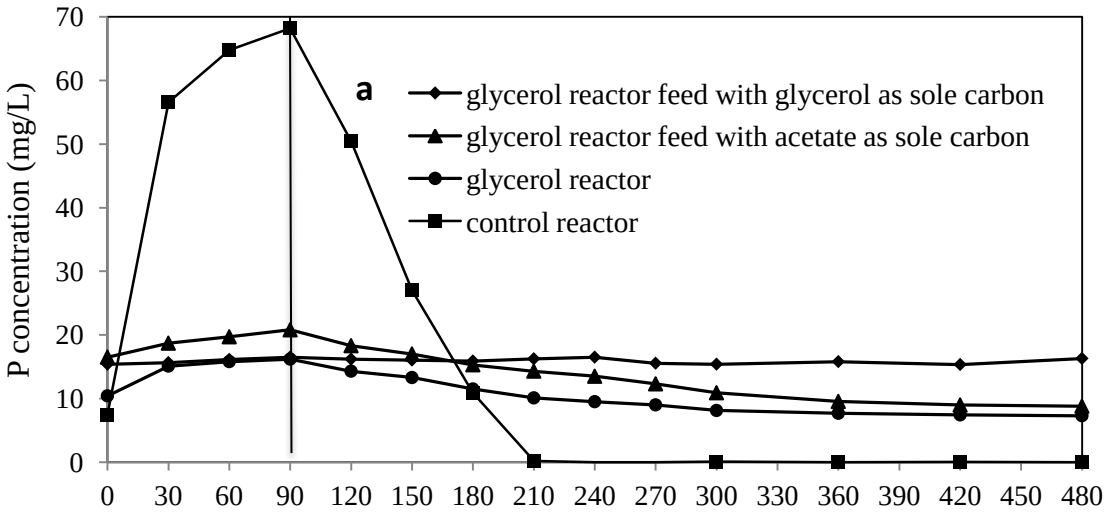
Figure 7-2 Effluent phosphate concentration in the reactors with and without glycerol as external carbon

To verify this hypothesis, a kinetic study of a regular reaction cycle was conducted for both control and glycerol testing reactors. In addition, a kinetic study monitoring the performance of the glycerol testing reactor using glycerol as well as acetate as the sole carbon source was also carried out (Fig. 7-3). As expected, the control reactor showed a typical EBPR process with A/O configuration. It was observed that in the control reactor a sharp anaerobic phosphate release occurred coupled with rapid COD uptake followed by a greater aerobic phosphate uptake (Fig. 7-3 a and 7-3 b).

For the glycerol testing reactor after glycerol feeding for 45 days, regardless of the carbon source, no significant change in the phosphorus profile was observed (Fig. 7-3 a and 7-3 b). When acetate was used as the sole carbon source, fairly small amounts of phosphate were released during the anaerobic phase and there was also little phosphate uptake during aerobic phase. This observation implies that most of the PAOs in the reactor were washed out from the system. Furthermore, when glycerol was used as sole

carbon source, the results showed that there was almost no phosphorus release and uptake which occurred. This confirmed that the microbes in this system could utilize glycerol as a carbon source (Fig 7-3 b) but not for phosphorus removal (Fig 7-3a).

Although the glycerol testing reactor had little phosphorus removal, it had a similar COD removal curve as the control reactor where EBPR occurred (Fig. 7-3 b). The majority of COD in the glycerol testing reactor was rapidly removed during the anaerobic phase regardless of the amount of phosphorus release. This phenomenon suggested that there was a population of facultative microorganisms in the system. These microorganisms were able to utilize COD under anaerobic conditions without releasing phosphorus, which led to negligible aerobic phosphate uptake. This experiment demonstrated that supplying an improper carbon source directly may cause a microbial population shift away from PAOs to other facultative microbes.



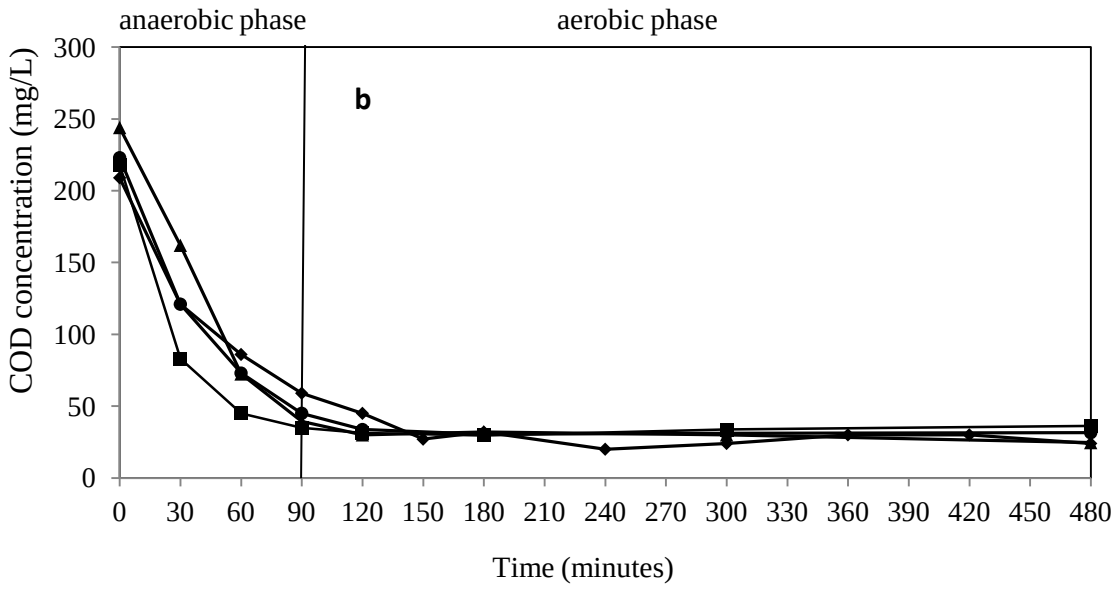


Figure 7-3 Phosphate (a) and COD (b) profiles with different carbon sources (Phase 1)

7.3.2 Phase 2 - Addition of glycerol co-fermented with WAS

7.3.2.1 VFA generation from co-fermentation of glycerol with WAS

It took about 12 days for the fermenter to reach stable operation at which time waste activated sludge from the mother reactor was effectively co-fermented with glycerol. The pH of the fermenter was between 6.4 - 6.7. Significant VFA production from the fermenter was observed with an average VFA concentration of 795 mg COD/L (shown in Fig. 7-4). An average VFA-to-alkalinity ratio of 2.3 was observed. This high ratio pointed to an effective generation of VFA in the fermenter.

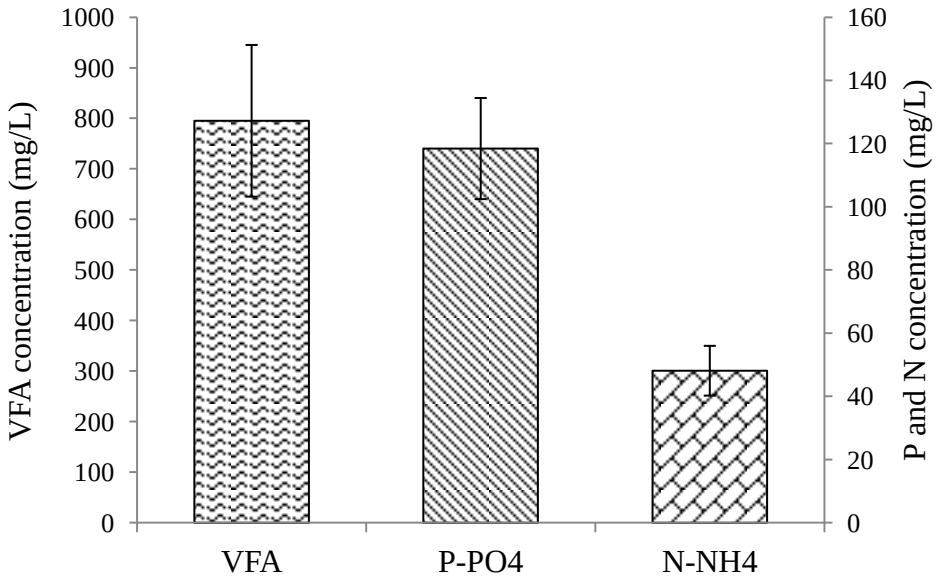


Figure 7-4 Nutrient concentration in the fermenter (average of 56 samples)

It was noticed that in terms of VFA composition, VFA generated from the co-fermentation of WAS with glycerol was quite different from the fermentation of WAS alone. Previous studies showed that acetate was the major VFA component from WAS fermentation, and it accounted for 66% of VFA generated (Figure 7-5) (Yuan et al, 2009). Whereas in this study, when WAS was co-fermented with glycerol, the primary product was propionic acid, which accounted for 67% of the VFA component, while acetic acid accounted for only 29%. The high propionic acid production was the result of a different fermentation pathway consistent with glycerol fermentation. Protein and polysaccharides are the primary compounds of WAS, which require a wide variety of enzymatic pathways to break them down to acetic acid and other VFAs, whereas glycerol is a simple sugar alcohol and is a more easily biodegradable substrate. VFA production from fermentation of 1.11 g VSS/L of WAS alone, in the previous study, yielded approximately 0.12g VFA/g VSS $\cdot d^{-1}$ –Fig. 7-5 (Yuan *et al*, 2009). In this study, when 0.3 g/d of glycerol was

fermented with 2.02 g VSS/L of WAS, VFA production of 0.36g VFA/ g VSS d^{-1} was achieved. This result illustrated that microorganisms can utilize glycerol more easily and more effectively than WAS. The fermentation of glycerol resulted in primarily propionic acid production (Fig. 7-5).

VFA were the major products of the fermentation, indicated by the high COD from VFA, to the soluble COD ratio. Theoretical conversion of VFA to COD showed that approximately 75% of soluble COD came from VFA. The substances which accounted for the remaining 25% of soluble COD, possibly other fermentation by-products, were not analysed further.

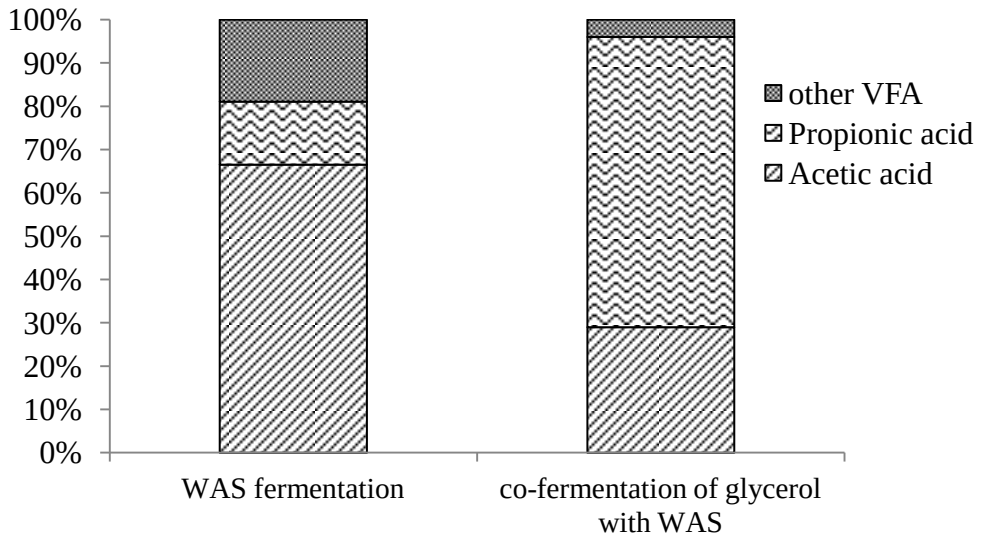


Figure 7-5 VFA composition during WAS fermentation alone and with glycerol. WAS fermentation data from Yuan et al. (2009)

The supernatant of the effluent from the fermenter contained higher soluble N, P and COD than the influent, as shown in Fig. 6, suggesting that the biomass from the mother reactor was partially solubilised during the fermentation. That the solubilisation led to degradation was also indicated by the 47% solids reduction achieved in the fermenter. As a result of solubilisation, nutrients were released into the fermenter and they became the nutrient source for the growth of glycerol utilizing microorganisms.

7.3.2.2 EBPR with external carbon addition from the fermenter

The solubilisation of the biomass increased the nutrient load, especially phosphorus, to the mother reactor, when the supernatant was used as the substrate for the external carbon supplement. Excellent phosphorus removal in the mother reactor (shown in Fig. 7-7 a) indicated that the VFA generated from the fermenter were sufficient to achieve EBPR despite the extra phosphorus loading. As shown above, propionic acid was the primary component of the VFA, and it was the major source of the external carbon. The results show that both propionic acid and acetic acid were good carbon sources for biological phosphorus removal which was consistent with other studies (Pijuan, et. al., 2003, Oehmen, et. al., 2005). It should be mentioned that nitrification was not negatively affected by the addition of the supernatant; both reactors had good nitrification, although the mother reactor exhibited a slower nitrification rate than the control reactor.

In contrast, the control reactor, which did not receive any VFA supplement, provided little phosphorus removal (Fig. 7-7 a). Nevertheless, this control reactor had similar COD consumption pattern (Fig. 7-7 b) as the glycerol testing reactor in Phase 1. Both reactors

had a sharp anaerobic COD uptake, with little phosphorus release and subsequent aerobic phosphorus uptake. This experiment reaffirms the importance of VFA for biological phosphorus removal.

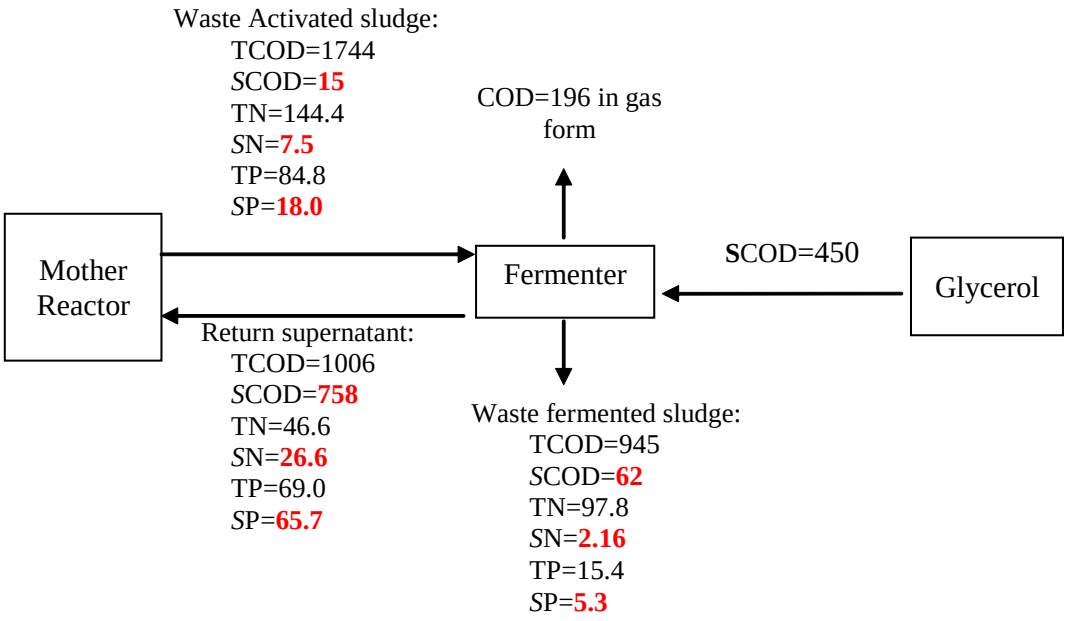
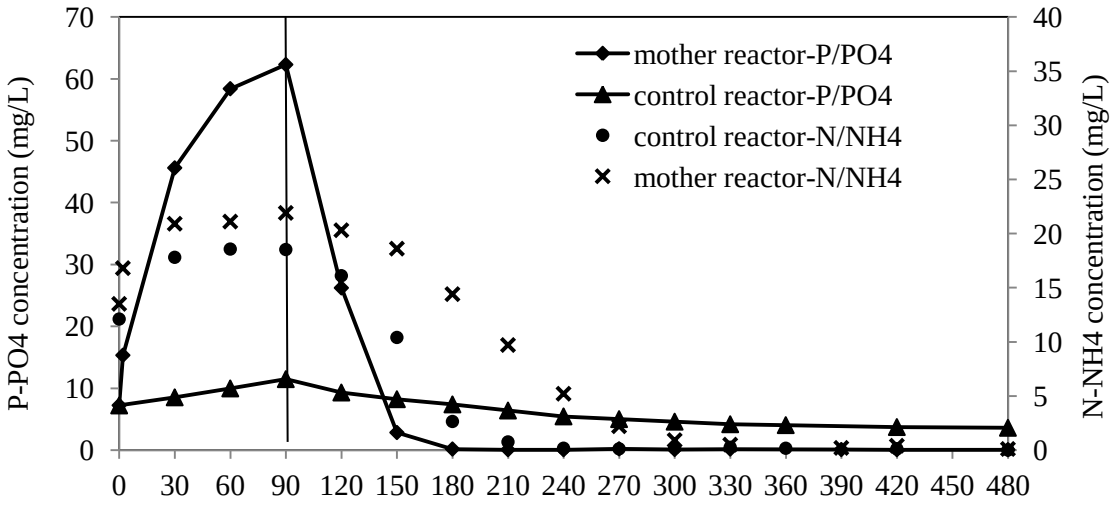


Figure 7-6 COD, N, P flows in the system. All numbers in milligrams per day (mg/d). SN, SP, SCOD– Soluble nitrogen, phosphorus, or COD. TN, TP, TCOD – Total nitrogen, phosphorus or COD

7.3.2.3 Sludge generation comparison

The VFA-rich supernatant addition resulted in a higher solids concentration in the mother reactor than that of the control reactor. The average total suspended solids concentration in the mother reactor and control reactor were 4.12 g/L and 2.39 g/L respectively. However, the co-fermentation of waste activated sludge from the mother reactor with glycerol effectively reduced the sludge volume wasted from the complete system; the average sludge generated from the system was about 10% less than that of the control reactor (Fig. 7-8) (two–tailed t-test, α=0.15). These results suggested that using glycerol as the external carbon source to enhance VFA production, did not increase the sludge handling and disposal needs.



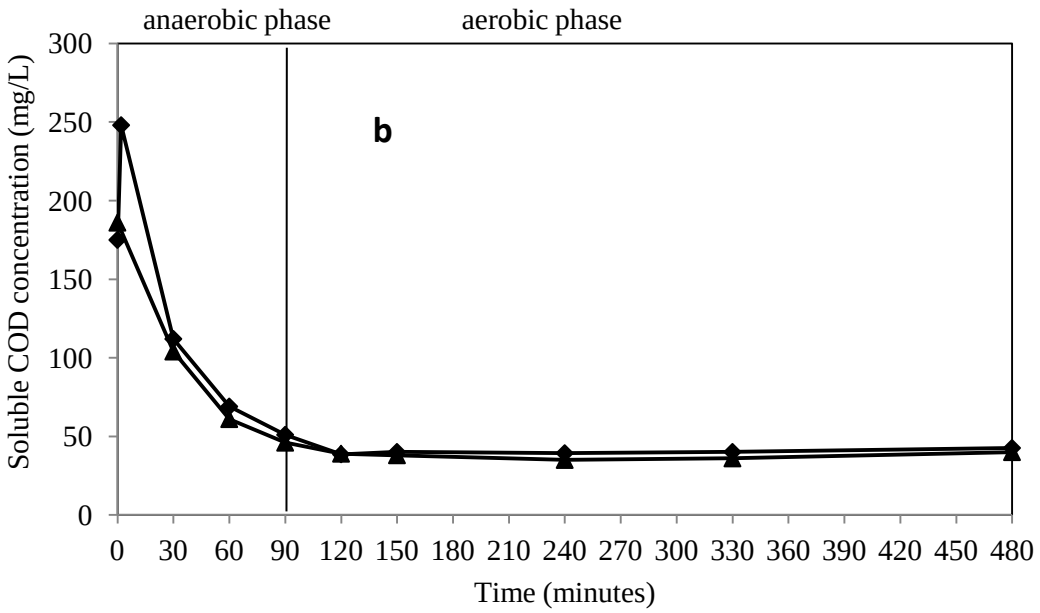


Figure 7-7 A typical phosphate, ammonium and COD profile in the control and mother reactors

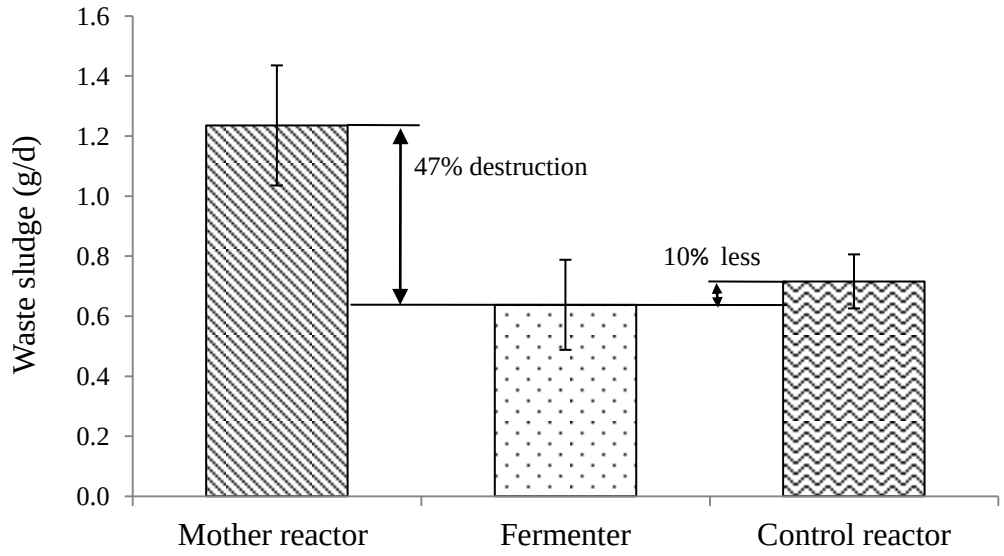


Figure 7-8 Average daily sludge generation from reactors and fermenter (56 samples)

7.4 CONCLUSIONS:

In the light of the results from this experiment, the following conclusions can be made:

i. When acetate was replaced by glycerol in an existing EBPR system where acetate was used as the primary carbon source, the system failed to maintain EBPR. This suggested that glycerol may not be a suitable direct external carbon source for these PAOs to perform biological phosphorus removal. The majority of COD was rapidly removed during the anaerobic phase, but it was coupled with little phosphorus release. This observation implied that an improper carbon source may cause a shift of the microbial community population away from PAOs.

ii. Significant amounts of VFA were produced when glycerol was co-fermented with WAS, indicating that glycerol is an excellent co-substrate for WAS fermentation. Propionic acid was the major product of this fermentation, followed by acetic acid.

iii. The addition of external VFA from the supernatant of the fermenter to the mother reactor, increased the mother reactor's sludge generation. However, the co-fermentation of glycerol with waste activated sludge resulted in a considerable solids reduction. This contributed to an average of 10% solids reduction overall in the system.

iv. The addition of the external VFA, primarily enriched with propionic acid from the fermentation of glycerol, assisted the system with EBPR and had no negative effects on nitrification.

v. The system failed to maintain EBPR when insufficient VFA was supplied, demonstrating that VFA was an important substrate for the system to achieve biological phosphorus removal.

Chapter 8 **Engineering Significance**

The research was aimed at resolving carbon deficiency issues in phosphorus removal processes as used in full scale biological nutrient removal plants. These included: the use of WAS fermentation and the co-fermentation of WAS and glycerol to generate VFA to augment phosphorus removal, the use of the WAS fermentation to reduce the sludge volume; the impact of temperature, mixing, SRT and solids concentration on WAS fermentation, as well as the recovery of P from the WAS fermentation supernatant. This chapter contains the engineering and economic significance of the key findings.

8.1 COST SAVINGS IN CARBON ADDITION

The presence of VFA is an important factor for both bio-P process and denitrification processes. Full scale plants that treat carbon-deficient wastewater usually purchase acetate and methanol as the source of soluble COD (sCOD). WWTP across America, such as Washington DC, Tampa FL, Arlington VA and Winnipeg MB, spend millions of dollars on methanol for denitrification. Primary sludge fermentation for on-site production of VFA is well established worldwide, and has been practiced for over 20 years. However, the reliability of VFA generation is often not adequate, particularly in flat terrain large sewer systems such as in St Paul-Minneapolis USA, Winnipeg South CDN or Gdansk PL. In a number of treatment plants the mass of VFA produced from primary sludge fermentation is frequently below that required to ensure efficient removal of both phosphorus and nitrate, for example in the Noosa AUS biological nutrient removal (BNR) WWTP (Thomas et al., 2003). Therefore, WAS fermentation to generate VFA can be supplemental in addition to primary sludge fermentation. Though there is a

capital and O&M cost associated with the WAS fermenter, the decrease in the costs of purchasing external carbon, especial the cost of sludge processing due to WAS minimization can compensate the costs of WAS fermenter. This research finding (Chapter 5) demonstrated that the co-fermentation of WAS and PS enhanced sCOD production. an increasing number of BNR plants in Western Canada, northern Europe and in USA have primary sludge fermenter. Therefore, for the plant with existing primary sludge fermenter, retrofit the PS fermenter to a co-fermentation fermenter will be an economical practice.

It was found through this research that approximately 7-18% of COD can be released through sludge fermentation (including co-fermentation of WAS and PS). The value of VFA production is approximately 100-180 mg COD/g TS. For a domestic WWTP, such as Winnipeg NorthEnd treatment plant, with an average COD of 400 mg/L, assuming that 120 g sludge is produced from 1 m^3 wastewater and the average VFA production from the sludge is 150 mg COD/g TS, the wastewater will be enriched with 18 mg COD/L. Additional 1.2 mg P/L or 3 mg NO_3/L can be removed. With the average flow at NorthEnd WWTP of 180 MLD, sludge fermentation can replace the use of 4.2 ton of Acetate or 1.71 m^3 methanol per day, which can be translated into annual savings of $3 M and $ 0.2 M of purchasing acetate and methanol, respectively. (Acetate @ $2,000/ton, Methanol @ $ 355 /m^3)

8.2 COST SAVINGS IN SLUDGE HANDLING

Another important benefit of sludge fermentation is the reduction of excess sludge production. With the increased cost of sludge handling, reduced sludge production is now an important topic in operating WWTP. At a WWTP, the cost of sludge handling is

around 50% of the total operational cost. The total, complete final disposal costs may range from $400 to $1000/t dry solids. From this research, the volatile solids destruction obtained from sludge fermentation was in the range of 21-38%. City Winnipeg has three WWTP, only North End WWTP (central plant) has the facility for sludge treatment. The sludge generated from SouthEnd and WestEnd WWTP needs to be hauled daily to the central plan for sludge treatment. If sludge can be fermented onsite in the holding tank before hauling, cost for hauling sludge will be reduced due to the reduction of sludge volume. In addition, the VFA generated from fermentation can further improve the performance of BNR processes at these plants.

8.3 REVENUES FROM PHOSPHORUS RECOVERY

The implementation of sustainable wastewater treatment requires not only removal of phosphorus from wastewater, but also recovers phosphorus. The value of the recovered P, in the form of struvite, tends to be higher than that of phosphate ore, due to higher quality, e.g. high purity and low heavy metal contamination (Forrest et al., 2007). In comparison to other conventional fertilizer, struvite has been identified as a slow-release fertilizer. This feature has increased the value of struvite during the last decades. Several fluidized bed reactors have been in full-scale production in Japan and there covered struvite has been sold as a fertilizer (Shimamura et al., 2003) at a price of approximately USD $250 per ton in 2001. Their market analysis demonstrates that, if distributed successfully, WWTP can generate revenue to offset operational costs, through struvite recovery processes, concurrently becoming more sustainable (Ueno & Fujii 2001). The Ostara Nutrient Recovery Technology that developed by University of British Columbia has the full scale demonstration at the City of Edmonton Gold Bar WWTP. Their analysis

showed that struvite recovery has the potential for offsetting meaningful amounts of greenhouse gas emissions through sustainable and energy efficient production of fertilizers (Britton, et al., 2009).

The commercial nutrient recovery technology can also be applied in the sludge fermentation process. In the case of NorthEnd WWTP, some 50% of phosphorus can be recovered through struvite formation. The estimated value of the struvite at $0.15/lb (based on an estimate of what a fertilizer company might pay for raw product at the farm, Westerman, et al. 2009), the revenues from struvite can be $ 1 M annually. Though net revenue will be less after considering the capital cost and operational cost, the positive impact of phosphorus recovery from wastewater on the environment has more meaning than the economic saving.

Chapter 9 **FUTURE WORK**

The ongoing research project shows that phosphorus removal and denitrification can be achieved simultaneously by a group of bacteria called denitrifying phosphorus removal organisms (DPAOs). The reference publications regarding DNPAOs are attached in the Appendices A and B. The objective of both researches is focusing on solving the carbon deficiency issue in biological phosphorus removal process, therefore, the future work can combine these two research topics as e.g. enhancement of P removal and recovery through WAS fermentation in the DNPAOs-enriched BNR process. The detailed research recommendations are following:

9.1 PILOT SCALE APPLICATION

The research of DNPAOs (see Appendix C) showed that biological nutrient removal can be achieved by DNPAOs in a continuous flow, one-biomass system at lab scale level. To become a more viable optional technology for application of DNPAOs in the BNR process, the configuration of this process needs to be tested in pilot scale. Since the DO in the RAS stream is the key for sustaining DNPAOs in the system, studies regarding the application of the minimization of DO technology would be implemented. Due to the limitation of lab scale experiment, DO in the RAS stream was eliminated by nitrogen gas, however, this can be expensive and impractical in the full scale application. It was proposed in the pilot scale test that a degasification technology such as BIOGRADEX should be used.

As addressed in Chapter 6, it was necessary to remove phosphorus, which was released during the fermentation process, in the WAS fermentation stream to reduce the P

load to the main stream. This provides an excellent opportunity for the phosphorus recovery. Currently, the technology that has been used at three WWTP is Ostara. The applicability of Ostara process in the WAS fermentation stream needs to be investigated through pilot scale tests. This study should mainly focus on the effect of P recovery process on the VFA quality and quantity in the fermentation stream, e.g. if VFA will be lost during the P recovery process. Subsequent question to be answered is if the modification of the recovery process is required to minimize the lost VFA.

9.2 MODELING THE PERFORMANCE AND FERMENTATION OF DNPAOS

Mathematical modeling is now an integral part of biological wastewater treatment, often for optimization and prediction of process performance, and as a design tool. The IWA ASM No. 2 (Gujer et al., 1995; Henze et al., 1995), and later, IWA ASM2d (Henze et al., 1999), have been most widely used to simulate full-scale EBPR processes. IWA ASM2d is an updated model of IWA ASM2, which incorporates the denitrification capability of PAOs. The yield coefficients in the IWA ASM2d as well as the other IWA ASM1 and IWA ASM3 models are determined experimentally. Due to the limited research on the DNPAOs, the availability of the stoichiometric and kinetic parameters is very uncertain. Therefore, the default stoichiometric and kinetic parameters provided by the models are needed to be fully-calibrated based on the process in order to model it accurately.

As addressed in appendix A, the competition of carbon source between DNPAOs and GAOs were the major reason for the failure of operation of the continuous flow system. However, the existence of GAOs was not considered in any of the IWA ASM models. It would be necessary to conduct further research to incorporate the growth and activity of

GAOs into the models. This is especially important for the predication of microbial population dynamics in the EBPR system where competition between PAOs and GAOs can occur.

Chapter 10 **Appendix A**

INTERACTION BETWEEN DENITRIFICATION AND PHOSPHORUS REMOVAL IN A SBR PHOSPHORUS REMOVAL SYSTEM [*]

10.1 INTRODUCTION

In a conventional biological nutrient removal (BNR) wastewater treatment plant, nitrogen removal is achieved through nitrification and denitrification. Although organic carbon is not required for nitrifying bacteria to complete nitrification, sufficient organic carbon is necessary for denitrifying bacteria to carry out denitrification. To achieve phosphorus removal, it is crucial to provide phosphorus accumulating organisms (PAOs) with anaerobic conditions to promote the consumption of readily bio-degradable COD (rbCOD). Thus, there is a competition for organic carbon between PAOs and denitrifying bacteria. The availability of biodegradable organic carbon is often the key factor for the BNR process where simultaneous nitrogen and phosphorus removal is required. This rbCOD availability is most important for a municipal wastewater treatment plant where organic carbon content is low. Morling (2001) pointed out that the enhanced biological phosphorus removal (EBPR) process often deteriorates when the availability of organic carbon is at a low level. At the same time, denitrification also requires a significant amount of organic carbon, often leading to the addition of an external carbon source (Claus and Kutzner, 1985). To address the problem of competition for the limited

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organic substrate, denitrifying phosphorus accumulating organisms (DNPAOs) have received considerable attention (Spagni, et al., 2001; Shoji, et al., 2003; Kuba, et al., 1996).

DNPAOs have been distinguished from aerobic PAOs by to their unique metabolic characteristic (Kerrn-Jespersen and Henze, 1993). The mechanism of anaerobic phosphate release of DNPAOs is the same as aerobic PAOs; specifically, external organic substrate is taken up and converted to polyhydroxyalkanoate (PHA) as a cell energy source. DNPAOs are different from aerobic PAOs in the manner of phosphate uptake. Aerobic PAOs can only use oxygen as an electron acceptor for cell respiration which promotes phosphorus removal; whereas DNPAOs can use nitrite or nitrate as an electron acceptor instead of oxygen (Meinhold, et al., 1999). In other words, DNPAOs can combine phosphorus removal and denitrification into one process using the same amount of organic substrate. In addition, less aeration is needed which translates into lower energy requirement. Research of Kuba et al. (1996) showed that DNPAOs can reduce \sludge generation by 30%, due to their low cell yield. These advantages of DNPAOs have steered researchers to improve biological nutrient removal systems. Single sludge systems of BCFS (Biological-chemical phosphorus and nitrogen removal) as well as two sludge system such as DEPHANOX process (De-nitrification and Phosphate accumulation in Anoxic) were developed for extensive utilization of DNPAOs.

To facilitate anoxic and aerobic phosphorous uptake Kuba et al. (1996) suggested that a predenitrification configuration is necessary. However, Comeau et al. (1986) reported ten years earlier that when nitrate concentration in return sludge was less than 5 mg/L, EBPR would not deteriorate. The aim of this study was to investigate the interaction

between denitrification and phosphorus removal. In addition, the relationship between denitrification and phosphorus uptake by DNPAOs will be evaluated.

10.2 METHODOLOGY

10.2.1 *Reactor setup and operation.*

Two sequencing batch reactors (SBR) with a 3 L working volume were seeded with activated sludge from a local domestic wastewater treatment plant, which operates an aerobic activated sludge process to remove COD. The reactors were operated under an anaerobic/aerobic configuration to enrich PAOs for one month. After enrichment one SBR (SBR1) was operated under an anaerobic/anoxic/aerobic configuration. The other reactor (SBR2) was operated with an anoxic/aerobic configuration for about 2 months. Anaerobic conditions were achieved by bubbling nitrogen gas into the bulk liquid. Aeration was accomplished by pumping air into the reactor, and dissolved oxygen was regularly measured to ensure it remained above 2 mg/L. Anoxic conditions were achieved by KNO_3 addition to attain a system concentration of 20 mg/L NO_3-N.

Each SBR was operated with an 8 h cycle and at the end of each cycle 2 L of effluent was decanted and new cycle started with filling the same volume of influent, thus, hydraulic retention time of 12 h was maintained. A solid retention time (SRT) of 10 days was obtained by removing 100 ml of mixed liquid from each cycle. The pH and reactor temperature were controlled between 7.0-7.5 and 20-22 ℃ respectively.

The reactors were fed with synthetic wastewater using acetate, yeast and beef extract as the carbon sources. The composition of synthetic wastewater is shown in Table 10-1. To

minimize nitrate residue in the system, ammonium was controlled in the feed to diminish nitrification. Nitrogen was supplied in form of beef extract and yeast extract.

Table 10-1 Synthetic wastewater composition

Synthetic wastewater		**Mineral solution**	
Ingredients	Concentration (mg/L)	**Ingredients**	Concentration (g/L)
NaAc	255	**$FeCl_3 \cdot 6H_2O$**	1.5
Beef extract	65	**H_3BO_3**	0.15
Yeast extract	65	**$CuSO_4 \cdot 5H_2O$**	0.03
$MgSO_4 \cdot 7H_2O$	170	**KI**	0.03
$CaCl_2 \cdot 2H_2O$	14	**$MnCl_2 \cdot 4H_2O$**	0.12
P (K_2HPO_4)	9	**$Na_2MoO_4 \cdot 2H_2O$**	0.06
TN (organic)	14-15	**$ZnSO_4 \cdot 7H_2O$**	0.12
		$CoCl_2 \cdot 2H_2O$	0.15
Mineral solution	0.3 ml	**EDTA**	10

10.2.2 Batch test.

Set I: Effect of nitrate on phosphorus uptake

Three batch tests, using the mixed liquor taken from SBR 1 (anaerobic\anoxic\aerobic), were conducted. The experiment was carried out under the following conditions: a 90 minutes anaerobic phase followed by a 180 minutes anoxic phase with a final 180 minutes aerobic phase. Three different amounts of potassium nitrate were added at the end of the anaerobic phase to achieve anoxic conditions with different nitrate concentrations.

Set II: Effect of nitrate addition point on phosphorus release and uptake

Three batch tests, using the mixed liquor taken from SBR2 (anoxic\aerobic), were conducted. The experiment was carried out under the following conditions: a 210 minutes anoxic phase followed by a 240 minutes aerobic phase. Three different amounts of potassium nitrate were added in the feed resulting in different nitrate concentrations.

Note: The feed for the batch tests was the same as the feed for the SBR systems (Table 10-1).

10.2.3 Analytical Methods.

Carbon content of the substrates was measured as dissolved organic carbon (DOC). A Phoenix 8000 TOC Analyzer (Tekmar Dohrmann, Mason, Ohio) was used for the determination of DOC. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) measurements were performed according to Standard Methods (APHA,2005) . Dissolved phosphate, nitrite and nitrate were measured by Lachat Instrument (Ontario, Canada) Quik Chem 8500, followed Quik Chem Method orthophosphate 10-115-01-1-O and nitrate/nitrite 10-107-01-1-A, respectively.

10.3 RESULTS AND DISCUSSION

10.3.1 Set I: Effect of nitrate on phosphorus uptake

The effects of nitrate concentration on denitrifying phosphorus removal under increasing NO_3-N concentrations of 13.3mg/L, 21.3mg/L, and 33.9 mg/L are shown in Fig. 10-1. The increased nitrate concentrations (Fig. 10-1b) resulted in an increased phosphorus uptake in the anoxic phase. The lowest concentration of NO_3-N resulted in a phosphorus uptake of only 22.3mg/L. Increasing the NO_3-N concentration provided 28.8 mg/L and 46.3 mg/L of phosphorus uptake, respectively. These levels corresponded to a 30%, 45%

and 67% uptake of the total amount of phosphorus released in the anaerobic phase, suggesting that denitrifying phosphorus removal increases with nitrate concentration. Nitrate is used by the DNPAOs as the electron acceptor to oxidize stored PHB/PHA which generates the required energy needed for phosphorus uptake. Therefore, an inadequate supply of nitrates slows down the rate of anoxic phosphorus uptake.

The denitrification profile of DNPAOs is shown in Figure 10-1a. Complete denitrification was obtained in 90 minutes when the concentration of nitrate added was 13.3 mg/L. Only about 0.3 mg/L of nitrite was detected during this denitrification process (data not shown). When nitrate addition was increased to 21.3 mg/L, 0.9 mg/L of nitrite was measured, and denitrification was completed in 150 minutes. With the highest NO_3-N addition of 33.9 mg/L, denitrification occurred during the entire 180 min anoxic phase but about 0.5 mg/L NO_3-N remained in the system at the end of anoxic phase. The highest nitrite levels of 2.5 mg/L were measured around 30 minutes after nitrate addition. In all cases no nitrite was detected at the end of the anoxic phase which suggests that there are DNPAOs in the system that can use both nitrite and nitrate as an electron acceptor for phosphorus uptake.

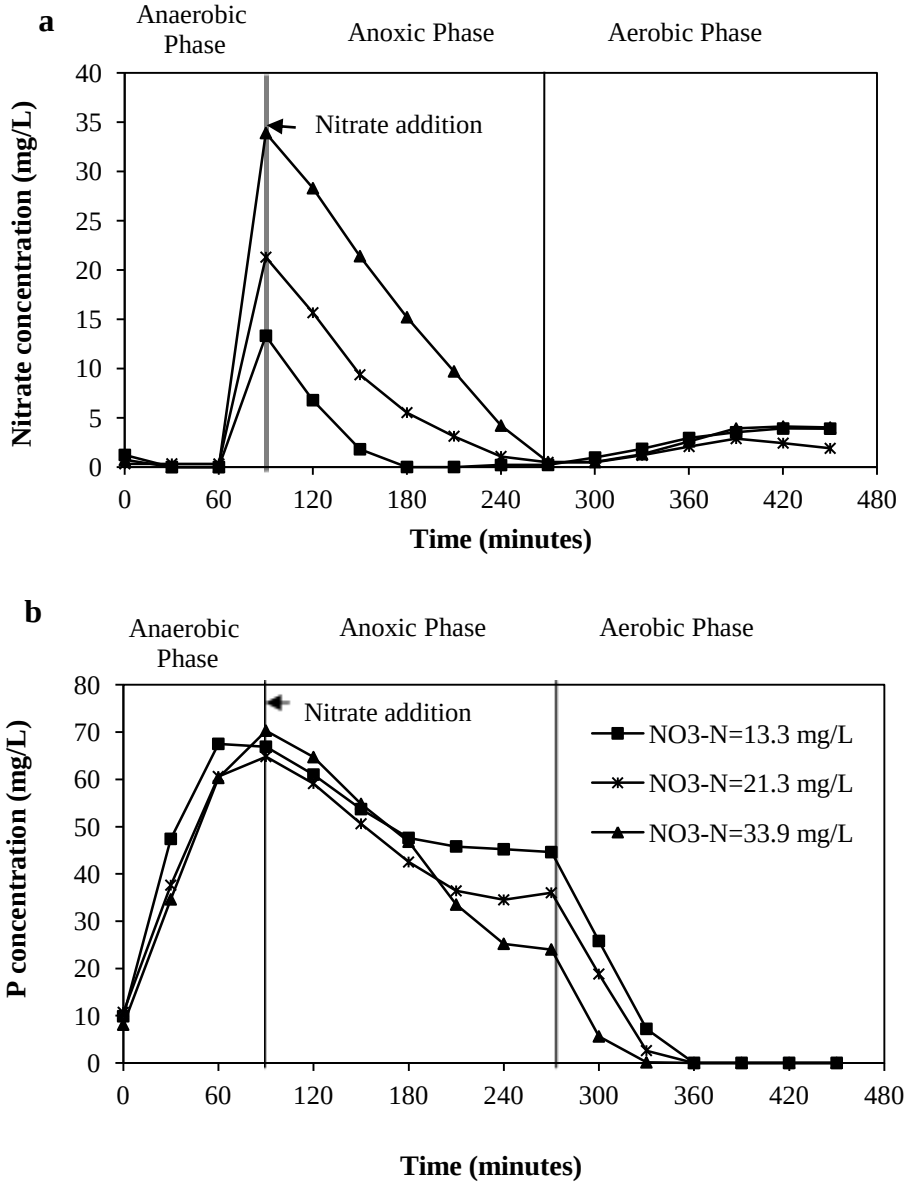

Figure 10-1Concentration profile for phosphorus and nitrate under A_2/O configuration

Figure 10-2 shows the comparison of Phosphorus Uptake Rate (PUR) under different conditions. The phosphate uptake rate is determined based on the initial linear part of the phosphate decrease curve (see Figure 10-1 b). It can be seen that with increasing nitrate

concentration, anoxic PUR increased slightly from 6.4 to 7.8 mg P /g VSS h. Under aerobic conditions the PUR decreases significantly with increasing nitrate concentrations; from 18.7 to 11.9 mg P /g VSS hr. One explanation is that when more PHB is oxidised in the anoxic phase, less PHB remains to be oxidised in the aerobic phase. This corresponded to a higher anoxic PUR followed by a lower aerobic PUR. Therefore high nitrate concentrations resulted in the highest anoxic PUR and the lowest aerobic PUR. It was also observed that aerobic PUR was always higher than anoxic PUR which suggested that, compared to $NO_{3,}$ oxygen is a more efficient electron acceptor for the metabolism of phosphorus accumulating microorganisms.

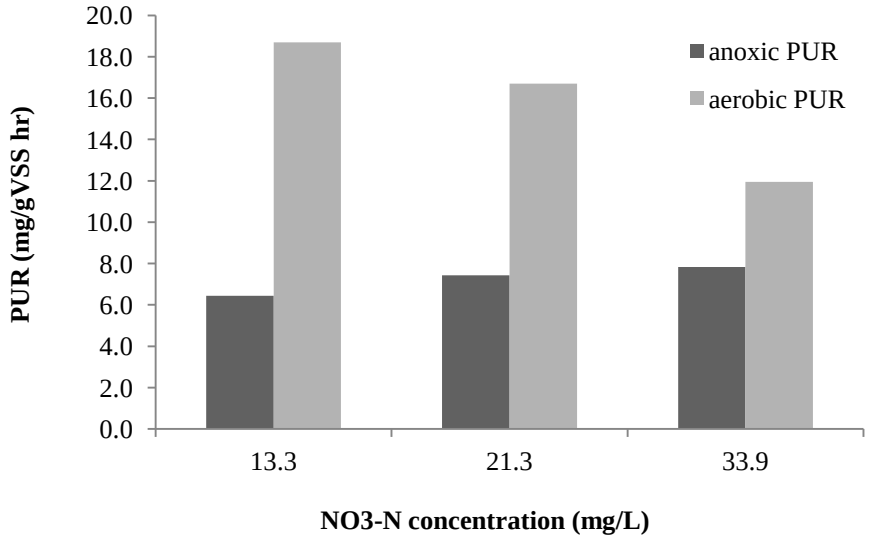

Figure 10-2 Anoxic and aerobic phosphorus uptake rate with different nitrate concentration

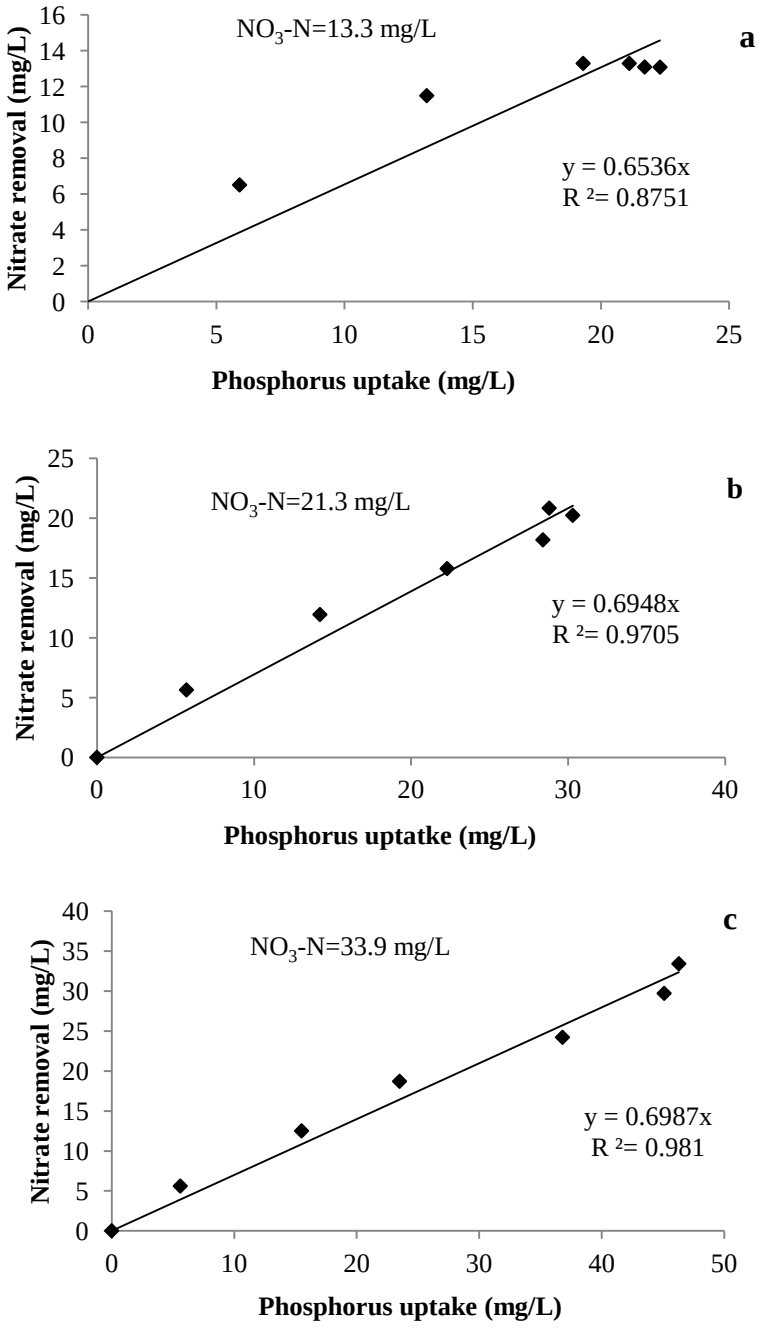

Figure 10-3Relationship between phosphorus uptake and nitrate removal

The correlation between P uptake and NO_3-N removal in the anoxic phase of the SBR 1 system indicates that P uptake under anoxic condition is proportional to nitrate removal – Fig.10-3. This experiment suggested that for one milligram of phosphate uptake approximately 0.7 mg NO_3-N was utilized by the DNPAOs. This can be translated into 0.13 mol P/mol e^- where NO_3 is used as the electron accepter. The results from this experiment were slightly lower than Kuba et al (1993) who reported 0.19 mol P/mol e^- in an anaerobic / anoxic SBR system. This difference may have been due to the aeration phase in this system resulting in different DNPAOs community and population.

10.3.2 Batch test to evaluate the DNPAOs fraction of total PAOs

Wachtmeriste r et al. 1997 proposed a method to estimate the DNPAOs fraction (X_{DNPAO}) of total PAOs (X_{PAO}) based on the phosphate uptake rate under different conditions:

$$\frac{X_{DNPAO}}{X_{PAO}} = \frac{P_{anoxic}}{P_{aerobic}} \bullet \eta G \qquad (1.1)$$

Where: ηG is a reduction factor for the lower energy gain in the anoxic condition compared to the aerobic energy gain, a typical value of 0.8 was used in this experiment. P_{anoxic} and $P_{aerobic}$ are the phosphorus uptake rate under anoxic and aerobic conditions respectively.

A batch test of anoxic and aerobic phosphate uptake rate was conducted to evaluate the DNPAOs fraction in SBR 1. The system was operated under the same anaerobic/anoxic/aerobic conditions as previous batch tests. Right after the anaerobic phase, the mixed liquor was divided evenly into 2 parts. One part was spiked with an

adequate amount of nitrate to ensure nitrate was not the limiting factor. The other part was aerated, and the DO level was maintained above 2 mg/L.

The total PAOs phosphate uptake under anoxic and aerobic condition was 17.7 mg/L h and 36.7 mg/L h, respectively – Fig. 10-4. Thus, the DNPAOs fraction was calculated to be 38% using the Wachemerister et al. method.

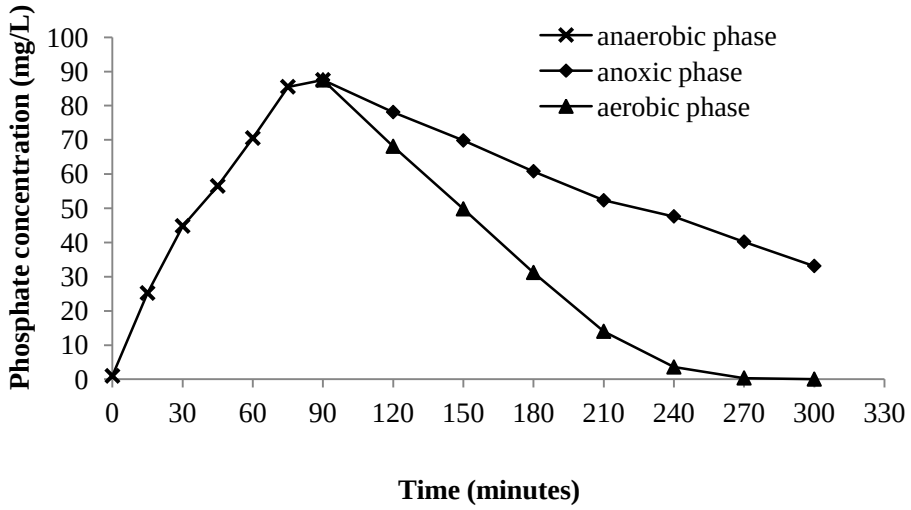

Figure 10-4Aerobic and anoxic phosphate uptake rate

10.3.3 Set II: Effect of nitrate addition configuration on phosphorus release and uptake

For Reactor 2, nitrate was added in the feed to study the effects of nitrate addition configuration on denitrifying phosphorus removal in the anoxic-aerobic sequence (Figure 10-5). Despite the presence of nitrate and regardless of the concentration, phosphorus release occurred as long as organic substrate was present and the maximum phosphorus concentration was reached after 60 minutes. The phosphorus release rate decreased

significantly when nitrate was present in the substrate (compared to Figure 10-1 b). The average phosphorus release rate shown in Figure 10-1 b was about 25 mg/g VSS h, whereas when nitrate was introduced together with the substrate, the phosphorus release rate decreased to 14, 13 and 8 mg/g VSS h with increasing nitrate concentrations from 17.7 mg/L, 25.5 mg/L to 33.6 mg/L, respectively. These observations point to a competition for the carbon source between the phosphorus removal bacteria and the denitrifying bacteria. The competition resulted in lower phosphorus release due to the decreased availability of the carbon source. These experiments emphasized that the presence of nitrate hinders phosphorus release.

With NO_3-N dosing at 17.7 mg/L, nitrate was completely removed from the system at 60 minutes while the released phosphorus in the system also reached the maximum concentration. There was also no significant change of phosphorus concentration until aeration began. When NO_3-N concentration was increased to 25.5 mg/L and 33.6 mg/L, there was significant denitrifying phosphorus uptake following the phosphorus release. This observation suggested that DNPAOs exist in the system. It was thought that phosphorus removal bacteria will out-compete the denitrifying bacteria in the presence of both nitrate and substrate. The result from this experiment proved that DNPAOs can grow in a system where nitrates are present during the anaerobic phase.

To evaluate the fraction of DNPAOs to total PAOs in the SBR 2 system, a batch test was carried out. The result showed that phosphate uptake under anoxic and aerobic condition was 13 g/L h and 33 mg/L h respectively. Therefore the DNPAOs fraction was estimated at 32%. Although this fraction was slightly lower than in SBR 1, this confirmed that DNPAOs can develop in a system with NO_3 present during the anaerobic phase.

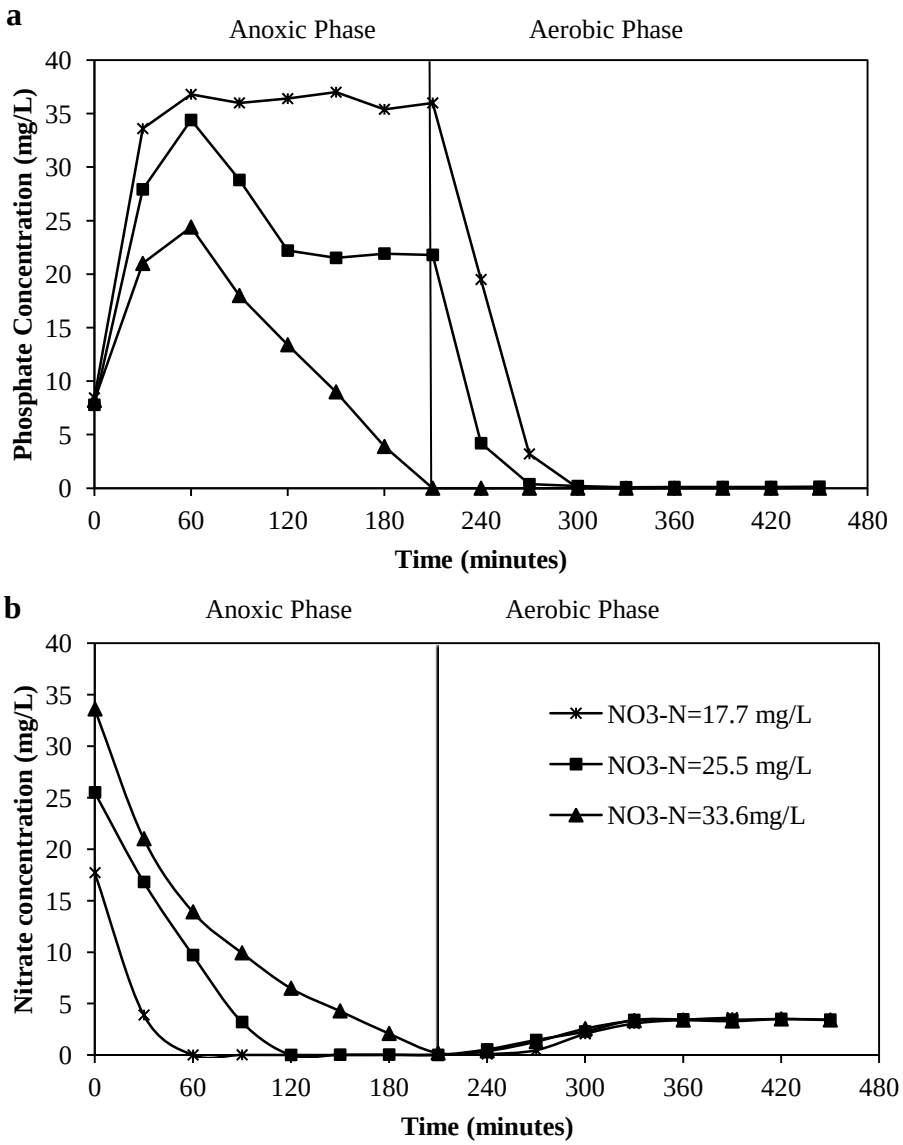

Figure 10-5Concentration profile for phosphorus and nitrate under anoxic phase

Higher carbon consumption rate during the anoxic phase in SBR 2 was expected due to simultaneous denitrification and phosphorus release. However, nearly the same amount of dissolved organic carbon (DOC) removal occurred regardless of the time of nitrate addition. As illustrated in Figure 10-6, DOC removal followed the same trend whether

the nitrate addition occurred before or after the anaerobic phase. This novel result can be attributed to the characteristics of the substrate. Two thirds of the COD supplement in the substrate was made up of acetate which can be utilized directly by the majority of the microorganisms. Acetate was rapidly removed in the first 30 minutes and corresponded to an approximately 67% reduction in DOC in both reactors. Beef and yeast extract made up the remaining one third of the COD supplement. Although both beef and yeast extracts are biodegradable, specific enzymes are required to hydrolyze them into small molecules for microorganism utilization. Therefore, this remaining portion of the COD supplement was gradually degraded and assimilated in the following 30 minutes and corresponded to the remaining 33% DOC removal. This experiment again showed that acetate is the best substrate for both PAOs and denitrifiers.

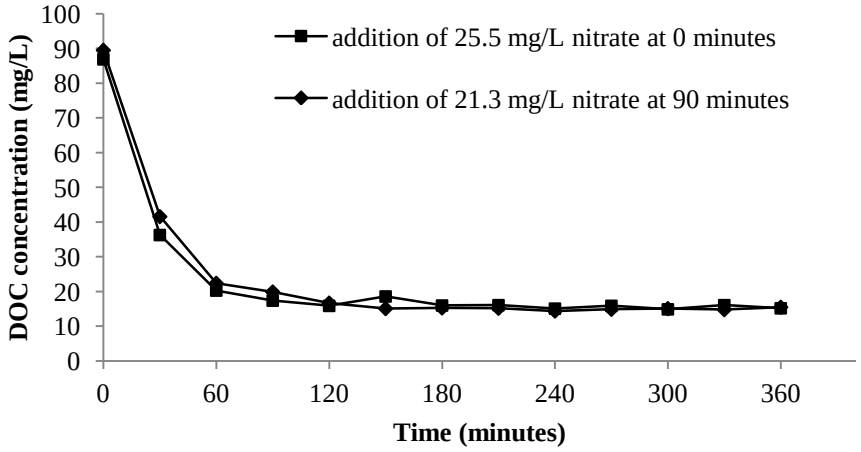

Figure 10-6 DOC reduction with different nitrate addition configuration

10.4 CONCLUSION

A series of batch tests were run on biomass from SBR operating in the anaerobic/anoxic/aerobic mode and anoxic/aerobic mode. The experiments focused on elucidating the role denitrifying phosphorus accumulating organisms (DNPAOs) play in the anaerobic phosphorus release and uptake when compared to aerobic phosphorus accumulating organisms (PAOs). It was conclude that:

- With the same amount of carbon source available to both the PAOs and the DNPAOs, the latter were capable of accomplishing denitrification and phosphorus removal simultaneously. In order to keep a good DNPAOs population in the system, adequate amounts of nitrate were required in the anoxic phase to promote DNPAOs denitrifying phosphorus uptake.
- Some DNPAOs were able to utilize nitrite as electron acceptor as evidenced by the lack of nitrite build up during the denitrifying phosphorus uptake phase
- The experiment showed that 0.7mg NO_3-N was removed for a 1 mg uptake of phosphorus by DNPAOs.
- Phosphorus release has been shown to occur as long as adequate amount of carbon substrate was present in the anaerobic phase, regardless of nitrate concentration. It was also shown that nitrate did hinder the release of phosphorus in the anaerobic stage due to denitrifiers competing with PAOs for their carbon source. DNPAOs remained competitive in a system with nitrate in the anaerobic phase.

The experiment confirmed that comparing to beef extract and yeast extract, acetate was the best substrate for both PAOs and denitrifiers.

Chapter 11 **Appendix B**

THE EFFECT OF DISSOLVED OXYGEN ON THE BIOLOGICAL NUTRIENT REMOVAL BY DENITRIFYING PHOSPHORUS ACCUMULATING ORGANISMS IN A CONTINUOUS FLOW SYSTEM*

11.1 INTRODUCTION

Successful biological nutrient removal depends on the availability of suitable organic carbon in the influent. For weak wastewater, such as many municipal wastewaters, the requirement for external carbon addition is becoming more common, especially when meeting the increasingly stringent effluent nutrient limits. In order to solve the problem of competition for the limited organic substrate, denitrifying phosphorus accumulating organisms (DNPAOs), with their lower overall carbon requirement, have received considerable attention. Research of DPAOs has demonstrated that under anoxic conditions, DNPAOs can use nitrate or nitrite as electron acceptors to uptake phosphorus which allows the removal of N and P simultaneously. In other words, DNPAOs can combine phosphorus removal and denitrification into one process using the same amount of organic substrate. In addition, less aeration is needed which translates into lower energy requirement. Research by Kuba et al (1996) has shown that DNPAOs can reduce sludge generation by 30%, due to their low cell yield.

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The recent studies of DNPAOs have often been focused on: 1) characterization of DNPAOs by using molecular methods.(Soiji, 2006, Ahn, 2002, Zeng, 2003); 2) Model development of DNPAOs by stoichiometry and kinetic study of anoxic p storage and anoxic PAOs growth (Mino, 1995, Murnleitner, 1997, Oehmen, 2010); 3) the factors that affect DNPAOs growth, such as the type of carbon source, nitrite, C/P, pH, etc (Carvalho, 2007, Zhou, 2010, Wang, 2007). However, most studies on DNPAOs were conducted in lab-scale sequencing batch reactors (SBR). The limitations of using SBR to study DNPAOs are: 1) anoxic condition was achieved by dosing NO_3 to simulate the Anaerobic/Anoxic/Aerobic or Anaerobic/Anoxic processes, which cannot be applied in the real practice; 2) in an SBR system the anoxic phase follows anaerobic phase which provides an ideal anoxic condition; whereas in a continuous flow system, the anoxic reactor receives the internal recycle flow from the aerobic reactor which contains oxygen. Lie & Welander (1994) found that oxygen had a negative effect on denitrification even at a lower concentration (<0.1 mg/L). Therefore, in a continuous flow system the oxygen from the nitrate recycle flow might have a negative impact on the denitrification activities of DNPAOs in the anoxic reactor. However, this has not been studied yet.

The limitation of the SBR requires the study of the performance of DNPAOs in a continuous flow system in order to understand and optimize the operational conditions for DNPAOs. The objective of this research was to study the effect of oxygen from the nitrate recycle stream in the anoxic reactor on the performance of DNPAOs in a continuous flow system (an A2O process).

11.2 METHODOLOGY

11.2.1 Reactor setup

A lab scale continuous flow system with Anaerobic\Anoxic\Aerobic configuration was set-up (Fig. 11-1). The reactor was seeded with the DNPAOs sludge from a Lab-scale SBR and was operated with SRT of 6 d and HRT of 8 h. Synthetic wastewater was used as feed with COD of 350 mg/L. Acetate, beef and yeast exact were used as carbon source. Acetate was the main carbon source for the phosphorus removal microorganism; Beef and yeast extract provided nutrient for the growth of the diverse microorganisms since they are mainly composed of protein and carbohydrate. Acetate provided 50% of COD in the feed. Beef and yeast extract provided the rest - 50% of COD. The composition of the synthetic wastewater was listed in Table 11-1. For the aerobic reactor, the air was supplied from a compression air pump with the flow of 2.5 LPM. This provided the dissolved oxygen (DO) concentration in the aerobic reactor of 5 ± 0.5 mg/L. The pH of each reactor was monitored regularly but not controlled since they were fairly neutral and stable (in the range of 6.9-7.5). The reactors were operated in a temperature-controlled room (20-22 ℃). In phase 1, in order to eliminate the dissolved oxygen being recycled back to the anoxic reactor, the mix liquor from the aerobic reactor was passed through a degas device before it was sent back to the anoxic reactor. The nitrogen gas was constantly flowed through the degas device at a rate of 100 ml/min to strip the oxygen. Phase 1 lasted for 75 days. In phase 2, the degas device was taken out of service to examine the effect of DO on the performance of DNPAOs in the anoxic reactor. Phase 2 lasted for 90 days.

Table 11-1 Synthetic wastewater composition

Synthetic wastewater		Mineral solution	
Ingredients	Concentration (mg/L)	**Ingredients**	Concentration (g/L)
NaAc	255	**$FeCl_3 \cdot 6H_2O$**	1.5
Beef extract	65	**H_3BO_3**	0.15
Yeast extract	65	**$CuSO_4 \cdot 5H_2O$**	0.03
$MgSO_4 \cdot 7H_2O$	170	**KI**	0.03
$CaCl_2 \cdot 2H_2O$	14	**$MnCl_2 \cdot 4H_2O$**	0.12
P (K_2HPO_4)	9	**$Na_2MoO_4 \cdot 2H_2O$**	0.06
TN (organic)	14-15	**$ZnSO_4 \cdot 7H_2O$**	0.12
		$CoCl_2 \cdot 2H_2O$	0.15
Mineral solution	0.3 ml	**EDTA**	10

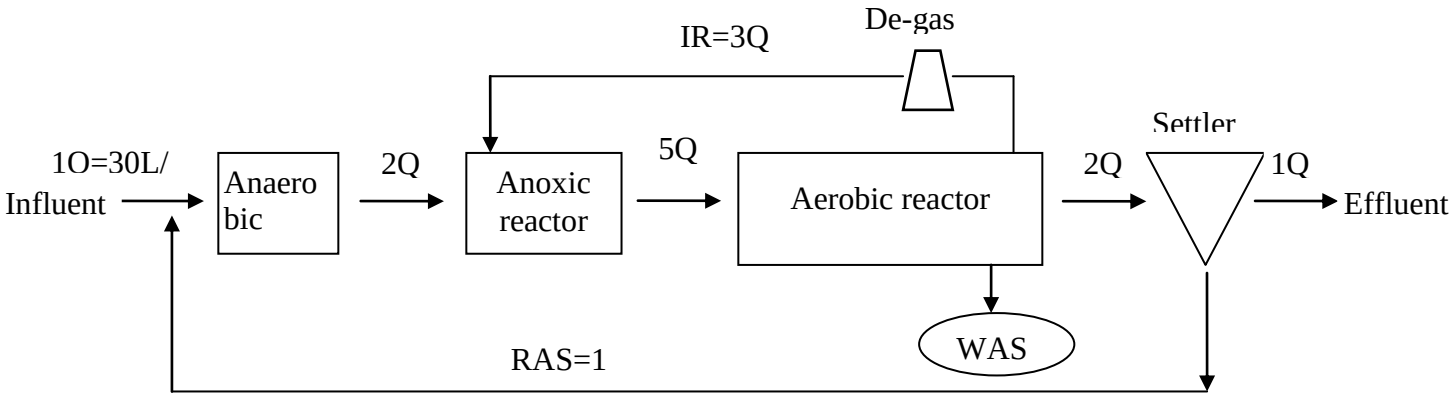

Figure 11-1Experiment set-up in the BNR process with the continuous flow system

11.2.2 Analytical methods

Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) measurements were performed according to Standard Methods (APHA, 1998). Hach COD digestion vials were used to measure COD. Dissolved phosphate and ammonium were measured using a Lachat Instrument Quik Chem 8500, following the Quik Chem orthophosphate method 10-115-01-1-O, and Quik Chem ammonia method 10-107-06-1-I. The analysis of acetate was conducted by means of a Varian CP-3800 Gas Chromatograph using a flame ionization detector (FID) and HP-FFAP capillary column (inner diameter of 0.25mm and length of 25m). Acetic acid concentration was converted to COD concentration by using the conversion factors of 1.07. Glycogen measurements were performed according to the Anthrone Method outlined in Manual of Methods for General Bacteriology (Gerhardt et al., 1981).

11.3 RESULTS AND DISCUSSION

11.3.1 Phase 1- the performance of EBPR

11.3.1.1 Anaerobic P release

As can be seen from Fig 12-2., in phase 1 the reactor achieved excellent P removal. The average P concentration in the effluent is approximately 0.5 mg/L. Full nitrification was also observed throughout the experimental period (data was not shown). In the anaerobic reactor, with approximate acetate COD of 5250 mg/d in the feed (Fig 3), the P released by the PAOs was approximately 2277 mg/d. The ratio between the anaerobic P release and acetate uptake (P/HAc) was 0.5 P-mol/C-mol acetate which was close to the value reported by Smolders et al (1994) from an SBR PAOs reactor. It was observed the COD

removal in the anaerobic zone was approximately 8620 mg which was higher than the acetate COD provided in the feed. This can be explained by two reasons: 1) the occurrence of denitrification in the return sludge cycle in the anaerobic reactor required COD; 2) there were some fermentative microorganisms in the system. These fermentative microorganisms can convert beef and yeast extract to some fermentation products such as lactate, ethanol, etc. which were consumed by other microorganisms under anaerobic condition.

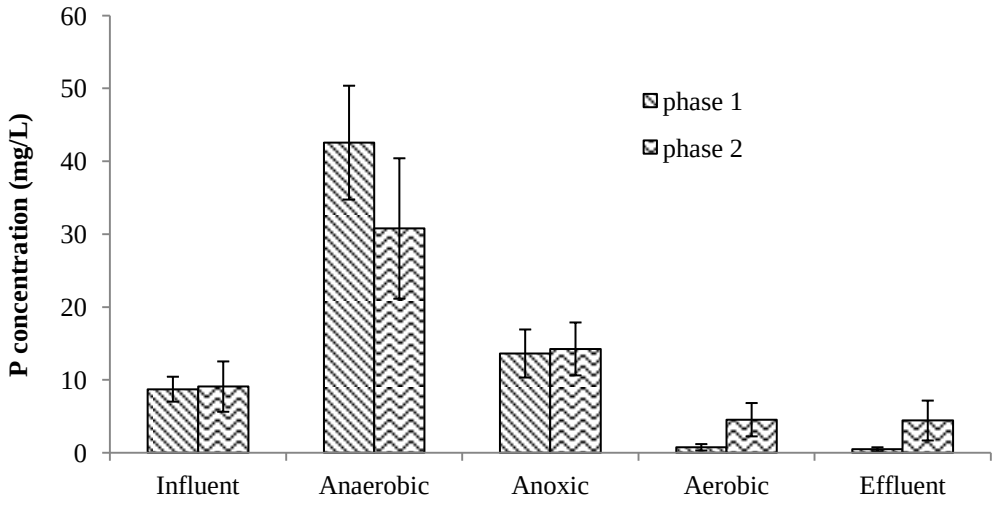

Figure 11-2P concentration at different point in phase 1 and 2

11.3.1.2 Anoxic and aerobic P uptake

As mentioned in the introduction, SBR reactors were the most widely used method to study DNPAOs in recent studies. In the SBR reactor, the anoxic phase follows the anaerobic phase and was achieved by dosing nitrate. Therefore, the anoxic condition was

ideal, whereas in a continuous flow system the oxygen will be introduced by the internal nitrate recycle from the aerobic reactor. To our best knowledge, the effect of DO in the internal nitrate recycle stream, and its effect on the growth of DNPAOs have not been studied yet. Therefore, in the current experiment, attempt was made to eliminate the DO in the anoxic reactor to optimize the anoxic condition for the denitrification process. A unique de-gas device was introduced. The nitrogen gas was constantly bubbled into this device while the recycle flow was passing through it to strip the oxygen. The DO concentration in the aerobic reactor was approximately 4.5-5.15 mg O_2/L, while after passing through the degas device it effectively dropped to 0.05-0.07 mg O_2/L. The DO in the anoxic reactor was measured as 0-0.02 mg O_2/L.

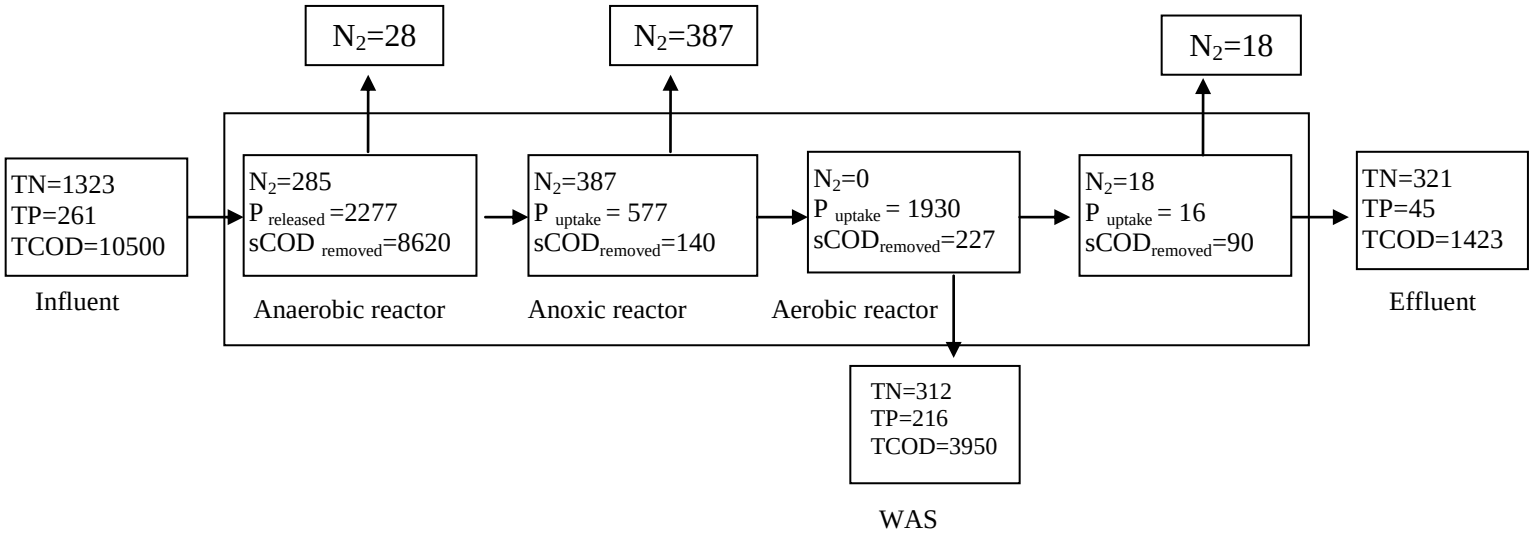

Figure 11-3N, P and COD flow (mg/d) in the continuous flow system in phase 1

It was observed that in the anoxic reactor with the removal of 387 mg of nitrate (Fig. 11-3), the DNPAOs up took 577 mg which was approximately 25% of the total P in the anaerobic reactor. This suggested that for DNPAOs to remove 1 mg N-NO_3, it uptake 1.5

mg $P\text{-}PO_4$. This value was very close to our previous SBR result. This observation suggested that at low DO of 0-0.02 mg/L DNPAOs can be sustain in the system. The NO_3 removal in the anoxic reactor is approximately 56% of the total NO_3 removed which suggested that 56% of the required COD for denitrification could be potentially saved by using DNPAOs.

To study the DNPAOs performance, the kinetic studied was carried out (Fig. 11-4). The anaerobic P release was observed as approximately 26.2 mg P/ g VSS hr which was much higher than the test conducted by Lopez-vazquez et al (2008) using the sludge from the full-scale plant. This was because the biomass from this experiment was acclimated and enriched with P removal microorganisms. The aerobic P uptake rate obtained from the kinetic study was 17.9 mg P/ g VSS hr while the anoxic P uptake rate was much lower with 6 mg P/g VSS hr. The denitrification rate was calculated as 4.2 mg N/ gVSS hr. The aerobic P uptake rate was similar to the literature value, however, the anoxic p uptake rate and denitrification rate were lower than the literature findings that were obtained from the SBR system and they were also lower than the value from the seeded SBR reactor (same substrate was used in the feed, but SRT=10days). This low anoxic P uptake rate and denitrification rate suggested a low DNPAOs population in the system. One possible reason would be the low SRT. In this study SRT was only 6 days, while SRT longer than 10 days was widely used in the literature studies. Since DNPAOs have a slow growth rate (cell yield) (Kuba et al., 1996) than PAOs, the low SRT would limit their growth in the system. It was suggested that the effect of SRT on DNPAOs growth should be investigated.

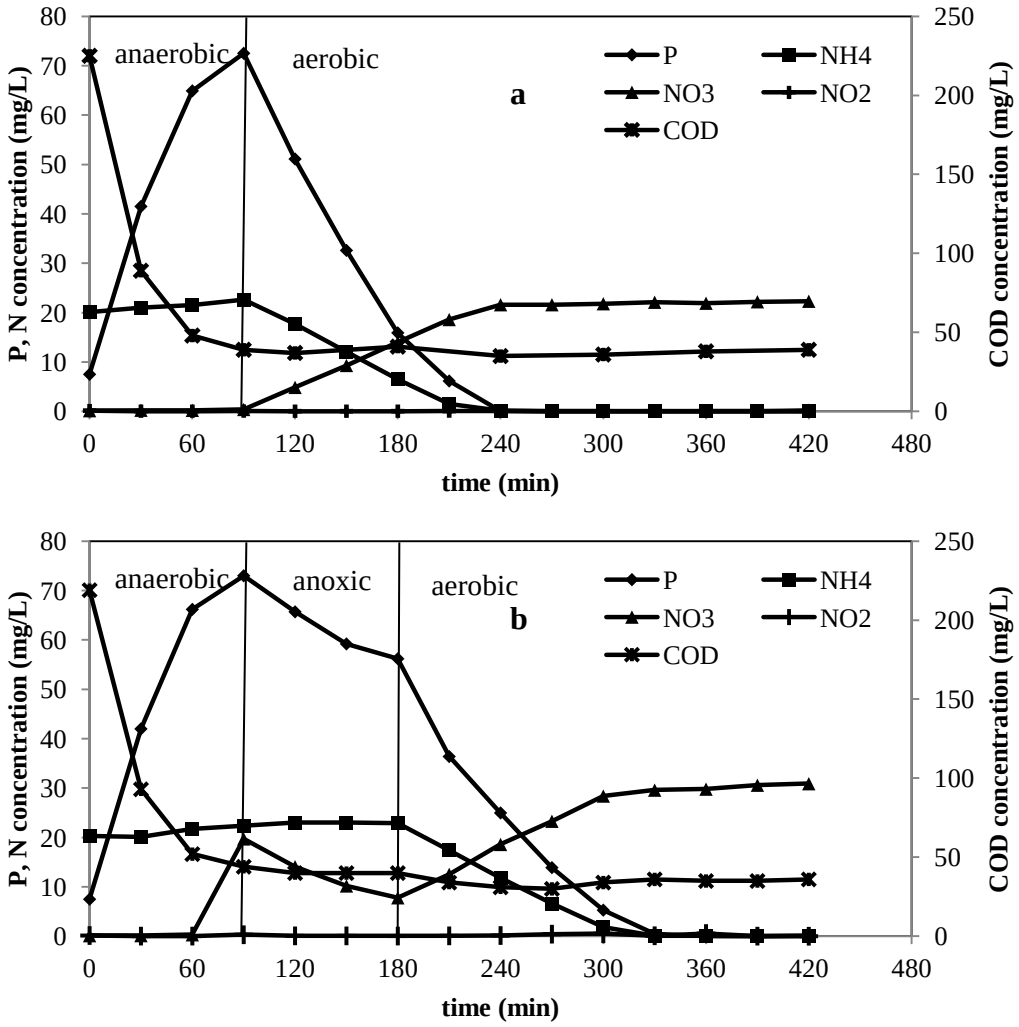

Figure 11-4Kinetic study of N and P profile in phase 1. (a) A/O configuration with 90 min of anaerobic phase and 330 min of aeration; (b) A2/O configuration, with 90 min of anaerobic phase, followed by 90 min of anoxic condition which was achieved by addition of NO_3. The aeration lasted 150 min.

Wachtmeister et al (1999) proposed a method to estimate the DNPAOs fraction of total PAOs based on the phosphate uptake rate under different conditions. The equation Wachtmeister proposed is shown below:

$$\frac{\text{XDPAO}}{\text{XPAO}} = \frac{\text{Panoxic}}{\text{Paerobic}} * \eta G$$

(ηG is a reduction factor for the lower energy gain in the anoxic condition compared to the aerobic energy gain, a typical value of 0.8 was used in this experiment)

The DNPAOs fraction was calculated as 27% of the total PAOs in the continuous flow reactors. It was lower than the SBR seeding reactor which was 38% (Yuan, 2010). One reason of for decreased DNPAOs fraction in the continuous flow reactor again can be explained by the shorter SRT. The SBR was operated at a longer SRT of10 days.

11.3.2 Phase 2- The failure of EBPR performance

11.3.2.1 Anaerobic P release

In order to examine the impact of the degas device on the anoxic reactor and on the denitrification activity, in phase 2, the degas device was removed from the system. It was observed that the P removal started to deteriorate after one week. After 3 SRT, the anaerobic P release became very unstable with the P concentration in the anaerobic reactor in the range from 49 to 13 mg/L and gradually decreased. The average concentrate of P in the anaerobic reactor decreased from 43 mg/L in phase 1 to 31 mg/L in phase 2 (Fig 11-2). The P release in the anaerobic reactor was 1468 mg/d which was about 35% lower than the phase 1 (Fig. 11-5). However, no significant difference in anaerobic COD removal was observed. Approximately 82% of COD in the feed was removed in the anaerobic reactor in both phases. It was speculated the growth of the GAOs population which compete with PAOs for carbon source.

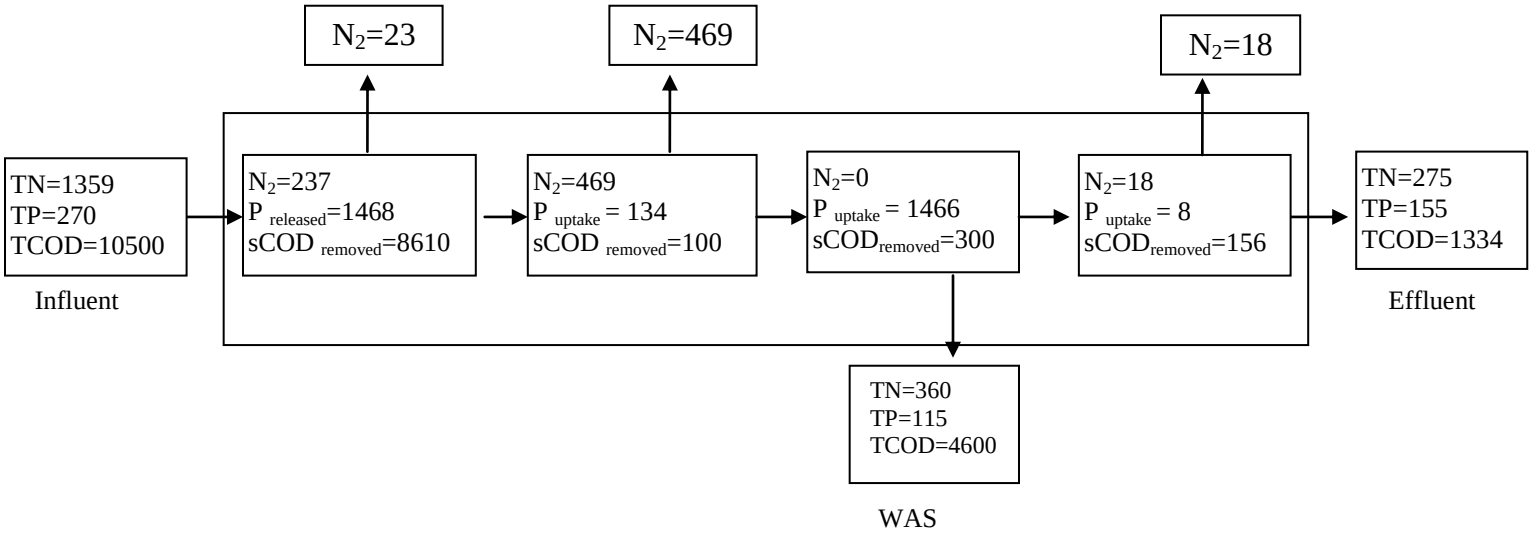

Figure 11-5N, P and COD flow (mg/d) in the continuous flow system in phase 2

The kinetic study (Fig. 11-6) of anaerobic P release in phase 2 showed that sharp P release occurred only in the first 30 min. with the release rate of 23 mg P/gVSS hr. however, in the following 30 min the P release rate significantly slowed down, almost no P release occurred after 60 min. While in phase 1, P release was in a constant rate during the first 60 min with the release rate of 27 mg P/gVSS hr and slowed down in the following 30 min. In terms of COD anaerobic removal, there was no significant difference observed between phase 1 and phase 2. The majority of COD was removed in 60 min. with a faster removal rate in the first 30 min. The acetate was fully removed in the first 30 min. (data was not shown). P release to VFA uptake ratio (P/VFA, P-mol/C-mol) reflects the growth of GAOs: the increase GAOs population will result in a decrease P/VFA ratio. In phase 2, the P/VFA ratio was found to be approximately 0.3 which was considerably lower than 0.5 from phase 1, this again suggested the proliferation of GAOs in phase 2.

In order to verify the hypothesis of the growing population of GAOs, the glycogen analysis was carried out. Due to the fact that the glycogen analysis data was not available from phase 1, glycogen analysis data of the biomass from the seeding SBR was used instead. The profile of glycogen in the SBR could be used as background information for the stage of the system start-up. It was assumed that the population of GAOs did not develop during phase 1 since the P/VFA ratio was not lower but slightly higher than the SBR. The glycogen activity can be reflected by the anaerobic glycogen hydrolysis value (C mol/C mol of acetate uptake). Since GAOs use glycogen as their sole energy source, with comparison to PAOs they tend to consume more glycogen at a given level of acetate uptake (Filipe et al., 2001). Therefore, the high level of a glycogen hydrolysis value will indicate presence of high level of GAOs activities. The value of 0.5 was used in the acetate PAOs model proposed by Smolder et al (1994) and 1.1 was used in the acetate GAOs model proposed by Zeng et al. (2002). For the SBR this value was calculated as 0.55 which was very close to the value used in the PAOs model. The value from the continuous flow reactor was calculated as 0.8 suggested of the relatively high activities of GAOs. The increase of anaerobic glycogen hydrolysis value in phase 2 indicated the development of the GAOs population in the continuous flow reactor.

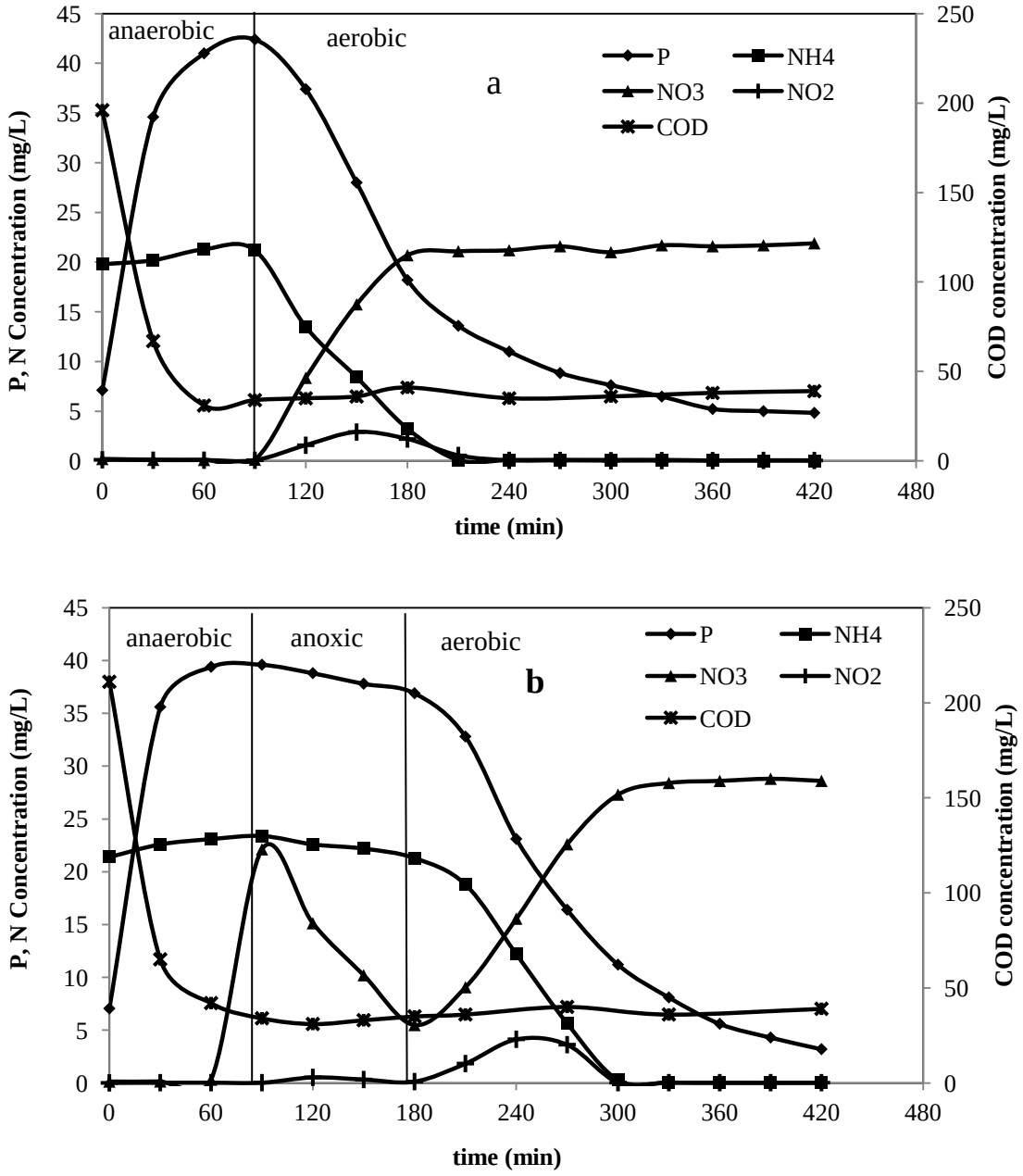

Figure 11-6Kinetic study of N and P profile in phase 2. (a) A/O configuration with 90 min of anaerobic phase and 330 min of aeration; (b) A2/O configuration, with 90 min of anaerobic phase, followed by 90 min of anoxic condition which was achieved by addition of NO3. The aeration lasted 150 min.

11.3.2.2 Anoxic P uptake

In phase 2, when the degas device was taken out of the line, in the anoxic reactor, the average DO was increased from 0.01±0.01 mg/L in phase 1 to 0.1±0.02 mg/L. The increased DO could be the result of the carryover of DO from the internal recycle. It was observed that P uptake was decreased significantly from 577 mg/d in phase 1 to 134 mg/d in phase 2. The decrease of the anoxic P uptake indicated the decrease of the DNPAOs population. Zeng et al (2003a) suggested that PAOs and DNPAOs were the same microorganisms since they found that the population enriched under Anaerobic/aerobic condition were capable of using nitrate after only a few hours to induce the denitrifying enzymes. If this is the case, then the presence of DO (in this experiment 0.1+0.01 mg/L) in the anoxic reactor may hinder the inducing of the denitrifying enzymes, therefore hindering the performance of the anoxic P uptake. Kinetic study (Fig 11-6b) also showed that much less anoxic P uptake occurred compared with phase 1 (4 vs 23 mg/L). The decreased anoxic P uptake indicated the decreased population of DNPAOs in phase 2. Using the above method proposed by Wachtmerister et al (1999) of estimating the DNPAOs fraction, it was calculated that the DNPAO fraction decreased to only 10%. It was expected the decreasing DNPAO population would result in a decrease of nitrate removal accordingly. In contrast, the nitrate removal was increased from 387 mg/d in phase 1 to 467 mg/d in phase 2 with no further COD removal (Fig.11-5). The nitrate concentration in the anoxic reactor was approximate 1.7 mg/L lower than the value that was obtained in phase 1 (Fig. 11-7). This observation suggested that there were a group microorganisms which could denitrify without using external carbon in the anoxic condition. The study of Zeng, et al. (2003b) demonstrated the existence of denitrifying

GAOs (DGAOs) in an Anaerobic/Anoxic activated sludge system. It was reported in their study that the stoichiometry and kinetic of DGAOs during the anaerobic stage was the same as GAOs. Both of them can take up acetate anaerobically and convert it to PHA by using glycogen as an energy source and reducing power. However, DGAOs would be able to use nitrate or nitrite as an electron acceptor to oxidize PHA to replenish their glycogen pull and grow anoxically, while GAOs can only grow aerobically. The observation in phase 2 was consistent with the characteristic of DGAOs as reported literature. Therefore, it was hypothesised the development of DGAOs occurred in the system in phase 2. The mechanism of the thriving DGAOs was not understood yet. Since the only operating condition difference between phase 1 and 2 was the anoxic DO concentration, it was hypothesised that the increased DO (in this experiment 0.1±0.02 mg/L) may favour DGAOs in the competition with DNPAOs in the anoxic reactor. However, further study should be carried out to investigate the effect of DO on the competition between DGAOs and DNPAOs.

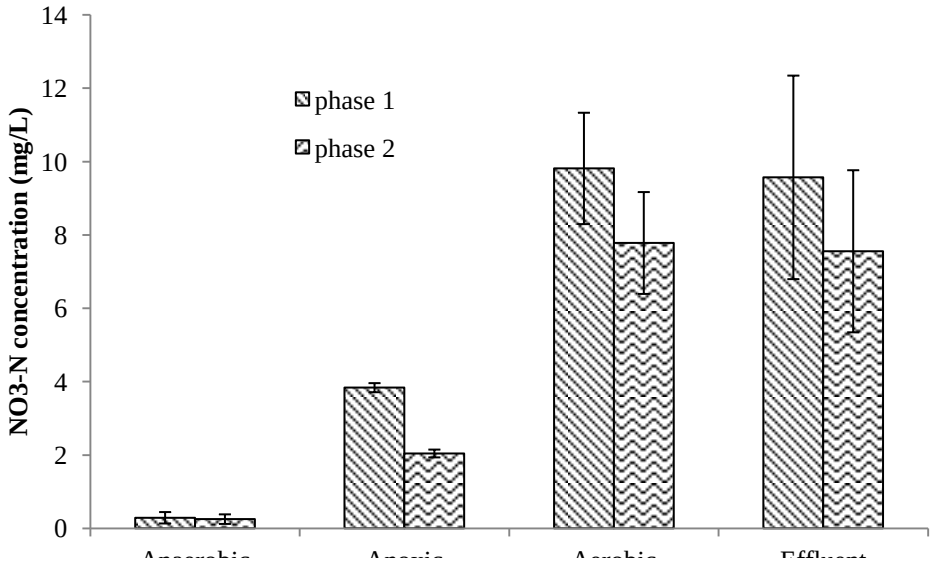

Figure 11-7Nitrate concentration at different points in phase 1 and 2

Another interesting observation was that, in phase 2 both aerobic and anoxic P uptake was significantly slowed down compared with phase 1. In phase 1, fast aerobic P uptake was observed. PAOs/DNPAOs in the system were able to uptake 72.5 mg/L P in only 2.5 hr, while in phase 2, with similar biomass concentration, only 40 mg/L P was removed during a 5.5 hr aeration period and the P uptake rate significantly slowed down after 2 hr aeration. The study of Petersen et al. (1998) found that the aerobic p-uptake rate was dependent on the available PHB that were present in the biomass. Therefore they suggested that it was important to couple the PHB degradation with P-uptake and not to waste the PHB by pure oxidation. In the current experiment, the low aerobic P-uptake rate can be caused by the low PHB content in the PAOs/DNPAOs biomass. This was due to the competition for carbon source with GAOs/DGAOs. Again, this demonstrated that the presence of GAOs/DGAOs would cause the deterioration of EBPR performance.

In this present experiment, it is demonstrated that the elimination of DO in the anoxic reactor is crucial for the growth and sustenance of DNPAOs in the system. The removal of DO in the recycle stream is achieved by nitrogen stripping. This method is somewhat impractical in a full-scale application. A vacuum method is used widely, on the other hand, to strip the gas in full-scale applications. It is suggested, therefore, that a vacuum chamber could be a practical solution.

11.4 CONCLUSION

This experiment demonstrated that DNPAOs can be sustained in a biological nutrient removal (BNR) process, in a one–biomass system with continuous flow. By means of

degas, the dissolved oxygen in the nitrate recycle stream was effectively decreased from 0.1 ± 0.01 to 0.01 ± 0.01 mg/L. This provided DNPAOs favourable conditions to grow anoxically. It was found that after the degas device was taken out from the system, the DO concentration in the anoxic reactor increased from 0.01 ± 0.01 to 0.1 ± 0.02 mg/L, and the proliferation of DGAOs population occurred. The EBPR process has deteriorated due to the increased population of DGAOs/GAOs which competed for the carbon source with DNPAOs/PAOs. The mechanism of DO influence on the growth of DNPAOs and DGAOs should be further studied.

Chapter 12 **Appendix C**

LOW TEMPERATURE BIOLOGICAL PHOSPHORUS REMOVAL AND PARTIAL NITRIFICATION IN A PILOT SBR SYSTEM*

12.1 INTRODUCTION

Eutrophication caused by nitrogen and phosphorus discharge into water bodies is becoming a global issue. Many wastewater treatment plants around the world are mandated to remove nutrients to meet discharge permits and there is a trend toward more stringent discharge limitations. In case of domestic wastewater, biological removal is the least expensive for both nitrogen and phosphorus removal. The organic carbon necessary for simultaneous nitrogen and phosphorus removal is typically lacking. Finding the solution for this carbon-limitation issue has become an important research objective. The research approach has been focused on the process that: 1) maximizes the carbon generation from the internal carbon source, 2) minimizes the usage of carbon source. The first approach for solving carbon deficiency is commonly used for enhanced biological phosphorus removal (EBPR) process. Volatile fatty acids (VFA) are the key substrate for phosphorus accumulating organisms (PAOs). Domestic wastewater typically lacks sufficient natural VFA (Barajas *et al*., 2002) to achieve efficient EBPR performance.

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VFA generation from internal sludge (primary and waste activated sludge) fermentation are the most common and sustainable methods and have been practiced worldwide.

The second approach aims to solve the carbon limitation issue of domestic wastewater by establishing a nitritation-denitritation sequence. One of the important advantages of nitritation-denitritation is considerable saving in the amount of carbon required for denitrification. The key to successful partial nitrification is that nitrite oxidizing bacteria (NOB) are washed out while ammonia oxidizing bacteria (AOB) are allowed to grow in the system. Several process parameters, such as dissolved oxygen (DO) concentration, temperature, solids retention time (SRT), substrate concentration, have been found to selectively affect the growth of NOB with respect to AOB. Up to now, most research focused on partial nitrification alone while overlooking biological phosphorus removal. Several researches concluded that the nitrite produced from partial nitrification will significantly inhibit P uptake which will have negative impact on the overall phosphorus removal (Sin, et al., 2008).

The objective of this study was to 1) examine the performance of phosphorus removal in a partial nitrification process at low temperatures, 2) investigate the operational conditions which inhibit the growth of NOB.

12.2 METHODOLOGY

12.2.1 The SBR set-up

Two identical 60 L pilot SBRs were set up at South End Wastewater Pollution Control Centre (SEWPCC) in Winnipeg, Manitoba. The SEWPCC is a carbon-removal only, high-purity oxygen activated sludge facility. The SBR were operated for both ammonia and phosphorus removal following an anaerobic/aerobic reaction sequence. The volumetric exchange rate (VER) was 0.66, which corresponds to 40 L of the filling volume in each cycle. The SBR had 3 cycles per day and a hydraulic retention time (HRT) of 12 hours. Each SBR cycle started with 20 min feeding, followed by 120 min of anaerobic phase, 240 min of aerobic, 60 min of settling and 30 min of decant. There were about 10 min of idle period between of each cycle. Compressed air pump was used to supply oxygen during the aerobic phase. The dissolved oxygen (DO) was not controlled but was regularly monitored to ensure the DO concentration was above 3 mg O_2/L. The excess sludge of 2.5 L was wasted at the end of aerobic phase of each cycle to control the SRT at 8 days.

The plant primary effluent used as feed is shown in Table 1. The reactors were operated at the temperature of the primary settling chamber which was controlled at 13-15 ℃. The reactors have operated for 8 months.

Table 12-1Primary effluent characteristics (average* with standard deviations)

TSS (mg/L)	97 ±48	TCOD (mg/L)	451 ±126
VSS (mg/L)	79 ±40	sCOD (mg/L)	214 ±57
NH_4-N (mg/L)	30.5 ±7.4	VFA (mg/L)	72 ±47
PO_4-P (mg/L)	4.9 ±1.5	pH	7.9 ±0.2

- 138 samples were used for Nitrogen and Phosphorus analysis and 48 samples were used for the rest.

For the kinetic study of the SBR, initial sample was taken at time 0 when the cycle started, and then samples were taken every 30 minutes until the end of the cycle. Temperature, DO and pH were measured directly from the reactor in the same time interval. Samples were measured for different parameters, such as PO_4-P, NH_3-N, NO_3-N, NO_2-N, sCOD, etc.

12.2.2 The effect of nitrite on anaerobic phosphorus release and aerobic phosphorus uptake batch test

The batch tests were performed in 1.5 L glass reactors. The biomass used for the tests was obtained from the pilot reactor. The batch test reactors were operated under anaerobic and aerobic configuration. The anaerobic periods were 90 minutes. The aerobic time was four hours. Different dosages of NO_2 were added at the beginning of the cycle to study the effect of nitrite on anaerobic P release and aerobic P uptake. Samples for the

anaerobic P release study were taken every 10 min and the samples for aerobic P-uptake study were taken every 30 min.

12.2.3 Microbiological analysis

To quantify the nitrifiers present in the reactor, fluorescence in situ hybridisation (FISH) technique was used. Sample preparation and hybridization were performed as described by)Jubany et al. (2009). Two groups of AOB (*Nitrosospira* and *Nitrosomonas*) and NOB (*Nitrospira* and *Nitrobacter*) were selected for FISH analysis. Specific oligonucleotide probes targeted for 16SrRNA sequences for AOB- *Nitrosospira* and *Nitrosomonas* detection were Nsv 443 and Nsm 156 respectively. Specific probes for NOB- *Nitrospira* and *Nitrobacter* detection were Ntspa 662 and NIT 3 respectively. Samples were stained with 4′, 6-diamidino-2-phenylindole (DAPI) to mark all bacteria. Each sample was analyzed separately for AOB fraction determination and for NOB fraction determination. AOB or NOB fraction was quantified as a percentage of all bacteria using 40 randomly chosen images from each sample. The microscopy was performed using Nikon Microscope Eclipse E400 with camera Olympus DP70. Image processing was performed with specific software program developed by Jubany et al. (2009) which used Matlab® Image processing Toolbox.

12.2.4 Analytical Methods

Total suspended solids (TSS) and volatile suspended solids (VSS) measurements were performed according to Standard Methods (APHA, 1998). Hach COD digestion vials were used to measure COD. The samples were filtered through 0.45 μm membrane filter

(Millipore) for measuring soluble COD. Dissolved phosphate and ammonium were measured using a Lachat Instrument Quik Chem 8500, following the Quik Chem orthophosphate method 10-115-01-1-O, and Quik Chem ammonia method 10-107-06-1-I. The analysis of VFA composition was conducted by means of a Varian CP-3800 Gas Chromatograph using a flame ionization detector (FID) and HP-FFAP capillary column (inner diameter of 0.25mm and length of 25m). VFA concentration was converted to COD concentration by using the following conversion factors: 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric and isobutyric acid, 2.04 for valeric and isovaleric acid, 2.21 for caproic and iso-caproic acid and 2.34 for heptanoic acid.

12.3 RESULT AND DISCUSSION

12.3.1 Enhanced biological phosphorus removal EBPR

Excellent biological phosphorus removal was achieved in less than 30 days (Fig. 12-1a). Since the performances of both SBRs were quite similar, the result of one SBR is presented and discussed here for simplification. From mid of Jan to the end of May, the average P concentration in the effluent was approximately 0.13 mg/L and this gave P removal efficiency of 98%. The relatively high VFA concentration (average 72 mg/L) in the plant's primary effluent (pilot reactor's influent) has played a key role in the rapid achievement of phosphorus removal. Interestingly, VFA in the raw wastewater was measured as approximately 25 mg/L, while after primary settling tank, it increased to 72 mg/L. It was concluded that primary sludge was fermented in the primary settling tank which led to the unprecedented increase in the VFA concentration. From June to the beginning of August – the rainy season of Winnipeg, the City experienced several heavy

storms which resulted in high flows to the WWTP. The high flows diluted the nutrient concentration in the wastewater while VFA concentration in the primary effluent decreased to 15 mg/L. This was probably due to the decrease of the HRT in the primary settling tank. The low concentration of VFA resulted in the unstable P removal, therefore, several spikes of high P (>1 mg/L) concentration in the effluent were observed during this period. Another time when high P concentration was detected in the effluent was in April (Fig. 12-1) when snow melting occurred. The resulting high flow led to low VFA concentration in the primary effluent that consequently led to poor P removal.

In order to further understand the performance of the SBR, kinetic study of one reaction cycle was carried out and the results are shown in Fig. 12-2. It was calculated that the anaerobic P release rate was 8.4 mg P/g VSS h. The released P during anaerobic period was fully taken up by PAOs during the 3 h aeration, which resulted in aerobic phosphorus uptake rate of approximately 5.3 mg/ g VSS h. Both anaerobic P release and aerobic P uptake rate from the full scale plants in literature (Kuba et al., 1997a, b, Meinhold et al.,1999a, and López-Vázquez et al, 2008) showed large variability 6 to 20.9 and 2.2 to 19 mg/g VSS h, respectively. The results from the present study represent the SBR performance under one set of conditions of the local wastewater, with much less variability.

12.3.2 Nitrogen removal through partial nitrification

During the first month of operation, in order to select for PAOs, SBRs were operated with relatively short SRT of 5 days and the aerobic SRT_a of only 2.5 days. Nitrification

was not observed at this low SRT (Fig.12-1b). To achieve nitrification, the SRT of SBR was therefore, increased to 8 days and SRT_a increased to 4 d. Nitrification gradually occurred. After 5 d SRT_a, full nitrification was achieved. However, during the rest of the experimental period variation of effluent NH_4–N concentration was detected – ammonia was not always completely removed. The average NH_4–N removal rate was approximately 72%. The failure of operational facilities could be the most important factor that caused this unstable and poor nitrification. From March till the end of this experiment, we experienced several operational problems, such as 1) the failure of decant pump which resulted in the loss of biomass 2) decreased room temperature due to the annual power equipment maintenance 3) the failure of air pump which caused no aeration supply for - a few days, etc. Though these operational issues had exerted a negative impact on biological P removal, the P removal recovered within 1 or 2 days. The nitrifiers took much longer (1 to 4 SRT) to recover. This indicated that, compared to PAOs, the nitrifiers were more fragile, sensitive to the environmental conditions and slow growing.

Partial nitrification was observed throughout this experiment (Fig. 12-1c). The average nitrite concentration of approximately 11 mg/L and nitrate concentration of 0.64 mg/L was detected in the effluent. This indicated that under the system's operational conditions NOB were washed out.

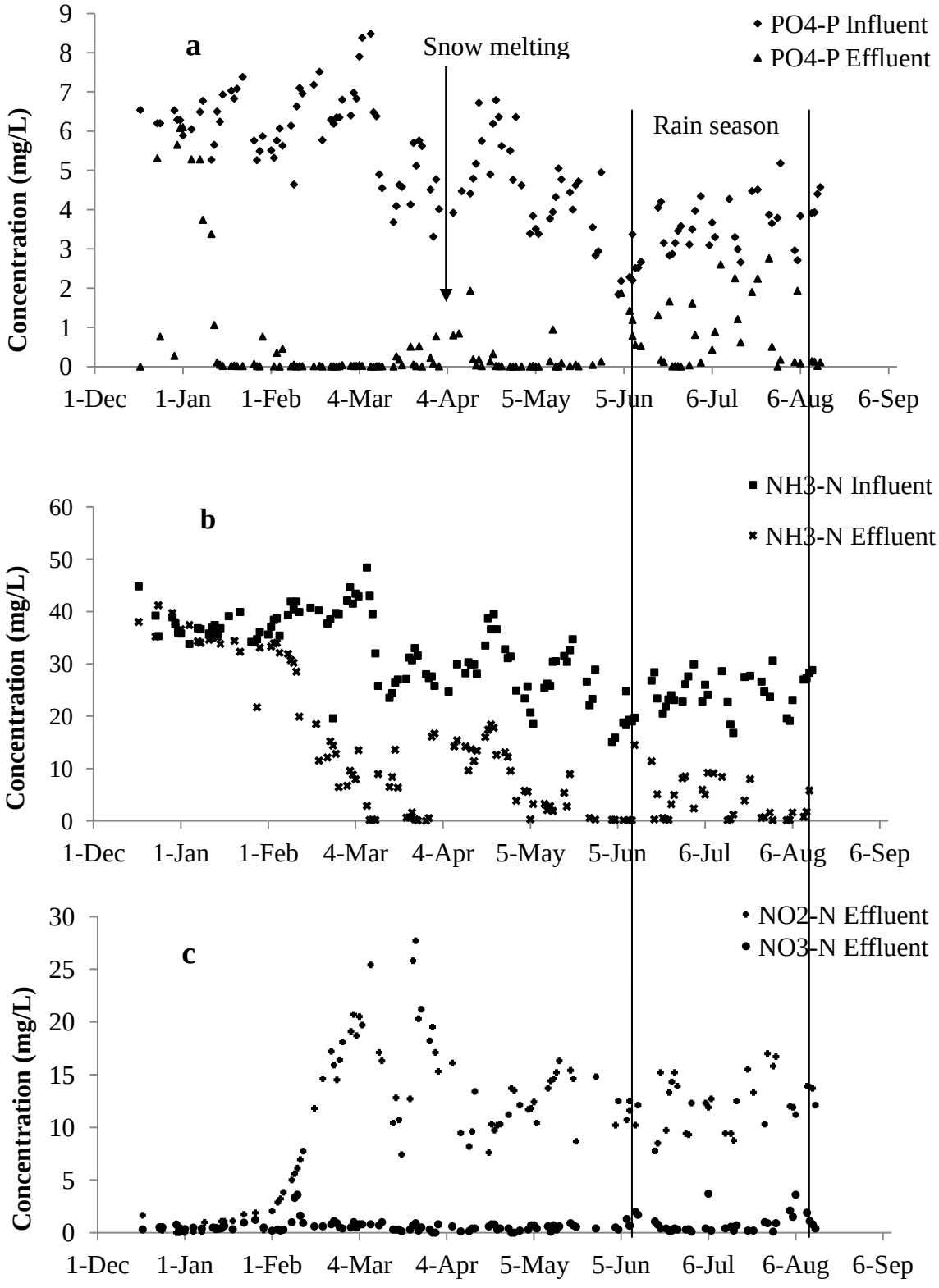

Figure 12-1 Influent and effluent N, P concentration during long term operation of the SBR

It is well accepted that low DO concentration is the key to selection of AOB over NOB since AOB have a stronger affinity to DO than NOB. In this experiment, the DO concentration in the reactor during the aerobic phase was measured above 3 mg/L. Nitritation occurred regardless of the high DO concentration. This observation demonstrated that the selection of AOB could be achieved under high DO concentration.

Kinetic studies (Fig. 12-2) were conducted to investigate the possible reasons for the wash-out of NOB (and AOB growth) at this relatively low average temperature of 15 ℃. Both DO and nitrite were not present when the aeration started. DO rapidly increased to 2 mg/L after 30' aeration eliminating the possibility of DO limitation for the growth. Nitrite, on the other hand, was almost undetectable after 30' aeration and slowly increased to only 1.9 mg/L after 60' aeration. This very low concentration of NO_2 in the first one hour aeration resulted in the substrate limitation for the growth of NOB at the beginning of the aerobic cycle. In other words, the effective aerobic SRT for NOB was approximately 1 hour less than the aerobic SRT. Whereas for AOB, there was no substrate limitation – average ammonia removal rate was 72%, and the effective SRT for AOB equalled to the aerobic SRT. Taking the solids in the effluent into consideration, the actual aerobic SRT was calculated as 3.6 days. Therefore, the SRT for AOB and NOB was 3.6 and 2.7 days, respectively.

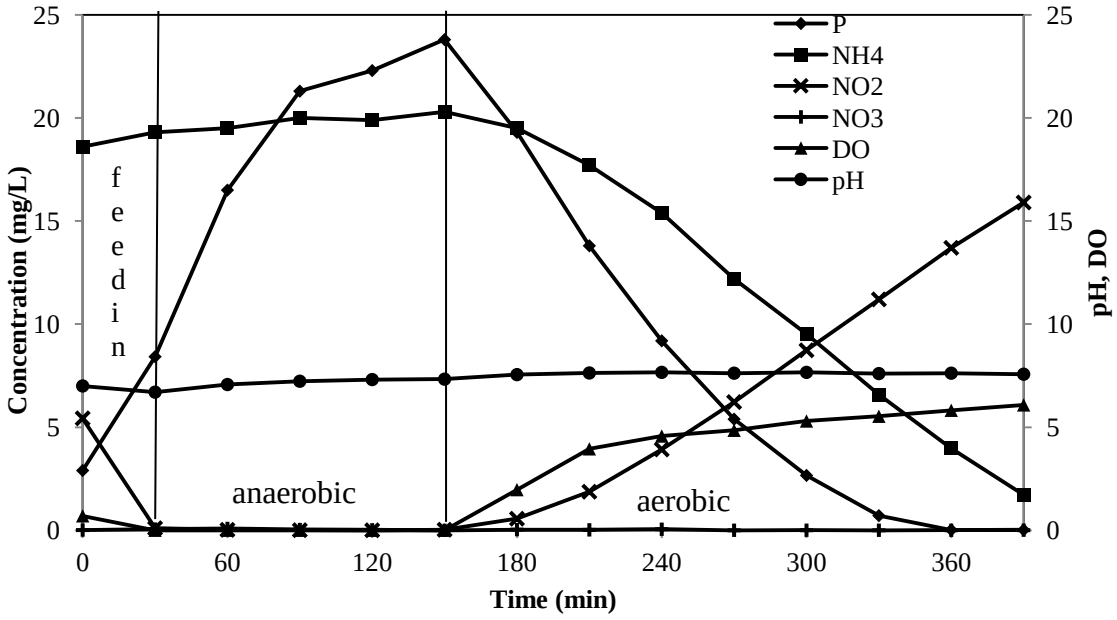

Figure 12-2 Reactor profile of one SBR reaction cycle

The default maximum growth rate for AOB and NOB at 20 ℃ used by Biowin (a modeling tool) is 0.9 and 0.7 d^{-1} respectively and the temperature coefficient –was 1.072 for AOB and 1.06 for NOB. The minimum SRT for AOB and NOB at 15 ℃ was therefore, calculated as 1.6 and 1.9 days, respectively. This suggests that at 15 ℃ the SRT required for NOB is longer than AOB. However, in this experiment, where the total SRT was 7.2 days, the SRT for AOB was longer than the NOB, which favoured the growth of the AOB. The short SRT of NOB could be the reason that caused the NOB wash-out from the reactor. The inhibition by free unionized ammonia (FA) was also been examined. The FA was in equilibrium with ionized form of ammonium and was dependent on the pH and temperature. Generally, FA is considered as toxic to microorganisms, due to its ease of diffusion through the membrane. Anthonisen (1976) reported that a possible FA inhibition threshold for AOB was higher than 10 mg N/L and for NOB was higher than 0.1-1.0 mg N/L. In this experiment, the average NH_4-N in the

reactor was lower than 20 mg N /L, which resulted in FA concentration often lower than 0.1 mg N/L under pH<7.5 at 15 ℃. This suggested that NOB in the reactor were not inhibited by FA.

Taking these factors into account, it was concluded that in an SBR system at lower temperature, with high DO, nitrogen removal can be achieved by partial nitrification. In a continuous system it was usually believed that nitritation will occur only with oxygen limitation (Park & Bae, 2009).

The operational parameters were also examined by using the default values of the kinetic and stoichiometric parameters from the Biowin modeling tool (graph not shown). The model fitted our results quite well under their default values of maximum growth rate and temperature coefficient. Based on a Biowin modeling exercise NOBs had lower maximum specific growth rate than AOB also at temperatures below 20 ℃, which was not in agreement with some literature studies (Beline et al., 2007). It is necessary to further study the specific growth rate of AOB and NOB associated with temperature to verify this assumption.

The microscopic (FISH) result showed that AOB in the system were mainly Nitrosomonas spp. which was approximately 1.7% of the total biomass. No noticeable NOB population was observed - which was consistent with the result, i. e the very low concentration of NO_3 detected in the effluent.

12.3.3 Effect of nitrite on P removal

Further batch test was conducted on the effect of nitrite on anaerobic P release and aerobic phosphorus uptake. The control reactor had 0 nitrite, while the other reactors had

different concentration of nitrite in the system when anaerobic and aerobic phase started. The C/P ratio of 11 was used in this test which was much lower than that of the pilot reactor. The C/P ratios in the pilot reactors were in the range of 25-50. The purpose of using such low C/P ratio was to examine the impact of nitrite on anaerobic P release under the lower carbon condition.

For all the reactors with nitrite addition, approximately 7-9 mg/L of NO_2-N was removed during 90 min anaerobic period with COD removal of 70-77 mg/L. Control reactor, where no nitrite was added, lesser COD removal (56 mg/L) was observed. It is well known that nitrate has negative impact on anaerobic phase release due to denitrifiers competing with PAOs for carbon source. Since denitritation, the second step of denitrification, also requires carbon, therefore, it is reasonable to assume that nitrite may also have negative impact on anaerobic phase release. Consequently, it is logical to assume that with similar nitrite addition dosage, nitrite addition at time 0 should have resulted in lower P release rate than addition at 30 min. However, the result obtained from this experiment showed nitrite addition time did not make significant difference in P release rate (Table 12-2).

Table 12-2Nitrite concentration vs. anaerobic P-release and aerobic P-uptake rate

Nitrite (mg N/L)	Anaerobic P release rate (mg P/g VSS ħ)	Nitrite (mg N/L)	Aerobic P uptake rate (mg P/g VSS ħ)
0.0 (control)	8.8	0	3.9
8.0 (addition at 0')	8.2	1.5	3.5
8.3 (addition at 30')	7.8	6.4	3.07
15.4 (addition at 0')	7.7	10.2	2.72
16.9 (addition at 30')	7.1		

It was observed that in all reactors P-release occurred immediately after feeding with a constant rate regardless of nitrite addition. In both reactors with nitrite addition at time 0, a lag phase of nitrite removal was observed - no denitritation occurred until 20 minutes after the feeding. This indicated that PAOs are strong competitors for the carbon source for the NO_2 removal microorganisms. Another interesting observation was that the most rapid of sCOD removal was observed in the first 20 minutes after feeding. Approximately 1/3 of sCOD removed during the anaerobic phase occurred in the first 20 minutes. No significantly increased sCOD removal rate was observed when NO_2 removal started.

Our previous study (Yuan and Oleszkiewicz, 2010) showed that with NO_3 addition at time 0, denitrification occurred immediately after feeding with significant decrease of P release rate. This was not the case in this experiment. It was therefore, hypothesised that converting NO_3 to NO_2 was the key step responsible for denitrifiers successfully

outcompeting PAOs for carbon source. This experiment demonstrated that the presence of NO_2 in the influent did not have significant impact on the anaerobic P release.

In terms of aerobic P uptake, it was observed that aerobic P uptake rate was decreased with the increased nitrite concentration (Table 12-2). At the highest nitrite concentration of 10.2 mg/L, the aerobic P uptake rate was approximately 30% lower than of that of control reactor. This observation agreed with findings of other researchers where nitrite inhibited the aerobic P uptake rate. Sin, et al. (2008) reported that the aerobic P-uptake of the biomass from SBR was inhibited by 65% at nitrite concentration of 10 mg N/L. Meinhold et al. (1999 b) observed that when biomass exposed to higher concentration of NO_2 levels (roughly equal to or higher than 8 mg N/L) the aerobic P uptake was inhibited severely. Similar observation was reported by Saito et al. (2004). However, the present experiment demonstrated that the degree of inhibition due to the presence of nitrite in the aerobic phase is not as significant as was reported in the literature. This difference of the observation might be due to the difference of the treatment process, wastewater characteristics and biomass properties. It was concluded the aerobic P uptake by PAOs was more sensitive to nitrite than anaerobic P-release.

12.4 CONCLUSION

Biological phosphorus removal and partial nitrification (nitritation) process was achieved in an SBR system operated at low temperature (15 ^{o}C), for 8 months on primary effluent. Sufficient supply of VFA was the key to successful phosphorus release and uptake. The nitritation was achieved at dissolved oxygen above 3 mg O_2/L. The NO_2-N substrate limitation at the beginning of the aerobic cycle resulted in the shorter SRT for the NOB.

This in turn could have been the primary factor that hindered the growth of NOB. The presence of nitrite hindered the aerobic P-uptake by PAOs. The aerobic P-uptake was more sensitive to nitrite than the process of anaerobic P-release.

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