**İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY**

# METABOLIC AND MATHEMATICAL MODELLING OF PHOSPHATE AND GLYCOGEN ACCUMULATING ORGANISMS BEHAVIOUR IN ENHANCED BIOLOGICAL PHOSPHATE REMOVAL SYSTEMS

**Ph.D. Thesis by**

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## NOMENCULATURE

| | |
|---|---|
| **3H2MV** | : 3-Hydroxy-2-Methylvalerate |
| **3HB** | : 3-Mydroxybutyrate |
| **3HV** | : 3-Mydroxyvalerate |
| **Ac** | : Acetate |
| **ASM1** | : Activated Sludge Model No.1 |
| **ASM2** | : Activated Sludge Model No.2 |
| **ASM2d** | : Activated Sludge Model No.2d |
| **ATP** | : Adenosine Tri Phosphate |
| **BOD** | : Biochemical Oxygen Demand |
| **CoA or CoASH** | : Coenzyme A |
| **COD** | : Chemical Oxygen Demand |
| **EBPR** | : Enhanced Biological Phosphorus Removal |
| **ED** | : Entner-Doudoroff |
| **EMP** | : Embden-Meyerhoff-Parnas |
| **FAD** | : Flavin Adenine Dinucleotide, Oxidized Form |
| **$FADH_2$** | : Flavin Adenine Dinucleotide, Reduced Form |
| **FID** | : Flame Ionization Detector |
| **GAO** | : Glycogen Accumulating Organism |
| **Gly** | : Glycogen |
| **Hac** | : Acetic Acid |
| **HRT** | : Hydraulic Retention Time |
| **MCRT** | : Mean Cell Residence Time |
| **MLSS** | : Mixed Liquor Suspended Solids |
| **MLVSS** | : Mixed Liquor Suspended Solids |
| **MLVSS** | : Mixed Liquor Volatile Suspended Solids |
| **$NAD^+$** | : Nicotinamide Adenine Dinucleotide, Oxidized Form |
| **NADH** | : Nicotinamide Adenine Dinucleotide, Reduced Form |
| **P** | : Phosphorus. |
| **PAO** | : Polyphosphate Accumulating organism |
| **PHA** | : Polyhydroxyalkanoates |
| **$PHA_{stored}$** | : Stored Polyhydroxyalkanoates |
| **PHB** | : Polyhydroxybutyrate |
| **$pH_{out}$** | : External pH |
| **PHV** | : Polyhydroxyvalerate |
| **$P_i$** | : Orthophosphate Ion |
| **PMF** | : Proton Motive Force |
| **Poly-P** | : Pyrophosphate |
| **$P_{rel}$** | : Phosphate Release |
| **$P_{upt}$** | : Phosphate Uptake |
| **SBR** | : Sequencing Batch Reactor |
| **SCFA** | : Short Chain Fatty Acids |
| **SRT** | : Solids Retention Time |

| | |
|---|---|
| **SS** | : Suspended Solids |
| **TCA** | : Tricarboxylicacid |
| **TP** | : Total Phosphorus |
| **UCT** | : University of Cape Town |
| **VFA** | : Volatile Fatty Acid |
| **$VFA_{upt}$** | : Volatile Fatty Acid Uptake |
| **VSS** | : Volatile Suspended Solids |

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# BİYOLOJİK AŞIRI FOSFOR GİDEREN SİSTEMLERDE FOSFOR VE GLİKOJEN DEPOLAYAN ORGANİZMALARIN METABOLİK VE MATEMATİK MODELLENMESİ

## ÖZET

Bu çalışmada, asetat ve propiyonat ile beslenen iki ardışık kesikli reaktörden elde edilen veriler, değişken atıksu fosfor/uçucu yağ asidi oranlarında farklı karbon kaynaklarının biyolojik aşırı fosfor giderimi ve depolama ürünleri üzerine etkisinin araştırılması amacıyla kullanılmıştır. Buna göre, fosfor salım/alım oranının karbon kaynağından bağımsız olarak 1.2-1.3 arasında olduğu bulunmuştur. Ayrıca, biyokütle içinde depolanan polihidroksialkanoat (PHA) kompozisyonunun uçucu yağ asidi tipine bağlı olarak değiştiği belirlenmiştir. Asetat için PHA'nın polihidroksibütirat (PHB) içeriği yüksek iken propiyonat için bu oranın daha düşük olduğu bulunmuştur. Farklı propiyonat oranlarında yürütülen kesikli deney sonuçları, asetata alışık biyokütlenin aklimasyon periyoduna ihtiyacı olacağını göstermiştir.

Bu çalışmada, fosfor depolayan organizma (PAO) ve glikojen depolayan organizma (GAO) karışık kültürünün anaerobik koşullarda asetat alımı için bir metabolik model önerilmiştir. Bu model, FDO'ların PHA sentezini gerçekleştirmek üzere gerekli indirgeme kuvvetlerini glioksilat metabolik yolunu kullanarak elde edebilecekleri esasına dayanmaktadır. Bu nedenle, model değişken bir stokiyometriye sahiptir. Önerilen model deneysel sonuçlar ile test edilmiş ve doğrulanmıştır. Deneysel veriler, biyolojik aşırı fosfor giderimini (BAFG) gerçekleştirmek üzere asetatın karbon kaynağı olarak kullanıldığı farklı giriş asetat/fosfor oranlarında işletilen bir ardışık kesikli reaktörden elde edilmiştir. Elde edilen deneysel veriler, tüm HAc/P oranlarında, özellikle fosfor kısıtlı koşullarda, GAO'ların varlığını öngören modelin geçerliliğini desteklemiştir. PHB üretimi, PHA kompozisyonu, glikojen tüketimi ve fosfor salımı parametreleri açısından deneysel değerler ve model öngörülerinin uyumlu oldukları gözlenmiştir.

Bu çalışmada, Aktif Çamur Modeli No.2d'nin öngörü kabiliyeti, değişken giriş fosfor yüklerinde işletilen bir ardışık kesikli biyolojik reaktörün (AKR) biyolojik aşırı fosfor giderim verimi açısından değerlendirilmiştir. Bu amaçla, esas karbon kaynağı olarak asetat içeren sentetik atıksu ile beslenen bir laboratuvar ölçekli AKR'den elde edilen veriler kullanılmıştır. Giriş atıksuyunda farklı fosfor/asetat oranlarına sahip dört farklı deney seti olarak gerçekleştirilen deneylerin sonuçları değerlendirilmiştir. Model, kararlı denge halinde temsil edici bir çevrim süresince ölçülen konsantrasyon profilleri esas alınarak değerlendirilmiştir. Hassasiyet analizleri esas alınarak bir iteratif kalibrasyon metodolojisi geliştirilmiştir. Bu metodoloji, dört farklı deney setinde, AKR verimini etkileyen model parametrelerine uygulanmıştır.

Bu çalışmada, ASM2d'nin, AKR'nin kararlı denge davranışını farklı model katsayı setleri ile kalibre edildiğinde belirleyebildiği ortaya konmuştur. Katsayıların düzenli değişken düzeni, AKR sistemde anaerobic koşullarda glikojenin bozunması ile elde ettiği enerjiyi kullanarak substrat depolayan, böylece düşük P/Ac oranlarında sistemde hakim olabilen glikojen depolayan organizmaların (GAO) AKR sisteminde varlığını açıklamak üzere kullanılmıştır.

Elde edilen sonuçlar, sistemde asetat ($S_A$) ve polihidroksialkanoat ($X_{PHA}$) profillerinin daha doğru öngörü ile elde edilebilmesi için glikojen ve GAO'ların model bileşenleri olarak PAO ve GAO proseslerine ilave edilmesi gerektiğini göstermiştir.

Ayrıca, biyolojik aşırı fosfor gideriminin modellenmesi, asetat için rekabet eden glikojen ve fosfor depolayan organizmaların mekanistik ifadesi ve daha sonra glikojen metabolizmasının modele ilave edilmesi ile geliştirilmiştir. Önceden geliştirilen metabolik kavram ve modellere dayanılarak karışık kültürler için yeni bir proses stokiyometrisi tanımlanmıştır. Önerilen model, asetat beslemesi ve P/Ac oranının 0.05 mgP/mgKOİ olması durumunda elde edilen deneysel verilerin test edilmesi ve kalibrasyonu için kullanılmıştır. Kalibrasyonun, test edilen tüm model katsayıları için kabul edilebilir sayısal sonuçlar verdiği ve proses stokiyometrisi açısından seçilen metabolik ilişkilerin geçerliliğini desteklediği görülmüştür. Ayrıca, proses davranışı ve verimi üzerine önemli etkisi olan glikojen depolayabilen organizmaların sistemde sağlanabilmesi için gerekli koşullar belirlenmiştir.

Ayrıca bu çalışmada, glikojen ve fosfor depolayan organizmalar arasındaki rekabet üzerine pH ve substratın etkisi değerlendirilmiştir. Bu amaçla, biyolojik aşırı fosfor giderimi yapan, farklı giriş asetat/fosfor oranlarına sahip ve karbon kaynağı olarak sadece asetat ile asetat ilave edilmiş evsel atıksu kullanılan bir ardışık kesikli reaktörden elde edilen veriler kullanılmıştır. Farklı giriş asetat/fosfor oranlarına sahip asetat ilaveli evsel atıksu kullanılarak, asetat ve evsel atıksu karışımının farklı başlangıç pH değerleri için kesikli deneyler yürütülmüştür. Elde edilen sonuçlar, tüm HAc/P oranlarında, özellikle asetatın karbon kaynağı olarak kullanıldığı fosfor kısıtlı koşullarda, GAO'ların varlığını destekler niteliktedir. Substrat alımı, glikojen depolaması ve fosfor salımı açısından asetat ilaveli atıksu sisteminde PAO oranının esas karbon kaynağını sadece asetat olduğu sisteme göre daha yüksek olduğu gözlenmiştir.

## METABOLIC AND MATHEMATICAL MODELLING OF PHOSPHATE AND GLYCOGEN ACCUMULATING ORGANISMS BEHAVIOUR IN ENHANCED BIOLOGICAL PHOSPHATE REMOVAL SYSTEMS


### SUMMARY

In this study, data obtained from two sequencing batch reactors fed with acetate and propionate was served to investigate effect of different carbonaceous substrates on biological phosphorus removal (EBPR) and on storage polymers whereas influent P/volatile fatty acid (VFA) ratios were changed. It is found that phosphate release and uptake values were ranged from 1.2 to 1.3 regardless of carbon source. It is determined that the composition of PHA stored in biomass vary depending on volatile fatty acid type. PHB fraction of PHA was higher for acetate, which was lower for propionate. The results of batch experiments with different fractions of propionate were implied that acetate enriched biomass need acclimation period.

In this study, a new metabolic model is proposed for acetate uptake by a mixed culture of phosphate and glycogen accumulating organisms (PAOs and GAOs) under anaerobic conditions. The model uses variable overall stoichiometry based on the assumption that PAOs may have the ability of using the glyoxylate pathway to produce the required reducing power for PHA synthesis.

The proposed model was tested and verified by experimental results. Experimental data were obtained from a sequencing batch reactor system operated for enhanced biological phosphorus removal (EBPR) with acetate as the sole carbon source at different influent acetate/phosphate (HAc/P) ratios. The resulting experimental data supported the validity of the proposed model, indicating the presence of GAOs for all tested HAc/P ratios, especially under P limiting conditions. Strong agreement is observed between experimental values and model predictions for all model components, namely PHB production, PHA composition, glycogen utilization and P release.

In this study, the prediction capability of Activated Sludge Model No.2d (ASM2d) is evaluated for the enhanced biological phosphorus removal (EBPR) performance of a sequencing batch reactor (SBR) receiving variable influent phosphate load. For this purpose, experimental data obtained from a laboratory-scale SBR operated with a synthetic feed containing acetate as the sole carbon source, was used. The results of experiments that conducted for four different Runs to ensure a range of different phosphate/acetate ratios in the influent were evaluated. Model evaluations were carried out on concentration profiles measured throughout a representative cycle at steady state. An iterative calibration methodology was developed based on sensitivity analysis and applied to four different sets of experimental data on relevant model parameters reflecting SBR performance.

ASM2d was not able to predict the steady-state behavior of the SBR system in this study, unless calibrated with a different set of model coefficients for each experimental Run. The regular changing pattern of the coefficients could be interpreted with the ability of the SBR system to sustain glycogen accumulating microorganisms, (GAOs), which can store substrate

under anaerobic conditions, without the polyphosphate energy but deriving energy from the degradation of glycogen, thus capable of prevailing at lower P/Ac ratios. The results indicate the need to include glycogen and GAOs as model components for processes involving both PAOs and GAOs, in order to obtain a better prediction of acetate ($S_A$) and polyhydroxyalkanoate ($X_{PHA}$) profiles in the system.

Besides, the modeling of enhanced biological phosphate removal is improved by introducing the mechanistic description of a mixed culture of glycogen and phosphate accumulating organisms competing for acetate, and glycogen metabolism of the latter. A new process stoichiometry is defined for the mixed culture based upon previously developed metabolic concepts and models.

The proposed model is tested with and used for the calibration of experimental data obtained from the Run having acetate and P/Ac ratio of 0.05 mgP/mgCOD in the influent. The calibration yielded acceptable numerical results for all the model coefficients tested and supported the validity of selected metabolic relationships for process stoichiometry. The defined conditions allowing the system to sustain glycogen accumulating organisms with significant impact on process behavior and performance are also defined.

In this study, effects of pH and substrate on the competition between glycogen accumulating (GAOs) and phosphorus accumulating organisms (PAOs) are evaluated. For this purpose, data obtained from a sequencing batch reactor system operated with acetate as the sole carbon source and acetate added domestic wastewater for EBPR at different influent acetate/phosphate ratios, was used.

Some batch tests were performed using acetate added domestic wastewater at different influent acetate/phosphate ratios, with different initial pH values of acetate and domestic wastewater mixture. The obtained experimental data supported the presence of GAOs for all tested HAc/P ratios, especially under P limiting conditions for acetate as sole carbon source. Strong evidence is observed that acetate added domestic wastewater system had higher PAOs fraction than acetate system as sole source carbon, with using model components, namely substrate uptake, glycogen utilization and P release.

# 1. INTRODUCTION

Discharge of phosphate into river and lakes promotes the excessive growth of algae, called as eutrophication, and thus change quality of water sources. Due to introduction of nutrients, excessive algae growth causes poorer quality of water and detrimental effect to aquatic life. Environmental objectives require improved sewage treatment, including nutrient removal as eutrophication affects public life such as causing problems on water supply and treatment systems. There are four main applications for phosphates: Agriculture, Food and animal feed supplements, Detergents and other industrial applications. Even most of recent wastewater treatment plants are designed for carbonaceous and nitrogenous materials; phosphorus removal from wastewaters should be performed in order to prevent natural bodies of water due to possible nutrient introduction processes such as nitrogen fixation by blue-green algae from the air, and contribution of non-point sources.

Due to strict discharge limitations in many countries, intensive afford have been made to understand mechanism of phosphorus removal, design of phosphorus removal systems and retrofitting of recent treatment plants. In order to maintain acceptable effluent nutrient concentrations, attention has been spent on better understanding of biochemical, microbiological and modeling aspects of enhanced biological phosphorus removal (EBPR), either in laboratory scale and plant scale.

Even phosphorus can be removed by using chemical methods, it is widely accepted that EBPR can be advantageous over chemical phosphorus removal as no chemicals are required, less excess sludge is produced, no additional chloride salts are discharged, and contamination of sludge by e.g. heavy metals derived from impurities in the added chemicals are avoided.

Biological phosphate removal occurs whereby phosphorus accumulating organisms (PAOs) incorporate internal polyphosphate pools that energy derived during storage of organics in anaerobic conditions. The process is performed in two stages. In the

first stage, PAOs take up volatile fatty acids (VFAs) and store it as polyhydroxyalkanoates (PHAs), mainly polyhydroxybutyrate (PHB) for acetate, and release phosphate to the liquid. During VFA uptake, internally stored glycogen that is stored under subsequent aerobic conditions served as a source of reducing power. And in the second stage, PAOs oxidize the stored PHA for the replenishment of the glycogen and polyphosphate pools, and growth.

The mechanism of phosphate uptake and release and storage of carbonaceous matter is still a topic of debate. Consequently, the main objective of this study was to investigate effect of P/VFA ratio and VFAs type on the performance of EBPR process. For this reason, two lab-scale sequencing batch reactors (SBRs) were operated with synthetic wastewater.

SBR was selected since it provides better controls and results in wastewater treatment and assure flexibility associated with working in a time rather than in a space sequence thereby facilitating successful growth of organisms which has poly-phosphate storage capability.

The relationship between acetic acid and propionic acid was considered as a main research subject in this study, which are generally regarded as the best carbon sources for EBPR. Experiments reported in this section were evaluated in terms of effect of VFA type on the performance of EBPR process. The effect of acetic acid and propionic acid on phosphate release and PHA storage was determined. In addition, the importance of acclimation period was investigated with batch experiments. The results compared with the results obtained from SBRs at steady-state conditions.

The data obtained from extensive experimental study served to develop a metabolic model for acetate uptake by a mixed culture of PAOs and glycogen accumulating organisms (GAOs) that present in EBPR systems and able to remove VFAs using glycogen as a source of energy and reducing power. The proposed metabolic model tested and verified under different phosphate inputs by experimental data.

In addition, results were used to investigate prediction capability of well recognized mathematical model for EBPR that is developed by the International Water Association (IWA) (formerly IAWQ) namely Activated Sludge Model No:2d (ASM2d) established for denitrifying phosphate accumulating organisms. This model

was selected because of biomass subjected to anoxic conditions at the beginning of mixing period of SBR. An iterative calibration methodology was developed based on sensitivity analysis. Model calibration procedure was defined for ASM2d and applied on SBR fed with acetate.

From the results obtained, the model revised to include possibility of the accumulation of GAOs and to illustrate competition between PAOs and GAOs. The model tested with adequate experimental data. A new process stoichiometry is defined for the mixed culture based upon previously developed metabolic concepts and models. Furthermore, conditions that allow the system to sustain glycogen accumulating organisms with significant impact on process behavior and performance were also defined.

Finally, as a part of research, effects of pH on the competition between GAOs and PAOs, as a key factor in the overall stoichiometry of anaerobic metabolism of organisms, was investigated. The dependency of P/VFA on pH in anaerobic stage was reported for uncontrolled pH system. The importance of pH that affects the balance between PAO and GAO populations in the EBPR system was evaluated.

# 2. LITERATURE REVIEW

## 2.1 History

It is first reported that the phenomenon of extensive phosphate uptake more than normal requirements for cell growth is observed by **Srinath et al. (1959)** in India. **Vacker et al. (1967)** observed same phenomenon in wastewater treatment plants in USA at the end of 1960s. These observations initiated a discussion on whether it is due to chemical precipitation or excessive phosphorus uptake by organisms under certain conditions.

**Levin and Shapiro (1965)** first investigated mechanism of biological phosphorus removal in activated sludge. **Shapiro et al. (1967)** assumed that some of organisms in biological activated sludge systems can store phosphate in the form of polyphosphate granules under aerobic conditions. Based on their observations, they suggested that redox potential causes phosphate release under anaerobic conditions. Then, these observations lead to obtain anaerobically high phosphate release in a separate tank, which can be followed by precipitation of phosphate. This is called as "Phostrip process" (**Levin et al., 1972**).

**Barnard (1975)** reported the importance of anaerobic/aerobic sequence to achieve enhanced biological phosphorus removal in biological nutrient removal activated sludge systems. Besides, it is emphasized that oxidized nitrogen entering anaerobic stage deteriorates enhanced biological phosphorus removal.

**Nicholls and Osborn (1979)** and **Marais et al. (1983)**, proposed biochemical explanations for phosphorus removal. They stated that volatile fatty acids are stored as internal storage materials in the cell by organisms under anaerobic stress conditions, and utilized for growth and replenishment of polyphosphate pools under aerobic conditions.

In this chapter, principles of enhanced biological removal (EBPR), factor affecting EBPR performance, and biochemical and mathematical models will be reviewed.

## 2.2 Principles of Enhanced Biological Phosphate Removal

Biological enhanced phosphate removal (EBPR) is based on the feature that dominance of certain organism under alternating anaerobic and aerobic conditions, which are called phosphate accumulating organisms (PAOs). PAOs are a group of organisms that possess ability to accumulate phosphate in the form of polyphosphate. Introducing sludge with wastewater under anaerobic conditions provides competitive advantages to PAOs over other microorganisms. The understanding of the EBPR removal mechanism was significantly advanced with the observations on storage of carbohydrate products within the cell in the anaerobic phase (USEPA, 1987). PAOs can utilize volatile fatty acids (VFAs) to store intracellular storage products within the cell as polyhydroxyalkanoates (PHA). The schematic presentation of the mechanism of enhanced biological phosphorus removal is shown in Figure 2.1. Higher phosphate removal is achieved by removing excess sludge with phosphate accumulates in the biomass.

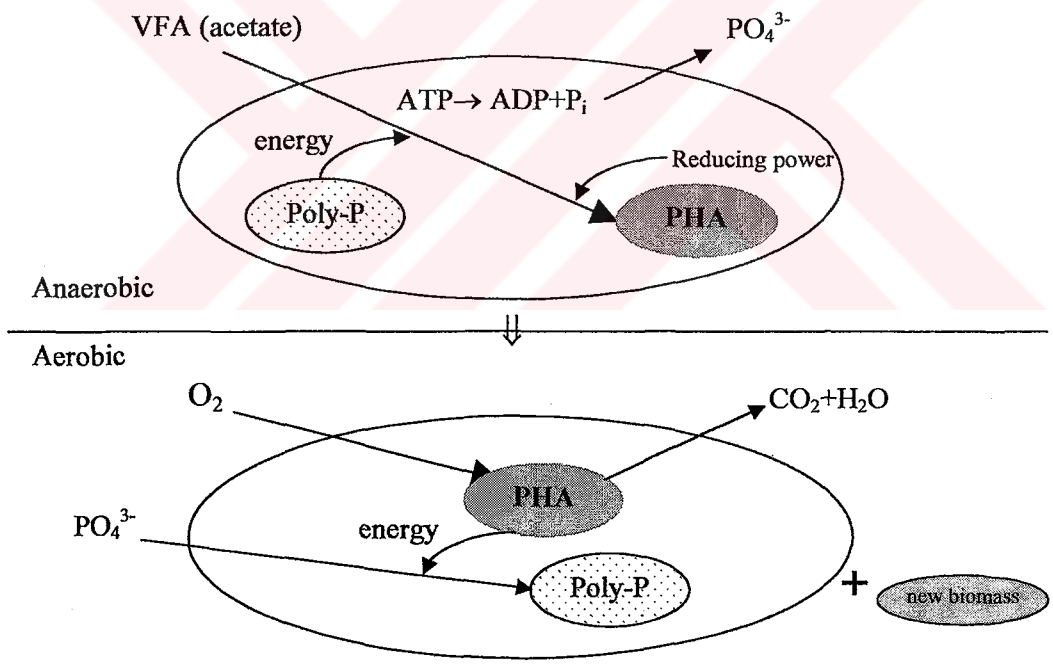



**Figure 2.1:** Schematic presentation of mechanism of enhanced biological phosphorus removal

Under anaerobic conditions, addition of carbon source (VFAs such as acetate, propionate, butyrate, lactate, glucose) results simultaneous substrate uptake and storage and triggers phosphate release to the liquid. In EBPR process, phosphate

uptake is higher than phosphate release. Abu-ghararah and Randall (1991) reported that phosphate uptake to phosphate release ratio is around 1.2.

Acetate metabolism under anaerobic conditions has been studied more since it has been well known that VFA is favorable carbon source for EBPR (**Wentzel et al., 1989**). According to this study, energy required for activation and transport of VFAs is derived from the internal polyphosphate pool by liberation of phosphate molecules from ATP. ATP is used for the uptake of VFAs and subsequent storage of PHA (**van Loosdrecht et al., 1997**). Under oxygen and nitrogen limited conditions, the assimilated carbon source is accumulated in the cell as an intermediate products, primarily poly-3-hydroxy-butyrate (PHB) (**Fukase et al., 1982, Deinema et al., 1985**). It is widely accepted that, internally stored glycogen under aerobic conditions serves as a source of reducing power ($NADH_2$) required for storage of PHA (**Mino et al., 1987; Smolders et al., 1994; Hesselman et al., 2000**). During subsequent aerobic or anoxic phase, when oxygen, nitrate or nitrite is present, bacterial growth, and polyphosphate and glycogen replenishment occur using PHA as the substrate to derive energy to uptake phosphate (**Kortstee et al., 2000**).

## 2.3 Microbiology of Enhanced Biological Phosphate Removal

*Acinetobacter spp.* was first proposed to be responsible for phosphorus removal (**Fuhs and Chen, 1975**). They demonstrated that *Acinetobacter* was able to take up phosphate and degrade PHB under aerobic conditions, and release phosphate under anaerobic conditions. However, recently some other species mentioned below were isolated from EBPR sludge and biological phosphate removal communities were identified with molecular biology tools.

**Wagner et al. (1994)** developed a genus-specific 26S rRNA-targeted oligonucleotide probe to investigate the role of *Acinetobacter spp.* in situ. Using molecular techniques, this research revealed that *Acinetobacter spp.* fraction of activated sludge samples taken from both anaerobic and aerobic phases constitutes only a small fraction (10%) of all bacteria. It is also observed from the results of total cell counts that dominant class was *Proteobacteria* with a fraction of up to 80%. It is likely supported by **Bond et al. (1999)** and **Liu et al. (2000)** that *Proteobacteria* and *Actinobacteria* were dominant in phosphate removing sludge. Kong *et al.* (2002) studied on community composition for acetate fed as a sole carbon source, while the

P/C ratio in the feed changed. The results of this study showed that when P/C ratio was decreased (from 1:10 to 1:50), the percentage of *Proteobacteria* decreased from 77% to 38%.This decrease was related to decrease in *Proteobacterial Phodocyclus*-related phosphorus accumulating bacteria. According to molecular analysis, no *Acinetobacter spp.* was detected in the phosphate removing sludge. Further, the study of **Mc Mahon et al. (2002)** was verified that the phosphate removing sludge was enriched in *Phodocyclus*-like *Proteobacteria* which was consisted of 80% of total bacteria. Likely, *Proteobacteria* group contains a PAO responsible for EBPR with up to 80% percent of the phosphate removing sludge achieved by increasing phosphate concentration in the influent, as suggested by **Crocetti et al. (2000)**.

**Nakamura et al. (1995)** isolated a new type of phosphate accumulating bacteria namely *Microlunatus phosphovorus* from a laboratory scale EBPR process. However, even that bacteria can accumulate phosphate under aerobic conditions, but does not convert acetate to PHA under anaerobic conditions.

Stante *et al.* (1997) reported that *Lampropedia spp.* can be classified amongst phosphate accumulating organisms. In this study, *Lampropedia spp.*was isolated from a laboratory scale EBPR activated sludge system operating on dairy and piggery wastewaters. It is observed that *Lampropedia spp.* stored acetate as PHB in aerobic growth test. Additional batch tests were revealed that *Lampropedia spp.* can sequestrate acetate and store as PHB under anaerobic conditions.

Currently, there is no enough information about whole phosphate removing community in EBPR sludge, but some of PAOs were defined as different groups of microorganisms so far as mentioned above. In summary, expected characteristics of PAOs reported in the literature is as given in Table 2.1 (**Mino et al., 1998**).

Those investigations attempting using molecular and conventional techniques on microbial communities in phosphate removing activated sludge indicated that not only single bacterium is dominant, but several bacterial strains are present, depending on wastewater characteristics and operation conditions of the system.

**Table 2.1:** Expected characteristics of PAOs (**Mino et al., 1998**)

| | Items | Characteristics of PAOs |
|---|---|---|
| Morphology | Cell shape etc. | Most probably short rod or cocci.<br>Frequently present in cluster. |
| | Gram stain | uncertain |
| | Neisser stain | strongly positive, after aerobic growth. |
| | PHB staining | strongly positive, after anaerobic subtrate uptake. |
| | Internal structure | Poly-P and PHA granules are visible. |
| Chemotaxonomy | Quinone | Q-8 or MK-8($H_4$) |
| | Fatty acids | 16:1 d9c, 16:0 or 18:1 d11c |
| Phylogenetics | | suspected to belong to Gram positive bacteris with a high G + C content or beta subclass of proteobacteria |
| Physiology | Anaerobic metabolism | take up acetate and convert it to PHA accompanied by poly-P an glycogen degradation and phosphate release.<br>take up wide range of low molecular organic compounds like sugars, carboxylic acids and amino acids.<br>enzymes for anaerobic metabolism are inducible. |
| | Aerobic/anoxic metabolism | convert internally stored PHA to glycogen, accumulate polyphosphate level and grow in the absence of external carbon source.<br>presence of carbon source inhibits uptake of phosphate.<br>some can metabolize nitrate as electron acceptor. |

## 2.4 Deterioration of Enhanced Biological Phosphate Removal

The failure was observed in enhanced biological phosphorus removal (EBPR) processes in the literature due to unknown reasons may be attributed to the domination of a group of bacteria rather than phosphate accumulating organisms in anaerobic-aerobic activated sludge process. These bacteria, called as glycogen accumulating bacteria (GAOs), dominate under certain operating conditions and with some of favoring wastewater characteristics. These types of organisms take up VFAs and store as PHA without need of energy from polyphosphate. **Satoh et al. (1992)** and **Jeon et al. (2001)** observed deterioration of phosphate removal which is attributed to the presence of GAOs in the system. **Mino et al. (1995)** stated that GAOs provide energy and reducing power only by glycogen degradation through glycolysis during anaerobic phase.

Under certain circumstances such as high temperature, longer unaerated detention times, low pH, GAOs can dominate PAOs in nutrient removal systems. GAOs compete with PAOs for the VFAs in the wastewater. **Filipe (1999)** stated that GAOs can compete very effectively with PAOs for the same substrate. With the experimental results, they determined the stoichiometry and kinetics of acetate uptake by GAOs and PAOs. The results also showed that GAOs have ability to use substrate faster than PAOs if pH of anaerobic zone was lower than 7.25, and at the pH of above 7.0 in the aerobic zone causes the displacement of the GAO community by the more desired PAO community.

**Satoh et al. (1994)** investigated deterioration of EBPR by the domination of microorganisms without polyphosphate accumulation. They found that dominant microorganisms in the sludge was consumed carbohydrates simultaneously with substrate uptake under anaerobic conditions and accumulated PHA. They observed no significant release of phosphate in deteriorated biological phosphate removal reactor. They also emphasized that the difference in the metabolic systems between PAOs and GAOs may give hints to avoid the deterioration of enhanced biological phosphorus removal. Therefore, it is essential to determine the operational conditions that minimize GAOs for higher EBPR performance in the system.

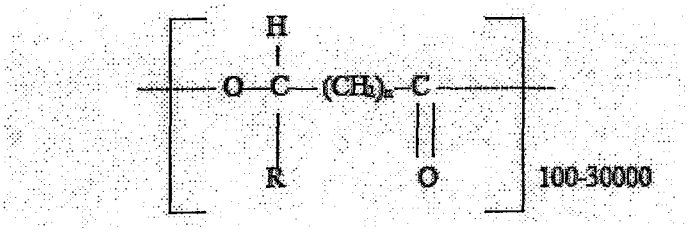
## 2.5 Storage Products

### 2.5.1 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are one of the intracellular storage materials in family of polyesters of 3-hydroxyacids synthesized by a wide variety of bacteria and found in granules in the cell as well as glycogen and polyphosphate. Storage of PHAs takes place during anaerobic phase by phosphorus and glycogen accumulating organisms. Composition and content of PHA stored in activated sludge show a discrepancy depending on wastewater characteristics and operation conditions of nutrient removal system. General structure of PHA is as given in Figure 2.2 (Punrattanasin, 2001).

PHAs are composed mainly of four types of 3-hydroxyalkanoates: 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV), 3-hydroxy-2-methybutyrate (3H2MB), 3-hydroxy-2-methyvalerate (3H2MV) (**Satoh et al., 1992**). Table 2.2 shows chemical structure and precursors of the PHA units given by **Satoh et al. (1992).**

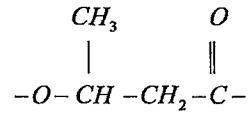
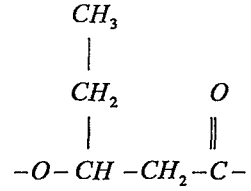
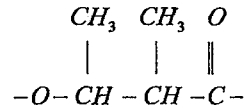
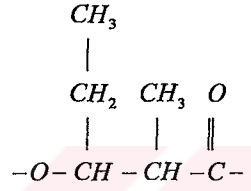
PHB and PHV are most common polymers of PHA. The PHB biosynthetic pathway starts with condensation of two Acetyl-CoA that is obtained from oxidation of pyruvate. These two condensated Acetyl-CoA form acetylacetyl-CoA. Acetylacetyl-CoA is reduced by enzyme to R-(-)-3-hydroxybutryl-CoA, and this is then polymerized to form PHB. This pathway requires reducing power. Likely, PHV is formed via polymerization of 3-hydroxyveleryl-CoA that is produces by condensation of Acetyl-CoA and Propionyl-CoA. Propionyl-CoA is produced from succinate over the meyhylmalonyl pathway.




n = 1 R = methyl: polymer = poly(3-hydroxybutyrate)
R = ethyl: polymer = poly(3-hydroxyvalerate)
n = 2 R = hydrogen: polymer = poly(4-hydroxybutyrate)
n = 3 R = hydrogen: polymer = poly(5-hydroxybutyrate)

**Figure 2.2:** General structure of PHA (**Punrattanasin, 2001**)

**Table 2.2:** Chemical structure and precursors of the PHA units (**Satoh et al., 1992**)

| Unit | Structure | Precursors |
|---|---|---|
| 3-hydroxybutyrate (HB) |  | 2 Acetyl-CoA |
| 3-hydroxyvalerate (3HV) |  | Acetyl-CoA + Propionyl-CoA |
| 3-hydroxy-2-methybutyrate (3H2MB) |  | Acetyl-CoA + Propionyl-CoA |
| 3-hydroxy-2-methyvalerate (3H2MV) |  | 2 Propionyl-CoA |

According to many investigators, acetate leads to the best PHA production compared to other VFAs used independently during the experiments. In recent experimental studies, it is observed that copolymer composition is primarily influenced by the substrate used as it is also formerly considered by **Doi (1990)**. For instance, depending on composition of carbon substrate supplied, the composition of the produced copolymers has been reported to vary from 0 to 47 mol% 3HV (**Doi, 1990**).

It is also reported that acetate consumption leads to the production of mainly PHB and partially PHV (**Comeau et al., 1987; Satoh et al., 1992; Smolders et al., 1994; Lemos et al., 1998**). When acetate is used as carbon source, PHB content of PHA was obtained by many researchers as 77% by **Comeau et al. (1987)**, 75.25% by **Lemos et al. (1998)**, 87% by **Satoh et al. (1992)**. Besides, there are few studies on other VFAs such as propionate, which is also most common in the domestic wastewater and one of most significant product of pre-fermentation. For propionate, varying PHB fractions in the sludge (10% by **Randall and Liu, 2002**, 26% by **Chen et al., 2004**; and 36% by **Pijuan et al., 2004**) was observed, where 3H2MV was not determined. According to **Satoh et al. (1996)**, PHA composition from propionate can be predicted theoretically based on the stoichiometry.

### 2.5.2 Glycogen

Glycogen is an intracellular carbohydrate present in cytosol in form of granules. It is a readily mobilized form of glucose. It serves as a carbon, energy and reducing power source for biological phosphate removing process.

**Mino et al. (1987)** reported a significant decrease and increase in carbohydrate concentration under anaerobic and aerobic conditions, respectively. Consequently, they proposed a biochemical model that incorporated this feature. According to this, under anaerobic conditions, phosphate accumulating organisms utilize glycogen as a provider of reducing power to kept redox balance unchanged, and energy to be supplied while VFAs was transporting into cell.

**Smolders et al. (1994)** and **Pereira et al. (1996)** incorporated glycogen as a component of their metabolic models. They reported that participation of glycogen in the metabolism has consequence for the polyphosphate degradation and phosphate release under anaerobic conditions.

Both the Mino and the Smolders models suggest that the reducing power for PHA formation is only generated by the breakdown of glycogen through glycolysis. Liu **et al. (1997)** reported that PAOs can store energy in the form of polyphosphate and that glycogen storage is of minor importance for EBPR.

Anaerobically consumed glycogen is replenished in the aerobic phase, whereas PHA is consumed. **Smolders et al. (1994)** developed a structured metabolic model of the aerobic phase including glycogen synthesis. According to this model, glycogen is produced from oxaloacetate in glyconeogenesis. Oxaloacetate is produced from PHA through the glyoxylate cycle.

## 2.6 Factors Influencing Enhanced Biological Phosphate Removal Process

### 2.6.1 Environmental factors

#### 2.6.1.1 Temperature effect

Temperature is generally considered as a parameter that must be taken into account, especially depending on climate of the location of biological nutrient removal plant in case of it may experience extreme low or high wastewater temperature.

Shapiro (1967) reported increasing phosphate release rate for activated sludge with increasing temperature from 10°C to 30°C. Likely, **McClintock et al. (1993)** observed that biological phosphate removal efficiency is improved at higher temperatures.

It is emphasized that temperature affects a variety of processes in activated sludge such as lysis, fermentation, nitrification (**Brdjanovic et al., 1997**). Therefore, it is difficult to distinguish exact impact on biological phosphate removal process in nutrient removal systems.

**Brdjanovic et al., 1997** studied on conversion of relevant compounds for EBPR at 5, 10, 20 and 30°C. They reported that the stoichiometry of the anaerobic process was insensitive to temperature changes, but some effects on aerobic stoichiomerty was observed. On the other hand, they found strong influence of temperature on the kinetics of the processes under anaerobic and aerobic conditions.

**Jones and Stephenson (1996)** studied on effect of temperature and concluded that the optimum temperature was 30°C for anaerobic release and aerobic uptake of phosphate. They also reported that efficiency of phosphate removal were lower at two extreme temperatures (5°C and 40°C).

**Erdal et al. (2002)** conducted experiments at temperature ranging from 5°C to 20°C in a lab scale modified UCT process. They found that even though the kinetic rates decrease as temperature decreases, EBPR systems perform better at colder temperatures. They stated that the reason for better system performance is apparently related to reduced competition among organisms in the system for substrate in the non-oxic zones.

#### 2.6.1.2 External pH effect

In anaerobic phase of a biological phosphate removal process, energy required for the transport of VFA is depends on external pH (**Smolders et al., 1994**). This dependency cause a wide range of P/VFA ratios as reported in the literature.

**Smolders et al. (1994)** studied on function of pH on phosphate release. They conducted a series of experiments at varying pH values in a SBR with a constant acetate concentration. The results of this study showed a linear relationship between pH and P/HAc ratio. They reported that phosphate/acetate ratio is varied as a

function of pH. They also suggested that glycogen degradation kinetics should be pH independent.

Filipe (1999) showed that pH plays a critical role in the competition between PAOs and GAOs. In order to achieve complete phosphate removal, external pH in the anaerobic phase should be higher than 7.25 (**Filipe et al., 1999**). This provides favoring conditions for PAOs to compete with GAOs for acetate. **Filipe et al. (1999)** also found that the stoichiometry of glycogen and PHA accumulation by PAOs was independent on the pH. **Filipe et al. (2001a)** and **Filipe et al. (2001b)** proposed a metabolic model for acetate uptake by PAOs and GAOs in which the kinetics of acetate uptake were modeled.

**Smolders et al. (1994)** and **Filipe et al. (1999)** explained dependency of P/HAc ratio with the external pH based on bioenergetics. According to this, cell spends energy for both maintenance of proton motive force (PMF) and activation of acetate. The PMF is a chemiosmotic gradient across the bacterial cytoplasmic membrane that can considered two distinct components: electrical potential arising from a net charge on the cytoplasmic side of the cell membrane as compared to outside, and pH gradient caused by the higher alkalinity of the bacterial cytoplasm (Comeau, 1989). According to Mino at al., (1987), activation of 1 mole-C acetate requires 0.25 mol of polyphosphate. Thus, P/PHAc ratio is equal to $0.25 + \alpha_{PAO}$. The relationship between pH and P/HAc ratio experimentally found by **Smolders et al. (1994)** was:

$$\frac{P}{HAc} = -0.85 + 0.19\ pH_{out} \qquad \left[\frac{mol-P}{mol-C}\right] \qquad \textbf{(2.1)}$$

The results of experimental study performed with GAO enriched culture by **Filipe et al. (1999)** alike with the **Smolders et al. (1994)** for PAOs in terms of findings on varying α values as a function of external pH. They suggested that the relationship between $\alpha_{GAO}$ and external pH is as given:

$$\alpha_{GAO} = 0.057\ pH_{out} - 0.34 \qquad \left[\frac{mol}{mol-C}\right] \qquad \textbf{(2.2)}$$

### 2.6.1.3 Dissolved oxygen

**Shön et al. (1993)** reported deterioration of phosphate uptake at lower dissolved oxygen concentrations (0.1-0.5 mg/l). Consequently, it is essential to provide

sufficient dissolved oxygen in aerobic phase for biological phosphate removal systems as well as aerobic systems. In the presence of adequate amount of oxygen in aerobic phase, phosphate accumulating organisms grow and phosphate uptake occurs.

In order to maximize phosphate removal, 2.0 mg/l or greater dissolved oxygen concentrations are recommended in the middle of aeration basin (**USEPA, 1987**), since it may affect the rate of phosphate uptake.

### 2.6.2 Design Parameters

#### 2.6.2.1 Sludge age

Biological phosphate removal is achieved removing excess sludge with high phosphate content and as a function of sludge age. As it is widely accepted that lower sludge ages result in higher sludge production with high amount of polyphosphate in the sludge.

**Marais and Jenkins (1992)** reported that PAOs can be wash-out of the system at low sludge ages. They suggested that stability of EBPR is independent from sludge age at sludge age of higher than 3 days at higher temperature conditions.

**Rodrigo et al. (1996)** reported that phosphate removal capacity is varying with sludge age. The results of this study demonstrated the effect of sludge age on the phosphate removal range. They found that phosphate removal decreases with increasing sludge age.

#### 2.6.2.2 Aerobic sludge age

**Brdjanovic et al. (1998)** developed a model for the prediction of the minimally required aerobic sludge age as a function of kinetic and process parameters. They suggested that aerobic sludge age should be long enough to oxidize the amount of PHA stored in the anaerobic phase.

### 2.6.3 Wastewater characteristics

An influent characteristic of wastewater is one of the most important factors for biological nutrient removal. The P/COD ratio, and presence and amount of volatile fatty acids have reported as crucial characteristics of wastewater. Consequently, presence of nitrates as electron acceptor at the beginning of anaerobic phase

observed in the literature reduces phosphate release and thus deteriorates phosphate removal.

### 2.6.3.1 P/COD ratio

The importance of P/COD ratio of wastewater is in substantial agreement in literature as affecting the performance of phosphate removal. It is observed that the amount of phosphate released increases as the P/COD ratio increases in the biological nutrient removal systems. In order to achieve complete phosphate removal in the system, the required amount of COD increases with the amount of phosphate in the wastewater. Thus, it is suggested that the ratio of P/COD have recommended to be higher than 1/20-1/25 (**Tetreault et al., 1986**).

**Carlsson et al. (1996)** conducted an experimental study on a pilot plant with domestic wastewater to study on EBPR operation in activated sludge process. They examined the relation between VFA, COD and phosphate in the influent wastewater at the plant. They found that high P/COD ratios in the influent increases phosphate concentration in the effluent. They also stated that low P/COD ratio may seem favorable for the EBPR even for COD concentrations as low as 100 mg/L.

The effect of COD/P ratio is investigated in a UCT pilot plant by **Kisoglu et al. (2000)**. They operated the system with varying COD/P ratios where acetate was sole carbon source and kept constant during the experiments. They observed that COD utilization, Phosphate uptake and release, glycogen storage and system performance differ depending on COD/P ratio. They concluded that COD/P ratio of 18 was also refers to phosphate limiting conditions as it is reported commonly as 20.

**Comeau et al. (1987)** investigated the relation between acetate uptake and phosphate release in anaerobic phase in batch experiments with sludge from a biological phosphate removal plant. They found that net phosphate release (mg/L) is equal to 0.781xAcetate Consumed (mg/L as HAc)-1.4.

**Chen et al. (2004)** obtained ratio of phosphate release to VFA for acetate and propionate. They calculated this ratio as 0.46 in both SBR and batch reactor for acetate, and as 0.45 in SBR and 0.41 in batch reactor for propionate.

Observed phosphate release to VFA uptake ratios were 0.72 mol/mol-C for acetate and 0.46 mol/mol-C for propionate as reported by **Pijuan et al. (2004)**. This

difference was referred to that more energy could be required per C-mol to activate acetate than to activate propionate.

**Abu-ghararah and Randall (1991)** investigated the effect of organic acid addition on the phosphate release. They found that measured ratios of phosphate release to six VFAs added. This ratio was 0.37 for acetic acid, 0.12 for propionic acid, 0.15 for butyric acid, 0.16 for isobutyric acid, 0.19 for valeric acid and 0.25 for isovaleric acid for unit of mg phosphate release to mg COD utilized in the anaerobic phase. They suggested that different amount of VFAs causes different amount of phosphate release, and the amounts were related to the number of carbon atoms.

P/COD ratio was proposed to be a key factor for the competition between PAOs and GAOs (**Liu et al., 1997; Filipe et al., 2001c**). **Liu et al. (1997)** stated that when excessive phosphate was provided (P/C=20/100), PAOs successfully out-compete GAOs. The results of this study showed that the ratios of phosphate release to acetate uptake observed in sludge with different phosphate contents were in accordance.

The values of phosphate release and acetate uptake reported in the literature were as given in Table 2.2. It must be noted that the observed P/Ac ratios reported in literature vary significantly under different experimental conditions.

### 2.6.3.2 Volatile fatty acids

Biological phosphate removal mechanism involves storage of volatile fatty acids during anaerobic phase to accumulate PHA required for subsequent excess phosphate in aerobic conditions. For the success of biological phosphate removal process, it is crucial to provide sufficient volatile fatty acid to the biological nutrient removal systems.

In order to assure complete phosphate removal, availability of readily biodegradable COD entering anaerobic zone considered to be maximized (**Siebritz et al., 1983**). This provides maximized carbon storage in anaerobic phase and thus subsequent aerobic phosphate uptake would be maximized due to the large carbon reserves in the sludge (**Comeau et al., 1987**).

**Table 2.3:** Observed phosphate release to acetate uptake ratios of EBPR sludge

| Reference | P/HAc (mol/mol) |
|---|---|
| **Fukase et al. (1982)** | 0.9 |
| **Arvin and Kristensen, (1985)** | 1.43 |
| **Wentzel et al. (1985)** | 0.4-1.0 |
| **Mino et al. (1987)** | 0.3-0.76 |
| **Arun et al. (1988)** | 0.4-0.76 |
| **Satoh et al. (1992)** | 0.68-0.98 |
| **Cech et al. (1993)** | 0-0.78 |
| **Kuba et al. (1993)** | 0.8-1.2 |
| **Satoh et al. (1994)** | 0.04 |
| **Satoh et al. (1992)** | 0.87 |
| **Smolders et al. (1995)** | 0.52-1.52 |
| **Pereira et al. (1996)** | 0.32 |
| **Kuba et al. (1997)** | 0.84 |
| **Hesselman et al. (2000)** | 0.74 |

**Carlsson et al. (1996)** confirmed that the VFA in the influent wastewater is mainly affect the release of phosphate in the system. The results of this study suggested that hydrolysis and/or fermentation processes have to be considered when describing phosphate release in the anaerobic reactor.

Acetic acid and propionic acid are the most common VFAs in domestic wastewater. Many researchers assumed that these acids are most preferable to stimulate large amount of phosphate removal (**Siebritz et al., 1983; Arvin and Kristensen, 1985 and Comeau et al., 1989**)

The effect of influent organic compounds was studied by **Abu-ghararah and Randall (1991)** using a pilot plant operated for biological nutrient removal. They considered six different organic compounds (formic, acetic, propionic, butyric, isobutyric, valeric and isovaleric acids) added separately into domestic wastewater. They state influence of substrate addition on phosphate release and uptake. According to experimental results of this study, acetic acid caused the greatest phosphate release and propionic acid caused the least. Likely, acetic acid produced the greatest phosphate uptake, and propionic acid the least.

**Satoh et al. (1992)** investigated anaerobic uptake mechanisms of acetate, propionate and lactate with batch experiments using polyphosphate accumulating sludge achieved in a laboratory scale continuous anaerobic-aerobic phosphate removal system. They quantified four storage materials of 3HB, 3HV, 3H2MV and 3H2MB in the sludge. They considered glycogen as a reserve material accumulated in aerobic phase and degraded as energy and reducing power source under anaerobic conditions by phosphate accumulating organisms. Consequently, they postulated a mechanism of anaerobic acetate and/or propionate uptake with the consumption of intracellular glycogen.

**Lemos et al. (1998)** conducted experiments to investigate how different carbon sources and their concentrations can influence of PHA by phosphate removing sludge. In this study, acetic, propionic and butyric acids were used to evaluate the effect of carbon source on phosphate release and production of PHA. They concluded that acetic acid leads to the best PHA production. They found that PHB content of the sludge was 75.25% for acetic acid, 28.06 for propionic acid and 59.68% for butyric acid. Therefore, they suggested that carbon source affects the composition of PHA. This is also in agreement with those reported by **Randall and Liu (2002), Randall et al. (2003),** and **Pijuan et al. (2004).**

**Hood and Randall (2001)** stated that acetic acid, isovaleric and succinic acids were significantly beneficial to phosphate removal. Even they observed low phosphate removal efficiency for propionic acid in batch experiments; they also found that propionic acid is a very efficient selector for EBPR in long term cultivation.

**Randall et al. (2003)** conducted batch experiments with increasing propionic fraction of VFA using biomass from two sequencing batch reactors fed with domestic wastewater spiked with additional VFAs. They found that PHV fraction of total PHA increases with increasing proponic acid fraction of total VFA and decreasing phosphate release. They also observed higher phosphate uptake for acetic acid than propionic acid.

#### 2.6.3.3 Nitrate effect

**Barnard (1976), Hascoet et al. (1985)** and **Comeau et al. (1985)** reported that the presence of nitrate causes detrimental effect on biological phosphorus removal and reduces phosphorus release in anaerobic stage. It is suggested that anaerobic

phosphate release reduces due to consumption of low fatty acids for denitrification (**Hascoet et al., 1985**). On the other hand, in some of studies on the effect of nitrate on phosphorus release, it is stated that if sufficient fatty acid is present, phosphorus release and denitrification can take place simultaneously (**Nicholls et al., 1985; van Starkenburg et al., 1993; Yagci et al., 2003**).

**Hascoet et al. (1985)**, **Gerber et al. (1987)**, **Artan et al. (1998)** and **Yagci et al. (2003)** shown that besides oxygen, nitrate can be utilized as an electron acceptor by PAOs to take up phosphate under anoxic conditions. **Artan et al. (1998)** stated that the phosphorus uptake was slower under anoxic conditions, which is attributed to that only a part of the PAOs takes up phosphate under anoxic conditions, whereas all the PAOs take up phosphate under aerobic conditions.

These observations in the literature lead to define two PAOs present in the EBPR systems. The one is non-denitrifying PAOs that can only use oxygen as terminal electron acceptor; the other is denitrifying-PAOs that can use both nitrate and oxygen as terminal electron acceptors.

**Filipe and Daigger (1999)** stated that the anoxic-aerobic population has a significant competitive disadvantage when competing with PAOs because of a lower thermodynamic efficiency of anoxic growth compared to aerobic growth. The authors confirmed that introduction of even a small aerobic zones in an EBPR system will strongly affect accumulation of denitrifying-PAOs with experimental observations.

## 2.7 Biochemical Models

During last two decades, different biochemical models have been developed for the mechanistic understanding of the biological phosphate removal process. These models given in the literature to describe the mechanism of phosphate and glycogen accumulating organisms individually, which compete for substrate in EBPR process, were summarized below.

### 2.7.1 Metabolic models for VFA uptake by PAOs

There are widely accepted metabolic models for phosphate accumulating organisms called the Comeau/Wentzel model, the Mino model and the Adapted Mino model.

Recently, there are few models which are modifications of these models proposed by **Smolders et al. (1994), Pereira et al. (1996), Maurer et al. (1997)** and **Hesselman et al. (2000).**

According to Comeau/Wentzel model required reducing equivalents for PHA synthesis are produced by oxidation of some of Acetyl-CoA through the citric acid **(Comeau et al., 1986; Wentzel et al., 1986). Comeau et al. (1987)** proposed that polyphosphate is used as an energy source for replenishment of proton motive force (PMF) besides acetate uptake under anaerobic conditions. **Wentzel et al. (1991a)** stated that TCA enzymes can operate under anaerobic conditions. Figure 2.3 illustrates the proposed metabolism of PAOs under anaerobic conditions by **Comeau et al. (1986).**

According to this model, acetate is taken up by organism with passive diffusion. Required energy for activation of VFA to Acetyl-CoA is obtained from the cleavage of phosphate molecules from polyphosphate pools. This results in release of some cations (mainly $K^+$ and $Mg^{++}$) and anion (orthophosphate) into liquid. A part of Acetyl-CoA enters to TCA cycle to obtain reducing power to store rest of the Acetyl-CoA as PHB in the cell. Thus, the stoichiometry of phosphate release to acetate uptake is 1:1 (mol/mol). The stoichiometric equation for Comeau/Wentzel Model is as follows:

$$9\ \text{Acetate} + 9\ \text{ATP} \rightarrow (C_4H_6O_2)_4 + 2\ CO_2 + 9\ \text{ADP} + 9\ \text{Pi} \qquad \textbf{(2.3)}$$

**Mino et al. (1987)** proposed a metabolic model based on observations of simultaneous decrease in carbohydrates with acetate during anaerobic phase. Consequently, they suggested that reducing power is generated by degradation of glycogen via Embden-Meyerhoff (EMP) instead of oxidation of Acetyl-CoA through TCA cycle, which supplies 3 mole ATP for glycolysis of 1 mole glycogen. Production of ATP via glycolysis lowers the required energy involvement from hydrolysis of polyphosphate. Thus, the stoichiometry of phosphate release to acetate uptake for "Mino model" is 1:2 (mol/mol). The stoichiometric equation for Mino Model is as follows:

$$6\ \text{Acetate} + \text{Glycogen} + 3\ \text{ATP} \rightarrow (C_4H_6O_2)_4 + 2\ CO_2 + 3\ \text{ADP} + 3\ \text{Pi} \qquad \textbf{(2.4)}$$

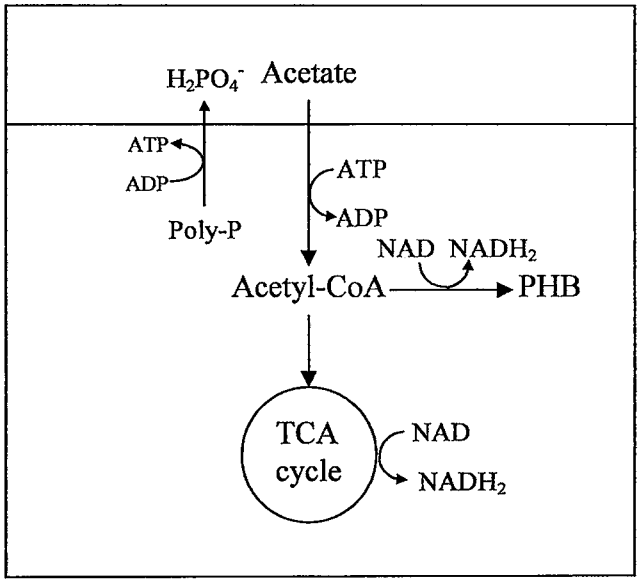



**Figure 2.3:** Schematic illustration of proposed Comeau/Wentzel Model for microorganisms under anaerobic conditions

Mino model is modified by **Wentzel at al. (1991a)** with a minor change. They proposed "modified Mino model" in which internally stored glycogen supplies 2 mole ATP through Enther-Doudoroff (ED) pathway. The stoichiometry of phosphate release to acetate uptake for "modified Mino model" is 2:3 (mol/mol), which is lower compared to Mino model.

Figure 2.4 illustrates the proposed metabolism of PAOs under anaerobic conditions by **Mino et al. (1987)** and **Wentzel et al. (1991a)**.

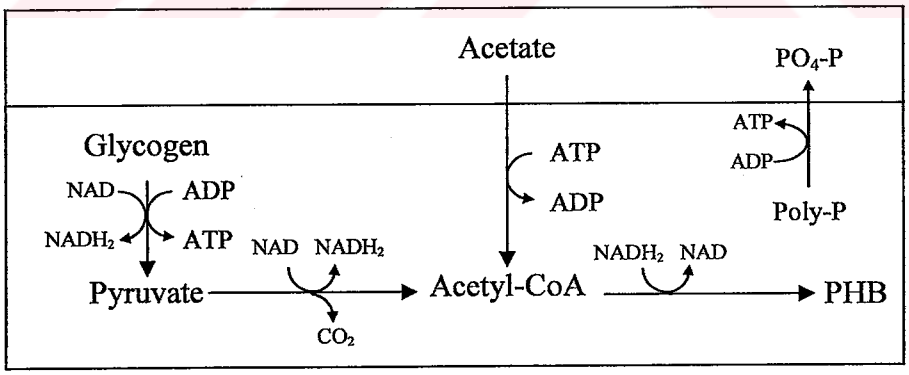



**Figure 2.4:** Schematic illustration of proposed Mino Model for microorganisms under anaerobic conditions

**Smolders et al. (1994)** proposed a metabolic model including glycogen that serves not only as a source of reducing equivalent but also as an energy source besides polyphosphate. They confirmed stoichiometry of phosphate, PHB and carbon dioxide to acetate of the proposed model with experimental results.

Recent models explaining the experimental results in terms of carbon, redox and energy balances are likely to explain the observed stoichiometry of the anaerobic phase of biological phosphate removal process (**Pereira et al., 1996; Hesselman et al., 2000**)

**Pereira et al. (1996)** proposed a biochemical model for the synthesis of polyhydroxyalkanoates from acetate and glycogen. They suggested that TCA cycle is involved in biological phosphate removal process as an additional source of reduction equivalents. Figure 2.5 shows presentation of proposed model by **Pereira et al. (1996)** is shown in.

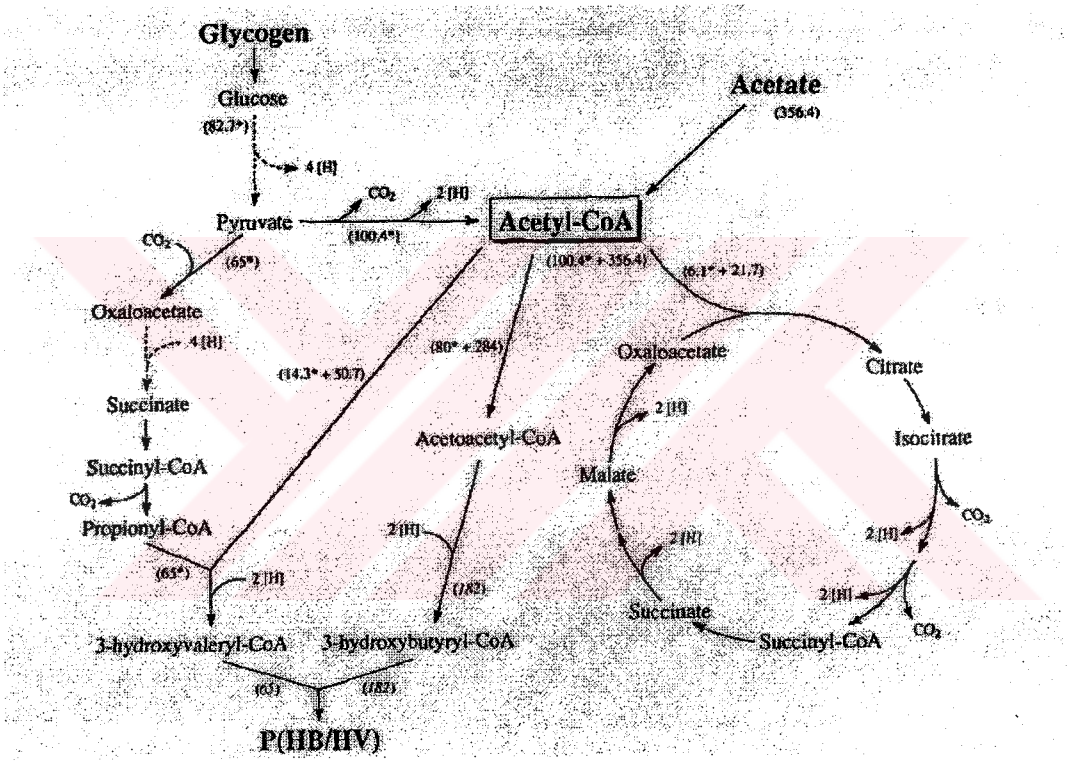



**Figure 2.5:** Proposed metabolic model by by **Pereira et al. (1996)**

**Maurer et al. (1997)** stated that glycogen is used anaerobically for energy production using a solid state NMR to track carbon flow in biological phosphate removal sludge. They suggested that the Entner-Doudoroff (ED) pathway is used and EMP pathway plays only a minor role for anaerobic degradation of glycogen. They concluded that the maintenance of glycogen and polyphosphate pool reduces the growth yield on substrate. Likely, **Louie et al. (2000)** suggested that the common enzymes to the TCA cycle and the glyoxylate pathway are necessary for anaerobic PHA sysnthesis.

**Hesselmann et al. (2000)** studied on stoichiometry and enzymatic reactions of anaerobic phase in a laboratory scale sequencing batch reactor with acetate. The results of this study showed that glycogen is degraded via the Entner-Doudoroff pathway. They explained varying phosphate release to acetate taken up ratio with polyphosphate and glycogen content of the sludge. They proposed operation of partial TCA cycle to explain the generation of extra reducing equivalent. In Figure 2.6 shows schematic presentation of combined operation of enzymes from the TCA cycle and a modified succinate-propionate pathway as proposed by **Hesselman et al. (2000)**.

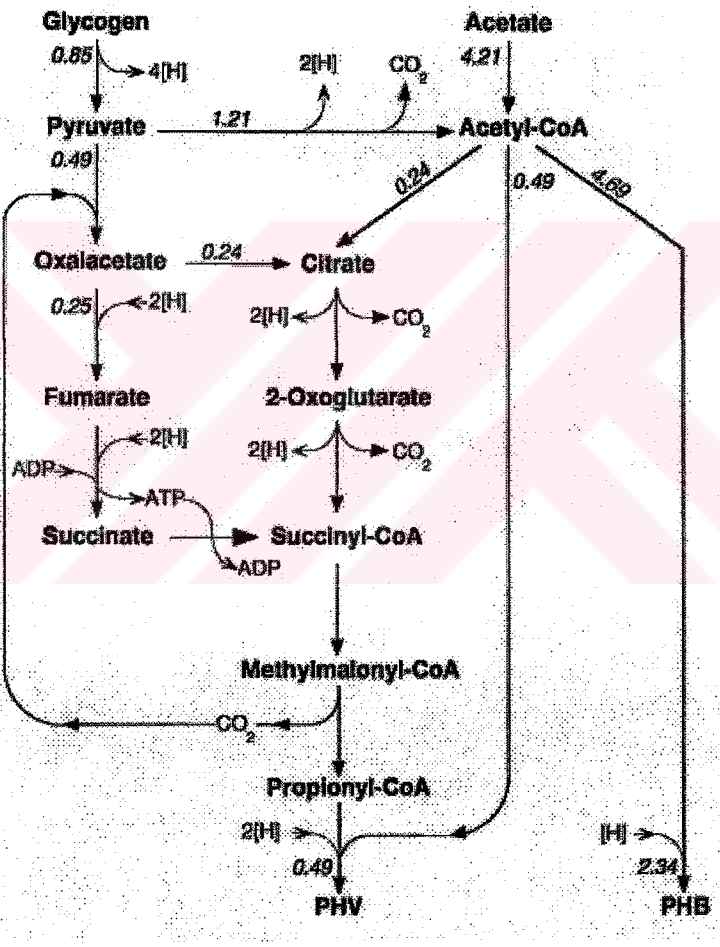



**Figure 2.6:** Anaerobic operation of TCA cycle (**Hesselman et al., 2000**)

**Kortstee et al. (2000)** proposed a tentative metabolic model based on the literature. The five characteristic features of this tentative scheme are (adapted from **Kortstee et al., 2000**): 1) conversion of glycogen into pyruvate via the the Entner.Doudoroff pathway [54]; 2) conversion of extracellular acetate into PHB via the well-known pathway and the conversion of acetyl-CoA plus propionyl-CoA into PHV; 3)

conversion of pyruvate (derived from glycogen) into propionyl- CoA might occur via transcarboxylation to oxaloacetate and subsequent reduction to methylmalonyl-CoA or via the first part of the citric acid cycle; 4) the citric acid cycle is incomplete since succinyl-CoA is converted into propionyl-CoA; 5) formation of propionyl-CoA from pyruvate via malate and fumarate requires reducing equivalents whereas formation of propionyl-CoA from pyruvate via the first part of the citric acid cycle is accompanied by the formation of reducing equivalents.

**Zeng et al. (2003a)** stated that PAO enrichment studies may create a significant amount of GAOs. They studied on PAO/GAO competition for acetate in a mixed culture. Consequently, they proposed a model-based data analysis method as an effective approach to determine the separate acetate uptake rates by PAOs and GAOs.

### 2.7.2 Metabolic models for VFA uptake by GAOs

It is first observed that decrease in observed phosphate release to acetate uptake ratios with substantial deterioration in phosphorus removal potential by Cech and Hartman (1990). Many attempts of researchers to explain varying parameters of phosphate, PHA, glycogen and carbon dioxide lead them to the presence of other types of bacteria that are capable of storing substrate under anaerobic conditions without using energy from phosphate release which is called glycogen accumulating organisms (GAOs).

Glycogen accumulating organisms (GAOs) take up acetate and stored as PHV enriched PHA, concurrently with significant consumption of glycogen (**Satoh et al., 1994; Liu et al., 1996; Filipe et al., 2001b**). These organisms provide energy and reducing power by glycogen degradation without any polyphosphate involvement.

**Satoh et al. (1994)** compared two energetic systems: polyphosphate and glycogen dependent systems. They found that glycogen is consumed simultaneously with acetate and propionate under anaerobic conditions by both systems. They suggested that the main role of the glycolysis is to supply reducing power in polyphosphate dependent system. However, glycogen is used as a source of both energy and reducing power in glycogen dependent system.

Based on the previous work of many researchers, a metabolic model for the stoichiometry of acetate uptake under anaerobic conditions by an enriched culture of

GAOs is developed by **Filipe et al. (2001b)**. They suggested that a fraction of the pyruvate produced by glycolysis is directed to the propionate-succinate pathway. Illustration of the metabolic model proposed by **Filipe at al. (2001b)** is given in Figure 2.7.

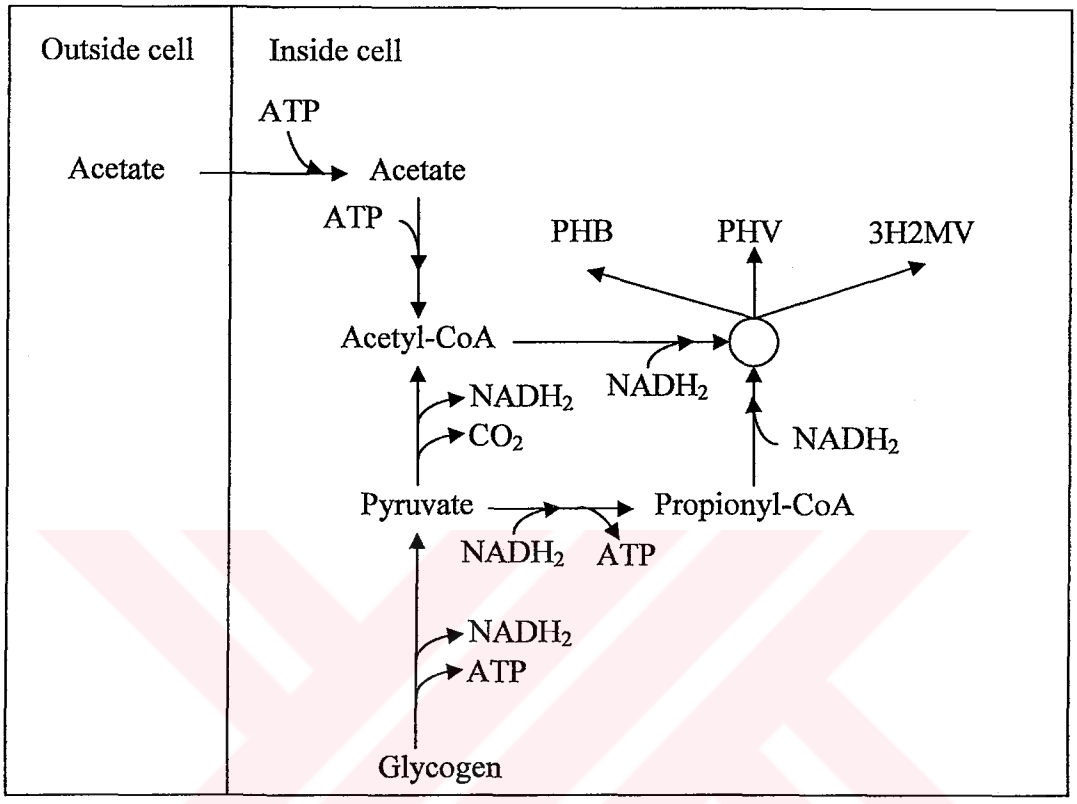



**Figure 2.7:** Schematic illustration of proposed metabolic model for glycogen accumulating organisms by **Filipe et al. (2001b)**

**Filipe et al. (2001b)** also observed that the stoichiometry for acetate uptake by GAOs was more favorable at lower pH values resembling the observations of Shuler and Jenkins (2002).

**Zeng et al. (2002)** described an anaerobic model for GAOs based on their metabolic pathways. The main difference between proposed models reported by **Zeng et al. (2002)** and **Filipe et al. (2001b)** is ATP production via propionate-succinate pathway. **Zeng et al. (2002)** did not taken into account consumption of one ATP from succinate to succinyl-CoA.

### 2.8 Mathematical Models

In order to assist the designers to predict performance of biological wastewater treatment systems under varying wastewater characteristics and operation conditions,

mathematic models were used as a powerful tool. Present kinetic models were developed and modified for better understanding of the processes in these systems, and evaluated against experimental data in the literature.

In 1983, the International Water Association (IWA) (formerly IAWQ) formed a task group to develop mathematical models for design and operation of biological wastewater treatment systems. Task Group for Mathematical Modeling for Design and Operation of Biological Wastewater Treatment proposed the Activated Sludge Model No.1 (ASM1) for organic matter and nitrogenous material removal by activated sludge systems which treat domestic wastewater (**Henze et al., 1987**). Many concepts of this model were adapted from the UCT (University of Cape Town) model, which is based on kinetic model developed by **Dold et al. (1980)** expended by **Van Haandel et al. (1981)** to include denitrification process.

The Task Group introduced biological phosphate removal organisms as a new group of organisms in the activated sludge into the ASM1 model based on mathematical model of EBPR developed by **Wentzel et al. (1986 and 1989)**. This model is published in 1995 and referred as Activated Sludge Model No.2 (ASM2) (**Henze et al., 1995**).

A general model for carbon, nitrogen and phosphorus removal is proposed by Barked and **Dold (1997)** by incorporating anoxic growth of PAOs. Denitrification is modeled with the assumption of that a fraction of the PAOs can use nitrate as an electron acceptor. Aerobic growth processes are duplicated for the anoxic conditions. Anoxic hydrolysis and lysis processes are considered. Fermentation of readily biodegradable organic matter is incorporated into this model. The authors divided the particulate non-biodegradable substrate into endogenous mass and particulate non-biodegradable matter. They stated that the particulate non-biodegradable substrate is correspondent to the inert, non-biodegradable component.

Researchers at the Technology University of Delft (TUD) developed a model for biological phosphorus removal, based on the bacterial metabolism (**Smolders et al., 1995; Murnleitner et al., 1997; van Veldhuizen et al., 1999**). **Smolders et al. (1995)** proposed a model, which includes glycogen metabolism of phosphorus accumulating organisms. **Murnleitner et al., (1997)** proposed a new kinetic structure that describes phosphorus removal under denitrifying and aerobic conditions with the

same kinetic equations and parameters. Conversion of glycogen to PHB is considered as a process under both denitrifying and aerobic conditions. In the TUD model, the maintenance concept is used and it is assumed that the organic substrate is consumed for growth and maintenance. The TUD model is verified over a range of sludge retention time values by **Smolders et al. (1995)** and different electron acceptors by **Murnleitner et al. (1997)** and validated with simulations of a sequencing batch reactor. **Brdjanovic et al. (1998)** combined the integrated model proposed by **Murnleitner et al. (1997)** and ASM2 model.

**Mino et al. (1995)** and **Manga et al. (2001)** introduced denitrification capability of PAOs by adding anoxic storage of polyphosphate (XPP) and anoxic growth of PAOs. In these studies, stoichiometry, kinetic equations and model coefficients of denitrification and related processes by PAOs were recommended. They set saturation coefficient for $S_{NO3}$ ($K_{NO3}$) at 0.50 $gN/m^3$ not only for denitrification by PAOs but also for denitrification by heterotrophs and anoxic polyphosphate storage. **Mino et al. (1995)** suggested that reduction factor for PAO growth ($\eta_{NO3}$) is equal to 0.60 gPP/gPAO.

ASM2 model subsequently extended to take into account denitrification capability of phosphate removal organisms based on the observations on anoxic phosphate uptake associated with denitrification in EBPR systems in the literature (**Kuba et al., 1996; Barker and Dold, 1996**). This model is published in 1995, which is called as Activated Sludge Model No.2d (ASM2d). This extension of ASM2 allows for improved modeling of the process, especially with respect to the dynamics of nitrate and phosphate (**Henze et al., 1999**).

These mechanistic models and proposed modifications of ASM2d were described and attempts to upgrade activated sludge model to include competition between phosphate and glycogen accumulating organisms based on evidences on existence of another bacteria in activated sludge systems, which store acetate under anaerobic conditions without providing energy from polyphosphate were reviewed below.

### 2.8.1 Activated sludge models for phosphate removal

#### 2.8.1.1 Activated Sludge Model No.2

The processes of three different organisms considered in ASM2 model: heterotrophic organisms, phosphorus accumulating organisms and nitrifying organisms (autotrophic organism). With the introduction of phosphorus accumulating organisms, cell internal storage products introduced particular components. These are $X_{PHA}$ (PHA, glycogen etc.) and $X_{PP}$ (polyphosphate), which occur only associated with PAOs ($X_{PAO}$).

VFAs widely accepted as major carbon source for PAOs in the literature. Therefore, readily biodegradable substrate ($S_S$) of ASM1 model divided into two separate soluble components as described fermentation products ($S_A$) and fermentable, readily biodegradable organic substrates ($S_F$) in ASM2 model. For the model, fermentation products considered to be acetate and fermentable, readily biodegradable organic substrates defined as that may serve as a substrate for fermentation.

Processes incorporated in ASM2 model were briefly described below.

- *Hydrolysis Processes*: ASM2d model incorporates aerobic, anoxic and anaerobic hydrolysis processes of colloidal materials ($X_S$) to fermentable, readily biodegradable organic substrates depends on the available electron acceptor by heterotrophic organisms.
- *Heterotrophic Organisms*: Growth and denitrification processes on fermentation products and fermentable, readily biodegradable organic substrates, and fermentation and lysis processes by heterotrophic organisms defined in the model. Fermentation is described as a simple transformation and not as a growth process.
- *Phosphorus Accumulating Organisms*: It is assumed that phosphate accumulating organisms store fermentation products in the form of polyhydroxyalkanoates ($X_{PHA}$) under anaerobic conditions. These organisms may store soluble phosphorus in the form of polyphosphate ($X_{PP}$) in the cell under aerobic conditions. This model assumes PAOs to grow only under aerobic conditions. Lysis process of storage materials ($X_{PHA}$ and $X_{PP}$) besides lysis of $X_{PAO}$ also described in the model.

- *Nitrification*: The phosphorus requirement for the growth is considered in the model. The model for nitrification is identical to ASM1 model.

### 2.8.1.2 Activated Sludge Model No.2d

Beyond the processes of phosphate accumulating organisms in ASM2 model, denitrifying fraction of PAOs introduced in Activated Sludge Model No.2d (ASM2d) in the light of experimental observations suggesting that PAOs can denitrify (**Mino et al., 1985; Kerrn-Jespersen and Henze, 1993**). ASM2d model is minor extension of ASM2 model (**Henze et al., 1999**).

It is assumed that PAOs can use cell internal storage products for denitrification and grow under anoxic conditions. Therefore, ASM2d model includes two additional processes: 1) Anoxic storage of $X_{PP}$ by PAOs, and 2) Anoxic growth of $X_{PAO}$.

Definitions of soluble and particulate components in ASM2d model are given in Table 2.4. Table 2.5, 2.6, 2.7 and 2.8 summarizes the stoichiometry of the hydrolysis processes, heterotrophic ($X_H$), phosphorus accumulating ($X_{PAO}$) and nitrifying ($X_{AUT}$) organisms, respectively.

**Table 2.4:** Definitions of soluble and particulate components in ASM2d model **(Henze et al., 1999)**

| Component | Definition | Unit |
|---|---|---|
| *Soluble components* | | |
| $S_A$ | Fermentation products, considered to be acetate | M(COD) $L^{-3}$ |
| $S_{ALK}$ | Alkalinity of the wastewater | mol($HCO_3^-$) $L^{-3}$ |
| $S_F$ | Fermentable, readily biodegradable organic substrate | M(COD) $L^{-3}$ |
| $S_I$ | Inert soluble organic material | M(COD) $L^{-3}$ |
| $S_{N2}$ | Dinitrogen, $N_2$ | M(N) $L^{-3}$ |
| $S_{NH4}$ | Ammonium plus ammonia nitrogen | M(N) $L^{-3}$ |
| $S_{NO3}$ | Nitrate plus nitrite nitrogen | M(N) $L^{-3}$ |
| $S_{O2}$ | Dissolved oxygen | M($O_2$) $L^{-3}$ |
| $S_{PO4}$ | Inorganic soluble phosphorus, primarily orthophosphate | M(P) $L^{-3}$ |
| $S_S$ | Readily biodegradable substrate | M(COD) $L^{-3}$ |
| *Particulate components* | | |
| $X_{AUT}$ | Nitrifying organisms | M(COD) $L^{-3}$ |
| $X_H$ | Heterotrophic organisms | M(COD) $L^{-3}$ |
| $X_I$ | Inert particulate organic material | M(COD) $L^{-3}$ |
| $X_{MeOH}$ | Metal-hydroxides | M(TSS) $L^{-3}$ |
| $X_{MeP}$ | Metal-phosphate, $MePO_4$ | M(TSS) $L^{-3}$ |
| $X_{PAO}$ | Phosphate accumulating organisms | M(COD) $L^{-3}$ |
| $X_{PHA}$ | A cell internal storage product of PAO | M(COD) $L^{-3}$ |
| $X_{PP}$ | Poly-phosphate | M(P) $L^{-3}$ |
| $X_S$ | Slowly biodegradable substrates | M(COD) $L^{-3}$ |
| $X_{TSS}$ | Total suspended solids, TSS | M(TSS) $L^{-3}$ |

**Table 2.5:** Stoichiometry of the hydrolysis processes (**Henze et al., 1999**)

| Process | $S_F$ | $S_{NH4}$ | $S_{PO4}$ | $S_I$ | $S_{ALK}$ | $X_S$ | $X_{TSS}$ |
|---|---|---|---|---|---|---|---|
| 1 Aerobic hydrolysis | $1-f_{SI}$ | $\nu_{1,NH4}$ | $\nu_{1,PO4}$ | $f_{SI}$ | $\nu_{1,ALK}$ | -1 | $\nu_{1,TSS}$ |
| 2 Anoxic hydrolysis | $1-f_{SI}$ | $\nu 2_{,NH4}$ | $\nu_{2,PO4}$ | $f_{SI}$ | $\nu_{2,ALK}$ | -1 | $\nu_{2,TSS}$ |
| 3 Anaerobic hydrolysis | $1-f_{SI}$ | $\nu_{3,NH4}$ | $\nu_{3,PO4}$ | $f_{SI}$ | $\nu_{3,ALK}$ | -1 | $\nu_{3,TSS}$ |

**Table 2.6:** Stoichiometry of the heterotrophic organisms (**Henze et al., 1999**)

| Process | $S_{O2}$ | $S_F$ | $S_A$ | $S_{NO3}$ | $S_{N2}$ | $X_I$ | $X_S$ | $X_H$ |
|---|---|---|---|---|---|---|---|---|
| 4 Aerobic growth on $S_F$ | $1-\frac{1}{Y_H}$ | $-\frac{1}{Y_H}$ | | | | | | 1 |
| 5 Aerobic growth on $S_A$ | $1-\frac{1}{Y_H}$ | | $-\frac{1}{Y_H}$ | | | | | 1 |
| 6 Anoxic growth on $S_F$ | | $-\frac{1}{Y_H}$ | | $-\frac{1-Y_H}{2.86\cdot Y_H}$ | $\frac{1-Y_H}{2.86\cdot Y_H}$ | | | 1 |
| 7 Anoxic growth on $S_A$, Denitrification | | | $-\frac{1}{Y_H}$ | $-\frac{1-Y_H}{2.86\cdot Y_H}$ | $\frac{1-Y_H}{2.86\cdot Y_H}$ | | | 1 |
| 8 Fermentation | | -1 | 1 | | | | | |
| 9 Lysis | | | | | | $f_{XI}$ | $1-f_{XI}$ | -1 |

**Table 2.7:** Stoichiometry of the phosphorus accumulating organisms (**Henze et al., 1999**)

| Process | $S_{O2}$ | $S_A$ | $S_{N2}$ | $S_{NO3}$ | $S_{PO4}$ | $X_I$ | $X_S$ | $X_{PAO}$ | $X_{PP}$ | $X_{PHA}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 Storage of $X_{PHA}$ | | -1 | | | $Y_{PO4}$ | | | | $-Y_{PO4}$ | |
| 11 Aerobic storage of $X_{PP}$ | $-Y_{PHA}$ | | | | -1 | | | | 1 | $-Y_{PHA}$ |
| 12 Anoxic storage of $X_{PP}$ | | | $-\nu_{12,NO3}$ | $\nu_{12,NO3}$ | -1 | | | | 1 | $-Y_{PHA}$ |
| 13 Aerobic growth of $X_{PAO}$ | $\nu_{13,O2}$ | | | | $-i_{PBM}$ | | | 1 | | $-1/Y_H$ |
| 14 Anoxic growth of $X_{PAO}$ | | | $-\nu_{14,NO3}$ | $\nu_{14,NO3}$ | $-i_{PBM}$ | | | 1 | | $-1/Y_H$ |
| 15 Lysis of $X_{PAO}$ | | | | | $\nu_{15,PO4}$ | $f_{XI}$ | $1-f_{XI}$ | -1 | | |
| 16 Lysis of $X_{PP}$ | | | | | 1 | | | | -1 | |
| 17 Lysis of $X_{PHA}$ | | 1 | | | | | | | | -1 |

**Table 2.8:** Stoichiometry of the nitrifying organisms (**Henze et al., 1999**)

| Process | $S_{O2}$ | $S_{NH4}$ | $S_{NO3}$ | $S_{PO4}$ | $X_I$ | $X_S$ | $X_{AUT}$ |
|---|---|---|---|---|---|---|---|
| 18 Aerobic growth of $X_{AUT}$ | $-\frac{4.57-Y_A}{Y_A}$ | $\nu_{18,NH4}$ | $\frac{1}{Y_A}$ | $-i_{PBM}$ | | | 1 |
| 19 Lysis | | $\nu_{19,NH4}$ | | $\nu_{19,PO4}$ | $f_{XI}$ | $1-f_{XI}$ | -1 |

Process rate equations are given in Table 2.9.

**Table 2.9:** Process rate equations for ASM2d (**Henze et al., 1999**)

| Process | Process rate equations $\rho_j$, $\rho_j \geq 0$ $[ML^{-3}T^{-1}]$ |
|---|---|
| *Hydrolysis Processes* | |
| 1 Aerobic Hydrolysis | $K_h \cdot \frac{S_{O2}}{K_{O2}+S_{O2}} \cdot \frac{X_S/X_H}{K_X+X_S/X_H} \cdot X_H$ |
| 2 Anoxic Hydrolysis | $K_h \cdot \eta_{NO3} \cdot \frac{K_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3}+S_{NO3}} \cdot \frac{X_S/X_H}{K_X+X_S/X_H} \cdot X_H$ |
| 3 Anaerobic Hydrolysis | $K_h \cdot \eta_{NO3} \cdot \frac{K_{O2}}{K_{O2}+S_{O2}} \cdot \frac{K_{NO3}}{K_{NO3}+S_{NO3}} \cdot \frac{X_S/X_H}{K_X+X_S/X_H} \cdot X_H$ |
| *Heterotrophic organisms: $X_H$* | |
| 4 Growth on fermentable substrates, $S_F$ | $\hat{\mu}_H \cdot \frac{S_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{S_F}{S_F+S_A} \cdot \frac{S_{NH4}}{K_{NH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot X_H$ |
| 5 Growth on fermentation products, $S_A$ | $\hat{\mu}_H \cdot \frac{S_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_A}{K_A+S_A} \cdot \frac{S_A}{S_F+S_A} \cdot \frac{S_{NH4}}{K_{NH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot X_H$ |
| 6 Denitrification with fermentable substrates, $S_F$ | $\hat{\mu}_H \cdot \eta_{NO3} \frac{K_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3}+S_{NO3}} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{S_F}{S_F+S_A} \cdot \frac{S_{NH4}}{K_{NH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot X_H$ |
| 7 Denitrification with fermentation products $S_A$ | $\hat{\mu}_H \cdot \eta_{NO3} \frac{K_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3}+S_{NO3}} \cdot \frac{S_A}{K_A+S_A} \cdot \frac{S_A}{S_F+S_A} \cdot \frac{S_{NH4}}{K_{NH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot X_H$ |
| 8 Fermentation | $q_{fe} \cdot \frac{K_{O2}}{K_{O2}+S_{O2}} \cdot \frac{K_{NO3}}{K_{NO3}+S_{NO3}} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot X_H$ |
| 9 Lysis | $b_H \cdot X_H$ |
| *Phosphorus accumulating products (PAO): $X_{PAO}$* | |
| 10 Storage of $X_{PHA}$ | $q_{PHA} \cdot \frac{S_A}{K_A+S_A} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot \frac{X_{PP}/X_{PAO}}{K_{PP}+X_{PP}/X_{PAO}} \cdot X_{PAO}$ |
| 11 Aerobic storage of $X_{PP}$ | $q_{PP} \cdot \frac{S_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_{PO4}}{K_{PS}+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}} \cdot X_{PAO}$ |
| 12 Anoxic storage of $X_{PP}$ | $\rho_{12} = \rho_{11} \cdot \eta_{NO3} \frac{K_{O2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3}+S_{NO3}}$ |
| 13 Aerobic growth on $X_{PHA}$ | $\hat{\mu}_{PAO} \cdot \frac{S_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{NH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} X_{PAO}$ |
| 14 Anoxic growth on $X_{PHA}$ | $\rho_{14} = \rho_{13} \cdot \eta_{NO3} \frac{K_{O2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3}+S_{NO3}}$ |
| 15 Lysis of $X_{PAO}$ | $b_{PAO} \cdot X_{PAO} \cdot S_{ALK} / (K_{ALK} + S_{ALK})$ |
| 16 Lysis of $X_{PP}$ | $b_{PP} \cdot X_{PP} \cdot S_{ALK} / (K_{ALK} + S_{ALK})$ |
| 17 Lysis of $X_{PHA}$ | $b_{PHA} \cdot X_{PHA} \cdot S_{ALK} / (K_{ALK} + S_{ALK})$ |
| Nitrifying organisms (autotrophic organisms): $X_{AUT}$ | |
| 18 Aerobic growth of $X_{AUT}$ | $\mu_{AUT} \cdot \frac{S_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_{NH4}}{K_{NH4}+S_{NH4}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot X_{AUT}$ |
| 19 Lysis of $X_{AUT}$ | $b_{AUT} \cdot X_{AUT}$ |

Typical concentrations of relevant model components in the wastewater are presented in Table 2.10. Typical values for the stoichiometric coefficients of ASM2d are given in Table 2.11.

**Table 2.10:** Short definition of model components and typical wastewater composition (**Henze et al., 1999**)

| $COD_{tot}$=260 $gCODm^{-3}$, TKN=25 $gNm^{-3}$, TP= 6$gPm^{-3}$ | | | |
|---|---|---|---|
| *Soluble components* | | | |
| $S_{O2}$ | Dissolved oxygen | 0 | g $O_2$ $m^{-3}$ |
| $S_F$ | Readily biodegradable substrate | 30 | g COD $m^{-3}$ |
| $S_A$ | Fermentation products (acetate) | 20 | g COD $m^{-3}$ |
| $S_{NH4}$ | Ammonium | 16 | g N $m^{-3}$ |
| $S_{NO3}$ | Nitrate (plus nitrite) | 0 | g N $m^{-3}$ |
| $S_{PO4}$ | Phosphate | 3.6 | g P $m^{-3}$ |
| $S_I$ | Inert, bon-biodegradable organics | 30 | g COD $m^{-3}$ |
| $S_{ALK}$ | Bicarbonate alkalinity | 5 | mol $HCO_3^-$ $m^{-3}$ |
| *Particulate components* | | | |
| $X_I$ | Inert, non-biodegradable organics | 30-150 | g COD $m^{-3}$ |
| $X_S$ | Slowly biodegradable substrate | 80-600 | g COD $m^{-3}$ |
| $X_H$ | Heterotrophic biomass | 20-120 | g COD $m^{-3}$ |
| $X_{PAO}$ | Phosphorus-accumulating organisms, PAO | 0-1 | g COD $m^{-3}$ |
| $X_{PP}$ | Stored poly-phosphate PAO | 0-0.5 | g P $m^{-3}$ |
| $X_{PHA}$ | Organic storage products of PAO | 0-1 | g COD $m^{-3}$ |
| $X_{AUT}$ | Autotrophic, nitrifying biomass | 0-1 | g COD $m^{-3}$ |

**Table 2.11:** Definition and typical values for the stoichiometric coefficients of ASM2d (**Henze et al., 1999**)

| Typical conversion factors for conservation equation | | | |
|---|---|---|---|
| Nitrogen: | | | |
| Soluble material: | | | |
| $i_{NSI}$ | N content of inert soluble COD $S_I$ | 0.01 | g N $g^{-1}$COD |
| $i_{NSF}$ | N content of fermentable substrates $S_F$ | 0.03 | g N $g^{-1}$ COD |
| Particulate material: | | | |
| $i_{NXI}$ | N content of inert particulate COD $X_I$ | 0.02 | g N $g^{-1}$ COD |
| $i_{NXS}$ | N content of slowly degradable substrate $X_S$ | 0.04 | g N $g^{-1}$ COD |
| $i_{NBM}$ | N content of biomass, $X_H$,$X_{PAO}$,$X_{AUT}$ | 0.07 | g N $g^{-}$ COD |
| Phosphorus: | | | |
| Soluble material: | | | |
| $i_{PSI}$ | P content of inert soluble COD $S_I$ | 0.00 | g P $g^{-1}$ COD |
| $i_{PSF}$ | P content of fermentable substrates $S_F$ | 0.01 | g P $g^{-1}$ COD |
| Particulate material: | | | |
| $i_{PXI}$ | P content of inert particulate COD $X_I$ | 0.01 | g P $g^{-1}$ COD |
| $i_{PXS}$ | P content of slowly degradable substrate $X_S$ | 0.01 | g P $g^{-1}$ COD |
| $i_{PBM}$ | P content of biomass, $X_H$,$X_{PAO}$,$X_{AUT}$ | 0.02 | g P $g^{-1}$ COD |
| Total Suspended Solids (TSS): | | | |
| $i_{TSSXI}$ | TSS to COD for $X_I$ | 0.75 | g TSS $g^{-1}$ COD |
| $i_{TSSXS}$ | TSS to COD ratio for $X_S$ | 0.75 | g TSS $g^{-1}$ COD |
| $i_{TSSBM}$ | TSS to COD ratio for $X_H$,$X_{PAO}$,$X_{AUT}$ | 0.90 | g TSS $g^{-1}$ COD |
| Typical stoichiometric parameters | | | |
| Hydrolysis | | | |
| $f_{SI}$ | Production of $S_I$ in hydrolysis | 0 | g COD $g^{-1}$ COD |
| Heterotrophic biomass: $X_H$ | | | |
| $Y_H$ | Yield Coefficient | 0.625 | g COD $g^{-1}$ COD |
| $f_{XI}$ | Fraction of inert COD generated in biomass lysis | 0.10 | g COD $g^{-1}$ COD |
| Phosphorus accumulating organisms :$X_{PAO}$ | | | |
| $Y_{PAO}$ | Yield Coefficient (biomass/ PHA) | 0.625 | g COD $g^{-1}$ COD |
| $Y_{PO4}$ | PP requirement ($PO_4$ release) per PHA stored | 0.40 | g P $g^{-1}$ COD |
| $Y_{PHA}$ | PHA requirement for PP storage | 0.20 | g COD $g^{-1}$P |
| $f_{XI}$ | Fraction of inert COD generated in biomass lysis | 0.10 | g COD $g^{-1}$ COD |
| Nitrifying organisms: $X_{AUT}$ | | | |
| $Y_A$ | Yield of autotrophic biomass per $NO_3^-$-N | 0.24 | g COD $g^{-1}$N |
| $f_{XI}$ | Fraction of inert COD generated in biomass lysis | 0.10 | g COD $g^{-1}$ COD |

Definition and typical values for the kinetic coefficients of ASM2d were reported as given in Table 2.12.

**Table 2.12:** Definition and typical values for the kinetic coefficients of ASM2d **(Henze et al., 1999)**

| | Temperature | 20° | 10° | Units |
|---|---|---|---|---|
| Hydrolysis of particulate substrate: $X_S$ | | | | |
| $K_h$ | Hydrolysis rate constant | 3.00 | 2.00 | $d^{-1}$ |
| $\eta_{NO3}$ | Anoxic hydrolysis reduction factor | 0.60 | 0.60 | - |
| $\eta_{fe}$ | Anaerobic hydrolysis reduction factor | 0.40 | 0.40 | - |
| $K_{O2}$ | Saturation/inhabitation coefficient for oxygen | 0.20 | 0.20 | $g\ O_2\ m^{-3}$ |
| $K_{NO3}$ | Saturation/inhabitation coefficient for nitrate | 0.50 | 0.50 | $g\ N\ m^{-3}$ |
| $K_X$ | Saturation coefficient for particulate COD | 0.10 | 0.10 | $g\ X_S\ g^{-1}\ X_H$ |
| Heterotrophic organisms: $X_H$ | | | | |
| $\mu_H$ | Maximum growth rate on substrate | 6.00 | 3.00 | $g\ X_S\ g^{-1}\ X_H\ d^{-1}$ |
| $q_{fe}$ | Maximum rate for fermentation | 3.00 | 1.50 | $g\ S_F\ g^{-1}\ X_H\ d^{-1}$ |
| $\eta_{NO3}$ | Reduction factor for denitrification | 0.80 | 0.80 | - |
| $b_H$ | Rate constant for lysis and decay | 0.40 | 0.20 | $d^{-1}$ |
| $K_{O2}$ | Saturation/inhabitation coefficient for oxygen | 0.20 | 0.20 | $g\ O_2\ m^{-3}$ |
| $K_F$ | Saturation coefficient for growth on $S_F$ | 4.00 | 4.00 | $g\ COD\ m^{-3}$ |
| $K_{fe}$ | Saturation coefficient for fermentation of $S_F$ | 4.00 | 4.00 | $g\ COD\ m^{-3}$ |
| $K_A$ | Saturation coefficient for growth on acetate $S_A$ | 4.00 | 4.00 | $g\ COD\ m^{-3}$ |
| $K_{NO3}$ | Saturation/inhabitation coefficient for nitrate | 0.50 | 0.50 | $g\ N\ m^{-3}$ |
| $K_{NH4}$ | Saturation coefficient for ammonium (nutrient) | 0.05 | 0.05 | $g\ N\ m^{-3}$ |
| $K_P$ | Saturation coefficient phosphate (nutrient) | 0.01 | 0.01 | $g\ P\ m^{-3}$ |
| $K_{ALK}$ | Saturation coefficient for alkalinity ($HCO_3^-$) | 0.10 | 0.10 | $mol\ HCO_3^-\ m^{-3}$ |
| Phosphorus accumulating organisms: $X_{PAO}$ | | | | |
| $q_{PHA}$ | Rate constant for storage $X_{PHA}$ ( base $X_{PP}$) | 3.00 | 2.00 | $g\ X_{PHA}\ g^{-1}\ X_{PAO}\ d^{-1}$ |
| $q_{PP}$ | Rate constant for storage $X_{PP}$ | 1.50 | 1.00 | $g\ X_{PP}\ g^{-1}\ X_{PAO}\ d^{-1}$ |
| $\mu_{PAO}$ | Maximum growth rate of PAO | 1.00 | 0.67 | $d^{-1}$ |
| $\eta_{NO3}$ | Reduction factor for anoxic activity | 0.60 | 0.60 | - |
| $b_{PAO}$ | Rate for Lysis of $X_{PAO}$ | 0.20 | 0.10 | $d^{-1}$ |
| $b_{PP}$ | Rate for Lysis of $X_{PP}$ | 0.20 | 0.10 | $d^{-1}$ |
| $b_{PHA}$ | Rate for Lysis of $X_{PHA}$ | 0.20 | 0.10 | $d^{-1}$ |
| $K_{O2}$ | Saturation/inhabitation coefficient for oxygen | 0.20 | 0.20 | $G\ O_2\ m^{-3}$ |
| $K_{NO3}$ | Saturation/inhabitation coefficient for nitrate | 0.50 | 0.50 | $G\ N\ m^{-3}$ |
| $K_A$ | Saturation coefficient for growth on acetate, $S_A$ | 4.00 | 4.00 | $G\ COD\ m^{-3}$ |
| $K_{NH4}$ | Saturation coefficient for ammonium (nutrient) | 0.05 | 0.05 | $G\ N\ m^{-3}$ |
| $K_{PS}$ | Saturation coefficient for phosphorus in storage of PP | 0.20 | 0.20 | $G\ P\ m^{-3}$ |
| $K_P$ | Saturation coefficient phosphate (nutrient) | 0.01 | 0.01 | $G\ P\ m^{-3}$ |
| $K_{ALK}$ | Saturation coefficient for alkalinity ($HCO_3^-$) | 0.10 | 0.10 | $mol\ HCO_3^-\ m^{-3}$ |
| $K_{PP}$ | Saturation coefficient for poly-phosphate | 0.01 | 0.01 | $G\ X_{PP}\ g^{-1}\ X_{PAO}$ |
| $K_{MAX}$ | Maximum ratio of $X_{PP}/X_{PAO}$ | 0.34 | 0.34 | $G\ X_{PP}\ g^{-1}\ X_{PAO}$ |
| $K_{IPP}$ | Inhibition coefficient for PP storage | 0.02 | 0.02 | $G\ X_{PP}\ g^{-1}\ X_{PAO}$ |
| $K_{PHA}$ | Saturation coefficient for PHA | 0.01 | 0.01 | $g\ X_{PHA}\ g^{-1}\ X_{PAO}$ |
| Nitrifying organisms (autotrophic organisms) :$X_{AUT}$ | | | | |
| $\mu_{AUT}$ | Maximum growth rate of $X_{AUT}$ | 1.00 | 0.35 | $d^{-1}$ |
| $b_{AUT}$ | Decay rate of $X_{AUT}$ | 0.15 | 0.05 | $d^{-1}$ |
| $K_{O2}$ | Saturation coefficient for oxygen | 0.50 | 0.50 | $g\ O_2\ m^{-3}$ |
| $K_{NH4}$ | Saturation coefficient for ammonium (substrate) | 1.00 | 1.00 | $g\ N\ m^{-3}$ |
| $K_{ALK}$ | Saturation coefficient for alkalinity ($HCO_3^-$) | 0.50 | 0.50 | $mol\ HCO_3^-\ m^{-3}$ |
| $K_P$ | Saturation coefficient for phosphorus (nutrient) | 0.01 | 0.01 | $g\ P\ m^{-3}$ |

### 2.8.2 Attempts to upgrade Activated Sludge Model to include glycogen storage and processes associated with glycogen accumulating organisms

**Mino et al. (1995)** proposed sub models to introduce glycogen storage by PAOs and processes related to glycogen storage including glycogen lysis processes to the ASM2 model. Glycogen involvement is modeled by **Mino et al. (1995)** introducing glycogen as a new parameter, which is accumulated under aerobic conditions and consumed as a carbon source under anaerobic conditions. Stoichiometric constant of fermentation products ($S_A$) requirement for PHA storage is defined as $Y_{SA}$ which is defined as equal to 1 in the ASM2 model. They estimated that the ratio of consumed glycogen to synthesized PHA, which is equal to ($1-Y_{SA}$), is 0.33 for acetate and 0.22 for propionate. The stoichiometry of the glycogen storage incorporated model for PAOs called as "the GLP model" is summarized in Table 2.13.

**Table 2.13:** Stoichiometry of processes associated with glycogen storing PAOs **(Mino et al., 1995)**

| Processes | $S_{O2}$ | $S_F$ | $S_A$ | $S_{PO4}$ | $X_{PAO}$ | $X_{PHA}$ | $X_{PP}$ | $X_{Gly}$ | $X_I$ | $X_S$ |
|---|---|---|---|---|---|---|---|---|---|---|
| PHA storage | | | $-Y_{SA}$ | $Y_{PO4}$ | | 1 | $-Y_{PO4}$ | $-(1-Y_{SA})$ | | |
| PAO growth | $1-\frac{1}{Y_{PAO}}$ | | | $-i_{PBM}$ | 1 | $-1/Y_{PAO}$ | | | | |
| PP storage | $-Y_{PHA}$ | | | -1 | | $Y_{PHA}$ | 1 | | | |
| Glycogen storage | | | | | | -1 | | 1 | | |
| Lysis of $X_{PAO}$ | | | | $v_P$ | -1 | | | | $f_H$ | $1-f_H$ |
| Lysis of $X_{PHA}$ | | | 1 | | | -1 | | | | |
| Lysis of $X_{PP}$ | | | | 1 | | | -1 | | | |
| Lysis of $X_{Gly}$ | | 1 | | | | | | -1 | | |

Rate equations for processes of the glycogen storage incorporated model for PAOs called as "the GLP model" is summarized in Table 2.14.

**Table 2.14:** Stoichiometry of processes associated with glycogen storing PAOs **(Mino et al., 1995)**

| Processes | Reaction rate equations |
|---|---|
| PHA storage | $q_{PHA} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_A}{S_A + S_F} \cdot \frac{X_{PP}/X_{PAO}}{K_{PP} + X_{PP}/X_{PAO}} \cdot \frac{X_{Gly}/X_{PAO}}{K_{Gly} + X_{Gly}/X_{PAO}} \cdot X_{PAO}$ |
| PAO growth | $\hat{\mu}_{PAO} \cdot M_{NH4} \cdot M_{ALK} \cdot M_{PO4} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA,PAO} + X_{PHA}/X_{PAO}} X_{pAO}$ |
| PP storage | $q_{PP} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_{PO4}}{K_P + S_{PO4}} \cdot \frac{K_{MAX} - X_{PP}/X_{PAO}}{K_{PHA,PP} + (K_{MAX} - X_{PP}/X_{PAO})} \cdot X_{PAO}$ |
| Glycogen storage | $q_{Gly} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA,Gly} + X_{PHA}/X_{PAO}} \cdot X_{PAO}$ |
| Lysis of $X_{PAO}$ | $b_{PAO} \cdot M_{ALK} \cdot X_{PAO}$ |
| Lysis of $X_{PHA}$ | $b_{PHA} \cdot M_{ALK} \cdot X_{PAO}$ |
| Lysis of $X_{PP}$ | $b_{PP} \cdot M_{ALK} \cdot X_{PAO}$ |
| Lysis of $X_{Gly}$ | $b_{Gly} \cdot M_{ALK} \cdot X_{PAO}$ |

$M_{NH4}$, $M_{ALK}$, $M_{PO4}$: Monod terms for nutrient dependency.

Values of proposed coefficients of glycogen storage incorporated model for PAOs called as "the GLP model" is summarized in Table 2.15. Other parameters were identical with the values of ASM2 model.

**Table 2.15:** Values of proposed coefficients for processes associated with glycogen storing PAOs (**Mino et al., 1995**)

| | Symbol | Parameters | Proposed | Unit |
|---|---|---|---|---|
| Stoichiometric constants | $Y_{SA}$ | $S_A$ requirement for PHA storage | 0.70 | gCOD/gCOD |
| Kinetic constants | $q_{Gly}$ | Rate constant for glycogen storage | 3.00 | gCOD/(gPAO•d) |
| | $K_{Gly}$ | Saturation coefficient for $X_{Gly}$ | 0.01 | gCOD/gPAO |
| | $K_{PHA/Gly}$ | Saturation coefficient for $X_{PHA}$ for $X_{Gly}$ storage | 0.10 | gCOD/gPAO |
| | $b_{Gly}$ | Decay rate constant of glycogen | 0.20 | 1/d |

**Mino et al. (1995)** also reported a modification to include processes associated with glycogen accumulating organisms, which is referred as "the GAO model". Characteristics of processes of GAOs are briefly defined as given below.

- Capability to take up $S_A$, $S_F$ and glycogen as carbon sources to store PHA without involvement of polyphosphate under anaerobic conditions,
- Capability to store glycogen using anaerobically stored PHA in the cell under aerobic conditions.

The stoichiometry of processes associated with glycogen accumulating organisms is summarized in Table 2.16.

**Table 2.16:** Stoichiometry of processes associated with glycogen accumulating organisms (**Mino et al., 1995**)

| Processes | $S_{O2}$ | $S_F$ | $S_A$ | $X_{GAO}$ | $X_{PHA}$ | $X_{Gly}$ | $X_I$ | $X_S$ |
|---|---|---|---|---|---|---|---|---|
| PHA storage on $S_A$ | | | $-Y_{SA}$ | | 1 | $-(1-Y_{SA})$ | | |
| PHA storage on $S_F$ | | $-Y_{SF}$ | | | 1 | $-(1-Y_{SF})$ | | |
| GAO growth | $1-\frac{1}{Y_{GAO}}$ | | | 1 | $-1/Y_{GAO}$ | | | |
| Glycogen storage | | | | | -1 | 1 | | |
| Lysis of $X_{GAO}$ | | | | -1 | | | $f_H$ | $1-f_H$ |
| Lysis of $X_{PHA}$ | | | 1 | | -1 | | | |
| Lysis of $X_{PGly}$ | | 1 | | | | -1 | | |

Rate equations for processes associated with glycogen accumulating organisms is summarized in Table 2.17.

**Table 2.17:** Rate equations for processes associated with glycogen accumulating organisms (**Mino et al., 1995**)

| Processes | Reaction rate equations |
|---|---|
| PHA storage on $S_A$ | $q_{PHA} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_A}{S_A + S_F} \frac{X_{Gly}/X_{GAO}}{K_{Gly} + X_{Gly}/X_{GAO}} \cdot X_{GAO}$ |
| PHA storage on $S_F$ | $q_{PHA} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_F}{S_A + S_F} \frac{X_{Gly}/X_{GAO}}{K_{Gly} + X_{Gly}/X_{GAO}} \cdot X_{GAO}$ |
| GAO growth | $\hat{\mu}_{GAO} \cdot M_{NH4} . M_{ALK} . M_{PO4} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot \frac{X_{PHA}/X_{GAO}}{K_{PHA,GAO} + X_{PHA}/X_{GAO}} X_{GAO}$ |
| Glycogen storage | $q_{Gly} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot \frac{X_{PHA}/X_{GAO}}{K_{PHA,Gly} + X_{PHA}/X_{GAO}} \cdot X_{GAO}$ |
| Lysis of $X_{GAO}$ | $b_{GAO} . M_{ALK} . X_{GAO}$ |
| Lysis of $X_{PHA}$ | $b_{PHA} . M_{ALK} . X_{GAO}$ |
| Lysis of $X_{Gly}$ | $b_{Gly} . M_{ALK} . X_{GAO}$ |

$M_{NH4}$, $M_{ALK}$, $M_{PO4}$: Monod terms for nutrient dependency.

In order to take into account competition between phosphorus and glycogen accumulating organisms in EBPR systems, **Manga et al. (2001)** proposed a modification to the ASM2 model to include processes of these organisms including glycogen storage by PAOs. The competition is attributed to the low phosphorus to acetate ratio in the influent by the authors.

In modified model proposed with the aim of upgrading ASM2 model by **Manga et al. (2001)**, new components of the model are defined as glycogen accumulating organisms ($X_{GAO}$), glycogen as a cell internal storage product of PAOs ($X_{GLY}$) and GAOs ($X_{GLY,G}$), and PHA as a cell internal storage product of GAOs ($X_{PHA,G}$). Five new processes (anoxic polyphosphate storage, anoxic growth of phosphorus accumulating organisms, aerobic glycogen storage, anoxic glycogen storage, lysis of

glycogen) are added to PAO metabolism and PHA storage process by PAOs is modified. Reaction rates for phosphorus and glycogen accumulating organism is given in Table 2.18 for this modified model proposed by **Manga et al. (2001)**.

**Table 2.18:** Process rates (**Manga et al., 2001**)

| Process | Process rate |
|---|---|
| *Phosphorus accumulating organisms (PAO):$X_{PAO}$* | |
| 1 Storage of $X_{PHA}$ | $q_{PHA} \cdot M_{SA} . M_{SALK} \cdot \frac{X_{PP}/X_{PAO}}{K_{PP}+X_{PP}/X_{PAO}} \cdot \frac{X_{GLY}/X_{PAO}}{K_{GLY}+X_{GLY}/X_{PAO}} . X_{PAO}$ |
| 2 Aerobic storage of $X_{PP}$ | $q_{PP} \cdot M_{SO2} \cdot M_{SPO4} \cdot M_{SALK} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA-P}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+(K_{MAX}-X_{PP}/X_{PAO})} \cdot X_{PAO}$ |
| 3 Anoxic storage of $X_{PP}$ | $q_{PP} \cdot \eta_{PAO} . N_{SO2} \cdot M_{SPO4} \cdot M_{ALK} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+(K_{MAX}-X_{PP}/X_{PAO})} \cdot X_{PAO}$ |
| 4 Aerobic growth | $\hat{\mu}_{PAO} \cdot M_{SO2} \cdot M_{NH4} \cdot M_{SPO4} \cdot M_{SALK} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} X_{PAO}$ |
| 5 Anoxic growth | $\hat{\mu}_{PAO} \cdot \eta_{PAO} . N_{SO2} \cdot M_{NO3} . M_{NH4} \cdot M_{SPO4} \cdot M_{SALK} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} X_{PAO}$ |
| 6 Aerobic growth of $X_{GLY}$ | $q_{GLY} \cdot M_{SO2} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA-GLY}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MG}-X_{GLY}/X_{PAO}}{K_{IG}+(K_{MG}-X_{GLY}/X_{PAO})} . X_{PAO}$ |
| 7 Anoxic growth of $X_{GLY}$ | $q_{GLY} \cdot h_{PAO} . N_{SO2} \cdot M_{NO3} . M_{SALK} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA-GLY}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MG}-X_{GLY}/X_{PAO}}{K_{IG}+(K_{MG}-X_{GLY}/X_{PAO})} . X_{PAO}$ |
| 8 Lysis of $X_{PAO}$ | $b_{PAO} . M_{SALK} . X_{PAO}$ |
| 9 Lysis of $X_{PP}$ | $b_{PP} . M_{SALK} . X_{PP}$ |
| 10 Lysis of $X_{PHA}$ | $b_{PHA} . M_{SALK} . X_{PHA}$ |
| 11 Lysis of $X_{GLY}$ | $b_{GLY} . M_{SALK} . X_{GLY}$ |
| *Glycogen accumulating products (GAO):$X_{GAO}$* | |
| 12 Storage of $X_{PHA,G}$ | $q_{PHA,G} \cdot M_{SA} . M_{SALK} \cdot \frac{X_{GLY,G}/X_{GAO}}{K_{GLY,G}+X_{GLY,G}/X_{GAO}} . X_{GAO}$ |
| 13 Aerobic growth | $\hat{\mu}_{GAO} \cdot M_{SO2} \cdot M_{NH4} \cdot M_{SPO4} \cdot M_{SALK} \cdot \frac{X_{PHA,G}/X_{GAO}}{K_{PHA,G}+X_{PHA,G}/X_{GAO}} X_{GAO}$ |
| 14 Anoxic growth | $\hat{\mu}_{GAO} \cdot \eta_{GAO} . N_{SO2} \cdot M_{NO3} . M_{NH4} \cdot M_{SPO4} \cdot M_{SALK} \cdot \frac{X_{PHA,G}/X_{GAO}}{K_{PHA,G}+X_{PHA,G}/X_{GAO}} X_{GAO}$ |
| 15 Aerobic growth of $X_{GLY,G}$ | $q_{GLY,G} \cdot M_{SO2} \cdot \frac{X_{PHA,G}/X_{GAO}}{K_{PHA,G-GLY}+X_{PHA,G}/X_{GAO}} \cdot \frac{K_{MG,G}-X_{GLY,G}/X_{GAO}}{K_{IG,G}+(K_{MG,G}-X_{GLY,G}/X_{GAO})} . X_{GAO}$ |
| 16 Anoxic growth of $X_{GLY,G}$ | $q_{GLY,G} \cdot h_{GAO} . N_{SO2} \cdot M_{NO3} . M_{SALK} \cdot \frac{X_{PHA,G}/X_{GAO}}{K_{PHA,G-GLY}+X_{PHA,G}/X_{GAO}} \cdot \frac{K_{MG,G}-X_{GLY,G}/X_{GAO}}{K_{IG,G}+(K_{MG,G}-X_{GLY,G}/X_{GAO})} . X_{GAO}$ |
| 17 Lysis of $X_{GAO}$ | $b_{GAO} . M_{SALK} . X_{GAO}$ |
| 18 Lysis of $X_{PHA,G}$ | $b_{PHA,G} . M_{SALK} . X_{PHA,G}$ |
| 19 Lysis of $X_{GLY,G}$ | $b_{GLY,G} . M_{SALK} . X_{GLY,G}$ |

The authors hypothesized that a fraction of the GAOs would be able to denitrify. Proposed processes of GAOs and stoichiometric matrix are given for phosphorus and glycogen accumulating organisms presented in Table 2.19.

**Table 2.19:** Stoichiometric matrix (**Manga et al., 2001**)

| No Process | $S_{O2}$ | $S_A$ | $S_{NO3}$ | $S_{PO4}$ | $X_I$ | $X_S$ | $X_{PAO}$ | $X_{PP}$ | $X_{PHA}$ | $X_{GLY}$ | $X_{GAO}$ | $X_{PHA,G}$ | $X_{GLY,G}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Phosphorus accumulating organisms: $X_{PAO}$* | | | | | | | | | | | | | |
| 1 Storage of $X_{PHA}$ | | $-Y_{SA}$ | | $Y_{PO4}$ | | | | $-Y_{PO4}$ | 1 | $-(1-Y_{SA})$ | | | |
| 2 Aerobic storage of $X_{PP}$ | $-Y_{PHA}$ | | | -1 | | | | 1 | $-Y_{PHA}$ | | | | |
| 3 Anoxic storage of $X_{PP}$ | | | $-\frac{Y_{PHA,NO}}{2.86}$ | -1 | | | | 1 | $-Y_{PHA,NO}$ | | | | |
| 4 Aerobic growth | $-\frac{(1-Y_{PAO})}{Y_{PAO}}$ | | | $-i_{PBM}$ | | | 1 | | $-\frac{1}{Y_{PAO}}$ | | | | |
| 5 Anoxic growth | | | $-\frac{(1-Y_{PAO,NO})}{2.86Y_{PAO,NO}}$ | $-i_{PBM}$ | | | 1 | | $-\frac{1}{Y_{PAO,NO}}$ | | | | |
| 6 Aerobic storage of $X_{GLY}$ | $-\frac{(1-Y_{GLY})}{Y_{GLY}}$ | | | | | | | | $-\frac{1}{Y_{GLY}}$ | 1 | | | |
| 7 Anoxic storage of $X_{GLY}$ | | | $-\frac{(1-Y_{GLY,NO})}{2.86Y_{GLY,NO}}$ | | | | | | $-\frac{1}{Y_{GLY,NO}}$ | 1 | | | |
| 8 Lysis of $X_{PAO}$ | | | | $v_{8,PO4}$ | $f_{XI}$ | $(1-f_{XI})$ | -1 | | | | | | |
| 9 Lysis of $X_{PP}$ | | | | 1 | | | | -1 | | | | | |
| 10 Lysis of $X_{PHA}$ | | 1 | | | | | | | -1 | | | | |
| 11 Lysis of $X_{GLY}$ | | 1 | | | | | | | | -1 | | | -(1-$Y_{SAG}$) |
| *Glycogen accumulating organisms: $X_{GAO}$* | | | | | | | | | | | | | |
| 12 Storage of $X_{PHA,G}$ | | $-Y_{SAG}$ | | | | | | | | | | 1 | |
| 13 Aerobic growth | $-\frac{(1-Y_{GAO})}{Y_{GAO}}$ | | | $-i_{PBM}$ | | | | | | | 1 | $-\frac{1}{Y_{GAO}}$ | |
| 14 Anoxic growth | | | $-\frac{(1-Y_{GAO,NO})}{2.86Y_{GAO,NO}}$ | $-i_{PBM}$ | | | | | | | 1 | $-\frac{1}{Y_{GAO,NO}}$ | |
| 15 Aerobic storage of $X_{GLY}$ | $-\frac{(1-Y_{GLY,G})}{Y_{GLY,G}}$ | | | | | | | | | | | $-\frac{1}{Y_{GLY,G}}$ | 1 |
| 16 Anoxic storage of $X_{GLY}$ | | | $-\frac{(1-Y_{GLY,GNO})}{2.86Y_{GLY,GNO}}$ | | | | | | | | | $-\frac{1}{Y_{GLY,GNO}}$ | 1 |
| 17 Lysis of $X_{GAO}$ | | | | $v_{17,PO4}$ | $f_{XI}$ | $(1-f_{XI})$ | | | | | -1 | | |
| 18 Lysis of $X_{PHA,G}$ | | 1 | | | | | | | | | | -1 | |
| 19 Lysis of $X_{GLY,G}$ | | 1 | | | | | | | | | | | -1 |

**Manga et al. (2001 and 2003)** calibrated their model using data from pilot plant and found that many of kinetic and stoichiometric parameters for PAOs are different compared to default values proposed in ASM2d (see Table 2.20 and Table 2.21).

**Table 2.20:** Stoichiometric parameters (**Manga et al., 2001**)

| Symbol | Characterization | Units | Value |
|---|---|---|---|
| *Phosphorus accumulating organisms* | | | |
| $Y_{SA}$ | $S_A$ requirement for PHA storage | gCOD/gCOD | 0.75 |
| $Y_{PO4}$ | PP requirement for PHA storage | gP/gCOD | 0.40 |
| $Y_{PHA}$ | PHA requirement for PP storage | gCOD/gP | 0.32 |
| $Y_{PHA,NO}$ | PHA requirement for anoxic storage of PP | gCOD/gP | 0.57 |
| $Y_{PAO}$ | Aerobic yield coefficient | gCOD/gCOD | 0.58 |
| $Y_{PAO,NO}$ | Anoxic yield coefficient | gCOD/gCOD | 0.47 |
| $Y_{GLY}$ | Aerobic glycogen yield coefficient | gCOD/gCOD | 1 |
| $Y_{GLY,NO}$ | Anoxic glycogen yield coefficient | gCOD/gCOD | 1 |
| *Glycogen accumulating organisms* | | | |
| $Y_{SA,G}$ | $S_A$ requirement for PHA,G storage | gCOD/gCOD | 0.75 |
| $Y_{GAO}$ | Aerobic yield coefficient | gCOD/gCOD | 0.58 |
| $Y_{GAO,NO}$ | Anoxic yield coefficient | gCOD/gCOD | 0.47 |
| $Y_{GLY,G}$ | Aerobic glycogen yield coefficient | gCOD/gCOD | 1 |
| $Y_{GLY,GNO}$ | Anoxic glycogen yield coefficient | gCOD/gCOD | 1 |

Although only limited studies reported in the literature, yet the prediction capability of Activated Sludge Model No.2d (ASM2d), for the enhanced biological phosphorus removal (EBPR) performance, and the proposed models deliberated to upgrade ASM2 model should be further investigated with appropriate data since very limited experimental validation was provided in all these models mentioned above.

Using the calibrated parameters obtained from the simulation of ASM2d, the problem addressed to the model should be further evaluated. Based on the stoichiometry and kinetics of denitrification, glycogen storage processes and processes associated with glycogen accumulating organism of proposed mathematical models given in the literature, the mechanistic description of a mixed culture of glycogen and phosphate accumulating organisms competing for acetate, and glycogen metabolism should be introduced to the ASM2d model and tested with experimental data profiles of the model components.

**Table 2.21:** Kinetic parameters (**Manga et al., 2001**)

| Symbol | Characteristics | Value | Units |
|---|---|---|---|
| Phosphorus accumulating organisms: $X_{PAO}$ | | | |
| $q_{PHA}$ | Rate constant for storage PHA | 3.60 | g COD/(gPAO*d) |
| $K_A$ | Saturation coefficient for $S_A$ | 3.63 | g COD/$m^3$ |
| $K_{PP}$ | Saturation coefficient for $X_{PP}$ for $X_{PHA}$ storage | 0.01 | gPP/gPAO |
| $K_{GLY}$ | Saturation coefficient for $X_{GLY}$ for $X_{PHA}$ storage | 0.001 | gCOD/gPAO |
| $q_{PP}$ | Rate constant for storage of PP | 2.8 | gPP/(gPAO*d) |
| $K_{O2}$ | Saturation coefficient for $S_{O2}$ | 0.20 | g COD/$m^3$ |
| $K_{PS}$ | Saturation coefficient for $S_{PO4}$ for $X_{PP}$ storage | 0.20 | g P/$m^3$ |
| $K_{ALK}$ | Saturation coefficient for alkalinity | 0.10 | mole $HCO_3/m^3$ |
| $K_{PHA-P}$ | Saturation coefficient for $X_{PHA}$ for $X_{PP}$ storage | 0.07 | gPHA/gPAO |
| $K_{MAX}$ | Maximum ratio of $X_{PP}/X_{PAO}$ | 0.28 | gPP/gPAO |
| $K_{IPP}$ | Inhibition coefficient for $X_{PP}$ storage | 0.001 | gPP/gPAO |
| $K_{NO3}$ | Saturation coefficient for nitrate | 0.50 | g N/$m^3$ |
| $K_{PHA}$ | Saturation coefficient for $X_{PHA}$ for growth | 0.08 | gPHA/gPAO |
| $\mu_{PAO}$ | Maximum growth rate | 0.84 | $d^{-1}$ |
| $K_{NH4}$ | Saturation coefficient for ammonium | 0.05 | g N/$m^3$ |
| $K_P$ | Saturation coefficient $S_{PO4}$ for growth | 0.01 | g P/$m^3$ |
| $h_{PAO}$ | Anoxic reduction coefficient | 0.48 | - |
| $q_{GLY}$ | Rate constant for storage of $X_{GLY}$ | 3.80 | g COD/(gPAO*d) |
| $K_{PHA-GLY}$ | Saturation coefficient for $X_{PHA}$ for $X_{GLY}$ storage | 0.12 | gPHA/gPAO |
| $K_{MG}$ | Maximum ratio of $X_{GLY}/X_{PAO}$ | 0.25 | gCOD/gPAO |
| $K_{IG}$ | Inhibition coefficient for $X_{GLY}$ storage | 0.015 | gCOD/gPAO |
| $b_{PAO}$ | Rate constant for lysis of $X_{PAO}$ | 0.08 | $d^{-1}$ |
| $b_{PP}$ | Rate constant for lysis of $X_{PP}$ | 0.08 | $d^{-1}$ |
| $b_{PHA}$ | Rate constant for lysis of $X_{PHA}$ | 0.08 | $d^{-1}$ |
| $b_{GLY}$ | Rate constant for lysis of $X_{GLY}$ | 0.08 | $d^{-1}$ |
| Glycogen accumulating organisms: $X_{PAO}$ | | | |
| $q_{PHA,G}$ | Rate constant for storage $X_{PHA,G}$ | 2.60 | g COD/(gGAP*d) |
| $K_A$ | Saturation coefficient for $S_A$ | 3.63 | g COD/$m^3$ |
| $K_{GLY,G}$ | Saturation coefficient for $X_{GLY,G}$ for $X_{PHA,G}$ storage | 0.001 | gCOD/gGAO |
| $\mu_{GAO}$ | Maximum growth rate | 0.80 | $d^{-1}$ |
| $K_{O2}$ | Saturation coefficient for $S_{O2}$ | 0.20 | g COD/$m^3$ |
| $K_{ALK}$ | Saturation coefficient for alkalinity | 0.10 | mole $HCO_3/m^3$ |
| $K_{PHA,G}$ | Saturation coefficient for $X_{PHA,G}$ for growth | 0.03 | gPHA/gGAO |
| $K_{NO3}$ | Saturation coefficient for nitrate | 0.50 | g N/$m^3$ |
| $K_{NH4}$ | Saturation coefficient for ammonium | 0.05 | g N/$m^3$ |
| $K_P$ | Saturation coefficient $S_{PO4}$ for growth | 0.01 | g P/$m^3$ |
| $h_{GAO}$ | Anoxic reduction coefficient | - | - |
| $q_{GLY,G}$ | Rate constant for storage of $X_{GLY,G}$ | 1.20 | g COD/(gGAO*d) |
| $K_{PHA,G-GLY}$ | Saturation coefficient for $X_{PHA,G}$ for $X_{GLY,G}$ storage | 0.008 | gPHA/gGAO |
| $K_{MG,G}$ | Maximum ratio of $X_{GLY,G}/X_{GAO}$ | 0.40 | gCOD/gGAO |
| $K_{IG,G}$ | Inhibition coefficient for $X_{GLY,G}$ storage | 0.015 | gCOD/gGAO |
| $B_{GAO}$ | Rate constant for lysis of $X_{GAO}$ | 0.08 | $d^{-1}$ |
| $b_{PHA,G}$ | Rate constant for lysis of $X_{PHA,G}$ | 0.08 | $d^{-1}$ |
| $b_{GLY,G}$ | Rate constant for lysis of $X_{GLY,G}$ | 0.08 | $d^{-1}$ |

# 3. THE EFFECT OF SUBSTRATE ON BIOLOGICAL PHOSPHORUS REMOVAL AND COMPOSITION OF POLYHYDROXYALKANOATES

## 3.1 Introduction

In order to assure biological phosphate removal in activated sludge systems, wastewater characteristics together with system configuration are important to satisfy effluent quality standards not to produce an undesirable disturbance to the water bodies. For this purpose, enhanced biological phosphate removal that can be achieved with pre-fermentation products in the domestic wastewater, namely acetate and propionate, which are most common VFAs, with the dominance of phosphorus accumulating organisms (PAOs) to provide sludge with higher phosphorus content compared to conventional aerobic activated sludge systems is considered as appropriate process to remove phosphate from wastewater.

According to great promising metabolic models, under anaerobic conditions, PAOs take up preferably volatile fatty acids (VFAs) and store them as polyhydroxyalkanoates (PHA), and utilize glycogen to provide reducing equivalent which is stored under aerobic conditions. This results in excess orthophosphate in the solution. Under aerobic conditions, orthophosphate is stored to replenish polyphosphate (poly-P) pools of the cell, while PHA is oxidized to provide carbon and energy, and glycogen stored. Net phosphate removal is achieved by wasting polyphosphate (poly-P) rich sludge at the end of aerobic phase.

Conversely, dominance of certain group of bacteria, named glycogen accumulating organisms (GAOs), that can store VFAs as PHA using glycogen as an energy source rather then poly-P, were reported preferably at low P/VFA ratios (**Yagci et al., 2003**), high sludge age and temperature (**Whang et al., 2001; Erdal et al., 2002**), longer anaerobic period (**Satoh et al., 1992**) and low pH (**Filipe et al., 2001**).

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Based on the observations that the activated sludge behavior for different VFAs and P/VFA ratios is not the same (**Abu-ghararah and Randall, 1991**; **Liu et al., 1997**), the importance of wastewater characteristics were considered by all means for the complete enhanced biological phosphate removal (EBPR). Intensive affords have been made to determine the effects of these factors contributing to competition between PAOs and GAOs in enhanced phosphate removal process.

In order to explain mechanism of this phenomena, there are some of mechanistic models attempts given in the literature, which are mostly on acetate (**Mino et al., 1987; Wentzel et al., 1991a; Satoh et al., 1992; Smolders et al., 1994; Pereira et al., 1996; Hesselman et al., 2000; Yagci et al., 2003**), and rarely on other VFAs, such as propionate (**Abu-ghararah and Randall, 1991; Lemos et al., 1998, Hood et al., 2001; Randall et al., 2003, Chen et al., 2004; Pijuan et al., 2004**).

In most of studies with synthetic wastewater, it is confirmed that phosphate release is lower with propionate compared to acetate (**Abu-ghararah and Randall, 1991; Hood and Randall, 2001; Randall and Liu, 2002; Chen et al., 2004; Pijuan et al., 2004**). **Abu-ghararah and Randall (1991)** investigated the effect of different VFAs on performance of the biological nutrient removal. A series of tests using a pilot-scale UCT process were conducted adding seven different VFAs, including acetate and propionate, into the domestic wastewater. A very good relationship between phosphate uptake and phosphate release (around 1.2 for all added organic substrates, except formic acid) was obtained. They suggested that this ratio is almost constant regardless of VFA type.

**Pijuan et al. (2004)** followed the microbial community developed in an EBPR system using propionate as the sole carbon source by using fluorescence in situ hybridization (FISH) techniques. They concluded that propionate is more beneficial than acetate to obtain as many PAOs as possible in the sludge.

In this study, it is aimed to investigate behavior of activated sludge while using single substrate or mixture of acetate and propionate as the sole carbon source with varying P/VFA ratio in the feed. Based on the results, anaerobic phosphate release rate and aerobic phosphate uptake rates were evaluated in terms of dependency on P/VFA ratio and substrate composition. Further analysis with varying compositions of feeding was presented to explore the acclimation, and effect on especially PHA

composition. SBR and batch test data were compared and evaluated in terms of effect of P/VFA ratio and substrate on relevant parameters.

## 3.2 Materials and Methods

Two sequencing batch reactors (SBRs) were operated to investigate effect of organic carbon and P/VFA ratio. In this chapter, operation of sequencing batch reactors, experimental set-up of continuous and batch experiments performed with activated sludge, and methods for analysis used throughout this research will be presented in details.

### 3.2.1 Origin of the seed

The SBR was initially seeded with sludge obtained from a nutrient removal pilot plant treating domestic sewage from Blacksburg-VPI Sanitation Authority nitrification-denitrification wastewater treatment plant (in USA) with acetate supplement.

### 3.2.2 Experimental design and operation of sequencing batch reactors

The two cylindrical bench-type reactors made of plexiglass equipped with mixers, aerators, timers for controlling all equipment and system operation electronically, and pumps for filling, dosage and discharge were operated in an anaerobic/aerobic sequence throughout this research. The SBR unit used in the research was a cylindrical tank with a working volume of 4 l. A fill volume, $V_F$ of 2.5 l was selected, leaving 1.5 l for the initial volume, $V_0$ and corresponding to a relatively low $V_0/V_F$ ratio of 0.6. Schematic presentation of SBRs with equipments is given in Figure 3.1. The picture of the SBRs is given in Figure 3.2.

Each reactor was set-up with identical operation conditions. The SBR operation for EBPR was designed for four cycles a day (a total cycle time, $T_C$ of 6 hours), where 5 h was devoted to the process phase, $T_P$. The remaining period of 1h after the process phase involved 30 min for settling ($T_S$), 15 min for the withdrawal ($T_W$) for the treated effluent and 15 min for the idle phase ($T_I$). The anaerobic phase was selected as 2 h ($T_{AN}$=2 h) with filling in the first 15 min ($T_F$=15 min) and the reactor was aerated for the remaining portion of the process phase ($T_A$=3 h). Total cycle time was defined as:

$$T_C = T_F + T_{AN} + T_{AE} + T_S + T_W + T_I \quad (3.1)$$

Operating conditions of the reactors are schematically given in Figure 3.3. The reactors kept in a constant temperature room adjusted to the temperature of 20±1°C during the experimental work.

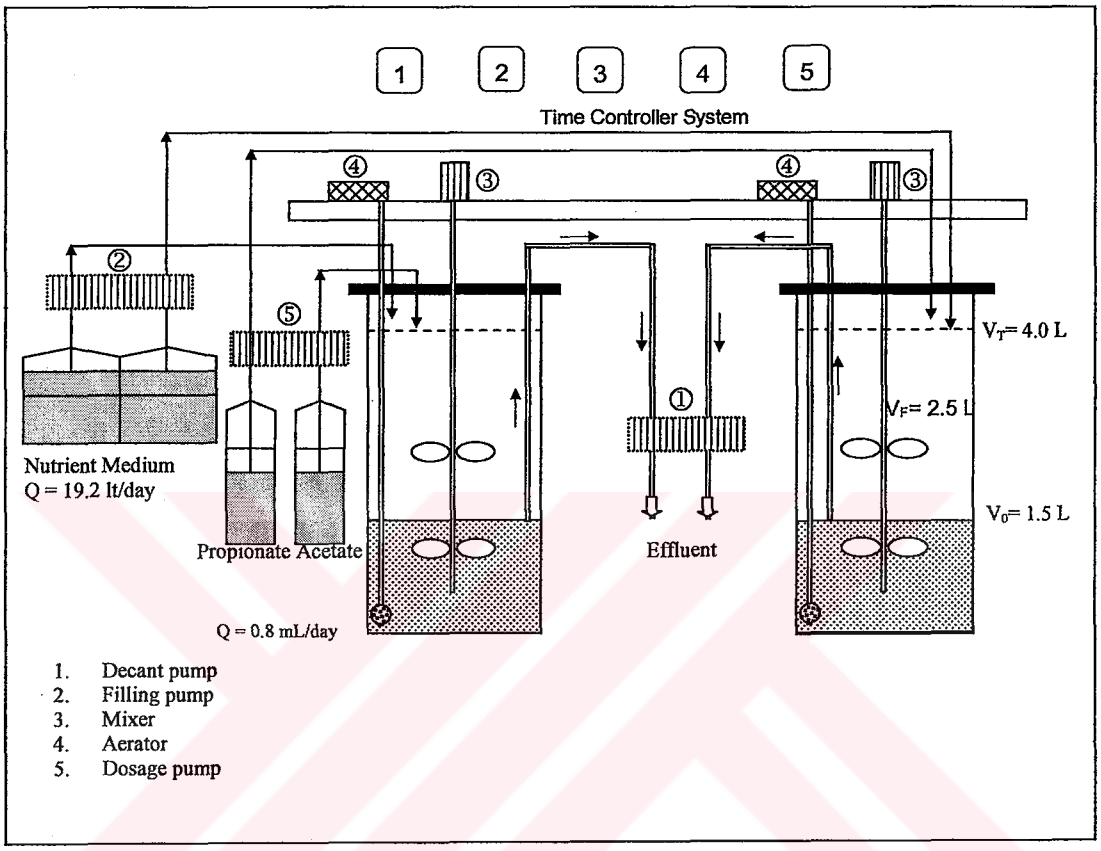


**Figure 3.1:** Schematic illustrations of SBRs with equipments

The system was ultimately adjusted to a total sludge age, $\theta_X$ of 10 days with appropriate sludge wasting at the end of the aeration phase. Operating parameters of SBRs were summarized in Table 3.1.

The experiments were carried out during the study in which pH was not controlled. pH was monitored at steady-state with detailed measurements in the system. The dissolved oxygen (DO) concentration was always above 2.0 $mgO_2\ l^{-1}$ after the commencement of aeration.

### 3.2.3 Characteristics of synthetic wastewater

The SBR was fed with synthetic wastewater containing single VFA and mixture of acetate and propionate as the sole carbon source: one was fed with acetate, and

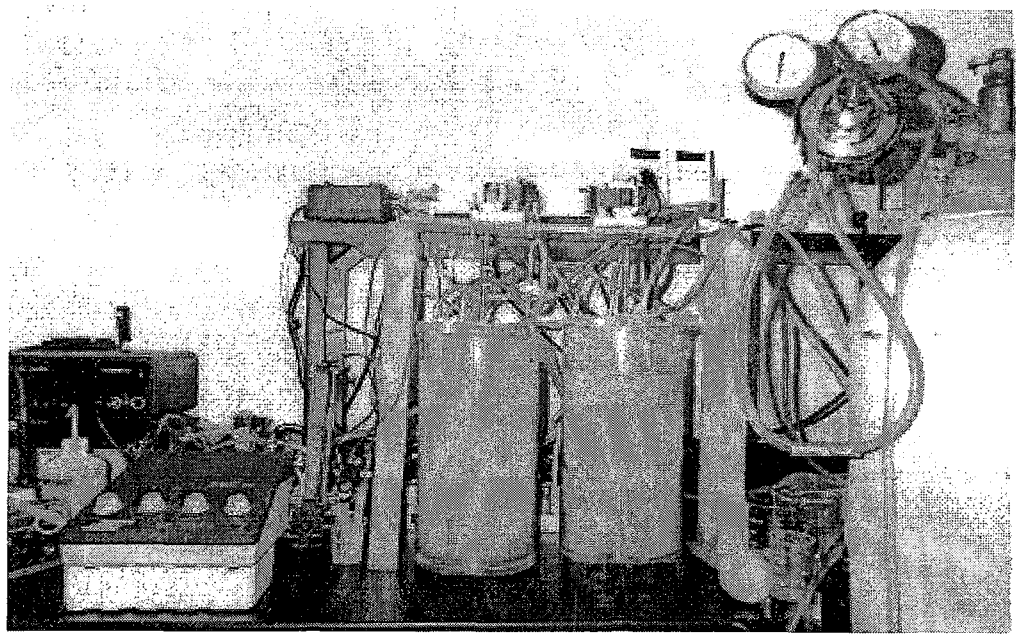

**Figure 3.2:** The picture of the SBRs

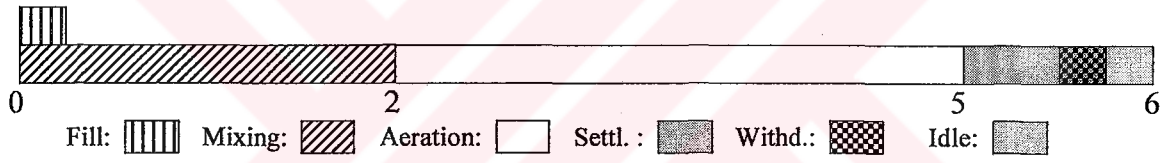


**Figure 3.3:** Operation conditions of the reactors

**Table 3.1:** Operating parameters of two SBRs

| Parameter | Symbol | Value | Unit |
|---|---|---|---|
| Cycle time | $T_C$ | 6 | h |
| Duration of filling period | $T_F$ | 15 | min |
| Duration of anaerobic (mixing) period | $T_{AN}$ | 2 | h |
| Duration of aerobic (aeration) period | $T_A$ | 3 | h |
| Duration of settling period | $T_S$ | 30 | min |
| Duration of withdrawn period | $T_W$ | 15 | min |
| Duration of idle period | $T_I$ | 15 | min |
| Initial volume | $V_0$ | 1.5 | L |
| Total volume | $V_T$ | 4.0 | L |
| $V_F/V_0$ | - | 0.6 | - |
| Hydraulic Retention Time | HRT | 9.6 | h |
| Sludge Retention Time | SRT | 10 | d |
| Temperature | - | 20±1 | °C |

propionate spiked with 25% of acetate, hereinafter called as "acetate-SBR". The other reactor was fed with propionate, and acetate spiked with 25% of propionate, hereinafter called as "propionate-SBR".

The study was conducted in five different Runs, (Run 1 to 5), where the VFA concentration was always kept as 400 mg $l^{-1}$ COD, while the influent phosphate concentration was increased gradually from 20 mg $l^{-1}$ in Run-1 to 35 mg $l^{-1}$ in Run-2, 45 mg in Run-3 and finally to 60 mg $l^{-1}$ in Run-4 and Run-5 to ensure a range of different influent P/Ac ratios of 0.05, 0.08, 0.12 and 0.15 respectively.

The SBR was fed with a mixture of organic and inorganic nutrient solutions pumped separately into reactors to avoid any biological activity in the feeding, which could be resulted in reduction in the influent COD.

The inorganic nutrient solution was prepared with nutrient mixture and tap water. Composition of stock solution consisting micro and macro nutrients is given in Table 3.2. This solution was prepared daily and deoxygenated by purging $N_2$ until the dissolved oxygen concentration in the influent, which enters the system through the anaerobic phase, fell below 0.5 mg/L. pH of the nutrient medium was around 7.1.

**Table 3.2:** Composition of macro and micro compounds in the stock solutions and influent

| Component | Compound | Stock (g/L) | Feed (ml/L) | Concentration |
|---|---|---|---|---|
| $NH_4Cl$ (or $NH_4SO_4$) | N | 25 (50) | 2.45 | 20 mg/L |
| $KH_2PO_4$, $K_2HPO_4$ | P | (10 + 12.8)* | 4.39 | 20 mg/L |
| $NaHCO_3$ | $CaCO_3$ | 50 | 2.8 | 100 mg/L |
| Yeast Extract | - | 1 | 3.4 | 3.4 mg/L |
| $CaCl_2$ | Ca | 41.63 | 1 | 15 mg/L |
| $MgSO_4$ | Mg | 49.4 | 1 | 10 mg/L |
| $FeSO_4.7H_2O$ (or $FeCl_3$) | Fe | 1.74 (1,02) | | 350 µg/L |
| $MnSO_4.H_2O$ | Mn | 0.55 | | 180 µg/L |
| $ZnCl_2$ | Zn | 0.27 | | 130 µg/L |
| $CoCl_2.H_2O$ | Co | 0.14 | | 35 µg/L |
| $CuSO_4.5H_2O$ | Cu | 0.14 | | 35 µg/L |
| $H_3BO_3$ | B | 0.12 | | 20 µg/L |
| KI | I | 0.05 | | 35 µg/L |
| $(NH_4)_6Mo_7O_{24}.4H_2O$ | Mo | 0.45 | | 35 µg/L |
| EDTA | - | 3 | | 3 mg/L |

*Changed to obtain different influent P concentrations

Carbon sources (acetate and propionate) were pumped into reactor from separate feeding bottles, which were prepared and neutralized daily and sterilized closing cap of bottles carefully to avoid contamination and minimize biological activity. $CaCl_2$

was added to carbon source bottle to avoid calcium phosphate precipitation in the inorganic nutrient medium. Theoretical COD concentration and phosphate concentration in the influent were as given in Table 3.3.

**Table 3.3:** Wastewater characteristics of two sequencing batch reactors

| | Run | Acetate (mgCOD/L) | Propionate (mgCOD/L) | Phosphate (mgP/L) |
|---|---|---|---|---|
| Acetate-SBR | 1 | 400 | 0 | 25 |
| | 2 | 400 | 0 | 35 |
| | 3 | 400 | 0 | 45 |
| | 4 | 400 | 0 | 60 |
| | 5 | 300 | 100 | 60 |
| Propionate-SBR | 1 | 0 | 400 | 25 |
| | 2 | 0 | 400 | 35 |
| | 3 | 0 | 400 | 45 |
| | 4 | 0 | 400 | 60 |
| | 5 | 100 | 300 | 60 |

### 3.2.4 Batch experiments

Eight batch tests were designed with either acetate-SBR sludge or propionate-SBR sludge, while phosphate and ammonia concentrations were around 20 mg/L.

Batch experiments were conducted with mixed liquors taken from the end of aerobic phase of each SBRs. Sludge was divided into four beakers. In order to simulate acetate and propionate SBRs, all beakers were equipped with stirrer and air stones and kept in a constant temperature (20±1°C) room. $V_0/V_F$ ratio was as it was for the SBRs. For all experiments, 3 h of aerated period was provided following 2 h of mixing period.

Inorganic nutrient solution was prepared to ensure similar concentration of inorganic nutrients in the influent of two SBRs. As summarized in Table 3.4, both sludges were exposed to acetate and propionate with varying fractions of total VFA, which were prepared in order to ensure 400 mg COD/L in the influent.

### 3.2.5 Experimental plan and data collection

During the experimental study, each run was monitored daily in the influent and effluent streams, and at the end of aerobic and anaerobic phases for each run. The performance of the SBR was further investigated by measuring the concentration profiles throughout a representative cycle during the steady state operation. During detailed cycle measurements, samples were taken from every 5, 10, 15 or 30 minutes

**Table 3.4:** Arrangement of batch tests

| Batch | Origin of the sludge | Acetate fraction (%) | Propionate fraction (%) |
|---|---|---|---|
| B1 | Acetate-SBR | 100 | - |
| B2 | | 75 | 25 |
| B3 | | 50 | 50 |
| B4 | | 25 | 75 |
| B5 | Propionate-SBR | 100 | - |
| B6 | | 75 | 25 |
| B7 | | 50 | 50 |
| B8 | | 25 | 75 |

of cycle. The data collection was done by means of taking samples from the mixed liquor during the phases of the SBR cycle.

During each batch test, the sludge was sampled at the end of anaerobic phase, 120$^{th}$ minute of aerobic phase and at the end of aerobic phase.

### 3.2.6 Analysis

The reactor was monitored through measurements of pH, dissolved oxygen (DO), acetate (HAc), chemical oxygen demand (COD), orthophosphate ($PO_4$-P), ammonia ($NH_4$-N), nitrate ($NO_3$-N), nitrite ($NO_2$-N), calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), potassium ($K^+$), sodium ($Na^{2+}$), sulfate ($SO_4^-$), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), glycogen, poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), 3-hydroxy-2-methylvalerate (3H2MV), sludge volume index (SVI) and oxygen uptake rate (OUR). Samples were taken from the mixed liquor into 25 ml graduated plastic tubes with conical bottoms. Then, tubes were sealed and centrifuged for 10 minutes immediately and supernatant was filtered through 0.45 micron disc filter (25 mm) paper using 25 mm easy pressure syringe filter holder for anion and cation analysis. Solids were dried for 24 hours at 100°C and kept in room temperature, then, weighed as they were placed into high pressure bottles (Wheaton V vials).

The cation and anion analyses of filtered samples were performed using DIONEX Ion Chromatograph (DX 300 for anions and DX 120 for cations) according to Standard Methods for the Examination of Water and Wastewater (1998).

PHB, PHV and 3H2MV standards were also weighed and placed into high pressure bottles. 3-hydroxybutyricacid-co-3-hydroxyvaleric acid and caproic acid sodium salt were used as standard.

Polyhydroxyalkonoates were estimated by gas chromotography according to the modified Braunegg method (**Braunegg et al., 1978**), and as modified by **Kisoglu (2000)** as described below:

- Prepare benzoic acid solution mixing 50 mg of benzoic acid into 100 mL of 3% sulfuric acid.
- Ad 2 ml benzoic acid solution and then 2 ml of chloroform into each vial.
- Lock the bottles by tightening the caps.
- Incubate the bottles in an oven at 100 °C for 3.5 hours.
- Allow the bottles to cool and add 1 ml of distilled water.
- Shake the bottles for 10 minutes and allow the organic and inorganic layers to mix properly.
- After the separation, pipette 1.25 ml from the organic phase (chloroform phase) into GC bottles and seal.
- Inject the samples into gas chromotograph with a 3 meter 2% Reoplex 400 Chromosorb GAW column attached to an FID (flame ionization detector).

The analysis was performed using a HP 5890 gas chromatograph equipped with auto-sampler and a flame ionization detector (FID) and a J&W DB-FFAP FID 0.53 mm wide bore column with film thickness of 1 μm, and length of 30 m. The carrier gas was nitrogen.

The injector and detector temperatures were 160 and 200°C, respectively. The oven temperature was started at 90°C, and kept at this temperature for 4 minutes. Then, temperature gradually increased from 90°C to 130°C in 2 minutes. Each run was continued for 11 minutes. First, chloroform was appeared. Retention times of PHB, PHV, 3H2MV and benzoic acid were about 7.3, 8.5, 8.7 and 10 minutes, respectively.

Benzoic acid was used as the internal standard to adjust each sample relatively. All measurements were made in duplicate. When different they were repeated until the discrepancy was within the limits of analytical accuracy.

Glycogen measurements were performed according to the anthrone method outlined in **Manual of Methods for General Bacteriology (1981)** with some modifications by Erdal (2002). Steps of the method followed for the measurement of carbohydrates

were described briefly as: Add 0.5 ml potassium hydroxide 30% w/v, lock the bottles by tightening the caps, incubate the bottles in an oven at 100°C for 3 hours, allow the bottles to cool and add 3 ml of distilled water and 8 ml ethanol, centrifuge tubes for 15 minutes and decant the supernatant, wash with 8 ml ice cold ethanol 60% (v/v), centrifuge tubes for 15 minutes and decant the supernatant, dry the solid in room temperature for 2 days, add 10 ml of distilled water and mix well on vortex mixer, pipette 1 ml into glass experiment tubes, use distilled water as a blank. Prepare 1 g/l stock glucose solution. Standards are typically glucose solution. Then, add 1 ml of phenol solution (5% w/w) and mix well, add 5 ml of sulphuric acid (reagent grade 95.5%) and mix immediately, wait for 20 minutes and read at 490 nm for hexoses and 480 nm for pentoses and uronic acids. Color is stable for a few hours.

## 3.3 RESULTS AND DISCUSSION

### 3.3.1 Effect of substrate on phosphate removal

To achieve storage of all available phosphate at the end of aerobic phase of each SBRs, influent phosphate concentration was increased in the feed during the experimental study. In each step, phosphate removal efficiencies with acetate and propionate for the same P/VFA ratios were compared. A complete utilization of VFAs in the anaerobic period for all runs was observed with measurements of acetate and COD. It should be noted that propionate was not monitored but assumed to be completely taken up by organisms with the confirmation of COD measurements during cycle.

In each run, influent phosphate concentrations, at the end of the anaerobic phase and aerobic phase were monitored at steady state conditions (Figure 3.4). In Table 3.5, calculated phosphate release values where VFA was completely depleted and phosphate uptake values together with phosphate removal efficiencies were also given for each run. As it can be seen from this table, the comparison of phosphate profiles for four runs, which were 100% acetate (Run 1 to 4) or propionate (Run 1 to 4), reveals that in contrast with the experimental studies where detrimental effect on phosphate release with propionate as a supplemental carbon source for domestic wastewater was reported (**Abu-ghararah and Randall, 1991**), propionate was more efficient in terms of phosphate removal even highest phosphate release was observed with acetate as substrate. This confirms the observations of that higher phosphate

removal for propionate considering that propionate is favorable substrate for EBPR systems by **Zeng et al. (2004)**, which is attributed to possible dominance of PAOs in the sludge.

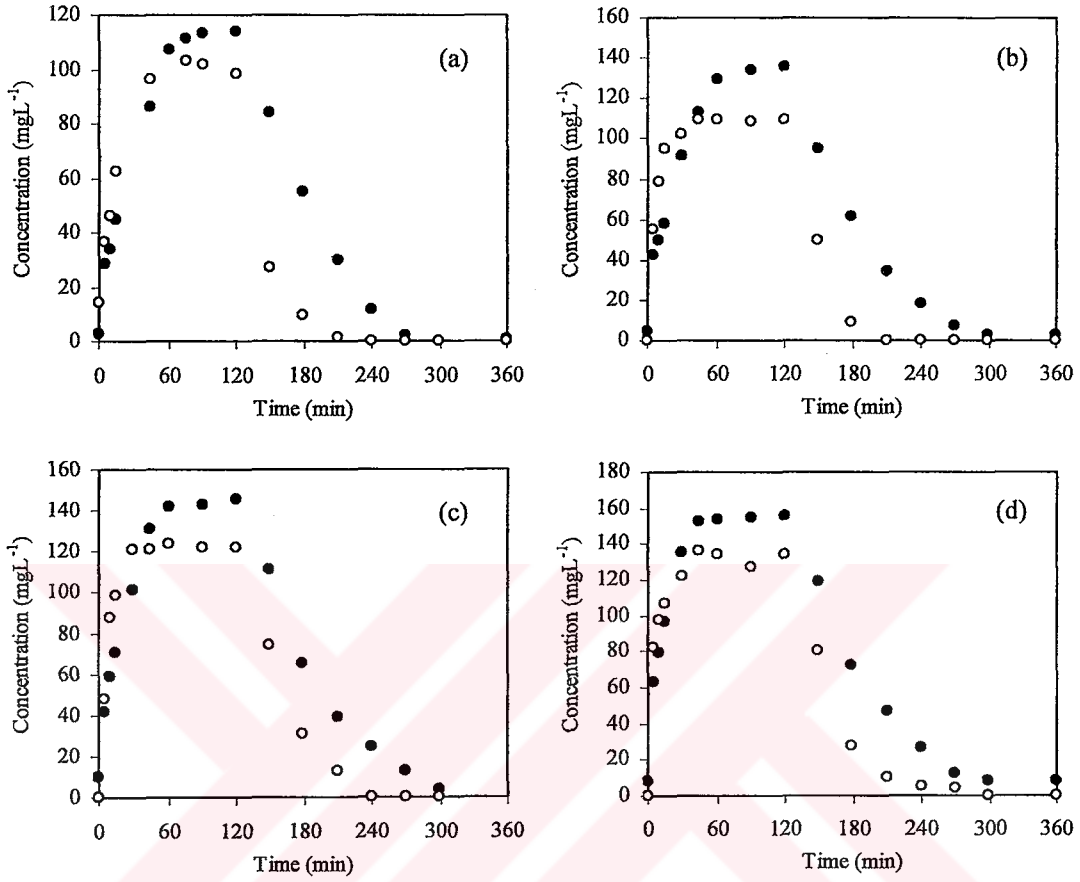


**Figure 3.4:** Phosphate profiles during complete cycle of acetate (Acetate-SBR) (O) and propionate (Propionate-SBR) (●) SBRs: (a) Run-1 (b) Run-2, (c) Run-3, and (d) Run-4

**Table 3.5:** Observed and calculated phosphate concentrations of two SBRs

| Substrate | Influent (mg-P/L) | Anaer. End (mg-P/L) | Aerob. End (mg-P/L) | $P_{rel}$ (mgP/L) | $P_{upt}$ (mgP/L) | Removal Efficiency |
|---|---|---|---|---|---|---|
| Acetate | 21.30 | 113.72 | 0.00 | 93.9 | 113.7 | 100% |
| Acetate | 35.41 | 135.41 | 2.99 | 105.6 | 132.4 | 91% |
| Acetate | 45.47 | 145.68 | 3.88 | 109.7 | 141.8 | 91% |
| Acetate | 60.87 | 155.46 | 8.35 | 112.1 | 147.1 | 86% |
| 75% Ac.Acid+25% Prop. Acid | 62.10 | 149.75 | 11.54 | 107.4 | 138.2 | 81% |
| Propionate | 21.30 | 94.06 | 0.00 | 80.2 | 97.2 | 100% |
| Propionate | 35.41 | 112.97 | 0.00 | 89.0 | 109.0 | 100% |
| Propionate | 45.47 | 122.00 | 0.00 | 95.1 | 122.0 | 100% |
| Propionate | 60.87 | 135.21 | 0.00 | 96.4 | 134.2 | 100% |
| 25% Ac.Acid+75% Prop. Acid | 62.10 | 141.16 | 1.38 | 102.7 | 139.8 | 98% |

Even released phosphate was lower for propionate in anaerobic phase; phosphate uptake rate was also lower in subsequent aerobic phase. Phosphate release rates were calculated between 15-30 min., which is giving linear correlation, as 1.83 and 0.86 mmol-P/gMLVSS.h for acetate and propionate SBRs, respectively. In the following aerobic phase, phosphate uptake rates between 120. and 180. min. were 1.04 and 1.43 mmol-P/gMLVSS.h for acetate and propionate SBRs, respectively, which indicates that propionate sludge has higher phosphate uptake capacity.

Phosphate release and uptake values for 100% acetate fraction in VFA were differed compared to values obtained for propionate. An additional observation was, in acetate SBR, when 25% of COD concentration was spiked with propionate, phosphate concentration was increased at the end of aerobic phase for the almost identical phosphate concentration.

In Table 3.6, phosphate uptake to phosphate release ($P_{upt}/P_{rel}$) ratios and variation of stoichiometry of phosphate release to VFA utilization ($P_{rel}/VFA_{upt}$) during mixing period were given for varying P/VFA ratios and substrate types in the feed. In this table, phosphate release and acetate uptake values reflect increasing trend with increasing P/VFA ratio in the feed for both substrate whereas they were lower for propionate.

**Table 3.6:** Phosphate uptake to phosphate release ($P_{upt}/P_{rel}$) ratios and stoichiometry of phosphate release to VFA utilization ($P_{rel}/VFA_{upt}$) during anaerobic phase

| Substrate | Influent P/VFA (mgP/mgCOD) | $P_{upt}/P_{rel}$ (mgP/mgP) | $P_{rel}/VFA_{ut}$ (mol-P/mol-C) | $K^+/Mg^{++}/P$ |
|---|---|---|---|---|
| Acetate | 0.05 | 1.21 | 0.41 | 0.37/0.29/1 |
| Acetate | 0.0875 | 1.25 | 0.45 | 0.24/0.28/1 |
| Acetate | 0.1125 | 1.29 | 0.47 | 0.24/0.27/1 |
| Acetate | 0.15 | 1.31 | 0.48 | 0.27/0.30/1 |
| 75% Ac.Acid+25% Prop. Acid | 0.15 | 1.29 | 0.48 | 0.33/0.31/1 |
| Propionate | 0.05 | 1.21 | 0.42 | 0.26/0.32/1 |
| Propionate | 0.0875 | 1.25 | 0.43 | 0.24/0.33/1 |
| Propionate | 0.1125 | 1.28 | 0.46 | 0.26/0.39/1 |
| Propionate | 0.15 | 1.32 | 0.47 | 0.26/0.37/1 |
| 25% Ac.Acid+75% Prop. Acid | 0.15 | 1.32 | 0.48 | 0.37/0.31/1 |

A good correlation between phosphate uptake and phosphate release was achieved for both organic acids (Figure 3.5). It was estimated that that ratio is around 1.2-1.3, which is close to the value reported by **Wentzel et al. (1985)** and **Abu-ghararah**

**and Randal (1991)** as it was ranged from 1.15 to 1.2 regardless of sludge age and substrate type. However, it should be noted that this ratio was lower for lower P/VFA ratios (around 1.1) in this study as reported by Rubens (1994) for phosphate concentration of 6-8 mg/l in the influent.

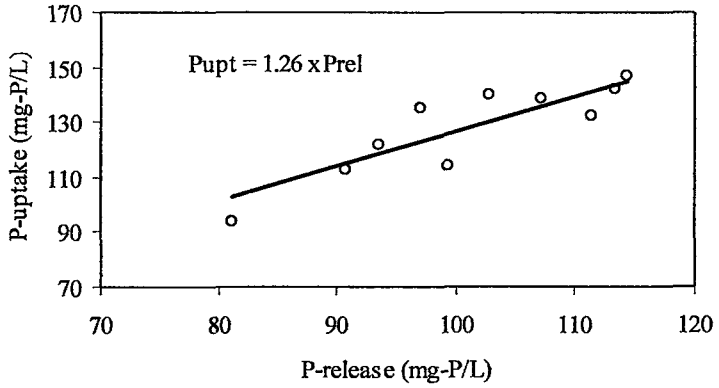


**Figure 3.5:** Relationship between phosphate release and uptake

It is suggested that some of metallic cations, namely potassium, magnesium and calcium, which could be co-transferred with phosphate (Figure 3.6). Thus, it is suggested that metalic cations are the components of Poly-P granules. Under anaerobic conditions, calculated $K^{+}/Mg^{++}/P$ ratios for all runs and batch tests as given below were almost the same regardless of substrate type and P/VFA ratio. It is concluded that these values indicate that the chemical composition of Poly-P granules can be assumed to be $MgK(PO_3)_3$ as confirmed by experimental studies by **Comeau et al. (1989), Schönborn et al. (2001), Christensen et al. (1998) and Hesselman et al. (2000).**

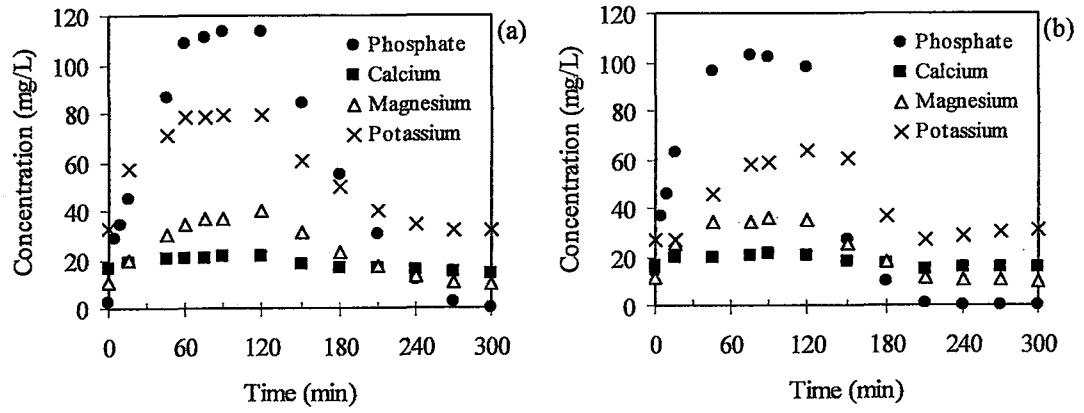


**Figure 3.6:** Profiles of phosphate, calcium, magnesium and potassium during detailed cycle of Run-1; (a) acetate-SBR, (b) propionate SBR

### 3.3.2 Effect of substrate on PHA composition

Figure 3.7 shows profiles of PHA and glycogen variations during the complete cycle in two SBRs. Observations of PHA and glycogen profiles showed that acetate and propionate were accumulated as PHA in the anaerobic phase and then PHA components were consumed in the aerobic phase while glycogen was consumed in the anaerobic phase to provide substantial energy and reducing equivalents by glycolysis, and replenished during the subsequent aerobic phase.

For all runs, concentrations of PHA component, stroichiometry of PHA storage and specific PHA storage under anaerobic conditions were as shown in Table 3.7. The highest PHA storage was formed with acetate. It is suggested that the clear difference between stored PHA concentrations while PHA/VFA ratios were almost the same can be attributed to the difference among the number of carbons of two VFAs and metabolic pathways followed by organisms to metabolize obtained organic acid.

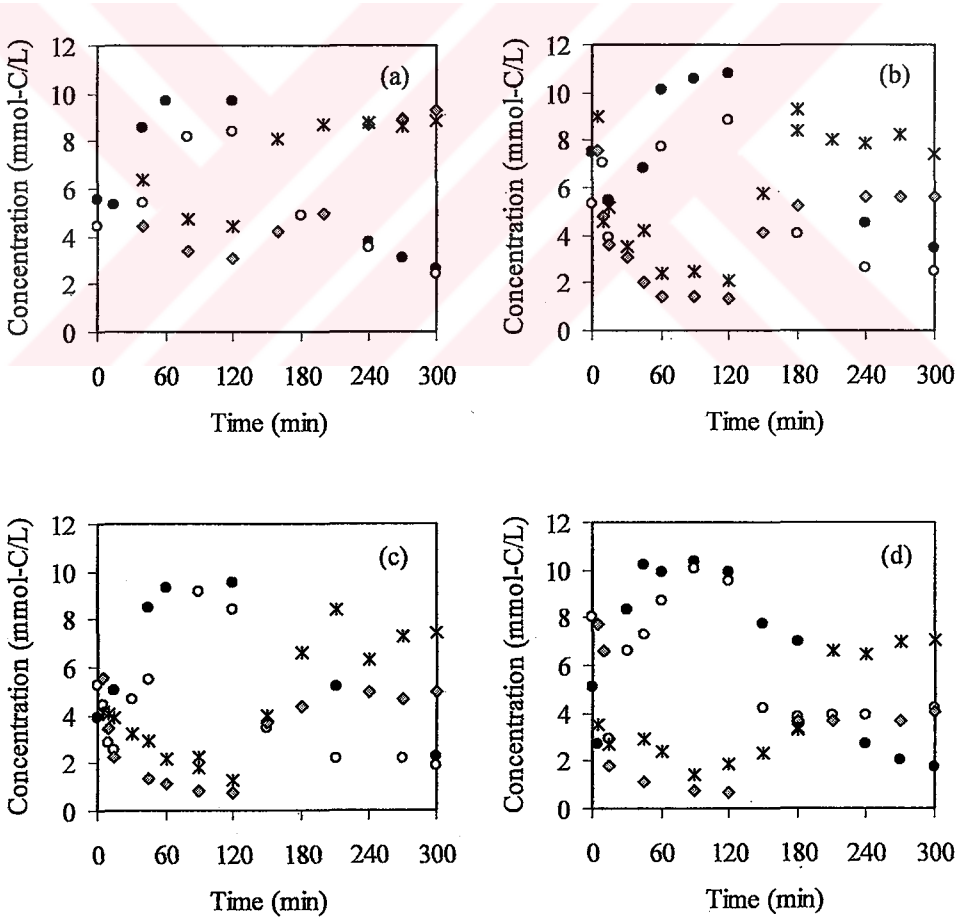


**Figure 3.7:** PHA profiles for acetate (●) and propionate (○) and glycogen profile for acetate ( ◇) and propionate (*); (a) Run-1, (b) Run-2, (c) Run-3 and (d) Run-4

**Table 3.7:** Measured PHA components, stroichiometry of PHA storage and specific PHA storage under anaerobic conditions

| | Run | 3H2MV | PHV | PHB | PHA | PHA/SA | 3H2MV | PHV | PHB | PHA |
|---|---|---|---|---|---|---|---|---|---|---|
| | | mmol-C | | | | mmol-C/mmol-C | mmol-C/g-MLVSS | | | |
| Reactor-I | 1 | 0.55 | 1.32 | 5.74 | 7.61 | 1.03 | 0.26 | 0.63 | 2.73 | 3.62 |
| | 2 | 0.42 | 1.02 | 6.56 | 8.00 | 1.06 | 0.18 | 0.44 | 2.79 | 3.40 |
| | 3 | 0.45 | 1.01 | 6.62 | 8.08 | 1.07 | 0.18 | 0.41 | 2.67 | 3.26 |
| | 4 | 0.26 | 0.99 | 6.77 | 8.02 | 1.07 | 0.10 | 0.38 | 2.56 | 3.04 |
| | 5 | 0.79 | 1.91 | 5.18 | 7.88 | 1.09 | 0.32 | 0.78 | 2.11 | 3.22 |
| Reactor-II | 1 | 3.04 | 2.21 | 1.23 | 6.48 | 1.05 | 0.97 | 0.71 | 0.39 | 2.07 |
| | 2 | 3.27 | 2.59 | 1.01 | 6.86 | 1.02 | 1.13 | 0.89 | 0.35 | 2.37 |
| | 3 | 3.67 | 2.61 | 0.94 | 7.21 | 1.07 | 1.40 | 1.00 | 0.36 | 2.75 |
| | 4 | 3.47 | 2.81 | 0.77 | 7.05 | 1.06 | 1.48 | 1.20 | 0.33 | 3.01 |
| | 5 | 3.15 | 2.21 | 1.85 | 7.21 | 1.05 | 1.14 | 0.80 | 0.67 | 2.62 |

In this study, a remarkable disparity among PHA composition of two sludges with change in VFA composition in the influent was observed. The results shown in Table 3.8 shows that propionate was likely stored as 3H2MV and as PHV while acetate was mostly stored as PHB.

**Table 3.8:** Stored PHA and utilized glycogen values and composition of PHA in the anaerobic phase of two SBRs

| | Run | Glycogen mmolC/L | PHB/PHA (molC/molC) | PHV/PHA (molC/molC) | 3H2MV/PHA (molC/molC) | PHB/ (PHB+PHV) |
|---|---|---|---|---|---|---|
| Reactor-I | 1 | 3.90 | 0.75 | 0.17 | 0.07 | 0.81 |
| | 2 | 3.73 | 0.84 | 0.10 | 0.05 | 0.86 |
| | 3 | 3.58 | 0.81 | 0.14 | 0.05 | 0.87 |
| | 4 | 3.53 | 0.83 | 0.15 | 0.02 | 0.87 |
| | 5 | 3.83 | 0.66 | 0.24 | 0.10 | 0.73 |
| Reactor-II | 1 | 4.31 | 0.19 | 0.34 | 0.47 | 0.36 |
| | 2 | 4.88 | 0.15 | 0.38 | 0.48 | 0.28 |
| | 3 | 5.17 | 0.13 | 0.36 | 0.51 | 0.26 |
| | 4 | 5.53 | 0.11 | 0.40 | 0.49 | 0.21 |
| | 5 | 4.42 | 0.27 | 0.32 | 0.41 | 0.46 |

In the literature, varying PHB fractions in the sludge between 10% and 36% were found for propionate (**Chen et al., 2004; Pijuan et al., 2003; Randall and Liu, 2002**) where 3H2MV was not determined. According to these studies while PHB was major polymer for acetate, PHV fraction in the sludge was higher for propionate.

In this study, PHB content of sludge was calculated in two ways. These were; PHA was assumed to be equal to sum of (1) three components, namely PHB, PHV and 3H2MV, and (2) only PHB and PHV. In Table 3.8, PHB contents of PHA for all runs were also illustrated which was calculated as a sum of PHB and PHV. Thus, PHB fraction of PHA was estimated to be between 21%-36% when PHA assumed to be

sum of PHB and PHV for different P/VFA ratios at the steady state conditions while this fraction was between 11%-19%.

Therefore, 3H2MV was appeared to be a component of PHA that should be taken into account for more accurate estimation of PHA concentration in the sludge. This result also suggests that, in most of the studies, present PHA contents as a sum of PHA and PHV probably do not give comparable results in terms of sufficient explanation of storage mechanism for especially propionate.

PHA compositions of both sludges were also compared for the same phosphate concentration. As shown in Figure 3.8, the comparison of PHA composition for acetate and propionate for these runs indicated that PHB content of PHA decreased with increasing propionate.

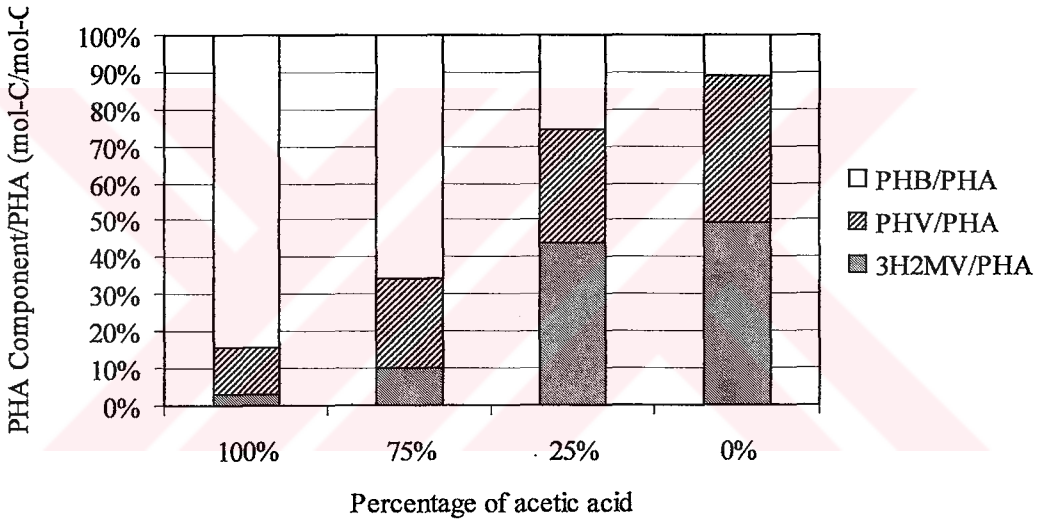


**Figure 3.8:** Changes in the composition of PHA with increasing acetate

### 3.3.3 Effect of VFA composition on acclimation

The results of batch experiments served to the purpose of understanding how two VFAs affected PHA composition and exploring the acclimation of biomass to different VFAs. The first significant observation was decreasing trend of phosphate release and uptake values and $P_{rel}/VFA_{upt}$ ratios with decreasing proportion of acetate (Table 3.9). However, it is surprisingly observed that phosphate release and uptake values of propionate sludge were considerably higher compared to acetate sludge.

**Table 3.9:** Effect of VFA on the performance of EBPR process in the batch experiments

| Proportion of acetate (%) | $P_{rel}$ (mg/l) | $P_{upt}$ (mg/l) | Effluent $N_{ox}$-N (mg/l) | $P_{upt}/P_{rel}$ (mg/mg) | $P_{rel}/VFA_{ut}$ (mg/mgCOD) | $K^+/Mg^{++}/P$ |
|---|---|---|---|---|---|---|
| *Acetate sludge* | | | | | | |
| 100 | 97.3 | 108.6 | 7.6 | 1.12 | 0.39 | 0.46/0.31/1 |
| 75 | 101.5 | 112.2 | 6.8 | 1.11 | 0.41 | 0.40/0.26/1 |
| 50 | 95.9 | 106.8 | 7.3 | 1.11 | 0.38 | 0.39/0.28/1 |
| 25 | 87.4 | 98.3 | 6.5 | 1.12 | 0.35 | 0.39/0.26/1 |
| *Propionate sludge* | | | | | | |
| 100 | 120.0 | 133.5 | 1.7 | 1.11 | 0.48 | 0.44/0.28/1 |
| 75 | 111.6 | 123.7 | 1.6 | 1.11 | 0.45 | 0.41/0.28/1 |
| 50 | 104.3 | 116.6 | 1.8 | 1.12 | 0.42 | 0.44/0.27/1 |
| 25 | 102.2 | 113.5 | 1.7 | 1.11 | 0.41 | 0.39/0.27/1 |

The PHB contents of SBR sludge were compared with the results obtained from batch experiments (Table 3.10). Utilization of acetate and propionate were resulted in different PHA compositions in the batch tests (Figure 3.9).

**Table 3.10:** Comparison of PHB contents for SBRs and batch tests

| Inf. P | Acetate (%) | PHB | Reactor |
|---|---|---|---|
| Acetate sludge | | | |
| 20 | 100 | 0.75 | SBR |
| 60 | 100 | 0.84 | SBR |
| 60 | 75 | 0.66 | SBR |
| 20 | 100 | 0.76 | Batch |
| 20 | 75 | 0.75 | Batch |
| 20 | 25 | 0.65 | Batch |
| Inf. P | Propionate (%) | | |
| Propionate sludge | | | |
| 20 | 100 | 0.19 | SBR |
| 60 | 100 | 0.11 | SBR |
| 60 | 75 | 0.26 | SBR |
| 20 | 75 | 0.34 | Batch |
| 20 | 25 | 0.66 | Batch |

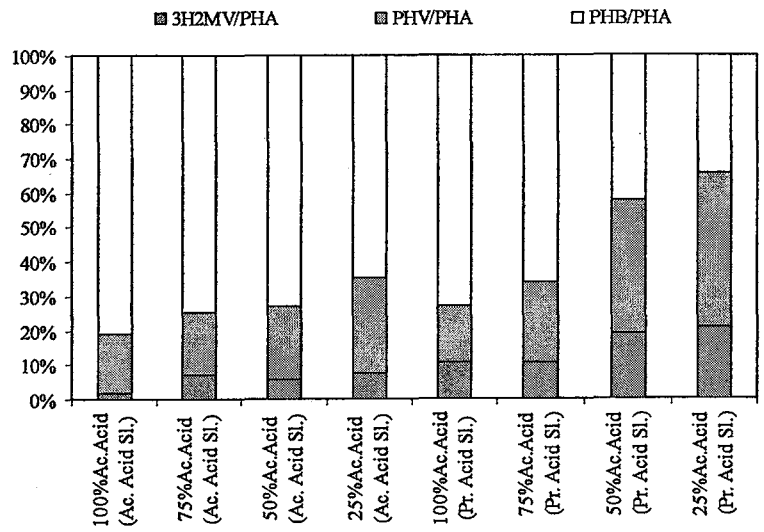


**Figure 3.9:** PHA compositions at the end of anaerobic phase in batch experiments

It is observed that the reduction in PHB content was unlikely obtained in batch experiments compared to SBR data when 25% of acetate spiked with propionate.

A slower response to propionate in terms of PHB content of sludge was found in batch experiments. In opposition to this, propionate enriched biomass showed rapid response to acetate addition. For instance, 19% of PHB content in SBR was increased to 34% in batch experiment whilst 25% of propionate fraction of VFA spiked with acetate while 11% of PHB content was increased to 26% for 60 mg/l phosphate concentration in the influent in SBR. That means, the expected storage fraction was achieved for increase in acetate content of VFA. This confirms the result of that propionate enriched biomass is able to use acetate immediately for EBPR by **Pijuan et al. (2004)**. It is proposed that this disparity may be due to dominance of different species or need of acclimation period to active unique enzyme for propionate to be metabolized by acetate enriched sludge.

# 4. A METABOLIC MODEL FOR ACETATE UPTAKE BY A MIXED CULTURE OF PHOSPHATE AND GLYCOGEN ACCUMULATING ORGANISMS UNDER ANAEROBIC CONDITIONS

## 4.1 Introduction

Since the recognition of enhanced biological phosphate removal (EBPR) in activated sludge systems (**Barnard, 1975; Fuhs and Chen, 1975**), major research effort has been devoted to the mechanistic understanding and modeling of the process. It is now widely accepted that EBPR occurs as a result of the predominance of a group of bacteria, commonly called phosphate accumulating organisms (PAOs), with the capability of storing polyphosphate within the cell. EBPR is only sustained when the activated sludge system is operated in an anaerobic/aerobic sequence and the biomass is fed with short chain fatty acids (SCFAs) like acetate, during the anaerobic phase. In this phase, PAOs activate the metabolic tools to take up acetate and to store it as polyhydroxyalkanoates (PHAs), mainly polyhydroxybutyrate (PHB). PHA storage proceeds using the internal polyphosphate (poly-P) pool as energy source resulting in a release of orthophosphate ($P_i$). In the subsequent aerobic stage, PAOs grow on the internally stored PHA and take up orthophosphate for the replenishment of poly-P reserves that increase the P content of sludge. Higher P removal is achieved by withdrawing excess sludge with high P-content. Because ordinary heterotrophs cannot take up acetate due to the lack of an energy source (**Wentzel et al., 1986**), the competition for substrate under anaerobic conditions favors the enrichment of PAOs within the activated sludge.

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Accurate interpretation of the basic stoichiometry is the key issue for the basic understanding and modeling of the anaerobic phase of the EBPR process. The ratio of $P_i$ release to acetate uptake (P/Ac) at constant pH is a significant and direct observation for this purpose; but it depends upon a number of fundamental parameters like the ATP balance, the source and the budget for reducing power and the magnitude and nature of internal storage compounds. It was first proposed that the required reducing power for PHB synthesis from acetate was supplied from the oxidation of acetate to $CO_2$ through the TCA cycle (**Comeau et al., 1986; Wentzel et al., 1986**). **Mino et al., (1987)** postulated the degradation of intracellular glycogen to acetyl-CoA and $CO_2$ through the EMP pathway, for the same purpose. The Mino model, which yields higher PHB production as compared to the Comeau-Wentzel model due to the glycogen involvement, has found wider theoretical and experimental support (**Mino et al., 1998**).

PHB was conceived to be the only organic storage polymer produced in the anaerobic metabolism of PAOs in these earlier metabolic models. Later, it was shown that the PHA contained 3-hydroxybutyrate (3HB) synthesized from acetyl-CoA and 3-hydroxyvalerate (3HV) synthesized from acetyl-CoA and propionyl-CoA as monomeric building units (**Comeau et al., 1987**). **Satoh et al. (1992)** further revealed the presence of two other monomers: namely, 3H2MB, composed of one acetyl-CoA and one propionyl-CoA; and 3H2MV involving two molecules of propionyl-CoA. **Mino et al. (1998)** have postulated a metabolic pathway to explain the conversion of acetate to 3HV and $CO_2$ via the glyoxylate cycle. These findings indicate that the composition of PHA could be a useful parameter for the identification of the relevant metabolic pathways. Radioactively labeled acetate as the carbon source for the EBPR process was also used for the same purpose (**Bordacs and Chiesa, 1989; Pereira et al., 1996**).

In the Mino model, less energy from poly-P degradation (consequently less P release) is required since a part of ATP needed for the uptake and activation of acetate is produced through glycogen degradation via the EMP pathway (yielding 3 ATP) or ED pathway (yielding 2 ATP) (**Wentzel at al., 1991a**). Consequently, the pathway used for the generation of reducing power as well as for the mechanism of substrate transport, has a considerable impact on the energy budget, and, thus, on the amount of phosphate released per acetate taken up.

The observed P/Ac ratios reported in literature vary substantially under different experimental conditions, far beyond the range that can be explained by the proposed models (**Liu et al., 1997; Smolders et al., 1994**). These observations may be related to the presence of other types of bacteria that are capable of storing substrate under anaerobic conditions without using energy from P release. This implies the involvement of an energy source other than poly-P in EBPR systems, thus leading to a drastic decrease in the P released/substrate uptake ratio. This phenomenon was first observed in EBPR systems fed with glucose together with substantial deterioration in phosphorus removal potential (**Cech and Hartman, 1990**). Under operating conditions favoring the dominance of this type of organisms, later called glycogen accumulating organisms (GAOs), acetate was still taken up and stored as 3HV enriched PHA, concurrently with significant consumption of glycogen (**Liu et al., 1994; Satoh et al., 1994**). It should be noted that during anaerobic acetate metabolism in GAOs, energy and reducing power are provided only by glycogen degradation without any poly-P involvement. This necessitates a metabolic pathway for the regeneration of the surplus $NADH_2$ produced during glycolysis to maintain the redox balance inside the cell. The presence of GAOs in EBPR systems influences the P removal performance significantly since the SCFA concentration in wastewater is very limited. Therefore, a better understanding of the competition for acetate between PAOs and GAOs is crucial to identifying the operational conditions that minimize GAOs, and thus obtain a stable EBPR system.

**Filipe et al. (2001a)** and **Shuler and Jenkins (2002)** suggested that pH may be a parameter that can be manipulated to minimize the presence of GAOs in EBPR systems, based on the observation that the stoichiometry for acetate uptake by GAOs was more favorable at lower pH values. **Erdal et al. (2002a)** have concluded that reduced competition for substrate at low temperatures increases the PAO population and EBPR efficiency, provided that the sludge retention time (SRT) in the system was above the critical washout SRT. Although factors influencing the dominance of GAOs are not so far well defined, in many studies it is demonstrated that GAOs may proliferate in anaerobic-aerobic activated sludge fed with a synthetic wastewater having higher acetate to phosphate ratio (**Liu et al., 1997; Hesselman et al., 2000**). The observations indicate that the microbial population sustained in EBPR systems is

possibly a mixture of PAOs and GAOs and may become GAO-enriched when the operation becomes P-limited.

The theoretically developed stoichiometry for earlier biochemical models does not fit very well the experimentally observed stoichiometric balances for anaerobic acetate uptake, PHA formation, glycogen utilization and $CO_2$ production by PAO enriched sludge. Recent models explaining the experimental results in terms of carbon, redox and energy balances tend to interpret the observed stoichiometry in terms of either PAO metabolism (**Pereira et al., 1996; Hesselman et al., 2000**) or GAO metabolism (**Filipe et al., 2001b**).

This chapter basically defines an EBPR system involving a dynamic balance between PAOs and GAOs and proposes a biochemical model for the acetate uptake by a mixed culture of PAOs and GAOs under anaerobic conditions. The proposed model is used to establish basic stoichiometric balances for organic carbon, ATP and reducing power, through appropriate metabolic pathways. The model was tested with experimental data obtained from an enriched culture of PAOs with varying fractions of GAOs. The overall performance and cyclic operation data of a sequencing batch reactor (SBR) system fed with acetate and operated at steady state with different acetate/P ratios in the influent was used for this purpose.

## 4.2 Metabolic Model Development

PAOs and GAOs have similar metabolism except for their internal energy sources. Both of them uptake acetate anaerobically and store it as PHA. During PHA storage, the redox balance is regulated by the consumption of glycogen. Their metabolisms basically differ in the energy source utilized: While PAOs have the metabolic complement to use both poly-P cleavage and glycolysis as their energy supply; GAOs solely depend on glycogen for this purpose.

### 4.2.1 Basic reactions

The metabolic model developed and proposed for acetate uptake by PAOs and GAOs under anaerobic conditions, consists of the following reactions. As outlined below, it

involves the same sequence for the two types of microorganisms, except for the formation of propionyl-CoA.

#### 4.2.1.1 Reaction 1: Acetate uptake and activation to Acetyl-CoA

One mole of acetate entering the cell is coupled with one mole of ATP hydrolyzed and activated to acetyl-CoA (**Gottschalk, 1986**). ATP is also necessary for substrate transport across the membrane. **Comeau et al. (1987)** assumes that 0.5 mole of ATP is utilized for the transport of one mole of acetate as contrasted to Wentzel's hypothesis of passive diffusion. **Smolders et al. (1994)** defined this value as $\alpha$ (mol ATP/C-mol of acetate) and showed that for PAOs the value of $\alpha$ was highly dependent on the external pH. Using the same approach and considering that there are two moles of carbon per mole of acetate, the overall equation can be expressed as:

$$\text{Acetate} + (1+2\alpha)\ \text{ATP} \rightarrow \text{Acetyl-CoA} \qquad \textbf{(4.1)}$$

#### 4.2.1.2 Reaction 2: Glycogen degradation to pyruvate

Glycogen degradation into glucose-1-phosphate, which is converted at no cost into glucose-6-phosphate, is considered to proceed without consumption of energy (**Stryer, 1995**). Glucose-6-phosphate can be degraded to pyruvate either via the EMP pathway or the ED pathway. The significant difference between the two pathways is that in the ED pathway, two ATP are produced during the conversion of glucose-6-phosphate to two moles of pyruvic acid, whereas three ATP are produced in the EMP pathway. It is still controversial whether EMP or ED pathway is used for glycolysis. In this study, the EMP pathway is adopted, based on the experimental study of **Filipe et al. (2001b)**, who detected no activity of glucose-6-phosphate dehydrogenase, an enzyme that catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconate.

$$\text{Glycogen} \rightarrow 2\ \text{Pyruvate} + 3\ \text{ATP} + 2\ NADH_2 \qquad \textbf{(4.2)}$$

#### 4.2.1.3 Reaction 3: Oxidative decarboxylation of pyruvate

Under anaerobic conditions, ordinary heterotrophs cannot generally undertake further pyruvate oxidation by reducing NAD, since its major problem is the oxidation of the $NADH_2$ already formed in the previous metabolic steps (**Gaudy and Gaudy, 1980**). However, in a cell that needs reducing power to store acetate as PHB, the further oxidation of pyruvate can be advantageous since it produces additional $NADH_2$. In such cells, pyruvate undergoes an oxidative decarboxylation to form acetyl-CoA.

$$\text{Pyruvate} \rightarrow \text{Acetyl-CoA} + CO_2 + NADH_2 \qquad \textbf{(4.3)}$$

#### 4.2.1.4 Reaction 4a: Formation of Propionyl-CoA from pyruvate

Experimental results show that the stored PHA inevitably includes the 3HV units derived from an acetyl-CoA and a propionyl-CoA, even if acetate is the sole carbon source available in the anaerobic phase (**Pereira et al., 1996; Hesselman et al., 2000**). Therefore, there should be a pathway or pathways to produce propionyl-CoA. **Satoh et al. (1992)** assumed partial conversion of pyruvate to propionyl-CoA via the succinate-propionate pathway. Succinate is formed as an intermediate in the cyclic pathway that leads to propionic acid, by the reversal of the TCA cycle reactions between succinate and oxaloacetate. When the cyclic pathway producing propionate is in operation, oxaloacetate is formed from pyruvate by the transfer of a $C_1$ fragment from methylmalonyl-CoA to pyruvate (**Gaudy and Gaudy, 1980**). In this study, this pathway is introduced as shown in Figure 4.1, indicating that the conversion of pyruvate to propionyl-CoA consumes reducing power. The ATP balance in this reaction would critically affect the overall stoichiometry; this reaction should require one mole of ATP provided that the conversion of fumarate to succinate does not yield one mole of ATP as shown in some of the previous studies (**Gaudy and Gaudy, 1980; Pramanik et al., 1998**). In this study however, the net zero ATP balance approach is adopted and reflected in reaction 4a, in accordance with **Gottschalk (1986)**, indicating that fumarate reduction coupled by $NADH_2$ regeneration yields one mole of ATP. This issue deserves further clarification.

$$\text{Pyruvate} + 2\ NADH_2 \rightarrow \text{Propionyl-CoA} \qquad \textbf{(4.4)}$$

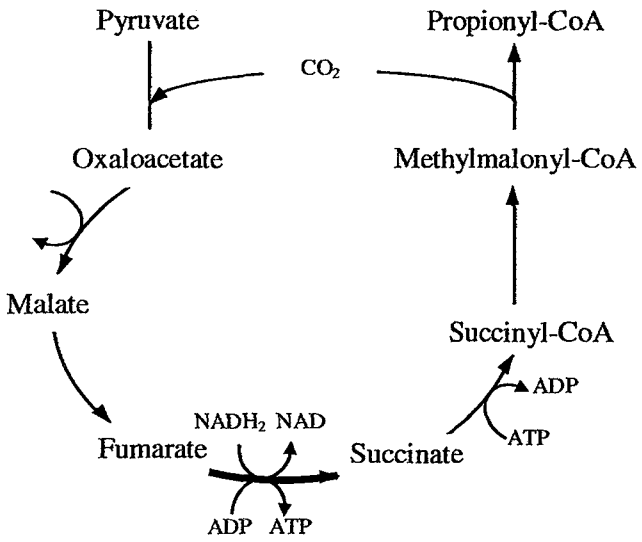


**Figure 4.1:** Succinate-Propionate Pathway

#### 4.2.1.5 Reaction 4b: Formation of Propionyl-CoA from Acetyl-CoA

Succinate can also be formed as an intermediate in the TCA cycle or glyoxylate bypass, which consequently leads to the formation of propionyl-CoA. Usually the TCA cycle is linked with respiration and operates only under aerobic or anoxic conditions since succinate dehydrogenase can not be operative under anaerobic conditions (**Mino et al.1998**). However, succinate can be produced by glyoxylate pathway bypassing the oxidative steps of the TCA cycle and forming oxaloacetate by condensation of two $C_2$ compounds, acetyl-CoA and glyoxylic acid. Acetyl-CoA enters the cycle by condensing with oxaloacetate to form citrate, which is then isomerized into isocitrate. The first of the bypass reactions is cleavage of isocitrate to glyoxylate and succinate. Glyoxylate can be converted to oxaloacetate by condensation with acetyl-CoA while succinate is converted to propionyl-CoA over the methylmalonyl-CoA pathway. Formation of propionyl-CoA from acetyl-CoA via the glyoxylate and the methylmalonyl-CoA pathways as conceived in the study is shown in Figure 4.2. The simplified format below shows that, unlike the alternative outlined in reaction 4a, this reaction generates additional reducing power:

$$2\ \text{Acetyl-CoA} \rightarrow \text{Propionyl-CoA} + CO_2 + NADH_2 \qquad \textbf{(4.5)}$$

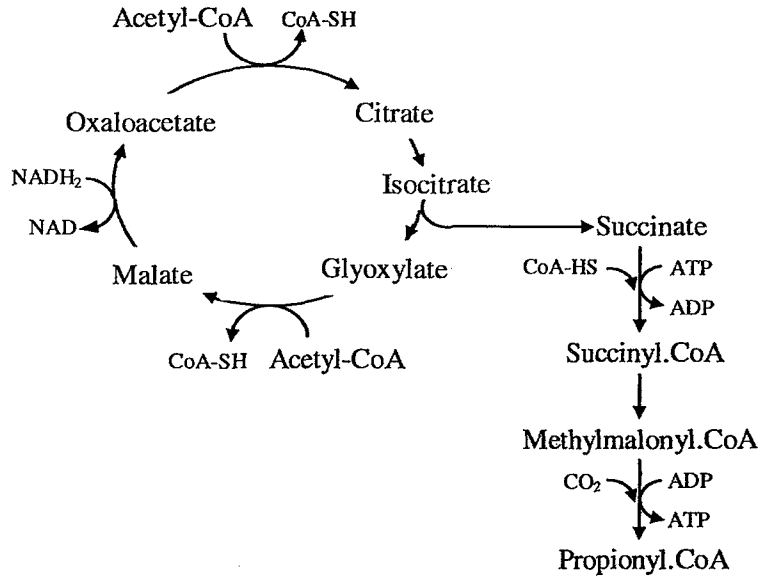


**Figure 4.2:** Glyoxylate and Methylmalonyl Pathway

#### 4.2.1.6 Reaction 5: PHB synthesis

PHB is a polymer of 3-hydroxybutyrate synthesized from acetyl-CoA. First, the intermediary acetoacetyl-CoA is produced from two molecules of acetyl-CoA and this is reduced to 3-hydroxybutyryl-CoA by consuming one molecule of $NADH_2$ per pair of acetyl-CoA.

$$2\ \text{Acetyl-CoA} + NADH_2 \rightarrow \text{3-hydroxybutyryl-CoA} \qquad \textbf{(4.6)}$$

#### 4.2.1.7 Reaction 6: PHV synthesis

In this study, it is assumed that propionyl-CoA produced either through Reaction 4a or 4b is exclusively devoted to the formation of the 3HV unit, which then is polymerized to PHV. This is basically to simplify the model, despite the fact that two molecules of propionyl-CoA may also be synthesized to 3H2MV. Generally the low detection levels of 3H2MV justify this assumption.

$$\text{Acetyl-CoA} + \text{Propionyl-CoA} + NADH_2 \rightarrow \text{3-hydroxyvaleryl-CoA} \qquad \textbf{(4.7)}$$

#### 4.2.1.8 Reaction 7: Poly-P cleavage

It is assumed that ATP regeneration from polyphosphates occurs by the activity of two enzymes: AMP phosphotransferase and adenylate kinase, yielding 1ATP per cleaved phosphate residue (**Van Groenestijn et al., 1987**).

$$\text{Poly-P}_n + \text{ADP} \rightarrow \text{Poly-P}_{n-1} + \text{ATP} \qquad \textbf{(4.8)}$$

### 4.2.2 Determination of the overall stoichiometry for GAOs

For substrate uptake and activation, GAOs have to be able to produce the required amount of energy through utilization of glycogen which also serves as the reducing power source for PHA storage. The basic stoichiometry relies on the balance of both ATP and $NADH_2$ generated through the conversion of glycogen to acetyl-CoA and consumed for PHB synthesis. This balance shows that all the $NADH_2$ produced during the conversion of glycogen to acetyl-CoA cannot be regenerated through PHB synthesis. Conversely, enough acetate cannot be uptaken and activated to acetyl-CoA with produced ATP via EMP pathway even if acetate is available. Therefore, GAOs would be unable to compete with PAOs for acetate unless the $NADH_2$ accumulating within the cell is regenerated through a pathway other than PHB synthesis. In this modeling approach, it is postulated that GAOs are capable of triggering the succinate-propionate pathway ($r_{4a}$) for excess $NADH_2$ regeneration. This way, a certain amount of pyruvate ($f_{SPP}$), produced via glycogen degradation ($r_2$), is converted to propionyl-CoA while the remaining undergoes an oxidative decarboxylation to form acetyl-CoA ($r_3$). It is assumed that all the propionyl-CoA produced from pyruvate leads to PHV, which is composed of one acetyl-CoA and one propionyl-CoA ($r_6$). The remaining acetyl-CoA, produced through glycogen degradation and acetate uptake and activation ($r_1$), will be stored as PHB ($r_5$). Figure 4.3 illustrates the proposed metabolism of GAOs under anaerobic conditions. Combining these reactions without producing net reducing power, the stoichiometric equation for acetate uptake by GAOs under anaerobic conditions is formulated as follows, as a function of $f_{SPP}$.

$$6\,(1\text{-}f_{SPP})\,\text{Acetate} + \text{Glycogen} \rightarrow 4\,(1\text{-}f_{SPP})\,\text{PHB} + f_{SPP}\,\text{PHV} + (2\text{-}f_{SPP})\,CO_2 \qquad \textbf{(4.9)}$$

If there is any energy source other than glycolysis, as in the PAO metabolism, the succinate-propionate pathway may not be operative. In fact, when $f_{SPP}$ is set to zero, equation 4.9 reverts back to reflect the original stoichiometry for PAOs proposed by the Mino model. On the other hand, if one out of two moles of pyruvate produced from one mole of glycogen unit undergoes the succinate-propionate pathway ($f_{SPP}$=1), glycogen is converted to PHV and $CO_2$ without acetate uptake producing extra ATP. This reaction can be considered as the maintenance reaction for GAOs.

$$\text{Glycogen} \rightarrow \text{PHV} + CO_2 + 3\text{ATP} \qquad \textbf{(4.10)}$$

The appropriate fraction of pyruvate that will undergo the succinate-propionate pathway ($f_{SPP}$) is determined by energy requirement for acetate uptake and activation. From ATP balance $f_{SPP}$ can be calculated depending on $\alpha_{GAO}$ as below:

$$f_{SPP} = \frac{1/2 + 2\alpha_{GAO}}{1 + 2\alpha_{GAO}} \qquad \textbf{(4.11)}$$

Incorporation of the value of $f_{SPP}$ from Equation 4.11 into Equation 4.9, and arranging for one mole of acetate, yields:

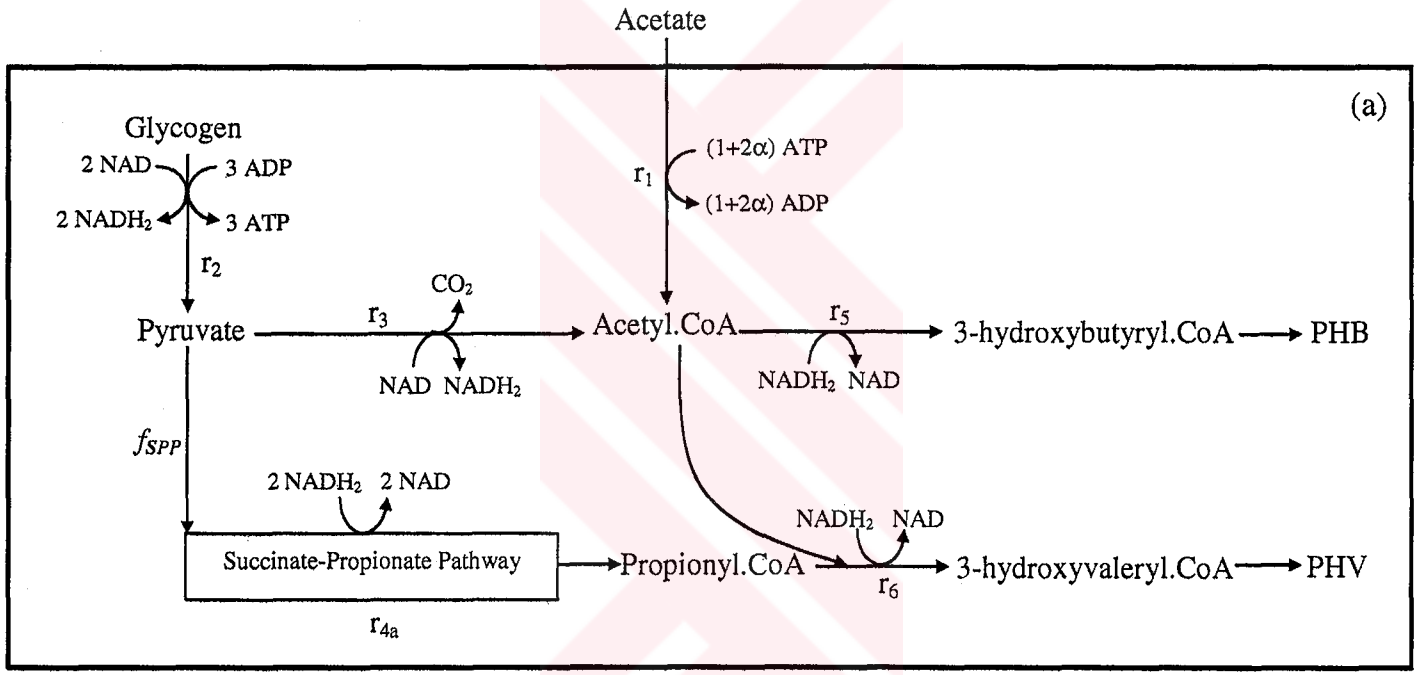


**Figure 4.3:** Proposed metabolisms of GAO under anaerobic conditions

$$\text{Acetate}+\left(\frac{1}{3}+\frac{2}{3}\alpha_{GAO}\right)\text{Glycogen} \rightarrow \frac{2}{3}\ \text{PHB}+\left(\frac{1}{6}+\frac{2}{3}\alpha_{GAO}\right)\ \text{PHV}+\left(\frac{1}{2}+\frac{2}{3}\alpha_{GAO}\right)CO_2 \quad \textbf{(4.12)}$$

The stoichiometry for GAOs given in the above equation maintains the redox and ATP balances in the cell without P release. Incorporating the value of $\alpha_{GAO}$=1/12 at pH= 7.4 as suggested by **Filipe et al. (2001b)**, the above equation may be expressed as:

$$\text{Acetate} + 0.39\ \text{Glycogen} \rightarrow 0.67\ \text{PHB} + 0.22\ \text{PHV} + 0.55\ CO_2 \quad \textbf{(4.13)}$$

### 4.2.3 Determination of the overall stoichiometry for PAOs

Metabolism of PAOs relies on poly-P degradation as energy source for anaerobic acetate uptake rather than glycolysis. Glycogen plays an important role for acetate uptake, serving as a means of maintaining the redox balance within the cell through production of $NADH_2$ only during glycolysis ($r_2$). PAOs oxidize pyruvate further to acetyl-CoA since their major problem is the production of $NADH_2$ to store acetate as PHA. When glycogen is limited, the cells should have another means of $NADH_2$ production. It is postulated that PAOs can produce additional reducing power by glyoxylate pathway ($r_{4b}$) through which a fraction of acetyl-CoA ($f_{GLX}$), produced from pyruvate via oxidative decarboxylation ($r_3$) or from external acetate ($r_1$), is converted to propionyl-CoA. It is assumed that all the propionyl-CoA combining with acetyl-CoA leads to PHV ($r_6$), while the remaining acetyl-CoA will be stored as PHB ($r_5$).

Figure 4.4 illustrates the proposed metabolism of PAOs under anaerobic conditions. Combining these reactions without producing net reducing power, a stoichiometric equation for acetate uptake by PAOs under anaerobic conditions is formed depending on $f_{GLX}$.

$$(6+3f_{GLX})\ \text{Acetate} + \text{Glycogen} \rightarrow 4\ \text{PHB} + f_{GLX}\ \text{PHV} + (2+f_{GLX})\ CO_2 \quad \textbf{(4.14)}$$

When glycogen is not limited for acetate uptake, the glyoxylate pathway may not be operative ($f_{GLX}$=0), yielding the stoichiometry proposed by the Mino model. A value

of $f_{GLX}$ higher than one indicates that a fraction of acetyl-CoA formed from external acetate is converted to propionyl-CoA.

ATP balance is regulated by the poly-P cleavage preceding P release. Arranging the above equation for one mole of acetate and adding the released $P_i$, complete stoichiometry for PAOs is determined as a function of $f_{GLX}$ as below.

$$\text{Acetate} + \frac{1}{6+3f_{GLX}}\,\text{Glycogen} \rightarrow$$

$$\frac{4}{6+3f_{GLX}}\,\text{PHB} + \frac{f_{GLX}}{6+3f_{GLX}}\,\text{PHV} + \frac{1}{3}\,CO_2 + \left[1+2\alpha_{PAO}-\frac{3}{6+3f_{GLX}}\right] P_i \qquad \textbf{(4.15)}$$

## 4.3 Materials and Methods

In this study, the experimental data for the evaluation of the proposed metabolic model for an enhanced biological phosphate removal activated sludge system was derived from lab-scale sequencing batch reactor, (SBR), operated at steady state in an anaerobic/aerobic sequence. The SBR operation for EBPR was designed for four cycles a day (a total cycle time, $T_C$ of 6 hours), where 5 h was devoted to the process phase, $T_P$. The remaining period after the process phase involved 30 min for settling, 15 min for the withdrawal for the treated effluent and 15 min for the idle phase ( $T_S+T_W+T_I=1$ h). The anaerobic phase was selected as 2 h ($T_{AN}=2$ h) with filling in the first 15 min ($T_F=15$ min) and the reactor was aerated for the remaining portion of the process phase ($T_A=3$ h).

The SBR unit used in the study was a cylindrical tank with a working volume of 4 l. A fill volume, $V_F$ of 2.5 l was selected, leaving 1.5 l for the initial volume, $V_0$ and corresponding to a relatively low $V_0/V_F$ ratio of 0.6. The unit was fed with acetate as the sole carbon source to achieve EBPR and initially seeded with biomass obtained from a pilot plant treating acetate supplemented domestic sewage for nutrient removal. The system was ultimately adjusted to a total sludge age, $\theta_X$ of 10 days with appropriate sludge wasting at the end of the aeration phase.

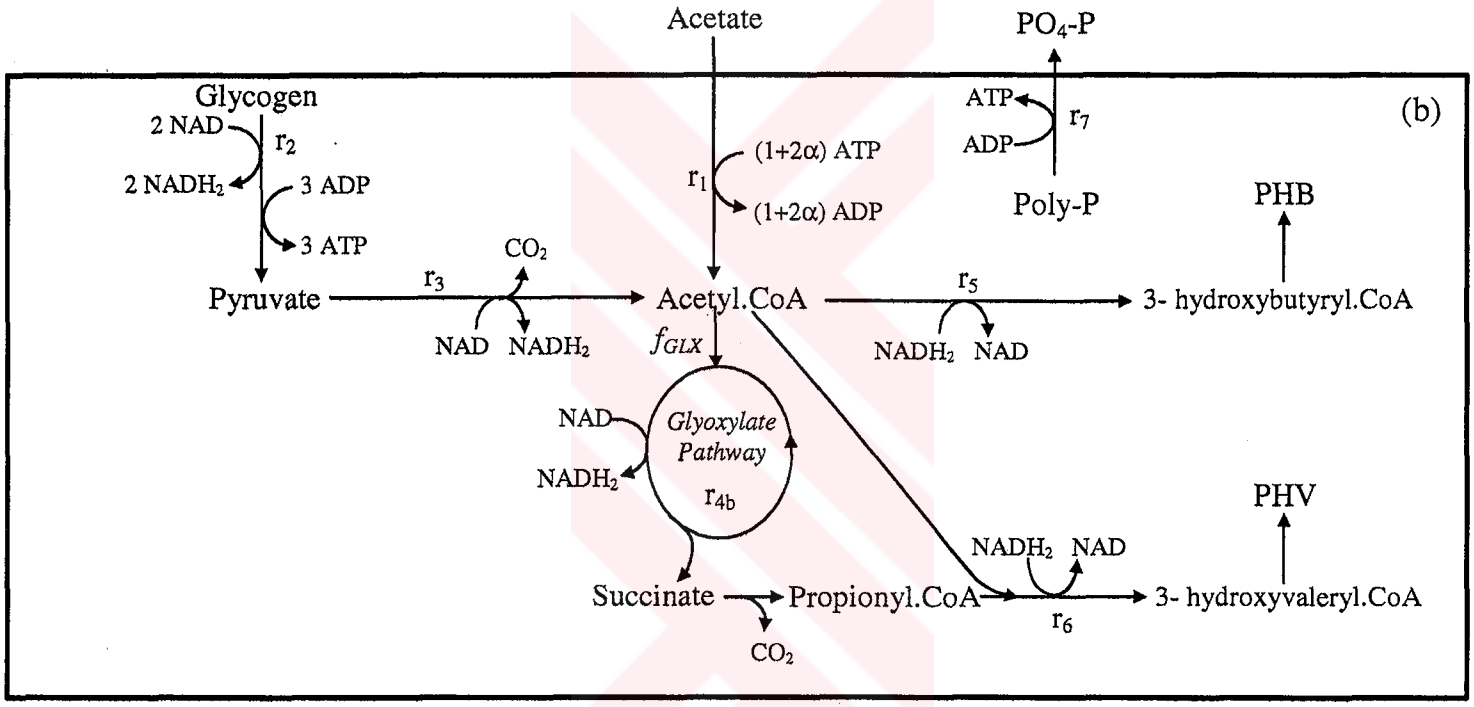


**Figure 4.4:** Proposed metabolisms of GAO under anaerobic conditions

The reactor was equipped with feeding and effluent pumps, air pump, stirrer and motor, and timers for controlling all equipment and system operation. The experiments were carried out during the study in which pH was not controlled in the system. Characteristics and operating conditions of the reactor are schematically given in Fig.4.5.

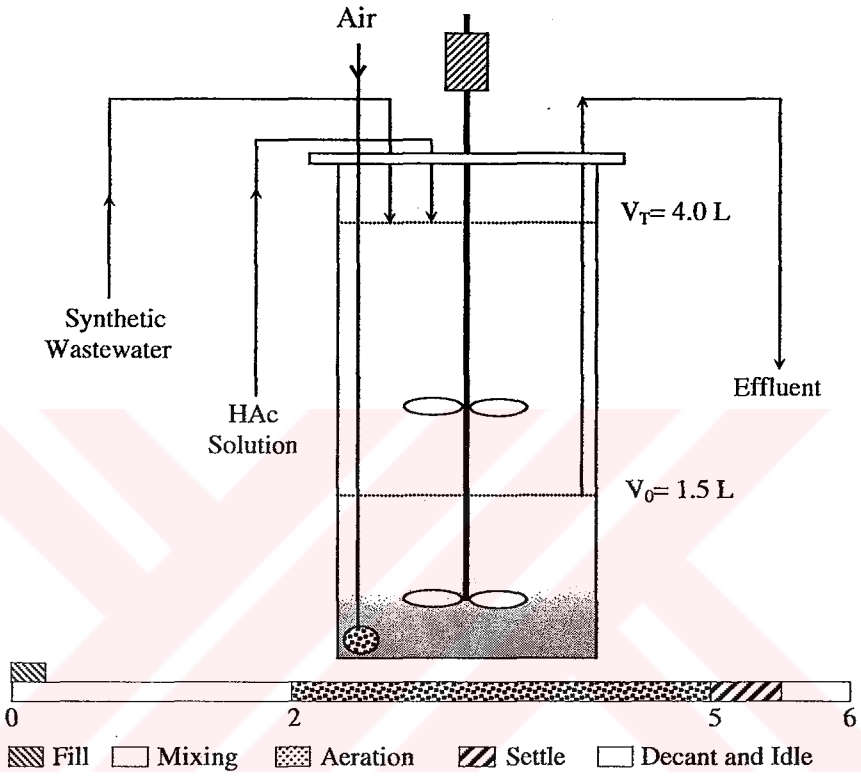


**Figure 4.5:** Schematic representation and operation condition of the SBR

### 4.3.1 Medium

The SBR was fed with a mixture of two feeding solutions, one an inorganic nutrient solution (prepared using nutrient mixture and tap water) and the other an acetic acid solution. Inorganic nutrient solution was prepared daily and deoxygenated by purging $N_2$. The acetic acid solution was also prepared daily and neutralized with NaOH. It was fed to the reactor from a daily-sterilized bottle with closed cap to avoid contamination and minimize biological activity. The synthetic wastewater was as follows: $CH_3COOH$, 400 mg-COD/L; $NH_4Cl$, 20 mg-N/L; $NaHCO_3$, 100 mg-$CaCO_3$/L; 3.4 mg/L yeast extract; and nutrient solution, $MgSO_4$, 10 mg-Mg/L; $FeSO_4.7H_2O$, 350 µg-Fe/L; $MnSO_4.H_2O$, 180 µg-Mn/L; $ZnCl_2$, 130 µg-Zn/L;

$CoCl_2.H_2O$, 35 µg-Co/L; $CuSO_4.5H_2O$, 35 µg-Cu/L; $H_3BO_3$, 20 µg-B/L; KI, 35 µg-I/L; $(NH_4)_6Mo_7O_{24}.4H_2O$, 35 µg-Mo/L; EDTA, 3 mg/L. $CaCl_2$, 15 mg-Ca/L, added to acetate solution to avoid calcium phosphate precipitation in the inorganic nutrient solution. $KH_2PO_4$ and $K_2HPO_4$ were added to increase the theoretical influent phosphate concentrations to 20, 35, 45 and 60 mg/L, respectively.

### 4.3.2 Analysis

The reactor was monitored daily through measurements of pH, HAc, COD, $PO_4$-P, $NH_4$-N, $NO_3$-N, $NO_2$-N, $Ca^{2+}$, $Mg^{2+}$, $K^+$, $Na^+$, $SO_4^{2-}$, MLSS, MLVSS, glycogen, PHB, PHV, and 3H2MV at the aerobic end of the previous cycle, influent, anaerobic end and aerobic end of cycle and effluent. All these parameters were measured during detailed cycle measurements in samples taken from every 5, 10, 15 or 30 minutes of cycle. Samples taken from the mixed liquor were centrifuged for 10 minutes immediately and supernatant was filtered through 0.45 micron filter paper.

The cation and anion analyses of filtered samples were performed using DIONEX Ion Chromatograph according to **Standard Methods for the Examination of Water and Wastewater (1998)**. Glycogen measurements were performed according to the Anthrone Method outlined in **Manual of Methods for General Bacteriology (1981)** with some modifications by Erdal (2002b). PHB, PHV and 3H2MV were analyzed according to **Kisoglu (2000)**. Benzoic acid was used as the internal standard throughout the entire extraction process. The analysis was performed using a HP 5890 gas chromatograph equipped with auto-sampler and a flame ionization detector (FID) and a J&W DB-FFAP FID 0.53 mm wide bore column with film thickness of 1 µm, and length of 30 m. The carrier gas was nitrogen. PHB, PHV and 3H2MV standards were 3-hydroxybutyricacid-co-3-hydroxyvaleric acid and caproic acid sodium salt.

All measurements were made in duplicate. When different they were repeated until the discrepancy was within the limits of analytical accuracy.

## 4.4 Experimental Results

### 4.4.1 SBR Performance at steady state

The laboratory-scale SBR reactor was operated at four different runs, all adjusted to a sludge age, $\theta_X$, of 10 days, mainly to visualize the effect of influent P/COD ratio on system performance. For this purpose, the acetate concentration in the feed was kept constant around 400 mgCOD/l while the $PO_4$-P concentration was gradually increased from 20 mgP/l in the first run to 60 mgP/l in the last run, corresponding to a three-fold change in the influent P/COD ratio in the range of 0.05 to 0.15. At steady state conditions in each run, influent characteristics and values of significant parameters at the end of the anaerobic and aerobic (effluent) stages of the operation were monitored as outlined in Table 4.1. These results first indicate that the selected influent P/COD ratios enabled to sustain two distinct performance patterns in terms of enhanced biological phosphorus removal mechanism involved: At the low P/COD ratio of 0.05, phosphate limiting conditions prevailed and controlled the system performance in the first run with total P removal at the end of the aerobic phase. Increasing P/COD ratios in the other runs, created a different stoichiometry where P was in excess and not totally removed by EBPR. P limitation obviously relates to the stoichiometry of P storage and specifically, to initial acetate/P ratio. In other words, if P is limiting, the existing organic carbon (acetate) is more than necessary to store all available P so that the net result is total P removal (Run 1); whereas when P is not limiting, the P storage capacity is limiting and there is always a net P level in the effluent escaping EBPR (other runs). It is important to note that in these runs, the P concentration at the end of the aerobic phase remained in the low range of 3–8 mg/l. The gradual increase in the influent P level did not proportionally reflect on the effluent and SBR basically responded to this increase by providing additional P removal potential.

**Table 4.1:** The characteristics of influent and end of anaerobic and aerobic periods of SBR

| | | pH | COD | $PO_4$-P | $K^+$ | $Mg^{2+}$ | $Ca^+$ | $NH_4$-N | $NO_3$-N | $NO_2$-N | MLSS | MLVSS/MLSS g/g | Pcontent %TSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mg/l | | | | | | | | | | |
| Influent | Run-1 | 7.1 | 387 | 21.3 | 34.4 | 14.1 | 15.8 | 21.2 | 0 | 0 | - | - | - |
| | Run-2 | 7.1 | 393 | 35.4 | 55.0 | 15.9 | 28.1 | 15.5 | 0 | 0 | - | - | - |
| | Run-3 | 7.1 | 400 | 45.5 | 77.4 | 14.4 | 27.2 | 21.5 | 0 | 0 | - | - | - |
| | Run-4 | 7.1 | 398 | 60.9 | 101.8 | 14.1 | 27.8 | 22.1 | 0 | 0 | - | - | - |
| An. End | Run-1 | 7.40 | 23 | 113.7 | 75.4 | 41.7 | 19.2 | 15.2 | 0 | 0 | 3050 | 0.73 | - |
| | Run-2 | 7.31 | 21 | 135.4 | 95.3 | 41.5 | 30.8 | 10.6 | 0 | 0 | 3750 | 0.66 | - |
| | Run-3 | 7.40 | 20 | 145.7 | 120.9 | 40.7 | 31.5 | 15.2 | 0 | 0 | 3450 | 0.72 | - |
| | Run-4 | 7.31 | 26 | 155.5 | 146.6 | 38.2 | 33.1 | 15.6 | 0 | 0 | 4700 | 0.58 | - |
| Ae. End | Run-1 | 8.50 | 27 | 0.0 | 23.5 | 8.1 | 15.6 | 0.0 | 3.0 | 3.0 | 3650 | 0.68 | 14.6 |
| | Run-2 | 8.92 | 15 | 3.0 | 47.5 | 9.5 | 18.5 | 0.0 | 1.5 | 1.5 | 4560 | 0.61 | 17.8 |
| | Run-3 | 8.91 | 16 | 3.9 | 65.8 | 7.0 | 17.6 | 0.0 | 3.1 | 3.1 | 4500 | 0.59 | 23.0 |
| | Run-4 | 8.94 | 18 | 8.4 | 81.9 | 7.2 | 17.0 | 0.0 | 3.8 | 3.8 | 4370 | 0.50 | 30.0 |

It is known that poly-P is complexed in bacteria almost exclusively with $K^+$ and $Mg^{2+}$, and sometimes slightly with $Ca^{2+}$. Thus, Poly-P metabolism in activated sludge is accompanied by the release/uptake of these ions. From observation with a variety of EBPR sludge, it appears that $K^+$ and $Mg^{2+}$, and sometimes $Ca^{2+}$ to a minor extent, are co-transported with P in a total molar ionic charge ratio of about one (**Comeau et al, 1986**). In this research, the molar $K^+$/P and $Mg^{2+}$/P ratios during P release were observed to be about 0.34 with a minor $Ca^{2+}$ concentration change in solution.

The remaining fraction of the ammonia nitrogen, $NH_4$-N, included in the feed stream as a basic nutrient for heterotrophic growth, was fully nitrified and also denitrified to the extent determined by the $V_O/V_F$ ratio in the SBR operation (**Murat et al., 2002**), so that an equilibrium $NO_3$-N concentration of 1.5–3.8 mgN/l was observed at the end of the aerobic phase of all runs. Denitrification in the early part of the anaerobic phase, although limited, needs to be considered for the necessary adjustment in the calculations of acetate uptake associated with P release.

The SBR was operated with average volatile suspended solids, (VSS), concentration of 2500 mg/l, in accordance with the selected sludge age. The VSS level in the reactor varied slightly in each run, depending on the relative magnitude of stored organics. The significant feature in the performance of different runs related to the fate of particulate matter was the increasing trend in the average suspended solids, (SS), concentration and the corresponding decrease in the VSS/SS ratio, parallel to the increase in the influent P levels. Moreover, the VSS/SS ratio was systematically lower in the aerobic phase as compared to the preceding anaerobic phase. The trend in the VSS/SS ratio, both at steady state and between the aerobic/anaerobic phases of each run should be regarded as typical indexes of the EBPR potential sustained in the SBR operation.

### 4.4.2 Fate of significant components through cyclic operation

Observation of the concentration profiles throughout a representative cycle is a powerful tool for the understanding of SBR behavior under corresponding conditions. When properly interpreted, it may be used to evaluate the fate of significant components and to indicate the required adjustments for optimizing

system operation. This approach is often adopted for SBR treatment of different wastewaters (**Tasli et al., 1999; Carucci et al., 1999**). In this research, phosphate and acetate concentrations together with concentration profiles of storage compounds, namely, PHB, PHV, 3H2MV and glycogen, measured within a selected complete cycle during the steady state operation of each SBR run are given in Figure 4.6.

The evaluation of the data presented in Figure 4.6 shows that, after an initial increase associated with reactor hydraulics during the fill phase, acetate concentration rapidly dropped to depletion within the first 60 min of the anaerobic phase. A gradual increase in the acetate uptake rate was observed from 205 mg HAc/l.h in Run 1 to 272 mg HAc/l.h in Run 4. Parallel to the trend in the acetate uptake, phosphate was released at an increasingly higher rate of 94 mg P/l.h in Run 1 to 112 mg P/l.h in Run 4. In all Runs, the pace of P release slowed down considerably after the depletion of acetate in the solution. As expected, accumulation of PHB and, to a much lesser extent, other PHA compounds, exhibited a similar trend with P release.

The fate of glycogen is also an important parameter for evaluation of the EBPR system sustaining a dual culture of PAOs and GAOs as tested in the experiments. The level of the glycogen concentration recorded at the beginning of the anaerobic phase of each run was observed to drop parallel to the increase in the P/COD ratio of the influent.

Glycogen consumption proceeded rapidly as long as acetate remained in solution and continued at a much slower rate after the complete removal of acetate. After the depletion of acetate, the minor glycogen consumption was coupled with a similar PHA (mainly PHV) storage, observed until the end of anaerobic period. In all runs, glycogen was not totally utilized with always a remaining concentration at the end of the anaerobic phase.

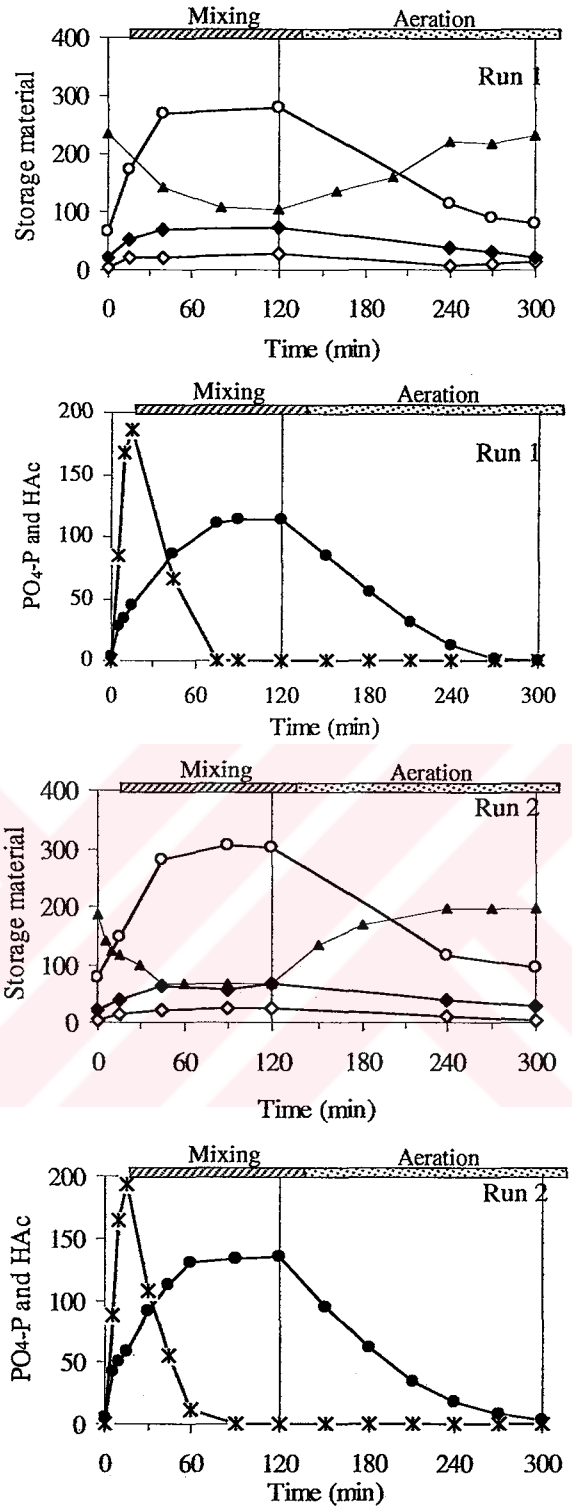


**Figure 4.6:** Variations of phosphate (●) and acetate (∗) (mg/L), PHB (○), PHV (◆), 3H2MV (◇) and glycogen (▲) (mg COD/L) concentrations during a typical cycle of each Run

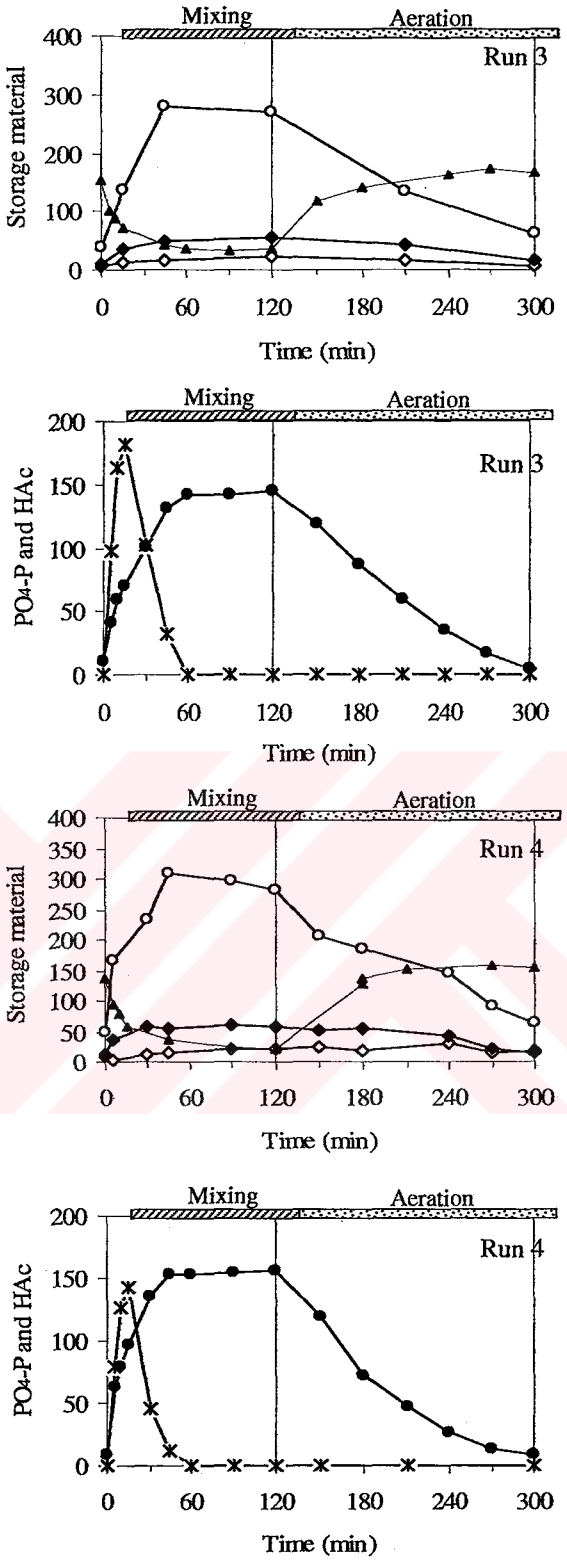


**Figure 4.6:** (continued) Variations of phosphate (●) and acetate (∗) (mg/L), PHB (○), PHV (◆), 3H2MV (◇) and glycogen (▲) (mg COD/L) concentrations during a typical cycle of each Run

### 4.4.3 Observed stoichiometry of acetate uptake

For a better understanding of the basic stoichiometry related to the metabolic activities of the mixed culture of PAOs and GOAs sustained in the SBR system, the magnitude of significant parameters, namely the amount of P release, acetate uptake, glycogen consumption and PHA generation, was calculated after the first 60 min within the anaerobic phase of each Run, a period corresponding to complete depletion of acetate. Computed values for these parameters are given in Table 4.2. Acetate consumption due to nitrate and dissolved oxygen recirculated into the anaerobic phase through mixing of the settled (initial) volume at the beginning of the cycle was taken into account for the correction of acetate uptake values.

**Table 4.2:** The observed values of phosphate release, glycogen consumption and PHA production associated with acetate uptake in the anaerobic phase

| | COD/TP Ratio | Phosphate Release | Acetate Uptake | PHB Prod. | PHV Prod. | 3H2MV Prod. | PHA Prod. | Glycogen Cons. |
|---|---|---|---|---|---|---|---|---|
| | mgCOD/mgP | mgP/L | mgCOD/L | | | | | |
| Run-1 | 20.0 | 93.9 | 235.4 | 206.5 | 50.7 | 22.0 | 279.2 | 124.8 |
| Run-2 | 11.4 | 105.6 | 240.8 | 222.8 | 29.1 | 15.6 | 267.5 | 119.4 |
| Run-3 | 8.9 | 109.8 | 242.6 | 229.5 | 42.3 | 14.7 | 286.5 | 114.5 |
| Run-4 | 6.7 | 112.1 | 240.9 | 238.0 | 45.2 | 8.0 | 291.2 | 113.1 |

The first important observation on the data in Table 4.2 relates to acetate uptake and phosphate release: The acetate uptake stayed constant around 240 mgCOD/l, as expected because the influent acetate concentrations was nearly constant for all SBR Runs, and full acetate removal was accomplished by each run, whereas phosphate release exhibited a 20% increase from 93.0 mg/l in Run 1 to 112.1 in Run 4. Consequently, the P/HAc increased in the range of 0.40 to 0.46 mgP/mgCOD in the same direction of the change in the feed composition. The results related to glycogen consumption and accumulation of PHA compounds should be differentiated for the first Run, where phosphate was lower and limiting EBPR, and the other Runs operated with increasing EBPR but always with excess phosphate. In this framework, glycogen consumption showed a gradual decrease with a markedly higher level of 124.8 mgCOD/l with P limitation as compared to a value around 114 mgCOD/l in Runs 3 and 4. As far as stored organics is concerned, the total PHA level seemed to remain constant around 280-290 mgCOD/l with PHB the major component in all the

Runs, whereas the PHB/PHA ratio of 0.73 in the first Run was lower than the 0.80-0.82 level associated with the other Runs.

### 4.5 Evaluation of Results

The evaluation will focus on data summarized in Table 4.3, reflecting the basic stoichiometry of acetate uptake under anaerobic conditions. The table expresses experimental results obtained in this research for major parameters as molar ratios with respect to acetate together with the corresponding values predicted in the proposed metabolic model; it also includes, for comparative evaluation, corresponding stoichiometric data derived from similar model predictions and experimental results reported in the literature.

As illustrated in Table 4.3, the significant feature of the proposed model for PAOs is a variable stochiometry reflecting the presumed ability of these types of microorganisms to trigger different metabolic pathways best suitable to environmental conditions. The earlier models however were structured to yield fixed stochiometric ratios for PHB production, glycogen consumption and phosphate release per mole of acetate uptake. They also postulated that all PHA generation could be expressed in terms of PHA whereas in this research PHV is measured and envisaged in the proposed model as well as PHB.

Recently a metabolic model for the stoichiometry of acetate uptake under anaerobic conditions by an enriched culture of GAOs has been developed by Filipe *et al.* (2001b) and modified by **Zeng et al. (2002)**. Although using the same pathway, the modified model resulted in a different stoichiometry than the one developed by Filipe *et al.* (2001b) postulating one mole of ATP generation in the succinate-propionate pathway. However, no net ATP generation can be associated with this pathway based on principles of bacterial metabolism. In this chapter, the metabolic pathways used in the modified model was adopted so the stoichiometry for GAOs is the same with the one proposed by **Zeng et al. (2002)** except for the combination of precursors in PHA.

The experimental results, both obtained in this research and reported in the literature in different EBPR systems fed with acetate, all reflect stoichiometric ratios quite

variable depending upon feeding and environmental conditions selected, with a strong indication of the presence of a mixed culture of PAOs and GAOs. This may be better evaluated by establishing a carbon and oxidation/reduction (O/R) balance with $CO_2$ production during the storage of acetate as PHA by PAOs and GAOs. Glycogen consumption should also maintain the ATP balance required for the anaerobic acetate uptake with or without P release; P release ratios may vary significantly both due to the presence of GAOs and to the differences in the ATP balance.

### 4.5.1 Carbon and O/R balance

No net oxidation can be achieved during anaerobic metabolism of acetate due to lack of external inorganic electron acceptor. This means that the oxidation level of all the products put together must be the same as that of the starting materials, acetate and glycogen. The oxidation-reduction state of an organic compound may be calculated by comparing it with that of water as defined by **Gaudy and Gaudy (1980)** where hydrogen is arbitrarily assigned a value of -0.5, oxygen a value of +1 and the oxidation state of water, or of any compound containing hydrogen and oxygen, in a ratio of 2:1 (e.g., that carbohydrates, is thus 0). Carbon and O/R balances may then be used as a check on the recovery of products. It is known that storage products, generally defined as PHA, consist of four monomers (**Satoh et al., 1992**). As PHA cannot be measured, most studies choose to process and evaluate PHB as PHA, leading to a serious impairment of carbon balance. This study did not measure one of the four monomers, 3H2MB and $CO_2$ generated during the anaerobic metabolism of glycogen or acetate. A joint carbon and O/R balance however can be used to provide a unique solution for the corresponding values of both 3H2MB and $CO_2$. Table 4.4 illustrates the calculation of these parameters based on experimental results from Run 1, yielding a 3H2MB/HAc ratio of 0.05 and a $CO_2$/HAc ratio of 0.39. Corresponding ratios calculated for the other Runs are given in Table 4.3. It is important to note that the level $CO_2$/HAc ratio, ranging from 0.36 to 0.39 for different Runs in the study, is higher than the value of 0.33 solely associated with the PAO metabolism, also a significant indication for the presence of GAOs.

**Table 4.3:** Theoretical and observed stoichiometry for uptake of 1 mol acetate

| $\frac{Gly}{HAc}$ | $\frac{PHB}{HAc}$ | $\frac{PHV}{HAc}$ | $\frac{3H2MV}{HAc}$ | $\frac{P}{HAc}$ | $\frac{CO_2}{HAc}$ | Reference |
|---|---|---|---|---|---|---|
| Biochemical Models | | | | | | |
| *Biochemical Models for PAOs* | | | | | | |
| - | 0.44 | - | - | 1.00 | 0.22 | **Comeau et al., 1986** |
| 0.17 | 0.67 | - | - | 0.50 | 0.33 | **Mino et al., 1987** |
| 0.17 | 0.67 | - | - | 0.67 | 0.33 | **Wentzel at al., 1991a** |
| 0.17 | 0.67 | - | - | $2\alpha_{PAO} + 0.5$ | 0.33 | **Smolders et al., 1994** |
| $\frac{1}{6+3f_{GLX}}$ | $\frac{4}{6+3f_{GLX}}$ | $\frac{f_{GLX}}{6+3f_{GLX}}$ | - | $2\alpha_{PAO}+\left(1-\frac{3}{6+3f_{GLX}}\right)$ | 0.33 | This study |
| *Biochemical Models for GAOs* | | | | | | |
| 0.33 | 0.68 | 0.15 | 0.008 | - | 0.50 | **Filipe et al., 2001b*** |
| 0.39 | 0.68 | 0.19 | 0.014 | - | 0.55 | **Zeng et al., 2002*** |
| 0.39 | 0.67 | 0.22 | - | - | 0.55 | This study* |
| Observed Stoichiometry | | | | | | |
| *This study* | | | | | | |
| 0.18 | 0.49 | 0.07 | 0.02 | 0.83 | 0.39** | Run 1 |
| 0.17 | 0.52 | 0.04 | 0.02 | 0.91 | 0.38** | Run 2 |
| 0.16 | 0.53 | 0.06 | 0.02 | 0.93 | 0.37** | Run 3 |
| 0.16 | 0.54 | 0.05 | 0.01 | 0.96 | 0.36** | Run 4 |
| *Results in the literature* | | | | | | |
| 0.20 | 0.65 | - | - | 0.87 | - | **Satoh et al. (1992)** |
| - | 0.60 | - | - | 0.52-1.52 | 0.36-0.44 | **Smolders et al. (1995)** |
| 0.23 | 0.51 | 0.18 | - | 0.32 | - | **Pereira et al. (1996)** |
| 0.19 | 0.66 | - | - | 0.84 | - | **Kuba et al. (1997)** |
| 0.20 | 0.56 | 0.12 | - | 0.74 | - | **Hesselmann et al. (2000)** |

*$\alpha_{GAO}$= 1/12; **Calculated from C and O/R balance

**Table 4.4:** Calculation of a C and O/R balance for Run-1

| Species | Formula | (1) No of C | (2) O/R State | (3) mol | (1)*(3) mol-C | (2)*(3) |
|---|---|---|---|---|---|---|
| Acetate | $C_2H_4O_2$ | 2 | 0 | 1 | 2.00 | 0.00 |
| Glycogen | $C_6H_{10}O_5$ | 6 | 0 | 0.18 | 1.07 | 0.00 |
| | | | | | 3.07 | |
| 3HB | $C_4H_8O_3$ | 4 | -1 | 0.49 | 1.96 | -0.49 |
| 3HV | $C_5H_{10}O_3$ | 5 | -2 | 0.07 | 0.35 | -0.14 |
| 3H2MV | $C_6H_{12}O_3$ | 6 | -3 | 0.02 | 0.12 | -0.06 |
| 3H2MB | $C_5H_{10}O_3$ | 5 | -2 | 0.05* | 0.25 | -0.10 |
| $CO_2$ | $CO_2$ | 1 | +2 | 0.39* | 0.39 | 0.78 |
| | | | | | 3.07 | |

*Calculated from C and O/R balance

Although the storage polymer consists of four monomeric units the proposed biochemical model accounts for only PHB (3HB unit) and PHV (3HV unit) since the main concern is the amount and composition of precursors (acetyl-CoA and propionyl-CoA) and $NADH_2$ balance. The calculated PHA unit (3H2MB) can be added to 3HV since they are isomers. The measured 3H2MV unit, which is composed of two molecules of propionyl-CoA, can be partitioned between PHB and PHV (twice the amount in moles of measured 3H2MV added to PHV, while the measured amount is subtracted from PHB). The moles of produced PHB* and PHV* corrected this way from measured 3H2MV values per mole of acetate are given in Table 4.5.

**Table 4.5:** Corrected PHA components and PHA composition

| | COD/TP | PHB*/HAc | PHV*/HAc | PHB*/HAc | PHV*/HAc | PHA*/HAc | PHB*/PHA* |
|---|---|---|---|---|---|---|---|
| | mgCOD/mgP | mol/mol | mol/mol | C-mol/C-mol | C-mol/C-mol | C-mol/C-mol | C-mol/C-mol |
| Run-1 | 20.00 | 0.47 | 0.17 | 0.94 | 0.42 | 1.36 | 0.69 |
| Run-2 | 11.43 | 0.50 | 0.13 | 1.00 | 0.32 | 1.32 | 0.76 |
| Run-3 | 8.89 | 0.51 | 0.11 | 1.02 | 0.29 | 1.31 | 0.78 |
| Run-4 | 6.67 | 0.53 | 0.10 | 1.06 | 0.24 | 1.30 | 0.82 |

### 4.5.2 PHB production and PHA composition

The model proposed in this study for the PHB production postulates a variable stoichiometry, introducing the *glyoxylate pathway* with a coefficient $f_{GLX}$ which indicates fraction of acetyl-CoA converted to propionyl-CoA as contrasted to earlier

models predicting a constant PHB/HAc ratio. According to the Mino model and its modifications (**Mino et al., 1987; Wentzel et al., 1991a; Smolders et al., 1994**) the PHB production ratio is 0.67, higher than that in the Comeau model due to the glycogen involvement. A PHB/HAc ratio lower than 0.67 as obtained in this study and in similar experimental work implies that a part of acetyl-CoA, either produced from glycogen or from external acetate, is used to produce reducing power via TCA cycle or other pathways yielding PHV and $CO_2$. In fact, this ratio was consistently lower than 0.67 in the experiments, ranging from 0.47 in Run 1 to 0.53 in Run 4, as given in Table 4.5.

The observed PHA production/acetate uptake ratios gradually decrease with increasing P input and the PHB fractions of PHA increase from 0.69 in Run 1 where the influent P was limited to 0.75 in Run 4, as can be seen from Table 4.5. This result implies that P limiting conditions trigger higher glycogen involvement in PHA synthesis and higher glycogen consumption by GAOs leads to PHV enriched carbon storage material.

$$\frac{PHB^*}{HAc} = f_{PAO}\left(\frac{4}{6+3f_{GLX}}\right) + 0.67(1-f_{PAO}) \qquad \textbf{(4.16)}$$

$$\frac{PHV^*}{HAc} = f_{PAO}\left(\frac{f_{GLX}}{6+3f_{GLX}}\right) + 0.22(1-f_{PAO}) \qquad \textbf{(4.17)}$$

Cycle measurements also show that the PHV fraction of produced PHA does not remain constant during the anaerobic period of a cycle at steady state (Figure 4.7). At the beginning, the PHV fraction is high and decreases with decreasing acetate concentration until acetate depletion at about 60 minutes. This result can be explained by a higher PHV production of PAOs via *glyoxylate pathway* to produce extra reducing power for storage of acetate when at high concentration. Figure 4.7 also shows that this observation is more pronounced in Run 4 presumably operating with a higher PAO population. The slight increase in the PHV fraction after depletion of acetate may be attributed to the maintenance reaction of GAOs.

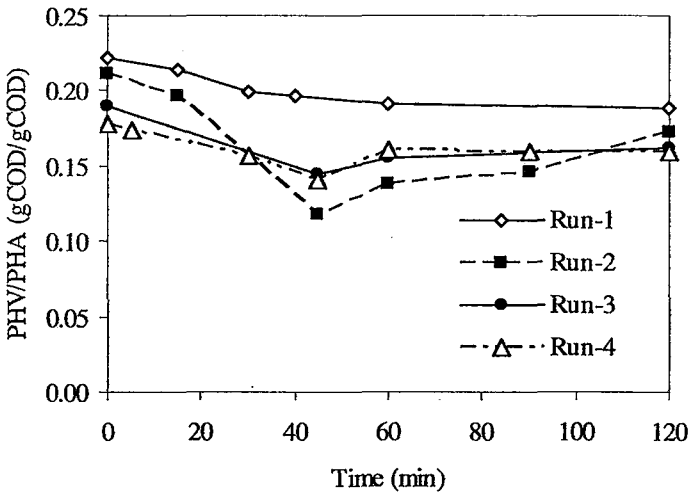


**Figure 4.7:** Changes in PHV fraction during the anaerobic period

### 4.5.3 Glycogen utilization

The proposed metabolic model also defines a variable stoichiometry for glycogen utilization. A glycogen utilization/acetate uptake ratio higher than 0.17 would strongly indicate the presence of GAOs, while a lower ratio may be regarded as the result of acetyl-CoA consumption for reducing power production. In fact, the presence of GAOs influences this ratio since they consume more glycogen than PAOs. However, glycogen utilization for reducing power by PAOs is not fixed based on the assumption that they can derive a part of the required reducing power from converting acetate to PHV via *glyoxylate pathway*. Thus, the Gly/HAc ratio in the model is affected not only by the fraction of PAOs ($f_{PAO}$) in the mixed culture but also by their reducing power source as shown in the following expression:

$$\frac{Gly}{HAc} = f_{PAO}\left(\frac{1}{6+3f_{GLX}}\right) + 0.39\,(1-f_{PAO}) \qquad \textbf{(4.18)}$$

In the SBR experiments, the level of glycogen utilization per acetate (Gly/HAc) increased with decreased P input, that is, with an increase in the influent COD/TP ratio. This trend is also supported by similar experimental results reported in the literature as plotted in Figure 4.8.

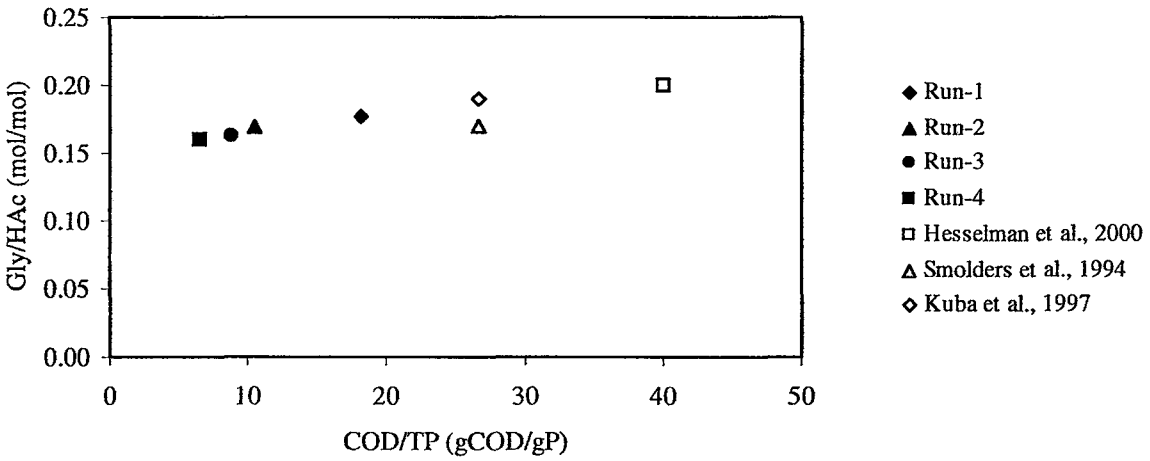


**Figure 4.8:** Effect of influent COD/TP ratio on GLY/HAc

### 4.5.4 Phosphate release

The observed ratio of phosphate released per acetate uptake (P/HAc) in this study and in similar experimental work derived from the literature exhibit a decrease with decreased P input as indicated in Figure 4.9, in an opposite trend associated with of glycogen utilization. This result is expected since glycogen is used for additional ATP generation. It also indicates that GAOs may affect system stoichiometry when they are sustained to an appreciable extent in the PAO enriched EBPR sludge, especially for a high influent COD/TP ratio.

In this framework, the measured P/HAc ratios may be interpreted using the following stoichiometric expression, accounting for the presence of GAOs in the mixed culture:

$$\frac{P}{HAc} = f_{PAO}\left((1+2\alpha_{PAO}) - \frac{3}{6+3f_{GLX}}\right) \qquad \textbf{(4.19)}$$

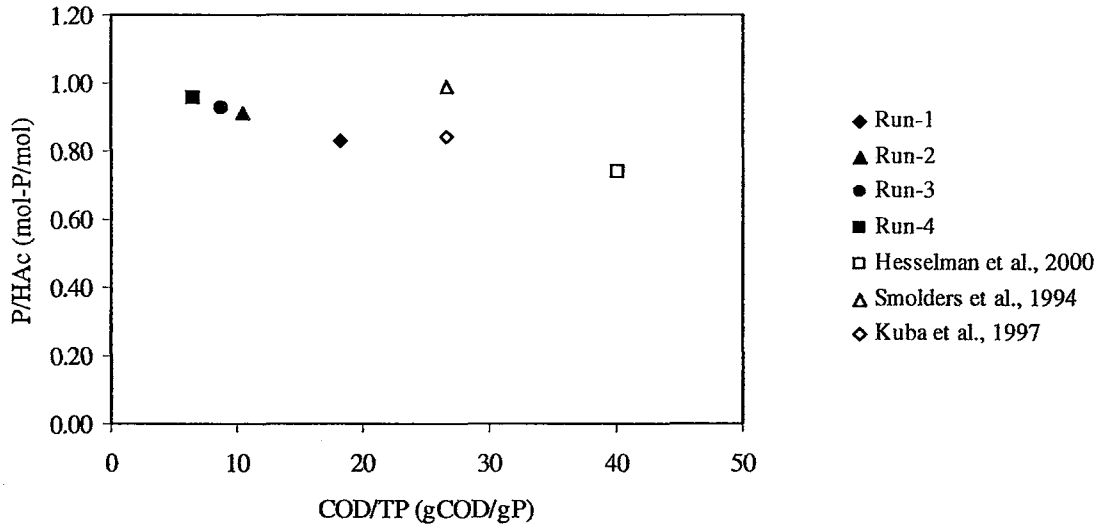


**Figure 4.9:** Effect of influent COD/TP ratio on P/HAc

### 4.5.5 Experimental verification of the proposed model

The proposed model describing the stoichiometry of anaerobic acetate metabolism by a mixed culture of PAOs and GAOs was tested with experimental data obtained in this study and given in the literature using different experimental EBPR systems. The fraction of PAOs, ($f_{PAO}$) and the fraction of acetyl-CoA converted to PHV by PAOs via the *glyoxylate pathway,* ($f_{GLX}$), were calculated from the measured PHB*/HAc and Gly/HAc values using equations (4.16) and (4.18) for each Run. A similar evaluation was also carried out using the data presented by **Pereira et al. (1996)** and **Hessselman et al. (2000)**. As listed in Table 4.6, calculated $f_{PAO}$ values ranged from 0.88 in Run 4 to 0.73 in Run 1, confirming the validity of a mixed culture and the presence of a significant GAO fraction under P limiting conditions. The $f_{PAO}$ values calculated for other studies varied over a wide spectrum of 0.29 to 0.99, underlining the impact of specific experimental conditions on population dynamics. Table 4.6 also indicates that $f_{GLX}$ values remained around 1.0 or below. An $f_{GLX}$ level below 1.0 indicates that the Propionyl moiety of PHV is not derived from extracellular acetate, but exclusively from the intracellular glycogen. This result is confirmed by **Pereira et al. (1996)** not detecting labelled propionate in PHV using (13C-2) acetate. On the other hand, decreasing $f_{GLX}$ values may be attributed to the fact that extra reducing power production from glycogen via the glyoxylate pathway decreases with

increasing P input, that is, glycogen consumption by PAOs per mole of acetate increases when P is not limited.

Using the calculated $f_{PAO}$ and $f_{GLX}$ values, $PHV^*$/HAc ratios were computed for each Run from equation (4.17) and listed in Table 4.6 to check with the corresponding values derived from the experiments. Comparison of data presented in Table 4.6 indicates full agreement between $PHV^*$/HAc ratios measured and calculated from the model, also showing that the PHA composition was correctly predicted by the proposed model. Furthermore, an additional check was made for the observed P release levels on the basis of the proposed stoichiometry. This check as listed in Table 4.6 indicates that the values of the P/HAc ratio derived from equations suggested by **Smolders et al. (1994)** and **Filipe et al., (2001a)** expressing P/HAc as a function of pH are quite compatible with those obtained in this study. This provides additional support for the validity of the proposed model advocating existence of a mixed culture of PAOs and GAOs since the above agreement was secured by correcting the measured P/HAc ratios by the $f_{PAO}$, the fraction of PAOs in the mixed population. Table 4.6 reflects a good agreement between proposed and observed stoichiometry except for the results of **Pereira et al. (1996)**, showing inconsistency in the ATP balance although C and redox balances were satisfied; glycogen consumption and P release given in this study cannot yield enough energy required for the acetate uptake.

Despite the fact that the glycogen metabolism used by GAOs is less efficient in terms of energy production as compared to poly-P metabolism of PAOs, GAOs can proliferate when EBPR systems are operated with P limiting conditions. In these systems, a part of acetate, $(1-f_{PAO})$, remains available for GAOs due to slow-down of acetate uptake by PAOs under P limitation. Understanding the mechanism of PAO-GAO competition under COD limiting conditions is also quite significant since COD limitation is likely to affect directly the level of effluent P concentration. Both aspects deserve further investigation.

**Table 4.6:** Verification of the proposed model

| | $f_{GLX}$ | $f_{PAO}$ | $[PHV^{\cdot}/HAc]$ mol/mol | | pH | $[P/HAc]$ mol/C-mol | | | $\frac{[P/HAc]_m}{f_{PAO}}$ |
|---|---|---|---|---|---|---|---|---|---|
| | - | - | Calculated[1] | Measured | - | Calculated[2] | Calculated[3] | Measured | - |
| Run-1 | 1.35 | 0.73 | 0.16 | 0.17 | 7.4 | 0.63 | 0.56 | 0.40 | 0.55 |
| Run-2 | 0.91 | 0.80 | 0.13 | 0.13 | 7.3 | 0.62 | 0.54 | 0.45 | 0.56 |
| Run-3 | 0.78 | 0.84 | 0.11 | 0.11 | 7.4 | 0.63 | 0.56 | 0.44 | 0.52 |
| Run-4 | 0.61 | 0.88 | 0.10 | 0.10 | 7.3 | 0.62 | 0.54 | 0.45 | 0.51 |
| **Hesselman *et al.* (2000)** | 0.56 | 0.73 | 0.11 | 0.12 | 7.0 | 0.57 | 0.48 | 0.37 | 0.51 |
| **Pereira *et al.* (1996)** | 1.54 | 0.54 | 0.18 | 0.18 | 7.0 | 0.57 | 0.48 | 0.16 | 0.30 |

[1] calculated from equation (4.17) using $f_{PAO}$ and $f_{GLX}$ values

[2] P/HAc = 0.16 pH - 0.55 (**Filipe et al., 2001c**)

[3] P/HAc = 0.19 pH - 0.85 (**Smolders et al., 1994**)

**Hesselmann et al. (2000)**, assume that propionyl-CoA is formed by PAOs via one of the two pathways (reaction 4a or the first part of the citric acid cycle accompanied by the formation of $NADH_2$) dependent upon the availability of reducing power. In their metabolic model approach, the possible contribution by GAOs are inherently accounted for, since the metabolic pathway used by GAOs to regenerate excess $NADH_2$ (reaction 4a) is included in PAO metabolism. This implies that PAOs and GAOs are possibly one group of organism. There is no experimental evidence that PAOs can perform the metabolism of GAOs under anaerobic conditions. Conversely, **Brdjanovic et al. (1998)** showed that no acetate is taken up under conditions of absence of poly-P and surplus glycogen content of the biomass. Their study suggested PAO can not use glycogen as the sole energy source under anaerobic conditions, which seems to be the restricted to GAOs. If PAOs and GAOs are different organisms and may coexist in EBPR sludge, as postulated and verified in our metabolic model, competition between PAOs and GAOs should be more significant for the determination of EBPR performance. This possibility increases the importance of proper definition of the environmental factors that increase the acetate uptake rate by GAOs more than that of PAOs.

# 5. A NEW INTERPRETATION OF ASM2d FOR MODELING AND CALIBRATION OF SBR BEHAVIOR FOR ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL

## 5.1 Introduction

Enhanced biological phosphorus removal, (EBPR), is one of the most intriguing metabolic activities of microbial cultures. It is commonly associated with a specific group of bacteria with the exclusive ability of converting short chain fatty acids, mainly acetate, into internal storage products with orthophosphate release, ($P_i$), under anaerobic conditions. These bacteria require a subsequent aerobic stage where they grow at the expense of the stored organics taking back excessive amounts of orthophosphate as compared to conventional heterotrophic growth. Although widely used as a biological treatment method for effective phosphorus removal from wastewaters, many aspects of the complex microbial mechanism in the process sequence still require better understanding and interpretation. Despite extensive research on the subject, there are still conflicting arguments concerning the basic stoichiometry and kinetics of the different processes involved (**Wentzel et al., 1990; Wentzel et al., 1991b; Smolders et al., 1994; Murnleitner et al., 1997; Filipe et al., 2001c**).

Modeling is now an integral part of biological wastewater treatment not only as a supporting tool for system design and performance prediction, but also as a valuable asset for a more accurate evaluation of the complex microbial mechanisms, which eventually leads to the development of more realistic models. Activated sludge as a wastewater treatment system has been the platform of a remarkable success on process modeling in the last two decades, first for organic carbon and nitrogen removal (**Dold et al. 1980; Ekama and Wentzel, 1990; Henze, et al., 1987**) and then

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for EBPR (**Wentzel et al., 1985; Wentzel et al., 1991b; Barker and Dold, 1997**). The accumulated scientific information has been the basis of Activated Sludge Model No.2, (ASM2) including the additional model components and processes defining EBPR for domestic sewage (**Henze et al., 1995**). Later, ASM2 was modified as ASM2d to include denitrification by PAOs with reduced P-uptake (**Henze et al., 1999**). In these models, (ASM2 and ASM2d), poly-hydroxyalkanoates, (PHA) are involved as the single carbonaceous polymer which is stored only by phosphate accumulating organisms, (PAOs), at the expense of acetate as the external fermentation product.

ASM2d, as well as other activated sludge models, are proposed with a numerical set of stoichiometric and kinetic coefficients suggested as default values, derived from full scale and laboratory experiments performed under conditions often favoring particular processes. Therefore, these parameters can be very case-specific and new values should be assigned for each specific wastewater based on pilot and/or full-scale data for the model calibration. The ASM2d provides an acceptable and reliable description for carbon removal and nitrogen transformations, but still experiences problems in predicting the fate of phosphate, especially because of the uncertainties associated with the definition of PAOs and the nature of storage polymers. Disagreements appear in the literature on the reported values of kinetic and stoichiometric parameters as a result of the complexity of the model due to the increased number of processes and coefficients it inevitably includes, the lack of sufficient experimental support and the unexpected uncertainties in full-scale cases. On the other hand, the success of the model for a particular application depends on the validity of the parameters suggested for the particular coefficients affecting the behavior of PAOs and the fate of significant model components, such as acetate, phosphate and PHA. This requires appropriate experimental design and reactor selection for parameter adjustment and model calibration with experimental data. In this respect, the sequencing batch reactor is a well studied biological treatment system in terms of its potential for nutrient removal (**Tasli et al., 2001; Artan et al., 2002; Artan et al., 2003**). It also offers a major advantage for the observation of cyclic experimental data on the fate of model components for model simulation and calibration.

In this context, the main objective of this research was to test and evaluate the predictive capability of ASM2d for the EBPR achieved with an SBR receiving variable influent phosphate loads. For this purpose, an iterative calibration methodology was developed based on sensitivity analysis and applied to four different sets of experimental data on relevant model parameters reflecting SBR performance. Differences in the values of calibrated parameters and biomass composition for different experimental conditions were interpreted accounting for the structure of ASM2d, which excludes some of the significant model components affecting, to a variable extent, system stoichiometry and kinetics.

## 5.2 Materials and Methods

### 5.2.1 Operation of sequencing batch reactor

The experimental data required for the evaluation and modeling of enhanced biological phosphorus removal was obtained from a lab-scale SBR with a working volume of 4 L, continuously operated at steady-state in an anaerobic/aerobic sequence with a pre-anoxic phase. The experimental work was carried out in a constant temperature room maintained at 20±1°C. The SBR was initially seeded with sludge obtained from a nutrient removal pilot plant treating domestic sewage with acetate supplement, and fed with acetate as the sole carbon source to achieve EBPR.

The SBR operation for EBPR involved four cycles a day (a total cycle time, $T_C$, of 6 hours), with a process phase, $T_P$, of 5 h in each cycle. An anaerobic phase of 2 h ($T_{AN}$ =2 h) was selected with filling in the first 15 min ($T_F$ =15 min) and the reactor was aerated for the remaining portion of the process phase ($T_A$ =3 h). The remaining period after the process phase involved 30 min for settling, 15 min. for the withdrawal for the treated effluent and 15 min for the idle phase ($T_S+T_W+T_I$ = 1 h). The SBR was operated with a relatively low $V_O/V_F$ ratio of 0.6, which was obtained by leaving 1.5 L for the initial volume, $V_0$, after a fill volume, $V_F$, of 2.5 L was decanted from the system. The total sludge age, $\theta_X$, of 10 days was sustained in the system with appropriate sludge wasting at the end of the aeration phase. The pH of the mixed liquor was around 7.3 under anaerobic conditions. The dissolved oxygen (DO) concentration was always above 2.0 $mgO_2/l$ after the commencement of

aeration. The SBR was fed with synthetic wastewater containing acetic acid, (Ac), as the sole carbon source that was prepared and sterilized daily and added separately to set the COD level in the feed at 400 mg/l COD. The composition of the nutrient medium is given in detail in **Yagci et al. (2003)**.

### 5.2.2 Experimental plan and data collection

The study was conducted in four different Runs, (Run 1 to 4), where the acetate concentration was always kept as 400 mg/l COD, while the influent phosphate concentration was increased gradually from 20 mg/l in Run-1 to 35 mg/l in Run-2, 45 mg in Run-3 and finally to 60 mg/l in Run-4 to ensure a range of different influent P/Ac ratios of 0.05, 0.08, 0.12 and 0.15 respectively. The four different runs (Run 1 to 4) of SBR operation were mainly designed to observe the effect of influent P/COD ratio on the system performance and its reflection on model calibrations. Each run was monitored for almost 40 days to ensure system operation at steady-state. During the experimental study, concentrations of acetate, phosphate, PHA, mixed liquor total and volatile suspended solids (MLSS and MLVSS), together with ammonia and nitrate, were monitored daily in the influent and effluent streams, and at the end of aerobic and anaerobic phases for each run. The performance of the SBR was further investigated by measuring the concentration profiles throughout a representative cycle during the steady state operation. The data collection was done by means of taking galiquots from the mixed liquor during the phases of the SBR cycle. The data intervals for different variables were adjusted in order to best define and reflect the cyclic trend of measurements representing the steady state conditions.

### 5.2.3 Model selection and simulation tool

The Activated Sludge Model No.2d, (ASM2d), as defined by **(Henze et al., 1999)** together with the process dynamics of SBR, formed the basis of model simulations which were performed by means of the AQUASIM computer program developed by **Reichert (1998)**.

## 5.3 Experimental Results of SBR Performance

Experimental data related to SBR performance were collected to define the system characteristics of a typical cycle at steady-state. For all different runs, cyclic trends in the phosphate, PHA, acetate, ammonia, nitrate and MLVSS were characterized based on in-cycle measurements. Following the feeding period of 15 min, acetate was consumed rapidly and converted into PHA, together with a corresponding phosphate release into the mixed liquor. After depletion of acetate, PHA storage and phosphate release were observed to continue at a reduced rate. The adopted operational conditions allowed for nitrogen removal through nitrification and denitrification. Nitrate remaining in the reactor at the end of the cycle was completely exhausted within the first 15 minutes of the next cycle, showing that pre-denitrification took place before the start of an anaerobic phase in all the Runs of SBR operation. Table 5.1 gives the concentrations of significant parameters measured in the influent, effluent and at the end of the anaerobic phase, together with significant conversion ratios and rates characterizing different phases of EBPR within the cycle.

Table 5.1 shows the significant effect of the influent P/COD ratio on the performance of SBR operation for EBPR. Run 1 illustrates a phosphate-limited operation resulting in total P removal at the end of the aerobic phase, while in other Runs phosphate was in excess and not totally removed by EBPR. The gradual increase in effluent phosphate concentration was parallel to the increase of the influent P/COD ratio from Run 2 to Run 4. Similarly, nitrogen is oxidized and reduced to around 3.5 mg N/l in the effluent for Runs with 21-22 mg/l of ammonia in the feed stream, while N balance results in an effluent nitrate concentration of 1.5 mg N/l in Run 2 operated with an influent ammonia concentration of 15.5 mg N/l. Acetate was consumed during the first hour of the mixing period with increasing consumption rates of 0.06 mgCOD/h in Run-1 to 0.09 mgCOD/h in Run 4. Acetate uptake values, calculated accounting for acetate consumption for denitrification, were almost constant for all runs. Phosphorus release values increased with increasing influent phosphate concentration. Accordingly, phosphorus release/acetate uptake ratios ($P_{rel}/HAc_{upt}$) varied depending on phosphate concentration in the feed. Increasing values of $P_{upt}/P_{rel}$ from Run 1 to Run 4 were mostly due to gradually increasing $P_{upt}$ levels, which always remained comparably higher than that of $P_{rel}$. Stored PHA values were

higher then acetate uptake values for all runs. MLVSS/MLSS ratios decreased from 68% to 50% because of higher polyphosphate accumulation as the phosphate concentration increased in the feed.

**Table 5.1:** Characterization of SBR performance at steady-state for EBPR

| Measured compound | Unit | Run-1 | Run-2 | Run-3 | Run-4 |
|---|---|---|---|---|---|
| Influent COD/TP | mgCOD/mgP | 400/20 | 400/35 | 400/45 | 400/60 |
| Influent $NH_4$-N | mgN/l | 21.2 | 15.5 | 21.5 | 22.1 |
| Effluent $N_{OX}$-N | mgN/l | 3.0 | 1.5 | 3.1 | 3.8 |
| Anaerobic end $PO_4$-P | mgP/l | 113.7 | 135.4 | 145.7 | 155.5 |
| Effluent $PO_4$-P | mgP/l | 0.0 | 3.0 | 3.9 | 8.4 |
| MLVSS | mg/l | 2590 | 2780 | 2640 | 2820 |
| Acetate uptake | mgCOD/l | 235.4 | 240.8 | 242.6 | 240.9 |
| Phosphate release | mgP/l | 100.4 | 113.3 | 117.3 | 117.5 |
| Phosphate uptake | mgP/l | 113.7 | 132.4 | 141.8 | 147.1 |
| PHA stored | mgCOD/l | 279 | 268 | 287 | 291 |
| $P_{rel}/HAc_{uptake}$ | gP/gCOD | 0.43 | 0.47 | 0.48 | 0.49 |
| $PHA_{stored}/ HAc_{uptake}$ | gCOD/gCOD | 1.19 | 1.21 | 1.18 | 1.21 |
| $P_{upt}/P_{rel}$ | g P/g P | 1.13 | 1.17 | 1.21 | 1.25 |
| MLVSS/MLSS | g/g | 0.68 | 0.61 | 0.59 | 0.50 |

Experimental assessment of SBR performance also involved measurements of the concentration profiles of components playing a major role in the EBPR process, namely $PO_4$-P, acetate and PHA together with other major parameters like MLVSS, $NO_3$-N, etc., throughout a representative complete cycle during the steady-state operation of each Run. These results will be presented and discussed in the model simulation and calibration section.

## 5.4 Calibration Study

### 5.4.1 Model description

ASM2d adopted in this chapter, involves, aside EBPR, all model components and processes defining organic carbon and nitrogen removal. Since EBPR is the main focus in this research, process stoichiometry and kinetics of phosphate accumulating organisms, $X_{PAO}$, as conceived in this model are briefly explained below. It should be remembered that ASM2d provides a useful but simplified account of the wide

array of complex biochemical processes taking part in EBPR, mainly for a reasonable interpretation of domestic sewage treatment.

As outlined in the commonly adopted matrix format (**Henze et al., 1999**) in Tables 5.2 and 5.3, EBPR involves three additional model components aside $X_{PAO}$ and $S_{PO4}$, namely, the acetate concentration, $S_A$, the polyphosphate concentration, $X_{PP}$, and the PHA concentration, $X_{PHA}$ accumulated in the biomass. These model components are considered with four main processes, storage of PHA, storage of polyphosphate, growth of PAOs on PHA under aerobic and anoxic conditions and lysis of particulate components.

In this framework, PAOs have two internal storage products namely the poly-hydroxyalkanoates, $X_{PHAs}$ and the polyphosphate, $X_{PP}$, pools. The $X_{PAOs}$ store acetate, $S_A$ in the form of poly-hydroxyalkanoates, $X_{PHA}$, with the stoichiometry of 1:1 in order to compete with ordinary heterotrophs, $X_H$ (Table 5.2-P1). The required energy for conversion of $S_A$ to $X_{PHA}$ is provided from orthophosphate liberation from $X_{PP}$ pools. As a result, the phosphate, $S_{PO4}$, released to bulk solution is defined by a stoichiometric coefficient; The corresponding reaction rate is determined by the rate constant of $X_{PHA}$ storage, $q_{PHA}$, and the double-Monod function based on acetate, $S_A$, and polyphosphate, $X_{PP}$, switches (Table 5.3-P1).

When oxygen and/or nitrate are available, the stored material $X_{PHA}$ is used up for both cell growth and phosphate uptake ($X_{PP}$) (Table 5.2, P2-P3). For the storage of polyphosphate, the required energy is gained from the oxidation of $X_{PHA}$, desribed by a stoichiometric coefficient, $Y_{PHA}$. Storage of polyphosphate is controlled by the rate constant, $q_{PP}$, the $X_{PHA}/X_{PAO}$ ratio and an inhibition term, which is the controlling parameter for the $X_{PP}$ storage and becomes active if $X_{PP}/X_{PAO}$ ratio approaches a maximum allowable value of $K_{MAX}$ (**Henze et al., 1995**). The reaction rate for growth, defined in terms of a surface saturation-type equation, is controlled by both the maximum growth rate ($\mu_{PAO}$) and the $X_{PHA}/X_{PAO}$ ratio. (Table 5.3-P4; P5).

The saturation coefficient, $K_{PHA}$ is a major parameter that is incorporated in the reaction rate expression of both growth and polyphosphate storage processes. This way $K_{PHA}$ affects the availability of internal substrate, PHA, to be used both in growth and phosphate uptake processes, by adjusting the $X_{PHA}/X_{PAO}$ level. **Mino et**

**al., (1995)** suggested that considering $K_{PHA}$ as two different parameters in these rate expressions would provide additional flexibility of interpretation and independent manipulation of PHA utilization via growth and phosphate uptake processes. Rate coefficients of the lysis process affect both PAO activity and the liberation of storage compounds into the bulk liquid (Table 5.3-P7; P8).

**Table 5.2:** The stoichiometry for Phosphorus Accumulating Organisms, $X_{PAO}$

| No | Process name | $S_{O2}$ | $S_A$ | $S_{NO3}$ | $S_{PO4}$ | $X_{PAO}$ | $X_{PP}$ | $X_{PHA}$ |
|---|---|---|---|---|---|---|---|---|
| P1 | Storage of $X_{PHA}$ | | -1 | | $Y_{PO4}$ | | $-Y_{PO4}$ | 1 |
| P2 | Aerobic storage of $X_{PP}$ | $-Y_{PHA}$ | | | -1 | | 1 | $-Y_{PHA}$ |
| P3 | Anoxic storage of $X_{PP}$ | | | $v_{3,NO3}$ | -1 | | 1 | $-Y_{PHA}$ |
| P4 | Aerobic growth of $X_{PAO}$ on $X_{PHA}$ | $1-\frac{1}{Y_{PAO}}$ | | | $-i_{PBM}$ | 1 | | $-1/Y_H$ |
| P5 | Anoxic growth of $X_{PAO}$ on $X_{PHA}$ | | | $v_{5,NO3}$ | $-i_{PBM}$ | 1 | | $-1/Y_H$ |
| P6 | Lysis of $X_{PAO}$ | | | | $v_{6,PO4}$ | -1 | | |
| P7 | Lysis of $X_{PP}$ | | | | 1 | | -1 | |
| P8 | Lysis of $X_{PHA}$ | | 1 | | | | | -1 |

$v_{3,NO3} = -Y_{PHA}/2.86$

$v_{5,NO3} = -(1-Y_H)/(2.86*Y_H)$

$v_{6,PO4} = i_{PBM}$

**Table 5.3:** Process kinetics for Phosphorus Accumulating Organisms, $X_{PAO}$

| No | Process name | Reaction rate ($\rho_i$) |
|---|---|---|
| P1 | Storage of $X_{PHA}$ | $q_{PHA} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{X_{PP}/X_{PAO}}{K_{PP} + X_{PP}/X_{PAO}} \cdot X_{PAO}$ |
| P2 | Aerobic storage of $X_{PP}$ | $q_{PP} \cdot \frac{S_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_{PO4}}{K_{PS}+S_{PO4}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} \cdot \frac{K_{MAX}-X_{PP}/X_{PAO}}{K_{IPP}+K_{MAX}-X_{PP}/X_{PAO}}$ |
| P3 | Anoxic storage of $X_{PP}$ | $\rho_3 = \rho_1 \cdot \eta_{NO3} \frac{K_{O2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3}+S_{NO3}}$ |
| P4 | Aerobic growth of $X_{PAO}$ on $X_{PHA}$ | $\hat{\mu}_{PAO} \cdot \frac{S_{O2}}{K_{O2}+S_{O2}} \cdot \frac{S_{PO4}}{K_P+S_{PO4}} \cdot \frac{X_{PHA}/X_{PAO}}{K_{PHA}+X_{PHA}/X_{PAO}} X_{PAO}$ |
| P5 | Anoxic growth of $X_{PAO}$ on $X_{PHA}$ | $\rho_5 = \rho_4 \cdot \eta_{NO3} \frac{K_{O2}}{S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3}+S_{NO3}}$ |
| P6 | Lysis of $X_{PAO}$ | $b_{PAO} . X_{PAO}$ |
| P7 | Lysis of $X_{PP}$ | $b_{PP} . X_{PP}$ |
| P8 | Lysis of $X_{PHA}$ | $b_{PHA} . X_{PHA}$ |

*alkalinity and nutrient nitrogen switch were not shown

### 5.4.2 Sensitivity analysis and selection of parameters

The preliminary part of this chapter was devoted to an extensive testing of the sensitivity of major model components to all the parameters involved in the different biochemical processes defining EBPR, in order to reduce the selection down to a limited number of significant parameters for obtaining a reasonable model fit. Based on steady state cyclic simulations of $S_{PO4}$, $X_{PHA}$, $S_A$ and MLVSS, the sensitivity of each model parameter was determined via repeating simulation for individual changes in the magnitude of related parameters. The preliminary testing identified $Y_{PO4}$, $q_{PHA}$, $q_{PP}$, $K_{PHA}$ and lysis rates as the most relevant parameters to form a subset for calibration and the sensitivity analysis was carried out with this subset. The effect of other parameters in the model, not reported in the paper, was observed to be low enough not to warrant their inclusion in the evaluation.

The sensitivity analysis was performed with the manual adjustment of parameters to evaluate their impact on the concentration profiles mentioned above, using operation conditions of SBR and wastewater characteristics for Run 4. The model simulations were carried out introducing wastewater characteristics and operating parameters of this Run to the simulator together with default values for the selected parameter subset, as suggested in ASM2d. The parameters pertaining to ordinary heterotrophic biomass were also set to their defaults as proposed in ASM2d. In order to have constant biomass composition in the aerobic phase, each simulation was carried out for around 40 days until steady-state conditions for all model components of interest prevailed. The individual effects of selected parameters on model components were investigated based on steady state simulations. Figure 5.1 shows the sensitivities of four parameters within the selected subset, namely, $q_{PHA}$, $q_{PP}$, $Y_{PO4}$ and lysis rates, with ±25% changes from their default levels on the $S_{PO4}$ profile.

As expected from the structural framework of ASM2d, and clearly depicted in Figure 5.1, all selected parameters exerted a significant effect on the $S_{PO4}$ profile, directly or through their impact on other model components. The effect of the rate constant for storage, $q_{PHA}$, was inevitably on the rate of P release during the anaerobic phase of the operation. A similar effect was also observed on $X_{PHA}$, expectedly due to the direct relation between $S_{PO4}$ release and $X_{PHA}$ storage. Moreover, $q_{PHA}$ had a direct impact on acetate concentration, $S_A$, for the same reason since the model assumes

that the amount of $X_{PHA}$ stored is stoichiometrically equal to acetate utilized. Therefore, in the adopted calibration procedure, the $S_A$ profile was calibrated through direct adjustment of $q_{PHA}$. In parallel, the $S_{PO4}$ simulation output was considered in the following calibration step as illustrated in Figure 5.1-a.

The sensitivity simulations obtained for $q_{pp}$ showed that the downhill slope of the $S_{PO4}$ profile could be simulated with the adjustment of this parameter during the aerobic phase (see Figure 5.1-b), giving a clear indication that, the trend of phosphate uptake under aerobic conditions requires the tuning of the rate constant of $X_{PP}$ storage, $q_{PP}$. As seen in Table 5.4, in order to make it comparable the $K_{PS}$ values for different runs all were set to the value of 0.5 mgP/l because of an expected correlation between $q_{PP}$ and $K_{PS}$ parameters.

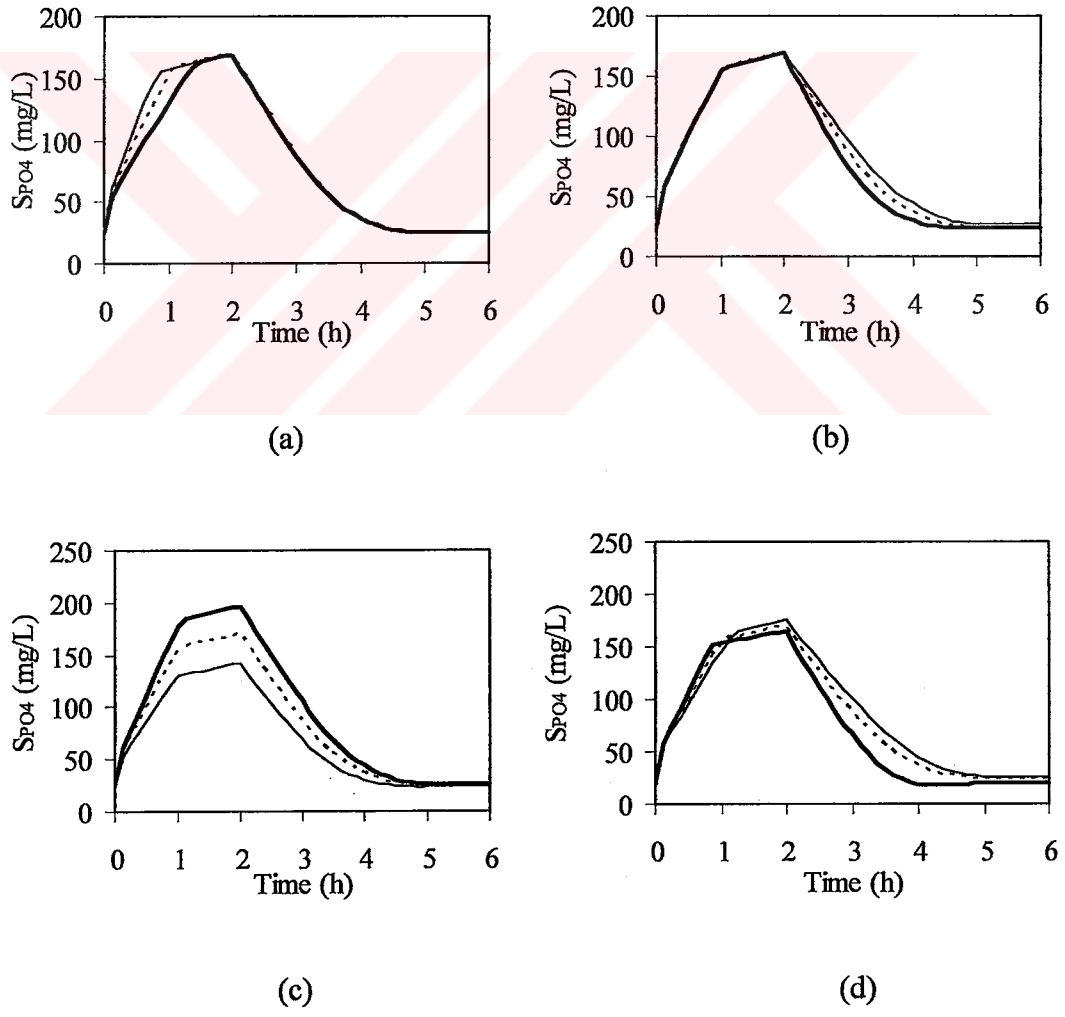


**Figure 5.1:** Sensitivity simulation for (a) $q_{PHA}$, (b) $q_{PP}$, (c) $Y_{PO4}$ and (d) lysis rates on $PO_4$-P profile (—:+25%; ---: default; —: -25%)

In order to represent the effect of $Y_{PO4}$, a set of sensitivity simulations were performed as illustrated in Figure 5.1-c by changing this parameter in the range of ±25% of the default value. After acetate depletion before the end of the anaerobic phase, a gradual increase in $S_{PO4}$ profile was obtained due to the endogenous metabolism of $X_{PP}$, characterized by the rate constant, $b_{PP}$, which also affected the maximum level of $S_{PO4}$, the $S_{PO4}$ profile in the aerobic phase and the effluent $S_{PO4}$ level (Figure 5.1-d).

The following step deals with the calibration of saturation constant, $K_{PHA}$, for $X_{PP}$ storage and growth of $X_{PAOs}$. According to the original ASM2d, the growth and phosphate storage processes are governed only by a single saturation constant, $K_{PHA}$. There are, however, arguments in the literature (**Mino et al., 1995; Manga et al., 2000**) that the rate expressions for the two processes should involve different values for the $K_{PHA}$ parameter, this way encouraging $X_{PHA}$ consumption in favor of $X_{PP}$ storage, or vice versa. In this approach, $K_{PHA}$ is regarded as two different parameters limiting independently (1) $X_{PP}$ storage and (2) growth of $X_{PAO}$. The merit of this argument is tested and validated in the sensitivity analysis by running the tests with two different parameters, namely $K_{PHAg}$ for growth and $K_{PHAs}$ for $X_{PP}$ storage. The analysis involved simultaneous evaluation of both $X_{PHA}$ and $S_{PO4}$ profiles. Figure 5.2 illustrates the results of two different sets of analysis: in the first set, the effect of $K_{PHAg}$ through growth process was investigated for a constant (default) value of $K_{PHAs}$ (Figure 5.2-a); in the second set, a variable $K_{PHAs}$ was adopted while keeping $K_{PHAg}$ constant (Figure 5.2-b). The simulation results justified selection of independent saturation coefficients, as; $X_{PHA}$ proved highly sensitive to $K_{PHAg}$ which in turn did not have an noticeable effect on the $S_{PO4}$ profile (Figure 5.2-a); conversely, variation of $K_{PHAs}$ could only predict a low $X_{PHA}$ profile, but significantly affected the effluent $S_{PO4}$ level.

### 5.4.3 Calibration steps

A stepwise manual calibration methodology previously proposed by (Insel *et al.*, 2004) was specifically adapted to EBPR as depicted in Figure 5.3 based on the results of the sensitivity analysis. The first step of the methodology involved introduction of the SBR operating parameters and the activated sludge model (with default values of parameters) to the simulator. This iterative calibration methodology was reassessed for the rest of

the calibrations after achieving a reasonable model fit. The calibration algorithm was reevaluated since a change in one parameter might affect the rest of the simulation outputs.

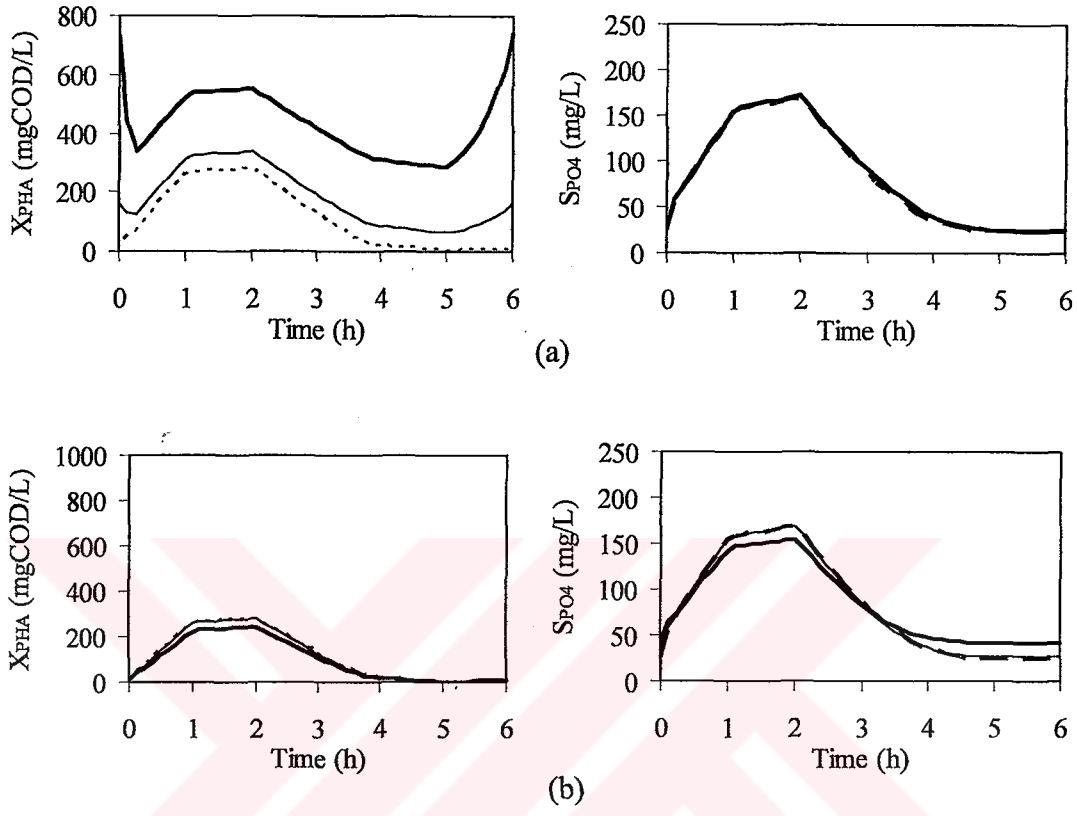


**Figure 5.2:** Sensitivity analysis for $K_{PHA}$ on $S_{PO4}$ and $X_{PHA}$: (a) $K_{PHAs}$ = default (b) $K_{PHAg}$ =default (---: default;—:+200%; —: +500%)

As a first step of the methodology, nitrogen data was adjusted before the model calibration for $X_{PAOs}$. The adjustment basically involved calibration of the nitrate nitrogen ($NO_3$-N) profiles within a representative cycle at steady-state for different Runs. For this purpose, the nitrogen content of the biomass, $i_{NBM}$, was increased from its default value of 0.07 to 0.09 gN/gMLVSS to reflect the measured total nitrogen content of mixed liquor (around 13% in dry weight) during the experiments. Furthermore, the maximum autotrophic growth rate, $\mu_A$, was set to 0.8 1/day to obtain a reasonable model fit. The selected values of $i_{NBM}$ and $\mu_A$ provided a good characterization of the experimental nitrogen data and no further calibration effort was required for the $NO_3$-N profiles associated with different Runs and reflecting different operation conditions. Figure 5.4 illustrates the simulation trajectories of nitrogen species and experimental data for Runs 2 and 4.

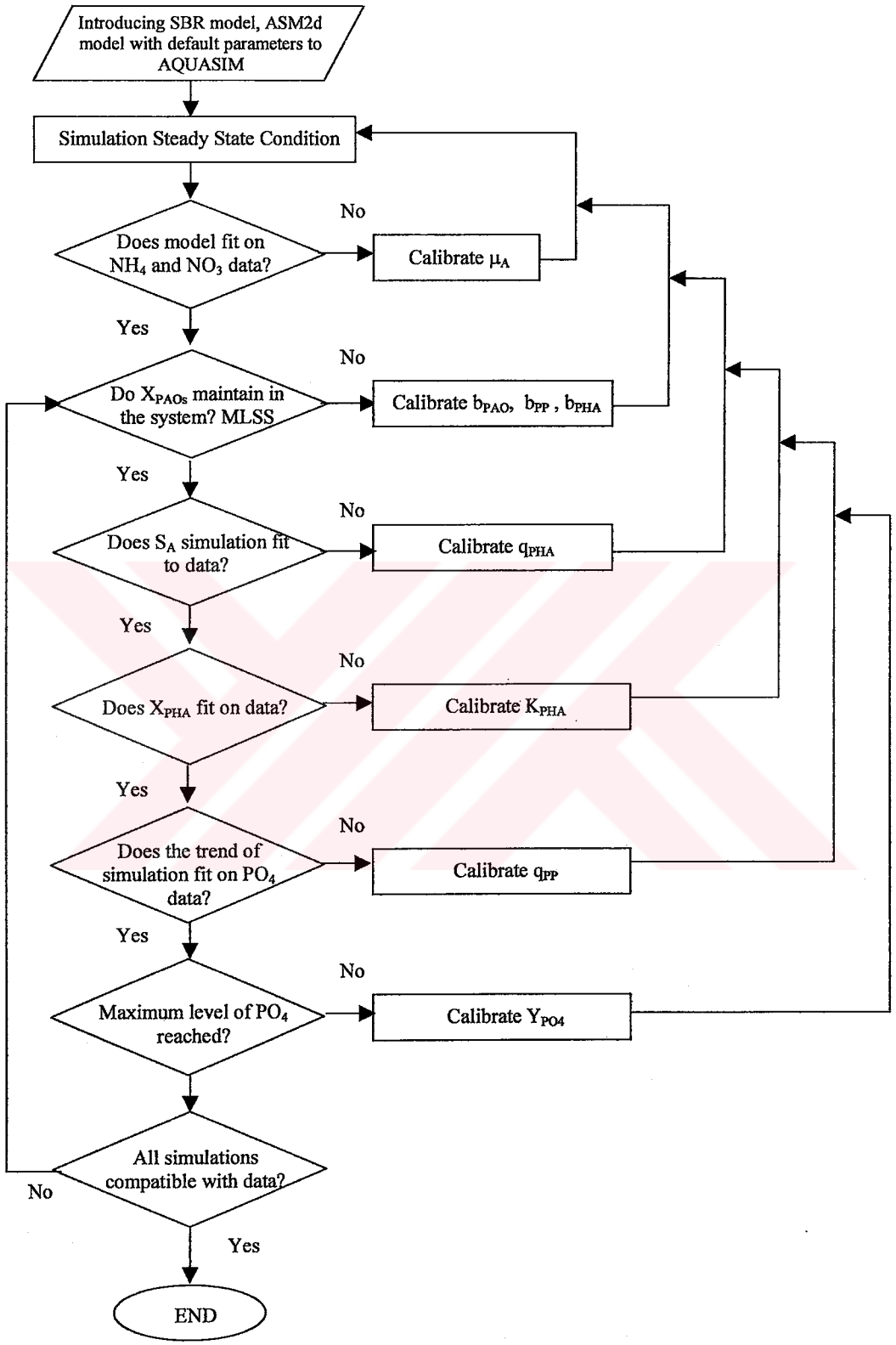


**Figure 5.3:** Proposed iterative calibration algorithm for EBPR

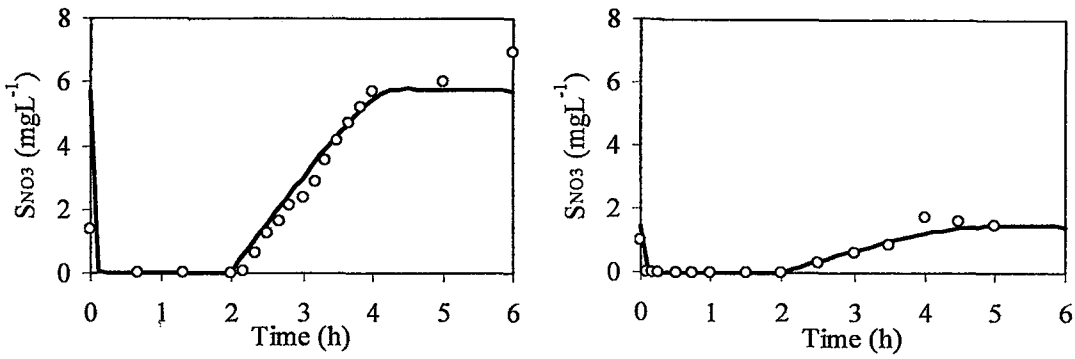


**Figure 5.4:** Simulation profiles and experimental data for nitrate, $S_{NO}$; (a) Run 4; (b) Run 2 (O Experimental data; — Simulation with calibrated parameters)

### 5.4.4 Calibration of Run-4

The model calibration was first initiated using the data of Run-4 obtained with the highest influent P/Ac ratio and therefore the maximum expected biomass activity for EBPR. The simulation profiles obtained with the default values of ASM2d and the calibrated parameters derived in this research together with the experimental concentration profiles of $S_{PO4}$, $S_A$, $X_{PHA}$ and VSS are illustrated in Figure 5.5. The proposed iterative procedure schematically defined in Figure 5.1 was followed for calibrating the model.

The calibration exercise first indicated that the default values of the lysis rates $b_{PAO}$, $b_{PP}$ and $b_{PHA}$, were appropriate for an accurate prediction of the $X_{PAO}$ level in the reactor (Figure 5.5-d). However, the simulated phosphate, acetate and PHA profiles using the default values of ASM2d were quite different from the experimental data. First, $q_{PHA}$ was changed from its default value of 3.0 $gX_{PHA}/gX_{PAO}.d$ to 5.3 $gX_{PHA}/gX_{PAO}$ d, basically to simulate the experimental $S_A$ profile, but also to obtain a model fit for the P release under anaerobic conditions.

The prediction potential of ASM2d was most deficient for the $X_{PHA}$ profile as defined with its default $K_{PHA}$, a substantially lower PHA level as compared to measured values during the cyclic SBR operation. Calibration for the experimental data required the selection of a value of 0.05 $gX_{PHA}/gX_{PAO}$ for the saturation coefficient for growth, $K_{PHAg}$, as previously observed in the sensitivity analysis.

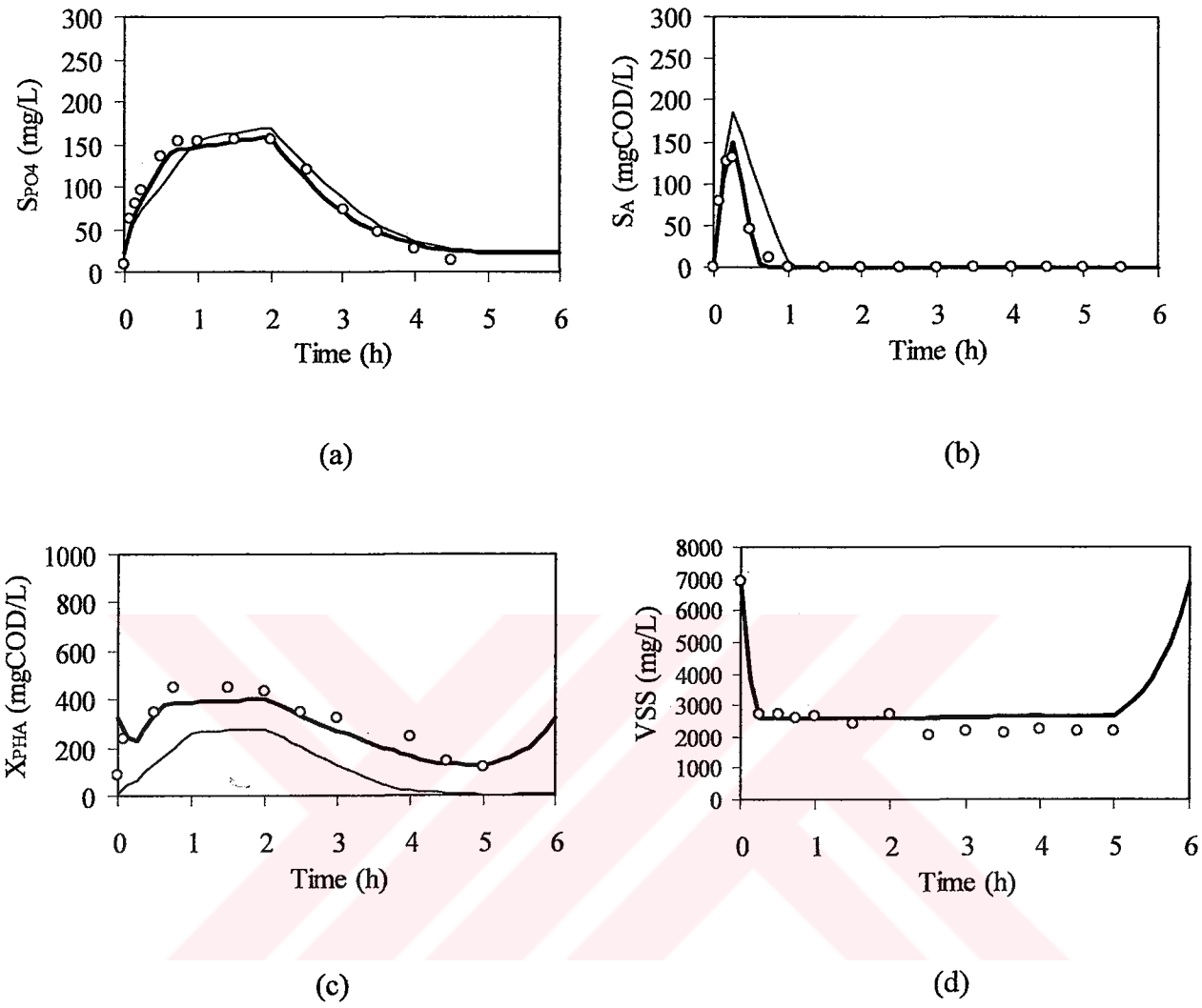


**Figure 5.5:** Simulation and experimental results of (a) $PO_4$-P, (b) $S_A$, (c) PHA and (d) VSS data for Run-4 (○ Experimental data; — Simulation with default parameter values of ASM2d; —Simulation with calibrated parameters)

The rate constant for storage, $q_{PP}$, was assigned a relatively higher level of 2.0 $gX_{PP}/gX_{PAO}.d$ to obtain the required steeper slope of the $S_{PO4}$ profile during the aeration phase. The simulation of the $S_{PO4}$ profile during this phase also necessitated the adjustment of the saturation coefficient for $X_{PP}$ storage, $K_{PHAs}$ to 0.02 $gX_{PHA}/gX_{PAO}$, quite different than its counterpart for growth.

Besides, the experimental profiles of $S_{PO4}$ and $X_{PHA}$ can only be fitted at once via calibrating the $K_{PHA}$ parameters. Finally, the $X_{PHA}$ saturation coefficients for $X_{PP}$ storage ($K_{PHAs}$) and for growth were adjusted to 0.02 and 0.05 $gX_{PHA}/gX_{PAO}$,

respectively, for Run-4. In fact, the model reasonably reflected the $S_{PO4}$ profile well when maintaining the $X_{PHA}$ at higher levels.

The ratio of the polyphosphate required for PHA storage, $Y_{PO4}$, was calibrated to mimic the maximum level of $PO_4$-P released at the end of the anaerobic phase. Accordingly, the yield coefficient for phosphate release, $Y_{PO4}$, was reduced from its default value of 0.4 to 0.35 gP/gCOD to match the maximum level of $S_{PO4}$ experimentally determined at the end of anaerobic phase for this run.

The simulation profiles obtained with the calibrated parameter values with respect to $S_{PO4}$, $S_A$, $X_{PHA}$ and MLVSS yielded, as shown in Figure 5.5-a, a reasonable model prediction except for the minor difference in $X_{PHA}$ profile for this run.

### 5.4.5 Calibration for Run 1-3

The second step involved the steady-state simulation of the experimental data obtained from Run 1, characterized by the lowest influent concentration of 20 mgP/l and the corresponding P/Ac ratio of 0.05 gP/gCOD, using the same calibrated parameters of Run 4. Figure 5.6 clearly indicate that simulated profiles generated with the stoichiometric and kinetic parameters adopted for Run 4 did not match the selected experimental concentration profiles. Then, the same iterative procedure was used for calibrating the model with parameters that are specifically appropriate for Run 1. A decrease was observed, as outlined in Table 5.4, in the values of these parameters when the P/Ac ratio decreased from 0.15 down to 0.05 gP/gCOD. In this context, the lysis rate for $X_{PAO}$ was set to 0.17 1/day, which is slightly lower than that of Run-4. Similarly, $q_{PHA}$ and $q_{PP}$ were determined as 3.30 and 1.30, respectively. On the other hand, the value of $Y_{PO4}$ was slightly increased to 0.42 in order to fit the $S_{PO4}$ level at the end of the anaerobic phase. It should be noted that simulating Run-1 did not require separating the $K_{PHA}$ parameter for the processes of growth and phosphate uptake. So, the $K_{PHA}$ parameter was set to 0.05 g XPHA/g$X_{PAO}$.

The same calibration exercise was performed for the experimental data of Runs 2 and 3. The calibration results of Run-2 operated with P/Ac ratio of 0.08 gP/gCOD showed that the profiles of experiments could be fitted by assigning $q_{PHA}$ and $q_{PP}$ the values of 4.40 and 1.65, respectively. However, compared to Run-1, it was required

to lower the value of $Y_{PO4}$ down to 0.39 to match the simulation with maximum measured $S_{PO4}$ level at the end of anaerobic phase. And the simulation profile reasonable predicted the fate of $X_{PHA}$ and $S_{PO4}$. Separate $K_{PHAs}$ and $K_{PHAg}$ coefficients of 0.04 and 0.05 $gX_{PHA}/gX_{PAO}$, respectively, were selected to simultaneously match the experimental $X_{PHA}$ and $S_{PO4}$ profiles. A satisfactory simulation of the $X_{PHA}$ profile could still not be achieved. The experimental data and simulation results are illustrated in Figure 5.7.

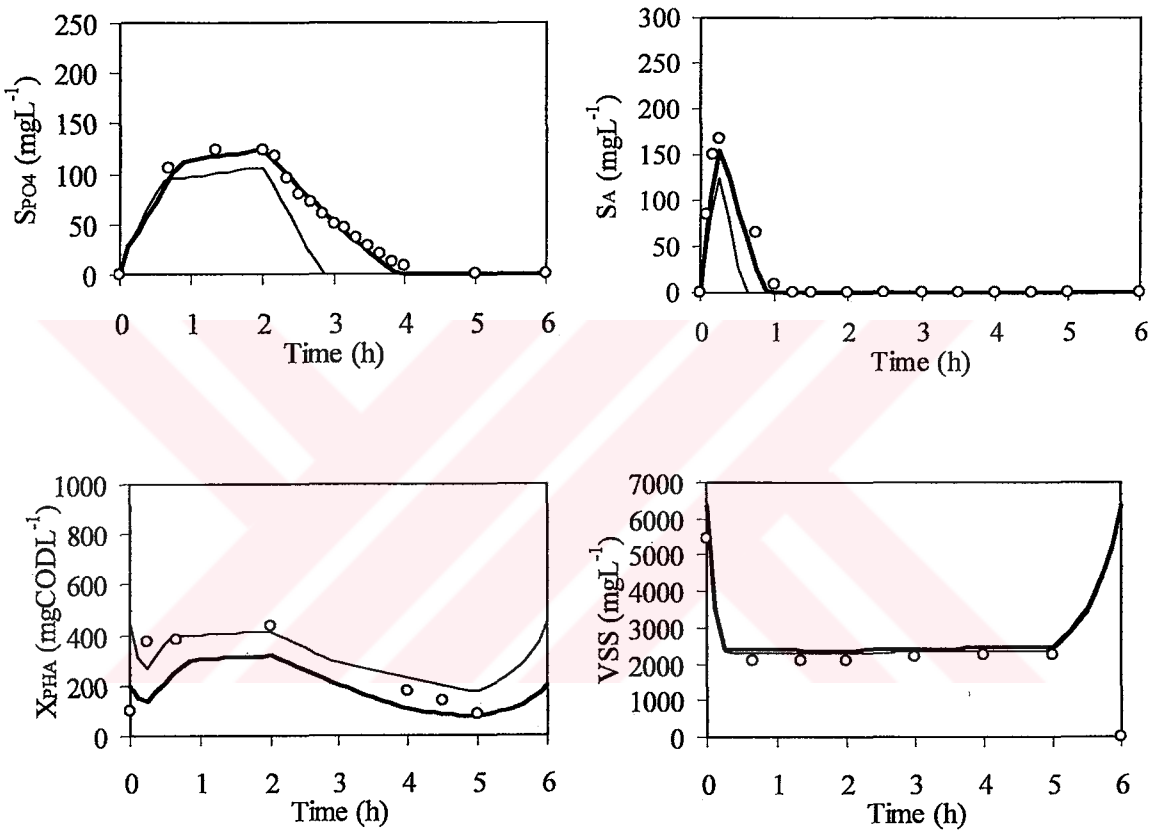


**Figure 5.6:** Simulation results and experimental data for Run-1 (○ Experimental data; — Simulation with calibrated parameter values for Run-4; — Simulation with calibrated parameters)

In the last step, the calibration study was carried out for Run-3 (P/Ac=0.12) using the same iterative procedure described in Figure 5.3. A slight increase for the lysis rate of $X_{PAO}$, $b_{PAO}$ to 0.18 1/day was required for meeting measured MLVSS together with decreasing the concentration of $X_{PAO}$. $X_{PHA}$ Values of $q_{PHA}$ and $q_{PP}$ were adjusted to 4.90 and 1.85, respectively, to accurately describe the trend of

experimental $S_{PO4}$ and $X_{PHA}$ data. For Run-3, the experimental and simulation results are shown in Figure 5.8.

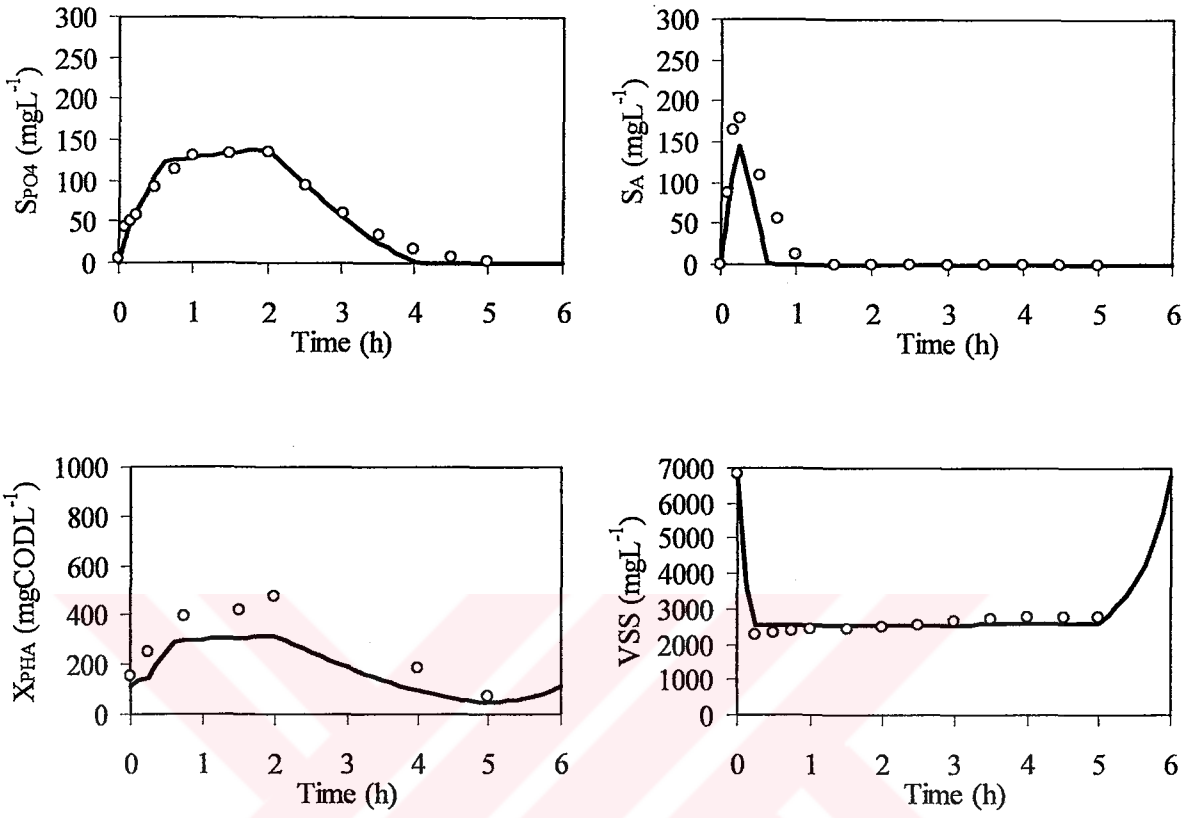


**Figure 5.7:** Simulation results and experimental data for Run-2 (O Experimental data; — Simulation with default parameter values of ASM2d; — Simulation with calibrated parameters)

## 5.5 Discussion

A review of the results obtained indicate that the experimental concentration profiles for a particular SBR Run could be simulated using the proposed iterative calibration methodology, which defined appropriate values for the significant model parameters of ASM2d. However, the same values of the parameter subset could not be used for the prediction of the in-cycle measurements for the other Runs operated under different P/Ac ratios. Hence, an additional recalibration effort was required for each experimental run. Therefore, ASM2d was not able to predict the steady-state behavior or the SBR system used for this research, and fed with acetate as the sole carbon source and at different P/Ac ratios.

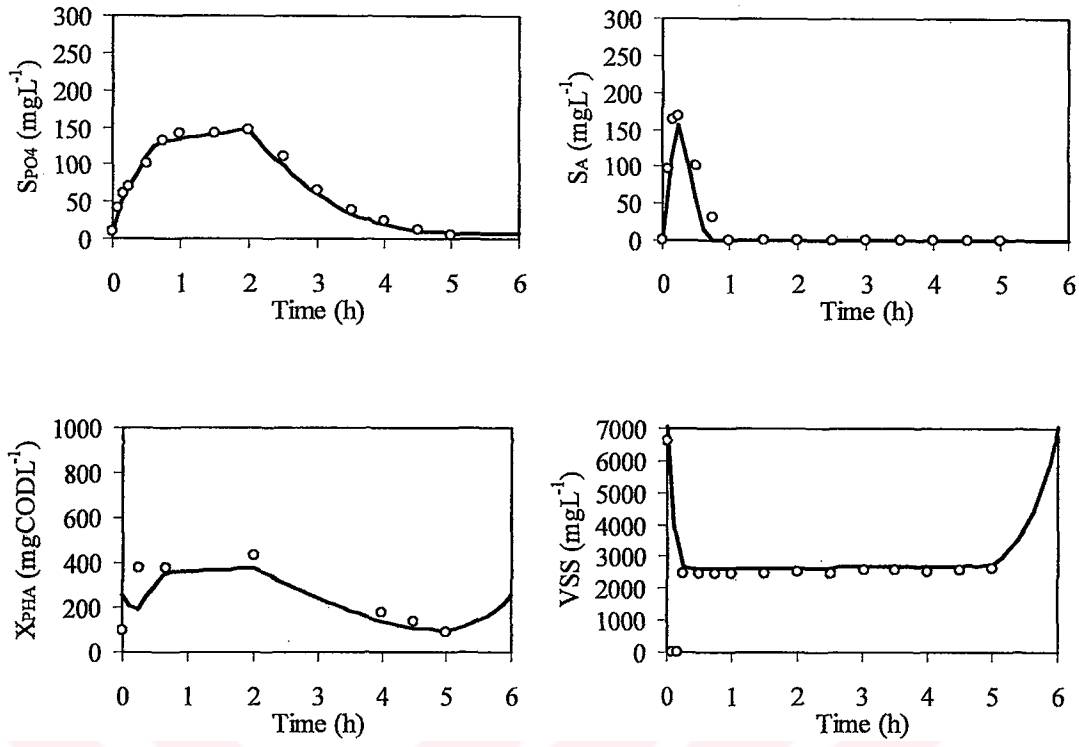


**Figure 5.8:** Simulation results and experimental data for Run-3 (O Experimental data; — Simulation with default parameter values of ASM2d; — Simulation with calibrated parameters)

Inspection of the model calibration results outlined in Table 5.4 shows that values of the model parameters, although different for each Run, exhibit a definite and meaningful changing pattern with respect to P/Ac ratios. A descending trend in the values of $q_{PHA}$, $q_{PP}$ and $b_{PHO}$ is observed as the P/Ac ratio decreases from 0.15 in Run 4 to 0.05 in Run 1. The results show an opposite ascending trend for $Y_{PO4}$. The $q_{PHA}$ value, defined as 5.3 $gX_{PHA}/gX_{PAO}$ in Run 4, gradually drops to 92%, 83% and 62% of this level in Runs 3, 2 and 1 respectively. Almost identical decreasing ratios apply for $q_{PP}$. Decrease in $q_{PHA}$ and $q_{PP}$ indicate a slower P release ($X_{PHA}$ storage) and P uptake of the active biomass for lower P/Ac ratios. It should be noted that according to ASM2d, both processes also depend on $X_{PAO}$ activity and the respective process rates are more correctly expressed as $q_{PHA}$ $X_{PAO}$ and $q_{PP}$ $X_{PAO}$. Therefore, the observed rate decrease is either due to a reduction factor that applies to $q_{PHA}$ and $q_{PP}$, or it may be attributed to the presence of another type of microorganism in the biomass, commonly defined in the literature as glycogen accumulating organisms, (GAOs), that have a slower acetate uptake and no P storage (**Liu et al., 1994; Satoh et al., 1994**). It also was previously reported that GAOs may proliferate in similar

activated sludge operation with synthetic wastewater having a low P/Ac ratio (**Liu et al. 1997; Hasselman et al., 2000**). A metabolic model developed on the same experimental results justified the existence of a mixed culture of PAOs and GAOs competing for the same substrate under anaerobic conditions and demonstrated that only 73% of the acetate could be stored by PAOs in Run 1 (**Yagci et al., 2003**). In fact, the findings **of Filipe et al. (2001b)** indicate that the acetate uptake rate of GAOs is lower as compared to PAOs, supporting the argument in this study that the observed decrease in the $q_{pp}$ level should be associated with the existence and impact of GAOs. It was also stated that GAOs have comparably lower endogenous rates ($b_{GAO}$=0,10) in order to maintain in the system (**Manga et al., 2001; Yagci et al., 2004**), explaining lower lysis rates corresponding to lower P/Ac ratios were obtained during model calibration.

In addition, descending trend of $Y_{PO4}$ indicates a lower $S_{PO4}$ release requirement for a unit amount of acetate consumed. This observation supports the argument that low P loading systems can favor GAO activity, which then affects EBPR in the presence of relatively high organic substrate levels, such as in Run-1. GAOs are capable of storing substrate under anaerobic conditions, without polyphosphate energy, but deriving energy from degradation of glycogen, and thus are capable of prevailing at a much lower P/Ac ratio. The result is a reduced phosphate release/acetate uptake ratio due to the presence of GAOs at lower influent P/Ac ratios, leading to the selection of lower $Y_{PO4}$ values to calibrate the experimental results using ASM2d.

The structure of ASM2d only accounts for PAOs and does not include the additional model components and processes to reflect the metabolic activities of GAOs that store PHA without polyphosphate accumulation (**Yagci et al., 2004; Manga et al., 2001; Sudiana et al., 1999**). As a result, the impact of population shifts in favor of GAOs at different P/AC ratios could only be compensated by assigning different values to process rate coefficients like $q_{PHA}$ and $q_{pp}$. This adjustment however affects the amount of $X_{PHA}$ used for the uptake and storage of $X_{PP}$ and creates a fictitious $X_{PHA}$ shift towards the growth process. Then, the balance between growth and P uptake requires selection of different $K_{PHAs}$ values which facilitates separate model fitting on $X_{PHA}$ and $S_{PO4}$ data (**Mino et al., 1995**).

**Table 5.4:** The default and calibrated parameters of ASM2d for different runs

| | | ASM2d | Run-1 | Run-2 | Run-3 | Run-4 | Unit |
|---|---|---|---|---|---|---|---|
| **Phosphorus accumulating organisms: $X_{PAO}$** | | | | | | | |
| *Stoichiometric parameter:* | | | | | | | |
| $Y_{PO4}$ | PP requirement for PHA storage | 0.40 | 0.42 | 0.39 | 0.36 | 0.35 | gP/gCOD |
| *Kinetic parameters:* | | | | | | | |
| $q_{PHA}$ | Rate constant for storage of $X_{PHA}$ | 3.00 | 3.30 | 4.40 | 4.90 | 5.30 | g $X_{PHA}$/g $X_{PAO}$.d |
| $q_{PP}$ | Rate constant for storage of $X_{PP}$ | 1.50 | 1.30 | 1.65 | 1.85 | 2.00 | g $X_{PP}$/g $X_{PAO}$.d |
| $b_{PAO}$ | Rate of lysis of PAO | 0.20 | 0.17 | 0.17 | 0.18 | 0.20 | 1/day |
| $b_{PP}$ | Rate of lysis of PP | 0.20 | 0.17 | 0.17 | 0.18 | 0.20 | 1/day |
| $b_{PHA}$ | Rate of lysis of PHA | 0.20 | 0.17 | 0.17 | 0.18 | 0.20 | 1/day |
| $K_{PS}$ | Sat. coeff. for P in storage of $X_{PP}$ | 0.20 | 0.50 | 0.50 | 0.50 | 0.50 | g $P/m^3$ |
| $K_{PHAs}$ | Sat. coeff. for PHA for $X_{PP}$ storage | 0.01 | 0.05 | 0.04 | 0.03 | 0.02 | g $X_{PHA}$/g $X_{PAO}$ |
| $K_{PHAg}$ | Sat. coeff. for PHA for growth | 0.01 | 0.05 | 0.05 | 0.05 | 0.05 | g $X_{PHA}$/g $X_{PAO}$ |
| **Nitrifying organisms: $X_A$** | | | | | | | |
| $\mu_{AUT}$ | Maximum growth rate of $X_A$ | 1.00 | | | 0.80 | | 1/day |

Model simulations displayed in Figures 5.5 to 5.8 show that the measured $S_{PO4}$ and $S_A$ profiles were well predicted with calibration using different parameter subsets. Even though the calibrated value of $X_{PHA}$ was picked up at the end of aeration phase by changing the $K_{PHAg}$ parameter for all runs, the magnitude of the measured PHA levels were still underestimated. So, $K_{PHAs}$ was additionally adjusted for fine-tuning of the $X_{PHA}$ level together with $S_{PO4}$. There was always a discrepancy between the measured and simulated PHA data at the end of anaerobic period. This discrepancy was highest in Run-1 having the lowest influent P concentration compared to Runs 3 and 4 with higher influent P concentrations. It was also impossible to obtain a data set providing best fit for both $S_{PO4}$ and $X_{PHA}$. This observation can be attributed to the existence of glycogen as another storage material, which is consumed to store more $X_{PHA}$ during the anaerobic phase and re-accumulated during the aerobic phase as was suggested by **Yagci et al. (2003)**. Similar observations were also explained by means of an additional glycogen metabolism by several researchers (**Satoh et al., 1992; Hesselman et al., 2000; Pereira et al., 1999; Yagci et al., 2003**). Therefore, the modeling efforts for the particular feeding and operating conditions in this research indicate the need to include glycogen and GAOs as model components for processes involving both PAOs and GAOs, in order to obtain a better prediction of $S_A$ and $X_{PHA}$ profiles in the system.

# 6. MODELLING AND CALIBRATION OF PHOSPHATE AND GLYCOGEN ACCUMULATING ORGANISM COMPETITION FOR ACETATE UPTAKE IN SEQUENCING BATCH REACTOR

## 6.1 Introduction

Substantial research effort has been devoted to the understanding the complex mechanisms governing enhanced biological phosphorus removal (EBPR) in biological wastewater treatment. ASM2d (**Henze et al, 1995**) providing a mechanistic explanation for phosphate accumulating organisms (PAOs) and later ASM2d, (**Henze et al., 1999**) incorporating denitrification by PAOs with reduced P-uptake emerged and met with wide acceptance and application. The models identified a single internal storage product of $X_{PHA}$ that could be taken up only by PAOs at the expense of short-chain volatile fatty acids (VFA) available as external substrate. Consequently, the models indicated that the amount of $X_{PHA}$ sequestered was stoichiometrically equal to VFA-COD utilized. This evidently is a very simplified mechanistic framework for EBPR, applicable mainly for domestic sewage commonly characterized by a low VFA (acetate-Ac) content and a relatively high P/Ac ratio. Therefore, they often proved inconsistent for interpreting experimental data obtained with different substrate composition and P/Ac ratio. This inconsistency between the experimental data and the proposed PAO-models was attributed to the presence of another type of bacteria, namely glycogen accumulating organisms, (GAOs), capable of storing substrate under anaerobic conditions, without the poly-P energy but deriving energy from degradation of glycogen, thus capable of prevailing at a much lower P/Ac ratio (**Cech and Hartman, 1993; Satoh et al., 1994; Liu et al., 1997; Yagci et al., 2003**).

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For experiments carried out at P/Ac ratios lower than that of domestic sewage, this inconsistency was also attributed to the glycogen-metabolism of PAOs, which is similar to that of GAOs, not accounted for in either ASM2 or ASM2d. Recently, the measurement of glycogen -mostly in lab-scale EBPR systems- emphasized the importance of this component on the process stoichiometry and kinetics especially for the PHA economy in relation to the overall P-removal efficiency. The amount of regenerated PHA pool was reported to be higher than the utilized VFA under anaerobic conditions (**Smolders et al., 1994; Mino et al., 1994**). Basically, it was argued that under anaerobic conditions, PHA pool was restored partially by the external COD and glycogen pool of biomass, itself. Concomitantly, the soluble phosphate was liberated into the bulk media. In the following aerobic or anoxic conditions, PHA was used up for the biomass synthesis, storage of poly-P and regeneration of glycogen pool (**Smolders et al., 1994**). In this context, **Mino et al. (1995) and Manga et al. (2001)** suggested modifications for ASM2d incorporating GAOs and glycogen metabolism by PAOs.

An EBPR model involves as in ASM2 and ASM2d, a vast spectrum of model coefficients. Suggested modifications while improving and refining conceptual framework for the clarification of relevant processes increase the complexity of the model by introducing new processes and coefficients. Therefore, the merit of the model chiefly also depends on the validity of the numerical values suggested for the set of coefficients. This on the one hand requires support derived from relevant metabolic principles and on the other hand necessitates model calibration with experimental data. Sequencing batch reactor (SBR) serves as an excellent instrument yielding cycles experimental data on the fate of model components for model simulation and calibration.

In this context, the objective of this study was to improve the mechanistic understanding of EBPR systems operated under varying P/Ac ratios by a model that accounts for the competition of PAOs and GAOs for acetate and incorporates glycogen metabolism of PAOs. This model was defined supplement to ASM2d with a new stoichiometry derived on the basis of new relevant metabolic concept. The proposed model was tested with and used for the calibration of experimental data generated by a sequencing batch reactor operated with acetate feeding at a P/Ac ratio of 0.05 mgP/mgCOD.

## 6.2 Model Development

The mechanistic approach proposed in widely accepted ASM2d is considered as a base-model since it basically embodies three different types of microorganisms: heterotrophs, autotrophs and phosphorus accumulating organisms describing the nutrient removal processes in activated sludge systems (**Henze et al., 1999**). In ASM2d, the phosphorus accumulating organisms, $X_{PAOs}$ takes up acetate in the availability of acetate and store only in the form of polyhydroxyalkanoate, $X_{PHA}$ with the stoichiometric ratio of HAc/PHA:1. In subsequent aerobic or anoxic conditions, the processes of $X_{PAOs}$ growth and the phosphate uptake utilize $X_{PHA}$. In this study, the stoichiometry and processes for the autotrophs and heterotrophs are taken as the same in ASM2d, accordingly. The modifications in the model structure are given as follows:

### 6.2.1 Glycogen metabolism for PAOs

The glycogen budget needed to be emphasized because of the fact that it plays an important role for the systems operated under high COD/TP loadings. On the basis of pioneering work of **Smolders et al., 1994** and **Mino et al., 1995**, the glycogen component in $X_{PAOs}$ metabolism is included in ASM2d model given in Table 6.1. Shortly, under anaerobic condition, the $X_{PHA}$ pool is replenished by the consumption of both external acetate, $S_A$ and glycogen, $X_{GLY}$ with the stoichiometry of $Y_{SA}$ and $1\text{-}Y_{SA}$ ($gCODg^{-1}COD$), respectively by assuming $X_{PHA}$ is equal to unit COD (P1). The kinetic equation describing the $X_{PHA}$ storage (for $X_{PAOs}$) is controlled by Monod-type switch functions for acetate, polyphosphate and glycogen. Any of those components, for instance the limitation of $X_{GLY}$ may limit or halt the reaction rate, if this is so (see Table 6.2).

**Table 6.1:** Model stoichiometry for $X_{PAOs}$ related to glycogen

| No. | Processes | $S_{PO4}$ | $S_A$ | $S_F$ | $S_{O2}$ | $X_{GLY}$ | $X_{PP}$ | $X_{PHA}$ |
|---|---|---|---|---|---|---|---|---|
| $P_1$ | Storage of $X_{PHA}$ | $Y_{PO4}$ | $-Y_{SA}$ | | | $-(1-Y_{SA})$ | $-Y_{PO4}$ | 1 |
| $P_2$ | Storage of $X_{GLY}$ | | | | $-\frac{1-Y_{GLY}}{Y_{GLY}}$ | 1 | | $-\frac{1}{Y_{GLY}}$ |
| $P_3$ | Lysis of $X_{GLY}$ | | | 1 | | -1 | | |

Under aerobic condition, the $X_{PHA}$ is converted to $X_{GLY}$, and the rest is oxidized in the availability of oxygen (Table 6.1). In the model definition of **Manga et al., 2001**, the stoichiometric constant of PHA requirement for GLY storage, $Y_{GLY}$ is set to 1.0 $gCODg^{-1}COD$ under the assumption that no energy is required to convert PHA to glycogen under aerobic condition. The storage rate equation of $X_{GLY}$ delineated with P2 is not only controlled by the availability of $X_{PHA}$ pool and also limited by the maximum glycogen content of active biomass (depicted by $K_{MaxGly}$) at a maximum rate of $q_{GLY}$. The glycogen is then subjected to lysis in parallel to the decay of endogenous biomass, $X_{PAOs}$ (Table 6.2-P3).

**Table 6.2**: Process rates for $X_{PAOs}$ related to glycogen

| No | Processes | Process Rate |
|---|---|---|
| $P_1$ | Storage of $X_{PHA}$ | $q_{PHA}\frac{S_A}{K_A+S_A}\frac{X_{PP}/X_{PAO}}{K_{PP}+X_{PP}/X_{PAO}}\frac{X_{GLY}/X_{PAO}}{K_{GLY}+X_{GLY}/X_{PAO}}X_{PAO}$ |
| $P_2$ | Storage of $X_{GLY}$ | $q_{GLY}\frac{X_{PHA}/X_{PAO}}{K_{PHAGLY}+X_{PHA}/X_{PAO}}\frac{K_{MaxGLY}-X_{GLY}/X_{PAO}}{K_{IGLY}+(K_{MaxGLY}-X_{GLY}/X_{PAO})}\frac{S_{O2}}{K_{O2}+S_{O2}}X_{PAO}$ |
| $P_3$ | Lysis of $X_{GLY}$ | $b_{GLY}\ X_{GLY}$ |

### 6.2.2 Modelling glycogen accumulating organisms, GAOs

The stoichiometric and kinetic trade-off between the substrate, internal storage materials and biomass is regarded as in the same way of PAOs by excluding the phosphorus release and uptake processes (**Mino et al. 1995; Manga et al., 2001**). In the matrix representation, the additional indice 'G' indicates the parameter(s) or state variable(s), which are related to the GAOs metabolism. Accordingly, the GAOs replenish their $X_{PHA(G)}$ pools by taking up acetate and consuming their glycogen pools without any phosphate release under anaerobic condition (G1). In the following aerobic or anoxic conditions, available $X_{PHA(G)}$ is consumed for bacterial growth and replenishment of glycogen without Poly-P, $X_{PP}$ storage (Table 6.3). The growth process of $X_{GAO}$ only consumes $X_{PHA(G)}$ pools under aerobic conditions. It should be stresses that the anoxic growth of $X_{GAO}$ process is not considered in this study as model simplicity.

According to Table 6.4, the storage of $X_{PHAG}$ is controlled by external acetate concentration, $S_A$ and the internally stored $X_{GLY}$ concentration described by double-monod equations (G1). Under aerobic condition, the cells restore their $X_{GLY}$ or $X_{GLYG}$ pools and perform growth by consuming $X_{PHA}$ pools. The main difference from the process of PAOs is that no $X_{PP}$ limitation exists (G1). The storage of glycogen, $X_{PHAG}$ is controlled by 2 switch functions. In the same way of storage products in $X_{PAOs}$, the GAOs are subjected to lysis as indicated by G4-G6 shown in Table 6.4. It should be noted that, no oxygen consumption takes place when $X_{PHAG}$ is converted to $X_{GLYG}$ under aerobic conditions (**Manga et al., 2001**).

### 6.2.3 Stoichiometry of acetate uptake for PAOs and GAOs

The stoichiometry of the model used in this study is based upon a recent metabolic model describing the relationship and stoichiometry among the acetate, internal storage pools and the polyphosphate under anaerobic conditions for a mixed culture of PAOs and GAOs (**Yagci et al. 2003**). Accordingly, the anaerobic stoichiometry for GAOs at pH= 7.4 is given in Eq. 6.1 as follows:

$$\text{Acetate} + 0.39\ \text{Glycogen} \rightarrow 0.67\ \text{PHB} + 0.22\ \text{PHV} + 0.55\ CO_2 \qquad \textbf{(6.1)}$$

From the equation above, it can be calculated that $Y_{SAG}$ = 0.46 gCOD of acetate is used to produce 1 gCOD of PHA synthesized by GAOs. However, the same study proposed variable stoichiometry for PAOs based on the assumption that PAOs may have the ability to use acetyl-CoA derived from the degradation of glycogen or extracellular-acetate via the glyoxylate pathway to produce reducing power (see Eq. 6.2).

$$(6+3f_{GLX})\ \text{Acetate} + \text{Glycogen} \rightarrow 4\ \text{PHB} + f_{GLX}\ \text{PHV} + (2+f_{GLX})\ CO_2 \qquad \textbf{(6.2)}$$

The proposed model describing the stoichiometry of anaerobic acetate metabolism by a mixed culture of PAOs and GAOs is tested with experimentally data determined for acetate uptake, phosphate release, glycogen consumption and PHA production rates and their composition. A fraction of acetyl-CoA converted to PHV by PAOs via the *glyoxylate pathway* ($f_{GLX}$) was calculated to be 1.35. Incorporation of the value of $f_{GLX}$

**Table 6.3**: Model stoichiometry for $X_{GAO}$

| No. | Processes | Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $S_{PO4}$ | $S_A$ | $S_F$ | $S_{O2}$ | $X_{GAO}$ | $X_{GLYG}$ | $X_{PHAG}$ | $X_S$ | $X_I$ |
| $G_1$ | Storage of $X_{PHAG}$ | | $-Y_{SAG}$ | | | | $-(1-Y_{SAG})$ | 1 | | |
| $G_2$ | Storage of $X_{GLYG}$ | | | | $-\frac{1-Y_{GLYG}}{Y_{GLYG}}$ | | 1 | $-\frac{1}{Y_{GLYG}}$ | | |
| $G_3$ | Growth of $X_{GAOs}$ | $-i_{PBM}$ | | | $-\frac{1-Y_{GAO}}{Y_{GAO}}$ | 1 | | $\frac{1}{Y_{GAO}}$ | | |
| $G_4$ | Lysis of $X_{GAO}$ | $v_{i,PO4}$ | | | | -1 | | | $1-f_{XI}$ | $1-f_{XI}$ |
| $G_5$ | Lysis of $X_{GLYG}$ | | | 1 | | | -1 | | | |
| $G_6$ | Lysis of $X_{PHAG}$ | | 1 | | | | | -1 | | |

**Table 6.4**: Process kinetics for $X_{GAO}$s

| No | Processes | Process Rate |
|---|---|---|
| $G_1$ | Storage of $X_{PHAG}$ | $q_{PHAG}\frac{S_A}{K_A+S_A}\frac{X_{GLYG}/X_{GAO}}{K_{GLY}+X_{GLYG}/X_{GAO}}X_{GAO}$ |
| $G_2$ | Storage of $X_{GLYG}$ | $q_{GLYG}\frac{X_{PHAG}/X_{GAO}}{K_{PHAGLYG}+X_{PHAG}/X_{GAO}}\frac{K_{MaxGLYG}-X_{GLYG}/X_{GAO}}{K_{IGLYG}+(K_{MaxGLYG}-X_{GLYG}/X_{GAO})}\frac{S_{O2}}{K_{O2}+S_{O2}}X_{GAO}$ |
| $G_3$ | Growth of $X_{GAO}$s | $\mu_{GAO}\frac{X_{PHAG}/X_{GAO}}{K_{PHAG_{growth}}+X_{PHAG}/X_{GAO}}\frac{S_{PO4}}{K_P+S_{PO4}}\frac{S_{O2}}{K_{O2}+S_{O2}}X_{GAO}$ |
| $G_4$ | Lysis of $X_{GAO}$ | $b_{GAO}\,X_{GAO}$ |
| $G_5$ | Lysis of $X_{GLYG}$ | $b_{GLYG}\,X_{GLYG}$ |
| $G_6$ | Lysis of $X_{PHAG}$ | $b_{PHAG}\,X_{PHAG}$ |

and adding the term of released $P_i$, a stoichiometric equation for acetate uptake by PAOs under anaerobic conditions is then formed as (Eq. 6.3):

$$\text{Acetate} + 0.10\ \text{Glycogen} \rightarrow 0.40\ \text{PHB} + 0.13\ \text{PHV} + 0.33\ CO_2 + [2\alpha_{PAO} + 0.7]\ P_i \qquad \textbf{(6.3)}$$

The ratio of acetate uptake to synthesized PHA by PAOs, $Y_{SA}$, can be determined from the above equation as 0.77gCOD(gCOD)$^{-1}$. The observed anaerobic stoichiometry also shows that 73% of the total acetate uptake was taken up by PAOs under these circumstances. The ratio of P release to acetate uptake by PAOs (0.53gP/gCOD) which was obtained from the observed value corrected by this factor is quite compatible with that expected at the pH level in the study. Furthermore, the value of phosphate release to synthesized PHA, calculated using the experimental data for mixed culture is corrected for PAOs for anaerobic storage of PHA. The model coefficient $Y_{PO4}$ then is equal to 0.41gP/gCOD. The stoichiometric coefficients of the model are given in Table 6.5.

### 6.3 Materials and Methods

The required data for calibration of the model is obtained from a laboratory-scale reactor, with 4 L of total volume, operated as an anaerobic-aerobic sequencing batch reactor (SBR). The temperature of 20°C was maintained keeping the SBR in the constant temperature room. The sludge used for the study was obtained from a pilot-plant activated sludge plant achieving nutrient removal, which was treating domestic wastewater and acetate. The $V_0/V_F$ ratio of 0.6 was obtained leaving initial volume of 1.5 L after withdrawal. The cycling operation of the reactor was controlled with time controllers. The total cycle time ($T_C$) was 6 h to obtain 4 cycles per day (m). The sludge exposed to 2 h ($T_M$) of mixing with 0.25 h ($T_F$) filling period and 3 h ($T_A$) of aerobic conditions following with 1 h settling, decanting and idle period. The total sludge age of $\theta_X$ of 10 days was obtained with appropriate sludge wasting at the end of the aeration phase. The overall hydraulic retention time was 0.4 d. The reactor was fed with synthetic wastewater containing acetic acid as the sole carbon source to obtain 400 mgCOD $L^{-1}$. $PO_4$-P and $NH_4$-N concentrations were 20 mgP $L^{-1}$ and 20 mgN $L^{-1}$, respectively. Nutrient medium was as detailed by **Yagci et al. (2003)**. The performance of the SBR

was further investigated by measuring the concentration profiles of relevant parameters, within a selected complete cycle during the steady state operation. The reactor was monitored with daily and detailed cycle measurements of HAc, $PO_4$-P, $NH_4$-N, $N_{OX}$-N, PHA components of PHB, PHV and 3H2MV, glycogen, MLSS, MLVSS and OUR. Detailed cycle measurement data was collected at steady state conditions. The measured data of the reactor served the purpose of verification and calibration of the model. The calibration efforts based on one of detailed cycle measurements, which were extremely identical and collected at steady state. Full EBPR was maintained in the reactor. Acetate was completely consumed in the first half of the anaerobic conditions. Approximately $PO_4$-P release of 109 $mgL^{-1}$ and $PO_4$-P uptake of 123 $mgL^{-1}$ obtained during the cycle, which is indicating that the release/uptake rate was 1.13. The measured MLSS and MLVSS concentrations were 3300 and 2250 $mgL^{-1}$, respectively, at the end of the aerobic phase of the selected detailed cycle. It is observed that PHA was still remained at the end of aerobic phase. It is also observed that full nitrification achieved and $NH_4$-N was completely consumed throughout the aerobic phase. Pre-anoxic conditions were maintained at the beginning of mixing period. Nitrogen content of the sludge was 12.6% per amount of VSS as calculated from TKN concentration at the end of the aerobic phase with sludge.

Simulations were performed by the AQUASIM software package (**Reichert, 1998**). The sequencing batch operation of the system was provided via definitions of variable volume type reactor and recirculation of particular components to the next cycle with initial volume.

## 6.4 Results and Discussion

### 6.4.1 Calibration study

A stepwise manual calibration methodology (**Vanrolleghem et al., 2003**) was selected for fitting the model outputs on the measured components of the ammonia, $S_{NH}$ and nitrate nitrogen, $S_{NO3}$, total polyhydroxyalkonates, $X_{PHA}$; total glycogen, $X_{GLY}$, orthophosphate, $S_{PO4}$, acetate, $S_A$, oxygen uptake rate, OUR and mixed liquor volatile suspended solids, MLVSS which were measured within one-SBR cycle after reaching

steady state conditions. Each simulation was carried out for 50 days (more than 3 times sludge age) in order to have constant biomass composition in the aerobic phase after changing some relevant parameters, iteratively. Due to the over-parameterized structure of the model, some parameters responsible for PAOs kinetics -especially the lyses terms- were also set to their default values as proposed in base-ASM2d model in order to decrease the number of parameters to be calibrated, manually. In this way, the number of calibrated parameters was decreased considerably (see Table 6.5). In the first step of calibration study, the aim is to maintain the biomass components ($X_A$, $X_H$, $X_{GAO}$ and $X_{PAO}$) in the system by simulating the same amount of MLVSS concentration in aerobic condition (Figure 6.1). The second step is to fit the model trajectories by the selection of other kinetic parameters on measured components. The ideas behind the selection of parameters for the processes will be addressed in the following sections.

### 6.4.2 Modeling of nitrogen removal and heterotrophic activity

In the modeling of biological nutrient removing SBRs, the starting point should be providing the autotrophic biomass growth (**Insel et al., 2003**). In this respect, the effluent ammonia should be reduced and nitrate nitrogen, $S_{NO3}$ has to become available to sustain heterotrophic growth under in the expense of substrate under anoxic conditions. However, in this calibration, the default values of the parameters responsible for autotrophic activity are not changed ($\mu_A$=1.0day$^{-1}$ and $b_A$=0.15day$^{-1}$) since the simulation trajectories of $S_{NH}$ and $S_{NOx}$ reflects the trend of measured values in a SBR cycle (Figure 6.2). The kinetic and stoichionmetric constants related to the heterotrophic biomass, $X_H$ are kept as default, as well. The concentration of heterotrophic biomass is simulated to be around 630 mgcellCOD/l at the end of aerobic phase (results not shown).

It should be noted that the acetate COD, $S_A$ is completely consumed in mixing/filling phase (see Figure 6.3-a). The simulation and measurements indicate that there is no COD leakage into the aerobic phase. So, the available COD (solely acetate) is distributed over heterotrophs, PAOs and GAOs in filling/mixing phase.

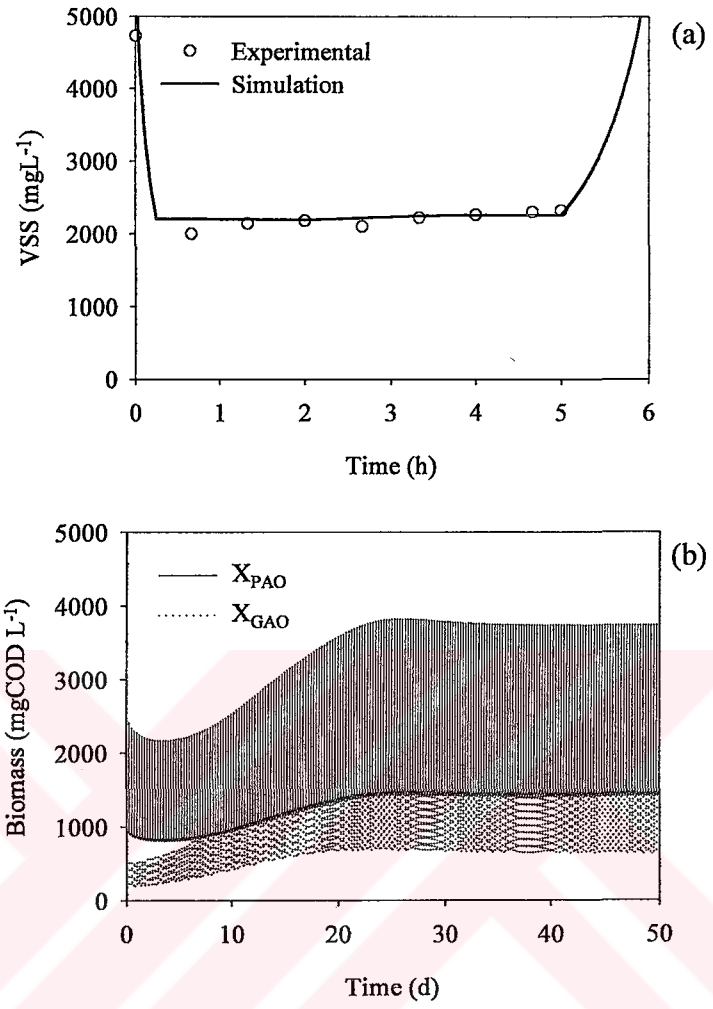



**Figure 6.1:** Simulated and measured MLVSS concentration (a) and simulation of GAOs/PAOs (b)

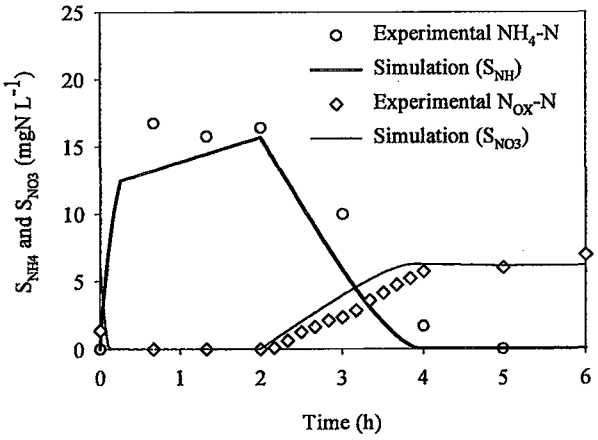



**Figure 6.2:** Nitrogen components in SBR cycle at steady state

### 6.4.3 Modeling of PAOs and GAOs

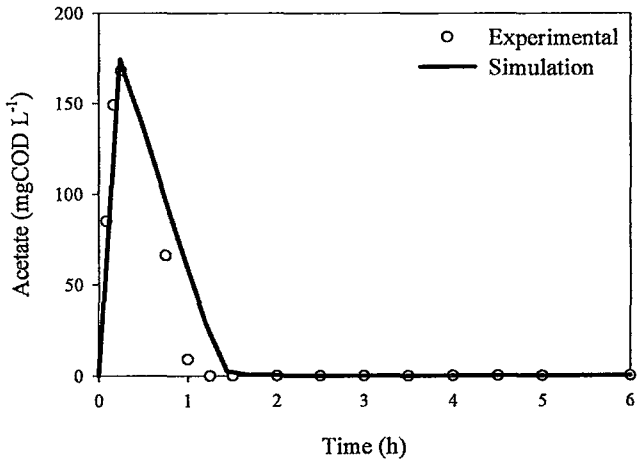
The important points in the modeling of PAOs and GAOs and $X_H$ are (a) to maintain the same total amount of MLVSS by sustaining the biomass composition of PAOs and GAOs and $X_H$ in the reactor (b) to allow sufficient growth material for $X_{PAO}$ and $X_{GAO}$ (c) to keep the biomass composition at a constant level, in turn, at a steady state level while after changing the relevant parameter(s) at each simulation run (d) the selection and manual calibration of the most important parameters in order to obtain a better fit on the measurements under predefined stoichiometry. The growth of GAOs and the measured MLVSS concentration at the end of aerobic phase are maintained by decreasing the lysis rate of GAOs down to 0.1 $day^{-1}$ (see Table 6.5). This situation is in agreement with the findings of **Cech and Hartman, (1993)** stating that PAOs might be predated more easily by the protozoan activity compared to GAOs since their cells are relatively smaller. On the other hand, while keeping $b_{PAO}$ around 0.2$day^{-1}$, the GAOs are washed out from the system It should be stressed here that the stoichiometry for $S_A$ requirement for PHA storage ($Y_{SA}$ and $Y_{SAG}$) parameters are adopted from the metabolic model proposed by **Yagci et al., (2003)** as previously discussed.

According to the model, the growth of PAOs and GAOs is associated with the consumption and replenishment of internal storage pools, $X_{PHA}$ and $X_{GLY}$ under anaerobic and aerobic conditions. In this respect, the growth is highly influenced by the parameters: storage rate of $S_A$, $q_{PHA(G)}$, the rate for storage of glycogen, $q_{GLY(G)}$ and the maximum glycogen capacity of the active biomass represented by $K_{MAXGLY(G)}$ terms. Hence, the calibration of $q_{PHA}$ plays an important role for appropriate distribution of $S_A$ over $X_{PAO}$ and $X_{GAO}$. However, the replenishment of internal storage product together with maintaining sufficient substrate pools are provided with the slight increase in the storage rate constant for PAOs, $q_{PHA}$ from 3.00 to 3.40 g COD $g^{-1}$ COD $d^{-1}$ which is found to be quite sensitive to biomass composition. At steady state, $S_A$ is completely consumed in the mid of fill/mixing phase resulting a phosphorus release up to 115 mgP/l due to Bio-P activity as shown in Figure 3-b. In the following aerobic phase, $S_{PO4}$ is completely consumed. Then, the maximum glycogen content of active biomass namely the $K_{MAXGLY(G)}$ are calibrated to obtain a fit in measured total glycogen concentration providing sufficient glycogen pools (Figure 6.4-a). In Figure 6.4, the terms of $X_{GLY}$ and

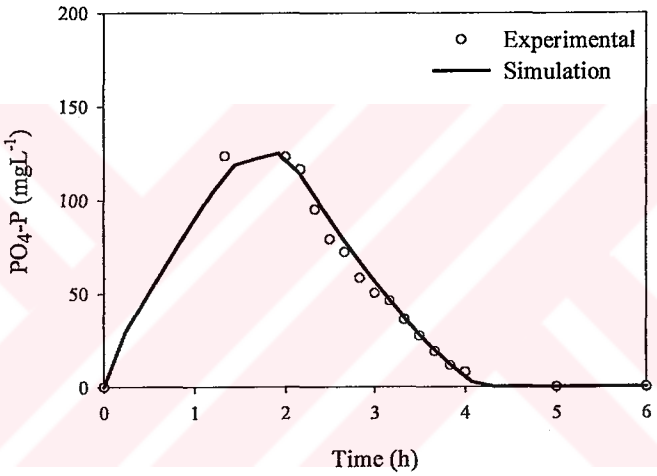
$X_{GLYG}$ stand for the glycogen content of PAOs and GAOs, respectively. Thus, $K_{MAXGLY(G)}$ is set to a higher value than that of $X_{PAO}$ since the energy requirement for the growth of GAOs is provided only by the internal storage pools, $X_{PHA}$ and $X_{GLY}$. In addition, a delicate balance between $X_{PHA}$ and $X_{GLY}$ is maintained at a rate of 2.7 g $CODg^{-1}CODd^{-1}$ same for $q_{GLY}$ and $q_{GLYG}$ which are in concert with the findings of **Manga et al., (2001).**

The saturation coefficient of $X_{PHA}$ for $X_{PP}$ storage, $K_{PHA}$ and growth of $X_{PAOs}$, $K_{PHAgrowth}$ are increased to 0.05 mgCOD/l in order to acquire smooth transition phase for $S_{PO4}$ as well as the $X_{PHA}$ variations under aerobic condition. With respect to the simulated and experimental evidence illustrated in Figure 6.4-a, the glycogen profiles and contribution of each processes reveal that glycogen content of $X_{PAO}$ is around 250 mgCOD/l comparably higher than $X_{GAO}$ has. The stoichiometry of acetate uptake does not provide sufficient carbon source for $X_{GAO}$ to store the glycogen although the maximum glycogen content of $X_{GAO}$ is high ($K_{MAXGLYG}$=0.30). It is important to note that the model stoichiometry and kinetics have to be testified under limited COD conditions. In this way, the trade-off between the internal substrate pools and phosphate for $X_{PAOs}$ will be no more limited by the phosphate available in bulk liquid. The same holds for the $X_{PHA}$ variations depicted in Figure 6.4-b where the major contribution is devoted to phosphorus accumulating organisms, $X_{PAOs}$. Under aerobic condition, $X_{GAOs}$ completely consumed its $X_{PHA}$ pools necessary for growth.

As shown in Figure 6.5, the simulation of oxygen uptake rate, OUR shows a similar trend compared to in-cycle measurements. After the commencement of aeration, the OUR increased up to 90 $mgO_2$/l.h and decreased gradually to a level of 20-25 $mgO_2$/l exerting a tailing. This transition in OUR is attributed to nutrient (N, P) limitations at the end of the phase based upon the model simulation. The calculation of cumulative oxygen consumption in a SBR cycle by integrating the OUR graph reveals that the phosphate uptake mechanism is only responsible for 20% of total oxygen consumption. The rest is the sum of OUR pertaining to the autotrophic activity, growth of PAOs and endogenous biomass.




(a)




(b)

**Figure 6.3**: Simulations for acetate-COD, $S_A$ (a) and phosphate profiles, $S_{PO4}$ (b)

**Table 6.5:** Comparison of stoichiometric and kinetic parameters for ASM2d and calibrated model

| | | ASM2d | PAO | GAO | Unit |
|---|---|---|---|---|---|
| Stoichiometric parameters | | | | | |
| $Y_{SA}$, $Y_{SAG}$ | $S_A$ requirement for PHA storage | - | 0.77 | 0.46 | g COD $g^{-1}$COD |
| $Y_{PO4}$ | PP requirement ($PO_4$ release) for PHA | 0.40 | 0.41 | - | g P $g^{-1}$COD |
| $Y_{PAO}$, $Y_{GAO}$ | Yield coefficient | 0.625 | 0.625 | 0.625 | g COD $g^{-1}$COD |
| $Y_{PHA}$ | PHA requirement for PP storage | 0.20 | 0.20 | - | g COD $g^{-1}$P |
| $Y_{GLY}$, $Y_{GLYG}$ | Glycogen yield coefficient | - | 1 | 1 | g COD $g^{-1}$COD |
| Kinetic parameters (20°C) | | | | | |
| $q_{PHA}$, $q_{PHAG}$ | Rate const. for storage of $X_{PHA}$ (base $X_{PP}$) | 3.00 | 3.40* | 3.00* | g COD $g^{-1}$ COD $d^{-1}$ |
| $q_{GLY}$, $q_{GLYG}$ | Rate constant for storage of $X_{GLY}$ | - | 2.70* | 2.70* | g COD $g^{-1}$ COD $d^{-1}$ |
| $q_{PP}$ | Rate constant for storage of $X_{PP}$ | 1.50 | 2.00* | - | g P $g^{-1}$ COD $d^{-1}$ |
| $\mu_{PAO}$, $\mu_{GAO}$ | Maximum growth rate of $X_{PAO}$ | 1.00 | 1.00 | 1.00 | $d^{-1}$ |
| $b_{PAO}$, $b_{GAO}$ | Rate of lysis of PAO | 0.20 | 0.2 | 0.1* | $d^{-1}$ |
| $b_{PP}$ | Rate of lysis of PP | 0.20 | 0.2 | | $d^{-1}$ |
| $b_{GLY}$, $b_{GLYG}$ | Rate of lysis of PP | 0.20 | 0.2 | 0.1* | $d^{-1}$ |
| $b_{PHA}$, $b_{PHAG}$ | Rate of lysis of PHA | 0.20 | 0.2 | 0.1* | $d^{-1}$ |
| $K_A$ | Saturation coefficient for acetate | 4.00 | 4.00 | 4.00 | g COD $m^{-3}$ |
| $K_{PS}$ | Saturation coeff. phosphorus storage ($X_{PP}$) | 0.20 | 0.50* | - | g P $m^{-3}$ |
| $K_P$ | Saturation coeff.for phosphorus (nutrient) | 0.01 | 0.01 | 0.01 | g P $m^{-3}$ |
| $K_{PP}$ | Saturation coeff.for poly-phosphate | 0.01 | 0.01 | - | g P $g^{-1}$ COD |
| $K_{MAX}$ | Maximum ratio of $X_{PP}/X_{PAO}$ | 0.34 | 0.34 | - | g P $g^{-1}$ COD |
| $K_{MAXGLY}$, $K_{MAXGLYG}$ | Max. ratio for $X_{GLY}/X_{PAO}$, $X_{GLY}/X_{GAO}$ | - | 0.15* | 0.30* | g COD $g^{-1}$ COD |
| $K_{IPP}$ | PP storage inhibition coefficient | 0.02 | 0.02 | - | g P $g^{-1}$ COD |
| $K_{IGLY}$, $K_{IGLYG}$ | GLY storage inhibition coefficient | 0.02 | 0.02 | 0.02 | g COD $g^{-1}$ COD |
| $K_{PHA}$ | Saturation coeff. for PHA for PP storage | 0.01 | 0.05* | - | g COD $g^{-1}$ COD |
| $K_{PHAgrowth}$, $K_{PHAGgrowth}$ | Saturation coeff.for PHA for growth | - | 0.05* | 0.01 | g COD $g^{-1}$ COD |
| $K_{PHAGLY}$, $K_{PHAGLYG}$ | Saturation coeff.for PHA for GLY storage | 0.01 | 0.01 | 0.01 | g COD $g^{-1}$ COD |
| $K_{GLY}$, $K_{GLYG}$ | Saturation coeff. for GLY | - | 0.001 | 0.001 | g COD $g^{-1}$ COD |

* Calibrated parameters

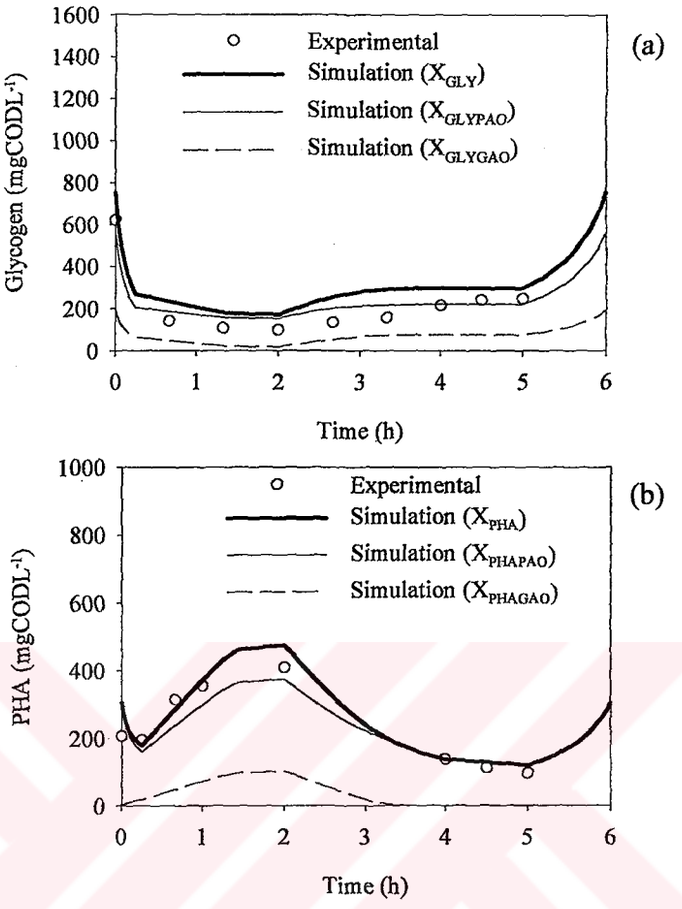



**Figure 6.4:** Simulation and measured (a) total glycogen, and (b)PHA concentration

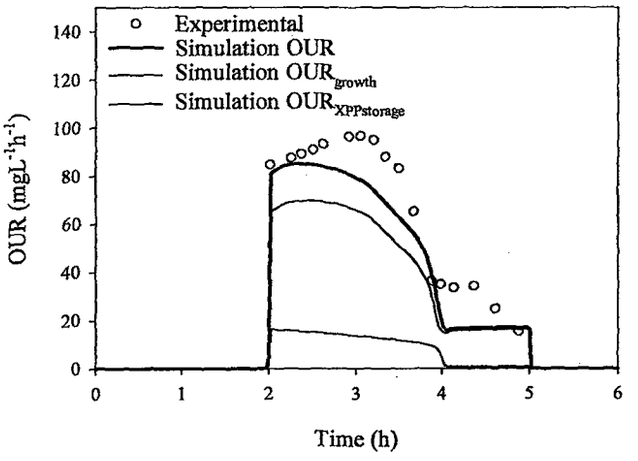



**Figure 6.5:** Oxygen uptake rate, OUR profile in SBR cycle

**Table 6.5:** Comparison of stoichiometric and kinetic parameters for ASM2d and calibrated model

| | | ASM2d | PAO | GAO | Unit |
|---|---|---|---|---|---|
| Stoichiometric parameters | | | | | |
| $Y_{SA}$, $Y_{SAG}$ | $S_A$ requirement for PHA storage | - | 0.77 | 0.46 | g COD $g^{-1}$COD |
| $Y_{PO4}$ | PP requirement ($PO_4$ release) for PHA | 0.40 | 0.41 | - | g P $g^{-1}$COD |
| $Y_{PAO}$, $Y_{GAO}$ | Yield coefficient | 0.625 | 0.625 | 0.625 | g COD $g^{-1}$COD |
| $Y_{PHA}$ | PHA requirement for PP storage | 0.20 | 0.20 | - | g COD $g^{-1}$P |
| $Y_{GLY}$, $Y_{GLYG}$ | Glycogen yield coefficient | - | 1 | 1 | g COD $g^{-1}$COD |
| Kinetic parameters (20°C) | | | | | |
| $q_{PHA}$, $q_{PHAG}$ | Rate const. for storage of $X_{PHA}$ (base $X_{PP}$) | 3.00 | 3.40* | 3.00* | g COD $g^{-1}$ COD $d^{-1}$ |
| $q_{GLY}$, $q_{GLYG}$ | Rate constant for storage of $X_{GLY}$ | - | 2.70* | 2.70* | g COD $g^{-1}$ COD $d^{-1}$ |
| $q_{PP}$ | Rate constant for storage of $X_{PP}$ | 1.50 | 2.00* | - | g P $g^{-1}$ COD $d^{-1}$ |
| $\mu_{PAO}$, $\mu_{GAO}$ | Maximum growth rate of $X_{PAO}$ | 1.00 | 1.00 | 1.00 | $d^{-1}$ |
| $b_{PAO}$, $b_{GAO}$ | Rate of lysis of PAO | 0.20 | 0.2 | 0.1* | $d^{-1}$ |
| $b_{PP}$ | Rate of lysis of PP | 0.20 | 0.2 | | $d^{-1}$ |
| $b_{GLY}$, $b_{GLYG}$ | Rate of lysis of PP | 0.20 | 0.2 | 0.1* | $d^{-1}$ |
| $b_{PHA}$, $b_{PHAG}$ | Rate of lysis of PHA | 0.20 | 0.2 | 0.1* | $d^{-1}$ |
| $K_A$ | Saturation coefficient for acetate | 4.00 | 4.00 | 4.00 | g COD $m^{-3}$ |
| $K_{PS}$ | Saturation coeff. phosphorus storage ($X_{PP}$) | 0.20 | 0.50* | - | g P $m^{-3}$ |
| $K_P$ | Saturation coeff.for phosphorus (nutrient) | 0.01 | 0.01 | 0.01 | g P $m^{-3}$ |
| $K_{PP}$ | Saturation coeff.for poly-phosphate | 0.01 | 0.01 | - | g P $g^{-1}$ COD |
| $K_{MAX}$ | Maximum ratio of $X_{PP}/X_{PAO}$ | 0.34 | 0.34 | - | g P $g^{-1}$ COD |
| $K_{MAXGLY}$, $K_{MAXGLYG}$ | Max. ratio for $X_{GLY}/X_{PAO}$, $X_{GLY}/X_{GAO}$ | - | 0.15* | 0.30* | g COD $g^{-1}$ COD |
| $K_{IPP}$ | PP storage inhibition coefficient | 0.02 | 0.02 | - | g P $g^{-1}$ COD |
| $K_{IGLY}$, $K_{IGLYG}$ | GLY storage inhibition coefficient | 0.02 | 0.02 | 0.02 | g COD $g^{-1}$ COD |
| $K_{PHA}$ | Saturation coeff. for PHA for PP storage | 0.01 | 0.05* | - | g COD $g^{-1}$ COD |
| $K_{PHAgrowth}$, $K_{PHAGgrowth}$ | Saturation coeff.for PHA for growth | - | 0.05* | 0.01 | g COD $g^{-1}$ COD |
| $K_{PHAGLY}$, $K_{PHAGLYG}$ | Saturation coeff.for PHA for GLY storage | 0.01 | 0.01 | 0.01 | g COD $g^{-1}$ COD |
| $K_{GLY}$, $K_{GLYG}$ | Saturation coeff. for GLY | - | 0.001 | 0.001 | g COD $g^{-1}$ COD |

* Calibrated parameters

# 7. EFFECTS OF pH AND SUBSTRATE ON THE COMPETITION BETWEEN GLYCOGEN ACCUMULATING AND PHOSPHORUS ACCUMULATING ORGANISMS

## 7.1 Introduction

Biological nitrogen and phosphorus removal (BNR) processes have been developed and are being used as an economical and effective method of treating municipal wastewater. The efficiency of enhanced biological phosphorus removal (EBPR) is influenced by several operational parameters such as the carbon source (**Lemos et al., 1998, Liu et al., 2000**), temperature (**Beatens et al., 1999, Erdal et al, 2002a**) **or pH (Smolders et al., 1994, Liu et al., 1997**). Good phosphorus removal is achieved when activated sludge is enriched with a population of phosphorus accumulating organisms (PAOs) (**Cech and Hartman, 1993, Mino et al., 1998**). Recently, many researchers have reported observations of glycogen accumulating organism (GAOs) in BNR systems. These GAOs take up substrate and synthesize PHA anaerobically in competition with PAOs. However, GAOs do not store polyphosphate and hence do not contribute to excess phosphorus removal. If a significant amount of GAOs accumulate, the percentage of VAFs available to PAOs is reduced, which decreases the phosphorus removal ability of the system. As a result, it is very crucial to identify operational conditions that maintain the competitive advantage necessary for domination of PAOs.

**Filipe et al. (2001)** and **Shuler and Jenkins (2002)** have suggested that pH may be a parameter that could be manipulated to minimize the presence of GAOs in EBPR systems, based on the observation that the stoichiometry for acetate uptake by GAOs

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was more favorable at lower pH values. **Erdal et al. (2002a)** have concluded that reduced competition for substrate at low temperatures increases the PAO population and EBPR efficiency, provided that the sludge retention time (SRT) in the system remained above the critical washout SRT for PAOs. Although factors influencing the dominance of GAOs are not well defined at present, in many studies it has been demonstrated that GAOs may proliferate in anaerobic-aerobic activated sludge fed with a synthetic wastewater having higher acetate to phosphate ratio (**Liu et al., 1997**; **Hesselman et al., 2000**). The observations indicate that the microbial population sustained in EBPR systems is possibly a mixture of PAOs and GAOs and may become GAO-enriched when the operation becomes P-limited.

The objectives of this chapter were to evaluate pH-controlled and pH-uncontrolled BNR systems; to interpret the effect of initial pH values of wastewater on biological excess phosphorus removal systems; and then to compare the effects of acetate as a sole carbon source with acetate supplemented domestic wastewaters on BNR systems, particularly on EBPR mechanisms.

## 7.2 Materials and Method

### 7.2.1 Operation of anaerobic/aerobic SBR

The experimental units used in the study were two identical lab scale sequencing batch reactors (SBR) with a total volume adjustable up to 4 liters. They were incubated at a constant temperature of 20°C and operated at steady state in an anaerobic/aerobic sequence. The reactors were equipped with both mechanical mixing and diffused aeration devices to create a sequence of anaerobic/anoxic and aerobic conditions. The operation was adjusted to 4 cycles a day at cycle times of 6 hours. Each cycle involved the regular consecutive sequence of the anoxic/anaerobic fill and mixing phase, the aerated reaction phase and the settle/idle phase where the treated effluent was discharged. Initial volume, $V_0$, and fill volume, $V_F$, were 1.5 and 2.5 liters, respectively. The units were fed with either acetate as the sole carbon source (acetate SBR) or acetate supplemented domestic sewage (acetate added domestic SBR), with acetate addition of 100 mg acetate/L as COD to achieve EBPR, and initially seeded with biomass obtained from a BNR pilot plant treating acetate

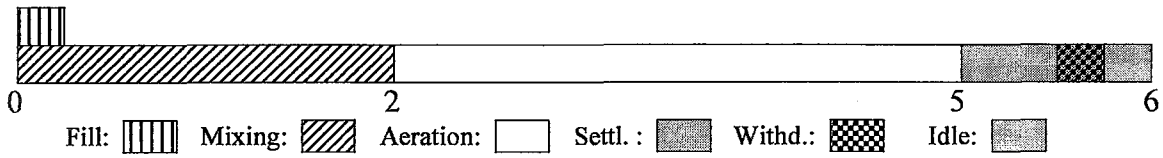
supplemented domestic sewage for nutrient removal. The SBRs were operated at a total sludge age, $\theta_X$, of 10 days with appropriate sludge wasting at the end of the aeration phase. The experiments were carried out during the study in which pH was not controlled in the system. Characteristics and operating conditions of the reactor are schematically given in Figure 7.1. Six stable EBPR experimental periods with influent phosphorus concentrations ranging from 13 to 61 mgP/L with 2 different wastewater compositions were studied.

### 7.2.2 Influent wastewater characteristics

During the study of the acetate SBR, the acetate concentration in the feed was around 400 mgCOD/l. The synthetic wastewater concentrations were as follows: $CH_3COOH$, 400 mg-COD/L; $KH_2PO_4$ and $K_2HPO_4$, 20 mgP/L; $NH_4Cl$, 20 mg-N/L; $CaCl_2$, 15 mg-Ca/L, $NaHCO_3$, 100 mg-$CaCO_3$/L; 3.4 mg/L yeast extract; and nutrient solution, $MgSO_4$, 10 mg-Mg/L; $FeSO_4.7H_2O$, 350 µg-Fe/L; $MnSO_4.H_2O$, 180 µg-Mn/L; $ZnCl_2$, 130 µg-Zn/L; $CoCl_2.H_2O$, 35 µg-Co/L; $CuSO_4.5H_2O$, 35 µg-Cu/L; $H_3BO_3$, 20 µg-B/L; KI, 35 µg-I/L; $(NH_4)_6Mo_7O_{24}.4H_2O$, 35 µg-Mo/L; EDTA, 3 mg/L. The acetate added domestic SBR system with an addition of 100 mg acetate COD/L was operated using domestic wastewater obtained from a main sewer of Blacksburg, VA, USA (**Cokgor et al., 2003**). To minimize the variation of wastewater characteristics from day to day, domestic wastewater was collected at approximately the same time each day. Sufficient $KH_2PO_4$ and $K_2HPO_4$ were added to increase the influent phosphate concentrations to 20, 35, 45 and 60 mg/l in the acetate SBR, and to 13 and 23 mg/l in the acetate added domestic SBR (**Cokgor et al., 2003**) (Table 7.1). At steady state conditions, values of significant parameters were monitored with detailed cycle measurements. Samples taken from the mixed liquor were centrifuged for 10 minutes immediately after collection and supernatant was obtained by filtering through 0.45 micron filter paper.

The cation and anion analyses of filtered samples were performed using a DIONEX Ion Chromatograph according to **Standard Methods for the Examination of Water and Wastewater (1999)**. Glycogen measurements were performed according to the anthrone method outlined in **Manual for General Bacteriology (1981)** with some modifications as described by **Erdal (2002b)**.

All measurements were made in duplicate. When different, they were repeated until the discrepancy was within the limits of analytical accuracy.




| Operation | Time (min) Acetate / Acetate Added Domestic (**Cokgor et al., 2003**) |
|---|---|
| 1. Anaerobic Phase* | 120/95 |
| 2. Aerobic Phase | 180/200 |
| 3. Settling | 30/30 |
| 4. Withdrawal | 15/15 |
| 5. Idle | 15/20 |

* with filling in the first 15 min/5 min for synthetic/domestic reactors

**Figure 7.1:** Characteristics and operating conditions of the anaerobic/aerobic SBRs

**Table 7.1:** Influent characteristics of the SBRs

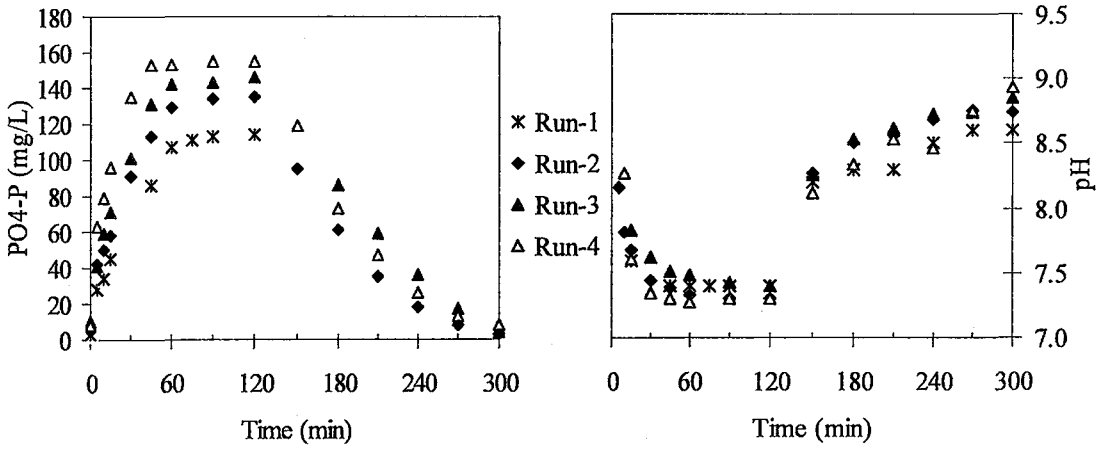
| Run No | Carbon Source | TCOD | TP |
|---|---|---|---|
| | | mgCOD/l | mg/l |
| Run 1 | Acetate | 400 | 21 |
| Run 2 | Acetate | 400 | 35 |
| Run 3 | Acetate | 400 | 46 |
| Run 4 | Acetate | 400 | 61 |
| Run 5 | Acetate added domestic | 400 | 13 |
| Run 6 | Acetate added domestic | 400 | 23 |

## 7.3 Experimental Results and Evaluation

### 7.3.1 The acetate SBR

Experiments were carried out during the research in which pH was not controlled in the system but was closely monitored. The pH profiles in the acetate SBR are presented in Figure 7.2 (b), and the phosphate concentration profiles in Figure 7.2 (a), during a cycle of the acetate SBR. As seen from the figure, a pH decrease was observed at the same time that acetate addition was started. A remarkable drop in pH was observed until the acetate concentration in the mixed liquor was depleted, and then the pH values stayed reasonably stable until the end of the anaerobic phase. The

P/Ac ratios under anaerobic conditions were calculated for each sampling point using the detailed cycle measurements performed for Runs 1 to 4 that had been obtained for comparison with the equations given in the literature for pH controlled systems, which represent pH as a function of P/Ac ratio.




(a) (b)

**Figure 7.2:** Phosphate and pH profiles of the acetate SBR

The relationship between pH and Prel/Acuptake were:

Prel/Ac= 0.19 pH – 0.85 (mol-P/mol-C) **(7.1)**

for pH controlled system **(Smolders et al., 1994)**

Prel/Ac= 0.16 pH – 0.55 (mol-P/mol-C) **(7.2)**

for pH controlled system **(Filipe et al. 2001)**

Prel/Ac= 0.12 pH – 0.44 (mol-P/mol-C) **(7.3)**

for pH uncontrolled system (This study)

The $P_{rel}$/Ac ratios obtained were not in accordance with the pH dependency suggested by **Smolders et al. (1994), Filipe et al. (2001)** and **Schuler and Jenkins (2002)** from batch tests in which the pH was maintained at constant values (Figure 7.3). Actually, the acetate SBR study was part of a comprehensive survey proposing a new metabolic

model for acetate uptake by a mixed culture of phosphate and glycogen accumulating organisms (PAOs and GAOs) under anaerobic conditions, (**Yagci et al., 2003**). The proposed model was experimentally tested and verified under different P inputs. Theoretical and experimental evaluations supported the presence of GAOs for all tested HAc/P ratios, and indicated a significant GAO fraction in the mixed culture for systems operated under P limiting conditions. In the mentioned study, the PAO fraction ($f_{PAO}$) values ranged from 0.88 in Run 4 to 0.73 in Run 1 for the acetate SBR, confirming the validity of a mixed culture and the presence of a significant GAO fraction under P limiting conditions. Using the $f_{PAO}$ values, Eq. 7.4 was obtained for the comparison.

$$(P_{rel}/Ac)/f_{PAO} = 0.16\ pH - 0.64\ (mol\text{-}P/mol\text{-}C) \qquad (7.4)$$

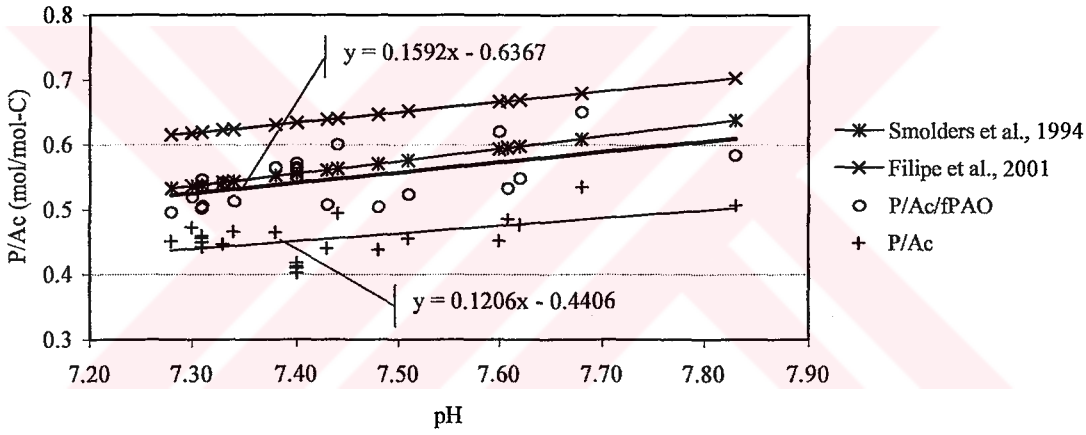



**Figure 7.3:** Relationship between P/Ac ratio and pH in the anaerobic phase

The model proposed by **Yagci, et al. (2003)** that included the PAO fractions, satisfactorily described the experimental results, including the pH effects on Prel/Ac ratios. A linear dependency of Prel/Ac on pH was observed in this study. The different equations reported **by Smolders et al. (1994)** and **Filipe et al. (2001)** are not identical, implying that pH is only one factor that determines the $P_{rel}$/Ac ratio, as suggested by **Yagci et al. (2003).**

### 7.3.2 The acetate added domestic SBR

In the experimental study conducted by **Cokgor et al. (2003)**, the domestic wastewater used was characterized by an average total COD of 275 mg/L, Ammonia

19.1 mg/L, Total Phosphorus 2.2 mg/L, VSS 45 mg/L, $NH_4$-N/TKN ratio 0.45 and VSS/SS ratio 0.90, and was fed to the system for a period of 13 months. To ensure good BNR performance, 100 mg acetate/l, as COD was added to the acetate added domestic SBR system. TP/TCOD ratios of 12.2 and 22.7 were used for Runs 5 and 6, respectively. Total phosphorus removal was around 82% and 93%, respectively, for these Runs at steady state conditions.

Batch tests were performed with identical acetate added domestic wastewaters by **Cokgor et al. (2003)** having different initial pH values using biomass obtained from the acetate added domestic SBRs, which were operated at 2 different TCOD/TP ratios. Initial pH was maintained at 6.0 (Run 5.1 and 6.1), 6.9 (Run 5.2 and 6.2), and 7.6 (Run 5.3 and 6.3), respectively, for the batch tests. pH and phosphorus concentrations obtained by the detailed cycle measurements are shown in Figure 7.4. Similar behavior for changes in the phosphorus concentration during the anaerobic phase could be seen with the acetate SBR system, but the pH changes were very different because of the buffering capacity of the domestic wastewater.

A comparison of $P_{rel}/TCOD_{uptake}$ versus pH was performed using the results from the acetate SBRs and the batch tests performed using the acetate added domestic SBR sludge. The results obtained are illustrated in Table 7.2 and Figure 7.5. It was noted that the $P_{rel}/TCOD_{uptake}$ ratios obtained for the acetate added domestic SBR batch tests were always higher than those from the acetate SBR results, even though they had the same total COD. The acetate added domestic SBR was fed just 100 mg acetate COD/L, added to around 300 mg COD/L of domestic wastewater that contained mostly slowly biodegradable COD. This is the first reported observation that PAOs were more dominant in a SBR system using acetate added domestic wastewater than in a SBR system using just acetate as a sole carbon source.

Observations supporting a higher PAO fraction in the acetate added domestic SBR compared to the acetate SBR:

1. When the TCOD/TP ratio and pH value in anaerobic phase for both systems were approximately the same, the $P_{rel}$ in the acetate added domestic SBR was higher than in the acetate only SBR.

2. In the acetate added domestic SBR system, glycogen consumption was lower than in the acetate SBR system.

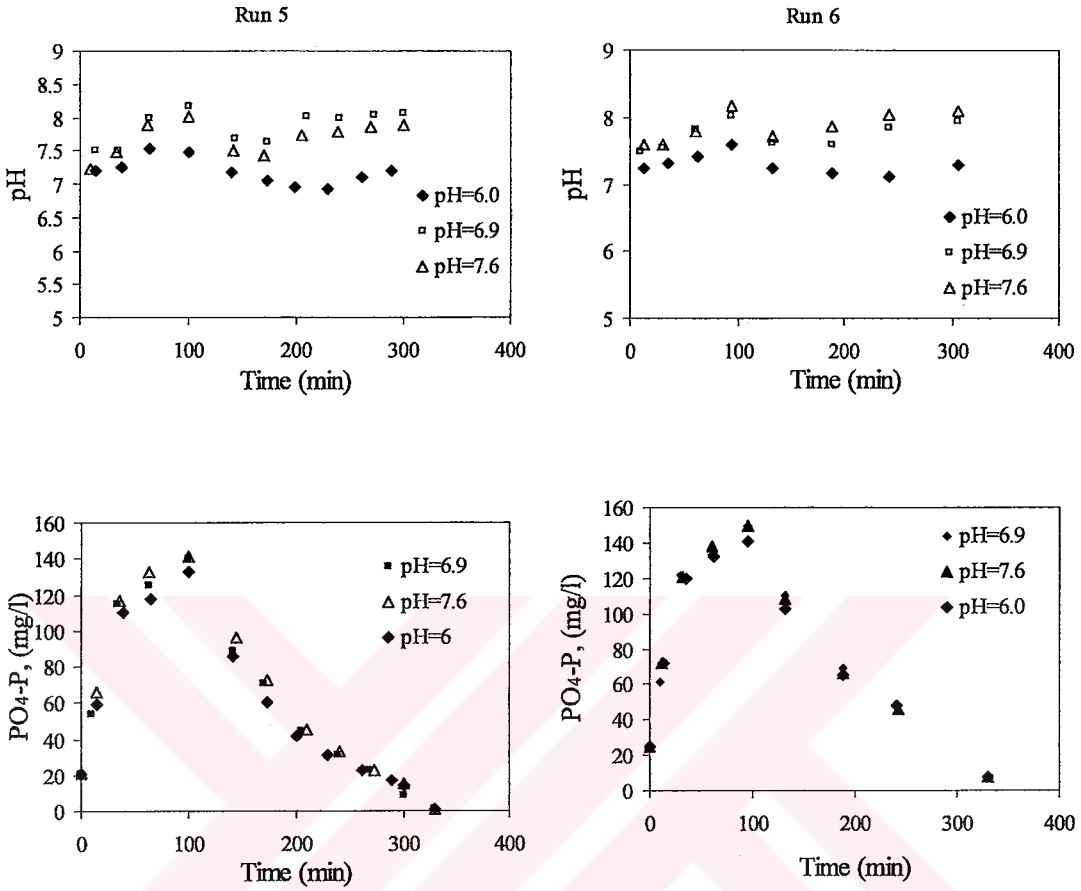



**Figure 7.4:** pH and phosphorus concentrations for the acetate added domestic SBR batch

**Table 7.2:** The observed values of phosphate release and glycogen consumption associated with acetate uptake in the anaerobic phase of an EBPR system

| Run | pH | Influent COD/TP | $COD_{upt}$ | $P_{rel}$ | $P_{rel}/TCOD_{upt}$ | $Gly_{cons}$ | P in sludge |
|---|---|---|---|---|---|---|---|
| | | mgCOD/mgTP | mgCOD/l | mgP/l | mgP/mgCOD | mgCOD/l | %TSS |
| Run 1 | 7.4 | 20 | 235 | 94 | 0.40 | 125 | 14.6 |
| Run 2 | 7.3 | 11.4 | 241 | 106 | 0.44 | 119 | 17.8 |
| Run 3 | 7.4 | 8.9 | 243 | 110 | 0.45 | 115 | 23 |
| Run 4 | 7.3 | 6.7 | 241 | 112 | 0.47 | 113 | 30 |
| Run 5.1 | 7.5 | 22.7 | 191 | 112 | 0.59 | 18 | 20 |
| Run 5.2 | 8.1 | 22.7 | 189 | 119 | 0.63 | 18 | 20 |
| Run 5.3 | 8.1 | 22.7 | 196 | 120 | 0.61 | 19 | 20 |
| Run 6.1 | 7.6 | 12.2 | 190 | 116 | 0.61 | 21 | 23 |
| Run 6.2 | 8.0 | 12.2 | 194 | 124 | 0.64 | 20 | 23 |
| Run 6.3 | 8.2 | 12.2 | 195 | 125 | 0.64 | 19 | 23 |

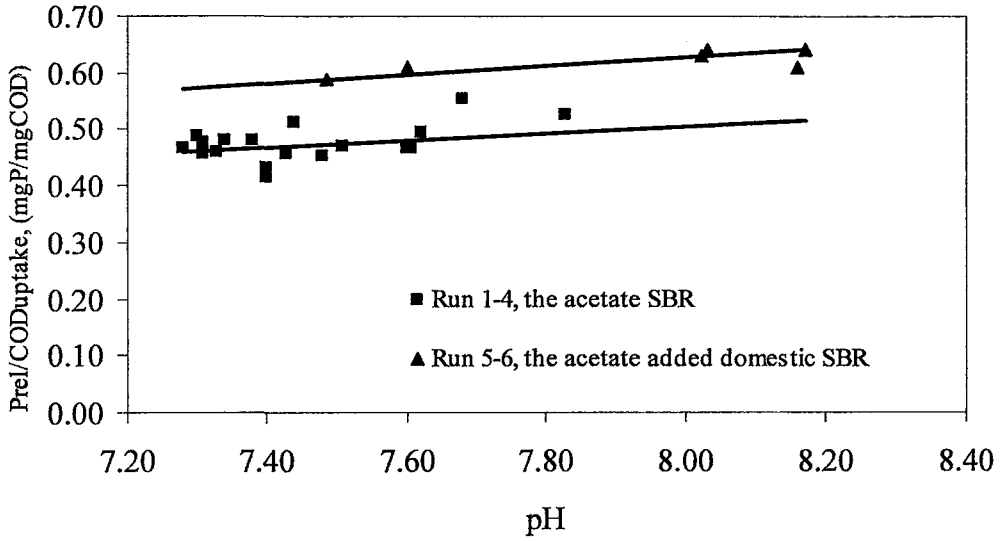



**Figure 7.5:** Comparison of $P_{rel}/COD_{uptake}$ ratio versus pH for the acetate and acetate added domestic SBRs

3. In the acetate added domestic SBR system, the TP content in the sludge was higher than in the acetate SBR system.

4. Evaluation of initial pH showed that as pH decreased, $P_{rel}$ decreased in anaerobic phase, as expected the results given in **Filipe et al., 2001** and **Schuler and Jenkins, 2002**.

## 8. CONCLUSIONS

In this study, two lab scale sequencing batch reactors (SBRs) were operated with synthetic wastewaters mainly to investigate effect of P/VFA ratio and VFAs type on the performance of EBPR process. The data obtained from two SBRs were served to evaluate effects of substrate on biological phosphorus removal and composition of polyhydroxyalkanoates, to develop a new metabolic model for acetate uptake by a mixed culture of phosphate and glycogen accumulating organisms (PAOs and GAOs) under anaerobic conditions, and to improve the modeling of enhanced biological phosphate removal by introducing the mechanistic description of a mixed culture of glycogen and phosphate accumulating organisms competing for acetate, and glycogen metabolism of the latter.

In this thesis, the following concluding remarks may be made based upon the results of the extensive experimental study:

The data obtained in this study showed that increased P/VFA ratios in the influent results in higher $P_{rel}/VFA_{upt}$ ratios. And, the highest phosphate removal efficiency was observed with propionate, even the lower phosphate release and uptake was observed with propionate in the influent. As it is clarified that increase in P/VFA ratio can be attributed to the increase in the number of PAOs in the sludge. Furthermore, the effects of pH and substrate on the competition between glycogen accumulating (GAOs) and phosphorus accumulating organisms (PAOs) were also evaluated in the thesis. A linear dependency of $P_{rel}/Ac$ on pH in anaerobic phase was observed for uncontrolled pH system. It is concluded that the influent HAc/P is an important parameter that affects the balance between PAO and GAO populations in the EBPR system.

In this study, it is estimated that correlation between phosphate release and uptake can be ranged from 1.2 to 1.3 regardless of substrate type.

Influence of carbon source was investigated on PHA concentration and composition. A clear disparity between PHB contents was observed especially for where the

organic carbon was propionate. Using propionate as the carbon source in the influent caused 3H2MV enrichment in the biomass.

The results of batch experiments showed that propionate addition to acetate enriched sludge was lead to lower PHB content compared to values obtained from SBR, which is attributed to possible available enzyme activity for acetate in propionate enriched biomass and likely dominance of PAOs.

This study has found its starting point from earlier similar research efforts which essentially related enhanced biological phosphorus removal systems to solely PAO activity and advocated metabolic models with a fixed stoichiometry, while at the same time reporting stoichiometric ratios and coefficients varying over a wide range depending upon different specific experimental conditions.

Behavior of enhanced biological phosphorus systems is better evaluated in terms of the resulting overall stoichiometry of a mixed culture of PAOs and GAOs competing for the same substrate under anaerobic conditions.

Experimental results are best evaluated by means of a metabolic capability of PAOs triggering the glyoxylate pathway for the conversion of acetyl-CoA to propionyl-CoA, an alternative means of producing the required reducing power for PHA synthesis.

A metabolic model is proposed for the acetate uptake of EBPR systems under anaerobic conditions for a mixed culture of PAOs and GAOs, also accounting for the postulated variable stoichiometry of PAOs. The model was experimentally tested and verified under different P inputs. Theoretical and experimental evaluations supported the presence of GAOs for all tested HAc/P ratios and indicated a significant GAO fraction in the mixed culture for systems operated under P limiting conditions. Strong agreement is observed between experimental values and model predictions for all model components, namely PHB production, PHA composition, glycogen utilization and P release.

The experimental data were also served to evaluate the prediction capability of Activated Sludge Model No.2d (ASM2d), for the enhanced biological phosphorus removal (EBPR) performance of a sequencing biological (SBR) reactor receiving variable influent phosphate load. In this study, ASM2d is basically structured to incorporate PAOs and related microbial processes for the evaluation and simulation

of EBPR from domestic sewage. It generally provides satisfactory interpretation and calibration of the behavior of biological systems for the overall character commonly associated with domestic sewage. However, for the SBR system used in this study, fed with acetate as the sole carbon source and operated for different influent P/Ac ratios, ASM2d fails to predict system performance and defines for each experimental Run operated under different P/AC ratios, a different set of values for the group of significant model coefficients selected for calibration. The shortcomings of ASM2d become most apparent in the calibration of the $X_{PHA}$ profiles.

And also, the regular changing pattern of the coefficients is explained by a mixed culture of phosphate and glycogen accumulating microorganisms, (GAOs), both responsible for acetate uptake under anaerobic conditions in this study. It is argued that the SBR system in this study sustained GAOs, which can store substrate under anaerobic conditions without using polyphosphate energy, but by deriving energy from the degradation of glycogen, thus making them capable of prevailing at lower P/Ac ratios. This argument is used to explain the discrepancies between model calibration and experimental results. It also indicates the need to include glycogen and GAOs as model components for processes involving both PAOs and GAOs, in order to obtain a better model prediction of $S_A$ and $X_{PHA}$ profiles in the system.

Using experimental data obtained in this study and in the light of the former evaluations on prediction capability of recent mathematical model (ASM2d) for nutrient removal systems, the mechanistic description of a mixed culture of glycogen and phosphate accumulating organisms competing for acetate, and glycogen metabolism of the latter were introduced to the model in order to improve the modeling of enhanced biological phosphate removal. As a results of this study, a bio-kinetic model based on the stoichiometry of metabolic models describing the acetate uptake by PAOs and GAOs was developed and tested successfully to provide better understanding of microbial competition. The structure of the model is similar to the ASM2d but includes glycogen metabolism of PAOs and GAO metabolism, individually. Model development mainly relied on accurate numerical assessment of critical coefficients based on proposed metabolic relationships related to the interactive growth of GAOs and PAOs in the mixed cultures.

Also it is concluded that the modified mathematical model including processes of GAOs and glycogen metabolism of PAOs is able to describe the split of acetate

utilization between PAOs and GAOs, accurately as predicted by related metabolic relationship. The proposed model structure and stoichiometry allows simulate the experimental data obtained from the long-term operation of the SBR. The calibration results justified the presence of GAOs in the mixed culture with a significant impact on process behaviour and performance despite an unfavorable acetate utilization stoichiometry ($Y_{SAG}<Y_{SA}$) and with a slower rate of PHA storage as compared to PAOs. This unfavorable process stoichiometry and kinetics associated with GAOs is observed to be compensated by a similarly slower endogenous decay rate ($b_{GAO}<b_{PAO}$) as also indicated and supported in the literature.

The resulting experimental data supported the presence of GAOs for all tested HAc/P ratios, especially under P limiting conditions for acetate as sole carbon source.

Strong evidence is observed that acetate added domestic wastewater system had higher PAOs fraction than acetate system as sole source carbon, with using model components, namely substrate uptake, glycogen utilization and P release.

It is recommended that further efforts to improve the existing mechanistic EBPR models relying solely on PAO activity should be devoted to incorporation of at least GAOs and glycogen as model components. For this purpose, additional information on the kinetics of PAOs and GAOs as well as on factors affecting GAO-PAO competition would be required.

In this study, it is concluded that the influent HAc/P is an important parameter that affects the balance between PAO and GAO populations in the EBPR system. On the other hand, the effect of other parameters deserves further investigation.

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# APPENDIXES

# APPENDIX A: DAILY MEASUREMENT DATA

**Table A.2:** Daily measurement data of acetate SBR (Day 12-19)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | Influent | 340 | 19.11 | 22.61 | 0.00 | 0.00 | 21.10 | 13.88 | 36.31 | 45.07 | 172.83 | 185.86 | - | - |
| | Vo | 30 | 3.43 | 0.00 | 9.13 | 0.00 | 19.36 | 10.69 | 32.94 | 42.84 | 182.55 | 185.41 | - | - |
| | Initial | 224 | 13.23 | 14.13 | 3.43 | 0.00 | 20.45 | 12.69 | 35.05 | 44.24 | 176.47 | 185.69 | - | - |
| | Anaerobic End | 23 | 67.37 | 15.35 | 0.00 | 0.00 | 21.81 | 25.98 | 54.41 | 41.46 | 172.64 | 186.18 | - | - |
| | Aerobic End | 23 | 4.81 | 0.00 | 12.22 | 0.00 | 21.16 | 11.41 | 32.70 | 43.51 | 180.71 | 186.73 | 2610 | 2030 |
| | Effluent | 16 | 5.14 | 0.00 | 12.48 | 0.00 | 21.37 | 11.32 | 32.00 | 44.79 | 183.00 | 190.88 | - | - |
| 17 | Influent | 420 | 18.62 | 21.46 | 0.00 | 0.00 | 17.18 | 13.22 | 31.62 | 39.88 | 118.95 | 185.56 | - | - |
| | Vo | 23 | 12.51 | 0.00 | 6.28 | 0.00 | - | 11.81 | 34.79 | 40.13 | 177.16 | 201.69 | - | - |
| | Initial | 271 | 16.33 | 13.41 | 2.35 | 0.00 | - | 12.69 | 32.81 | 39.97 | 140.78 | 191.61 | - | - |
| | Anaerobic End | 58 | 79.96 | 15.11 | 0.00 | 0.00 | 22.40 | 29.21 | 58.90 | 40.47 | 130.62 | 202.61 | - | - |
| | Aerobic End | 16 | 14.01 | 0.00 | 12.85 | 0.00 | 18.63 | 14.54 | 35.50 | 39.46 | 132.61 | 197.95 | 3170 | 2460 |
| | Effluent | 16 | 13.76 | 0.00 | 13.87 | 0.00 | 18.94 | 14.60 | 35.70 | 39.68 | 133.71 | 198.89 | - | - |
| 18 | Influent | - | 18.45 | 20.79 | 0.00 | 0.00 | 18.81 | 12.62 | 33.96 | 40.99 | 119.61 | 192.02 | - | - |
| | Vo | 16 | 3.40 | 0.00 | 6.08 | 0.00 | 21.65 | 10.35 | 32.21 | 40.73 | 192.61 | 266.93 | - | - |
| | Initial | - | 12.81 | 12.99 | 2.28 | 0.00 | 19.87 | 11.77 | 33.30 | 40.89 | 146.99 | 220.11 | - | - |
| | Anaerobic End | - | 80.73 | 14.61 | 0.00 | 0.00 | 22.90 | 27.79 | 56.46 | 40.98 | 159.28 | 233.12 | - | - |
| | Aerobic End | - | 4.41 | 0.00 | 13.89 | 0.00 | 19.91 | 11.03 | 31.82 | 40.95 | 168.02 | 229.90 | 3080 | 2450 |
| | Effluent | 5 | 4.35 | 0.00 | 13.38 | 0.00 | 20.12 | 10.70 | 31.10 | 40.90 | 162.14 | 225.54 | - | - |
| 19 | Influent | 415 | 18.07 | 21.44 | 0.00 | 0.00 | 18.71 | 13.25 | 33.91 | 39.81 | 178.24 | 184.25 | - | - |
| | Vo | 5 | 0.00 | 0.00 | 1.05 | 0.00 | 19.88 | 9.75 | 32.17 | 13.75 | 192.61 | 168.34 | - | - |
| | Initial | 261 | 11.29 | 13.40 | 0.39 | 0.00 | 19.15 | 11.94 | 33.26 | 30.04 | 183.63 | 178.28 | - | - |
| | Anaerobic End | 23 | 87.31 | 15.10 | 0.00 | 0.00 | 20.67 | 29.86 | 62.71 | 39.10 | 186.59 | 198.71 | - | - |
| | Aerobic End | 10 | 0.00 | 0.00 | 12.93 | 0.00 | 20.46 | 9.42 | 30.35 | 39.54 | 190.84 | 193.12 | 3050 | 2310 |
| | Effluent | 10 | 0.00 | 0.00 | 13.66 | 0.00 | 20.26 | 9.40 | 30.34 | 39.20 | 191.12 | 193.71 | - | - |

**Table A.3:** Daily measurement data of acetate SBR (Day 24-35)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | Influent | 395 | 22.92 | 20.42 | 0.00 | 0.00 | 18.01 | 13.12 | 33.38 | 36.52 | 166.85 | 147.58 | - | - |
| | Vo | 16 | 0.00 | 0.00 | 5.36 | 0.00 | 18.36 | 8.79 | 31.49 | 41.85 | 176.29 | 205.54 | - | - |
| | Initial | 253 | 14.33 | 12.77 | 2.01 | 0.00 | 18.14 | 11.50 | 32.67 | 38.51 | 170.39 | 169.32 | - | - |
| | Anaerobic End | 23 | 107.35 | 14.85 | 0.00 | 0.00 | 20.98 | 33.74 | 70.18 | 41.72 | 173.05 | 212.31 | - | - |
| | Aerobic End | 19 | 0.00 | 0.00 | 10.57 | 0.00 | 16.95 | 8.79 | 29.59 | 39.53 | 177.31 | 189.90 | 3070 | 2090 |
| | Effluent | 10 | 0.00 | - | 11.29 | 0.00 | - | - | - | 40.47 | - | 195.02 | - | - |
| 25 | Influent | 400 | 21.61 | 21.26 | 0.00 | 0.00 | 19.00 | 13.91 | 33.05 | 37.44 | 158.08 | 157.89 | - | - |
| | Vo | 19 | 0.00 | 0.00 | 6.05 | 0.00 | 19.50 | 10.23 | 31.83 | 43.70 | 173.60 | 205.59 | - | - |
| | Initial | 257 | 13.51 | 13.29 | 2.27 | 0.00 | 19.19 | 12.53 | 32.59 | 39.79 | 163.90 | 175.78 | - | - |
| | Anaerobic End | 20 | 106.55 | 15.70 | 0.00 | 0.00 | 21.81 | 35.24 | 70.39 | 43.00 | 168.83 | 214.30 | - | - |
| | Aerobic End | 16 | 0.00 | 0.00 | 12.22 | 0.00 | 18.57 | 10.26 | 30.70 | 42.49 | 173.99 | 199.76 | 3780 | 2610 |
| | Effluent | 5 | 0.00 | - | 12.84 | 0.00 | - | - | - | 43.09 | - | 200.52 | - | - |
| 26 | Influent | 372 | 19.69 | 20.66 | 0.00 | 0.00 | 14.12 | 13.34 | 32.82 | 22.05 | 160.96 | 153.77 | - | - |
| | Vo | 20 | 0.00 | 0.00 | 2.58 | 0.00 | 15.31 | 9.01 | 33.24 | 27.38 | 173.28 | 206.04 | - | - |
| | Initial | 240 | 12.31 | 12.91 | 0.97 | 0.00 | 14.56 | 11.72 | 32.97 | 24.05 | 165.58 | 173.37 | - | - |
| | Anaerobic End | 23 | 105.56 | 15.11 | 0.00 | 0.00 | 17.08 | 34.73 | 69.55 | 24.24 | 166.99 | 200.84 | - | - |
| | Aerobic End | 20 | 0.00 | 0.00 | 10.38 | 0.00 | 13.33 | 9.20 | 29.45 | 25.11 | 172.55 | 191.01 | - | - |
| | Effluent | 5 | 0.00 | - | 11.27 | 0.00 | - | - | - | 26.01 | - | 192.10 | - | - |
| 35 | Influent | 403 | 19.92 | 22.19 | 0.00 | 0.00 | 21.04 | 14.24 | 36.26 | 53.71 | 34.83 | 222.11 | - | - |
| | Vo | 20 | 2.67 | 0.00 | 7.07 | 0.00 | 16.39 | 10.81 | 32.58 | 25.84 | 151.68 | 208.86 | - | - |
| | Initial | 259 | 13.45 | 13.87 | 2.65 | 0.00 | 19.29 | 12.96 | 34.88 | 43.26 | 78.65 | 217.14 | - | - |
| | Anaerobic End | 23 | 122.70 | 15.94 | 0.00 | 0.00 | 21.16 | 40.19 | 79.14 | 27.70 | 35.60 | 219.16 | - | - |
| | Aerobic End | 26 | 0.00 | 0.00 | 11.30 | 0.00 | 15.01 | 10.06 | 31.70 | 29.74 | 38.21 | 209.78 | 3750 | 2590 |
| | Effluent | 26 | 0.49 | | 11.41 | 0.00 | - | - | - | 29.11 | - | 206.61 | - | - |

**Table A.4:** Daily measurement data of acetate SBR (Day 41-49)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^+$ mg/L | $Cl^-$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | Influent | 405 | 20.38 | 22.22 | 0.00 | 0.00 | 16.52 | 15.25 | 38.14 | 42.55 | 43.99 | 217.81 | - | - |
| | Vo | 20 | 0.00 | 0.00 | 5.23 | 0.00 | - | 9.65 | 31.12 | 31.51 | 185.51 | 211.55 | - | - |
| | Initial | 261 | 12.74 | 13.89 | 1.96 | 0.00 | - | 13.15 | 35.50 | 38.41 | 97.06 | 215.46 | - | - |
| | Anaerobic End | - | 106.45 | 16.59 | 0.00 | 0.00 | 20.37 | 40.11 | 74.13 | 31.14 | 197.34 | 222.00 | 3540 | 2720 |
| | Aerobic End | - | 0.00 | 0.00 | 8.81 | 0.00 | 17.71 | 9.52 | 32.04 | 32.93 | 197.88 | 212.80 | 3800 | 2590 |
| | Effluent | - | 0.00 | 0.00 | 8.74 | 0.00 | 17.32 | 9.40 | 31.45 | 32.18 | 195.46 | 212.80 | 14 | - |
| 47 | Influent | 398 | 20.68 | 20.95 | 0.00 | 0.00 | 15.80 | 13.99 | 34.16 | 41.69 | 38.19 | 218.47 | - | - |
| | Vo | 23 | 0.00 | 0.00 | 2.96 | 0.56 | 17.54 | 9.78 | 28.88 | 33.39 | 171.56 | 218.81 | 7700* | 5360* |
| | Initial | 257 | 12.92 | 13.09 | 1.11 | 0.21 | 16.45 | 12.41 | 32.18 | 38.58 | 88.21 | 218.59 | - | - |
| | Anaerobic End | 12 | 113.89 | 15.25 | 0.00 | 0.39 | 19.94 | 38.34 | 68.20 | 31.74 | 167.27 | 225.83 | 3000 | 2430 |
| | Aerobic End | 19 | 0.00 | 0.00 | 6.11 | 2.01 | 16.13 | 9.18 | 28.35 | 32.99 | 169.76 | 209.71 | 3410 | 2410 |
| | Effluent | - | 0.00 | - | 7.25 | 1.03 | - | - | - | 33.75 | - | 213.61 | - | - |
| 48 | Influent | 400 | 20.99 | 20.99 | 0.00 | 0.00 | 15.99 | 14.11 | 33.63 | 41.69 | 38.66 | 218.47 | - | - |
| | Vo | 16 | 0.00 | 0.00 | 2.88 | 0.00 | 18.06 | 9.68 | 28.44 | 34.99 | 171.86 | 220.19 | 7160 | 4730 |
| | Initial | 256 | 13.12 | 13.12 | 1.08 | 0.00 | 16.77 | 12.45 | 31.68 | 39.18 | 88.61 | 219.11 | - | - |
| | Anaerobic End | 23 | 115.92 | 15.66 | 0.00 | 0.41 | 20.88 | 39.15 | 69.19 | 33.32 | 169.73 | 235.21 | 2945 | 2180 |
| | Aerobic End | 15 | 0.00 | 0.00 | 7.61 | 0.65 | 16.94 | 9.44 | 28.88 | 34.85 | 172.67 | 219.07 | 3310 | 2240 |
| | Effluent | - | 0.00 | - | 7.38 | 0.62 | - | - | - | 32.54 | - | 210.16 | - | - |
| 49 | Influent | 396 | 20.53 | 21.53 | 0.00 | 0.00 | 15.72 | 14.23 | 35.30 | 40.13 | 40.08 | 236.54 | - | - |
| | Vo | 23 | 0.00 | 0.00 | 3.72 | 0.12 | 17.22 | 9.91 | 29.28 | 30.24 | 181.12 | 234.87 | 8080 | 5460 |
| | Initial | 256 | 12.83 | 13.46 | 1.40 | 0.00 | 16.28 | 12.61 | 33.04 | 36.42 | 92.97 | 235.91 | - | - |
| | Anaerobic End | 23 | 123.10 | 16.39 | 0.00 | 0.00 | 20.87 | 42.20 | 76.27 | 29.68 | 200.97 | 291.42 | 3050 | 2360 |
| | Aerobic End | 27 | 0.00 | 0.00 | 7.04 | 0.00 | 15.84 | 9.34 | 29.40 | 30.08 | 201.94 | 290.52 | 3260 | 2180 |
| | Effluent | - | 0.00 | - | 6.93 | 0.00 | - | - | - | 33.13 | - | 277.61 | - | - |

**Table A.5:** Daily measurement data of acetate SBR (Day 58-77)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 58 | Influent | - | 20.27 | 21.16 | 0.00 | 0.00 | 25.84 | 14.11 | 34.36 | 41.17 | 38.98 | 224.49 | - | - |
| | Anaerobic End | - | 111.50 | 35.19 | 0.00 | 0.00 | 19.19 | 41.69 | 75.37 | 25.25 | 171.65 | 238.55 | 3050 | 2230 |
| | Aerobic End | - | 0.00 | 0.00 | 1.80 | 0.00 | 15.61 | 8.10 | 23.50 | 25.97 | 120.00 | 219.99 | 3650 | 2480 |
| 66 | Anaerobic End | - | - | - | 0.00 | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 5.60 | - | 0.43 | - | - | - | - | - | - | - | - | - |
| 67 | Anaerobic End | - | - | - | 0.00 | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 6.23 | - | 0.47 | - | - | - | - | - | - | - | - | - |
| 68 | Anaerobic End | - | 139.01 | - | 0.00 | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 2.48 | - | 0.44 | - | - | - | - | - | - | - | 4500 | 3000 |
| 69 | Influent | - | 35.05 | - | - | - | - | - | - | - | - | - | - | - |
| | Anaerobic End | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 3.30 | - | 0.25 | - | - | - | - | - | - | - | 4750 | 3040 |
| 70 | Influent | - | 34.74 | - | | - | - | - | - | - | - | - | - | - |
| | Anaerobic End | - | 150.37 | - | 0.00 | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 3.35 | - | 0.49 | - | - | - | - | - | - | - | 4525 | 2850 |
| 74 | Influent | | 35.74 | 15.52 | 0.00 | 0.00 | 13.08 | 15.92 | 54.99 | 15.99 | 45.40 | 157.93 | - | - |
| | Vo | 17 | 4.78 | 0.00 | 0.21 | 0.00 | 22.47 | 11.66 | 52.70 | 42.95 | 198.56 | 152.65 | 11050 | 6850 |
| | Initial | | 24.13 | 9.70 | 0.08 | 0.00 | 16.60 | 14.32 | 54.13 | 26.10 | 102.83 | 155.95 | - | - |
| | Anaerobic End | 21 | 140.00 | 10.55 | 0.00 | 0.00 | 30.83 | 41.46 | 95.28 | 45.40 | 195.27 | 150.26 | 3750 | 2480 |
| | Aerobic End | 15 | 2.99 | 0.00 | 0.53 | 0.00 | 18.54 | 9.46 | 47.47 | 44.91 | 194.28 | 148.12 | 4560 | 2780 |
| | Effluent | 18 | 2.72 | 0.00 | 0.49 | 0.00 | 18.24 | 9.46 | 47.31 | 44.70 | 196.10 | 149.75 | - | - |
| 76 | Influent | - | 45.18 | 21.24 | 0.00 | 0.00 | 9.60 | 25.83 | 76.02 | 14.31 | 82.34 | 234.62 | - | - |
| | Anaerobic End | - | 145.09 | 339.22 | 0.00 | 0.62 | 31.52 | 74.85 | 120.56 | 50.84 | 370.99 | 235.97 | - | - |
| | Aerobic End | - | 6.96 | 338.44 | 3.21 | 0.39 | 18.29 | 12.34 | 67.52 | 52.13 | 370.14 | 232.27 | - | - |
| 77 | Influent | - | 44.76 | 21.67 | 0.00 | 0.00 | 8.58 | 24.45 | 76.04 | 14.54 | 83.93 | 238.83 | - | - |
| | Anaerobic End | - | 146.51 | 328.05 | 0.00 | 0.55 | 28.91 | 78.18 | 119.94 | 46.09 | 358.77 | 227.39 | - | - |
| | Aerobic End | - | 15.29 | 335.60 | 3.68 | 0.40 | 15.36 | 14.70 | 68.74 | 47.75 | 367.03 | 227.56 | - | - |

**Table A.6:** Daily measurement data of acetate SBR (Day 78-97)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 78 | Influent | - | 44.82 | 21.34 | 0.00 | 0.00 | 10.38 | 27.18 | 75.79 | 14.78 | 82.76 | 237.93 | - | - |
| | Anaerobic End | - | 132.52 | 330.97 | 0.00 | 0.72 | 29.60 | 81.39 | 121.73 | 46.35 | 361.97 | 236.94 | - | - |
| | Aerobic End | - | 12.93 | 327.58 | 3.49 | 0.40 | 15.70 | 14.87 | 67.25 | 45.71 | 358.26 | 233.02 | - | - |
| 81 | Influent | - | 44.69 | 21.53 | 0.33 | 0.00 | 12.15 | 14.39 | 77.36 | 6.09 | 43.99 | 143.33 | - | - |
| | Vo | 16 | 10.14 | 0.00 | 2.11 | 0.00 | 21.63 | 9.61 | 74.47 | 19.69 | 192.41 | 138.25 | 11300 | 6600 |
| | Initial | - | 31.73 | 13.45 | 1.00 | 0.00 | 15.71 | 12.60 | 76.28 | 11.19 | 99.65 | 141.43 | - | - |
| | Anaerobic End | 20 | 157.30 | 15.23 | 0.00 | 0.60 | 31.50 | 40.65 | 120.90 | 19.38 | 191.59 | 158.73 | 3450 | 2410 |
| | Aerobic End | 16 | 9.61 | 0.00 | 3.10 | 0.56 | 17.60 | 7.01 | 65.80 | 20.04 | 190.53 | 141.73 | 4500 | 2640 |
| | Effluent | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 84 | Influent | - | 61.84 | - | 0.31 | 0.00 | - | - | - | 12.78 | - | 193.88 | - | - |
| | Aerobic End | - | 12.07 | - | 4.04 | 0.00 | - | - | - | 39.46 | - | 186.60 | - | - |
| 85 | Influent | - | 62.91 | - | 0.31 | 0.00 | - | - | - | 12.18 | - | 196.48 | - | - |
| | Aerobic End | - | 7.13 | - | 3.91 | 0.00 | - | - | - | 39.57 | - | 193.11 | - | - |
| 86 | Influent | - | 59.53 | - | 0.32 | 0.00 | - | - | - | 11.26 | - | 183.40 | - | - |
| | Aerobic End | - | 7.53 | - | 3.12 | 0.00 | - | - | - | 39.34 | - | 180.74 | - | - |
| 88 | Influent | - | 60.62 | 22.08 | 0.00 | 0.00 | 27.83 | 14.14 | 101.75 | 40.54 | 44.78 | 166.95 | - | - |
| | Vo | 24 | 8.18 | 0.00 | 4.53 | 0.00 | 16.66 | 7.02 | 90.46 | 41.25 | 161.26 | 163.23 | 12800 | 6930 |
| | Initial | - | 40.96 | 13.80 | 1.70 | 0.00 | 23.64 | 11.47 | 97.51 | 40.81 | 88.46 | 165.55 | - | - |
| | Anaerobic End | 26 | 172.86 | 15.63 | 0.00 | 0.00 | 33.06 | 29.35 | 146.60 | 40.96 | 157.45 | 170.66 | 3730 | 2410 |
| | Aerobic End | 18 | 8.35 | 0.00 | 3.80 | 0.00 | 16.95 | 5.55 | 81.90 | 39.30 | 151.27 | 157.43 | 3370 | 1800 |
| | Effluent | - | 8.54 | 0.00 | 4.02 | 0.00 | 16.99 | 5.60 | 85.58 | 40.96 | 159.20 | 163.29 | - | - |
| 97 | Vo | - | - | 0.00 | - | - | 25.34 | 3.69 | 103.22 | - | 180.90 | - | - | - |
| | Anaerobic End | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 14.04 | 0.00 | - | - | 19.40 | 2.12 | 96.58 | - | 179.02 | - | - | - |

**Table A.7:** Daily measurement data of acetate SBR (Day 99-102)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99 | Influent | - | 61.21 | 25.66 | 0.00 | 0.00 | 12.78 | 14.88 | 112.36 | 15.81 | 41.66 | 200.68 | - | - |
| | Vo | - | 31.20 | 0.00 | 1.88 | 0.00 | 22.87 | 5.27 | 102.61 | 44.19 | 178.46 | 222.31 | - | - |
| | Initial | - | 49.96 | 16.04 | 0.71 | 0.00 | 16.56 | 11.28 | 108.70 | 26.45 | 92.96 | 208.79 | - | - |
| | Anaerobic End | - | 176.92 | 15.84 | 0.00 | 1.22 | 37.89 | 34.80 | 154.44 | 43.41 | 173.00 | 229.13 | 4000 | 2245 |
| | 150' | - | 161.90 | - | 0.09 | 1.03 | - | - | - | 43.09 | - | 229.20 | 4200 | 2350 |
| | 180' | - | 126.97 | 10.27 | 0.47 | 1.03 | 21.51 | 18.96 | 126.88 | 42.24 | 184.38 | 235.03 | 4460 | 2475 |
| | 210' | - | 83.57 | 6.11 | 0.66 | 1.52 | 20.14 | 12.50 | 110.48 | 41.68 | 181.21 | 225.16 | 4660 | 2550 |
| | 240' | - | 59.19 | 2.12 | 1.72 | 1.38 | 18.86 | 7.66 | 100.15 | 43.11 | 178.91 | 223.07 | 4430 | 2340 |
| | Aerobic End | - | 11.26 | 0.00 | 2.08 | 0.00 | 18.03 | 3.18 | 90.47 | 44.02 | 180.42 | 223.57 | 4840 | 2575 |
| | Effluent | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 100 | Influent | - | 59.66 | 26.37 | 0.00 | 0.00 | 13.08 | 15.71 | 116.63 | 13.93 | 46.36 | 222.49 | - | - |
| | Vo | - | 38.00 | 0.00 | 1.25 | 0.00 | 26.06 | 5.90 | 102.38 | 44.24 | 177.29 | 217.24 | - | - |
| | Initial | - | 51.54 | 16.48 | 0.47 | 0.00 | 17.95 | 12.03 | 111.28 | 25.29 | 95.46 | 220.52 | - | - |
| | Anaerobic End | - | 170.48 | 8.27 | 0.00 | 0.00 | 30.30 | 28.34 | 137.09 | 44.88 | 163.14 | 222.93 | - | - |
| | Aerobic End | - | 15.11 | 0.00 | 2.03 | 0.00 | 19.01 | 3.71 | 94.44 | 44.51 | 176.97 | 217.78 | 5860 | 3050 |
| | Effluent | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 101 | Influent | - | 60.27 | 26.46 | 0.00 | 0.00 | 13.50 | 15.42 | 116.45 | 18.72 | 46.93 | 238.18 | - | - |
| | Vo | - | 22.59 | 0.00 | 2.07 | 0.00 | 18.00 | 4.19 | 96.40 | 48.29 | 177.81 | 214.61 | - | - |
| | Initial | - | 46.14 | 16.54 | 0.77 | 0.00 | 15.19 | 11.21 | 108.93 | 29.81 | 96.01 | 229.34 | - | - |
| | Anaerobic End | - | 172.36 | 13.67 | 0.00 | 1.27 | 34.29 | 32.47 | 150.41 | 43.19 | 164.91 | 217.50 | 6380 | 3650 |
| | Aerobic End | - | 13.20 | 0.00 | 1.56 | 0.00 | 17.76 | 3.07 | 92.78 | 45.81 | 174.80 | 210.81 | 6700 | 3400 |
| | Effluent | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 102 | Influent | - | 60.60 | 22.20 | 0.00 | 0.00 | 25.31 | 13.45 | 106.14 | 48.25 | 41.93 | 207.99 | - | - |
| | Vo | 21 | 9.84 | 0.00 | 2.32 | 0.00 | 16.78 | 3.31 | 91.17 | 48.54 | 173.10 | 221.20 | 22240 | 11760 |
| | Initial | - | 41.57 | 13.87 | 0.87 | 0.00 | 22.11 | 9.65 | 100.53 | 48.36 | 91.12 | 212.94 | - | - |
| | Anaerobic End | 22 | 167.29 | 15.91 | 0.00 | 0.95 | 38.04 | 35.63 | 157.17 | 45.65 | 170.96 | 228.05 | 4580 | 2560 |
| | Aerobic End | 21 | 11.54 | 0.00 | 1.83 | 0.00 | 19.82 | 2.12 | 86.55 | 48.30 | 169.44 | 212.08 | 7990 | 4050 |
| | Effluent | 21 | 11.18 | 0.00 | 1.92 | 0.00 | 19.42 | 2.04 | 86.68 | 47.93 | 170.79 | 211.60 | - | - |

**Table A.8:** Daily measurement data of propionate SBR (Day 1-11)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^+$ mg/L | $Cl^-$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | START-UP | | | | | | | | | | | | | |
| 3 | Influent | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Vo | - | 13.69 | 0.00 | 0.00 | 0.00 | 21.41 | 12.76 | 35.03 | 91.19 | 159.42 | 92.67 | - | - |
| | Anaerobic End | - | 30.57 | 9.68 | 0.00 | 0.00 | 24.68 | 17.30 | 42.22 | 98.01 | 127.91 | 104.23 | - | - |
| | Aerobic End | - | 13.21 | 0.00 | 1.01 | 0.00 | 22.41 | 12.72 | 35.86 | 89.91 | 125.34 | 99.89 | 1930 | 1620 |
| | Effluent | - | 12.90 | 0.00 | 0.90 | 0.00 | 22.83 | 13.07 | 36.60 | 92.07 | 128.74 | 102.55 | - | - |
| 4 | Influent | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Vo | - | 13.42 | 0.00 | 0.00 | 0.00 | 23.44 | 13.89 | 37.62 | 93.48 | 167.89 | 57.01 | - | - |
| | Anaerobic End | - | 31.05 | 9.27 | 0.00 | 0.00 | 24.10 | 17.61 | 42.01 | 98.43 | 126.15 | 59.68 | - | - |
| | Aerobic End | - | 12.04 | 0.00 | 0.00 | 0.00 | 22.20 | 13.41 | 35.99 | 91.41 | 126.98 | 55.74 | 1970 | 1590 |
| | Effluent | - | 13.28 | 0.00 | 0.00 | 0.00 | 21.96 | 13.12 | 35.86 | 91.43 | 127.57 | 56.69 | - | - |
| 5 | Influent | - | 13.34 | 23.01 | 0.17 | 0.00 | 22.01 | 11.40 | 38.90 | 41.23 | 125.33 | 240.90 | - | - |
| | Vo | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Anaerobic End | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Effluent | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 7 | Influent | - | 17.85 | 21.79 | 0.00 | 0.00 | 19.63 | 13.81 | 34.76 | 52.51 | 154.20 | 178.74 | - | - |
| | Vo | - | 13.79 | 0.00 | 0.00 | 0.00 | 19.42 | 13.44 | 32.98 | 36.30 | 136.25 | 173.69 | - | - |
| | Initial | - | 16.33 | 13.62 | 0.00 | 0.00 | 19.55 | 13.67 | 34.09 | 46.43 | 147.47 | 176.85 | - | - |
| | Anaerobic End | - | 52.15 | 15.82 | 0.00 | 0.00 | 21.76 | 22.62 | 45.52 | 45.67 | 153.19 | 184.82 | - | - |
| | Aerobic End | - | 12.29 | 0.00 | 9.18 | 0.00 | 19.75 | 13.93 | 33.19 | 38.48 | 155.64 | 182.60 | 1840 | 1530 |
| | Effluent | - | 10.10 | 0.00 | 7.81 | 0.00 | 18.40 | 12.31 | 28.32 | 34.88 | 134.98 | 158.24 | - | - |
| 11 | Influent | 340 | 18.32 | 22.22 | 0.00 | 0.00 | 20.93 | 13.54 | 35.86 | 52.66 | 174.45 | 183.59 | - | - |
| | Vo | 34 | 6.61 | 0.00 | 0.00 | 0.00 | 18.54 | 10.97 | 18.95 | 29.32 | 106.74 | 116.44 | - | - |
| | Initial | 225 | 13.93 | 13.89 | 0.00 | 0.00 | 20.03 | 12.57 | 29.52 | 43.91 | 149.06 | 158.41 | - | - |
| | Anaerobic End | 23 | 70.61 | 15.42 | 0.00 | 0.00 | 21.43 | 27.14 | 49.61 | 36.95 | 152.97 | 164.06 | 2840 | 2210 |
| | Aerobic End | 20 | 3.73 | 0.00 | 12.22 | 0.00 | 19.72 | 9.59 | 24.86 | 37.21 | 117.17 | 155.96 | - | - |
| | Effluent | 16 | 2.88 | 0.00 | 12.82 | 0.00 | 20.21 | 11.11 | 27.10 | - | - | - | - | - |

**Table A.9:** Daily measurement data of propionate SBR (Day 12-19)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | Influent | 350 | 18.94 | 22.07 | 0.00 | 0.00 | 21.13 | 13.85 | 36.01 | 53.55 | 119.65 | 185.10 | - | - |
| | Vo | 30 | 0.00 | 0.00 | 4.08 | 0.00 | 21.94 | 9.92 | 28.20 | 42.43 | 122.49 | 179.35 | - | - |
| | Initial | 230 | 11.84 | 13.79 | 1.53 | 0.00 | 21.43 | 12.38 | 33.08 | 49.38 | 120.72 | 182.94 | - | - |
| | Anaerobic End | 30 | 82.28 | 15.54 | 0.00 | 0.00 | 24.13 | 30.20 | 56.89 | 43.13 | 121.38 | 186.90 | - | - |
| | Aerobic End | 20 | 0.00 | 0.00 | 13.51 | 0.00 | 21.65 | 9.85 | 31.24 | 43.97 | 125.65 | 184.61 | 3950 | 2980 |
| | Effluent | 23 | 0.00 | 0.00 | 13.71 | 0.00 | 21.35 | 9.76 | 30.41 | 44.67 | 126.24 | 187.84 | - | - |
| 17 | Influent | - | 18.57 | 21.41 | 0.00 | 0.00 | 18.96 | 13.79 | 34.83 | 39.83 | 190.45 | 231.29 | - | - |
| | Vo | 23 | 1.29 | 0.00 | 0.44 | 0.00 | 19.70 | 10.71 | 26.60 | 3.12 | 188.61 | 160.61 | - | - |
| | Initial | - | 12.09 | 13.38 | 0.16 | 0.00 | 19.24 | 12.63 | 31.75 | 26.06 | 189.76 | 204.79 | - | - |
| | Anaerobic End | 23 | 120.02 | 15.67 | 0.00 | 0.00 | 22.26 | 39.64 | 69.63 | 38.52 | 196.65 | 247.66 | - | - |
| | Aerobic End | 16 | 13.91 | 0.00 | 12.70 | 0.00 | 19.33 | 10.68 | 29.79 | 39.43 | 201.57 | 198.05 | 3450 | 2430 |
| | Effluent | 10 | 2.28 | 0.00 | 12.90 | 0.00 | 19.05 | 10.67 | 30.11 | 39.82 | 198.04 | 118.20 | - | - |
| 18 | Influent | 405 | 18.21 | 20.20 | 0.00 | 0.00 | 18.58 | 12.55 | 33.63 | 39.99 | 202.17 | 252.14 | - | - |
| | Vo | 23 | 0.00 | 0.00 | 3.33 | 0.00 | 20.54 | 10.12 | 26.21 | 40.57 | 133.11 | 206.62 | - | - |
| | Initial | 262 | 11.38 | 12.62 | 1.25 | 0.00 | 19.32 | 11.64 | 30.85 | 40.21 | 176.27 | 235.07 | - | - |
| | Anaerobic End | 30 | 118.37 | 14.77 | 0.00 | 0.00 | 22.58 | 39.73 | 69.67 | 38.41 | 177.16 | 241.36 | - | - |
| | Aerobic End | 23 | 0.00 | 0.00 | 4.52 | 0.00 | 19.27 | 9.67 | 29.62 | 38.80 | 181.21 | 231.77 | 3470 | 2490 |
| | Effluent | 12 | 0.00 | 0.00 | 5.62 | 0.00 | 18.97 | 9.59 | 29.37 | 39.05 | 178.65 | 233.08 | - | - |
| 19 | Influent | 370 | 18.22 | 20.50 | 0.00 | 0.00 | 18.34 | 13.01 | 33.59 | 40.52 | 123.86 | 209.53 | - | - |
| | Vo | 5 | 1.42 | 0.00 | 1.74 | 0.00 | 20.35 | 10.65 | 26.43 | 40.01 | 135.09 | 220.34 | - | - |
| | Initial | 233 | 11.92 | 12.81 | 0.65 | 0.00 | 19.09 | 12.13 | 30.91 | 40.33 | 128.08 | 213.58 | - | - |
| | Anaerobic End | 10 | 91.70 | 15.27 | 0.00 | 0.00 | 21.96 | 32.05 | 59.72 | 39.88 | 130.91 | 221.45 | - | - |
| | Aerobic End | 5 | 0.00 | 0.00 | 7.72 | 0.00 | 19.03 | 9.75 | 29.81 | 39.86 | 131.84 | 211.95 | 3590 | 2540 |
| | Effluent | 5 | 0.00 | 0.00 | 7.84 | 0.00 | 18.05 | 9.28 | 28.39 | 39.70 | 132.31 | 214.61 | - | - |

**Table A.10:** Daily measurement data of propionate SBR (Day 24-35).

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^+$ mg/L | $Cl^-$ mg/L | $Na^{2+}$ mg/L | $SO4^=$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | Influent | 404 | 23.34 | 21.56 | 0.00 | 0.00 | 18.42 | 13.13 | 32.55 | 51.05 | 112.72 | 158.43 | - | - |
| | Vo | 16 | 0.00 | 0.00 | 1.01 | 0.00 | 18.61 | 10.76 | 22.70 | 43.89 | 119.25 | 204.95 | - | - |
| | Initial | 259 | 14.59 | 13.48 | 0.38 | 0.00 | 18.49 | 12.24 | 28.85 | 48.37 | 115.17 | 175.88 | - | - |
| | Anaerobic End | 10 | 93.94 | 15.14 | 0.00 | 0.00 | 21.18 | 32.41 | 58.03 | 41.99 | 117.87 | 211.95 | - | - |
| | Aerobic End | 10 | 0.00 | 0.00 | 3.93 | 0.00 | 17.19 | 9.29 | 28.69 | 41.91 | 119.19 | 192.85 | 3700 | 2580 |
| | Effluent | 5 | 0.00 | - | 4.55 | 0.00 | - | - | - | 41.98 | - | 193.37 | - | - |
| 25 | Influent | 382 | 21.43 | 20.10 | 0.00 | 0.00 | 19.33 | 14.16 | 33.46 | 50.54 | 110.83 | 158.00 | - | - |
| | Vo | 19 | 0.00 | 0.00 | 1.53 | 0.00 | 19.58 | 11.42 | 23.06 | 44.27 | 119.74 | 205.30 | - | - |
| | Initial | 246 | 13.39 | 12.56 | 0.57 | 0.00 | 19.43 | 13.14 | 29.56 | 48.19 | 114.18 | 175.74 | - | - |
| | Anaerobic End | 10 | 96.27 | 15.45 | 0.00 | 0.00 | 21.88 | 33.81 | 58.60 | 43.21 | 116.75 | 215.29 | - | - |
| | Aerobic End | 10 | 0.00 | 0.00 | 4.42 | 0.00 | 17.98 | 9.93 | 28.84 | 42.41 | 118.36 | 195.65 | 4560 | 3270 |
| | Effluent | 10 | 0.00 | - | 4.92 | 0.00 | - | - | - | 42.21 | - | 195.15 | - | - |
| 26 | Influent | 402 | 19.73 | 20.27 | 0.00 | 0.00 | 13.36 | 12.87 | 32.15 | 39.13 | 109.08 | 153.55 | - | - |
| | Vo | 19 | 0.00 | 0.00 | 0.00 | 0.00 | 15.42 | 10.19 | 24.40 | 27.55 | 118.29 | 205.77 | - | - |
| | Initial | 258 | 12.33 | 12.67 | 0.00 | 0.00 | 14.13 | 11.86 | 29.24 | 34.79 | 112.53 | 173.13 | - | - |
| | Anaerobic End | 10 | 94.74 | 15.30 | 0.00 | 0.00 | 17.20 | 32.90 | 58.90 | 24.86 | 118.45 | 207.60 | - | - |
| | Aerobic End | 10 | 0.00 | 0.00 | 3.60 | 0.00 | 13.94 | 9.20 | 29.14 | 25.43 | 120.13 | 193.04 | 3560 | 2490 |
| | Effluent | 16 | 0.00 | - | 3.95 | 0.00 | - | - | - | 26.18 | - | 194.53 | - | - |
| 35 | Influent | 402 | 19.92 | 22.19 | 0.00 | 0.00 | 21.04 | 14.24 | 36.26 | 53.71 | 34.83 | 222.11 | - | - |
| | Vo | 20 | 4.49 | 0.00 | 1.33 | 0.00 | 16.71 | 11.82 | 26.66 | 24.72 | 31.77 | 212.11 | - | - |
| | Initial | 259 | 14.13 | 13.87 | 0.50 | 0.00 | 19.42 | 13.34 | 32.66 | 42.84 | 33.68 | 218.36 | - | - |
| | Anaerobic End | 15 | 98.30 | 15.27 | 0.00 | 0.00 | 20.28 | 35.02 | 64.07 | 26.70 | 34.31 | 219.03 | - | - |
| | Aerobic End | 23 | 0.00 | 0.00 | 3.41 | 0.00 | 15.56 | 9.89 | 30.94 | 26.82 | 34.47 | 208.43 | 4640 | 3200 |
| | Effluent | - | 0.00 | 0.00 | 3.55 | 0.00 | - | - | - | 26.70 | - | 211.77 | - | - |

**Table A.11:** Daily measurement data of propionate SBR (Day 41-49)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | Influent | 412 | 20.38 | 22.22 | 0.00 | 0.00 | 16.52 | 15.25 | 38.14 | 42.55 | 43.99 | 217.81 | - | - |
| | Vo | 24 | 0.00 | 0.00 | 3.65 | 0.00 | 18.86 | 10.08 | 25.57 | 30.27 | 151.68 | 208.86 | - | - |
| | Initial | 267 | 12.74 | 13.89 | 1.37 | 0.00 | 17.40 | 13.31 | 33.42 | 37.95 | 84.37 | 214.46 | - | - |
| | Anaerobic End | - | 85.23 | 16.29 | 0.00 | 0.00 | 22.86 | 35.83 | 40.72 | 31.76 | 135.90 | 219.16 | 4860 | 3680 |
| | Aerobic End | - | 0.00 | 0.00 | 5.93 | 0.00 | 17.10 | 9.56 | 31.64 | 31.24 | 134.48 | 209.78 | 4610 | 3290 |
| | Effluent | - | 0.00 | 0.00 | 5.77 | 0.00 | 17.08 | 9.26 | 30.46 | 30.73 | 130.62 | 206.61 | - | - |
| 47 | Influent | 415 | 20.68 | 20.95 | 0.00 | 0.00 | 15.80 | 13.99 | 34.16 | 41.69 | 38.19 | 218.47 | - | - |
| | Vo | 23 | 0.00 | 0.00 | 2.96 | 0.56 | 17.54 | 9.78 | 28.88 | 33.39 | 171.56 | 218.81 | 11120* | 7800* |
| | Initial | 268 | 12.92 | 13.09 | 1.11 | 0.00 | 16.45 | 12.41 | 32.18 | 38.58 | 88.21 | 218.59 | - | - |
| | Anaerobic End | 12 | 92.44 | 15.25 | 0.00 | 0.39 | 19.94 | 38.34 | 68.20 | 31.74 | 167.27 | 225.83 | 4910 | 3680 |
| | Aerobic End | 19 | 0.00 | 0.00 | 6.11 | 2.01 | 16.13 | 9.18 | 28.35 | 32.99 | 169.76 | 209.71 | 4710 | 3350 |
| | Effluent | - | 0.00 | - | 7.25 | 1.03 | - | - | - | 33.75 | - | 213.61 | - | - |
| 48 | Influent | 419 | 20.99 | 20.99 | 0.00 | 0.00 | 15.99 | 14.11 | 33.63 | 41.69 | 38.66 | 218.47 | - | - |
| | Vo | 19 | 0.00 | 0.00 | 1.03 | 0.45 | 17.97 | 10.90 | 21.85 | 34.70 | 123.50 | 222.10 | 12780 | 8550 |
| | Initial | 269 | 13.12 | 13.12 | 0.38 | 0.00 | 16.74 | 12.91 | 29.21 | 39.07 | 70.48 | 219.83 | - | - |
| | Anaerobic End | 16 | 97.21 | 15.31 | 0.00 | 0.47 | 20.09 | 33.14 | 55.94 | 32.54 | 123.24 | 232.72 | 3945 | 2880 |
| | Aerobic End | 26 | 0.00 | 0.00 | 5.89 | 0.48 | 15.89 | 9.39 | 27.85 | 33.56 | 122.49 | 224.66 | 4280 | 2850 |
| | Effluent | - | 0.00 | - | 5.59 | 0.36 | - | - | - | 33.42 | - | 221.86 | - | - |
| 49 | Influent | 396 | 20.53 | 21.53 | 0.00 | 0.00 | 15.72 | 14.23 | 35.30 | 40.13 | 40.08 | 236.54 | - | - |
| | Vo | 19 | 0.00 | 0.00 | 1.99 | 0.00 | 16.79 | 10.70 | 22.12 | 30.30 | 128.84 | 237.32 | 12110 | 8580 |
| | Initial | 255 | 12.83 | 13.46 | 0.75 | 0.00 | 16.12 | 12.90 | 30.36 | 36.44 | 73.37 | 236.83 | - | - |
| | Anaerobic End | 19 | 109.90 | 15.84 | 0.00 | 0.00 | 19.72 | 35.20 | 62.31 | 29.16 | 141.84 | 246.04 | 4180 | 3220 |
| | Aerobic End | 19 | 0.00 | 0.00 | 6.07 | 0.00 | 14.83 | 8.97 | 28.40 | 28.81 | 124.38 | 230.76 | 4280 | 2900 |
| | Effluent | - | 0.00 | - | 5.90 | 0.00 | - | - | - | 33.51 | - | 224.63 | - | - |

**Table A.12:** Daily measurement data of propionate SBR (Day 58-77)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 58 | Influent | | 20.27 | 21.16 | 0.00 | 0.00 | 25.84 | 14.11 | 34.36 | 41.17 | 38.98 | 224.49 | - | - |
| | Anaerobic End | | 86.80 | 23.87 | 0.00 | 0.00 | 16.23 | 22.32 | 45.18 | 26.21 | 120.77 | 229.01 | 3160 | 2340 |
| | Aerobic End | | 0.00 | 0.00 | 0.35 | 0.00 | 16.06 | 8.50 | 24.40 | 23.02 | 121.43 | 196.09 | 3690 | 2520 |
| 66 | Anaerobic End | - | 122.24 | - | 0.00 | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 0.00 | - | 0.00 | - | - | - | - | - | - | - | - | - |
| 67 | Anaerobic End | - | - | - | | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 0.00 | - | 0.00 | - | - | - | - | - | - | - | - | - |
| 68 | Anaerobic End | - | 112.60 | - | 0.00 | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 0.00 | - | 0.00 | - | - | - | - | - | - | - | 4450 | 3030 |
| 69 | Influent | - | 35.05 | - | | - | - | - | - | - | - | - | - | - |
| | Anaerobic End | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 0.00 | - | 0.00 | - | - | - | - | - | - | - | 4990 | 3280 |
| 70 | Influent | - | 34.74 | - | | - | - | - | - | - | - | - | - | - |
| | Anaerobic End | - | 122.06 | - | 0.00 | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 0.00 | - | 0.00 | - | - | - | - | - | - | - | 4980 | 3190 |
| 74 | Influent | | 35.74 | 15.52 | 0.00 | 0.00 | 13.08 | 15.92 | 54.99 | 15.99 | 45.40 | 157.93 | - | - |
| | Vo | 21 | 0.00 | 0.00 | 0.00 | 0.00 | 26.44 | 8.63 | 38.55 | 42.48 | 138.85 | 149.01 | 12320 | 7870 |
| | Initial | | 22.34 | 9.70 | 0.00 | 0.00 | 18.09 | 13.19 | 48.82 | 25.92 | 80.45 | 154.59 | - | - |
| | Anaerobic End | 17 | 119.94 | 9.65 | 0.00 | 0.00 | 29.94 | 35.02 | 76.26 | 47.29 | 135.97 | 149.37 | 4660 | 2940 |
| | Aerobic End | 8 | 0.00 | 0.00 | 0.39 | 0.00 | 23.76 | 8.22 | 44.81 | 46.59 | 137.52 | 148.41 | 4670 | 3130 |
| | Effluent | 12 | 0.00 | 0.00 | 0.34 | 0.00 | 23.66 | 7.49 | 45.04 | 46.23 | 139.10 | 148.13 | - | - |
| 76 | Influent | - | 45.18 | 21.24 | 0.00 | 0.00 | 9.60 | 25.83 | 76.02 | 14.31 | 82.34 | 234.62 | - | - |
| | Anaerobic End | - | 116.87 | 239.48 | 0.00 | 0.60 | 29.70 | 62.81 | 101.05 | 51.19 | 261.91 | 234.29 | - | - |
| | Aerobic End | - | 0.00 | 236.59 | 1.87 | 1.55 | 22.70 | 5.74 | 62.82 | 50.45 | 258.75 | 224.91 | - | - |
| 77 | Influent | - | 44.76 | 21.67 | 0.00 | 0.00 | 8.58 | 24.45 | 76.04 | 14.54 | 83.93 | 238.83 | - | - |
| | Anaerobic End | - | 116.83 | 243.22 | 0.00 | 0.54 | 28.20 | 62.24 | 103.83 | 49.05 | 266.00 | 231.88 | - | - |
| | Aerobic End | - | 0.00 | 235.65 | 1.96 | 3.52 | 20.28 | 1.88 | 55.48 | 51.59 | 257.72 | 220.81 | - | - |

**Table A.13:** Daily measurement data of propionate SBR (Day 78-97)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 78 | Influent | - | 44.82 | 21.34 | 0.00 | 0.00 | 10.38 | 27.18 | 75.79 | 14.78 | 82.76 | 237.93 | - | - |
| | Anaerobic End | - | 120.51 | 250.07 | 0.00 | 0.51 | 29.44 | 64.74 | 105.28 | 46.90 | 273.49 | 235.75 | - | - |
| | 2h 25` | | 0.00 | 239.24 | 1.71 | 2.68 | 19.73 | 5.40 | 60.01 | 49.05 | 261.64 | 223.32 | | |
| | Aerobic End | - | 0.00 | 239.24 | 1.71 | 2.68 | 19.73 | 5.40 | 60.01 | 49.05 | 261.64 | 223.32 | - | - |
| 81 | Influent | - | 44.69 | 21.53 | 0.33 | 0.00 | 12.15 | 14.39 | 77.36 | 6.09 | 43.99 | 143.33 | - | - |
| | Vo | 30 | 0.00 | 0.00 | 0.00 | 0.00 | 24.51 | 4.64 | 62.35 | 17.52 | 140.50 | 130.43 | 4690 | 2640 |
| | Initial | - | 27.93 | 13.45 | 0.21 | 0.00 | 16.79 | 10.73 | 71.73 | 10.38 | 80.18 | 138.49 | - | - |
| | Anaerobic End | 30 | 132.66 | 15.29 | 0.00 | 0.53 | 30.93 | 32.98 | 106.76 | 19.11 | 139.60 | 150.30 | 4300 | 2600 |
| | Aerobic End | 23 | 0.00 | 0.00 | 2.65 | 3.25 | 21.51 | 3.09 | 63.41 | 19.58 | 138.00 | 133.78 | 5400 | 2900 |
| | Effluent | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Release | - | 104.73 | - | - | - | 14.14 | 22.24 | 35.03 | - | - | - | - | - |
| | Uptake | - | 132.66 | - | - | - | 9.41 | 29.89 | 43.35 | - | - | - | - | - |
| | upt/rel | - | 1.27 | - | - | - | 0.67 | 1.34 | 1.24 | - | - | - | - | - |
| 84 | Influent | - | 61.84 | - | 0.31 | 0.00 | - | - | - | 12.78 | - | 193.88 | - | - |
| | Aerobic End | - | 0.00 | - | 3.19 | 3.00 | - | - | - | 41.18 | - | 183.78 | - | - |
| 85 | Influent | - | 62.91 | - | 0.31 | 0.00 | - | - | - | 12.18 | - | 196.48 | - | - |
| | Aerobic End | - | 0.00 | - | 3.39 | 2.88 | - | - | - | 41.10 | - | 183.45 | - | - |
| 86 | Influent | - | 59.53 | - | 0.32 | 0.00 | - | - | - | 11.26 | - | 183.40 | - | - |
| | Aerobic End | - | 0.00 | - | 3.09 | 1.96 | - | - | - | 39.74 | - | 171.47 | - | - |
| 88 | Influent | - | 60.62 | 22.08 | 0.00 | 0.00 | 27.83 | 14.14 | 101.75 | 40.54 | 44.78 | 166.95 | - | - |
| | Vo | 24 | 0.32 | 0.00 | 3.99 | 2.84 | 19.03 | 2.94 | 85.08 | 44.84 | 116.22 | 159.31 | 14880 | 7780 |
| | Initial | - | 38.01 | 13.80 | 1.49 | 0.00 | 24.53 | 9.94 | 95.50 | 42.15 | 71.57 | 164.08 | - | - |
| | Anaerobic End | 23 | 149.41 | 15.69 | 0.00 | 0.00 | 35.16 | 25.77 | 136.86 | 44.18 | 114.38 | 166.66 | 4750 | 2490 |
| | Aerobic End | 21 | 0.00 | 0.00 | 4.56 | 2.77 | 18.82 | 2.45 | 82.80 | 43.10 | 114.00 | 159.96 | 5740 | 2820 |
| | Effluent | - | 0.00 | 0.00 | 4.68 | 2.68 | 18.53 | 2.47 | 81.97 | 45.96 | 113.79 | 159.03 | - | - |
| 97 | Vo | - | 52.58 | 0.00 | - | - | 36.25 | 3.31 | 112.72 | - | 155.65 | - | - | - |
| | Anaerobic End | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Aerobic End | - | 10.24 | 0.00 | - | - | 21.91 | 0.79 | 96.00 | - | 153.17 | - | - | - |

**Table A.14:** Daily measurement data of propionate SBR (Day 99-102)

| day | | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^+$ mg/L | $Cl^-$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99 | Influent | - | 61.21 | 25.66 | 0.00 | 0.00 | 12.78 | 14.88 | 112.36 | 15.81 | 41.66 | 200.68 | - | - |
| | Vo | - | 77.78 | 0.00 | 0.00 | 0.00 | 32.17 | 8.11 | 119.70 | 45.37 | 157.91 | 218.06 | - | - |
| | Initial | - | 67.42 | 16.04 | 0.00 | 0.00 | 20.05 | 12.34 | 115.11 | 26.90 | 85.25 | 207.20 | - | - |
| | Anaerobic End | - | 173.75 | 15.22 | 0.00 | 1.03 | 40.39 | 33.73 | 155.75 | 45.50 | 150.67 | 226.26 | 4400 | 2680 |
| | 150' | - | 164.50 | 14.71 | 0.34 | 1.33 | 25.35 | 28.66 | 142.64 | 42.81 | 178.66 | 228.85 | 5310 | 3200 |
| | 180' | - | 130.40 | - | 1.18 | 2.21 | - | - | - | 42.99 | - | 225.06 | 5740 | 3420 |
| | 210' | - | 87.60 | - | 1.88 | 3.68 | - | - | - | 43.41 | - | 225.82 | 6030 | 3560 |
| | 240' | - | 46.80 | - | 2.72 | 4.38 | - | - | - | 43.86 | - | 222.07 | 6200 | 3550 |
| | Aerobic End | - | 17.78 | 0.00 | 4.50 | 2.06 | 17.20 | 3.87 | 93.33 | 44.33 | 153.93 | 214.81 | 6210 | 3500 |
| 100 | Influent | - | 59.66 | 26.37 | 0.00 | 0.00 | 13.08 | 15.71 | 116.63 | 13.93 | 46.36 | 222.49 | - | - |
| | Vo | - | 89.69 | 0.00 | 0.30 | 0.00 | 33.73 | 10.31 | 119.79 | 46.98 | 155.45 | 214.52 | - | - |
| | Initial | - | 70.92 | 16.48 | 0.11 | 0.00 | 20.83 | 13.68 | 117.81 | 26.32 | 87.27 | 219.50 | - | - |
| | Anaerobic End | - | 178.05 | 15.59 | 0.00 | 1.26 | 41.92 | 35.39 | 154.58 | 47.21 | 150.31 | 227.46 | - | - |
| | Aerobic End | - | 14.04 | 0.00 | 3.60 | 1.15 | 17.25 | 3.89 | 92.24 | 46.81 | 155.03 | 213.03 | 7130 | 4050 |
| 101 | Influent | - | 60.27 | 26.46 | 0.00 | 0.00 | 13.50 | 15.42 | 116.45 | 18.72 | 46.93 | 238.18 | - | - |
| | Vo | - | 62.93 | 0.00 | 0.42 | 0.00 | 29.40 | 8.69 | 109.97 | 48.81 | 155.31 | 211.40 | - | - |
| | Initial | - | 61.27 | 16.54 | 0.16 | 0.00 | 19.46 | 12.89 | 114.02 | 30.01 | 87.57 | 228.13 | - | - |
| | Anaerobic End | - | 175.33 | 14.58 | 0.00 | 1.17 | 40.22 | 32.85 | 155.56 | 46.73 | 148.22 | 219.53 | 5200 | 3160 |
| | Aerobic End | - | 17.54 | 0.00 | 3.48 | 1.94 | 17.07 | 3.33 | 114.51 | 47.35 | 153.90 | 210.98 | 6760 | 3800 |
| 102 | Influent | - | 60.60 | 22.20 | 0.00 | 0.00 | 25.31 | 13.45 | 106.14 | 48.25 | 41.93 | 207.99 | - | - |
| | Vo | 111 | 2.01 | 0.00 | 3.17 | 0.00 | 16.35 | 6.05 | 95.93 | 48.82 | 148.87 | 208.53 | 15040 | 8730 |
| | Initial | - | 38.63 | 13.87 | 1.19 | 0.00 | 21.95 | 10.67 | 102.32 | 48.46 | 82.03 | 208.19 | - | - |
| | Anaerobic End | 25 | 167.35 | 15.58 | 0.00 | 1.12 | 37.49 | 35.11 | 158.54 | 47.41 | 148.86 | 223.25 | 4360 | 2650 |
| | Aerobic End | 20 | 1.38 | 0.00 | 2.87 | 1.86 | 16.68 | 6.01 | 92.78 | 49.40 | 148.22 | 208.19 | 5620 | 3150 |
| | Effluent | 27 | 1.28 | 0.00 | 3.60 | 3.02 | 16.64 | 6.27 | 93.79 | 49.36 | 147.33 | 208.41 | - | - |

# APPENDIX B: DETAILED MEASUREMENT DATA
## DAILY MEASUREMENT DATA

**Table B.1:** Detailed measurements data of acetate SBR (Run-1/35.day)

| t (min) | pH | $S_A$ | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Cl^-$ | $Na^{++}$ | $SO_4^=$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| influent | nd | nd | 386 | 21.30 | 22.19 | 0.00 | 0.00 | 21.51 | 14.24 | 36.26 | 53.75 | 34.83 | 222.11 | - | - |
| $V_0$ | nd | 0 | 20 | 2.67 | 0.00 | 7.07 | 0.00 | 16.39 | 10.81 | 32.58 | 25.84 | 33.20 | 213.81 | nd | nd |
| 5 | nd | 85 | 95 | 28.27 | nd | 0.63 | 1.22 | nd | nd | nd | 26.72 | nd | 218.13 | nd | nd |
| 10 | nd | 149 | 170 | 34.17 | nd | 0.23 | 1.28 | nd | nd | nd | 34.80 | nd | 220.67 | nd | nd |
| 15 | 7.60 | 168 | 190 | 44.85 | 17.67 | 0.00 | 0.00 | 18.64 | 19.58 | 57.40 | 30.02 | 38.57 | 221.14 | 3530 | 2470 |
| 45 | 7.40 | 66 | 72 | 86.42 | 16.91 | 0.00 | 0.00 | 20.09 | 29.84 | 71.30 | 28.59 | 36.73 | 225.14 | 3380 | 2500 |
| 60 | 7.40 | 9 | 50 | 108.20 | 16.48 | 0.00 | 0.00 | 20.13 | 34.10 | 78.20 | 28.29 | 34.89 | 224.97 | 3230 | 2500 |
| 75 | 7.40 | 0 | 30 | 111.15 | 16.33 | 0.00 | 0.00 | 20.34 | 36.61 | 78.20 | 28.29 | 36.35 | 224.33 | 3300 | 2470 |
| 90 | 7.40 | 0 | 15 | 113.11 | 16.00 | 0.00 | 0.00 | 20.98 | 36.66 | 78.90 | 27.18 | 34.92 | 223.79 | 3250 | 2440 |
| 120 | 7.40 | 0 | 23 | 113.67 | 15.94 | 0.00 | 0.00 | 21.16 | 40.19 | 79.14 | 27.70 | 35.60 | 227.82 | 3360 | 2480 |
| 150 | 8.20 | 0 | 23 | 84.27 | 12.03 | 0.79 | 0.00 | 18.22 | 30.65 | 60.02 | 26.88 | 34.55 | 223.32 | 3380 | 2500 |
| 180 | 8.30 | 0 | 20 | 55.01 | 8.77 | 1.83 | 4.27 | 16.44 | 22.94 | 49.99 | 26.19 | 33.65 | 222.25 | 3550 | 2510 |
| 210 | 8.30 | 0 | 23 | 30.02 | 4.93 | 3.98 | 5.99 | 16.03 | 17.48 | 40.23 | 26.03 | 33.45 | 220.61 | 3660 | 2560 |
| 240 | 8.50 | 0 | 21 | 11.55 | 0.00 | 7.32 | 4.50 | 15.19 | 13.19 | 34.12 | 26.63 | 34.22 | 218.49 | 3680 | 2540 |
| 270 | 8.60 | 0 | 15 | 2.16 | 0.00 | 10.03 | 1.20 | 14.56 | 10.80 | 31.49 | 29.85 | 38.36 | 214.48 | 3850 | 2580 |
| 300 | 8.60 | 0 | 26 | 0.00 | 0.00 | 11.30 | 0.00 | 14.00 | 10.06 | 31.70 | 29.74 | 38.21 | 217.51 | 3750 | 2590 |
| Effluent | nd | nd | nd | 0.49 | nd | 11.41 | 0.00 | nd | nd | nd | 29.11 | nd | 220.95 | nd | nd |

nd: not determined

Notes

| Time: | 0' | 10' | 120'30" | 121' | 121'30" | 122' | 123' | 130' |
|---|---|---|---|---|---|---|---|---|
| DO: | 6.00 | 0.50 | 1.20 | 2.10 | 3.50 | 4.20 | 5.20 | 6.20 |

TKN of sludge (at the end of aerobic phase): 355 mgN/L
Sludge N Content = 14.00 %

**Table B.2:** Detailed measurements data of acetate SBR (Run-1/47.day)

| t (min) | pH | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^{+}$ | $Cl^{-}$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| influent | nd | 406 | 20.68 | 20.95 | 0.00 | 0.00 | 15.80 | 13.99 | 34.16 | 41.69 | 38.19 | 218.47 | - | - |
| 300 (prev.cycle) | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 2890 | 2010 | - |
| $V_0$ | 7.7 | 23 | 0.00 | 0.00 | 2.96 | 0.56 | 17.54 | 9.78 | 28.88 | 33.39 | 171.56 | 218.81 | nd | nd |
| 40 | 7.6 | 156 | 81.38 | 16.00 | 0.00 | 0.49 | 19.75 | 29.39 | 57.26 | 32.95 | 168.23 | 222.69 | 3210 | 2440 |
| 80 | 7.5 | 85 | 112.80 | 15.66 | 0.00 | 0.45 | 20.26 | 37.65 | 67.26 | 32.24 | 169.78 | 224.81 | 3100 | 2480 |
| 120 | 7.5 | 12 | 113.89 | 15.25 | 0.00 | 0.39 | 19.94 | 38.34 | 68.20 | 31.74 | 167.27 | 225.83 | 3000 | 2430 |
| 160 | 8.5 | 23 | 58.31 | 11.55 | 0.44 | 1.58 | 17.20 | 24.89 | 48.77 | 31.76 | 163.67 | 221.30 | 2575 | 1880 |
| 200 | 8.6 | 16 | 27.62 | 7.56 | 1.54 | 3.37 | 16.15 | 16.55 | 36.90 | 32.85 | 168.74 | 215.12 | 2960 | 2100 |
| 240 | 8.5 | 19 | | 2.78 | nd | nd | 15.66 | 10.93 | 28.09 | nd | 168.83 | nd | 3275 | 2260 |
| 280 | 8.6 | 19 | 4.12 | 0.00 | 3.46 | 4.61 | 16.04 | 9.26 | 27.99 | 33.55 | 168.28 | 207.90 | 3405 | 2350 |
| 300 | 8.6 | 19 | 0.00 | 0.00 | 6.11 | 2.01 | 16.13 | 9.18 | 28.35 | 32.99 | 169.76 | 209.71 | 3395 | 2410 |
| Effluent | nd | nd | 0.00 | nd | 7.25 | 1.03 | nd | nd | nd | 33.75 | nd | 213.61 | 9 | - |

nd: not determined

Notes

| | | | | | |
|---|---|---|---|---|---|
| Time: | 8' | 10' | 13' | 15' | 17' |
| DO: | 2.00 | 1.50 | 1.00 | 0.70 | 0.50 |

**Table B.3:** Detailed measurements data of acetate SBR (Run-1/48.day)

| t (min) | pH | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $N_{OX}$-N | $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Cl^-$ | $Na^{++}$ | $SO_4^=$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| influent | nd | 398 | 20.68 | 20.99 | 0.00 | 0.00 | 0.00 | 15.99 | 14.11 | 33.63 | 41.69 | 38.66 | 218.47 | - | - |
| $V_0$ | nd | 16 | 0.00 | 0.00 | 2.88 | 0.00 | 2.88 | 18.06 | 9.68 | 28.44 | 34.99 | 171.86 | 220.19 | 7160 | 4730 |
| 40 | 7.6 | 111 | 82.07 | 16.41 | 0.00 | 0.00 | 0.00 | 19.84 | 31.42 | 54.55 | 33.53 | 167.62 | 224.64 | 2700 | 2000 |
| 60 | 7.5 | 60 | 94.30 | 16.30 | 0.00 | 0.00 | 0.00 | 19.88 | 35.50 | 60.12 | 33.10 | 170.11 | 228.45 | 2850 | 2080 |
| 80 | 7.5 | 31 | 109.67 | 16.01 | 0.00 | 0.00 | 0.00 | 19.91 | 37.12 | 66.53 | 32.73 | 171.09 | 233.38 | 2890 | 2140 |
| 120 | 7.4 | 23 | 115.92 | 15.66 | 0.00 | 0.00 | 0.00 | 20.88 | 39.15 | 69.19 | 33.32 | 169.73 | 235.21 | 2945 | 2180 |
| 160 | 8.5 | 23 | 63.90 | 11.79 | 0.56 | 1.86 | 1.68 | 17.92 | 25.39 | 50.00 | 33.03 | 170.98 | 228.54 | 2960 | 2100 |
| 200 | 8.5 | 23 | 26.04 | 7.38 | 2.37 | 3.51 | 4.48 | 16.35 | 16.24 | 35.74 | 33.93 | 168.39 | 221.79 | 3170 | 2220 |
| 240 | 8.5 | 15 | 3.26 | 2.64 | 3.91 | 4.49 | 6.60 | 16.37 | 10.98 | 27.83 | 34.84 | 171.50 | 220.07 | 3325 | 2260 |
| 280 | 8.5 | 15 | 0.00 | 0.00 | 6.21 | 2.05 | 7.44 | 16.58 | 9.55 | 28.43 | 33.93 | 172.56 | 219.66 | 3430 | 2300 |
| 300 | 8.5 | 15 | 0.00 | 0.00 | 7.33 | 0.65 | 7.72 | 16.94 | 9.44 | 28.88 | 34.85 | 172.67 | 219.07 | 3460 | 2320 |
| Effluent | nd | nd | 0.00 | nd | 7.38 | 0.62 | 7.75 | nd | nd | nd | 32.54 | nd | 210.16 | - | - |

nd: not determined

Notes
TKN of sludge (at the end of aerobic phase) = 302 mgN/L
Sludge N Content = 13.40 %

**Table B.4:** Detailed measurements data of acetate SBR (Run-1/49.day)

| t (min) | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $N_{OX}$-N | $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Cl^-$ | $Na^{++}$ | $SO_4^=$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| influent | 412 | 22.44 | 20.53 | 0.00 | 0.00 | 0.00 | 15.72 | 14.23 | 35.30 | 40.13 | 46.51 | 236.54 | - | - |
| $V_0$ | 23 | 0.00 | 0.00 | 1.30 | 0.12 | 1.37 | 17.22 | 9.91 | 29.28 | 30.24 | 181.12 | 234.87 | 8080 | 5460 |
| 40 | 85 | 105.81 | 16.73 | 0.00 | 0.00 | 0.00 | 19.74 | 32.47 | 64.13 | 30.05 | 194.60 | 292.14 | 2750 | 2060 |
| 80 | 23 | 123.60 | 15.75 | 0.00 | 0.00 | 0.00 | 19.53 | 40.20 | 72.52 | 29.29 | 198.49 | 288.63 | 2700 | 2090 |
| 120 | 23 | 123.10 | 16.39 | 0.00 | 0.00 | 0.00 | 20.87 | 42.20 | 76.27 | 29.68 | 200.97 | 291.42 | 2700 | 2090 |
| 130 | nd | 116.60 | nd | 0.09 | 0.00 | 0.09 | nd | nd | nd | 30.32 | nd | 292.92 | nd | nd |
| 140 | nd | 95.10 | nd | 0.19 | 0.69 | 0.60 | nd | nd | nd | 30.27 | nd | 302.72 | nd | nd |
| 150 | nd | 79.21 | nd | 0.40 | 1.40 | 1.24 | nd | nd | nd | 33.98 | nd | 282.36 | nd | nd |
| 160 | nd | 72.34 | nd | 0.53 | 1.83 | 1.62 | nd | nd | nd | 33.93 | nd | 283.84 | nd | nd |
| 170 | nd | 58.57 | nd | 0.77 | 2.23 | 2.10 | nd | nd | nd | 32.69 | nd | 277.24 | nd | nd |
| 180 | 23 | 50.30 | 9.97 | 0.98 | 2.26 | 2.33 | 16.90 | 22.65 | 46.80 | 30.78 | 200.05 | 291.98 | 3100 | 2212 |
| 190 | nd | 46.10 | nd | 1.34 | 2.49 | 2.83 | nd | nd | nd | 34.79 | nd | 285.31 | nd | nd |
| 200 | nd | 36.18 | nd | 1.73 | 3.06 | 3.56 | nd | nd | nd | 33.25 | nd | 282.17 | nd | nd |
| 210 | nd | 27.41 | nd | 2.04 | 3.48 | 4.13 | nd | nd | nd | 33.92 | nd | 283.08 | nd | nd |
| 220 | nd | 18.98 | nd | 2.45 | 3.76 | 4.71 | nd | nd | nd | 32.63 | nd | 281.11 | nd | nd |
| 230 | nd | 11.50 | nd | 2.92 | 3.80 | 5.20 | nd | nd | nd | 32.80 | nd | 280.70 | nd | nd |
| 240 | 23 | 7.94 | 1.67 | 3.43 | 3.82 | 5.72 | 15.82 | 11.89 | 30.26 | 30.12 | 202.19 | 289.56 | 3250 | 2240 |
| 300 | 27 | 0.00 | 0.00 | 6.00 | 0.00 | 6.00 | 15.84 | 9.34 | 29.40 | 30.08 | 201.94 | 290.52 | 3300 | 2250 |
| Effluent | nd | 0.00 | nd | 6.93 | 0.00 | 6.93 | nd | nd | nd | 33.13 | nd | 277.61 | - | - |

nd: not determined

Notes

TKN of sludge (at the end of aerobic phase) = 278 mgN/L

Sludge N Content = 12.35 %

**Table B.5:** Detailed measurements data of acetate SBR (Run-1/58.day)

| t (min) | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^{+}$ | $Cl^{-}$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | 406 | 21.27 | 21.16 | 0.00 | 0.00 | 15.84 | 14.11 | 34.36 | 41.17 | 38.98 | 224.49 | - | - |
| Vo | 23 | 0.00 | 0.00 | 1.72 | 0.00 | 14.66 | 9.91 | 22.28 | 30.24 | 181.12 | 234.87 | nd | nd |
| 120 | nd | 113.72 | 15.19 | 0.00 | 0.00 | 19.19 | 41.69 | 75.37 | 25.25 | 171.30 | 238.55 | 3050 | 2230 |
| 135 | nd | 98.84 | 10.41 | 0.08 | 0.60 | 18.03 | 33.80 | 61.73 | 25.52 | 165.70 | 231.00 | 3190 | 2300 |
| 150 | nd | 76.53 | 8.87 | 0.20 | 1.01 | 16.48 | 28.45 | 56.71 | 27.33 | 157.80 | 229.70 | 3300 | 2360 |
| 165 | nd | 59.64 | 7.64 | 0.22 | 1.25 | 16.10 | 24.89 | 51.00 | 27.84 | 157.84 | 229.10 | 3350 | 2380 |
| 180 | nd | 40.00 | 5.65 | 0.41 | 1.65 | 15.50 | 22.50 | 47.39 | 27.84 | 149.00 | 226.80 | 3400 | 2380 |
| 210 | nd | 12.70 | 3.00 | 1.73 | 1.09 | 14.88 | 16.89 | 37.05 | 26.55 | 134.00 | 222.90 | 3480 | 2400 |
| 240 | nd | 0.00 | 1.27 | 1.90 | 1.01 | 14.57 | 9.80 | 27.05 | 26.33 | 123.03 | 217.73 | 3600 | 2450 |
| 300 | nd | 0.00 | 0.00 | 2.95 | 0.00 | 15.61 | 8.10 | 23.50 | 25.97 | 120.00 | 219.99 | 3650 | 2480 |

nd: not determined

**Table B.6:** Detailed measurements data of acetate SBR (Run-2)

| t (min) | pH | $S_A$ | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $N_{OX}$-N | $Ca^{++}$ | $Mg^{++}$ | $K^{+}$ | $Cl^{-}$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| influent | nd | nd | 403 | 35.41 | 15.52 | 0.00 | 0.00 | 28.08 | 15.92 | 54.99 | 42.62 | 45.40 | 157.93 | - | - |
| $V_0$ | nd | 0 | 17 | 4.78 | 0.00 | 1.00 | 1.00 | 22.47 | 11.66 | 52.70 | 42.95 | 189.47 | 152.65 | nd | nd |
| 5 | 8.16 | 88 | 115 | 42.43 | 7.89 | 0.00 | 0.00 | 25.70 | 16.22 | 57.37 | 44.40 | 191.49 | 152.11 | nd | nd |
| 10 | 7.82 | 165 | 169 | 49.87 | 10.55 | 0.00 | 0.00 | 25.64 | 18.34 | 60.85 | 45.74 | 191.78 | 152.30 | nd | nd |
| 15 | 7.68 | 178 | 200 | 58.14 | 11.98 | 0.00 | 0.00 | 26.47 | 20.41 | 63.29 | 46.87 | 190.85 | 152.72 | 3400 | 2250 |
| 30 | 7.44 | 108 | 106 | 91.33 | 11.58 | 0.00 | 0.00 | 28.63 | 27.98 | 74.97 | 46.13 | 196.09 | 151.27 | 3490 | 2330 |
| 45 | 7.38 | 55 | 53 | 112.76 | 11.40 | 0.00 | 0.00 | 29.81 | 33.31 | 81.01 | 46.43 | 194.10 | 149.49 | 3570 | 2350 |
| 60 | 7.33 | 11 | 27 | 129.50 | 11.26 | 0.00 | 0.00 | 29.77 | 37.34 | 85.65 | 45.15 | 195.80 | 147.71 | 3610 | 2410 |
| 90 | 7.31 | 0 | 23 | 133.54 | 10.96 | 0.00 | 0.00 | 31.39 | 42.02 | 95.56 | 45.64 | 197.10 | 150.53 | 3680 | 2430 |
| 120 | 7.31 | 0 | 21 | 135.41 | 10.55 | 0.00 | 0.00 | 30.83 | 41.46 | 95.28 | 45.40 | 198.32 | 150.26 | 3750 | 2480 |
| 150 | 8.27 | 0 | 18 | 94.56 | 7.72 | 0.15 | 0.28 | 20.42 | 28.99 | 78.96 | 46.61 | 195.74 | 151.95 | 3960 | 2550 |
| 180 | 8.50 | 0 | 16 | 61.06 | 5.15 | 0.39 | 0.64 | 16.98 | 20.86 | 67.57 | 46.69 | 194.32 | 150.83 | 4160 | 2660 |
| 210 | 8.56 | 0 | 14 | 34.52 | 0.00 | 0.69 | 0.89 | 15.94 | 15.37 | 58.76 | 46.18 | 196.58 | 150.69 | 4480 | 2730 |
| 240 | 8.68 | 0 | 15 | 17.98 | 0.00 | 1.78 | 1.78 | 14.97 | 11.97 | 52.52 | 46.04 | 196.03 | 150.81 | 4510 | 2760 |
| 270 | 8.76 | 0 | 15 | 7.56 | 0.00 | 1.62 | 1.62 | 15.27 | 9.51 | 47.75 | 44.69 | 194.63 | 150.47 | 4590 | 2780 |
| 300 | 8.74 | 0 | 15 | 2.99 | 0.00 | 1.53 | 1.53 | 18.54 | 9.46 | 47.47 | 44.91 | 194.28 | 148.12 | 4560 | 2780 |
| Effluent | nd | nd | 18 | 2.72 | 0.00 | 1.49 | 1.49 | 18.24 | 9.46 | 47.31 | 44.70 | 196.10 | 149.75 | nd | nd |

nd: not determined

**Table B.7:** Detailed measurements data of acetate SBR (Run-3)

| t (min) | pH | $S_A$ | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Cl^-$ | $Na^{++}$ | $SO_4^=$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| influent | | nd | 365 | 45.47 | 21.53 | 0.33 | 0.00 | 27.15 | 14.39 | 77.36 | 32.72 | 43.99 | 143.33 | - | - |
| $V_0$ | nd | 0 | 16 | 10.14 | 0.00 | 1.50 | 0.00 | 21.63 | 9.61 | 74.47 | 19.69 | 193.15 | 138.25 | nd | nd |
| 5 | nd | 97 | 120 | 41.36 | 12.99 | 0.58 | 0.00 | 24.44 | 13.14 | 77.32 | 20.19 | 188.44 | 142.95 | nd | nd |
| 10 | nd | 163 | 167 | 58.96 | 15.67 | 0.68 | 0.00 | 26.08 | 16.51 | 81.88 | 20.06 | 189.43 | 142.09 | nd | nd |
| 15 | 7.83 | 169 | 200 | 70.72 | 17.13 | 0.00 | 0.00 | 27.29 | 19.17 | 85.20 | 20.58 | 189.74 | 147.70 | 3950 | 2460 |
| 30 | 7.62 | 102 | 105 | 101.40 | 16.49 | 0.00 | 0.00 | 28.49 | 25.22 | 93.59 | 20.57 | 191.37 | 150.16 | 3850 | 2480 |
| 45 | 7.51 | 32 | 66 | 131.10 | 16.04 | 0.00 | 0.00 | 29.32 | 31.60 | 104.09 | 21.34 | 191.19 | 153.85 | 3600 | 2460 |
| 60 | 7.48 | 0 | 53 | 141.97 | 16.26 | 0.00 | 0.00 | 30.65 | 35.76 | 110.07 | 20.30 | 193.76 | 155.28 | 3650 | 2450 |
| 90 | 7.43 | 0 | 32 | 142.56 | 15.62 | 0.00 | 0.00 | 31.15 | 40.65 | 118.49 | 19.26 | 186.57 | 155.51 | 3500 | 2480 |
| 120 | 7.40 | 0 | 20 | 145.68 | 15.23 | 0.00 | 0.00 | 31.50 | 40.65 | 120.90 | 19.38 | 183.52 | 158.73 | 3450 | 2500 |
| 150 | 8.26 | 0 | 18 | 111.50 | 13.19 | 0.00 | 0.87 | 23.91 | 29.58 | 104.44 | 18.62 | 188.52 | 146.79 | 3800 | 2475 |
| 180 | 8.53 | 0 | 16 | 64.90 | 10.82 | 0.38 | 1.09 | 19.33 | 21.86 | 97.49 | 19.31 | 191.98 | 149.65 | 4350 | 2550 |
| 210 | 8.62 | 0 | 18 | 39.30 | 8.27 | 1.48 | 1.09 | 17.58 | 16.27 | 86.59 | 19.56 | 190.13 | 143.95 | 4350 | 2590 |
| 240 | 8.72 | 0 | 20 | 24.00 | 5.25 | 1.59 | 1.65 | 17.10 | 11.86 | 76.82 | 19.31 | 190.48 | 144.76 | 4300 | 2530 |
| 270 | 8.74 | 0 | 18 | 12.80 | 1.98 | 2.52 | 1.24 | 17.50 | 8.93 | 69.10 | 19.39 | 189.53 | 138.27 | 4280 | 2580 |
| 300 | 8.85 | 0 | 16 | 3.88 | 0.00 | 3.10 | 0.56 | 17.60 | 7.01 | 65.80 | 20.04 | 190.53 | 141.73 | 4500 | 2640 |
| 1.stage | 7.83 | nd | nd | 7.41 | 0.00 | 3.16 | 0.00 | 20.87 | 7.60 | 66.65 | 19.79 | 189.94 | 158.98 | nd | nd |
| 2.stage | 7.09 | nd | nd | 7.56 | 0.00 | 2.57 | 0.00 | 28.06 | 8.04 | 65.17 | 19.74 | 188.96 | 200.59 | nd | nd |

nd: not determined

Notes

pH of the reactor adjusted two times following aeration period:

1st stage; pH adjusted to pH of about 8 with NaOH.

2nd stahe; pH adjusted to pH of about 7 with NaOH.

**Table B.8:** Detailed measurements data of acetate SBR (Run-4)

| t (min) | pH | $S_A$ | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $N_{OX}$-N | $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Cl^-$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| influent | nd | nd | 385 | 60.87 | 22.08 | 0.00 | 0.00 | 27.83 | 14.14 | 101.75 | 40.54 | 41.93 | 166.95 | - | - |
| $V_0$ | nd | 0 | 24 | 8.18 | 0.00 | 1.70 | 1.70 | 16.66 | 9.12 | 90.46 | 41.25 | 153.60 | 163.23 | nd | nd |
| 5 | nd | 79 | 99 | 62.73 | 12.05 | 0.65 | 0.65 | 27.21 | 19.57 | 102.29 | 44.51 | 156.73 | 160.62 | nd | nd |
| 10 | 8.27 | 126 | 134 | 79.17 | 14.55 | 0.23 | 0.23 | 28.27 | 23.26 | 105.13 | 44.43 | 156.46 | 165.55 | nd | nd |
| 15 | 7.61 | 130 | 162 | 96.50 | 16.69 | 0.00 | 0.00 | 28.19 | 26.61 | 108.59 | 40.69 | 157.61 | 165.19 | 4500 | 2700 |
| 30 | 7.34 | 46 | 77 | 134.80 | 16.45 | 0.00 | 0.00 | 29.62 | 32.50 | 117.97 | 40.99 | 157.55 | 167.57 | 4370 | 2700 |
| 45 | 7.30 | 12 | 47 | 152.63 | 16.28 | 0.00 | 0.00 | 31.53 | 36.75 | 127.85 | 40.98 | 159.19 | 169.42 | 5200 | 2650 |
| 60 | 7.28 | 0 | 39 | 153.24 | 16.12 | 0.00 | 0.00 | 32.20 | 38.68 | 135.21 | 40.96 | 159.66 | 170.25 | 4900 | 2640 |
| 90 | 7.31 | 0 | 24 | 154.62 | 15.66 | 0.00 | 0.00 | 32.68 | 38.88 | 145.51 | 40.66 | 157.20 | 170.61 | 4100 | 2640 |
| 120 | 7.31 | 0 | 26 | 155.46 | 15.63 | 0.00 | 0.00 | 33.06 | 38.15 | 146.60 | 40.96 | 151.40 | 170.66 | 3600 | 2650 |
| 150 | 8.12 | 0 | nd | 119.20 | 12.87 | 1.68 | 1.68 | 27.23 | 29.76 | 124.12 | 38.78 | 153.30 | 160.02 | 3480 | 2740 |
| 180 | 8.34 | nd | nd | 72.53 | 10.84 | 1.53 | 1.90 | 21.82 | 24.55 | 122.21 | 41.55 | 156.31 | 164.89 | 4200 | 2740 |
| 210 | 8.54 | 0 | nd | 46.55 | 7.40 | 1.96 | 2.54 | 18.21 | 18.19 | 108.93 | 41.50 | 156.86 | 167.01 | 4350 | 2770 |
| 240 | 8.47 | nd | nd | 26.17 | 5.34 | 2.51 | 2.90 | 18.06 | 15.30 | 103.14 | 42.17 | 159.26 | 166.30 | 5220 | 2800 |
| 270 | 8.75 | nd | nd | 12.70 | 0.00 | 3.57 | 3.78 | 16.98 | 9.49 | 88.43 | 40.79 | 156.62 | 168.84 | 5200 | 2820 |
| 300 | 8.94 | 0 | 18 | 8.35 | 0.00 | 3.80 | 3.80 | 16.95 | 7.21 | 81.90 | 39.30 | 151.27 | 157.43 | 5670 | 2820 |
| Effluent | nd | nd | nd | 8.54 | 0.00 | 4.02 | 4.02 | 16.99 | 7.28 | 85.58 | 40.96 | 159.20 | 163.29 | nd | nd |

nd: not determined

**Table B.9:** Detailed measurements data of acetate SBR (Run-5)

| t (min) | pH | $S_A$ mg/L | COD mg/L | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{++}$ mg/L | $Mg^{++}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{++}$ mg/L | $SO_4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Influent | nd | nd | 415 | 62.10 | 22.20 | 0.00 | 0.00 | 27.11 | 13.45 | 106.14 | 43.88 | 41.93 | 207.99 | - | - |
| Vo | nd | 0 | 21 | 9.84 | 0.00 | 1.32 | 0.00 | 16.78 | 3.31 | 91.17 | 48.54 | 173.10 | 221.20 | 15800 | 8400 |
| 5 | 7.93 | 87 | 104 | 53.46 | 12.00 | 0.43 | 0.00 | 28.10 | 11.58 | 104.49 | 48.85 | 169.41 | 222.87 | nd | nd |
| 10 | 7.71 | 122 | 145 | 66.75 | 15.33 | 0.11 | 0.00 | 28.79 | 15.22 | 105.68 | 49.55 | 166.44 | 219.07 | nd | nd |
| 15 | 7.47 | 117 | 160 | 88.17 | 16.43 | 0.00 | 0.00 | 31.00 | 20.17 | 109.89 | 48.89 | 171.16 | 228.40 | 4380 | 2350 |
| 30 | 7.38 | 61 | 82 | 124.66 | 16.44 | 0.00 | 0.00 | 33.53 | 28.33 | 122.50 | 49.27 | 171.81 | 227.15 | 4200 | 2320 |
| 45 | 7.29 | 33 | 46 | 136.54 | 16.08 | 0.00 | 0.00 | 34.65 | 34.36 | 135.23 | 48.30 | 173.63 | 230.59 | 4140 | 2330 |
| 60 | 7.27 | 0 | 30 | 149.87 | 15.72 | 0.00 | 0.00 | 34.49 | 35.54 | 144.84 | 47.91 | 173.40 | 230.16 | 3940 | 2450 |
| 90 | 7.25 | 0 | 25 | 148.65 | 15.69 | 0.00 | 0.00 | 36.12 | 35.37 | 152.84 | 46.37 | 171.14 | 230.80 | 3960 | 2340 |
| 120 | 7.25 | 0 | 22 | 149.75 | 15.91 | 0.00 | 0.00 | 38.04 | 35.63 | 157.17 | 45.65 | 170.27 | 228.05 | 3930 | 2470 |
| 150 | 8.04 | 0 | 21 | 118.92 | 13.19 | 0.00 | 1.36 | 30.11 | 25.85 | 137.72 | 45.44 | 170.23 | 224.90 | 3770 | 2470 |
| 180 | 8.48 | 0 | 21 | 82.97 | 9.66 | 0.21 | 1.14 | 21.10 | 15.20 | 124.13 | 48.35 | 170.21 | 219.98 | 4700 | 2540 |
| 210 | 8.66 | 0 | 21 | 54.60 | 6.57 | 0.51 | 1.16 | 18.93 | 11.50 | 113.02 | 48.16 | 171.82 | 220.38 | 4980 | 2570 |
| 240 | 8.81 | 0 | 21 | 27.97 | 2.77 | 1.05 | 1.05 | 17.40 | 6.48 | 98.20 | 46.02 | 171.58 | 217.68 | 5150 | 2680 |
| 270 | 9.14 | 0 | 21 | 16.77 | 0.00 | 2.16 | 0.00 | 16.65 | 3.07 | 89.37 | 48.70 | 170.24 | 213.64 | 5400 | 2820 |
| 300 | 9.14 | 0 | 21 | 11.54 | 0.00 | 2.88 | 0.00 | 19.82 | 2.12 | 86.55 | 48.30 | 169.44 | 212.08 | 5700 | 2890 |
| Effluent | nd | 0 | 21 | 11.18 | 0.00 | 2.92 | 0.00 | 19.42 | 2.04 | 86.68 | 47.93 | 170.79 | 211.60 | nd | nd |

nd: not determined

Notes

| Time: | 130' | 137' | 160' | 167' |
|---|---|---|---|---|
| pH: | 7.59 | 7.75 | 8.24 | 8.33 |

SVI= 16 ml/g

**Table B.10:** Detailed measurements data of propionate SBR (Run-1/35.day)

| t (min) | pH | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^{+}$ | $Cl^{-}$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | nd | 405 | 19.94 | 22.19 | 0.00 | 0.00 | 21.51 | 14.24 | 36.26 | 53.75 | 34.83 | 222.11 | - | - |
| $V_0$ | nd | 4 | 14.13 | 0.00 | 1.33 | 0.00 | 16.71 | 11.82 | 26.66 | 24.72 | 31.77 | 212.11 | nd | nd |
| 5 | nd | 37 | 36.54 | nd | 0.00 | 0.60 | nd | nd | nd | 27.62 | | 214.61 | nd | nd |
| 10 | nd | 46 | 46.03 | nd | 0.00 | 0.59 | nd | nd | nd | 30.42 | | 215.73 | nd | nd |
| 15 | 6.90 | 63 | 62.65 | 16.19 | 0.00 | 0.00 | 19.56 | 25.41 | 26.66 | 30.53 | 39.23 | 219.04 | 4480 | 3020 |
| 45 | 7.20 | 96 | 95.94 | 15.51 | 0.00 | 0.00 | 19.69 | 34.68 | 45.36 | 26.89 | 34.55 | 223.01 | 4230 | 3070 |
| 75 | 7.20 | 103 | 102.78 | 15.40 | 0.00 | 0.00 | 20.02 | 34.27 | 58.25 | 26.67 | 34.27 | 217.55 | 4030 | 3130 |
| 90 | 7.20 | 102 | 101.87 | 15.69 | 0.00 | 0.00 | 21.52 | 35.83 | 58.79 | 25.89 | 33.27 | 221.56 | 4110 | 3150 |
| 120 | 7.20 | 98 | 98.30 | 15.27 | 0.00 | 0.00 | 20.28 | 35.02 | 64.07 | 26.70 | 34.31 | 219.03 | 4110 | 3160 |
| 150 | 7.40 | 27 | 27.22 | 12.75 | 0.00 | 1.63 | 18.01 | 25.38 | 60.44 | 28.77 | 36.98 | 213.32 | 4230 | 3300 |
| 180 | 8.10 | 10 | 9.82 | 9.40 | 0.35 | 1.66 | 16.74 | 17.75 | 36.45 | 25.53 | 32.81 | 214.98 | 4380 | 3400 |
| 210 | 8.10 | 1 | 1.18 | 4.22 | 0.97 | 1.37 | 15.00 | 11.16 | 26.55 | 25.62 | 32.92 | 211.00 | 4650 | 3500 |
| 240 | 8.10 | 0 | 0.00 | 0.00 | 2.68 | 0.00 | 15.70 | 10.37 | 28.87 | 28.80 | 37.00 | 209.31 | 4720 | 3580 |
| 270 | 8.10 | 0 | 0.00 | 0.00 | 3.43 | 0.00 | 15.83 | 10.31 | 30.24 | 26.99 | 34.68 | 209.25 | 4630 | 3550 |
| 300 | 8.20 | 0 | 0.00 | 0.00 | 3.41 | 0.00 | 15.56 | 9.89 | 30.94 | 26.82 | 34.47 | 208.43 | 4640 | 3550 |
| Effluent | nd | 0 | 0.00 | nd | 3.55 | 0.00 | nd | nd | nd | 26.70 | nd | 211.77 | nd | nd |

nd: not determined

Notes

| Time: | 125' | 135' | 145' | 150' | 180' | 300' |
|---|---|---|---|---|---|---|
| DO: | 2.25 | 1.75 | 2.25 | 3.00 | 6.10 | 6.50 |

TKN of sludge (at the end of aerobic phase): 280 mgN/L
Sludge N Content = 8.80 %

**Table B.11:** Detailed measurements data of propionate SBR (Run-1/47. day)

| t (min) | pH | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^{+}$ | $Cl^{-}$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | nd | 415 | 20.68 | 20.95 | 0.00 | 0.00 | 15.80 | 13.99 | 34.16 | 41.69 | 38.19 | 218.47 | - | - |
| 300 (prev.cycle) | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 4170 | 2920 | nd |
| Vo | 7.4 | 19 | 0.00 | 0.00 | 1.33 | 0.27 | 17.42 | 11.27 | 22.90 | 31.09 | 120.78 | 216.30 | - | - |
| 40 | 7.3 | 27 | 90.69 | 15.69 | 0.00 | 0.28 | 20.09 | 32.37 | 54.14 | 31.65 | 120.61 | 230.00 | 5095 | 3770 |
| 80 | 7.3 | 19 | 93.21 | 15.38 | 0.00 | 0.38 | 19.72 | 32.38 | 55.42 | 33.10 | 120.49 | 229.67 | 5025 | 3770 |
| 120 | 7.3 | 19 | 92.44 | 15.02 | 0.00 | 0.29 | 19.69 | 32.57 | 55.87 | 32.70 | 121.84 | 232.50 | 4910 | 3680 |
| 160 | 8.5 | 19 | 53.03 | 11.64 | 0.34 | 1.32 | 16.82 | 23.34 | 46.34 | 32.28 | 155.16 | 225.26 | 3865 | 2820 |
| 200 | 8.0 | 19 | 0.00 | 6.24 | 1.04 | 2.36 | 15.99 | 10.18 | 26.66 | 31.80 | 120.65 | 215.94 | 4145 | 2900 |
| 240 | 8.2 | 23 | 0.00 | 0.00 | 3.71 | 1.86 | 15.99 | 9.63 | 27.48 | 32.37 | 121.01 | 216.78 | 4230 | 2960 |
| 280 | 8.3 | 19 | 0.00 | 0.00 | 3.77 | 0.36 | 15.66 | 9.32 | 28.24 | 23.48 | 120.21 | 160.72 | 4325 | 3070 |
| 300 | 8.5 | 19 | 0.00 | 0.00 | 5.34 | 0.37 | 15.71 | 9.34 | 29.01 | 32.50 | 122.50 | 215.31 | 4415 | 3100 |
| Effluent | nd | nd | 0.00 | nd | 5.53 | 0.39 | nd | nd | nd | 32.46 | nd | 219.81 | 16 | nd |

nd: not determined

**Table B.12:** Detailed measurements data of propionate SBR (Run-1/48. day)

| t (min) | pH | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^{+}$ | $Cl^{-}$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | nd | 420 | 20.68 | 20.99 | 0.00 | 0.00 | 15.99 | 14.11 | 33.63 | 41.69 | 38.66 | 218.47 | - | - |
| $V_0$ | nd | 19 | 0.00 | 0.00 | 1.03 | 0.45 | 17.97 | 10.90 | 21.85 | 34.70 | 123.50 | 222.10 | 12780 | 8550 |
| 40 | 7.4 | 31 | 89.15 | 15.10 | 0.00 | 0.44 | 19.34 | 32.21 | 51.79 | 32.13 | 119.61 | 237.04 | 3875 | 2750 |
| 80 | 7.3 | 15 | 93.08 | 15.49 | 0.00 | 0.54 | 20.18 | 32.88 | 55.55 | 32.91 | 123.72 | 239.88 | 3835 | 2800 |
| 120 | 7.2 | 16 | 94.06 | 15.31 | 0.00 | 0.47 | 20.09 | 33.14 | 55.94 | 32.54 | 123.24 | 232.72 | 3945 | 2880 |
| 160 | 8.5 | 23 | 28.68 | 10.54 | 0.19 | 1.10 | 16.65 | 17.39 | 34.93 | 32.36 | 121.52 | 231.09 | 3970 | 2780 |
| 200 | 7.9 | 23 | 0.00 | 5.75 | 1.84 | 2.84 | 16.06 | 10.30 | 25.31 | 31.93 | 122.07 | 224.93 | 4145 | 2860 |
| 240 | 8.1 | 23 | 0.00 | 0.00 | 4.39 | 1.84 | 15.99 | 9.75 | 26.05 | 32.01 | 121.00 | 221.21 | 4200 | 2900 |
| 280 | 8.1 | 26 | 0.00 | 0.00 | 5.49 | 0.58 | 15.83 | 9.34 | 26.60 | 32.08 | 120.85 | 222.43 | 4300 | 2920 |
| 300 | 8.2 | 26 | 0.00 | 0.00 | 5.89 | 0.48 | 15.89 | 9.39 | 27.85 | 33.56 | 122.49 | 224.66 | 4350 | 2915 |
| Effluent | nd | nd | 0.00 | nd | 5.59 | 0.36 | nd | nd | nd | 33.42 | nd | 221.86 | nd | nd |

nd: not determined

Notes

TKN of sludge (at the end of aerobic phase): 343 mgN/L

Sludge N Content = 12.04 %

**Table B.13:** Detailed measurements data of propionate SBR (Run-1/49. day)

| t (min) | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^{+}$ | $Cl^{-}$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | 395 | 22.44 | 20.53 | 0.00 | 0.00 | 15.72 | 14.23 | 35.30 | 40.13 | 40.08 | 236.54 | - | - |
| Vo | 19 | 0.00 | 0.00 | 1.99 | 0.00 | 16.79 | 10.70 | 22.12 | 30.30 | 128.84 | 237.32 | 12110 | 8580 |
| 40 | 19 | 106.67 | 15.64 | 0.00 | 0.00 | 19.54 | 34.03 | 57.40 | 29.77 | 128.12 | 234.14 | 4100 | 3020 |
| 80 | 19 | 107.35 | 15.70 | 0.00 | 0.00 | 19.66 | 33.38 | 58.20 | 29.10 | 125.94 | 233.22 | 4050 | 3050 |
| 120 | 19 | 109.90 | 15.84 | 0.00 | 0.00 | 19.72 | 35.20 | 62.31 | 29.16 | 141.84 | 246.04 | 4150 | 3060 |
| 130 | nd | 83.42 | nd | 0.00 | 0.00 | nd | nd | nd | 32.92 | nd | 228.48 | nd | nd |
| 140 | nd | 66.32 | nd | 0.16 | 0.00 | nd | nd | nd | 33.36 | nd | 229.45 | nd | nd |
| 150 | nd | 46.77 | nd | 0.02 | 0.00 | nd | nd | nd | 33.03 | nd | 228.35 | nd | nd |
| 160 | nd | 32.51 | nd | 0.20 | 0.81 | nd | nd | nd | 33.31 | nd | 226.11 | nd | nd |
| 170 | nd | 20.90 | nd | 0.28 | 1.07 | nd | nd | nd | 31.69 | nd | 229.01 | nd | nd |
| 180 | 19 | 9.70 | 8.29 | 0.63 | 1.26 | 15.24 | 12.58 | 29.70 | 29.29 | 125.25 | 233.03 | 4700 | 3156 |
| 190 | nd | 3.55 | nd | 0.83 | 1.93 | nd | nd | nd | 31.37 | nd | 223.90 | nd | nd |
| 200 | nd | 0.00 | nd | 1.29 | 2.60 | nd | nd | nd | 31.42 | nd | 225.98 | nd | nd |
| 210 | nd | 0.00 | nd | 1.90 | 3.00 | nd | nd | nd | 31.06 | nd | 222.01 | nd | nd |
| 220 | nd | 0.00 | nd | 2.85 | 2.97 | nd | nd | nd | 31.13 | nd | 223.07 | nd | nd |
| 230 | nd | 0.00 | nd | 3.55 | 2.27 | nd | nd | nd | 31.42 | nd | 222.56 | nd | nd |
| 240 | 16 | 0.00 | 0.00 | 4.80 | 1.32 | 15.73 | 9.63 | 27.82 | 29.35 | 126.66 | 232.98 | 4800 | 3200 |
| 300 | 19 | 0.00 | 0.00 | 6.07 | 0.00 | 14.83 | 8.97 | 28.40 | 28.81 | 124.38 | 230.76 | 4800 | 3200 |
| Effluent | nd | 0.00 | nd | 5.90 | 0.00 | nd | nd | nd | 33.51 | nd | 224.63 | nd | nd |

nd: not determined

Notes

TKN of sludge (at the end of aerobic phase): 325 mgN/L

Sludge N Content = 10.15 %

**Table B.14:** Detailed measurements data of propionate SBR (Run-1/58. day)

| t (min) | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^{+}$ | $Cl^{-}$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | nd | 21.27 | 21.16 | 0.00 | 0.00 | 15.84 | 14.11 | 34.36 | 41.17 | 38.98 | 224.49 | - | - |
| $V_0$ | 20 | 0.00 | 0.00 | 2.11 | 0.00 | 16.01 | 9.98 | 26.88 | 23.30 | 128.84 | 137.32 | nd | nd |
| 120 | nd | 94.30 | 17.24 | 0.00 | 0.00 | 16.23 | 32.32 | 55.18 | 26.21 | 120.77 | 229.01 | 3160 | 2340 |
| 135 | nd | 85.80 | 16.06 | 0.00 | 0.00 | 16.89 | 21.20 | 42.40 | 27.20 | 120.62 | 236.00 | 3180 | 2358 |
| 150 | nd | 60.45 | 14.08 | 0.16 | 0.00 | 16.03 | 20.00 | 39.50 | 28.19 | 134.78 | 231.02 | 3280 | 2400 |
| 165 | nd | 33.63 | 10.91 | 0.38 | 1.29 | 15.01 | 18.10 | 38.57 | 26.55 | 140.18 | 225.98 | 3310 | 2420 |
| 180 | nd | 12.32 | 9.13 | 1.13 | 1.32 | 14.86 | 16.09 | 36.67 | 25.69 | 146.00 | 219.11 | 3440 | 2450 |
| 210 | nd | 0.00 | 3.28 | 2.38 | 2.12 | 13.87 | 10.43 | 27.64 | 27.59 | 156.00 | 222.74 | 3540 | 2480 |
| 240 | nd | 0.00 | 0.00 | 3.54 | 0.99 | 14.31 | 9.40 | 25.20 | 26.98 | 164.49 | 223.80 | 3650 | 2500 |
| 300 | nd | 0.00 | 0.00 | 4.35 | 0.52 | 16.06 | 8.50 | 24.40 | 23.02 | 121.43 | 196.09 | 3690 | 2520 |

nd: not determined

**Table B.15:** Detailed measurements data of propionate SBR (Run-2)

| t (min) | pH | $S_A$ | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Cl^-$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | nd | nd | 425 | 35.41 | 15.52 | 0.00 | 0.00 | 28.08 | 15.92 | 54.99 | 42.62 | 45.40 | 157.93 | - | - |
| $V_0$ | nd | nd | 21 | 0.00 | 0.00 | 0.99 | 0.00 | 26.44 | 8.63 | 38.55 | 42.48 | 138.85 | 149.01 | nd | nd |
| 5 | 7.56 | nd | 38 | 55.02 | 7.52 | 0.12 | 0.00 | 29.17 | 19.33 | 51.51 | 46.73 | 136.74 | 150.00 | nd | nd |
| 10 | 7.36 | nd | 60 | 78.42 | 9.87 | 0.00 | 0.00 | 29.38 | 25.47 | 55.24 | 47.89 | 135.29 | 149.48 | nd | nd |
| 15 | 7.28 | nd | 46 | 94.56 | 10.96 | 0.00 | 0.00 | 29.79 | 30.14 | 59.29 | 49.02 | 135.97 | 148.54 | 4500 | 2880 |
| 30 | 7.14 | nd | 36 | 102.56 | 10.32 | 0.00 | 0.00 | 30.41 | 36.10 | 71.61 | 47.35 | 137.67 | 149.40 | 4440 | 2920 |
| 45 | 7.10 | nd | 21 | 108.97 | 10.40 | 0.00 | 0.00 | 30.64 | 36.06 | 74.53 | 46.95 | 136.39 | 148.25 | 4380 | 2890 |
| 60 | 7.08 | nd | 18 | 111.15 | 10.14 | 0.00 | 0.00 | 30.17 | 35.46 | 74.73 | 46.91 | 135.15 | 146.65 | 4220 | 2900 |
| 90 | 7.06 | nd | 17 | 112.54 | 10.05 | 0.00 | 0.00 | 30.05 | 35.68 | 76.96 | 47.43 | 136.40 | 148.77 | 4120 | 2950 |
| 120 | 7.05 | nd | 17 | 112.97 | 9.65 | 0.00 | 0.00 | 29.94 | 35.02 | 76.26 | 47.29 | 135.97 | 149.37 | 4110 | 2950 |
| 150 | 8.00 | nd | 17 | 50.13 | 5.45 | 0.05 | 0.00 | 22.36 | 19.66 | 57.22 | 47.39 | 139.08 | 150.42 | 4350 | 2960 |
| 180 | 8.69 | nd | 17 | 9.08 | 2.10 | 0.20 | 0.00 | 18.69 | 10.35 | 44.63 | 47.13 | 136.75 | 148.30 | 4560 | 3000 |
| 210 | 8.74 | nd | 16 | 0.00 | 0.00 | 0.94 | 0.08 | 22.07 | 8.05 | 43.31 | 47.14 | 137.45 | 150.07 | 4630 | 3040 |
| 240 | 8.88 | nd | 16 | 0.00 | 0.00 | 0.99 | 0.62 | 23.16 | 7.74 | 44.02 | 47.60 | 139.05 | 148.15 | 4700 | 3035 |
| 270 | 8.55 | nd | 12 | 0.00 | 0.00 | 1.70 | 0.42 | 23.62 | 7.64 | 44.67 | 45.82 | 138.71 | 147.58 | 4660 | 3060 |
| 300 | 8.52 | nd | 8 | 0.00 | 0.00 | 2.16 | 0.00 | 23.76 | 8.22 | 44.81 | 46.59 | 137.52 | 148.41 | 4670 | 3070 |
| Effluent | nd | nd | 12 | 0.00 | 0.00 | 0.43 | 0.00 | 23.66 | 7.49 | 45.04 | 46.23 | 139.10 | 148.13 | nd | nd |

nd: not determined

Notes
Time: 138'
DO: 7.00

**Table B.16:** Detailed measurements data of propionate SBR (Run-3)

| t (min) | pH | $S_A$ | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Cl^-$ | $Na^{++}$ | $SO_4^{=}$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | nd | nd | nd | 45.47 | 21.53 | 0.33 | 0.00 | 27.15 | 14.39 | 77.36 | 32.72 | 43.99 | 143.33 | - | - |
| $V_0$ | nd | nd | 30 | 0.00 | 0.00 | 2.16 | 0.00 | 24.51 | 5.57 | 62.35 | 17.52 | 140.50 | 130.43 | nd | nd |
| 5 | nd | nd | 61 | 48.05 | 12.02 | 0.00 | 0.00 | 28.00 | 16.90 | 68.47 | 18.34 | 134.17 | 135.37 | nd | nd |
| 10 | nd | nd | 58 | 87.53 | 14.87 | 0.00 | 0.00 | 30.09 | 29.48 | 81.06 | 18.71 | 139.38 | 142.37 | nd | nd |
| 15 | 7.45 | nd | 85 | 98.60 | 16.11 | 0.00 | 0.00 | 29.30 | 30.36 | 78.59 | 19.42 | 136.44 | 139.63 | 4370 | 2600 |
| 30 | 7.36 | nd | 30 | 120.87 | 16.01 | 0.00 | 0.00 | 30.53 | 40.43 | 94.07 | 18.63 | 139.62 | 147.76 | 4390 | 2660 |
| 45 | 7.29 | nd | 30 | 121.53 | 15.73 | 0.00 | 0.00 | 30.82 | 39.93 | 101.42 | 18.86 | 138.53 | 148.60 | 4260 | 2590 |
| 60 | 7.26 | nd | 30 | 123.56 | 15.36 | 0.00 | 0.00 | 30.62 | 39.59 | 103.35 | 19.14 | 138.71 | 149.50 | 4300 | 2620 |
| 90 | 7.21 | nd | 30 | 121.88 | 15.34 | 0.00 | 0.00 | 31.05 | 39.13 | 105.50 | 19.16 | 138.60 | 147.81 | 4350 | 2650 |
| 120 | 7.18 | nd | 30 | 122.00 | 15.29 | 0.00 | 0.00 | 30.93 | 39.57 | 106.76 | 19.11 | 139.60 | 150.30 | 4300 | 2650 |
| 150 | 8.33 | nd | nd | 73.87 | 12.02 | 0.00 | 0.62 | 25.87 | 24.47 | 89.38 | 19.09 | 138.83 | 144.70 | 4800 | 2740 |
| 180 | 8.64 | nd | 34 | 30.89 | 9.13 | 0.27 | 0.54 | 21.94 | 16.19 | 82.26 | 19.79 | 138.80 | 141.91 | 5000 | 2760 |
| 210 | 8.78 | nd | nd | 13.09 | 6.77 | 0.66 | 1.14 | 20.31 | 9.44 | 71.84 | 19.73 | 138.11 | 138.88 | 5050 | 2780 |
| 240 | 8.76 | nd | 30 | 0.00 | 4.08 | 1.20 | 2.85 | 20.15 | 4.41 | 61.37 | 19.21 | 134.54 | 127.46 | 5250 | 2840 |
| 270 | 8.40 | nd | nd | 0.00 | 1.61 | 2.00 | 3.85 | 21.43 | 3.88 | 63.09 | 19.44 | 138.18 | 130.47 | 5350 | 2910 |
| 300 | 8.55 | nd | 23 | 0.00 | 0.00 | 2.65 | 3.25 | 21.51 | 3.71 | 63.41 | 19.58 | 138.00 | 133.78 | 5400 | 2910 |
| 1.stage | 7.58 | nd | nd | 0.00 | 0.00 | 2.67 | 3.15 | 22.32 | 3.97 | 62.81 | 19.45 | 141.70 | 147.79 | nd | nd |
| 2.stage | 7.02 | nd | nd | 0.00 | 0.00 | 2.33 | 1.16 | 22.99 | 4.22 | 61.24 | 18.44 | 140.81 | 165.80 | nd | nd |

nd: not determined

Notes

pH of the reactor adjusted two times following aeration period:

1st stage; pH adjusted to pH of about 8 with NaOH.

2nd stahe; pH adjusted to pH of about 7 with NaOH.

**Table B.17**: Detailed measurements data of propionate SBR (Run-4)

| t (min) | pH | $S_A$ | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Cl^-$ | $Na^{++}$ | $SO_4^=$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | nd | nd | 425 | 60.87 | 22.08 | 0.00 | 0.00 | 27.83 | 14.14 | 101.75 | 40.54 | 44.78 | 166.95 | - | - |
| $V_0$ | nd | nd | 24 | 0.32 | 0.00 | 2.01 | 0.00 | 19.03 | 4.12 | 85.08 | 44.84 | 116.22 | 159.31 | nd | nd |
| 5 | nd | nd | 113 | 81.91 | 12.00 | 0.91 | 0.00 | 33.64 | 20.61 | 106.48 | 50.25 | 125.41 | 160.77 | nd | nd |
| 10 | 7.13 | nd | 109 | 97.97 | 13.95 | 0.11 | 0.00 | 33.61 | 25.92 | 104.50 | 48.34 | 120.03 | 156.36 | nd | nd |
| 15 | 7.18 | nd | 81 | 107.06 | 15.64 | 0.00 | 0.00 | 31.49 | 29.07 | 105.93 | 43.56 | 110.74 | 158.98 | 4590 | 2340 |
| 30 | 7.17 | nd | 21 | 121.54 | 16.01 | 0.00 | 0.00 | 33.73 | 37.25 | 118.31 | 42.48 | 114.55 | 164.75 | 4450 | 2320 |
| 45 | 7.11 | nd | 28 | 125.84 | 16.03 | 0.00 | 0.00 | 33.95 | 36.75 | 126.49 | 42.39 | 113.56 | 165.12 | 4580 | 2360 |
| 60 | 7.10 | nd | 24 | 134.52 | 15.43 | 0.00 | 0.00 | 33.26 | 35.28 | 124.01 | 40.69 | 109.48 | 158.18 | 4480 | 2340 |
| 90 | 7.10 | nd | 21 | 135.56 | 15.78 | 0.00 | 0.00 | 34.85 | 36.00 | 135.33 | 42.37 | 113.26 | 166.17 | 4420 | 2400 |
| 120 | 7.10 | nd | 23 | 135.21 | 15.69 | 0.00 | 0.00 | 35.16 | 36.08 | 136.86 | 44.18 | 114.38 | 166.66 | 4580 | 2490 |
| 150 | 7.90 | nd | nd | 79.98 | 12.15 | 0.88 | 0.95 | 30.69 | 26.60 | 117.96 | 30.43 | 112.61 | 131.45 | 5420 | 2770 |
| 180 | 8.15 | nd | nd | 27.36 | 9.27 | 0.93 | 0.85 | 27.06 | 22.09 | 112.71 | 29.83 | 113.43 | 131.45 | 5450 | 2770 |
| 210 | 8.40 | nd | nd | 10.34 | 5.86 | 1.66 | 2.06 | 23.30 | 15.81 | 101.19 | 42.83 | 113.37 | 162.74 | 5680 | 2860 |
| 240 | 8.61 | nd | nd | 5.38 | 3.62 | 1.16 | 3.50 | 21.85 | 11.33 | 93.66 | 28.90 | 113.96 | 130.40 | 5740 | 2820 |
| 270 | 8.71 | nd | nd | 4.23 | 0.00 | 2.55 | 2.11 | 19.47 | 5.83 | 84.77 | 43.07 | 112.62 | 161.18 | 5780 | 2900 |
| 300 | 8.84 | nd | 21 | 0.00 | 0.00 | 2.56 | 3.01 | 18.82 | 3.43 | 82.80 | 43.10 | 114.00 | 159.96 | 5740 | 2820 |
| Effluent | nd | nd | nd | 0.20 | 0.00 | 1.69 | 2.85 | 18.53 | 3.46 | 81.97 | 45.96 | 113.79 | 159.03 | nd | nd |

nd: not determined

**Table B.18:** Detailed measurements data of propionate SBR (Run-5)

| t (min) | pH | $S_A$ | COD | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Cl^-$ | $Na^{++}$ | $SO_4^=$ | MLSS | MLVSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L |
| Influent | nd | nd | 435 | 60.10 | 22.20 | 0.00 | 0.00 | 27.11 | 13.45 | 106.14 | 43.88 | 41.93 | 207.99 | - | - |
| $V_0$ | nd | nd | 111 | 2.01 | 0.00 | 1.17 | 0.00 | 16.35 | 6.05 | 95.93 | 48.82 | 148.87 | 208.53 | nd | nd |
| 5 | 7.42 | nd | 104 | 91.66 | 11.85 | 0.00 | 0.00 | 33.34 | 19.58 | 115.84 | 50.91 | 150.89 | 230.23 | nd | nd |
| 10 | 7.29 | nd | 130 | 98.26 | 14.76 | 0.00 | 0.00 | 33.19 | 22.77 | 117.01 | 53.52 | 151.18 | 223.35 | nd | nd |
| 15 | 7.20 | nd | 37 | 82.72 | 16.40 | 0.00 | 0.00 | 33.42 | 25.54 | 120.22 | 52.16 | 150.82 | 219.04 | 5670 | 3300 |
| 30 | 7.12 | nd | 67 | 126.54 | 15.42 | 0.00 | 0.00 | 31.88 | 32.44 | 131.68 | 46.48 | 145.40 | 216.28 | 4650 | 2750 |
| 45 | 7.16 | nd | 30 | 139.54 | 15.81 | 0.00 | 0.00 | 35.47 | 36.14 | 149.37 | 47.95 | 165.52 | 254.36 | 4660 | 2760 |
| 60 | 7.12 | nd | 25 | 141.02 | 15.60 | 0.00 | 0.00 | 36.28 | 35.01 | 149.98 | 46.51 | 154.17 | 232.01 | 4560 | 2750 |
| 90 | 7.06 | nd | 22 | 141.11 | 15.63 | 0.00 | 0.00 | 38.85 | 35.62 | 160.91 | 47.83 | 151.54 | 220.96 | 4400 | 2700 |
| 120 | 7.09 | nd | 25 | 141.16 | 15.58 | 0.00 | 0.00 | 37.49 | 35.11 | 158.54 | 47.41 | 148.86 | 223.25 | 4360 | 2650 |
| 150 | 7.83 | nd | 20 | 98.21 | 12.44 | 0.29 | 1.77 | 33.63 | 28.44 | 139.62 | 48.11 | 147.29 | 219.21 | 5220 | 3100 |
| 180 | 8.07 | nd | 20 | 38.50 | 9.28 | 0.78 | 2.42 | 29.65 | 23.79 | 131.52 | 48.56 | 149.59 | 218.41 | 5720 | 3350 |
| 210 | 8.26 | nd | 20 | 17.20 | 6.06 | 1.27 | 3.74 | 24.23 | 18.19 | 118.49 | 47.87 | 146.52 | 212.72 | 5660 | 3250 |
| 240 | 8.48 | nd | 20 | 7.32 | 3.39 | 1.82 | 4.91 | 22.70 | 14.31 | 108.19 | 47.70 | 147.00 | 210.98 | 5680 | 3200 |
| 270 | 8.91 | nd | 20 | 3.25 | 1.83 | 2.75 | 4.05 | 19.81 | 10.49 | 104.53 | 48.46 | 149.86 | 209.69 | 5600 | 3150 |
| 300 | 9.02 | nd | 20 | 1.38 | 0.00 | 3.56 | 3.22 | 16.68 | 6.01 | 92.78 | 49.40 | 148.22 | 208.19 | 5620 | 3150 |
| Effluent | nd | nd | 27 | 1.28 | 0.00 | 3.60 | 3.02 | 16.64 | 6.27 | 93.79 | 49.36 | 147.33 | 208.41 | nd | nd |

nd: not determined

Notes
SVI= 62 ml/g

# APPENDIX C: BATCH TEST DATA

**Table C.1:** Batch test data - sludge from acetate SBR (100% acetate and 50%acetate+50%propionate addition)

| | t | $PO_4$-P | $NH_4$-N | $NO_3$-N | $NO_2$-N | $Ca^{2+}$ | $Mg^{2+}$ | $K^+$ | $Cl^-$ | $Na^{2+}$ | $SO4^{=}$ | MLSS | MLVSS | VSS/SS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | mg/L | % |
| Vo | | 0.00 | 0.00 | 1.53 | 0.00 | 27.05 | 9.82 | 31.98 | 45.44 | 196.09 | 210.85 | 9370 | 5900 | 63 |
| 1) 100% Acetate Addition | | | | | | | | | | | | | | |
| Influent | | 17.07 | 24.21 | 0.00 | 0.00 | 26.47 | 14.98 | 36.16 | 46.28 | 245.57 | 392.77 | | | |
| Initial | 0 | 11.28 | 15.13 | 0.56 | 0.00 | 26.41 | 12.95 | 34.26 | 45.50 | 224.97 | 322.35 | | | |
| Anaerobic End | 120 | 108.58 | 16.74 | 0.00 | 0.00 | 31.07 | 43.21 | 78.83 | 42.08 | 225.74 | 335.19 | 4160 | 3140 | 75 |
| Aerobic Phase (2. h) | 240 | 0.00 | 0.00 | 5.72 | 1.06 | 24.36 | 9.71 | 34.65 | 43.74 | 2222.78 | 323.65 | 4420 | 2960 | 67 |
| Aerobic End | 300 | 0.00 | 0.00 | 7.64 | 0.00 | 25.46 | 8.85 | 34.92 | 44.46 | 224.23 | 346.37 | 4890 | 3030 | 62 |
| *Release* | | *97.30* | | | | *4.66* | *30.26* | *44.57* | | | | | | |
| *Uptake* | | *108.58* | | | | *5.61* | *34.36* | *43.91* | | | | | | |
| *Uptake/Release* | | *1.12* | | | | *1.20* | *1.14* | *0.99* | | | | | | |
| 2) 50% Acetate + 50% Propionate Addition | | | | | | | | | | | | | | |
| Influent | | 16.46 | 24.15 | 0.00 | 0.00 | 25.77 | 14.71 | 38.28 | 49.37 | 251.46 | 475.51 | | | |
| Initial | 0 | 10.89 | 15.09 | 0.56 | 0.00 | 25.97 | 12.77 | 35.59 | 47.42 | 228.66 | 374.06 | | | |
| Anaerobic End | 120 | 106.78 | 16.72 | 0.00 | 0.00 | 30.19 | 38.06 | 74.25 | 41.75 | 229.31 | 391.60 | 4000 | 2880 | 72 |
| Aerobic Phase (2. h) | 240 | 0.00 | 0.00 | 6.36 | 0.00 | 26.10 | 8.45 | 35.43 | 42.13 | 229.51 | 377.33 | 4370 | 2910 | 66 |
| Aerobic End | 300 | 0.00 | 0.00 | 7.33 | 0.00 | 27.70 | 8.34 | 37.79 | 42.27 | 231.42 | 382.99 | 4710 | 2990 | 63 |
| *Release* | | *95.90* | | | | *4.22* | *25.29* | *38.66* | | | | | | |
| *Uptake* | | *106.78* | | | | *2.49* | *29.72* | *36.46* | | | | | | |
| *Uptake/Release* | | *1.11* | | | | *0.59* | *1.18* | *0.94* | | | | | | |

**Table C.2:** Batch test data - sludge from acetate SBR (75% acetate+25% propionate and 25%acetate+75%propionate addition)

| | t min | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L | VSS/SS % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vo | | 0.00 | 0.00 | 1.53 | 0.00 | 27.05 | 9.82 | 31.98 | 45.44 | 196.09 | 210.85 | 9370 | 5900 | 63 |
| 3) 75% Acetate + 25% Propionate Addition | | | | | | | | | | | | | | |
| Influent | | 16.22 | 24.22 | 0.00 | 0.00 | 25.58 | 14.75 | 38.54 | 46.20 | 256.84 | 434.99 | | | |
| Initial | 0 | 10.72 | 15.14 | 0.56 | 0.00 | 25.85 | 12.80 | 35.75 | 45.44 | 232.02 | 348.74 | | | |
| Anaerobic End | 120 | 112.24 | 16.76 | 0.00 | 0.00 | 29.94 | 40.91 | 75.78 | 41.47 | 234.87 | 379.44 | 2690 | 2020 | 75 |
| Aerobic Phase (2.h) | 240 | 0.00 | 0.00 | 5.08 | 0.00 | 27.04 | 9.32 | 35.51 | 42.93 | 235.12 | 366.18 | 3450 | 2310 | 67 |
| Aerobic End | 300 | 0.00 | 0.00 | 6.82 | 0.00 | 25.47 | 8.90 | 34.88 | 42.03 | 236.08 | 367.24 | 3870 | 2440 | 63 |
| *Release* | | *101.51* | | | | *4.09* | *28.11* | *40.03* | | | | | | |
| *Uptake* | | *112.24* | | | | *4.47* | *32.01* | *40.90* | | | | | | |
| *Uptake/Release* | | *1.11* | | | | *1.09* | *1.14* | *1.02* | | | | | | |
| 4) 25% Acetate + 75% Propionate Addition | | | | | | | | | | | | | | |
| Influent | | 16.46 | 24.20 | 0.00 | 0.00 | 25.56 | 14.70 | 38.50 | 50.87 | 185.17 | 325.35 | | | |
| Initial | 0 | 10.89 | 15.13 | 0.56 | 0.00 | 25.84 | 12.77 | 35.72 | 48.36 | 187.22 | 280.21 | | | |
| Anaerobic End | 120 | 98.32 | 16.09 | 0.00 | 0.00 | 30.34 | 35.73 | 69.54 | 41.46 | 189.89 | 302.28 | 3720 | 2750 | 74 |
| Aerobic Phase (2.h) | 240 | 0.00 | 0.00 | 5.47 | 0.00 | 26.24 | 8.35 | 35.75 | 42.53 | 190.93 | 294.86 | 4220 | 2870 | 68 |
| Aerobic End | 300 | 0.00 | 0.00 | 6.51 | 0.00 | 27.45 | 8.28 | 37.54 | 41.97 | 193.38 | 296.01 | 4760 | 3000 | 63 |
| *Release* | | *87.44* | | | | *4.50* | *22.96* | *33.82* | | | | | | |
| *Uptake* | | *98.32* | | | | *2.89* | *27.45* | *32.00* | | | | | | |
| *Uptake/Release* | | *1.12* | | | | *0.64* | *1.20* | *0.95* | | | | | | |

**Table C.3:** Batch test data - sludge from propionate SBR (100% acetate and 50%acetate+50%propionate addition)

| | t min | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^{+}$ mg/L | $Cl^{-}$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L | VSS/SS % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vo | | 0.00 | 0.00 | 0.09 | 0.00 | 26.32 | 13.01 | 26.14 | 47.77 | 146.91 | 237.54 | 13560 | 8880 | 66 |
| 1) 100% Acetate Addition | | | | | | | | | | | | | | |
| Influent | | 20.50 | 22.37 | 0.00 | 0.00 | 24.97 | 17.19 | 45.64 | 47.27 | 177.87 | 221.23 | | | |
| Initial | 0 | 13.55 | 13.98 | 0.03 | 0.00 | 25.20 | 15.49 | 38.06 | 46.96 | 164.73 | 224.87 | | | |
| Anaerobic End | 120 | 133.52 | 16.15 | 0.00 | 0.00 | 29.75 | 49.17 | 90.88 | 43.65 | 164.60 | 237.37 | 4100 | 3100 | 73 |
| Aerobic Phase (2.h) | 240 | 0.00 | 1.97 | 1.00 | 0.00 | 20.98 | 12.36 | 32.07 | 43.85 | 160.64 | 224.96 | 4240 | 2900 | 68 |
| Aerobic End | 300 | 0.00 | 0.00 | 1.74 | 0.00 | 23.05 | 11.06 | 35.72 | 43.70 | 159.99 | 224.28 | 4410 | 2990 | 68 |
| *Release* | | *119.97* | | | | *4.55* | *33.68* | *52.82* | | | | | | |
| *Uptake* | | *133.52* | | | | *6.70* | *38.11* | *55.16* | | | | | | |
| *Uptake/Release* | | *1.11* | | | | *1.47* | *1.13* | *1.04* | | | | | | |
| 2) 50% Acetate + 50% Propionate Addition | | | | | | | | | | | | | | |
| Influent | | 18.57 | 20.53 | 0.00 | 0.00 | 22.31 | 15.08 | 37.20 | 51.28 | 157.32 | 233.60 | | | |
| Initial | 0 | 12.28 | 12.83 | 0.03 | 0.00 | 23.54 | 14.17 | 32.78 | 49.47 | 151.89 | 232.61 | | | |
| Anaerobic End | 120 | 116.62 | 15.99 | 0.00 | 0.00 | 28.15 | 43.12 | 75.31 | 44.13 | 151.92 | 244.12 | 3730 | 2720 | 73 |
| Aerobic Phase (2.h) | 240 | 0.00 | 0.52 | 1.29 | 0.00 | 22.78 | 11.29 | 33.56 | 44.76 | 151.60 | 232.05 | 4160 | 2840 | 68 |
| Aerobic End | 300 | 0.00 | 0.00 | 1.79 | 0.00 | 22.53 | 10.85 | 35.30 | 44.24 | 158.62 | 225.31 | 4290 | 2900 | 68 |
| *Release* | | *104.34* | | | | *4.61* | *28.95* | *42.53* | | | | | | |
| *Uptake* | | *116.62* | | | | *5.62* | *32.27* | *40.01* | | | | | | |
| *Uptake/Release* | | *1.12* | | | | *1.22* | *1.11* | *0.94* | | | | | | |

**Table C.4:** Batch test data - sludge from propionate SBR (75% acetate+25%propionate and 25%acetate+75%propionate addition)

| | t min | $PO_4$-P mg/L | $NH_4$-N mg/L | $NO_3$-N mg/L | $NO_2$-N mg/L | $Ca^{2+}$ mg/L | $Mg^{2+}$ mg/L | $K^+$ mg/L | $Cl^-$ mg/L | $Na^{2+}$ mg/L | $SO4^{=}$ mg/L | MLSS mg/L | MLVSS mg/L | VSS/SS % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vo | | 0.00 | 0.00 | 0.09 | 0.00 | 26.32 | 13.01 | 26.14 | 47.77 | 146.91 | 237.54 | 13560 | 8880 | 66 |
| 3) 75% Acetate + 25% Propionate Addition | | | | | | | | | | | | | | |
| Influent | | 18.33 | 21.03 | 0.00 | 0.00 | 23.49 | 16.54 | 39.52 | 50.50 | 171.64 | 231.83 | | | |
| Initial | 0 | 12.13 | 13.14 | 0.03 | 0.00 | 24.28 | 15.08 | 34.23 | 48.98 | 160.83 | 231.50 | | | |
| Anaerobic End | 120 | 123.69 | 15.53 | 0.00 | 0.00 | 27.89 | 45.29 | 82.91 | 45.45 | 152.52 | 244.65 | 3580 | 2580 | 72 |
| Aerobic Phase (2.h) | 240 | 0.00 | 2.14 | 0.99 | 0.00 | 22.21 | 12.63 | 32.81 | 43.92 | 160.61 | 225.84 | 3930 | 2610 | 66 |
| Aerobic End | 300 | 0.00 | 0.00 | 1.57 | 0.00 | 23.56 | 11.10 | 34.69 | 44.06 | 150.98 | 228.41 | 3990 | 2710 | 68 |
| *Release* | | *111.57* | | | | *3.61* | *30.21* | *48.68* | | | | | | |
| *Uptake* | | *123.69* | | | | *4.33* | *34.19* | *48.22* | | | | | | |
| *Uptake/Release* | | *1.11* | | | | *1.20* | *1.13* | *0.99* | | | | | | |
| 4) 25% Acetate + 75% Propionate Addition | | | | | | | | | | | | | | |
| Influent | | 17.09 | 21.31 | 0.01 | 0.00 | 23.59 | 16.27 | 40.79 | 49.08 | 168.94 | 228.56 | | | |
| Initial | 0 | 11.30 | 13.32 | 0.04 | 0.00 | 24.34 | 14.91 | 35.02 | 48.09 | 159.15 | 229.45 | | | |
| Anaerobic End | 120 | 113.50 | 15.88 | 0.00 | 0.00 | 29.14 | 42.75 | 74.50 | 46.08 | 152.84 | 246.50 | 5100 | 3570 | 70 |
| Aerobic Phase (2.h) | 240 | 0.00 | 1.56 | 1.57 | 0.55 | 23.51 | 11.39 | 32.66 | 45.44 | 145.45 | 233.85 | 5430 | 3640 | 67 |
| Aerobic End | 300 | 0.00 | 0.00 | 1.70 | 0.00 | 23.50 | 11.04 | 35.48 | 44.55 | 150.97 | 228.64 | 5360 | 3700 | 69 |
| *Release* | | *102.20* | | | | *4.80* | *27.84* | *39.48* | | | | | | |
| *Uptake* | | *113.50* | | | | *5.64* | *31.71* | *39.02* | | | | | | |
| *Uptake/Release* | | *1.11* | | | | *1.18* | *1.14* | *0.99* | | | | | | |

## CURRICULUM VITA

Nevin Yağcı was born in 1971 in Haskova, Bulgaria. She received a Bachelor of Science degree in Environmental Engineering in 1993 and Master of Science degree in Environmental Engineering in 1996 from Istanbul Technical University. She first started to work as a research associate at the Department of Environmental Engineering Department in Trakya University. She is working in the same position in Istanbul Technical University since 1997. Upon the completion of her Masters degree, she continued at the same department where she completed her Ph.D. studies in Environmental Engineering in the autumn semester of 2004.

She won research scholarship from TINCEL Foundation and studied on biological phosphorus removal under supervision of Prof. Clifford W. Randall at the Department of Civil and Environmental Engineering at Virginia Tech. She also studied on biocides effect on biofilm formation at the Cardiff University in UK under supervision of Julian Wimpenny with a scholar of "*Long Term Research Program*" that is supported by Istanbul Technical University.

Her major research and professional interests is on biotechnology for nutrient removal and modeling. She has so far published 8 scientific papers in journals indexed by SCI. She attended to "10. International Environmental Pollution and Its Impact on Life in the Mediterranean Region" in 1999, "The 5th Specialized Conference on Small Water And Wastewater Treatment Systems" in 2002, "International Conference on Environmental Problems of the Mediterranean Region" in 2002, and "International Conference on Wastewater Treatment For Nutrient Removal & Reuse" in 2004 as a presenter and "Final Meeting on COST Action 624 on Optimal Management of Wastewater Systems" in 2004. She also authored 5 research report including results of projects supported by *Scientific and Research Council of Turkey* (TUBITAK). She has been the editor of proceedings published with papers presented in technical conference on "National Industrial Pollution Control" in Turkish.

She is a member of "National Water Pollution and Control Committee" and working for "Journal of Water Pollution Control" as a member of group of preparation for publication.