İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

EFFECTS OF INTERMITTENT FEEDING CONDITIONS ON MICROBIAL GROWTH KINETICS

M.Sc. Thesis by
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Programme : Environmental Biotechnology

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DEC 2010

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Date of submission : **07 May 2010**
Date of defence examination: **27 December 2010**

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MAY 2010

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

KESİKLİ BESLEME KOŞULLARININ MİKROBİYAL ÇOĞALMA KİNETİĞİ ÜZERİNE ETKİLERİ

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Tezin Enstitüye Verildiği Tarih : 07 Mayıs 2010
Tezin Savunulduğu Tarih : 27 Aralık 2010

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MAYIS 2010

FOREWORD

I would like to express my deepest appreciation and thanks; to my advisor, Assoc. Prof. Dr. Güçlü İNSEL for his valuable guidance and advice; Prof. Dr. Emine Ubay ÇOKGÖR for his valuable suggestions and encouragement; İlke PALA and Tuğçe KATİPOĞLU for their assistance in the laboratory and also for sharing information with me. I would like to express my gratitude to Murat Cem ERTÜRK for his valuable aids during this painful process. Last but not least, I would like to thank Filiz EREZ for supporting me with her endless love, patience and understanding.

May 2010

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ABBREVIATIONS

ADP **:** Adenosine Di-Phosphate
ARP : Available Reaction Potential
ATP **:** Adenosine Tri-Phosphate
COD **:** Chemical Oxygen Demand
DNA **:** Deoxyribonucleic acid
EMP **:** Embden–Meyerhof-Parnas
GRH **:** Growth Rate Hysteresis
OUR **:** Oxygen Uptake Rate
PHB **:** Polyhydroxybutyrate
PSS **:** Protein Synthesis System
RNA **:** Ribonucleic acid
SS **:** Suspended Solids
TCA **:** Tricarboxylic acid
VSS **:** Volatile Suspended Solids

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EFFECTS OF INTERMITTENT FEEDING CONDITIONS ON MICROBIAL GROWTH KINETICS

SUMMARY

The Monod Kinetic Theory of steady state microbial growth on substrates is used to predict the performance of biochemical unit processes. However, the inputs to the most wastewater treatment systems are time-variant, indicating that the steady states are rarely achieved. Furthermore activated sludge systems are alternately exposed to environments with alternating substrate availability. Consequently, their metabolic state is in a constant state of flux that cannot truly be represented by a steady state theory. The difference between the experimental data and simulation results occurs because those circumstances. This difference which is called transient response can be observed especially at the starting period of the respirometric analysis. The transient response cannot be explained by the Monod Kinetic Theory using the same set of kinetic and stoichiometric model parameters.. Hence, it is difficult to evaluate transient response with standard modelling approach. Mechanisms such as enzymatic response, substrate uptake rate and storage must be integrated in the models for a better assessment of the growth kinetics of biomass subjected to variable short-term feeding conditions.

In order to eliminate this difference, there is a need for a better understanding of the dynamics of microbial growth on biodegradable substrate with taking substrate uptake rate and storage materials into account by incorporating those variables in the mathematical models. In this study, growth and storage kinetics of a biomass sample exposed to feast and famine conditions monitored.

The results showed that repeated substrate additions to the biomass at endogenous respiration phase showed different OUR profiles. The difference in the profiles can be seen at the initial responses. Another difference is observed during the depletion of substrate.

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ÖZET

Biyokimyasal proseslerin denge koşulları altındaki performanslarını belirlemek için Monod tarafından geliştirilen kolay ayrışabilir substrat üzerinden mikrobiyal büyüme kinetiği kullanılmaktadır. Ancak birçok arıtma sistemine giren kirletici yükü zamana bağlı olarak değişkendir. Sistem denge koşullarına nadiren ulaşır. Ayrıca aktif çamur sistemleri sürekli değişen koşullara maruz kalmaktadır. Bu nedenle mikroorganizmaların metabolizmaları sürekli değişim halindedir ve bu durum denge koşulları altında geçerli olan Monod kinetiği ile açıklanamamaktadır. Yukarıda belirtilen nedenlerden dolayı simülasyon sonuçları ile deneysel veriler arasında farklılıklar gözlenmektedir. Özellikle deneyin başlangıcında gözlemlenen bu farklılıklara "geçici tepki (transient response)" adı verilmektedir. Monod Kinetik Teorisi bakterilerin geçici tepkisini açıklamakta yetersiz kalmaktadır. Bu nedenle standard modelleme yaklaşımı ile büyüme kinetiğinin değerlendirilmesi zor olmaktadır. Büyüme kinetiğinin daha iyi anlaşılabilmesi için enzimatik tepkiler, substrat alım hızı ve depolama gibi mekanizmalar da modellerin yapısına entegre edilmelidir.

Bu farklılığı ortadan kaldırmak için kolay ayrışabilir substrat üzerinden mikrobiyal büyüme dinamiğinin bakterinin substrat alım hızı ve depolama ürünlerinin kullanılmasının da modele birlikte dahil edilmesi gerekmektedir. Bu çalışmanın genel amacı, değişen besleme koşulları altında bakterilerin metabolik değişiminin incelenerek mikrobiyal büyüme dinamiğinin daha iyi anlaşılmasını sağlamaktır.

İçsel solunum yapmakta olan biyokütleye yapılan arka arkaya yapılan beslemelerde oluşan oksijen kullanım hızı profilleri farklılık göstermektedir. Bu farklılıklar ilk beslenme anında ve substratın tamamen tükenmesi sırasında görülebilmektedir.

1 INTRODUCTION

The kinetic theory of steady state microbial growth on soluble substrates is well developed and routinely used to predict the steady-state performance of biochemical unit processes in wastewater treatment systems. However the inputs to most wasewater treatment systems are time-variant, indicating that steady-states are seldom achieved. Consequently, realistic prediction of the performance of the biochemical unit processes requires that the dynamic response of the microbial culture to be consiederd. Even when the inputs to and outputs form a biochemical processes are constant, steady-state kinteics may not truely reflect what is occuring within the system. In several plant configurations, the biomass grows under transient (unsteady) conditions, even though the overall process can be considered under steady-state conditions. In configurations where a concentration gradient of the substrate is produced (like plug-flow configuration of the aeration tanks) a transient condition is introduced for the biomass. To a greater extent, transient conditions are typical for configurations where biomass experiences alternately (vs time or flow direction) high and low substrate concentrations, like in sequencing batch reactors, intermittently-fed reactors, contact-stabilization processes or selectors for bulking control.

Under such transient conditions, growth becomes unbalanced and the storage of substrates as internal polymers becomes of importance as a mechanism of response to the transient condition. When the biomass experiences a sudden increase of the available substrate (the transient condition which causes unbalanced growth) two main types of physiological adaptation can occur, depending on the nature of the biomass, on the nature of the substrate and on operating conditions (Daigger and Grady, 1982a): the biomass can adapt itself to the new conditions by increasing the growth rate from the previous level (growth response) and/or by rapidly storing the available substrate (storage response). Even though the substrate uptake rate is increased and new solids are formed in both cases, the solid composition and the observed yield are different. Because the storage response is faster (less

physiological adaptation is required), the more the microorganisms are able to store substrates during imposed transient conditions and subsequently reuse them for growth, the more they have a competitive advantage.

Substrate storage under dynamic conditions is regarded as a significant process for activated sludge systems. Substrate concentration gradients are always present in wastewater treatment plants, which are caused by the dynamic conditions due to changes in wastewater composition, treatment plant scheme, reactor hydraulics and variations in the modes of operation of treatment plants. Activated sludge culture adapts to the dynamic conditions by storing the substrate when available and surviving on the stored substrate when external substrate is not present.

Altough substrate storage response was already a known phenomena, its signifcance in the modelling and design of activated sludge systems has recently been recognised. Substrate storage is incorporated into activated sludge modelling with Avtivated Sludge Model No. 3 (ASM3) It was explained as all readily biodegradable substrate is first being stored inside the cells and then storage products being reused for growth without addition of external substrate under both aerobic and anoxic conditions. The experiments conducted in activated sludge systems, operating under dynamic conditions (sequencing batch reactors and aeration tanks with plug-flow configuration or with selector for bulking control); indicate that storage of internal polymers is usually the main mechanism for the removal of readily biodegradable carbon sources.

1.1 Aim of the Thesis

The aim of this thesis is to fill the gap in the literature regarding the substrate uptake rate and storage materials during transient response using experimental data. The experimental data will be used for development of a new model in order to explain transient phenomena.

1.2 Scope of the Thesis

In this study, the activated sludge taken from a lab-scale reactor fed with acetate is used for acclimation in order to assess the effects of intermittent feeding conditions on microbial growth kinetics. The acclimation process was monitored through respirometric studies. In parallel chemical oxygen demand, suspended solids, volatile suspended solids analysis were performed. The acclimation process and experimental studies were repeated for activated sludge in a rapidly growing system having smaller sludge age, which is 2 days. After the acclimation process, intermittent feeding conditions were applied during respirometric studies with different F/M ratios. The response of activated sludge is observed during respirometric studies by monitoring soluble COD, PHB and VSS measurements. The same procedure is followed for a fast growing system with a sludge age of 2 days. The results of batch tests will be used to develop dynamic mathematical model characterizing the short term response of acclimated biomass.

2 LITERATURE REVIEW

2.1 Definition of Storage

It is generally assumed that carbon sources are used for growth and respiration (Gujer and Henze, 1991), however accumulation of internal storage polymers was observed in a number of studies (Van den Eynde *et al*., 1984).

Activated sludge systems are highly dynamic with respect to the feeding regime. Wastewater treatment processes consist of feast and famine periods and this regime causes substrate storage in the system. These concepts were explained as: when external carbon source is available only short periods of time, microorganisms consume substrate and produce storage polymers in feast period whereas no external substrate is found and consumption of stored polymer occurs for growth in famine period (van Loosdrecht *et al*., 1997). If there was no external substrate, bacteria would undergo long starvation periods. Owing to storage ability, microorganisms have a competitive advantage over the other bacteria without the capacity of substrate storage. If the bacteria can not store the substrate; extra energy is needed for rapid growth in feast period. In case of all substrate being taken up, maintenance problems will occur in bacteria to keep the proper shape of cell structure when new substrate is added to the system. Bacteria which can store substrate will be dominant since more or less constant relatively low growth rate can be maintained and the viability of the cells is conserved in case of external substrate depletion.

Formations of storage polymers rely on the external carbon source. Mainly three types of organic storage polymers were reported as PHB (poly-β-hydroxy butyrate), glycogen and lipids (Zevenhuisen and Ebbnik, 1974). These polymers are considered as carbon and energy stores with respect to endogenous substrates of bacteria. Lipids which present constant amounts in all bacteria usually do not function as carbon and energy stores and have to be accepted as structural components of membranes as phospholipids and of cell walls as lipopolysaccharides.

Many substrates are degraded into acetyl-CoA as intermediate in the cell. Since PHB is directly formed from the central metabolite Acetyl-CoA (Doi, 1990), it is considered the most important storage polymer. PHAs (Poly hydroxyl alkanoates) are accumulated by most bacteria as carbon and energy storage material when a carbon source is available in excess or when other nutrients are limiting. Storage Polymers can be depolymerized and then metabolized as carbon and energy source if limited nutrient is provided (Merrick and Doudoroff, 1964).

The formation of storage polymers is a newly introduced phenomenon to standard aerobic and anoxic COD removal processes. Recent studies with respirometry and pure substrates gave obvious results showing the formation of storage polymers. Amount of oxygen utilized per amount of substrate removed is lower in mixed cultures than the yield found in pure culture studies, which indicates the presence of storage process.

2.2 Formation of Storage Polymers

Storage polymers are formed while excess amount of substrate which can not be used for growth and maintenance process is available. In other words, since the uptake rate of external substrate exceeds the conversion rates of these substrates into cell components, storage is occurs.

It is reported that when activated sludge culture is fed with volatile fatty acids (VFAs) the storage polymers are PHAs and if the system is fed with only acetate the PHA formed is mostly poly-β-hydroxy butyrate (PHB). When activated sludge is fed with glucose the storage polymer is reported as glycogen (Majone et al., 1999).

PHB is present as granules enclosed by a membrane in the cytoplasm of the cells. The granules of PHB have typical diameters of 0.2 to 0.5 μm. The composition of PHB is shown in **Figure 2.1** below.

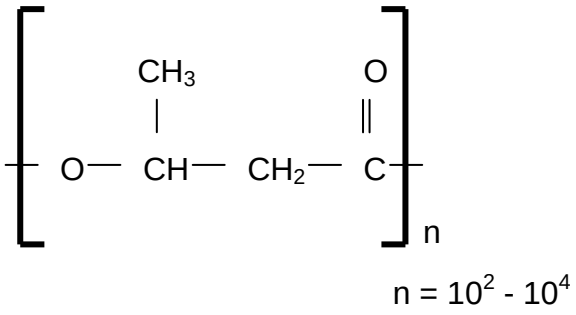

Figure 2.1 : The composition of PHB

PHB is synthesized with a cyclic metabolic pathway (Dawes and Senior, 1973; Doi, 1990). Acetate is used as a model substrate since this compound is one of the most important substrates in wastewater. After bacteria adapts to acetate, it is converted into acetyl-CoA. While produced acetyl-CoA is primarily used in TCA cycle to produce ATP, $NADH_2$, the remaining acetyl-CoA molecules are coupled to form of acetoacetyl-CoA. This condensation reaction is catalysed by β-ketothiolase and free coenzyme A (CoA) is released. The acetoacetyl-CoA is reduced to (R)-3-hydroxybutyryl-CoA. This reaction is catalysed by NADPH-dependent acetoacetyl-CoA reductase and (R)-3-hydroxybutyryl-CoA molecules synthesize PHB by PHB synthesis and free CoA is released at last. The cycle of PHB synthesis is shown in **Figure 2.2**.

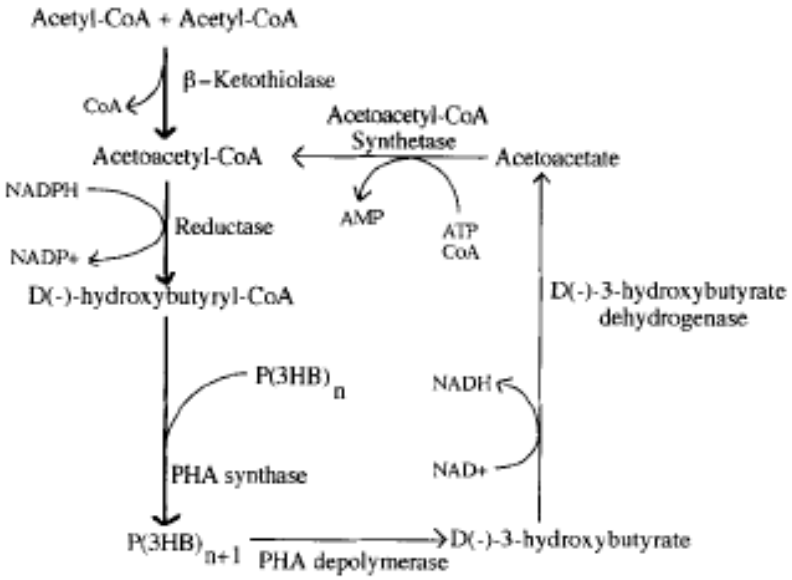

Figure 2.2 : PHB synthesis

When PHB is used by biomass while no external substrate is available, PHB is converted to D(-)-3-hydroxybutyrate by PHB depolymerase and then produced D(-)-3-hydroxybutyrate is converted to acetoacetate by D(-)-3-hydroxybutyrate dehydrogenase. Finally, acetoacetyl-CoA is formed by Acetoacetyl-CoA synthetase. Metabolic pathway involved in the synthesis and degradation of PHB require energy and reducing power.

The reaction of acetoacetyl-CoA formation is not preferred due to the equilibrium constant of 6.10^{-5} (Reich and Sel'kov, 1981). When excess amount acetate is available, the acetyl-CoA is produced. Therefore, equilibrium reaction of β-ketothiolase is derived into the way of PHB synthesis. Similarly, since increases of acetyl-CoA are lead to increases of ratio acetyl-CoA/ free CoA, bacteria produce more anabolic enzymes and then increase their growth rate which is called growth response (Daigger and Grady, 1982b).

When the excess external substrate is available in a long period, the specific growth rate of biomass will be increase and the PHB synthesis rate will be decreased. On the contrary, if the period of excess external substrate availability is faster than time needed for the growth of biomass, PHB synthesis rate will be increase while growth rate will not be affected. For this reason, when bacteria can not adapt the physiological state to fast availability of substrate, storage mechanism is used to keep growth rate constant with time.

The regulation of PHB synthesis is controlled at the enzymatic level (Senior and Dawes, 1971) and mainly depends on the intracellular concentration of acetyl-CoA and free coenzyme A (Haywood *et al*., 1988). It was reported that high intracellular concentration of NAD(P)H and high ratios of NAD(P)H/ NAD(P) arouse PHB synthesis (Lee *et al*., 1995). Also, high amount of NADH and NADPH inhibit the citrate synthase in TCA cycle and then it is possible that all acetyl-CoA will be available for PHB synthesis. According to this opinion, PHB synthesis process is controlled with citrate synthase since PHB accumulation is improved by the metabolic flux of acetyl-CoA to the PHB synthesis pathway (Henderson and Jones, 1997).

According to these phenomena, a numerous pure culture studies were conducted. It is observed that the floc forming bacteria, A. globiforbis showed a larger overcapacity for substrate uptake, a larger accumulation of storage polymers and a more efficient

mobilization of these polymers than the filamentous species *Spaerotilus natans* (van den Eynde *et al*., 1984). Intermittently fed activated sludge systems had a low SVI and high storage capacity and continuously fed systems had a high SVI and low capacity for the accumulation of storage polymers. Whenever an amount of substrate is suddenly added to the steady state cultures, there is an immediate increase in substrate uptake rate, the growth rate (μ) not being able to switch to higher values. It was observed that pulse feeding has a profound influence on the metabolism of the bacteria (van den Eynde *et al*., 1983). Where the Monod equation would suggest an immediate increase of μ_{max} with higher concentrations of substrate, a completely different behavior was found. Very small or no immediate increase in growth rate can be explained in numbers of ribosomes engaged in protein synthesis. The μ_{max} can only be attained after a lag phase during which RNA and more ribosomes must be synthesized. According to this mechanism the initial substrate uptake rate is too high and the excess of substrate is accumulated as reserves or excreted as overflow metabolites. The main features of *A. globiformis* and *Spaerotilus natans* during a shift-up were the high substrate uptake rates compared to the previous growth rate (overcapacity), the lag phase in protein synthesis and the metabolism of reserve materials.

2.3 Storage in Activated Sludge Processes

The behavior of bacteria with respect to carbon source is the main subject in activated sludge process. Bacteria store carbon source and then growth under dynamic conditions while overall process is considered as steady state (Majone et al, 1999).

Microorganism adapts its cell composition (RNA, protein etc.) to the environment and reaches to a balanced growth, which shows that growth of cell is in optimum level and no further adaptation occurs (Roels, 1983; Grady *et al*, 1996). If the culture is adopted to grow under a substrate-limited condition, available protein synthesis of the culture will not be enough to increase the growth rate when the limitation is removed. Therefore the protein synthesis and the specific growth rate will increase only gradually. However, if the adaptation of the culture to the old environment is not complete, there will be an extra protein synthesis available so the culture will increase its specific growth rate.

Storage process is very significant in activated sludge systems under dynamic conditions. In storage response, substrate uptake rate increases quickly and new solids are formed. While all components are formed during growth, mainly storage polymers (generally polysaccharides and lipids) are formed during storage. This leads to different kinetic and stoichiometric coefficients because synthesis of storage polymers is simpler than that of the whole cell due to less physiological adaptation is required and also storage is faster than growth.

2.4 Modeling Activated Sludge Storage

Several metabolic models have proposed that enmeshment, sorption and accumulation as dominant mechanisms instead of storage process. While these models have shown that very little or no energy was required for these processes, storage process requires significant amount of energy for removal of soluble substrate.

Activated Sludge Model No. 3, ASM3, (Gujer *et al.*, 2000) proposed that all readily biodegradable substrate is firstly stored inside the cells and then storage products are reused for growth without addition of external substrate, however, biochemical modeling approaches accepted that growth and storage occur simultaneously (**Figure 2.3**).

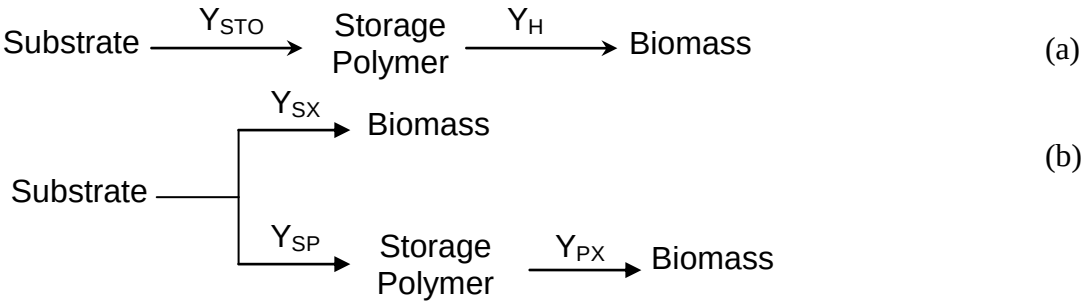

Figure 2.3 : (a) Composition of biomass in ASM3 (b) Composition of biomass in biochemical approach

2.5 Metabolic Models

A metabolic model is based on the principle that the metabolism of organism is composed of a limited number of metabolic pathways which results in more or less constant energy requirement and ultimately constant stoichiometry (Roels, 1983).

Metabolic pathways are described with internal reactions that are combined with reaction stoichiometry and degree of reduction balances to derive the linear equation for description of involved metabolism.

A metabolic model was proposed that acetate is aerobically stored as PHB under dynamic conditions by van Aalst-van Leeuwen et al., (1997). This metabolic model reduces the number of unknown parameters and describes the observed kinetics of PHB formation and consumption by selected microorganism.

The substrate uptake rate will be larger than required for growth when carbon source is given periodically. It was observed that the fast uptake of substrate results in the formation of $NADH_2$ which is consumed by oxidative phosphorylation and leading to ATP formation. If the energy needed for growth is limited, ATP will accumulate which is lead to accumulation of $NADH_2$. This explains that the production of a more reduced storage polymer PHB (requires $NADH_2$) is more likely to be compared with the production of glycogen (leads $NADH_2$ formation).

van Aalst-van Leeuwen *et al*., (1997) used acetate limited continuous culture of *Paracoccus pantotrophus sp.* in order to observe the metabolism of a microorganisms capable of producing and consuming which was described by seven internal reaction. The reactions are shown in Figure 2.4 schematically. Biomass is assumed to contain two different parts as an active biomass compartment ($1-f_{PHB}$ = capable of reproduction and growth) and PHB fraction (f_{PHB} = used as storage for carbon and energy).

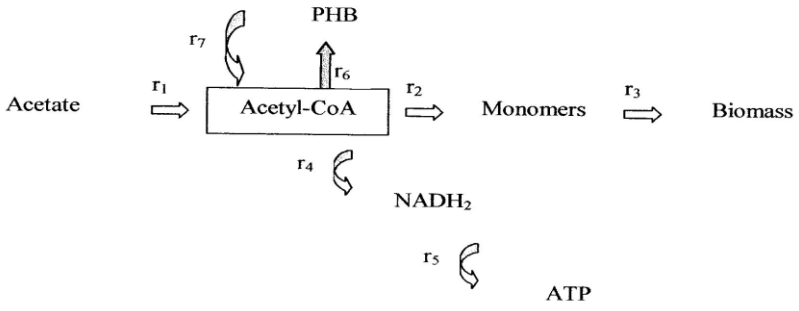

Figure 2.4 : Schematic representation of the metabolism of an organism capable of forming and consuming PHB (van Aalst-van Leeuwen *et al.*, 1997)

Synthesis of Acetyl-CoA from Acetate, (r_1): Acetate is taken into cell by active transport, Acetyl-P is formed and at last, conversion to Acetyl-CoA is completed.

Synthesis of biomass monomers from Acetyl-CoA, (r_2): This is the first part of the synthesis of active biomass.

Polymerization of biomass precursors and maintenance, (r_3): Biomass formation is completed by this reaction.

Carbon source catabolism, (r_4)

Oxidative phosphorylation, (r_5): ATP is produced from $NADH_2$.

Synthesis of the storage product PHB from Acetyl-CoA, (r_6): The substrate is both used for the synthesis of biomass and PHB.

Synthesis of Acetyl-CoA from PHB, (r_7): In the absence of acetate, the microorganisms utilize the intracellular accumulated PHB as carbon and energy source. The storage polymer is hydrolyzed and converted into Acetyl-CoA.

The metabolic model results in two equations describing the conversion processes for feast and famine phases. For the feast period, the linear equation describing acetate - uptake, biomass growth, PHB production and maintenance is defined as below (Equation 2.1).

$$(-r_S) = \frac{1}{Y_{SX}^{\max}} r_X + \frac{1}{Y_{SP}^{\max}} r_P + m_S C_X \qquad \textbf{(2.1)}$$

As similar, PHB consumption, biomass growth, and maintenance are expressed with the following equation for the famine period (Equation 2.2):

$$(-r_P) = \frac{1}{Y_{PX}^{\max}} r_X + m_P C_X \qquad \textbf{(2.2)}$$

where,

$$Y_{SX}^{\max} = \frac{4\delta - 2}{4.13\delta + 4.32} \qquad \textbf{(2.3)}$$

$$Y_{SP}^{\max} = \frac{4\delta - 2}{4.5\delta} \qquad \textbf{(2.4)}$$

$$Y_{PX}^{\max} = \frac{4.5\delta - 0.5}{4.13\delta + 4.32} \qquad \textbf{(2.5)}$$

$$m_S = \frac{m_{ATP}}{2\delta - 1} \quad \textbf{(2.6)}$$

$$m_P = \frac{m_{ATP}}{4.5\delta - 0.5} \quad \textbf{(2.7)}$$

The linear equations contain two unknown parameters namely, the ratio between - ATP produced and electrons transferred from $NADH_2$ to an electron acceptor (δ) and the ATP consumption due to maintenance processes (m_{ATP}). Substition of $r_X=\mu C_X$, $r_S=q_S.C_X$, $r_P=q_P.C_X$ followed by division by biomass concentration, C_X gives the following Equation (2.8):

$$(-q_S) = \frac{1}{Y_{SX}^{\max}}\mu + \frac{1}{Y_{SP}^{\max}} q_P + m_S \quad \textbf{(2.8)}$$

From earlier experiments (Pot, 1995), it was observed that maximum yield of biomass on acetate, Y_{SX}^{max}= 0.45 [C-mol/C-mol] and maintenance coefficient for growth on acetate m_S = 0.038 [C-mol/C-mol.h]. The P/O ratio, δ= 1.84 [mol ATP/mol $NADH_2$] and the ATP maintenance coefficient, m_{ATP} = 0.102 [mol ATP/C-mol.h] were calculated by using the related equations and above values, also these parameters are considered to be constant within the experimental range. Maximum yield of PHB on acetate, Y_{SP}^{max} = 0.648 [C-mol/C-mol] was found by using the Equation 2.4. Substituting these yield coefficients in Equation 2.8 gives (Equation 2.9):

$$-qs = 2.22\mu. + 1.544q_P + 0.038 \quad \textbf{(2.9)}$$

From the carbon balance and the degree of reduction balance relations for the specific carbon dioxide production rate (q_C) and for the specific oxygen consumption rate (q_O) can be derived as a function of μ and q_P (Equation 2.10 and 2.11):

$$q_C = (-q_S) - \mu - q_P = 1.22\mu + 0.544q_P + 0.038 \quad \textbf{(2.10)}$$

$$(-q_O) = 1.188\mu + 0.419q_P + 0.038 \quad \textbf{(2.11)}$$

Stoichiometry of the kinetic model for the PHB production can be based upon Equations (2.9- 2.11).

Maximum yield of biomass on PHB, Y_{PX}^{max} = 0.653 [C-mol/C-mol] and maintenance coefficient for growth on PHB, m_P = 0.0131 [C-mol/C-mol.h] were calculated for the famine phase. Below equations were derived by using these values:

$$(-q_P) = \frac{1}{Y_{PX}^{\max}} \mu + m_P \quad \textbf{(2.12)}$$

$$-q_P = 1.532\mu + 0.0131 \quad \textbf{(2.13)}$$

$$q_C = 0.532\mu + 0.0131 \quad \textbf{(2.14)}$$

$$(-q_O) = 0.693\,\mu + 0.0147 \quad \textbf{(2.15)}$$

Stoichiometry of the kinetic model for the PHB consumption can be based upon Equations (2.12 -2.15).

It was observed from experimental and modeling results that, the substrate uptake rate reaches its maximum value immediately after the pulse addition. However, the growth rate is only influenced by maximal growth rate, and slowly decreases during growth on PHB presumably being related to the reduction in PHB amount (van Aalst- van Leeuwen *et al.*, 1997). According to the standard theory, the substrate uptake rate in a chemostat is found by the multiplication of the maximum uptake rate and Monod factor for the substrate relationship. This is an evidence for that the organisms will induce a maximal level of substrate uptake enzymes, while the enzyme system for cell growth will not be completely induced. So, even though the cells are cultivated close to growth rate of zero, the maximum substrate uptake activity will be maintained. Since the conditions are firmly different from a chemostat in wastewater treatment processes under dynamic conditions, it can be discussed that under such conditions microorganisms that have a fully induced substrate uptake system, accumulate more substrate and out compete organisms that do have lower substrate uptake rates.

The maximum substrate uptake rate of an organism is generally independent of the, real growth rate of that organism (Roels, 1983). In the model of van Aalst-van Leeuwen *et al*. (1997), it was proposed that PHB is generally used as a buffer for the substrate taken up but not directly used for growth. The storage polymers can play a significant role in microbial growth under unbalanced conditions (van den Eynde et *al.*, 1984; van Loosdrecht *et al.*, 1997).

A metabolic model for PHB metabolism in aerobic, slow growing, activated sludge culture has been proposed by Beun *et al*., (2002) for definition of relation between

the PHB storage and activated sludge models. The metabolic model is the same as described by van Aalst- van Leeuwen *et al*., (1997) with the exception of biomass composition. It is found that bacteria can balance their growth rate respect to dynamic substrate feeding, using the PHB metabolism and regulate the substrate uptake rate. Therefore, bacteria can compete effectively for substrate. While acetate is taken up with maximum substrate uptake rate, PHB is produced with a constant rate in the feast period. Biomass growth on PHB leads to only 4-10% lower biomass yield then the direct utilization of acetate for growth. Whereas PHB synthesis process consumes 66-100% of the acetate, the rest of the acetate is consumed for growth and maintenance.

According to another proposed hypothesis by van Loosdrecht and Heijnen (2002), the growth rate will depend on the level of the protein synthesis system includes RNA and anabolic enzymes and separate biomass compartments for storage polymers. The main objective of the proposed model is microbial ecology principle that bacteria compete on substrate uptake rate rather than on growth rate. In general, when the substrate is available, most RNA and enzymes will be induced for balancing the level of microbial protein synthesis system. Since these cellular components are subjected to, a constant decay processes at the same time, the anabolic enzymes are induced and the enzyme fraction in the cell will increase when external substrate is available. Due to the enzyme decay and dilution of the newly generated cell material, the level of RNA and enzymes in the cells will reduce. The balance between enzyme synthesis and decay leads to the level of induction of protein synthesis system and consequently to the actual growth rate in the availability of external substrate.

A model is formulated by van Loosdrecht and Heijnen (2002) based on the model presented by Kompala *et al*., (1984) to describe enzyme-structured biomass. The main difference is the absence of a precisely expressed maximum enzyme amount. It is assumed that the balance between production and decay will give a maximum enzyme level as occurring in real cells. Proposed model is based on the metabolic model of Beun *et al*., (2002) developed for PHB formation and consumption by mixed cultures. Conversion of acetate to PHB and new biomass in feast phase is described in Equations 2.16 and 2.17 while equation 2.18 shows the growth on PHB in famine phase as below:

$$r_S = \frac{1}{Y_{SP}} r_{STO} + \frac{1}{Y_{SX}} r_X + m_S X_H \quad \textbf{(2.16)}$$

$$r_{STO} = Y_{SP} r_S + \frac{Y_{SP}}{Y_{SX}} r_X - m_S Y_{SP} X_H \quad \textbf{(2.17)}$$

$$r_X = Y_{PX} r_{STO} - m_P Y_{PX} X_H \quad \textbf{(2.18)}$$

The model is presented in Table 2.1. The actual growth rate on a substrate is related to the level of enzyme induction for growth on that substrate (Kompala *et al.*, 1984). This general approach is also used in this model but there is a critical difference. While the above authors assume the organisms try to grow as quick as possible, it was assumed that the protein synthesis system is only induced to competition of organisms effectively on substrate uptake rate when there is an external substrate available in this model. There is no stoichiometry for the enzyme conversion process since there is not sufficient information on yield coefficients or concentrations of enzymes. Reactor conditions and kinetic values reported by Beun *et al.,* (2001) were used for the model for aerobic, pulse fed, sequencing batch reactors.

The formation and decay rates of the protein synthesis system almost has not been studied and certainly not under conditions related to activated sludge systems. It is possible that the formation and decay rates of enzymes are similar for all bacteria since the cell internal processes are generally similar for various microorganisms. This means that consumption rate of storage polymers should be used instead of growth rate in mathematical models like ASM3. The consumption rate of storage polymers should be described by a first order rate instead of saturation kinetics (van Loosdrecht and Heijnen 2002).

Table 2.1 : Matrix representation of proposed model structure (van Loosdrecht and Heijnen, 2002)

	Acetate S_{Hac}	Ammonium S_N	Oxygen S_O	Biomass X_H	PHB X_{PHB}	Anab. Enz. X_e	Rate
Feast Phase – aerobic							
Acetate Uptake	−1		$-(1-Y_{sp})$		Y_{sp}		$q^{max}_{HAc} \cdot M_{HAc} \cdot M_o \cdot X_H$
Growth 1		$-i_N$	$-\left(\frac{Y_{SP}}{Y_{SX}}-1\right)$	1	$-\frac{Y_{SP}}{Y_{SX}}$		$\mu^{max} \cdot \frac{\mu^{max}+\beta}{\alpha+\alpha^*} \cdot f_e \cdot M_{HAc} \cdot M_O \cdot M_N \cdot X_H$
Endogenous Rate			$-Y_{sp}$		$-Y_{sp}$		$m_S \cdot M_{HAc} \cdot M_o \cdot X_H$
Famine Phase – aerobic							
PHB consumption		$-i_N \cdot Y_{PX}$	$-(1-Y_{PX})$	Y_{PX}	−1		$k_{PHB} \cdot f_{PHB} \cdot I_{HAc} \cdot M_o \cdot M_N \cdot X_H$
Endogenous Rate		$-i_N \cdot Y_{PX}$	$-Y_{PX}$	Y_{PX}	−1		$m_{PHB} \cdot I_{HAc} \cdot M_o \cdot X_H$
Feast Phase – anoxic			Nitrate S_{NO}				
Acetate Uptake	−1		$-(1-Y_{SP})/2.86$		Y_{SP}		$q^{max}_{HAc} \cdot M_{HAc} \cdot M_o \cdot X_H$
Growth		$-i_N$	$-\left(\frac{Y_{SP}}{Y_{SX}}-1\right)/2.86$	1	$-\frac{Y_{SP}}{Y_{SX}}$		$\mu^{max} \cdot \frac{\mu^{max}+\beta}{\alpha+\alpha^*} \cdot f_e \cdot M_{HAc} \cdot M_O \cdot M_N \cdot X_H$
Endogenous Rate			$-Y_{SP}/2.86$		$-Y_{SP}$		$m_e \cdot I_{HAc} \cdot M_o \cdot X_H$
Famine Phase – anoxic							
PHB consumption		$-i_N \cdot Y_{PX}$	$-(1-Y_{PX})/2.86$	Y_{PX}	−1		$k_{PHB} \cdot f_{PHB} \cdot I_{HAc} \cdot M_o \cdot M_N \cdot X_H$
Endogenous Rate		$i_N \cdot Y_{PX}$	$-Y_{PX}/2.86$	$-Y_{PX}$			$m_{PHB} \cdot I_{HAc} \cdot M_o \cdot X_H$
Protein synthesis system							
Rate						1	$(\alpha^* M_{HAc}{}^* M_o{}^* M_N - \beta^* f_e + \alpha^*)^* X_H$

$f_{PHB} = \frac{X_{PHB}}{X_H}$ $f_e = \frac{X_e}{X_H}$ $M_i = \frac{S_i}{S_i + K_i}$ $I_i = \frac{K_i}{K_i + S_i}$ $\mu^{max} = Y_{SX} * q^{max}_{HAc}$

$Y_{SX} = \frac{4 \cdot \delta - 2}{4.2 \cdot \delta + 4.32} + \frac{4.2}{4}$; $Y_{SP} = \frac{4 \cdot \delta - 2}{4.5 \cdot \delta} + \frac{4.5}{4}$; $Y_{PX} = \frac{4.5 \cdot \delta - 0.5}{4.2 \cdot \delta + 4.32} + \frac{4.2}{4.5}$ Aerobic growth: δ = 2.0 molATP / $2e^-$; Anoxic growth : δ = 1.2 molATP / $2e^-$

$m_S = \frac{m_{ATP}}{2 \cdot \delta - 1} + \frac{4}{4.2}$; $m_P = \frac{m_{ATP}}{2.25 \cdot \delta - 0.25} + \frac{4.5}{4.2}$; m_{ATP} = 0.02 molATP / C - mol.h

2.6 Activated Sludge Model No:3

ASM3 has been proposed by IAWQ Task Group to establish the new concept in activated sludge modeling. While component and process are similar to other activated sludge models, ASM3 includes storage process and products and attain to estimate the oxygen consumption, sludge production, nitrification, and denitrification of activated sludge systems. In addition, ASM3 contains nitrogen and alkalinity limitation for growth of microorganisms with description of ammonification kinetics by assuming constant N and COD ratio.

The hydrolysis process has been very important in predicting the electron acceptor under aerobic and anoxic conditions. Whereas the quantification of the kinetic parameters for hydrolysis process was difficult, ASM3 simplified hydrolysis process with only one process independent of the electron acceptor. The model has also included the differences in the decay rates of nitrifiers under aerobic and anoxic conditions.

2.7 Components and Processes of the Model

ASM3 integrated seven soluble and six particulate components to characterize the wastewater and activated sludge. Soluble and particulate components were distinguished by 0.45 μm membrane filtration.

The first component is dissolved oxygen (So), which can be directly measured and is subject to gas exchange. Inert soluble organic material (S_I) is a part of influent and may be produced by the hydrolysis of X_S. Readily biodegradable organic substrates (S_S) are first taken up by heterotrophic organisms and stored as XSTO, then used for growth, according to ASM3. S_S and S_I are approximately equal to the total soluble COD determined by filtration from 0.45 μm membrane filters. Ammonium plus ammonia nitrogen (S_{NH}), dinitrogen (S_{N2}) and nitrite plus nitrate nitrogen (S_{NO}) is given as fractions of nitrogen. Alkalinity of the wastewater (S_{ALK}) is used to approximate the conservation of ionic charge in biological reactions.

Inert particulate organic matter (X_I) is a part of influent and may be produced as a result of biomass decay. Bacteria cannot directly use the colloidal and particulate organic substrates, because they have high molecular weights. Therefore, slowly

biodegradable substrates (X_S) must undergo hydrolysis before it is used by the biomass. The products of the hydrolysis are assumed to be readily biodegradable or soluble inert substrate. It must be reminded that all X_S is contained in the influent and not involved in the filtrate of 0.45 μm membrane filters in ASM3. Heterotrophic organisms (X_H) may grow both aerobically and anoxically. Hydrolysis does not consume any electron acceptor in ASM3. Active biomass is responsible for the hydrolysis of XS and storage process. Internal storage products (X_{STO}) consist of PHA, glycogen etc., which is only defined as a functional component required for modeling but is not directly identifiable chemically. X_A represents autotrophic organisms responsible for nitrification. They oxidize the ammonium directly to nitrate; nitrite is not considered in ASM3. Last component considered in model is the total suspended solids, X_{TS} that may be used for the modeling of volatile suspended solids by some special ratios. There are twelve processes taken into account in ASM3.

Hydrolysis: This process makes all of the slowly biodegradable substrates in the influent with converting to available soluble substrates. This process is independent from electron acceptor and therefore is different when compared to ASM1.

Aerobic storage of readily biodegradable substrate: it is assumed that all readily biodegradable substrate is stored as storage products (X_{STO}), and then ready to be used for the growth of biomass. This conversion required energy produced by aerobic respiration in the form of ATP.

Anoxic storage of readily biodegradable substrate: This process similar to aerobic storage process but energy requirement of this process is supplied by anoxic respiration. Due to lower amount of denitrifiers, the process rate is naturally smaller than aerobic storage rate.

Aerobic growth of heterotrophs: It is assumed that the stored products are used as substrate for the growth of heterotrophs.

Anoxic growth of heterotrophs: This process identical with aerobic growth but it is based on anoxic respiration.

Aerobic endogenous respiration: This process was explained as all forms of biomass loss and energy requirements not related to growth. The process consist of decay,

maintenance, endogenous respiration, lysis, predation, motility and death which are related to aerobic respiration

Anoxic endogenous respiration: This process is similar to aerobic endogenous respiration, but occurs at a slower rate. Especially protozoa predation is considerably less active under anoxic conditions than under aerobic conditions.

Aerobic respiration of storage products: X_{STO} decays together with biomass like endogenous respiration.

Anoxic respiration of storage products: Denitrifying conditions are applied for the similar process of aerobic respiration of storage products.

Aerobic growth of autotrophs: Nitrifiers oxidize ammonium directly to nitrate, nitrite as an intermediate component, is not considered.

Aerobic endogenous respiration for autotrophs: Apart from description for nitrifiers, it takes place similar to aerobic endogenous respiration.

Anoxic endogenous respiration for autotrophs: It is similar to aerobic endogenous respiration for autotrophs, but it occurs at a slower rate.

2.8 Process Stoichiometry

The net (true) yields of heterotrophic biomass (X_H) produced per unit of readily biodegradable substrate (S_S) removed in ASM3 are found from following equations.

$$Y_{net,O2} = Y_{STO,O2}.Y_{H,O2} \quad \textbf{(2.19)}$$

$$Y_{net,NO} = Y_{STO,NO}.Y_{H,NO} \quad \textbf{(2.20)}$$

All stoichiometric parameters of ASM3 are identified together with their units and a typical value in Table 2.2. According to these values, a numeric example is introduced for typical applications of ASM3. This stoichiometric matrix is given in Table 2.3.

The composition of all organic fractions relative to Theoretical Oxygen Demand (ThOD) is assumed be unity. ThOD is the conservative form of COD. In most cases, ThOD of organic compounds may analytically be estimated by standard COD analysis. ThOD effectively accounts for the electrons involved in the biological redox processes.

The stoichiometric coefficient for S_{N2} is the negative of the coefficient for SNOX in any denitrification process. The composition coefficients for ThOD for S_{N2} (-1.71 g ThOD/ gN2), S_{NOX} (-4.57 g ThOD/ g NO_3--N) and S_{O2} (-1 g ThOD/ g O2) are negative for electron donors corresponding to the redox reference for ThOD.

Table 2.2 : Typical stoichiometric and composition parameters for ASM3

Symbol	Characterization	Value	Units	
f_{SI}	Production of S_I in hydrolysis	0	g COD_{SI}/(g COD_{XS})	
$Y_{STO,O2}$	Aerobic yield of stored product per S_S	0.85	g COD_{XSTO}/(g COD_{SS})	
$Y_{STO,NO}$	Anoxic yield of stored product per S_S	0.80	g COD_{XSTO}/(g COD_{SS})	Equation 2.21
$Y_{H,O2}$	Aerobic yield of heterotrophic biomass	0.63	g COD_{XH}/(g COD_{XSTO})	
$Y_{H,NO}$	Anoxic yield of heterotrophic biomass	0.54	g COD_{XH}/(g COD_{XSTO})	Equation 2.22
Y_A	Yield of autotrophic biomass per NO_3^-N	0.24	g COD_{XA}/(g N_{SNOX})	
f_{XI}	Production of X_I in endogenous respiration	0.20	g COD_{XI}/(g COD_{XBM})	
$I_{N,SI}$	N content of S_I	0.01	g N/(g COD_{SI})	
$I_{N,SS}$	N content of S_S	0.03	g N/(g COD_{SS})	
$I_{N,XI}$	N content of X_I	0.02	g N/(g COD_{XI})	The values below are suggested if X_{SS} is used to model VSS rather than SS:
$I_{N,XS}$	N content of X_S	0.04	g N/(g COD_{XS})	
$I_{N,BM}$	N content of biomass, X_H, X_A	0.07	g N/(g COD_{XBM})	
$I_{SS,XI}$	SS to COD ratio for X_I	0.75	g SS/(g COD_{XI})	0.75 g VSS/(g COD_{XI})
$I_{SS,XS}$	SS to COD ratio for X_S	0.75	g SS/(g COD_{XS})	0.75 g VSS/(g COD_{XS})
$I_{SS,BM}$	SS to COD ratio for biomass, X_H, X_A	0.90	g SS/(g COD_{XBM})	0.75 g VSS/(g COD_{XBM})

Table 2.3 : Stoichiometric matrix of ASM3 based on the stoichiometric parameters in Table 2.2

	Compound i →	1	2	3	4	5	6	7	8	9	10	11	12	13
j	Process	S_{O2}	S_I	S_S	S_{NH2}	S_{N2}	S_{NOX}	S_{ALK}	X_I	X_S	X_H	X_{STO}	X_A	X_{SS}
↓	Expressed as →	O_2	COD	COD	N	N	N	Mole	COD	COD	COD	COD	COD	SS
1	Hydrolysis		0	1	0.01			0.001		-1				-0.75
Heterotrophic organisms, aerobic and denitrifying activity														
2	Aerobic storage of S_S	-0.15		-1	0.03			0.002				0.85		0.51
3	Anoxic storage of S_S			-1	0.03	0.07	-0.07	0.007				0.80		0.48
4	Aerobic growth of X_H	-0.60			-0.07			-0.005			1	-1.60		-0.06
5	Anoxic growth (denitrification)				-0.07	0.30	-0.30	0.016			1	-1.85		-0.21
6	Aerobic Endogenous Respiration	-0.80			0.066			0.005	0.20		-1			
7	Anoxic Endogenous Respiration				0.066	0.28	-0.28	0.025	0.20		-1			
8	Aerobic respiration of X_{STO}	-1										-1		
9	Anoxic respiration of X_{STO}					0.35	-0.35	0.025				-1		
Autotrophic organisms, nitrifying activity														
10	Aerobic growth of X_A	-18.04			-4.24		4.17	-0.600					1	
11	Aerobic Endogenous Respiration	-0.80			0.066			0.005	0.20				-1	
12	Anoxic Endogenous Respiration				0.066	0.28	-0.28	0.025	0.20				-1	

Since the biochemical energy (ATP) yield of anoxic respiration is smaller than in aerobic respiration, aerobic yield coefficients ($Y_{STO,O2}$ and $Y_{H,O2}$) surpass the anoxic yield coefficients ($Y_{STO,NO}$ and $Y_{H,NO}$). Anoxic energy yield is assumed η_{anoxic}= 0.70 of the aerobic energy yield in ASM3 with following energy relationships (Equation 2.21 and 2.22):

$$\frac{1-Y_{STO,O2}}{Y_{STO,O2}} = \frac{\eta_{anoxic}\left(1-Y_{STO,NO}\right)}{Y_{STO,NO}} \qquad \textbf{(2.21)}$$

$$\frac{1-Y_{O2}}{Y_{O2}} = \frac{\eta_{anoxic}\left(1-Y_{NO}\right)}{Y_{NO}} \qquad \textbf{(2.22)}$$

It is suggested that Equation (2.21) and (2.22) are used to relate anoxic and aerobic yields in ASM3.

2.9 Process Kinetics

In relation to removal of all soluble compounds, the kinetic expressions of ASM3 are relied on switching functions, which are hyperbolic or saturation terms, Monod equations, S/(K+S). Similarly, the switching functions are affected for particulate compounds by the ratio of X_{STO}/X_H and X_S/X_H. All kinetic parameters of ASM3 are given in Table 2.4. The units and typical values at 10^0C and 20^0C for these kinetic parameters are identified in Table 2.5.

Interpolation of kinetic parameters k for different temperatures is proposed with the following temperature equation.

$$k(T) = k\left(20^0 C\right).\exp\left(\theta_T.\left(T-20^0 C\right)\right) \qquad \textbf{(2.23)}$$

where θ_T (in ^{0}C)may be found from

$$\theta_T = \frac{\ln\left(k(T_1)/k(T_2)\right)}{T_1 - T_2} \qquad \textbf{(2.24)}$$

Table 2.4 : Kinetic rate expressions ρ_j for ASM3 All $\rho_j \geq 0$

j	Process	Process rate equation ρ_j, for all $\rho_j \geq 0$
1	Hydrolysis	$k_H \cdot \frac{X_S / X_H}{K_X + X_S / X_H} . X_H$
Heterotrophic organisms, aerobic and denitrification		
2	Aerobic storage of COD	$k_{STO} \cdot \frac{S_O}{K_O + S_O} \cdot \frac{S_S}{K_S + S_S} . X_H$
3	Anoxic storage of COD	$k_{STO} . \eta_{NO} \cdot \frac{K_O}{K_O + S_O} \cdot \frac{S_{NO}}{K_{NO} + S_{NO}} \cdot \frac{S_S}{K_S + S_S} . X_H$
4	Aerobic growth	$\mu_H \cdot \frac{S_O}{K_O + S_O} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \cdot \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \cdot \frac{X_{STO} / X_H}{K_{STO} + X_{STO} / X_H} . X_H$
5	Anoxic growth (denitrification)	$\mu_H . \eta_{NO} \cdot \frac{K_O}{K_O + S_O} \cdot \frac{S_{NO}}{K_{NO} + S_{NO}} \cdot \frac{S_{NH}}{K_{NH} + S_{NH}} \cdot \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \cdot \frac{X_{STO} / X_H}{K_{STO} + X_{STO} / X_H} . X_H$
6	Aerobic endogenous respiration	$b_{H,O2} \cdot \frac{S_O}{K_O + S_O} . X_H$
7	Anoxic endogenous respiration	$b_{H,NO} \cdot \frac{K_O}{K_O + S_O} \cdot \frac{S_{NO}}{K_{NO} + S_{NO}} . X_H$
8	Aerobic respiration of PHA	$b_{STO,O2} \cdot \frac{S_O}{K_O + S_O} . X_{STO}$
9	Anoxic respiration of PHA	$b_{STO,NO} \cdot \frac{K_O}{K_O + S_O} \cdot \frac{S_{NO}}{K_{NO} + S_{NO}} . X_{STO}$
Autotrophic organisms, nitrification		
10	Nitrification	$\mu_A \cdot \frac{S_O}{K_{A,O} + S_O} \cdot \frac{S_{NH}}{K_{A,NH} + S_{NH}} \cdot \frac{S_{HCO}}{K_{A,HCO} + S_{HCO}} . X_A$
11	Aerobic endogenous respiration	$b_{A,O2} \cdot \frac{S_O}{K_O + S_O} . X_A$
12	Anoxic endogenous respiration	$b_{A,NO} \cdot \frac{K_O}{K_O + S_O} \cdot \frac{S_{NO}}{K_{NO} + S_{NO}} . X_A$

According to ASM3, readily biodegradable substrate (S_S) is best determined from bioassay in wastewater. Kinetics of the storage and growth processes are related to rapid uptake of oxygen after addition of wastewater to biomass. So, the yield of storage process must be used to relate oxygen uptake to substrate consumption. This

can be simulated with batch experiment for estimation of S_S in ASM3 by following equation:

$$S_S(batch) = \int \Delta r_{S_S}.dt = \frac{\upsilon_{S_S}}{\upsilon_{SO_2}} \int \Delta r_{SO_S}.dt = \int \frac{\Delta r_{SO_2}.dt}{1 - Y_{O_2,STO}} \qquad \textbf{(2.25)}$$

Table 2.5 : Typical values of kinetic parameters for ASM3

Symbol	Characterization	Temperature 10 °C	Temperature 20 °C	Units
k_H	Hydrolysis rate constant	2	3	g COD_{XS}.(g.COD_{XH})$^{-1}$.d^{-1}
K_X	Hydrolysis saturation constant	1	1	g.COD_{XS}.(g.COD_{XH})$^{-1}$
Heterotrophic organisms X_H, *aerobic and denitrifying activity*				
k_{STO}	Storage rate constant	2.5	5	g COD_{SS}.(g.COD_{XH})$^{-1}$.d^{-1}
η_{NO}	Anoxic reduction factor	0.6	0.6	-
K_{O2}	Saturation constant for S_{NO2}	0.2	0.2	g O_2 m^{-3}
K_{NO}	Saturation constant for S_{NOX}	0.5	0.5	g NO_3^--N m^{-3}
K_S	Saturation constant for substrate S_S	2	2	g COD_{SS} m^{-3}
K_{STO}	Saturation constant for X_{STO}	1	1	g.COD_{XSTO}.(g.COD_{XH})$^{-1}$
μ_H	Heterotrophic maximum growth rate of X_H	1	2	d^{-1}
K_{NH4}	Saturation constant for ammonium, S_{NH4}	0.01	0.01	g N m^{-3}
K_{ALK}	Saturation constant for alkalinity for X_H	0.1	0.1	g HCO_3^- m^{-3}
$b_{H,O2}$	Aerobic endogenous respiration rate of X_H	0.1	0.2	d^{-1}
$b_{H,NO}$	Anoxic endogenous respiration rate of X_H	0.05	0.1	d^{-1}
$b_{STO,O2}$	Aerobic respiration rate for X_{STO}	0.1	0.2	d^{-1}
$b_{STO,NO}$	Anoxic respiration rate for X_{STO}	0.05	0.1	d^{-1}
Autotrophic organisms X_A, *nitrifying activity*				
μ_A	Autotrophic maximum growth rate of X_A	0.35	1.0	d^{-1}
$K_{A,NH4}$	Ammonium substrate saturation for X_A	1	1	g N m^{-3}
$K_{A,O2}$	Oxygen saturation for nitrifiers	0.5	0.5	g O_2 m^{-3}
$K_{A,ALK}$	Bicarbonate saturation for nitrifiers	0.5	0.5	g HCO_3^- m^{-3}
$b_{A,O2}$	Aerobic endogenous respiration rate of X_A	0.05	0.15	d^{-1}
$b_{A,NO}$	Anoxic endogenous respiration rate of X_A	0.02	0.05	d^{-1}

2.10 Modifications of ASM3

Krishna and van Loosdrecht (1999) make the first modeling study for the evaluation of ASM3, with measurement the conversion of acetate, ammonium, oxygen, biomass and PHB at different temperatures in laboratory SBR system. These measurements are compared to simplified version of ASM3 for the case of only aerobic heterotrophic conversions and acetate as the only substrate. All expressions and parameters were chosen according to the description of Gujer *et al.,* (1999), except the conversion of particulate COD components to TSS and fraction of nitrogen in the biomass.

First simulation results were not so successful in predicting the observed experimental results due to it gave too low acetate uptake rate, very high PHB turnover, low MLSS concentrations and low OUR and growth rate for feast period. Therefore, in case of evaluation of the poor model prediction, the stoichiometric coefficients of simplified ASM3 were changed with earlier experiments of van Aalst-van Leeuwen *et al.,* (1997) while kinetic parameters remained the same. Results of new simulation did not supply much improvement in the acetate uptake rate, the PHB and growth rate.

Finally, it was tried to calibrate the kinetic coefficients for storage and growth (k_{STO} and μ) in order to remove the differences between the model prediction and experimental results. Alternative model structure, gave good accordance in OUR while the growth rate still shows great difference. Matrix representation of simplified ASM3 is shown in Table 2.6. As a result, simplified ASM3 gives a reasonable description of the studied SBR process, but the discontinuity in growth rate between feast and famine period cannot be described. Therefore, a new model structure is proposed (Table 2.7) based on experimental observations (Krishna and van Loosdrecht 1999).

Karahan-Gül *et al.,* (2003) is made a similar modification of ASM3 with the assumption of simultaneous growth and storage on the primary external substrate. According to the proposed modified version, direct heterotrophic growth occurs on the primary external substrate and after the depletion of external substrate; growth of biomass takes place on the internal storage product in the model. Kinetic and stoichiometric coefficients, which are used in model for acetate are given Table 2.7.

Table 2.6 : Matrix representation of simplified ASM3 (Krishna and van Loosdrecht 1999)

Component / Process	1 S_O	2 S_S	3 S_{NH}	4 X_I	5 X_H	6 X_{STO}	7 X_{TS}	8 Process rate
1 Aerobic storage of COD (PHB storage)	$-(1-Y_{STO})$	-1				Y_{STO}		$k_{STO}.\frac{S_O}{K_O+S_O}.\frac{S_S}{K_S+S_S}.X_H$
2 Aerobic growth on PHB	$-\frac{1-Y_{H2}}{Y_{H2}}$				1	$-\frac{1}{Y_{H2}}$		$\mu_{H2}.\frac{S_O}{K_O+S_O}.\frac{S_S}{K_S+S_S}.\frac{S_{NH}}{K_{NH}+S_{NH}}.\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}.X_H$
3 Aerobic endogenous respiration	$-(1-f_I)$			f_I	-1			$b_{H,O2}.\frac{S_O}{K_O+S_O}.X_H$
4 Aerobic respiration of PHB	-1							$b_{STO,O2}.\frac{S_O}{K_O+S_O}.X_H$
5 Aerobic growth on acetate	$-\frac{1-Y_{H1}}{Y_{H1}}$	$-\frac{1}{Y_{H1}}$			1			$\mu_{H1}.\frac{S_O}{K_O+S_O}.\frac{S_S}{K_S+S_S}.\frac{S_{NH}}{K_{NH}+S_{NH}}.X_H$

Table 2.7 : Model structures of modifications of ASM3 (Krishna and van Loosdrecht 1999, Karahan-Gül *et al.* 2003)

Parameter	Proposed model results of Krishna and van Loosdrecht (1999) (Model A)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	Units
b_H	0.2	0.24	0.24	d^{-1}
b_{STO}	0.2	0.24	0.24	d^{-1}
K_I	0.1	-		g COD.m^{-3}
K_{NH}	0.01	-	0.01	g N.m^{-3}
K_O	0.2	-	0.2	g O2.m^{-3}
K_S	0.1	3	4	g COD.m^{-3}
k_{STO}	10	14	16	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	d^{-1}
μ_{H2}	2	3	3&1.8	d^{-1}
Y_{H1}	-	0.65	0.67	g cell COD.g^{-1} COD
Y_{H2}	-	0.63	0.75	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	g COD.g^{-1} COD
f	-	0.20	0.20	g COD.g^{-1} COD

The proposed model consists of hydrolysis, endogenous respiration of biomass and respiration of storage products processes as described in ASM3. Storage of readily biodegradable substrate and primary growth processes were described as simultaneous processes competing for substrate and electron acceptor. Both processes have reaction rates according to Monod kinetics where growth processes has ammonia nitrogen and bicarbonate limitations. Secondary growth processes is inhibited when primary substrate is present in the system, uses products as substrate and processes kinetics was defined as surface reaction kinetics similar to ASM3.

OUR experiments were carried out for acetate and results were compared with the OUR curve of the model proposed simulation. The results have provided strong indication that there was a need for considering direct growth on primary substrate as a significant biological mechanism (Karahan-Gül *et al*., 2003). Same experiments were also carried out for glucose. The proposed model (Table 2.8) was simulated for the experimental conditions of the SBR system using AQUASIM (Reichert *et al.*, 1998).

Table 2.8 : Matrix representation of the proposed model structure (Karahan-Gül *et al*. 2003)

Component / *Process*	S_O O_2	S_I COD	S_S COD	X_I COD	X_S COD	X_H COD	X_{STO} COD	*Rate*
Hydrolysis		f_{SI}	$1-f_{SI}$		-1			$k_H \frac{X_S/X_H}{K_X + X_S/X_H} X_H$
Aerobic Storage of COD	$-\frac{1-Y_{STO}}{Y_{STO}}$		$-1/Y_{STO}$				1	$k_{STO} \frac{S_O}{K_O + S_O} \frac{S_S}{K_S + S_S} X_H$
Growth on S_S	$-\frac{1-Y_{H1}}{Y_{H1}}$		$-1/Y_{H1}$			1		$\mu_{H1} \frac{S_O}{K_O + S_O} \frac{S_{NH}}{K_{NH} + S_{NH}} \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \frac{S_S}{K_S + S_S} X_H$
Growth on X_{STO}	$-\frac{1-Y_{H2}}{Y_{H2}}$					1	$-1/Y_{H2}$	$\mu_{H2} \frac{S_O}{K_O + S_O} \frac{K_S}{K_S + S_S} \frac{S_{NH}}{K_{NH} + S_{NH}} \frac{S_{HCO}}{K_{HCO} + S_{HCO}} \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} X_H$
Endogenous Respiration	$-(1-f_I)$			f_I		-1		$b_{H,O2} \frac{S_O}{K_O + S_O} X_H$
Respiration of X_{STO}	-1						-1	$b_{STO,O2} \frac{S_O}{K_O + S_O} X_{STO}$

2.11 Experimental Studies

The experiments carried out in activated sludge systems, operating under dynamic conditions indicate that storage of internal polymers is usually the main mechanism for the removal of readily biodegradable carbon sources. Bacteria can grow on external substrate and on internal substrate under dynamic conditions. It has been reported that bacteria use external substrate preferentially for cell growth (i.e. optimize the growth rate) or for storage processes (i.e. optimize the substrate uptake rate).

As reported by Chudoba *et al.*, (1985), the yield of biomass on glucose for pure cultures was found to be 0.64 g COD/g COD in plug-flow and completely mixed reactors. This yield is calculated from the amount of oxygen consumed until all glucose is converted while the theoretical yield for the formation of the glycogen is 0.96 g COD/g COD. The difference between these yields significantly indicates the formation of storage polymers. Based on thermodynamic interpretation, growth process requires a standard amount of energy utilization and the yield for aerobic growth can be evaluated as 0.5 g COD/g COD for a wide range of aerobic bacteria and substrates (Smolders *et al*, 1994).

General aspects of storage polymer formation have been improved in consequence of research carried out by van Aalst-van Leeuwen *et al.*, (1997). According to this research, all substrate was transformed to biomass at a constant rate and no biopolymers were formed in steady-state continuous culture. This steady state continuous culture was concerned by a pulse of substrate (acetate) added, so the organism has taken up the substrate at a high rate. A direct increase in growth rate was not observed due to the change in substrate uptake rate. Polymer formation was occurred as the conversion of excess acetate to PHB. When the external substrate was fully consumed, the organism started to grow on the stored PHB. The growth rate on the stored polymer is definitely lower than it is on the original influent substrate. The organism can keep its balanced metabolism by forming PHB when there is a sudden change in substrate addition. The uptake rate is limited by the substrate concentration therefore when organisms are growing under limited substrate conditions; they need comparatively large quantity of substrate uptake

enzymes. A rapid uptake of the substrate can be observed when the substrate concentration increases unexpectedly.

The faster substrate uptake rate ($-q_S$) has been usually interpreted as indirect evidence of more relevant presence of a storage response in activated sludge process. Microorganisms maximize their substrate uptake rates rather than the growth rates (van Loosdrecht *et al*., 1997). Substrate uptake rates were found as 0.54-0.31 g COD/ (g COD.h) for different sludge retention time (SRT) when acetate was used as carbon source (Beun *et al*., 2002) and 1.75-1.53 g COD/ (g COD.h) for different SRT when glucose was used as substrate (Dirks *et al*., 2000). These results have shown that substrate uptake rate is only affected the content of storage compound and not affected SRT or growth rate of bacteria.

The difference between substrate uptake rate and growth rate leads to substrate storage. The experiments observed that while substrate uptake rate was linearly decreased, storage compound production rate (q_P) was linearly increased in feast period. The storage compound production rates were reported as 0.77 g COD/ (g COD.h) in sludge storing acetate as PHB (Beun *et al*., 2002) and 2.02 g COD/ (g COD.h) in sludge storing glucose as glycogen (Dirks *et al*., 2000). This observation has shown that production rate of storage compound is only limited by the substrate type and addition rate.

The storage yields directly calculated from the fraction of removal substrate to produce PHB ($q_P^{pro}/-q_S$), which is recovered as storage compound. Although, ASM3 suggested the default value of storage yield as 0.85 g COD/ g COD, different values have been remarked for storage yield from experimental studies. Studies showed that storage yield is significant parameter in the removal of the substrate as a fast and high yield response that found ranges from 0.32 to 0.79 g COD/g COD. The PHB storage from acetate have been observed as 0.73 g COD/g COD (van Aalst-van Leeuwen *et al*., 1997) for pure culture, *Paracoccus pantotrophus* and 0.69 g COD/g COD (Beun *et al*., 2002) for activated sludge. The diversity of storage yields based on using different model than ASM3, where the readily biodegradable substrate is used for growth and storage at the same time.

Storage yields for glycogen storage from glucose has been founded as 0.96 g COD/g COD (Chudoba *et al.*, 1985) and accepted as 0.9 g COD/g COD (Goel *et al*., 1999). This difference in the storage yield of two type of substrate is explained that the

formation of glycogen from glucose requires less energy than the PHB formation from acetate. While storage products are producing at a constant rate in the beginning, the rate of storage decreases due to reaching maximum storage capacity in time. This causes the overall and observed storage yields to vary over time on the F/M ratio of the experiment. On the other hand, it was observed that F/M ratios did not affected the storage yields that were reported as 0.78 g COD/gCOD for acetate and 0.87 g COD/g COD for glucose (Karahan-Gül *et al*., 2003).

In addition, studies were carried out to detect the impact of the initial acetate concentration on production of PHB. According to Serafim *et al*., (2004), while storage yield increased with acetate concentration, the growth rate decreased. The maximum storage yield and growth yield were found as 0.93 and 0.80 g COD/g COD in this study. These results indicate that the smaller part of substrate consumed for growth and bigger portion of substrate used for storage polymer formation after high amount of acetate is given to reactor. In relation to higher acetate concentration, the specific storage rate slightly increased found as 0.43-0.6 g COD/ (g COD.h). This observation is in agreement with the previous study of Beun *et al*., (2002) which was found qP varied from 0.56-0.68 g COD/ (g COD.h). However, it was reported that the specific acetate uptake rate was not affected by the substrate concentration. The range of acetate uptake rates were obtained as 0.83-1.14 g COD/ (g COD.h) (Serafim *et al*., 2004) and 0.89-1 g COD/ (g COD.h) (Beun *et al*., 2002).

Although, storage yield increases with substrate concentration, such high substrate concentrations were observed as inhibitory for PHB formation which causes a decrease in the storage yield (Dionisi *et al*., 2001; Serafim *et al*., 2004). The obstruction of inhibitory affect was attempted with applying continuously and three pulse feeding the same amount substrate (Serafim *et al*., 2004). In continuously feeding, the concentration of substrate in the reactor was always zero and substrate limitation may be main factor responsible for the low PHB storage rate as 0.6 g COD/ (g COD.h) than the obtained from inhibited sludge as 0.72 g COD/ (g COD.h). In three pulse experiment, while storage rate was found as 0.7 g COD/ (g COD.h) in first pulse, it was observed that the storage rate increased in the subsequent two pulse as 0.85 and 0.84 g COD/ (g COD.h). The increases in the storage rate were explained with ammonia depletion, which caused more carbon being available for the storage process in the further pulses.

The relation with storage yield and ammonia concentration was observed under aerobic conditions. Since it can be considered that ammonia is only used for growth under aerobic conditions, the fraction of substrate obtained for cell growth could be controlled with limitation of ammonia. Related to this hypothesis, when the ammonia concentration is increased, the more carbon is used for growth and storage process is being less important. This was specified that the storage yield decreased with ammonia concentration, while the growth yield increased (Serafim *et al*., 2004). With respect to this observation, the maximum storage yield was found as 0.98 g COD/g COD where ammonia was not given to the reactor.

The faster uptake rates and higher observed yields have been shown in intermittently feeding than continuously feeding. Due to the capacity of storage is higher than accumulation response, substrate uptake is quickly decrease and than constant (Cech and Chudoba, 1983). In respect of this approach, while both mechanisms are performing for intermittently feeding, only storage occurs when microorganism is fed continuously.

The removal of substrate was recognized based on COD balance under transient conditions. COD balance consists of the substrate removed from the liquid phase was recovered as transformed into solids, as PHB and active biomass, or oxidized for energy needs. In addition, it was proposed that the small amount of substrate was used to produce other cellular components since it was observed the population mainly composed of bacteria belonging to the *Zoogloea* genus under feast and famine conditions. These bacteria are floc formers, which store high amounts of PHB, and produce an exopolysaccharide termed as Zooglan, mainly composed of glucose and galactose in a 2:1 proportion (Guillouet *et al*., 1999).

Another view for completing the COD balance was given by Dionisi *et al.*, (2001). These authors explain the lack of substrate in the COD balances with accumulation. It is proposed that after the substrate transported into the cell, it is first accumulated and then stored as a polymer; there is a time shift between acetate uptake and PHB production.

2.12 Transient Phenomena

Transient phenomena can be divided into two general classes: growht responses and storage reponses. The difference between the two is best illustrated by considering their basic nature. Microbial growth involves the traansport of extracellular substrates into the cell, oxidation of a portion to obtain energy, and the use of another portion to synthesyze all components of the biomass in the proper porportion. Storage involves the transport of substrates, the oxidation of a smaller portion, and the synthesis primarily of the storage polymers (carbohydrate and lipid).

2.13 Growth Response

One type of growth response is called growth rate hysteresys (GRH). This response, originally deduced theoretically by Perret (1960), and can be described as the affinity during periods when the substrate concentration changes, for the specific growth rate of a microbial culture to lag behind the value predicted by the steady-state specific growth rate – substrate relationship. During periods of increasing substrate concentration the specific growth rate will be less than predicted, while during periods of decreasing concentration it will be greater (Daigger and Grady, 1982a, Insel et al., 2007, Ferenci, 1999). GRH has also been observed experimentally by Storer & Gaudy (1969). They applied a 3 fold step increase in the influent substrate concentration to a natural community which had been selected in a glucose-limited chemostat, and monitored the response. The specific growth rates and substrate concentrations measured during the transient were then compared to the steady-state relationship, and the results clearly indicated that GRH has occured.

The second type of growth response, called available reaction potential (ARP), which has been defined as the ability of a microbial culture to rapidly increase its growth and substrate removal rates during transient response. It has been proved that the cause of ARP is the increases in the substrtae concentration (Daigger and Grady, 1982b).

Microbial cultures are capable of showing both GRH and ARP during a transient response. Daigger and Grady (1982b). Many authors showed that the specific growth rate immediately after the start of a transient was grater than the rate before but less than the maximum which the culture could achieve. The specific growth rate increased with time and reached the maximum rate. (George &Gaudy, 1975), (Krishna & Gaudy, 1976) and Saleh & Gaudy, 1978).

2.14 Storage Response

Storage has been observed most commonly during periods when growth of a culture is limited by a biosynthetic restriction even though readily biodegradable carbon and energy source is available. It has also been observed, however, during transient growth situations. Observations of storage during transient responses have generally been made in fill-and-draw activated sludge units growing on readily utilizable carbon and energy sources (most of the glucose). Good examples are the studies of Lavallée *et al.* (2005), and Vanrolleghem *et al*. (1998). During periods of high substrate concentration the cultures were capable of accumulating high levels of glycogen and/or PHB which were subsequently metabolized after depletion of the exogenous substrate (Daigger and Grady, 1982a, İnsel et al.2007). Substantial lags in cell growth were also observed, as evidenced by the fact that increases in DNA did not occur until several hours after the cultures were fed. Storage rate and capacity were influenced by the net specific growth rate of the culture and the nature of the substrate. The fraction of substratre stored increased initially as the net specific growth rate was decreased, but eventually decreased at very low net specific growth rates. The highest storage rates and capacities were observed for glucose, and they decreased when acetate, glutamic acid, or peptone was substitued.

Storage has also been observed in steady state chemostat cultures subjected to shift-ups. Examples with pure cultures have been given by Tempest *et al.* (1967) while Gaudy (1974) has presented examples with natural microbial communities. Gaudy (1974) has also demonstrated that storage will not necessarily be the primary response exhibited during shift-ups of natural microbial communities selected on a readily utilizable carbon and energy source; a growth response may also be observed. Saleh & Gaudy (1978) developed natural populations in glucose limited chemostats and monitored their responses to shift-ups. When two cultures were selected under identical conditions and subjected to identical shift-ups, one showed a growth response while the other gave a storage response.

The occurance of storage during transient responses is consistent with the principles of physiological adaptation. First consider the situation in which an excess of a readily utiliable carbon and energy source is available to a culture that is prevented from growing at its maximum rateby a biosynthetic growth limitation (e.g. nutrient defciency). Altough the substrate may be transported into the cell and metabolized rapidly, the biosynthetic growth limitation prevents all of the energy generated by its oxidationfrom being used. This leads to an increase in the adenylate energy charge, which in turn, leads to storage of the substrate. Second consider the situation in which storage has been observed during transients which are not associated with an external biosynthetic limitation. In those cases it seems likely that the biosynthetic limitation is internal. It can be understood from the discussion of the growth response that lags in the specific growth rate occur and that they result from physiological adaptations of the protein synthesizing machinery, enzyme levels and the concentration of intracellular metabolites to low growth rate environments. If the cells contain excess catabolic enzymes, energy would be released faster than it could be used, leading to storage. Such scenario is consistent with the lags in cell growth observed during storage reponses.

3 MATERIALS AND METHODS

For the observation of behavior of microorganisms under intermittent feeding conditions, experimental studies mainly consisted of acclimation period and batch tests. For this purpose, two fill and draw reactors were used for the acclimation of activated sludge.

Activated sludge taken lab scale reactor fed with acetate used for acclimation purposes. Activated sludge was fed with acetate solution having 200 mgCOD /L in fill & draw reactors, which had a working volume of 14 and 8 L. All necessary macro and micronutrients were added in sufficient quantities for biological growth. The temperatures of systems were kept constant at 20 ^{O}C. Dissolved oxygen concentration in the reactors was also kept at minimum of 3 mg/L. The reactors were operated at a sludge age of 10 days (14 L) and 2 days (8 L) and a hydraulic retention time of one day. During acclimation period same feeding pattern, which is pulse feeding, is used. Acetate was given during 1 minute in pulse feeding and after 24 hours the sludge left for settling. The system was operated until steady state were reached. After the acclimation period, effect of intermittent feeding conditions to activated sludge was investigated.

Thus 7 sets of batch experiments were conducted in order to investigate the effects of intermittent feeding patterns. The measurements of these samples gave information about the substrate removal performances, acetate uptake rates and storage stoichiometry and kinetics of the system for each feeding period.

3.1 Experimental Setup

Steady – state conditions were obtained with both fill and draw reactors which was operated at sludge ages of 10 days and 2 days. Acetate, which is readily biodegradable (Henze, 1992), was used as carbon source. The batch experiments are summarised in Table 3.1.

Table 3.1 : Summary of experimental set-up

SETS		COD	VSS	Sludge Age	Duration Between Feedings
SET1	**SET1.1**	200	410	10	2 Hours
	SET1.2	200	410	10	
SET2	**SET2.1**	200	410	10	15 Minutes
	SET2.2	200	410	10	
	SET2.3	200	410	10	
SET3		150	600	10	-
SET4		100	600	10	-
SET5		50	600	10	-
SET6	**SET6.1**	200	410	2	2 Hours
	SET6.2	200	410	2	
SET7	**SET7.1**	200	410	2	15 Minutes
	SET7.2	200	410	2	
	SET7.3	200	410	2	

Experiments representing the same conditions in respirometric tests were conducted in parallel. Sets 1 and 6 for both reactors were conducted with an intermittent feeding of acetate solution. Biomass left for 2 hours in endogenous decay phase then the acetate is added to the reactor in order to reach 200 mg COD/l. OUR curve decreased to endogenous decay level in approximately one hour. The biomass left again in endogenous phase for two hours and the second addition performed.

.The reactors were monitored with measurements of COD and PHB. Since the formation of PHB from the central metabolite acetyl-CoA, is the main storage polymer, PHA measurements mainly consisted of PHB, and therefore the results of experiments in fact represent PHB as storage compound.

Sets 2 and 7 were performed following the same procedure except the waiting time is reduced to 15 minutes between two feedings. During experiments response of the activated sludge system monitored by measuring the change of PHB and COD levels.

The duration between the batch tests were kept as minimum 1 week to ensure the steady state conditions in the parent reactor are achieved.

3.2 Analytical Procedures

Suspended solids, Volatile Suspended Solids, COD and pH analysis were performed in order to monitor and control reactor operation. In the experiments, analyses of pH and COD parameters were performed as defined in the Standard Methods (1998). On the other hand, COD samples were filtered through 0, 45 μm membrane filters and performed as described in the method proposed by ISO 6060 (1986). pH measurements were performed by a 520Aplus pH meter. Respirometric tests were performed with Applitek RA respirometer with PC connection for overall evaluation and modelling purposes). PHB samples were taken into 2x10 ml centrifuge tubes containing 2 drops formaldehyde for preventing the biological activity. The PHB content of the washed (K-P buffer solution) and freeze-dried biomass was subjected to extraction, hydrolization, and esterification in a mixture of hydrochloric acid, 1-propanol, and dichloroethane at 100°C (Beun *et al*., 2002). The resulting organic phase was extracted with water to remove free acids. The propylesters were analyzed by gas chromatograph (Agilent 6890N). Benzoic acid was used as an internal standard throughout the procedure.

4 RESULTS AND DISCUSSION

4.1 Conceptual Approach

In order to investigate intermittent feeding pattern of activated sludge, samples form a parent reactor under steady state conditions. Respirometric analyses were performed while disturbing steady-state conditions. Samples for PHB measurements were taken in order to observe the intermittent feeding conditions on storage process. Modelling study was performed for parameter estimation. The parameter estimation study provided a basis for observing the change in the kinteic parameters of activated sludge while adopting to different conditions. In this context simplified ASM 3 model (Orhon et.al., 2009) modified for simultaneous growth and storage is used. Model matrix is given in Table 4.1

Table 4.1 : A simplified ASM3 matrix for aerobic and anoxic processes

j ↓ Process / Expressed as →	Component i→	1 S_S COD	2 S_O COD	3 S_{NO} N	4 X_P COD	5 X_H COD	6 X_{STO} COD	Process rate equation, ρ_j, all $\rho_j \geq 0$
1	Aerobic storage of COD	-1	$-(1-Y_{STO})$				Y_{STO}	$k_{STO}\frac{S_S}{K_S+S_S}X_H\frac{S_O}{K_O+S_O}$
3	Aerobic growth	$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{Y_H}$			1		$\mu_H\frac{S_S}{K_S+S_S}X_H\frac{S_O}{K_O+S_O}$
3	Aerobic growth on X_{STO}		$-\frac{1-Y_H}{Y_H}$			1	$-\frac{1}{Y_H}$	$r_{GS}\frac{X_{STO}/X_H}{K_{STO}+X_{STO}/X_H}X_H\frac{S_O}{K_O+S_O}$
5	Aerobic endogenous respiration		$-(1-f_{EX})$		f_{EX}	-1		$b_H X_H\frac{S_O}{K_O+S_O}$

4.2 SET 1

Experiments representing the same conditions in respirometric tests were conducted in parallel. SET 1 was conducted with an intermittent feeding of acetate solution. Biomass left for 24 hours in endogenous decay phase then the acetate is added to the reactor in order to reach 200 mg COD/l. OUR curve decreased to endogenous decay level in approximately one hour. The biomass left again in endogenous phase for two hours and the second addition performed. The result of respirometric test of SET 1 is shown in Figure 4.1**.**

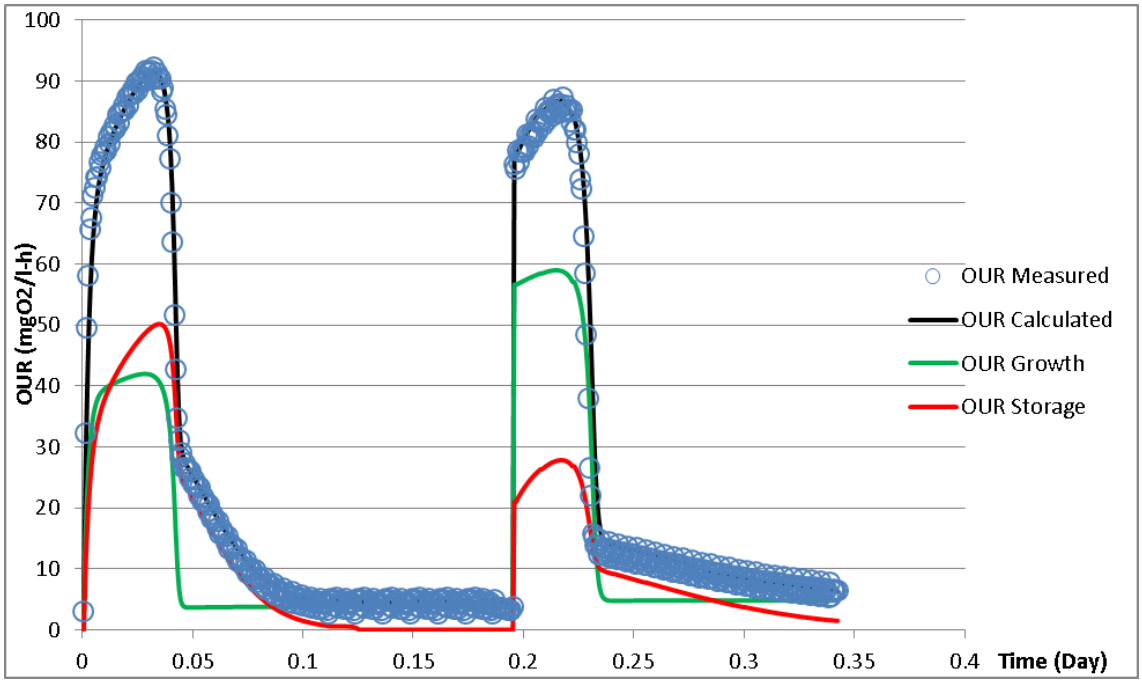

Figure 4.1 : The respirometric test results of SET1

The OUR curve reaches a maximum level of 95 mg O2/L h in 37 minutes. After the second addition OUR curve reaches a maximum level of 90 mg O2/L h in 33 minutes. On the other hand the trend of the OUR profile has changed. The SET 1 characterized with sludge age of 10 days was started with an initial VSS concentration (X0) of 410 mg/L. The resulting S0/X0 ratio was 0.50 mg COD/mg VSS and was so selected to approximate the steady-state conditions in the fill and draw reactor. As previously explained, two parallel batch reactors were operated in each run, one for the observation of the PHB profile and the other for respirometric analysis yielding the resulting oxygen uptake rate (OUR) profile. Figure 4.2 gives the obtained PHB profile.

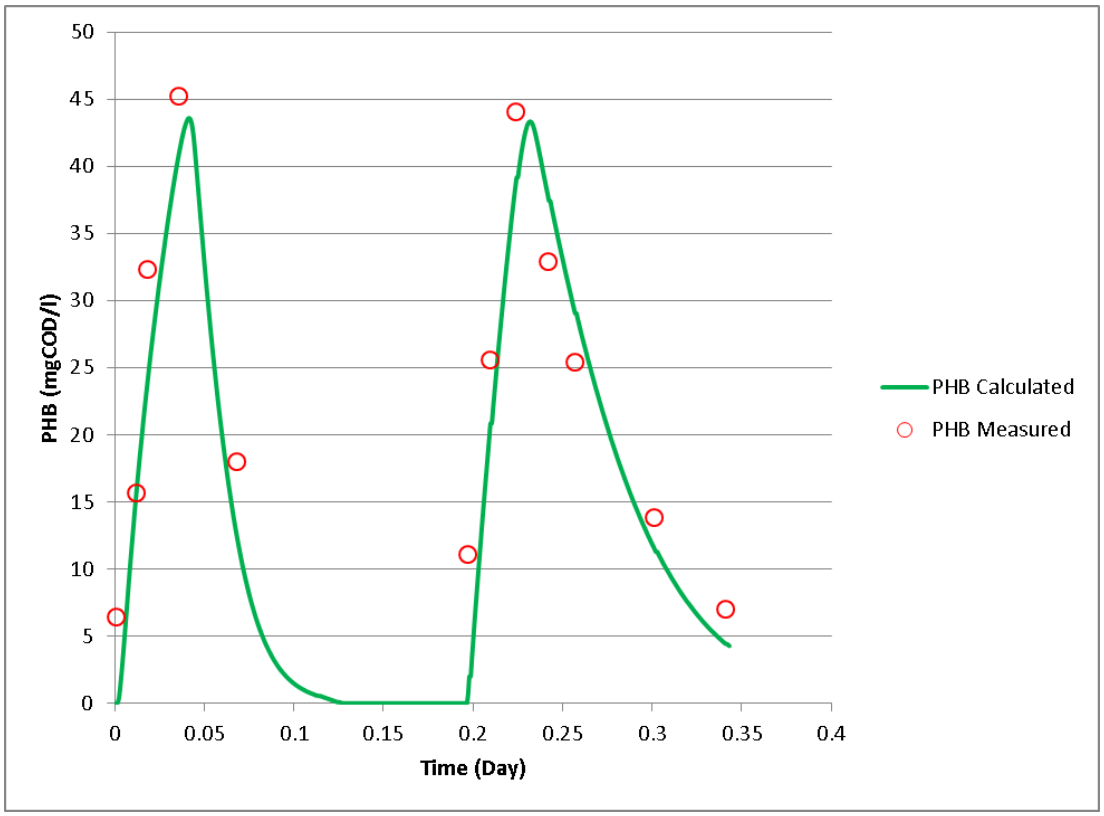

Figure 4.2 : PHB profile of SET 1

As it can be seen in Figure 4.2, the stored PHB amounts are approximately same for first and second additions. However maximum stored PHB level of 54 mg COD/l is reached in 57 minutes at the first addition and 50 minutes in the second addition. The modelling results also indicate that approximately %25 of the acetate is stored as PHB.

4.3 SET 1.1

The OUR and PHB profiles for first substrate addition (SET1.1) is given in Figure 4.3 and Figure 4.4 respectively.

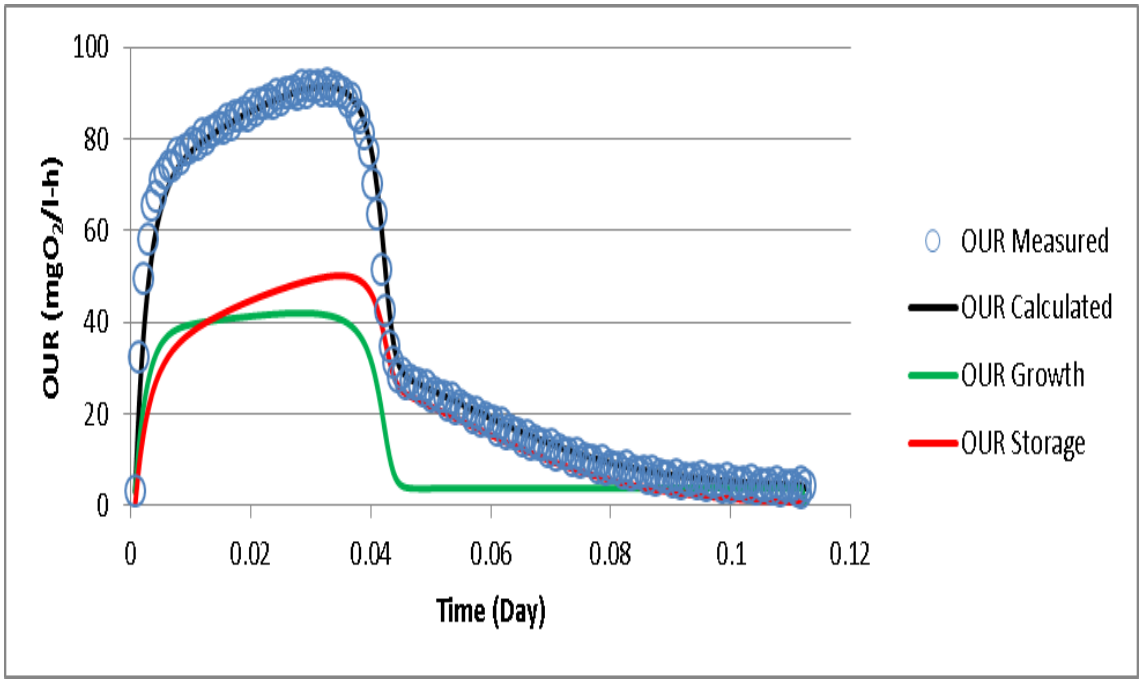

Figure 4.3 : OUR profile of SET 1.1

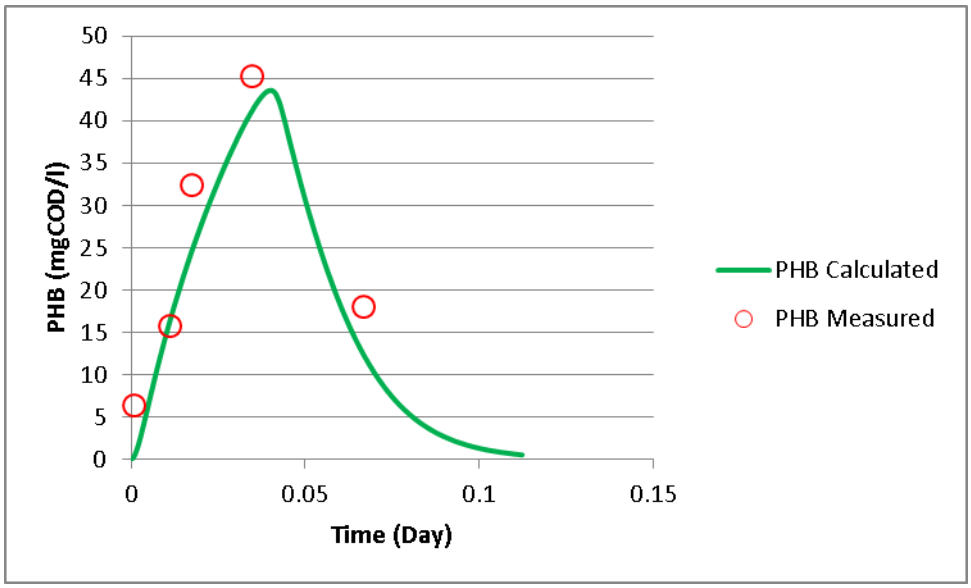

Figure 4.4 : PHB profile of SET1.1

The results of the modelling Study for SET1.1 is given in Table 4.2

Table 4.2 : Estimated stoichiometric and kinteic parameters for SET 1.1

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 1.1	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	6.13	g COD.m^{-3}
k_{STO}	10	14	16	6.21	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	3.72	d^{-1}
μ_{H2}	2	3	3&1.8	4.34	d^{-1}
Y_H	-	0.65	0.67	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

4.4 SET 1.2

The OUR and PHB profiles for the second substrate addition (SET1.2) is given in Figure 4.5 and Figure 4.6 respectively

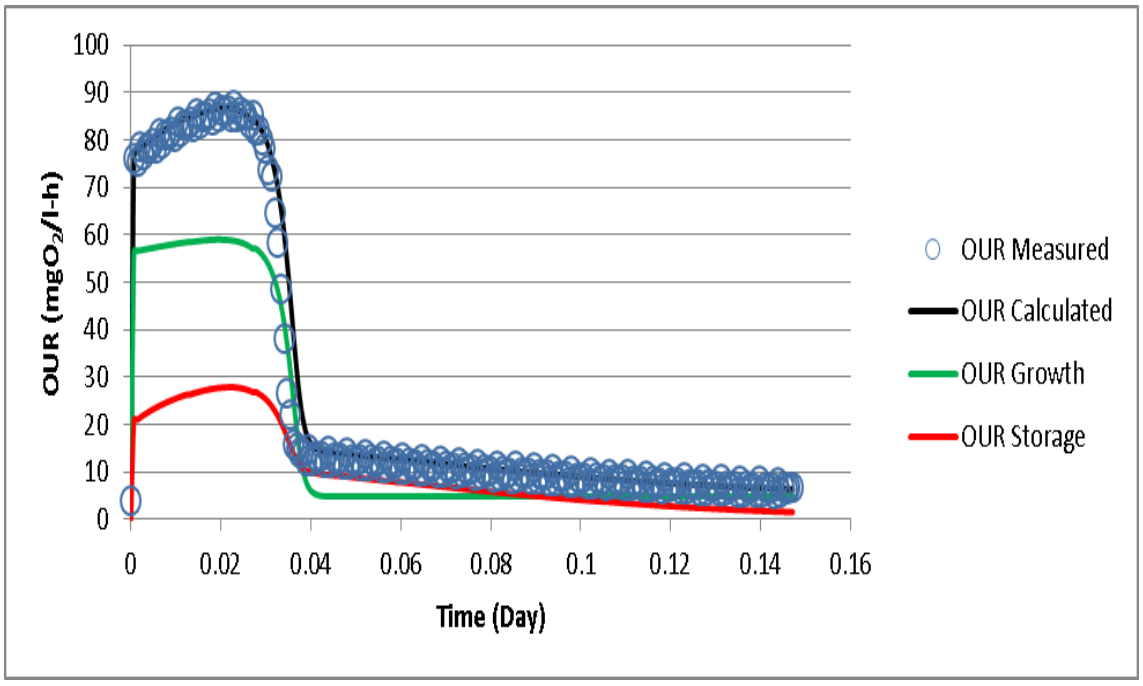

Figure 4.5 : OUR profile of SET 1.2

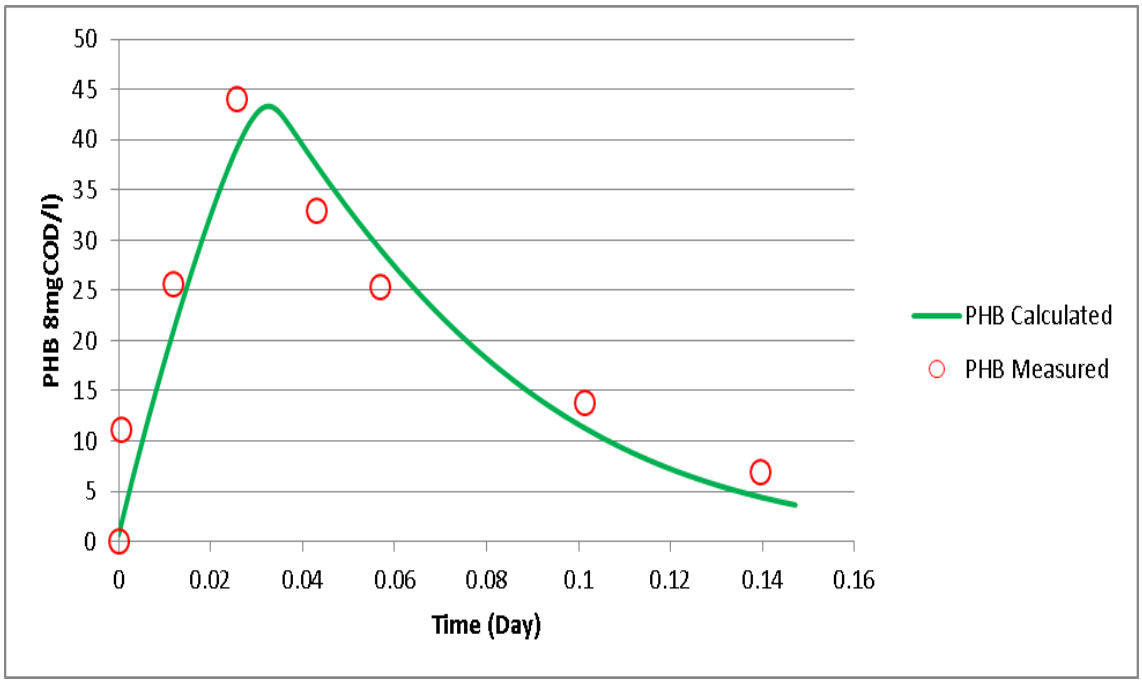

Figure 4.6 : PHB profile of SET1.2

The results of the modelling study for SET1.2 is given in Table 4.3.

Table 4.3 : :Estimated stoichiometric and kinteic parameters for SET 1.2

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 1.2	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	6.04	g COD.m^{-3}
k_{STO}	10	14	16	4.49	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	4.35	d^{-1}
μ_{H2}	2	3	3&1.8	1.79	d^{-1}
Y_H	-	0.65	0.67	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

The results of the modelling study for SET 1 is given in Table 4.4

Table 4.4 : :Estimated stoichiometric and kinteic parameters for SET 1

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 1.1	SET1.2	Units
b_H	0.2	0.24	0.24	0.22	0.22	d^{-1}
K_S	0.1	3	4	6.13	6.04	g COD.m^{-3}
k_{STO}	10	14	16	6.21	4.49	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	3.72	4.35	d^{-1}
μ_{H2}	2	3	3&1.8	4.34	1.79	d^{-1}
Y_H	-	0.65	0.67	0.66	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	0.2	

As it can be seen from the Table 4.4 after the second addition of acetate, growth rate on X_{STO} decreases significantly from 4.34 d^{-1} to 1.79 d^{-1} while direct growth on external substrate increases from 3.72 d^{-1} to 4.35 d^{-1}. The maximum storage rate decreases from 6.21 to 4.49. These results indicate that the metabolism of the activated sludge is shifting from storage to growth

4.5 SET 2

Experiments representing the same conditions in respirometric tests were conducted in parallel. SET 2 was conducted with an intermittent feeding of acetate solution. Biomass left for 24 hours in endogenous decay phase then the acetate is added to the reactor in order to reach 200 mg COD/l. OUR curve decreased to endogenous decay level in approximately one hour. The biomass left again in endogenous phase for fifteen minutes and the second addition performed. The result of respirometric test of SET 2 is shown in Figure 4.7**.**

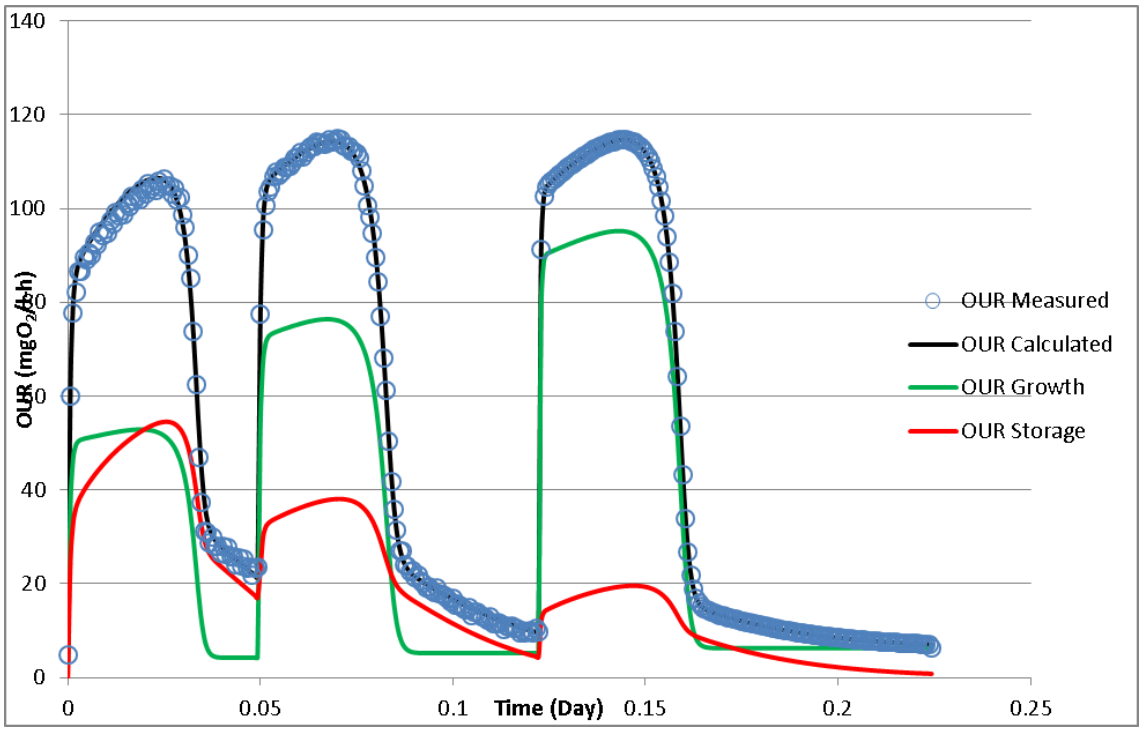

Figure 4.7 : The respirometric test results of SET2

The SET 2 which is also characterized with sludge age of 10 days was started with an initial VSS concentration (X0) of 410 mg/L. The resulting S0/X0 ratio was 0.50 mg COD/mg VSS and was so selected to approximate the steady-state conditions in the fill and draw reactor. As previously explained, two parallel batch reactors were operated in each run, one for the observation of the PHB profile and the other for respirometric analysis yielding the resulting oxygen uptake rate (OUR) profile. Figure 4.8 gives the obtained PHB profile.

The OUR curve reaches a maximum level of 105 mg O2/L h in 30 minutes. After the second addition OUR curve reaches a maximum level of 114 mg O2/L h in 25 minutes. After the third addition the maximum OUR level of 114 mg O2/L is maintained however it was reached in 23 minutes. The changing OUR profile trend is also observed in SET 2 as in SET 1.

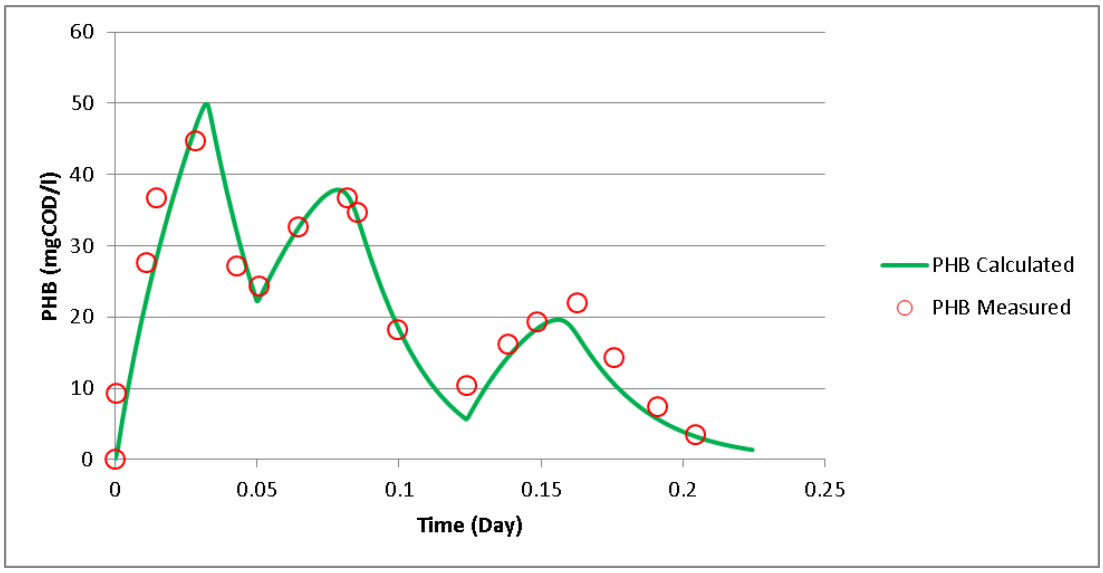

Figure 4.8 : PHB profile of SET 2

As it can be seen in Figure 4.8, the amount of stored PHB decreases after each addition significantly. On the other hand, the biomass reaches its maximum amount of stored PHB in approximately same duration. After the first addition the maximum stored PHB level of 50 mgCOD/l is reached after 46 minutes. After the second addition the maximum stored PHB level of 37 mgCOD/l is reached after 45 minutes and after the third addition the maximum stored PHB level of 20 mgCOD/l is reached after 47 minutes

4.6 SET 2.1

The OUR and PHB profiles for the first substrate addition (SET 2.1) is given in Figure 4.5 and Figure 4.6 respectively. The results of the modeling study for SET 2.1 is given in Table 4.5 **:**

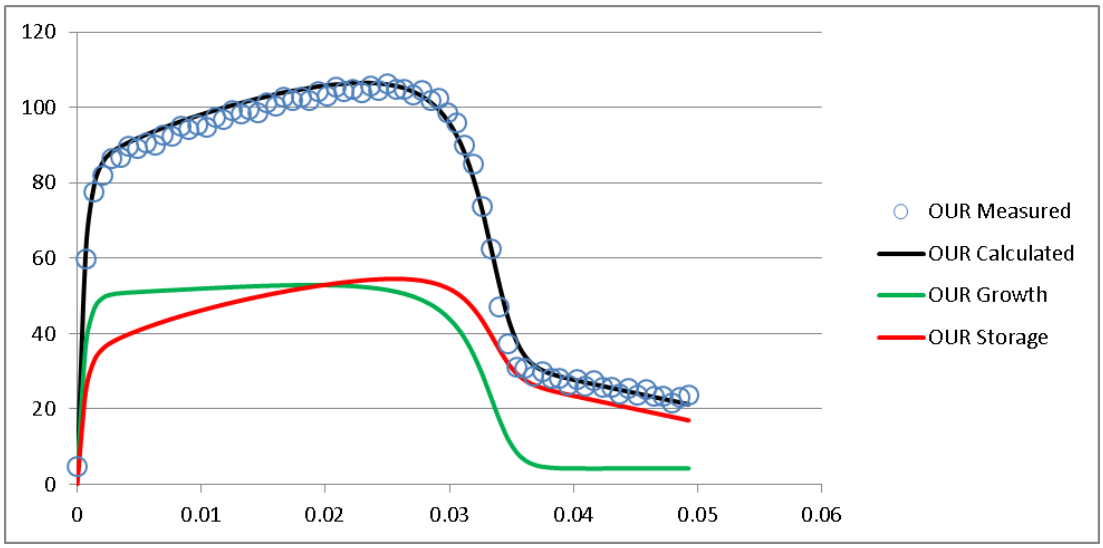

Figure 4.9 : OUR profile of SET 2.1

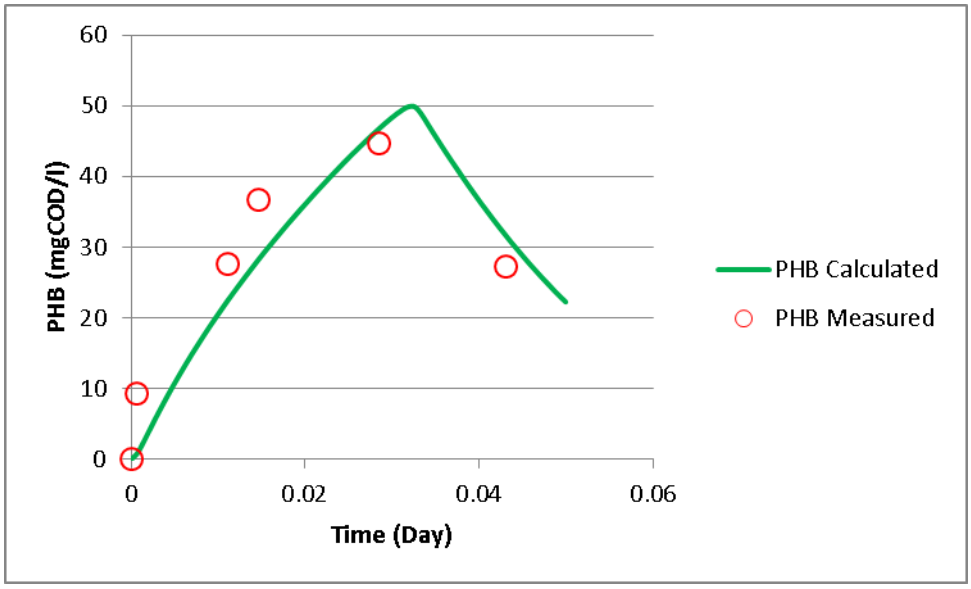

Figure 4.10 : PHB profile of SET 2.1

Table 4.5 : Estimated stoichiometric and kinteic parameters for SET 2.1

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 2.1	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	8.87	g COD.m^{-3}
k_{STO}	10	14	16	6.28	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	4.15	d^{-1}
μ_{H2}	2	3	3&1.8	4.77	d^{-1}
Y_H	-	0.65	0.67	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

4.7 SET 2.2

The OUR and PHB profiles for the second substrate addition (SET2.2) is given in Figure 4.5 and Figure 4.6 respectively. The results of the modeling study for SET 2.1 is given in Table 4.5 **:**

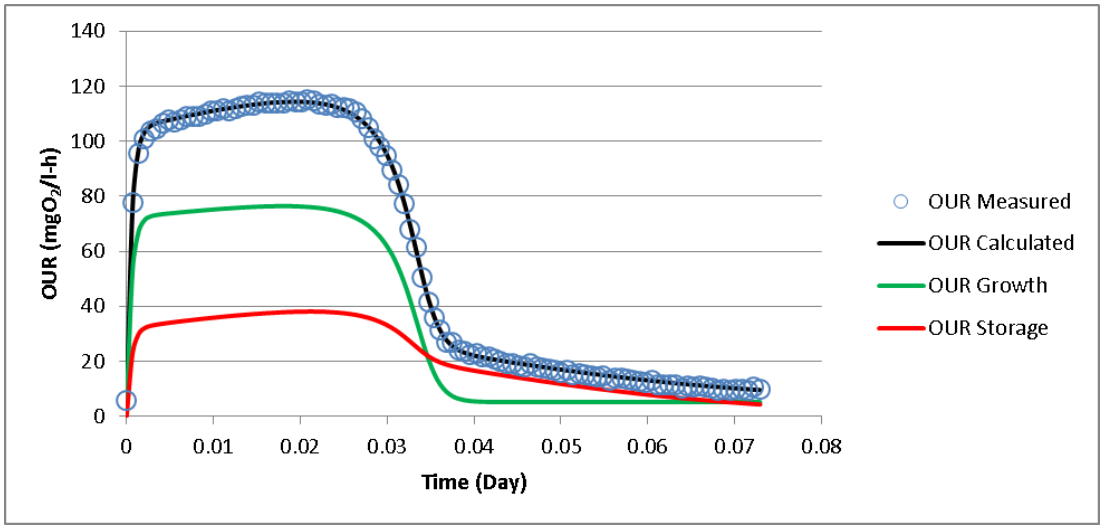

Figure 4.11 : OUR profile of SET 2.2

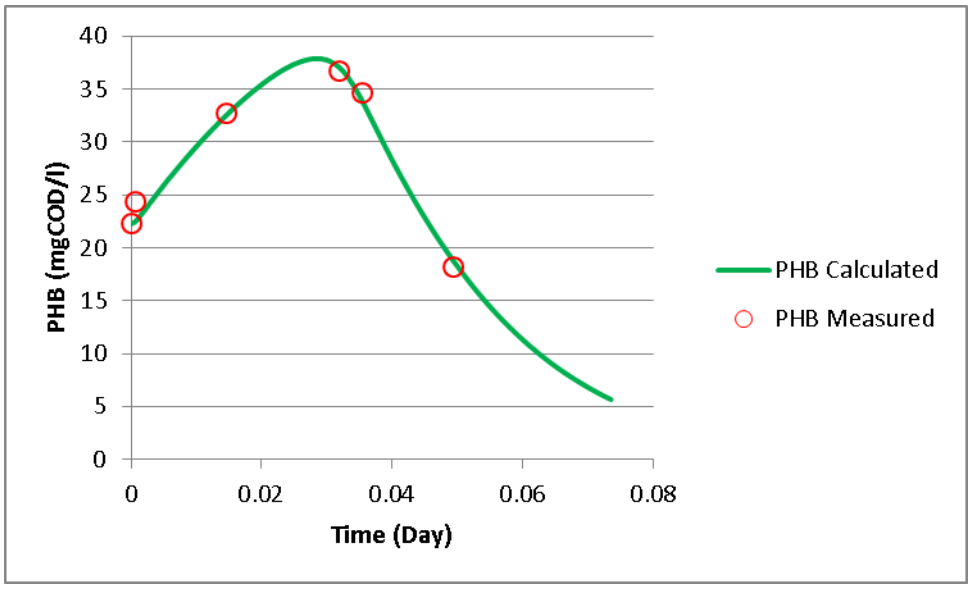

Figure 4.12 : PHB profile of SET 2.2

The results of the modeling study for SET 2.1 is given in Table 4.6.**Table 4.5 :**

Table 4.6 : Estimated stoichiometric and kinteic parameters for SET 2.2

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 2.2	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	9.05	g COD.m^{-3}
k_{STO}	10	14	16	3.6	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	4.96	d^{-1}
μ_{H2}	2	3	3&1.8	4.72	d^{-1}
Y_H	-	0.65	0.67	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

4.8 SET 2.3

The OUR and PHB profiles for the third substrate addition (SET2.3) is given in Figure 4.13 and Figure 4.14 respectively. The results of the modelling study for SET 2.1 is given in Table 4.7.

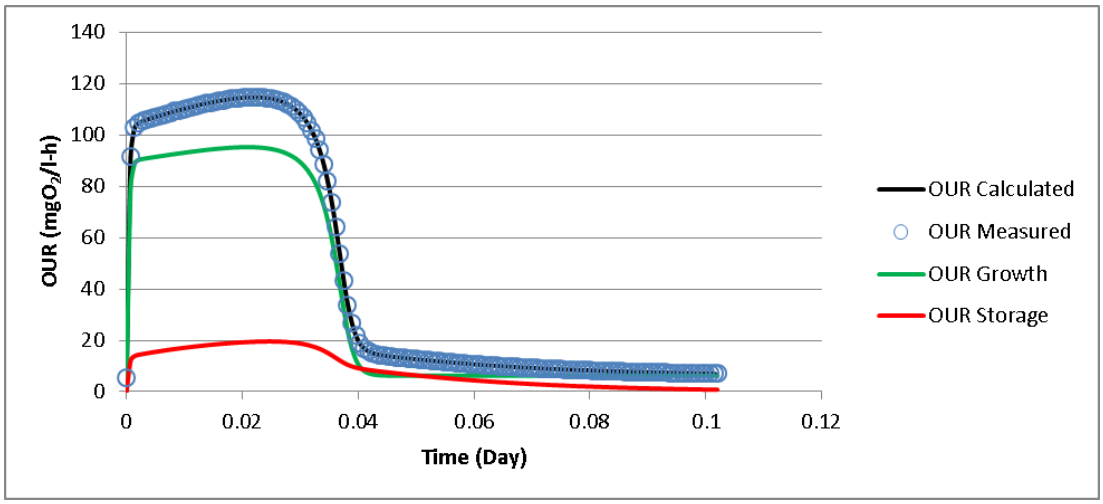

Figure 4.13 : OUR profile of SET 2.3

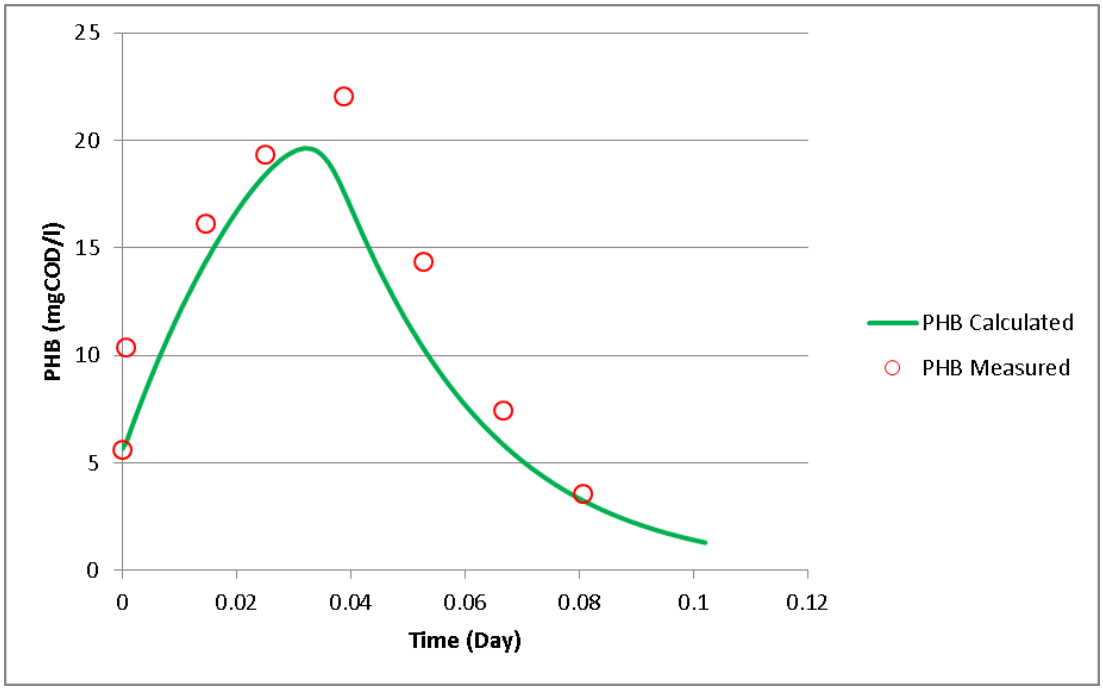

Figure 4.14 : PHB profile of SET 2.3

Table 4.7 : Estimated stoichiometric and kinteic parameters for SET 2.3

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 2.3	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	9.13	g COD.m^{-3}
k_{STO}	10	14	16	1.63	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	5.02	d^{-1}
μ_{H2}	2	3	3&1.8	2.92	d^{-1}
Y_H	-	0.65	0.67	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

The results of the modelling study for SET 1 is given in Table 4.4

Table 4.8 : :Estimated stoichiometric and kinteic parameters for SET 2

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 2.1	SET2.2	SET2.3	Units
b_H	0.2	0.24	0.24	0.22	0.22	0.22	d^{-1}
K_S	0.1	3	4	8.87	9.05	9.13	g COD.m^{-3}
k_{STO}	10	14	16	6.28	3.6	1.63	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	4.15	4.96	5.02	d^{-1}
μ_{H2}	2	3	3&1.8	4.77	4.72	2.92	d^{-1}
Y_H	-	0.65	0.67	0.66	0.66	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	0.8	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	0.2	0.2	

As it can be seen from the Table 4.8, after the second addition of acetate, growth rate on X_{STO} decreases slowly from 4.77 d^{-1} to 4.72 d^{-1}. However after the third addition growth rate on X_{STO} significantly decreases to 2.92 d^{-1}. On the other hand, direct growth on external substrate increases from 4.15 d^{-1} to 4.96 d^{-1} after the second addition eventually reaching to 5.02 d^{-1} after the third addition.. The maximum storage rate decreases significantly after each addition from 6.28 to 3.6 and 1.63 These results indicate that the metabolism of the activated sludge is shifting from storage to growth

4.9 SET 3

Experiments representing the same conditions in respirometric tests were conducted in parallel. SET 3 was conducted with an pulse feeding of acetate solution. Biomass left for 24 hours in endogenous decay phase then the acetate is added to the reactor in order to reach 150 mgCOD/l. The result of respirometric test of SET 3 is shown in Figure 4.15**.**

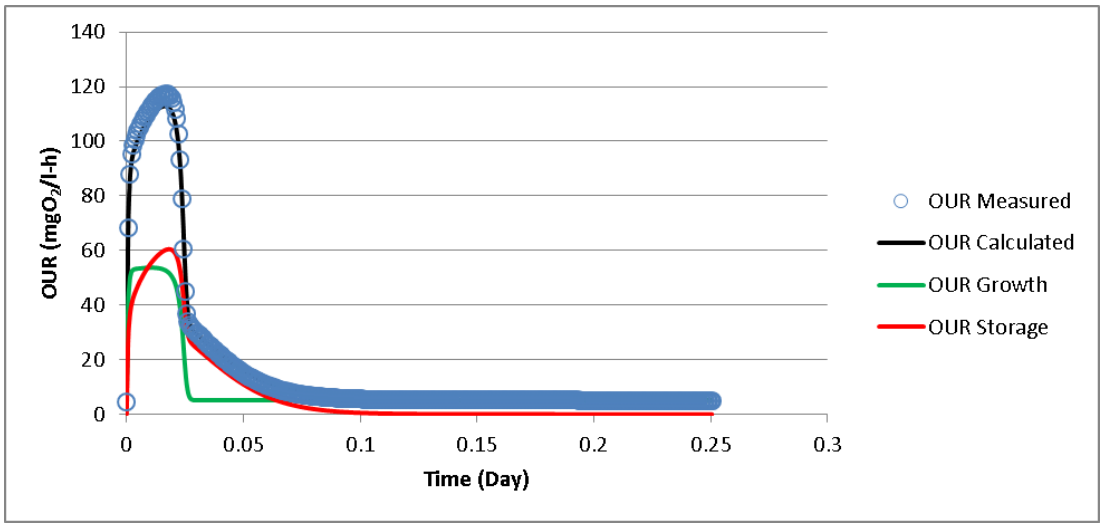

Figure 4.15 : OUR profile of SET 3

The SET 3 which is also characterized with sludge age of 10 days was started with an initial VSS concentration (X0) of 600 mg/L. The resulting S0/X0 ratio was 0.25 mg COD/mg VSS. As previously explained, two parallel batch reactors were operated in each run, one for the observation of the PHB profile and the other for respirometric analysis yielding the resulting oxygen uptake rate (OUR) profile. Figure 4.16 gives the obtained PHB profile. The OUR curve reaches a maximum level of 117 mg O2/L h in 25 minutes.

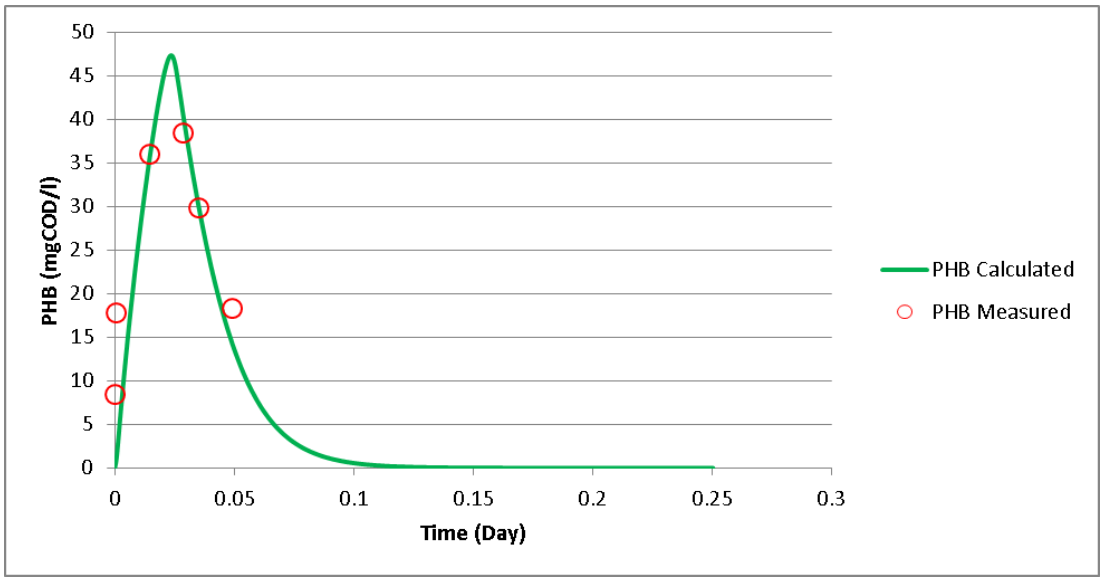

Figure 4.16 : PHB profile of SET 3

As it can be seen in Figure 4.16, the maximum stored PHB level of 47 mgCOD/l is reached after 33 minutes. The results of the modelling study for SET 3 is given in Table 4.9.

Table 4.9 : Estimated stoichiometric and kinteic parameters for SET 3

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 3	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	6.12	g COD.m^{-3}
k_{STO}	10	14	16	6.26	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	3.27	d^{-1}
μ_{H2}	2	3	3&1.8	4.42	d^{-1}
Y_H	-	0.65	0.67	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

As it can be seen from the Table 4.9, growth rate on X_{STO} is estimated as 4.42 d^{-1} and direct growth rate on external substrate is estimated as 3.27. Both values are slightly smaller than the values estimated for SET 1.1.

4.10 SET 4

Experiments representing the same conditions in respirometric tests were conducted in parallel. SET 4 was conducted with an pulse feeding of acetate solution. Biomass left for 24 hours in endogenous decay phase then the acetate is added to the reactor in order to reach 100 mgCOD/l. The result of respirometric test of SET 4 is shown in Figure 4.17**.**

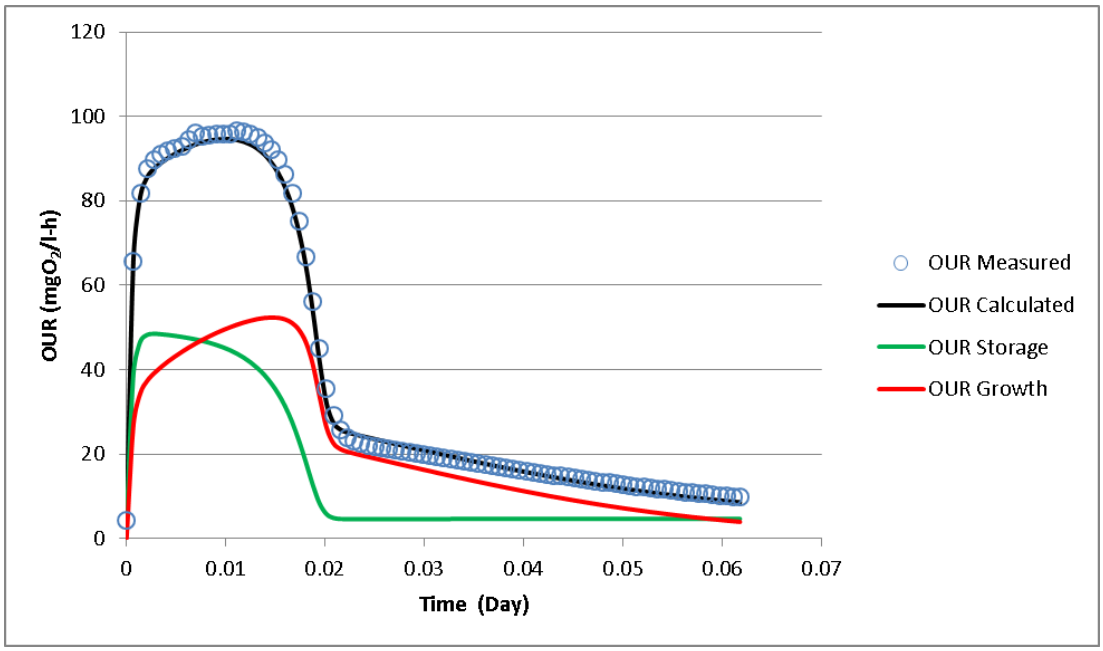

Figure 4.17 : OUR profile of SET 4

The SET 4 which is also characterized with sludge age of 10 days was started with an initial VSS concentration (X0) of 600 mg/L. The resulting S0/X0 ratio was 0.17 mg COD/mg VSS. As previously explained, two parallel batch reactors were operated in each run, one for the observation of the PHB profile and the other for respirometric analysis yielding the resulting oxygen uptake rate (OUR) profile. Figure 4.18 gives the obtained PHB profile. The OUR curve reaches a maximum level of 96 mg O2/L h in 17 minutes.

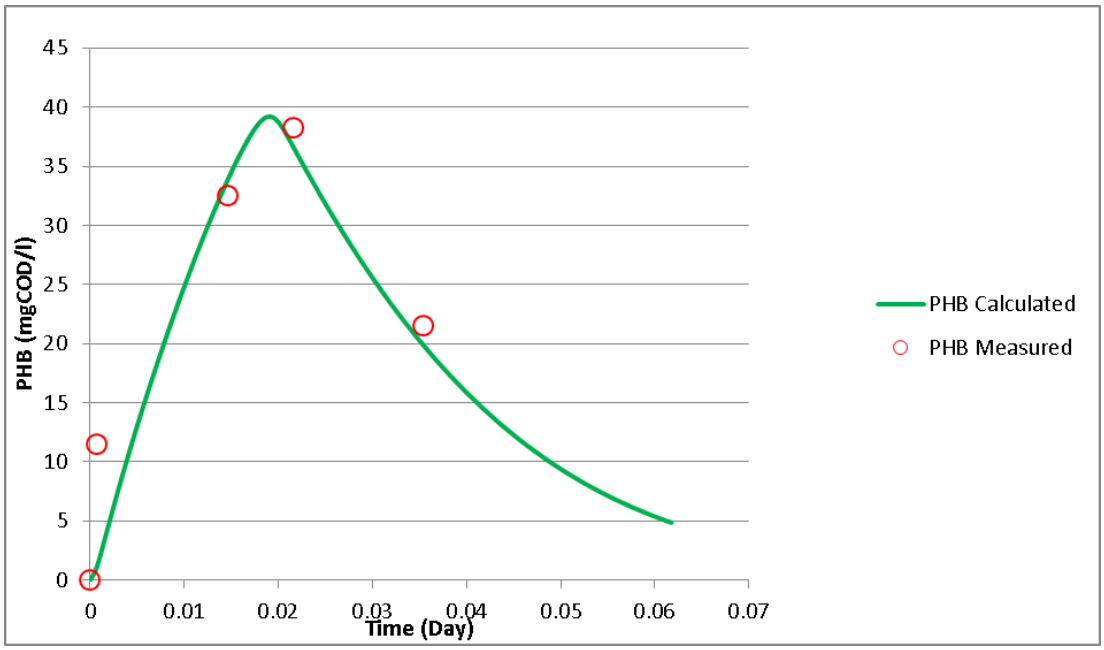

Figure 4.18 : PHB profile of SET 4

As it can be seen in Figure 4.18, the maximum stored PHB level of 39 mgCOD/l is reached after 37 minutes. The results of the modelling study for SET 4 is given in Table 4.10.

Table 4.10 : Estimated stoichiometric and kinteic parameters for SET 4

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 4	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	5.99	g COD.m^{-3}
k_{STO}	10	14	16	6.14	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	3.49	d^{-1}
μ_{H2}	2	3	3&1.8	3.42	d^{-1}
Y_H	-	0.65	0.67	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

As it can be seen from the Table 4.10, growth rate on X_{STO} is estimated as 3.42 d^{-1} and direct growth rate on external substrate is estimated as 3.49.

4.11 SET 5

Experiments representing the same conditions in respirometric tests were conducted in parallel. SET 5 was conducted with an pulse feeding of acetate solution. Biomass left for 24 hours in endogenous decay phase then the acetate is added to the reactor in order to reach 50 mgCOD/l. The result of respirometric test of SET 5 is shown in Figure 4.19**.**

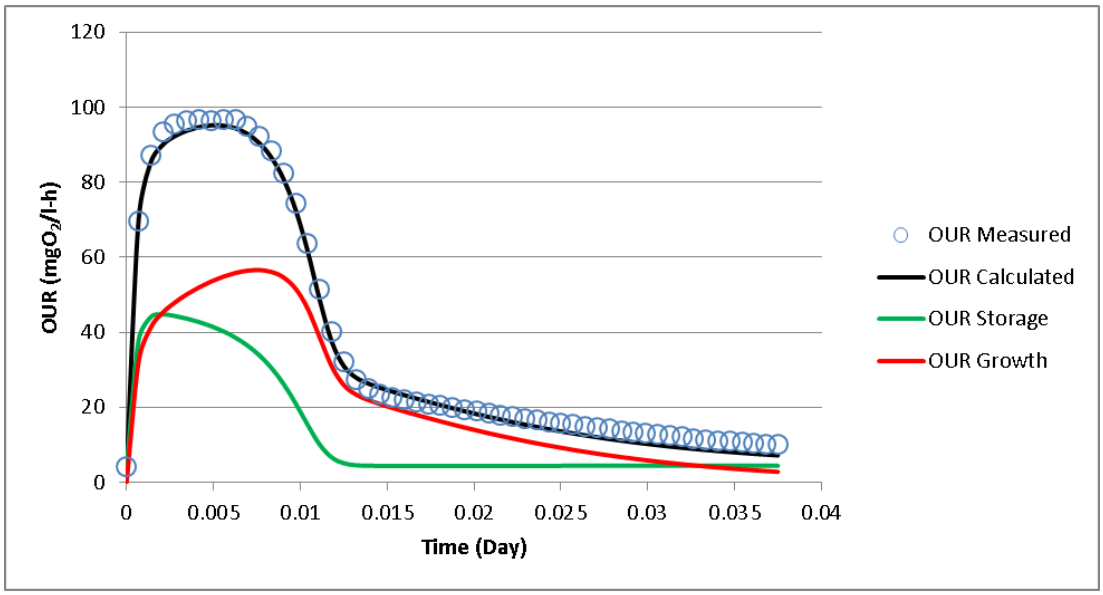

Figure 4.19 : OUR profile of SET 5

The SET 5 which is also characterized with sludge age of 10 days was started with an initial VSS concentration (X0) of 600 mg/L. The resulting S0/X0 ratio was 0.08 mg COD/mg VSS. As previously explained, two parallel batch reactors were operated in each run, one for the observation of the PHB profile and the other for respirometric analysis yielding the resulting oxygen uptake rate (OUR) profile. Figure 4.20 gives the obtained PHB profile. The OUR curve almost immediately reaches a maximum level of 96 mg O2/L h in 7 minutes.

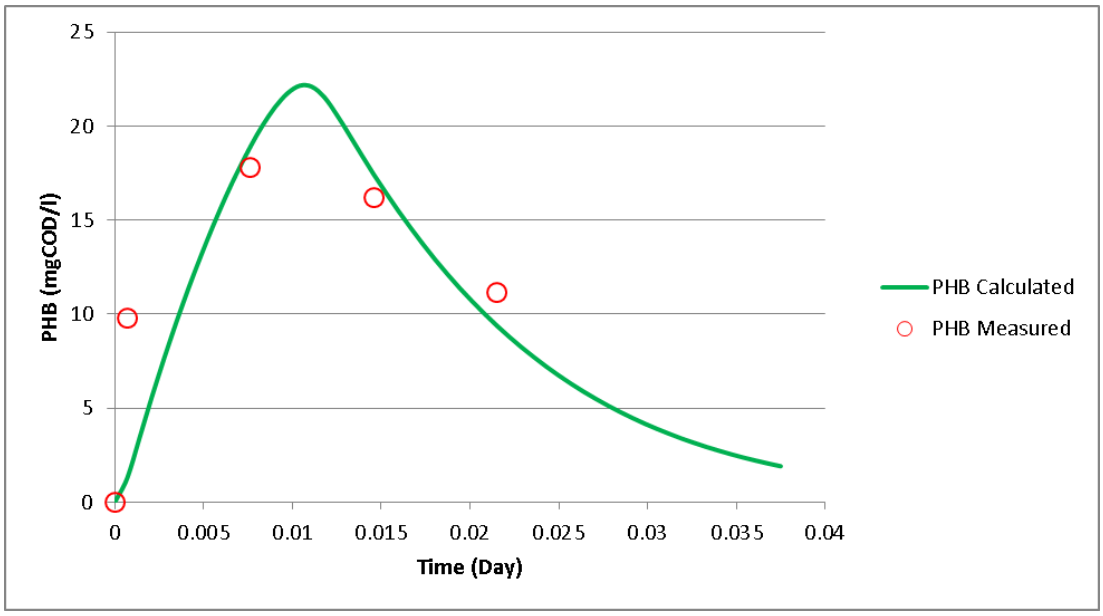

Figure 4.20 : PHB profile of SET 5

As it can be seen inFigure 4.20, the maximum stored PHB level of 22 mgCOD/l is reached after 15 minutes. The results of the modelling study for SET 5 is given in Table 4.11.

Table 4.11 : Estimated stoichiometric and kinteic parameters for SET 5

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 5	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	5.95	g COD.m^{-3}
k_{STO}	10	14	16	6.60	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	3.95	d^{-1}
μ_{H2}	2	3	3&1.8	5.14	d^{-1}
Y_H	-	0.65	0.67	0.66	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

4.12 SET 6

Experiments representing the same conditions in respirometric tests were conducted in parallel. SET 6 was conducted with an intermittent feeding of acetate solution. Biomass left for 24 hours in endogenous decay phase then the acetate is added to the reactor in order to reach 200 mg COD/l. OUR curve decreased to endogenous decay level in approximately one hour. The biomass left again in endogenous phase for two hours and the second addition performed. The result of respirometric test of SET 6 is shown in Figure 4.21**.**

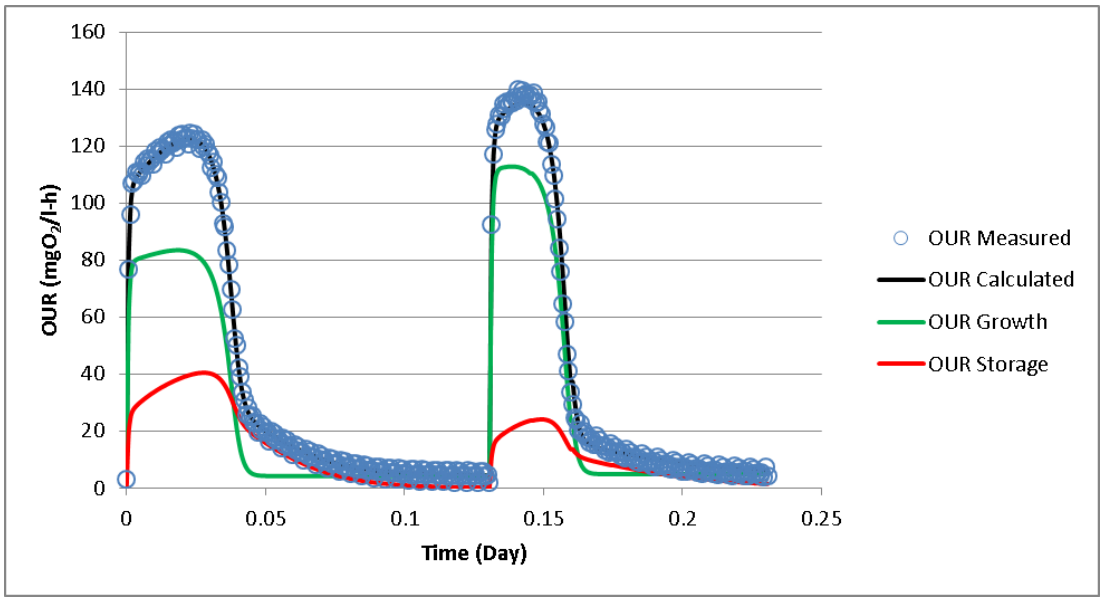

Figure 4.21 : OUR profile of SET 6

The SET 6 characterized with sludge age of 2 days was started with an initial VSS concentration (X0) of 410 mg/L. The resulting S0/X0 ratio was 0.50 mg COD/mg VSS and was so selected to approximate the steady-state conditions in the fill and draw reactor. As previously explained, two parallel batch reactors were operated in each run, one for the observation of the PHB profile and the other for respirometric analysis yielding the resulting oxygen uptake rate (OUR) profile. Figure 4.22 gives the obtained PHB profile.

The OUR curve reaches a maximum level of 124 mg O2/L h in 29 minutes. After the second addition OUR curve reaches a maximum level of 139 mg O2/L h in 15 minutes. On the other hand the trend of the OUR profile has changed. Comparison of SET 6 with SET 1 showed that activated sludge sample with smaller sludge age reached the maximum level of OUR quicker after the second addition and showed much significant transient response.

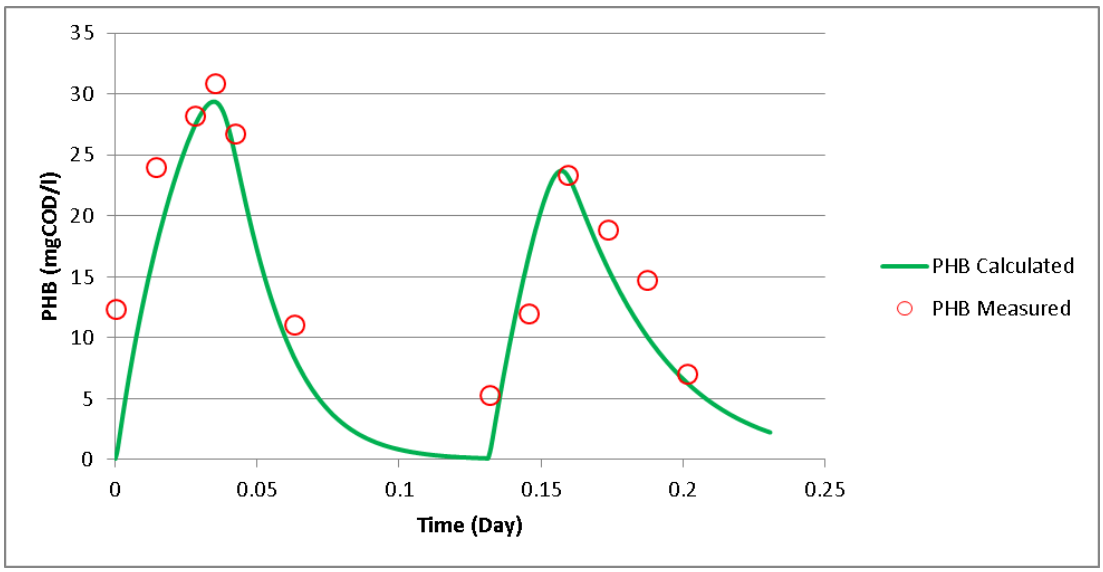

Figure 4.22 : PHB profile of SET 6

As it can be seen in Figure 4.22, the stored amount of PHB decreased after the second addition of acetate. Moreover maximum stored PHB level of 31 mg COD/l is reached in 51 minutes at the first addition and stored PHB level of 24 mg COD/l in 37 minutes in the second addition.

Comparison of SET 6 with SET 1 in the scope of stored PHB amounts showed that activated sludge sample with smaller sludge age stored less PHB in approximately same duration after the first addition. However, after the second addition stored amount of PHB decreases and the duration for reaching maximum level of PHB shortens.

4.13 SET 6.1

The OUR and PHB profiles for first substrate addition (SET6.1) is given in Figure 4.3 and Figure 4.24 respectively. The results of the modelling study for SET 6.1 is given in Table 4.12.

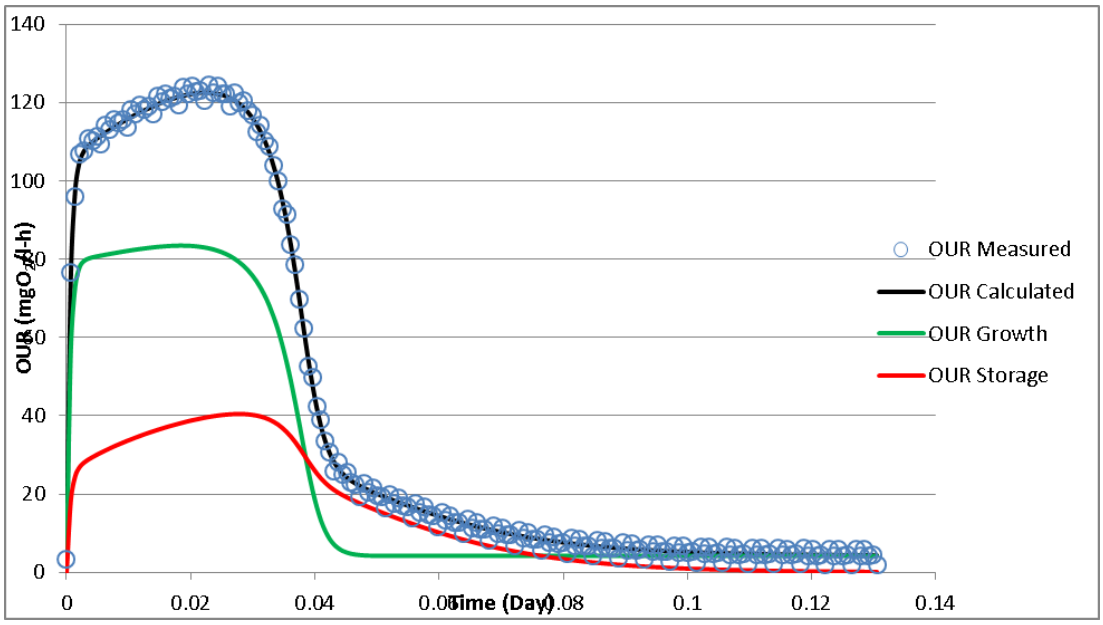

Figure 4.23 : OUR profile of SET 6.1

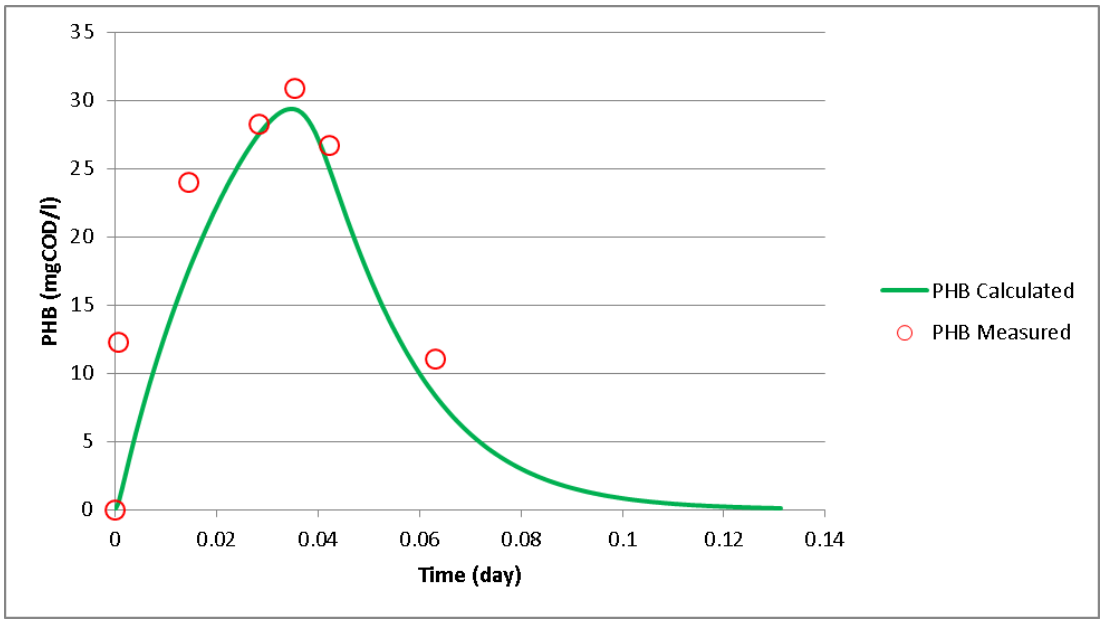

Figure 4.24 : PHB profile of SET 6.1

Table 4.12 : Estimated stoichiometric and kinteic parameters for SET 6.1

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 6.1	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	15.51	g COD.m^{-3}
k_{STO}	10	14	16	4.3	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	5.5	d^{-1}
μ_{H2}	2	3	3&1.8	3.92	d^{-1}
Y_H	-	0.65	0.67	0.6	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

4.14 SET 6.2

The OUR and PHB profiles for the second substrate addition (SET6.2) is given in Figure 4.25 and Figure 4.26 respectively. The results of the modelling study for SET 6.1 is given in Table 4.12

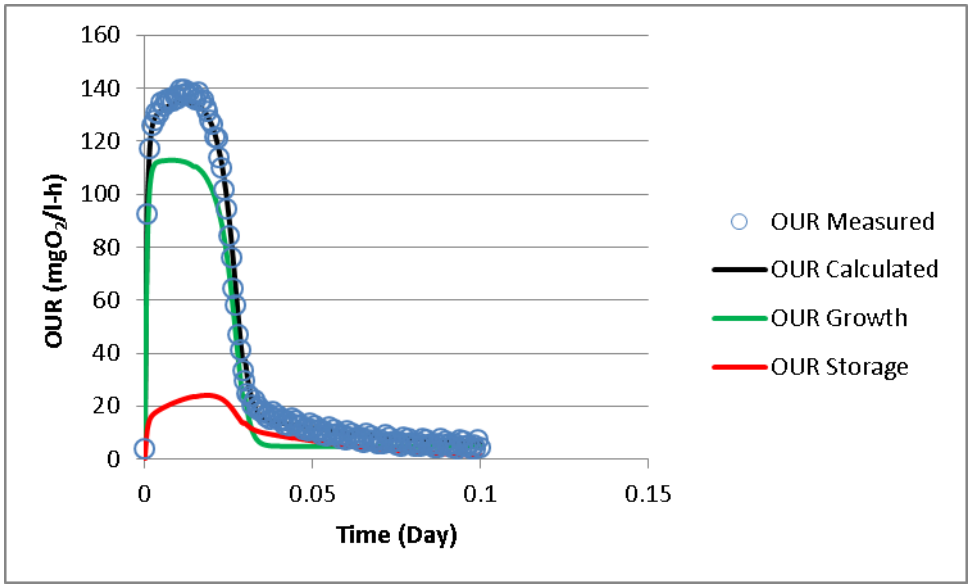

Figure 4.25 : OUR profile of SET 6.2

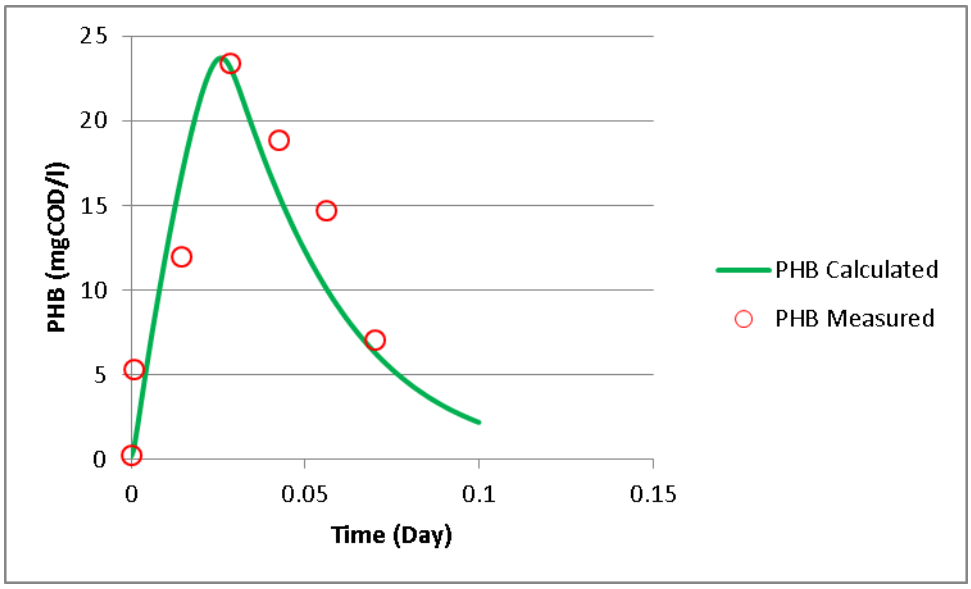

Figure 4.26 : PHB profile of SET 6.2

Table 4.13 : Estimated stoichiometric and kinteic parameters for SET 6.2

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 6.2	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	15.62	g COD.m^{-3}
k_{STO}	10	14	16	3.02	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	6.3	d^{-1}
μ_{H2}	2	3	3&1.8	2.23	d^{-1}
Y_H	-	0.65	0.67	0.6	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

The results of the modelling study for SET 1 is given in Table 4.14.

Table 4.14 : :Estimated stoichiometric and kinteic parameters for SET 6

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 6.1	SET6.2	Units
b_H	0.2	0.24	0.24	0.22	0.22	d^{-1}
K_S	0.1	3	4	15.51	15.62	g COD.m^{-3}
k_{STO}	10	14	16	4.3	3.02	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	5.5	6.3	d^{-1}
μ_{H2}	2	3	3&1.8	3.92	2.23	d^{-1}
Y_H	-	0.65	0.67	0.6	0.6	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	0.2	

As it can be seen from the Table 4.14 after the second addition of acetate, growth rate on X_{STO} decreases significantly from 3.92 d^{-1} to 2.23 d^{-1} while direct growth on external substrate increases from 5.6 d^{-1} to 6.3 d^{-1}. The maximum storage rate decreases from 4.3 to 3.02. These results indicate that the metabolism of the activated sludge is shifting from storage to growth

4.15 SET 7

Experiments representing the same conditions in respirometric tests were conducted in parallel. SET 7 was conducted with an intermittent feeding of acetate solution. Biomass left for 24 hours in endogenous decay phase then the acetate is added to the reactor in order to reach 200 mgCOD/l. OUR curve decreased to endogenous decay level in approximately one hour. The biomass left again in endogenous phase for fifteen minutes and the second addition performed. The result of respirometric test of SET 7 is shown in Figure 4.27**.**

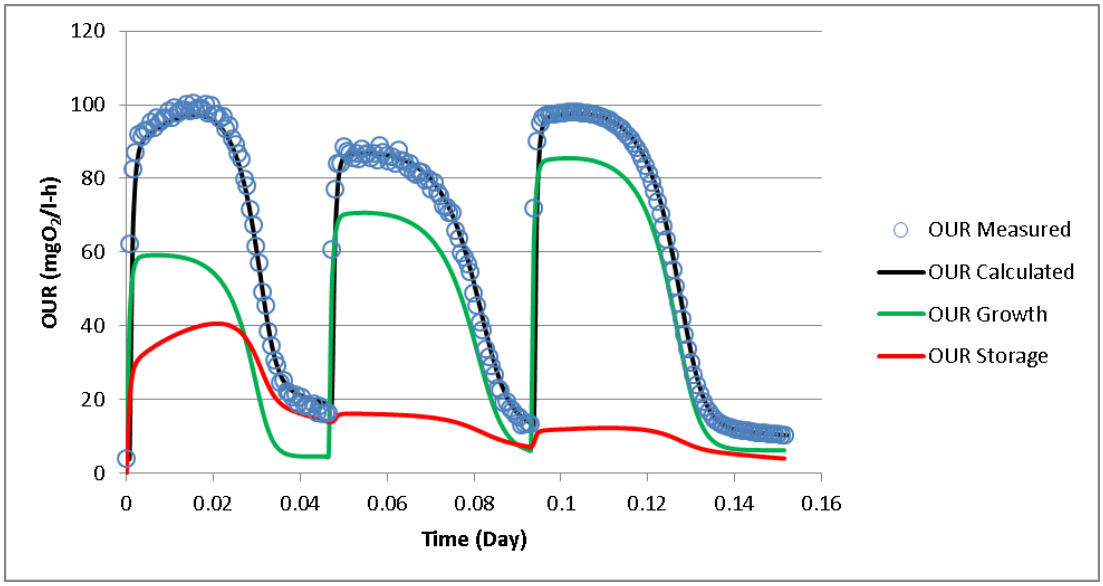

Figure 4.27 : OUR profile of SET 7

The SET 7 which is also characterized with sludge age of 2 days was started with an initial VSS concentration (X0) of 410 mg/L. The resulting S0/X0 ratio was 0.50 mg COD/mg VSS and was so selected to approximate the steady-state conditions in the fill and draw reactor. As previously explained, two parallel batch reactors were operated in each run, one for the observation of the PHB profile and the other for respirometric analysis yielding the resulting oxygen uptake rate (OUR) profile. Figure 4.28 gives the obtained PHB profile.

The OUR curve reaches a maximum level of 100 mg O2/L h in 20 minutes. After the second addition OUR curve reaches a maximum level of 88 mg O2/L h in 5 minutes. After the third addition the maximum OUR level of 97 mg O2/L is maintained however it was reached in 5 minutes. The changing OUR profile trend is also observed as in SET 1 and SET 2.

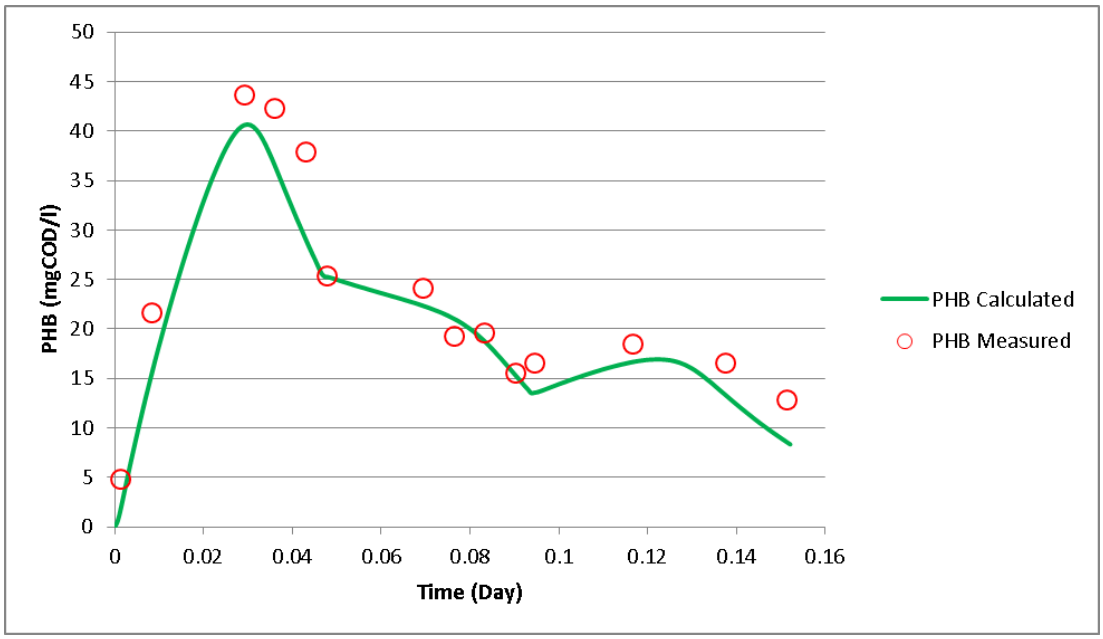

Figure 4.28 : PHB profile of SET 7

As it can be seen in Figure 4.28, the amount of stored PHB decreases after each addition significantly. Moreover after second addition the biomass nearly stops storage process. After the third addition stored amount of PHB increases again.

4.16 SET 7.1

The OUR and PHB profiles for the first substrate addition (SET 7.1) is given in Figure 4.29 and Figure 4.30 respectively. The results of the modelling study for SET 7.1 is given in Table 4.15.

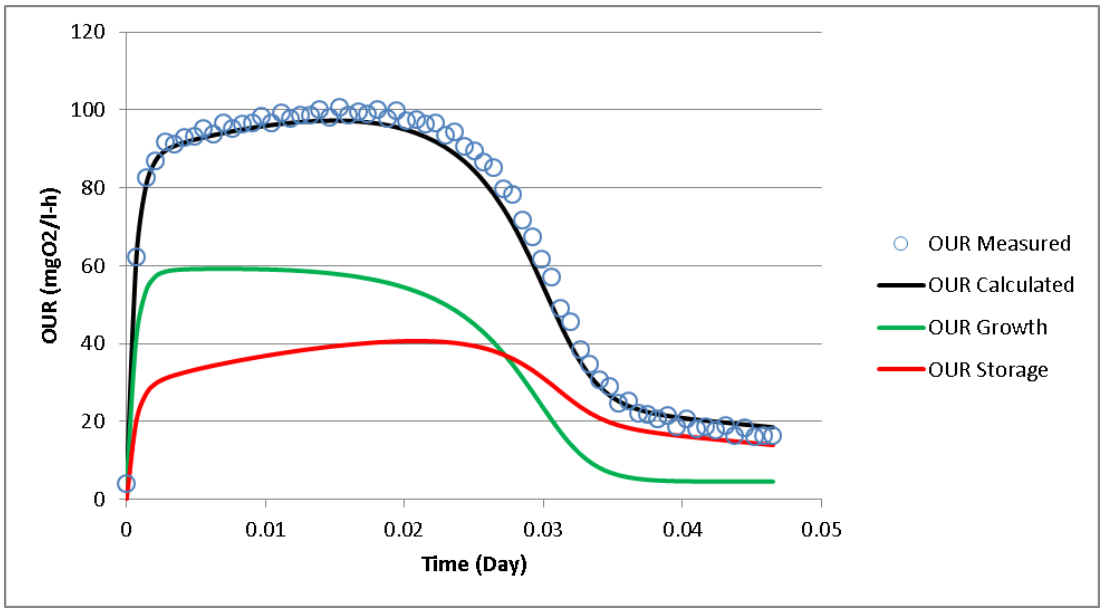

Figure 4.29 : OUR profile of SET 7.1

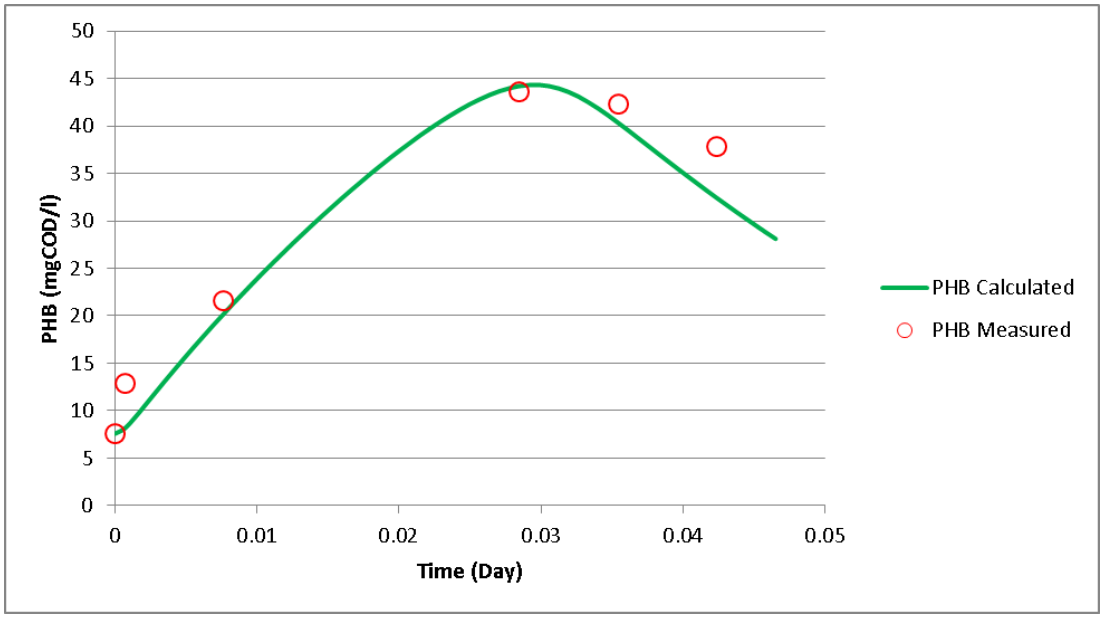

Figure 4.30 : PHB profile of SET 7.1

Table 4.15 : Estimated stoichiometric and kinteic parameters for SET 7.1

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 7.1	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	20	g COD.m^{-3}
k_{STO}	10	14	16	5.57	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	5.20	d^{-1}
μ_{H2}	2	3	3&1.8	3.35	d^{-1}
Y_H	-	0.65	0.67	0.6	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

4.17 SET 7.2

The OUR and PHB profiles for the second substrate addition (SET 7.2) is given in Figure 4.31 and Figure 4.32 respectively. The results of the modelling study for SET 7.2 is given in Table 4.16.**Table 4.5 :**

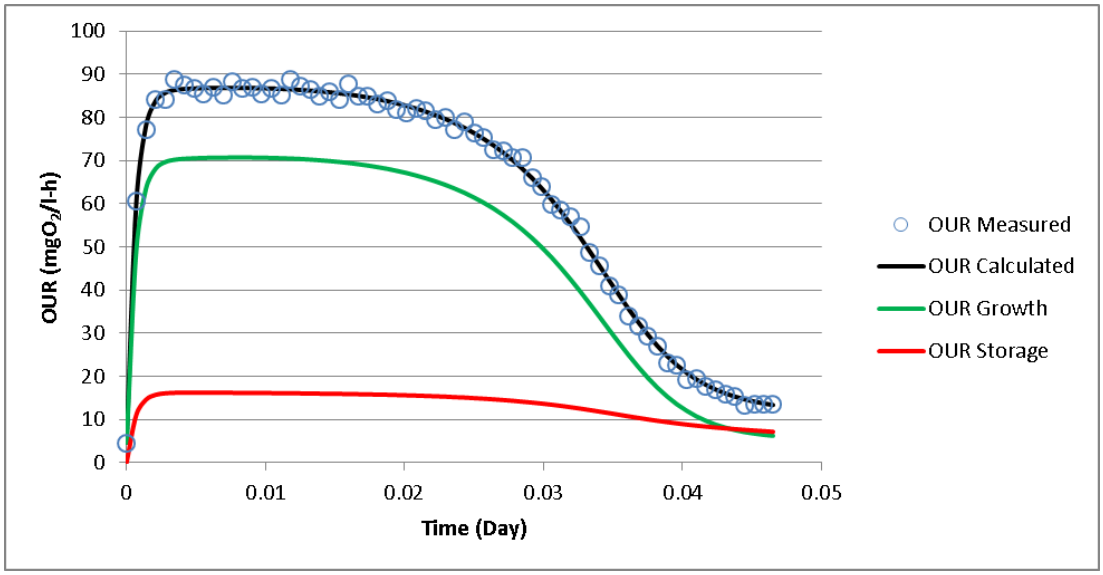

Figure 4.31 : OUR profile of SET 7.2

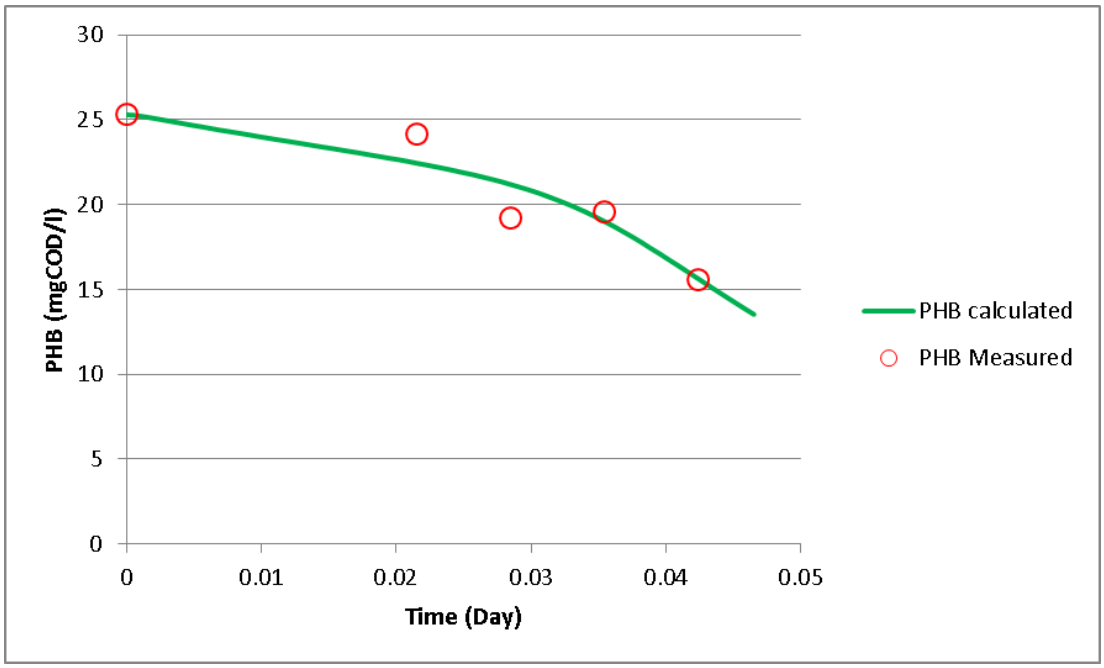

Figure 4.32 : PHB profile of SET 7.2

Table 4.16 : Estimated stoichiometric and kinteic parameters for SET 7.2

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 7.2	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	20	g COD.m^{-3}
k_{STO}	10	14	16	1.22	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	5.31	d^{-1}
μ_{H2}	2	3	3&1.8	2.45	d^{-1}
Y_H	-	0.65	0.67	0.6	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

4.18 SET 7.3

The OUR and PHB profiles for the third substrate addition (SET 7.3) is given in Figure 4.33 and Figure 4.34 respectively. The results of the modelling study for SET 7.3 is given in Table 4.17.**Table 4.5 :**

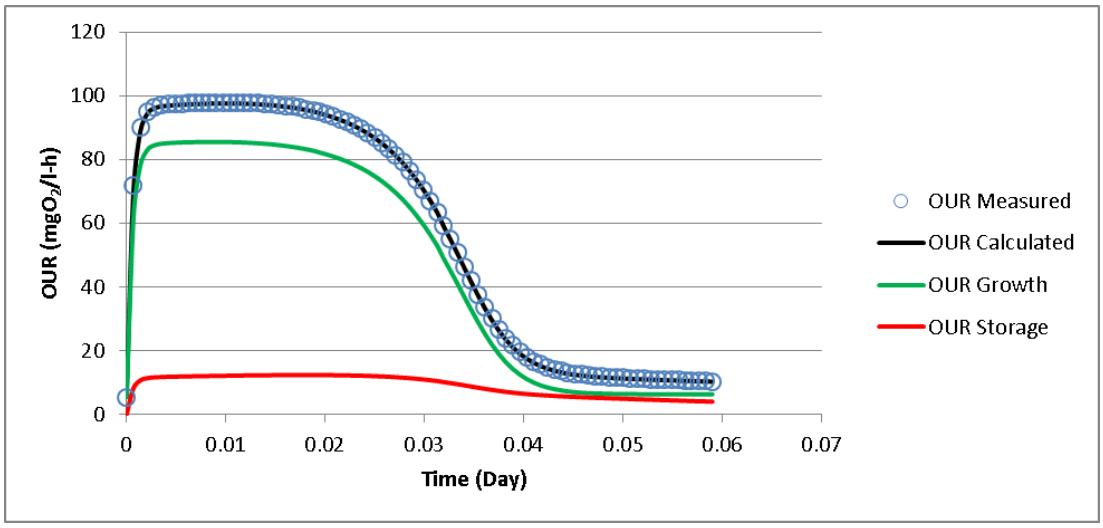

Figure 4.33 : OUR profile of SET 7.3

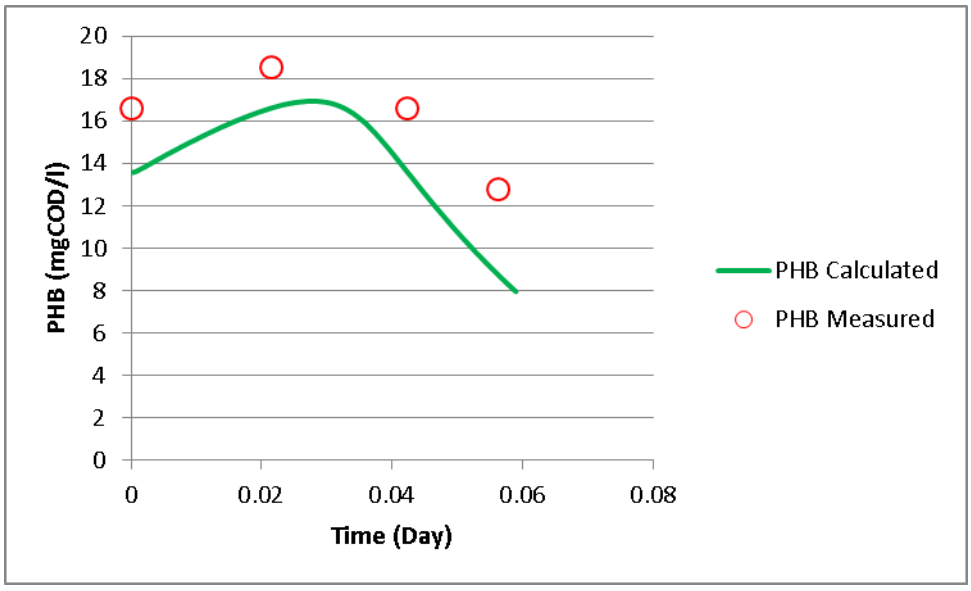

Figure 4.34 : PHB profile of SET 7.3

Table 4.17 : Estimated stoichiometric and kinteic parameters for SET 7.3

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 7.3	Units
b_H	0.2	0.24	0.24	0.22	d^{-1}
K_S	0.1	3	4	20	g COD.m^{-3}
k_{STO}	10	14	16	1.01	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	5.36	d^{-1}
μ_{H2}	2	3	3&1.8	1.92	d^{-1}
Y_H	-	0.65	0.67	0.6	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	g COD.g^{-1} COD

The results of the modelling study for SET 7 is given in Table 4.18.

Table 4.18 : :Estimated stoichiometric and kinteic parameters for SET 7

Parameter	Proposed model results of Krishna and van Loosdrecht (1999)	Proposed model results of Karahan-Gül *et al.*, (2003)	ASM3	SET 7.1	SET7.2	SET7.3	Units
b_H	0.2	0.24	0.24	0.22	0.22	0.22	d^{-1}
K_S	0.1	3	4	20	20	20	g COD.m^{-3}
k_{STO}	10	14	16	5.57	1.22	1.01	g SS.g^{-1} X_H.d^{-1}
K_{STO}	1	1	1	1	1	1	g X_{STO}.g^{-1} X_H
μ_{H1}	4	4	2	5.20	5.31	5.36	d^{-1}
μ_{H2}	2	3	3&1.8	3.35	2.45	1.92	d^{-1}
Y_H	-	0.65	0.67	0.6	0.6	0.6	g cell COD.g^{-1} COD
Y_{STO}	-	0.80	0.80	0.8	0.8	0.8	g COD.g^{-1} COD
f	-	0.20	0.20	0.2	0.2	0.2	

As it can be seen from the Table 4.18 after the second and the third addition of acetate, growth rate on X_{STO} decreases significantly from 3.35 d^{-1} to 2.45 d^{-1}. and to 1.92 d^{-1}. On the other hand, direct growth on external substrate increases from 5.20 d^{-1} to 5.31 d^{-1} after the second addition eventually reaching to 5.36 d^{-1} after the third addition.. The maximum storage rate decreases significantly after each addition from 5.57 to 1.22 and 1.01 These results indicate that the metabolism of the activated sludge is shifting from storage to growth

5 CONCLUSIONS AND RECOMMENDATIONS

The results showed that repeated substrate additions to the biomass at endogenous respiration phase showed different OUR profiles. The difference in the profiles can be seen at the initial responses. Another difference is observed during the depletion of substrate.

For Sets 1 and 2, OUR profile indicates the depletion of storage material after the first addition. After the second addition storage cannot be observed. For Sets 3, 4 and 5 storage can be seen after the two additions but not after the third. These results indicate that microorganisms adjusted their metabolism to the changing conditions and the response of the biomass is shifting form storage to growth.

The results of model evaluation provides support to the findings of Daigger and Grady (1982a) who stated that, physiological adaptation of microbial cultures to environmental conditions triggers the regulation of complex biochemical components directly affecting the specific growth rate. They further suggested that in microbial cells, the protein synthesis system, which is directly related to RNA levels in the cells, plays important role on the bacterial anabolism/catabolism during cultivation.

The maximum growth conditions are a direct measure of the RNA level of the cell which trigger the protein synthesis mechanism. Accordingly, a decrease in RNA content indicates a reduction of protein synthesis, thus a parallel reduction in the maximum growth rate (Daigger and Grady, 1982a).

As further support to variable maximum specific growth rate, Kovarova-Kovar and Egli (1998) stated that the microbial cells could adjust their kinetic properties within a window of μ_H-Ks plane depending upon the type of the bacteria having oligotrophic and copiotrophic properties. The oligotrophic properties correspond to lower maximum μ_H-K_S domain, however, the copiotrophic properties indicates higher μ_H-Ks levels. For instance, microorganisms are able to increase their affinity (<<Ks) during long-term adaptation from high to low substrate concentration in such way that glucose is transported via high affinity galactose binding protein/maltose system rather than the glucose phosphotransferase system (Lengeler, 1993; Kovarova- Kovar and Egli, 1998). Simultaneously, when long term adapted cells are removed from chemostat to batch reactors at high glucose concentrations, the maximum growth rate are reduced by the factor of 60%.

Feast and famine periods also play an important role on the selection of kinetic properties due to the adjustment of substrate transport system(s) as well as variations in catabolic/anabolic properties (Dircks et al., 2001).

Alternating loads and flows to a biological treatment system results in the rapid change of kinetic parameters. As a future perspective there is a need for cybernetic models which can adopt to the changing conditions in order to simulate biological treatment systems more accurately.

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