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EFFECT OF F/M RATIO ON SUBSTRATE STORAGE MECHANISM IN ACTIVATED SLUDGE SYSTEMS

M.Sc. THESIS

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To my hero, my father, and to my angle, my mother,

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June 2013 Yester BİROS

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ABBREVIATIONS

COD : Chemical Oxygen Demand
F/M : Food to Microorganism Ratio
SS : Suspended Solids
VSS : Volatile Suspended Solids
PHA : Poly-hydroxyl-alkanoate
PHB : Poly-β-hydroxy-butyrate
SRT : Sludge Retention Time
OUR : Oxygen Uptake Rate
ASM3 : Activated Sludge Model No: 3
SBR : Sequencing Batch Reactor
ATP : Adenosinetriphosphate
CSTR : Continuously Stirred Tank Reactor

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EFFECT OF F/M RATIO ON SUBSTRATE STORAGE MECHANISM IN ACTIVATED SLUDGE SYSTEMS

SUMMARY

The behavior of bacteria with respect to carbon source is the main subject in activated sludge process. Storage of intracellular biopolymers is now recognized as a significant auxiliary process during substrate utilization by microbial cultures. Substrate is either directly introduced into the microbial growth mechanism, or it may be diverted to storage. This mechanism is mainly observed under transient feeding conditions, where sequential *feast* and *famine* phases trigger substrate storage. Transient conditions are inherent characteristics of some of the biological treatment schemes such as *sequencing batch reactors, intermittent aeration*, etc.; they are also sustained in most experimental systems. Dynamic conditions sustained in batch reactors approximate pulse feeding and induce a process physiological adaptation for the microbial community, often leading to substrate storage.

The storage mechanism is often studied with simple, readily biodegradable substrates like acetate or glucose, generating *polyhydroxybutyrate* (PHB) and glycogen as storage products. Reported results suggest that up to 70% of the simple substrate could be converted to storage products under pulse feeding. They also indicate that the magnitude of storage is likely to exhibit significant variations depending on the nature of substrate, the feeding regime and culture history – i.e. sludge age of the microbial culture.

The aim of this study is to investigate the effect of F/M ratio on the formation of storage polymers in aerobic conditions under pulse feeding. For this purpose, a laboratory scale fill and draw parent reactor was established and operated at 2 days of sludge age and under aerobic conditions as a control reactor for other experiments which will be done to evaluate the effect of F/M ratio on storage response of biomass. The experimental works covered (i) determination of the process performance sustained at steady-state, (ii) respirometric tests, (iii) batch experiments, (iv) evaluation of the stored amount of PHB.

Six different F/M ratios of 0.22, 0.38, 0.98, 1.80, 2.95, and 4.54 gCOD/gVSS were tested by conducting batch tests. The biomass concentration of the control reactor was stabilized approximately 255mgVSS/L, yielding the F/M ratio of 0.98 gCOD/gVSS. This control reactore had COD removal efficiency of 0.87. The maximum amount of stored PHB was 126 mgCOD/L (ΔPHB) that is the 63% of the feeded acetate. The effect of loading rate 1.80 gCOD/gVSS was investigated in a batch reactor which was started with an initial VSS concentration of 255 mg/L and 460 mg/l COD was fed to the system by pulse feeding after the endogenous decay level was observed. 47 % of the feeded substrate was converted to storage polymer and 172 mgCOD/L PHB (ΔPHB) was stored. This percentage was almost remain constant for F/M ratios of 4.54 and 2.95gCOD/gVSS. The effect of a loading rate lower than the control reactor, 0.38 gCOD/gVSS was investigated in a batch reactor

that was started with an initial VSS concentration of 280 mg/L and 105 mg/L COD. 79 % of the feeded substrate was converted to storage polymer and 66 mgCOD/L PHB (ΔPHB) was stored. This percentage was almost remained constant for F/M ratio of 0.22gCOD/gVSS. Shortly the stored substrate as PHB (ΔPHB) decreased from 393mgCOD/L to 37mgCOD/L while PHA/AcCOD ratio increased 0.39mgCOD/mgCOD to 0.65mgCOD/mgCOD together with the reduction of F/M ratio.

In addition, respirometric analyses were performed to correctly evaluate microorganism's energy consumption and substrate utilization mechanism. The OUR curves which were obtained from the respirometric analyses were used to model the microorganisms metabolic mechanisms at different F/M ratios. The maximum specific growth rate (μ_{H1}) decreased from 4.6 day^{-1} to 2 day^{-1} and the maximum storage rate of substrate (k_{STO}) increased 9 day^{-1} to 11 day^{-1} and then 14 day^{-1} in the experiments with the reduction of F/M ratio. In addition, other kinetic and stoichiometric parameters remain constant.

Experimental findings in this study have shown that the ratio of initial substrate to biomass (S_0/X_0) was the key parameter for respirometric experiments directly affecting the shape and the order of magnitude of the respirometric profile. The experiments also shown that, the biomass is able to switch its substrate utilization mechanism to growth or storage when it is subjected to unsteady feast conditions. The fast growing systems (low sludge age) gives much faster response than that of high sludge age. The results of this study indicates that, when the biomass face with higher concentrations of substrate feeding than the study state conditions, it slows down the storage mechanism and increases the growth rate. The enzymatic levels controlling growth and storage kinetics could be the reason of the biomass response in activated sludge systems.

AKTİF ÇAMUR SİSTEMLERİNDE F/M ORANININ SUBSTRAT DEPOLAMA MEKANİZMASI ÜZERİNE ETKİSİ

ÖZET

Aktif çamur sürecinin ana konusu bakterilerin karbon kaynağına karşı verdiği tepkidir. Günümüzde hücre içi biopolymer depolanması, mikrobial kültürlerin substrat gideriminde kullandığı önemli bir yardımcı süreç olarak kabul edilmektedir. Hücre içerisine alınan substrat direkt olarak bakterilerin çoğalma mekanizmasına dâhil olabileceği gibi, depo maddesine de dönüştürülebilir. Bu durum bakterinin maruz kaldığı koşullara göre farklılık gösterir. Substratın depolama maddesine dönüştürülmesi en çok kesikli, düzensiz beslemeye tabi tutulan sistemlerde gözlenmektedir. Bu tip besleme koşullarında; açlık (famine) ve ziyafet (feast) olarak adlandırılan iki ayrı faz meydana gelmektedir. Açlık fazında ortamda karbon kaynağı bulunmazken; ziyafet fazında ortamda karbon kaynağı mevcuttur. Bu faz ayrımı ve ortamda devamlı substrat bulunmayışı bakterileri depolamaya yönlendirmektedir. Kesikli havalandırma, ardışık kesikli reaktörler, vb. bazı biyolojik arıtma sistemlerinin doğal özellikleri arasında, depolanma sürecini ortaya çıkaran kesikli koşulların oluşması yer almaktadır. Kesikli reaktörlerde meydana gelen dinamik koşullar bakterileri fiziksel bir adaptasyon sürecine girmek zorunda bırakmakta ve bunu bir sonucu olarak da çoğunlukla karbon kaynağının depolanması gözlenmektedir.

Substrat depolama mekanizması çalışmaları, çoğunlukla asetat ve glikoz gibi; basit ve kolay ayrıştırılabilen substratlar kullanılarak yapılmıştır. Bu tip karbon kaynaklarının kullanımı sonucu oluşturulan depo maddeleri de polihydroksibütrat (PHB) ve glikojendir. Araştırma sonuçlarına göre anlık beslemeye tabi tutulan ve basit substratlar ile beslenen sistemlerde ortamdan hücre içerisine alınan substratın %70'i depolanmaktadır. Ayrıca depolanma miktarı pek çok faktöre göre değişkenlik gösterebilmektedir. Bunlar; beslenen karbon kaynağının yapısı, beslenme şekli ve mikrobial kültürün geçmişi ya da mikrobial kültürün çamur yaşı olarak sıralanabilir. Evsel atık su gibi kompleks substratların karışımından oluşan besleme koşullarında depo polimeri miktarının oldukça düşük olması beklenmektedir. Bunun nedeni beslenen karbon kaynağında yer alan, hücre içi depo maddesine dönüştürülebilen hızlı ve kolayca ayrışabilen karbon kaynağı miktarının oldukça az olmasıdır.

Aktif çamur sistemlerinde dinamik koşullarda önemli proseslerden biri de depolama mekanizmasıdır. Depolama mekanizmasında, substrat giderim hızı kısa sürede artar ve yeni maddeler üretilir. Her türlü yapıtaşı çoğalma fazında üretilirken, depolama sırasında sadece depo polimerleri sentezlenir. Bu durum farklı sitokiometrik ve kinetik sabitlerin oluşmasına sebep olur çünkü depo polimerlerinin sentezi tüm hücrenin sentezine göre oldukça basit ve daha az bir fiziksel adaptasyon süreci gerektirmektedir. Ayrıca depolama çoğalmaya oranlar oldukça hızlı gerçekleşir.

Bu çalışmanın amacı aerobik koşullarda ve kesikli besleme uygulanarak farklı F/M oranlarının substrat depolama mekanizması üzerine etkisinin incelenmesidir. Bu amaçla, laboratuvar ölçekli doldur-boşalt bir ana reaktör kurulmuş ve 2 çamur

yaşında işletilmiştir. Bu ana reaktör daha sonra F/M etkisi test etmek amacıyla yapılacak olan kesikli reaktörlerdeki deneysel çalışmalarda kullanılacak çamuru elde etmek amacıyla kurulmuştur. Deneysel çalışmaların kapsamında; (i) karalı dengeye ulaşan ana reaktör sisteminin performans analizi, (ii) kesikli reaktör deneyleri, (iii) respirometric deneyler ve (iv) depolanan PHB miktarlarının ölçülmesi ile değerlendirilmesi yer almaktadır.

Ana reaktördeki biokütle konsantrasyonu yaklaşık 255mgCOD/L'de sabitlenmiş ve F/M oranı 0.98 gCOD/gVSS olacak şekilde kararlı dengeye ulaşana kadar sistem gözlemlenmiştir. Ana reaktör sistemi kararlı denge koşullarına ulaştıktan sonra uygun miktarlarda çamur alınarak 6 farklı F/M oranının test edileceği kesikli reaktör deneyleri yapılmıştır. Bu farklı F/M oranları 0.22, 0.38, 0.98, 1.80, 2.95 ve 4.54 gCOD/gVSS'dir. Kesikli reaktörler COD giderim performansı ve PHB üretim kapasitesi açısından gözlemlenmiştir. Kontrol reaktörünün COD giderim performansı %87'dir.Maksimum depolanan substrat miktarı 126 mgCOD/L (ΔPHB) olup bu da sisteme beslenen substrat miktarının % 63'üne denk gemektedir. Başlangıç F/M oranı kontorl reaktörüne gore daha yüksek bir seviyeye çıkarıldığında bu yüzdede bir düşüş gözlemlenmektedir. F/M oranı 1.80 gCOD/gVSS olarak çalıştırılan kesikli reaktörde başlangıç substrat mikatrı 460 mgCOD/L ve başlangıç biyokütle kosantrasyonu 255 mgVSS/L 'dir. Beslenen Substratın %47'si depolanmış ve net depolanan PHB miktarı 172 mgCOD/L (ΔPHB) olarak hesaplanmıştır. Yani F/M oranının artışı beslenen substrat miktarının depolamaya dönüştürülen kısmında bir azakmaya neden olmuştur. Bu belirtilen %47 lik oran 4.54 ve 2.95 gCOD/gVSS değerlerindeki F/M oranlarında da sabit kalmış büyük bir değişim gözlenmemiştir. Tam tersi olarak kontrol reaktöre göre daha düşük bir F/M oranı seçildiğinde ise sonuçlar tersi bri netice almıştır. F/M oranı 0.38 gCOD/gVSS olan kesikli reaktörde, 66 mgCOD/L PHB (ΔPHB) depolanmış ve bu oranın beslenen asetat miktarının %79'unu oluşturduğu gözlemlenmiştir. Bu yüzde F/M oranı 0.22 gVOD/gVSS olan kesikli reaktörde de sabit kalmıştır. Kısacası F/M oranı azaldıkça; PHB olarak depolanan madde miktarı (ΔPHB) 393mgCOD/L'den 37mgCOD/L'ye düşerken, PHB/AcCOD oranı 0.28mgCOD/mgCOD'den 0.65mgCOD/mgCOD'ye yükselmiştir.

Bu çalışamalra ek olarak, respirometre kullanımı ile respirometrik deneyler de yapılmıştır. Bu deneylerin amacı mikroorganizmalrın enerji tüketimin ve substrat giderim mekanizmaları doğru olark tespit etmektir. Ayrıca respirometreden elde edilen bakterilerin oksijen tüketim hızlarını (OUR) gösteren grafikler kullanılarak farklı F/M oranlarında mikroorganizmaların metabolik mekanizmalarının modellemesi de yapılmıştır. F/M oranı azaldıkça; maksimum çoğalma hızı (μ_{H1}) 4.6 gün^{-1}'den 2 gün^{-1}'e düşerken, maksimum substrat depolama hızı ise 9gün^{-1}'den 11 gün^{-1} 'e ve son olarak da 14 gün^{-1}'e yükselmiştir. Bununla beraber diğer kinetik ve sitokiometrik parametreler sabit kalmıştır.

Bu çalışmanın sonuçlarına göre; başlangıç substrat miktarı ve biokütle konsantrasyonu arasındaki oran (S_0/X_0) respirometrik deneyler için kilit bir parametre olup direkt olarak oksijen tüketim hızı grafiğini etkilemekte ve respirometrik profilin düzen ve şeklini değiştirmektedir. Ayrıca deneysel sonuçlara göre, biyokütlenin düzensiz kesikli koşullara maruz bırakıldığı takdirde istediği şekilde depolama ya da büyüme yönünde metabolizmasını değiştirebilme yetisine sahip olduğu söylenebilir. Hızlı çoğalan sistemlerde (düşük çamur yaşında çalışan sistemlerde) mikroorganizmların bu gibi değişken koşullara tepki vermesi daha çabuk olmaktadır.

Bu çalışmadan ortaya çıkan diğer bir sonuç ise şöyle açıklanabilir: Mikroorganizmalar alıştığı koşullara göre yüksek konsantrasyonda substrat ile karşılaştığında, depolama mekanizmasının yavaşlatıp çoğalmaya doğru yönelirken; düşük konsantrasyonda substrat ile karşılaştığında ise çoğalma mekanizmasının yavaşlatıp metabolizmasını depolama yönünde harekete geçirmektedir. Mikroorganizmanın bu tepkisi enzimatik seviyede kontrol edilen çoğalma ve doplama mekanizmalarında kullanılan enzim miktarlarındaki değişimlerden ileri gelebilmektedir.

1. INTRODUCTION

1.1 Aim and Scope of the Study

Storage of intracellular biopolymers is now recognized as a significant auxiliary process during substrate utilization by microbial cultures. Substrate is either directly introduced into the microbial growth mechanism, or it may be diverted to storage. This mechanism is mainly observed under transient feeding conditions, where sequential *feast* and *famine* phases trigger substrate storage. Transient conditions are inherent characteristics of some of the biological treatment schemes such as *sequencing batch reactors*, *intermittent aeration*, etc.; they are also sustained in most experimental systems. Batch reactors are often selected as the most suitable experimental tool for investigating different aspects of substrate biodegradation, mainly because they offer accurate evaluation of transient responses and resulting concentration profiles of major parameters. Dynamic conditions sustained in batch reactors approximate pulse feeding and induce a process physiological adaptation for the microbial community, often leading to substrate storage.

The storage mechanism is often studied with simple, readily biodegradable substrates like acetate or glucose, generating *polyhydroxybutyrate* (PHB) and glycogen as storage products. Reported results suggest that up to 70% of the simple substrate could be converted to storage products under pulse feeding. They also indicate that the magnitude of storage is likely to exhibit significant variations depending on the nature of substrate, the feeding regime and culture history – i.e. sludge age of the microbial culture. Expected storage would be substantially lower in complex substrate mixtures such as domestic sewage where only a small percent of the organic matter consist of readily biodegradable compounds favoring formation of intracellular biopolymers.

Disregarding storage in organic substrate removal, as generally adopted in traditional studies and earlier activated sludge models involves a significant risk of eclipsing the correct mechanism and may distort results of kinetic evaluation. Therefore, the

magnitude of overall storage and the composition of generated biopolymers need to be assessed for each case.

In essence, storage results from an imbalance between removal of available substrate and microbial growth potential while substrate may be removed, limitations on the metabolic reactions leading to growth may prevent consumption of all energy and divert a fraction of the substrate for generating intracellular biopolymers. In biological systems, substrate loading or the food to microorganism (F/M) ratio defines the stoichiometric balance between the growth rate and the amount of substrate that should be available for maintaining the selected growth rate. This is obviously an average value, defining in theory the balance for substrate utilization at steady state. In real systems however, substrate feeding fluctuates with time around the average level, causing perturbations of the substrate/growth balance. These perturbations obviously affect substrate utilization dynamics and the storage mechanism. In other words, the amount of storage products generated would also exhibit a similar fluctuation along with the variable substrate feeding regime. This information is needed for an accurate understanding of system operation.

In this context, the objective of the study was to evaluate the effect of substrate loading on the generation storage biopolymers. For this purpose a continuously fed fill and draw reactor was operated at steady state at a sludge age of 2days. A faster growing microbial community and a low sludge age were selected to better evaluate the growth requirements of the community compared with simultaneous storage. Then a series of batch experiments were conducted with biomass taken from the fill and draw reactor and therefore acclimated to fast growth conditions. One of the batch reactors duplicated the substrate loading in the main reactor. The other were started with a range of substrate loadings, i.e. different food to microorganism ratios both in the lower and higher ranges with respect to the one representing the operating conditions in the fill and draw reactor.

Acetate was selected as the sole organic carbon source, mainly because it is a well known substrate for generating a typical storage product, namely *polyhydroxybutyrate,* (PHB), and to be able to compare the results with previous findings in similar studies. Batch reactors were basically monitored for the fate of PHB and respirometric analyses yielding the corresponding oxygen uptake rate (OUR) profiles. The experimental data obtained were used for the calibration of an

appropriate model, yielding values of model coefficients associated with different experimental conditions.

The aim of this study is to investigate the effect of F/M ratio on the formation of storage polymers in aerobic conditions under pulse feeding. For this purpose, a laboratory scale fill and draw parent reactor was established and operated at 2 days of sludge age and under aerobic conditions as a control reactor for other experiments which will be done to evaluate the effect of F/M ratio on storage response of biomass. Six different F/M ratios were tested by conducting batch tests. Batch reactors were monitored for COD removal efficiencies and PHB storage capacity. In addition, respirometric analyses were performed to correctly evaluate microorganism's energy consumption and substrate utilization mechanism. The OUR curves which were obtained from the respirometric analyses were used to model the microorganisms metabolic mechanisms at different F/M ratios.

2. SUBSTRATE STORAGE PHENOMENA

2.1 Description of Storage Phenomena

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).

Storage response has been usually associated with the highly dynamic feeding conditions in activated sludge systems. Studies generally interpret a transient substrate feeding regime in wastewater treatment processes, creating a sequence of feast (presence of external substrate) and famine (absence of external substrate) conditions, as the major factor for the generation of intracellular storage biopolymers (Majone et al., 1999; Beun et al., 2000; Ciğgin et al., 2011a, 2012). These concepts were explained as when external carbon source is available only short periods, 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 were no external substrate bacteria would undergo long starvation periods. In case of starvation by substrate for a certain period, the amount of intracellular components especially the enzymes and RNA that need for cell growth can decrease. After this period when cells faced with substrate, most of the RNA and enzymes are induced and this induction may cause to an imbalance between substrate uptake rate and growth rate. Cell compensates this imbalance by storing the excess substrate as storage polymer. Thus, storage occurs preferentially instead of cell growth because the amount of enzymes required for storage is lower than the enzymes needed for growth at maximum rate. Bacteria that own ability of storage have a competitive advantage over the other bacteria without the capacity of storage of substrate. If bacteria cannot store the substrate, extra energy is needed for rapid growth in feast period (van Loosdrecht et al., 1997). Bacteria that 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.

The presence and relative amount of the storage phenomena are dependent on the type of the carbon source. Mainly three types of organic storage polymers were reported as polyhydroxybutyrate (PHB), glycogen and lipids (Zevenhuisen and Ebbnik, 1974). PHB is directly formed out of the central metabolite acetyl-Coenzyme A (acetyl-CoA), while glycogen is formed when sugars are present in the feed (Carta et. al., 2001). These polymers are energy and carbon storage materials synthesized by numerous microorganisms. Lipids are usually accepted as structural components of membranes as phospholipids and of cell walls as lipopolysaccharides, so that present large amounts in all bacteria but do not function as carbon and energy store.

Many substrates are degraded into acetyl-CoA as intermediate in the cell since polyhydroxyalkanoates (PHAs) are directly formed from the central metabolite acetyl-CoA (Anderson and Dawes, 1990; Doi, 1990). Storage polymers can depolymerize and then metabolized as carbon and energy source if limited nutrient was provided (Merrick and Doudoroff, 1964).

Recent studies with pure substrates and respirometer 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

Research results has clearly shown that when volatile fatty acids, i.e., acetate, propionate, etc. are fed to an activated sludge system they are stored as polyhydroxyalkanoates and when saccharides like glucose, maltose, starch, etc. are fed, the storage polymers are glycogen or glycogen like sugars (Karahan et.al., 2008). If the system is fed with only acetate, the PHA formed is mostly poly-β-hydroxy-butyrate (PHB).

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. In addition, PHB is insoluble and relatively resistant to hydrolytic degradation.

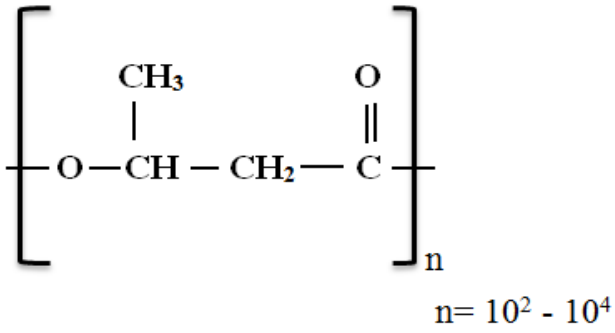

Figure 2.1: The composition of PHB.

PHB formation occurs in a cyclic metabolic pathway as it shown in Figure 2.2. Acetate molecule used in model pathway since it is one of the most known compounds in wastewaters as substrate for cells. After bacteria intake acetate to the cell, it is directly converted to acetyl-CoA and two molecules of produced acetyl-CoA are condensed to form acetoacetyl-CoA and release a CoA. An enzyme called 3-ketothiolase catalyzes this condensation reaction. The acetoacetyl-CoA is reduced to (R)-3-hydroxybutyryl-CoA. The enzyme acetoacetyl-CoA reductase, an NADPH dependent enzyme is catalyzed this reduction reaction and PHB is formed from (R)-3-hydroxybutyryl-CoA 3 molecules with release of a free CoA.

PHB degradation occurs while no excess external substrate is available for bacteria as an energy and carbon source. PHB is converted to (D)-3-hydroxybutyrate by an enzyme called PHB depolymerase. The enzyme (D)-3-hydroxybutyrate dehydrogenase catalyzed the conversion reaction of (D)-3-hydroxybutyrate to acetoacetate. 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 regulation of PHA synthesis is controlled at enzymatic level and mainly depends on the intracellular concentration of free CoA and acetyl-CoA. The production of storage polymers is thought as serving a NADPH overflow mechanism to control the redox state of heterotrophic cells during unbalanced growth conditions (Van Niel et al,.1995).

When there is unfavorable unbalanced growth conditions the acetate that is taken into the cell directly converted to acetyl-CoA but acetyl-CoA do not enter its usual pathway the tricarboxylic acid (TCA) cycle to produce $NADH_2$, ATP and to form

biomass. Instead of that, it is converted to PHB by the cyclic metabolic pathway that is described above. The reason of this change in metabolic pathway in the cell is the high concentration of NADH that is inhibit the generation of citrate synthase, one of the key enzymes of TCA cycle, leading to an increase in the level of acetyl-CoA.

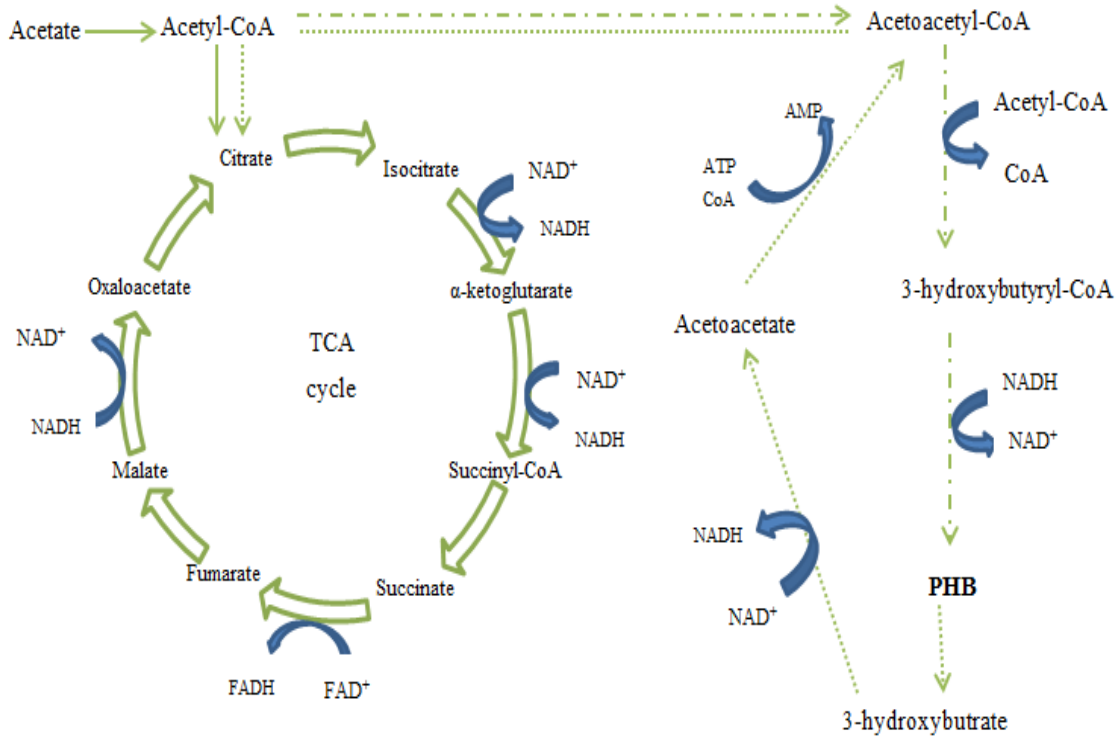

Figure 2.2: PHB production pathways in feast/famine conditions (adapted from Salehizadeh and Van Loosdrecht, 2004.).

The increase in the concentration of acetyl-CoA drives the equilibrium reaction of 3-ketothiolase into the direction of PHB synthesis. According to this opinion, PHB synthesis process is controlled by citrate synthase since PHB accumulation is improved by the metabolic flux of acetyl-CoA to the PHB synthesis (Henderson and Jones, 1997).

Subsequently, PHB is accumulated in order to keep the concentration of intermediates at a balanced level in the cells. Similarly, since increases of acetyl-CoA lead to increase of ratio acetyl-CoA/free CoA, bacteria produce more anabolic enzymes and then increase their growth rate which called growth response (Daigger and Grady, 1982). If the period of excess external substrate avaliability is long enough, the specific growth rate of the biomass will be increase to its maximum and the PHB synthesis will slow down (Cığgın et al., 2011). On the contrary, if the period

of excess external substrate availability is faster than time need for the growth of biomass, PHB synthesis rate will be increase while growth rate will not be affected. For this reason, when bacteria cannot adapt the physiological state of the fast availability of substrate, storage mechanism is used to keep growth rate constant with time.

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 storage is faster than growth.

3. ACTIVATED SLUDGE MODELLING WITH SUBSTRATE STORAGE

Models are essential to provide estimations of important but otherwise inaccessible process/physiological parameters, or to support the design of control strategies. The identification and interpretation of major biochemical reactions occurring in the specific conditions can be achieved with a modeling approach. Storage process is a major mechanism that occurs under unbalanced feeding conditions and it is necessary to determine kinetic parameters for accurate description of the process exposed to new operational conditions or for accurate description of the new process configuration (Van Loosdrecht and Heijnen, 2002).

Several metabolic models have proposed different dominant mechanisms like enmeshment, sorption and accumulation 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.

Subsequently, Activated Sludge Model No.3, ASM3, was then proposed introducing biochemical storage as the only way for the utilization of the readily biodegradable substrate (Gujer et al.,2000). According to ASM3, biodegradable substrate is firstly stored inside the cells and then storage products are reused for growth without additional external substrate. However, biochemical models on pure substrates showed that there was substantial evidence for simultaneous utilization of primary substrate for growth and storage for pure cultures (van Aalst-van Leeuwen et al., 1997) and for mixed cultures (Beun et al., 2000; Dricks et al., 2001). Therefore, biochemical-modeling approaches accepted that growth on external substrate and storage of external substrate occur simultaneously (Figure 3.1).

There have been three yield factors for simultaneous storage and growth, which are the yield for growth of biomass on substrate (Y_{SX}), the yield of storage on substrate (Y_{SP}) and the yield of growth on the stored substrate (Y_{PX}). If there is no simultaneous storage and growth, it is not necessary to define the yield for growth on external substrate.

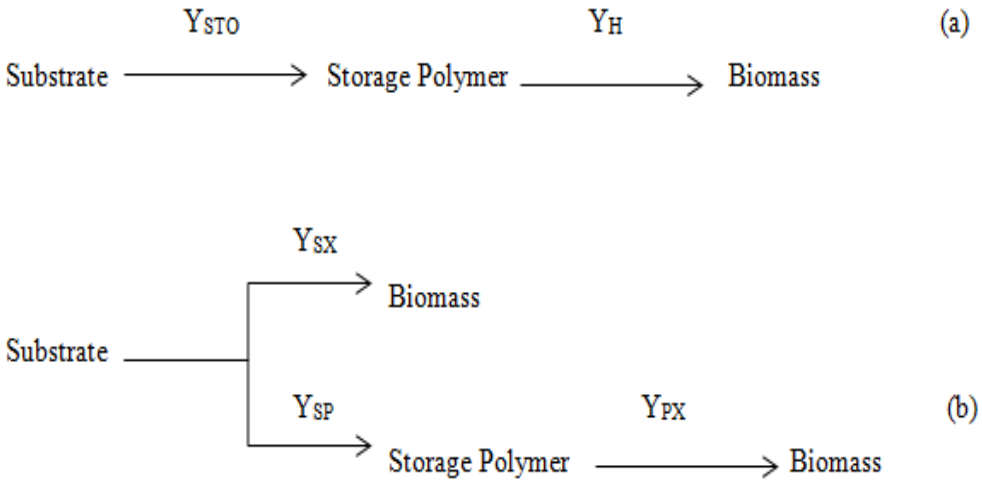

Figure 3.1: Metabolic route of biomass (a) in ASM3 (b) in biochemical approach.

3.1 Metabolic Models for Substrate Storage

A metabolic model is based on the principles that the metabolism of organism is composed of a limited number of metabolic pathways which result 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.

van Aalst-van Leeuwen et al. (1997a) proposed a metabolic model in that acetate is aerobically stored as PHB under dynamic conditions. 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).

In the metabolic model proposed by van Aalst-van Leeuwen et al., (1997) acetate limited continuous culture of *Paracoccus pantotrophus sp.* was used in order to

observe the metabolism of a microorganism capable of producing and consuming PHB which was described by seven internal reactions **(**Figure 3.2). Biomass is assumed to contain two different parts as an active biomass compartment (1-f_{PHB} = capable of production and growth) and PHB fraction (f_{PHB} = used as storage for carbon and energy).

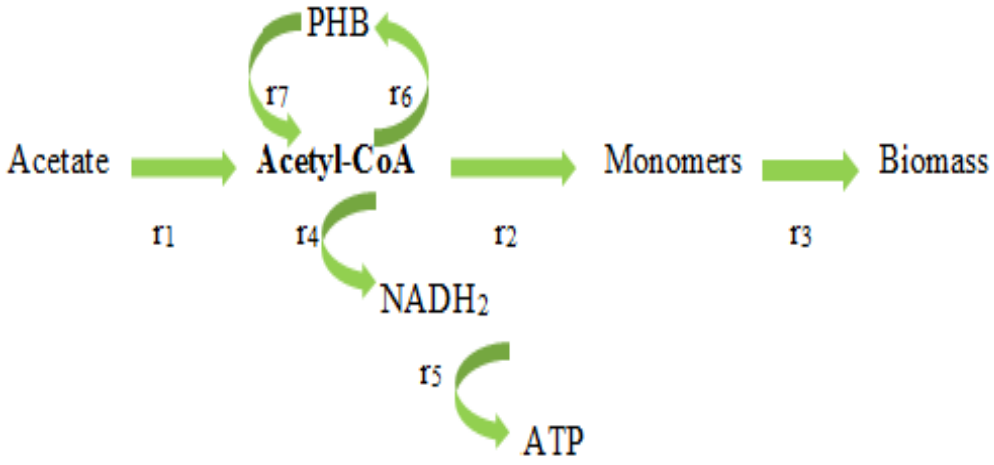

Figure 3.2: Schematic representation of the metabolism of an organism capable of forming and consuming PHB (van Aalst –van Leeuwen et al.,1997).

- **Synthesis of acetyl-CoA from acetate, (r_1):** Acetate is taken into cell by active transport, with the addition of one mole Pi to the acetate Acetyl-P formed. At last, Acetyl-P converted to Acetyl-CoA and reaction is completed.
- **Synthesis of biomass monomers from acetyl-CoA, (r_2):** Anabolism, synthesis of active biomass, starts from the synthesis of monomers. This is the first step of biomass formation.
- **Polymerization of biomass precursors and maintenance, (r_3):** This is the second and last step of biomass production process. Precursors are polymerized into active biomass.
- **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 is describing acetate uptake, biomass growth, PHB production and maintenance are defined as below (Equation **3.1**).

$$(-r_S) = \frac{1}{Y_{SX}^{max}}\, r_X + \frac{1}{Y_{SP}^{max}} r_P + m_S C_x \qquad \textbf{(3.1)}$$

As similar, PHB consumption, biomass growth, and maintenance are expected with the following equation for the famine period (Equation **3.2**).

$$\left(-\mathrm{r_p}\right) = \frac{1}{\mathrm{Y_{PX}^{max}}}\, \mathrm{r_X} + \mathrm{m_P C_x} \qquad \textbf{(3.2)}$$

Where,

$$\mathrm{Y_{SX}^{max}} = \frac{4\delta - 2}{4.13\delta + 4.32} \qquad \textbf{(3.3)}$$

$$\mathrm{Y_{SP}^{max}} = \frac{4\delta - 2}{4.5\delta} \qquad \textbf{(3.4)}$$

$$\mathrm{Y_{PX}^{max}} = \frac{4.5\delta - 0.5}{4.13\delta + 4.32} \qquad \textbf{(3.5)}$$

$$\mathrm{m_S} = \frac{\mathrm{m_{ATP}}}{2\delta - 1} \qquad \textbf{(3.6)}$$

$$\mathrm{m_P} = \frac{\mathrm{m_{ATP}}}{4.5\delta - 0.5} \qquad \textbf{(3.7)}$$

The linear equations contain two unknown parameters namely, the ratio between ATP produced and electron 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 (**3.8**)

$$(-q_S) = \frac{1}{Y_{SX}^{max}}\, \mu + \frac{1}{Y_{SP}^{max}} q_P + m_S \qquad \textbf{(3.8)}$$

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 coefficent for growth on acetate m_S =0.038 [C-mol/C-mol.h]. The P/O ratio, δ= 1.84 [molATP/molNADH$_2$] and the ATP maintenance coefficient, m_{ATP} =0.102

[molATP/Cmol.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 **3.4**. Substituting these yield coefficients in Equation **3.8** gives Equation **3.9**:

$$-q_S = 2.22\ \mu + 1.544\ q_P + 0.038 \quad \textbf{(3.9)}$$

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 **3.10** and **3.11**).

$$q_C = (-q_S) - \mu - q_P = 1.22\ \mu + 0.544 q_P + 0.038 \quad \textbf{(3.10)}$$

$$-q_O = 1.188\ \mu + 0.419 q_P + 0.038 \quad \textbf{(3.11)}$$

Stoichiometry of the kinetic model for the PHB consumption can be based upon Equations (**3.9-3.11**).

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:

$$(-q_P) = \frac{1}{Y_{PX}^{max}}\ \mu + m_P \quad \textbf{(3.12)}$$

$$-q_P = 1.532\mu + 0.0131 \quad \textbf{(3.13)}$$

$$q_C = 0.532\ \mu + 0.0131 \quad \textbf{(3.14)}$$

$$(-q_O) = 0.693\ \mu + 0.0147 \quad \textbf{(3.15)}$$

Stoichiometry of the kinetic model for the PHB consumption can be used upon Equations (**3.12-3.15**).

It was observed from experimental and modeling results that, the substrate uptake rate reaches its maximum value immediately after the pulse addition of acetate. 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 Loosdrecht et al., 1997).

3.2 Activated Sludge Model No: 3 (ASM3)

A new concept in activated sludge modeling based on storage phenomena of readily biodegradable substrate Activated Sludge Model No.3 (ASM3) was proposed by IWA Task Group to take into account the endogenous decay process. ASM3 assumes that storage is the sole initial biochemical mechanism for the utilization of readily biodegradable COD (Gujer et al., 2000). According to the new model approach, all readily biodegradable COD (S_S) is initially converted to internal storage products (X_{STO}), either directly or through preliminary hydrolysis, and growth occurs only at the expense of stored polymers during the famine phase.

Components and processes similar to other activated sludge models, ASM3 contains storage process and attain to estimate oxygen utilization, sludge production, nitrification, and denitrification of the systems. The reaction kinetics and stoichiometry of ASM3 simplified for organic carbon removal is given in Table 3.1. Whereas the quantification of the kinetic parameters for hydrolysis process, 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. In addition, ASM3 contains alkalinity and

nitrogen limitations for growth of microorganisms with description of ammonification kinetics by assuming constant N and COD ratio.

The full ASM3 model accounts for the extince of two group of organisms (heterotrophic and autotrophic organisms) and attempts to simultaneously describe the sludge production, nitrification and denitrification processes, as well as the storage of organic substrates, either through aerobic or anoxic storage of COD.

Table 3.1: Simplified ASM3 for organic carbon removal under aerobic conditions.

a) Components of the model				
Component / **Process**	**S_O**	**Ss**	**$X_{STO,PHA}$**	**X_H**
Storage of Ss	$-(1-Y_{STO})$	-1	Y_{STO}	
Growth on X_{STO}	$-\frac{1-Y_H}{Y_H}$		$-\frac{1}{Y_H}$	1
Endogenous respiration	$-(1-f_{ES}-f_{EX})$			-1
Respiration of X_{STO}	-1		-1	
b) Process rates of the model				
Process	**Process rate**			
Storage of Ss	$k_{STO} \cdot \frac{S_S}{K_S + S_S} \cdot X_H$			
Growth on X_{STO}	$\mu_H \cdot \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} \cdot X_H$			
Endogenous respiration	$b_H . X_H$			
Respiration of X_{STO}	$b_{STO} . X_{STO}$			

3.2.1 Components and processes of the model

ASM3 includes seven soluble and six particulate components to characterize the wastewater and activated sludge. The first soluble component is dissolved oxygen (S_O) which can directly be measured. Dissolved oxygen consumption occurs during the mechanisms of storage, growth, endogenous decay and respiration of storage products. Inert soluble organics (S_I), the second component of this new model, can be formed by the hydrolysis of X_S, produced by endogenous decay of biomass and it

is a part of the influent. S_I and S_S are approximately equal to the total soluble COD determined by filtration from 0.45μm membrane filters. The three nitrogen fractions; ammonium plus ammonia nitrogen (S_{NH}), dinitrogen (S_{N2}) and nitrite plus nitrate nitrogen (S_{NO}) are the other components of the model. In addition, alkalinity of the wastewater (S_{ALK}) is used to approximate the conservation of ionic charge in biological reactions.

Inert particulate organic matter (X_I), slowly biodegradable substrate (X_S), heterotrophic organisms (X_H), internal storage products (X_{STO}), autotrophic organisms (X_A) and total suspended solids (X_{TS}) are the six particulate components of the model. Inert particulate organics are a part of influent and can be produced by endogenous respiration of biomass. Xs cannot be directly metabolized by the biomass since it is not possible for high molecular weight molecules pass the cell membrane before hydrolysis. Therefore, slowly biodegradable substrates 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. Hydrolysis does not consume any electron acceptor in ASM3. X_{STO} consist of PHA, glycogen etc., which is only defined as a functional component required for modeling but is not directly identifiable chemically. Autotrophic organisms are responsible from nitrification process. They oxidized ammonium directly to nitrate; nitrite is not considered in ASM3. X_{TS} may be used for the modeling of volatile suspended solids by some special ratios.

ASM3 integrated twelve processes that are summarized below:

- **Hydrolysis:** All of the slowly biodegradable substrates in the influent convert to readily biodegradable substrate by hydrolysis process. This process does not consume any electron acceptor.
- **Aerobic storage of readily biodegradable substrate:** In ASM3, it is accepted that all readily biodegradable substrate first stored as X_{STO} and then consumed for the generation of new biomass. This storage process needs ATP that produced by oxidative phosphorylation.
- **Anoxic storage of readily biodegradable substrate:** This process is similar to aerobic storage of readily biodegradable substrate but energy requirement is less than the aerobic one and supplied by anoxic respiration. The process

rate is naturally slower than the aerobic storage rate, since the amount of denitrifiers is low.

- **Aerobic growth of heterotrophic organisms:** It is assumed that the stored products are the only substrate that used for the growth of heterotrophic biomass. The energy required for this process is supplied by aerobic respiration.
- **Anoxic growth of heterotrophic organisms:** This process is identical with aerobic growth but it is based on anoxic respiration.
- **Aerobic endogenous respiration:** The all forms of biomass loss occur in this process. The process consists of decay, endogenous respiration, lies, maintenance, predation, motility and death of biomass.
- **Anoxic endogenous respiration:** This process is similar to aerobic endogenous respiration but occurs at a slower rate.
- **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 autotrophic organisms:** Nitrifiers oxidized ammonium directly to nitrate.
- **Aerobic endogenous respiration for autotrophic organisms:** Apart from description of nitrifies, it takes place similar to aerobic endogenous respiration.
- **Anoxic endogenous respiration for autotrophic organisms:** It is similar to aerobic endogenous respiration for autotrophs, but it occurs at a slower rate.

3.2.2 Process stoichiometry

The net (true) yields of heterotrophic biomass (X_H) produced per unit of readily biodegradable substrate removed in ASM3 is found from the equations below:

$$Y_{net} = Y_{STO} . Y_H \quad \textbf{(3.16)}$$

$$Y_{NO} = Y_{STO,NO} . Y_{H,NO} \quad \textbf{(3.17)}$$

Table 3.2 shows all stoichiometric parameters of ASM3 with their units and typical values. The composition of all organic fractions relative to Theoretical Oxygen

Demand (ThOD) is assumed be unity. ThOD is 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 stoichiometry coefficient for S_N is the negative of the coefficient for S_{NOX} in any denitrification process. The composition coefficients for ThOD for S_{N2} (-1.71g ThOD /gN_2), S_{NOX} (-4.57 gThOD/ gNO3-N) and S_{O2} (-1g ThOD/gO2) are negative for electron donors corresponding to the redox reference for ThOD.

3.2.3 Process kinetics

In relation to removal of all soluble compounds, the kinetic expressions of ASM3 rely on switching functions, which are hyperbolic or saturation terms, Monod equations, S/(Ks+S). Similarly, the switching functions are affected for particulate compounds by the ratio of X_{STO}/X_H and X_S/X_H. The units and typical values at 10ºC and 20ºC for these kinetic parameters are listed in Table 3.3.

Table 3.2: Typical stoichiometric paremeters of ASM3.

Symbol	Characterization	Value	Units
f_{SI}	Production of S_I in hydrolysis	0	$gCOD_{SI}/gCOD_{XS}$
Y_{STO}	Aerobic yield of stored product per Ss	0.85	$gCOD_{XSTO}/gCOD_{SS}$
$Y_{STO, NO}$	Anoxic yield of stored product per Ss	0.80	$gCOD_{XSTO}/gCOD_{SS}$
Y_H	Aerobic yield of heterotrophic biomass	0.63	$gCOD_{XH}/gCOD_{XSTO}$
$Y_{H, NO}$	Anoxic yield of heterotrophic biomass	0.54	$gCOD_{XH}/gCOD_{XSTO}$
Y_A	Yield of autotrophic biomass per NO_3^--N	0.24	$gCOD_{XA}/gN_{SNOX}$
f_{XI}	Production of S_I in endogenous respiration	0.20	$gCOD_{XI}/gCOD_{XBM}$
$I_{N, SI}$	N content of S_I	0.01	$gN/gCOD_{SI}$
$I_{N, SS}$	N content of S_S	0.03	$gN/gCOD_{Ss}$
$I_{N, XI}$	N content of X_I	0.02	$gN/gCOD_{XI}$
$I_{N, XS}$	N content of X_S	0.04	$gN/gCOD_{XS}$
$I_{N, BM}$	N content of biomass X_H, X_A	0.07	$gN/gCOD_{XBM}$
$I_{SS, XI}$	S_S to COD ratio for X_I	0.75	$gSs/gCOD_{XI}$
$I_{SS, XS}$	S_S to COD ratio for X_S	0.75	$gSs/gCOD_{XS}$
$I_{SS, BM}$	S_S to COD ratio for biomass X_H, X_A	0.90	$gSs/gCOD_{XBM}$

Table 3.3: Typical values for kinetic parameters of ASM3.

Symbol	Characterization	Temperature		Units
		10°C	20°C	
k_H	Hydrolysis rate constant	2	3	$gCOD_{XS}/gCOD_{XH}.d^{-1}$
K_X	Hydrolysis saturation constant	1	1	$gCOD_{XS}/gCOD_{XH}$
k_{STO}	Storage rate constant	2.5	5	$gCOD_{SS}/gCOD_{XH}.d^{-1}$
η_{NO}	Anoxic reduction function	0.6	0.6	-
K_{O2}	Saturation constant for S_{NO2}	0.2	0.2	gO_2/L
K_{NO}	Saturation constant for S_{NOX}	0.5	0.5	gNO_3^-N/L
K_S	Saturation constant for substrate S_S	2	2	$gCOD_{SS}/L$
K_{STO}	Saturation constant for X_{STO}	1	1	$gCOD_{XSTO}/gCOD_{XH}$
μ_H	Heterotrophic maximum growth rate of X_H	1	2	1/d
K_{NH4}	Saturation constant for ammonium, S_{NH4}	0.01	0.01	gN/L
K_{ALK}	Saturation constant for alkalinity for X_H	0.1	0.1	$gHCO_3^-/L$
$b_{H,\,O2}$	Aerobic andogenous respiration rate of X_H	0.1	0.2	1/d
$b_{H,\,NO}$	Anoxic andogenous respiration rate of X_H	0.05	0.1	1/d
$b_{STO,\,O2}$	Aerobic respiration rate of X_{STO}	0.1	0.2	1/d
$b_{STO,\,NO}$	Anoxic respiration rate of X_{STO}	0.05	0.1	1/d

3.2.4 ASM3 modification/ calibration studies

Krishna and van Loosdrecht (1999) have observed that ASM3 failed to model two significant experimental observations: (*i*) the constancy of the growth rate of biomass observed experimentally either in feast and famine phases and (ii) it required prediction of higher levels of internal storage polymers than measured to fit the oxygen consumption during feast and famine phases. Afterwards, to evaluate storage mechanism and ASM3, Krishna and van Loosdrecht have proposed the first modeling study, Activated Sludge Model for Growth and Storage (ASMG), with the measurement the conversion of acetate, ammonium, oxygen, biomass and PHB at different temperatures in laboratory SBR system. Matrix representation of this new approach is shown in Table 3.4.

Table 3.4: Matrix representation of simplified ASM3 (Krishna and van Loosdrecht, 1999).

Component / **Process**	**S_O**	**S_S**	**X_I**	**X_H**	**X_{STO}**	**X_{TS}**	**Process rate**
Aerobic storage of COD (PHB storage)	-(1-Y_{STO})	-1			Y_{STO}		$k_{STO} \cdot \frac{S_S}{K_S + S_S} \cdot X_H$
Aerobic growth on PHB	$-\frac{1 - Y_{H2}}{Y_{H2}}$			1	$-\frac{1}{Y_{H2}}$		$\mu_{H2} \cdot \frac{S_S}{K_S + S_S} \cdot \frac{X_{STO}/X_H}{K_{STO} + X_{STO}/X_H} \cdot X_H$
Aerobic endogenous respiration	-(1- f_I)		f_I	-1			$b_{H..} X_H$
Aerobic respiration of PHB	-1						b_{STO}. X_H
Aerobic growth on acetate	$-\frac{1 - Y_{H1}}{Y_{H1}}$	$-\frac{1}{Y_{H1}}$		1			$\mu_{H1} \cdot \frac{S_S}{K_S + S_S} \cdot X_H$

In ASMG, aerobic heterotrophic conversion is expected to simplify ASM3 and acetate is chosen as the only carbon source. All expressions and parameters were selected 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. As a result, simplified ASM3 gives a reasonable description of the studied SBR process, but the constancy of the growth rate of biomass observed experimentally either in feast and famine phases cannot be described.

Orhon et al. (2009) also evaluated that modeling of peptone biodegradation with simultaneous PHA storage was basically constructed on the same modified ASM1 template utilized in the earlier part of the experimental evaluation. The adopted model structure (growth-storage model) included a new model component reflecting the concentration of the storage products, X_{PHA} and two new processes, namely storage and growth on stored PHA, as given in matrix representation in Table 3.5. The storage process was conventionally defined in terms of a similar Monod-type of an expression where k_{STO} denotes the maximum storage rate. In this study, peptone was selected as carbon source because it is very similar to domestic swage in terms of biodegradation characteristics. In addition, it contains a similar balance between readily biodegradable COD and slowly biodegradable COD fractions. The kinetic and stoichiometric parameters are given in Table 3.7.

Cığgın et al. (2011) proposed a new model for acetate utilization. The model adopted for this study involved the basic template of ASMG and implemented for evaluation the utilization mechanism of starch (Karahan et al.2006). This model is a combination of ASM1 and ASM3 necessary for the removal selected readily biodegradable substrate, acetate. Respiration of X_{PHB} as suggested in ASM3 was also added to be compatible with the template of the ASM3 structure. Generation of microbial products was also taking into account as part of endogenous respiration with the simplifying assumption of decay-associated processes adopted in many similar studies (Orhon et al., 1994). Switching functions of ammonia nitrogen, dissolved oxygen and alkalinity were neglected in the rate expressions, because they were maintained in excess of rate limiting levels in the SBR reactor fed with the synthetic substrate feed solution. A matrix format of model components, processes and processes' rates are given in Table 3.6. The kinetic and stoichiometric parameters for other related studies are given in Table 3.7.

Table 3.5: Matrix representation of simplified ASM3 (Orhon et al., 2009).

Component / **Process**	**S_{O2}**	**S_S**	**S_{H1}**	**S_{H2}**	**X_H**	**X_{STO}**	**X_P**	**Process rate**
Hydrolysis of S_{H1}		1	-1					$k_h \cdot \frac{S_{H1}/X_H}{K_X + S_{H1}/X_H} \cdot X_H$
Hydrolysis of SH2		1		-1				$k_{hx} \cdot \frac{S_{H2}/X_H}{K_{XX} + S_{H2}/X_H} \cdot X_H$
Storage of PHA	-(1-Y_{STO})	-1			1	Y_{STO}		$k_{STO} \cdot \frac{S_S}{K_S + S_S} \cdot X_H$
Growth on X_H	$-\frac{1-Y_H}{Y_H}$				1			$\mu_H \cdot \frac{S_S}{K_S + S_S} \cdot X_H$
Growth on PHA	$-\frac{1-Y_H}{Y_H}$				-1	$-\frac{1}{Y_H}$		μ_{STO}. X_{STO}
Decay of X_H	-(1- f_P)						f_P	$b_{H..}X_H$

Table 3.6: Matrix representation of the model structure for simultaneous growth and storage (Cığgın et al., 2011).

Component / **Process**	S_{AC}	X_{PHB}	X_H	S_P	X_P	S_O	**Process rate**
Growth on S_{AC}	$-\frac{1}{Y_H}$		1			$-\frac{1-Y_H}{Y_H}$	$\mu_{H1} \cdot \frac{S_{AC}}{K_{S1}+S_{AC}} \cdot X_H$
Storage of S_{AC}	-1	Y_{STO}				$-(1-Y_{STO})$	$k_{STO} \cdot \frac{S_{AC}}{K_{S2}+S_{AC}} \cdot X_H$
Growth on PHB		$-\frac{1}{Y_H}$	1			$-\frac{1-Y_H}{Y_H}$	$\mu_{H2} \cdot \frac{K_{S1}}{K_{S1}+S_{AC}} \cdot \frac{X_{PHB}/X_H}{K_{STO}+X_{PHB}/X_H} \cdot X_H$
Endogenous respiration			-1	f_{ES}	f_{EX}	$-(1-f_{ES}-f_{EX})$	$b_H . X_H$
Endogenous respiration of X_{PHB}		-1				-1	$b_{STO} . X_{STO}$

Table 3.7: Kinetic and stoichiometric parameters of ASM3 and modified ASM3.

Parameters	Insel et. al, 2012		Ozkok Pala et al., 2012		Cokgor et. al, 2011	Cığgın et al. 2011		Orhon et. al, 2009		Karahan et.al, 2008	Insel et. al, 2007	Karahan et.al, 2006	Karahan-Gül et.al, 2003		Krishna and van Loosdrecht, 1999	
Model Type	Modified ASM3		Modified ASM3		Adopted model	Modified ASM3		ASM3		Modified ASM3	Proposed Model	Modified ASM3	ASM3	Modified ASM3	ASM3	Modified ASM3
SRT (day)	10	2	10	2	10	2	8	10	2	-	5	5	20		2.5	
Carbon Source	Acetate		Peptone		Peptone	Acetate		Peptone		Acetate	Glucose	Starch	Acetate		Acetate	
μ_{Hmax} (1/day)	3.9	6.8	5.2	7.2	4.1	2.5	1.5	4.1	6.1	-	15.1	3	-	4	-	2
μ_{STO} (1/day)	4.7	2.2	0.8	0.0	1.25	2.0	1.5	1.25	1.10	5	-	4	3 & 1.8	3	4	3.5
K_S (mgCOD/L)	6	25	24.0	30.0	4.0	10.0	5.0	4	2.3	5	16	20	4	3	1	0.1
K_{STO} (mgCOD/L)	1	5	0.5	0.0	0.41	0.5	0.5	-	-	0.5	-	0.4	1	0.4	1	1
bH (1/day)	0.22	0.22	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.15	0.1	0.24	0.24	0.2	0.2
bSTO (1/day)	-	-	-	-	-	0.1	0.1	-	-	0.05	-	0.05	0.24	0.24	0.2	0.4
kSTO (1/day)	5.7	6.3	1.2	0.0	0.90	6.5	8.0	0.9	0.45	15	1.4	25	16	14	10	10
YH (mgCOD/ mgCOD)	0.66	0.66	0.6	0.6	0.60	0.7	0.7	0.6	0.6	-	0.47	0.79	-	0.65	-	-
YSTO (mgCOD/ mgCOD)	0.80	0.80	0.8	0.0	0.8	0.8	0.8	0.8	0.8	0.76	0.9	0.91	0.80	0.8	-	-
kh (1/day)	-	-	5.2	4.0	4.34	-	-	4.34	5.95	-	-	30	-	-	-	-
K_X (mg COD/ mgCOD)	-	-	0.15	0.15	0.03	-	-	0.03	0.05	-	-	0.15	-	-	-	-
f_{ES}			0.05	0.05	0.05	0.1	0.1	0.05	0.05	-	0.05	0.05	0.05	0.05	-	-
f_{EX}			0.15	0.15	0.15	0.1	0.1	0.15	0.15	-	0.15	0.1	0.15	0.15	-	-
SBR cycles	1	1	1	1	1	6	6	1	1	6	1	6	1	1	6	6

3.2.5 Effects of SRT on storage process

The type and the extent of storage response of a mixed microbial culture in an activated sludge system can depend on the physiological state of any microorganism in the consortium, which is also affected by the operating conditions, especially sludge residence time (SRT) (Çığgın et al., 2011a). Although, ASM3 suggested the default value of storage yield as 0.85 gCOD/g COD, extensive experimental studies showed a large range of different storage yield values. Authors proposed different yield values for the same substrate but at different SRTs. These yield values are listed in Table 3.8.

Table 3.8: Storage yield (Y_{STO}) values caluculated at different SRTs.

Reference	**Growth Conditions of Activated Sludge**		**Carbon Source**	**Y_{STO}**
	SRT (days)	**Feed Composition (Ratio, COD based)**		**(gCOD/gCOD)**
Beun et al.(2000)	3.8	Acetate	Acetate	0.69
Beun et al.(2002)	2-20	Acetate	Acetate	0.68
Carta et al.(2001)	6.1	Acetate + Glucose (1:1)	Acetate Glucose	0.6-0.7
Cığgın et al (2011a)	2-8	Acetate	Acetate	
Dricks et al.(2001)	7.7	Glucose	Glucose	0.91
Goel et al. (1998)	10	Acetate+ Glucose + Peptone+ Yeast extract (2:1:1:1)	Glucose Acetate Starch	0.68 0.45 0.36
Karahan et al. (2006)	10	Starch	Starch	0.91
Krishna and van Loosdrecht (1999)	2.5	Acetate	Acetate	0.73
Martins et al. (2003)	7-10	Acetate	Acetate	0.47-0.59
Van Aalst-van Leeuwen et al. (1997)	0.5	Acetate	Acetate	0.73

The yield of PHB storage is directly calculated from the ratio between removal substrate and produced PHB at feast phase ($q_P/-q_S$) (Dricks et al., 2001). The faster substrate uptake rate has been usually interpreted as indirect evidence of more relevant presence of a storage response in activated sludge process (van Loosdrecht et al., 1997). In activated sludge system, the sludge age is the defining parameter for culture history of the enriched microbial community (Frigon et al., 2006), and hence it affects the metabolic activities of biomass related to growth and storage. The longer the SRT value results with an slow growth rate of activated biomass that leads to the conclusion amount of the maximum substrate uptake rate is higher than the needed amount for average biomass growth rate, since a part of the uptaken substrate is stored and the other part is directly used for growth.

Shortly, experiments showed that under the operating conditions with low SRT, high growth rate of biomass, the substrate removal rate is higher at feast period when external substrate is available with an extra concentration. The substrate uptake rate is limited by the substrate concentration. Even though a linear relation exists between substrate uptake rate and biomass growth in the systems where only biomass growth is observed, more complicated relations are present between the substrate uptake rate, biomass growth and PHB production.

Studies on the storage process with pure cultures at high specific growth rate- i.e. low SRT- gave the result that storage was correlated with the fraction between the substrate uptake rate and its utilization rate for growth (van Aalst-van Leeuwen et al, 1997). Under the operating conditions of a biomass culture with a growth rate close to its maximum substrate uptake rate, storage was observed to be negligible; in this range, the lower the SRT, the less substrate was converted into storage polymers (van Loosdrecht and Heijnen 2002).

According to Beun et al. (2002), the ratio ($q_P/-q_S$) has a constant value for dynamically fed mixed microbial culture systems operated at a sludge age higher than 2 days (SRT>2d). This value is 0.6 Cmol/Cmol (0.68 mgCOD/mgCOD) under aerobic conditions.

van Aalst- van Leeuwen et al. (1997) observed that faster growing organisms accumulated less PHB. In addition to the observation, Majone et al. (2007) showed that the overall transient response increased as SRT decreased in their pure culture

study. van Loosdrecht and Heijnen (2002) made the same statement that the shorter SRT, the higher growth rate and the less substrate is converted into storage polymers. Krishna and van Loosdrecht (1999) reported the same result that the ratio of storage to the overall substrate removal was reduced when the sludge age of an SBR was lowered from 9.5 days to 3.8 days.

In a study done by Beun et al. (2000), they investigated storage in a SBR system involving pulse feeding of acetate at three different sludge ages. For SRT of 3 days PHB/acetate ratio was 0.46 mgCOD/ mgCOD and it increased to 0.69 mgCOD/ mgCOD for 10 days and 0.70 mgCOD/ mgCOD for 20 days. The reason for these changes is the difference in specific growth rate between the feast and famine period decreases with increasing the SRT. At lower SRT, the specific growth rate in the feast phase is significantly increased relative to the specific growth rate in the famine period. In addition, specific PHB production rates were almost constant at different sludge ages. Higher substrate uptake rate was observed in the acclimated culture.

Çığgın et al. (2011b) presented that the sludge age induced a decreasing effect on the storage of PHB when the system was operated at low sludge age. This study is conducted by an SBR that is fed with acetate as sole carbon source under two different sludge ages (2 and 8 days). PHB storage capacity of biomass is increased when the system sludge age was lowered to 2 days. The authors were explained this change, with a corresponding increase in the portion of acetate consumed for directly growth process, probably because of the higher maximum growth rate obtained at sludge age of 2 days.

In addition, Dricks et al. (2001), run batch experiments with pulse addition to activated sludge taken from full-scale nutrient removal treatment plants. They found that the rate of substrate utilization and PHB formation was much slower in real wastewater treatment system. The experiments yielded a PHB/acetate ratio of 0.69 mgCOD/ mgCOD for the plant operated at SRT of 4 days and 0.73 mgCOD/ mgCOD for SRT of 21 days. According to the study, PHB degradation at the famine phase depends on the amount of PHB present inside the cells at the end of the feast period. They concluded that 90% of microbial growth occurs in the famine period on stored PHB. The biomass with the SRT of 4 days was found to degrade PHB faster than the sludge with the SRT of 10 days.

3.2.6 Effect of feeding pattern on storage process

The feeding pattern affects occurrence of storage and release of metabolic intermediates through the selection of microbial species and the regulation of enzyme synthesis (Daigger and Grady, 1982). The intermittent feeding regime gives favorable conditions for microorganisms capable of storing and releasing metabolic intermediates, which hereby can take up available external substrate very fast and utilize it to gain balance growth. In addition, these microorganisms will have a competitive advantage in an environment where the feast and famine phases present, and thus they will be selected.

When the excess external substrate is available for a long period (under continuous feeding), the specific growth rate of biomass will be increased and the synthesis rate of storage polymer will be decreased. However, no competitive advantage is associated with the ability to form storage products or the release of metabolic intermediates, when feeding is continuous. Consequently, biosynthesis of storage products and release of metabolic intermediates are more likely to occur when the excess external substrate present for short period of time (pulse feeding). Therefore, high storage capacity has been observed in pulse fed activated sludge systems. Dionisi et al. (2001) proposed that the feast period should be no more than 25-30% of overall reaction period in order to have a strong storage response.

A study conducted by Majone et al (1996), to investigate the conversion of substrates to storage polymer under continuous feeding. In this study, two parallel continuously stirred reactors (CSTR) were operated at the SRT of 3 days with the continuously and intermittently acetate addition. Although, two different dominant microbial groups were observed as a result, significant amount of PHB storage observed in both feeding regimes. The observation of the study shows that the storage of PHB is in general the prevailing mechanism of substrate removal; even the dominant groups are change according the feeding pattern of the system. The microbial groups were named filamentous bacteria in continuously fed CSTR and floc forming bacteria in pulse fed (2 min.) CSTR. Baccari et al. (1998) was also studied the storage capability of the filamentous bacteria under pulse feeding. The two studies were explained the important role of the initial inoculum on the microbial composition.

Studies on the storage process with mixed cultures confirmed that the ability of microorganisms to easily and fastly increase their substrate removal rate and shift it toward more storage give them a competitive advantage. van Loosdrecht et al. (1997) tested pulse feeding of acetate on the system previously operated at steady state with continuous feeding and observed immediate PHA formation. Besides, several pure culture studies were also conducted and all of the studies reached the same results that more or less strong dynamic conditions affect the storage response of biomass, even if all of the operating conditions, e.g. organic loading rate and culture residence time, are the same in the system. Also, short term and long term effects to different feeding patterns was evaluated by several authors. It is easy to observe a short term dynamic effect of a system by a single substrate addition with pulse feeding to a culture that previously adapted to growth under continuously feeding. Martins et al. (2004) has conducted a study in a continuously fed SBR under anoxic conditions and observed PHA storage when pulse feeding applied in a single cycle. Another study conducted by Cığgın et al (2011b) showed that when the feeding pattern change from continuous to pulse just for one cycle typical feast and famine conditions were created and more PHA storage was observed. On the contrary, when the biomass acclimated to pulse feeding was disturbed by using continuous feeding, a decrease in PHA concentration was observed although the culture was simultaneously using acetate. The reason of this behavior can be explained that the specific substrate uptake rate is high during the feast phase as well as PHA consumption is fast during the famine phase because the biomass is likely to be acclimated pulse feeding.

Clearly, the effect of intermittent conditions on the physiological state and related transient response of single microorganisms can be different from species to species (Majone and Tandoi, 2002). Other pure culture studies have shown that the role of storage (rate and yield) is also depending on operating conditions (organic loading rate and culture residence time), even with well adapted microorganisms (Dionisi et al., 2005; Majone et al., 2007).

The observed yields and rates for the biomass acclimated under different feeding length are shown in Table 3.9. As shown in Table 3.9, periodically fed systems typically have faster substrate uptake rate and higher observed yields than the systems fed with continuously.

Table 3.9: The rates and yields obtained from different feeding patterns.

References	SRT (days)	Feeding/Cycle Length (min/min)	q_S	q_P	Y_{STO}
			(mgCOD/gCOD.h)		(gCOD/gCOD)
Beccari et al., 1998	3	2/360	740-920	510-610	0.69
	3.8	3/240	538	210	0.38
Beun et al., 2000	9.5	3/240	362	210	0.57
	19.8	3/240	311	179	0.56
Krishna and van Loosdrecht, 1999	2.5	55/240	203	112	0.55
Majone et al., 1996	3	continuous	200-260	n.r.	0.35
		2/240	700-800	420-640	0.6- 0.8
Beun et al.,2002	4	3/240	715	421	0.56
Martins et al., 2003	6.9	3/240	361	156	0.47
	10.4	90/240	655	327	0.59
Martins et al., 2010	10	3/240	529	371	-
		50/240	79	11	-

Conversely, Majone et al. (1996) and van Loosdrecht et al. (1997) have shown that the storage response is the main mechanism for both long and short fed sludge. Carbon limited systems were also shown storage response (van Aalst-van Leeuwen et al. 1997; Krishna and Loosdrecht, 1999). The production of PHB even under carbon limited chemostat could be an indication of that the storage response is an instrict part of microbial growth physiology (van Aalst-van Leeuwen et al., 1997).

4. MATERIALS AND METHODS

To investigate the effect of F/M ratio on storage potential of microorganisms under aerobic conditions, experimental studies start up with acclimation period of parent reactors and continued with two sets of experimental runs. For this purpose, two lab-scales fill and draw reactors were set-up as parent reactors for the acclimation period of activated sludge.

Biomass was acclimated to pulse feeding pattern under aerobic conditions and carbon source was added to systems once a day. Acetate was chosen as sole carbon source for parent reactors and acclimation period was continued until steady state conditions were reached. After steady state conditions were established, activated sludge culture was taken to perform respirometric tests and batch experiments to evaluate the effect of F/M ratio on storage mechanism of microorganisms.

Hence, a set of experimental runs were conducted to observation of storage behavior of microorganisms in different F/M ratios, in addition to experiments conducted during steady state operation of parent reactors. In addition, respirometric experiments were conducted to see oxygen uptake rate profiles of biomass at different initial COD concentrations (different F/M ratios) for model evaluation of substrate storage mechanism in batch tests and parent reactors. 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.

4.1 Reactor Set-up

The experiments were started with the set-up of the parent reactors. Parent reactors were inoculated with activated sludge taken from another study, which acclimated to anaerobic-aerobic conditions to remove excess phosphorus. Reactors had working volume of 4L and operated at a sludge age of 2 days (Figure 4.1). As sole carbon source acetate was chosen, because of its representativeness of readily biodegradable substrates. The initial carbon source concentration was selected as 250 mg COD/L,

pH was kept in the range of 6.0-8.0, suitable for biological activity and the temperature was maintained at 20 ± 1ºC during the operation of parent reactors. Reactors were continuously aerated with the help of air diffusers and the oxygen concentration in the reactor kept above 2 mg/L to maintain aerobic conditions. Two mechanic stirrers were also provided with the reactor in order established well-mixed liquor in the system. During each daily feeding period, reactors were decanted until 2 L, according to selected sludge age, while reactor mixing was continued. Reactors were operated until steady state conditions, which was monitored by suspended solids (SS), volatile suspended solids (VSS) and chemical oxygen demand (COD) measurements. At the steady state, biomass concentration of the reactor was stabilized approximately 255mg VSS/L.

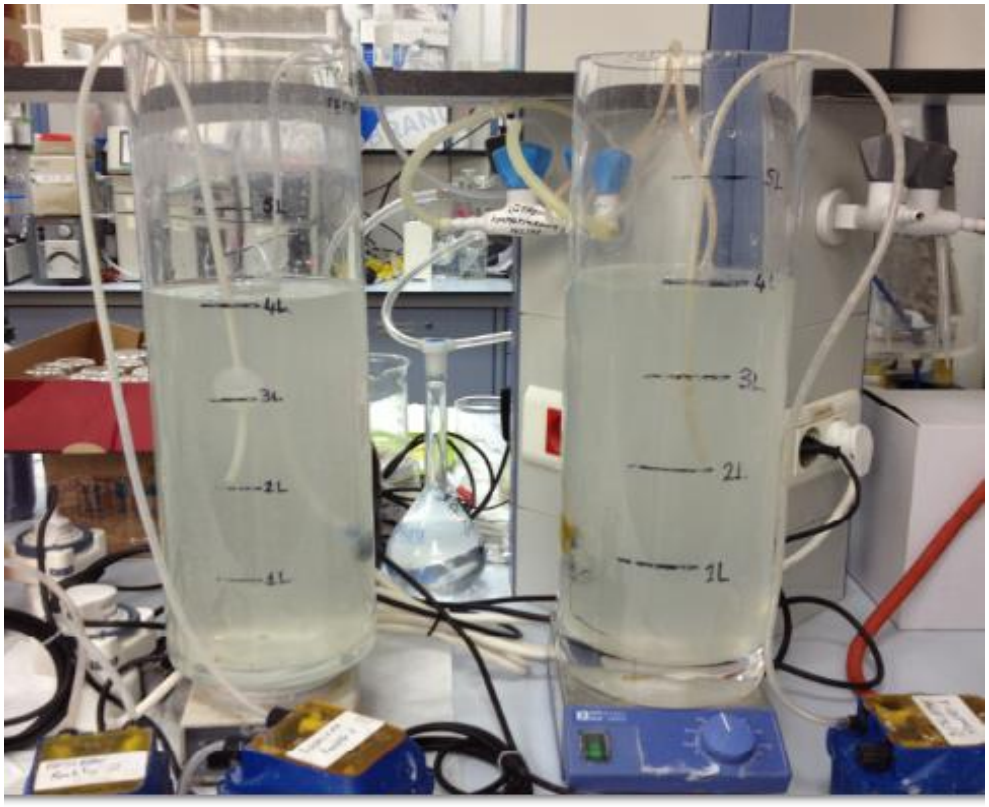

Figure 4.1: Laboratory scale parent reactors.

The substrate feed was prepared by diluting a stock solution of sodium acetate trihydrate (CH_3COONa) in distilled water. Additionally for the nutrient requirements of activated sludge macro and micronutrients solutions, namely Solution A and Solution B (O'Connor, 1972) were prepared and fed with carbon source. Besides supplying essential nutrients for biomass, these nutrient solutions also provide enough buffer capacity to the reactor so that pH is kept around 7.0. The composition

of these solutions is given in Table 4.1. In the reactor, the amount of Solution A and Solution B were adjusted to supply 10mL of solution for 1000mg COD/L of carbon source (O'Connor, 1972).

Table 4.1: Composition of nutrient solutions.

Solution	Component	Amount of Component (g/L)
Solution A	NH_4Cl	120
	KH_2PO_4	160
	K_2HPO_4	320
Solution B	$MgSO_4.7H_2O$	15
	$CaCl_2.7H_2O$	2.65
	$FeSO_4.7H_2O$	0.5
	$ZnSO_4.7H_2O$	0.5
	$MnSO_4.H_2O$	0.41

4.2 Experimental Procedure

After sludge reached steady state conditions, 2L activated sludge taken from the mixed liquor to conduct batch tests. The batch reactor was initially operated at an idle period for 10 minutes and then fed according to selected initial acetate COD concentration that will determine the F/M ratio. The batch experiments are summarized in Table 4.2. The reactors were monitored with measurements of COD, acetate, PHB, SS and VSS. 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 storage compound.

Respirometric analyses were also conducted to understand the change in respiration capacities of activated sludge at different F/M ratios. Oxygen uptake rate (OUR) profiles should be comprehended for the evaluation of substrate removal mechanisms. Experiments representing the same conditions with batch tests were conducted in a respirometer. In these experiments, biomass is left for half hour in endogenous decay phase then the selected initial acetate concentration added to the respirometer tank. The respirometric analyses were continued until the supplied

carbon source was totally depleted. The OUR profiles, VSS, SS, soluble COD and PHA measurements obtained from these experiments were used for the modeling of activated sludge system.

Table 4.2: Summary of experimental setup.

RUNS	Acetate	Biomass	S_0/X_0
	mgCOD/L	mgVSS/L	gCOD/gVSS
Run 1 (control)	250	255	0.98
Run 2	460	255	1.80
Run 3	105	280	0.38
Run 4	57	255	0.22
Run 5	650	220	2.95
Run 6	1000	220	4.54

In this study, OUR measurements were performed with an Applitek RA-Combo-1000 (Applitek Co., Nazareth, Belgium) continuous respirometer with PC connection (Figure 4.2). During each experiment, 1.04 g Allyl Thio Urea (ATU) (Formula 2533TM, Hach Company) was added to the OUR reactors as a nitrification inhibitor to prevent any possible interference induced by nitrification. The respirometer used in the experiments involved a continuous flow-through measurement using the dissolved oxygen concentration in the liquid phase to calculate the respiration rate of the activated sludge (Spanjers et al, 1996). In principle, the activated sludge was continuously transferred to the respirometer with a peristaltic pump. After passing through the respirometer vessel (0.75 L), where the dissolved oxygen at the inlet and outlet was measured by a single dissolved oxygen (DO) probe, the sample returned to the OUR reactor. Hereby the activated sludge was continuously recirculated. Due to the use of a single DO-electrode, the measuring frequency was limited by the response rate of the DO-electrode (Spanjers et al, 1996).

4.3 Analytical Procedure

Volatile suspended solids (VSS), suspended solids (SS), COD and pH analysis were performed in order to control and monitor reactor operation. In the experiments, pH was kept in the range of 7.0-8.0, suitable for biological activity. Temperature of all experiments was maintained 20°C ± 1 and minimum dissolved oxygen concentration of 2 mg/L was supplied in the system. During the experiments, an Orion 520 pH meter was used for pH measurements and before each usage of the device, the pHmeter was calibrated. SS and VSS analysis were performed as described in Standard Methods (APHA). SS and VSS measurement were carried out after filtration of the sample from Millipore AP40 glass fiber filters with an effective por size of approximately 1.2µm. Samples, which were taken for soluble COD measurements, were filtered through polyvinylidene fluoride (PVDF) syringe filters. COD measurements were performed as described in the ISO 6060 Method (ISO 6060, 1989).

Figure 4.2: Applitek RA-Combo-1000 continuous respirometer.

Samples for PHA analysis were taken into 30 ml centrifuge tubes and containing 3-4 drops of formaldehyde for preventing the biological activity. The PHA content of the washed (K-P buffer solution) and freez 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., 2000; Cığgın et al., 2009). The resulting

organic phase was extracted with water to remove free acids. The extracted propylesters were analyzed by gas chromatograph (Agilent 6890N) equipped with a flame-ionisation detector and capillary column (INNOWAX 19095N-123) where benzoic acid was used as an internal standard for the determination of PHB and PHV contents. The injection split (2:1 ratio) temperature and the FID detector temperature were 50°C and 250°C, respectively. Helium was used as the carrier gas. In the detector, the hydrogen flow was set as 30mL/min, airflow to 400mL/min, and the make-up flow (helium) to 25mL/min. The oven temperature was started at 90°C and kept at this temperature for 0.10 minutes. Then oven temperature was gradually increased to 150°C, kept at this temperature for 5 minutes, and increased to 210°C for 5 minutes.

Acetate samples were also analyzed by gas chromatograph (Agilent 6890N) equipped with a flame-ionisation detector and capillary column (DB-FFAP 125-3232). 1.6 mL of acetate samples filtered through 0.22µm PVDF syringe filters were transferred into a gas chromatography vial and 0.2mL of 10M phosphoric acid (H_3PO_4) was added to each vial. For the acetate analysis, the temperature of the injection port and detector were 230°C and 250°C, respectively. The sample is injected with splitless injection. The oven temperature reached 100°C in first 5 minutes and then 160°C; it was kept at this temperature for 5 minutes and fixed at 230°C in 3 minutes. Helium was the carrier gas at 45mL/min. In addition, hydrogen gas was used at 40mL/min flow rate.

5. RESULTS AND DISCUSSION

5.1 Acclimation Studies of Control Reactor

A laboratory-scale fill and draw reactor, namely "parent reactor" a net volume of 4L was operated at sludge age of 2 days with the initial carbon source concentration of approximately 250mgCOD/L. Acetate representing readily biodegradable substrate was chosen as sole carbon source. pH was kept in the range of 7.0-8.0, suitable for biological activity and the temperature was maintained at 20 ± 1ºC during the operation of reactor. At steady state conditions, biomass concentration of reactor was stabilized approximately 255 mg VSS/L, yielding the S_0/X_0 ratio of 0.98mgCOD/mgVSS.

In summary, the parent reactor characteristics at steady state conditions were given in Table 5.1. The SS, VSS, VSS/SS ratio, and effluent COD of the control reactor were monitored. Reactor monitoring data can be seen in Figure 5.1, Figure 5.2, and Figure5.3.

Table 5.1: Steady state characteristics of the control reactor.

Carbon Source	**SS**	**VSS**	**VSS/SS**	**S_0/X_0**	**COD inf.**	**COD eff.**	**Removal efficiency**
	mgSS/L	mgVSS/L	-	mgCOD/ mgVSS	mg/L	mg/L	%
Acetate	320	255	0.85	0.98	250	32	87

5.2 Experimental Results

5.2.1 Evaluation of control experiment in the parent rector

The first run of the experimental setup was performed with using the acetate as sole carbon source and biomass taken from the parent reactor in order to investigate the biomass behavior under steady state conditions. Respirometric test was started with the approximately same loading ratio (S_0/X_0) with control reactor. For this reason

VSS and substrate concentrations were diluted by 1/2 ratio. After observation of the endogenous decay level in terms of straight line, the acetate was added to OUR reactor of respirometry as carbon source.

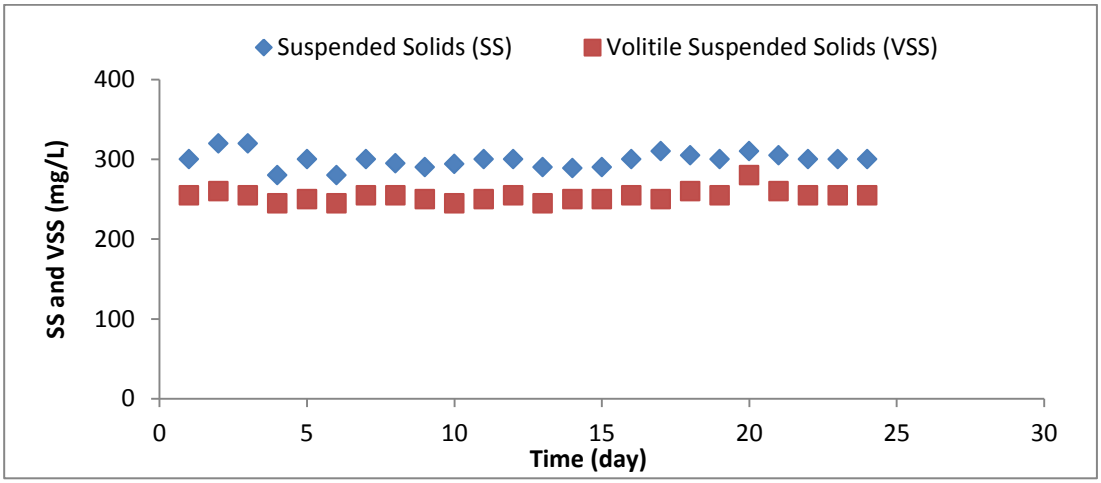

Figure 5.1: SS and VSS results of the control reactor.

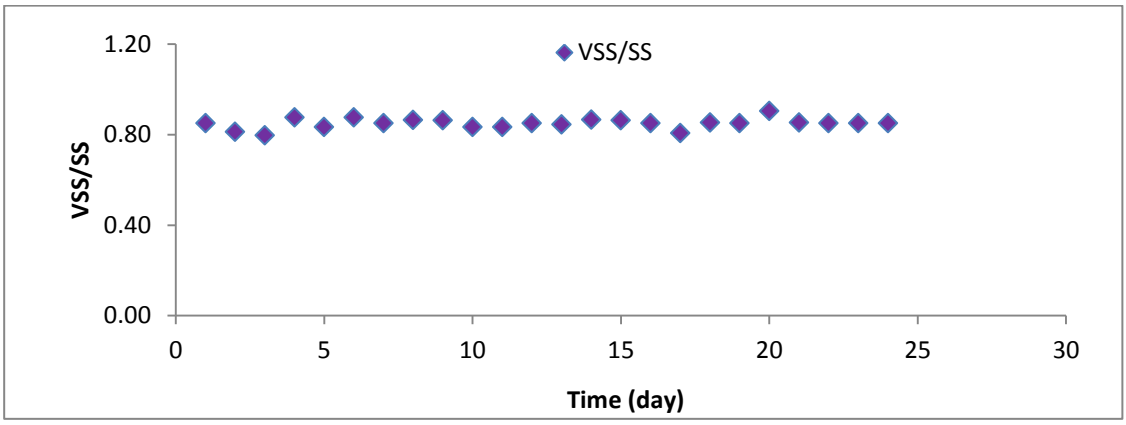

Figure 5.2: VSS/SS ratio of the control reactor.

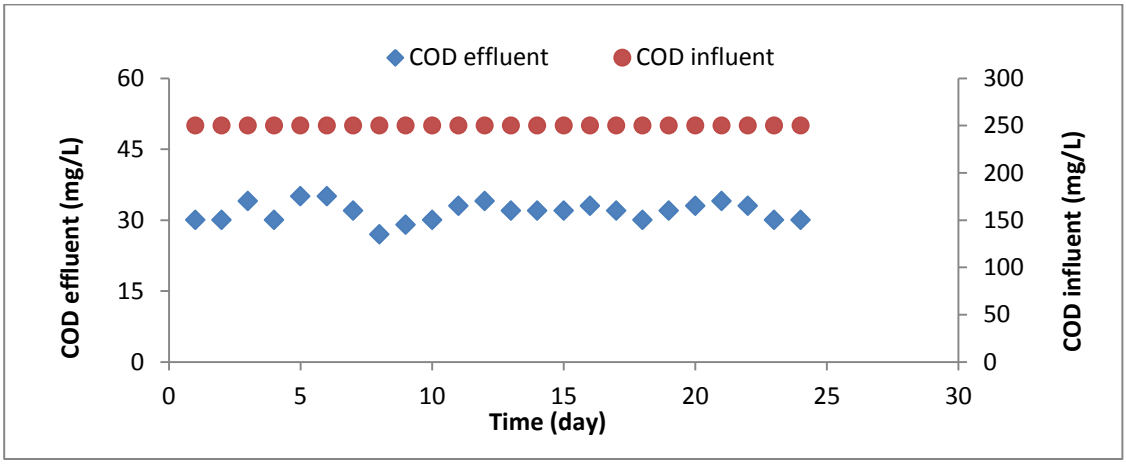

Figure 5.3: Influent and effluent soluble COD concentrations of the control reactor.

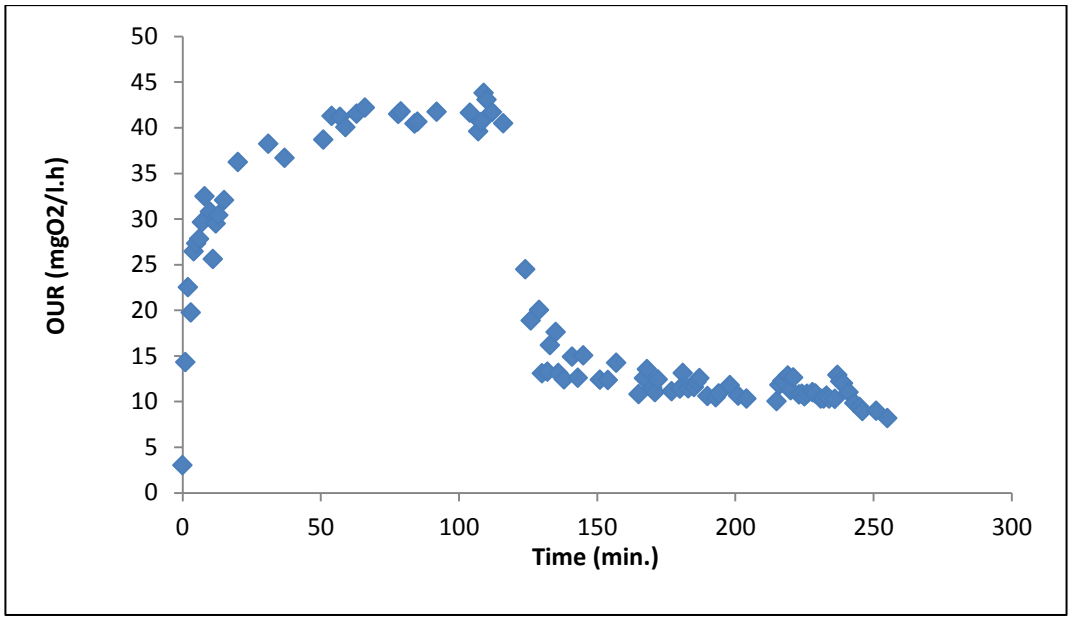

Figure 5.4: OUR profile of the control experiment (Run1).

The oxygen utilization rate (OUR) curve obtained from biodegradation of acetate was used as control for evaluating the F/M ratio effect on biomass storage response (Figure 5.4). In the other words, the impact was interpreted by changes inflicted by F/M ratio on the shape of the initial OUR profile obtained in the control test conducted at the same F/M ratio. The OUR, defined as the rate of oxygen utilization in biochemical processes in the activated sludge models, is the change observed in the dissolved oxygen concentration (So) in time due to biochemical transformations. Thus, OUR is an overall process rate reflecting the cumulative impact of all oxygen/energy consuming reactions. The OUR curve obtained from the biodegradation reaction of acetate in Run 1 is shown in Figure 5.4. The maximum oxygen uptake rate of the biomass gave the first peak around 43 mg/L.h in 120 min., which is due to readily biodegradable COD component in the system. The area under the OUR curve has shown that the total oxygen consumption for defined period (i.e. one minute).

The COD removal efficiency of biomass in Run 1 is shown in Figure 5.5, which indicates removal of all influent acetate in the control reactor during a period. The organic substrate (acetate) used in experiments is by nature totally biodegradable; this is one of the main reasons for its selection and recommendation as the standard substrate for biodegradation experiments. Because the biodegradable substrate in the control reactor was completely depleted after the OUR profile dropped to the initial

endogenous respiration level. The COD in the reactor first reach the level at the endogenous decay phase with the degradation of the total acetate but a little increase observed after that because of the synthesized inert microbial products.

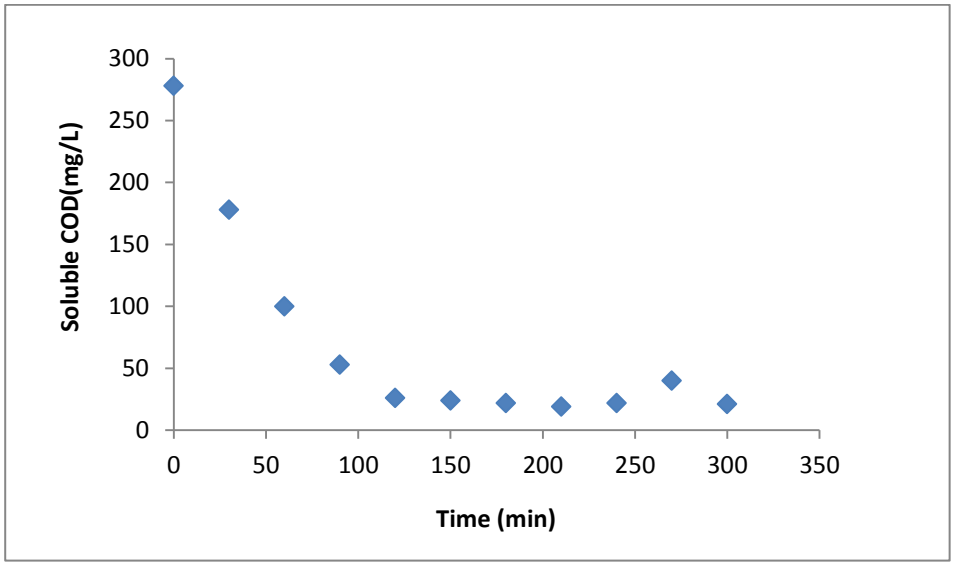

Figure 5.5: Soluble COD profile of the control experiment (Run1).

The substrate storage response of biomass in Run 1 is shown in Figure 5.6, which indicates storage of acetate as PHB in the control reactor during a period. As an initial PHB content 12 mgCOD/L, PHB was measured at the beginning of the experiment and with acetate addition to the system this level was started to increase by time. The maximum PHB storage was observed around 110 mgCOD/L in 120 min.

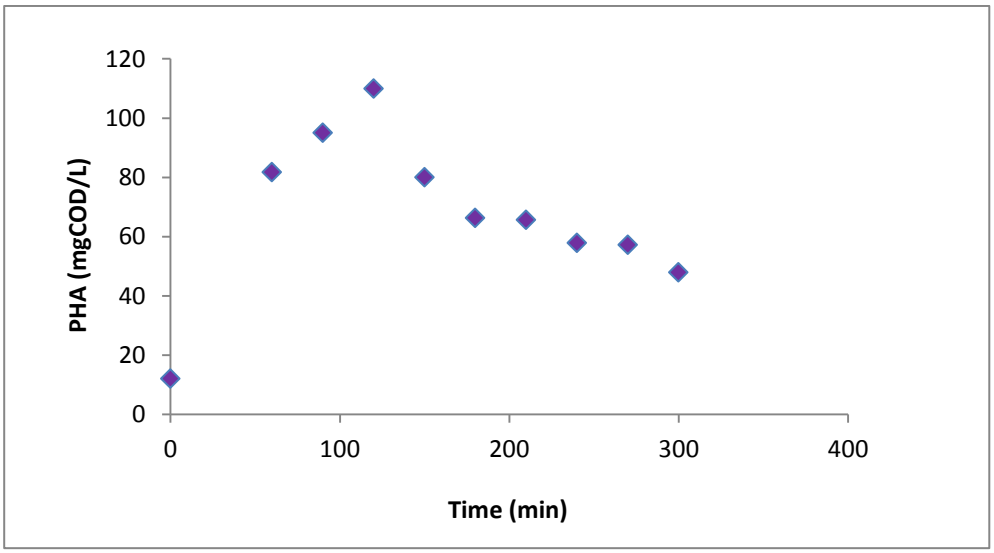

Figure 5.6: PHB profile of the control experiment (Run 1).

5.2.2 Evaluation of batch experiments

These experiments were conducted to determine the different F/M ratio effects on substrate storage on the activated sludge. For this purpose, after observing the response of biomass under steady state conditions in the control experiment, additional 5 runs were conducted under different F/M ratios. OUR measurements were started with same initial X_0 amount but after observation of the endogenous decay level in terms of straight line, the acetate was added to the systems in different concentrations to create different initial loading ratios (S_0/X_0).The OUR tests were conducted with 5 different F/M ratios; 1.80, 0.38, 0.22, 2.95 and 4.54 gCOD/gVSS.

The effect of loading rate 1.80 gCOD/gVSS was investigated in Figure 5.7. Run2 was started with an initial VSS concentration of 255 mg/L and 460 mg/l COD was fed to the system by pulse feeding after the endogenous decay level was observed. The OUR curve reaches a maximum level of 74mg/L.h in 140 minutes.

Addition of different concentration of acetate to the system changes the PHB storage amount. The stored amount of PHB was increased but the amount of acetate that used for storage is lower than the amount in the system fed with 250mgCOD/L. Figure 5.8 shows the stored PHB amount in the system fed with initial acetate concentration of 460mgCOD/L. The PHB concentration at the begging was 12mgCOD/L and it increases up to 143mgCOD/L in 150 minutes, with the storage of added substrate.

OUR profile of 105 mgCOD/L acetate feed was displayed in Figure 5.9. Run3 was started with an initial VSS concentration of 280mg/L. The F/M ratio for this system was 0.38gCOD/gVSS, lower than the control experiment Run1. The OUR curve reaches a maximum level of 44mg/L.h in 38 minutes. The substrate storage as PHB is shown in Figure 5.10. This loading rate had a positive effect on the percentage of acetate that converted to PHB. Even the amount of stored PHB is lower than the control experiment because the available substrate is low; the amount of acetate that is used for growth is lower than Run1 the control reactor that had a higher loading rate (0.98gCOD/gVSS). The PHB concentration at the begging was 10mgCOD/L and it increases up to 70mgCOD/L in 30 minutes, with the storage of added substrate.

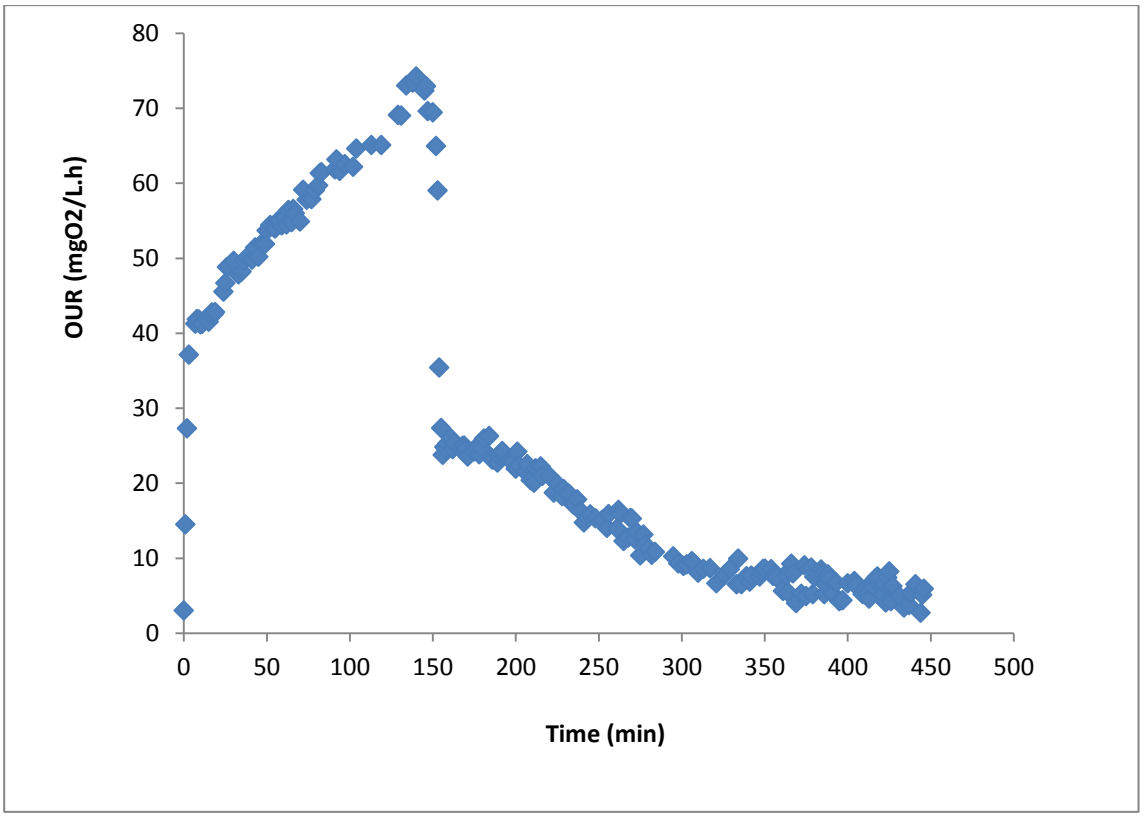

Figure 5.7: OUR profile of the experimental Run2.

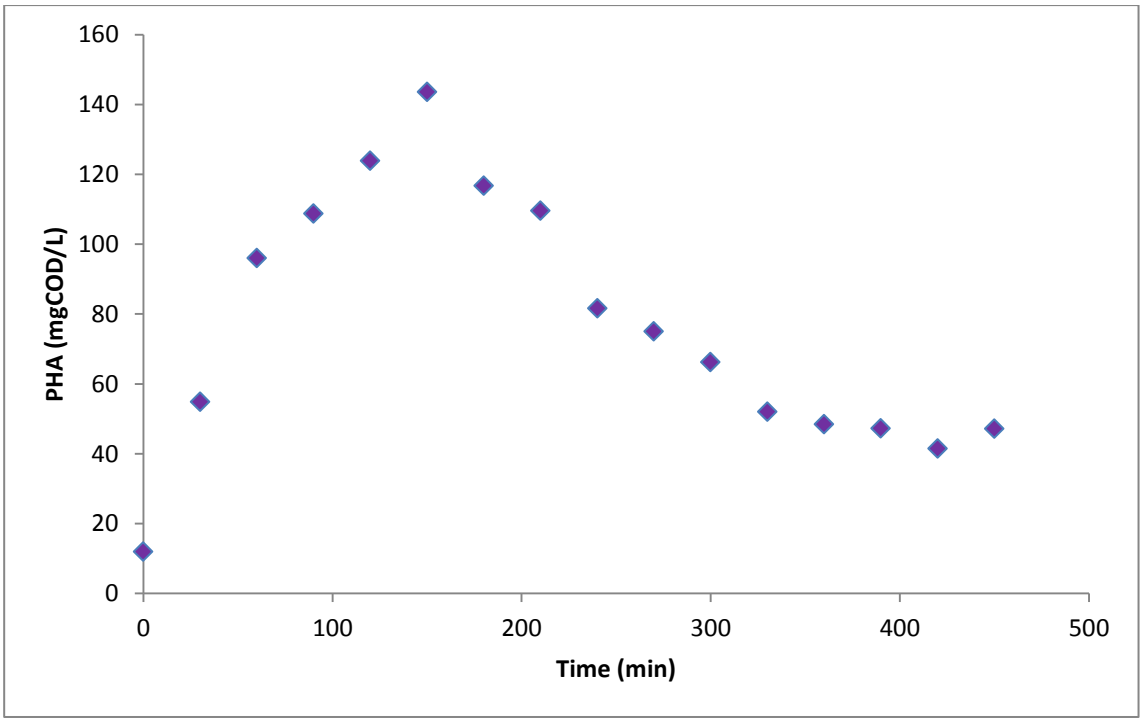

Figure 5.8: PHB profile of the experimental Run2.

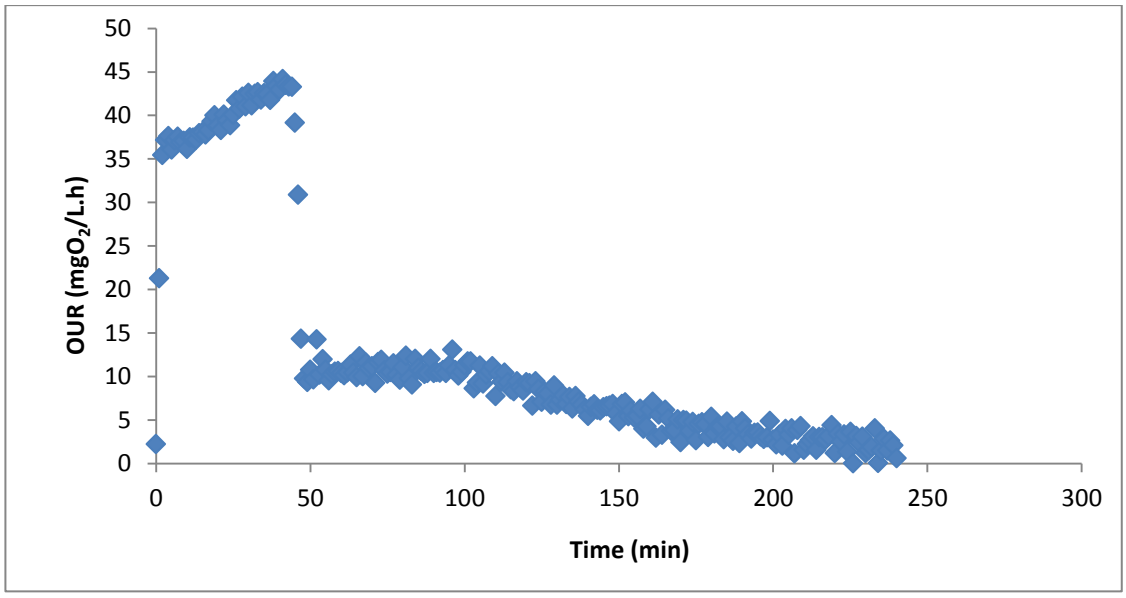

Figure 5.9: OUR profile of experiment Run3.

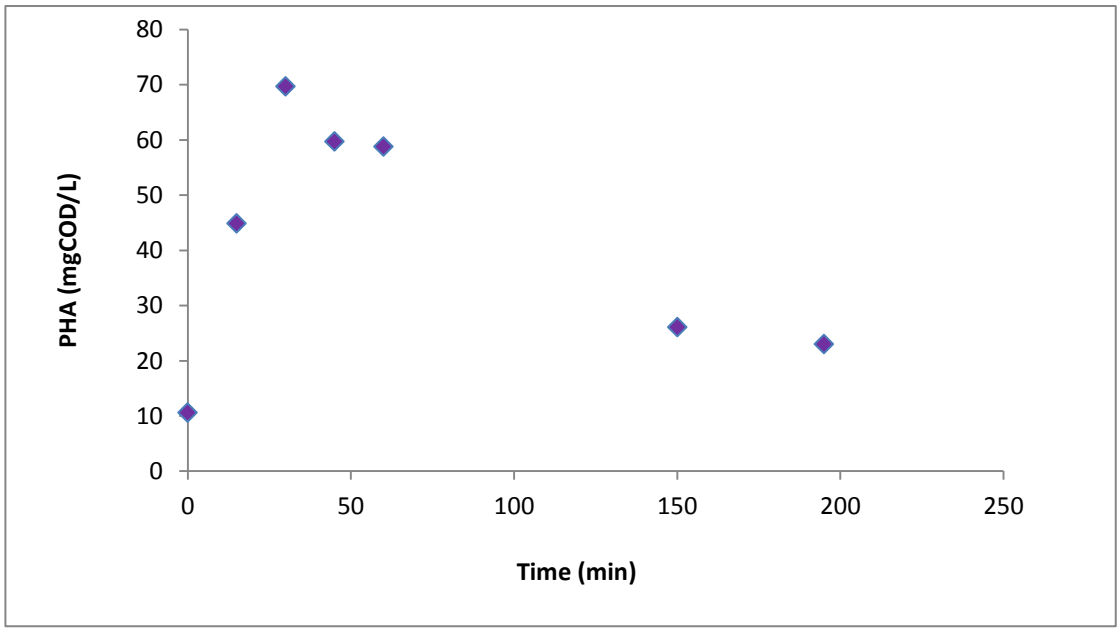

Figure 5.10: PHB profile of experiment Run3.

Loading rate of 0.22 gCOD/gVSS was tested in Run4 with an initial biomass concentration of 255mgVSS/L. Run4 was carried out addition of 57 mgCOD/L acetate to the system. The OUR curve of experiment is shown in Figure 5.11. The maximum point of the OUR curve is 36mg/L.h. This peak level was reached at 26 minutes. PHB storage amount is shown in Figure 5.12. The lower the F/M ratio from 0.38 to 0.22 increased the amount of acetate converted to PHB. The maximum PHB storage amount is 44mgCOD/L in the experiment Run 4 and observed at 15minutes.

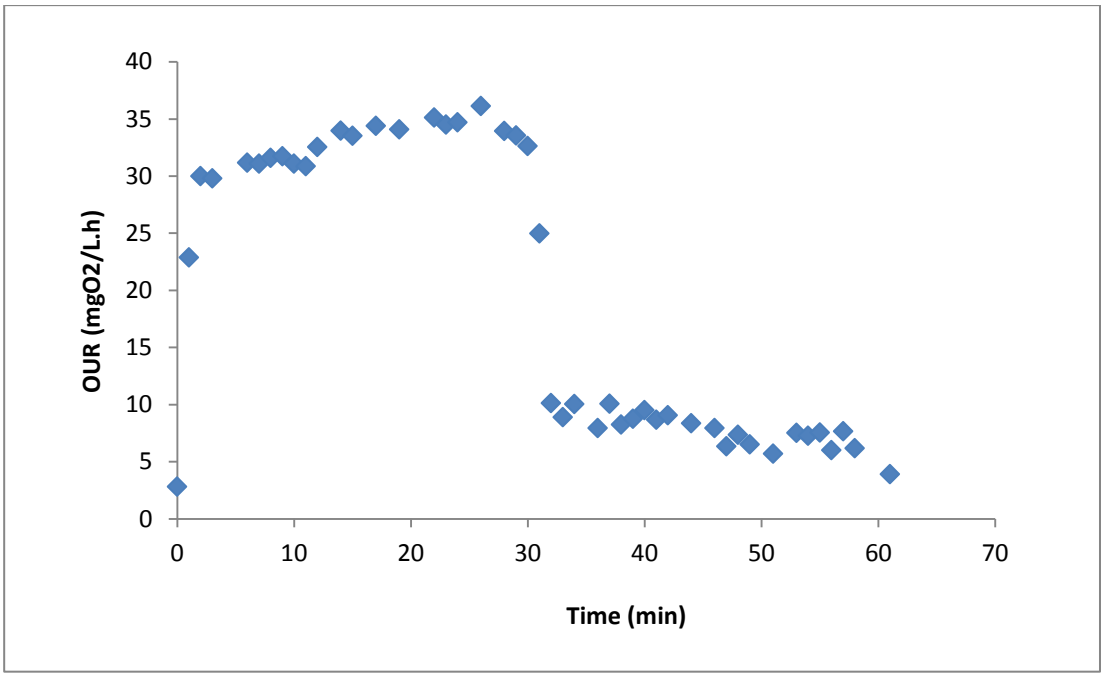

Figure 5.11: OUR profile of experiment Run4.

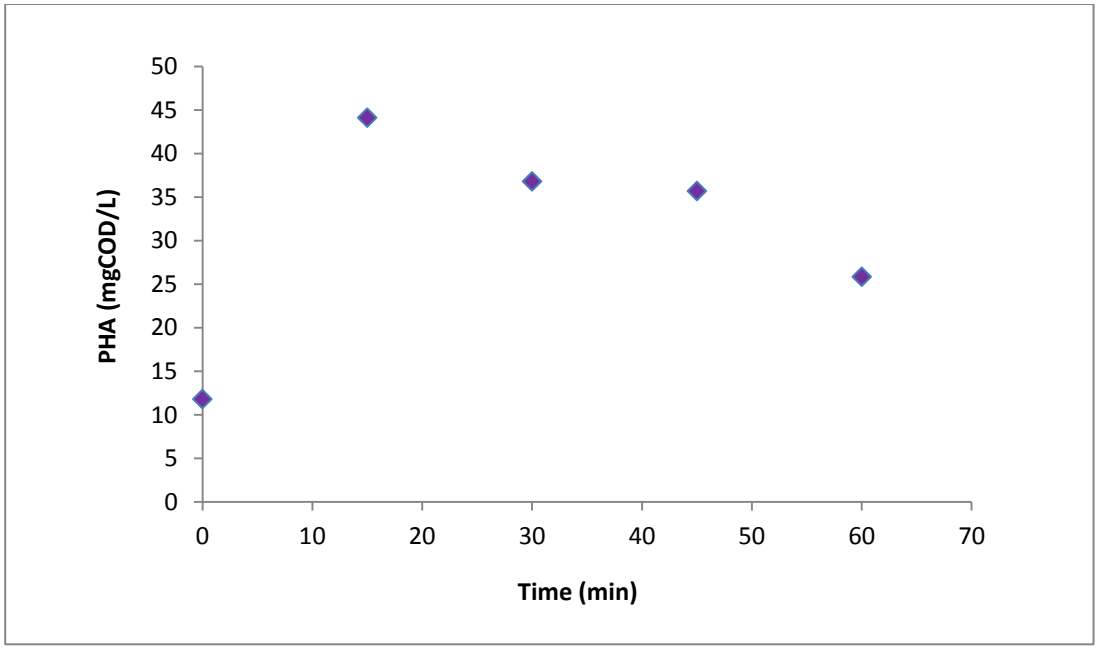

Figure 5.12: PHB profile of experiment Run4.

The effect of loading rate 2.95 gCOD/gVSS was investigated in Figure 5.13. Run5 was started with an initial VSS concentration of 220 mg/L and 650 mg/l COD was fed to the system by pulse feeding after the endogenous decay level was observed. The OUR curve reaches a maximum level of 64mg/L.h in 218 minutes. PHB storage amount of biomass at the F/M ratio of 2.95 gCOD/gVSS is shown in Figure 5.14.

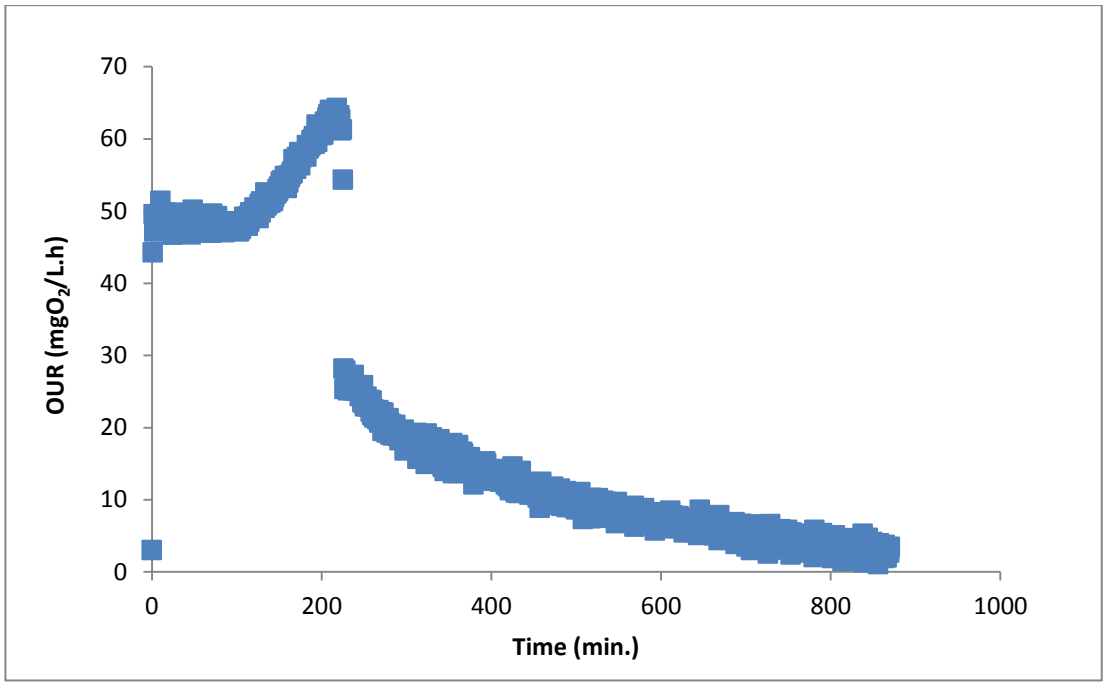

Figure 5.13: OUR profile of experiment Run5.

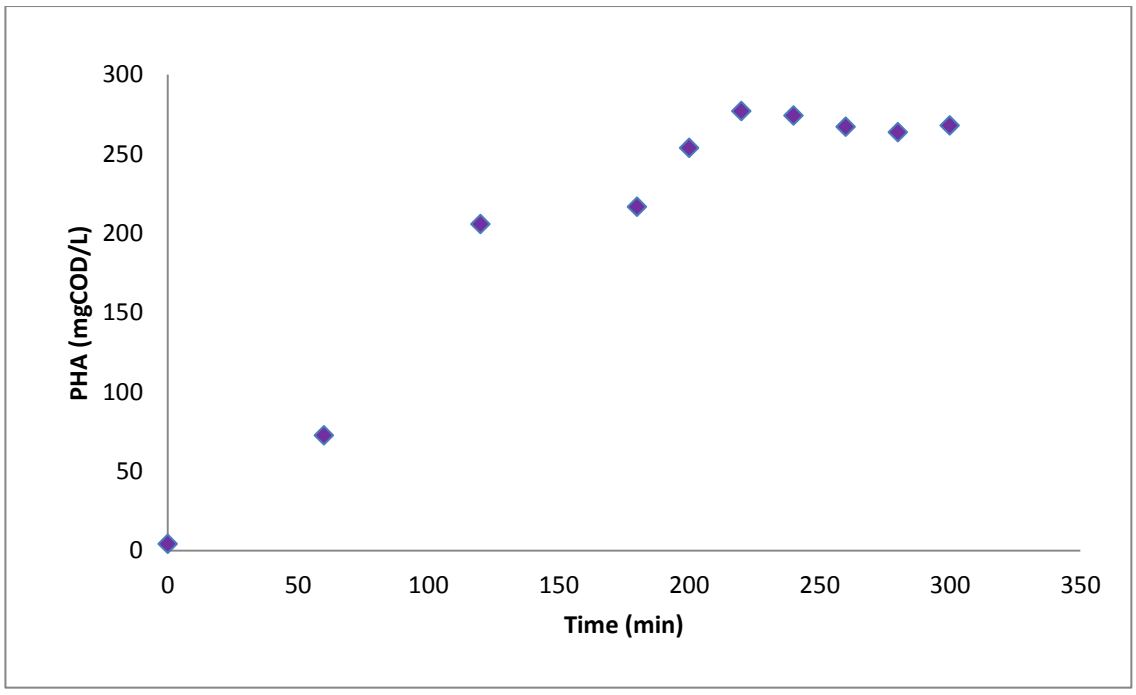

Figure 5.14: PHB profile of experiment Run5.

Loading rate of 4.54 gCOD/gVSS was tested in Run6 with an initial biomass concentration of 220mgVSS/L. Run4 was carried out addition of 1000mgCOD/L acetate to the system. The OUR curve of experiment is shown in Figure 5.15. The maximum point of the OUR curve is 36mg/L.h. This peak level was reached at 26 minutes. PHB storage amount is shown in Figure 5.16.

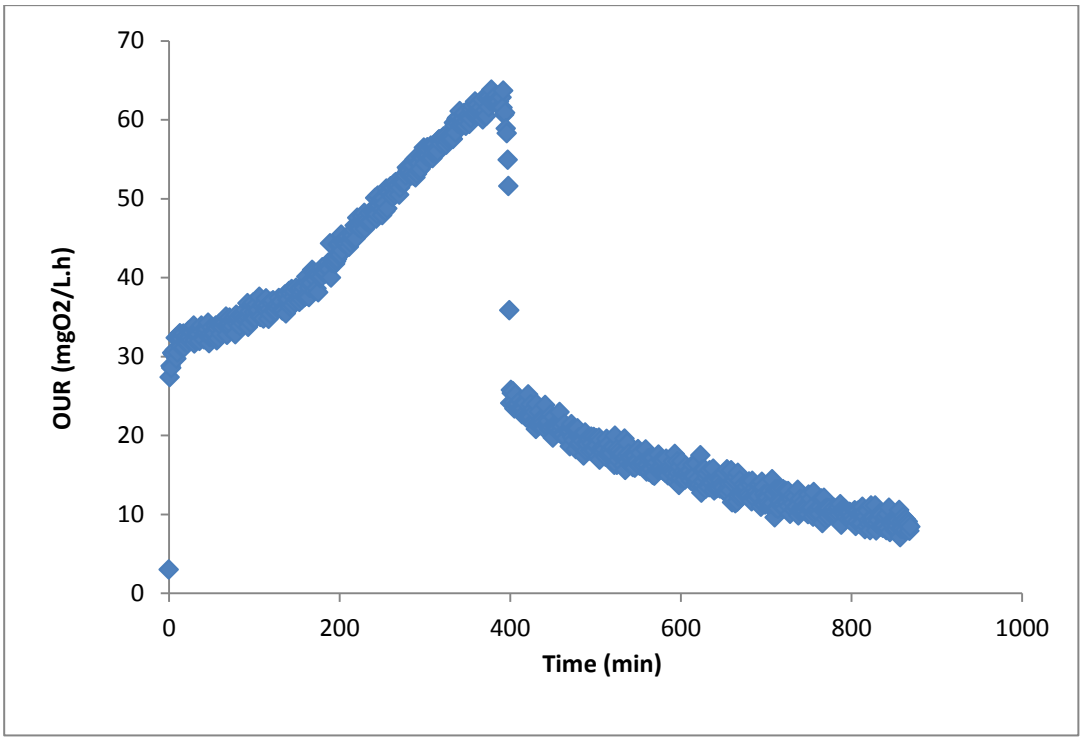

Figure 5.15: OUR profile of experiment Run6.

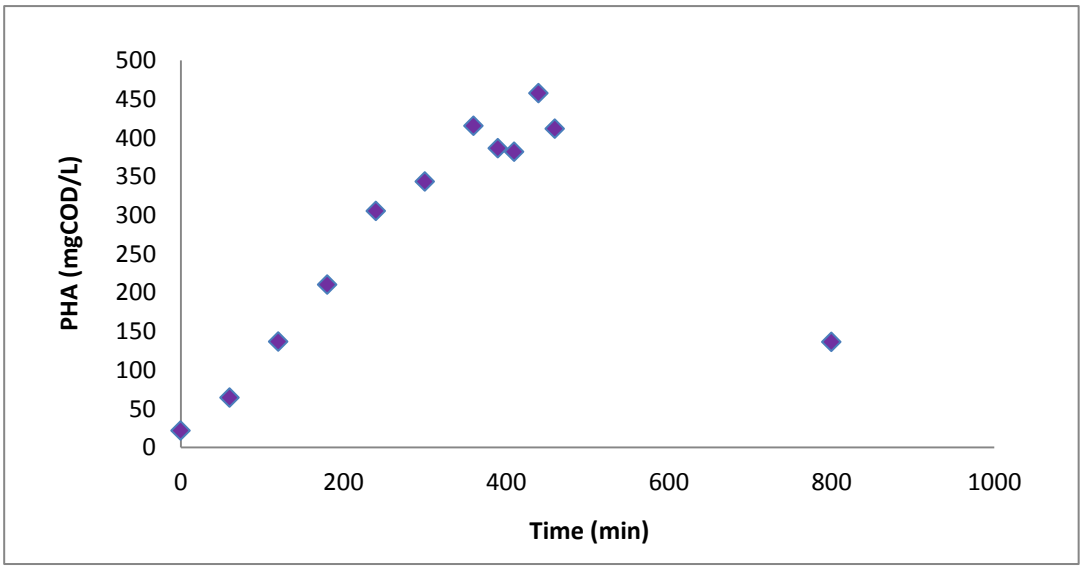

Figure 5.16: PHB profile of experiment Run6.

The summary of experimental results is shown in Table 5.2. Evaluated of the stored substrate as PHB (ΔPHB) and the PHB/AcCOD ratio in the experiments, ΔPHB decreased from 393mgCOD/L to 37mgCOD/L while PHB/AcCOD ratio increased 0.39mgCOD/mgCOD to 0.65mgCOD/mgCOD together with the reduction of F/M ratio.

Table 5.2 : Summary of experimental results.

RUNS	Initial Acetate COD	Initial Biomass	F/M	∆PHB	PHB/ AcCOD	Acetate to PHB	Acetate to Storage
	mg/L	mgVSS/L	gCOD/ gVSS	mgCOD/ L	mgCOD/ mgCOD	mgCOD/ L	%
Run 6	1000	220	4.54	393	0.39	491	49
Run 5	650	220	2.95	246	0.38	307.5	47
Run 2	460	255	1.80	172	0.37	215	47
Run 1 (control)	250	255	0.98	126	0.50	157.5	63
Run 3	105	280	0.38	66	0.63	82.5	79
Run 4	57	255	0.22	37	0.65	46	81

Beun et al. (2000) investigated storage in a SBR system involving pulse feeding of acetate at three different sludge ages (Θx). For Θx of 3 days PHB/acetate ratio was 0.46 mgCOD/mgCOD and it increased to 0.69 mgCOD/mgCOD for 10 days and 0.70 mgCOD/mgCOD for 20 days. Majone et al., (1996) has demonstrated that the feeding regime had a significant influence on the magnitude of storage. They conducted batch experiments using two parallel CSTR systems sustained at a sludge age of 3 days, one with continuous and the other with intermittent feeding , While the PHB/acetate ratio was only 0.37 mgCOD/mgCOD for continuous CSTR biomass, it went up to 0.78 mgCOD/mgCOD for the intermittent CSTR. Beun et al. (2001) performed a study with an SBR under pulse feeding of acetate at Θx of 4 days and found a PHB /acetate ratio of 0.7mgCOD/mgCOD that confirming the previous results. They also investigate the fate of PHB storage under pulse feeding of 8 times higher acetate addition. This time the PHB/acetate ratio was increased to 0.76mgCOD/mgCOD. Carta et al (2001) run an experiment with five time higher feeding than the control experiment. They found out that the PHB/acetate ratio was increased to 0.79 mgCOD/mgCOD. According to these results, it is clear to say that the observations of this study are not similar to the ones in literature. In this study,

when the biomass faces with higher concentrations of substrate feeding than the study state conditions, it slows down the storage mechanism and increases the growth rate.

5.3 Modelling of Experimental Results

Modeling is now regarded as a useful instrument for understanding and interpreting biodegradation and related mechanisms of substrate utilization. Recent models structured for this purpose operate with two major conceptual developments. (i) they use chemical oxygen demand (COD) and the organic carbon parameter and include COD fractions with different biodegradation characteristics based on the pioneering works of Dold et al. (1980) and Ekama et al. (1986); (ii) they incorporate dissolved oxygen concentration (SO) as a significant model component which allow to calibrate and evaluate oxygen uptake rate profiles (Cokgor et al, 2011).

Modeling consist of analyzing and calibrating the OUR and PHB profiles. In this content, a mechanistic model involving model components and kinetic and stoichiometric parameters was developed for acetate utilization. Modified ASM3 model was used for this study. The model structure involved four model components, namely readily biodegradable COD, Ss; active heterotrophic biomass, X_H; storage products, X_{STO} and dissolved oxygen, So. Generation of residual microbial products (Xp) as a decay-associated process is implicitly involved in the model by means of the coefficient fp that acts as a correcting factor for oxygen consumption due to endogenous respiration. The model structure consists of four microbial processes: growth on Ss; growth on stored PHB and decay of X_H by means of endogenous respiration (Orhon et al, 2009). The growth process associated with X_H was defined as conventionally adopted Monod type equations. Both endogenous respiration mechanisms were described as first-degree reactions with respect to biomass concentrations. The storage process was defined in terms of a similar Monod type of an expression where k_{STO} denotes the maximum storage rate (Karahan et al, 2006). The matrix representation of modified ASM3 structure was indicated in Table 5.3.

Table 5.3:Matrix representation of modified ASM3 model structure.

Component / **Process**	**S_{O2}**	**S_S**	**X_H**	**X_{STO}**	**X_P**	**Process rate**
Storage of PHA	-(1-Y_{STO})	-1		Y_{STO}		$k_{STO} \cdot \frac{S_S}{K_S + S_S} \cdot X_H$
Growth of X_H	$-\frac{1-Y_H}{Y_H}$	$-\frac{1}{Y_H}$	1			$\mu_H \cdot \frac{S_S}{K_S + S_S} \cdot X_H$
Growth on PHA	$-\frac{1-Y_H}{Y_H}$		1	$-\frac{1}{Y_H}$		$\mu_{STO} \cdot \frac{X_{STO}}{K_{STO} + X_{STO}} \cdot X_H$
Decay of X_H	-(1- f_P)		-1		f_P	$b_{H..}X_H$

Kinetic and stoichiometric parameters for the modified ASM3 model were estimated using the AQUASIM simulation program (Reichert et al, 1998). In addition, modeling study was also performed for parameter estimation. The parameter estimation study provided a basis for observing the change in the kinetic parameters of activated sludge while adapting to different conditions (Insel et al, 2012).

Model simulation results for OUR profile and PHB data of control experiment (Run1) were shown in Figure 5.17 and Figure 5.18, respectively. The initial biomass concentration was determined as 255mgVSS/L and acetate concentration was 250mgCOD/L. The F/M ratio was 0.98mgCOD/mgVSS. The calibration of initial endogenous respiration part of the OUR curve yielded an endogenous decay coefficient, b_H of 0.22day^{-1}. According to simulation results, the model was calibrated with a maximum specific growth rate, μ_{Hmax} of 2day^{-1}. The model calibration was performed with the assumption of yield coefficient, Y_H of 0.66 mgcellCOD/mgCOD and a storage yield coefficient, Y_{STO} of 0.80 mgcellCOD/mgCOD (Yavasbay, 2010). The storage yield coefficient, Y_{STO} of 0.80 mgcellCOD/mgCOD was used in the studies of Insel et al. (2012), Cokgor et.al (2011), Çığgın et al. (2012), and Karahan-Gül et. al (2003). The coefficient of residual products generation, f_P was selected as 0.20 in the all experiments (Orhon et al. 2009).

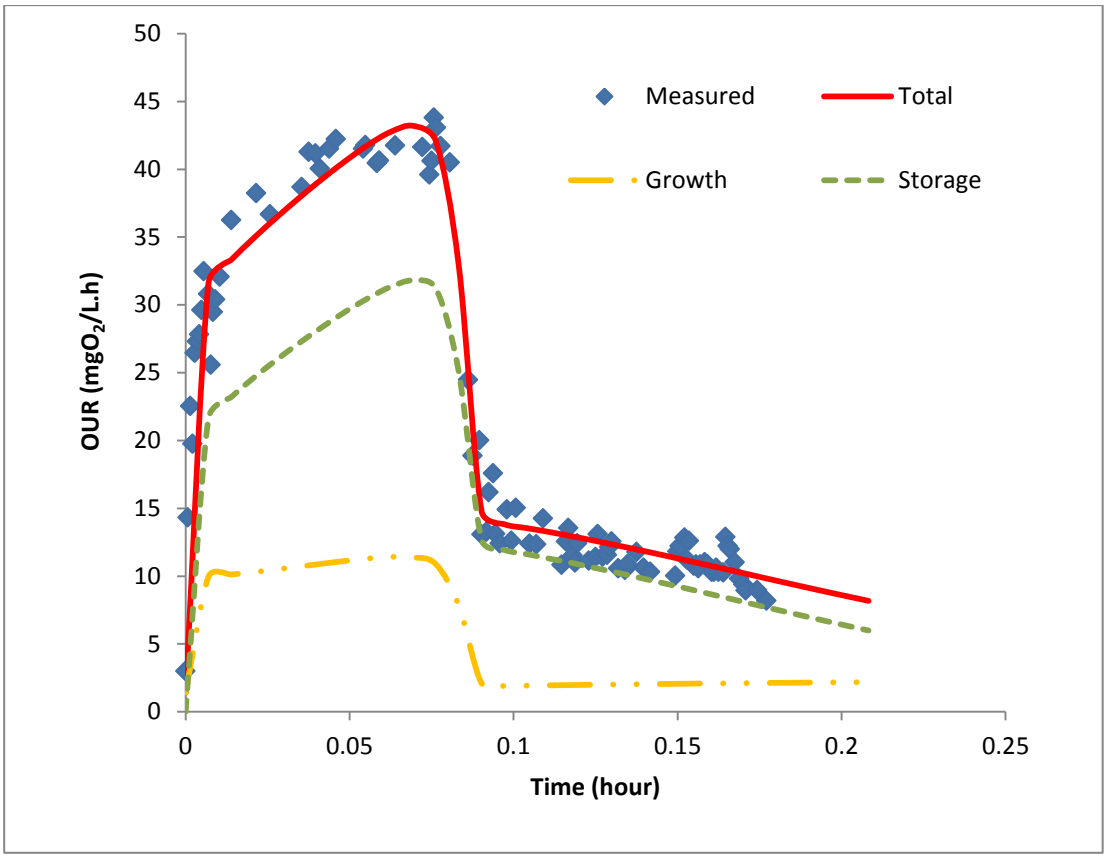

Figure 5.17: Model simulation of OUR profile for Run1.

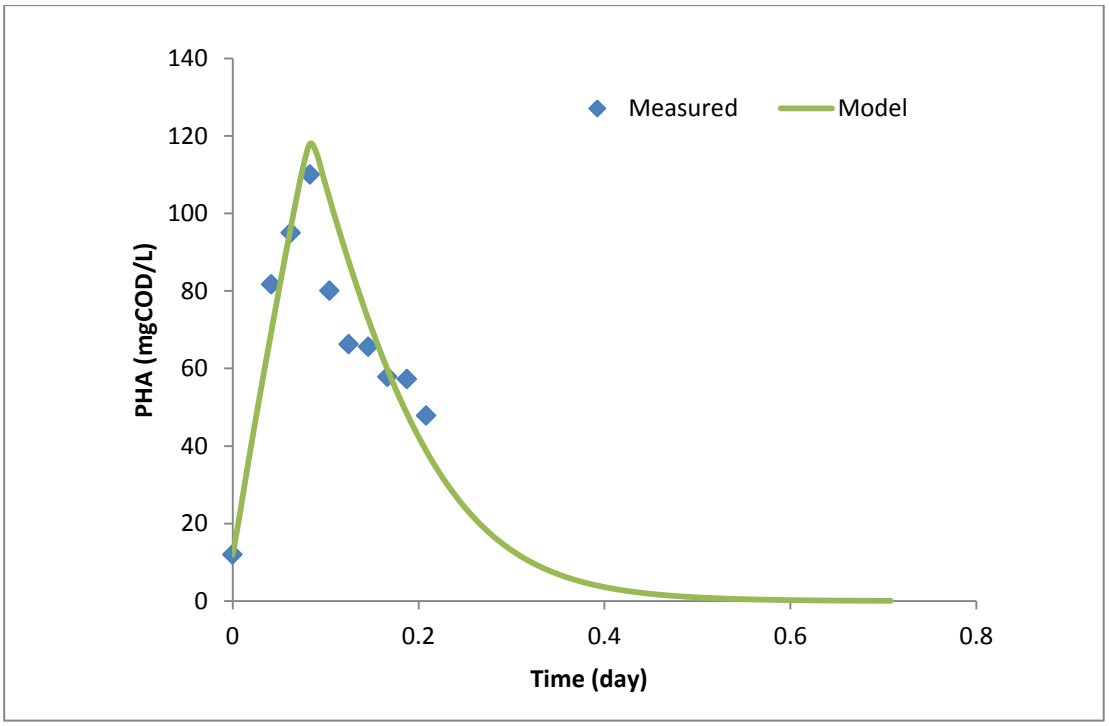

Figure 5.18: Model simulation of PHB data for Run1.

Model simulation results showed that the half saturation constant for growth (Ks) was found as 5mgCOD/L with the F/M ratio of 0.98gCOD/gVSS under 2 days of sludge age. In another study conducted by Insel et al. (2012), the Ks value was decreased from 25 to 6mg COD/L with the increasing the sludge age (SRT) from 2 to 10 days. Cığgın et al. (2012) stated that the reducing the sludge age from 8 to 2 days increased the half saturation constant for growth (Ks) from 5 to 10mg COD/L. In another study, this value was found 5mgCOD/L by Karahan et al (2008).

Simulation results reflected the PHA storage, yielding with a k_{STO} value of $11day^{-1}$. Almost similar results were found in another study conducted by Krishna and van Loosdrecht (1999) with a SBR system of 6 cycles under the sludge age of 2.5 days. They found k_{STO} of $10day^{-1}$, both for ASM3 and for modified ASM3 models. For all experimental runs, the half saturation constant for storage, K_{STO} was obtained as 0.54mgCOD/L. This value was nearly similar to the results that observed in another study done by Çığgın et al. (2012). They found the K_{STO} value of 0.5mgCOD/L.

After control experiment's simulation, three different experimental runs were conducted to observe the effect of F/M ratio on storage mechanism's kinetic and stoichiometric parameters. Model simulation was applied to F/M ratios of 1.80, 0.38, and 0.22. Model simulation results of OUR profile and PHA data for Run 2 (F/M ratio of 1.80gCOD/gVSS) was shown in Figure 5.19 and Figure 5.20, respectively. According to Run 2, half saturation constant of growth and half saturation constant for storage were not change and the values were 5mg COD/L and 0.54mg COD/L, respectively. In addition, the maximum growth rate on storage products, μ_{STO} of 4.8 day^{-1} and the maximum decay rate, b_H of 0.22 day^{-1} were remain constant. On the other hand, the maximum specific growth rate on external substrate, μ_H was 4.6 day^{-1} whereas it was 2.0 day^{-1}in the control experiment. In the Run1, the substrate storage rate, k_{STO} was also changed. It was decreased from $11day^{-1}$ to 9 day^{-1} while F/M ratio increased.

Model simulation results of OUR profile and PHB data for Run 3 (F/M ratio 0.38gCOD/gVSS) were displayed in Figure 5.21 and Figure 5.22, respectively. Run 3 modeling results showed that K_S, K_{STO}, μ_{STO}, μ_H and b_H values were remain as same in the control experiment. Only the maximum substrate storage rate, k_{STO} was changed with the effect of low F/M ratio than control experiment. The k_{STO} value was measured as $14day^{-1}$ that was higher than the control experiment k_{STO} of $11day^{-1}$.

The maximum growth rate of biomass was remain constant with the value of 2.0day^{-1} according to control experiment but it was different from the Run 1 μ_{STO} of 4.6day^{-1}.

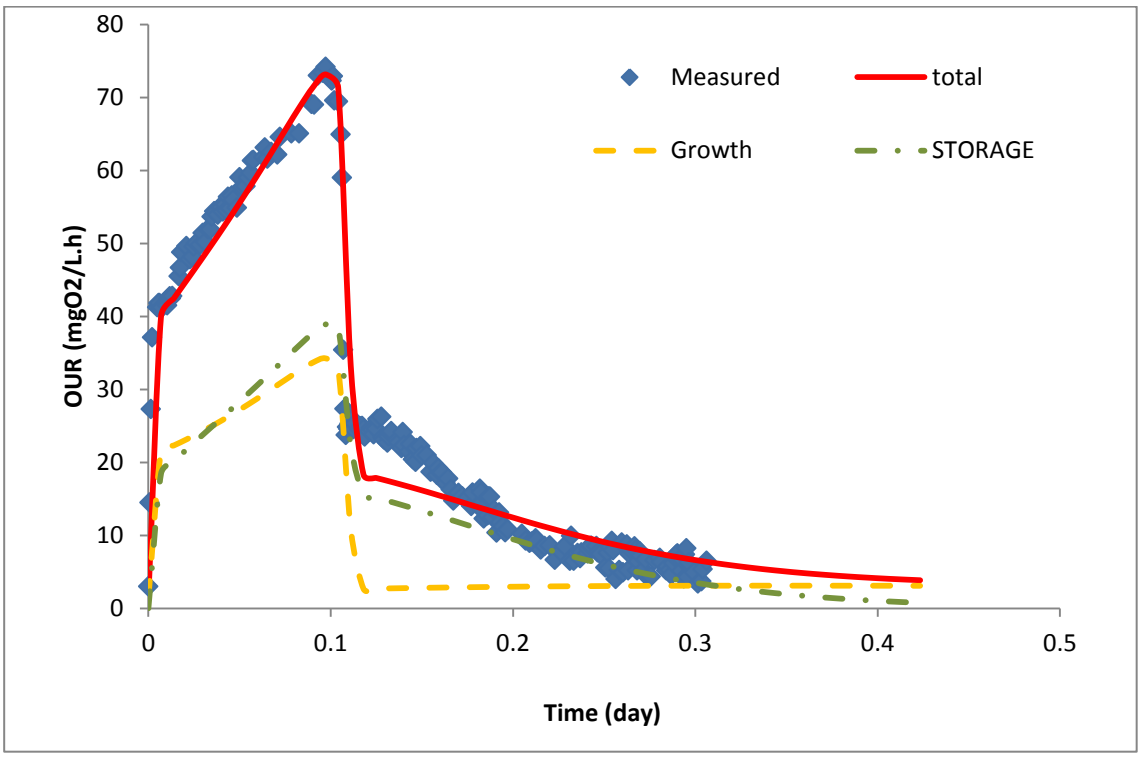

Figure 5.19: Model simulation of OUR profile for Run2.

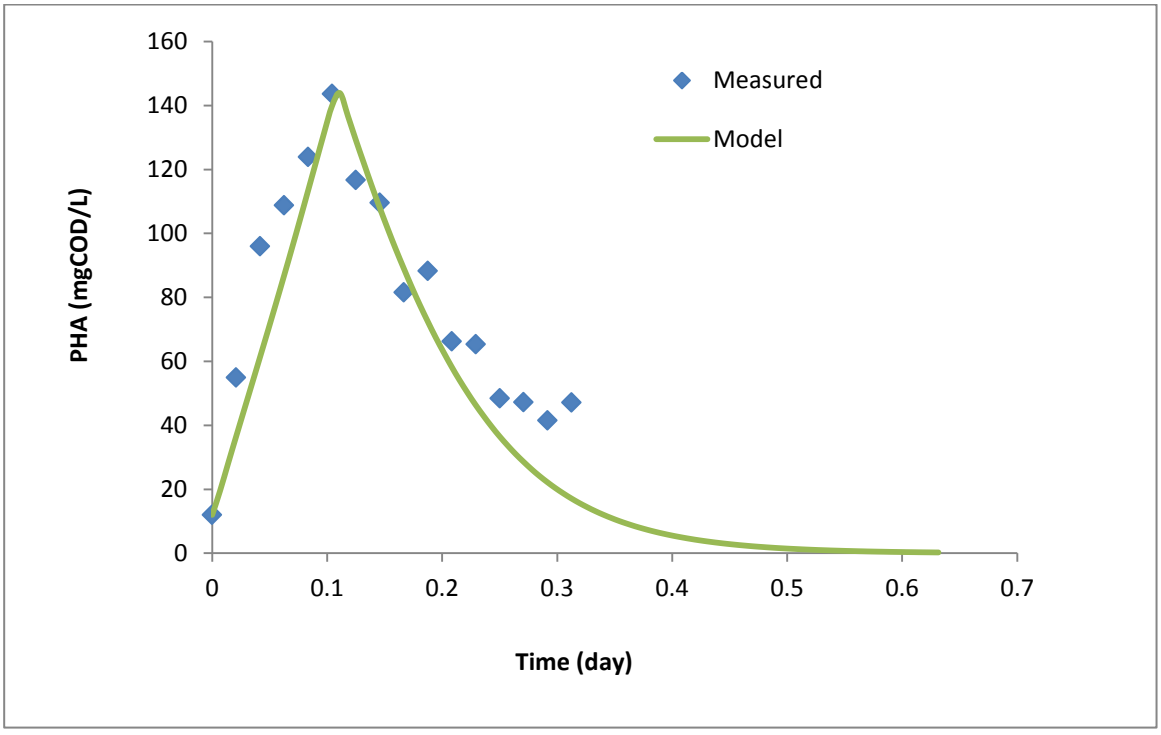

Figure 5.20: Model simulation of PHB data for Run2.

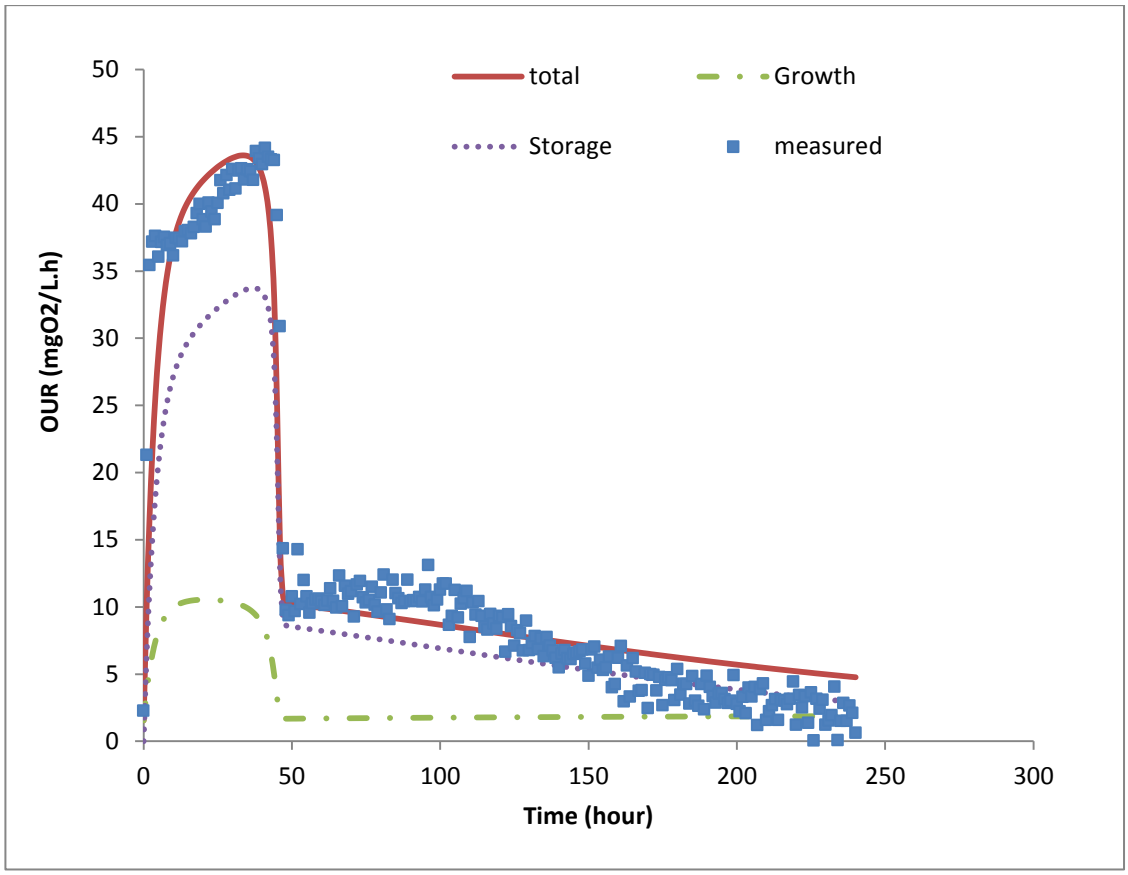

Figure 5.21: Model simulation of OUR profile for Run3.

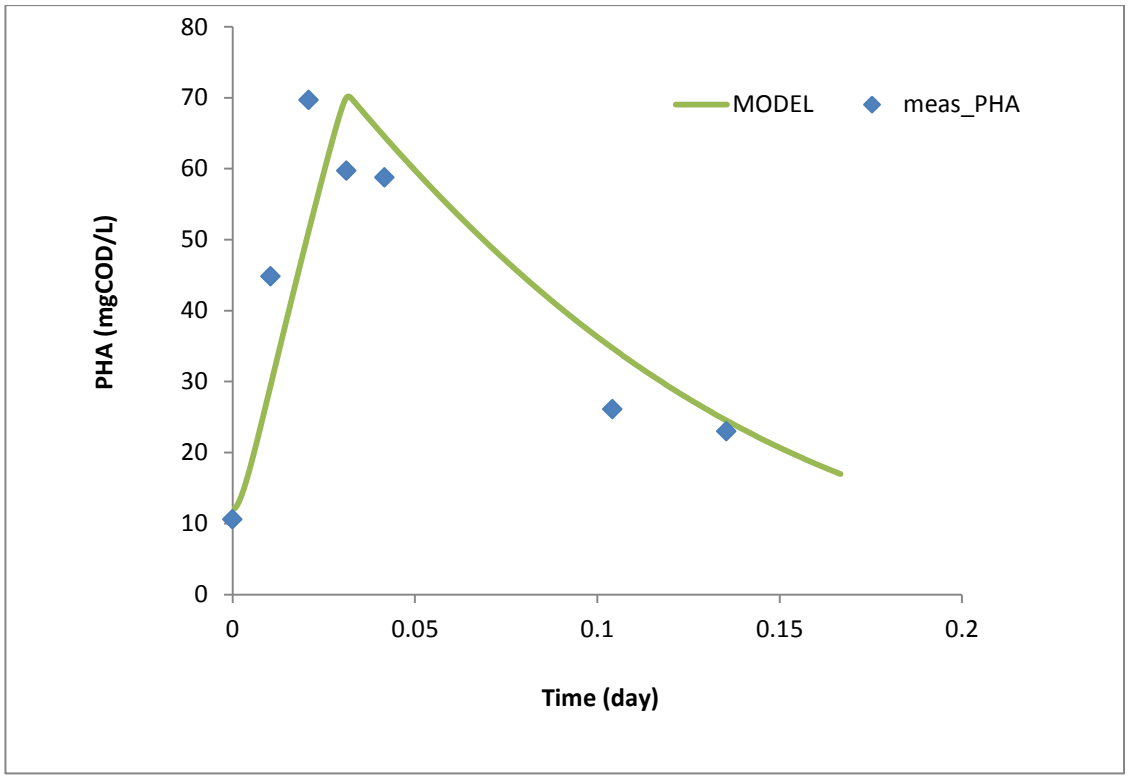

Figure 5.22: Model simulation of PHB data for Run3.

Figure 5.23 and Figure 5.24 were illustrated the model simulation results of OUR profile and PHB data for Run 4. In the Run 4 (F/M ratio 0.22) again K_S, K_{STO}, μ_{STO}, μ_H and b_H values were remain as same in the control experiment. Only the maximum substrate storage rate, k_{STO} was changed with the effect of low F/M ratio than control experiment. On the other hand as it observed also in Run 3 the K_{STO} value was increased with the decreased in F/M ratio. It increased up to 14 day^{-1} like in the Run3.

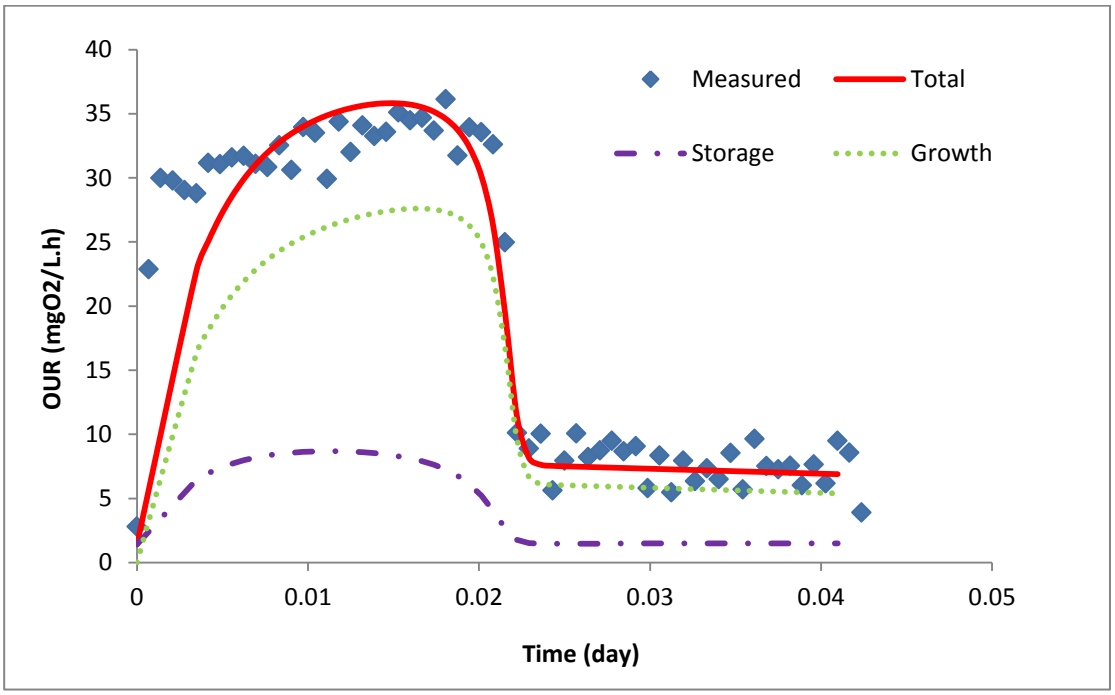

Figure 5.23: Model simulation of OUR profile for Run4.

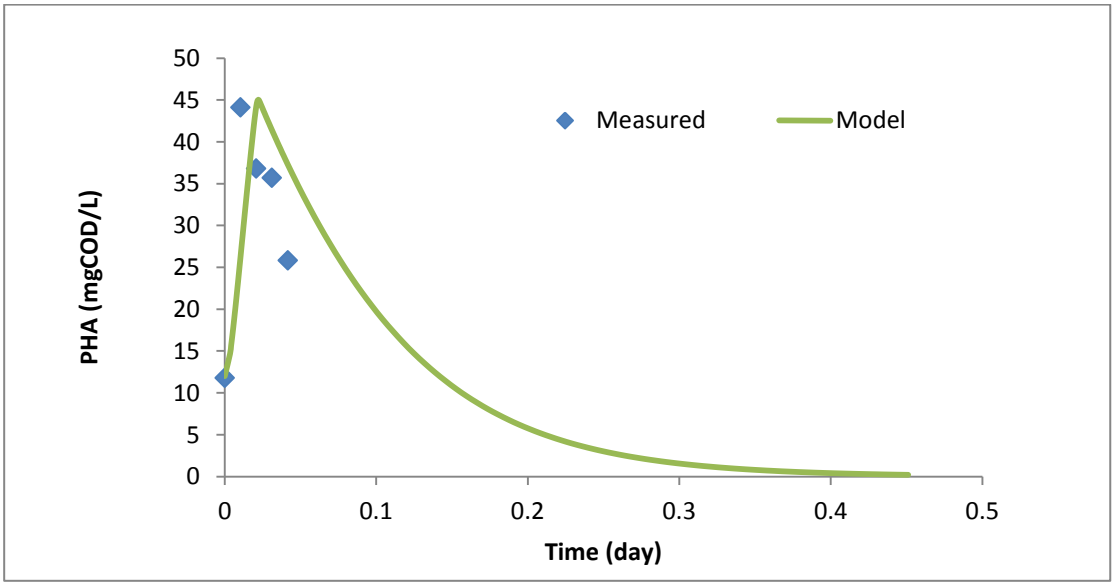

Figure 5.24: Model simulation of PHB data for Run4.

Table 5.4: Results of model calibration for the different S_0/X_0 ratios experiments.

Model Parameter	**Unit**	**S_0/X_0 (gCOD/gVSS)**			
		Run 1	Run 2	Run 3	Run 4
		1.80	0.98	0.38	0.22
$\hat{\mu}_{H1}$	1/day	4.6	2.0	2.0	2.0
K_{S1}	mg COD/L	5	5	5	5
k_{STO}	1/day	9	11	14	14
$\hat{\mu}_{STO}$	1/day	4.8	4.8	4.8	4.8
K_{STO}	mg COD/L	0.54	0.54	0.54	0.54
b_H	1/day	0.22	0.22	0.22	0.22
Y_H	g COD/g COD	0.66	0.66	0.66	0.66
Y_{STO}	g COD/g COD	0.8	0.8	0.8	0.8
f_{ES}	-	0.08	0.08	0.08	0.08
f_{EX}	-	0.15	0.15	0.15	0.15
State variables	**Units**				
Total biomass	mg VSS/L	255	255	280	255
X_{H1}	mg COD/L	200	200	220	200
Activity	%	55	55	55	55
X_{STO}	mg COD/L	12	12	12	10
S_{S1}	mg COD/L	460	250	105	57

The model simulation results based on modified ASM3 were given in Table 5.4 in details. Evaluated of the maximum specific growth rate (μ_{H1}) and the maximum storage rate of substrate (k_{STO}) in the experiments, μ_{H1} decreased from 4.6 day^{-1} to 2 day^{-1} while k_{STO} increased 9 day^{-1} to 11 day^{-1} and then 14 day^{-1} together with the reduction of F/M ratio. Insel et al (2012), observed the similar results like this study. They worked with low sludge age (SRT of 2days) and lower the F/M ratio from 0.71gCOD/gVSS to 0.49gCOD/gVSS; μ_{H1} decreased from 7.1 day^{-1} to 6.8 day^{-1} while k_{STO} increased 4.2 day^{-1} to 6.3 day^{-1} and then 14 day^{-1}. According to Grady et al. (1996) the maximum specific growth rate (μ_{H1}), is closely related to the r-RNA level of the cell as it controls the protein synthesis mechanism. As related to this subject, Insel et al. (2012) argued that the biomass is able to increase its primary

growth metabolism and reduce its maximum storage rate when it is subjected to temporal feast conditions (high substrate concentration). In addition, the culture history (sludge age, dilution rate etc.) which regulates the biochemical mechanisms within the cell is likely to effect the growth kinetics (Orhon et al, 2009). Furthermore, a recent study has shown that substrate utilization highly influenced by the total enzyme level, which regulates the protein synthesis and substrate transforming, and the r-RNA level (Lavallée et al, 2005).

6. CONCULUSION

The objective of this study was to evaluate the effect of substrate loading on the generation of storage biopolymers. For this purpose, this research focused on PHB that represent the storage polymer. A faster growing microbial community and a low sludge were selected to better evaluate the growth requirements of the community compared with simultaneous storage. Then a series of batch experiments were conducted with biomass taken from the fill and draw reactor and therefore acclimated to fast growth conditions. Six different initial substrate concentrations were applied to same initial biomass concentration for the investigation of the effect of F/M ratio on substrate storage in SBR. Respirometric tests were also performed for the assessment of the substrate biodegradation and PHB storage behavior of the biomass.

The maximum specific growth rate (μ_{H1}) decreased from 4.6 day^{-1} to 2 day^{-1} and the maximum storage rate of substrate (k_{STO}) increased 9 day^{-1} to 11 day^{-1} and then 14 day^{-1} in the experiments with the reduction of F/M ratio. In addition, other kinetic and stoichiometric parameters remain constant.

The stored substrate as PHB (ΔPHB) decreased from 393mgCOD/L to 37mgCOD/L while PHB/AcCOD ratio increased 0.39mgCOD/mgCOD to 0.65mgCOD/mgCOD together with the reduction of F/M ratio. The estimated results were not similar with the observed results in the literature. Beun et al. (2000) investigated storage in a SBR system involving pulse feeding of acetate at three different sludge ages (Θx). PHB/acetate ratio increased from 0.46mgCOD/mgCOD to 0.69 mgCOD/mgCOD and then 0.70 mgCOD/mgCOD for increasing Θx values. Carta et al (2001) run an experiment with five time higher feeding than the control experiment. They found out that the PHB/acetate ratio was increased to 0.79 mgCOD/mgCOD.

Experimental findings in this study have shown that the ratio of initial substrate to biomass (S_0/X_0) was the key parameter for respirometric experiments directly affecting the shape and the order of magnitude of the respirometric profile. The experiments also shown that, the biomass is able to switch its substrate utilization

mechanism to growth or storage when it is subjected to unsteady feast conditions. The fast growing systems (low sludge age) gives much faster response than that of high sludge age. The results of this study indicates that, when the biomass face with higher concentrations of substrate feeding than the study state conditions, it slows down the storage mechanism and increases the growth rate. The enzymatic levels controlling growth and storage kinetics could be the reason of the biomass response in activated sludge systems.

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