EFFECTS OF DIFFERENT LOADING RATES ON SUBSTRATE STORAGE PHENOMENA

M.Sc. Thesis by
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Department: Environmental Engineering
Programme: Environmental Biotechnology

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HAZİRAN 2005
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EROL ÇAVUŞ
3.5.1 Conceptual basis

4. RESULTS and DISCUSSIONS

4.1 Reactor Analysis

4.2 Respirometric Results and Evaluation

5. CONCLUSION

REFERENCES

CURRICULUM VITAE
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Representative Matrix of Activated Sludge Model No.3</td>
<td>13</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Operation phases of working SBR</td>
<td>24</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Operating parameters of SBR</td>
<td>24</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Stock Macro and Micro Nutrients</td>
<td>25</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>Stock solution of feeding COD</td>
<td>25</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Result of Monitoring the Steady State in SBR systems having 4 cycles in a day</td>
<td>29</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Experimental conditions</td>
<td>33</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Modeling Results</td>
<td>36</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Page No

Figure 2.1: The metabolic events taking place inside the cell ......................... 22
Figure 2.2: Proposed Models for storage..................................................... 8
Figure 2.3: Schematic representation of glycogen ranching............................... 10
Figure 2.4: The metabolic model of glycogen metabolism with its six internal reactions .............................................................................................. 21
Figure 2.5: Representation of a conventional activated sludge system for carbon removal using ASM3 components. ........................................... 12
Figure 3.1: A simple illustration of SBR............................................................ 23
Figure 3.2: COD Stoichiometry of aerobic storage in ASM3 ............................. 28
Figure 4.1: Monitoring the Suspended Solids (SS) and Volatile Suspended Solids ................................................................................................. 30
Figure 4.2: Monitoring the Effluent COD parameters........................................... 31
Figure 4.3: In-cycle observation in the SBR system............................................. 32
Figure 4.4: Respirometric OUR profile (Run)...................................................... 33
Figure 4.5: COD, glycogen and glucose concentrations changing with time (Run1) ................................................................................................. 35
Figure 4.6: COD and Efficiency analysis during the batch test for Run1.............. 35
Figure 4.7 OUR profiles of the experimental Runs in batch tests....................... 35
Figure 4.9: Modeling of Batch system of F/M=1................................................... 37
Figure 4.10 Modeling all Runs according to ASM3 .......................................... 38
NOTATION LIST

Ks : Half saturation constant of substrate [ML\(^{-1}\)]
Ss : Readily biodegradable substrate [MCODL\(^{-1}\)]
X_H : Heterotrophic biomass [ML\(^{-1}\)]
\(\mu_H\) : Specific growth rate [T\(^{-1}\)]
OUR : Oxygen uptake [ML\(^{-1}\) T\(^{-1}\)]
b_H : Endogenous respiration rate[ML\(^{-1}\)]
COD : Chemical oxygen demand [ML\(^{-1}\)]
F/M Ratio : Food to microorganism ratio
Y_H : Growth yield [MCODMCOD\(^{-1}\)]
MLVSS : Mixed liquor volatile suspended solids
MLSS : Mixed liquor suspended solids
Y_STO : Storage yield [MCODMCOD\(^{-1}\)]
GC : Gas chromatograph
HPLS : High performance liquid chromatograph
DEĞİŞİK YÜKLEME ORANLARININ SUBSTRAT DEPOLAMA MEKANİZMASINA OLAN ETKİSİ

ÖZET

Günümüzde kadar aktif çamur sistemlerinin depolama metabolizması detaylı olarak açıklanamamıştır. Depolama prosesinin net olarak anlaşılabilmesi ve elde edilecek bilgilerin atıksu arıtma tesislerinin tasarımının optimizasyonunda kullanılabilmesi için, depolama prosesinin farklı metabolik koşullarda incelenmesi gerekmektedir.

EFFECTS OF DIFFERENT LOADING RATES ON SUBSTRATE STORAGE PHENOMENA

SUMMARY

So far, the storage metabolism of activated sludge systems has not yet been explained in detail. In order to understand the storage process clearly and to use the information obtained for the optimization of wastewater treatment plant design, it is necessary to investigate the storage process under different metabolic conditions.

The aim of the study is to improve the understanding of biochemical storage process in activated sludge systems. In this respect, the effects of the important parameters of biochemical storage, which is different feeding patterns, on this process will be investigated in detail. For this aim, Sequencing Batch Reactor type has been selected for system operations. The aim covers microbial behaviors of the activated sludge and measurements of storage molecules at different F/M ratios in the present study. The respirometric measurements were executed and these measurement data were simulated with the ASM3. Coefficient estimations were obtained from simulated ASM 3. The coefficient results were compared with the literature values. A better-understood storage process will improve the biological treatment efficiency and form a basis for plant-scaled applications.
1. INTRODUCTION

1.1 The Aim of the Study

The sewage originated from industrial and domestic use of water is undesired wastewater that consist of harmful and pathogenic microorganisms, carbohydrates, fatty acids, amino acids, volatile fatty acids, detergents, amino sugars etc. Additionally wastewaters also contain nutrients like, nitrogen, phosphorus, sulfur etc. For this reason wastewater should be treated before to be given in to receiving body.

Among the wastewater systems designed for wastewater treatment, biological activated sludge systems are attributed much of the success. First of all, these systems are easy to manage. Moreover these systems are cheaper and have a good efficiency that makes them more desirable for the use of treatment.

On the other hand, activated sludge system plants are always facing dynamic condition. This means, these systems are always subject to concentration gradient, reactor hydraulics and variation in the modes of operating conditions. Therefore, these concentrations gradients and changing modes of operation force activated sludge cultures to develop a storage response for their sake of survival. Activated sludge cultures adapt to the dynamic conditions by storing the substrate when it is available in the medium. When there is lack of substrate on the external medium, using the stored material for their maintenance and growth. This put the storage phenomena in the research field. Need of understanding the storage phenomena is essential, because it is important tool for design of better activated sludge systems.

Substrate storage is defined and added to activated sludge the modeling by the Activated Sludge Model No: 3(ASM3). Thus, biological transformation and storage inside the biomass has been taken into account together with the already present substrate in the extra cellular medium.
It has been observed that under dynamic condition microorganisms produce various storage polymers like glycogen and PHB (Van Loosdrecht et al, 1997). The metabolism of various storage polymers in mixed cultures under different feeding condition and under different reactor types were investigated. However, how different substrate under different feeding patterns are affect microorganisms is still stays unclear for the researchers. It is not well understood how microorganisms shift to storage response to growth situation. Therefore, the aim of this study is to improve the understanding of storage process in activated sludge under aerobic condition. To evaluate kinetic and stoichiometric parameters that is mentioned in the biological processes. The study intends to investigating effect of feeding patterns on aerobic substrate storage by observing: pulse feeding response and long-term feeding response of the activated sludge system. Moreover, present study investigates effect of F/M ration on storage mechanism. As a final step, all observed date will be modeled mathematically according to the proposed model ASM3. And results will be compared with the literature.

Sequencing Batch Reactor (SBR) type is chosen as a reactor type, in the research. The reason of this is: in the literature researchers mostly emphasized on batch reactor types. SBR, simply, is an activated sludge system that works under a fill-and-draw basis. The SBR process is essentially composed for a single tank. The process operations consist of its semi-batch operation and the fact that biological conversion and settling take place in the same reactor. SBR process is a cyclic operation: Fill time, waste is fed into the tank and mixed with already present biomass. React time, sufficient time is allotted for biological conversion. Then microorganisms are separated from treated waste, this is settling time. Wastewater is emptied in decant time. And reactor is left idle before being refill.

In the present study, reactor is fed with sole glucose. At this condition it is expected that glucose to be stored by microorganisms. Biomass is expected to store glucose in feasting times and use it in the fasting period when external glucose is absent in the media.
2. LITERATURE SURVEY

2.1 The Importance of the Storage Phenomena

Researchers have well documented the ability of microorganisms to store polymers in internal cell for some conditions (Chudoba et al, 1973; Van den Eijnde et al., 1984). Activated sludge processes are often operated under non steady conditions such as, changes in feed flows rates and concentrations. Even if the overall process can be considered under steady state, microorganisms are cautiously recycled among the zones which have concentrations gradients and different environmental conditions.(Beccari el al, 2002). Consequently, microbial response to the dynamic conditions can be a simple increase in cell numbers (growth), and other substrate removal activities, i.e. internal cell reactions like sorption, accumulation and storage (Majone et al., 1999). When microorganisms are exposed to feast conditions, the response of the microorganisms is to accumulate the storage polymers. In the absence of substrate, when the famine period occurs these storage polymers are considered to be used for growth and microorganismal survival. Microorganisms that adopt the conditions by accumulating their substrate in the feast period, and then grow in the famine conditions have very good competitive advantage over organisms without ability of storage (Satoh et al., 1999).

It is obvious that, formation of the storage molecule in the bacteria gives the advantage of a balance the metabolism when sudden changes occur. When the substrate uptake is limited, the organisms would need a large amount of substrate uptake enzymes. Therefore the decrease of the amount of enzymes would be uncontrolled (Akkaya et al., 2004). Rising of the substrate concentration in the medium will cause a rapid uptake of the substrate due to large amount of enzymes. However, the growth process of the microorganisms can not directly consume this rapid uptake amount of substrate and this may cause imbalance in the cell.
Consequently, the storage is related with growth, in other word, storage can not be defined as an independent mechanism from growth (van Loosdrecht et al., 1997).

2.2 Microbial Response

Microbial response to dynamic condition on the environment could include several different ways like, growth and storage response (Daigger and Grady, 1982a). These behaviors will be discussed in the following section.

2.2.1 Growth response

Cells are considered to be the fundamental units of life. Cell itself is a dynamic unit which means it undergoes changes and replaces its part continuously. At the beginning of the cultivation of a cell in the culture, a cell tends to adapt itself physically to the new environment. Therefore an adaptation occurs for the cell. Adaptation is essential for the microorganisms because it directly affects the ability to survive in substrate gradients, different reactor hydraulics and changing modes of operational modes, i.e. dynamic conditions.

All cells contain macromolecules, in particular proteins, nucleic acids, lipids and polysaccharides. Some changes in the cellular composition such as deoxyribonucleic acids (DNA), ribonucleic acids (RNA) and proteins are indicators of an adaptation process. As soon as the medium in which cell sustain reach a steady state, the protein sentences reach an optimal level for growth (Roels, 1983; Grady et al., 1996). Assuming there is not substrate limitation in the environment for the microorganism, the growth occurs in the constant rate. However, the growth response varies if the microorganism is in the medium that is substrate limited. The microbial culture being adapted to growth under substrate limiting conditions will be slow to synthesize protein, i.e. sustain growth, even if substrate limitation is not present. Thus, the specific growth rate will increase gradually, and if the time of dynamic condition is long fair enough.

Likewise any life form, microorganisms need an energy for sustaining growth, making storage particles and accumulating them within the internal cell boundaries. Required energy is generally obtained from previously conserved and stored forms of
chemical adenosine triphosphate (ATP). ATP is commonly used as an energy carrying and storing particle between energy generating reactions (catabolism) and energy consuming reactions (anabolisms) in the cell (Gottschalk, 1985). (Figure 2.1)

2.2.2 Storage response

In most activated sludge process, the biomass grows under unsteady, i.e. dynamic, conditions, even if the overall process can be assumed to be in steady state condition (Aulenta et al., 2003). In activated sludge systems the biomass is consciously recycled between the settler tank to the biological tank. Thus, the microbial community is exposed to the dynamic conditions such as concentration gradients, presence of electron acceptor or not etc. dynamic condition also is present in SBRs where spatial sequences of steps are replaced by periodic time sequences in a single tank (Aulenta et al., 2003).

It is well recognized that in activated sludge and mixing cultures under dynamic conditions a storage response is usually triggered (Majone et al., 1999). The substrate is removed and transformed into internal polymers that can be later used as a carbon source for growth. The kinetics and yield in the storage process are different than the growth. While new forms are formed within the cell, the substrate uptake rate is rapidly increased. The stored solid composition is differentiating because of the initial carbon source i.e. substrate and because of storage mechanism. Main storage polymers are considered to be polysaccharide and lipids in general. As it is apparent, because synthesis of storage polymers are more simple than the synthesis of whole complete cell, less psychological adaptation is required and storage response can be considered as more fast/rapid than growth response itself.

Storage products composition depends on the carbon source (feed). Among the organic storage compounds PHB is probably the most dominant polymer as it is formed directly of acetyl-CoA. However, glycogen is detected to be form only when the sugar is present in the influent. On the literature, there is few reports on presence of the lipids as a storage molecules. They are observed in small, constant amount in all bacteria, and these amounts are mostly considered as structural components of membranes and cell walls.
In the studies, organic and inorganic storage metabolites have been reported. PHB, glycogen and lipids are reported to be most important organic storage metabolites. On the other hand, polyphosphates are considered to be inorganic storage particles (Zevenhuizen and Ebbink, 1974).

A study was conducted by Beccari et al., (2002) about the effect of different substrate on storage. The aim of the study was to provide a deeper insight about substrate removal mechanisms by a real activated sludge of a municipal WWTP with some synthetic substrates (ethanol, acetate, glutamic acid) and with wastewater (raw and filtered), with the main focus on the role of storage. The results were as follow:

Acetate study: Acetate is removed at a constant rate, OUR increases immediately and remains more or less until acetate is present in the medium, PHB is stored. After acetate depletion PHB is consumed as internal carbon and energy source. Among the three synthetic substrates, acetate is the one removed at the highest specific rate. 75% of the COD is removed (overall).

Ethanol test: Ethanol as a carbon source is used: Behaviour is similar to the acetate. The substrate profile is linear, associated oxygen consumption is low; PHB is formed in significant amounts. However, On the other hand, the contribution of PHB storage in substrate removal is lower than in the test with acetate (25% vs 45%), whereas the growth is higher (26% vs 13%). Overall COD percent of 80 % were removed.

Test with glutamic acid: lag phase of about 30 minutes is observed, after addition of the feed. After this lag phase, glutamic acid is consumed and the corresponding OUR increases. No significant storage occurs for PHB or other polyhydroxyalkanoates (PHAs) that could confirm a more relevant role of growth. An hypothesis to explain this behavior is that a fraction of the removed glutamic acid is not used for active biomass synthesis but it is stored inside the cell in a form other than PHA (e.g. a polyaminoacid, as already reported under anaerobic conditions by Satoh et al., (1998) or simply accumulated inside cells.

Wastewater Test: The filtered and raw wastewater show a similar OUR pattern: a first phase of high and quickly decreasing OUR, a second phase of lower and more or less constant OUR. The main differences in the OUR profiles are the length of the second phase at constant OUR (which is clearly longer with raw wastewater,
corresponding to the higher COD) and the decrease to the endogenous value which is much slower in the test with raw wastewater. The presence of a second OUR phase in the test with filtered wastewater seems to indicate that a significant fraction of the analytically soluble COD is actually slowly biodegradable, as already reported in previous studies (Carucci et al., 2001). This second phase could in principle be attributed to consumption of storage product (Dircks et al., 1999; Goel et al., 1999).

Under the assumption that all soluble COD is also readily biodegradable. A more quantitative evaluation of the role of growth in substrate removal can be done only by using a mathematical model. Thus, researchers proposed mathematical models as follows:

**ASM3 Model:**

```
Soluble Readily Stored compound Active biomass
Biodegradable
Substrate
```

**Proposed Model 1:**

```
Soluble Readily Stored compound
Biodegradable
Substrate
  
Active biomass
```

```
Proposed Model 2:

![Diagram showing Proposed Model 2]

Figure 2.2: Proposed Models for storage by Beccari et al., (2002)

Aulenta et al (2003) states that, in transient conditions all substrate removal was due to the oxidation and storage. At acetate depletion PHB storage was 50-71% of removed acetate and 73-95% of overall observed yield. A longer famine period caused a higher capacity of cells to answer quickly to sudden change of substrate availability.

Carta et al., (2000) tried to evaluate simultaneous PHB and glycogen storage in an enriched culture. They focused on describing the performance of an acetate/glucose fed SBR process and comparing it with SBR processes fed with a single substrate. In the first 10min the liquid (1L) was mixed and re-aerated so the DO increased. At the moment of substrate addition the OUR increased (and the DO decreased) immediately to a maximum due to substrate consumption. After the depletion of glucose and acetate, the OUR immediately decreased (and the DO increased). These changes in the profiles were a clear observation to define the transition of the feast to the famine period, considering the end of the feast period when all the substrates (glucose plus acetate) were consumed. In the feast period, glucose was extremely rapid consumed and converted into glycogen. The acetate concentration decreased linearly, and a linear increase in PHB concentration was measured. In the famine period, glycogen and PHB were consumed simultaneously. It can be seen that a large fraction of the external substrate is stored as PHB or glycogen. In the system with a mixed substrate, the conversion of acetate and glucose in PHB and Glycogen, respectively, is not different. Moreover it is reported that the storage compounds had
no influence on each others observed degradation rates. The study has concluded also that the glucose consumption rate is difficult to be measured because glucose itself is depleted rapidly.

Martins et al.,(2003) tried to seed the effect of the storage on setleability of an system. Since the growth of filamentous bacteria seems to be connected to the feeding pattern of the system. This study was to evaluate some of the general theory about proliferation of filamentous bacteria, under controlled lab conditions. Researchers choose a fully aerobic SBR type reactor for experiment. Increasing the length of the feeding period has made a strong negative effect of the setleability of the system, i.e. SVI is improving while fill time period in SBR is shortened.

Martins et al.,(2003) also report that: the maximum specific acetate uptake rates and maximum specific PHB production rates of bad settling sludge and well settling sludge were similar. They observed that in systems that are pulse fed, more than 80% of the acetate was used for synthesis of PHB and the remainder was used for growth and maintenance processes. However, in continuously feed systems where biomass growth predominates, a linear relation between substrate uptake rate and biomass growth exists. At low bulk liquid substrate concentrations filamentous bacteria was easily grown, on the other hand, at high bulking liquid there was no substrate advantage and for filamentous microorganisms. No significant differences were observed in the maximum PHB production rates for different sludge types. They concluded that there is no for different between the kinetics parameter of flock and filamentous bacteria.

Substrate storage was also accepted as a key process for biological phosphorus removal (Wentzel et al., 1986; Mino et al., 1987). Recently a new model for activated sludge cultures has been introduced (ASM3), the model includes the storage phenomena in to the mathematical modeling (Gujer et al., 2000).

2.2.3. Accumulation response

A quick removal of the substrate could be achieved by accumulation (Grau et al., 1982). In this context, the substrate is transformed into the cell and maintained in
internal cell in almost unchanged form are sometimes transformed into low molecular weight metabolic intermediates for some reasons like, unfavorable concentration gradients and osmotic pressure, this can be done efficiently to much more limited extent than the case of storage where molar concentration of substrate or intermediates is reduced by polymerization (Majone et al., 1999).

Consequently, storage can be a good alternative mechanism for preventing the accumulation of internal metabolites at toxic level during the growth process.

### 2.3. Storage Metabolism

#### 2.3.1. Glycogen metabolism

Different from PHB, glycogen is a straight-chained polymer of linear $\alpha$-1,4 linked D-glucose subunit with some $\alpha$-1,6 branches. Branching increases the number of terminal points at which the degradation enzymes can bind meanwhile enhancing the rate of glycogen synthesis and degradation (Stryer, 1995. Dircks et al., 2000) demonstrated that, the branching also increases the density of glycosyl units in the ties as glycogen molecule grows in size. The free space available for the enzymes to attack the bonds is dramatically reduces after the ties of bonds increase.

![Glycogen representation](image_url)

Figure 2.3. Schematic representation of glycogen ranching (Gottschalk, 1985)
2.3.2. Biochemical pathway and regulation of glycogen production and consumption

In general, organic storage polymers are separated into two major groups which are; polysaccharides and lipids. Different metabolites cause the different storage polymers. When primary substrate is glucose i.e. sugar, or in more general; a compound that could be converted to private with an increase in reducing power; for instance, different carbohydrates, glycerol or proteins, glycogen is formed as the storage metabolite. The rate of glycolysis will be reduced when the NADH\textsubscript{2} level increased in the internal cell.

There have been a recant studies aiming to describe the aerobic metabolism of glycogen in the activated (Dircks et al., 2000). In this study Dircks et al., (2000) compared their data with the metabolisms of PHA which has been identified by Beun et al., (2000). As a result following metabolic model was proposed.

Glucose metabolism (glycolysis) will be discussed later. Simply, the cells take up the glucose and phosphorylate and convert it into glucose-6-phospahte (G6P) by utilizing ATP (R1). The sufficient amount of G6P is used for the synthesis of new cells (R2). NADH\textsubscript{2} produced in the catabolism reactions is used (R3) as a electron transporter phosphorilation(R4) in order to gain energy for cells. Synthesis of glycogen from G6P requires 1/6ATP per G6P (Stryer, 1995). Figure 4.4 illustrate the mechanisms.

When the external glucose is finished, microorganisms utilize the stored glycogen as an energy and carbon source. The phosphorolyses of glycogen into the G6P is assumed to be without consuming energy. However, degradation of glycogen is assumed not to occur when there is a substrat in the external cell medium available.

Dircks et al., (2001) have reported the storage yield of glycogen as 0,91 gCOD/gCOD and growth yield in the feast phase as 0,57 gCOD/gCOD. The growth rate in the famine phase is 0,6 gCOD/gCOD. Karahan-Gul et al., (2002) have examined the storage phenomena and modeled their result according to ASM3 model. They found storage yield defined by ASM3 (Y\textsubscript{sto}) 0,87 gCOD/gCOD, 0,78
gCOD/gCOD and 0.96 gCOD/gCOD for acetate, glucose and domestic wastewater respectively.

2.4 Evaluation of ASM3 Stoichiometry for Organic and Carbon Removal

Activated sludge model was recently developed for carbon and nitrogen removal as an alternative to prior models of ASM 1. The importance and difference of the model comparing to other models are the concept of the storage metabolism. In the model the main postulation is that: all soluble biodegradable substrate is first converted to storage polymers, which are used for growth in the lack of external substrate. Thus, the storage phenomena are added to the activated system sludge modeling. Table 3.1 are illustrated the matrix representation of ASM3.

The conventional activated sludge systems consist of an aerobic reactor and a clarifier. The aeration reaction is assumed to be a completely mixed tank with no oxygen limitation, and clarifier is assumed to be settling tank where no biological conversions takes place. Fill schematic illustration of the system with the model components are illustrated in Fig 2.5. Although the particulate fractions are discharged from bottom of the clarifier a fictive portion of the particulate matter depending on the hydraulic retention time and sludge retention time are assumed to leaving the system with the effluent. This will be added to calculation to achieve an accurate COD removal.

Parameters Present in the Reactor

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S_{HCO}</td>
</tr>
<tr>
<td></td>
<td>S_{S1}</td>
</tr>
<tr>
<td></td>
<td>S_{S}</td>
</tr>
<tr>
<td></td>
<td>S_{H}</td>
</tr>
<tr>
<td></td>
<td>S_{01}</td>
</tr>
<tr>
<td></td>
<td>S_{NH1}</td>
</tr>
<tr>
<td></td>
<td>S_{HCO1}</td>
</tr>
<tr>
<td></td>
<td>X_{S1}</td>
</tr>
<tr>
<td></td>
<td>X_{STO1}</td>
</tr>
<tr>
<td></td>
<td>X_{H}</td>
</tr>
<tr>
<td></td>
<td>X_{H1}</td>
</tr>
<tr>
<td>Effluent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q</td>
</tr>
<tr>
<td></td>
<td>QR</td>
</tr>
<tr>
<td></td>
<td>QW</td>
</tr>
<tr>
<td></td>
<td>P_{XT}</td>
</tr>
<tr>
<td>Reactor</td>
<td></td>
</tr>
</tbody>
</table>

\[ S_{S} \quad X_{S} \]
\[ S_{1} \quad X_{STO} \]
\[ S_{0} \quad X_{I} \]
\[ S_{NH} \quad X_{H} \]
Figure 2.5. Representation of a conventional activated sludge system for carbon removal using ASM3 components.

Table 2.1 Representative Matrix of Activated Sludge Model No.3

<table>
<thead>
<tr>
<th>COMPONENT PROCESS</th>
<th>SO</th>
<th>SS</th>
<th>XI</th>
<th>XH</th>
<th>XSTO</th>
<th>RATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage of Ss</td>
<td>O2</td>
<td>COD</td>
<td>COD</td>
<td>COD</td>
<td>COD</td>
<td>( k_{STO} \frac{S_0}{K_0 + S_0} \frac{S_s}{K_s + S_s} X_H )</td>
</tr>
<tr>
<td>Growth of XSTO</td>
<td>-(1-YSTO)</td>
<td>1</td>
<td></td>
<td></td>
<td>YSTO</td>
<td>( \mu_{H} \frac{S_0}{K_0 + S_0} \frac{X_{STO}}{X_H + X_{STO}} / X_H )</td>
</tr>
<tr>
<td>Endogenous Respiration</td>
<td>-(1-fI)</td>
<td>fI</td>
<td>-1</td>
<td></td>
<td></td>
<td>( b_{H} \frac{S_0}{K_0 + S_0} X_H )</td>
</tr>
<tr>
<td>Respiration of XSTO</td>
<td>-1</td>
<td></td>
<td>-1</td>
<td></td>
<td></td>
<td>( b_{STO} \frac{S_0}{K_0 + S_0} X_{STO} )</td>
</tr>
</tbody>
</table>
2.4.1 Steady state solutions and mass balance

It is very important to obtain the steady state solutions of mass balance on the model, thus each parameter behavior will be described within the system. The mass balance is involves the input, output and generation of parameters are under concern and there is no accumulation predicted.

Heterotrophic biomass is one of the important parameters within the system. Biomass enters system with influent, $X_{W1}$, and leave the system with the sludge waste, $X_{W1}$. Biomass is generating in the system as a result of growth, and is reduced as a result of endogenous decay.

$$QX_{W1} - Q_w X_w + \nu \mu H X_H - Vb_H X_H = 0$$ \hspace{1cm} (2.1)

The heterotrophic biomass consecration in the influent is very low, therefore it is assumed to be zero. Thus, this equation is going to the simpler and it indicates the sludge age.

$$\frac{1}{\theta_x} = \mu_H - b_H$$ \hspace{1cm} (2.2)

The sludge age is traditionally defined related to the volume of the aeration tank divided by daily sludge waste.

$$\theta_x = \frac{V}{Q_T}$$ \hspace{1cm} (2.3)

Rewriting the equation 2 by adding the heterotrophic growth, hence,

$$\frac{1}{\theta_x} + b_H = \mu_H \frac{X_{STO}/X_H}{K_{STO} + (X_{STO}/X_H)}$$ \hspace{1cm} (2.4)

Rearranged as
\[
\frac{\mu_H \theta_X}{1 + b_H \theta_X} = \frac{K_{STO} X_H + X_{STO}}{X_{STO}}
\]

(2.5)

Above expression can be solved for \(X_H\), so the heterotrophic growth can be derived as

\[
X_H = \frac{\mu_H \theta_X - (1 + b_H \theta_X)}{K_{STO}(1 + b_H \theta_X)} X_{STO}
\]

(2.6)

The heterotrophic growth concentration depends on the stored polymers, \(X_{STO}\) in the system according to the model.

Readily biodegradable substrate \(S_s\) comes to the system through influent, \(S_{s1}\) and leaves to the system with effluent discharge. Readily biodegradable COD is consumed and converted to the storage polymers according to the ASM 3 model assumption. Therefore the mass balance can be written as,

\[
Q S_{s1} - Q_W S_s + V k_{STO} \frac{S_s}{K_s + S_s} X_H = 0
\]

(2.7)

Total consumed \(S_s\) can be defined as;

\[
\Delta S = S_{s1} - S_s
\]

(2.8)

According to the ASM3 model, the amount of the substrate consumed is the amount of the substrate converted into storage polymers. From mass balance equation, we can derive amount of the substrate consumption like;

\[
\Delta S_s = V k_{STO} \frac{S_s}{K_s + S_s} X_H
\]

(2.9)

The most critical parameter is \(X_{STO}\), because it appears both in storage and growth processes.
Storage products can inflow the system by influent as $X_{STO1}$, but it happens at very low concentrations, therefore it is assumed to be zero. A relatively small amount of the stored COD leaves the system with particulate fractions. Thus, it is generated in the system and consumed by heterotrophic growth and endogenous decay. Bearing these in mind the mass balance equation of $X_{STO}$ written like:

$$QX_{STO} - Q_w X_{STO} + VY_{STO}k_{STO} \frac{S_s}{S_s + K_s} X_h - V \frac{\mu_H}{Y_H} X_h - Vb_{STO}X_{STO} = 0$$  \tag{2.10}$$

The inflow storage is neglected due to low consent rations. Hence equation is derived as;

$$- Q_w X_{STO} + VY_{STO}k_{STO} \frac{S_s}{S_s + K_s} X_h - V \frac{\mu_H}{Y_H} X_h - Vb_{STO}X_{STO} = 0$$  \tag{2.11}$$

Above equation is solved taking $X_{STO}$ into account. Therefore the equation becomes

$$X_h = \frac{Y_H}{1 + b_H \theta_X} \left[ Y_{STO} \Delta S_S - (1 + b_{STO} \theta_X) \frac{\theta_h}{\theta_X} X_{STO} \right] \frac{\theta_X}{\theta_h}$$  \tag{2.12}$$

Combining equation 6 and 12 will give the description of storage products concentrations.

$$\frac{\mu_H \theta_X - (1 + b_H \theta_X)}{K_{STO}(1 + b_H \theta_X)} X_{STO} = \frac{Y_H}{1 + b_H \theta_X} \left[ Y_{STO} \Delta S_S - (1 + b_{STO} \theta_X) \frac{\theta_h}{\theta_X} X_{STO} \right] \frac{\theta_X}{\theta_h}$$  \tag{2.13}$$

$$\frac{\mu_H \theta_X - (1 + b_H \theta_X)}{K_{STO}(1 + b_H \theta_X)} X_{STO} + (1 + b_{STO} \theta_X) X_{STO} = \left[ Y_{STO} \Delta S_S \frac{\theta_X}{\theta_h} \right]$$  \tag{2.14}$$

$$\frac{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \theta_X)}{K_{STO}(1 + b_H \theta_X)} X_{STO} = \left[ Y_{STO} \Delta S_S \frac{\theta_X}{\theta_h} \right]$$  \tag{2.15}$$

$$X_{STO} = \frac{K_{STO} Y_H}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO}(1 + b_H \theta_X)} Y_{STO} \Delta S_S \frac{\theta_X}{\theta_h}$$  \tag{2.16}$$
In order to simplify the definition, some stoichiometric expression is defined;

\[ A(\theta_X) = \frac{K_{STO}Y_H}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO}(1 + b_H \theta_X)} \]  

(2.17)

The expression for the heterotrophic biomass given in equation 6 now could be rewritten as;

\[ X_{STO} = [1 - (1 + b_{STO} \theta_X)A(\theta_X)] \frac{Y_H}{(1 + b_H \theta_X)} Y_{STO} \Delta S_S \theta_X \theta_h \]  

(2.18)

Here another expression is defined

\[ B(\theta_X : \theta_h) = [1 - (1 + b_{STO} \theta_X)A(\theta_X)] \frac{Y_H}{(1 + b_H \theta_X)} Y_{STO} \theta_X \theta_h \]  

(2.19)

By using equation 2.20 below, equation 9 can be re arranged as it is in eq. 2.21.

\[ \theta_h = \frac{V}{Q} \]  

(2.20)

\[ \Delta S_S = \theta_h k_{STO} \frac{S_S}{K_S + S_S} X_H \]  

(2.21)

Using the simplified expression of 2.22, readily biodegradable substrate is redefined as it is in equation 2.23;

\[ X_H = B(\theta_X : \theta_h) \Delta S_S \]  

(2.22)

\[ S_S = \frac{K_S}{\theta_h k_{STO} B(\theta_X : \theta_h) - 1} \]  

(2.23)

Putting the extended expression of B in the equation;
\[ S_S = \frac{K_{STO}(1 + b_H \theta_X)}{k_{STO} \theta_X Y_H Y_{STO}} \left[ 1 - (1 + b_{STO} \theta_X) A(\theta_X) \right] - (1 + b_H \theta_X) \]  

(2.24)

\( X_S \), slowly biodegradable fraction of the substrate, hydrolyses is identified with non-linear expression in the ASM3 model. This expression may be simplified using first order kinetics. Mass balance is like below;

\[ QX_{S1} - QW X_S - Vk_H X_S = 0 \]  

(2.25)

The slowly biodegradable COD fraction is described as it is illustrated in the following equation.

\[ X_S = \frac{\theta_X}{\theta_H} \frac{X_{S1}}{1 + k_H \theta_X} \]  

(2.26)

### 2.4.2 Removal of COD and production of sludge

The total COD removal is thought as sum removal of the slowly biodegradable and readily biodegradable COD.

\[ \Delta C_S = C_{S1} - C_S = (S_{S1} + X_{S1}) - (S_S + \frac{\theta_H}{\theta_X} X_S) \]  

(2.27)

The total COD removal is rewritten as below by substituting the equation 26 in the expression.

\[ \Delta C_S = C_{S1} - C_S = (S_{S1} + X_{S1}) - (S_S + \frac{X_{S1}}{1 + \theta_X k_H}) \]  

(2.28)

The daily sludge production will be derived only by removal of readily biodegradable COD

\[ P_{S_H} = Q \frac{Y_H}{1 + b_H \theta_X} \left[ Y_{STO} \Delta S_S - (1 + b_{STO} \theta_X) \frac{\theta_H}{\theta_X} X_{SSTO} \right] \]  

(2.29)
Using the net heterotrophic growth yield as it is given in equation 30, the expression above can be rearranged like;

\[ Y_{NH} = \frac{Y_H}{1 + b_H \theta_X} \]  

(2.30)

\[ P_{NH} = Q Y_{NH} \left[ Y_{STO} \Delta S_S - (1 + b_{STO} \theta_X) \frac{\theta_h}{\theta_X} X_{STO} \right] \]  

(2.31)

A factor, \( f \), is defined as the fraction of storage products which are not consumed for growth. However, this fraction is retained in within the system because of operating conditions or endogenous respiration.

\[ f = \frac{(1 + b_{STO} \theta_X) \frac{\theta_h}{\theta_X} X_{STO}}{Y_{STO} \Delta C_S} = \frac{K_{STO} Y_H (1 + b_{STO} \theta_X)}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \theta_X)} \]  

(2.32)

\[ f = (1 + b_{STO} \theta_X) A(\theta_X) \]  

(2.33)

the daily net amount of heterotrophic biomass generated within the system is identified as a fraction of stored polymers which are produced as a fraction of the total COD removed

\[ P_{NH} = Q Y_{NH} (1 - f) Y_{STO} \Delta C_S \]  

(2.34)

The sludge production of the stored polymers can be expressed as;

\[ P_{STO} = Q \frac{K_{STO}}{\mu_H \theta_X - (1 + b_H \theta_X) + K_{STO} Y_H (1 + b_{STO} \theta_X)} Y_{STO} \Delta C_S \]  

(2.35)

Substituting the variations \( A(\theta_X) \) and \( f \), in the above equation. The daily sludge production of \( X_{STO} \) is expressed as below:
\[ P_{XSTO} = QA(\theta_X)Y_{STO}\Delta C_s = Qf \frac{Y_{STO}}{1 + b_{STO}\theta_X} \Delta C_s \]  
\( (2.36) \)

Hence the net yield is described as

\[ Y_{NSTO} = \frac{Y_{STO}}{1 + b_{STO}\theta_X} \]  
\( (2.37) \)

Thus, equation becomes,

\[ P_{XSTO} = Qf Y_{STO} \Delta C_s \]  
\( (2.38) \)

The net sludge production coming from the slowly biodegradable COD fraction is illustrated in below equation

\[ P_{XS} = Q \frac{X_{s1}}{1 + k_{H}\theta_X} \]  
\( (2.39) \)

The inert COD in the system is the sum of influent inert CO and microbial products

\[ X_I = X_{I1} \frac{\theta_X}{\theta_H} + f_I b_H X_{H} \theta_X \]  
\( (2.41) \)

By definition the total sludge production of the system is given as

\[ P_X = P_{XH} + P_{XSTO} + P_{XS} + P_{Xf} = \frac{VX_H}{\theta_X} + \frac{VX_{STO}}{\theta_X} + \frac{VX_S}{\theta_X} + \frac{VX_f}{\theta_X} \]
\( (2.42) \)
Figure 2.4 The metabolic model of glycogen metabolism with its six internal reactions (Dircks et al. 2000)
CATABOLISM

ANABOLISM

Figure: 2.1 The metabolic events taking place inside the cell (Gottoschalk, 1985)
3. MATERIAL AND METHODS

3.1 Reactor Operation

A cylindrical bench-type reactor made of plexiglas equipped with mixer, aerator, timers for controlling time period of cycles and pump for filling, were operating in a fully aerobic mode throughout this study. The SBR unit in this research was a cylindrical tank with working volume of 7.3 l. A fill volume, \( V_F \), of 4.6 l was selected and leaving a 2.7 l for initial volume, \( V_0 \). Therefore, corresponding of relatively low \( V_0/V_F \) ratio of 0.58. In the figure below SBR and its units are schematically illustrated.

![SBR apparatus diagram]

**Figure: 3.1 A simple illustration of SBR**

The SBR operations were designed for 4 cycles per day, i.e. a total cycle time, \( T_C = 6 \) hours. The process phase (Reaction), \( T_R \), was selected to be 5 h. Consequently, the remaining time of 1 h right after process phase was devoted to settling (\( T_S \)), decant
(T_D) and idle times (T_I) for 35 min, 15 min and 5 min respectively. Hence, total cycle time was calculated as:

$$T_C = T_F + T_S + T_D + T_I$$

Operation conditions of the reactor were given schematically in Table 3.1. The reactor was kept in a constant room adjusted temperature of 20°C during the whole experimental work.

Table 3.1 Operation phases of working SBR

<table>
<thead>
<tr>
<th></th>
<th>Volume (l)</th>
<th>Aeration and Mixing</th>
<th>Feeding</th>
<th>Discharge</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fill</td>
<td>2.7</td>
<td>NO</td>
<td>YES</td>
<td>NO</td>
<td>5</td>
</tr>
<tr>
<td>React</td>
<td>7.3</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
<td>300</td>
</tr>
<tr>
<td>Settle</td>
<td>7.3</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>35</td>
</tr>
<tr>
<td>Decant</td>
<td>7.3</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>15</td>
</tr>
<tr>
<td>Idle</td>
<td>2.7</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3.2 Operating parameters of SBR

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbol</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle Time</td>
<td>T_C</td>
<td>H</td>
<td>6</td>
</tr>
<tr>
<td>Duration of filling time</td>
<td>T_F</td>
<td>Min</td>
<td>5</td>
</tr>
<tr>
<td>Duration of aerobic period</td>
<td>T_R</td>
<td>H</td>
<td>5</td>
</tr>
<tr>
<td>(mixing)</td>
<td>T_S</td>
<td>Min</td>
<td>35</td>
</tr>
<tr>
<td>Duration of settling period</td>
<td>T_D</td>
<td>Min</td>
<td>15</td>
</tr>
<tr>
<td>Duration of idle period</td>
<td>T_I</td>
<td>Min</td>
<td>5</td>
</tr>
<tr>
<td>Initial Volume</td>
<td>V_0</td>
<td>L</td>
<td>2.7</td>
</tr>
<tr>
<td>Total Volume</td>
<td>V_T</td>
<td>L</td>
<td>7.3</td>
</tr>
<tr>
<td>Filling Volume</td>
<td>V_F</td>
<td>L</td>
<td>4.6</td>
</tr>
<tr>
<td>Vo/V_F</td>
<td>-</td>
<td>-</td>
<td>0.58</td>
</tr>
<tr>
<td>Hydraulic Retention Time</td>
<td>HRT</td>
<td>H</td>
<td>-</td>
</tr>
<tr>
<td>Sludge Retention Time</td>
<td>SRT</td>
<td>d</td>
<td>10</td>
</tr>
<tr>
<td>Temperature</td>
<td>-</td>
<td>°C</td>
<td>20</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>-</td>
<td>7.14</td>
</tr>
</tbody>
</table>

The stirrer speed was kept at 200 rpm. Dissolved oxygen was maintained higher than 2 mg O_2/l by airflow generator. pH was monitored at steady state with detailed measurement which indicate average pH of 7.15. Feeding and drawing were performed with a pomp and solenoid van respectively. Stirring and aerating, feeding and discharging were connected to timer. The reactor was operated at sludge age of
10 days, for 7 months. SBR was fed with 1000 mgCOD/l cycle glucose. Firstly a stock solution has been prepared shown in the Table 3.4, after that the appropriate volume has been added to the reactor. The need of trace elements macro and micro nutrients were satisfied by adding appropriate solutions i.e. solution A and solution B. The solutions were illustrated in Table 3.3.

Table 3.3 Stock Macro and Micro Nutrients

<table>
<thead>
<tr>
<th>Solution</th>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>K$_2$HPO$_4$</td>
<td>320 g/l</td>
</tr>
<tr>
<td></td>
<td>KH$_2$PO$_4$</td>
<td>160 g/l</td>
</tr>
<tr>
<td></td>
<td>NH$_4$Cl</td>
<td>120 g/l</td>
</tr>
<tr>
<td>B</td>
<td>MgSO$_4$.$7$H$_2$O</td>
<td>15 g/l</td>
</tr>
<tr>
<td></td>
<td>FeSO$_4$.$7$H$_2$O</td>
<td>0.5 g/l</td>
</tr>
<tr>
<td></td>
<td>ZnSO$_4$.$7$H$_2$O</td>
<td>0.5 g/l</td>
</tr>
<tr>
<td></td>
<td>MnSO$_4$.3H$_2$O</td>
<td>0.5 g/l</td>
</tr>
<tr>
<td></td>
<td>CaCl$_2$</td>
<td>2.0 g/l</td>
</tr>
</tbody>
</table>

Table 3.4. Stock solution of feeding COD

<table>
<thead>
<tr>
<th>Feed Component</th>
<th>Concentration</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>48 g/l</td>
<td>Glucose</td>
</tr>
</tbody>
</table>

3.2 Batch Experiments

Batch experiments were conducted with mixed liquors taken directly from the SBR at the end of the cyclic period i.e. when aeration and mixing has just stopped. In order to simulate glucose fed SBR, batch reactor was fed with 1000 mgCOD/l and was equipped with stirrer and aeration also kept 20 °C temperatures constantly. To obtain a desired F/M ratio, needed MLVSS amount were taken directly from mixed liquor VSS of the SBR right after VSS of the SBR is measured. Therefore, desired F/M ratios are adjusted for the experimental work.

Inorganic nutrient solutions were prepared to ensure similar concentrations that is present in the SBR. Used nutrients had been given in Table 3.3.
3.3 Analytical Techniques

In this study, analytical techniques could be grouped in two simple groups; techniques related to the control of the system and techniques related to measurement of the microbial activity level during the oxygen uptake. First group techniques include determination of suspended solids (SS), volatile suspended solids (VSS) effluent COD measurement and pH. All these analyses were performed as they are defined in Standard Methods (1998), except the measurement of COD. COD samples were filtrated through 0.45 μm membrane filters, and afterwards COD of the samples were measured according to the defined method proposed by ISO 6060 (1986). Second group techniques were related to measurement of the activity level of microbial behavior when oxygen uptake is plotted by respirometer.

Glycogen and glucose analyses were performed by using a BIORAD HPX87H column in Shimadzu HPLC system. The system is equipped with a Shimadzu SCL-10A system controller, a LC-10A vp pump, a DGU-14A degasser, a SPD-10A vp UV-Vis detector, RID-10A refractive index detector, SIL-10AD vp auto injector, CTO-10A cp oven, Class-VP software. Volatile fatty acids (VFA) composition was analyzed by gas chromatography (GC) (Agilent 6890 N) using a flame ionization detector (FID) with a HP-FFAP capillary column with column diameter 0.53mm and length 10 mm respectively.

3.4 Respirometric Analysis

Applatech RA-1000 respirometer with PC connection has been used for all respirometric measurements. All respirometric measurements were conducted by using the relevant SBR grown biomass. At the initial stage only calculated biomass were added to the system with the needed nutrients and other buffers used for SBR. Mixed liquor was taken at the end of the reaction phase, right before settling. Desired F/M ratio was adjusted by assuming the same initial feeding concentration as SBR, 1000 mgCOD/l and needed VSS concentration was derived from mixed liquor. After adjusting F/M ratio the respirometer was started without initial feed to be added. Thus, the endogenous respiration could be marked on the respirometer by following the OUR measurements. No nitrification inhibitor was added to the system, because
there was no NO₃ and/or NO₂ seen in the effluent discharge. That will be given in more detail in the next chapter.

3.5 Experimental Assessment of Bacterial Storage Yield

3.5.1 Conceptual basis

Batch experiments tests performed on readily biodegradable substrate have two phases of respiration: readily biodegradable and slowly biodegradable substrate (Dircks et al., 1999). ASM 1 is unable to simulate this behavior which is caused by microbial growth and microbial storage polymers of original readily biodegradable substrate. However, ASM3 could simulate this kind of outputs. ASM3 includes the concept of COD fractionations with two differences according to ASM1. first of all, readily biodegradable substrate, Ss, is defined as the biodegradable fraction of soluble COD (Gujer et.al., 2000). While, is recommended to estimate the amount of Ss in wastewater by respirometric tests to decrease model uncertainty in WWTP simulations (Koch et al., 2000). Secondly, ASM3 also assumed that all biodegradable COD is converted into storage products and growth occurs only by utilization of these products. Storage is a faster process compared to the growth and could be defined as dominant process in the system. The reaction kinetics and Stoichiometry of the mentioned ASM3 model of organic carbon removal, simplified for soluble COD as a sole carbon source as evaluated in this study, is given in Table 2.1

The basic Stoichiometry between the readily biodegradable COD consumed storage products generated and dissolved oxygen utilized under aerobic conditions could be schematized in the Figure 2.1. Where,

\[ \Delta S_{Sto} = (1 - Y_{Sto}) \Delta S_s \]  \hspace{1cm} (3.1)

\[ \Delta X_{Sto} = Y_{Sto} \Delta S_s \]  \hspace{1cm} (3.2)

The mass balance according to the figure for amount of consumed oxygen could be as below;

\[ \Delta O_{Sto} = (1 - Y_{Sto}) \Delta S_s \]  \hspace{1cm} (3.3)
At the depletion of the all initially available readily biodegradable substrate, $S_s$, (COD) the above equation could be manipulated to give the storage yield, $Y_{STO}$, of ASM3 model

Figure 3.2 COD Stoichiometry of aerobic storage in ASM3 (Karahan, Ö., G., et all, 2003)
4. RESULTS and DISCUSSIONS

4.1 Reactor Analysis

Mixed culture bacteria taken from biological activated sludge plant in Ataköy was used as an initial seed for sequencing batch reactor, (SBR). Some indicator parameters were taken for monitoring the reactor for 7 months. These parameters are listed below in the Table 4.1.

Table 4.1 Result of Monitoring the Steady State in SBR systems having 4 cycles in a day

<table>
<thead>
<tr>
<th>Substrate Type</th>
<th>Reactor</th>
<th>F/M</th>
<th>SS</th>
<th>VSS</th>
<th>VSS/SS</th>
<th>COD_{inf}</th>
<th>COD_{eff}</th>
<th>Eff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>SBR</td>
<td>0.588</td>
<td>8480</td>
<td>6800</td>
<td>0.8</td>
<td>1000</td>
<td>42</td>
<td>0.958</td>
</tr>
</tbody>
</table>

Steady state is very important condition state, because all calculation about mass balance calculations is made for system in the steady state condition. The mass balance involves the input, output and generation of parameters are under concern and there is no accumulation predicted. Knowing the importance of the steady state, monitoring must be executed very carefully and in detail. To examine the steady state condition, samples of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) from the SBR reactor have been taken and have been analyzed as described by Standard Methods (1998). The result of the monitoring is illustrated in Figure 4.1. For further monitoring the effluent COD parameters also have been taken with intervals. As it is demonstrated in the Figure 4.2 effluent COD result also point out an expected steady state condition is achieved.

Some calculations have been done to control the steady state condition according to conventional system.

\[
X_H = \frac{\theta_X Y_H (S_I - S)}{\theta_X (1 + b_H \theta_H)} = \frac{10}{0.40} \frac{0.70(1000 - 50)}{1 + 0.15 \times 10} = 6650 mgCOD/l
\]  

(4.1)
$$X_P = f_{EX}b_H \theta_X X_H = 0.2 \times 0.15 \times 10 \times 665 = 1995 \text{mgCOD/l}$$ (4.2)

Glycogen concentrations are measured as $X_{GLY} = 1100 \text{mgCOD/l}$.

$$X_T = X_H + X_{GLY} + X_P = 6650 + 1100 + 1995 = 9745 \text{mgCOD/l} = 6863 \text{mgVSS/l}$$ (4.3)

The calculated $X_T$ gave reliable results when compared with the measured VSS concentrations.

Figure 4.1: Monitoring the Suspended Solids (SS) and Volatile Suspended Solids (VSS)
Figure 4.2: Monitoring the Effluent COD parameters

Several measurements were conducted thorough a cycle. Sample taking have been done with time intervals. Sampling days are chosen randomly to see the reactor condition. The aim of the sampling was not only to know weather SBR is in steady state but also to have a general clue about the utilization of glucose during whole cycle time. As it can be seen from the Figure 4.3, nearly 75% of the glucose, 1000 mg COD/l, is depleted at first 30 min. after 60 minutes, whole glucose is utilized, and there is only soluble microbial product, S_p, left in the SBR. Experimental conditions are tabulated in Table 4.2.
4.2 Respirometric Results and Evaluation

Four sets of respirometric batch tests were conducted at various $S_0/X_0$ ratios, with glucose. MLVSS were taken directly from SBR in appropriate concentrations to fit the ratio. The response of the biomass acclimated to glucose was also evaluated in terms of respirometric measurements. Tests were started with biomass seeding alone to obtain first the oxygen uptake rate, (OUR), attributed to the level of initial endogenous respiration of the biomass. Then substrate samples were added on the biomass in the reactor for the monitoring of induced OUR profile. The experimental condition and initial conditions of batch test are tabulated in Table 4.2. OUR profile and the result of the test are illustrated in Figure 4.4. At specific points, sampling was executed to examine the behavior of the biomass. As it is plotted in the Figure 4.5, and Figure 4.6 more detailed, after 5 min the COD drops to 800 mgCOD/l. And after 90 mins the COD is almost depleted (Run 1).
Table 4.2 Experimental conditions.

<table>
<thead>
<tr>
<th>Set No</th>
<th>Reactor</th>
<th>Volume</th>
<th>Composition</th>
<th>Biomass mgVSS/l</th>
<th>Substrate mgCOD/l</th>
<th>S₀/X₀ Ratio COD/VSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>2</td>
<td>Glucose</td>
<td>1000</td>
<td>1000</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Run 2</td>
<td>2</td>
<td>Glucose</td>
<td>1000</td>
<td>2000</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Run 3</td>
<td>2</td>
<td>Glucose</td>
<td>500</td>
<td>1000</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Run 4</td>
<td>2</td>
<td>Glucose</td>
<td>1000</td>
<td>250</td>
<td></td>
<td>0.25</td>
</tr>
</tbody>
</table>

Figure 4.4: Respirometric OUR profile (Run 1)
For Run 1, maximum stored glycogen value was found as averaged 53% (mg glycogen (COD)/mg glucose (COD)), with a completely COD and glucose removal, illustrated in Figure 4.5.
Figure 4.7 show the OUR profiles for Run 1-4. According to the figure, it is seen clearly that F/M ratios affect overall trend of OUR curve.

![Graph showing OUR profiles for RUN 1 to RUN 4 over time with data points]

Figure 4.7 OUR profiles of the experimental RUNs in batch tests.

Calibration of the experimental OUR data was performed by means of model simulation using the AQUASIM program developed by Reichert [32]. Modified ASM3 model were used for model evaluations. For comparison, a fill&draw reactor data was used for modelling (Özdemir, 2005). The mentioned fill&draw reactor fed with 1000 mg COD/l as glucose had a hydraulic retention time of 1 day, a sludge age of 10 days. Maximum glycogen storage values was approx. 80% as mg Glycogen COD/mg Glucose COD. Both system had the same amount of COD of 1000 mg/l at the beginning but SBR system was stored as 535 mg glycogen/l while the fill&draw stored 800 mg glycogen/l at the end of the storage period. Modelling results are illustrated in Figure 4.8 and Figure 4.9. Model simulation results for four different sets of experiments are also shown in Figure 4.10. As seen from the figures modelling results present acceptable prediction of the respiration behaviour obtained in respirometric tests. The kinetic and stoichiometric parameters obtained for each F/M combination are given in Table 4.3.
### Table 4.3 Modelling Results

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Run 1</th>
<th>Özdemir (2005)</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{STO}$</td>
<td>20</td>
<td>40</td>
<td>1/d</td>
</tr>
<tr>
<td>$\mu_{H1}$</td>
<td>13</td>
<td>3</td>
<td>1/d</td>
</tr>
<tr>
<td>$\mu_{H2}$</td>
<td>3</td>
<td>0.5</td>
<td>1/d</td>
</tr>
<tr>
<td>$K_{S1}$</td>
<td>200</td>
<td>200</td>
<td>mg/l COD</td>
</tr>
<tr>
<td>$K_{S2}$</td>
<td>200</td>
<td>200</td>
<td>mg/l COD</td>
</tr>
<tr>
<td>$K_{STO}$</td>
<td>0.6</td>
<td>0.6</td>
<td>mg/l COD</td>
</tr>
<tr>
<td>$Y_{H1}$</td>
<td>0.74</td>
<td>0.70</td>
<td>gcellCOD/gCOD</td>
</tr>
<tr>
<td>$Y_{H2}$</td>
<td>0.74</td>
<td>0.70</td>
<td>gcellCOD/gCOD</td>
</tr>
<tr>
<td>$Y_{STO}$</td>
<td>0.94</td>
<td>0.94</td>
<td>gcellCOD/gCOD</td>
</tr>
<tr>
<td>$b_{STO}$</td>
<td>0.1</td>
<td>0.1</td>
<td>1/d</td>
</tr>
<tr>
<td>$b_{H}$</td>
<td>0.1</td>
<td>0.1</td>
<td>1/d</td>
</tr>
</tbody>
</table>

From the modeling result, it can be seen clearly that storage capacity for the fill&draw systems was higher that SBR systems. Growth on external substrate for SBR system was more dominate than for the fill&draw system according to the model and experimental evaluation.
Batch system and SBR system fed with the same initial feed, 1000mg COD/l, and in same F/M ratio experiments were conducted. Plotted OUR trends of SBR and batch systems and the model output of ASM3 fitting with the OUR data are illustrated in the Figure 4.8 and Figure 4.9 respectively.
All runs were modeled with the accordance of ASM3 for SBR, is shown in the Fig.4.10. Further evaluations and experiments will be carried out later to verify and adjust documented results in the present work.

Figure 4.10 Modeling all Runs according to ASM3
5. CONCLUSION

SBR reactor was fed with initial feed of 1000mgCOD/l/ cycle. Cycles were chosen to be 4 per day which indicate a 6 hour cyclic time. COD removal efficiency of 96% was measured at the steady state conditions. System was fed with the sole carbon source of glucose, needed macro and micro nutrients were added in appropriate concentrations to maintain growth. MLVSS and MLSS concentrations were 6800 mgVSS/l and 8480mg SS/l respectively in steady state condition.

To determine the effect of the F/M ration on the substrate storage mechanism, several experiments were executed with different F/M ratios. In the F/M= 1 (Run 1) experimental work storage and COD consumptions were measured during time, resulting of 53% mg Glycogen/mg Glucose. This result was very different from the fill&draw experiment executed by Özdemir since it was approx 80% mg Glycogen/mg Glucose (2005). Also OUR profiles were obtained for 4 different F/M rations i.e. for Run 1, Run2, Run3, and Run4. These data were modeled by using the ASM3. Coefficient values estimated by model were compared with the Özdemir (2005). Results showed a difference with the mentioned study, indicating a different microbial behavior for storage between SBR and Fill&draw systems. To verify and adjust the values documented in the present work, several more experiments should be carried out, thus, more detailed models should be executed and coefficients should be estimated.
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Erol Çavuş was born in Sofia, Bulgaria. He finished primary education in Bulgarian elementary school. He immigrated to Turkey, Istanbul at 1992. He was accepted in ITU at 1998 to study B.Sc in Environmental engineering. He completed the course in 2004 and, immediately applied to the M.Sc in the same university. He was accepted in Environmental Biotechnology program. He has been studying in the same program till June 2005.
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