ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

EFFECT OF DECREASING SETTLING TIME ON SETTLEABILITY PROPERTIES OF BIOMASS IN AN ANAEROBIC /AEROBIC SEQUENCING BATCH REACTOR SYSTEM

M.Sc. THESIS

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ÇÖKELME SÜRESİNİN KADEMELİ OLARAK AZALTILMASININ ANAEROBİL/AEROBİK ARDIŞIK KESİKLİ REAKTÖR SİSTEMİNDEKİ BİOKÜTLENİN ÇÖKELME ÖZELLİKLERİNE ETKİSİ

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EYLÜL 2013

FATEMEH SADRZADEH, a **M.Sc.** student of ITU **Graduate School of Engineering and Technology** student ID 501101801, successfully defended the **thesis/dissertation** entitled "**Effect of Decreasing Settling Time on Settleability Properties of Biomass in an Anaerobic/Aerobic Sequencing Batch Reactor System**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 03 MAY 2013
Date of Defense : 10 SEPTEMBER 2013

To my family,

I would especially like to thank my amazing family for the love, support, and constant encouragement I have gotten over the years. In particular, I would like to thank my mother and my brothers. You are the salt of earth, and I undounbtedly could not have done this without you.

FOREWORD

To My Supervisor
The thesis is carried out at the Laboratory Of Envioromental Engineering Department of ISTANBUL TECHNICAL UNIVERSITY, in close collaboration with my supervisor Assist.Prof.Dr.Ebru Dülekgürgen. I would like to thank my supervisor for guiding and supporting me over the thesis. You have set an example of excellense as a researcher, Mentor, Instructor and Role model.

I would like to thank my professors,Özlem Karahan and Nevin özgür Yağcı for all of their guidance, constant enthusiasm and encouragement.

SEPTEMBER 2013

Fatemeh Sadrzadeh
(Enviromental Biotechnology)

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ABBREVIATIONS

MLSS : Mixed liquor suspended solids
MLVSS : Mixed liquor volatile suspended solids
SVI : Sludge volume index
COD : Chemical oxygen demand
BOD : Biochemical oxygen demand
ESS : Effluent suspended solids
EVSS : Effluent volatile suspended solids
PHA : Polyhydroxy alkonate
GAS : Aerobic granular activated sludge
Ts : Settling time
BioWWT : Biological wastewater treatment
AS : Activated sludge
SBR : Sequencing batch reactor
GC : Gas chromatography
HCL : Hydrochloric
PHB : Polyhydroxy butyrate
PHV : Polyhydroxy valerate
PHA : Polyhydroxyalkonate
HPLC : High performance liquid chromatography
OUR : Oxygen uptake rate

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EFFECT OF DECREASING SETTLING TIME ON SETTLEABILITY PROPERTIES OF A BIOMASS IN AN ANAEROBIC/AEROBIC SEQUENCING BATCH REACTOR SYSTEM

SUMMARY

Biological wastewater treatment(BioWWT) is an important and integral part of wastewater treatment plants that treats wastewater municipalities or industries having soluble/particulate organic impurities or a mix of the two types of wastewater sources thus treated effluent can separate from the biomass. The obvious economic advantage, both in terms of capital investment and operating costs of biological treatment over treatment processes like chemical oxidation; thermal oxidation etc; has cemented its place in any integrated wastewater treatment plant. Biological treatment using aerobic activated sludge(AS) process has been in practice for well over a century.

One of the most important factors that will affects the design and operation of AS system is settleability of biomass. Aerobic granular microorganisms are dominant over the conventional floccular structure in Aerobic Granular activated sludge (AerGAS) systems and where granules have excellent settling properties. Due to excellent settling capacity, the time and working volume which are required for separation of sludge-liquid phase decrease thus costs are reduced. Also; in such sytems high biomass concentration obtained. Therefore, settling capacity of biomass acts as a crucial factor in the design and operation of AS system.

According to the "Selection Pressure Theory" it is possible to select for microbial populations with certain desired properties by imposing external stress on biomass and creating hydraulic, kinetic, or metabolic selection pressures . Among mentioned factors; selecting time(Ts) acts as one of the main hydraulic selection pressures for washing out slowly settling flocks thus help to retain rapidly settling biomass in sequencing batch reactors.

In this thesis, the effect of gradually decreasing settling time on settling properties of biomass in an anaerobic/ aerobic SBR system was examined. The reactor was fed with starch and acetate solution having a soluble COD of 854 mg/L. For washing out microorganisms which had a slow settling capacity ; the settling time was gradually decreased from 45 to 30,15,5 and finally to 1 minute and the system was monitored for biomass production (MLSS and MLVSS), biomass washout(ESS) , settling capacity of biomass (SVI) and COD removal for 10 month.
Standard methods were used for measurement of MLSS, MLVSS, SVI, COD, ESS, EVSS were measuredaccording to the ISO6060 protocols, PHA and glycogen were measured using internationally accepted and widely used methods.
Monitoring results showed that, a significant biomass washout occured each time the settling time was decreased to 5 minutes, biomass production in the system stabilized, average biomass concentration was 10g MLVSS/L , SVI_{10} and SVI_{30} values stabilized at 20 and 18 ml/g, and COD removal efficiency was 95%.
Despite biomass escaping to the effluent, good SVI values and high biomass concentrations were obtained in the system. Results for operation with one min settling time was similar. Furthermore, results highlited the importance of minimum

settling velocity (V_{vmin}) along with the settling time(Ts) as the hydraulic pressure for washing out slowly settling flocks and maintaining rapidly settling biomass and good effluent quality.

ÇÖKELME SÜRESİNİN KADEMELİ OLARAK AZALTILMASININ ANAEROBİK/AEROBİK ARDIŞIK KESİKLİ REAKTÖR SİSTEMİNDEKİ BİOKÜTLENİN ÇÖKELME ÖZELLİKLERİNE ETKİSİ

ÖZET

Hızlı nüfus artışı ve sanayileşme sonucunda oluşan atık sular tabiatın özümleyebileceği miktarı aşmış ve alıcı ortamları kirlenme tehlikesi ile karşı karşıya bırakmıştır. Doğadaki ekolojik dengeyi olumsuz yönde etkileyebilecek ve diğer faydalı kullanımlarını engelleyecek bu durumun önüne geçebilmek için atıksuları uzaklaştırmadan önce arıtma zorunluluğu doğmuştur.Biolojik atıksu arıtımının amacı; atıksudakı çökelemeyen kolloidal ve organik maddeleri farklı mikrooganizmalar yardımıyla gidermek ve kararlı hale getirmektir. Biyolojik arıtmanın temeli tek hücreli canlılardır.Yani mikroorganizmalardır.

Mikroorganizmalar da diğer canlılar gibi doğar, beslenir, solunum yapar, ürer ve ölür.Mikroorganizmalar gerekli şartların sağlanması durumunda çok hızlı bir şekilde üreyebilirler. Bu şartlar tüm canlılar için gerekli olan oksijen ve besin maddesidir.Bakteriler gıda ihtiyacı olarak atıksuda fazlaca mevcut bulunan ve çevreye verildiğinde kirlilik yaratan organik maddeleri kullanırlar.Bakterilerin solunum yapması ve çoğalması için suda bulunan çözünmüş oksijen yeterli olmaz. Bunun için dışarıdan oksijen verilmesi gerekmektedir. Dışarıdaki havayı suya veren mekanik ekipmanlar sayesinde gerekli oksijen temin edilmiş olur.Böylece atıksu içerisindeki mikroorganizmalar gelişerek suda kirliliğe sebep olan tüm organik madde ve kirletici maddeleri yok ederler. Bu işlem sonunda atıksu içindeki organik maddeler biyolojik olarak ayrıştırılır.

Seleksiyon teorisine göre, biokütleye dış stres faktörleri uygulayarak ve hidrolik, kinetik veya metabolik seleksiyon baskıları yaratarak istenen belirli özelliklerde mikrobiyal popülasyonların seçimi mümkündür(wanner, 1994). Bu dış arasında, çökelme süresi(Ts;dk) yavaş flokları yıkayıp uzaklaştıran ve dolaysıyla ardışık kesikli reaktör (AKR) sistemlerinde hızlı çökelen biokütlenin üretilmesine yardımcı olan ana hidrolik seleksiyon baskılarından biri olarak edilmektedir (Qin et al 2004).

Proje önerisi kapsamında karbon kaynağı olarak çözünmüş nişasta ve asetat ile beslenen labratuvar ölçekli ve 2L çalışma hacimli bir AKR'de elde edilen biokütlenin çökelme kapasitesi ve organik madde giderim verimleri araştırılmıştır. Biokütlenin çökelme özelliklerinin iyileştirilmesi hedefli temel strateji olarak reaktördeki çökelme süresi kademeli olarak azaltılan sistemden toplanan örneklerde askıda katı madde(AKM) , çamur hacim indeksi, KOİ giderim verimi, hücre-içi karbon depolama polimerleri olan PHA'lar ve glikojenlerin ölçümü ile sistem karakterize edilmiştir.

Bu araştırmada sistem asetat ve çözünmüş nişasta ile beslenen sistemden elde edilen numunelerde aşağıda sıralı parametreler ölçülmüş.

Parametre birim açıklamalar aşagıda acıkladıgım gibidir:

AKM mg/l reaktör içerisindeki toplam askıda katı madde

UAKM mg/l reaktör içerisindeki biokütle knstrasyonunu gösteren uçucu askıda katı madde

ÇHİ ml/g biokütlenin çökelme kapasitesinin göstergesi olan çamur hacim indeksi

D-AKM mg/l çıkış suyu kalitesi açısından cıkış suyu toplam askıda katı madde miktarı

D-UAKM mg/l çıkış suyu kalitesi açısından çıkış suyu uçucu askıda katı madde miktarı

KOİ mg/l çıkış suyu kalitesi ve sistemin organik madde giderim verimi ile ilgili olarak giriş ve çıkış suyu süzülmüş KOİ değerleri

PHB ve PHV cinsinden PHA mg/l KOİ ekivanlanı farklı işletme koşullarında biokimyasal dönüşüm süreçlerinin irdelenmesi için gerçekleştirilen çevrim-içi deneylerin ve bunlara paralel yürütülen respirometrik deneylerin bazılarında AKR ve respirometreden tam karışım örnekleri toplanır ve hücre-içi karbon depolama süreçlerinin aydınlatılabilmesi amacıyla PHA (polihidroksialkonat) ölçümleri Gaz Kromatografi ile gerçekleştirilmiş.

AKM ve UAKM deneyde AP40 filtreden kullanılmıştır. Filtreyi distile su ve musluk suyla yıkanıp 1saat etüvde (105 ˚C) kurutmuştur. Sonra etüvden çıkarıp nemden etkilenmemesi için 30 dakikada desikatörde beklenmıştır. 1saat beklenip desikatöre konululdu. Desikatörde 30 dakika beklenildi. Sonra tartım yapıldı. Bu filtreyi 15 dakika için 550'de konuldu. Yarım saat beklenip tartım yapıldı. Sonra standard metode göre hesaplamalar yapıldı.

KOI deney için numune hacmi seçildi. Her numune için çift çalışıldı. Sonra su ile 5ml'ye tamamlandı. Üzerine 1.5 ml dikromat ve 3.5 ml asit eklendi(standart metode göre). 2 saat 150 C'de kaynatıldı. Sonra titrasyon yapıldı. Hesaplamalar standard metode göre yapıldı.

PHA deneyi için reaktörden 25-30 ml numune alındı. Sentrifüjlendı. Üst faz dökülünecek. 4.5 ml fosfat buffer eklendi. Yine sentrifujlendi. Freeze-dry yapıldı. 48 saat sonra freeze dry dan çıkarıp tartım yapıldı. Tartımlar kaydedildi. Standardlar eklenip kaynatıldı. Üst faz alındı ve GC'de ölçüldü. Glikojen parçalama işlemi için 4.5 ml numune alındı. Üzerine 0.5 ml HCL eklendi. Numuneler 5saat için kaynatıldı. Pastor pipet ile HPLC viallara aktarıldı. Sonra okulamalar HPLC ile yapıldı.

Sonuç olarak, çökelme süresi azaltınca iyi bir biokütle giderimi elde edildi. Çökelme süresi 5 dakikaya gelince, biökütle miktari reaktörde sabitlendi. Ortalama olarak, elde edilen biokütle miktari 10g AKM/L, çamur hacim indeksi($ÇHI_{1O}$ ve $ÇHI_{30}$) 20 ve 18 ml/g ve KOI giderimi 75%di. Biokütle giderimi rağmen, iyi bir çamur hacim indeksi ve yüksek biokütle konstrasyon systemde elde edildi.

Analizlerin sonuçta minimum çökelme hızının, çökelme süresinden daha etkili olduğu isbat edildi. Minimum çökelme hızı, yavaş çökelen biokütlelerin systemde kalmasına etkilidir.

Sonuç olarak, anaerobik fazda PHB,PHV,PHA ve glikoz üretimi artar çünkü systeme besleme yapılır ve bu besleme hücre içinde depolanır çünkü elektron verici vardir ama elektron alıcı olmadığı için bu beslemeyi kullanamaz oyüzden depolanır ve aerobik fazda kullanılır çünkü aerobik fazda oxyjen (elektron alıcı) vardır ve bu enerjiyi mikroorganizmalarin büyümesi için kullanacak buyüzden PHB,PHV,PHA ve glykoz'un konsantrasyonu aerobik fazında azaltır.

1. INTRODUCTION

It is one of the basic concepts of wastewater treatment that sludge settling is exceptionally important to maintain good effluent quality made by biological nutrient removal. Many researchers had developed various diagnosis tools and models to detect setlleability[1].

This thesis aims to evaluate:

1) Effect of gradually decreasing settling time on settleability properties of biomass.

2) While obtaining a good COD removal efficiency.

3) Investment the intracellular C-storage and growth phenomena of biomass in an anaerobic/aerobic SBR.

To achive this goal, the methodologies for evaluation of mixed liquor suspended solids(MLSS) and mixed liquor volatile suspended solids(MLVSS) , effeluent suspended solids(ESS), effluent volatile suspended solids(EVSS),chemical oxygen demand(COD), sludge volume index(SVI), polyhydroxy butyric acid(PHA) and glycogen test were applied.

2.LITERATURE

Sludge can also be characterized by how well it settles. Most settling test to measure the sludge settleability is developed by Mohlman (1934) and called the Sludge Volume Index(SVI).

SVI is conducted with a homogenous sludge mixture. It is defined as " the volume in ml occupied by 1 g activated sludge after settling the aerated liquor for 30 min"[7].

SVI is a very important indicator that determines your control or rate of desludging on how much sludge is to be returned to the aeration basin and how much to take it out from the system. It actually serves as a very important empirical measurement that can be used as a guide to maintain sufficient concentration of activated sludge in the aeration basin whereby too much or too little can be considered determinetal to the system's overall health.

Effect of decreasing settling time(which measured by SVI) is on acting as hydraulic pressure for washing out slowly flocks and obtain rapidly settling biomass and good effluent quality[2].

Two commonly measures developed to quantify the settling characteristics of activated sludge are SVI and the zone settling rate (WEF,1998). The SVI is the volume of ml occupied by 1g of suspension after 30min of settling. Although SVI is not theoretically supported, experience has shown it is useful in routine process control. SVI typically is used to monitor the settling characteristics of activated sludge and can impact on return sludge rate and MLSS. SVI is calculated from the laboratory test results of the suspended solids concentration of a well mixed sample of the suspension and the 30-min settled sludge volume. The sludge volume is measured by filling a 1-liter graduated cylinder to the 1.0 liter mark, allowing settling for 30 min, and then reading the volume of settled solids. The mixed liquid suspended solids is determined by filtering, drynig, and weighting a sample of the

mixed liquor with standard methods which is glass-fiber filter disk. The value of SVI can be calculated by the following formula(Standard methods-APHA et al.,1998).

$$SVI = \frac{\text{Settled volume of sludge}, \frac{\text{ml}}{\text{L}} \quad \frac{10^3 \text{mg}}{\text{g}}}{\text{Suspended solids}, \frac{\text{mg}}{\text{L}}} = \frac{\text{ml}}{\text{g}} \qquad \textbf{(1)}$$

For example, a mixed-liquor sample with a 3000 mg/L TSS concentration that settles to a volume of 300 mL in 1-L cyclinder would have an SVI of 100 mL/g. A value of 100 mL/g is considered a good settling sludge (SVI values below 100 are desired). SVI values above 150 are typically associated with aboundant filamentous microorganisms.

Typically values of SVI for domestic activated-sludge plants operating with an MLSS concentration of 2000 to 3500 mg/L range from 80 to 150 mL/g.

Because the SVI test is empirical, it is subject to significant errors. For example, if sludge with a concentration of 10.000 mg/L did not settle at all after 30min, the SVI value would be 100. To avoid erroneous results and to allow for a meaningful comparison of SVI results for different sludges, the diluted SVI(DSVI) test has been used effluent until the settled volume after 30 min is 250 Ml/L or less. The standard SVI test is then followed with the sample.

Many SVI tests at wastewater treatment plants are done in a 2-L settleometer that has a larger diameter than 1- or 2-L graduated cylinders. To eliminate effects on solids settling in a small settling in a small diameter test apparatus, use of slow-speed stiring device is encouraged . The test is called a stirred SVI when a stirring device is used. The stirred SVI test is used frequently in Europe.

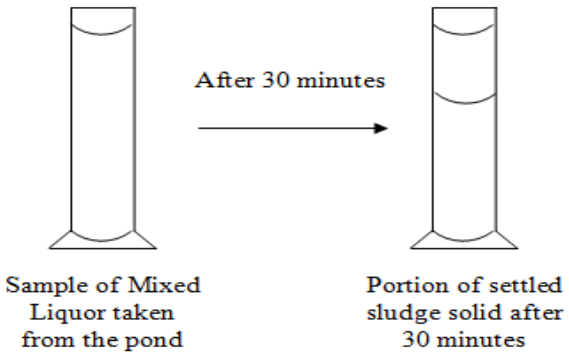

Figure 2.1: SVI test.

Typically SVI values and their meaning are and corresponding sludge properties given in Table 2.1.

Table 2.1 : Typically SVI values and corresponding sludge propeties (Metcalf &Eddy, Wastewater engineering treatment).

SVI Range	**Activated Sludge Properties**
Less than 50 ml/g	Pin floc
50 to 100 ml/g	Good range Potential
100 to 150 ml/g	Filamentous growth
150 to 200 ml/g	Bulking at high flows
200 to 300 ml/g	Bulking
Higher than 300 ml/g	Severe Bulking

Many authors recognise SVI as the parameter best characterising sludge settling properties. SVI is also a good indicator of sludge bulking. In practice, SVI can vary from 30 to 400 ml g-1[3,4].However, it usually does not exceed the value of 150 ml g-1 which is an indicator of good settling properties of the sludge. Palm and Jenkins reported that sludge of the SVI over 150 ml g-1 is often classified as bulking sludge[5]. The same authors also pointed out the dangers associated with too low SVI in the conventional wastewater treatment systems. In the thesis; we have found out that quickly settling sludge(SVI below 50 ml g-1) can be the reason for granular microorganisms are produced more than flocks structure in the system and the produced granular have good settling capacity which can settle fast.

A proper SVI value, especially below 100 mlg-1 , is of major importance in the activated sludge method. Although Rensink and Donker have demonstrated that organic compounds are better removal by well-settling sludge(of low SVI[6].

The use of SVI is controversial. According to several authors , a bad correlation with the settleability characteristics of aerobic sludge[7-10]. The latters authors also agreed that a more accurate procedure for the evaluation of the settleability seems to be the one develped by settleability which is based on the relationship between the solid zone settling velocity and the sludge concentration. This method generates two

emprical parameters, namely ‘K’ and Us,o which provide insight into the hydrodynamics of the aerobic sludge flocks[11]. However, the use of this kind of test for anaerobic sludge is so far not reported. In this case, other methods were used to access the settleability by using SVI [12-16].

2.1 Effect of settling time on settling properties of biomass

The biological treatment of wastewater in the sewage treatment plant is often accomplished by using conventional activated sludge system. These seystems generally require large surface areas for treatment and biomass seperation units due to the generally poor settling properties of the sludge. In recent years, new technologies have been developed to improve settleability. The use of aerobic granular sludge technology is one of them. As a self immobilized biotechnology for wastewater treatment, aerobic granular is characterized by outstanding excellent settling properties(below 100 ml/g) just in a single granule [17-19] , while the mechanism behind aerobic granulation in SBR are not fully understood yet[20,21].

The physical settling-washing out action was a pure screening step without a demand for the microbes to respond to or to make changes upon the settling time carryout, hence having different intended meaning by the biological evaluation theory. In SBR operation, only particles thet settle within a given time frame could be retained in the reactor, while those with poor settleability were washed out from the system. According to biological evaluation theory, this physical screening step was considered to provide a ‘’selection pressure’’ to the biomass in the reactor, and only those which adapted to this challenge (to become big and dense enough to settle fast) would survive and be retained in the reactor. Tay et al studied nitrifying bacterial granulation at different selection pressure and concluded the need of high selection forces for granulation. The required selection pressure had been created by keeping the constant column height and varying the discharge port height. The low settling time in this thesis was to use to obtain aerobic granular sludge[22].

In recent years, many researchers have focused on reducing the settling time required for activated sludge flocs by forming dense flocks. The settling time acts as a major hydraulic selection pressure on microbial community[23-26]. A short settling time selects for the growth of fast settling bacteria and the sludge with a poor settleability is washed out. Wang Q.et al concluded that aerobic granular sludge was

cultivated by means of decreasing sedimentation time to wash out poor-settling biomass and increasing organic loading rate ; Kg COD/m3.day(OLR) to ensure sufficient new biomass growth, granules started to appeared on the 67th days[27]. Qin et al reported that aerobic granules were successfully cultivated and became dominant only in the SBR operated with a settling time of 5minutes mixture of aerobic granules and flocks were observed inthe SBRs run at settling time of 20, 15 and 10 mintes[28]. Therefore, choice of an optimal settling time was very important in aerobic granulation. Short settling time caused poorly settled suspended biomass washout and retained only well settled granules. Studies have indicated that short settling time could enhance aerobic granulation. McSwain et al demonstrated that after 2 minutes of settling time, the mature granules were well[29].

2.2 Aerobic Granulation

Aerobic granulation is a process of cell autoaggregation through cell-to-cell immobilization to form a stable, contiguous, multicellular association. These aggregated granules have a compact structure as compared to suspended sludge flocs[30]. Studies showed that aerobic granulation is a gradual process from seed sludge to compact aggregates, further to granular sludge and finally to mature granules[31]. Aerobic granules have been successfully cultivated with a wide variety of organic substrates in SBR, including glucose, acetate, ethanol, ohenol, particulate organic matter-rich wastewater and both simulated and real municipla wastewater

[17,22,25], while nitrifying and phosphorous accumulating granules have also been developed [24].These seem to indicate that the formation of aerobic granules is a process independent of or insensitive to the characteristics of the feed wastewater, while evidence of mature aerobic granules are closely related to the type of substrates used [22].

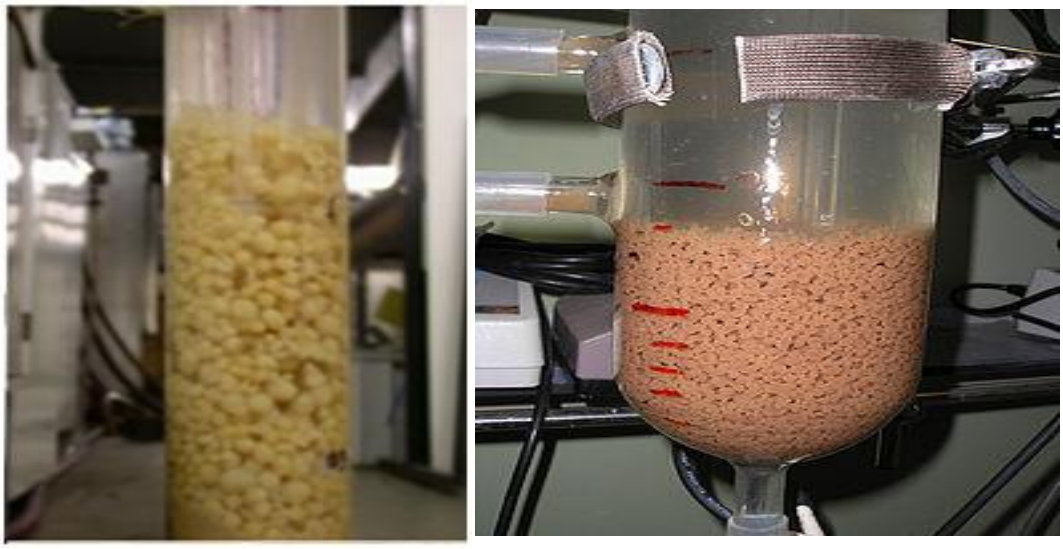

Figure 2.2: SBR reactors with aerobic granules(Left; Dulekgurgen, 2006; right: Mosquerra-Coral).

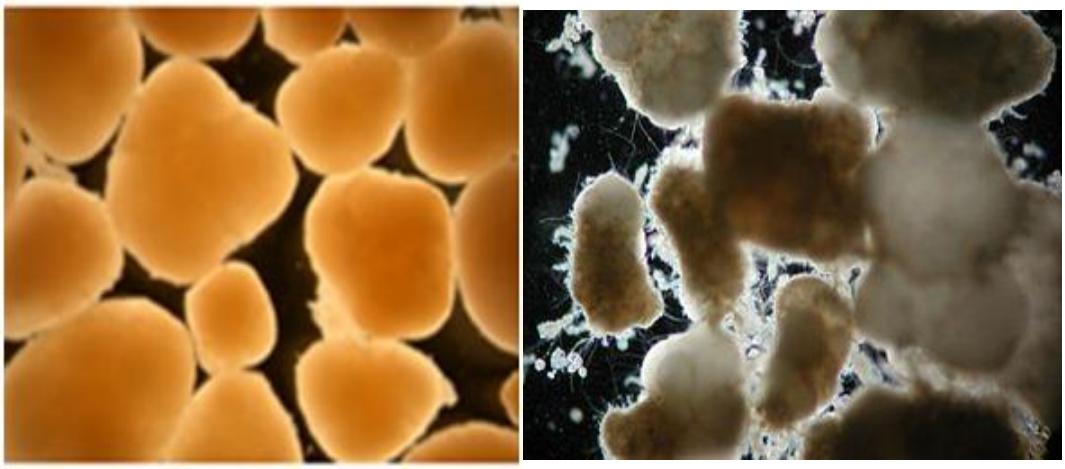

Fig 2.3: Aerobic granules treating synthetic wastewater(Left; Dulekgurgen, 2006) and municipal wastewater (right; de Kreuk et al.,2004).

Several factors can affect the process of cell aggregation such as feeding composition and the reactor design, which affects the hydrodynamic shear forces and the extracellular polymeric substances secreted by cells [32]. To achieve aerobic granulation of the biomass, reactors are operated in cycles comprising short feeding and settling periods and long aerated periods. Selection of slow growing granular biomass is achieved by the feast and famine regime and the well settling biomass selection by the use of short settling periods.

Granular biomass is developed in sequencing batch reactors (SBR) and without carrier materials. These systems fulfill most of the requirements for their formation such as:

1) Hydrodynamic shear force: Evidenceshow that the applicationof high shear force favours the formation of aerobic granules and the physical granule integrity. It was found that aerobic granules could be formed only above a threshold shear force value in terms of superficial upflow air velocity above 1.2 cm/s in a column SBR, and more regular , rounder, and more compact aerobic granules were developed at high hydrodynamic shear force(Tay et al,2001)[33] on the other hand; Dulekgurgen et al(2008) showed that external shear force due to a combination of mechanical

moving & upflow anaerobic prevented progress of aerobic granulation and it was only after decreasing those shear force effects that aerobic granulation were formed in the system.

2) Feast -Famine regime: In fully aerated systems, short feeding periods must be selected to create feast and famine periods, characterized by the presence or absence of organic matter in the liquid media, respectively[25]. With this feeding stategy the selection of the appropriate microorganisms to form granules is achieved. When the substrate concentration in the bulk liquid is high, the granule former organism can store the organic matter in form of poly-B- hydroxybutyrate to be consumed in the famine period, giving an advantage over filamentous organisms.

3) Metabolic selection: Feeding under anaerobic conditions(long & continous or short & rapid) , then applying antron(Dulekgurgen 2003, de kreuk 2004 et al).

Usually aerobic granulation is achieved in SBRs with a high height to diameter (H/D) ratio mixed by the application of air [34] . The hydrodynamic conditions that are created by the air depend on the aeration flow and are described by the upflow air velocity. In these systems the reactor configuration has an impact on the flow patterns of liquid and microbial aggregates inside the reactor. The upflow pattern of the air favours the motion of biomass granules which are constantly subjected to circular hydraulic attrition thus increased shearing forces [30].

4) Cycle time: Basically , the SBR cycle time represents the duration of the substrate oxidation reaction; and it is interelated to the hydraulic retention time. If the SBR is run at an extremely short cycle time, microbial growth can be hindered by an insufficient reaction time for micro-organisms to break down substrates. As a result, the sludge loss due to hydraulic washout from the system cannot be compensated by the growth of bacteria. Previous research showed that when a complete washout of sludge blanket occured, a failure of nitrifying granulation resulted at a very short cycle time [2002]. In contrast, if the cycle time is kept much longer than that requiered for abiological reaction, hydrolysis of the biomass would eventually cause a negative effect on microbial aggregation [39]. The cycle time of SBR should be short to suppress biomass hydrolysis, but long enough for biomass growth and accumulation in the system. Recent research clearly demonstrated that, for SBRs operated at the optimum cycle time determined previously, aerobic granules still

could not form if the settling time was kept longer than 15min, as discussed in the next section [28]. This implies that cycle time is not a decisive factor for aerobic granulation in SBR.

5) Exchange ratio: The exchange ratio in SBR is defined as the liquid volume withdrawn at the end of the given settling time over the total reactor working volume. For column SBR with the same diameter, the exchange ratio is proportionally correlated to L, the height from the discharging port to the water surface. To date, very limited research effort has been given to looking into importance of the exchange ratio in aerobic granulation in SBR. Just recenly, Wang (2004) seriously studied aerobic granulation in SBR run at different exchange ratios in the range of 20-80% while the settling time was kept constant at 5 min. Findings demonstrated that a larger exchange ratio is associated with a higher L. The fraction of aerobic granules in the total biomass was found to be proportionally related to the exchange ratio, e.g. only in the SBR run at the higher exchange ratios of 60% and 80% were aerobic granules dominant; and a mixture of aerobic granules and suspended sludge instead of pure aerobic granules developed at the smaller exchange ratios of 40% and 20%. In addition, Zhu and Wilderer (2003) successfully developed aerobic granules at a fixed exchange ratio of 75%. These results provide experimental evidence that aerobic granulation is highly dependent on the exchange ratio of SBR.

The feasibility and efficiency of other types of bioreactors, such as completely stirred tank reactor, applied to the development of aerobic granular sludge have not been sufficiently demonstrated. The different features of these systems rely not only on the hydrodynamics, in terms of interactive patterns between flow and microbial aggregates, but also on the active selection of the biomass during the settling period of the operational cycle, which allows for different minimum settling velocities for the biomass to be retained inside the reactor (Liu & Tay 2002)[30]. In this aspect , the coulumn type upflow reactor with high H/D can provide an optimal interactive pattern between flow and microbial aggregates for granulation while systems with low H/D ratio and mechanical stirring provide different hydrodynamic conditions (Dulekgurgen 2008; rome SBR Congresses, 2nd paper). Furthermore a high H/D results in a reactor with a small footprint **,**This may be a major reason for granular sludge formation in column-type upflow reactors[25,35]. In an engineering sense, the

desirable interactive patttern between flow and aggregates might be achived by controlling reactor configurations and operation strategy. Consequently , it is interesting to research new types of reactors to study their feasibility to produce granular sludge.

Studying the possibility of forming granules on wastewater with low organic matter composition in a SBR was a logical step in the scalling-up process and development of this technology [36]. De kreuk & van loosdrecht studied the formation of aerobic granules with domestic sewage and they found that the chemical oxygen demand (COD) load could be a critical factor for granulation[37]. Domestic sewage typically has much lower organic matter content than industrial wastewaters do and this will influence the granule formation process[38]. Tay et al, demonstrated that the organic loading rate (OLR) affected the formation of aerobic granules[39]. They found that there were no granules formed under the OLR of 1 kg COD/m3 d. Instead , flocs with rather loose structure dominated reactor mixed-liquor. They also found that the biomass was always a mixture of granules and flocs when the OLR appeared to be unfavorable for the formation of a compact sludge bed, and further, for maintaining the stability of the reactor.

2.3 Effect of settling time on aerobic granulation and sludge settleability

SVI has been commonly used to describe the sludge settleability and compactness. Lei Qin , Yu Lin et al. Examined the effect of settling time on sludge settleability. They operated four SBRs with four column(R1-R4) which were 127 cm in height and 5cm in diameter, each with a working volume of 2.51 L.

R1-R4 were operated at arespective settling times of 20,15,10 and 5min, while the other operation conditions were kept the same(4h of cycle time, 5min of filling, and 5 min of effluent withdrawl). The remaining time in one cycle is the aerobic reaction period. Fine air bubbles were introduced at an air flow rate of 0.3/min through a dispenser located at the bottom of reactors,which gives a dissolve oxygen concentration of above 50% saturation. A hydraulic retention time of 8 h was maintained in all reactors. The sequential operation of the reactors was automatically controlled by timers, while two peristaltic pumps were employed for influent filling and effluent withdrawal. In order to look into the effect of settling time on aerobic granulation , in the first phase of the study, R1-R4 were run at respective settling

times of 20,15,10 and 5min. After the stabilization of four reactors, the settling times in R1-R3 were further shortened to 5,2 and 1min in the second phase of the study.

According to Qin, the seed sludge had a mean floc size of 110μm, and SVI of 230 ml/g. After 7days of operation ,aerobic granules were firstly observed in R4 operated at the settling time of 5min. On day 10,tiny aggregates appeared in R1-R3 run at a respective settling times of 20,15 and 10 min. After three-week operation , four reactors reached steady state. The biomass concentration in steady states R1-R4 was 5.3, 4.9,5.5 and 5.4 g. According to results which showed morphology of aerobic granules developed in R1-R4 evaluated that those aerobic granules had a very regular and spherical outer shape indicates by an aspect ratio higher than 0.78. Due to results which obtained with measuring fraction of aerobic granules concluded that size of granules are above 0.35mm in steady state R1-R4. It found that the fraction of aerobic granules only at the shortest settling time (R4) which was 5min was about 10%. These indicates that a mixture of aerobic granules and suspended sludge were developed in R1-R3 instead of aerobic granules as observed in R4. The fraction of aerobic granules in the reactors seems to be related to the settling time.

The fraction of aerobic granules decreased with decreasing settling time thus concluded that the SVI was closely related to the settling time.

3.HYPOTHESIS

It is inferrable from the literature reviews, that it is poosible to use methods to measure the effect of decreasing settling time on properties of biomass in an anaerobic/aerobic SBR system. For example, different settling times in a gradually decreasing order might be applied as a strategy to examine if settling properties of biomass are improved or not. A short settling time selects for the growth of fast settling bacteria [27]. Wang et al. concluded that aerobic granules obtained by decreasing settling time because it washed out poorly settling bacteria forming flocks. Qin et al. 2004 reported that aerobic granules were dominant in the SBR which operated at settling time of 5 minutes[28].

4. MATERIALS AND METHODS

4.1 Design of SBR System

Experiments were done at Istanbul Technical university Enviromental Engineering and Genetic Laboratory.

The experiment were conducted with an anaerobic/aerobic sequencing batch reactor(SBR) system which the working volume was 2L and Hw/D (height to diameter) was 1.2. The bioreactor was involved with a BiostatBplus which is connected to a software for collecting measured data, mechanical stirrer which used for mixing, temperature probes which measured inside temperature in order to whether inside temperature is appropriate microorganisms or not, PH probe, dissolved oxygen (DO) inside the reactor was controlled by a mass flow meter which has to measure 0% saturation during anaerobic phase and 40% during aerobic phase always.

Figure 4.1: The SBR used in the study,Biostat B plus,Sartorious Stedim,Germany).

acetate solution: sodium Acetate was used as the rapidly biodegradable , soluble organic matter(carbon) source.

starch solution : soluble starch was used as the rapidly hydrolysable and slowly biodegradable organic matter(carbon source)

Solution A :Nutrient (N and P) source with NH4Cl, KH2PO4, K2HPO4

Solution B :Trace elements source with MgSO4, CaCl2, MnSO4, FeSO4, ZnSO4

The treatment system with the SBR model was designed which has five sequencing steps for seven month. The five steps are: Fill, React(Anaeobic phase+Aerobic), Settle, With-Draw and Idle. The operated five sequencing steps were different during different settling time. For instance; the cycle configuration for 45min is as belows: feeding(Tf): 5.5min, nitrogen purging(T_{N2}):15min, mechanical mixing(Tstir): 99.5min, anaerobic period (Tanaer): 120min(Tanaer=Tf+T_{N2}+Tstir), aerobic period(Taer):180min, settling time(Ts):45min, discharge(Tw): 6min, idle period (Ti): 9min. This cycle configuration for Ts=45min is schematically presented in Figure 4.2.The full treatment cycle lasted approximately 360 minutes(6 hours).

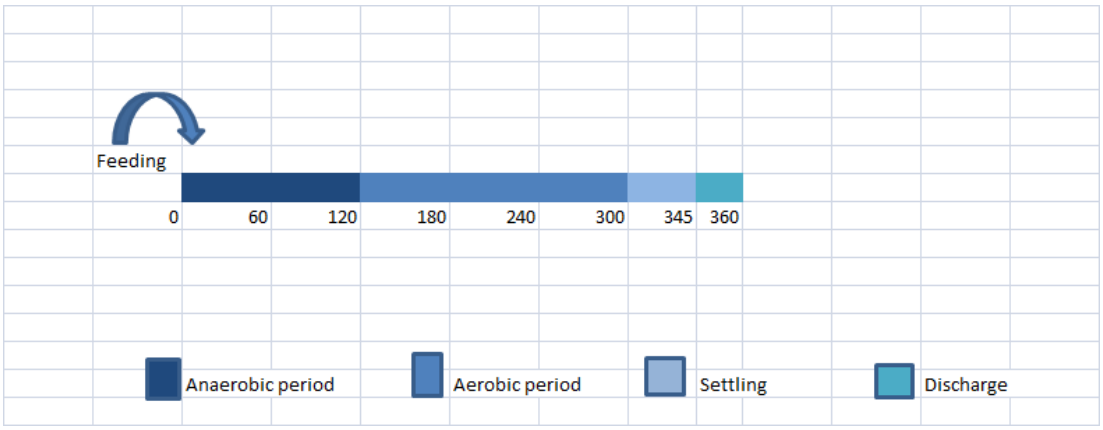

Figure 4.2: The cycle configuration for 45 minutes(Ts=45min).

Table 4.1 : Changes in cycle configuration throughout the study(duration cycle Periods in minutes).

Phases(weeks)	Days	Tf	TN2	Tstir	Tanaer	Taer	Ts	Tw	Ti	Tc
I(3.5)	1-25	5.5	15	99.5	120	180	45	6	9	360
II(5.5)	64	11.7	15	93.3	120	180	30	10.6	20	361
III(4)	94	11.3	15	93.7	120	195	15	11.0	18	359
IV(7)	145	11.5	15	113.5	140	190	5	10.5	14.5	360
IV(6)	189	11.5	15	303.5	140	190	5	10.5	14.5	360
V(3)	210	11.5	15	303.5	140	190	1	10.5	18.5	360

4.2 Test Setup

4.2.1 Testing Procedure

Samples from reactor were taken 2-3 times in a week for the period of 240 days approximately(from August until April 2012). Samples which taken were mixed liquor suspended solids(MLSS) and mixed liquor volatile suspended solids(MLVSS) , sludge volume index at 10 minutes and 30 ($SVI_{10}$and SVI_{30}) ,suspended solids and volatile suspended solids in the effluent(ESS and EVSS), effluent and influent chemical oxygen demand (COD) and samples were taken by cycle evaluation. Each samples were measured with standard methods(**APHA et al.2005**).

4.2.2 Measurement Of MLSS and MLVSS

Measurement of MLSS and MlVSS samples done with standard methods. The standard methods which measured MLSS and MLVSS was glass fiber filter technique.

Calculation of MLSS and MLVSS:

$$= (A-B)*1000 \div Vsample(ml) \quad \textbf{(2)}$$

A: Weight of filter and dried residue(mg)

B: Weight of Filter

Figure 4.3: Left: Glass fiber filter disks sludge sample filtered; right:105˚ C oven.

4.2.3 Measurement Of SVI

Sludge Volume Index(SVI) defined as the volume in ml occupied by 1g activated sludge after settling the aerated liquor for 30 minutes. In this thesis SVI10 and SVI30 were measured by putting 100ml of sample in cylender and then read settle volume after 10minutes and 30 minutes then multiply it in 10(normaly SVI operate with 1L volume but because our sample volume is not so enough thus SVI measured with 100ml Sample volume).

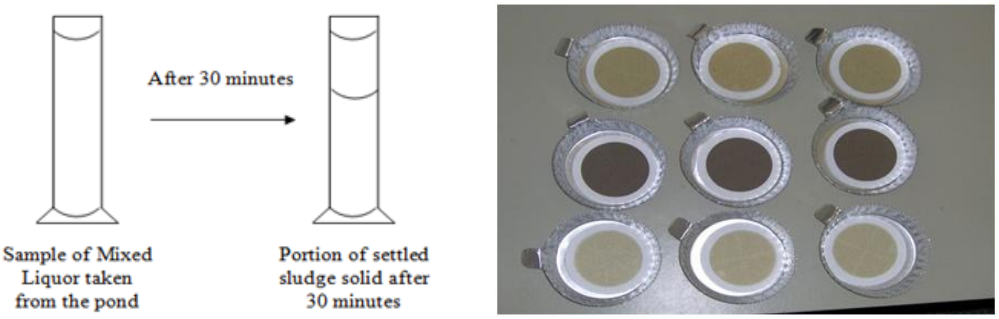

Figure 4.4: Standard SVI test.

4.2.4 Effluent COD

The chemical oxygen demand (COD) test is widely used as a means of measuring the organic strength of domestic and industrial wastes. Measurement of Effluent COD is done by standard method. The standard method which measures COD is closed reflux method(ISO6060).

4.2.4.1 Reagents and Materials

This method involves the handling and boiling of strong solutions of sulfuric acid and dichromate. Protective clothing, gloves and full face protection are necessary

a) Materials

GL 18 shot tubes: This tubes with their covers should be buy before starting the test.

Heater block (150 ˚ C): This instrument can boil samples to 150□ C. For avoiding the outside temperature to the samples; the shot tubes should be closed well.

b) Reagents

Standard potassyum dichromate solution, 0.01667 M:

For preparing this solution ; 4.903 g $K_2Cr_2O_7$, 167 ml H_2S0_4 and 33.3 g $HgSO_4$ will add to 500 ml distilled water. When solution comes to the room temperature, can add distiled water to solution until 1L volume.

Acid Sulfuric: Dissolve 20.24 g Ag_2SO_4 in H_2SO_4. Wait to completely dissolve it.

Ferroin Indicator Solution: Dissolve 1.485 g1,10- fenantrolin monohidrat ve 695 mg $FeSo_4.7H_20$ and complete it to 100ml. This solution should be dilute (4+1) factor.

Standart ferrous amonyum sulfate: Dissolve 0.10 M; 39.2 $Fe(NH_4)2.(SO_4)2.6H_20$ and H_2SO_4 together and complete it to 1 litre. This solution should be set in the day that titration used.

4.2.4.2 Procedure

Transfer 2.5 ml of sampleas to the GL18 shot tubes and add 2.5 distilled water to it. Then add 1.5 ml dichromate solution and 4.5 acid solution. Then boil it for 2 hours at heater blocks(150 C). Wait to cool down and then titrate it.

Calculation of COD [COD(mg/l)]= (A-B)*M*8000/Volume of sample **(3)**

A: Utilized DAS which used for Blank

B: Utilized DAS which used for distilled water

M: Normalite of DAS

8000: Equivalent of oxygen

Figure 4.5: Thermal Block.

Figure 4.6: Titration for COD.

4.2.5 Measuremnt For Glycogen

4.2.5.1 Materials

- Destruction tubes
- Heating block(the same equipment that is used for PHB)
- 1 and 5 ml autopipets
- Glycogen (glycogen standard for Microbiology Merck 4202 ya da sigma G1507)

4.2.5.2 Reagents

- 6M HCL (37% HCL and demineralised water- mix in ratio 1:1)
- Glycogen standards:
  1g/L standard: 0.1 g dried glycogen in 100 ml detemineralised Water

  0.5 g/L standard: 4.5 ml of the 1g/L standard is mixed with 4.5 ml demineralised water in destructio
  0.25 g/L standard: 4.5 ml of the 0.5 g/L standard is mixed with 4.5 ml demineralised water in destruction tube

  0.125 g/L standard: 4.5 ml of the 0.25 g/L
  All standards are then mixed with 0.5 ml 6M HCL per 4.5 ml standard. This dilution should take into account when calculating the centration of glycogen in the sample.

4.2.5.3 Sample procedure

Take 4.5 ml mixed from reactor and respirometer. Then put 0.5 ml 6 M HCL in destruction tubes. Hereafter put the tubes directly into the freezer.

4.2.5.4 Hydrolysis Procedure

The samples were heated for 5 hours at 100 □ C. Then the tubes were cold down and then the supernatant transferred into sample tubes by pasteur pippetes. For each sample should use a new pipet. Then the tubes labled fully. Send the samples to Genetic Laboratory for measuring.

4.2.5.5 Results

Values from the analist can be directly used to calculate the C-moles of glycogen as they are expressed as mg glucose/L taking into account the dilution of the sample by the addition of acid. The glycogen standards used to check the yield.

4.2.6 PHB measurement

4.2.6.1 Standards

- Weigh: 0.5,1.0,1.5,2.0 mg PHB standards
- Standard: SIGMA Polyhydroxybutiricacid

4.2.6.2 Internal Standard

- 20 mg benzoic acid in 1 ml 1-propanol.
- Internal standards should be added as 10% of PHB content.
- Pipet 50 mikro liter internal standards to each sample and standards.

4.2.6.3 Acid

- HCL +1-propanol mixture:1:4(v/v)
- Add 1.5 ml of the mixture in each sample and standard

4.2.6.4 Solvent

- 1.5 ml of the mixture in each sample and standard

4.2.6.5 Sample preparation

- Take 25-30 ml samples
- Centrifuge it and discard the liquid phase
- Add 4.5 ml phosphate buffer(PO4 buffer:K_2HPO_4=0.58g/L + KH_2PO_4=0.23g/l)
- Vortex vigourously and centrifuge.
- Collect sludge pellets in a sample tube and freeze at -20 C
- Frreze-dry for at least 48 hours at -50 C
- Crush freeze-dried sludge pellets
- Weight 20-30 mg with a microgram balance and record exact amount
- Put weighed amount of sample in glass tubes with PTFE lined screw caps(Schott GL18 Max 200 C)
- Standards should be kept in the freezer
- Prepare 3 standards each 2-3 mg for each series of 15 samples.
- Follow the extraction procedure for samples and standards

4.2.6.6 Extraxtion

- Pipet 50 micro liter of internal standards to each sample and standard
- Add 1.5 ml of the acid mixture in each sample and standard
- 1.5 ml 1,2 dichloro ethane is added to each standard and sample

- Boil samples and standards for 2 hours at 100 C. shake every 15 minutes
- Cool down samples
- Wait for phase seperation for a few minutes
- Take around 1 ml from the lower organic phase

4.2.6.7 Preparation For GC Vials

- Put glass fiber in blue pipet tips and sodium sulfate
- Let the sample (1 ml organic phase) to filter from sodium sulfate column
- Measure in GC

5. RESULTS AND DISSCUSION

The measured values of SVI, compared different settling time involved 45 minutes for 25 days, 30 minutes for 30days, 15 minutes for 30days, 5minutes for 95days and 1 minute for 60days. The SVI values obtained in the experiment are much lower compared to those reported for flocular sludge by other authors and comparable to the SVI values reported for aerobic granular sludge . This short time (5minutes and 1 minute) and thus high hydraulic selection pressure on the microbial community allowed the retention of well settling biomass inside the reactor while flocculent biomass was washed out and the sludge observed in the effluent.

5.1 Effect of gradually decreasing the settling time on biomass production

5.1.1 Observed changes when the settling time is 45 minutes (Ts=45min)

Figure 5.1 showed the biomass production in the system during approximately 10month. It can be concluded that from starting day of the system until 25th days which applied settling time is 45 minutes(Ts=45min), the concentration of mixed liquor suspended solids(MLSS) and mixed liquor volatile suspended solids(MLVSS) increased from 8g/L to 36g/L and from 4g/L to 12 g/L respectively which indicates that given feed consumed for producing biomass and showes the system works well for biomass production.

5.1.2 Observeed changes when the settling time is 30minutes (Ts=30min)

According to figure 5.1 which showes biomass production, it can observe very sharply decreasing in concentration of MLSS from 35g/L to 9g/L. Although concentration of MLVSS decreased from 13g/L to 5g/L too. It should remember that half of biomass concentration was removed from the system in the end of Ts=45min at refrigerator in this period of time because producted biomass did not have good settling capacity and good effluent quality , thus concentration of biomass decreased suddenly.

5.1.3 Observed changes when the settling time is 15minutes (Ts=15min)

According to figure 5.1 which showes biomass production due to Ts=15min; it can be demonstrate that with decreasing settling time biomass production increased from about 9g/L to 21g/L due to approximately 13 days which production of biomass indicates that the system worked well. At the end of this period (Ts=15min); biomass production is decreased from 18g/L to 12 g/L which emphasized that the system does not work well for production of biomass.

5.1.4 Observed changes when the settling time is 5minutes (Ts=5min)

According to figure 5.1 which showes biomass production that obtained with applying 5min settling time; it can demonstrate that mixed liquor suspended solids (MLSS) concentration increased from 12 g/L to 15 g/L for approximately 3 days (from start of applying Ts=5min until 3^{rd} day of it) where mixed liquor volatile suspended solids (MLVSS) concentration decreased from 8 g/L to 7g/L which emphasized that volatile percent is lower thus can not evaporate so much when put the samples in 550 ° C oven. After passing 3 days (after 98^{th} day) for 6-7 days a slightly decreasing from 15g/L to 13g/L in MLSS concentration observed which demonstarte that biomass production decreased during this days but volatile percent increased from 6g/L to 8 g/L. After almost 102^{th} ; the concentration of MLSS suddenly increased from 13g/L to 14g/L and MLVSS concentrayion is almost fixed which indicate that biomass production during this period of time(between 102^{th} day and 106^{th} day) increased which showes the system worked well for biomass production. Between 106^{th} day and 109^{th} day; the concentration of MLSS and MLVSS changed from 14g/L to 16g/L and from 7g/L to 7.2g/L which demonstrate that the biomass concentration increased during this days and the system works well but volatile percent is still low. Between 109^{th} day and 117^{th} day; the concentration of MLSS and MLVSS increased from 16g/L to 17 g/L and from 7g/L to 8g/L respectively which indicates that biomass production increased during passing time. From 117^{th} day until 123^{th} day; a slightly decreasing in MLSS and MLVSS concentration from 17g/L to 14g/L and from 8g/L to 6g/L observed which demonstrate that biomass production decreased during this period of time.

Between 123th day and 130th day, the concentration of MLSS and MLVSS increased from 14g/L to 25g/L and from 6g/L to 10g/L respectively which demonstrate that biomass production increased that showes the system still worked well for biomass production.

Between 130th and 159th day; the concentration of MLSS and MLVSS decreased from 25g/L to 19g/L and from 10g/L to 8g/L which indicates that biomass concentration decreased during this period of time because of some problems happened in this days such as reactor was not received feed for 2 days(starvation) and also it had aeration mixing problem in next days which biomass left unmixed for most of the aeration period. Between 159th and 162th day; MLSS and MLVSS concentration sharply increased from 19g/L to 31g/L and from 7g/L to 12g/L which demonstrate that biomass production increased because the system fed and starvation finished and also aeration mixing problem is finished thus microorganisms have good condition to grow up and produce more therefore the concentration of biomass increased. Between 162th and 167th day; the concentration of MLSS and MLVSS decreased from 31g/L to 26g/L and from 12g/L to 10g/L which demonstrate that biomass production decreased because the sample got exactly after the reactor faced with starvation problem during weekend thus biomass could not produce as much as it should be. Between 167th and 172th days ; the concentration of MLSS and MLVSS increased from 26g/L to 34g/L and from 11g/L to 13g/L respectively which indicates that biomass production increased because starvation problem solved and the system fed well thus biomass production started again. Between 172th and 180th day; the MLSS and MLVSS concentration decreased from 34g/L to 23g/L and from 13g/L to 10g/L which demonstrate that biomass production decreased.

Finally; at the end of Ts=5min which is between 180th and 187th dat, the biomass production increased from 23g/L to 25g/L which showes the system works well for biomass production.

5.1.5 Observed changes when the setlling time is 1minute(Ts=1min)

According to figure 5.1 which showes biomass production ; it demonstrate that MLSS and MLVSS concentration decresed from 26g/L to 20g/L and from 13g/L to 9g/L respectively which indicates that biomass production decreased. After 210th day, reciepie of feed changed and instead of 65% acetate and 35% starch solution fed just with acetate solution (100% acetate). Between 210th and 249th day, biomass production increased from 19 g/L to 71g/L which emphasized that given feed act well for production of biomass. Then between 249th day and 272thday which is end of the experiments, biomass production decreased from 71g/L to 32 g/L. Although, the biomass production decreased at the end of thesis but concentration of biomass production is still good.

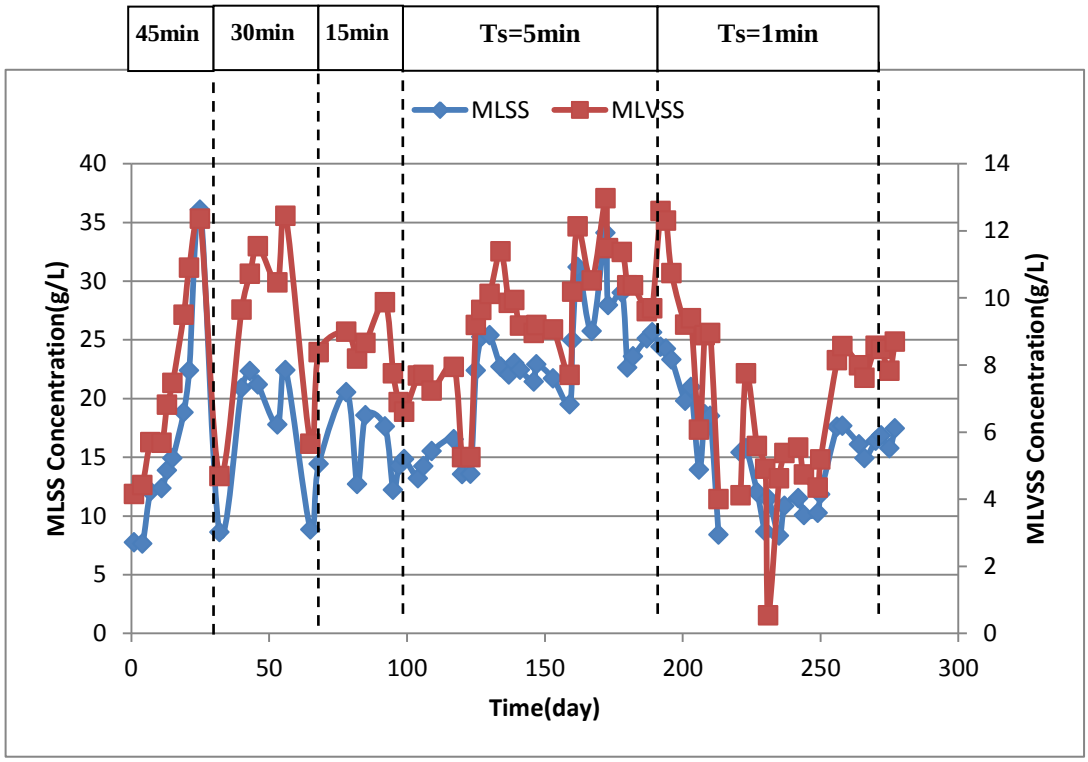

Figure 5.1: Biomass production during reactor operation with gradually decreasing settling time.

5.1.6 Calculating SRT in the system

Calculating SRT in the system is done by this formula:

$$SRT(day) = MLSS(mg/l)/EVSS(mg/l)*1/N*Vw(ml)/V_{F(ml)} \quad \textbf{(4)}$$

Where N: Number of cycle in day which is 4 cycle

Vw: Working volume which is 2000ml(2L)

V_F: Amount of feeding which is 200 mg per each cycle

Table 5.1: SRT in the SBR system.

Phases	Ts(min)	MLSS(mg/l)	EVSS ave	SRT(day)
I	45	min=7620,max=36070	4930	min=4,max=18
II	30	min=8600,max=24420	min=1615,max=2030	min=13,max=30
III	15	Min=8840,max=20520	-	-
IV(1)	5	19115	5088	9
IV(2)	5	25470	772	82
V	1	21045	1930	27

5.2 Effect of gradually decreasing the settling time on the settling capacity of biomass

5.2.1 Observed changes when the settling time was 45minutes (Ts=45min)

According to figure 5.2 and 5.3 which showes settling capacity of biomass during the thesis; during this settling time the sludge volume index(SVI) gradually decreased from approximaltely 120ml/g to 44 and 16ml/g(SVI10 and SVI30) which emphasized that eventhough biomass production is high in the system the producted biomass had good settling capacity to settle fast.

5.2.2 Observed changes when the setlling time was 30minutes (Ts=30min)

According to figure 5.2 and 5.3; SVI concentration decreased from about 61 mg/l to 45 mg/l which indicates that with decreasing biomass, the settling capacity of biomass will improved because at the end of settling time 45 minutes (Ts=45min) half of biomass removed from the reactor and stored in the refrigerator thus less concentration of biomass can settle faster than higher one .

5.2.3 Observed changes when the settling time was 15mintes (Ts=15min)

Eventhough MLSS and MLVSS concentration is decreased which indicate that biomass production decreased produced biomass does not have good settleability property according to figure 5.2 and 5.3 which showes the settling capacity of biomass because SVI increased from 65 mg/l to 71mg/l. Finally, at the end of this settling time SVI concentration decreased from 65 mg/l to 54 mg/l which indicates that the settling time capacity of biomass works well in this system.

5.2.4 Observed changes when the settling time was 5minutes (Ts=5min)

Despite biomass production increased in this period of time (anout 3 days) , SVI concentration declined from 77 ml/g to 64 ml/g which concluded that producted biomass has good sludge capacity that can settle fast. After passing 3 days (after 98^{th} day) for 6-7 days SVI concentration increased from 64 mg/l to 71mg/l which indicates that the sludge capacity is not very good during these days. After almost 102^{th} ; SVI concentration decreased from 71mg/l to 67 ml/g which emphasized that the settling capacity of biomass improved during this period of time. Between 106^{th}

day and 109th day; according to figure 5.2 and 5.3 which show the settling capacity of biomass during 10 month non-stop experiments, the SVI concentration decreased from 66 ml/g to 29 ml/g between this days which demonstrate that producted biomass has good settling property during this days.

Between 109th day and 117th day; SVI concentration (SVI10 and SVI30) increased from 29 ml/g to 46ml/g and from 38 ml/g to 57 ml/g respectively which demonstrate that the settling capacity of biomass get damaged and microorganisms can not settle fast during this days. From 117th day until 123th day; SVI concentration decreased from 57ml/g and 46ml/g(SVI 10 and SVI30) to 25 and 19ml/g which emphasized that producted biomass obtained very good settling capacity which can settle fast eventhough with settling time of 5 minutes(Ts=5min) which is very short settling time.

From 117th day until 123th day, a siginificant improvement in the settling capacity of biomass obtained according to figure 5.2 and 5.3 which indicates that the system worked for settling property. Between 123th day and 130th day, SVI concentration decreased from 19 ml/g to 17ml/g(SVI30) and from 26ml/g to 18ml/g(SVI10) which emphasized that the producted biomass has good settling capacity during this days. Between 130th and 159th day; SVI concentration decreased from 26ml/g to 16ml/g (SVI10) and from 15 ml/g to 11ml/g(SVI30) between 130th day and 139th days which demonstrate that with decreasing in biomass concentration due to problems which told, the sludge capacity of biomass can settle faster than high one because they are not dense. After that; concentration of SVI increased from 11ml/g to 20ml/g (SVI10 and SVI30) which can be say that the settling capacity of biomass damaged a little between 139th and 146th day then from 20ml/g increased to 28ml/g at the end of 159th day.

Between 159th and 162th day; The concentration of SVI decreased from 28ml/g to 15ml/g (SVI10) and from 22ml/g to 12ml/g(SVI30) which demonstrate that produced biomass have good settling quality that can settle fast. Between 162th and 167th day; despite decreasing in biomass production SVI concentration is unchangeable during this days which indicates that the system still has good settling capacity. Between 167th and 172th days ; eventhough the biomass production increased SVI concentration remains the same which emphasized that produced

biomass still has good settling capacity whose does not change the settling capacity eventhough concentration of produced biomass increased.

Between 172th and 180th day; SVI concentration increased from 15ml/g to 22ml/g where indicates that the settling capacity of biomass got damaged during this day but also still is low which emphasized that biomass has good settling capacity.

Finally; at the end of Ts=5min which is between 180th and 187th day, SVI concentration decreased from 26ml/g to 23ml/g which indicates that the settling capacity of biomass is excellent with this settling time.

5.2.5 Observed changes when the settling time was 1minute (1min)

According to figure 5.2 and 5.3 which show settling capacity of biomass, SVI concentration stabilized at 18ml/g(SVI10) and 16ml/g(SVI30) which emphasized that with decreasing settling time produced biomass obtained excellent settling capacity with passing time. After 210th day, the reactor only fed with acetate solution thus SVI10 and SVI30 increased from 31 to 89ml/g and from 18 to 71ml/g respectively. Then SVI10 and SVI30 decreased from 89 to 49ml/g and from 71 to 32ml/g so it demonstrate that the system works well for settling capacity of biomass.

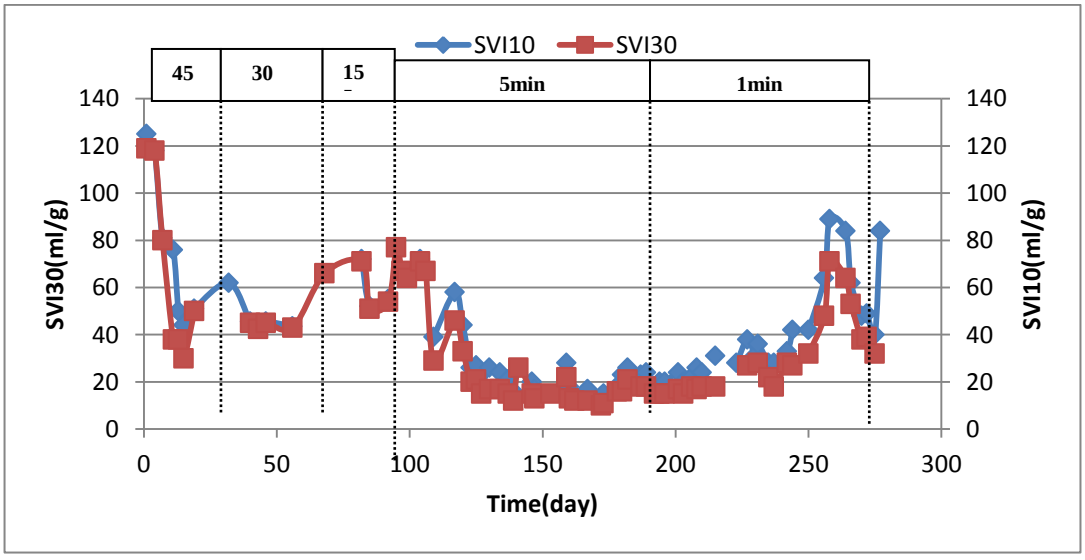

Figure 5.2 : Settling capacity of biomass during reactor operation with gradually decreasing settling time.

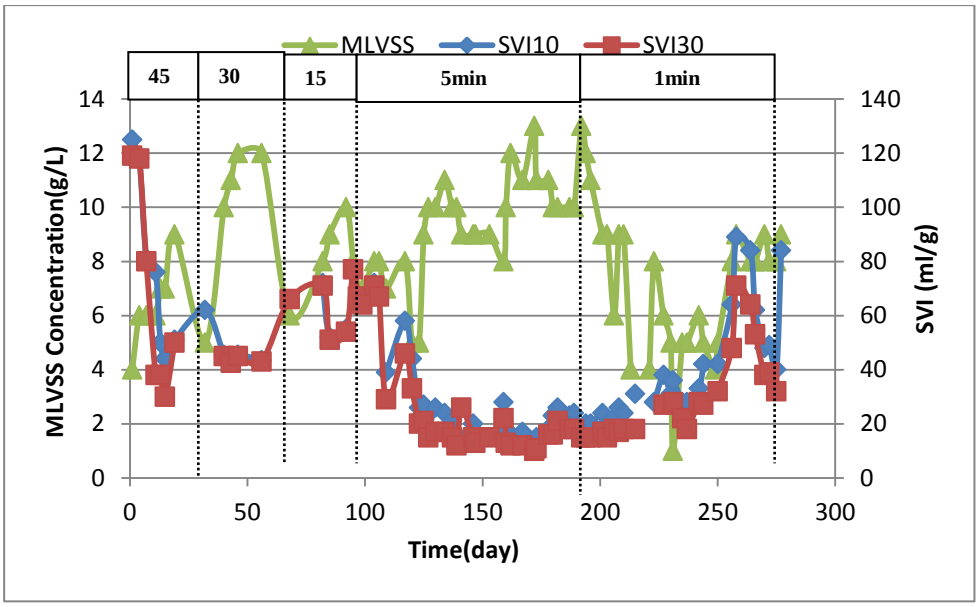

Figure 5.3: Settling capacity of biomass during reactor operation with gradually decreasing settling time.

5.3 Effect of gradually decreasing the settling time on effluent quality

5.3.1 Observed changes when the settling time is 45minutes (Ts=45min)

Despite good biomass production and settling capacity, Effluent quality of biomass (ESS) was not good in the system according to figure 5.4 which showes effluent-biomass washout in the system. According to fogure 5.4; from the starting day of the system until 15th day of the system concentration of ESS increased sharply from 5000mg/l to 8000mg/l because the settling time was 45min thus they don't need to settle fast therefore they observed in the effluent as a result concentration of effluent increased. After that, the effluent concentration decreased from 8000mg/l to 3000mg/l which means settling capacity of biomass improved and the biomass can settle well so they will not observe so much in the effluent but between 20th and 25th day which are the last days of Ts=45min, concentration of ESS increased from 3000mg/l to 20.000 mg/l which is because of scaping high concentration of biomass to the effluent. Consequently, despite good biomass production and sludge capacity the system could not work well for effluent washout therefore half of biomass was removed from the reactor at the end of this settling time(Ts=45min) and stored in the refrigerator and add tap water instead of it.

5.3.2 observed changes when the settling time is 30minutes (Ts=30min)

Due to figure 5.4 which showes effluent biomass washed out during applied different settling time, demonstrates that with removing half of biomass at the end of Ts=45min from the system, effluent concentration decreased sharply from 20.000mg/l to 3360 mg/l between 25^{th} and 45^{th} days which demonstrate that concentration of scaped biomass to the effluent decreased but between 45^{th} and 55^{th} days the concentration increased which can concluded that the biomass concentration which scaped to the effluent is increased during this period of time.

5.3.3 Observed changes when the settling time is 15minutes (Ts=15min)

ESS concentration decreased from 4705 mg/l to 4028 mg/l in this period of time which showes the system obtained better effluent quality than Ts=30min with decreasing settling time.

5.3.4 Observed changes when the settling time is 5minutes (Ts=5min)

The concentration of discharged effluent (ESS) increased from 12346 mg/l 15833 mg/l during this period of time which the reason is some biomass escaped to the effluent. After almost 102^{th} ; slightly increasing in ESS concentration from 12346 mg/l to 15833mg/l observed which indicates that some biomass escaped to the effluent. Between 106^{th} day and 109^{th} day; effluent quality of biomass decreased because with improving settling capacity the microorganisms can settle fast eventhough with Ts=5min which is very short settling time therefore they do not observe in the effluent thus effluent concentration decreased from 15833mg/l to 11333mg/l.

Between 109^{th} day and 117^{th} day; Despite biomass production and SVI concentration increased, the effluent qulaity(ESS) is still good and decreased sharply from 11333mg/l to 5050 mg/l which demonstrate that despite the settling capacity decreased the biomass did not escaped to the effluent. From 117^{th} day until 123^{th} day, very sharply decreasing in ESS and EVSS concentration from 11333mg/l to 2670mg/l and from 4016 mg/l to 1080 mg/l observed which demonstrate that effluent quality of biomass improved significantly and microorganisms whose settle fast increased during this period of time thus they can settle good with Ts=5min therefore they do not observe in the effluent anymore. Moreever; the filtrated sample volume increased with effluent quality improvement from 5ml to 10 ml because the ESS

sample is more transparent and does not involve so much biomass in it so it can filter easily and do not create clogging problem. Between 123th day and 130th day, ESS concentration increased from 2670 mg/l to 3095 mg/l which indicates that a slightly increasing in microorganisms whose settle slow can be happened. However; the concentration of this slow microorganisms is not so much thus can not effect on settling property of the system. Between 130th and 159th day; ESS concentration decreased from 3095mg/l to 1405mg/l which demonstrate that the biomass obtained good effluent quality. Between 159th and 162th day; ESS concentration slightly increased from 1405 mg/l to 1935mg/l which can be because of some biomass escaped to the effluent. Between 162th and 167th day; The effluent concentration is stabilized during this day which indicates that the system obtained to stabilized condition. Between 167th and 172th days ; ESS concentration remains the same which indicates that produced biomass has good settling capacity thus they can settle fast in the reactor therefore they do not observe in the effluent. Between 172th and 180th day; ESS concentration decreased from 1770mg/l to 1460mg/l which indicates that the system has good effluent quality. Finally; at the end of Ts=5min which is between 180th and 187th day, the effluent quality is still low in the system.

5.3.4 Observed changes when the settling time is 1minute (Ts=1min)

Due to this settling time, effluent quality of biomass still is low which indicate tha the system has good effluent quality. When feed receipe changed to just with acetate, does not any significant effect on effluent quality and it is still low.

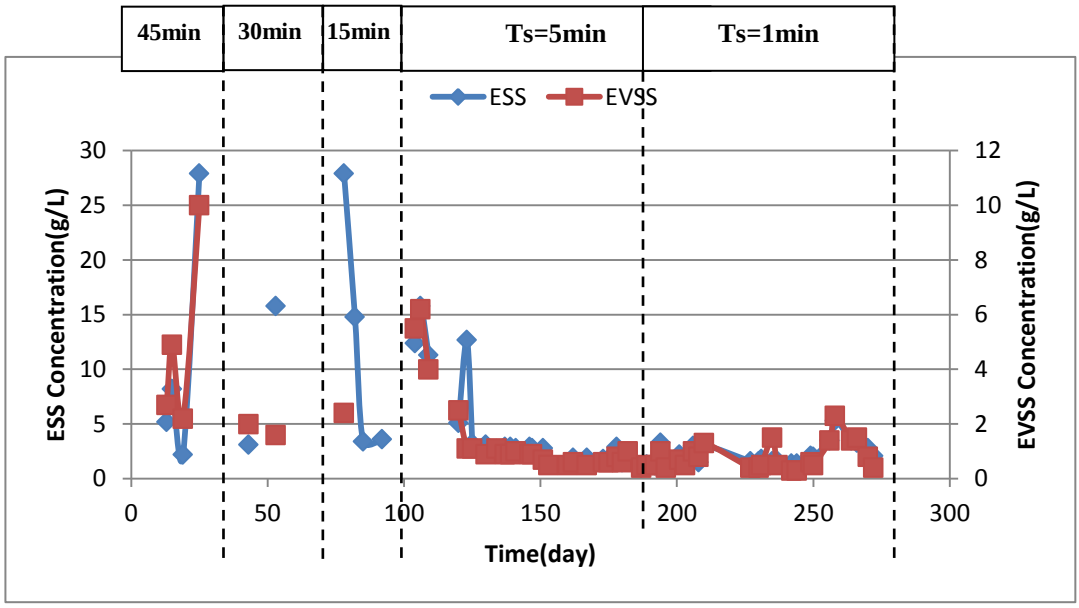

Figure 5.4 : Effluent-biomass washout during reactor operation with gradually decreasing settling time.

5.4 Effect of gradually decreasing the settling time on COD performance removal of the system

5.4.1 Observed changes when the settling time is 45minutes (Ts=45min)

According to figure 5.5 and 5.6 which show biomass maintenance and COD removal performance due to 10 month experiment, and biomass concentration and organic matter removal efficiency respectively, it can demonstrate that from the starting day of the reactor until 25th day (the end day of Ts=45min) high increasing in COD concentration from 429 mg/l to 1912mg/l observed which demonstrate that this system does not work well for COD removal because the concentration of COD fed to the system was ~ 8500 mg/L but concentration of effluent COD is 1912mg/l which emphasized that the system can not remove COD as well as it should. Furthermore; performance of COD removal decreased from 95% to 90% which is another reason to system does not work well for removal of COD. If COD concentration be high in the system, it means that the microorganisms consume dissolve oxygen in the reactor so it will lack of oxygen concentration with passing time thus it needs to give oxygen to the system otherwise microorganisms will die. Due to oxygen is expensive element so this system will cost for us. Consequently, it should change the operation of system to obtain low COD concentration.

5.4.2 Observed changes when the settling time is 30minutes (Ts=30min)

According to figure 5.5 which showes COD removal performance, it can observe that effluent COD concentration decreased from 1912 mg/l to 1289 mg/l at the end of Ts=30min which indicates that with removing half of biomass from the reactor, the COD concentration in the effluent decreased thus COD removal performance improved from 78% to 85%.

5.4.3 Observed changes when the settling time is 15minutes (Ts=15min)

During this settling time, concentration of COD decreased from 387 mg/l to 280mg/l with increasing biomass production which demonstrate that producted biomass does not need to give high amount of oxygen concentration that is good for the system because the system will not cost for us. Also; performance of COD removal increased from 95% to 97% in this period of time(13days) which indicates that the system has good removal performance. . After that; for a very short time (about 5days) COD concentration decreased from 335 mg/l to 230 mg/l during passing time

which can be indicates that with decreasing biomass concentration demand of oxygen will be decreased too. Due to decreasing in COD concentration, performance of COD increased from 96% to 97% which demonstrate that the system has good removal performance.

Finally; at the end of this period (Ts=15min); COD concentration decreased from 388mg/l to 230mg/l where perforamnce of COD removal increased from 95% to 97%.

5.4.4 Observed changes when the settling time is 5minutes (Ts=5min)

Despite biomass production and effluent concentration increased, the effluent COD concentration decreased from 289 mg/l to 248 mg/l therefore it causes to increase the performance of COD removal from 96% to 97% which showes the system still works well for COD removal. After passing 3 days (after 98^{th} day) for 6-7 days ; COD concentration and performance of it changed from 289 mg/l to 247 mg/l and from 96% to 97% respectively which emphasized that when concentration of effluent COD decreases, the performance of it will increase that shows the system acts very well for COD removal. After almost 102^{th} ; COD concentration declined from 289mg/l to 248 mg/l where performance of COD removal increased from 96% to 97% which emphasized that the system works well for COD removal. Between 106^{th} day and 109^{th} day; concentration of effluent COD decreased from 247mg/l to 201mg/l where performance of COD removal increased from 97% to 98% which emphasized that the system works good for COD removal during this period of time.

Between 109^{th} day and 117^{th} day; the effluent COD concentration increased from 201 mg/l to 478mg/l where performance of COD removal sharply decreased from 98% to 94% which demonstrate that the system does not work good for COD removal during this period of time.

From 117^{th} day until 123^{th} day; the effluent COD concentration concentration decreased from 478mg/l to 237mg/l where the performance of it decreased from 94% to 97% which demonstrate that the system works good for COD removal thus it won't need to give extra oxygen. Between 123th day and 130^{th} day, effluent COD concentration increased from 168mg/l to 523mg/l where COD removal efficiency

declined from 98% to 94% which demonstrate that performance of COD removal decreased in the system that is not good effect on the system.

Between 130th and 159th day; The effluent COD concentration decreased from 523mg/l to 221mg/l where the performance of it increased from 94% to 97% which indicates that the system works good for COD removal. Also; it can say that the performance of COD removal is stabilized from this days until the end of this settling time (Ts=5min).

Between 159th and 162th day; the effluent COD concentration does not have siginificant change during this period of time so the performance removal of it is stabilize. Between 162th and 167th day; the effluent COD concentration is stabilized too.

Between 167th and 172th days ; the effluent COD concentration increased from 215mg/l to 300mg/l that cause in decreasing the performance of COD removal from 97% to 96% which indicates that microorganisms consumed more dissolved oxygen that was in the reactor and also COD can not remove well during when in compares with another days.

Between 172th and 180th day; The effluent COD concentration decreased from 300mg/l to 235mg/l where the performance removal of it increased from 96% to 97% which emphasized that the system acts well for COD removal.

Finally; at the end of Ts=5min which is between 180th and 187th day, COD concentration is still low during this days.

5.4.4 Observed changes when the settling time is 1minute (Ts=1min)

effluent COD concentration stabilized at the end of Ts=5min is still continue until the end of Ts=1min which is about 270mg/l where COD performance removal is allmost 97% that showes the system works excellent for COD removal.

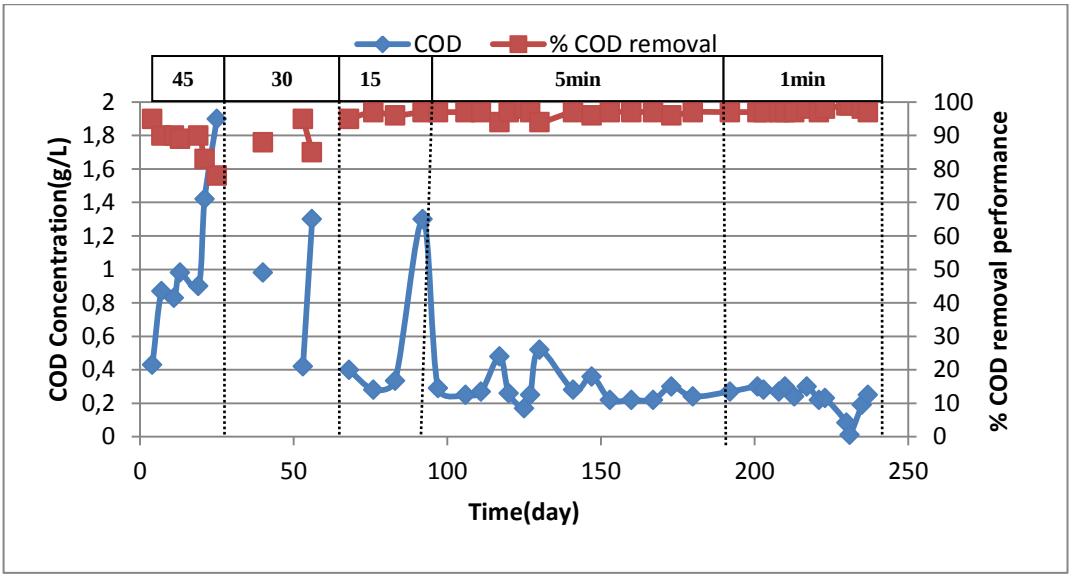

Figure 5.5: Biomass maintenace and COD removal performance during reactor operation with gradually decreasing settling time.

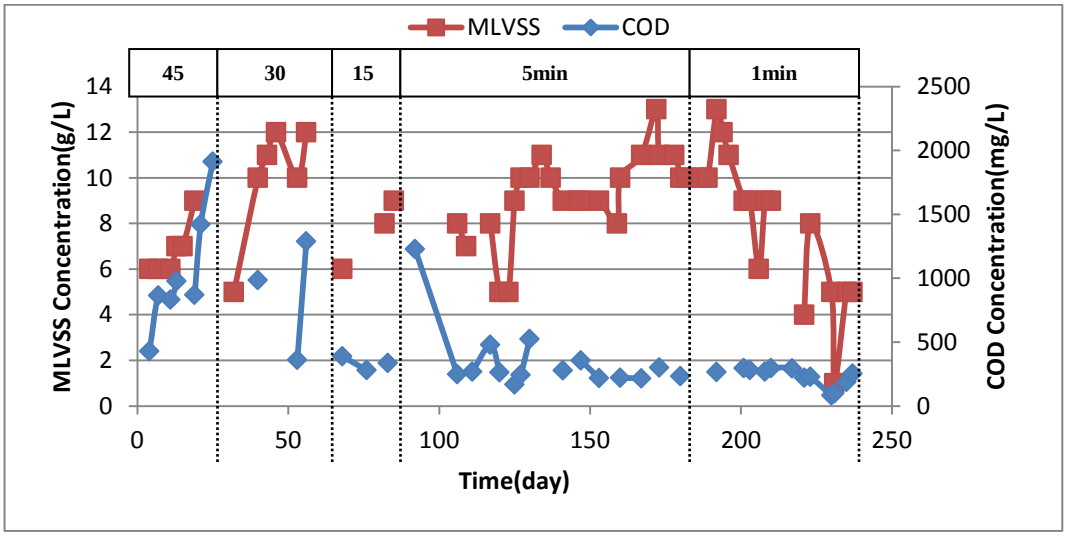

Figure 5.6 : Biomass concentration and organic matter removal efficiency during operation with gradually decreasin settling time.

5.5 Overal system performance

5.5.1 Overall performance due to settling time 45minutes (Ts=45min)

According to Table 5.2 which showes all parameters due to settling time 45minutes, it can demonstrate that the system has good biomass production but despite high biomass production in the system the SVI concentration is not so high which emphasized that the producted biomass has good settling capacity. According to this table; concentration of ESS and COD is so high which the reason is high concentration of biomass scaped to the effluent and COD and system can not remove COD well. Thus ; removing of half of biomass applied in the end of this settling time(Ts=45min).

Table 5.2: Measured ranges of all significant system parameters when Ts=45min.

Parameter	Unit	Max	Min	Standard deviation
MLSS	mg/L	36070	7620	8325
MLVSS	mg/L	12630	4150	2590
SVI10	ml/g	119	44	31
SVI30	ml/g	118	29	26
ESS	mg/L	27850	3140	9825
EVSS	mg/L	10020	2160	3114
COD eff	mg/L	1912	429	445
COD influ	mg/L	8540	-	-
COD removal	%	95	78	5.2

5.5.2 Overall performance due to the settling time 30minutes (Ts=30min)

According to Table 5.3; it is demonstrated that COD removal efficiency increased with decreasing settling time. Also; SVI values(SVI10 and SVI30) decreased with decreasing settling time which indicates that with decreasing settling time, settling capacity of biomass improved. Furthermore , the decreasing in the SVI can be because of removing half of biomass at the end of Ts=45min.

This table did not involve ESS, EVSS and EVSS/ESS parameters because this parametrs had only two data which max and mean for two data is meaningless thus they neglected.

Table 5.3: Measured ranges of all significant system parameters when Ts=30min.

Parameter	Unit	Max	Min	Standard deviation
MLSS	mg/L	22420	8600	5525
MLVSS	mg/L	11540	4680	2750
SVI10	ml/g	62	43	7.1
SVI30	ml/g	45	42	1.1
COD influ	mg/l	8540	-	-
COD efflu	mg/l	1289	420	360
COD removal	%	95	85	4.2

5.5.3 Overall performance due to settling time 15minutes (Ts=15min)

According to Table 5.4 which showes the system's performance for Ts=15min ; it can be demonstrate that COD removal efficiency improved with decreasing settling time where ESS concentration did not show any significant difference with the last applied settling time (Ts=30min). howeever; SVI concentration decresed which demonstrate that settling property of biomass improved with decreasing settling time.

Table 5.4: Measured ranges of all significant system parameters when Ts=15min.

Parameter	Unit	Max	Min	Standard deviation
MLSS	mg/L	20520	8840	3890
MLVSS	mg/L	9870	5650	1300
SVI10	ml/g	72	52	8.8
SVI30	ml/g	71	51	8.3
ESS	mg/l	27850	3360	1005
COD influ	mg/l	8540	-	-
COD efflu	mg/l	1230	280	390
COD removal	%	97	95	0.83

5.5.4 Overall system performance due to settling time 5minutes (Ts=5min)

According to Table 5.5; it demonstarte that with decreasing settling time the settling capacity of biomass decrease eventhough the biomass production is high in the reactor which indicates that produced biomass have good settling property which can settle fast. Also ; COD removal performance increase with decreasing settling time that shows system acts good for removal of COD.

Table 5.5: Measured ranges of all significant system parameters when Ts=5min.

Parameter	Unit	Max	Min	Standard deviation
MLSS	mg/L	34110	12220	5670
MLVSS	mg/L	12970	5250	1880
SVI10	ml/g	77	14	17
SVI30	ml/g	72	10	20.6
ESS	mg/L	15835	1010	4435
EVSS	mg/L	6217	400	1620
COD influ	mg/L	8540	-	-
COD efflu	mg/L	525	168	94.3
COD removal	%	97	94	0.013

5.5.5 Overall system performance due to settling time 1minute (Ts=1min)

According to Table 5.5; it can demonstrate that despite high biomass production SVI values are low in the system which indicate that producted biomass has good effluent quality which can settle fast or it can be because of they are heavy microoraganisms which settle fast thus SVI is low. Moreever, effluent quality is good in the system thus it can discharge.

Table 5.6: Measured ranges of all significant system parameters when Ts=1min.

Parameter	Unit	Max	Min	Standard deviation
MLSS	mg/L	25635	13920	3530
MLVSS	mg/L	12585	6067	1852
SVI10	ml/g	26	18	2.5
SVI30	ml/g	18	15	1.35
ESS	ml/g	2160	1150	820
EVSS	ml/g	1290	420	282
COD influ	ml/g	8540	8540	-
COD efflu	ml/g	300	240	20
COD removal	ml/g	97	97	0

5.6 INTERNAL CELLULAR STORAGE COMPOUNDS

For evaluating internal cell storage compunds , polyhydroxyalkanoates (PHAs) and glycogen tests applied. PHAs are biologically produced, biodegradable thermoplastics with many potential commercial applications(Chen2009). Bacteria synthesize PHAs, which are carbon and energy storage reserves, as cytoplasmic granules from organic carbon when subject to stressful enviromental conditions (Lee 1996; Serafim et al.2008).For evaluating that the system produced PHA and glycogen respirometric test was used. Respirometry is a measurement and interpretation of the biological oxygen consumption rate(aerobic conditions) under well defined condition. Sampling was taken from both respirometer and bioreactor at the certain minutes. Sampling in biorector are shown in Table 5.7:

Table 5.7: Sampling in biorector.

Time	PHA	Glycogen	COD
-30	+	-	-
18	-	-	+
24	-	-	+
30	+	+	-
31	-	-	+
45	-	+	-
50	-	-	+
60	-	+	-
70	-	-	+
90	+	+	+
110	+	+	
120	-	-	+
132	-	+	-
138	-	-	+
155	+	+	-
170	+	+	-
180	-	-	+
200	-	+	+
210	-	-	+
260	-	+	-
290	-	-	+
332	-	-	+

Sampling from respiro are shown in Table 5.8:

Table 5.8: Sampling in reaspiro.

Time	PHA	Glycogen
5	+	-
15	+	+
30	+	-
45	+	+
60	+	-
95	+	+
140	-	-
190	+	+
240	+	+

For evaluating PHB,PHV, PHA and glycogen respirometric test and cycle evaluation is done. According to figure 5.7 which showes cycle evaluation test, it can demonstrated that at the start of test until 139 minutes which is at anaerobic phase the production of PHB and PHV is high which emphasized that the given feed used for internal cellular storage but production of PHB is higher than PHV becuasue internall cellular storage basically stored as PHB. Because PHB and PHV concentration is high thus PHA production is high too because PHA is sum of PHB and PHV. Furthermore, the production of glycogen is so high(about 8000mg/L) which demonstrated that internal storage production as glycogen and PHA is high in the system. After finishing anaerobic phase, aerobic phase started which from figure 5.7 demonstrate that concentration of PHB,PHV,PHA and glycogen decreaased because this molecules act as storage molecule thus they deplete at aerobic phase and microorganisms consume energy from depleting them that stored in this molecules at anaerobic phase for growth phenomena. If we look to the cycle evaluation graph and respirometric the concentration of producted internal cellular storage molecules (PHB,PHV,PHA and glycogen) is lower at respirometric test because at respirometric test the respirometer fed with diluted feed where at cycle evaluation test fed directly from feed bucket.

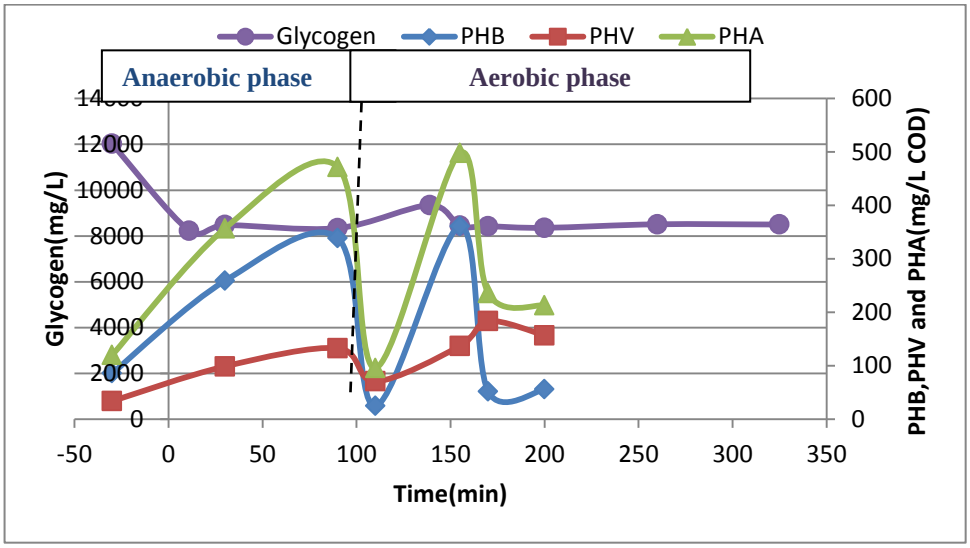

Figure 5.7: Internal cellular storage due to Cycle Evaluation test.

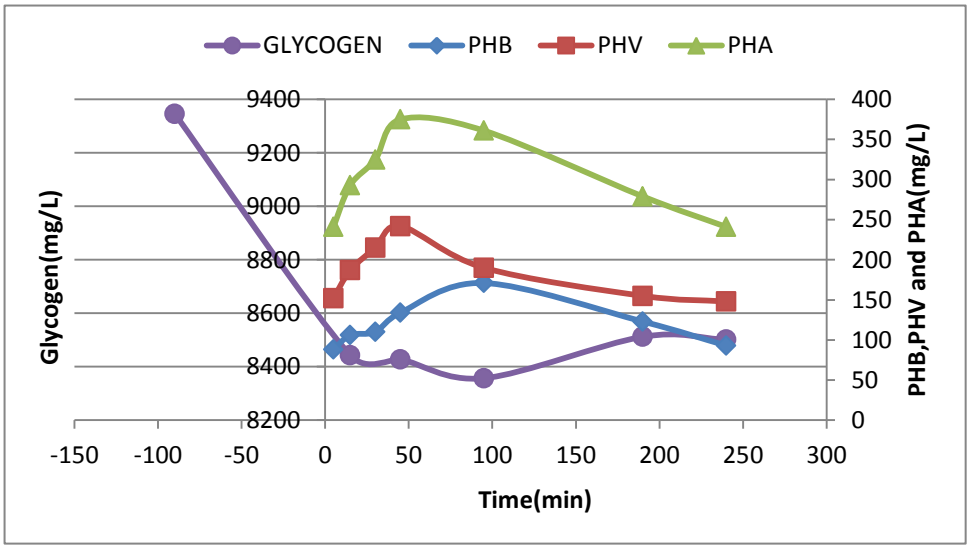

Figure 5.8 : Internal cellular storage due to Respirometric test.

At the start of test which is anaerobic phase for both Cycle evaluation and respirometric test , COD concentration is high because given feed has high amount of COD (9000mg/l) which 1/10 dilution goes to the reactor(About 900mg/L) thus it is reasonable that COD is high but after that the microorganisms consumed the feed thus the concentration of COD decreased at aerobic phase. But the COD concentration in respirometric test is lower than cycle evaluation because in respirometric feed diluted with distilled water where in cycle evaluation fed directly from feed bucket.

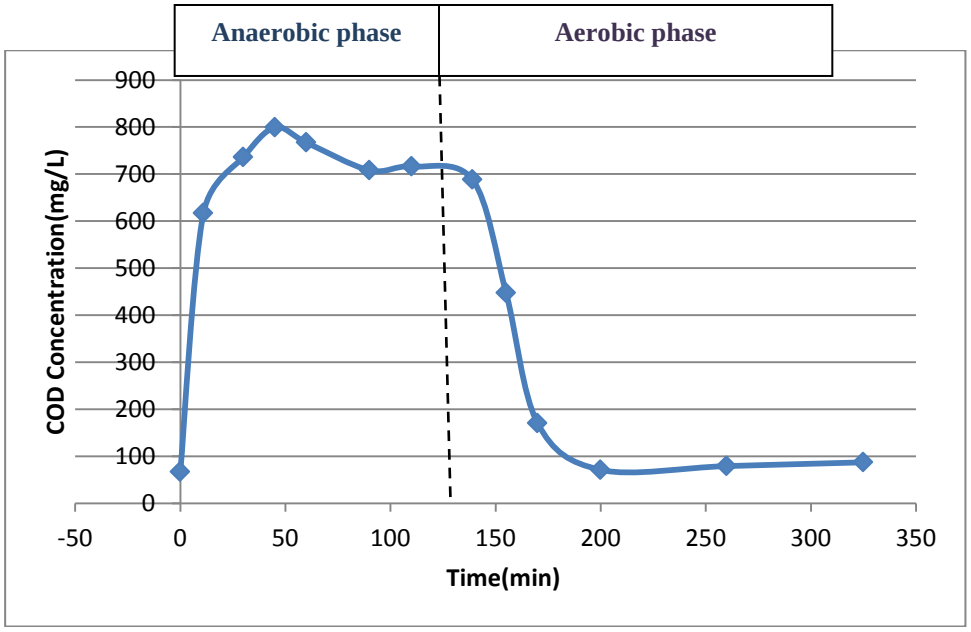

Figure 5.9 : COD removal due to Cycle Evaluation test.

Acoording to figure 5.10 which showes COD removal according to respirometric test, it can demonstrate that COD concentration after feeding increase shaply because given feed has high concentration but after passing approximately 20 minutes it decrease because the microorganisms consumed it. Also, according to this figute, it can conclued that oxugen uptake rate increase as same as COD but after 20minutes it starts to decrease because microorganisms consumed oxygen for growth. After almost passing 10minutes until end of test the OUR concentration stabilized which means endogenous decay happened.

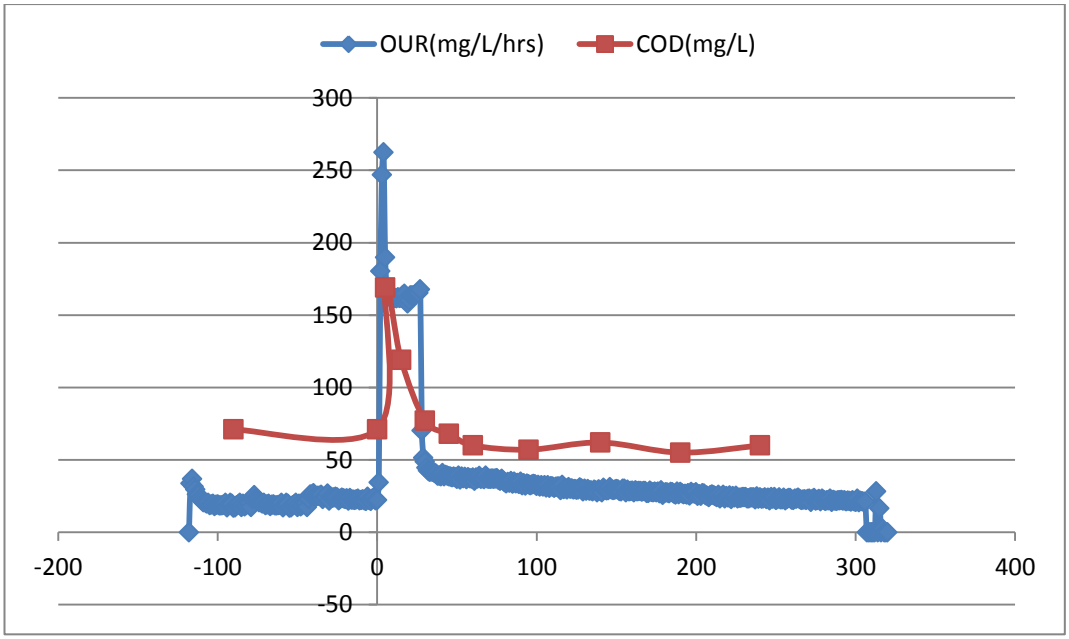

Figure 5.10: COD removal & OUR profile due to respirometric test.

6. CONCLUSION

This dissertation contains an experimental study on the effect of decreasing settling time on properties of a biomass in an anaerobic/aerobic sequencing batch reactor. Parameters such as MLSS and MLVSS, SVI, ESS and COD which can be effect by decreasing settling time were tested during this thesis.

According to the results, a significant biomass washed out ech time when the settling time(Ts) decreased to the next lower level (From 45 to 30,15,5 and 1). A stable biomass concentration obtained in the system when Ts decreased to 5 min. Average biomass concentartion which obtained in the system was 10 g MLVSS/L, SVI_{10} and SVI_{30} values stabilized at 20 and 18 ml/g and COD removal efficiency was more than 95%.

Despite biomass escaping to effluent, good SVI values and high biomass concentration were obtained in the system. According to the findings, it can be demonstrate that the importance of minimum settling velocity (Vmin) rather than settling time(Ts) as the hydraulic pressure for washing out slowly settling flocks and maintain rapidly settling biomass and good effluent quality.

According to the results, when the system stored given feed as internal cellular storage molecules such as PHA and glycogen and deplete this molecules at aerobic phase to consume the release energy for microbial growth. The concentration of internal cellular storage molecules is higher when the system fed with acetate & starch solution than only starch solution. COD concentraion is high after feeding but it deacresd after ending anaerobic phase and starting aerobic phase.

ACKNOWLEDGEMENT

TÜBITAK(Türkiye Bilimisel Ve Teknolojik Araştırma Kurumu) or (The Scientific and Technological Research Council Of Turkey).

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