Nutrient removal from wastewater by aerobic sludge

Master of Science Thesis in the Master's Programme Environmental Measurements and Assessments

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Water Environment Technology
CHALMERS UNIVERSITY OF TECHNOLOGY
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Preface

This Master Thesis project is a part of aerobic granular sludge project which was initiated by Associate Professor Britt-Marie Wilén at the Water Environment Technology Division at the Department of Civil and Environmental Engineering at Chalmers University of Technology. The aerobic granular sludge project was started in September 2008, while my thesis work was started in January 2010. Two student participants were carrying out the project before my work.

My Master Thesis work was supervised and guided by Associate Professor Britt-Marie Wilén. On this occasion, I'd like to express my sincere appreciation to her. Without out her help, I would not to be able to finish my thesis and I have also gained a lot useful knowledge about wastewater treatment technology. Thanks for her patience and kindness. It was a nice experience for me to work on the project.

I also want to acknowledge Mona Pålsson of Environmental Chemistry Laboratory who gave me the practical help during the experiment and staff at GRYAAB wastewater treatment plant who help with a part of analytic work during the experiment period.

Finally, I want to express my thanks to my parents and my friends, their encouragement supported me to tide over some difficult periods.

GÖTEBORG, May 2011
Shuang Qi

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Abstract

Aerobic granular sludge is a new and advanced biological wastewater treatment technology typically applied in sequencing batch reactor (SBR) systems. Compared to conventional activated sludge systems, aerobic granular sludge has good biomass retention, good settling properties and good simultaneous COD, and biological nutrient removal.

This thesis mainly investigated nutrient removal from synthetic wastewater by aerobic granular sludge in lab-scale SBR. The characteristics of aerobic granules at different COD: N: P ratios from 100: 10.47: 0.8 to 100: 2.62: 0.8 and superficial up flow air velocity of 1.47 and 0.82cm/s while organic loading rate (OLR) was 1.5 kg/(m^3.d) were investigated. The pH, reduction-oxidation potential and Dissolved Oxygen (DO) conditions were also analyzed during operation.

The analysis results showed aerobic granular sludge with high removal degree of COD and nutrients. The conversion rate of COD was approximately 90%, and for nitrogen it was 85-95%. At the end of operation period, phosphorus removal reached finally 70-90%. Aerobic granules developed with high N/COD ratio had more compact, stronger structure, smaller size and better settleability. Low superficial up flow air velocity led to larger size and poorer properties of aerobic granules. Under alternating anaerobic-aerobic condition, nitrification and denitrification was achieved by aerobic granules in SBR, especially in the reactor with higher nitrogen load. Enhanced biological phosphorus removal (EBPR) also occurred in the alternating anaerobic-aerobic SBR system.

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Terminology and abbreviations

DO	dissolved oxygen
EBPR	enhanced biological phosphorus removal
EPS	extracellular polymeric substance
OLR	organic loading rate
OUR	oxygen uptake rate
PAG	phosphorus-accumulating granules
SBR	sequencing batch reactor
SND	simultaneous nitrification and denitrification
SOUR	specific oxygen uptake rate
SRT	solids retention time

1. Introduction

The biological treatments of wastewater are usually divided into three types according to their different microorganism growth: flocculated sludge (flocs), the activated sludge is the typical microorganism flocculation growth condition; biofilm, its chief feature is that the microorganisma adher to the solid carrier surface; granular sludge (biogranules), its major characteristic is the microorganism granulated aggregation, has very high biological activity and good settling properties.

The flocculated microbial sludge is the conventional treatment technique used in waste water treatment plants. This conventional technology has some limitations. Conventional activated sludge—based treatment system always require large area containing both nitrification and denitrification reactors. Since the large sludge production and poor sludge settleability of activated sludge, extra large sedimentation tanks (i.e. secondary sedimentation tanks) is needed, which require large construction space and cost. Most treatment systems are not very efficient in biological nutrient removal (including nitrification, denitrification and enhanced biological phosphorus removal (EBPR)), for example nitrification requires a long sludge age simultaneously as flocs are easy to wash out from the system.

Aerobic granular sludge is a new and advanced biological wastewater treatment technology developed at the end of 1990s (Morgenroth, Sherden et al. 1997; Beun, Hendriks et al. 1999). Compared to conventional sludge flocs, the granulated sludge systems have special characteristics such as: good settleability, short settling times, good liquid-solid separation, high biomass retention and the ability of treating high strength waste water and toxic components (Tay, Liu et al. 2001; Moy, Tay et al. 2002; Arrojo, Mosquera-Corral et al. 2004; Tay, Moy et al. 2005; Zheng, Yu et al. 2005; Liu and Liu 2008). Due to its superiority, aerobic granular sludge should be applied to municipal sewage and industrial wastewater treatment with the purposes of avoiding or diminishing secondary settling ponds, consequently reducing the investment and running cost of treatment system.

The aerobic granular sludge technique is a sequencing batch reactor (SBR)-based technology. Column-type upflow SBR supply circular hydraulic attrition to facilitate microbial granulation(Liu and Tay 2002). In the past few years, this SBR-based aerobic granule sludge technique was studied as a hot spot issue owing to its ability of organic carbon removal as well as nitrogen and phosphors removal(Beun, Hendriks et al. 1999; Beun, Heijnen et al. 2001; Etterer and Wilderer 2001; Yang, Tay et al. 2003; De Kreuk and Van Loosdrecht 2004; Qin and Liu 2006). In Yang's study (Yang, Tay et al. 2003), simultaneous removal of organics and nitrogen was feasibly achieved by microbial granules; and in Qin's study, simultaneous nitrification and denitrification occurred under alternating aerobic–anaerobic conditions (Qin and Liu 2006), and a complete denitrification was achieved when external carbon source was added (Qin, Liu et al.

2005). The compact granular systems is particularly suitable for simultaneous COD and nutrient removal.

Therefore, the aim of this study is to analyze COD removal, nitrification and denitrification, as well as biological phosphorus removal under alternating aerobic–anaerobic conditions, and also increasing the knowledge about granule morphology at different COD/N/P loading ratios.

2. Aims and Objectives

The specific aim of this thesis is to achieve stable nutrient removal from synthetic wastewater by aerobic granular sludge in laboratory scale sequencing batch reactors (SBR). The development of aerobic granular sludge was analyzed by operating the system at: 1) different COD and N loading rates; and 2) different superficial up flow air velocity. The feasibility of achieving nitrification and denitrification in SBR under alternating anoxic-aerobic conditions and the occurrence of enhanced biological phosphorus removal in system was assessed.

The main research questions to be answer in the thesis are:

1. How would superficial up flow air velocity impact aerobic granule characteristics?
2. How would different COD/N/P loading ratios affect the nitrogen and organic carbon removal as well as phosphorous elimination?
3. How would different COD/N/P loading ratios affect the granule characteristics?
4. Can nitrification/denitrification and enhanced biological phosphorus removal be achieved under alternating anoxic-aerobic conditions in SBR?

3. Theoretical background

3.1 Sequencing Batch Reactor Technology

Sequencing batch reactor (SBR) technology is an intermittent aeration process applied by activated sludge systems for wastewater treatment. As opposed to the conventional wastewater technique, SBR technology separate operation processes by time instead of by space. In the aerobic granular sludge system, SBR is reported as the exclusive technique to cultivate aerobic granules.

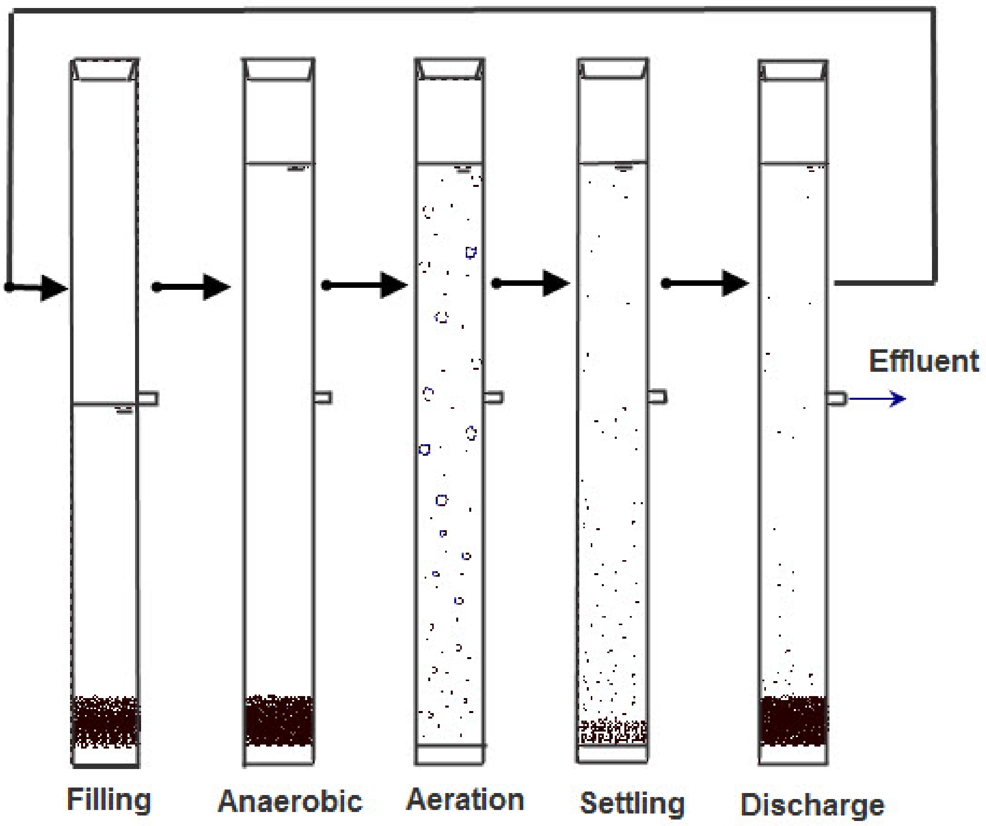


Figure 3-1 Alternating aerobic-anaerobic SBR processing

Figure 3-1 shows an alternating aerobic-anaerobic SBR system which is widely applicable to aerobic granulation. Alternating aerobic-anaerobic SBR processes usually include several stages in one process cycle: filling feed solution; anaerobic rest; air flow aeration; sludge settling and effluent discharge.

Anaerobic phase starts after feeding. In this stage, anoxic condition as well as sufficient COD improves denitrifying bacteria to oxidize nitrate to nitrogen gas following the reaction above (acetate as carbon source for instance):

$$5CH_3COOH + 8NO_3^- \rightarrow 4N_2 \uparrow + 10CO_2 + 6H_2O + 8OH^-$$

The phosphorus-accumulating granules (PAGs) are also working to release phosphorus and remove COD in this anaerobic stage.

After the anaerobic phase, an aeration stage is introduced in the SBR. The mixed liquid in the SBR is aerated by air bubbles from the bottom of the column. In this aerobic phase, COD will be removed rapidly and nitrifying bacteria will start to oxidize ammonium to nitrate and nitrite by two types of bacteria working in cooperation. The total reaction can be presented according to the following equation:

$$\mathrm{NH_4^+ + 1.86O_2 + 1.98HCO_3^- \rightarrow 0.02C_5H_7NO_2 + 0.98NO_3^- + 1.88H_2CO_3 + 1.04H_2O}$$

The reaction shows that alkalinity is an important reagent in nitrification process. So that pH and alkalinity values should be optimize in SBR in order to achieve nitrification.

In the aerobic stage, P-accumulating granules (PAG) take up the phosphorus content in the liquid bulk phase, thus phosphorus will be eliminated in the SBR. The reaction for biological phosphorus removal is described as in the following equation:

$$\mathrm{40.4g\ P - P + 1g\ HAc - COD \rightarrow 0.4g\ PO_4^{-3} - P + 1g\ PHA - COD + 0.04g\ H^+}$$

Therefore, enhanced biological phosphorus removal can be developed in alternating aerobic-anaerobic SBR systems.

3.2 Aerobic granulation

As was mentioned in the previous chapter, aerobic granular sludge technology is a new, advanced biological wastewater treatment technique compared with conventional activated sludge which is generally used in municipal sewage systems. In order to distinguish aerobic biogranules from the conventional flocculated sludge, the following definition came out during the first aerobic granular sludge workshop in 2004 (De Kreuk, Kishida et al. 2007):

Granules making up aerobic granular activated sludge are to be understood as aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear, and which settle significantly faster than activated sludge flocs.

The characteristics of aerobic granules in SBR were extensively researched by many leading researchers in this field. In this chapter, the formation mechanism, affecting factors, nutrition removal and other characteristics of aerobic granules will be briefly introduced.

2.2.1 Mechanism

Aerobic granulation is recognized as a process of microbial self-immobilization without the support of carrier (Tay, Liu et al. 2001). This process is regarded as a multistage formation process consisting of multiple physical, chemical and biological actions. The general immobilization mechanism of aerobic granulation, which is similar with to formation of biofilm and anaerobic granular reported by Liu al. (Liu and Tay 2002), is including the following four steps:

Step1: *Physical movement to initiate bacterium-to-bacterium contact or bacterial attachment onto nuclei*
Step2: *Initial attractive forces to keep stable bacterium-to-bacterium contacts*
Step3: *Microbial forces to make cell aggregation mature*
Step4: *Stable three-dimensional structure of microbial aggregate shaped by hydrodynamic shear forces*

In this four-step model, a number of physical, chemical and biochemical forces interact to form microbial granules, e.g. hydrodynamic force, thermodynamic force, van der Waals forces, hydrogen liaison, formation of ionic pairs and cellular surface dehydration and so on. Hydrodynamic shear force finally shapes the microbial granules and also effect the outer shape and size of granules associated with microbial species, substrate loading rate and other factors (Liu, Joo-Hwa Tay et al. 2006)s.

Extracellular polymeric substances (EPS) is a sticky macromolecule matter secreted by bacterial cells, which should form a protective capsular matrix, and play a important role in adhesion of microorganism cell, formation and stability of microbial granules structure, and also be able to improve and strengthen of granules structure and enhance granules surface physical property (Tay, Liu et al. 2001; Liu, Liu et al. 2004; McSwain, Irvine et al. 2005). In microbial granulation, EPS, which has been observed in both aerobic and anaerobic granules, works as bridges among individual bacterial cells, supplying spacious surface area for microorganism adhesion.

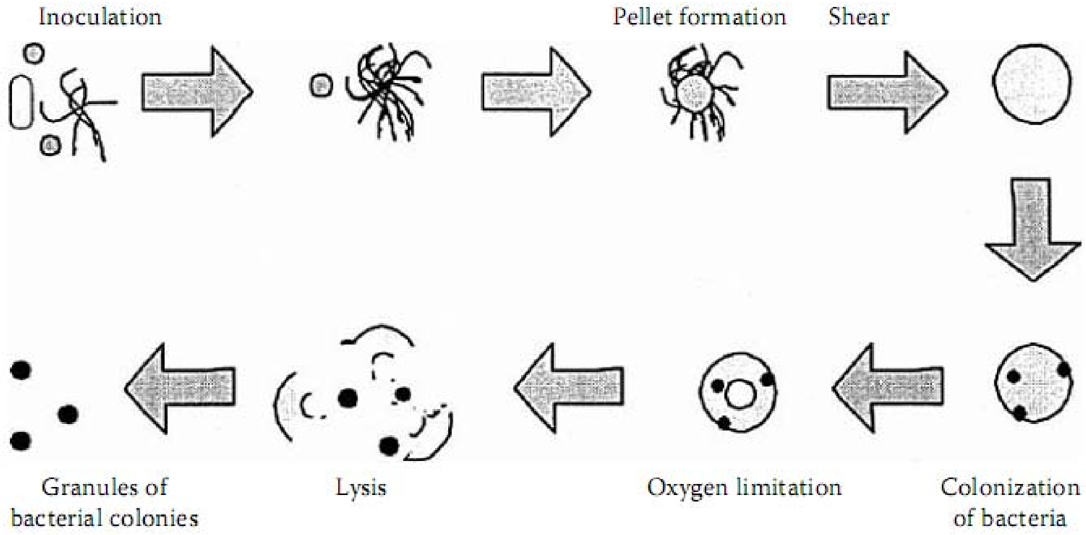


Figure 3-2 Illustration of aerobic granulation proposed by Beun et al., (1999)

Figure 3-2 demonstrate the mechanism of aerobic granulation in SBR investigated by Beun et.al (Beun, Hendriks et al. 1999).

2.2.2 Affecting factors

Aerobic granulation is a microbial aggregation process by cell to cell immobilization. Many factors are able to impact cells aggregation in a culture. Expecting to form dense, compact, stable and strong aerobic granules, some affecting factors have been investigated:

- **Substrate composition and loading**

  Studies show that aerobic granulation can be cultivated in many different organic substrates in SBR, e.g. both of synthetic and real municipal wastewater, glucose, acetate, ethanol and so on (Morgenroth, Sherden et al. 1997; Beun, Hendriks et al. 1999; Peng, Bernet et al. 1999; Arrojo, Mosquera-Corral et al. 2004). Glucose as well as acetate are general substrates that are most widely applied. Granules cultivated by glucose substrate gives more filamentous structures, whereas the acetate-fed granules have more dense and non-filamentous structure (Tay, Liu et al. 2001). Aerobic granules can also grow at large range of substrate loading rates, from 2.0 to 15 kg COD $m^{-3}day^{-1}$ (Moy, Tay et al. 2002; Liu, Tay et al. 2003). And nitrifying bacteria and phosphorous-accumulating bacteria can also form independently of either substrate composition or substrate loading rate (Tsuneda, Nagano et al. 2003). Therefore, feed composition and organic loading rate are not the essential determinant in aerobic granules formation process, only some physical characteristics e.g. microbial structure depend on substrate type and load.

- **Hydrodynamic shear force**

  The hydrodynamic shear force in column SBR is created by the superficial upflow air velocity produced during aeration. Evidence shows aerobic granules formation is not decided by hydrodynamic shear force level, whereas the microbial structure of aerobic granules is correlated with it. High shear force, referred to superficial upflow air velocity, is capable of creating more compact, denser, stronger and smaller granules (Liu and Tay 2002; Tay, Liu et al. 2004).

- **Feast-famine regimen**

  The aeration process in SBR includes two different periods: degradation period to exhaust organic feed; aerobic starvation period when no external carbon source exists. This process is called periodic starvation, i.e. periodic feast-famine regime (Tay, Liu et al. 2001). When periodic feast-famine occurs, bacteria will improve its cell hydrophobicity to resist starvation (Liu, Yang et al. 2004). Due to the fact listed above, feast-famine regime should be an affecting factor in cell surface property selection, however, aerobic granules cannot be cultivated successfully without managing settling time as periodic feast-famine occurs (Qin, Tay et al. 2004).

- **Feeding strategy**

According to McSwain's study, SBR filling time is an unnecessary factor in aerobic granulation, simply high feast-famine ratio or pulse feeding lead to more compact and denser granular structure (McSwain, Irvine et al. 2004).

- **Dissolved oxygen**

Aerobic granules can be cultivated at different dissolved oxygen concentration, from high level 2-6 mg l^{-1} to low concentration 0.7-1.0 mg l^{-1} (Peng, Bernet et al. 1999; Qin, Tay et al. 2004). It implies that dissolved oxygen concentration has little impact in aerobic granulation.

- **Settling time**

According to the preceding section, water treatment in SBR contains the following phases: feeding, aeration, settling and discharge. During the settling phase, if sludge is not settling out within a certain settling time, it could be washed out when the discharge phase begins. Only aerobic granules with good settleability are able to settle down within short settling time, thus settling time becomes a crucial parameter for aerobic granules formation. Qin et al. found that aerobic granules cannot form unless settling time is controlled to less than 5min (Qin, Liu et al. 2004; Qin, Tay et al. 2004). Under short setting time condition, sludge flocs with poor settleability will be eliminated by discharging; good settling granules will be reserved in SBR, hence improve aerobic granulation process (Liu, Wang et al. 2005). Therefore, settling time plays a significant role in aerobic granulation. Optimizing settling time is an essential work to operate aerobic granulation.

- **Exchange ratio**

Exchange ratio in SBR is described as the liquid volume withdrawn when settling stage finish divided by total working liquid volume in reactor. As illustrated in Figure 3-3, when reactor diameter is constant, exchange ratio is proportionally related to L, i.e. height from liquid surface to discharge port. So exchange ratio can be defined as following equation:

$$\text{Exchange ratio} = L/H$$

Where L is height of discharge port to water surface, and H is the total height of working liquid volume in reactor.

Investigation exhibited that by keeping settling time at 5min in SBR, only high excharge ratios of 60% and 80% had aerobic granules dominant

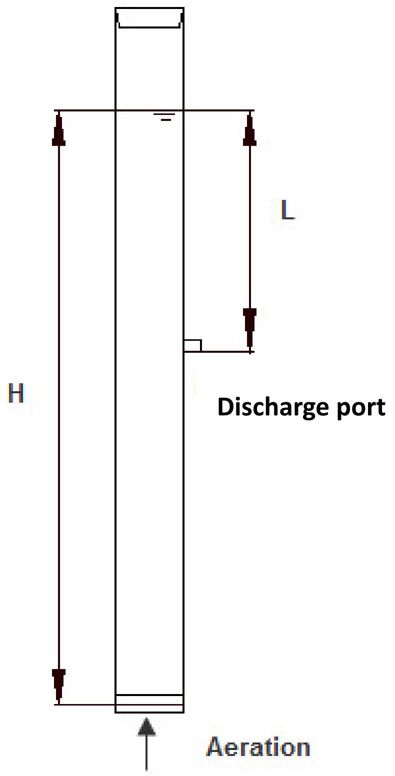


Figure 3-3 Schematic of a column-type SBR for aerobic granulation

(Wang, Liu et al. 2006). It seems excharge ratio is regarded as a decisive parameter to aerobic

granules formation.

According to the affecting factors discussed above, settling time and exchange ratio were considered as the crucial selection pressure for the aerobic granulation process.

2.2.3 COD, nutrition removal and heavy metal biosorption by aerobic granules

As was mentioned in the previous section, aerobic granular sludge can be applicable at COD loading as high as 15 kg $m^{-3}day^{-1}$, or low loading to about 2.0 kg $m^{-3}day^{-1}$.

Simultaneous removal of COD and nitrogen by aerobic granules is also expected in SBR. Yang et al. explored its feasibility and found simultaneous removal was able to operate at different substrate N/COD ratios, from 5/100 to 30/100 (Yang, Tay et al. 2003). In alternating aerobic-anaerobic condition, organic carbon and nitrogen removal in SBR was also investigated by Qin et al. (Qin and Liu 2006). Under alternating aerobic-anaerobic condition, denitrification will be well developed with external carbon source added, hence simultaneous nitrification and denitrification (SND) is possible to achieve.

Studies of phosphorous removal by aerobic granules at variable P/COD ratios in SBR were developed by Lin and Liu et al (Lin, Liu et al. 2003; Liu, Lin et al. 2005). Investigations indicated that phosphorous is eliminated by P-accumulating granules (PAG) cultivated in aerobic granules in SBR are the same as can be found in conventional activated sludge, i.e. release of phosphorous and removal of COD at anaerobic condition while phosphorous is taken up at aerobic conditions. Compared to conventional flocculated sludge, microbial granules have high P uptake capacity about 1.9-9.3% by weight.

The feasibility of heavy metal biosorption by aerobic granules was discussed by Liu et al (Liu, Joo-Hwa Tay et al. 2006). Studies explored biosorption profiles of Zn^{2+} and Cd^{2+} by granules and found aerobic granules biosorption capacity is correlated with initial concentration of metal ions and initial aerobic granules concentration. The maximum adsorption capacity of Zn^{2+} was 270mg/g while it was 566mg/g of Cd^{2+}.

4. Materials and methods

4.1 Reactor set up

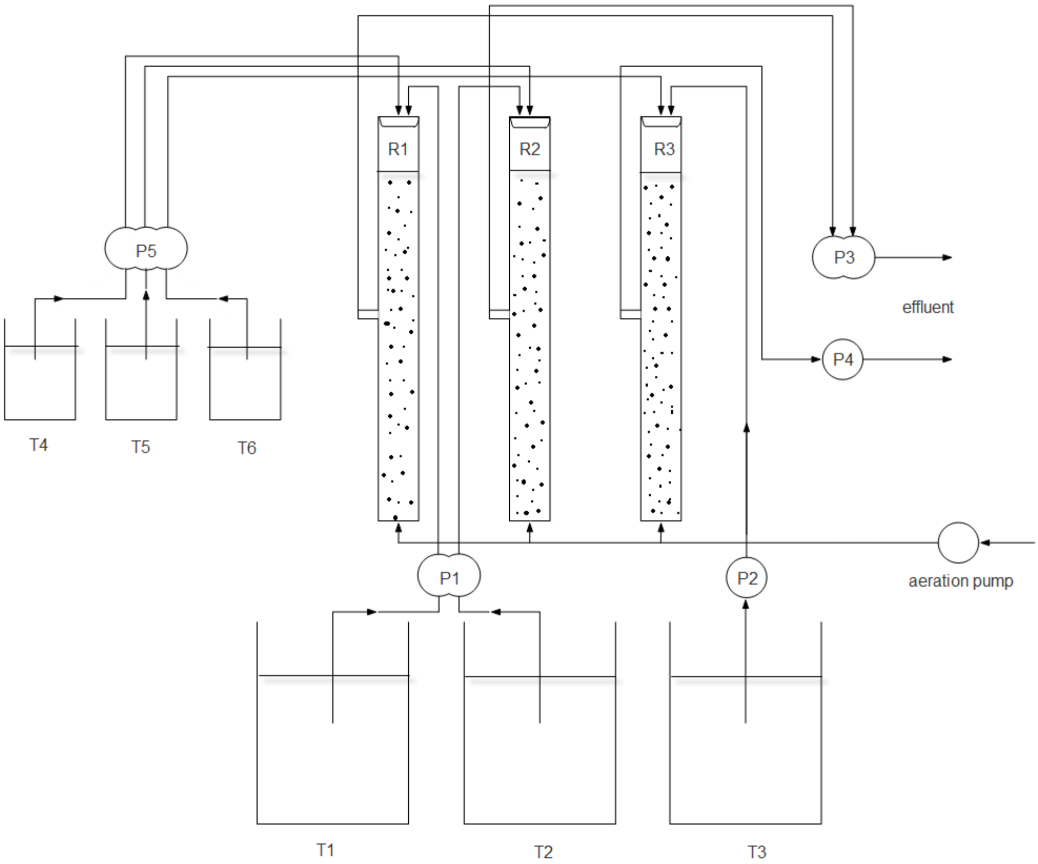
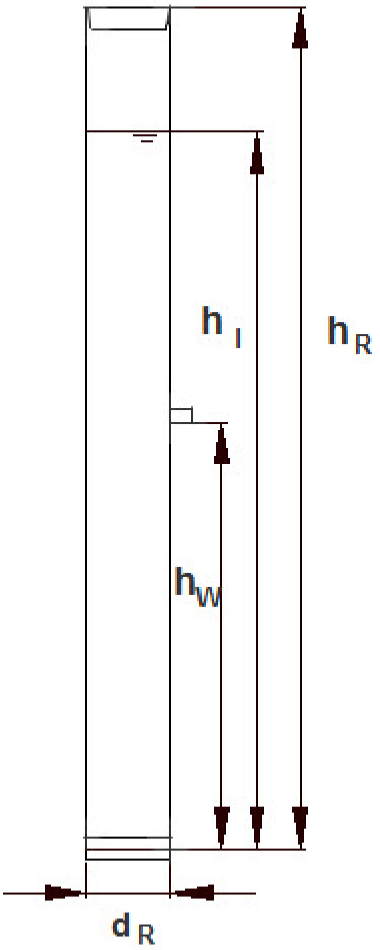


Figure 4-1 Experimental reactor set up

The lab-scale experiment system is designed as shown in Figure 4-1. The synthetic wastewater with different nitrogen load was stored in tanks T1, T2 and T3 used as feed. The volume of each feed tanks is 100 liter. Sodium acetate solution served as carbon source was kept in three glass bottles, i.e. T4, T5 and T6. The reason acetate solution was kept separately from the feed is to prevent carbon source from degrading or leading to precipitation in the large feed tanks. P1 and P2 are two peristaltic pumps in charge of pumping synthetic wastewater from T1, T2 and T3 through inlet pipe to supply Reactor 1, 2 and 3 (in terms of R1, R2 and R3). P3 and P4 pump out effluent from three reactors from discharge ports. P1 and P3 are two-channel peristaltic pumps while P2 and P4 are single types. Acetate solution is pumped by a three-channel peristaltic pump i.e. P5 to provide carbon source to Reactor 1-3. The feed from tanks T1-T6 were inserted in two separate tubes inserted in each reactor and the feed entered the columns at the bottom of the reactors. This was to achieve good mixing during feed through a stagnant bed of settled granules. Aeration was performed by inserting compressed air through glass filters at the bottom of the column reactors. The pump is controlled by magnetic valves and the air flow is regulated by a flow meter.

4.2 Reactor design and operation condition



Reactor dimension design of the air bubble column SBR is illustrated in Figure 4-2. The total height of the reactor (h_R) is 1.32m. The height of discharge (withdrawn) port (h_w) is 0.63m. And the water level in reactor after feeding (h_l) is 1.1m. The water level was kept lower than the total reactor height to make sure liquid in reactor will not overflow when aeration start. During aeration the water level increased by 8%. The inner diameter of the reactor (d_R) is 0.06m and the total liquid volume after feeding is 3.05l. Synthetic wastewater feed, sodium acetate solution and aeration air flow are pumped through rubber tubes respectively to the bottom of reactor.

The exchange ratio in this column SBR is calculated by the equation mentioned in chapter 2, i.e. $1- h_w/ h_l \approx 0.43$.

The experiment temperature was kept at room temperature during the whole operation period.

Figure 4-2 Reactor dimensions

The aeration air flow is regulated and controlled by a flow meter. In the first period of the experiment (from 2009/09/08 to 2010/02/08), the air flow at the bottom of the reactor was Q_{air}=2.5 l/min. The superficial up flow air velocity can be recalculated estimated to 1.47 cm/s according to the equation:

$$v = \frac{Q_{air}}{A_R} = \frac{Q_{air}}{(\pi/4) \cdot d_R^2} \quad [cm/s]$$

In the second period (from 2010/02/08 to 2010/08), the air flow was reduced to Q_{air}=1.4 l/min, as well as the superficial up flow air velocity was reduced to 0.82 cm/s correspondingly.

4.3 Cycle time

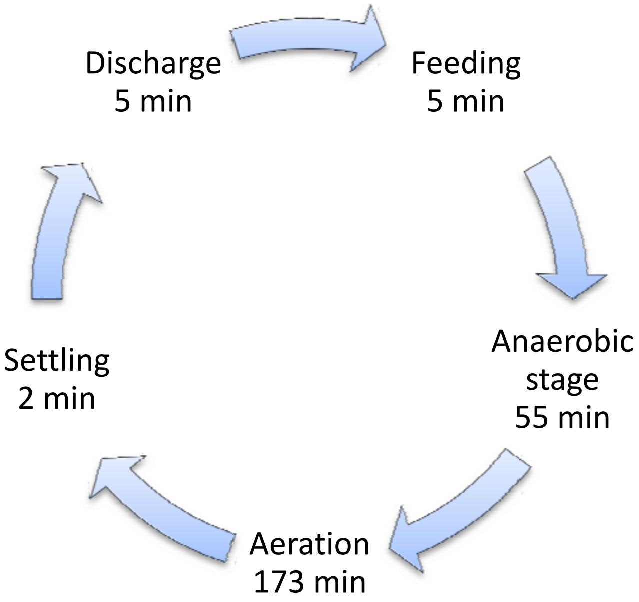


Figure 4-3 Stages in one cycle

In this experimental alternating aerobic-anaerobic SBR, the cycle time was fixed at 4 hours. During one 4-hour-cycle, 5 min feeding time, 55 min anaerobic stage, 173 min aeration phase, 2 min settling time and 5 min water withdrawn were included. When a new cycle started, 57% liquid in reactor remained from previous cycle.

4.4 Medium

The synthetic wastewater consists of the following chemical substances: sodium acetate (CH_3COONa), NH_4Cl (nitrogen source), K_2HPO_4 (P source), $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$. The chemical composition in all reactors is in same loading rate except NH_4Cl. The NH_4Cl concentration in reactors decreased gradually to provide different N/COD ratio in SBR. Micronutrients were added to each feed tank 100 ml per 100 l. The chemical composition of the three solutions and the micronutrient solution is listed in Table 4-1 and Table 4-2. The recipe composition of the synthetic wastewater refer to Liu and Tay (Liu and Tay 2007), while the recipe of microelement solution is based on Tay et.al. (Tay, Liu et al. 2001).

The composition of synthetic wastewater was diluted by deionized water and stored in 100 l tank. 1.31 l synthetic wastewater was fed to the SBR in each cycle. The COD concentration in the feed for all reactors was 500 mg/l resulting in an organic loading rate of 1.5 kg/(m^3.d).

From 2010/02/05, the pH in acetate solution was adjusted to 6 by 37% HCl in order to reduce the pH increase in the reactors.

Table 4-1 Composition of the synthetic wastewater

Unit (mg/l)	Reactor 1	Reactor 2	Reactor 3
CH_3COONa	649.7	649.7	649.7
$MgSO_4 \cdot 7H_2O$	12.5	12.5	12.5
$CaCl_2 \cdot 2\ H_2O$	15	15	15
FeSO4·7H2O	10	10	10
NH_4Cl	200	100	50
K_2HPO_4	22.5	22.5	22.5
COD: N: P	100: 10.47: 0.8	100: 5.24: 0,8	100: 2.62: 0.8
N loading rate(kg/(m^3.d))	0.135	0.068	0.034

Table 4-2 Composition of the microelement solution

Microelements	g/l
H_3BO_3	0.5
$ZnCl_2$	0.5
$CuCl_2 \cdot 2H_2O$	0.038
$MnSO_4 \cdot H_2O$	0.5
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.5
$AlCl_3$	0.5
$CoCl_2 \cdot 6H_2O$	0.5
$NiCl_2$	0.5

4.5 Analytical methods

- **pH/ DO/ conductivity:** the pH and DO were measured by electronic probes (SATRON) connected to a data logger (INTAB) connected to a computer.. The logging program is Easy View 5, the logging interval was 1 s but only an average from 1 min was stored. Conductivity measurement is performed by conductivity electrode combined with a WTW Multiline P4 device.

- **COD/PO$_4$:** to analyze chemical oxygen demand (COD) and phosphate content (PO$_4$), water samples were filtered through 0.45μm micropore filters, then followed HACH method. The COD measurement was based on HACH COD vial method, which is sensitive for COD concentrations between 0-150 mg/l. While the PO$_4$ was analyzed by HACH PERMACHEN PhosVer 3 method applied to PO$_4$ concentrations between 0 to 2.5 mg/l.

- **NO$_3$/NO$_2$/NH$_4$:** nitrate-nitrogen, nitrite-nitrogen and ammonium-nitrogen concentrations in the filtered effluent sample were measured by GRYAAB wastewater treatment plant by using the photometric method (FIAstar 500 instrument), according to FOSS Tecator AB.

- **MLSS/MLVSS:** mixed liquor suspended solids (MLSS) is a measurement of biomass content in activated sludge while mixed liquor volatile suspended solids (MLVSS) is regarded as amount of organic matters in the solid fraction of the sludge. According to *Standard Method for the Examination of Water and Wastewater* (APHA 1998), MLSS and MLVSS were measured by weighting. Filtered solid samples were dried at 105°C. The MLSS was calculated as:

  $$MLSS = \frac{m_{MLSS} - m_{Initial}}{sample\ volume}\ [g/l]$$

  Heating the dried samples at 550°C burning chamber with filters, MLVSS equals to:

  $$MLVSS = \frac{m_{MLSS} - m_{MLVSS}}{sample\ volume}\ [g/l]$$

  The volatile organic compounds ratio in biomass is presented as volatile fraction:

  $$Volatile\ fraction = \frac{MLVSS}{MLSS} \cdot 100\ [\%]$$

- **SVI/SSV:** the measurement of sludge volume index (SVI) and settled sludge volume (SSV) followed *Standard Method for the Examination of Water and Wastewater* (APHA 1998). SVI and SSV values give indication of the settleability and compactness of granular sludge. SVI is a ratio of settled sludge volume (SSV) and mixed liquor suspended solids (MLSS):

  $$SVI = \frac{SSV}{MLSS}\ [ml/g]$$

  Where settled sludge volume (SSV) is defined as:

  $$SSV = \frac{suspension\ volume\ after\ 30\ min}{100\ ml} \cdot 10\ [ml/l]$$

  This experiment was performed as: taking 100 ml mixed sample, settling in a 100 ml cylinder for 30min. The settled height of granules was also measured after 5 minutes. For granular sludge the SVI measured at 5 and 30 minutes should be roughly the same.

- **Microscopy:** the microscopic images of aerobic granules were taken by a Olympus BX60 light microscope at 2X magnification without cover glass.
- **Settling velocity:** taking granules with different sizes, drop them into a 250 ml cylinder filled with room temperature tap water. Select a certain height, measure the settling time of granules through this height by stopwatch, then calculate the settling velocity.
- **Hydrophobicity:** the measurement of hydrophobicity in this experiment referred to Rosenberg et al (Rosenberg, Gutnick et al. 1980). Measure the absorbency of treated samples by UV spectrometer, cell surface hydrophobicity of aerobic granules is calculated as:

  $$Hydrophobic\ Fraction = \left(1 - \frac{ABS_{Initial} - ABS_{Separation}}{ABS_{Initial}}\right) \cdot 100\ [\%$$

  Where $ABS_{Initial}$ is the absorbance of the diluted sample and $APS_{Separation}$ is the absorbance of water phase after separation. The measurement was always made in triplicate.

- **Oxygen uptake rate (OUR):** refer to OUR/SOUR test in *Standard Method,* testing method in this experiment was planning as: prepare an Oxygen meter YSI5100 with an oxygen probe, six 300 ml BOD bottles (2 for each reactor). Add 300 ml feed solution that is saturated with oxygen to 30 ml of sludge suspension taken from reactor. Add 3 ml of acetate solution (sodium acetate of 32.5 g/l). Insert the oxygen probe and start measuring the decrease in DO concentration every 15 s for about 10 min. Do the same with the second bottle but add 100 µl of 3.3 g/l ally-thiourea to stop nitrification. Measure the MLSS and VSS after the measurements.

  The OUR ratio is equal to:

  $$OUR = \frac{DO_{start} - DO_{end}}{t_{elapsed}} \cdot \frac{3600sec}{1\ h} \cdot \frac{total\ volume}{sample\ volume}\ [mgO2/l/h]$$

- **Nitrification rate**

  Reagents

  Feed from large feed reactor 900ml; granule solution from the different reactors 100ml; Compressed air.

  Procedure

  Mix feed solution (previously saturated with air) with granule suspension on a magnetic stirrer. Add compressed air through stone diffuser. Measure the temperature (room temperature ca 20 C). Take samples for ammonium along the experiment. Run the experiment for 2 hours and take samples for nitrate (10ml) every 15 minutes the first hour, then every 30 minutes and filter immediately through 0.45 µm filter. Dilute the nitrate samples 10 times as for normal analysis (3.5 ml filtered sample plus 31.5 ml MQ water). Take samples for granule concentration sometime under the experiment.

- **Denitrification rate**

Reagents

Feed from large feed reactor 900ml; granule solution from the different reactors 100ml; acetate stock solution 32.5 g/l: add 9 ml to 1000 ml; nitrogen gas; stock solution of nitrate 26g/l as NO_3-N: add 2 ml to get an initial concentration of 52.4 mg NO_3-N/L.

Procedure

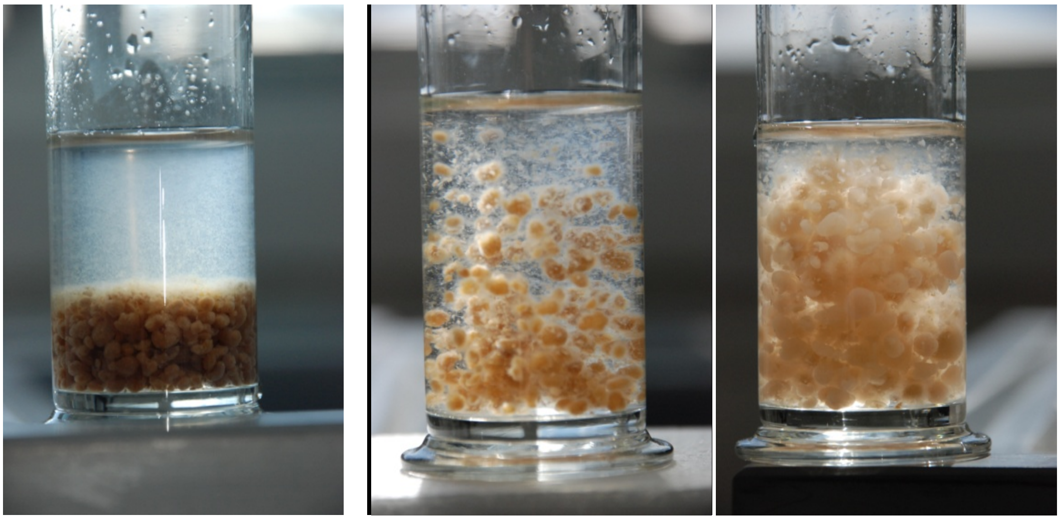
Mix feed solution and granules suspension on a magnetic stirrer. Add nitrogen gas and leave for 30 minutes. Measure the temperature (room temperature ca 20 C).Take samples for COD and nitrate along the experiment. Run the experiment for 2 hours and take samples for nitrate (10ml) every 15 minutes the first hour, then every 30 minutes and filter immediately through 0.45 µm filter. Take samples for COD with the same frequency. Dilute the nitrate samples 10 times as for normal analysis (3.5 ml filtered sample plus 31.5 ml MQ water). Take samples for granule concentration sometime under the experiment.

5. Result and discussion

5.1 General

The aerobic granule experiment started in September 2009, and continued to August 2010. The author of this thesis participated in the experiment operating from January 2010 to May 2010. All the operation parameters were the same as in the beginning except for aeration air flow. The aeration air flow, i.e. superficial up flow air velocity, was decreased from 1.47 to 0.82 cm/s in 2010/02/08. Theoretically, granule size is supposed to increase while superficial up flow air velocity is decreased.

As shown in Figure 5-1, the properties of the granules in R1, R2 and R3 had large difference. The granules in reactor 1 were deep brown, had denser structure and smaller size than in other reactors. Granules of R2 were light brown and had a filamentous surface structure. In reactor 3, granules had pink color and gel-like matrix.



COD/N: 100/10.47 SRT: 62 days

COD/N: 100/5.24 SRT: 40 days

COD/N: 100/2.62 SRT: 33 days

Figure 5-1 granules properties of R1, R2 and R3 (2010/05/03)

According to the different N/COD loading rate, aerobic granules were supposed to display that the granules size gradually increased as well as compactness gradually decreased while N/COD loading rate decreased (Liu, Joo-Hwa Tay et al. 2006).

The solids retention time (SRT), so-called sludge age, was 62, 40 and 33 days in reactor 1, 2 and 3 respectively, due to mixed liquid withdrawn approximately 39.2 ml/day of each reactor. SRT was calculated by the following equation:

$$STR = \frac{V_R \cdot MLSS_R}{Q_W \cdot MLSS_R + Q_E \cdot MLSS_E}\,[d]$$

Where V_R is total operating mixed liquid volume in reactor; $MLSS_R$ is average mixed liquid suspended solids in reactor; Q_W is mixed liquid withdrawn volume per day; Q_E is effluent volume per day; $MLSS_E$ is average mixed liquid suspended solids in effluent.

Since aerobic granules in reactor 1 had very long solids retention time, granules in R1 had darker color than in the other reactors. Granules in R3 had shortest sludge age, reasonably they had a pinkish color.

Because the experiment was operating by other participant before Jan. 2010, the result of that period will not present in the following part. Some time data and results from that operation period need to be mentioned, it will be named as period I.

5.2 Mixed liquid suspended solids

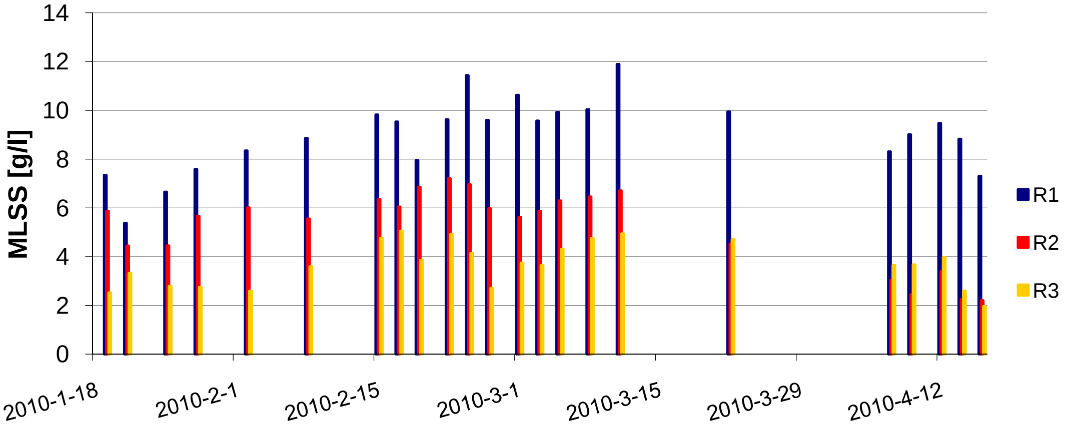


Figure 5-2 Mixed liquid suspended solids in reactors

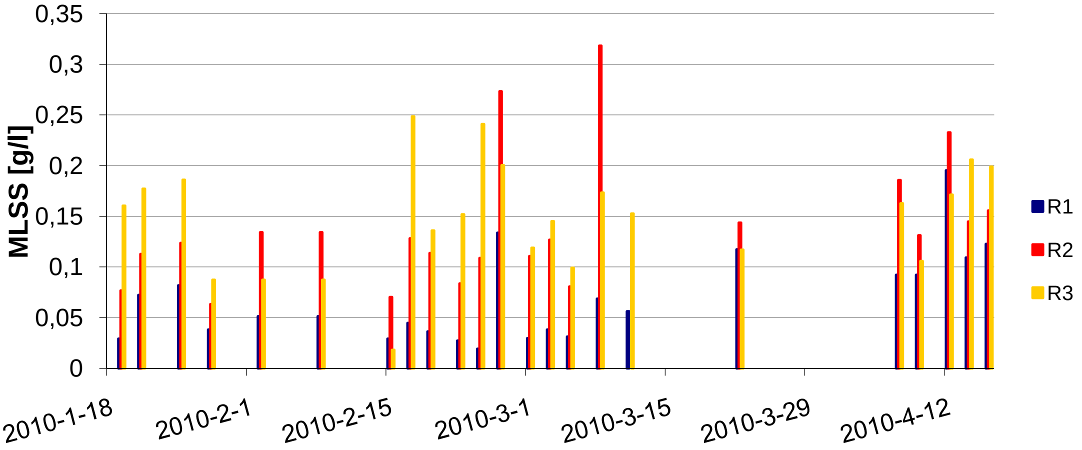


Figure 5-3 Mixed liquid suspended solids in effluent

Figure 5-2 illustrates the mixed liquid suspended solids from January to April 2010.

Before the reduction of aeration air flow in 2010/02/08, the MLSS in all three reactors was increasing as the granules were formed and matured. After that the air flow changed, the MLSS in each reactor was stable in a range for a period. The MLSS in reactors kept the same tendency with R1>R2>R3 until middle of March. The MLSS trend in the reactors was corresponding to the N/COD loading rate in different reactors. From middle of March, MLSS in R2 dropped a lot and also in R3. The exact reason to the drop in MLSS is not known but some disturbances might have occurred due to less sampling and hence less granules were removed from the system. From the Figure 5-3, MLSS in effluent increased after the aeration air flow reduction, it maybe because the poorer settleability caused by lower hydrodynamic shear force. Aerobic granules with poor settleability could not settle within 2 min settling time and was washout by effluent withdrawn.

5.3 Mixed liquid volatile suspended solids

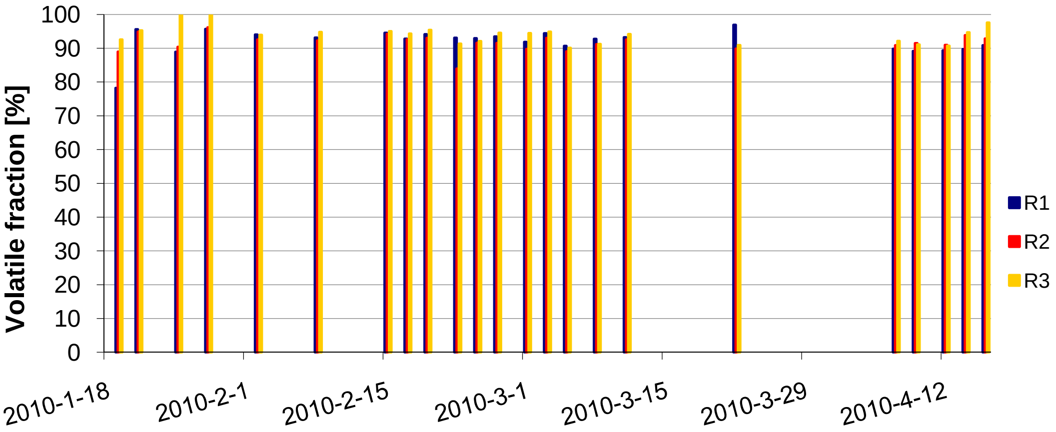


Figure 5-4 Volatile fraction in reactors

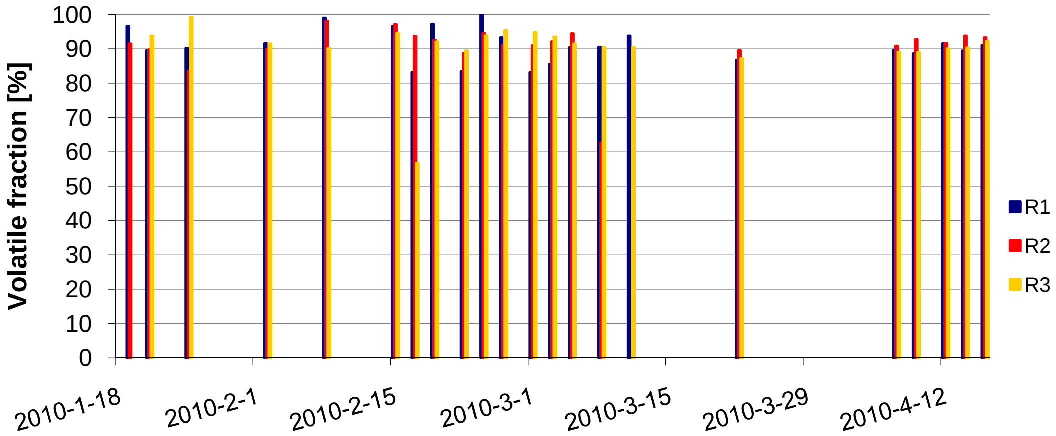


Figure 5-5 Volatile fraction in effluent

Mixed liquid volatile suspended solids in all reactors and effluent exhibited a similar tendency as the mixed liquid suspended solids, see appendix A. According to the method mentioned in the last chapter, the volatile fraction was calculated as:

$$Volatile\ fraction = \frac{MLVSS}{MLSS} \cdot 100\ [\%]$$

The results of the volatile fraction are shown in figure 5-4 and 5-5. The volatile fraction in reactors was as high as approximately 90%, which indicated that organics content in granules was very high. And the volatile fraction was almost no change after air flow reduction, seems hydrodynamic shear force did not effect on volatile fraction in granules.

5.4 Sludge Volume Index

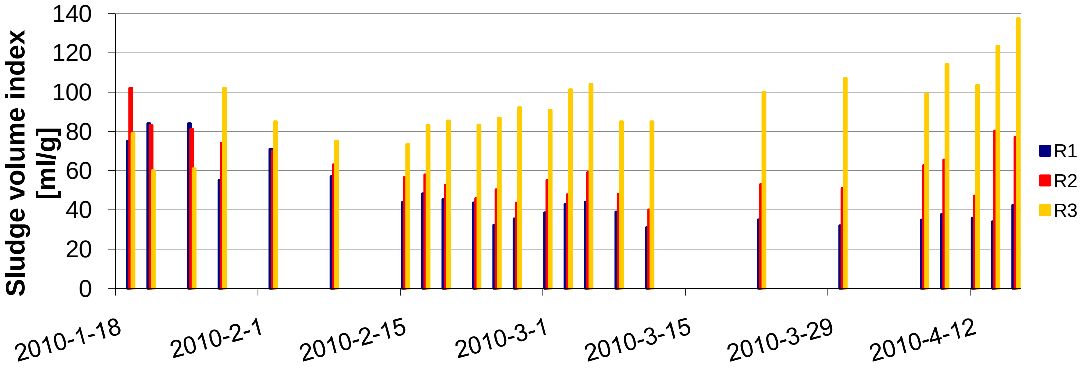


Figure 5-6 Sludge volume index during operation

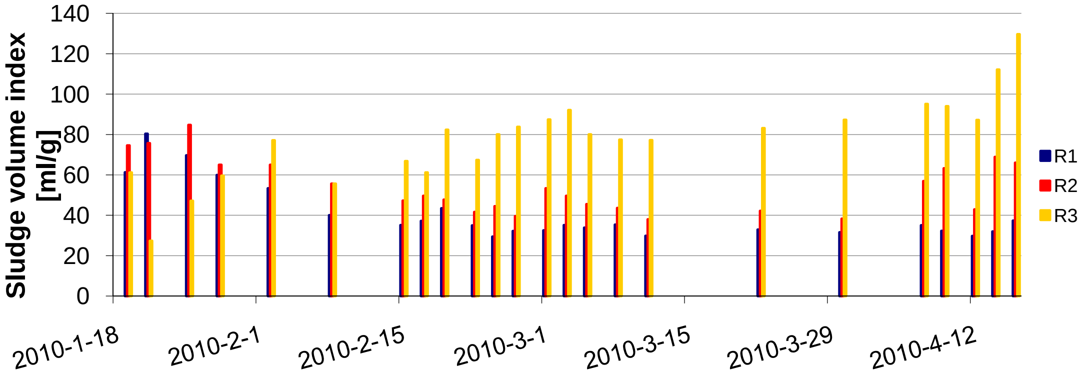


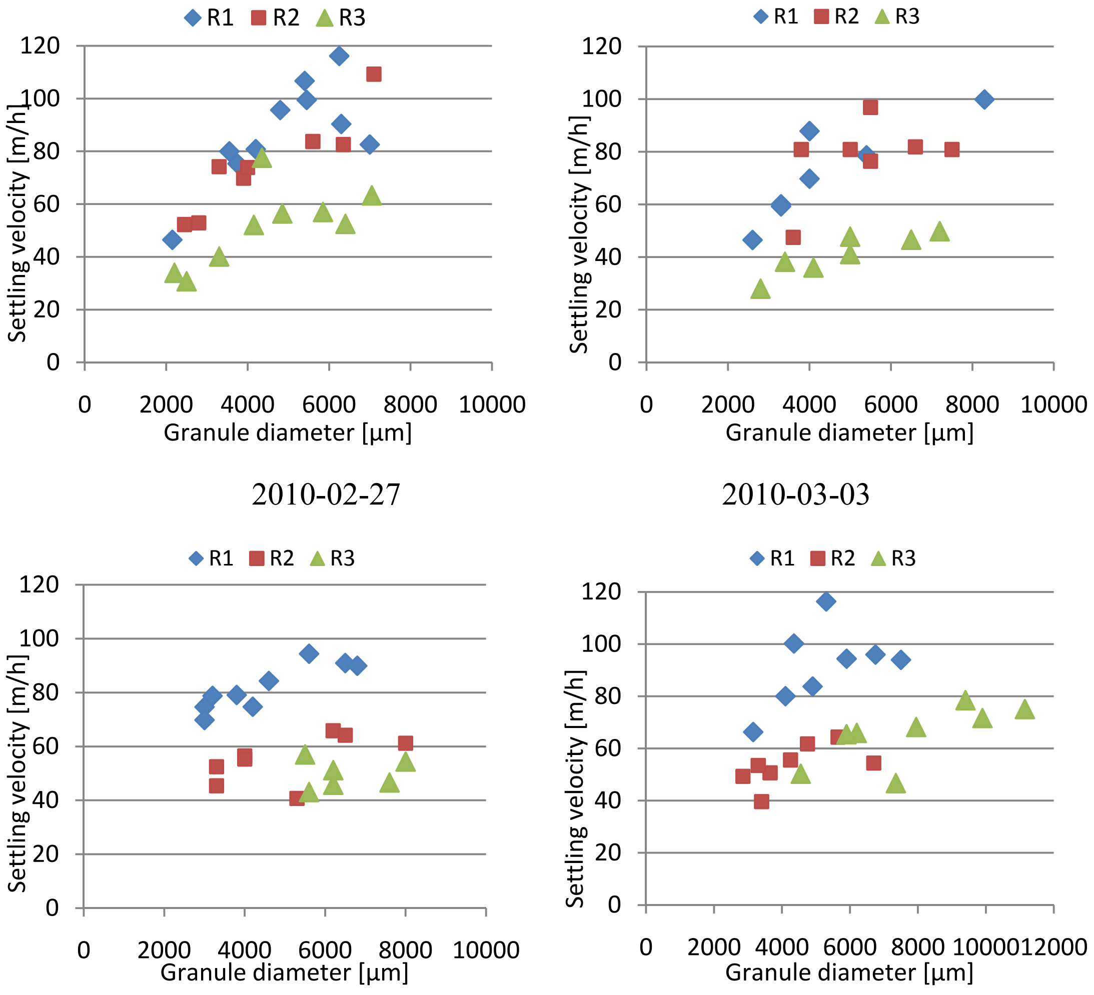
Figure 5-7 Adjusted sludge volume index

Sludge volume index (SVI) is a measure of the settling property of granular sludge. According to the calculation formula mentioned in the last chapter, sludge volume index is correlated with settled sludge volume (SSV) and mixed liquid suspended solids concentration. Data of the settled sludge volume was shown in Appendix A.

During high aeration air flow period, SVI was decreasing gradually. When air flow was reduced, SVI value is supposed to become greater. Referring to Figure 5-6, sludge volume index in reactor 3 increased rapidly and kept increasing until the end of operation. In R2, the SVI increasing rate after air flow change was slower. However, sludge volume index in R1 was nearly constant. The possible reason was the slow growing nitrifying bacteria were forming in SBR. Nitrifying granules has more compact, stronger structure, smaller size and higher MLSS concentration.

The sludge volume index demonstrated by Figure 5-6 based on settled sludge volume which was measured in 100 ml cylinders. In small cylinders the wall effects can be significant and this effect is smaller when the diameter of the cylinder is getting larger. Hence, in order to correct the SVI results for wall effects, settled sludge volume was also measured directly in the reactors. Figure 5-7 showed the adjusted SVI value, which indicated the same trend but lower than unadjusted SVI.

5.5 Settling velocity



2010-02-27

2010-03-03

2010-04-10

2010-05-03

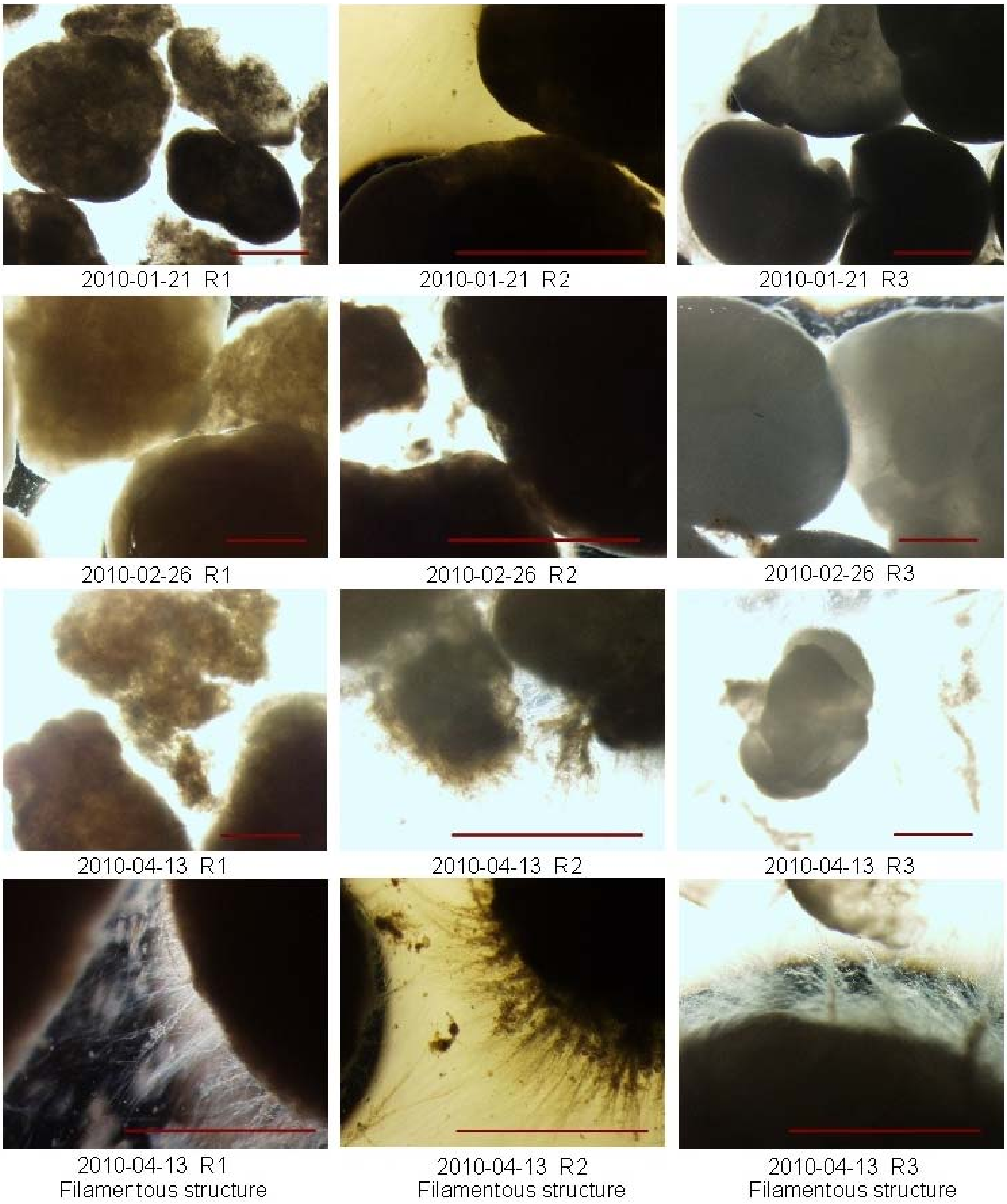
Figure 5-8 Settling velocity of granules with different diameter

Settling velocity indicates the settleability of aerobic granules. Aerobic granules with different diameters were selected in the reactors, and their settling velocity was measured individually. The results are illustrated in Figure 5-8. Observing from the charts, granular size lead to increased settling velocity, but settling velocity was not strictly granular size-dependent. All the charts showed that the granule with the highest settling velocity was always not the one with largest size. That maybe because aerobic granules with large size have a hollow and anaerobic inner structure, hence the compactness and settleability is decreased. The settling velocity of granules in reactor 2 was decreasing by time, according to the microscope in last section, aerobic granules in R2 presented a filamentous surface structure during operation processing, and the filamentous structure would impact on granular settleability.

Granules in reactor 1, which had the largest N load, had higher settling velocity than those in reactor 2&3 (greater than 60 m/h, mostly). Since granules with higher N load have more compact, denser and stronger microbial structure, N load can impact on settling velocity to some extent. Figure 5-8 shows that settling velocity of granules with the same size in the three reactors followed the trend in N load, i.e. R1>R2>R3.

Due to method limitations, the selected granular size was not representative for all the granular size ranges in the reactors. But since granular sizes in the measurements were in similar range as dominated in reactors, the result could give a rough estimation of the average diameter of aerobic granules development in the reactors.

5.6 Microscopy

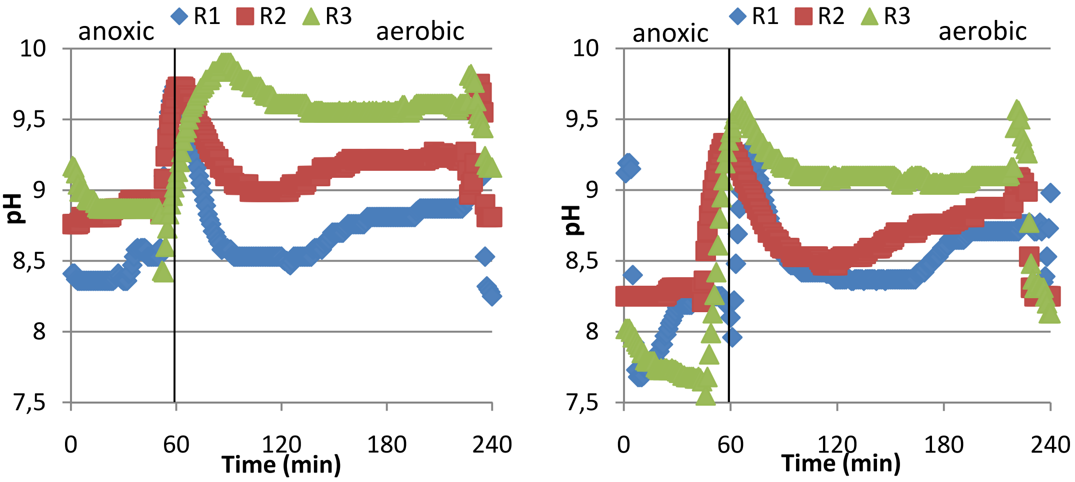


2010-01-21 R1
2010-01-21 R2
2010-01-21 R3
2010-02-26 R1
2010-02-26 R2
2010-02-26 R3
2010-04-13 R1
2010-04-13 R2
2010-04-13 R3
2010-04-13 R1
Filamentous structure
2010-04-13 R2
Filamentous structure
2010-04-13 R3
Filamentous structure

Figure 5-9 Microscope of aerobic granules (bar: 1 mm)

After aerobic granules were cultivated in period I, granules under microscopy displayed a round, potato-like shapes with mean size around 3 mm in January 2010. During one more month of operation, granule size kept increasing to 5 mm of mean value and granules structure became weak and more gel-like. Since superficial up flow air velocity was decreased from 1.47 to 0.82 cm/s, the granules became larger and weaker as expected according to the lower hydrodynamic shear force. In the middle of April, the properties of the granules were very poor, fragments of granules were easy to find under the microscopy, granules structure turned less compact and filamentous. From the images of filamentous structure in Figure 5-9, it was observed that filamentous bacteria were growing on the aerobic granules surface. The filamentous bacteria should have an impact on granules properties like SVI and settling velocity.

5.7 pH



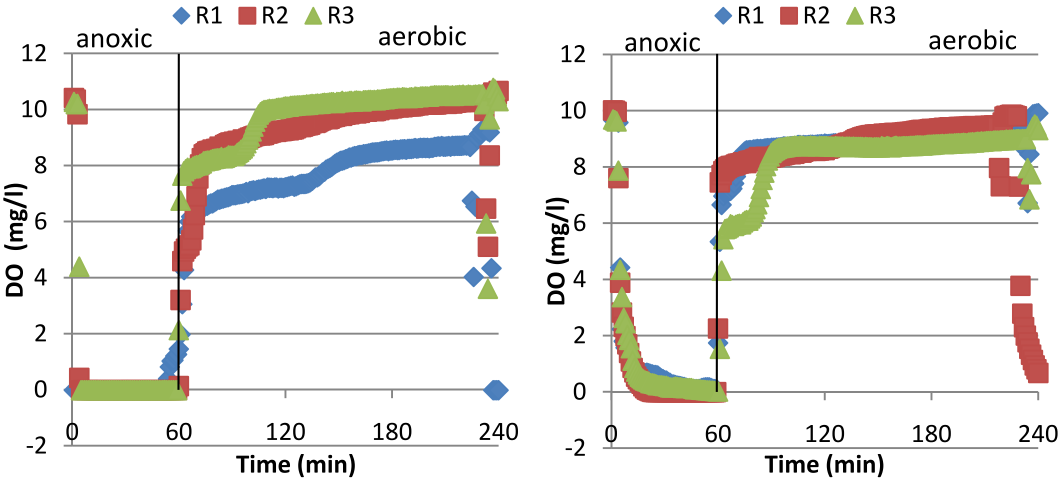
a.2010/01/25 b.2010/03/09

Figure 5-10 pH value during one single

pH value in reactors was high at the beginning of operation (2010/01/25). Since the optimum pH for nitrification was 8-9 as well as 7-9 for denitrification, pH value during one cycle (Figure 5-10, a) was probably too high for good nitrification and denitrifition, and had to be adjusted. At the end of January, pH of acetate solution was adjusted to 6 by 37% HCl. After adjustment, pH value in SBR system was reduced, the pH variation during one cycle was illustrated as Figure 5-10, b. It was, however, observed that if the pH probe was measuring in the reactors at the end of the aeration phase when the aeration was turned off, lower pH values were recorded. The average pH was 8.57, 8.82 and 8.94, for reactor 1, 2 and 3, respectively. The on-line recordings give higher values which can be an affect of the air bubbles that disturb the pH electrode.

Figure 5-10 presented the typical pH variation during an alternating anaerobic-aerobic cycle. The pH variation during one cycle was dependent on the biological processes in anaerobic and aerobic phases. pH kept low value at anaerobic stage without aeration. Denitrification dominated anaerobic phase led to pH value increase in reactor 1&2. It seems that denitrification occurred in Reactor 3 during anaerobic period. The rapid pH rise at the beginning of the aerobic stage was caused by carbon dioxide stripping. The carbon dioxide produced by organics degradation in anaerobic phase would separate from liquid in reactors and left the SBR system with the air due to aeration. All CO_2 was stripped out within about 30min after aeration, and then pH decreased since nitrification and biological phosphate removal dominated in this period. When nitrification and phosphate removal were completed, pH increased again by organics degradation in aerobic period. In R3, since no evident nitrification occurred in aerobic phase, pH value kept stable after carbon dioxide stripping during aeration period.

5.8 Dissolved Oxygen



a.2010/01/25 b.2010/03/09

Q_{air} = 2.5 l/min Q_{air} = 1.4 l/min

Figure 5-11 Dissolved Oxygen concentration during one cycle

Figure 5-11 demonstrate typical dissolved oxygen concentration curves during an alternating anaerobic-aerobic cycle. DO concentration was nearly zero during anaerobic period. When aerobic phase started, the DO concentration raised rapidly since aeration air flow supplied oxygen for the SBR system. The dissolved oxygen concentration at the first half hour of the aerobic phase has a lower increasing rate, because COD removal consumed Dissolved Oxygen within this half hour. After COD removal was completed, DO increased again and kept on until a saturation state was reached in the reactors at the end of the cycle.

Figure 5-11 a and b show the DO content variation under different aeration air flow. After aeration flow reduction, DO concentration in reactors showed almost no change compared to at high aeration flow. It implies that the varying aeration flow had no impact on DO concentration. Furthermore, according to the high DO concentration, it seems DO in reactors was saturated after the first half hour in aerobic phase no matter how air flow varied. The difference of concentration only related with operating temperature in reactors.

5.9 Nutrient removal

5.9.1 Chemical oxygen demand (COD)

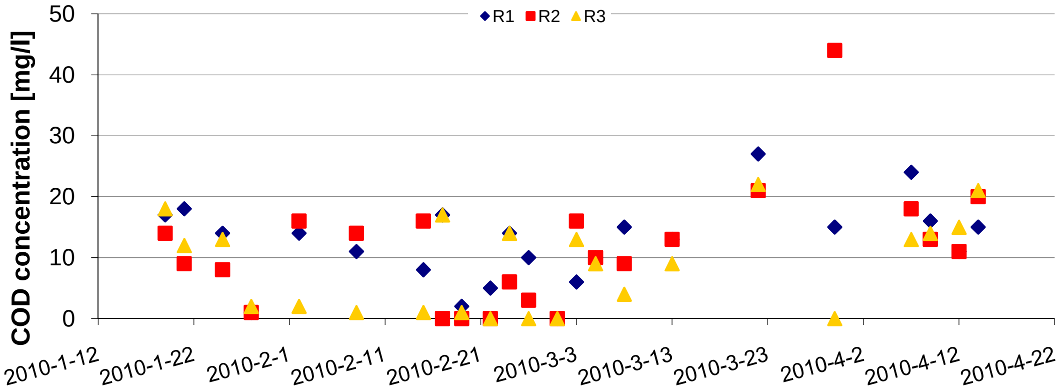


Figure 5-12 Chemical oxygen demand concentration

The theoretical COD in the feed was 500 mg/l, the relative COD conversion rate was calculated as following equation:

$$rel.conversion = \left(1 - \frac{c_E{}'}{0.43 \cdot c_F + (1-0.43) \cdot c_E}\right) \cdot 100\ [\%]\ \textit{(eq.x)}$$

Where $c_E{}'$ is the concentration in the effluent of the measured cycle, c_F is the current concentration in the feed, and c_E is the concentration in the effluent during the previously measured cycle. The value 0.43 represents the volumetric exchange ratio.

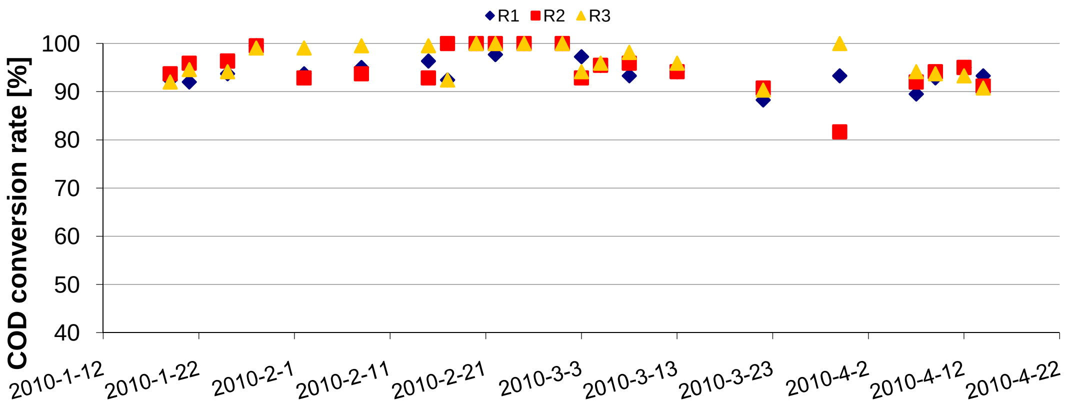


Figure 5-13 Relative chemical oxygen demand conversion

The calculation results of relative COD conversion rate are illustrated in Figure 5-13. COD removal in the three reactors was very efficient. COD concentration in effluent was less than 30 mg/l most of time. COD conversion rate was usually more than 90%. The greatest conversion rates in all three reactors reached 100%. The lowest conversion rates in reactors were 88.3% (R1, 2010-03-22), 81.7% (R2, 2010-03-30), and 90.3 (R3, 2010-03-22), respectively. Although the theoretical COD in feed was 500 mg/l in all three reactors, the actual COD concentration pumped into reactors was hard to control accurately and keep constant every time. The fluctuating outcomes of COD in effluent were considered reasonable.

5.9.2 Phosphate content

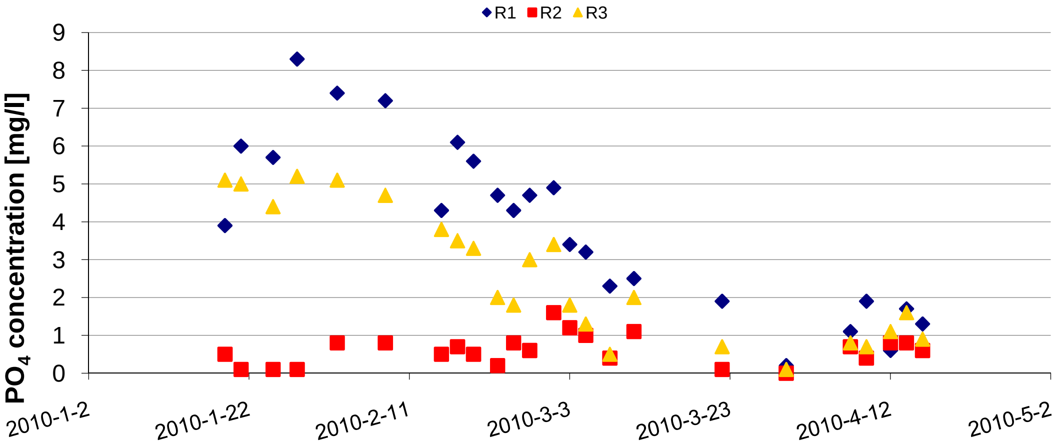


Figure 5-14 Phosphate content in effluent

The relative phosphate conversion rate was calculated with the same equation as that for COD conversion.

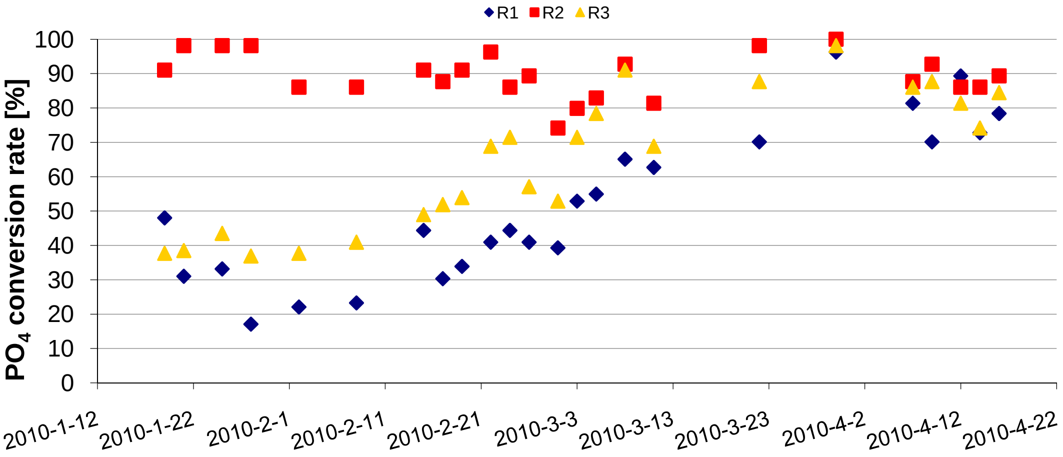


Figure 5-15 Relative phosphate conversion rate

Observing Figure 5-15, phosphate content removal was enhancing by time during operating period in reactor 1&3. The relative phosphate conversion rate in reactor 1 increased from 20% to 90%, while in Reactor 3 increased from 35% to 90%. Phosphate conversion in reactor 2 kept stable high rate around 90%. The theoretical starting concentration of PO_4^{3-} is approximately 5.28 mg/l if no PO_4^{3-} would be present in the bulk water from previous cycle. In reactor 1, substantially higher concentrations could be observed which indicate some accumulation of PO_4^{3-}. Due to the increasing phosphate conversion rate, phosphorus-accumulating granules (PAGs) were probably forming to enhance phosphate content elimination.

5.9.3 Nitrogen

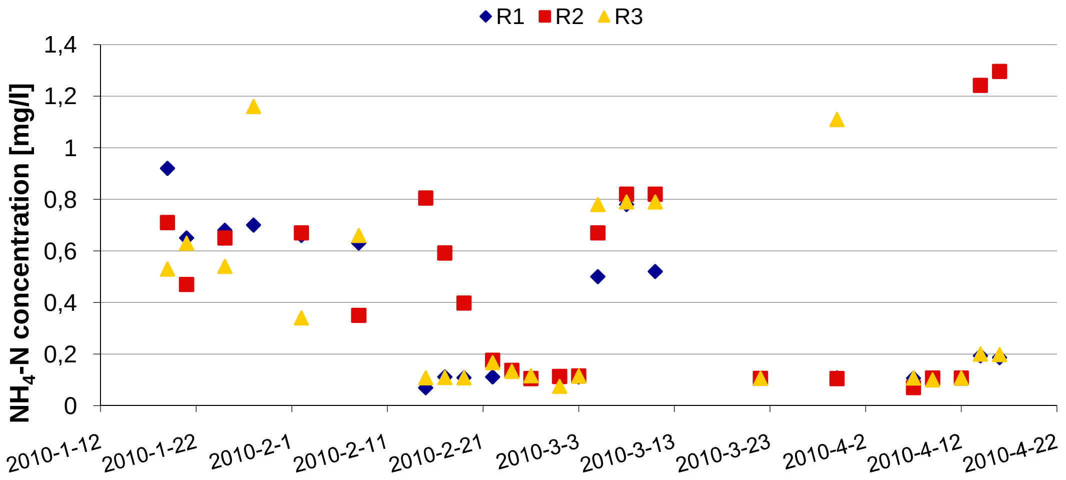


Figure 5-16 Ammonium-nitrogen concentration in effluent

The relative ammonium-nitrogen conversion rate was calculated with the same equation as for COD conversion.

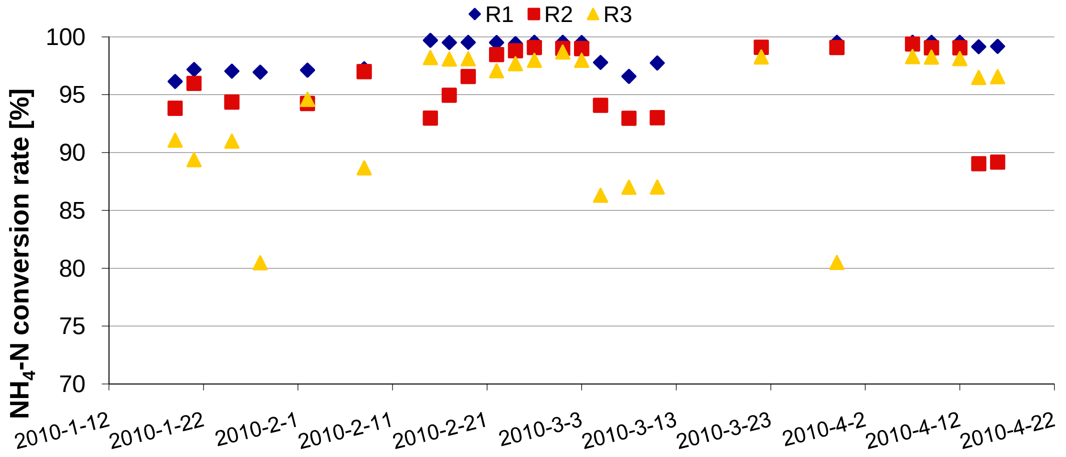


Figure 5-17 Relative ammonium-nitrogen conversion rate

Figure 5-17 illustrate the relative ammonium-nitrogen conversion rate during operation period. Ammonium-nitrogen conversion rate kept high value during operating time, larger than 80% in all three reactors. In Reactor 1, aerobic granules had extremely high conversion rate greater than 95%. However, according to Figure 5-16, ammonium-nitrogen content in effluent was in same concentration range (0-1.3 mg/l). Refer to equation of ammonium-nitrogen conversion rate, the mean initial ammonium-nitrogen concentration in reactors were approximately 22.7, 11.5 and 5.8 mg/l respectively. Since the low N loading in R3, ammonium-nitrogen removal by aerobic granules was less efficient than in R1 and R2. High N/COD loading favor nitrifying bacteria forming in aerobic granules. Granules in R1 had a comparatively high N/COD load with 10/100 mg/mg, so that aerobic granules had high ammonium-nitrogen conversion rate in R1 which was greater than 95%.

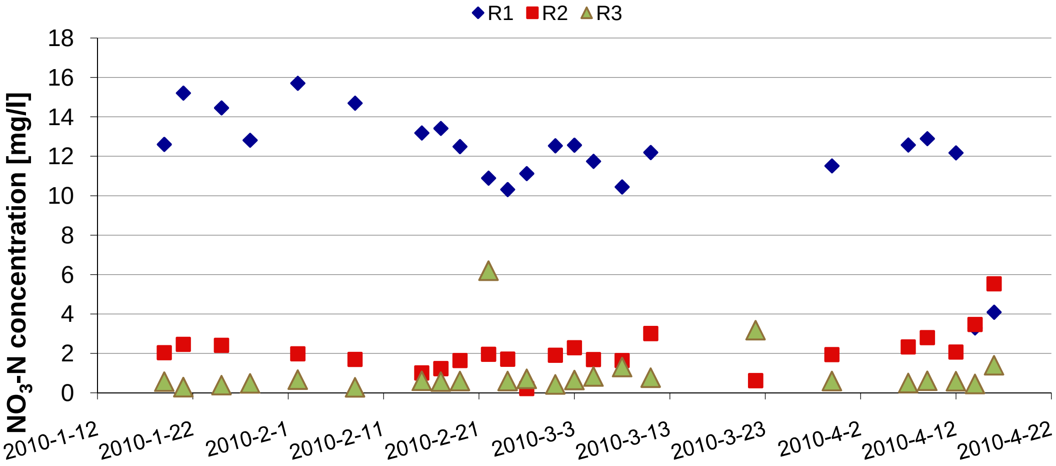


Figure 5-18 Nitrate-nitrogen concentration in effluent

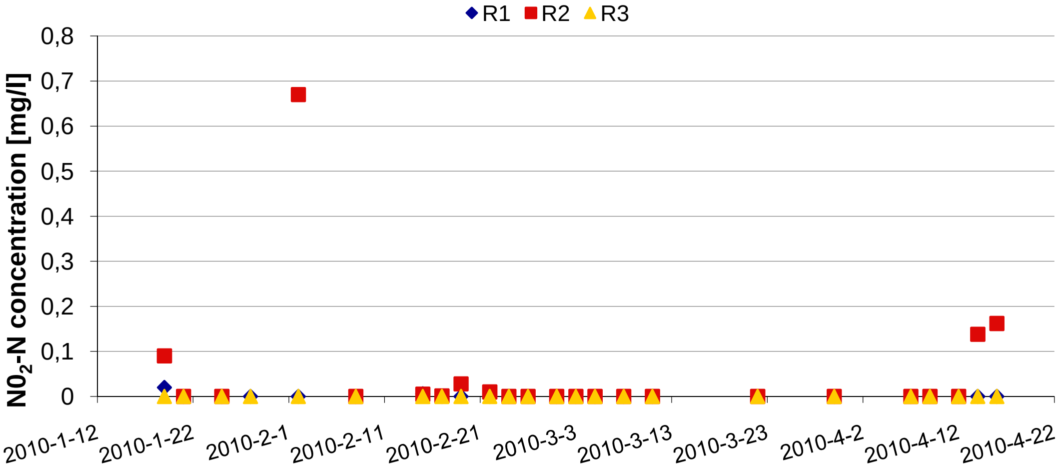


Figure 5-19 Nitrite-nitrogen concentration in effluent

Nitrite-nitrogen content scarcely occurred in the effluent (measured at the end of the cycle) from the three reactors (shown as Figure 5-19). This is reasonable as nitrite is oxidized to nitrate during the aeration stage in SBR. Nevertheless, nitrate-nitrogen concentration was also extremely low at some dates of measurement. If nitrification occurs, nitrate-nitrogen concentration in effluent is supposed to be corresponding to the converted ammonium-nitrogen concentration during operation. Nitrate-nitrogen content was less than the converted ammonium-nitrogen content, possibly due to consumption of nitrogen content by heterotrophic microorganisms to cultivate microbial granules. Hence, during the operation period, nitrification in Reactor 1 and 2 was successfully developed. But in Reactor 3, it seems no or very little nitrification occurred. Referring to the pH section, the pH value in R3 was mostly higher than 9, and therefore ammonium could have been converted to ammonia gas which could be stripped out of the system. According to (Anthonisen et al. 1976) the percentage of the total ammonium that is in the form of ammonia can be estimated as:

$$\%ammonia = \frac{10^{pH}}{EXP^{\left(\frac{6344}{(272+20)}\right)} + 10^{pH}} \cdot 100\%$$

From this it can be calculated that at 20ºC the percentage of ammonium that is in the ammonia form is around 28%. For pH 8 and 8.5 the corresponding values are 3.8 and 11%, respectively. That is probably why ammonium removal rate was high in R3 but nitrate concentration was very low in effluent. Free ammonia can also inhibit nitrification at concentrations above 15 mg/l (Anthonisen et al. 1976). However, since that initial concentrations of ammonium is relatively low in these reactors even at a pH of 9 the ammonia concentrations would not reach inhibiting concentrations. Another feasible reason which led to the low nitrate concentration in SBR system may be occurrence of simultaneous nitrification and denitrification (SND) in the aeration phase. Along with aerobic granules size increase, granules with large diameters could have an inner zone with zero dissolved oxygen concentration during the aeration phase, which induce an anaerobic layer inside the granules. The microorganisms in this anaerobic layer can perform denitrification if denitrifying bacteria occurs and if organic carbon is available and thus nitrate-nitrogen is eliminated. Carbon source could exist in the form of stored polymers or degradation of EPS.

5.9.4 Cycle analysis for nutrient removal

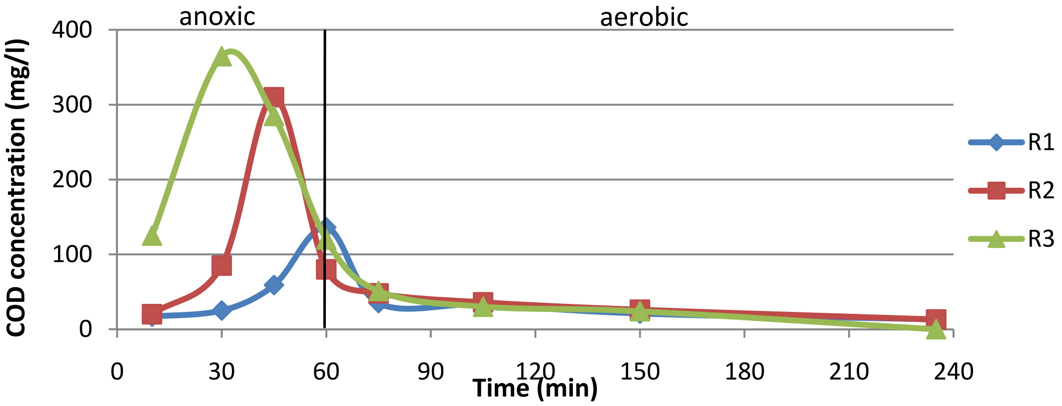


Figure 5-20 COD concentration during one cycle

To further assess the processes occurring during the different phase cycles studies were performed. In the four hours alternative anaerobic-aerobic condition (1hour anaerobic/anoxic phase plus 3 hours aerobic phase), COD concentration was varied as shown in Figure 5-20. In anoxic stage, COD concentration increased in R1, and had a rapid increase and decrease in R2 and R3. No aeration in anoxic period led to poor liquid mixture during feeding, thus COD concentration was low in three reactors at the beginning and had high value even greater than theoretical COD concentration (around 220 mg/l) in R1&R2. The acetate was fed through a tube at the bottom of the reactor and the samples for the concentrations were taken in the middle of the column. Hence, the reactors were not completely mixed during feeding. The feeding from the large tanks with nutrients was also from the reactor bottoms and it was noticed that the granules lifted a bit more in reactor 2 and 3 compared to in reactor 1 since these granules were less dense. At the end of anaerobic phase, the COD concentration in each reactor was much lower than the theoretical value. In the anoxic period, denitrification and biological phosphorus removal should consume COD for their processes. When the aerobic stage started, COD was mainly removed in the first half an hour. At the end of cycle period, COD conversion rate approached 95% in R1 and R2, and 100% in R3.

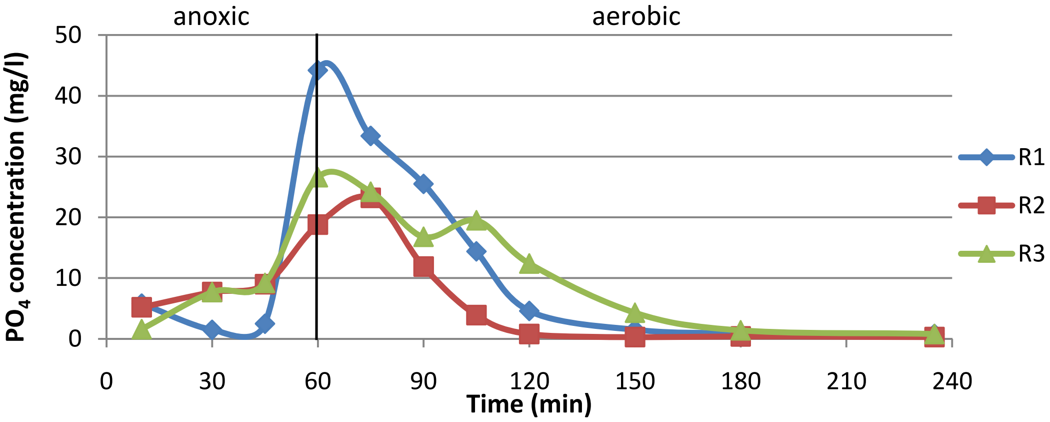
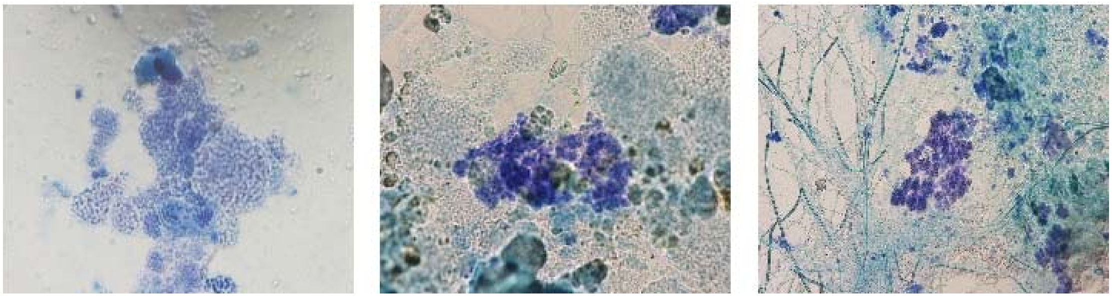


Figure 5-21 Phosphate concentration during one cycle

Observing from Figure 5-21, enhanced biological phosphorus removal was achieved by the alternating anaerobic-aerobic cycle operating. Under anaerobic conditions, certain groups of bacteria containing bound P-accumulating granules (PAGs) release phosphate from stored polyphosphates in their cells. Therefore, in the SBR systems, the phosphate concentration was much higher than theoretical value (5.28 mg/l) in the reactors. Under aerobic conditions, phosphate uptake was achieved by P-accumulating organisms (PAOs), and phosphate content was removed in reactors to achieve P-content elimination at the end of the cycle where the conditions were aerobic.



R1 R2 R3

Figure 5-22 Examples of Methylene blue staining

Figure 5-22 show micrographs of granule samples which are methylene blue stained. Purple cells contain polyphosphates (Tykesson 2005). The microscope images of phosphate containing cells further illustrate that enhanced biological phosphorus removal occurred in the operating SBR system.

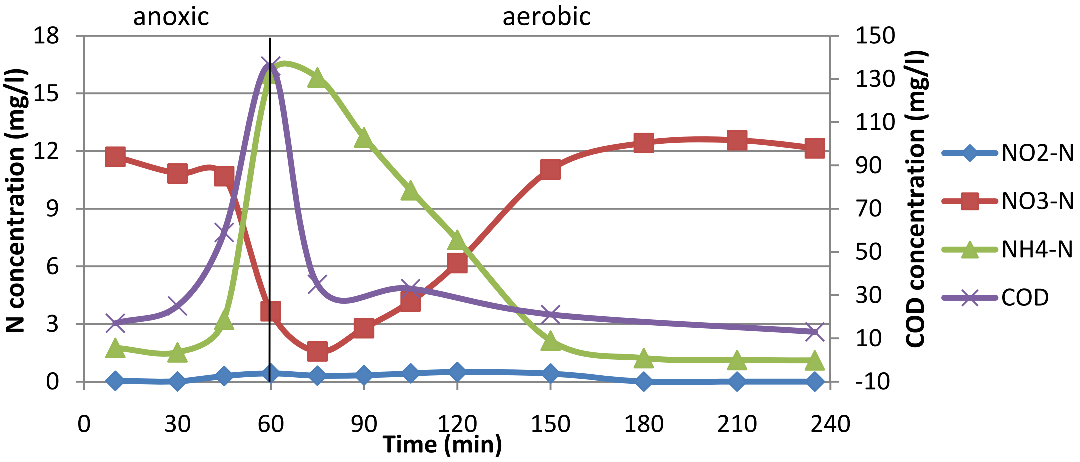


Figure 5-23 Nitrogen concentration in R1 during one cycle

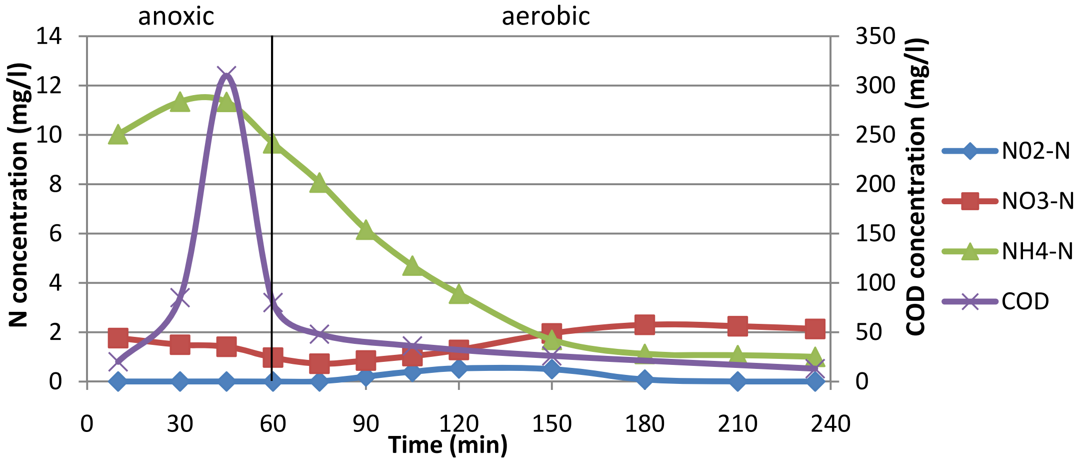


Figure 5-24 Nitrogen concentration in R2 during one cycle

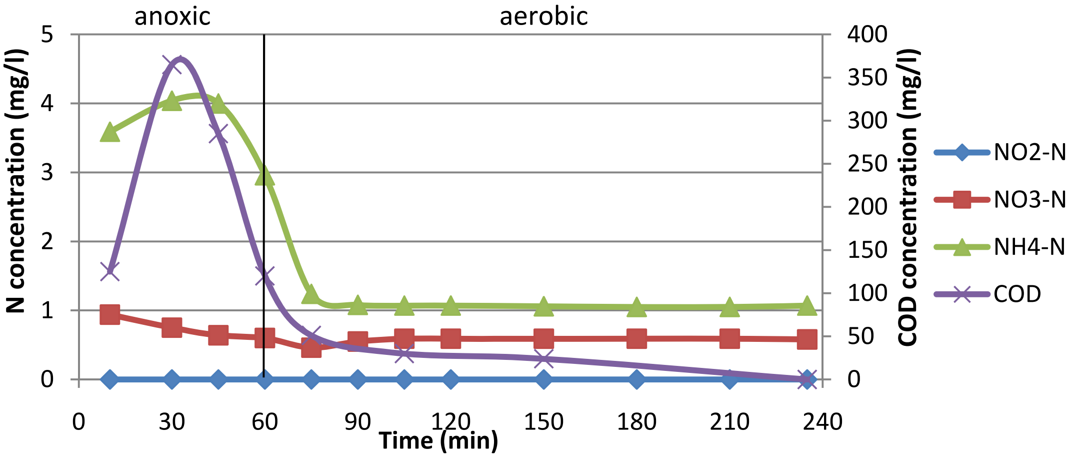


Figure 5-25 Nitrogen concentration in R3 during one cycle

Figure 5-23~25 indicated the nitrogen components variation during one cycle in each reactor. In R1, according to the nitrate-nitrogen curve, both denitrification and nitrification occurred in the anaerobic-aerobic cycle period. Qin et.al. studied nitrification and denitrification under alternating aerobic-anaerobic condition and found external carbon source should be added to achieve complete dentritrification in anaerobic phase (Qin and Liu 2006). In this thesis project, pre-anaerobic was applied to the SBR process and carbon source was plenty for denitrification. This setting avoided external carbon source adding process and made operation easy to control. The nitrification and denitrification rate in R2 was very low. Since nitrogen load in R2 was low, no high nitrification and denitrification rate was expected in R2. There was almost no nitrification and denitrification happened in R3, however, the ammonium removal rate in R3 was high. According to the pH value during one cycle (Figure 5-26), pH in R3 was around 9 during aerobic period. Ammonium at high pH value (>9) will convert to ammonia gas and stripping out of the system. That's the possible reason why ammonium was eliminated without nitrification.

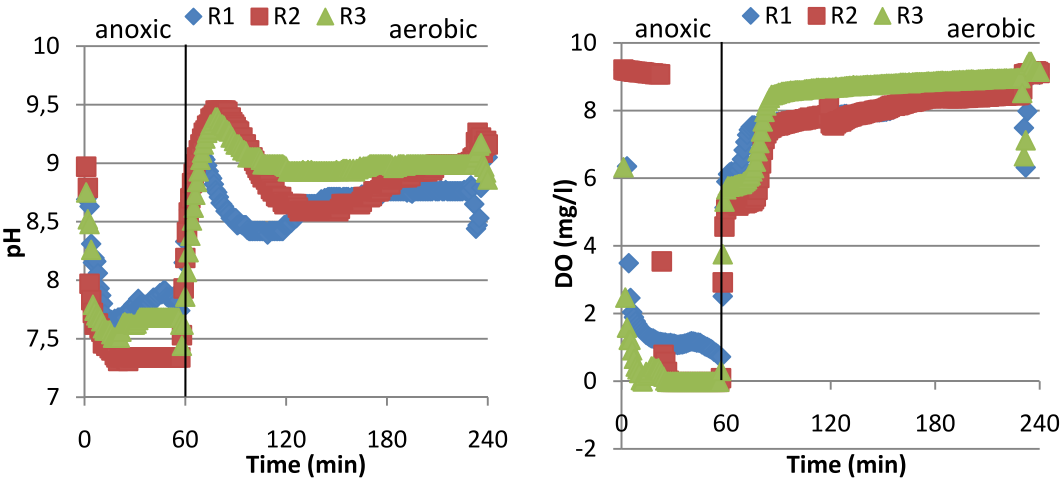


Figure 5-26 pH and DO condition during one cycle

The pH value at the beginning of aerobic phase was higher than 9 in each reactor by carbon dioxide stripping. Since optimum pH for nitrification is 8-9, nitrification process happened after carbon dioxide stripping. This time pH value dropped back to lower than 9, refer to the nitrate curves presented in Figure 5-23~25, nitrification started after the first half hour of aeration period. The DO curves at beginning of aerobic phase also indicated COD and nutrient removal consuming oxygen in the first half hour. In addition, owing to the online measurement limitation, the pH and DO conditions were not measured in the specific cycle period as for the cycle analysis for nutrient removal. That may lead to that the actual pH and DO values in the cycle analysis were not perfectly corresponding to the curves illustrated in Figure 5-26. However, since their measuring time was close in time, pH and DO condition which are demonstrated in Figure 5-26 was representative to describe pH and DO variation in the cycle analysis.

5.10 Hydrophobicity

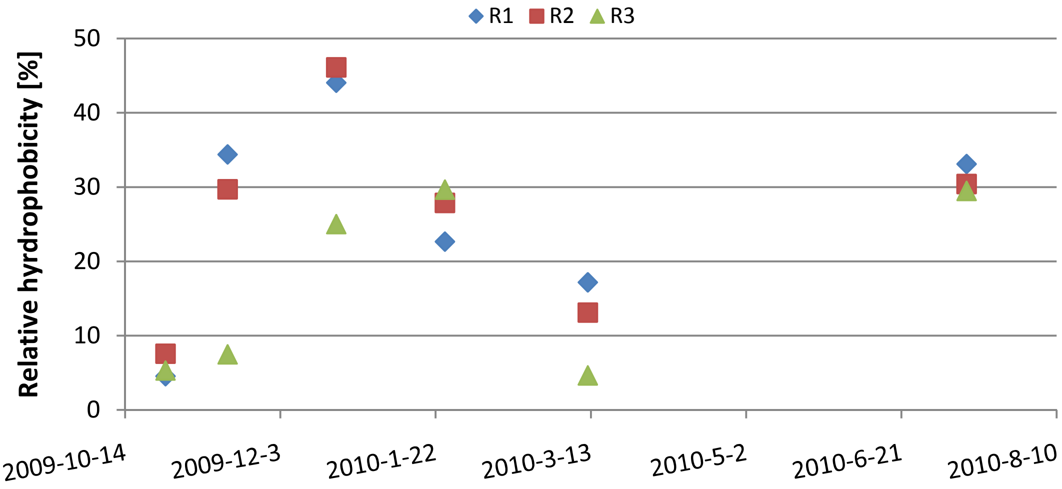


Figure 5-27 Relative hydrophobicity of aerobic granular sludge

Cell hydrophobicity of aerobic granules is considered as a significant factor for aerobic granulation. Based on Liu et al., cell hydrophobicity of aerobic granules in acetate-fed was reported as high as 73% (Liu, Yang et al. 2004). However, as Figure 5-27 shown, the relative hydrophobicity in this experiment was very low. In period I, cell hydrophobicity increased according to aerobic granules grow up, whereas the greatest value was below 50%. From January, cell hydrophobicity of aerobic granules in Reactor 1&2 decreased, only increased a bit in R3, and in 2010-03-12, cell hydrophobicity granular sludge were very low in all three reactors (<20%). In this period, the aerobic granules had poor properties, exhibited as filamentous growth on granules surface and visible dark anaerobic inner structure. That may cause the small value of cell hydrophobicity. At the ending period of experiment, the relative hydrophobicity increased dependent on granular sludge property improved. In addition, analytical method limitation should be a reason for low cell hydrophobicity. The method requires homogenization of the granules and the measured hydrophobicity is therefore an average value for all cells. It was observed that it was very difficult to achieve homogenization by sonication and that the cells tended to reflocculate easily.

5.11 Nitrification rate/denitrification rate

The results of nitrification and denitrification rate tests are shown in Table 5-1~2.

Table 5-1 Nitrification rate

Nitrification rate	**R1**	**R2**	**R3**
Granules cultivating N loading rate (kg/(m^3.d))	0.135	0.068	0.034
Initial NH_4-N load (mg/l)	47.097	47.097	47.097
Nitrate forming rate (mg NO_3-N/gMLSS/min)	0.015	0.010	0.005
Ammonium removal rate (mg NH_4-N/gMLSS/min)	0.019	0.051	0.039
SOUR- nitrification (mg O_2/gMLSS/min)	0.762	3.419	4.280

The nitrification rate was measured as either removal of ammonium or as production of nitrate (Table 5-1). There was no correlation between the load of nitrogen and the removal rate of ammonium. The obtained nitrification rates were relatively low compared to literature values; 0.145-0.236 mg N/gMLSS/min for activated sludge (Parker and Wanner 2007) and 0.3 mg N/gMLSS/min for autotrophic aerobic granules(Shi, Sheng et al. 2010). The low nitrate forming rate in relation to ammonium removal rate suggests that some other processes than nitrification removed some of the ammonium, such as stripping of ammonium due to the high pH in the reactors or due to simultaneous nitrification/denitrification or anammox processes.

Theoretically, SOUR should be high when N loading rate was high. However, in this study, the higher SOUR for lower COD/N ratios can be due to a higher concentration of heterotrophic microorganisms present in R3 compared to in R2 and R1.

Table 5-2 Denitrification rate

Denitrification rate	**R1**	**R2**	**R3**
Granules cultivating N loading rate (kg/(m^3.d))	0.135	0.068	0.034
Initial NO3-N load (mg/l)	52.4	52.4	52.4
Denitrification rate (mg N/gMLSS/min)	0.240	0.444	0.310
External COD load (mg/l)	292	292	292

The denitrification rates obtained were in the same range as found in other studies of similar systems (Yang et al, 2003;Qin and Liu, 2006).

6. Conclusions and outlook

During the experiment operating period, aerobic granular sludge showed ability of high degree of COD and nutrient removal, under the operation condition of different COD: N: P ratios from 100: 10.47: 0.8 to 100: 2.62: 0.8 and superficial up flow air velocity of 1.47 and 0.82cm/s. The COD removal rate was approximately 90% while nitrogen removal rate was 85-95%. At the end of operation period, phosphorus removal reached finally 70-90%.

The main conclusions obtained from this study were:

- Aerobic granules developed with high N/COD ratio had more compact, stronger structure, smaller size and better settleability because of the characteristics of nitrifying bacteria. Therefore, nitrifying granules were successfully cultivated in R1, which had better nitrogen removal rate than in the other reactors. Phosphate content elimination was gradually increasing and reached finally a high level at the end of the operational time.
- Low superficial up flow air velocity, producing lower hydrodynamic shear force, led to larger granule size and poorer settling properties of the aerobic granules. Nitrification in the reactor with high N/COD ratio was also reduced a little at low superficial up flow air velocity condition.
- Nitrification and denitrification was achieved by aerobic granules in SBR, under alternating anaerobic-aerobic condition, especially in the reactor with higher nitrogen load. In reactor 3, nitrification and denitrification rate was extraordinary low owing to its low N/COD, and ammonium was stripping out according to high operation pH value. Enhanced biological phosphorus removal (EBPR) also occurred in alternating anaerobic-aerobic SBR system.

The significant outcome of the experiment was the dominant affect of N/COD ratio. Aerobic granules cultivated at high N/COD ratio had better settleability, stronger structure and higher nitrogen removal rate. However, since the nitrogen, phosphate content and organic load of the SBR system was low, high level of nutrient elimination was not expected. In future studies, more complex and more concentrated wastewater should be applied in the SBR systems to investigate the impact it has on the characteristics of the aerobic granules.

The processes of granulation were found complicated to assess due to high concentration gradients inside them and the actual environmental conditions were not known. Although the optimum size and property of the aerobic granules was well known, the properties of the granules cannot be controlled. Development of mathematical models describing the processes in the system and hence facilitate control of the aerobic granule properties should be considered in further studies.

In this experimental SBR system, the pH value in operation was not controlled and it was hard to keep it at an optimum levels. According to the nitrogen removal section mentioned previously, ammonium will convert to ammonia gas and strip out from the system when pH value is higher than 9. Also high concentrations of ammonia can inhibit nitrification. Since the pH value cannot be regulated in SBR during operating period, nitrogen removal in reactor 3, which had pH value around 9, was becoming unreasonable to analyze. Since the pH value is a very important parameter for biological nutrient removal, controlling and regulating pH in SBR system as well as in granules should be aimed for in the future in order to get valid experimental results.

In this experiment, air flow reduction was not only expected to get lower hydrodynamic shear force, but also achieve DO concentration reduction. However, the DO concentration in the SBR system was not impacted by air flow to a large extent. Therefore, an impactful method to reduce DO should be found to assess the characteristics of aerobic granules under low DO condition.

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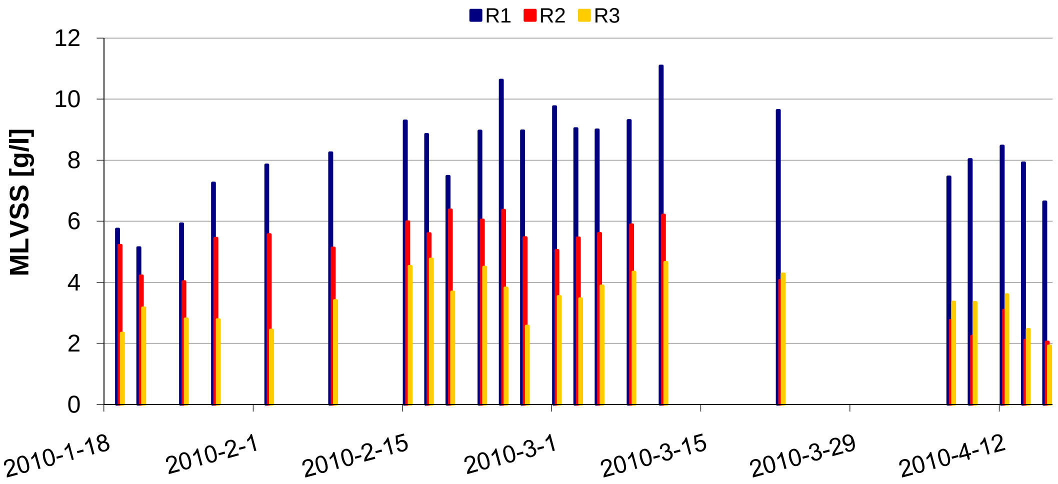
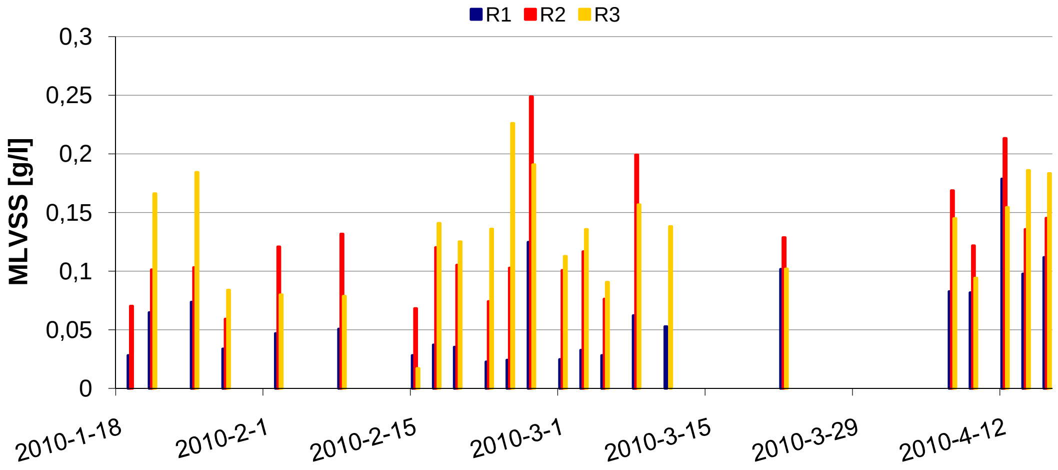
Appendix A

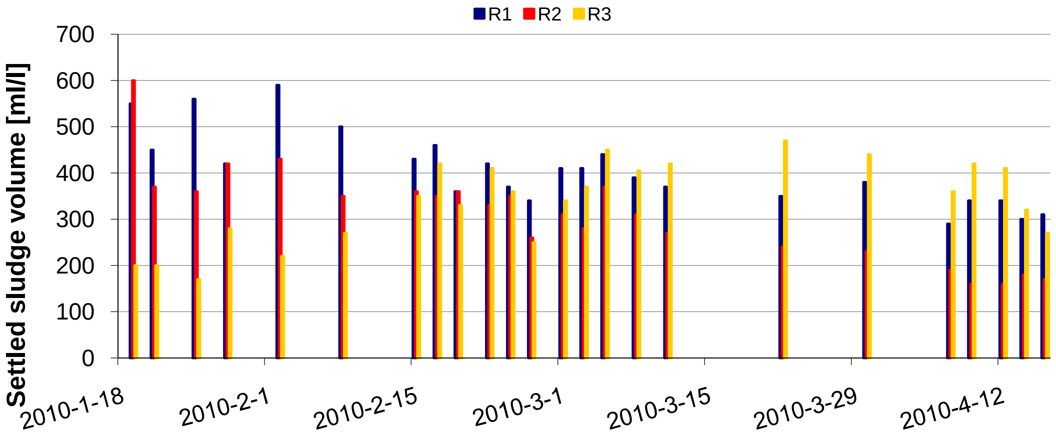
MLVSS in the reactor – diagram



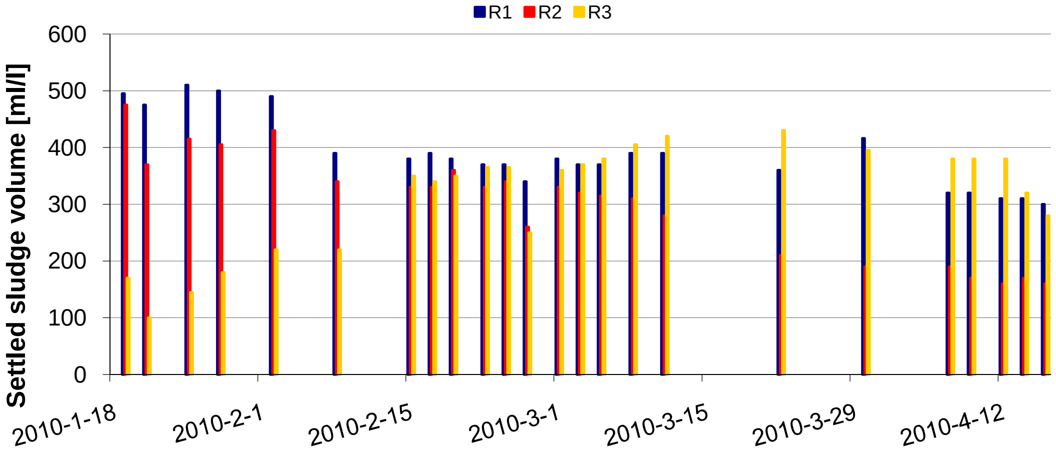
MLVSS in the effluent – diagram



SSV in the cylinder– diagram



SSV in the reactor– diagram



Appendix B

Settled sludge volume and sludge volume index in the cylinder

Date	SVI(ml/g)			SSV(ml/l)		
	R1	**R2**	**R3**	**R1**	**R2**	**R3**
2010-1-19	75	102	79	550	600	200
2010-1-21	84	83	60	450	370	200
2010-1-25	84	81	61	560	360	170
2010-1-28	55	74	102	420	420	280
2010-2-2	71	70	85	590	430	220
2010-2-8	57	63	75	500	350	270
2010-2-15	43.77	56.66	73.47	430	360	350
2010-2-17	48.25	57.91	83.1	460	350	420
2010-2-19	45.32	52.52	85.4	360	360	330
2010-2-22	43.64	45.81	83.27	420	330	410
2010-2-24	32.26	50.33	86.87	370	350	360
2010-2-26	35.44	43.52	92.11	340	260	250
2010-3-1	38.59	55.12	90.81	410	310	340
2010-3-3	42.82	47.75	101.26	410	280	370
2010-3-5	44	59	104	440	370	450
2010-3-8	39	48	85	390	310	405
2010-3-11	31	40	85	370	270	420
2010-3-22	35	53	100	350	240	470
2010-3-30	32	51	107	380	230	440
2010-4-7	34.92	62.62	99.06	290	190	360
2010-4-9	37.76	65.47	114.32	340	160	420
2010-4-12	35.89	47.14	103.43	340	160	410
2010-4-14	34	80.21	123.36	300	180	320
2010-4-16	42.44	77.13	137.47	310	170	270

Settled sludge volume and sludge volume index in the reactor

	SVI(ml/g)			SSV(ml/l)		
Date	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
2010-1-19	67.4	82	67.35	495	475	170
2010-1-21	88.39	83.26	30.08	475	370	100
2010-1-25	76.65	93.17	51.9	510	415	145
2010-1-28	65.93	71.5	65.6	500	405	180
2010-2-2	58.72	71.5	84.81	490	430	220
2010-2-8	44.05	61.22	61.21	390	340	220
2010-2-15	38.68	51.94	73.47	380	330	350
2010-2-17	40.91	54.6	67.27	390	330	340
2010-2-19	47.83	52.52	90.58	380	360	350
2010-2-22	38.45	45.81	74.13	370	330	365
2010-2-24	32.36	48.89	88.08	370	340	365
2010-2-26	35.44	43.52	92.11	340	260	250
2010-3-1	35.77	58.68	96.15	380	330	360
2010-3-3	38.65	54.57	101.26	370	320	370
2010-3-5	37.28	50.05	88.09	370	315	380
2010-3-8	38.87	48	85.19	390	310	405
2010-3-11	32.79	41.77	84.95	390	280	420
2010-3-22	36.2	46.42	91.41	360	210	430
2010-3-30	34.75	42.09	96.01	416	190	395
2010-4-7	38.54	62.62	104.57	320	190	380
2010-4-9	35.54	69.56	103.43	320	170	380
2010-4-12	32.72	47.14	95.86	310	160	380
2010-4-14	35.13	75.76	123.36	310	170	320
2010-4-16	41.07	72.6	142.57	300	160	280

MLSS, MLVSS and volatile fraction in the reactor

	MLSS (g/l)			MLVSS (g/l)			Volatile fraction (%)		
Date	R1	R2	R3	R1	R2	R3	R1	R2	R3
2010-1-19	7.34	5.86	2.52	5.75	5.22	2.34	78.2	88.9	92.6
2010-1-21	5.37	4.44	3.32	5.14	4.22	3.17	95.6	94.9	95.2
2010-1-25	6.65	4.45	2.79	5.92	4.03	2.81	88.9	90.4	100.4
2010-1-28	7.58	5.66	2.74	7.26	5.45	2.78	95.7	96.2	101.2
2010-2-2	8.34	6.01	2.59	7.85	5.57	2.44	94.0	92.6	93.9
2010-2-8	8.85	5.55	3.59	8.25	5.13	3.41	93.1	92.3	94.8
2010-2-15	9.82	6.35	4.76	9.29	5.99	4.53	94.6	94.2	95.0
2010-2-17	9.53	6.04	5.05	8.85	5.60	4.77	92.8	92.6	94.3
2010-2-19	7.94	6.85	3.86	7.48	6.38	3.69	94.1	93.0	95.4
2010-2-22	9.62	7.20	4.92	8.96	6.05	4.50	93.1	83.9	91.3
2010-2-24	11.43	6.95	4.14	10.63	6.37	3.82	92.9	91.5	92.1
2010-2-26	9.59	5.97	2.71	8.97	5.47	2.57	93.5	91.5	94.5
2010-3-1	10.62	5.62	3.74	9.76	5.05	3.54	91.8	89.8	94.4
2010-3-3	9.57	5.86	3.65	9.04	5.46	3.47	94.4	93.0	94.9
2010-3-5	9.92	6.29	4.31	9.00	5.61	3.89	90.6	89.1	90.1
2010-3-8	10.03	6.45	4.75	9.31	5.89	4.34	92.7	91.2	91.2
2010-3-11	11.89	6.70	4.94	11.09	6.21	4.66	93.2	92.6	94.2
2010-3-22	9.94	4.52	4.70	9.64	4.07	4.28	96.9	89.9	90.9
2010-4-7	8.30	3.03	3.64	7.46	2.76	3.36	89.8	90.8	92.1
2010-4-9	9.00	2.44	3.67	8.03	2.24	3.35	89.1	91.5	91.1
2010-4-12	9.47	3.39	3.96	8.47	3.09	3.60	89.4	90.9	90.7
2010-4-14	8.82	2.24	2.59	7.92	2.11	2.46	89.7	93.9	94.7
2010-4-16	7.30	2.20	1.96	6.64	2.05	1.92	90.9	92.8	97.6

MLSS, MLVSS and volatile fraction in the effluent

	MLSS (g/l)			MLVSS (g/l)			Volatile fraction (%)		
Date	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
2010-1-19	0.03	0.08	0.16	0.03	0.07	-	96.6	91.5	-
2010-1-21	0.07	0.11	0.18	0.06	0.10	0.17	89.6	89.8	93.8
2010-1-25	0.08	0.12	0.19	0.07	0.10	0.18	90.2	83.5	99.1
2010-1-28	0.04	0.06	0.09	0.03	0.06	0.08	88.2	93.7	96.1
2010-2-2	0.05	0.13	0.09	0.05	0.12	0.08	91.6	89.9	91.5
2010-2-8	0.05	0.13	0.09	0.05	0.13	0.08	99.0	98.1	90.1
2010-2-15	0.03	0.07	0.02	0.03	0.07	0.02	96.6	97.2	94.5
2010-2-17	0.04	0.13	0.25	0.04	0.12	0.14	83.2	93.8	56.7
2010-2-19	0.04	0.11	0.14	0.04	0.11	0.13	97.2	92.5	92.1
2010-2-22	0.03	0.08	0.15	0.02	0.07	0.14	83.5	88.6	89.5
2010-2-24	0.02	0.11	0.24	0.02	0.10	0.23	126.0	94.5	93.9
2010-2-26	0.13	0.27	0.20	0.12	0.25	0.19	93.3	91.0	95.3
2010-3-1	0.03	0.11	0.12	0.02	0.10	0.11	83.2	91.0	94.8
2010-3-3	0.04	0.13	0.15	0.03	0.12	0.14	85.6	92.1	93.5
2010-3-5	0.03	0.08	0.10	0.03	0.08	0.09	90.4	94.4	91.4
2010-3-8	0.07	0.32	0.17	0.06	0.20	0.16	90.5	62.6	90.4
2010-3-11	0.06	-	0.15	0.05	-	0.14	93.8	-	90.4
2010-3-22	0.12	0.14	0.12	0.10	0.13	0.10	86.8	89.6	87.2
2010-4-7	0.09	0.19	0.16	0.08	0.17	0.14	89.7	90.8	89.1
2010-4-9	0.09	0.13	0.11	0.08	0.12	0.09	88.6	92.8	89.0
2010-4-12	0.20	0.23	0.17	0.18	0.21	0.15	91.5	91.6	90.1
2010-4-14	0.11	0.14	0.21	0.10	0.14	0.19	89.5	93.8	90.3
2010-4-16	0.12	0.16	0.20	0.11	0.15	0.18	91.0	93.3	92.2

Settling velocity

	R1			**R2**			**R3**		
Distance (cm)	**Diameter** (µm)	**Time** (s)	**Velocity** (m/h)	**Diameter** (µm)	**Time** (s)	**Velocity** (m/h)	**Diameter** (µm)	**Time** (s)	**Velocity** (m/h)
20100227									
20	5450	7.24	99.45	5600	8.6	83.72	5850	12.63	57.01
20	4200	8.91	80.81	3900	10.31	69.84	4850	12.75	56.47
20	3750	9.56	75.31	2800	13.62	52.86	4150	13.82	52.10
20	2150	15.5	46.45	7100	6.59	109.26	2500	23.43	30.73
20	7000	8.72	82.57	6350	8.72	82.57	2200	21.21	33.95
20	6250	6.2	116.13	4000	9.75	73.85	4350	9.28	77.59
20	6300	7.97	90.34	3300	9.71	74.15	7050	11.37	63.32
20	5400	6.75	106.67	2450	13.78	52.25	6400	13.71	52.52
20	4800	7.53	95.62				3300	17.94	40.13
20	3550	9	80.00						
20100303									
22.1	5400	10.12	78.62	7500	9.84	80.85	7200	15.97	49.82
22.1	4000	9.06	87.81	5500	8.22	96.79	3400	20.82	38.21
22.1	4000	11.41	69.73	5500	10.41	76.43	5000	16.66	47.76
22.1	3300	13.25	60.05	6600	9.72	81.85	5000	19.34	41.14
22.1	3300	13.43	59.24	3600	16.75	47.50	4100	22.07	36.05
22.1	2600	17.12	46.47	5000	9.84	80.85	2800	28.41	28.00
22.1	8300	7.97	99.82	3800	9.84	80.85	6500	17.07	46.61
20100410									
22.1	5600	8.43	94.38	3300	17.53	45.39	7600	17.06	46.64
22.1	6500	8.75	90.93	6200	12.09	65.81	6200	15.53	51.23
22.1	6800	8.85	89.90	4000	14.09	56.47	6200	17.37	45.80
22.1	3800	10.06	79.09	4000	14.38	55.33	5600	18.47	43.08
22.1	3000	10.66	74.63	5300	19.54	40.72	8000	14.62	54.42
22.1	4200	10.65	74.70	6500	12.4	64.16	5500	13.94	57.07
22.1	3200	10.1	78.77	8000	13	61.20			
22.1	4600	9.44	84.28	3300	15.16	52.48			
22.1	3000	11.4	69.79						
20100503									
22.1	7500	8.47	93.93	6700	14.63	54.38	11150	10.59	75.13
22.1	6750	8.29	95.97	4750	12.88	61.77	9900	11.1	71.68
22.1	5900	8.43	94.38	5650	12.36	64.37	9400	10.13	78.54
22.1	5300	6.84	116.32	4250	14.31	55.60	7950	11.66	68.23
22.1	4350	7.94	100.20	3650	15.71	50.64	7350	17.03	46.72
22.1	4900	9.5	83.75	3400	20.09	39.60	6200	12.06	65.97
22.1	4100	9.94	80.04	2850	16.13	49.32	5900	12.15	65.48
22.1	3150	12	66.30	3300	14.88	53.47	4550	15.81	50.32

COD

Date	COD in the effluent (mg/l)			COD conversion rate (%)		
	R1	**R2**	**R3**	**R1**	**R2**	**R3**
2010-1-19	17	14	18	92.4	93.7	92.0
2010-1-21	18	9	12	92.0	95.9	94.6
2010-1-25	14	8	13	93.7	96.4	94.2
2010-1-28	1	1	2	99.5	99.5	99.1
2010-2-2	14	16	2	93.7	92.9	99.1
2010-2-8	11	14	1	95.0	93.7	99.5
2010-2-15	8	16	1	96.4	92.9	99.5
2010-2-17	17	0	17	92.4	100.0	92.4
2010-2-19	2	0	1	99.1	100.0	99.5
2010-2-22	5	0	0	97.7	100.0	100.0
2010-2-24	14	6	14	93.7	97.3	93.7
2010-2-26	10	3	0	95.5	98.6	100.0
2010-3-1	0	0	0	100.0	100.0	100.0
2010-3-3	6	16	13	97.3	92.9	94.2
2010-3-5	10	10	9	95.5	95.5	95.9
2010-3-8	15	9	4	93.3	95.9	98.2
2010-3-13	13	13	9	94.2	94.2	95.9
2010-3-22	27	21	22	88.3	90.7	90.3
2010-3-30	15	44	0	93.3	81.7	100.0
2010-4-7	24	18	13	89.5	92.0	94.2
2010-4-9	16	13	14	92.9	94.2	93.7
2010-4-12	11	11	15	95.0	95.0	93.3
2010-4-14	15	20	21	93.3	91.2	90.7

Phosphate

Date	PO_4 in the effluent (mg/l)			PO_4 conversion rate (%)		
	R1	R2	R3	R1	R2	R3
2010-1-19	3.9	0.5	5.1	48.0	91.0	37.7
2010-1-21	6	0.1	5	31.0	98.1	38.5
2010-1-25	5.7	0.1	4.4	33.2	98.1	43.5
2010-1-28	8.3	0.1	5.2	17.1	98.1	36.9
2010-2-2	7.4	0.8	5.1	22.1	86.1	37.7
2010-2-8	7.2	0.8	4.7	23.3	86.1	41.0
2010-2-15	4.3	0.5	3.8	44.4	91.0	49.0
2010-2-17	6.1	0.7	3.5	30.3	87.7	51.9
2010-2-19	5.6	0.5	3.3	33.9	91.0	53.9
2010-2-22	4.7	0.2	2	41.0	96.3	68.8
2010-2-24	4.3	0.8	1.8	44.4	86.1	71.5
2010-2-26	4.7	0.6	3	41.0	89.3	57.1
2010-3-1	4.9	1.6	3.4	39.3	74.2	52.9
2010-3-3	3.4	1.2	1.8	52.9	79.9	71.5
2010-3-5	3.2	1	1.3	55.0	82.9	78.4
2010-3-8	2.3	0.4	0.5	65.1	92.7	91.0
2010-3-11	2.5	1.1	2	62.7	81.4	68.8
2010-3-22	1.9	0.1	0.7	70.1	98.1	87.7
2010-3-30	0.2	0	0.1	96.3	100.0	98.1
2010-4-7	1.1	0.7	0.8	81.4	87.7	86.1
2010-4-9	1.9	0.4	0.7	70.1	92.7	87.7
2010-4-12	0.6	0.8	1.1	89.3	86.1	81.4
2010-4-14	1.7	0.8	1.6	72.8	86.1	74.2
2010-4-16	1.3	0.6	0.9	78.4	89.3	84.5

Ammonium- nitrogen

Date	NH_4-N in the effluent(mg/l)			NH_4-N conversion rate (%)		
	R1	R2	R3	R1	R2	R3
2010-1-19	0.92	0.71	0.53	96	94	91
2010-1-21	0.65	0.47	0.63	97	96	89
2010-1-25	0.68	0.65	0.54	97	94	91
2010-1-28	0.7	-	1.16	97	-	80
2010-2-2	0.66	0.67	0.34	97	94	95
2010-2-8	0.63	0.35	0.66	97	97	89
2010-2-15	0.069	0.805	0.108	100	93	98
2010-2-17	0.111	0.592	0.109	100	95	98
2010-2-19	0.108	0.398	0.108	100	97	98
2010-2-22	0.111	0.176	0.167	100	98	97
2010-2-24	0.133	0.137	0.133	99	99	98
2010-2-26	0.106	0.105	0.116	100	99	98
2010-3-1	0.107	0.113	0.075	100	99	99
2010-3-3	0.111	0.115	0.115	100	99	98
2010-3-5	0.5	0.67	0.78	98	94	86
2010-3-8	0.78	0.82	0.79	97	93	87
2010-3-11	0.52	0.82	0.79	98	93	87
2010-3-22	-	0.106	0.106	-	99	98
2010-3-30	0.107	0.105	1.11	100	99	81
2010-4-7	0.106	0.07	0.107	100	99	98
2010-4-9	0.105	0.107	0.1	100	99	98
2010-4-12	0.107	0.107	0.107	100	99	98
2010-4-14	0.193	1.242	0.2	99	89	96
2010-4-16	0.186	1.296	0.198	99	89	97

Nitrite- and nitrate-nitrogen

	NO_2-N in the effluent [mg/l]			NO_3-N in the effluent [mg/l]		
Date	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
2010-1-19	0.02	0.09	0	12.6	2.04	0.57
2010-1-21	0	0	0	15.2	2.46	0.29
2010-1-25	0	0	0	14.45	2.41	0.39
2010-1-28	0	-	0	12.81	-	0.48
2010-2-2	0	0.67	0	15.7	1.981	0.67
2010-2-8	0	0	0	14.69	1.7	0.28
2010-2-15	0.006	0.005	0	13.18	1.01	0.61
2010-2-17	0	0.0012	0	13.41	1.23	0.57
2010-2-19	0	0.028	0	12.49	1.64	0.6
2010-2-22	0	0.01	0	10.89	1.96	6.2
2010-2-24	0	0	0	10.31	1.71	0.6
2010-2-26	0	0	0	11.12	0.217	0.71
2010-3-1	0	0	0	12.53	1.91	0.43
2010-3-3	0	0	0	12.56	2.29	0.65
2010-3-5	0	0	0	11.74	1.69	0.83
2010-3-8	0	0	0	10.44	1.64	1.31
2010-3-11	0	0	0	12.19	3.01	0.77
2010-3-22	-	0	0	-	0.62	3.18
2010-3-30	0	0	0	11.51	1.94	0.6
2010-4-7	0	0	0	12.57	2.33	0.5
2010-4-9	0	0	0	12.89	2.8	0.61
2010-4-12	0	0	0	12.17	2.07	0.59
2010-4-14	0	0.138	0	3.29	3.47	0.45
2010-4-16	0	0.162	0	4.09	5.53	1.4

Cycle analysis

Phosphate and COD

Time (min)	PO4 concentration (mg/l)			COD concentration (mg/l)		
	R1	R2	R3	R1	R2	R3
10	5.8	5.2	1.6	17	20	125
30	1.5	7.7	7.7	25	85	365
45	2.5	9	9.2	59	310	285
60	44.2	18.8	26.6	136	80	120
75	33.4	23.2	24.2	35	48	51
90	25.5	11.9	16.8			
105	14.4	3.9	19.5	33	36	30
120	4.6	0.8	12.4			
150	1.5	0.3	4.3	21	26	24
180	0.9	0.4	1.4			
235	0.8	0.3	0.8	13	13	0

Nitrogen

Time (min)	NO_2-N (mg/l)			NO_3-N (mg/l)			NH_4-N (mg/l)		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
10	0.04	0	0	11.7	1.76	0.94	1.76	10.03	3.59
30	0.01	0	0	10.83	1.49	0.75	1.53	11.35	4.04
45	0.29	0	0	10.69	1.41	0.64	3.22	11.34	4
60	0.43	0	0	3.66	0.97	0.6	16.03	9.66	2.96
75	0.31	0	0	1.57	0.72	0.46	15.83	8.07	1.24
90	0.33	0.2	0	2.78	0.85	0.55	12.71	6.16	1.08
105	0.43	0.39	0	4.17	1.03	0.59	9.96	4.7	1.07
120	0.5	0.53	0	6.17	1.28	0.59	7.38	3.56	1.07
150	0.42	0.5	0	11.04	1.95	0.59	2.14	1.69	1.06
180	0	0.08	0	12.42	2.3	0.59	1.23	1.12	1.05
210	0	0	0	12.56	2.24	0.59	1.13	1.07	1.05
235	0	0	0	12.14	2.13	0.58	1.11	1	1.07