# AEROBIC GRANULATION COUPLED MEMBRANE BIOREACTOR

by

Bui Xuan Thanh

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Engineering

Examination Committee: Prof. C. Visvanathan (Chairman)
Dr. Oleg V. Shipin
Dr. Thammarat Kootatep

Nationality: Vietnamese
Previous Degree: Bachelor of Chemistry and Food Engineering
Ho Chi Minh City University of Technology
Scholarship Donor: Netherlands Government

Asian Institute of Technology
School of Environment, Resources and Development
Thailand
May 2005

**Acknowledgements**

The author would like to start this thesis with profound gratitude, great appreciation and indebtedness to his advisor, Prof. C. Visvanathan, for his valuable guidance, encouragement, support and stimulating ideas. His enthusiasm and systematic approach in completing work challenged his to do his best. Prof. C. Visvanathan not only gives academic assistant but also shares knowledge and professional experience to solve the problems that happened throughout the study period.

Special thanks are extended to Dr. Oleg V. Shipin and Dr. Thammarat Koottatep, the members of the exam committee, for their useful suggestions and comments, which have been a great help in achieving the objectives of this study.

Acknowledgements are brought to Emeritus Prof. Roger Ben Aim for his valuable recommendations and Dr. Mathieu Spérandio for his intensive training and Doctoral students at GPI lab, INSA – Toulouse – France.

The author wishes to present deepest sincere thanks to Dr. Nguyen Phuoc Dan for his continuous encouragement and academic advices.

Words of thanks must be also conveyed to Asian Institute of Technology (AIT) and AIT-INSA project for providing scholarship that made him able to pursue the master program at AIT. He also gives his deepest appreciation to staff members of the AIT Environmental Engineering Program, both laboratory and secretary sections.

Finally, the author would like to express his incisive gratitude and dedicate this research work to his parents, sisters and friends, whose love, assisted the author through difficult times and contributed to the success of this study.

## Abstract

Granular activated sludge was formed in two laboratory scale Sequencing Batch Airlift Reactors (SBAR) using two types of support media. One reactor was cultivated with bivalve shell carrier media (CR) and the other with anaerobic granules media (AR). First, these reactors were operated at loading rate of 2.5 kg COD/$m^3$.day for granular sludge formation and characterization with glucose as sole carbon source. Second, applied loading rate could be increased for estimating maximum and optimum loading rate of granules and contemporarily carrying out more granule characterization. Organic loading rate was varied from 2.5 to 30 kg COD/$m^3$.day. The optimum loading rate was found to be 10 kg COD/$m^3$.day and bivalve shell carrier was identified to be a good media for granule formation due to more compact (settled biomass concentration of 21-49 mg/$L_{granule}$), higher settability (mostly SVI of less than 26 mL/g, settling velocity of 21-103 m/h), and shock loading suffering ability. Granule size was in range of 0.5-4 mm at all loading rates. CR granules were “smoother” than AR one with smaller cavities.

At each loading rate, supernatant of both reactors was also analyzed for its fouling potential and compared with that of conventional sequencing batch reactor. It was found that supernatant of carrier reactor was least fouling with MFI of $1.9x10^3$ and $130.7x10^3$ $s/L^2$ at loading rate of 2.5 and 5 kg COD/$m^3$.day, respectively. Fouling potential was identified due to cake layer formation on membrane surface and soluble polysaccharides of supernatant.

Finally, two treatment sequences, internal (continuous mode) and external (batch mode) membrane modules were coupled with SBARs to find out suitable application of aerobic granules for membrane bioreactor. The internal one which was totally as same configuration as conventional submerged membrane bioreactor made granules worn after two days of operation and granules characteristics became like conventional activated sludge. In addition, maximum fouling rate of internal system was 79.7 kPa/day that was three fold more than the external one of 24.8 kPa/day with permeate flux of 8.7 L/$m^2$.h for both systems. Fouling time was 1 and 7 days for internal and external membrane module, respectively.

However, the external system was much more suitable with granule membrane bioreactor. It produced high effluent quality with low turbidity of 0.8 NTU, COD of less than 20 mg/L at loading rate of 10 kg COD/$m^3$.day. Moreover, unsettled biomass of supernatant was also biodegraded as substrate in membrane reactor and this could reduce sludge production and prolong operating duration of membrane.

## Table of Contents

## List of Table

## List of Figure

## List of Abbreviations

| | |
|---|---|
| HRT | Hydraulic Retention Time |
| SBAR | Sequencing Batch Airlift Reactor |
| MLSS | Mixed Liquor Suspended Solids |
| MLVSS | Mixed Liquor Volatile Suspended Solids |
| COD | Chemical Oxygen Demand |
| SVI | Sludge Volume Index |
| BAS | Bio-film Airlift Suspension Reactor |
| SBR | Sequencing Batch Reactor |
| EPS | Extra-cellular Polymeric Substances |
| SBBC | Sequencing Batch Bubble Column |
| BAS | Bio-film Airlift Suspension Reactor |
| FISH | Fluorescent In Situ Hybridisation |
| USBR | Upflow Sequencing Batch Reactor |
| PS/PN | Polysaccharides to Protein Ratio |
| SOUR | Specific Oxygen Utilization Rate |
| MBR/GMBR | Membrane Bioreactor/Granule Membrane Bioreactor |
| SRT | Solid Retention Time |
| MF | Micro-filtration |
| NF | Nano-filtration |
| MWCO | Molecular Weight Cut-off |
| VLR | Volumetric Loading Rate (Kg COD/m3.day) |
| OLR | Organic Loading Rate |
| WW | Wastewater |
| CR/AR | Bivalve Shell Carrier Reactor/Anaerobic Granule Reactor |
| SBR | Sequencing Batch Reactor |
| CRi/ARi | sample of CR/AR at loading rate i |
| TMP | Transmembrane Pressure |

# Chapter 1

# Introduction

## 1.1 Background

Biological processes have been the major unit processes in most of wastewater treatment plants especially conventional activated sludge process (CASP). Nowadays, due to extensive interest in wider applications of wastewater reuse and recycle, necessitate produce effluent of high quality, which cannot be obtained by conventional biological process such CASP so the application of conventional activated sludge faces some difficulties because of its low treatability (0.5-2 kg COD/$m^3$.day) and high suspended solids in effluent. Moreover, settleability of conventional activated sludge is rather low so this made the construction and sludge treatment cost increased. In addition, CASP needs high surface area for its full construction including large clarifier that is impossible for somewhere that land is unavailable or high price.

After CASP, Membrane Bioreactor (MBR) appeared as a significant change of application of activated sludge in water and wastewater treatment field which has much more advantages than CASP. MBR has higher volumetric loading rate, good treated water quality, long sludge retention time, ability to withstand with toxic substances and less space requirement but there are two problems: (1) the sludge settling ability is very weak due to fine size of sludge flocs. Sludge volume index is usually higher than 200 mL/g in most of MBR and (2) membrane fouling is an unavoidable problem with those kinds of systems using conventional activated sludge.

From the above limitations of conventional activated sludge, one kind of aerobic granular sludge which has excellent characteristics has been found by Tijhuis et al, 1994. Aerobic granular sludge has many advantages in comparison with other the types of conventional activated sludge in terms of settled biomass concentration, size, shape, regularity and settling ability. Especially, it has very good settling ability (settling velocity greater than 10 m/h, sludge volume index (SVI) up to 30 mL/g (Linlin et al.., 2005)) and very high organic and nitrogenous loading rate so the size of treatment plant will be very compact. With this kind of granule, organic loading rate (OLR) could reach to more than 9 kg COD/$m^3$.day (Tay et al., 2003) and 15 kg COD/$m^3$.day (Moy et al. 2002) that was seven-fold higher in comparison with CASP. In addition, when creating aerobic granules in Sequencing Batch Airlift Reactor (SBAR), this reactor is operated as two in one that it happens as both aeration tank and settling tank in one unit. SBAR has very good mixing condition by even current convection from the top to the bottom vice versa.

The appearance of aerobic granules could break through a new trend of wastewater treatment when combining with MBR. As known, MBR had many advantages in comparison with other processes. Depending on advantages and disadvantages of membrane bioreactor with conventional activated sludge and specific characteristics of granular sludge, application of aerobic granules coupled with MBR was investigated in this research. This application had ability to maintain advantages of MBR and also overcome its disadvantages. Some of advantages of the Granule Membrane Bioreactor (GMBR) that can be foreseen are (1) high OLR (greater than 30 kg COD/$m^3$.day); (2) high settling ability of granules; (3) reduction of membrane clogging and increase, prolongation of membrane lifetime; (4) ability for nitrogen removal; (5) and other advantages as conventional MBR.

Based on advantages of aerobic granules and MBR, if aerobic granules are able to combine effectively with MBR and overcome these current disadvantages, it will be the most attractive alternative in the future.

From the above advantages of GMBR, this research could find out the new technique for the applications of GMBR in field of wastewater treatment. In this study, SBAR was used to cultivate aerobic granules by synthetic wastewater and then formed granules were investigated characteristics such as settled biomass concentration, granule settling velocity, SVI, Extracellular Polymeric Substance (EPS) of granules and supernatant, hydrophobicity, granule bioactivity, kinetic data, etc. When coupling with membrane, some more parameters were measured as fouling rate, fouling index, and EPS especially with polysaccharides of supernatant for fouling estimation.

## 1.2 Objectives of study

The main objectives of this study were to focus on investigating of aerobic granules and its application with types of MBR. These include:

1. To study the creation of aerobic granules with types of support media including bivalve shell carrier and anaerobic granules in SBAR;

2. To determine the characteristics of types of aerobic granules with two different support media;

3. To investigate the performance of aerobic granular reactor in terms of OLR for SBAR and also select fine media and optimum OLR for coupling with MBR;

4. To investigate the coupling of aerobic granulation reactor with types of Membrane Bioreactor.

## 1.3 Scope of study

This study used SBAR (batch system) to cultivate aerobic granules with superficial air velocity of 95 m/h and observed the development of granules in reactors. Glucose was sole source of organic matter of synthetic wastewater. COD of synthetic wastewater for granule cultivation was 600 mg/L (loading rate of 2.5 kg COD/$m^3$.day). Granule characteristics was investigated by measuring parameters such as COD, biomass concentration, settled biomass concentration, hydrophobicity, extracellular polymeric substances, sludge volume index, settling velocity, etc. After matured granules formed, loading rate of system was increased (up to 30 kg COD/$m^3$.day) to determine maximum, optimum level of loading and bioactivity of matured granules. Finally, optimum loading rate and optimum carrier were selected to connect with membrane units for more investigation with membrane. Parameters were measured as membrane fouling index, fouling rate, etc.

1. Formation of aerobic granules by SBAR at organic loading rate of 2.5 kg COD/($m^3$.day) with two support media (bivalve shell and anaerobic granules);

2. Investigation of maximum organic loading rate with matured aerobic granules;

3. Investigation of bioactivity and some kinetic parameters of aerobic granules;

4. Investigation of application of aerobic granules with types of MBRs.

# Chapter 2

# Literature Review

## 2.1 Introduction

Most wastewater treatment systems have some disadvantages like high surplus biomass production, low flexibility with respect to fluctuating loading rates, a large area requirement for reactors and especially sedimentation tank and rather low OLR (0.5-2 kg COD/($m^3$.day) for conventional activated sludge or biofilm process).

For anaerobic processes much more efficient reactors have been developed (up to 40 kgCOD/($m^3$.day) for UASB (Upflow Anaerobic Sludge Blanket) reactor. Moreover no settlers are necessary because sludge separation is integrated in the UASB itself. Despite the widespread use of UASB processes the mechanism of granulation is still subject of discussion. For methanogenic granules it is hypothesized that the microorganisms grow in a granule because of specific synthrophic bacterial interactions. Effectively granular growth is just a special case of biofilm growth. Recently, Some authors found the granulation in aerobic condition such as Tijhuis et al., 1994; Beun et al, 1999 & 2002; Tay et al., 2001 & 2002; Arrojo et al., 2004; Wang et al., 2004; Jang et al., 2003; Qin et al., 2004; Linlin et al.., 2005; Liu et al., 2003; Yang et al., 2004; Zheng et al., 2004; Toh., 2003. Granulation of types of microorganisms has been observed such as acidifying bacteria, nitrifying bacteria, denitrifying bacteria (Beun et al., 1999; Beun et al., 2002; Tsuneda et al., 2004; McSwain et al., 2004) and aerobic heterotrophs (Tijhuis et al., 1994b; Van Benthum et al., 1996; Yang et al., 2004). These aerobic granules occurred in continuous operated biofilm airlift reactors.

For many applications it was found that a discontinuous system is more advantageous than continuous one in cultivating aerobic granules. It has been showed that aerobic granular sludge can be cultured in a SBR (Morgenroth et al., 1997; Heijnen and Van Loosdrecht, 1998; McSwain et al., 2004; Tay et al., 2004; Schwarzenbeck et al., 2004) or in SBAR (Beun et al., 1999; Beun et al., 2002). But the SBAR is preferred due to its good mixing conditions and high shear stress. Moreover, critical factors are the establishment of well-settling characteristic of sludge, high organic and nitrogenous loading rate.

Because of continuous depletion of fresh water resources, focus has shifted more toward water recovery, reuse and recycling, which required alternative treatment systems. This is currently a major problem for environmental managers and engineers. Nowadays because of high organic and nitrogenous strength of wastewater, it needs a new aerobic treatment technology that has higher loading rate, high settling ability of sludge and high toleration with toxic substances. To meet this need, biological process using aerobic granular system could be an attractive alternative because of its above advantages. If this process is coupled with membrane technology, this problem could be solved easily. Moreover, when granular sludge combines with membrane process, the traditional problem of membrane fouling could be reduced.

## 2.2 Production and Characteristics of Aerobic Granular Sludge

### 2.2.1. Carbon sources

The carbon sources used to culture granular sludge are usually acetate, glucose, both acetate and glucose or real wastewater. The carbon sources are shown in table 2.1.

**Table 2.1 Carbon sources used to cultivate granular sludge**

| Carbon source | OLR kg/$m^3$.d | Granule diameter (mm) | SVI (mL/g) | Settled biomass conc. (gVSS/L) | Reactor | Formation time | Reference |
|---|---|---|---|---|---|---|---|
| Acetate | 2.3 | 1 | | 11.9 | SBAR | 50 days | Beun et al., 1999 |
| Acetate | 2.5 | 2.5 | - | 60 | SBAR | > 63 days | Beun et al., 2002 |
| Acetate | - | 1.2 | 30-40 | - | SBR | 50 days | Linlin et al.., 2005 (*) |
| Acetate | 6.0 | 0.35 | 50 | - | SBR | Cycle 42; 4h/cycle | Tay et al., 2002 |
| Acetate | 5 | 0.35(**) | | 15-20 | BAS | - | Tijhuis et al., 1994 |
| Acetate | 6 | 0.33 - 0.39 | 46-62 | 40-60 | SBR | 21 days | Tay et al., 2004 |
| Acetate; glucose & peptone | 3.6 | 1.1-6.5 | - | - | SBR | 56 days | Etterer & Wilderer, 2001 |
| Barley dust WW | 3.4 | 2-4 | 30-40 | - | SBR | 4 weeks | Swazenbeck et al., 2004 |
| Dairy WW | 7 | 0.25-4 | 60 | 10-15 | SBR | 60 days | Arrojo et al., 2004 |
| Ethanol | - | 0.4-1.9 | - | - | SBR | 40 days | Yang et al., 2003 |
| Glucose | 4.8 | 6-9 | 40 | - | SBR | 67 days | Wang et al., 2004 |
| Glucose | 5 | 1.2 | < 65 | 45.2-45.7 | SBSR | 50 days | Cai et al., 2004 |
| Glucose & acetate | 2.5 | 1.0-1.3 | 70-90 | - | SBR | 50 days | Jang et al. 2003 |
| Glucose & peptone | 2.4 | | 46-114 | | SBR | 120 days | McSwain et al., 2004 |
| Molasses | 2.9 | 2.35 | - | - | SBR | 40 days | Morgenroth et al., 1997 |
| Sodium acetate | - | 0.35 | 50-140 | - | SBR | 3 weeks | Qin et al., 2004 |
| Sodium acetate | - | 0.25-0.32 | - | - | SBR | 4 weeks | Yang et al., 2004 |
| Sucrose | - | 0.5-1.2 | 23 | - | SBAR | 68 days | Zheng et al., 2004 |
| Phenol | < 2.5 | | 40-65 | | SBR | - | Jiang et al., 2004 |
| Sodium acetate | 4 | 1.2-1.8 | - | - | SBR | - | Liu et al., 2005 |

(*) Seeding sludge is anaerobic sludge; (**) including carrier diameter $d_c$ = 0.26 mm; WW: wastewater; SBSR: Sequencing Batch Shaking Reactor.

From the table 2.1, firstly it shows that aerobic granule could be cultured with the carbon sources such as acetate, glucose, both acetate and glucose or industrial wastewaters. This means that granule was able to form with any carbon sources that can be biodegradable or even with high suspended solid wastewater (Arrojo et al., 2004, Schwarzenbeck et al., 2004). Secondly, granules could be operated at higher loading rate (2.3-14 kg COD/$m^3$.d) than conventional activated sludge process (0.5-2 kg COD/$m^3$.d). Thirdly, granule size was normally from 0.3 to 9 mm that can be seen by naked eyes like anaerobic granules. When the granules formed, it had excellent settling ability, high biomass retention, high bioactivity and an ability to withstand high organic loading rate. Totally SVI parameter was lesser than 100 mL/g and sometime decreased up to 30 mL/g so this meant granular sludge had very good settling ability. Moreover with the very good settling ability if a

clarifier was necessary, its volume would be much smaller than conventional activated sludge settling tank. Excess granular sludge treatment could also be easier with this characteristic.

### 2.2.2. Reactor configuration

From above research, granules could be formed in batch system such as SBAR (Sequencing Batch Airlift Reactor), SBR (Sequencing Batch Reactor) and continuous system such as BAS (Biofilm Airlift Suspension Reactor) after certain period of time and cultivation method. Normally, it was formed after 40 days. But it was more efficient when culturing aerobic granule in batch system (Beun et al., 1999) especially with SBAR. SBAR had excellent mixing condition, simple design, and possibility for easily deal with peak-load and reactor was more compact because of high ratio of height to diameter (H/D). The comparison among types of reactor to cultivate granule showed in the table 2.2:

**Table 2.2. Comparison among the SBAR, SBBC and BAS**

| SBAR (Beun et al., 2000) | SBBC (Beun et al., 1999) | BAS (Tijhuis et al., 1994) |
|---|---|---|
| Discontinuous system | Discontinuous system | Continuous system |
| No external settler needed | No external settler needed | No external settler needed |
| Riser needed | Riser needed | Riser, 3 phase separator needed |
| No carrier needed | No carrier needed | Carrier needed |
| Settling time is selection variable | Settling time is selection variable | HRT is selection variable |
| Detachment determined by hydrodynamic conditions | Detachment determined by hydrodynamic conditions | Detachment determined by bare carrier concentration |
| Nitrification and denitrification occurred | - | No denitrification occurred |
| Density $\rho = 48 g/L_{granules}$ | $\rho = 12 g/L_{granules}$ | $\rho = 15 g/L_{granules}$ |
| Granule diameter d = 1.0 mm | d = 2.0 mm | d = 0.35 mm ($d_{carrier}$ = 0.26 mm) |

SBAR: Sequencing batch airlift reactor; SBBC: sequencing batch bubble column; BAS: Bio-film airlift suspension reactor;

From table 2.2, SBAR was more efficient because this can create granule with dense, smaller granules so this type is much suitable for the research. Moreover, old conventional activated sludge process can be upgraded to be SBAR or SBR for treatment improvement. The used SBAR would be described in details in methodology part.

### 2.2.3. Seed sludge

Seed sludge to create granule could be started from aerobic conventional activated sludge (Tay et al., 2001; Beun et al., 1999; Jang et al., 2003; Arrojo et al., 2004; Wang et al., 2004; Qin et al., 2004; Schwarzenbeck et al., 2004) or anaerobic sludge (Linlin et al. al, 2005). Therefore, aerobic granules could be cultivated by either conventional activated sludge or anaerobic granules.

### 2.2.4. Microbial immobilization

***Classification of cell immobilization:***

Cell immobilization technology has been used in bioengineering and environmental engineering areas for decades. In general, cell immobilization is known as microbial aggregation, and can be roughly classified into three categories:

- Biofilm: Microorganisms are immobilized or attached onto a solid surface, such as activated carbon, basalts, plastics, polymers, ceramics and others (Liu and Tay, 2002).
- Microbial aggregates and granular sludge: aerobic and anaerobic granules have been successfully developed. Microbial granulation can be regarded as a self-immobilization community of bacteria.
- Entrapped microorganisms: Microorganisms may be entrapped in hydrophobic gels of photo-crosslinked polymers or in other types of gels, such as polyacrylamide (Liu and Tay, 2002).

***Cell immobilization process:***

Biofilm and granular sludge indeed can be regarded as different forms of cell immobilization. So far, it has been recognized that the formation of biofilms and microbial aggregates is a multiple-step process, to which physico-chemical and biological forces make significant contributions (Beun et al., 1999; Tay et al., 2001; Tay et al., 2004). Based on previous studies, it is encouraged to propose that cell immobilization can be roughly described as a four-step process as follows:

*Step 1: Physical movement to initiate cell-to-cell contact or bacterial attachment onto a solid surface. The forces involved in this step are:*

- Hydrodynamic force;
- Diffusion force;
- Gravity force;
- Thermodynamic force;
- Cell mobility

*Step 2: Initial attractive forces to keep stable bacteria solid surface and multicellular contacts. Those attractive forces are:*

- Physical forces:
- Van der Waals forces;
- Electrostatic forces;
- Thermodynamic forces;
- Hydrophobicity
- Filamentous bacteria can link or bridge cell together.
- Chemical forces:
- Hydrogen liaison
- Formation of ionic pairs;
- Formation of ionic triplet;
- Interparticulate bridge and so on.
- Biochemical forces:
- Cellular surface dehydration;

- Cellular membrane fusion;

*Step 3: Microbial forces to make attached bacteria or aggregated bacteria nature:*

- Production of extracellular polymer such as exopolymer saccharides etc;
- Growth of cellular cluster;
- Metabolic change and genetic competence induced by environment, which facilitate and further strengthen the cell-cell interaction and result in the high density of adhering cells.

***Step 4:*** *Steady state three-dimensional structure of microbial aggregate shape by hydrodynamic shear force. The outer shape and size of microbial aggregates are finally determined by the interactive strength between aggregate and shear force, microbial species, and substrate loading rate and so on.*

### 2.2.5. Characteristics of aerobic granular sludge

Granular sludge had much more advantages than conventional activated sludge. These characteristics between granular sludge and floc-like sludge are shown in the figure 2.1.

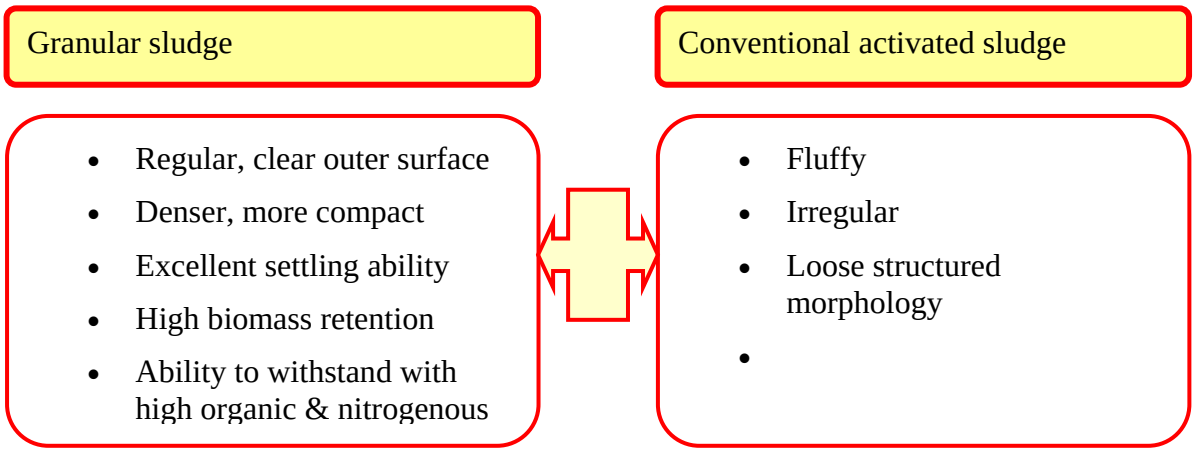



**Figure 2.1. Characteristics of granular sludge and conventional activated sludge**

Some authors (Tijhuis et al., 1994; Jang et al., 2003) have regarded the granules as a suspended spherical biofilm including microbial cells, inert particles, degradable particles and extracellular polymeric substances (EPS). Among other particles, an aqueous matrix of EPS allowed various microbial species to form stable aggregates (Jang et al., 2003).

Depending on spherical dense structure, aerobic granules have specific characteristic of aerobic sludge in outer part and anaerobic sludge in inner part so nitrogen could be easily removed if oxygen diffusion is limited or diameter of granule is large enough. Hereafter there were two conditions of aerobic granules. Anaerobic condition was in central core and aerobic condition in outer part. The trend of substrate concentrations happen inside aerobic granules is described in the figure 2.2.

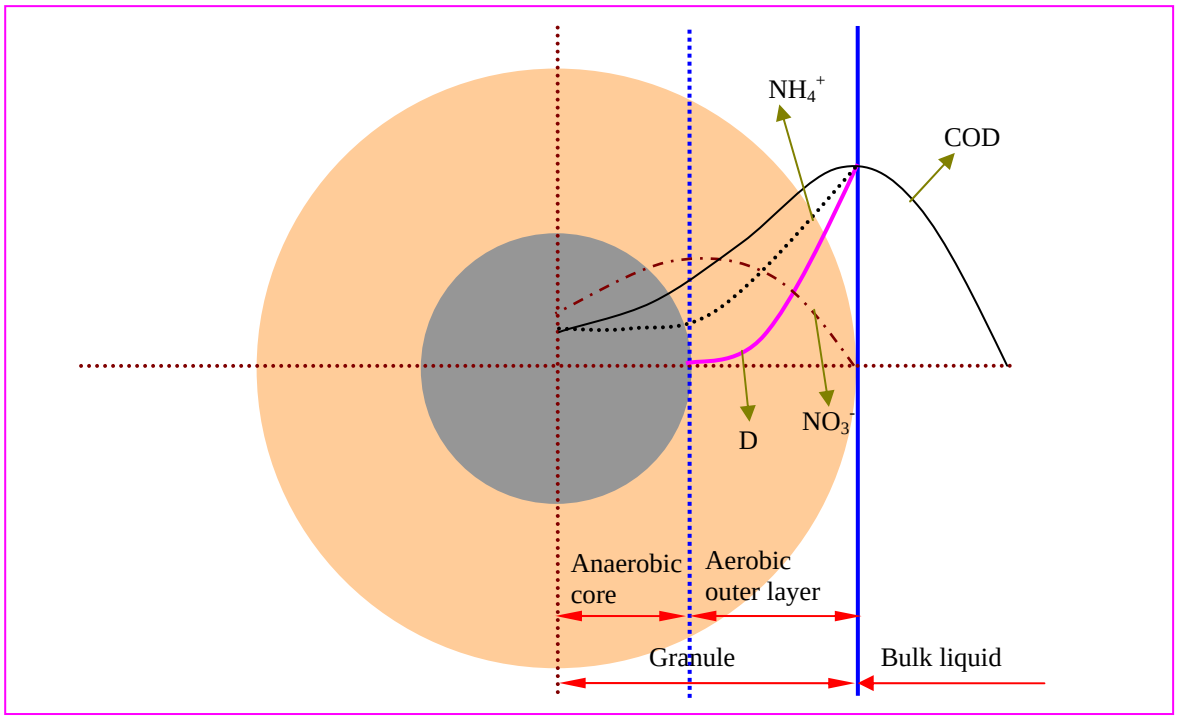



**Figure 2.2. Diagram of substrate concentration in aerobic granules**

Rod bacteria were predominant in granules, and lots of cavities were present. These cavities could enhance substrates transfer from the bulk liquid to granules and intermediate or by-product, product easily transfer form inside granules to the bulk (Tay et al., 2002).

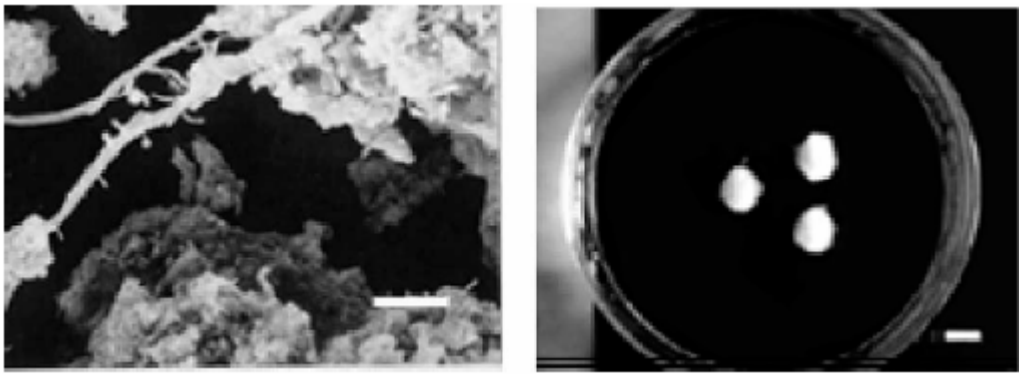


**Figure 2.3 Microscopy image of the seed sludge (left), bar = 5 μm, filamentous sludge; aerobic granules (right) at steady-state, bar = 8 mm (Wang et al., 2004)**

***The granule size:***

The granule size was very important for substrate, nutrients, oxygen accessibility and product releasing, which also had great impact on microbial viability, microenvironment and the microstructure of the microbial community. Granule size also decided the nitrification and denitrification ability and anaerobic decomposition due to limitation of oxygen diffusion. Normally depth of oxygen penetration was from 100-150μm (Tijhuis et al., 1994) so if the granule radius is higher than this, denitrification and anaerobic degradation can be achieved in process. Hence, granule size is the eminent factor in molding the physical performance and characteristics of aerobic granules (Linlin et al., 2005).

***Water content:***

The water content in aerobic granules was 94.3%. The water content in anaerobic granules was about 97.2% (Linlin et al., 2005).

***Settling velocity:***

The settling velocity of cultivated aerobic granules was in the range of 22-60 m/h, the average was 38.4 m/h, compared with 72 m/h for anaerobic granules. The settling velocity was lower because of increasing of water content in aerobic granule (Linlin et al., 2005).

***VSS/SS ratio:***

VSS/SS in aerobic granule was 0.71; compared with anaerobic one was 0.57. However, this ratio was lower that of normal activated sludge (0.85) (Linlin et al., 2005).

***Density of granule:***

Density of granules was equal to density of discrete bacterial cells but granule showed much better settling properties because of their larger sizes. Density of granule could reach to 60 mg/$L_{granule}$ (Beun et al., 2002).

***Cell surface hydrophobicity:***

Cell surface hydrophobicity in granule was rather different from conventional floc-like sludge. There is a significant difference in cell hydrophobicity was observed before and after the formation of aerobic granules. It increased from a value of 50.6% in the period before granulation to 75.1% after granulation, i.e. 50% higher than for aerobic granular sludge. It appeared that the formation of aerobic granules was coupled to an increase in the cell hydrophobicity. Hydrophobicity of cell surface had generally been considered to play an important role in the self-immobilization and attachment of cells to a surface, i.e. cell-to-cell attachment (Tay et al., 2002).

***Exopolysaccharides production:***

The exopolysaccharides could mediate both cohesion and adhesion of cells, and played a crucial role in maintaining structural integrity of the biofilm matrix. The content of biofilm polysaccharides (PS) was at least 4.5-fold higher than that of biofilm-proteins (PN) (data from three-phase fluidizedbed reactor of Lertpocasombut (Liu and Tay, 2002)) but this was till on research in case of aerobic granule. When superficial air velocity increases, this ratio is also proportional to shear stress.

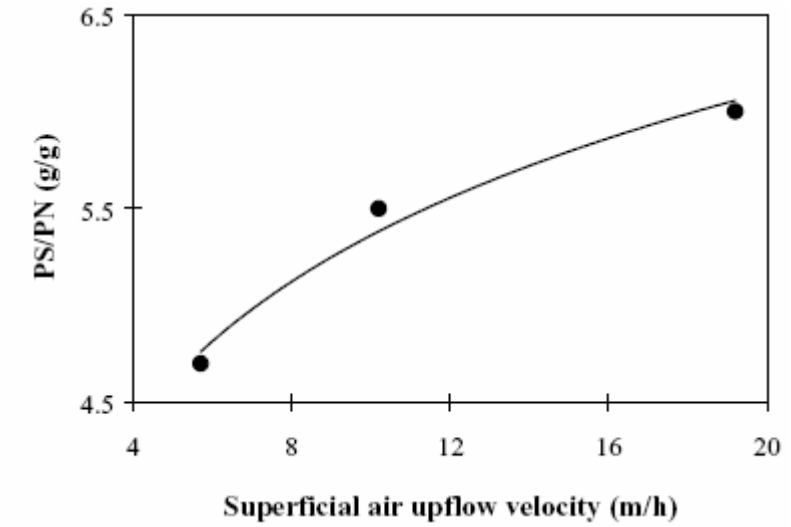



**Figure 2.4 Effects of superficial air upflow velocity on the ratio PS/PN in steady state three-phase fluidizedbed reactor (data from Lertpocasombut). (Liu and Tay, 2002)**

***Specific gravity:***

Specific gravity of granular sludge also increases after granulation. It was 1.0008 at the beginning and increased to an average value of 1.0069 kg/L during the granulation period. The specific gravity of the sludge reflects the compactness of microbial community. The significant improvement of specific gravity for granular sludge indicated its highly compact structure (Tay et al., 2002).

### 2.2.6. Support media for aerobic granules

Another effective way of increasing the settleability and enhancing the formation of the sludge granules was the use of support media. Various support media have been suggested such as basalt (Tijhuis et al, 1994), sponge, sand, plastic bead, shells, etc (as table 2.3). These support media acted as a seed for the sludge granule formation and also aided in settleability.

Study conducted by Tijhuis, et al (1994), suggested the application as basalt as the support media. Basalt was commonly found solidified lava, a type of igneous rock mainly comprising of calcium rich feldspar and pyroxene. The carrier material had a rough surface which resulted in good potential for biofilm development. Calcium was also said to play an important role in cultivation of aerobic sludge granule (Wang et al, 2004), making the use of calciferous shells and basalt advantageous. The particle size density of basalt used in the study was 3 kg/L with a mean diameter of 260μm, Carrier concentration was 5% of the volume, which was about 150 g/L. Basalt was suspended homogenously in the airlift reactor. The surface area of the biofilm could be thus increased with a larger diameter than the carrier.

Types of support media could be used as support for granule are list in table 2.3.

**Table 2.3. Type of studied support media (Modified from Harendranath et al., 1996)**

| Support materials | Morphological features | Macro-structure | Porosity | Surface area (m2/ m3) | Reference |
|---|---|---|---|---|---|
| Smooth | | | | | |
| P.V.C | Even surface; pore absent | Size, shape variable | 0.96 | 140 | Kenedy & Droste, 1983 |
| P.A.C | Smooth surface; pores absent | Fine power | - | - | Ng et al, 1988 |
| Porcelain | Smooth surface even; pore absent | Size, shape variable | - | - | - |
| Perspex | | | | | |
| Uneven | | | | | |
| Nylon | Densely distributed | Size, shape variable | - | - | - |
| Stone | Highly corrugated with randomly distributed crevices | Amorphous | 0.42-0.53 | - | Henze & Harremoes, 1983 |
| Sand | Crests and troughs | ~ 0.7 mm | 0.38-0.43 | 2500-4000 | Henze & Harremoes, 1983 |
| Sintered glass | Rough, jagged surface, shallow pore up to 20μm width, loosely distributed | Open-pore structures | 0.57 | 90,000 | Anderson et al., 1994 |
| Porous | | | | | |
| Basalt | Rough surface | 0.26 mm | - | 1150 | Tijhuis et al., 1994 |
| Bivalve shell (*) | Crests and troughs with deep pores, 10μm with densely distributed | Concave, convex | 0.77-0.82 | | Henze & Harremoes, 1983 |
| Granulated clay | A moisiac of particulates, 1-10μm width, pores of 5μm width, uniformed distributed | Amorphous aggregate | ~ 0.7 | 53-397 | Henze & Harremoes, 1983 |
| Gravel | Pores of 5μm, loosely distributed, irregular ridges present | Amorphous | 0.4 | - | Henze & Harremoes, 1983 |
| Ceramic | Thorny surface with polygonal pores 1-10μm width | Variable size and shape | 0.6 | 274 | Cordora & sinerriz, 1990 |
| Refractory bridge | Crystalline, pointed structure and pores, densely distributed | Amorphous | - | 149 | Henze & Harremoes, 1983 |
| Diatomaceous earth | Deep, minute pores 5μm width, densely distributed, | Variable size and shape | - | - | - |
| GAC | Rough surface with 3 dimensional pore distribution, pore size up to 250μm | Amorphous | 0.6 | 5469 | Henze & Harremoes, 1983 |
| Limestone | Highly porous structures, composed of crystalline unit 2-5μm length, densely distributed deep pores 10 μm width | Variable size and shape | 0.49 | 5,000 – 10,000 | Henze & Harremoes, 1983 |
| Sponge | Labyrinth of pores 200-500μm width, hexa or pentagonal in shape | Highly compressible variable size and shape | - | - | - |

(*) In this study, bivalve shell carrier used has size of 0.15-0.22 mm

## 2.3 Factors stimulating aerobic granulation formation

Aerobic granular sludge cultivation depended on various factors such as hydrodynamic shear force, preset settling velocity of sludge particles, EPS percent of sludge, cell hydrophobicity of sludge, type of reactor, seed sludge characteristics, organic loading rate, composition of feed wastewater, operational conditions, inhibition substances, etc. All of factors contributed to granule formation and aerobic granule characteristics. Although these factors have not documented much but some main factors has been surveyed as follows:

### 2.3.1. Hydrodynamic shear force

Biofilm and granular sludge processes included many advantages as previous discussion. The formation, structure and metabolism of immobilized microbial community were associated very closely with hydrodynamic shear force in reactors. Hydrodynamic shear forces were created by superficial air velocity in the reactor. More compact, stable and denser than biofilms, aerobic and anaerobic granules form at relatively higher hydrodynamic shear force. It was clearly shown that shear force has significant influences on the structure, mass transfer, production of exopolysaccharides, metabolic/genetic behaviour of biofilm, aerobic and anaerobic granules.

Aerobic granulation is a type of self-immobilization process. Similar to formation of biofilm, hydrodynamic shear force would have a significant effect on the formation and structure of granules. According to previous research, when upflow sequencing batch reactor (USBR) was operated at a low superficial air velocity of 0.008 m/s, no granules were observed in system but only fluffy flocs (figure 2.5a). On the contrary, when it was of high superficial velocity of 0.025 m/s, regular shaped granules were successfully developed in the USBR (figure 2.5b) (Tay et al., 2001).

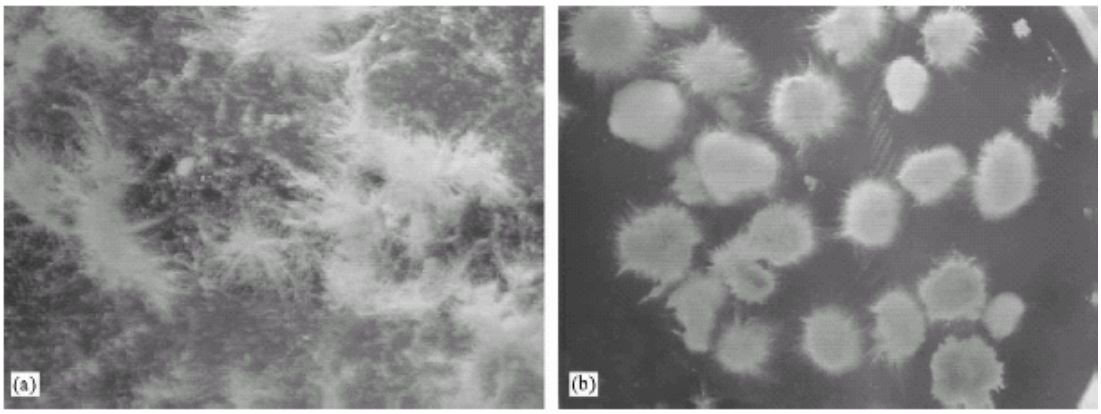



**Figure 2.5. Bioflocs cultivated at a superficial air velocity of 0.008 m/s (a); and granules formed at a velocity of 0.025 m/s in USBR (b) (Tay et al., 2001).**

The same result as Tay et al. (2001), Beun et al. (1999) also observed that at low superficial air velocity did not lead to the formation of granules in USBR.

It was found that specific gravity of aerobic granules increased with the increase of hydrodynamic shear force, while SVI decreased (figure 2.6). In fact, higher granule density associated with a low SVI could ensure a more efficient biosolid-liquid separation, which is important in wastewater treatment systems for the successful operation of the process and production of high quality effluent.

It was also pointed out that at a superficial air velocity of 0.3 cm/s, aerobic granules did not occurred and only conventional bioflocs as figure 2.5 (a).

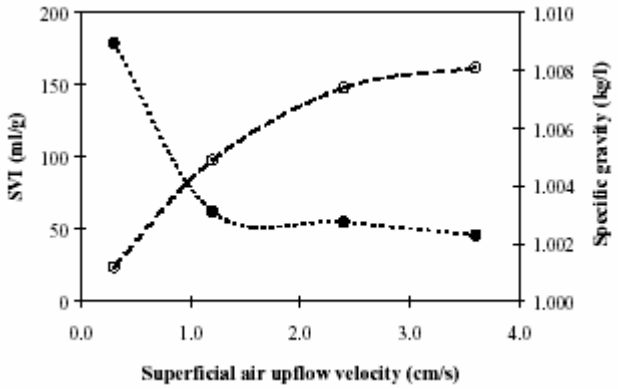



**Figure 2.6. Effects of superficial air upflow velocity on the specific gravity and SVI of aerobic granules developed in USBR. (•): SVI; (■): specific gravity (Liu et al., 2002)**

Moreover, hydrodynamic shear stress also caused the increase of hydrophobicity and polysaccharides (Tay et al., 2004).

From the previous research, it was proved clearly that hydrodynamic shear force affects significantly to aerobic granulation and granule characteristics. And superficial air velocity had to be greater than 1.2 cm/s (43.2 m/h) to form aerobic granule (Tay et al., 2001). So this was the most really important factor in creating aerobic granules.

### 2.3.2. Settling velocity

Settling velocity was the key factor to maintain aerobic granules in reactor. This decided which particles were maintained in reactor and which particles were washed out. In conventional system, settling velocity of flocs was usually lesser than 10 m/h but in the granular sludge system the settling velocity of granules must be much higher than this value for washing out most of flocs, sludge having low settling velocity.

In aerobic granule cultivation process, the settling velocity of particles was usually chosen first to calculate settling time for system (Beun et al., 2002).

Settling time (h) = [ settling height (m) / chosen settling velocity (m/h)]

If the settling velocity was chosen 10 m/h and settling height was 50 cm, the settling time would be 3 min. It meant that granular sludge had smallest settling velocity of 10 m/h.

Settling velocity or settling time affected to granulation. Fraction of aerobic granules in reactor and settling ability of granules were high when settling time was short (Qin et al., 2004). Therefore, settling time was a trigger factor for cultivating aerobic granules.

**Table 2.4. Settling velocity, superficial air velocity and diameter of granule.**

| References | Beun et al., 2000 | Etterer and Wilderer, 2001 | Morgenroth et al., 1997 | Jang et al., 2003 | Wang et al., 2004 |
|---|---|---|---|---|---|
| Settling velocity (m/h) | 16.2 | 12.6-64.8 | 30-40 | 25.2-28.8 | > 32.7 |
| Superficial air velocity (m/h) | 86.4 | 72 | - | - | 63 |
| Granule size (mm) | 1 | 1.1-6.5 | 2.35 | 1.1-1.3 | 6-9 |
| Initial formation time (days) | 30 | 56 | 40 | 30 | 67 |

### 2.3.3. Extracellular Polymeric Substances

The abbreviation "EPS" has been used for "extracellular polymeric substances", "extracellular polysaccharides", "exopolysaccharides" and "exopolymers". However, The composition of the EPS matrix in biofilms and activated sludge flocs has been reported to be very complex, mainly consisting of protein, polysaccharides, nucleic acids, lipids, various heteropolymers and humic substances as table 2.5 (Wingender, 1999).

**Table 2.5. Composition of EPS (Modified from Wingender et al., 1999)**

| EPS | Principle components (subunits, precursors) | Main types of linkage between subunits | Structure of polymer backbone |
|---|---|---|---|
| Polysaccharides | Monosaccharides<br>Uronic acids<br>Amino sugars | Glycosidic bonds | Linear, branch |
| Proteins | Amino acids | Peptides bonds | Linear |
| Nucleic acids | Nucleotides | Phosphodiester bonds | Linear |
| Lipids (phosphor) | Fatty acids<br>Glycerol<br>Phosphate<br>Ethanolamine<br>Serine<br>Chlorine<br>Sugars | Ester bonds | Side-chains |
| Humic substances | Phenolic compounds<br>Simple sugars<br>Amino acids | Ether bond, C-C bonds, peptide bonds | |

The exopolysaccharides could mediate both cohesion and adhesion of cells, and played a crucial role in maintaining structural integrity of biofilm matrix. EPS formed a three-dimensional gel-like, highly hydrated and often charged biofilm matrix, in which the microorganisms were embedded and more or less immobilized as figure 2.7. In general, the proportion of EPS in biofilms could be varied between roughly 50 and 90% of the total organic matters (Wingender et al., 1999; Liu and Tay, 2002). EPS included bound EPS attaching to cell wall and soluble EPS suspending in bulk liquid (Winderer et al., 1999). Carbohydrate and protein were usually found as the major EPS components having a protein to carbohydrate ratio between 0.2 and 5 (w/w) (Frølund et al., 1996).

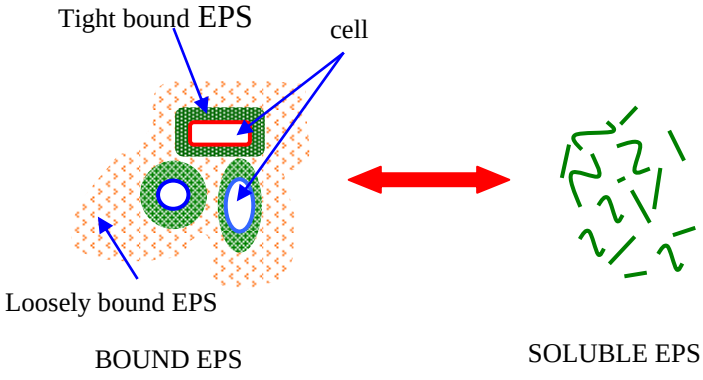



**Figure 2.7. Definition of cell biomass and EPS**

In granular sludge the content of polysaccharides was much higher than that of proteins (Liu and Tay, 2002; Yang et al., 2004). Vandevivere and Kirchman (1993) also found that

the content of exopolysaccharides normalized to proteins was 5-fold greater for attached cells than for free-living cells. These in turn implied that the polysaccharides would highly play an important role in attachment and self-immobilization processes of bacteria and the contribution of cellular proteins to the structure and stability of granule-associated bacteria would be less important (Wingender et al., 1999).

The ratio of polysaccharides to proteins (PS/PN) depended on hydrodynamic shear force as figure 2.4 (Tay and Liu, 2002), and inhibitor such as ammonia (Yang et al., 2004), etc. Superficial velocity increased, the ratio was also higher (Tay et al., 2004) and when ammonia concentration increased, the ratio was lower (Yang et al., 2004).

### 2.3.4. Cell hydrophobicity

Cell hydophobicity could be induced by culture conditions, and in turn initiates cell-to-cell aggregation that is a crucial step towards biogranulation. More recent research showed that cell hydrophobicity-induced by culture conditions could serve as triggering force of aerobic granulation (Liu et al., 2003). In fact, the physico-chemical properties of cell surface have profound effects on the formation of biofilms (Liu and Tay, 2002; Liu et al., 2003). When the bacteria became more hydrophobic, cell-to-cell adhesion was increased (Liu et al., 2003).

In the sense of process thermodynamics, microbial aggregation is driven by decreases of free energy, thereby increasing the hydrophobicity of cell surfaces would cause a corresponding decrease in the excess Gibbs energy of the surface, which in turn promotes cell-to-cell interaction and further serves as inducing force for cells to aggregate out of hydrophilic liquid phase.

Cell hydrophobicity can be determined by contact angle (CA) measurement, microbial adhesion to hydrocarbon in forms of liquid or solid (Liu et al., 2004). Cell hydrophobicity was classified into three categories:

- $CA > 90^o$ : hydrophobic surface
- $50^o < CA < 60$ : medium hydrophobic surface
- $CA < 40^o$ : hydrophilic surface

Cell hydrophobicity depends on types of carbon source. Cell hydrophobicity measured by the method of microbial adhesion to hydrocarbon was 68% for glucose-fed aerobic granules, and 73% for aerobic granules grown on acetate as sole carbon source, while the cell hydrophobicity of suspended seed sludge was only about 39%, indicating that the cell hydrophobicity of aerobic granules was nearly two times higher than that of the suspended seed sludge (Tay et al., 2003). Besides, nitrifying bacteria that were exposed to high free ammonia concentrations, nitrifying bacteria could not form granules, and a low surface hydrophobicity of the nitrifying biomass was detected.

Some studies showed that starvation conditions could induce cell surface hydrophobicity that in turn facilitated microbial adhesion and aggregation (Tay et al., 2001; Liu et al., 2004). It is most likely that microorganisms can change their surface properties when faced with starvation, and such changes can contribute to their ability to aggregate.

### 2.3.5. Organic loading rate

High organic loading rates (OLRs) are possible with aerobic granules. This points out the trend for development of aerobic granule based systems for high-strength wastewater (Moy et al., 2002; Tay et al., 2003)

Substrate loading rate also influence to aerobic biogranulation. Tay et al. (2003) carried out this study with OLR of 8, 4, 1 kg COD/($m^3$.day). The result is shown in the table 2.6.

**Table 2.6. The characteristics of aerobic granules/aggregates with superfical air velocity of 0.041 m/s (Tay et al., 2003)**

| Reactor | R1 | R2 | R3 |
|---|---|---|---|
| OLR, kg COD/($m^3$.day) | 8 | 4 | 1 |
| SOUR, mg $O_2$/(mg VSS.h) | 148 | 131 | 82 |
| Mean diameter by number, mm | 8.8 | 5.4 | 4 |
| Granule roundness | 1.49 | 1.29 | 2.23 |
| Specific gravity, kg/L | 1.024 | 1.034 | 1.011 |
| SVI, mL/g | 65 | 50 | 138 |
| COD removal efficiency | 0.79 | 0.99 | 0.95 |
| VSS/SS ratio, % | 0.91 | 0.87 | 0.88 |

Depending on table 2.6, the optimum OLR that can create the best aerobic granule is at loading rate of 4 kg COD/($m^3$.day). This OLR has the stabilized granules with the size of 5.4 mm, roundness of 1.29, SOUR of 118 mg $O_2$/(mg VSS.h), SVI of 50 mL/g, COD removal rate of 99%. Too high or too low of OLR appeared to be unfavourable for the formation of a compact sludge bed, and further, for maintaining the stability of reactor performance. Granule size decreased with the OLR applied, the roundness of granule was the smallest at the OLR of 4 kg COD/($m^3$.day). Under the OLR of 1 kg COD/($m^3$.day), only the patchy flocs were produced. If the OLR is higher than 8 kg COD/($m^3$.day), both granules and fluffy flocs also co-existed. This also contained a relatively smaller amount of EPS and its strength rather weaker (Tay et al., 2003).

Moy et al. investigated the effects of OLR with the physical characteristics of aerobic sludge granules. Acetate substrate can create the compact spherical morphology at OLR of 6 and 9 kg COD/($m^3$.day) and at low OLR granules exhibited a loose fluffy morphology dominated by filamentous bacteria.

### 2.3.6. Mineral cations

Mineral cations tend to complex with EPS, affecting bioflocculation, settling and dewaterability of the sludge (Liu and Fang, 2003). There are two bioflocculation models: double layer compression and cation bridging.

In the cation-bridging model (Liu and Fang, 2003; Tezuka, 1969; Forster and Lewin, 1972; Bruus et al., 1992; Higgins and Novak, 1997a), cations serve as a bridge between negatively charged EPS of neighbouring microbial cells. The bridging stabilizes the floc network and thus improves sludge bioflocculation, settling and dewaterability. And

Calcium may create a matrix for granulation of sludge (Liu and Fang, 2003; Van der Hoek, 1987).

Calcium ion were suggested either to stimulate granulation by neutralizing negative charges on bacterial surfaces as a result of relatively strong Van der Waals attractive forces, or to function as cationic bridges between bacteria since most of microorganisms are negatively charged at usual pH. Consequently, the calcium-induced cell fusion might initiate the formation of cell cluster, which acted as microbial nuclei of further granulation (Liu et al., 2003).

Wang et al., 2004 found that most of the metal elements in the sludge changed significantly during the start-up operation because of the differential chemical composition of the influents. Calcium and potassium amount are increased in matured aerobic granules. Therefore, Calcium may play an important role in the cultivation of aerobic granular sludge, the same as that for anaerobic granules. The change of granule color from brown to white was probably due to the change of the biomass composition, especially for decrease in the content of iron, magnesium, copper and cobalt in the sludge.

**Table 2.7. The metal elements in the sludge (mg/g) (Wang et al., 2004)**

| Type | **K** | Na | **Ca** | Mg | Fe | Cu | Mn | Co | Zn |
|---|---|---|---|---|---|---|---|---|---|
| Seed sludge | 9.60 | 9.16 | 30.20 | 5.93 | 26.40 | 0.23 | 0.37 | 0.024 | 1.12 |
| Matured sludge | 43.58 | 8.00 | 45.70 | 2.58 | 0.76 | 0.01 | 0.01 | 0.012 | 0.17 |

### 2.3.7. Suspended solids and support media

Suspended particles in feed wastewater could be a factor for enhancing aerobic biogranulation because of its available surface area for cell attachment. Firstly, with it presence, it also increase shear stress in reactor which is the main factor for granulation (Tay et al., 2001). Secondly, exopolysaccharides has a trend to be produced on the surface of any support media and exopolysaccharides is the bridging factor of cell (Wingender et al., 1999; Liu and Tay, 2002). For example, Arrojo et al. (2004) and Schwarzenbeck et al., 2004 could form aerobic granules with the concentration of suspended particles of 1.2 g/L and 0.95g/L. So support media – inorganic or organic suspended solids also play an important role in enhancing aerobic granules due to above reasons.

## 2.4 Aerobic granular sludge formation

### 2.4.1. Aerobic granule formation from anaerobic sludge process

Aerobic granular sludge can be formed by conventional aerobic activated sludge (Beun et al., 2000; Jang et al., 2003; Tay et al., 2002; Etterer and Wilderer, 2001); Morgeroth et al., 1997; Wang et al., 2004; Arrojo et al., 2004; Schwarzenbeck et al., 2004; etc) or anaerobic sludge (Linlin et al.., 2005). It means that the seeding sludge is not the limitation of cultivating aerobic granules. However, the cultivation of aerobic granules from anaerobic and aerobic sludge is rather different from their mechanisms.

***The granule formation process suggested by Linlin et al. al., (2004) as follows:***

Firstly, the anaerobic granular sludge disintegrated under aerobic conditions after inoculation, forming irregular and small flocs, and highly filamentous granules. These granules were not stable at all and broke up into pieces after a few days. Subsequently,

large part of the biomass was washed out remaining debris from the disintegrated granules recombined under aerobic conditions; and finally the granules grew up, resulting in the formation of aerobic granular sludge. The granules formed in this stage hardly contained any filament and consist of dominantly bacteria. The disintegrated anaerobic sludge may play a role of nucleus for the granulation of aerobic sludge (Linlin et al.., 2005). The formation process can be described by the figure 2.8.

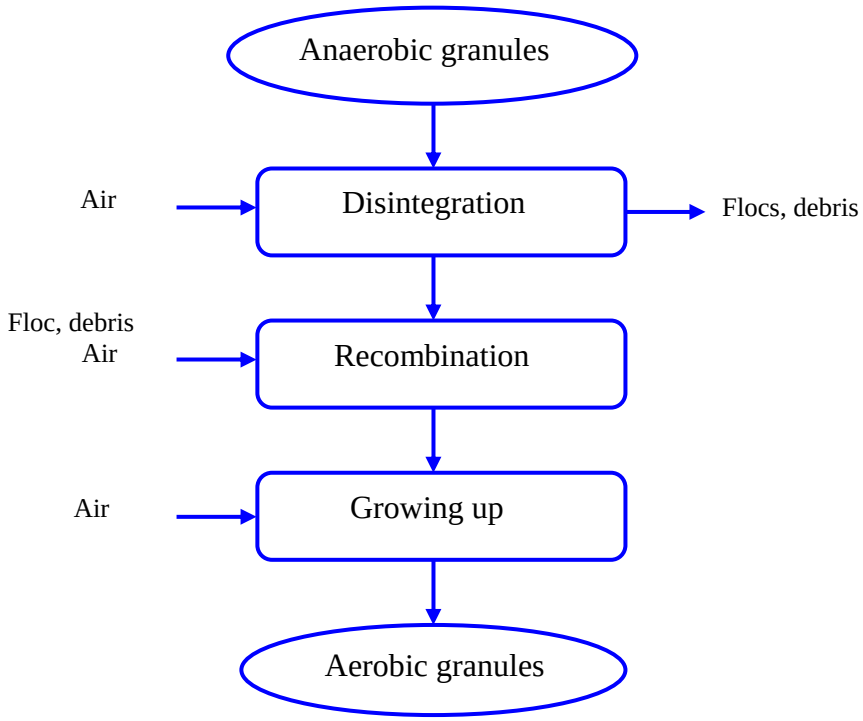



**Figure 2.8. Formation process of aerbic granule from anaerobic granular sludge**

The morphological change of granules in the reactor shown in the figure 2.9

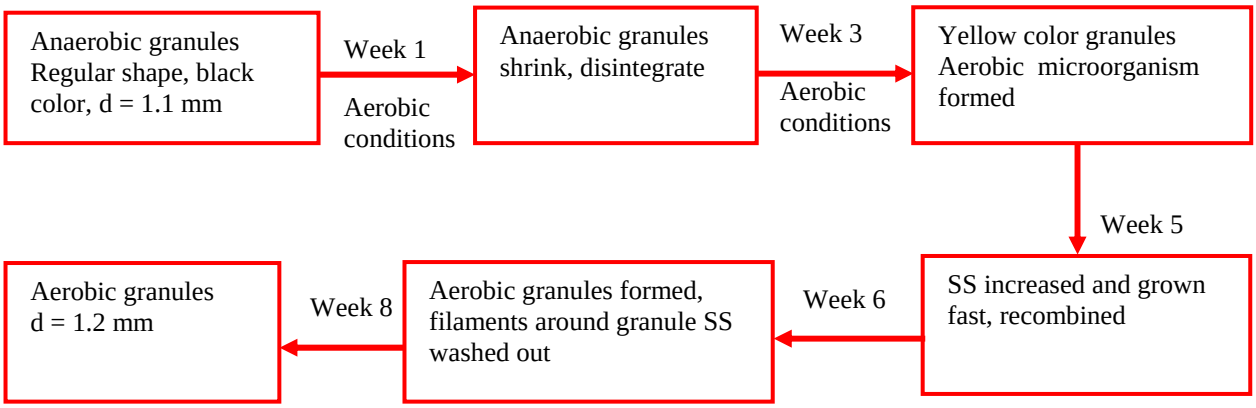



**Figure 2.9. The morphorlogical change of granules (modified from Linlin et al., 2005)**

The figure 2.10 shows the process of morphological change of granules in the reactor.

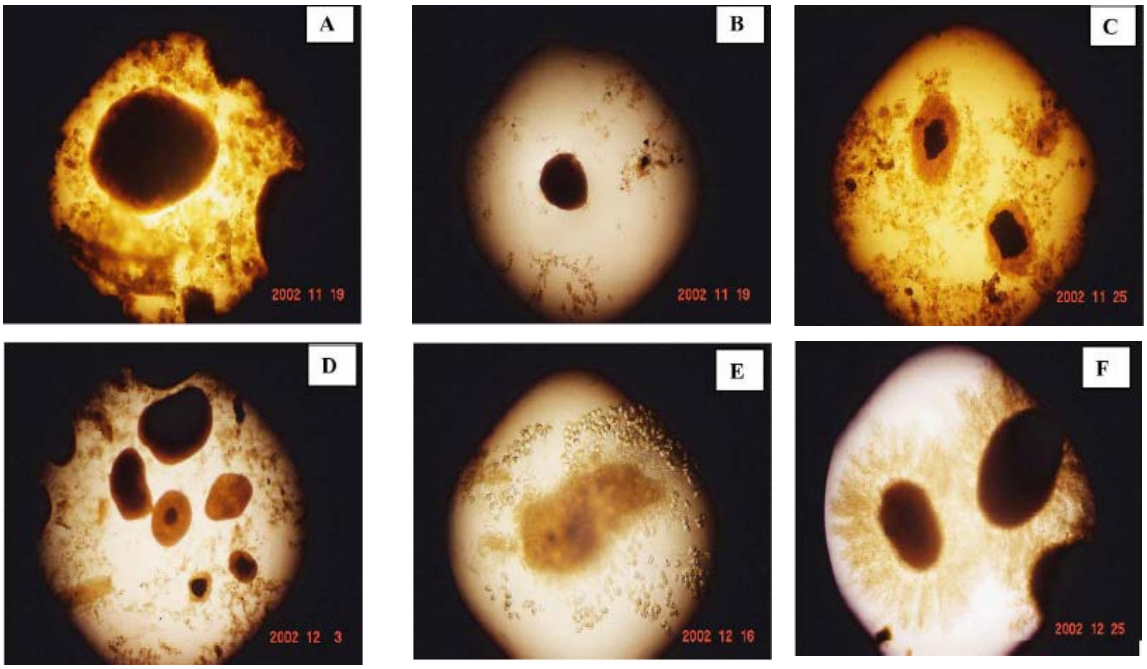



**Figure 2.10. The morphological variation of granular sludge during the experimental period (40x). (A) Seed anaerobic granule; (B) after 1 week; (C) after 2 weeks; (D) after 3 weeks; (E) after 5 weeks and (F) after 5 weeks (Linlin et al., 2005)**

### 2.4.2. Aerobic granule formation from conventional aerobic activated sludge process

Wang et al. (2004) found that the granulation process of sludge can also be categorized into three phases: acclimation, granulation and maturation. The granules were first initiated as mycelial pellets in the reactor and began to accelerate growth, the 'granules initiated'. The corresponding period from the start-up operation to the 'granules initiated' was the sludge acclimation phase. Similarly, the initial granules could grow out fully and the biomass concentration was not changed, the matured point. The granulation phase was that corresponding from the 'granules initiated' to the granules matured point. Based on the above categorization, the granulation process was initiated and then matured in the reactor.

The sludge inoculated in the SBR was a mixture of filamentous sludge with brown colour and was loose and difficult in settling. Since the settling time was kept short, a washout of biomass took place. The sludge concentration in the reactor decreased from this wash out and more sludge was observed in the effluent because of bad settling ability. During this time, most of the sludge in the reactor changed to flocs. Over the next 8 weeks, the floc-like sludge gradually changed to granular sludge with time. After 67 days operation, granular sludge began to appear whereas flocs still remained dominant in the reactor. The initial granular sludge formed in the SBR was smaller in size, had fluffy edges.

The small granules grew rapidly in the following weeks, while more floc-like sludge was washed out, resulting in the accumulation of the granules. Eleven weeks after inoculation, the sludge in the reactor was nearly completely granulized, and visually no suspended biomass was present. Due to the intensive mixing by aeration, the granular sludge became spherical with a smooth surface. The diameter of the granular sludge increased to 6.0–9.0 mm. Most of the biomass was kept in the reactor due to the good settleability.

After the granules matured point, the granules were stable and dynamically balanced in the maturation phase. In this phase, the granular size might still be shifting mainly between 6.0

and 9.0 mm, but slowly and slightly, depending on the change of operational conditions. The matured granule colour was white and somewhat transparent (Figure 2.11a)

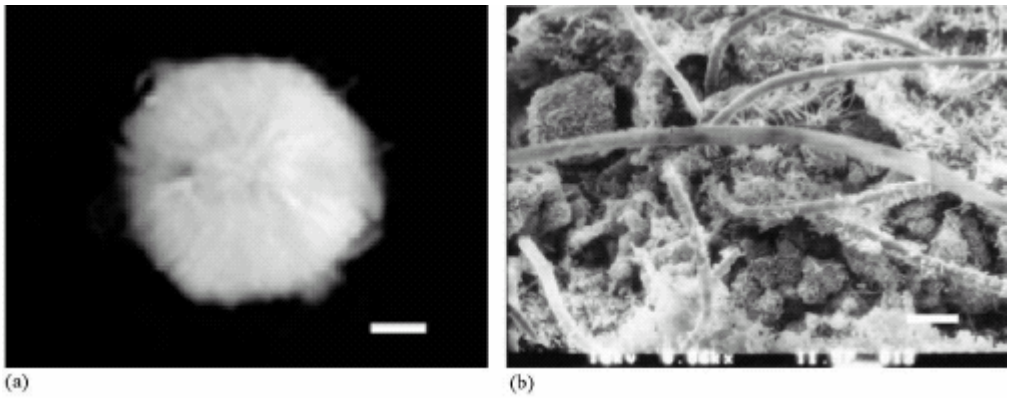



**Fig. 2.11. Microscopy images of mature granules after 120 days. (a) Microscope overview image, bar = 2 mm, (b) SEM of the granules surface, bar =11 µm.**

From the above research result, the granule formation process can be described as the figure 2.12.

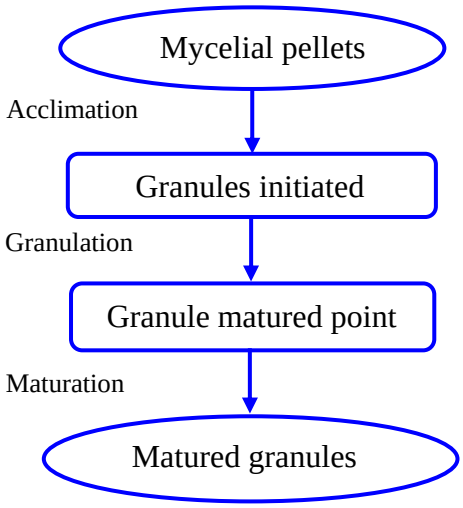



**Figure 2.12. Formation process of aerobic granule (Modified from Wang et al., 2004)**

Jang et al., (2003) found that aerobic sludge granules can be cultured in SBR. The initial seed sludge has size of 0.08-0.18 mm and SVI of 210-230 ml/g. After 50 days, granules formed with the size of 0.95-1.35 mm and SVI of 70-90 ml/g. The floc-like sludge changed gradually to granules over time. Granulation of the seed sludge can be achieved through accumulation by interparticle bridging under a condition of turbulent flow mixing. After 40 days of operation, the seed sludge in reactor was nearly totally granularized. First, the seed sludge was not in the form of large flocs, rather irregular and unstable filaments ware dominant. The particles eventually started to join together to form biomass aggregates and the aerobic floc-like sludge form was accomplished within 10 days. Second, the aerobic floc-like sludge was heterogeneous mixed, with irregular and soft granules that started to appear around 30 days. After 40 days, the aerobic floc-like sludge formed. At this time most of granules had an uneven surface and soft texture. Finally, the irregular granules became stable and were smoother and round-shaped with a solid surface after 50 days. This process is shown in the figure 2.13.

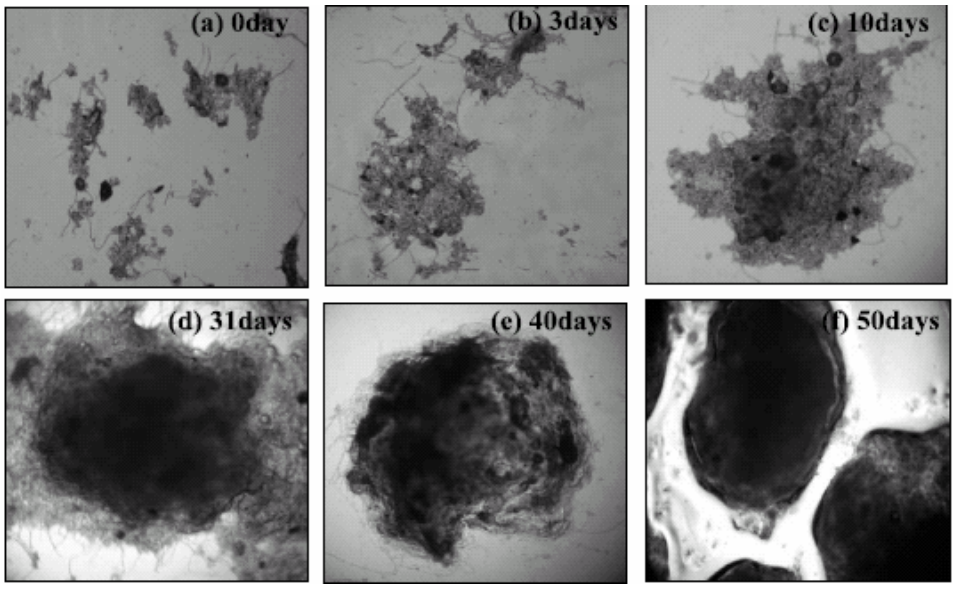



**Fig. 2.13. Time dependent development of granules, extending from the seed sludge to granules, of: (a) 0 day, seed sludge; (b) 3 days; (c) 10 days; (d) 31 days, flocs-like; (e) 40 days and (f) 50 days, granular sludge (Jang et al., 2003)**

From Jang's research, the granulation process can be described like the following scheme as figure 2.14.

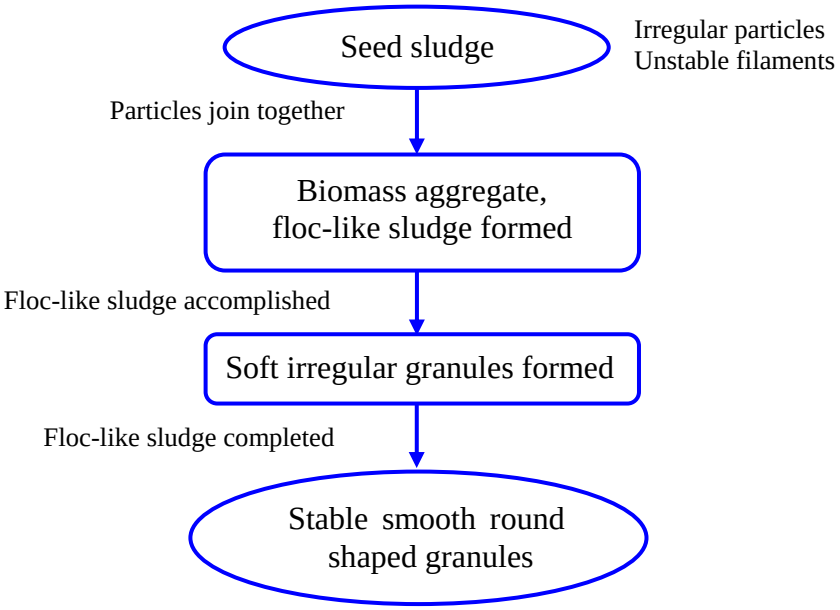



**Figure 2.14. Formation process of aerobic granule (Based on Jang et al., 2003)**

Etterer and Wilderer, (2001) found that when keeping the short settling time, biomass in the SBR was washed out during the start-up period. First, the filamentous granules appeared after 10 to 15 days whereas flocs still remained dominant. In the following weeks granules accumulated and three or four weeks after inoculation, biomass in reactor consisted of mainly aerobic granules. Due to the intensive mixing with suitable hydrodynamic shear force by aeration, granules became spherical with smooth surface. Besides, it indicated that fungi and filamentous organisms in general were present in the overall structure of the aggregates when observing granules by light microscope but when

using fluorescent in situ hybridisation (FISH), only filaments were possibly detected. The formation process of granules by these authors is shown in figure 2.15.

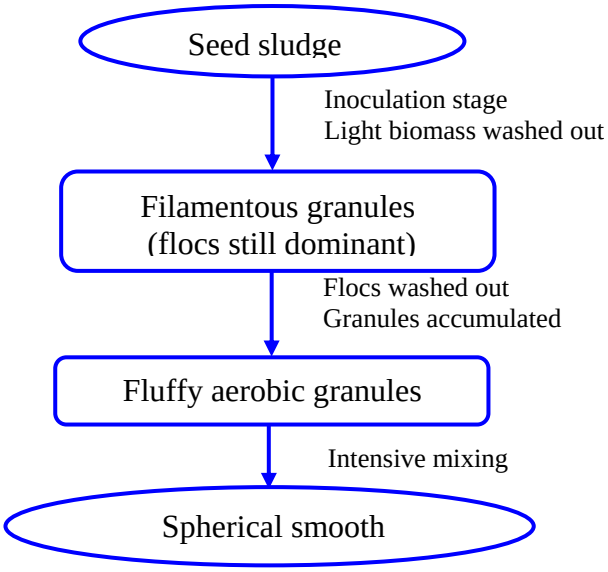



**Figure 2.15. Formation process of aerobic granule (Etterer and Wilder, 2001)**

Beun et al. (1998) suggested the mechanism of aerobic granulation by the following scheme

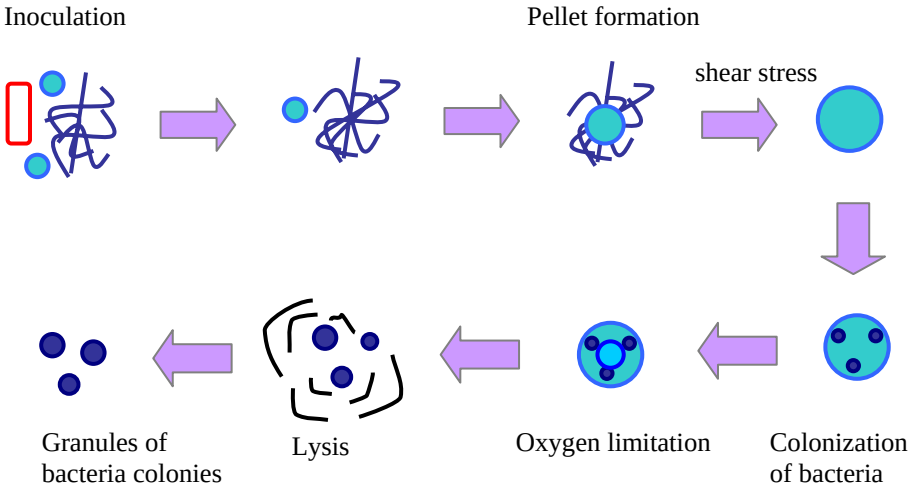



**Figure 2.16. Mechanism of granulation of aerobic granule (Beun et al., 1998)**

After inoculation with bacterial sludge fungi become dominating. Fungi easily form mycelial pellets, which settle very well and can be retained in the reactor. Bacteria do not possess that property and will be washed out almost completely. Therefore, during the start-up, the biomass in reactor will consist of mainly filamentous mycelial pellets. Due to the shear in the reactor, detachment of the filaments on the surface of pellets takes place and the pellets become more compact. The pellets grow out to diameter of 5-6 mm and then they lyse probably due to oxygen limitation in the inner part of the pellets. The mycelial pellets seem to function as an immobilization matrix in which bacteria can grow out to colonies. When the mycelial pellets fall apart due to lysis of inner part of the pellets,

the bacterial colonies can maintain themselves because now they are large enough to settle. These colonies grow out to granules.

## 2.5 Factors inhibiting aerobic granulation formation

### 2.5.1. Free amonia

Similar to other biological process, the aerobic granulation is affected by organic loading rate, hydrodynamic shear force, toxic substances, etc. When looking into the ability of developing aerobic granules for the simultaneous organics removal and nitrification, the role of free ammonia must be taken in account.

Free ammonia is inhibitor for most of microbial community at a high concentration. Free ammonia form concentration depends on pH and temperature of wastewater. The free ammonia concentration is calculated by the following formulae:

$$FA(mg/L) = \frac{[NH_4 - N]x10^{pH}}{\exp[6334/(273+T)]+10^{pH}}$$

Yang et al., (2004) found that when cultivating aerobic granules and determining effects of ammonia into granule formation with optimum conditions for nitrification (pH = 7.8-8, DO ≥ 2 mg/L) and N/C ratio is shown in the table 2.8, aerobic granules could not be formed at the concentration of free ammonia is higher than 23.5 mg/L.

**Table 2.8: Effects of free ammonia to aerobic granular sludge (Yang et al., 2004)**

| Reactor | R1 | R2 | R3 | R4 | R5 |
|---|---|---|---|---|---|
| N/C ratio | 5/100 | 10/100 | 15/100 | 20/100 | 30/100 |
| Free ammonia (mg N/L) | 2.5 | 9.2 | 18.0 | 23.5 | 39.6 |
| Granular size (mm) after 4 weeks | 0.51 | 0.32 | 0.25 | - | - |
| Morphology | Smooth, regular shaped dense | Smooth, regular shaped dense | Less smooth than R1, R2 | - | - |

High free ammonia causes influences to nitrification, cell hydrophobicity, production of extracellular polysaccharides, nitrifying activity. Especially, it reduces cell hydrophobicity and extracellular polysaccharides so the granulation process is restricted.

***Effect on cell hydrophobicity:***

Cell hydrophobicity plays an important role in the formation of biofilm and granules (Tay et al., 2001). The cell hydrophobicity decreased from 70.6% to 40.6% with the increase of the free ammonia concentration from 2.5 mg/L to 39.6 mg/L (Yang et al., 2004) (figure 2.10). Consequently, low cell hydrophobicity resulted from the free ammonia inhibition would lead to the failure of aerobic granulation.

***Effect on production of polysaccharides:***

Polysaccharides also play an important role in the cell immobilization (Wingender et al., 1999; Liu and Tay, 2002). Extracellular polysaccharides can contribute to the formation

and architect of biofilm, anaerobic and aerobic granules and their stability (Tay et al., 2001). Increase of free ammonia concentration led to decrease of synthesis the cell polysaccharides. The ratio of polysaccharides to protein (PS/PN) in R4 and R5 is 0.62 and 0.58, which is compared with that of seed sludge (PS/PN = 0.55), no granule is observed in the reactors. The PS/PN ratio decreases from 2.8 to 0.55 when free ammonia concentration increases from 2.5 to 39.6 mg/L (Yang et al., 2004) (figure 2.17).

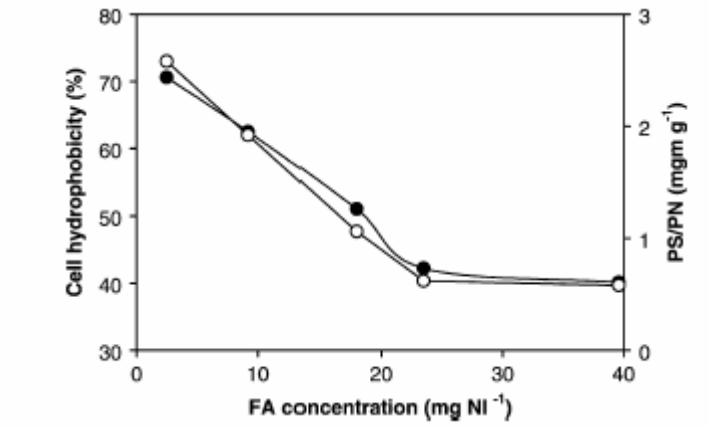



**Figure 2.17 Effect of free ammonia on cell hydrophobicity and PS/PN ratio after four-week operation (●) hydrophobicity; (♦) PS/PN ratio (Yang et al., 2004).**

***Effect on Nitrification:***

Free ammonia inhibition threshold is 10-150 mg/L for Nitrosomonas and 0.1-4 mg/L for nitrobacter (Liu and Tay, 2002).

***Effect on activity of heterotrophic and nitrifying bacteria:***

High free ammonia concentration exhibits the activities of nitrifying bacteria and also represses the energy metabolism of heterotrophs. Metabolic activities of heterotrophic bacteria are quantified by the specific oxygen utilization rate (SOUR). When free ammonia increases, SOUR decreases (Yang et al., 2004).

Deflocculation was observed when the aerobic microbial activities were exhibited (Wilén and Nielsen, 2000) while inhibition of energy-generating function would prevent the development of competence for cell aggregation (O'Toole, 2000). Consequently, the reduced microbial activity that results from the free ammonia inhibition is partially responsible for no aerobic granulation (Yang et al., 2004).

### 2.5.2. Settling time

The initial selected settling time must be considered carefully when cultivating aerobic granules. This will decide granules formed or not. If the settling time is too high, it was observed that only flocculated biomass is formed. In contrary, it did not lead to accumulate of sufficient granules in the reactor (Linlin et al., 2005). So the settling time must be chosen that the settling velocity of particle must be higher than 10 m/h for granules accumulated in the reactor (Beun et al., 2002).

## 2.6 Application of MBR process in wastewater treatment

### 2.6.1. MBR

Membranes are natural or artificial, two dimensional objects that separate fluids with different compositions from each other (Visvanathan et al., 2000). The special quality of membranes allows the transport of only specific matters or materials groups. So membranes can be combined with water and wastewater treatment facilities.

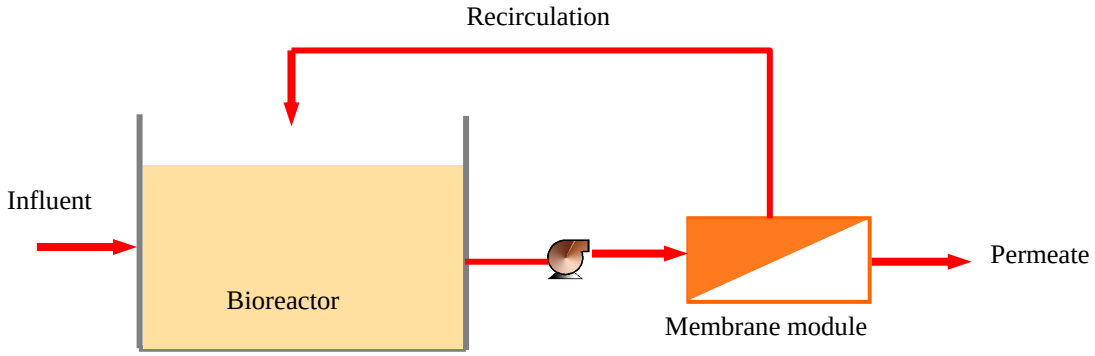
The many different membrane processes resulted from the different demands on the separation process. Subdivision of the different processes occurs according to:

- The driving force behind the filtration process;
- The type of the inserted membrane;
- The kind of the matters to be separated.

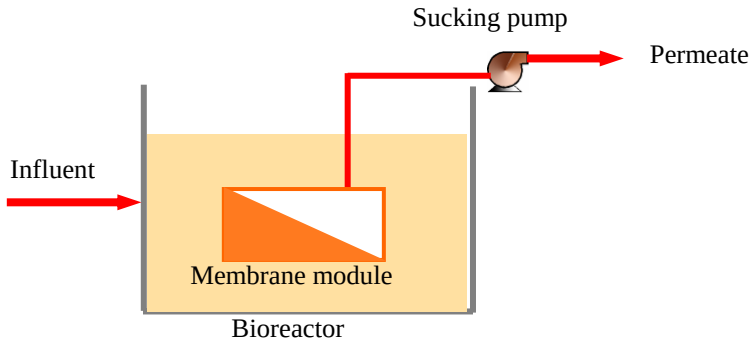
Combining membrane technology with biological reactors for the treatment of wastewater has led to the development of three generic MBRs:

- Separation and retention of solids;
- Bubbleless aeration within bioreactor;
- Extraction of priority organic pollutants from industrial wastewater.

The first one, separation and retention of biosolids, is the most widely studied and has found full-scale applications in many countries (Visvanathan et al., 2000). Solid/liquid separation bioreactors employ micro- or ultrafiltration modules for the retention of biomass for this purpose. The membranes can be placed in the external circuit of bioreactor or they can be submerged directly into bioreactor (figure 2.18)




a. Membrane in external circuit



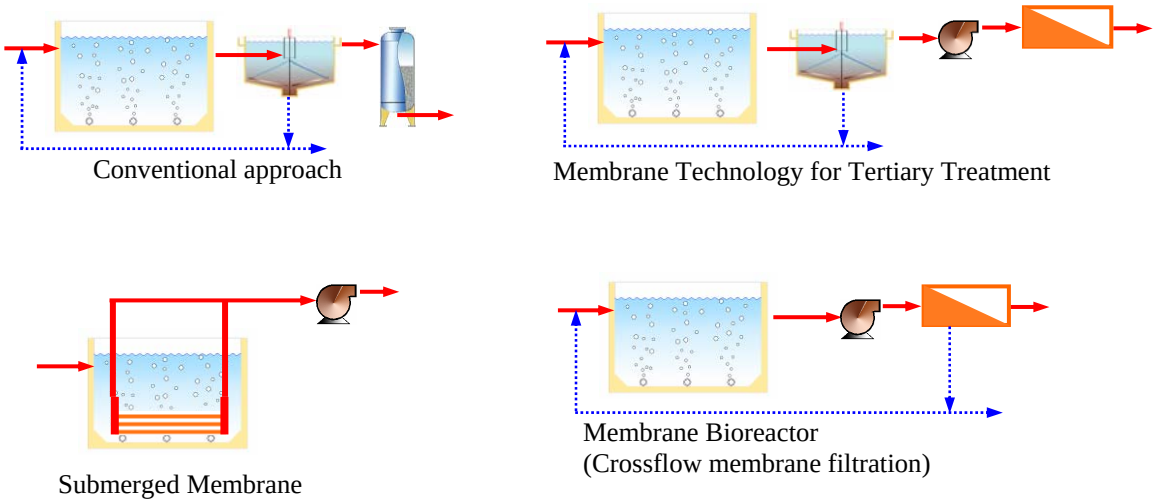

b. Membrane in external circuit

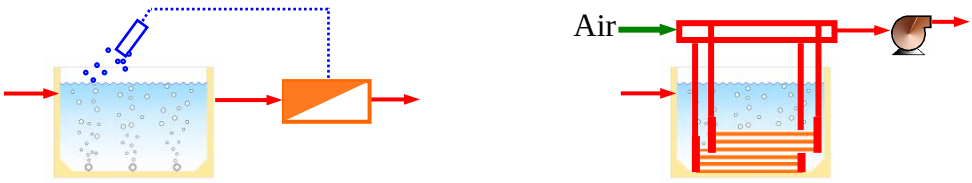
**Figure 2.18 Solid/liquid separation MBR (Visvanathan et al., 2000)**

A submerged membrane should be outer-skinned. In general, permeate is extracted by suction or, less commonly, by pressurizing the bioreactor. In the external circuit, the membrane can be either outer- or inner skinned, and permeate is extracted by circulating the mixed liquor at high pressure along the membrane surface.

Gas permeable porous membranes can be used to aerate the mixed liquor in the aeration tank by bubbleless oxygen mass transfer. In certain case, the membrane can act as support for biofilm development, with direct oxygen transfer through the membrane wall in one direction and nutrient diffusion from the bulk liquid phases into the biofilm in the other direction. Because the membrane can form bubble-free or fine bubble mass transfer, the efficiency is very high (Visvanathan et al., 2000).

Membranes have been finding wide application in water and wastewater treatment ever since the early 1960s when Loeb and Sourirajan invented an asymmetric acetate membrane for reverse osmosis. Many combinations of membrane solid/liquid separators in biological treatment processes have been studied since. The trends that led to the development of today's MBR are depicted in figure 2.19.






Plunging Liquid Jet Aeration

Membrane as solid/liquid separator & Air Diffuser

**Figure 2.19 Trends in MBR development (Visvanathan et al., 2000)**

### 2.6.2. Advantages of MBR process

MBR process has been proved to have many advantages in comparison with conventional biological processes. The main advantages are high quality of treated water, small size of treatment plant, less sludge production and flexibility of operation (Visvanathan et al., 2000).

- High rate decomposition: Treatment efficiency is also improved by preventing leakage of undecomposed polymer substances. If these polymers are biodegradable, they can be broken down with a reduction in the accumulation of substances within the treatment process. Other dissolved organic substances with low molecular weights, which can be eliminated by membrane separation alone, can be broken down and gasified by microorganisms or produced new bacteria cells. Most MBR studies indicate the effluent $BOD_5$ is below 5 mg/L (Buisson et al., 1997; Parameshwaran and Visvanathan, 1998) and based on reactor volume, MBR process is 15 to 20 fold higher in substrate conversion rate in comparison with conventional activated sludge process (Buisson et al., 1997; Stephenson et al., 2000).
- Treated water quality: In conventional technology, treated quality strongly depends on settling ability in sedimentation tank. In MBR process, solid/liquid separation is conducted by membrane filtration. Therefore, the final effluent does not contain suspended matter, and almost bacteria. This enables the direct discharge of the final effluent into the surface water and the reuse of the effluent for cooling, toilet flushing, lawn watering or process water.
- Flexibility in operation: Solid retention time (SRT) can be controlled completely independent from hydraulic retention time (HRT). So the system can be run at very long SRT providing favourable conditions for the growth of slow-growing microorganisms, which are able to degrade biorefractory compounds.
- Compact plant size: Because the MBR process is independent upon sludge settling quality, high biomass concentration can be maintained up to 40 g/L in the reactor. Therefore, the system can stand for high volumetric loading rate resulting in the reduced size of the bioreactor. In addition, secondary settling tank, filter, sludge thickener or post treatment for further BOD, SS removal are not necessary in MBR process, thus the plant becomes more compact (Visvanathan et al., 2000).
- Low sludge production rate: In real MBR sludge production rate is very low. Excess sludge from MBR process is much lower than conventional activated sludge process about one fifth fold. Low F/M ratio and longer sludge age (from

50 to 100 days) in the reactor may be the reason for low sludge production rate. In addition, the microscopic observation on microorganism population indicates that with increased sludge age, reduction in filamentous bacteria increased rotifiers and nematodes (Visvanathan et al., 2000).

- Disinfection and odour control: In membrane filtration, the removal of bacterial and viruses can be achieved without any chemical addition (Visvanathan et al., 2000). All the process equipment can also tightly close, no odor dispersion occurs.

### 2.6.3. Membrane fouling

Membrane fouling is one of the obstacles of this application because of flux reduction, cause of short membrane life and impairment of fractionation capability of membrane. As the resistance increases the flux will decline. This increase in resistance may be due to changes $R_m$, $R_c$ or $R_f$ or all of three (equation 2.2). If the flux decline is not reversible by simply alternating operating conditions, it is termed fouling (Fane et al., 1989). Membrane fouling can result from the precipitation of less soluble inorganic species (scaling), adsorption of organic substances (organic fouling), and adhesion and growth of microbial cells at the membrane surface (biofouling).

Membrane fouling can result from the formation of a polarization cake layer and the plugging of membrane pores (figure 2.20).

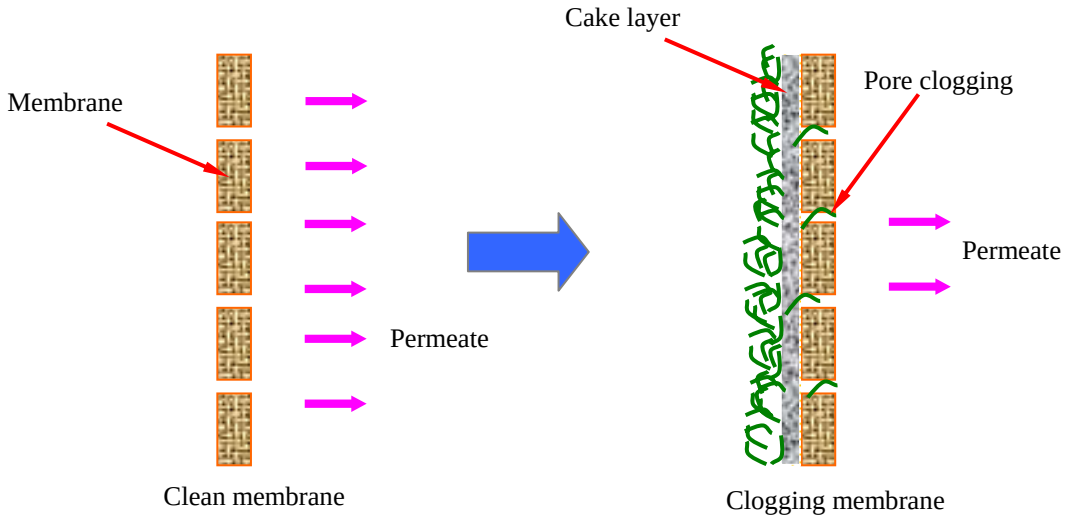



**Figure 2.20 Membrane fouling**

Effect of membrane fouling on the decline of permeate flux can be explained by using the resistance-in-series model. According to this model, the relationship between permeate flux and transmembrane pressure (TMP) is described by equation 2.1.

$$J = \frac{\Delta P}{\mu * R_t} \qquad \text{(Eq. 2.1)}$$

J: Permeate flux ($m^3/m^2.s$)

$\Delta P$: Transmembrane pressure (Pa)

$\mu$: Viscosity of the permeate (Pa.s)

$R_t$: Total resistance for filtration (1/m)

$R_t = R_m + R_c + R_f$ (Eq. 2.2)

$R_m$: Intrinsic membrane resistance

$R_c$: Cake layer resistance

$R_f$: Fouling resistance due to irreversible and pore plugging

Intensive researchers have been conducted to understand the mechanisms and causes of membrane fouling. Current trends of controlling membrane fouling are focus on (1) controlling the production of extracellular polymeric substances (EPSs) in bioreactor and (2) reducing the cake layer resistance.

EPSs are the substances excreted by microorganism. These compounds comprises of polysaccharides, protein, nucleic acid and lipid. EPS in microbial flocs have been reported as a major foulant in the membrane bioreactor system (Chang et al., 1996; Nagaoka et al., 1996) as they occupy the pores of the membrane. Among different approaches to control of EPS production have been investigated is control of nutrient composition in the reactor and development attached growth for MBR system (Kim et al., 1998).

Characteristics of the cake layer play an important role in membrane fouling. Effects of cake layer characteristics can be described by the Carman-Kozeny equation as follows (Liew et al., 1995):

$$P_h = \frac{d_p^2 * \varepsilon^3}{180(1-\varepsilon)}$$

$P_h$ : Hydraulic permeability through the cake layer

$d_p$: Particle diameter

$\varepsilon$: Porosity of the cake layer

Based on this model, one can derive that the greater the particle size and porosity, the higher permeability. Many attempts have been conducted to improve the permeability of the cake layer by the addition of filter aids such as metal-based coagulants into reactor (Visvanathan et al., 2000). These filter aids are expected to form a dynamic cake layer on the membrane surface. The permeability of the dynamic cake layer is thought to be higher due to larger particle size and porosity. Schematic diagram of the dynamic cake layer is illustrated in figures 2.21. The porous layer also plays as a filter layer to retain soluble organic compounds preventing them to contact and plug in the membrane pores. Dan (2002) reported a different approach as developing a yeast culture for MBR system. Due to larger size of yeast cells in comparison with bacteria cells, the yeast cells play as a porous layer and therefore permeate flux can be enhanced.

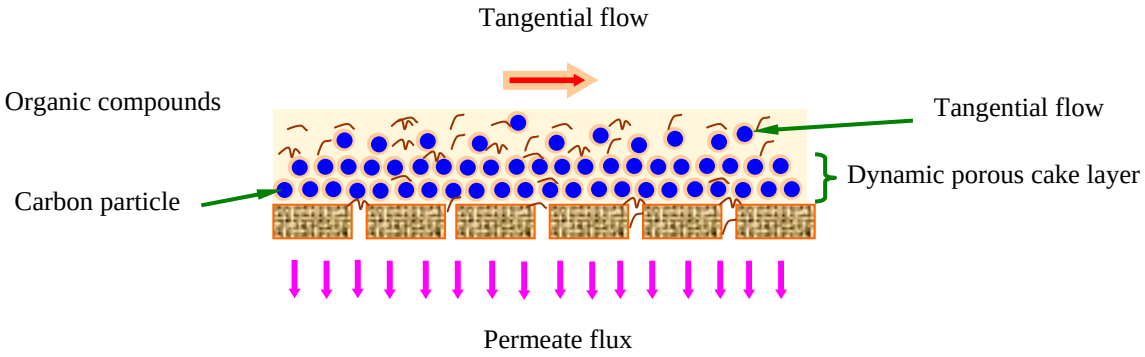



**Figure 2.21 Schematic diagram of dynamic porous PAC layer in crossflow membrane filtration from Pirbazari et al., 1996 (Tri, 2002)**

# Chapter 3

# Methodology

## 3.1 Materials and Microorganisms

### 3.1.1. Feed Wastewater

The experimental runs were conducted using synthetic wastewater. In the previous research (Beun et al., 2000; Jang et al., 2003; Wang et al., 2004), glucose and acetate were usually selected as carbon source for aerobic granule cultivation.

In this study, glucose was the carbon source for granule cultivation and influent COD was 600 mg/L and COD:N:P = 100:6.5:1.6. Components of feed wastewater were in table 3.1.

**Table 3.1 Components of feed wastewater**

| Medium | Component | Concentration (mg/L) |
|---|---|---|
| Medium A | Glucose | 664.3 |
| Medium B | $NaHCO_3$ | 450.0 |
| Medium C | $NH_4Cl$ | 150.0 |
| Medium D | $KH_2PO_4$ | 43.0 |
| Medium E | $CaCl_2.2H_2O$ | 30.0 |
| | $MgSO_4.7H_2O$ | 12.0 |
| | $FeCl_3$ | 3.6 |
| Medium F – Trace solution 1ml/L (Wang et al., 2004) | $H_3BO_3$ 0.15 g/L; $CoCl_2.6H_2O$ 0.15 g/L; $CuSO_2.5H_2O$ 0.03 g/L; $FeCl_3.6H_2O$ 1.5 g/L; $MnCl_2.2H_2O$ 0.12 g/L; $Na_2Mo_4O_{24}.2H_2O$ 0.06 g/L; $ZnSO_4.7H_2O$ 0.12 g/L; KI 0.03 g/L; | |

When increasing loading rate, most of medium were proportional to organic matter except micronutrients including medium E and medium F. Initially, these medium were used as concentration as table 3.1.

### 3.1.2. Carrier/Support Media

This study used bivalve shell carrier and anaerobic granules as the support media to cultivate aerobic granules. The first one, calciferrous shell carrier was made of bivalve shell of white rose cockle. This material was rich in Calcium that could be support media for microbial adhesion and granulation. The support media was produced in AIT laboratory. First, the selected bivalve shells were dried to remove all organic constituents. Second, it was grinded into power form with the certain size. The size of carrier was selected in the range of 150-212 μm with the sieves No.70 and No.100. At last, carrier was washed by tap water for dirt, colloid particle removal and then dried at 105$^oC$ during 24 hours before use. The physical characteristic of this carrier is presented in table 3.2. The second one, anaerobic granules had diameter in range of 1 – 2.5 mm (figure 3.1d).

**Table 3.2 Physical characteristics of support media**

| Characteristics | Value |
|---|---|
| **Bivalve shell carrier (media I):** | |
| Density | 1.45 g/cm$^3$ |
| Settling velocity | 55-300 m/h |
| Colour | White |
| Size | 0.150-0.212 mm |
| Components | Ca, Fe, Mg |
| Loss weight (550$^o$C, 20 min) | 2% |
| **Anaerobic granule (media II): from UASB** | |
| Size | 1-2.5 mm |

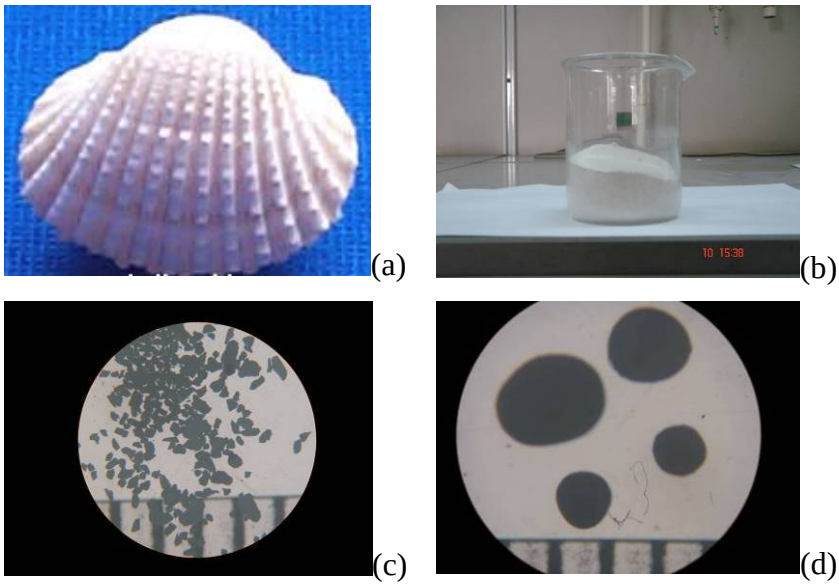



**Figure 3.1. Types of support media (white rose cockle (a); grinded carrier (b); bivalve shell carrier (c); anaerobic granules (d)**

### 3.1.3. Microbial Seed

Seed sludge was taken from conventional activated sludge process of Thammasat University's wastewater treatment plant at Pathumthani province, Thailand. The characteristics of the seed sludge were determined prior to start-up of lab scale experiments. The initial seed sludge of the first batch runs was 6 gSS/L.

## 3.2 Overall experiment course

The experiment work of this research was divided into 2 main parts: (1) cultivation, characterization of granular sludge in SBAR and (2) determination of membrane fouling behaviour of granule SBAR supernatant. In the first part, main objective was to characterize types of aerobic granules with physical chemical and biological characteristics at different OLRs. In the second part, optimum granular sludge with selected support media was used for coupling with MBR and membrane fouling behaviour was investigated in term of membrane fouling index, soluble EPS (protein and polysaccharides) of supernatant and transmembrane pressure (TMP). Moreover, a conventional SBR was run with the same

feed to take supernatant for comparison with that of granule SBAR. Two treatment sequences were used for membrane connection as figure 3.7 and 3.8.

The process could be described as the following figure 3.1:

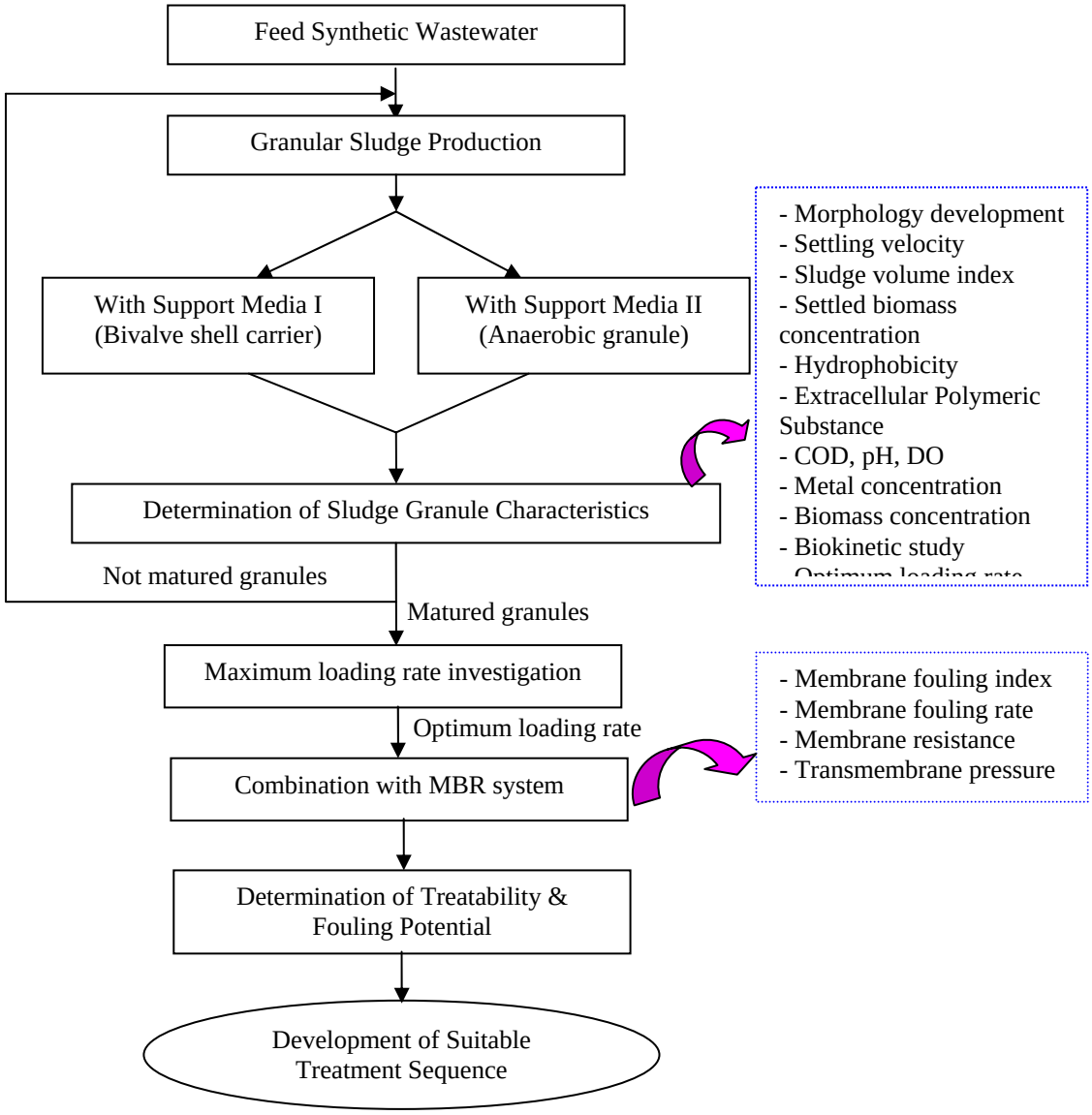



**Figure 3.2. Experimental process**

## 3.3 Cultivation of Granular Sludge

### 3.3.1. Reactor set-up and operation

The type of batch system chosen was Sequencing Batch Airlift Reactor (SBAR) because of throughout mixing in the reactor and discontinuous system was more advantageous than continuous one in granule formation (Beun et al., 1999).

The SBAR had working volume of 2.5 L (figure 3.3). The internal diameter of the down-comer was 6.2 cm. The riser was 90 cm in height had an internal diameter 4 cm, and was positioned at a distance of 1.5 cm from the bottom of the down-comer.

The HRT was 5.5 hour and initial OLR was 2.5 kg COD/($m^3$.day). Air was introduced by a fine bubble aerator at the bottom of the reactor at a superficial air velocity of 95 m/h (air flow of 4.5 L/min). The air-flow rate was controlled by an air-flow controller and solenoid valve. Feed wastewater was maintained at neutral pH = 7.2±0.2. Reactor was well-mixed and highly turbulent by strong flow convection of airlift configuration.

The reactor was operated in successive cycles of 3 hours each. Initially, one cycle consisted of 5 minutes influent addition, 170 minute aeration, 3 minute settling, and 2 minute effluent withdrawal. Effluent was withdrawn by effluent valve, 50 cm from the bottom of reactor. When carrying out effluent withdrawal during manual operation time at two first weeks, the highest to the lowest valve was opened for gradual discharge. The effluents were stored in the holding tanks. The settling time was initially chosen such that only particles with a settling velocity higher than 10 m/h was effectively retain in the reactor. When matured granules formed, settling time was reduced progressively down to 3 minute to retain only high settling granules. When system reached to steady state of OLR of 5 kg COD/$m^3$.day, settling time was reduced to 2 minutes to remain only fast settling granules in reactors.

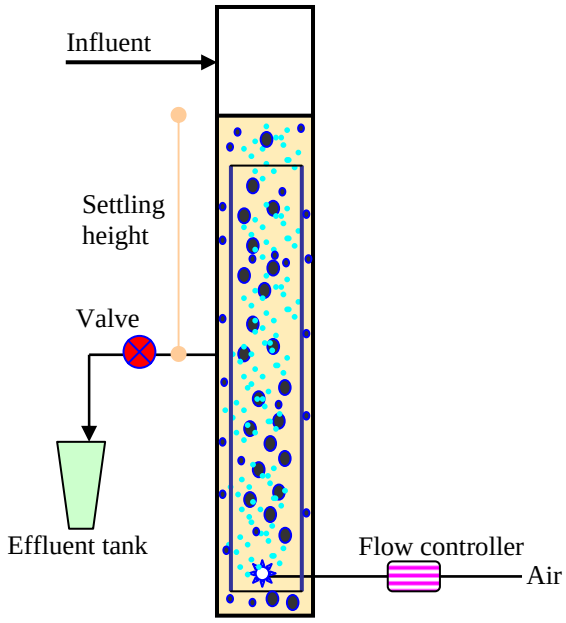



**Figure 3.3 Shematic representation of the SBAR**

### 3.3.2. Operational conditions

The research was carried out at AIT's ambient lab. The operation temperature was ambient temperature from 27-32$^{o}$C. On day 1 the reactor was inoculated with one litter of activated sludge (total 6 gSS) taken from the conventional activated sludge process. The certain

settling time was applied as table 3.3 during the first two weeks after acclimatization to prevent washout of all the biomass. After two weeks, the settling period was decreased to 3 minutes until matured granules formed in both reactors. On week 15, settling time was reduced to 2 minutes to maintain only high settling granules in reactors. When reactor walls became completely covered with attached biomass and then it was manually removed. To avoid competition between attached growth and granulation, the walls of the reactor were cleaned at day 6 and each month during the remaining of the operation period if any (modified from Beun et al. (2002)).

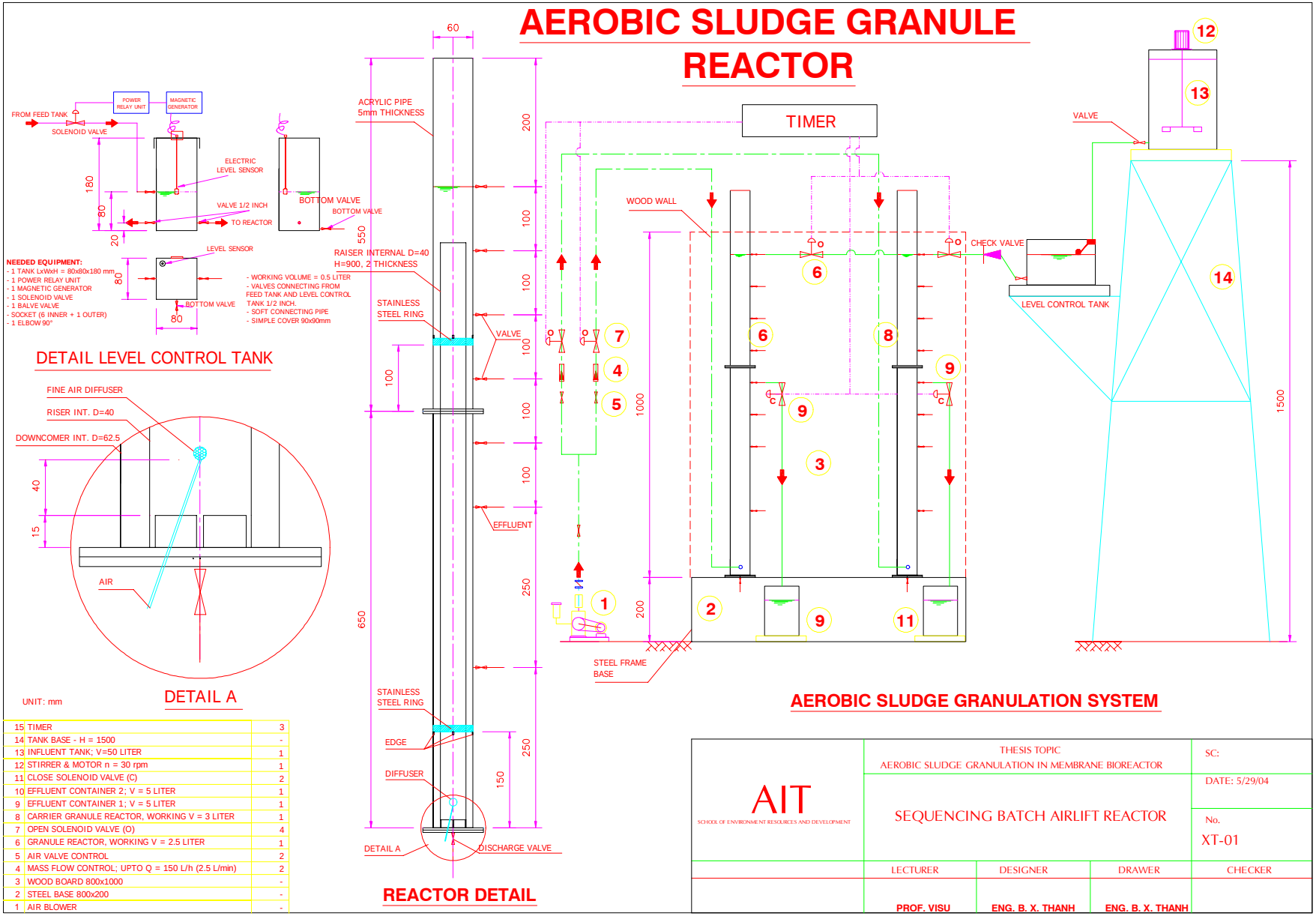



**Figure 3.4 Detailed drawing of SBAR**

**Table 3.3 Operational conditions for SBAR**

| Stage/week | Action | Observation |
|---|---|---|
| **Start-up stage** (2 weeks) | - Inoculation with fresh activated sludge<br>- Acclimatize with suggested synthetic WW<br>- Biomass was settled and returned to reactor | COD removal greater than 95%<br>(System was run manually) |
| **Washout stage** (onward) | | Granulation stage<br>(System was controlled automatically) |
| Week 1 | - Add support media<br>- Settling period reduced from 10 to 9 min | Significant biomass growth on the walls of reactors<br>Biomass was washed out |
| Week 2 | - Settling period reduced from 9 to 3 min | Biomass was being washed out |
| Week 3-week 11 | - Settling period 3 min | Matured granules formed on week 6 |
| Week 15-onward | - Settling period 2 min | Granules became bigger and matured |

Sources: Modified from Beun et al. (2002)

The settling time was fixed of 3 minutes at steady state to ensure that only particles that had settling velocity higher than 10 m/h retained in the reactor. And all of filaments and flocs were washed out because of slow settling velocity less than 10 m/h. Since OLR of 10, settling time was reduced to 2 minutes to maintain mostly granules in reactors.

The H/D ratio (column height to column diameter) was high enough to improve selection of granules by difference in settling velocity (H/D = 20).

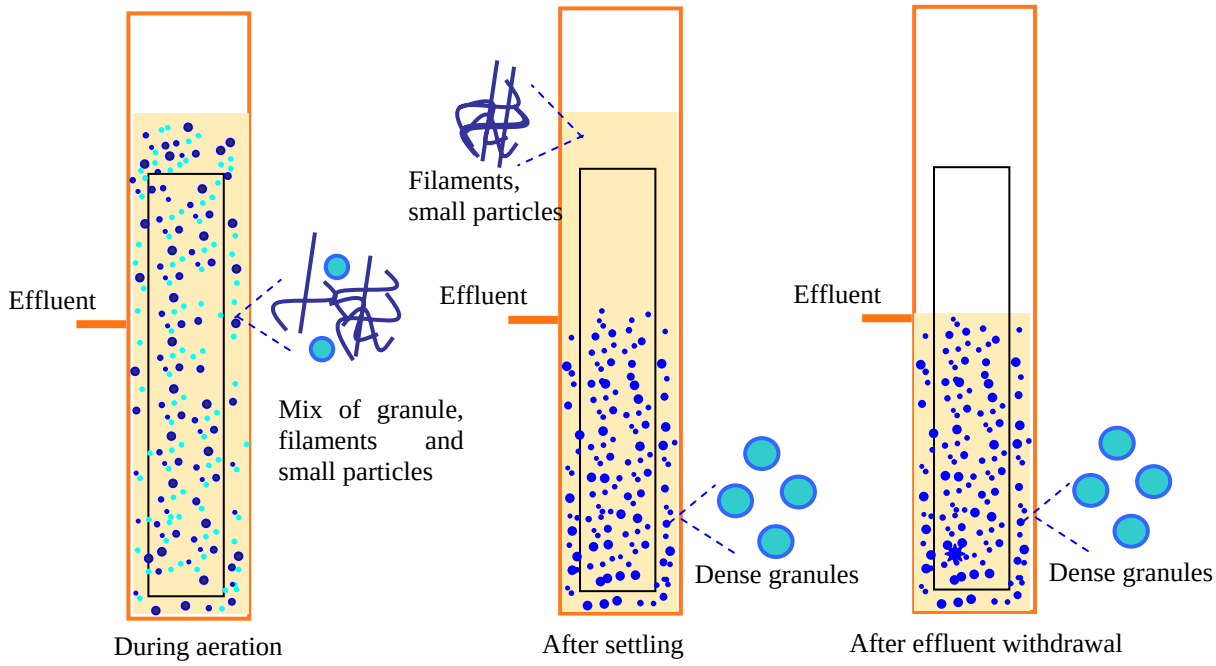



**Figure 3.5 Selection of well settling, dense granules in the SBAR**

### 3.3.3. Granular Sludge Production

Two SBAR were acclimatized with conventional activated seed sludge during two first weeks. After acclimatizing, one was started creating granules with bivalve shell carrier (calciferous shells) that was rich in Calcium as support media and the other with anaerobic granules. This type of support media had rough surface which resulted in good potential for biofilm development. Calcium was also said to play an important role in cultivation of

aerobic sludge granule (Wang et al, 2004), thus use of calciferous shells and basalt as support media was advantageous in granule formation. The other was started forming granules with anaerobic granules that were a type of organic support media for microbial growth.

Anaerobic granules were taken from UASB of treatment plant of Pepsi Company. These had diameter in range of 0.10 – 0.25 mm (figure 3.1d). Carrier was produced by grinding bivalve shell of white rose cockle and sieved to select the size of carrier in range of 150-220 μm (figure 3.1a,b,c).

These two reactors were operated with the same operational conditions as mentioned above in table 3.3 to investigate characteristics of types of granules. From this result, the optimum OLR with good support media was selected to couple with MBR to survey efficiency of granule membrane bioreactor (GMBR).

## 3.4 Granule Membrane Bioreactor

### 3.4.1. Experimental Set-up

Two laboratory scale SBARs coupled with MBR was used in this study. The schematic diagram of the experiment set-up was presented in figure 3.7, 3.8. Reactors were made by transparent acrylic plastic. The operational condition of SBAR in these systems was the same with previous SBAR for granule cultivation.

The first system, treatment sequence I, external MBR, CR1, A hollow fibre MF ceramic membrane module was used in the separate tank. The SBAR was the reactor that used for previous granule cultivation. The flow rate of SBAR, flow rate of MBR, was 10 L/day. Effluent of SBAR after each batch settled and stored in a settling-holding tank with total working volume of 6 L (4 and 2 litter for settling and holding tank respectively). Excess compacted sludge was discharged intermittently through a bottom valve of holding tank at the end of day. Influent of MBR was pumped from holding tank and permeate was sucked by another pump. These pumps were operated with 6 on/4 off minute filtration cycle with flowrate of 12 mL per minute.

The second system, treatment sequence II, internal MBR, CR2, was operated continuously with a hollow fibre MF ceramic membrane module submerged in raiser tube of SBAR. Flow rate was also 10 L/day and permeate was sucked by a pump with 6 on/4 off minute filtration cycle. And light fraction of generated biomass was discharged intermittently once per day. Sludge was removed by open the effluent valve of SBAR after 2 minutes of settling to maintain same sludge retention time as treatment sequence I.

The diagram was described in figure 3.6 and figure 3.7.

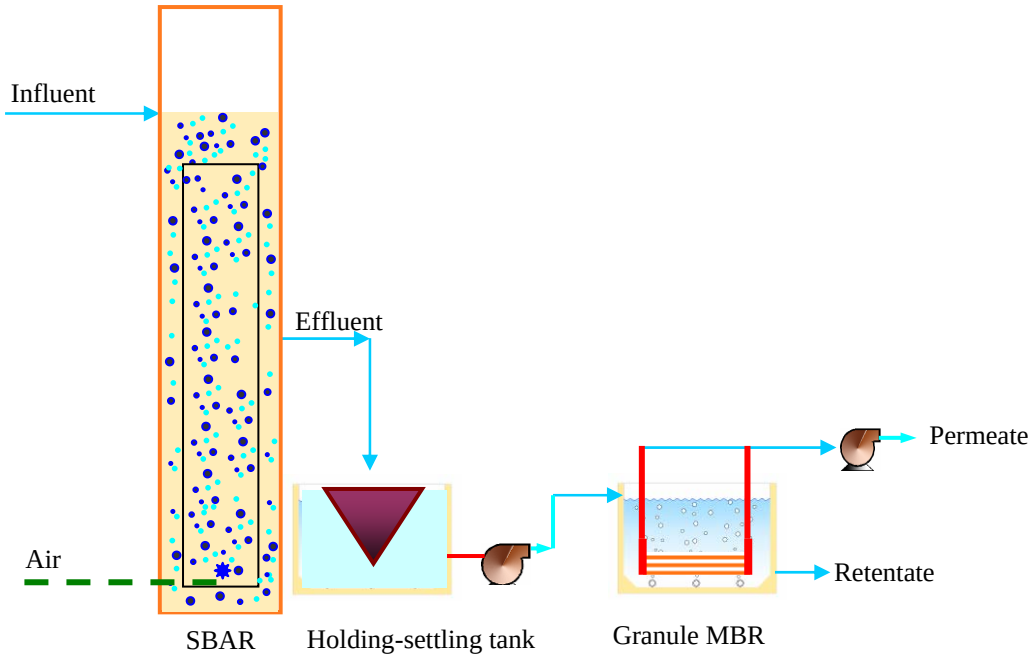



**Figure 3.6 Treatment sequence I of granule MBR**

The second system, submerged hollow fibre ceramic membrane was installed in the inner pipe of SBAR as figure 3.8.

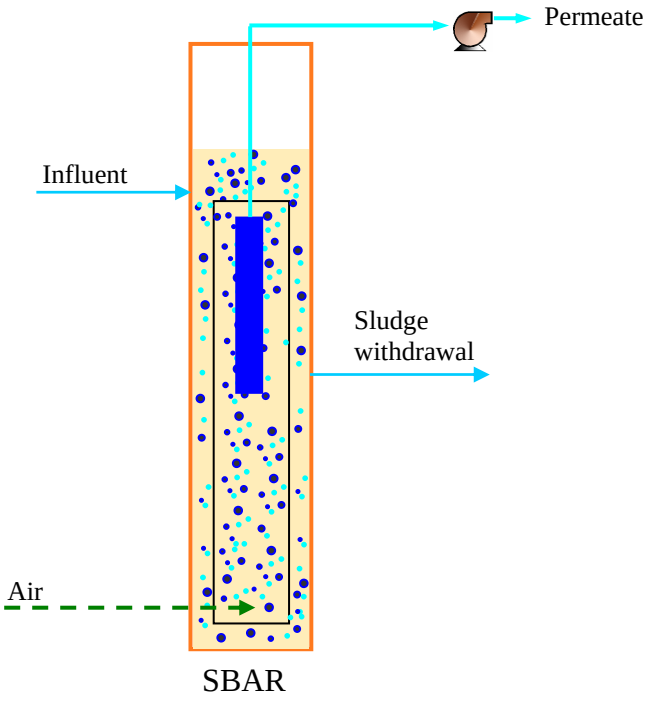



**Figure 3.7 Treatment sequence II of granule MBR**

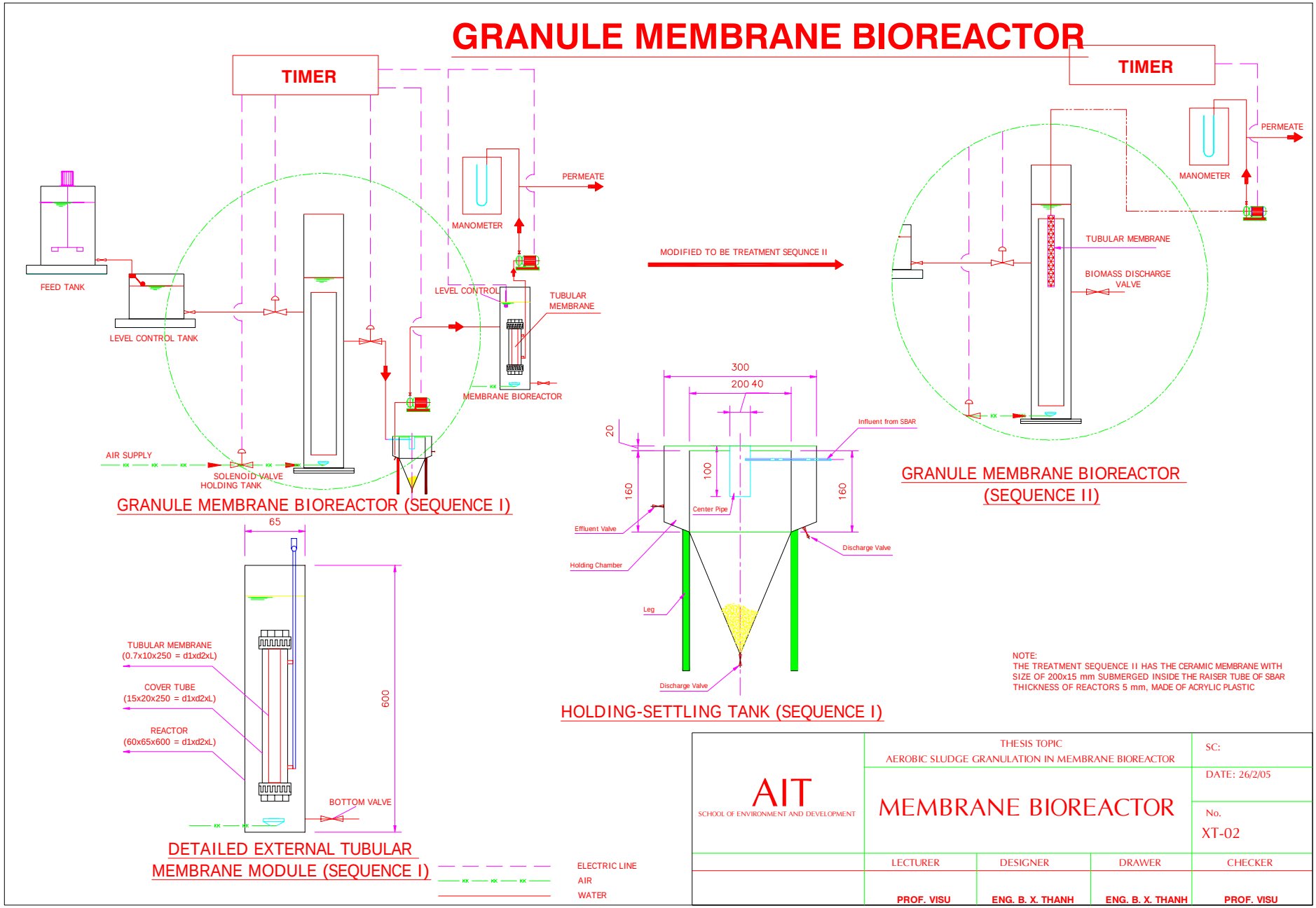



**Figure 3.8 Detailed drawing of granule MBR system**

The membranes used in two treatment sequences were described in table 3.4.

**Table 3.4. Membrane module characteristics**

| Specification | Characteristics |
|---|---|
| | For treatment sequence I & II |
| Membrane materials | Ceramic |
| Membrane type | Hollow fibre – MF |
| Pore size | 0.1 µm |
| Surface area | 0.05 $m^2$ |
| Dimension of membrane | LxD = 250x25mm |
| Length x outer diameter of a fibre | 250 x 2.1 mm |
| Number of fibres | 30 pieces |

MF: Micro-filtration; L: Length, D: diameter.

### 3.4.2. Operational Conditions

In the treatment sequence I, SBAR had operational conditions as same as previous granule creating reactor (batch mode) and effluent from SBAR was settled and filtered with 6 on/4 off minute filtration cycle by hollow fibre ceramic MF membrane module as figure 3.7.

In the second system, submerged hollow fibre ceramic MF membrane module was installed in the raiser tube of SBAR. Wastewater was fed continuously into reactor (continuous mode). This membrane was connected with the permeate pump that operated intermittently with 6 on/4 off minute filtration cycle. Excess biomass was discharged by discharging from the effluent valve daily (figure 3.7).

In run 3 with membrane bioreactors, biomass concentration in SBAR was maintained at steady state amount of OLR of 10 kg COD/$m^3$.day with steady state biomass concentration (biomass concentration was controlled by settling time). In addition, applied OLR for granule MBR was the optimum one, 10 kg COD/$m^3$.day which was selected after aerobic granule characterization (run 1 and run 2).

**Table 3.5 Operational conditions for the Treatment sequence I - granule MBR**

| Period | Time | Remark |
|---|---|---|
| Influent addition | 5 min | |
| Aeration | 170 min | |
| Settling | 2 min | |
| Effluent withdrawal | 3 min | Supernatant was settled and pumped to MBR |
| Membrane operation | 6 min of suction | 3 seconds for air release |
| regulation | 4 min of stand-by | Hollow fibre ceramic MF membrane |

In addition, a conventional SBR was run at the same time to take supernatant for comparison with that of granule SBAR. The cycle was total 225 minutes including 10 minutes of feeding, 170 minutes of aeration, 40 minutes of settling and 5 minute of withdrawal. Supernatant of SBAR was taken to measure membrane fouling index, treatment efficiency, EPS, SVI, etc for comparing fouling ability between granular sludge and conventional activated sludge. SBR was operated at OLR of 2.5 to 5 kg COD/$m^3$.day.

### 3.4.3. Experimental runs

The research was divided into 3 runs:

The first was acclimatizing process and creating aerobic granules with two types of support media in SBAR at OLR of 2.5 kg COD/$m^3$.day with influent COD of 600 mg/L. During this stage, each aerobic sludge granules characteristics were investigated with parameters such as granule morphology, MLSS/MLVSS, settled biomass concentration, settling velocity, bound EPS, hydrophobicity, COD, pH, DO, granule formation time.

The second was to investigate treatment efficiency and characteristics of each type of granules by increasing loading rate of system (by changing COD). In each loading rate, those previous parameters were also measured for selecting type of media and optimum loading rate to couple with MBR in run 3. Biokinetic data and metal concentration was implemented with granules at the optimum loading rate.

The last one, optimum loading rate and selected support media was used for both treatment sequence I and treatment sequence II. In this stage, parameters such as membrane fouling index, TMP, membrane resistance, and all parameters of run 1 and run 2 were measured for studying membrane fouling behaviour of granular sludge to find out suitable treatment sequence for aerobic granulation coupled MBR.

The process of experiment was described in figure 3.9.

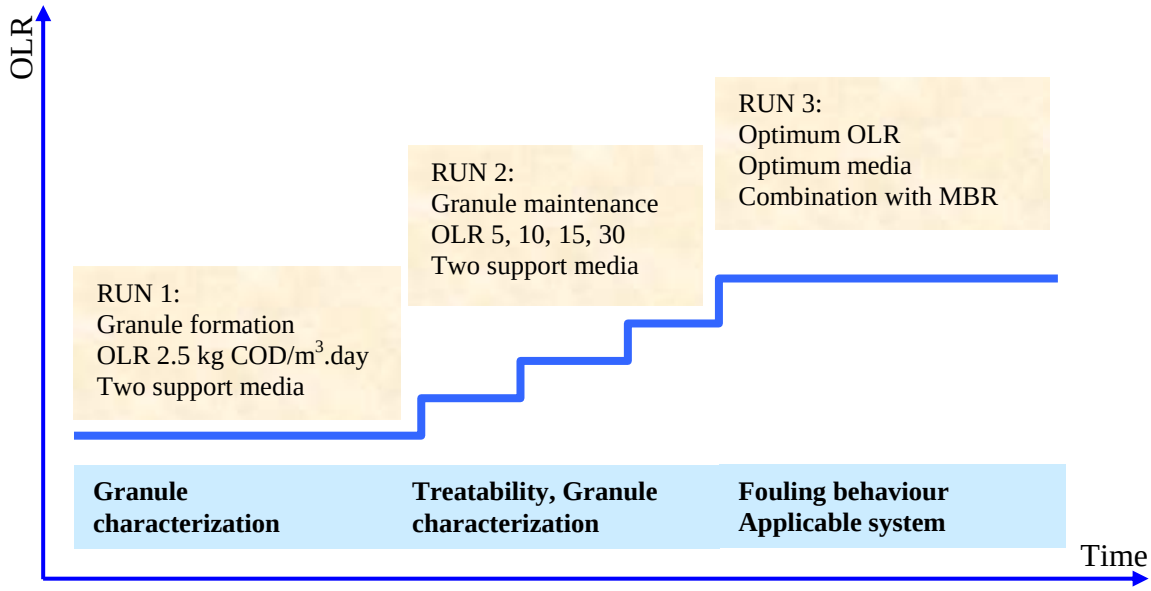



**Figure 3.9 Process of granule MBR experimental runs**

### 3.4.4. Membrane Cleaning

The membrane after each run was taken out of the reactor for cleaning. First, the membrane was flushed with tap water to remove the cake layer attaching on the membrane surface. Second, the membrane was soaked in NaOH 4 % and chlorine 3000 mg/L (Sodium hypochlorite having effective chlorine concentration around 3000 mg/L). Chlorine in aqueous solution was not stable so the percentage of chlorine in sodium hypochlorite solution was had to check when using. Third, the membrane was washed with tap water

and soaked in $HNO_2$ 1% solution or HCl 2% solution for 4 hours. Finally, the membrane was subsequently washed with tap water again and measured membrane resistance before use in the next run.

If transmembrane pressure (TMP) was increased up to 60 kPa, membrane cleaning was required.

* Membrane cleaning procedure:

- Disconnect the suction line and other lines from membrane module;
- Take out the membrane from the reactor;
- Wash the membrane by spraying pressurized water to remove cake formation;
- Immerse the membrane in a chemical cleaning tank (Chlorine in base solution);
- Leave for 6-24 h;
- Rinse with water to remove the chemicals and chlorine residual;
- Immerse the membrane in acid solution for 2-15 h (Remove clogging by salts);
- Rinse with sufficiently water to remove the chemicals;
- Measure membrane resistance ($R_m$);

Membrane resistance after chemical cleaning should be closely to initial resistance (Recovery > 80%).

### 3.4.5. Membrane Resistance

Membrane resistance was measured by using the resistance-in-series model (Choo and Lee, 1996) according to equation 2.1 and 2.2.

Applying this model, membrane resistance was measured by filtrating with pure water at different filtration fluxes and recording the corresponding transmembrane pressures. Membrane resistance was derived from the slope of the linear curve of ΔP versus J as described by the equation 3.1.

* Membrane resistance measurement procedure:

- Take out the membrane from the reactor
- Measure membrane resistance ($R_t$) with membrane resistance measurement
- Wash the membrane by spraying pressurized water to remove cake formation
- Measure membrane resistance ($R_f$+$R_m$)
- Carry out chemical cleaning
- Measure membrane resistance ($R_m$)
- The membrane resistance is derived from the slope of linear curve of ΔP versus permeate flux (J) as described by the equation 3.1.

$$J = \frac{\Delta P}{\mu * R_t} \Rightarrow \Delta P = J * \mu * R_t \qquad \text{(Eq. 3.1)}$$

Where:

J: permeate flux ($L/m^2.h$)

ΔP: transmembrane pressure (kPa)

μ: Viscosity of the permeate (Pa.s);

$R_t$: total resistance (1/m); $R_t = Rm + Rc + R_f$

$R_m$ : intrinsic membrane resistance

Rc: Cake resistance from by the cake layer

$R_f$: fouling resistance caused by solute adsorption into the membrane pore

### 3.4.6. Membrane Fouling Index

Membrane fouling index (MFI) was determined by measurement of volume of filtrate versus with time to volume ratio. So the unit of MFI was $T/L^6$ (often $s/L^2$). In this research, MFI was measured by stirred cell, pressure filter holder made by Germany.

* Membrane fouling index measurement procedure:

- Adjust air flow rate from the compressed nitrogen container to create 1 bar by 3 valves V-1, V-3, V-4 (figure 3.10). Close V-2 and V-4 & Adjust V-1 and V3 to get constant pressure of 1 bar maintaining in system.
- Prepare distilled water and real samples with volume of 100 mL each;
- Fill sample into Filter Holder;
- Insert membrane(*) and other membrane support layers as the below picture;
- Leave Filter Holder on a magnetic stirrer:
- Prepare a beaker 250 mL on a balance connected with PC for weighing filtrate;
- Activate weighing software to start data recording;
- Open V- 4 to start measurement of filtrate volume versus time;
- Stop measurement at the time of constant weight recorded;
- Close V-1 to stop gas supplied from cylinder;
- Open V-2 for air released and close V-4 before reinstalling Filter Holder;
- Reinstall and clean equipment.

(*) Membrane that used for distilled water can be used for real sample.

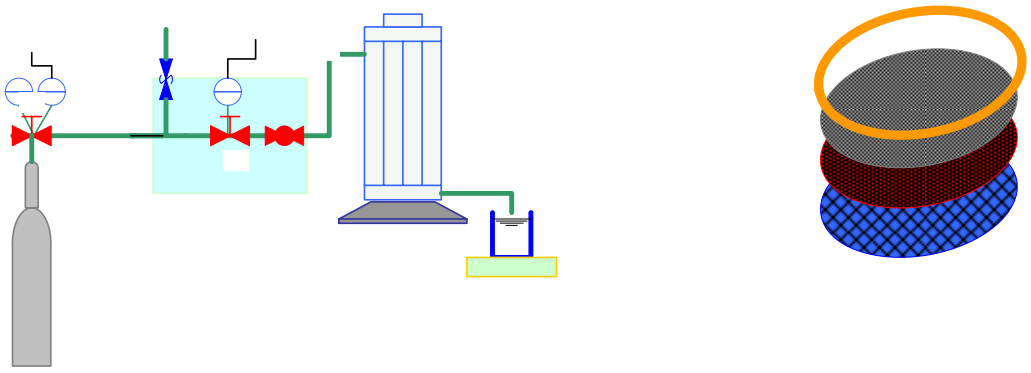


**Figure 3.10 MFI measurement set-up**

## 3.5 Analytical methods

### 3.5.1. Hydrophobicity

The relative hydrophobicity of the sludge floc was measured as adherence to hydrocarbons as mention by Jin *et al*., (2003) and Rosenberg *et al*., (1980). Tris buffer pH 7.1 was used for microbe stability. The process of hydrophobicity was described in figure 3.11.

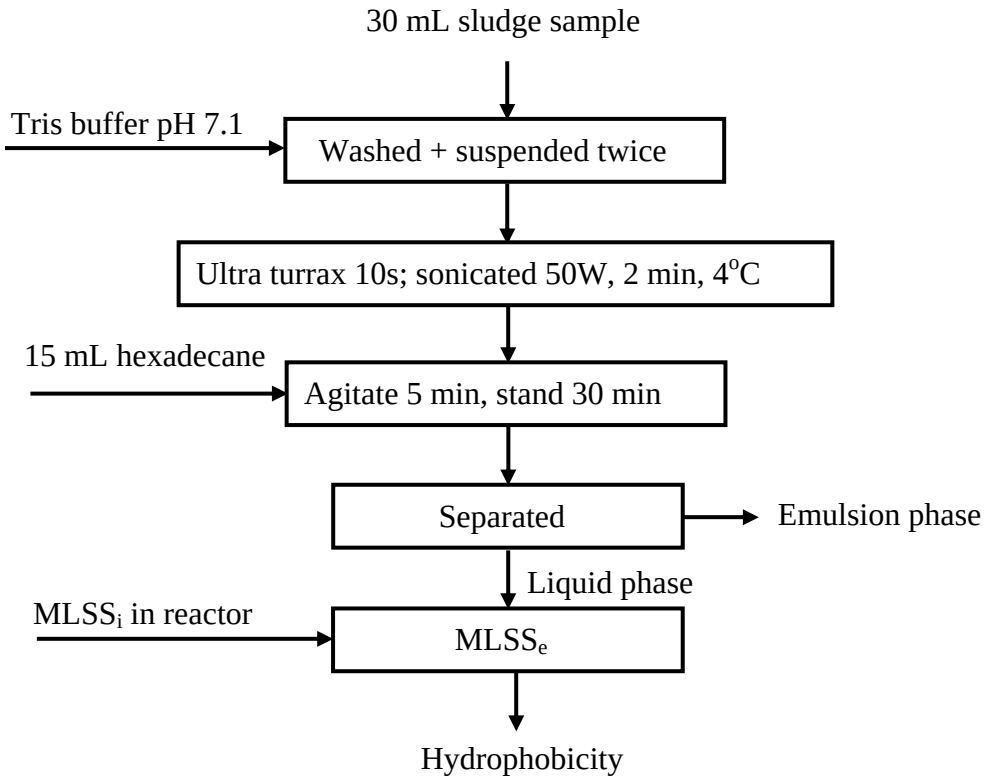



**Figure 3.11. Hydrophobicity determination process**

The relative hydrophobicity was expressed as a ratio of MLSS concentration in the aqueous phase after emulsification ($MLSS_e$) to the MLSS concentration in the aqueous phase before emulsification ($MLSS_i$).

$$Hydrophobicity(\%) = \left[1 - \frac{MLSS_e}{MLSS_i}\right] \times 100$$

### 3.5.2. Extracellular polymeric substance

There were two types of EPS as soluble EPS and bound EPS. The sample was determined EPS in term of polysaccharides (PS) and protein (PN) per milligram of VSS so MLVSS must be determined in this analysis. Normally, only bound EPS was considered in granular sludge. The EPS determining process was as figured 3.12.

***The procedure for PS determination:***

- Pipet sample and adjust volume with distilled water to 2 mL solution into tube;
- Add 1 mL of phenol solution 5%  and 5 mL of sulphuric acid;
- Allow the tubes to stand 10 min;
- Shake, place in water bath for 15 min;

- Read $A_{490}$ after 2 minutes but before 1 hour $\Rightarrow$ sample concentration has A mg/L.

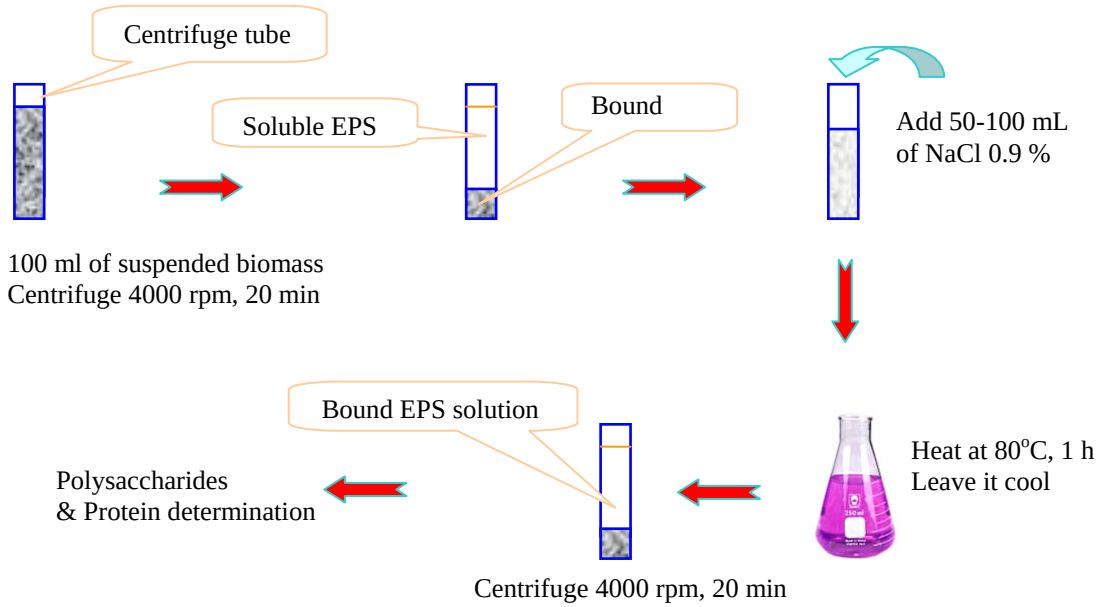



**Figure 3.12 Scheme of sample preparation for bound EPS determination**

***Determine PS (mg/gVSS):***

EPS extracted solution X L has PS = AX mg
MLVSS: =
B mg/L
Take mixed liquor C L so MLVSS = BC mg

$$PS(\frac{mg}{gVSS}) = \frac{PS(mg)}{MLVSS(mg)} * 1000 = \frac{AX}{BC} * 1000$$

***The procedure for PN determination:***

- Bring sample solution to 0.5 mL with distilled water
- Add 2.5 mL of solution C (*)
- Vortex and let stand at room temperature for 5-10 min
- Add 0.25 mL of solution D (*) and vortex
- After 20-30 min, read A750

Solution A: 100 mL of (0.5 g $CuSO_4.5H_2O$ + 1 g $Na_3C_6H_5O_7.2H_2O$); Solution B: 1000 mL of (20 g $Na_2CO_3$ + 4 g NaOH); Solution C: 1 mL of solution A + 50 mL of solution B; Solution D: 10 mL of Folin-Ciocalteu phenol reagent + 10 mL of deionized water.

*Determine PN (mg/gVSS):*

Pipet sample 0.5 mL has Protein = A mg/L
EPS extracted solution X L has protein = AX mg

MLVSS:

= B mg/L
Take mixed liquor C L
so MLVSS = BC mg

$$PN(\frac{mg}{gVSS}) = \frac{PN(mg)}{MLVSS(mg)} * 1000 = \frac{AX}{BC} * 1000$$

### 3.5.3. Settling velocity

A plastic cylinder (6 cm in diameter and 90 cm in height) filled with clear liquid phase of the reactor was used for free-settling test. Single granule was put in the cylinder and could reach their final settling velocity in the upper 30 cm of the water column. Then settling time for the distance of 50 cm will be taken manually with accuracy of ± 0.5 s. All settling test was carried out twice and the average was recorded (Etterer and Wilderer, 2001).

### 3.5.4. Settled biomass concentration ("biomass density" as other publications)

The settled biomass concentration of granules was determined as follows:

From a sample of granules (50 mL), it was left for settling for 30 minutes in centrifugal tube. The total volume of granules could now be determined by reading the total volume of granules (or sludge). Sample was treated with ultra turrax 10 seconds for separating carrier and biomass. After that, carrier was separated by vortex three times and biomass was transferred to another centrifugal tube. This was repeated thrice to make sure most of carrier removed (or separate carrier and biomass by filtering sample with 100 µm metal grid). Hereafter the dry weight of these granules was determined by drying the sample for at least 24 hours at 120$^{o}$C. Finally, settled biomass concentration could then be calculated by dividing the dry weight of the granules by the total volume of the granules (Modified from Tijhuis et al., 1994; Beun et al, 1999).

### 3.5.5. Granule morphology

Granule development was observed and determined by microscope Olympus DF Plan 1X and camera Nikon CoolPix 995 3.34 Mega pixels at AIT's lab. This microscope had maximum magnification of 64x. Types of microbes were observed by biological microscope Olympus BH2-RFCA with maximum magnification of 20,000x.

Matured granule morphology was observed by Scanning Microscope Electron (SEM), JSM-6301F Scanning Microscope Oxford Instrument.

Granule sample for SEM observation was fixed using the fixative reagent (25% solution Glutaraldehyde 10 ml; 0.2 M cacodylic acid sodium salt trihydrate 40 ml and distilled water 50 ml), washed in buffer solution 1:1 (0.2 M cacodylic acid sodium salt trihydrate + 0.5 M Sucrose) and then dehydrated by ethanol series from 20-100% ethanol. Then specimens were placed in the critical point drying (CPD) where the solvent was exchanged with liquid carbon dioxide. Raising the temperature of the sealed "bomb" raised the liquid carbon dioxide pasted the critical point, where the density of the liquid phase was equal to that of the vapor phase. This process could avoid the surface tension effects of normal drying and so preserved the natural structure of the sample. After that, specimens were coated with gold by sputter coater before SEM observation.

### 3.5.6. Other parameters

To determine sludge characteristics and development needed measure the following parameters in table 3.7.

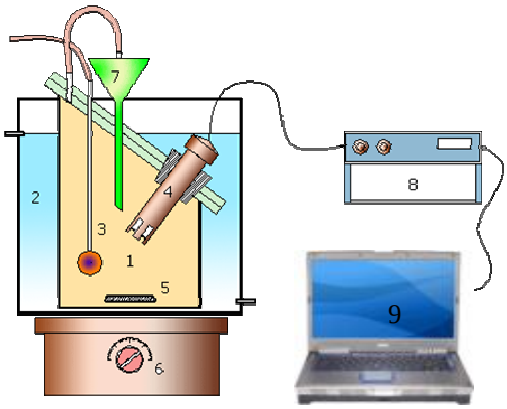
**Table 3.6 Other measured parameters**

| Parameters | Analytical method | Analytical equipment | Interference | Range | Reference |
|---|---|---|---|---|---|
| pH | pH meter | pH meter | | 0-14 | |
| DO | DO meter | Hach Potable LDO | $H_2S$, $N_2$, etc | | APHA et al., 1989 |
| COD | Dichromate closed reflux | | $NO_2^-$, $Cl^-$, $Br^-$, $F^-$ | 40-400 mg/L | APHA et al, 1989 |
| Turbidity | | | Setteable solids | | APHA et al, 1989 |
| Biomass concentration | TOC | TOC-$V_{CSN}$ | Suspended solids | 0-500 mgC/L | Tijhuis et al., 1994. |
| Metals | Atomic absorbance | Hitachi Z8230 polarized Zeeman Atomic Absorbance Spectrophotometer | | 0.5-10 ppm | APHA et al., 1998 |
| SVI | | | High sludge concentration | | APHA et al., 1989 |
| MFI | | Pressure Filter Holder | | | As above |
| Membrane fractionation | | Pressure Filter Holder or Gel Chromatography | | 1 – 300 kDa | Huang et al., 2000 |
| Membrane resistance | Gravitational method | Manometer, balance, stop watch | | | Choo and Lee, 1996 |

### 3.5.7. Kinetic study

#### *3.5.7.1 Respirometer and DO meter*

The configuration of respirometer was shown in figure 3.13. It had working volume of 0.9 L and a fluorescent DO meter which could measure at each 10 second interval was inserted into bulk liquid. Dissolved oxygen was recorded automatically and extracted after each test.




1. Respirometer
2. Water Jacket
3. Air diffuser
4. DO probe
5. Magnetic bar
6. Magnetic machine
7. Expansion funnel
8. DO meter
9. Computer

**Figure 3.13 Respirometer configuration**

#### *3.5.7.2 Analytical procedure*

Experimental procedure was as follows:

a. DO probe preparation

b. Filtered effluent of reactor was used for dilution liquid media (Mathieu and Etienne, 2000).

c. Mixed liquor of granule was aerated for 30 minutes prior to testing to oxidize residual substrate remained. Test was conducted at temperature of 20$^{o}$C.

d. Biomass concentration in respirometer was measured in term of mg VSS/L.

e. Granular sludge was aerated at least one hour until endogenous respiration reached. OUR of endogenous phase was recorded ($r_{x,e}$) and DO in respirometer was saturated in range of 6-8.5 mg/L.

f. An accurate amount of concentrated substrate was added to obtain ration of $S_o/X_o$ =0.01 - 0.2 mg COD/mgVSS (Cech et al., 1984; Chudoba et al., 1992). Total OUR was recorded ($r_{x,t}$). DO in respirometer must be maintained higher than 2 mg/L.

g. DO reduction due to microbial respiration was monitored every ten seconds by DO meter. DO changed and OUR, SOUR were calculated. When one dose of substrate finished, the new one was injected into the cell and the new respirogram was recorded. The typical program was shown in figure 3.14.

h. Kinetic data was calculated by the equation section 3.5.7.3.

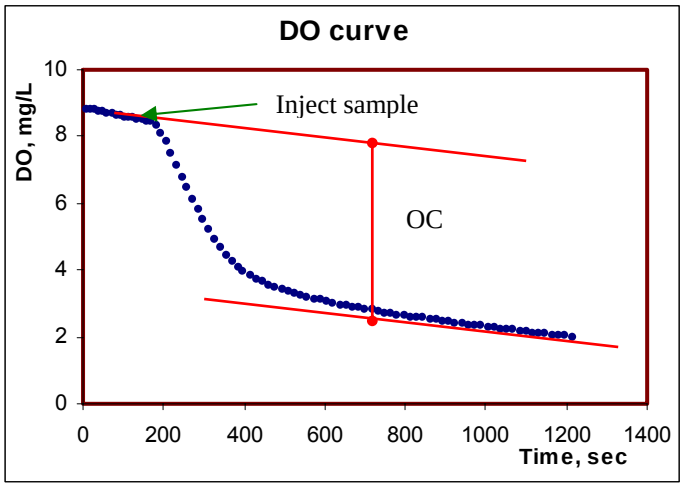



**Figure 3.14. Typical respirogram chart**

### *3.5.7.3 Determination of kinetics data*

Evaluating the respirogram, these data could be achieved:

Endogenous respiration rate : $r_{x,e}$

Total respiration rate : $r_{x,t}$

Net oxygen concentration : OC

Using these data, the following rates and coefficients can be computed.

Specific substrate oxidation rates at concentration S:

$$r_{x,ox} = r_{x,t} - r_{x,e} \quad \text{(Eq. 3.2)}$$

Specific substrate removal rate at concentration S:

$$r_x = \frac{r_{x,ox}}{OC/S} \quad \text{(Eq. 3.3)}$$

Coefficient of substrate oxidation:

$$1 - Y = \frac{OC}{S} \quad \text{(Eq. 3.4)}$$

Coefficient of biomass yield:

$$Y = 1 - \frac{OC}{S} \quad \text{(Eq. 3.5)}$$

Specific growth rate:

$$\mu = Y . r_x \quad \text{(Eq. 3.6)}$$

Half saturation constant, $K_S$, and maximum specific growth rate was determined by Monod equation (for model without inhibition substance):

$$\mu = \mu_m \frac{S}{K_S + S} \quad \text{(Eq. 3.7)}$$

# Chapter 4

## Results and Discussions

In this chapter, there were three parts such as aerobic granule characterization, supernatant characterization and granule membrane bioreactor. The first considered as a main part was to cultivate aerobic granule at OLR of 2.5 kg COD/$m^3$.day with two kinds of support media (bivalve shell carrier, anaerobic granules). After matured granule formed at this loading rate, OLR was increased until maximum value (30 kg COD/$m^3$.day). During this process, physical chemical biological characteristics of granules were investigated such as cell surface hydrophobicity, settled biomass concentration, SVI, settling velocity, bound EPS, granule morphology, granule size development, and also granule treatability in term of OLR. After reaching to maximum OLR, the optimum OLR was selected with optimum support media for coupling with MBR. The second, supernatant from SBAR and control SBR was collected at different OLR to investigate its fouling ability in term of membrane fouling index, soluble EPS (polysaccharides and protein). The last, optimum OLR (10 kg COD/$m^3$.day) and bivalve shell carrier media was selected to combine with MBR. There were two treatment sequence I, II (external system-CR1, internal system-CR2) as batch and continuous system. The purpose of this run was to find out the suitable system for real application on water reuse and reclamation with granular sludge.

### 4.1 Aerobic Granule Characterization

#### 4.1.1. System Start-up

Two Sequencing Batch Airlift Reactors (SBARs) were started up with synthetic wastewater with glucose as sole carbon source and used seed sludge collected from conventional activated sludge process. These two reactors were used to culture aerobic granules with two types of support media at OLR of 2.5 kg COD/$m^3$.day at superficial air velocity of 95 m/h (4.5 NL/min). After acclimatizing and gaining high organic removal efficiency, the first reactor (CR) was added bivalve shell carrier media made of white rose cockle and the second one (AR) was added anaerobic granule media collected from Pepsi wastewater treatment process.

Seed sludge was inoculated in both reactors with MLVSS was about 2000 mg/L in both reactors. In the first two weeks of start-up stage, these reactors were operated manually. COD removal efficiency and COD effluent was demonstrated in figure 4.1a and b.

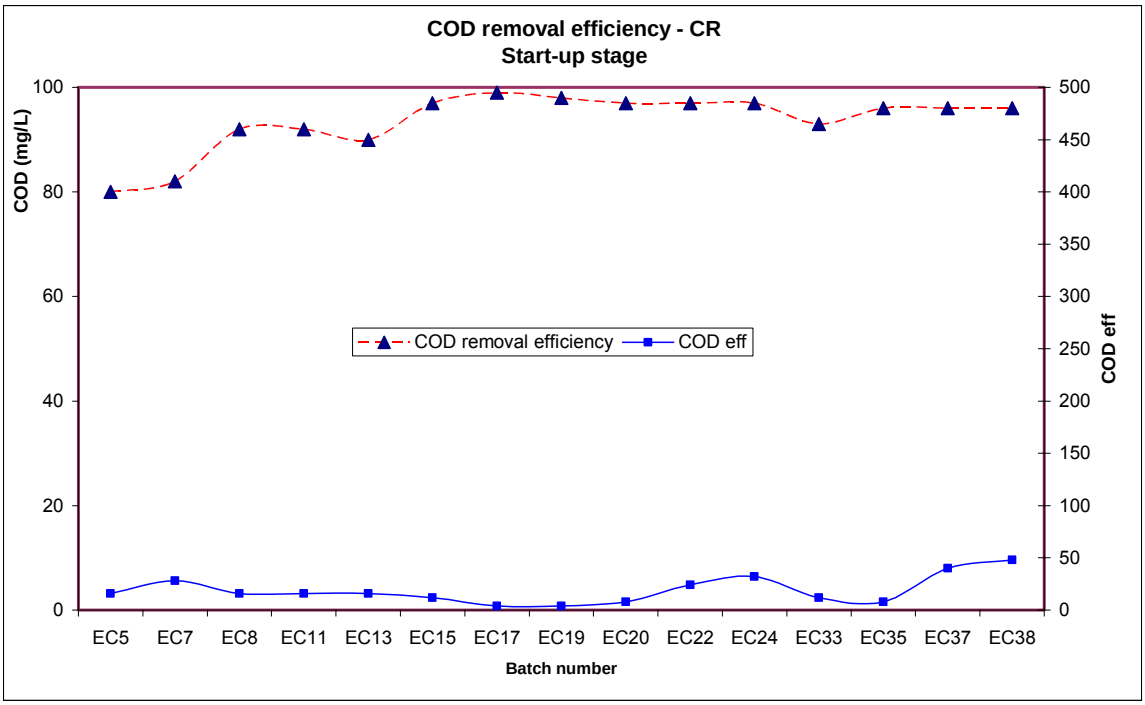



**Figure 4.1a. COD removal efficiency at start-up stage - CR**

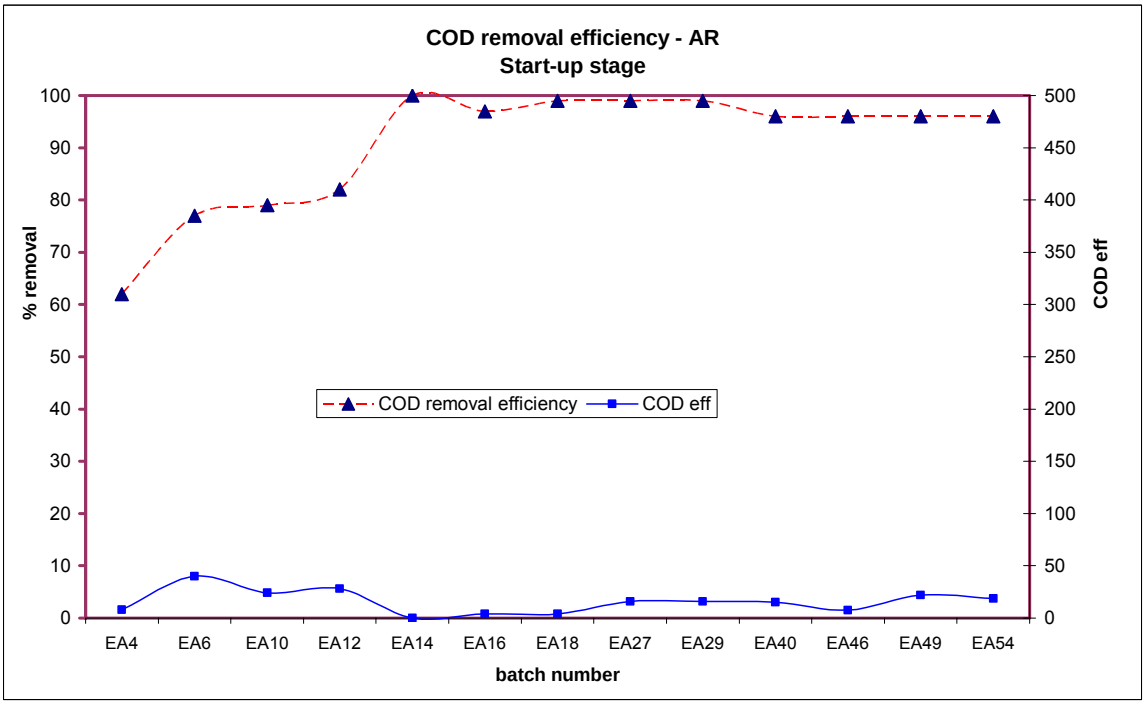



**Figure 4.1b. COD removal efficiency at start-up stage - AR**

From the figure 4.1a and b, after two weeks of start-up, COD removal in both reactors was greater than 95 percent and effluent COD less than 20 mg/L in both reactors. The color of seed sludge changed from blackish brown to yellow during the first week of acclimatization. Bigger flocs appeared in form of particles that could be observed by naked eyes. The settling ability increased a little bit.

After two weeks the microbes in seed sludge seemed to be acclimatized with the new feeding and had high enough organic removal efficiency. Then carrier and aerobic granules were added to stimulate microbial attached growth on media surface.

- Total bivalve shell carrier used: 20 g/L * 2.5 L = 50 g
- Total anaerobic granules used = 135 mL

When putting support media, the color of CR became pale yellow (light yellow) and that of AR were yellowish brown. The color of anaerobic granules became paler and paler. In addition, the settling ability of sludge in two reactors increased. Moreover, carriers helped to make the reactor's walls clean and biofilm was automatically removed due to friction between carrier and reactor walls. Potentially, this could also enhance microbial aggregate.

When stopping air supply for settling, the small particles from the bottom moved up automatically so that the heavy fraction came down. This meant light fractions of biomass could be selected to be washed out automatically when withdrawing supernatant.

### 4.1.2. Aerobic granulation

At the beginning of washout stage (automatically control), biomass concentration was 4950 and 4240 mg/L in CR and AR, respectively. After adding support media, the granulation was started by performing washing out light fraction of biomass due to changing the settling time progressively from 10 to 3 minutes. The gradual decrease of settling time was to avoid washing out most of biomass in reactors. So, biomass concentration in reactors reduced day by day until only particle had settling velocity greater than 10 m/h retained in reactors.

| Week | 1 | | | | | | | 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 onward |
| Settling time (min) | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 3 |

After acclimatizing stage, so many new microbes appeared in both reactors such as rotifers, ciliates, flagellate, nematodes, protozoa colonies, etc. These kinds of microbes had very good settling ability (figure 4.2 and 4.3). These appeared and attached around the support media surface.

At beginning of week 2 (day 8 of washout with settling time of 8 minutes), biomass in CR seemed to be linking with carrier surface. In AR, biomass totally attached with anaerobic granules and made anaerobic granule color became pale brown.

At early of week 3, biomass seemingly covered shell carrier and anaerobic granules with thin layer of microorganism presented in figure 4.2 and 4.3. Progressively, anaerobic granules became brighter and smaller as well because of disintegration until disappeared by violent aeration. After aerobic granules disappeared, thin layer of biofilm formed on the wall of AR but not for CR. So, biofilm was removed manually to enhance granulation process.

At the end of week 3, sludge characteristics changed significantly. SVI was 24 mL/g in CR and 43 mL/g in AR but the seed sludge 243 mL/g. It was also more and more compacted. Settled biomass concentration was 20.8 and 23.8 $g/L_{granule}$ in CR and AR in comparison with seed sludge 2.7 $g/L_{sludge}$. Hydrophobicity was 65 percent in for both granules and 31 percent for seed sludge. COD was reduced very quickly during the first five minutes of aeration stage illustrated in figure 4.7 and 4.8.

At the end of week 4, some of initial granules could be observed by naked eyes in both reactors but it was more clearly in AR. At this moment, these granules were touched as "hard particles".

At week 5, granules appeared with a majority and could be recognized while moving. The microbial aggregate continuously happened in reactors and until week 6 big granules appeared. They became hard with diameter from 0.1 to 0.5 mm in CR and from 0.2 to 1 mm in AR. Hereafter; granules became more and more matured by improving physical chemical characteristics gradually (see details in figure 4.5, 4.11, 4.12a, 4.13, 4.15 or appendix 3).

Until week 9, end of run 1, at this time aerobic granules was really matured with hydrophobicity of 84 and 86 percent for CR and AR, SVI of 18 and 35 mL/g and settled biomass concentration of 25 and 24 $g/L_{granules}$. At this moment, the color of granules in CR was reddish yellow and bright yellow in AR.

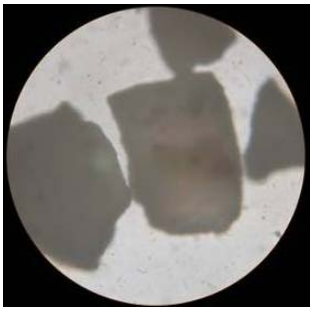
From week 10 to 16 during run 2, OLR was increased to maximum value of 30 kg $COD/m^3$.day. During this run, hydrophobicity was in range of 51-81% for both reactors with lower hydrophobicity at higher OLR (figure 4.12a and 4.12b), SVI was less than 26 and 39 mL/g for CR and AR respectively (figure 4.13), settled biomass concentration was from 25 to 49 $g/L_{granules}$ for CR and from 20 to 62 for AR (figure 4.11). Settled biomass concentration was high at higher OLR. Settling velocity was in range of 56-103 m/h for CR and 15-49 m/h for AR (figure 4.15). Granule size in this run was 0.5 – 2 mm and 0.3 – 4 mm respectively for CR and AR (figure 4.5).

From week 17 to the end during run 3, only carrier granules was coupled with MBR at OLR of 10 kg $COD/m^3$.day with two treatment sequences (detail in section 4.3).

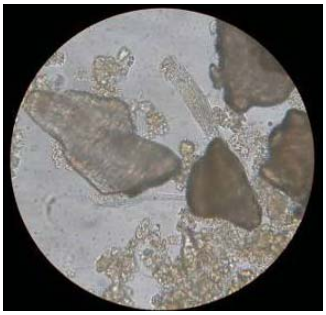
### 4.1.3. Microbial Cultures and Granule Morphology

At first, seed sludge was black color and contained a lot of filamentous bacteria (figure 4.2a). After one week of operation sludge color became dark yellow and light yellow after three weeks. At the second week, it was interesting that majority of big microorganisms appeared including protozoa, rotifers, protozoa colonies, ciliates, flagellate, nematodes, spirillum, etc. Since the third week, most of spirillum cultures that was small and swam very fast disappeared gradually due to its bad settling ability in both reactors. At the forth week, in both reactors there were a lot of red nematodes but in AR it was much more than in CR. From this week, aerobic granules were already formed in both reactors. From week 7, most of separate microbes such as rotifers, protozoa, nematodes, ciliates, flagellates, etc were gradually disappeared from bulk liquid in both reactors as figure 4.2i and 4.3f. This could be explained that microbes have been aggregated to be aerobic granules. Until week 11, OLR of 5 kg $COD/m^3$.day, there were only some nematodes and rotifers in bulk liquid.

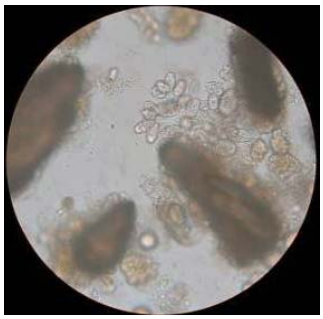
a. Seed sludge (x20)



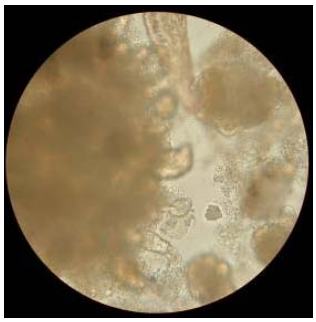
b. Dry carrier



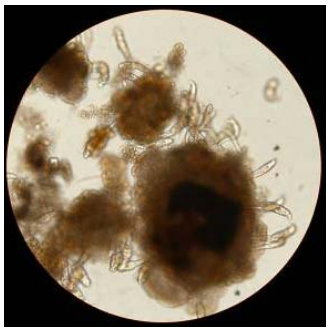
c. Carrier surface, rotifers (x20)



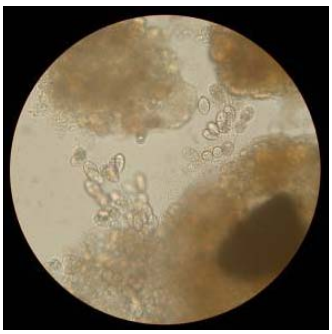
d. Thin biofilm layer on carrier



e. Rotifers, protozoa, nematodes

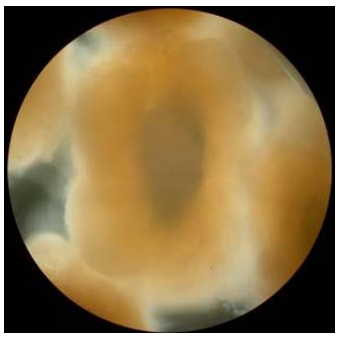


f. Initial granules



g. Granules with & without carrier

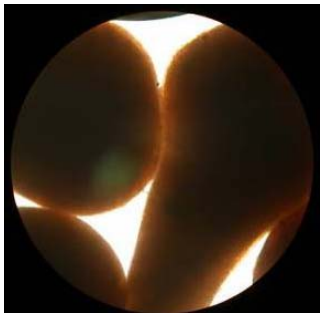
h. Carrier granules with nematodes



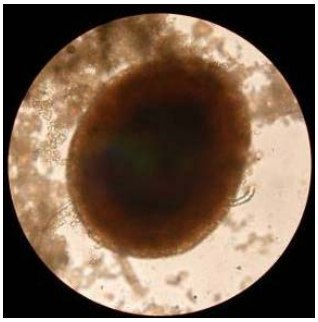
i. Matured CR granules

**Figure 4.2. Microbial cultures and granule morphology in CR**

Based on figure 4.2, microbes were slowly covered on carrier to form carrier granules and granules became predominant from week 6. Moreover, microbes themselves were also granulated without carrier like in AR. Therefore, there were granules two types of granules with and without carrier in this reactor.

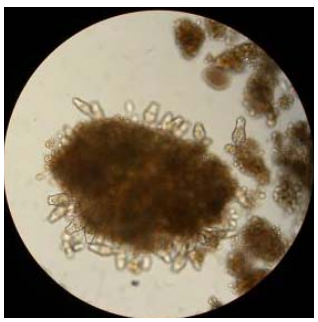


a. Original anaerobic granule with clear outer

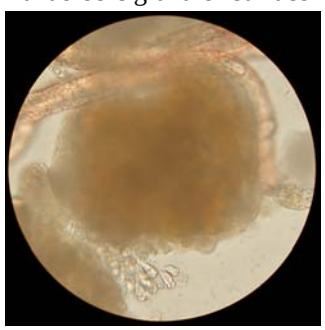


b. Signal of microbial attachment on "anaerobic granule" surface

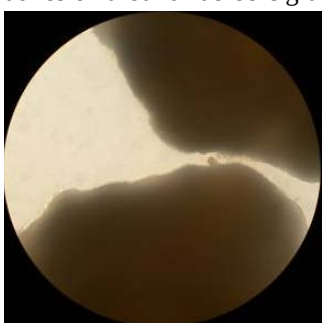
c. Initial granule with microbial adhesion around "aerobic granule"



d. Initial microbial adhesion



e. Granules, nematodes, ciliates



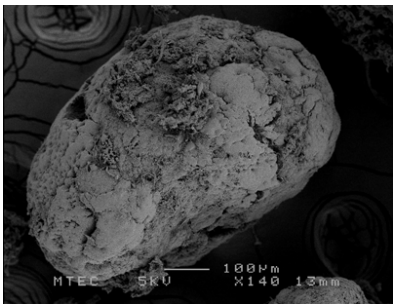
f. Matured AR granules

**Figure 4.3. Microbial cultures and granule morphology in AR**

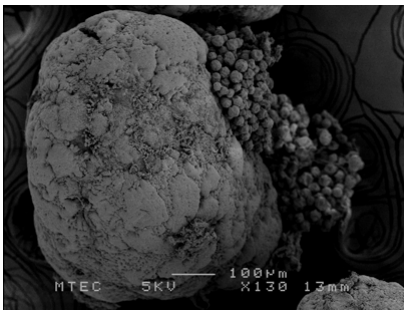
Depending upon figure 4.3, microbes seemed to attach on the anaerobic granule surface shown in figure 4.3b and gradually anaerobic granules were worn until disappeared and slowly new aerobic granules were formed. This granulation mechanism was totally as same as granulation of conventional activated sludge. More details of microbial development were in appendix 3.

Granule morphology of matured granule was observed by SEM presented in figure 4.4.

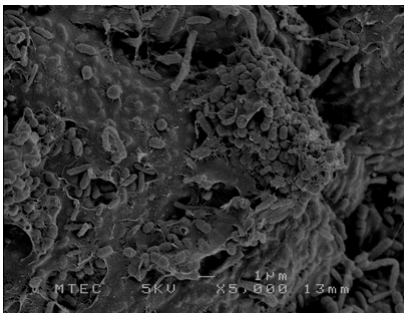
**A. SEM of CR granules**



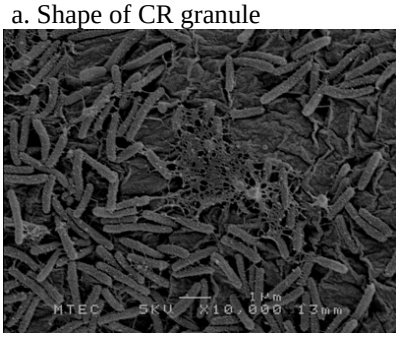

a. Shape of CR granule



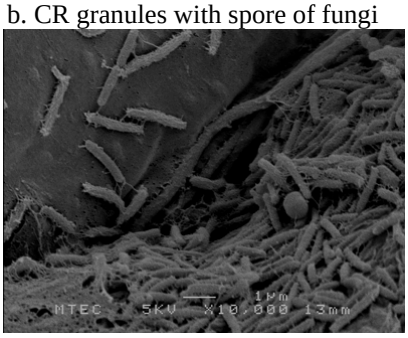

b. CR granules with spore of fungi



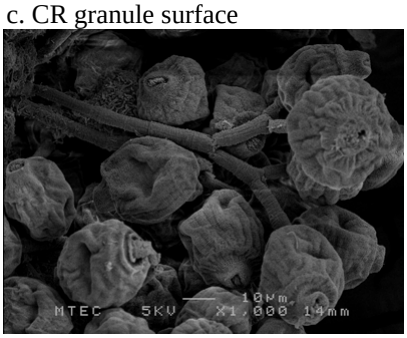

c. CR granule surface




d. Surface with cocci and rod shape bacteria

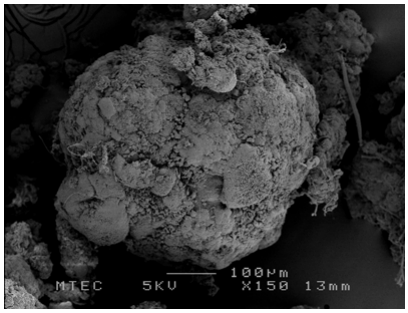



e. Surface at granule cavity

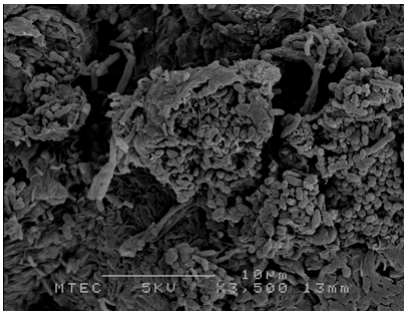



f. Fungi attached to granules

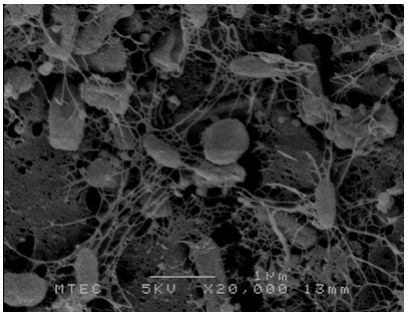
**B. SEM for AR granules**




g. AR granule surface




h. AR granule morphology




i. Cocci and rod shape bacteria with cobweb linkage

**Figure 4.4. Granule morphology of CR, AR by SEM**

In both types of granules, there was a lot of cavities (as figure 4.4 a,b,e,g) that could enhance substrate, nutrient, oxygen diffusion into cores of granules. Cocci, rod shape bacteria, fungi, filamentous organisms coexisted in structure of the granules shown in figure 4.4c,d,e,h,i. Respectively, Rod shape and cocci bacteria were dominant in both granules. Microbial aggregation was linked together by cobweb-like material as figure 4.4d,i. This material was EPS (polysaccharides and protein) and acted as bridging factor

for cell to cell adhesion as same as result of Morgenroth et al., 1997; Tay et al., 2001; Linlin et al., 2005; Wang et al., 2004; Jang et al., 2004.

All in all, there was difference between two types of granules:

(1) CR granules surface seemed to be "smoother" with smaller cavities and more compact relatively so it could be explained why CR granule could suffer better with shock loading as section 4.1.8 and settled biomass concentration of CR was often higher than that of AR.

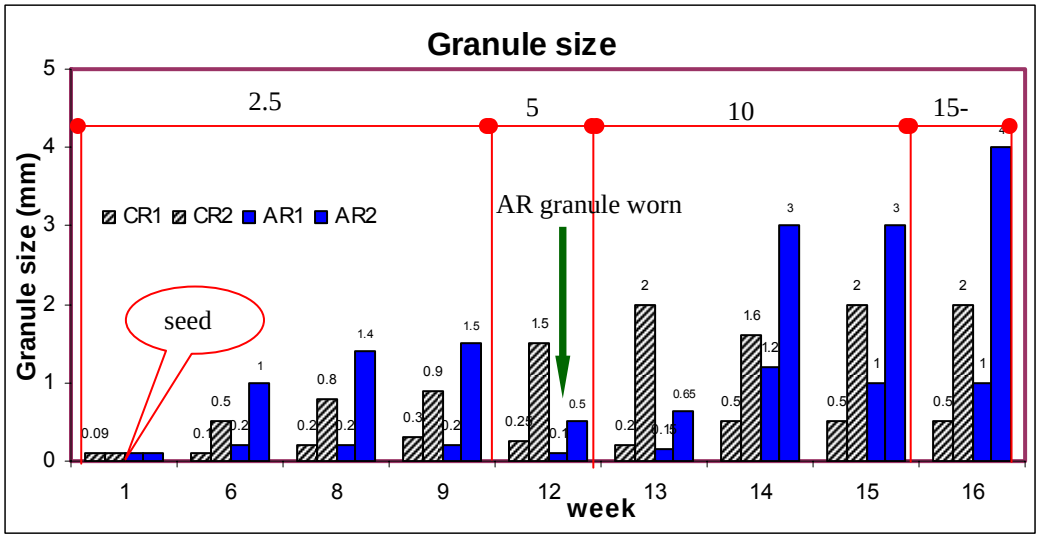
(2) CR granule was predominant with rod shape more than cocci bacteria.

### 4.1.4. Granule Size Development

From figure 4.5, seed sludge had diameter less than 90 µm and anaerobic granules were 1-2 mm. The granule size was slowly developed in both reactors. The initial granules with the size of less than 0.1 mm appeared in AR after anaerobic granule (support media) vanished totally on week 2. At this moment, thin biofilm was covered around bivalve shell carriers in CR. From week 3, initial granules could be observed by naked eyes from reactor walls. From week 6, granules could be easily seen and appeared with majority in reactors. The size in AR was bigger than that in CR but less compact. At week 9, matured granules had diameter from 0.3 to 0.9 mm and from 0.2 to 1.5 mm, settled biomass concentration of 25 and 24.1 g/$L_{granules}$, SVI of 18 and 35 mL/g in CR and AR, respectively.

When increasing OLR up to 5 kg COD/$m^3$.day, the size of granule in CR was continuously increased until 2 mm but that in AR was reduced less than 0.5 mm due to applying shock loading into system by sharply increasing loading rate from 2.5 to 5 kg COD/$m^3$.day. At steady state of this loading, settled biomass concentration also increased up to 32.1 and 28.3 g/$L_{granule}$ in CR and AR. This value was less than 48 g/L (as Beun et al, 1999) and higher than 10-15 g/L (as Arrojo et al., 2004). Sludge settling ability of CR, AR in terms of SVI seemed to be constant with 22-26 and 33-39 mL/g at steady condition of this loading.

After shock loading, AR was run continuously to OLR of 10 kg COD/$m^3$.day and biofilm was removed. After two days of biofilm removal, granule size in AR was suddenly grown up bigger and bigger up to 3 mm quickly at the end of loading rate.




*Range of granule size of CR and AR (from CR1 to CR2) and (from AR1 to AR2)

**Figure 4.5. Granule size development in CR and AR**

In the same manner, when increasing up to OLR of 15, 30 kg COD/$m^3$.day, granule size seemed to be proportional to COD and reached the maximum size of 0.5-2 and 0.3-4 mm respectively for both CR and AR. This confirmed that granulation was more suitable with high loading rate as same results of Tay et al., 2001.

### 4.1.5. Role of Support Media and Granule Mechanism

#### *4.1.5.1 Role of support media*

Firstly, after acclimatization stage, there was biofilm layer formed in both reactor walls. When support media were added, the biofilm was automatically removed by friction force between moving media and reactor walls. The following day after adding support media, reactor walls became clear and transparent like initial time. After week 3, all of anaerobic granules disappeared and biofilm grew up again in AR reactor walls but not for CR. Obviously, bivalve shell carrier played a wonderful role in removing attached growth on reactor walls and let it develop only on the carrier surface. In addition, this could also avoid the competition between biofilm and granule formation. It created the priority for granule development only.

Secondly, carriers also increased shear stress due to its movement combining with hydraulic shear force formed by air intensity (physical force). Shear stress was one of the vital factors considered to create granules that were suggested by Tay et al., 2004 and others. So, in this study Carrier was found to enhance granule development by its supplementary shear stress.

Thirdly, bivalve shell carrier made aerobic granules in CR more regular, rounder and denser than that in AR even at the beginning granules in CR had diameter smaller than in AR.

Eventually, due to high density of carrier, it also enhanced granule settling velocity.

#### *4.1.5.2. Granule mechanism*

Based on the granulation observation, in CR granule was formed by shear stress of hydraulic shear force of air intensity and also of movement of carrier media. Shear force made sludge characteristic become more and more hydrophobic under starvation condition and rough surface of carrier was also attractive for biomass coverage. Biomass included bacteria, protozoa, flagellate, ciliates, nematodes, etc. Cell surface hydrophobicity became high, so cells could be easily gathered together by separating with water phase. Moreover, at this time polysaccharides and/or divalent cations played a vital role of bridging agents to form a cell matrix. Since that time, initial granules formed and became bigger and bigger gradually. With more and more intensive mixing, granules would become more and more matured. Detail of granule formation in CR was presented in figure 4.6.

The mechanism of granule formation in AR was mostly similar with the one suggested by Wang et al., 2004; Tay et al., 2001; Tay et al., 2002; Tay et al., 2004; Jang et al., 2003; Etterer and Wilder 2001 as granule cultivation by conventional activated sludge that was illustrated in section 2.4.2 of literature review. It was not similar with suggestion of Linlin et al., 2005 that granule was only cultivated directly from anaerobic granules because in this research almost anaerobic granules disintegrated and washed out through effluent. So, the mechanism was as same as one of formation from conventional activated sludge. However, the presence of anaerobic granules in reactor at the beginning could also help remove biofilm and increase shear stress for stimulating granule formation.

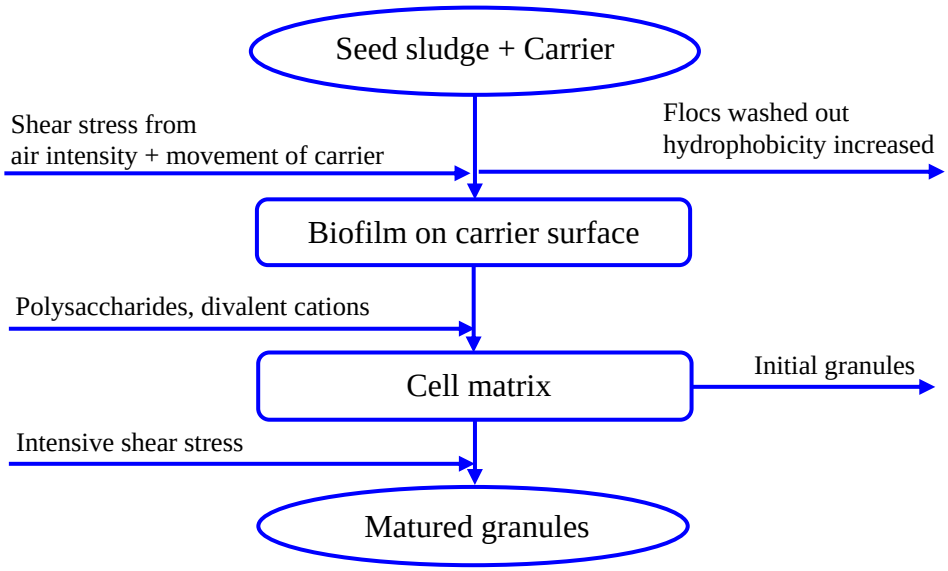



**Figure 4.6. Granule formation mechanism in CR**

### 4.1.6. Granular Sludge characterization

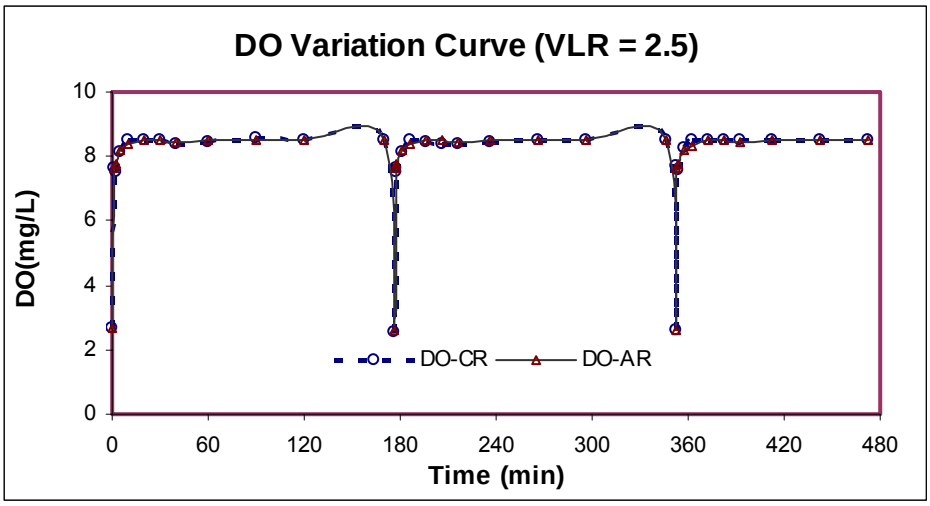
#### *4.1.6.1. pH*

During the operation, there was no pH adjustment. Feed wastewater was maintained in range of 7.2 ± 0.2 by adjusting $NaHCO_3$ dose. The variation of pH in reactors was usually in the range of 7.2 - 8.7. pH was usually close to 7.2 at the beginning and close to 8.7 at the end of aeration stage. The value of settled effluent pH was often lower than pH in bulk liquid around 7.5± 0.2, so effluent from aerobic granulation reactor could meet the need of effluent standards of most of Asian countries.

#### *4.1.6.2 Dissolved Oxygen*

Oxygen concentration in reactors was often at saturated value due to high flow rate of air supply, except first few minutes because of available abundant organic matters. After organic matters were almost removed from bulk liquid (absorbed or biodegraded) DO reached to saturated level (8.6 ± 0.1 mg/L).

Figure 4.7 and 4.8 showed DO variation of both reactors at different OLRs. From the curves of DO and COD, it was said that when DO increased, COD in bulk liquid seemed closely equal to effluent COD at that moment. When COD reached to effluent value, DO become saturated after two and ten minutes at loading rate of 2.5 and 5 kg COD/$m^3$.day, respectively. The first stage with high COD and the second one with low COD were called feast and famine period (Beun et al., 2002). After feast period, it was the starvation stage that was good condition for granulation (Tay et al., 2001; 2002; 2003; 2004).



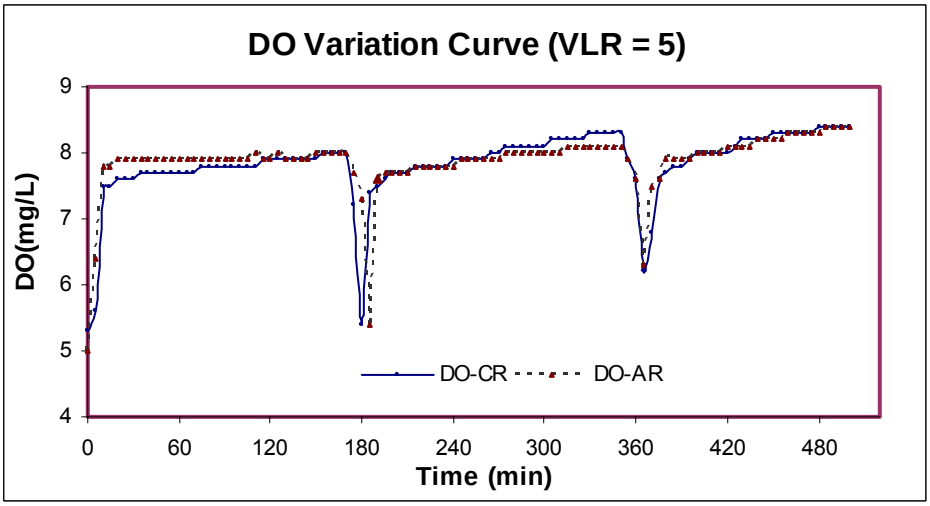
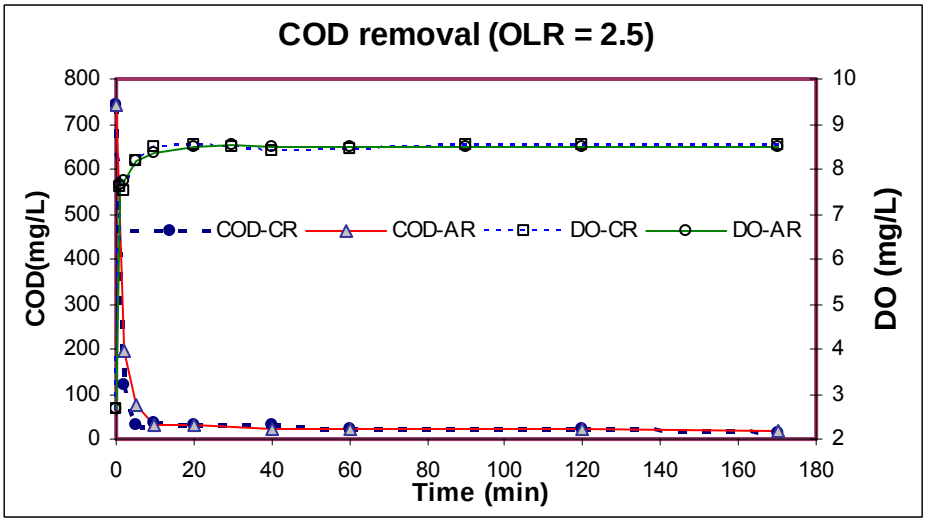




**Figure 4.7 DO variation curve in CR & AR**



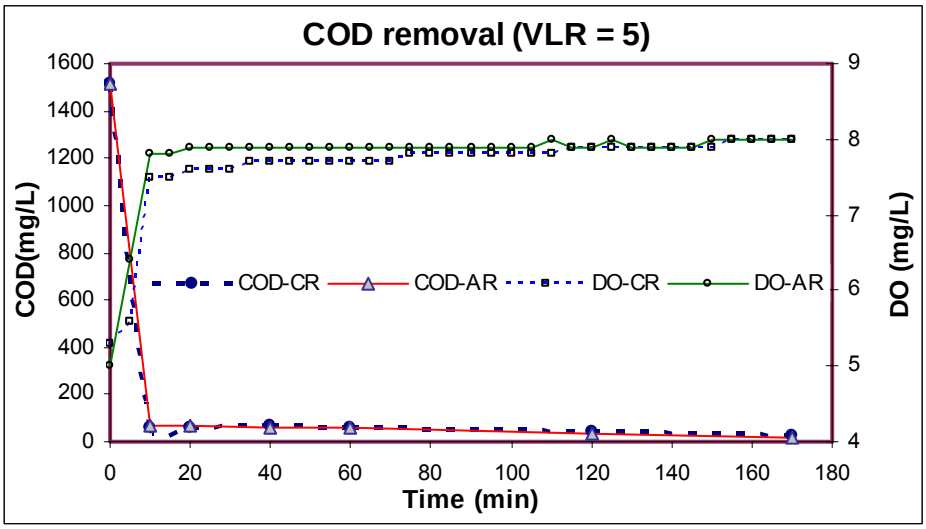



**Figure 4.8 Correlation between DO and COD removal in CR & AR**

As shown in figure 4.8, it is tangible that the rate of organic matter consumption of granules was extremely fast. Majority of soluble COD was biodegraded or absorbed by granules within first ten minutes. As a result, granule was much higher bioactivity than conventional activated sludge in terms of specific oxygen uptake rate as section 4.19 and figure 4.24.

### *4.1.6.3 Biomass Concentration*

After acclimatization stage, biomass concentration in CR and AR ranged from 4900 to 4200 mg/L. As figure 4.9, most of sludge particles had settling velocity less than 10 m/h (settling time of 3 minutes) would be washed out and only heavy fraction with high settling rate was retained. Until fifth week, most of light biomass was washed out and biomass accumulation started increasing in the reactors till loading rate of 5 kg COD/$m^3$.day. Duration from the fourth to the fifth week, it was called “washout valley”. At this loading rate granules in AR was worn and became less than 0.5 mm but that in CR was the same. This time, biomass in AR was reduced strongly to 3200 mg/L (figure 4.9). However, COD removal efficiency was almost higher than 96 percent and the physical chemical characteristics of AR such as settled biomass concentration, hydrophobicity, SVI, settling velocity were not varied much as figure 4.11, 4.13 and 4.15.

At week 11 at OLR of 5 kg COD/$m^3$.day, settling time was reduced to 2 minutes to retain only big granules in reactors. Therefore, there was a reduction in biomass at week 11-12.

When loading rate reached to 30 kg COD/$m^3$.day, biomass concentration was positively proportional to the time.

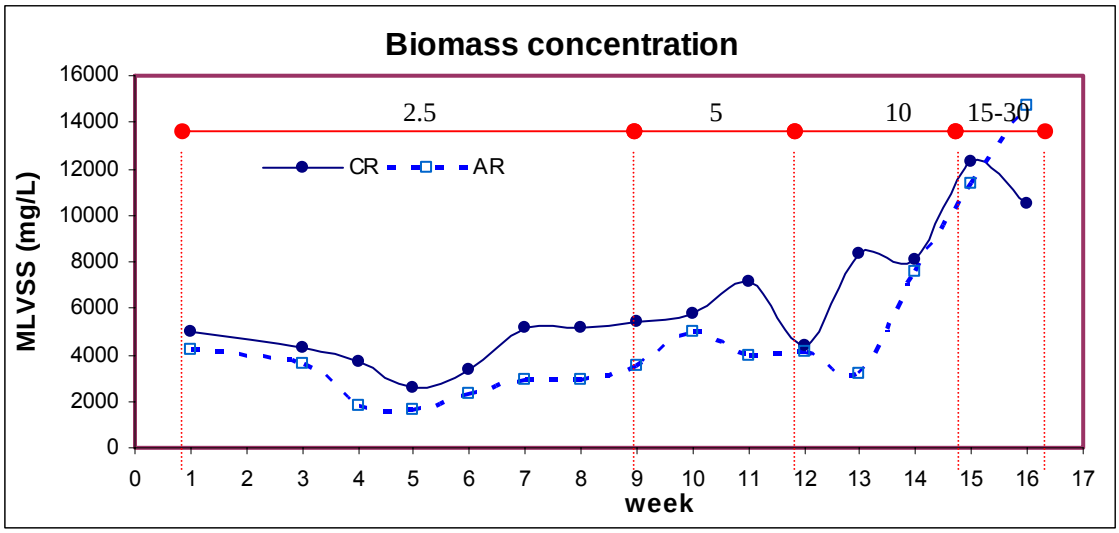



**Figure 4.9. Biomass concentration in CR & AR**

Figure 4.10 reveals that at loading rate of 2.5 kg COD/$m^3$.day biomass concentration was within 80 - 250 mg/L. When loading rate increased, effluent biomass was also increased due to higher organic matter amount in feed.

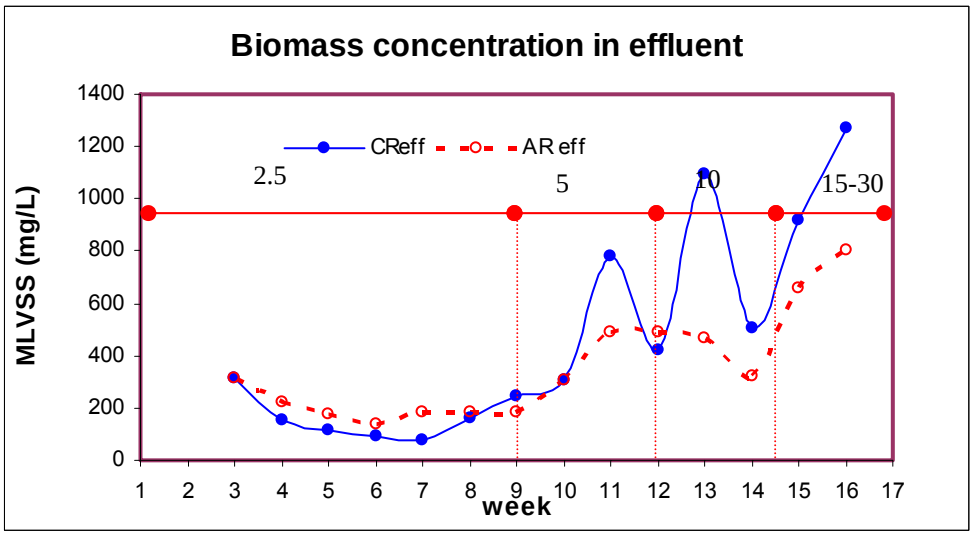



**Figure 4.10. Effluent biomass concentration**

Effluent biomass concentration was too high from 300 to 1200 mg/L at different OLRs so that this led to low sludge retention time in granule SBAR. Moreover, supernatant would need post treatment before discharging such as settling or filtration. Therefore, membrane filtration could be attractive solution for effluent biomass removal of granulation system.

#### *4.1.6.4 Settled biomass concentration (or biomass density in other publications)*

Settled biomass concentration of both reactors significantly increased after three weeks of operation. It reached from 2.7 g/$L_{sludge}$ of seed sludge to greater than 20 g/$L_{granule}$ at third week. From then on, it kept constant around 25 g/$L_{granule}$ in the reactors until matured granules formed on week 9. This value was lower than Beun et al., 1999 & 2002 (48 & 60 g/L) but higher than Arrojo et al., 2004 (10-15 g/L). But when reaching to higher loading rates from week 9 onward, settled biomass concentration of CR seemed to be slightly

higher than that of AR and also became denser in comparison with OLR of 2.5 kg COD/$m^3$.day. Furthermore, this proved positive effect of carrier media.

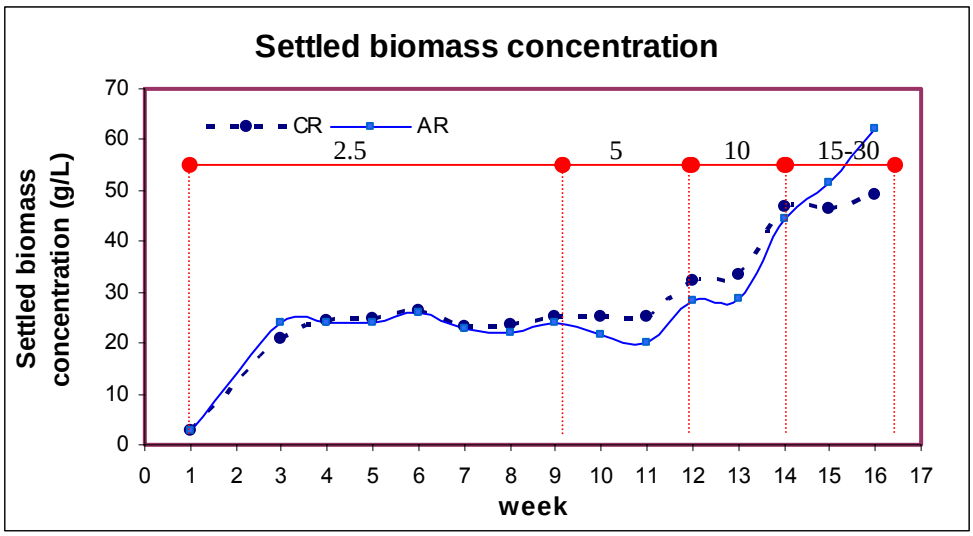



**Figure 4.11. Settled biomass concentration of CR & AR**

As biomass concentration, settled biomass concentration was also proportional to the increase of OLR. At OLR of 30 kg COD/$m^3$.day, settled biomass concentration was 49 and 62 g/$L_{granule}$ for CR and AR, respectively. This could be understood that high OLR made granules more compacted.

### *4.1.6.5 Hydrophobicity:*

Seed sludge had hydrophobicity of 31 percent and after 3 weeks of operation, it reached to 65 percent in the reactors. And this became greater and greater until matured granules formed. The stable value was in range of 73-81 percent CR and AR. The increase of hydrophobicity of granule at third week showed that sludge characteristics started changing and granules would be readily created. Hydrophobicity of cells increased due to high shear stress of aeration and system was in starvation phase. Under famine condition there was no substrate supply caused increase of cell surface hydrophobicity as Tay et al., 2001.

When increasing loading rate to 5 kg COD/$m^3$.day, there was a shock with microbes so, hydrophobicity was suddenly fell down but after one week it was recovered closely to the stable value again.

When OLR reached to greater than 10 kg COD/$m^3$.day, cell hydrophobicity was reduced. This reduction was due to slightly high organic substrate in bulk liquid. At OLRs of 15-30 kg COD/$m^3$.day, COD in effluent was about 100 mg/L so starvation condition did not exist in reactors so hydrophobicity was decreased to 51 and 53 percent respectively for CR and AR at OLR of 30 kg COD/$m^3$.day as figure 4.12b.

Moreover, the phenomenon of high hydrophobicity could be recognized by observation of settling sludge. When settling, the sludge linked together to form large groups and settled. It was never observed in conventional activated sludge. Figure 4.12b showed the correlation between OLR and hydrophobicity at each loading rate.

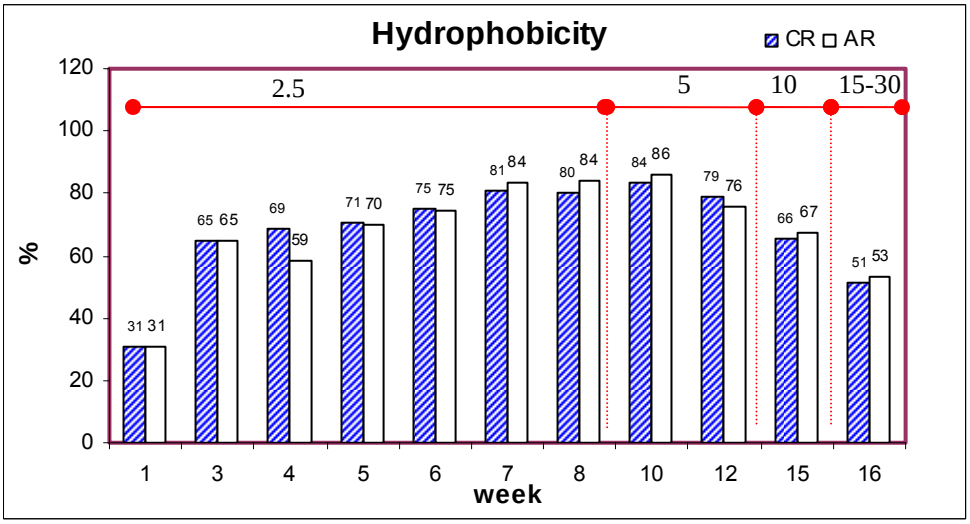



**Figure 4.12a. Hydrophobicity of CR & AR**

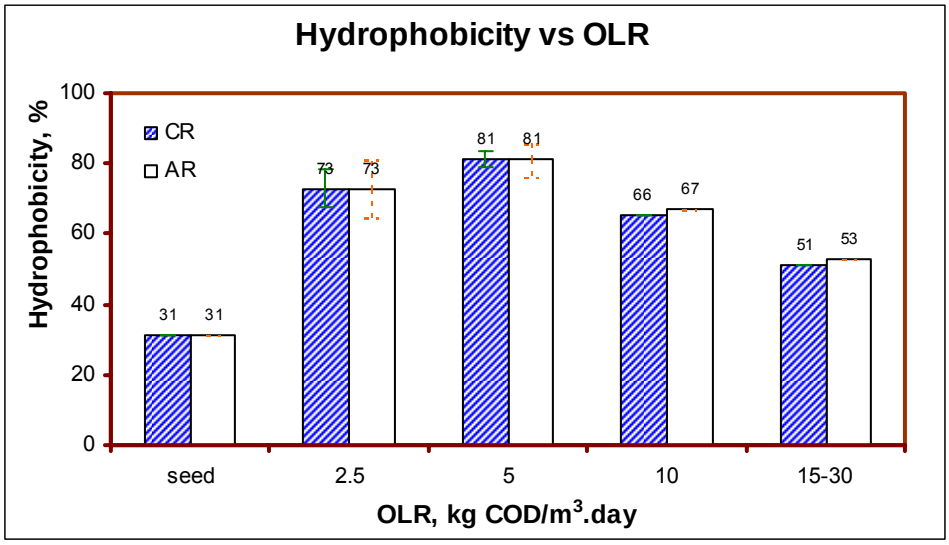



**Figure 4.12b. Hydrophobicity vs OLR of CR & AR**

### *4.1.6.6. Setting Ability*

When there was a signal of granules formed at the third week, SVI significantly decreased from 243 mL/g of seed sludge to 24 and 43 mL/g of CR and AR (figure 4.13). Thenceforth, SVI was always lower than 30 and 40 mL/g in CR and AR. When matured granules formed, SVI was 18 and 35 mL/g in CR and AR. These values showed the excellent settling ability of granules. When OLR increased up to 30 kg COD/$m^3$.day, SVI still decreased to 14 and 19 mL/g for CR and AR.

Especially, carrier media enhanced settling ability of granules significantly due to its original great settling ability.

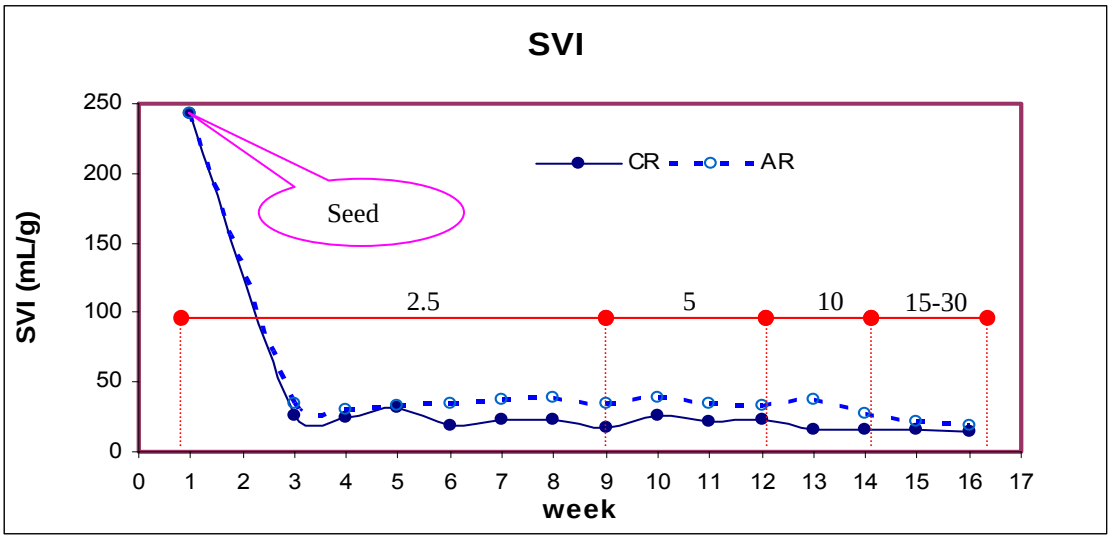



**Figure 4.13. Sludge volume index of CR and AR**

Moreover, zone settling velocity was also very high so that time duration for sludge zone settled to get minimum volume was 30 minutes in activated sludge but it was only 5-7 minutes (figure 4.14 a and b). Sludge volume decreased from 100 mL to 18 ml and 22 for CR and AR (at OLR 2.5 kg COD/$m^3$.day) during five minutes of settling and after 30 minutes the settling zone also did decrease a little bit but not significant. It meant the settling time of granule was only equal 17 percent of that of activated sludge to achieve the same compact sludge volume. As a result, when measuring SVI of granular sludge, the volume that was recorded after 10 minutes would be equal to the volume noted after 30 minutes.

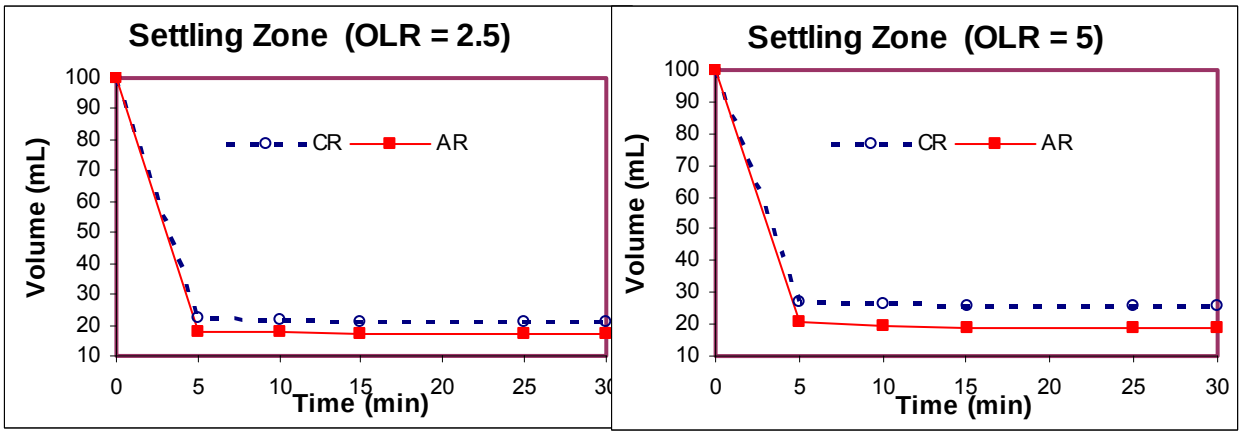



**Figure 4.14 Zone settling volume**

The settling velocity of both types of granules was much higher than that of conventional activated sludge which was less than 10 m/h as Beun et al., 2002. The velocity was measured by testing with about 20 typical granules. At steady state of OLR of 2.5 kg COD/$m^3$.day, settling velocity was 21 and 16 m/h in CR and AR. When raising OLR, granule settleability increased and the highest value was 103 and 51 m/h for CR and AR.

Velocity of CR granules was mostly higher than AR ones due to great settling ability of carrier and more compacted biomass. On the same manner, carrier showed the important role in granule cultivation and maintenance.

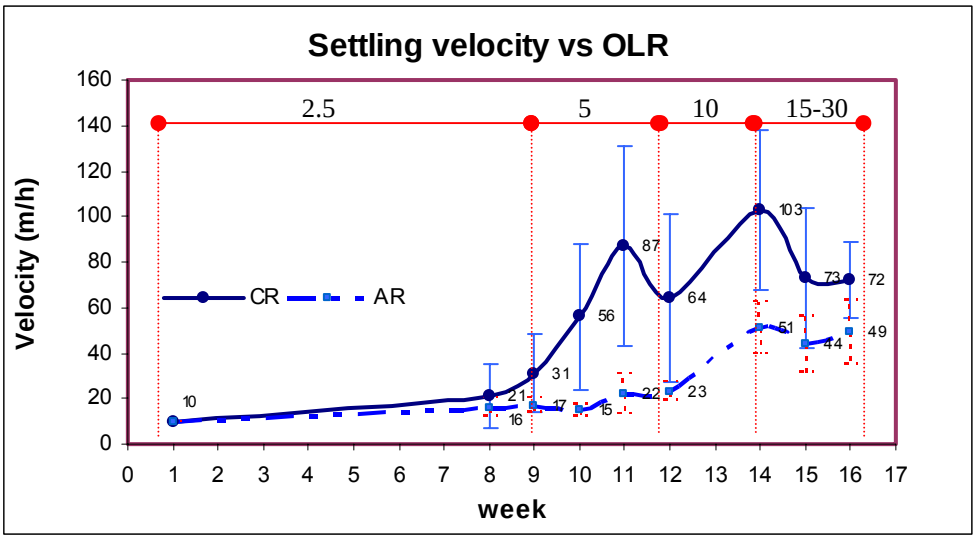



**Figure 4.15 Granule settling velocity vs OLR**

### *4.1.6.7. Sludge Retention Time*

Figure 4.16 described relationship between SRT and OLR in which SRT of CR was slightly higher than that of AR at loading rate less than 5 kg COD/$m^3$.day and vice versa at higher one. So, SRT in granule SBAR was not high due to washing out process to maintain granule formation. That's why biomass in effluent was rather high.

In most cases, SRT was not over 8 days (typical CASP) and normally 3 or 4 days because generated biomass' settling ability was lower than granules'. So most of generated biomass was often washed out and made SRT low.

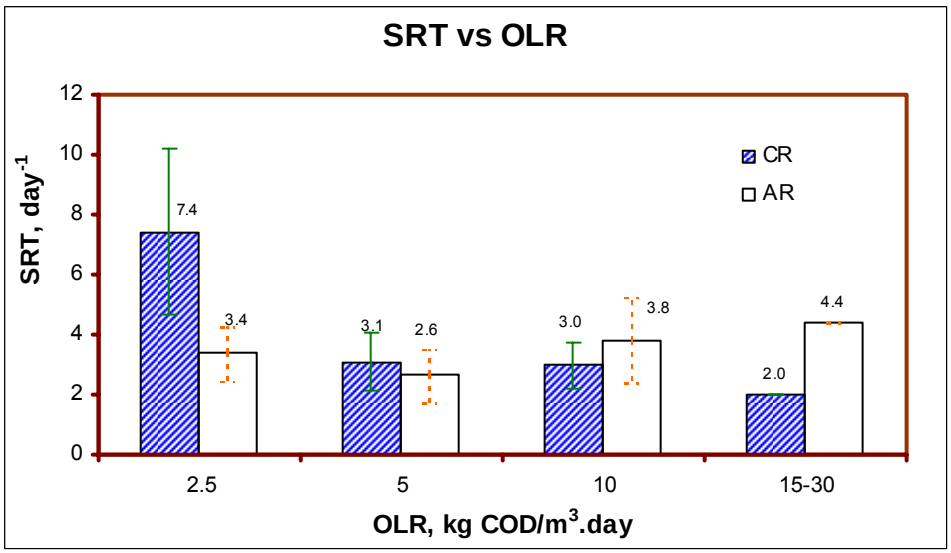



**Figure 4.16. Sludge retention time vs OLR**

### *4.1.6.8. Extracellular Polymeric Substances:*

From the beginning to steady state of granule formation i.e. week 9 on ward as figure 4.17a, Bound polysaccharides (PS) significantly increased from 20.6 mg/gVSS (seed sludge) to range of 42 to 89 mg/gVSS at different levels of OLR in both reactors. This stated that polysaccharides played a role in granule formation as bridging factor of cell

aggregation. This could be suggested that PS was generated by creating linkage of cells for granule formation. However, Protein (PN) varied slightly according to the OLR in comparison with seed sludge. It was from 90.2 mg/gVSS (seed sludge) to range of 45 to 120 mg/g VSS and little bit low at high OLRs.

Those above confirmed the role of extracellular polymeric substance as cohesion, adhesion and maintaining biofilm matrix which were the same with results carried out by Liu and Tay, 2002. Especially, Polysaccharides found to be the main bridging factor for biomass granulation.

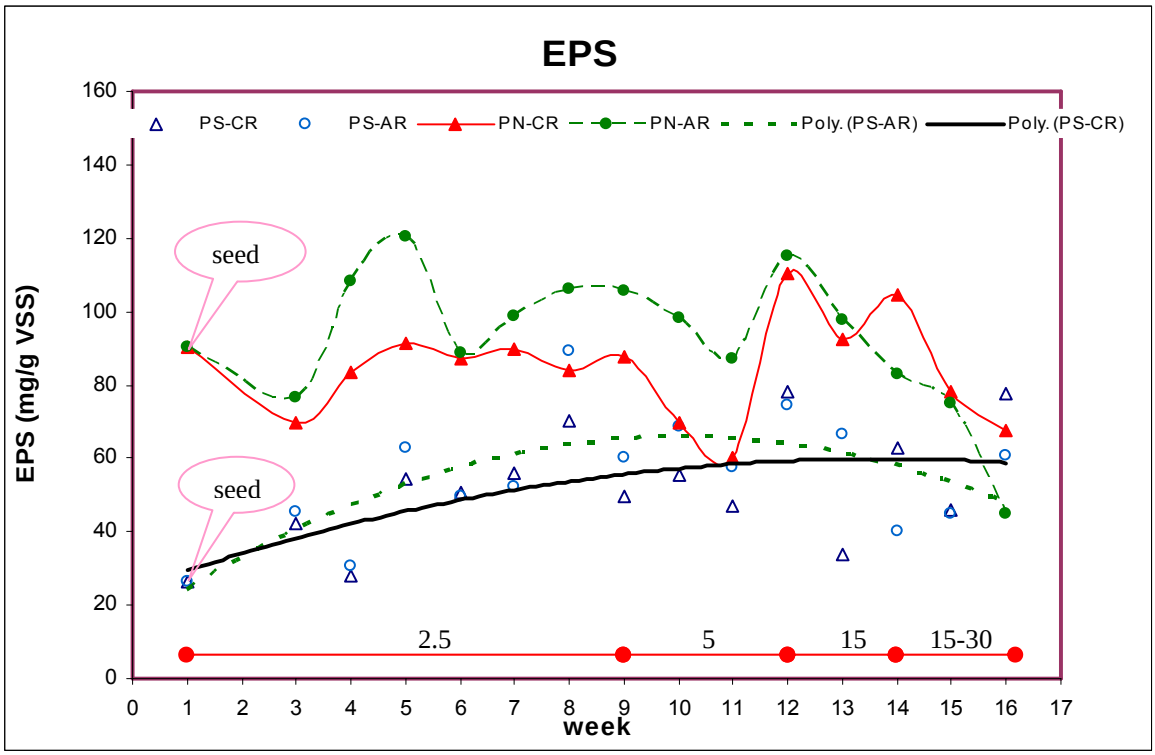



**Figure 4.17a. Bound EPS in CR & AR**

Figure 4.17b,c,d showed relationship of EPS component with OLR. Likewise, figure 4.17c showed difference between bound PS of seed sludge and granular sludge. Bound PS of granule was estimated about two fold higher than that of seed sludge at each OLR while bound PN was almost same.

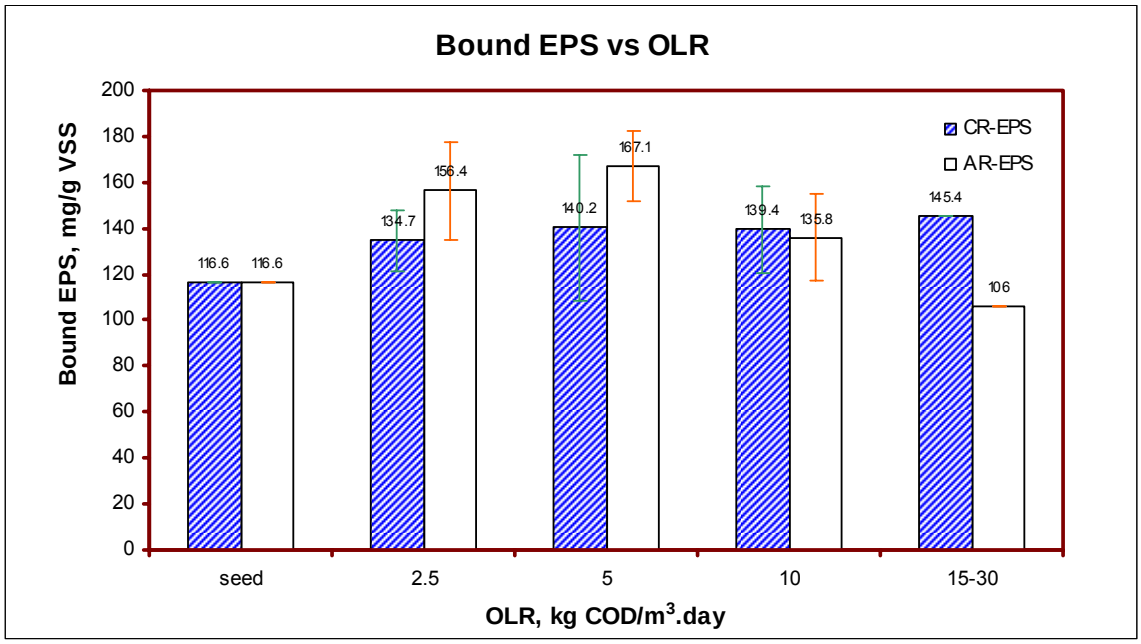



**Figure 4.17b. Bound EPS vs OLR in CR & AR**

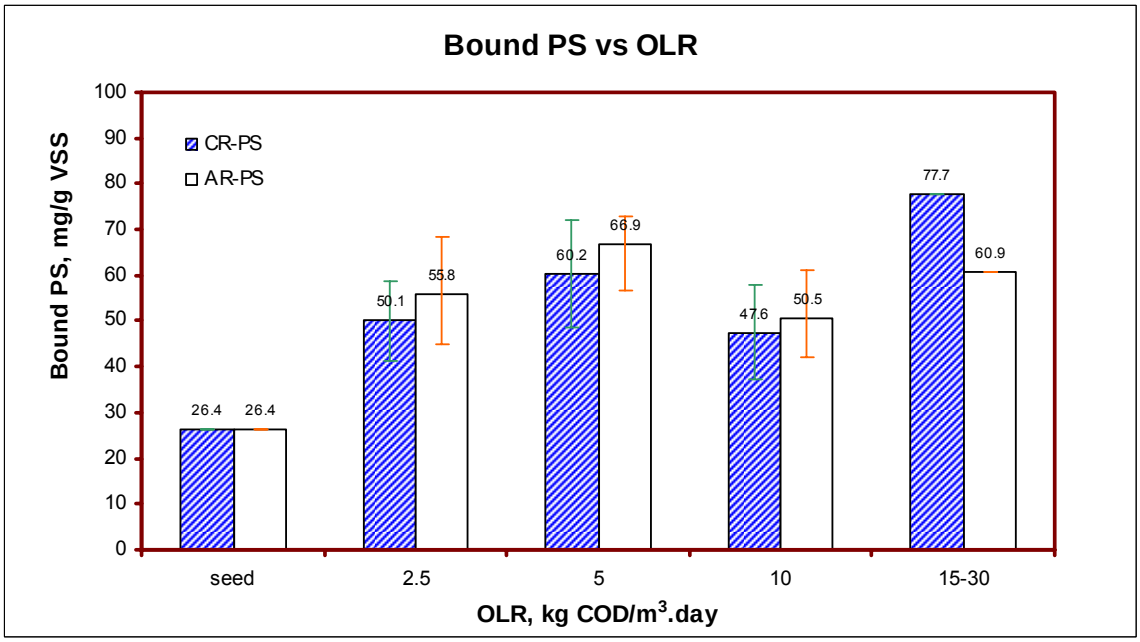



**Figure 4.17c. Bound PS vs OLR in CR & AR**

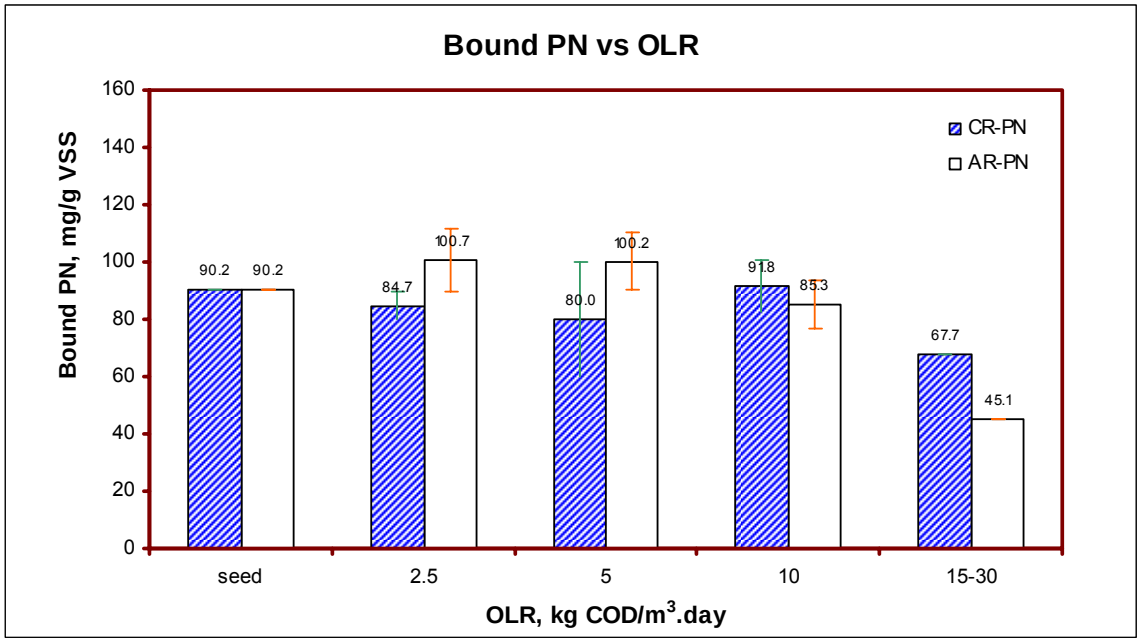



**Figure 4.17d. Bound PN vs OLR in CR & AR**

Even though, there was a difference in values of PS and PN in CR and AR throughout the time but the ratios of PS to PN (PS/PN) were not different in both reactors as figure 4.18. The ratio seemed to increase as matured granules formed (seed sludge 0.3 and when OLR of 30 kg COD/$m^3$.day, it was from 0.5 to 0.9). The PS/PN ratio increased due to the increase of PS because PN was almost constant according to the time. This could confirme that under high shear conditions polysaccharides was generated to link cell to cell to form granules (modified from Liu and Fang, 2002).

When OLR reached to 30 kg COD/$m^3$.day, the PS/PN increased 1.1 and 1.4 for CR and AR respectively. It happened due to PN decreased while PS did not change much as figure 4.17. The PS/PN ratio in this research was almost lower than 2 which was less than some results of Tay. It was low due to the heat extraction method that was recognized to have protein leakage from cells (Frølund et al., 1996; Wingender et al., 1999). When protein was high due to cell leakage, it made PS/PN ratio low.

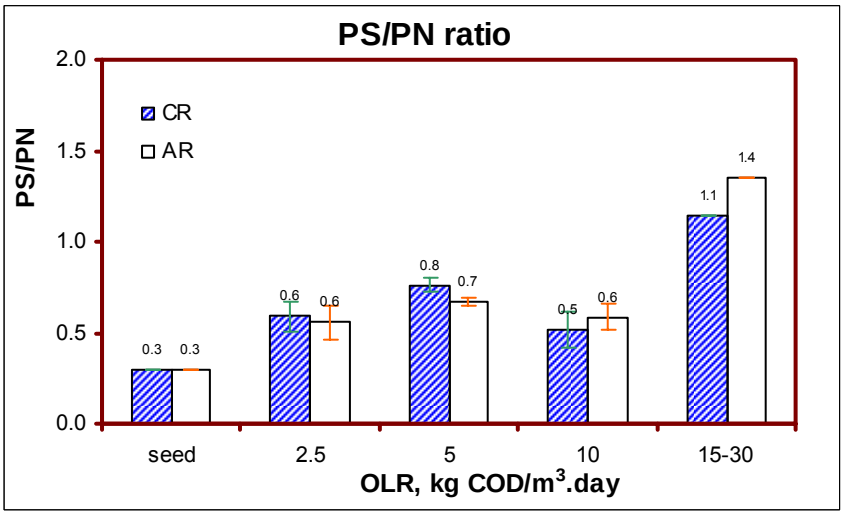



**Figure 4.18. PS/PN ratio in CR & AR**

#### *4.1.6.9. Metal Element in Granular Sludge*

**Table 4.1. Metal elements in granules**

| Reference | Sludge | Ca (mg/g) | Mg (mg/g) | K (mg/g) | Na (mg/g) | Fe (mg/g) |
|---|---|---|---|---|---|---|
| This study | Seed | 3.4 | 0.9 | 2.7 | 1.0 | 5.1 |
| | CR 2.5 | 15.5 | 2.4 | 6.9 | 1.2 | 6.4 |
| | AR 2.5 | 4.1 | 1.8 | 5.3 | 0.8 | 7.3 |
| | CR 5 | 22.3 | 3.7 | 9.9 | 9.9 | 1.0 |
| | AR 5 | 3.4 | 1.4 | 7.6 | 3.2 | 5.7 |
| | CR 15 | 3.3 | 0.9 | 6.0 | 3.2 | 1.0 |
| | AR 15 | 1.5 | 0.9 | 6.4 | 4.5 | 0.6 |
| Liu et al., 2003 | C:N=100:5 | 4.2 | 1.3 | 3.0 | 3.3 | 1.8 |
| Liu et al., 2003 | C:N=100:10 | 2.3 | 0.7 | 1.6 | 1.1 | 0.43 |
| Wang et al., 2004 | | 45.7 | 2.58 | 43.58 | 8 | 0.76 |
| Qin et al., 2004 | | 20.4-187.6 | - | - | - | - |

From table 4.1, Calcium in granules was higher than that in seed sludge at any loading rate. Iron and Magnesium decreased when loading rate increased. Potassium was same as each loading rates and almost higher than seed sludge. Sodium in granules seemed higher than that in seed sludge.

Based upon the results of this study as well as other authors, Calcium was no longer the main factor taking a main part in granule formation but it could accumulate very high in granules accounted up to 187.6 mg/g VSS (as Qin et al., 2004). Finally, granule formation was due to shear stress of air flowrate and settling time of granulation reactor.

### 4.1.7. Aerobic Granule Treatability

Organic matter as glucose was used to feed to culture aerobic granules in CR and AR at loading rate 2.5 kg COD/$m^3$.day. After that, organic loading was increased to 5, 10, 15, and 30 kg COD/$m^3$.day. In case of loading rate of 2.5, 5 kg COD/$m^3$.day correlative with influent COD of 600 and 1200 mg/L, effluent COD was always less than 30 mg/L (figure 4.19). The efficiency at these OLRs was always greater than 96 percent and normally 98 percent (figure 4.20). Moreover, most of organic matter was biodegraded or absorbed by granules only after ten minutes of aeration in both reactors. When increasing to OLR of 10, 15, 30 kg COD/$m^3$.day respectively with influent COD of 3600, 5400, 7200 mg/L, COD removal efficiency was also about 96-99 percent. In fact, system could be operated higher than OLR of 30 kg COD/$m^3$.day but the system was often got clogging due to high viscous and concentrated feeding. So that OLR was stopped at value of 30 kg COD/$m^3$.day.

This meant that aerobic granules had excellent treatability in comparison with conventional activated sludge which was only suitable with loading rate less than 2 kg COD/$m^3$.day. All in all, the effectiveness of granular sludge was 15 fold of conventional activated sludge.

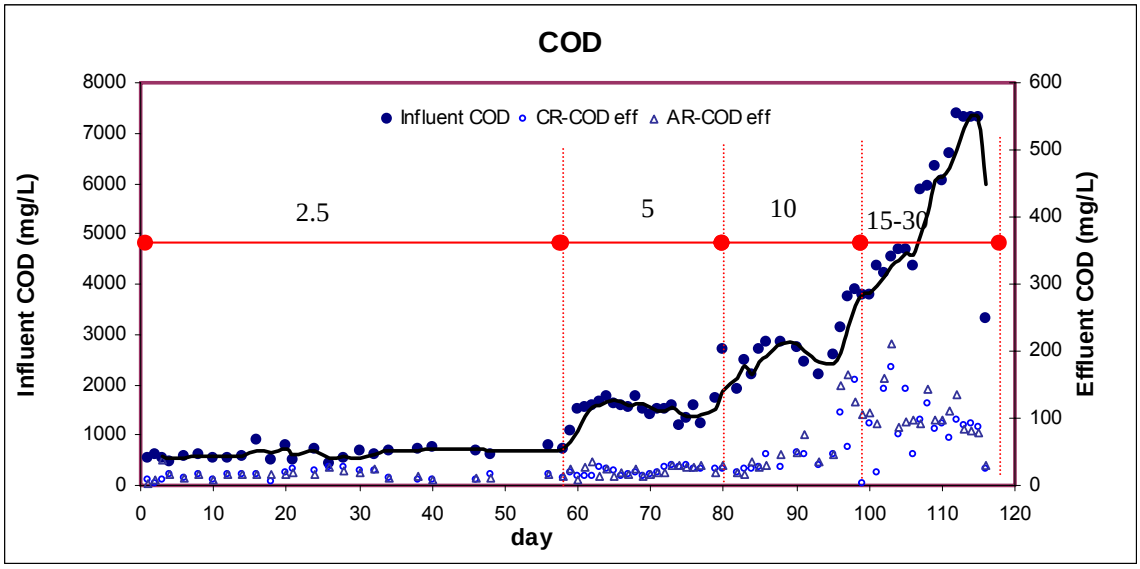



**Figure 4.19. Feed COD and effluent COD in reactors**

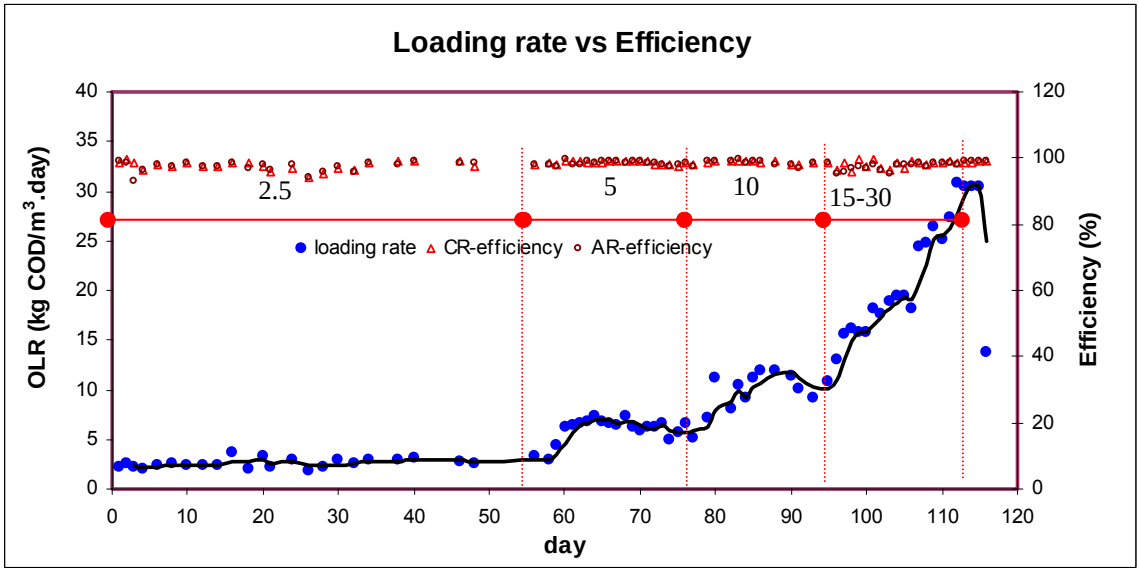



**Figure 4.20 Loading rate vs efficiency**

The correlation between OLR and biomass in SBARs was described in table 4.2 and figure 4.21. On average, F/M ratio in both reactors was within 0.57-2.4 kgCOD/kgVSS.day$^{-1}$ which was relatively seven fold higher than that in conventional SBR (0.05-0.3 kgBOD/kgVSS.day$^{-1}$, Metcaft and Eddy, 1991). The ratio was almost proportional to OLR in both reactors.

**Table 4.2. Food to Microorganism Ratio at each OLR**

| OLR gCOD/$m^3$.day | $S_o$ mg COD/L | Biomass conc. mg/L | | F/M $day^{-1}$ | | | |
|---|---|---|---|---|---|---|---|
| | | CR | AR | CR | average | AR | average |
| 2.5 | 616 | 5124 | 2947 | 0.50 | 0.57 | 0.87 | 0.96 |
| | 752 | 5169 | 2905 | 0.61 | | 1.08 | |
| | 790 | 5450 | 3484 | 0.60 | | 0.94 | |
| 5 | 1516 | 5805 | 4988 | 1.09 | 0.97 | 1.26 | 1.26 |
| | 1346 | 7135 | 3943 | 0.78 | | 1.42 | |
| | 1100 | 4414 | 4167 | 1.04 | | 1.10 | |
| 10 | 2438 | 8086 | 7551 | 1.25 | 1.07 | 1.34 | 1.15 |
| | 2591 | 12303 | 11346 | 0.88 | | 0.95 | |
| 15-30 | 6038 | 10479 | 14696 | 2.40 | 2.40 | 1.71 | 1.71 |

Based on table 4.2, Under OLR of 30 kg COD/$m^3$.day, F/M = 2.4 was rather high because at this moment reactor was clogged and sludge could not circulate so that amount of granule was withdrawn for system operation. It was explained that at this OLR MLVSS was reduced from 12303 to 10479 mg/L. Therefore, it made F/M ratio high.

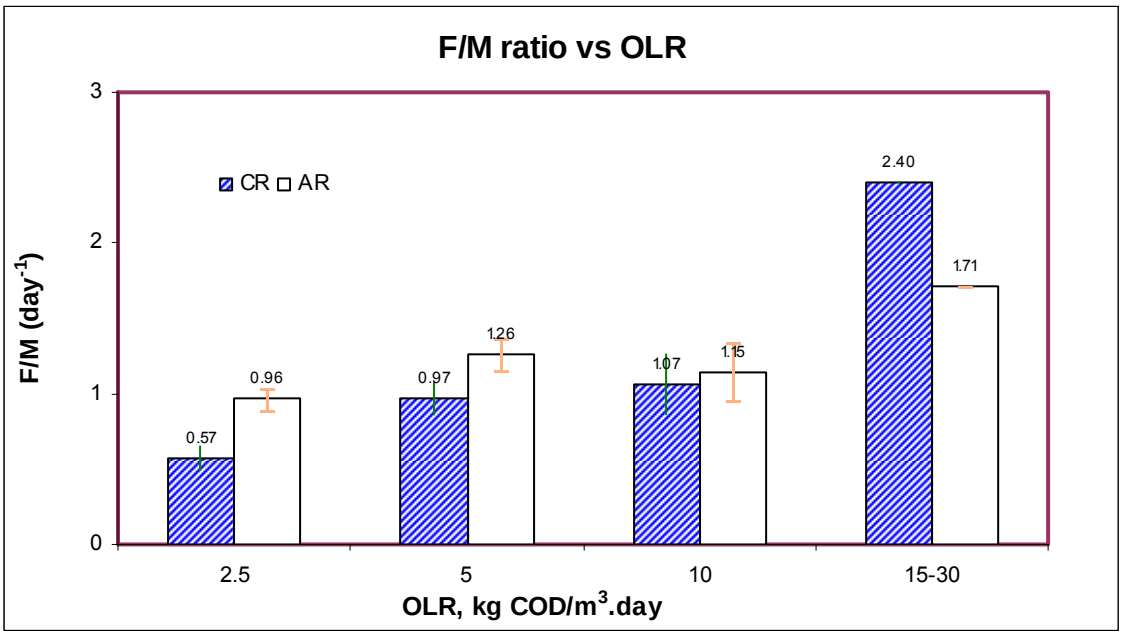



**Figure 4.21 Food to Microorganism ratio at different OLRs (kg COD/$m^3$.day)**

The result revealed that granular sludge could work from low to high F/M ratio. The ratio was rather high with COD removal efficiency greater than 96% in all cases. It explained why granular sludge could reach to OLR of 30 kg COD/$m^3$.day.

Moreover, It was also confirmed by biological activity of granular sludge in section 4.19 that specific oxygen uptake rate of granular sludge was at least 3 fold greater than conventional activated sludge. Because of this, the applied F/M ratio of granular sludge was no longer similar with conventional activated sludge. This meant that whenever carrying out design for granulation system, F/M design parameter would be in range of 0.57-2.4 kg COD/kgVSS.$day^{-1}$ for aerobic granular sludge system.

### 4.1.8. Shock loading in Granule Reactor

Aerobic granular sludge was operated with high loading rate up to 9 kg COD/$m^3$.day (Tay et al., 2001) and 15 kg COD/$m^3$.day (Moy et al., 2002). All these results were conducted in normal loading condition that loading rate was increased progressively in step by step. However, in order to identify the effect of shock load with granules. These series of experiment was conducted within OLR of 2.5 to 10 kg COD/$m^3$.day. The test was performed by changing the organic loading in terms of glucose COD. Loading was sharply increased from OLR of 2.5 to 5 kg COD/$m^3$.day but gradually from OLR of 5 to 10 kg COD/$m^3$.day.

Once matured granules were major in reactors, COD of feed wastewater was increased sharply from 600 to 1200 mg/L and slowly step by step from 1200 to 2400 mg/L.

There was a significant change with AR granule characteristics. AR granules were worn, mostly disintegrated (granule diameter was reduced from 0.2-1.5 mm) to (0.1-0.5 mm). Therefore, biomass was washed out significantly and settled biomass concentration decreased from 25 to 20 g/$L_{granule}$ when increasing to loading rate of 5 kg COD/$m^3$.day but CR granules was normal. But when increasing to 10 kg COD/$m^3$.day with progressive increase of COD, AR granules had signal of recovery. At this moment, AR granule size was about 0.15 – 0.65 mm and biofilm was generated and covered on reactor walls. Then biofilm was removed manually, after that 2 days AR granules suddenly became bigger and major with size within 1.2 – 3 mm (average 1.8 mm). The detail presented in table 4.3.

**Table 4.3. Shock loading test of granular sludge**

| OLR (kgCOD/$m^3$.day) | Observation | CR | AR |
|---|---|---|---|
| Steady state of 2.5 (Matured granules in reactors) | Granule size (mm) | 0.3 – 0.9 | 0.2 – 1.5 |
| | BM conc. (g/L) | 25 | 24.1 |
| | SVI, mL/g | 18 | 35 |
| | Hydrophobicity (%) | 84 | 86 |
| | Color | reddish yellow | Pale reddish yellow |
| From 2.5 to 5 (Increased sharply) | Granule size (mm) | 0.25 – 1.5 | 0.1 – 0.5 |
| | BM conc, g/L | 25.2 | 20 |
| | SVI, mL/g | 23 | 33 |
| | Hydrophobicity (%) | 79 | 76 |
| | Color | reddish yellow | bright yellow |
| | Phenomenon | Normal development condition | Foaming produced strongly Granules worned, smaller, biomass washed out significant |
| From 5 to 10 (Increased slowly step by step) | Granule size (mm) | 0.5 – 1.6 | 1.2 – 3 |
| | BM conc. (g/L) | 46.5 | 51.4 |
| | SVI, mL/g | 15 | 21 |
| | Hydrophobicity (%) | 66 | 67 |
| | Color | reddish yellow | bright white yellow (palest) |
| | Phenomenon | Granule was in normal development conditions | Granules grew very fast after 2 days of biofilm removal, even size distribution |

* COD removal efficiency greater than 97 %; BM conc.: Settled biomass concentration

Based on above test, it could be stated that:

(1) Both types of granules could suffer with high loading rate but CR granule could withstand with sharp shock loading in term of increase of COD more effectively than AR ones. AR was only suitable for progressive increase of loading rate.

(2) There was a clear competition between granulation and attached growth (same result with Beun et al., 2002). After biofilm was removed for two days, AR granules grew significantly fast and significantly as observation at OLR of 10 kg COD/$m^3$.day (granule diameter increased from 0.1-0.5 mm to 1.2-3 mm). So to stimulate aerobic granule formation, biofilm should be removed to avoid the competition between granulation and biofilm development. Moreover, granules could recover quickly after disturbance.

(3) Granule cultivation was more suitable with high OLR as same as Tay et al., 2001.

### 4.1.9. Kinetic data of granules

Specific growth rates (μ), yield coefficient (Y) of granules were measured by respirometric method. Biokinetic study was conducted for matured granules at OLR of 10 kg COD/$m^3$.day. The results were shown in table 4.4, 4.5 and figure 4.22, 4.23.

**Table 4.4. Kinetic data for CR granules**

| Substrate volume, mL | | 1.25 | 1.50 | 1.75 | 2.00 | 3.00 | 5.00 | 6.00 |
|---|---|---|---|---|---|---|---|---|
| S | mg COD/L | 10.7 | 12.7 | 14.8 | 16.8 | 25.2 | 41.5 | 49.3 |
| S/X | mg/mg | 0.003 | 0.003 | 0.004 | 0.004 | 0.007 | 0.011 | 0.013 |
| Rx,e | mg $O_2$/L.h | 6.8 | 7.2 | 6.8 | 6.8 | 6.8 | 6.7 | 6.7 |
| Rx,t | mg $O_2$/L.h | 26.5 | 29 | 31.2 | 34.9 | 37.3 | 35.7 | 36.8 |
| Rx,ox | mg $O_2$/L.h | 19.7 | 21.8 | 24.4 | 28.1 | 30.5 | 29 | 30.1 |
| Rx,ox | mg $O_2$/g.h | 5.2 | 5.8 | 6.5 | 7.5 | 8.1 | 7.7 | 8.0 |
| OC | mg $O_2$/L | 1.9 | 2.2 | 2.3 | 2.6 | 3.4 | 4.9 | 6 |
| OC/S | mg$O_2$/mgCOD | 0.18 | 0.17 | 0.16 | 0.15 | 0.13 | 0.12 | 0.12 |
| Rx | mg COD/gVSS.h | 110.94 | 125.85 | 157.01 | 181.57 | 226.06 | 245.61 | 247.32 |
| Y | mg VSS/mg COD | 0.58 | 0.58 | 0.59 | 0.60 | 0.61 | 0.62 | 0.62 |
| μ | $day^{-1}$ | 1.54 | 1.76 | 2.24 | 2.59 | 3.31 | 3.66 | 3.67 |

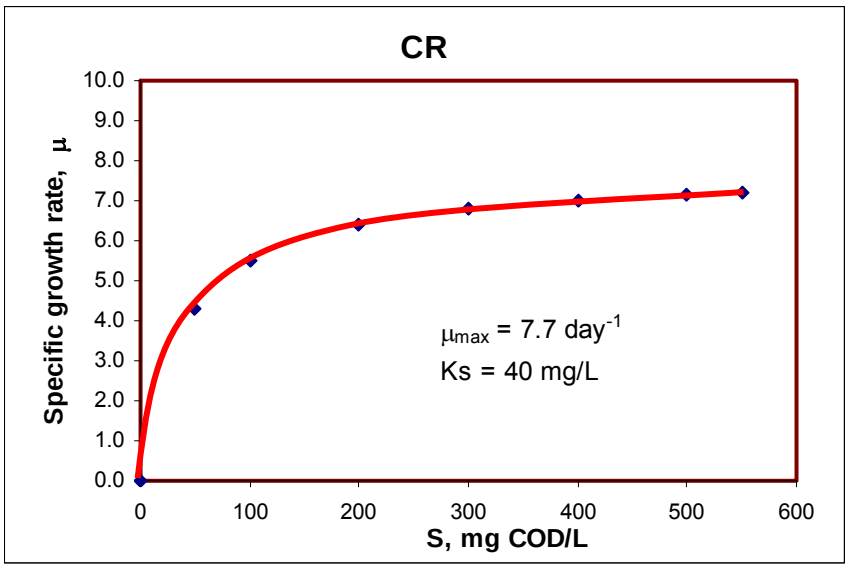



**Figure 4.22. Relationship between substrate and specific growth rate of CR**

**Table 4.5. Kinetic data for AR granules**

| Substrate volume, mL | | 0.50 | 0.75 | 1.00 | 1.25 | 1.50 | 1.75 | 2.00 | 5.00 |
|---|---|---|---|---|---|---|---|---|---|
| S | mg COD/L | 4.3 | 6.4 | 8.5 | 10.6 | 12.7 | 14.8 | 16.6 | 42 |
| $S_o/X_o$ | mg/mg | 0.002 | 0.004 | 0.005 | 0.006 | 0.007 | 0.008 | 0.009 | 0.023 |
| Rx,e | mg $O_2$/L.h | 6.7 | 6.8 | 6.7 | 6.8 | 6.8 | 6.8 | 6.2 | 6.7 |
| Rx,t | mg $O_2$/L.h | 19 | 21.7 | 22.8 | 26.04 | 31 | 31.8 | 31.3 | 54.6 |
| Rx,ox | mg $O_2$/L.h | 12.3 | 14.9 | 16.1 | 19.24 | 24.2 | 25 | 25.1 | 47.9 |
| Rx,ox | mg $O_2$/g.h | 6.75 | 8.18 | 8.84 | 10.57 | 13.29 | 13.73 | 13.78 | 26.30 |
| OC | mg $O_2$/L | 1.06 | 1.6 | 1.9 | 2.4 | 2.8 | 2.82 | 3.3 | 5 |
| OC/S | mg$O_2$/mgCOD | 0.25 | 0.25 | 0.22 | 0.23 | 0.22 | 0.19 | 0.20 | 0.12 |
| Rx | mg COD/gVSS.h | 49.90 | 59.60 | 72.03 | 84.98 | 109.76 | 131.21 | 126.26 | 402.36 |
| Y | mg VSS/mg COD | 0.53 | 0.53 | 0.55 | 0.54 | 0.55 | 0.57 | 0.56 | 0.62 |
| μ | $day^{-1}$ | 0.64 | 0.76 | 0.95 | 1.11 | 1.45 | 1.80 | 1.71 | 5.99 |

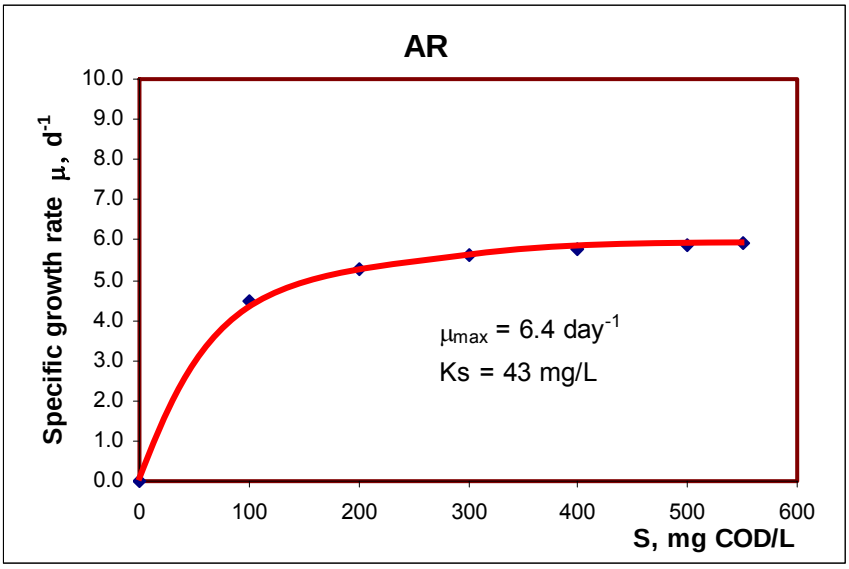



**Figure 4.23. Relationship between substrate and specific growth rate of AR**

**Table 4.6 Biokinetic data of granules and activated sludge**

| Coefficient | CR | AR | granules, Tijhuis et al., 1994 | AS, Metcalf and Eddy, 2003 |
|---|---|---|---|---|
| Y, mgVSS/mg COD | 0.58-0.62 | 0.53-0.62 | 0.34 | 0.4-0.8 |
| Ks, mg COD/L | 40 | 43 | - | 15-70 |
| $\mu_{max,}$ $day^{-1}$ | 7.7 | 6.4 | 6 | - |
| Note | OLR of 10 kgCOD/$m^3$.day | | OLR of 5 kgCOD/$m^3$.day | |

As presented in table 4.6, yield coefficient (Y) of two types of granules was almost similar and fluctuated from 0.53 to 0.62 mgVSS/mgCOD. Those values were higher than that of Tijhuis et al., 1994 because substrate to biomass ratio was rather high (F/M = 0.24 kgCOD/kgVSS.$day^{-1}$ at OLR of 10 kg COD/$m^3$.day). According to Chudoba et al., 1992, it was stated that low yield observed at higher initial $S_o/X_o$ ratio. At OLR of 30 kgCOD/$m^3$.day, $S_o/X_o$ was 0.5 mgCOD/mgVSS which was less than 2 mg/mg so yield coeficient could be rather low. The real yield was 0.44 (as below).

Maximum specific growth rate of CR granules ($\mu_{CR}$ = 7.7 $day^{-1}$) was slightly higher than that of AR ones ($\mu_{AR}$ = 6.4 $day^{-1}$) and both of these granules could have been fixed with Monod's kinetic model without inhibition substances.

**Sludge production:**

Sludge produced = sludge accumulated in reactor + sludge washed out through effluent

Sludge production test was carried out by measuring initial biomass in CR of day 1, day 2, effluent biomass concentration and COD of feed at OLR of 10 kg COD/$m^3$.day. Additional data was as follows:

| | | |
|---|---|---|
| VSS1 | VSS of day 1 | 8099 mg/L |
| VSS2 | VSS of day 2 | 9110 mg/L |
| VSSe | VSS of effluent | 790 mg/L |
| CODo | COD of influent | 2410 mg/L |
| CODe | COD of effluent | 40 mg/L |
| Remarks | Reactor volume V = 2.5 L; influent flowrate Q= 1.3 L/batch; 8 batches/day | |

Biomass generated per day = (9110-8099) mg/L * 2.5 L + 8 b/d * 1.3 L/b * 790 mg/L = 10,744 mg/day

COD supplied per day = 8 b/d * 1.3 L/b * (2410-40) mg/L = 24,648 mg COD/day

Observed yield was calculated as follows:

$Y_{obs}$ = (10,744 mgVSS/day)/(24,648 mgCOD/day) = 0.436 mgVSS/mgCOD

Assume decay coefficient $k_d$ = 0.06 $day^{-1}$, HRT = θ =5.6 h = 0.233 days, Yield coefficient:

$$Y_{obs} = \frac{Y}{1 + k_d * \theta} \Rightarrow Y = Y_{obs} * (1 + k_d * \theta) = 0.436 * (1 + 0.06 * 0.223) = 0.442 mgVSS / mgCOD$$

Real yield was 0.44 which was less than average value obtained by respirometric method (0.6 mg/mg) but rather close to value of Tjhuis et al, 1994 (0.34 mg/mg) in table 4.6. This difference could be error due to limitations of the used respirometer. This confirmed that aerobic granule operated at high OLR produced sludge as same as conventional activated sludge at OLR of 10 kg COD/$m^3$.day.

**Specific oxygen uptake rate (SOUR) of granules was shown in figure 4.24**

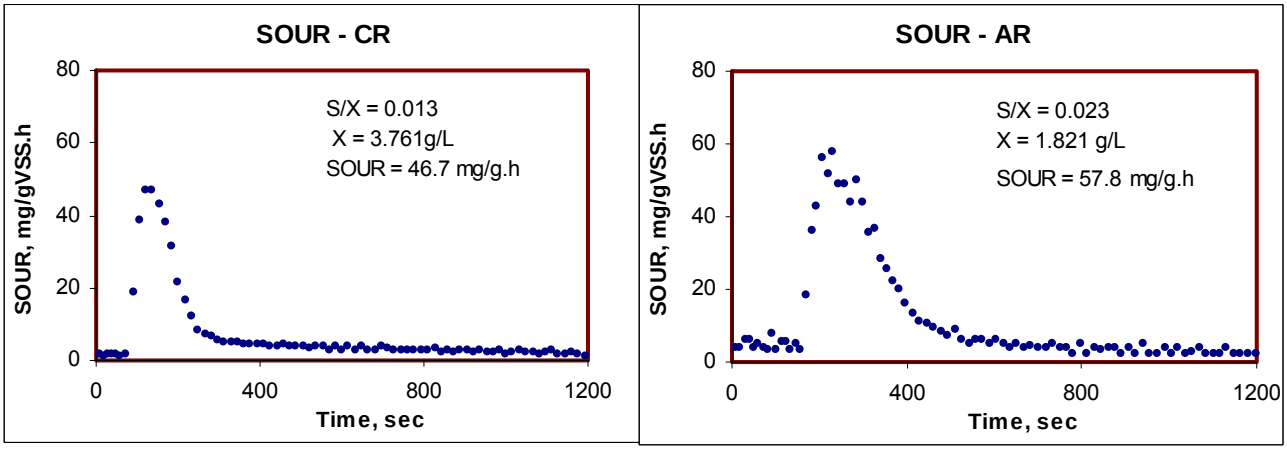



**Figure 4.24. Specific oxygen uptake rate curves of granules**

Based on figure 4.24, SOUR of CR and AR was nearly equivalent with the value of 46.7 and 57.8 mg/g VSS.h. So, carrier did not affect much to granule bioactivity.

**Table 4.7. Comparison of SOUR value**

| Sludge | CR | AR | Granule | AS | Granule |
|---|---|---|---|---|---|
| SOUR, mg/gVSS.h | 46.7 | 57.8 | 41.9 | 18.32 | 69.4 |
| Reference | This study | | Liu et al., 2005 | | Tay et al., 2001 |

From table 4.7, it was critical that bioactivity of aerobic granule was approximately 3 fold greater than that of conventional activated sludge in term of oxygen uptake rate per unit of biomass. This explained why aerobic granules were able to suffer with high OLR up to 30 kg COD/$m^3$.day mentioned above.

## 4.2 Supernatant characterization

In this section, supernatant of granule SBAR and of SBR (Conventional Activated Sludge) was investigated to find out its specific fouling potential with membrane filtration. Parameters such as membrane fouling index (MFI) and soluble EPS (PS, PN) were measured for characterization.

### 4.2.1. Soluble EPS in supernatant

During granule formation process (Run 1, OLR of 2.5 kg COD/$m^3$.day), soluble PS in both reactors was always less than 8 mg/L and PN was nearly zero in effluent. When increasing loading rate, soluble PS and PN of both reactors started increasing. At loading rate of 5 kg COD/$m^3$.day, soluble PN was still zero but PS seemed to increase around 12 mg/L.

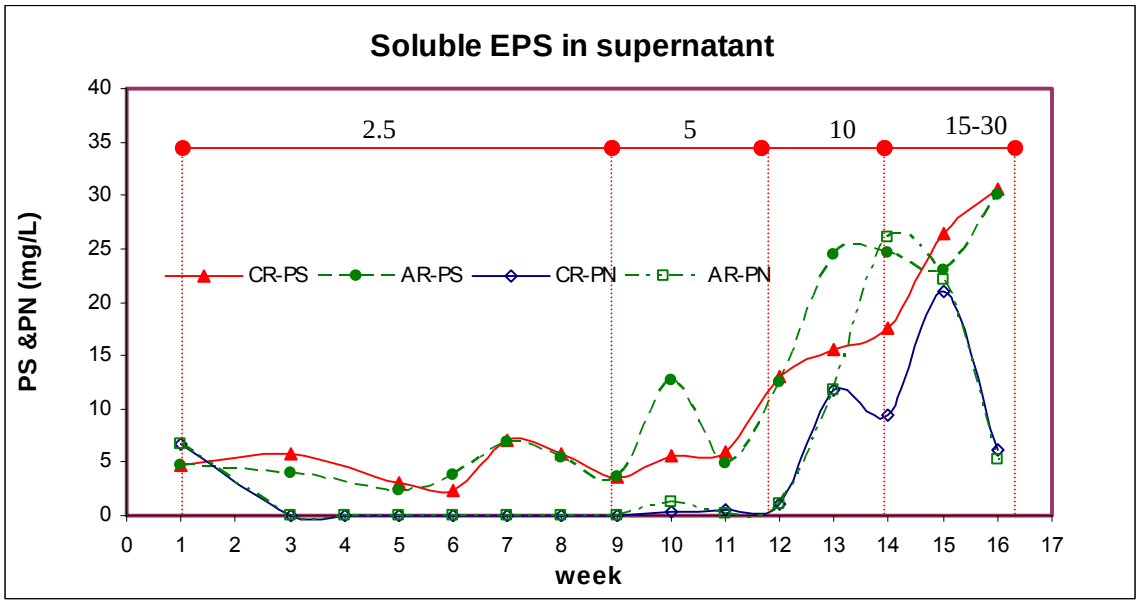



**Figure 4.25. Soluble EPS in supernatant of CR & AR**

### 4.2.2. Membrane Fouling Index

Fouling potential of supernatant at different OLRs was performed to estimate fouling behavior of supernatant. Effluent was settled 45 minutes and supernatant was decanted for membrane fouling index measurement.

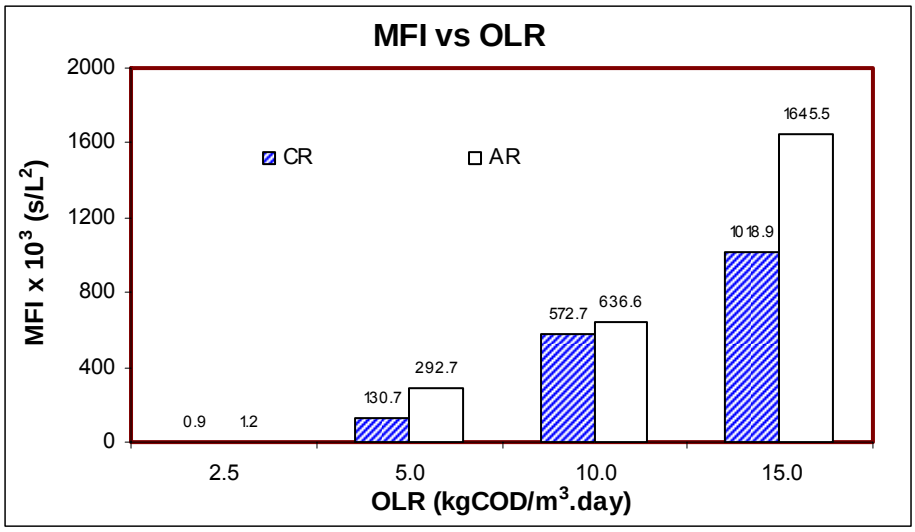



**Figure 4.26. Membrane fouling index of supernatant of CR & AR**

**Table 4.8. Comparison of soluble EPS in supernatants at different OLRs**

| OLR | 2.5 | | 5 | | 10 | | 15 | |
|---|---|---|---|---|---|---|---|---|
| Reactor | CR | AR | CR | AR | CR | AR | CR | AR |
| PS (soluble) | 4.6 | 4.4 | 8.3 | 12.5 | 19.9 | 24.4 | 30.6 | 30 |
| PN (soluble) | 0 | 0 | 0.6 | 0.8 | 14.1 | 20.0 | 6.2 | 5.3 |
| EPS (soluble) | 4.6 | 4.4 | 8.9 | 13.3 | 34.0 | 44.4 | 36.8 | 35.3 |
| % PS/EPS | 100 | 100 | 93 | 94 | 59 | 55 | 83 | 85 |
| | 84 ± 13 % | | | | | | | |

* PS, PN is average value at OLRs, unit mg/L

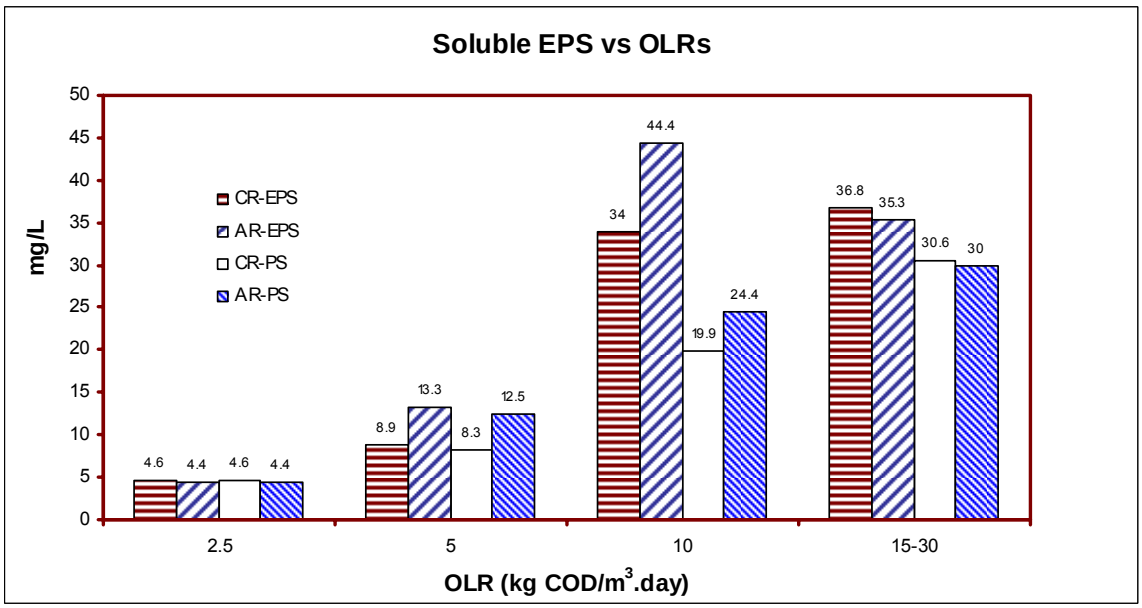



**Figure 4.27. Soluble EPS of supernatant at OLRs**

From figure 4.27, it was stated that:

(1) Fouling potential was proportional with the increase of organic loading rate. At loading rate 2.5 kg COD/$m^3$.day, there was almost no fouling happened because MFI was $0.9x10^3$ and $1.2x10^3$ s/$L^2$ for CR and AR, respectively. These values were very close to the value of distilled water ($0.2x10^3$ s/$L^2$) (figure 4.26). In fact, fouling caused by both soluble EPS (polysaccharides, protein) and cake layer of new generated biomass that were increased with the increase of loading rate. Moreover, Table 4.8 showed that when OLR increased, EPS increased respectively with majority of PS which was 84 ± 13 % of soluble EPS. It could state that fouling was mainly caused by soluble Polysaccharides.

(2) Fouling of CR was always less than that of AR at any loading rate (MFI less than 10-48%). This could be explained due to higher settling ability of CR effluent biomass, so after settling duration, suspended biomass in CR supernatant was less than that in AR and cake layer on membrane of CR was less than that of AR. Therefore, fouling of CR supernatant was lesser than AR one. Moreover, soluble EPS in both supernatant was nearly equal so that it did not affect much fouling potential in this case.

(3) Based on this result, it could be found that carrier also played an important role in fouling reduction.

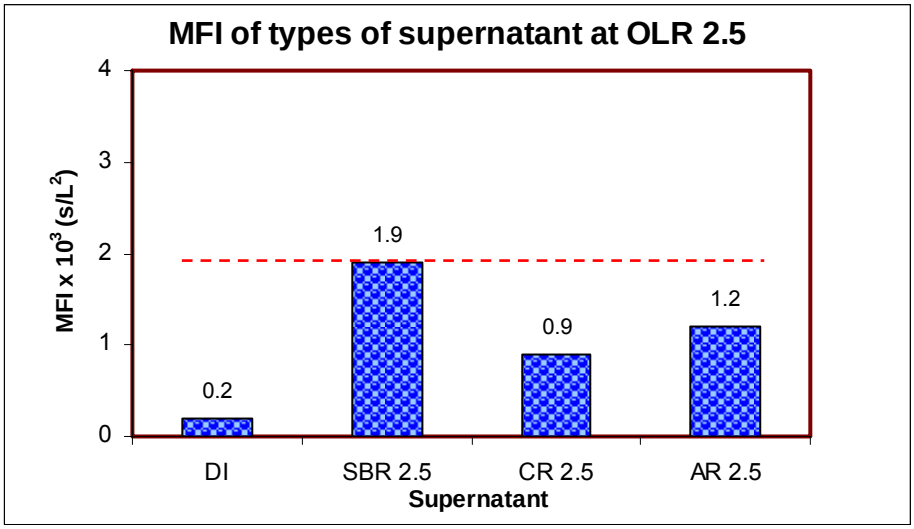



**Figure 4.28. MFI of type of supernatant at OLR 2.5 & distilled water**

Figure 4.28 showed that at OLR of 2.5 kg COD/$m^3$.day, supernatant of SBR (supernatant from conventional activated sludge process) caused more fouling than that of CR and AR but those supernatant had less fouling in comparison with other loading rates (5, 10, 15-30 kg COD/$m^3$.day). At low loading rate, effluent biomass concentration was lower than 300 mg/L in both reactors and effluent PS was lower than 8 mg/L.

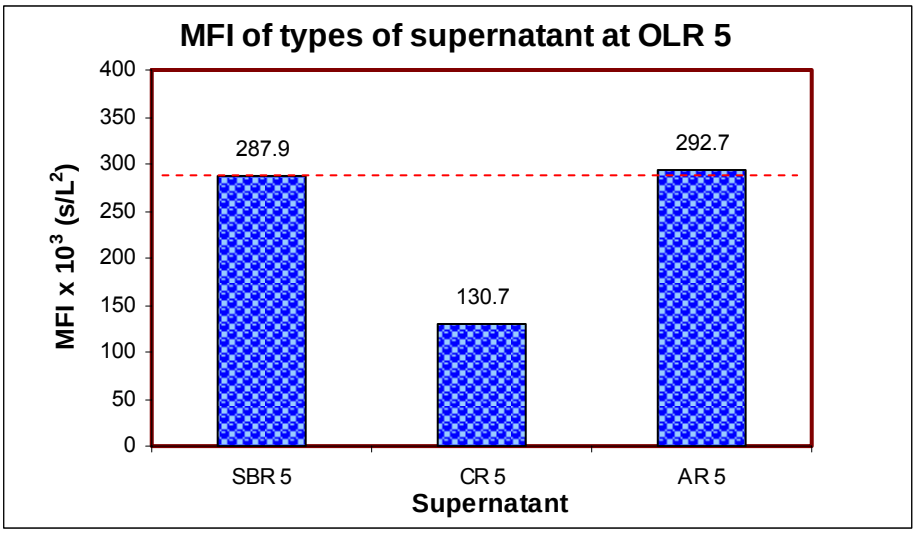



**Figure 4.29. Membrane fouling index of type of supernatant at OLR 5**

At OLR of 5 kg COD/$m^3$.day (figure 4.29), MFI of SBR supernatant was higher than that of CR but nearly similar with that of AR. So, it was clearly that at loading rate less than 5 kg COD/$m^3$.day (maximum loading rate of conventional activated sludge process), supernatant of carrier granular sludge had least fouling effect as compared with granular sludge without carrier and conventional activated sludge.

**Table 4.9. Comparison of soluble EPS in supernatants at OLR of 5 kg COD/$m^3$.day**

| Reactor | CR5 | AR5 | SBR5 |
|---|---|---|---|
| PS (soluble) | 8.3 | 12.5 | 24.2 |
| PN (soluble) | 0.6 | 0.8 | 0.44 |
| EPS (soluble) | 8.9 | 13.3 | 24.6 |

* PS, PN is average value at OLR of 5 kg COD/$m^3$.day, unit mg/L

The reason why CR, AR supernatant was less fouled than SBR (conventional activated sludge process) could be explained as follows:

(1) Soluble EPS in supernatant including polysaccharides and protein of CR, AR was less than that of SBR (table 4.3). Polysaccharides (Frank and Belfort, 2003) and protein (Chan and Chen, 2004) caused membrane fouling.

(2) Effluent biomass of CR and AR was seemingly higher settling ability than that of SBR so that after settled, most of biomass was removed out of supernatant and this led to reducing cake layer on membrane surface.

In addition, there was correlation between EPS, PS, PN and fouling potential at each OLR as illustrated in figure 4.30:

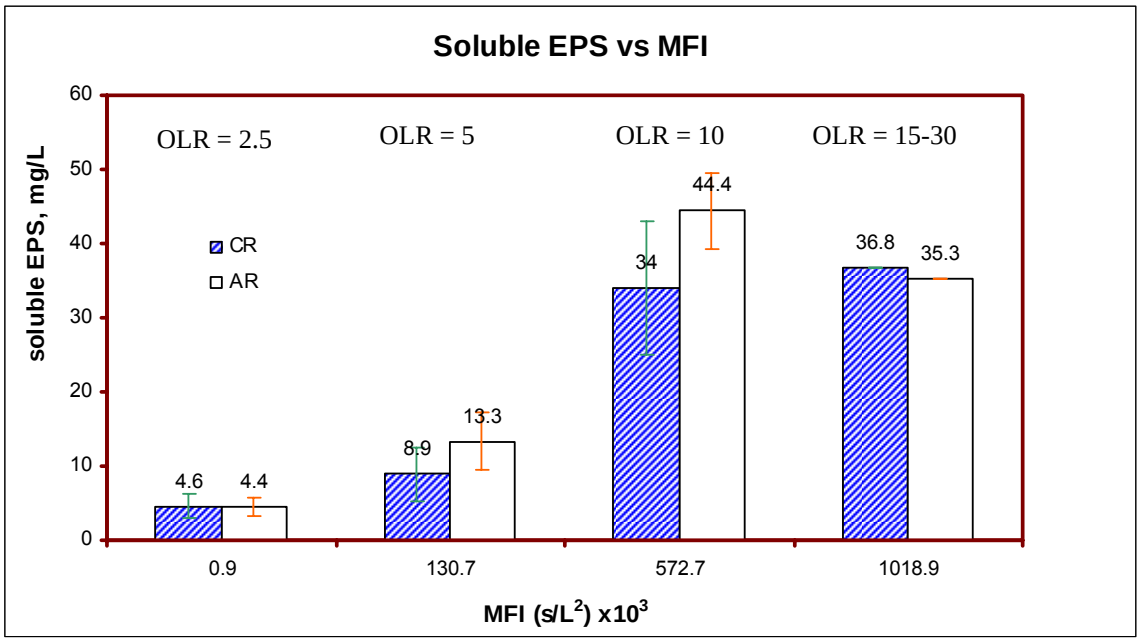



**Figure 4.30: Correlation between soluble EPS and MFI at each OLR**

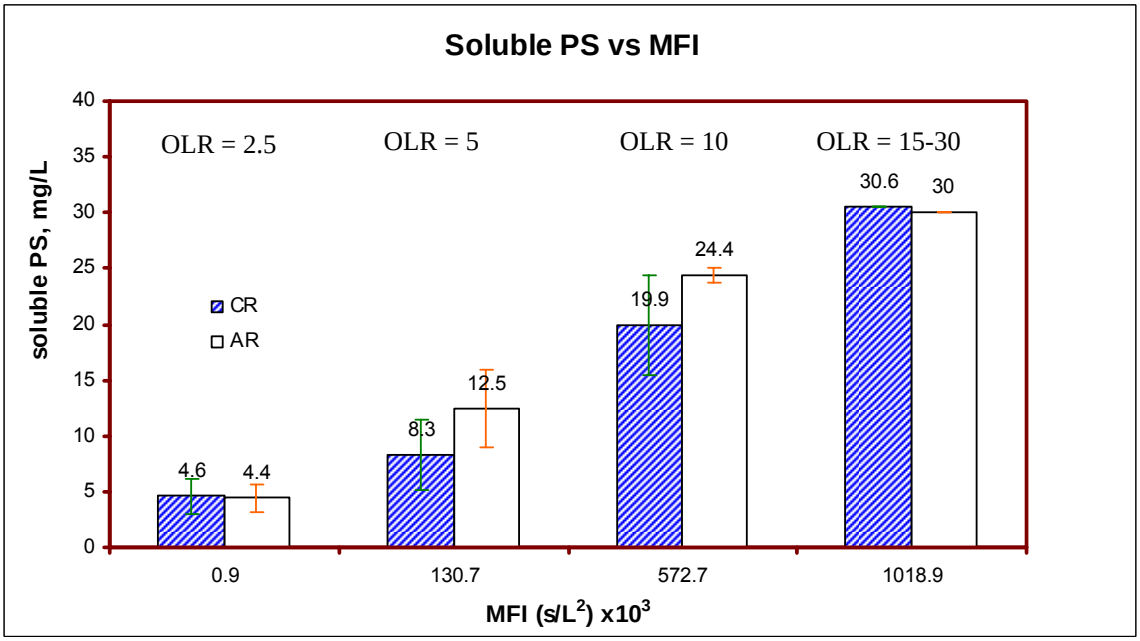



**Figure 4.31: Correlation between soluble PS and MFI at each OLR**

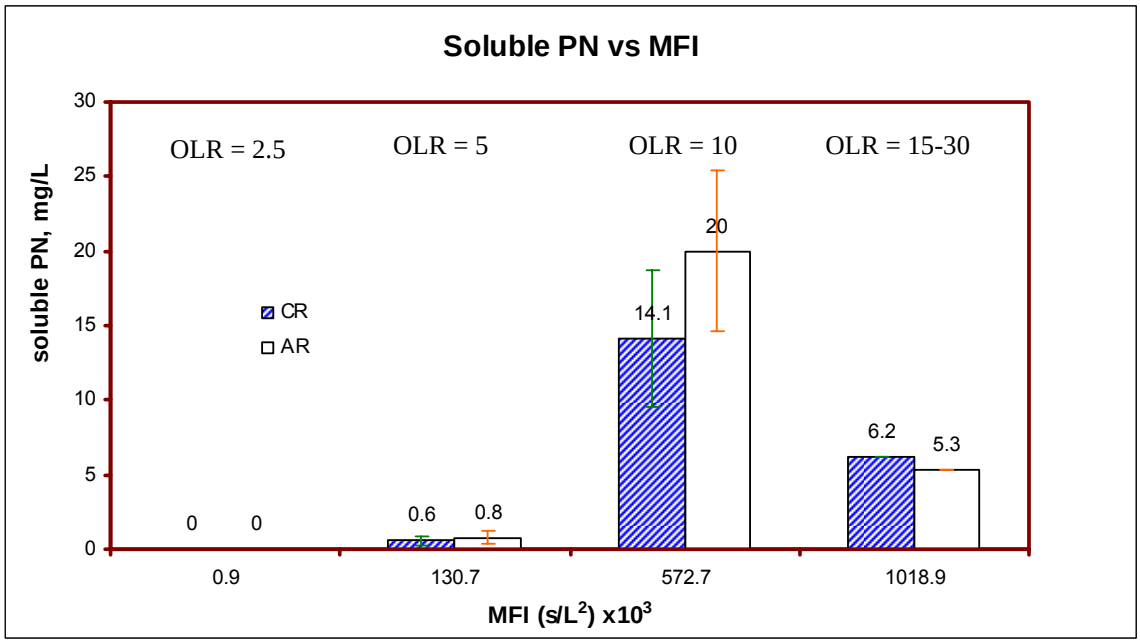



**Figure 4.32: Correlation between soluble PN and MFI at each OLR**

Based on figure 4.30, 4.31 and 4.32, there was an obvious correlation between MFI and EPS, PS, PN, especially MFI with EPS and PS. MFI was proportional to the increase of EPS and PS as well when OLR was rising. From figure 4.27 and table 4.9, PS occupied 84 ± 13 percent of EPS. Therefore, PS was considered as significant foulant among EPS components.

Membrane fouling index of CR1, CR2 supernatant when starting with MBR in table 4.10:

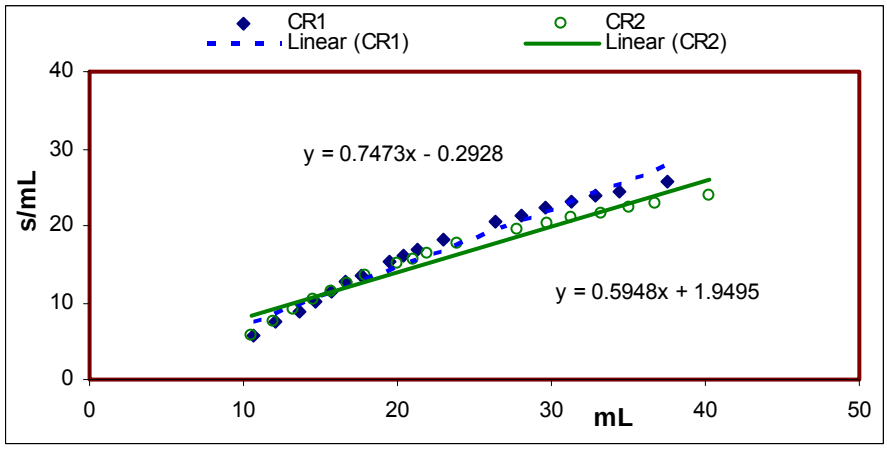



**Figure 4.33. Membrane fouling index of CR1, CR2 supernatant at OLR 10**

**Table 4.10: MFI of settled supernatant of CR1, CR2**

| Reactor at OLR of 10 kg COD/$m^3$.day | MFI, $s/L^2$ |
|---|---|
| CR1 | 747.3 x $10^3$ |
| CR2 | 594.8 x $10^3$ |

## 4.3 Aerobic Granule Membrane Bioreator

In this section, membrane units were connected with SBAR to examine treatment ability of granule coupling with membrane bioreactor. Here onward, bivalve shell carrier was selected as optimum support media with OLR of 10 kg COD/$m^3$.day in both reactors.

Treatment sequence I, CR1, worked as batch system and supernatant from SBAR was settled and pumped through filtration unit and Treatment sequence II, CR2, did as continuous system which membrane was inserted inside the raiser tube of SBAR and sucked as conventional submerged membrane.

The both membranes used were ceramic hollow fibre micro-filtration type with the same surface area and filtration cycle was six minutes ON and four minutes OFF (details as section 3.4.2 of Methodology). Membrane fouling and treatment ability were investigated as below.

### 4.3.1. Granulated Biomass and Suspended Biomass

The test conducted in treatment sequence I, CR1, was to find out the fraction between granulated biomass and total biomass in reactor at run 3. Granules and suspended were separated by leaving sample for settling 10 seconds in centrifugal tube of 50 mL. Hereafter, both of fractions were measured biomass concentration. Data was measured on week 2 and week 3 at steady state of OLR 10 kg COD/$m^3$.day (at run 3)

**Table 4.11: Granulated biomass fraction for CR1 at OLR of 10 kg COD/$m^3$.day**

| Fraction | week 2 | week 3 |
|---|---|---|
| Granulated biomass | 6285 mgVSS/L | 9326 mgVSS/L |
| Suspended biomass | 1059 mgVSS/L | 463 mgVSS/L |
| % granulated biomass over total biomass | 86 % | 95 % |

From table 4.11, most of biomass in reactor at steady state was more than 95 percent of granular sludge and the rest was new biomass generated and “initial granules”. As a matter of fact, more amount of suspended biomass was also initial granules but not matured granules yet. Majority of suspended biomass was washed out at the end of each batch amount of about 600-1200 mg/L due to less settling ability. Hence, it was assumed that most of biomass in reactor was granular sludge.

### 4.3.2. Treatment Ability of Two Treatment Sequences

Treatment sequence I, CR1, was operated in the same manner with granule SBAR in batch mode. Effluent of SBAR was settled and filtered by MF membrane. Here, effluent quality was improved in terms of suspended biomass, turbidity, COD removal (figure 4.34 and table 4.12). Moreover, suspended biomass from settled SBAR effluent was also oxidized as a substrate in external membrane filtration unit, which was considered as membrane bioreactor with a low biomass concentration.

Treatment sequence II, CR2, worked with continuous feeding and membrane was submerged in raiser tube of SBAR. In this case, turbidity and COD removal of CR2 were not much less than CR1 but the fouling happened more seriously (figure 4.36) and almost granules were disappeared after one week of operation.

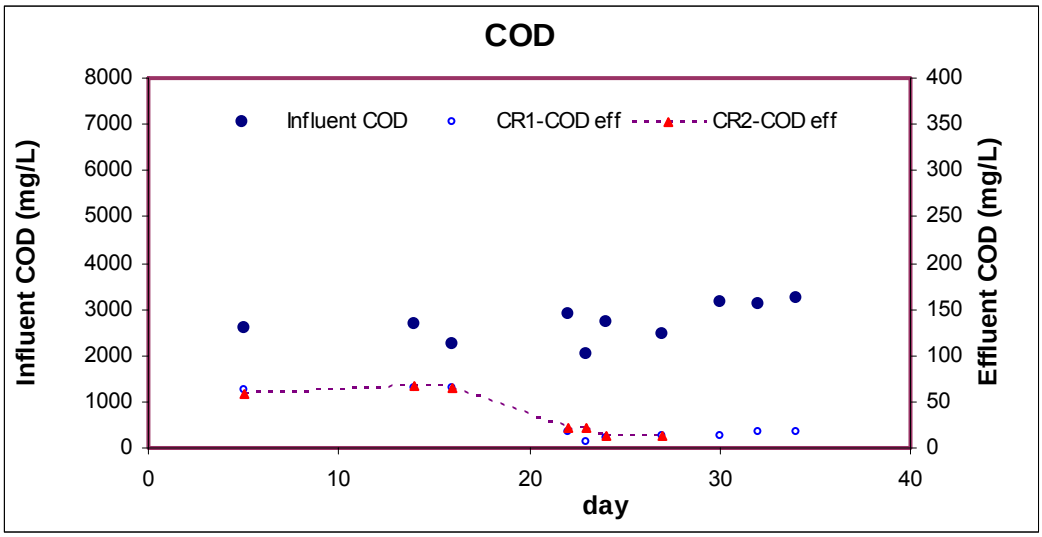



**Figure 4.34. COD influent and effluent CR1, CR2**

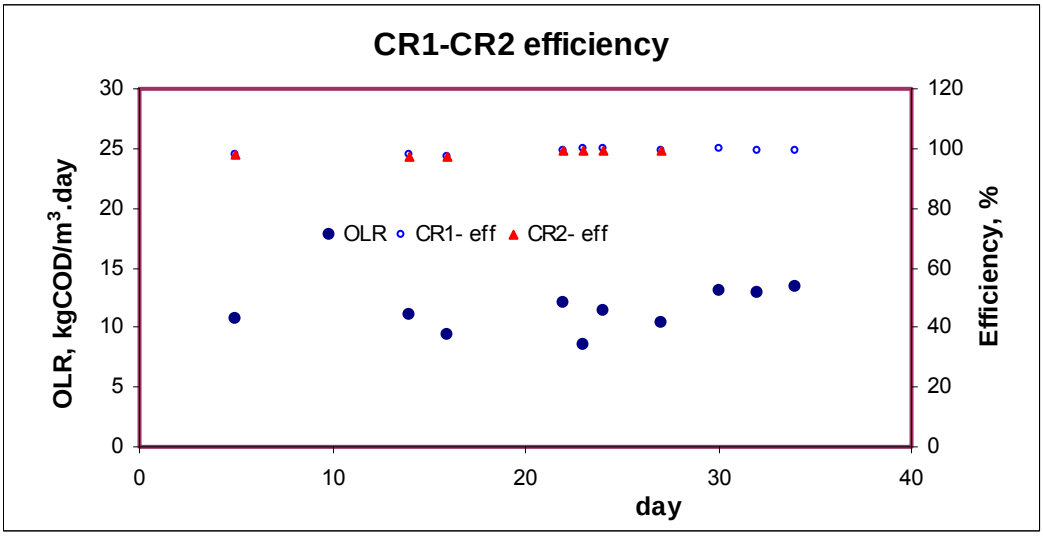



**Figure 4.35. COD removal efficiency of CR1, CR2**

Depending upon figure 4.34 and 4.35, COD removal efficiency was higher than 97 percent and effluent turbidity was often less than 0.8 NTU when coupled with MBR. It meant that any configuration could achieve high efficiency in term of COD removal and no suspended solids. Both systems could be suitable for water reuse and reclamation in real.

### 4.3.3. Granule Characteristics in MBR

The granule characteristics were not changed in CR1 but in CR2. After one week of operation, most of granules were worn and became conventional activated sludge as biomass in submerged membrane bioreactor (conventional MBR). Detail of granule size change in systems is presented in table 4.12.

**Table 4.12. Granule size change in MBRs**

| Sequence | CR1 | CR2 | Remark |
|---|---|---|---|
| time | mm | mm | |
| week 1 | 0.5 – 2.0 | 0.5 – 2.0 | no membrane inserted |
| week 2 | 0.5 - 2.5 | **< 0.1** | granule worn after 2 days |
| week 3 | 0.6 - 4.0 | - | stop CR2 |
| week 4 | 0.6 - 4.5 | - | stop CR2 |

When coupling with MBR, granule size in external system, CR1 became bigger and bigger according to the time. However, in internal system, CR2 it was less than 0.1 mm after two days of operation. This happened due to disturbance of membrane module which was inserted into the raiser pipe of SBAR. This created high physical friction between granules and membrane module and destroyed granules. After that, granular sludge became conventional activated sludge so treatment sequence II, CR2 was stopped.

**Table 4.13. Granule characteristics change in MBRs**

| Parameter | Settled BM conc. ($g/L_{sludge}$) (*) | | SVI (mL/g) | | Settling velocity (m/h) | | Remark |
|---|---|---|---|---|---|---|---|
| Sequence | CR1 | CR2 | CR1 | CR2 | CR1 | CR2 | |
| week 1 | 39 | 38 | 18 | 20 | 75 | 70 | no membrane |
| week 2 | 39 | 7 | 28 | 122 | 51 | < 10 | granules disappeared |
| week 3 | 41 | - | 20 | - | 62 | - | stop CR2 |
| week 4 | 48 | - | 21 | - | 65 | - | stop CR2 |

(*) BM conc.: Biomass concentration;

Moreover when granule worn, settled biomass concentration was also reduced to 7 $g/L_{sludge}$ which was nearly similar with seed sludge of 2.7 $g/L_{sludge,}$ SVI increased up to 122 mL/g and settling velocity was less than 10 m/h. Because of this, granules in CR2 was completely became conventional activated sludge. From this result, the fourth objective was obtained and the external system, CR1 became much more attractive for sludge granulation coupled MBR.

### 4.3.4. Fouling Behaviour

Fouling rate of system was shown in term of variation of transmembrane pressure (TMP). In figure 4.36, TMP of CR1 was increased to 60 kPa after 7 days but that of CR2 to 95 kPa after only one day. It was clearly shown that CR1, external membrane system was much more attractive for coupling with granulation reactor. Filtration flux of membrane was 14.4 $L/h.m^2$ with cycle 6 ON/4 OFF.

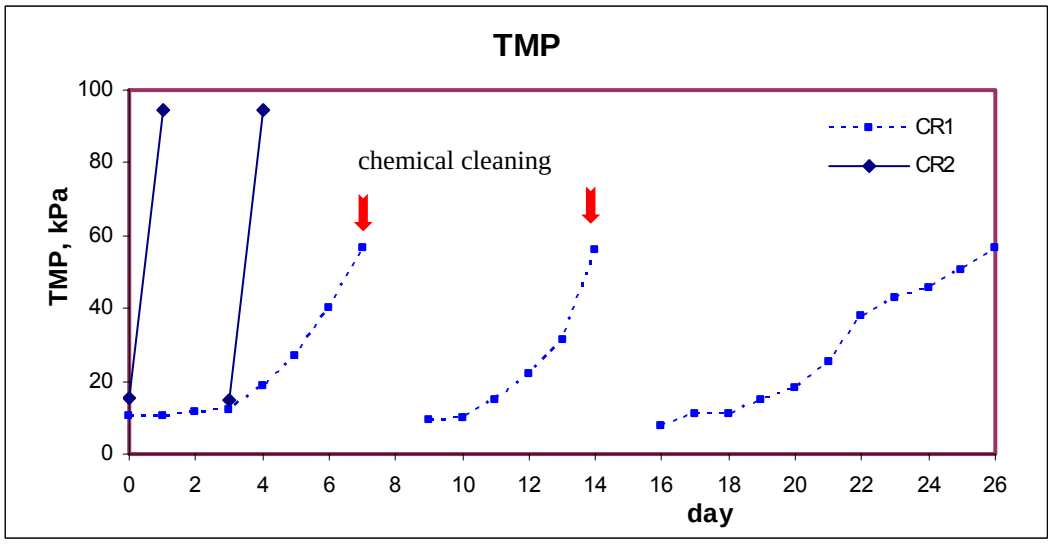



**Figure 4.36. Transmembrane pressure variation of CR1 & CR2**

In the third cycle in figure 4.36, the fouling time increased up to 10 days because at this time the settler worked well due to avoiding the phenomenon of sludge rising causing by anaerobic condition at the bottom of the settler.

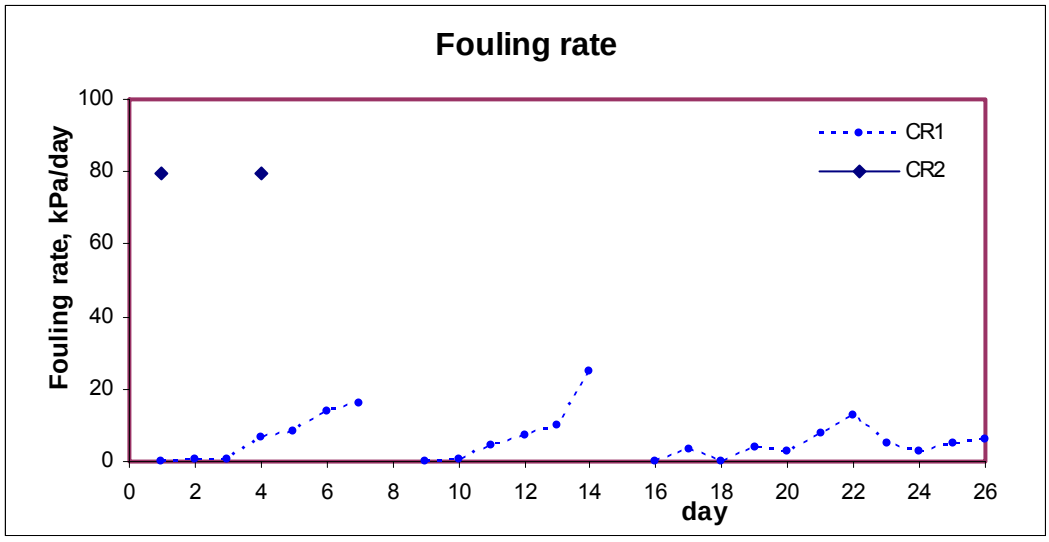



**Figure 4.37. Fouling rate of CR1 & CR2**

Figure 4.37 presented fouling rate of system in term of kPa/day, maximum fouling rate of CR1 and CR2 were 79.7 and 24.8 kPa/day, respectively. Fouling rate of CR1 was 3 fold less than that of CR2 so external granule MBR was an attractive alternative for new treatment technology.

### 4.3.5. Membrane resistance

**Table 4.14 Membrane resistance of treatment sequence**

| Resistance | CR1, $m^{-1}$ | % | CR2, $m^{-1}$ | % |
|---|---|---|---|---|
| $R_m$ | 2.16 $x10^{12}$ | 12.9 | 1.86 $x10^{12}$ | 2.1 |
| $R_t$ | 16.78 $x10^{12}$ | | 86.87 $x10^{12}$ | |
| $R_c$ | 14.62 | 87.1 | 59.42 $x10^{12}$ | 68.4 |
| $R_f$ | | | 25.59 $x10^{12}$ | 29.5 |

$R_m$: initial resistance; $R_t$: total resistance; $R_c$: cake resistance; $R_f$: irreversible resistance

From table 4.15, CR2 membrane was much more fouled than CR1 one. CR2 membrane was seriously clogged after 1 day of operation. For CR1 membrane, there was a very thin cake layer (CR2 with thick cake layer on membrane surface) so it was considered that fouling was mainly caused by irreversible fouling (87.1%). On the contrary, CR2 membrane was 68.4 % fouled by cake formation and 29.5% by irreversible foulant. At last, CR1 was mainly fouled by irreversible resistance and CR2 by reversible one.

# Chapter 5

## Conclusions and Recommendations

In this study, aerobic granules were cultivated with different support media and granulation reactor was combined with membrane technology. This research included three parts: (1) Aerobic granules were created with two support media, bivalve shell carrier and anaerobic granule, at OLR of 2.5 kg COD/$m^3$.day. (2) Organic loading was varied from 2.5 to 30 kg COD/$m^3$.day to find out granule treatability and to select optimum OLR. Later, this value was used for membrane bioreactor set-up. Suitable support media was also selected for the optimum run. The optimum OLR was 10 kg COD/$m^3$.day and bivalve shell carrier was identified as a good media for granule formation. During the experimental runs, granule characteristics were investigated and supernatant of each OLR was also analysed for its membrane fouling potential. (3) Two treatment sequences, batch (external MBR) and continuous (internal MBR) system, were installed to develop a suitable treatment sequence for aerobic granule membrane bioreactor. Contingent upon those experimental results, the followed conclusions could be drawn hereafter:

### 5.1 Conclusions

1. Aerobic granule could be cultivated at either OLR of 2.5 kg COD/$m^3$.day or higher with both types of support media. Especially it is preferable at high OLRs. This could meet the first and second objective.

2. Both support media, either bivalve shell or anaerobic granule carrier could form aerobic granules but carrier had much more advantages as follows:

- Increase shear stress by carrier movement to stimulate granule formation. Strong shear stress is one of the key factor for granule formation as Tay et al., 2001;
- Cleanse biofilm automatically on reactor walls by its friction force to avoid the competition between granulation and attached growth;
- Work as support media for microbial aggregation on the carrier surface and porosity;
- Create denser, more compact and more regular granules;
- Have “smoother” surface with smaller cavities in comparison with AR granules;
- Increase settling ability of granules by its high original density. Settling velocity of bare carrier is in range of 100 – 300 m/h;
- Suffer sharp shock loading applied in granulation reactor;
- Reduce membrane fouling due to less EPS, PS in supernatant.
- Based on above advantage of carrier, it is really good media for granule cultivation. This could fulfil one part of the third objective of this research.

3. Granule diameter was in range of 0.5-4.0 mm during the runs. The size became bigger with higher OLRs. CR granules surface seemed to be “smoother” with smaller cavities and more compact relatively so that was the reason why CR granule could suffer better with shock loading and settled biomass concentration of CR was often higher than that of AR.

4. Settled biomass concentration increased with the increased of OLRs. It was in range of 20-25 $g/L_{granule}$ and 49-62 $g/L_{granule}$ at OLR of 2.5 and 30 kg $COD/m^3$.day, respectively. This was much higher than value of conventional activated sludge of 2.7 $g/L_{sludge}$.

5. Hydrophobicity increased when granules formed but decreased when famine condition did no longer exist in reactor. In most OLRs, hydrophobicity was higher than 50% in comparison with activated sludge of 31%. High hydrophobicity was stimulated microbial aggregation due to reducing negative charge on cell surface. In the thermodynamic sense, the increase of hydrophobicity could reduce excess Gibbs energy of the surface which promoted self-aggregation as same as Liu et al., 2003.

6. Bound polysaccharides increased when granule formed and this was a key bridging factor in microbial aggregation. It was changed from 26 mg/gVSS in seed sludge to 42-90 mg/g VSS in granules.

7. Aerobic granule treatability could reach to rather high OLR as equal as anaerobic process. The applied OLR could be higher than 30 kg $COD/m^3$.day with F/M ratio in range of
0.57-2.4 $day^{-1}$ with COD removal efficiency greater than 96% in all cases (F/M of conventional SBR was 0.05-0.3 $day^{-1}$). As already stated, treatability of aerobic granulation systems was 15 fold higher than that of conventional activated sludge process in term of OLR. Moreover, granules had excellent settling ability, dense biomass, high bioactivity and also high biomass accumulation in reactor. This could fulfill the third objective of this study.

8. Bioactivity of aerobic granule was at least 3 fold greater than conventional activated sludge in term of oxygen uptake rate. It was 46.7 $mgO_2/gVSS.h$ and 57.8 $mgO_2/gVSS.h$ for CR, AR granules, respectively. This explained why aerobic granules could operate at high OLRs up to 30 kg $COD/m^3$.day.

9. At high OLR of 10 kg $COD/m^3$.day, yield coefficient of granular sludge was 0.44 mgVSS/mgCOD so it was rather similar with conventional activated sludge.

10. Supernatant of carrier reactors caused less fouling than that of anaerobic granule reactor and conventional SBR. Fouling index of settled supernatant increased with the increase of OLR. At loading of 5 kg $COD/m^3$.day, MFI of CR, AR, SBR was $130.7x10^3$; $287.9x10^3$; $292.7x10^3$ $s/L^2$, respectively.

11. Fouling potential of supernatant was mainly caused by cake layer of new generated biomass and polysaccharides. For polysaccharides, it relatively contributed more than 82% total EPS in most of OLRs.

12. The treatment sequence I, continuous mode, internal MBR, could not maintain granules in reactors. Because submerged membrane inserted in the raiser tube of SBAR disturbed the reactor structure and this generated extreme shear stress to destroy granules. Moreover, due to low substrate gradient in continuous reactor, this eliminated substrate diffusion depth into inner core. Those made granules worn in submerged granule MBR.

13. The treatment sequence II, batch mode, external MBR, was found to be suitable alternative for MBR coupled with granular sludge. System was compact, high OLR, high effluent quality.

14. The fouling rate of external MBR was less than that of internal one 3 fold so external MBR could be the attractive alternative for water reuse and recycling. Furthermore, this could obtain the last objective of this research.

15. Finally, discharged sludge from granulation system was also compact, dense and high settlability so this would be easier in sludge treatment in comparison with MBR sludge or conventional sludge.

## 5.2 Recommendations for future study

This research could contribute some certain know-how about aerobic granule formation. However, there should be much more deep research about this especially its application with membrane. Hereafter, some recommendations for future study are:

1. Find out the method to create aerobic granule with optimum diameter for getting simultaneous nitrification/denitrification. The diameter should be in range of 0.3 – 1.2 mm to make sure anoxic zone exists in inner granule core even during aeration time.

2. Investigate about organic absorbance (organic matters) and inorganic accumulation (toxic metals) ability in aerobic granules.

3. Carry out granulation with some types of industrial wastewater having high COD and nitrogen wastewater such as seafood, beer processing, wheat flour processing, alcohol processing and new leachate. Especially new leachate, which is very high COD and nitrogen also free ammonia which was considered to inhibit granulation if its concentration is greater than 23 mg/L (Yang et al., 2004). From here, it might be interesting if one could find out the conditions to form granules even in high free ammonia wastewater.

4. Find out another method that is technically economic to create granule without high air velocity like current condition. This could be tried to enhance shear stress by some types of carrier and reduce superficial air velocity for aeration energy save.

5. Implement FISH analysis for more understanding about microbial cultures in granular sludge.

6 Study about fouling due to EPS components with the variation of nutrient to organic matter ration (N:C = 5:100; 10:100; 15:100; 20:100; 30:100). Nutrient was in doubt to affect to EPS quantity which was one of fouling factor in MBR.

7. Optimize treatment sequence I, external membrane system, with single compact MBR. A baffle reactor with membrane module located in the last chamber could be attractive solution for granule MBR. This solution could save pump energy.

8. Study more deeply about fouling behaviour of supernatant and new generated biomass which is positively hydrophobicity. High hydrophobic of effluent biomass might cause less fouling with some hydrophilic membrane.

9. Focus on characterizing effluent of granulation reactor to optimize filtration process to prolong fouling time of membrane by installing an efficient settler (lamella settler) and hydrophilic membrane.

10. Try to form aerobic granule by the continuous system, Biofilm Airlift Suspension Reactor (BAF), like Tjhuis et al., 1994 and couple with MBR. This could solve the overflow of supernatant in batch system such SBAR. Moreover, supernatant of batch and continuous system should be characterized for its fouling potential.

11. Try to form aerobic granule with bubble column which was thought to be less expensive than airlift reactor.

12. Granular sludge treatment. Find out technical economic solution for its treatment and make a comparison with traditional MBR and conventional activated sludge. It was

predicted that sludge treatment system for discharged granular sludge more compact and economic.

## References


APHA, AWWA, WPCF (1992). Standard methods for the Examination of Water and Wastewater, 18th Edition, Washington DC: APHA.

Bartell, T. (1986). *Summary Report: Sequencing Batch Reactor*. US-EPA: EPA/625/8-86/011.

Batstone, D.J., Keller, J., Blackall, L.L. (2004). The Influence of Substrate Kinetics on the Microbial Community Structure in Granular Anaerobic Biomass, *Water Research*, 38, 1390-1404.

Beun, J.J., Hendriks, A., Van Loosdrecht, M.C.M., Morgenroth, E.P.A., Heijnen, J.J. (1998). Aerobic Granulation in a Sequencing Batch Reactor, *Water Research*, 33 (10), 2283-2290.

Beun, J.J., van Loosdrecht M.C.M, Heijnen, J.J. (2002). Aerobic Granulation in a Sequencing Batch Airlift Reactor, *Water Research*, 36 (4-5), 702-712.

Beun, J.J., van Loosdrecht, M.C.M., Heijnen, J.J. (2000). Aerobic Granulation. *Water Science and Technology*, 41, No 4-5, 41-48.

Boerlage, S.F.E, Kennedy, M.D, Aniye, M.B, Abogrean, E., Tarawneh, Z.S, Schippers, J.C. (2003). The MFI-UF as a water quality test and monitor, *Journal of Membrane Science*, 211, 271–289.

Boerlage, S.F.E, Kennedy, M.D, Dickson, M.R, El-Hodali, D.E.I, Schippers, J.C. (2002). The modified fouling index using ultra filtration membranes (MFI-UF): characterization, filtration mechanisms and proposed reference membrane, *Journal of Membrane Science*, 197, 1–21.

Boerlage, S.F.E., Kenedy, M.D., Aniye, M.P., Abogrean, E.M., El-Hodali, D.E.Y, Tarawneh, Z.S., Schippers, J.C. (October, 2000). Proceedings of the Conference on Membranes in Drinking and Industrial Water Pollution: Modified Fouling $Index_{ultrafiltration}$ to Compare Pre-treatment Process of Reverse Osmosis Feed water. *Desalination Publications*, Italy, 1, 397-410.

Boerlage, S.F.E., Kennedy, M., Aniye, M.B., Schippers, J.C. (2003). Applications of the MFI-UF to measure and predict particulate fouling in RO systems, *Journal of Membrane Science*, 220, 97–116

Cai, C., Xu, F., Zhu, N., Cai, W. (2004). Cultivation of Aerobic Granules in a Sequencing Batch Shaking Reactor, *Environmental Technology*, 25, 937-944.

Cech, J.S., Chudoba, J., Grau, P. (1984). Determination of Kinetic Constants of Activated Sludge Microorganisms, *Water Science and Technology*, 17, 259-272.

Chang, I.S., Kim, J.S., Lee, C.H. (1996). Membrane Biofouling Characteristics in Membrane Coupled Activated Sludge System, In Proc. of ICOM' 96: 926-927. Yokohama, Japan.

Cho, J.W., Ahn, K.-H, Lee, Y.H., Lim, B.-R., Kim, J.Y. (2004). Investigation of Biological and Fouling Characteristics of submerged Membrane Bioreactors Process for Wastewater Treatment by Model Sensitivity Analysis, *Water*

*Science and Technology,* 49, 245-254.

Choi, J.-G., Baea, T.-H., Kim, J.-H, Tak, T.-M. (2002). The Behavior of Membrane Fouling Initiation on The Crossflow Membrane Bioreactor System, *Journal of Membrane Science,* 203, 103–113.

Chudoba, P., Capdeville, B., Chudoba, J. (1992). Explanation of Biological Meaning of the $S_o/X_o$ Ratio in Batch Cultivation, *Water Science and Technology,* 26, 743-751.

Clair N. Sawyer, Perry L. McCarty, Gene F. Parkin (1994). *Chemistry for Environmental Engineering.* 4th ed. New York, McGRAW-HILL: ISBN 0-07-113908-7.

Cole, A.C., Shanahan, J.W., Semmens, M.J., LaPara, T.M. (2002). Preliminary Studies on The Microbial Community Structure of Membrane-Aerated Biofilms Treating Municipal Wastewater, *Desalination,* 146, 421-426.

Dahm, K., Hanus D., Semmens, M., Department of Civil Engineering, University of Minesota (2000). *Membrane Technology: An Innovative Alternative In Wastewater Treatment.* Water Environment Research Foundation: ISBN 1-893664-25-2.

Dammel, E.E., Schroeder, E.D. (1991). Density of Activated Sludge Solids, *Water Research*, 25, 841-846.

Dangcong, P., Bernet, N., Delgenes, J.-P., Moletta, R. (1998). Rapid Communication Aerobic Granular Sludge - a Case Report, *Water Research*, 33, No. 3, 890-893.

De Bruin, L.M.M., De Kreuk, H.F.R., Van der Roest, H.F.R., Uijterlinde, C., Van Loosdrecht, M.C.M. (2004). Aerobic Granular Sludge: an Alternative to Activated Sludge?, *Water Science and Technology*, 49, 1-7.

De Kreuk, M.K. and De Bruin, L.M.M (2004). Aerobic Granule Reactor Technology. Stowa-Foundation for Applied Water Research. IWA: ISBN 1-84339-067-1.

De Kreuk, M.K., Van Loostdrect, M.C.M. (2004). Selection of Slow Growing Organisms as a Means For Improving Aerobic Granular Sludge Stability, *Water Science and Technology*, 49, 9-17.

Eckenfelder, W.W.J. (2000). Industrial Water Pollution Control. 3rd Edition. Boston:USA:McGraw-Hill: ISBN 0-07-116275-5.

Etterer, T., Wilderer, P.A. (2001). Generation and Properties of Aerobic Granular Sludge. *Water Science and Technology,* 43 (3), 19-26.

Frølund, B., Griebe, T., Nielsen, P.H. (1995). Enzymatic Activity in the Activated Sludge Floc Matrix, *Applied Microbiology and Technology*, 43, 755–761.

Frølund, B., Palmgren, R., Keiding, K., Nielsen, P.H. (1996). Extraction of Extracellular Polymer from Activated Sludge Using a Cation Exchange Resin. *Water Re*search, 30, 1749-1758.

Ganczarczyk, J.J. (1983). *Activated Sludge Process: Theory and Practice.* Marcel Dekker, INC: ISBN 0-8247-1758-9.

Gunder, B. (2001). The Membrane-Coupled Activated Sludge Process in Municipal Wastewater Treatment. Technomic Publishing Co., USA: ISBN 1-56676-959-0.

Hoa, P.T., (2002). Effect of Nutrient On Extracellular Polymeric Substance Production and Sludge Characteristics, AIT Dissertation No. EV-02-20.

Hodgson, P.H., Leslie, G.L., Schneider, R.P., Fane, A.G., Fell, C.J.D, Marshall, K.C. (1993). Cake Resistance and Solute Rejection in Bacterial Microfiltration: The Role of The Extracellular Matrix, *Journal of Membrane Science,* 79, 53–35.

Huang, X., Liu, R., Quian., Y. (2000). Behaviour of soluble microbial products in a membrane bioreactor, *Process Biochemistry*, 36, 401-406.

Hulshoff Pol, L.W., De Castro Lopes, S.I., Lettinga, G. (2004). Anaerobic Sludge Granulation, *Water Research*, 38, 1376-1389.

Jang, A., Yoon, Y.H., Kim, I.S., Kim, K.S., Bishop, P.L. (2003). Characterization and Evaluation of Aerobic Granules in Sequencing Batch Reactor, *Journal of BioTechnology,* 105, 71-82.

Jefferson, B., Brookes, A., Le Clech, P., Judd, S.J. (2004). Method for Understanding Organic Fouling in MBRs, *Water Science and Technology,* 49, 237-244.

Jimenez, B., Noyola, A., Capdeville, B., Roustan, M., Faup, G. (1998). Destran Blue Colorant As a Reliable Tracer in Submerged Filter, *Water Research*, 22, 1253-1257.

Jin, B., Wilen, B.-H, Lant, P. (2003). A Comprehensive Insight into Floc Characteristics and Their Impact on Compressibility and Settlability of Activated Sludge, *Chemical Engineering Journal,* 95, 221-234.

Judd, S. (2004). A Review of Fouling of Membrane Bioreactors in Sewage Treatment, *Water Science and Technology*, 49, 229-235.

Le-Clech, P., Jefferson, B., Judd, S.J. (2003). Impact of Aeration, Solids Concentration and Membrane Characteristics on The Hydraulic Performance of a Membrane Bioreactor, *Journal of Membrane Science,* 218, 117–129.

Lee, D. J., Chen, G.W., Liao, Y.C., Hsieh, C.C. (1996). On The Free-Settling Test for Estimating Activated Sludge Floc Density, *Water Research*, 30, 541-550.

Lee, N., Amy, G., Croue, J.-P., Buisson, H. (2004). Identification and understanding of fouling in low-pressure membrane (MF/UF) filtration by natural organic matter (NOM) , *Water Research*, 38, 4511-4523.

Lim, B.-R., Ahn, K.-H., Songpraset, P., Cho, J.W., Lee, S.H. (2004). Microbial Community Structure of Membrane Fouling Film in an Intermittently and Continuously Aerated Submerged Membrane Bioreactors Treating Domestic Wastewater, *Water Science and Technology*, 49, 255-261.

Lin, Y.-M., Liu, Y., Tay, J.-H. (2003). Development and Characteristics of Phosphorus-Accumulating Microbial Granules in Sequencing Batch Reactors, *Applied Microbial and Cell Physiology*. 62, 430–435.

Linlin, H., Jianlong, W., Xianghua W., Yi, Q. (2005). The Formation and

Characteristics of Aerobic Granules in Sequencing Batch Reactor (SBR) by Seeding Anaerobic Granules, *Process Biochemistry*, 40, 1-7.

Liu, L., Wang, Z., Yao, J., Sun, X., Cai, W. (2005). Investigation on the properties and kinetics of glucose-fed aerobic granular sludge, *Enzyme and Microbial Technology*, 36,307-313.

Liu, Y., Fang, H.H.P. (2003). Influences of Extracellular Polymeric Substances (EPS) on Flocculation, Settling, and Dewatering of Activated Sludge, *Environmental Science and Technology*, 33,237-273.

Liu, Y., Tay, J.-H. (2002). The Essential Role of Hydrodynamic Shear Force in The Formation of Biofilm and Granular Sludge, *Water Research*, 36, 1653-1665

Liu, Y., Yang, S.F., Qin, L., Tay, J.H. (2003). A Thermodynamic Interpretation of Cell Hydrophobicity in Aerobic Granulation, *Applied Microbial and Cell Physiology*, 64, 410–415.

Liu, Y., Yang, S.F., Tay, J.H. (2003). Elemental Compositions and Characteristics of Aerobic Granules Cultivated at Different Substrate N/C Ratios. *Applied Microbial and Cell Physiology*, 61, 656–561.

Liu, Y., Yang, S.F., Tay, J.-H. (2004). Improved Stability of Aerobic Granules by Selecting Slow-Growing Nitrifying Bacteria. *Journal of BioTechnology*, 108, 161-169.

Liu, Y., Yang, S.-H., Tay, J.-H., Liu, Q.-S., Qin, L., Li, Y. (2004). Cell Hydrophobicity is a Triggering Force of Biogranulation, *Enzyme and Microbial Technology*, 34,371-379.

Mallevialle, J., Odendaal, P.E. Wiesner, M.R., American Water Works Association Research Foundation (1996). *Water Treatment Membrane Process.* New York, McGRAW-HILL: ISBN 0-07-001559-7.

Mathieu, S., Etienne, P. (2000). Estimation of Wastewater biodegradable COD Fraction by Combining Respirometric Experiments in Various S0/X0 Ratios, *Water Research*, 34, 1233-1246.

McSwain, B.S., Irvine, R.L., Wilderer, P.A. (2004). The Effect of Intermittent Feeding on Aerobic Granule Structure, *Water Science and Technology*, 49, 19-25.

Metcalf and Eddy (1991). Wastewater Engineering: Treatment Disposal Reuse. 3rd ed. McGRAW-HILL: ISBN 0-07-100824-1.

Metcalf and Eddy (2003). *Wastewater Engineering: Treatment and Reuse*. 4th ed. McGRAW-HILL: ISBN 0-07-112250-8.

Meyer, R.L., Saunders, A.M., Zeng, R.J., Keller, J., Blackall, L.L. (2003). Microscale Structure and Function of Anaerobic - Aerobic Granules Containing Glycogen Accumulating Organisms, *FEMS Microbiology Ecology*, 45, 253-261.

Morgenroth, E., Sherden, T., Van Loosdrecht, C. M., Heijnen, J.J., Wllderer, A. (1997). Rapid Communication: Aerobic Granular Sludge in a Sequencing Batch Reactor, *Water Research*, 31, No. 12, 3191-319.

Moy, B.Y., Tay, J.H., Toh, S.K., Liu, Y., Tay, S.T. (2002). High organic loading influences the physical characteristics of aerobic sludge granules, *Letter of Applied Microbiology*, 34, 407-412.

Nicolella, C., van Loosdrecht, M.C.M., Heijnen, S.J. (2000). Particle-Based Biofilm Reactor Technology, *Tibtech*, 18, 312-320.

Novak, L., Larrea, L., Wanner, J. (1994). Estimation of Maximum Specific Growth Rate of Heterotrophic and Autotrophic Biomass: A Combined Technique of Mathematical Modelling and Batch Cultivations, *Water Science and Technology*, 30, 171-180.

Ognier, S., Wisniewski, C., Grasmick, A. (2002), Influence of Macromolecule Adsorption During Filtration of a Membrane Bioreactor Mixed Liquor Suspension, *Journal of Membrane Science,* 209, 27–37.

Ognier, S., Wisniewski, C., Grasmick, A. (2004), Membrane Bioreactor Fouling in Sub-Critical Filtration Conditions: A Local Critical Flux Concept, *Journal of Membrane Science,* 229, 171–177.

Oron, G., Bick, A. (September, 2001). Proceedings of the International Conference on Membrane Technology for Wastewater Reclamation and Reuse. Tel-Avis, Israel. Ben-Gurion University of the Negev.

Penya-roja, J.M, Seco, A., Ferrer, J., Rodrigo, M.A. (in press). A Procedure for Determination of High Influence Parameters of ASM No.2 IAWQ.

Reddy, M., (1994). *Biological and Chemical Systems for Nutrient Removal.* Alexandria, USA, Water Environment Federation: ISBN 1-57278-123-8.

Reid, G., Cuperus, P. L., Bruce, A.W., Van der Mei, H.C., Tomeczek, L., Khoury, A. H., Busscher, H.J (1992). Comparison of Contact Angles and adhesion to Hexadecane of Urogenital, Dairy, and Poultry Lactobacilli: Effect of Serial Culture Passages, *Applied and Environmental Microbiology*, 1549-1533.

Rodrigo, M.A., Seco, A., Ferrer, J., Penya-roja, J.M. (in press). A Procedure for Determination of High Influence Parameters of ASM No.1 IAWQ.

Roorda, J. H., Van der Graaf, J.H.J.M. (2001). New Parameter for Monitoring Fouling During Ultrafiltration of WWTP Effluent, *Water Science and Technology*, 43, 241-248.

Ros, M., Dular, M., Farkas, P.A. (1988). Measurement of Respiration of Activated Sludge, *Water Research*, 22, 1405-1411.

Schwarzenbeck, N., Erley, R., Wilderer, P.A. (2004). Aerobic Granular Sludge in an SBR-system Treating Wastewater Rich in Particulate Matter, *Water Science and Technology*, 49, 41-46.

Scott, K., Hughes, R. (1996). Industrial Membrane Separation Technology. Blackie Academic & Professional: ISBN 0-7514-0338-5.

See, H.J., Vassilliadis, V.S., Parks, G.T. (2004). Design of Reverse Osmosis (RO) Water Treatment Networks Subjects To Fouling, *Water Science and Technology*, 49, 263-270.

Shin, H.-S., Lim, K.-H., Park, H.-S. (1992). Effect of Shear Stress on

Granulation in Oxygen Aerobic Upflow Sludge Bed Reactor, *Water Science and Technology*, 26, 601-605.

Shufang, Y., (2004). Development of Aerobic Granules for Simultaneous Organic Carbon and Nitrogen Removal, Nanyang Technology University's Dissertation.

Spanjers, H., Vanrolleghem, P.A., Olsson, G., Dold, P.L. (1998). Scientific and Technical Report No. 7: Respirometry in Control of the Activated Sludge process: Principles. IAWQ: ISBN 1-900222043.

Stephenson T, Judd S., Jefferson B, Brindle K. (2000). Membrane Bioreactor for Wastewater treatment, IWA Publishing.

Stephenson, T., Judd, S., Jefferson, B., Brindle, K. (2000). Membrane bioreactors For Wastewater Treatment. Industrial Membrane Separation Technology. IWA Publishing, Alliance House, London: ISBN 1-900222-078.

Tay, J.-H, Liu, J., Sun, D.D. (2003), Quantification of membrane fouling using thermogravimetric method, *Journal of Membrane Science*, 217, 17–28.

Tay, J.-H., Liu, Q.S., Liu, Y. (2001). Microscopic observation of aerobic granulation in sequential aerobic sludge reactor, *Journal of Applied Microbiology*, 91, 168-175.

Tay, J.H., Liu, Q.S., Liu, Y. (2002). Aerobic Granulation in Sequential Sludge Blanket Reactor, *Water Science and Technology*, 46, No 4-5, 41-48.

Tay, J.-H., Liu, Q.S., Liu, Y. (2004). The Effect of Upflow Air Velocity on The Structure of Aerobic Granules Cultivated in a Sequencing Batch Reactor, *Water Science and Technology*, 49, 35-40.

Tay, J.-H., Pan, S., Tay, S.T.L., Ivanov, V., Liu, Y. (2003). The Effect of Organic Loading Rate on Aerobic Granulation: The Development of Shear Force Theory, *Water Science and Technology*, 47, 235-240.

Tay, S.T.L., Ivanov, V., Yi, S., Zhuang, W.Q., Tay, J.-H. (2002). Presence of Anaerobic Bacteroides in Aerobically Grown Microbial Granules, *Microbial Ecology*, 44, 278-285.

Tijhuis, L., Van Benthum, W.A.J., Van Loosdrecht, M.C.M., Heijnen, J.J. (1994). Solid Retention Time in Spherical Biofilm in a Biofilm Airlift Suspended Reactor, *Biotechnology and Bioengineering*, 44, 595-608.

Tijhuis, L., Van Loosdrecht, M.C.M., Heijnen, J.J. (1994). Formation and Growth of Heterotrophic Aerobic biofilms on Small Suspended Particles in Airlift Reactors, *Biotechnology and Bioengineering*, 44, 595-608.

Toh, S.K., Tay, J.-H., Moy, B.Y.P., Ivanov, V., Tay, S.T.L. (2003). Size-Effect on The Physical Characteristics of The Aerobic Granule in a SBR, *Applied Microbial and Cell Physiology*, 60, 867–879.

Tri, P.T., (2002). Oily Wastewater Treatment By Membrane Bioreactor Process Coupled With Biological Activated Carbon Process, AIT Dissertation No. EV-02-21.

Tsuneda, S., Ejiri, Y., Nagano, T., Hirata, A. (2004). Formation Mechanism of Nitrifying Granules Observed in an Aerobic Upflow Fluidized Bed (AUFB)

Reactor, *Water Science and Technology*, 49, 27-34.

Tsuneda, S., Nagano, T., Hoshino, T., Ejiri, Y., Noda, N., Hirata, A. (2003). Characterization of Nitrifying Granules Produced in an Aerobic Upflow Fluidized Bed Reactor, *Water Research*, 37, 4965-4973.

Uyanik, S., Sallis, P.J., Anderson, G.K. (2002). The Effect of Polymer Addition on Granulation in an Anaerobic Baffled Reactor (ABR). Part II: Compartmentalization of Bacterial Populations, *Water Research*, 36, 994-955.

Vanrolleghem, P.A, Spanjers, H., Peterson, B., Ginestet, P. (1998). *Estimation (Combination of) Activated Sludge Model No.1 Parameters and Components by Respirometry*. IAWQ Specialed Seminar on Modelling and microbiology, Kollekolle, Denmark.

Visvanathan, C., Ben Aim, R., Parameshwaran (2000). Membrane Separation Bioreactors for Wastewater Treatment, *Environmental Science and Technology*, 30, 1:1-48.

Visvanathan, C., Ben Aim, R., Parameshwaran, K. (2000). Critical Review in Environmental Science and Technology: Membrane Separation Bioreactors for Wastewater Treatment, *Environmental Science and Technology*, 30, 1-48.

Wang, Q., Du, G., Chen, J. (2003). Aerobic Granular Sludge Cultivated Under The Selective Pressure as a Driving Force, *Process Biochemistry*, 39, 557-563.

Wijeyekoon, S., Mino, T., Satoh, H., Matsuo, T. (2004). Effect of Substrate Loading Rate on Biofilm Structure, *Water Research*, 38, 2479-2488.

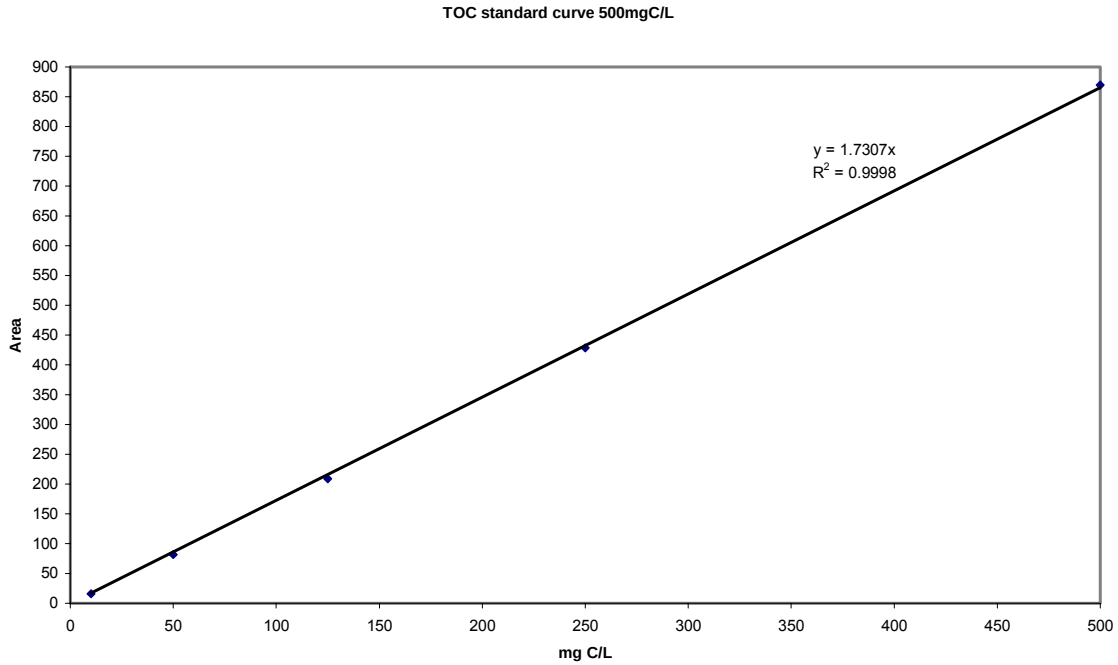
Wingender, J., Neu, T.R., Flemming, H.-C. (1999). Microbial Extracellular Polymeric Substances: Characterization, Structure and Function. Springer: ISBN 3-540-65720-7.

Yang, S.F., Tay, J.-H., Liu, Y. (2003). Inhibition of Free Ammonia to The Formation of Aerobic Granules, *Biochemical Engineering Journal*, 17, 41–48.

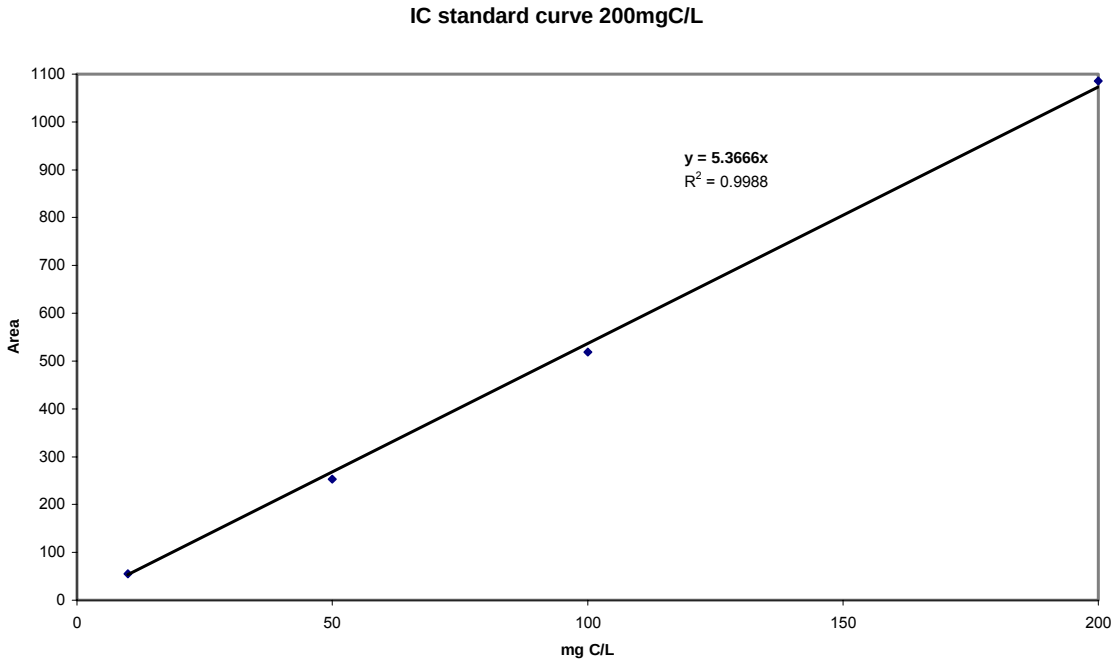
Zheng, Y.-M., Yu, H.-Q., Sheng, G.-P. (2004). Physical and Chemical Characteristics of Granular Activated Sludge from a Sequencing Batch Airlift Reactor, *Process Biochemistry*, In-press.

# Appendix 1

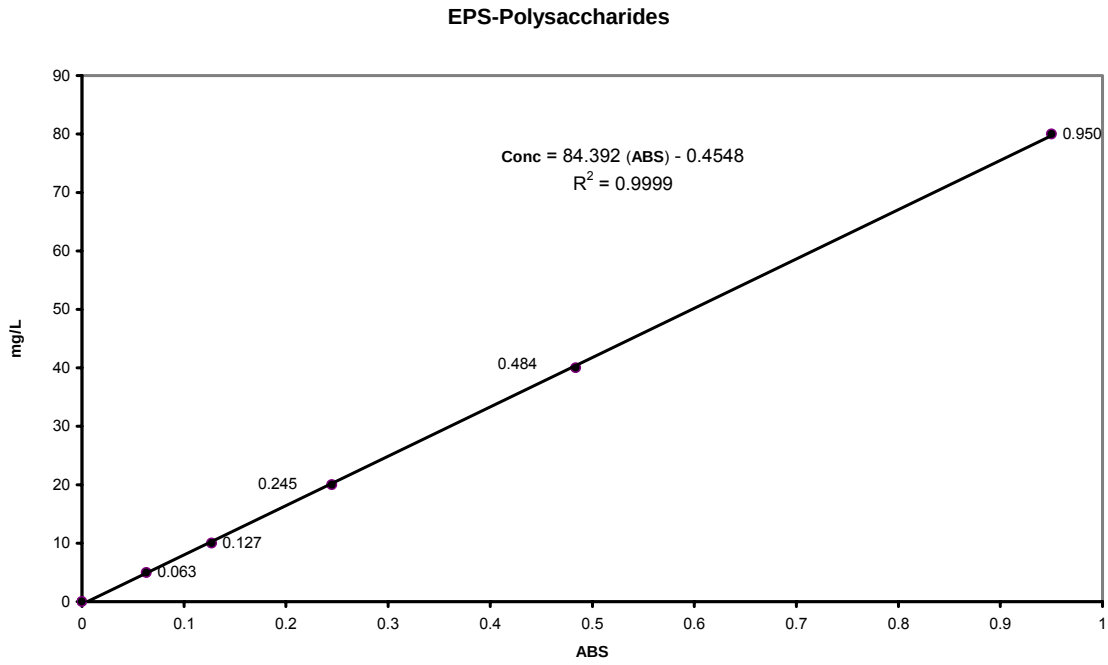
## TOC Standard curve maximum concentration 500 mg C/L



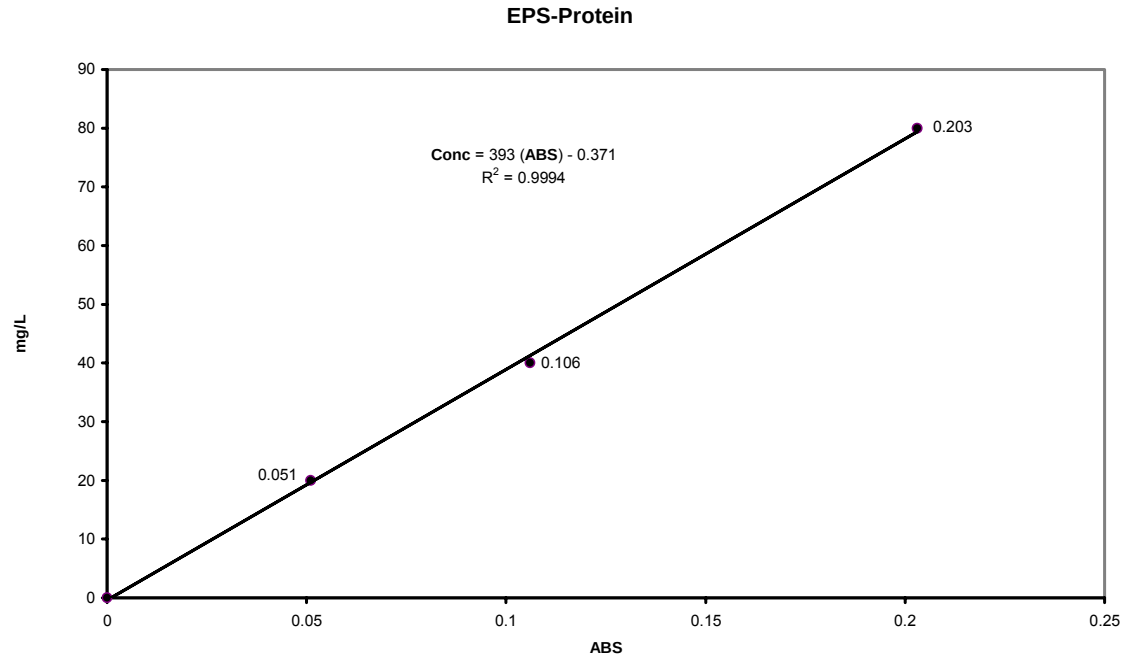

## IC Standard curve maximum concentration 200 mg C/L



## Polysaccharides Standard Curve – EPS determination




## Protein Standard Curve – EPS determination



## Appendix 2

## Calculation sample

**F/M ratio:**

$S_o$ = COD = 2438 mg/L

X = MLVSS = 8086 mg/L

Q = 1.3 L/batch; 8 batch/day; V = 2.5 L

$$F/M = \frac{Q*S_o}{V*X} = \frac{1.3*8*2438}{2.5*8086} = 1.25$$

**SRT:**

X = MLVSS = 8086 mg/L

$X_e$ = MLVSS in effluent = 504 mg/L

$$SRT = \frac{V*X}{Q*X_e} = \frac{2.5*8086}{1.3*8*504} = 3.86day$$

**OLR:**

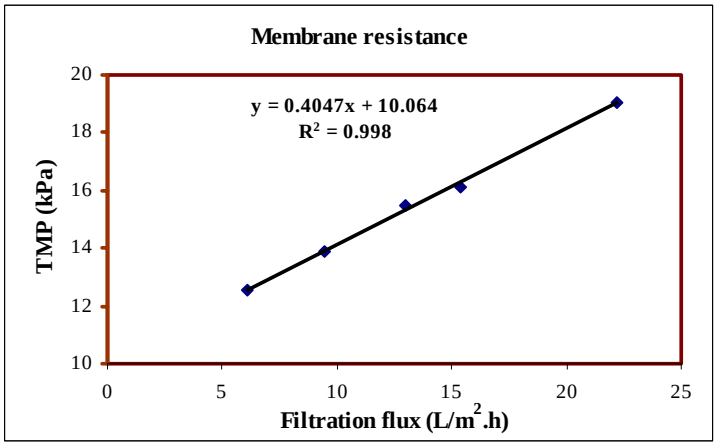
S = COD influent = 7396 mg/L

$$OLR = \frac{Q*S}{V} = \frac{1.3L/batch*8batch/day*7396mg/L*10^{-3}g/mg}{2.5L} = 30.7kgCOD/m^3.day$$

**Membrane resistance:**

Viscosity of water at 30°C, $\mu = 0.798*10^{-3}$ N.s/$m^2$

| Flux (ml/min) | TMP (mm Hg) | Filtration flux | TMP |
|---|---|---|---|
| S =0 .05$m^2$ | | (L/$m^2$.h) | kPa |
| 5.1 | 94 | 6.1 | 12.5 |
| 7.9 | 104 | 9.5 | 13.9 |
| 10.8 | 116 | 13.0 | 15.5 |
| 12.8 | 121 | 15.4 | 16.1 |
| 18.5 | 143 | 22.2 | 19.1 |



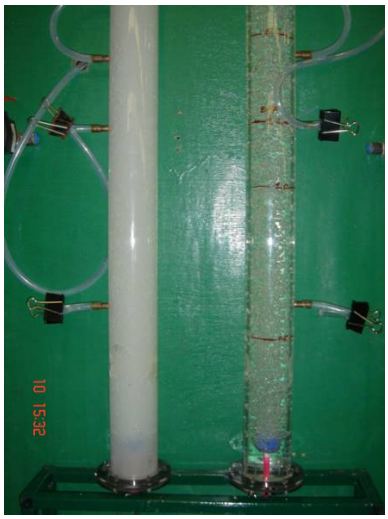

$R_m$=0.4047kPa/(L/$m^2$.h)*$10^3$kPa/Pa*3600s/h*1000L/$m^3$/0.798*$10^{-3}$Ns/$m^2$ = 1.826*$10^{12}$ $m^{-1}$

## Appendix 3

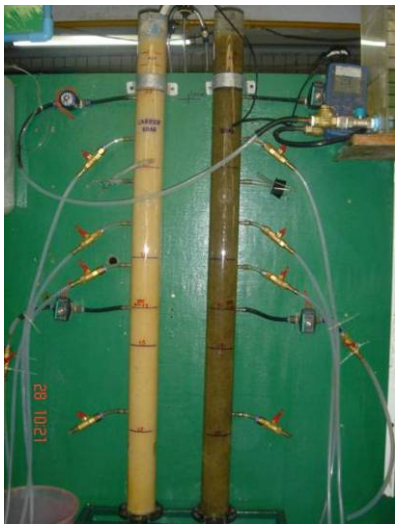
## Pictures in Research


a. Bivalve shell carrier producing equipment

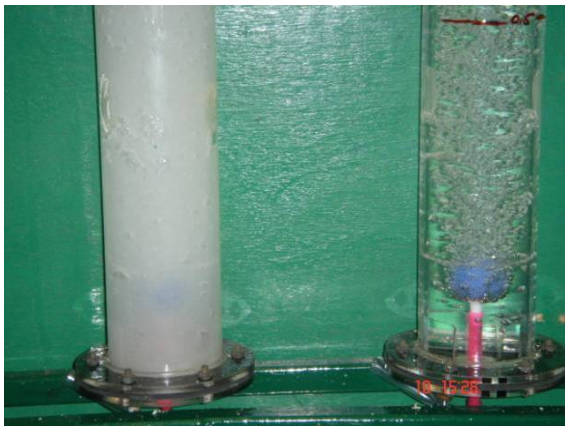

b. Used Calciferrous Carrier



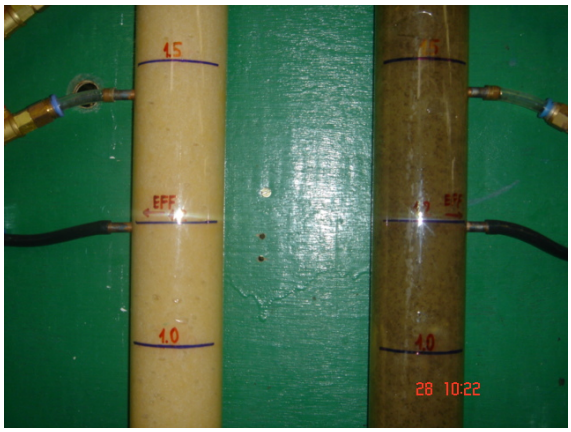

c. Reactor with bivalve shell carrier




d. Reactors with support media



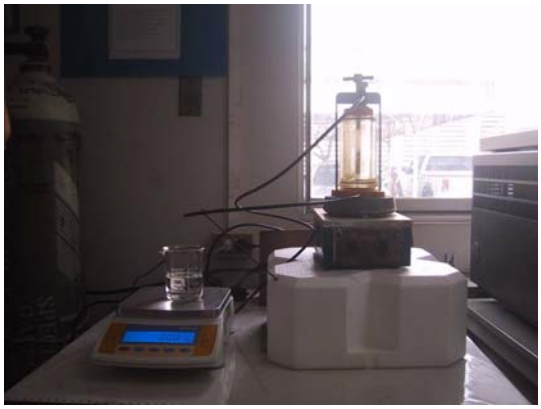
e. Bottom of reactor tested with water



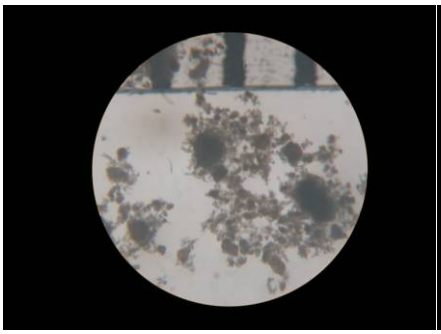

f. Effluent of running reactors

E

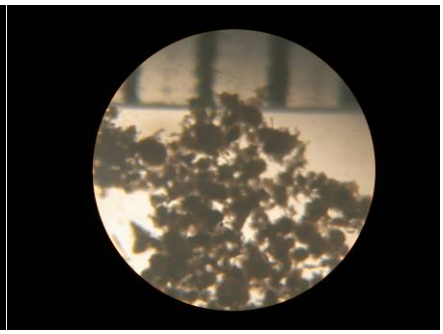
Kinetic data measurement



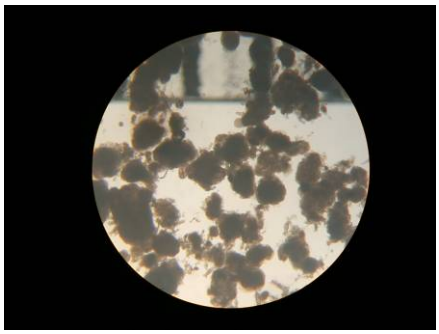
Membrane fouling index test



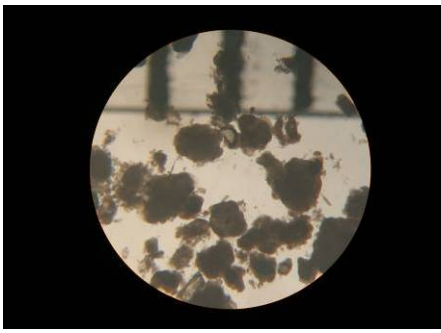
a. week 3 – initial granules



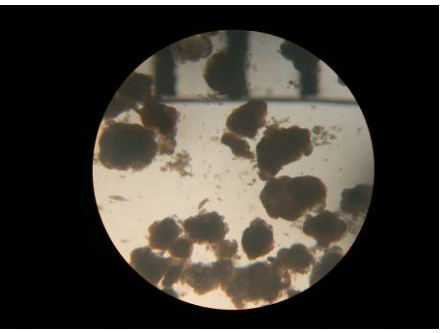
b. week 4 – mostly granulated



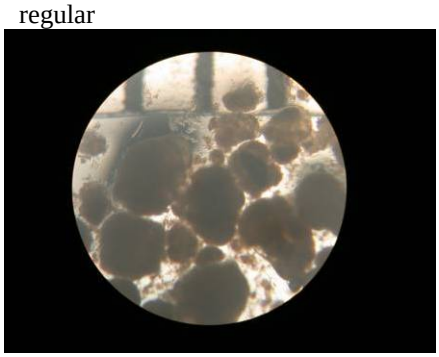
c. week 5 – became bigger, more regular



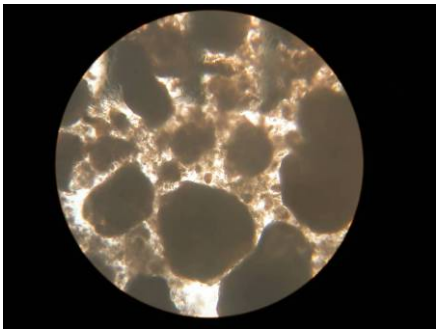
d. week 7 - growing granules



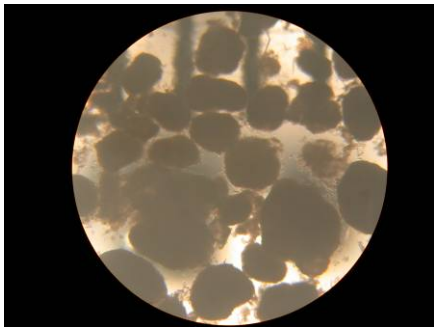
e. week 8 – matured granules



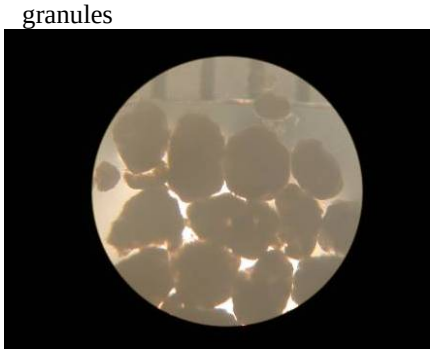
f. week 11 – denser, more regular granules



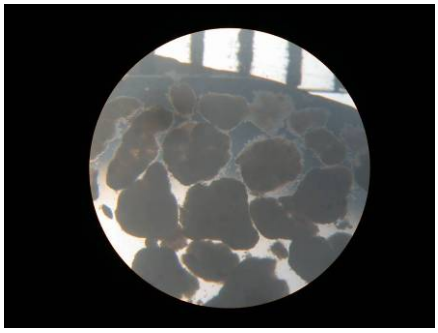
g. week 12 – some granules became smaller



h.Week 14 steady state of loading rate 10, some nematodes but not red

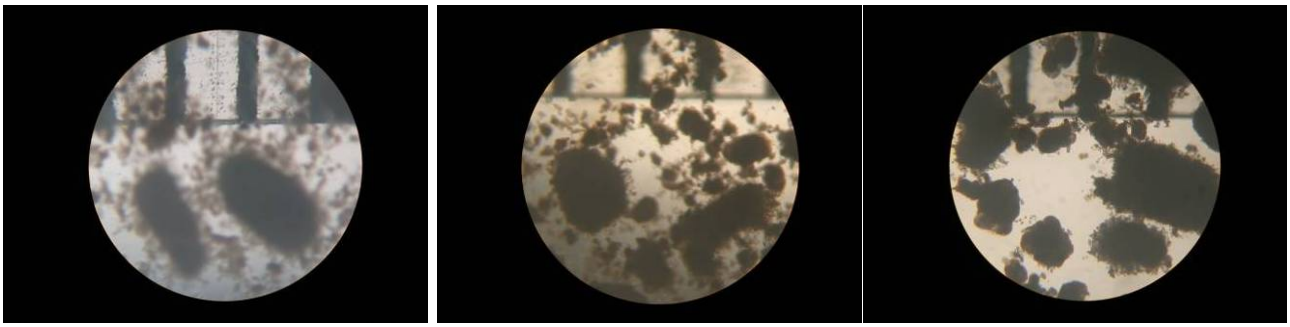


k. Week 15 - granule became even and clear outer (0.5-2mm)



l. Week 16-granule size 0.5-2 mm

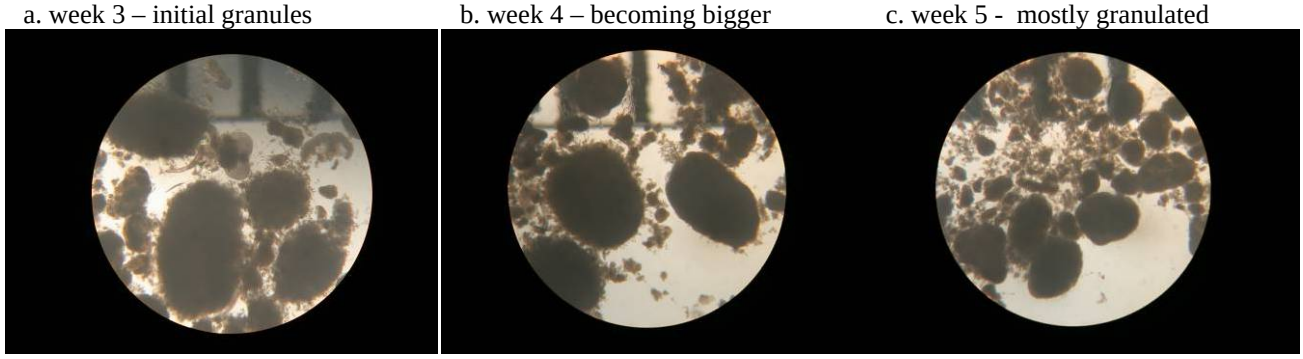
**Granule size development in CR by microscopic observation**



a. week 3 – initial granules

b. week 4 – becoming bigger

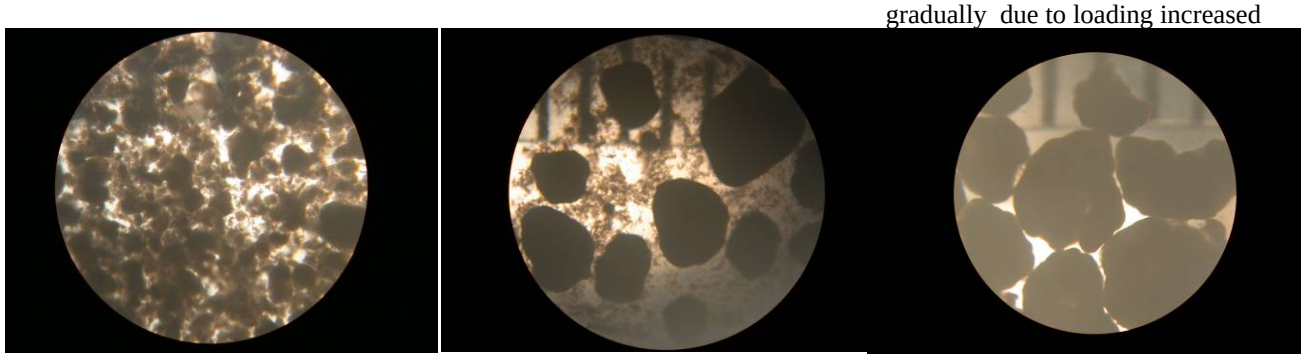
c. week 5 - mostly granulated



d. week 7 – becoming matured

e. week 8 – matured granules

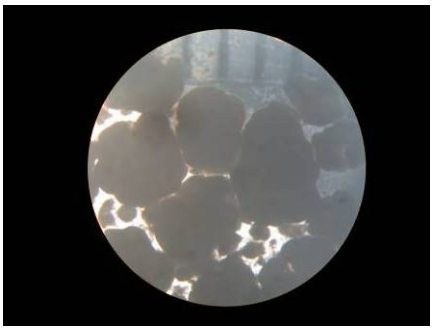
f. week 11 – granules worn gradually due to loading increased



g. week 12 – granules became smaller

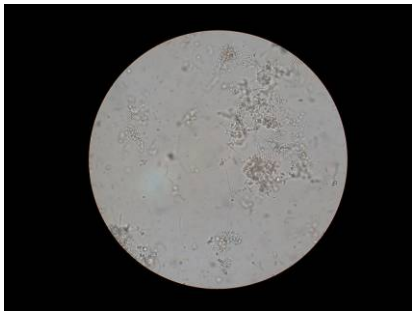
* h. week 14 – granules suddenly became bigger after removing biofilm 2 days (up to 3 mm)

k. Week 15 – granule became even and clear outer (1-3 mm)

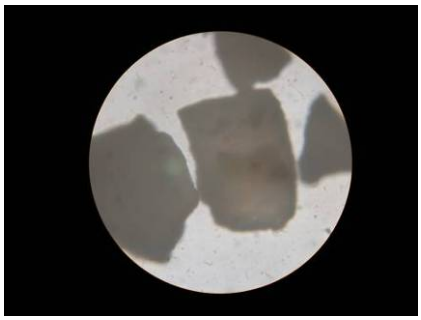


l.Week 16-granule size 0.3-4mm

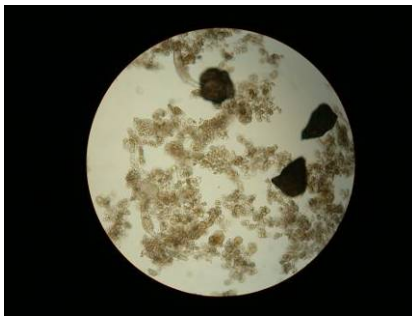
**Granule size development in AR by microscopic observation**



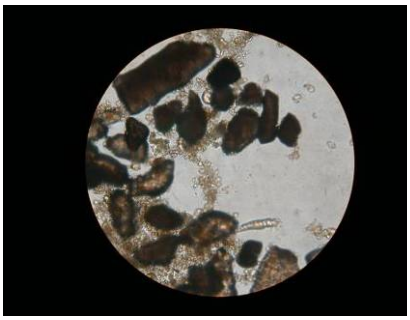
a. Seed sludge (x20)



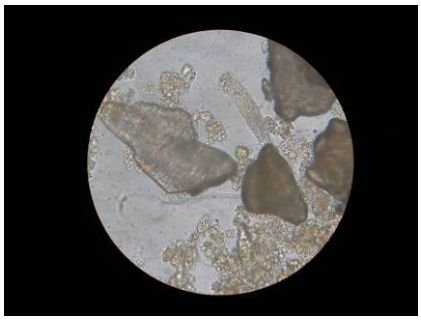
b. Dry carrier



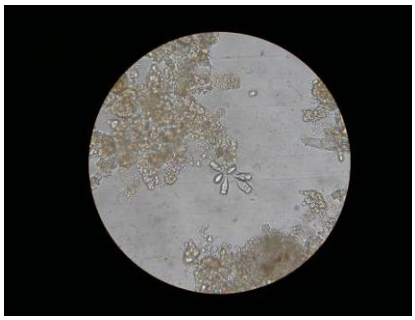
Carrier surface at week 1 (x10)



a. Carrier surface at week 2 (x10)

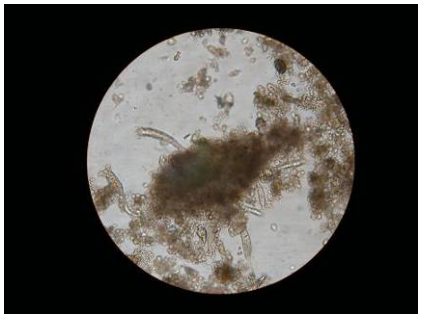


b. Carrier and rotifers surface at week 2 (x20)



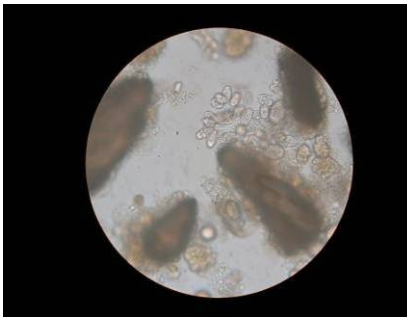
c. Microbial growth in CR at week 2

d. Microbial growth at week 2

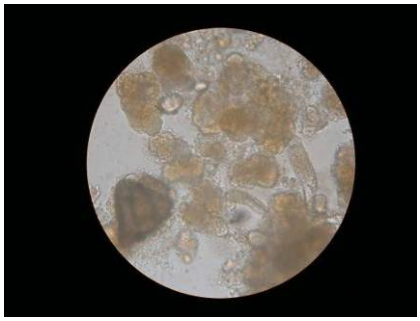


e. Signal of microbial aggregate at week 2

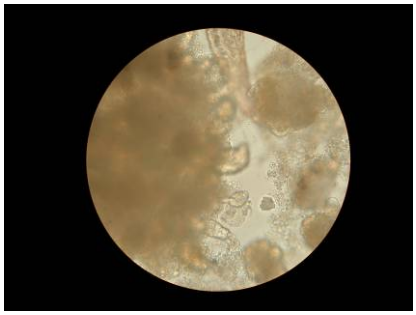
f. Microbial growth with rotifers at week 2



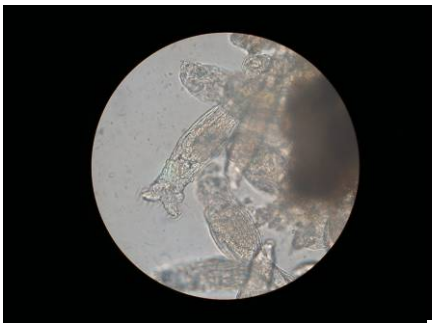
g. Thin biofilm layer on carrier surface at week 3



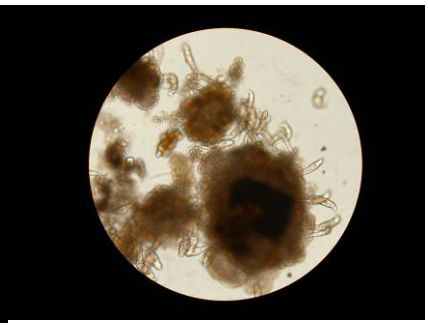
h. Carrier, initial granules and microbes at week 3



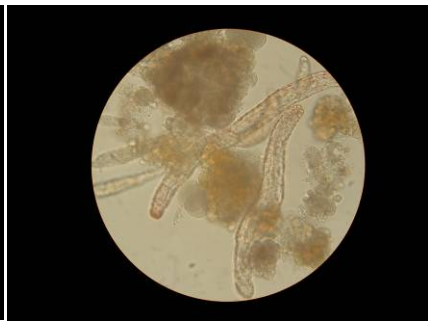
i. Microbial aggregate with rotifers, protozoa colonies, nematodes at week 3



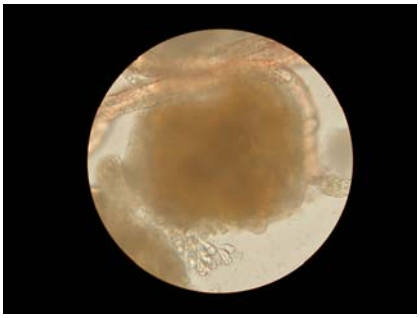
j. Carrier, rotifers, protozoa colonies at week 3



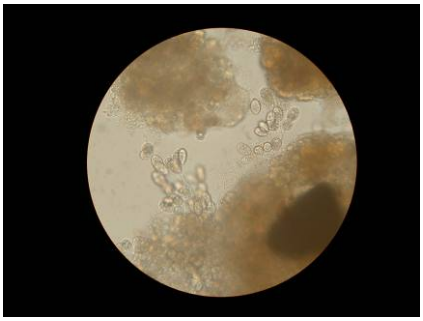
k. Initial granules with layer of microbes around at week 4



l. Nematodes, rotifers with initial granules at week 4

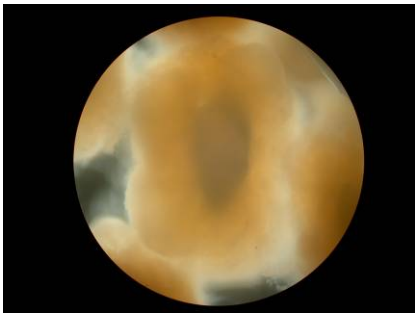


m. Nematodes, ciliates, rotifers and granules at week 5

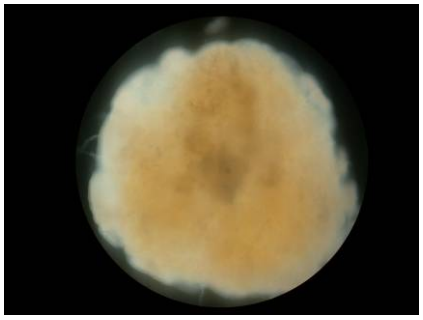


n. Granules with and without carrier at week 5

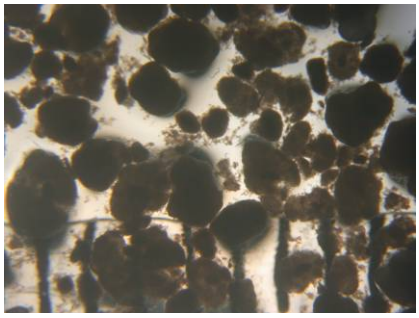
o. Carrier granules with nematodes at week 7. al most separate microbes disappeared from bulk liquid



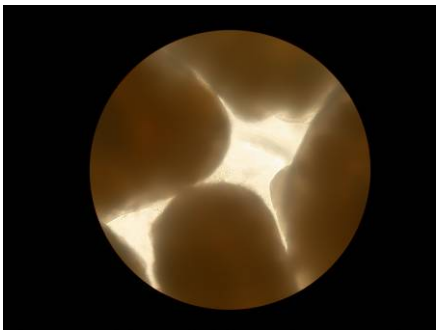
p. Carrier granules at week 11



q. carrier granule morphology at week 11

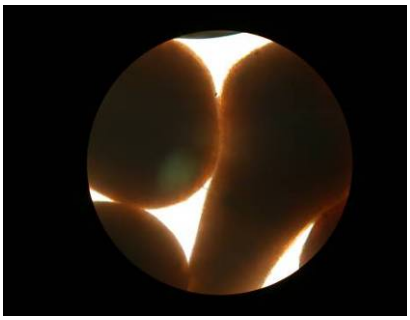


r. Granule size at week 12 (distance between two line 1 mm)

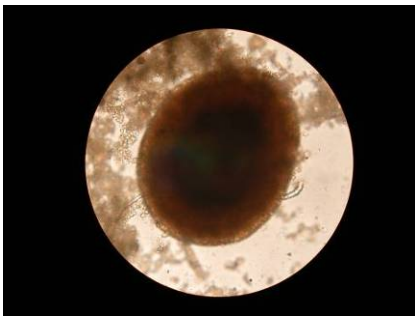


s. Carrier granule at week 15 (clear outer surface)

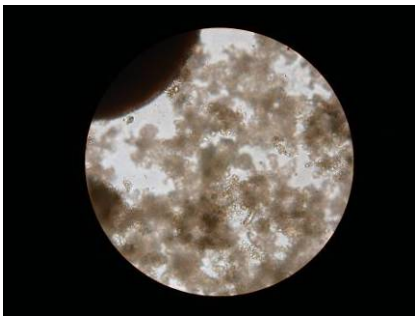
**Microbial cultures and granule morphology in CR**



a. Original anaerobic granule with clear outer

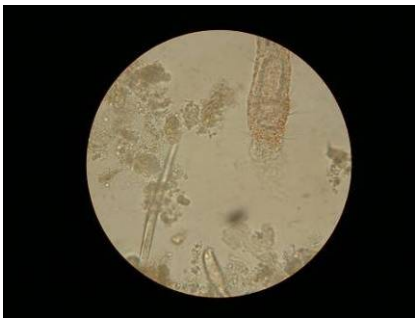


b. Signal of microbial attachment on granule surface at week 2

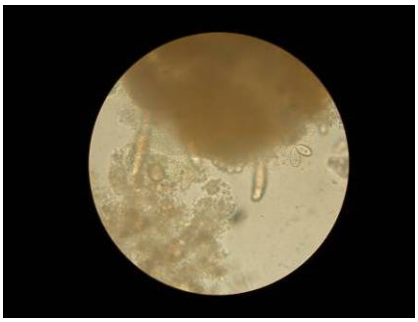


c. Granule and microbial growth at week 2

d. Rotifers at week 2 in AR

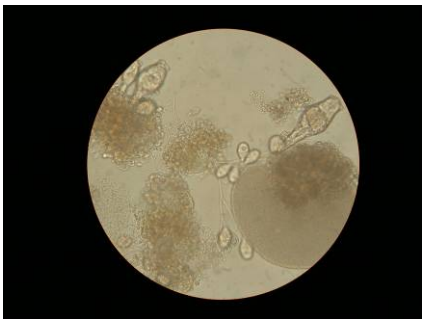


e. Nematodes, rotifers, protozoa colonies a week 3 & anaerobic granules disappeared

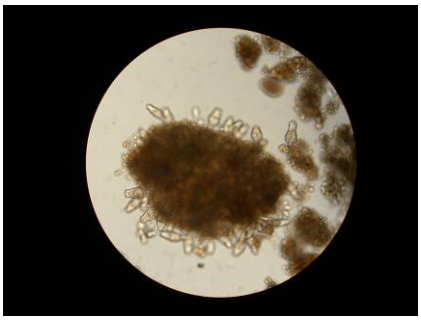


f. Initial granule with microbial adhesion around “aerobic granule” at week 3

g. Nematodes in AR at week 3

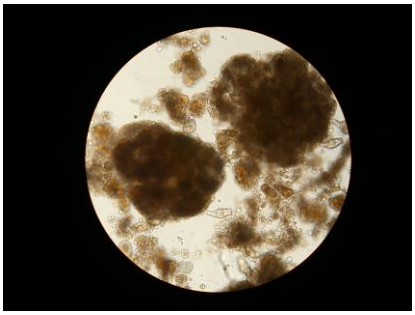


h. Rotifers, protozoa, ciliates at week 3

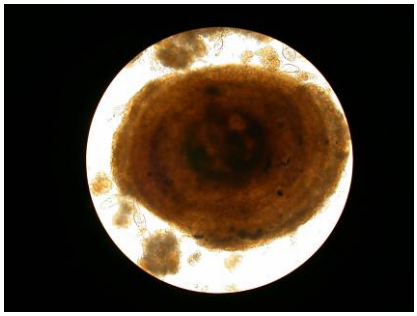


i. Initial granules with microbial adhesion at week 4

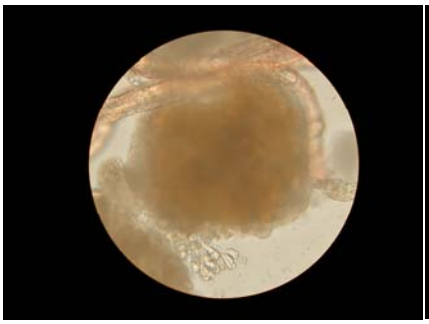
j. Nematodes and granules at week 4



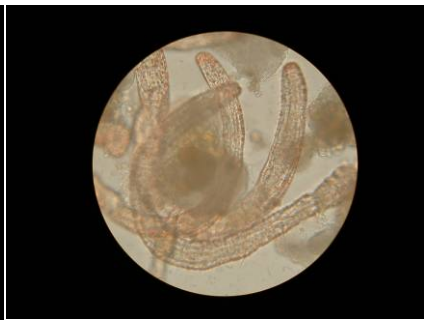
k. Initial granules and signal of granulation



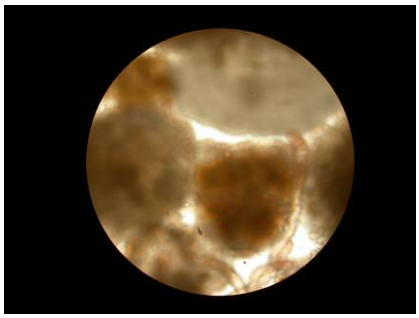
l. Granule morphology at week 4



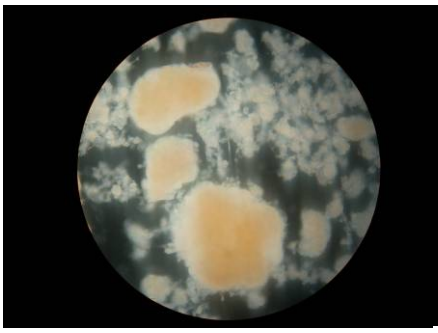
m. Granules, nematodes, ciliates, rotifers at week 5



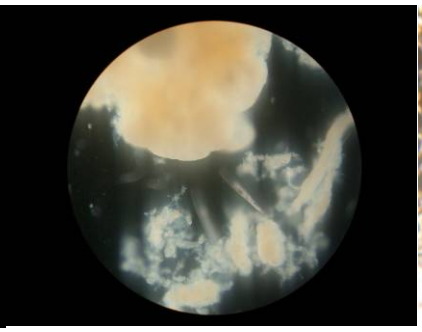
n. A large number of nematodes generated



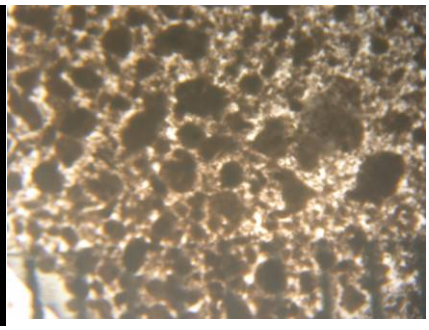
o. Granules and nematodes at week 7



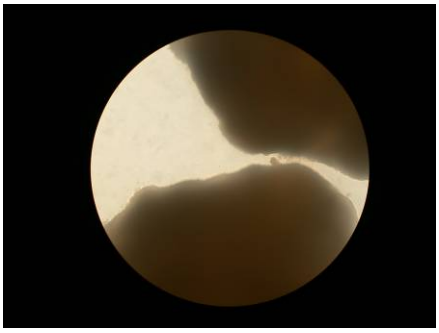
p. Granule disruption at loading 5 kg COD/$m^3$.day at week 11



q. Granule with less protozoa, nematodes



r. Granule size at week 12 (distance between two line is 1 mm)



s. Granules at week 15 with clear outer surface

**Microbial cultures and granule morphology in CR**

## Appendix 4

## Result of Sludge Granulation

### A. COD removal efficiency at start-up stage

COD removal efficiency of CR at start-up time

| batch | EC5 | EC7 | EC8 | EC11 | EC13 | EC15 | EC17 | EC19 | EC20 | EC22 | EC24 | EC33 | EC35 | EC37 | EC38 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| COD Inf | | 576 | | | | | 620 | | | | | | | 1060 | |
| COD eff | 16 | 28 | 16 | 16 | 16 | 12 | 4 | 4 | 8 | 24 | 32 | 12 | 8 | 40 | 48 |
| COD Efficiency | 80 | 82 | 92 | 92 | 90 | 97 | 99 | 98 | 97 | 97 | 97 | 93 | 96 | 96 | 96 |

COD removal efficiency of AR at start-up time

| batch | EA4 | EA6 | EA10 | EA12 | EA14 | EA16 | EA18 | EA27 | EA29 | EA40 | EA46 | EA49 | EA54 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| COD in feed | | 576 | | | | | 620 | | | 1060 | | | |
| COD eff | 8 | 40 | 24 | 28 | 0 | 4 | 4 | 16 | 16 | 15 | 7.5 | 22 | 18.8 |
| COD Efficiency | 62 | 77 | 79 | 82 | 100 | 97 | 99 | 99 | 99 | 96 | 96 | 96 | 96 |

**B. COD removal efficiency of run 1, run 2**

COD removal for CR

| day | 1 | 2 | 3 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample name | CR28 | CR29 | CR30 | CR31 | CR2 | CR4 | CR6 | CR8 | CR10 | CR12 | CR14 | CR16 | CR17 |
| COD in feed, mg/L | 533 | 627 | 549 | 470 | 580 | 627 | 554 | 554 | 570 | 884 | 488 | 792 | 518 |
| COD eff, mg/L | 8 | 4 | 8 | 16 | 12 | 16 | 8 | 15 | 15 | 15 | 6 | 19 | 23 |
| Removal Efficiency (%) | 98 | 99 | 99 | 97 | 98 | 97 | 99 | 97 | 97 | 98 | 99 | 98 | 96 |
| loading rate, kg COD/$m^3$.day | 2.2 | 2.6 | 2.3 | 2.0 | 2.4 | 2.6 | 2.3 | 2.3 | 2.4 | 3.7 | 2.0 | 3.3 | 2.2 |
| | | | | | | | | | | | | | |
| day | 24 | 26 | 28 | 30 | 32 | 34 | 38 | 40 | 46 | 48 | 56 | 58 | |
| sample name | CR20 | CR22 | CR24 | CR26 | CR28 | CR30 | CR4 | CR6 | CR12 | CR14 | CR22 | CR24 | |
| COD in feed | 703 | 445 | 534 | 688 | 614 | 702 | 716 | 752 | 670 | 616 | 790 | 710 | |
| COD eff | 22 | 26 | 26 | 22 | 21 | 11 | 7 | 7 | 7 | 17 | 15 | 10 | |
| Removal Efficiency (%) | 97 | 94 | 95 | 97 | 97 | 98 | 99 | 99 | 99 | 97 | 98 | 99 | |
| loading rate | 2.9 | 1.9 | 2.2 | 2.9 | 2.6 | 2.9 | 3.0 | 3.1 | 2.8 | 2.6 | 3.3 | 3.0 | |
| | | | | | | | | | | | | | |
| day | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 |
| sample name | CR26 | CR27 | CR28 | CR29 | CR30 | CR31 | CR1 | CR2 | CR3 | CR4 | CR5 | CR6 | CR7 |
| COD in feed | 1065 | 1516 | 1565 | 1581 | 1645 | 1772 | 1613 | 1581 | 1548 | 1774 | 1516 | 1398 | 1515 |
| COD eff | 20 | 13 | 14 | 13 | 26 | 24 | 22 | 14 | 16 | 19 | 14 | 15 | 18 |
| Removal Efficiency (%) | 98 | 99 | 99 | 99 | 98 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| loading rate | 4.4 | 6.3 | 6.5 | 6.6 | 6.8 | 7.4 | 6.7 | 6.6 | 6.4 | 7.4 | 6.3 | 5.8 | 6.3 |
| | | | | | | | | | | | | | |
| day | 72 | 73 | 74 | 75 | 76 | 77 | 79 | 80 | 82 | 83 | 84 | 85 | 86 |
| sample name | CR8 | CR10 | CR11 | CR12 | CR13 | CR14 | CR16 | CR17 | CR19 | CR20 | CR21 | CR22 | CR24 |
| COD in feed | 1515 | 1577 | 1200 | 1346 | 1600 | 1230 | 1730 | 2692 | 1923 | 2500 | 2192 | 2692 | 2845 |
| COD eff | 27 | 30 | 27 | 31 | 23 | 23 | 23 | 23 | 19 | 23 | 23 | 27 | 46 |
| Removal Efficiency (%) | 98 | 98 | 98 | 98 | 99 | 98 | 99 | 99 | 99 | 99 | 99 | 99 | 98 |
| loading rate | 6.3 | 6.6 | 5.0 | 5.6 | 6.7 | 5.1 | 7.2 | 11.2 | 8.0 | 10.4 | 9.1 | 11.2 | 11.8 |
| | | | | | | | | | | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| day | 88 | 90 | 91 | 93 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | 103 |
| sample name | CR26 | CR28 | CR29 | CR31 | CR2 | CR3 | CR4 | CR5 | CR6 | CR7 | CR8 | CR9 | CR10 |
| COD in feed | 2845 | 2743 | 2438 | 2210 | 2591 | 3124 | 3734 | 3886 | 3774 | 3776 | 4377 | 4226 | 4528 |
| COD eff | 27 | 50 | 46 | 30 | 45 | 107 | 57 | 156 | 4 | 91 | 19 | 143 | 177 |
| Removal Efficiency (%) | 99 | 98 | 98 | 99 | 98 | 97 | 98 | 96 | 100 | 98 | 100 | 97 | 96 |
| loading rate | 11.8 | 11.4 | 10.1 | 9.2 | 10.8 | 13.0 | 15.5 | 16.2 | 15.7 | 15.7 | 18.2 | 17.6 | 18.8 |
| | | | | | | | | | | | | | |
| day | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 |
| sample name | CR11 | CR12 | CR13 | CR14 | CR15 | CR16 | CR17 | CR18 | CR19 | CR20 | CR21 | CR22 | CR23 |
| COD in feed | 4679 | 4679 | 4377 | 5887 | 5962 | 6340 | 6038 | 6585 | 7396 | 7320 | 7330 | 7300 | 3320 |
| COD eff | 76 | 143 | 45 | 98 | 121 | 83 | 91 | 70 | 98 | 90 | 91 | 86 | 25 |
| Removal Efficiency (%) | 98 | 97 | 99 | 98 | 98 | 99 | 98 | 99 | 99 | 99 | 99 | 99 | 99 |
| loading rate | 19.5 | 19.5 | 18.2 | 24.5 | 24.8 | 26.4 | 25.1 | 27.4 | 30.8 | 30.5 | 30.5 | 30.4 | 13.8 |

COD removal for AR:

| day | 1 | 2 | 3 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample name | AR28 | AR29 | AR30 | AR31 | AR2 | AR4 | AR6 | AR8 | AR10 | AR12 | AR14 | AR16 | AR17 |
| COD in feed | 533 | 627 | 549 | 470 | 580 | 627 | 554 | 554 | 570 | 884 | 488 | 792 | 518 |
| COD eff | 4 | 8 | 39 | 16 | 12 | 16 | 8 | 15 | 15 | 15 | 15 | 15 | 19 |
| Removal Efficiency (%) | 99 | 99 | 93 | 97 | 98 | 97 | 99 | 97 | 97 | 98 | 97 | 98 | 96 |
| loading rate | 2.2 | 2.6 | 2.3 | 2.0 | 2.4 | 2.6 | 2.3 | 2.3 | 2.4 | 3.7 | 2.0 | 3.3 | 2.2 |
| | | | | | | | | | | | | | |
| day | 24 | 26 | 28 | 30 | 32 | 34 | 38 | 40 | 46 | 48 | 56 | 58 | |
| sample name | AR20 | AR22 | AR24 | AR26 | AR28 | AR30 | AR4 | AR6 | AR12 | AR14 | AR22 | AR24 | |
| COD in feed | 703 | 445 | 534 | 688 | 614 | 702 | 716 | 752 | 670 | 616 | 790 | 710 | |
| COD eff | 15 | 26 | 22 | 19 | 25 | 11 | 14 | 7 | 10 | 10 | 17 | 13 | |
| Removal Efficiency (%) | 98 | 94 | 96 | 97 | 96 | 98 | 98 | 99 | 99 | 98 | 98 | 98 | |
| loading rate | 2.9 | 1.9 | 2.2 | 2.9 | 2.6 | 2.9 | 3.0 | 3.1 | 2.8 | 2.6 | 3.3 | 3.0 | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| day | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 |
| sample name | AR26 | AR27 | AR28 | AR29 | AR30 | AR31 | AR1 | AR2 | AR3 | AR4 | AR5 | AR6 | AR7 |
| COD in feed | 1065 | 1516 | 1565 | 1581 | 1645 | 1772 | 1613 | 1581 | 1548 | 1774 | 1516 | 1398 | 1515 |
| COD eff | 25 | 7 | 28 | 35 | 13 | 24 | 13 | 18 | 16 | 23 | 14 | 16 | 19 |
| Removal Efficiency (%) | 98 | 100 | 98 | 98 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 | 99 |
| loading rate | 4.4 | 6.3 | 6.5 | 6.6 | 6.8 | 7.4 | 6.7 | 6.6 | 6.4 | 7.4 | 6.3 | 5.8 | 6.3 |
| | | | | | | | | | | | | | |
| day | 72 | 73 | 74 | 75 | 76 | 77 | 79 | 80 | 82 | 83 | 84 | 85 | 86 |
| sample name | AR8 | AR10 | AR11 | AR12 | AR13 | AR14 | AR16 | AR17 | AR19 | AR20 | AR21 | AR22 | CR24 |
| COD in feed | 1515 | 1577 | 1200 | 1346 | 1600 | 1230 | 1730 | 2692 | 1923 | 2500 | 2192 | 2692 | 2845 |
| COD eff | 19 | 30 | 31 | 27 | 27 | 31 | 19 | 29 | 19 | 15 | 35 | 27 | 31 |
| Removal Efficiency (%) | 99 | 98 | 97 | 98 | 98 | 97 | 99 | 99 | 99 | 99 | 98 | 99 | 99 |
| loading rate | 6.3 | 6.6 | 5.0 | 5.6 | 6.7 | 5.1 | 7.2 | 11.2 | 8.0 | 10.4 | 9.1 | 11.2 | 11.8 |
| | | | | | | | | | | | | | |
| day | 88 | 90 | 91 | 93 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | 103 |
| sample name | AR26 | AR28 | AR29 | AR31 | AR2 | AR3 | AR4 | AR5 | AR6 | AR7 | AR8 | AR9 | AR10 |
| COD in feed | 2515 | 2743 | 2438 | 2210 | 2591 | 3124 | 3734 | 3886 | 3774 | 3776 | 4377 | 4226 | 4528 |
| COD eff | 46 | 50 | 76 | 35 | 45 | 149 | 164 | 123 | 106 | 109 | 91 | 159 | 211 |
| Removal Efficiency (%) | 98 | 98 | 97 | 98 | 98 | 95 | 96 | 97 | 97 | 97 | 98 | 96 | 95 |
| loading rate | 10.5 | 11.4 | 10.1 | 9.2 | 10.8 | 13.0 | 15.5 | 16.2 | 15.7 | 15.7 | 18.2 | 17.6 | 18.8 |
| | | | | | | | | | | | | | |
| day | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 |
| sample name | AR11 | AR12 | AR13 | AR14 | AR15 | AR16 | AR17 | AR18 | AR19 | AR20 | AR21 | AR22 | AR23 |
| COD in feed | 4679 | 4679 | 4377 | 5887 | 5962 | 6340 | 6038 | 6585 | 7396 | 7320 | 7330 | 7300 | 3320 |
| COD eff | 87 | 94 | 98 | 91 | 143 | 98 | 98 | 110 | 136 | 84 | 80 | 78 | 30 |
| Removal Efficiency (%) | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 99 | 99 | 99 | 99 |
| loading rate | 19.5 | 19.5 | 18.2 | 24.5 | 24.8 | 26.4 | 25.1 | 27.4 | 30.8 | 30.5 | 30.5 | 30.4 | 13.8 |

## C. Statistics of physical chemical biological characteristics of granules

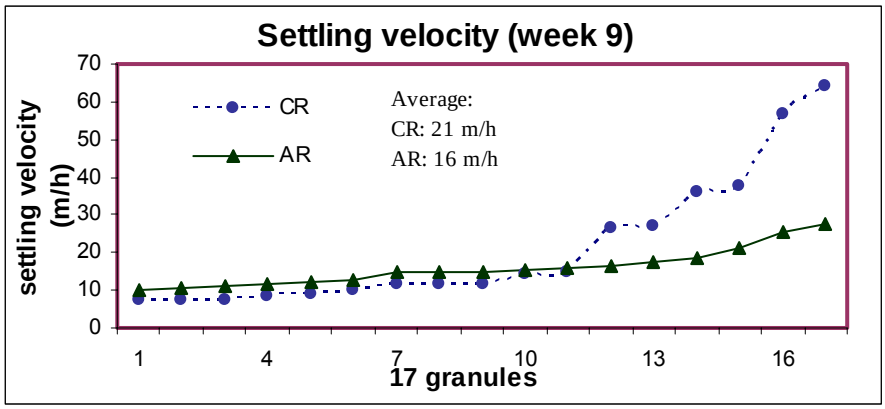
| | | hydrophobicity | | EPS-PS (mg/g) | | EPS-PN (mg/g) | | PS/PN | | PS eff (mg/L) | | PN eff (mg/L) | | MLVSS | | MLVSS in effluent | | OLR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| week | day | CR | AR | CR | AR | CR | AR | CR | AR | CR | AR | CR | AR | CR | AR | CR | AR | |
| 1 | seed | 31 | 31 | 26.4 | 26.4 | 90.2 | 90.2 | 0.29 | 0.29 | 4.8 | 4.8 | 6.7 | 6.7 | 4947 | 4243 | | | |
| 1+2 | 1 - 7 Nov | | | | | | | | | | | | | | | | | 2.5 |
| 3 | 8 - 14 Nov | 65 | 65 | 42.2 | 45.6 | 69.5 | 76.4 | 0.61 | 0.60 | 5.8 | 4.0 | 0.0 | 0.0 | 4278 | 3653 | 316 | 313 | |
| 4 | 15-21 Nov | 69 | 59 | 27.8 | 30.5 | 83.4 | 108.1 | 0.33 | 0.28 | | | 0.0 | 0.0 | 3697 | 1813 | 151 | 225 | |
| 5 | 22-28 Nov | 71 | 70 | 54.3 | 62.6 | 91.6 | 120.2 | 0.59 | 0.52 | 3.1 | 2.3 | 0.0 | 0.0 | 2584 | 1640 | 112 | 173 | |
| 6 | 29 -5 Dec | 75 | 75 | 50.5 | 49.8 | 86.9 | 88.9 | 0.58 | 0.56 | 2.3 | 3.8 | 0.0 | 0.0 | 3385 | 2358 | 91 | 136 | |
| 7 | 6 -12 Dec | 81 | 84 | 55.9 | 52.5 | 89.9 | 98.9 | 0.62 | 0.53 | 7.0 | 6.9 | 0.0 | 0.0 | 5169 | 2905 | 80 | 183 | |
| 8 | 13 - 19 Dec | 80 | 84 | 70.3 | 89.3 | 84.0 | 106.3 | 0.84 | 0.84 | 5.8 | 5.5 | 0.0 | 0.0 | 5124 | 2947 | 164 | 181 | |
| 9 | 20 - 26 Dec | | | 49.5 | 60.2 | 87.4 | 105.8 | 0.57 | 0.57 | 3.7 | 3.6 | 0.0 | 0.0 | 5450 | 3484 | 247 | 180 | |
| 10 | 27 - 2 Jan | 84 | 86 | 55.6 | 68.7 | 69.9 | 98.1 | 0.80 | 0.70 | 5.7 | 12.7 | 0.3 | 1.2 | 5805 | 4988 | 309 | 303 | 5 |
| 11 | 3 - 9 Jan | | | 47.2 | 57.7 | 60 | 87.3 | 0.79 | 0.66 | 6.0 | 4.8 | 0.5 | 0.2 | 7134 | 3943 | 784 | 491 | |
| 12 | 10 - 16 Jan | 79 | 76 | 77.9 | 74 | 110.1 | 115.3 | 0.71 | 0.64 | 13.1 | 12.5 | 1.1 | 1.1 | 4414 | 4167 | 421 | 491 | |
| 13 | 17 - 23 Jan | | | 33.8 | 66.3 | 92.5 | 97.9 | 0.37 | 0.68 | 15.5 | 24.4 | 11.8 | 11.8 | 8378 | 3198 | 1092 | 465 | |
| 14 | 24 - 29 Jan | | | 62.9 | 40.3 | 104.6 | 82.9 | 0.60 | 0.49 | 17.6 | 24.7 | 9.5 | 26.0 | 8086 | 7551 | 504 | 320 | |
| 15 | 30 - 6 Feb | 66 | 67 | 46 | 45 | 78.3 | 75.2 | 0.59 | 0.60 | 26.5 | 22.9 | 21.0 | 22.0 | 12303 | 11346 | 918 | 656 | 10 |
| 16 | 7 - 20 feb | 51 | 53 | 77.7 | 60.9 | 67.7 | 45.1 | 1.15 | 1.35 | 30.6 | 30.0 | 6.2 | 5.3 | 10479 | 14696 | 1269 | 807 | 15-30 |

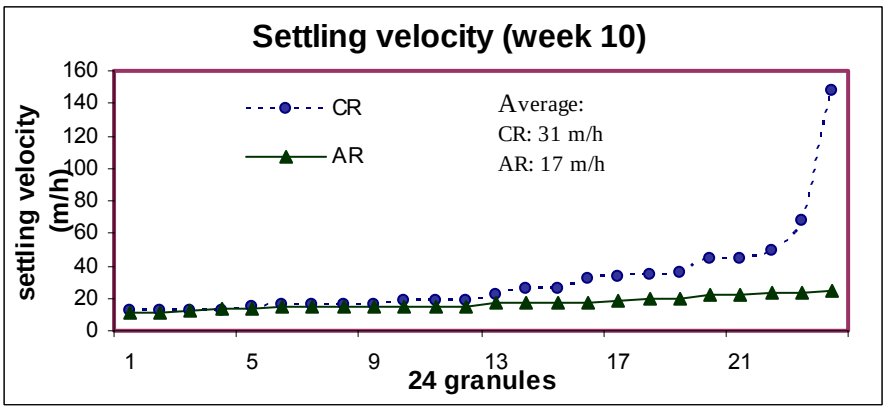
| | | SRT | | BM density (g/L) | | Carrier conc | SVI (mL/g) | | settling velocity (m/h) | | granule size (mm) | | OLR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| week | day | CR | AR | CR | AR | | CR | AR | CR | AR | CR | AR | |
| 1 | seed | | | 2.7 | 2.7 | g/L | 243 | 243 | 10 | 10 | | | |
| 1+2 | 1 - 7 Nov | | | | | | 24 | 43 | <10 | <10 | | | 2.5 |
| 3 | 8 - 14 Nov | 3.3 | 2.8 | 20.8 | 23.8 | 2.3 | 26 | 34 | | | | | |
| 4 | 15- 21 Nov | 5.9 | 1.9 | 24.4 | 23.9 | 1.4 | 24 | 30 | | | | | |
| 5 | 22 - 28 Nov | 5.5 | 2.3 | 24.8 | 23.8 | 5.9 | 32 | 32 | | | | | |
| 6 | 29 - 5 Dec | 8.9 | 4.2 | 26.3 | 26.1 | 7.7 | 19 | 34 | | | 0.1-0.5 | 0.2-1.0 | |
| 7 | 6 -12 Dec | 15.5 | 3.8 | 23.1 | 22.8 | 5.9 | 23 | 37 | | | * | * | |
| 8 | 13 - 19 Dec | 7.5 | 3.9 | 23.5 | 22.2 | 8.8 | 23 | 39 | | | 0.2-0.8 | 0.2 -1.4 | |
| 9 | 20 - 26 Dec | 5.3 | 4.7 | 25.0 | 24.1 | 8.5 | 18 | 35 | 21 | 16 | 0.3-0.9 | 0.2-1.5 | |
| 10 | 27 - 2 Jan | 4.5 | 4.0 | 25.0 | 21.8 | 10.9 | 26 | 39 | 31 | 17 | | | 5 |
| 11 | 3 - 9 Jan | 2.2 | 1.9 | 25.2 | 20.0 | 4.07 | 22 | 35 | 56 | 15 | | | |
| 12 | 10 - 16 Jan | 2.5 | 2.0 | 32.1 | 28.3 | | 23 | 33 | 87 | 22 | 0.25-1.5 | 0.1-0.5 | |
| 13 | 17 - 23 Jan | 1.8 | 1.7 | 33.5 | 28.7 | | 15 | 37 | 64 | 14 | 0.2-2 | 0.15-0.65 | |
| 14 | 24 - 29 Jan | 3.9 | 5.7 | 46.8 | 44.3 | | 15 | 28 | 103 | 51 | 0.5-1.6 | 1.2-3.0 | |
| 15 | 30 - 6 Feb | 3.2 | 4.2 | 46.5 | 51.4 | | 15 | 21 | 73 | 44 | 0.5-2.0 | 1.0-3.0 | 10 |
| 16 | 7 - 20 feb | 2.0 | 4.4 | 49.2 | 62.1 | | 14 | 18.7 | 72 | 49 | 0.5-2.0 | 0.3-4.0 | 15-30 |

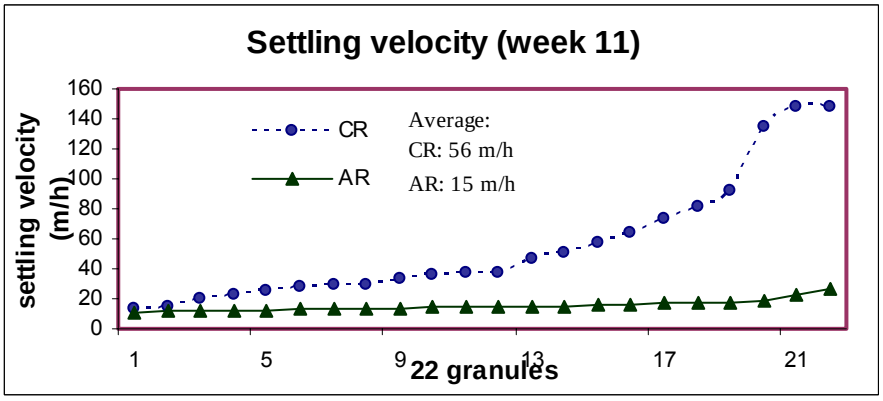
## D. Metal elements in granules

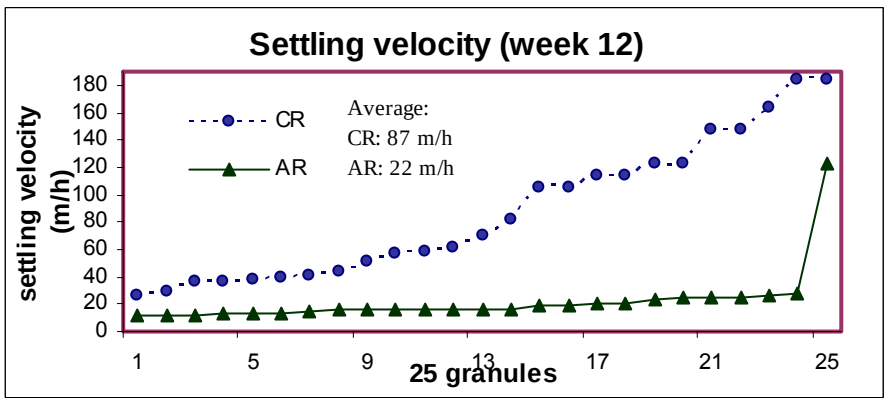
| Sludge | weight (g) | Ca (mg/l) | Ca (mg/g VSS) | Mg (mg/L) | Mg (mg/g VSS) | K (mg/L) | K (mg/g VSS) | Na (mg/L) | Na (mg/g VSS) | Fe (mg/L) | Fe (mg/g VSS) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| seed | 0.1000 | 6.708 | 3.4 | 1.828 | 0.9 | 5.463 | 2.7 | 2.017 | 1.0 | 10.254 | 5.1 |
| CR15 | 0.1052 | 6.850 | 3.3 | 1.838 | 0.9 | 12.549 | 6.0 | 6.719 | 3.2 | 2.026 | 1.0 |
| AR15 | 0.0993 | 2.911 | 1.5 | 1.808 | 0.9 | 12.733 | 6.4 | 8.932 | 4.5 | 1.273 | 0.6 |
| CR5 | 0.0212 | 9.459 | 22.3 | 1.552 | 3.7 | 4.178 | 9.9 | 4.208 | 9.9 | 0.419 | 1.0 |
| AR5 | 0.0636 | 4.299 | 3.4 | 1.815 | 1.4 | 9.695 | 7.6 | 4.060 | 3.2 | 7.194 | 5.7 |
| CR2.5 | 0.0384 | 11.869 | 15.5 | 1.880 | 2.4 | 5.269 | 6.9 | 0.925 | 1.2 | 4.952 | 6.4 |
| AR 2.5 | 0.0502 | 4.128 | 4.1 | 1.813 | 1.8 | 5.369 | 5.3 | 0.764 | 0.8 | 7.344 | 7.3 |

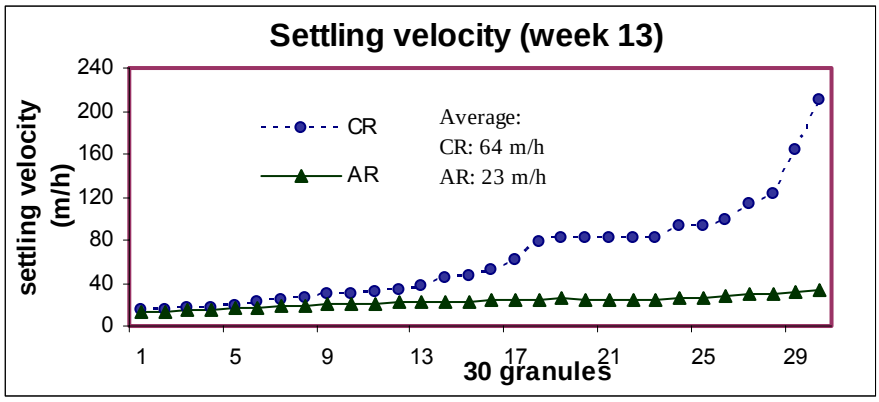
**E. Settling velocity of granules at different week (OLRs)**

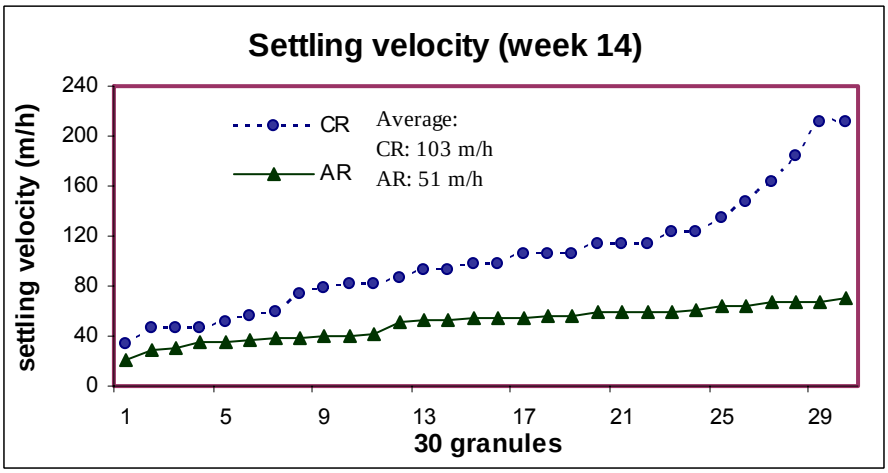


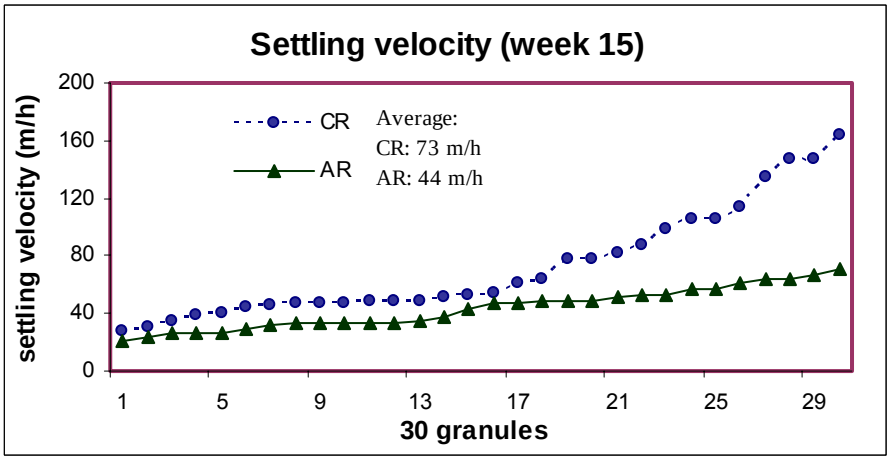



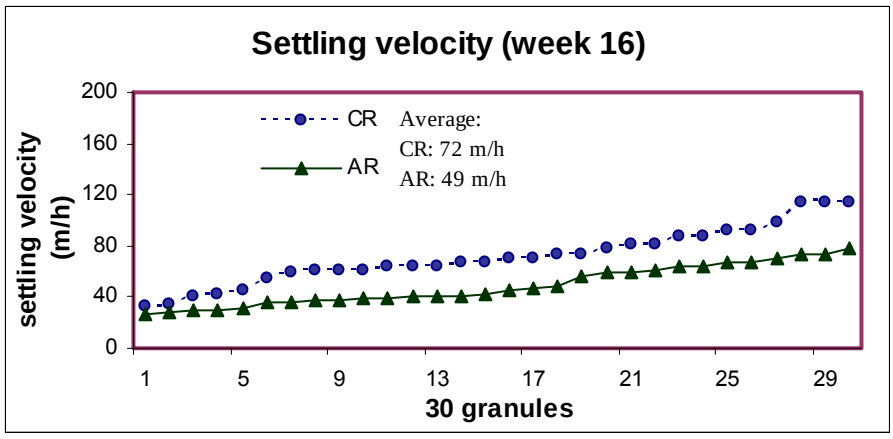




Settling velocity (week 13)
settling velocity (m/h)
240
200
160
120
80
40
0
CR
AR
Average:
CR: 64 m/h
AR: 23 m/h
1
5
9
13
17
21
25
29
30 granules

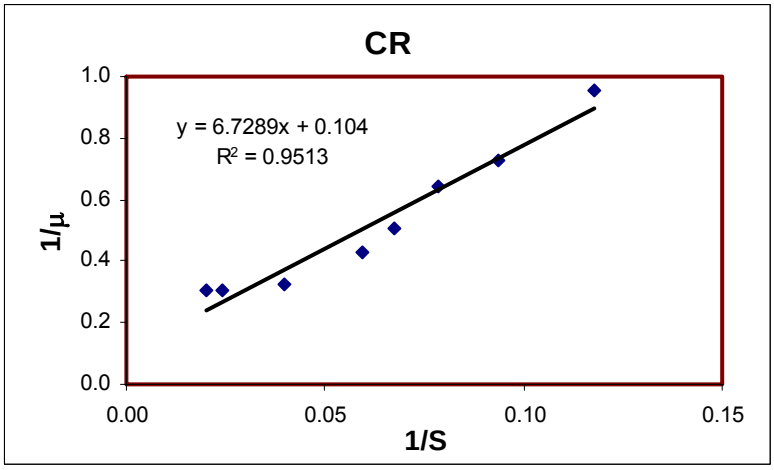

Settling velocity (week 14)
settling velocity (m/h)
240
200
160
120
80
40
0
CR
AR
Average:
CR: 103 m/h
AR: 51 m/h
1
5
9
13
17
21
25
29
30 granules

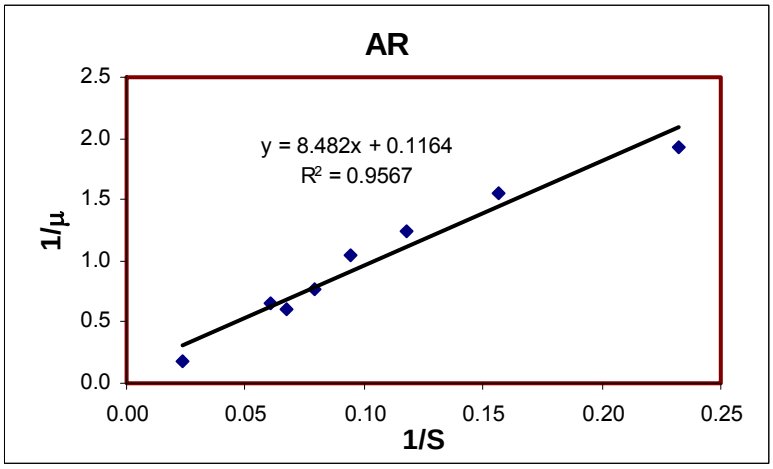

Settling velocity (week 15)
settling velocity (m/h)
200
160
120
80
40
0
CR
AR
Average:
CR: 73 m/h
AR: 44 m/h
1
5
9
13
17
21
25
29
30 granules


Settling velocity (week 16)
settling velocity (m/h)
200
160
120
80
40
0
CR
AR
Average:
CR: 72 m/h
AR: 49 m/h
1
5
9
13
17
21
25
29
30 granules

## F. Kinetic data of granules

Maximum specific growth rate and haft saturation coefficient of granule determination:



## Data for SOUR, OUR of CR at ratio S/X = 0.013

| S/X = 0.013 | | | | | |
|---|---|---|---|---|---|
| TIME | duration, sec | time, sec | DO, mg/L | OUR, mg/L.h | SOUR, mg/g.h |
| 5:38:23 PM | | | 8.63 | | |
| 5:38:38 PM | 15 | 15 | 8.6 | 4.5 | 1.2 |
| 5:38:54 PM | 16 | 31 | 8.58 | 69.6 | 18.5 |
| 5:39:09 PM | 15 | 46 | 8.29 | 146.3 | 38.9 |
| 5:39:25 PM | 16 | 62 | 7.64 | 175.5 | 46.7 |
| 5:39:41 PM | 16 | 78 | 6.86 | 175.5 | 46.7 |
| 5:39:57 PM | 16 | 94 | 6.08 | 162.0 | 43.1 |
| 5:40:13 PM | 16 | 110 | 5.36 | 144.0 | 38.3 |
| 5:40:28 PM | 15 | 125 | 4.76 | 119.3 | 31.7 |
| 5:40:44 PM | 16 | 141 | 4.23 | 80.5 | 21.4 |
| 5:41:01 PM | 17 | 158 | 3.85 | 63.0 | 16.8 |
| 5:41:17 PM | 16 | 174 | 3.57 | 45.0 | 12.0 |
| 5:41:33 PM | 16 | 190 | 3.37 | 31.5 | 8.4 |
| 5:41:49 PM | 16 | 206 | 3.23 | 27.0 | 7.2 |
| 5:42:05 PM | 16 | 222 | 3.11 | 24.8 | 6.6 |
| 5:42:21 PM | 16 | 238 | 3 | 21.6 | 5.7 |
| 5:42:36 PM | 15 | 253 | 2.91 | 19.1 | 5.1 |
| 5:42:53 PM | 17 | 270 | 2.82 | 18.0 | 4.8 |
| 5:43:09 PM | 16 | 286 | 2.74 | 18.0 | 4.8 |
| 5:43:25 PM | 16 | 302 | 2.66 | 15.8 | 4.2 |
| 5:43:41 PM | 16 | 318 | 2.59 | 15.8 | 4.2 |
| 5:43:57 PM | 16 | 334 | 2.52 | 15.8 | 4.2 |
| 5:44:13 PM | 16 | 350 | 2.45 | 15.8 | 4.2 |
| 5:44:29 PM | 16 | 366 | 2.38 | 13.5 | 3.6 |
| 5:44:45 PM | 16 | 382 | 2.32 | 13.5 | 3.6 |
| 5:45:01 PM | 16 | 398 | 2.26 | 15.8 | 4.2 |
| 5:45:17 PM | 16 | 414 | 2.19 | 13.5 | 3.6 |
| 5:45:33 PM | 16 | 430 | 2.13 | 14.4 | 3.8 |
| 5:45:48 PM | 15 | 445 | 2.07 | 13.5 | 3.6 |
| 5:46:04 PM | 16 | 461 | 2.01 | 12.7 | 3.4 |
| 5:46:21 PM | 17 | 478 | 1.95 | 13.5 | 3.6 |
| 5:46:37 PM | 16 | 494 | 1.89 | 13.5 | 3.6 |
| 5:46:53 PM | 16 | 510 | 1.83 | 11.3 | 3.0 |
| 5:47:09 PM | 16 | 526 | 1.78 | 14.4 | 3.8 |
| 5:47:24 PM | 15 | 541 | 1.72 | 11.3 | 3.0 |
| 5:47:40 PM | 16 | 557 | 1.67 | 13.5 | 3.6 |
| 5:47:56 PM | 16 | 573 | 1.61 | 11.3 | 3.0 |
| 5:48:12 PM | 16 | 589 | 1.56 | 13.5 | 3.6 |
| 5:48:28 PM | 16 | 605 | 1.5 | 11.3 | 3.0 |
| 5:48:44 PM | 16 | 621 | 1.45 | 10.6 | 2.8 |
| 5:49:01 PM | 17 | 638 | 1.4 | 13.5 | 3.6 |
| 5:49:17 PM | 16 | 654 | 1.34 | 12.0 | 3.2 |
| 5:49:32 PM | 15 | 669 | 1.29 | 11.3 | 3.0 |
| 5:49:48 PM | 16 | 685 | 1.24 | 11.3 | 3.0 |
| 5:50:04 PM | 16 | 701 | 1.19 | 11.3 | 3.0 |
| 5:50:20 PM | 16 | 717 | 1.14 | 10.6 | 2.8 |
| 5:50:37 PM | 17 | 734 | 1.09 | 11.3 | 3.0 |
| 5:50:53 PM | 16 | 750 | 1.04 | 10.6 | 2.8 |

| | | | | | |
|---|---|---|---|---|---|
| 5:51:10 PM | 17 | 767 | 0.99 | 12.0 | 3.2 |
| 5:51:25 PM | 15 | 782 | 0.94 | 9.0 | 2.4 |
| 5:51:41 PM | 16 | 798 | 0.9 | 11.3 | 3.0 |
| 5:51:57 PM | 16 | 814 | 0.85 | 9.0 | 2.4 |
| 5:52:13 PM | 16 | 830 | 0.81 | 11.3 | 3.0 |
| 5:52:29 PM | 16 | 846 | 0.76 | 11.3 | 3.0 |
| 5:52:45 PM | 16 | 862 | 0.71 | 9.0 | 2.4 |
| 5:53:01 PM | 16 | 878 | 0.67 | 11.3 | 3.0 |
| 5:53:17 PM | 16 | 894 | 0.62 | 9.0 | 2.4 |
| 5:53:33 PM | 16 | 910 | 0.58 | 9.0 | 2.4 |
| 5:53:49 PM | 16 | 926 | 0.54 | 11.3 | 3.0 |
| 5:54:05 PM | 16 | 942 | 0.49 | 7.2 | 1.9 |
| 5:54:20 PM | 15 | 957 | 0.46 | 8.5 | 2.3 |
| 5:54:37 PM | 17 | 974 | 0.42 | 11.3 | 3.0 |
| 5:54:53 PM | 16 | 990 | 0.37 | 9.0 | 2.4 |
| 5:55:09 PM | 16 | 1006 | 0.33 | 9.0 | 2.4 |
| 5:55:25 PM | 16 | 1022 | 0.29 | 6.7 | 1.8 |
| 5:55:41 PM | 16 | 1038 | 0.26 | 9.0 | 2.4 |
| 5:55:57 PM | 16 | 1054 | 0.22 | 9.6 | 2.6 |
| 5:56:12 PM | 15 | 1069 | 0.18 | 6.4 | 1.7 |
| 5:56:29 PM | 17 | 1086 | 0.15 | 7.2 | 1.9 |
| 5:56:44 PM | 15 | 1101 | 0.12 | 9.0 | 2.4 |
| 5:57:00 PM | 16 | 1117 | 0.08 | 6.4 | 1.7 |
| 5:57:17 PM | 17 | 1134 | 0.05 | 4.5 | 1.2 |
| 5:57:33 PM | 16 | 1150 | 0.03 | 2.4 | 0.6 |
| 5:57:48 PM | 15 | 1165 | 0.02 | 2.1 | 0.6 |
| 5:58:05 PM | 17 | 1182 | 0.01 | 0.0 | 0.0 |
| 5:58:21 PM | 16 | 1198 | 0.01 | 0.0 | 0.0 |

## Data for SOUR, OUR of AR at ratio S/X = 0.023

| S/X = 0.023 | | | | | |
|---|---|---|---|---|---|
| TIME | duration, sec | time, sec | DO, mg/L | OUR, mg/L.h | SOUR, mg/g.h |
| 7:54:07 PM | | | | | |
| 7:54:17 PM | 10 | 10 | 8.82 | 7.20 | 4.0 |
| 7:54:27 PM | 10 | 20 | 8.80 | 7.20 | 4.0 |
| 7:54:37 PM | 10 | 30 | 8.78 | 10.80 | 5.9 |
| 7:54:47 PM | 10 | 40 | 8.75 | 10.80 | 5.9 |
| 7:54:57 PM | 10 | 50 | 8.72 | 7.20 | 4.0 |
| 7:55:07 PM | 10 | 60 | 8.70 | 9.00 | 4.9 |
| 7:55:19 PM | 12 | 72 | 8.67 | 7.20 | 4.0 |
| 7:55:29 PM | 10 | 82 | 8.65 | 6.55 | 3.6 |
| 7:55:40 PM | 11 | 93 | 8.63 | 14.40 | 7.9 |
| 7:55:50 PM | 10 | 103 | 8.59 | 6.55 | 3.6 |
| 7:56:01 PM | 11 | 114 | 8.57 | 9.82 | 5.4 |
| 7:56:12 PM | 11 | 125 | 8.54 | 9.82 | 5.4 |
| 7:56:23 PM | 11 | 136 | 8.51 | 6.55 | 3.6 |
| 7:56:34 PM | 11 | 147 | 8.49 | 9.00 | 4.9 |
| 7:56:46 PM | 12 | 159 | 8.46 | 6.00 | 3.3 |
| 7:56:58 PM | 12 | 171 | 8.44 | 33.00 | 18.1 |
| 7:57:10 PM | 12 | 183 | 8.33 | 66.00 | 36.2 |
| 7:57:22 PM | 12 | 195 | 8.11 | 77.54 | 42.6 |
| 7:57:35 PM | 13 | 208 | 7.83 | 102.00 | 56.0 |
| 7:57:47 PM | 12 | 220 | 7.49 | 94.15 | 51.7 |
| 7:58:00 PM | 13 | 233 | 7.15 | 105.23 | 57.8 |
| 7:58:13 PM | 13 | 246 | 6.77 | 88.62 | 48.7 |
| 7:58:26 PM | 13 | 259 | 6.45 | 88.62 | 48.7 |
| 7:58:39 PM | 13 | 272 | 6.13 | 79.71 | 43.8 |
| 7:58:53 PM | 14 | 286 | 5.82 | 91.38 | 50.2 |
| 7:59:06 PM | 13 | 299 | 5.49 | 79.71 | 43.8 |
| 7:59:20 PM | 14 | 313 | 5.18 | 64.80 | 35.6 |
| 7:59:35 PM | 15 | 328 | 4.91 | 66.86 | 36.7 |
| 7:59:49 PM | 14 | 342 | 4.65 | 51.43 | 28.2 |
| 8:00:03 PM | 14 | 356 | 4.45 | 46.29 | 25.4 |
| 8:00:17 PM | 14 | 370 | 4.27 | 40.80 | 22.4 |
| 8:00:32 PM | 15 | 385 | 4.10 | 36.00 | 19.8 |
| 8:00:46 PM | 14 | 399 | 3.96 | 29.25 | 16.1 |
| 8:01:02 PM | 16 | 415 | 3.83 | 24.00 | 13.2 |
| 8:01:17 PM | 15 | 430 | 3.73 | 20.25 | 11.1 |
| 8:01:33 PM | 16 | 446 | 3.64 | 19.20 | 10.5 |
| 8:01:48 PM | 15 | 461 | 3.56 | 16.94 | 9.3 |
| 8:02:05 PM | 17 | 478 | 3.48 | 14.82 | 8.1 |
| 8:02:22 PM | 17 | 495 | 3.41 | 13.50 | 7.4 |
| 8:02:38 PM | 16 | 511 | 3.35 | 15.75 | 8.6 |
| 8:02:54 PM | 16 | 527 | 3.28 | 11.25 | 6.2 |
| 8:03:10 PM | 16 | 543 | 3.23 | 9.60 | 5.3 |
| 8:03:25 PM | 15 | 558 | 3.19 | 11.25 | 6.2 |
| 8:03:41 PM | 16 | 574 | 3.14 | 11.25 | 6.2 |
| 8:03:57 PM | 16 | 590 | 3.09 | 9.00 | 4.9 |
| 8:04:13 PM | 16 | 606 | 3.05 | 11.25 | 6.2 |
| 8:04:29 PM | 16 | 622 | 3.00 | 9.00 | 4.9 |

| | | | | | |
|---|---|---|---|---|---|
| 8:04:45 PM | 16 | 638 | 2.96 | 7.20 | 4.0 |
| 8:05:00 PM | 15 | 653 | 2.93 | 9.00 | 4.9 |
| 8:05:16 PM | 16 | 669 | 2.89 | 6.75 | 3.7 |
| 8:05:32 PM | 16 | 685 | 2.86 | 8.47 | 4.7 |
| 8:05:49 PM | 17 | 702 | 2.82 | 6.75 | 3.7 |
| 8:06:05 PM | 16 | 718 | 2.79 | 6.75 | 3.7 |
| 8:06:21 PM | 16 | 734 | 2.76 | 9.00 | 4.9 |
| 8:06:37 PM | 16 | 750 | 2.72 | 6.75 | 3.7 |
| 8:06:53 PM | 16 | 766 | 2.69 | 7.20 | 4.0 |
| 8:07:08 PM | 15 | 781 | 2.66 | 4.50 | 2.5 |
| 8:07:24 PM | 16 | 797 | 2.64 | 9.00 | 4.9 |
| 8:07:40 PM | 16 | 813 | 2.60 | 4.50 | 2.5 |
| 8:07:56 PM | 16 | 829 | 2.58 | 6.75 | 3.7 |
| 8:08:12 PM | 16 | 845 | 2.55 | 6.35 | 3.5 |
| 8:08:29 PM | 17 | 862 | 2.52 | 6.75 | 3.7 |
| 8:08:45 PM | 16 | 878 | 2.49 | 7.20 | 4.0 |
| 8:09:00 PM | 15 | 893 | 2.46 | 4.50 | 2.5 |
| 8:09:16 PM | 16 | 909 | 2.44 | 6.75 | 3.7 |
| 8:09:32 PM | 16 | 925 | 2.41 | 4.50 | 2.5 |
| 8:09:48 PM | 16 | 941 | 2.39 | 9.00 | 4.9 |
| 8:10:04 PM | 16 | 957 | 2.35 | 4.24 | 2.3 |
| 8:10:21 PM | 17 | 974 | 2.33 | 4.50 | 2.5 |
| 8:10:37 PM | 16 | 990 | 2.31 | 6.75 | 3.7 |
| 8:10:53 PM | 16 | 1006 | 2.28 | 4.50 | 2.5 |
| 8:11:09 PM | 16 | 1022 | 2.26 | 6.75 | 3.7 |
| 8:11:25 PM | 16 | 1038 | 2.23 | 4.50 | 2.5 |
| 8:11:41 PM | 16 | 1054 | 2.21 | 4.80 | 2.6 |
| 8:11:56 PM | 15 | 1069 | 2.19 | 6.75 | 3.7 |
| 8:12:12 PM | 16 | 1085 | 2.16 | 4.50 | 2.5 |
| 8:12:28 PM | 16 | 1101 | 2.14 | 4.50 | 2.5 |
| 8:12:44 PM | 16 | 1117 | 2.12 | 4.50 | 2.5 |
| 8:13:00 PM | 16 | 1133 | 2.10 | 6.75 | 3.7 |
| 8:13:16 PM | 16 | 1149 | 2.07 | 4.50 | 2.5 |
| 8:13:32 PM | 16 | 1165 | 2.05 | 4.50 | 2.5 |
| 8:13:48 PM | 16 | 1181 | 2.03 | 4.24 | 2.3 |
| 8:14:05 PM | 17 | 1198 | 2.01 | 4.50 | 2.5 |
| 8:14:21 PM | 16 | 1214 | 1.99 | | |

## Supernatant Characterization

### F. Membrane Fouling Index of supernatant at different loading rate:

| OLR (kgCOD/$m^3$.day) | 2.5 | 5 | 10 | 15 | 15 |
|---|---|---|---|---|---|
| MFI *$10^6$ | | | | | |
| day | (21/12) | (10/1) | (3/2) | (10/2) | (14/1) |
| CR *$10^6$ | 0.0009 | 0.1307 | 0.5727 | 1.0189 | 0.1717 |
| AR *$10^6$ | 0.0012 | 0.2927 | 0.6366 | 1.6455 | 0.3319 |
| CR *$10^3$ | 0.9 | 130.7 | 572.7 | 1018.9 | 171.7 |
| AR *$10^3$ | 1.2 | 292.7 | 636.6 | 1645.5 | 331.9 |

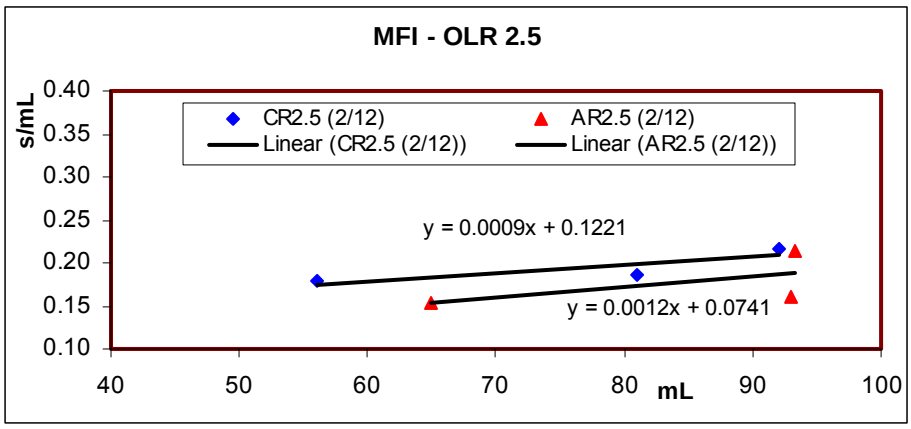
### G. Comparison of Membrane fouling index between distilled water, SBR, SBAR at OLR of 2.5 kg COD/$m^3$.day:

| | OLR | | MFI (x$10^6$) | s/$L^2$ | MFI (x$10^3$) |
|---|---|---|---|---|---|
| DI | | | 0.0002 | | 0.2 |
| SBR 2.5 | 2.5 | 07-Feb | 0.0019 | | 1.9 |
| CR 2.5 | 2.5 | 21-Dec | | | 0.9 |
| AR 2.5 | 2.5 | 21-Dec | | | 1.2 |

### H. Data for Membrane fouling Index at different OLRs:

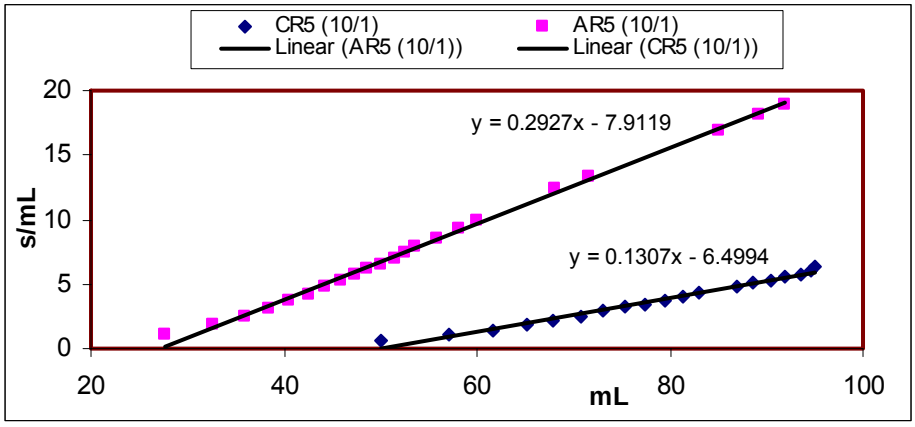
### Loading rate 2.5 kg COD/$m^3$.day:

| t (sec) | CR2.5 (21/2) | t/v | t (sec) | AR2.5 (21/2) | |
|---|---|---|---|---|---|
| s | mL | s/mL | s | mL | s/mL |
| 10 | 56 | 0.18 | 10 | 65 | 0.15 |
| 15 | 81 | 0.19 | 15 | 93 | 0.16 |
| 20 | 92 | 0.22 | 20 | 93.3 | 0.21 |



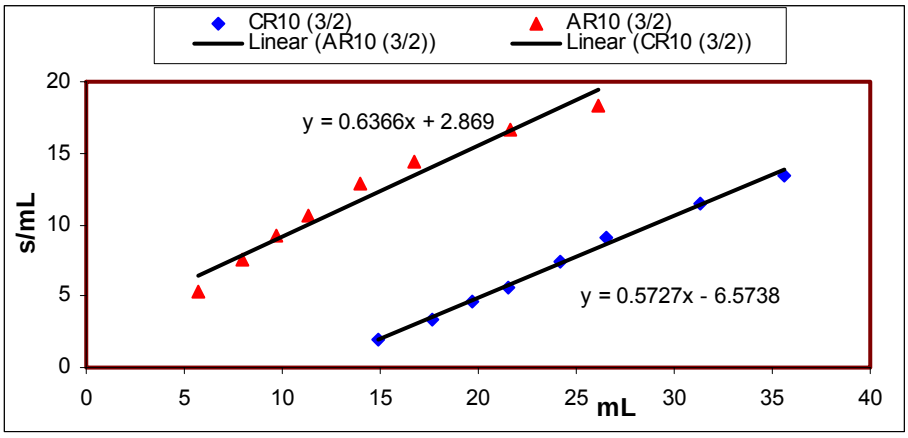

### Loading rate 5 kg COD/$m^3$.day:

| t (min) | CR5 (10/1) | t/v (s/ml) | | t (min) | AR5 (10/1) | t/v (s/ml) |
|---|---|---|---|---|---|---|
| min | ml | s/ml | | min | ml | s/ml |
| 0.5 | 50.0 | 0.60 | | 0.5 | 27.6 | 1.09 |
| 1.0 | 57.0 | 1.05 | | 1.0 | 32.6 | 1.84 |
| 1.5 | 61.6 | 1.46 | | 1.5 | 35.9 | 2.51 |
| 2.0 | 65.1 | 1.84 | | 2.0 | 38.5 | 3.12 |
| 2.5 | 67.9 | 2.21 | | 2.5 | 40.6 | 3.69 |
| 3.0 | 70.7 | 2.55 | | 3.0 | 42.6 | 4.23 |
| 3.5 | 73.0 | 2.88 | | 3.5 | 44.3 | 4.74 |
| 4.0 | 75.4 | 3.18 | | 4.0 | 45.9 | 5.23 |
| 4.5 | 77.5 | 3.48 | | 4.5 | 47.4 | 5.70 |
| 5.0 | 79.5 | 3.77 | | 5.0 | 48.7 | 6.16 |
| 5.5 | 81.4 | 4.05 | | 5.5 | 50.1 | 6.59 |
| 6.0 | 83.0 | 4.34 | | 6.0 | 51.5 | 6.99 |
| 7.0 | 86.9 | 4.83 | | 6.5 | 52.5 | 7.43 |
| 7.5 | 88.7 | 5.07 | | 7.0 | 53.6 | 7.84 |
| 8.0 | 90.4 | 5.31 | | 8.0 | 55.9 | 8.59 |
| 8.5 | 91.9 | 5.55 | | 9.0 | 58.1 | 9.29 |
| 9.0 | 93.5 | 5.78 | | 10.0 | 60.1 | 9.98 |
| 9.5 | 94.6 | 6.03 | | 14.0 | 68.0 | 12.35 |
| 10.0 | 95.0 | 6.32 | | 16.0 | 71.6 | 13.41 |
| | | | | 24.0 | 85.0 | 16.94 |
| | | | | 27.0 | 89.3 | 18.14 |
| | | | | 29.0 | 92.0 | 18.91 |



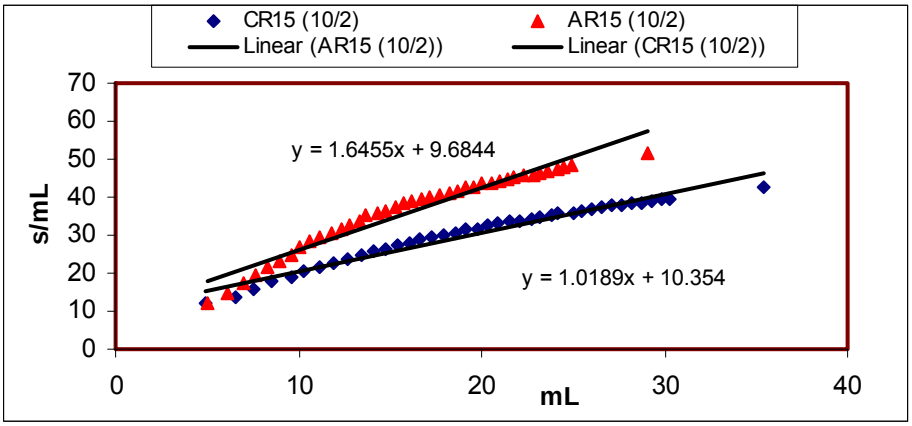
**Loading rate 10 kg COD/m$^3$.day:**

| t (min) | CR10(3/2) | t/v | t (min) | AR10(3/2) | t/v |
|---|---|---|---|---|---|
| min | mL | s/mL | min | mL | s/mL |
| 0.5 | 14.9 | 2.01 | 0.5 | 5.7 | 5.26 |
| 1 | 17.7 | 3.39 | 1.0 | 8.0 | 7.50 |
| 1.5 | 19.7 | 4.57 | 1.5 | 9.7 | 9.28 |
| 2 | 21.5 | 5.58 | 2.0 | 11.3 | 10.62 |
| 2.5 | 22.9 | 6.55 | 3.0 | 14.0 | 12.86 |
| 3 | 24.2 | 7.44 | 4.0 | 16.7 | 14.37 |
| 3.5 | 25.5 | 8.24 | 6.0 | 21.6 | 16.67 |
| 4 | 26.5 | 9.06 | 8.0 | 26.1 | 18.39 |
| 4.5 | 27.9 | 9.68 | 13.0 | 36.6 | 21.31 |
| 5 | 29.3 | 10.24 | 17.0 | 44.0 | 23.18 |
| 6 | 31.3 | 11.50 | 20 | 49.2 | 24.39 |
| 7 | 33.5 | 12.54 | 26 | 59.0 | 26.44 |
| 8 | 35.6 | 13.48 | 28 | 62.2 | 27.01 |
| 9 | 37.4 | 14.44 | 30 | 65.4 | 27.52 |
| 10 | 39.3 | 15.27 | | | |
| 11 | 41.2 | 16.02 | | | |
| 12 | 43.0 | 16.74 | | | |
| 13 | 44.8 | 17.41 | | | |
| 14 | 46.5 | 18.06 | | | |
| 15 | 48.3 | 18.63 | | | |
| 16 | 49.9 | 19.24 | | | |
| 20 | 56.6 | 21.20 | | | |
| 24 | 62.1 | 23.19 | | | |
| 30 | 70.9 | 25.39 | | | |



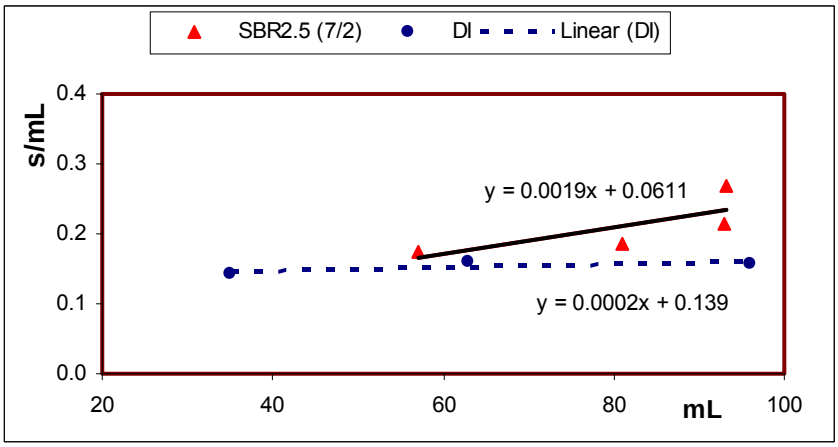
**Loading rate 15 kg COD/$m^3$.day:**

| t (min) | CR15(10/2) | t/v (s/ml) | t (min) | AR15(10/2) | t/v (s/ml) |
|---|---|---|---|---|---|
| min | ml | s/ml | min | ml | s/ml |
| | | | | | |
| 1.0 | 4.9 | 12.24 | 1.0 | 5.0 | 12.00 |
| 1.5 | 6.6 | 13.64 | 1.5 | 6.1 | 14.75 |
| 2.0 | 7.5 | 16.00 | 2.0 | 7.0 | 17.14 |
| 2.5 | 8.5 | 17.65 | 2.5 | 7.7 | 19.48 |
| 3.0 | 9.6 | 18.75 | 3.0 | 8.3 | 21.69 |
| 3.5 | 10.3 | 20.39 | 3.5 | 9.0 | 23.33 |
| 4.0 | 11.2 | 21.43 | 4.0 | 9.6 | 25.00 |
| 4.5 | 11.9 | 22.69 | 4.5 | 10.1 | 26.73 |
| 5.0 | 12.7 | 23.62 | 5.0 | 10.6 | 28.30 |
| 5.5 | 13.4 | 24.63 | 5.5 | 11.2 | 29.46 |
| 6.0 | 14.1 | 25.53 | 6.0 | 11.8 | 30.51 |
| 6.5 | 14.7 | 26.53 | 6.5 | 12.3 | 31.71 |
| 7.0 | 15.4 | 27.27 | 7.0 | 12.8 | 32.81 |
| 7.5 | 16.1 | 27.95 | 7.5 | 13.3 | 33.83 |
| 8.0 | 16.6 | 28.92 | 8.0 | 13.7 | 35.04 |
| 8.5 | 17.3 | 29.48 | 8.5 | 14.3 | 35.66 |
| 9.0 | 17.9 | 30.17 | 9.0 | 14.8 | 36.49 |
| 9.5 | 18.6 | 30.65 | 9.5 | 15.3 | 37.25 |
| 10.0 | 19.1 | 31.41 | 10.0 | 15.7 | 38.22 |
| 10.5 | 19.8 | 31.82 | 10.5 | 16.2 | 38.89 |
| 11.0 | 20.3 | 32.51 | 11.0 | 16.7 | 39.52 |
| 11.5 | 20.9 | 33.01 | 11.5 | 17.2 | 40.12 |
| 12.0 | 21.5 | 33.49 | 12.0 | 17.7 | 40.68 |
| 12.5 | 22.1 | 33.94 | 12.5 | 18.2 | 41.21 |
| 13.0 | 22.7 | 34.36 | 13.0 | 18.7 | 41.71 |
| 13.5 | 23.2 | 34.91 | 13.5 | 19.1 | 42.41 |
| 14.0 | 23.8 | 35.29 | 14.0 | 19.6 | 42.86 |
| 14.5 | 24.2 | 35.95 | 14.5 | 20.0 | 43.50 |
| 15.0 | 25.0 | 36.00 | 15.0 | 20.5 | 43.90 |
| 15.5 | 25.5 | 36.47 | 15.5 | 21.0 | 44.29 |
| 16.0 | 26.0 | 36.92 | 16.0 | 21.4 | 44.86 |
| 16.5 | 26.6 | 37.22 | 16.5 | 21.8 | 45.41 |
| 17.0 | 27.1 | 37.64 | 17.0 | 22.3 | 45.74 |
| 17.5 | 27.7 | 37.91 | 17.5 | 22.8 | 46.05 |
| 18.0 | 28.2 | 38.30 | 18.0 | 23.2 | 46.55 |
| 18.5 | 28.7 | 38.68 | 18.5 | 23.6 | 47.03 |
| 19.0 | 29.3 | 38.91 | 19.0 | 24.1 | 47.30 |
| 19.5 | 29.8 | 39.26 | 19.5 | 24.5 | 47.76 |
| 20.0 | 30.3 | 39.60 | 20.0 | 24.9 | 48.19 |
| 25.0 | 35.4 | 42.37 | 25.0 | 29.1 | 51.55 |




**MFI of SBR at OLR 2.5 kg COD/m$^3$.day and distilled water:**

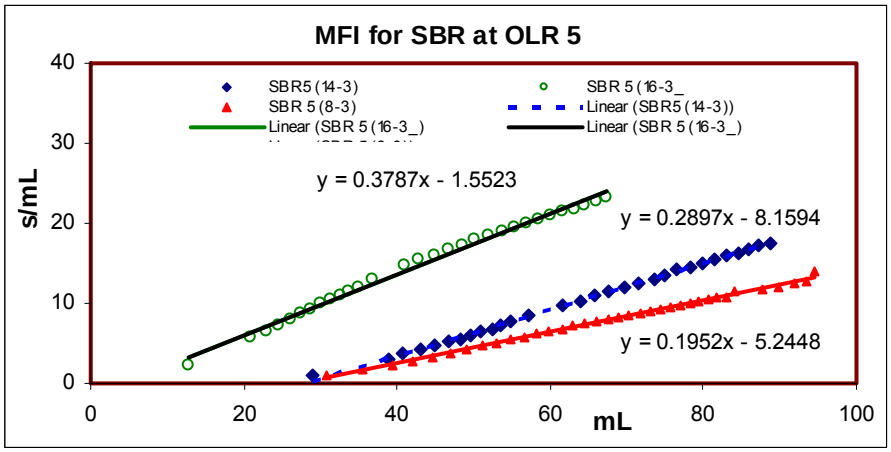
| t (sec) | SBR2.5 (7/2) | t/v | t (sec) | DI | t/v |
|---|---|---|---|---|---|
| s | mL | s/mL | s | mL | s/mL |
| 10 | 57 | 0.18 | 5.0 | 35.0 | 0.1 |
| 15 | 81 | 0.19 | 10.0 | 63.0 | 0.2 |
| 20 | 92.9 | 0.22 | 15.0 | 96.0 | 0.2 |
| 25 | 93.1 | 0.27 | | | |




**MFI of SBR at OLR 5 kg COD/m$^3$.day**

| t (min) | SBR (8/3) | t/v | t (min) | SBR5 (14/3) | t/v | SBR5 (16/3) | t/v |
|---|---|---|---|---|---|---|---|
| min | mL | s/mL | | mL | s/mL | mL | s/mL |
| 0.5 | 30.9 | 0.97 | 0.5 | 28.9 | 1.04 | 12.8 | 2.34 |
| 1.0 | 35.6 | 1.69 | 2.0 | 39.0 | 3.08 | 21.0 | 5.71 |
| 1.5 | 39.4 | 2.28 | 2.5 | 40.8 | 3.68 | 22.9 | 6.55 |
| 2.0 | 42.1 | 2.85 | 3.0 | 43.0 | 4.19 | 24.5 | 7.35 |
| 2.5 | 44.6 | 3.36 | 3.5 | 45.0 | 4.67 | 26.0 | 8.08 |
| 3.0 | 46.9 | 3.84 | 4.0 | 46.7 | 5.14 | 27.5 | 8.73 |
| 3.5 | 49.1 | 4.28 | 4.5 | 48.2 | 5.60 | 28.8 | 9.38 |
| 4.0 | 51.1 | 4.70 | 5.0 | 49.6 | 6.05 | 30.1 | 9.97 |
| 4.5 | 52.9 | 5.10 | 5.5 | 51.0 | 6.47 | 31.4 | 10.51 |
| 5.0 | 54.8 | 5.47 | 6.0 | 52.4 | 6.87 | 32.6 | 11.04 |
| 5.5 | 56.6 | 5.83 | 6.5 | 53.6 | 7.28 | 33.7 | 11.57 |
| 6.0 | 58.2 | 6.19 | 7.0 | 54.8 | 7.66 | 35.0 | 12.00 |
| 6.5 | 59.8 | 6.52 | 8.0 | 57.2 | 8.39 | 36.9 | 13.01 |
| 7.0 | 61.5 | 6.83 | 10.0 | 61.7 | 9.72 | 41.0 | 14.63 |
| 7.5 | 63.0 | 7.14 | 11.0 | 63.9 | 10.33 | 42.9 | 15.38 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8.0 | 64.5 | 7.44 | 12.0 | 65.7 | 10.96 | 44.9 | 16.04 |
| 8.5 | 66.1 | 7.72 | 13.0 | 67.7 | 11.52 | 46.7 | 16.70 |
| 9.0 | 67.5 | 8.00 | 14.0 | 69.6 | 12.07 | 48.5 | 17.32 |
| 9.5 | 68.9 | 8.27 | 15.0 | 71.5 | 12.59 | 50.2 | 17.93 |
| 10.0 | 70.3 | 8.53 | 16.0 | 73.5 | 13.06 | 51.9 | 18.50 |
| 10.5 | 71.7 | 8.79 | 17.0 | 75.0 | 13.60 | 53.7 | 18.99 |
| 11.0 | 73.0 | 9.04 | 18.0 | 76.4 | 14.14 | 55.3 | 19.53 |
| 11.5 | 74.5 | 9.26 | 19.0 | 78.2 | 14.58 | 56.8 | 20.07 |
| 12.0 | 75.6 | 9.52 | 20.0 | 80.0 | 15.00 | 58.4 | 20.55 |
| 12.5 | 76.9 | 9.75 | 21.0 | 81.5 | 15.46 | 60.0 | 21.00 |
| 13.0 | 78.2 | 9.97 | 22.0 | 83.0 | 15.90 | 61.6 | 21.43 |
| 13.5 | 79.4 | 10.20 | 23.0 | 84.6 | 16.31 | 63.1 | 21.87 |
| 14.0 | 80.6 | 10.42 | 24.0 | 85.9 | 16.76 | 64.6 | 22.29 |
| 14.5 | 81.8 | 10.64 | 25.0 | 87.3 | 17.18 | 66.0 | 22.73 |
| 15.0 | 83.0 | 10.84 | 26.0 | 88.7 | 17.59 | 67.4 | 23.15 |
| 16.0 | 84.2 | 11.40 | | | | | |
| 17.0 | 87.7 | 11.63 | | | | | |
| 18.0 | 89.9 | 12.01 | | | | | |
| 19.0 | 91.8 | 12.42 | | | | | |
| 20.0 | 93.6 | 12.82 | | | | | |
| 22.0 | 94.5 | 13.97 | | | | | |




**MFI of types of supernatant at OLR 2.5 kg COD/m$^3$.day:**

| | OLR | | MFI (x10$^6$) s/L$^2$ | MFI (x10$^3$) s/L$^2$ |
|---|---|---|---|---|
| DI | | | 0.0002 | 0.2 |
| SBR 2.5 | 2.5 | 07-Feb | 0.0019 | 1.9 |
| CR 2.5 | 2.5 | 21-Dec | 0.0009 | 0.9 |
| AR 2.5 | 2.5 | 21-Dec | 0.0012 | 1.2 |
| SBR 5 | 5 | 08-Mar | 0.1952 | 195.2 |
| CR 5 | 5 | 10-Jan | 0.1307 | 130.7 |
| AR 5 | 5 | 11-Jan | 0.2927 | 292.7 |

**MFI for CR1, CR2 at OLR of 10 kg COD/m$^3$.day (Run 2):**

| t (min) | CR1-10 (8/3) | t/v | CR2-10 (8/3) | t/v | t (min) | CR1-10 (8/3) | t/v | CR2-10 (8/3) |
|---|---|---|---|---|---|---|---|---|
| | mL | s/mL | mL | s/mL | | mL | s/mL | mL |
| 1.0 | 10.6 | 5.66 | 10.5 | 5.71 | 16.0 | 37.5 | 25.60 | 40.3 |
| 1.5 | 12.1 | 7.44 | 11.9 | 7.56 | 18.0 | 40.6 | 26.60 | 43.7 |
| 2.0 | 13.7 | 8.76 | 13.3 | 9.02 | 19.0 | 42.1 | 27.08 | 45.4 |
| 2.5 | 14.7 | 10.20 | 14.6 | 10.27 | 21.0 | 45.1 | 27.94 | 48.8 |
| 3.0 | 15.7 | 11.46 | 15.7 | 11.46 | 22.0 | 46.5 | 28.39 | 50.5 |
| 3.5 | 16.6 | 12.65 | 16.8 | 12.50 | 23.0 | 48.0 | 28.75 | 52.0 |
| 4.0 | 17.6 | 13.64 | 17.9 | 13.41 | 24.0 | 49.4 | 29.15 | 53.7 |
| 5.0 | 19.5 | 15.38 | 20.0 | 15.00 | 26.0 | 52.3 | 29.83 | 56.6 |
| 5.5 | 20.4 | 16.18 | 21.0 | 15.71 | 28.0 | 55.2 | 30.43 | 60.0 |
| 6.0 | 21.3 | 16.90 | 22.0 | 16.36 | 29.0 | 56.7 | 30.69 | 61.4 |
| 7.0 | 23.0 | 18.26 | 23.9 | 17.57 | 30.0 | 58.0 | 31.03 | 63.0 |
| 9.0 | 26.4 | 20.45 | 27.8 | 19.42 | 36.0 | 66.1 | 32.68 | 72.0 |
| 10.0 | 28.0 | 21.43 | 29.7 | 20.20 | 37.0 | 67.5 | 32.89 | 73.5 |
| 11.0 | 29.6 | 22.30 | 31.3 | 21.09 | 38.0 | 68.9 | 33.09 | 75.0 |
| 12.0 | 31.3 | 23.00 | 33.2 | 21.69 | 39.0 | 70.2 | 33.33 | 76.4 |
| 13.0 | 32.8 | 23.78 | 35.0 | 22.29 | 40.0 | 71.6 | 33.52 | 77.8 |
| 14.0 | 34.4 | 24.42 | 36.8 | 22.83 | 46.0 | 79.0 | 34.94 | 83.4 |

## Granule Membrane Bioreactor (Run 3)

### K. COD removal at run 3

| date | 5-Mar | 14-Mar | 16-Mar | 22-Mar | 23-Mar | 24-Mar | 27-Mar | 30-Mar | 2-Apr | 4-Apr |
|---|---|---|---|---|---|---|---|---|---|---|
| day | 5 | 14 | 16 | 22 | 23 | 24 | 27 | 30 | 32 | 34 |
| COD in feed, mg/L | 2598 | 2667 | 2257 | 2906 | 2051 | 2735 | 2483 | 3160 | 3096 | 3225 |
| CR1- COD eff, mg/L | 62 | 65 | 65 | 17 | 7 | 10 | 13 | 13 | 18 | 17 |
| CR2-COD eff, mg/L | 58 | 68 | 65 | 21 | 21 | 14 | 13 | | | |
| Efficiency CR1, % | 98 | 98 | 97 | 99 | 100 | 100 | 99 | 100 | 99 | 99 |
| Efficiency CR2, % | 98 | 97 | 97 | 99 | 99 | 99 | 99 | | | |
| OLR, kgCOD/$m^3$.day | 10.8 | 11.1 | 9.4 | 12.1 | 8.5 | 11.4 | 10.3 | 13.1 | 12.9 | 13.4 |

### L. Fouling behavior of granule MBR (CR1, CR2)

| | TMP | | | | | | | | fouling rate | |
|---|---|---|---|---|---|---|---|---|---|---|
| day | CR1 | | TMP | flux | CR2 | | TMP | flux | CR1 | CR2 |
| | TMP1 | TMP2 | CR1 | mL/min | TMP1 | TMP2 | CR2 | mL/min | | |
| 0 | 286 | 365 | 10.5 | 13 | 270 | 385 | 15.3 | 12 | | |
| 1 | 286 | 366 | 10.7 | 12.5 | 0 | 710 | 94.7 | 4 | 0.1 | 79.3 |
| 2 | 283 | 369 | 11.5 | 12.5 | | | | | 0.8 | |
| 3 | 280 | 371 | 12.1 | 12 | 269 | 381 | 14.9 | 12 | 0.7 | |
| 4 | 256 | 395 | 18.5 | 12 | 2 | 712 | 94.7 | 4.5 | 6.4 | 79.7 |
| 5 | 226 | 426 | 26.7 | 12 | stop CR2 | | | | 8.1 | |
| 6 | 174 | 476 | 40.3 | 12 | | | | | 13.6 | |
| 7 | 115 | 538 | 56.4 | 12 | | | | | 16.1 | |
| 8 | | | | | | | | | | |
| 9 | 293 | 363 | 9.3 | 12 | | | | | 0 | |
| 10 | 290 | 365 | 10.0 | 11.5 | | | | | 0.7 | |
| 11 | 272 | 382 | 14.7 | 11.5 | | | | | 4.7 | |
| 12 | 250 | 413 | 21.7 | 11.5 | | | | | 7.1 | |
| 13 | 208 | 444 | 31.5 | 11.5 | | | | | 9.7 | |
| 14 | 115 | 537 | 56.3 | 11.5 | | | | | 24.8 | |
| 15 | | | | | | | | | | |
| 16 | 298 | 357 | 7.9 | 11.5 | | | | | 0.0 | |
| 17 | 284 | 368 | 11.2 | 11 | | | | | 3.3 | |
| 18 | 284 | 368 | 11.2 | 11 | | | | | 0.0 | |
| 19 | 270 | 382 | 14.9 | 11 | | | | | 3.7 | |
| 20 | 259 | 393 | 17.9 | 11 | | | | | 2.9 | |
| 21 | 230 | 421 | 25.5 | 11 | | | | | 7.6 | |
| 22 | 182 | 467 | 38.0 | 11 | | | | | 12.5 | |
| 23 | 164 | 486 | 42.9 | 11 | | | | | 4.9 | |
| 24 | 154 | 496 | 45.6 | 11 | | | | | 2.7 | |
| 25 | 135 | 515 | 50.7 | 10.5 | | | | | 5.1 | |
| 26 | 114 | 538 | 56.5 | 11.5 | | | | | 5.9 | |