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ENHANCED AEROBIC SLUDGE GRANULATION IN CYCLIC AEROBIC GRANULAR SLUDGE BIOREACTOR (CAgSBio) BY Mg^{2+} AUGMENTATION

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ABSTRACT

Two sequencing batch reactors (SBRs) known as Cyclic Aerobic Granular Sludge Bioreactor (CAgSBio) were concurrently operated to investigate the effect of Mg^{2+} augmentation on aerobic granulation. Augmentation with 10 mg/l Mg^{2+} in R2 significantly decreased the sludge granulation (defined as that over 15% of granules were larger than 0.6 mm) time from 32 days to 18 days, at the same time, the mean diameter of the granules in R2 was 2.9 mm after the granulation, which was consistently larger than that (1.8 mm) in R1. Mg^{2+}-fed granules were denser and more compact, showed better settling and had higher polysaccharide contents, but it did not result in a difference in microbial morphology. The results demonstrated that Mg^{2+} enhanced the sludge granulation process in the sequencing batch reactor.

ABSTRAK

Dua buah Reaktor Kelompok Berjujukan (SBRs) iaitu *Cylic Aerobic Granular Sludge Bioreactor (CAgSBio)* yang sama beroperasi untuk mengkaji pengaruh penambahan Mg^{2+} pada granular aerobik. Penambahan 10mg/l Mg^{2+} di R2 signifikan dengan pengurangan granular enapcemar (ditentukan apabila lebih daripada 15% granular lebih besar dari 0.6 mm) sepanjang 32 hari kepada 18 hari, pada masa yang sama, diameter purata granular di R2 adalah 2.9 mm selepas penggumpalan, secara keseluruhannya granular tersebut lebih besar dari granular (1.8mm) di R1. Granular dengan penambahan Mg^{2+} didapati lebih padat dan lebih mampat, menunjukkan enapan yang lebih baik dan mempunyai kandungan polisakarida yang lebih tinggi, walau demikian, tidak menimbulkan perbezaan morfologi mikrob. Keputusan kajian menunjukkan bahawa Mg^{2+} meningkatkan proses granulasi enapcemar dalam Reaktor Kelompok Berjujukan.

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CHAPTER 1

INTRODUCTION

Aerobic granulation as a novel environmental biotechnology has been extensively reported in sequencing batch reactors (Beun et al., 1999; Tay et al., 2001; Su and Yu, 2005; Bathe et al., 2005). Compared to the conventional activated sludge flocs, aerobic granular sludge features a number of advantages, such as a denser and stronger microbial structure, a better settling ability, more effective sludge–effluent separation, greater biomass retention and enrichment, and a greatly improved capability to withstand shock loadings (Etterer and Wilderer, 2001; De Kreuk and van Loosdrecht, 2004). From an engineering and economic point of view, aerobic sludge granulation is a promising process with the potential to lead the next generation of biological wastewater treatment technologies.

Despite the advantages and potential of the aerobic sludge granulation process, the mechanisms of aerobic granulation were not well understood and aerobic granules showed a poor stability (Liu et al., 2004; Schwarzenbeck et al., 2005; Tay et al., 2004a,b; Zheng et al., 2006), which limited their application in wastewater treatment practice. The formation of aerobic granules was very crucial for their applicability in wastewater treatment while this process took several weeks to start-up an aerobic granular system from the conventional activated sludge (Beun et al., 1999; Tay et al., 2001; Moy et al., 2002; Wang et al., 2007). Such long start-up times may pose a problem in deploying aerobic granulation for industrial application. Previous researches had revealed that granulation might be initiated by bacterial

adsorption and adhesion to inert matters, inorganic precipitates and/or to each other through physico-chemical interactions and syntrophic associations (Fang et al., 1995; Schmidt and Ahring, 1996).

These substances served as initial precursors (carriers or nuclei) for the new bacterial growth, and those loosely adhered bacterial aggregates were strengthened by extracellular polymers excreted by bacteria and form firmly attached initial granules (Schmidt and Ahring, 1996; Yu et al., 2000). There were a number of factors, such as the influent substrate, the selective pressure, the COD loading rate and the type of reactor, which had been shown to have important influences on sludge granulation. It was also reported that divalent metal ions, such as Ca^{2+} and Fe^{2+} could enhance the granulation (Yu et al., 2000, 2001; Jiang et al., 2003) for their important roles in the self-immobilization of microbial biomass, since extracellular polymers preferred to bind multivalent metals due to the formation of stable complexes (Rudd et al., 1984). However, the studies on diva-lent mental ions had been focused on the effect of Ca^{2+} (Jiang et al., 2003); information about the effect of Mg^{2+} is still sparse. This study was conducted to evaluate whether Mg^{2+} augmentation can lead to improvements in the process of granule formation in SBR. The work could be useful for the development of aerobic granule-based systems for full-scale application.

CHAPTER 2

LITERATURE REVIEW

2.1 Conventional Activated Sludge System: Main Features and Weaknesses

Activated sludge systems are still the most commonly used systems for biological wastewater treatment. In these systems, a mixed culture of suspended biomass is grown and organic carbon and nutrients were removed from the influent. Activated sludge systems consist of two basic treatment steps: (a) aeration tank in which biochemical processes take place and (b) a settling tank, in which the treated effluent is separated from the biomass. The following sub-section will describe more about what are the main features and some common issues that underlines the weaknesses of this conventional system.

2.1.1 Characteristics of Conventional Activated Sludge System

An activated sludge process uses microorganisms to feed on organic contaminants in wastewater, producing a high quality effluent. The basic principle behind all activated sludge processes is that as microorganisms grow, they form particles that clump together. These particles (floc) are allowed to settle to the

bottom of the tank, leaving a relatively clear liquid free of organic material and suspended solids (Metcalf and Eddy, 2003). Screened wastewater is mixed with varying amounts of recycled liquid containing a high proportion of organisms taken from a secondary clarifying tank, and it becomes a product called mixed liquor. This mixture is stirred and injected with large quantities of air, to provide oxygen and keep solids in suspension. After a period of time, mixed liquor flows to a clarifier where it is allowed to settle. A portion of the bacteria is removed as it settles, and the partially cleaned water flows on for further treatment. The resulting settled solids, the activated sludge, are returned to the first tank to begin the process again. Figure 2.1 shows a typical activated sludge process.

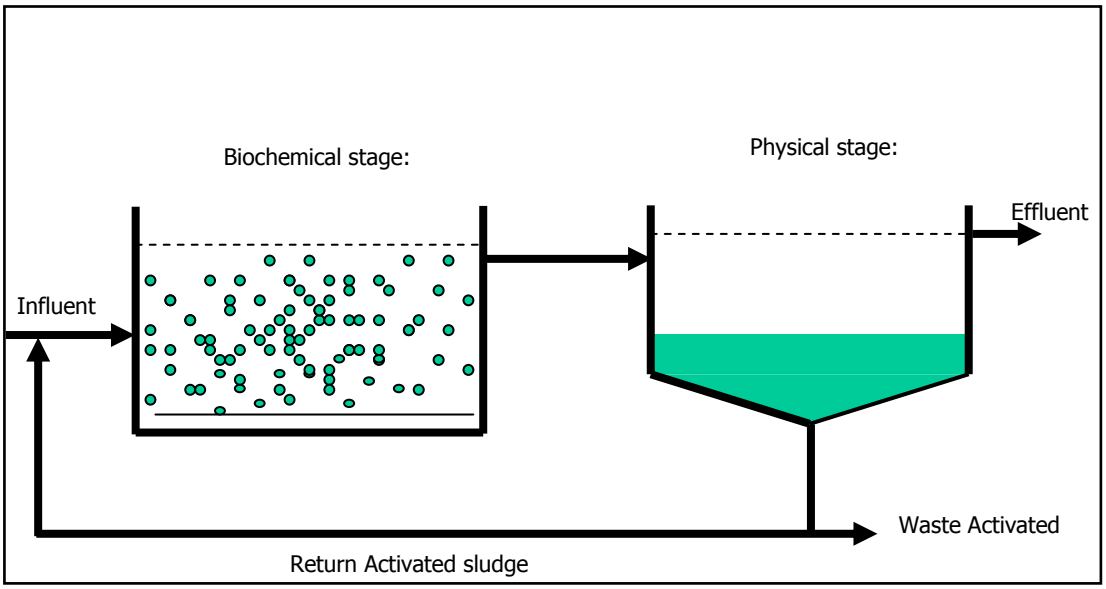

Figure 2.1 Typical activated sludge process

A basic activated sludge process consists of several interrelated components (Jeyanayagam and Venner, 2007):

i. An aeration tank where the biological reactions occur

ii. An aeration source that provides oxygen and mixing

iii. A tank, known as the clarifier, where the solids settle and are separated from treated wastewater

iv. A means of collecting the solids either to return them to the aeration tank, (returned activated sludge [RAS]), or to remove them from the process (waste activated sludge [WAS])

The activated sludge process is widely used by large cities and communities where large volumes of wastewater must be extensively treated. Activated sludge process plants are good choices too for isolated facilities, such as hospitals or hotels, cluster situations, subdivisions, and small communities.

2.1.2 Aerobic Biological Oxidation

Soluble organic matter in domestic sewage can be roughly divided into carbohydrates, fats and proteins. Most heterotrophic organisms can directly oxidize the organic carbon to carbon dioxide using oxygen or nitrate/nitrite (denitrification) as an electron acceptor. Part of the organic carbon will be assimilated to new biomass. Particulate and colloidal organic carbon must be hydrolyzed first before the bacteria can use it for their metabolism. Biologically non-degradable carbon will be incorporated in the activated sludge flocs and removed from the system with the excess sludge (Jeyanayagam and Venner, 2007).

Using a selector (continuously operated system) or a pulse dosing (Sequencing Batch Reactor), the biomass will experience a situation with high

concentrations of organic substrates followed by a situation without external organic substrate. Under these circumstances, microorganisms are able to accumulate substrate as internal storage products in their cells. Usually these storage products are glycogen, lipids or polyhydroxyalkanoates (PHA). Organisms can use the stored substrates to oxidize during famine periods and to regulate their growth rate (van Aalst-Van Leeuwen *et.al*., 1997; Beun, 2001; Martins *et.al*., 2004). A special form of storing organic carbon before it is used in the metabolism is performed by phosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs). Their main characteristics will be explained in the sub-section about phosphate removal.

2.1.3 Biological Nitrification and Denitrification

Nitrification is the oxidation of ammonia into nitrite and nitrite into nitrate. A group of bacteria known as Nitrosomonas performs the first conversion while a group of bacteria known as Nitrobacter does the second step. Nitrifying bacteria are autotrophic organisms (use carbon dioxide [CO_2] as carbon-source) and characterized by their low growth rate (de Kreuk *et al.*, 2005).

The latter is a major concern in activated sludge plants, since this highly determines the solid retention time (sludge retention time or sludge age) in the system (Jeyanayagam and Venner, 2007). The second step of the nitrogen removal is the denitrification, in which nitrite or nitrate is used as electron acceptor for the oxidation of organic carbon and is converted to nitrogen gas. This process takes place under anoxic conditions. Nitrification and denitrification can occur simultaneously within one sludge floc, as long as the oxygen penetration depth in the

floc is limited and a substrate-rich interior is present for denitrification (Pochana and Keller, 1999; Satoh *et al.*, 2003; Third *et al.*, 2003).

2.1.4 Biological Phosphorus Removal

To achieve enhanced biological phosphorus removal (EBPR), bacteria have to be exposed to changing anaerobic and aerobic conditions, where readily biodegradable substrate must be supplied under anaerobic conditions. During the anaerobic period, easily degradable substrates such as acetate are taken up in the cell and stored as polyhydroxyalkanoates (PHA). Acetate will be stored as poly-b-hydroxybutyrate (PHB). The energy of this transport and storage is supplied by the hydrolysis of intracellular-stored polyphosphate to orthophosphate, which is released from the cell into the liquid. The reduction equivalents required for the conversion of acetate to PHB are supplied by the conversion of glycogen, which is stored during the aerobic period from PHB (Mino *et al.*, 1998; Blackall *et al.*, 2002). The anaerobic and aerobic metabolisms are schematically shown in Figure 2.2.

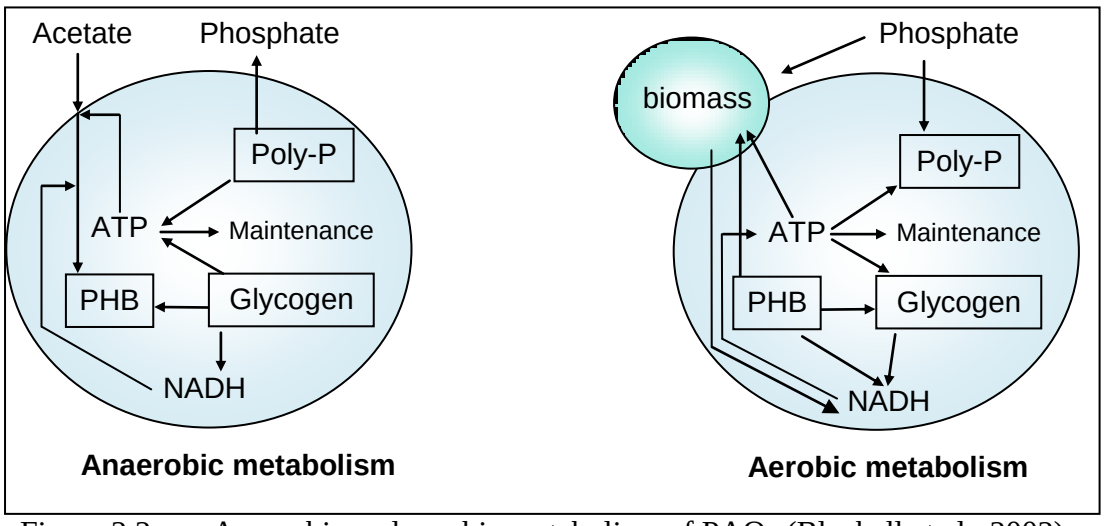

Figure 2.2 Anaerobic and aerobic metabolism of PAOs (Blackall et al., 2002)

In the aerobic phase, without the presence of an external substrate, PHB is used as substrate for cell growth, polyphosphate synthesis and glycogen formation. Glycogen accumulating organisms (GAOs) are able to compete for organic substrate with the phosphate accumulating organisms (PAOs) during anaerobic periods. The only metabolic difference between those organisms is the uptake of phosphate, which does not occur during the metabolism of GAOs. However, using acetate as the main substrate favours the development of the PAOs (Hong *et al*., 2007).

2.1.5 Biomass and Effluent Separation

In wastewater treatment, biomass and treated effluent need to be separated before effluent can be discharged to the surface water. This is conducted in a secondary clarifier, settling tank or thickener that produces clarified final effluent and a thickened flow of sludge that is returned to the activated sludge tank. If the settling tank fails, for example due to sludge bulking, excessive loss of sludge deteriorates effluent quality and could lead to uncontrolled low sludge ages and reduction of efficiency in the aeration tanks (Jeyanayagam and Venner, 2007).

Due to the lack of shear stress in the clarifier with no mixing, the suspended biomass usually aggregates to form sludge flocs. These sludge flocs settle relatively slow (velocities < 1 mh^{-1}), which leads to long needed hydraulic retention times (HRT) in the settling tanks before clear effluent can be released (Mulder *et al*., 2001). The sludge volume index (SVI), volume occupied per unit mass of biomass determines the size of the settling tanks. Well-settling sludge from activated sludge systems, usually have a SVI of 90 to 150 mLg^{1} (Metcalf and Eddy, 2003).

2.2 Issues on Design, Operation and Maintenance of Conventional Activated Sludge System

Various operational problems occur in a wastewater treatment plant based on activated sludge operations that adversely affect effluent quality. The most common issues/problems that are usually encountered in the activated sludge process are sludge bulking and foaming, rising sludge, high surplus biomass production, long processing time, substantial land requirements and high cost. The following sub-section shall provide a more detailed discussion of these issues. Nowadays, the need for 'smart' systems may solve these issues and the increasing attention for environmental impact issues make the search for new wastewater treatment technologies an important objective to reach.

2.2.1 Sludge Bulking

Sludge bulking is a common and serious problem in an activated sludge operation, affecting most activated sludge plants at one time or another. Filamentous bulking is the number one cause of effluent non-compliance.

A bulking sludge is defined as one that settles and compacts slowly. An operational definition often used is a sludge with a sludge volume index (SVI) of >150 mLg^{-1}. However, each plant has a specific SVI value where sludge builds up in the final clarifier and is lost to the final effluent, which can vary from a SVI <100 mLg^{-1} to >300 mLg^{-1}, depending on the size and performance of the final clarifiers and hydraulic considerations (Richard, 2003). Thus, a bulking sludge may or may

not lead to a bulking problem, depending on the specific treatment plant's ability to contain the sludge within the clarifier.

A bulking sludge can result in the loss of sludge inventory to the effluent, causing environmental damage and effluent violations. In severe cases, loss of the sludge inventory can lead to a loss of the plant's treatment capacity and failure of the process. Additionally, the excess solids present during bulking can compromise disinfection of the treated wastewater. In less severe cases, bulking leads to excessive return sludge recycle rates and problems in waste activated sludge disposal. Many problems in waste sludge thickening are sludge/filamentous bulking problems (Crocetti *et al.,* 2008).

Sludge bulking can also have wider implications than difficult secondary settling. Low settleability may result in poor effluent quality due to a high solids carry-over from the secondary settlers. The high effluent solids can be described to incomplete settling as well as anoxic conditions developing in the settler due to sludge accumulation. Denitrification occurs and the resulting nitrogen bubbles cause sludge particles to float. The poorly compacted sludge results in excessive waste sludge volumes usually with poor thickening properties with respect to gravity settling and dissolved air flotation (Bratby, 1977) and poor dewaterability in centrifuges and belt presses (Osborn *et al.,* 1986). Most importantly, the poorly compacted sludge results in much thinner sludges being returned to the aeration tank with a low mixed liquor suspended solids (MLSS), which leads to difficulty of maintaining the desired operational MLSS in the aeration tank with a subsequent fall in effluent quality. Attempts to control the height of the sludge blanket within the sedimentation tank by wasting more sludge than normal, results in the MLSS concentration in the aeration tank rapidly declining.

2.2.2 Sludge Foaming

Foaming is also one of the most common problems encountered in the activated sludge process. Filamentous organisms are most commonly responsible in this case. A brief review of activated sludge foams and their causes is given in Table 2.1.

Table 2.1: Description and causes of activated sludge foams (Richard, 2003)

Foam Description	Cause (s)
Thin, white to grey foam	Low cell residence time or "young" sludge (start-up foam)
White, frothy, billowing foam	Once common due to non-biodgradable detergents (now uncommon)
Pumice-like, grey foam (ashing)	Excessive fines recycle from other processes (e.g. anaerobic digesters)
Thick sludge blanket on the final clarifier	Denitrification
Thick, pasty or slimy, greyish foam (industrial systems only)	Nutrient-deficiet foam; foam consists of polysaccharide material released from the floc
Thick, brown, stable foam enriched in filaments	Filament-induced foaming, caused by *Nocardia, Microthrix* or type 1863

Generally, sludge foaming problems make the maintenance works of the treatment plant become difficult and dangerous. Foaming in closed aeration tanks reduces the available headspace and has even reduced the available hydraulic head for gravity flow through the tank. In addition, the trapped mixed liquor suspended solids (MLSS) in the foam cause a dark brown colour. Between thirty to fifty percent (30-50 %) of the total activated sludge can be entrained in the foam, making the plant operationally difficult to maintain an adequate MLSS for continuous treatment.

Sludge foaming also creates odour problems especially during summer/hot weather (Richard, 2003).

2.2.3 Rising Sludge

Unlike bulking, the problem of rising sludge is only seen in the final settling tank and has definite operational causes and it can be corrected through an understanding of the system and defined management practices (Pastor *et al.*, 2008). Rising sludge is caused by denitrification in which nitrites and nitrates in the wastewater are reduced to nitrogen gas. Denitrification occurs in the sludge layer in the secondary clarifier when conditions become anaerobic or nearly anaerobic. As the nitrogen gas accumulates, the sludge mass becomes buoyant and rises or floats to the surface (Pitt and Jenkins, 1990). Rising sludge can easily be differentiated from a bulking sludge by noting the presence of small gas bubbles attached to the floating solids and by microscopic examination. This problem can be overcome by increasing the removal rate of the sludge from sludge-collecting mechanism, by regulation of the flows (loading) and monitoring of the dissolved oxygen levels in the final settling tank.

2.2.4 Surplus Sludge Production

Another important disadvantage related to the conventional activated sludge systems is a high surplus biomass production (sludge treatment and disposal thus become an important economical aspect). Usually, additional treatments are required

for this sludge, in which treating excess sludge produced may represent 50 percent of the total operation and maintenance cost of a treatment plant (Metcalf and Eddy, 2003). Sludge treatment generally consists of three steps: stabilization, dewatering and utilization or disposal. However, there are some potential limitations on these methods which were described by Badreddine (2008) as shown in Table 2.2.

Table 2.2 : Types of sludge treatment , advantages and some potential limitation (Badreddine, 2008).

Types of Sludge Treatment	Advantages	Potential Limitations
Aerobic Digestion	i. Low odour potential ii. Simple and reliable process iii. No chemicals are required	i. Very high power consumption ii. Aeration system requires high maintenance iii. Larger-volume tank required for cold climate application iv. There is potential for foaming
Lime Stabilization	i. Low capital cost ii. Ease of use iii. Improved pathogen reduction iv. Lime stabilization also serves as an ideal temporary or interruptible supplemental process for periodically overloaded existing digestion systems	i. Inability of the process to reduce the sludge mass ii. Large increase in additional inert solids for dewatering and disposal. iii. The benefits of this process are usually lost after two weeks of storage.
Dewatering Beds	i. Minimal skill and operation attention ii. Low construction costs	i. Covering the beds may be required in cold or wet climates ii. Potential odour and vector problems iii. Considerable land requirements
Land Application	i. Simplicity ii. Low cost iii. Stabilised sludge can be utilized as a soil conditioner and source of organic matter and nutrients	i. Care must be taken to avoid inappropriate application of sludge containing heavy metals, the presence of which must be monitored (though few sludge contain metals that are at level which will cause a problem) ii. Storage requirements may be considerable to accommodate sludge generation during wet, frozen soil conditions iii. Costs my increase substantially if large storage volumes are required. iv. Suitable areas for land spreading of stabilized sludge are becoming quires less energy difficult to find in urbanized areas of the state and country.

Sludge Composting	i. Less expensive and requires less energy than incineration ii. Produces a more manageable product for land application iii. It is a more productive use than land filling iv. Well-managed composting facilities can benefit public relations and community pride	i. Severe odour problems in poorly managed or designed facilities ii. Public relations problems related to any odor problems iii. The need to site the facility in a sparsely populated area.

2.2.5 Processing Time

Usually, the conventional activated sludge system took a long time to treat a large volume of wastewater, especially in the big cities. As an example, the normal processing time for an extended aeration system (one of the systems that is based on activated sludge technology) is 24 hours to turn out effluent from a high quality wastewater system that can be put back into the ground (after chlorination) (Marilyn, 2003). The relatively poor settling characteristics of conventional activated sludge floc also lead to the system facing the time-consuming problem of biomass and effluent separation in a secondary clarifier, which took more than an hour (Hastings *et al.,* 2007). Therefore, an efficient system with short processing time and efficient is required to improve the existing technology.

2.2.6 Large Footprint and Other Issues

Activated sludge plants are expensive to build and occupy substantial land areas. They rely upon utilization of large and costly settling tanks and aeration basins to “settle out” and “digest” biosolid in the waste stream as well as the introduction of bacteria, chemicals, or membranes to treat the sewage and reduce the amount of leftover sludge (Metcalf and Eddy, 2003).

High-energy costs are involved in operating most activated sewage treatment plants, due to pumping oxygen into aeration tanks, operating moving screens or filters, etc. Activated sludge treatment plants also produce significant amounts of sludge, that must be further treated and disposed of at tremendous costs.

Most importantly, the local odour emission regulations require that no gas or odour emissions may be released within 100 meters of residential areas (Badreddine, 2008). The only way that activated sludge treatment can resolve this is through the addition of an odour control system. This will not only be expensive in equipment, maintenance and labor, but will also consume a large amount of space and energy.

Therefore, nowadays most researchers are looking for new alternative technologies for improving wastewater treatment system, which have smaller footprints/land area requirements, less energy requirements, lower sludge generation, enhance odour control and reduce processing time. During the last decade, several types of compact treatment systems have been developed such as sequencing batch reactors, membrane bioreactors and biofilm / bio-granulation reactors.

2.3 Compact Wastewater Treatment Systems

2.3.1 Sequencing Batch Reactors

The sequencing batch reactor (SBR) is considered a fill-and-draw activated sludge system. The processes of equalization, aeration, and clarification are all achieved in the same tank, unlike a conventional activated sludge system, in which the same processes are accomplished in separate tanks (Morgenroth and Wilderer, 2000). Wastewater is added to the tank, treated to remove undesirable components, and then discharged. SBR systems consist of five common steps carried out in sequence: (1) fill, (2) react (aeration), (3) settle (sedimentation/ clarification), (4) draw (the effluent is decanted), and (5) idle. Sludge wasting usually occurs during the settling phase. The SBR acts as an equalization basin when filling with wastewater, enabling the system to tolerate peak flows or loads (Irvine and Ketchum, 1989; Guo *et al.*, 2007). Figure 2.3 shows a typical SBR operation for one cycle.

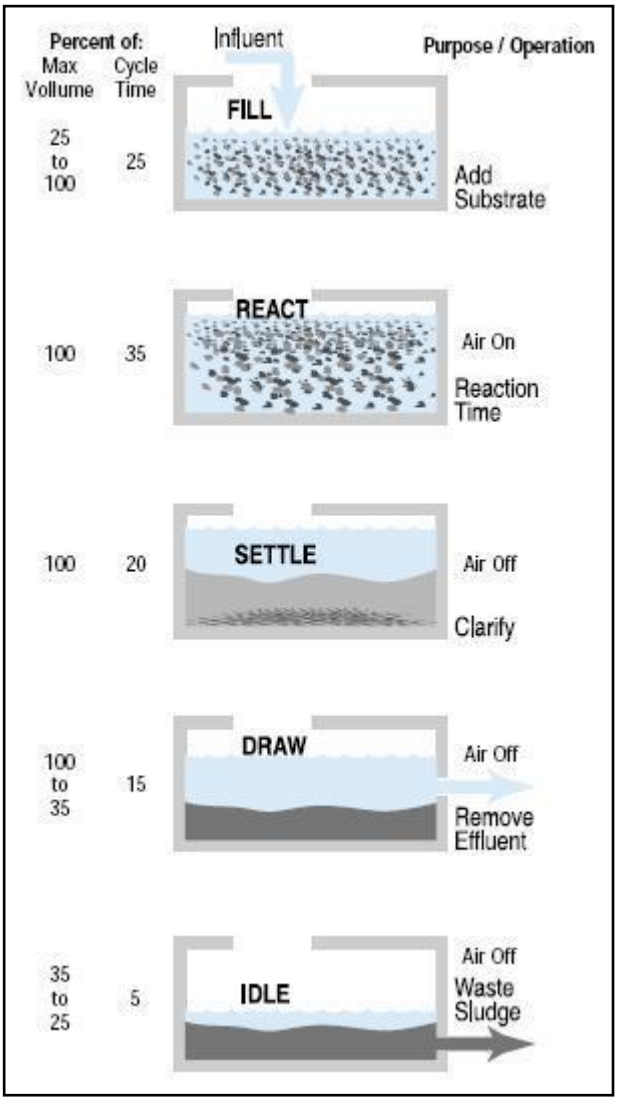

Figure 2.3 Typical sequencing batch reactor operation for one cycle (Wang *et al.*, 2006)

SBRs are typically used where flow rates are five million gallons per day or less. Due to their relatively small footprints, they are useful in areas where available land is limited. In addition, it is easy to modify cycles within the system for nutrient removal if necessary. SBRs are also cost effective if treatment beyond biological treatment, such as filtration, is required. SBRs also offer a potential capital cost savings by eliminating the need for clarifiers (Wang *et al.*, 2006). SBRs require a sophisticated level of maintenance due to the timing units and controls. Depending

upon the downstream processes, it may be necessary to equalize effluent after leaving the SBR.

2.3.2 Membrane Bioreactors

Membrane bioreactor (MBR) are based on a combination of activated sludge processes and membrane filtration in one treatment step. An ultrafiltration or microfiltration membrane separates the activated sludge from the effluent. The membrane can be applied within the bioreactor (submerged configuration) or externally through recirculation. Since external settlers, or any other post treatment step, become superfluous by using a membrane for the suspended solid and effluent separation, the required space for an installation is small and sludge concentration in the aeration tanks can be two to three times higher than in conventional systems. Furthermore, the effluent quality is significantly better as all suspended and colloidal material such as micro contaminants, bacteria and viruses are removed (Ujang and Anderson, 2000; Trussell *et al.*, 2005;).

Biological processes in a MBR are often comparable or better than in conventional activated sludge systems (Ujang *et al.*, 2005 a,b and c). Due to the long sludge ages, N-removal is more efficient because the slow growing autotrophs bacteria are kept efficiently in the system. Denitrification can occur by introducing anoxic tanks or intermittent aeration (Drews *et al.*, 2005; Gander *et al*., 2000). Figure 2.4 shows a typical MBR system.

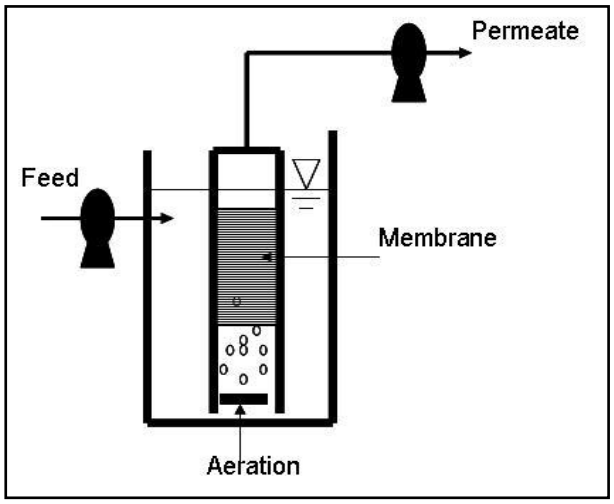

Figure 2.4 Typical membrane bioreactor system (Ujang and Anderson, 2000)

Despite the excellent effluent quality, a breakthrough for the MBR technology is lacking. This is mainly due to the costs involved with membrane modules (Ujang *et al.,* 2007). Due to the high biomass concentration in the system, in combination with the appearance of the activated sludge (more suspended growth) aeration is inefficient. Furthermore, the present generation of membranes shows low permeability due to fouling, operation of membranes is energy demanding and, although prices are decreasing, membranes are still relatively expensive (Brouwer *et al.,* 2005). In order to make MBR technology attractive for a wide range of wastewater treatment applications, these drawbacks should first be eliminated.

2.3.3 Particle-Based Biofilm (Bio-granulation) Reactor

Biofilm reactors, also known as bio-granulation reactors, are used in situations wherein the reactor capacity obtained by using freely suspended organisms is limited by the biomass concentration and hydraulic residence time (van Loosdrecht

and Heijnen, 1993). This can be the case either for slow-growing organisms (e.g. nitrifiers, methanogens), whose growth in suspension requires long residence time, or for diluted feed streams (often present in wastewater treatment processes), in which only a very low biomass concentration can be achieved without biomass retention. In these cases, biofilm reactors are an effective solution to successfully retain biomass in the reactors and to improve the volumetric conversion capacity.

Biofilm reactors are not particularly useful when fast-growing organisms (i.e. with a specific maximum growth rate > 0.1 h^{-1}) or concentrated feed streams are used (e.g. in industrial fermentation processes). In these situations, sufficient biomass will be formed to metabolize the substrate with relatively short residence time without the need for any form of retention; it is the oxygen supply to the liquid phase, not the biomass concentration, which is often the limiting factor. For this reason, in the majority of industrial fermentation processes where high substrate concentrations are used, biofilm formation is either unnecessary or even disadvantageous, and the range of applications of immobilized-cell systems in industry is mainly limited to wastewater treatment processes (Nicolella *et al.,* 2000).

Biofilms are extensively used in environmental biotechnology because biofilm reactors can be operated at high biomass concentrations to treat the large volumes of dilute aqueous solutions that are typical of industrial and municipal wastewaters without the need for separating the biomass and the treated effluent (Nicolella *et al.,* 2000, Alvarado-Lassman *et al.,* 2008). Figure 2.5 shows types of particle-based biofilm reactors. The main reactor types that are applicable for the suspension of particle-based biofilms are the upflow sludge blanket (USB), biofilm fluidized bed (BFB), expanded granular sludge blanket (EGSB), biofilm airlift suspension (BAS) and internal circulation (IC) reactors. In USB, BFB and EGSB reactors, the particles are kept fluidized by an upward liquid flow. In BAS reactors, a suspension is obtained by pumping air into the systems, and in IC reactors, the gas

produced in the system drives the circulation and mixing of the liquid and solids in an airlift-like reactor.

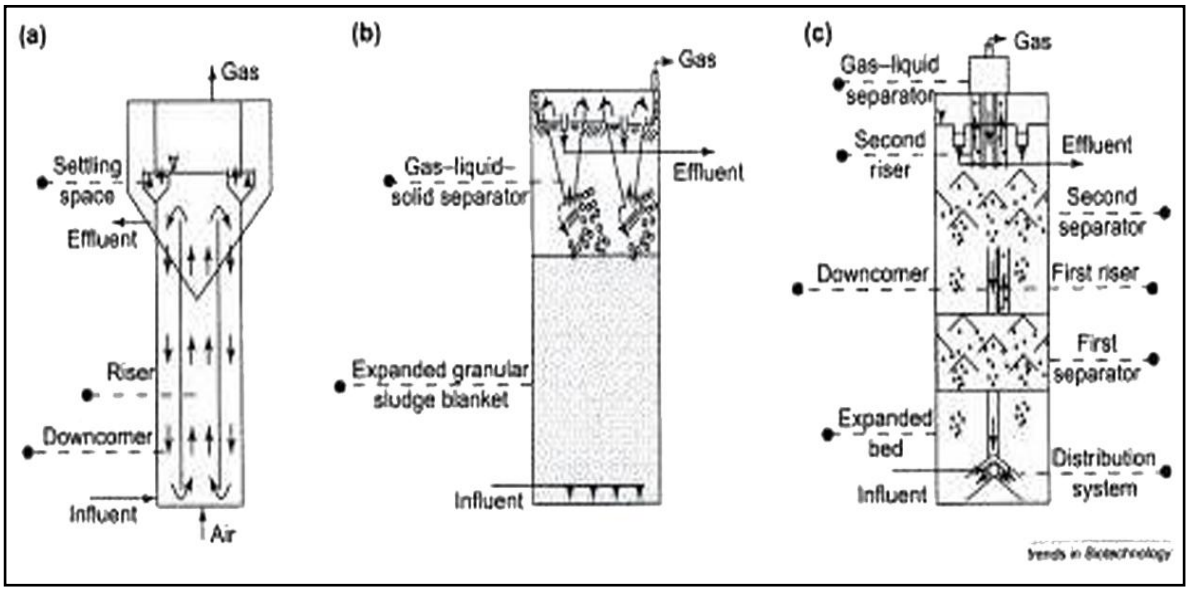

Figure 2.5 Types of particle-based biofilm reactors: (a) biofilm airlift suspension, (b) expanded granular sludge blanket and (c) internal circulation reactors (Nicolella *et al.*,2000)

2.4 Bio-granulation Technology

Bio-granulation involves cell-to-cell interactions that include biological, physical and chemical phenomena. Bio-granules form through self-immobilization of microorganisms (Liu and Tay, 2004; Tay *et al*. 2006). These granules are dense microbial consortia packed with different bacterial species and typically contain millions of organisms per gram of biomass. Compared to the conventional activated sludge, they enable high biomass retention and withstand high-strength wastewater and shock loadings. Bio-granulation can be classified as aerobic and anaerobic

granulation. The following sub-section shall provide a more detailed discussion of both types of bio-granulation.

2.4.1 Anaerobic Granulation

An anaerobic granule has been extensively studied and is probably best recognized in the upflow anaerobic sludge blanket (UASB) reactor (Ramakrishnan and Gupta, 2006). Many wastewater treatment plants already apply anaerobic granulation technology (Alves *et al*., 2000, Tay *et al*. 2006).

The feasibility and efficiency of UASB reactors and their various modifications (e.g., the internal circulation (IC) reactor) for removing biodegradable organic matter from municipal and industrial wastewater have been successfully demonstrated (Lettinga *et al*., 1980; Fang and Chui, 1993; Schmidt and Ahring, 1996).

Anaerobic granular sludge is a dense microbial community that typically includes millions of organisms per gram of biomass. None of the individual species in these micro-ecosystems is capable of completely degrading the influent wastes. Complete degradation of industrial waste involves complex interactions between the resident species. Thus, granular sludge reactors are desirable in wastewater biological treatment processes because a very high number of organisms can be maintained in the bioreactor. This in turn implies that contaminant transformation is rapid and highly concentrated; therefore, large volumes of waste can be treated in compact bioreactors.

In granular sludge reactors, the large size and relatively high density of individual granules causes them to settle rapidly, which simplifies the separation of treated effluent from the biomass. Anaerobic granular sludge has proved capable of treating high-strength wastewater contaminated with soluble organic pollutants. However, the anaerobic granulation technology has some drawbacks. These include the need for a long start-up period, a relatively high operation temperature and unsuitability for low-strength organic wastewater (Liu and Tay, 2004). In addition, anaerobic granulation technology is not suitable for the removal of nutrients (nitrogen and phosphorus) from wastewater. In order to overcome those weaknesses, research has been devoted to the development of aerobic granulation technology.

2.4.2 Aerobic Granulation

Granulation in aerobic systems has been extensively studied (Mulder *et al.*, 2001; Beun *et al.*, 2002; Tay *et al.*, 2002a,b; de Kreuk *et al.*, 2005; Boonyarit *et al.*, 2008). Most flocs granulation processes have been observed to occur in sequencing batch reactor mode with a short fill time (Beun *et al.*, 1999; de Kreuk *et al.*, 2005; McSwain *et al.*, 2004). AGS can form with various substrates (Jiang *et al.*, 2002, 2004; Morgenroth *et al.*, 1997; Peng *et al.*, 1999; Tay *et al.*, 2001, 2002c, 2003a; Tsuneda *et al.*, 2003, Sun *et al.*, 2006, Zitomer *et al*, 2007; Nancharaiah *et al.*, 2008). The granules are typically dense in structure and settle fast in a water pool (Liu and Tay, 2004; Tay *et al*., 2006).

The presence of exopolysaccharide and the application of high shear force promote granulation (Liu and Tay, 2002; Tay *et al.*, 2003b). McSwain *et al.* (2005)

proposed that protein is enriched in the core of the granule, whose presence facilitates granulation and promotes stability.

In addition, AGS or activated sludge flocs are micro-reactors that adsorb and oxidize organic pollutants in wastewater. The AGS system yields a very high biomass concentration (up to $15gL^{-1}$) (di Iaconi *et al*., 2005) and has a capacity to degrade high-strength wastewater (up to 15 kgCOD/ m^3d) (Moy *et al*., 2002), representing a compact and highly efficient wastewater treatment alternative over the conventional activated sludge system.

2.5 Aerobic Granulation Technology

2.5.1 Formation of Aerobic Granular Sludge

Sludge is the microbial biomass that utilizes nutrient substrates present in wastewater. Microbial granules can be regarded as compact and dense microbial aggregates with a spherical outer shape (de Kreuk *et al.*, 2005; Sunil *et al.*, 2008). The growth of AGS is sometimes regarded as a special case of biofilm development (Liu and Tay, 2002; Yang *et al.*, 2004, Tay *et al.*, 2006). In fact, microbial granulation is quite fundamental in biology and cell aggregation can be defined as the gathering together of cells to form a fairly stable, contiguous, multicellular association under physiological conditions (Calleja, 1984). Each AGS is an enormous metropolis of microbes containing millions of individual bacteria. Almost all AGS have been cultivated in SBR. The SBR system is a modified design of the conventional activated sludge process and has been widely used in municipal and industrial wastewater treatment. Aerobic granulation may be initiated by microbial

self-adhesion. Bacteria are not likely to aggregate naturally because of the repulsive electrostatic forces and hydration interactions among them.

Tay *et al.* (2001a) used different microscopic techniques to investigate how AGS formed from seed sludge. For comparison, granules were cultivated in two reactors fed with glucose in one case and acetate in the other case, as sole carbon sources. The results showed that the seed sludge had a very loose and irregular structure, dominated by filamentous bacteria. After operation in SBR for one week, compact aggregates appeared. The filamentous bacteria gradually disappeared in the the acetate-fed reactor; however, in the the glucose-fed reactor, filamentous bacteria still prevailed. Two weeks after the start-up, the AGS with clear round outer shape was formed in both reactors.

Although the filamentous bacteria disappeared completely in acetate-fed reactor, they were still predominant in glucose-fed reactor. This may imply that a high-carbohydrate feed composed of glucose supports the growth of filamentous bacteria as reported in the activated sludge process previously (Chudoba, 1985). After operation for three weeks, AGS matured in both reactors. At this stage, both glucose-and acetate-fed granules had a very regular round-shaped outer surface. The average aspect ratio of glucose-fed granules was 0.79 and 0.73 for acetate-fed granules (aspect ratio of a particle is the ratio of the lengths of minor axis and major axis of an ellipse that is equivalent to the particle). Compared to acetate-fed granules, glucose-fed granules had a fluffy outer surface because of the predominance of filamentous bacteria as shown in Figure 2.6.

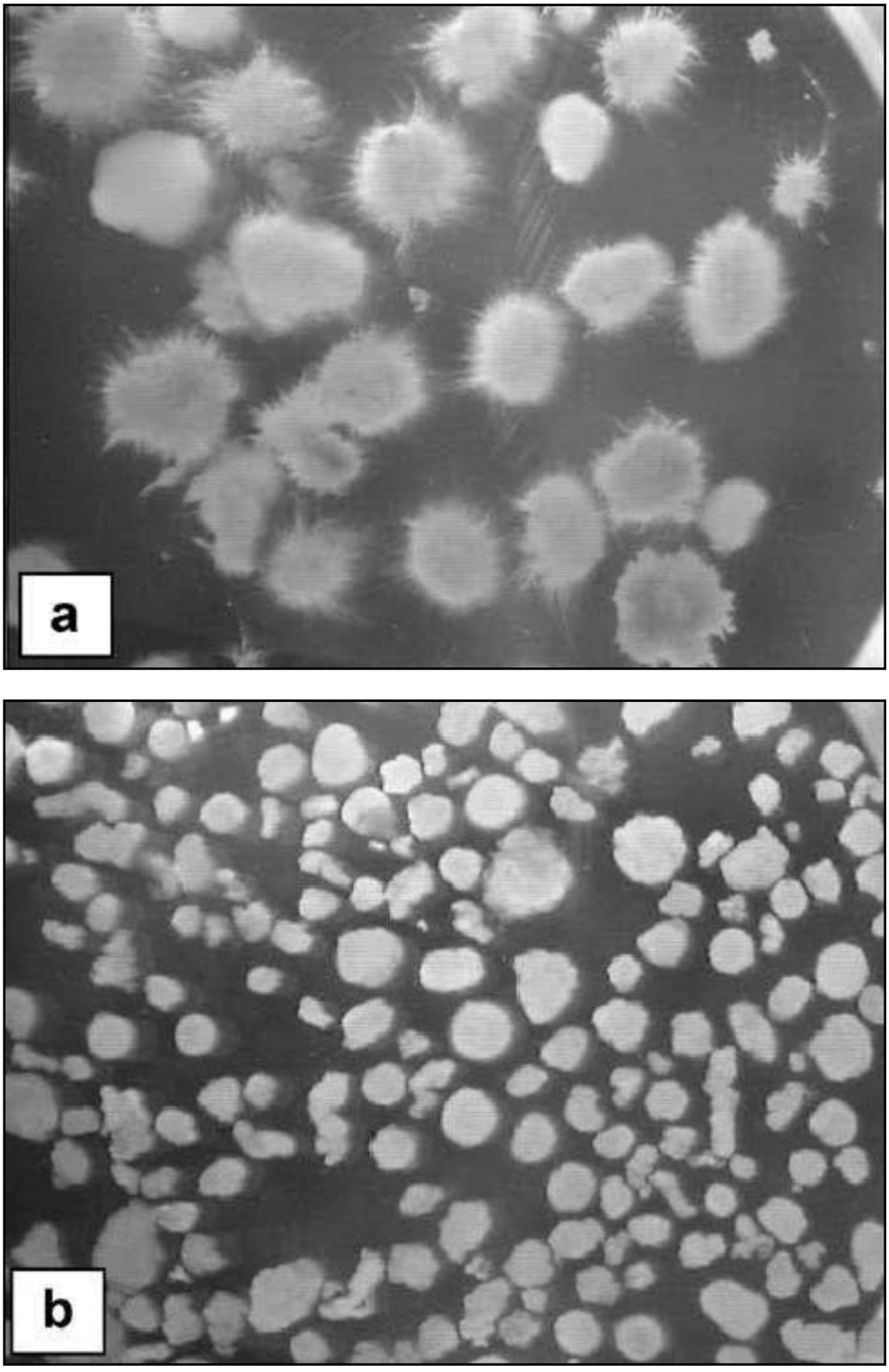

Figure 2.6 Macrostructures of glucose-fed (a) and acetate-fed (b) aerobic granules (Tay *et al*., 2001a).

2.5.2 Factors Affecting Aerobic Granulation

2.5.2.1 Substrate Composition

AGS have been successfully cultivated with a wide variety of substrates including glucose, acetate, ethanol, phenol, and synthetic wastewater (Beun *et al.*, 1999; Peng *et al*., 1999; Tay *et al.*, 2001a; Moy *et al.*, 2002; Jiang *et al*., 2002; Schwarzenbeck *et al.*, 2004, de Kreuk *et al.*, 2005; Sunil *et al.*, 2008). However, AGS microstructure and species diversity appear to be related to the type of carbon source.

The glucose-fed AGS have exhibited a filamentous structure, while acetate-fed AGS have had a nonfilamentous and very compact bacterial structure in which a rod-like species predominated. AGS have also been cultivated with nitrifying bacteria and an inorganic carbon source (Tay *et al*., 2002b; Tsuneda *et al.*, 2003). These nitrifying AGS showed excellent nitrification ability. More recently, AGS were also successfully developed in a laboratory-scale SBR for treating particulate organic matter-rich wastewater (Schwarzenbeck *et al*., 2004).

2.5.2.2 Organic Loading Rate

The essential role of organic loading rate (OLR) in the formation of anaerobic granules has been recognized (Ramakrishnan and Gupta, 2006). Relatively high organic loading rates facilitate the formation of anaerobic granules in upflow-anaerobic sludge blanket (UASB) systems. In contrast to anaerobic granulation, the accumulated evidence suggests that AGS can form across a very wide range of

organic loading rates from 2.5 to 15 kg Chemical Oxygen Demand (COD)/m^3.day (Moy *et al.,* 2002; *Liu et al.,* 2003a; Tay *et al.,* 2005). It seems that aerobic granulation is not sensitive to the organic loading rate. Although the effect of organic loading rate on the formation of AGS is insignificant, the physical characteristics of AGS depend on the organic loading rate. The mean size of aerobic AGS increased from 1.6 to 1.9 mm with the increase of the organic loading from 3 to 9 kg COD/m^3.day (Liu *et al.,* 2003a).

A similar trend was also observed in anaerobic granulation (Grotenhuis *et al*., 1991). The effect of organic loading rate on the morphology of AGS in terms of roundness was found to be insignificant, while the AGS developed at different organic loading rates exhibited comparable dry biomass density, specific gravity, and SVI. The physical strength of AGS decreased with the increase of OLR (Liu *et al.,* 2003a; Tay *et al.,* 2005).

Similarly, in anaerobic granulation, a high organic loading rate has been found to reduce strength of anaerobic granules; that is, partial loss of structural integrity and disintegration can occur at high OLR (Morvai *et al.*, 1992; Quarmby and Forster, 1995). It should be stressed that an increased organic loading rate can raise the biomass growth rate and this in turn reduces the strength of the three-dimensional structure of the microbial community (Liu *et al*., 2003b).

2.5.2.3 Hydrodynamic Shear Stress

Evidence shows that a high shear stress favours the formation of AGS and AGS stability (Shin *et al.,* 1992; Chisti, 1999; Tay *et al.,* 2001a; Liu and Tay, 2002;

Elenter *et al.*, 2007). It was found that AGS could be formed only above a threshold shear force value in terms of superficial upflow air velocity above 1.2 cms^{-1} in a column SBR, and more regular, rounder, and compact AGS were developed at high hydrodynamic shear force (Tay *et al.*, 2001a).

The AGS density and strength were also proportionally related to the shear force applied (Tay *et al.*, 2003c). These observations may imply that the hydrodynamic shear stress present in a bioreactor mainly determines the structure of AGS. However, it is well known that extracellular polysaccharides can mediate both cohesion and adhesion of cells and play a crucial role in maintaining the structural integrity in a community of immobilized cells.

Tay *et al*. (2001a) reported that the production of extracellular polysaccharides was closely associated with the shear stress and the stability of AGS was found to be related to the production of extracellular polysaccharides (Tay *et al*., 2001c). The extracellular polysaccharides content normalized to protein content, increased with the shear force estimated in terms of superficial upflow air velocity. Thus, a high shear stress stimulated bacteria to secrete more extracellular polysaccharides. In fact, shear stress-induced production of extracellular polysaccharides has been observed in biofilms (Ohashi and Harada, 1994). Consequently, the enhanced production of extracellular polysaccharides at high shear can contribute to the compact and stronger structure of AGS.

2.5.2.4 Settling Time

In a SBR system, wastewater is treated in successive cycles each lasting a few hours. At the end of every cycle, the biomass is settled before the effluent is withdrawn. The settling time acts as a major hydraulic selection pressure on microbial community. A short settling time preferentially selects for the growth of fast settling bacteria and the sludge with a poor settleability is washed out.

Qin *et al*. (2004) and de Kreuk (2006) reported that AGS were successfully cultivated and became dominant only in the SBR operated at a settling time of 5 minutes. Mixtures of AGS and suspended sludge were observed in the SBRs run at settling times of 20, 15, and 10 minutes. The production of extracellular polysaccharides was stimulated and the cell surface hydrophobicity improved significantly at short settling times. These findings illustrate the fact that AGS granulation is driven by selection pressure and the formation and characteristics of the AGS may be controlled by manipulating the selection pressure. Therefore, choice of an optimal settling time is very important in AGS granulation.

Generally, the mature AGS tend to settle within 1 minute, leaving a clear supernatant in the reactor (Tay *et al*., 2001a). The easily retainable biomass in the reactor ensures a faster and more efficient removal of organic pollutants in wastewater. AGS with excellent settling properties are essential for the effective functioning of biological systems treating wastewater.

2.5.2.5 Hydraulic Retention Time

In AGS granulation, the light and dispersed sludge is washed out and the relatively heavy granules are retained in the reactor. The SBR cycle time represents the frequency of solids discharge through effluent withdrawal, or the so-called washout frequency, and it is related to the HRT at a given exchange ratio (de Kreuk, 2006). The latter is defined as the volume of effluent discharged divided by the working volume of the SBR (Liu and Tay, 2004; Tay *et al.*, 2006).

A short cycle time would suppress the growth of suspended solids because of frequent washout of the suspended material. If the SBRs are run at an extremely short cycle time, sludge loss has been observed through hydraulic washout because bacterial growth has been unable to compensate. As a result, a complete washout of sludge blanket occurs and leads to a failure of microbial granulation. Thus, the HRT should be short enough to suppress the suspended growth, but long enough for microbial growth and accumulation.

By its nature, a SBR is cyclic in operation. The SBR cycle time can serve as a main hydraulic selection pressure on the microbial community in the system. Tay *et al*. (2002b) investigated the effect of hydraulic selection pressure on the development of nitrifying granules in column-type sequencing batch reactors. No nitrifying granulation was observed in the SBR operated at the longest cycle time of 24 hours because of a weak hydraulic selection pressure. Excellent nitrifying granules were successfully developed in the SBR operated at cycle times of 3, 6 and 12 hours. A short cycle time stimulates microbial activity and production of cell polysaccharides and improves the cell hydrophobicity. These hydraulic selection pressure-induced microbial changes favour the formation of nitrifying granules (Tsuneda *et al.*, 2005).

2.5.2.6 Aerobic Starvation

The SBR operation is a sequencing cycle of feeding, aeration, settling, and discharging of supernatant fluid (Guo *et al.*, 2007). As a result, microorganisms growing in the SBR are subject to periodic fluctuations in the environmental conditions. During operation cycles, an important period of aerobic substrate starvation has been identified (*Tay et al.*, 2001a). The waste degradation time required tends to reduce with the increase in the number of operation cycles. The aeration period of the operation actually consists of two phases: a degradation phase in which the substrate is depleted to a minimum, followed by an aerobic starvation phase in which the external substrate is no longer available.

Under starvation conditions, bacteria became more hydrophobic which facilitates microbial adhesion. It is likely that aggregation is a strategy of cells against starvation. It appears that the microorganisms are able to change their surface characteristics when they face starvation (Tay *et al*., 2001a).

Bossier and Verstraete (1996) reported that under starvation conditions, bacteria become more hydrophobic which likely facilitates adhesion or aggregation. Such changes contribute to microbial ability to aggregate. Thus, starvation plays a role in the microbial aggregation process and leads to stronger and denser granules. Although the periodical starvation in SBR is important for microbial aggregation, the contribution of other operation conditions should not be neglected.

2.5.2.7 Presence of Calcium Ion in Feed

Jiang *et al.* (2003) reported that addition of calcium ions (Ca^{2+}) accelerated the AGS granulation process. With addition of 100 mg $Ca^{2+}L^{-1}$, the formation of AGS took 16 days compared to 32 days in the culture without Ca^{2+} added. The Ca^{2+} augmented AGS also showed better settling and strength characteristics and had higher polysaccharides contents. It has been proposed that Ca^{2+} binds to negatively-charged groups present on bacterial surfaces and extracellular polysaccharides molecules and thus acts as a bridge to promote bacterial aggregation. Polysaccharides play an important role in maintaining the structural integrity of biofilmS and microbial aggregates, such as AGS, as they are known to form a strong and sticky non-deformable polymeric gel-like matrix (Tay *et al.*, 2006).

2.5.2.8 Dissolved Oxygen

Dissolved oxygen (DO) concentration is an important variable that influences the operation of aerobic wastewater treatment system. AGS has formed at DO concentration as low as 0.7 to 1.0 mgL^{-1} in a SBR (Peng *et al.*, 1999, de Kreuk *et al.*, 2005). In addition, AGS have been successfully developed at DO concentrations of > 2 mgL^{-1} (Tay *et al*., 2002c; Yang *et al.*, 2003a; de Kreuk *et al.*, 2005). It appears, therefore, that DO concentration is not a decisive variable in the formation of AGS.

2.5.2.9 pH

Concerning the roles of the reactor pH on AGS granulation, detailed studies are lacking. For most of the research studies on sludge granulation, the pH was controlled at neutral (pH 7 ± 0.1) via acid and base, during reactor operation (Schwarzenbeck *et al.,* 2004, de Kreuk *et al.,* 2005; Sunil *et al.,* 2008). Only one study conducted by Mosquera-Corral *et al*., (2005), study the granulation process without pH control, which varied between 7.4 and 8.5. Therefore, Tay *et al.,* (2006) suggests that these effects are not as important in aerobic granulation as they are in anaerobic granulation.

2.5.2.10 Temperature

Many laboratory studies (among others Morgenroth *et al.,* 1997; Beun *et al.,* 1999; Tay *et al.,* 2002a; de Kreuk *et al.,* 2005; Tay *et al.,* 2006; Sunil *et al.,* 2008), a feasibility study (de Bruin *et al.,* 2004) and a pilot study (de Bruin *et al*., 2005) showed the potential of AGS developed at low temperature (8-15^{o}C) and room temperature (20-25^{o}C). Table 2.3 summarised recent studies conducted on AGS granulation. The specific process parameters such as temperature condition are also highlighted in Table 2.3. Unlike anaerobic granules, information about the physical characteristics of AGS developed at high temperature is still scarce.

2.5.2.11 Seed Sludge

AGS developed in SBRs have been seeded with conventional activated sludge while in anaerobic granulation, there is evidence that the characteristics of the seed sludge profoundly influence the formation and properties of anaerobic granules. The important factors that determine the quality of seed sludge for AGS granulation appear to include the macroscopic characteristics, settleability, surface properties (a high surface hydrophobicity and low surface charge density are preferred), and microbial activity (Zheng and Yu, 2007). Little information is available on the role of seed sludge in AGS granulation (Liu and Tay, 2004; Tay *et al*, 2006).

2.5.2.12 Reactor Configuration

In almost all cases reported, AGS were produced in column-type upflow reactors. Reactor configuration has an impact on the flow pattern of liquid and microbial aggregates in the reactor (Beun *et al*., 1999; Liu and Tay, 2002) Column-type upflow reactor and completely-mixed tank reactor (CMTR) have very different hydrodynamic behaviours in terms of the interaction between flow and microbial aggregates.

The air or liquid upflow in column reactors can create a relatively homogenous circular flow and localized vortexing along the reactor's axis and microbial aggregates are constantly subject to a hydraulic attrition (de Kreuk, 2006). The circular flow apparently forces the microbial aggregates to adapt a regular granular shape that has a minimum surface free energy. In a column-type upflow

reactor, a high ratio of reactor height to diameter (H/D) can ensure a longer circular flow trajectory, which in turn provides a more effective hydraulic attrition to microbial aggregates.

However, in CMTR microbial aggregates stochastically move with dispersed flow in all directions. Thus, microbial aggregates are subject to varying localized hydrodynamic shear force, upflow trajectories and random collisions. Under such circumstances, only flocs with irregular shape and size instead of regular granules occasionally form (Liu and Tay, 2002). For practical applications, the SBR should have a high H/D ratio to improve selection of granules by the difference in settling velocity (Beun *et al.*, 1999). A high H/D ratio and the absence of an external settler result in a reactor with a small footprint.

2.6 Characteristics of Aerobic Granular Sludge

Compared to the loose, fluffy, and irregular conventional activated sludge floc (Liu and Tay, 2004; de Kreuk *et al*., 2005; Tay *et al*., 2006; Pratt *et al*., 2007 and Chen *et al.*, 2008) , the AGS has the following characteristics:

i. Dense and strong microbial structure;

ii. Regular, smooth and round shape with a clear outer surface;

iii. Visible as separate entities in the mixed liquor during both the mixing and the settling phases;

iv. Have a high biomass retention and excellent settleability;

v. Capable to withstand high flow rates;

vi. Able to withstand high organic loading rates;

vii. Less vulnerable than the suspended sludge to the toxicity of organic chemicals and heavy metals in wastewater.

The excellent settling characteristic of AGS simplifies the separation of treated effluent from the sludge (biomass). A detailed description about AGS characteristics are discussed in the following sub-sections.

2.6.1 Morphology

Microscopic examination shows that the morphology of the AGS is completely different from conventional activated sludge floc. The shape of the AGS is nearly spherical with a very clear outline (Pratt *et al*., 2007 and Chen *et al.*, 2008). The size is an important parameter in the characterization of AGS granulation. The average diameter of AGS varies in the range of 0.2 to 5 mm. (Peng *et al.*, 1999; Tay *et al*., 2001a,b,c; Zhu and Wilderer, 2003). This is mainly due to a balance between growth and abrasive detachment due to the relatively strong hydrodynamic shear force in aerobic reactors (Liu and Tay, 2002; Liu *et al.*, 2003c). Hydrodynamic shear forces are known to control the prevailing size of the suspended bio-solids in many situations (Chisti, 1999a). Chisti (1999a) has discussed methods of estimating the magnitudes of these forces under various conditions of operation.

2.6.2 Settling Characteristics

The settling characteristics (settleability) of AGS determine the efficiency of solid–liquid separation that is essential for the proper functioning of wastewater treatment systems. The SVI of AGS can be lower than 50 mLg^{-1}, which is much lower than that of conventional bioflocs (Liu *et al*., 2003c; Qin *et al*., 2004). This implies that from an engineering perspective, the settleability of sludge can be improved significantly through the formation of AGS so that it can be settled in a more compact clarifier.

The settling velocity of AGS is associated with granule size and structure and is as high as 30 to 70 mh^{-1}. This is comparable with that of the UASB granules, but is at least three times higher than that of activated sludge flocs (typical settling velocity of around 8 to 10 mh^{-1}. The high settling velocities of AGS allow the use of relatively high hydraulic loads to the reactors without having to worry about washout of biomass (Beun *et al.*, 2000; Tay *et al*., 2001b). Thus, AGS granulation can lead to more biomass retention in the reactor and this can enhance the performance and stability of the reactor. A high concentration of the retained biomass ensures a faster degradation of pollutants and relatively compact reactors (Zitomer *et al*., 2007; Chen *et al.*, 2008)

2.6.3 Density, Strength and Stability

AGS with a high physical strength withstand high abrasion and shear. The physical strengths of aerobic and anaerobic granules are comparable. AGS with smaller sizes tend to be more compact compared to larger aerobic granules (Toh *et*

al., 2003; Yang *et al*., 2004). The studies found that there is a relationship between AGS strength and density (Gjatelma *et al.*, 1997; Beun *et al*.,1999; Villaseñor *et al.*, 2000 and Tay *et al*., 2005, Ghangrekar *et al.*, 2005) The more dense the granules, the stronger the granules. Meanwhile, Tay *et al*. (2005) found that AGS (fed with glucose and acetate) developed in the laboratory-scale reactor was stronger than those in the pilot-scale reactor.

The physical strength, expressed as integrity coefficient, was 96% for the pilot-scale reactor and 96.9% for the laboratory-scale reactor. The larger the integrity coefficient, the higher is the strength of granules. The method to measure the strength followed the method suggested by Ghangrekar *et al.,* (2005). However, the method is not strong enough to justify the stability of AGS against shear stress, since the shear effects introduced by the platform shaker is not comparable with the shear effects introduced by mechanical or aerated-mixing. It is suggested that a new procedure has to be developed to evaluate the strength of AGS. Chapter 5 in this thesis shall discuss in detail on this issue.

2.7 Applications of Aerobic Granulation Technology

The performance of a biological system for wastewater treatment depends significantly on the active biomass concentration, the overall biodegradation rates, the reactor configuration, and the feeding rates of the pollutants and oxygen (Metcalf and Eddy, 2003). Process efficiency of large-scale treatment plants can be improved by using AGS in ways that allow high conversion rates and efficient biomass separation to minimize the reactor volume. Treatment capacities can be easily varied to accommodate varying loading rates, wastewater composition, and treatment goals by bio-augmentation with specifically developed AGS (Tay *et al.*, 2006).

2.7.1 High-Strength Organic Wastewater

Granulation of the sludge can lead to high biomass retention in the reactor because of the compact and dense structure of the AGS. Biomass concentrations as high as 6.0 to 12.0 gL^{-1} have been obtained in SBRs operated with a volumetric exchange ratio of 50% (Tay *et al*., 2002a,c). The feasibility of applying AGS granulation technology for the treatment of high-strength organic wastewaters was demonstrated by Moy *et al.* (2002), who examined the ability of AGS to sustain high organic loading rates by introducing step increases in organic loading only after the COD removal efficiencies had stabilized at values of >89% for at least 2 weeks. AGS cultivated this way on glucose were exposed to organic loading rates that were gradually raised from 6.0 to 9.0, 12.0, and 15.0 kg COD/m^3.day. AGS were able to sustain the maximum organic loading rate of 15.0 kg COD/m^3.day while removing more than 92% of the COD.

The granules initially exhibited a fluffy loose morphology dominated by filamentous bacteria at low loadings and evolved into smooth irregular shapes. These irregularities were thought to allow for better diffusion and penetration of nutrients into the interior of the granule (Chen *et al.*, 2008)

2.7.2 Simultaneous Organic and Nitrogen removal

Complete nitrogen removal involves nitrification and denitrification. Nitrite and nitrate produced from nitrification are reduced to gaseous nitrogen by denitrifiers. Yang *et al*. (2003a, 2004) investigated the simultaneous removal of organics and nitrogen by aerobic granules. Heterotrophic, nitrifying, and denitrifying

populations were shown to successfully coexist in microbial granules. Increased substrate of nitrogen and organic ratio (N/COD) led to significant shifts among the three populations within the granules. Co-existence of heterotrophic and nitrifying populations in aerobic granules was also observed by Jang *et al.* (2003).

Enhanced activities of nitrifying and denitrifying populations were achieved in granules developed at high substrate N/COD ratio; however, the heterotrophic populations in granules tended to decrease with the increase of substrate N/COD ratio. Dissolved oxygen (DO) concentration had a pronounced effect on the efficiency of denitrification by microbial granules and a certain level of mixing was necessary for ensuring sufficiency of mass transfer between the liquid and granules during denitrification (Yang *et al.,* 2003b). Similar phenomena were reported by Beun *et al.* (2001). It appears that complete organics and nitrogen removal can be efficiently and stably achieved in a single granules-based SBR(de Kreuk and van Loosdrecht, 2006 ; Pratt *et al*., 2007; Zheng *et al*, 2007 and Chen *et al*., 2008)

2.7.3 Phosphorus Removal

Environmental regulations in many countries require a reduction of phosphorus concentration in wastewater to levels of 0.5 to 2.0 mgL^{-1} before discharge (Sunil *et al.,* 2008). Enhanced biological phosphorus removal (EBPR) process removes phosphorus (P) without the use of chemical precipitation and is an economical and reliable option for P removal from wastewater. The EBPR process operates based on alternating anaerobic and aerobic conditions with substrates feeding limited to the anaerobic stage.

Most EBPR processes are based on suspended biomass cultures and require large reactor volumes. Although full-scale experience shows a strong potential of the EBPR, difficulties in assuring stable and reliable operation have also been recognized. Often, the reasons for failure of biological phosphorus removal are not clear (Barnard *et al.*, 1985; Bitton, 1999). In attempts to overcome the problems associated with the conventional bio-removal of P, Lin *et al.* (2003) successfully developed phosphorus-accumulating microbial granules in a sequencing batch reactor operated at substrate of phosphorus and organic ratio (P/COD) ranging from 1/100 to 10/100 by weight. The soluble chemical oxygen demand (COD) and phosphate (PO_4–P) profiles showed that the AGS had typical P-accumulating characteristics, with concomitant uptake of soluble organic carbon and the release of phosphate in the anaerobic stage, followed by rapid phosphate uptake in the aerobic stage.

The size of phosphate accumulating granules exhibited a decreasing trend with the increase of substrate P/COD ratio. The structure of the AGS became more compact and dense as the substrate P/COD ratio increased (Lin *et al.*, 2003; Cassidy and Belia, 2005). The P uptake by AGS was in the range of 1.9% to 9.3% by weight, or comparable to that of the conventional enhanced biological phosphorus removal (EBPR) processes. These results will certainly spur the further development of novel granule-based EBPR technologies.

2.7.4 Simultaneous Organic, Nitrogen and Phosphorus Removal

de Kreuk *et al.* (2005) investigate the important factors for simultaneous organic, nitrogen and phosphate removal by AGS in SBR systems and reported simultaneous nutrient removal was possible, because of heterotrophic growth inside

the granules (denitrifying phosphate accumulating organisms [DPAO]). At low oxygen saturation (20%), high removal efficiencies were obtained; 100% COD removal, 94% phosphate removal and 94% total nitrogen removal (with 100% ammonia removal).

Enrichment of phosphate accumulating organisms (PAO) in AGS by introducing alternating anaerobic feeding and aeration periods, resulted in stable granules at low dissolved oxygen concentrations (< 2 mgL^{-1}). Furthermore, high phosphate removal efficiency (94%) was achieved by these PAO enriched granules (de Kreuk *et al.*, 2005). Besides improved granule formation and phosphate removal, also the problems of pulse feeding at full-scale installations, which are among others oversized pump capacity and large buffer tanks, are solved.

It is also reported by de Kreuk *et al.* (2005), that with 60 minute anaerobic feed, followed by an aerobic period with oxygen saturation of 20% resulted in maximum simultaneous organic (COD) (100%, acetate), phosphate (94%) and nitrogen removal (100% ammonia removal by nitrification and 94% total nitrogen removal). The biomass concentration that can be maintained in this type of SBR reactors with an exchange ratio of 50% was around 5 times higher than in an activated sludge system with flocculated biomass. Because of high biomass concentrations in combination with the extraordinary settling capacity of granular sludge (no external settler needed and high height/diameter ratio possible), aerobic granular sludge systems can be built very compact (de Bruin *et al.*, 2004). These results showed the potential of this process for wastewater treatment systems.

The use of biological phosphate removal can simplify the simultaneous nitrification and denitrification (SND) process. Figure 2.7 clearly shows a layered structure within the granules, with a mixture of heterotrophic PAO and autotrophic organisms in the outer layers of the granule and PAO inside the granule.

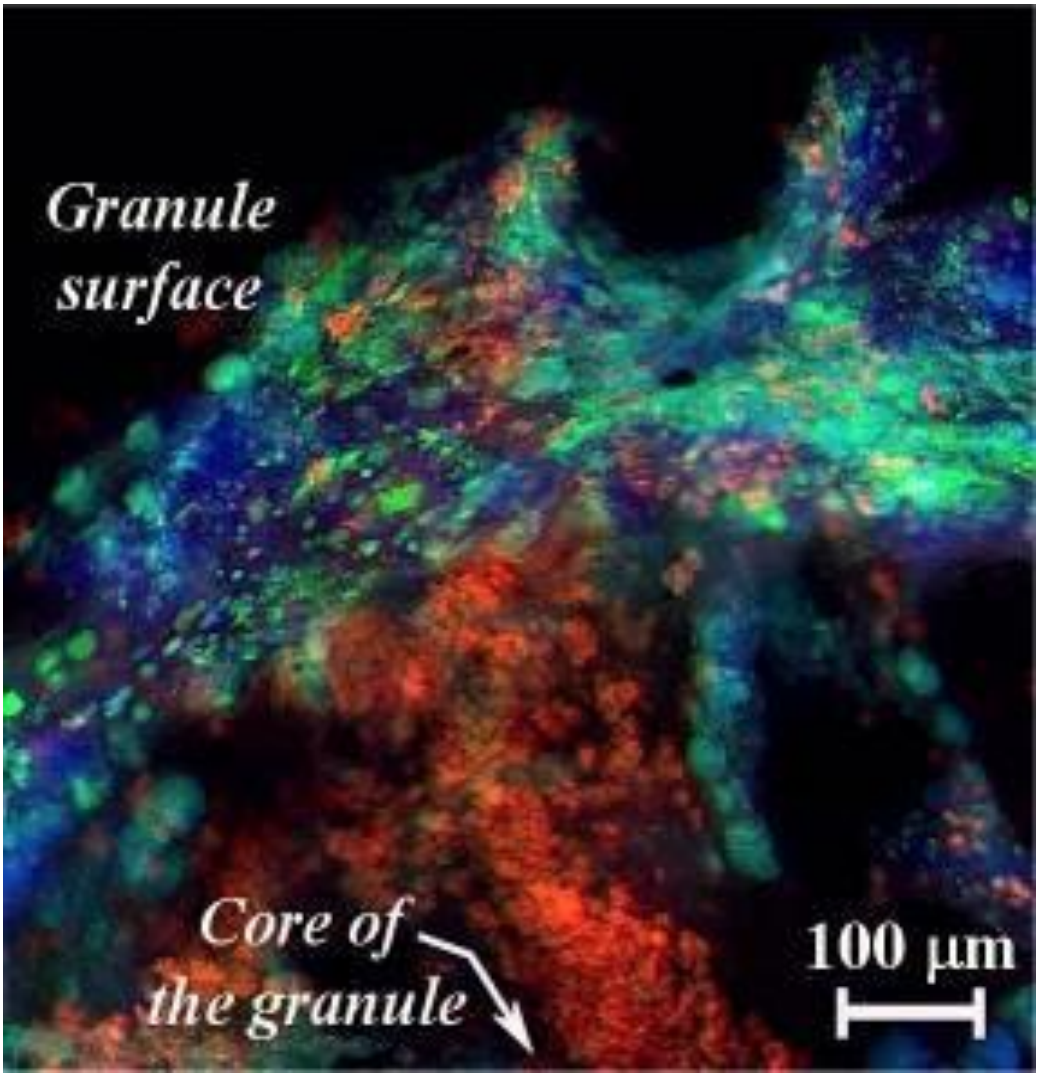

Figure 2.7 Impression of the layered structure of the granule (20% oxygen saturation in bulk liquid) by applying FISH techniques (green = ammonium oxidising bacteria; blue = eubacteria; red = PAO (de Kreuk, 2006).

Distribution of heterotrophic and autotrophic organisms in AGS plays an important role in SND. During the feast period (feeding time), the concentration of external carbon is high. This substrate will diffuse into the granules completely and will be anaerobically stored by PAO and aerobically or anoxically stored by heterotrophs as a poly-b-hydroxybutyrates (PHB). During the famine

period (non-feeding time), cell-internally stored substrate is available throughout the granule as schematically shown in Figure 2.8. Since autotrophic organisms need oxygen, they will exist in the aerobic layers of the granule. In this layer, ammonium will be converted to nitrate. The nitrate can penetrate to the interior of the granule where the stored substrate can serve as carbon source for denitrification. Optimal nitrogen removal in the system will occur when the aerobic and anoxic volume are well balanced throughout the aeration period (Beun *et al.*,2001 and de Kreuk, 2006).

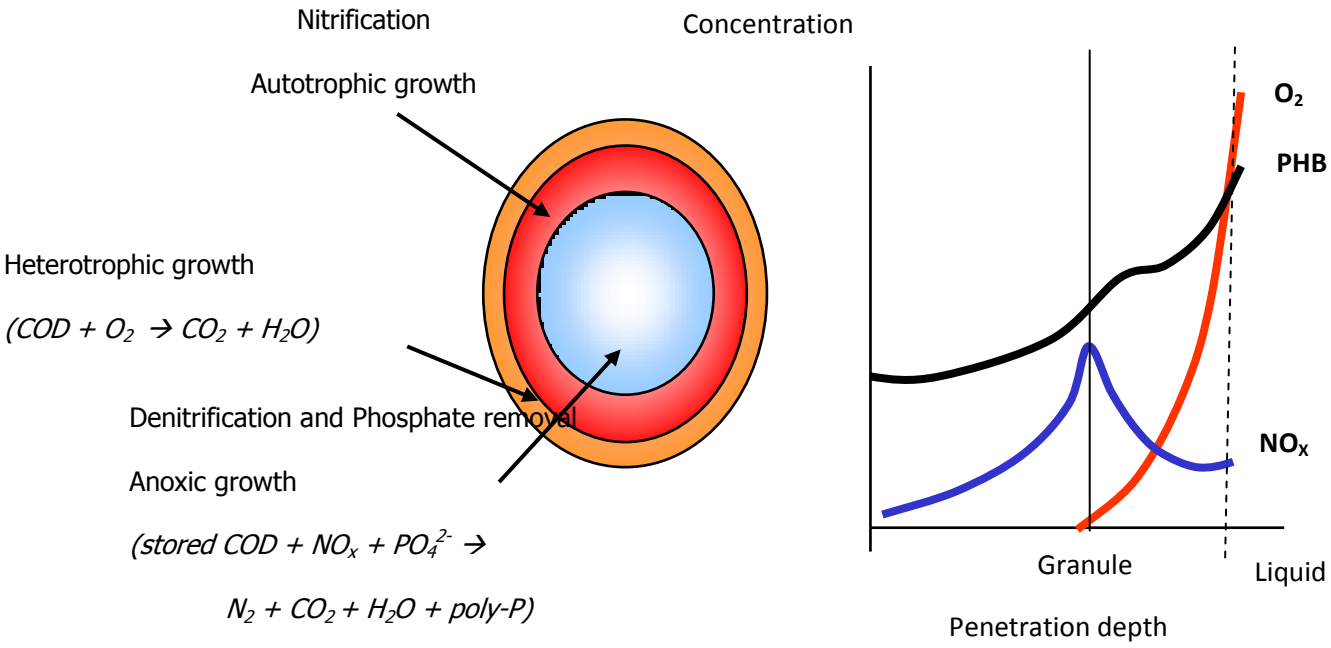

Figure 2.8 Schematic representation of the layered structure of AGS and of the substrate and electron acceptor concentrations inside the AGS during the famine phase (de Kreuk, 2006).

According to a study conducted by de Kreuk *et al*, (2005), during steady state operation at 20% oxygen saturation resulted in the highest nitrogen removal efficiency. With AGS sizes larger than 1.3 mm, the anoxic volume containing active DPAO inside the granule is large enough for denitrification, leading to 94% nitrogen

removal and stable granules. Therefore, AGS technology offers a possibility to design compact wastewater treatment plants based on simultaneous COD, nitrogen and phosphate removal in one sequencing batch reactor.

2.7.5 Wastewater with High Particulate Matter

Schwarzenbeck *et al*., (2004) investigated the feasibility to develop AGS in SBR systems treating malting wastewaters with a high content of particulate organic matter. The study concluded that protozoa play an important role in the removal of particulate matter from wastewaters in AGS reactors. Investigations of the spatial distribution of particles by means of confocal laser scanning (CLSM), epifluorescence and phase contrast microscopy after nucleic acid staining showed that protozoa growing on the AGS surface almost exclusively ingest particles. Scanning of regions more distant from the AGS surface showed that no particles attached to the biofilm aggreagate (granule). Protozoa were hence concluded to be the location of the primary particle uptake.

2.7.6 Phenolic Wastewater

Phenol is a toxic and inhibitory substrate, but also a carbon source for the bacteria. The consequence of the presence of phenol in biological wastewater treatment is process instability, which can lead to the washout of the microorganisms (Allsop *et al.*, 1993). In low concentrations, phenol is biodegradable, but high concentrations can kill phenol-degrading bacteria. Industrial wastewaters from fossil

fuel refining, pharmaceutical and pesticide processing are the major sources of phenolic pollution. Jiang *et al.* (2002, 2004) investigated the feasibility of treating phenol-containing wastewater with AGS.

AGS is less susceptible to toxicity of phenol because much of the biomass in the granules is not exposed to the same high concentration as present in the wastewater. The phenol-degrading AGS displayed an excellent ability to degrade phenol (Jiang *et al.*, 2002 and 2004). For an influent phenol concentration of 500 mgL^{-1}, a stable effluent phenol concentration of less than 0.2 mgL^{-1} was achieved in the AGS reactor (Jiang *et al.*, 2002 and 2004). The high tolerance of AGS to phenol can be exploited in developing compact high-rate treatment systems for wastewaters loaded with a high concentration of phenol. AGS may prove powerful bio-agents for removing other inhibitory and toxic organic compounds from high-strength industrial wastewaters. AGS appear to be highly tolerant of toxic heavy metals (Xie, 2003).

2.7.7 Biosorption of Heavy Metals

Heavy metals are often found in a wide variety of industrial wastewaters. More stringent metal concentration limits are being established in view of their relatively high toxicity. Many biomaterials have been tested as biosorbents for heavy metal removal. These include marine algae, fungi, waste activated sludge, and digested sludge (Lodi *et al*., 1998; Taniguchi *et al*., 2000; Valdman and Leite, 2000). In view of the physical characteristics of aerobic granular sludge (AGS) as discussed earlier, these AGS are ideal biosorbent for heavy metals (Tay *et al.*, 2006). The AGS are physically strong and have large surface area and high porosity for adsorption.

In addition, the AGS can be easily separated from the liquid phase after biosorption capacity is exhausted. The biosorption of Zn^{2+} and Cd^{2+} by AGS has been reported (Liu *et al.*, 2002, 2003a,b,c). The biosorption of Zn^{2+} was shown to relate to both the initial Zn^{2+} and granule concentrations (Liu *et al.*, 2002). The concentration gradient of Zn^{2+} was the main driving force for Zn^{2+} biosorption by the AGS. The maximum biosorption capacity for Zn^{2+} was 270 mgg^{-1} of granules. For Cd^{2+}, this capacity was 566 mgg^{-1} (Liu *et al*., 2003c).

CHAPTER 3

METHODOLOGY

3.1 Reactor setup and operation

Experiments were performed in parallel using two identical sequencing batch reactors for 52 days. The SBR had a working volume of 10 l with the size of 18 cm length, 18 cm width and 35 cm height. The influent was added from the top of the reactor and the air was introduced from the bottom of the reactor. The air-flow rate was controlled by a gas-flow controller. The temperature of the reactor was maintained at 25 LC using a ribbon heater and a temperature controller. The reactors were operated in successive cycles of 4 h each. One cycle consisted of 10 min of influent addition, 150 min of aeration, 60 min of settling and 20 min of reaeration, resettling and effluent withdrawal. Effluent was discharged 15 cm above the reactor bottom at a volumetric exchange ratio of 50%.

3.2. Wastewater and seed sludge

One reactor (R1) without adding $MgSO_4 \cdot 7H_2O$ was served as control, while $MgSO_4 \cdot 7H_2O$ was added to the other reactor (R2) with an influent 10 mg/l of Mg. Synthetic wastewater with the following composition was used: glucose 600 mg/l, NH_4CL 130 mg/l, K_2HPO_4 20mg/l, $CaCl_2$ 6 mg/l, trace element solution 1.0 ml/l. The composition of the trace element solution was EDTA 30 mg/l, $FeSO_4.7H_2O$ 1.5 mg/l, $ZnSO_4.7H_2O$ 1.5 mg/l, $(NH_4)_6Mo_7O_{24}$ 0.18 mg/l, $CoCl_2.6H_2O$ 0.15 mg/l, $MnCl_2.2H_2O$ 0.12 mg/l, $CuSO_4.5H_2O$ 0.03 mg/l. The buffer capacity was provided by the addition of $NaHCO_3$. Activated sludge from Changsha Wastewater Treatment Plant, Hunan, China, was used as seed sludge.

3.3 Analytical methods

The measurement of COD, sludge volume index (SVI), mixed liquor suspended sludge (MLSS) and specific gravity was con-ducted according to the Standard Methods (APHA, 1998). PH in reactor was measured by pH meter. Microbial observation was conducted by using either common optical microscope (Leica, Ger-many) or scanning electron microscope (SEM) (TSM-6360LV, Japan). To estimate the size distribution, the sludge samples taken from the bottom sampling points were classified into four fractions using laboratory sieves with various openings (0.2 mm, 0.6 mm, 1.0 mm, 1.5 mm). The sludge particles were first placed in the sieve with the biggest opening. The particles were gently submerged in water and shaken to let the smaller particles pass through. The procedures were repeated until the three sieves were used.

3.4. Extraction and analysis of extracellular polymeric substances (EPS)

Twenty millilitre sludge sample was centrifuged at 3000g for 15 min and washed gently in 40 ml PBS (0.05 mol/l sodium phosphate, pH 7, and 0.15 mol/l NaCl). Then the sludge sample was bro-ken with ultrasonic cell-break method (35 W, 4 LC, 4 min). After the sonication, the samples were centrifuged (20,000g, 4 LC) for 20 min, and filtered through a 0.22 lm cellulose acetate membrane to remove residual cells (Azeredo et al., 1998). The polysaccharide content in EPS (glucose equivalent) was determined using the phenol–sulphuric method, with glucose as the standard (Dubois et al., 1956). The contents of protein in EPS were measured by the modified Lowry method using bovine serum albumin as standard.

CHAPTER 4

RESULT AND DISCUSSION

The initial seed sludge was grayish brown and showed a fluffy, irregular and loose-structural morphology. After 4 days of operation, aerobic granules were firstly observed in R2, and then gradually became much denser and bigger. In R1, no granules were found until day 17. On day 18, the majority of granules had an uneven surface and soft texture in R2. After 35-day operation, the irregular granules became stable and were smoother and round-shaped with a solid surface in R2. Its colour changed from brown to white, and then to yellow at the end of the experimental process in R2. The reactor R1 had a granulation process similar to that in R2, but it had a slower granulation process compared with R2. The specific gravity of the granules in R1 and R2 were 1.008 kg/ l and 1.002 kg/l. It clearly showed that Mg^{2+} argumentation affected the compactness of a microbial community.

The detailed microstructure of the aerobic granules taken from the reactors was further examined using scanning electron microscopic (photos not shown). SEM observation revealed that the sludge from the reactors had a similar composition and morphology. The granules in both two reactors had a dense and compact bacterial structure, with most of rods and cocci bacterial cells in the inner surface, and filamentous bacteria on the outer surface of granules, which implied that the addition of Mg^{2+} did not result in any difference in microbial morphology, despite the fact that the addition of Mg^{2+} accelerated the granulation process and led to the formation of larger size granules through physico-chemical functions.

At the beginning of operation, MLSS in the two reactors was 3.2 g/l and the SVI of seed sludge was 235 ml/g. Changes of MLSS and SVI are shown in Figure 4.1. Initially, the biomass was loose and expanded easily. The sludge concentration in the reactors decreased due to the washing out of flocs in the first period, and then it in-creased as the granules dominated in the reactor. The steady-state biomass concentrations in R2 were high and reached the value of 7.6 g/l SS. Augmentation with Mg^{2+} also led to significant increases in the biomass concentrations in R2. On the other hand, the bio-mass concentrations in R1 were low and stabilized at 6.8 g/l SS. With the progress of the experiment, the SVI of the reactors generally kept decreasing, but the SVI of R1 decreased slightly slower than that of R1. Along with reaching the stable MLSS in the reactors, the value of SVI became stable at 20–25 ml/g, indicating that the mature granular sludge had an excellent settling property.

The influent COD of the reactors was kept at 640 mg/l. Initially the COD removal efficiencies of the reactors were low. With the progress of the experiment, the COD removal efficiencies of the reactors generally kept increasing. During the first 45 days, the COD removal efficiencies of R2 slightly higher than that of R1. At the last week, the two reactors achieved almost the same level of COD removal efficiencies. When the experiment was terminated on day 52, the effluent COD in R2 averaged 56 mg/l with a removal efficiency of 91%, while the effluent COD in R1 averaged 57 mg/l with the removal efficiency of 90%. This may suggest that the COD removal efficiencies had no direct correlation with the augmentation of $Mg^{2+.}$

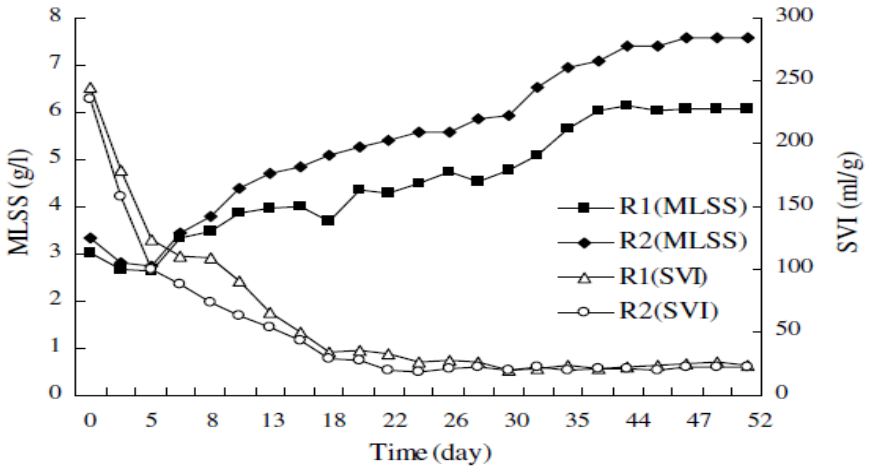

Figure 4.1 Changes in the process of aerobic granulation.

Granule size is a direct parameter to show the growth and aging process in the sludge granulation. The granule size distributions of the reactors were illustrated in Fig. 4.2. In R1, no granules were found until day 17. On day 20, the mean diameters of the two reactors were 0.25 mm and 0.68 mm, respectively. After 30 days approximately 35% of the granules from R1 were in the range of 0.2–1.5 mm and only 5% of the sample was above 1.5 mm. By day 50, approximately 20% of granules in R1 had a diameter over 1.5 mm. The granule size distribution for R2 was significantly different from that for R1 on any given day. For R2, 45% of the samples measured above 0.6 mm on day 30. The size of the granules in R1 and R2 stabilized at 1.8 mm and 2.9 mm finally, respectively. Except for the first few days, the mean sizes of biomass in R2 were consistently larger than in R1. This result implied that the presence of Mg^{2+} had promoted granule formation by allowing aggregates to form earlier and to achieve a larger size.

For the purpose of comparison, the ‘‘time needed to accomplish granulation” in this study is defined as when over 15% of granules were larger than 0.6 mm in the reactor. Accordingly, the granulation was achieved in R2 after 18 days, while granulation was achieved in R1 within 32 days. R2 had a higher biomass concentration and had visible granules earlier compared with R1. The average size

of granules in R2 was larger on any given day. These results clearly indicated that 10 mg/l Mg^{2+} improved the biomass retention and achieved a fast granulation process.

Figure 4.3 illustrated the main components of EPS in the reactors, taken on day 10, 20, 30, 40 and 50. At the beginning of operation, the extracellular protein content determined in the seed sludge was 50.4 mg/g MLSS. On day 10, the value was doubled to 99.7 mg/g MLSS for the sludge sample with the appearance of granules in the reactors. However, when the granules formed and matured, the content of extracellular protein increased slightly. After the complete granulation of sludge in the reactors, the extracellular protein contents in the aerobic granules were increased to 140.3 mg/g MLSS. No differences in extracellular protein contents of the sludge were observed in the reactors.

This may suggested that the extracellular protein content had no direct correlation with the augmentation of Mg^{2+}. The variation of extracellular polysaccharide throughout the operation times is shown in Figure 4.3. It can be seen that during the operation times there was sharp increase of extracellular polysaccharide from 30.7 mg/g MLSS to 71.4 mg/g MLSS in R2, however, the content of extracellular polysaccharide changed slightly in R1. It clearly showed that Mg^{2+} augmentation had no effect on the amount of extracellular proteins, but produced a greater increase in the amount of extracellular polysaccharides.

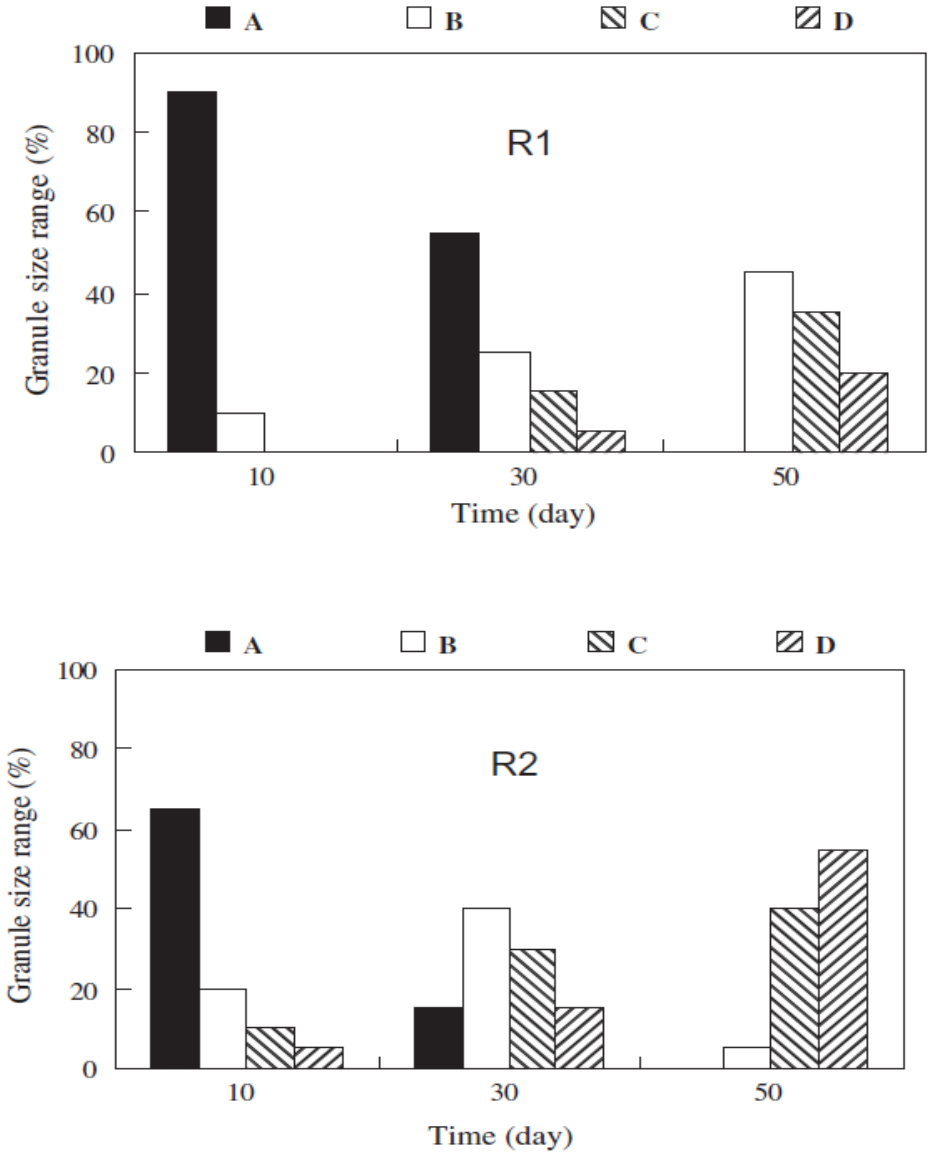

Figure 4.2 Size distributions (by weight) of granules of each reactor A: d < 0.2; B: 0.2 < d < 0.6; C: 0.6 < d < 1.5 (unit in mm)

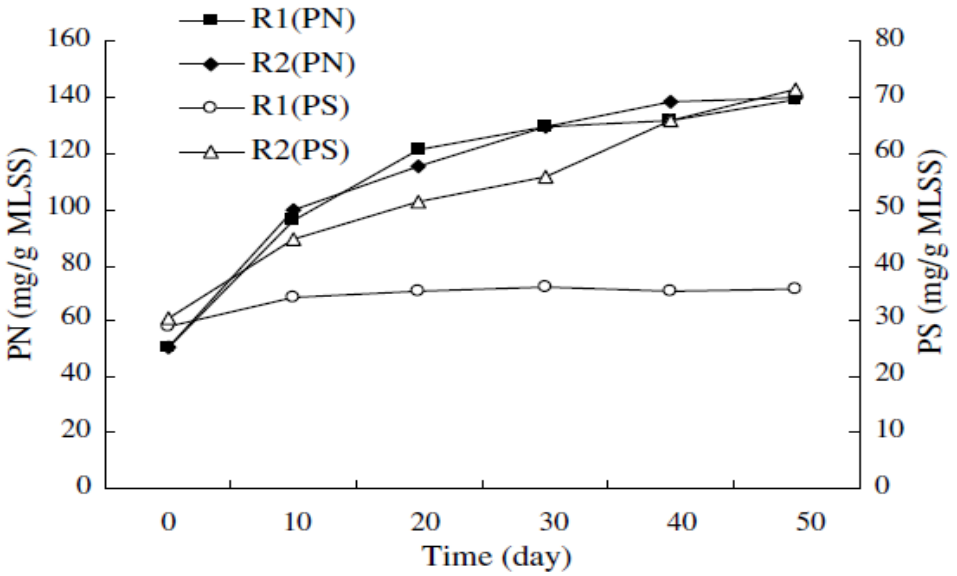

Figure 4.3 Changes of protein (PN) and polysaccharide (PS) in EPS during aerobic granulation.

So far, it had been well known that polysaccharides can mediate both cohesion and adhesion of cells, and play an important role in maintaining the structural integrity of biofilms and anaerobic granules (Fletcher and Floodgate, 1973; Christensen, 1989; Liu et al., 2004). Similar observations of high polysaccharide levels had been reported for other biological systems such as anaerobic granules and aerobic granules, in which the carbon utilization shifts towards polysaccharide production in the presence of excess divalent ions such as Fe^{2+}, Mg^{2+} and Ca^{2+} (Shen et al., 1993; Jiang et al., 2003; Veiga et al., 1997).

Polysaccharides can form a strong and sticky framework and it was likely this helped in the formation and maintenance of a stable granular structure in the aerobic granules (Christensen, 1989; Sutherland, 2001). The secondary functional groups in the polysaccharides, such as OH_3, could also interact with Mg^{2+} to form a rigid, non-deformable polymeric gel-like matrix (Sutherland, 2001; Costerton et al., 1987) and further enhance the structural stability of the aerobic granules.

Comparison between the present study and the study regarding the effect of Ca^{2+} on sludge granulation showed that, Mg^{2+} had a positive effect quite similar to Ca^{2+} (Mahoney et al., 1987; Shen et al., 1993; Jiang et al., 2003). Both ions promoted granule formation by allowing aggregates to form earlier and to achieve a larger size, and resulted in a faster granulation process and a shortened start-up period for SBR. Besides, the addition of the two ions did not lead to a difference in predominant microorganisms. These suggested that both ions enhanced the sludge granulation process through the same physico-chemical functions. Ca^{2+} and Mg^{2+} probably moderate the aerobic granulation process in two ways (Jiang et al., 2003).

Firstly, both ions could be bound to the negatively charged groups presented on bacterial surfaces and extracellular polysaccharides molecules, and acted as a bridge to interconnect these components (Costerton et al., 1987; van Loosdrecht et al., 1987; Bruus et al., 1992) and promote bacterial aggregation. Secondly, granules augmented with the two ions produced higher amounts of polysaccharides without any corresponding increase in protein content.

CHAPTER 5

CONCLUSION

Mg^{2+} augmentation can be beneficial to aerobic granulation. Augmentation with 10 mg/l Mg^{2+} in R2 significantly decreased the sludge granulation time from 32 days to 18 days, at the same time, the mean diameter of the granules in R2 was 2.9 mm after the granulation, which was consistently larger than that (1.8 mm) in R1. Mg^{2+}-fed granules were denser and more compact, showed better settling and had higher polysaccharide contents, but it did not result in a difference in microbial morphology. These findings would be useful for the development of aerobic granule-based systems, where rapid reactor start-up and system stability were key considerations.

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