Wastewater Purification

Aerobic Granulation in Sequencing Batch Reactors
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Edited by Yu Liu
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Preface

Biogranulation is a process of microbial self-immobilization, and it can be divided into two general groups, that is, anaerobic and aerobic granulation. Anaerobic granulation has been studied extensively for decades, whereas the interest in aerobic granulation was started just a few years ago. Aerobic granulation is an environmental biotechnology developed for the purpose of high-efficiency wastewater treatment. The distinguishing characteristics of aerobic granules attribute superiority to this technology in comparison with the conventional activated sludge processes. Thus far, intensive research has been conducted to understand the mechanism of aerobic granulation in sequencing batch reactor (SBR) and its application in treating a wide variety of municipal and industrial wastewater. Obviously, the basic research of aerobic granulation has promoted this technology from laboratory study to the present pilot- and full-scale applications. This book aims to discuss the up-to-date research and application of this environmental biotechnology tailored for enhanced wastewater purification.

First, chapter 1 presents experimental evidence showing that aerobic granulation in SBR is indeed insensitive to the substrate type and its concentration applied, although the carbon source seems to influence the physical properties and microbial diversity of mature aerobic granules. It appears from this chapter that aerobic granulation technology is applicable to the purification of a wide spectrum of wastewater. Hydrodynamic shear force resulting from intensive aeration in SBR plays an essential role in aerobic granulation. Chapter 2 elaborates on how hydrodynamic shear force would influence aerobic granulation, with special focus on shear force-associated changes in microbial activity, cell surface property, and production of extracellular polysaccharides. Hitherto, almost all successful aerobic granulations are achieved in SBR that is featured by its cyclic operation. Chapter 3 further looks into the role of SBR cycle time in aerobic granulation.

Chapter 4 focuses on understanding the role of settling time in aerobic granulation, which is a unique operating parameter of SBR as compared to conventional activated sludge reactors. Settling time is shown as an essential driving force of aerobic granulation. Aerobic granulation would fail if settling time is not properly controlled. Aerobic granulation seems to be an effective defensive or protective strategy of the microbial community against external selection pressure. Chapter 5 identifies the volume exchange ratio and discharge time of SBR as two other possible driving forces of aerobic granulation in SBR. Further, chapter 6 shows that all the major selection pressures identified so far can be unified to an easy concept of the minimal settling velocity that ultimately determines aerobic granulation in SBR. This selection pressure theory offers useful guides for up-scaling, manipulating, and optimizing aerobic granular sludge SBR.

Aerobic granulation is a gradual process that can be quantitatively described as change in granule size in the course of SBR operation. In this regard, some kinetic
models have been developed and presented in chapter 7. Because of the large size of the aerobic granule, mass diffusion limitations exist in the aerobic granule. Chapter 8 looks into the diffusion behaviors of substrate and dissolved oxygen in aerobic granules and presents a comprehensive modeling system, which describes the dynamic diffusion of substrate and oxygen in various-sized aerobic granules. This model system can provide an effective and useful tool for predicting and optimizing the performance of aerobic granular sludge SBR.

It is believed that cell-to-cell self-aggregation initiates aerobic granulation. Cell surface hydrophobicity serves as an essential affinity force that initiates the first contact of cell to cell. Existing evidence shows that a number of culture conditions can induce cell surface hydrophobicity. Chapter 9 discusses the factors known to influence cell surface hydrophobicity. Furthermore, a thermodynamic interpretation of the role of cell surface hydrophobicity in aerobic granulation is also given. The enrichment culture of highly hydrophobic bacteria thus appears to greatly facilitate aerobic granulation. Chapter 10 discusses the essential roles of extracellular polysaccharides in the formation and maintenance of structural stability of aerobic granules. It appears that both the quantity and the quality of extracellular polysaccharides determine the matrix structure and integrity of aerobic granules.

Chapter 11 reveals that the internal structure of the aerobic granule experiences a shift from homogenous to heterogeneous as the aerobic granule grows to a big size due to mass diffusion limitation. Uneven distributions of granule biomass, extracellular polysaccharides, and cell surface hydrophobicity are also discussed in chapter 11. Chapter 12 mainly focuses on biodegradability of extracellular polysaccharides produced by aerobic granules. Only nonbiodegradable extracellular polysaccharides can play a crucial protective role in the granule integrity stability, while biodegradable extracellular polysaccharides accumulated at the central part of the aerobic granule can serve as an additional energy reservoir when an external carbon source is no longer available for microbial growth. Chapter 13 provides a plausible explanation for the observed high calcium accumulation in acetate-fed aerobic granules from both experimental and theoretical aspects. It is shown that the calcium ion may not be an essential element required for successful aerobic granulation.

Unlike the continuous activated sludge process, a substrate periodic starvation exists in aerobic granular sludge SBR due to its cyclic operation. Chapter 14 discusses different, even controversial, views with regard to the role of such a periodic starvation in aerobic granulation. As filamentous growth has been frequently observed in aerobic granules, chapter 15 looks into causes and control of filamentous growth in aerobic granular sludge SBRs. In view of its industrial application, long-term stability of aerobic granular sludge SBR remains a main concern. For this purpose, chapter 16 sheds light on the possible operation strategy that can help improve the stability of aerobic granules, including the selection of slow-growing bacteria and control of granule age. After nearly ten years of laboratory research, aerobic granulation technology has achieved pilot- and full-scale applications. Chapter 17 shows that successful aerobic granulation can be achieved in pilot-scale SBR using fresh or stored aerobic granules as seeds.
This book presents readers all aspects of aerobic granulation in SBR. The successful test of this technology in pilot-scale study foresees its promising application in practical wastewater treatment. I sincerely hope that the publication of this book will provide a platform for the further development of this technology and promote its quick application in the wastewater treatment industry.

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1 Aerobic Granulation at Different Carbon Sources and Concentrations

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1.1 INTRODUCTION
Granulation is a process in which microorganisms aggregate to form a spherical, dense biomass. Granules have been grown successfully in either anaerobic or aerobic...
environments (Lettinga et al. 1984; Morgenroth et al. 1997; Beun et al. 1999; J. H. Tay, Liu, and Liu 2001; Su and Yu 2005). The characteristics of the substrate have been considered to influence the formation and structure of anaerobic granules (Wu 1991; Chen and Lun 1993). Filamentous anaerobic granules developed on volatile fatty acids (VFAs) tend to be mechanically fragile and larger in size, whereas more robust, rod-type anaerobic granules were grown on sugar beet or potato processing wastewater (Adebowale and Kiff 1988). However, the formation of aerobic granules seems to be independent of the characteristics of the organic substrate (J. H. Tay, Liu, and Liu 2001).

Another important parameter that affects the anaerobic granulation process and the characteristics of anaerobic granules is the substrate concentration (Hulshoff Pol, Heijnekamp, and Lettinga 1988; Campos and Anderson 1992). An appropriate substrate concentration is critical to the microbial granulation in anaerobic systems. Morvai et al. (1990) found that anaerobic granulation developed well in upflow anaerobic sludge blanket (UASB) reactors fed with influent chemical oxygen demand (COD) concentrations of 1000 to 3000 mg L\(^{-1}\), but not in a reactor with influent concentration of 500 mg L\(^{-1}\). The substrate concentration also has direct impact on the biofilm structure where high surface loading rate leads to the increase of the average biofilm thickness (van Loosdrecht et al. 1995; Tijhuis et al. 1996; Kwok et al. 1998). This chapter discusses the effect of substrate carbon source and its concentration on the formation and characteristics of aerobic granules.

1.2 AEROBIC GRANULATION WITH ACETATE AND GLUCOSE

1.2.1 MICROSCOPIC OBSERVATION OF AEROBIC GRANULATION

J. H. Tay, Liu, and Liu (2001) investigated the evolution process of aerobic granulation in two sequencing batch reactors (SBRs) that were fed with glucose and acetate, respectively, and monitored by means of optical microscope, image analysis (IA) technique, and scanning electronic microscope (SEM), and found that aerobic granulation is a gradual process from seed sludge to aggregates and finally to compact mature granules.

1.2.1.1 Seed Sludge

Microscopic examination of seed sludge taken from a sewage treatment plant showed a typical morphology of conventional activated sludge, in which filaments were observed (figure 1.1). A SEM micrograph further revealed that seed sludge had a very loose and irregular three-dimensional structure (figure 1.1C). The average floc size of the seed sludge was about 70 \(\mu\)m, with a sludge volume index (SVI) value of 280 mL g\(^{-1}\), which suggests filamentous bacteria were predominant in the seed sludge due to its high SVI value (Crites and Tchobanoglous 1998).

1.2.1.2 Formation of Compact Aggregates after Operation for One Week

One week after the reactor startup, filamentous bacteria gradually disappeared in the acetate-fed SBR, but still prevailed in the glucose-fed SBR. Figure 1.2A shows the
morphologies of 1-week-old sludge in the glucose-fed SBR observed by imagine analysis. The compact and dense sludge aggregates can be seen and at this stage, the sludge aggregates exhibited much more compact and denser structure than the seed sludge.

1.2.1.3 Formation of Granular Sludge after Operation for Two Weeks

Figure 1.2B shows the sludge morphology after operation for 2 weeks. It is clear that granular sludge with a clear round outer shape was formed. Filamentous bacteria were still predominant in the reactor fed with glucose, while filaments completely disappeared in the reactor fed with acetate after operation for 2 weeks. It is known that a high-carbohydrate substrate composed of glucose or maltose supports the growth of filamentous bacteria (Chudoba 1985). This might be the reason for the filamentous-dominant situation in the glucose-fed sludge. As can be seen in figures 1.2A and 1.2B, the major differences between microbial aggregates and granular sludge can be attributed to their sizes, compactness, and outer shapes. It should be realized that the evolution of sludge in both the glucose- and acetate-fed SBRs indeed followed a similar evolution pattern in the course of operation. These indicate that the carbon source has an insignificant influence on the formation of aerobic granules in SBR.
Mature aerobic granules were obtained after 3 weeks of operation (figure 1.2C). Aerobic granules had an average roundness of 0.79 in terms of the aspect ratio, defined as the ratio between the minor axis and the major axis of the ellipse equivalent to the granule. Mature granules had a much more regular, homogeneous and clearer outer morphology than the granular sludge observed after operation for 2 weeks. Figure 1.2 clearly exhibits the visual evolution track of the aerobic granulation process. The SEM micrograph further shows the detailed microstructures of glucose- and acetate-fed aerobic granules (figure 1.3). Glucose-fed granules had a filaments-dominant outer surface, whereas the acetate-fed granules showed a very compact bacterial structure, in which rod bacteria, tightly linked cell to cell, were found to be predominant. Such a tight cellular structure was not found in the seed sludge.
It can be seen from figures 1.1 and 1.2 that the formation of aerobic granules is a gradual process from seed sludge to dense aggregates, then to granular sludge, and finally to mature granules. Microscopic observations clearly revealed that microbial structure could be significantly strengthened, and further shaped, that is, they became more and more regular and dense, as the granulation process proceeded. In fact, the sludge-settling property could be improved significantly after granulation. Seed sludge for the reactor startup had a SVI value of 280 mL g\(^{-1}\) with many filamentous bacteria present (figure 1.1). However, an average SVI of 50 to 85 mL g\(^{-1}\) was achieved for granules formed from both substrates, which is almost three times higher than the original seed sludge. It is clear that granulation leads to a significant improvement in the sludge settleability. The granulation process could take 1 to 2 weeks or even a few more weeks depending on the substrate and the condition of operation. The process will normally take longer for slow-growing bacteria, for example nitrifying bacteria, and for toxic wastewater (Tsuneda et al. 2003; S. T. L. Tay, Zhuang, and Tay 2005; Yi et al. 2006). Aerobic granules can form with different carbon sources. It seems that the formation of aerobic granules is a process independent of or insensitive to the characteristics of the substrate (J. H. Tay, Liu, and Liu 2001). However, the substrate component has a profound impact on the microbial structure and the diversity of mature granules, as discussed above. In fact, the microstructure of anaerobic granules formed in UASB reactors is also strongly associated with the substrates (Wu 1991).

### 1.2.2 Characteristics of Glucose- and Acetate-Fed Aerobic Granules

The physical characteristics of aerobic granules were more compact compared with the sludge flocs, while the microbial activity was comparable or somewhat lower compared with sludge flocs, depending on the size and structure of the granules. The characteristics of granules cultivated from glucose and acetate substrate are compared in the following section.

#### 1.2.2.1 Morphology

The photographs by image analysis exhibited that mature granules formed from both glucose and acetate substrates had a regular round-shaped structure with an average roundness of 0.79 in terms of aspect ratio for glucose-fed granules, and 0.73 for acetate-fed granules (table 1.1). The glucose-fed granules had a mean diameter of 2.4 mm, whereas the granules grown on acetate had a mean diameter of 1.1 mm. The glucose-fed granules had filamentous bacteria extruding out from the surface (figure 1.3C and D). However, the acetate-fed granules had a smooth surface with a very compact bacterial structure and few filaments were observed (figure 1.3A and B).

#### 1.2.2.2 Sludge Settleability

The sludge-settling property is a key operation factor that determines the efficiency of solid–liquid separation, which is essential for the proper functioning of a wastewater treatment system. The settleability of aerobic granules was much better than the sludge flocs of a conventional activated sludge process. The sludge volume
The sludge volume index (SVI) of the mature granules was 51 to 85 mL g⁻¹ for glucose-fed granules and 50 to 80 mL g⁻¹ for acetate-fed granules (Table 1.1). The low SVI values indicated the high compactness of the granules. Compared with the seed sludge of SVI 280 mL g⁻¹, it is obvious that the settleability of sludge had improved significantly for aerobic granules. The average settling velocity of glucose-fed granules was 35 m h⁻¹, and 30 m h⁻¹ for acetate-fed granules. Such settling velocities of aerobic granules are

<table>
<thead>
<tr>
<th>Items</th>
<th>Glucose-Fed Granules</th>
<th>Acetate-Fed Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average diameter (mm)</td>
<td>2.4 (± 0.71)</td>
<td>1.1 (± 0.43)</td>
</tr>
<tr>
<td>Aspect ratio</td>
<td>0.79 (± 0.06)</td>
<td>0.73 (± 0.04)</td>
</tr>
<tr>
<td>Sludge volume index (mL g⁻¹)</td>
<td>51–85</td>
<td>50–80</td>
</tr>
<tr>
<td>Settling velocity (m h⁻¹)</td>
<td>35 (± 8.5)</td>
<td>30 (± 7.1)</td>
</tr>
<tr>
<td>Granule strength (%)</td>
<td>98 (± 0.9)</td>
<td>97 (± 1.2)</td>
</tr>
<tr>
<td>Biomass density (g L⁻¹)</td>
<td>41.1 (± 6.9)</td>
<td>32.2 (± 9.1)</td>
</tr>
<tr>
<td>Hydrophobicity (%)</td>
<td>68 (± 3.9)</td>
<td>73 (± 5.3)</td>
</tr>
<tr>
<td>Specific oxygen uptake rate (mg O₂ g⁻¹ h⁻¹)</td>
<td>69.4 (± 8.8)</td>
<td>55.9 (± 7.1)</td>
</tr>
</tbody>
</table>

**FIGURE 1.3** Scanning electron micrographs of aerobic granule cultivated from acetate substrate (A) and its surface microstructure (B), and granule cultivated from glucose substrate (C) and its surface microstructure (D). (From Liu, Q. S. 2003. Ph.D. thesis, Nanyang Technological University, Singapore. With permission.)
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comparable with that of anaerobic granules cultivated in UASB (Hulshoff Pol et al. 1986; Beeftink 1987), and at least three times higher than those of activated sludge flocs having a settling velocity of less than 10 m h\(^{-1}\). In fact, high settling velocity of 72 m h\(^{-1}\) was also reported for aerobic granules (Etterer and Wilderer 2001). It can be understood that the settling velocity will be influenced by the size and compactness of the aerobic granules.

1.2.2.3 Granule Physical Strength and Biomass Density

The physical strength of aerobic granules, expressed as the integrity coefficient (%), which is defined as the ratio of residual granules to the total weight of the granular sludge after 5 min of shaking at 200 rpm on a platform shaker (Ghangrekar et al. 1996), was 98% for glucose-fed granules and 97% for acetate-fed granules. The higher the integrity coefficient, the higher is the physical strength of granules. A high integrity coefficient represents the granule’s ability to withstand high abrasion and shear. Aerobic granules cultivated in both substrates had a high strength. Meanwhile, the mature granules had a dry biomass density of 41.1 g L\(^{-1}\) for glucose-fed granules, as determined by the method of Beun et al. (1999), while it was 32.2 g L\(^{-1}\) for acetate-fed granules. The higher biomass density of aerobic granules reflects a denser microbial structure. The better settling ability of aerobic granules is consistent with higher biomass density, which is the result of a denser microbial structure.

1.2.2.4 Cell Surface Hydrophobicity

The seed sludge flocs had a cell surface hydrophobicity of 39% measured by the hydrocarbon partitioning method of Rosenberg, Gutnick, and Rosenberg (1980). After the formation of aerobic granules, the respective hydrophobicity of the cell surface increased to 68% for glucose-fed granules and 73% for acetate-fed granules. The hydrophobicity of aerobic granules was nearly twice higher than that of the seed sludge. High cell surface hydrophobicity favors cell attachment and then the aggregation of the sludge. Cell surface hydrophobicity is considered an important affinity force in cell attachment and self-immobilization (Del Re et al. 2000; Y. Liu et al. 2003).

1.2.2.5 Microbial Activity

The glucose-fed granules had a microbial activity expressed by specific oxygen uptake rate (SOUR) at 69.4 mg O\(_2\) g\(^{-1}\) MLVSS h\(^{-1}\), and 55.9 mg O\(_2\) g\(^{-1}\) MLVSS h\(^{-1}\) for acetate-fed granules. The microbial activity of the granules would be strongly associated with the granule size and structure, which influence the oxygen and substrate transfer. The most beneficial aspect of aerobic granules is their excellent physical characteristics, which could lead to a high biomass concentration in the reactor, and subsequently smaller footprint for the reactor system.

1.2.2.6 Storage Stability of Aerobic Granules

aerobic granules cultivated from both glucose and acetate substrates had little reduction in microbial activity after 10 days of storage at 4°C in a refrigerator (figure 1.4). The SOUR then gradually decreased for both types of granules, from the initial value of 57 mg O₂ g⁻¹ biomass h⁻¹ to 6 mg O₂ g⁻¹ biomass h⁻¹ for acetate-fed granules at day 77, while the SOUR only decreased to 24 mg O₂ g⁻¹ biomass h⁻¹ for glucose-fed granules after 137 days, that is, a 60% reduction after more than 4 months of storage. Glucose-fed granules could be stored longer than those acetate-fed granules with less SOUR reduction. The loss in microbial activity would be associated with the length of storage time, the type of feed carbon, and the culture history. Both types of granules were visually in good granular shape after being stored for 4 months, and no disintegration was observed. Compared with the fresh granules, the strength of the stored granules decreased from 98% to 91% for glucose-fed granules, and from 97% to 89% for acetate-fed granules. Therefore, aerobic granules could still maintain good physical strength. Similar to the anaerobic granules, stored aerobic granules could be used as the seeding material for reactor startup because of its long storage ability. In fact, Zhu and Wilderer (2003) found that after 7 weeks of storage

![Figure 1.4](image-url)
of aerobic granules in ambient environment, these aerobic granules could regain their microbial activity in less than a week.

1.3 AEROBIC GRANULATION ON OTHER CARBON SOURCES

Aerobic granules can be formed with different organic carbon sources of acetate and glucose as discussed above, while nitrifying granules can be formed with various N/COD ratios, as discussed above. In fact, aerobic granulation has been demonstrated in a variety of substrates, including organic/inorganic carbon, toxic wastewater, real municipal and industrial wastewater, for example, sucrose (Zheng et al. 2006), ethanol (Beun et al. 1999), phenol (Jiang, Tay, and Tay 2002; J. H. Tay, Jiang, and Tay 2004; Jiang et al. 2006), pentachlorophenol (Lan et al. 2005), and tert-butyl alcohol (S. T. L. Tay, Zhuang, and Tay 2005; Zhuang et al. 2005); particulate organic matter-rich wastewater (Schwarzenbeck, Borges, and Wilderer 2004), domestic sewage (de Kreuk and van Loosdrecht 2006), and industrial wastewater (Arrojo et al. 2004; Inizan et al. 2005; Schwarzenbeck, Erley, and Wilderer 2005; Su and Yu 2005; Wang et al. 2007). Phosphorus-accumulating granules have also been developed in the SBR (Y. Liu, Lin, and Tay 2005).

It was reported that nitrifying aerobic granules cultivated possess excellent nitrification ability (J. H. Tay, Yang, and Liu 2002), while aerobic granules grown on phenol can enhance the ability of bacteria to tolerate the toxic effect of phenol (Jiang, Tay, and Tay 2002). It is believed that cell immobilization is a useful strategy for bacteria to overcome the substrate inhibition associated with high-strength phenolic wastewater. It was shown that the kinetic behaviors of the phenol-degrading granules are subject to the Haldane model, indicating that the phenol-degrading aerobic granules could counteract the adverse effects of phenol inhibition (Jiang, Tay, and Tay 2002). The aggregation of microbial cells into compact granules may serve as an effective protection against the high phenol concentration. Aerobic granules would be powerful bioagents for the removal of inhibitory or toxic organic compounds present in industrial wastewater.

A novel strategy to add the benign co-substrate to toxic substrate to accelerate the granulation process and the performance of the granular sludge was proposed (Yi et al. 2006). It was found that with the addition of glucose to a toxic substrate,  \( p \)-nitrophenol (PNP), the PNP metabolic activity could be enhanced through the formation of granules. The improvement of metabolic activity is most likely due to the retention of specific PNP-degrading microorganisms in the granules. This could be the result of syntrophic interactions between the community members in the granules, while the metabolic enhancement could result from the increase of specific degradation activity through the exchange of genetic material among the bacteria in granules. Use of a co-substrate strategy in the granulation process could improve the biodegradation of toxic and recalcitrant organic compounds. This shows another beneficial aspect of applying aerobic granulation technology in wastewater treatment.

1.4 AEROBIC GRANULATION AT DIFFERENT COD CONCENTRATIONS

studied the effect of substrate concentration on the formation, structure, and characteristics of aerobic granules and found that granules can be successfully formed with COD concentrations from 500 to 3000 mg L\(^{-1}\), corresponding to an organic loading rate of 1.5 to 9.0 kg COD m\(^{-3}\) d\(^{-1}\).

The formation of aerobic granules at different COD concentrations was a gradual process from seed sludge to the mature granules, with the same process as discussed earlier. It was found that a fast increase in sludge particle size was observed in the reactors supplied with high COD concentrations, for example, the size increased to 0.7 mm at day 10 of the operation in the reactor fed with influent COD of 500 mg L\(^{-1}\), while it was 1.0, 1.1, and 1.4 mm for reactors fed with influent COD of 1000, 2000, and 3000 mg L\(^{-1}\), respectively. It appeared that high substrate concentration favored a fast increase in granule size. This would be due to the fact that a high substrate concentration can sustain fast microbial growth.

The sludge particle size and SVI variation with the operation time in the reactor supplied with the highest substrate COD concentration of 3000 mg L\(^{-1}\) is shown in figure 1.5. It can be seen that the size of sludge particles gradually increased from 0.09 mm to a stable value of 1.9 mm after 20 days of operation. With an increase in size, the sludge SVI decreased from 208 mL g\(^{-1}\) to about 35 mL g\(^{-1}\) accordingly. These results clearly indicated that the settleability of aerobic granules was much better than that of seeding sludge bioflocs. Figure 1.6 compares the size distributions of seed sludge and the granular sludge cultivated, indicating that over 90% of the seed sludge particles had a size less than 0.2 mm, while more than 98% of the granular sludge fell in the size range of 0.4 mm to 3.2 mm. It is clear that the formation of aerobic granules seems to be independent of substrate concentration in the range from 500 to 3000 mg COD L\(^{-1}\) as discussed above. In fact, other researchers also suggest that aerobic granules can be formed in a wide COD range (Moy et al. 2002; J. H. Tay et al. 2004). This is probably due to the nature of aerobic bacteria. Compared to the biofilm process, aerobic granulation is a phenomenon of cell-to-cell self-immobilization instead of cell attachment to a solid surface. Similar to biofilm, it seems that substrate concentration is not a governing factor for the formation of aerobic granules.

1.4.1 Effect of COD Concentration on the Properties of Aerobic Granules

A comparison of the average size of aerobic granules formed at different substrate concentrations found that the granule size slightly increased with the increase of substrate concentration (figure 1.7). The granule size was 1.57 mm at 500 mg COD L\(^{-1}\), while it increased to 1.79 mm at 1000 and 2000 mg COD L\(^{-1}\), and further increased to 1.89 mm at 3000 mg COD L\(^{-1}\). Moy et al (2002) also found the size of aerobic granules increased when the loading rate was increased. A similar phenomenon was observed in anaerobic granulation (Grotenhuis et al. 1991). This can be easily understood because high substrate concentration would lead to the fast biomass production, and finally to a large size. It can be expected that the granules either of aerobic or anaerobic would have similar growth pattern in relation to the substrate concentration.
The relationship between substrate concentration and granule morphology expressed by the roundness and aspect ratio found that granules become more irregular under high substrate concentration, as shown in figure 1.8. The average roundness of granules was 0.69 at substrate concentration of 500 mg COD L\(^{-1}\), while it was 0.66, 0.67, and 0.64 at substrate concentrations of 1000, 2000, and 3000 mg COD L\(^{-1}\). The irregularity of the granule surface at high substrate concentration could be due to the fast growth rate. In fact, a heterogeneous and porous biofilm structure with extrusion was observed under high loading rate condition (van Loosdrecht et al. 1995; Kwok et al. 1998).

As shown in figure 1.9, the granule strength decreased with the increase of substrate concentration. The granule strength, expressed as integrity coefficient, was 97% at 500 and 1000 mg COD L\(^{-1}\), but dropped to 95% at 2000 mg COD L\(^{-1}\), and
**FIGURE 1.6** Comparison of size distribution by volume between seed sludge (A) and granular sludge (B) cultivated in a reactor fed with influent COD of 3000 mg L\(^{-1}\). (From Liu, Q. S., Tay, J. H., and Liu, Y. 2003. *Environ Technol* 24: 1235–1242. With permission.)

further to 87% at 3000 mg COD L\(^{-1}\). The granules formed at the highest substrate concentration have a very loose structure. The lower strength of aerobic granules at high substrate concentrations could also possibly be due to the high biomass production rate. This has been reported in anaerobic granules where a high substrate concentration/loading rate resulted in a reduced strength of anaerobic granules, that is, the granules would easily lose their structural integrity, and disintegration would occur (Quarmby and Forster 1995). Morvai, Mihaltz, and Czake (1992) also found that increased loading rate raises the biomass growth rate, and high growth rate of anaerobic microorganisms would reduce the strength of the three-dimensional structure of a microbial community.

The specific gravity and density of sludge reflect the compactness of a microbial community. The specific gravity of granular sludge formed in various substrate COD


![Figure 1.8](image)

FIGURE 1.9 Effect of substrate concentration on the granule polysaccharides contents (white bar) and granule strength (dark bar) (From Liu, Q. S. 2003. Ph.D. thesis, Nanyang Technological University, Singapore. With permission.)

![Figure 1.9](image)
TABLE 1.2
Characteristics of Aerobic Granules Cultivated at Different Substrate Concentrations

<table>
<thead>
<tr>
<th>Parameters</th>
<th></th>
<th>Influent Substrate COD Concentration (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed Sludge</td>
<td>500</td>
</tr>
<tr>
<td>Size (mm)</td>
<td>0.09</td>
<td>1.57 (± 0.14)</td>
</tr>
<tr>
<td>SVI (mL g⁻¹)</td>
<td>208</td>
<td>41 (± 4.6)</td>
</tr>
<tr>
<td>Biomass density (g L⁻¹)</td>
<td>~</td>
<td>54.3 (± 6.3)</td>
</tr>
<tr>
<td>Specific gravity of sludge (kg L⁻¹)</td>
<td>1.001</td>
<td>1.01 (± 0.001)</td>
</tr>
<tr>
<td>Biomass concentration in reactor (g L⁻¹)</td>
<td>~</td>
<td>8.4 (± 1.0)</td>
</tr>
<tr>
<td>Cell surface hydrophobicity (%)</td>
<td>49.4</td>
<td>81.1 (± 4.1)</td>
</tr>
<tr>
<td>Effluent COD concentration (mg L⁻¹)</td>
<td>~</td>
<td>27 (± 9.4)</td>
</tr>
<tr>
<td>COD removal efficiency (%)</td>
<td>~</td>
<td>95 (± 1.7)</td>
</tr>
</tbody>
</table>

Concentrations of 500 to 3000 mg L⁻¹ was around 1.010 kg L⁻¹, which is a significant increase compared with the seed sludge flocs of 1.001 kg L⁻¹ (table 1.2). The SVI of 30 and 40 mL g⁻¹ for granular sludge also improved significantly as compared with that of seed sludge of 208 mL g⁻¹. The granule density was around 55 g L⁻¹ at different substrate concentrations. However, it seems that substrate concentration has an insignificant effect on the settleability of the granules.

The effect of substrate concentration on cell polysaccharides contents of aerobic granules is shown in figure 1.9. The cell polysaccharides content of aerobic granules formed at 500 mg COD L⁻¹ was 93.6 mg g⁻¹ VSS, while it was 91.6, 84.7, and 85.2 mg g⁻¹ VSS at 1000, 2000, and 3000 mg COD L⁻¹, respectively. There is a slight decrease in polysaccharide content with the increase of substrate concentration. However, compared with the cell polysaccharide content of seed sludge of 60.9 mg g⁻¹ VSS, a very significant increase was observed between the seed sludge and granular sludge. It should be pointed out that the lower physical strength of aerobic granules observed at high COD concentration could be reasonably attributed to its lower polysaccharides content. It has been generally agreed that cell polysaccharides are important in maintaining the structural integrity of a cell-immobilized community. It has also been reported that the production of cell polysaccharides was closely associated with hydrodynamic shear force, as discussed in chapter 2. It is not surprising that there is no significant change in cell polysaccharide content with varied substrate concentration because of its similar shear condition in the reactors.

Cell surface hydrophobicity of granular sludge seems to have no relationship to the substrate concentration, as listed in table 1.2. The cell surface hydrophobicity of aerobic granules was 81% at 500 mg COD L⁻¹, and 84%, 78%, and 79% at 1000, 2000, and 3000 mg COD L⁻¹, respectively. However, cell surface hydrophobicity
of aerobic granules was much higher than that of seed sludge. Cell surface hydrophobicity has been generally considered to be an important affinity force in the self-immobilization and attachment of cells, for example biofilm and anaerobic granules (Del Re et al. 2000; J. H. Tay, Xu, and Teo 2000). The role of cell surface hydrophobicity in aerobic granulation will be discussed in detail in chapter 9. It can be considered that hydrophobicity might be the force for the initiation of aerobic sludge granulation. However, the substrate concentration did not seem to affect the cell surface hydrophobicity.

1.4.2 Effect of COD Concentration on the Reactor Performance

The reactor performance, in terms of the effluent COD concentration, exhibited an increased trend with the substrate concentration. An average effluent COD of 27 mg L\(^{-1}\) was obtained in the reactor fed with 500 mg L\(^{-1}\) influent COD, while it was 48, 68, and 156 mg L\(^{-1}\) for reactors fed with 1000, 2000, and 3000 mg L\(^{-1}\), respectively. However, COD removal efficiency showed a comparable level in all reactors, which was above 95%. It should be mentioned that a high biomass concentration of 8.4 to 12.3 g L\(^{-1}\) was achieved among different reactors at steady state after granulation. Aerobic granulation could lead to more biomass being retained in the reactor due to good settling property of granular sludge. The high biomass concentration in reactors favored the performance and stability of biological reactors.

1.5 Aerobic Granulation at Different Substrate N/COD Ratios

Yang, Tay, and Liu (2005) investigated the effect of substrate N/COD ratio on the formation and physical characteristics of aerobic granules, and found that aerobic granules can be formed successfully in a relatively wide range of N/COD ratios, from 5/100 to 30/100. Figure 1.10 further illustrates the size change of microbial aggregates in the course of SBR operation. It can be seen that the mean size of microbial aggregates cultivated at different substrate N/COD ratios gradually increased and stabilized over the culture time, while small aerobic granules were obtained at the high substrate N/COD ratio. Figure 1.10 clearly shows that aerobic granular sludge reactors can be started up within about four weeks. Figure 1.11 further displays a correlation of SVI of mature aerobic granules with substrate N/COD ratio, indicating that SVI tended to decrease with the increase of the substrate N/COD ratio and the lowest SVI of 51 mL g\(^{-1}\) was found at the highest substrate N/COD ratio of 30/100. It is reasonable to consider that the substrate N/COD ratio would have a significant effect on the structure of microbial granules, that is, a more compact microbial structure could be expected at a higher substrate N/COD ratio.

It seems certain that the use of aerobic granules for upgrading the existing wastewater treatment plants towards simultaneous organics removal and nitrification would be feasible and beneficial, meanwhile such granules can also be used to bioaugment municipal wastewater treatment plant in which washout of nitrifying biomass is encountered.
1.5.1 Effect of N/COD Ratio on the Properties of Aerobic Granules

The changes of SVI with the time of SBR operation at different N/COD ratios are shown in figure 1.12. It clearly indicates that after the formation of aerobic granules on day 20 onwards, the SVI was found to be as low as 50 mL g⁻¹. Moreover, it appears from figures 1.10 and 1.12 that smaller and denser aerobic granules would be formed at higher substrate N/COD ratios. This seems to imply that one may expect to manipulate the size of aerobic granules by controlling substrate N/COD ratio. Figure 1.13 reveals that the specific gravity of aerobic granules seems to be positively related to the applied substrate N/COD ratio, that is, aerobic granules developed at higher substrate N/COD ratios would have a much more compact and denser structure. In fact, this observation is consistent with the SVI trend as shown in figure 1.11.

**FIGURE 1.10** Change in aggregate size versus time in reactors operated at different substrate N/COD ratios. (From Yang, S. F., Tay, J. H., and Liu, Y. 2005. *J Environ Eng* 131: 86–92.)

**FIGURE 1.11** Sludge volume index of aerobic granules developed at different substrate N/COD ratios. (From Yang, S. F. 2005. Ph.D. thesis, Nanyang Technological University, Singapore. With permission.)
Y. Liu et al. (2005) proposed the following equation to describe the settling velocity of bioparticles:

\[ V_s = \alpha \frac{d_p^2}{SVI} e^{-\beta X} \]  \hspace{1cm} (1.1)

where \( V_s \) is the settling velocity of bioparticles, \( d_p \) is the diameter of the particle, SVI stands for sludge volume index, \( X \) is biomass concentration, \( \alpha \) and \( \beta \) are two constant coefficients. It is obvious that the settling velocity of aerobic granules would be determined by the size of the granule, SVI, and biomass concentration of granules. A smaller SVI would lead to a higher settling velocity of bioparticles or an improved settleability.

Yang, Tay, and Liu (2005) found that cell surface hydrophobicity at steady state was positively correlated to the applied substrate N/COD ratio (figure 1.14). In fact,
as revealed in figure 1.15, the activities of both ammonia oxidizer and nitrite oxidizer were proportionally related to the substrate N/COD ratio, that is, when the substrate N/COD ratio was increased, the nitrifying population would be enriched in the aerobic granules. Previous research reported that nitrifying bacteria would have a higher hydrophobic interaction than that of activated sludge (Sousa et al. 1997; Kim, Stabnikova, and Ivanov 2000). Thus, the enriched nitrifying population in aerobic granules cultivated at high substrate N/COD ratio would be responsible for the high cell surface hydrophobicity observed in figure 1.14.

Figure 1.16 exhibits the direct effect of substrate N/COD ratio on the ratio of sludge polysaccharides to sludge protein (PS/PN). It was found that the PS/PN ratio increased significantly along with the formation of aerobic granules. These results imply that extracellular polysaccharides could contribute, at least partially, to microbial aggregation. Meanwhile, increased substrate N/COD ratio would result in a low PS/PN ratio. In fact, other studies found a similar phenomenon, that a reduced substrate N/COD ratio could stimulate the production of extracellular polysaccharides, leading to an improved bacterial attachment to solid surfaces (Schmidt and Ahring 1996; Durmaz and Sanin 2001). There is evidence that cell carbohydrate content increased and protein content decreased in a very significant way as the substrate N/COD ratio decreased (Durmaz and Sanin 2001). It can be reasonably considered that nitrifying bacteria would produce much less extracellular polysaccharides than heterotrophic bacteria. For example, Tsuneda et al. (2001) used extracellular polysaccharides produced by heterotrophic bacteria to enhance the formation of nitrifying biofilm.

### 1.5.2 Effect of N/COD Ratio on Population Distribution

The activity of ammonium oxidizers and nitrite oxidizers as measured by specific ammonium oxygen utilization rate ($SOUR_{NH4}^+$) and specific nitrite oxygen utilization...
rate (SOUR)_{NO_2} and specific heterotrophic oxygen utilization rate (SOUR)$_h$ showed that higher (SOUR)$_{NH_4}$ and (SOUR)$_{NO_2}$ were obtained at the higher substrate N/COD ratios, while the (SOUR)$_h$ exhibited a decreasing trend with the increase in the substrate N/COD ratio (figure 1.16). It indicates that the nitrifying population in aerobic granules was greatly sustained at the high substrate N/COD ratios. In fact, a similar finding was also reported in biofilm reactors (Moreau et al. 1994; Ochoa et al. 2002). Evidence shows that bacteria can sense and move towards nutrients (Prescott,
Harley, and Klein 1999). As shown in figure 1.15, the activity of nitrifying population over heterotrophic population in the aerobic granules grown at the substrate N/COD ratio of 5/100 was very low as compared to the granules developed at high substrate N/COD ratios. At the high substrate N/COD ratio, competition between nitrifying and heterotrophic populations on nutrients would be significant.

It is clear that aerobic granules can be developed at a relatively wide range of substrate N/COD ratios. The substrate N/COD ratios had an insignificant effect on the formation of aerobic granules, but would determine microbial and physicochemical characteristics of aerobic granules.

1.6 CONCLUSIONS

Aerobic granulation is a gradual process from seed sludge to compact aggregates, further to granular sludge, and finally to mature granules. The formation of aerobic granules is insensitive to the substrate carbon source because granules can be formed with organic or inorganic carbon source, or toxic wastewater. However, substrate characteristics had a profound impact on the microbial structure and the diversity of aerobic granules. Aerobic granules can be formed in a relatively wide range of substrate concentrations and substrate N/COD ratios. This would make aerobic granular sludge technology applicable to low-strength wastewater, such as sewage, and to high-strength wastewater treatment, such as industrial waste.


**Inoculation of microbial granular sludge under aerobic conditions.** Ph.D. thesis, Nanyang Technological University, Singapore.


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