Fundamental and application of aerobic granulation technology for wastewater treatment

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Abstract

Biogranulation has been studied widely and successfully industrialized for the last two decades. Biogranulation technology for wastewater treatment includes anaerobic and aerobic granulation processes. Even though anaerobic granulation has been relatively well studied and known, studies on aerobic granulation have begun recently. The aerobic granular sludge is known to have denser and stronger microbial structure. It has regular, smooth round shape, and a clear outer surface, while conventional activated sludge flocs is loose, fluffy, and irregular. Since formation and characteristics of aerobic granulation are affected by many factors. Studying the models of aerobic granulation, major factors affecting aerobic granulation, characteristics of aerobic granules, microbial structure and diversity, and their industrial applications are important for a comprehensive understanding of aerobic granulation.

Keyword

Biogranulation; Aerobic granules; Aerobic granulation; Microbial structure; Diversity; Mechanism of granulation; Operatinal condition.

Introduction

Aerobic and anaerobic granulations involve cell-to-cell interactions that contain biological, physical and chemical phenomena. In selective environment, microorganisms are capable of attaching to each other and aggregate. Through self-immobilization of microorganisms, granules are formed. These dense microbial aggregates consist of various bacterial species and typically contain millions of organisms per gram of biomass. These bacteria are expected to play different roles in degrading wastewater containing various organic chemicals and removing of nutrients. As compared to conventional activated sludge flocs, granular sludge has regular, denser and stronger microbial structure and good settling ability. These characteristics result in high biomass retention and withstand high-strength wastewater and shock loadings.

In the Netherlands, granular sludge was discovered in 1976 in a 6m^3 pilot plant at the CSM sugar factory in Breda. Granular sludge with good settling ability and methanogenic activity has now been extensively applied to anaerobic wastewater treatment system and studied in the upflow anaerobic sludge blanket (UASB) reactor. 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 microecosystems 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. Therefore, large volumes of waste can be treated in compact bioreactors. However, some drawbacks of the anaerobic granulation technology include the need for a long start-up period, a relatively high operation temperature and unsuitability for low strength organic wastewater. Anaerobic granulation technology is not suitable for the removal of nutrients from wastewater. In order to overcome those weaknesses, research has been devoted to the development of aerobic granulation technology.

The development of aerobic granules was first reported by Mishima and Nakamura (1991) in a continuous aerobic upflow sludge blanket reactor. Aerobic granules with diameters of 2 to 8 mm were developed, with good settling properties. Aerobic granulation has been observed in sequencing batch reactors (SBRs) (Morgenroth et al., 1997; Beun et al., 1999; Peng et al., 1999; Etterer and Wilderer, 2001; Tay et al., 2001a; Liu and Tay, 2002). It has been utilized in treating high-strength wastewaters containing organics, nitrogen and phosphorus, and toxic substances (Jiang et al., 2002; Moy et al., 2002; Tay et al., 2002e; Lin et al., 2003). However, the granulation process of aerobic sludge has not been well studied.

Aerobic granulation

Sludge may be defined as the microbial biomass utilizing nutrient substrates present in wastewater. Microbial granules are characterized as dense and compact microbial aggregates with a spherical outer shape. The concept of aerobic granular sludge reactor has been developed in recent years. Aerobic granules are self-immobilized spherical aggregates of microorganisms. The growth of aerobic granules were considered to be a special case of biofilm growth (Liu and Tay, 2002; Yang et al., 2004a). Most of aerobic granules have been cultured in sequencing batch reactors (SBRs) only. The SBR system is a modified design of the conventional activated sludge process and has been commonly used for industrial and municipal wastewater treatment (Fig. 1). The sequential steps of feeding, aeration, settling and discharge of supernatant fluid in a SBR were conducted in the same tank (Metcalf and Eddy, 2003).Compared to the conventional activated sludge, dense and strong microbial structure, good settling ability, high biomass retention and ability to withstand a high organic loading rate are advantages of aerobic granules formed in SBRs. Aerobic granulation can be initiated by microbial self-adhesion while bacteria are not likely to aggregate naturally due to the repulsive electrostatic forces and hydration interactions among them.

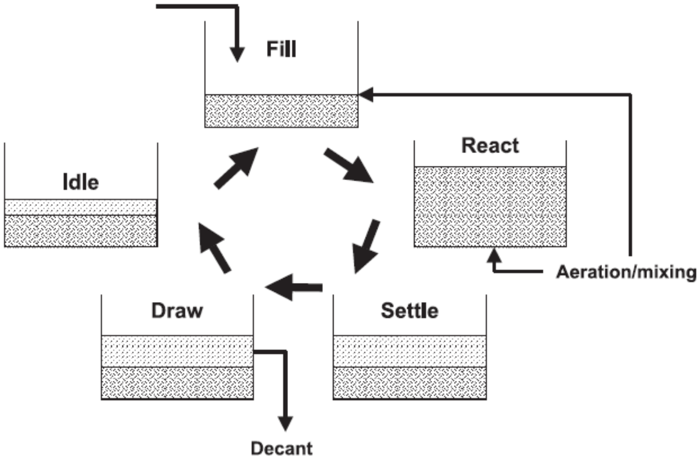

Fig. 1. Sequencing batch reactors (SBRs) design principle (EPA., 2000).

Tay et al. (2001a) investigated formation of an aerobic granule from seed sludge. Granules cultivated in two reactors fed with glucose and acetate, as sole carbon sources, were compared with microscopic techniques. The seed sludge with fluffy, loose and irregular structure was dominated by filamentous bacteria. Aerobic granules matured in both reactors after operation in SBR for 3 weeks. At this stage, both glucose-fed and acetate-fed granules had a very regular round-shaped outer surface. Compared to acetate-fed granules, filamentous bacteria dominant in glucose-fed granules made a fluffy outer surface of granules (Fig. 2).

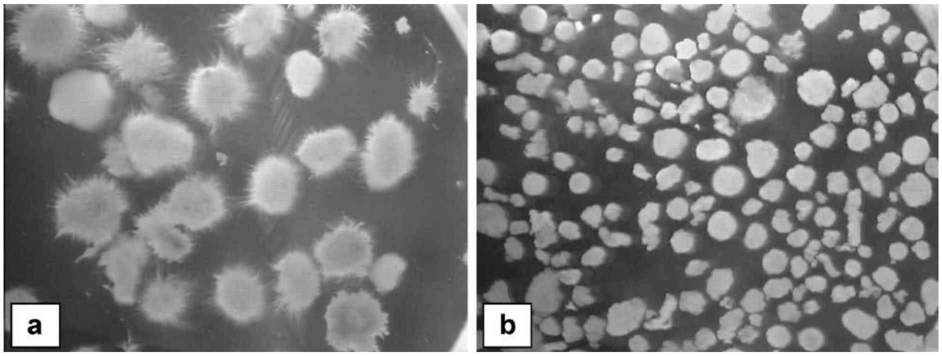

Fig. 2. Macrostructures of glucose-fed (a) and acetate-fed (b) aerobic granules (Tay et al., 2001a).

In scanning electron microscope (SEM), it was observed that a filamentous dominant outer surface in the glucose-fed aerobic granules. The acetate-fed aerobic granules had a very compact microstructure in which cells were compactly connected together and rodlike bacteria were predominant (Fig. 3). It implied that aerobic granulation is a gradual process involving the progression from seed sludge to compact aggregates, further to granular sludge and finally to mature granules.

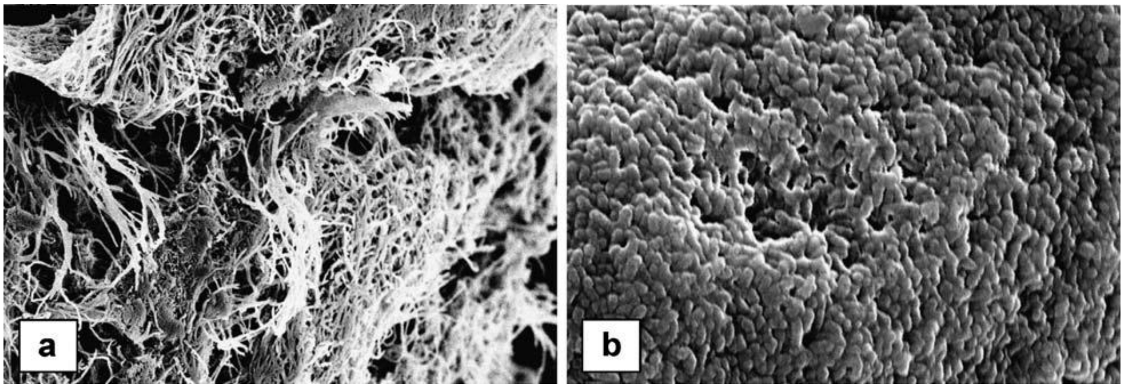

Fig. 3. Microstructures of glucose-fed (a) and acetate-fed (b) aerobic granules (Tay et al., 2001a).

Factors affecting aerobic granulation

Composition of substrate

The cultivation of aerobic granules have been reported using various 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., 2003). Aerobic granules have been also cultivated with nitrifying bacteria and an inorganic carbon source (Tay et al., 2002b; Tsuneda et al., 2003). These nitrifying granules showed excellent nitrification ability. Aerobic granules were also successfully cultivated in laboratory-scale SBR for treating particulate organic matter rich wastewater (Schwarzenbeck et al., 2003). In recent years it was studied that the feasibility of aerobic granular sludge formation on domestic sewage occurs in a SBR system as a logical step in the scaling-up process (De Kreuk and van Loosdrecht, 2006). The glucose-fed aerobic granules have exhibited a filamentous structure, while acetate-fed aerobic granules have had a nonfilamentous and very compact bacterial structure in which a rodlike species predominated. It suggested that granule microstructure and species diversity depend on the type of carbon source.

Organic loading rate

Loading rates are important operational parameters in conventional activated sludge processes. Studies on biofilm have demonstrated that the structure of biofilm was closely related with organic loading rate (OLR) (Liu et al., 2007). In the formation of anaerobic granules, organic loading rate is one of the most critical factors. Increased organic loading rates enhance the formation of anaerobic granules in UASB systems. However, aerobic granule 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. (2004) reported that it was difficult to form granules with organic loading rates lower than 2 kg COD m^{-3} day^{-1}. The study about effects of organic loading rates on the formation and stability of granules showed that the lower organic loading rates resulted in slower formation of granules and took longer time to reach the steady state. Even though the formation of aerobic granule is not significantly affected by organic loading rate, the physical characteristics such as the mean size of aerobic granules depend on the organic loading rate. Liu et al. (2003a) reported that the mean size of aerobic granules was increased with increase of organic loading rate. An increased organic loading rate can enhance the biomass growth rate, therefore the weakness of the three-dimensional structure of the microbial community can occur (Liu et al., 2003c).

Hydrodynamic shear force

The formation of aerobic granules and granule stability was improved at a high shear force (Shin et al., 1992; Tay et al., 2001a). Low shear stress on the granules was discussed as one of the most important factors influence the formation of aerobic granules. Higher local shear forces in the SBR resulted in more dense granules with a smaller diameter (De Kreuk and van Loosdrecht, 2006). Tay et al. (2001a) operated four parallel SBRs with increasing aeration rates, showing that granules formed only in reactors with a superficial gas velocity greater than 1.2 cm $\sec^{-1}$. More regular, rounder, and compact aerobic granules were developed at high hydrodynamic shear force. It was reported that the production of extracellular polysaccharides was closely associated with the shear force and the stability of aerobic granules was found to be related to the production of extracellular polysaccharides (Tay et al., 2001c). Since extracellular polymeric substances (EPS) are a major component of cell flocs and biofilms, they are hypothesized to play a dominant role in all types of biofilm formations, including flocculation and granulation. The aerobic granules had higher cell surface hydrophobicity than the seed sludge. The cell surface hydrophobicity was positively related to an increase of the extracellular protein content in sludge EPS. Some researchers have suggested polysaccharides in EPS played a major role in the formation of these microbial aggregates. The enhanced production of extracellular polysaccharides at high shear can contribute to the compact and stronger structure of aerobic granules. Zhang et al. (2007) reported that the extracellular protein may play a most important role in controlling the formation and stability of aerobic granules. Many studies have reported that the extracellular protein was more abundant than polysaccharides and even predominated in EPS preparations from several sources. The presence of protein in the extracellular fraction suggests it may play a major in the granulation. In addition, since protein has a high content of negatively charged amino acids, protein is more involved than sugars in electrostatic bonds with multivalent cations, a key factor in stabilizing aggregate structure.

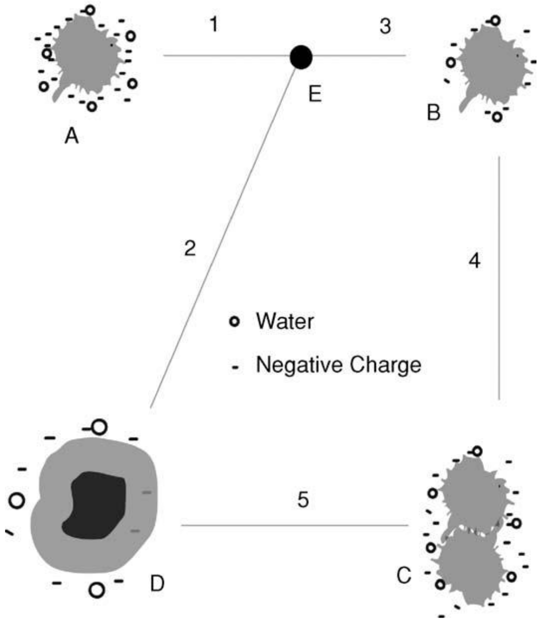

Fig. 3. Illustration of EPS effect on the formation and growth of aerobic granules (Li et al., 2006b).

Settling time

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) reported that aerobic granules were successfully cultivated and became dominant only in the SBR operated at a settling time of 5 min. Mixtures of aerobic granules and suspended sludge were observed in the SBRs run at settling times of 20, 15, and 10 min. The production of extracellular polysaccharides was stimulated and the cell surface hydrophobicity improved significantly at short settling times. These findings illustrate the fact that aerobic granulation is driven by selection pressure and the formation and characteristics of the granules may be controlled by manipulating the selection pressure. Therefore, choice of an optimal settling time is very important in aerobic granulation. Granules with excellent settling properties are essential for the effective functioning of biological systems treating wastewater.

Hydraulic retention time

Washing out flocs is one of the essential operational conditions for aerobic granulation, since flocs can easily obtain substrate and then suppress granules. A short cycle time stimulates microbial activity and production of cell polysaccharides and also improves the cell hydrophobicity. These hydraulic selection pressure-induced microbial changes favor the formation of nitrifying granules. Therefore, short settling time and hydraulic retention time (HRT) are usually applied for the selection of granules.

Aerobic starvation

Starvation phase plays a significant role on the formation of aerobic granules. The sequential steps of feeding, aeration, settling and discharge were conducted in the SBR. Therefore, microorganisms in the SBR are supposed to experience fluctuations periodically. As the number of operation cycles increased, the waste degradation time required is decreased. The formation of aerobic granule is initiated by starvation and cooperated by shear force. It makes bacteria more hydrophobic which promote the granulation from flocs (Tay et al., 2001a; Li et al., 2006b). The aggregation is regarded as a strategy of cells against starvation. It was reported that bacteria become more hydrophobic which facilitates adhesion or aggregation under starvation conditions (Bossier and Verstraete., 1996; Tay et al., 2001a). These changes result in the microbial aggregation process and lead to stronger and denser granules. (Fig. 3).

Other factors affecting aerobic granulation

Presence of calcium ion in feed enhances the aerobic granulation process. Improved settling and strength characteristics and more polysaccharides were founded in aerobic granules with Ca^{2+} added (Jiang et al., 2003). Ca^{2+} may binds to negatively charged groups present on bacterial surfaces and extracellular polysaccharides molecules and thus acts as a bridge to promote bacterial aggregation. As mentioned above, periodic starvation can be occurred during operation of SBR (Tay et al., 2001a). Under starvation conditions, bacteria could become more hydrophobic and this in turn encouraged microbial aggregation and adhesion (Bossier and Verstraete, 1996). McSwain et al. (2003) reported that a high feast-famine ratio, or pulse feeding provided by dump fill in the SBR, was required for the formation of compact, dense granules. Intermittent feeding affected the selection and growth of floc-forming and filamentous organisms, which affected the structure of aerobic granules. Studies about the effect of temperature, pH and dissolved oxygen on the aerobic granulation process are lacking. Liu et al. (2007) reported that acetate-fed granules cultivated at 25˚C could be successfully applied to start-up reactor operation for treating low-strength domestic wastewater with COD removal and nitrification at moderate (25˚C) and high temperature (35˚C). Peng et al. (1999) reported that aerobic granules have cultivated at DO concentration as low as 0.7 to 1.0 mg/l in a SBR. Studies about the role of seed sludge in aerobic granulation are also needed. The formation and properties of anaerobic granules is strongly influenced by the characteristics of the seed sludge. Aerobic granular sludge SBRs have been seeded with conventional activated sludge. Inoculating reactor with granular sludge has been proven to favor and accelerate the start-up process of aerobic granular system and anaerobic granular system (Liu et al., 2005). Yang et al. (2004a, b) researched the inhibitory effect of free ammonia on aerobic granulation in a SBR fed with acetate as the sole carbon source. Free ammonia could hinder the formation of aerobic granules by inhibiting the energy metabolism of microorganisms.

Characteristics of aerobic granules

Morphology

The shape of the granules is nearly spherical with a very clear outline (Peng et al., 1999; Tay et al., 2001a, c; Zhu and Wilderer, 2003). The average diameter of aerobic granules varies in the range of 0.2 to 5 mm. 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., 2003g).

Density and strength

The specific gravity of aerobic granules typically ranges from 1.004 to 1.065 (Etterer and Wilderer, 2001; Tay et al., 2001a). The granules with a high physical strength withstand high abrasion and shear. The physical strengths of aerobic and anaerobic granules are comparable. Aerobic granules with smaller sizes tend to be more compact compared to larger aerobic granules (Toh et al., 2003; Yang et al., 2004a).

Specific oxygen utilization rate

Microbial activity of microorganisms is characterized by the specific oxygen utilization rate (SOUR). A very wide range of SOUR values for aerobic granules have been reported (Morgenroth et al., 1997; Tay et al., 2001b; Yang et al., 2003b; Zhu and Wilderer, 2003). The biochemical reactions associated with bacterial metabolism show in an approximately linear relationship between oxygen utilization and carbon dioxide production: that is, relatively less cell mass is produced at high oxygen utilization as the metabolism is faster and more of the substrate is converted to carbon dioxide. The microbial activity represented by SOUR is inversely related to the hydraulic selection pressure in terms of the settling time (Qin et al., 2004). SOUR is an important characteristic for assessing the ability of aerobic granules to handle high-strength industrial wastewaters.

Settleability

The sludge volume index (SVI) of aerobic granules can be lower than 50 ml/g, which is much lower than that of conventional bioflocs (Liu et al., 2003f; Qin et al., 2004). This implies that from an engineering perspective, the settleability of sludge can be improved significantly through the formation of aerobic granules so that it can be settled in a more compact clarifier. The settling velocity of aerobic granules is associated with granule size and structure and is as high as 30 to 70 m/h. 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 m/h).

Thus, aerobic granulation can lead to more biomass retention in the reactor and this can enhance the performance and stability of the reactor.

Cell surface hydrophobicity

Cell surface hydrophobicity is an important affinity force in cell self-immobilization and attachment processes (Pringle and Fletcher, 1983; Kos et al., 2003; Liu et al., 2003b). The hydrophobicity of granular sludge was nearly twofold higher than that of conventional bioflocs.

Storage stability

The loss of granule stability and activity during an extended idling period is associated with the storage temperature. A high storage temperature accompanied with the absence of external substrate can lead to endogenous respiration and a rapid disintegration of the granules. The loss of granule activity and structural integrity during storage depend on the storage temperature, duration, the storage medium, and the characteristics of the granules. Compared to fresh granules, the strength of the stored granules has been observed to decrease by 7–8% for glucose- and acetate-fed aerobic granules after 4 months storage at 4 ℃ (Tay et al., 2002c).

Microbial structure and diversity

Microbial structure

Confocal laser-scanning microscopy (CLSM) has been used with different oligonucleotide probes, specific fluorochromes, and fluorescent microspheres for studying the microstructure of aerobic granules (Tay et al., 2002d, 2003a; Toh et al., 2003; Jang et al., 2003; Meyer et al., 2003). The obligate aerobic ammonium-oxidizing bacterium Nitrosomonas spp. was found mainly at a depth of 70 to 100 µm from the granule surface, and aerobic granules contained channels and pores that penetrated to a depth of 900 µm below the granule surface. The anaerobic bacterium Bacteroides spp. also detected at a depth of 800 to 900 µm from the granule surface (Tay et al., 2002e), while a layer of dead microbial cells was located at a depth of 800 to 1000 µm (Toh et al., 2003). Consequently, smaller granules will be more effective for aerobic wastewater treatment as these granules have more live cells within a given volume of granules.

Microbial diversity

Microbial diversity of aerobic granules has been studied by molecular biotechnology techniques (Yi et al., 2003; Tay et al., 2002d; Jang et al., 2003; Meyer et al., 2003; Tsuneda et al., 2003). Heterotrophic, nitrifying, denitrifying, P-accumulating bacteria, and glycogen- accumulating bacteria have been identified in aerobic granules developed under different conditions (Jang et al., 2003; Meyer et al., 2003; Tsuneda et al., 2003; Lin et al., 2003; Liu et al., 2003f; Yang et al., 2003b). The microbial diversity of aerobic granules is closely related to the composition of culture media, in which they are developed and structure of aerobic granules. The presence of anaerobic bacteria in aerobic granules is likely to result in the production of organic acids and gases within the granules. These end products of anaerobic metabolism can destroy the granules or at least diminish their long-term stability.

Mechanisms of aerobic granulation

i. Physical movement to initiate bacterium-to-bacterium contact. The factors involved in this step are hydrodynamics, diffusion mass transfer, gravity, thermodynamic effects, and cell mobility.

ii. Stabilization of the multicell contacts resulting from the initial attractive forces. These attractive forces are physical forces (e.g., Van der Waals forces, opposite charge attraction, thermodynamically driven reduction of the surface free energy, surface tension, hydrophobicity, filamentous bacteria that can bridge individual cells), chemical forces, and biochemical forces including cell surface dehydration, cell membrane fusion, signaling, and collective action in bacterial community.

iii. Maturation of cell aggregation through production of extracellular polymer, growth of cellular clusters, metabolic change, environment-induced genetic effects that facilitate the cell–cell interaction and result in a highly organized microbial structure. Shaping of the steady state three-dimensional structure of microbial aggregate by hydrodynamic shear forces (Chisti, 1999a).

Applications of aerobic granulation technology

Aerobic granules can be used for not only carbon removal but also nitrogen removal and phosphorus removal. After several years of studies on operation conditions for granule formation and characterization of granule properties with synthetic wastewater, some studies are currently focused on the application of this technology to real wastewater with different types such as dairy wastewater, livestock wastewater and industrial wastewater. Acetate-fed granules cultivated can be successfully applied to start-up reactor operation for treating low-strength domestic wastewater with COD removal and nitrification at moderate and high temperature (Liu et al., 2007).

- High-strength organic wastewater treatment
- Simultaneous organic and nitrogen removal
- Phosphorous removal
- Phenolic wastewater treatment Tay et al. (2004) demonstrated that their granules degraded phenol at a specific rate exceeding 1 g phenol g^{-1} VSS d^{-1} at 500 mg l^{-1} of phenol, or at a reduced rate of 0.53 g phenol g^{-1} VSS d^{-1} at 1900 mg l^{-1} of phenol.
- Biosorption of heavy metals by aerobic granules. Soluble Cd^{2+}, Cu^{2+} and Ni^{2+} ions could be removed by aerobic granules through three mechanisms, i.e., ion exchange, binding to extracellular polymers and chemical precipitation

Conclusion

The advantages of aerobic granules formed in SBRs are dense and strong microbial structure, good settling ability, high biomass retention and ability to withstand a high organic loading rate. Aerobic granules can be used in treating high-strength wastewaters containing organics and toxic substances. Especially, nitrogen removal and phosphorus removal are also achieved by using aerobic granules. However, only in SBRs aerobic granulation has been observed. More studies on the feasibility of developing aerobic granulation in continuous culture systems are need to be investigated. As compared to anaerobic granules, aerobic granules have relatively low stability because of their fast growth rate. Therefore, it is required that research for improving the stability of aerobic granules by manipulating operational conditions or through selecting for slow-growth bacteria.

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