A proposed aerobic granules size development scheme for aerobic granulation process

Farrah Aini Dahalan a,b,⇑, Norhayati Abdullah c, Ali Yuzir b, Gustaf Olsson d, Salmiati b, Myzairah Hamdzah b, Mohd Fadhih Mohd Din b, Siti Aqlima Ahmad e, Khalilah Abdul Khalil f, Aznah Nor Anuar b, Zainura Zainon Noor b, Zaini Ujang b

The School of Environmental Engineering, Universiti Malaysia Perlis, Kompleks Pengajian Kejuruteraan Jejawi 3, 02600 Arau, Perlis, Malaysia
b Institute of Environmental and Water Resource Management (IPASA), Universiti Teknologi Malaysia, 81310 Skudai, Johor Bahru, Malaysia
c Palm Oil Research Centre, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia
d Department of Industrial Electrical Engineering and Automation, Lund University, S-22100 Lund, Sweden
e Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
f Department of Biomolecular Sciences, Faculty of Applied Sciences, Universiti Teknologi MARA, 40150 Sec. 2, Shah Alam, Selangor, Malaysia

HIGHLIGHTS

- We propose illustrative scheme for mechanism of aerobic granule size development.
- Proposed scheme delineates the formation of aerobic granule size development.
- Different types of aerobic granules follows similar size development scheme.
- Stability of granules was enhanced through idle phase in SBR cycle time.
- Mature granules favour further aggregation with newly augmented sludge flocs.

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 9 January 2015
Accepted 14 January 2015
Available online 28 January 2015

Keywords:
Psychochemical
Aerobic granular sludge
Phototrophic
Sequencing batch reactor
Mechanism

ABSTRACT

Aerobic granulation is increasingly used in wastewater treatment due to its unique physical properties and microbial functionalities. Granule size defines the physical properties of granules based on biomass accumulation. This study aims to determine the profile of size development under two physicochemical conditions. Two identical bioreactors namely Rnp and Rp were operated under non-phototrophic and phototrophic conditions, respectively. An illustrative scheme was developed to comprehend the mechanism of size development that delineates the granular size throughout the granulation. Observations on granules' size variation have shown that activated sludge revolutionised into the form of aerobic granules through the increase of biomass concentration in bioreactors which also determined the changes of granule size. Both reactors demonstrated that size transformed in a similar trend when tested with and without illumination. Thus, different types of aerobic granules may increase in size in the same way as recommended in the aerobic granule size development scheme.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Aerobic granulation is a combination of chemical engineering and biological processes to develop a compact form of activated sludge with high settleability properties. Transformation of seeded activated sludge into the form of aerobic granular sludge in a sequencing batch reactor (SBR) is determined by biomass increment in a granulation reactor system. Increase of biomass concentration is a result of dynamic microorganisms association and aggregation of minerals to form dense, strong, and spherical biomass aggregates (Simoes et al., 2009). According to Toh et al. (2003), the biomass distribution pattern may justify the changing physical properties of a granule as they change in size. Toh et al. (2003) further explained that as size increased, parameters such as the settling velocity, total and biomass densities may also
increase although not in parallel with the size increment whilst the granule strength, specific hydrophobicity and sludge volume index (SVI) may decreased accordingly.

Aerobic granular sludge has been successfully cultivated in size ranging between 0.02 mm (Liu et al., 2005) to 9.0 mm (Wang et al., 2004). In average, aerobic granular sludge is commonly featured in diameters of between 0.1 mm and 3.0 mm (Li et al., 2008; Verawaty et al., 2013; Liu et al., 2012; Abdullah et al., 2013; Li et al., 2014). When compared to bioflocs, aerobic granules also featured a well-defined appearance and shape.

Granule size, although understudied, verifies the changes in physiological and physical performances of granules. Granule size may limit mass transport and diffusion due to the porosity of the structures which decreased with size increment. Porosity of granules also determined nutrient accessibility as well as seepage of unwanted products. These conditions are size-dependent which would impact the microenvironment of granules. Hence, granule size is an important factor in shaping the physical performance and determining the characteristics of aerobic granules.

Generally, it is well-understood that the size of granules which increases throughout the aerobic granulation process is related to the SBR operational conditions. Operational parameters such as substrate loading, dissolved oxygen, settling time, shear stress and so forth may affect the performance of aerobic granular sludge which is directly indicated through the morphology and structure of granules. For example, an increase in shear stress which largely controls the aerobic granulation system produced granules in the range of 0.28–0.35 mm with good sludge volume index (SVI) value (Tay et al., 2002).

However, understanding on the transition phase where smaller sludge particles aggregate with medium size granules, and medium size granules combined together to form larger size granules seems lacking. This knowledge is critical to ensure successful granulation as biomass retention in aerobic granulation environment is influenced by granule biomass density which is associated with the size of granules.

This study aims to investigate the profile of the granule size distribution during granulation period under phototrophic condition supplied by continuous illumination which supports the growth of photosynthetic microorganisms. The knowledge and understanding of illumination might be useful for aerobic granulation development under phototrophic conditions for several wastewater conditions. A size development scheme was developed to explain the phenomenon of granule formation. The knowledge and understanding of granule size would be useful for granule size-range that selects for optimal aerobic granular sludge system.

2. Methods

2.1 Reactor set-up and operations

Two identical reactors with internal diameters of 65 mm and effective height to diameter (H/D) ratio of 17 namely Rnp and Rp, were setup and operated under non-phototrophic and phototrophic conditions at a working volume of 3 L each, respectively. Each reactor was equipped with an influent feeding tube located at the bottom of the reactor, an effluent port located at mid-height of the reactor yielding a volumetric exchange rate (VER) of 50% and sampling ports for mixed liquor and granular sludge samplings.

A constant aeration was supplied by means of air bubble diffusers similar to Abdullah et al. (2013) through compressed air distribution at a flow rate of 4 L/min to promote the formation of fine bubbles to ensure that the sludge was homogenised. Dissolved oxygen (DO) pattern in the reactor was continuously monitored during aeration period of SBR cycles. The pH and temperature were monitored using a pH meter (Orion 2 Star Benchtop pH). These probes were located at midpoint height of both reactors. Illumination to Rp was provided under constant supply of light/dark cycles of 16/8 h using 3 6 ft Phillips Cool white tubes and 3 6 ft Phillips warm white tubes placed around reactor Rp. Incoming light energy was measured at 70.9 μmol m⁻² s⁻¹ using a LI-250 Light meter connected to LI-192 Quantum sensor (LI-COR, Lincoln, USA).

The reactors were operated in successive SBR cycles for a continuous operation of 24 h. The operational period and phases of the SBRs in granulation of aerobic granular sludge under phototrophic condition (AGSp) and non-phototrophic condition (AGSnp) is shown in Table 1.

2.2 Wastewater preparation

Synthetic wastewater was fed from the bottom of both Rp and Rnp respectively. For each cycle, 150 ml of medium organic (O) and nutrient (N) were added to the reactors together with 1200 ml of distilled water. The COD-load with medium was 1.6 g COD/L/d and the COD/N ratio was 8.3. The composition of synthetic wastewater in this study is given in Table 2.

2.3 Experimental procedures

The formation of aerobic granules was closely monitored using scanning electron microscopy (FESEM-Zeiss Supra 35 VPFESEM). Prior to gold sputter coating, the sampled granules were left to dry at room temperature (Biorad Polaron Divisions SM Coating System) as previously explained in Abdullah et al. (2013).

The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations were determined according to Standard Methods 2540-E (APHA, 2005). The morphological and structural observations of granules were measured by using an image analysis system (PAX-ITv6, ARC PAX-CAM). Prior to microscopic examinations, the granules were distributed based on sizes using a mesh sieve (0.2, 0.4, and 0.6 mm) to differentiate granules into 3 different categories; small, medium, and large size granules.

3. Results and discussion

3.1 Biomass concentrations

In this study, AGSnp refers to aerobic granular sludge developed under non-phototrophic conditions while AGSp are granules cultivated under phototrophic conditions. The biomass concentrations of AGSnp was compared with the one of AGSp. The profiles of biomass concentrations throughout the granulation process for both AGSp and AGSnp are shown in Fig. 1. The initial concentrations of biomass were at 3 gMLSS/L for both types of aerobic granules. After 4 days of reaction in the Rp, an increase of 5.5 gMLSS/L in the biomass concentration was observed for the AGSp. However,
increased further to 8.8 gMLSS/L. However, the was still very low during the first 10 days. This could be due to conversion of substrate to CO₂ which

system consisting of a small amount compared to featured cul-

and H₂. Growth of AGSnp continued to grow in slower rate. At this stage, the ''win-

increased sharply. As a result, stable flocs with compact microbial structure were formed at the end of this maturing stage.

On day-26, the biomass concentrations of AGSp increased sharply to 13.5 gMLSS/L (Fig. 1). At this stage, mature aerobic granules were seen to have supplementary structure formed on the surface of the granules due to new flocs attachment. The morphology of aerobic granules appeared as dark brown colored with an additional structure of yellowish brown floc patches fixed firmly on the granules surface. Over time, all the aerobic granules were transformed into yellowish brown structures, indicating that the facultative anaerobic microorganisms have gradually become inadequate. Thus the aerobic microorganisms dominated the outer part of the aerobic granules. This phenomenon was discovered and well documented by Hu et al. (2005).

In addition, unusually mature AGSp with sizes larger than 5 mm were unexpectedly discovered from the bed of reactor Rp which was operated under phototrophic conditions, having the capability to sustain the structural characteristics even after 90 days of prolonged granulation. Despite the large size, the AGSp featured an oval asymmetrical-shaped representation with brownish color and a well-defined outer surface. New flocs tend to affix onto the AGSp, enveloping the outer surface of the mature granules (Fig. SM 1-b). Based on microscopic examinations, the mature
AGSnp differed by 2 mm when compared to mature AGSp which reached larger size of up to 5 mm (Fig. SM 1-a), indicative of different morphology and structural characteristics of granules cultivated at different phototrophic conditions. The common sizes for aerobic granules ranges in between 2 mm and 4 mm as previously reported by Liu et al. Besides, the mature AGSp also favoured further aggregation with newly augmented sludge flocs to attach on the granule surface and formed irregular shape of aerobic granules. Although AGSnp was able to expand the granule size to 5.0 mm, the ideal operational size-range for optimal performance and economically effective aerobic granules for SBR treatment should be in between 1.0 mm and 3.0 mm (Toh et al., 2003). Song et al. (2010) suggested that mature aerobic granules can be treated as seed sludge to serve as substratum for new episode of granulation. These granules have been applied and demonstrated good performance in bioremediation such as biodegradation of dye and heavy metals (Muda et al., 2010; Sun et al., 2010, 2008a,b).

A significant decrease of the biomass concentration of AGSnp was seen on day-35 of experiments. This was due to granules disintegration, similar to what occurred earlier during the granulation of AGSp. Microscopic and visual observations indicated that mature granules disintegration resulted from cleaving of cracked granules’ structure. Cleavage of these granules revealed the internal structure which consisted of a central core of bacterium-encased cavities surrounded by the presence of a large diversity of bacterial morphotypes. Disintegration of granules was observed upon cleaving of granules’ structure followed by splitting of the mature granules into smaller fragments (Fig. SM 2). This profile suggests that similar behaviour followed the event as experienced by AGSp in between day-10 and day-18 of experiments. Related phenomenon has been found in another study by Liu et al. (2012). As the AGS possessed late disintegration phase, this may also suggest that a longer period is needed to develop the granules. This hypothesis was regarded previously by Nor-Anuar et al. (2007), describing that aerobic granular sludge (AGS) had been cultivated within 90 days of granulation.

As for AGSp, the increase of biomass concentration was slowed down again for the second time. Thereafter, no significant change of the granules occurred. At this stage, the aerobic microorganisms began to grow, and the total suspended solids increased and reached the highest value of 14 gMLSS/L. As granulation is a continuous process that involves evolution of suspended flocs to compact aggregates and then to compact granules, it is difficult to determine the turning point when aerobic granules are significantly enlarged. Thus, it was assumed that granulation was completed when clear boundary and regular shape of granules formed as described by Liu et al. (2008).

Overall, all the AGSnp granules formed were in a stable, regular, dense, and compact state in which facilitate good settling ability compared to AGSnp. Both the MLVSS concentrations of AGSnp and AGSnp increased substantially in both bioreactors namely Rp and Rnp, indicating biomass enrichment during sludge granulation, respectively. Based on the biomass concentration monitoring and microscopic observations in this study, a mechanism for the formation of aerobic granules in Rp by acclimatising activated sludge in phototropic condition may be proposed in the following section as the seed sludge underwent a series of morphological and physiological changes.

Initially, the seed sludge developed into “stable flocs” after inoculation. After a while, the number of phototrophic facultative anaerobic bacteria increased and congregated at the interior part of the granules together with the less capable and low oxygen tolerant bacteria and allowed the most capable, high oxygen tolerant and aerobic bacteria to reside on the outer side of the granules. Then the granules tended to disintegrate to form irregular, small flocs and particles. The flocs and particles from the disintegrated granules recombined under aerobic conditions allowing the granules to increase in size, resulting in the formation of mature AGSnp as previously observed by Sturm and Irvine (2008) and Hu et al. (2005). The development of AGSnp followed the same manner but a longer time was needed due to various physicochemical circumstances.

The results from this study reveals the possibility to cultivate aerobic granules in SBR under phototrophic condition. Besides that, the granules produced in Rp also provide acceptable and satisfactory outcome over the AGSnp. The idle phase of 35–40 min applied on the bioreactor configuration during granulation also indicates that longer starvation time is required to form AGSp with high biomass concentration in the Rp. This is in agreement with the studies by Liu and Tay (2004a,b) and Li et al. (2006). With the high biomass concentration over the AGSnp, it is supposed that the phototrophic facultative anaerobic bacteria have comfortably resided in the inner part of granules. It is obvious that a shorter starvation time leads to the production of lower biomass concentration of AGSp and the difference of the formation time is also significant, also observed by Pijuan et al. (2009) and Li et al. (2006).

### 3.2. Size development and distribution of granules

Fig. 2 gives the profiles of size distributions of aerobic granules in both Rp and Rnp, respectively. Both the AGSnp and AGSnp were developed from sludge that possessed unstable, loose, and bulky flocs with an average size of less than 0.4 mm. Over time, episodes of granulation were observed for both AGSp and AGSnp throughout the experimental period, and both exhibited similar size distribution schemes. Small size flocs were diminished, and they aggregated each other to form larger size of aerobic granules. The behaviour of the decreasing fraction of the small size flocs (AGSp: AGSnp: 0.2–0.4 mm) was clearly shown from the size distribution profile as shown in Fig. 2. The decreasing number of flocs was presumably due to the formation of larger granules of between 0.4 and 0.6 mm.

On day-10 of experiments, an increased size of AGSnp was observed. Despite the size increment, well-defined AGSnp has not formed yet but remained as flocs which featured a net-like loose structure that was easily washed-out, which confirms the observation made earlier by Liu and Tay (2006). Some flocs were larger than the opening gap on the sieve, which has caused them to

![Fig. 2. Aerobic granule size distribution in SBRnp and SBRp.](image-url)
remain on the sieve. Therefore, the remaining flocs on the sieve were counted for the size distribution determination.

On day-19, the net-like structure flocs were seen to form more stable flocs with smoother external morphology. At this stage, both types of granules uniformly formed in both Rp and Rnp, but the granules' structure remained ill-defined. The size fraction volume showed that AGSp possessed higher percentage than the AGSnp for the granules size >0.6 mm. This was due to longer period needed for AGSnp to increase the biomass concentration. Similar phenomenon was also observed by Wang et al. (2008) where concentration of inoculated seed sludge did not show significant change within 24 days for granule sizes of larger than 0.6 mm. However, the biomass concentration showed rapid increase between day-24 and day-45, and stabilised between day-45 and day-70 of experiments.

There was only a small difference of 0.2 mm between the AGSp and AGSnp indicative of not much difference on the size of granules cultivated in both reactors. The existing 0.4–0.6 mm size fraction granules fused with the small size fraction granules (>0.2 < 0.4 mm) to form >0.6 mm granules. As a result of these mechanisms, the net change of the 0.4–0.6 mm size flocs was negligible. Flocs were formed from the smaller sizes, but flocs were also combined with smaller flocs to form the large ones (>0.6 mm).

Gradually, on day-26 of experiments, the AGSp size fraction volume continued to increase more and eventually reached over 50% of the total volume for >0.6 mm of granule size. At this point, the AGSp had around 1.5 times higher size fraction ratio than the AGSnp. Throughout these experimental observations, an illustrative scheme was developed to show the mechanism of aerobic granule size development as shown in Fig. 3. The proposed scheme gives a simple overview on the phenomenon of aerobic granule size development in order to provide simple concept of aerobic granulation process.

The transformation from bulky seed sludge to AGSp in the Rp has resulted in granule sizes of between 0.5 and 5 mm. The average granule sizes were between 1.5 and 2 mm. In Rp, the granule sizes were between 0.5 mm and 1.2 mm. The average granule sizes were 1–2 mm, which are similar to the AGSnp cultivated in previous study conducted by Nor-Anuar et al. (2007). Based on the “theory of sedimentation”, the sizes obtained in this study for AGSp and AGSnp are considered as granules. The theory has assigned the minimum diameter of bioparticles, which is defined as granule diameter of at least 0.34 mm (Bhunia and Ghangrekar, 2007).

The sizes of the cultivated granules in this study were in the common range frequently observed for aerobic granules. Zheng and Yu (2007) have categorised aerobic granule sizes into three groups: 0.2–0.6 mm (small), 0.6–0.9 mm (medium), and 0.9–1.5 mm (large). Verawaty et al., 2013 had cultivated small, medium, and large size fractions of aerobic granules with the size of 425 mm, 900 mm, and 1125 mm. In this case, the AGSp and AGSnp can be categorised as large size granules (Zheng and Yu, 2007). In most cases, however, aerobic granules range between as low as 0.02 mm (Liu et al., 2005) to as high as 9.0 mm (Wang et al., 2004). The largest aerobic granules reported were 10 mm in diameter (Liu et al., 2010). In the study, the 10 mm granules were specially developed in order to study the mechanism of stability and disintegration purposes. Therefore, the size range of granular sludge is wider in an SBR system than in a conventional activated sludge system.

Generally, larger size granules are prone to mixing that may cause disintegration and small size granules tend to have low settleability. For those reasons, the significant magnitude of aerobic granulation should not be only restricted on the increase of granule size. In this case, size does not matter. The indication of a successful granulation is preferably based on the compactness and the settling ability of the granules. However, in the cultivation of aerobic granules it is important to keep the aeration rate at a controlled level in order to provide appropriate shear force for mixing for

<table>
<thead>
<tr>
<th>Items</th>
<th>Criteria/Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size:</td>
<td>Small</td>
</tr>
<tr>
<td>Granule size:</td>
<td>Medium</td>
</tr>
<tr>
<td>Size development:</td>
<td>Large</td>
</tr>
<tr>
<td>1) Non-steady state:</td>
<td>0.2 - 0.4</td>
</tr>
<tr>
<td>2) Steady-state:</td>
<td>0.4 - 0.6</td>
</tr>
<tr>
<td>3) Combined scheme:</td>
<td>&gt; 0.6</td>
</tr>
<tr>
<td>Flocs were formed from the smaller sizes, but flocs were also combined with smaller flocs to form the large ones (&gt;0.6 mm).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fig. 3. Proposed aerobic granules size development scheme.</td>
</tr>
</tbody>
</table>
the prevention of the development of too large or too small aerobic granule size (Liu and Tay, 2004a,b). Hu et al. (2005) have suggested that granule size is an eminent factor in designing the physical performance and characteristics of aerobic granules. The limitation of mass transfer and diffusion are influenced by the porosity in the interior part of the granular structures, and increases along with an increase of size and age.

The increase in the size of AGSP was presumably due to the increase of biomass concentration, which leads to habitability of facultative anaerobic microorganisms that have growth preference in the inner part of granules, and assemblage of minerals forming precipitates that build and strengthen the structure of the granules (Juang et al., 2010; D’Abzac et al., 2010; Tay et al., 2002; Liu et al., 2003). For example, SEM-EDX analysis revealed that solid particles of mineral fractions such as CaCO₃ and CaOH₂PO₄ are formed and bound within the EPS surrounding the aerobic granules (D’Abzac et al., 2010). Furthermore, the minerals also coalesce with various metallic elements, i.e. Al, Fe, Cu, Mn) to dedicate strong structure to the aerobic granules. Juang et al., 2010 suggested that calcium and/or iron precipitates in the granule interior have enhanced the structural stability of aerobic granules.

4. Conclusion

The granule size development is a key parameter that describes the biomass concentrations during aerobic granulation. This study gives simplified understanding on the formation of aerobic granules based on granule size transformation. Different types of aerobic granules formation follows similar size development scheme despite differing physicochemical conditions. Aerobic granules may increase in size in the same way as recommended in the aerobic granule size development scheme with or without illumination. This study reveals the possibility to cultivate aerobic granules in SBR under phototropic condition.

Acknowledgements

The authors wish to thank the Government of Malaysia and Ministry of Education Malaysia (Research Grant: 79004; 9016-00013; 9017-00009; 9003-00386), Institute of Environmental and Water Resource Management – Universiti Teknologi Malaysia (UTM), Universiti Malaysia Perlis (UniMAP), Lund University (Sweden), Universiti Putra Malaysia (UPM), Universiti Teknologi Mara (UiTM) for the supports.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.01.062.

References