



## A proposed aerobic granules size development scheme for aerobic granulation process



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### 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.

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### 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  $R_{np}$  and  $R_p$  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.

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### 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

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increase although not in parallel with the size increment whilst the granule strength, specific hydrophobicity and sludge volume index (SVI) may decrease 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  $R_{np}$  and  $R_p$ , 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  $R_p$  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  $R_p$ . Incoming light energy was measured at  $70.9 \mu\text{mol m}^{-2} \text{s}^{-1}$  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 (AGS<sub>np</sub>) is shown in Table 1.

### 2.2. Wastewater preparation

Synthetic wastewater was fed from the bottom of both  $R_p$  and  $R_{np}$ , 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, AGS<sub>np</sub> refers to aerobic granular sludge developed under non-phototrophic conditions while AGSp are granules cultivated under phototrophic conditions. The biomass concentrations of AGS<sub>np</sub> was compared with the one of AGSp. The profiles of biomass concentrations throughout the granulation process for both AGSp and AGS<sub>np</sub> 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  $R_p$ , an increase of 5.5 gMLSS/L in the biomass concentration was observed for the AGSp. However,

**Table 1**  
The SBR operations for aerobic granulation of AGSp and AGS<sub>np</sub>, respectively.

Bioreactor operation phase	AGSp	AGS <sub>np</sub> (Nor-Anuar et al., 2007)
	Time (min)	Time (min)
Aeration	120	110
Settling	5	5
Discharge	5	5
Feeding	10	60
Idle	35–40	0
Total cycles per day	8	8

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