

## Granulation of biological flocs under elevated pressure: characteristics of granules

Yang-Min Liang, Jie-Yuan Chen, Gaëtan Marchesini, Chi-Wang Li and Shiao-Shing Chen

### ABSTRACT

Aerobic granules (AG) have good settling ability and are relatively insensitive to the variation of organic loading rate. When sizes of granules become bigger, substrate and oxygen become limited in the granule core, leading to cell lysis and disintegration of granules. The higher the dissolved oxygen, the deeper the oxygen penetration inside AG. AG operated under elevated pressure might be a possible way to maintain long-term stability of granules. In this study, formation and characteristics of AG in the reactor operated under elevated high pressure (HP) and ambient pressure (AP) are investigated. Results show that both systems removed an average 95% of total organic carbon. Sludge volume index at 5 and 30 min settling times under HP are 35% smaller those under AP, indicating that HP granules have a better settling ability and a denser structure than AP granules. The granule size in the HP system is very uniform, while size distribution in the AP system is broader, indicating that the AP system contains flocculent sludge. Extracellular polymeric substances and polysaccharides (PS) are almost the same for HP and AP; however, exopolymeric protein (PN) is very different. PS/PN ratio for HP sludge is four times that of AP. The result is consistent with sludge settleability, which is improved with increasing PS/PN ratio.

**Key words** | aerobic granulation, elevated pressure, organic loading rate

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### INTRODUCTION

Aerobic granules (AG) are formed gradually, evolving from fluffy seed sludges to dense granules without the help of carriers, i.e., a microbial self-immobilization process (Liu & Liu 2006; Lee *et al.* 2010). AG have been employed to treat various kinds of wastewater under a wide range of organic loading rates (OLR) (Abdullah *et al.* 2011; Duque *et al.* 2011; Figueroa *et al.* 2011; Wang *et al.* 2011; Coma *et al.* 2012). The formation and stability of AG depend on OLR, hydraulic shear force (Adav *et al.* 2008; Chen *et al.* 2008; Sturm & Irvine 2008), selection pressure (Ni *et al.* 2009), starvation time (Liu & Tay 2007), and concentration of divalent ions (Liu *et al.* 2010). Although hydraulic shear force has often been cited by researchers to be an important parameter to control the formation of granules, it might be dissolved oxygen (DO) that is the underlying factor affecting the formation of granules. Strong shear force not only provides enough force to compact aggregates into granules, but also provides sufficient oxygen to suppress filament growth

(Adav *et al.* 2008). By operating a sludge blanket reactor with the same shear force but with different oxygen concentration, Sturm & Irvine (2008) concluded that oxygen concentration is a more important factor than shear force for formation of AG.

Although AG could be operated at extremely high OLR, loss of granule stability during long-term operation is still a major barrier for practical application of AG (Lee *et al.* 2010). Several reasons have been cited for the cause of losing granule stability, including outgrowth of filamentous organisms (Liu & Liu 2006) and hydrolysis in the granule core due to anaerobic condition (Lee *et al.* 2010). According to Liu & Liu (2006), the possible causes of filamentous growth include long sludge retention time, low substrate concentration in the liquid phase, high substrate gradient within granule, DO and nutrient deficiency inside the granule, temperature shift and flow patterns. It was shown that substrate and oxygen become

limited in the core of granules due to the growth of granule size, leading to cell lysis at the core (Chiu *et al.* 2006; Chen *et al.* 2008; Lee *et al.* 2010) and disintegration of the granule (Sturm & Irvine 2008; Lee *et al.* 2010). The higher the DO in the reactor, the deeper the oxygen penetration inside the AG. According to Henry's law, DO increases with increasing oxygen pressure in gas phase. In our previous study (Liang *et al.* 2012b), a biological process was operated under intermittent high pressure (HP), and DO ranging from 16 to 10 mg/L was achieved with aeration cycle varying from 3 to 15 min. Thus, operation of AG under elevated pressure might be a possible way to maintain the long-term stability of granules. In this study, reactors were operated under HP and ambient pressure (AP) to compare treatment efficiency and characteristics of granules under HP and AP systems.

## MATERIALS AND METHODS

### Experimental setup and materials

Two identical reactors made of PVC pipes with diameter of 10.16 cm and height of 70 cm were operated in parallel as shown in Figure 1. The working volume was 1.7 L. One was operated under HP of 3 bars and the other was under AP. Effluent was discharged from a port on the reactor wall with a volumetric exchange ratio of 50%. Both systems were operated on a 2-h cycle with hydraulic retention time of 4 h. Each cycle consisted of 4 min of feeding, 110 min

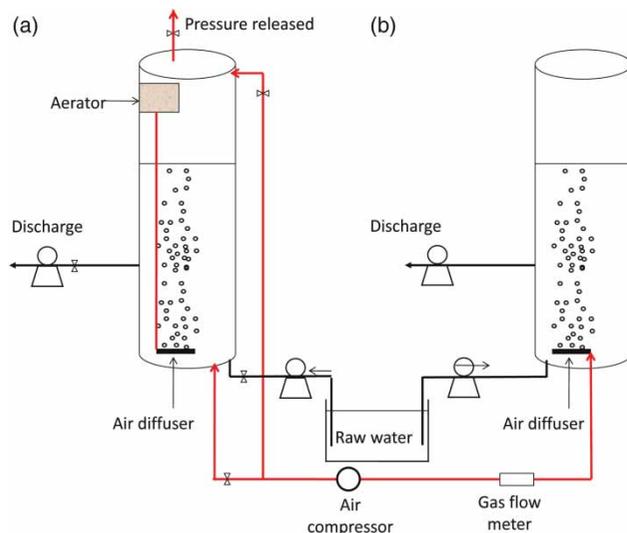


Figure 1 | (a) High pressure system and (b) ambient pressure system.

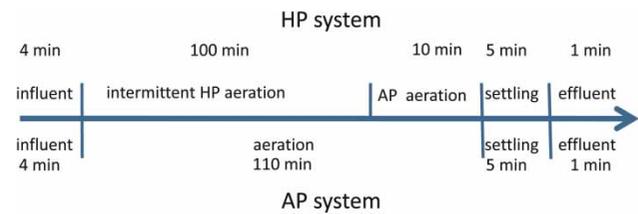


Figure 2 | Operation sequence for a 2-h cycle.

of aeration, 5 min of settling, and 1 min of discharging (see Figure 2). For the HP system, the aeration period consisted of an intermittent HP aeration of 100 min, and 10 min of aeration under AP. During intermittent HP aeration step, the pressure release valve was opened periodically for 1 second every minute. During pressure release, the reactor pressure dropped from 3 to around 2 bars. Before the settling step, the HP system was aerated under ambient condition for 10 min to remove any fine bubbles generated during transition from HP to AP. The formation of fine bubbles might attach to sludge flocs/granules, resulting in sludge flotation during settling.

The aeration flow was controlled at the superficial upflow velocity (SUV) of 1.2 cm/s for the AP system corresponding to an airflow rate of 5.9 L/min. For the HP system, the aeration was provided by two sources. One was provided by an air pump installed inside the pressurized reactor, and the SUV was 0.6 cm/s. The other was provided during intermittent HP aeration step where around 3 L of air was exchanged during each pressure release, corresponding to a maximum SUV of 5.1 cm/s. The combined total airflow rate for the HP system was also 5.9 L/min. Reactors were operated at room temperature, which is around 25–30 °C, and pH was around 7.0–7.5. No sludge was wasted during the operation except for samples taken for mixed liquor suspended solids (MLSS) analysis.

Sugar-containing waste solution with chemical oxygen demand (COD) of more than a hundred thousand mg/L was obtained from a local candy plant, and was added with nutrients to increase N, P, and Fe to the C:N:P:Fe ratio of 100:5:1:0.5 along with other minor nutrients. The solution was then diluted with tap water as the influent to obtain total organic carbon (TOC) of 550–600 mg/L corresponding to OLR of 3.3–3.6 kg TOC/m<sup>3</sup>·d. Both reactors were seeded with granular sludge from our previous study focusing on the effect of OLR (Liang *et al.* 2012a). MLSS and 30-min sludge volume index (SVI<sub>30</sub>) of seed sludge were 5,000 mg/L and 30–50 mL/g suspended solids (SS), respectively.

## Analytical methods

Samples were periodically collected and analyzed for COD, pH, SVI, SS, and MLSS according to *Standard Methods* (1998). Methods for soluble microbial products (SMP) and extracellular polymeric substances (EPS) were modified from the thermo-extraction method of Wang et al. (2006). A 20-mL sample of granular sludge was centrifuged at 5,000 rpm for 15 min; the supernatant was collected and analyzed for TOC, polysaccharides (PS), and exopolymeric protein (PN) content which is defined as SMP. The sludge settled at the bottom of the centrifuge tube was mixed with 30 mL of phosphate-buffered saline solution (containing  $\text{NaH}_2\text{PO}_4$  of 6 g/L and NaCl of 8 g/L) and heated for 1 h in a water bath with temperature set at 80 °C. The mixture was then centrifuged at 5,000 rpm for 15 min and the supernatant was collected and analyzed for TOC (EPS content), PS, and PN. Zeta potential of granules was analyzed using a microelectrophoresis analyzer (Zetasizer Nano ZS, Malvern, UK). The particle size distribution was analyzed using a laser scattering particle size distribution analyzer (Horiba LA-300 particle analyzer). Since the particle size analyzer can only detect particles with size less than 600  $\mu\text{m}$ , sludge samples were first sieved by a #30-mesh sieve using the sieving method proposed by Xuan et al. (2010). For each sample, three measurements were taken and averaged.

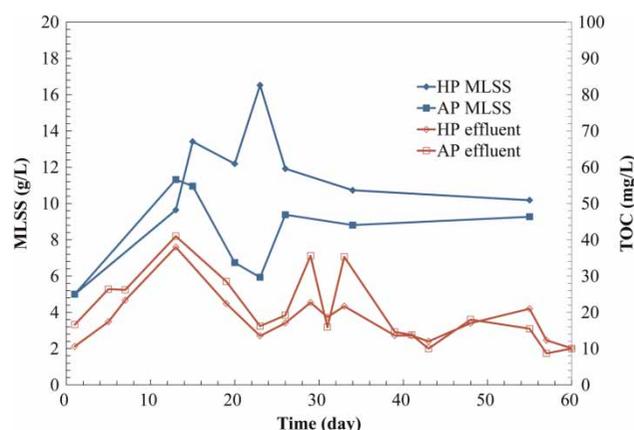
## RESULTS AND DISCUSSION

### Reactor performance

MLSS and TOC of effluent under HP and AP are shown in Figure 3. For both systems, TOC of effluent is between 10 and 40 mg/L, corresponding to average TOC removal of 95%. The extent of granule formation can also be observed through the evolution of MLSS. As shown in Figure 3, MLSS in HP system are higher than those in AP system during the course of the experiment. While MLSS concentration increases rapidly to 16,000 mg/L at day 21 for HP, the concentration of MLSS in AP system reaches the highest point of 11,000 mg/L at day 13. Both systems more or less stabilized at MLSS of around 10,000–11,000 mg/L after day 30.

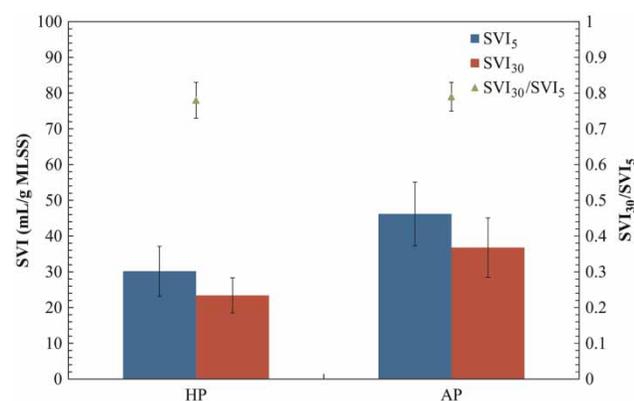
### Characteristics of granules

SVI is a good indicator for the extent of sludge granulation (Qin et al. 2004; Liu & Tay 2007; Song et al. 2009; Liu



**Figure 3** | MLSS and TOC as a function of operation time for HP and AP systems operated at TOC loading of 3.3–3.6  $\text{kg}/\text{m}^3\cdot\text{d}$ .

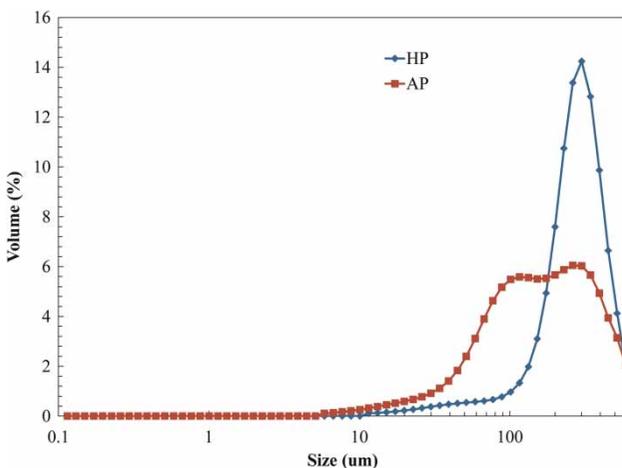
et al. 2010). Regardless of the particle size of granular sludge, values of  $\text{SVI}_{30}$  for mature granules have been reported ranging from 20 to 60 mL/g-SS (Jiang et al. 2002; McSwain et al. 2004; Chen et al. 2008). Schwarzenbeck et al. (2004) reported that the difference between  $\text{SVI}_5$ ,  $\text{SVI}_{10}$  and  $\text{SVI}_{30}$  (SVI at 5, 10 and 30 min settling time) of granular sludge are smaller than those of flocculent sludge. Therefore, the ratio of  $\text{SVI}_{30}/\text{SVI}_5$  can be used as an index for the extent of sludge granulation. A ratio of close to one indicates the sludge is in granular form and has a very good settling property. As shown in Figure 4, values of  $\text{SVI}_{30}/\text{SVI}_5$  for both systems are between 0.78 and 0.79 showing no significant differences. It indicates that both systems have a very high degree of granulation. However,  $\text{SVI}_5$  and  $\text{SVI}_{30}$  under HP are 35% smaller than those under AP. Those values are 30.16 and 26.39 mL/g-SS, respectively, for HP, and are 46.2 and 36.77 mL/g-SS, respectively, for AP.



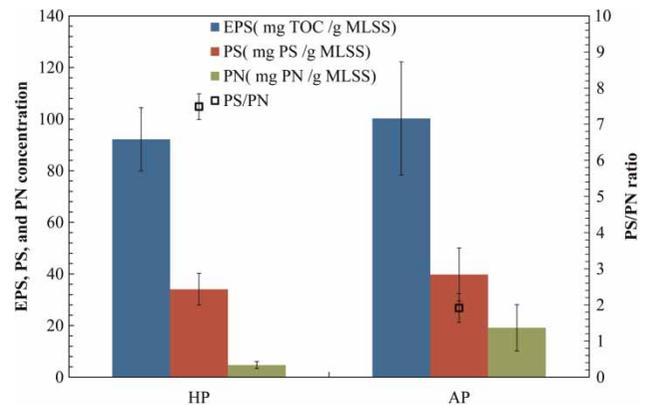
**Figure 4** |  $\text{SVI}_5$ ,  $\text{SVI}_{30}$ , and ratio of  $\text{SVI}_{30}/\text{SVI}_5$  for HP and AP systems operated at TOC loading of 3.3–3.6  $\text{kg}/\text{m}^3\cdot\text{d}$ .

The result indicated that HP granules have a better settling ability and a denser structure than AP granules.

Since the particle size analyzer can only detect particles with size less than 600  $\mu\text{m}$ , sludge samples were first sieved by a #30-mesh sieve, and those particles with size smaller than 600  $\mu\text{m}$  were then analyzed using a particle size analyzer. The percentage of sludge with size greater than 600  $\mu\text{m}$  under the AP and HP systems was 58.3 and 0%, respectively. Figure 5 shows particle size distribution of those granules with size of less than 600  $\mu\text{m}$ . The granule size in the HP system is very uniform, ranging from 200 to 450  $\mu\text{m}$ . On the other hand, the distribution of granules with size of less than 600 in the AP system is broad ranging from 50 to 450  $\mu\text{m}$ , indicating that the AP system still contains flocculent sludge. In general, granules in the AP system are bigger than those in the HP system. However, according to SVI data shown in Figure 4, it can be concluded that HP granules have a denser structure than AP granules. The reason that sludge in the HP system is more uniform and smaller in general than those in AP is the action of pressurization and depressurization during intermittent HP aeration, which produced very intense aeration with SUV as high as 5.1 cm/s compared to SUV of 1.2 cm/s for the AP system. Geng & Hall (2007) reported that average particle size in a membrane bioreactor is around 100  $\mu\text{m}$ , which is smaller than in a conventional activated sludge process (Le-Clech *et al.* 2006). Sludge particles broken up by shear stress resulting from intensive aeration velocity for controlling membrane fouling is the most cited reason. In the current study, although the airflow rate of 5.9 L/min applied is much higher than the airflow rate of 1.5 L/min used by Geng & Hall (2007), the selection process, i.e.,



**Figure 5** | Particle size distribution of granules less than 600  $\mu\text{m}$  for HP and AP systems operated at TOC loading of 3.3–3.6  $\text{kg}/\text{m}^3\text{-d}$ . Samples were taken at day 55.



**Figure 6** | EPS and the composition of EPS for HP and AP systems operated at TOC loading of 3.3–3.6  $\text{kg}/\text{m}^3\text{-d}$ .

short settling time, applied in this study is able to overcome the stress imposed by aeration flow, and is still able to form granules with size in the range of 200–450  $\mu\text{m}$ .

Figure 6 shows the results of PN, PS, PS/PN, and EPS for AG cultivated under HP and AP conditions. For both systems, values of EPS and PS are almost the same whereby EPS are between 90 and 100 mg TOC/g MLSS and PS are between 30 and 40 mg/g MLSS. However, values of PN are very different. They are 4.7 and 19.15 mg/g MLSS, respectively, for HP and AP. The value of PS/PN in HP sludge is four times higher than that in AP sludge. PS/PN ratio under HP and AP are 7.49 and 1.92, respectively. This result is consistent with that of Qin *et al.* (2004) who investigated the effects of settling time on granular sludge and observed that sludge settleability improved when PS/PN ratio increased. Yang *et al.* (2005) studied the effect of substrate nitrogen/COD ratio on the formation of granules, reporting that both PS/PN ratio and granule settleability increased with increasing nitrogen/COD ratio. Zhu *et al.* (2012) have pointed out that PN content is an important factor affecting the granulation process. PN content and PN/PS ratio are positively correlated to hydrophobicity and surface charge of granules. In this study, zeta potential of granules was analyzed using a microelectrophoresis analyzer, and the result indicated that on average the zeta potential of granules in HP is around  $-17.3$  mV and is  $-20.7$  mV for those in the AP system. This is consistent with the higher PN content of granules in the AP system.

## CONCLUSION

Effects of operating pressure (HP and AP systems) on granule formation and characteristics of the granule were

studied. Results show that both systems achieved average TOC removal of 95%, and MLSS under the HP system are higher than those under the AP system during the course of the experiment.  $SVI_5$  and  $SVI_{30}$  under HP are 35% smaller than those under AP, indicating that HP granules have a better settling ability and a denser structure than AP granules. The granule size in the HP system is very uniform, while particle size distribution in the AP system is broader. The result indicates that the AP system still contains flocculent sludge. Values of EPS and PS are almost the same in both HP and AP systems; however, values of PN are very different. The value of PS/PN for HP sludge is four times that of AP sludge. PS/PN ratio under HP and AP are 7.49 and 1.92. Zeta potential of granules on average is around  $-17.3$  mV in HP and is  $-20.7$  mV in the AP system. The result is consistent with the sludge settleability which is improved with increasing PS/PN ratio and decreasing zeta potential.

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