Performance of aerobic granular sludge at variable circulation rate in anaerobic–aerobic conditions

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ABSTRACT

Aerobic granular sludge (AGS) has been applied to treat a broad range of industrial and municipal wastewater. AGS can be developed in a sequencing batch reactor (SBR) with alternating anaerobic–aerobic conditions. To provide anaerobic conditions, the mixed liquor is allowed to circulate in the reactor without air supply. The circulation flow rate of mixed liquor in anaerobic condition is the most important parameter of operation in the anaerobic-AGS processes. Therefore, this study investigates the effect of circulation rate on the performance of the SBR with AGS. Two identical reactors namely R1 and R2 were operated using fermented soy sauce wastewater at circulation rate of 14.4 and 36.0 l/h, respectively. During the anaerobic conditions, the wastewater was pumped out from the upper part of the reactor and circulated back into the bottom of the reactor for 230 min. A compact and dense AGS was observed in both reactors with a similar diameter of 2.0 mm in average, although different circulation rates were adopted. The best reactor performance was achieved in R2 with chemical oxygen demand removal rate of 89%, 90% total phosphorus removal, 79% ammonia removal, 10.1 g/l of mixed liquor suspended solids and a sludge volume index of 25 ml/g.

Key words | aerobic granular sludge, anaerobic–aerobic conditions, circulation rate, fermented soy sauce wastewater, granular sequencing batch reactor, nitrification

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INTRODUCTION

Aerobic granular sludge (AGS) is a dense self-immobilized microbial consortium and one of the huge achievements in wastewater treatment in the twentieth century. It has advantages of high biomass retention and effective sludge settlement, and its successful application in sequencing batch reactors (SBRs) for over a decade attracted many researchers to focus in the field of aerobic granulation technology. However, due to strict legislation on wastewater pollution discharge, significant attention has been focused on compact high-rate bioreactors, which combine the AGS system with anaerobic processes in a single reactor. A combination of anaerobic and aerobic processes in a single reactor is capable of enhancing the simultaneous organic and nitrogen removal efficiency. The efficient collision between liquid flow and granules is a main factor for both the anaerobic–aerobic bioreactors and aerobic bioreactors, which induces the development, mass transfer, EPS production, molecular biology, structure and stability of aerobic and anaerobic granules (Ni & Yu 2010). This highlights the significance of hydrodynamic conditions in the cell aggregation and self-immobilization process. However, the understanding of hydrodynamics is still unclear except for hydrodynamic shear force, which is a key factor that influences granulation. Qin *et al.* (2004), Wu *et al.* (2012) and Liu & Tay (2002) showed that compact granules were developed under high hydrodynamic shear force and it takes a long period for seed sludge to develop into granular from under low hydrodynamic shear force whether in anaerobic or aerobic reactors.

Studies have shown that the circulation process for anaerobic phase in an anaerobic–aerobic bioreactor can provide anaerobic conditions and develop hydrodynamic conditions as well as simultaneous mixing between microbe and substrate (Hano *et al.* 1992; Muda *et al.* 2010). It has been applied to improve the contact between substrate and biomass within the treatment system by expanding

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the hydrodynamic conditions either in aerobic phase by aeration intensity or in anaerobic phase by the circulation process, which intensifies hydraulic mixing and results in better performance and stability. Therefore, circulation rate of mixed liquor in a reactor is an essential operation parameter in the anaerobic–aerobic processes. The aim of this study is to analyse granular sludge formation in an anaerobic–aerobic SBR at two different circulation rates, in order to optimize the development of AGS with good ability to degrade substrate, a stable structure and excellent settling properties. It is expected that this study will contribute to a better understanding of the role of circulation rate for anaerobic conditions in aerobic granulation technology.

MATERIALS AND METHODS

Experimental setup

Experiments were performed in two identical laboratory scale SBRs (100 cm in height, 6.5 cm in diameter and 15.4 of height:depth ratio) with a working volume of 3 L. The influent was fed into the bottom of the reactor and the effluent was discharged via an outlet port at 50 cm above the reactor bottom, resulting in a volumetric exchange ratio of 50% (Table 1). Same concentrations of seed sludge were inoculated in both reactors. The reactors were operated with alternating anaerobic–aerobic conditions in a continuous cycle of 8 h at 3.5–7.5 kg chemical oxygen demand (COD)/(m^3·d) of organic loading rate. In both reactors, each cycle consisted of 5 min of feeding, 120 min of circulation, 340 min of aeration, 5 min of settling time and 5 min of discharging. During circulation phase, the wastewater in both reactors was circulated from the top of the column to the bottom at a circulation rate of 14.4 l/h (R1) and 36.0 l/h (R2), respectively, which results in superficial upflow velocity of 0.24 cm/s for R1 and 0.60 cm/s for R2 to intensify hydraulic mixing in the reactor. The circulation process was carried out using a peristaltic pump (Cole-Parmer System Model, 6-600 rpm). Air was supplied in a subsequent stage at a superficial air-upflow velocity of 1.5 cm/s in the aerobic phase, which gave a dissolved oxygen (DO) concentration of about 50% oxygen saturation. Air was supplied through a bubble stone at the reactor bottom by an air pump and the airflow rate was controlled by a gas-flow controller. The reactors were operated at room temperature (25–30 °C) where pH and DO were continuously monitored. The seed sludge used in both reactors was obtained from a municipal sewage treatment plant located in Johor Bahru, Malaysia. The mixed liquor suspended solids (MLSS) concentration was 8,100 mg/l and sludge volume index (SVI) was 99.27 ml/g at pH of 6.95.

Table 1 | Characteristics of soy sauce wastewater

Parameter	Unit	Soy sauce wastewater
pH	–	6
COD	mg/l	5,400
5-day biochemical oxygen demand	mg/l	2,620
Colour	ADMI	>600
Suspended solids	mg/l	480
Turbidity	–	304
Total dissolved solids	mg/l	4,050
Ammonia-N	mg/l NH_3-N Ness	21
Total nitrogen	mg/l N-TNT	70
TP	mg/l PO_4^{3-}-TNT	55

Analytical methods

The parameters of wastewater samples were analysed for COD, MLSS, mixed liquor volatile suspended solids (MLVSS), ammonium (NH_4) and total phosphorus (TP) using standard methods (APHA 2005). Physical characteristics of the aerobic granule such as particle size distribution, settling velocity (SV) and SVI were measured. The particle size distributions of aerobic granules were analysed throughout the experiment based on the method proposed by Laguna *et al.* (1999), in which the dry weight of the granules that passed through different-sized humid sieves was calculated as a percentage of the total volume of the granule. The SVI_5 was determined based on Beun *et al.* (1999) where the bed volume was obtained by measuring the bed height of the sludge that settled within 5 min after the aeration phase stopped in the reactor and multiplying by the surface area of the reactor column. The SV of an AGS was determined as the time taken for an individual granule to settle at a certain height in a glass column filled with tap water. Then, the obtained bed volume was divided by the dry weight of the biomass in the reactor. Photographs of the granules were taken with a digital camera (Canon IXUS 100S, Japan). A scanning electronic microscope (FESEM-Zeiss Supra 35 VPFESEM) was used to examine the external morphology of the granule. The pH, DO and oxidation–reduction potential were monitored by a pH/DO probe meter (Orion 3-Star Benchtop pH/DO meter).

RESULTS AND DISCUSSION

Morphology and size distribution

The morphology of aerobic granules for R1 and R2 are shown in Figures 1(a) and 1(b), respectively. At day 60, aerobic granules in both reactors were analysed for the size distribution. The granule size distribution histogram shows that, at day 60, the seed sludge had become matured granules in both reactors. At this stage, the average granule diameter was around 1.0–1.5 mm in both R1 and R2. The results indicated that the granules in both reactors had a similar morphological structure and almost had a similar size distribution. As both reactors were initially seeded with activated sludge, the progression of the granule percentage in both reactors was a good indicator of sludge properties. Apart from that, it is a direct indicator showing the growth and aging process in a microbial organization and a decisive parameter for building the physical performance and characteristics of aerobic granules (Wang *et al.* 2005). As can be seen (Figure 1(b)), developed aerobic granules in both reactors had a clear contour without any filamentous outgrowths. The outer shape of granules was spherical or elliptic by an aspect ratio higher than 0.9. The result showed that as the value of aspect ratio of granules from R1 and R2 became close to 1.0, the granules have a shape close to a circle. From the observations, granules developed in R1 presented a smooth round surface similar to the granules from R2. As explained by Liu & Tay (2002), shaping of the three-dimensional structure of AGS is driven by hydrodynamic shear forces to form a certain structured community where the outer shape and size of microbial aggregates are finally determined by the interactive strength between aerobic granules and hydrodynamic shear force. Apart from that, AGS could become denser under high shear force, due to the bridging process among extracellular polymeric substances (EPS) and the growth of aerobic microorganisms and their attachment and detachment processes (Su & Yu 2005).

The size distribution of the mature AGS is shown in Figure 2. The size distribution is related to the circulation rate. The AGS with a size range of 1.0–2.0 mm contributed

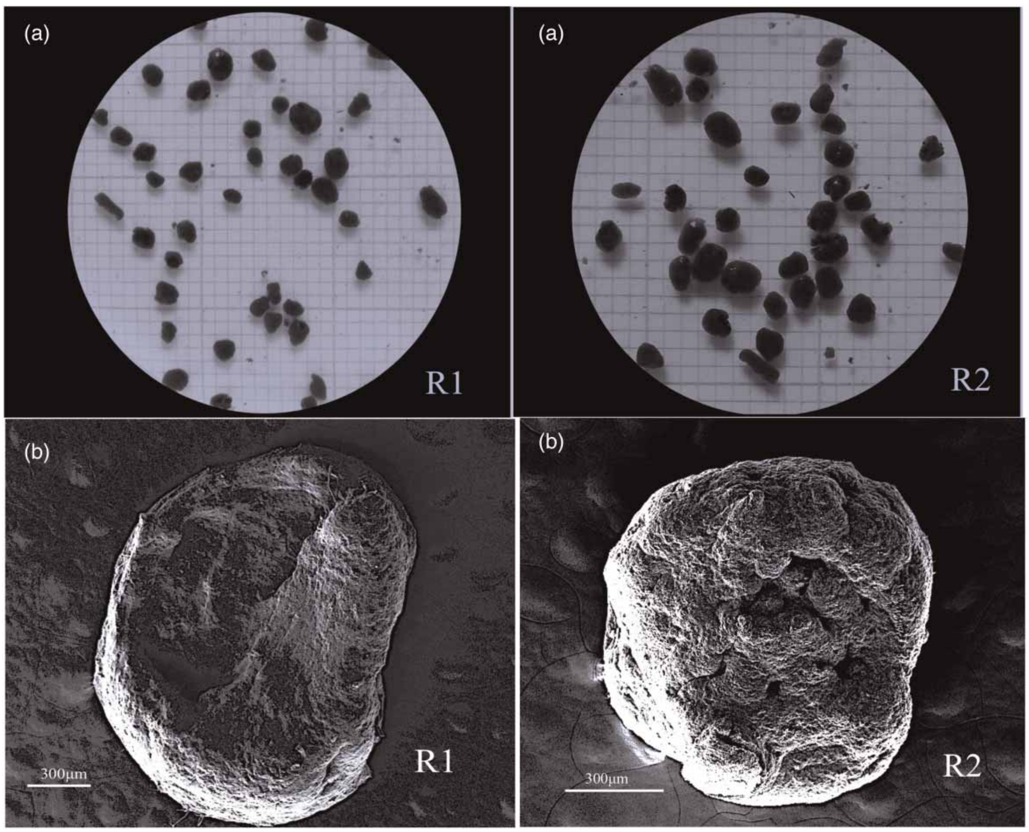

Figure 1 | Image analysis of AGS after 60 days' operation: (a) photograph of AGS and (b) SEM image of the non-filamentous AGS in R1 and R2.

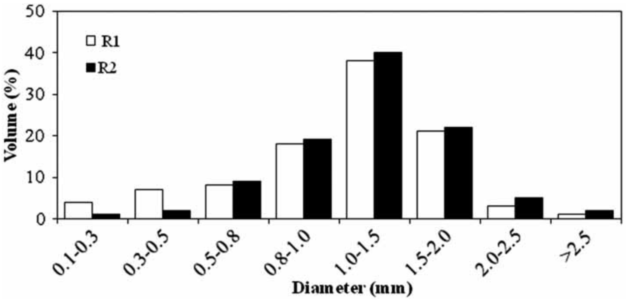

Figure 2 | Size distributions of aerobic granules at day 60.

about 59% of the total volume of sludge from R1 while in R2 about 62% of the granules are inside the size range. Thus, the aerobic granules at lower circulation rate had a high volume percentage of small granular size range (0.1–0.5 mm), while the granules at higher circulation rate had a high volume percentage of larger size (>0.8 mm) and became more compact. In view of the hydraulic characteristics, the higher circulation rate could lead to more frequent collision between granules and stronger friction between granules and liquid, and the aerobic granule formation could be enhanced through a purely physical aggregation. This situation also improved the size of the aerobic granules as observed in the case of R2. Therefore, a certain shear force in the granulation system is necessary in order to form dense aggregates (Liu & Tay 2002). The characteristic of dense aggregates was associated with the presence of EPS, as the EPS production may strengthen the structure of aerobic granules and it is important in building up and maintaining the structure of aerobic granules (Tay *et al*. 2001). However, aerobic granules that were exposed to the lower circulation rate tended to have lower shear force and less friction between granules and liquid. As a result, less dense granules developed where the cells loosely grown on the surface are removed, and the size was reduced or disintegrated.

Biomass profile and physical characteristic of aerobic granules

R1 and R2 were started with same seed sludge, and the biomass concentration was relatively similar, that is, 25.8–28.1 g MLSS/l and 13.3–15.6 g MLVSS/l, with poor settling properties (initial SVI was close to 50 ml/g). At the end of the operation, the MLSS and MLVSS in R1 gradually accumulated up to 8.5 g MLSS/l and 7.2 g MLVSS/l while in R2, higher values of MLSS and MLVSS were achieved (10.1 g MLSS/l and 9.0 g MLVSS/l). SV and SVI could show the settling ability of AGS. The SV of aerobic granules in R1 and R2 was 47 and 40 m/h, respectively. The SVI of aerobic granules in R1 (30 ml/g) was higher than R2 (25 ml/g). The reduction of SVI and increment of MLSS both resulted from the development of compact aerobic granules where the shear force has been sufficiently involved in the formation of aerobic granules. From the results, it appeared that the settling ability of AGS was partially affected when the circulation process was applied during the inoculation process.

The sludge properties for MLVSS/MLSS in R1 and R2 are shown in Figure 3. The ratio of MLVSS to MLSS of seed sludge was low at the start for R1 and R2 (51.6% and 55.4% respectively) due to the low substrate/biomass (food/microorganism: F/M) ratio, which was only 0.10 g COD/(g SS·d) in both reactors. On day 25, the MLVSS/MLSS in R1 was 20% lower than R2. The accumulation of inert substances in R1 resulted in a decrease of the MLVSS/MLSS where MLVSS represents the volatile (organic) fraction of the MLSS, in the lower circulation rate reactor. In time, the F/M ratio increased gradually and reached 75.5% (R1) and 76.7% (R2) of MLVSS/MLSS on day 35. Thereafter, they became stable at the end of the operation. The high MLVSS/MLSS ratio showed that the inorganic substance concentration in R2 was less than in R1. It may also be related with the high organic loading rate supplied into the reactor. In R1, the ratio of MLVSS to MLSS increased continuously from 51.6% of seed sludge to 84.9% of granules, while in reactor R2 the MLVSS/MLSS ratio initially increased from 55.4% and then progressively increased up to 89.0%.

Performance of AGS under different circulation rate

From day 35 of the operation, the COD removal was increasing in both reactors until the end of the study (60 days) as shown in Figure 4(a). Within 2 weeks of operation,

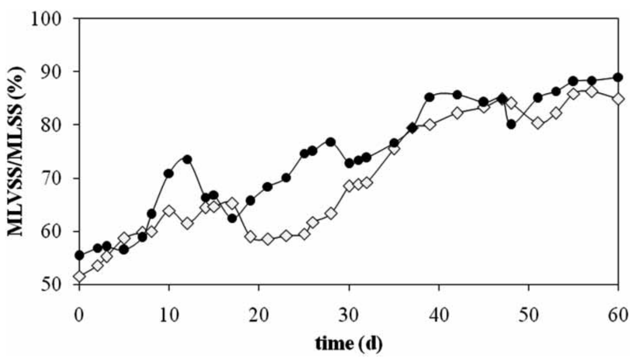

Figure 3 | Evolution of MLVSS/MLSS in both reactors R1 (◇) and R2 (•).

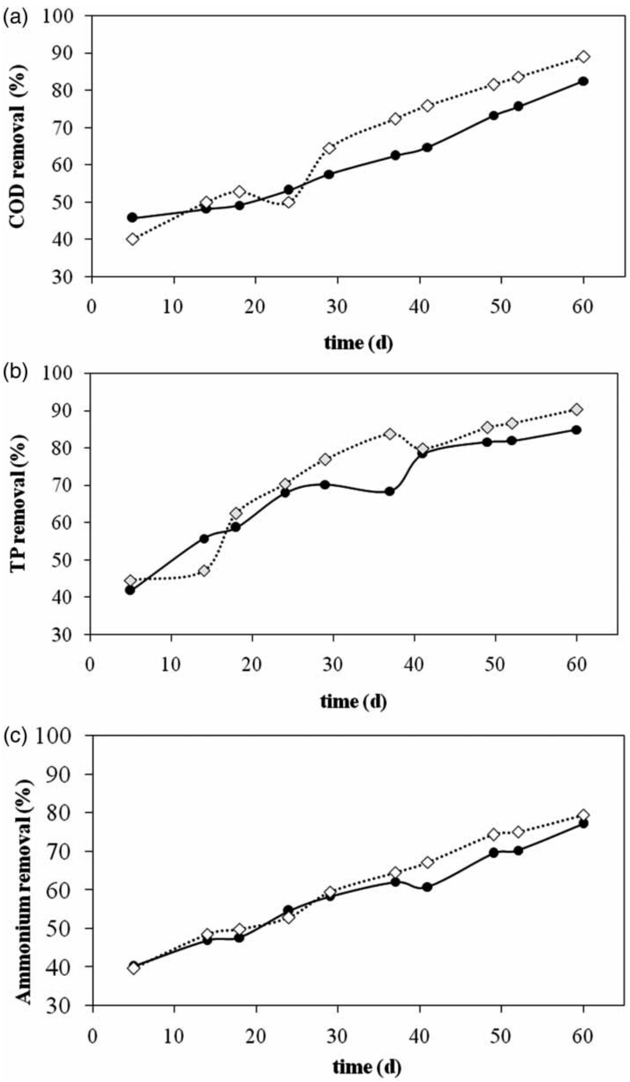

Figure 4 | Profile of removal performances in AGS reactor: (a) COD, (b) TP and (c) ammonia. (•) R1, (◊) R2.

the COD removal efficiency in both reactors reached 50% when the influent COD was around 5,400 mg/l. Furthermore, an increase in the COD removal efficiency was observed in R1 from day 37 to 41. During this period, the effluent COD increased from 1,414 to 1,746 mg/l. These results suggest that a moderate circulation rate reduced mutual collisions among sludge granules, resulting in unstable granular sludge. At the end of the operation, around 82 and 89% of COD removal efficiency was observed in R1 and R2, respectively. These results indicated that minor differences in terms of COD removal in both reactors as the circulation rate varied and the appropriate hydrodynamic shear force would favour the formation of dense aerobic granules with compact microbial communities. The compact aerobic granules had a good settleability and an efficient solid–liquid separation which could ensure the responsible microorganism for COD treatment was maintained and retained in the reactors to continue the biodegradation process of the organic materials.

TP removals in the anaerobic–aerobic granular system kept rising with time with a good performance under either high or low circulation rate (Figure 4(b)). The removal of TP gradually increased to about 78% in both reactors. In R2, the TP removal oscillated between 80 and 86% at day 41 to 49 of operation with a mean efficiency of 73%. At the end of operation, R1 achieved 84.9% TP removal, which is lower than the TP removal rate in R2 (90.3%). The effluent TP achieved in both reactors was below 10 mg/l and it meets the Malaysia legislation for environmental requirement. From the experiment, the circulation rate did not have an obvious effect on the TP removal in the granular system. As reported by Marcelino *et al.* (2011), Liu *et al.* (2012) and Pan *et al.* (2013), a single SBR system with a combination of anaerobic and aerobic reaction in the operating conditions is an efficient degradation pathway for phosphorus. Furthermore, the mixed liquor circulation could enhance the mass transfer between the substrate and granules during the anaerobic phase, thus accelerating the granulation and degradation process (Zhang *et al.* 2009). However, low external mass transfer due to the lower circulation rate limits the performance of the treatment, including microbial growth and biodegradation.

The NH_4 (40 mg/l influent) was almost removed within 8 h of operation in the anaerobic–aerobic granular system. The percentage of NH_4 removal increased to 77% in R1 and 79.5% in R2 towards the end of the experiment from 40.4% (R1) and 39.7% (R2) at the beginning of operation as shown in Figure 4(c). From the result, at 24 days of operation, the NH_4 removal percentage was only 54.6% in R1 and 52.8% in R2. At this stage, controlling circulation rate favours the enhancement of the microbial community, but the effect on the NH_4 removal performance was not significant, even though the aerobic granules enriched in the reactors were capable of denitrification and removal of NH_4. The higher NH_4 removal performance may be attributed to the fact that the speed of nitrification and denitrification became faster with an increase in circulation rate and hydrodynamic shear force. Tsuneda *et al.* (2001) reported that the existence of aerobic granules in the reactor promotes the retention of large amounts of nitrifying bacteria, which guarantees highly efficient nitrification in the system.

CONCLUSIONS

This study aimed to evaluate the influence of circulation rates in anaerobic phase on AGS formation and performance, which operated in an anaerobic–aerobic SBR system. A compact and dense AGS could be developed in both reactors, with a clear, round outer shape under higher (36.0 l/h) and lower (14.4 l/h) circulation rate because the hydrodynamic shear force created by the circulation process in both reactors enhanced friction and collision between liquid and granules. From the results, we found that the circulation rates induced granulation in both reactors, but showed different effects on the removal performance of effluent and characteristics of aerobic granules. The aerobic granules under higher circulation rate in R2 had better settling ability with 25 ml/g of SVI and had higher MLVSS/MLSS ratio (89%). The high MLVSS/MLSS ratio in R2 was due to high potential biomass retained in the reactor. The excellent removal performance in R2 confirmed that high circulation rate could favour the formation of granular sludge in the anaerobic–aerobic SBR system, and average removal efficiencies from day 50 onwards of 86% COD, 89% TP and 77% NH_4 (77%) were achieved.

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