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Characterization of Aerobic Granular Sludge Developed under Variable and Low Organic Loading Rate

(Pencirian Enap Cemar Berbutir Aerobik Dibangunkan di bawah Pemboleh Ubah dan Kadar Pemuatan Organik Rendah)

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ABSTRACT

Understanding the formation of aerobic granules sludge (AGS) under the variations of organic loading rate (OLR) could give a different insight on AGS stability, which had become the bottleneck for practical application in sewage treatment. This study demonstrates the formation of AGS that had previously been stored for eight months at 5°C in sequencing batch reactor (SBR) with sewage as substrate. Despite being redeveloped under variable OLR of 0.26 to 0.81 kg CODs/m³ d and low superficial air velocity (SAV) of 1.33 cm/s, the loose structure of AGS during storage can be recovered within 46 days of formation process. Variations in OLR intrude the formation process particularly during low OLR, resulting in longer period to achieve mature AGS or full granulation of biomass in reactor. The next-generation sequencing (NGS) analysis indicated that the shift in microbial community from Rhodocyclaceae to Comamonadaceae class for denitrification process was accommodated with the changes in the AGS size from 326 µm to more than 600 µm.

Keywords: Aerobic granular sludge; next generation sequencing; sewage; stability

ABSTRAK

Memahami pembentukan enap cemar berbutir aerobik (AGS) di bawah variasi kadar pembebanan organik (OLR) boleh memberikan pandangan yang berbeza pada kestabilan AGS, yang menjadi masalah utama untuk aplikasi praktik dalam rawatan kumbahan. Kajian ini menunjukkan pembentukan semula AGS yang sebelum ini telah disimpan selama 8 bulan pada 5°C, dalam reaktor penjujukan berkumpulan (SBR) dengan kumbahan sebagai substrat. Walaupun dibangunkan semula di bawah pemboleh ubah OLR daripada 0.26 kepada 0.81 CODs kg/m³ d dan halaju udara cetek rendah (SAV) 1.33 cm/s, struktur longgar AGS semasa penyimpanan dapat dipulihkan dalam tempoh 46 hari daripada proses pembentukan semula. Variasi dalam OLR mengganggu proses pembentukan terutamanya semasa OLR rendah dan menyebabkan tempoh yang lebih lama untuk mencapai AGS matang atau pembutiran penuh biojisim dalam reaktor. Analisis penjujukan generasi akan datang (NGS) menunjukkan pertukaran komuniti mikrob daripada kelas Rhodocyclaceae kepada Comamonadaceae untuk proses denitrifikasi adalah disebabkan oleh perubahan dalam saiz AGS daripada 326 µm kepada lebih 600 µm.

Kata kunci: Enap cemar berbutir aerobik; kestabilan; kumbahan; penjujukan generasi akan datang

INTRODUCTION

Organic loading rate (OLR) is an important operational parameter of a bioreactor, which is also an indicator to evaluate the processing capacity of the reactor (Long et al. 2015). In a sequencing batch reactor system (SBR), OLR is dependent upon substrate concentration and hydraulic retention time (HRT) (Ni et al. 2009). OLR exert the granulation process by selecting and enriching different microbial species and thus, influencing the size, morphology and kinetic behaviour of aerobic granules (Moy et al. 2002). This is in agreement with the work by Awang and Shaaban (2016), which indicated that biomass production or bacterial growth proliferates with the increase in OLR.

Filamentous bacteria will have a higher specific growth rate compared to floc forming bacteria at a low OLR. The outgrowth of filamentous bacteria will subsequently cause aerobic granules instability, and subsequently lead to poor separation of treated water from the biomass (Aqeel et al. 2016). Under low OLR, diffusion of substrate will become a limiting factor in aerobic granules formation (Li et al. 2008), thus tend to decrease the substrate removal rate. Peyong et al. (2012) indicated that compact aerobic granules subjected to low OLR conditions will end up as loose structure and cause the existence of filamentous. This might be attributed to the nature of filamentous to excellently fit in the loose structure and lead to the development of irregular shape. Although these abovementioned of low OLR could retard aerobic granulation in a varied degree, report by Corsino et al. (2017) proved that low OLR and long reaction time had stimulate the stability of aerobic granules by producing more storage products to sustain metabolic activity while the substrate availability is lower.

Controlling the OLR in the operation of real wastewater is challenging and attributable to the variation in influent

parameters (Awang & Shaaban 2016). The majorities of previous studies on aerobic granules treating low strength wastewater were carried out solely using synthetic wastewater with fixed chemical oxygen demand (COD) concentration and were put under high shear force. In a column-type upflow reactor like SBR, hydrodynamic shear force effect is often governed by the strength of superficial upflow air velocity (SAV). A higher shear force is preferable for the development of regular, rounder, stronger and more compact granules (Tay et al. 2002). In practice, high SAV will subsequently lead to the increase in operational cost for aeration energy requirement. Although Pronk et al. (2015) have successfully implemented aerobic granules technology for the treatment of sewage at full-scale with detailed description of the process, conversions, energy usage and design consideration, relatively, the obtained information regarding the characteristics of AGS with respect to OLR variations were insufficient. Therefore, further enhancements to characterize the behaviour of AGS developed in real wastewater with respect to variable and low OLR have to be established.

In this context, we studied the characteristics of aerobic granules to understand the behaviour of aerobic granules during the formation process. The dynamic granulation process under OLR range of 0.26 to 0.81 kg CODs m^{-3} d^{-1} and low SAV of 1.33 cm s^{-1} was evaluated based on the physical size, morphology, biomass production and reactor performance. The shift in the microbial community was analysed using polymerase chain reaction (PCR) coupled with next-generation sequencing (NGS) method. NGS is a new method that helps to identify microbial community based on percentage quantity rather than similarity.

Materials and Methods

Reactor Setup, Seed Preparation and Sewage Collection

A lab-scale SBR with a working volume of 4.0 L and H/D ratio of 10 was used for aerobic granules formation process. The operating strategies for SBR comprised of two phases. During Phase 1, cycle time was 4 hour with filling, reaction, settling and discharge time fixed to 30, 170, 30 and 6 min, respectively. During Phase 2, the cycle time was changed to 3 h with filling times of 30-10 min, reaction times of 110-145 min and settling times of 30-13 min; the adjustment was made according to the performance of AGS. Effluent standpipe was fixed for a volumetric exchange rate of 50%. Aeration was supplied from the bottom of SBR at an air flow rate of 4 L/min resulting in SUAV of 1.33 cm/s. The SBR was operating at room temperature without pH control. During the start-up, 1.0 L of aerobic granules which had been stored for eight months at 5ºC were used as seeds instead of activated sludge. The seeds with size ranging from 154 to 374 µm were re-activated by means of 1 day aeration, resulting in an initial concentrated mixed liquor suspended solids (MLSS) of 0.7 g/L. The raw sewage was collected from Damansara STP and taken directly from primary clarifier. Sewage was stored at 5ºC and used within 18 days. Beyond 18 days, the COD concentration will drop significantly. During this time, the settled suspended solids from sewage tanks were removed. Influent for the SBR was replaced every two to three days. Sewage characteristic is highly dependable on the weather during collection time.

Analytical Methods

Parameters such as MLSS, mixed liquor volatile suspended solids (MLVSS), CODs, suspended solids (SS), volatile suspended solids (VSS) and sludge volume index (SVI), were analysed in accordance with APHA standard methods (2005). Ammonium, nitrite and nitrate were determined by photometric determination using Merck test kit. Spectrophotometer Spectroquant® Pharo 100 with a wavelength range of 320-1100 µm was used to measure the photometric absorbance. The pH was measured just before the reaction time ended. Measurements of all parameters were carried out two to three times per week.

Morphology and Size Measurement

Aerobic granules sizes were measured under a digital microscope, DSX500 Opto with 5× magnification lenses. Geometric shapes and angles on screen were measured automatically with an image acquisition of 2D. Microscope slide with double concave (Ø15 - 18 mm) was used, since samples in wet condition tend to shrink or hydrated on normal microscope slide. The morphology of aerobic granules surface was observed with field emission scanning electron microscope (FESEM) of Quanta FEG-450 model. Sample preparation of aerobic granules for FESEM was described in previous report (Awang & Shaaban 2015).

Microbial Community Structure by NGS

The 10 000 g of biomass were collected at the end of the reaction phase was centrifuged at room temperature for 30 s. Water was removed to provide approximately 0.25 g of biomass or aerobic granules samples. DNA was isolated from aerobic granules using PowerSoil®DNA isolation kit (catalogue no. 12888, MoBio). The pure extracted DNA was kept at -20ºC until microbial identification was carried out. The platform for NGS used was based on Illumina Miseq. Method described for DNA quality control, bioinformatics analysis and taxonomic classification was provided by Sango Biotech (Shanghai) Co., Ltd.

DNA concentration and purity was measured using agarose gel electrophoresis technique. DNA concentration required for PCR reaction was quantified using Qubit 2.0 DNA kit. The primer for PCR was designed using a blend of universal primer of Illumina MiSeq sequencing platform. Agarose gel electrophoresis was run to test the PCR products and DNA was recovered by Sangon agarose recover kit. Qubit 2.0 was used to quantify the recovered products based on a mixture of 1:1 ratio with DNA concentration. Mixed sample was given a shake and later used for subsequent sample library construction and sequencing.

PRINSEQ software was used to truncate data with low-quality. Flash software was used to fuse dual-terminal sequences and then split the data to samples by barcodes. Primer and barcodes were then trimmed from the resulting sequences using PRINSEQ software. UCHIME software and sequence in Silva data as template was used to filter out PCR chimeras. Only samples with sequences length of over 450 bp were used for taxonomic classification analysis. The sequences reads were clustered into operational taxonomic units (OTU) based on the distance between sequences. OTU clustering software used was UCLUST. Sequence similarity was set at 0.97 and OTU was considered to be close to genus probably. The representative sequences from each OTU were subjected to RDP Classifier software and Greengenes database were used to classify taxonomic assignment based on Bergey's taxonomy.

RESULTS

AGS SIZE AT DIFFERENT PHASES OF FORMATION PROCESS

Figure 1 exhibits the evolution of inoculated seed on day 0 to mature aerobic granules on different days of formation period. The deviation in aerobic granules size at different days which was reflected on the length of the box plot was presented in Figure 2(a). As shown in day 0, the inoculated seeds that were previously kept in cold room at temperature of 5ºC for eight months are aerobic granules with median size of 245 μm and fuzzy structure. Disintegration of aerobic granules occurred within 24 h of start-up resulting in the formation of pin flocs on day 1. It appears that the aggregation of pin flocs started from day 1 and by day 8, the median size of pin flocs promptly increased from 13 to 326 μm. Although the aerobic granules' size continues to increase, most of the sludge was washed out from SBR from day 1 due to the excessive disintegration process resulting in a rapid decrease in MLSS from 0.45 to 0.1 g/L (Figure 2b). A sharp decrease in CODs influent from 231 mg/L on day 8 to 93 mg/L on day 13, once again, had resulted in low MLSS and MLVSS.

The deviation of aerobic granules' size on day 32 which is between 482 to 1396 μm is within the range of aerobic granules size on day 18, which is 395 to 2137 μm. This indicates that the granulation process for fluffy aerobic granules on day 18 has started. This result is in line with the pattern of SVI profile (Figure 3a) that illustrates a continuous increase in SVI_{30} from 25 mL/g on day 18 to 83 mL/g on day 22 onwards. MLSS decreases following the decrease in CODs influent from 161 mg/L on day 27 to 86 mg/L on day 32, thereby leads to a reduction of around 50% of SVI_{30} on day 36 and 41. This probably suggests that small aerobic granules with minimum size of 482 μm are predominant in the reactor on day 32 and were washed out afterwards due to substrate limitation. In order to ensure high availability of substrate, cycle time was adjusted from 4 to 3 h (Phase 2) after analysis on day 33 with other operating conditions remained unchanged.

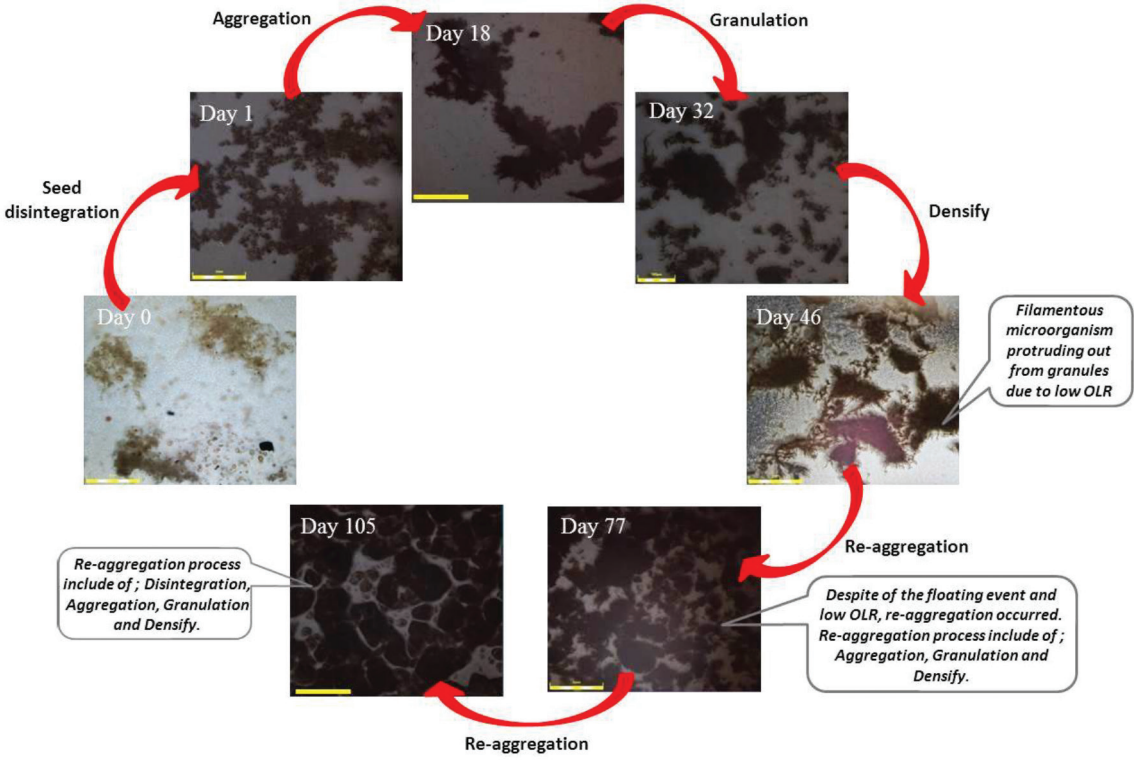

FIGURE 1. Evolutions of aerobic granules develop in variable and low OLR. Bar = 1 mm for day 0, 1, 77 and 105. Bar = 500 μm for day 18, 32 and 46

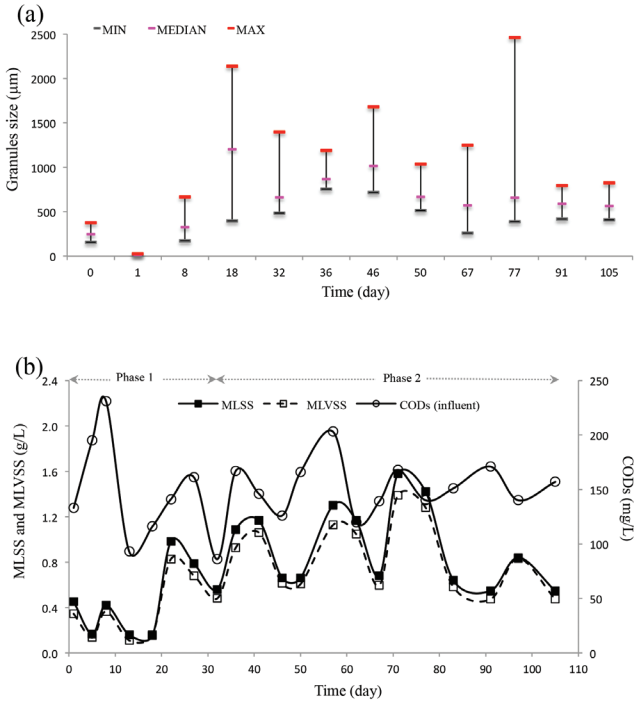

FIGURE 2. (a) Deviation in aerobic granules size (b) Profile of biomass (MLSS and MLVSS) and CODs influent

Afterwards, the predomination of dense aerobic granules with sizes between the range of 754 and 1188 µm on day 36 exhibited a starting point for densification process. This was supported with the SVI profile that showed a small deviation between 5 min of intervals of settling time and a continuous increase of MLSS from 0.56 g/L on day 32 to 1.09 g/L on day 36 onwards. Microscopic image of aerobic granules on day 46 indicated an apparent existence of filamentous microorganism protruding from aggregates and this pattern continues until day 50 and 67 when CODs influent was reduced to around 144 mg/L. This result is in line with a study by Peyong et al. (2012) which indicated that the compact granules subjected to low substrate conditions will end up with loose structure and the existence of filamentous. This might be attributed to the nature of filamentous that excellently fit in loose structure leading to the development of irregular shape.

It appears that some aerobic granules were floating and entrapped into effluent discharge point during feeding time on day 57. In turn, SVI_{30} was noticeably reduced from 70 mL/g on day 57 to 53 mL/g on day 62. This was expected due to the long feeding time of 30 min which may create an anoxic condition inside the reactor and helps to govern the denitrification process that releases nitrogen gas within the sludge layer. From observation, aerobic granules started to float after 10 min of feeding time. Similar floating event also occurred on day 71 resulting in decreased SVI_{30} and MLSS on day 77. Hence, in order to avoid the floating event, the feeding time was changed from 30 to 10 min on day 82. Settling time was also changed from 30 to 15 min on day 82 after considering the SVI profile for settling properties of aerobic granules and high CODs influent.

Despite the floating event, aerobic granules size continued to increase on day 77 indicating the occurrence of re-aggregation process. The significant change in feeding and settling time contributed to a sharp decrease of MLSS on day 83 which was from 1.42 g/L on day 77 to 0.64 g/L. Anyhow, MLSS kept increasing afterwards. Large aerobic granules with maximum size of 2463 µm on day 77 were disintegrated due to the change in operating conditions which in turn led to the fraction of aerobic granules with 400 to 800 µm size ranges on day 91 and 105, respectively. The results showed that MLSS was only reduced to 0.55 g/L in line with the decrease in CODs influent and change in operating conditions. This suggests that aerobic granules system is able retain sufficient amount of biomass or active microbial to recover from shock loading and change in operating conditions within a short time. Biomass profile analysis showed that the produced aerobic granules were highly dependent on CODs influent attributed by size and the maturity state of aerobic granules.

As shown in Figure 3(b), food to microorganism (F/M) ratio did not substantially increase with significant changes in CODs influent and MLSS between days 40 and 83. This indicates that the SBR system is in stable conditions and comprises mature aerobic granules that could easily adapt and stabilize in various substrate concentrations and change in operating conditions. In addition, SRT during stable conditions was between 5 to 10 days which are suitable for the aerobic granules system.

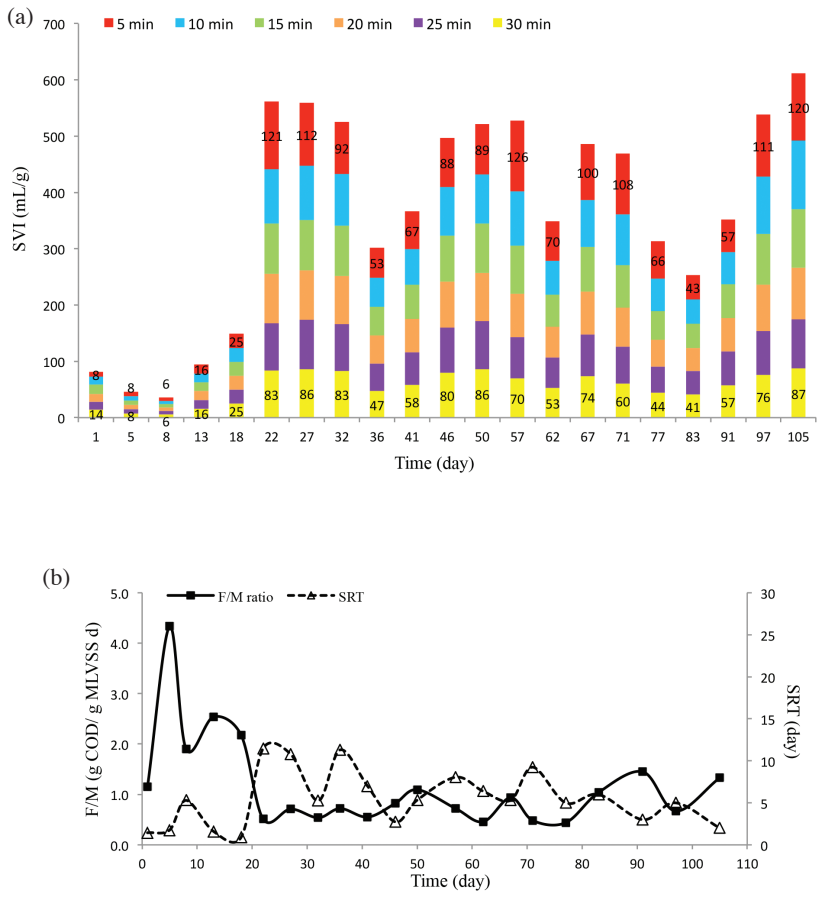

FIGURE 3. (a) Profile of SVI at 5 min intervals of settling time (b) Profile of F/M ratio and SRT

REACTOR PERFORMANCES

During the formation process, the performances of aerobic granules in terms of CODs, SS and nitrogen removal were determined. Figures 4(a) and 4(b) depict the influent and effluent concentrations as well as the removal efficiency of CODs, SS and ammonium during the re-formation process. During the first 18 days, aerobic granules were in aggregation stage and poor CODs and SS removal were expected. Negative removal for SS at an early stage indicates that biomass was excessively being washed out from the reactor. Afterwards, aerobic granules' performance improved consistently at stable condition whereby the highest removal of CODs, SS and ammonium was marked at day 57 with 69%, 72% and 83%, respectively. As for the concentrations of nitrite and nitrate in the effluent during stable periods, the respective average values are 17 and 11 mg/L. Poor CODs removal and decrease in pH at the end of reaction time to 5.8 was attributed by low COD/ammonium ratio during Phase 1 and Phase 2, which were 3.4 and 3.9, respectively. CODs removal was at its lowest when CODs influent decreased sharply on days 13, 32, 46, 62 and 77.

MICROBIAL COMMUNITY STRUCTURE OF AGS

In order to demonstrate the evolution of microbial community, 55,495 totals of effective, qualified sequences were assigned to known phylum, class, order, family and genus based on Bergey's taxonomy system. Analysis was carried out on day 0, 8 and 50 to represent seed, aggregation and granulation phase while day 77 and 105 denote re-aggregation phase. Most of the sequence reads were affiliated with two major phyla out of 25 detected phyla in total, namely *Proteobacteria* and *Bacteroidetes*. The similarity of microbial community at class level from day 0 (seed) to the end of formation process is shown in Figure 5. The *Betaproteobacteria* and *Sphingobacteriia,* which belong to the *Proteobacteria* and *Bacteroidetes* phyla, dominated after eight days of formation process, although they are not the dominant groups in seed. Total sequences percentage for the first five taxonomic classifications (*Sphingobacteriia, Betaproteobacteria, Alphaproteobacteria, Gammaproteobacteria* and *Flavobacteriia*) from *Proteobacteria* and *Bacteroidetes* phyla indicate a shift in microbial domination in seed 52% to 86%, 92%, 94% and 93% on days 8, 50, 77 and 105, respectively. While, *Gammaproteobacteria* was a subdominant class on day 8 with sequences percentage of 25% and promptly decreased to 10% on day 105, suggesting that this class is beneficial to the aggregation process at an early stage.

At genus level, pyrosequencing taxonomic classification of bacterial community (Figure 6) showed that the seed and aerobic granules on day 8 contained a greater microbial

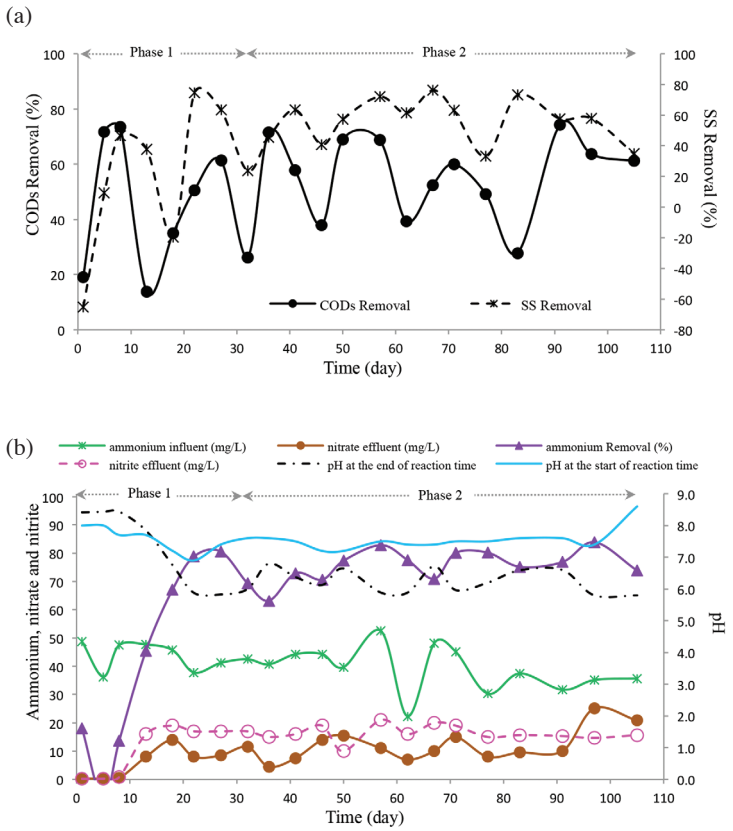

FIGURE 4. (a) VSS effluent, SS and CODs removal (b)Profile of nitrogen effluent and pH at the end of reaction time

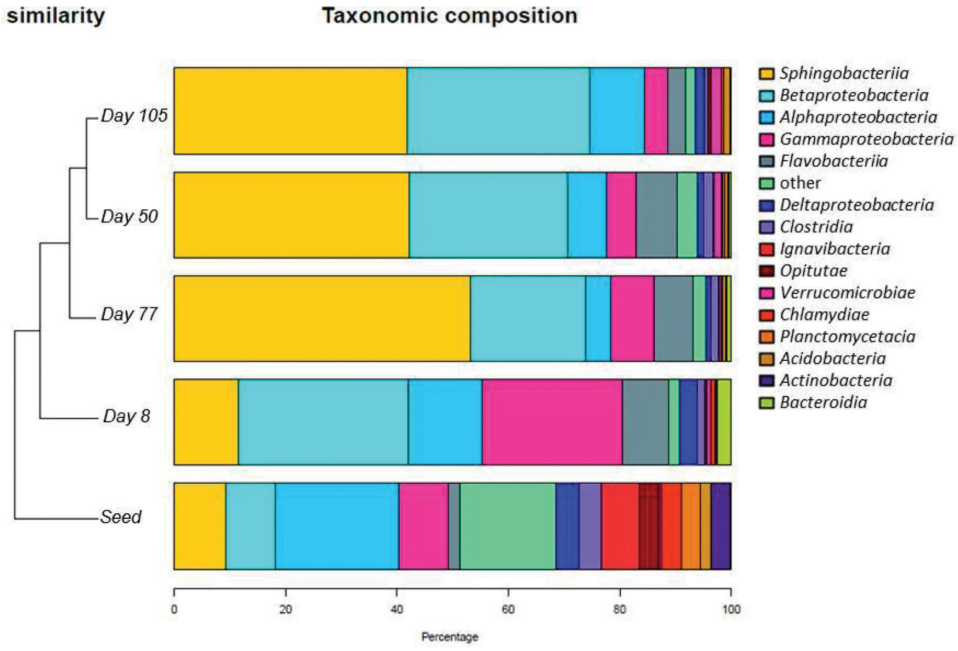

FIGURE 5. Cluster analysis for the first 15 taxonomic classifications from bacterial community of seed, day 8, 50, 77 and 105 at the class level

diversity compared to the developed aerobic granules on days 50, 77 and 105. Aside from several dominant genus (*unclassified_Chitinophagaceae*, *unclassified_Saprospiraceae*, *Alicycliphilus* and *Comamonas*), most of the genus existing in seed on day 8 were suppressed to less than 1% of abundance sequences. *Ignavibacterium,* which

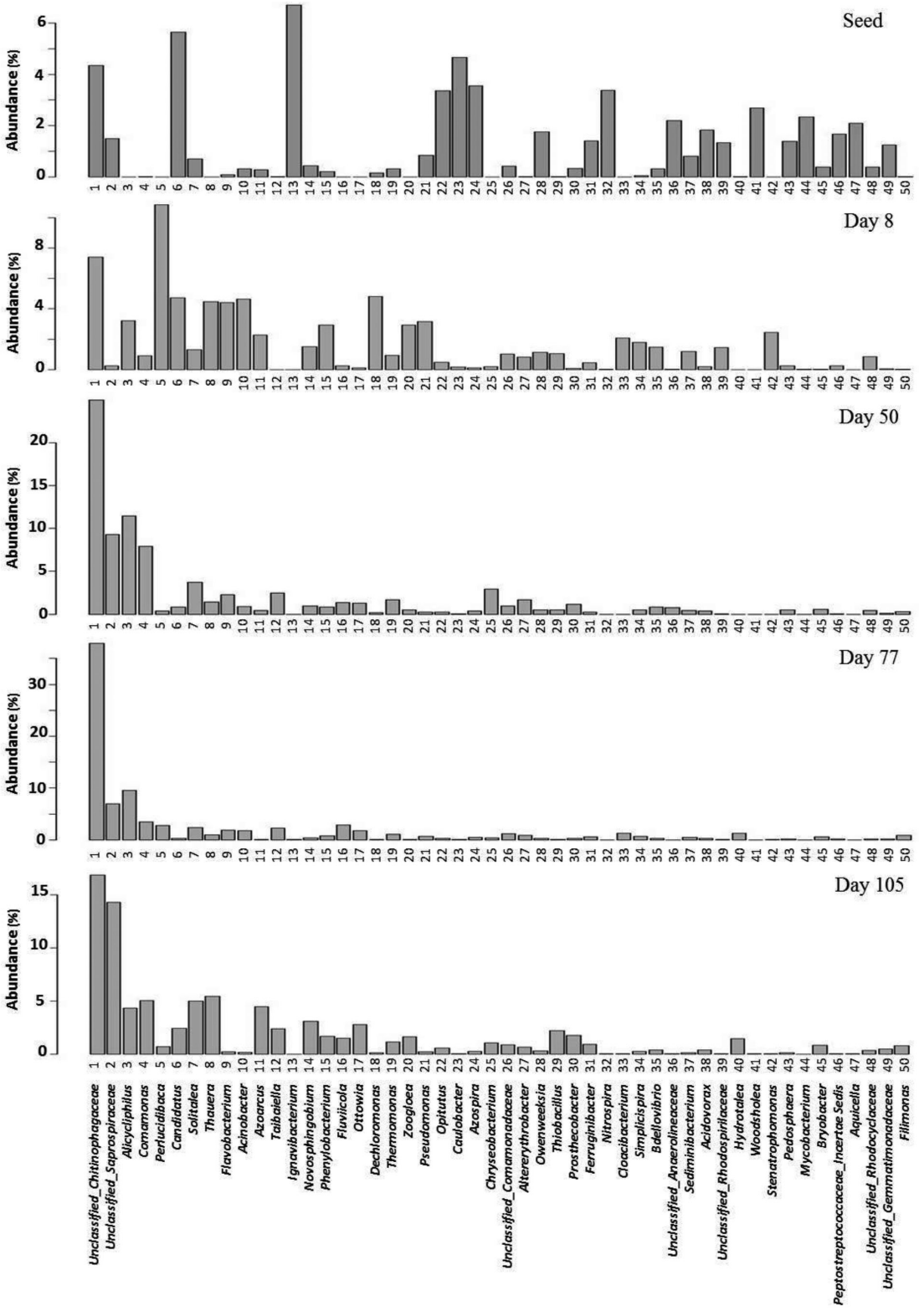

FIGURE 6. Taxonomic classification of pyrosequences from bacterial community of seed, day 8, 50, 77 and 105 at the genus level

belongs to *Chlorobi* phyla, appears to be most dominant in seed and followed by *Candidatus*, *Caulobacter* and *unclassified_Chitinophagaceae,* which accounted for 6.7%, 5.6%, 4.7% and 4.3% of abundance sequences, respectively. *Ignavibacterium* was known as facultative anaerobic organism suggesting its survival as heterotrophic bacteria (Rosenberg et al. 2014) during long term storage. Other genus like *Caulobacter* and *Nitrospira* (3.4%) were also significantly detected only in seed showing that this genus could thrive under condition of comparatively

scarce resource (Rosenberg et al. 2014) due to long term storage. The abundance of *Perlucidibaca*, *Acinetobacter* and *Pseudomonas* genus from *Gammaproteobacteria* class were dominant on day 8, while it was almost non-existent in the late reformation process.

The total abundance of *Thauera*, *Dechloromonas* and *Zoogloea* genus of the *Rhodocyclaceae* class were prevalent on day 8 with 12.4% when the median aerobic granules size was 326 µm and was reduced to less than 3% on days 50 and 77 when median aerobic granules size was more than 600 µm. *Comamonas* and *Alicycliphilus* genus from the *Comamonadaceae* class increased with a decrease in *Rhodocyclaceae* class on days 50 and 77 with total abundance accounted at 19.8% and 13%, respectively. As fraction of aerobic granules size on day 105 ranging from 410 to 825 µm, *Rhodocyclaceae* and *Comamonadaceae* class were nearly in balance with 7.2% and 9.5% of the total abundance. Thus, it can be concluded that the shift in microbial community from *Rhodocyclaceae* to *Comamonadaceae* class for the denitrification process can accommodate with the changes in aerobic granules size. The results from this study also suggested that the given stability period within day 46 to 83 is contributed by *Comamonas*. This is based on the finding by Lv et al. (2013) on the re-cultivation of drying aerobic granules, where polar flagellum of *Comamonas* might have contributed to the stability of aerobic granules. The result suggested that the dominance of particular bacteria is not dependable on the period of aerobic granules formation process. The results confirmed an increasing trend in the dominant abundance of *unclassified_Chitinophagaceae* and *unclassified_Saprospiraceae* from *Sphingobacteriia* class throughout the formation period. Further inquiry on the phylogenetic analysis using MEGAN software showed a shift in *Chitinophagaceae* family, from *Ferruginibacter* genus in seed, to *Sediminibacterium* genus on day 8 and *Taibaiella* genus afterwards. *Solitalea* genus, which belongs to *Sphingobacteriaceae* family, was clearly present on day 105 with total abundance of 5%.

SURFACE MORPHOLOGY OF AGS

Figure 7 shows the morphological evolution of inoculated seed to mature aerobic granules at different days of formation phase. Analysis was carried out on days 32 and 50 to represent aggregation and granulation phases, while days 77 and 105 denoted re-aggregation phase. The closed up images on the right hand side of Figure 7 were taken between 5 and 40 µm image scales. Long term storage of aerobic granules had resulted in excessive cell lysis for seed. Aerobic granules on day 32 exhibited an irregular surface and this pattern continues to day 50, whilst aerobic granules on day 77 displayed a smooth surface and at day 105, the cauliflower-like structure can be observed. Similar cauliflower-structure was observed by Lemaire et al. (2008) when the aerobic granules developed at substrate COD/N ratio of less than five.

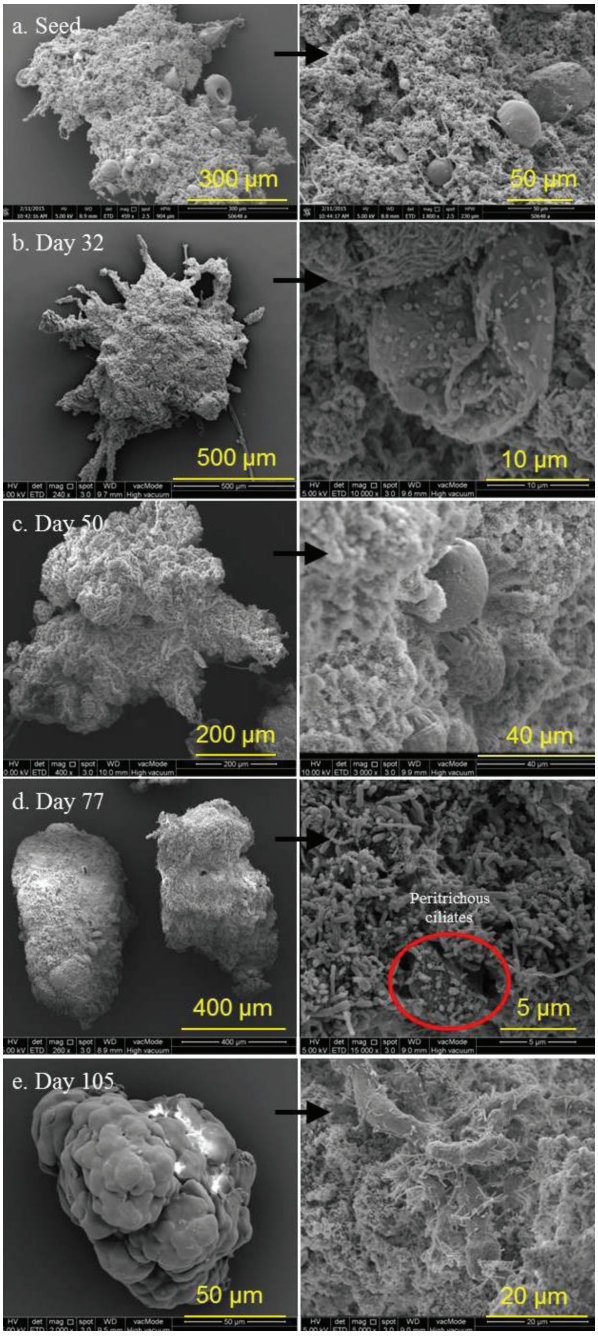

FIGURE 7. FESEM images of aerobic granules

The closed up images demonstrates the appearance of protozoa vorticella (seed and at day 50) and ciliates (day 77) which was previously proven to be beneficial to aerobic granules formation by Li et al. (2013). A closed up image on day 77 demonstrated peritrichous ciliate stalks serve as backbone in the granule-forming process since bacteria use them as a substratum to grow and this is in line with the report by Weber et al. (2007). Lemaire et al. (2008) and Weber et al. (2007) remarked that ciliates contributed to a reduction of SS effluent and turbidity by removing certain amount of non-flocculated bacteria and fine suspended biomass particle.

Discussion

In this study, microscopic observation of aerobic granules size evolution is in accordance with the dynamic granulation model developed by Zhou et al. (2014). The model helps to explain the enormous deviation in aerobic granules size obtained in this study which might be caused from the self-aggregation of aerobic granules and re-granulation of the detached bioflocs, newborn cells and crushed aged aerobic granules. The final aerobic granules sizes obtained in this study were in the range of 410 to 825 μm and were comparable with the results demonstrated by Ni et al. (2009), Peyong et al. (2012) and Su et al. (2012) for aerobic granules developed in sewage. The typical size of mature granules developed in sewage was assumed to be around 750 to 800 μm and can reach up to 2000 μm, depending on the selection of operating conditions. The variations in OLR will intrude the formation process particularly during low OLR and resulting in longer period to achieve mature aerobic granules or full granulation of biomass in the reactor. The produced aerobic granules exhibited a tiny oval or rod shape form instead of the desired sphere shaped due to the occurrence of substrate limitation before aerobic granules were well developed.

Comparing the result of microbial community in this study with other research works further prove that the dominance of particular bacteria is governed by substrate composition (type of wastewater) and concentration (OLR). For example, the domination of *Sphingobacteriia* class in this study is similar to the work by Zhou et al. (2014) on the formation of aerobic granules in sewage. On the other hand, findings by Adav et al. (2009) suggested that the aerobic granules cultivated with acetate as carbon source demonstrated dominancy genus of *Zoogloea,* whilst granules cultivated in swine slurry characterized by high carbon to nitrogen ratio were mainly composed of genus *Nitrosomonas* (Morales et al. 2013). The diversity and dominancy of the bacteria species were caused by the physiological characteristics of the bacteria. Zhou et al. (2014) has confirmed that microbial selection pressure was not a prerequisite for aerobic granulation from both the dynamic granulation steps and molecular biology aspects.

Despite the increasing number of studies that have been carried out on aerobic granules worldwide, many aspects of aerobic granules' formation and stability for real wastewater treatment remained uncertain. Characterization of aerobic granules is crucial to understand the intermolecular interaction of different microbial community that keep the aerobic granules stable (Awang & Shaaban 2016). Future studies should not focus solely on the formation of aerobic granules using synthetic wastewater. Since all of the theories regarding the formation of aerobic granules had been well documented and described using synthetic wastewater, the technology must be ready to be taken to a pilot scale treating real wastewater and finally to a full scale implementation.

Conclusion

Aerobic granular sludge was successfully developed under the variable OLR ranging from 0.26 to 0.81 kg CODs/m^3 d and low SUAV of 1.33 cm/s. Low CODs/N ratio of 3.4 for Phase 1 and 3.9 for Phase 2 contributed to the inhibition of pollutant removals with the highest CODs, SS and ammonium removals that were marked at day 57 with 69%, 72% and 83%, respectively. Variation in OLR intrudes the development of aerobic granules, resulting in longer period to achieve the stable conditions which is after 40 days of formation process. The result of this study had successfully demonstrated that system operation was not in total failure conditions when aerobic granules started to disintegrate due to the variations in OLR.

Acknowledgements

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