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Cultivation of aerobic granular sludge by modification of seeding condition

Laila Dina Amalia Purba[a], Ali Yuzir[b], Arash Zamyadi[c,d], Mohd Hakim Ab Halim[a], Ecaterina Daniela Zeca[e], Norhayati Abdullah[b,f,*]

[a]*Malaysia-Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100 Kuala Lumpur, Malaysia, emails: amalia.l@graduate.utm.my (L.D.A. Purba), mohdhakim@utm.my (M.H. Ab Halim)*
[b]*Department of Chemical and Environmental Engineering, Malaysia-Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100 Kuala Lumpur, Malaysia, emails: norhayati@utm.my (N. Abdullah), muhdaliyuzir@utm.my (A. Yuzir)*
[c]*Water Research Australia, Melbourne Water, 250 Victoria Square, Adelaide, SA 5000, email: a.zamyadi@unsw.edu.au*
[d]*Australia Water Research Centre, School of Civil and Environmental Engineering, University of New South Wales (UNSW), Sydney, NSW 2052, Australia, email: a.zamyadi@unsw.edu.au*
[e]*Universitatea "Dunărea de Jos" din Galați, Strada Domnească 47, Galați, Romania, email: Daniela.Zeca@ugal.ro*
[f]*UTM International, Jalan Sultan Yahya Petra, 54100, Kuala Lumpur, Malaysia, email: norhayati@utm.my*

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ABSTRACT

This study aims to cultivate aerobic granular sludge by using low-strength domestic wastewater. The cultivation was conducted by modifying the seeding condition whereby the seed sludge was mixed with anaerobic granular sludge. Sequencing batch reactor system consisted of two columns were used namely R_c as control reactor and R_e containing anaerobic granular sludge were utilized to produce aerobic granular sludge. The results indicated that anaerobic granular sludge may be used to induce rapid granulation process, resulting in aerobic granular sludge with more desirable characteristics as compared to the conventional activated sludge. The developed granular sludge exhibited excellent settling velocity (74.6 m/h) and low SVI_{30}. Total phosphorus removal was also enhanced in reactor R_e. Cocci-shaped bacteria were mainly observed on the granular surface along with few of rod-shaped and filamentous bacteria. Moreover, Proteobacteria dominated the microbial population in the aerobic granules. This study demonstrated the possibility to achieve rapid granulation using anaerobic granules and activated sludge as the seeding materials.

Keywords: Aerobic granulation; Biological nutrient removal; Domestic wastewater treatment; Low-strength wastewater

1. Introduction

As an advancing technology in the wastewater treatment system, aerobic granular sludge may be developed using various types of wastewater, including high-strength agro-based wastewater [1], piggery wastewater [2], dairy wastes [3], municipal wastewater and low-strength domestic wastewater [4,5]. The granulation system has been applied at various full-scale municipal and industrial wastewater treatment plants [6]. Nevertheless, the formation of aerobic granular sludge is a complex mechanism involving the interaction of microbial cells through physical, chemical and biological events [7]. The development of aerobic granular sludge is reported to be more favored in wastewaters characterized by high organic concentrations (chemical oxygen demand, COD) due to the high availability of readily biodegradable organic content [5].

* Corresponding author.

Ni et al. [8] were among the first to report the development of aerobic granular sludge in low-strength municipal wastewater with COD concentration ranging from 95–200 mg COD/L. Aerobic granular sludge was developed from conventional activated sludge after a 300 d start-up period by using low-strength municipal wastewater. The development of aerobic granular sludge started with the initial cell-to-cell attachment due to the neutralization of microbial cell's surface charge [9]. The attached microbial cells gradually develop into smaller bioflocs followed by the extensive production of extracellular polymeric substances (EPS), which may take longer than 200 d in order to achieve mature granules [6].

Numerous researches have been conducted in order to improve the development of aerobic granular sludge, including modification of reactor configuration, organic loading rate (OLR) and microbiological alteration [10,11]. A study on the modification of height to diameter (H/D) ratio towards aerobic granulation was reported [10] and it was concluded that a higher H/D ratio of a column may be used to induce granulation in the wastewater treatment process. Moreover, the addition of an external carbon source to increase the OLR of low-strength domestic wastewater was reported to shorten the start-up period of the aerobic granular sludge system [11]. However, these modifications may not be economically feasible especially during the full-scale application of aerobic granular sludge system.

Another approach to improve granulation in low-strength wastewater is to apply different seeding conditions, such as introducing crushed aerobic granules [12] and utilizing anaerobic granular sludge [13]. Pijuan et al. [12] investigated aerobic granulation by using crushed aerobic granules. The investigation on the development of aerobic granular sludge from crushed aerobic granules showed that it was able to induce a faster granulation process and reduce the risk of biomass washout. However, it is worth noting that aerobic granular sludge is not widely commercialized. Therefore, utilization of anaerobic granular sludge as the seeding may become an alternative for the development of aerobic granular sludge. A study by Hu et al. [13] reported the successful formation of aerobic granular sludge from anaerobic granules, whereby the anaerobic granules were firstly disintegrated into smaller bioflocs and act as a core for the development of aerobic granular sludge. Nevertheless, the development of aerobic granular sludge from anaerobic granules by using low-strength domestic wastewater requires further study, as actual domestic wastewater may have varied concentrations depending on the time and season of the year.

This study aims to utilize locally sourced domestic wastewater stream to cultivate aerobic granular sludge using different seeding conditions. A mixture of anaerobic granular sludge and conventional activated sludge was used as the seed sludge to achieve rapid aerobic granulation in low-strength domestic wastewater. This study focuses on the development of aerobic granular sludge using low-strength domestic wastewater, followed by the detailed characterization of developed aerobic granules, including the physical, chemical and microbiological features. This study also demonstrates the capability of aerobic granular sludge to be implemented in full scale domestic wastewater treatment plant (WWTP).

2. Materials and methods

2.1. Experimental set-up

Two custom-made sequencing batch reactor (SBR) columns with a working volume of 1.5 L were used in this study (modified from Arrojo et al. [3]). The first column or referred as R_c was seeded with activated sludge sourced from local WWTP, whilst another column namely R_e was seeded with a mixture of anaerobic granular sludge and activated sludge at a 1:1 ratio (v/v). Different seeding conditions was applied to enhance aerobic granulation in the SBR system [9,13]. The height to diameter ratio (H/D) was 17 as shown in Fig. 1. A 50% volumetric exchange rate (VER) was applied to the columns and aeration was provided from the air compressor (Atman, China) using a microsparger (Dwyer, USA) at the bottom of the reactor with a superficial air velocity of 1.74 cm/s. Both columns were operated in 3 h cyclic time, consisted of 5 min feeding, 161 min aeration, 10 min settling, 2 min effluent withdrawal and 2 min idle periods. During the feeding stage, 750 mL of domestic wastewater was introduced to each column using a peristaltic pump (Watson-Marlow, UK). Meanwhile, during the effluent withdrawal stage, 750 mL of treated wastewater was discharged from each column through a port located at 25 cm height of the column. Initially,

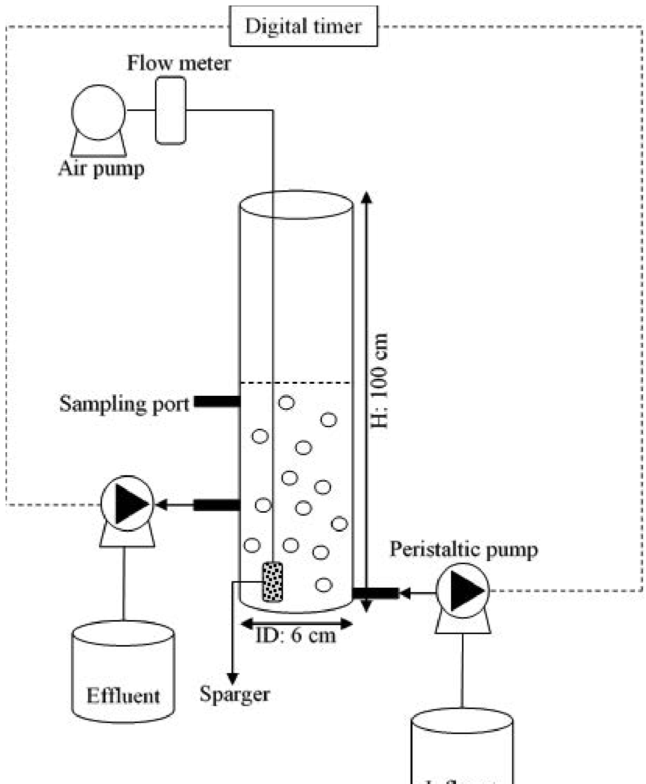

Fig. 1. Diagram of SBR column used in this study with H/D ratio of 17.

750 mL of seed sludge was introduced into both columns, whereby each column contained different seed sludge.

2.2. Characteristics of wastewater and seed sludge

In this study, locally sourced domestic wastewater and conventional activated sludge were utilized, whereby both samples were collected from Bunus wastewater treatment plant (WWTP). This particular treatment plant was chosen as it has adopted the SBR system. The wastewater and activated sludge samples were filtered through a 1 mm sieve in order to remove unwanted large debris that might clog the piping system in the reactor. Throughout the study, a total of 18 wastewater samples were collected (from the WWTP. Wastewater samples were characterized in terms of COD, total phosphorus (TP), NH_3–N, NO_3^-–N and NO_2^-–N prior to being used as influent. Table 1 summarizes the characteristics of domestic wastewater used in this study.

Meanwhile, the sample of the conventional activated sludge system was aerated in an incubator for at least 24 h for acclimatization purposes. In reactor R_c, conventional activated sludge was directly used as the seeding, while in reactor R_e, the conventional activated sludge was mixed with anaerobic granular sludge. Commercialized anaerobic granular sludge was obtained from a WWTP in Thailand and directly used as seeding. The anaerobic granular sludge was round-shaped, black colored with a diameter ranging between 4–5 mm.

2.3. Analytical method

Evaluation of the wastewater treatment efficiency was conducted by analyzing the effluent from the SBR system. The effluent sample was collected once every 2 d from the effluent tank. The samples were analyzed immediately, whereby the analyses include COD, TP and NH_3–N. The analyses were conducted using a UV-Vis spectrophotometer (DR5000, HACH, US) following the Standard Methods [14]. Meanwhile, observation on granular development was performed by conducting analyses of biomass concentration (mixed liquor suspended solids, MLSS) and settleability of the granular sludge (sludge volume index, SVI_{30}).

Characterization of aerobic granular sludge was conducted by the end of the study after the sludge harvesting procedure. Characterization of aerobic granular sludge includes physical characteristics and chemical characteristics. In terms of physical characteristics, the morphology of granules was observed using a digital microscope (SZX7 Olympus, Japan) as well as a field emission scanning electron microscope (FESEM, JSM-7800F, JEOL, Japan). The samples were prepared accordingly prior to FESEM analysis following method by Dahalan [15] which included the chemical fixation using 2.5% glutaraldehyde and drying process using gradient concentration of ethanol. Auto fine coater was chosen for 60 s with coating current 20 mA for scanning electron microscope imaging purposes. Moreover, granular diameters were determined from the microscopic images. The aspect ratio was calculated as the ratio of the shortest and longest dimension of the granular sludge using Eq. (1), where 0 equals to line meanwhile 1 equals to circle [16].

$$\text{Aspect ratio} = \frac{F_{\min}}{F_{\max}} \tag{1}$$

where $F_{\min}$: smallest diameter of granular sludge; $F_{\max}$: largest diameter of granular sludge.

The analysis on the settling velocity was conducted by observing the average time taken for individual granules to settle at a certain height of a glass column filled with tap water. The settling velocity of the granular sludge was estimated based on the time taken to reach the bottom of the column. In terms of chemical characterization, aerobic granular sludge was analyzed using energy-dispersive X-ray (EDX) attached to the FESEM. The elemental composition of aerobic granular sludge was reported from a random granular sludge sample.

Table 1
Characteristics of domestic wastewater sample

Elements	Concentration (mg/L)[a]
Chemical oxygen demand (COD)	177
Total phosphorus (TP)	25
Ammoniacal nitrogen (NH_3–N)	31.7
Nitrate nitrogen (NO_3^-–N)	19.4
Nitrite nitrogen (NO_2^-–N)	0.18
pH	7.27

[a]Based on average of 18 collected samples.

2.4. Molecular analyses

Molecular analyses were performed to study the microbial composition of aerobic granular sludge. Molecular analyses included the extraction of genomic DNA (gDNA) from aerobic granular forming sludge, polymerase chain reaction (PCR) to amplify the targeted DNA sequence and next-generation sequencing (NGS) to investigate the microbial community of aerobic granular sludge. Upon gDNA extraction, gel electrophoresis was conducted to confirm the presence of the extracted DNA. 1% TAE agarose gel was used and 50 ng of gDNA samples were run at 100 V for 60 min. The extracted gDNA were subjected to PCR to amplify targeted 16 s rRNA genes in V3 and V4 region by using forward primer (5′-CCTAYGGGRBGCASCAG) and reverse primer (3′-GGACTACNNGGGTATCTAAT). The PCR products were then used for library preparation. The library preparation and NGS were conducted using Illumina MiSeq (USA).

3. Results and discussion

3.1. Development of aerobic granular sludge

Both reactors R_c and R_e were inoculated with 1,500 mg/L seed sludge despite different seeding condition in each reactor, whereby reactor R_c was seeded with activated sludge and reactor R_e was seeded with a mixture of activated sludge and commercialized anaerobic granular sludge. The development of aerobic granular sludge in reactor R_c can be divided into two phases as shown in Fig. 2a. In reactor R_c, the seed sludge exhibited poor settling properties with SVI_{30} value of 147 mL/g. Throughout phase 1, biomass washout continuously occurred as the less dense sludge with poor settling properties was decanted together with the treated wastewater due to the poor settleability of the sludge. This phenomenon resulted in the fluctuation of biomass concentration and unstable reactor performance in the first 60 d of experimental period.

In phase 2, the activated sludge started to flocculate forming small bioflocs as depicted in Fig. 3a. This phenomenon was indicated by the stable increase of MLSS and low SVI_{30} values, whereby low SVI_{30} values demonstrate an excellent settleability of aerobic granular sludge. In phase 2, SVI_{30} values always remained below 100 mL/g. Moreover, by the end of the experimental period, the aerobic granular sludge has reached its maturation stage. Fig. 3b shows the mature aerobic granular sludge with a diameter ranging between 5–6 mm. The SVI_{30} and MLSS values reached 29 and 7,000 mg/L, respectively.

Development of aerobic granular sludge in reactor R_e can be divided into three phases as shown in Fig. 2b. Phase 1, which was the acclimatization period of the seed sludge, was indicated by the fluctuating values of both MLSS and SVI_{30}. In contrast to reactor R_c, the acclimatization period in reactor R_e took significantly less amount of time which may be due to the presence of anaerobic granular sludge as the seeding. Within 30 d of the acclimatization period, anaerobic granular sludge was disintegrated into smaller bioflocs as shown in Fig. 3c. This was aligned with previous research by Hu et al. [13] which also utilized anaerobic granular sludge as seeding in the aerobic granulation process. In phase 2, a significant decrease in SVI_{30} value was observed as the granulation process occurred. During this phase, the activated sludge is attached to the smaller bioflocs that act as a core and grow as aerobic granular sludge [13]. The SVI_{30} values throughout this period was found to be always lower than 20 mL/g. Moreover, mature aerobic granular sludge was observed with an average diameter of 6 mm as depicted in Fig. 3d.

However, after maturation phase was achieved, aerobic granular sludge exhibited disintegration process (phase 3). Starting from day-120, the significant increase in SVI_{30} value was observed from 14 to 48 mL/g, which indicated the deteriorating of settling properties in aerobic granular sludge. Moreover, the biomass concentration was also decreased throughout this period. However, there has been no clear evidence towards the factors affecting disintegration of aerobic granular sludge. The segregation might be due to the increase in granular size as the microbial growth is increasing rapidly in aerobic granules [9]. Therefore, further study may be conducted in order to fully understand the mechanisms of granular disintegration and the affecting factors to it. After 140 d of experimental period, the MLSS and SVI_{30} values were found to be 5,400 mg/L and 45 mL/g, respectively.

It may be concluded that, in reactor R_c with activated sludge as the seeding, the development process of aerobic

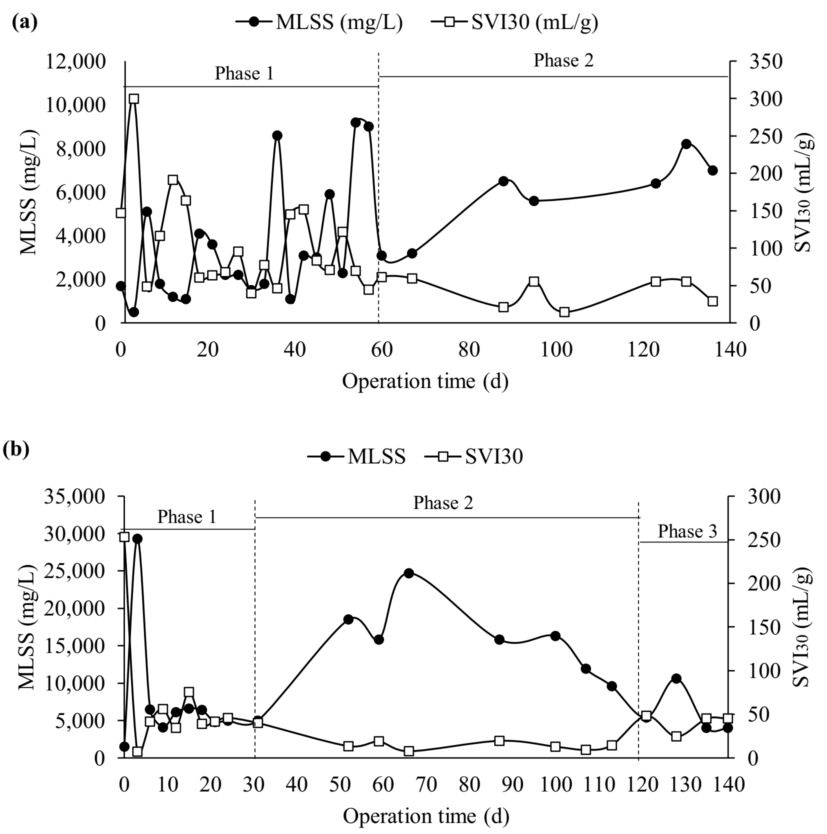

Fig. 2. Development of aerobic granular sludge in (a) R_c and (b) R_e.

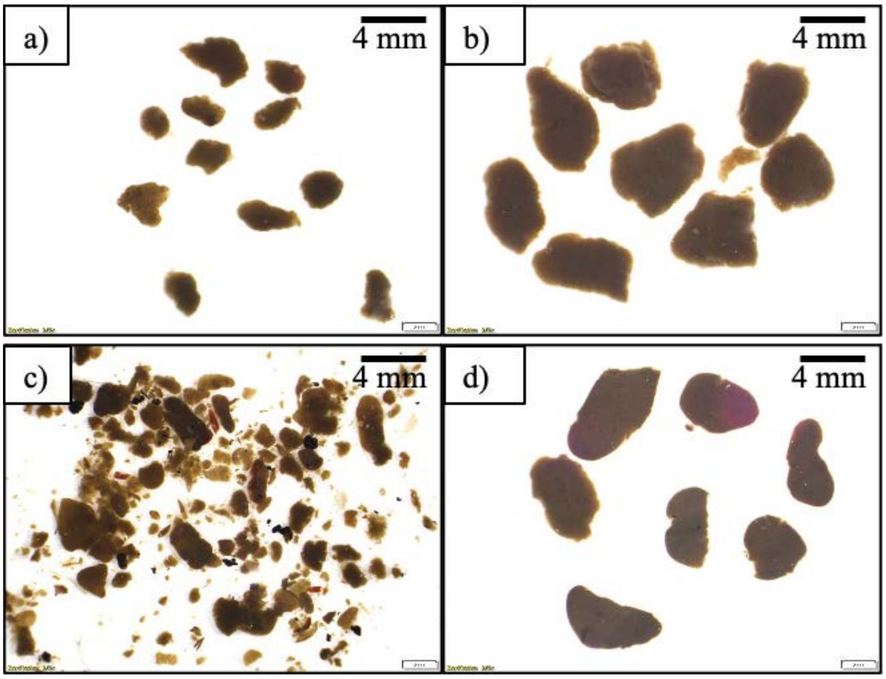

Fig. 3. (a) Small bioflocs in reactor R_c on day-60, (b) mature aerobic granular sludge in reactor R_c, (c) disintegrated anaerobic granular sludge in reactor R_e on day-30, and (d) mature aerobic granular sludge in reactor R_e (Magnification: 2.5×).

granular sludge took longer period of time. Nevertheless, the developed aerobic granular sludge demonstrated excellent properties and granular stability. In reactor R_e, the initiation of granulation process was more rapid due to the presence of anaerobic granular sludge. Although the granulation process was faster and the settling properties of the aerobic granular sludge was found to be better than R_c, the developed aerobic granular sludge was found to be disintegrated towards the end of the experiments.

3.2. Physical and morphological characterization

Aerobic granular sludge was harvested from both reactors R_c and R_e after day-140 of experimental period. The harvested aerobic granular sludge was separated based on size, namely R_c-1, R_c-2 and R_c-3 for granular sludge from reactor R_c; and R_e-1, R_e-2 and R_e-3 for granular sludge from reactor R_e, whereby 1 indicates the smallest granular sludge and 3 indicates largest granular sludge. During harvesting process, the separation of aerobic granular sludge based on the size was conducted in order to simplify the physical characterization process of granular sludge sample. During the characterization process, five random granular sludge sample from each R_c-1, R_c-2, R_c-3, R_e-1, R_e-2 and R_e-3 were analyzed accordingly. The smallest diameter of aerobic granular sludge from reactor R_c was averaging at 2.8 mm, while the largest diameter of granular sludge from reactor R_c was 6.2 mm. The microscopic picture of mature aerobic granular sludge from reactor R_c is shown in Fig. 3b. Meanwhile, the largest diameter of aerobic granular sludge harvested from reactor R_e was 6.3 mm in average. The mature aerobic granular sludge from reactor R_e is shown in Fig. 3d. Therefore, no significant difference was observed on the diameter of granular sludge despite the different seeding conditions applied to both reactors.

The aspect ratio of aerobic granular sludge was also observed as it shows the capability to develop round-shaped aerobic granules. It was found that the aspect ratio of aerobic granular sludge in R_c (0.94) was better than R_e (0.8). The aspect ratio of ideally round-shaped granular sludge is 1. Better aspect ratio may indicate better microbial structure of aerobic granular sludge with the presence of distinguished layer of aerobic and anoxic zones inside the granules [17]. However, there has been no clear relationship between the aspect ratio and the performance of aerobic granular sludge in wastewater treatment system.

The observation on settling velocity of aerobic granular sludge was performed and it was demonstrated that settling velocity was proportionally aligned with the diameters of aerobic granular sludge. A larger diameter of aerobic granular sludge is always associated with higher settling velocity. The conventional activated sludge used as the seeding exhibited poor settling velocity at 11.4 m/h. The mature granular sludge from reactor R_c and R_e exhibited excellent settling velocity averaging at 58.8 and 74.6 m/h, respectively. The settling velocity in this study was found to be higher than the previous reports treating different types of wastewater [18,19]. Rosman et al. [18] reported the development of aerobic granular sludge using rubber wastewater and was able to achieve settling velocity of 33 m/h. Meanwhile, Gonzalez-Martinez et al. [19] reported settling velocity of 51.8 m/h after 240 d development of aerobic granular sludge using synthetic wastewater. The aerobic granular sludge from both reactors reflected a significant improvement of settling velocity compared to the seed sludge. Table 2 listed the physical characteristics of developed aerobic granular sludge in both reactors R_c and R_e.

Table 2
Summary on physical characteristics of developed aerobic granular sludge

Sample name	Average diameter (mm)	Aspect ratio	Settling velocity (m/h)
R_c-1	2.8	0.75	35.7
R_c-2	4.6	0.78	48.9
R_c-3	6.2	0.94	58.8
R_e-1	2.4	0.42	37.3
R_e-2	4.6	0.75	59.9
R_e-3	6.3	0.8	74.6

Detailed morphological observation of mature aerobic granular sludge after day-140 of experimental period was conducted by FESEM observation. The outer layer of aerobic granular sludge from both reactors R_c and R_e were dominated with Cocci-shaped bacteria while several rod-shaped bacteria were also present in the surface of granular sludge. Cocci-shaped bacteria were proven to act as a support in microbial attachment process of aerobic granulation [10,20]. Meanwhile, rod-shaped bacteria are known to enhance the bonding of microbial matrix in granulation process [22]. However, as can be seen in Fig. 4d, numerous of filamentous bacteria was also detected on the surface of aerobic granular sludge from reactor R_e indicated by the presence of flagella on the granular outer layer. Meunier et al. [23] proved that the number of filamentous bacteria in aerobic granules directly correlate with the granular stability. This may explain the phenomenon of disintegration of aerobic granular sludge in reactor R_e. Therefore, in future, the growth of filamentous bacteria can be controlled by means of various parameters, including pH value and the VER in the system.

Observation using FESEM also showed the presence of micropores or also sometimes referred as cavities. Micropores are essential as a mean of transportation for substrates into the inner layer of granular sludge and for the metabolic products to be excreted from the granules [24]. EPS that act as glue-like substance that holds microbial cells together were also observed surrounding the micropores as shown in Figs. 4e and 4f. EPS characterization from the developed aerobic granular sludge may be conducted in future research.

3.3. Chemical elemental composition

FESEM-EDX analyses were performed for mature aerobic granular sludge from both reactors R_c and R_e. Table 3 summarizes the chemical elements detected in granular sludge sample. Based on the FESEM-EDX analyses, dominant elements found in aerobic granular sludge comprised of oxygen and carbon. Although the mass percentage of carbon in R_c (51%) was higher as compared to R_e (29%), the percentage of oxygen in both granular samples showed no significant difference. Furthermore, the significant difference found in granular sludge from R_c and R_e was the absence of some chemical elements in the granular sludge from R_e, including sodium, magnesium, phosphorus, zirconium, iron, copper and nitrogen.

Table 3
Elemental composition of aerobic granular sludge from reactor R_c and R_e

Elements	R_c (%)	R_e (%)
C	51	29
O	33	36
Na	0.28	n/a
Mg	0.32	n/a
Al	1.08	3.25
P	0.89	n/a
K	0.077	0.085
Ca	0.90	17
Zr	1.75	n/a
Si	6	15
S	0.81	0.41
Fe	0.95	n/a
Cu	0.26	n/a
N	3	n/a

n/a as in not applicable as the elements were not detected in the sample.

However, the reason and evidence behind the absence of referred elements is still yet to be discovered. Meanwhile, the other chemical elements present in R_e were significantly higher as compared to R_c. For example, mass percentage of Aluminum are 3 times higher in R_e than R_c. High concentration of aluminum directly correlates with fast settling properties of granular sludge [25]. Moreover, the concentration of calcium in R_e was 16 times higher than in R_c. This indicates the capability of aerobic granular sludge to absorb the calcium. Calcium is also known to initiate granulation process by neutralizing the negative charge on bacterial cell surface, thus enhancing the attachment of microbial cells [26]. In addition, high concentration of silicon was detected in R_e (15%), that may associate with the growth of microbial metabolism and contribute to the strength of granular sludge [16].

3.4. Microbial community in aerobic granular sludge

The sequencing results revealed a slightly different number of operational taxonomic unit (OTUs) in R_c and R_e of 2,495 and 2,573, respectively. It was highlighted that the dominant phylum in both samples from R_c and R_e was Proteobacteria as depicted in Fig. 5. Major groups of phylum detected were Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria and Chloroflexi in both samples. The relative abundance of Proteobacteria in this study was slightly lower than the previous reports [27–29]. Zhang et al. [29] reported almost 60% relative abundance of Proteobacteria, meanwhile Lv et al. [27] reported more than 60% relative abundance of Proteobacteria in the granular sludge. The phylum of Proteobacteria in the granular sample consisted of four major classes, namely Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria. Gammaproteobacteria was dominant in R_c as compared to R_e. Meanwhile, Deltaproteobacteria that is known for its ability

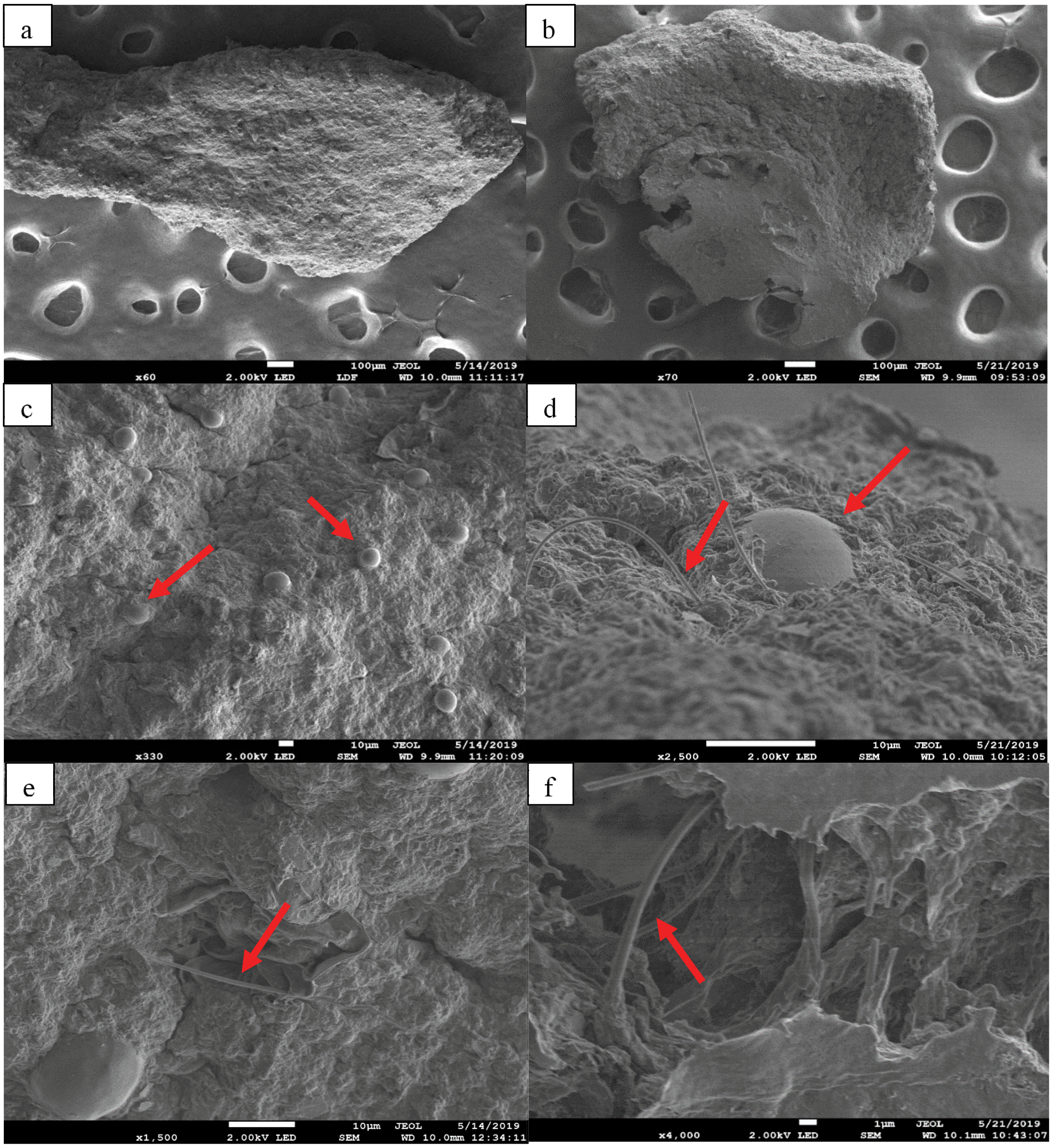

Fig. 4. (a) Aerobic granular sludge from R_c, (b) aerobic granular sludge from R_e; Cocci-shaped bacteria on granular sludge from R_c (c) and R_e (d); glue-like substances (EPS) on the granular sludge from R_c (e) and R_e (f).

to reduce sulfur had higher relative abundance in R_e (7%) than R_c (2%) [30]. Meanwhile, Betaproteobacteria or tentatively named as *Candidatus Accumulibacter phosphatis* or also can be generally referred as phosphate accumulating organisms (PAOs) are known for its ability to accumulate phosphate from the wastewater [31]. Proteobacteria was found to be slightly higher in R_c as compared to R_e. Furthermore, Gammaproteobacteria that produced EPS in granulation processes was also higher in R_c than R_e [29].

Bacteroidetes that were the second phylum with highest relative abundance consisted of the class Sphingobacteriia, which are capable of producing sphingolipids and also involved in biofilm formation [32], Saprospirales, which are highly abundant in soil [33] and Flavobacteriales, that can degrade a varieties of polycyclic aromatic hydrocarbon [34] were also detected. Sphingobacteriia was slightly higher in R_e at 6% relative abundance as compared to R_e at 4% relative abundance. However, the relative abundance of Bacteroidetes in this study was lower than previous report by Chen et al. [35] that identified more than 20% relative abundance of Bacteroidetes in the granular sludge. Another commonly retrieved bacteria in WWTP and soil sample were also detected in the both granular sludge sample, such as Actinobacteria and Clostridia [37,38]. It is also worth noting that some acidophilic and thermophilic groups of bacteria were detected at a smaller relative abundance, including Acidimicrobiales, Acidobacteria and Thermoleophilia.

From a total number of more than 2000 OTUs, the alpha diversity of the microbial community in aerobic granular sludge was analyzed. Table 4 tabulates the alpha diversity among two samples, R_c and R_e, according to Shannon, Simpson, ACE and Chao1 indices along with the previous reports. Simpson and Shannon index represent the species diversity by taking into account the species richness and evenness in a community. Meanwhile, ACE and Chao1 indicate only the species richness of the community. The Shannon index in R_e was found to be slightly higher than R_c. These results indicate that the species diversity in this study was higher than the previous reports. Meanwhile, the results of ACE index were aligned with the previous reports as shown in Table 4. Nevertheless, the alpha diversity indices in this study highlighted that the aerobic granular sludge developed in both R_c and R_e were high in microbial richness. Furthermore, it may be concluded that aerobic granular sludge from R_e has higher diversity index compared to R_c.

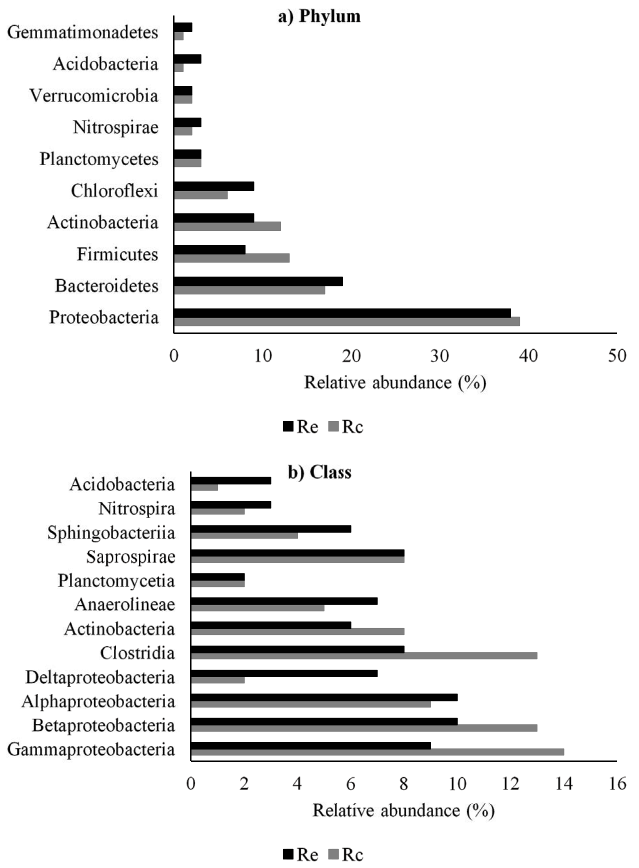

Fig. 5. Relative abundance of the phylum and class in aerobic granular sludge from R_c and R_e.

3.5. Reactor performance

Treatment efficiency of low-strength actual domestic wastewater was reported in terms of COD, ammoniacal nitrogen and total phosphorus removals, as summarized in Table 5. Throughout 140 d of experimental period, the average COD removal efficiency were found to be 72% and 67% in R_c and R_e, respectively. It was found that during acclimatization phase (phase 1) of both reactors, the COD removal efficiency was more stable in reactor R_c as compared to reactor R_e. This might be influenced by the different seeding condition applied to both reactors, whereby it was reported that application of anaerobic granular sludge in treating low-strength wastewater was not efficient [9]. Thus, the seeding condition in R_e may not be beneficial towards the COD removal efficiency in the beginning stage. During phase 2, both reactors exhibited stable COD removal efficiency at more than 70%. Nevertheless, due to the granular disintegration in reactor R_e, indicated in phase 3, the COD removal efficiencies decreased up to below 60%. The statistical analysis revealed that there was a significant difference ($p < 0.05$) in COD removal efficiency of both reactors.

The ammoniacal nitrogen removals throughout 140 d experimental periods were found to be stable in both reactors R_c and R_e with no significant difference ($p > 0.05$). Concentration of ammoniacal nitrogen in the effluent of reactors R_c and R_e were 8.5 and 8.9 mg/L, respectively. These results indicated that ammonia-oxidizing and nitrification occurred in both reactors. Specifically, in R_e, this result implied that aerobic layer was successfully attached to the anaerobic granular sludge. Meanwhile, average TP concentrations in the effluent after day-140 were 11 and 7 mg/L in R_c and R_e, respectively with an average of removal percentage at 34% in R_c and 59% in R_e. TP removal

Table 4
Alpha diversity index of the granular sludge in this study and previous reports

Total OTU	Shannon	Simpson	ACE	Chao1	References
2,495	5.9	0.98	2,639.75	2,633.94	Reactor R_c
2,573	6.1	0.99	2,727.46	2,725.03	Reactor R_e
–	3.78	0.05	390.36	429.06	[35]
2,013	5.51	0.024	4,665.5	3,734.5	[38]
2,216	7.76	0.0178	–	2,217.3	[39]

Table 5
Summary on the bioreactor performance in this study along with the previous reports

COD removal	Ammonia removal	TP removal	References
72% (R_c)	73% (R_c)	34% (R_c)	This study
67% (R_e)	72% (R_e)	59% (R_e)	This study
87%	97%	90%	[6]
82%	99%	13%	[39]
95%	–	64%	[41]

was significantly better in R_e as compared to R_c ($p < 0.05$). Anaerobic granular sludge used as the seeding in R_e may improve the TP removal as anoxic layer was formed rapidly and activated sludge may attach to the core and form aerobic layer on the granules. Thus, the growth of PAOs that is anoxic was favored [40].

Nevertheless, the removal efficiencies of TP in the system were found to be lower than in previous study [6]. The TP concentration in the raw wastewater used in this study was varied significantly since actual sewage was directly utilized to feed the columns, this has posed a challenge to achieve stable removal efficiencies in the system. This study was able to achieve a higher TP removal compared to a study by Wang et al. [39] that treated actual sewage containing 6.8 mg/L TP concentration.

4. Conclusion

Development of aerobic granular sludge can be achieved rapidly by utilizing anaerobic granular sludge as the seeding, whereby the anaerobic granular sludge might act as a core zone for the activated sludge to attach. The developed aerobic granular sludge in both reactors R_c and R_e exhibited excellent settling properties with highest settling velocity recorded at 74.6 m/h. The microbial community analysis revealed high microbial richness in developed aerobic granular sludge, with dominance of group of Proteobacteria. Moreover, TP removal efficiency was also improved in R_e due to the presence of anaerobic granular sludge in the seeding. However, the overall treatment efficiency in this study was found to be lower than the previous reports. In future, scaling-up of this system may be conducted by using the relevant information provided from the lab-scale study.

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