Original Article

Rapid Development of Microalgae-Bacteria Granular Sludge Using Low-Strength Domestic Wastewater


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ABSTRACT

Recently, the symbiosis between microalgae and bacteria for wastewater treatment system receives more attention as microalgae and bacteria coexist symbiotically under suitable environmental condition. Moreover, the microalgae and bacteria interaction in granular form had been considered as an environmental friendly alternatives due to the synergistic cooperation between microalgae and bacteria in treating wastewater. This study aims to develop microalgae-bacteria aerobic granular sludge using low-strength domestic wastewater. A mixture of Scenedesmus obliquus and activated sludge at ratio of 17% microalgae to 83% activated sludge (v/v) was used as the seed sludge to develop microalgae-bacteria aerobic granular sludge. Upon 30 days of experimental period, granular sludge was successfully developed with largest granular diameter of 6 mm. The developed granules exhibited excellent settling properties with 62.8 m/h settling velocity. Better granular settleability indicated by low sludge volume index (SVI30) was detected at 8 mL/g. Observation using field-emission scanning electron microscope (FESEM) showed the attachment of microalgae cells on the outer layer of granular sludge. Moreover, microalgae-bacteria aerobic granular sludge demonstrated good COD and ammoniacal nitrogen removal at 72% removal efficiency.

Keywords: microalgae, granular sludge, domestic wastewater, nutrient removal, photo-sequencing batch reactor

INTRODUCTION

Aerobic granular sludge is an innovative technology that composed of self-immobilized microbial cells held together by physical, chemical and biological forces [1]. Aerobic granular sludge was successfully developed in various types of wastewater, including industrial [2–4], municipal [5] and domestic wastewaters [6]. In comparison with conventional activated sludge, aerobic granular sludge requires shorter settling time due to its compact structure, thus eliminating the needs of separate settling tank in wastewater treatment plant [7]. It was reported that aerobic granular sludge system may reduce up to 80% area requirement of a wastewater treatment plant (WWTP) [8].

Recently, the symbiosis between microalgae and bacteria for wastewater treatment system receives more attention as microalgae and bacteria coexist symbiotically under suitable environmental condition [9]. Moreover, the microalgae and bacteria interaction in granular form had been considered as an environmental friendly alternatives due to the synergistic cooperation between microalgae and bacteria in treating wastewater. For example, the exchange of organic and in-
organic nutrients through photosynthesis and respiration allows simultaneous growth of microalgae and bacterial cells [10].

Microalgae-bacteria granular sludge possessed several advantages compared to conventional aerobic granular sludge, such as less energy demand, enhanced nutrient removal, possibility for resources recovery and production of valuable metabolites [11]. There are two approaches in developing microalgae-bacteria granular sludge, first is by utilizing indigenous microalgae in the wastewater treatment system and second is by introducing targeted microalgae species. Huang et al. (2015) [12] reported the development of microalgae-bacteria granular sludge by natural sunlight-exposure using synthetic wastewater (chemical oxygen demand (COD): 600 mg/L). Meanwhile, Liu et al. (2017) [13] reported the use of targeted microalgae, i.e. Scenedesmus and Chlorella to induce the development of microalgae-bacteria granular consortia using synthetic wastewater (COD: 300 mg/L). The previous studies have reported excellent performance of wastewater treatment using microalgae-bacteria granular sludge. Nevertheless, most of the studies were focusing on treatment of synthetic wastewater while the utilization of actual wastewater is still limited, specifically on low-strength domestic wastewater.

This research aims to cultivate microalgae-bacteria granular sludge using actual low-strength domestic wastewater. Microalgae species Scenedesmus obliquus was used as targeted microalgae in the granular consortia. The respective microalgae species was chosen due to its extensive application in wastewater treatment system, thus the inoculation of this microalgae species was expected to enhance the wastewater treatment efficiency while promoting the granulation process [14]. It was also reported that inoculated microalgae species may attached to the outer layer and inner layer of the granules and lead to faster biomass production [15,16]. Moreover, the performance of biological nutrient removal in photo-sequencing batch reactor (PSBR) was evaluated. The developed microalgae-bacteria granular sludge was analyzed in terms of the morphological, physical and chemical characteristics while the results were compared with the conventional activated sludge system. This study demonstrates the feasibility of microalgae-bacteria granular consortia to be developed in actual low-strength domestic wastewater.

MATERIALS AND METHODS

Reactor set-up and operational conditions

A PSBR column with a diameter of 6 cm and a height of 100 cm was used to develop microalgae-bacteria aerobic granular sludge. The reactor had a working volume of 1.5 L with 50% volume exchange ratio (VER). The schematic diagram of PSBR is shown in Fig. 1. A 3 hour cyclic time was applied to PSBR system, consisting of 5 minutes feeding, 161 minutes aeration, 10 minutes settling, 2 minutes effluent discharge and 2 minutes idle time. An LED light was set above the reactor to provide continuous and uniform illumination at 54 µmol/m²s light intensity, whereby the light intensity was measured at the middle height of the reactor using a luminometer. Aeration was introduced by air pump and the flow rate was set to 2.5 L/min by an air flowmeter. The reactor was inoculated with 750 mL of seed sludge containing activated sludge and targeted microalgae inoculum. In each cycle, 750 mL of domestic wastewater was introduced to the reactor and by the end of the cycle, 750 mL of treated wastewater was discharged from the reactor. The reactor was operated at room temperature (24 ± 3°C).

Seed sludge and domestic wastewater sample

The seed sludge used in this study consisted of activated sludge collected from a local WWTP and targeted microalgae species, Scenedesmus obliquus. The collected activated sludge was filtered to remove large debris that might clog the tubing in the reactor and followed by pre-conditioning where the activated sludge was aerated for at least 24 hours in a room temperature. Scenedesmus obliquus was chosen as targeted microalgae due to its ability to treat various types of wastewater [17]. Microalgae strain was cultured in an aseptic condition to eliminate contamination for 2 weeks to reach the exponential phase of microalgae growth. Once the exponential phase of microalgae culture was achieved, the microalgae was inoculated into activated sludge at designated volumetric ratio of 17% microalgae to 83% activated sludge (v/v) [18]. A 750 mL of seed sludge was prepared containing 125 mL microalgal culture and 625 mL activated sludge. The biomass concentration of the seed sludge was 8,000 mg/L while the sludge volume index (SVI30) was found to be 44 mL/g.

Domestic wastewater sample was collected from the same local WWTP. The wastewater sample was kept at 4°C until further utilization. Wastewater was also filtered by using 1 mm pore size filter to remove unwanted debris prior to be introduced to the reactor. The domestic wastewater was characterized in terms of the chemical oxygen demand (COD), total nitrogen (TN), ammoniacal nitrogen and total phosphorus (TP) concentrations. The wastewater sample on average contained 189 mg/L COD, 26 mg/L TN, 24 mg/L.
ammoniacal nitrogen and 6.2 mg/L TP (average of 4 batches of collected wastewater samples).

Analytical methods
The analyses for wastewater and sludge including COD, TN, ammoniacal nitrogen (NH$_3$-N), nitrate nitrogen (NO$_3$-N), nitrite nitrogen (NO$_2$-N), total phosphorus (TP), mixed liquor suspended solids (MLSS) and SVI$_{30}$ were conducted in accordance with the standard methods [19]. The sample of treated effluent was collected every two days and analyzed immediately for better results. The effluent sample was not filtered prior to be analyzed. Characterization of the granular sludge was conducted for the morphological, physical and chemical characteristics. Morphological characterization was performed using two types of microscopes, stereomicroscope (SZX7, Olympus, Tokyo, Japan) and field emission scanning electron microscope (FESEM, JSM7800F, Jeol, Tokyo, Japan). The granular sludge sample was prepared accordingly prior to FESEM observation which included fixation process using 2.5% glutaraldehyde and drying process using gradient concentration of ethanol [20]. Auto fine coater was chosen with coating current of 20 mA for imaging purposes.

Physical characterization of the microalgae-bacteria aerobic granular sludge was conducted by analyzing the aspect ratio and settling velocity of the granular sludge. Aspect ratio that indicates the roundness of the granular sludge was calculated as the ratio of the shortest and longest dimension of the granular sludge. Meanwhile, analysis on settling velocity was conducted by observing the average time taken for the granular sludge to reach the bottom of a cylindrical column filled with tap water. Chemical characteristic of the granular sludge was analyzed using FESEM energy dispersive X-ray (FESEM-EDX) to detect the organic and inorganic elements concentration in the granular sludge.

Fig. 1 PSBR column used to develop microalgae-bacteria aerobic granular sludge.
RESULTS AND DISCUSSION

Development of microalgae-bacteria aerobic granular sludge

Morphological observation

The seed sludge which consisted of a mixture of activated sludge and microalgae culture had an irregular and fluffy structure with little to none bioflocs observed. After 7 days of reactor operation, the sludge started to form small bioflocs and the color shifted to lighter brown due to the growth of microalgae. The average size of bioflocs after 7 days of operation was ranging from 1–2 mm. During the experimental period, it was clearly observed that the granular sludge started to be dominant with dense and compact structure. Upon 30 days of reactor operation, mature aerobic granular sludge was found in the reactor with average granular size of 4–6 mm. The development of mature microalgae-bacteria aerobic granular sludge is shown in Fig. 2.

Furthermore, morphological observation using FESEM revealed that the granular sludge surface was dominated with cocci-shaped bacteria as depicted in Fig. 3 (a) and (b). Cocci-shaped bacteria is known to play important roles in microbial attachment and granular formation process [2,21]. In addition to that, small number of filamentous bacteria was found in the granular sludge. The presence of filamentous bacteria directly correlates with granular stability [22]. Microalgae cells were also detected on the outer layer of the granules as shown in Fig. 3 (c) and (d). This proved the co-habitation of microalgae and bacteria in aerobic granular sludge. Liu et al. (2017) [13] demonstrated that the surface of microalgae-bacteria aerobic granular sludge was dominated with the inoculated microalgae species (Chlorella and Scenedesmus). On the surface of granular sludge, micropores may also be observed as shown in Fig. 3 (e). Micropores or also called cavities are essential in granular sludge as it acts as mean of transportation for substrate into the inner layer of the granular sludge as well as to excrete metabolic products such as carbon dioxide and lactate from the microbial cells [23]. Furthermore, the glue-like substances or extracellular polymeric substances (EPS) also appeared surrounding the microbial cells on granular surface as shown in Fig. 3 (f).

Previous studies have proven the dominance of inoculated microalgae species in microalgae-bacteria aerobic granular sludge [13,17], whereby two microalgae species were used, namely Chlorella and Scenedesmus. It was reported that Scenedesmus present in the granular sludge at higher relative abundance of 61% compared to Chlorella at 38% [17]. Meanwhile, another study utilized the indigenous microalgae present in the activated sludge and reported the dominance of Chlorophyceae class from the phylum of Chlorophyta [11]. Microalgae species of Chlorella was successfully isolated from microalgae-bacteria granular consortia [24]. The
isolated microalgae species exhibited excellent nitrogen and phosphorus removal efficiency at 93% and 96%, respectively. Previous study suggested that inoculation of suitable microalgae species may lead to the rapid formation of granular sludge while achieving stable operation in a short experimental period. In future, analysis on the microbial composition of the microalgae-bacteria aerobic granular sludge may be conducted to provide detailed information on the effect of *Scenedesmus obliquus* towards microalgae composition in the granules.

**Biomass growth and settleability**

Initial MLSS of the seed sludge was 8,000 mg/L with SVI$_{10}$ value of 44 mL/g. The analyses of biomass growth and sludge settleability was performed weekly throughout the experimental period as shown in Fig. 4. During first 7 days

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**Fig. 3** FESEM observation of a) overview of microalgae-bacteria granular sludge; b) cocci-shaped bacteria on the granular surface; c) and d) microalgae cells on the outer layer of granular consortia; e) Micropores or cavities; and f) EPS attached to a microbial cell on the granular surface.
of experimental period, MLSS increased to 9,300 mg/L. However, from day 7–21 the MLSS significantly decreased due to severe biomass washout in the reactor. This phenomenon usually appeared as less dense sludge with poor settling properties was discharged from the reactor along with the treated effluent [7]. This selection process retained sludge with fast settling properties to adapt and form granular sludge. Therefore, the biomass concentration successfully recovered from day 21 to 30 with final MLSS of 7,400 mg/L.

Another important characteristic to observe is the settleability of granular sludge represented by SVI30 value. The initial SVI30 value of 44 mL/g indicated poor settling properties of the seed sludge. Throughout the reactor operation, SVI30 value was decreasing as the seed sludge transformed into the granular sludge. Although the SVI30 value was slightly increased on day 21 to 20 mL/g due to the biomass washout, it was able to recover and achieved 8 mL/g SVI30 after 30 days of experimental period. This result was aligned with previous study that proved that microalgae and filamentous bacteria are two important factors in achieving rapid granulation [25].

The settleability of microalgae-bacteria aerobic granular sludge in this study was significantly better than previous studies that reported SVI30 value of 24 mL/g [17] and 71 mL/g [16]. Liu et al. (2018) [17] used mature aerobic granular sludge with addition of Chlorella and Scenedesmus as the seed sludge. Meanwhile, Zhang et al. (2020) [16] used mature algal-bacteria aerobic granular sludge as the seeding. Microalgae-bacteria aerobic granular sludge is able to achieve better settleability due to its more compact structure and higher lipid concentration in the microbial cells that may lead to higher biomass density [14].

It was argued that bacteria is the initiator for microbial attachment in development process of microalgae-bacteria granular sludge [26]. Bacteria help in protecting microalgae cells from predators such as zooplankton. Furthermore, there is evidence that bacterial EPS play important roles in shaping granular sludge and enhance settling ability [27]. However, lysis of microalgae cells can help accelerate granulation process as it supplies more nutrients for bacterial growth [28]. In another study by Liu et al. (2017) [13], it was found that the seed strains remained dominant in the granular sludge. However, photoheterotrophic bacteria and microalgae would compete with each other for the surface space of the granules. Nevertheless, till date, there has been no conclusive evidence on the effect of seed strains towards the performance of microalgae-bacteria aerobic granular sludge. It was also worth noting that mature microalgae-bacteria aerobic granular sludge was able to be harvested in a relatively short experimental period of 30 days, in comparison with previous studies that need more than 50 days to achieve similar biomass concentration and granular settleability [29].
Physical and chemical characteristics

The analyses of physical characteristics of aerobic granular sludge included both aspect ratio and settling velocity of the matured granules. The aspect ratio of microalgae-bacteria aerobic granular sludge was found to be 0.9 that proved the ability to rapidly produce a round-shaped granular sludge in PSBR within 30 days of experimental period using low strength domestic wastewater. In addition to that, analysis of settling velocity revealed that microalgae-bacteria aerobic granular sludge exhibited excellent settling velocity of 62.8 m/h. This result directly correlated with the low value of SVI30 which indicates fast settling properties of granular sludge. Nonetheless, there are very limited reports on the settling velocity of microalgae-bacteria aerobic granular sludge. Arcila and Buitron (2017) [30] reported the formation of microalgae-bacteria aggregates with settling velocity of 18 m/h using municipal wastewater while Cai et al. (2019) [31] found an increase settling velocity of microalgae-bacteria aerobic granular sludge up to 12.8 m/h. Therefore, this study was able to achieve higher settling velocity of the granular sludge. Nevertheless, further study is required to extend the knowledge of settling velocity and other physical characteristics of microalgae-bacteria granular sludge, including the factors affecting these features.

Based on the FESEM results, EDX analyses was performed to analyze the elemental composition of microalgae-bacteria granular sludge. As depicted in Fig. 5, carbon and oxygen were the highest elements present in the granular sludge. When compared with the elemental composition in activated sludge sample (50%), the relative mass percentage of the carbon in the microalgae-bacteria aerobic granular sludge was found to be less (35%). Meanwhile, relative mass concentration of oxygen was significantly increased to 48% from initially 33% in the activated sludge sample. This demonstrated the capability of microalgae cells in the granules to produce oxygen which aid in supporting bacterial metabolism [32]. Moreover, high concentration of phosphorus was found in the microalgae-bacteria granular sludge at 2% compared to 0.9% in activated sludge sample. This result indicates the accumulation of phosphorus in the granular consortia. Phosphorus can be directly assimilated by microalgae and stored in the microalgae cells through phosphorylation [10]. The result proved the assumptions that the phosphorus removal in this study occurred based on the biomass uptake only. Furthermore, calcium was found at high concentration (3%) in microalgae-bacteria granular sludge consortia. This proved that calcium can enhance the initial microbial attachment process and contributes towards the strength of aerobic granules [33]. Moreover, high concentration of zirconium was detected at 9% mass percentage in microalgae-bacteria granular sludge. However, no study was reported to elaborate the effect of high zirconium content in aerobic granular sludge.

![Fig. 5 Composition of chemical elements in the microalgae-bacteria granular sludge.](image-url)
Performance of microalgae-bacteria granular sludge COD removal

Stable COD removal was observed throughout 30 days of experimental period as depicted in Fig. 6 (a). Despite the event of biomass washout in the system, COD removal was maintained at more than 70%. When biomass washout was observed, the COD removal was performed by mainly aerobic bacteria in activated sludge with the aid of heterotro-
phic metabolism by microalgae cells [34]. After 30 days of experimental period, the average COD removal was 72% resulting in 52 mg/L COD concentration in the effluent. These results were found to be lower than previous reports treating higher concentration of COD [11,26]. Zhang et al. (2018) [11] reported 96% of COD removal from synthetic wastewater containing 1,200 mg/L COD content. Meanwhile, Zhu et al. (2019) [34] was able to achieve 82% COD removal efficiency from synthetic wastewater containing 1,130 mg/L COD content. Nevertheless, when final COD concentration was compared, this study was able to produce effluent with lower COD concentration than previous study by Zhu et al. (2019) [34]. Previous study discovered that high concentration of carbon sources can enhance the aggregation process and lead to more stable performance of microalgae-bacteria aerobic granular sludge [35]. Moreover, during the treatment of low-strength wastewater, it was concluded that bacteria will undergo endogenous metabolisms due to the lack of readily biodegradable organic matters and resulting in the release of organic matters to the wastewater [34]. Therefore, it is still challenging to treat actual low-strength domestic wastewater by using microalgae-bacteria aerobic granular sludge. Table 1 summarizes the wastewater treatment performances of this study along with the previous reports.

### Biological nutrient removal

Biological nutrient removal from domestic wastewater was observed based on the removal of TN, TP, NH₃-N, NO₃-N and NO₂-N. Concentration of TN in the effluent throughout 30 days of experimental period was found to be stable as shown in Fig. 6 (b). After 30 days of reactor operation, average of TN concentration in the effluent was 22 mg/L with 20% removal efficiency. The result of TN removal in this study was lower than previous reports with higher initial TN concentration. For instance, Liu et al. (2017) [13] reported removal of TN from synthetic wastewater containing 35 mg/L and successfully achieved 50% TN removal efficiency. A study by Petrini et al. (2020) [15] reported treatment of municipal wastewater using microalgae-bacteria aerobic granular sludge. The study was able to achieve 52% TN removal with addition of *Chlorella vulgaris* in the seeding. However, by comparing the concentration of TN in the effluent, current study was able to achieve lower TN concentration than previous report with 27 mg/L TN concentration in the effluent.

In order to study the detail of TN removal, analyses of NH₃-N, NO₃-N and NO₂-N were conducted, and the results are depicted in Fig. 6 (c). The biomass washout in the system lead to the significant increase in all nitrogen variations in the effluent. However, the system was able to recover and low concentration of all nitrogen variations were observed. In the end of the reactor operation, average NH₃-N removal was 72%. This result was aligned with previous report by Zhu et al. (2019) [34] that reported 75% NH₃-N removal efficiency from synthetic wastewater. However, when final concentration of NH₃-N was compared, current study was able to achieve lower concentration of NH₃-N at around 6 mg/L. Meanwhile, a study by Zhu et al. (2019) [34] produced effluent with 65 mg/L NH₃-N concentration. A good removal efficiency of NH₃-N by using microalgae-bacteria aerobic granular sludge might be influenced by the ability of microalgae cells to directly assimilate ammoniacal nitrogen in wastewater [17].

The nitrification and denitrification processes were observed based on the concentration of NO₃-N and NO₂-N in the treated effluent. After 30 days of experimental period, the NO₃-N and NO₂-N concentrations were found to be 5.5 mg/L and 0.04 mg/L, respectively. A considerably high NO₃-N and NO₂-N indicated the inefficient denitrification process which may be due to the lack of development in the anoxic zone inside granular consortia. These results also demonstrated that microalgae and bacteria co-exist in a spatially close microstructure in a granular sludge, thus, the granular sludge has

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<td>72%</td>
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<td>40–92%</td>
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higher number of aerobic micro-zones and limited anoxic zone [34]. Furthermore, microalgae cells prefer to consume ammonia rather than nitrate, whereby nitrate consumption may occur once ammonia is almost fully consumed, which did not achieved in this study [36]. Despite the poor removal efficiencies, the concentration of NO$_3$-N in the effluent was significantly lower than the previous study by Zhang et al. (2018) [11] that reported an accumulation of NO$_3$-N up to 26.8 mg/L by using microalgae-bacteria granular consortia. The concentration of NO$_3$-N was also found to be lower than the previous study that produced effluent with 15 mg/L NO$_2$-N concentration [17]. Therefore, this study exhibited better NO$_3$-N and NO$_2$-N concentrations in the effluent with similar ability to remove NH$_3$-N with the previous studies. Further improvement in TN removal efficiency needs to be investigated, such as by applying intermittent aeration strategy to induce to formation of anoxic zone in granular sludge [37].

The concentration of TP in treated effluent was stable as shown in Fig. 6 (d). Although the biomass washout affects the TP removal in negative fashion, TP concentration in the effluent remained low at around 6 mg/L. Average of TP concentration in the effluent after 30 days of reactor operation was 7 mg/L resulting in 5% TP removal efficiency. Poor TP removal efficiency was observed in this study which may be due to the absence of sludge retention time (SRT) control in the system, therefore no intentional biomass discharge was conducted. Moreover, low organic loading rate (OLR) in the system may not be beneficial for the nutrient removal in this study. Nevertheless, this study was able to achieve similar concentration of TP in the effluent with previous report by Ahmad et al. (2017) [38] and lower than reports by Zhu et al. (2019) [34] as summarized in Table 1. The low pH level in PSBR system (5–6) eliminated the possibility of abiotic precipitation of phosphorus, thus phosphorus removal in this study was solely based on biological absorption [39]. In future, optimization process is required to achieve optimal performance of microalgae-bacteria aerobic granular sludge in treating low strength domestic wastewater. Optimization process may include aeration strategy, SRT value and pH level. Moreover, long-term operation of wastewater treatment using microalgae-bacteria aerobic granular sludge should be investigated to prove the feasibility of this system to be applied in full-scale treatment plant.

CONCLUSIONS

Microalgae-bacteria aerobic granular sludge was successfully developed using low-strength domestic wastewater within 30 days of experimental period. The developed granular sludge exhibited excellent settling properties with 62.8 m/h settling velocity and SVI30 value of 8 mL/g. Performance of microalgae-bacteria granular sludge was found to be stable with COD removal efficiency of 72% and ammonia nitrogen removal efficiency of 72%. Further research related to the performance of microalgae-bacteria granular sludge in treating actual low strength domestic wastewater is still required.

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