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# Characteristics and performance of aerobic granulation seeded with anaerobic bioflocs for treatment of domestic wastewater

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**Abstract.** In the present study, aerobic granular sludge was developed using a combination of anaerobic bioflocs and activated sludge. The granulation process and removals of organic compound (COD and ammoniacal nitrogen) were observed for 140 days of experimental period. The granulation process occurred after 30 days of the experiments, indicated by the decrease in sludge volume index (SVI) and increase of biomass concentration. The COD and ammoniacal nitrogen removal efficiencies were 67% and 72%, respectively. Mature aerobic granules were developed with diameter ranging from 4 to 6 mm with excellent settling velocity at 75 m/h. The results indicated the possibility to use anaerobic bioflocs as seeding to develop aerobic granular sludge for domestic wastewater treatment.

## 1. Introduction

The advancing technology of aerobic granular sludge has been applied for treatment of various types of wastewater. Aerobic granular sludge involves the interaction between microbial cells through physical, chemical and microbiological forces, thus allowing the microbial attachment, forming a granular shaped structure [1]. Due to the unique structure of aerobic granular sludge, a distinguished layer of aerobic and anoxic zones may be observed in the granular sludge, whereby the aerobic layer was found on the outer layer and anoxic zone was observed in the inner layer of the granules [2]. The special features of aerobic granular sludge allow this system to perform better as compared to conventional activated sludge, including better settleability, indicated by lower sludge volume index (SVI<sub>30</sub>) and ability to perform both aerobic and anoxic reaction in a single tank.

Aerobic granular sludge has been cultivated using different types of wastewater, such as high strength agro-based wastewater [3], piggery wastewater [4], municipal wastewater [5] and low strength domestic wastewater [6]. However, previous studies suggested a high organic loading rate (OLR) is crucial to cultivate aerobic granular sludge as the number of readily degradable organic content is high. Low OLR has been proven to produce smaller granules with loose and porous structure [7]. Therefore, successful cultivation of aerobic granular sludge using low strength domestic wastewater is challenging.

To enhance aerobic granulation using domestic wastewater, different seeding conditions may be applied to the system. Previous study has conducted aerobic granulation seeded with 10% crushed aerobic granules and 90% activated sludge [8]. The results proved that the addition of crushed granules might enhance granulation process as well as the performance of biological nutrient removal. Meanwhile, Linlin et al. (2005) utilized anaerobic bioflocs as the seeding in aerobic granulation processes [9]. According to this study, anaerobic bioflocs firstly disintegrated under aerobic conditions,



then the flocs and debris from the disintegrated granules attached and aerobic granular sludge was formed. However, the study of aerobic granulation by utilizing different seeding conditions are still lacking.

This study aims to develop and characterize aerobic granular sludge using locally sourced domestic wastewater. Different seeding conditions was applied, whereby the seed sludge was a mixture of anaerobic bioflocs and activated sludge. The developed aerobic granular sludge was characterized for the morphological, physical and chemical characteristics. Moreover, the overall performance of aerobic granular sludge in treating low-strength domestic wastewater was analyzed.

## 2. Materials and methods

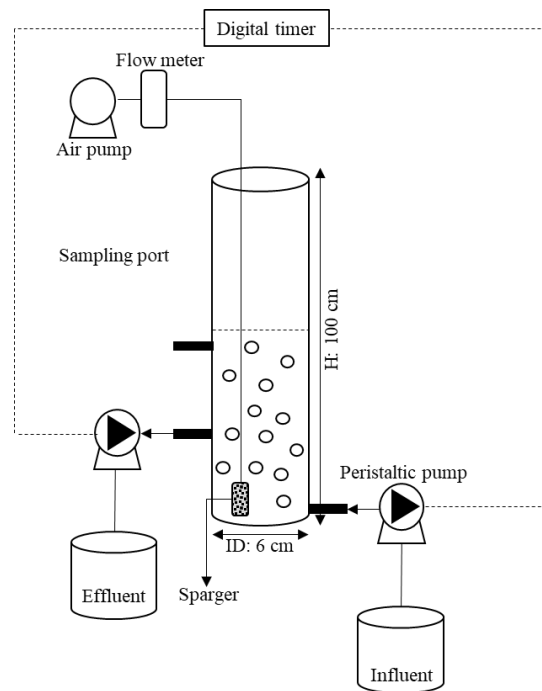
### 2.1. Experimental set-up and operational conditions

A cylindrical glass column sequencing batch reactor (SBR) with a working volume of 1.5 L was used to cultivate aerobic granular sludge. Diameter of the SBR column was 6 cm with 100 cm height as shown in figure 1, resulting in H/D ratio of 16.67. A relatively high H/D ratio was applied to the reactor to enhance the granulation process. The reactor was operated continuously at room temperature (24-27°C) at 3 hours cyclic time, consisted of 5 minutes feeding, 161 minutes aeration, 10 minutes settling, 2 minutes effluent withdrawal and 2 minutes idle time. Moreover, air was introduced through a microsparger located at the bottom of the column. Aeration was maintained using a flow meter at 2.5 L/min airflow rate. SBR column was seeded with 325 mL anaerobic bioflocs and 325 mL activated sludge. Anaerobic bioflocs was used to induce rapid granulation in the system [9]. Activated sludge was collected together with domestic wastewater sample from a local wastewater treatment plant (WWTP). The SBR column was seeded with 1,500 mg/L seed sludge and the initial SVI<sub>30</sub> value of the seed sludge was 147 mL/g, indicating a poor settleability of the seed sludge. Meanwhile, 750 mL of domestic wastewater was introduced in each cycle, resulting in 728 mg COD/L.d OLR. Table 1 summarizes the characteristics of domestic wastewater used in this study.

**Table 1.** Characteristics of domestic wastewater sample

Elements	Concentration (mg/L)*
COD	177
Total Nitrogen	27
Total Phosphorus	25
Ammoniacal nitrogen	31.7
Nitrate nitrogen	19.4
Nitrite nitrogen	0.18
pH	7.27

\*Average of 18 sampling times



**Figure 1.** Experimental set-up of SBR column for granulation purposes

## 2.2. Analytical methods

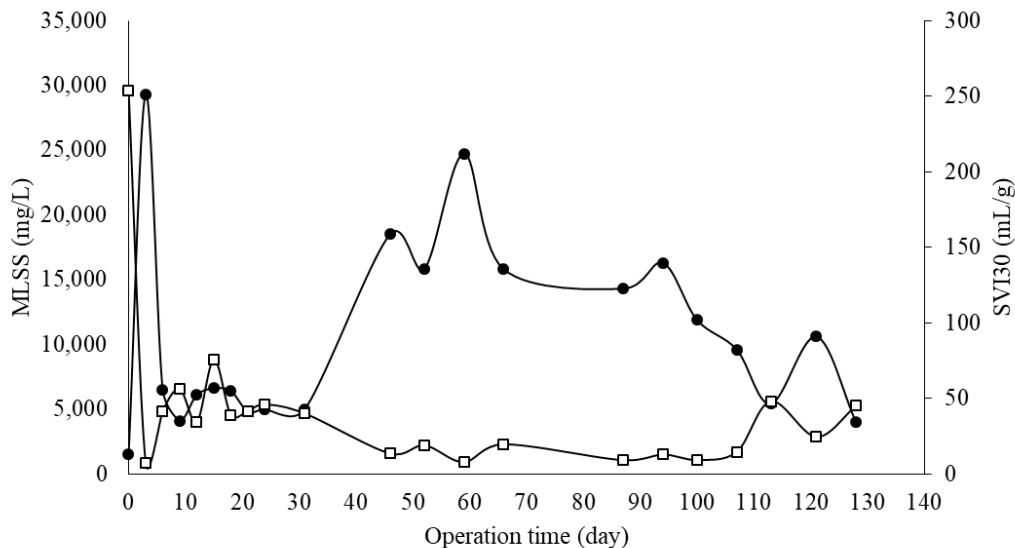
Domestic wastewater and effluent samples were collected every two days and analyzed directly after sampling. Parameters that reflect the bioreactor performance including COD and ammoniacal nitrogen were analyzed along with the  $SVI_{30}$  and mixed liquor suspended solids (MLSS). All analyses were conducted following Standard Methods for the Examination of Water and Wastewater [10]. A stereomicroscope (Olympus SZX7, Japan) was used to examine the morphology and diameters of developed granular sludge. Moreover, a field emission scanning electron microscope-energy dispersive X-Ray (FESEM-EDX; Jeol JSM7800F, Japan) was used to observe the microscopic structure and chemical elemental composition of aerobic granular sludge. Before the FESEM-EDX analysis, the granular sample was fixated using 2.5% glutaraldehyde and dried using gradient concentration of ethanol (10%, 30%, 50%, 70% and 100%). Therefore, the selection of granular sample was critical to ensure the successful preparation phase.

## 3. Results and Discussion

### 3.1. Development of aerobic granular sludge

In the seed sludge, activated sludge sourced from local WWTP exhibited irregular shape with poor settling properties. Meanwhile, the anaerobic bioflocs were spherical shaped and black colored. A mixture of anaerobic bioflocs and activated sludge resulted in  $SVI_{30}$  value of 147 mL/g, meanwhile the  $SVI_5$  was 264 mL/g, which indicated poor settleability of the seed sludge. In the first 30 days, biomass washout occurred in the system, causing fluctuation in both MLSS and  $SVI_{30}$  values. Due to this condition, settling time was prolonged from 10 to 15 minutes to retain biomass concentration in the SBR columns. Settling time was gradually decreased as stable concentration of MLSS was observed. In the first stage, anaerobic bioflocs were disintegrated into smaller granules. This phenomenon was aligned with previous reports by Linlin et al. (2005). Color shift was also observed in the anaerobic bioflocs from black to brownish color. The color changes indicated successful attachment of aerobic layer on anaerobic bioflocs.

After 30 days of experimental period, stable increase of MLSS and decrease of SVI<sub>30</sub> values may be observed in the system as depicted in figure 2. By day-110, the MLSS value was 11,900 mg/L with SVI<sub>30</sub> around 10 mL/g. However, towards the end of the experiments, the MLSS decreased to 4,000 mg/L and SVI<sub>30</sub> increased to 45 mL/g. These signified disintegrations of aerobic granular sludge in the system. However, further study is required to fully understand the disintegration mechanisms of aerobic granular sludge along with the governing factors for granular strength.

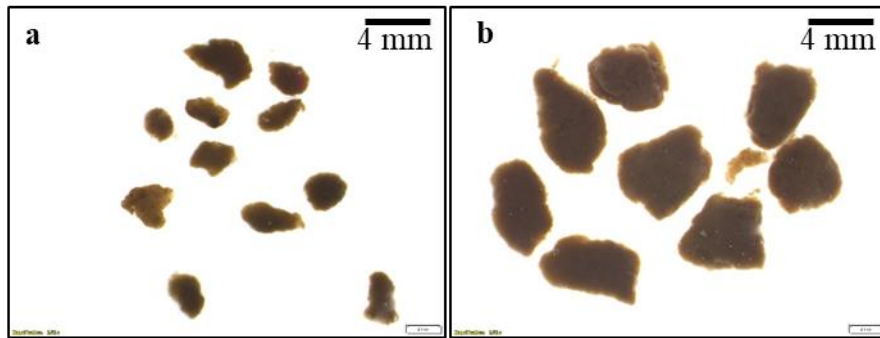


**Figure 2.** Variation of MLSS and SVI<sub>30</sub> value throughout the development of aerobic granular sludge

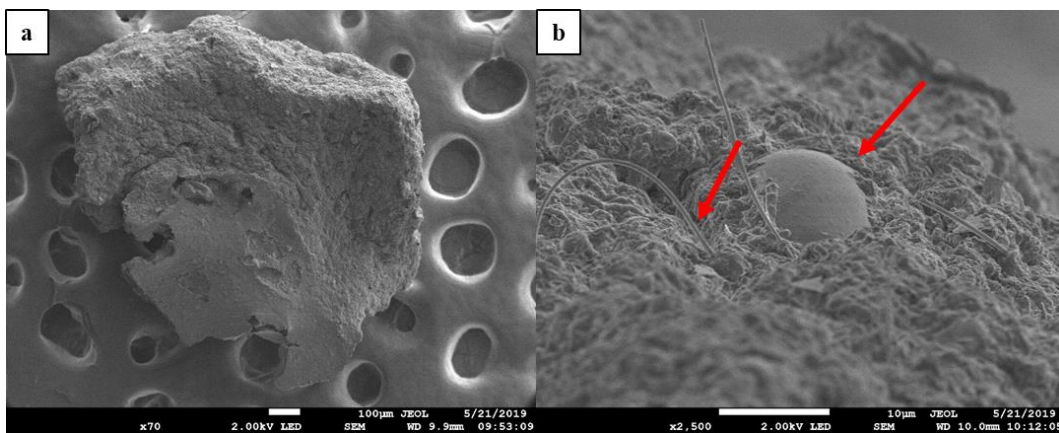
### 3.2. Characteristics of aerobic granular sludge

#### 3.2.1. Morphological and physical characteristics

Developed aerobic granules were harvested from the SBR column and observed using stereomicroscope. Aerobic granular sludge sample was separated based on its size, from smallest to largest, accordingly. The largest diameter of granular sludge was found to be 6.3 mm, meanwhile, the smallest diameter of granular sludge was 2.8 mm. Mature aerobic granular sludge showed a smooth borderline and regular spherical structure as shown in figure 3. FESEM analysis revealed a high number of cocci-shaped bacteria residing on the outer layer of aerobic granules as depicted in figure 4. Cocci-shaped bacteria were proven to act as a support in microbial attachment process in aerobic granulation [11]. Moreover, few of rod-shaped bacteria were also observed on the surface of aerobic granules as they can enhance the bonding of microbial matrix in granulation process [12]. Micropores that are essential as a mean of transportation for substrates and metabolic products were also found on the granular surface [13].



**Figure 3.** Mature aerobic granular sludge harvested after 140 days of experimental period (Magnification: 2.5 $\times$ ) whereby a) smallest aerobic granules and b) largest aerobic granules



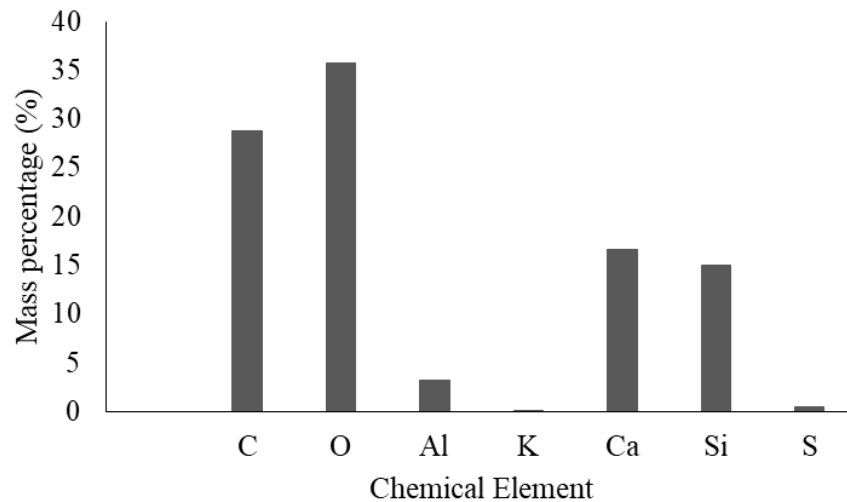
**Figure 4.** a) Overview of aerobic granular sludge observed using FESEM; b) Cocci-shaped bacteria residing on outer layer of aerobic granules

In comparison with conventional activated sludge, aerobic granular bioflocs is known to have excellent settling properties. The largest granular sludge exhibited excellent settling velocity of 75 m/h, which was almost 7-times higher than the settling velocity of conventional activated sludge used in this study (11 m/h). The settling velocity in this study was found to be higher than previous reports treating different types of wastewater [14,15]. A study by Rosman et al. (2013) demonstrated aerobic granulation using rubber wastewater, resulting in 33 m/h settling velocity. Meanwhile, Gonzalez-Martinez et al. (2017) used synthetic wastewater and reported 51.8 m/h settling velocity after 240 days development of aerobic granular sludge. It may be concluded that the anaerobic bioflocs in the seeding has increased the cell density of granular sludge, therefore, resulting in higher settling velocity.

### 3.2.2. Chemical elemental compositions

Composition of chemical elements in aerobic granular sludge was affected by many factors, such as granular age and operational temperature [16]. In this study, the results demonstrated a dominant percentage of carbon and oxygen in the granular sludge at 29% and 36%, respectively, as shown in figure 5. High mass percentage of calcium and silicon were also detected in the granular sample. High calcium content indicates the capability of aerobic granular sludge to absorb calcium from the wastewater. Moreover, calcium is also useful to initiate granulation process due to its capability to neutralize negative charge on microbial surface, thus enhancing the cell-to-cell attachment [17]. Silicon was detected at 15% in the granular sample. Although low concentration of silicon has previously been reported [18], it is worth noting that high silicon composition may assist the growth of microbial

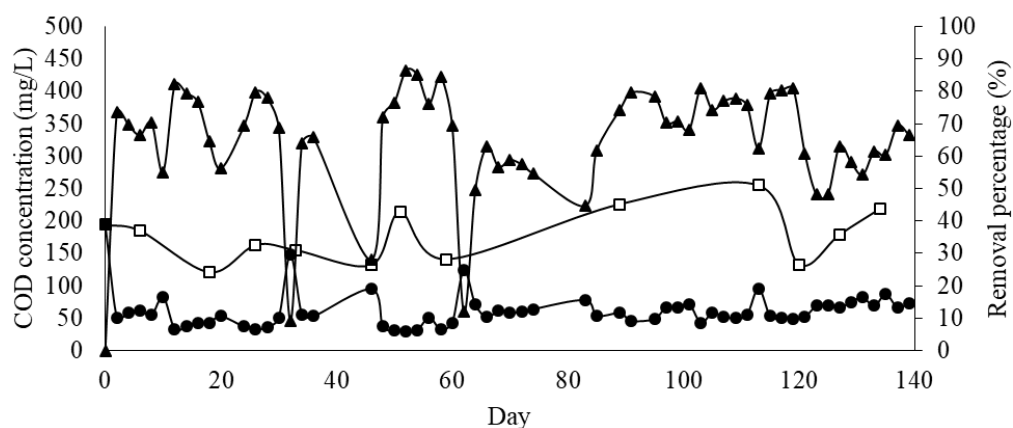
metabolism and contribute to the strength of granular sludge [19]. Moreover, aluminum was detected at 3.25%, which directly correlates with excellent settling features [20].



**Figure 5.** Chemical elemental composition of aerobic granular sludge

### 3.3 Performance of domestic wastewater treatment

Performance of aerobic granular sludge in treating domestic wastewater was observed based on the COD and ammoniacal nitrogen removals. COD removal was achieved at 67% with 58 mg/L COD concentration in the effluent after 140 days of experimental period. COD concentration in the effluent was decreasing in the first 20 days as depicted in figure 6, however, due to unpredictable weather conditions, the COD concentration in the raw wastewater decreased, causing unstable COD concentration in the effluent. Since open pond system is widely used in local WWTP, heavy rainfall may directly affect the concentration of organic content in the wastewater stream. Moreover, some mechanistic issue also occurred throughout the experimental period, specifically on day 32 and 62. These issues caused the fluctuation in the COD removal efficiency. Nevertheless, despite the technical issues, the reactor was able to maintain its performance after the operation was resumed.

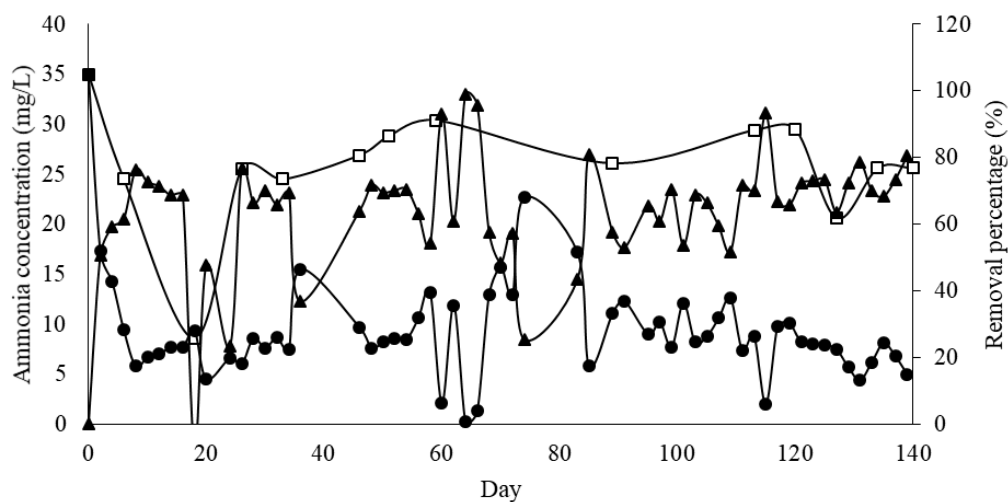


**Figure 6.** COD removal in the SBR system using aerobic granular sludge (●: COD concentration in the effluent, □: COD concentration in the influent, ▲: COD removal percentage)

Although aerobic granulation was successfully developed into the full-scale treatment plant [21] and in laboratory scale [8,22], the concentration of COD in the raw wastewater used in this study was much lower and varied than the previous studies. For example, Coma et al. (2012) used domestic wastewater with COD concentration of 326 mg/L. On the other hand, de Kreuk and van Loosdrecht (2006) used domestic wastewater with COD concentration of 160 mg/L. Domestic wastewater, in general, has lower organic content than industrial wastewater, such as piggery wastewater; 418-1600 mg/L tCOD [23], livestock wastewater; 3600 mg/L [24] and rubber wastewater; 1850 mg/L [14].

The COD removal efficiency in this study was found to be lower than the previous reports. Therefore, it may be concluded that the addition of anaerobic bioflocs in the seeding may not be beneficial for COD removal efficiency. Proteobacteria which was known to play dominant roles in COD removal is aerobic bacteria, thus the mechanisms of COD removal mostly require aerobic microorganisms. Nevertheless, the results in the present study implied that aerobic granular sludge might be used in long-term operation in domestic wastewater with stable biological activities. These results are complementary to previous studies treating low-strength wastewater [6,25].

The concentration of ammoniacal nitrogen was also observed throughout the study and it was found that removal of ammoniacal nitrogen was relatively stable at 72%, which depicted in figure 7. This result indicated that ammonia-oxidizing and nitrification occurred in the reactor. Moreover, this result demonstrated that aerobic layer was successfully attached to anaerobic bioflocs. However, the removal of ammoniacal nitrogen in this study was found to be lower than the previous reports that reached more than 90% ammoniacal nitrogen removal [26].



**Figure 7.** Ammoniacal nitrogen removal in SBR system using aerobic granular sludge

#### 4. Conclusion

Aerobic granular sludge was successfully developed using anaerobic bioflocs as the seeding. This seeding condition might induce rapid granulation process using low strength domestic wastewater. Granulation started after 30 days of experimental period and aerobic layer attached to the anaerobic bioflocs. Mature granular sludge was found to be spherical-shaped with dominant of cocci-shaped bacteria. Moreover, mature granular sludge has excellent settling properties compared to conventional activated sludge. However, the performance of domestic wastewater treatment in terms of COD and ammoniacal nitrogen removal was found to be lower than the previous study. Factors governing the efficiency of domestic wastewater treatment may be optimized in further study.



## Acknowledgements

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