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# Development and characteristics of microalgae-bacteria aerobic granules treating low strength municipal wastewater

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# Development and characteristics of microalgae-bacteria aerobic granules treating low strength municipal wastewater

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**Abstract.** This study aims to assess the feasibility of cultivating microalgae-bacteria aerobic granular sludge using low-strength municipal wastewater (<200 mg COD/L) as a substrate. A laboratory-scale photo-sequencing batch reactor was inoculated with activated sludge and fed with municipal wastewater. The development and characteristics of microalgae-bacteria aerobic granular sludge were observed for 90 days. The average COD removal efficiency of this system was 71%. The granulation process was observed to occur on day 28, indicated by a high biomass accumulation and decreasing sludge volume index (SVI). After 90 days of cultivation, MLSS value was found to be 3.7 g/L and $SVI_{30}$ at 18.9 mL/g. In addition, the diameter of the granules was significantly increased. Mature microalgae-bacteria aerobic granules were developed with an average and a maximum diameter of 0.65 mm and 6 mm, respectively. Moreover, cocci-shaped bacteria and microalgae cells were observed to reside on the granular surface during observation using field emission scanning electron microscopy (FESEM). The granules exhibited an excellent settling velocity at 80 m/h. The findings suggest the possibility of using low-strength wastewater to develop microalgae-bacteria aerobic granular sludge.

## 1. Introduction

Aerobic granular sludge is a dense and self-immobilized aggregate consisting of microbial consortiums that concurrently remove carbon, nitrogen, phosphorus, and other contaminants only in a single reactor [1]. It has been shown that aerobic granular sludge is a more efficient and novel method of treating wastewater than conventional activated sludge [2,3]. Aerobic granular sludge system demonstrated several advantages such as high rates of organic matter and nutrient removals, lower land footprint, less investment cost, lower energy, and better effluent quality [4,5]. At present, aerobic granular sludge has been successfully developed using various wastewater sources such as domestic, municipal, and industrial wastewaters, including palm oil mills effluent (POME), paper mills effluent, dairy, textile, and leachate [6]. Currently, several full-scale aerobic granular sludge technologies have been developed and are mainly used to treat municipal wastewaters with medium to high COD concentration (>500 mg/L) [7]. However, a full-scale aerobic granular sludge system for the treatment of low-strength wastewater remains understudied [8].

Low COD concentrations due to rain and sewer leakage are prevalent in countries with combined sewer systems, such as China [9]. Similarly, low influent COD concentrations of 300–400 mg/L were frequently reported in Malaysia [8]. However, the implementation of aerobic granular sludge for low-strength municipal wastewater remains a challenge due to the long-term granular stability and the lengthy start-up phases. While the latter problem may be solved by adding biodegradable organic substrates or/and inoculating with mature granules, complete knowledge of the fundamental factors that affect granule stability is still inadequate, posing the primary disadvantage of long-term aerobic granular sludge application for the treatment of actual low-strength wastewater.

Recent research has shown that microalgae can be integrated into aerobic granular sludge systems, namely the microalgae-bacteria aerobic granular sludge system [10]. This newly established microalgae-bacteria aerobic granular sludge system was reported to enhance system stability, induce the rapid formation of aerobic granular sludge, and require less carbon source [11,12]. Up to the present, several studies have developed microalgae-bacteria aerobic granular sludge systems using low-strength and synthetic wastewater [12,13]. However, limited studies have been conducted to investigate the feasibility of developing microalgae-bacteria aerobic granular sludge using actual low-strength wastewater as a substrate. Synthetic wastewater may not accurately represent how the system will perform when using actual wastewater, as actual wastewater is more likely to contain complex organic compounds. Moreover, actual wastewater has varied concentrations that can affect system stability. Therefore, the purpose of this study is to develop and characterize microalgae-bacteria aerobic granular sludge using actual low-strength municipal wastewater as an influent.

## 2. Materials and methods

### *2.1. Reactor design and operational conditions*

A single cylindrical column type photo-sequencing batch reactor (PSBR) column with a total working volume of 1.5 L and a height/diameter ratio of 16.67 (6 cm of internal diameter and 100 cm of height) was employed to develop microalgae-bacteria aerobic granular sludge. A microsparger at the bottom of the reactor was used to introduce air at a rate of 2.5 L/min. The reactor was operated at room temperature, and illumination was provided at 113 µmol/m$^2$s with 12hr:12hr (light:dark) photoperiod. The batch cycle lasted 6 hours, comprising 5 min feeding period, 232-247 min reaction period (90 min of non-aeration and 227-242 min of aeration), 15-20 min settling period, and 3 min period of effluent discharge. The column was seeded with 750 ml of activated sludge and was fed with municipal wastewater collected from a local wastewater treatment plant (WWTP). The characteristics of municipal wastewater employed in this study are listed in Table 1.

**Table 1.** Characteristics of municipal wastewater

| Elements | Concentrations (mg/L) |
|---|---|
| COD | 184 ± 73 |
| BOD | 67.9 ± 6.9 |
| Total Nitrogen (TN) | 16.6 ± 2.3 |
| Total Phosphorus (TP) | 2.4 ± 1.5 |
| pH | 6.9 ± 0.1 |

### *2.2. Analytical methods*

Parameters indicating the development of microalgae-bacteria aerobic granular sludge were evaluated, including sludge volume index ($SVI_{30}$), mixed liquor suspended solids (MLSS), and chemical oxygen demand (COD) removal efficiency, respectively. All analyses were carried out in compliance with the Standard Methods for the Examination of Water and Wastewater [14]. The shape and diameters of microalgae-bacteria aerobic granules were determined using a stereomicroscope (LEICA EZ4W, Germany). Additionally, aerobic granular sludge's microscopic structure and chemical elemental content were observed using a field emission scanning electron microscope-energy dispersive spectroscopy (FESEM-EDS; JSM-7800F, Japan). Prior to FESEM-EDS analysis, the granules were fixated with 2.5

percent glutaraldehyde mixed with phosphate buffer saline (PBS) for 2 hours and then dried with ethanol gradients (10%, 30%, 50%, 70%, and 100%).

## 3. Results and discussions

### *3.1. Development of microalgae-bacteria aerobic granular sludge*

Figure 1 depicts the changes in MLSS concentration and $SVI_{30}$ values throughout the study. When the PSBR was first inoculated with activated sludge and fed with municipal wastewater, the initial MLSS was 2.1 g/L with an $SVI_{30}$ of 166.67 mL/g, indicating poor settleability. MLSS values were first observed to increase but then sharply decreased due to biomass washout after day-14. Moreover, the decrease in biomass concentration may be attributed to a scarcity of carbon sources. To combat this issue, settling time was increased from 10 minutes to 15 minutes. MLSS concentration was observed to gradually increase after day-35, and 3.7 g/L of MLSS was obtained at the end of the experiment. The MLSS concentration in this study is comparable to previous reports in treating low-strength municipal and domestic wastewater [15,16]. Following a similar pattern, the $SVI_{30}$ value fluctuated until day 35; then, a decreasing trend was observed as MLSS concentration was stably increasing. A final $SVI_{30}$ value of 18.9 mL/g was recorded, indicating an excellent settleability. An $SVI_{30}$ value of 30-80 mL/g is generally reported for the settleability of mature aerobic granules [5].

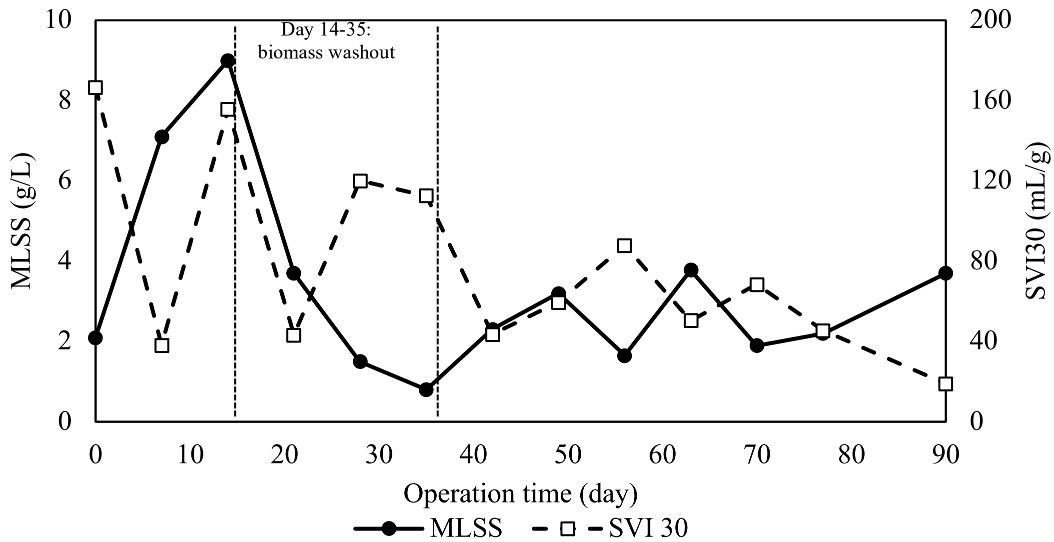


**Figure 1.** MLSS and SVI30 value throughout the microalgae-bacteria aerobic granular sludge system.

As this study utilized actual low-strength wastewater, the influent COD concentration (184 ± 73 mg/L) fluctuated during the entire experimental period, causing the fluctuation in effluent COD concentration. The municipal wastewater taken from a local WWTP used an open tank system; therefore, unforeseen weather circumstances such as heavy rainfall can directly affect the organic content of the wastewater. However, the effluent COD concentration was consistently less than 50 mg/L, albeit greater in some cases, as shown in figure 2. The average COD removal and effluent COD content after 90 days of the experimental period were 71% and 48.5 mg/L, respectively. This result is in accordance with Malaysia Sewage and Industrial Effluent Discharge Standard A (≤50 mg/L) [17].

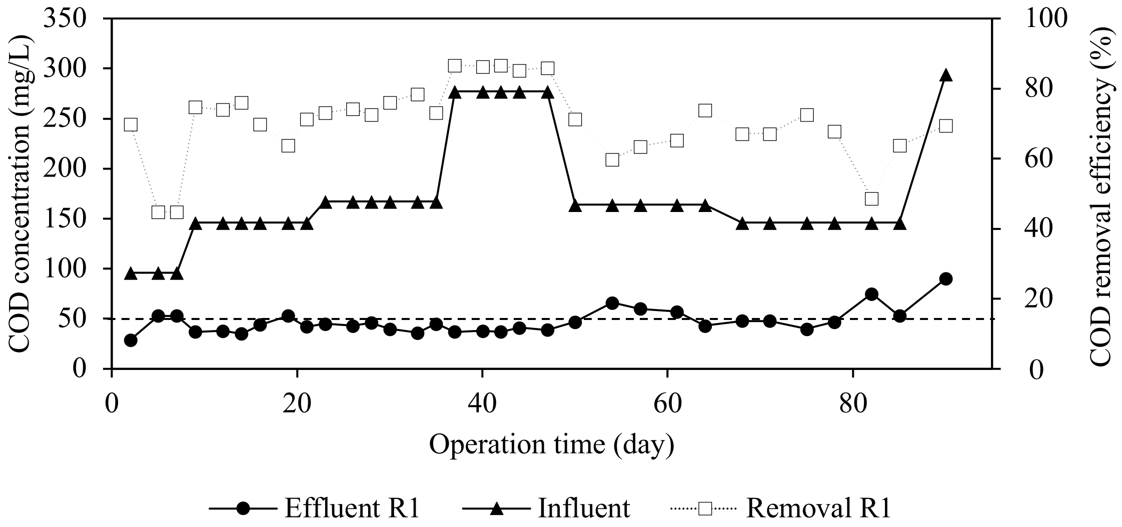


**Figure 2.** COD removal in the microalgae-bacteria aerobic granular sludge system.

In this study, the COD removal efficiency is reported to be lower than prior findings in treating actual low-strength wastewater at ≈80-85% removal efficiency [5,15,18,19]. However, it should be emphasized that the COD concentration of the municipal wastewater employed in this study (184 mg/L) is much less than the previous study (218-600 mg/L). This shows that microalgae-bacteria aerobic granular sludge may be suitable for treating very low-strength municipal wastewater.

### *3.2. Characteristics of microalgae-bacteria aerobic granular sludge*

#### *3.2.1. Morphological and physical characteristics*

The mean size of the granules was increased significantly after day 28 and was stable at around 0.6 mm, as shown in figure 3. This indicates that granulation occurs roughly on day 28, and this observation is supported by the gradual increase in MLSS value and the decrease in $SVI_{30}$ value after day 30. Similarly, a rather small granule size of 0.25-0.63 mm was reported by Sguanci et al. [18]. When the diameter of aerobic granules reaches above 1 mm during low-strength wastewater treatment, they become loose, porous, and unstable; therefore, they are more likely to undergo disintegration [20,21].

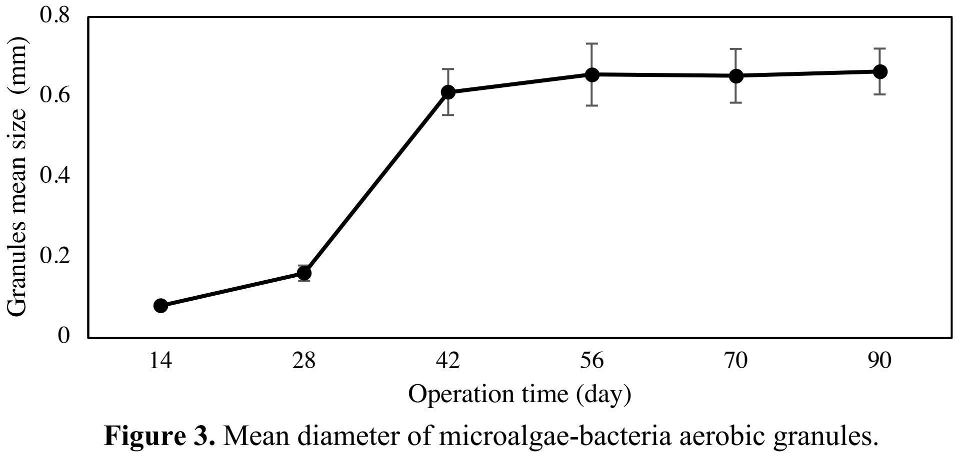


**Figure 3.** Mean diameter of microalgae-bacteria aerobic granules.

At the end of the experiment, microalgae-bacteria aerobic granules were harvested from the PSBR column. Figure 4 shows the notable difference between the size of the granules on day-0 and at the end of the experimental period. The obtained mature microalgae-bacteria aerobic granular sludge had a maximum diameter of approximately 6 mm. Furthermore, the microalgae-bacteria aerobic granular sludge has a remarkable settling velocity of 80 m/h, which is about ten times faster than the settling velocity of conventional activated sludge used in this study (7.9 m/h). This corresponds to the low $SVI_{30}$ value at the end of the experiment, indicating an excellent settleability as well as full granulation.

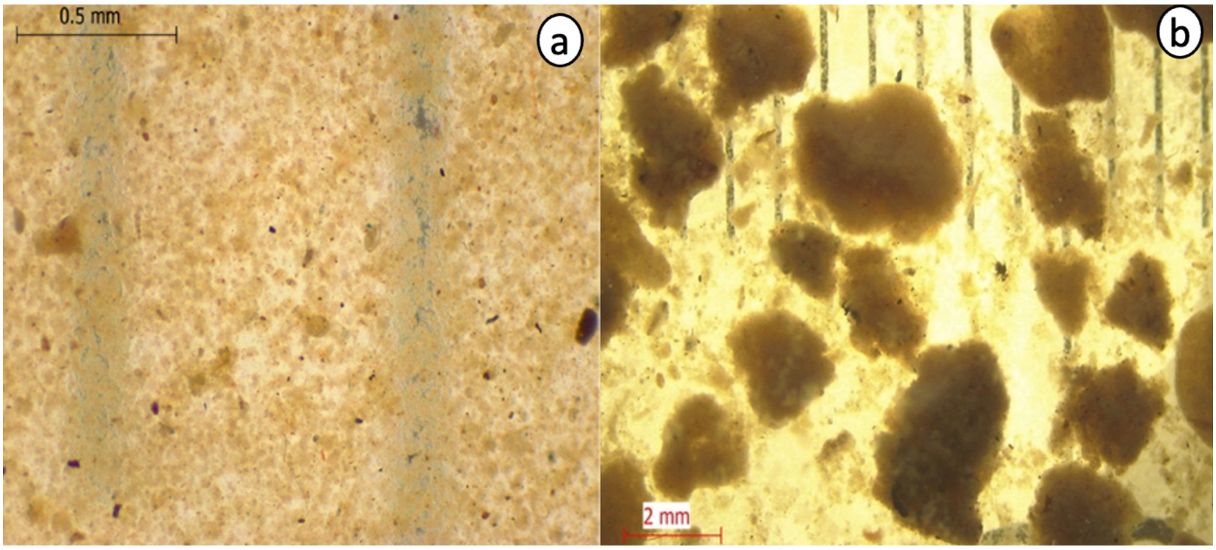


**Figure 4.** (a) seeding sludge on day-0 (b) Mature microalgae-bacteria aerobic granular sludge harvested after 90 days.

*3.2.2. SEM images and elemental composition*

FESEM analysis revealed many cocci-shaped bacteria residing on the exterior layer of microalgae-bacteria aerobic granules as shown in figure 5 (a). Cocci-shaped bacteria have been shown to function as a support in the aerobic granulation's microbial attachment process [22]. In addition, microalgae cells and a glue-like material termed extracellular polymeric substances (EPS) were also found to be embedded on the surface of microalgae-bacteria aerobic granules, as indicated in figure 5 (b). EPS is a complex substance consisting of a mixture of different macromolecules such as carbohydrates, proteins, lipids, nucleic acids and glycoproteins. EPS has been recognized as the primary component leading to the production of mature granules because it serves as a 'glue' to trigger biofilm formation among microorganisms [23]. Moreover, numerous different-sized micropores were seen on the granular surface. Micropores are required for substrate transportation into the inner layer of the granules as well as for excretion of the metabolic product out from the granules [24].

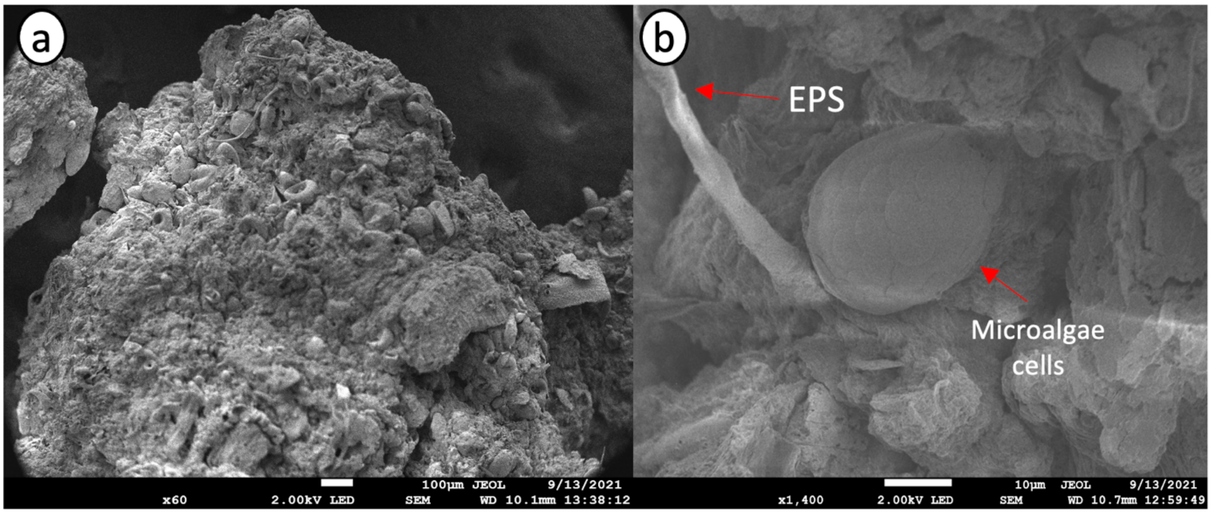


**Figure 5.** (a) Overview of microalgae-bacteria aerobic granular sludge (b) EPS and microalgae cells.

EDS analysis detected 9 types of elements (figure 6); carbon (C), oxygen (O), iron (Fe), sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), potassium (K), and calcium (Ca). The results showed a dominating proportion of carbon and oxygen in the microalgae-bacteria aerobic granular sludge of 44% and 26%, respectively. Calcium, potassium, and magnesium ($Ca^{2+}$, $K^+$, and $Mg^{2+}$) are all strongly linked to the composition of the EPS matrix. [25]. $Ca^{2+}$ is required for the gelation of the alginate-like exopolysaccharides (ALE) and ALE provides mechanical stability and elasticity to the microalgae bacteria aerobic granular sludge [26]. Potassium content in microalgae-bacteria aerobic granular sludge was 0.6%, and none was detected in the activated sludge. However, calcium and magnesium content in microalgae-aerobic granular sludge was significantly lower than in activated sludge. These findings contradict the excellent settleability showed by microalgae-bacteria aerobic granular sludge and the significant increase in granule diameter. Therefore, complementary spectroscopic analysis such as x-ray photoelectron spectroscopy (XPS) is recommended to confirm and elucidate the elemental composition of microalgae-aerobic granular sludge.

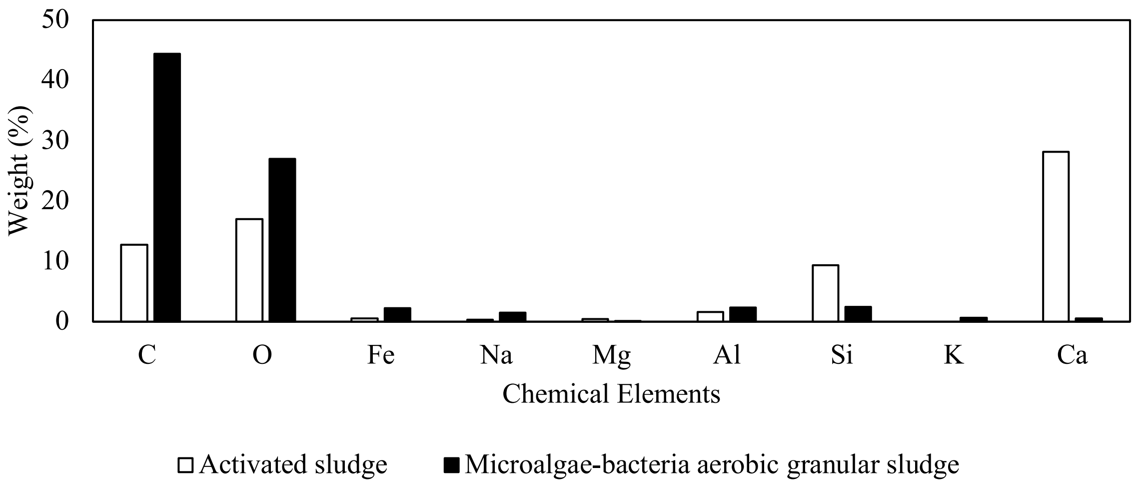


**Figure 6.** Elemental composition of microalgae-bacteria aerobic granular sludge.

## 4. Conclusion

Microalgae-bacteria aerobic granular sludge was successfully developed using low-strength municipal wastewater. Granulation started after 28 days of the experimental period, indicated by a stable increase of MLSS concentration up to 3.7 g/L, excellent settleability indicated by 80 m/h settling velocity and low SVI of, as well as a significant increase in granule diameter. The average effluent COD concentration and COD removal efficiency is >50 mg/L and 71% respectively, indicated the capability of microalgae-bacteria aerobic granular sludge to be used for the treatment of low-strength wastewater. Finally, complementary spectroscopic analysis is recommended to characterize microalgae-aerobic granules' element composition in detail.

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## References

[1] Nancharaiah Y v. and Kiran Kumar Reddy G 2018 Aerobic granular sludge technology: Mechanisms of granulation and biotechnological applications *Bioresource Technology* **247** 1128–43
[2] Boltz J P, Johnson B R, Takács I, Daigger G T, Morgenroth E, Brockmann D, Kovács R, Calhoun J M, Choubert J M and Derlon N 2017 Biofilm carrier migration model describes reactor performance *Water Science and Technology* vol 75 (IWA Publishing) pp 2818–28
[3] Sarma S J and Tay J H 2018 Aerobic granulation for future wastewater treatment technology: Challenges ahead *Environmental Science: Water Research and Technology* **4** 9–15
[4] Pronk M, Abbas B, Al-zuhairy S H K, Kraan R, Kleerebezem R and van Loosdrecht M C M 2015 Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge *Applied Microbiology and Biotechnology* **99** 5257–68
[5] Derlon N, Wagner J, da Costa R H R and Morgenroth E 2016 Formation of aerobic granules for the treatment of real and low-strength municipal wastewater using a sequencing batch reactor operated at constant volume *Water Research* **105** 341–50
[6] Purba L D A, Ibiyeye H T, Yuzir A, Mohamad S E, Iwamoto K, Zamyadi A and Abdullah N 2020 Various applications of aerobic granular sludge: A review *Environmental Technology and Innovation* **20**
[7] Sepúlveda-Mardones M, Campos J L, Magrí A and Vidal G 2019 Moving forward in the use of aerobic granular sludge for municipal wastewater treatment: an overview *Reviews in Environmental Science and Biotechnology* **18** 741–69
[8] We A C E, Aris A and Mohd Zain N A 2020 A review of the treatment of low-medium strength domestic wastewater using aerobic granulation technology *Environmental Science: Water Research and Technology* **6** 464–90
[9] Cao Y, van Loosdrecht M C M and Daigger G T 2020 The bottlenecks and causes, and potential solutions for municipal sewage treatment in China *Water Practice and Technology* **15** 160–9

[10] Guo D, Zhang X, Shi Y, Cui B, Fan J, Ji B and Yuan J 2020 Microalgal-bacterial granular sludge process outperformed aerobic granular sludge process in municipal wastewater treatment with less carbon dioxide emissions *Environmental Science and Pollution Research*
[11] Zhang Y, Dong X, Liu S, Lei Z, Shimizu K, Zhang Z, Adachi Y and Lee D J 2020 Rapid establishment and stable performance of a new algal-bacterial granule system from conventional bacterial aerobic granular sludge and preliminary analysis of mechanisms involved *Journal of Water Process Engineering* **34** 101073
[12] Yang X, Zhao Z, Zhang G, Hirayama S, Nguyen B van, Lei Z, Shimizu K and Zhang Z 2021 Insight into Cr(VI) biosorption onto algal-bacterial granular sludge: Cr(VI) bioreduction and its intracellular accumulation in addition to the effects of environmental factors *Journal of Hazardous Materials* **414**
[13] Dong X, Zhao Z, Yang X, Lei Z, Shimizu K, Zhang Z and Lee D J 2021 Response and recovery of mature algal-bacterial aerobic granular sludge to sudden salinity disturbance in influent wastewater: Granule characteristics and nutrients removal/accumulation *Bioresource Technology* **321** 124492
[14] Anon 1999 *Standard Methods for the Examination of Water and Wastewater* APHA/AWWA/WEF 2012 Standard Methods for the Examination of Water and Wastewater *Standard Methods* **541**
[15] Yu C, Wang K, Tian C and Yuan Q 2021 Aerobic granular sludge treating low-strength municipal wastewater: Efficient carbon, nitrogen and phosphorus removal with hydrolysis-acidification pretreatment *Science of the Total Environment* **792**
[16] Campo R, Sguanci S, Caffaz S, Mazzoli L, Ramazzotti M, Lubello C and Lotti T 2020 Efficient carbon, nitrogen and phosphorus removal from low C/N real domestic wastewater with aerobic granular sludge *Bioresource Technology* **305**
[17] Ministry of Environment and Water 2009 Environmental Quality Sewage Regulations *Environmental Quality Act 1974* P.U.A 432-2009
[18] Sguanci S, Lubello C, Caffaz S and Lotti T 2019 Long-term stability of aerobic granular sludge for the treatment of very low-strength real domestic wastewater *Journal of Cleaner Production* **222** 882–90
[19] Petrini S, Foladori P, Beghini F, Armanini F, Segata N and Andreottola G 2020 How inoculation affects the development and the performances of microalgal-bacterial consortia treating real municipal wastewater *Journal of Environmental Management* **263**
[20] Zhang H, Dong F, Jiang T, Wei Y, Wang T and Yang F 2011 Aerobic granulation with low strength wastewater at low aeration rate in A/O/A SBR reactor *Enzyme and Microbial Technology* **49** 215–22
[21] Peyong Y N, Zhou Y, Abdullah A Z and Vadivelu V 2012 The effect of organic loading rates and nitrogenous compounds on the aerobic granules developed using low strength wastewater *Biochemical Engineering Journal* **67** 52–9
[22] Zhang H, Dong F, Jiang T, Wei Y, Wang T and Yang F 2011 Aerobic granulation with low strength wastewater at low aeration rate in A/O/A SBR reactor *Enzyme and Microbial Technology* **49** 215–22
[23] Tay J H, Liu Q S and Liu Y 2001 The effects of shear force on the formation, structure and metabolism of aerobic granules *Applied Microbiology and Biotechnology* **57** 227–33
[24] Xu G, Xu X, Yang F and Liu S 2011 Selective inhibition of nitrite oxidation by chlorate dosing in aerobic granules *Journal of Hazardous Materials* **185** 249–54
[25] Dahalan F A, Mohamed Najib M Z, Salim M R and Ujang Z 2015 Characteristics of developed granules containing phototrophic aerobic bacteria for minimizing carbon dioxide emission *International Biodeterioration and Biodegradation* **102** 15–23
[26] Lin Y M, Sharma P K and van Loosdrecht M C M 2013 The chemical and mechanical differences between alginate-like exopolysaccharides isolated from aerobic flocculent sludge and aerobic granular sludge *Water Research* **47** 57–65