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Development of Microalgae-Bacteria Aerobic Granular Sludge for Landfill Leachate Treatment

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Abstract. Over the past few decades, aerobic granular sludge has become favorable biotechnology in wastewater treatment. Combining the AGS process with microalgae technology is desired to achieve higher treatment efficiency, especially for high-strength wastewater. This study aims to investigate the effectiveness of microalgae-bacteria AGS in treating landfill leachate. The photo-sequencing batch reactor, inoculated with *Acutodesmus obliquus*, was fed with old synthetic leachate, and run for 30days. The result showed that chemical oxygen demand (COD) removal up to 96%. The initial ammonia concentration was 130mg/L and achieved an efficiency of up to 80.77%. At the same time, the average removal of the phosphorus was 19.5mg/L, equal to 48.68%. The result of microalgae-bacteria AGS can produce the fast-settling properties of the granules throughout the experiment.

1. Introduction

Malaysia hugely relies on landfilling as a primary disposal method in dealing with municipal solid waste (MSW), which has become one of the most significant environmental issues. There were 300 operated and non-operated solid waste disposal sites in Malaysia, and only 13 were classified as sanitary landfills [1]. Approximately 95% of the collected waste was disposed in open-dumpling and landfill sites, making most sites exceed their full operating capacity [2]. Later, the degradation of the waste in landfills over time releases gaseous products and liquid waste called leachate. Leachate is considered a very toxic and highly contaminated solution product, and its composition varies widely depending on landfill's age, climate condition, type of waste [3].

The constituent of leachate consists of ammonia, dissolved solids, heavy metals, biodegradable, and refractory organic matter. Most importantly, landfills will continue to produce leachate for 30 to 50 years even after the landfills site is closed [4]. It is significant to collect leachate and treat to prevent the impact on the environment and human health, usually by removing the hazardous substances before discharging them to the water system. It is pretty challenging to treat leachate due to its variety of characteristic composition and quantity [5]. Several different technologies, such as chemical, biological, physiochemical, and physical methods, are available to treat landfill leachate to achieve the standards established by legislation. However, these conventional methods generally require various processes which are expensive and complicated [6].

Despite the limitation, the biological treatment process is still used worldwide to treat leachate. Though it is only effective for young leachate as it contains an easily biodegradable organic matter, old leachate is predominantly constituted by refractory organic matter and high ammonia nitrogen concentration which is not easily degraded [6]. In the meantime, microalgae are receiving great attention due to their ability to produce valuable metabolites and perform wastewater treatment at once. The worthiness of microalgae is due to their capability to uptake metal, time and energy-saving, eco-friendliness, and enable easy recovery [7]. Nevertheless, harvesting is one of the most challenging aspects of microalgae biotechnology, accounting for 20 to 30 percent of total production expenses [8]. Recent studies discovered that microalgae could integrate into the aerobic granular sludge system to induce its formation and stable performance. Therefore, cultivating microalgae with the AGS system was favored. Aerobic granulation for wastewater treatment is a recent innovation inside biofilm reactors constitutes a providential technology that does not require material support for biofilm growth which is considered a particular type of biofilm compromised of self-immobilized cells [9]. It is known to have a strong microbial structure, compact and dense, with excellent settleability and high biomass retention, which is suitable for treating toxic substrates and a wide range of wastewater types [10]. However, the ability of AGS to treat leachate wastewater that might contain toxic substrates is still unknown.

Considering the information of leachate treatment by microalgae-bacteria AGS is lacking. The purpose of this study is to cultivate microalgae-bacteria aerobic granular sludge for landfill leachate treatment. *Acutodesmus obliquus* was used as a supplementary microalga in this investigation. The inclusion of microalgae species was intended to improve treatment efficiency while also promoting the granular process.

2. Materials and methods

2.1. Reactor setup and operational condition

The PSBR, which consists of a plexiglass cylinder with a diameter of 60mm and a height of 650 mm, was utilized to develop microalgae-bacteria aerobic granular sludge. The given diameter and height provide the H/D ratio of 10.83, and the total volume of the reactor is 1.8 L. The reactor had a working volume of 1L and a volume exchange ratio of 50 percent (VER). This PSBR system was run in an eight-hour cyclic time, consisting of 10 minutes feeding, 90 minutes of non-aeration, 365 minutes of aeration, settling time of 10 minutes settling time, and 5 minutes for wastewater to be discharged [11]. The reactor was illuminated by white LED light at 130 μmol/m^{2}s light intensity under constant dark/night cycles of 12h/12h and operated at room temperature (25 ± 2°C). The diagram of the SBR is shown in figure 1. A 500 mL seed sludge, including activated sludge and microalgae, was used to inoculate the reactor. 500 mL of wastewater was supplied and released at the beginning and end of each cycle.

2.2. Seed sludge

In the study, seed sludge consisted of activated sludge from Indah Water Konsortium (IWK) Sewage Treatment Plant in Bunus, Kuala Lumpur, and target microalgae species *Acutodesmus obliquus* from algae biomass ikozha, Malaysia-Japan International Institute of Technology. The collected activated sludge was filtered to eliminate massive residue to avoid clogging the reactor tube, followed by pre-conditioning, which involved acclimating the activated sludge for 24 hours at room temperature. *Acutodesmus obliquus* was selected regarding its remarkable growth rate in the presence of leachate and positive growth rate in all nutrient-rich settling [12]. The microalgae were cultured in AF6-medium for two weeks to reach the exponential phase of microalgae growth. [13]. Subsequently, it was inoculated with activated sludge with approximately 10^8 cells. The seed sludge biomass initial concentration was 9575mg/L, and the sludge volume index was (SVI_{30}) was 69.8mL/g.

2.3. Wastewater preparation

This synthetic wastewater was prepared to follow the characteristic of old leachate. This leachate was formulated with tannic acid to stimulate the refractory organic matter contained in old leachate. The ingredients to prepare 1 liter of synthetic old leachate were listed as follows (mg/L): tannic acid 200, NaCl 2000, $CaCl_2$ 700, $NaHCO_3$ 2000, NaOH 297, K_2HPO_4 32.5, NH_4Cl 120-150, and trace metal 0.02mL ($MnSO_4.7H_2O$ 500mg, $CoSO_4.7H_2O$ 150mg, $NiSO_4.6H_2O$ 500 mg, $CuSO_4.5H_2O$ 40mg, H_3BO_4

50mg, $ZnSO_4.7H_2O$ 50mg, $(NH_4)_6Mo_7O_{24}.4H_2O$ 50mg, $Al_2(SO_4)_3.16H_2O$ 30mg, and 96%H_2SO_4 1ml) [14].

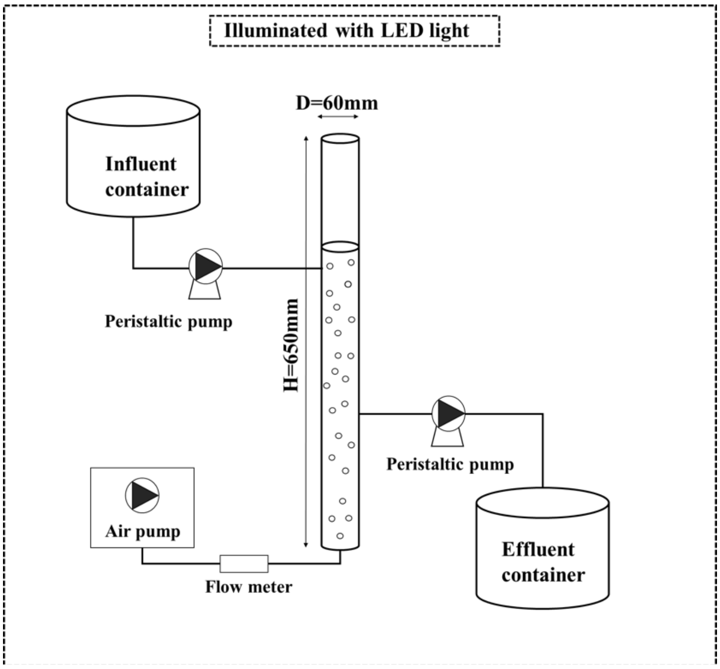

Figure 1. The simplified diagram of PSBR.

2.4. Analytical methods
The wastewater and sludge analyses were carried out using parameters including chemical oxygen demand (COD), total phosphorus (TP), and ammoniacal nitrogen (NH3-N). The sample was taken twice a week and analysed immediately for a better result, and the effluent was not filtered before being analysed. The system's development was evaluated on mixed liquor suspended solids (MLSS) and sludge volume index (SVI_{30}) following standard methods [15]. The granular sludge settling velocity and aspect ratios were used to undertake physical characterization of the microalgae-bacteria aerobic granular sludge. The average time it took granular sludge to reach the bottom of a cylindrical column filled with tap water was used to calculate velocity. At the same time, the aspect ratios were determined as the ratio of the granular sludge's shortest and longest dimensions to reflect the granular sludge's roundness.

3. Results and Discussion

3.1. Development of microalgae-bacteria aerobic granular sludge

3.1.1. Morphological observation. The initial seed sludge was microalgae culture, and the activated sludge had an uneven form and flossy structure with extremely tiny bioflocs. After seven days, the sludge began to form minute granules ranging from 1.3 to 2.3mm. Due to the sheer presence of microalgae, the colour of the sludge also changed to light brown. On day 14, the granule size was slightly more noticeable, with diameters ranging from 1.45 to 2.61mm. It is obvious during the trial period that the granule size began to dominate with a more compact shape on day 30th. In the reactor, granular sludge with an average size of 1.54 to 2.41mm and a dark brown colour was observed. Figure 2 depicts the maturation of the granular sludge.

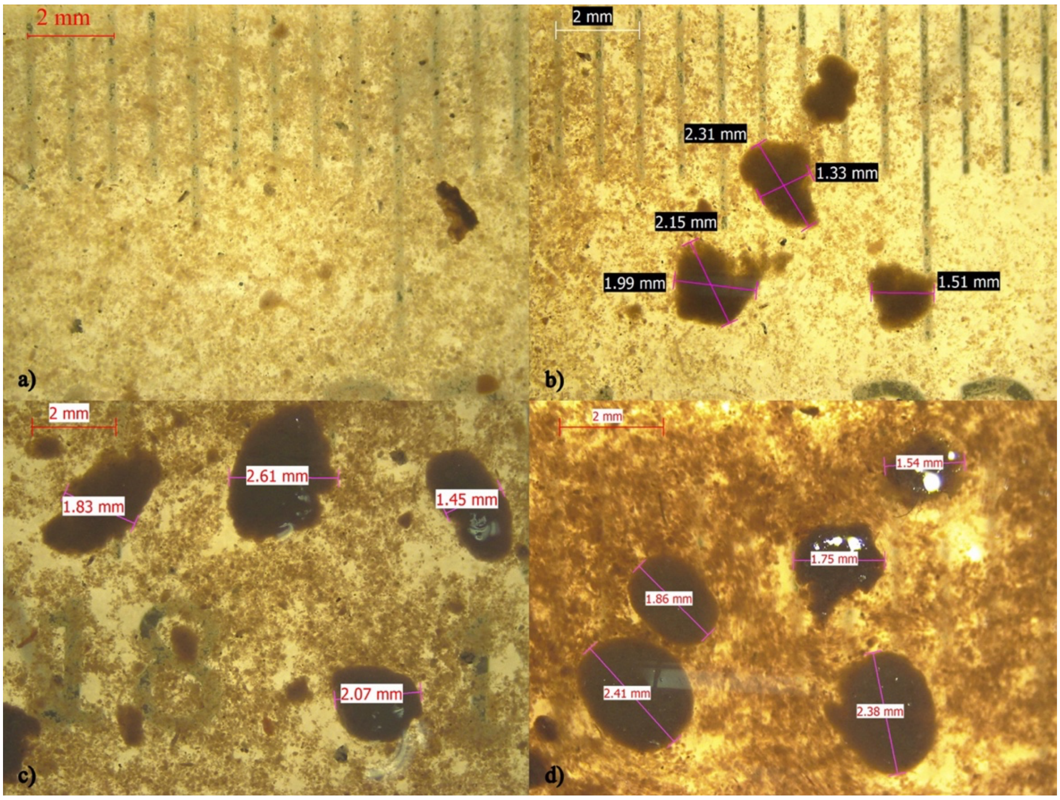

Figure 2. The development of microalgae-bacteria AGS inside the PSBR system **a)** Seed sludge; **b)** At 7th day of operation; **c)** At the 14th day of operation; and **d)** At 30th day of the experiment.

3.1.2. Biomass growth and settleability. The seed sludge contained activated sludge and microalgae at an initial MLSS of 9575mg/L. During the experimental period, weekly evaluations of biomass growth and settleability were performed, as shown in figure 2. During the first week, the MLSS dropped considerably to 4200mg/L. It seemed that less compacted sludge with poor settling abilities was discharged with the wastewater. This process happened as a result of the desire to keep sludge with strong settling capabilities in order to adapt and develop a granular sludge [13]. Hence, the MLSS concentration started to recover from day 7 to day 21 with an average concentration of 5525mg/L. Despite that, the final MLSS remained only 3350mg/L by day 30. It appeared that some granules might be too big and started to disintegrate and wash out through the effluent. Moreover, the amount of sludge in the reactor was not monitored during the experimental study. Therefore, it is essential to pay attention to the sludge contained in the reactor to avoid the major drop for the whole development.

The settleability of the granular sludge is represented by the sludge volume index for 30mins (SVI_{30}). The initial value of SVI_{30} was 69.8mL/g, which suggested poor settling properties of the seed sludge. The SVI_{30} rapidly dropped to 29.07 mL/g over the first 14 days of reactor operation as the seed sludge changed into granular sludge. The result showed the importance of microalgae and filamentous bacteria in the granulation process [16]. Even though the SVI_{30} was minor increased on day 21 to 37.21 mL/g due to the disintegration of the granule and biomass washout, the SVI_{30} was able to attain 35.82 mL/g by day 30 of the experiment.

3.1.3. Physical characteristic. The physical characteristic of aerobic granular sludge was addressed for the mature granules' settling velocity and aspect ratio. The average aspect ratio of microalgae-bacteria aerobic granular sludge was 0.8, demonstrating the capacity to swiftly produce a round-shaped granular sludge utilizing synthetic leachate over the 30-day experimental period. Additionally, the settling

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velocity of the mature granule found after 30 days achieved 40.65m/h, which was higher than a previous study from Arcila and Buitrón [17] with the formation of microalgae-bacteria aggregate for treating municipal wastewater of 18m/h. At the same time, Cai et al [18] achieved a settling velocity of 12.8m/h of the granules treating synthetic domestic wastewater. Thereby, this result could indicate the fast-settling velocity of the mature granules. Consequently, the result of both SVI_{30} and settling velocity could show excellent settling properties throughout the experiment. Regarding this result, it is worth mentioning that although microalgae-bacteria AGS can produce the fast-settling properties of the granules, it is also essential to maintain the concentration of MLSS along with time.

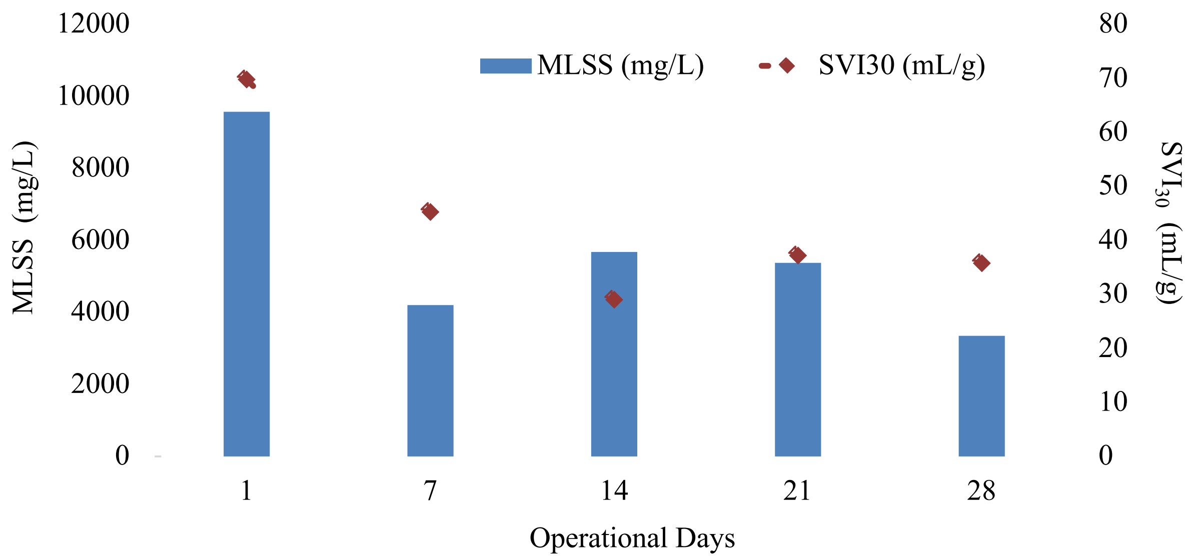

Figure 3. Dynamic of the biomass concentration (MLSS) and settle ability (SVI_{30}) throughout the 30 days of experiment.

3.2. Performance of microalgae-bacteria granular sludge

3.2.1. COD removal. The initial concentration of COD in the influent was 3690mg/L. As shown in figure 4 (a), stable COD removal was observed throughout the 30days of the experimental period. Despite a biomass washout at the beginning of the experiment, COD removal remained greater than 90%. The excellent potential for organic removal was performed mainly by the bacterial consortium in the activated sludge, which converts organic carbon as an energy source for their growth and proliferation. As a result, the heterotopic bacteria proliferate faster than microalgae, implying that the removal of organic matter was mostly attributable to bacteria in the co-culture system [19]. By the end of the experiment, the average COD removal rate was 96 percent, resulting in a COD concentration of 160.61 mg/L in the effluent. This finding was shown to be comparable to prior publications dealing with lower COD concentrations [20-22]. According to Huang *et al.* [20], COD removal from synthetic wastewater containing 600mg/L was around 96 percent, with a final COD content of less than 30mg/L.

In comparison, Zhang *et al*. [21] achieved 96% COD removal with the initial concentration of 1200mg/L COD. A prior investigation utilizing synthetic wastewater yielded a similar result, with an average COD removal effectiveness ranging from 72-89% [13, 19, 23]. According to the findings of this investigation, granular sludge has a high potential for organic removal.

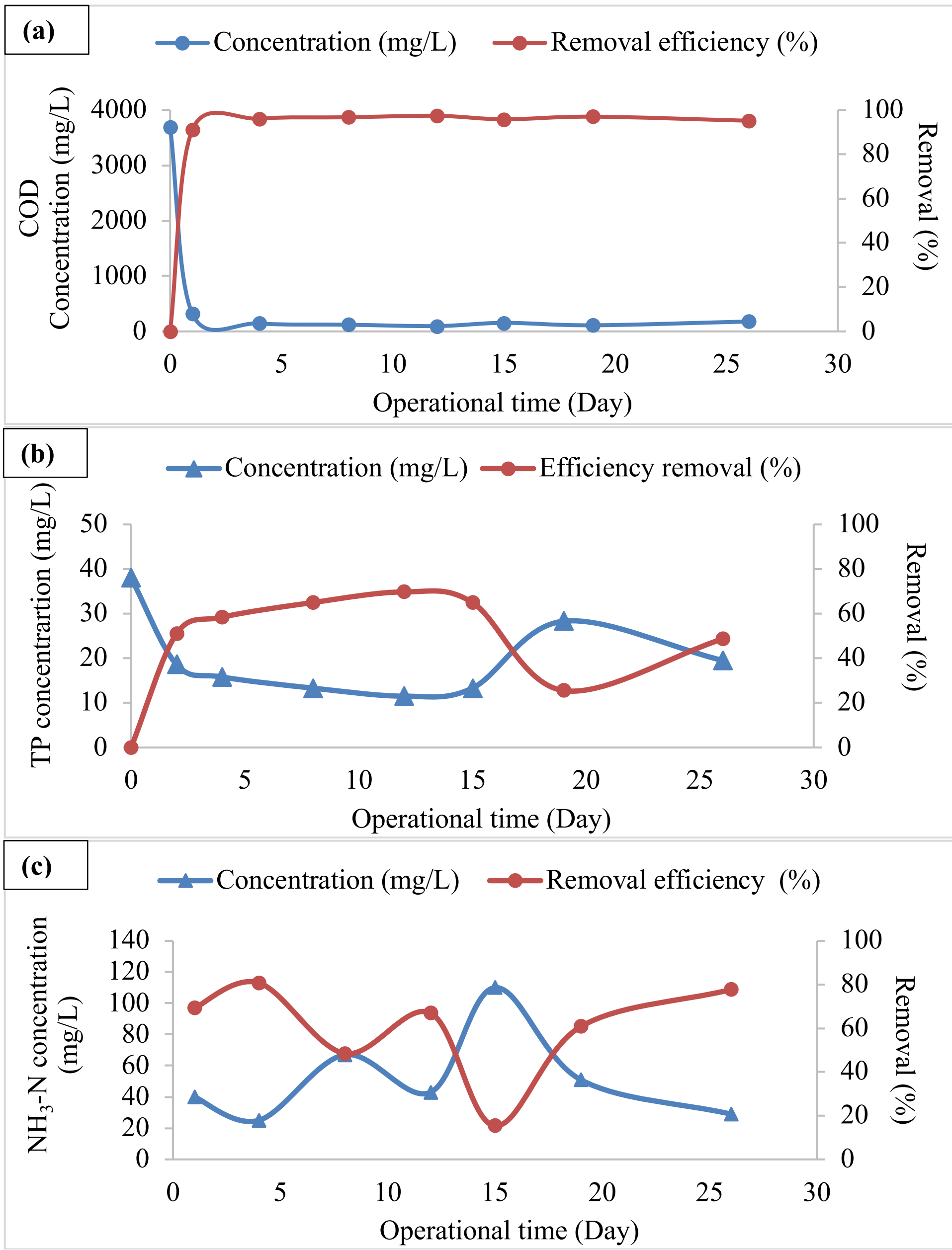

Figure 4. (a) COD concentration and percentage removal in PSBR system; **(b)** TP concentration of the effluent in PSBR; **(c)** Ammonia concentration of the effluent in PSBR; throughout 30days experiment.

3.2.2. Performance of nutrient removals. The biological nutrient removal from synthetic leachate wastewater was determined in terms of TP and ammonia. The concentration of influent P was 38mg/L; thus, TP removal can be analyzed by PO_4-P removal. The result shows that the average effluent PO_4-P concentration during the first 15 days was 13.3mg/L, equal to 65% removal efficiency. Subsequently, from day 15 to 20, PO_4-P in the effluent was slightly increased to 28.3mg/L, equivalent to 25.53% in removal efficiency. However, by day 30, this concentration remains only 19.5mg/L, equal to 48.68%. The Average PO_4-P concentration was 16.8mg/L resulting in 55.79% PO_4-P removal from synthetic leachate wastewater. These findings were found to be lower than previous studies using synthetic wastewater [19, 21]. Zhang *et al.* [21] and Zhu *et al.* [19] achieved PO_4-P removal to 89% and 100%, respectively. According to Purba *et al.* [13], poor PO_4-P removal efficiency may be attributable to the absence of sludge retention time (SRT) control in the system; therefore, no intentional biomass discharge was conducted. Furthermore, the organic loading rate (OLR) in the system is not favorable for nutrient removal in this study.

The ammonia concentration in the effluent was unstable throughout the experiment, as illustrated in figure 4 (c). The initial concentration of ammonia in synthetic leachate was 130mg/L. In the first 5 days of operation, the removal efficiency of ammonia in the treated effluent was 80%. However, the result varied and achieved the lowest removal efficiency after 15 days at a concentration of 110mg/L, corresponding to 15% removal efficiency. The ammonia fluctuations in the effluent greatly increase due to biomass washout in the system [13]. However, at the end of the experiment, the removal efficiency was able to reach 78%, which was equal to 29mg/L. Through 30days experimental period, the average concentration of ammonia in the effluent was 52.14mg/L with a removal efficiency of 60%. As indicated in table 1, the outcome of ammonia removal in this study was lower than in earlier publications. Meanwhile, Zhu *et al*. [19] demonstrated an ammonia removal efficiency of 75%, corresponding to an ammonia concentration of 65mg/L. According to Liu *et al* [24], the ability of microalgae cells to directly assimilate ammoniacal nitrogen in wastewater has the potential for microalgae-bacteria aerobic granular sludge to attain optimum efficiency.

Table 1. Summary of wastewater performance using microalgae-bacteria aerobic granular sludge.

Wastewater type	Percentage removal of the effluent			
	COD	TP	Ammonia	References
Synthetic leachate	96% (160.61mg/L)	55.79% (16.8mg/L)	60% (52.14mg/L)	This study
Synthetic domestic wastewater	95.2% (<30mg/L)	44%	_	[20]
Domestic wastewater	72%	5% (7mg/L)	72% (6mg/L)	[13]
Synthetic domestic wastewater	–	17-35%	40-92%	[25]
Municipal wastewater	88-89%	20-33%	99%	[23]
Synthetic wastewater	96%	89%	99%	[21]
Synthetic municipal wastewater	–	34%	98%	[24]
Domestic wastewater	96%	44%	99%	[22]
Synthetic wastewater	82%	100%	75%	[19]

4. Conclusions

Microalgae-bacteria aerobic granular sludge was successfully developed using old synthetic leachate within 30days of the experiment. The produced granules had a satisfactory settling property, with a settling velocity of 40.65m/h and an SVI_{30} value of 35.82mL/g. Additionally, the wastewater treatment performance of microalgae-bacteria aerobic granular sludge remained steady during the whole experiment, with a COD removal efficiency of 96%, PO_4-P removal efficiency of 55.79%, and an ammoniacal nitrogen removal efficiency of 60%. A further study should focus on higher ammonia nitrogen and heavy metal removal from the system. Moreover, the chemical characteristic of the granule should also be analysed.

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