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Microalgal Growth and Nutrient Removal Efficiency in Non-Sterilised Primary Domestic Wastewater

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Abstract: Microalgae biomass can produce high quantities of biochemicals that can be used in various applications such as biodiesel, biogas, and aquaculture feed. The potential of sterilizing wastewater for microalgae-based wastewater treatment on a lab scale is well introduced. However, the operation cost for large-scale microalgae cultivation in wastewater treatment plants is high if using sterilising wastewater as the growth medium. The present study aimed to evaluate the growth of *Scenedesmus* sp., *Chlorococcum aquaticum*, *Ankistrodesmus augustus*, and *Haematococcus pluvialis* in non-sterilised domestic wastewater and their potential for pollutant removal in wastewater. The microalgae were cultivated in different concentrations of non-sterilised domestic wastewater, collected from a primary wastewater plant of a national sewerage company in Malaysia. Each species' capacity for growth and the removal of pollutants were assessed. The results showed that the cell density, maximum biomass productivity, and biomass concentration of *H. pluvialis*, *Scenedesmus* sp., and *C. aquaticum* in 100% wastewater were significantly higher than the standard medium. Higher biomass concentration was obtained from *H. pluvialis* and *C. aquaticum* in 100% wastewater (815 g/L and 775.83 mg/L); nevertheless, *Scenedesmus* sp. in 100% wastewater yielded the highest specific growth rate (0.798 d^{-1}) and the maximum biomass productivity (99.33 mg/L/day). *Scenedesmus* sp. in 100% wastewater also achieved better removal efficiency of total nitrogen (TN), total phosphorus (TP), and ammonia ($N\text{-}NH_4$) with more than 90%. All tested microalgae species successfully remove nitrogen, ammonium, and phosphorus and reach the concentration limits set by the Department of the Environment, Malaysia. This study demonstrated that microalgae can grow well in non-sterilised domestic wastewater while simultaneously removing nitrogen and phosphorus effectively.

Keywords: domestic wastewater treatment; microalgal biomass; biomass energy; non-sterile microalgae growth; nitrogen removal; *Scenedesmus*; phosphorus removal

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1. Introduction

Wastewater usually contains various wastes, especially nitrogen and phosphorus. When these excessive nutrients are released into the waterbody, they induce the rapid growth of algae and aquatic plants, leading to a phenomenon that is known as an algal bloom or eutrophication [1]. Even though algae and aquatic plants are not harmful in normal situations, they have devastating effects on aquatic ecosystems during eutrophication. The overgrowth of these organisms reduces the oxygen level, increases water acidity, produces cyanotoxins, and blocks sunlight penetration [2–4]. This, in turn, causes marine life to be unable to survive, therefore leading to biodiversity and economic loss [3,5]. In addition to that, the toxin produced during eutrophication can become accumulated in fish and shells. Consumption of these aquatic products is hazardous to human health [6,7]. To minimize the occurrence of eutrophication, it is vital to reduce the level of nitrogen and phosphorus in wastewater effluents before releasing them into the natural environment. In this scenario, wastewater treatment plays an important role in removing these pollutants from wastewater before discharging them into natural water bodies.

Conventional wastewater treatment systems usually have two stages: primary treatment, which separates out most of the solids; secondary treatment, which biologically removes degradable organic matter [8]. However, secondary wastewater still has a significant quantity of nitrogen and phosphorus, which often leads to eutrophication in aquatic environments. In this regard, tertiary wastewater treatment is implemented to further reduce nitrogen and phosphorus to 0.3 mg/L [9]. Together with chemical and physical processes, these activities depend on microbial activity to decrease nitrogen and phosphorus. However, conventional wastewater treatments possess several limitations such as the consumption of a huge amount of chemicals, generation of greenhouse gases, high operational costs, high construction costs, and high power consumption [8,10]. Activated sludge processing technology that is usually employed in secondary treatment also comes with the cons of sludge disposal that might render environmental problems [11]. With an increase in the human population in the future, it is expected that the volume of wastewater will be gradually increasing, which in turn may cause more nutrients to be released to the water surface [12]. More wastewater treatment plants, especially tertiary treatment plants or more advanced facilities, will be necessary for the future. In some developing and lower-income countries, the investment and upgrading of treatment facilities have not grown at the same pace as urbanization and population growth because they cannot afford the continually increasing investment cost of treatment facilities [13]. On the other hand, dealing with the continually rising operational and maintenance costs of treatment facilities is another challenging task for developing countries [14]. Hence, it is critical to develop wastewater treatment systems with low capital requirements and good performance. Numerous technologies have been reported to remove phosphate and nitrogen from wastewater, such as the utilization of composite adsorbent [2], ligand-based conjugate materials [15], membrane contactors [16], and microalgae [17,18]. The use of microalgae for removing phosphate and nitrogen from wastewater has received increasing interest due to their varied advantages. [19]. Microalgae are a class of unicellular organisms that vary in size from a few to a few hundred micrometres and are present in many aquatic ecosystems. Compared to conventional wastewater treatment, microalgae-based wastewater treatment is less expensive, with lower greenhouse gas production, less capital cost, and lower energy consumption [20]. Many studies have demonstrated the capability of microalgae in treating different types of wastewater with high nutrient removal efficiency [21–24]. As the biodiversity of microalgae species is huge, different microalgae can be cultivated in various kinds of wastewater with complex characteristics. According to the studies, the capability of carbon dioxide uptake and the high carbon dioxide fixation potential of microalgae can help to mitigate atmospheric carbon dioxide while growing in wastewater [25,26]. The microalgae biomass obtained from wastewater contains many micro and macro metabolites such as proteins, lipids, carbohydrates, and pigments [27,28]. These metabolites have been demonstrated to have potential use in different applications such as biodiesel, biogas, and aquaculture feed [29–31]. Using wastewater as a microalgae culture medium also reduces the high cost of feedstock and the significant consumption of freshwater in biofuel production [32]. Olabi et al. [33] indicated that the integration of microalgae cultivation with wastewater treatment could help to achieve various Sustainable Development Goals including "Clean water and sanitation", "Affordable and clean energy", and "Climate action".

Wastewater usually contains bacteria, pathogens, and other microorganisms. The microbial communities in the wastewater could compete for nutrients with the microalgae or produce extracellular substances that kill microalgae, thereby reducing microalgal growth [34]. In many studies, the wastewater was sterilised before microalgae cultivation to eliminate microorganisms [35,36]. This practice might be effective on a small scale but will increase the operational cost in large-scale production [18]. On the other hand, some studies report the relationship of mutualism between microalgae and other microorganisms [37,38].

Microorganisms such as bacteria can help to degrade complex substances into phosphate, ammonium, and carbon dioxide, which can easily be used by microalgae. Hence, prior to large-scale cultivation, it is important to evaluate microalgal growth in non-sterilised wastewater. However, studies using non-sterilised wastewater for cultivating microalgae are still limited.

Among the microalgae species, *Chlorella* is used in most often used in wastewater treatment studies because of its fast growth, high nutrient removal efficiency, and simple cultivation [39]. Other microalgal species such as *Chlorococcum*, *Ankistrodesmus*, *Haematococcus*, and *Scenedesmus* are not commonly used in wastewater treatment studies, although some of them have been reported to have high biochemical compositions under certain growth conditions, which are suitable for biofuels and other applications [40–42]. *Chlorococcum*, *Ankistrodesmus*, *Haematococcus*, and *Scenedesmus* belong to the same class of green algae as *Chlorella* [43,44], these microalgae should be able to grow in wastewater.

In Malaysia, studies related to microalgae-based wastewater treatment are limited. Several studies in Malaysia have reported microalgae could achieve high growth using palm oil mill effluent, municipal wastewater sludge, and wet market wastewater as culture mediums [45–47].

In this study, domestic wastewater collected from a primary wastewater treatment plant of a national wastewater company in Malaysia was used as the culture medium for cultivating microalgae. The collected wastewater sample, without sterilization, was used to cultivate four selected microalgae species, namely *Scenedesmus* sp., *C. aquaticum*, *A. augustus*, and *H. pluvialis*. The growth of the four selected microalgae in different concentrations of non-sterilised domestic wastewater was studied and the ability of each species on removing nitrogen and phosphorus from non-sterilised domestic wastewater was evaluated.

2. Materials and Methods

2.1. Microalgal Species and Culture Conditions

Microalgae strains *Scenedesmus* sp. (UTEX 1589), *C. aquaticum* (UTEX 2222), *A. augustus* (UTEX 189), and *H. pluvialis* (UTEX 2505) were purchased from University of Texas (UTEX), United States of America. The microalgae were grown and sustained in 250 mL Erlenmeyer flask containing 200 mL autoclaved Basal Bold Medium (BBM). The cultures were maintained at room temperature (25 ± 2 °C) with 2000 ± 200 lux, 16-h, cool-white fluorescent light illumination. To avoid microalgae adherence and congregation, manual aeration was performed thrice per day.

2.2. Domestic Wastewater Sources

Domestic wastewater was collected from primary sewage treatment plant located in Indah Water Konsortium (IWK), Titiwangsa, Kuala Lumpur, Malaysia. The concentrations of chemical oxygen demand, total suspended solids, ammonium ($N\text{-}NH_4$), total nitrogen (TN), and total phosphorus (TP) were measured using the Hach methods [48] and the results are presented in Table 1. The large solid particles were filtered from wastewater samples using Whatman grade 1 qualitative filter papers. The filtered wastewater was stored in a refrigerator at 4 °C prior to use.

Table 1. Characteristics of domestic wastewater collected from IWK.

Characteristics	Concentration (mg/L)
pH	7 ± 0.5
Chemical oxygen demand	120 ± 20
Total suspended solids	45 ± 5
Ammonia	29 ± 0.3
Total nitrogen	27 ± 1.1
Total phosphorus	12 ± 0.5
pH	7 ± 0.5

2.3. Microalgae Cultivation Using Domestic Wastewater

The experiments were carried out in 250 mL flasks with 245 mL medium consisting of different concentrations of non-sterilised wastewater (0%, 10%, 25%, 50%, 75%, and 100%, *v*/*v*). The diluted wastewater medium was prepared from 100% wastewater and distilled water without any additional nutrients. The microalgae were also cultivated in the autoclaved BBM and used as control culture. The inoculum added in the wastewater mediums and control culture was 1% (*v*/*v*) culture of exponentially growing microalgae described in Section 2.1. Both the control and experimental cultures were incubated in the conditions described in Section 2.1. The microalgae growth was measured every 2 days.

2.4. Harvesting and Drying Microalgae

Microalgal cells were collected at the same time of day every two days. Then, the cells were separated from medium by vacuum filtration using mixed cellulose ester membrane filters with absorbent pads. The cell residues were then dried in an oven at 70 °C until constant weight.

2.5. Determination of Microalgal Growth

The cell density of each culture was measured on the initial day of cultivation and every two days using hemocytometer (Marienfeld-Superior, Neubauer-improved, Lauda-Königshofen, Germany) with a light microscope (Eclipse E-100 LED, Nikon, Tokyo, Japan).

The biomass concentration was measured using the following equation:

$$\text{Biomass concentration (mg/L)} = (\text{DW}_\text{T} - \text{DW}_0)/\text{volume of sample} \quad (1)$$

where DW_T and DW_0 were the dry cell weight at time T and 0, respectively. The biomass productivity and specific growth rate were measured using the following equations:

$$\text{Biomass productivity, P}_\text{biomass}\text{ (mg/L/d)} = (\text{X}_\text{T} - \text{X}_0)/(\text{t}_\text{T} - \text{t}_0) \quad (2)$$

$$\text{Specific growth rate, } \mu\text{ (d}^{-1}) = (\ln \text{X}_\text{T} - \ln \text{X}_0)/(\text{t}_\text{T} - \text{t}_0) \quad (3)$$

where X_T and X_0 were the biomass concentrations at time T_t and T_0, respectively.

2.6. Nutrient Removal Analysis

Wastewater was sampled at the start and end of the experiments for the analyses of $N\text{-}NH_4$, TN, and TP. To separate wastewater samples from the biomass, samples were vacuum filtered using mixed cellulose ester membrane filters with absorbent pads. TN, TP, and $N\text{-}NH_4$ were digested and analyzed using Hach digestion kits [48]. The digestion of TN, TP, and $N\text{-}NH_4$ was performed using persulfate digestion method, acid persulfate digestion method, and Nessler method, respectively. The concentration of each sample was measured by a program set in Hach spectrophotometer DR5000 (Hach, Ames, IA, USA).

2.7. Statistical Analysis

All experiments were carried out in triplicate. One-way analysis of variance (ANOVA) with Tukey's HSD test at $p < 0.05$ was carried out to determine the significance of differences between variables.

3. Results and Discussion

3.1. Microalgal Growth in Wastewater Medium and BBM

Six different domestic wastewater concentrations (0%, 10%, 25%, 50%, 75%, and 100%, *v*/*v*) were used for the cultivation of *H. pluvialis*, *Scenedesmus* sp., *C. aquaticum*, and *A. augustus*. Figures 1 and 2 show the cell density and biomass concentrations of microalgae at different concentration levels during the 16 days of the experimental period.

The results showed that all tested microalgal species were able to grow in non-sterilised wastewater medium.

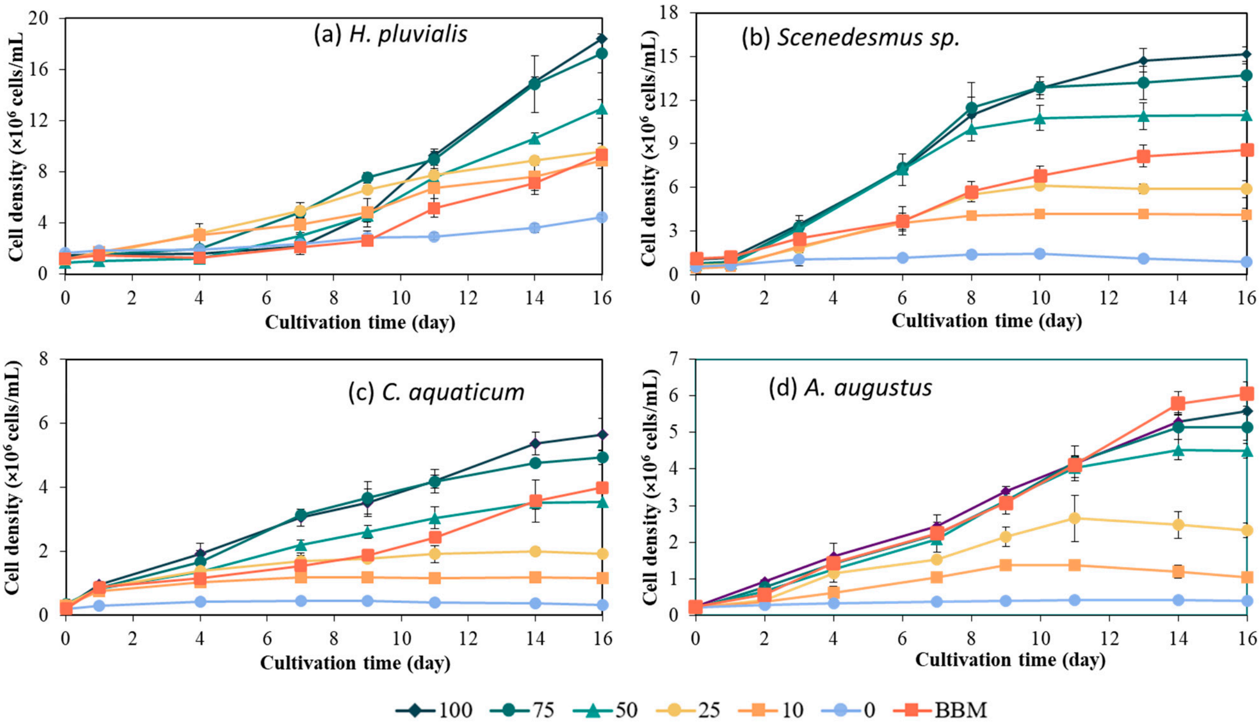

Figure 1. Cell density growth of microalgae in different concentrations of wastewater medium and BBM (n = 3).

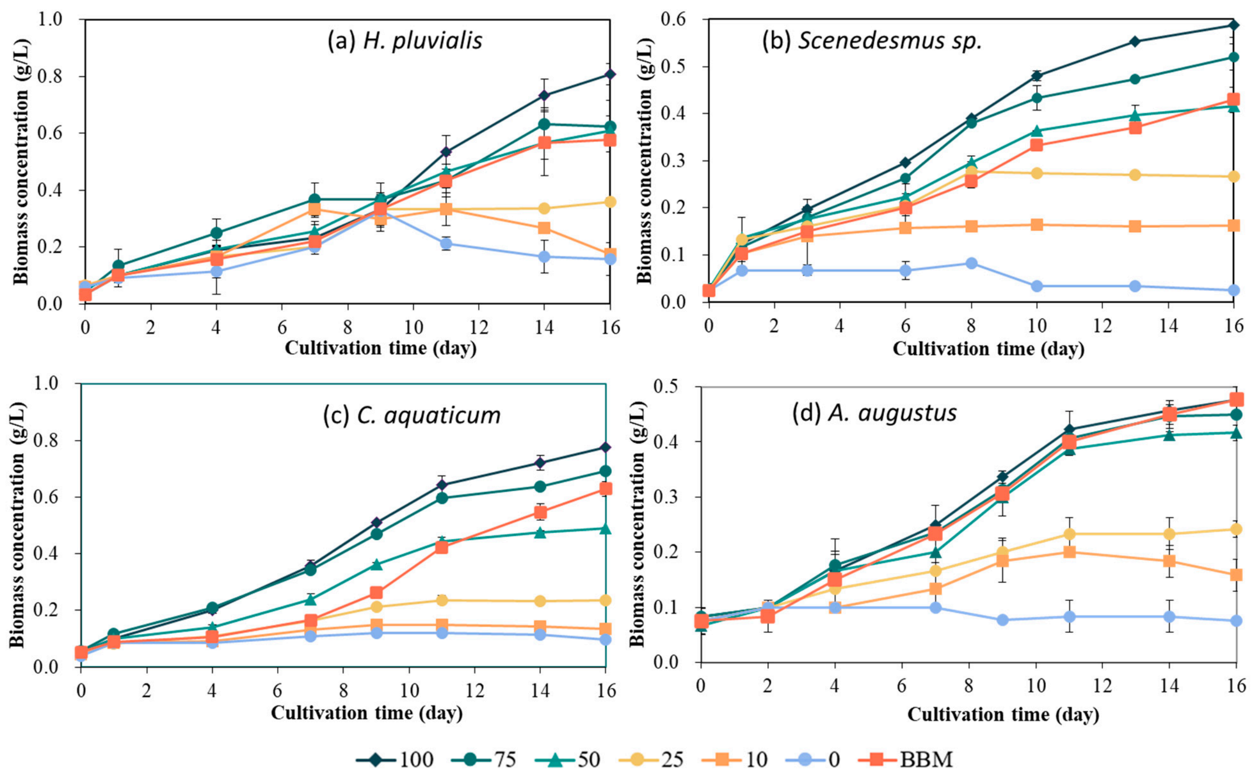

Figure 2. Biomass growth of microalgae in different concentrations of wastewater medium and BBM (n = 3).

The cell density and biomass concentrations of all microalgae increased with the concentration levels of the wastewater. The biomass concentration for *H. pluvialis*, *Scenedesmus* sp., and *C. aquaticum* in 100% wastewater at the end of the cultivations were 815.00, 588.60, and 775.83 mg/L, respectively, which was 47%, 37%, and 23% higher than the biomass concentration of microalgae cultivated in BBM. The biomass concentration of *A. augustus* in 100% wastewater was 476.67 mg/L, which was 1% lower than BBM. In contrast, the growth of all microalgae species declined after eight days with 10% and 25% wastewater due to the depletion of nutrients. This result agreed well with Silambarasan et al. [49] and Miao et al. [50], where the biomass concentration of microalgae in a high concentration (75–100%) of non-sterilised domestic wastewater was better than the biomass concentration in a low concentration (25–50%) of non-sterilised domestic wastewater and standard medium. Gupta et al. [51] observed that microalgae show a low value of quantum efficiency (Fv/Fm) of reaction centres in PS-II of chlorophyll when cultured in low-concentration wastewater after several days. The low value in Fv/Fm indicates that the microalgae were undergoing physiological stress therefore they declined in growth.

Compared to BBM, the cell density of *H. pluvialis*, *Scenedesmus* sp, and *C. aquaticum* in 100% wastewater at day 16 was 18.42×10^6, 15.16×10^6, and 5.65×10^6 cells/mL, respectively, which was 47.26%, 43.27%, and 29.33% higher, respectively, whereas the cell density of *A. augustus* in 100% wastewater at day 16 was 5.58×10^6 cells/mL, which was 8.6% lower. Several studies also reported a better growth of Scenedesmus sp. and *H. pluvialis* in a wastewater medium than in a standard synthetic medium [52–54]. Microalgae grew better in wastewater medium probably because the nutrient content in wastewater medium was more suitable to grow in. In comparison to *H. pluvialis* and *Scenedesmus* sp., the cell density of *C. aquaticum* was much lower while the maximum biomass concentration of these microalgae was comparable. This was probably due to the larger cell size of *C. aquaticum*; the cell size of *C. aquaticum* was larger than 5 µm, whereas the cell size of *H. pluvialis* and *Scenedesmus* sp. were smaller than 5 µm (see Appendix A). Aketo et al. [55] observed that smaller microalgal species can reach higher cell densities than larger microalgal species while the biomass concentration of both species could be similar. On the other hand, the biomass concentration and cell density of *A. augustus* were much lower than other microalgae. Likewise, the growth of *Ankistrodesmus* sp. in a previous study was much lower than other green microalgae species when cultivated in secondarily treated urban wastewater [56]. Suboptimal environmental factors such as light intensity, photoperiod, and pH might reduce microalgal growth [57].

Table 2 shows the maximum biomass productivity, maximum biomass concentration, and specific growth rate of *H. pluvialis*, *Scenedesmus* sp., *C. aquaticum*, and *A. augustus* cultivated in wastewater medium and BBM. Excluding Scenedesmus sp., the specific growth rate of other microalgae species cultivated in 100% and 75% wastewater showed no significant difference ($p < 0.05$) compared to BBM. In terms of maximum biomass productivity and biomass concentration of *H. pluvialis*, *Scenedesmus* sp., and *C. aquaticum*, the values obtained in 100% and 75% wastewaters were significantly higher ($p < 0.05$) than BBM. In the study of *A. augustus*, the maximum biomass productivity in BBM showed no significant difference ($p > 0.05$) compared to 100% and 75% wastewater while the maximum biomass concentration was significantly lower than 100% and 75% wastewater ($p < 0.05$). In addition to that, when observing under the light microscope, only tested microalgae species existed. No other microalgae grazer was observed. These results indicate the non-sterilised domestic wastewater without dilution can serve as a growth medium for *H. pluvialis*, *Scenedesmus* sp., *C. aquaticum*, and *A. augustus*. Previous studies also revealed other kinds of non-sterilised wastewater have the potential for microalgal cultivation [58]. Khatiwada et al. [59] indicated that *Chlorella* sp. in non-sterilised 70% malted wastewater yielded higher dry biomass than standard medium. Ziganshina et al. [60] showed that *Chlorella sorokiniana* in non-sterilised wastewater anaerobically digested agricultural waste by 10%.

Table 2. Maximum biomass productivity, maximum biomass concentration, and specific growth rate of all species in BBM and wastewater. Mean values ± standard deviation.

	Maximum Biomass Productivity ($P_{biomass}$, mg/L/day)	Maximum Biomass Concentration (X_{max}, g/L)	Specific Growth Rate, μ (d^{-1})
H. pluvialis			
BBM	45.83 ± 3.61 [a]	554.17 ± 38.19 [a]	0.563 ± 0.148 [a]
100%	51.19 ± 3.72 [b]	815.00 ± 37.75 [b]	0.588 ± 0.114 [a]
75%	54.94 ± 3.34 [b]	733.33 ± 57.74 [b]	0.589 ± 0.005 [a]
Scenedesmus sp.			
BBM	39.17 ± 7.64 [a]	430.00 ± 26.46 [a]	0.580 ± 0.026 [a]
100%	99.33 ± 5.13 [b]	588.60 ± 26.01 [b]	0.798 ± 0.023 [b]
75%	91.67 ± 5.77 [b]	520.00 ± 27.04 [c]	0.770 ± 0.025 [b]
C. aquaticum			
BBM	36.43 ± 1.14 [a]	629.17 ± 26.03 [a]	0.326 ± 0.037 [a]
100%	54.11 ± 2.35 [b]	775.83 ± 1.44 [b]	0.406 ± 0.039 [a]
75%	49.17 ± 1.14 [b]	693.33 ± 11.55 [c]	0.335 ± 0.029 [a]
A. augustus			
BBM	33.70 ± 4.85 [a]	483.29 ± 13.28 [a]	0.238 ± 0.013 [a]
100%	30.91 ± 3.88 [a]	476.67 ± 2.89 [a]	0.263 ± 0.016 [a]
75%	28.64 ± 1.20 [a]	447.67 ± 2.52 [b]	0.222 ± 0.021 [a]

Different lowercase letters in the same column indicate significant differences as determined by Tukey's test ($p < 0.05$).

Among all the species, Scenedesmus sp. in 100% wastewater yielded the highest specific growth rate and maximum biomass productivity. Although the cell density and biomass concentrations of *H. pluvialis* in 100% wastewater were the highest, its specific growth rate and maximum biomass productivity were lower than *Scenedesmus* sp. *Scenedesmus* sp. has the highest specific growth rate, probably due to its smaller cell size. Álvarez-Díaz and colleagues [56] reported that Scenedesmus obliquus in wastewater medium achieved a higher specific growth rate and maximum biomass productivity than *Ankistrodesmus falcatus.* Similarly, Kim et al. [52] reported that *Scenedesmus* sp. produced a comparable-or-higher maximum biomass concentration, specific growth rate, and biomass productivity compared to the other three microalgal species in the wastewater medium. On the other hand, the maximum biomass concentration, maximum biomass productivity, and specific growth rate of *A. augustus* were the lowest. This is probably because the environmental factors are not favoured for *A. augustus*; as the wastewater is not sterilized, the microorganisms existing in the wastewater probably inhibited the growth of *A. augustus*.

3.2. Nutrient Removal Efficiency

To assess the potential of selected microalgae as replacements for wastewater treatments, the nutrient removal efficiency in high (100%), medium (50%), and low (10%) concentration wastewaters was evaluated on day 16 of cultivation. As illustrated in Figure 3, all microalgae species achieved more than 90% of nitrogen, ammonium, and phosphorus removal in 50% and 10% wastewater. While *H. pluvialis*, *Scenedesmus* sp., *C. aquaticum*, and A. augustus remove 99.62%, 98.88%, 99.28%, and 98.78% of $N\text{-}NH_4$, respectively, in 100% wastewater, 92.26%, 94.81%, 88.91%, and 62.07% of TN, respectively, were removed in 100% wastewater. These results were similar to other studies [61,62] that reported microalgae preferred ammonium rather than other forms of nitrogen as nitrogen sources. This preference can be explained by the direct assimilation and lower energy of the metabolism of ammonium. Other nitrogen sources, such as nitrate and organic nitrogen, are reduced into ammonium before utilization, which consumes more energy than the metabolism of ammonium [63]. In addition, the presence of ammonium as an end product of nitrate reduction can inhibit nitrate uptake from the feedback inhibition effect [62].

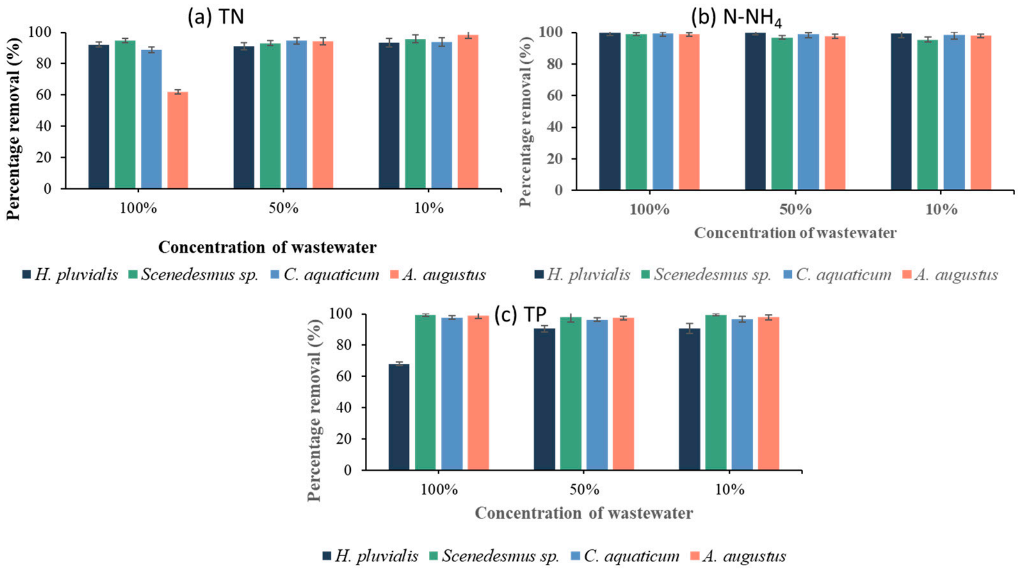

Figure 3. Nutrient removal of four microalgae in high (100%), medium (50%), and low (10%) concentrations of domestic wastewater at day 16 (n = 3).

The TN removal efficiency of *A. augustus* was lower than the other three microalgae species. The low growth rate could be contributing to this result. Additionally, the N-NH_4 removal efficiency of *A. augustus* was higher than TN removal efficiency, indicating *A. augustus* consumed N-NH_4 before using other nitrogen sources.

H. pluvialis, *Scenedesmus* sp., *C. aquaticum*, and *A. augustus* reduce TP in 100% wastewater samples with 68.02%, 99.50%, 97.66%, and 97.97% removal efficiency, respectively. Phosphorus is crucial for nuclei acid synthesis and energy storage in microalgae, therefore, microalgae can remove phosphorus effectively from wastewater [64]. Previous studies found that although microalgae species need small quantities of phosphorus at early growth, they absorb a large amount of phosphorus at the earlier stages of cultivation and accumulate phosphorus in the form of intracellular polyphosphate [65,66]. Among all the species, *H. pluvialis* achieved the lowest TP removal efficiency, only at 68.02%. The low removal efficiency of *H. pluvialis* may be due to the lower N:P ratio of 2.3 in the wastewater. Some phosphorus biochemicals, such as ribosomal RNA, require nitrogen for synthesis; hence, limited nitrogen in the medium may reduce the utilization of phosphorus [67].

In summary, *C. aquaticum* and *Scenedesmus* sp. attained better removal performance of TN, N-NH_4, and TP among the four tested microalgae species. From the literature reviews, it has been shown that few research studies have been published referring to the utilization of microalgae for the removal of phosphate and nitrogen from non-sterilised wastewater. The results varied between the studies. In Walls et al. study [35], *Scenedesmus* sp., cultivated in non-sterilised undiluted domestic wastewater, achieved a removal rate of only 60% for nitrate, 53% for N-NH_4, and 46% for orthophosphate. In another study conducted by Agustin et al. [68], the microalgae consortium removed 86.2% N-NH_4 and 4.4% orthophosphate from non-sterilised domestic wastewater. The nutrient removal efficiency of *C. aquaticum* and *Scenedesmus* sp. in this study was higher. The different results between these studies may be attributed to several reasons such as different microalgae strains, light intensity, initial inoculum, and the carbon: nitrogen: phosphate ratios in the wastewater [58,69,70].

Table 3 summarizes the comparison between characteristics of the treated wastewater using microalgae and the limit concentrations of N-NH_4, TN, and TP set by Malaysia and other developed countries for wastewater discharge into the aquatic environment. Interestingly, *H. pluvialis* and *A. augustus* removed TP and TN with less than 70% removal but the obtained results satisfy the requirements set by Malaysia, the European Union (EU), and Japan. The TN, TP, and N-NH_4 concentrations of all the treated wastewater in this study were lower than the limit concentrations set by the Malaysian Department of the Environment [71], the EU [72], and Japan [73] for wastewater discharge into the aquatic environment. This result indicates that these four microalgae species have the potential for treating domestic wastewater. Besides N-NH_4, TN, and TP, these regulations also set the limit values for biochemical oxygen demand, chemical oxygen demand, metals, certain organic compounds, and pathogens, and another analysis should be carried out on the treated wastewater before discharge or reuse.

Table 3. Comparison of treated wastewater by microalgae and wastewater discharge standards in Malaysia and other developed countries.

Pollutant	Initial Residue (mg/L) [a]	After Treatment (mg/L)				Malaysia [c] (mg/L)		EU [f] (mg/L)	Japan [h] (mg/L)
		HP [b]	S [b]	CA [b]	AA [b]	Std A [d]	Std B [d]		
N-NH_4	29.28 ± 0.38	0.10 ± 0.17	0.37 ± 0.05	0.23 ± 0.03	0.33 ± 0.06	5	5	- [g]	-
TN	26.75 ± 1.09	1.53 ± 0.31	1.47 ± 0.06	2.90 ± 0.33	10.67 ± 1.35	20 (10) [e]	50 (10) [e]	10	60
TP	12.45 ± 0.45	3.62 ± 0.02	0.03 ± 0.06	0.26 ± 0.02	0.25 ± 0.12	5	10	1	8

[a] the concentration is calculated using the average of each nutrient concentration in 100% wastewater for each microalgae species. [b] HP: *H. pluvialis*; S: *Scenedesmus* sp; CA: *C. aquaticum*; AA: *A. augustus*. [c] Data from Industrial Effluent Regulation 2010 set by the Department of Environment, Ministry of Natural Resources and Environment, Malaysia. [d] Standard A (std A) is applicable for discharges into any inland waters within catchment areas listed by the Malaysian government, while Standard B (std B) is applicable to any other inland waters or Malaysian waters. [e] the concentration not in the brackets is the specific limits on concentration that can be discharged into rivers while the concentration in the brackets is the specific limits on concentration that can be discharged into an enclosed water body. [f] Data from the European Environment Agency, 1991. [g] no specific limits on concentrations in effluent discharge. [h] Data from the Uniform National Effluent Standards, 2015, published by the Ministry of the Environment, Japan.

4. Conclusions

The present study demonstrated that non-sterilised wastewater can serve as a nutrient medium for the cultivation of *H. pluvialis*, *Scenedesmus* sp., *C. aquaticum*, and *A. augustus*. *H. pluvialis*, *Scenedesmus* sp., and *C. aquaticum* cultivated in 100% wastewater yielded higher cell density, higher biomass concentrations, higher biomass productivity, and a higher specific growth rate compared to the microalgae cultivated in standard medium. For *A. augustus*, its cell density, biomass concentration, biomass productivity, and specific growth rate in 100% wastewater were close to microalgae that were cultivated in a standard medium. The results also confirmed these microalgae can remove nitrogen and phosphorus effectively and meet the limit concentration set by the Department of the Environment, Malaysia, and other developed countries; therefore, these microalgae have the potential to be used in wastewater treatment. Among the microalgae species tested in the wastewater, *Scenedesmus* sp. had the highest capabilities of utilizing non-sterilised 100% wastewater as a growth medium and removing pollutants from wastewater because of its high growth and nutrient removal efficiency. Although the TN, TP, and N-NH_4 removal efficiency of microalgae is confirmed, other concentrations of pollutants in wastewater such as metals and organic compounds must be analysed to ensure they are safe for reuse or discharge.

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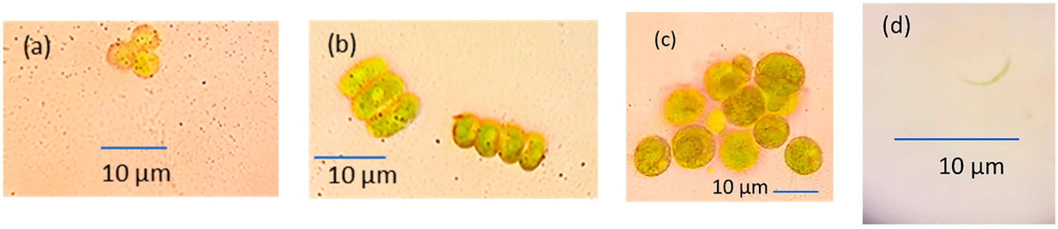
Appendix A



(**a**) (**b**) (**c**) (**d**)

Figure A1. Cell size of (**a**) *H. pluvialis*, (**b**) *Scenedesmus* sp., (**c**) *C. aquaticum* and (**d**) *A. augustus*.

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