Pertanika J. Sci. & Technol. 30 (1): 565 - 580 (2022)

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Assessment of Domestic Wastewaters as Potential Growth Media for *Chlorella vulgaris* and *Haematococcus pluvialis*

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ABSTRACT

Domestic wastewater contains chemical compounds that can be used as nutrients for microalgae. Removing these chemical compounds from wastewater by microalgae might help in reducing the operation cost of wastewater management while minimizing the cultivation cost for large-scale microalgae metabolite production. In this study, domestic wastewater collected from Indah Water Konsortium (IWK), Kuala Lumpur, Malaysia, was assessed as growth media for two types of microalgae, namely *Chlorella vulgaris* and *Haematococcus pluvialis*. The biomass growth and nutrient removal efficiency of total nitrogen (TN), total phosphorus (TP), and total ammonia (TAN) in different concentrations of diluted wastewater were measured. The results showed that biomass concentration (0.227 g/L), biomass productivity (0.029 g/L/day), and specific growth rate (0,284 d^{-1}) yielded by *C. vulgaris* in 14 days of 80% wastewater were comparable to those microalgae grew in standard Bold's Basal medium (BBM). Besides, *C. vulgaris* grew in 50% wastewater to remove TN, TP, and TAN with the highest removal efficiency (>88%). For *H. pluvialis*, the biomass concentration in all wastewater concentrations was lower than BBM. The removal efficiencies of TN and TP were lower than 55%, but more than 80% for removal efficiency of TAN in 50% and 80% wastewater. Hence, *C. vulgaris* has better growth performance and nutrient removal efficiency than *H. pluvialis*. These findings indicated that IWK domestic wastewater could be used as growth media for microalgae, especially *C. vulgaris*.

Keywords: Chlorella vulgaris, *Haematococcus pluvialis,* microalgae, nutrient removal, wastewater treatment

ARTICLE INFO

Article history:
Received: 1 July 2021
Accepted: 13 October 2021
Published: 10 January 2022

DOI: https://doi.org/10.47836/pjst.30.1.31

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ISSN: 0128-7680
e-ISSN: 2231-8526

INTRODUCTION

Wastewater generated from industrial, agricultural, and domestic activities often contains organic matters, metals, and toxic chemicals (Abdullah et al., 2017; Qi et al., 2020). Therefore, wastewater must be treated by sewage treatment plants prior to discharge into the environment. Sewage service needs huge costs on replacement, maintenance, and building of new sewage plant (Aliman, 2019). While the wastewater treatment companies charge expensive bills to the local authorities and clients, they can reduce the operation cost by introducing biological processes on the treatment plant.

Several studies have indicated that microalgae are potential candidates for wastewater treatment due to their ability to utilize the organic and inorganic matters in wastewater (Ramsundar et al., 2017; Kotoula et al., 2020; Umamaheswari et al., 2020). Moreover, the biomolecules produced by microalgae, such as lipid, can be transformed into biodiesel. However, microalgae-based biodiesel is not available in the market today due to its high cultivation cost using freshwater. Therefore, Lu et al. (2015) suggested that harnessing wastewater as a microalgae cultivation medium can minimize the cost of producing the desired products.

Different wastewater has distinct compositions based on their sources. Therefore, the nutrient removal efficiencies vary among different microalgae in the same type of wastewater (Bhatnagar et al., 2011; Ling et al., 2019). Furthermore, even the microalgae strain that showed high nutrient removal efficiency in one wastewater might show different characteristics in another wastewater. For instance, Odjadjare et al. (2018) cultivated *Neochloris aquatica* in two domestic wastewaters collected from different treatment plants. The results showed that the dry biomass weight and metabolite amount were significantly different due to the different physicochemical profiles of wastewater. Therefore, before developing microalgae-based wastewater treatment for a certain area, it is important to study and select the correct type of microalgae for the local wastewater to maximize nutrient removal efficiency and biomass production.

Chlorella vulgaris was commonly used in the study of wastewater treatment due to its high growth rate and high nutrient removal efficiency (Cheah et al., 2018; Wang et al., 2015). The harvested biomass could be further used as feedstock for biodiesel, pharmaceutical medicines, and biofertilizers (Ru et al., 2020). *Haematococcus pluvialis* is a high-valued microalga due to its bestowed ability to produce precious astaxanthin, which has a strong antioxidant capacity (Shah et al., 2016). It was reported that astaxanthin has a high market value (USD 2000/kg), and the demand is expected to increase in the future (Ren et al., 2021). However, large-scale *H. pluvialis* cultivation is limited due to the high cost. Shah (2019) suggested that wastewater treatment integration with *H. pluvialis* is a great option in reducing cultivation costs. However, the biomass production by *H. pluvialis* using wastewater was different (Shah, 2019). Hence, the study should be conducted to assess the wastewater from the certain treatment plant is suitable for *H. pluvialis* to grow to serve its purpose to produce high biomass and remove nutrients from wastewater simultaneously.

The study on the assessment of domestic wastewater collected from Indah Water Konsortium (IWK) as potential growth media for *C. vulgaris* and *H. pluvialis* had not been reported in Malaysia. In this study, *C. vulgaris* and *H. pluvialis* were cultivated in different concentrations of diluted IWK wastewater (10%, 20%, 50%, and 80% of wastewater). The biomass concentration, biomass productivity, and specific growth rate of both microalgae species in each concentration of diluted wastewater were compared to those microalgae grew in standard Bold's Basal medium (BBM). The diluted wastewater samples that stimulated the high biomass concentration and cell density were collected for nutrient removal analysis. The nutrient removal efficiencies of total nitrogen (TN), total phosphorus (TP), and total ammonia (TAN) of both microalgae species were compared to determine the suitable species for IWK water treatment and to access the suitability of IWK domestic wastewater as growth media for *C. vulgaris* and *H. pluvialis*.

MATERIALS AND METHODS

Microalgae Strain Cultivation

Microalgae strain *C. vulgaris* was purchased from Culture Collection of Algae and Protozoa (CCAP), United Kingdom, while *H. pluvialis* was attained from the University of Texas (UTEX), United States of America. Both microalgae were initially maintained in Bold's Basal medium (BBM). Then, microalgae cultures were cultivated at room temperature under illumination from cool-white fluorescent tubes with 16 hours of light and 8 hours of the dark cycle. During the cultivation, manual aeration was done twice a day. Hemocytometer (Marienfeld-Superior, Neubauer) with a light microscope (Eclipse E-100 LED, Nikon) was used to investigate the growth phases of the cells by determining the cell density.

Microalgae Cultivation with Wastewater

Wastewater was collected from Indah Water Konsortium (IWK), Kuala Lumpur, Malaysia. Indah Water Konsortium is a national sewerage and sanitation company in Malaysia. The wastewater sample was filtered to remove solid particles prior to use. The initial pH, total nitrogen (TN), total phosphorus (TP), and total ammonia (TAN) of wastewater was measured according to the method described in the "nutrient removal efficiency" section, and they were approximately 7.33, 33.2 mg/L, 43.8 mg/L, and 10.08 mg/L, respectively. A simple preliminary study was conducted, and the result showed that the original wastewater was not suitable for the direct cultivation of both microalgae due to the high amount of various nutrients. Then, the wastewater was diluted with distilled water to 10%, 20%, 50%, and 80% of wastewater percentages without further process. When *C. vulgaris* and

H. pluvialis reached the log phase, 4 mL of the culture (cell density 6.21×10^6 cells/mL and 2.73×10^5 cells/mL) were transferred into 250 mL of diluted wastewater. Microalgae cultivation with BBM and deionized water were positive and negative control, respectively. The microalgae were cultivated in the 500-mL conical flask at the conditions as previously described in the "Microalgae Strain Cultivation" section.

The cell density and biomass concentration of each sample were measured on the initial day of cultivation and every two days using the method as described in the sections of "Microalgae Strain Cultivation" and "Determination of Microalgae Biomass" until day 14. The microalgae in wastewater during day 0 and day 14 were filtered through a vacuum filter, and the filtrates were collected. The wastewater that had high biomass concentration and cell density was further analyzed to determine the nutrient removal efficiency of TN, TP, and TAN as described in the section of "Nutrient Removal Efficiency."

Determination of Microalgae Biomass

The volume of 10 mL aliquots of culture was filtered using mixed cellulose ester membrane filters with absorbent pads. The loaded filter was dried in an oven at 70°C until constant weight. The dry cell weight (DCW) of the microalgae biomass was obtained by subtracting the dry weight of the blank membrane filter with the dry-loaded membrane filter.

The DCW was used to calculate the microalgae growth by biomass concentration, biomass productivity, and specific growth rate using Equations 1, 2, and 3 below:

$$\text{Biomass concentration} = DCW_t/\text{volume of aliquots} - DCW_0/\text{volume of aliquots} \quad [1]$$

$$\text{Biomass productivity, } Pb = (X_f - X_0)/(t_f - t_0) \quad [2]$$

$$\text{Specific growth rate, } \mu = (\ln X_f - \ln X_0)/(t_f - t_0) \quad [3]$$

Where

DCW_t and DCW_0 are dry cell weight (g) on the final day of cultivation (t_f) and initial day of cultivation (t_0)

X_f and X_0 are the biomass concentration (g/L) on day t_f and day t_0.

Nutrient Removal Efficiency

The samples for the determination of TN, TP, and TAN were digested and treated according to methods 10071, 8190, and 8038, respectively, described by Hach (Hach, 2021). TN was measured based on the persulfate digestion method using Nitrogen, Total, LR, Test 'N Tube™ reagent set while TP was measured based on PhosVer ® 3 with acid persulfate digestion method using Phosphorus (Total) TNT Reagent Set. TAN was measured based on the Nessler method using the Hach type Nessler nitrogen-ammonia reagent set. The digested samples were put into Hach spectrophotometer DR5000, and the concentration of each sample was measured using a program set in DR5000. The sample was diluted with deionized water if the concentration was too high to be measured (Hach, 2021).

The nutrient removal efficiency (%) was calculated using Equation 4 below:

$$\text{Nutrient Removal Efficiency (\%)} = (C_0 - C_t) / C_0 \times 100\% \quad [4]$$

C_0 and C_f are the nutrient concentration (mg/L) on day t_0 and day t_f.

All experiments were repeated in triplicates, and data were presented as means ± standard error of the mean. Significance of results, differences between the strains of the microalgae and the wastewater concentrations were evaluated for a duplicate set of data by using one-way analysis of variance (ANOVA) with Post-hoc Turkey's test.

RESULTS AND DISCUSSIONS

Effect of Wastewater Concentration on Microalgae Growth

The cell density of *C. vulgaris* and *H. pluvialis* in different wastewater concentrations is shown in Figure 1. The cell density of *C. vulgaris* in 50% and 80% wastewater was notable compared to other wastewater concentrations. The maximum cell density obtained in 50% wastewater was 1.014×10^7 cells/mL, and 80% wastewater was 1.102×10^7 cells/mL, respectively. These results were comparable to those microalgae that grew in BBM (1.132×10^7 cells/mL). Whereas *C. vulgaris* grew in deionized water, 10% and 20% wastewater reached 1.28×10^6, 2.38×10^6, and 4.31×10^6 cells/mL, respectively. From Figure 1, the lag phase of *C. vulgaris* in all wastewater concentrations was very short, and after that, the microalgae cells initiated the log phase at day 3 with a high specific growth rate. The growth of *C. vulgaris* reached the stationary phase after 7 and 10 days of cultivation in 10%, 20%, 50%, and 80% wastewater, respectively, which were similar to Ryu et al. (2014).

For *H. pluvialis*, the maximum cell density obtained from the culture in BBM was recorded as the highest among all wastewater concentrations on day 14, which is 2.503×10^7 cells/mL. The maximum cell density in all wastewater concentrations on day 14 was lower than BBM. On day 3, red nonmotile aplanospore were microscopically observed in 10% and 20% wastewater. The cell number of aplanospore has surged, whereas green vegetative motile cells and palmella cells were diminished along with the increasing cultivation period. The stressed environment, such as nutrient deprivation, prompted the transformation of green vegetative motile cells and palmella cells into red nonmotile aplanospore (Shah et al., 2016). Aplanospore is resting vegetative cells, which means their metabolism rate slows down. The nutrients in 10% and 20% wastewater were lower and probably deprived during the initial cultivation period. As a result, some green vegetative cells were transformed into red nonmotile aplanospore; therefore, cell density increased slowly.

The biomass concentration of *C. vulgaris* and *H. pluvialis* in different wastewater concentrations during 14 cultivation days is illustrated in Figure 2. Although the biomass concentration of *C. vulgaris* in 80% wastewater showed a similar biomass concentration produced in BBM, there is no significant difference (P > 0.05) between these two culture mediums after 14 days of cultivation. It is probably due to rich nutrients in 80% wastewater (Figure 3). Previous studies have reported that *C. vulgaris* survived in the presence of an inflated concentration of nitrogen and reached high biomass concentration (Zhang et al., 2016; Li et al., 2019; Trivedi et al., 2019). However, a high concentration of phosphorus is not favorable for *C. vulgaris* growth through exorbitant nitrogen was available (Zhang et al., 2016).

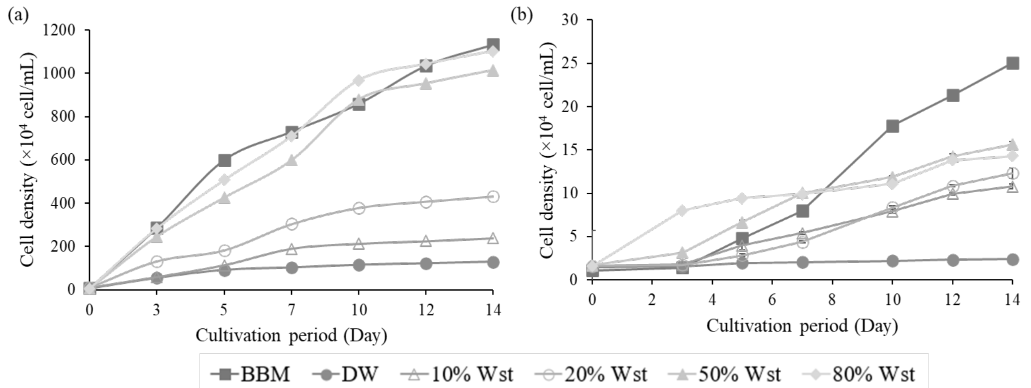

Figure 1. The cell density of (a) *C. vulgaris* and (b) *H. pluvialis* in BBM, deionized water (DW), and different wastewater concentrations (n = 3). 10% Wst: 10: 90 (v/v) of wastewater and distilled water; 20% Wst: 20: 80 (v/v) of wastewater and distilled water; 50% Wst: 50: 50 (v/v) of wastewater and distilled water; 80% Wst: 80: 20 (v/v) of wastewater and distilled water.

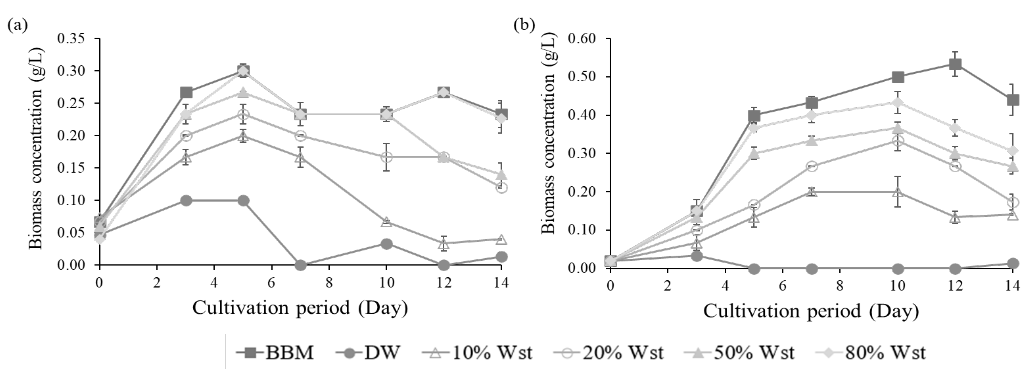

Figure 2. Biomass concentration of (a) *C. vulgaris* and (b) *H. pluvialis* in BBM, deionized water (DW), and different wastewater concentrations (n = 3).

The biomass concentration of *C. vulgaris* in 10% and 20% wastewater was low during the cultivation period. This result was consistent with another study, which too diluted wastewater was not favorable for *C. vulgaris* growth (Wang et al., 2015; Nam et al., 2017). These results indicated that the nutrients in the wastewater must be monitored to obtain a high biomass concentration of *C. vulgaris*.

Figure 2(b) shows that the highest biomass concentration of *H. pluvialis* was observed in BBM standard control media. Although the biomass concentration and cell density increased rapidly from day 2 to day 5, biomass concentration in all wastewater concentrations was significantly lower than in BBM. Moreover, the presence of aplanospore, as described earlier, indicated that *H. pluvialis* was in a stressful environment. However, other studies reported that *H. pluvialis* was able to produce a high amount of biomass concentration (> 600 mg/L) in wastewater containing more than 40 mg/L of total nitrogen and 4.4 mg/L of total phosphorus (Kang et al., 2006; Liu & Yildiz, 2019). Therefore, the low performance of *H. pluvialis* in this study is probably due to inhibitors, such as metals and growth inhibitors in wastewater samples.

Table 1 indicates the biomass concentration, average biomass productivity, and specific growth rate of *C. vulgaris* and *H. pluvialis* in BBM, deionized water, and different concentrations (10%, 20%, 50%, 80%) of wastewater after 14 days of cultivation. The results showed that the biomass concentration, average biomass productivity, and specific growth rate of *C. vulgaris* and *H. pluvialis* in 80% wastewater are comparable to the production in BBM standard culture medium (Table 1). However, compared to *C. vulgaris*, *H. pluvialis* showed higher biomass concentration and productivity. It is probably because of the different cell sizes of microalgae. The cell size of *C. vulgaris* was 2-10 µm (Weil et al., 2017), whereas *H. pluvialis* was 8-50 µm (Li et al., 2019), respectively. Therefore, the larger cell size of *H. pluvialis* probably has a higher weight than *C. vulgaris,* resulting in higher biomass concentration and productivity.

Table 1

Biomass concentration (BC), average biomass productivity (P_b), and specific growth rates (µ) of C. vulgaris *and* H. pluvialis *in BBM, deionized water (DW), and different wastewater concentrations (n = 3) after 14 days of cultivation.*

C. vulgaris				
Medium		BC (g/L)	P_b (g/L/day)	$\mu(d^{-1})$
BBM		**0.233 ± 0.024[a]**	**0.030 ± 0.011**	**0.212 ± 0.028**
DW		0.013 ± 0.033[b]	0.002 ± 0.001	0.062 ± 0.005
Wastewater	10%	0.040 ± 0.058[b]	0.011 ± 0.004	0.079 ± 0.005
	20%	0.120 ± 0.057[c]	0.020 ± 0.001	0.164 ± 0.015

Table 1 *(Continue)*

C. vulgaris				
Medium		BC (g/L)	P_b (g/L/day)	μ(d^{-1})
Wastewater	50%	0.140 ± 0.010[c]	0.025 ± 0.009	0.204 ± 0.038
	80%	**0.227± 0.010[a]**	**0.029 ± 0.003**	**0.284 ± 0.043**
H. pluvialis				
Medium		BC (g/L)	P_b (g/L/day)	μ(d^{-1})
BBM		**0.440 ± 0.085[d]**	**0.050 ± 0.024**	**0.421± 0.060**
DW		0.013 ± 0.015[e]	0.001 ± 0.002	0.028 ± 0.002
Wastewater	10%	0.140 ± 0.071[f]	0.016 ± 0.008	0.272 ± 0.040
	20%	0.173 ± 0.048[f]	0.026 ± 0.005	0.330 ± 0.020
	50%	0.267 ± 0.059[g]	0.036 ± 0.008	0.380 ± 0.025
	80%	**0.307± 0.012[g]**	**0.043 ± 0.014**	**0.404 ± 0.057**

Values within the same row having different letters are significantly different ($P < 0.05$).

Total Nitrogen, Total Phosphorus, and Total Ammonia Removal Efficiency

The nutrient removal efficiency of C. vulgaris and H. pluvialis in wastewater was assessed to evaluate their potential in wastewater treatment. Samples were taken at the time of inoculation and the end of the cultivation period and analyzed for total residual nitrogen, phosphate, and ammonia. As shown in Figure 3, both microalgae were able to remove TN, TP, and TAN efficiently from 10% wastewater, in which TN, TP, and TAN were reduced more than 85% during the 14^{th}-day cultivation of both microalgal species. Compared to H. pluvialis, C. vulgaris can remove more than 88% of TN, TP, and TAN in 50% wastewater. Interestingly, the TN and TP removal efficiency of both microalgal species in 80% wastewater was the lowest even though their biomass concentrations are the highest compared to other wastewater concentrations. The amount of phosphorus uptake by C. vulgaris in 50% and 80% wastewater were 21.9 mg/L and 25.5 mg/L, respectively, resulting in removal efficiency of 100% and 72.89%. These results correspond with the findings from the other studies wherein mediocre removal efficiency of TN (49–60%) and TP in minimally diluted wastewater (Deng et al., 2017; Wen et al., 2017). The low removal efficiency is probably because the phosphorus requirement for C. vulgaris has reached a saturated point. Besides, the low nitrogen concentration in the wastewater medium is probably attributed to this result. Phosphorus uptake was mitigated in a low nitrogen environment as protein and ribosome synthesis was reduced (Loladze & Elser, 2011).

Previous work has shown that the unbalance N/P ratio, especially low nitrogen has critical effects on removal efficiency and cell growth (Lee et al., 2013; Whitton et al., 2016; Huang et al., 2021). Beuckels et al. (2015) reported that under a low nitrogen environment, the uptake of TP by microalgae into the biomass remains low regardless of the TP concentration in the medium. Sufficient nitrogen is essential to ensure no restriction on protein and ribosome synthesis. Alketife et al. (2017) also indicated that TP removal efficiency by *C. vulgaris* was reduced in the medium containing the initial TP concentration of more than 19 mg/L. A longer cultivation period enhanced TN concentration, or higher initial inoculum density probably could enhance the removal efficiency of TP.

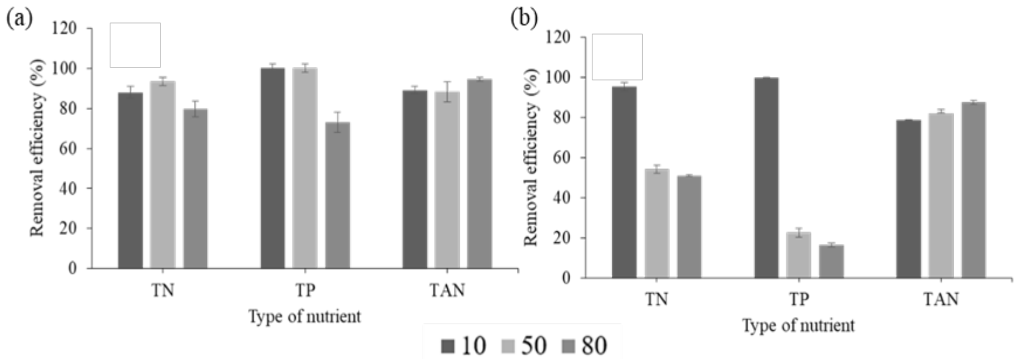

Figure 3. Removal efficiency of (a) *C. vulgaris* and (b) *H. pluvialis* in 10%, 50% and 80% wastewater after 14-day cultivation (n = 3). TN: total nitrogen; TP: total phosphorus; TAN: total ammonia.

For *H. pluvialis*, the results showed that only 5 mg/L and 5.77 mg/L of phosphorus were taken in 50% and 80% wastewater, resulting in removal efficiency of 22.83% and 16.48%, respectively, which much lower than *C. vulgaris*. The reason is underlying these results perhaps due to the slow metabolism of *H. pluvialis*. Pan et al. (2021) revealed that *H. pluvialis* required eight days to reduce 16 mg/L of initial nitrate and 3 mg/L of initial TP to below one mg/L in potato juice wastewater. In another study, 30 mg/L of TN and 4.7 mg/L of TP to 0 mg/L and 0.49 mg/L, respectively, in primary effluent by *H. pluvialis* after 35 days of cultivation (Sato et al., 2015). A longer time is needed to achieve high removal efficiency in low diluted wastewater.

TN removal efficiency of *H. pluvialis* in 50% and 80% wastewater is also lower *than C. vulgaris*. *H. pluvialis* took up 9 mg/L and 13.56 mg/L TN in 50% and 80% wastewater during 14^{th} day cultivation, resulting in removal efficiency of 54.52% and 51.05%, respectively. On the one hand, the TAN removal efficiency of *H. pluvialis* in 50% and 80% wastewater was 82.14% and 87.72%, respectively, comparable to the *C. vulgaris*. The preference of nitrogen sources of *H. pluvialis* is varied among the strains. Sipaúba-Tavares et al. (2015) showed that *H. pluvialis* prefers to utilize nitrate, whereas Cifuentes et al. (2003) and Ledda et al. (2016) revealed *H. pluvialis* prefers to consume ammonium. In another study, Wang et al. (2019) pointed out that *H. pluvialis* JNU35 prefers to use urea instead of nitrate and ammonium. In this study, the removal efficiency of TAN is higher than the removal efficiency of TN in both 50% and 80% wastewater for *H. pluvialis*. Therefore *H. pluvialis* prefers to utilize ammonium in this study. Besides strain, light intensity, turbidity, and medium pH might influence the uptake preference of nitrogen sources (Cifuentes et al., 2003; Wang et al., 2019). More research is required to clarify the relationship and mechanisms of these factors on the nitrogen sources uptake.

The amount of TN, TP, and TAN taken by *H. pluvialis* in 50% wastewater was almost closed to 80% wastewater. It is probably because *H. pluvialis* has reached the maximum uptake amount in 50% wastewater. Therefore, the cell density and biomass concentration in 50% and 80% wastewater are also similar. Kang et al. (2006) also observed that the cell density of *H. pluvialis* in two- and four-fold diluted wastewater was higher compared to eight-fold diluted wastewater, but their removal efficiency was much lower than eight-fold diluted wastewater.

However, the removal efficiency of TAN was from 78.67% to 94.54% in all diluted wastewater for microalgal species. It is important to know that the removal of ammonium ions is influenced by confounding factors, including microalgae, volatilization, air-stripping, and the presence of nitrifying and denitrifying bacteria (Podevin et al., 2015; Tao et al., 2017; Tan et al., 2020). Wastewater without sterilization may consist of nitrifying and denitrifying bacteria that convert ammonium ions into nitrogen gas. Previous research also reported that in the presence of various forms of inorganic nitrogen, ammonium is generally favored by many microalgae (Wu et al., 2013; We et al., 2013) because nitrite or nitrate must be converted into ammonium prior to its utilization while no requirement of redox reaction during ammonium assimilation as it can be directly absorbed into amino acids inside the cells (Kim et al., 2016).

Nutrient removal efficiency and biomass productivity by different microalgae grown in domestic wastewater from previous studies were compared in Table 2. Although the biomass productivity of *C. vulgaris* in this study was low, the removal efficiency of TN, TP, and TAN was higher than in other studies. For *H. pluvialis,* the nutrient removal efficiency of TN, TP, and TAN in 10% wastewater were comparable to other studies.

Overall, *C. vulgaris* and *H. pluvialis* achieved high biomass concentration and nutrient removal efficiency in proper dilution of wastewater compared to other microalgae species. Based on the results, *C. vulgaris* shows the better potential for IWK domestic wastewater treatment and simultaneously produces high biomass concentration in 14 days cultivation period.

Table 2

Comparison of nutrient removal efficiency and biomass productivity (mg/L/day) by different microalgae grown in domestic wastewater from previous studies.

Type of system	Treatment	Removal efficiency (%)			P_b	Day of cultivation	Reference
		TN	TP	TAN			
C. vulgaris	50% diluted	93.37	100	88.29	0.029	14	This study
H. pluvalis	10% diluted	95.48	100	78.67	0.040	14	This study
C. vulgaris	0.02 v/v diluted	85	35	-	0.040	12	Lam et al. (2017)
C. vulgaris	50% diluted	83	100	-	-	18	Thomas et al. (2016)
Chlorella sp.	Autoclaved	-	53.77	95.90	-	10	Kiran et al. (2014)
C. vulgaris	50% autoclaved diluted	73.9	45.4	-	-	12	Pacheco et al. (2021)
H. pluvalis	25% diluted	100	100	-	-	8	Kang et al. (2006)
H. pluvalis	-	93.8	97.3	-	0.028	15	Wu et al. (2013)
Chlorella sorokiniana	municipal wastewaters	28.67	83.3	94.29	0.077	14	Ramsundar et al. (2017)
Scenedesmus acutus	Autoclaved	42.9	92.4	64.3	0.048	16	Alva et al. (2013)

CONCLUSION

The present study demonstrated that unsterilized IWK domestic wastewater could be used as growth media to cultivate *C. vulgaris* and *H. pluvialis*. The cell density, biomass concentration, average biomass productivity, and specific growth rate of *C. vulgaris* in 80% wastewater were comparable to those grown in BBM but not for *H. pluvialis*. However, the removal efficiencies of TN, TP, and TAN of *C. vulgaris* in 80% wastewater were lower than in 50% wastewater. Nevertheless, the removal efficiency of TN, TP, and TAN of *C. vulgaris* was higher than *H. pluvialis* in 50% and 80% wastewater. Hence, *C. vulgaris* has better growth performance and nutrient removal efficiency than *H. pluvialis*. These findings suggested that *C. vulgaris* is a more suitable candidate for IWK domestic wastewater treatment compared to *H. pluvialis*.

ACKNOWLEDGEMENTS

The authors want to express their appreciation to the Ministry of Higher Education Malaysia, MOHE: FRGS/1/2020/STG02/UNITEN/02/1 (60%) and UNITEN BOLD Research Grant 2020: RJO10517844/044 (40%) for the financial support and thank Indah Water Konsortium for wastewater samples.

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