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Integrated Growth Potential of *Scenedesmus sp.* using Public Market Wastewater via Phycoremediation Process

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Abstract: Malaysia has been experiencing water pollution crisis over a decade and one of the causes is the direct discharge of public market wastewater (PMWW) into the drains. Therefore, phycoremediation of wastewater especially from public market sources with microalgae is proposed. The current work aimed to investigate the potentially of Scenedesmus sp. to remove nutrient from PMWW. Nutrient profile and the ability of Scenedesmus sp. to phycoremediate was tested for five (5) different concentrations of PMWW (10, 25, 50, 75, and 100%) during 19 days of treatment process. The process involves growing the Scenedesmus sp. in the PMWW which is contributing to utilize the nutrients to grow. The results indicated that Scenedesmus sp. is effective to reduce total nitrogen (TN) (91.51%), total phosphorus (TP) (92.67%), and total organic carbon (TOC) (90.20%) for 50% concentration of PMWW. Meanwhile, for the 100% of PMWW concentration Scenedesmus sp. capable to remove TN (57.49%), TP (62.92%) and TOC (50.10%). At 50% PMWW, the maximum removal of heavy metals was also recorded which were 87.21 for Fe, 86.07% for Zn, 91.47 for Cr, and 88.97% for Cd. It concluded that Scenedesmus sp. can grow effectively in the 50% PMWW than in the 100%PMWW but still be able to reduce the rate of nutrient for all samples. These findings revealed the applicability of PMWW as a medium and economical way for microalgae biomass production.

Keywords: Public market wastewater, *Scenedesmus* sp., phycoremediation

1. Introduction

In the past two decades, bioremediation of wastewater to remove unwanted nutrients using microorganisms has been widely applied throughout the world [1]. Some of the important advantages of using microalgae include the relative safety of the microorganism, the efficient removal of the primarily nitrogen and phosphorous due to its rapid growth rate, and the ability to utilize whereby the resulting algae has the potential to become as a food source for fish or farm animals [2], [3]. Public market wastewater (PMWW) can make a significant contribution to the biotreatment, because it is rich in nutrients such as nitrogen, phosphorus, and organic matters [4]. Nevertheless, it has received relatively little attention. Besides that, it has low toxic components that needs to be removed owing due to its organic sources [5].

Besides, wastewater from public markets generate a bad smell and the effluent is usually discharged directly into the river body without any proper treatment [6]. PMWW is well known to have pollutants that can negatively impact if not controlled and stabilized the pollutant load removal. PMWW contained pollutants, nutrients and microbes in the form of dissolved or suspended solids and causes to various health problems for humans which also hazardous to our

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environment condition [5], [7]. PMWW also contained high nitrogen, phosphorus, and organic matter in both soluble and solid that is often derived from manure, seafood or animal waste [8]. In addition, PMWW may also contain heavy metals that occur poisoning in the food chain of aquatic and terrestrial ecosystem and posing health hazard. Thus, discharging PMWW into the water body without a proper treatment may lead to pollutants load and environment problems which can cause long-term problems such as eutrophication that stimulates algae bloom in the water.

In spite of the high nutrient concentration, microalgae would become potential candidate as bioremediation agent with efficient growth on agricultural waste. Microalgae have the ability to assimilate pollutant in different environmental conditions and have the capabilities in removing nutrients as well as heavy metals [9 -10]. The use of microalgae for nutrient and pollutants removal is known as phycoremediation. Phycoremediation is an effective technique that treat wastewater using algae with an offer of a very simple method comparing to the other conventional treatments [11]-[13].

Phycoremediation can be classified as sustainable method and cost saving improves the economic perspective and environmental outline of the whole process [12], [13]. Thus, coupling algae with wastewater can give significant benefits on wastewater bioremediation, nutrient reuse and carbon dioxide fixation [11]. Hence, the conventional wastewater treatment has extensive of chemical usage result in huge amount of sludge which forms the hazardous solid waste [12]. Therefore, the phycoremediation process is one of the most appropriate methods for removing of nutrients. The advantages of microalgae technology include the low cost due to utilization of natural sunlight, fixation of CO2, and economically profitable application of harvested algal biomass in several industries [14], [15].

Scenedesmus sp. has good adaptation in removing nutrients from wastewater such as nitrogen and phosphorus. Zhang et al. [16] reported that *Scenedesmus* sp. cultivated in wastewater that was discharged from domestic area in Beijing, China achieved the highest removal of nutrients, total nitrogen (TN), total phosphorus (TP) and dissolved organic carbon (DOC) were 92.9%, 99.2% and 72.1% respectively. Mohamed et al. [17] investigated that microalgae *Scenedesmus* sp. utilized and removed TN, phosphate (PO4-3) total organic carbon (TOC) by 100%, 95.4%, and 85% respectively from cafeteria wastewater which suggested that *Scenedesmus* sp. has the capability in degrading nutrients [16], [17].

Remarkable efforts have been conducted into research on microalgae cultivation using wastewater. However, the optimization of phycoremediation process with different concentrations of PMWW is still lacking. The major goal of this work effort was to determine the ability of the microalgae *Scenedesmus* sp. to remove nutrient, heavy metals from PMWW and the effectiveness on the growth rate as well as the potential of *Scenedesmus* sp. derived from PMWW.

2. Material and Method

2.1 Microalgae *Scenedesmus sp.* Culturing

Microalgae *Scenedesmus* sp. that has been used in this present laboratory was obtained from the tropical rainforest located in the southern region of Peninsular Malaysia and identified based on the morphological and molecular analysis using 16S rRNA sequencing. The microalgae inoculum was organized using sub-culturing of *Scenedesmus* sp. in Bold Basal Medium with the stock solution composition as described by Bishoff and Bold [18] of the following: 25.0 g/L NaNO3, 2.5 g/L, CaCl2.2H2O, 7.5 g/L, MgSO4.7H2O, 7.5 g/L, K2HPO4, 17.5 g/L, KH2PO4, 2.5 g/L, NaCl, 50.0 g/L, EDTA, 31.0 g/L, KOH, 4.98 g/L, FeSO4.7H2O, 1.0 ml/L, H2SO4, 11.42 g/L, H3BO3, 8.82 g/L, ZnSO4.7H2O, 1.44 g/L, MnCl2.4H2O, 0.71 g/L, MoO3, 1.57 g/L, CuSO4.5H2O, 0.49 g/L, Co(NO3)2.6H2O. Then, the medium was placed under the sunlight for 12 days to observe the cultivation of microalgae prior to experiment. The *Scenedesmus* sp. growth was harvested by centrifugation at 4000 rpm for 10 min and washed with 10 mL sterile physiological saline (PS; 0.85% NaCl solution, pH 7.0) and transferred with a sterilized pipette to sterilize bottles to suspend in sterilize bottles for 10mL suspension physiological saline. Then the cells of *Scenedesmus* sp. in the suspension was counted using Haemocytometer counter cover. In brief; Haemocytometer chamber and glass cover slip was cleaned with 70% ethanol. Next, the *Scenedesmus* sp. cells suspension was shaken for 1 min and 10 µL cell suspension was transferred using sterilized micropipette into each side of Haemocytometer counter cover. The Scenedesmus sp. cells count was enumerated under a light microscope (x40, Olympus, Model CX22LED) The cells located in the central on the right or top line and ranged from 50-100 cells of each five squares were counted on both sides of the Haemocytometer counter. The *Scenedesmus* sp. cells were calculated according to Eq. (1). The *Scenedesmus* sp. cells count was expressed as cells mL^{-1}.

$$\text{Number of cell/ml} = \text{Average number of cell per 1 mm}^2/10^4 \text{ x sample dilution} \quad (1)$$

The preparation of different *Scenedesmus* sp. inoculum was conducted according to Eq. (2).

$$C_1 \times V_1 = C_2 \times V_2 \quad (2)$$

2.2 Wastewater Sampling

The PMWW samples were collected at Pasar Borong Rengit, Batu Pahat, Johor (1.6776° N, 103.1455° E). All wastewater sampling was done between 7.00 am. to 1.00 pm. The collected samples were collected using acid washed bottles and immediately transported to the laboratory in ice box before being preserved in the laboratory chiller at a temperature of below 4°C. and then subjected for the chemical and physical analysis within 24 hrs. The characteristics of the waste samples were carried out according to APHA 2012 [19]. Details in laboratory analysis which is for the standard method and equipment used for each parameter are shown in Table 1.

Table 1 - Standard method of parameter

Parameter	Method APHA (2012)	Equipment
pH	Method 4500-H-B	pH meter
BOD	Method 5210-B	-
TSS	Method 2540-D	-
COD	Method 8000	
TP	Method 4110B	Ion Chromatography
TN	Method 5310B	TOC Analyzer
TOC		
Fe	Method 3111D	AAS
Zn, Cd and Cr	Method 3125B	ICP-MS

**AAS: Atomic Absorption Spectrometer, ICP-MS: Inductively Coupled Plasma-Mass Spectrometry.*

2.3 Experimental Set Up

Phycoremediation experiment was conducted in 500 mL Erlenmeyer flasks containing 400mL of wastewater. The wastewater media was cultured with five different concentrations. Subsequently, the volume of the culture broth was added with 10%, 25%, 50%, 75%, and 100% of PMWW and organic free water. The medium broth (BBM) was used as control group to compare the difference in nutrient removal between the microalgae grown culture and the medium itself. The experiment flasks were cultured with the initial concentration of 1×106 cells/mL. The flask was sealed with a good cloth and exposed to direct sunlight. The flasks were shaken 2 times daily for homogenized purposes. The growth of biomass was measured daily for 19 days at a wavelength of optical density (OD) 650nm using DR6000 spectrophotometer (Janway, USA).

Meanwhile, the TP, TN and TOC in removing nutrient a fixed volume of (30 mL) of the sample was taken from each sample at the beginning and at the end of experiment. The concentrations of TN, TP and TOC were carried out according to APHA [19]. Therefore, the percentage of the removal values was calculated as given in Eq. (3).

$$\text{Removal percentage} = \frac{\text{Initial value} - \text{Final value}}{\text{Initial value}} \times 100 \tag{3}$$

3. Results and Discussion

3.1 Characteristic of PMWW

A total of 6 parameters were tested to characterize the raw public market wastewater samples. The chosen parameters are important in order to compare the parameters with the wastewater effluents standard. These parameters values need to meet the Standard A and B that has been established by the Malaysia Wastewater Effluent Discharge (Sewage and Industrial Effluents Regulation, DOE, 2009) [20] guidelines prior being released into water bodies as shown in Table 2.

Table 2 - Characteristics of PMWW compared to effluent standard A and B

Parameter	PMWW	Effluent standard (Environmental Quality Act, 1974)	
		Standard A	Standard B
pH	8.1	6-9	5.5-9
BOD	1220.6	20	50
COD	2860.88	80	200
TN	968	-	-
TP	178	-	-
TOC	1798.22	-	-
Zn	2858	0.312	65
Fe	2628	1.071	55.4
Cr	194	-	-
Cd	14.2	-	77.0
Zn	2858	0.312	65

These results showed that the characteristic of PMWW was highly polluted compared to the regulation standard. It can be clearly seen that the raw PMWW has high concentration of BOD and COD with a value of 1220.6 mg/L and 2860.88 mg/L respectively. The concentration of BOD in this study were high compared with the study by Jais et al. [21], Zulkifli et al. [22] and Rawat et al. [11] with the comparison values of 500–540 mg/L, 71–122 mg/L and 89 mg/L, respectively. The concentration of BOD and COD were increasing based on the increment of wastewater medium percentage and it's probably occurred because the organic materials contain dirt and seafood entrails during activities at the market that make the water bodies to become contaminated [2].

This can be proven by looking at concentration of total organic carbon (TOC) in this study which is up to 1798.22 mg/L. The difference in value of TOC in each sample was due to the different amount of suspended solid in the wastewater sample. These phenomena may be due to the different concentration of public market wastewater used and also affected by the nutrients concentration in wastewater itself. As for other nutrients; total nitrogen (TN) and total phosphorus (TP) was also found to be highly content with value of 178 mg/L and 1798.22 mg/L/.

3.2 Microalgae *Scenedesmus* sp. Growth

Microalgae *Scenedesmus* sp. growth was tested for five concentrations of PMWW which were 10%, 25%, 50%, 75%, and 100% PMWW from 0 to 19th day during cultivation process by measuring optical density (OD) at a wavelength of 650nm. Fig. 1 shows that 50% PMW has the highest growth at day 14 with the OD of 650nm was increased from 0.112 to 0.2923 and the growth were decreased at day 19th with OD of 0.228. The present study agrees with Zhu et al. [23] whom reported the optimum microalgae growth using only 50% of piggery wastewater for *Chlorella zofingiensis*. These findings were also similar to *Scenedesmus* sp. that grew in 50% of tannery wastewater concentration done by Ajayan et al. [9]. Thus, high concentration of nutrient that exceeds the needs of microalgae required nutrient will give bad effect on the microalgae growth tolerance [24].

Meanwhile, for 25% and 75% PMWW also showed a good increment for microalgae growth at maximum value. of 0.266 and 0.271 at day 11th respectively and decreased after day 13th as shown in Fig. 1. On the other hand, the growth for 10% and 100%PMWW concentration decreased after day 11th and 6th, where the growth was 0.182 and 0.169 respectively. The reason for this situation might be due to the less (10% PMWW) and excessive (100% PMWW) nutrients concentration as shown previously in Table 2. Hence, this PMWW contained of an extremely high concentration, and thus had to be diluted before microalgae cultivation for biomass production. Therefore, microalgae growth in different concentrations of PMWW has been successful examined.

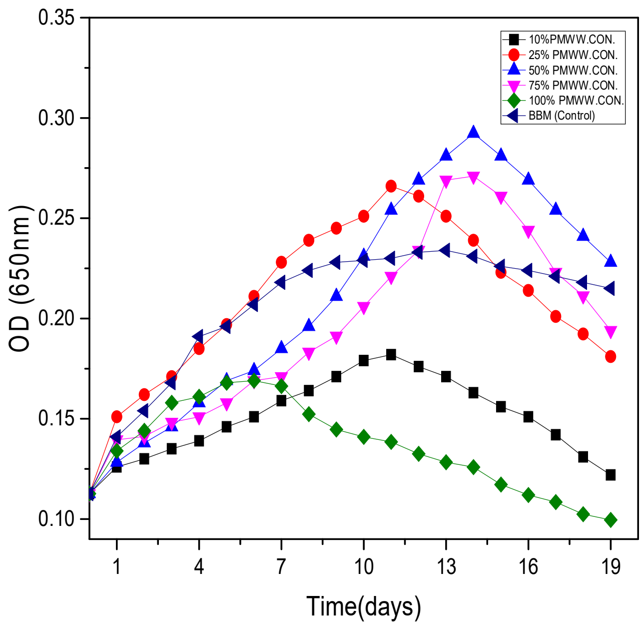

Fig. 1 - Microalgae *Scenedesmus* sp. Growth

Besides that, BBM (control) sample grown up to 0.228 at day 19th the maximum value was 0.234 at day 13th. It shows that this control group are lower than in PMWW concentration except 10% and 100%PMWW. Therefore, microalgae growth in different concentrations of PMWW in the current work has shown that 50%PMWW achieved high performance for *Scenedesmus* sp. growth.

3.3 Nutrient Removal Analysis

Three types of nutrient had been investigated in this study which is total nitrogen (TN), total phosphorus (TP), and total Organic Carbon (TOC). These three nutrients were measured based on five (5) different mediums of public market wastewater which were 10%PMWW, 25%PMWW, 50%PMWW, 75%PMWW, and 100%PMWW for 19 days as shown in Table 3 and Fig. 2. The highest removal of TN from PMWW was 91.51% in 50%PMWW concentration while in control sample was around 14.68%. Moreover, other concentrations of PMWW which were 10%PMW, 25%PMWW, and 75%PMWW the removal was 85.30%, 88.60%, and 70.7% respectively. While the lowest removal was 57.49% in 100%PMWW. This situation happened might due to the high concentration of TN and affect the algae growth condition [2].

Table 3 - Nutrient removal analysis of PMWW

PMW Conc.	Initial Conc. (ppb)	Final Conc. (mg/L)	Removal Efficiency (%)
Total Nitrogen, (TN)			
10 %	223	32.76	85.30
25 %	396	45.13	88.60
50%	613	52	91.51
75 %	758	222.07	70.7
100 %	968	411.43	57.49
Total Phosphorus, (TP)			
10 %	33.08	8.07	75.60
25 %	58.96	5.20	91.169
50%	111.84	8.19	92.67
75 %	158.03	32.64	79.34
100 %	178	66	62.92
Total Organic Carbon, (TOC)			
10 %	389	74	80.97
25 %	697	94.02	86.51
50%	1022.03	100.08	90.20
75 %	1458	322.01	77.91
100 %	1798.22	897.23	50.10

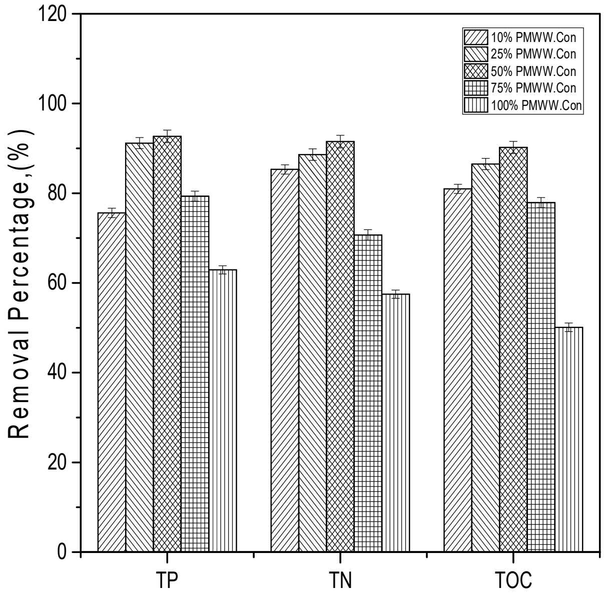

Fig. 2 - Nutrient removal percentage from PMWW

According to Fig. 2 the result for 50%PMWW in TP removal was 92.671, while the removal percentage for 10%PMWW, 25%PMWW, 75%PMWW, and 100%PMWW were 75.6%, 91.17%, 79.34%, and 62.92% respectively. On the other hand, the removal percentage for TOC which also success whereby the total removal was 80.97%, 86.51%, 90.20%, 77.91%, and 50.10% in 10% PMWW, 25% PMWW, 50% PMWW, 75%PMWW, and 100%PMWW concentrations.

Overall, based on the trends produced, the *Scenedesmus* sp. has reveal a great removal in 50% of PMWW concentration which is the total reduction of TN, TP and TOC were 91.51%, 92.67% and 90.20 respectively. Meanwhile, TN, TP and TOC removal for 100%PMWW were 57.49%, 62.92% and 50.10% respectively and slightly lower than in 50%PMWW and 75%PMWW. This condition was expected since the microalgae growth was higher in 50%PMWW and 75%PMWW as can be seen in Figure 1. It also indicates that *Scenedesmus* sp. has the ability to adapt nutrient in 100% of PMWW, however the total removal of TN, TP and TOC can be increased if prolonged the phycoremediation periods.

3.4 Heavy Metals Removal Analysis

In this study, there were four elements of heavy metals that had been analyzed for this wastewater sample which is Zinc (Zn), Iron (Fe), Chromium (Cr) and also Cadmium (Cd). Table 4 shows the initial and final concentration of heavy metals whereby the highest removal was found to be 50% and 75%PMWW with more than 70% of removal efficiency. The removal of (Zn) from PMWW was 86.07% in 50%PMWW higher than in PMWW concentration of 10%, 25% and 75%, which has the total removal of 50.75%, 67.99% and 71.96% respectively and the lowest removal was 30.49% was in 100%PMWW.

Table 4 - Heavy metals percentage removal

PMWW Con.	Initial Conc. (mg/L)	Final Conc. (mg/L)	Removal Efficiency (%)	Control (%)
		Chromium (Cr)		
10 %	52.3	23.5	55.06	13.76
25 %	70.8	17.55	75.21	13.41
50 %	77	6.56	91.47	16.49
75 %	160	38.45	75.96	16.83
100 %	194	149.2	23.09	11.03
		Iron (Fe)		
10 %	966	346.99	64.07	18.32
25 %	1770	376	78.75	6.77
50 %	1900	243	87.21	15.26
75 %	2200	428	80.54	10.5
100 %	2628	1724	34.39	11.94
		Cadmium (Cd)		
10 %	5.43	2.21	59.30	14.91
25 %	6.04	1.77	70.59	17.549
50 %	7.35	0.81	88.97	7.75
75 %	11.2	2.55	77.23	11.78
100 %	14.2	10.09	28.94	13.94
		Zinc (Zn)		
10 %	817	402.33	50.75	15.62
25 %	1100	352.06	67.99	14.10
50 %	1580	220	86.07	15.62
75 %	2240	628	71.96	14.10
100 %	2850	1981	30.49	15.62

Accordingly, Zn had been reduced from (1580 ppb to 220 ppb) for 50% PMWW concentration (Fig. 3(a)). However, the reduction of (Fe) in 50% PMWW concentration was up to 87.21% (from 1900 ppb to 243 pbb), while the presence of (Fe) in 10%, 25%, 75% and 100%PMWW concentration was able to be taken up about 64.07%, 78.75%, 80.54%, 34.39%, respectively (Fig. 3(b)).

Meanwhile, as for the highest removal in (Cr), 50%PMWW was reduced from (77 ppb to 6.56 ppb) which equivalent to 91.47% of total removal. Meanwhile, (Cr) for 10%, 25% and 75% PMWW were 55.06%, 75.21% and 75.96% respectively (Fig. 4(c)). (Cr) removal for 100% PMWW was the lowest which was reduced from (194 ppb to 149.2 ppb) 23.09%. The (Cd) for 50% was reduced from (7.35 ppb to 0.81ppb) which was equivalent to 88.97 of total removal (Fig. 4(d)).

On the other hand, the total removal for (Cd) in 10%, 25% and 75%PMWW were 59.30%, 70.59% and 77.23% respectively. Cd removal for 100%PMWW was the lowest which was reduced from (14.2 mg/L to 10.09 mg/L) 28.94%. This situation (low reduction of Zn, Fe, Cr, Cd in 100%PMWW) can be explained by the growth affect in 100%PMWW as shown in Fig. 3. The present study was in the line and similar with previous study done by Ajayan et al. [9]. which able to remove Zn (78%), Cr (70%), Cu (85%) and Pb (62%) in 50% of tannery wastewater using microalgae *Scenedesmus* sp. However, the reduction of heavy metals in the present study was also success in 75%PMWW.

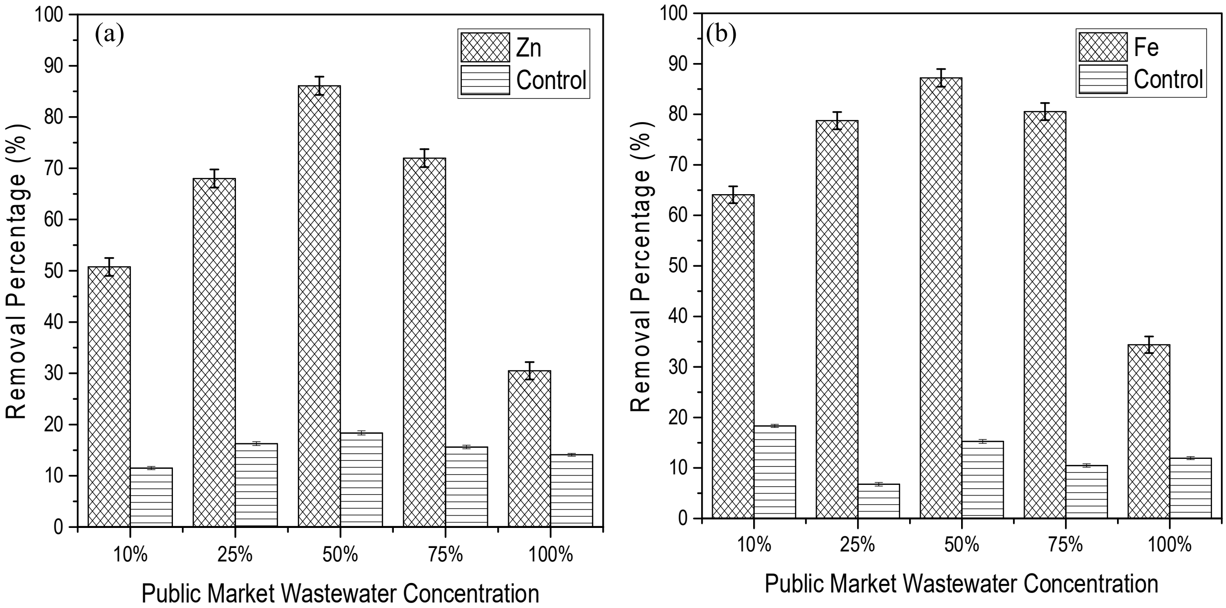

Fig. 3 - Percentage removal of (a) Zn and (b) Fe of PMWW

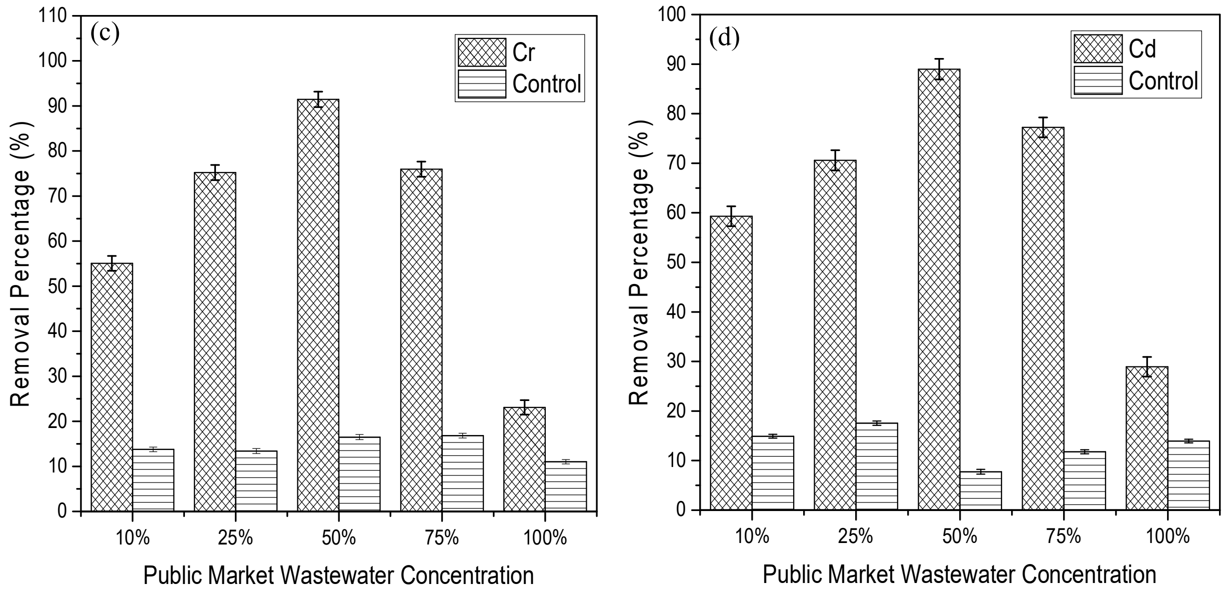

Fig. 4 - Percentage removal of (c) Cr and (d) Cd of PMWW

Overall, the application of microalgae *Scenedesmus* sp. in removing heavy metals has succeeded. It can be indicated that different concentration of PMWW plays an important role for microalgae growth but still *Scenedesmus* sp. has the potential in treating high nutrient wastewater prior discharge to the ecosystem.

3.5 Composition of Biomass Yield

Although the main focus in this study was the use of PMWW for phycoremediation using microalgae *Scenedesmus* sp. and to evaluate its capability for the nutrient and heavy metals removal. It is a consideration to produced biomass after wastewater treatment as the view of sustainable process. Therefore, many researchers had studied on exploiting biomass yield in term of protein and lipid from nutrient and heavy metals removal [25]-[27].

The proximate composition of protein and lipid profile from the biomass yield that has been produced in public market wastewater and BBM was illustrated in Table 5 and Fig. 5. These data show a contradict result that higher nutrient content in wastewater could generate higher protein and lipid production. It can be noted that the total protein and lipids contents in the biomass yield produced during the phycoremediation of PMWW was more than that in the biomass produced in the BBM (35.7 vs. 32.4 and 19.8 vs. 18.6%, respectively).

Table 5 - Biomass production (protein and lipid) compare WMWW and control (BBM)

Sample	Protein (%)	Lipid (%)
PMWW	35.7 ± 1.01	19.8± 0.98
BBM	32.4 ± 0.25	18.6 ± 0.71

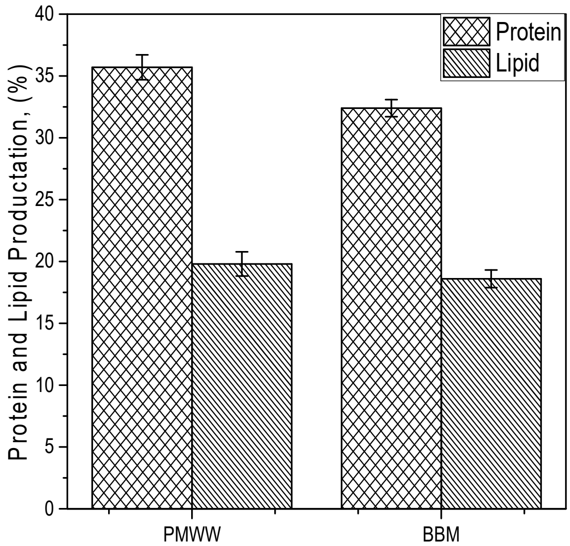

Fig. 5 - Biomass production (protein and lipid) compare (PMWW) and (BBM)

However, it is not surprising because the optimal growth and nutrient removal were the respond for protein and lipid production whereby the highest growth rate and nutrient removal was in the PMWW. This may imply also that the stress condition was triggered from the existence of nutrient in BBM as the stress condition is tightly linked to the amount of nitrogen, phosphorus and the presence of other microorganisms [3], [28]. It also proved that *Scenedesmus* sp. highest growth rate might the dominant factor for the results of protein and lipid content. Additionally, Sheng et al. [25] reported that, micronutrient deficiency and some other stress factor such as higher nitrogen involvement achieved higher lipid productivity, whereas PMWW has high level of nutrient which explained these results.

4 Conclusion

This study reveals that *Scenedesmus* sp. is suitable to be as phycoremediation agent to bio-transform the pollutant load into microalgae cell. This study also proven that the growth of microalgae *Scenedesmus* sp. was affected by the concentration of nutrients in the 100%PMWW sample. The capability of *Scenedesmus* sp. was more efficient in 50%PMWW where the removal reached up to 90%. In addition, the TN, TP and TOC concentration was also successfully reduced more than 50% total removal in raw (100%PMWW). Moreover, the highest removal percentage for heavy metals (Fe, Zn, Cr and Cd) were belong to sample of 50% PMWW which were 87.21, 86.07, 91.47 and 88.97% respectively.

From overall finding, it indicates highly difference of value by each parameter since the beginning at day 0 until day 19^{th} of phycoremediation, this study also has a promising way to reduce the cost of algae technologies since using PMWW as medium can replace the expensive synthetic chemical with cheap resources as nutrient source for microalgae growth. Therefore, from this study it can be said that microalgae represent the best biological treatment process for wastewater because it does not incur high treatments cost.

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