Phycoremediation of Heavy Metals in Wet Market Wastewater

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Abstract. The efficiency of phycoremediation using microalgae for removing nutrients and heavy metals from wastewaters has been proved. However, the differences in the composition of wastewaters as well as microalgae species play an important role in the efficient of this process. Therefore, the present study aimed to investigate the effectiveness of Scenedesmus sp. to removal of heavy metals from wet market wastewater. Scenedesmus sp. was inoculated with 10^6 cells/mL into each wet market wastewater concentration included 10, 25, 50, 75 and 100% and incubated for 18 days. The highest growth rate was recorded in 50% WM with a maximum dry weight of 2006 mg L^-1 which subsequently removed 93.06% of Cd, 91.5% of Cr, 92.47% of Fe, 92.40% of Zn. These findings reflected the high potential of Scenedesmus sp. in the treatment of wet market wastewater and production microalgae biomass.

1. Introduction
In developing countries, more than 2 tons millions of sewage, agricultural (70% contribution) and industrial wastes are dumped untreated into waters bodies equivalent through the escalating population of 7 billion people where potentially pollute the usable water supply [1]. Thus, Malaysian population is to be estimated around 31.7 million people in 2017 with 1.5% population growth rate from the previous year as stated in Department of Statistics Malaysia [2]. Many of researchers had studied on biological treatment to reduce pollutants using natural processes involving bacteria, and other microorganism such as microalgae since it is cost effective, easy to get and less maintenance [3,4]. Furthermore, the most popular place used to Malaysian to buy their food supply and groceries is wet market. Presently, Malaysia is experiencing the effect from untreated of wet market wastewater release freely to the ecosystem which carrying a large number of organic and inorganic pollutants [5,6].

Wet market wastewater were reported as a significant risks on human health from the pathogenic bacteria or trigering allergic reactions. Wet market wastewater and its inappropriate disposal are one of the major concerns that need to be aware especially when the market had none of wastewater treatment system. Generally, wet market wastewater contains high levels of contaminants and nutrients such as biological and chemical oxygen demand, nitrogen [7]. Other than that, heavy metals also contain in wet market wastewater which has turned into one of the negative issues since wet market dealing with seafood entrails, meat cutting and chicken slaughtering [8]. In order to meet the allowable standard for effluent discharge, these contaminants must be removed from wet market wastewater before discharged into environments.

The use of microalgae for agro-industrial wastewater treatment has proved to be an efficient alternative and make them as an attractive means for sustainable and low cost wastewater treatment. Furthermore, algae based technology is known to be versatile approach since it’s easily to be adapted with any environmental conditions [9]. Although Scenedesmus sp. has been widely used on the wastewater treatment, there has been limited use on wet market wastewater treatment for heavy metal. So, it is necessary to do deep investigation on phycoremediation of heavy metals presence in this wastewater and highly potential to be coupled with biomass production. Wastewater phycoremediation using microalgae offers a lot of advantages compared to conventional method as intensively described by previous studies [10-12,19]. Phycoremediation process is cost effective, environmental friendly, high-efficiency in reduce nutrient and heavy metals, potential production; biodiesel, fish feed. Hence,
the objective of present study is to investigate the potential of *Scenedesmus* sp. in removing selected heavy metals contain in wet market wastewater as influenced by different ratio of wastewater concentrations.

### 2. Methodology

#### 2.1 Microalgae *Scenedesmus* sp. culturing

A local microalgae *Scenedesmus* sp. was collected and isolated from freshwater temporary pond located at Endau Rompin National Park, Johor, Malaysia [10]. Isolated cells were sub-cultured in Bold Basal’s Media (BBM). Bold Basal Medium with the stock solution composition of the following: 25.0 g/L NaNO₃, 2.5g/L CaCl₂·2H₂O, 7.5g/L MgSO₄·7H₂O, 7.5g/L K₂HPO₄, 17.5g/L KH₂PO₄, 2.5g/L NaCl, 50.0g/L EDTA, 31.0g/L KOH, 4.98g/L FeSO₄·7H₂O, 1.0ml/L H₂SO₄, 11.42g/L H₃BO₃, 8.82g/L ZnSO₄·7H₂O, 1.44gMnCl₂·4H₂O, 0.71g/LMoO₃, 1.57g/LCuSO₄·5H₂O, 0.49g/LCo(NO₃)₂·6H₂O [13]. The culture medium was then placed under the sunlight as source of illumination for 12 days prior to experiments [11].

#### 2.2 Phycoremediation experiment

The phycoremediation experiment was set up using 500mL of Erlenmeyer flasks containing 400mL of wastewater. In order to prepare the sample, wet market wastewater (WM) was filtered using GF/C (Whatman) filter to remove suspended solid and then diluted with distilled water at 10%, 25%, 50%, 75%, and 100% (WM). The sample flask without microalgae (the same ratio of wet market wastewater) was assumed as control sample. The experiment flasks were cultured with the initial concentration of 1× 10⁶ cells/mL. The phycoremediation process had been conducted for 18 days. The inoculated flasks sample were shaken twice a day and concealed with sterile cotton plugs. The growth of biomass (dry weight per liter) was measured daily according to a calibration curve as describe by Eq. (1).

\[
\text{Dry weight (mg/L)} = 837.79 \times \text{OD650} + 0.02229
\]

\[R^2 = 0.9866\]  

(1)

#### 2.3 Heavy metals analysis

The experiment was cultured for 18 days and fixed volume of 10 mL was taken out under sterilized conditions after 18 days of treatment. The sample was then diluted with deionized water up to 50mL and analyzed for heavy metals content using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The removal efficiency was calculated according to Eq. (2)

\[
E = \frac{(C_i - C_e)}{C_i} \times 100
\]

(2)

where Ci and Ce are the initial and equilibrium values of evaluated heavy metals

### 3. Results and discussion

#### 3.1 Growth rate of *Scenedesmus* sp. in different concentration of wet market wastewaters

The effect of microalgae *Scenedesmus* sp. with different concentration of wet market wastewater (WM) is presented in Fig 1. These figure illustrated the growth trend of *Scenedesmus* sp in five different of wet market diluted media (10%, 25%, 50%, 75%, and 100% WM) and subsequently, found that 50%WM has the highest growth with a dry weight of 2006 mg L⁻¹. Unfortunately, microalgae growth of wet market concentration in the production medium for 100%WM was negatively affect as the growth curve started to level off at day 3 onwards. This situation is similar to a previous study done by Ajayan et al. [14] which was obtained 1180 × 10⁴ cells/mL in 50% and has lesser growth in 75% and 100% of tannery wastewater. This condition was probably due to the excessive nitrogen (N) and phosphorus (P) concentration in wastewater nutrient content [15]. However, as for other concentration was positively
grown and increased rapidly at day 4. At 10%WM, Scenedesmus sp, began to increase at the beginning but steadily decreased after the day 9 which indicated that the growth potential of this microalgae with different concentration were effective between 25% and 50%WM of wet market concentration. It seems that the wet market wastewaters are capable to be used as an alternative medium for microalgae production. Different types of wastewaters such as domestic wastewater, meat processing wastewater, food wastewater have been investigated as a production medium for microalgae and exhibited high productivity of biomass yield [16-17].

Figure 1. Growth rate of Scenedesmus sp. in different concentration of wet market wastewater

3.2 Heavy metal removal efficiency
Four heavy metals were analysed i.e. Cadmium (Cd), Chromium (Cr), Ferum (Fe) and, Zinc (Zn) are shown in table 1 and figure 2. Table 1 shows the initial and final concentrations of heavy metals found in wet market wastewater. The best initial concentration for all heavy metals that has been removed successfully was found to be in 50%WM with more than 80% of removal efficiency. Accordingly, Cr had been reduced from 77 ppb to 6.9 ppb with 91.5% removal efficiency in 50%WM while the removal percentage in the control was 19%. As for Cd (7.3 ppb to 0.51 ppb), Fe (1900 ppb to 143 ppb) and Zn (1580 ppb to 120 ppb) in 50%WM. This finding was similar to study done by Ajayan et al. which constantly remove Cr (70%), Cu (85%), Pb (62%) and Zn (78%) from 50% of tannery wastewater using microalgae Scenedesmus sp. [14]. However, the reduction of heavy metals in higher concentration of wet market wastewater (100%WM) was observed to be lower than all the wet market concentration for Cd, Cr, Fe and Zn. This could be explained by the growth condition in 100% was miserable as shown in Fig 1. On the other hand, Cd, Cr, Fe and Zn in 75%WM was able to be removed slightly same as in the 50%WM which eliminated up to 82.6%, 83.8%, 78.6%, 92.6% respectively (Fig.2.). Based on study conducted by Sengar et al. [18], microalgae Scenedemus sp. removed 100% of Cu from wastewater at 20th day of treatment. The complete removal of heavy metals might be achieve if the treatment day to be prolonged.
Table 1. The initial and final concentrations of the investigated heavy metals found in wet market wastewater

| Wet Market wastewater concentration | In. (ppb) | F. (ppb) | R.e.% | In. (ppb) | F. (ppb) | R.e.% | In. (ppb) | F. (ppb) | R.e.% |
|------------------------------------|----------|----------|-------|----------|----------|-------|----------|----------|-------|
| 10% WM Control                     | 5.43     | 1.01     | 89.05 | 52.3     | 7.5      | 85.7  | 817      | 158      | 80.66 |
| 25% WM Control                     | 6.04     | 0.661    | 89.05 | 57.8     | 1.1     | 91.4  | 1100     | 152      | 86.18 |
| 50% WM Control                     | 7.3      | 0.51     | 93.06 | 77       | 6.9      | 91.5  | 1580     | 120      | 92.40 |
| 75% WM Control                     | 11.2     | 1.95     | 82.6  | 120      | 19.4     | 83.8  | 2040     | 150      | 92.64 |
| 100% WM Control                    | 17.2     | 9.09     | 47.2  | 194      | 89.2     | 54.02 | 4850     | 2340     | 51.75 |

*(In: Initial, F: Final and, R.e%: Removal efficiency %)

Figure 2. The comparison of heavy metal removal efficiency between wet market wastewater and control; (a) Cadmium, (b) Chromium, (c) Ferum and (d) Zinc
Overall, phycoremediation using microalgae Scenedesmus sp. in removing heavy metals from wet market wastewater was successfully achieved. In another study, Onalo et al. [17] obtained only 2% of Cd removal using Botryococcus sp. while the other study done by Gani et al. [18] removed 83.5% (Cd) and 71.5% (Zn) using Botryococcus sp. in domestic wastewater. Meanwhile, Latiffi et al. [12] reported that Scenedesmus sp. was capable of reducing Fe concentration in food stall wastewater up to 88.22%, Cu at 60% and Zn at 76.63% respectively. It can be indicated that heavy metals can be removed from wet market wastewater by Scenedesmus sp. However, these wastes should be diluted in order to reduce the substances which might inhibit the growth rate of Scenedesmus sp. and thus reduce the removal efficiency. Moreover, Scenedesmus sp. could be a potential in treating prior discharge to the ecosystem.

4. Conclusion
This study has shown that microalgae Scenedesmus sp. offer a promising alternatives method in the treatment of wet market wastewater and removing heavy metals. The capability of Scenedesmus sp. was more efficient in 50% WM, the removal percentage reached more than 90%. Moreover, the investigation of phycoremediation of wet market wastewater using microalgae can be combined with biomass production and also motivated for researcher in implementation of treating wastewater. It is also an eco-friendly process with no secondary pollution as long as the biomass produced is reused and allows efficient nutrient recycling. This study has thrown up many questions in need of further investigation. Further work needs to be done to implement this approach in real system of wet market wastewater treatment in the field.

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