HEAVY METALS IN MICROALGAE BIOMASS ADDED WITH DIFFERENT CONCENTRATION OF WET MARKET WASTEWATER

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ABSTRACT

The aim of this study is to assess heavy metals content namely arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) in microalgae Scenedesmus sp. biomass cultivated with wet market wastewater. Microalgae Scenedesmus sp. is added to wet market wastewater which contains high nutrient level that has a potential to be made into fish supplement products. However, this process depends on the level of heavy metals in the wastewater. Different concentrations of wet market wastewater were tested in the dilution of 10% (10WM), 15% (15WM), 20% (20WM) and 25% (25WM) during the cultivation to measure the potential by-product quality. As, Cd, Pb and Hg heavy metals in dry biomass of Scenedesmus sp. were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Mercury Analyser. Heavy metals content (ug/kg in ppb) in the Scenedesmus sp. were found in the range of 182.5-7.74 for As, 0.985-3.111 for Cd, 4.95-2.375 for Pb and 9.774-1.037 for Hg. Arsenic was the highest trace metal (182.5 ppb) found in 25WM and the lowest (0.311 ppb) was Cadmium from Bolt’s Basal Medium (BBM) sample. The level of heavy metals in all microalgae biomass samples did not exceed the maximum concentration set up by the European Commission Regulation. Therefore, the biomass from microalgae Scenedesmus sp. cultivated with wet market wastewater has acceptable concentration of As, Cd, Pb and Hg. Thus, it has a great potential to be processed into bioproducts such as fish feed and fish supplement.

Keywords: Heavy metals, Scenedesmus sp., Wet market wastewater, Heavy metals concentration

INTRODUCTION

Consumers can buy fresh produce in various places, including the wet market. Unfortunately, wet market wastewater which consists of various components such as nitrogen and phosphorus are commonly discharged directly to the drainage without any treatment [1]. In Malaysia, most of the wastewater will be channeled into the drainage and end up flowing into a river. This phenomenon escalates the pollution in Malaysian rivers. For example, a river in Perlis, Malaysia, has been polluted with wastewater coming mostly from the effluent of food-based wastewater which includes wet market with 58.30 (polluted), 61.87 (slightly polluted) and 41.64 (polluted) water quality index [2]. Wastewater from wet market contains nutrients and heavy metals derived from fresh foodstuff, waste scraps of seafood and fish entrails. The wastewater discharged contains various wastes such as organic materials, suspended solids, fat, and toxic compounds such as heavy metals. The content of organic matter found in wastewater from wet market is three times more than in solid waste [3]. Although low concentration of heavy metals is harmless, yet high concentration of this substance can potentially be toxic.

For the past few years, a lot of researches were carried out to study the value potential of dual application of microalgae for wastewater treatment and biomass production [4, 5]. Gani et al. [6] studied the use of microalgae and successfully removed up to 93.3% of total organic carbon from wastewater, and the algae became a potential candidate for biodiesel production. Wastewater treatment by using microalgae is eco-friendly and offers the advantage of a cost effective way of nutrient removal and biomass production [7]. Since microalgae have the ability to absorb nitrogen and phosphorus [8], instead of the nutrients being wasted; this will provide nutrients for the microalgae to grow which in turn becomes valuable biomass products [9-10]. Since microalgae have been recognized as a very favorable source of biomass, it can also be used as an economical alternative to treat wastewater efficiently. Microalgae are also the best candidate to decrease heavy metals concentration from ppm to ppb level and known to be resilient to the contaminants of high concentrated wastewater [11].

There are few types of toxic heavy metals that may impact human and animal health. The content of toxic heavy metals will affect the biomass quality. Heavy metals in biological organisms are dangerous because the concentration tend to bioaccumulate over time compared to static concentration found in the environment [12]. This study has selected four toxic heavy metals namely Arsenic (As), Cadmium (Cd), Lead (Pb) and Mercury (Hg) to be studied. To evaluate and remove
the heavy metals selected, the process of bioremediation technology was applied in this study [13]. The existence of heavy metals in the wastewater may affect the characteristic compound of the microalgae which will automatically give negative impact to the main food source for bivalve mollusks at their growth stages (zooplankton; larvae stages of crustacean and fish species) [14]. Furthermore, these toxic metals in microalgae could be a threat to the ecosystem and public health and must be treated. Therefore, this study is to evaluate the heavy metals (As, Cd, Pb and Hg) contents from biomass derived from different concentrations of wet market wastewater.

MATERIALS AND METHODS

Wastewater Characterization and Experimental Procedure

Untreated wet market wastewater (WM) was collected from Pasar Borong Rengit, Batu Pahat, Johor, Malaysia (N 1° 40' 39.5144" E 103° 8' 43.7844"). Wet market wastewater has been taken by grab sampling according to the standard of APHA (2002) [15]. The main organic waste fraction was from seafood and fish entrails and market’s operations. Wastewater was transported to the laboratory and went under sterilization using autoclave at 120°C for 15 minutes. Wet market wastewater sample was filtered with GF/C (Whatman) filter to remove suspended solids and diluted with distilled water into four concentrations 10%, 15%, 20% and 25% in triplicate to investigate the effect of different concentrations in order to support microalgae growth. Bolt Basal Medium (BBM) was used as control media. The experiment was conducted in duplicate for each concentration. Physical and chemical parameters were analyzed according to the standard method given in APHA (2002) [15]. Samplings for parameters analysis were collected before and after the treatment. Total parameter removal efficiencies was calculated in accordance with Eq. (1).

\[
\text{Removal} \% = \frac{\text{Initial conc. (mg/L)} - \text{Final conc. (mg/L)}}{\text{Initial conc. (mg/L)}} \times 100
\]

Eq. (1)

Cultivation of Scenedesmus sp. Using Wet Market Wastewater

Microalgae Scenedesmus sp. was obtained from the culture collection of Faculty of Science, Technology and Human Development, Universiti Tun Hussein Onn Malaysia. The inoculum of Scenedesmus sp. was maintained in BBM prior to experiment. The experiment was carried out using 2L Erlenmeyer flask containing 1.5 L of wastewater and BBM. BBM medium was used as control media. Microalgae were cultivated under outdoor sunlight with continuous aeration at 3.2 L/min to ensure the distribution of nutrients using atmospheric air from an aquarium pump through air sparger. The experiment flask was inoculated with the same initial microalgae cells of 10^6 cells/mL during the start of the experiment. The biomass was harvested immediately after 12 days by centrifugation at 4000 rpm for 5 min. The sample was then kept and stored at -20°C until further analysis. The cultivation batches were carried out in duplicate.

Sample Preparation and Analysis

The frozen microalgae sample was melted individually and dried at 105°C to a constant weight. Dried microalgae were then finely crushed and homogenized using mortar and pestle and about 0.5g of the homogenized sample was digested in 10ml of HNO₃ (nitric acid) at 160°C. The solution was diluted with deionized water up to 50ml and analyzed for heavy metals content using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for As, Cd, Pb and Hg and Mercury Analyser for Hg [16].

RESULTS AND DISCUSSION

Wastewater Characteristics

Wet market wastewater remediated with Scenedesmus sp. was evaluated for pollutants parameter removal of biochemical oxygen demand (BOD), chemical oxygen demand (COD) and turbidity. The percentage removal before and after the microalgae Scenedesmus sp. cultivation was shown in Table 1. Scenedesmus sp. was able to efficiently remove BOD and COD from the wastewater; that is 83.6% BOD from 25MW, and 94.7% COD from 15MW. Compared to the result from another study by Violeta et al. (2011) [17], this study achieved slightly higher BOD removal at 83.6% from wet market wastewater compared with Violeta et al.’s (2011) 92.12% from Scenedesmus sp. cultivation using sewage wastewater. The reduction of BOD and COD by microalgae cultivation happens because microalgae absorbs carbon dioxide and convert BOD and COD concentration into their carbon sources of living [18-19]. Meanwhile, the pH parameter in each sample slightly decreased and did not exceed the standard A & B [20]. Assimilation of photosynthetic activity reduces the pH levels because the ammonia concentration acts as a nitrogen source for microalgae to grow [9, 21].
Heavy Metals Content in Microalgae Biomass

According to the Table 2, the highest heavy metals concentration is mostly dominated by Arsenic (As) with the value of 182.5 ± 7.18 ppb from the biomass of 25WM. This amount may due to high concentration of wastewater medium (25% of wet market wastewater) during the microalgae cultivation process, compared to biomass from BBM (control) (7.74 ± 2.7 ppb) and 10WM (76.86 ± 10.5 ppb). The next highest value is Lead (Pb) with a total of 4.95 ± 4.82 from 15WM. Next, the lowest metal is Cadmium (Cd) with a total of 0.985 ± 0.124 from the biomass of 20WM. For BBM biomass, the total value of all metals is very low compared to other biomass samples, verifying that this microalgae (Scenedesmus sp.) is non-toxic and harmless. Low levels of trace metals in this study could be from the dilution due to low parameter content in the wastewater.

Table 1 Percentage Removal of Wet Market Wastewater Characteristic

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before (MSC)</th>
<th>After (MSC)</th>
<th>%</th>
<th>Before (MSC)</th>
<th>After (MSC)</th>
<th>%</th>
<th>Before (MSC)</th>
<th>After (MSC)</th>
<th>%</th>
<th>Before (MSC)</th>
<th>After (MSC)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.36</td>
<td>7.0</td>
<td>-</td>
<td>7.41</td>
<td>6.4</td>
<td>-</td>
<td>7.49</td>
<td>6.18</td>
<td>-</td>
<td>7.52</td>
<td>6.79</td>
<td>-</td>
</tr>
<tr>
<td>BOD</td>
<td>349</td>
<td>74</td>
<td>78</td>
<td>375</td>
<td>78</td>
<td>79.2</td>
<td>512</td>
<td>87</td>
<td>83</td>
<td>566</td>
<td>93</td>
<td>83.6</td>
</tr>
<tr>
<td>COD</td>
<td>574</td>
<td>32</td>
<td>94.4</td>
<td>639</td>
<td>34</td>
<td>94.7</td>
<td>647</td>
<td>46</td>
<td>92.9</td>
<td>689</td>
<td>56</td>
<td>91.9</td>
</tr>
</tbody>
</table>

*MSC: Microalgae Scenedesmus sp. cultivation

Table 2 Heavy Metals Content in Microalgae Biomass Compared with the Maximum Content of the European Commission Regulation (2015)

<table>
<thead>
<tr>
<th>Heavy Metals</th>
<th>Microalgae Biomass Concentration (ug/kg as in ppb)</th>
<th>Remarks</th>
<th>Max. content in ug/kg (ppb) (EU No 186/2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BBM (control) 10WM 15WM 20WM 25WM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenic (As)</td>
<td>7.74 ± 2.7 76.86 ± 10.5 107.7 ± 3.68 117 ± 11.98 182.5 ± 7.18</td>
<td>Low</td>
<td>25000</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.311 ± 3.9 0.319 ± 3.25 0.515 ± 3.12 0.985 ± 0.124 0.413 ± 2.03</td>
<td>Low</td>
<td>10000</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>2.695 ± 5.1 3.36 ± 0.343 4.95 ± 4.82 3.195 ± 1.57 2.375 ± 5.59</td>
<td>Low</td>
<td>200</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>1.44 ± 0.00 9.774 ± 0.00 3.502 ± 0.00 1.033 ± 0.00 2.422 ± 0.00</td>
<td>Low</td>
<td>25000</td>
</tr>
</tbody>
</table>

The tests deduced that the biomass cultivation of Scenedesmus sp. microalgae using four different mediums of wet market wastewater is harmless and non-toxic where all the values of heavy metal concentrations are below the limits set by the European Commission Regulation (2015) [22]. In order to protect public health, it is appropriate to limit the maximum levels of metals that are safe for human/animal exposure in foodstuffs [22, 23]. This experiment proved that the production of non-toxic algae biomass cultivated from wet market wastewater carry a great potential to be used in future bioproducts such as fish feed, food supplements for humans and animals, as well as fertilizers. This experiment proved that the production of non-toxic algae biomass cultivated from wet market wastewater carry a great potential to be used in future bioproducts such as fish feed, food supplements for humans and animals, as well as fertilizers.
CONCLUSION

The cultivation of Scenedesmus sp. microalgae in wet market wastewater is one of the alternatives to treat wastewater before it can be discharged to the drainage. The result of metal concentration in dry biomass in each sample is mostly acceptable under the European Commission Regulation (2015), making it a potential means to produce bioproducts. It can be said that the Scenedesmus sp. microalgae has potential in absorbing pollutants from wastewater. Although the levels of heavy metals in this study were below the standard regulation (EU No 186/2015) (Table 2), monitoring on more contaminated wet market wastewater should be necessary since wet market wastewater has high nutrients and pollutants parameter when the sample is not diluted. Thus, Scenedesmus sp. microalgae extracted from wet market wastewater in this study is non-toxic and can be used to produce various bioproducts such as fish feed, fish supplement and much more.

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REFERENCES


