Green Approach in the Bio-removal of Heavy Metals from wastewaters

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Abstract. Cultivation of microalgae has been suggested as a green approach for a sustainable wastewater treatment especially heavy metal bioremediation. This study investigated the bio-removal of zinc (Zn), iron (Fe), cadmium (Cd) and manganese (Mn) from domestic wastewater (DW) and food processing wastewater (FW) using green microalgae, *Botryococcus* sp.. The total of five treatments represented by five different cell concentrations (1×10^3, 1×10^4, 1×10^5, 1×10^6 and 1×10^7 cells/mL) of *Botryococcus* sp. in the wastewaters medium. The results revealed high removal efficiency of Zn, Fe, Cd and Mn after 18 days of the culture compared to control (wastewaters without algae). In DW , Zn, Fe, Cd and Mn were successfully removed at the highest efficiencies up to 71.5%, 51.2%, 83.5% and 97.2%, respectively while in FW, the same metal concentrations were reduced by up to 64.4%, 53.3%, 52.9% and 26.7%, respectively. Overall, most of the algae cell concentrations tested were successfully reducing the metals contaminant presence in both wastewaters and provides a baseline for further phycoremediation coupled with biomass production.

1 Introduction

Rapid population growth and industrial development are expected to contribute extremely to the generation of waste, especially wastewater. Increasing in population normally will be accounted to the production of domestic wastewater which is highly contaminated and harmful to the environment. Similarly, the booming food industry in Malaysia is becoming one of the leading contributors of wastewater that pollutes the receiving water bodies [1]. Both domestic and food industry wastewaters contained a high amount of organic and inorganic nutrients which are suitable for growth of microalgae [2]. The growing microalgae can then be used to carry out the phycoremediation process [1, 2]. However,

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these wastewaters contained not only nutrients but also harmful heavy metals that threaten the aquatic life and public health.

The heavy metals present in domestic wastewater (DW) is mainly contributed by pipe sedimentation, corrosion and domestic product usages such as medicine, caulking products, cleansing product cosmetics and others [4]. Meanwhile, metals concentration in the food wastewaters (FW) were mainly contributed by fertilisers and pesticides used during the production and growing stage of the fruit plants. Conventional techniques to remove heavy metal are often ineffective and very expensive. In addition, these methods are used to reduces heavy metal contaminations at low concentrations [5]. In fact, most of the conventional techniques provided incomplete metals removal, require high energy and used a significant amount of reagent, and had limited tolerance to pH changes [5]. Hence, the green approach has been introduced to address these problems. What is meant by green approach is to apply the cultivation technology of microalgae using wastewater and simultaneously reduce the concentration of hazardous metals indirectly [6]. This approach has a promising potential to contribute to achieving this goal in an environmentally friendly manner. Moreover, green technologies comprise low-cost, high-efficiency technique for heavy metals bio-removal from wastewaters [5–9]. However, the effectiveness of metal removal using this approach depends on microalgal species, environmental factors and microalgae concentrations [2, 10, 11]. As highlighted earlier, there has been few information exist on the potential of microalgae, *Botryococcus* sp. in the removal of heavy metals from wastewater. Previously, Onalo et al. [12] grew microalgae, *Botryococcus* sp. in heavy metal contaminated textile industry wastewater for the potential Cr, Cu, As and Cd bio-removal. They found that *Botryococcus* sp. effectively reduce Cr, Cu, As and Cd up to 94%, 45%, 9% and 2%, respectively. Soeprobowati and Hariyati [14] reported using *Chaetoceros calcitrans* to remove Pb, Cd, Cu and Cr from synthetic wastewater while Worku and Sahu [15] also used synthetic wastewater to phytoremediate the Cr, Ni, Fe and Hg using microalgae, *Synechocystis salina*. In 2012, Krustok and Nehrenheim [16] had demonstrated the cultivation of microalgae (species not specify) for the reduction of heavy metals present in domestic wastewater. Accordingly, the objective of the present work is to investigate the capability of *Botryococcus* sp. in bio-removal of selected heavy metal present in domestic wastewater and food processing wastewater with different cell concentrations under indoor culture condition.

2 Materials and Methods

2.1 Wastewaters sampling and analytical analysis

The domestic wastewater (DW) was obtained from wastewater treatment plant located in the main campus of Universiti Tun Hussein Onn Malaysia, Johor (N 01° 51' 55.224" E 103° 5' 21.183"). This plant receives daily wastewater generated from different activities in the campus area of which student's residential college and campus cafeterias contributed the most. Meanwhile, the food industry wastewater (FW) was collected from Azhar Food Manufacturing Industry located in Rengit, Johor (N 01° 41' 13.2" E 103° 07' 43.6"). The industries carry out processing activities and the production of snack foods such as potato chips, banana chips and cookies. Both wastewater samples were collected using acid washed sample bottles at the site and immediately transferred to the laboratory and preserved at temperatures below 4°C in a refrigerator [17]. The chemical oxygen demand (COD) and total phosphorus (TP) were measured using DR 6000 Spectrophotometer (Hach, USA) according to Method 8000 and Method 10127, respectively. Meanwhile, the biochemical oxygen demand (BOD) and total suspended solid (TSS) were analysed

according to the standard method [17]. For the heavy metal (Zn, Fe, Cd and Mn) analysis, the inductively coupled plasma spectrometry (ICP-MS) was used.

2.2 Experimental design

The microalgae namely *Botryococcus* sp. used in this study was obtained from tropical rainforest located in the southern region of Peninsular Malaysia. The preparation of *Botryococcus* sp. inoculum follows Gani et al. [3]. This was followed by cell observation and cell concentration count using Neubauer haemocytometer chamber.

Indoor culture condition was employed in the current study. The experiments were conducted in an environmental chamber (Wisd – ThermoStable SWGC) with control temperature (33°C) light intensity (243 μmol m^{-2}s^{-1}) and 24:0 hours of photoperiod [6]. These factors were considered to be optimal for this microalgae species according to our preliminary biomass productivity examination (data not shown). There are five initial microalgae cell concentration were tested (1×10^3, 1×10^4, 1×10^5, 1×10^6, and 1×10^7 cells/mL) excluding control sample [3].

The wastewater samples were filtered using a membrane filter (Whatman) with a 0.45μm pore size to remove other microorganisms and suspended solids before commencing the experiments. A total of 36 Erlenmeyer flasks (500 mL) were filled with 350 mL of prepared DW, and FW were used in this study. These 36 flasks correspond to 12 different treatments including control in triplicates. Each treatment represents initial concentrations of microalgae at the beginning of the experiment. The culture flasks were exposed to controlled indoor condition. The flasks were covered with sterile cotton plugs and repeatedly shaken four times daily to ensure homogenised cells in the wastewaters [18]. The experiment was carried out for 18 days in total. Total removal efficiencies calculated following to Equation 1.

$$\mathrm{Re}\,moval\,,\% = \frac{Initial \text{ conc. } (ppb) \text{ - Final conc. } (ppb)}{Initial \text{ conc. } (ppb)} \times 100 \qquad (1)$$

2.3 Statistical analysis

Data analyses of average, mean differences, standard deviation, and significant different level (95%) for heavy metals removal were performed accordingly using analysis of variance (One-way ANOVA) SPSS 20. Basically, this statistical analysis served to test the mean difference of the dependent variable for three or more microalgae concentrations simultaneously.

3 Result and discussion

3.1 Characteristic of wastewaters

Wastewater characterization is important for determining the organic and inorganic nutrient supplements required for microalgae growth during bioremediation process. The FW showed higher COD, BOD and TSS compared to DW (Table 1). Similarly, heavy metals, zinc, cadmium and manganese were found higher in FW than DW (Table 1). The physicochemical parameters of both wastewaters were found to be above the limit set by the Environmental Quality Act of Malaysia 1974 [19] (Table 1). On the other hand, the heavy metal (Zn, Fe, Cd and Mn) contents of both DW and FW were still within the allowable limit (Table 1).

Table 1. Raw characteristics of domestic wastewater (DW) and food processing wastewater (FW) compared to effluent standard

Parameter	[a] Concentration		Effluent standard (Environmental Quality Act, 1974)	
	Domestic wastewater (DW)	Food processing wastewater (FW)	Standard A	Standard B
Physiochemical, mg/L				
Chemical oxygen demand (COD)	129.00±2.35	2838.62±793.46	50	100
Biochemical oxygen demand (BOD)	71.08±2.80	793.89±110.20	20	50
Total phosphorus (TP)	8.99±0.53	6.81±1.93	5	10
Total suspended solid (TSS)	2158.00±10.32	12418.67±8989.20	50	100
pH	6.96±0.31	5.86±0.75	6.0 – 9.0	5.5 – 9.0
Heavy metal, ppb				
Zinc, Zn	334.6±30.0	670.9±290.0	1000	1000
Ferum, Fe	891.5±30.0	685.2±120.0	1000	5000
Cadmium, Cd	0.7±0.00	1.3±0.00	10	20
Manganese, Mn	159.3±20.0	219.3±170.0	200	1000

* All unit in mg/L except for pH and heavy metal (ppb)
[a] All experiments conducted in replicates (*n*=3)

In general, the characteristics of the DW (Table 1) were highly variable but comparable to the range reported in previous studies. The concentration of COD and BOD were 129 mg/L and 71.08 mg/L, respectively; this concentration was different from that used in other research paper. For instance, Gani et al. [3] used untreated DW containing 76.1 mg/L of COD and 44 mg/L of BOD to cultivate green microalgae *Botryococcus* sp. Zhang et. al. [20] cultivated mixotrophic microalgae strain in DW containing 142 mg/L of COD while Mostafa et. al. [21] used DW containing 50 mg/L of COD and 15 mg/L of BOD to grow cyanobacteria and *Chlorella vulgaris*. Nevertheless, the wastewaters used in this study contained 8.99 mg/L of TP 6.96 of pH value, indicating an acceptable concentration compared to the effluent standard and suitable enough for microalgae cultivation [22].

In terms of metal elements, manganese shows higher concentration compared to effluent Standard A (Table 1), while the rest of the elements studied were within allowable limit as stated in the standard (Table 1). Although the selected metal elements studied were still within the range of standard effluent limit, however, they still need to be reduced considerably if not completely removed before discharged to the environment. The need for this action is because heavy metal have the capacity to bioaccumulate in living freshwater organisms habitating the receiving water bodies, hence a threat to public health [12]. Thereby, this study may allow the use of DW and FW for the development of *Botryococcus* sp. culture and simultaneously to perform the bio-removal process of metals.

3.2 Heavy metal bio-removal efficiency

The removal of zinc (Zn), ferum (Fe), cadmium (Cd) and manganese (Mn) using cultivation of microalgae, *Botryococcus* sp. from domestic wastewater (DW) and food processing wastewater (FW) with different cells concentration are successfully performed (Table 2 and Fig. 1). Statistically, there is a significant effect ($P<0.05$) of microalgae concentrations on the bio-removal of Zn, Fe, Cd and Mn from both DW and FW under indoor culture

condition. The highest removal of Zn from DW and FW was 71.5% and 64.4%, respectively. Accordingly, Zn had been reduced from 156.67 ppb to 44.7 ppb for DW while 141 ppb to 50.23 ppb for FW at the concentration of 1×10^4 cell/mL and 1×10^6 cell/mL, respectively (Table 2). However, the reduction of Fe in DW was up to 51.2% (from 302.19 ppb to 147.59 ppb) at microalgal cell concentration of 1×10^5 cell/mL; while the presence of Fe in FW was able to be taken up about 53.4% at the concentration of 1×10^6 cell/mL (reduced from 455.33 ppb to 212.67 ppb). The Cd ion in DW was reduced from 0.34 ppb to 0.056 ppb which is equivalent to 83.5%. Meanwhile, Cd in FW reduced from 0.18 ppb to 0.085 ppb (52.9%). Other toxic such as Mn in DW was able to be removed optimally about 97.2% at the lowest microalgae cell concentration applied in which reduced from 112.33 ppb to 3.15 ppb. Whereas, in FW, Mn could be eliminated up to 26.7% only under indoor culture condition.

Table 2. The bio-removal efficiency of heavy metal from domestic wastewater (DW) and food processing wastewater (FW) using *Botryococcus* sp.

Microalgae cells concentration, cell/mL	Initial concentration, ppb		Final concentration, ppb		Removal efficiency, %	
	DW	FW	DW	FW	DW	FW
Zinc, Zn						
1×10^3	156.67± 3.06		49.3±3.4	61.20±3.02	68.53±1.97	56.60±3.06
1×10^4			44.7±4.49	57.77±2.54	71.47±2.83	59.03±2.66
1×10^5		141± 3.00	33.83±3.82	58.37±2.10	71.00±2.42	58.61±2.33
1×10^6	116.67± 3.51		48.7±3.04	50.23±3.02	58.26±3.13	64.37±2.89
1×10^7			63.93±2.61	60.70±2.52	45.20±0.59	56.95±2.70
Control			80.51±2.27	94.79±2.86	30.99±4.05	32.78±0.81
Ferum, Fe						
1×10^3	333.67± 3.51		174.45±3.09	215±5.00	47.72±1.24	52.78±1.26
1×10^4			172.95±2.62	218.67±12	48.17±0.49	51.98±2.44
1×10^5		455.33± 2.52	147.59±2.51	218±13.89	51.16±0.34	52.13±2.84
1×10^6	302.19± 3.02		192.45±3.09	212.7±11.0	36.31±0.70	53.29±2.43
1×10^7			166.19±3.38	300±6.00	45.00±0.86	34.11±1.56
Control			175.45±1.50	443.67±5.5	41.94±0.12	2.56±1.42
Cadmium, Cd						
1×10^3	0.48± 0.03		0.2±0.0095	0.115±0.01	57.87±1.90	36.11±1.45
1×10^4			0.179±0.005	0.085±0.01	62.36±1.78	52.89±2.04
1×10^5		0.18± 0.002	0.056±0.004	0.10±0.004	83.52±2.69	43.89±1.63
1×10^6	0.34± 0.04		0.093±0.004	0.103±0.00	72.48±3.18	42.78±2.30
1×10^7			0.1463±0.01	0.096±0.01	56.97±3.54	46.41±3.61
Control			0.206±0.004	0.152±0.01	38.71±7.78	15.37±4.48
Manganese, Mn						
1×10^3	112.33± 5.51		3.15±1.23	50.13±2.01	97.20±1.14	26.60±1.08
1×10^4			3.66±1.52	50.33±2.52	96.74±1.22	26.31±4.63
1×10^5		68.3± 1.75	4.55±0.51	50.73±2.61	91.75±1.00	25.72±3.06
1×10^6	55.17± 0.76		7.4±0.53	50.07±1.10	86.58±0.97	26.70±1.74
1×10^7			7.62±0.54	60.13±2.01	86.18±1.15	11.96±0.72
Control			18.65±0.57	66.13±2.73	66.20±1.48	3.17±1.92

[*]All experiments conducted in replicates (*n*=3)

The present study, however, makes several noteworthy contributions to the application of green approach in the removal of metals in wastewater using microalgae, *Botryococcus* sp. is highly influenced by the different microalgae concentration employed. Obviously,

each metal element study required a different amount of microalgae cell to perform the bio-removal process effectively. These phenomena may be due to the difference species of microalgae used and also affected by the metal concentration itself in wastewater [23].

Overall, cultivation of *Botryococcus* sp. in contaminated DW for biomass production and simultaneously remove some metal elements (Zn, Fe, Cd, and Mn) was successfully performed. Previously, El-Sheekh et al. [9] found that freshwater microalgae *Chlorella vulgaris* was able to remove Zn, Mn, and Fe concentration in sewage water up to 64.96%, 100%, and 100%, respectively while *Chlorella salina* successfully reduced about 15.6-28.5%, 89.94-93.71%, and 97.24%, respectively after 10 days of treatment. In another study, *Chlorella vulgaris* also successfully reduced the concentration of Zn, Fe, and Mn up to 80.1%, 100%, and 100%, respectively from domestic secondary effluent [24].

In 2014, Onalo et al. [12] investigated the removal of Cd in textile wastewater using *Botryococcus* sp. only up to 2%. Meanwhile, Chan et al. [13] reported that *Chlorella vulgaris* and *Spirulina maxima* capable of reducing Zn concentration in DW up to 96.3% and 94.9%, respectively. Other than that, Hamouda et al. [25] studied removal of heavy metals in industrial wastewater using green microalgae and found that *Scenedesmus obliquus* able to reduce Cd about 70% in the light and dark culture condition. Therefore, heavy metals removal in wastewater mostly depending on the species of microalgae used in which has different accumulation affinities towards the tested elements. Moreover, it is also relying on the nature and charge of the cell wall polysaccharides of microalgae [9]. In fact, green microalgae cells cultivated in wastewater with high metal contents also reduced higher metal concentration [26]. In other words, the metal accumulate was independent on the external metal concentration [9].
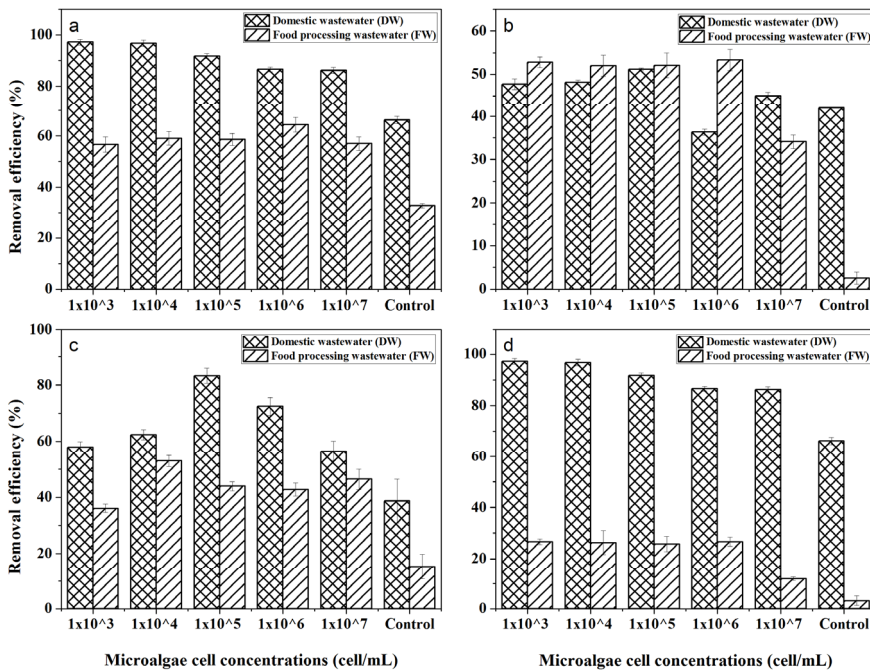

Fig. 1. The comparison of heavy metal removal efficiency between domestic wastewater and food processing wastewater; (a) Zinc, (b) Ferum, (c) Cadmium and (d) Manganese

4 Conclusions and recommendations

The purpose of the present study was to investigate the effect of microalgae; *Botryococcus* sp. cell concentration on the bio-removal of heavy metal from domestic wastewater and food processing wastewater under indoor culture condition. The capability of *Botryococcus* sp. in metals removal is clearly supported by the current findings. However, removal efficiencies are highly depending on the microalgae concentration applied. Most of all concentration employed were successfully removed Zn, Fe, Cd and Mn between 11.96% and 97.2%. More broadly, the investigation is also needed to determine the influence of the application of outdoor culture condition on the bio-removal of metal ions coupled with potential precious biomass production.

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