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Growth of Freshwater Microalga, Botryococcus sp. in Heavy Metal Contaminated Industrial Wastewater

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Abstract

The aim of this study was to determine the growth and the bioremoval capacity of the green microalga, Botryococcus sp. grown in industrial wastewater contaminated with heavy metals. The freshwater green microalga, Botryococcus sp. was cultured in different concentrations of wastewater (25%, 50% and 100%) with an initial cell concentration of 1000 cells/ml for a period of 12 days. Bold basal medium and sterile distilled water were used as positive and negative control, respectively. The Botryococcus sp. grown in Bold's basal medium showed the highest ($P<0.05$) average growth rate (7.8 × 106 cells/ml) after a period of 12 days, whereas, the lowest ($P<0.05$) growth was observed in 50% concentration of wastewater (4.8 × 104 cells/ml). Similar results were obtained for the specific growth rate (μ/day) with an average of 1.93μ/day and 1.22μ/day for the positive control and the 50% concentration respectively. Highest reduction of heavy metals was achieved for chromium which is equivalent to 94%, followed by copper (45%), arsenic (9%) and cadmium (2%). The results of this study suggest the potential of Botryococcus sp. as bioremediator of wastewater contaminated with heavy metals.

Keywords: industrial wastewater, heavy metals, microalgae, botryococcus sp

1. INTRODUCTION

The introduction of untreated industrial wastewater containing toxic elements (such as the heavy metals) into the water bodies has become a great deal of concern to the environment [1]. Heavy metals contained in industrial effluents are dangerous particularly in human health perspective due to their toxicity and bio-accumulating effects [2,3,4]. Some of the conventional methods which are still used in the treatment of wastewaters such as ion exchange and chemical precipitation [5], do not produce significant or desired results when the concentration of heavy metal present is relatively high [6]. In addition, they are also expensive to manage and could sometimes lead to an unfavourable condition such as the formation of toxic chemical sludge as a result of the many chemicals involved in the process. The application of bioremediation in heavy metal removal from wastewater has offered some advantages over the use of conventional wastewater treatment technologies. These include cost effectiveness, high efficiency, renewability of materials and no generation of additional pollutants [1,7,8]. Microalgae have shown high heavy metal binding affinity primarily due to short-chain amino acids they possess which are linked by peptide bonds [9,10]. It has also been found that they possess a large surface area-to-volume ratio that tends to be accessible for contact with the environment within as well as their accumulation ability which makes them potential remediating agents [11]. Considering the toxicity of heavy metals, there is therefore a need to treat industrial effluents before discharging into the waterways with a cost effective and environmental friendly approach like the use of biosorbents (such as the microalgae). The aim of this study was to determine the growth efficiency and the bioremoval capacity of the green microalga, Botryococcus sp. in industrial wastewater contaminated with heavy metals.

2 MATERIALS AND METHODS

2.1 Industrial Wastewater

Industrial wastewater was obtained from an industry in Batu Pahat, (1º 51’ 0” North, 102º 56’ 0” East) Johor, Malaysia in acid washed sample bottles and was transferred to the laboratory. Immediately upon arrival in the laboratory, the water quality parameters of raw wastewater were measured (BOD, COD, Temp, pH, DO, Conductivity, TN, TP, TOC, TDS, TSS and heavy metals) following the standard method for water and wastewater [12]. The wastewater was kept in a cold room (4ºC) before the commencement of the experiment. Prior to the experiment, the wastewater was filtered first with 0.45μm membrane filter (Whatman) and then diluted with distilled water at the desired concentrations (25%, 50% and 100%).

2.2 Preparation of Botryococcus sp. inoculum

The Botryococcus sp. isolates was obtained from microbiology laboratory of the Faculty of Science, Technology and Human Development, University Tun Hussein Onn Malaysia. Prior to the experiment, the isolate was cultured for 10 days in 1 litre Bold's basal media [13,14]. Algal cells were harvested with centrifugation set at 5000 rpm for 10 minutes followed by the determination of cell concentration. The cells of Botryococcus were counted using the Neubaeur haemocytometer with the aid of a compound microscope.

2.3 Experimental Setup

A total of 15 Erlenmeyer flasks (500ml capacity) filled with 125ml of distilled water, growth media and wastewater diluted at different concentrations were used for this experiment. The growth media was used as the positive control and distilled water was used as the negative control. Three treatments corresponding to different concentrations of wastewater, 25% (25% wastewater and 75% distilled water), 50% (50%wastewater and 50% distilled water) and 100% (pure wastewater). All treatments were replicated three times. The treatment flasks were inoculated with the same initial microalgal cell concentrations (1000 cells/ml) at the start of the experiment and were covered with sterile cotton plugs and kept at room temperature all throughout the experimental period. Microalgal sample collection for growth monitoring started at day one and daily until day 12. Collections of samples were done under sterile condition. Daily growth rate of the microalga was monitored by counting the density under compound microscope using haemocytometer.

2.4 Data Analysis

2.4.1 Specific Growth Rate (μ/day)

The specific growth rate (μ/day) of the microalga was calculated using the following equation [42].

$$SGR(\mu / day) = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

Where;
X_2 = final algal concentration
X_1 = initial algal concentration
t_2 = final time (day 4)
t_1 = initial time (day 0)

2.4.2 Statistical Analysis

The statistical analysis using ANOVA with Turkey's-b test were performed on the bioassay data to determine significant differences between treatments at $p<0.05$. All analysis was done using Statistical Package for Social Sciences version 20.0.

3. RESULTS AND DISCUSSION

3.1 Wastewater Parameters

There are established standards for effluents before discharging into the water bodies [15]. These standards are to be met in the level of effluents that are released and are ready to be discharged to the environment which is the responsibility of either the company or the treatment plants. In this study the concentrations of heavy metals arsenic (21.99 mg/L) and cadmium (0.081 mg/L) were found to be above permissible limits for water effluent standard (Table 1). Similarly, two of the most important water parameters which are the Chemical oxygen demand (COD) and Biochemical oxygen demand (BOD) at the concentration of 3847 mg/L and 167.16 mg/L, respectively were found to be above the permissible limits (Table 1). In addition, total suspended solids (240 mg/L) were also found to be above permissible limits (Table1).

The results obtained from the analysis of the raw wastewater in this study were a clear indication and in fact evident that wastewater coming from the industry has to be first treated before releasing into the environment. In particular, the heavy metals arsenic and cadmium which were found present in the wastewater could post harm to the receiving ecosystem due to their toxic effect. Heavy metal pollution in the aquatic biota has severe effects that threaten all the living organisms in the receiving water bodies [16,17,18]. Specifically, their persistence in the environment which is as a result of non-biodegradable nature and their bioaccumulation potential in the food chain make heavy metals very dangerous [19,20,21]. According to Rani et al (2010), toxic metallic elements such as cadmium, chromium and copper are exceptionally toxic to biological and ecological activities.) [22]. Heavy metals are considered the most important form of pollution particularly the arsenic and cadmium which are exceptionally toxic to humans [23]. It is said that heavy metal bioaccumulation is dependent on the type of metal, however, all heavy metals are known to disseminate throughout the trophic level of a specific ecosystem [24]. Heavy metals are regarded pervasive, highly soluble and are therefore promptly taken in by aquatic life [25]. According to Mansour and Sidky (2002), in the aquatic food chain, fishes are most times at the top and they can accumulate significant concentrations of these heavy metals in their tissues [26]. Considering the regularity of fish in human diet can bring about exposure which may have detrimental effect as heavy metals accumulate in the system [27,23].

Table 1: Characteristics of raw wastewater

Effluent Parameter	Value present in wastewater	Standard for effluent parameter (Environmental Quality Act, 1974)	
		A	B
Dissolved Oxygen (DO)	3.98 mg/L	-	-
Temperature	18oC	40oC	40oC
Conductivity	51.2 Ms/cm	-	-
pH	9.94	6.90-9.0	5.5-9.0
Total Phosphorus (PO43-)	10.20 mg/L	-	-
Total Nitrogen (TN)	655.3 ppm	-	-
Total Organic Carbon (TOC)	210.5 ppm	-	-
Chemical Oxygen Demand (COD)	3847 mg/L	50mg/L	100mg/L
Biochemical Oxygen Demand (BOD5)	167.16 mg/L	20mg/L	50mg/L
Turbidity	46 NTU	-	-
Total Dissolved Solids (TDS)	31, 550 mg/L	-	-
Total Suspended Solids (TSs)	240 mg/L	50mg/L	100mg/L
Total Solids (TSS)	31, 790 mg/L	-	-
Inorganic Carbon	1.503 ppm	-	-
Total Carbon	212.0 ppm	-	-
Chromium	0.018 mg/L	0.05mg/L (Chromium Hexavalent) 0.02mg/L (Chromium Trivalent)	0.05mg/L (Chromium Hexavalent) 1.0 mg/L (Chromium Trivalent)
Cadmium	0.081 mg/L	0.01 mg/L	0.02 mg/L
Copper	0.099 mg/L	0.20 mg/L	1.0 mg/L
Arsenic	21.99 mg/l	0.05 mg/L	0.10 mg/L

3.2 Microalgal Ggrowth in Wastewater

The growth pattern of the microalga in wastewater and in the control was the same which peak on the 4th day (Figure 1). The growth rate of Botryococcus sp. was found highest in the positive control (BBM), with an average density of 7.8 × 106 cells/ml whereas, the lowest growth rate was found in treatment 2 (50% wastewater) with an average cell density of 4.8 × 104 cells/ml. Generally, since the nutrient required by the algal cell for normal growth are provided in the growth media, the microalga growth rate is expected to be highest in comparison to wastewater medium [28]. Although the growth rate of Botryococcus sp in wastewater treatment were lower, the fact that pattern of growth was similar with that of the control, suggest that this microalga was able to utilize both the nutrients and heavy metals present in the wastewater. This result proved the potential of this microalgal species in bioremediation.

The specific growth rate (SGR) of microalgae ranged from 1.20 µ/day - 1.43 µ/day being highest ($p<0.05$) in BBM, while lowest ($p<0.05$) in 50% concentration (Figure 2). The high SGR in BBM was expected as this media is supposed to provide the necessary nutrients for the normal growth of microalgal cell. On the other hand, the SGR of Botryococcus sp. was found to be most affected by the 50% wastewater concentrations as show in the lowest SGR value (Figure 2). In fact, the SGR was even higher ($p<0.05$) in pure wastewater as compared to 50% wastewater. Perhaps in this case, higher nutrient present in the undiluted wastewater provided an advantage over those that are diluted. Higher nutrient concentrations were readily available for microalgal utilization, hence rapid growth which peaked on the 4th day of culture was observed in this study. According to De la Noue et al (1992), wastewater is regarded as a potential and sustainable media of growth for algal feedstock [29]. Many microalgae, specifically Botryococcus braunii has been effectively grown in municipal sewage wastewater, agricultural wastewater and industrial wastewater [30,31,32]. Moreover, microalgae have also shown high adaptability to a variety of environmental conditions such as industrial and domestic effluent dumping site [11] which probably explained their ability to grow even in the medium containing toxic heavy metals.

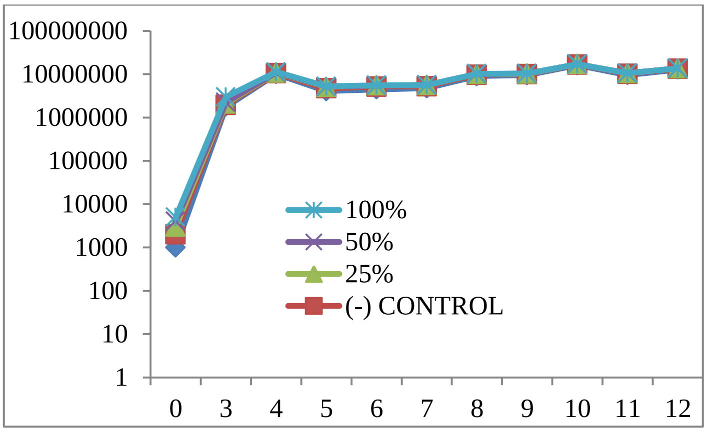

Figure 1: Daily growth rate (x 103) cells/ml of Botryococcus sp. in different concentration of the wastewater (25%, 50% and 100%), distilled water and BBM.

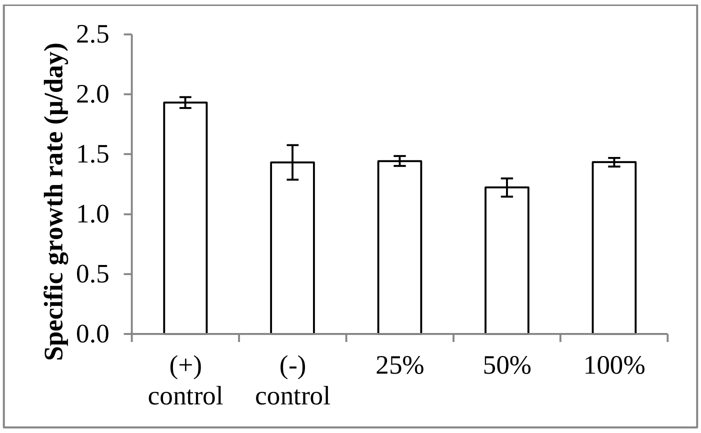

Figure 2: Specific growth rate (μ/day)

3.3 Heavy Metal Bioremoval

The heavy metal concentration was determined before and after the growth study of Botryococcus sp. in the industrial wastewater containing heavy metals to observe the reduction in the concentration of the elements. Four different heavy metals were studied in the course of these experiments which include cadmium, copper, arsenic and chromium (Figure 3). Botryococcus sp. efficiently reduced chromium from 0.018 to 0.001 which is equivalent to 94% and copper from 0.099 to 0.054 equal to 45% (Figure 3). Whereas, arsenic and cadmium were reduced to less than 10% (Figure 3). According to Jordanova et al (1999) and Bajguz (2000), different species of microalgae differ in their heavy metal accumulation potential in as much as many parameters have roles to play in the bioaccumulation process [33,34]. The use of active metabolic Botryococcus braunii in the removal of copper from solution has been reported by Areco et al (2013) [35]. Similarly, utilization of Botryococcus as an effective biological agent in the reduction of heavy metals in industrial wastewater has been reported earlier [32,36].

The application of biological system in the treatment of wastewater containing heavy metals is regarded as pertinent, cost effective and an efficient approach [37]. In particular, the use of microalgae in the removal of contaminants from industrial wastewater can go a long way in helping to lessen pollutant load in the environment [38]. The bioaccumulation potential of microalgae can be explained by their resistance mechanism against heavy metal toxicity [11]. According to Mehta and Gaur (2005), the most common and widely used resistance mechanism by microalgae to counter the toxic effects of heavy metals even at the dominance of ion exchange is the complexation and microprecipitation of the metallic ions [6]. Other known mechanisms include the regulation of metal concentration within the cells by the development of energy-driven flux pump, enzymatic and intracellular conversion of toxic metal to a non or less toxic form [6].

Although it has been recognised for many years that microalgae are indeed capable of absorbing metals from the environment, and in fact, microalgae are known to play a major role in controlling metal concentration in lakes and oceans [39,40]. However, it has to be understood as well that the tolerance of microalgae to heavy metal toxicity depend greatly on their defence responses [41] and that different species of microalgae have different bioaccumulation capacity against different heavy metal species.

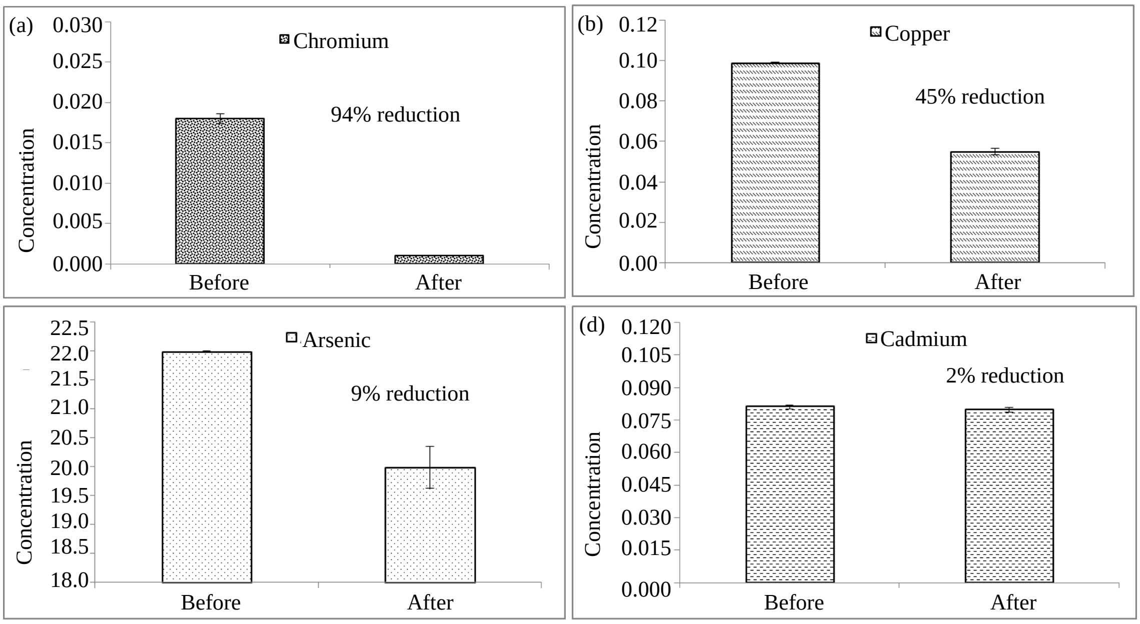

Figure 3: Heavy metal (a) Chromium, (b) Copper, (c) Arsenic and (d) Cadmium concentration before and after the treatment with Botryococcus sp

4. CONCLUSION

This study demonstrated the applicability of a freshwater microalga, Botryococcus sp. as a potential bioremediator of industrial wastewater contaminated with heavy metals. Botryococcus sp grown in different concentrations of wastewater showed similar growth pattern with those grown in synthetic growth media BBM. In addition, Botryococcus sp. significantly reduced the concentration of heavy metal chromium to up to 94% and 45% of the heavy metal copper.

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