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BIOREMOVAL OF LEAD IN INDUSTRIAL WASTEWATER BY MICROALGAE

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Abstract

The removal of heavy metals from our environment especially wastewater is now shifting from the use of conventional removal method such as chemical precipitation, coagulation and membrane filtration to the use of bioremoval method. The presence of heavy metals in the environment is of major concern because of their toxicity, bioaccumulating tendency, and threat to human life and the environment. In recent years, many low cost sorbents such as microalgae, fungi bacteria and lignocellulosic agricultural by-products have been investigated for their biosorption capacity towards heavy metals. In this project, the focus is on bioremoval of heavy metals in wastewater using marine microalgae. The study will be emphasize on the efficiency of two marine microalgae named *Nannochloropsis oculata* and *Tetraselmis chuii* in treating the Lead (Pb) content in industrial wasterwater. An experiment on the effect of various Pb concentration (10/20/40/60/80/100mg/L) towards the microalgae has been studied. The obtained result showed that the content of chlorophyll-A in the microalgae sample, after 7 days of exposures to Pb, decreased as the Pb concentration increased. Besides that, *Tetraselmis chuii* was found to be more sensitive compared to *Nannochloropsis oculata* where both were able to tolerate the Pb concentration of up to only 20mg/L and 60mg/L, respectively.

Keywords: Microalgae, Wastewater, Heavy metals, Bioremoval, Chemisorption.

1. Introduction

Rapid industrialization causes constant release of contaminants into wastewater which cause serious harm to human health. Among all water contaminations, heavy metal ions have high toxic and nonbiodegradable properties which may cause severe health problems in animals and human beings [1]. Some of the heavy metals, such as copper (Cu), nickel(Ni), and zinc(Zn) at very low concentrations essential for life (also termed microelements or trace elements) because they play important roles in metabolic processes taking place in living cells [2].

However, elevated levels of these metal ions are toxic to most prokaryotic and eukaryotic organisms. Other heavy metals such as cadmium (Cd), lead (Pb), and mercury(Hg) are nonessential and are known to cause severe damage in organisms even at very low concentrations. Pb is a heavy metal element which forms complexes with oxo-groups in enzymes to affect virtually all steps in the process of heamoglobin synthesis and porphyrin metabolism [3]. Toxic levels of Pb in man have been associated with encephalopathy, seizures and mental retardation [4]. Wastewater from many industries, including chemical manufacturing, battery manufacturing industries, metallurgical, leather tanning, and mining, contain lead [5]. These wastewater with heavy metal ions are discharged into natural water directly, not only threat the aquatic organisms, but may be enriched by precipitation, adsorption, and harmed human health through the food chain.

Heavy metal ions could be eliminated by several traditional techniques, including chemical precipitation [6], reverse osmosis [7], electrochemical treatment techniques [8], ion exchange [9], membrane filtration [6], coagulation [7], extraction [10], irradiation [11], and adsorption [12]. Amongst all the treatments proposed, adsorption using sorbents is one of the most popular methods since proper design of the adsorption process will produce high-quality treated effluents. Adsorption is a well-known equilibrium separation process. It is now recognized as an effective, efficient and economic method for water decontamination applications and for separation purposes. The adsorbents may be of mineral, organic or biological origin. Several types of non-biological materials, such as activated carbons [13], clay minerals [14], chelating materials [15], and chitosan/natural zeolites [16] have been researched to adsorb metal ions from aqueous solutions. Although non-biological sorbents could remove heavy metal ions from wastewater, the high cost, low sorption capacities and efficiencies limits their application.

Recently, numerous approaches have been studied for the development of cheaper and more effective adsorbents from natural products. Biosorption is a physiochemical technique that uses biomass as adsorption medium. It involves the detoxification of hazardous substances instead of transferring them from one medium to another by means of microbes and plants. This process is characterized as less disruptive and can be often carried out on site, eliminating the need to transport the toxic, materials to treatment sites. Due to the high uptake capacity and very cost-effective source of the raw material, biosorption is a progression towards a perspective method. Various biomaterials have been examined for their biosorptive properties and different types of biomass have shown levels of metal uptake high enough to warrant further research. There were a number of studies being carried out using biosorbents such as algae [17], saw dust [18], sugar beet pulp [19], papermill sludges [20], corncob [12}, wheat straw [18], baker's yeast [21] and fungal biomass [17].

A special attention was focused on microalgae based sorbents, due to their abundant availability in the natural environment and are well adapted to a wide range habitats, for example fresh and seawater, domestic and industrial effluent, salt marshes, and constructed wetlands. They have a remarkable ability to take up and accumulate heavy metals from their surrounding environment [22 - 24].

Hence, adsorption on microalgae shows potential as one of the most efficient methods for the treatment and removal of heavy metals over other methods because of its simple design and can involve low investment in term of both initial cost and land requires. Besides, the increasing number of publications on adsorption of toxic compounds by various microalgae shows that there is a recent interest in the design of new adsorption system for heavy metal removal using this cost-effective green technology. Marine microalgae, the largest primary biomass, have been attracting attention as resources for new metabolites and biotechnologically useful genes especially for wastewater treatment. The current study will be conducted using two marine microalgae named *Nannochloropsis oculata* and *Tetraselmis chuii* to identify their efficiency as prospective biosorbent on Pb in industrial wastewater effluent. The most notable structural alteration in microalgae cells treated with heavy metals ions was chloroplasts which appeared to be the primary target of metal contamination. Therefore, in this study the content of chlorophyll-A was investigated as the function of various Pb concentrations.

2. Materials and Methods

2.1. Microalgae culture

Nannochloropsis oculata and *Tetraselmis chuii* obtained from Malacca straits were cultured in F2 medium which is a common and widely used general enriched seawater medium designed for growing coastal marine algae, especially diatoms. The microalgae from exponential phase were introduced to the standard culture media. These microalgae cultures were incubated at 24 ±2oC under continuous illumination and regularly subculture until used. The microalgae sample that will be introduced to Pb solution for biosorption was collected from the exponential phase of growth (9-12days).

2.2. Heavy metal sample preparation

Stock solution of the heavy metal was prepared by adding 100mg Pb(NO3)2 in 1L distilled water to obtain the final concentration of 100mg/L. From this stock solution, serial dilution was carried out to obtain the test concentrations of 0, 10, 20, 40, 60, 80 and 100mg/L where the final volume of each flask was 20 ml.

2.3. Measurement of microalgae growth

A known volume of microalgae culture was transferred into each flask containing Pb solution. The samples placed and maintained at light intensity of 30µE/m2s under room temperature for 7 days. The chlorophyll-A content in each sample were analysed by measured the absorbance at 688nm using a UV-Vis Spectrophometer (Perkin-Elmer Lambda 35, Waltham, US)

3. Results and Discussion

According to Vogel [24], absorbance above 0.1 at 688nm (chlrophyll-A content) shows the ability of a microalgae to survive in a medium up to the respective heavy metal concentration. The data in Fig. 1 illustrated that, *Nannochloropsis Oculata* and *Tetraselmis chuii* able to tolerate Pb concentration up to 60mg/L and 20mg/L, respectively (absorbance>0.1). Cellulose is a polymer constituted of linked monomeric glucose units b-1,4 the structure of which can provide a series of hydroxyl groups as potential binding sites for Pb uptake. Several published works on the use of green algae have already shown a high bioaccumulation of metals, the process being attributed to the displacement of hydrogen ions from the algal structure [25]. A similar behaviour should have occurred with *Tetraselmis chuii* and *Nannochloropsis Oculata* cells, through polysaccharides of similar chemical structures. Nevertheless, it is also possible that a series of proteins was also responsible for *Tetraselmis chuii* and *Nannochloropsis Oculata* to withstand in a high Pb concentration. These proteins are associated with structural polysaccharides in a unique network forming the cell wall. Their constituent amino acid composition provides additional binding sites for the uptake of heavy metals.

However, from Fig. 1 it's proves a strong inhibition of chlorophyll-A biosysnthesis in *Tetraselmis Chuii* compared to *Nannochloropsis Oculata*. A complete destruction of algal cell at concentration above 20 mg/l and 60 mg/l were found in *Tetraselmis Chuii* and *Nannochloropsis Oculata*, respectively. This means that the efficiency of the photosynthesis apparatus seemed to be less affected by Pb on *Nannochloropsis Oculata* and severely altered on *Tetraselmis Chuii*. The significant reduction in chlorophyll-A content in microalgae shows that the particular heavy metal concentration started to exhibit stimulatory effect to the microalgae growth.

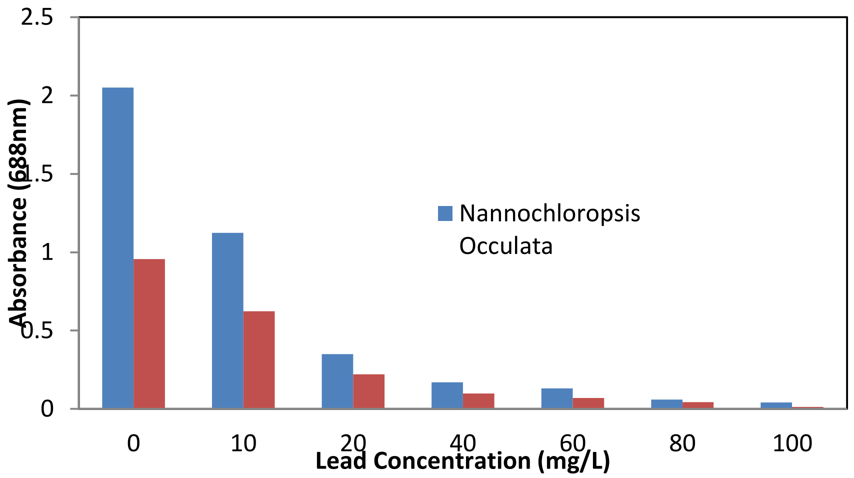

Fig. 1. The optical density at 688nm (chlorophyll-A content) of *Nannochloropsis Occulata* and *Tetraselmis Chuii* as a function of the initial lead concentration in the growth medium.

Besides, the graph configuration also clearly illustrated that the increase of Pb concentrations in medium causes the decrease of the chlorophyll-A content. The result is in parallel with the result reported by Shjarul [26] and Antonio [27] who studied the effect of Pb ion on the chlorophyll-A content of *Tetraselmis Chuii.* Wang and Dei [28] and Inthorn [29] reported that the addition of Pb ion caused the decrease of photosynthesis process and nutrient uptake. It can be seen that the decreasing chlorophyll-A pattern should be associated with a gradual saturation of the binding sites in the surface layers of the algae; for each initial Pb concentration, a solid/liquid equilibrium is obtained, and this equilibrium between the solid phase (*Tetraselmis chuii* and *Nannochloropsis Oculata*) and the liquid phase (Pb solutions) determines the amount of accumulated metal, if one considers that the bioaccumulation process is restricted to surface processes. However, when the most concentrated Pb solution was used, a significant decrease in the chlorophyll-A was observed. This decrease is probably associated with damage in the cell wall. The specific mode of action of Pb upon the microalgae certainly depends on the detoxification level promoted by the cells; that is, it depends on the amount of Pb accumulated by the cells. The literature reports that the bioaccumulation of heavy metals can cause pronounced modifications in the cell wall, changing cell morphology [30]. These modifications may affect the permeability of the cell wall, altering the transport of liquids in the cells and increasing the condensation of external structural components. Thomas [31] described the toxic effect of Pb on *Thalassiosira aestivalis* cells, reporting the formation of cell clumps owing to the presence of heavy metals, thereby indicating that Pb promoted marked structural changes as well as substantial effects on chlorophyll-A content.

4. Conclusions

The efficiency of two marine microalgae named *Nannochloropsis oculata* and *Tetraselmis chuii* in treating the Pb content in industrial wasterwater were indicated and explained based on their behaviour on chlorophyll-A content under controlled light and temperature conditions. *Tetraselmis chuii* was found to be more sensitive compared to *Nannochloropsis oculata* which proved by their capacity to tolerate the Pb concentration up to 20mg/L and 60mg/L, respectively. The response of *Nannochloropsis oculata* in this study shows its prospect to work as an effective biosorbents in wastewater treatment particularly for Pb removal.

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