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ADSORPTION OF Cd AND Pb USING BIOMASS OF MICROALGAE SPIRULINA PLATENSIS

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ABSTRACT: Microalgae is one of the most common sources of biomass for heavy metal removal from wastewater. *Spirulina platensis* is one of Indonesia's blue-green algae species found in Indonesian waters and has the ability to adsorb heavy metals, but very little is used for metal adsorption. As a cost-effectiveness approach, *S. platensis* was selected in this study which aimed to determine the effectiveness of Cd and Pb uptake by *S. platensis* for 10 days experiment, using Dutatonic solution as microalgae growth medium. The method used was complete random sampling with 3 concentration variations and 3 repetitions on each metal. The concentration variations for Cd were 0.1, 1, 5 mg/L, and 1, 2, 4 mg/L for Pb. The optimum adsorption concentration was tested by AAS. Biomass and cell size (length and width) were also measured to understand the physiological effects of Cd and Pb exposure on *S. platensis*. The results showed that Cd was more effectively adsorbed by *S. platensis* than Pb which reached 91.8% (day 5) at concentrations of 1 mg/L Cd and 84.3% (day 5) at concentrations of 1 mg/L Pb. The biomass and cell size of *S. platensis* were not influenced by the variation of Cd concentration ($p>0.05$), while Pb was significantly at the 5% significance level ($p <0.05$). It can be assumed that Pb was more toxic than Cd for *S. platensis* because the weight of biomass decreased in increasing Pb concentration.

Keywords: Biosorbent, Cadmium, Lead, Microalgae Spirulina platensis

1. INTRODUCTION

Industrial wastewater without pre-treatment has resulted in many heavy metals pollution problems in aquatic ecosystems [1], [2] including ground water [3] and ocean [4], [5] or even in soil [6]. This is the major issue in Indonesia was reported the serious effects to organisms [7], [8]. Heavy metals like cadmium and lead are very toxic even at low concentrations [9], [10]. Due to the toxic effects to organisms, cadmium and lead need to be removed before they accumulate in the environment and finally will pass into the human food chain which could result in health problems.

Biological agent, such as algae had been studied extensively as biosorbent due to their ubiquitous occurrence in nature [11]-[15] and high efficiency in removing metals through an ion-exchange mechanism [16]-[18] *Spirulina platensis* is one of microalgae belonging to *Cyanobacteria* has high adaptive ability because it is not affected by fluctuating environmental changes in physical and chemical parameters [19]. Many studies had been carried out using *S. platensis* on heavy metal removal [9], [20], [21]-[23]. However, none of them observed the effectsof heavy metals to density and cell size of microalgae biomass. Moreover, dead cells of *S. platensis* were mostly used in many studies [20], [21], [23], but less studies used living cells.

The study using local strain of *S. platens is* for heavy metal adsorption is also limited. Therefore, this study used living cells of *S. platensis* from local strain-INK to observe the adsorption ability of Cd and Pb in aqueous solution. The efficiency of Cd and Pb ions removal were also analyzed associated with biomass and size of cells.

2. MATERIALS AND METHODS

The algae species used in this study was *Spirulina plantesis* obtained from the Laboratory of Limnology, Cibinong LIPI Bogor. The microalgae culture was maintained for mass multiplication in modified commercial leaf fertilizer (Dutatonik H-16) and incubated at 25ºC in a growth chamber for 8h-16h and 3000-3500 lux light using fluorescent tube lamps.

Stock solutions of the heavy metals Cd and Pb were prepared, from which concentrations 0 (control), 0.1, 1, 5 mg/L, and 0 (control),1, 2, 4 mg/L were used in case of algae tolerance experiments, respectively. A density 500,000 cells/ml of *S. platensis* was inoculated and exposed to Cd and Pb, with each concentration then incubated for 5 and 10 days, in triplicate. Physical measurement was also observed including temperature, light intensity, humidity and pH of the media.

Each incubation period, day 5 and day 10, 25 ml of solution was stirred at 4000 rpm for 10 minutes, then the supernatant was measured using Atomic Absorption Spectrophotometer Perkin Elmer Analyst 700 to obtain the metal concentration in each solution treatments. The bioremoval efficiency of metals by *S. platensis* algae was calculated by the formula adapted from Clesceri et al.[24]:

$$S = \frac{(Ci-Ce)}{Ci} \times 100\% \quad (1)$$

where, S: biosorption efficiency (biosorption efficiency) (%); C_i: initial metal concentrations in aqueous solution (mg/L); C_e: equilibrium metal concentrations in aqueous solution (mg/L).

To understand the effect of Cd and Pb on *S. platensis* cells, biomass and size of cell were observed. Weight of biomass was counted every day for 10 days observation from 3 ml of solution. The solution was filtered using filter paper and dried in the oven 105°C for two hours. The biomass is the difference between the weight of filter paper with dried sample on it and the weight of filter paper. The cell size consists of length and width of cells which were counted using SEM (*Scanning Electron Microscopy*) with magnification of 10x40. All the data were analyzed by Pearson's Correlation using Minitab version 16 to determine correlation of each variable, including percentage of Cd and Pb adsorption, biomass, and cell size.

3. RESULTS AND DISCUSSION

In this study the biosorption of Cd and Pb from aqueous solution was examined using the local algal strain-NKI of Indonesia *Spirulina platensis* at different variation of initial concentration. The biosorption of Cd and Pb after 5 and 10 days of incubation period with *S. platensis* is given in Fig. 1 and Fig. 2.

Figure 1 shows that the highest Cd adsorption by *S. platensis* was 91.8% at concentration 1 mg/L of Cd, in day 5 of incubation (H5). It was supported with decreasing equilibrium concentration at each initial concentration. This rapid Cd adsorption for *S. platensis* was due to the abundant availability of active binding sites on microalgae such as –COOH, –OH and $-NH_2$ [25]. After day 5, cadmium adsorption tended to decline as saturation had already occurred.

The highest adsorption of Pb by *S. platensis* was 84.3% lower than that of Cd which also occurred in

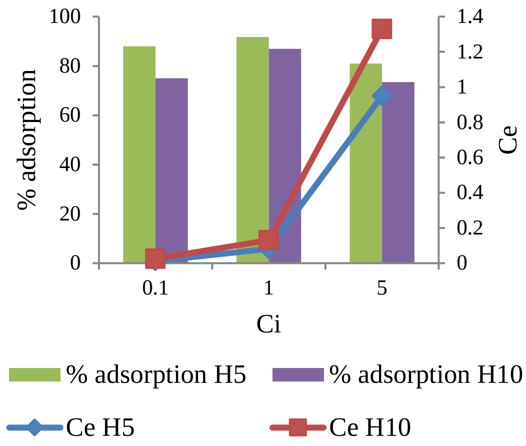

Fig.1 Percentage of adsorption and equilibrium concentration of Cd

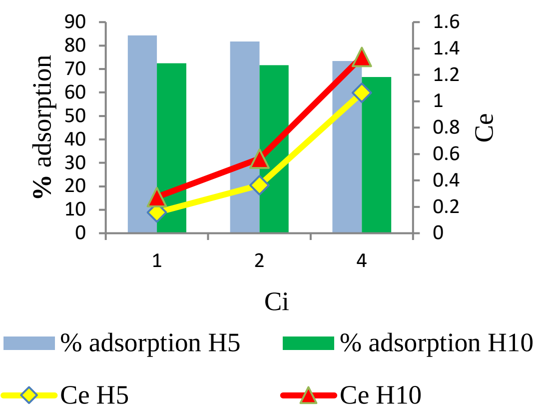

Fig.2 Percentage of adsorption and the equilibrium concentration of Pb

day 5 of incubation. This confirms that Cd ions bond more selective to *S. platensis* than Pb ions.This is related to the mechanism for intracellular metal detoxification in living organisms which is the formation of metal-binding peptides or proteins such as metallothioneins [26],[27]. Since *S. platensis* was exposed to Cd ions, they secrete proteins from intracellular or extracellular spaces to bind the toxic metals, so toxic effect is inhibited [26]. Although increasing Pb concentration, algae are not all died, in opposite some of algae cells are still alive and can still adsorp more ions. Whereas decreasing adsorption ability of metals at day 10 was due to toxicity effect of metals in *S. platensis*. It also could be caused by the large surface area and the presence of various active sites in the cell walls which is dependent on a number of parameters: pH [28], heavy metal type [28], algae type [29], [30] and concentration of biomassa [28]. The active sites binding was also confirmed in other bio-adsorbent such as banana peel Hossain.al [31], macroalgae [32] and mushroom [33].

The adsorption of metals are also related to biomass which provides more availability of active sites for binding of ions and this was proved by Huang and Lin [34], Putri et al. [35] and Soeprobowati & Hariyati [9]. The studies showed decreasing biomass at the longer incubation time, after rapid growth at first period of incubation. Figure 3 and 4 exhibit the trend ofbiomass in variety of incubation time.It shows that biomass increased until day 5 and 6, then decreased slightly with longer incubation time. Increasing initial concentration of the metals also affected the biomass decline after *S. platensis* was exposed to Pb but not in Cd exposure. Statistically, the percentage of Pb exposure to *S. platensis* was significantly correlated to biomass (p<0.01, coefficient correlation r = -.473), but not for Cd (p>0.05, coefficient correlation r = -.259).

This is related to toxic effect of metals [35]. It can be assumed that Pb ions were more toxic for *S.platensis* than Cd ions. However, the toxicity of metals could be different for other microalgae

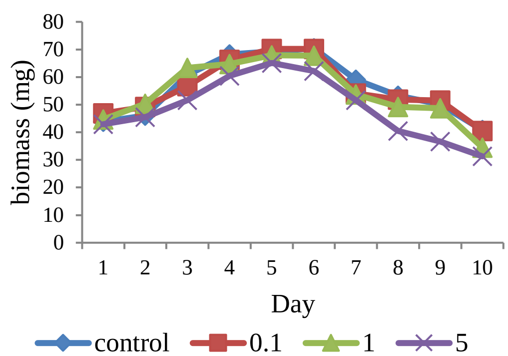

Fig.3 Biomass of *S. platensis* exposed to Cd

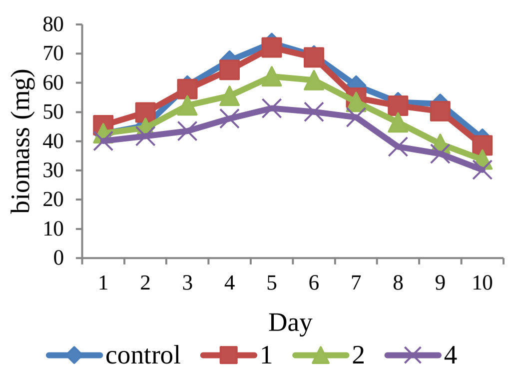

Fig.4 Biomass of *S. platensis* exposed to Pb

depending on the presence of various active sites inthe cell walls [25].

Biomass of *S. platensis* followed the exponential pattern which showed growth phase of cell including phase of lag, exponential, stationary and death [36]. The lag phase occurred at day 1 followed by exponential phase at day 2 to 5 when cells grew increasingly and biomass increased. After day 5 to 7, cells were in stationary phase marked by maximum density of cells. Death phase occurred from day 8 to 10 and cells decreased slightly.

The highest biomass was 70.2 mg at 0.1 mg/L of Cd concentration on day 5, whereas 72.1 mg of cells was obtained on day 5 at 1 mg/l of Pb concentration. It proves that biomass can also used to analyze metals adsorption by living cells such as microalgae. The living cells can adapt well on the treatment medium since availability of nutrients in medium was maintained. Nutrients [37] and rapid growth ability of living cells are the main advantage of biosorption technology.

On day 10, biomass decreased both in Cd and Pb exposures which also occurred in higher concentration of metal ions. This showed that the higher concentration of metal ion, the more toxic metal ions which further caused cell dead and poisoned. Only surviving cells were able to form colonies and continue in cell division.

Biosorption ability of *S. platensis* to adsorb metal ions was also supported by pH. During the 10 days of observation, pH of observed culture medium was fluctuated. The highest adsorption of Cd and Pb were in pH 8.8 and 8.79 respectively. Increasing pH value on observed medium was caused by the breakdown of proteins and other nitrogen compounds which was ammonium (NH_4^+) as a form of organic compounds which proceed decomposition [38]. This compound will accumulate and settle in the the bottom of culture medium which in further will poison cells and cells died. It also interferres the adsorption of dissolved oxygen and nutrients which is conducted by the cells are still alive [39].

However, alkaline pH is not suitable for the adsorption of Pbdue to precipitation of ions. This was supported by Al-Homaidan et al. [22] which reached up to 91% lead adsorption at acidic pH 3, while less adsorption of lead obtained in this study, only 84.3% at pH 8.79.

For Cd adsorption, it showed higher adsorption at alkaline pH 8.8. This result was the same as reported by Al-Homaidan et al. [21]. The control of pH in culture medium is very important to maintain the balance of cell growth of *S. platensis*. Temperature and light is able to accelerate the metabolism of *S. platensis* in absorbing metal ions,

[40]. In this study, the physical condition of culture room demonstrated suitable conditions for growth of *S. platensis*. The temperature was at 26.6°-27.9ºC, the humidity ranged 71-79%, and the light intensity of the culture was around 3008-3014 Lux.

Other parameter used in this study was cell size to analyze the adsorption effects of Cd and Pb in *S. platensis*. The cell size was differed from length and width of cells given in Fig. 5 and 6.

The length and width of *S. platensis* had similar trend both in Cd and Pb exposures which increased slightly at day 5 of incubation, then decreased at day 10. It exhibited the same pattern as growth of *S. platensis* which was exponential pattern. In this study, it was not found a significant correlation (at p>0.05, coefficient correlation r= -0.160 (Cd), coefficient correlation r = -0.150 (Pb)) between cell size and percentage of Cd and Pb adsorption to *S. platensis*.

Higher concentration of Cd and Pb were resulted in shorter length and width of cell and lower biomass.

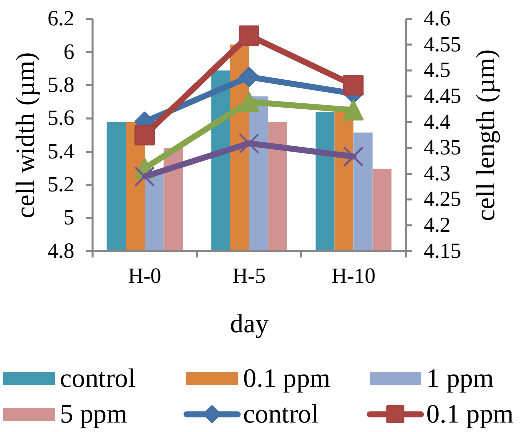

Fig.5 Cell size of *S. platensis* exposed to Cd

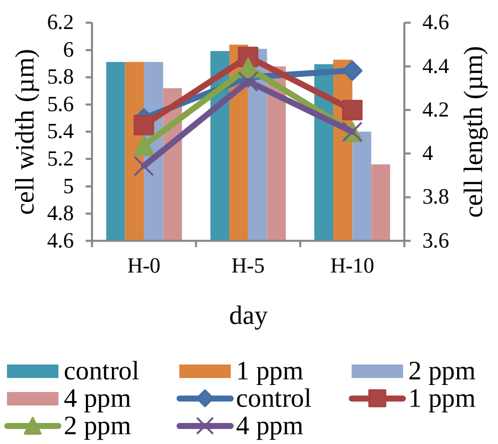

Fig. 6 Cell size of *S. platensis* exposed to Pb

This is presumably due to the excessive concentration of metals which can damage the chloroplast, since the chloroplast is the most sensitive part of cell to heavy metals [36]. Destruction of chloroplast implies loss of pigment and will disturb photosynthetic activity which occurred in thylakoid membranes [41]. Broken chloroplast will inhibit respiration process in cells so that the ability of cells to proliferate is reduced. This led to the increase in number of cells to be blocked. If protein in *S. platensis* cells is damaged then nutrient transport into the cells was inhibited, so that most of the *S. platensis* cells die [42].

4. CONCLUSION

Spirulina platensis, the local algae strain-INK was proved effectively remove Cd and Pb ions from aqueous solution and indicated a very good candidate as biosorption agent of heavy metals. The highest biosorption of Cd and Pb were 91.8% and 84.3% both at 1 mg/L of initial concentration on day 5, respectively. Only biomass of *S. platensis* can be considered as adsorption parameter of heavy metals removal in water or wastewater, since in this study biomass showed significant correlation with Pb ions adsorption (p<0.01), but not with Cd ions. It is related to toxicity of heavy metal of which Pb is more toxic than Cd.

Biomass is associated with heavy metal toxicity. Only cells exposed to metals with low toxicity can survive and then continue to divide, resulting in increased biomass, but do not affect the size of cells. The availability of nutrients is a factor has to be considered for maintaining cells growth which further affects cell size. This study proves that cell size was not correlated to Pb ions adsorption and to Cd as well (p>0.05).

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