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Phycoremediation of Pb^{+2}, Cd^{+2}, Cu^{+2}, and Cr^{+3} by *Spirulina platensis* (Gomont) Geitler

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Abstract: Phycoremediation is an application of algae for remediate environmental pollution by reducing or eliminating toxicity of such components in waste water. One of environmental problem in Indonesia is heavy metals pollution. Heavy metals are required by microalgae in a trace concentration, but in a high concentration heavy metals are toxic. *Spirulina platensis* is a microalgae belong to Cyanobacteria that has been used as a single cell protein, feed fish, and supplement. Many researchs on *S. platensis* had been done to optimize the product. Research on the use of *S. platensis* for remediation environmental pollution were also had been conducted. But, research on the use of local *S. platensis* to reduce the concentration of Pb^{2+}, Cd^{2+}, Cu^{2+} and Cr^{2+}, had not yet well developed. Therefore, this research was conducted in order to find out the effect of 1, 3, and 5 mg/L concentrations of Pb^{2+}, Cd^{2+}, Cu^{2+} and Cr^{2+} on the *S. platensis* population growth and its accumulation. A laboratory experiment was developed with those different concentrations with 3 replications and control. The initial concentration of heavy metals were measured as well as the 7 and 15 days treatment to determine the bioaccumulation of heavy metals by *S.platensis* . Based on this research, the concentration of 1 mg/L heavy metals may induced the population growth of *S. platensis,* due to improvement enzymatic process*,* the concentration of 3 mg/L heavy metals inhibit population growth, and the concentration of 5 mg/L heavy metals was toxic for *S. platensis.* Since *S. platensis* was found high tolerance to Pb^{2+}, Cd^{2+}, Cu^{2+} and Cr^{2+}at low concentration, it was recommended that for phycoremediation process using *S. platensis* more appropriate to be applied for low concentration contaminated water. It was suggested that *S. platensis* can be used as a metal absorbent and can be cultivated from wastewaters after phycoremediation process for other uses such as fertilizer, with the note that heavy metal contamination in waste water should not exceed 1 mg/L. *S. platensis* will be better if apply 15 days in contaminated wastewater to get the highest removal heavy metals.

Keywords: Phycoremediation, Heavy Metals, Pb^{2+}, Cd^{2+}, Cu^{2+} and Cr^{2+} *Spirulina platensis,* Bio Concentration Factor (BCF)

1. Introduction

Nowsday water quality and water pollution are major issues in Indonesian aquatic ecosystem. The pollutant enters to food chain and accumulated to the organisms. One of environmental problem is heavy metals pollution due to their serious effects to organism. Lead (Pb), Cadmium (Cd), Chromium (Cr) and Cooper (Cu) concentration were often exceeded the Indonesian Water Quality Standard for drinking water, agriculture and/or fisheries [[1,2]].

Heavy metals is a trace element with the density of ≥ 3 g/cm^3 that was required by organism in the low concentration, and will be toxic in a higher than physiological concentration [[3]]. Lead (Pb^{+2}) is a neurotoxic, that induce anemia due to the small red blood production. Pb^{+2} concentration in the blood was biomarker of a high Pb in the environment. The mobility Pb^{+2} in the soil and plant tends to slow, with the normal concentration of 0.5 – 3 ppm. The toxicity of Pb^{+2} was affected by pH, alkalinity, and dissolved oxygen[[3]].

Cadmium (Cd) is easier to accumulate by plant rather than Pb. Toxicity of Cd was influenced by pH, Zn, and Pb in the water [[4]]. Althouh the mobility of Cromium (Cr) in the plant was low, but Cr had as high toxicity to the plant. The toxicity of Cr was influenced by temperature, pH, and oxidation [[3]].

Copper (Cu^{+2}) was essential heavy metals for algae for plastocyanine development which has a function for electron transport on photosynthesis[[5]]. Cu was found as a superoxide dismutase enzyme which carried oxygen for hematocyanine pigment [3]. However, in the concentration of more than 0.1 ppm, Cu was toxic. Algacide contains of copper ($CuSO_4.5H_2O$) that was used to kill algae mass.

Soluble heavy metals can not be seperated physically. Physicochemical methods such as precipitation, oxidation or reduction, electrochemical treatment, evaporation, filtration, ion exchange or membrane technologies had been used to remove heavy metals ion from industrial waste water, but those methods were ineffective and expensive, especially, when the concentration of heavy metals were very high [6]. Phycoremediation is the process in which algae are used to clean up the environment from pollutant [7]. Soeprobowati and Hariyati (2013) tent to used the term of phycoremediation for the used of microalgae to remediate environmental pollution [8]. Biological methods such as biosorption or bioaccumulation promote an advantages to physico-chemical methods on reducing the contaminant from the industrial effluent [9]. The advantages of phycoremediation were easily found in a big amount, low operational cost, minimum sludge, and no need additional nutrition [9], simple and flexible in the application, and low maintenance [10]. As a developing technology, research on phycoremediation has to be developed to overcome complex environmental problems [11].

S. platensis is microalgae belong to Cyanobacteria. It has been reported there are about 2,000 species of Cyanobacteria that have wide distribution from freshwater to marine ecosystem. Cyanobacteria are easy to aquaculture. Currently, Cyanobacteria was cultivated in large scale systems related to its use for nutrient and consumer products. *Spirulina* reach optimum population in the day of 7 (11,698 x 10^3 unit/ml) [12]. Single cell cell protein from Cyanobacteria have many advantages over other organisms because its rapid growth and high content of macro minerals such as calcium, sodium, potassium; micro mineral of iron; and nutrient such as protein (58.5%), ash (12%), carbohydrate (7.5%), lipid (7%) and crude fiber (0.95%) [13].

On the other side, there were also many researchs on the use of Cyanobacteria to remediate environmental pollution. *Spirulina maxima* accumulated Cd on different layers of cell wall. *Sargassum* was used on the continuous process for metal uptake as a good immobilizator for *S. maxima.* Immobilization efficiency of *S. maxima* was 85% [14]. *Microcystis. aeruginosa* has a high affinity to remove Cd^{2+} (90%), Hg^{2+} (90%), and Pb^{2+} (80%), repectively. The presence of Cd^{2+} interfered only slightly with the uptake of Hg^{2+}, as Pb^{2+} and Hg^{2+} did with Cd^{2+}. In contrast, Hg^{2+} sorption was affected by Pb^{2+} to a great extent [15]. *Spirulina* was efficient biosorbant, the process equilibrium was reach 5-10 min with the maximum contribution of physical adsorption was 3.7%. The process of biosorption by *Spirulina* was strongly affected by pH [16]. Living *Oscillatoria* is more efficient than dead biomass in Cr^{6+} removal. Removal Cr^{6+} by *Oscillatoria* involves bioreduction and biosorption. The highest removal through biosorption for *Oscillatoria* was achieved between pH 5 and 5.9 and for dead biomass at pH 2. The maximum removal was on the tenth day of exposure. Cr^{6+} removal increased from 0.2 to 0.4 g/ L of culture biomass with a decrease with further increase in biomass. An increased of Cr^{6+}concentration decreases growth of *Oscillatoria* over time[17].

Basically, heavy metals enter to the Cyanobacterial cell by active uptake (biosorption) or passive uptake (bioaccumulation). Biosorption is the ability of organism to bound toxic heavy metals on their cell wall [6]. Bioaccumulation is an increase of heavy metals concentration in the cell body from air, water, contaminated food and chemically elimination process through respiratory exchange, fecal egesting, chemical parent substance biotransformation, and increasing of tissue volume [18].

Biosorption and bioaccumulation can be applied to reduce heavy metal concentration from effluent. *Spirulina* capable to bound heavy metals (in order from high) of ionic Cr^{+3}, Cu^{+2} and Cd^{+2}. In comparison to macroalgae, *Spirulina* had biosorption capacity much more higher than macroalgae [9]. The aims of this research was to study the population growth of *S. platensis* for 15 days treatment of 1, 3, 5 mg/L Pb, Cd, Cr, and Cu and its bioaccumulation.

2. Material and Methods

S. platensis stock was collected from Main Center Brackish Water Aquaculture Development, Jepara - Indonesia. All equipments had been sterilized with chlorox 150 ppm for 1 hour and dilute with boiled water. 1 liter sea water with a salinity of 15 ppt that enriched with Walne fertilizer was used as a culture medium. During the treatments, pH, temperature, salinity, and light intensity were maintain to be stabile on 7-8, 28-32^oC, 32-34 ppt, and 4,200 lux, respectively.

S. platensis requires trace heavy metals concentration, however, there were many industries that discharged their wastes in a high concentration above the waste standard criteria. Indonesia Government Regulation by Ministry of Environment Criteria for industrial waste stated maximum concentration of Pb, Cd, Cu, and Cr were 1, 0.1, 2 mg/L. The Regulation Number 5, year 2012 stated that the maximum concentration of Pb, Cd, Cu, and Cr of liquid waste from metal plating in Central Java, Indonesia were 0.1, 0.05, 0.6, and 0.5 mg/L, respectively. Belokobylsky (2004) used Cr^{+3} concentration of 3 mg/L to determine the accumulation on the *S. platensis* [19].

The trace elements of Pb, Cd, Cu, and Cr were added to the culture media in the form of $Pb(NO_3)_2$, $3CdSO_4.8H_2O$, $CuCl_2.2H_2O$, and $CrCl_3.6H_2O$. The 1, 3, and 5 mg concentrations of Pb^{+2}, Cd^{+2}, Cu^{+2}, and Cr^{+3} were exposed to the *S. platensis* culture, respectively. These concentrations were arranged based on preliminary research [20]. The concentrations of Pb, Cd, Cu and Cr were measured at the initial day, day of 7 and day of 15. The initial concentrations were heavy metal concentrations in the sea waters which were added with treatment concentrations. These initial concentrations had used for the following calculation, and mention as 1, 3, or 5 mg/L of heavy metals treatment. Algae cultured in the Walne medium without heavy metals addition was served as a control. All experiment was performed in triplicates. The heavy metals concentrations in media culture and in the *S. platensis* were measure with AAS.

A reduction of heavy metals (percentage of removal) was calculated as well as *S. platensis* population. BioConcentration Factor (BCF) was calculated to determine the accumulation of heavy metals in the *S. platensis*. BCF is a comparison between heavy metal concentrations on the *S. platensis* with the concentration on the aqueous environment [21].

$$BCF = C_{org} / C_{medium} \qquad [2.1]$$

C_{org} was heavy metals concentration in *S. platensis*

C_{media} was heavy metals concentration in the culture media

3. Result and Discussion

S.platensis was grown under Walne media that treat with Pb^{+2}, Cd^{+2}, Cu^{+2}, and Cr^{+3} with different respons. Based on this research Cr^{+3} and Pb^{+2} had inhibit population growth of *S. platensis* (Figure 1). Under the treatment of Pb^{2+}, there was a similar population trend on the day 1 – 6. The concentration 1, 3, 5 mg/L Pb^{2+} had reduced the population growth of *S. platensis*. However, on the day 8 – 10, under the concentration of 3 and 5 mg/L Pb^{2+}, there was a sharp increase of *S. platensis* over the control. This trend was similar to *Chaetoceros calcitrans* under the same treatment, that was in the first week, the population below control [22].

In a low concentration of heavy metal in the medium the population growth increases. Based on their research, Arunakumara et al (2008) found that the growth rate of *S. platensis* was influenced by Pb^{2+} concentration, at low concentration Pb could stimulate its growth. *S. platensis* tolerated to a high Pb^{2+} concentration, therefore able to remediate contaminated waters[23].

Soeprobowati and Hariyati (2013) had shown that the population growth of *Porphyridium cruentum* was high in the concentration of 1 mg/L Pb^{2+}, Cd^{2+}, and Cu^{2+}. In the concentration of 3 mg/L Pb^{2+}, Cd^{2+}, and Cu^{2+}. had reduced the population and in the concentration of 5 mg/L had limited the population growth related to the toxicity of those heavy metals [3].

There was an evidence from this research that Cd^{2+} had toxicity higher than other heavy metals, indicated by low population growth during 15 days treatments. The population growth of *S. platensis* inhibition increased at higher Cd^{2+} concentration. Slight inhibition population growth occurred at low Cd^{2+} concentration (Figure 1). At the concentration 1 mg/L the competitive inhibitation may take place between cadmium and sodium (from Walne medium), however, on the concentration 3 and 5 mg/L Cd^{2+} were more dominant than sodium leading to reducing growth rate. This was similar to the research conducted by Kiran et al (2012), that found at the concentration of 10 mg/L Cd^{2+} is highly toxic for the growth of *S. indica*. At the concentration 1 mg/L, rapid biosorption of *S. indica* was 74% cadmium adsorbed in 6 minutes, and 93.9% cadmium adsorbed within 24 hours. Bioaccumulation of cadmium follows monod kinetics with competitive inhibition mechanism at low concentrations and the toxicity of Cd^{2+} at the concentration >3.21mg/L [24].

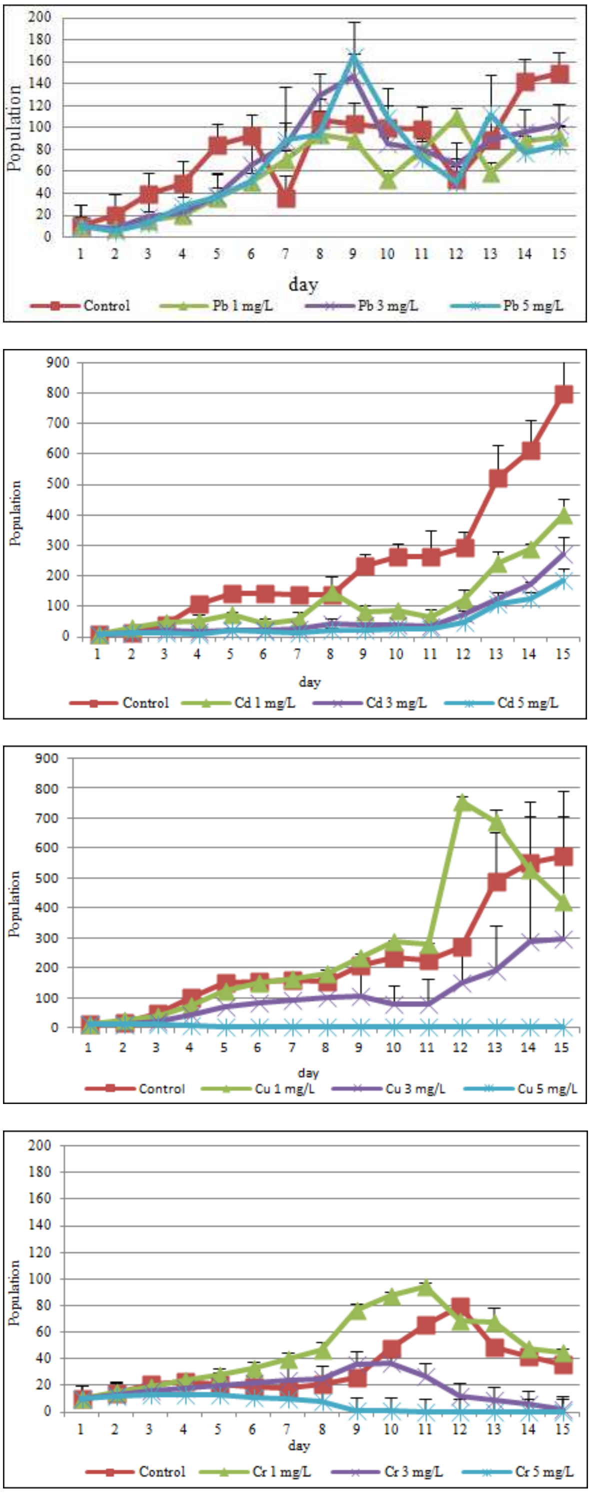

Figure 1. *Population growth (×1,000 ind/L) of S. platensis on the treatments of Pb, Cd, Cu and Cr 1, 3, and 5 mg/L*

Cu^{2+} and $^{Cr3}+$ ions showed different trend, that on the concentration of 1 mg/L Cu^{2+} and $^{Cr3+}$ induced population growth of *S. platensis* as shown in Figure 1, especially after 7 days. However, concentration of 3 and 5 mg/L Cu^{2+} reduced population growth of S. platensis population. This result was similar to the respond of *C. vulgaris* [22].

S. platensis rapidly accumulated heavy metals in the first phase of incubation and was slow down later. *S. platensis* still be alive at 1 mg/L but there was no growth when copper contamination was over than 1 mg/L [25]. Disyawongs (2002) used Zarrouk's media, and found that the optimum growth of *S. platensis* was 16 days without exhibiting a lag phase in 30 days. The accumulation of copper and lead by *S. platensis* had rapidly increased up to 3 days and rapidly decreased afterwards. But this research, used Walne's media and copper contamination of 1 mg/L, the population growth of *S. platensis* sharly increased until 11 day, and decreased afterwards as well. Metal ions affect the disruption of many physiological functions such as water uptake, respiration, mineral nutrient uptake and photosynthesis [26]. Cu is a micronutrient required by microalgae growth and plays an important role as an enzymatic cofactor and electron carrier in the photosynthetic and respiratory processes [27]. Cu inhibited photosynthetic electron transport which in turn decreased the assimilation of CO_2 by influencing the activities of photosynthetic carbon reduction cycle enzymes [28].

The population growth of *S. platensis* was very influence by chromium. At 1 mg/L concentration, Cr^{2+} induced population growth of *S. platensis*, but at higher concentration had reduced its population. This was related to the absorption and fluorescence emission of phycobiliproteins i.e., phycocyanin that was drastically decreased with increasing concentration of Cr^{2+}. A decrease in phycocyanin absorption and alteration in peak position due to alteration in apoprotein and chromophore interactions. In turn, this affect the electron transport multiple sites and alter the energy transfer [29]. Cr^{2+} suppressed the intensity of fluorescence emitted from phycocyanin at room temperature and induce blue shift in the emission peak suggestive of changes in energy transfer within the phycobilisomes.

In the day 7 and 14, the percentage of removal heavy metals from media culture of *S. platensis* was measured. From this research it was shown that there was a decrease on the heavy metals concentration in the media culture of *S. platensis*. The highest removal was on the concentration of 1 mg/L heavy metals. In 7 days, the removal of heavy metals was less than 50%, whereas in 15 days, the removal was more than 80% (Figure 2). This result was supported by Bio Concentration Factor (BCF) value that also high for concentration 1mg/L in 15 days (Figure 3). BCF was calculated as the ratio of the trace element concentration in the microalgae at harvest to the concentration of the element in the external environment [30]. For this laboratory experimental research, heavy metals accumulation in microalgae come up from water, that is why BCF was used to determine heavy metals accumulation in microalgae. Bioaccumulation was usually used to identify worse impact of the environment to organism [31]. Hyperaccumulator are plants (including microalgae) which have the potential to uptake and retain large quantities of metals into their cell quite suitable for phycoremediation process.

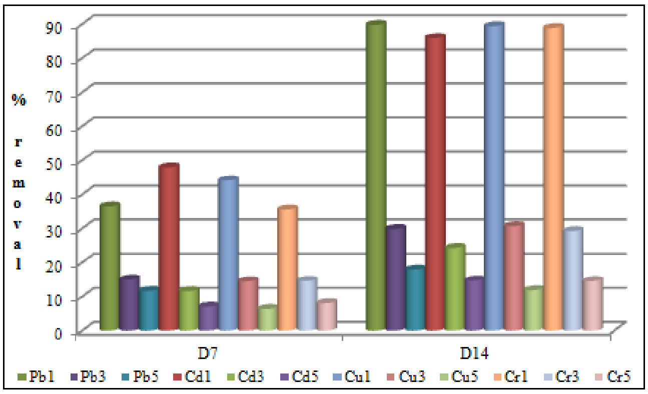

Figure 2. *Percentage heavy metals removal from media culture of S. platensis in day 7 and day 14*

A hyperaccumulator should be able to concentrate at least 1mg/g Ni, Co, Cr, Cu, Pb and 0.1 mg/g As and Cd in their cells [31]. As seen in Figure 3, *S. platensis* has high a BCF of Pb^{2+}, Cd^{2+}, Cu^{2+} and Cr^{3+}. In 7 days treatment with those heavy metals concentration of 1, 3 and 5 mg/L, the BCF was low. However, in 15 days treatment, the BCF were sharply increase, particularly in the 1 mg/l concentration was Pb > Cu > Cr > Cd. This means that for phycoremediation process, if the wastewater contain more than 1 mg/L it will be better if the wastewater was diluted first into 1 mg/L to gain high heavy metals removal.

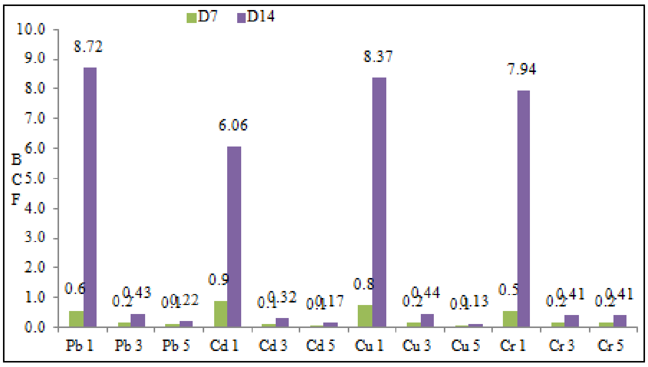

Figure 3. *Bio Concentration Factor of Pb, Cd, Cu, and Cr concentration 1, 3, 5 mg/L in day 7 and day 14*

For *Porphyridium cruentum,* the removal of heavy metals was highest on Cu^{2+} treatment (92% in the day of 15). On the 0.5 mg/L of Pb, Cd, Cu, and Cr treatments, *P. cruentum* had shown the highest reduction of Cu and Cd concentrations of 96% and 70%, respectively [22]. On the higher treatment concentrations on the further research, the concentration of 3 and 5 mg/L had reduced population growth and *P. cruentum* shown the highest reduction of Cu^{2+} concentration compare with others, therefore *P. cruentum* was good phycoremediator for Cu pollution [22]. Phycoremediation Pb by *C. calcitrans* was better when apply 14 days (92% reduction) rather than 9 days (48% reduction), related reduction of heavy metals in the culture medium that was accumulated in the cell of *C. calcitrans* [27].

There was 3 important finding from this research. First, Since *S. platensis* was found high tolerance to Pb^{2+}, Cd^{2+}, Cu^{2+} and Cr^{3+} at low concentration, it was recommended that for phycoremediation process using *S. platensis* more appropriate to be applied for low concentration contaminated water. Second, it was suggested that *S. platensis* can be used as a metal absorbent and can be cultivated in wastewaters for other uses such as fertilizer, with the note that heavy metal contamination in waste water should not exceed 1 mg/L. Third, *S. platensis* will be better if apply 15 days in contaminated wastewater to get the highest removal heavy metals.

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