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Bioaccumulation of Pb, Cd, Cu, and Cr by *Porphyridium cruentum* (S.F. Gray) Nägeli

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Abstract The red microalgae *Porphyridium cruentum* (S.F. Gray) Nägeli usually was used as feeds, a pigment for food and cosmetic, and antiviral activity that might be became industrial interest. Similar to another microalgae, *P. cruentum* has an ability to remediate heavy metals pollution, however research on it still limited. This research was conducted in order to find out the accumulation of Pb, Cd, Cr, and Cu on the *P. cruentum.* A laboratory experiment were developed with different concentrations. Based on this research, *P. cruentum* with the treatment of 1 mg/L had reduced higher Cu, Pb, Cd, and Cr concentrations rather than 3 and 5 mg/L concentrations, respectively. This was also similar to the BCF, that in day 8 in order of Cu > Cr > Cd > Pb, respectively; however, in day 15 was Cu > Pb > Cd > Cr. The length of treatment influenced BCF value. *P. cruentum* was good for bioremediation of heavy metal pollution, with the advantage of the short of accumulation time.

Keywords Bioaccumulation; Heavy metal; *Porphyridium cruentum*; Microalgae; Bioremediation; BCF

Introduction

The concentration of heavy metals in the environment tend to increase due to the industrial development. Heavy metals is a trace element with the density of ≥ 3 g/cm^3, which on the low concentration was required by organism, but toxic in the higher concentration for physiological organism (Banvalvi, 2011). One of the water pollution problem in Indonesia was heavy metals, particularly Lead (Pb), Cadmium (Cd), Chromium (Cr) and Cooper (Cu) that often exceeded the Water Quality Standard for drinking water, agriculture and/or fisheries (Soeprobowati et al., 2001; Soeprobowati et al., 2012). Heavy metals in the environment cannot be degraded and tent to accumulate in the organism. This heavy metals pollution can be solved by bioremediation technique.

Bioremediation is the clean up process of the environment biologically from polluted materials by organism that can be done *in-situ* or *ex-situ* of polluted sites (Crawford and Crawford, 2005). In the earlier development, bioremediation only applied microbes, furthermore, it is more wider application of organism to remediate freshwater, marine, even terrestrial ecosystems. Bioremediation offer effectiveness, low cost and low impact on ecosystem rather than physical and chemical remediation (Leung, 2004). Phytoremediation was more effective and efficient compare with bacteria-based remediation due to no need oxygen and less odor problem (Dwivedi, 2012). Microalgae have potential use to sink or to remove some toxic substances such as heavy metal by accumulate, adsorb or metabolize into substantial level (Priyadarshani et al., 2011). Phycoremediation (remediation that use microalgae) has advantages to remediate heavy metal since these microalgae can be used as fertilizer after remediation process (Riesing, 2006) or biofuels (Priyadarshani et al., 2011; Kumar et al., 2013); the low cost, simple and flexible in the application, and low maintenance (Emienour, 2012). The disadvantages of using microalgae for heavy metal remediation were require of energy for drying when using dead microalgae, need to be immobilized, and has limited application in the batch systems (Brinza et al., 2007).

Bioaccumulation is an absorption process of chemical compound from the environment by organism through respiratory surface and dietary uptake, and chemically elimination process through respiratory exchange, fecal egestion, chemical parent substance biotransformation, and increasing of tissue volume (Arnot and Gobas, 2006). Bioaccumulation can be used for environmental pollution monitoring since there was a correlation between bioaccumulation capacity with polluted environment or waste concentration. Both biosorption and bioaccumulation can be applied to reduce contaminant from the effluent (Chojnacka, 2009).

Microalgae are microscopic lower plants that have an important role in aquatic ecosystem as the largest primary producers and source of oxygen (Priyadarshani et al., 2011). Microalgae were good biosorption due to functional ion that able to bound ionic metal, especially carboxyl, hydroxylamine, sulphudrile immadazole, sulphate, and sulphonate that located on the cell wall (Volesky, 2007); easily found in a big amount, low operational cost, minimum sludge, and no need additional nutrition (Wang and Chen, 2009). However, microalgae has weaknesses due to a small size, low mass index, and easily degraded by microorganism.

Many researches had been conducted for the use of microalgae for environmental remediation, such as bioaccumulation of Cd by *Tetraselmis chuii* and *Spirulina maxima* (Costa and Franca, 2003); biosorption of Pb, Cd, Hg by *Microcystis aeruginosa* (Chen et al., 2005), biosorption Cd, Cr, Cu by *Spirulina* (Chojnacka et al., 2005); bioaccumulation of Pb and Cd by *Chladophora* (Lamai et al, 2005); biosorption of Cu by *Chlorella vulgaris* (Al-Rub et al., 2006); the application of *Chlorella vulgaris* to remediate textile wastewaters (Lim et al., 2010); bioremediation of Hg, Cd, Pb by *Dunaliella* (Imani et al., 2011); toxicity, transformation and accumulation arsenic in *Scenedesmus* (Bahar et al., 2012); Zn and Pb resistance of two ecotype *Eustigmatos* sp. (Trzeinska and Pawlik-Skowronska, 2012); Cr^{6+} bioremediation efficiency of *Oscillatoria* (Miranda et al.; 2012). However, researches on the use of *Porphyridium* for remediation were still limited. Preliminary study had shown, that *Porphyridium* had a potential to use in heavy metals bioremediation (Soeprobowati and Hariyati, 2012).

P. cruentum (S.F.Gray) Nägeli is the primitive micro red algae that can be found to live in variety habitats such as sea water, fresh water, or on the surface of moist soil that form a reddish layer, but prefer to live in a saline habitat. The red color of *P. cruentum* is coming from phycoerythrine pigment, its big chloroplast is surrounded by sulphate polysaccharide; single cell but able to form colonies (Arrad, 1992). *P. cruentum* had been used for antiviral (Huleihel et al., 2001). *P. cruentum* had also been used for nutrition source, particularly of polysaccharides, unsaturated fatty acids, carotenoids, and phycobiliproteins. The phycobiliproteins content were phycoerythrin, R-phycocyanin, and allophycocyanin that were affected by sodium bicarbonate (Velea et al., 2011).

P. cruentum consisted of proteins (28%~39%), polysaccharides (40%~57%) and lipids (9%~14%) subsumed into dry algal mass (Velea et al., 2011); phycobiliproteins, exopolysaccharides, long-chain polyunsaturated fatty acids, carotenoids (zeaxanthin, tocopherol, etc.) and vitamins (Wang et al., 2007; Huang and Chen, 2005). The biomass (w/w) contains of 32.1% available carbohydrates, and 34.1% crude protein. 100 g dried *P. cruentum* biomass contains of 4,960 mg Ca; 1,190 mg K; 1,130 mg Na; 629 mg Mg and 373 mg Zn. A short residence times in the bioreactor, the biomass were rich in protein and eicosapentaenoic acid (Fuentes et al., 2000).

P. cruentum was qualified for bioremediation due to the absence of toxic production, easily to be cultured, ability to grow in extremes of salinity, pH, and temperature, rapid grow in defined media, ability to achieve a high population, and easily of harvesting (Wilde et al., 1988). *P. cruentum* is promising for bioremediation of heavy metals since it provides double solution to overcome environmental pollution and energy alternative. *P. cruentum* had a higher tolerance for the agitation than *Phaeodactylum tricornutum*. The cell damage was related to the rupture of small gas bubbles at the surface of the culture. An increase of agitation rate had reduced the bubble size to produce damaging (Sobczuk et al., 2006).

1 Result and Discussion

P. cruentum tolerated to a high concentration of heavy metal, as seen in Figure 1. After a second peak population growth on day of nine, it seems that the concentration of 5 mg/L Pb had reduced population growth, meanwhile the lower concentration tent to increase its population. This trend was similar to Cd and Cu treatments. On the concentration of 1 mg/L Cu had induced *P. cruentum* population growth, however,

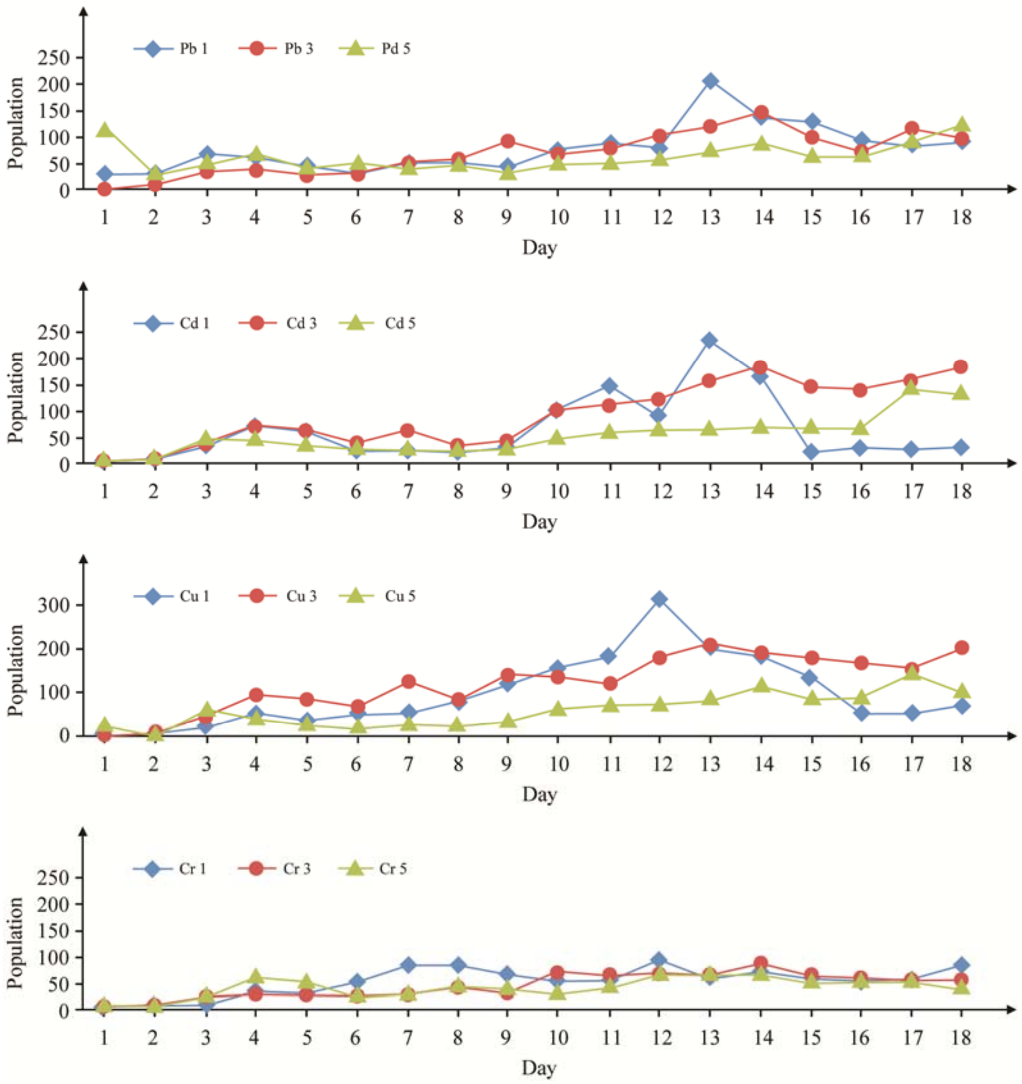

Figure 1 Population (×1 000 ind/L) of *Porphyridium cruentum* (S.F.Gray) Nägeli on the treatment of Pb, Cd, Cr, and Cu, on concentration 1, 3, and 5 mg/L

on the concentration of 3 and 5 mg/L had lowered *P. cruentum* populations (Figure 1). The concentrations of heavy metals in the sea water before treatments were 0.17 mg/L Pb, 0.01 mg/L Cd, 0.15 mg/L Cu, and 0.03 mg/L Cr. Therefore, the initial concentration of heavy metals was the concentration of treatments added with the sea water concentration.

On the preliminary research, the population growth of *P. cruentum* on the 0.5 mg/L concentration of Pb, Cd, Cu, and Cr were fluctuated, the peak population growth were on the day of 4, 10, and 13 (Soeprobowati and Hariyati, 2012). The life cycle of *Porphyrydium* tent to go a day forward in the higher heavy metal concentrations, but with the lower population.

Generally, the culture media of *P. cruentum* with Pb, Cd, Cu, and Cr 1 mg/L had the highest reduction of heavy metals than on the concentrations of 3 and 5 mg/L, respectively. For *P. cruentum,* the percentage of heavy metals concentration reduction was highest on Cu treatment (92% in the day of 15, Figure 2). On the 0.5 mg/L of Pb, Cd, Cu, and Cr treatments, *P. cruentum* culture had shown the highest reduction of Cu and Cd concentrations of 96% and 70%, respectively (Soeprobowati and Hariyati, 2012). On the higher treatment concentrations of this research, the concentration of 3 and 5 mg/L had reduced population growth, and *P. cruentum* shown the highest reduction of Cu concentration compare with others, therefore *P. cruentum* was good to remediate Cu pollution.

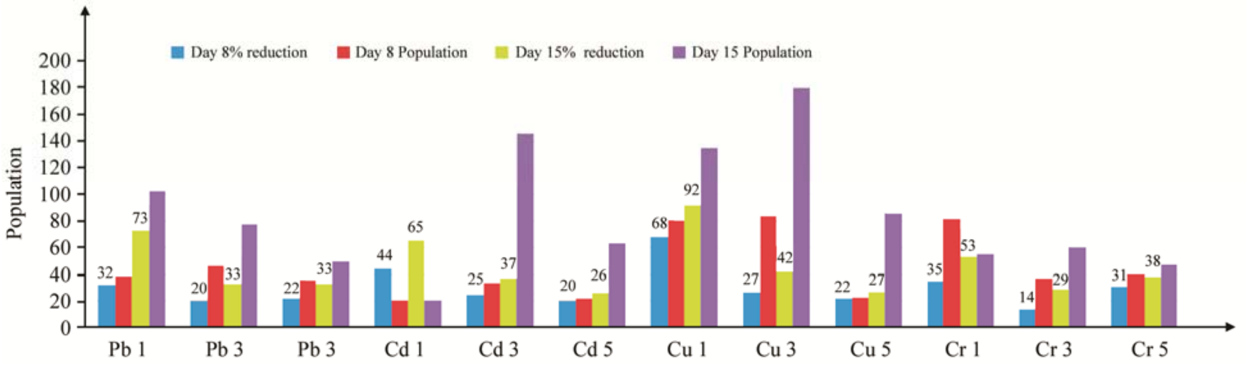

Figure 2 The percentage of Pb, Cd, Cu, and Cr reduction on the culture media of *Porphyridium cruentum* (S.F. Gray) Nägeli in day 8 and 15 treatment concentration 1, 3, 5 mg/L

Heavy metals toxicity can be study by BCF approach. The highest BCF occurred for heavy metals treatment on the concentrations of 1 mg/L (Figure 3). However, the length of treatment influenced BCF value. *P. cruentum* shown the higher toleration on Cu than Pb, Cd, and Cr. BCF of *P. cruentum* in day of 8 from high was in order of Cu > Cr > Cd > Pb, respectively; however, in day 15 was Cu > Pb > Cd > Cr.

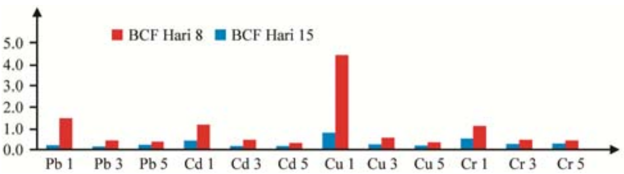

Figure 3 BCF (ppm) of Pb, Cd, Cr, Cu on the concentration 1, 3, and 5 mg/L

Heavy metals bioconcentration on the *P. cruentum* able to figure out the environmental impact of heavy metals. Based on the trend in Figure 3, it seems that Pb required longer time to accumulate, whereas Cu was more faster.

BCF had calculated as a homeostatically ratio of heavy metal concentration on the *P. cruentum* with heavy metal concentration of media. Microalgae had a protection mechanism against heavy metals by development of heavy metals complex with cellular protein without change its activity (Wang and Chen, 2009; Girard, 2010). On a high concentration, heavy metals had reduced the population or cell growth because *P. cruentum* can not counterbalance the heavy metals toxicity.

The mechanism of heavy metal entering to the cell was affected by the concentration difference, the negative charged of the surface cell wall and the positive charged metal ion on the microalgal medium. It was shown from this study that *P. cruentum* demonstrated successfully in the sorption and removal of heavy metals ion from the water, the highest affinity towards Cu which in the day of 8 and/or 15. A reduction of Cu concentration was higher following the time exposure, which accumulate in the cell wall. This was related to the Cu release rate that relatively lower than the Cu absorption. The heavy metals absorption occurred in 2 ways i.e. heavy metal ionic change with cell wall caption, or development covalent bound between heavy metals with active ionic of cell wall. *P. cruentum* cell wall consists of organic protein, polysaccharide, alginate acid and urinate acid which were able to bind with heavy metals (Wang and Chen, 2009).

Heavy metal accumulation will increase H^+ ion concentration. Therefore, an increase of pH media will increase H^+ ion production, which in turn will increase heavy metal absorption by *Porphyridium*. So, heavy metals bioremediation by *P. cruentum* will be optimum on the alkaline pH (7-8) condition.

Many researches had been done on the effect of heavy metals on the microalgae. A high concentration of Pb and Cd had decreased *Cladophora fracta* growth, due to induction of peroxides enzyme activity that had an important role on the indoneacetic acid (IAA) degradation. IAA was a hormone that stimulates the growth and vision of microalgae (Lamai, et al., 2005). *Chlamydomonas reinhardtii*, *Chlorella salina*, *Chlorella sorokiniana*, *Chlorella vulgaris*, *Chlorella miniata*, *Chlorococcum* sp., *Cyclotella cryptica*, *Lyngbya taylorii*, *Phaeodactylum tricornutum*, *Porphyridium* *purpureum*, *Scenedesmus abundans*, *Scenedesmus quadricauda*, *Scenedesmus subspicatus*, *Spirogyra* sp., *Spirulina platensis*, *Stichococcus bacillaris* and *Stigeoclonium tenue* were a good biosorbant heavy metal ion (Brinza et al., 2007). *Spirulina* sp was biosorbant of Cr^{3+}, Cd^{2+} and Cu^{2+} ions (Chojnacka et al., 2005).

Bioaccumulation is a process of chemical absorption by organisms from all routes in the environment, including from food and chemical elimination from organism through respiratory, fecal excretion, and metabolic biotransformation and growth emasculation (Arnot dan Gobas, 2006). Bioaccumulation can be used to identify the negative impact of environmental degradation to the organism (McGeer et al., 2003). Aquatic organism could accumulate chemical compound directly form the environment through skin and intestinal digestion surface, or indirectly from chemical compound accumulation from food (Ivanciuc et al. , 2006).

Bioaccumulation Factor (BAF) is the ratio of accumulated chemical compound in the organism and the concentration of chemical compound of the environment. There was a fundamental difference between BCF and BAF. BCF is the degree of absorption process of chemical compound by organism from environment through respiration or skin surface. That's why BCF was used to determine bioaccumulation under controlled laboratory experiment. BAF is similar to BCF, but with dietary chemical exposure, usually measured under field condition (Arnot and Gobas, 2006).

BCF had been used for fish, but it was possible to calculate BCF of microalgae. The concentration of heavy metals on the microalgae reflected the heavy metal concentration on its environment. Research that had been done in the Uganda's river was similar to the result of this laboratory experiment, that microalgae, particularly *P. cruentum* was a bioaccumulator of Cu > Pb > Cd (Sekabira et al., 2011).

The BCF value that greater than 1 ppm indicated heavy metal accumulator, however, it will be a good bioaccumulator when the BCF was greater than 1,000 ppm (Conti and Cecchetti, 2003). Based on the Conti and Cecchetti (2003) criteria, result of this research shown that *P. cruentum* was only in the category of bioaccumulator, rather than good bioaccumulator similar to the result of Sekabira et al (2011). However, it does not mean that *P. cruentum* do not good bioaccumulator, since Conti and Cecchetti (2003) developed BCF for fish, therefore it was difficult to gain the criteria of good bioaccumulator for microalgae due to the microscopic size that was very difficult to reach BCF of more than 1,000 ppm. However, the short of accumulation time was the advantages of *P. cruentum* as a good organism to remediate heavy metal pollution.

2 Materials and Methods

P. cruentum stock was collected from Main Center Brackish water Aquaculture Development, Jepara-Indonesia. All equipments had been sterilized to eliminate or minimize the presence of microorganisms or substances bullies on tools and cultivation media during the study. 1 liter sea water with a salinity of 28 ppt that enriched with Walne medium was used as a culture media. During the treatments, pH, temperature, salinity, and light intensity were maintained to be stabile on 7~8, 28℃ ~32℃, 32~34 ppt, and 4,200 lux, respectively.

Porphyridium requires trace heavy metals concentration; however, there were many industries that discharged their wastes in high concentration above the waste standard criteria. Indonesia Government Regulation of Ministry of Environment Criteria for industrial waste stated maximum concentration of Pb, Cd, and Cu were 1, 0.1, 2 mg/L. Costa and Franca (2003) used the concentration of 42.3±2.0 and 61.2±1.1 mg/L to determine the Cd uptake by *Tetraselmis chuii*. Belokobylsky (2004) used Cr concentration of 3 mg/L to determine the accumulation on the *Spirulina platensis*. Syahputra (2008) treated *Chlorella pyrenoidosa* with 3.29 mg/L Cu from metal plated industry.

The 1, 3, and 5 mg concentrations of Pb, Cd, Cu, and Cr were exposed to the *P. cruentum* culture, respectively. These concentrations were arranged based researches mentioned above, and on the preliminary study on the 0.5 mg/L that induced alga growth (Soeprobowati and Hariyati, 2012).

The trace elements were added to the culture media in the form of Pb (NO_3), 3Cd $SO_4.8H_2O$, $CuCl_2.2H_2O$, and $CrCl_3.6H_2O$. The initial concentration on the culture media was measured as well as on the day of 7 and 14. The initial concentrations were heavy metal concentrations in the sea waters added with treatment concentration. These initial concentrations had used for the following calculation, and mention as 1, 3, or 5 mg/L of heavy metals treatment. However, the initial heavy metals of the cell concentration do not measure. It was assume that there were no heavy metals concentration in the initial cell, caused of the stock had been collected from under control nutrients and environments condition. For further research, the measurement of initial cell concentration will provide more detail data that able to be compared.

Algae cultured in the Walne medium without heavy metals served as controls. All experiments were performed in triplicates. Every day the population was counted for 14 days. In the beginning, day of 8 and end of experiment the concentration of Pb, Cd, Cu, and Cr was measured with AAS.

A reduction of heavy metals was calculated as well as *P. cruentum* population. Bioconcentration Factor (BCF) was calculated to determine the accumulation of heavy metals in the *P. cruentum*. BCF is a comparison between chemical concentrations on the organism with the concentration on the environment (Ivanciuc et al., 2006).

$BCF = C_{org}/C_{media.}$

C_{org} was heavy metals concentration in *Porphyridium cruentum* (S.F. Gray) Nägeli

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

Author's Contribution

Authors worked together in the laboratory experiment. TRS was responsible on the *P. cruentum* population growth and RH for water quality control. TRS drafted the manuscript, and authors read approved the final manuscript.

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