Phycoremediation of Pb, Cd, Cu, and Cr by *Chaetoceros calcitrans* (Paulsen) Takano

Tri Retnaningsih Soeprobowati, and Riche Hariyati

Abstract— Bacillariphyte of *Chaetoceros calcitrans* (Paulsen) Takano usually was used as live feed in aquaculture due to protein, lipid, and vitamins contents that was required by larvae. The use of marine microalgae for remediation had already been reported. However, the use of *C. calcitrans* for Pb, Cd, Cu, and Cr has not yet well understood. Therefore, this research was conducted in order to find out the effect of different concentration of Pb, Cd, Cr, and Cu on the *C. calcitrans* population growth and its accumulation. A laboratory experiment were developed with different concentrations. Generally, on the concentration of 1mg/L heavy metals had the highest reduction of heavy metals on the culture medium *of C. calcitrans. C. calcitrans* was phycoremediator for Cu and Cr in day 8, but was Pb > Cu > Cr > Cd in day 15, respectively. Based on this research, the length of treatment influenced BCF value of *C. calcitrans.*

Keywords—Bioaccumulation, heavy metal, *Chaetoceros calcitrans*, microalgae, bioremediation, BCF

I. Introduction

PHYCOREMEDIATION is the clean up process of the polluted environment by microalgae [1]. Microalgae play an important role in controlling heavy metals concentration, since their functional ion that located on the cell wall, able to bound ionic metal [2]; sink or remove it by accumulate, adsorb or metabolize into substantial level [3]. The advantages of phycoremediation were easily found in a big amount, low operational cost, minimum sludge, and no need additional nutrition [4]; simple and flexible in the application, and low maintenance [5]. After remediation process, harvested microalgae can be used as fertiliser [6] or biofuels [3,7]. The disadvantages of using microalgae for heavy metal remediation were require of energy for drying when using dead microalgae, need to be immobilised, and has limited application in the batch systems [8]; have potential use to sink or to remove some toxic substances such as heavy metal by accumulate, adsorb or metabolize into substantial level [3]; its small size, low mass index, and easily degraded by microorganism [1].

Nowsday water quality and water pollution are major issues in aquatic ecosystem. The pollutant enter to food chain and accumulated to the organisms. One of environmental problem is heavy metals pollution due to their serious effect 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 [9], [10].

Chaetoceros calcitrans (Paulsen) Takano was marine planktonic microalgae of Bacillariophytes or diatom, has sizes of 6-8 µm [11]. *C. calcitrans* is abundant in Indonesia, easily culture with high growth rate. Usually *C. calcitrans* was mass culture for live feeds, however, since it was known that diatom's food storage was lipid, then many explorations had developed to proof that diatoms were one of potential source of fatty acids [12], [13]. There were evidences that diatoms have potential to remediate heavy metals. *Dunaliella* had ability in bioremediation of Hg, Cd, Pb [14]. However, research on the use of *C. calcitrans* for remediation were still limited. Preliminary study had shown, that *C. calcitrans* had a potencial to use in heavy metals bioremediation [15]. The optimum accumulation occured after 15 minutes at pH of 8 on the concentration of 1055.27 mg/g *C. calcitrans*.

II. Methods

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

The 1, 3, and 5 mg concentrations of Pb, Cd, Cu, and Cr were exposed to the *C. calcitrans* culture, respectively. These concentrations were arranged based on preleiminary researchs (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, day 7 and day 14 of concentration Pb, Cd, Cu, and Cr on the culture medium were measured. 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. Algae

Tri R. Soeprobowati is with Department Biology, Diponegoro University, Semarang, Indonesia (corresponding author's phone:62-2476480923; e-mail: trsoeprobowati@yahoo.co.id).

Riche Hariyati is with Department Biology, Diponegoro University, Semarang, Indonesia (e-mail:riche.hariyati@gmail.com).

cultured in the Walne medium without heavy metals served as controls. All experiment were performed in triplicates.

A reduction of heavy metals was calculated as well well as *C. calcitrans* population. BioConcentration Factor (BCF) was calculated to determine the accumulation of heavy metals in the *P. cruentum*. BCF is a comparison between chemical concentration on the organism with the concentration on the aqueous environment [16] .

$$BCF = C_{org} / C_{medium} \quad \text{......[16]}$$

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

C_{medium} was heavy metals concentration in the culture medium.

III. Result And Discussion

The population of *C. calcitrans* on the pre treatment shown that on the concentration of 0.5 mg/L of heavy metals, the first peak population had occured on the day 4, then reach second peak on day 9. The highest population had occured similar to the initial population, indicated that Pb, Cd, and Cu had reduced *C. calcitrans* population [15] .

C. calcitrans had fluctuated population growth on the heavy metals treatments. The peak population growth of *C. calcitrans* occured in day of 6 and sharply reduced in day of 8 and start to remain stable in the day of 9 with different population on the different heavy metals treatments (Fig 1). At the first 5 days, a high concentration of Pb had reduced the population growth of *C. calcitrans*. The population growth of *C. calcitrans* on the Pb 1 mg/L treatment was higher than on the concentration of 5 and 3 mg/L treatment, although its population was below the control. The first peak population growth was occured in day 3 (control and Pb 1), but for concentration of 3 and 5 mg/L the population were reduce sharply below control and Pb1. After reach peak in day 6, the population of *C. calcitrans* were remain stable but below the initial population. It seems that from the day of 8, the concentration of Pb in the culture medium were not as toxic as in the first week. This was indicated by the lowest population for control compare the others. A reduction of population might be corelated with limited nutrient.

In the first four days treatment of Cadmium (Cd) on the *C. calcitrans*, *it* shown that Cd was toxic for *C. calcitrans*, indicated by low population growth on the concentration of 1, 3, 5 mg/L, respectively, compare to the control. However, in the day of 8 and forward, the population of *C. calcitrans* control was below the Cu treatment. 1 ppm Cd on the culture medium of C. calcitrans had increased the growth rate, the number of cells, the dried weight and the chlorophyl-A content [17].

An interesting trend was shown on the Copper (Cu) treatment. In the 6 days treatment, the population of *C. calcitrans* was in order of control>1>5>3 mg/L Cu, respectively. In the days of 8-14, Cu had induced population growth (1>3>5>control) which was higher than initial population for the treatment of Cu 1 and 3 mg/L.

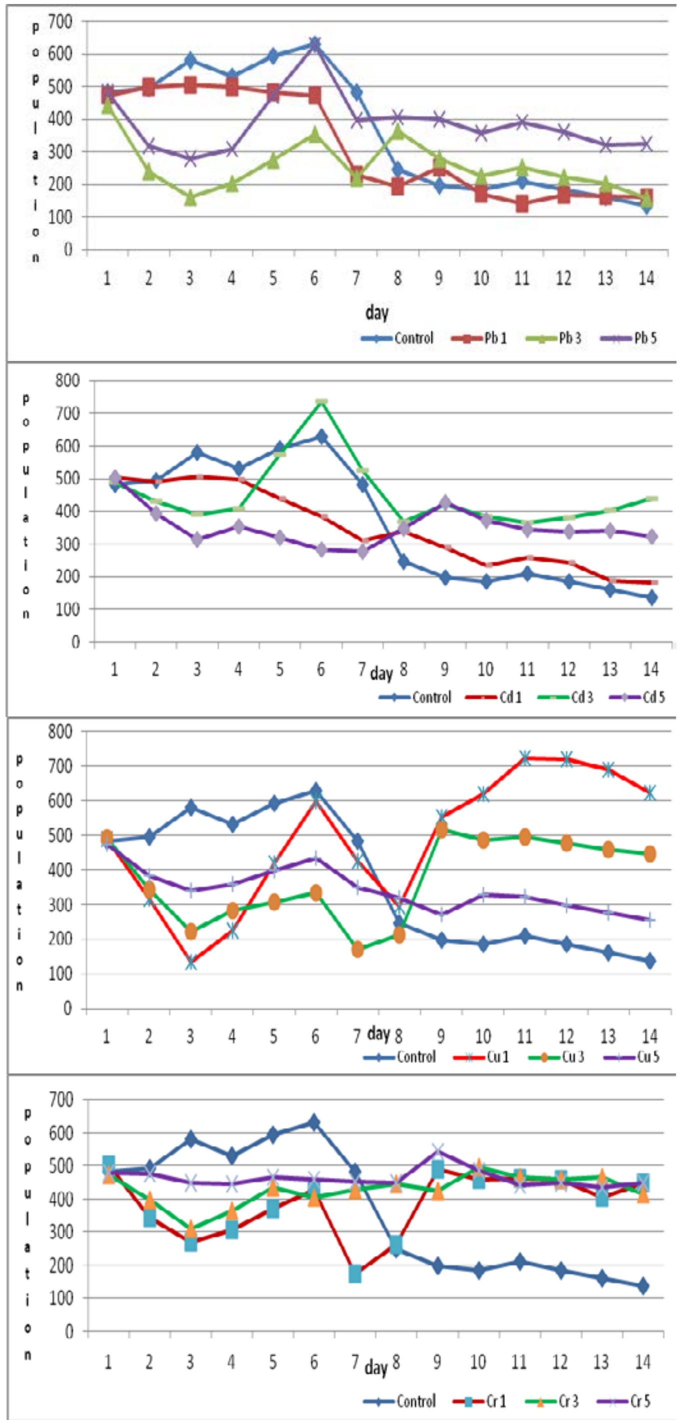

Fig. 1 Population (x 1,000 ind/L) of *Chaetoceros calcitrans* on the Pb, Cd, Cr, and Cu concentration of control, 1, 3, dan 5 mg/L

On the Cr treatments in day 1 – 6, the population of *C. calcitrans* were control > 5 > 3 > 1 mg/L, respectively, in the day of 7 had recuded, and in the days of 8-14 the population of *C. calcitrans* were in order of treatment 5>3>1>control, respectively, whereas the population of control very low (Fig 1). [18] Setyaningsih *et al.* (2013) found that after 22 days treatment with Pb, Cd, Cu, and Cr, the population of *Chlorella vulgaris* sharply increased although the culture was not added with fertiliser. This was due to decomposition of dead *C. vulgaris* in the culture medium and provide component that was required for population growth.

Generally, on the concentration of 1mg/L heavy metals had the highest reduction of heavy metals on the culture medium *of C. calcitrans.* In the day of 9, its reduction was in order of Cu>Cr>Pb>Cd. However, in the day of 14 its reduction was in order of Pb>Cu>Cr>Cd. It was also seen that a high of heavy metals reduction on the culture medium usually had a low population of *C. calcitrans* (Fig 2). Pb was requred for peroxidase enzyme activity which was induce Indone Acetic Acid (IAA), the hormone that stimulate growth and fission of microalgae [19]. The functional groups that involved in the bioaccumulation of Cd in *C. calcitrans* are C=C, C=O,M-S, O-H and S-S [17].

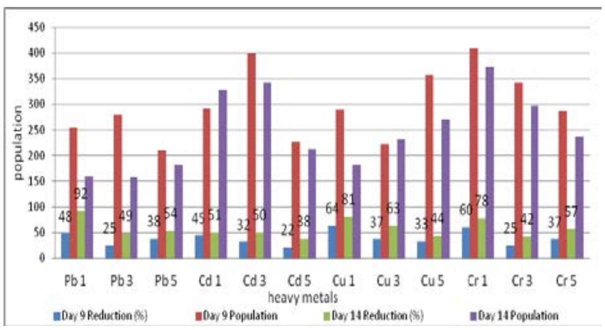

Fig. 2 The percentage reduction of Pb, Cd, Cu, dan Cr on the culture medium and population (X1,000 ind/L) of *C. calcitrans* for treatment of 1, 3, 5 mg/L in the day of 9 and 14

BioConcentrationFactor (BCF) on the *C. calcitrans* in the day of 9 was lower than in the day of 14. In the day of 9, the BCF of *C. calcitrans* was in order of Cr>Cu>Cd>Pb. However, in the day of 14, the BCF of *C. calcitrans* was in order of Pb>Cr>Cu>Cd (Fig 3). *C. calcitrans* 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 [2]; easily found in a big amount, low operational cost, minimum sludge, and no need additional nutrition [4]

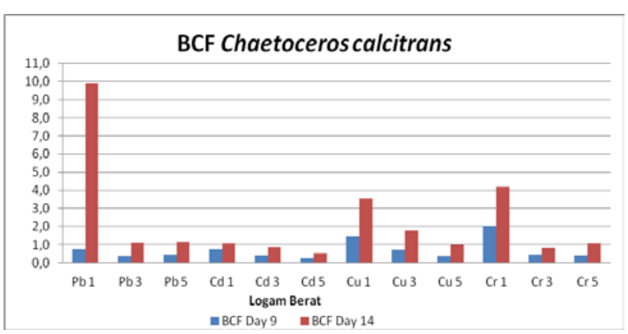

Fig. 3 BCF (ppm) of Pb, Cd, Cr, Cu on the concentration 1, 3, dan 5 mg/L for *C. calcitrans*

Heavy metal has a high electromagnetivity and low radii ion that induce *C. calcitrans* able to absorb it. That is why the heavy metal concentration in the day of 9, *C. calcitrans* has a high BCF for heavy metals treatment of 1 mg/L in order of Cr>Cu,Cd>>P, whereas in the day of 14 gthe BCF was in order of Pb>Cr>Cu>Cd, respectively (Fig 3). The BCF for *Porphyridium cruentum* in day of 8 in order of Cu > Cr > Cd > Pb, respectively; however, in day 15 was Cu > Pb > Cd > Cr [1]. Conti and Cecchetti [20], stated that BCF > 1 ppm indicated that fish was bioaccumulator, and will be stated as a good bioaccumulator if has BCF > 1.000 ppm. Regarding to the BCF value for *C. calcitrans* that was not in the stage of good bioaccumulator in the criteria developed by Conti and Cecchetti [20] but the shorth accumulation time was advantaged microalgae as a good bioremediator [1]. Bioaccumulation studies re-veal the accumulation of the contaminant in the organism via uptake of food or water containing the contaminant [21].

IV. Conclusion

B ased on this research Pb was the heavy metal that was more toxic than other for *C. calcitrans* indicated by low population but highest reduction of heavy metals in the culture medium. In the day of 9, the BCF of *C. calcitrans* was in order of Cr>Cu>Cd>Pb. However, in the day of 14, the BCF of *C. calcitrans* was in order of Pb>Cr>Cu>Cd.

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