NUSANTARA BIOSCIENCE
Vol. 7, No. 2, pp. 177-179
November 2015

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n070220

Short Communication: The growth response of indigenous microalga *Synechococcus* sp. to different concentrations of a heavy metal Cd

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Manuscript received: 19 July 2015. Revision accepted: 30 November 2015.

Abstract. *Gunawan, Muhamat. 2015. The growth response of indigenous microalga* Synechococcus *sp. to different concentrations of a heavy metal Cd. Nusantara Bioscience 7: 177-179.* Microalgae are aquatic organisms having the capability of absorbing heavy metals. The objective of this study was to analyze the response of microalga species *Synechococcus* sp. to different concentrations of a heavy metal Cd and its ability to absorb Cd. The microalga used in this study was isolated from a pond in coal mined land. This experiment was conducted using a completely randomized design with five treatments and three replications. The microalga was grown in one liter of BG 11 medium, with Cd concentrations of 0.56 mg/L, 1 mg/L, 1.8 mg/L, 3.2 mg/L, and 5.6 mg/L. The microalga was grown for 14 days and the growth pattern was analyzed. The heavy metal analysis was done using Inductively Coupled Plasma. The numbers of microalga cells after 14 days of culture were 1.015 cell/mL, 866 cell/mL, 800 cell/mL,768.50 cell/mL, and (440 cell/mL) at Cd concentrations of 0.56 mg/L, 1 mg/L, 1.8 mg/L, 3.2 mg/L, and 5.6 mg/L respectively. The concentrations of heavy metal in the medium at the end of experiment were 0.350 mg/L, 0.615 mg/L, 0.471 mg/L, 0.828 mg/L, and 1.456 mg/L, respectively declining from the initial concentrations of 0.56 mg/L, 1 mg/L, 1.8 mg/L, 3.2 mg/L, and 5.6 mg/L. The decline of Cd concentration in the medium at the end of experiment showed that *Synechococcus* sp. had the capability of absorbing heavy metals from its environment.

Keywords: growth response, cadmium absorption, microalga *Synechococcus* sp.

INTRODUCTION

Environmental pollution is a big problem for community because it has negative impact on life. Currently, industries are source of pollutant, because they dispose of liquid, gas and solid wastes to the environment. The liquid industrial waste discharged to water is usually contaminated with heavy metals, such as Cd, Cr, Hg and Pb which will have deleterious impact to the environment.

The concentration of heavy metals in the environment can be reduced using chemical process, but this method is expensive and tends to create new problems, such as accumulation of chemicals in sediments and aquatic organisms. (Suhendrayatna 2001). Hussein et al. (2004) says that heavy metals can be removed from water using algae which can absorb heavy metals through bioaccumulation. Microalgae have the ability to accumulate heavy metals in their cells. Therefore, they can be used as bioabsorbent for cleaning water from heavy metal contamination (Hala et al. 2012).

Removing heavy metal using microalgae through bioaccumulation process has some advantages. First, it is more effective than ion exchange and reverse osmosis. Second, it is easy to do because microalgae grow fast. Some microalgae such as *Chlorella* sp., *Nannochloropsis* sp., *Chaetoceros gracilis* and *Chaetoceros calcitrans* are known to have the capability of absorbing heavy metals (Inthorn et al. 2001).

Cadmium (Cd) is a heavy metal element toxic to organisms, usually resulted from coal mining at concentration of 4.2 mg/L. This concentration is higher than the threshold level permitted for coal mining waste, which is 0.05 mg/L. The objective of this study was to analyze the response of microalga species *Synechococcus* sp. to different concentrations of Cd and its ability to absorb Cd as indicated by decline of Cd concentration in the culture medium at the end of the experiment.

MATERIALS AND METHODS

Materials and equipment

Synechococcus sp. was inoculated from a pond in a coal mined land, using a serial dilution method. The microalga was grown in one liter of BG 11 medium in a mini bioreactor. The solution of cadmium was made from $CdSO_4$ (Cadmium Sulfate), at five concentrations, namely: 0.56 mg/L, 1 mg/L, 1.8 mg/L, 3.2 mg/L, and 5.6 mg/L (Setiawati 2009).

The experimental design

This experiment was conducted using a completely randomized design with five treatments and three replications.

The working procedures and data analyses

The pattern of microalga growth at different Cd concentrations

The pattern of microalga growth was analyzed based on number of cells. The cell number was counted every day using hemocytometer observed under a microscope. The cell density was determined using this formula:

Cell density (cell/mL) = N X 10^4

Where N is the number of counted cells (Fachrullah 2011)

The concentrations of Cadmium (Cd)

Microalga was mixed with five concentrations of Cd and then diluted to 500 mL. Then it was shaken in a shaker at a speed of 200 rpm for 24 hours. Then, the biomass of *Synechococcus* sp. was separated from the medium. The biomass of *Synechococcus* sp. was placed in the oven for 24 hours, and then the cells were destroyed. The concentration of Cd in the medium and in the biomass of *Synechococcus* sp. was measured at the same time using Inductively Coupled Plasma.

RESULTS AND DISCUSSION

The growth pattern of *Synechococcus* sp

The pattern of growth was observed based on the change of the number of cells within 12 days. In general, at all Cd concentrations, the number of cells increased over time until the 10^{th} or 11^{th} day (Figure 1). The growth pattern indicates that the microalga was undergoing adaptation from normal to heavy metal-contaminated environment. The adaptation phase was indicated by the slow increase of cell number from the first day to the forth day. Soeprobowati et al. (2013) say that slow grow of microalgae is caused by environmental changes.

The exponential phase started at the fifth day, as indicated by the fast increase of cell number, which was1.015 x 10^4 cell/mL at a Cd concentration of 0.5 mg/L, and the phase ended at the 10^{th} day. At the following day *Synechococcus* sp. was at stationer phase, followed by death phase at the 11^{th} day as indicated by the decline of cell number. The declining of growth was caused by the increase of cell density coupled by the decline of nutrient. Wood et al. (2005) say that the growth rate of microalgae at culture media is positively correlated with the nutrient content of the media.

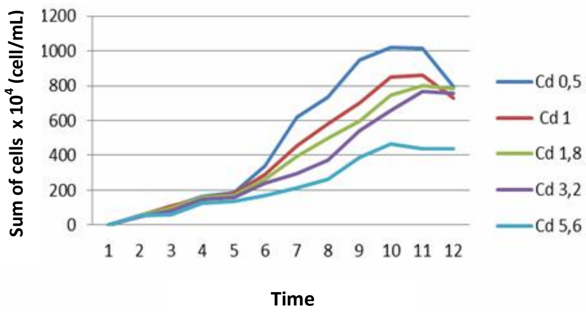

Figure 1. The growth of microalga *Synechococcus* sp within 12 days of observation

At Cd concentration of 5.6 mg/l, the microalga had the lowest number of cells. In general, microalgae have defense mechanism against heavy metal contamination. However, too high concentration of heavy metals can impede the growth of microalgae, because the defense mechanism is no longer effective in preventing toxicity from the heavy metals (Wang and Chen 2009; Hala et al. 2012). Cadmium is a very toxic heavy metal element which can accumulate in microalgae cells, slowing the growth and even killing the microalgae (Arunakumara and Zhang 2008). The death or damage of cells of microalgae begins with the damage of chloroplast structure, impairing photosynthesis process, which leads to the reduced ability of microalgae for reproduction. In addition, respiration is also impeded (Pinto et al. 2003).

The decline of Cadmium concentration in the culture medium

The data of Cd concentrations in the medium at the end of experiments showed that the magnitude of decline in the medium was positively correlated with the initial concentration of Cd in the medium: the higher the initial concentration the larger the decline was. The highest average of Cd concentration at the end of experiment (3.444 mg/L) was found at the medium with initial Cd concentration of 4.9 mg/L and the lowest one (0.210 mg/L) was found at the initial Cd concentration of 0.56 mg/L. The average decline of absorbed Cd concentration, the Cd concentration at the end of experiment, and the concentration of Cd at the biomass of *Synechococcus* sp. are presented in Table 1.

Table 1. The average decline of absorbed Cd concentration, the Cd concentration at the end of experiment, and the concentration of Cd at the biomass of *Synechococcus* sp.

Initial concentration of Cd (mg/L)	Actual concentration Cd (mg/L) *	Control (mg/L) *	Average concentration of Cd in the water at the end of experiment (mg/L)*	Average decline of Cd concentration (mg/L)**	Average concentration of CD at the biomass of *Synechococcus* sp. (mg/g)*
0.56	0.56	0.55	0.210	0.350	0.11
1	0.92	0.98	0.305	0.615	0.31
1.8	1.6	1.8	1.129	0.471	0.95
3.2	3.2	3.1	2.372	0.828	2.29
5.6	4.9	5.4	3.444	1.456	3.34

Note: *The numbers in these columns were the results of analyses. **The numbers of this column were the differences between the initial Cd concentration and the Cd concentration at the end of experiment.

The concentration of heavy metal in media affects the absorption of the heavy metal by microalgae (Syauqiah et al. 2011). The ability of microalgae to absorb heavy metal improves with the increased concentration of heavy metals in the media, because the stress due to heavy metal concentration increases ionic transfer which in turn increases adsorption of heavy metal ions (Davis et al. 2003). Heavy metal will accumulate in microalgae cells and it will be bound to heavy metal-binding proteins such as metallothioneins and phytochelatins, and it will accumulate in vacuoles (Niess 1999).

Generally, Cadmium impairs the functioning of enzymes in the microalgae cells and Cd^{2+} disturbs redox reactions in microalgae cells (Kusuma and Zulaika 2014). Carfagna et al. (2013) explain that proteins and enzymes in microalgae cells are negatively charged, while Cd^{2+} is positively charged; so, if they bind to each other, the functioning of enzymes will be impaired.

The mechanism of heavy metal absorption by microalgae consists of two processes, namely ion exchange and ion binding by functional groups in the cell surface. Usually, the microalgae cell walls are composed of cellulose which has functional groups, such as hydroxyl, capable of binding heavy metals. The absorption of heavy metals by microalgae is affected by several factors, such as pH, temperature, light, the presence of other ions and chelating agents (Fauziah 2011). It is also affected by the size and species of microalgae (Levy et al. 2007). Nacorda et al. (2007) say that different species of microalgae have different ability to absorb and accumulate heavy metals.

Beside the increase of heavy metal concentration, the increase of temperature also increases the absorption of heavy metals by microalgae because temperature affects the speed of metabolisms such as enzymatic activities and active transport. Light also influences Cd absorption, because Cd absorption is done through active transport, and this active transport is powered by energy from light-dependent process of photosynthesis. In addition, microalgae are capable of detoxifying heavy metals by converting toxic metals into non toxic one. Detoxification occurs inside and outside the cells (Fauziah 2011). This study showed that microalgae *Synechococcus* sp isolated from a pond in coal mined pond was capable of absorbing heavy metal from the environment; so, this species can be used to reduce the concentration of Cd in the environment.

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