

Removal of Mercury, Arsenic, and Cadmium in Synthetic Wastewater by Cyanobacterium *Aphanothece halophytica*

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ABSTRACT

Aphanothece halophytica is a halotolerant cyanobacterium that can grow under sodium chloride concentration range of 0.2 – 3.0 M and can tolerate to mercury, arsenic, and cadmium concentration at 4, 40, and 10 mg/L, respectively. The aim of this study was to investigate the reduction of mercury (Hg^{2+}), arsenic (As^{3+}), and cadmium (Cd^{2+}) in synthetic wastewater by halotolerant cyanobacterium *A. halophytica*. These organisms were able to remove Hg^{2+} , As^{3+} , and Cd^{2+} in the synthetic wastewater about 70%, 55%, and 50%, respectively at pH 7.5 and ambient temperature. The optimal pH to reduce all heavy metals is 6.0 at ambient temperature. By increasing the content of cyanobacterial cell used in the experiment, the results showed that heavy metal removal depended on cell concentration. Temperature has no significant effect to remove all heavy metals. This work demonstrated that the efficiency of Hg^{2+} , As^{3+} , and Cd^{2+} removal by *A. halophytica* in these conditions depended on pH and cell concentration, respectively.

Keywords: Cyanobacteria, synthetic wastewater, heavy metal removal.

INTRODUCTION

Heavy metal ions (Hg^{2+} , As^{3+} , and Cd^{2+}) are found in effluents discharged from various industries; for example batteries, metal plating, and mining, respectively (Hutton *et al.*, 1987). Mercury, cadmium, and arsenic ions can not degrade and these heavy metals can accumulate in living organisms and can cause a possible human health risk (Hardman *et al.*, 1993). Toxicity of heavy metals may result in various effects, which depend on the type of organisms, the nature and concentration of heavy metals as well as the environmental conditions (Satoh *et al.*, 2005).

The heavy metal contaminations were continuous increasing as a serious environmental problem while researchers try to find out new mechanisms to remove these pollutants. There are several techniques used to remove heavy metals from contaminated medium such as ion exchange, precipitation, filtration, and ion

transformation which are commercially high cost for operating system (Ahluwalia and Goyal, 2007; Patterson *et al.*, 1998).

Biosorption and bioprecipitation were techniques to select for heavy metal removal by using living or dead microorganisms. Microorganism was used to eliminate the toxic metals such as bacteria, algae and cyanobacteria (Satoh *et al.*, 2005; Sridokchan *et al.*, 2005; Sirisopana *et al.*, 2008).

There are many reports on the use of cyanobacteria to remove of heavy metals. Cyanobacterium, one of the micro living materials, has good potential for eliminating the heavy metal as shown in Table 1. *A. halophytica* had been reported that could remove zinc, lead, and chromium, respectively (Incharoensakdi, A. and Kitjaharn, P., 2002; Sirisopana *et al.*, 2008).

Table 1 Removal of mercury, arsenic, and cadmium by cyanobacteria

Cyanobacterial name	Types of metals removed	References
<i>Spirulina platensis</i>	Hg	Cain <i>et al.</i> , 2008
<i>Aphanothece flocculosa</i>		Slotton <i>et al.</i> , 1989
<i>Phormidium</i> sp. <i>Nostoc</i> sp <i>Anabaena</i> sp. <i>Nostoc</i> sp-1	As	Shaheen <i>et al.</i> , 2007
<i>Nostoc linckia</i> , <i>Nostoc rivularis</i> , <i>Tolypothrix tenuis</i> , <i>Synechocystis</i> PCC7942	Cd	El-Enany and Issa, 2000 Inthorn <i>et al.</i> , 1996 Gaede-Torresdey <i>et al.</i> , 1998

Aphanothece halophytica is a cyanobacterium found in marine water (Dead Sea, Israel). *A. halophytica* accumulates glycine betaine under high salinity medium (2 – 3 M NaCl) and alkaline pH (8 - 10) (Laloknam *et al.*, 2006; Waditee *et al.*, 2005). The previous study had evaluated the effect of chromium, arsenic, cadmium and mercury on growth and chlorophyll contents and showed that *A. halophytica* can tolerate to mercury, arsenic, cadmium, and chromium concentration at 4, 40, 10, and 50 mg/L, respectively (Laloknam *et al.*, 2008).

These cells were able to remove Cr(VI) in the synthetic wastewater about 30% at pH of 7.5 and room temperature. The optimal pH to reduce chromium is 6.0 at room temperature (Sirisopana *et al.*, 2008). The aim of this study was to reduce mercury, arsenic, and cadmium from synthetic wastewater by bioabsorption using cyanobacterium *A. halophytica*.

METHODOLOGY

Chemicals

Hg^{2+} , Cd^{2+} , and As^{3+} (metals under experiments) stock solutions were prepared by dissolving their corresponding salts of HgCl_2 , CdCl_2 , and NaAsO_2 (Sigma, USA) in distilled water.

Culture conditions

A. halophytica cultures were grown in BG_{11} medium, pH 7.5 containing 0.5 M NaCl (Laloknam *et al.*, 2008). The cyanobacteria were cultured aerobically at room temperature under continuous fluorescent white light ($30\mu\text{Em}^2\text{s}^{-1}$). The growth of the cultures was determined by measuring the optical density of the culture at 750 nm using a Spectronic spectrophotometer Model Jenway 6405 until mid log phase of cell growth (10 days) as shown in Figure 1. After 10 days, the cell pellet was obtained by centrifugation of cyanobacterial culture at 4,000 rpm for 10 min. Before the heavy metal removal experiment, the cell concentration was adjusted to 2 mg of protein per ml cell suspension. Protein concentration was determined by the Bradford method (Bradford, 1976).

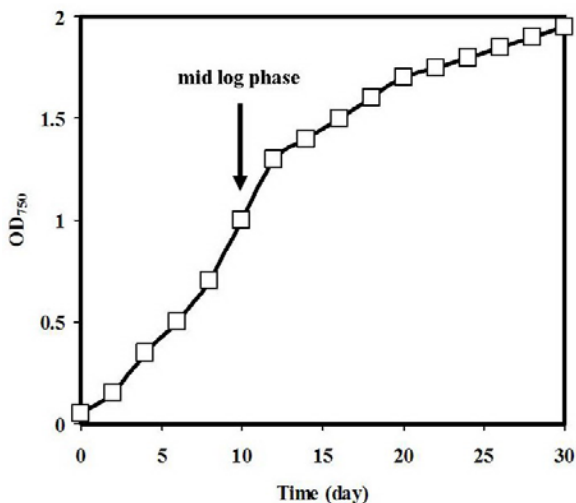


Figure 1 Growth curve of *A. halophytica*. Cells were cultivated in BG_{11} medium containing 0.5 M NaCl and followed cell growth by using UV-visible spectrophotometry at 750 nm.

Removal of mercury, arsenic, and cadmium in synthetic wastewater

Synthetic waste water in this experiment depends on limitation of growth of *A. halophytica*. Also, synthetic wastewater is the BG_{11} medium containing 0.5 M NaCl at pH 7.0 supplemented with mercury, arsenic, or cadmium at 4, 40, and 10 mg/l, respectively (Laloknam *et al.*, 2008). Heavy metal removal was determined by measuring the heavy metal loss in the culture medium after 30, 60, and 120 min of incubation at room temperature. The cyanobacterium were

suspended in 50 ml (2 mg protein per ml) then harvested by centrifugation at 4,000 rpm for 10 min at interval time. Removal of heavy metal by *A. halophytica* was determined by measuring the heavy metal contents in the supernatant by atomic absorption spectrophotometer Model AA 280 FS (Varian). Each experiment was repeated three times.

Effect of pH, temperature, and cell contents

The effect of pH (6.0, 7.5, and 9.0) was investigated by adjusting pH of the medium using cells of the cyanobacterium. The removal test was conducted on a shaker (200 rpm) at room temperature for 30, 60, and 120 min. The supernatants were analyzed for the remaining heavy metals in the solution after medium was centrifuged. The percentages of each metal removal calculate based on its initial concentration. The effect of temperature was studied at 15, 30, 45, and 60°C, respectively. The cell contents were varied at the concentration range of 1 – 10 mg protein/ml in medium. The optimum pH, temperature and bacterial contents were selected for applications. Each experiment was repeated three times.

RESULTS AND DISCUSSION

Removal of mercury, arsenic, and cadmium in synthetic wastewater

Figure 2 shows the contents of heavy metal under examination. The heavy metal concentration at room temperature was decreased in 1 hour and remained constant at 30%, 45%, and 50% for mercury (Figure 2A), arsenic (Figure 2B), and cadmium (Figure 2C), respectively. *Synechocystis* PCC7942 could remove cadmium from synthetic wastewater at normal condition by bioabsorption and showed 80% of heavy metal remaining after investigation (Gaede-Torresdey, 1998).

Effect of pH, temperature, and cell content

The effects of pH were studied at 6.0, 7.5, and 9 were shown in Figure 2. The optimal pH for removal of all heavy metal in this experiment is 6.0. Temperature was studied at 15, 30, 45, and 60°C and results indicated that temperature has no significant effect on all heavy metal removal (data not shown). Figure 4 showed that cell contents had effect on all heavy metal removal in synthetic wastewater and the 5, 80, and 10 mg protein/ ml is the optimized condition for mercury, arsenic, and cadmium, respectively.

It has been reported that *Synechococcus* PCC 7942 could remove some heavy metal such as lead, cadmium, chromium, and nickel at acidic pH range of 5 – 6 (Gaede-Torresdey *et al.*, 1998). Previous studies on removal of chromium metal showed optimum pH at 6.0 (Sirisopana *et al.*, 2008). Removal of heavy metals might be obtained by cell precipitation because carboxylic group of cell wall or plasma membrane. The acid dissociation constants for carboxyl groups have been reported whereas pH increases; these groups become deprotonated and attract the positively charged metal ions (Gaede-Torresdey *et al.*, 1998). Temperature has no effect on removal of heavy metals. These might be demonstrated that the functional groups of protein could tolerate within temperature under these experiments.

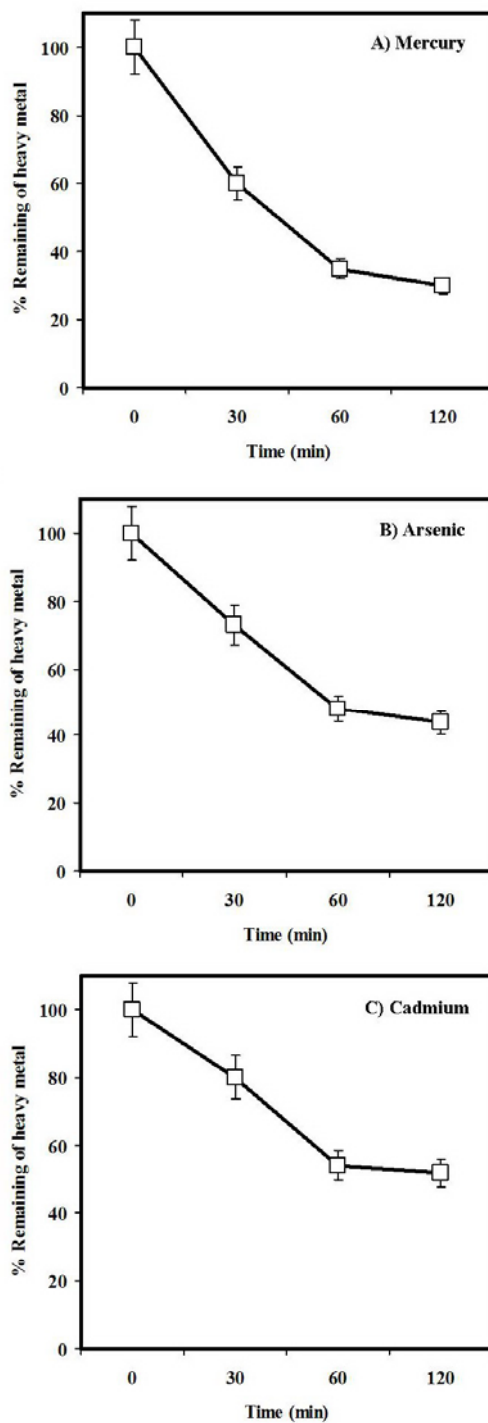


Figure 2 Reduction of heavy metal by *A. halophytica*. A) Mercury, B) Arsenic, and C) Cadmium.

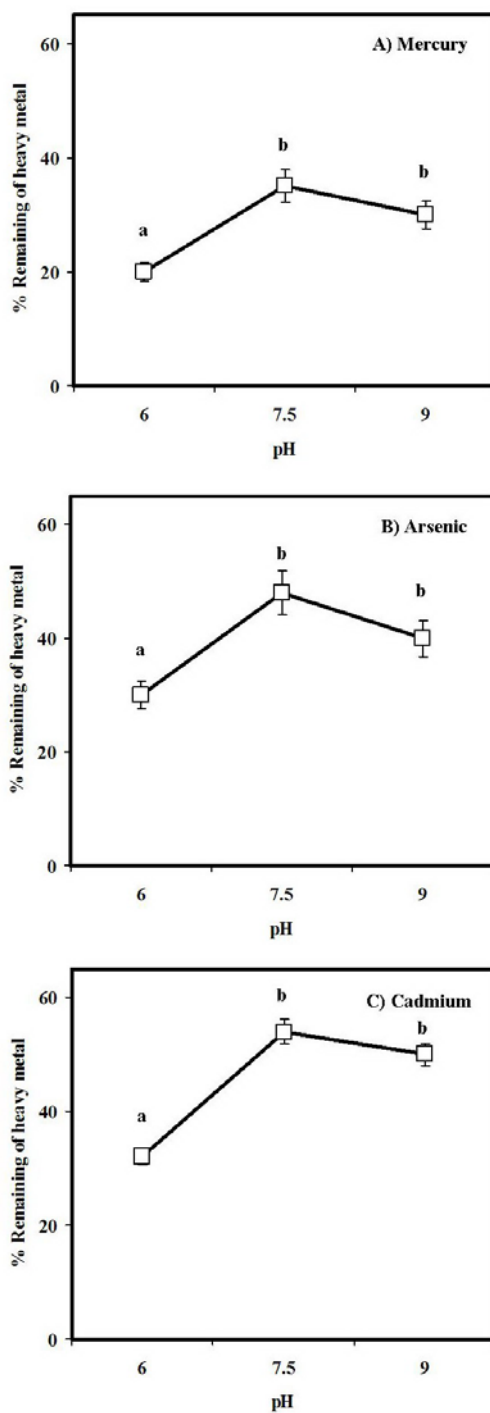


Figure 3 Effects of pH on removal of heavy metals of *A. halophytica*. A) Mercury, B) Arsenic, and C) Cadmium.

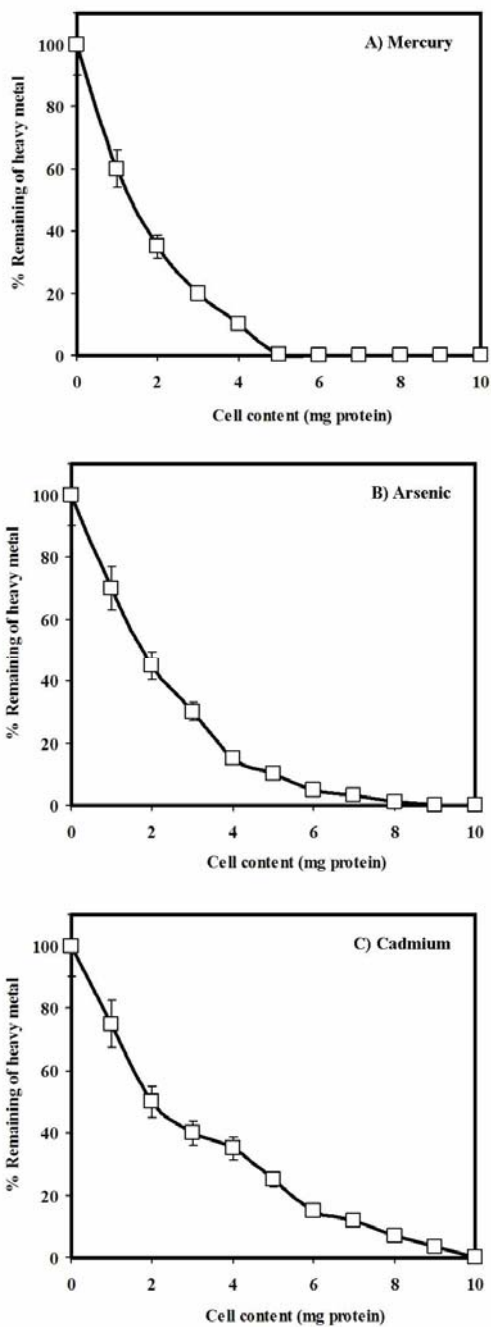


Figure 4 Effects of cell content on removal of heavy metals of *A. halophytica*. A) Mercury, B) Arsenic, and C) Cadmium.

Increasing of cell contents has a positive effect on the reduction of all heavy metals. This can be explained that increasing of cell contents can increase the capacity to absorb heavy metal per cell contents. Mehta and Gaur (2001) have shown that increasing the biomass concentration by 100- fold enhanced Ni and Cu removal from medium. Increased metal removal at higher biomass concentration is a simple method due to greater availability of metal binding sites (Mehta and Gaur, 2001).

CONCLUSION

Cyanobacterium *A. halophytica* is one type of microbe that has some advantages to remove some heavy metals (chromium, mercury, cadmium, arsenic) from synthetic waste water which can improve the wastewater quality. The kinetic and mechanism on heavy metal removal by this cyanobacterium might be necessary for future study to understand these mechanisms. For the future work, the heavy metal uptake will be studied to determine the absorption of heavy metal by *A. halophytica*. After the uptake concentration of heavy metals by this cyanobacterium is known, we can use this organism for the environmental purposes, such as the reduction of heavy metals from the contaminated areas or industries.

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