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Preliminary Study for the Arsenate Removal in a Synthetic Wastewater by Acclimated Cultures

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Abstract: Many exhaustive studies of health risks associated with low-level arsenic have been recently published. Arsenic contamination of groundwater in Bangladesh provides an unfortunate reminder of the scale this problem can attain. The increasing awareness of toxicity and pollution of arsenic led to a quest for environment-friendly remediation processes in water treatment system at discharge of arsenic to acceptable level. The main objective of this paper is to evaluate the possibility to remove arsenate from water by using phototropic organisms inoculated from the sludge collected from local wastewater treatment plant. The best arsenate removal efficiency was 25.7% in the time period of 14 days. Although the removal efficiency was not high enough to become an independent process, these promising results obtained in laboratory scale experiments still proved that it is feasible to remove arsenate from wastewater by acclimated cultures. This study also offers the results of effects of environmental parameters on arsenate removal. These preliminary results could offer the data for the wastewater treatment plant to evaluate the possibility to combine this bioremediation technology into their existing treatment processes to increase the total arsenic removal efficiency or decrease the load of expensive processes applied to the arsenic removal.

Key words: Arsenate, algae, phototrophic, bioremediation.

Introduction

Recently, arsenic has become one of the major environmental concerns in the world as millions of people have been exposed to low-level inorganic arsenic through contaminated materials and suffer from the diseases caused by the exposure to arsenic. The toxic effects caused by exposure of low-level arsenic include cardiovascular, neurological, diabetic, immunological, hematological, dermal and respiratory effects (Hertz-Picciotto et al., 2000; Milton and Rahman, 2002; Tseng et al., 2002; Rodr´iguez et al., 2003). Also, epidemiological studies indicate that arsenic is a potent carcinogen, co-carcinogen, or promoter (Vaha¨kangas, 2003; Moore et al., 2004; Rossman et al., 2004).

Source of arsenic pollutant includes natural and anthropogenic inputs. The natural source of arsenic enters the environment primarily by leaching from geological formation. In past few decades, arsenic contamination of the environment arises as a result of many human activities, such as mining, smelting, glass manufacturing, pharmaceutical industry, electronics, agriculture, disposal of sewage, and chemical warfare agents (NRC, 2001).

Due to the increasing arsenic contamination of environment and emerging health problems linked to arsenic, the removal of arsenicals from water is important in the protection of the human health. Recently, many researchers tried to develop environment-friendly arsenic remediation processes to replace or improve the efficiency of processes now-a-days used in water treatment plant (Shih, 2005). Bioremediation has the

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potential to greatly contribute to the achievement of these goals. Bioremediation is defined as the removing or recovering of the pollutants from aqueous solutions by the use of biological materials based on the mechanisms of uptake of pollutants by microbial cells. There are many different microorganisms that can be used for the purpose of bioremediation (Hobson and Poole, 1988; Wood, 1992). The phototrophic organisms have received attention recently, since these microorganisms can use light as an energy source in the absence of organic carbon sources and can be easily cultivated in open ponds of water treatment plant. The present work was aimed to use the phototrophic microorganisms inoculated from acclimated aerobic sludge to verify the possibility for the improvement of arsenic removal efficiency or replacement of water treatment processes now-a-days used in the water treatment plant.

Previous studies on relationship between arsenate and algae are mainly focussed on isolation and characterization of arsenate-sensitive algae and biotransformation of arsenate (Fujiwara et al., 2000; Murry et al., 2003). The interactions of arsenate removal efficiency and environmental factors have received little attention. In fact, growth and nutrient uptake of phototrophic organisms are probably affected by environmental parameters such as temperature, air, light and phosphate. The environmental parameters that are able to affect the arsenic removal efficiencies would be investigated here. The results of these experiments might be used to evaluate the feasibility and operation environments for the application of phototrophic organisms in wastewater treatment plant.

Experimental Processes

Material and Methods

The sludge used for this research was collected from aerobic basin in the local wastewater treatment plant. In order to obtain the phototrophic microorganisms, the aerobic sludge was inoculated in a modified version of the Modified-Detmer culture medium (Maeda et al., 1983) without the addition of carbon source. Sodium arsenate was added into medium to acclimate the microorganism populations in the sludge. The medium consists of the following in g/L: KNO_3, 1; $CaCl_2$, 0.1; $MgSO_4.7H_2O$, 0.25; NaCl, 0.1; $K_2HPO_4.3H_2O$, 0.19; $FeSO_4.7H_2O$, 0.02; Trace elements, 1 ml. The trace elements consists of the following in g/L: H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.22; $CuSO_4.5H_2O$, 0.08; $Na_2MoO_4.2H_2O$, 0.2467; Conc. H_2SO_4, 0.1 ml. The pH values of medium were adjusted to 8.

A series of procedures would be used to isolate and inoculate the phototrophic microorganisms with a competitive growth advantage. First, two litres of sludge and two litres of synthetic medium were mixed and transferred into a 10-litre working volume fermentor, which was exposed to indirect sunlight, and operated at temperature of 21±2°C. After three days, 400 ml of fresh synthetic medium was continuously pumped into the fermentor while 400 ml of effluent was drained from the fermentor each day. This process would be operated for one month. Finally, 1000 ml of solution was taken from the fermentor and 1000 ml of synthetic medium was added every seven days repeatedly. After three months of operation, the remaining microorganisms were those with a competitive growth advantage.

The culture used for all batch studies was taken directly from the fermentor described above. All batch experiments were carried out in a 500 ml reactor containing 270 ml of fresh synthetic medium and 30 ml of inoculums. To prove that the microbial cell was required to ensure the removal of arsenate, a batch experiment with cell-free filtrate was used. Also, a batch experiment with dead cells was proceeded to ensure that the removal of arsenate was biological.

A series of experiments were set up and used to investigate the effect of the cultivation parameters on arsenate removal efficiency. These parameters included temperature, light, oxygen, phosphate (from 15 mg/L to 813 mg/L) and pH (pH4, pH6, pH8 and pH10). 30 ml of inoculums and 270 ml fresh synthetic medium were mixed in a 500 ml conical flask equipped with air distributors used as the basic reactors for all these environment parameter experiments. Samples were withdrawn periodically.

Analytical Methods

The pH values of solution were measured directly with a pH probe (cole-parmer and chemcadet). The cultures were observed with a microscope (Olympus, BH-2). For measurement of soluble arsenic, 10 ml sample was previously centrifuged, then filtered with Whatman GF/C paper and Gelman Science GN-6 grid 0.45 μm sterilized membrane filter paper. The filtrate was stored at 0°C until measured by an atomic absorption spectrophotometer (AAS) (Perkin-Elmer Zeeman furnace 5100 ZL, Norwalk, Conn.).

Results and Discussions

Evidence of Biological Removal of Arsenate

One of the purposes of this research was to verify the possibility to remove arsenic from aqueous solutions. No attempts were made to identify and isolate the microorganisms responsible for arsenate removal from aqueous solutions in this preliminary research. These arsenate-acclimated cultures were observed by microscope, and were found to be the mixed cultures, especially alga. This observation also indicated that all these microorganisms inoculated in this research were able to survive and tolerate to high concentration of arsenate. Few experiments were set up and proceeded to prove that these acclimated cultures were not only able to tolerate to high concentration of arsenate, but also were able to remove arsenate from water. The results are shown in Figure 1. Figure 1 shows that the arsenic concentration decreased from 92 ppm to around 83 ppm in the existing acclimated cultures. There were no changes of arsenic concentration in the cell-free filtrate flask. The results of Figure 1 clearly indicate that the arsenic removal was solely occurring in the existing cell. Also, the experiment results showed the concentration of arsenic increased in day 4 and day 8 (Figure 1). The rising of concentration could be related to the release of organometallic forms of arsenic, since the atomic absorption (AA) spectrophotometer only could detect the total concentration of arsenic. This assumption was made and supported by some literature surveys. From the literatures, it was postulated that inorganic arsenic was accumulated in freshwater algae and transformed into organometallic forms (Maeda et al., 1983; Maeda et al., 1985; Suhendrayatna et al., 1999). The transformation process involved both reduction and methylation (Maeda et al., 1987). Algae used this mechanism of transformation as a method to detoxify the inorganic arsenic, since the organometallic forms of arsenic were generally less toxic than the inorganic arsenicals. These arsenate-acclimated cultures probably used the same or similar mechanisms to remove arsenate from solution.

Metal uptake using non-living biomass of algae was observed in some reports (Jalali et al., 2002). From Figure 1, it is shown that the removal of arsenic only occurs in live cell, since the flask with heat-killed mixed culture did not show any arsenic removal. These results indicated the removal of arsenate not only adsorbed by cell, but these processes of adsorption also involved bioreaction occurred in living cell.

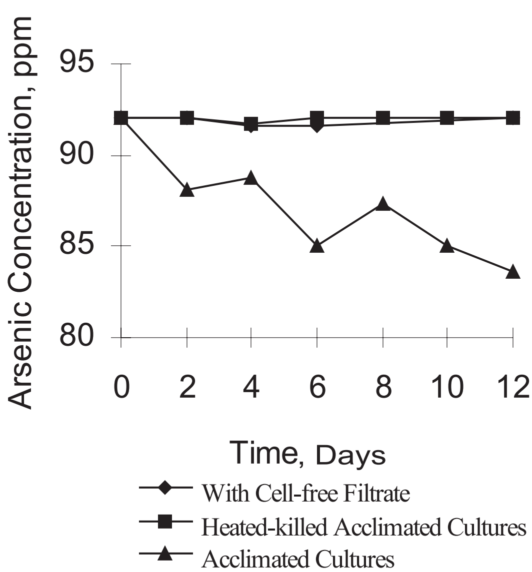

Figure 1: Arsenic removal by acclimated culture with and without heat treatment, and with cell-free filtrate.

Effect of Environmental Parameters on Arsenate Removal

The influence of pH was investigated in separate conical flask equipped with air distributors. From the experimental results shown in Figure 2, the arsenic concentration of arsenate decreased from the initial concentration of 94 ppm to 81 ppm in the flask with setting of pH 8. The arsenic concentration of the flasks with pH setting of 4, 6, and 10 decreased from 94 ppm to around 87 ppm. These experimental results showed that the acclimated cultures were able to remove arsenate in acid and base solution and the optimal environmental pH value for the arsenate removal would be pH 8.

The experimental results of temperature effect on arsenate removal are shown in Figure 3. The results showed a minor arsenate reduction at 12°C. From Figure 3, it showed the arsenate concentration retained in the water being decreased with the increasing temperature, and having a maximum arsenate removal efficiency at temperature 32°C. From the data, it showed that the

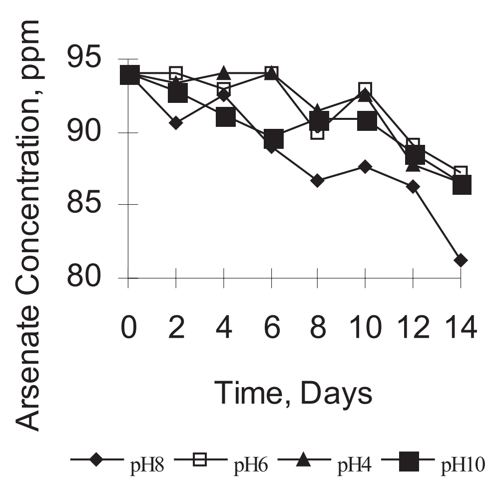

Figure 2: The influence of pH on biological removal of arsenate.

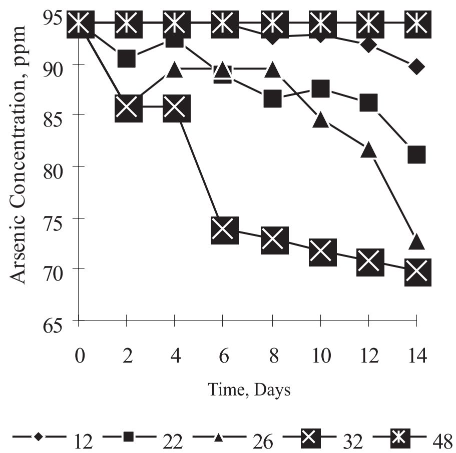

Figure 3: Influence of temperature on biological removal of arsenate.

arsenate removal did not occur at temperature 48°C. Probably this high temperature killed the mixed cultures or suppressed the growth of cell, since the removal of arsenate only occurred in live cell. These results indicated that the water treatment plant located in high temperature areas would be the better locations to use this method to remove arsenic from water, since these areas did not need to use any heating equipments to raise the pond's temperature for the optimal operational temperature. Southeast Asia and Africa will be the better locations, since the annual average temperatures of Southeast Asia and Africa were over 25°C.

The influence of air was investigated in separate conical flask equipped with and without air distributors. The data from Figure 4 showed that the arsenic was almost retained in the water at anoxic condition even when the flask was under a lighting condition. These microorganisms obviously needed oxygen to survive or consume the arsenate from water. Therefore, the water treatment plant needed to pump air into the pond or used rotating biological contactor to offer enough air for microorganisms to reach better arsenic removal efficiency. The effect of light was also investigated in different conical flasks and is shown in Figure 5. The data from Figure 5 indicated that these microorganisms needed light to survive or consume the arsenate from water.

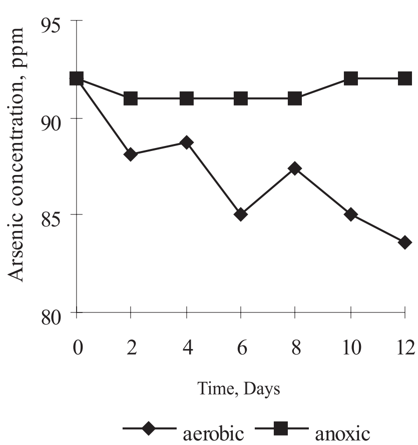

Figure 4: Arsenic removal in acclimated cultures with and without air.

Arsenic (As) is a member of the group VA in the periodic table as phosphorus. Therefore, arsenate and phosphate might play a similar role in the metabolism of microorganisms; phosphate might competitively inhibit arsenic reduction. The experiment data of different phosphate levels is shown in Figure 6. Low phosphate (7.9 ppm) and high phosphate concentration (500 ppm) experiments had bad removal efficiency. Maeda and

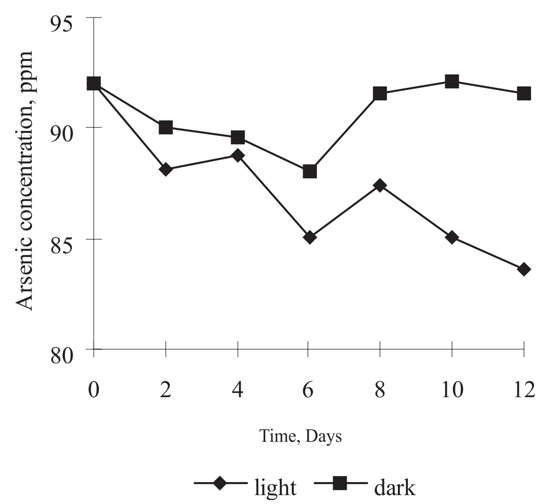

Figure 5: Arsenic removal in acclimated cultures with and without light.

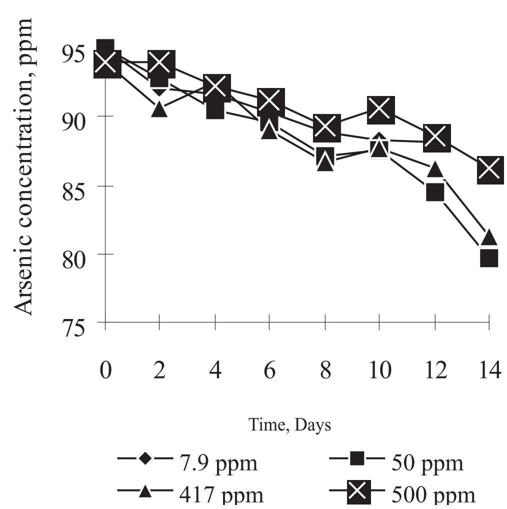

Figure 6: The influence of phosphate on biological removal of arsenate.

coworkers (Maeda et al., 1985; Maeda et al., 1987) had published some similar results. They found that the phosphate reduced the arsenic accumulation. Some other researchers also had similar results (Planas and Healey, 1978; Budd and Craig, 1981; Baker et al., 1983). Our experimental results matched these researchers' experiment results.

Conclusions and Perspective

In this study, aerobic sludge obtained from local municipal wastewater treatment faculties was acclimated to arsenate in a synthetic medium. The experimental results indicated these acclimated cultures as capable of arsenate removal ability. Also, it was reasonable to assume that the alga were the primary species responsible for the arsenate removal in these experiments based on the observation of microscope, design of medium, experimental processes, and literature survey. However, the total arsenate removal efficiency was low. The best arsenate removal efficiency was only around 25.7% in the time period of 14 days from the present work. There are some literatures which could be used to explain that the low arsenate removal efficiency was reasonable and offer further references to obtain a better removal efficiency. In 1995, Hammouda et al. (1995) found the chromium removal efficiency less than 15% in the first 14 days and over 75% after 49 days operation by using algae to remove 0.4 ppm chromium from wastewater. Pena-Castro et al. (2004) used microalga to remove 3.5 ppm cadmium and found cadmium removal efficiency less than 30%. In present research, the initial arsenate concentration was over 90 ppm and the whole experiment time for each experiment was 14 days. Comparing to the literatures' experimental conditions and pollutant concentration, it was found that the 14 days might be not long enough to obtain good removal efficiency. Also, the high initial concentration might slow down the removal rate. It was reasonable to assume that a longer operation time and lower initial arsenic concentration would be helpful to obtain the better arsenic removal efficiency for further study or application.

The positive results of this experiment presented a potential application for the removal of arsenic from water by using domestic wastewater sludge. This method could be applied in some processes, which are already used in the wastewater treatment plant, to increase the total arsenic removal efficiency or decrease the load of expensive equipments or processes applied to the arsenic removal. The only requirements for the application of acclimated cultures were air and light. There are many different processes able to offer air and light in the water treatment plant, such as aerated equalization basin, the aeration basin, oxidation ditch, aerobic lagoon, and rotating biological contactors (RBC).

The results of this experiment found that these microorganisms preferred the operational temperature higher than 26°C but less than 48°C. Therefore, the wastewater treatment plant located in warm weather area would be able to obtain a better removal efficiency than the cold area. Influent wastewater pH not always had similar values. The experimental results showed that the acclimated cultures were able to survive and reduce arsenate in acid or base conditions. The tolerance of high arsenate concentration made these microorganisms to be used in high arsenic pollution areas. The investigations of effects of environmental parameters on arsenate removal were able to offer the data for the wastewater treatment plant to evaluate the possibility to combine the acclimated cultures technology into their treatment processes. These preliminary experimental results also verified the possibility of bioremediation for arsenate removal and many further researches could be continued to proceed based on this research.

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