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Uptake of Heavy Metals by Microorganisms: An Experimental Approach

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Contamination in drinking water is the most common form of environmental problems encountered in water resources management. Some contaminants, present accidentally in drinking water, are very difficult to remove, such as heavy elements that are products of industrial waste. Lead is one of the most difficult-to-remove elements. This paper proposes a novel process for removal of lead compounds contaminants from water. The proposed method shows great efficiency.

The technique uses thermophilic bacteria found in the United Arab Emirates near Al-Ain town located in Abu-Dhabi Emirates. These bacteria were isolated and used in a reactor coupled with a membrane system. The bacteria, the stirrer and the membrane housed in the reactor are arranged in a distinctive way to form the novel bio-stabilization process proposed in this research. This proposed technique could be used at low cost and with great confidence in the purification of drinking water. The system was found to be adequate for concentrations of lead in the range of 5–40 ppm. At the end of the operation the lead concentration reaches the level allowed by the World Health Organization regulations.

Keywords bioremediation, lead, membrane reactor, thermophilic bacteria

Environmental problems associated with heavy metals are very difficult to solve in contrast to organic matters because incineration or biodegradation can transform the latter. As a fact, most of heavy metals have toxic effects on living organisms when exceeding a certain concentration. Furthermore, some heavy metals are being subject to bioaccumulation and may pose a risk to human health when transferred to the food chain (USEPA, 1987). The scientific literature shows that lead is one of the heavy metals that has been recognized as a potent human toxin with reports of many diseases, such as brain damages and mental disabilities associated with ingestion dating to the last century. In the last two decades environmental interests has induced much research concentrated and focused on the effects of toxic metals on the ground water because they ultimately reach

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and accumulate in plants and animals tissues (Environ, 1988). According to the water standards used by the World Health Organization, levels of heavy metals such as lead ions in wastewater must be controlled and reduced to set value (USEPA,1986). It is recognized that mine wastes have been generated for several centuries, and mining activity has accelerated significantly during the 20th century. Many studies were aimed at the following points:

1. Understand mechanisms of lead toxicity, using a variety of research tools, that may aid in extension of the observed human findings when extrapolation is required;
2. Measure exposures of a specific population to lead from various sources to allow better definition of cumulative exposures to lead;
3. Improve methods for analyzing and monitoring lead in drinking water; and
4. Develop a process that can remove lead from drinking water.

The last point is especially related to this research; therefore, a review of the studies in this area is extremely of great benefit. Chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis and solvent extraction are the most commonly used techniques for the removing of heavy metals ions from dilute solutions. Furthermore, these techniques of purification are often inappropriate or expensive, particularly when unwanted heavy metals are present in very low concentration or in large solution volume. For instance, some of the proposed methods for removal of heavy metals, like adsorption on zeolites, clay minerals, and synthetic organic ion exchangers, have its shortcomings. Zeolites tend to decompose in the presence of high pH; clays cannot sustain selectivity at high sodium concentration (Amphelett, 1964). Furthermore, these traditional techniques present significant disadvantages, such as energy requirements, and are very expensive when the contaminant contents are in the range of 10–100 mg/l (Wilde and Benemann, 1993). Thus, for many years chemical engineers have been attracted to new techniques of water treatment requiring less energy and less pollutant. One of these techniques is the use of micro-algae and microorganisms as biosorbents to degrade or detoxify hazardous. Avery and Tobin (1992) reported the use of Saccharomyces Cervisiae in order to adsorb divalent metal cations in dilute solutions into the cell walls, both alive or denatured. They reported particularly high selectivity for Sr^2 atoms. Khoshmanesh and co-workers (1995, 1997) used green microalgae in the removal of heavy metals from polluted waters. Maeda and Skaguchi (1990) have indicated the importance of algae and their role in the bioremediation of toxic metals. Aladhab and co-workers (1997) used the same technique as part of a process that was proposed to remove traces of radioactive elements found in water. The algae applied in the process were collected from fresh water coming from a hot spring located in Al-Ain in the Emirate of Abu Dhabi.

It has been known for a long time that various living and dead microorganisms can remove heavy toxic ions from solutions (Sterritt and Lester, 1996). In addition, their applications are important in the general environment and in areas where potential exists for both clean wastewater and heavy metals recovery (Khummongkol, 1982). The methods are now recognized not only as viable alternatives but a desirable alternative and/or addition to the traditional remediation technologies. The biosorption of heavy metals ions by microorganisms is a promising property with a great potential for industrial applications. The mechanisms of the biosorption are beyond the scope of this research and can be found elsewhere (Chang et al., 1995). Furthermore, a great deal of research has been conducted in the area of mesophilic and thermophilic bacteria in the context of leaching and other forms of mineral extraction (Gilbert et al., 1988). At least 25% of all

copper produced worldwide, for instance comes from bioprocessing with mesophilic or thermophilic bacteria (Moffet, 1994). Metals in insoluble minerals are solubilized either directly through mineral metabolic activities or indirectly by chemical oxidation brought on by products of metabolic activity, mainly acidic solutions of iron (Marsden, 1992). Most of these bacteria prefer low pH conditions. One of the best-known mesophiles is the Thiobacilli family. These bacteria are capable of catalyzing mineral oxidation reaction (Hughs and Poole, 1989) is the most studied organism relevant to leaching of metal sulfides. This strain of bacteria is most active in the pH range 1.5–3.5, with an optimum pH of 2.3. Even though it is generally recognized that Thiobacillus ferrooxidans bacteria can survive at temperatures ranging from 30–37°C, no effort has been apparently made in trying to identify this stain of bacteria in hot climate areas. However, if themophiles can survive in the presence of heavy metals contaminants, their usefulness can be extended to bio-remediation. Leighton and Forster (1998) studied the effect of heavy metals on a thermophilic methanogenic up flow sludge blanket reactor. They showed that lead has the greatest effect on biogas production and, on the basis of the volatile fatty acid production, appeared to act immediately on acidogenesis and move slowly on the methanogenic bacteria. The present work is part of the studies carried out at the U.A.E. University by Al-Maghribi et al. (1998, 1999) and Zekri and Chaalal (2000) in the field of using themophilic bacteria to reduce asphaltene content in heavy oil. The investigation is aimed at the development of a new process for the reduction of lead concentration in drinking water employing the same thermophillic bacteria isolated from the UAE environment. It introduces a novel technique that uses a combination of a membrane system and thermophilic bacteria. The technique was tested and found to remove lead and reduce its concentration to an acceptable level.

Material and Methods

Bacteria

Two strains of bacteria, both belonging to the Bacillus family, were isolated from the local United Arab Emirates hot water streams. The temperature of the water in this region varies from 30 to 70°C. These bacteria were the only kind that survived the harsh temperature and salinity conditions prevailing in the environment.

Medium

The medium growth similar to the medium quoted in reference (Al-Maghrabi et al., 1999) was prepared in 100-ml sterilized bottle. It was composed of 1 ml of indigenous thermophilic bacteria solution, 90 ml of double distilled water. One g of nutrient (extract of yeast powder) was added to the medium. The medium was well mixed, but was not subjected to any further agitation. Also, the culture medium was exposed to different temperature conditions (25–35°C).

Chemicals

Riedel de Haen Germany and Lead (II) acetate supplied by Fluka Chemika Switzerland supplied the Lead (II) nitrate. The stock solutions of lead were prepared by dissolving the chemicals in doubly distilled water and the pH of the two solutions was measured before and after the addition of the bacterial suspension.

Computer Image Analyzer

A computer image analyzer was used to measure concentrations of bacteria in the culture. Several methods are available to determine the growth of bacteria in the culture (Seeley et al., 1991). The simplest method is by microscopic enumeration. This process is commonly used because it uses ordinary laboratory equipment, is inexpensive, and is relatively fast. However, this method has been recently found to be prone to errors (Livingston, 1994; Kaleli and Islam, 1997). In order to count bacteria in this study, an Image Analysis System (IAS) was used. The basic system consists of a high resolution video camera mounted on an optical microscope, an image processor, a Pentium PC, a high resolution image monitor, and high resolution text monitor. The image is visualized with the video camera through a microscope lens. The signal from the video camera is in analogue form and must be digitized so the computer is able to store the image in the library. Therefore, the signal has to be processed by an analogue to digital converter. However, the signal has to be converted into its analogue form in order for the image to be produced in the monitor. In addition, the IAS has the facility of sharpening, edge detection, threshold function, transition filter, chord sizing, erosion and dilatation. Once binary images are produced from an accepted microphotography, a feature count is performed. This simply means that the desired bit plan is selected and the feature count option is activated. A count instantly appears on the screen. Also shown is the area covered by the microscope, enabling one to determine the volume by knowing the depth of the slide. The depth can assumed to be the same as the one used for microscopic enumeration, viz. 0.100 mm. In addition, a correlation must be found between microscopic enumeration and IAS count. This was done by Livingston (1994) and was used by Kaleli and Islam (1997). The same technique was employed in this study.

Atomic Absorption

Concentrations of lead were measured using the Atomic Absorption (AA) technique. The equipment (spectrophotometer Perken_Elmer Model 2380) was available in the Department of Chemical Engineering of the UAE University.

Membrane Reactor

An outline scheme for the experimental facility built for this study is shown in Figure 1. The bioreactor is a very successful stirred reactor that was built specially to be used for this research. It consists of two cylindrical cell compartments of 150-ml volume each separated by a membrane system. The membrane system consisted of two kinds of membranes. The first membrane was a polypropylene flat sheet membrane of a thickness 70–110 μm and pore size 0.1-micron supplied by Enka AG, Germany. The second membrane 1 was a Millipore HA type (0.45 μM). The two membranes were housed in a box having a wall thickness of 1 mm. The box was placed in a groove through which it could be sled in and out. Turbidity test showed that the membrane system stopped any infiltration of bacterial suspension into the cell that contains no bacterial solution. Surface plugging did not occur because of the limited amount of bacterial suspension through the body of the membrane system. The active membrane system had a plane area of 16 cm^2 and a thickness of 0.3 mm. The whole system was perfectly sealed with special glue in order to avoid any eventual leakage. A mechanical stirrer continuously agitates the cell that contains a known population of thermophilic bacteria mixed with

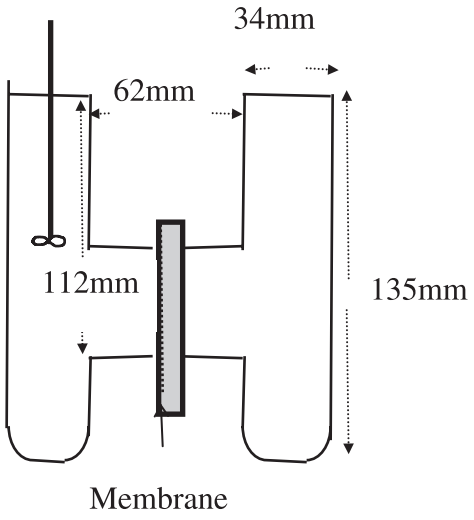

Figure 1. Schematic diagram of two compartments bioreactor.

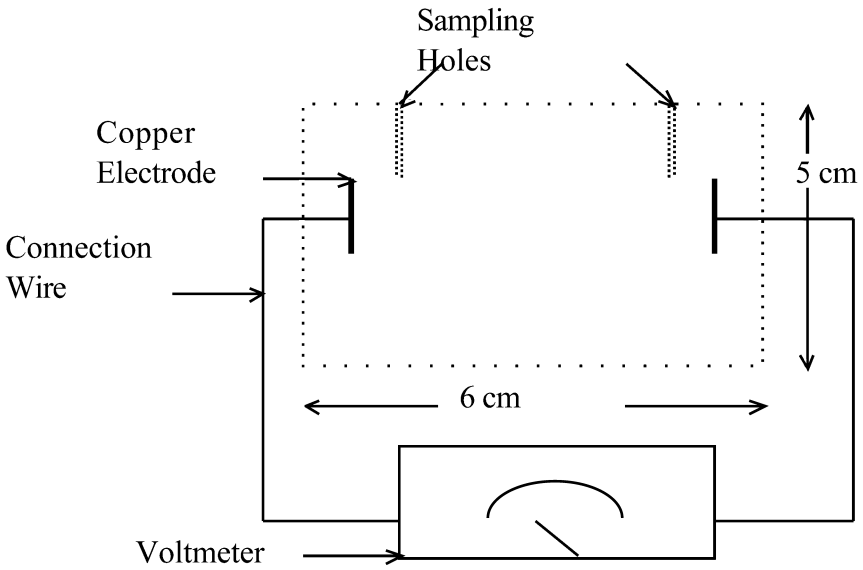

Figure 2. Schematic diagram of the electrical cell.

the lead solution whereas the other cell contains only the lead solution, which was not submitted to any kind of mixing.

Electrical Cell

The electrical cell, constructed from perspex and glass, consisted of a small rectangular box of internal rectangular cross section 10 cm $\times$ 1.5 cm and 7 cm deep as shown in Figure 2. The current is supplied to the cell through two copper electrodes of 0.5 cm^2

fixed against the small walls. The box lid was sealed with a neoprene rubber gasket and was held in place by special glue. The lid has two glass ports 0.5-mm diameter fitted with a quickfit screw closure for sampling by means of a siring.

Procedure

The tests were carried out in 3 steps as follows:

Step 1

In this experiment, 90 ml of the bacteria solution containing a known population of bacterial suspension was investigated in the electrical cell. Five volts were maintained across the cell. Samples were withdrawn from the cell by means of a 1 mm syringe near the electrodes via the ports, and microphotographs were taken for these samples at different times in order to visualize the charge of the bacteria type and concentrations at different electrodes.

Step 2

This step elaborates the growth of bacteria when the reactor contains a lead solution. For this case, a solution containing 30 ppm of lead was prepared from double distilled water and lead acetate. Both cells of the reactor were filled with approximately 130 ml of this solution then 10 ml of thermophilic bacterial suspension were added into cell 1 (10 ml of bacterial suspension represents a bacteria count equivalent to 12×10^3 cells/ml). In order to maintain cell 1 well agitated, a mechanical stirrer rotating at a speed of 50 rpm (this rate was found suitable for the mixing in cell 1) was introduced in cell 1 at a position located at 62 mm from the top, as shown in Figure 1. The solution of lead in cell 2 was not subject to any agitation. Even though the concentration of lead was high, it was needed to observe biodegradation of lead and the resistance of bacteria to lead. During the entire operation of the reactor, microphotographs were randomly taken from both cells. Samples were withdrawn and tested by atomic absorption for lead content, and were observed under the microscope in order to asses the growth mechanism, as well as to determine the bacteria growth rate and the rate of lead removal.

Step 3

This step was conducted to observe the rate of lead removal as function of initial lead concentration. The investigation of the rate of lead removal by the bacteria was carried out in two sets of experiments. The first set implicated the study of four solutions containing initial lead concentration of 7, 12.5, 20, and 30 ppm, respectively. The two cells of the reactor were filled with the same lead solution. A two-ml of bacterial suspension (3×10^3 cells/mil) was added to cell 1, which was continuously agitated. Samples were withdrawn from both cells and analyzed with atomic absorption as function of time. The same batch of experiments were repeated using 10 ml of bacteria (10 ml of bacteria equivalent to 12×10^3 cells/ml).

Results and Discussion

The reduction of lead (II) concentration by thermophilic bacteria is influenced by several factors. This embodies the bacteria population, the surface property of the bacteria and

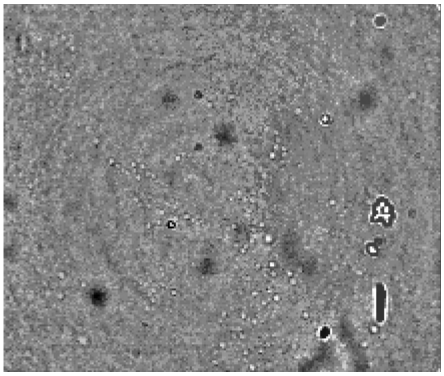

Figure 3. Electron photo of water samples obtained from the bottom of bioreactor at the end of run #1 (×1000).

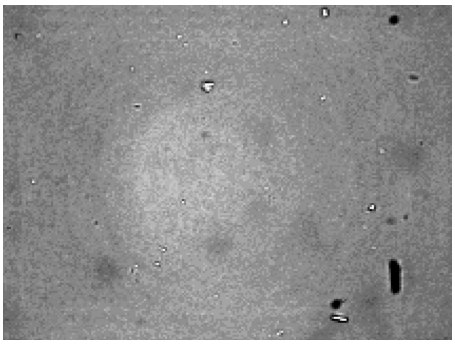

Figure 4. Bacteria concentration at the top of the bioreactor at the end of run 1 (×1000).

its electrical charge, the temperature of the solution, the pH and the initial concentration of lead (II). The results obtained in this study are discussed as follows:

The Behavior of the Bacteria in an Electrical Field

The microphotographs taken in step one are presented in Figures 3 and 4. These microphotographs indicated that the negative pole attracted all the rod shaped bacteria, whereas the rounded shape bacteria were gathered near the positive pole. This phenomenon clearly showed that the round shaped bacteria are carrying a negative charge and the rod type bacteria is carrying a positive charge. Therefore, the lead ions must be attracted by the rounded bacteria and repulsed by the rod type bacteria.

Bacteria Growth in 30 ppm Lead Solution

The bacteria growth and the rate of lead removal are shown in Figures 7 and 8 for a 30-ppm lead solution in the presence of 10-ml bacterial suspension. Figures 7 and 8 represent the bacteria activity in cell 1 and cell 2, respectively. Even though the lead concentration dropped considerably in both cells after 24 hours, the growth rate in cell 1 showed both viability and enhanced bacteria growth in the presence of high lead concentration with a decrease in the bacteria count at the beginning of the experiment. This

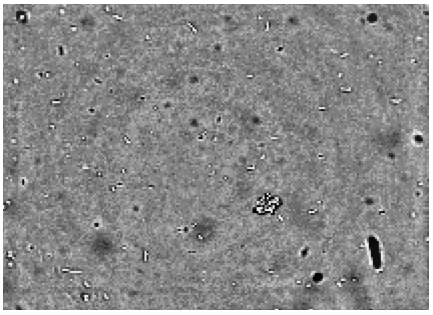

Figure 5. Microphotograph showing the gathering of the rod-shape bacteria near the negative electrode ($\times 1000$).

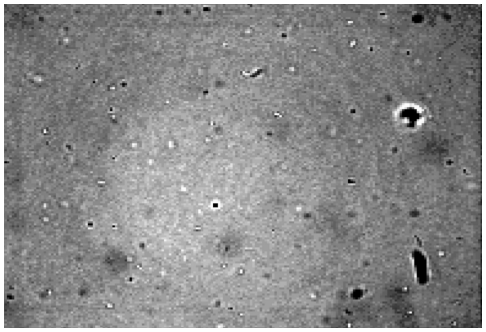

Figure 6. Microphotograph showing the gathering of round bacteria near the positive electrode ($\times 1000$).

phenomenon leads one to believe that the bacteria are experiencing an adaptation period to the lead solution (adaptation period that lasted for almost 1 hour). After this period, a significant exponential increase in bacteria count has been noticed with a maximum growth equals to $17 * 10^3$ cells/ml after 8 hours followed by a constant growth for the remaining time. At the same time, the lead concentration profile showed an exponential decrease during the first 8 hours, reaching a value of 6 ppm followed by a constant decrease that reached 1.6 ppm after 24 hours. These observations are consistent and prove the existence of a significant transfer of lead from the solution to the bacteria cells if the bacteria can survive for a longer period. Furthermore, these observations also showed that the amount of bacteria in the solution is a limiting factor in removal of lead from the solution. Hence, a higher bacteria count is needed to reduce the lead content to the desired level and to diminish operation period to an accepted time. The same behavior was observed in Figure 6 but at lower magnitude because the bacteria count in cell 2 was very low. Furthermore, the microphotographs 3 and 4 where taken at the end of the process in order to visualize the bacteria presence. Microphotograph 3 shows the bacteria activity at the bottom of cell 1. Note in this microphotograph all the bacteria are observed to be located at the bottom near the membrane. Microphotograph 4 showed few bacteria present at the top of the cell. All the bacteria observed under the microscope were alive and moving around freely in the lead environment. Consequently, they must be lead resistant and responsible of the lead decrease in the bioreactor.

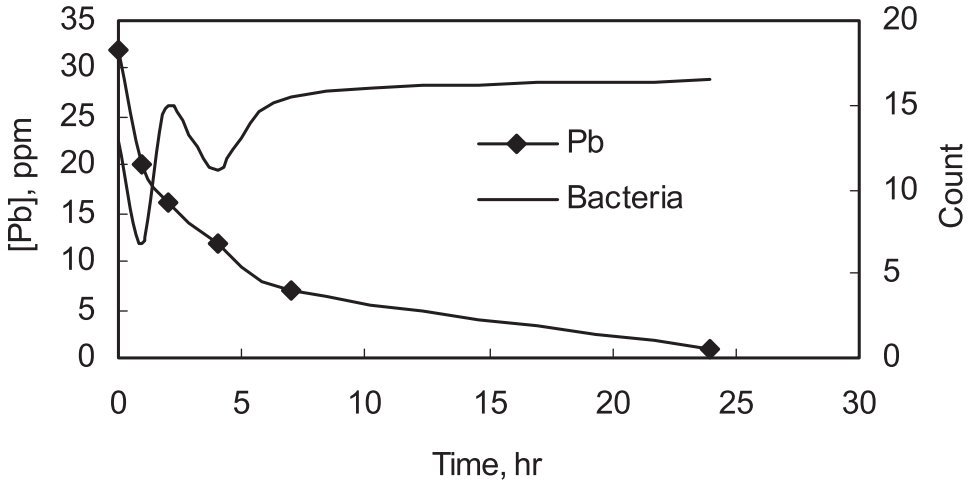

Figure 7. $[Pb]$ concentration and bacteria growth in cell 1 in 38 ppm Pb solution, 10 ml bacteria.

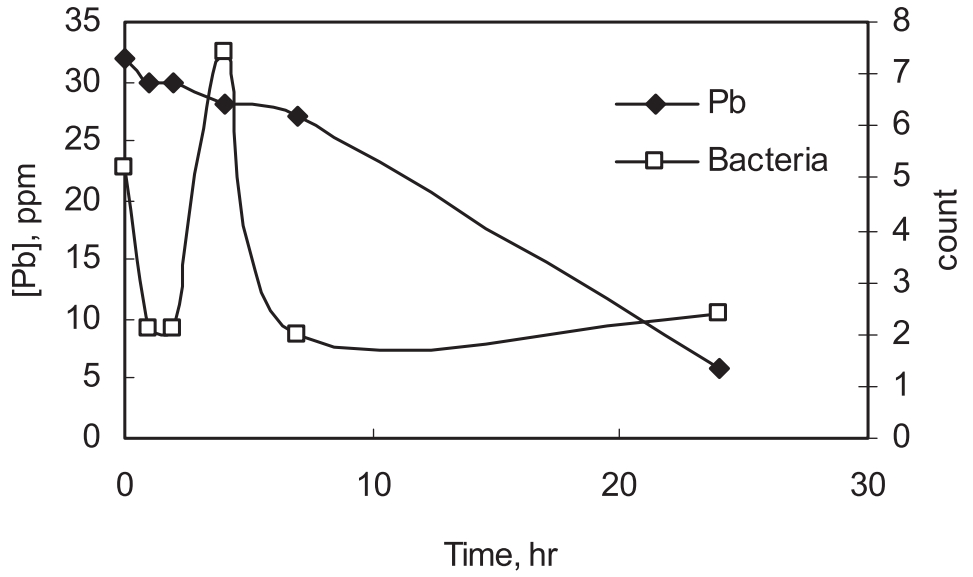

Figure 8. $[Pb]$ concentration and bacteria growth in cell 2 in 38 ppm solution, 10 ml bacteria.

The Rate of Lead Removal

To discuss the rate of lead removal meaningfully, it has to be defined precisely. The rate of lead removal is a positive quantity that expresses how the concentration of the lead changes with time. To illustrate what this means, consider the adsorption process of lead by the bacteria.

$$[BA] + [pb^{2+}] \quad [BApb^{2+}]$$

where $[BA]$, $[pb^{2+}]$, and $[BApb^{2+}]$ are bacteria concentration, lead concentration in the solution and the lead concentration on the bacteria. As we can see from the above

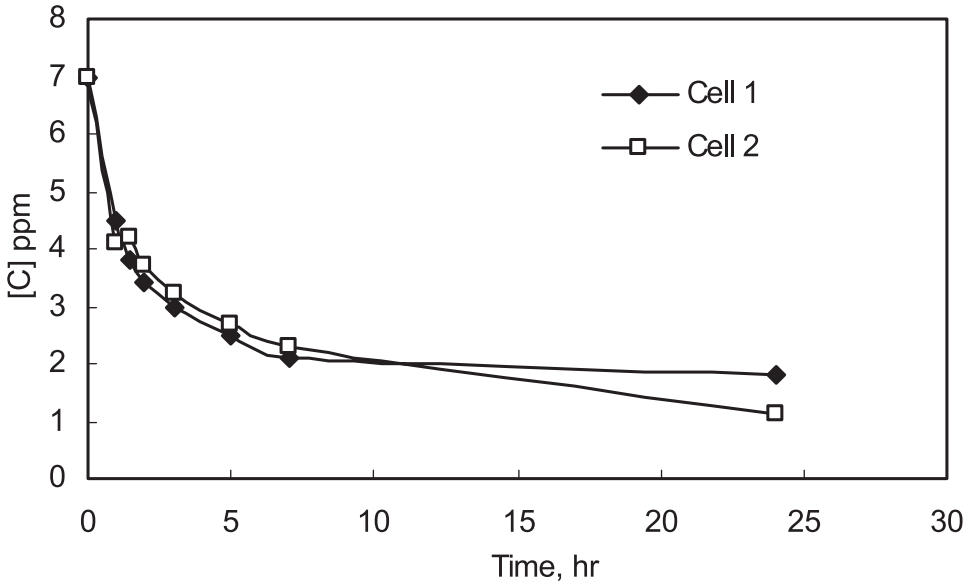

Figure 9. Lead concentration vs. time, $Pb = 7$ ppm 2 ml bacteria ($3 * 10^3$ cell/ml).

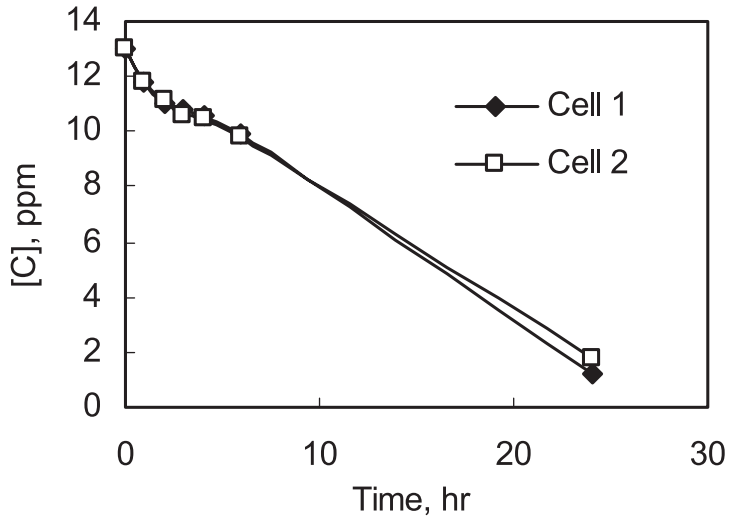

Figure 10. Lead concentration vs. time, $Pb = 12.5$ ppm 2 ml bacteria ($3 * 10^3$ cell/ml).

equation, the concentration of lead in the solution decreases at a rate r. Rate r can be expressed as $-\Delta[pb^{2+}]/\Delta t$ where $\Delta[pb^{2+}]$ refers to the change in concentration in ppm, and the minus sign in front of the lead term takes account of the fact that the lead decreases in the solution as the process progresses. The results of rate investigation are shown in Figures 9, 10, 11, and 12. Note that all the figures demonstrate the lead concentration in the solution at various times when 2 ml of bacterial suspension was added. In Figure 9, the lead concentration decreased from 7 ppm to 4.5 ppm after one hour, which corresponds to an average rate of lead up take of 0.36 mg/h. In the following

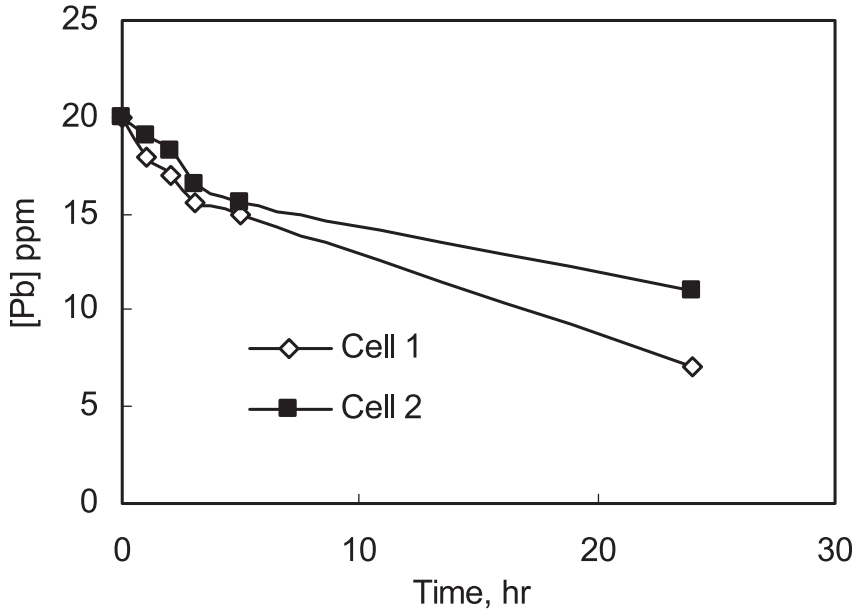

Figure 11. Lead concentration vs. time, $Pb = 20$ ppm 2 ml bacteria ($3 * 10^3$ cell/ml).

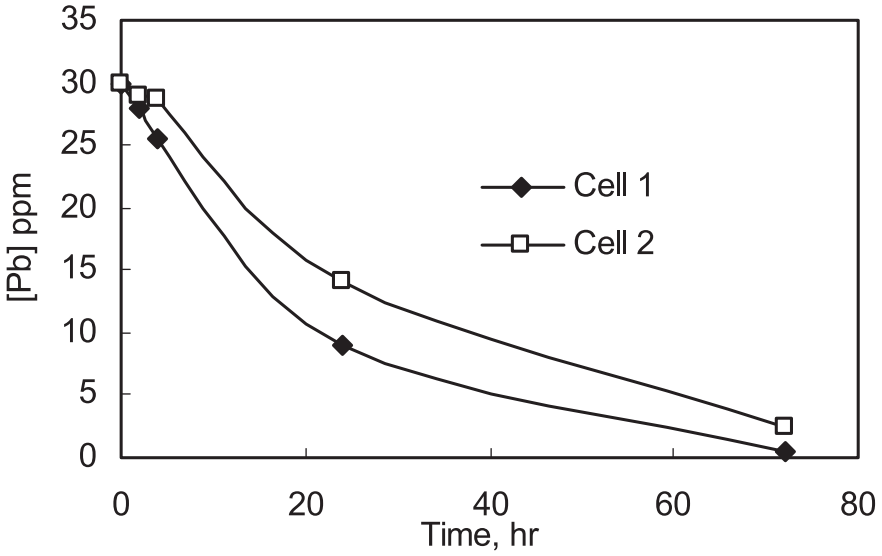

Figure 12. Lead concentration vs. time, $Pb = 30$ ppm 2 ml bacteria ($3 * 10^3$ cell/ml).

seven hours, the rate was slow and reached a constant value equal to 0.1 mg/h. When the concentration of lead was increased to 12.5 ppm, as shown in Figure 10, a linear decrease in lead concentration was observed. The lead removal rate was constant and equal to 0.2 mg/h. Furthermore when solutions of lead containing 20 ppm was investigated, as shown in Figure 11, the final lead concentration has dropped to 11 ppm in cell 1 and 13 ppm in cell 2 after 24 hours. However, using 30 ppm solution the final concentration

reached 10 ppm in cell 1 and 14 ppm in cell 2 after 12 hours. The final concentration of 2 ppm in cell 1 and 4 ppm in cell 2 was obtained after 72 hours, as shown in Figure 12.

Effect of pH, Bacteria Population and Initial Lead Concentration

Effect of pH. The two lead solutions from two different lead sources are lead nitrate and lead acetate. The lead nitrate solution was acidic and lead acetic solution was basic. All the experiments performed using acidic solutions did not give any positive response because the growth of bacteria in such solutions was difficult to achieve. Hence, it was decided to use the basic lead solution (lead acetate) and the experimental results have shown that the bioreactor exhibits excellent performance when lead concentrations in the range 7–30 ppm were employed (pH in the range 7–8).

Effect Bacteria Population. The effect of the bacteria population was investigated in 30-ppm and 38-ppm lead solutions. Figure 13 shows the effect of 2 ml ($3 * 10^3$ cell/ml) and 10 ml ($12 * 10^3$ cell/ml) bacteria solutions on the lead concentration as function of time. Comparing the two curves, it is clear that the lead concentration decreases with increasing bacteria population for a fixed less concentration and time. The 10 ml bacterial suspension system showed an exponential decrease; meanwhile the 2 ml bacteria suspension exhibit nearly a linear decrease as function of time.

Effect of Initial Lead Concentration. The overall efficiency of separation processes is directly related to the cost of the operation. In the case under investigation, it was found that the final concentration and the time taken to reach it were dependent of initial concentration. Thus, the results in Figure 14 shows the percent removal of lead at different

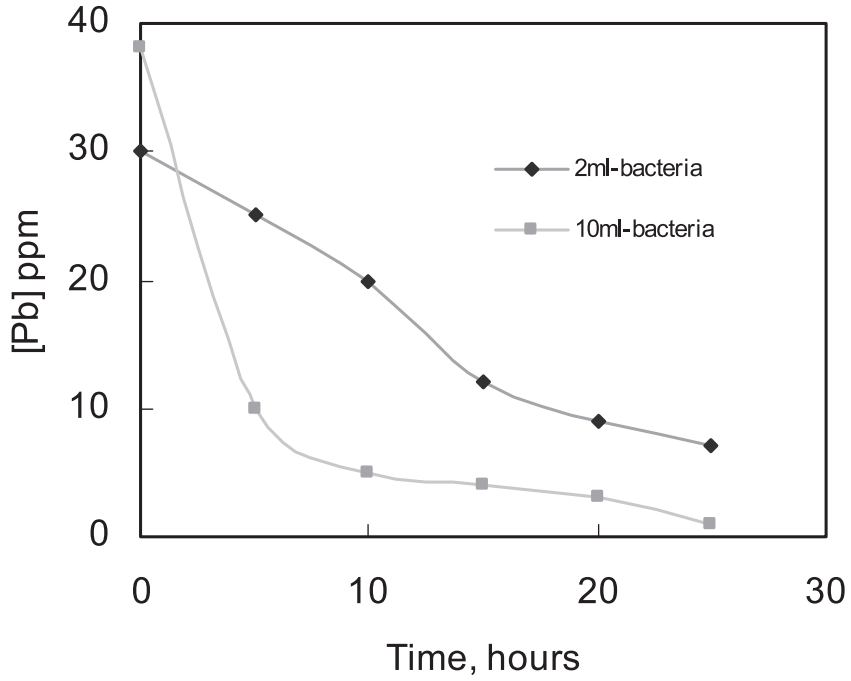

Figure 13. Effect of bacteria concentration on lead concentration.

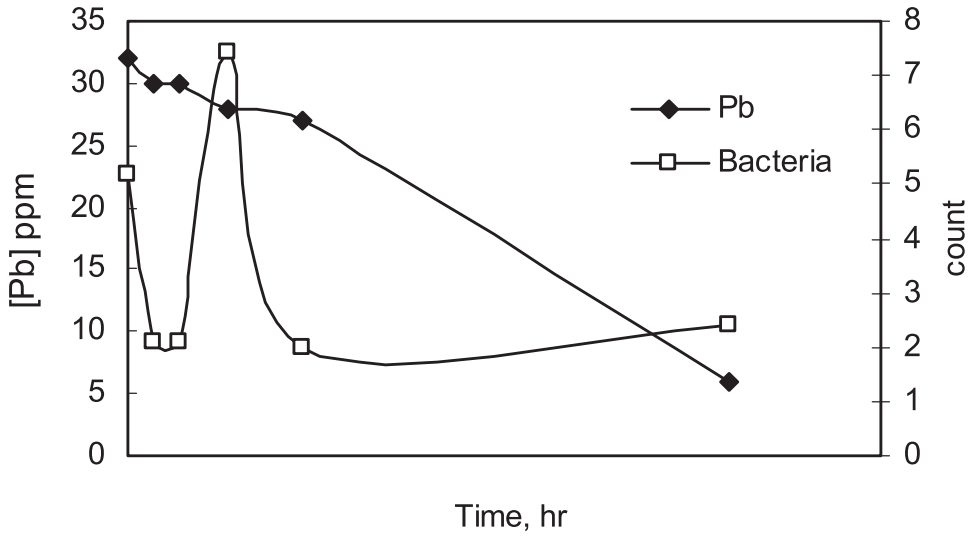

Figure 14. The effect of initial cell lead concentration on % reduction of cell concentration, 25 hours basis.

initial lead concentrations and amount bacterial. The system exhibits a small reduction in lead concentration at a low initial lead concentration of less than 7 ppm. At initial concentrations higher than 7 ppm, increasing the initial lead concentrations resulted in a significant reduction of lead concentration for both bacteria populations employed in this study. The optimum initial lead concentration for the system containing 2 ml of bacterial suspension was around 13 ppm and corresponds to 92% reduction in lead concentration. On the other hand, when the bacterial suspension was increased to 10 ml the optimum initial lead concentration for the same percent of lead removal of the system was found to be around 35 ppm.

Conclusion

A novel technique was developed in this project for water purification of lead. Laboratory studies showed that the bioreactor has the potential for significant reduction in equipment volume and weight and also in its simplicity compared to other devices used to remove heavy metals from water. Performance is dependent on the lead concentration and amount of bacteria used in the experiment.

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