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Bioleaching of heavy metals contaminated sediment by pure and mixed cultures of *Acidithiobacillus* spp.

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ABSTRACT

The aim of this study was to investigate the effect of bacterial strains on the bioleaching of Cr, Cu, Pb, and Zn from contaminated sediment samples taken from a stationary point in Izmir Inner Bay. Single and mixed cultures of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* were used separately to achieve metal solubilization in suspension. Of the three trials tested, single culture of *A. thiooxidans* decreased the pH to 0.7, resulting in the highest metal solubilization ratios. Solubilization efficiencies of Cr, Cu, and Zn were high (>80%) with *A. thiooxidans* but Pb could be solubilized to a ratio of 63%. The efficiency of metal solubilization from contaminated sediment in decreasing order is: Zn>Cu>Cr>Pb. In order to check for the mass balance, metals remaining in the residual sediment were also determined. The effect of bioleaching on sequentially extracted (chemically distributed) metals was investigated in addition to the metal solubilization efficiencies. For most of the trials, it is observed that, the stable forms of heavy metals were transferred and accumulated in more soluble forms during bioleaching and they directly solubilized in the water when they were finally transferred to the exchangeable and/or reducible form.

1. Introduction

Metal contaminated sediments are considered to be one of the main sources of pollution in aquatic environments. They undergo a number of reactions in the system including sorption and precipitation, and they are greatly influenced by redox conditions in the sediments [1]. Under certain conditions, metals in sediments can be released to overlying waters and taken up by the organisms. Since metals discharged in to water bodies present a major problem in aquatic environments, remediation technologies of metal contaminated sediments have gained great consideration through environmental studies.

Treatment of metal contaminated sediments can be achieved by either physical or chemical methods. Although these techniques have been extensively applied in practice, they show some limitations such as low efficiency and high cost. The bioremediation of heavy metals has received a great deal of attention in recent years, not only as a scientific novelty but also for its potential application in industry [2]. Bioremediation primarily uses microorganisms or microbial processes to degrade and transform environmental contaminant into harmless and less toxic forms [3].

Bioleaching is an innovative, environmentally friendly, simple, economical and effective method, which has been adapted from the mining industry and used in various metal removal operations from soils, sediments, and sludges. The mechanism takes place under aerobic conditions, and the bacterial activity either leads to the production of sulfuric acid, resulting in the acidification of the sediments and extraction of heavy metals adsorbed on sediment particles (indirect mechanism), or to the direct solubilization of metal sulfides by enzymatic oxidation stages (direct mechanism) [4].

The bacteria most active in bioleaching belong to the genus *Acidithiobacillus*. These are gram-negative, non-spore forming rods, which grow under aerobic conditions. The thiobacilli are mesophilic bacteria, which grow best at temperatures of 25–35 °C. Most thiobacilli use carbon dioxide from the atmosphere as their carbon source for the synthesis of new cell material. The energy derives from the oxidation of reduced or partially reduced sulfur compounds; including sulfides, elemental sulfur, and thiosulfate, where the final oxidation product is sulfate [5]. The three important environmental conditions for the active growth of thiobacilli are the acid pH values (to support acidification), high redox potential (maintained by aeration), and the availability of substrate (sulfur) [4].

The most widely used bacteria in bioleaching are *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* [6]. Since bacterial leaching is carried out in an acid environment (at pH 1.5–3) where most ions remain in solution, the acidophilic species *A. ferrooxidans* and *A. thiooxidans* are of particular importance. Other thiobacilli are also able to oxidize sulfur and sulfide but they grow only at higher pH values at which metal ions are not maintained in solution [7]. Among the above mentioned species, *A. thiooxidans* use reduced forms of inorganic sulfur but not ferrous iron energy sources. In addition, they are highly acidophilic (pH 0.5 to 5.5, optimum pH 2 to 3.5), and may

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decrease the pH in the medium to 1.5 to 1 and even lower [8]. On the other hand, *A. ferooxidans* differ from all other thiobacilli by the fact that besides deriving energy from the oxidation of reduced sulfur compounds, they use ferrous iron as the electron donor. The cells are mesophilic (10 to 37 °C, optimum 30 to 35 °) and acidophilic (pH 2.3 to 4.5, optimum pH 2.5 to 2.8) species. In the absence of oxygen, *A. ferooxidans* are still able to grow on reduced inorganic sulfur compounds using ferric iron as an alternative electron acceptor [7].

Various studies have been carried out by scientists investigating the effects of thiobacilli species on bioleaching efficiency. In some studies only mixed cultures of *A. thiooxidans, T. thioparus,* and *A. ferooxidans* were used [9–11] while some of the studies were conducted by using single cultures of *A. thiooxidans* and *A. ferooxidans* [12–14]. The studies are either completed by using treatment sludge and metal ores, or they are made by using sediment but the cultures are used individually or mixed only. Since sediment is something else than a metal ore or sludge, and the behavior of the bacteria may differ, especially if more than one heavy metals are considered. The objective of this work was to study and compare the bioleaching efficiencies for Cr, Cu, Pb and Zn from sediment taken from the Izmir Inner Bay in Turkey. Different from previous works, treatment efficiencies using both mixed and pure cultures of two thiobacilli species, *A. thiooxidans* and *A. ferooxidans,* were investigated for the same sediment, under the same conditions. There are very few studies including the metals partitioning in the sediments before and after bioleaching and also investigating the metals mass balance in the system during bioleaching. In this study, metals remained in the sediments were determined as well as the metals solubilized in water, to check for the mass balance over the process. In addition to the detection of total metal removal efficiencies at the end of the process, changes in the chemical distribution of the metals remaining in the sediments were also measured; and it was possible to make a more realistic estimate of the bioleaching efficiencies of the bacterial strains for the metal contaminated sediments.

2. Experimental methods

2.1. Sediment

The sediment sample was taken from a station with a 15 m water depth in the Izmir Inner Bay located on the eastern zone of Aegean Sea (the coordinates were noted as 38° 26′ 18″ N and 27° 06′ 06″ E). A Van Veen Grab sampler was used for sediment sampling. In order to determine the recent pollution on the sediment surface, 10-cm thick top layer of the sediment samples was collected with spatulas and deposited into plastic bags. Samples were dried, and granulated to <60 μm in order to provide homogenization for further chemical operations.

2.2. Chemical analysis

Wet sediment sample was used to determine the pH, water content, organic matter content, and grain size distribution. The pH of the sediment sample was determined according to the EPA Method 9045C [15]. Water and organic matter contents were calculated via gravimetric analysis. The grain size distribution of the sediment sample was determined via wet sieving by using 4 sieves with different hole sizes.

The dried and granulated bulk sediment sample was characterized for its total and chemically distributed metal concentrations. A Questron MicroPrep Q20 Microwave Digestion System with four digestion vessels was used for the digestion. The metals in the sample were extracted by using a mixture of concentrated HNO_3, HF, and HCl, and the accuracy of the heating protocol was tested with Estuarine Sediment-1646-A as the standard reference material [16].

Chemical partitioning of Cr, Cu, Pb and Zn was determined by using a scheme developed by the European Commission for Standards, Measurement and Testing (ECTS&T) [17] that offers a practical procedure using three main stages. In this technique, metals are divided into four fractions such as: exchangeable and acid soluble, reducible, oxidizable, and residual forms which indicate the binding properties of metals on sediment particles. The exchangeable and acid soluble phase presents the loosely bound, labile or exchangeable metals, and these metals are extracted from the sediment mass by the application of CH_3COOH (0.11 mol/L acetic acid). The reducible fraction of the metals are the metals bound to iron and manganese oxides which may be released if the sediment changes from oxic to anoxic state. This phase is determined by using $NH_2OH \cdot HCl$ (0.5 mol/L hydroxylamine hydrochloride) as extraction chemical. The third stage consists of the application of H_2O_2 (30% hydrogen peroxide) and CH_3COONH_4 (1 mol/L ammonium acetate solution). This step allows the extraction of oxidizable fraction (metals bound to organic matter). Finally, the difference between the total metal concentration and the sum of the three fractions above gives the metals in the residual fraction. These are the metals bound within the crystal matrix, and they are not expected to be released under normal conditions in nature [18]. The procedure started with the extraction of 1 g of dry sediment sample, and each sequential extraction was applied the solid residue remaining from the previous stage. The "selective" extractions were conducted in 50-mL polypropylene centrifuge tubes to prevent or minimize losses of solid material.

2.3. Microorganisms

The microbial cultures of *A. ferrooxidans* (11477) and *A. thiooxidans* (11478) were supplied by DSMZ (Deutche Sammlung von Mikroorganismen und Zellkulturen GmbH) and used throughout this study. The liquid media-Medium 271 [19] was used to cultivate both species. The media were composed of 2 g $(NH_4)_2SO_4$, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of KCl, 0.01 g of $Ca(NO_3)_2$ and 1000 mL of distilled water. In addition, 8 g of $FeSO_4 \times 7\ H_2O$ were used for *A. ferrooxidans*, and 10 g of elemental sulfur was added to the medium as the substrate for *A. thiooxidans*. The pH was adjusted to 2.0 with dilute H_2SO_4 (1 mol/L). The basal medium, ferrous sulfate, and elemental sulfur were sterilized separately, cooled and mixed. The cultures were inoculated for growth of single and mixed subcultures in 500 mL shaker flasks at 170 rpm and 30 °C before being used in leaching experiments.

Plate count method on sterile Petri plates was used for the enumeration of the bacteria. TSM 1 Medium and Medium of Starkey [20] were prepared separately and poured in to the Petri plates on diluted cultures of *A. ferooxidans* and *A. thiooxidans*. The cultures were then incubated at 30° C and the cell concentrations were counted at the end of the incubation time.

2.4. Bioleaching experiments

Before the bioleaching experiments, the subcultures of the microorganisms were acclimated to the sediment sample. The inoculum of 1% (v/v) of bacteria (*A. ferrooxidans, A. thiooxidans* and the mixed culture) was each transferred to 150 mL of water containing 2% (w/v) total solids of autoclaved sediment. A 0.5% (w/v) elemental sulfur was added to the suspensions to be used as substrate, and the cultures were incubated in 500 mL flasks in the shaking incubator at 30 °C. The pH values were determined during the acclimation process by taking samples from the mixtures at regular intervals. The acclimation process ended when the pH in the mixtures dropped below 2 [9].

The bioleaching experiments were carried out in 1000 mL flasks with 250 mL reaction volume containing the dilution medium necessary for the bacterial growth. Two of the bacterial cultures