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Bioremediation of zinc using *Desulfotomaculum nigrificans*: Bioprecipitation and characterization studies

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ABSTRACT

Desulfotomaculum nigrificans, a typical sulfate reducing bacterium (SRB) was successfully grown in the presence of 12–210 mg/L of zinc. Complete bioremoval of zinc was achieved in 2 days for 12 mg/L while the bioremoval efficiency was about 70% in 40 days in the presence of 210 mg/L initial concentration of zinc, attesting to the inhibition of bacterial cell growth at higher zinc concentrations. The bioremoval mechanism was predominantly governed by bioprecipitation with biosorption contributing to a minor extent. The amount of protein present in the extracellular secretions obtained by growth of SRB in modified Baars' medium devoid of iron was the highest followed by those obtained in the presence of zinc or iron, in that order. Bioremediation studies carried out using a specially designed set-up, facilitating the transfer of biogenically produced hydrogen sulfide gas to a separate precipitation assembly, confirmed that zinc could be successfully precipitated from its corresponding sulfate solution, varying in concentration from 10 to 20,000 mg/L. Detailed characterization of the various zinc sulfide precipitates by EDAX and X-ray diffraction analysis conformed to wurtzite structure. The isoelectric points of high purity zinc sulfide and that of chemically synthesized, biogenically produced and zinc sulfide precipitated using bacterially produced hydrogen sulfide gas (BPH-ZnS) were located at pH 3, 7.8, 2.8 and 8, respectively.

1. Introduction

Zinc is one of the metals found in effluents discharged from industries involved in galvanization, electroplating, manufacture of batteries and other metallurgical industries. Zinc in its metallic form has limited bioavailability and poses no ecological risk. However, zinc can react with other chemicals like acids and oxygen to form compounds, which can be potentially toxic and can cause serious damage to biological systems (Fosmire, 1990).

Although the removal of toxic metals from industrial wastewaters has been practised for several decades, the cost-effectiveness of the commonly used treatment technologies such as oxidation–reduction, filtration, electrochemical treatment, evaporation, ion exchange and reverse osmosis processes is limited. High reagent requirement and unpredictable metal removal are disadvantages of these methods. Further, strong and contaminating reagents are used for desorption, resulting in toxic sludge and secondary environmental pollution. Bioremediation, which involves the use of microbes to detoxify and degrade environmental contaminants, has received increasing attention in recent times to clean up a polluted environment (Iwamoto and Nasu, 2001; Kakonsen et al., 2003; Malik, 2004; Watanabe and Baker, 2000). Bioremediation, being in situ treatment, offers several advantages over the conventional chemical and physical treatment technologies, especially for diluted and widely spread contaminants (Eccles, 1995). The recent advances in

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molecular microbial ecology have provided a further impetus to environmental biotechnological approaches.

Treatment of metal containing wastewaters with sulfate reducing bacteria (SRB) is a promising alternative over chemical methods (Christensen et al., 1996). The major application of SRB to wastewater treatment is based on their ability to reduce sulfate to sulfide, which then reacts with most metals to form insoluble sulfides (Lyew and Sheppard, 2001). The potential advantages of metal sulfide precipitation include production of lower sludge volume and lower solubility products as compared to hydroxide precipitation (Peters et al., 1985; Venkatachalam, 1998). In addition, valuable metals can be recovered from metal sulfide sludges (Boonstra et al., 1999).

A scrutiny of the literature indicates that there is a paucity of fundamental data on the application of bioremediation techniques for the decontamination of zinc utilizing anaerobic microorganisms. Further, very few systematic studies have been carried out to understand the changes in the bacterial growth characteristics and the bioremoval mechanisms consequent to interaction with toxic heavy metals. In the present investigation, with the above objectives in mind, a typical sulfate reducing bacterium, namely *Desulfotomaculum nigrificans (Dsm. nigrificans)*, has been chosen for the bioremediation of synthetic solutions of zinc sulfate.

2. Materials and methods

2.1. Analytical reagents

Analytical grade $ZnSO_4.7H_2O$ was procured from E.Merck, Germany. All the chemicals used in the various analytical estimations and for the preparation of media were of analytical reagent grade and deionised water obtained from a Milli-Q system (Millipore, USA) was used in all the experiments. The resistivity of the water was $>10\,M\Omega\,cm$.

2.2. Analytical methods

The concentration of zinc was determined using a Thermo-Jarrell Ash, Video 11E atomic absorption spectrophotometer in an air–acetylene flame at 213.6 nm wavelength. The bacterial cell number was determined using a Petroff-Hausser counter with a Leitz phase contrast microscope (Laborlux K Wild MPS12). pH and Eh changes were also monitored periodically using a Systronics digital pH meter with appropriate electrodes. The reference electrode used was saturated calomel electrode and the redox potential values so obtained (E_{SCE}) were converted to Eh by adding 244.4 mV. Samples for determination of cell number and concentration of zinc were drawn by a sterile hypodermic syringe and needle and assayed immediately.

2.3. Organism and growth conditions

A pure strain of *Dsm. nigrificans* (NCIM 2834, NCIB 8788) species was obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. *Dsm. nigrificans* occurs as straight or curved rods and is an endospore forming heterotroph (Postgate, 1984). It was grown in modified Baars' medium (MB medium) devoid of iron of the following composition: K_2HPO_4-0.5 g/L; NH_4Cl-1 g/L; $CaSO_4$-2 g/L; $MgSO_4$-4 g/L; Sodium citrate (dihydrate)-5 g/L; Sodium lactate (60%)-46 mL; Yeast extract-0.5 g/L; $(NH)_4SO_4.FeSO_4.6H_2O$-0.5 g/L; $C_2H_3NaO_2S$-0.05 g/L. The pH of the medium was adjusted to 7 ± 0.05 using 1N KOH. All the procedures during preparation of the medium and inoculation were performed according to the modified Hungate's method for anaerobes (Miller and Wolin, 1974). Sodium thioglycollate was filter sterilized using 0.2 μm membrane filter and added to the medium just before inoculation. The microorganism was grown anaerobically in sealed and crimped serum bottles under nitrogen atmosphere at $35 \pm 2\,^{\circ}C$. The bottles were incubated in a Remi orbital shaker at 200 rpm (2.24 g).

2.4. Bioremoval studies

Bioremoval studies were carried out using *Dsm. nigrificans* cells in MB medium devoid of iron in the presence of 12 mg/L of zinc. The cells present in 25 mL of the fully grown culture in the exponential phase of growth were harvested by centrifuging at 12,000 rpm (8050 g) at 4 °C in a Sorvall RC-5B refrigerated superspeed centrifuge for 20 min. The cell pellet was washed with Milli-Q water twice and again centrifuged. The cell pellet so obtained was dispersed in fresh medium for all experiments.

The cell pellet used for the bioremoval experiments containing 12 mg/L of zinc was obtained from a zinc free culture grown in MB medium devoid of iron. The parameters such as pH, Eh, cell number and zinc concentration were monitored regularly. For the measurement of Eh, pH and zinc concentration, 1.5 mL aliquots of the sample were withdrawn periodically while purging nitrogen gas using a sterile hypodermic syringe and the crimped rubber septum was covered with parafilm in the mid region. The samples, which were withdrawn were subjected to analysis and were not put back into the serum bottles. Precautions were taken to avoid ingestion of air into the serum bottle. Samples for zinc analysis were filtered through 0.2 μm membrane filter and analyzed. After withdrawing the samples, the bottles were purged with nitrogen, sealed and incubated at 35 °C. Studies on the bioremoval of 63, 108 and 210 mg/L of zinc were carried out using cells adapted to 12 mg/L zinc. A zinc free culture was maintained as a control sample. Zinc sulfide precipitated during the bioremoval studies as described above in the presence of *Dsm. nigrificans* has been termed as biogenic zinc sulfide.

2.5. Biosorption studies

The bacterial cell pellet obtained as per the procedure outlined earlier was used as a substrate for the biosorption of 12 mg/L of zinc. The cell pellet was dispersed in 100 mL of 10 mg/L zinc solution in an Erlenmeyer flask at pH 6.2 and equilibrated in a Remi orbital shaker for a specified time at 200 rpm (2.24 g) at 35 °C. The residual zinc concentration in the solution obtained after filtration through 0.2 μm membrane filter was analyzed using a Thermo-Jarrell Ash, Video