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Biological Strategy for Heavy Metal Removal from Industrial Wastewater

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ABSTRACT

Industrial wastewater containing substances that may affect human health and are difficult to degrade is a major health concern, where examples include effluents containing heavy metals. The contamination of receiving water bodies by heavy metals constitutes a major environmental concern as these contaminants are extremely toxic, recalcitrant, and exhibit a tendency to bio-accumulate. Although heavy metals could be removed from industrial wastewater by a range of physicochemical treatment technologies such as precipitation, ion exchange, adsorption, electrochemical processes, and membrane processes; however, regulatory standards are not always sufficient. The objective of this paper is to study the amenability of using the bacterial strain of *Bacillus Subtilis* isolated from the surface of natural Egyptian phosphate as an alternative for the conventional techniques of treatment. It is found that Zn removal increased from 80% to 93%, while Pb removal varied from 95.55% to 99.34 % by the addition of 10-20 ml of bacterial strain (Cell + Product) in a solution having 50 mg/l Zn^{2+} and 30 mg/l pb^{2+} ions. The relative removal capacity for metals was in the order Pb >Zn.

1. Introduction

Water contamination due to increased population and industrial activities is one of the most challenging issues to the researchers, because it is continuously increasing threats to both human health and the environment. This pollution mainly caused by waste water drain from different industries e.g. metal plating, mining processes, fertilizer industries, tanneries, batteries, paper industries and pesticides, etc. [1-2]. This wastewater contains mainly heavy metals that their concentrations increase especially in developing countries. Heavy metals are well known as non-biodegradable unlike the organic contaminants. These toxic heavy metals can accumulate in living organisms cause severe illness such as hypertension, anemia, cancer, renal kidney disease, nervous system damage and mental disorder [3-4]. Copper and lead are the most hazardous heavy metals, which take intensive attention as popular containments of industrial wastewaters. Owing to the standard limits of water, it generates a strong demand to improve the efficiency of existing methods for removing Pb and Zn from the water. Many techniques are used for the effective removal of toxic heavy metals from aqueous solutions such as adsorption, flocculation, coagulation, membrane filtration, electrochemical process, solvent extraction, chemical precipitation, ion exchange, as well as various other processes [5-6]. However, most of these techniques have several disadvantages such as production of secondary pollution, high cost, high levels of energy and chemicals needed, and weak treatment operation at low metal concentrations. [7-8]. biosorption method is an effective and economic way for heavy metal wastewater treatment. This technique shows an operation flexibility and usually produce high quality treated effluent.

The aim of this paper is to use the bacterial strain of *Bacillus Subtilis* isolated from the surface of Egyptian phosphate rock for the uptake of heavy metals of Zn and Pb ions from industrial wastewater.

2. Materials and Methods

2.1. Isolation and Growing of Bacteria

Bacterial strain was isolated from surface of Egyptian phosphate ore through vigorous agitation of phosphate sample with 0.4% sodium chloride, NaCl, solution for 30 min on a rotary shaker at 30°C, and allowed to settle. The supernatant obtained was serially diluted with sterile water and spread on the surface of nutrient agar plates which were incubated at 30 ºC. About eight bacterial isolates were isolated, purified by streaking on nutrient agar plates, then transferred to nutrient agar slopes stored at 4°C and subcultured monthly. The efficiency of these isolates was screened using a laser particle size analyzer **[9, 10]**. Based on the later test, the most promising bacterial isolate has been selected to conduct this study.

2.2. Morphological and Gram Staining Identification

Microscopic examination and gram staining of the selected bacterial isolate were carried out.

2.3. Bio-Chemical Identification

The selected bacterial isolate was identified using the BIOLOG GEN III Micro-plate microbial identification system. A pure culture was grown on biolog recommended agar media and incubated at 30° C. Inoculum were prepared where the cell density was in the range of 90-98%T. precisely 100 µl of the cell suspension was transferred by multichannel pipette into the wells of biolog micro-plate. The plates were incubated for 36 hours at 30° C into the Omni-Log incubator/reader. The biolog micro-plate tests the

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ability of an organism to utilize or oxidize a pre-selected panel of 95 different carbon sources. The dye tetrazolium violet is used to indicate utilization of substrates. A panel of 95 different substrates gives a very distinctive and repeatable pattern of purple wells for each organism in which the manufacturers literature terms a "Metabolic Fingerprint". Finally; micro plate was read using Biolog's Microbial Identification Systems software through biology reader **[10, 11]**.

2.4. Microorganism Growth and Preparation for Biosorption

The nutrient broth was prepared using the prescribed growth medium containing beef extract 1.0g, yeast extract 0.1g, peptone 5.0g, sodium chloride 5.0g and distilled water 1.0 liter. The bacterial culture was sterilized in an autoclave maintained at 15 lbs for 15 minutes and maintained as per the guidelines of MTCC.

2.5. Separation Experiments

Metal solutions with known and different concentrations of Zn and Pb were prepared by dissolving their salts in double distilled water (Synthetic) and the removal efficiency was evaluated under different operating conditions as pH, heavy metal concentration, mixing speed and bacterial concentration. All the experiments of filtration were carried out at time of 20 min. at room temperature (25±3) and the filtrate analyzed for metal ions concentration using Atomic absorption. The conditioning process for 20 ml of a solution containing industrial wastewater with certain concentration of bacterial cultured broth in 100 ml liquid (distilled water with additives like cultured broth).

3. Results and Discussion

3.1. Identification of Bacterial Isolates

Biolog identification indicated that bacterial isolate is Bacillus subtilis. Microscopic examination revealed that cells are Gram-positive, catalase-positive bacterium. Bacillus Subtilis is rod-shaped, and can form a tough, protective endospore, Fig. 1. Colonies of Bacillus Subtilis are yellowish, flat, opaque, and dry, with lobate or crenate edges, Fig.2.

Fig 1. Gram stain of B. Subtilis .

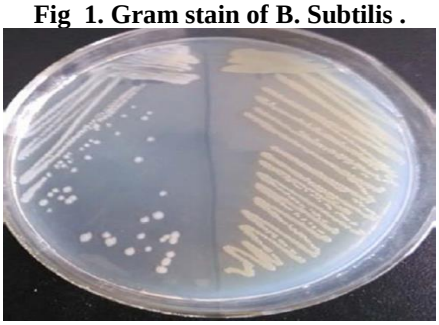

Fig.2. Colonies of *B. Subtilis*

3.2. Removal of Heavy Metals (Pb and Zn ions)

To study the effect of pH on biosorption, the pH of the metal ion solution was adjusted to values in the range of (2–10) by the addition of pH regulators prior to the experiment. The pH should be above 6.0 and below 8.0. The effect of the bacterial strain concentration of *Bacillus Subtilis* on the removal of (Zinc and Lead) ions is depicted in Table 1 for varied bacterial strain doses of 10, 15, and 20 g/l. Zn removal using modified Glauconite increased from 80% to 93%, while Pb removal varied from 95.55% to 99.34%.

Table 1. Removal of Zinc and Lead ions.

Metal ions	Adsorbent Dose g/l	Inlet concentration mg/l	Outlet concentration mg/l	Removal efficiency %
Zn	10	50	10	80.00
	15	50	5.0	90.00
	20	50	3.5	93.00
Pb	10	30	1.335	95.55
	15	30	0.7	97.67
	20	30	0.2	99.34

4. Conclusions

1 The Research team succeeded for the first time to use naturally isolated bacterial strain of Bacillus Subtilis in an economic process for the removal of heavy metals from industrial wastewater.

2 The maximum Zn and Pb ions removal was 93% and 99.34% with initial concentration 50 mg/l and 30mg/l, respectively.

3 The relative removal capacity for metals was in the order Pb >Zn.

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