Studies on Biological Removal of Plumb (Pb) by *Bacillus subtilis*

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ABSTRACT
Waste from industries is a serious and growing problem in most developing countries. Some heavy metals are known to cause damage to living organisms including human beings. Their detoxification efficiency indicates good potential for application in bioremediation of toxic heavy metals. In those heavy metals, Plumb in soil is influenced to a great extent by microbial activity. Microorganisms have ability to absorb Pb from a medium. The biological removal of Pb ions from aqueous solutions by using the bacterial culture was investigated. The growth of the *Bacillus subtilis* culture was noticed by Pb concentration in growth medium, thus about 20% of the bacteria can grow up to 50 mg Pb/L medium. The results revealed that the living biomass of the cultures were more efficient to absorb Pb. The formulation of yeast, peptone medium fortified the cultures by ingredients favoured the best growth yields that have the highest Pb absorption. The absorption of Pb by *Bacillus subtilis* was considerably influenced by the pH value of the absorption medium, contact time, biomass levels and Pb concentration. Thus, 91% of Cd was absorbed in medium containing 10 mg Cd.

KEYWORDS
Bacillus subtilis, Biomass, Bioremediation, Biosorption, Plumb.

INTRODUCTION
A broad attention has been kept on management of environmental pollution and its control for last two decades due to hazardous materials such heavy metals. During this period, removal of heavy metals from the soil and water around industrial plants has been a challenge. In those toxic heavy metals, mercury, lead and cadmium are in the limelight due to their major impact on the environment [1]. The harmful effects of cadmium include a number of acute and chronic disorders, such as *itai-itai* disease, renal damage, emphysema, hypertension, and testicular atrophy [2].

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Conventional techniques for the removal of heavy metals from wastewater, such as chemical precipitation, ion exchange, activated carbon adsorption and separation processes have limitations and become inefficient and expensive especially when the heavy metal concentration is less than 100 ppm[3]. The uses of microorganism or its biomass or its products are for the recovery of metals from waste streams [4, 5]. Bioremediation is the cost effective and environmental friendly process, which has been widely accepted by microbial metal reabsorption [6]. That's why there is a need to search such metal tolerant, metal absorbent for biodegradation process. Bacterial genes determine transport systems for maintaining equilibrium intracellular concentrations balancing needs and toxicity, and for elimination of purely toxic elements such as Hg, Pb, As, Cr, Cd, and Ag [7, 8, and 9]. Bacillus *subtilis c*an survive in different habitats, including water, soil, and plants [10, 11]. However, microorganisms absorb the heavy metal or transform it through enzymatic reactions and cause its binding to metabolites or other chemicals in the soil. Several authors consider microorganisms as natural "biosorbants" [12, 13, and 14]. Microorganisms indigenous to heavy metal-containing environments have evolved several distinct mechanisms of heavy metal tolerance [15, 16]. Microbial biodegradation of oil in the environment is slow process and it is influenced by a number of factors which include the population of oil bio degraders, temperature and nutrient availability [17, 18]. Strains of *Bacillus subtilis* grew on a large number of hydrocarbon compounds as a source of carbon and energy demonstrating these strains might be efficient hydrocarbon degraders [19, 20].In this study, we attempted to determine the absorption ability of Bacillus *subtilis* to remove Pb from a liquid medium.

MATERIALS & METHODS

Bacillus subtilis Cultures were obtained from Microbial Type Culture Collection. In this study, the cultures were grown in water soluble medium containing Beef extract, Yeast extract, Peptone, and NaCl. The salts of Lead (Lead Carbonate) were added to the medium at concentrations of 0mg, 10mg, 20mg, 30mg, and 40mg per litre respectively. The pH of the medium was adjusted to 7.0 and sterilized in an autoclave at 121^{o}C pressure for 15min. *Bacillus subtilis* Cultures were inoculated into the medium. Then about 2ml of the culture of the medium was incubated at 30^{0}c for 7days. The medium with different concentrations of Pb and bacteria were kept for treatment and medium without Lead salts and organism served as control. Then the tubes were analyzed for the growth of organism at different concentrations of Pb, absorption of metal by organism and concentrations of metal present after treatment in an atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

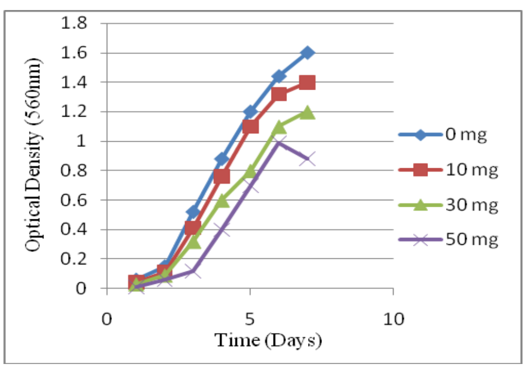

Figure 1: Growth of Bacillus subtilis in the presence of Plumb

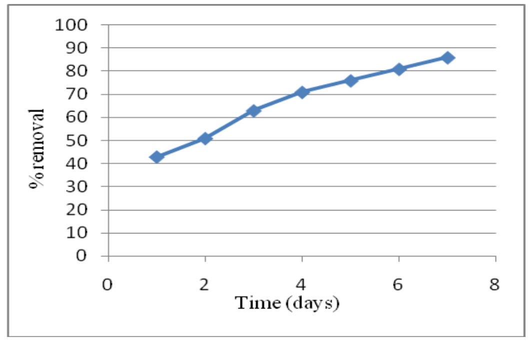

Figure 1 : % removal of Pb by B. subtilis

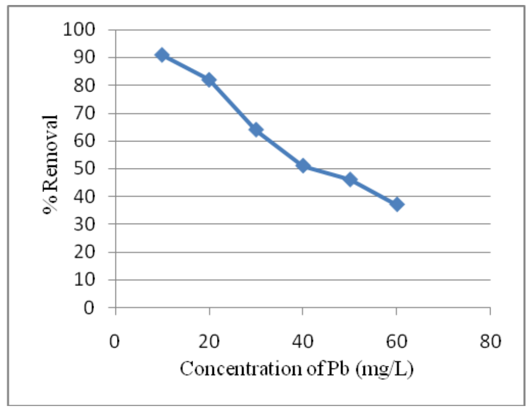

Figure 2: Pb uptake by B. subtilis at different concentrations

Bacillus *subtilis* resistance was investigated for Plumb. Live cells of B. *subtilis* were cultured in solutions containing metal ion. Growth curve of B. *subtilis* in the presence of various concentrations of Pb is represented in Figure 1. Results have demonstrated that the inhibitory effects of Pb increased as the concentration of this element was elevated in the culture medium. As stated in Figure 1, 50 mg/l Pb caused death in many of the bacterial cells on the first day. During the rapid growth, the ions are uptaken at the surface of microorganism. An effect of the amount of B. *subtilis* on the absorption rate of tested metals is demonstrated in Figure 2. Utilized biomass concentration of bacteria is varied between 10^{5} and 5×10^{5} cell/ml. Results have demonstrated that the amount of bacteria saliently affected the metal absorption rate. As the bacterial biomass increases the heavy metal-ions absorbs rate increases too. Impact of starting concentration of Pb ions upon bioremediation is visualized in Figure 3. Initial concentrations of Pb ions highly affect the absorption rate in equilibrium phase. It is identified that at the concentration of 10 mg/l Pb ions, the ion bioremediation rate increases higher than 90%. But at more than 30 mg/l concentration of all ions, uptake rate decreases. This kind of drop might be caused by the saturation of absorption sites of the microorganism (Figure 3). Bioremediation technology uses microorganisms to reduce and eliminate contaminants present in soils, sediments, water, and air [21]. Bioremediation can be a cost efficient and reliable method for removing hazardous waste from the heavily contaminated sites [21, 22, 23]. A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation, and complexation, and changes in valence state. These transformations affect the mobility and bioavailability of metals. At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species [24]. Fortunately, microorganisms can affect the reactivity and mobility of metals. Microorganisms that affect the reactivity and mobility of metals can be used to detoxify heavy metals and prevent further metal contamination [25, 26].

CONCLUSION

B. *subtilis* was the most tolerant to Pb level up to 50 mg/ L medium. The bacterial culture was more efficient to uptake Pb. The absorption of Pb by B. *subtilis* was considerably influenced by the composition of the growth medium. The removal of Pb by B. *subtilis* was considerably influenced by its treatment, pH value of the absorption medium, contact time, biomass level and Pb concentration under these conditions 91% of Pb was uptaken. In summary, the study establishes the role and efficiency of B. *subtilis* in the absorption, accumulation, degradation and detoxification of Plumb from environment.

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