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Effect of pH, Temperature and biomass on biosorption of heavy metals by *Sphaerotilus natans*

P. Ashokkumar*, V. Meena Loashini, V. Bhavya

Department of Microbiology, Sengunthar Arts and Science College, Tamil Nadu, India

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Abstract

Microbial remediation is a technique of eradication of hazardous substances that is organic and inorganic xenobiotic substances and also heavy metals from the environment with the help of microbes. Industrializations have increased in the concentration of heavy metals that is cadmium, lead, iron, copper, chromium. The biosorption by *S. natans* was carried out for three different heavy metals: copper, chromium, lead. Various physical and chemical factors affect the sorption capacity by the microorganisms. The pH for the removal of heavy metals at 7.0 was Cu (48%), Pb (75%) and Cr (52%). Optimum temperature for the removal of heavy metals was at 37°C. Maximum sorption was observed, Cu (58%), Cr (60%) and Pb (82%).

* **Corresponding Author:** P. Ashokkumar ✉ mvmeena6@gamil.com

Introduction

Nowadays metal played a large role in industrial development and technological advance. Gold, Nickel, Copper, Lead, chromium, Iron, Manganese, Zinc, Platinum group of metals and diamonds are more mined minerals. Heavy metals are the most dangerous substances in the environment due to their high level of durability and toxicity. Metal contamination has led to different types of medical problems also such as birth defects, cancer, skin lesions, grown retardation to name a few. Dietary intake of heavy metals through consumption of plants has long term effects of human health (Raja Rajeshwari and Namburu, 2014). Cadmium is toxic to extremely low levels. Cadmium and Lead result in various clinical problems including hepatic, degradation of basal ganglia of brain etc.

Bioremediation is a suitable strategy that utilises the metabolic potential of microorganism and plants to clean up contaminated environments. Bioremediation is a cost effective, eco-friendly means of healing nature with nature. This technology may be applied in the removal of xenobiotic compounds from agrochemical, petrochemical industries, oil spills etc. In this process, microorganism uses the contaminants as nutrient or energy source (Valentine and Umrania, 2006).

Microbial bioremediation is defined as process by which microorganisms rapidly degrade the contaminants in environment to safe levels in soil, water etc. Algae, bacteria, fungi, and yeast have proved to be potential metal biosorbents due to metal sequestering properties and can decrease the concentration of heavy metals. Studies on interaction of microorganisms with heavy metals have an increasing interest in recent years. Microbial metal uptake can either occur actively (bioaccumulation) or passively (biosorption) (Fourest and Roux, 1992).

In past few decades metal treatment is based on biosorption have been explored using both dead and living microbial biomass. Biosorption is basically the selective sequestering of metal soluble species that result in immobilisation of microbial cells. The major advantage of biosorption include: low cost, high efficiency, no additional requirement of nutrients, regeneration of biosorbents and possibility of metal recovery (Kumar *et al.*, 2010).

Iron bacteria have potential importance in scavenging of inorganic pollutants. Biosorption of pollutants on to the bacterial surface, sheath of iron bacteria has been identified as a potential application in the removal of heavy metals. The potential applications of iron bacteria in bioremediation process in both natural and anthropogenic ecosystems (Marina *et al.,* 2013).

The features of iron bacteria motivated to study the efficient removal of heavy metal from aqueous solution. This study deals with biosorption of copper, lead and chromium by cell broth of iron bacteria. Effects of pH and temperature on metal removal were also studied. Our study also aimed at determination of heavy metal tolerance of the organisms using the metal ions.

Materials and methods

Isolation

Soil samples were collected from Kanjamalai hills, Salem, Tamilnadu and preserved in polythene bags and stored at 4°C until processed. Cells were cultured in citrate agar medium: ammonium sulphate 5.0g/L, sodium nitrate 5.0g/L, magnesium sulphate 5.0g/L, dipotassium sulphate 5.0g/L, calcium chloride 2.0g/L, ferric ammonium citrate 10.0g/L and pH 6.6. Strain was maintained by subculturing on citrate agar. The culture was stored at 4°C between transfers and subcutured before experimental use. Microscopic and biochemical tests were performed according to Bergy's Manual of Systematic Bacteriology to determine the isolate.

Metal solution

Experimental metals used in the study were lead, cadmium and iron in the form of their respective metal solutions.

A synthetic multi-element standard solution of liquid media (1% tryptone, 0.5% yeast extract, 0.5% NaCl) containing 100 ppm of each Cd, Fe and Pb, was prepared from their respective stock solutions (1000 mg L^{-1}). Stock solutions of these metals were prepared using deionized water and autoclaved separately. Prior to addition of stock solution of metals the liquid media were autoclaved at 121°C for 20min. All the additions were performed aseptically (Yu- Chun *et al.,* 2005).

Effect of pH on biosorption

The experiments were carried out in the batch mode for the measurement of biosorption capabilities. Medium containing synthetic multi-element solution (200ppm of each metal) in 500ml Erlenmeyer flask was used. Before the addition of the biosorbent inoculum to the solution the samples were adjusted to different pH values viz. pH- 3.5, 7.0 and 8.0. The pH of solution was adjusted with 1M HCl and 1M NaOH solutions. Samples were inoculated with 10% overnight grown culture of bacterial isolates and then incubated at 120rpm for 48hrs. The inoculated samples were then incubated at 37°C for 48hrs. After the incubation period the cells were harvested by centrifugation for 30min at 5000rpm. Total metal concentration biosorbed by organism was analysed using Atomic Adsorption Spectrophotometer (Ritixa Patel and Monika Chandel, 2013).

Effect of temperature on biosorption

A set of three samples was prepared and pH was adjusted to 7.0 for all samples. After inoculation, samples were incubated at temperatures i.e. 10°C, 37°C, 50°C respectively and incubated at 120rpm for 48 hrs. Total metal concentration biosorbed by the organism was analysed using Atomic Adsorption Spectrophotometer (Ritixa Patel and Monika Chandel, 2013).

Determination of minimum inhibitory concentration

Five sets of experiments were prepared using liquid media. They had varying concentrations (200ppm to 1000ppm) each cadmium, iron and lead ion respectively.

The pH was maintained at 7.0 for all sets. 10% overnight grown culture of isolate was inoculated in these samples and incubated at 37°C, 120rpm for 48hrs. Growth was determined by measuring absorbance at 540nm. Samples showing zero absorbance were further confirmed for growth by examining total viable count (TVC). Maximum inhibitory concentration (MIC) of heavy metal was designated as the highest concentration of heavy metal that allowed growth after 48h. Samples were analysed to determine the dissolved ion concentration by an atomic absorption spectrophotometer (Schmidt and Schlegal, 1994).

Effect of biomass concentration

Biomass was centrifuged at 9000rpm and different weights of the biomass ranging from 0.5 to 2.5mg/ml were dispersed in solutions containing the 200ppm metal concentration. The solutions were adjusted to the optimum pH in which maximum biosorption of the metal ion occurred. Flasks were left for equilibration. The solutions were later centrifuged at 9000 rpm and the metal ion concentrations were determined using the atomic adsorption spectrophotometer.

Results

Isolation

The organism isolated from soil sample was identified as *Sphaerotilus natans* according to Bergy's Manual of Systematic Bacteriology was used for the biosorption of heavy metals: copper, chromium and lead. Effect of pH, temperature and biomass concentration was studied on removal efficiency of the isolated organism. Table 1 shows the biochemical test results for isolated bacterial strain.

Table 1. Biochemical characteristics of *S.natans.*

Test	Results
Indole	-ve
MR	-ve
VP	-ve
Citrate	+ve
Nitrate	-ve
Casein	-ve
Starch	-ve
Oxidase	+ve
Catalase	+ve
ONPG	+ve
Lysine decarboxylase	-ve

Effect of pH

Fig 1 shows percentage removal of heavy metals of 48%, 52% and 75% of copper, chromium and lead respectively by *Spherotilus natans*. The maximum removal percentage for Chromium, Copper and Lead was found to be 7.0 by the *Sphaerotilus natans.* At optimum pH 7, *S. natans* removed 75% of lead which was found to be the highest percentage removal than other two heavy metals.

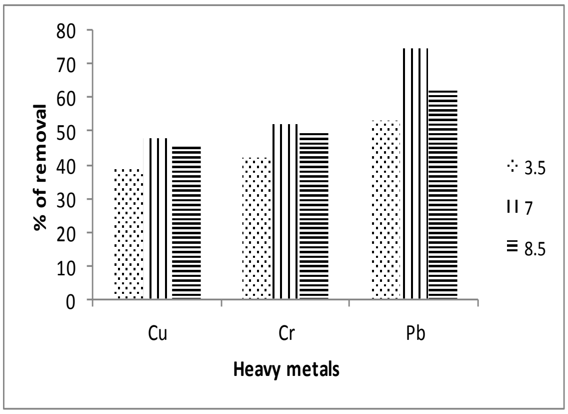

Fig. 1. Effect of pH on biosorption.

Effect of Temperature

The effect of temperature on heavy metals that is copper, chromium and lead were investigated at 10ºC–50ºC.The maximum removal was obtained at optimum temperature 37ºC with percentage removal 60%, 82% and 58% by Cr, Pb and Cu respectively (Fig 2). No significant differences were observed at high temperature. There was only slight difference in removal of heavy metals between temperature 37°C and 50°C.

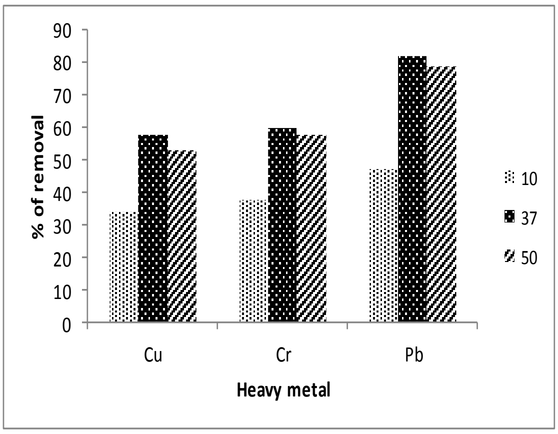

Fig. 2. Effect of temperature on biosorption.

Determination of minimum inhibitory concentration

The effect of copper, chromium and lead concentrations was investigated at the concentration range of 200-1000ppm and optimum temperature and pH of 37ºC and 7 respectively. *Sphaerotilus natans* for chromium, lead and copper showed remarkable ability to remove these metal ions.

They could reduce at 79%, 80% and 85% at 200ppm for chromium, copper and lead respectively (Fig 3). As the concentration increased, there was decrease in the removal of heavy metals.

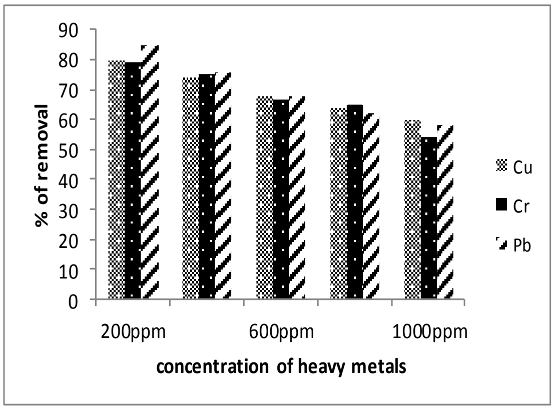

Fig. 3. Determination of MIC on biosorption.

Effect of Biomass concentration on Biosorption of isolates

The influence of biomass concentration on the percentage sorption of heavy metals: Cr, Cu and Pb by *S. natans* were studied in metal solutions of the same initial.

Metal ion concentration (200mg[1]) at optimum temperature and pH of 37ºC and 7.0 respectively. *S. natans* was grown and fixed with chromium, copper and lead of 200ppm. at optimum pH 7 and temperature 37ºC. *Spherotilus*

Natans showed percentage removal of 75%, 79% and 78% chromium, copper and lead respectively at 2.0mg/ml biomass concentration (Fig 4).

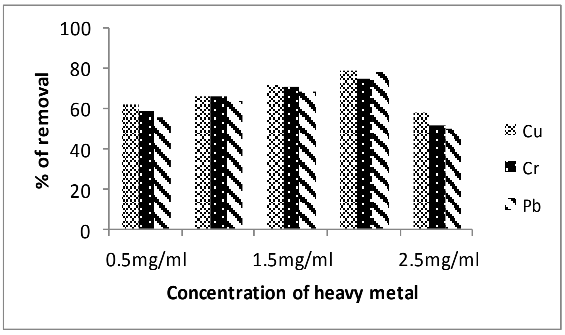

Fig. 4. Effect of biomass concentration on biosorption.

Discussion

The iron bacteria isolated from soil sample around Kanjamalai hills exhibited noticeable growth characteristics which differentiate it from other iron bacteria investigated by other researchers. In a study by Mahbudar *et al.,* (2010) reported other iron bacteria like , *Thiobacillus, Sulfobacillus, Gallionella, Metallogenium* and *Leptospirillum,* which are morphologically very different from the organism of the present work.

The pH of the solution is the most critical parameter for metal biosorption as it influences both the bacterial surface chemistry as well as solution chemistry of soluble metal ion. In the present study the optimum pH for biosorption of metal ions is 7.0 by *S. natans,* this is in accordance with the results obtained by Sultan and Hasnain (2007). In another study by Lopez *et al.,* (2000), the maximum biosorption of lead by *Pseudomonas fluorescens* was at pH 7 which is in agreement with the present study. At alkaline pH 8.5 heavy metals react more easier with H^+ therefore the removal capacity is reduced (Tatiana *et al*., 2007) and at acidic pH 3.5 there is decrease which is because at low pH the hydrogen ion has to compete for the active sites on the biosorbent and because of weak acidic nature on the active sites of the adsorbent.

Temperature has a significant role on biosorption. Temperature change will affect a number of factors which are important in heavy metal biosorption.

The maximum removal of heavy metal was at optimum temperature 37°C by *S. natans.* A study by Daghistani (2012) demonstrated the extent of biosorption of heavy metals with temperature range 37-50°C for *B.subtilis* spp. In an another study by Aravind *et al.,* (2015) demonstrated that the strains *A. towneri* and *R. eutropha*, the biosorption of chromium was at optimum temperature 37°C which is very well supported the present study. There was no much difference in the biosorption of heavy metals between the temperature 37°C and 50°C in the present study. This may be due to environmental factors. As the temperature decreases there is a decrease in metal uptake capacity which may be due to shrinkage of cells which will reduce the surface area of contact (Yuan *et al.,* 2009).

The phenomenon of copper, lead and chromium was examined at the concentration range of 200-1000ppm at optimum temperature and pH of 37°C and 7 respectively. *S. natans* could efficiently remove 85% of lead at 200ppm from the medium followed by 60% of chromium and 58% of copper. In a study by Saleh (2005) showed that there was no increase in metal uptake with increase in metal concentration by *K. pneumoniae* which is accordance with the present study. Thacker *et al.,* (2007) demonstrated that heavy metal uptake was 2-3 times higher than the present study. This difference may be due to the different growth media used. Zakaria *et al.*, (2007) reported that TSM, which is rich salt-based minimum media have 2-3 times higher MIC values than Nutrient media. Due to complexation between the metal cations and components of the growth media.

Biomass is a significant parameter which determines the biosorption capacity for removal of heavy metal concentration. *S. natans* was grown and fixed with chromium, copper and lead of 200ppm at optimum pH 7 and temperature 37º C. At 2.0mg/ml maximum uptake of heavy metals by *S. natans* was observed.

This was in accordance with the study by Tarangini *et al.,* (2009) who reported 80% of heavy metals was biosorbed by *Bacillus* spp. at 2.0mg/ml. In a study by Esposito *et al*., (2001) explained that increasing biomass concentration decrease in metal uptake may be because biomass can undergo different modifications depending on the experimental conditions such as pH, ionic strength, temperature and metal ion concentrations. Jayanthi *et al.,* (2013) demonstrated that chromium biosorption by *Staphylococcus aureus* was at 1ml of biomass concentration, whereas in *Pseudomonas aeruginosa* the highest biosorption was at 3ml. This difference may be due to the parameters like temperature, time, pH and other factors might affect the biosorption of heavy metals.

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