INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 11, No. 1, p. 234-239, 2017
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Biosorption of lead, cadmium and iron by *Sphaerotilus natans*

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

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

Article published on July 30, 2017

Key words: Lead, Iron, Cadmium, Biosorption, Bioremediation

Abstract

Heavy metal contamination is potentially environmental issue and certainly a major health concern in many parts of the world, primarily due to various anthropogenic activities. The heavy metals selected for this studies that is lead, iron and cadmium have no positive role in living systems. All these heavy metals are highly toxic. The biosorption by the culture were carried out for three different heavy metals: Lead, Cadmium and iron. Various physical and chemical factors affect the sorption capacity by the microorganisms. The optimum pH for the removal of heavy metals was found to be 7.0. Maximum percentage removal of heavy metals at pH 7.0, Pb (75%), Cd (53%). Optimum temperature for removal of heavy metals was at 37° C. Maximum sorption was observed at Pb (82%), Cd (66%) and Fe (89%).

***Corresponding Author:** V. Bhavya ✉ bhavyakrish97@gmail.com

Introduction

Heavy metal pollution is one of the major environmental problems these days. Heavy metal creates hazardous problems when pollution takes place in environment. Industrialization and extraction of natural resources have resulted in large scale environmental contamination and pollution. There has been a considerable increase in the discharge of industrial waste to environment mainly to soil and water. Contamination of soil, ground water, sediments, surface water and air with hazardous heavy metals and toxic chemical is one of the major threats facing the world as the cannot be broken down to nontoxic forms and have long lasting effects of eco system. Industrial effluents are a major cause of heavy metal concentration.

A survey conducted by U. S EPA showed that heavy metals were the most common contaminants in the 395 remedial action sites in U.S (U.S EPA, 1984). Metal contamination has led to different types of medical problems like birth defects, cancer, skin lesions and growth retardation leading to disabilities, heptorenal and other maladies (Raja Rajeshwari and Namburu, 2014). Accumulation of heavy metal in the environment is not only a serious problem to the animals and human beings but also to the underlying ecosystem as well. Consequently, ecosystems functioning can be seriously perturbed and long term soil fertility may be threatened due to such heavy metal contamination. Therefore, it can be understood, how much necessary it is to get rid of heavy metal pollution as far as possible also for the betterment of the ecosystem (Mohammad *et al.,* 2008).

Physico-chemical methods such as chemical precipitation, oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, ion-exchange and membrane technologies are widely used to remove heavy metal ions. However, applications of these treatment processes have been found to be sometimes restricted, because of investment, operational costs and potential generation of secondary pollution. Biological methods solve these drawbacks since they are easy to operate, do not produce secondary pollution (Ritixa and Monika 2013).

In the past few decades, metal treatment and recovery is based on biosorption have been explored using both dead and living microbial biomass with remarkable efficiency. Biosorption can be defined as the selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells. The technique of biosorption utilises the characteristics of microorganisms to absorb in a commercial manner. The major advantage of biosorption over conventional treatment methods include: low cost, high efficiency, no additional requirements of nutrients, regeneration of biosorbents and possibility of metal recovery (Kumar *et al.,* 2010, Ahaly).

The aim of the present study was to evaluate the possibility of the *S. natans* to biosorb heavy metals and to be used as bioremediating agent. The effect of pH and temperature on metal biosorption was also examined.

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 20 min. 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 (200 ppm of each metal) in 500 ml 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 1 M HCl and 1 M NaOH solutions. Samples were inoculated with 10% overnight grown culture of bacterial isolates and then incubated at 120 rpm for 48 hrs. The inoculated samples were then incubated at 37°C for 48 hrs. After the incubation period the cells were harvested by centrifugation for 30 min at 5000 rpm. 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 120 rpm 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, 120 rpm for 48 hrs. Growth was determined by measuring absorbance at 540 nm. 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 9000 rpm and different weights of the biomass ranging from 0.5 to 2.5 mg/ml were dispersed in solutions containing the 200 ppm 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 metal ions at pH 4.5, 7.0 and 8.5. The maximum biosorption for Iron, Cadmium and Lead ions was at pH 7 with the removal ability of 80%, 53% and 75% respectively. The results recommended that pH 7 was optimum for biosorption of these heavy metals by *S. natans*. Among the three metals, removal ability for iron (50%-80%) was found to be maximum.

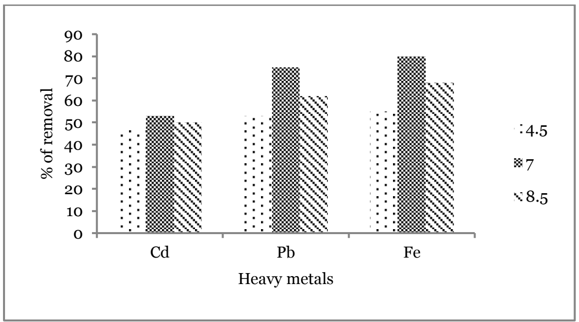

Fig. 1. Effect of pH on biosorption.

Effect of temperature

Fig 2 depicts the effect of temperature on heavy metal biosorption. The effect of temperature on percentage removal of metal ions were investigated at 10° C - 50° C. Maximum biosorption for Iron, Cadmium and Lead was at 37° C at 89%, 66% and 82% respectively by *S. natans*. At temperature 10°C and 50°C, the percentage of biosorption was decreased. Iron was maximally biosorbed with 89% removal other than the other two heavy metals.

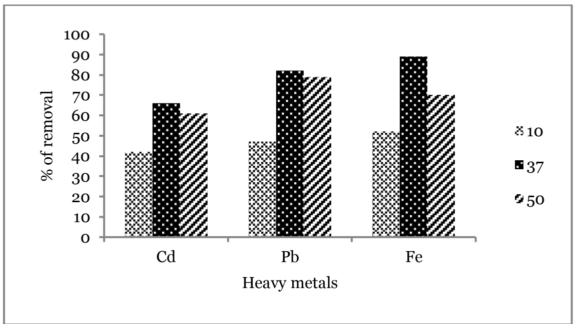

Fig. 2. Effect of temperature on biosorption.

Determination of Minimum Inhibitory Concentration

The effect of Iron, Cadmium and Lead concentration was investigated at the concentration range of 200-1000ppm and optimum temperature and pH of 37° C and 7 respectively. *S. natans* could efficiently remove 82% of Cd, 92% of Fe and 85% of Pb at 200ppm (Fig 3). The results showed that *S. natans* has high metal inhibition for iron and lead as compared to cadmium.

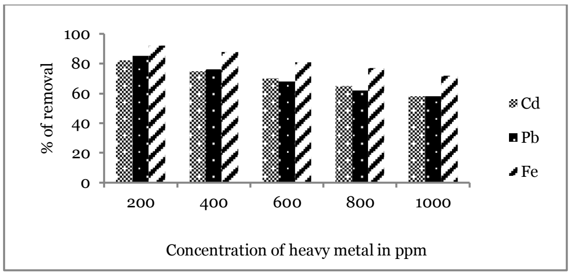

Fig. 3. Determination of MIC on biosorption.

Effect of Biomass concentration

The influence of biomass concentration on the percentage biosorption of heavy metals Iron, Cadmium and lead were studied in their respective metal solutions of concentration 200mg^{-1} and optimum temperature and pH of 37° C and 7 respectively. The maximum uptake by Cadmium Iron and Lead at 2 mg/ml biomass concentration is 86%, 90% and 78% respectively. (Fig 4) Above 2.0 mg/ml of biomass concentration there was decrease in the percentage removal capacity of the *Spherotilus natans*.

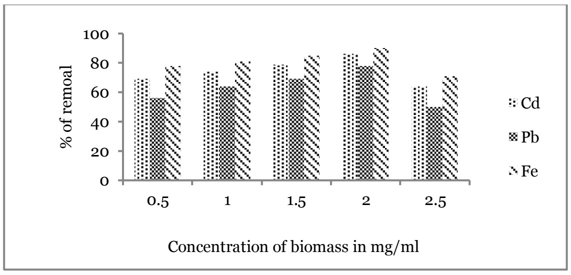

Fig. 4. Effect of biomass concentration on biosorption.

Discussion

The iron oxidising bacteria isolated from the iron rich soil samples around Kanjamalai hills showed very distinct growth characteristics which distinguish it from the other known iron oxidising bacteria's reported by other researches. Unlike most of the other iron oxidising bacteria, this strain is aerobic. Other iron bacteria which have been isolated to date are *Gallionella, Metallogenium* and *Leptospirillum* among others, which are morphologically very distinct and different from the isolates of the present study (Nahima Begum *et al.*, 2010).

The pH of solution have been examined as one of the most significant parameter influencing the biosorption process, it influences both the dissociation of functional group on the active sites of the biosorbant as well as solution ion chemistry. Different metal shows different pH optima for their biosorption (Saeed and Iqbal, 2003). In the present study the optimum pH was 7 for the biosorption of cadmium, lead and iron which was in accordance with the study of by Lopez *et al.,* (2000) for *Pseudomonas fluorescens*. At higher pH (>8) biosorption of metal ions was lowered because solubility of ions was decreased (Saleh, 2005). At pH 3.5 there was drastic decrease in removal of metal ions which is because at low pH the hydrogen ion had to compete for the active sites of biosorbant (Pagnanelli *et al.,* 2003).

Temperature changes will affect a number of factors which are important in heavy metals biosorption (Ajay Kumar *et al.,* 2009). In the present work the optimum temperature was 37°C for heavy metals with the removal % of 66%, 82% and 89% of Cd, Pd and Fe respectively. A study by Kumar (2014) showed that maximum removal of cadmium by *Pseudomonas aeruginosa* JN102340 was observed at 35°C which is also similar to the present study. Rate of metabolism of bacterial cells is depressed, as below optimum temperature, growth rate decreases and thermal death may occur at above optimal temperature (Pelczar *et al.,* 2004).

The effect of iron, cadmium and lead was investigated at the concentration range of 200ppm-1000ppm. *S.natans* could efficiently remove heavy metals at 200ppm concentration. As the concentration increased there was decrease in the removal of heavy metal ions. From the results it can be concluded that the percentage of heavy metal removal by *S.natans* decreased with the increasing concentartaion (200>400>600>800>1000ppm) with the metal uptake of Fe>Pb>cd. *S.natans* showed higher metal uptake of metal ions than other organisms, such as *B.subtillis*, *E.coli* and *Pseudomonas* spp (Ruparlia *et al.,* 2008). Our findings were in accordance with Shetty and Rajkumar (2009) who reported that strains of *Pseudomonas* spp at concentrations 100 ppm and 200 ppm beyond which saturation was achieved.

Biomass is a key factor which influences the biosorption activity for removal of heavy metals concentration (Suryan and Ahluwalia 2011). The maximum uptake of metal ions ws at 2mg/ml by *S.natans* In a study by Olukami *et al*., (2014) the heavy metal uptake by Pseudomonas aeruginosa was achieved between the biomass concentration of 0.5-2.0mg/ml above which there was no effect on biosorption which is in accordance with the present study. Above 2.0 mg/ml of biomass concentration there was decrease in the percentage removal capacity of the *Spherotilus natans*. This must be due to over populated cultures in medium (Schnepf *et al.,* 1998).

References

Ajay Kumar AV, Naif AD, Hilal N. 2009. Study of various parameters in the biosorption of heavy metals on activated sludge. World applied Science Journal **5**, 32-40.

Kumar RR. 2014. Isolation, Molecular identification of metal tolerant bacteria and its heavy metal removal capacity. International Journal of Micro. Biochem. Mol Bio 1-7.

Kumar, Ashok, Bisht BS, Joshi VD. 2010. Biosorption of heavy metals by four accimilated microbial species, *Bacillus* sp, *Pseudomonas* sp., *Staphylococcus* sp and *Aspergillus niger*. Journal of Environmental Sciences **5(12),** 97-108.

Lopez A, Lazaro N, Priego JM, Marques AM. 2000. Effect of pH on the biosorption of nickel and other heavy metals by *Pseudomonas fluorescens* 4F39. Journal of Indian Microbiology and Biotechnology **24,**146-151.

Mohammad Iqbal Lone, Zhen-Li HE, Peter Stoffella J, Xiao-e Yang. 2008. Pytoremediation of heavy metal polluted soils and water. Progresses and Perspectives. J Zhejiang Univ Science **9(3)**, 210-220.

Nahmina Begum, Mahbubar Rahman Khan, Mihir Lal Saha, Mohammad Nurul Islam and Sirajul Hoque. 2010. Isolation and characterization of bacteria from rusted iron materials. Bangladesh. J. Bot **39(2),** 185-191.

Olukanni DO, Agunwamba JC, Ugwu EI. 2014. Biosorption of heavy metals in industrial waste water using microorganisms. Americal Journal of Scientific and Industrial research **5(2),** 81-87.

Pagnanelli F, Esposito A, Toro L. 2003. Metal speciation and pH: Effect of Pb, Cu, Zn and Cd biosorption onto *Sphaerotilus natans*: Langmuir-type empirical model. Water research **37,** 627-633.

Pelczar MJ, Chan ECS, Kring NR. 2004. Microbiology 5th edition. Tata Mc. Graw-Hill publishing Co. ltd. New Delhi, India.

Raja Rajeswari, Namburu Sailaja. 2014. Impact of heavy metals on environmental pollution, national seminar on impact of toxic metals, minerals and solvents leading to environmental pollution. Journal of Chemical and Pharmaceutical Sciences **3,** 175-181

Ritixa Patel, Monika Chandel. 2013. Effect of pH and temperature on the biosorption of heavy metals by *Bacillus licheniformis*. International Journal of Science and Research (IJSR) 2319-7064.

Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. 2008. Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomaterials **4(3),** 707-716.

Saeed A, Iqbal M. 2003. Bioremoval of cadmium from aqueous solution by black gram husk (*Cicer arientum*). Water resources **37**, 3472-3480.

Saleh M, Al. Garni. 2005. Biosorption of lead by gram negative capsulated and non-capsulated bacteria. Water SA **31(3),** 345-350.

Schmidt T, Schlegel HG. 1994 Combined nickel-cobalt-cadmium resistances encoded by the ncc locus of *Alcaligens xylosoxidans* 31A. Journal of Bacteriology 7054-7054.

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev **62 (3)**, 775–806.

Shetty R, Rajkumar SH. 2009. Biosorption of Cu (II) by metal resistant *Pseudomonas* sp. Int. J. Env. Resource **3(1),** 121-128.

Suryan S, Ahluwalia SS. 2011. Biosorption of heavy metals by paper mill waste from aqueous solution. International Journal of Environmental Sciences **2(3)**, 1331-1343.

U.S Environmental Protection Agency.1984 Summary report: Remedial response at hazardous waste site. EPA-540/2-84-002a, Washington, DC.

Yu-Chun QN, Guan Xia-Hui, Rong YN, Wang Lrwen, de-Zhou WEI. 2005. Preliminary research on cadmium removal from waste water by *Sphaerotilus natans. Applied* Journal of Microbiology **21(6)**, 654-657.