J. Sci. Trans. Environ. Technov. 2013, 7(2) : 118-120

Scientific Transactions in Environment and Technovation

Biosorption of heavy metals by using free and immobilized cells of Bacillus subtilis and *Pseudomonas aeruginosa*

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Abstract

The level of heavy metals is constantly increasing in drinking water, air and soil. Three heavy metals such as copper, chromium and mercury were used in the present study and the heavy metal biosorption was analyzed using free and immobilized cells of *Bacillus subtilis* and *Pseudomonas aeruginosa,* isolated from industrial waste water. The results of the present study suggest that the biosorption of heavy metal could be achieved by using *Bacillus subtilis* and *Pseudomonas aeruginosa* and they could be successfully used for the treatment of waste water.

Keywords : *Bacillus subtilis,* Biosorption, Heavy metals, *Pseudomonas aeruginosa,* Waste water.

INTRODUCTION

Heavy metals are abundant in drinking water, air and soil. They are present in virtually every material used as part of consumerism, such as cosmetics, medicines, processed foods, fuel sources, destructions appliances and personal care products.The most common heavy metals to which the human beings exposed include aluminium, arsenic, cadmium, lead and mercury. They can directly influence the behaviour by impairing mental and neurological function, influencing neurotransmitter production, and utilization and altering numerous metabolic body process. More over the heavy metal pollution in the aquatic environment has become a serious threat today and of great environmental concern as they are non-biodegradable and the problem of bio accumulation is also a serious one. The present paper deals with the removal of heavy metals through the process of biosorption using microorganism such as *B.subtilis* and *P. aeruginosa*.

MATERIALS AND METHODS

The industrial waste water was collected in sterile 500 ml conical flasks and brought to the laboratory and were stored in specific aseptic condition at 4°C. 1 ml of the sample was serially diluted with distilled water (10^4-10^7) and plated on nutrient agar by spread plate technique. 0.1 ml aliquote was spread on each nutrient agar plate, and *Pseudomonas* agar plate, and incubated at 37°C for 24-48 h. After incubation,the colonies developed were observed (Aneja, 2002)

The isolated organisms were identified by Gram's staining, motility test and biochemical test , and using the manual of " Bergey's Manual of Determinative Bacteriology". The organisms selected for the present study included *B. subtilis* and *P. aeruginosa* (Bailey and Scott,1966).

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Screening of Metal Resistance

The isolates of *P. aeruginosa* and *B. subtilis* were screened for tolerance of copper, chromium and mercury. A loopfull of isolated colonies were streaked over nutrient agar containing 100 µg/ml of the metals and incubated at 37°C for three days. The organisms that survived were considered tolerant to the corresponding heavy metal. The tolerant isolates were selected for the determination of biosorption of copper, mercury, chromium with initial metal concentration at 10mg/ml (Leung *et al*., 2000)

The stock solutions for copper, mercury, chromium were prepared by dissolving 1g/l of each $Cuso_4$, $Hgcl_2$ and chromium respectively in 15% NaCl solution prepared in distilled water. The exact dilution of respective stock solutions were used for the biosorption studies.

Immobilization

P. aeruginosa and B. subtilis cells were entrapped in sodium alginate pellets. Entrapment of cells in non toxic alginate is one of the simplest, cheapest and most frequently used techniques for immobilization (Kierstan and Buckec, 1977).Sodium alginate and calcium chloride were used to prepare the alginate beads containing the whole cells for biosorption studies.

The contents were stirred vigorously for 10 minutes to obtain thick uniform slurry without any undissolved lumps and then sterilized by autoclaving. The slurry was taken into a sterile syringe, added drop wise into 2% $CaCl_2$ solutions to get approximately 500 immobilized cell composites. Approximately 100 composites were used for the sorption studies.

10µl of bacterial biomass was suspended in 10µl of metal solution in 100ml of conical flask and incubated at 35°C. Sample withdrawn at definite intervals were centrifuged at 4000rpm for 20mins and the supernatant was analyzed for residual metal content.

The residual metal content was determined by using gravimetric method.

Gravimetric Estimation

10ml of reaction solution was centrifuged at 4000 rpm for 20 min. To 5ml of supernatant solution, 5ml of 10% precipitating agents were added. The resultant solutions were centrifuged at 4000rpm for 20 minutes. Then the precipitates were collected and dried in hot a oven and weighed in an electronic balance. Percentage of sorption was calculated as follows(Puri *et al*.,2003).

Calculation:

$$\% \text{ of sorption} = \frac{(w_1 - w_f) \times 100}{w_1}$$

W_1 = Initial Weight

W_f = Final Weight

RESULTS AND DISCUSSION

Heavy metals released into the environment through different portals pose many health and environmental problems. Removal of heavy metals through biosorption has been recognized as one of the effective methods of removal of heavy metals from the environment. In the present study, many microorganisms were isolated and among them *P. aeruginosa and B. subtilis* were selected and they were identified by Gram's staining, motility and biochemical characteristics and confirmed with Bergey's Manual of Systematic Bacteriology. Based on the comparison, the two colonies were confirmed as *P. aeruginosa and B. subtilis.*

The biosorption of heavy metals was analyzed using both free and immobilized cells of *P. aeruginosa and B. subtilis* by molybdenum blue methods.

Removal of three different heavy metals was analysed using free and immobilized cells *P. aeruginosa and B. subtilis* by gravimetric method. From the study, more heavy metal removal was recorded with *P. aeruginosa* than *B. subtillis* in both free and immobilized cells. Both organisms highly removed the chromium, when compared to other metals (Table 1-3).

It has been reported that higher temperature could enhance biosorption of heavy metals by nonliving biomass of *Chlorella*. So any residual heat in industrial waste water enhance biosorption. But temperature dependence of adsorption is a complex process (Aksu *et al*., 1992). Thermodynamic parameter like heat adsorption and the energy of activation play an important role in predicting the adsorption behaviour and both are shortly temperature dependent (Jasuya *et al*., 1997). This present study also suggest that the effect of operating condition on biosorption performance of *P. aeruginosa and B. subtilis*, and also could be implemented practically for the treatment of waste water by providing the optimum conditions that favour the process of adsorption of heavy metals.

Table 1 : Effect of initial biomass concentration on sorption of heavy metal

S.No	Heavy Metal (PPM)	Biomass Concentration (mg/ml)	% of Sorption	
			Pseudomanas aeruginasa	Bacillus subtilis
1	Copper	0 2 4 6 8 10 12	0 15 29 37 45 54 53	0 20 30 35 42 55 60
2	Chromium	0 2 4 6 8 10 12	0 28 46 64 70 75 78	0 24 40 52 60 70 72
3	Mercury	0 2 4 6 8 10 12	0 20 22 35 40 45 50	0 30 35 45 54 67 69

Table 2 : Effect of on sorption of heavy metals

S.No	pH	Heavy Metal (PPM)	% of Sorption	
			Pseudomonas aeruginosa	Bacillus subtilis
1.	4	Copper Chromium Mercury	12 11 8	7 8 11
2.	5	Copper Chromium Mercury	9 11.5 20	8.5 10 17
3.	6	Copper Chromium Mercury	23 14.5 42	12 34.5 21
4.	7	Copper Chromium Mercury	11 34 21	12 21 34.5
5.	8	Copper Chromium Mercury	25 52.5 32	15 27 42
6.	9	Copper Chromium Mercury	25 32 52.5	22 30 47.5

Table 2 : Effect of temperature on biosorption of heavy metal

S.No	Temperature	Heavy Metal (PPM)	% of Sorption	
			Pseudomonas aeruginosa	Bacillus subtilis
1.	25°C	Copper Chromium Mercury	7 8.5 14	4 7.5 10
2.	30°C	Copper Chromium Mercury	25 32.5 42.5	22 32.5 39
3.	35°C	Copper Chromium Mercury	27 35 45	32 30 42
4.	40°C	Copper Chromium Mercury	20 20 30	17 19 22
5.	45°C	Copper Chromium Mercury	10 20 26	7.5 11.5 20

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