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Removal of Cu^{2+} Ions from Aqueous Solutions Using Copper Resistant Bacteria

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Abstract
Biosorption using resistant microorganisms is an effective process for treatment of industrial effluents and removal of heavy metals from contaminated sites. In this study copper resistant bacteria from fireworks industrial contaminated soil were isolated, enumerated and characterized. The tolerances of the organisms decreased with increase in the copper concentration. The isolated bacterial strain was identified as *Bacillus* sp. and was resistant to 300 mg/L of copper concentration. Biosorption studies were carried out in the *Bacillus* sp. The optimum temperature was determined to be 35°C and pH was 8.0. The maximum removal of 88% was obtained at optimum conditions when the initial copper ion concentration was 100 mg/ L. Hence the *Bacillus* sp. isolated proved to be an efficient biosorbent.

***Key words*:** Biosorption, *Bacillus* sp., fireworks, heavy metal.

Introduction

Heavy metals are metallic chemical elements that have relatively high density and are toxic at low concentrations. This term is used as an alternative for toxic metals, for which no consensus of exact definition exists either (John, 2002).

Copper is used as a thermal conductor, an electrical conductor, a building material, and a constituent of various metal alloys (Noyce *et al.*, 2006). Generally the toxicity of heavy metals is produced by forming complexes or 'ligands' with organic compounds (Hoekman *et al.*, 1995). Binding of metals to these groups may inactivate important enzyme systems, or affect protein structure. Plants are very sensitive to Cu toxicity, displaying metabolic disturbances and growth inhibition at Cu contents in the tissues only slightly higher than the normal levels (Fernandes and Henriques, 1991). Copper salts are also toxic to aquatic invertebrates, such as crab, shrimp, and oysters (Johnson and Finley, 1980). Higher concentrations caused some behavioral changes like secretion of mucous, and discharge of eggs and embryos. Ingesting high levels of copper can cause headaches, dizziness, nausea, vomiting, and diarrhea. If drinking water contains higher than normal levels of copper, one can experience vomiting, diarrhea, stomach cramps, and nausea. Very high doses of copper can cause damages to liver and kidney, and can even cause death

(Flemming and Trevors, 1989). The wastes and effluents from fireworks industry contaminate the environment with heavy metals barium, strontium, copper and sodium salts. The copper salts also used in fireworks as copper acetoarsenate, copper arsenate, Copper carbonate, copper chloride, copper nitrate, copper oxide, copper oxychloride, copper sulphate and copper sulphide (Pyrocreations.com).

Biosorption is uptake of metal ions by microorganisms which may involve several chemical processes including adsorption, ion exchange, covalent binding and co-ordination (Yuhaya *et al.*, 2009). Biosorption process is a low cost and effective process which requires no additional requirement of nutrients and where regeneration of biosorbent and metal recovery can be done (Ahalya *et al.*, 2003). In this study, we have evaluated the removal efficiency of the copper resistant bacteria isolated from fireworks industry chemical exposed contaminated soil.

Materials and methods

Sample collection and analysis

The soil sample were collected from Fireworks Industry, Sivakasi, Virudhunagar District, Tamilnadu, India and stored at -4°C.

Screening for copper tolerant bacteria

Luria Bertani Broth was prepared by adding Casein enzymatic hydrolysate (10 g), Yeast extract (5 g) and Sodium chloride (10 g) to 1000 ml of distilled water. For solid media 25 g/l of agar was added. Luria Bertani Agar plates with different concentrations of copper from 25 to 250 ppm were prepared. 0.1ml of the serially diluted sample (10^{-4} dilution) was taken and each plate was inoculated by standard spread plate method. The plates were incubated at 37°C for 24 hours. The pure cultures were isolated from the plate by inoculating the individual colonies into the sterile Luria Bertani Agar plates. The plates were incubated at 37°C for 24 hours. Identification of the bacteria was done by performing Gram Staining and various biochemical tests (Tab. 1).

Table 1. Morphological, physiological and biochemical characteristics of the isolated bacterial species.

Morphological, physiological and biochemical characteristics	Isolated bacterial species
Gram's staining	+ (rod)
Indole production test	-
Methyl red test	-
Voges- Proskauer test	+
Citrate test	-
Triple sugar iron test	K/A
Gas production	-
Urease test	+
H_2S production	-
Motility test	-
Catalase test	-
Oxidase test	-
ONPG test	-
Nitrate test	+
Gelatin hydrolysis test	+
Starch hydrolysis test	+
Strain name	*Bacillus* sp.

Tolerance test

The isolated and characterized bacterial strain was inoculated in 5 ml sterilized LB broth containing different concentrations of copper from 100 to 1000 ppm. The test tubes were incubated for 24 hours. After incubation the tubes were observed for bacterial growth.

Heavy metal assay and biomass quantification

About 0.025 g of bicyclohaxanone oxalyldihydrazone was dissolved in 2.5 ml of ethanol and 2.5 ml of hot water and diluted to 50 ml using sterile distilled water for the preparation of copper reagent. Copper reagent of 1.25 ml was added to a suitable aliquot of the sample solution. Concentrated ammonia of 0.25 ml was added to the mixture and made up to 25 ml with sterile distilled water (Vogel, 1989). Absorbance was measured after 15 minutes of incubation at 600 nm against a reagent blank in using UV- Vis spectrophotometer (Shimadzu, Japan). The linear regression of the standard graph for the estimation of Cu is 0.994. Biomass was determined at 595 nm (Rajeshkumar *et al*., 2010).

Optimization of temperature for metal removal

The bacterial isolate was inoculated in 25 ml of sterilized LB broths containing 100mg/L of copper at different temperatures such as 25, 30, 35, 40 and 45°C. The flasks were incubated for 24 hours at 120 rpm agitation speed.

Optimization of pH for metal removal

The bacterial isolate was inoculated in 25 ml of sterilized LB broths containing 100 mg/L of copper at different pH such as 2, 4, 6, 8 and 10. The flaks were incubated for 24 hours at 35°C and 120 rpm agitation speed.

Measurement of the kinetics of broth cellular growth and heavy metal removal

The bacterial isolate was inoculated in 25 ml of sterilized LB broths containing 100 mg/L of copper. The flasks were incubated for 24 hours at optimum pH and temperature. Bacterial growth and copper removal were observed for every 2 hours. The percentage removal was calculated using the following formula,

$$\%\ \text{removal of copper} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

Results and discussion

Isolation of copper tolerant bacteria

The concentration of 10^{-4} sample was inoculated in LB plates with different concentrations of copper for enumeration. The number of colonies decreased with increase in copper concentration. This shows the toxic nature of the metals (Rajeshkumar *et al.*, 2011). The isolated bacterial culture was tolerant to a copper concentration of 100 ppm. It was further characterized and used in Cu removal batch mode studies.

Identification of copper tolerant bacterial species

Identification of the isolated bacterial species was done by various biochemical tests and by using Bergeys Manual of systemic bacteriology (Tab. 1).

Optimization of temperature for removal of copper

The biomass growth and removal of copper with respect to temperatures (25-45°C) was shown in figure 1. The optimum temperature for growth was found to be 35°C and the same was found to be optimum for copper removal. Hence copper removal appears to be temperature dependent. This optimum temperature was comparable with the room temperature (30-35°C) which might have influenced the selection of these isolates. The temperature of the adsorption medium could be important for initial metal adsorption by

microbial cells, energy dependent mechanisms may be affected by the temperature of the adsorption medium. Temperature can affect the stability of the cell wall, its configuration and can also cause ionization of chemical moieties. The binding sites on the isolated bacterial species might be simultaneously affected by these factors and may cause reduction in metal removal (Rajeshkumar *et al.*, 2011). Energy-independent mechanisms are less likely to be affected by temperature since the processes responsible for removal are largely physiochemical in nature (Gulay and Yakup, 2003). Mostly adsorption is an exothermic process (Martins *et al.*, 2006), whereas, some examples of endothermic adsorption have also been reported (Davis *et al.*, 2003).

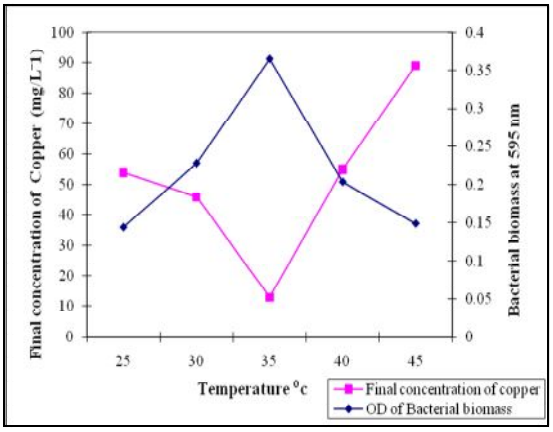

Figure 1. Cellular growth and copper removal by *Bacillus* sp. in response to various temperatures. Concentration of Cu= 100 mgL^{-1}, incubation time= 24 h

Optimization of pH for removal of copper

The biomass growth and removal of copper with respect to pH (2-10) was shown in figure 2. The optimum pH for growth was found to be 8 and the same was found to be optimum for copper removal. The percentage copper removal was 87%. The optimum pH of bacterial biomass was seen at temperature 35°C which is shown in figure 2. The proton concentration is high at

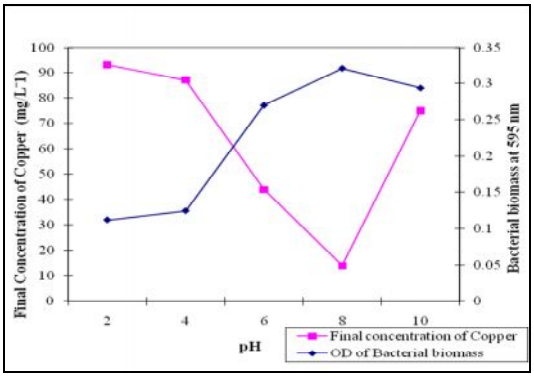

Figure 2. Cellular growth and copper removal by *Bacillus* sp. in response to various pH. Temperature= 35°C, incubation time= 24 h, concentration of Cu= 100 mgL^{-1}

lower pH (<2) and heavy metal biosorption decreases due to the positive charge density on metal binding sites, i.e. hydrogen ions compete effectively with metal ions in binding to the sites. The negative charge density on the cell surface increases with increasing pH due to deprotonation of the metal binding sites. The metal ions then compete more effectively for available binding sites, which increase biosorption (Kapoor *et al.*, 1999). Decrease in biosorption at higher pH (>6) is due to the formation of soluble hydroxylated complexes of the metal ions and their competition with hydroxyl ions for active sites. Beyond pH 8.0, precipitations of the ions as hydroxides occur (Martins *et al.*, 2006). Most of the microbial surfaces are negatively charged due to the ionization of the functional groups, thereby contributing

to metal binding. The pH of the biosorption medium affects the solubility of the metal ions and the ionization state of the functional groups (Rajeshkumar *et al.*, 2011).

Measurement of the kinetics of broth cellular growth and heavy metal removal

The time-course data for heavy metal removal and cellular growth were observed under its optimal pH and temperature conditions (Fig. 3). The removal of copper

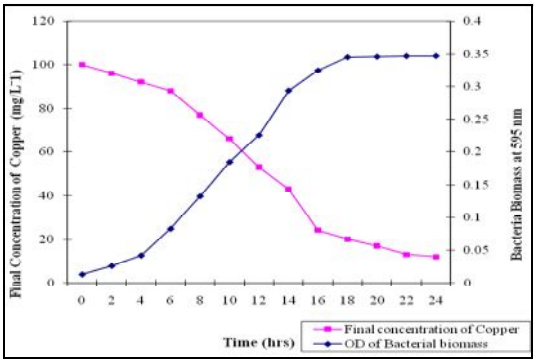

Figure 3. Kinetics of cellular growth and copper removal by *Bacillus* sp. Concentration of Cu= 100 mgL^{-1}, pH= 8, temperature= 35°C

was increasing steadily till 18 hours and then reached equilibrium. The maximum removal of heavy metal was 88% during 24 hr of incubation. The specific metal bioaccumulation increased when cells were in stationary phase. The information regarding the effect of growth phase will be useful in designing wastewater treatment plants for removal of heavy metals from industrial wastewaters. Because of adaptation to the environment by the bacterial isolate, the growth rate during lag phase was very low. They grew in logarithmic form using the nutrients in the next stage. The number of living and dead cells was fixed in the third stage. In the beginning, due to the availability of abundant active sites, the removal efficiencies by the biosorbents increased rapidly and with gradual occupancy of these active sites, sorption became less efficient later stages (Shankar *et al*., 2007).

Conclusion

From the study it becomes evident that the copper resistant bacteria capable of biosorption can be used in treatment of industrial effluents containing copper contamination. It can be used in immobilized form for easy recovery and maintenance. It can be spread over the contaminated soils to avoid the effect of the heavy metals on plants. Reactor vessels can be designed for biosorption of metals and also desorption process is possible, thus the heavy metals can be retrieved in metal extraction processes. Since the microbes were isolated from fireworks industry contaminated soil, where many heavy metals were present, the isolated organisms may be resistant to others heavy metals also. Remediation of contaminated soils is thus possible with the bacterial isolate.

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