Available online at www.elixirpublishers.com (Elixir International Journal)

Pollution

Elixir Pollution 40 (2011) 5529-5533

ISSN: 2229-712X

Enhanced copper sorption from solutions by cyanobacterial isolates exposed to electric field

Gaurav Pant[1], G. V. N. S. Deviram[1], Md. Asif[2] and R. Gyana Prasuna[1]

[1]Department of Microbiology, GITAM Institute of Science, GITAM University, Visakhapatnam - 530 045, Andhra Pradesh, India.

[2]Department of Chemistry, GITAM Institute of Science, GITAM University, Visakhapatnam - 530 045, Andhra Pradesh, India.

ARTICLE INFO

Article history:

Received: 11 September 2011;

Received in revised form:

7 November 2011;

Accepted: 19 November 2011;

Keywords

Biosorption,

Anabaena variabilis,

Cyanobacteria,

Externally applied electricity,

Cu sorption,

Neocuproine method.

ABSTRACT

The aim of the present study is the development of an ecofriendly approach to treat sewage or industrial waste containing metal contaminants. The approach utilized cyanobacterial strain "*Anabaena variabilis*", raised using exposure to externally applied electricity. Experimental algal cells were used to test for Copper sorption experiments, which were carried out as a function of pH, initial amount of biosorbtant and contact time. At pH 6 the maximum Cu sorption efficiency of live 1g (20.55%), 3g (34.28%) and 5g (51.43%) test sample were observed after 24 hours. Further studies showed a sharp increase in Cu sorption at the end of 4th day incubation. At the end of 4th day uptake of copper ions were found to be maximum for 1g (39.74%), 3g (55.47%) and 5g (71.62%) of test cells. Such findings show the possibility of manipulating or over expressing existing resistance mechanisms and the use of such organisms to remove harmful metals from the environment.

Introduction

Pollution is one of the major problems facing mankind, with water pollution being an important subgroup. Water is an important and essential factor for life. Metal pollution is the major and most hazardous source of water pollution due to the mobilized nature of pollutants (Dixit and Tiwari, 2008; Mensi et al., 2008; Abdel-Ghani, 2007). Over a long period of time, metals are released into water bodies by various anthropogenic activities. These metals damage the environment and have toxic effects on human and other forms of life, even at low concentrations. They have ability to disrupt the function of essential biological molecules, such as protein, enzyme and DNA, as they can bind with protein molecules and prevent replication of DNA and thus subsequent cell division (Alluri *et al*., 2007). As per health concern, it is necessary to remove these toxic metals from effluents before disposal.

Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe) and the platinum group elements (Duruibe et al. 2007). Copper is a reddish metal, having an atomic number of 29, average atomic weight of 63.54, with specific gravity of 8.94. It occurs naturally in rock, soil, water, sediment and air, with two naturally occurring stable isotopes 63Cu and 65Cu, having a ratio of approximately 7:3. There are two radioactive isotopes of copper, 64Cu and 67Cu, which have been useful for clinical and experimental purposes (Marceau *et al*., 1970; Strickland *et al*., 1972). Copper can be emitted into the environment by both natural and anthropogenic activities which include mining of copper and other metals (Hutton and Symon, 1986; Battarbee et al., 1988; Nriagu, 1989), industrial effluents, domestic waste water, phosphate fertilizer production, oxidation of fossil fuels and wastes etc. Copper is then transported to human beings by the means of contaminated soil, water and breathing air or dust containing copper (ATSDR, 1990; Nriagu, 1988). The phenomenon of bio-magnification is another important source of transportation when the mobilized metal is carried into food web (Paknikar et al., 1997; Paknikar et al., 1999).

After copper intake, it rapidly enters the bloodstream and is distributed throughout the body. Certain substances in food enhance the amount of copper that enters the bloodstream from the gastrointestinal tract. High levels can cause vomiting, diarrhea, stomach cramps and nausea. Hematological investigations includes reduced hemoglobin percentage, hematocrit, mean cell volume, mean cell hemoglobin and plasma and liver iron levels (Gipp *et al*., 1973). Hepatocellular necrosis and structural damage to proximal convoluted tubules in the kidneys are common results due to very high level of copper in body, which causes liver and kidney failure and finally lead to death (Haywood, 1985).

Cyanobacteria (also known as blue-green algae) are the largest and most diverse group of photosynthetic prokaryotes. Their habitats vary from fresh and marine water to terrestrial environments. They are oxygen-evolving organisms that respond to stress conditions such as light deprivation (Borbely et al., 1990). They are often used in phytotoxicity test for environmental monitoring (Boswell et al., 2002). These algae are known to fix atmospheric nitrogen and convert insoluble phosphorous into soluble phosphate (Irisarri et al., 2001). Live cells develop natural methods for responding metals such as copper, lead, and cadmium as they contain intracellular polyphosphates and extracellular polysaccharides (Zhang and Majidi, 1994; Kaplan et al., 1987; Van Eykelenburg, 1978). Phenomena of metal sequestration and to chelate or bind metal ions results through passive accumulation of metals in cells and through surface binding to various functional groups (Rai et al.,

Tele: +91 8374600018

E-mail addresses: rgauravpant@gmail.com

2000; Bender et al., 1994). Presence of mucilaginous sheath (Wang et al., 1998) and carboxyl groups present on the cell biomass such as poly-uranic acid and proteins (Gardea-Torresdey et al., 1990 ; Jorge L et al., 1996) are important factors for sorption and enhance the bioremediation capacity (Gamila and Nagalaa, 1999), and helps in maintaining the equilibrium of aquatic bodies (Campanella et al.,2001).

Keeping in view the above, experiments were conducted to study the copper absorption capacity of isolated cyanobacterial strain, *Anabaena variabilis*. An effort was also made to increase this capacity by developing improved strains showing resistance to externally applied electricity. Effect of externally applied electric field on the morphology, growth, survival and differentiation of heterocyst in cyanobacterial strain were studied. Comparative studies with effects of pH, exposure time, and amount of inoculums on the copper absorption capacity by the live non treated strains with live post electricity treated cells were analyzed. Such findings can help to remove harmful metals from the environment.

Materials and methods

Collection and isolation of cyanobacterial strains

The filamentous and heterocystous cyanobacterial strains were collected from the Visakhapatnam Steel Plant effluent, Dist- Visakhapatnam, AP, India during September 2010. The samples were brought to the Laboratory 1, of Department of Microbiology, Institute of Science, GITAM University, Visakhapatnam, AP, India for further processing. *Anabaena variabilis* culture were isolated and purified by repeated plating on solid Chu 10 medium and colonies of different morphologies were identified according to morphological properties and pigment composition (Desikachary, 1959, Sinha & Hader, 1996; Ferris & Hirsch, 1991; Reynaud & Franche, 1986).

Pure culture were maintained by subculturing in 250 ml Erlenmeyer flasks containing 100 ml of sterile Chu 10 medium and incubated under florescent light (3000 lux) at a temperature of 25 +/- 1°C. The cultures were harvested by centrifugation (4000 rpm for 15 minutes) after 15 days of inoculation. The dehydrated living algal pellet was added in 7.5 ml of Chu 10 medium to obtain a final O.D. of 0.15 at 560 nm. This suspension was further used for the electric shock treatment.

Design of electric chamber

Medium sized Electrophoresis Chamber was used as electric chamber (Zha et al., 2006; Reid et al., 2005; Foulds & Barker, 1983). A perforated diaphragm was used to separate the stage in the chamber. The perforation allows the medium and electric current to circulate within the chamber. Anode and cathode terminals of the chamber were joined to the anode and cathode end of the electrophoresis power supply unit respectively. Amount of electric current which has to be used for the experiment was adjusted by the means of fine and coarse current setting knobs. Algal culture was added in the center of the stage.

Electric shock treatment method

The electric chamber was filled with 142.5 ml of Chu 10 medium. 5% of cyanobacterial culture (OD: 0.15 at 560 nm) was transferred in the center of the stage. The sample was treated with 10 amperes of current for 2 hours by the means of electrophoresis power supply. During the treatment 1 ml of treated sample was transferred aseptically (from 0 to 120 minutes at an interval of 5 minutes) in a test tube containing 4 ml of Chu 10 medium. The tubes were incubated under ideal growth conditions for 15 days and further studied morphologically.

Experimental work

1ml of (10 amperes - 80 minutes) electricity treated cyanobacterial culture was taken as test sample while untreated culture was taken as control for copper profile. The copper sorption by cyanobacterial strain (Control and Test) was determined at pH values of 2, 4, 6 and 8. Stock solution of copper was prepared by dissolving 200mg of copper wire into a solution having 10 ml of redistilled water with 5 ml of concentrated HNO_3. For complete dissolution of copper, gentle warming was done followed by a step of boiling to expel oxides of nitrogen. The solution was transferred to 1 liter volumetric flask and diluted to the mark with redistilled water. The concentration of copper in 1 ml of solution will be 200 µg. All the experiments were performed under batch conditions for which stock solution of copper were added in Chu 10 medium to obtain 2 mg/l concentration of copper and used for further studies.

Standard acid and base solutions (0.1N H_2SO_4 and NaOH solutions) was used for pH adjustment. The experiments were performed in at room temperature (28 ± 2ºC). 1ml of live cyanobacterial samples (Control and test) were inoculated in 100 ml of sterile Chu 10 medium having 2mg/l copper concentration and incubated in ideal conductions for 24 hours. The biomass was removed by filtration through a 0.45 µm membrane filter (Millipore) and the concentration of copper was spectrphotometricaly determined by UV- spectrophotometer (Model-U-2900) with neocuproine method (Larsen 1974).

In order to investigate the effect of different initial Cyanobacterial concentration and time-dependency for copper binding were performed under batch conditions. The time intervals tested were 1, 2, 3, 4, 5, and 6 days at optimum conductions with 1, 3 and 5 g of initial amount of cyanobacterial samples (Control and Test). Growth medium with 2mg/l copper concentrations were prepared by the stock copper solution, remainder of the procedure were analogous to that reported previously in pH profile. All experiments were performed in triplicates and statistical analysis (average, standard deviation and correlation – coefficient values) were reported.

Results and discussion

On the basis of triplicate results, readings were taken from the fourth day to the fifteenth day. Algal cells were found to survive and grow upto 80 minutes of the treatment, whereas, further exposure inhibited the growth. Abnormal cells were found surviving till upto 5 days, at 85 and 90 minutes treatment but all cells were killed by an exposure of 95 minutes and above. Therefore 80 minutes post electric treated cells were taken as test cells for further studies. Microscopic examination showed increase in frequency and size of heterocysts when control were compared to test sample.

Role of hydrogen ion on biosorption

Biosorption of metal is a pH dependent phenomenon (Zhou, 1999). pH is an important factor that influences uptake of metal ions from aqueous solutions (King et al., 2006) because it affects the solubility of metal ions, degree of ionization of the absorbate during reaction and concentration of the counter ions.

The cell wall of Cyanobacteria consists of mucilaginous sheath (Wang et al., 1998) and carboxyl groups such as poly-uranic acid and proteins (Gardea-Torresdey et al., 1990 ; Jorge L et al., 1996) which are available for making coordinate bonds with metal ions such as copper (II), lead (II) and chromium (IV)

(Saifuddin, 2007). In acidic medium quantity of positive charged hydrogen ion is more which increase the competition with metal ions, as a result fewer metal ions binds to ligands present on the cell wall (kaewsarn, 2002) while, when pH of the medium is towards basic, concentration of hydrogen ion is low which leads to decrease in the competition and finally enhance biosorption capacity.

In the experiment maximum Cu absorption was observed to be 19.55% and 20.55% at pH 6 respectively for control and test cells and then declining at higher pH. The decrease of biosorption levels at lower pH can be explained to be due to increasing competition between protons and metal ions for capturing same sites, on the cell wall, which reduces sorption phenomena. Hence for all other studies pH 6 was considered as optimum pH for biosorption of copper. Correlation Coefficient between pH of the medium and percentage of copper removal by 1g of control and test sample after 1 day at 2mg/l concentration of copper were reported as + 0.84 and + 0.83 respectively .

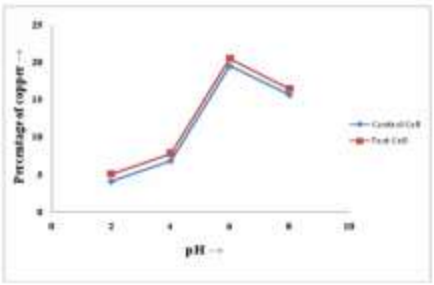

Figure 1: Effect of pH on the percentage removal of copper

Effect of initial amount of biosorbent

The concentration of biosorbent is a significant factor for effective biosorption. Metal ion concentration and concentration of biosorbent determines the sorbent/sorbate equilibrium of the system. Biosorption of Cu with varying biosorption concentration is shown in fig. 2. Copper uptake rose with increase in concentration from 19.55% at 1g biomass to 32.97% at 3g to 49.91% at 5g biomass of control cells whereas in test cells it increase from 20.55% at 1g to 34.28% at 3g to 51.43% at 5g, on the end of 24 hours. This appears to be due to the increase in the available binding sites on the cell wall for the complexation of copper. However some of the other studies show decrease in metal sorption with increase of the concentration of biosorbent, this can be explained by the fact that, increase of concentration of biosorbent causes reduction in inter cellular distance and cell agglomeration. Correlation Coefficient between initial amounts of cyanobacterial samples (Control and Test) and percentage of copper removal after 24 hours at optimum conduction were reported + 0.99 for both.

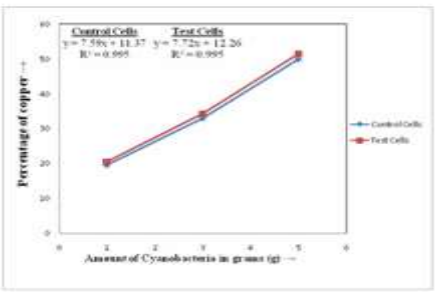

Figure 2: Effect of amount of biosorbent on the percentage removal

Effect of contact time

Copper sorption as a function of contact time was studied for determining the equilibration time. Effect of contact time on amount of Cu uptake with cyanobacterial samples (Control and Test) is shown in figure 3. Contact time was varied from 1 to 6 day. Percentage removal of copper increased with contact time. A sharp increase was observed at around the 4th day of incubation. Greater availability of various functional groups on the surface of algal cell, which are required for interaction with anions and cations, significantly improved the binding capacity and the process proceeded rapidly. This result is important, as equilibrium time is one of the important parameters for an economical wastewater treatment system. Correlation Coefficient between contact time and percentage of copper removal by 1g of control and test sample after 24 hours at 2mg/l concentration of copper were reported as + 0.77 and + 0.78 respectively .

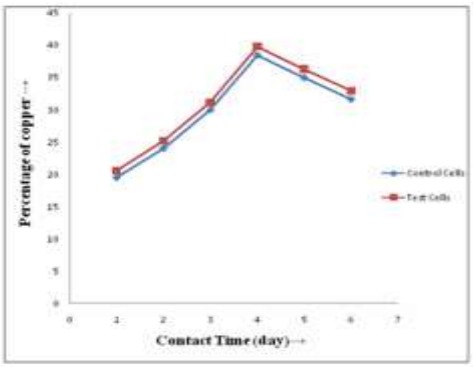

Figure 3: Effect of contact time on percentage of copper removal

Combined studies of biosorption with initial amount of biosorbent and contact time

In order to investigate the effect of initial amount of biosorbent with contact time, on the metal sorption 1, 3, and 5g of cyanobacterial live cells (Control and Test) were inoculate in growth medium having 2mg/l concentration of copper for 1, 2, 3, 4, 5 and 6 days at optimum conditions.

At the end of 4th day uptake of copper ions were found to be maximum for control as well as for test cells, however concentration varies from 1 to 5g as shown in fig. 4.

The roles of contact time on biosorption of copper ion by live cyanobacterial biosorbent were studied. In both types of cells biosorbents, copper sorption was increased upto 4th day. Based on these results equilibrium time of 4th day was chosen for further experiments.

Absorption rate were found to be decreased after 4th day and can be explained due to the fact that initially a large number of vacant surface sites were present and after that it may be difficult to occupy binding sites due to repulsive forces between the solute molecules of the solid and bulk phage.

The diminishing removal with increasing time may be due to intarparticle diffusion process dominating over absorption. The absorption process is actually a surface interaction with vary rapid sorption of ions by microbial surface.

Similar work has investigated by Gaur N and Dhankhar R (2009) with zinc ions. Another reason for the observed effects could be the start of deterioration of some of the filaments or cells.

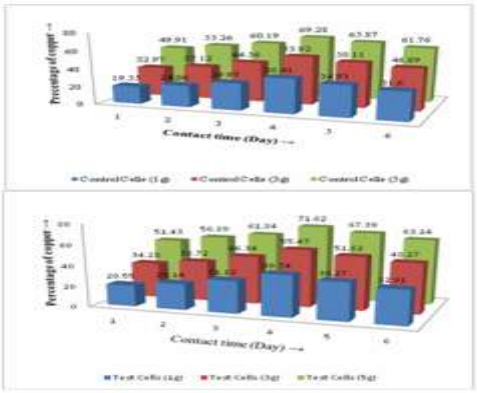

Figure 4: Combine effect of amount of biosorbent and contact time on percentage of copper removal

Conclusions

Heavy metals are important in many respects to human race, but the biotoxic effects, when unduly exposed to them could be potentially life threatening hence, cannot be neglected. The study showed the ability of isolated cyanobacterial strains to absorb and remove copper from solution. There was an increase in this absorption by the cyanobacterial strains resistant to applied electricity. Such finding has shown the possibility of manipulating or over expressing existing resistance mechanisms and the use of such organisms to remove harmful metals from the environment. Laboratory trials do show their potential for commercialization since it is technically feasible, ecofriendly with good metal binding capacity and can contribute pollution control mitigation.

Acknowledgement

This work was funded by the ICMR (Indian Council of Medical Research) New Delhi, India. Authors are grateful to ICMR and sincere thanks to management and staff of GITAM University, Visakhapatnam, A.P., India for providing facility for the research.

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