ORIGINAL ARTICLES

Adsorption Studies on Heavy Metals by Isolated Cyanobacterial Strain (Nostoc sp.) From Uppanar Estuarine Water, Southeast Coast of INDIA

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ABSTRACT

Microorganisms play a significant role in heavy metal adsorption from contaminated waste water. In the present study cyanobacterial isolates were picked up from the contaminated water and purified, identified by morphological and cultural characteristics. Each isolate was determined by the dilution method, Ni, Cd, Fe, Pb, and Zn were used in varying concentrations (10 to 1000 µL⁻¹). Anabaena sp., Trichodesmium sp., Oscillatoria sp., Cylindrospermopsis sp. and Nostoc sp. were showed minimum inhibitory concentration (MICs) to heavy metals. From this Nostoc sp. showed high resistant so this strain use for further studies. One ml were collected each Three days interval for analyze the growth, pH and adsorbed heavy metals analyzed by ICP-OES. During this experiment 95.4% of Cd, 97.7% of Fe, 99.6% of Pb, 88.23% of Ni and 75.3% of Zn was removed by the Nostoc sp. From this experiment Nostoc sp. showed high efficiency to remove the heavy metals and this experiment may be helpful to bioremediation research in future.

Key words: Cyanobacterial isolates, Nostoc sp., dilution method, ICP-OES, heavy metals.

Introduction

Heavy metals have been released into the environment over long periods of time by natural processes and manmade activities generate anomalous concentrations of metals. Once the metals have been released into the environment, they are difficult to be removed by physical or chemical means and most of them exhibit toxic effects on organisms. A great deal of interest has recently been generated by using microbes as biosorbents for metal removal such as bacteria, fungi, yeasts, and plants, have been employed to investigate effective biosorption systems (Shuttleworth and Unz, 1993; Nedelkoska and Doran, 2000). The role of microorganisms in the mobilization and immobilization of heavy metals in the environment spans from the biogeochemical cycles to their biotechnological applications in bioremediation (White et al., 1997; Gadd, 2000). A number of different uptake and resistance mechanisms have been identified and recently reviewed (Bruins et al., 2000; Valls and de Lorenzo, 2002). Several investigations concerning the interactions between heavy metal ions and photosynthetic microorganisms such as green algae Chlamydomonas reinhardtii (Danilov and Ekelund, 2001), Chlorella vulgaris (Rai et al., 1994; 1994b), cyanobacteria Spirulina platensis (Singh and Kumar, 1994), Microchaete tenera (Zaccaro et al., 2001), Microcystis sp. (Pradhan and Rai, 2001), Gloeothecae magna (Mohamed, 2001), Nostoc linckia (El-Enany and Issa, 2000) and proteobacteria R. spheroids (Moore and Kaplan, 1992), Rhodospirillum rubrum (Watt and Ludden, 1999) have been reported. The aim present investigation was isolate and identifies the heavy metals tolerant cyanobacterial strains from the effluent water and study the adsorption ratio of the heavy metals.

Materials and Methods

Sample Collection and Cyanobacterial Isolation and Identification:

Sample was collected from the estuarine region of Uppanar River (Lat. 11/ 43’N, Long. 79/ 46’ E). It runs behind the SIPCOT (State Industrial promotion Corporation of Tamilnadu Limited) industrial complex. This consists of many chemical and Pharmaceutical industries. Effluent water brought to the laboratory in the insulated bottle. 1 ml of water sample serially diluted with 99 ml of 50% aged seawater and 0.1 ml of water was spread on plated with sterilized basal agar medium (BG-11) at pH 8.5 using standard isolation was followed by the method of Stanier et al., (1971) in brief. The algal cultures were maintained at a light intensity of 3000 lux...
using cool fluorescent tubes at 28 ± 3°C in a culture room. Cyanobacterial isolates were picked up and purified by repeated streaking on basal agar medium and culturing techniques on nitrogen supplemented BG-11 medium (Kaushik, 1987). The morphology of cells and filaments was studied using an Olympus BX60 light microscope with a digital camera.

**Minimum Inhibitory Concentration (MICs) Assay:**

The minimum inhibitory concentration of the metals for each isolate was determined by the plate dilution method as adopted by Malik and Jaiswal, (2000) and Aleem et al., (2003). The metals Ni²⁺, Cd²⁺, Fe²⁺, Pb²⁺ and Zn²⁺ were used as NiCl₂, CdCl₂, FeSO₄, PbSO₄ and ZnCl₂ in varying concentrations ranging from 10 to 1000 μgL⁻¹. Stock solutions of the metal salts were prepared in sterile Milli-Q water and added to the basal agar medium (BG-11) in various concentrations which were then spot inoculated with approximately 3×10⁴ organisms. The algal cultures were maintained at a light intensity of 3000 lux using cool fluorescent tubes at 28 ± 3°C in a culture room. This test was carried for three times to conform MIC of the isolated strains.

**Biomass Preparation:**

Most heavy metal tolerant strain was cultured for 10 days in basal agar medium (BG-11) and incubated in a culture room. The initial heavy metal concentrations of freshly collected estuarine water were noted then only basal agar medium (BG-11) ingredients were added to the collected estuarine water and sterilized by autoclave at 120°C for 20 min then isolated cyanobacterial strain (1% of the effluent volume) were inoculated and maintent under continuous light intensity at 28 ± 3°C by shaking at 200 rpm/min for 15 days. In 3 days interval 1 ml sample were collected for analyze the growth of cyanobacterial culture and pH of the each sample.

**Growth and pH Assessment:**

The pH of the collected samples measured by using pH pen (Eco testr) then centrifuged with 8000 rpm for 10 min at 4°C with cooling centrifuge and supernatants were collected to analyse the heavy metals and the pellets also collected to analyze the heavy metals and growth of the cells. The chlorophyll a content in the cells was extracted by standard acetone extraction method described by Clesceri et al., (1999). Extraction of chlorophyll a was determined by spectrophotometrically at 460 nm by HITACHI-220S UV spectrophotometer.

**Determination of Accumulated Heavy Metals:**

Harvested cells and collected supernatants were kept in oven at approximately 90°C for at least 12 hrs then preserved by acidification with conc. HNO₃. The digested mixture was centrifuged, the supernatant was filtered and analysed by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrophotometer) using a specific lamp and at specific wavelengths. This analyze was carried for three times to conform the absorption.

**Results and Discussions**

**Cyanobacterial Isolation and Identification:**

In present investigation only five strains were tolerant to the heavy metals concentration of the basal agar medium (BG-11). *Anabaena* sp., *Trichodesmium* sp., *Cylindrospermopsis* sp., *Oscillatoria* sp. and *Nostoc* sp. were showed MIC to heavy metals. These isolates were identified by microscopic examination by morphological features of the cells. From these strains *Nostoc* sp. showed most resistant to heavy metals so this strain was taken for further absorption studies.

**Growth and pH Assessment:**

In growth assessment study, isolated *Nostoc* sp. showed high growth rate up to 12 day (Fig. 1 confirmed by the OD value) and the pH decrease according to the time duration (Fig. 2).
Determination of Accumulated Heavy Metals:

In this adsorption study the accumulated heavy metals analyzed by ICP-OES and compared with treated effluent sample. In Fig. 3 the absorption percentage of heavy metals by the cyanobacterial cells (*Nostoc* sp.) were noted. The graphs (Fig. 4 - 8) shows adsorbed heavy metals ratio of the cyanobacterial cells according to the metals and time duration. During experiment 95.4% of Cd, 97.7% of Fe, 99.6% of Pb, 88.23% of Ni and 75.3% of Zn were removed by the isolated cyanobacterial strain.
Discussion:

The aim of the trial was to assess the effectiveness of a microbial, made up with native selected strains, as a bioaugmentation agent for the adsorption of contaminated estuarine water. In principle we expect that native strains of co-contaminated matrices, already shaped by selective pressure, could take advantage with respect to sensitive strains in accomplishing biodegradation, when heavy metals are present. This way, they could help to overcome an important limitation in bioremediation applications. Two factors can be considered to account for toxicity: (a) this metal is not an essential trace element for organisms and (b) the low complexing capacity of the culture medium used in this assay leaves almost completely bioavailable (Ceretti et al., 2006). The metal removal capacity of an organism touches its peak at these high metal concentrations. At low metal
concentrations (as encountered in effluent samples) the biosorption capacity of the biosorbent is not fully utilized (Rani and Harapriya, 2003).

In present investigation five strains were tolerant to the heavy metals concentration of the basal agar medium (BG-11). The following parameters were analysed for identify the strains: length and width of vegetative cells; morphology of the terminal cell; the presence or absence of heterocysts, akinetes and gas vesicles; the distance between heterocysts and the distance between a heterocyst and the nearest akinete; and finally, the shape of filaments and their potential aggregation into colonies.

In growth assessment study, isolated *Nostoc* sp. showed high growth rate up to 12 day. In the present study the growth of strain was increased with decreased of pH in the effluent medium. The pH plays a very critical role in microbial metal uptake by influencing the metal speciation and solution chemistry as well as surface properties of cyanobacterial cells. pH was evaluated since it affects the number of cellular surface sites available to bind cations, as well as metal speciation (Yan and Viraraghavan, 2003). At very low pH values, metal cations and protons compete for binding sites on the cell walls, which results in higher uptake of metal. As pH levels are increased, more ligands with negative charge would be exposed with a subsequent increase in attraction for positively charged metals ions. The fig. 2 shows the pH decrease according to the time duration.

In this biosorption studies the accumulated heavy metals analyzed by ICP-OES and compared with treated effluent sample. The graphs (Fig. 4 - 8) shows adsorbed heavy metals ratio of the cyanobacterial cells according to the metals and time duration. During experiment 95.4% of Cd, 97.7% of Fe, 99.6% of Pb, 88.23% of Ni and 75.3% of Zn were removed by the isolated cyanobacterial strain. By initially the collected water contain 0.3254 ± 0.00026 µg/L of Cd, 0.9309 ± 0.00026 µg/L of Fe, 2.18 ± 0.005 µg/L of Pb, 0.071 ± 0.0019 µg/L of Ni, 3.257 ± 0.00016 µg/L of Zn. During this experiment 1st sample was collected at 3 days interval, in this 0.0664 ± 0.0012 µg/L of Cd, 0.1045 ± 0.045 µg/L of Fe, 12.58 ± 0.013 µg/L of Pb, 0.036 ± 0.0016 µg/L of Ni, 0.988 ± 0.0094 µg/L of Zn were accumulated by cyanobacterial cells. Whereas the 2nd sample was collected at 6th day interval, accumulated heavy metals noticed 0.098 ± 0.0008 µg/L of Cd, 0.29 ± 0.0047 µg/L of Fe, 14.41 ± 0.043 µg/L of Pb, 0.44 ± 0.0005 µg/L of Ni, 1.4 ± 0.024 µg/L of Zn. In 9th day sample was recorded 0.203 ± 0.033 µg/L of Cd, 0.55 ± 0.025 µg/L of Fe, 18.67 ± 0.3 µg/L of Pb, 0.06 ± 0.015 µg/L of Ni, 1.75 ± 0.025 µg/L of Zn were accumulated. 4th day sample was showed 0.277 ± 0.114 µg/L of Cd, 0.65 ± 0.015 µg/L of Fe, 19.64 ± 0.096 µg/L of Pb, 0.057 ± 0.001 µg/L of Ni, 2.33 ± 0.35 µg/L of Zn. As like 15th day sample also showed 0.3099 ± 0.05 µg/L of Cd, 0.887 ± 0.007 µg/L of Fe, 21.51 ± 0.245 µg/L of Pb, 0.0593 ± 0.0007 µg/L of Ni, 2.455 ± 0.24 µg/L of Zn (Fig.4, 5, 6, 7 and 8).

From the above details shows the isolated cyanobacteria (*Nostoc* sp.) was accumulated more than 91% of the heavy metals from the estuarine water. Comparing these results with similar studies confirmed that our selected organism is efficient as absorption of heavy metals. They exhibited more efficiency and faster removal rates than did other local species isolated from Qaroun [Lyngbya sp. (99.9%), Oscillatoria sp. (96.16%), *Anabaena* sp. (80.8%), *Oscillatoria* sp. (93.86%), *Lyngbya* sp. (87.20%), *Spirulina* sp. (91.30%)], where 2 weeks were needed for removal of lower lindane concentrations (0.5 mg ml⁻¹-10-90 ng ml⁻¹) (Final Report, 2003). These environmental species are also more efficient and possess more bio detoxification capabilities than the wild or mutant *Anabaena* sp. strain PCC7120 and *Nostoc ellipsiporum* that removed only one chlorine atom, producing g-pentachlorocyclohexane (Kuritz et al., 1997).

Therefore it can be recommended this to be employed in the purification of waste contaminated with these heavy metals. The present results were also coincident with other authors. Ma Clean et al., (1972) was the first reported that presence of Cd-binding material in a fresh blue-green algae (e.g *Anacystis nidulans*). Chong and Volesky, (1996) reported that brown algal biosorvents (FCAN2) have the binding preference in the order of Cu>Cd>Zn. The selective preference of a desorbent by the bound metal ion can be explained by the stability constants derived from the metal ligand formation (Andrea and Benno, 1995). Similarly, copper (II), nickel (II) and lead (II) ions can be efficiently removed from *Synechococcus* sp. with 0.1 M HCl (Gardea-Torresdey and Arenas, 1996a). Similar studies using *Oscillatoria* sp., *Nostoc* sp., *Gloeocapsa* sp. and *Synechococcus* sp. of cyanobacteria for bioremediation of effluents containing Cd have earlier been reported by (Swamy, 2000; Stanley Abraham and Swamy, 2001). All these information clearly reveals the existence of a finite heavy metal reduction capacity possibly due to heavy metal toxicity toward cells and the results of this study indicated that the biomass of *Nostoc* sp. was proved the development of efficient for the removal and recovery of Cd, Ni, Fe, Pb and Ni from estuarine water.

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References


