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Utilization of Biomass of *Nostoc* Species for Production of Pigments and Adsorption of Heavy Metal Ions Cu(II), Cd(II) and Cr(VI) from Aqueous Solution

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ABSTRACT

The dried biomass of species of cyanobacteria *Nostoc entophytum*, *N. punctiforme* and *N. ellipsosporum* was assessed for phycobiliprotein and carotenoid content. The biomass left after extraction of pigments was utilized for the adsorption of heavy metal ions. The phycobiliprotein content of these species ranged from 32.1–51.1 mg g^{-1} on a dry weight (dw) basis, while the total carotenoids content varied from 9.7 to 14.5 mg g^{-1} dw. The pH-dependant adsorption of metal ion showed that optimum adsorption of Cu(II) and Cd(II) takes place at pH 4-6, and that of Cr(VI) at pH 1-2. Kinetics of adsorption of metal ions on biomass of these species showed that initial rate of adsorption of metal ions was fast and within 120 min in which 78-88% of Cu(II), 56-62% of Cd(II) and 52-64% of Cr(VI) was adsorbed by the *Nostoc* species biomass. For more than 90% adsorption, the time required was about 6 h for Cu(II), 36-48 h for Cd(II) and 48-60 h Cr(VI). Column experiments showed that the biomass of these species act as efficient adsorbents of Cu(II), Cd(II) and Cr(VI) with more than 30 mg g^{-1} adsorption capacity towards Cu(II) by the biomass of all three species.

Keywords: carotenoids, cyanobacteria, phycobiliproteins, toxic heavy metals

INTRODUCTION

Research and development in utilization of cyanobacteria has gained much importance in recent years as they are a potential source of single cell proteins and food supplement such as colouring agents, vitamins, bioactivators and bio-antioxidants (Bhat *et al*. 2000). Phycobiliproteins are blue (phycocyanin and allophycocyanin) or red (phycoerythrin) coloured fluorescent proteins used as fluorescent markers in fluorescence immunoassay and microscopy, flow cytometry, and detection of reactive oxygen species (Glazer 1994). Carotenoids are yellow orange or red coloured pigments used mainly as food colorant or as feed additive.

Heavy metals are widespread pollutants. Some heavy metals are essential in trace amounts for living organisms but beyond a certain concentration they are generally hazardous (De 1994a). Their incremental accumulation in food chain leads to poisoning, brain damage and even cancer in human beings (Kiran *et al*. 2007). Removal of heavy metals from waste water by conventional physico-chemical methods is generally cost intensive (Noraho and Gaur 1996). In this sense use of microbial biomass has emerged as an option for developing economic and eco-friendly procedures for removal of toxic heavy metals (Wilde 1993). *Nostoc* is a photoautotrophic, filamentous, heterocystous, and nitrogen-fixing cyanobacterium that may serve as an economically feasible and efficient alternative for the production of phycobiliproteins and removal and recovery of heavy metal from waste water (El-Enany and Issa 2000). Therefore in this study we evaluated the biomass of three *Nostoc* species for the production of pigments and the biomass left after the extraction of pigments for adsorption of heavy metals Cu(II), Cd(II) and Cr(VI), which are common in effluents of electroplating, paint and pigment, plastic, alloy preparation, mining and fertilizers industries and silver-Cd batteries (Wodzki *et al.* 1999; Wanatabe *et al.* 2003; Zayed and Terry 2003; Herrero *et al.* 2006).

METHODS

Nostoc species – cultures, inoculation and culture conditions

Pure cultures of *Nostoc entophytum*, *N. punctiforme* and *N. ellipsosporum* were procured from the National Centre for Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune (India) and cultures were established in glass bottles (400 ml capacity) containing BG-11 medium (Stainer *et al.* 1971). The chemicals used for preparation of nutrient media were of analytical reagent grade and purchased from Merck, India, Ltd. Biomass production of the above mentioned species was carried out in polyethylene trays (25 × 17 × 5 cm) containing 400 ml sterilized modified BG-11 medium (KNO_3 – 0.8 g l^{-1}, $MgSO_4 \cdot 7H_2O$ – 0.1 g l^{-1}, KH_2PO_4 – 0.1 g l^{-1}, $CaCl_2$, 0.05 g l^{-1}, $FeSO_4 \cdot 7H_2O$ 0.006 g l^{-1} with 0.075 g Na_2EDTA, Na_2CO_3 – 0.1 g l^{-1}, NaCl – 0.1 g l^{-1}, micronutrient solution – 1 ml (micronutrient stock solution consists of H_3BO_3 – 0.286 g, $MnCl_2$ $4H_2O$ – 0.181 mg, $ZnSO_4$ $7H_2O$ – 0.022 g, Na_2MoO_4 $2H_2O$ – 0.039 g, $CuSO_4$ $5H_2O$ – 0.008 g, $CoNO_3$ $6H_2O$ – 0.005 g and distilled water – 100 ml)). The pH of the medium was adjusted to 7.5 ± 0.2 with 1 N NaOH prior to autoclaving at 1.06 kg cm^{-1} pressure for 20 min. In each tray 1 ± 0.1 g of fresh biomass (in the form of cell suspension) of each species were inoculated separately in the laminar air flow cabinet. All the trays were incubated in a culture room where diffused sunlight of about 44 μmole m^{-2} s^{-1} was adjusted by using semitransparent roof at environmental condition of temperature (22 ± 4°C) and at 80 to 90% relative humidity. At 3-day intervals 50 ml of fresh nutrient medium was added in each tray to compensate the volume of water lost by evaporation and the concentration of nutrients used by the growing cultures. In the preliminary experiments, opti-

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mum growth was observed between 16 to 21 days, therefore in the later experiments the biomass was harvested after 18 days. The harvested biomass was pressed between flaps of the filter papers to remove excess water. Initially the biomass was dried for about 6 hrs in the dark at room temperature using air blower followed by drying in an oven at 45°C for 3 h.

Isolation of phycobiliproteins

100 g dried and powdered biomass was suspended in 250 ml phosphate buffer of pH 7.0 for 10 min and stirred using a magnetic stirrer for 10 min. The resulting suspension was centrifuged at 5000 rpm for 10 min followed by extraction of pellet with the same buffer until the extract was colorless. The phycobiliproteins were isolated from supernatant using an ammonium sulfate precipitation method (Soni *et al*. 2008) and the content of phycobiliproteins was estimated by the method of Bennet and Bogorad (1973).

Isolation of carotenes

The biomass remained after extraction of phycobiliproteins was treated for 20 min with 4%, 250 ml hot methanolic KOH, cooled and carotenoids were extracted in to light petroleum ether until the ether layer became colourless (Harborne 1988). The petroleum fraction was treated with anhydrous sodium sulfate and then distilled under N_2 atmosphere to recover carotenoids. The carotenoid fraction was dried in vacuum, weighed and quantified by a spectrophotometric method (Gowenlock *et al.* 1988). HPLC (Younglin) analysis of carotenoids was done by using ODS C–18 column (Chemsil), UV-Visible detector (440 nm) and isocratic pump with methanol as mobile phase.

Biomass preparation for adsorption of heavy metal ions

The biomass remained after extraction of pigments was washed with distilled water until free from alkali; water was allowed to drain off completely and then it was dried in an oven at 60-65°C. The resultant dry biomass was crushed to powder (60-100 mesh size) and used for adsorption study of heavy metal Cu(II), Cd(II) and Cr(VI).

Preparation of metal ion solution

Solutions containing Cu(II), Cd(II) and Cr(VI) at a concentration of 0.1 mg ml^{-1} (100 ppm) were prepared in distilled water by using A. R. grade $CuSO_4 \cdot 5H_2O$, $Cd(NO_3)_2 \cdot 2H_2O$ and $K_2Cr_2O_7$, respectively.

pH dependant adsorption of Cu(II), Cd(II) and Cr(VI)

The influence of pH on adsorption of heavy metal ions Cu(II), Cd(II) and Cr(VI) was carried out analogous to Blanco *et al.* (1999) and Gardea *et al.* (1998). 500 mg of dry, powdered biomass of each species was suspended separately into 50 ml of each metal ion solution. The pH of the suspensions from different flasks was adjusted to 1 to 7 (at one unit intervals) by adding 0.1 M HCl. These suspensions were stirred for 2 h on a shaker at 30 rpm. Later these suspensions were filtered through a sintered glass crucible and the filtrates were analyzed for metal ion content.

Kinetics of adsorption

Kinetic experiments for adsorption of metal ions were carried out by following the method described by Kiran *et al*. (2007). A suspension of 0.5 g dry biomass of each species was made separately in 50 ml of metal ion solution (Cu(II), Cd(II), and Cr(VI) separately) at constant temperature (25 ± 0.2°C). The pH of suspensions was adjusted to optimum adsorption pH of respective metal ion with 0.1 M HCl and stirred on shaker at 30 rpm. Filtrates of these suspensions were obtained at 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 6, 12, 24, 36 and 48 h contact time and were analyzed for metal ion content. Percentage of metal ion adsorbed calculated by equation: % metal adsorbed = mg of metal adsorbed × 100/5.

Column experiments

4 g dry biomass (60–100 mesh size) of respective species was soaked into distilled water for 1 h and then packed into a chromatography column 18 mm diameter. The column was equilibrated with HCl solution of pH at which metal shows optimum adsorption and then 100 ml metal ion solution (pH adjusted to optimum adsorption pH of respective metal ion) was passed through the column at a rate of ~1.5 ml min^{-1}. Column effluents were collected and analyzed for metal ion content. Desorption of metal ions from biomass was achieved by washing the column with 25 ml of 0.2 M HCl and the column effluents were analyzed for metal ion content. For reuse, column was washed with 25 ml 0.1 N NaOH followed by distilled water until the washings were alkali free. Per gram adsorption capacity of biomass of each species was determined following the method for ion exchange resins (Vogel 1964). Percentage of metal ion adsorbed calculated by equation: % metal adsorbed = mg of metal adsorbed×100/10.

Quantification of metal ions

The concentrations metal ions in aqueous solutions were estimated by spectrophotometric methods. Cu(II) by using 8-hydroxy quinoline reagent (Jahagirdar 2003), Cd(II) with dithiozone reagent and Cr(VI) with diphenyl carbazide reagents (De 1994b).

Experimental design and statistical analyses

All the experiments were in a completely randomized design (CRD) carried out in triplicate. Data were analyzed for variance by performing an ANOVA test in MS Excel Program. The data were represented as mean ± standard deviation. Means differing significantly were compared using Duncan (1955) multiple range test (DMRT) at $p = 0.05$.

RESULTS AND DISCUSSION

In order to achieve maximum utilization of biomass of *Nostoc* species, the biomass was used first for the isolation of phycobiliproteins and later for isolation of carotenoids. After extraction of the pigments, the rest of the biomass applicability was studied for Cu(II), Cd(II), and Cr(VI) adsorption from aqueous solution. In reports of Olvera-Ramirez *et al.* (2000) and Moreno *et al.* (2003) biomass of the species *Calothrix* and *Anabaena* was evaluated either for content of phycobiliproteins or biomass of the species *N. muscorum*, *Phormidium laminosum* directly for the adsorption of heavy metal ions (Blanco *et al.* 1999; Prasad and Pandey 2000). No attempts to use biomass of cyanobacteria have yet been reported for both pigment production and heavy metal adsorption. For the present study, three common species of cyanobacterium *Nostoc*, *N. punctiforme*, *N. ellipsosporum* and *N. entophytum*, were chosen. Modified BG-11 culture medium was selected as growth medium for these species based on two years of testing in our laboratory in search of a suitable growth medium.

Phycobiliprotein content

The three *Nostoc* species were found to contain a variable amount of total phycobiliproteins (**Table 1**). The observed phycobiliprotein content (on a dw basis) in biomass of *N. punctiforme* was 5.11 ± 0.21%, which is significantly higher than phycobiliprotein content of the species *N. ellipsosporum* (3.40 ± 0.12%) and *N. entophytum* (3.21 ± 0.27%). However, a higher phycobiliprotein content (13.85%) was reported in *Nostoc* strain UAM 206 (Cesar *et al.* 2001) and in other species of cyanobacteria such as *Anabaena* sp. ATCC 33047 (12.3-18.9%) (Moreno *et al.* 2003). Phycobiliproteins are divided into three main classes according to their structure: phycoerythrins (PE), phycocyanin (PC) and allophycocyanins (APC). In the present study PC content of *N. punctiforme* (2.59 ± 0.35%) was found to be significantly higher than the PE and APC contents. A similar trend was observed in *N. ellipsosporum* and *N. entophytum* al-

Table 1 Total phycobiliprotein and carotenoid content of *Nostoc* species on dw basis.

Species name	PE (%)	PC (%)	APC (%)	Total phycobiliproteins (%)	Total carotenoids (%)	% β-Carotene*
N. ellipsosporum	0.43 ± 0.04 b	1.65 ± 0.09 b	1.32 ± 0.11 b	3.40 ± 0.12 b	1.17 ± 0.15 ab	41.61
N. entophytum	0.24 ± 0.08 c	1.79 ± 0.37 ab	1.18 ± 0.28 b	3.21 ± 0.27 b	1.45 ± 0.09 a	43.10
N. punctiforme	0.74 ± 0.07 a	2.59 ± 0.35 a	1.68 ± 0.11 a	5.11 ± 0.19 a	0.97 ± 0.12 b	35.57

PE - Phycoerythrin, PC - Phycocyanin, APC – Allophycocyanin
* Expressed as % contribution of β-Carotene to the total carotenoids
Values are mean ± SD of three independent experiment
Means within a column followed by the same letters are not significantly different at p = 0.05 by DMRT.

though the content of total phycobiliprotein was low compared to *N. punctiforme*. Analogous to *N. punctiforme*, a similar nitrate concentration in medium comparable PC content (2.32%) was observed in *Calothrix* sp. (Olvera-Ramirez *et al.* 2000). However, the PC content of *Spirulina* sp. (Vonshak 1993) was 4.7 times higher than that of *Nostoc* species. The results of the present study on *Nostoc* sp. shows the lower content of phycobiliproteins and PC than *Nostoc* strain UAM 206, *Anabaena* sp. and *Spirulina* sp. which might be due to variation in growth conditions (Olvera-Ramirez *et al.* 2000). The cultures of *Nostoc* strain UAM 206, *Calothrix*, *Anabaena* and *Spirulina* were grown in culture media by mixing pure CO_2, air and N_2 under continuous stirring and controlled conditions of temperature and light. In our study biomass of *Nostoc* species was produced using a tray culture method without stirring and without mixing pure CO_2, air and N_2 in culture media at ambient environmental temperature.

Total carotenoid content

The carotenoid content (dw basis) of *N. entophytum* (14.5 ± 0.9 mg g^{-1}) was significantly higher than that of *N. ellipsosporum* (11.7 ± 1.5 mg g^{-1}) and *N. punctiforme* (9.7 ± 1.2 mg g^{-1}) (**Table 1**). The total carotenoid content in the biomass of *N. entophytum* was 2.7 times higher than that of *Spirulina platensis* (Marquez *et al.* 1995). However, in general total carotenoid content of cyanobacterial biomass may range up to 2% on a dw basis (Becker 1994). In the present study, HPLC analysis showed that β-carotene was the major component along with different carotenoids present in the biomass of *Nostoc* species (**Table 1**).

pH-dependent adsorption of metal ions

The adsorption of metal ions by biomass of algae is governed by pH of the metal ion solution. For the optimum adsorption of Cr(VI) on sodium alginate immobilized biomass of *Lyngbya putealis* (Kirn *et al.* 2007) the required pH of the aqueous solution was 1–3. According to Zhou *et al.* (1998) pH 5 was optimum for maximum adsorption of Cu(II) using *Laminaria japonica* biomass and pH 6.7 using *Spirulina* sp. biomass. The maximum adsorption of Cd(II) was observed on biomass of *Fucus vesiculosus* (Sandau *et al.* 1996) and *Lyngbya* sp. (Klimmek *et al.* 2001) in a pH range of 3 to 7. Analogous to these results, a similar pH range is required for adsorption of Cu(II), Cd(II), and Cr(VI) by biomass of *Nostoc* species included in study (**Fig. 1**). The maximum adsorption of Cu(II) and Cd(II) occurred by biomass in the range of pH 4–6 while that of Cr(VI) occurred in the range of pH 1–2.

These results indicate that optimum adsorption Cr(VI) by *Nostoc* biomass required low pH compared to Cu(II) and Cd(II). This variation in pH might be due to involvement of different functional groups in adsorption of Cu(II) or Cd(II), and Cr(VI). Different metal binding functional groups are present in cyanobacterial cell biomass, such as –COOH, $–PO_4^{3–}$, $–NH_2$, –OH, sulphahydryl, etc. (Crist *et al.* 1981; Romero *et al.* 2001). In the present study the alkali treatment given to the biomass for carotenoid extraction might have resulted in the dissociation of groups like –COOH (Gardea-Torresdey *et al.* 1998). The optimum pH for the adsorption of Cu(II) and Cd(II) was 4–6 (relatively low H^+ ion concentration) may have prevented protonation of groups like $–NH_2$ and –SH. Both conditions result in a net negative charge on the surface of biomass (Mehta and Gaur 2005) and promote the adsorption of Cu(II) and Cd(II) ions which exist as positively charged hydrated ions in aqueous solution. The protonation of groups like amine, imidazole, sulphahydryl, etc. takes place at low pH (high H^+ ion concentration) giving rise to a net positive charge on cell biomass and helps to adsorb Cr(VI) which exists as negatively charged $Cr_2O_7^{2-}$ in solution.

Possible reactions taking place at cell surface with respect to pH of solution

$$–COOH + –NH_2 + OH^– \rightarrow –COO^– + –NH_2 + H_2O$$

(possible reaction of –COOH and $–NH_2$ group with alkali during carotenoid extraction)

$$–NH_2 + –COO^– + H^+ (pH = 1–2) \rightarrow –NH_3^+ + –COOH$$

(at low pH, protonation of $–NH_2$ group is possible which may be give rise to +ve charge on cell surface)

$$–NH_2 + –COO^– + H^+ (pH = 4–7) \rightarrow –COO^– + –NH_2$$

(at pH 4 - 7 protonation of $–COO^–$ group may not be taking place which can give rise to –ve charge to cell surface).

Kinetics of adsorption

This experiment was carried out to evaluate the rate of adsorption and time required for more than 90% adsorption of Cu(II), Cd(II) and Cr(VI) from aqueous solution by the biomass of *Nostoc* species. The kinetics of adsorption of Cu(II), Cd(II) and Cr(VI) by biomass of *Nostoc* species (**Fig. 2**) show that in the beginning, adsorption takes place at an exponential rate. Thus, at the end of the exponential phase (45 min), maximum adsorption of Cu(II) occured on *N. entophytum* biomass (3.6 ± 0.3 mg) and less than this on *N. punctiforme* biomass (3.5 ± 0.2 mg) and *N. ellipsosporum* biomass (3.1 ± 0.2). These results indicate that about 62 to 72% of Cu(II) was adsorbed by the biomass of *Nostoc* species within this time.

The quantity of Cd(II) adsorbed at the end of the exponential (60 min) by biomass of *N. ellipsosporum* was 2.5 ± 0.2 mg which is not significantly different than adsorption of Cd(II) by biomass of *N. punctiforme* (2.3 ± 0.1 mg) and *N. entophytum* (2.2 ± 0.1 mg). The same type of results were recorded for Cr(VI) where the quantity of Cr(VI) adsorbed on biomass of *Nostoc* sp. lies in the range of 2.1 ± 0.1 mg to 2.6 ± 0.2 mg, showing that 44 to 50% of Cd(II) and 42 to 52% of Cr (VI) was adsorbed by the biomass of *Nostoc* sp. within the exponential phase of adsorption. Upon reaching a well established adsorption-desorption equilibrium (at about 120 min), quantities of Cu(II) adsorbed were 4.4 ± 0.2, 4.3 ± 0.1 and 3.9 ± 0.3 mg respectively by the biomass of *N. punctiforme*, *N. ellipsosporum* and *N. entophytum* (about 78-88% of initial quantity). Within same time, quantities of Cd(II) adsorbed were 2.8 ± 0.2, 3.1 ± 0.2, and 3 ± 0.3 mg, respectively (56-62% of initial quantity). The quantities of Cr(VI) adsorbed were 3.2 ± 0.1, 2.8 ± 0.2 and 2.6 ± 0.2 mg (52-64% of initial quantity). These results

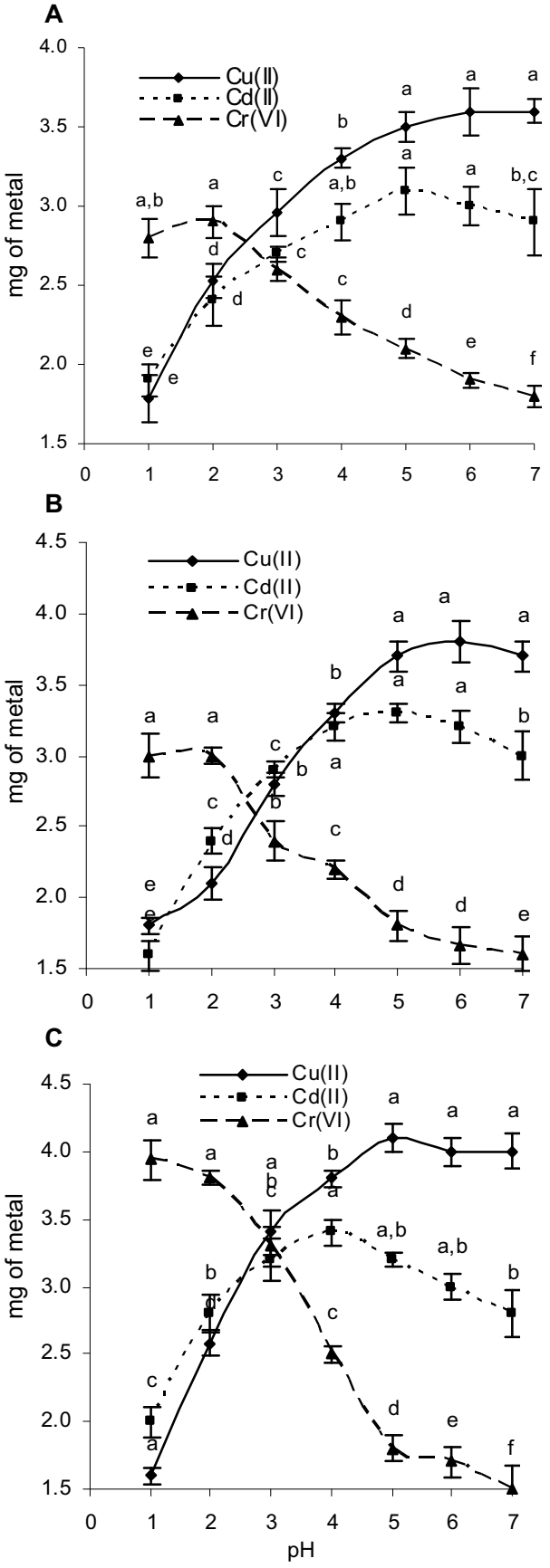

Fig. 1 pH-dependant sorption of Cu(II), Cd(II) and Cr(VI) by dry biomass. (**A**) *N punctiforme*, (**B**) *N. entophytum* and (**C**) *N. ellipsosporum.*

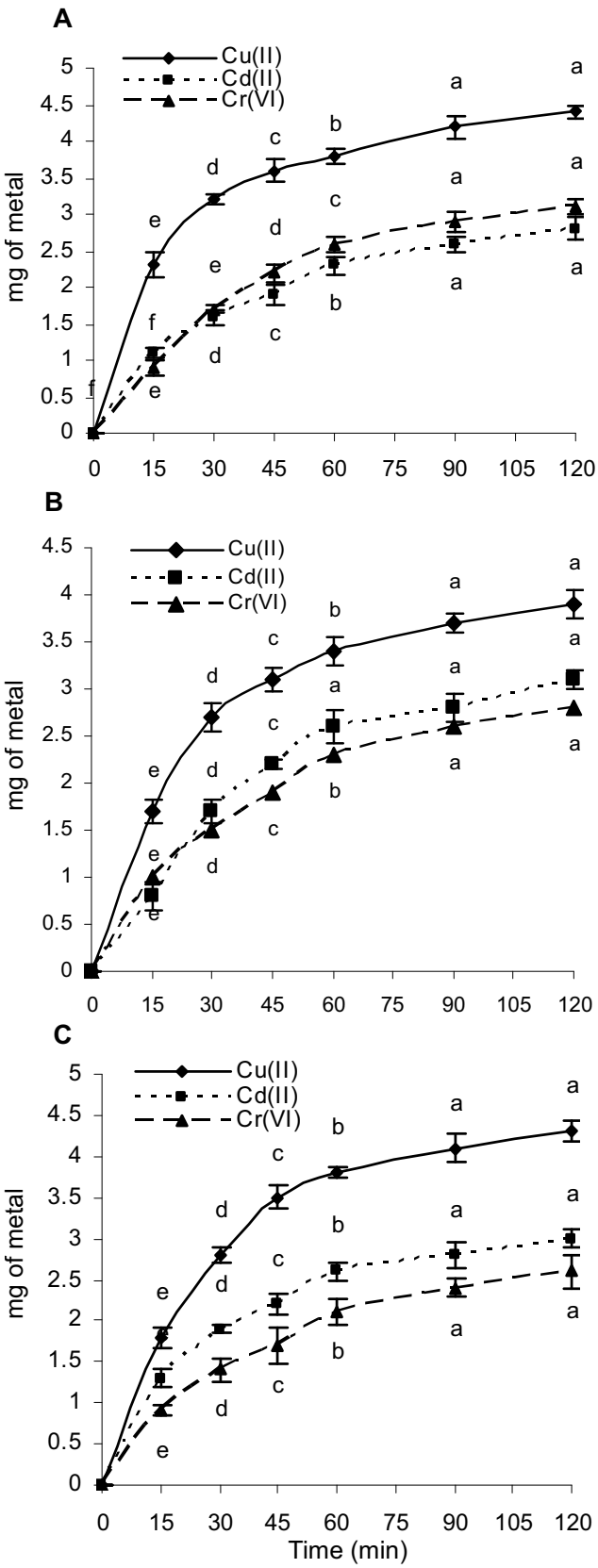

Fig. 2 Kinetics of sorption of Cu(II), Cd(II) and Cr(VI) on dry biomass (A) *N punctiforme* (B) *N ellipsosporum*, and (C) *N entophytum*. Error bars represent standard deviations (n = 3). Similar letters on line indicate the means which are not significantly different at p = 0.05 by DMRT.

indicate that the relative rate of adsorption of Cu(II) > Cd(II) > Cr(VI) by the biomass of these *Nostoc* species.

The kinetic experiment was continued to evaluate time required for more than 90% adsorption of Cu(II), Cd(II) and Cr(VI) by biomass of *Nostoc* species. These results show that more than 90% adsorption of Cu(II) was occurred on cell biomass of *N. punctiforme*, *N. ellipsosporum* and *N. entophytum* within 6 h while adsorption of a similar quantity of Cd(II) and Cr(VI) required about 36-48 and 48-60 h, respectively (**Fig. 3**). Similar to our results, Blanco *et al.*

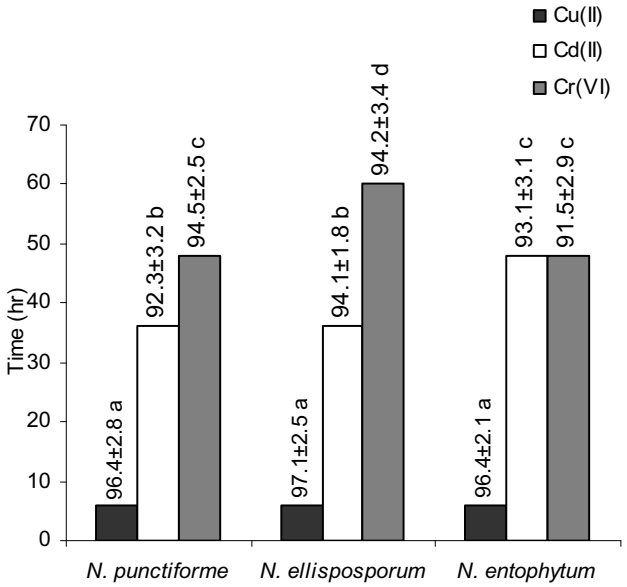

Fig. 3 Time required for more than 90% adsorption of metal ion Cu(II), Cd(II) and Cr(VI) by biomass of *N punctiforme*, *N entophytum* and *N ellipsosporum*. Values along with rows shows exact quantity of metal adsorbed within time indicated by the bar. Similar letters following the % of metal ion adsorbed shows the mean time are not significantly different at p = 0.05 by DMRT.

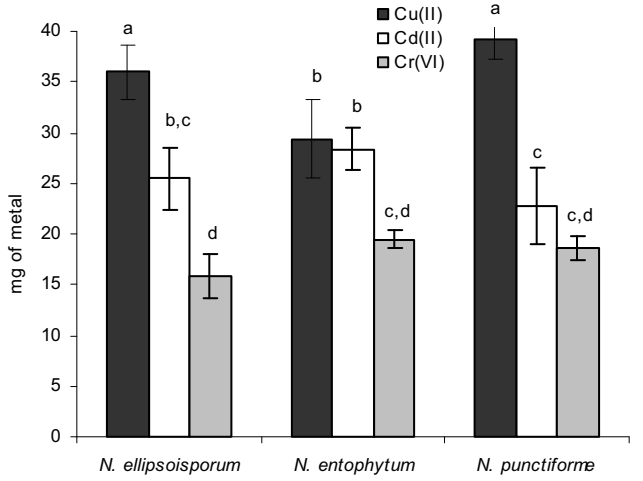

Fig. 4 Per gram adsorption capacity of biomass of *N punctiforme*, *N entophytum* and *N ellipsosporum* towards Cu(II), Cd(II) and Cr(VI). Error bars represent standard deviations (n = 3). Similar letters on line indicate the means which are not significantly different at p = 0.05 by DMRT.

(1999) reported 38–65% adsorption of Cu(II) within 1 h which then continued at a slower rate for several hours by the immobilized biomass *Phormidum laminosum*. Kiran *et al.* (2007) reported 60–75% adsorption of Cr(VI) by immobilized biomass of *Lyngbya* sp. within 120 min. However, 90% adsorption of Cd(II) was possible within 25 min by the dead biomass of *Fucus* sp. (Herrero *et al*. 2006). This difference in adsorption kinetics might be due to variation in composition of cell biomass of algal species.

Column experiments

The results of the column experiment (**Table 2**) show that when 100 ml aqueous solution containing 10 mg Cu(II) was passed through the column packed with biomass of *N. ellipsosporum* 9.7 ± 0.2 mg was adsorbed by biomass which is not significantly higher than *N. punctiforme* (9.4 ± 0.1 mg) but significantly higher than *N. entophytum* (9.1 ± 0.1 mg) showing that % adsorption of Cu(II) from aqueous solution lies in the range of 91 to 97%. The maximum adsorption of Cd(II) was occurred in a column packed with biomass of *N. punctiforme* (9.1 ± 0.2 mg) followed by *N. entophytum* (8.5 ± 0.5 mg) and *N. ellipsosporum* (7.7 ± 0.7 mg). Thus % adsorption of Cd(II) remained in the range of 77 to 91%. Quantities of Cr(VI) adsorbed were not found significantly different by biomass of *Nostoc* species and lies in the range of 8.5 ± 0.6 mg to 8.8 ± 0.5 mg showing 85 to 88% adsorption of Cr(VI) from aqueous solution. When column was washed with 0.2 M HCl, 80 to 90% of the adsorbed metal ions were recovered in to solution (**Table 2**). Though % desorption of metal ions lies in the range of 80 to 90% of adsorbed quantity, % desorption of Cu(II) and Cd(II) was significantly low than adsorbed quantities. These results indicate that the biomass of these *Nostoc* species behave like ion exchange resins and can be used as adsorbent of heavy metal ions from aqueous solution. Analogous results were reported by Gardea *et al.* (1998) on biomass of *synechoccus* sp. for the heavy metals Cu(II), Pb(II) and Ni(II) with 98.5% recovery.

The per gram adsorption capacity of biomass of *N. punctiforme* (39.2 ± 1.9 mg) and *N. entophytum* (36.0 ± 2.7 mg) towards Cu(II) was found significantly higher than biomass of *N. ellipsosporum* (29.4 ± 3.9 mg) (**Fig. 4**). These results on *Nostoc* biomass resembles with other algal biomass of *Chlorella minimata* (Lau *et al.* 1999) and *Spirulina platensis* (Zhou *et al.* 1998), however significantly higher per gram adsorption capacities were reported for the biomass of alga *Chlorella vulgaris* (Mehta and Gaur 2001c) and *Laminoria japonica* (Lee *et al*. 2004). Quantities of Cd(II) adsorbed by the biomass of *N. entophytum* was 28 ± 2.0 mg, *N. punctiforme* 25.5 ± 3.1 mg and *N. ellipsosporum* 22.8 ± 3.8 mg showing comparable adsorption capacities towards Cd(II). Compare to the *Nostoc* sp. biomass, a higher per gram adsorption capacity was reported for Cd(II) by *Spirulina platensis* (Zhou *et al.* 1998) and *Lyngbya taylorii* (Klimmek *et al.* 2001). A significant difference was not observed in the per gram adsorption capacity of Cr(VI) by biomass of the three species of *Nostoc* which remained in the range of 15.9 ± 2.2 to 19.5 ± 0.9 mg. However, a higher adsorption capacity was reported for Cr(VI) by the sodium alginate immobilized biomass of *Lyngbya putealis* (Kiran *et al.* 2007).

CONCLUSIONS

The biomass of *Nostoc* species can be utilized for the production of commercially and nutritionally important pigments, phycobiliproteins and carotenoids. The biomass left after extraction of pigments can be utilized effectively for removal of heavy metals Cu(II), Cd(II) and Cr(VI). However, the results indicate that the biomass of these species was superior for adsorption of Cu(II) than of Cd(II) or Cr(VI).

Table 2 Amount of metal ions Cu(II), Cd(II) and Cr(VI) adsorbed and desorbed in column experiments.

Metal ion	*Nostoc punctiforme*		*Nostoc entophytum*		*Nostoc ellipsosporum*	
	mg Adsorbed	mg Desorbed	mg Adsorbed	mg Desorbed	mg Adsorbed	mg Desorbed
Cu(II)	9.4 ± 0.1 a	8.5 ± 0.3 bc	9.1 ± 0.1 b	7.6 ± 0.3 de	9.7 ± 0.2 a	8.6 ± 0.9 bce
Cd(II)	9.1 ± 0.2 a	7.8 ± 0.1 de	8.5 ± 0.5 ab	7.3 ± 0.3 d	7.7 ± 0.7 bd	7.1 ± 0.6 d
Cr(VI)	8.8 ± 0.5 c	7.3 ± 0.3 d	8.7 ± 0.6 cb	7.0 ± 0.2 d	8.5 ± 0.6 bc	7.4 ± 0.6 de

Values are mean ± SD of three independent experiment
Means within a column and row followed by the same letters are not significantly different at p = 0.05 by DMRT.

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