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Chromium Tolerance and Bioremoval by Cyanobacteria Isolated from Textile Mill Oxidation Pond in Pure and Consortia Form

[1]*ANUBHA KAUSHIK; [1]KAMRA ANJANA; [1]KAUSHIK C P; [1]BALA KIRAN
Department of Environmental Science and Engineering
Guru Jambheshwar University of Science & Technology, Hisar -125001, INDIA

ABSTRACT: Two cyanobacterial species *Nostoc calcicola* HH-12 and *Chroococcus minutus* HH-11 isolated from a textile mill oxidation pond were examined individually and as consortium for their chromium(VI) tolerance and bioremoval from aqueous solutions. Both species were tolerant to the metal and showed significant increase ($p<0.01$) in concentration of chlorophyll and carotenoids at higher concentration of chromium, and the tolerance further in the consortium. Concentration of phycobilins also increased in the pure culture of *Chroococcus* and consortium was also higher in response to increasing chromium concentration in the medium. Chromium bioremoval potential was high in both species and the consortium was even more efficient in metal removal. Probable antioxidative role of carotenoids in metal tolerance have also been discussed. @JASEM

Metal contamination of fresh water ecosystems is of major environmental concern due to non-biodegradable nature of the metals which leads to their bioaccumulation in the food chain. Chromium is one such toxic non-essential metal discharged from leather tanning, textile, pigment and electroplating industries. It exists in many oxidation states of which Cr(III) and Cr(VI) are more common and stable, the latter being more toxic due to its oxidizing nature that can modify DNA transcription process, causing chromosomal aberrations in living organisms (Cieslak-Golonka, 1996).

Cyanobacteria have several advantages over other microorganisms due to their simple growth requirements, N_2 fixing ability in many, generally non-toxic nature, large size and greater biomass as compared to other microbes. Though there are lot of reports on heavy metal effects and biosorption by cyanobacteria, very little information exists on cyanobacterial interactions with chromium (Cervantes *et al*., 2001). Some of the recent reports on cyanobacteria and Cr biosorption include that on *Spirulina* (Chojnacka *et al*., 2005) and by the authors on *Lyngbya* (Kiran *et al*., 2008). An earlier study by the authors (Anjana *et al*., 2007) shows that the dry biomass of cyanobacteria (*Nostoc calcicola* & *Chroococcus minutus*) isolated from oxidation ponds showed very good Cr biosorption potential in immobilized form.

It is therefore worthwhile to explore whether these species could also be exploited for bioremoval of Cr(VI) from aqueous solution in live form. The present study was therefore, undertaken to study tolerance of the above two cyanobacterial isolates to Cr(VI) and to examine bioremoval of the metal. Consortium of the two strains was also studied to see whether live biomass of designed consortia could be used more effectively for bioremoval of Cr from contaminated wastewaters.

MATERIALS AND METHODS

The cyanobacterial strains *Nostoc calcicola* HH-12 and *Chroococcus mintus* HH-11were isolated from oxidation pond of a textile mill in Haryana, India. Pure cultures of these strains were obtained by streaking on basal agar medium (BG-11) at pH 8.5 (buffered with 0.1M tris amminomethane) using standard isolation and culturing techniques (Kaushik, 1987) under fluorescent light (56.85 $\mu molm^2sec^{-1}$) with 12/12 h light and dark photoperiod at 28±3°C temperature. *Nostoc calcicola*, was grown in nitrogen free medium whereas for *Chroococcus minutus*, nitrogen supplement was given in the form of $NaNO_3$ in the medium for growth. The consortium consisted of mixture of the two species (1:1) grown in broth culture under above conditions.

Stock solution (1000 mg/L) of aqueous Cr (VI) was prepared using potassium dichromate (AR grade Merck) which was further diluted with distilled water to 5, 10, 15 and 20 mg/L, the range recorded generally in textile effluent of the region. Cyanobacterial cultures (single and consortium) were grown in 250 ml Erlenmeyer flasks each containing 100ml of BG-11 culture medium (buffered at pH 8.5) with 0.1ml inoculum of 15d old culture (300 mg dry wt. approx.) spiked with different concentrations of Cr(VI).

Two sets of each treatment were taken, one each for estimation of pigments and for exoploysaccharides. Photosynthetic pigments chlorophyll, carotenoids, phycobilins and biomass (dry weight) of the cyanobacteria were determined at 7d interval using static cultures of the pure and consortial forms. Dry weight was recorded by collecting algal pellets on a pre-weighed filter paper followed by oven drying at 80°C to constant weight. Chlorophyll was estimated spectrophotometrically following hot extraction with methanol (Tandeau de marsac and Houmard, 1988), carotenoids following (Jensen, 1978) and

*Email: *aks_10@yahoo.com, Tel: +91 1662 263153, Fax: +91 1662 277942*

phycobiliproteins following (Bennett and Bogorad, 1971).

Cr removal by the cyanobacteria was determined as the difference between the initial and the residual Cr concentration in the medium after a particular time period (7, 14, 21d). Concentration of Cr(VI) in the medium was estimated spectrophotometrically at 540nm using 1, 5-diphenyl carbazide following (Clescri *et al*., 1996). All the experiments were performed in triplicate and variability was statistically analyzed using t-test and three way analysis of variance to test the significance of differences due to various factors viz. metal concentration, cyanobacterial species and growth stage (age in days) following (Coolidge, 2000).

RESULTS AND DISCUSSION

The cyanobacterial isolates tolerated Cr(VI) in the range 5-20 mg/L (Table 1.). Interestingly, in both the strains chlorophyll concentration tended to increase with increasing concentration of chromium, showing a significant increase ($P<0.01$) at M_{20}. The consortium showed significantly higher chlorophyll concentration at all concentrations of chromium as compared to control. Variations were, however, not statistically significant ($p>0.05$) between cyanobacterial strains indicating their similar response to different Cr concentrations. Carotenoid concentration increased significantly in *Nostoc* at M_{20} and at all concentrations in *Chroococcus* and the consortium. Response of phycobilins to Cr(VI) differed in the two species. In *Chroococcus* it increased significantly (120-156 %) in response to Cr whereas in *Nostoc* it declined significantly (34 %) beyond M_5 and the consortium showed increase in phycobilins. The cyanobacterial biomass was not affected significantly upto M_{15}, but declined significantly ($p<0.05$) at M_{20} in *Nostoc* and at M_{15} in case of *Chroococcus* and the consortium.

Table 1. Effect of different concentrations of chromium (M_0 to M_{20}; indicating concentration of the metal in mg/L) on photosyntheticpigments of cyanobacteria (C_I - *Nostoc calcicola*, C_{II} - *Chroococcus minutus*, C_{I+II} - Consortium) at the peak growth stage (14 days)

TT (mg/L)	Chlorophyll (µg/mg)			Carotenoids (µg/mg)			Phycobilins (µg/mg)		
	C_I	C_{II}	C_{I+II}	C_I	C_{II}	C_{I+II}	C_I	C_{II}	C_{I+II}
M_0	$1.017^{a,x}$ ± 0.189	$1.169^{a,x}$ ± 0.187	$0.977^{a,x}$ ± 0.141	$2.482^{a,x}$ ± 0120	$1.037^{a,y}$ ± 0.0875	$1.608^{a,x,y}$ ± 0.252	$9.931^{a,x}$ ± 0.120	$1587^{a,y}$ ± 0.55	$4.968^{a,z}$ ± 0.079
M_5	$0.875^{a,x}$ ± 0.07	$1.076^{a,x}$ ± 0.163	$1.383^{b,x}$ ± 0.9	$3.15^{a,x}$ ± 0.337	$1.145^{a,y}$ ± 0.163	$2.467^{a,z,x}$ ± 0.05	$10.581^{a,x}$ ± 1.40	$3.942^{b,y}$ ± 0.354	$7.217^{b,z,x}$ ± 0.583
M_{10}	$1.106^{a,x}$ ± 0.187	$1.305^{a,x}$ ± 0.268	$1.66^{b,x}$ ± 0.15	$3.058^{a,x}$ ± 0.225	$2.480^{b,x}$ ± 0.205	$3.28^{b,x}$ ± 0.28	$8.030^{b,x}$ ± 1.500	$5.134^{c,x}$ ± 0.53	$7.56^{b,x}$ ± 1.56
M_{15}	$0.968^{a,x}$ ± 0.158	$1.498^{a,x}$ ± 0.106	$1.759^{b,x}$ ± 0.145	$2.980^{a,x}$ ± 0.441	$2.874^{b,x}$ ± .211	$3.478^{b,x}$ ± 0.269	$6.790^{b,x}$ ± 2.186	$5.097^{b,x}$ ± 0.459	$6.977^{a,x}$ ± 0.807
M_{20}	$1.125^{b,x}$ ± 0.406	$1.970^{b,x}$ ± 0.214	$2.748^{b,c,x}$ ± 0.300	$4.518^{b,x}$ ± 0.299	$3.811^{b,c,x}$ ± 0.278	$4.942^{b,c,x}$ ± 0.138	$8.137^{b,x}$ ± 0.471	$5.995^{b,y}$ ± 0.236	$7.690^{a,x,y}$ ± 0.900

values in each column followed by different superscript (a, b, c) and in each row followed by different superscript (x, y, z) for each parameter indicate statistically significant ($p<0.05$) differences between those values (based on t-test) at different metal concentrations (columns) and different cyanobacterial strain (rows), respectively.

Percent removal of Cr by the cyanobacteria was found to be influenced both by age and initial metal concentration as depicted Fig 1. Metal removal was maximum by the 14d old cultures, usually followed by a slight decline in 21d old cultures, presumably due to desorption/release of metal ions as a consequence of cellular lyses in ageing cultures. Percent metal removal was many fold more at M_{15} and M_{20} (70-80%) as compared to that at M_5 (30-40%), indicating more removal at higher metal concentration.

Bioremoval of Cr by the cyanobacteria (Table 3.) varied significantly ($p<0.01$) between different cultures and also due to initial metal ion concentration as shown by Analysis of variance (Table 2). Since the main factor effects due to metal concentration and strain were statistically significant and interaction non-significant, therefore no multiple comparison tests were performed.

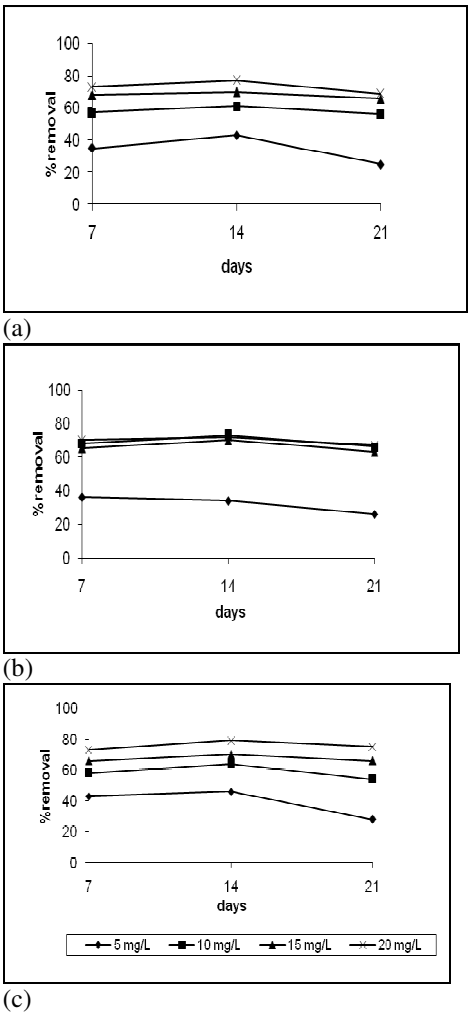
Substantially high carotenoid concentrations in these cyanobacteria developed in the presence of Cr(VI) seem to prevent oxidative damage to the cells due to the antioxidant properties (Salguero, 2003). Phycobilisomes which are the major accessory light harvesting complexes in cyanobacteria, have been recently shown to be highly mobile with great diffusibilty on the thylakoid membrane surface and under oxidative stress are involved in energy

quenching and prevents oxidative damage (Joshu and Mullineaux, 2004).

Table 2. Effect of different concentrations of chromium (M_0 to M_{20}; indicating concentration of the metal in mg/L) on biomass (C_I - *Nostoc calcicola*, C_{II} - *Chroococcus minutus*, C_{I+II} - Consortium) at the peak growth stage (14 days)

Treatment (mg/L)	Biomass (mg)**		
	C_I	C_{II}	C_{I+II}
M_0	$5.80^{a,x} \pm 1.02$	$8.0^{a,x} \pm 1.05$	$6.34^{a,x} \pm 0.58$
M_5	$5.33^{a,x} \pm 1.15$	$7.33^{a,x} \pm 0.58$	$6.0^{a,x} \pm 1.0$
M_{10}	$5.33^{a,x} \pm 0.57$	$6.33^{a,x} \pm 1.53$	$5.0^{a,c,x} \pm 1.0$
M_{15}	$5.67^{a,x} \pm 0.57$	$5.67^{b,x} \pm 0.58$	$4.83^{b,c,x} \pm 0.58$
M_{20}	$4.67^{b,x} \pm 0.58$	$4.67^{c,x} \pm 0.58$	$4.33^{b,c,d,x} \pm 0.58$

**Values in each column followed by different superscript (a, b, c) and in each row followed by different superscript (x, y, z) for each parameter indicate statistically significant ($p<0.05$) differences between those values (based on t-test) at different metal concentrations (columns) and different cyanobacterial strain (rows), respectively.



(a)

(b)

(c)

Fig 1. Chromium (VI) removal by (a) *Nostoc calcicola* (b) *Chroococcus minutus* (c) Consortium

It is quite interesting that the present strains showed increased chlorophyll concentration in Cr spiked medium. Development of some derivatives of chlorophyll in response to some other metals due to the presence of certain oxidative species has been reported earlier (Kato and Shimizu, 1985) which needs to be further investigated for the present species.

Reduced dry weight beyond M_{15} does not necessarily imply that the metal leads to deficient chemical energy utilization for CO_2 fixation. Under stress conditions, energy is also required for control and maintenance of cellular homeostasis (Perales-vela *et al*., 2007). Reduced biomass of the cyanobacteria at higher metal concentrations seems to be correlated with high EPS production by the cyanobacterial cells resulting in loss of dry weight under these conditions.

Using live cyanobacterial biomass for the purpose may have been added advantage that BOD load, if any, in the wastewater would be lowered due to oxygen evolutions by the microbes during photosynthesis. Designed consortia developed after exploring combinations of taxonomically distant species of cyanobacteria showing less competition and more synergism would be important in improving metal removal from wastewaters.

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