



**BIODETOXIFICATION OF HEXAVALENT CHROMIUM USING
CYANOBACTERIA**

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ABSTRACT

Chromium is one of the most toxic heavy metals abundant in the effluent of chromite mines of Odisha. An investigation was carried out to select local algal strains for their possible use in bioremediation application and to assess the hexavalent chromium detoxification potential using fast growing indigenous cyanobacteria *Anabaena cylindrica*, *Nostoc commune*, *Chroococcus sp* and integration of hexavalent chromium treatment with algal biomass production. Samples of chromium effluent were collected in wide mouthed sterilized glass bottles of 1 liter capacity from Kalaringatta Chromite Mines (M/S FACOR), Ostapala, district Jajpur. The physico-chemical parameters (pH, total suspended solid (mg/l) BOD₅(mg/l), hexavalent chromium(mg/l) of the effluent were analyzed. Each culture vessel containing 100ml of chromium effluent 5ml of the algal organism was inoculated and allowed to grow for 20 days. The evaluation of Cr (VI) reduction was carried out on 4,8,12 and 16, 20 days of observation of the treated samples. The evaluation tests evidenced the depletion and detoxification of the Cr(VI) content in the three experimental algal cultures. The degree of efficiency was observed as *Anabaena cylindrica*> *Nostoc commune*>Chroococcus sp. With higher growth rate, higher protein and lipid content *Anabaena cylindrica*, could stand out as an excellent candidate for biodegradation of hexavalent chromium.

**Keywords: Biodegradation, Toxicity, Hexavalent Chromium, Cyanobacteria,
Biosorption**

INTRODUCTION

A large volume of contaminated water from various industries and mining operations are discharged to the coastal rivers and associated wetlands with increasing risk of health hazards. Metals discharged into water bodies are not biodegraded but undergoes chemical or microbial transformation, creating large impact on the environment and public health (Volesky, 1995). Chromium is a transition metal located in group VI B of the periodic table. In nature it exists in several oxidation states ranging from -2 to +6. The most stable and common form are the trivalent (CrIII) and the hexavalent (CrVI) which display different chemical properties. The hexavalent form of the metal is considered to be more toxic than the relatively less motile Cr(III), (Panda & Patra, 1997). Presently contamination of the environment by Cr(VI) has become a major area of concern due to its large scale use in different industries and its recovery by open cast mining activities. The discharge of several industries containing high amount of chromium leads to high toxicity in plants and animals which come in contact with chromium contaminated waste water or sludge (Guruprasad, Nandakumar, 1983). Natural ecosystems adjacent to chromite mining and industrial activities in Odisha are under severe stress and in

absence of urgent correction measures it may lead to permanent and irreparable damage (Pujari & Patra, 1999). At many such dumping and overburden sites leaching and seepage of Cr(VI) from soil and ground water possess a considerable health hazards. Brahmani and Baitarani are two rivers of Odisha state located at Eastern Ghat region which passes through the adjoining regions of chrome mining and industrial operations before entering into coastal plains. A large volume of contaminated water from various industries and mining operations available to these coastal rivers and associated wetlands with increasing risk of health hazards. Elevated concentration of hexavalent chromium pollution and contamination has contributed a major health hazard affecting more than 2 lakh mine workers and inhabitants residing in the Sukinda chromite mine of Odisha (Dash and Singh, 2011) Hence in view of this critical situation of serious chromium pollution considerable efforts have been made for the use of biological or phyto remediation technology for the cleaning up of chromium contaminated soils and water. Algae particularly cyanobacteria are abundant source of protein, vitamin, lipids and trace elements. Thus cyanobacteria can play an important role in pollution

abatement program and particularly for implementation of biodegradation of effluent.

OBJECTIVES

The objectives of the study were

- To investigate the integration of hexavalent chromium treatment with algal biomass production using the fast growing indigenous cyanobacteria.
- Screening of fast growing cyanobacteria and their growth characterization.
- Scaling of cyanobacterial culture for mass production.
- Morphological, physiological and biochemical studies of three selected cyanobacterial strains.
- Biosorption and potential studies of selected strains in hexavalent chromium.
- Suggestion for effective implementation of the findings in large scale in the industrial sites for safe disposal.

MATERIAL AND METHODS

Samples of chromium effluent were collected in wide mouthed sterilized glass bottles of 1 liter capacity. The physico-chemical parameters (pH, total suspended solid (mg/l), BOD₅(mg/l), hexavalent chromium(mg/l) of the effluent collected

from the site were analysed. The cyanobacterial samples collected from in and around the industrial site were identified using algal monograph and three cyanobacterial strains out of the fifteen strains maintained in pure condition at Centre of Environmental Studies Laboratory were utilized in this project. Basing on their growth rate three cyanobacteria namely *Anabaena cylindrica*, *Nostoc commune*, and *Chroococcus sp* have been employed in the detoxification experiment.

Scaling of culture was undertaken from 250 ml capacity to 5litre capacity in different culture vessels which lasted for one month. Once the pure cultures were obtained the cultures were transferred to liquid and solid medium to develop small scale culture. When the cultures starts growing exponentially growing algal strains were transferred to yield a large volume of healthy cells into a flask and then in large sized carboy cultures. Each algal strain was used for detoxification of hexavalent chromium in different dilutions of 10%, 50%,100% of the effluent. The experiment was conducted in triplication. In each culture vessel containing 100ml of chromium effluent 5ml of the algal organism was inoculated and allowed to grow for 20days. The algal experiment was conducted at room temperature $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$

and light intensity 2240lux. They could tolerate hundred percent of the chromium effluent.

For determination of hexavalent chromium in the filtrate, the filtrate (passed through 0.22µm Milipore filter) was taken out and residual trivalent chromium estimation was done spectrophotometrically by 1,5 Diphenyl Carbazide Method (American Public Health Association, 1992). A control set was simultaneously maintained to know the amount of hexavalent chromium removal. The evaluation of Cr (VI) reduction was carried out on 4, 8, 12 and 16, 20 days of observation of the treated samples (Table 6). After one month of treatment the hexavalent chromium were converted to nontoxic forms.

RESULT AND DISCUSSION

Three strains *Anabaena cylindrica*, *Nostoc commune*, and *Chroococcus sp* with higher growth rate, higher carbohydrate, protein and lipid content were selected for the study.

Table 7 Shows the physico chemical analysis of the sample collected from the kalarangiatta chromite mine inlet. It shows pH of the sample as 8.4, total suspended solid 46.0mg/l, Biological oxygen demand 3.6mg/l, hexavalent chromium 0.152. Table 7 Shows the physico chemical parameters of the sample after treating with algal isolates of *Anabaena cylindrica*. It shows pH of the sample as 7.31, total suspended solid 5.2 mg/l, Biological oxygen demand 1.2mg/l, hexavalent chromium 0.038.

Table 1: Hexavalent chromium exposure and diseases

Route of Exposure	Mode of intake	Health hazards
Air	Breathing	Nasal irritation, nasal ulcer, respiratory tract cancer, lung cancer, tuberculosis, cough and cold, etc
Water	Drinking and eating	Stomach cancer, diarrhea, bronchospasm and pneumonia, etc.
Dermal	Skin penetration	Dermatitis, irritation, skin lesions

Table 2: Specific growth (µ/d) for different microalgae

Micro Algae	4 days	8days	12 days	16 days	20days
<i>Anabaena cylindrica</i>	0.320	0.350	0.462	0.475	0.485
<i>Nostoc commune</i>	0.360	0.395	0.412	0.430	0.451
<i>Chroococcus sp</i>	0.310	0.329	0.362	0.375	0.385

Table 3: Total carbohydrate (%) for different microalgae

Micro Algae	4 days	8days	12 days	16 days	20days
<i>Anabaena cylindrica</i>	17.10	18.71	21.07	27.01	29.01
<i>Nostoc commune</i>	18.01	20.08	21.12	23.18	24.08
<i>Chroococcus sp</i>	16.80	17.70	19.03	20.07	21.80

Table 4: Protein (%) of different micro algae

Micro Algae	4 days	8days	12 days	16 days	20days
<i>Anabaena cylindrica</i>	12.01	14.20	15.50	17.15	20.85
<i>Nostoc commune</i>	11.12	13.02	15.16	17.03	19.02
<i>Chroococcus sp</i>	10.11	12.03	13.16	16.56	17.80

Table 5: Lipid (%) of different microalgae

Micro Algae	4 days	8days	12 days	16 days	20days
<i>Anabaena cylindrica</i>	21.73	22.15	27.11	30.10	32.12
<i>Nostoc communeae</i>	18.22	19.01	20.15	23.15	28.00
<i>Chroococcus sp</i>	12.15	13.10	18.15	20.17	21.78

Table 6: Cr(VI) removal percentage in different incubation period for different micro algae.

Micro Algae	4 days	8days	12 days	16 days	20days
<i>Anabaena cylindrica</i>	02	28	48	62	81
<i>Nostoc communeae</i>	01	16	36	50	68
<i>Chroococcus sp</i>	01	14	22	48	56

Table 7: Physico-chemical parameters of chromium effluent

Parameter	At the inlet point	After treating with <i>Anabaena cylindrica</i>
pH	8.4	7.31
Total suspended solid(mg/l)	46.0	5.2
BOD(mg/l)	3.6	1.2
Cr (VI)	0.152	0.038

DATA ANALYSIS

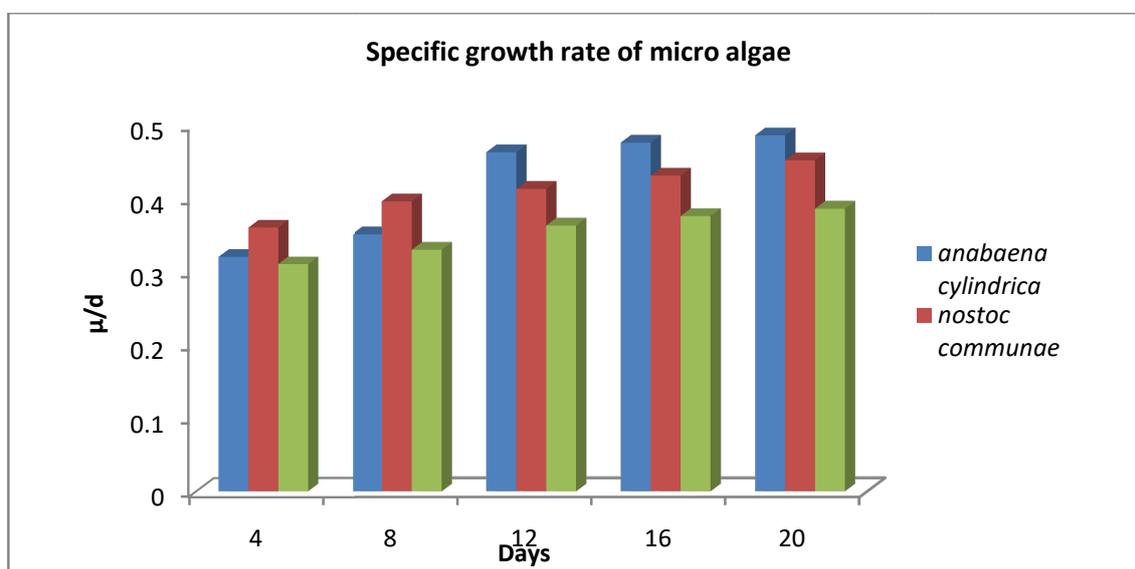


Fig.1: Specific Growth rate of micro algae

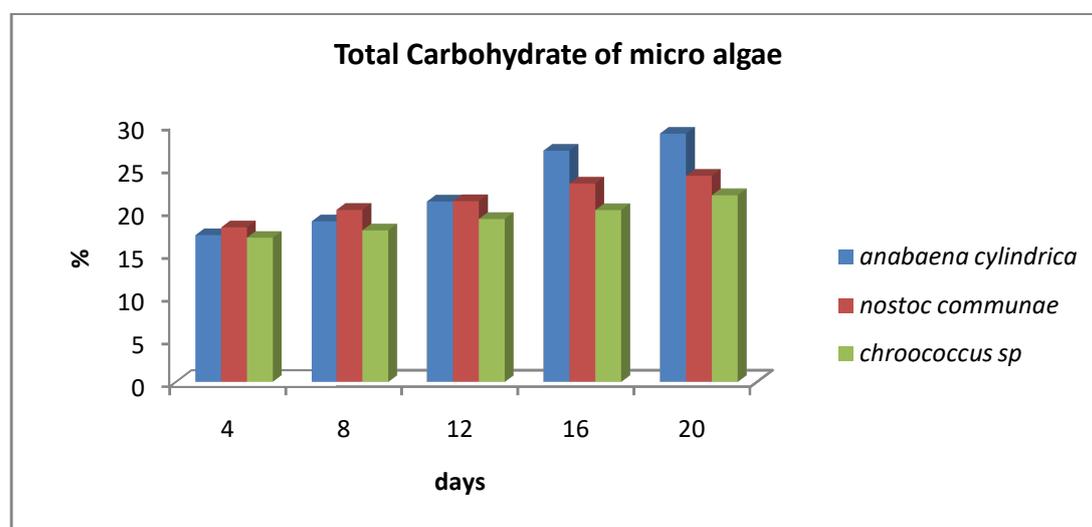


Fig.2: Total Carbohydrate of micro algae

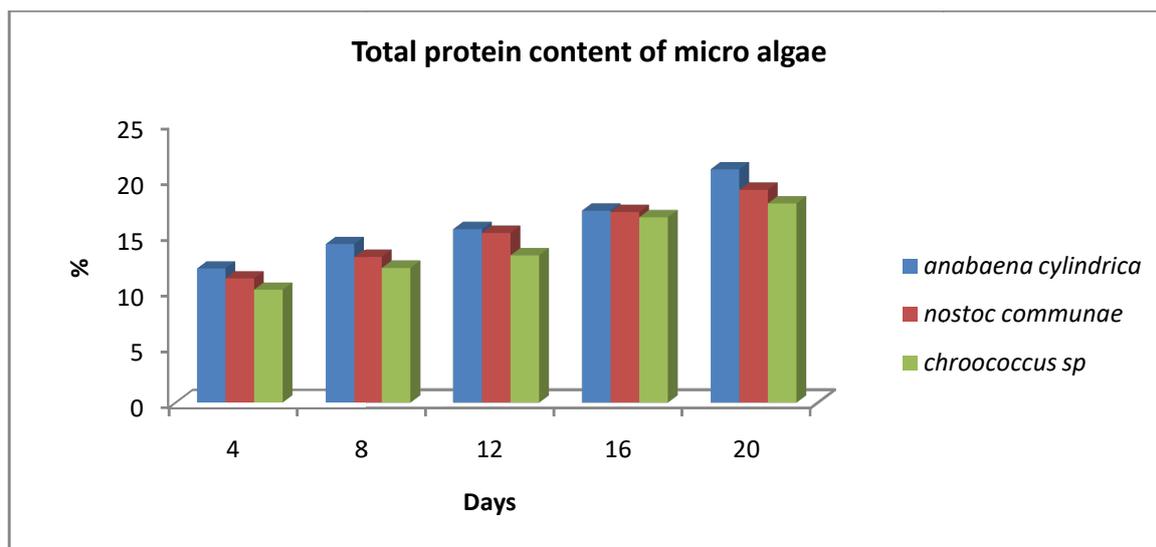


Fig.3: Total protein content of micro algae

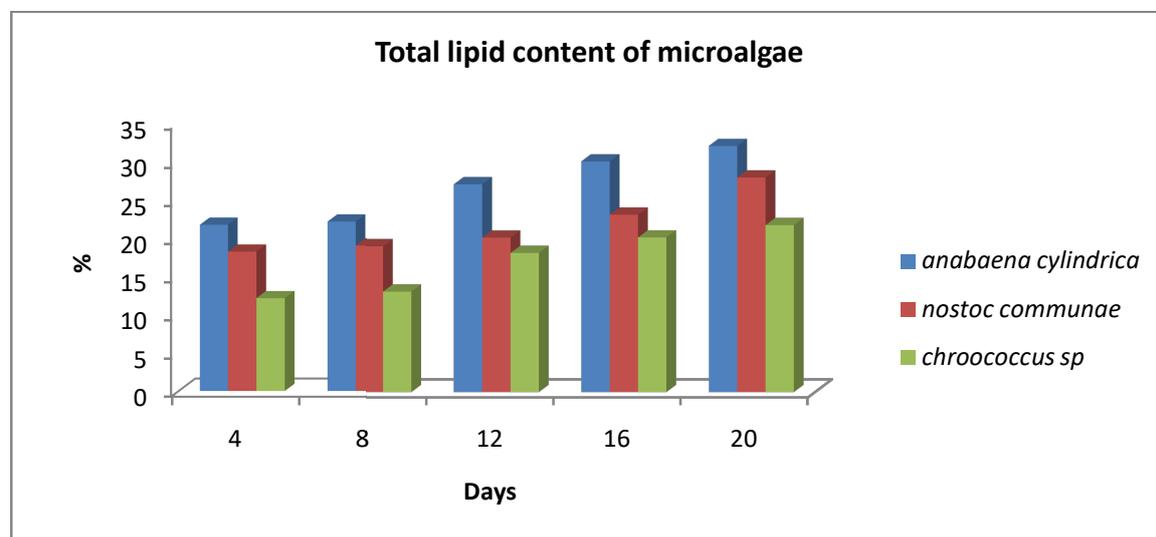


Fig.4: Total lipid content of micro algae

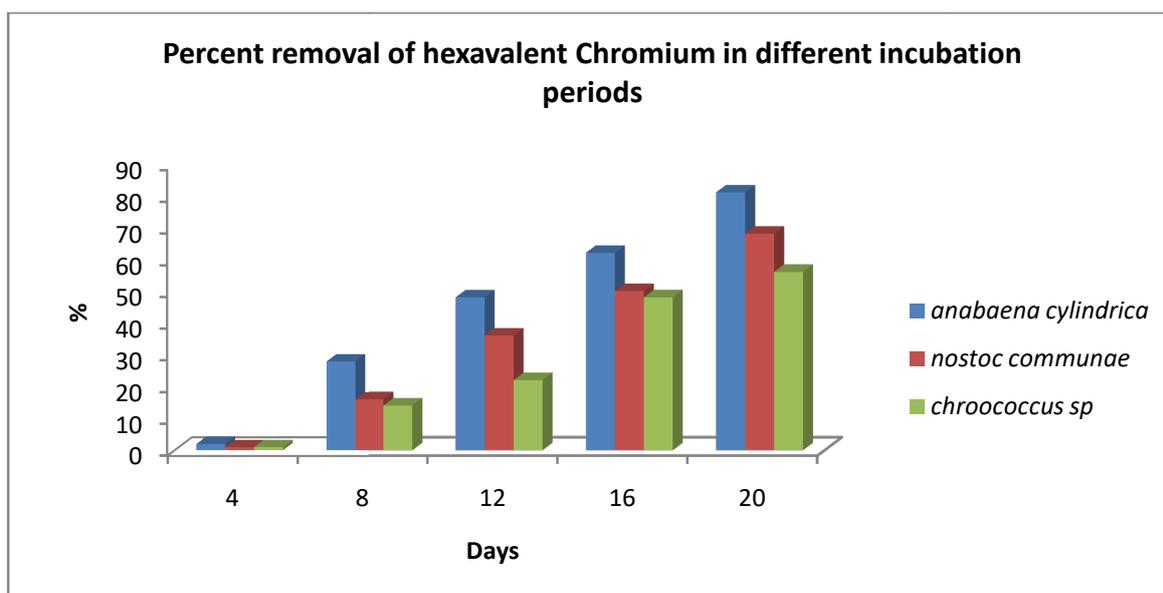


Fig. 5: Percent removal of hexavalent Chromium in different incubation periods

Findings

- a) The evaluation tests evidenced the depletion of the Cr(VI) content in cultures with the increase in the algal biomass. The degree of efficiency was observed as *Anabaena cylindrica* > *Nostoc commune* > *Chroococcus sp* (Fig5) .
- b) The blue green algae (cyanobacteria) Cr(VI) resistant strains, particularly *Anabaena cylindrica* showed high levels of growth (Fig.1), carbohydrate (29.01%) (Fig. 2), protein (17.80%) (Fig 3), lipid content (21.78% (Fig4)) after 20 days of inoculation.

Advantages

- a) Cyanobacteria have the ability to grow in stress condition and can degrade the hexavalent chromium to trivalent form.
- b) It is an environmental friendly technology with no leakage of CO₂ or contaminants.
- c) A low cost technology with safe disposal for a sustainable environment.

CONCLUSION

The present research highlights the capacity of wastewater grown cyanobacteria to grow at higher rates and with higher biomass productivity and store substantial amount

of protein and lipid. With higher growth rate, lipid and protein content cyanobacteria strain particularly *Anabaena cylindrica*, could stand out as an excellent candidate for detoxification of hexavalent chromium.

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