Development of Cyanobacterial Consortia in a Photobioreactor

Chaitali Urewar[1,*], Tapas Nandy[2], Kunal Roychoudhury[3]

[1]Project Assistant, [2]Chief Scientist, National Environmental Engineering Research Institute, Nagpur
[3]Professor & HOD, S. K. Porwal College, Kamptee

***Corresponding Author:**
Email: chaitali.urewar@gmail.com

ABSTRACT

The cyanobacterial consortia was grown and developed under laboratory conditions. In the present work we used equal proportion of three cyanobacterial strains i.e. Anabaena sp., Nostoc sp., and Oscillatoria sp. together to form consortia. A specially designed batch photo bioreactor was used for cultivation of consortia. Nutrient medium was modified according to the growth requirement of consortia. Several parameters like wet mount microscopy, dry weight and chlorophyll a content were used to monitor consortia growth.

Keywords: *Cyanobacterial consortia, batch photo bioreactor, media modification, MLSS and Chlorophyll-a*

Access this article online	
Quick Response Code:	**Website:** www.innovativepublication.com
	DOI: 10.5958/2394-5478.2015.00008.4

INTRODUCTION

Heavy metal pollution is one of the most important environmental problems today. Various industrial activities produces and discharge wastes containing different heavy metals into the environment such as electroplating, metallurgy, leatherworking, mining, energy and fuel production, fertilizer, pesticide and many more. Increasing contamination of aquatic resources with a host of pollutants including heavy metals is not only endangering the aquatic biota but creating a worldwide shortage of recreational and drinking waters. Conventional methods for removing metal ions from aqueous solutions include chemical precipitation, filtration, ion exchange, electrochemical treatment, and reverse osmosis which is expensive and ineffective when metal ions in the aqueous solution are in the range of 1 – 100 mg /L (Leusch et.al. 1995). Biological approaches are now being considered as an alternative for the removal of heavy metal contamination. Biosorption and or bioaccumulation have emerged as cost effective and efficient alternative methods. Biosorption is the passive process of adsorbing metal ions by metabolically inactive biomass, and is dependent on the affinity between the metallic species or its ionic forms and the binding sites on the molecular structure of the cellular wall (Pardo et.al.2003).The phenomena of adsorption has been described in a wide range of living biomass like fungi (Das and santra, 2008), bacteria (Chang et.al. 1997), bryophytes (Low and Lee, 1991), aquatic plants (Narain et.al. 2011) and algae (Kaewsarn, 2002). Algae and cyanobacteria are among the most promising biosorbents (Hussain 2009). Cyanobacteria are photosynthetic oxygen evolving prokaryotes that react to stress conditions. These cells can spontaneously respond to heavy metals through passive accumulation in cells and through surface binding to various functional groups (Gardea-Torresdey et.al., 1998). Functional groups within the cell wall provide the amino, carboxylic, sulfydryl, phosphate and thiol groups that can bind metal ions and sorption depends on the nature and composition of the cell wall, therefore among different biological substrates studied, algal biomass received much attention due to cost saving, low sensitivity to environmental and impurity factors, possible contaminant recovery from biomaterial and its elevated adsorption capacity, comparable to those of synthetic ion exchange resin, cheap availability, high surface area and high uptake capacity. Being autotrophic, cyanobacteria have particular nutrient requirements and shows drastic behaviour with changes in environment.

In this study, an attempt has been made to grow three cyanobacterial species together as consortia in a laboratory condition. This will help in better understanding of consortia behaviour and extracting good qualities of three cyanobacteria together. This study can be further extended for metal removal ability of consortia.

MATERIALS AND METHODS

Collection and characterization of cyanobacteria: The wild type species of *Anabaena*, *Nostoc* and *Oscillatoria* were obtained from Department of Botany, RTM Nagpur University, Nagpur. The cultures were transported to lab in a dark bottle to prevent exposure to light. The culture stocks were

immediately transferred and maintained in sterile BG-11 broth media (ATCC 616) at pH 7.1 at 25^0 centigrade under 1500 lux with a photoperiod of 10:15 h (light: dark).

Morphological characterization: Morphological study of each species of cyanobacteria was done by wet mount microscopy. Cultural studies were also carried out by pour plate method on 1% BG11 agar.

Wet mount microscopy: A loop full of culture was mixed with a drop of sterile saline solution. Culture was teased slowly and a cover slip was placed without letting air bubble in and microscopically viewed fewer than 40X objective (NIKON H600L).

Cultural studies: Sterile BG-11 agar plates were prepared by adding 1% agar in modified BG 11 medium. All the pure cultures were serially diluted up to 10^3 in a sterile isotonic saline. 1 ml homogenous culture was inoculated in media by pour plate method. All plates are incubated at temperature 25^0 centigrade under light irradiance of 1500 lux with a photoperiod of 10:14 h (light: dark) for 24-48 hrs.

Growth medium: All the pure cultures were grown in sterilized BG-11 media which was slightly modified for cyanobacterial consortia to enhance their growth requirements. This medium is used successfully for most cyanobacteria. The medium composition for standard BG11 and modified BG11 is given in Table No.1and 4.

All the three cyanobacterial cultures were grown and maintained in the standard BG11 medium (ATCC 616). Pure cultures were maintained at temperature 25^0 C-28^0 C under light irradiance of 1500 lux with a photoperiod of 10:14 h (light: dark). Culture flasks were shaken twice a day to release gases generated. After every 15 days the cultures were transferred into fresh sterile nutrient medium.

Table 1:Composition of BG 11 medium

Sr. No.	Ingredients	Quantity For Cyanobacteria pure Culture
1.	Sodium nitrate	-
2.	di-potassium hydrogen phosphate	0.04 g/L
3.	Magnesium sulphate	0.075 g/L
4.	Calcium chloride	0.036 g/L
5.	Citric acid	0.006 g/L
6.	Ferric ammonium citrate	0.006 g/L
7.	EDTA disodium salt	0.001 g/L
8.	Sodium carbonate	0.02 g/L
9.	Distilled water	1 Litre
10.	Trace metal solution	1 ml/L
Trace metal solution		
11.	Boric acid	2.86g
12.	Manganese chloride	1.81g
13.	Zinc sulphate	0.222g
14.	Sodium molybdate	0.39g
15.	Copper sulphate	0.079g
16.	Cobaltous nitrate	49.4mg
17.	Distilled water	1Litre

Photo bioreactor: Photo bioreactor was made up of transparent Perspex material. It is a cylindrical vessel. Total volume of reactor was 10 lit. Whereas working volume was 5 lit. More headspace was provided for gaseous exchange. Photo bioreactor was provided with a propeller with blades having speed of 30 rpm for gentle mixing of contents. Cool white circular tube light was provided for even distribution of light. Light intensity was measured by digital luxmeter (METRAVI 1330) and calculated as 1500 lux. Photo bioreactor was operated in batch mode.

Consortia development: 50 ml fully grown concentrated culture suspension, each of *Anabaena sp.*, *Nostoc sp.* and *Oscillatoria sp.* were taken and mixed together to form cyanobacterial consortia. 150 ml of this consortium of MLSS 2500 mg /L and *Chlorophyll a* of 4.8 mg/l was taken in a batch photobioreactor with working volume of 5 litre containing BG-11 medium.

RESULTS AND DISCUSSION

The results of morphological and cultural studies are shown in table 2, plate1, 2, photograph 1.

Table 2: Morphological and cultural characteristics of cyanobacterial species

Sr. No.	Species	Wet mount observation	Cultural characteristics
1.	*Anabaena**	Bluish green coloured, long filamentous cells with tapering ends. Heterocyst cells.	Flat ,filamentous, green coloured, colonies with high stickiness to agar
2.	*Nostoc**	Small green coloured, wavy shaped. Slimy cell surface	Flat ,filamentous, green coloured, colonies with high stickiness to agar
3.	*Oscillatoria*	Green coloured, flat, thin, long filaments, sluggishly motile. Cells with thick cell boundary.	Flat ,filamentous, green coloured, colonies with high stickiness to agar

Note:* observed under dark field microscopy

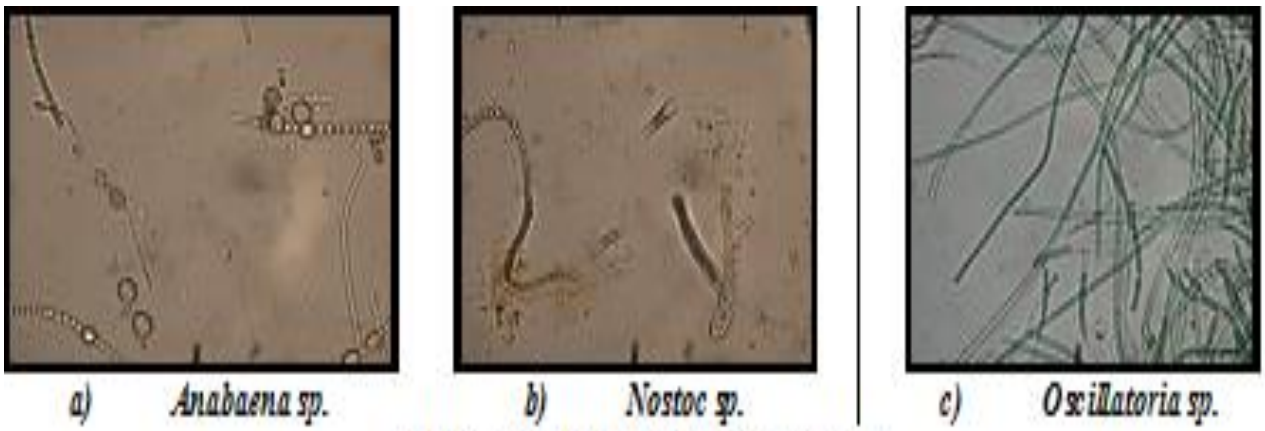

Plate 1: Wet Mount Microscopy results

The wet mount observations are similar to the observations of Ahmad A issa et.al. 2014. The growth on BG11 supplemented with 1% agar is similar as reported by Naoki Sato et.al.2014 and Jessica K Cole et.al 2014.The above observations thus confirm that the species are *Anabaena*, *Nostoc* and *Oscillatoria*.

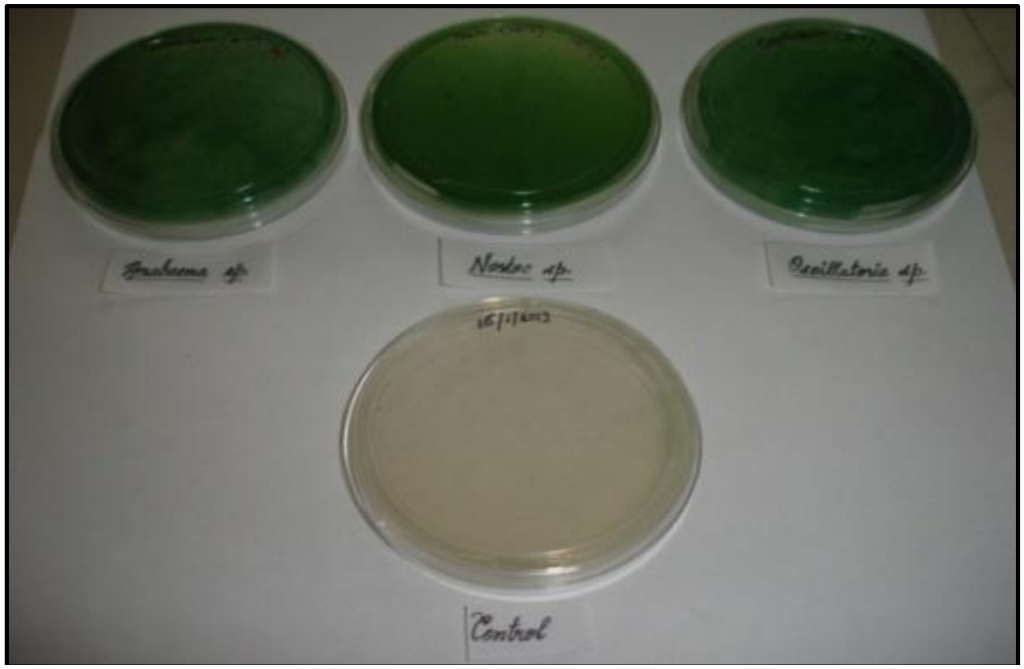

Fig. 1: Growth of Anabaena, Nostoc, and Oscillatoria on BG11 agar plates

Consortia development and modification in growth medium for consortia

All the three cyanobacterial cultures were grown and maintained in the standard BG11 medium. As *Anabaena sp.* and *Nostoc sp.* are nitrogen fixing bacteria, media was modified to make it nitrogen free. It helps in purification of culture. Whereas slight modification in the ingredients of BG11 was done to reach the growth requirements of cyanobacterial consortia. Initially unmodified Bg11 medium was fed to the consortia in reactor. In batch reactor when the consortia were exposed to the atmosphere, it responded quickly with the changes in temperature and carbon. The BG 11 medium fed initially to the consortia was found to contain less inorganic carbon. Also, magnesium sulphate concentration was on higher side for optimum growth of consortia. Because of high magnesium ion concentration, a white powdered layer was formed over the surface of aqueous medium within the reactor system. This layer may have created a barrier for environmental gaseous exchange. Because of this, the consortia added, started turning yellow in colour rather than lavish green because of deficiency in the medium for carbon and nitrogen. The dry weight and chlorophyll a of consortia started decreasing and reached up to 1200-1380 mg/L and 0.6-0.72 mg/L respectively. Consortia a characteristic with MLSS and chlorophyll a profile is shown in table 3. The concentration of sodium nitrate was regulated as the consortium contains *Anabaena sp*. and *Nostoc sp*. which can fix atmospheric nitrogen. Therefore, less quantity of sodium nitrate was added to support the growth of *Oscillatoria sp.* Potassium dihydrogen orthophosphate and dipotassium hydrogen orthophosphate were added to supply phosphorus ions and to buffer the medium as well. Likewise, the medium was modified for consortia as given in table 4. The medium modification helped in achieving stable growth of cyanobacterial consortia with 3500 mg/L MLSS. The microscopic images also supported that the consortia became healthy, with young filamentous cells. The stages of consortia development are shown in photograph 2.

Table 3: Consortia characteristics with changes in medium composition

Sr. No.	Media	Consortia characteristics	% increase in MLSS	% increase in *Chl-a*
1.	BG-11 Standard	Did not support growth No increment in MLSS and *Chlorophyll-a*	Decrease from 2500 mg/L to 1380 mg/L	Decrease from 4.8 mg/L to 0.6-0.72 mg/L
2.	BG-11 Modified (less inorganic carbon, high Magnesium sulphate)	White powdered layer has formed over liquid surface, deficient in carbon and nitrogen, Consortia became yellow colour	27.53-33.33	47.2-66.6
3.	BG-11 Remodified (Supplementation of Sodium nitrate and $KH_2 PO_4$)	Green coloured consortia with young filamentous cells	60.57-62.5	86.6-88.4

Table 4: Composition of Modified BG 11 medium

Sr. No.	Ingredients	Quantity
1.	Na_2CO_3	0.02 g/L
2.	$NaHCO_3$	0.5 g/L
3.	$NaNo_3$	0.05 g/L
4.	K_2HPO_4	0.04 g/L
5.	$KH_2 PO_4$	0.04 g/L
6.	$MgSO_4.7H_2O$	0.02 g/L
7.	Citric acid	0.006 g/L
8.	Ferric ammonium citrate	0.006 g/L
9.	$CaCl_2.2H_2O$	0.02 g/L
10.	Trace metal solution	1 ml
11.	Distilled water	1 Litre
Trace Metal Solution		
12.	H_3BO_3	2.86g
13.	$MnCl_2.4H_2O$	1.81g
14.	$ZnSO_4.7H_2O$	0.222g
15.	$NaMoO_4.2H_2O$	0.39g
16.	$CuSO_4.5H_2O$	0.079g
17.	$Co(NO_3)_2.6H_2O$	49.4mg
18.	Distilled water	1Litre

Stages of consortia development

Fig. 2: Stages of Consortia Development a) initial stage b) biomass deterioration c) fully grown consortia in batch reactor

CONCLUSION

Based on the results, a fully grown consortium has been developed using three cyanobacterial species. Nutrient medium modification has helped in cultivation of consortia with increase in MLSS up to 62.5% and chlorophyll a concentration up to 88.4%. Under these conditions of operation the consortia is fully stable and can be used in future to study the adsorption of metals.

REFERENCES

1. Ahmed A. Issa, Mohamed Hemida Abd-Alla and Takuji Ohyama dvances in Biology and Ecology of Nitrogen Fixation, chapter 2, Nitrogen Fixing Cyanobacteria: Future Prospect, pg no. 23-48.
2. American Public Health Association (APHA), (1992), Standard method for examination of water and wastewater analysis (18th ed.), American Public Health Association, Washington D. C.
3. ATCC Catalogue of Bacteria & Bacteriophages 18th edition, 1992.
4. Bergey's manual of determinative bacteriology 9th edition.
5. Chang, J., Law, A.R., Chang, C.C., (1997), Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. Water Research, 31, pp 1651.
6. Das, S., Santra, S. C., (2008), Characterization of simultaneous bio-accumulation and bio-reduction of hexavalent chromium by an *Aspergillus flavus* isolate (ESTF –241). Journal of Mycopathological Research, 46(1), pp 77-80.
7. Gardea-Torresdey, J. L., Arenas, J. L., Francisco, N.M.C., Tiemann, K. J., Webb, R., (1998), Ability of immobilized cyanobacteria to remove metal ions from solution and demonstration of the presence of metallothionein genes in various strains, Journal of Hazardous Substances Research, 1, pp 21-218.
8. Hussain, M. A., Salleh A., Milow, P., (2009), Characterization of the Adsorption of the Lead (II) by the Nonliving Biomass *Spirogyra neglecta* (Hasall) Kützing. American Journal of Biochemistry and Biotechnology, 5(2), pp 75-83.
9. Jessica K. Cole, Janine R. Hutchison, et. al. (2014), Phototrophic bio film assembly in microbial mat derived unicyanobacterial consortia: model systems for the study of autotroph-heterotroph interactions, frontiers in microbiology, vol.5 article 109.
10. Kaewsarn, P., (2002). Biosorption of copper (II) from aqueous solutions by pre-treated biomass of marine algae *Padina* sp. Chemosphere, 47: 1081-1085.
11. Leusch A., Z.R. Holan and B. Volesky, J. Chem. Tech. Biotechnol.62, 279-288 (1995).
12. Low, K. S. and Lee, C. K., (1991), Cadmium uptake by the moss *Calymperes delessertii* Besch. Bio resource Technology, 38, pp 1-6.
13. Naoki Sato, Yutaro Katsumata, Kaoru Sato and Naoyuki Tajima (2014), Cellular Dynamics Drives the Emergence of Supracellular Structure in the Cyanobacterium, *Phormidium* sp. KS, life 2014,4 pp 819-836.
14. Narain, S., Ojha, C.S.P., Mishra, S. K., Chaube, U.C. and Sharma, P.K.(2011), Cadmium and chromium removal by aquatic plant, International Journal of Environmental Sciences, 1(6), pp 1297-1304.
15. Pardo, R., Herguedas, M., Barrado, E., Vega, M., (2003), Adsorption of cadmium, copper, lead (II) and zinc by inactive biomass of *Pseudomonas putida*, Analytical and Bioanalytical Chemistry, 376, pp 26-32.

How to Cite this Article: Urewar C, Nandy T, Roychoudhury K. Development of Cyanobacterial Consortia in a Photobioreactor. Indian J Microbiol Res 2015; 2(3): 172-176.