

Annexure-VIII

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110002

**PROFORMA FOR SUBMISSION OF INFORMATION AT THE FINAL
REPORT OF THE WORK DONE ON THE PROJECT**

1) NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR :-

Dr. Kunal Roychoudhary

Office :- Department of Microbiology, Seth Kesarimal Porwal College of Arts and Science and Commerce, Kamptee 441001, Dist – Nagpur

Residential : 319, Atharva Veda, Dixit Nagar, Nari Road, Nagpur-440026.

2) NAME AND ADDRESS OF INSTITUTION :-

Seth Kesarimal Porwal College of Arts and Science and Commerce, Gutam Nagar, Kamptee 4410001, Dist – Nagpur

3) UGC APPROVAL NO. AND DATE :- F. No. 41-1168/2012 (SR)

Dt. 26th July 2012.

4) DATE OF IMPLEMENTATION :- July 2012

5) TENURE OF PROJECT :- July 2012 to December 2015 (With further Extension)

6) TOTAL GRANT ALLOTTED :- Rs. 10,25,000/- (Ten Lakh Twenty Five Thousad only)

7) TOTAL GRANT RECEIVED :- Rs. 10,05,000/- (Ten Lakh Five Thousand only)

1st Installment Rs. 9,25,000/- (Nine Lakh Twenty Thousand only)

2nd Installment Rs. 80,000/- (Eighty Thousand only)

8) FINAL EXPENDITURE Rs. 10,09,202 (Ten Lakh Nine Thousand Two Hundred Two only)

9) TITLE OF THE PROJECT :- PCR BASED CHARACTERIZATION OF CYNOBACTERIAL CONSORTIA USED IN METAL REMOVAL FROM WASTE WATER BY CONTINUOUS FLOW BIOREACTOR

10) OBJECTIVES OF THE PROJECT :-

- a) Isolation and Purification strains of cyanobacteria capable of chromium removal.
- b) Studies on chromium removal from synthetic waste water using photo bioreactor.
- c) PCR amplification of the available species of cyanobacteria.
- d) Sequencing on outsourcing basis and analysis of species characterization.

11) WHETHER OBJECTIVES WERE ACHIEVED :- Yes, all the objectives was achieved

12) ACHIEVEMENTS FROM THE PROJECT :- Three Species of Cynobacteria capable of chromium removal isolated and characterised (Gene Bank Accession No. Ky 421771, Ky421772, Ky421773 can be used for pilot studies.

13) SUMMARY OF THE FINDINGS :- See summary report attached as Annexure-A

14) CONTRIBUTION TO THE SOCIETY

The results obtained from the project is useful for treatment water bodies containing chromium VI as Pollutant. Culture can be used in low cost technology.

15) WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT :-

Based on this project, Geetesh Shukla was awarded Ph.D. by RTM, Nagpur University in 2019.

16) NO. OF PUBLICATION OUT OF THE PROJECT (REPRINTS ATTACHED IN FINAL REPORT):-

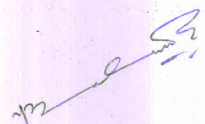
- 1) Geetesh Shukla, Sachin Yerpude, K. Roychoudhary, "Biosorption of hexavalent chromium using continuously Fed fixed Bed Photobioreactor System", Bionano Frontier 10(1), 128-136, 2017.
- 2) Geetesh Shukla, K. Roychoudhary, " Morphometric, Biochemical and Microscopic Characterization of Cyanobacterial Species". Bionano Fronier 10(1) 123-127, 2017.

POSTER PRESENTATION :-

Removal of hexavalent chromium by Cyanobacteria, Annual Conference of Association of Microbiologists of India (AMI-2013), Association of Microbiologist of India (AMI), 17-20 Nov. 2013, International.



Signature of Principal Investigator



Principal

Annexure – A

**PCR BASED CHARACTERIZATION OF
CYANOBACTERIAL CONSORTIA USED
IN METAL REMOVAL FROM WASTE
WATER BY CONTINUOUS FLOW
BIOREACTOR**

**Major Research Project
Summary Report**

Vide F. No. 41-1168/2012 (SR)

Submitted By
Dr. K. Roychoudhary
(M.Sc. Ph.D. Microbiology)

**Set Kesarimal Porwal College of Arts & Science & Commerce,
Kamptee**

Major Research Project Summary Report

PREAMBLE :-

The pollution of the natural environment, including land and water bodies by metals such as lead, arsenic, mercury, chromium, cadmium, nickel, copper and zinc etc. has become a serious problem not only in industrialized countries but also in developing countries. Both the surface and ground water bodies are under the threat of such pollution. Since water is the elixir of life and its availability as potable water is becoming scarce hence, better low cost technologies are required to be developed for rendering polluted water bodies to be safe

AIMS AND OBJECTIVE :-

- a) Isolation and Purification strains of cyanobacteria capable of chromium removal.
- b) Studies on chromium removal from synthetic waste water using photo bioreactor.
- c) PCR amplification of the available species of cyanobacteria.
- d) Sequencing on outsourcing basis and analysis of species characterization.

STRATEGY AND WORK PLAN :-

In the present study a specific strategy was employed for the development of reactor system so as to favour the increase of cell surface area during the exposure to chromium. During the complete work plan the light intensity was maintained and kept constant at 30 to 35 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$. Similarly the temperature of the system was kept at room temperature. The other parameters supply of carbon source was kept unchanged during all the experiment. Such a strategy was helpful in the comparative study of the different systems.

SAMPLE :-

Cyanobacteria are special class of prokaryotes which require distinct methods and procedures for their isolation, purification, characterization and cultivation. Being photoautotrophic they require special media for growth and usually proper intensity of light with a specified Light : Dark Period of exposure improves growth of these bacteria. Various methods employed in the present study are standard procedures used by various workers in their studies.

Considering the diversity of habitat of cyanobacteria species it was decided to select different sampling points for isolation of cyanobacterial species. Three sites selected for sampling are as follows :-

- a) Lonar Lake was selected for its diverse microbiological population and a distinctly different physicochemical nature of the water in the lake.
- b) A fresh water lake at Kunwara Bhimsen selected on the basis of its being a very vast stretch of water body with almost natural physicochemical parameters.
- c) Banks of Nag River passing through Nagpur city having extremely high organic and inorganic pollution.


STUDIES UNDERTAKEN :-

1. Primary Screening
2. Growth on solid media and further screening
3. Purification of isolates
4. Screening of isolates for removal of chromium (VI)
5. Chromium removal studies
6. Development of consortia
7. Morphometric characterization
8. Heterocyst and akinete studies
9. Gram Staining
10. Phase contrast microscopy
11. Scanning Electron Microscopy
12. Cultural characterization
13. Growth on nitrogen free BG11 medium
14. Growth on nitrogen supplemented BG11 medium
15. Biochemical characterization of cyanobacterial species
16. Estimation of chlorophyll a content
17. Estimation of accessory pigments.
18. Estimation of total carotenoides
19. Estimation of Phycobiliproteins
20. Estimation of total protein
21. Estimation of total free amino acids
22. Isolation of DNA
23. PCR amplification and sequencing
24. Development of static culture
25. Optimization of light intensity

26. Effect of light intensity on growth of cyanobacterial species
27. Effect of chromium (VI) concentration on percent removal of chromium
28. Effect of contact period on percent removal of chromium
29. Effect of chromium (VI) concentration on percent removal of chromium by consortia
30. Effect of contact period on percent removal of chromium by cyanobacterial consortia
31. Comparative analysis of percent removal of chromium (VI) by individual species and consortia
32. The design of the Continuously Fed Fixed Bed Reactor System
33. Growth and hydraulic retention time optimization
34. Removal of chromium (VI) by cyanobacterial species
35. Removal of chromium (VI) by cyanobacterial consortia
36. Studies on adsorption of chromium on cyanobacterial surfaces
37. Studies on Langmuir adsorption isotherm model
38. Freundlich adsorption isotherm
39. Scanning Electron Microscopy studies of cyanobacterial species post chromium (VI) treatment
40. EDX studies of cyanobacterial species post chromium (VI) treatment

OUTCOME OF STUDIES :-

- All the three sampling spots namely, Lonar Lake, Kunwara Bhimsen and Nag River showed the presence of cyanobacterial species capable of removing chromium at different levels ranging from 10ppm to 25ppm of chromium (VI) exposures.
- The three species isolated from the three sampling sites namely Cy 6, Cy7 and Cy40 could be confirmed by conventional methods of characterization and also by PCR based characterization to be Anabaena species, Nostoc species and Leptolyngbya species respectively.
- Out of the large numbers of the samples three cyanobacterial species were able to remove chromium (VI). Anabaena (N), Nostoc (N), Oscillatoria (N), Anabaena (C), Nostoc (C), and Leptolyngbya were able to remove chromium to the level of 85 to 93% at in initial exposure of 10ppm chromium (VI) in static culture while at 25ppm of chromium (VI) exposure the percent removal varied from 78 to 90%.
- The six species under study in continuously fed fixed bed reactor system exposed with 255ppm showed more than 97% removal of chromium within seven days. It was also found that all these six species were able to remove more than 90% chromium (VI) in 72 hours.
- All the species under study showed that adsorption of chromium occurred in the cell surface obeying Langmuir and Freundlich adsorption isotherms. Their observed r^2 values for all the cases were found to be more than 0.99 except Leptolyngbya where in r^2 was found to be more than 0.98 in exercise for Langmuir adsorption isotherm.
- The cyanobacterial consortia were able to remove chromium (VI) to the level of 93% in 24 hours where as it removed 99.4% within seven days in continuously fed fixed bed reactor system.
- Based on this studies Geetesh Shukla was awarded Ph.D. by RTM Nagpur University, Nagpur in 2019.


K. Roychoudhury