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Occurrence of Cyanotoxins and their Removal by Oxygen Evolving Bacteria with Implication to Fish productivity

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A B S T R A C T

The presence of blue-green toxins in surface and running waters used for drinking water source and recreation is receiving increasing attention around the world as a public health concern and fish productivity. However, potential risks from exposure to these toxins in contaminated health food products that contains BGA have been largely ignored, In the present study screening, clear-up, identification, quantitative and qualifitative analysis of microcystin were described. The Oxygen Evolving Bacteria (OEB) *Pseudomonas, Oscillatoria rukbescens* DC (singly) and *Pseudomonas* and *Oscillatoria rubescens* DC (mixed) were utilized for the treatment of running water and also detoxication of Cyanotoxins (MC) level under laboratory conditions. Result obtains are indicative of factors that *Pseudomonas* and *Oscillatoria rubescens* DC (mixed) was much better than *Pseudomonas, Oscillatoria rubescens* DC (singly) for treatment of polluted water in terms of reducing MC level, pH, BOD, Free-CO_2 and Sulphate while DO increased. *Oscillatoria rukbescesn* DC was very useful to reduce the level of N, P and N/P ratio as compare another. The present finding suggest that the detoxications of Microcystin (MC) may reduce 20-55% by mixed form of OEB and successful cultivation of *Labeo rohita* and *Catla catla* were also achieved.

Introduction

Blue-green algae or cyanobacteria exhibit remarkable ecological adaption and diversification in response to global evolutionary change, including the development of a modern day toxic biosphere. Algae often serve as excellent indicators of pollution and as they respond typically to many toxicants (Oh *et al.,* 2001; Dwivedi and Pandey, 2001, Dwivedi and Pandey, 2002 a, b; Pandey and Dwivedi, 2002). The algal blooms of the cynobacteria are ubiquitous phenomena in eutrophic water reservoir in many countries of the world. Many strains of *Microcystis* are known to produce cyanotoxin (microcystin-MC) which are unfavorable for the human health and fish productivity (Duncan *et al.,* 2000). The use of algae in water/ waste water treatment could prove beneficial in different ways since they will bring about oxygenation and mineralization, in addition to serving as a food source for aquatic

species (Subramanian and Uma, 1996; Perkins and Hunter, 2000). Blue green algae are ideally suited to perform these functions by virtue of their flexibility to adapt to varied environments and their known nutritional and fertilizers value. Algae-bacterial symbiosis has long been proved to be an inexpensive process for the reclamation of waste water. Despite the very widely recognized importance of Oxygen evolving microorganism (indicative bacteria) as fundamental to the appraisal of water quality (Tranter et al. 1996; Dwivedi and Pandey 2003a), very little research has been conducted on the efficiency of OEB treatment system in the removal of cyanotoxin and improvement of water quality from water bodies with respect to utilization of water/ waste water bodies for fish production. In view of the above, this study was undertaken OEB as a simple, cost-effective, self-sustaining alternative to alter the Microcystin (MC) level and the improvement of water quality of Yamuna river at Allahabad city under laboratory conditions.

Materials and Methods

In the present study extraction, isolation and purification of toxin producing algal species (*Microcystis* sp., *M. protocystis M.aeruginosa, Oscillatoria* sp., *O. princeps,. Lyngbya sp., Nostoc* sp. and *Anabaena* sp.) were carried in the laboratory from Yamuna river at around Allahabad city. Water sample have been taken from different sources where more scum of bloom and maked a composite sample for accurate monitoring following (APHA, 2010). These isolated and purified toxin producing algal species particularly (axenically) and mixed (mass culture) forms were transfered (inoculated) in 50 ml of BG-11 medium with special precautions to maintain the purity of the culture. They were incubated in

125-ml culture flask in culture cabinet at 25^0C under continuous illumination of intensity (2000-3000 lux- Philips TDL 18WX3 fluoresent tubes). The culture was aerated with ambient air and that was filtered through activated carbon and sterilized cotton.

Analysis of Microcystin

In order to analyse micorcystin (MC) from the sample, dry weight of the sample were determined by filtering 1 to 10 ml portion of cultures through pre weighed cellulose nitrate filters (pore size, 0.8 ☐ m; Sartorius). The filters were dried to constant weight at room temperature in a silica gel desicator. Samples (50 to 300 ml) were collected and frozen at 20^0 C. After thawing, glacial acetic acid was added to a final concentration of 5%. The sample were then extracted for 90 min with stirring at 40^0C. After centrifugation (9000 rpm for 10 min), the pellets were re extracted with 5% acetic acid, and the pooled supernatant were applied to activated C_{18} cartridges. The cartridges were rinsed with water and eluted with 5 of 10 ml of methanol. The toxin content was quantified by using HPLC performed with an internal surface reverse phase column (Meriluoto and Eriksson, 1992; Harada *et al.,* 1988). The mobile phase was 12% aceto nitrate-88% 0.1 M KH_2PO_4 (pH 6.8), and the flow rate was I ml/ h. The detector set at 238 nm. All peaks were tested toxin by using a standard mouse bioassay. The toxin was quantified by using purified toxin standards obtained for *M. aeruginosa*.

Culture of Oxygen Evolving Bacteria (OEB)

Primary inoculation of *Pseudomonas* (x) (5.5 x10^5 cells/ l), *Oscillatoria rubescens* DC (y) (0.05 μg Chl/ l) singly and

Pseudomonas, *Oscillatoria rubescens* DC in mixed form (z) (4.9×10^5 cells/ l & 0.05 µg Chl/ l) developed separetaly in 5 lites of BG-11, and were multiplied in separate aquarium of 2500 liters capacity. Urea (265 mg) and superphosphate (30 mg/l) were used for the multiplication of BGA. After four days, successfully inoculate 6.2×10^5 cells/ l, 0.57 µg Chl/ l and 5.8×10^5 cells/ l and 0.51 µg Chl/ l of x, y and z, respectively were pumped to their respective aquarium with composite water sample under laboratory conditions. The samples were adjusted to totally replace the medium and give a retention time of four days in each aquarium. The water quality factors were estimated initially before and after treatment and also at regular intervals.

Fish Growth Productivity

Fresh sample of two commercially important fishes *Labeo rohita* and *Catla catla* (mixed sex, same age group) were obtained from the Yamuna river during study period. These fishes were acclimated in the laboratory condition for one month. Length and weight (L-w) were measured by a Planimeter and top balance (GDR), respectively. The fishes were measured and weighted in the fresh condition with in three hours after capture. Total length of fish was measured to the nearest centimeter and the weight was recorded to the nearest gram. The L-W relationship of fishes and all procedure for culturing and exposure OEB following (Dwivedi and Pandey, 2002b). The data was presented as the average of the three replicates in each case.

Results and Discussion

The Yamuna river water was characterized by high level of Ammonia, nitrate, Suphate, Free-CO_2, BOD, Chloride, TDS and Phosphate (Table-I). In order to improve of

Yamuna river water quality, oxygen evolving bacteria were developed and evaluated as either singly, *Pseudomonas* (x), *Oscillatoria rubescens* DC (y) of mixed culturally, *Pseudomonas* and *Oscillatoria rubescens* DC (z), with or without toxin producing algal species under laboratory conditions. Data on MC concentration and alteration in toxicity MC level of Yamuna river water following exposure of OEB was presented in Table-2. The correlation–coeffcient (r) values between microcystin concentration and physic-chemical parameters has been presented in Table-3.

The quality of aquatic ecosystem depends on the physic-chemical nature of waters and also on the biological diversity of the system (Lund *et al.,* 2001; Dwivedi *et al.,* 2012, Dwivedi 2015; Dwivedi *et al.,* 2016). Generally bacteria may assimilate added carbonaceous material and produce carbon dioxide which accelerates algal photosynthetic (oxygen evolving) and increased O_2, (Uma and Subramaniam, 1990), these practiced reclamate the water quality and toxin level as green clean. Following four to six days days exposure of *Oscillatoria rubescens* DC was reduced 49% BOD, 30% Chloide, 36% Suphate, 30% Free- CO_2, 52% nitrate, 45% ammonia and 46% Photophate (Table-I). Mixture of *Pseudomonas* and *Oscillatoria rubescens* DC brought down 14% pH, 63% BOD, 30% Chloride, 47% Suphate, 42% Free-CO2, 50.0% Nitrate, 50% Ammonia and 48% Phoshate (Table-I). In the present study the performance of *Pseudomonas* both in terms of their growth and reduction of TDS, and Chloride was efficient than *Oscillatoria rubescens* DC. Mixed cultured on the other hand reduces much more BOD, Free-CO2, pH, and Suphate as compared with *Pseudomonas*, and *Oscillatoria rubescens* DC singly, but did not more influence the TDS, and Chloride in running water of

Yamuna river. The result showed that *Oscillatoria rubescesn* DC was very useful to reduce the level of Nitrate, Ammonia, Photophate and N/P ratio as compare to *Pseudomonas* singly and *Pseudomonas*, *Oscillatoria rubescesn* DC in mixed form. In the present study MC level were detected in the particulate and dissolved form, these biotoxin level/ concentration in particulate and dissolved form were found to be 92.2-245 and 14.90- 63.0 ng/1, respectively during the study period. This difference was

due to a combination of various complex factors such as nutrient concentration by evaporation as shown in other reservoirs (Lahti *et al.,* 1997; Oh *et al.,* 2000). The nutrient, as an easy way to determine the trophic status of water bodies, which was either directly or indirectly related to the chl-a concentration, toxin producing algal species biomass and MC concentration (Dwivedi and Pandey, 2003a, b). Similar observation were made by Lee *et al.,* (2001); Oh *et al.,* (2001) in case of other reservoirs.

Table.1 Reduction of Running Water (Yamuna River) Quality Parameters (Except DO i.e. Increased) following Exposure of Oxygen Evolving Bacteria (x- *Pseudomonas*, y- *Oscillatoria rubescens* DC and z- Mixed Culture of *Pseudomonas and Oscillatoria rubescens*)

S.No	Parameters	Control	Experimental		
			X	y	z
1	pH	08.09	07.17 (12)	7.05 (13)	7.02 (14)
2	DO*	05.09	06.69	7.19	8.09
3	BOD	08.09	04.24 (47)	4.02 (49)	3.09 (63)
4	Chloride	46.30	25.54 (45)	31.41 (32)	32.34 (30)
5	Free-CO_2	32.88	22.80 (33)	20.01 (39)	19.41 (42)
6	TDS	253.14	151.8 (40)	164.84 (34.9)	172.14 (32)
7	Sulphate	02.09	01.46 (26)	1.24 (36)	0.89 (47)
8	Ammonia	01.78	00.96 (27)	0.82 (50)	1.01 (45)
9	Nitrate	02.09	01.09 (49)	0.99 (52)	1.10 (48)
10	Phosphate	01.95	00.99 (45)	01.06 (48)	0.96 (46)

Except pH all values are in mg/l (Mean of three replicates); Figure in parentheses indicate % reduction of water quality parameters, * increased

Table.2 Reduction In Concentration of Microcystin (Ng/L) of Particulate (1) and Dissolved (2) in Yamuna River Following Exposure of Oxygen Evolving Bacteria (X- *Pseudomonas*, Y- *Oscillatoria rubescens* Dc and Z- Mixed Culture of *Pseudomonas* and *Oscillatoria rubescens*)

S.No.	Species	Control		Experiment					
				X		**Y**		**Z**	
		1	2	1	2	1	2	1	2
1.	*Microcystis sp.*	192.0	43.0	169.0	29.00	156.0	33.00	149	26.8
2.	*M. protocystis*	183.5	38.8	155.0	21.90	138.0	23.0	129	19.8
3.	*M. aeruginosa*	245.9	63.0	186.0	33.00	176.0	43.00	109	29.8
4.	*Oscillatoria* sp.	101.1	20.3	82.00	14.90	87.16	13.90	69.0	12.8
5.	*O. princeps*	124.2	20.2	119.1	16.00	102.2	16.90	97.0	12.9
6.	*Lyngbya* sp.	138.2	28.3	119.0	18.90	112.2	18.90	69.0	14.9
7.	*Nostoc sp.*	98.20	19.0	66.12	13.14	62.11	12.98	56.0	12.4
8.	*Anabaena sp.*	**92.20**	14.9	79.90	13.90	72.00	13.10	51.0	13.9

Table.3 Correlation Coefficient (R) Values Between Physic-Chemical Parameters and MC Concentration of Toxin Producing Algal Species

Parameters	A	B	c	D	e	F	g	H
pH	0.42	0.22	0.61	0.23	0.16	0.63	0.05	0.15
DO	0.39	0.81	0.88	0.21	0.32	0.79	0.21	0.15
BOD	0.43	0.76	0.87	0.21	0.76	0.68	0.19	0.32
Chloride	0.53	0.62	0.72	0.14	0.17	0.51	0.09	0.31
Free-CO_2	0.61	0.82	0.67	0.32	0.32	0.73	0.12	0.09
TDS	0.47	0.72	0.87	0,42	0.31	0.68	0.15	0.18
Sulphate	0.53	0.36	0.82	0.23	0.41	0.50	0.23	0.42
Ammonia	0.72	0.49	0.72	0.64	0.23	0.51	0.23	0.39
Nitrate	0.29	0.67	0.65	0.42	0.31	0.53	0.09	0.21
Phosphate	0.23	0.63	0.65	0.34	0.15	0.61	0.09	0.15

Abbreviation *a-Microcystis*, b- *M. protocystis*, c. *M. aeruginosa*, d. *Oscillatoria*, e. *O. princeps*, f. *Lyngbya* sp., g. *Nostoc* sp., h. *Anabaena* sp

Table.4 Percentage of Length Frequency Distribution In *Labeo Rohita & Catla Catla* Following One Month Exposure of Oxygen Evolving Bacteria (X- *Pseudomonas*, Y- *Oscillatoria rubescens* Dc and Z- Mixed Culture of *Pseudomonas* and *Oscillatoria rubescens*)

Length gp	*Labeo rohita*				*Catla catla*			
	Control	Exp.-x	Exp.- y	Exp.-z	Control	Exp.- x	Exp.- y	Exp.- z
10-15	-	-	-	-	08.80	-	-	
16-20	04.11		-	-	16.66	16.04	18.55	12.88
21-25	19.21	17.00	15.00	12.77	15.58	12.40	12.66	13.13
26-30	20.00	19.06	11.77	12.88	16.77	21.88	14.77	14.67
31-35	16.00	14.00	15.50	14.08	13.77	18.88	21.11	23.06
36-40	14.00	16.00	17.99	13.02	15.66	16.00	21.00	22.60
41-45	15.81	18.04	18.87	20.16	12.76	14.80	11.91	13.66
46-50	10.87	15.90	20.87	27.09	-	-	-	-

Negative sign (-) shows that particular fish was not found in respective length groups

Amongs 8 toxin producing algal species, 5 species (*Microcystis* sp., *M. protocystis, M. aerugnosa, Oscillatoria princeps*; and *Lyngbya* sp.) were found to be relatively high microcystin concentration (ng/1) throughout the study period in Yamuna river. It is well known that higher organic matter and low DO influencing the MC concentration and promote cyanotoxin in Yamuna river, stretched up to its last phase at Allahabad city. Out of three oxygen evolving bacteria (x, y, & z), mixed culturly have shown better performance for water quality improvement and detoxication of cyanotoxin, followed by *Pseudomonas* and *Oscillatoria rubescesn* DC singly.

The correlation-coeffcient (r) between physic-chemical parameters DO, BOD, chloride, sulphate, free-CO_2, TDS, phosphate, and MC concentration of *Microcystis* , *M. protocystis*, M. *aeruginosa* and *Lyngbya* sp. Showed positive correlation while *Nostoc* and Ansbaena sp showed in-significant correlation with ph , Do, BOD, TDS , nitrate and phosphate in Yamuna river before exposure of Oxygen evolving bacteria (Table-3).

The fish size indicate 10-15 and 16-20 cm were found to be less and consisting of 04.11, 08.80% length frequency distribution in case of *Labeo rohita* and *Catla catla*, respectively (Table-4). Following one month exposure OEB x it was found to be 15.90% in the highest group (46-50 cm) case of *Labeo rohita*, while exposure of y and z in the same group the growth rate was more and considered 20.87 and 27.09, respectively comparision with control (10.87 %). In case of *Catla catla* were 14.80, 11.91 and 13.66 % existed in the length frequency group (41-45 cm) after exposure of x, y and z, respectively, and showed less L-W frequency when compared with *Labeo rohita* (Table-4). It is evident from earlier study the growth rate of *Catla catla* was comparatively less than *Labeo rohita* before and after exposure of OEB and coinciding this the negative bearing on growth rate of both fishes due to nutrient influx and highly microcystin concentration (Dwivedi and Pandey, 2002b). Moreover, OEB efficiency was strongly, successful productivity in terms of length-weight (L-W) relationship of *Labeo rohita* and *Catla catla* fishes.

This study indicates that the high nutrient and pollution loaded river water lead to generate/ release of high MC concentration in river system and reduced fish production. This may serve ecological consequences in long run if proper regulatory measure are not taken. The use of OEB based system may prove as a highly cost- effective and sustainable aquaculture technology for treatment of water/ waste water especially in subtropical developing nations, including biomanipulation, for restoration of eutrophic reservoirs in general and running water at Allahabad city in particular.

References

APHA, AWWA and WPCT,, 2010. Standard methods for examination of waste water, 27th edition American health association water works association and water pollution control federation, New York.

Dwivedi, B K, G C Pandey, 2001. Seasonal dynamics of cyan bacterial toxin producing algal species of two water ponds. Aquacult. 2, 141-146.

Dwivedi, B K, G C Pandey, 2002a. Physico-chemical Factors and Algal Diversity of Two Pond (Girija kund and Maqubara pond) Faizabad India, Poll. Res. 21, 361-370.

Dwivedi, B K, G C Pandey, 2002b. Length-weight relationship and relative condition factors of Labeo rohita and Catla catla in Cyanotoxin Environment and its mitigation through Photosynthetic bacteria, Pro. Zoological Society of India. 1, 9-16.

Dwivedi, B K, G C Pandey, 2003a. Complex dynamic of toxin producing algal species and primary productivity in two ponds of Faizabad. Env. Biol. 24(1), 55-61.

Dwivedi, B K, G C Pandey, 2003b. An approach to improve water quality through photosynthetic bacteris. Nature Environment and and Pollution Technology, 2(2), 145-152.

Dwivedi, B K, S P Tiwari, D K Parihar, 2012. Biological Surveillance of running water at Allahabad city. Bioherald 2, (1), 35-40.

Dwivedi, B K, 2015. Current status of fresh water (running) in relations to primary productivity and ecological integrity. 85th NASI KIIT University, Bhubaneswar. 06- 08 Dec.

Dwivedi, B K, Divya Raghuvanshi, Neelam Shukla, 2016. River Water Quality in Relation to Primary Productivity and Pollution. Water Research, (under process).

Duncan, G J.,, K W Kenneth, H A Ronald, X Huang , CS Fun, 2000. Assessing Potential Health Risks from Microcystin Toxins in Blue- Green Algae Dietary Supplements. Environmental Health Perspectives, 108, (51), 435.439.

Pandey, G. C.,, B K Dwivedi, 2002. The Toxin of Cyan bacteria, emerging water quality problem, 395-405 in: Ecoogy of Polluted Water. (Ed Kumar) A P C New Delhi.

Subramanian, G.,, L Uma, 1996. Cyanobacteria in pollution contero, Journal of Scientifi & Industrial Research. 55685-692. Tranter J Hunter C J Gunn J and Perkins J 1996 The bacterial quality of an upland stream J. of the Institution of water and Enironmental

management, 10, 273-279 .

Utkilen, H.,, N Gjolme, 1992. Toxin Production by *Microcystis aeruginosa* as a Function of Light in Continuous Cultures and Its Ecological Significance, Appl. Environ. Microbio., 58, 1321-1325.

Oh, H M.,, S J Lee, M H Jand, B D Yoon, 2000. Microystin Production by *Microystis eruginosa* in a Phosphorous- Limited, Chemostat. Applied and Environmental Microbiology, 66, 176-179.

Oh, H M, S J Lee, H S Kim, B D Yoon, 2001. Seasonal variation and indirect monitoring of Microystin concentrations in Daechung reservoir Korea. Applied and Environmental and Microbiology, 4, 1484-1489 .

Tranter J, C J Hunter, J Gunn, J Perkins, 1995. The bacterial quality of an upland stream. J. of the Chartered Institution of Water and Environmental Management, 10, 273-279.

Joy Perkins, Colin Hunter, 2000. Removal of enteric bacteria in a surface flow constructed wetland in Yorkshire, England. Wat. Res. 34 (6), 1941-1947.

Meruluoto, J H O, J E Eriksson, 1992. Rapid anlaysis of peptide toxinsin cyanobacteri, J. Chromatogr, 438, 93-99 .

Harada K I, K Matsura, M Suzuki, H Oka, M F Watanable, S Oishi, A M Dahlem, V R Beasley, W W Carmichael, 1988. Analysis and and purification of toxic peptides from cyanobacteria by reversed-phase high performance liquid chromatography, J. Chromatogr. 118 275-283.

Lahti, K, J Rapala, M Farding, M Niemela, K Sivonen, 1997. Persistence of cyan bacterial hepatotoxin, microcystin-LR in particulate material and dissolved in lake water. Water Res. 31, 1005-1012.

Lund, M A, J A Davis, 2000. Seasonal dynamics of plankton communities and water chemistry in a eutrophic wetland (Lake Monger, Western Australia): implication for biomanipulation. Mar. freshwater Res., 51, 321-332.

Lee, S J, M H Jang, H S Kim, B D Yoon, M H Oh, 2001. Variation of Microcystin content of *Microcystis aeruginosa* relative to medium N:P ration and growth satge. J. Appl. Microbiol, 89, 323-329.