

Original Research Article

Studies on Nutrient Removal by Cyanobacterial Consortia Developed in a Photobioreactor

Chaitali Urewar^{1*}, Tapas Nandy¹ and Kunal Roychoudhury²

¹National Environmental Engineering Research Institute, Nagpur-440 020, India

²Department of Microbiology, S.K. Porwal College, Kamptee-441 001, India

*Corresponding author

ABSTRACT

Keywords

Cyanobacterial consortia, Hydraulic retention time, Nutrient removal, Photo-bioreactor

A cyanobacterial consortium was developed consisting of *Anabaena* sp., *Nostoc* sp. and *Oscillatoria* sp. This consortia was used for the nutrient removal i.e. nitrogen and phosphorous ion supplemented in modified BG-11 media using photobioreactor of 5 L working volume capacity, 1500 lux light intensity, and light to dark ratio of 10:14 hrs. The reactor was operated with different MLSS concentration 1500, 2500 and 3000 mg/L. pH was also monitored. Study was carried out for 7 days at each MLSS concentration. The consortia was able to remove at max 16.07% P and 16.77% N at 1500 mg/L MLSS, 27.20% P and 38.65% N at 2500 mg/L MLSS, 32.38% P and 17.69% N at 3000 mg/L MLSS. Maximum removal of nutrient was observed at 3 days and at 2500 mg/L MLSS concentration.

Introduction

The removal of nitrogen and phosphorus from wastewater has become an emerging worldwide concern because these compounds cause eutrophication in natural water. Moreover, nitrate is a risk to human health, especially as a possible cause of infant methaemoglobinaemia (B., 1952). Most of the nitrogen and phosphorous compounds from wastewater are not removed by conventional treatment processes. The presence of these nutrients leads to uncontrolled growth of aquatic weeds and algae (Rolf Eliassen, 1969). The increased growth of this phytoplankton disturbs entire wastewater treatment process. Cyanobacteria have nowadays attracted

biologists because of rapid utilization of nitrogen and phosphorous (Luuc and Mur, 1999) as cyanobacteria are photosynthetic prokaryote. Because of presence of specialized cell wall structure and minimum growth requirement; cyanobacteria are opted over many other bacteria (Selatnia, 2004), fungi (Das, 2008), bryophyte (Low, 1991) and pteridophyte (Dhir, 2010) for biosorption. Algae and cyanobacteria are also among the most promising biosorbents reported for efficient metal removal from wastewater (Hussain, 2009) Also the incomplete removal of nutrients during modern secondary sewage treatment necessitates second stage biological

treatment (Gates, 1964). Nutrient removal rate in a photobioreactor was studied by many investigators whereas only limited study has been carried out for hydraulic retention time. Hydraulic retention time is one of the important parameter in operating bioreactors. It is the actual residence time of influent in the reactor system at which maximum target pollutant removal efficiency is observed.

In present study three cyanobacterial species are mixed together to form consortia. The fully grown consortia was then studied for phosphorous and nitrogen removal at different MLSS concentration. The hydraulic retention time was then derived.

Materials and Methods

Collection and cultivation of cyanobacteria

The wild type species of *Anabaena*, *Nostoc* and *Oscillatoria* were obtained from Department of Botany, RTM Nagpur University, Nagpur. Three pure cultures of cyanobacterial species were cultured and grown together to form mixed consortia. ATCC 616 BG-11 medium was used for cultivation of cyanobacterial pure culture (Collection, 1992). It was grown in a photobioreactor with working volume capacity of 5.0L and developed in modified BG11 medium. The composition of modified BG-11 is given in table 1. Temperature was in the range of 28°C–30°C under light irradiance of 1500 lux with a photoperiod of 10:14 h (light: dark).

Growth Monitoring

Growth of cyanobacterial consortia was determined in terms of dry weight i.e. MLSS and *chlorophyll a* content as per standard methods (APHA, 1992)

Photobioreactor

Photobioreactor 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. Photobioreactor 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 luxmeter (METRAVI 1330) and calculated as 1500 lux. Photobioreactor was operated in batch mode.

Total Phosphorous and Nitrate Nitrogen estimation

Both influent and effluent samples were filtered by Whatmann filter paper. Phosphorous was measured by vanadomolybdophosphoric acid colorimetric method and nitrogen was measured by trophotometric screening. APHA standard method was followed (APHA, 1992).

Results and Discussion

Nutrient removal study

Nutrient removal was studied with sludge concentration of 1500mg/l, 2500mg/l and 3000 mg/l (Tables 2–4). At 1500 mg/L MLSS concentration, maximum removal efficiency was observed on 3rd day for both nutrients. Similarly at 2500 mg/L MLSS concentration, 27.20% P and 32.36% N removal efficiency was observed on 3rd only. At higher MLSS concentration i.e. 3000 mg/L removal efficiency was found to be decreased at 3rd day i.e. 7.74 % P and 14.15% N. pH of effluent was found to be more than 8 in all the three cases.

Table.1 Composition of modified BG 11 medium

Sr. No.	Ingredients	Quantity
1.	Na ₂ CO ₃	0.02 g/L
2.	NaHCO ₃	0.5 g/L
3.	NaNO ₃	0.05 g/L
4.	K ₂ HPO ₄	0.04 g/L
5.	KH ₂ PO ₄	0.04 g/L
6.	MgSO ₄ .7H ₂ O	0.02 g/L
7.	Citric acid	0.006 g/L
8.	Ferric ammonium citrate	0.006 g/L
9.	CaCl ₂ .2H ₂ O	0.02 g/L
10.	Trace metal solution	1 ml
11.	Distilled water	1 Litre
Trace Metal Solution		
12.	H ₃ BO ₃	2.86g
13.	MnCl ₂ .4H ₂ O	1.81g
14.	ZnSO ₄ .7H ₂ O	0.222g
15.	NaMoO ₄ .2H ₂ O	0.39g
16.	CuSO ₄ .5H ₂ O	0.079g
17.	Co(NO ₃) ₂ .6H ₂ O	49.4mg
18.	Distilled water	1Litre

Table.2 Reactor performance of nutrient removal at 1500 mg/L MLSS concentration

Sr. No.	Sample	pH	Total P (mg/L)	Total N (mg/L)	RE of P (%)	RE of N (%)
1.	Inlet	7.1	15.81	15.92	-	-
2.	Outlet	8.80	14.12	14.70	10.69	7.66
3.	Outlet	9.24	13.70	16.50	13.35	-3.64
4.	Outlet	9.45	13.27	13.32	16.07	16.33
5.	Outlet	9.96	14.12	12.25	10.69	23.05
6.	Outlet	9.96	14.12	12.70	10.69	20.23
7.	Outlet	8.46	14.12	13.83	10.69	13.13
8.	Outlet	8.86	14.12	14.23	10.69	10.62
MLSS- 1500mg/l; Chlorophyll- 3.98 mg/l; temperature 26⁰C-30⁰C RE= Removal Efficiency, P= Phosphorous, N= Nitrogen						

Table.3 Reactor performance of nutrient removal at 2500 mg/L MLSS concentration

Sr. No.	Sample	pH	Total P (mg/L)	Total N (mg/L)	RE of P (%)	RE of N (%)
1	Inlet	7.20	16.62	15.11	-	-
2	Outlet	8.93	15.1	12.65	9.15	16.28
3	Outlet	9.2	14.26	10.24	14.20	32.23
4	Outlet	8.56	12.1	10.22	27.20	32.36
5	Outlet	8.66	12.9	9.27	22.38	38.65
6	Outlet	8.47	13.36	9.44	19.61	37.52
7	Outlet	8.58	13.5	9.34	18.77	38.19
8	Outlet	8.76	14.2	10.02	14.56	33.69

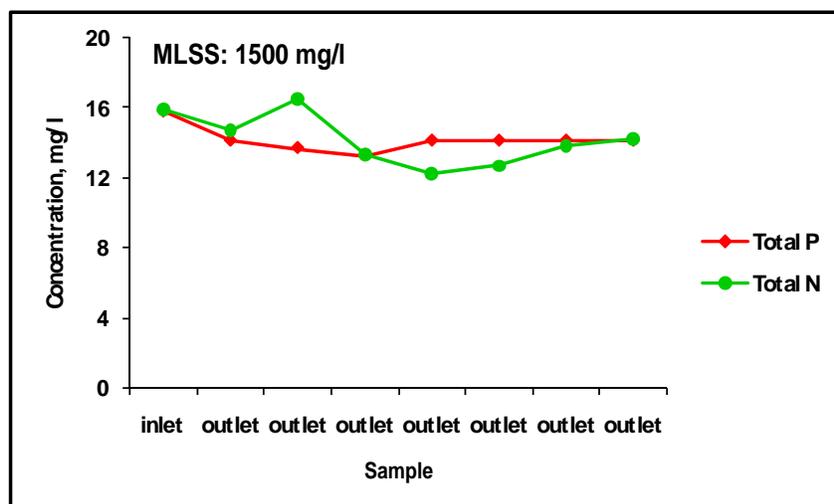
MLSS- 2560mg/l; Chlorophyll- 4.02 mg/l; temperature -30⁰C-32⁰C
RE= Removal Efficiency, P= Phosphorous, N= Nitrogen

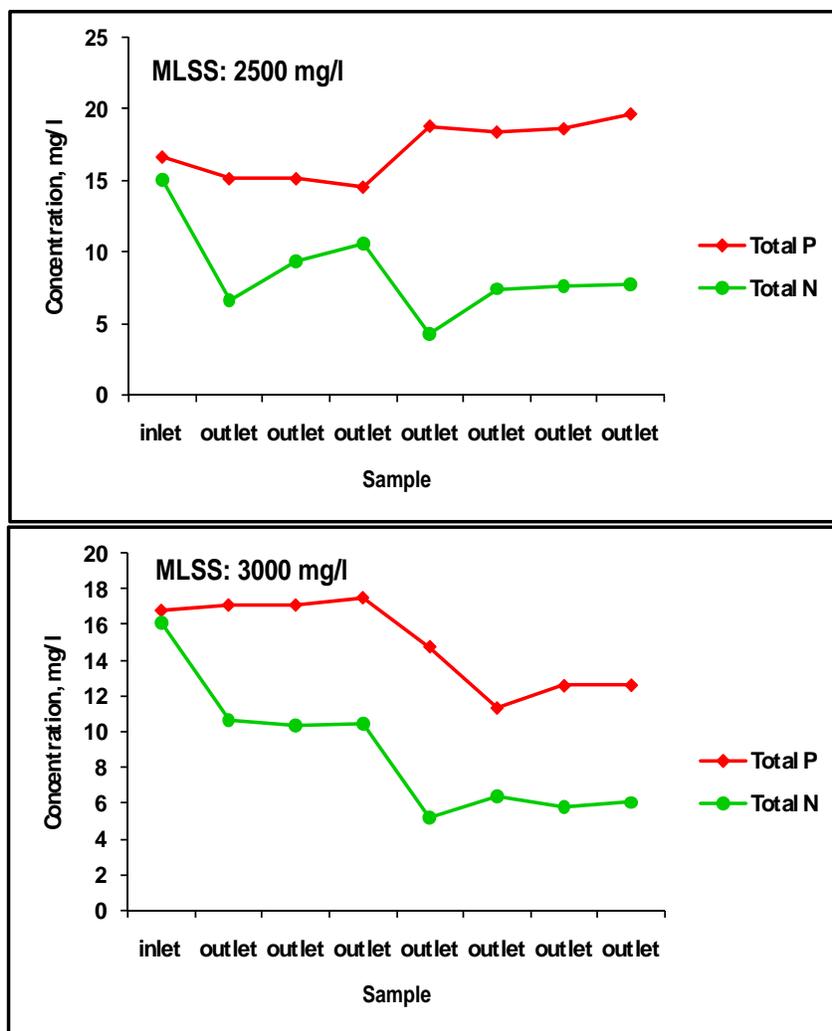
Table.4 Reactor performance of nutrient removal at 3000 mg/L MLSS concentration

Sr. No.	Sample	pH	Total P (mg/L)	Total N (mg/L)	RE of P (%)	RE of N (%)
1	Inlet	7.28	16.8	16.11		
2	Outlet	8.93	16.2	14.62	3.57	9.25
3	Outlet	9.2	16.5	14.52	1.79	9.87
4	Outlet	8.56	15.5	13.83	7.74	14.15
5	Outlet	8.66	15.82	13.26	5.83	17.69
6	Outlet	8.47	11.36	13.78	32.38	14.46
7	Outlet	8.58	12.6	14.12	25.00	12.35
8	Outlet	8.76	12.63	15.2	24.82	5.65

MLSS- 3000mg/l; Chlorophyll- 5.2 mg/l; temperature -30⁰C-32⁰C
RE= Removal Efficiency, P= Phosphorous, N= Nitrogen

Figure.1 Nutrient removal study at various MLSS concentration





HRT optimization

HRT was determined using following equation:

$$HRT \text{ (days)} = \frac{\text{Void Volume (L)}}{\text{Flow Rate (l/d)}}$$

where flow rate was calculated by measuring the feed flow pumped per unit time using peristaltic pump.

Figures 1–3 illustrates the performance of cyanobacterial consortia at different sludge concentration for HRT optimization.

The maximum removal of nutrients i.e. phosphate and nitrogen were observed on

third and fourth day at various MLSS concentration, with the maximum removal being observed at MLSS concentration of 2500 mg/l.

Thus, the batch photobioreactor was operated on 3 days HRT. Anand Prakash *et al.* (1999) have similar findings for the total ammonia removal in a 2 L photobioreactor using batch and semi batch modes of operation. They have used cyanobacterium *Plectonema boryanum* and observed ammonia removal at 100 mg/L and greater. Kwong-Yu-Chan *et al.* (1979) reported nitrogen and phosphorous removal from sewage effluent with high salinity. They have observed 86% to 100% NH₃-N, 98%

NO₃⁻-N, and 98% PO₄³⁻-P removal with 8 days of retention time with the use of controlled *Chlorella salina* CU1 cultures.

In conclusion, Maximum nutrient removal efficiency of 27.20 % of phosphorous and 32.36% of Nitrogen was observed at 2500 mg/L MLSS concentration at 3rd of operation. Therefore, hydraulic retention time was optimized at 3 days with maximum nutrient removal.

Limitations and Recommendations

The present study deals with the nutrient removal efficiency of cyanobacterial consortia. Individual cyanobacterial species may also be tested for its nutrient removal potential. A comparative study of individual and consortia for nutrient uptake may be analyzed in future. The fully grown consortia can be further tested for its metal removal ability under optimized conditions.

References

- Anand Prakash, Argyrios Margaritis, Richard C. Saunders, Sivaraman Vijayan, 1999. High concentrations ammonia removal by the cyanobacterium *Plectonema boryanum* in a photobioreactor system. *Can. J. Chem. Eng.*, 77: 99–106.
- APHA, A. 1992. Standard method for examination of water and wastewater analysis, 18 edn. American Public Health Association, Washington D.C.
- B., C.W. 1952. Methaemoglobinaemia due to nitrates in well water. *Braz. Med. J.*, 2: 371–373.
- Collection, A.T. 1992. ATCC Catalogue of bacteria and bacteriophages, 18 edn.
- Das, S.S. 2008. Characterization of simultaneous bio-accumulation and bio-reduction of hexavalent chromium by an *Aspergillus flavus* isolate (ESTF–241). *J. Mycopathol. Res.*, 46(1): 77–80.
- Dhir, B.A. 2010. Adsorption of heavy metals by salvinia biomass and agricultural residues. *Int. J. Environ. Res.*, 4(3): 427–432.
- Gates, W.A. 1964. Nitrogen and phosphorus extraction from domestic wastewater treatment plant effluents by controlled algal culture. *J. Water Pollut. Control Federat.*, 36: 443–462.
- Hussain, M.A. 2009. Characterization of the adsorption of the lead (ii) by the nonliving biomass *Spirogyra neglecta* (Hasall) Kützing. *Am. J. Biochem. Biotechnol.*, 5(2): 75–83.
- Kwong-Yu-Chan, K.H., Wong, P.K., Wong 1979. Nitrogen and phosphorous removal from sewage effluent with high salinity by *Chlorella salina*. *Environ. Pollut.*, 18: 139–146.
- Low, K.A. 1991. Cadmium uptake by the moss *Calymperes delessertii* Besch. *Bioresource Technol.*, 38: 1–6.
- Luuc, R., Mur, O.M. 1999. Toxic Cyanobacteria in water: A guide to their public health consequences, monitoring and management, Ingrid Chorus, J.B. (Ed.), WHO.
- Rolf Eliassen, G.T. 1969. Removal of nitrogen and phosphorous from waste water. *Environ. Sci. Technol.*, 3(6): 536–541.
- Selatnia, A.B. 2004. Biosorption of Fe³⁺ from aqueous solution by bacterial dead *Streptomyces rimosus* biomass. *Process Biochem.*, 39(11): 1643–1651.