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Phycoremediation of effluent from a soft drink manufacturing industry with a special emphasis on nutrient removal – a laboratory study

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Abstract
Effluent from a soft drink manufacturing industry situated in Ahmedabad, India, was treated with micro algae for reduction of major parameters like total hardness, calcium, magnesium, sulphates, BOD, COD etc. Special emphasis was given for rapid removal of nitrate and phosphate both in the laboratory conditions and out-door conditions. The selected micro algae viz., *Chlorococcum* sp, *Chlorella conglomerata* and *Desmococcus* sp. were able to remove nitrate and phosphate very rapidly both under the laboratory conditions and under the outdoor conditions. These micro algae can be employed at large scale effluent treatment systems for effective remediation.

Key words: *Chlorococcum humicola*, *Chlorella conglomerata*, *Desmococcus olivaceous*, nitrate removal, phosphate removal, industrial effluent, phycoremediation.

Introduction

Phycoremediation is employed to remediate environmental pollution using algae. Olguin (2003) defines phycoremediation involves the use of macro algae or microalgae for effective removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO_2 from waste air. Large-scale phycoremediation of industrial effluent is being implemented successfully in a number of industries in India (Sivasubramanian, 2006; Sivasubramanian *et al.,* 2009; Sivasubramanian, 2010; Sivasubramanian *et al.,* 2010; Hanumantha Rao *et al.*, 2010). Using algae based treatment technology efficient pH correction, sludge reduction and reduction of BOD and COD could be achieved by avoiding toxic chemicals by these industries. During effluent treatment process huge amount of valuable algal biomass is also being generated by these industries. One of the major applications of algae based remediation is the rapid removal of nutrients from industrial effluents (Sivasubramanian, 2011). The present study deals with a successful development of micro algae based treatment technology to remove nitrate and phosphate rapidly from an effluent of soft drink manufacturing industry situated at Ahmedabad, India.

Materials and methods

Effluent

Soft drink manufacturing units in India use ground water for the production. The ground water is filtered and softened using chemicals and sent to R/O and nano filtration (N/F) for further TDS reduction. The effluent generated by these units include R/O reject, reject from N/F , utilities, cleaning, softener regeneration, bottle wash and cleaning in process (CIP). In some units the treated effluent is sent to R/O for recycling. The effluent is treated with conventional chemical and physical methods. Removal of nutrients esp. nitrates and phosphates is the major problem faced by this industry. Data on physic-chemical analysis is presented in Table 1.

Analytical methods:

Effluent parameters were analyzed by employing APHA Standard methods (2000). Growth was measured by counting cells using haemocytometer and using the formula: $\frac{\log N - \log N_0}{\log 2 \, x \, t}$

Nitrate and phosphate analysis

Nitrate Estimation was done by Optical method for nitrate estimation (Collos *et. al*., 1989). Nitrite was estimated by the diazo-coupling method of Strickland and Parsons, (1972). Nitrate was estimated as nitrite after reduction by passage through a Cd-Cu column (Strickland and Parsons, 1972). Phosphate was estimated by the method of Murphy and Riley (1962)..

Table 1 Physico – chemical characteristics of effluent from soft drink industr

Parameters	Raw Effluent
Turbidity NTU	17.8
Total solids mg/L	3960
Total Dissolved Solids (TDS) mg/L	3864
Total suspended solids (TSS) mg/L	96
Electrical conductivity (micro mho/cm)	5496
pH	7.21
Alkalinity pH (as Ca CO_3) mg/L	0
Alkalinity total (as Ca CO_3) mg/L	1296
Total Hardness (as Ca CO_3) mg/L	1220
Calcium (as Ca) mg/L	320
Magnesium (as Mg) mg/L	101
Sodium (as Na) mg/L	690
Potassium (as K) mg/L	20
Iron (as Fe) mg/L	2.91
Manganese (as Mn) mg/L	Nil
Free ammonia (as NH_3) mg/L	21.28
Nitrite (as NO_2) mg/L	Nil
Nitrate (as NO_3) mg/L	21
Chloride (as Cl) mg/L	792
Flouride (as F) mg/L	1.08
Sulphate (as SO_4) mg/L	288
Phosphate (as PO_4) mg/L	8.05
Tidy’s test (as O) mg/L	106
Silica (as SiO_2) mg/L	44.18
BOD mg/L	360
COD mg/L	998
Total Kjeldhal Nitrogen mg/L	22.4
Copper (as Cu) mg/L	0.00321
Zinc (as Zn) mg/L	0.148
Chromium (as Cr) mg/L	0.00236

Laboratory growth conditions

The cultures were grown at 24 ± 1°C in a thermo-statically controlled environmental chamber illuminated with cool white fluorescent lamps (Philips 40w, cool daylight, 6500k) at an intensity of 2000 LUX in a 12/12 h light/dark cycle. Bold basal medium was used for maintaining micro algae (Nichols and Bold, 1965)

Screening of micro algae for remediation

The micro algae have been inoculated into raw effluent of soft drink industry to study the growth rates. 20mL of soft drink industrial effluents was taken in 50mL test tubes. The micro algae *Chlamydomonas pertusa, Chlorella conglamerata, Chlorella vulgaris, Chlorococcum humicolo, Dactylococcopsis raphioides, Desmococcus olivaceus, Oocystis borgei* and *Scenedesmus dimorphus* were used for screening. After an incubation period of one-week, pH, electrical conductivity and cell counts were recorded.

Results:

Growth of various micro algae in soft drink industrial effluent

Chlorococcum humicolo responded very well and showed a rapid maximum growth rate (0.1374 divisions/day). *Chlorella conglamerata* also showed a growth rate of 0.0762 divisions/day. *Chlamydomonas* sp. and *Desmococcus* sp. showed poor growth rate of 0.0159 and 0.0254 divisions/day respectively. Other micro algae namely *Chlorella vulgaris*, *Dactylococcopsis raphioides*, *Oocystis* sp., *Scenedesmus* sp., did not show any growth. The results are given in Table 2. Soft drink industrial untreated effluent, when subjected to phycoremediation there was an increase in pH from 7.6 to 9.4, while the electrical conductivity remained stable till the end of the experimental period. (Table 3).

Table 2 Growth of various micro algae in soft drink industrial effluent

S.No	Micro algae	Growth rate (Divisions/day)
1	*Chlamydomonas pertusa*	0.0159
2	*Chlorella conglamerata*	0.0762
3	*Chlorella vulgaris*	0.0000
4	*Chlorococcum humicolo*	0.1374
5	*Dactylococcopsis raphioides*	0.0000
6	*Desmococcus olivaceus*	0.0254
7	*Oocystis borgei*	0.0000
8	*Scenedesmus dimorphus*	0.0000

Table 3 pH and conductivity changes of soft drink industrial effluent treated with micro algae

S.No	Micro algae	pH		Conductivity (mmhos/cm)		Growth Rate (divisions/day)
		Initial	Final	Initial	Final	
1	*Chlamydomonas pertusa*	7.63	9.34	5.3	5.0	0.0159
2	*Chlorella conglomerata*	7.63	9.4	5.3	5.0	0.0762
3	*Chlorella vulgaris*	7.63	9.27	5.3	5.3	0.0000
4	*Chlorococcum humicolo*	7.64	9.3	5.3	5.0	0.1374
5	*Dactylococcopsis raphioides*	7.65	9.35	5.3	5.0	0.0000
6	*Desmococcus olivaceus*	7.67	9.27	5.2	5.1	0.0254
7	*Oocystis borgei*	7.64	9.35	5.3	5.0	0.0000
8	*Scenedesmus dimorphus*	7.63	9.35	5.3	5.0	0.0000

Studies of nitrate removal by micro algae from soft drink industrial effluents

Micro algae remove nutrients from sewage and wastewater very efficiently and utilize them for their metabolic needs. Micro algae take up nutrients through two mechanisms: 1. Active uptake and 2. Passive diffusion. Active uptake is a carrier mediated energy requiring process. Passive diffusion does not require a carrier or energy. The energy needed for the active uptake is derived from both photosynthetic and respiratory pathways. Micro algae can take up nutrients by employing both the mechanisms simultaneously. Whether active uptake is more or passive diffusion is more depends on physico-chemical and biological factors (Doran and Boyle 1979).

Methodology

Short-term removal of nutrients (nitrate and phosphate) from soft drink industrial effluents was studied employing selected micro algae. The micro algae viz., *Chlorococcum* sp, *Chlorella conglomerata* and *Desmococcus* sp. were selected based on a preliminary screening experiment. They were grown in BBM medium and transferred to BBM medium without phosphate and nitrate and incubated for 3 days to starve the algal cells before using them for a short – term uptake study. The starved micro algae were centrifuged and suspended in the effluent. Two sets of flasks were prepared. One set of flasks containing effluent with suspended algae was incubated in the laboratory condition (2 KLUX light intensity) and the second set was incubated in the direct sunlight (40 KLUX). The flasks incubated in the direct sunlight were kept in a tray of water to prevent the flasks from getting heated. The levels of nitrate (UV method) and phosphate (molybdate method) were recorded at equal intervals (30 min) up to 4 hrs. The cell density of individual species of algae was selected based on their maximum biomass in the laboratory and field conditions. (*Chlorococcum humicola* = 650 X 10^4 cells/ml, *Chlorella conglomerata* = 175 X 10^4 cells/ml and *Desmococcus olivaceus* = 165 X 10^4 cells/ml).

Nitrate removal by micro algae from soft drink industrial effluents

The micro algae took up nitrate rapidly within 30 min of incubation both in the lab and outdoor (76 to 96 % in the lab and 85 to 95 % in outdoor). Although there was no significant difference in the removal percentage in the lab and outdoor conditions, the nitrate removal was slightly better in the outdoor (reaching 90%). The results are given in the Tables 4 and 5.

Table 4 Nitrate removal by micro algae from soft drink industrial effluent: In the lab

S. No	Micro algae	Nitrate level in effluent (mg/L)								
		Time in minutes								
		0	30	60	90	120	150	180	190	240
1	*Chlorococcum humicolo*	60.13	9.53 (84.2)*	9.50 (84.2)	9.00 (85.0)	8.90 (85.2)	8.45 (85.9)	8.45 (85.9)	8.34 (86.1)	8.30 (86.2)
2	*Chlorella conglomerata*	60.13	10.31 (82.9)	9.97 (83.4)	9.17 (84.7)	9.10 (84.9)	9.00 (85.0)	9.00 (85.0)	8.90 (85.2)	8.70 (85.5)
3	*Desmococcus olivaceus*	60.13	14.33 (76.2)	13.09 (78.2)	12.33 (79.5)	12.00 (80.00)	11.87 (80.3)	11.28 (81.2)	11.00 (81.7)	11.00 (81.7)

Table 5 Nitrate removal by micro algae from soft drink industrial effluent: Outdoor

S.No	Micro algae	Nitrate level in effluent (mg/L)								
		Time in minutes								
		0	30	60	90	120	150	180	190	240
1	*Chlorococcum humicolo*	60.13	5.61 (90.7)*	5.60 (90.7)	5.20 (91.4)	5.10 (91.5)	5.00 (91.7)	4.50 (92.5)	2.45 (95.9)	1.48 (97.5)
2	*Chlorella conglomerata*	60.13	9.44 (84.3)	9.40 (84.4)	9.30 (84.5)	9.12 (84.8)	9.00 (85.0)	8.90 (85.2)	8.30 (86.2)	7.56 (87.4)
3	*Desmococcus olivaceus*	60.13	8.99 (85.0)	8.02 (86.7)	8.00 (86.7)	7.80 (87.0)	7.58 (87.4)	7.49 (87.5)	7.45 (87.6)	7.43 (87.6)

*(Values in parenthesis denote percent removal)

Phosphate removal by micro algae from soft drink industrial effluent

The micro algae took up phosphate rapidly within 30 min of incubation both in the lab and outdoor (46 to 57 % in the lab and 43 to 77 % in outdoor). But maximum removal was observed after 1 hr of incubation. Outdoor condition favored a better removal of phosphate. The results are given in the Tables 6 and 7.

Table 6 Phosphate removal by micro algae from soft drink industrial effluent: In the lab

S.No	Micro algae	Phosphate level in effluent (mg/ml)								
		Time in minutes								
		0	30	60	90	120	150	180	190	240
1	*Chlorococcum humicolo*	2.31	1.10 (52.4)*	0.44 (80.9)	0.17 (92.6)	0.14 (93.9)	0.11 (95.2)	0 (100)	0 (100)	0 (100)
2	*Chlorella conglomerata*	2.31	1.09 (52.8)	0.30 (87.0)	0.20 (91.3)	0.14 (93.9)	0.11 (95.2)	0 (100)	0 (100)	0 (100)
3	*Desmococcus olivaceus*	2.31	1.23 (46.8)	0.28 (87.9)	0.17 (92.6)	0.12 (94.8)	0.10 (95.7)	0 (100)	0 (100)	0 (100)

*(Values in parenthesis denote percent removal)

Table 7 Phosphate removal by micro algae from soft drink industrial effluent: Outdoor

S.No	Micro algae	Phosphate level in effluent (mg/ml)								
		Time in minutes								
		0	30	60	90	120	150	180	190	240
1	*Chlorococcum humicolo*	2.31	1.30 (43.7)*	1.30 (43.7)	0.18 (92.2)	0.18 (92.2)	0.13 (94.4)	0 (100)	0 (100)	0 (100)
2	*Chlorella conglomerata*	2.31	1.03 (55.4)	1.02 (55.8)	0.89 (61.5)	0.28 (87.9)	0.20 (91.3)	0 (100)	0 (100)	0 (100)
3	*Desmococcus olivaceus*	2.31	1.25 (45.9)	0.78 (66.2)	0.77 (66.6)	0.40 (82.7)	0.30 (87.0)	0 (100)	0 (100)	0 (100)

*(Values in parenthesis denote percent removal)

The selected micro algae viz., *Chlorococcum* sp, *Chlorella conglomerata* and *Desmococcus* sp. were able to remove nitrate and phosphate very rapidly both under the laboratory conditions and under the outdoor conditions. Phosphate uptake showed positive response to high light conditions. The micro algae were grown in the laboratory conditions (2000 LUX light intensity) and used for this short-term nutrient uptake studies. If the micro algae are grown under direct sunlight (high light adapted) they might exhibit a different response in the outdoor conditions. Micro algae adapt to incident light quality and intensity by changing their biochemistry and physiology. Probably these micro algae, when grown under direct sunlight, may show better nutrient removal efficiency.

Physical-chemical examination of soft drink industrial effluents treated with micro algae

The micro algae *Chlorococcum humicola* and *Chlorella conglomerata* were inoculated in the conical flask with two liters of raw effluent and incubated for 10 days. After 10 days the physicochemical parameters of the effluents were analyzed and tabulated (Tables 8 and 9).

Phycoremediation of soft drink industrial effluents treated with *Chlorococcum humicola*

Chlorococcum humicola was able to reduce BOD and COD by 86.1% and 85.9% respectively. Total hardness was reduced by 70.5%, calcium by 74.7% and magnesium by 64.2%. The other chemical parameters like phosphate, nitrate and sulphate were also reduced to more than 50%. The pH increased from 7.21 to 8.78. The results are given in Table 8.

Phycoremediation of soft drink industrial effluents treated with *Chlorella conglomerata*

Chlorella conglomerata was able to reduce BOD and COD by 77% and 77.7% respectively. Total hardness was reduced by 73.8%, calcium by 77.5%, magnesium by 66.3% and silica by 63%. Free ammonia and nitrate were reduced very appreciably by 89.47% and 81% respectively. The other chemical parameters like phosphate and sulphate were also reduced by 50% and 54.9% respectively. The pH increased from 7.21 to 8.75. The results are given in Table 9.

Table 8 Physico - chemical examination of effluents from soft drink industry treated with *Chlorococcum humicola*

Parameters	Effluent		
	Initial	Final	% reduction
PHYSICAL EXAMINATION			
Turbidity NTU	17.8	40	-
Total solids mg/L	3960	3621	**8.6**
Total Dissolved Solids (TDS) mg/L	3864	3518	**8.9**
Total suspended solids (TSS) mg/L	96	103	-
Electrical conductivity (micro mho/cm)	5496	4872	**10.9**
CHEMICAL EXAMINATION			
pH	7.21	8.78	-

Alkalinity pH (as Ca CO_3) mg/L	0	36	-
Alkalinity total (as Ca CO_3) mg/L	1296	1325	-
Total Hardness (as Ca CO_3) mg/L	1220	360	**70.5**
Calcium (as Ca) mg/L	320	81	**74.7**
Magnesium (as Mg) mg/L	101	36	**64.4**
Sodium (as Na) mg/L	690	690	0
Potassium (as K) mg/L	20	40	-
Iron (as Fe) mg/L	2.91	3	-
Manganese (as Mn) mg/L	Nil	Nil	-
Free ammonia (as NH_3) mg/L	21.28	32.8	-
Nitrite (as NO_2) mg/L	Nil	Nil	-
Nitrate (as NO_3) mg/L	21	8	61.9
Chloride (as Cl) mg/L	792	760	4.0
Flouride (as F) mg/L	1.08	1	-
Sulphate (as SO_4) mg/L	288	121	**58**
Phosphate (as PO_4) mg/L	8.05	3	62
Tidy's test (as O) mg/L	106	13.2	-
Silica (as SiO_2) mg/L	44.18	24	45.7
BOD mg/L	360	50	**86.1**
COD mg/L	998	141	**85.9**
Total Kjeldhal Nitrogen mg/L	22.4	50	-
Copper (as Cu) mg/L	0.00321	0.0003	-
Zinc (as Zn) mg/L	0.148	0.1	-
Chromium (as Cr) mg/L	0.00236	0.0001	-

Table 9 Physico - chemical examination of effluents from soft drink industry treated with *Chlorella conglomerata*

Parameters	**Effluent**		
	Initial	**Final**	**% reduction**
PHYSICAL EXAMINATION			
Turbidity NTU	17.8	24	-
Total solids mg/L	3960	3670	**7.3**
Total Dissolved Solids (TDS) mg/L	3864	3640	**5.8**
Total suspended solids (TSS) mg/L	96	30	68.75
Electrical conductivity (micro mho/cm)	5496	4912	**10.6**
CHEMICAL EXAMINATION			
pH	7.21	8.75	-
Alkalinity pH (as Ca CO_3) mg/L	0	32	-
Alkalinity total (as Ca CO_3) mg/L	1296	1293	0.2
Total Hardness (as Ca CO_3) mg/L	1220	320	**73.8**
Calcium (as Ca) mg/L	320	72	**77.5**
Magnesium (as Mg) mg/L	101	34	**66.3**
Sodium (as Na) mg/L	690	699	-
Potassium (as K) mg/L	20	50	-
Iron (as Fe) mg/L	2.91	2	-

Manganese (as Mn) mg/L	Nil	Nil	-
Free ammonia (as NH_3) mg/L	21.28	2.24	89.47
Nitrite (as NO_2) mg/L	Nil	0.02	-
Nitrate (as NO_3) mg/L	21	4	**81**
Chloride (as Cl) mg/L	792	750	5.3
Flouride (as F) mg/L	1.08	1	-
Sulphate (as SO_4) mg/L	288	130	**54.9**
Phosphate (as PO_4) mg/L	8.05	4	50
Tidy's test (as O) mg/L	106	22	-
Silica (as SiO_2) mg/L	44.18	16.35	**63**
BOD mg/L	360	84	**77**
COD mg/L	998	223	**77.7**
Total Kjeldhal Nitrogen mg/L	22.4	6.62	70.44
Copper (as Cu) mg/L	0.00321	0.00028	-
Zinc (as Zn) mg/L	0.148	0.1	-
Chromium (as Cr) mg/L	0.00236	0.0005	-

Discussion

Microalgae have the capacity to tolerate and take up nutrients from several domestic and industrial wastewaters (Lee, 2001). In general, both nitrogen and phosphorus are the major source of eutrophication, therefore, high concentrations of nitrogen or phosphorus can cause algal bloom other hazardous environmental problems (Shelef *et al.,* 1969). The use of microalgae for nutrients removal from different wastes has been described by number of workers (Benemann *et al.,* 1980; Garett and Fallowfield, 1981; Robinson, 1997; Lukavski, 1986; Mitchel and Richmond, 1987; De la Noüe and De Pawn, 1988; Oswald, 1988; Gantar *et al.,* 1991). It has gained importance in this decade than the old conventional chemical methods which generate secondary pollutants in the environment (Patterson, 1978). Micro algae have been used for removing nitrogen and phosphorus from wastewater and have the potential to be used to remove various pollutants including oxides of nitrogen (NO_x) (Nagase *et al.,* 2001). In the softdrink industrial effluent, the selected micro algae viz., *Chlorococcum* sp, *Chlorella conglomerata* and *Desmococcus* sp. were able to remove nitrate and phosphate very rapidly both under the laboratory conditions and under the outdoor conditions. Phosphate uptake showed positive response to increasing light intensity. These micro algae can be successfully employed at large scale treatment systems for rapid removal of nutrients of industrial effluents and wastewater and the algal biomass produced can be utilized for various applications including bioenergy, biofertilzers, aquaculture feed and as source of valuable biochemicals.

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