	Nature Environment and Pollution Technology An International Quarterly Scientific Journal	Vol. 8	No. 1	pp. 187-192	2009
Orio	ninal Research Paper				

# Phycoremediation of Sewage Using Microalgae Chlorella Sp.

### S. Kalaivani, A. Mahalakshmi Priya, S. S. Sudha\* and S. Balasubramaniyan\*\*

Department of Biotechnology, Dr. N. G. P. Arts and Science College, Coimbatore-641 035, T.N. \*Department of Microbiology, Dr. N. G. P. Arts and Science College, Coimbatore-641 035, T.N. \*\*ITC Ltd. and Paper Board and Speciality Paper Division, Mettupalayam-641 113, T.N.

Key Words: Phycoremediation Municipal sewage *Chlorella* sp. Physico-chemical Microbiological parameters

## ABSTRACT

Phycoremediation is a novel technique that uses algae to cleanup contaminated soil and water. It takes advantage of natural ability of algae to take up, accumulate, and sometimes degrade constituents that are present in their growth environment. The present work deals with the study of remediation of sewage water using Chlorella, which is an alga, isolated from Kalapatti pond, Coimbatore. The remediation study was carried out in the sewage water collected from Thudialur municipal sewage. The physicochemical and microbiological parameters like temperature, pH, turbidity, total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen, organic carbon (OC), chloride (CI), hardness and calcium were analysed in raw sewage as well in treated sewage. Sewage water was diluted to different concentrations as 25%, 50%, 75% and 100%. The inoculum was taken in different concentrations for the study (10%-T1), (20%-T2). The work was carried out for a period of four months and the readings were taken for every fifteen days interval throughout the study. There was a high reduction in all the physico-chemical parameters except DO. The treated sewage was observed to be clear, but there was was no significant reduction in the microbiological parameters. The use of disinfectant after separation of the algae from treated sewage will be a complete treatment. Thus, the green algae Chlorella is effective in reducing the physico-chemical parameters of the sewage. The sewage after the treatment attained the ISO permissible limit. The treated water can be used for irrigation, fisheries, etc. The algae can be reused for further remediation process.

## INTRODUCTION

Environment over the years has become a 'receptacle' for a myriad of chemicals of truly anthropogenic origin, threatening the existence of terrestrial and aquatic biota and causing irreparable damage to ecosystems. There are many expensive methods practiced, hence, the emphasis has been shifted to the concept of 'bioremediation' making effective use of microorganisms and macroorganisms (Matsumura 1989).

Bioremediation is emerging as most ideal alternative and ecologically sound technology for removing pollutants from the environment, restoring contaminated sites and preventing further pollution. Phycoremediation, a branch of bioremediation, is a novel technique that uses algae to clean up contaminated soil and groundwater. It is a process similar to phytoremediation and applied to the removal of nutrients from animal wastewater and other high organic content wastewater with a great potential and demand considering that surface and underground water bodies in several regions of the world are suffering from eutrophication (Kari Dresback 2001). Algal growth rate controls directly and indirectly the nitrogen and phosphorus removal efficiency. Algal strains are used in many

#### S. Kalaivani et al.

areas of research with special attributes such as tolerance to extreme temperature, chemical composition with predominance of high value added products, a quick sedimentation behaviour, or a capacity for growing mixotrophically. An appropriate starting point of the philosophy of 'ecodesign' is bringing together of what is technologically possible and what is ecologically necessary, and the growing demand for a safer, cost efficient method of cleaning up existing contaminated soil and water throughout the world makes phycoremediation a popular and reasonable alternative. Algal species are relatively easy to grow, adapt and manipulate within a laboratory setting, therefore, appear to be an ideal organism for use in remediation studies. The use of such eco-friendly technologies could be used for waste minimization, waste conversion to resource, waste treatment to render the waste less toxic, and for effective waste disposal (Mallick 2002).

Today million of litres of sewage or domestic waste, industrial and agricultural effluents containing different harmful substances are being added every year to various water resources. Sewage is a combination of liquid waste conducted away from residences, institutions and business buildings, wastes from industrial establishments; surface, ground and storm water may also find its way or be admitted into sewers (Ingram 1989). The human quest for material development, seriously threatens the fragile ecosystem. Most of our present day environment sufficiently can be said to originate from man's ecological misbehaviour (Desai et al. 1990).

If the sewage is directly discharged into natural water bodies like rivers, it will create a high pollution and the water sources become hazardous. Thus, sewage has to be properly treated before it is charged into water bodies or on land. The present study deals with the effectiveness of *Chlorella* in the treatment of sewage water.

## MATERIALS AND METHODS

The pond water sample was collected from the surface of the pond located at Kalapatti pond, Coimbatore, from which the algal species were isolated, identified and mass propagated for the sewage treatment. The sewage water samples were collected by keeping the container mouth open against the flow of sewage water at Thudialur municipal sewage outlet, Coimbatore. The physical, chemical and microbiological parameters like temperature, pH, turbidity, total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen, organic carbon (OC), chloride (Cl), hardness and calcium were analysed in the raw sewage and the treated sewage following the standard methods (APHA 1998). Different dilutions of sewage sample such as 25%, 50%, 75% and 100% were studied and the test inoculum was taken in two different concentrations as 10%-T1 (Treatment 1) and 20%-T2 (Treatment 2).

## **RESULTS AND DISCUSSION**

The data on the physico-chemical and bacteriological analysis of raw sewage and sewage treated with the alga *Chlorella* are given in Table 1. The characteristics were examined in the light of IS: 2296:1982 standards prescribed by IS permissible limit for inland surface water and for irrigation purposes. The IS standards are given in Table 2.

The treated sewage, both in T1 and T2, was odourless and clear at the end of the treatment. The effect of temperature controls the chemical reactions; solubility of substances, chemical and biological reactions of the organisms in water and the growth of nuisance organism are enhanced by warm water condition and could lead to the development of unpleasant taste and odour (Sacramento 1963,

Mechalas et al. 1972). Temperature ranged from 29.8°C to 32.8°C. There was no significant difference in temperature of the treated sewage water. In natural water, pH changes diurnally and seasonally due to variation in photosynthetic activity, which increases the pH due to consumption of  $CO_2$  in the process. The microbiological integrity of water also depends upon its pH value (Bouwer 1978, Yadav et al. 1987). pH values ranged from 6.6 to 8.8. At the end of the treatment the pH was found to be within the permissible limit (IS 2296:1982) and reached neutral. The treated sewage showed effective reduction in turbidity. The values were 33 NTU and 78 NTU in T1 and T2. Percentage reduction was maximum in T2 (73.75 %) (Fig. 1). According to Rao et al. (1979), solids in water samples vary directly with the alkaline nature. The total solids comprising of TSS and TDS showed reduction only to certain extent but are within the permissible limit (IS 2296:1982). The minimum values of TSS and TDS in T1 and T2 were 452 mg/L and 425mg/L respectively with percentage reduction of about 48.4% in T2 and 46.2% in T1 (Fig. 2).

Maintenance and distribution of biota in aquatic ecosystems depends upon DO concentrations to a great extent. High dissolved oxygen content is an indication of healthy system (Bilgrami & Datta Munshi 1979, Sreenivasan 1970, Jindal & Vashist 1985). In raw sewage DO was nil but at the end of the treatment in both T1 and T2 it showed high increase to 6.25mg/L and 6.9 mg/L respectively (Fig 3).

BOD test is useful in evaluating the self-purification capacity of streams, which serves as a measure to assess the quantity of wastewater, which can be safely assimilated by a stream (Trivedy & Goel 1984). According to Lens et al. (1995) COD values do not differentiate stable and unstable organic pollution, therefore, it is more than BOD values. There was gradual reduction in BOD and COD (156.1mg/L and 252.3mg/L) respectively. The maximum percentage reduction was about 60% in T2 of BOD and 64.5% in T2 of COD (Figs. 4 and 5). Initially the chloride content and hardness in sewage water was high. The chloride content in all types of water is due to the excretion of very high

Parameters	Initial	June			July				August		September						
	value	2nd We	eek	4 <sup>th</sup> we	eek	2nd we	eek	4 <sup>th</sup> we	eek	2nd we	eek	4 <sup>th</sup> we	eek	$2^{nd} w$	eek	$4^{th}w$	eek
		S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D
Temperature (°C)	32.8	32.6	32.5	32.4	32.1	31.8	31.7	32.1	32.3	31.7	31.8	30.8	30.6	29.8	29.7	30.2	30.8
pH	8.75	8.8	8.7	8.6	8.5	8.4	7.9	7.2	7.1	6.9	6.8	6.5	6.6	7.5	7.8	7.1	7.2
Turbidity (NTU)	80	78	76	71	69	67	60	59	60	54	56	49	38	42	30	33	21
TSS (mg/L)	352	349	332	328	296	298	271	273	247	254	218	226	212	216	201	210	196
TDS (mg/L)	489	479	458	458	432	410	389	368	348	326	301	295	272	269	243	242	229
TS (mg/L)	841	828	790	786	728	708	660	641	595	580	519	521	484	485	444	452	425
DO (mg/L)	0	0.9	0.92	1.3	1.25	1.6	1.8	2.1	2.5	2.6	2.9	3.5	4.8	4.7	5.9	6.25	6.9
BOD (mg/L)	426.5	418.2	410.6	396.4	384.1	352.6	348.6	321.6	293.1	283.1	258.2	248.3	218.3	219.3	189.4	188.8	156.1
COD(mg/L)	712.3	708.1	696.2	689.2	641.6	646.4	569.6	598.4	493.1	596.8	548.2	548.4	483.6	496.1	308.1	403.2	252.3
Cl (mg/L)	70.2	68.9	66.4	67.8	62.4	66.4	56.2	63.7	51.8	59.2	43.4	51.6	38.1	48.7	30.6	34.8	25.2
Hardness (mg/L)	251.8	248.1	241.6	234.6	228.9	228.4	213.4	192.7	169.8	181.3	146.1	173.8	128.2	167.4	111.6	133.4	92
Total N (mg/L)	68.1	65.6	61.2	60.4	55.1	56.0	49.6	52.4	42.4	47.1	36.1	43.8	31.9	39.6	24.8	31.4	16.2
Org. carbon (mg/L)	14.7	13.2	13.0	11.2	12.5	9.8	9.0	8.6	8.8	8.9	7.2	8.4	6.1	8.2	5.8	9.7	4.9
Calcium (mg/L)	76	73.4	71.4	68.1	67.8	62.8	60.9	59.6	52.1	51.8	45.7	48.1	38.2	44.6	32.6	41.4	29.3
$SPC \times 10^{5}/mL$	108	112	110	105	98	95	86	89	81	82	79	76	67	72	62	69	58
MPN of colifroms																	
$\times 10^{4}/100 mL$	275	278	276	274	270	270	261	268	253	258	241	249	229	234	213	221	196
MPN of faecal coli-																	
$forms \times 10^4 / 100 mL$	228	227	226	220	218	216	182	204	176	198	161	183	154	172	148	169	132

Table 1: Physico-chemical and microbiological parametres of sewage treated with test organism *Chlorella*. S-Single inoculum, D-double inoculum.

Nature Environment and Pollution Technology 

Vol. 8, No. 1, 2009

#### S. Kalaivani et al.

Table 2: Tolerance limits for discharge in inland surface waters and for irrigation purposes (IS: 2296-1982).

Sl.No	Characteristic	Tolerance limit for inland surface waters	Tolerance limit irrigation purposes					
1	Temperature°C	Shall not exceed 5°C above						
		the receiving water temperature	-					
2	pH value	6.5-8.5	5.5-9.0					
3	Total dissolved solids, mg/L Max.	1500	2100					
4	Total suspended solids, mg/L	100	200					
6	Dissolved oxygen, mg/L, Min.	4	-					
7	Biochemical oxygen demand	3	100					
	(5 days at 20°C), mg/L, Max.							
8	Chemical oxygen demand, mg/L, max.	250	-					
9	Chlorides (as Cl), mg/L, Max.	1000	600					
10	Total Kjeldahl nitrogen (as N);	100	-					
	mg/L, max.							
11	Total hardness mg/L, max.	600	-					
12	Calcium~(as Ca) mg/L, Max.	200	-					
13	Total coliform organisms,	5000	-					
	MPN/100 mL, Max.							



Fig. 1: % reduction of turbidity using the test organism *Chlorella* sp.



Fig. 2: Percentage reduction of TS using test organism *Chlorella* sp.







Fig. 4: Percentage reduction of BOD using the test organism *Chlorella* sp.



Fig. 5: Percentage reduction of COD using the test organism *Chlorella* sp.



Fig. 6: Percentage reduction of chloride using the test organism *Chlorella* sp.

#### Vol. 8, No. 1, 2009 • Nature Environment and Pollution Technology

190



Fig. 7: Percentage reduction of hardness using the test organism *Chlorella sp.* 



Fig. 8: Percentage reduction of organic carbon using the test organism *Chlorella sp.* 



Fig. 9: Percentage reduction of total nitrogen using the test organism *Chlorella sp.* 



Fig.10: Percentage reduction of calcium using the test organism *Chlorella sp.* 



Fig. 11: Percentage reduction of SPC×10<sup>5</sup>/mL using the test organism *Chlorella sp.* 



Fig. 12: Percentage reduction of MPN total coliform×10<sup>5</sup>/mL using the test organism *Chlorella sp.* 



Fig. 13: Percentage reduction of MPN faecal coliform  $\times \, 10^5 / mL$  using the test organism Chlorella sp.

Nature Environment and Pollution Technology 

Vol. 8, No. 1, 2009

S. Kalaivani et al.

quantities of chloride together with nitrogenous compounds by man and other animals (Gondave 1985, Daborn 1976). In the treated sewage the minimum and maximum values of chloride ranged from 25.2mg/L to 68.9mg/L, and the hardness from 92.0mg/L to 248.1mg/L (Figs. 6 & 7). The percentage reduction of organic carbon, total nitrogen and calcium was maximum in T2 (66.6%, 76.2% and 61.4%) respectively, which attained permissible limit values (Figs. 8-10). There was only minor reduction in the microbiological parameters. Maximum percentage reductions of SPC, MPN of total coliforms and MPN of faecal coliforms in T1 and T2 were 36.1% and 46.2%, 19.6% and 28.7%, and 25.8 and 42.1% respectively (Figs. 11-13).

#### REFERENCES

- APHA 1998. Standard Methods for Examination of Water and Wastewater, 16th edn. American Public Health Association, Washington DC.
- Bilgrami, K.S. and Datta Munshi, J.S. 1979. Limnological Survey and Impact of Human Activities on the River Ganges (Barauni to Farakka range). A Technical Report. Post-Graduate Deptt. of Botany, Bhagalpur University, Bhagalpur. Bouwer, H. 1978. Ground-Water Hydrology. McGraw Hill Inc., Tokyo and Japan.
- Daborn, G.R. 1976. Physical and chemical factors of vernal temporary pond in western Canada. Hydrobiologia, S1(1): 33-38. Desai, P.V., Godase, S.J. and Halkar, S.G. 1990. Physico-chemical characteristics of Ambazari lake water. Environmental

Health, 6: 186-188. Gondave, E.S. 1985. Supply of chloride in groundwater, 2<sup>nd</sup> World Congress on Engg. and Environment. 7-9, New Delhi. Ingram, W.J. 1989. In: Encyclopedia of Environmental Science, McGraw Hill Publication, II ed., 663-665.

Jindal, R. and Vasisht, H.S. 1985. Ecology of freshwater pond at Nabha Punjab (India). Zoological Orientals, 18: 74-85. Kari Dresback 2001. Phycoremediation of trichloroethylene (TCE). Physiol. Mol. Biol. Plants, 7(2): 117-123.

- Lens, P.N., Poorter, M.P., Croneberg, C.C. and Vestraete, W.H. 1995. Sulphate reducing and methane producing bacteria in aerobic wastewater treatment systems. Wat. Res., 29(3): 871-880.
- Mallick, N. 2002. Biotechnological potential of immobilized algae for metal removal: A review. Bio. Metals, 15(4): 377-390.
  Matsumura, F. 1989. Biotic Degradation of Pollutants. Ecotoxicology and Climate. Published by John Wiley & Sons Ltd., 79-89.

Mechalas, B.J., Hekimian, K.K., Schinazi, L.A. and Dudley, R.H. 1972. An investigation into recreational water quality. Water Quality Data Book, 4, US Environmental Protection Agency.

- Rao, S.S., Kwiatkowski, R.E. and Turkovie 1979. Distribution of bacteria and chlorophyll a at a near shore station in lake Ontario. Hydrobiologia, 66: 33-39.
- Sacramento, C.A. 1963. Water Quality Criteria, 2nd ed. California State Water Quality Control Board, 263.
- Sreenivasan, A. 1970. Limnology of tropical impoundments, a comparative study of the major reservoirs in Madras state (India). Hydrobiologia, 36(3-4): 443-469.
- Trivedy, R.K. and Goel, P.K. 1984. Chemical and Biological Methods for Water Pollution Studies. Environmental Publication, Karad: 1-251.
- Yadav, Y.S., Singh, R.K., Choudry, M. and Kolekar 1987. Limnology and productivity of Dighali bheel (Assam) India. Trop. Ecol., 28(2): 137-147.