

## **Bioremediation of Sewage Wastewater Using Selective Algae for Manure Production**

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### **Abstract**

Phycoremediation is the process of employing algae for removing excess nutrient load from wastewater and subsequently diminish the pollution load. It is an alternative technology of treating sewage wastewater compare to conventional treatment process in economical and sustainable way. Therefore, in the present investigation we had made an effort to phycoremediate the IARI's sewage wastewater with different microalgae viz. *Chlorella minutissima*, *Scenedesmus* spp & BGA (*Nostoc*) and their consortium. Results showed that these algae were very effective in reduction of BOD<sub>5</sub>, COD, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup> and TDS in sewage wastewater. Further, it has been observed that *Chlorella* was having best phycoremediation potential as well as manure production among all three microalgae and even better than consortium. Among the potential uses of algal biomass from such systems is its use as a slow release fertilizer. After 20 days microalgae were harvested using muslin cloth and fresh and dry weigh were determined. The maximum biomass was observed in *Scenedesmus* spp and *Chlorella minutissima* while percentage of nitrogen and phosphorus was highest in *Chlorella minutissima*. So we could conclude that *Chlorella minutissima* has the best manurial potential. The results of this study suggest that growing algae in nutrient-rich sewage wastewater offers a new option of applying algae to manage the nutrient load and after phycoremediation the biomass itself can be utilized for manure application in agriculture, serving the dual roles of nutrient reduction and valuable manure feedstock production.

**Keywords:** Phycoremediation, Microalgae, Wastewater, Manure.

## 1. Introduction

Wastewater treatment in Waste Stabilization Ponds (WSPs) is "green treatment" achieved by the mutualistic growth of microalgae and heterotrophic bacteria. The algae produce oxygen from water as a by-product of photosynthesis. This oxygen is used by the bacteria as they aerobically bio-oxidize the organic compounds in the wastewater. An end-product of this bio-oxidation is carbon dioxide, which is fixed into cell carbon by the algae during photosynthesis. Worldwide, there is a continuous interest in algal-based waste stabilization pond systems that are inexpensive and are known for their ability to achieve good removal of pathogens and organic pollutants (Zimmoet *al.*, 2002). Cyanobacteria and microalgae play an important role in the system since they supply molecular oxygen to heterotrophic partners and thus support the initial steps of degradation (Cerniglia, 1992). Nutrient removal with aid of algae compares favorably with other conventional technologies (De la Noue *et al.*, 1992; Raghukumaret *al.*, 2001; Muthukumaran *et al.*, 2005). It also found, some cyanobacteria and algae might remove xenobiotics from the environment by sorption, transformation and degradation (Olguín, 2003). Several attempts have been made to explore the efficiency of microalgae for metal removal (Wilde and Benemann, 1993; Zayed and Terry, 2003; Chu and Hashim, 2004).

Dry microalgae as soil additives improve plant nutrient, which in turn enhances all the physiological reactions that lead to a good growth. A quantity of 2 and 3 g dry algae per kg soil can improve soil fertility, plant nutrient required for obtaining good yields and leads to less environmental pollution (Fayza A., Faheed and Zeinababd-el Fattah, 2008). The large amount of algal sludge represents a potential source of fuel and recovered N and P fertilizer (Mulbry *et al.*, 2005).

The aim of the present work was to evaluate phycoremediation potential in the short term, using semi continuous cultures of different microalga and their consortia. Further the biomass was harvested and characterized for the nutrient potential.

## 2. Methods

Microalgal strain *Chlorella minutissima*, BGA (*Nostoc*) & *Scenedesmus* spp was obtained and cultured at National Centre for BGA, IARI (New Delhi, India). Treatments were as follows:

- To: Control
- T1: Waste water having *Chlorella minutissima*
- T2: Waste water having *Scenedesmus* spp
- T3: Waste water having BGA (*Nostoc*)
- T4: Waste water having Consortium

The samples of IARI (primary treated) wastewater were collected in October 2012 and January 2013. Samples were analysed within 2 days of sampling at laboratory of Centre for Environment Science and Climate Resilient Agriculture (CESCRA), IARI New Delhi. This was characterized for physico-chemical characteristics. Microalgae

had grown on sewage water in WSPs under controlled conditions. Response of microalgae on the waste was measured by analyzing the growth by taking optical density of waste water at different times. The accumulation of free nutrients, salts and other chemical elements by microalgae was done by characterizing the residual wastewater. The difference between the first and second depiction gave the estimate of phycoremediation by microalgae. The biomass was harvested and characterized for the nutrient potential. On 20<sup>th</sup> day of incubation algal biomass was harvested by muslin cloth 2-3 times followed by centrifugation and processed for manure production. The fresh weight and dry weight of different algal biomass is given in (Table 3.1) while total chlorophyll growth rate were analyzed at a 5 days interval shown in (Table 3.2). Harvested algal biomass was analyzed for Nitrogen (Kjelplus micro kjeldhal apparatus), phosphorus (spectrophotometer at 420 nm) and potassium (Flame photometer) for manure value.

**Table 3.1:** Biomass content in algae after harvesting.

Parameters	Chlorella	Scenedesmus	Nostoc	Consortium	C.D.
Fresh wt(g/l)	1.12±0.02	1.33±0.03	0.89±0.02	1.05±0.03	0.03
Dry wt (g/l)	0.78±0.01	0.79±0.02	0.66±0.01	0.68±0.02	0.05

**Table 3.2:** Total Chlorophyll ( $\mu\text{g/ml}$ ).

Treatment	Initial	5th day	10th day	15th day	20th day
Chlorella	6.19	8.47	11.44	9.64	7.51
Scenedesmus	5.47	9.45	17.62	10.83	8.63
Nostoc	3.09	3.18	5.35	3.45	2.42
Consortium	5.56	6.66	10.66	7.10	6.05

### 3. Result and Discussion

Preliminary analysis of the water sample revealed the pH of the water samples 8.01, presence of 3.68 ppm phosphorus, 2.38 ppm nitrates, 39.5 ppm  $\text{NH}_4^+\text{-N}$  and TDS in the range of 1700 -2500 mg/l dissolved Oxygen of the water sample was in range of 1.8 to 4.3 mg/l (Table 3.3). Change in the water quality after treatment with algae was checked in an interval of 5 days for a period of 20 days. pH of the water sample treated with microalgae showed a drift towards alkalinity. pH has been changed from 8.01 to 9.32. pH was drifted from 8.01 to 8.82 in the wastewater sample treated with *Chlorella minutissima*. pH has been changed from 8.01 to 9.09 in the wastewater sample treated with *Scenedesmus*. (Fig 3.1)

The amount of phosphorus in the water sample treated with *Chlorella minutissima* reduced from 4.47 ppm to 1.15 ppm (fig 3.1). There was 87.63% reduction in the

phosphate content in the water sample treated with consortium. The concentration of TDS in the wastewater sample treated with *Chlorella minutissima* has been decreased from 2196 to 82 ppm (fig 3.1). The reduction in TDS was due to consumption of dissolved solid from wastewater which was nutrient rich for the growth of microalgae. It was observed that maximum TDS reduction found in *Chlorella minutissima* 97.57% followed by consortium 96.27% (fig 3.2). 3.6 ppm of nitrate had been reduced to 0.3 ppm in the water sample treated with *Chlorella minutissima*. (fig 3.1). 91.49% reduction in the nitrate content has been noted. 45.68% decrease in the nitrate content has been observed in the wastewater sample treated with BGA (fig 3.2). Reduction in nitrate comparatively lesser in wastewater treated with BGA, might be due to nitrification process. More than 90%  $\text{NH}_4^+\text{-N}$  in the wastewater sample had been absorbed by the microalgae (fig 3.2). We knew that algae prefer to assimilate N in the form of ammonia because it is a passive way of assimilation and energetically less expensive than uptake of nitrate.

The present investigation endow with the substantiation for efficient removal rate of TDS, BOD, COD, nitrogen, and phosphorus by green alga *Chlorella minutissima*, *Scenedesmus spp.*, & BGA (*Nostoc muscorum*), without an adverse effect on the growth rate. In the present investigation *Chlorella minutissima* was found to remove about 97% TDS, 90% nitrogen, 70% phosphorus and 95% BOD and 90 % COD from the wastewater. The growth of the all the three microalgae was best up to the 10-12 days after that its start decreasing as the nutrients in the wastewater were consumed by microalgae. These results have also shown that the removal of ammonical nitrogen is preferred over that of the nitrate nitrogen. The residual  $\text{NH}_4^+\text{-N}$  concentration vanished fast as compare to nitrate. The depletion of ammonium from the synthetic wastewater (94% and 96% by *C. vulgaris* and *S. rubescens*, respectively, within 9 days) was comparable to studies conducted by Martínez et al. (2000), who described elimination of ammonia (between 79% and 100%) after 188.25 h (about 8 days), and González et al. (1997), who reported ammonium removal efficiencies of 90% from agro-industrial wastewater after 216 h (9 days). There are obvious advantages of eliminating ammonium from wastewater using microalgae: (1) it does not generate secondary pollution by generation of  $\text{NH}_3$  and (2) the microalgal biomass can be harvested and used as a slow-release fertilizer or soil conditioner (de la Noüe et al. 1992; Mallick 2002; Mulbry et al. 2005). In order to give the phosphate industry and agriculture a sustainable future, it has been advocated that phosphate should be recycled. Here, nearly 75 to 90 %  $\text{PO}_4^{3-}\text{P}$  removal was achieved in sewage wastewater while doing phycoremediation with these microalgae.

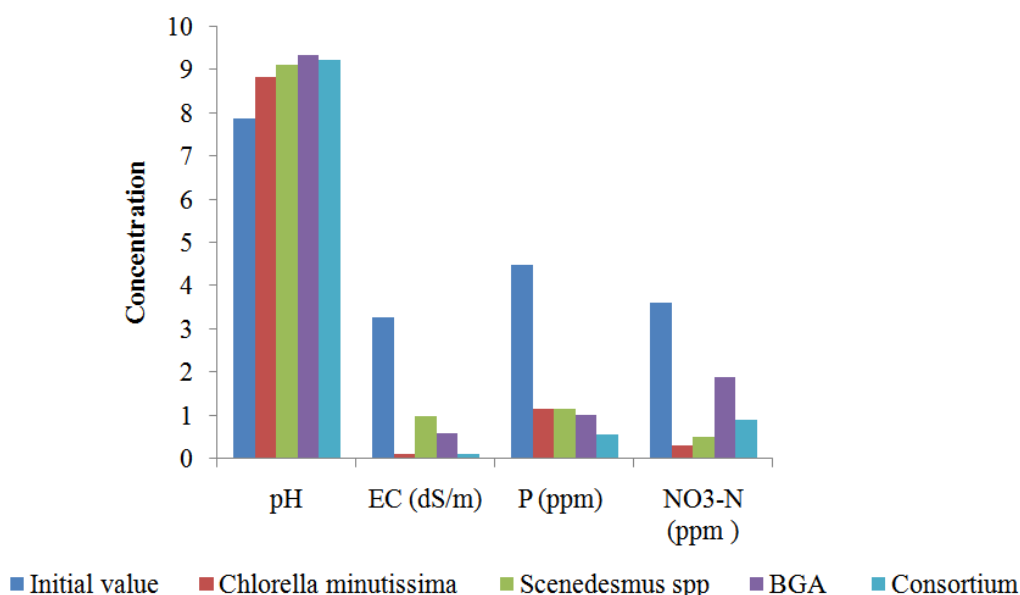
Travieso et al. (1996) reported that increasing the light intensity can lead to a higher microalgal activity and an increased removal of nutrients from wastewater. In the present study, the wastewater treatment was conducted in WSPs under natural illumination. It should be noted that in WSPs, due to the climatic condition and better sunshine condition in the month of October light is absorbed much more efficiently by microalgae than in the month of January. Hence, the experiment executed in October

has given 20% better results in terms of phycoremediation as well as biomass production.

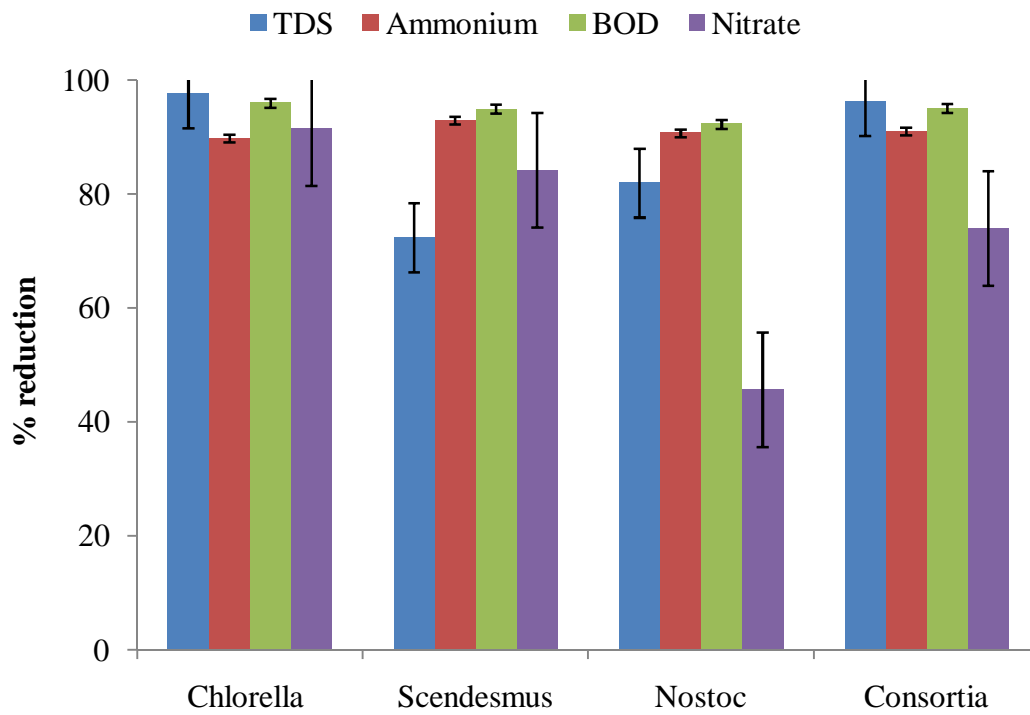
In this study, the resulting dry algal biomass typically contained 2.2 to 3.5% N, 0.3 to 1% P and about 0.3% K (fig 3.3). Moreover, algal N would have much less potential for leaching or for loss in run-off since only about 5% of the algal N would be available as mineral N at the time of application. This benefit may allow algal biomass to be side-dressed into established crops. The results of this study suggest that growing algae in nutrient-rich sewage wastewater offers a new option of applying algae to manage the nutrient load and after phycoremediation the biomass itself can be utilized for manure application in agriculture, serving the dual roles of nutrient reduction and valuable manure production

**Table 3.3:** Physico-chemical composition of sewage wastewater of IARI.

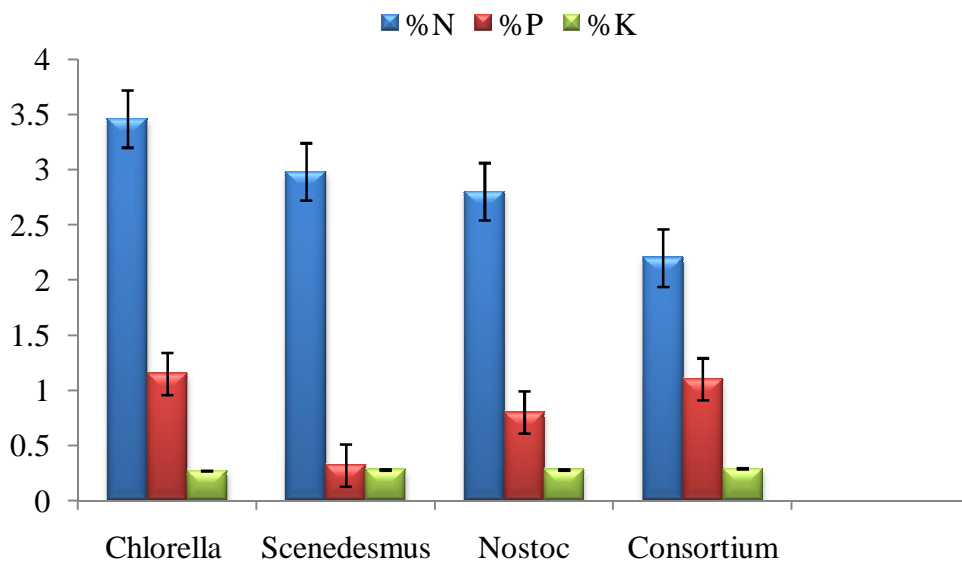
S. No.	Parameters	Range	Average ± Stand. dev.
1	pH	7.8-8.2	8.01±0.175
2	TDS (ppm)	1700 - 2500	2107±356.90
3	Phosphorus (ppm)	2.5-4.43	3.68±0.83
4	NH4+-N (ppm)	25-50	39.5±10.8
5	NO3-N (ppm)	0.7-4.8	2.38±1.97
6	DO (mg/l)	1.8-4.3	2.6±1.15
7	BOD5 (mg/l)	78-125	99.5±21.8
8	COD (mg/l)	117-187.5	149.75±32.78



**Fig 3.1:** Initial and final values.



**Fig 3.2:** Percentage reduction.



**Fig 3.4:** Percent NPK content in harvested algae biomass.

#### 4. Conclusion

Stimulating the natural process of phycoremediation offers an opportunity for reducing the environmental impact of various pollutants. This forms an effective and economic biological treatment of polluted waters. The intimate association which the algae have with the aquatic habitat makes them an interesting tool for such studies. The three microalgae employed were found efficient in phycoremediation. *Chlorella minutissima*, *Scenedesmus spp* & BGA (*Nostoc*) grow well in polluted habitats and can be used for phycoremediation purposes. The removal efficiency *Chlorella* was very high. It shows that *Chlorella minutissima* is more efficient than *Scenedesmus spp* and *Nostoc muscorum* for phycoremediation purposes. Moreover, algal N would have much less potential for leaching or for loss in run-off since only about 5% of the algal N would be available as mineral N at the time of application. This benefit may allow algal biomass to be side-dressed into established crops.

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