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Phytoremediation of domestic waste water of Gandhi Nagar, Vellore using microalgae *Chlorella vulgaris*

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Abstract
The accumulation of wastes of domestic and industrial processes in the nearby water bodies results in water pollution. The wastewater discharged into the water bodies are hazardous to environment and cause various health problems in human beings. Eutrophication is one such major environmental problem caused due to the discharge of nutrient rich wastewater into the nearby water bodies. Excessive pollutants including nutrients affect aquatic lives and environment in various ways. There are certain plants capable of removing pollutants from water. Phytoremediation is an alternate way to reduce nutrients from contaminated medium. Microalgae can be used for phytoremediation to reduce the nutrient content in the wastewater due to the algae's ability to assimilate nutrients into the cells. The microalga *Chlorella vulgaris* can utilize the nitrogen and phosphorus in wastewater for its growth. Hence in the present study, microalga *Chlorella vulgaris* was used to determine the removal efficiencies of pollutants, such as chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP). The *Chlorella vulgaris* was cultured in the shake flasks that contained wastewater in the presence of artificial light in the laboratory. It removes the maximum percentage of TN and TP were within 82.1% and 90.9%, respectively. The *Chlorella vulgaris* which could not only bioremediate the wastewater, but also produce plenty of the microalga biomass that could be used for the exploitation of fertilizers, feed additives and biofuels. The optimum detention period for the maximum phytoremediation is found to vary within 10 and 14 days. Based on the laboratory scale study under controlled environment, it can be concluded that *Chlorella vulgaris* has the potential to reduce nutrient content of wastewater.

Keywords: Domestic waste water, phytoremediation, *Chlorella vulgaris*

Introduction
Biological treatment of nitrate and phosphorus in wastewater have been widely studied, with various organisms namely bacteria, fungi, protozoa and microalgae identified as potent agents for their uptake. The concept of utilizing microalgae for the removal of high concentrations of nutrients in wastewater (Phytoremediation) has been development for decades (Oswald *et al*. 1957, de la Noue *et al*. 1992) [17, 7]. Phytoremediation studies have repeatedly shown high efficiencies (70-99.8%) of nitrogen, phosphorus and heavy metal removal (Oswald 1988, Muttamara *et al*. 1995, Hoffmann 1998, Chinnasamy *et al*. 2010, Wang *et al*. 2010) [18, 14, 12, 6, 23]. The algal process is an effective and low technology process which offers inherent cost savings and provides a more appropriate method of water treatment for developing countries (Pittman *et al*. 2011) [20]. One of the major advantages of algal processes over conventional treatment is the ability to recycle the nutrients forming high value products, such fertilizers, pharmaceutical products, food additives and biofuels (Benemann *et al*. 1977, Mata *et al*. 2010, Rawat *et al*. 2011) [5, 13, 22].
Alage are photosynthetic, pigment-producing, protein-rich microorganisms especially play vital role for treating wastewater treatment systems for their unique ability to generate their own carbon source and oxygen, greater visibility that aids growth monitoring, and high commercial value. These traits excellently complement their notable capacity in nitrogen and phosphorus uptake for synthesis of cellular proteins and other essential biomolecules. Microalgae such as *Chlorella* sp. and *Scenedesmus* sp. are commonly sighted at treatment tanks, especially in warmer climates, naturally colonizing wastewater postsecondary treatment at high rate and possessing high nutrient removal capabilities.

In Hong Kong, investigated the feasibility of cultivating *Chlorella pyrenoidosa* in primary settled sewage for inorganic nutrient removal, and up to 80% of nitrogen was removed from the settled sewage, whereas phosphorus content was reduced to 1-2 mg/L following a week of culture.

Thus, the use of microalgae for removal of nutrients from different wastes has been described by a number of authors (Beneman *et al*., 1980; De-Bashan *et al*., 2002; Gantar *et al*., 1991; Queiroz *et al*., 2007) [3, 8, 11, 21]. Microalgae have been used for removing nitrogen and phosphorus from wastewater (Oswald, 1988) [17] and have the potential to be used to remove various pollutants, including oxides of nitrogen (NOx) (Nagase *et al*., 2001) [15]. Similarly, degradation of complex organic carbon substrates in tannery wastewater has been attempted in high-rate algal ponding systems (Dunn, 1998) [10]. Microalgae assimilate a significant amount of nutrients because they require high amounts of nitrogen and phosphorus for the synthesis of proteins (45-60% of micro algal dry weight), nucleic acids and phospholipids. Algal species are relatively easy to grow, adapt and manipulate within a laboratory setting and appear to be ideal organisms for use in remediation studies (Dresback *et al*., 2001) [9]. In addition, Phytoremediation has advantages over other conventional physico-chemical methods, such as ion-exchange, reverse osmosis, dialysis and electro-dialysis, membrane separation, activated carbon adsorption, and chemical reduction or oxidation, due to its better nutrient removal efficiency and the low cost of its implementation and maintenance. In the present study, the green micro alga, *Chlorella vulgaris*, was used to phytoremediate the domestic sewage water of Gandhinagar, Vellore in the laboratory condition.

Materials and methods

Isolation and culturing of microalgae

Chlorella vulgaris was isolated from the standing pond water using serial dilution, standard plating, colony isolation and culture techniques. The monograph of Philipose (1967) [19] was used for the identification of the microalga. An axenic culture of *C. vulgaris* was maintained in Bold's basal medium (BBM) (Nichols and Bold, 1965) [16] at 24±1°C in a thermostatically controlled environmental chamber illuminated with cool white fluorescent lamps (Philips 40 W, cool daylight, 6 500 K) at an intensity of 2 000 lux in a 12/12 h light/dark cycle.

Laboratory trials

The exponential phase cells of *C. vulgaris* (growth determined by cell count method using a haemocytometer) were centrifuged and the washed pellet was re-suspended in 2 ℓ of the diluted effluent (5 times with BBM) in a conical flask bioreactor and incubated in the above-mentioned conditions for 7 days.

The Domestic waste water sample was analysed for physico-chemical parameters before and after treatment according to the method of APHA (2000) [1]. In the treated effluent, algal cells were separated by centrifugation before analysis.

Statistical analysis

Values of all data are expressed as mean ± SD. The one-tailed paired Student's *t*-test was used to determine statistical significance between the untreated and treated parameters at $P<0.05$. All analyses were carried out in triplicate.

Results

Phytoremediation of domestic sewage – laboratory study

The results of the laboratory study are presented in Table 1, after multiplying the values by the dilution factor. At the end of the Phytoremediation using *C. vulgaris*, i.e., on day 11, pH of the effluent increased from 7.6 to 8 and maintained thereafter, while total dissolved solids (TDS) was slightly reduced by 1.3%. The microalgal treatment resulted in a significant reduction in total hardness by about 50%. Calcium and magnesium also followed a similar trend with 63% and 50% reductions respectively. Sodium and potassium, which form an important constituent of inorganic TDS, were only reduced to a small extent, by around 14% and 18%, respectively. Free ammonia levels were reduced by 80%, nitrite levels by 89%, nitrates by 29% and total Kjeldahl nitrogen (TKN) by 73%. Phosphate levels were drastically reduced by 94%. Reductions in biochemical oxygen demand (BOD) and chemical oxygen demand (COD) levels were 22% and 38%, respectively, during the Phytoremediation process.

Plate 1: Microalgae *Chlorella vulgaris* cultures.

Plate: 2 showing the dissolved Domestic sewage solid before and after Phytoremediation. Before treatment, the dissolved Domestic sewage solid, which was black in colour, turned to green due to rich algal growth.

Plate 3: Collection of Algae *C.vulgaris*

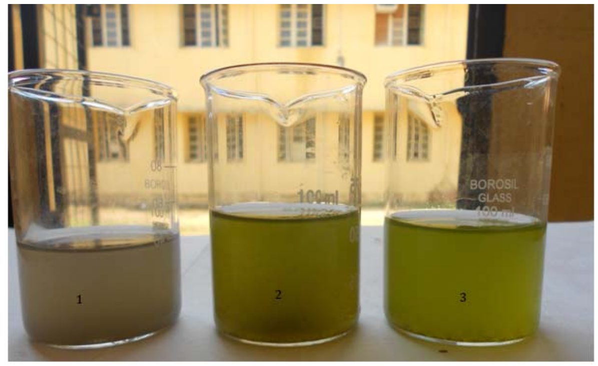

Plate 4: stages of phytoremediation of domestic sewage water using *C.vulgaris.*

Table 1: Physico-chemical analysis of domestic sewage – Phytoremediation using *C.vulgaris*

Physico-chemical analysis of domestic sewage – Phytoremediation using *C.vulgaris*			
S.No	**Parameters**	**Day 0**	**Day 11**
1	TDS (mg/ℓ)	1340±6.0828	1322.5± 6.0828
2	Electrical conductivity (µmho/cm)	1568±6.5574	1432± 6.5574
3	pH	7.6±0.1323	8.0± 0.1323
4	Total hardness (as CaCO3) mg/ℓ	255± 19.975	127± 10.5357
5	Calcium (as Ca) mg/ℓ	45±12.53	19±13.7477
6	Magnesium (as Mg) mg/ℓ	15±5.2915	7.4±4.3589
7	Sodium (as Na) mg/ℓ	225±13.4536	198± 15.1328
8	Potassium (as K) mg/ℓ	15±4.3589	12.4±4.1342
9	Total Kjeldahl Nitrogen (TKN) mg/ℓ	1.25±3.6056	0.37 ±3.2021
10	Free ammonia (as NH3) mg/ℓ	1.5±1.5133	0.3±1.2110
11	Nitrite (as NO2) mg/ℓ	0.005±0.0002	0.0005±0.0001
12	Nitrate (as NO3) mg/ℓ*	2±1	1.42±1.01
13	Phosphate (as PO4) mg/ℓ	1.421± 1.3159	0.085± 0.3201
14	BOD (mg/ℓ)	347±7.5498	270.6± 3.3159
15	COD (mg/ℓ)	692±13.2288	429.0±14.1137

All values are presented as mean SD of triplicate analysis,
Initial and final concentration are statistically significant except nitrate (*-not significant (NS)) at $P<0.05$ according to one –tailed paired Student's t-test.
The non-significance is due to the low initial concentration of nitrate.

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