Phycoremediation study for growth potential of Chlorella minutissima on wastewater for biomass and bioenergy production

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ABSTRACT

The use of microalgae for the treatment of sewage wastewater has been subject of research and development for several decades. However, the development of more efficient, integrated and sustainable system requires further research in this area. Hence, the experiment was set up to study the phycoremediation potential of Chlorella minutissima to remove the pollution load from wastewater and evaluation of biomass for fuel after harvesting. The physical and chemical parameters of wastewater quality such as nitrate, phosphate, potassium, EC, TDS, BOD, COD, etc., were studied. After phycoremediation, it was found that it removed about 90-98% TDS, 70-80% nitrogen, 60-70% phosphorus and 45-50% potassium from the wastewater. The level of BOD and COD were reduced by 60 and 75%, respectively. The algal biomasses were harvested for biodiesel extraction. The results of this study show that algal strain C. minutissima is not only an agent for remediation of pollutant load, but it can also be used as a potential agent for biodiesel production.

Keywords: Phycoremediation, wastewater, microalgae, biomass and biodiesel

I.INTRODUCTION

Water is an essential resource required for sustaining life and livelihoods. Wastewater reuse has recently been looked up as potential options to cope up with the increasing water stress. The focus on wastewater treatment has recently shifted from pollution control to resource exploitation in view of technical feasibility, economics, societal needs and sustainable development. Many bioprocesses can provide bioenergy while simultaneously achieving the objective of pollution control which might reduce the cost of wastewater treatment, and reduce dependence on fossil fuels [1]. Phycoremediation is the technology in which macro-algae or micro-algae use to remove or transform the pollutants and xenobiotics compounds from wastewater, polluted soil and CO_2 from the waste air. Wastewater phycoremediation is an eco-friendly process with no secondary pollution (sludge) as long as the biomass produced is reused and allow efficient nutrient recycling[2,3]. Wastewater is considered to be the potentially sustainable growth medium for the algal feedstock.

Microalgae are considered one of the most promising feedstocks for biodiesel production for higher growth rate and oil productivity compared to other oil crop plants. Microalgae are predicted to have a lower cost per yield and have the potential to reduce GHG emission through the replacement of fossil fuels [4-14]. According to CPCB report [15], only 30% of total sewage wastewater is collected and less than 20% undergoes treatment process. There is an urgent need for phycoremediation process that can treat the wastewater sustainably and further the biomass can be exploited for the fuels or other value-added products. The idea of using microalgae as a source of bioremediation and fuel is not new and is taken seriously because of their promising prospects [13]. However, efforts are required to reduce the high cost of biofuels. Keeping in view the high cost of biomass production and environmental concerns, the present study is an effort to remediate the wastewater of Indian Agricultural Research Institute (IARI) and Common Effluent Treatment Plant (CETP), Mayapuri by unicellular algal strain *Chlorella minutissima* and its potential application for biodiesel extraction. The present study was undertaken to (1) evaluate the physicochemical properties of IARI wastewater and CETP wastewater during algal growth, (2) assessed its biomass productivity and (3) biomass was analyzed for its recovery of biodiesel.

II. METHODS

2.1 Organism and growth condition

The *C. minutissima* algal species used in this study were procured from Center for Blue Green Algae, Indian Agricultural Research Institute (IARI), New Delhi. An axenic culture was established in BG 11 medium [16]. The composition of medium includes nutrients such as $NaNO_3$ (1.5 g), K_2HPO_4 (0.04 g), $MgSO_4.7H_2O$ (0.075 g), $CaCL_2.2H_2O$ (0.036 g), $NaCO_3$ (0.02 g), Agar (if needed) (10 g), Citric acid (6 mg), ferric ammonium citrate (6 mg), EDTA disodium salt) (1 mg), and trace metal mix A5 (1 ml). The pH of BG 11 medium was maintained at 7.1 after sterilization. The stock cultures were incubated at 27 ± 1° C at the 12-hours light, 12-hours dark cycle on tissue culture rack for 10 days.

2.2 Wastewater samples

The IARI (Indian Agricultural Research Institute) and Mayapuri CETP (Common Effluent Treatment Plant) situated in south-west Delhi in North India were chosen as the study sites (Fig. 1). The IARI campus is traversed by a network of sewage drains whose total discharge amounts to about 20 MLD. These sewage drains receive domestic and industrial effluents generated by the IARI campus and complex combination of industrial, commercial, agricultural and dwelling units around IARI micro-watershed. The wastewater entering in IARI is treated only for primary treatment and intermittently applied for irrigation for crop fields in IARI. The wastewater of Mayapuri industrial area is a mixture of wastewater, discharged from small-scale industries, residence and commercial buildings. The main types of industries present in Mayapuri include electroplating, anodizing, heat treatment automobile service station, drying, pickling, washing, powder coating textile etc. The samples of IARI (primary treated) and CETP Mayapuri (tertiary treated) wastewater were collected throughout the year in the morning from the final outlet (of CETP) and wastewater irrigation channel of IARI. The samples were characterized for Electrical conductivity (EC_w), pH, Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Major ions (Na^+, Ca^{+2}, NO^-_3, K^+, & NH^+_4). Trace metals (Fe, Cu, Cd, Zn, & Pb) according to standard methods given in APHA in the laboratory of Division of Environmental Sciences, IARI New Delhi. Conductivity and pH were analyzed within two hours of sampling by digital Conductivity and pH meter. BOD, TDS, NO^-_3 analysis was done on the same day of sampling, while other samples were kept at 4°C in the refrigerator with requires preservatives and were analyzed within 6 days of sampling for the determination of rest parameters. All parameters were analyzed in triplicate. The physicochemical characteristics of both IARI and CETP wastewater are shown in Table 1 at the zero-day of the experiment.

2.3 Optimization of algal growth on wastewater

The algal strain was grown at different concentrations (25%, 50%, 75% and 100%) of wastewater. The experiment was set up in a 5-litre capacity laboratory grade plastic tray containing 3 litres of varying concentration of wastewater. Centrifuged and homogenous algal culture of 2 ml was used to inoculate each tray and mixed thoroughly. Just after the inoculation of algae, we have taken 5 ml sample from each tray and centrifuged at 10000 rpm for 5 minutes. The supernatant was thrown and 5 ml of acetone was added to each tube and kept in the refrigerator for 24 hours. The chlorophyll content was measured using a visible spectrophotometer at 650 nm and 665 nm. This sampling is done at the 1-day interval for 10-12 days. The chlorophyll was calculated by formulae

$$\textit{Chlorophyll content (mg/ml)} = [(2.55 \times OD_{650}) + (0.4 \times OD_{665})]/100$$

2.4 Phycoremediation of wastewater by C. minutissima

This step of an experiment carried out in a 5-litre capacity laboratory grade plastic tray containing 3 litres of wastewater. Most favourable concentration (100%) of wastewater (IARI and CETP) for algal growth was taken for treatment experiment. Homogenous algal suspension (2 ml) was taken for inoculation. The water quality parameters were analyzed on the 12th day of inoculation to check the reduction in pollution load as given in Fig. 3.

2.5 Biomass cultivation

After 12 days the biomass of the trays was harvested by the centrifugation method and processed for biodiesel production. The wastewater and algae grown in trays were collected in 2 litres capacity sampling bottle. Centrifugation was done in 40 ml centrifugation tube for each tray separately at 10000 rpm for 5 minutes. Algal pellets where collected in 15 ml plastic tubes. The fresh weight and dry weight of algal biomass are given in table 2.

2.5 Biodiesel production

Algal biomass was ground with motor and pestle as much as possible. Further, it was dried for 20 minutes at 80 °C in an incubator for releasing water. The oil extraction from the powdered algal biomass was carried out by using n-hexane and methanol. The hexane (875.5 g) extract was mixed with ether (99%) for separation of the algal oil. The mixture was kept for 24 hrs for settling. The oil appeared in form of an upper layer and the residue was settled down at the bottom. The solvent was removed by placing the flask containing algal oil on a continuous rotator shaker (200 rpm). After removal of solvent, a mixture of catalyst (NaOH) (0.25 g) and methanol (24 ml) was poured into the flask containing the algal oil. The whole content was then kept for 3 h on continuous rotator shaker (200 rpm) to allow the completion of reaction (transesterification). After three hours, the biodiesel formed an upper layer and the pigment along with glycerin settled down at the bottom. Biodiesel was separated with the help of separating funnel.

III.RESULTS AND DISCUSSION

The experiment was carried out in triplicate in four different dilutions, in addition, to control (without inoculum). Treatment tray of 100% wastewater and further dilution of 75%, 50% and 25% were taken (Fig 2). Each tray was filled with 3 litres of wastewater as per treatment. After 10 days *Chlorella minutissima* was ready for inoculation. We have taken 200 ml stock culture in the 250 ml. Further then the cultures were taken in the 40 ml centrifuge tube and centrifuged at 4500 rpm for 10 minutes and the whole culture was concentrated to 25 ml. The dehydrated living algal pellet was used for the experiment. 2 ml of this pellet was inoculated in each tray at the centre and mixed thoroughly. Just after the inoculation of algae, we have taken 5 ml sample from each tray and centrifuged at 10000 rpm for 5 minutes. The supernatant was thrown and 5 ml of acetone was added to each tube and kept in the refrigerator for 24 hours. The chlorophyll content was measured using a UV/Visible spectrophotometer at 650 nm and 665 nm. This sampling is done at 1-day interval for 10-12 days. After 10-12 days the biomass of the trays was harvested by the centrifugation method (wastewater and biomass were centrifuged at 10,000 rpm for 5 minutes to separate the biomass from water). The wastewater on which algae was grown was back characterized for the physicochemical parameters after harvesting of chlorella. The *Chlorella* was harvested with the centrifugation method and leftover wastewater was characterized (Fig 3). Conductivity and pH were analyzed within two hours of sampling by digital conductivity and pH meter. BOD, TDS, NO^{-3} analysis was done on the same day of sampling, while other samples were kept at 4°C in the refrigerator with required preservatives and were analyzed within 6 days of sampling for the determination of rest parameters. APHA, AWWA, WEF [17] standard methods of analysis of water and wastewater and AOAC [18] official methods of analysis were followed for physicochemical analysis of collected samples. Each analysis was done in triplicate and the mean value was taken. The analytical data quality was ensured through careful standardization, procedural black measurements and duplicate sample.

The level of EC_w and Total Dissolved Solids (TDS) in the wastewater were reduced by 98 – 99 ± 2.5%. ECw and TDS were reduced due to the absorption of salts and solids present in wastewater. The reduction of TDS was formed 2200 ppm to 11.5 ± 1.99 ppm in IARI samples and that of CETP was from 1578 ppm to 7.45 ± 0.707 ppm. The reduction of EC_w in IARI sample was from 2.059 dS/m to 0.01 ± 0.004 dS/m while in CETP was reduced from 1.33 dS/m to 0.01 ± 0.0007 dS/m (Fig 4, 5).

The reduction of pH on an average was 5.5 - 7.4 % in all the samples. The reduction in pH was from 8.76 to 8.102 ± 0.144 for IARI samples and that of CETP was from 7.68 to 7.21 ± 0.071 (Fig 4, 5). Considerable reduction in BOD and COD of wastewater was noted in residual water. Nearly 27.11% COD and 31% BOD removal were achieved. The reductions in P, K, Na and Ca + Mg an average were 60 – 80%, 50 – 80%, 21 – 70% and 90 – 95% respectively (Fig 4, 5). The reduction in P was from 1.55 ppm to 0.855 ± 0.828 ppm, reduction in K was from 15.9 ppm to 7.537± 2.11 ppm, reduction in Na was from 17.17 ppm to 11.855 ± 1.8 ppm, and reduction in Ca +Mg was from 89.150 meq/l to 9.758 ± 1.6 meq/l in IARI samples and reduction in CETP samples were P from 3.02 ppm to 0.43 ± 0.07 ppm, reduction in K was from 0.62 to 0.29 ± 0.055 ppm, reduction in Na was from 19.33 to 15.54 ± 1.7 ppm, and reduction in Ca + Mg was from 20.48 to 1.19 ± 0.13 meq/l (Table 1, 2). The reductions in wastewater nitrogen concentration (NH^{+}_{4}-N and NO^{-}_{3}) in all the treatment were noted. The residual NH_4^{+}-N concentration vanished in IARI wastewater while in CETP samples it ranges from 50 – 90 %. The NH^{+}_{4}-N were 100% removed in the IARI wastewater just because of higher pH of IARI wastewater and better growth of microalgae. The NO^{-}_{3} concentration was reduced to 50% in IARI wastewater and 39% reduced in CETP wastewater (Fig 4, 5).

IV.CONCLUSION

Microalgae have been used in past to recycle some of the nutrients within the wastewater sources and also as a step in the wastewater treatment. The outcome of research had suggested the potential of specific microalgae to reduce the BOD, COD and pollution load of the sewage wastewater. In the present investigation *Chlorella, minutissima* was found to remove about 98-99% TDS, 70-80% nitrogen, 60-70% phosphorus and 45-50% potassium from the wastewater. It has also been revealed from the research that *Chlorella minutissima* could remove the higher concentration of Cu and Pb. The initial TDS value of sewage wastewater was in the range of about 1500 to 2300 ppm and after the phycoremediation by *Chlorella*, it is reduced to the range of 10 to 20 ppm. Hence *Chlorella* could be used for the remediation of sewage wastewater due to potential ability to reduce the TDS and pollution load and can further utilize the safe irrigation of crops. Inferring from the study it could be safely said that the use of algae for treatment of wastewater can help in reducing pollution load on the environmental sector and on the other side of coin recycle the wastewater for the agricultural and others use.

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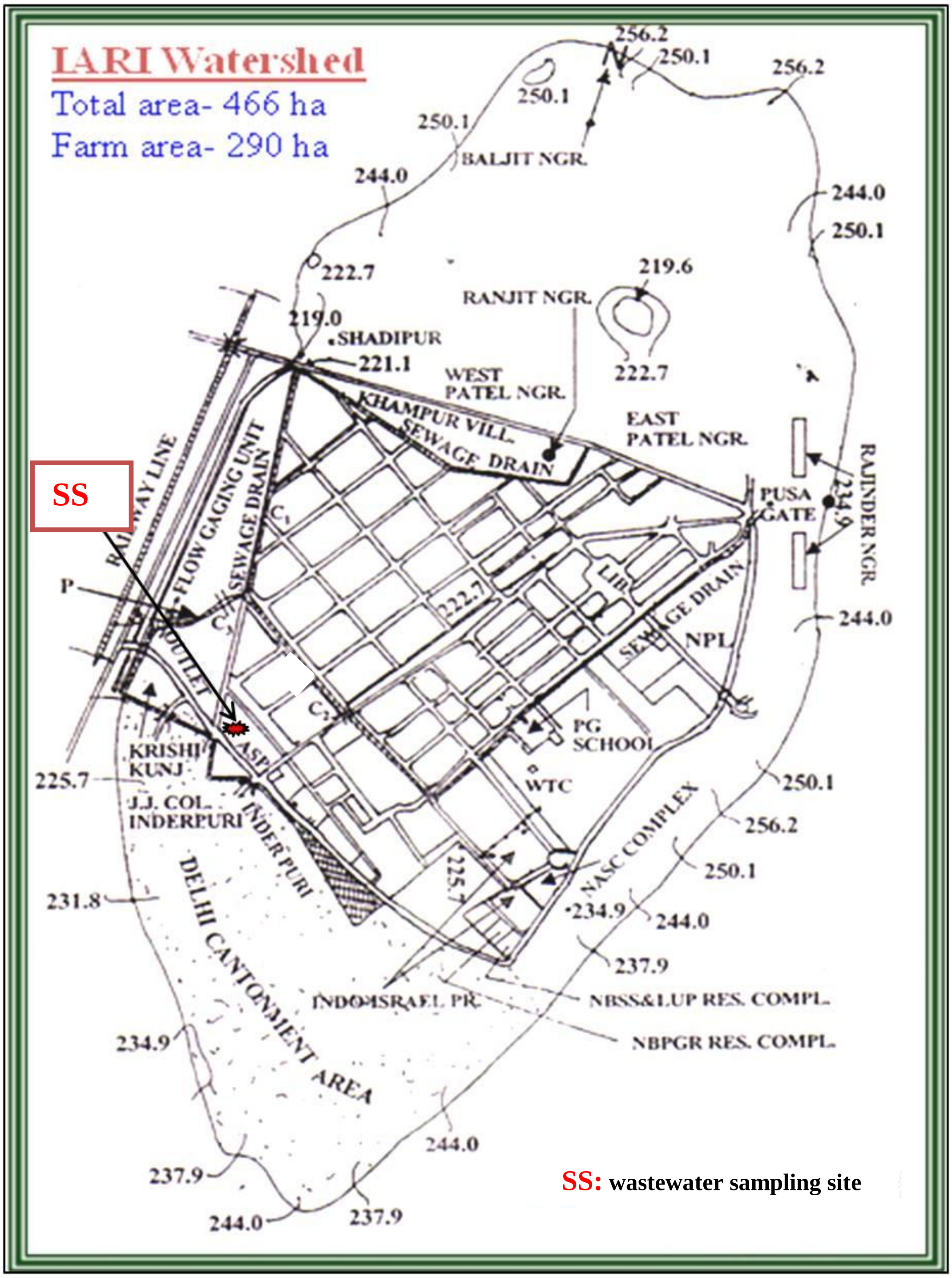

Figure 1 IARI watershed map showing site of sampling of wastewater

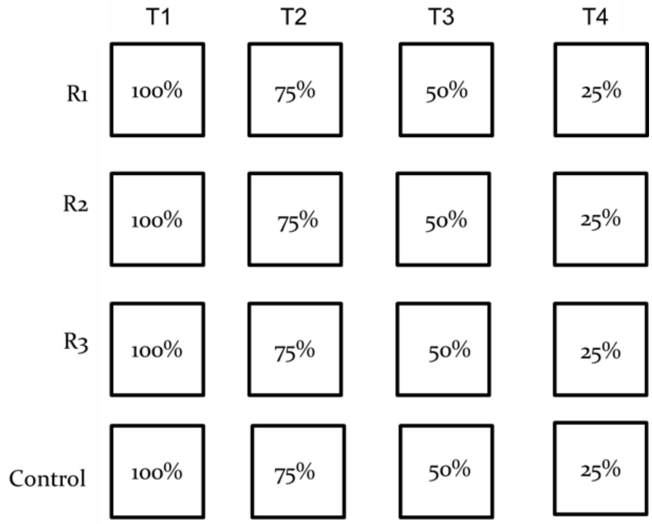

Figure 2 Treatment layout

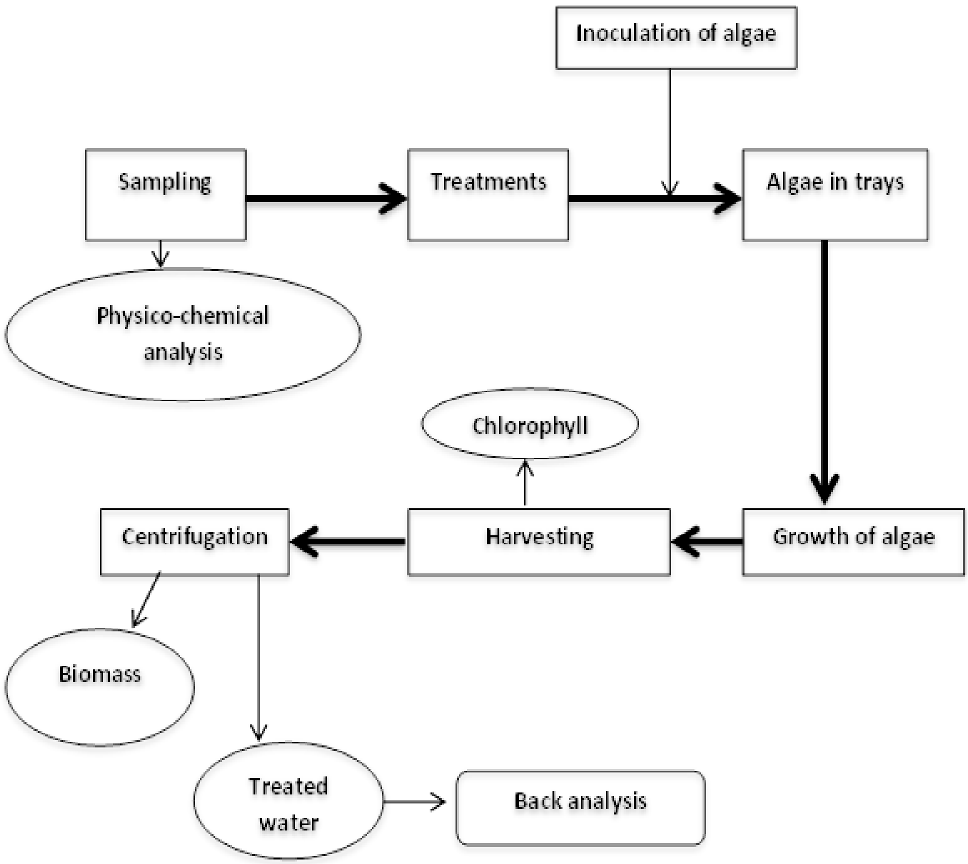

Figure 3 Schematic of experiment setup

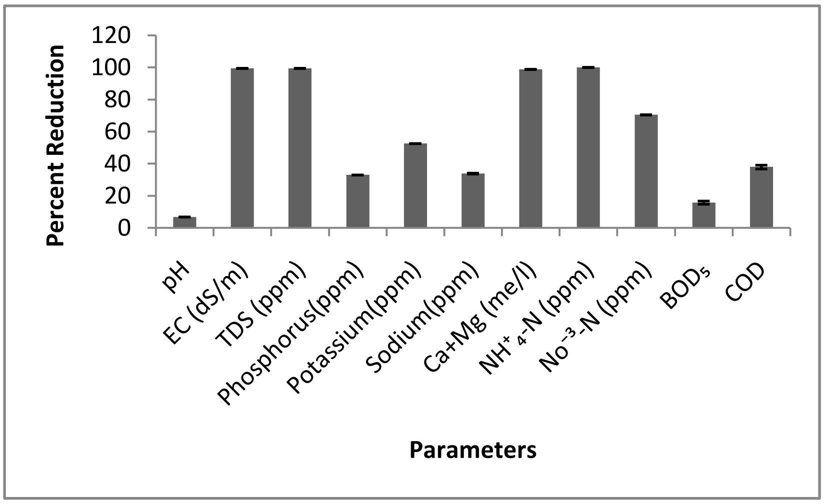

Figure 4 Percent reduction in pollutant load over after growth of algae in IARI

Table 1 Physico-chemical composition of effluent of CETP

PARAMETER	CETP	
	Initial	Final
pH	7.68	7.21
EC (dS/m)	1.33	0.01
TDS (ppm)	1578.67	7.45
Phosphorus(ppm)	3.02	0.43
Potassium(ppm)	0.62	0.29
Sodium(ppm)	19.33	15.54
Ca+Mg (meq/l)	20.48	1.19
NH_4^+-N (ppm)	36.84	14.74
NO_3^--N (ppm)	1.29	0.76
BOD_5	73.58	49.29
COD	223.13	168.20

Table 2 Physico-chemical composition of effluent of IARI

PARAMETER	IARI	
	Initial	Final
pH	8.79	8.102
EC (dS/m)	2.05	0.010
TDS (ppm)	2079.33	11.410
Phosphorus(ppm)	1.55	0.855
Potassium(ppm)	15.96	7.537
Sodium(ppm)	17.17	11.855
Ca+Mg (me/l)	89.50	9.758
NH_4^+-N (ppm)	20.43	0
NO_3^--N (ppm)	6.19	2.137
BOD_5	80.51	64.41
COD	181.29	177.46

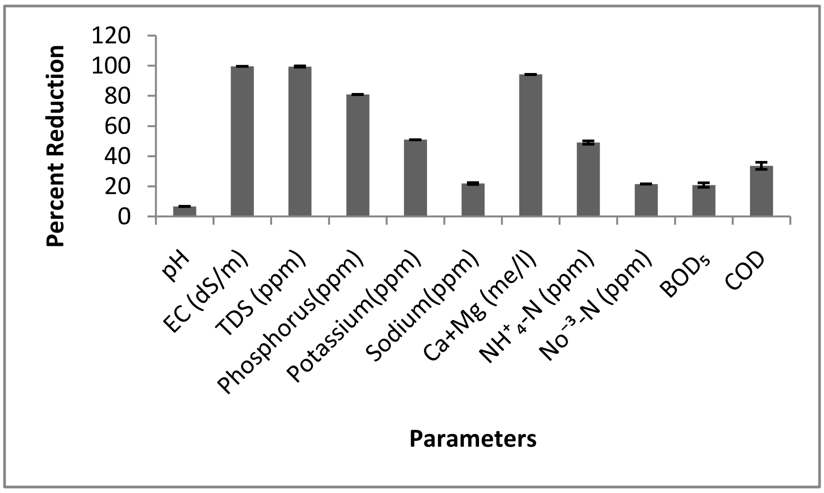

Figure 5 Percent reduction in pollutant load over after growth of algae in CETP