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Phycoremediation Potential of *Scenedesmus obliquus* on Tertiary Treated CETP Effluent and Lipid Production

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Abstract- *Microalgae based wastewater treatment and biodiesel production is gaining attention in recent years. Present research study highlights the phycoremediation potential of Scenedesmus obliquus for removal of contaminants present in CETP treated effluent. CETP treated effluent was collected from kalol Gandhinagar and was characterized for various physicochemical parameters i.e. nitrate, phosphate, pH, EC, TDS, BOD, COD, Chloride, ammonia etc. Scenedesmus obliquus showed nutrient removal efficiency i.e. 46.1% TDS, 52% Nitrate, 21.5% Phosphorous, 45% BOD, 40.1% COD, 42.8% ammonia, 10.3 % Chloride respectively. The algal strain characterized indicates presence of maximum lipid productivity 6.43±0.77 mgl^{-1}d^{-1}; Lipid content 27.4±1.46 % and biomass productivity 23.48±3.5 mgl^{-1}d^{-1} at the end of the experiment. The result obtained from the research study showed effective nutrient removal from wastewater and the biomass obtained can also be used as a source for biodiesel production.*

Key words- *Phycoremediation, Wastewater treatment, biodiesel production, Microalgae, Sustainable approach.*

I. Introduction

Industries in particular chemical industries produce wastewater containing organic and inorganic compounds. The wastewater generated from large scale industries are treated fully before discharge whereas the middle scale industries treat the generated wastewater partially and let out to the CETP or discharge into soil water environment. The small scale industries do not have treatment facilities so they become member of CETP and without treatment of wastewater, the wastewater generated is let out to the CETP. The common effluent treatment plant receives complex wastewater from various industries involves primarily, primary, secondary and tertiary treatment before discharge. Inspite of the present treatment employed in common effluent treatment plant the effluent discharged to the water bodies found to contain persistent organic and inorganic pollutants. In present research study the treated effluent from CETP plant was collected from the discharged site and characterized for physicochemical and microbial status. The persistent contaminants were found to be present in higher concentration than the prescribed standards. Therefore the lab technique has been developed wherein the different dilutions of effluent was subjected to algal bioremediation using microalgae *Scenedesmus obliquus* under laboratory conditions. The algal growth was monitored consistently throughout the experiment by maintaining the environmental conditions. The remediation is studied to assess the effectiveness of the microalgae *Scenedesmus obliquus* at laboratory scale. The biomass was characterized for dry weight, biomass productivity, lipid content and lipid productivity etc. The lipid content observed was reported which can be further used for biodiesel production, hence the microalgae can be a source of bioremediation and algal biomass after harvesting, utilized for renewable energy generation.

II. Material methods

A. Isolation and identification of microalgae

Water samples collected from Vinzol lake located in Ahmedabad, Gujarat India was further characterized and used for isolation of microalgae *Scenedesmus obliquus*. 15 ml of collected water samples was inoculated in 500 ml Erlenmeyer flask containing 300 ml BG11 medium ($NaNO_3$, 1.5 g/L; K_2HPO_4, 0.04 g/L; $MgSO_4{\cdot}7H_2O$, 0.075 g/L; $CaCl_2{\cdot}2H_2O$, 0.036 g/L; citric acid, 0.006 g/L; ferric ammonium citrate, 0.006 g/L; EDTA(disodium salt), 0.001 g/L; Na_2CO_3, 0.02 g/L; 1 mL trace elements solution (H_3BO_3, 2.86 g/L; $MnCl_2{\cdot}4H_2O$,1.81 g/L;$ZnSO_4{\cdot}7H_2O$, 0.222 g/L; $NaMoO_4{\cdot}2H_2$,O 0.39 g/L; $CuSO_4{\cdot}5H_2O$,0.079 g/L; $Co(NO_3)_2{\cdot}6H_2O$,0.0494 g/L),with a pH of 7.0±1) and were incubated at 28±1ºC in a 16:8 light-dark cycles for two weeks. Further algal growth was examined under optical microscope and then the samples were serially diluted in BG11 medium. These samples were subculture by inoculating 100µL culture in 100 ml of conical flask supplemented with 50 ml of BG11 medium. The developed culture was plated on algae agar medium and incubated at 28±1ºC for one week, after which the isolated microalgae were re-streaked for axenic culture identification. The isolated microalgae were identified on the basis of morphological characteristics and 28S rDNA technique.

B. Collection of and Characterization of the CETP effluent

The CETP effluent sample from kalol, Gandhinagar was collected from final outlet (tertiary treated). The sample was assessed for pH, electrical conductivity, total dissolve solids, alkalinity, total phosphorous, nitrate, ammonia, Sulphate, COD, BOD, chloride according to standard methods[1]. pH and Electrical conductivity were analyzed by digital pH and conductivity meter. BOD, COD, Nitrogen and TDS were analyzed on the same day and remaining sample was preserved in the refrigerator at 4˚C for further analysis. All parameters were analyzed in triplicate (Table 1).

Table: 1 Physico-chemical composition of tertiary treated CETP wastewater

Parameters	Before treatment	CETP Discharge standards to inland surface water[EPA 1986]
pH	7.71	5.5-9
EC (ms)	19.49 ± 0.006	-
TDS (mg/L)	6430 ± 2.1	2000 mg/L
Alkalinity (mg/l)	1000 ± 0.14	-
TP (mg/L)	7.1 ± 0.74	5 mg/L
Nitrate (mg/l)	38.2 ± 3.4	-
Ammonia (mg/l)	73.8 ± 8.91	50 mg/L
Sulphate (mg/l)	1324.1 ± 3.64	1000 mg/L
COD (mg/l)	764 ± 9.39	250 mg/L
BOD (mg/l)	158.31 ± 3.21	30 mg/L
Chloride (mg/l)	1242.5 ± 1.56	1000 mg/L
Cr (ppm)	1.45	2 mg/l
Mn (ppm)	2.261	2 mg/l
Co (ppm)	0.0060	-
Ni (ppm)	0.0483	3 mg/l
Cu (ppm)	0.0363	3 mg/l
Zn (ppm)	0.166	5 mg/l
As (ppm)	0.0028	0.02 mg/l
Cd (ppm)	1.91	1 mg/l
Pb (ppm)	0.345	0.1 mg/l

C. Experimental methodology for phycoremediation of effluent by *Scenedesmus obliquus*

The nutrient removal efficiency of *Scenedesmus obliquus* was assessed by growing the algal strain in 500 ml conical flask supplemented with 250 ml of CETP effluent diluted at different concentration (0%, 25%, 50%, 75% and 100%). Prior to inoculation the pre-cultured microalgae sample was centrifuged for 5 min at 4000 rpm and the cell pellet was washed with sterile distilled water and re-suspended for inoculation. All flasks were incubated at 28±3°C temperature under photoperiod of 16:8 light-dark cycles for a period of 25 days.

D. Measurements of algal growth

The growth rate of isolated freshwater Microalgae used in experimental setup was monitored by observing the optical density (680 nm) using UV-VIS spectrophotometer (Dynamica HB-20). A known volume of algal culture from experimental setup was filtered through pre-weighed glass fiber filter paper and dried overnight in hot air oven at 60°C and measured as g/L. Optical density and dry biomass of algae was found to be equivalent to 0.61g/L from calibration curve, thus the regression equation $y=0.613x$ ($R^2=0.98$), was used for calculation of biomass concentration during study; where x is the absorbance at 680 nm and Y is the DCW g/L (dry cell weight).

E. Kinetics study of microalgae growth

Microalgae are fastest growing unicellular photoautotrophs with great potential of generation of biomass at high rate in comparison to other phosynthetic organisms. Therefore microalgal growth rate kinetics was measured by Logistic growth kinetics model; Specific growth rate was calculated by using eq. (1):

$$\mu = \ln(N_o - N_t)/(t_1 - t_0) \quad (1)$$

Where, μ is the specific growth rate of *Scenedesmus obliquus* (mg/L/d), calculated by using the Graph Pad Prism Software (Version 6) by applying the commonly used exponential eq. (2),

$$Nt = No \exp(r * t) \quad (2)$$

Where N_t is the microalgae population at time *t* and ***No*** is the initial population of microalgae; ***r*** is the microalgae growth rate and ***t*** represents the time period up till experiment was carried out used for calculation.

F. Lipid extraction

The lipid content in the biomass was determined in control and CETP cultivated microalgae according to Modified Bligh and Dyer[2]. A mixture of 0.5 ml of PBS solution (phosphate buffer saline) containing 8 mM Na_2HPO_4, 140 mM NaCl, 2mM NaH_2PO_4, pH 7.4, glass beads (0.5mm) and algal cells was taken in the centrifuge tubes and cells were disintegrated by high speed centrifugation for 4 minute, after centrifugation 3 mL of Methanol and chloroform solution (1:2 ratio, v/v) was added to the above mixture and again centrifuged (10 min. at 3000 rpm) and incubated at room temperature for 18 hrs. After 18 hrs. of interval the phase separation was carried out by adding distilled water and the mixture was again centrifuged at 3000 rpm for 10 min. The lower supernatant layer with lipid was collected in pre-weighed vial and remaining solvent was evaporated in hot air oven at 50°C. Lipid content was calculated as % dry cell weight as given in eq. 3

$$\text{Lipid content } (\%) = \left(\frac{\text{Dry cell weight}}{\text{Lipid weight}}\right) * 100 \quad (3)$$

III. Results and discussion

A. Effect of CETP effluent concentration on algal growth kinetics

Microalgal growth was observed at different concentration of CETP effluent diluted with distilled water (0%, 25%, 50% and 100%). The results (Fig: 1) showed increase in algal growth with increasing concentration of effluent i.e. 50% and further growth was found decreasing with increase in the effluent concentration which revealed that algal growth pattern is influenced by culture medium conditions and its composition. The experimental data of growth study was observed for logistic growth kinetics. Data for variables of growth kinetics such as O.D. (a), rate constant (K) and doubling time (μ) are provided in Table 2.

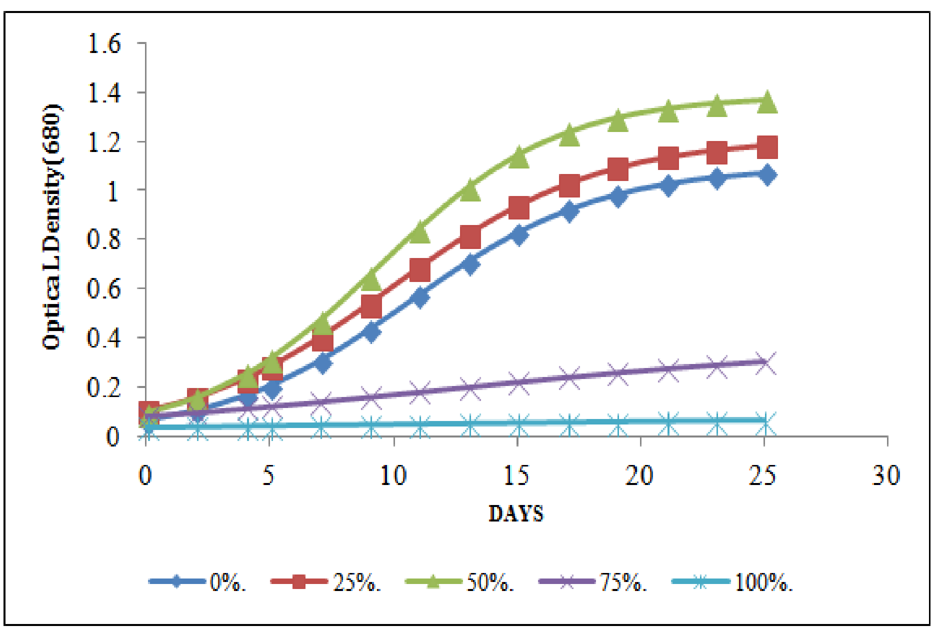

Fig.1.Logistic growth kinetics of *Scenedesmus obliquus* at different concentrations of CETP

The increase in CETP effluent concentration (below 50% dilution) was found to have negative effect on algal growth, which indicates high pollutants load has inhibitory effect on microalgal growth. Similar findings were also observed on limited growth of *Chlorella* sp. with increasing concentrations of industrial wastewater[3]. The stress conditions, such as a decrease in nutrient content appeared to accelerate neutral lipid accumulation in algal biomass. Based on the results of previous studies[4] increase in lipid accumulation leads to a decrease in growth rate but increased production of oil.

Table.2. Summary of growth kinetics for Scenedesmus obliquus grown at different concentrations of CETP

	0%	25%	50%	75%	100%
a	1.1	1.21	1.38	0.307	0.081
xc	10.66	9.95	9.43	11.86	3.11
k	0.259	0.241	0.278	0.107	0.06
t	10.76	11.76	11.29	18.28	30.28

B. Nutrient removal potential of *Scenedesmus obliquus*

The microalgae *Scenedesmus obliquus* has been widely used in wastewater treatment. Many co-workers have reported, the nutrient removal efficiency of microalgae from different type of waste water[5, 6, 7]. The reduction in various physicochemical parameters from 50% diluted CETP wastewater by *Scenedesmus obliquus* is shown in **(Table-3)** respectively.

Table: 3 Physico-chemical composition of CETP wastewater with 50 % dilution (after treatment)

S.N.	Parameters	Before treatment	After treatment	Percent removal (%)
1	**pH**	7.5	8.9	-
2	**EC (ms)**	13.61 ± 0.7	12.8 ± 0.31	5.8
3	**TDS (mg/L)**	4324 ± 12.8	2330.6 ± 0.5	46.1
4	**Alkalinity (mg/l)**	422 ± 5.6	284.8 ± 2.1	32.5
5	**TP (mg/L)**	3.7 ± 0.92	2.9 ± 0.3	21.5
6	**Nitrate (mg/l)**	19.3 ± 0.52	9.23 ± 0.14	52
7	**Ammonia (mg/l)**	55.3 ± 6.4	31.63 ± 2.6	42.8
8	**Sulphate (mg/l)**	783.5 ± 8.6	563.3 ± 1.2	28.1
9	**COD (mg/l)**	513.9 ± 3.9	307.28 ± 12.8	40.1
10	**BOD (mg/l)**	94.5 ± 1.7	51.97 ± 4.9	45
11	**Chloride (mg/l)**	784.15 ± 21.4	703.38 ± 7.1	10.3

After 25 days of algal cultivation in waste water, change in the pH of the effluent from 7.5 to 8.9 was observed; this could be attributed to photosynthetic activity during which the dissolved CO_2 was assimilated. Similar results have been reported by Elumalai et al.[8] where initial pH of tannery effluent was 6.2, which increased steadily to 8.42 in 21 days after phycoremediation with *S.obliqus*. 41.6% Total Dissolved Solid (TDS) from the CETP effluent was removed, which may be due to utilization of various nutrients by microalgae. The reduction in COD and BOD was found to be 40.1 % and 45% respectively; this could be due to high photosynthetic activity and intense algal growth[9]. Malla et al.[10] reported considerable reduction in the COD (27%) and BOD (31%) concentration in CETP effluent by *C. minutissima* after 12th day of inoculation. However Elumalai et al.[8] reported about 90% reduction in the COD and BOD levels of textile dye effluent by *Scenedesmus obliquus*. The nitrate content was reduced to 52% while phosphorous was found to reduce by 21%. It has been reported that the total nitrogen content was reduced from 53 mgL^{-1} to 22 mgL^{-1} and total phosphorus was reduced from 7.1 mg/L to 5.4 mg/L after 20 days of *S. Obliquus* cultivation in piggery wastewater[11]. The amount of Sulphate, chloride and ammonia was also reduced to 28.1%, 10.3% and 42.8% significantly. Reduction in EC from 13.6 to 12.8ms (5.8%) was observed during Phycoremediation. Significant reduction in COD, BOD, Ammonia, Sulphate, TDS, N and P content in the CETP effluent is given in the figure below **(Fig-2)**.

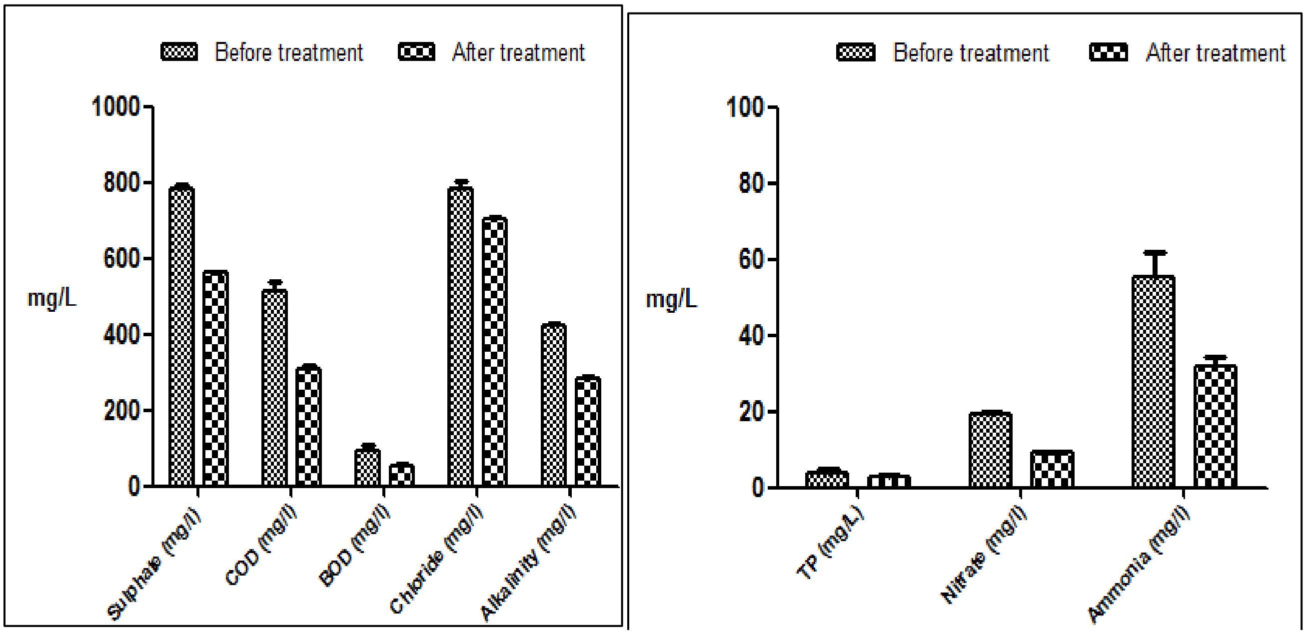

Fig-2 Removal of different pollutants during phycoremediation of CETP effluent

C. Growth properties, biomass and lipid productivity of *Scenedesmus obliquus*

In the present research study the growth of S. *Obliquus* was slow at initial stage as algal cells undergoes acclimatization phase due to change in nutrient profile i.e. from BG11 to CETP effluent. After 21 days, the maximum growth rate was observed in 50% dilution. Therefore 50% diluted CETP effluent can be used successfully for algal growth, as algal cell growth was supported in lower concentration of CETP effluent whereas 75% and 100% effluent inhibits the growth as well as biomass production of the S. *Obliquus*. The growth rate of *S. Obliquus* was analyzed after every 24 hrs. by measuring optical density at OD at 680 nm over a period of 25 days (**Fig: 1**). At the end of the experiment dry cell weight was 0.587 gm /L. However Malla et al.[11] had reported maximum dry cell weight 0.43 g/L (*Chlorella minutissima)* in 100% CETP wastewater while Ji et al.[12] reported 0.31 g/L Dry cell weight of S. *Obliquus* in Tertiary treated municipal wastewater. After 25 days of phycoremediation study the biomass productivity and lipid productivity of *Scenedesmus obliquus* was found to be 23.48 mg/l/d, 6.43mg/l/d respectively (**Table-3)**.

Table. 3 Dry wt., biomass productivity and lipid productivity in grown on different dilution of CETP effluent

Parameters	**control**	**25%**	**50%**	**75%**	**100%**
Dry wt. gm/L	0.472±0.058	0.5±0.43	0.587±0.55	0.45±0.69	0.064±0.51
Lipid (%)	23.1± 1.1	24.3±3.7	27.4±1.46	21.6±2.3	18.9±2.8
Biomass productivity(mg/l/d)	18.8±2.6	20±1.8	23.48±3.5	18±3.2	2.56±2.7
Lipid productivity(mg/l/d)	4.36±0.78	4.86±0.86	6.433±0.77	3.88±0.98	0.48±.83

IV. Conclusion

The present study highlights the advanced treatment of CETP effluent at the discharged sites. The algal strain *Scenedesmus obliquus* has been identified as a potential source for decontamination of CETP discharged effluent. In the present research study *S. obliquus* showed efficient removal of nutrients from tertiary treated CETP effluent i.e. 5.8 % EC, 46.1% TDS, 52% Nitrate, 21.5%, Phosphorous, 45% BOD, 40.1% COD, 42.8% ammonia, 10.3 % Chloride respectively. The research indicate that CETP effluent persistent pollutants can be further treated if residual contaminant found after final treatment before discharge using water bodies such as pond or extended lagoon wherein algal culture can be used to remove residual toxicants to permissible levels to the related act. The surface water bodies having algal biomass can be removed and utilized for biodiesel production so that decontaminated effluent can be discharged to soil water environment to maintain environmental standards.

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