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Phycoremediation of effluent using a culture of *Tetradesmus dimorphus* (Turpin) M. J. Wynne: A case study on Indian Oil's first public sector Refinery

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ABSTRACT

In the present study, phycoremediation of the Guwahati Refinery effluent, Indian Oil's first public sector refinery was studied using a pure culture of *Tetradesmus dimorphus* (Turpin) M. J. Wynne. Sampling was done at a monthly interval for a period of six months i.e., September, 2018 to March, 2019. Twelve physicochemical parameters of the refinery effluent were examined in the wastewater treated without test organism (T_0) as well as in the wastewater treated with test organism (T_1) at a sampling interval of 0 days, 10 days and 20 days. In every parameter being analysed, T_1 showed better results than T_0. The phycoremedial efficiency of T_1 was recorded to be the highest on 20^{th} day of incubation.

Key words: *Refinery effluent, Phycoremediation, Algae, Guwahati Refinery*

Introduction

Petroleum is a crucial source of energy, whose demand has been increasing at an alarming rate with the increase in population size, and this has led to the global problem of environmental pollution. Presence of excess nutrients such as nitrogen and phosphorus in untreated wastewater also results in algal blooms, eutrophication, oxygen depletion, which ultimately leads to total degradation of water bodies (Khan and Ansari, 2005).

Effluents can be treated by physical, chemical and biological methods (Bhaskaran, 1977). But since the physical and chemical methods are costly and most chemical methods increase the overall load of dissolved matter in the wastewater, therefore biological treatment of wastewater is a better option.

Algae is an important representative group of the microbes (Sen *et al*., 2013). These organisms include photoautotrophic, eukaryotic microalgae and prokaryotic cyanobacteria, having a wide range of diversity in their habitat and thallus organization and distributed in both fresh and marine aquatic environments (Lee, 2008). Their vast diversity and ability for adaptation in extreme and unfavourable habitats led scientists to screen and identify potential microalgae and to develop efficient microalgae-based technologies for the treatment of wastewater (Fouilland, 2012).

Phycoremediation is an innovative technology in the field of environmental studies. It is a silent promising trend which would solve all the ecological miseries. Phycoremediation is a novel, low cost, effective, continuous treatment technique that uses algae to clean up polluted water (Oswald, 2003).

The use of different species of micro and macro algae for the treatment of different types of wastewaters has been a subject of research and development for several decades (Renuka *et al*., 2015). Thus, the application of phycoremediation technology has been made on several sources of wastewater which includes food processing wastewater, sewage wastewater, industrial effluents (Chemical, Distilleries, Textile dye, Oil refinery) and Dairy effluents.

The present endeavour was aimed to examine the efficiency of some selected microalgae strains to decontaminate refinery wastewater with the following objectives:

a) To analyse the physico-chemical parameters of refinery effluent.
b) To ascertain phycoremediation potential of *Tetradesmus dimorphus* (Turpin) M. J. Wynne, a microalga isolated from refinery effluent.

Materials and Methods

Collection of wastewater/algal samples

Samples for the study were collected from Guwahati refinery located at Noonmati area in Guwahati city of Assam, India, geographically located between 26°11′4"N latitude and 91°48′41"E longitude (Figure 1). The wastewater samples were collected at a monthly interval from September, 2018 to March, 2019 from a polluted drain receiving effluents from Guwahati Refinery. Collection and preservation of wastewater samples were done following standard procedures of APHA (2012).

Experimental design

Development of Algal culture

A pure culture of isolated *Tetradesmus dimorphus* (Turpin) M. J. Wynne was maintained in BG-11 medium as the test organism in order to fulfil the objective of our study. Nomenclature of the test organism were confirmed by consulting Guiry and Guiry (2018).

Studying physico-chemical parameters of refinery effluent

Several important physico-chemical parameters of water body such as conductivity, pH, Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), calcium, chloride, hardness, oil and grease, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were examined following APHA (2012) and Trivedy and Goel (1986). Calculated values were compared with the Central Pollution Control Board (CPCB, 1995) permissible limits.

Ascertaining phycoremediation potential of selected algae

In order to study the role of the selected microalgae in the refinery drain wastewater, the following protocols were employed. i) The wastewater treated without algae (T_0) and ii) The wastewater treated with algae (T_1).

Analysis of each of the physico-chemical parameters for both T_0 and T_1 were conducted in three sets and repeated three times. For T_1, 0.5 ml of uniform algal suspension was inoculated into the flasks containing a total volume of 300 ml wastewater. The physico-chemical parameters of both T_0 and T_1 were examined at a sampling interval of 0 days, 10 days and 20 days of phycoremediation. All the experimental values were taken in triplicates and the values were expressed as mean ± SD.

Fig. 1a

Fig 1b

Fig. 1a. Drain receiving refinery effluent; b: Collection of algal samples

Results and Discussion

Impact of algal sample on physicochemical parameters

The physico-chemical parameters under control (T_0) and treatment (T_1) were found to display quantitative percentage change in their values. The result is shown in Table 1.

Conductivity measurement provides a quick assessment of potential water quality problems. In our study, the electrical conductivity of the wastewater sample was recorded as 710 ± 0.00 (µS/cm), which was well above the permissible limit of CPCB (1995) i.e., 400 µS/cm. Such high level of conductivity might be due to the use of inorganic chemicals in the oil refinery. Similar observations were also recorded in wastewater treatment with *Chlorella vulgaris* where the EC of the wastewater decreased by 51.43 % after 7 days of incubation (Fathi *et al*., 2013). Moreover, EC in the oil refinery effluent treated with *Scenedesmus obliquus* was also seen to decrease from 2625 ± 11.95 (µS/cm) to 2430 ± 10.7 (µS/cm) (Rajasulochana *et al*., 2009).

TDS of water forms an important parameter for its examination. Its value in the refinery effluent was recorded to be 520 ± 0.6 (mg/l). This value showed a reduction of 16.92 % and 23.07 % in T_0 at the interval of 10 days and 20 days respectively. The reduction value was almost doubled in T_1, ranging from 34.61 % in 10th day and 44.23 % in 20th day. Similar type of result was noted in wastewater grown with *Chlorella* sp., in which the value decreased upto 43.84 % after 7 days (Fathi *et al*., 2013). In another study conducted on dairy effluent, *Nostoc* sp. could reduce about 20.21 % of TDS (Kotteswari *et al*., 2012). *Scenedesmus obliquus* also showed a reduction of 6.10 % TDS in oil refinery effluent after 15 days of incubation (Rajasulochana *et al*., 2009).

TSS also forms a very important physical parameter of water body. In our study, TSS value of 130 ± 5.0 (mg/l) was recorded, which was above the permissible limit value (100 mg/l) of CPCB (1995). High amounts of suspended solids were also reported earlier by several workers (Sinha, 1993; Amudha and Mahalingam, (1999); Sundaramoorthy *et al*., 2000). Our results were concordant to the study conducted on the treatment of dairy effluent with *Nostoc* sp., which revealed a reduction of 53.93 % TSS after 15 days (Kotteswari *et al*., 2012).

Table 1. Percentage change in physico-chemical parameters with (T_1) and without treatment (T_0)

Parameter	Treatment	0th day	10th day	%Change	20th day	% Change
Conductivity (mS/cm)	T_0	710 ± 0.00	673.3 ± 1.2	-5.2	601 ± 1.2	-15.35
	T_1	710 ± 0.00	600 ± 0.00	-15.5	502.3 ± 1.0	-29.3
TS (mg/l)	T_0	650 ± 1.52	540 ± 3.60	-16.9	420 ± 5.0	-35.38
	T_1	650 ± 1.52	470 ± 5.29	-27.69	310 ± 15.0	-52.3
TDS (mg/l)	T_0	520 ± 0.6	432 ± 2.0	-16.92	340 ± 10	-34.61
	T_1	520 ± 0.6	400 ± 10	-23.07	290 ± 5.0	-44.23
TSS (mg/l)	T_0	130 ± 5.0	108 ± 4.35	-16.92	80 ± 5.0	-38.46
	T_1	130 ± 5.0	70 ± 8.66	-46.15	20 ± 5.0	-84.61
pH	T_0	6.3 ± 0.06	6.5 ± 0.06	3.07	6.8 ± 0.00	7.35
	T_1	6.3 ± 0.06	7.6 ± 0.00	17.1	8.77 ± 0.06	28.16
Calcium (mg/l)	T_0	111.31 ± 0.43	109.91 ± 1.09	-1.25	108.71 ± 0.28	-2.33
	T_1	111.31 ± 0.43	80.08 ± 0.35	-28.1	31.23 ± 0.10	-71.9
Chloride (mg/l)	T_0	301.57 ± 0.14	299.40 ± 0.24	-0.72	274.91 ± 0.53	-8.84
	T_1	301.57 ± 0.14	204.93 ± 0.43	-32.04	159.95 ± 0.15	-47
Hardness (mg/l)	T_0	301.65 ± 0.2	299.5 ± 1.5	-0.71	298.5 ± 1.1	-1.04
	T_1	301.65 ± 0.2	290 ± 2.0	-3.9	270 ± 2.0	-10.5
Oil and grease (mg/l)	T_0	7970 ± 13.22	5631 ± 6.55	-29.34	3108 ± 10.01	-61
	T_1	7970 ± 13.22	3560 ± 17.43	-55.33	10 ± 0.20	-99.87
DO(mg/l)	T_0	1.88 ± 0.104	4.63 ± 0.34	59.39	9.56 ± 0.12	80.3
	T_1	1.88 ± 0.104	21.26 ± 0.8	91.16	37.3 ± 0.6	95
BOD(mg/l)	T_0	82.68 ± 0.27	64.02 ± 0.42	- 22.56	48.01 ± 0.84	- 41.9
	T_1	82.68 ± 0.27	29.86 ± 3.2	- 63.88	11.77 ± 0.33	- 85.76
COD (mg/l)	T_0	121.6 ± 3.32	94.16 ± 6.78	- 22.56	70.61± 5.033	- 41.94
	T_1	121.6 ± 3.32	43.92 ± 6.11	- 63.88	8.01 ± 6.244	- 93.41

Although the values of pH in the discharged effluent indicated that it was well within the permissible limits of CPCB (1995), but, as per our protocols, we analysed the pH of both T_0 and T_1 on the 10th and 20th day of the incubation period. The results of our study revealed that T_1 had more tendency towards alkalinity in comparison to T_0. This increased tendency towards alkalinity might be due to increased photosynthetic activity of the algae or some chemical properties of water (Fathi *et al*., 2001). In a dairy effluent treated with *Nostoc* sp., the pH increased from 5.15 to 7.83 (Kotteswari *et al*., 2012). Increase in pH from 7.5 to 8.1, 9.0 and 8.7 with *Chlorella*, *Scenedesmus* and *Nostoc* respectively was also recorded on treatment of sewage wastewater after 24 days of incubation (Sethupathy *et al*., 2015).

Calcium content of the refinery wastewater was recorded as (111.31 ± 0.43 mg/l), which is well above the permissible limit of CPCB (1995) i.e., 100 mg/l. Similar observations were also made in wastewater treated with *Chlorella* sp., in which Calcium content reduced by 65.19 % after 7 days (Fathi *et al*., 2013). Another study on treatment of oil refinery by *Scenedesmus obliquus* showed calcium value reduction by 10.83 % after 15 days (Rajasulochana *et al*., 2009). Reports on decrease in calcium content by 67.10 % exists in the study on purification of river water by algae (Sengar *et al*., 1990).

The concentration of chloride in the present study was recorded to be (301.57 ± 0.14 mg/l). It was reduced to 32.04 % and 47 % on 10th and 20th day respectively in T_1. The value also showed slight reduction of 0.72 % on 10th day and 8.84 % on 20th day in T_0. A reduction of 7.06 % of Chloride was also recorded in wastewater treated with *Chlorella* sp. (Fathi *et al*., 2013). About 12 - 17 % reduction in value was also noted in the ossein effluent treated with cyanobacteria (Uma and Subramanian, 1990).

Analysis of total hardness in the Refinery wastewater recorded a value of (301.65 ± 0.2 mg/l), which was much above the desirable limit of 250 mg/l (CPCB, 1995). Similar results were also obtained on treatment of oil refinery effluent by *Scenedesmus obliquus* where a reduction of 6.34 % after a period of 15 days was noted (Rajasulochana *et al*., 2009).

The Oil and Grease content was recorded to be significantly high, i.e. (7970 ± 13.22 mg/l). Results on T_0 revealed a reduction of 29.34 % in 10 days and 61 % in 20 days. In T_1, test organism could reduce the oil and grease content by 55.33 % in just 10 days, which drastically reduced to 99.87 % in 20 days, with a value of only 10 ± 0.20 (mg/l), which is the permissible limit value by CPCB (1995). Our findings were concordant to the study conducted on the oil effluent treated with *Scenedesmus obliquus*, which showed a reduction value of 99.85 % after 15 days (Rajasulochana *et al*., 2009). The results were also comparable to another study conducted on treatment of textile dye industrial effluent with a consortium of *Chlorella vulgaris* and *Scenedesmus obliquus*, in which oil and grease content reduced by 53.65 % in 21 days (Elumalai *et al*., 2013).

Low DO is an indicator of polluted water. Study of the refinery wastewater recorded a very low content of DO. Its value was calculated to be (1.88 ± 0.10 mg/l), which was well below 4 mg/l, the permissible limit value of CPCB (1995). Our results were similar to the study conducted on wastewater sample treated with *Chlorella* sp., where the DO content increased by 50 % after an interval of 7 days (Fathi *et al*., 2013).

A high value of Biochemical Oxygen Demand (BOD) indicates the polluted status of a water body. In the present study, BOD of the refinery wastewater was recorded to be very high, (64.02 ± 0.42 mg/l), which was also much higher in comparison to the permissible limit (30 mg/L) (CPCB, 1995). The use of *Botryococcus* sp. for the treatment of food processing wastewater showed 61.11 % BOD reduction on the 15th day (Gani *et al*., 2016). There were also reports on treatment of oil effluent by *Scenedesmus obliquus*, where BOD reduced to 16.66 % after 15 days of incubation (Rajasulochana *et al*., 2009).

COD of the refinery wastewater was recorded to be (121.6 ± 3.32 mg/l). Percentage change of BOD and COD content on the 10th day was the same in both T_0 and T_1. Examination of COD on T_0 revealed a reduction of 22.56 % in 10 days and 41.93 % in 20 days of incubation. Whereas, on T_1, the value decreased upto (43.92 ± 6.11 mg/l) on 10th day, which further drastically reduced to 8.01 ± 6.244 (mg/l) on 20th day. These results could be compared to the study conducted on oil effluent by *Scenedesmus obliquus*, where COD was reduced to 82.80 % (Rajasulochana *et al*., 2009). Our findings were also concordant to the study conducted on wastewater with *Chlorella vulgaris* and *Scenedesmus quadricauda*, which could reduce 80.64 % and 70.97 % of COD content at the end of 15 days (Ayodhya, 2013).

From the results of the present study, it can be inferred that phycoremediation is an economically viable and effective practice to curtail toxicity of refinery effluents. The test organism *Tetradesmus dimorphus* (Turpin) M. J. Wynne can thus be recommended for use as a substitution for the conventional physical and chemical wastewater treatment methods. Further, proper screening of the test organism is suggested in order to ascertain their phycoremedial prospective in terms of heavy metal removal.

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Conflict of interest

The authors declare no conflict of interest.

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