Production of biodiesel based on the bioremediative potential of algae on industrial effluent

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Abstract: **The present study is aimed to check the bioremediative potential of microalga on industrial effluent with potential application on enhancement of lipid content for production of biodiesel. Isolation and identification of microalga from domestic pond, which is treated with textile effluent. The process optimization study using response surface methodology (RSM) with central composite design (CCD) to optimize the factors for textile effluent degradation by microalga where in pH, inoculum concentration and dilution factor were used as independent variables and decolourization, chemical oxygen demand (COD) reduction as dependent variables (response). Characteristics of untreated textile effluent were found to be highly pollution in comparison to TNPCB permissible limits. The maximum decolorization and COD reduction efficiency of *Oedogonium hatei* were found to be 95.16% and 90.74% respectively in the optimized condition of pH-6, inoculum concentration-1% and dilution factor-60% within 7 days. About 650mg of algal biomass was harvested from the optimized condition and it was utilized for biodiesel production. From this biomass 12.307% of lipid was extracted which was transesterified into 91.25% of biodiesel. Hence, it was concluded that *Oedogonium hatei* can be potentially applicable for the bioremediation and biodiesel production in the future with less manufacturing process & less cost effective.**

Keywords: **Microalgae, Bioremediation, Biodiesel, Chemical oxygen demand reduction, Decolourization, Response surface methodology.**

INTRODUCTION

Textile industries are responsible for major environmental pollution problems in the world for the reason of they release undesirable dyes, metals and other pollutants. The textile industry affects environment by its huge water consumption and the chemicals used in the manufacturing processes[1]. The textile industry wastewater contains high concentration of BOD, COD, TDS and pH after dyeing process[2]. Wastewater from textile industries needs proper treatment before its discharge into water bodies. The untreated or not properly treated textile effluent can be harmful to both aquatic and terrestrial life it affects natural ecosystem[3]. There are many processes for removal of pollutants from water that include physicochemical, biological and combined treatment processes. These effluents are not easy to treat using common physicochemical treatment methods which give good removal efficiency of suspended materials but these methods has some limitation such as its low efficiency of COD removing[4]. However, Biological treatment is found to be an efficient and economical process for the removal of pollutants such as dyes and color from wastewater, which is considered more cost-effective and environment-friendly[5].

In this context algae have good potential to treatment of textile wastewater and biomass production for sustainable biodiesel production. Microalgae can convert light, carbon dioxide, water and nutrients into energy and use that in cell development. The removal of organic pollutants by the algae is a promising bioremediation technology. Efficiency of algae for nutrient removal also varies with the type of wastewater and species selected for the remediation[6]. The advantages of algae-based bio treatment of wastewater are greater production biomass and it has high ability to accumulate, detoxify or degrade xenobiotics and pollutant load[7]. In addition, the biomass produced in bioremediation could be used in the form of bioenergy such as biodiesel[8]. Microalgae have 20%-80% of oil content in its dry weight[9]. The algae growth depends on the presents of nutrients in the textile wastewater. Phosphorous play an important role on algal growth that are support to formation of lipids and ATP[6]. Burning of petroleum based fuels causes accumulation of carbon dioxide in the environment and fuel price is increasing day by day. Petroleum oil spills may constitute a major source of contamination of the aquatic and terrestrial eco systems. Biodiesel combustion is more effective because of its higher oxygen content and its use in diesel engines shown great reduction of carbon monoxide, sulphur, hydrocarbons, smoke and noise[10].

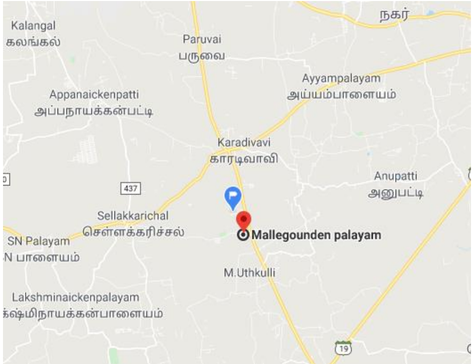
Thus, the current study is involved in environmental management for the protection of natural resources from hazardous and noxious agents. This work is design in such a manner that the algae can simultaneously degrade the toxic end product of the textile industry as well as generate alternative value added product like biodiesel keep this in mind we have developed this idea.

Materials and Methods

Collection, Isolation and Identification of algae sample

The algae sample was collected from domestic wastewater located at Mallegounden Palayam, Palladam, Tirupur, Tamil Nadu, India (10°56'56.9"N 77°11'45.8"E) (fig 1). Samples were collected using plastic container and the samples were transferred to the laboratory.

Figure 1: Location of algae sample collection

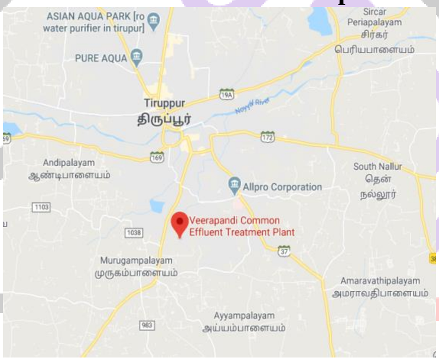


The algae sample were serially diluted for the isolation process and the pure culture were identified under microscopy. The microscopic mounts were prepared and examined under compound microscope (40X and 100X objectives) and inverted microscope to identify the shape, structure and other morphological characteristics. The isolated alga was cultivated using BG11 medium with pH 7.0[11].

Collection & Characterization of textile effluent

The textile waste effluent was collected from Veerapandi common effluent treatment plant, Tirupur, Tamil Nadu, India (11°04'28.7"N 77°20'56.7"E) (fig 2). The effluent was stored in refrigerator 4°C for further processing. The physio-Chemical parameters such as pH, Turbidity, Color, TDS, TSS and COD analyses of the water sample were carried out immediately according to APHA[12]. These parameters are compared with TNPCB standard values.

Figure 2: Location of effluent sample collection



Experimental design for optimization

Design Expert software (version 11.1.0.1) was used in this study to design the experiment and to optimize variables affecting decolourization and COD reduction. The experimental design employed in this work was a Response surface methodology with Central composite design (CCD). pH (A), Inoculum Concentration(B) and Dilution factor(C) were selected as independent variables and Decolourization, COD reduction used as response for the optimization study. The low and high levels of independent variables are for pH (3-9), Inoculum concentration (1-3) and Dilution factor (60%-100%). Table 1 shows that values of the set of experiments and this experimental plan used for to perform the optimizing study. The whole setup is kept in light and incubated for 7 days period. Color and COD were measured at initial and at the end of each run.

Decolourization and COD reduction percentage was calculated by following equation,

$$\text{Decolourization } \% = \frac{\text{Initial absorbance -final absorbance}}{\text{Initial absorbance}} \times 100$$

$$\text{COD reduction } \% = \frac{\text{Initial value -final value}}{\text{Initial value}} \times 100$$

Table 1: Experimental plan for optimizing study

Run	Factor 1 pH	Factor 2 Inoculum Conc. %	Factor 3 Dilution factor %
1	6	1	80
2	9	2	80
3	6	2	80
4	3	3	100
5	3	2	80
6	6	1	60
7	9	1	60
8	9	3	60
9	9	1	100
10	3	3	60
11	6	3	80
12	6	2	60
13	6	2	100
14	9	3	100

Statistical analysis

Statistical analysis was performed using the Design expert software. Data were analyzed by the analysis of variance (ANOVA), and *p*-value lower then 0.05 was considered to be significant in surface response analysis. The three-dimensional response surface methodology with central composite design analysis of independent variables (pH, Inoculum concentration and Dilution factor) and response factors were used to estimate the optimal values of the factors that affecting the degradation.

Phytotoxicity analysis of untreated and treated textile effluent

The optimized run and untreated effluent were subjected to phytotoxicity analysis for analyzing the production of Toxic end products in the treated wastewater. Plant growth parameters namely germination percentage, seedling survival and seedling height have been taken as criteria to assess plant response to specific pollutants. Untreated effluent had an inhibitory effect on seed germination and seedling growth because of its toxicity. The 20 seeds of sorghum (*Sorghum bicolor*) were collected. The seeds were cultivated on pots and watered with treated textile effluent and untreated effluent samples. The germination of seeds was observed and root length, shoot length and seedling length were measured after 10 days.

Biodiesel Production

Biomass cultivation

The algal cultures grown under the optimized condition (pH-6, Inoculum concentration-1% and Dilution factor-60) and incubate 30 days. After incubation, biomass was collect through centrifugation.

Lipid extraction

The cultivated biomass was dried for releasing water. Then dried alga was ground with mortar and pestle as much as possible. The algal powder was weighed and placed in glass vessel. It was homogenized with chloroform/methanol (2:1 v/v). The whole mixture is blended for 15-20 minutes at room temperature. The homogenate is centrifuged to recover the liquid phases. To this added 0.2 volume of water and the mixture is centrifuged at low speed (2000rpm) to separate the two phases. After centrifugation, remove the upper phase and the lower chloroform phase containing lipids. The solvent was evaporated and placed into a previously weighed glass vessel and the vessel was weighed again to obtain the lipid content of the sample[13].

The oil yield efficiency of microalga was then calculated by following equation,

$$\text{Oil yield (\%)} = \frac{\text{Mass of oil extracted (g)}}{\text{The total mass of dried alga (g)}} \times 100$$

Acid Catalyzed Transesterification

The algal oil was measured and mixed with two volume of methanol and 3.5% of concentrated H_2SO_4 as catalyst and it was reflux at 60°C for 90 minutes. The mixture was then allowed to settle down for 12 hours. There is two distinct liquid phases occurred. Crude ester (biodiesel) phase at the top and glycerol phase present at the bottom[14].

Quality of biodiesel

The quality of biodiesel was examined by visual examination, checking its neutral pH. For wash test, unwashed biodiesel was allowed to settle for 12 hours in room temperature. From glycerol layer, biodiesel was separate and to this equal volume of water added. This mixture was placed in table for separation of water from biodiesel. After separation, the pH of the biodiesel was determined. For methanol test, one part of biodiesel mixed with nine parts of methanol and stirred for 2 minutes. Then this mixture was allowed to settle for 30 minutes and checked for clearance of mixture and methanol solubilization in biodiesel. If methanol is completely soluble in biodiesel it indicates there is no undissolved materials was produced at the bottom and the mixture was given a single phase[15].

Yield of biodiesel

After separation and purification of biodiesel, the yield of biodiesel is estimated by following equation,

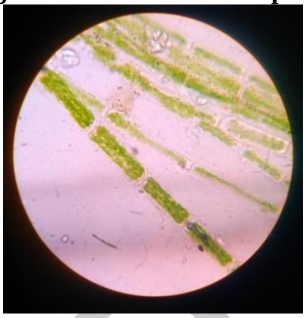
$$\text{Yield of biodiesel (\%)} = \frac{\text{Weight of the biodiesel produced (g)}}{\text{Weight of the algal oil used (g)}} \times 100$$

Result and Discussion

Identification of algae

The isolated microalga was identified under the microscope as *Oedogonium hatei* (fig.3). *Oedogonium hatei* is a multicellular alga which is green, filamentous and possess unbranched plant body, cylindrical cells with reticulate chloroplast and it was authenticated (BSI/SRC/5/23/2020/Tech/489) by Botanical Survey of India (South Zone), Coimbatore.

Figure 3: *Oedogonium hatei* under compound microscope



Characteristics of textile industry wastewater

Table 2: Initial values of the untreated textile wastewater and TNPCB standard values

Parameters	Measurements	TNPCB Standard
Chemical Oxygen demand (mg/l)	1440	250
Color (absorbance at 490nm)	0.876	-
Total dissolved solids (mg/l)	18.64	2100
Total suspended solids (mg/l)	5.4	100
Turbidity (660nm)	0.6922	-
pH	9.5	5.5-9

Immediately after collection of the effluent sample, characteristics of wastewater such as COD, Color, pH, Turbidity, TDS and TSS was calculated and it was compared to TNPCB standard values. Textile effluent was dark greenish in color with high pungent smell. Data in Table 2 indicates some initial analyses of textile wastewater, which was generally characterized by high chemical oxygen demand (1440 mg/l) and pH (9.5). Total dissolved solids, Total suspended solids and turbidity were found to be 18.64mg/l, 5.4mg/l and 0.6922 respectively. pH of the wastewater was found very strongly alkaline, because in textile industries mostly alkaline substances are used during manufacturing process and the other parameters like Chemical oxygen demand were found above permissible limit. It was having organic load as the COD was five times higher than the discharge limit. Thus, the collected textile wastewater indicates highly pollution.

Analysis of CCD trials

Design expert software gave as set of 14 runs at different range of pH, Inoculum concentration and dilution factor. Table 3 shown that the experimental design matrix along with predicted and actual values of response (Decolourization% & COD reduction %). Figure 4 (a&b) showed the color reduction of textile effluent before and after treatment. The concentration of both color and COD recorded at the end of all runs were decreased than initial concentration. Above 70% of COD reduction was obtained in all runs, it indicates toxic substances decreased in all runs after algal treatment. Microalgae produce oxygen by photosynthesis, which enhances the degradation of organic matter present in textile effluent and decreases its toxicity[16].

Considering three different independent variables based on the 14 runs, the result of all the 14 runs are discussed as follows,

Run 1: (pH- 6, Inoculum concentration- 1%, Dilution factor- 80%)

For run 1, decolourization and COD reduction was found to be 53.94% and 73.4% respectively. It proclaimed that having the lack of algal cells while the effluent concentration high would result in unutilized nutrients in effluent. However, the degradation efficiency of alga increased in minimum concentration of effluent than maximum concentration even the algal biomass is low.

Run 2: (pH- 9, Inoculum concentration- 2%, Dilution factor- 80%)

In these conditions, 68.59 % of color reduction and 84.51 % of COD reduction was achieved. Alga was effectively reduced the COD of effluent due to the adaptation and growth of algae in strong alkaline condition and 80% concentration of effluent. Nevertheless, the color reduction process is not effective because of presence of toxic dyes in high concentration of effluent decrease the proliferation of alga.

Run 3: (pH- 6, Inoculum concentration- 2%, Dilution factor- 80%)

The reduction rate of color and COD was found to be 58.02 % and 80.47 % respectively. High concentration of wastewater having enormous toxicity it is negatively impact on decolourization process by inhibiting the growth of algae even the pH is 6. It indicates the degradation ability of algae is depending on the relationship between concentration of effluent and inoculum concentration.

Run 4: (pH- 3, Inoculum concentration- 3%, Dilution factor- 100%)

Percentage removal of color and COD was found to be 81.89% and 88.34% respectively under this condition. Acceptable reduction rate of color and COD was found in maximum concentration of both inoculum and dilution factor even the pH is strong acidic condition, but it was not much effective than minimal effluent concentration and pH 6.

Run 5: (pH- 3, Inoculum concentration- 2%, Dilution factor- 80%)

The color and COD removal efficiencies at these conditions were 78.09% and 85.52% respectively. There is dilution factor is 80% moreover pH is strong acidic condition this combined effect negatively affect the enzymatic activity in decolourization and cellular growth of algae.

Run 6: (pH- 6, Inoculum concentration- 1%, Dilution factor- 60%)

The maximum reduction efficiency of microalga was observed in this design of experiment. It was provided the maximum decolourization of 95.17% and COD reduction of 90.74%. It can be concluded that pH 6, inoculum concentration of 1% and dilution factor of 60% was found to be favorable condition for the excellent reduction of color and COD. Alaguprathana and Poongothai (2019)[17] found 97% of decolourization at pH 6 for *Oedogonium subplagiostomum* AP1. This agrees with the findings of our study where the pH 6 is more favorable condition for cellular growth of *Oedogonium sp.* This microalga was effectively reducing organic matter and uptake dyes of effluent in these optimized conditions.

Run 7: (pH- 9, Inoculum concentration- 1%, Dilution factor- 60%)

Removal efficiencies of color and COD were found to be 64.21 % and 83.79 % respectively in this run. Microalga could tolerate and grown in this condition but the rate of biomass decreased as a result both COD reduction and decolourization efficiency was declined.

Run 8: (pH- 9, Inoculum concentration- 3%, Dilution factor- 60%)

About 88.91% and 89.35% of decolourization and COD reduction obtained respectively for this run. Higher rate of algal cells can effectively degrade organic matters present in effluent even the pH is strong alkaline condition. Aragaw and Asmare (2018)[18] mentioned that biological treatment of effluent could effectively decolorize in pH range of 6-9. This could imply maximum initial inoculum with minimum dilution factor significantly affects the reduction of both color and COD, resulting in bioremediation of wastewater.

Run 9: (pH- 9, Inoculum concentration- 1%, Dilution factor- 100%)

The minimum reduction of both color and COD was found to be 28.17% and 72.08% respectively in this run. In this run, the bioremediation efficiency of alga hugely affected due to the negative influence of process variables. From this result, it can be conclude that degradation ability of alga decreased at increase in concentration of effluent, strong alkaline condition with minimum initial inoculum concentration. Lim et al., (2010)[19] observed that the growth of *C. vulgaris* decreases when the concentration of textile wastewater increases.

Run 10: (pH- 3, Inoculum concentration- 3%, Dilution factor- 60%)

The reduction of color and COD was found to be 71.81 % and 84.73 % respectively. In this run, acceptable remediation efficiency was obtained even the pH is 3 for the reason of presence of high concentration of initial inoculum and the effective growth of algal cells occurs than other dilution range of effluent.

Run 11: (pH- 6, Inoculum concentration- 3%, Dilution factor- 80%)

For run 11, the removal percentage of color and COD was obtained 55.19% and 75.42% respectively. Interaction effect of maximum algal inoculum concentration and high wastewater concentration causes decrease the development of algae. Hence, it was not provided effective elimination of color and COD compared than 60% concentration of effluent and 1% algal inoculum. Moreover, maximum concentration of algal inoculum which leads to self-shading, an accumulation of auto-inhibitors and a reduction in photosynthetic efficiency[20].

Run 12: (pH- 6, Inoculum concentration- 2%, Dilution factor- 60%)

Under these conditions, COD reduction and decolourization found to be 90.27 % and 92.89 % respectively. Alga grows well in this condition and satisfactory degradation was obtained it shows the elimination of organic matter and utilization of dyes from waste effluent owing to favorable condition. pH 6 is more favorable than pH of 3 or 9 and it can increases degradation ability of microalgae.

Run 13: (pH- 6, Inoculum concentration- 2%, Dilution factor- 100%)

About 68.31 % of decolourization and 83.73 % of COD reduction was recorded in these conditions. The concentration of effluent mostly affects the decolourization process than COD reduction even the pH is favorable it was because of the more toxicity of the dyes in maximum concentration.

Run 14: (pH- 9, Inoculum concentration- 3%, Dilution factor- 100%)

The reduction rate of color and COD was found to be 80.79 % and 85.36 % respectively. This run shows an acceptable range of degradation but not much effective than at pH is 6. Therefore, the degradation ability of microalga was depending on the relationship between the factors of pH, Inoculum concentration and dilution range of effluent.

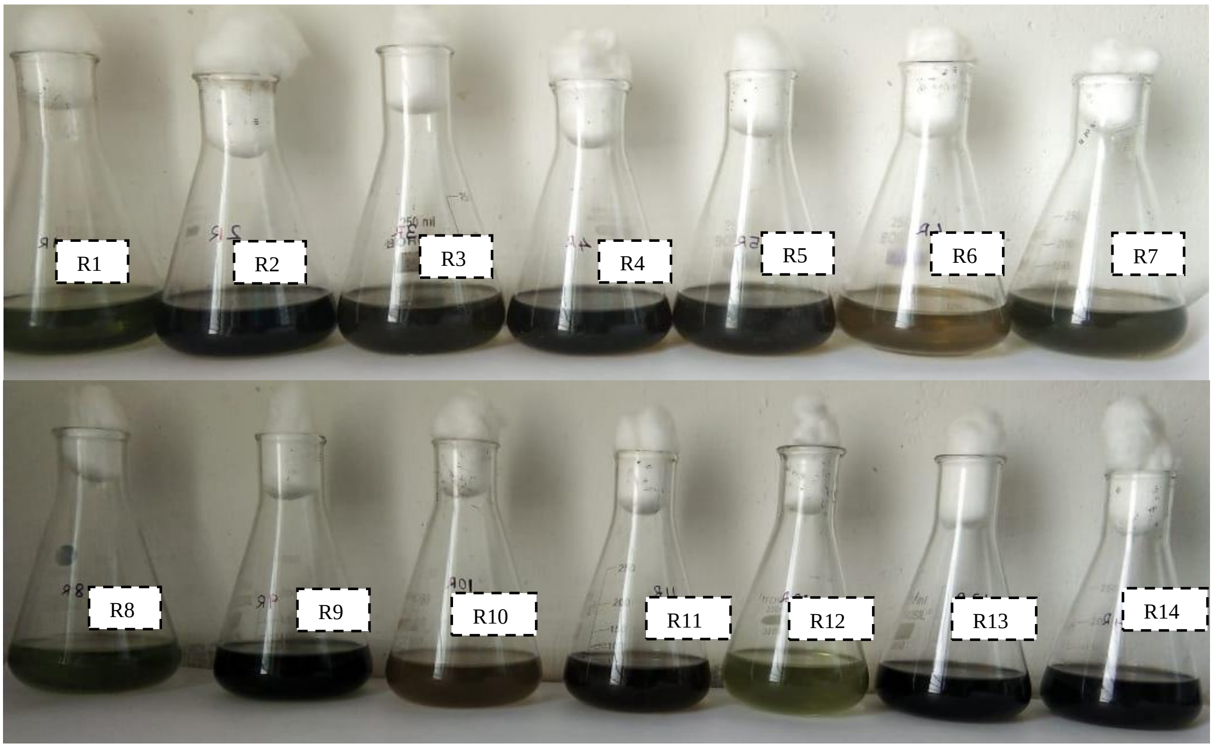
Effects of three different process parameters: pH, Inoculum concentration and dilution factor were investigated. Based on the above results it was found that the optimal range of pH-6, Inoculum concentration-1% and dilution factor-60% was found to be run 6. Where there is a reduction in around 90.74% of COD and 95.17% of decolourization efficiency, which we have taken it for the further analysis and biodiesel production.

Table 3: Design matrix with corresponding results of Decolourization% and COD reduction% based on the experimental and predicted values

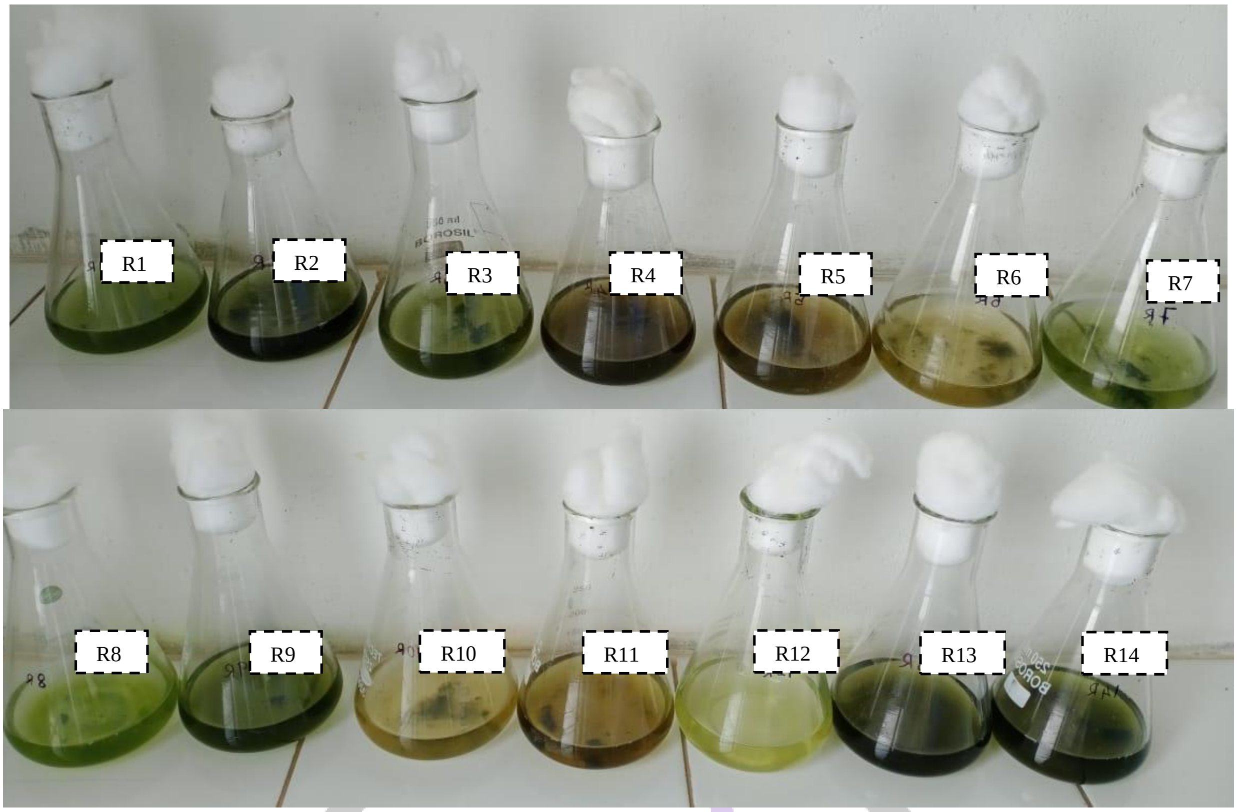
Run	Factor 1 pH	Factor 2 Inoculum Conc. %	Factor 3 Dilution factor %	Response 1 Decolourization%		Response 2 COD reduction%	
				Actual	*Predicted*	*Actual*	*Predicted*
1	6	1	80	53.94	53.00	73.4	74.30
2	9	2	80	68.59	59.42	84.51	81.63
3	6	2	80	58.02	63.42	80.47	80.57
4	3	3	100	81.89	77.41	88.34	86.89
5	3	2	80	78.09	81.81	85.52	86.85
6	**6**	**1**	**60**	**95.17**	89.67	**90.74**	87.73
7	9	1	60	64.21	71.42	83.79	86.38
8	9	3	60	88.91	88.90	89.35	88.99
9	9	1	100	28.17	27.39	72.08	71.59
10	3	3	60	71.81	72.57	84.73	84.86
11	6	3	80	55.19	56.19	75.42	75.97
12	6	2	60	92.89	90.42	90.27	90.92
13	6	2	100	68.31	70.83	83.73	84.54
14	9	3	100	80.79	83.53	85.36	86.50

Figure 4: Decolourization of textile effluent – (a) Before and (b) after treatment

a)



b)



Statistical analysis

The results obtained by Analysis of variance (ANOVA) for response surface model indicates that it was a significant model (Table 4 (a) & (b)). The Model F-value was found to be 7.04 and 6.16 for decolourization and COD reduction respectively and it implies the model is significant. There is only a 3.79% chance for decolourization and 4.78% chance for COD reduction that an F-value this large could occur due to noise. P values less than 0.05 indicate model terms are statistically significant. In this case, the model terms are A, C, AB, BC, C^2 had significant effects on the decolourization and C, B^2, C^2 had significant effects on the COD reduction. It was implies that these factors/interactions have significant effect on decolourization and COD reduction. The coefficient of determination (R^2) of 0.9406 & 0.9327means 94.06% & 93.27% of variation in response to decolourization and COD reduction respectively. Adjusted R^2 of the model was found to be 0.8069 & 0.7813 for decolourization and COD reduction respectively, which implies the relationship between independent variables and response, by regression model. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 9.390 for decolourization and 8.109 for COD reduction indicates an adequate signal. Analysis of the results showed that the significance and adequacy of the model. Scatter plot (fig 5 a&b) showed a satisfactory correlation between actual and predicted values of percentage of decolourization and COD reduction, wherein the points cluster around regression line, which indicates the good fit of the model, since the deviation between the actual and predicted values was low.

Table 4 (a). Analysis of variance (ANOVA) for response surface model (Response 1: Decolourization)

Source	Sum of Squares	Df	Mean Square	F-value	Prob>F
Model	3995.42	9	443.94	7.04	0.0379 (significant)
A-pH	499.21	1	499.21	7.91	0.0482
B-Inoculum Conc.	11.46	1	11.46	0.1816	0.6919
C-Dilution factor	673.53	1	673.53	10.68	0.0309
AB	782.55	1	782.55	12.41	0.0244
AC	30.18	1	30.18	0.4784	0.5272
BC	488.02	1	488.02	7.74	0.0497
A^2	118.42	1	118.42	1.88	0.2425
B^2	193.49	1	193.49	3.07	0.1548
C^2	736.46	1	736.46	11.67	0.0269
Residual	252.32	4	63.08		
Cor Total	4247.75	13			
R^2-0.9406 **Adjusted R^2**- 0.8069 **Predicted R^2**- -0.1635 **Adeq Precision**-9.3896					

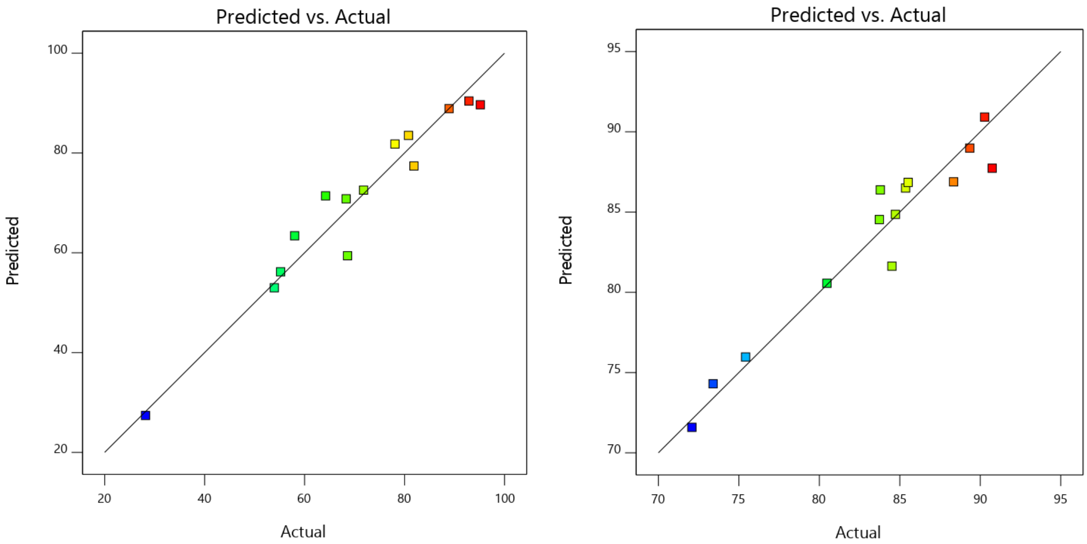
Table 4 (b). Analysis of variance (ANOVA) for response surface model

(Response 2: COD reduction)

Source	Sum of Squares	Df	Mean Square	F-value	Prob>F
Model	441.06	9	49.01	6.16	0.0478 (Significant)
A-pH	27.10	1	27.10	3.41	0.1387
B-Inoculum Conc.	3.13	1	3.13	0.3929	0.5648
C-Dilution factor	71.47	1	71.47	8.98	0.0400
AB	34.80	1	34.80	4.37	0.1046
AC	5.91	1	5.91	0.7430	0.4373
BC	49.46	1	49.46	6.22	0.0672
A^2	30.83	1	30.83	3.88	0.1204
B^2	73.27	1	73.27	9.21	0.0386
C^2	127.53	1	127.53	16.03	0.0161
Residual	31.82	4	7.96		
Cor Total	472.88	13			
R^2-0.9327 **Adjusted R^2**- 0.7813 **Predicted R^2**- -1.4377 **Adeq Precision**-8.1092					

Figure 5: Scatter plot showing Actual vs Predicted values of percentage of (a) color reduction and (b) COD reduction

(a) **(b)**



Optimization of factors for decolorization & COD reduction

The three-dimensional response surface graph (fig 6,7,8 (a) & (b)) shows the significant relationship between independent variables (A, B & C) and response (Decolourization & COD reduction) in graphically. The factors (pH, Inoculum concentration and dilution factor) were optimized for maximum decolourization and COD reduction of the effluent. The interaction effect of pH and Inoculum concentration on response of decolourization and COD reduction was shown in fig 6. The relationship between inoculum concentration and dilution factor on both responses was depicted in fig 7 were inoculum concentration of 1% and dilution factor of 60% was providing the maximum reduction in color and COD in the wastewater. The minimum concentration of dilution factor was shows significant influence of decolourization and COD reduction. From this experiments, the result is obtained shows that pH of 6, Inoculum Concentration of 1% and Dilution factor of 60% were the best conditions to obtain maximum reduction of both color and COD of 95.16% and 90.74% respectively.

Figure 6: 3D surface plot of Interaction effect of pH & Inoculum concentration on influence of (a) Decolourization and (b)

COD reduction

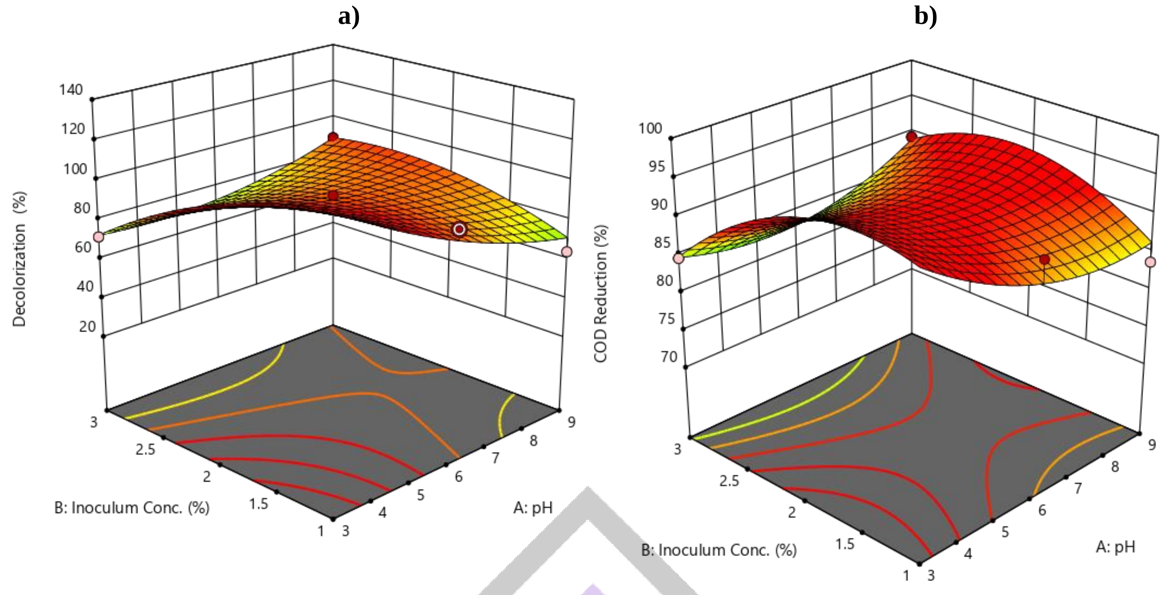


Figure 7: 3D surface plot of Interaction effect of Inoculum concentration & Dillution factor on influence of (a) Decolourization and (b) COD reduction

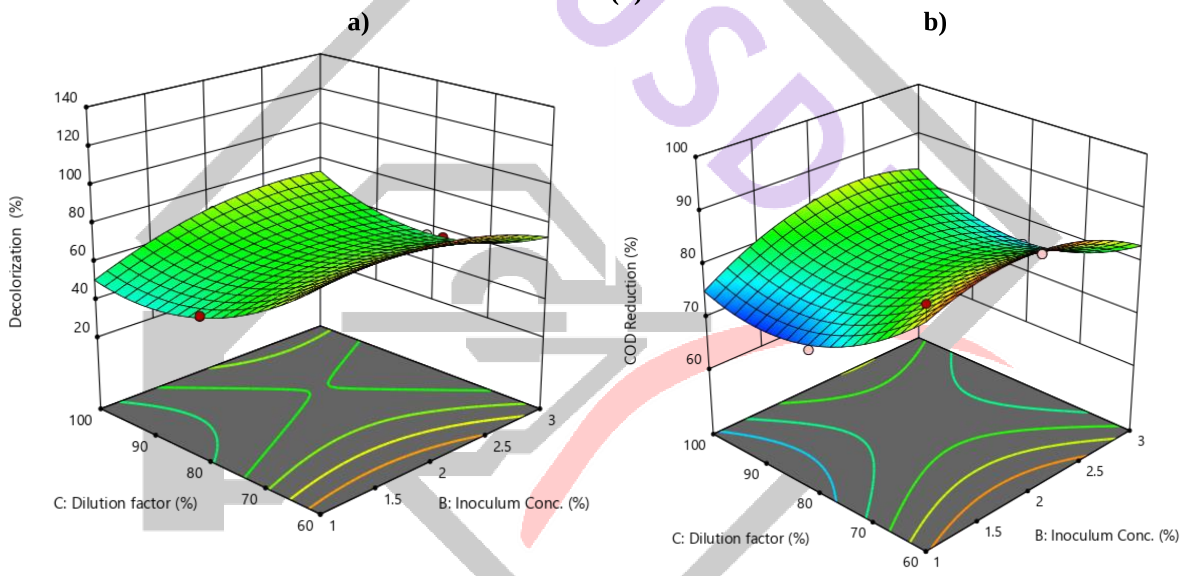
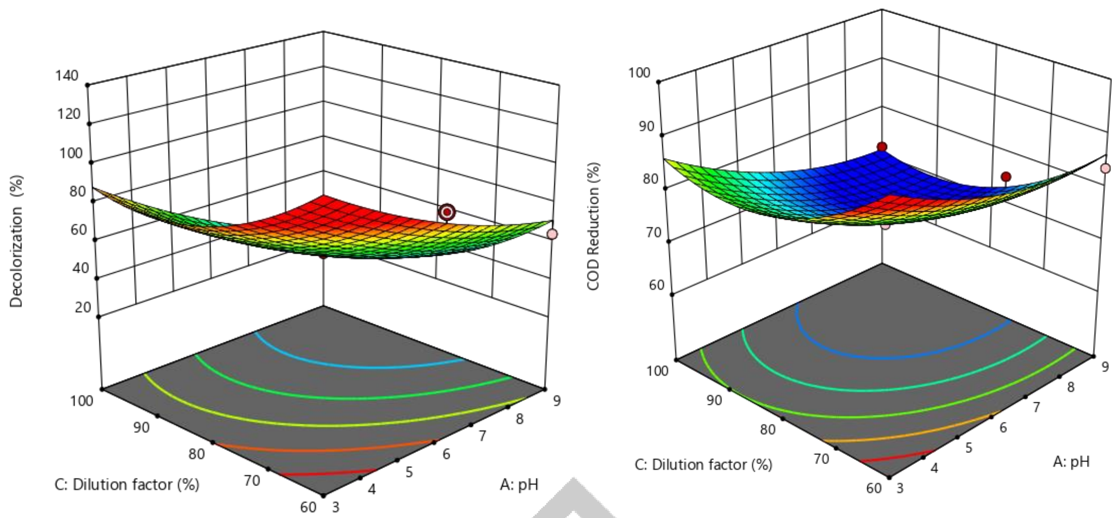


Figure 8. 3D surface plot of Interaction effect of pH & Dilution factor on influence of (a) Decolourization and (b) COD reduction

a) b)



Phytotoxicity analysis

The plant growth parameters of germination percentage, seedling length, root length and shoot length were observed and measured after 10 days. The parameters of plants watered with treated and untreated effluent was found to be 70%, 4.5cm, 3.0cm, 0.1cm and 50%, 2.0cm, 2.0cm, 0.1cm respectively. The treated effluent was achieved better germination percentage, seedling length and root length than untreated effluent. Toxicants present in the untreated effluent were inhibiting the plant growth. Generally, algae do not produce toxic substances unlike other microorganisms such as bacteria and fungi[20]. This shows that algal bioremediation could act as efficient tool whereby the algae is develop detoxifying toxic end products and reduce organic content by more than 90% which is found to be efficient model in treating the textile dyes in an insitu model. This shows that algal bioremediation has the potential to clean up hazardous compounds at the riverbeds.

Biodiesel production

Biomass harvesting

After 30 days of incubation, the algal biomass was harvested from optimized condition of wastewater. About 650mg of dry weight of alga was obtained in 6th run of experiment.

Lipid extraction

About 650mg of algal powder was applied to folch method to extract the lipid content in alga. After lipid extraction, the solvents were evaporated and the weight of algal oil was found to be 0.08gram. The percentage of algal oil was calculated. About 12.307% of oil extracted from 650mg of algal powder. Adegoke et al., (2018)[14] observed that *Oedogonium capillare* contains 8.18% of oil in its dry weight which differs from in this research. It was because of the lipid content of algae varies from each strain. Moreover, under stress conditions many algae are alter their lipid biosynthetic pathways towards the formation of triacylglycerol (TAG)[21].

Quality of Biodiesel

Biodiesel was subjected to wash test and methanol test. The biodiesel was purified by washing with water to remove all the residual by-products like excess alcohol, excess catalyst and glycerin, it was found very less and biodiesel phase from water separated in less than 15 minutes. Methanol also completely dissolved in biodiesel and there is no undissolved materials found at the bottom. The pH of the biodiesel found to be neutral.

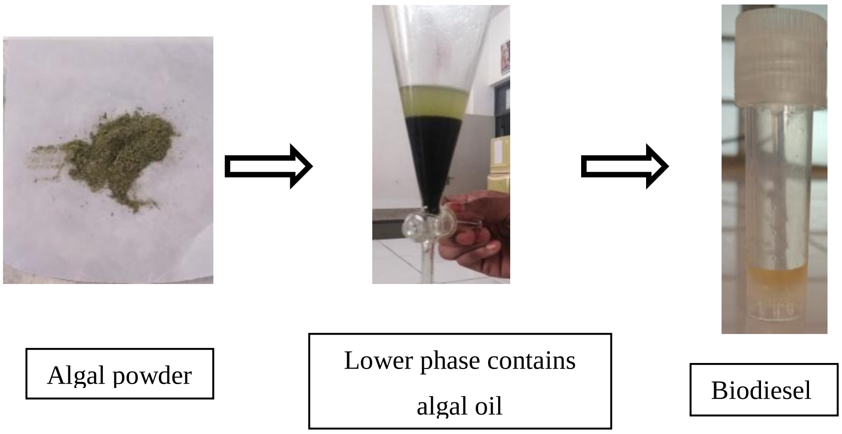
Yield of biodiesel

About 0.08g of algal oil was transesterified into 0.073g of biodiesel (fig. 9). The percentage of biodiesel yield was calculated. Table 5 shows that 91.25% yield of biodiesel was obtained for 0.08 gram of lipid from *Oedogonium hatei*.

Table 5: Biodiesel yield from *Oedogonium hatei*

Alga	Dry weight of alga (g)	Extracted oil (g)	Extracted biodiesel from oil (g)	Yield of biodiesel %
Oedogonium hatei	0.65	0.08	0.073	91.25

Figure 9. Biodiesel production



Conclusion

The proper treatment of wastewater is necessary for clean environment. The isolate *Oedogonium hatei* was selected for the treatment of textile wastewater and potential application of biodiesel production. In this work, the algae successfully utilized dyes and degraded organic matter present in wastewater for its metabolic process supporting to increase biomass as well as lipid synthesis and reduced color, COD in the effluent. The factors (pH, Inoculum concentration and dilution factor) were optimized for maximum decolourization and COD reduction of the effluent. In the range of pH-6, inoculum concentration-1% and dilution factor-60% was provided the maximum COD and color reduction within 7 days. The treated effluent was achieved better germination percentage, seedling length and root length than untreated effluent. Toxicants present in the untreated effluent were inhibiting the plant growth. Therefore, *Oedogonium hatei* was highly potential to remove the toxicants from effluent. About 650mg of dry weight algae was harvested from the optimized condition and it was yields 0.08 g of oil, which was transesterified into 0.073g of biodiesel. The present study was showed the bioremediative potential of *Oedogonium hatei* on industrial effluent with sustainable biodiesel production. It was reduced the cost for biodiesel production and wastewater treatment. Production of biodiesel from *Oedogonium hatei* will be a good source for the better yield with minimum manufacturing process as well as cost effective. This could be alternative way for the treatment of industrial wastewater into beneficial application.

Acknowledgement

The authors are grateful to DST-FIST Scheme, DBT-Star Scheme, Management, Principal and Deans of Dr. N.G.P Arts and Science College (Autonomous) for their extended support of this work**.** Communication number: DrNGPASC 2020-21 BS024.

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