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Biodiesel Production By Using Native Micro Algae From Food Processing Wastewater In Shake Flask Cultures

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Abstract
The present study describes the biodiesel production from food processing wastewater by using Chlorella vulgaris, Botryococcus braunii and mixed algae (Chlorella vulgaris, Botryococcus braunii, Nostoc and Anaebena). Continuous monitoring of parameters like pH, Volatile fatty acid, Chemical oxygen demand, Biochemical oxygen demand, Alkalinity, Sulphates were carried out to asses the efficiency of the treatment process. The micro algal oil was extracted with different solvents like hexane, benzyl alcohol, Iso amyl alcohol, dichloro methane, dichloro ethane, proponal and methanol using distillation. The catalyst concentration (0.00, 0.50, 1.00, 1.50, 1.50 and 2.00%) and Catalyst type (sodium hydroxide, potassium hydroxide, sodium methoxide and potassium methoxide) were studied for the production of biodiesel. The experimental results showed that more than 88% of COD and BOD were removed. Benzyl alcohol and hexane were found to be the best solvents for extraction of algal oil. The biodiesel was characterized by using gas chromatography with mass spectrophotometer. Sodium and potassium hydroxide offered the higher yields when compared to their corresponding methoxides. The results clearly indicated that the optimum concentration of NaOH required for effective transesterification of algal oil was 1.00% for the production of biodiesel. The properties of FAME investigated in this study satisfied nearly all prescribed ASTM D6751.
Keywords: Biodiesel, Transesterification, Microalgal oil, Alkali catalyst, Chlorella vulgaris, Botryococcus braunii

1.0 **Introduction**

Continuous exploitation of fossil fuels is now widely recognized as unsustainable. The ever growing concerns on the rapid depletion of fossil fuels, climate change and global warming necessitates the need for renewable and carbon neutral transport fuels for both environmental and economic sustainability. Biodiesel is commercially produced currently from plant and animal oils, but not from micro algae which are photosynthetic microorganisms that are ubiquitous in any aquatic ecosystem. High quality biodiesel production from micro algae appears to be a major renewable source of energy capable of meeting the global demand for transport fuels. Micro algae exceed both in terms of efficient utilization of solar radiation and productivity of the best producing oil crops [1,2]. It also contributes no net carbon dioxide or sulfur to the atmosphere and emits less gaseous pollutants than conventional diesel fuel [3]**.**

If the transport fuel in India has to be replaced totally with biodiesel, oil crops, waste cooking oil and animal fat alone cannot realistically meet this demand. Even meeting only half of the existing transport needs would require large areas for cultivation of major oil crops. Apparently, oil crops cannot significantly contribute to replacing petroleum derived liquid fuels in near future. This scenario can be changed dramatically, if micro algae are used to produce biodiesel. In view of the above facts, micro algae appear to be the only viable biodiesel that has the potential to completely replace fossil fuel. Micro algae, unlike other oil crops, grow extremely rapidly and many species are exceedingly rich in oil. They commonly double their biomass within 24 h. However, biomass doubling times during exponential growth are commonly as short as 3.5 h [4]. The oil content in micro algae at times can exceed 80% by weight of dry biomass [5] while oil levels of 20-50% are quite common.

Micro algae have been suggested as very good candidates for fuel production because of their advantages of higher photosynthetic efficiency, higher biomass production and faster growth compared to other energy crops [6, 7]. Heterotrophic growth of some micro algae has been used for efficient production of biomass and some

metabolites such as lipid [8], which can reduce the cost of micro algal biomass production and micro algal oil production. However, most of the research has concentrated on biodiesel production from vegetable oil such as soybean oil, sunflower oil, palm oil and rapeseed oil [9, 10, 11, 12]. Till now to our best knowledge, no experimental studies have been carried out on the production of biodiesel from native micro algae like chlorella.vulagaris, Botryococcus.braunii and mixed algae from food processing wastewater. Hence in the present study an attempt has been made to evaluate the efficiency of microalgae for the production of biodiesel and simultaneous treatment of food processing wastewater. The specific objectives of the present study includes

- Isolation of micro algae like Chlorella vulgaris, Botryococcus braunii from contaminated lake.
- Treatment of food processing wastewater using isolated algae (Chlorella vulgaris, Botryococcus braunii) and mixed algae(Chlorella vulgaris, Botryococcus braunii, Nostoc and Anaebena).
- Separation of algal oil using different solvents like hexane, benzyl alcohol, Iso amyl alcohol, dichloro methane, dichloro ethane, proponal and methanol
- Optimization of catalyst concentration (0.00, 0.50, 1.00, 1.50, 1.50 and 2.00%) and Catalyst type (sodium hydroxide, potassium hydroxide, sodium methoxide and potassium methoxide) for the production of biodiesel.

2.0 Materials and Methods

2.1 Isolation and purification

The samples were collected from contaminated Lake of Hyderabad, India and cultured in different selective medias of their respective particular algae. The algae were subjected to purification by serial dilution followed by plating. The individual colonies were isolated and inoculated into selective liquid medium and incubated at 25 ± 1ºC under 1.2 ± 0.2 Klux light intensity with 16:8 hrs light photoperiod. The purity of the culture was ensured by repeated plating and by regular observation under microscope.

2.2 Cultivation:

The above isolated and purified algae will be cultivated initially in a 250ml Erlenmeyer flask containing 150ml (100ml food processing wastewater + 50ml culture) medium for a period of three weeks. The culture flasks were incubated at 25 ±1°C klux light intensity with 16:8 hours light and dark cycle. The pH was maintained at 7.0 by the addition of carbon dioxide. The experimental setup is presented in Fig.1. The conical flasks are placed on a magnetic stirrer for continuous and homogenous mixing. Dissolved oxygen concentration was controlled by increasing agitation speed and airflow. Aeration rate and the agitation speed were variable and initially set at 0.5 vvm and 300 rpm. Temperature was controlled 28±1 ◦C.

2.3. Wastewater source and characterization

The wastewater for this study was obtained from the Food processing industry in Hyderabad and characterized using standard methods [13]. The characteristics of food processing wastewater used for the study were (mg/l): colour (orange), total dissolved solids (1700), total suspended solids (260-270), COD (3500-4000), BOD (1250-1300), volatile fatty acids (30-35), alkalinity as CaCO3 (210-220), chlorides (140-160), nitrates (120-170), sulphates (300-450), phosphates (40-50). The pH of the food processing wastewater was 7.0–7.5. The BOD: COD ratio of the wastewaters was in the range 0.45–0.6, which is amenable to biological treatment. All the chemicals used were of analytical reagent grade. Water used in all the experiments was. Laboratory distilled water with pH (7.2–8.0), Alkalinity (40–120 mg/l), chlorides (20–30 mg/l).

2.4. Analytical methods

Analysis of Alkalinity, phosphates, sulphates, BOD, Chlorides, Total suspended solids (TSS), nitrates and COD were conducted in accordance with Standard Method [13].

2.4.1 Biomass estimation

The cultures were harvested and the cells were washed with distilled water after centrifugation at 5000 rpm. Then the pellet was freeze dried. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms of dry weight.

2.4.2 Chlorophyll estimation

A known volume of culture was centrifuged (8000 rpm) for 10 min and the pellet was treated with known volume of methanol and kept in water bath for 30 min at 60ºC. Absorbance of the pooled extracts was measured at 652 and 665 nm and chlorophyll (a + b) was estimated [14].

4.4.4 Protein estimation

Protein content in the cell free medium was analyzed by Bradford protein assay [15].

4.4.5 Separation of Algal oil

The algae cells were harvested by centrifugation, washed with distilled water, and then dried in a freeze dryer. Micro algal oil was prepared by pulverization of heterotrophic cell powder in a mortar and extracted with different solvents like hexane, benzyl alcohol, iso amyl alcohol, dichloro methane, dichloro ethane, proponal and methanol using distillation. The

extraction was executed on a water bath for 6h with 1L of solvent. The solvent of distilled off under vaccum in a rotary evaporator at 45°C and the algal oil was recovered.

4.4.6 Transesterification of Algal oil

Experiments were designed to ascertain the effect of catalyst type and concentration on the transesterification of algal oil. The catalysts types were varied as sodium hydroxide, potassium hydroxide, sodium methoxide and potassium methoxide. The catalysts concentrations were used as 0.00, 0.50, 1.00, 1.50, 1.50 and 2.00%. The reaction temperature was kept constant at 60°C. The oil/methanol ratio was set at 1:6 and the stirring intensity was kept at 200 rpm. The trasesterification was carried out using a 1L round bottomed reactor, equipped with thermostat mechanical stirrer, sampling outlet and condensation systems. A fixed amount of freshly prepared alcoholic solutions of catalyst were added in to the algal oil established for each experiment and mixed, taking this movement as time of zero of the reaction. Each experiment was allowed to prolong for 120 minutes conduct to ensure the complete conversion of fatty acids in to FAMEs. After cooling and filtrating, two phases were formed. The upper phase consisted of methyl esters and the lower phase contained glycerol and the excess methanol. After separation of two layers by sedimentation, the methyl esters were purified by distilling the residual methanol at 65˚C.

$$\text{Yeild of Methyl esters} = \frac{\text{Grams of methyl esters produced}}{\text{Grams of oil taken for reaction}} \times 100$$

3.0 Results and discussion

3.1 Isolation and purification

It has been reported that, *Botryococcus braunii* exists in the form of blooms in fresh water bodies like ponds, lakes and reservoirs [16]. The samples collected in contaminated lake (Hussain sagar, Hyderabad, India) were initially cultured in modified Chu 13 medium and were purified by serial dilution fallowed by plating. The microscopic observations of Botryococcus.braunii of the isolated alga revealed its colonial existence and pyramid shape. Chlorella.vulgaris contains cup shaped chloroplast in its identification [17].

3.2 Cultivation of micro algae in conical flasks

As shown in Fig. 2a, the cell growth reached maximum value 3.59 g L−1 of chlorella.vulgaris, 3.79 g L−1 of Botryococcus.braunii and 3.98 g L−1 of mixed algae after 160 h culture with the substrate of food processing wastewater. These values are similar to that of Dayananda et al., 2007 for the autotrophic cultivation of Botryococcus braunii for the production of hydrocarbons and exopolysaccharides in various media [18]. It indicated that, it was feasible to use food processing wastewater as organic carbon to cultivate Chlorella.vulgaris, Botryococcus.braunii and mixed algae. The lipid content of microalgae during the treatment of food processing wastewater is shown in Fig.2b. Lipid content in the algal cells was 20%, 40% and 35% was observed in chlorella.vulgaris, Botryococcus.braunii and mixed algae respectively. These values are similar to that of Zhi-Yuan Liu et al., 2008 with out any addition of iron for growing of Chlorella.vulgaris. This indicated food processing wastewater is used as a best carbon source for the micro algae [19]. The main chemical components of micro algae before and after treatment are shown in Table 1. The chemical components of micro algae after treatment has been increased, this indicates that food processing waste water is used as a best substrate.

3.3. Treatment of food processing wastewater by using microalgae

The COD removal and BOD removal of food processing wastewater is shown in (Fig. 3a and Fig.3b). It was observed that after 120hrs, the COD reduction increased to 75%, 80% and 82% was observed in chlorella.vulgaris, Botryococcus.braunii and mixed algae respectively. The BOD reduction increased to 75%, 80% and 82% was observed in chlorella.vulgaris, Botryococcus.braunii and mixed algae respectively. The physico-chemical analysis of food processing wastewater before and after treatment is shown in Table 2. Almost all nutrients can be utilized by the micro algae, they assimilate a significant amount of nutrients because they require high amounts of nitrogen and phosphorus for proteins (45 to 60% of micro algae dry weight) , nucleic acids and phospholipid synthesis. Nutrient removal can also be further increased by NH_3 stripping or phosphorus pre precipitation due to the raise in pH associated with photosynthesis [20]. it indicates that food processing wastewater is used as a best substrate for micro algae. After the treatment studies, the grown biomass is subjected to oil extraction by using different solvents like hexane, benzyl alcohol, iso amyl alcohol, dichloro methane, dichloro ethane, proponal and methanol using distillation. The amount of algal oil with different solvents is shown in Table.3. Benzyl alcohol and hexane are the best solvents which is used for the extraction of algal oil. The higher oil yield was as obtained with benzyl alcohol in Botryococcus braunii

species. The oil yield was as per 100 gms of biomass, 27ml in Botryococcus braunii. The next good solvent was hexane which has provided 25ml oil yield with the same. Infact the other strains also have given better oil yields next to Botryococcus braunii. These values are nearly similar to that of D.Sreekanth et al., 2009, which is used for the oil extraction by using microalgae in domestic sewage [21].

3.4 Optimization of the catalyst type and concentration

The effectiveness of catalyst types and concentration towards transesterification of algal oil was evaluated to find the catalyst with the best catalytic activity. The most commonly used catalysts (sodium hydroxide, potassium hydroxide, sodium methoxide and potassium methoxide) were tried in the study. The screening data for the tested catalysts are presented in Fig.4,5 and 6 . Reaction products are presented as a percentage of methyl esters of fatty acids in the reaction mixture. Fig.4, 5 and 6 shows that, among the tested catalysts, potassium hydroxide exhibited the highest yield of methyl esters during the transesterification of algal oil with methanol. Moreover, it can be observed that sodium and potassium hydroxide offered the higher yields when compared to their corresponding methoxides. These values are similar to that of Xioling Miao et al., 2006 and Grace Pokoo-Aikins et al., 2009 revealed that, during the transesterification of Chlorella protothecoids algal oil with potassium hydroxide and sodium hydroxide exhibited the best catalyst activity. The concentration of the catalyst was an important parameter studied [22,23]. The catalyst NaOH concentrations opted in this study were 0.00 to 2.00% (on the basis of weight of raw oil). The operational conditions for the production of biodiesel from algal oil during the whole transesterification reaction were fixed at 60°C, a reaction time of 120 minutes, an oil/methanol molar ratio at 1:6 and the rate of stirring at 200 rpm. The results clearly indicated that the optimum concentration of NaOH required for effective transesterification of algal oil was 1.00%. It was observed that if the NaOH concentration was decreased below or increased above the optimum, there was no significant increase in the biodiesel yield; nevertheless, there was an increased formation of glycerol and emulsion. This might be attributed to the free acid content of the oil. With the increase in the concentration of catalyst, there was a decrease in the yield of methyl esters. This values are similar to that of Umer Rashid., et al 2008 who reported that the formation of soap in the presence of high amount of catalysts increases the viscosity of the reactants, thus resulting in a lower yield [24].

3.5. Biodiesel produced from treated microalgae

The important fuel properties such as density, viscosity, flash point, cold filter plugging point, solidifying point, and heating value of FAME as measured according to accepted ASTM methods and are depicted in Table.4. These properties of FAME investigated in this study satisfied nearly all prescribed ASTM D6751 and EN 14214 specifications, where applicable. A comparison of these properties of diesel fuel [25, 26, 27, 28, 29], biodiesel from microalgal oil and ASTM biodiesel standard is shown in Table 4. Most of these parameters comply with the limits established by ASTM related to biodiesel quality (Antolin et al., 2002).The physical and fuel properties of biodiesel from microalgal oil in general were comparable to those of diesel fuel.

4. Conclusions

A maximum of 85% removal of COD & BOD was achieved using mixed algae and Botryococcus braunii while only 72% removal was achieved with Chlorella vulgaris

Benzyl alcohol and Hexane were used as best solvents for extraction of algal oil from Chlorella vulgaris, Botryococcus braunii and mixed algae. Sodium and potassium hydroxide offered the higher yields when compared to their corresponding methoxides. The results clearly indicated that the optimum concentration of NaOH required for effective transesterification of algal oil was 1.00% for the production of biodiesel.

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Figure 6: % Yeild of methyl esters of mixed algae with different catalysts

Tables

Component	Chlorella vulgaris		Botryococcus braunii		Mixed Algae	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Lipid	**18.10 ± 0-16**	**48.64 ± 0.10**	**25.23 ± 0.65**	**55.12 ± 0.21**	**19.23 ± 0.26**	**49.12 ± 0.12**
Carbohydrate	**10.01 ± 0.14**	**15.34 ± 0.16**	**12.12 ± 0.24**	**16.02 ± 0.24**	**11.12 ± 0.34**	**14.14 ± 0.34**
Protein	**50 ± 0.12**	**12.14 ± 0.01**	**45.12 ± 0.10**	**18.01 ± 0.12**	**48.02 ± 0.12**	**16.23 ± 0.04**
Ash	**7.1 ± 0.09**	**6.01 ± 0.04**	**8.1 ± 0.16**	**4.2 ± 0.04**	**7.12 ± 0.08**	**5.61 ± 0.07**
Moisture	**6.4 ± 0.06**	**2.8 ± 0.12**	**7.4 ± 0.15**	**2.6 ± 0.11**	**7.10 ± 0.11**	**2.4 ± 0.19**
Others	**11.12 ± 0.05**	**13.2 ± 0.12**	**12.14 ± 0.01**	**14.1 ± 0.16**	**12.42 ± 0.14**	**14.12 ± 0.12**

Table1:The main chemical components of algae before and after treatment

S. No	Solvents	Mixed algae	Chlorella vulgaris	Botryococcus braunii
1.	Benzyl alcohol	19.5	17.6	27.0
2.	Iso amyl alcohol	3.5	4.0	8.4
3.	Hexane	19.0	17.0	25.0
4.	Methanol	6.0	5.19	8.9
5.	Proponal	5.8	7.0	9.19
6.	Dichloro methane	2.0	3.14	6.74
7.	Dichloro ethane	1.8	2.9	4.5

Table 2:**Amount of algal oil (ml) for 100 gms of Biomass with different solvents.**

S. No	Parameter	Before treatment	After treatment
1.	pH	7.0 – 7.5	7.0 – 7.4
2.	Total Dissolved Solids	1700-1800	800 – 850
3.	Chemical Oxygen demand	3500 – 4000	350 – 380
4.	Biological Oxygen Demand	1250 – 1300	80 – 100
5.	Volatile Fatty Acids	30 – 35	10 – 15
6.	Alkalinity	210 – 220	70 – 80
7.	Chlorides	140 – 160	90 – 100
8.	Nitrates	120 – 170	100 -120
9.	Sulphates	200-250	30 – 35
10.	Phosphates	40 – 50	Nil
11.	Total Hardness	210 – 220	100 – 110
12.	Total Solids	380 – 390	150 – 200
13.	Total Suspendes Solids	260 – 270	100 – 110
14.	Ammonical Nitrogen	20 - 30	Nil

All the values are expressed as mg/l except pH.

Table 3: The characteristics of food processing waste waters before and after treatment

Fuel property	**Chlorella vulgaris**	**Botryococcus braunii**	**Mixed algae**	**ASTM standard**	**ASTM method used**
Flash point	110	105	104	Min 100	D93
Viscosity/mm²/s,cst at 40ºC	4.9	5.12	4.8	3.5 – 5.0	D445
Density					
Heating value (MJ/Kg)	0.864	0.85	0.84	0.86 – 0.90	D5002
Cold filter plugging point (ºC)					
Solidifying point (ºC)	41	42	41	45.2	D4868
Acid value (mg KOH/g)					
Sulfur (%)	-11	-12	-11	0 < -15	D6371
Total glycerin					
Water (%)	-12	-14	-13	-	D4120
	0.312	0.301	0.314	- 0.05	D664
	0.0111	0.0123	0.0112	0.24	D4294
	0.215	0.214	0.204	0.05	D6584
	<0.01	<0.01	<0.01		D95

Table 4: Comparision of biodiesel characteristics with ASTM biodiesel standards

Figure 1: Experimental setup of algae for biodiesel production.

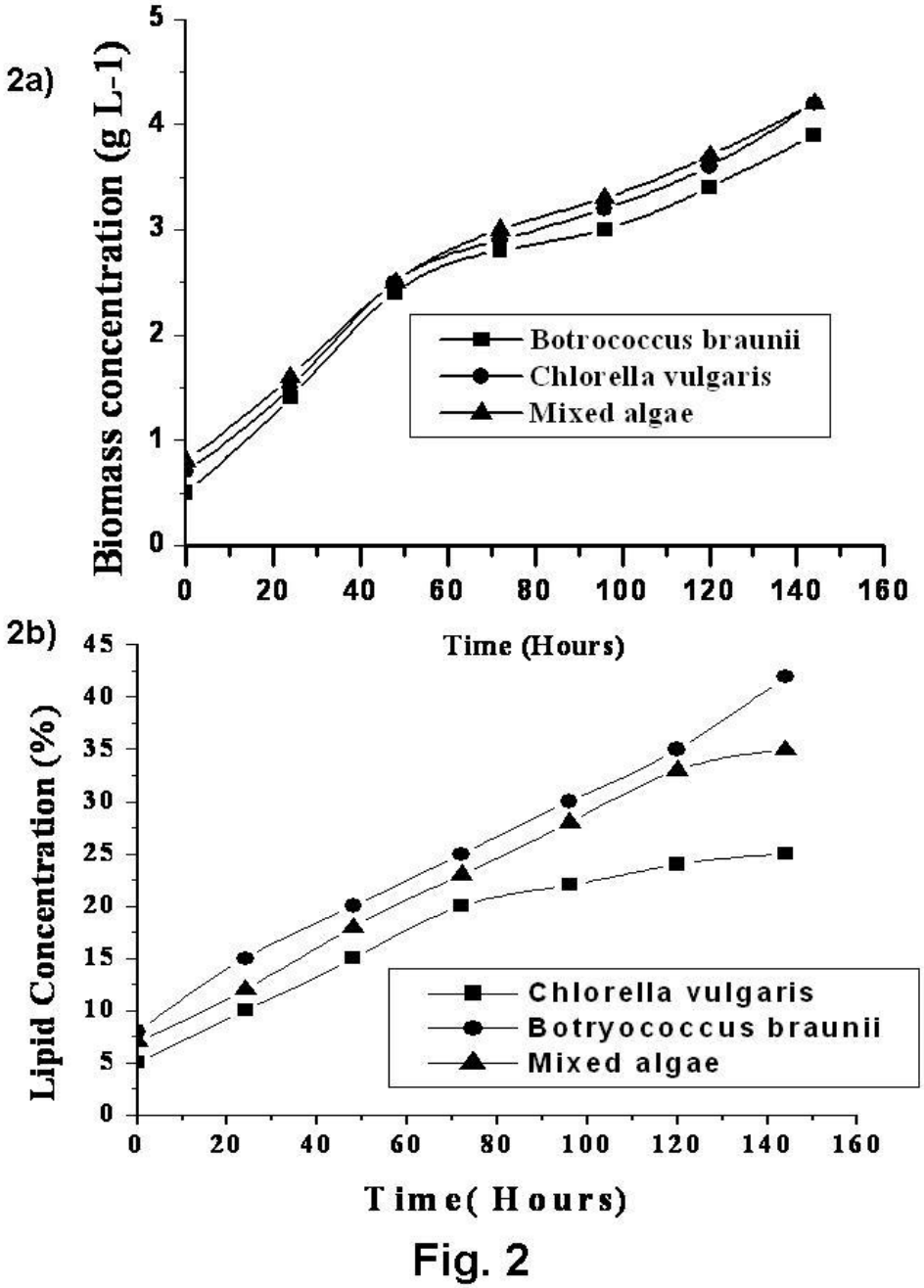
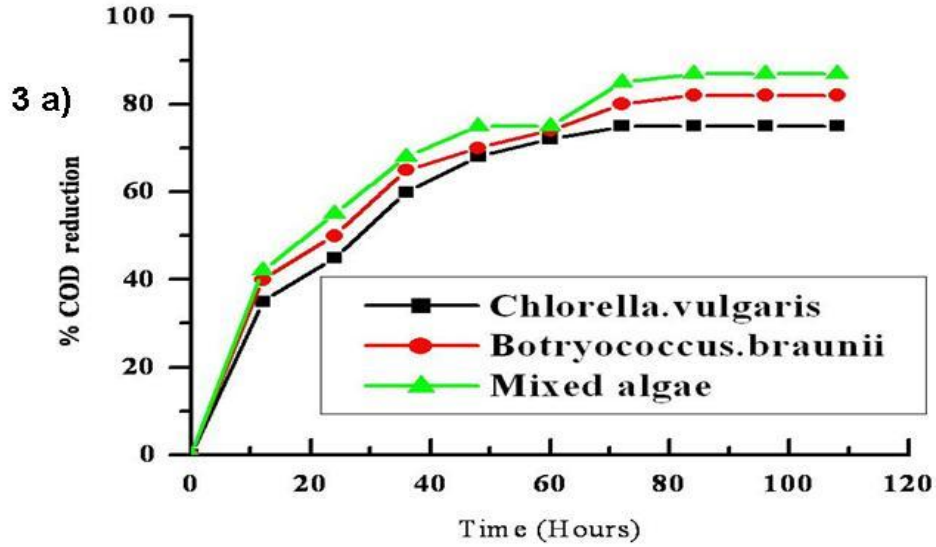


Figure 2: 2 a) Biomass concentration of micro algae during the treatment process.
2 b) Lipid concentration of micro algae during the treatment process



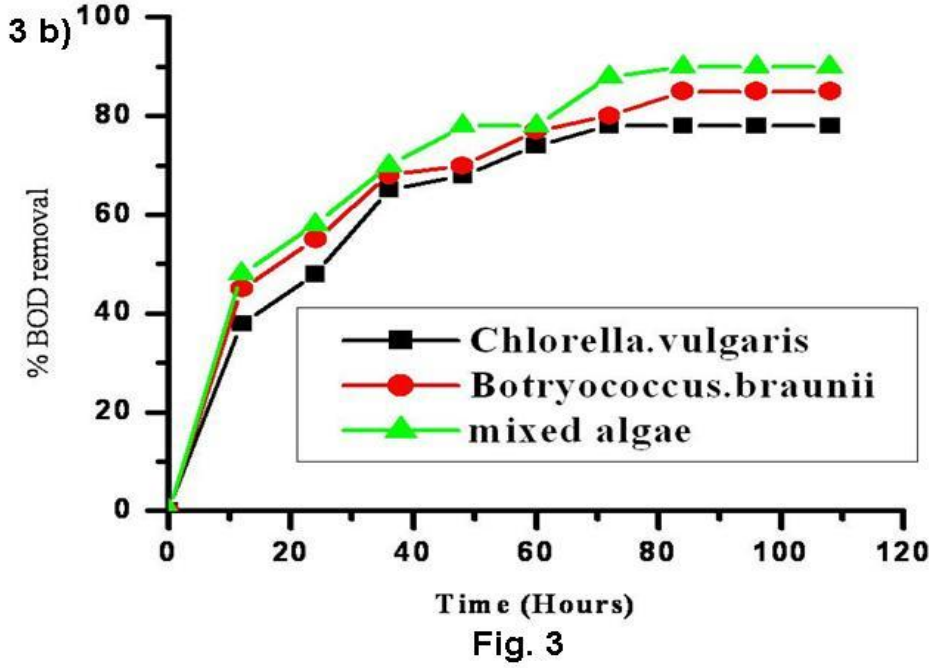


Fig. 3

Figure 3: 3 a) % of COD reduction of microalgae during the treatment process
3 b) % of BOD reduction of microalgae during the treatment process

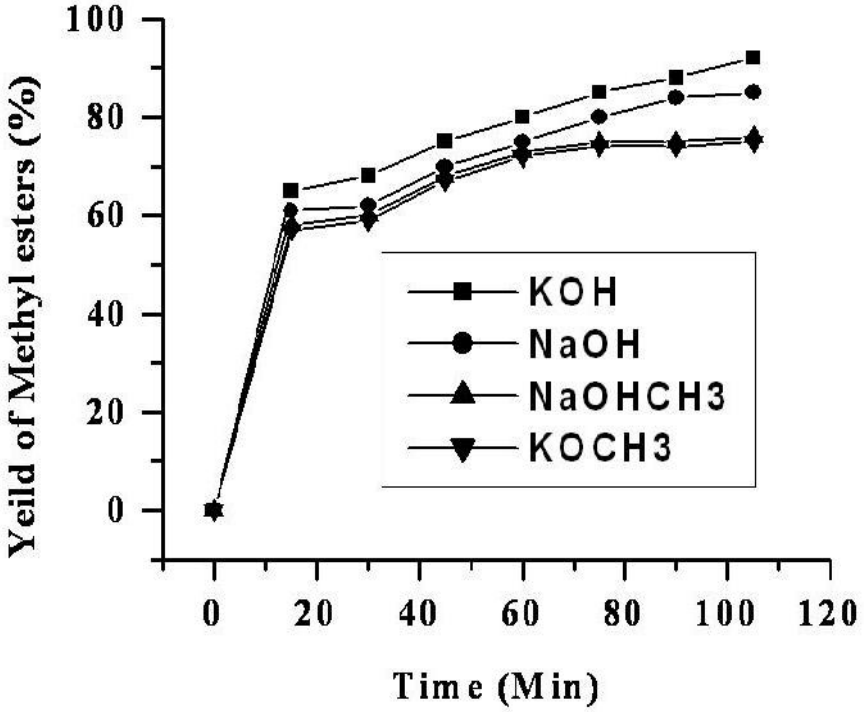


Fig. 4

Figure 4: % Yeild of methyl esters of Chlorella vulgaris with different catalysts

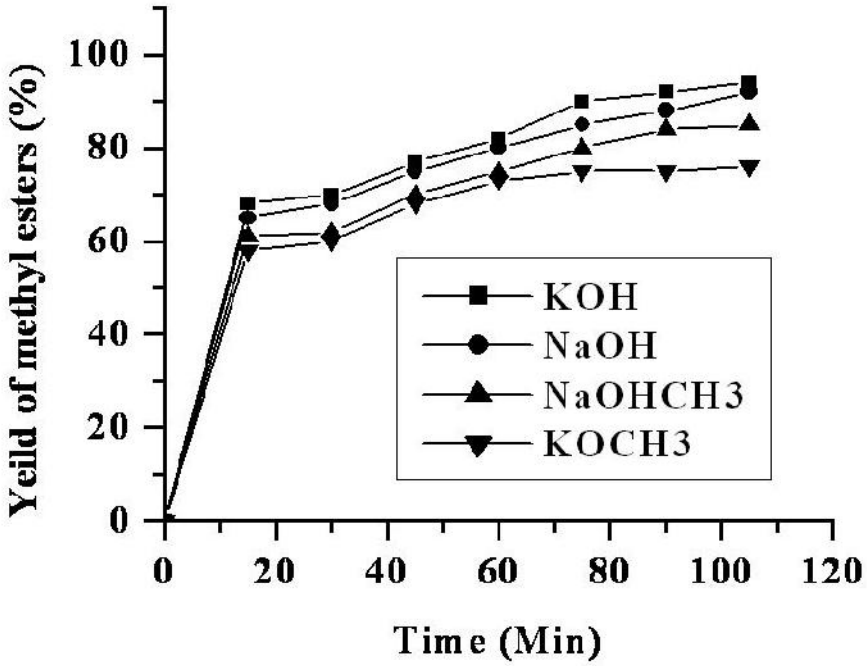


Fig. 5

Figure 5: % Yeild of methyl esters of Botryococcus braunii with different catalysts

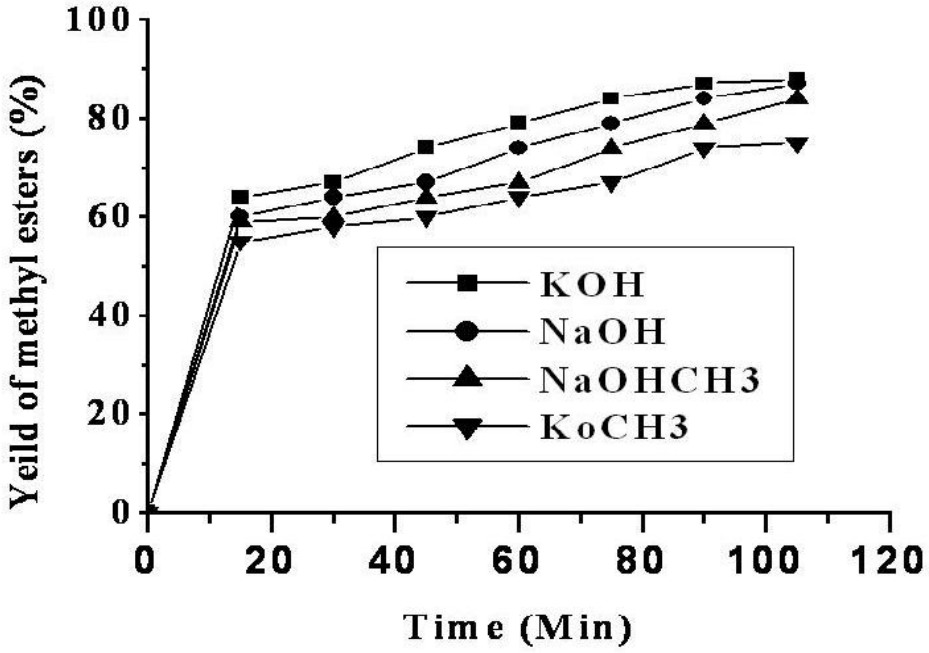


Fig. 6

Figure 6: % Yeild of methyl esters of mixed algae with different catalysts