

# *In-Situ Dodecylbenzenesulfonic acid -Catalyzed Transesterification of Micro Algae Chlorella Sp. for Biodiesel Production*

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**Abstract**— Reducing the cost for kg of biodiesel produced from micro-algae species is an important issue and In situ transesterification process can be the key for the reduction of the overall cost. The yield of biodiesel from microalgae biomass was compared using consecutive steps (extraction-transesterification) and In situ transesterification processes. Chlorella sp. microalgae were investigated as a biomass for biodiesel production. The Dodecylbenzenesulfonic acid activity as a catalyst was tested for the two processes. DBSA catalyzed the transesterification reaction, catalyzed the esterification reaction, reduced the formation of soap and had a hydrophobic structure. DBSA was verified to be a highly efficient catalyst for the Chlorella sp transesterification process. Methyl and ethyl alcohols were used in the processes. The parameters affecting on biodiesel yield and acid value for the In situ transesterification were optimized. The yield of biodiesel for the In situ transesterification process was higher than the yield for the two-step process (extraction-transesterification). The optimal conditions for the in situ transesterification process were found to be (alcohol to biomass ratio (v/w): 15, temperature: 338.15 K for methanol and 348.15 K for ethanol, catalyst ratio: 25%, reaction time: 4 h, mixing rate: 600 RPM).

**Keywords**— *Dodecylbenzenesulfonic acid catalyst, Microalgae Chlorella sp, In-situ transesterification, Biodiesel.*

## I. INTRODUCTION

Recently, micro algal biodiesel fuel (Third generation biofuel) has gained a wide attention in the researches and development in the industrial production world widely. Due to the higher rate of growth, the productivity of lipids and the efficient photosynthesis process for the microalgae species in compared to the other types of algae species and most of the earthly plants [1,2]. Exploring the catalytic-transesterification of microalgae still a vital point and need to be verified with many types of homogeneous and heterogeneous catalyst [3]. High free fatty acids of the microalgae lipids poses many problems in the conventional alkali catalyzed processes [4,5]. Hydrolysis, reducing catalyst activity, increase catalyst consumption, saponification and decreasing the biodiesel yield

were the main disadvantages of using basic catalysts [6]. The two-step acid-alkali catalyzed transesterification of microalgae are widely used for the production of biodiesel, investigated in many researches, and proved that it takes long time for the extraction, esterification and transesterification steps. marine macroalgae *E. compressa* was used as raw material for biodiesel production through the consecutive esterification and transesterification processes and the optimum conditions were explored [7]. Many acid catalysts such as  $H_2SO_4$ ,  $BF_3$  and  $HCl$  were used for the transesterification of microalgae and it is not new to use it in the literature [8]. The direct transesterification process of algae also has been used in the literature and showed a less time consuming and high yield of biodiesel [9]. Therefore, direct or in-situ transesterification process still needs to be investigated using other catalysts.

The present study examines for the first time, the DBSA-catalyzed in-situ transesterification of microalgae *Chlorella sp.* The present work provides an experimental investigation for the parameters affecting the transesterification process, the optimum condition for the reaction yield and the acid value of the final biodiesel product. The result was compared with the consecutive esterification and transesterification processes.

## II. EXPERIMENTAL

### A. Materials

Dodecylbenzenesulfonic acid (>0.99) was procured from Shanghai Hanhong Scientific Co., Ltd. Anhydrous  $MgSO_4$  (99.8 wt. %), chloroform (99.8), methanol (99.9%), ethanol (99 %), hexane (98.8%) and gas chromatography (GC) standard methyl heptadecanoate for the quantitative analysis of FAME were provided from Sigma-Aldrich. All the chemical materials used were analytical reagents. *Chlorella sp.* extract is supplied from Shaanxi Jintai Biological Engineering Co., Ltd. The microalgae are dried at 343.15 K. The independent fatty acids analysis verified that the lipid consists primarily as follow:

(C16:0, C18:3, C18:2, C16:3, C16:2, and C18:0). As shown in table (1).

TABLE I. FATTY ACID TYPE OF THE LIPID

Ester Type	C16:0	C16:2	C16:3	C18:0	C18:2	C18:3	others
percent	7.5	0.4	0.8	1.2	74.8	14.6	0.7

### B. Measuring and Procedure

The lipid extracted from microalgae was tested for calculating acid value (mg KOH/g) using the standard titration method according with the approved method of American Oil Chemists Society (AOCS). Each test was conducted in triplicate. The analysis of biodiesel produced at the optimum conditions for the free fatty acid profile and quantification are done using GC-2010-flame ionization detector (Shimadzu, Japan) armed with a split-splitless injector (oven temperature of 483.15 K, injection volume=2  $\mu$ L, split injection temperature of 513.15 K, split ratio=1:50, flow rate=1ml/min, column specification of 30 m length-0.25 mm i.d.-0.25 film thickness, analysis time of 30 min and detector temperature of 523.15 K.

### C. Extraction-Transesterification

A 0.5 L rounded flask was used for the extraction of oil by mixing 100g (dry *Chlorella* sp.) with a mixture of 300 ml (chloroform / methanol) as a solvent and conducted for 3h at 25°C under magnetic stirring of 600 RPM. The resulted mixture then was filtered, evaporated and finally dried at 60°C. The transesterification reaction was done using DBSA (10% based on the lipid mass) as a catalyst. The reaction was conducted in a 0.5L rounded flask armed with a condenser under constant stirring of 600 RPM. The optimum conditions for the reaction were as follow (alcohol to lipid ratio of 30:1, temperature of 333.15 K, reaction time of 4 h and stirring rate of 600 RPM). Afterwards, the evaporation of excess methanol was conducted using a rotary evaporator. Finally, the product was filtrated in a vacuum system and the filtrated product was dried over anhydrous  $MgSO_4$ . The extraction-transesterification process was conducted according to reference [10]. Figure 11 shows the steps of the process.

### D. In situ Transesterification

Direct transesterification was conducted in a 0.5L rounded flask using different conditions (alcohol to lipids ratio:10-50:1, catalyst amount: 5-45 % (based on the lipid mass), reaction temperature: 303.15-343.15 K, reaction time: 0.5-6 h and stirring rate: 200-1200 RPM, solvent type). 10 g of dry *Chlorella* sp. with a suitable volume of solvent were mixed with a suitable amount of DBSA as a catalyst. The final product was obtained using the same procedure as mentioned in the previous section [10]. GC-FID was used for the characterization of the product obtained from both extraction-transesterification and In situ transesterification process. The tests were conducted in triplicate. The yield of microalgae biodiesel was estimated according to the following equation [11]:

$$\text{Yield (wt.\%)} = \frac{\text{Biodiesel in each sample (g)}}{\text{Maximum biodiesel yield (g)}} \times 100 \quad (1)$$

## III. RESULTS AND DISCUSSION

A comparison was done between the In-situ transesterification yield with that one of the extraction-transesterification process for both the methyl and ethyl biodiesel. The yield of the extraction-transesterification was lower than the yield of the In-situ transesterification process.

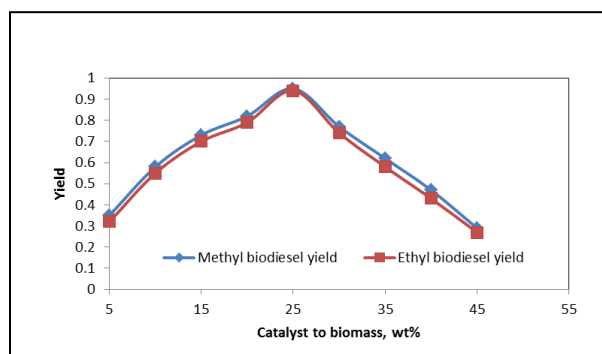


Fig. 1. Influence of catalyst ratio on the yield of biodiesel.

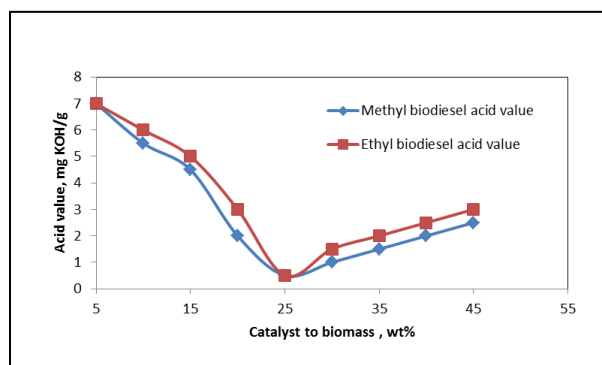


Fig. 2. Influence of catalyst ratio on acid value.

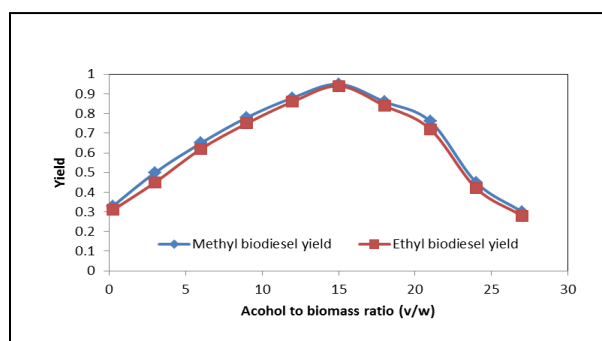


Fig. 3. Influence of alcohol/biomass ratio (v/w) on the yield of biodiesel.

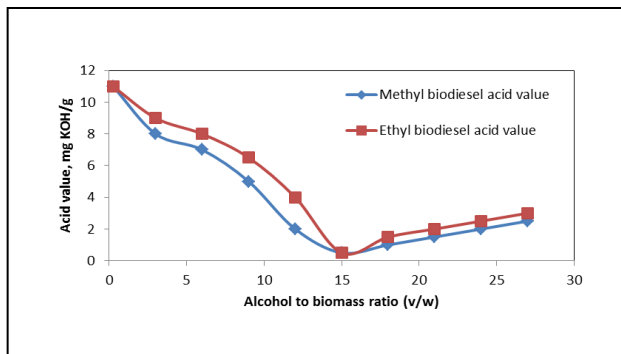


Fig. 4. Influence of alcohol/biomass ratio (v/w) on acid value.

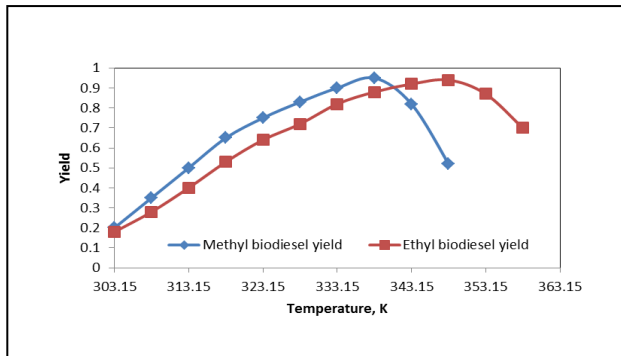


Fig. 5. Influence of reaction temperature on the yield of biodiesel.

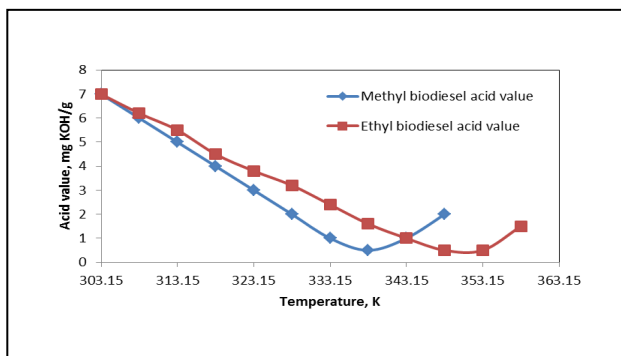


Fig. 6. Influence of reaction temperature on acid value.

#### A. Extraction-Transesterification

After extraction using chloroform: methanol solvent, The *Chlorella* sp. extract has a high acid value of 35 mg KOH/g. With the aim of reducing the acid value of *Chlorella* sp. extract, esterification reaction of free fatty acid in the *Chlorella* sp. extract was done using DBSA as a catalyst. The reversible esterification reaction was directed in the forward using DBSA catalyst and increase the yield of biodiesel in the transesterification step. Using methanol as a reagent in the transesterification step under the temperature of 25 °C for 3 h, and the chloroform-methanol solvent, the methyl biodiesel

yield was 89%, while the ethyl biodiesel yield produced by using ethanol as a reagent was 88%. The ethanol reagent was extracted less amount of lipids than methanol.

#### B. In situ Transesterification

In-situ transesterification of the *Chlorella* sp. (dry powder) was conducted using ethanol and methanol at different conditions and the parameters affecting the reaction was optimized as follows:

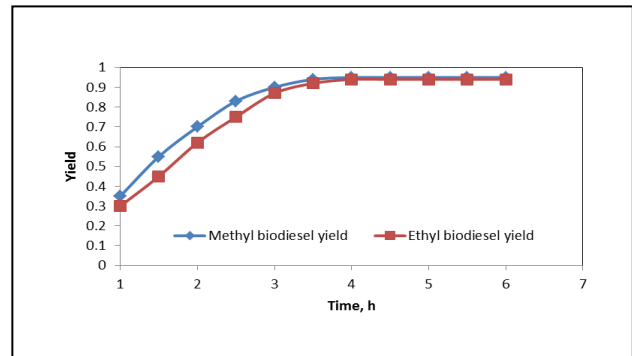


Fig. 7. Influence of reaction time on the yield of biodiesel.

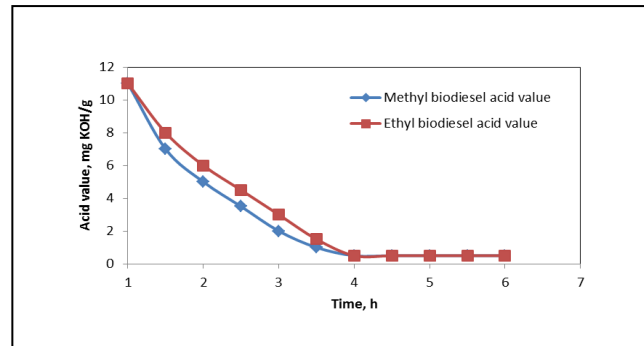


Fig. 8. Influence of reaction time on acid value.

#### C. Catalyst Amount

Acid catalyst could solve the problem of high FFA content of microalgae. DBSA catalyst accelerates the transesterification reaction, catalyze the esterification reaction, reduce the formation of soap and has a hydrophobic structure. DBSA was found to be a highly active catalyst for the transesterifying of *Chlorella* sp. compared to the activity of  $H_2SO_4$ . For the initial conditions of (alcohol to biomass ratio (v/w): 15, temperature: 338.15 K for methanol and 348.15 K for ethanol, reaction time: 4 h, mixing rate: 600 RPM) the catalyst ratio was investigated. Figure 1 shows the change in the yield of biodiesel with catalyst ratio. Increasing the catalyst amount from 10 to 45% showed that the optimum catalyst ratio

is 25%. Moreover, increasing the catalyst amount beyond that ratio would participate in increasing the viscosity of the mixture and led to mass transport limiting step in the process [12]. The presence of impurities like free fatty acids, chlorophylls, etc. decreased the biodiesel yield in the present work. A comparable results were showed for the *Chlorella pyrenoidosa* transesterification process which extracted using Chloroform/Methanol [13]. The influence of catalyst ratio on the acid value is shown in figure 2. The lowest acid value is obtained at a 25% catalyst ratio. Any increase in the catalyst ratio beyond that value may lead to hydrolyzing of triglyceride in the presence of DBSA catalyst.

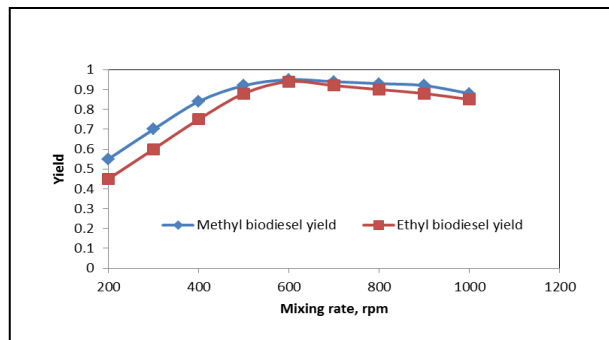


Fig. 9. Influence of mixing rate on the yield of biodiesel.

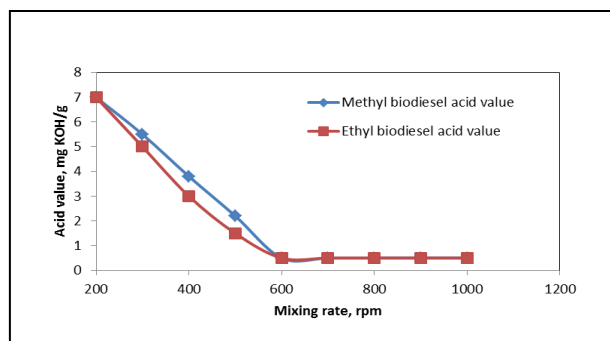


Fig. 10. Influence of mixing rate on acid value.

#### D. Alcohol to Biomass Ratio (v/w)

Theoretically, the molar ratio (alcohol to oil) of 3:1 for conversion of triglycerides to biodiesel is the suitable ratio for converting triglyceride to biodiesel and is equivalent to only 1:100 (v/w) ratio. The ratio was calculated assuming that the percent of lipid in the dry microalgae is 14% and 50% of it can be converted to biodiesel. Moreover, increasing the ratio beyond the ratio of 3:1 would increase the rate of reaction in the forward direction and would increase the yield of biodiesel [14]. Figure 3 shows the change in the yield of biodiesel with alcohol/biomass ratio (v/w). The optimum value of the alcohol/biomass ratio (v/w) was proved to be 15:1 in both cases of using methanol and ethanol and it is far greater than the theoretical ratio. The lower ratios of alcohol to oil (v/w) may

slow down the transesterification reaction [15-17]. Increasing the alcohol to biomass ratio (v/w) more than 15:1 would decrease the biodiesel yield due to the dissolving of biodiesel yield in the excess methanol amount. The influence of alcohol/biomass ratio (v/w) on biodiesel yield may be explained as follows. Increasing the ratio would increase the reaction rate in general. At the beginning, the reaction may take place in the oily phase, which is saturated with alcohol and any increase in alcohol would not increase the reaction rate. As the reaction proceeds, increasing the ratio would leave the methanol concentration constant in the oily phase, leaving the catalyst outside the oily phase and led to a decrease in reaction rate. Figure 4 shows the variation of acid value with the alcohol to biomass ratio (v/w). The acid value was the lowest value at a ratio of 15:1. Increase the alcohol to biomass ratio (v/w) beyond the value of 15:1 would not further decrease acid value due to water formation. The biodiesel yield would be decreased due to the decreasing the esterification reaction rate.

#### E. Reaction Temperature

Temperature is a main factor affecting the biodiesel yield. For the conditions of (alcohol to biomass ratio (v/w): 15, catalyst ratio: 25%, reaction time: 4 h, mixing rate: 600 RPM), the temperature of the reaction was examined for a range of 303.15-348.15 K for methanol and 303.15-358.15 K for ethanol. Figure 5 shows the influence of reaction temperature on the biodiesel yield. Increase the temperature of alcohol-oil mixture would increase the solubility of molecules. The extreme biodiesel yield of 95% in the case of methanol was obtained at a temperature of 338.15 K and about 94% in the case of ethanol at a temperature of 348.15 K. Increase the temperature above the boiling point of alcohol could increase the possibility of soap production. Moreover, the decrease in biodiesel yield with rising the reaction temperature can be clarified due to the increase the miscibility of biodiesel and glycerol which promote the backward reaction [18-19]. Figure 6 shows the change of acid value with the reaction temperature. Further increase in temperature beyond 338.15 K in case of methanol and 348.15 K in case of ethanol would not affect the acid value.

#### F. Reaction Time

Normally, the acid catalyzed transesterification reaction taking a long time for completion and causes a phase immiscibility for a longer time. For the conditions of (alcohol to biomass ratio (v/w): 15, temperature: 338.15 K for methanol and 348.15 K for ethanol, catalyst ratio: 25%, mixing rate: 600 RPM), the influence of reaction time was examined for the scope of time (1-6 h). Figure 7 displays the change in the yield of biodiesel over the range of time. The extreme yield of biodiesel was achieved after a time of 4 h. The acid value change with reaction time is shown in the figure 8. The decrease of acid value with the reaction time is continued till 4 h. After that, any decrease in acid value was not detected due to the creation of water. The acid value of oil decreased from 11 to 0.5 mgKOH/g.

## G. Mixing Rate

For the conditions of (alcohol to biomass ratio (v/w): 15, temperature: 338.15 K for methanol and 348.15 K for ethanol, catalyst ratio: 25%, reaction time: 4 h), the effect of mixing rate was investigated. Figure 9 shows the change in the yield of biodiesel with mixing rate. Increasing the mixing rate provide a suitable interaction area between the catalyst and the mixture of alcohol-oil. The optimum mixing rate was found to be 600 RPM. Increasing the mixing rate beyond the value of 600 RPM would decrease the yield of biodiesel due to the inadequate mixing and the interference between the two layers of biodiesel and glycerol [20]. Figure 10 shows the variation of acid value due to the change of mixing rate in the range of 200-1200 RPM. Changing the rate of mixing between 200-600 RPM would decrease the acid value from 7 to 0.5 mgKOH/g. A further increase in the mixing rate beyond 600 RPM there was no change in acid value.

## IV. CONCLUSION

One step transesterification process of *Chlorella* sp. using DBSA catalyst proved to be an efficient process for the biodiesel production. A 95% and 94% biodiesel yield was obtained using the In situ transesterification process with methanol and ethanol, respectively. Ethanol can be used instead of methanol for the biodiesel production and make the process eco-friendly, efficient process unlike methanol. DBSA catalyst accelerates the transesterification reaction, catalyze the esterification reaction, reduce the formation of soap and has a hydrophobic structure. DBSA is a commercial product used massively in the detergent industry. Full economical study of the biodiesel production from *Chlorella* sp. microalgae using DBSA as a catalyst need to be investigated. DBSA was found to be a highly active catalyst for transesterifying the *Chlorella* sp. compared to the activity of  $H_2SO_4$ . Alcohol/oil ratio was proved to be an obstacle in the way of reaction rate at lower conversions. Compared to the extraction-transesterification process, the direct transesterification process provides a higher biodiesel yield.

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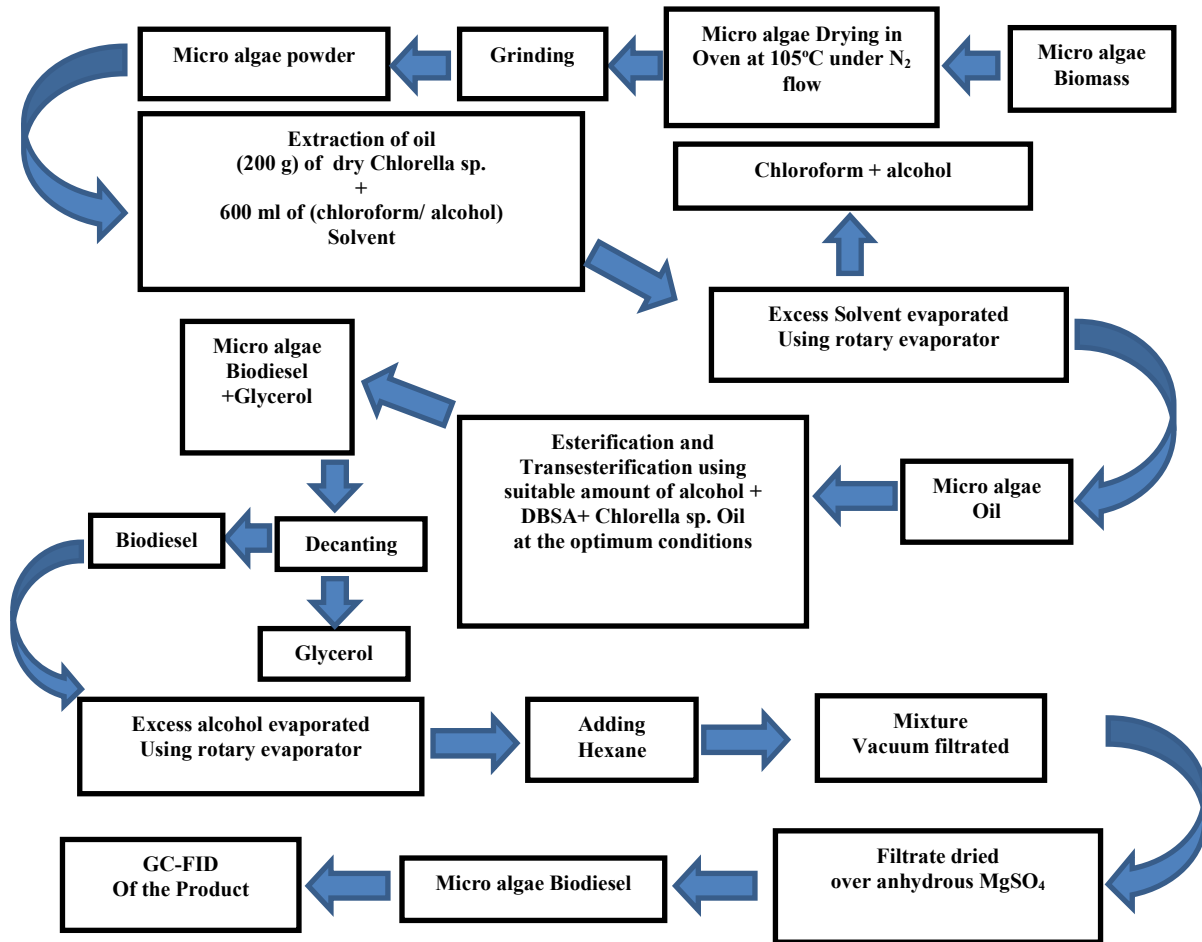


Fig. 11. Schematic diagram for the extraction-transesterification steps.