

PRODUCTION OF OIL FROM ALGAE AND ITS UPGRADATION USING HETEROGENEOUS CATALYST

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CANDIDATE'S DECLARATION

I hereby declare that the work presented in this thesis entitled "Production of oil from algae and its upgradation using heterogeneous catalyst" submitted towards the completion of dissertation in final semester of IDD (CHH) at the Indian Institute of Technology Roorkee, is an authentic record of my original work carried out under the guidance of Dr. P. Mondal, Assistant Professor, Department of Chemical Engineering, IIT Roorkee.

I have not submitted the matter embodied in this report for the award of any other degree.

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DEDICATION

This work is dedicated to my family

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ABSTRACT

Algae is considered as 3rd/4th generation feedstock for bio-oil production. It has good potential for bio-oil production since some of the algal species, particularly micro algal species have high growth rate with significantly high lipid content. Out of around 50,000 algal species only few have been exploited. Bio-oil produced from algal biomass basically contains fatty acids and has high viscosity, thus, are not suitable for direct application in combustion engines. Due to this reason upgradation of bio-oil is essential. In the present work native algal biomass collected from Solani River, Roorkee (mainly consisting of Hydrodictyon and Ulotrichalean strains of green algae) has been used. The algal biomass has been found to have ~ 14 % lipid content.

Oil from the dried algal biomass has been extracted using hexane as solvent in soxhlet apparatus. Oil was then upgraded to bio-diesel using heterogeneous catalyst to achieve high yield of biodiesel and avoid saponification. Heterogeneous catalysts consisting of various molar ratios of TiO₂ and CaO (0.25, 0.5, 1, 2) were prepared using conventional solid state reaction. Mixture with TiO₂:CaO molar ratio of 0.25 calcinated at 700°C was found to be the most active.

Optimization and modelling of the transesterification reaction was done using Design Expert software version 8.0.7.1 trial. Box-Behnken model using 3 factors (methanol to oil ratio, catalyst doze and reaction time) with 3 levels each was used to fix the experimental conditions. Various levels are methanol to oil ratio (12, 15, 18), catalyst conc. (3, 5, 7 wt. %) and reaction time (6, 8, 10 h). Total 17 reaction conditions were obtained to conduct experiments. The reactions were carried out at boiling temperature of methanol under reflux. The optimized conditions that were obtained by response surface methodology are: methanol to oil ratio 15.68, catalyst concentration 5.12 wt. % and reaction time 8.5h. Further, the catalyst was characterized by using X-Ray diffraction technique, thermo gravimetric analysis method and SEM. The proposed model is suitable to predict the biodiesel yield within the design space with error limit of ±1.5 %.

Algal oil and algal biodiesel were analyzed for determining various properties such as viscosity, cloud and pour point, density etc. Properties of bio-diesel matched as those prescribed in IS-15607:2005. GC-MS analysis of bio-diesel was also performed which detected more than 15 compounds ranging from C14 to C29.

CHAPTER 1 INTRODUCTION

Renewable sources of energy have been receiving significant focus from governments, academic institutions, corporates etc. in the recent years. The depleting and declining quality of fossil fuels, increased emissions of greenhouse gases (GHG) and ever increasing energy demand can be named as the three most important reasons for this focus. Researchers around the world are becoming more interested in finding out the economic routes, optimum process variables, and commercial feasibility for harnessing energy from the renewable sources of energy.

In the next 20 years, the energy consumption of world is estimated to grow at 1.6% per year, adding 39% to global consumption by 2030. By 2030, China and India will be the world's largest and 3rd largest economies respectively, jointly accounting for about 35% of global population, gross domestic product (GDP) and energy demand. Rapid economic development means industrialisation, urbanisation and motorisation. Over the next 20 years, China and India combined will account for all the net increase in global coal demand, 94% of net oil demand, 30% of gas, and 48% of the net growth in non-fossil fuels demand [1].

‘Projections made in the Integrated Energy Policy Report (IEPR), on the basis of the excerpt from the 11th Five Year Plan of India, reveal that to achieve its development goals, India would need to rely increasingly on imported oil, gas, and coal in the medium term (2032). Under this backdrop, the role of new and renewable energy assumes added significance, irrespective of whether it replaces coal or oil. In this regard, IEPR recognizes ‘the need to maximally develop domestic supply options as well as the need to diversify energy sources . . .’ [2]. Further, Govt. of India in its ‘National Policy of Biofuels’ has proposed 20% blending of biofuels/bioethanol in fuels by 2017 [3].

Increase in the consumption of fossil fuels is accompanied by increased emissions in the atmosphere, which raises the concern about their negative effects on the environment. The emissions include greenhouse gases such as carbon dioxide and carbon monoxide, particulate matter, sulphur and nitrogen oxides. These emissions pollute the environment and causes greenhouse effect which leads to increase in global warming.

Renewable energy sources can be helpful in reducing or controlling the intensity of above stated problems. These are the resources which have the capability to replace non-renewable energy sources efficiently. Various sources include sun, wind, algae and vegetable biomass etc. Biodiesel is obtained from the bio-oils extracted from algal/vegetable biomass, by transesterification in presence of a solvent and a catalyst. Biodiesel has the capability to act as an alternative fuel. It is carbon neutral and the source of biodiesel is of plant origin and therefore, it is renewable in nature. Using pure biodiesel reduces engine performance due to its high viscosity and hence it is blended with the conventional fuels. Most common are the B5 and B20 (BXX where XX represents the percentage of biodiesel in the mixture). It is important to note that the biodiesel used in the blend should be of pure quality and should meet the standards such as IS-15607 (Indian), ASTM D-6751 (American), EN-14214 (European) etc.

1.1. Biodiesel

Biodiesel, the renewable liquid fuel has received a great deal of attention. Biodiesel as defined by the World Customs Organization (WCO) is “a mixture of mono-alkyl esters of long-chain [C16-18] fatty acids derived from vegetable oils or animal fats. It is a domestic renewable fuel for diesel engines and meets the international specifications (ASTM D 6751).” Biodiesel can be obtained from oils and fats of plants and lipids of algae [4], [5], [6], [7] by transesterification with methanol, ethanol or any other suitable alcohol in presence of a catalyst.

Biodiesel offers many advantages over conventional fuel such as reduction in carbon dioxide and carbon monoxide emissions since it is of biomass origin and biomass can be replenished, reduction of particulate matter emissions, higher oxygen content in fuel leading to complete oxidation, high flash point making it less difficult to handle. Table 1.1 shows the physical and chemical properties of biodiesel.

Table 1.1: Physical and chemical properties of biodiesel [8]
(Reproduced by permission)

Name	Biodiesel
Chemical name	Fatty acid methyl ester
Chemical formula range	C14-C24 methyl esters
Kinematic viscosity range	3.3-5.2
Density range (kg/m ³)	860-894
Boiling point range (K)	>475
Flash point range (K)	430-455
Distillation range (K)	470-600
Vapour Pressure (mmHg at 295K)	<5
Solubility in water	Insoluble in water
Physical appearance	Light to dark yellow transparent liquid
Odour	Light soapy and oily odour
Biodegradability	More than conventional diesel
Reactivity	Stable, avoid strong oxidize agents

1.2. Algae as a feedstock for biodiesel

Algae are unicellular or multi cellular autotrophic organisms that uses CO₂ to produce potential biofuels, foods and feed, and high value bio-actives [9], [10]. Algae are classified as macro or micro according to their cell size. Micro algae can grow rapidly under harsh conditions and typically contains more oil than macro algae. According to estimates, more than 50,000 species of micro-algae exists but only 30,000 have been studied and analysed as potential species all over the world [11]. Oil from algae is extracted using various methods like expeller, ultrasonic extraction, hexane solvent, soxhlet etc.

1.3. Transesterification

The transesterification reaction involves 3 steps to give esters and glycerol as end products with the help of catalyst. Catalyst can be present in the same or different phase as the reactants. When present in the same phase, it is known as homogeneous catalyst whereas when present in different phase, it is known as heterogeneous catalyst. Homogeneous catalyst includes the strong basic and acidic solutions such as sodium hydroxide, potassium hydroxide, sulphuric acid, hydrochloric acid etc. These types of catalyst pose difficulty in separation of end products. The glycerol obtained is of poor quality and further separation process has to be applied for purification. This in turn increases the cost of biodiesel and glycerin. On the other hand, heterogeneous catalysts are easy to separate as they do not get mixed with the alcohol. Moreover, these are recyclable and water produced by them during the reaction is negligible or very less.

In addition to homogeneous and heterogeneous catalyst, enzyme catalysts are also being studied due to their high tolerance levels of free fatty acids and water. But, due to their slow reaction rate they become less attractive to be pursued commercially.

Table 1.2 provides the comparison of various types of catalyst. The comparison shows that heterogeneous base catalysts have the advantage over all the other. Hence, it makes them a popular choice to study among the researchers.

1.4. Objectives

- 1.4.1. Collection, pre-treatment and extraction of oil from algal biomass.
- 1.4.2. Synthesis of catalysts for biodiesel production and their characterization.
- 1.4.3. Selection of best catalyst for biodiesel production.
- 1.4.4. Optimization of process parameters for biodiesel production.
- 1.4.5. Modelling of the process.
- 1.4.6. Analysis of properties of bio-oil and biodiesel produced.

Table 1.2: Comparison of homogeneous, heterogeneous and enzyme transesterification catalysts. [12], [13], [14], [15]

Type of catalyst → ↓ Factor(s)	Homogeneous base catalyst	Heterogeneous base catalyst	Homogeneous acid catalyst	Heterogeneous acid catalyst	Enzyme
	Reaction rate	Very Fast	Fast	Very Slow	Very Slow
After treatment (other than product purification)	Neutralization Required	Not required	Neutralization Required	Not required	Not required
Processing methodology	Batch only	Batch/Continuous	Batch	Batch/Continuous	Batch
Presence of water/free fatty acids	Sensitive	Sensitive	Insensitive	Insensitive	Insensitive
Catalyst reuse	Not possible	Possible	Not Possible	Possible	Possible
Cost	Comparatively Costly	Relatively Cheaper	Comparatively Costly	Costly	Very High Cost
Other	Soap formation likely but Less energy intensive	Soap formation likely but Less energy intensive	Less energy intensive	Energy intensive and severe reaction conditions required	Sensitive to alcohol, typically methanol that can deactivate the enzyme

CHAPTER 2 LITERATURE REVIEW

Biodiesel is a non-toxic, biodegradable eco-friendly fuel which can be obtained using oils of various feed stocks. But, in present economic conditions biodiesel is expensive but would be more cost-effective if it can be produced cheap feed stocks which do not present challenges to human being. This chapter will focus upon algae as a prospective feedstock for producing biodiesel, merits of using heterogeneous catalyst and vastness of the field of heterogeneous catalyst for transesterification.

2.1. Biofuel feedstock

Bio-oils can be obtained from many feed stocks such as corn, soybean etc. But using food crops always invites the question of usage in food or fuel. Table 2.1 shows the comparison of algae with food crops in terms of oil content, oil yield, land use and biodiesel productivity

Table 2.1: Comparison of algae with food crops in terms of oil content, oil yield, land use and biodiesel productivity [16] (Reproduced by permission)

Plant Source	Seed oil content (% oil by wt in biomass)	Oil yield (L oil/ ha year)	Land use (m ² year/kg biodiesel)	Biodiesel productivity (kg biodiesel/ha year)
Corn/Maize (<i>Zea mays</i> L.)	44	172	66	152
Soybean (<i>Glycine max</i> L.)	18	636	18	562
Jatropha (<i>Jatropha curcas</i> L.)	28	741	15	656
Canola/Rapeseed (<i>Brassica napus</i> L.)	41	974	12	862
Sunflower (<i>Helianthus annuus</i> L.)	40	1070	11	946
Palm oil (<i>Elaeis guineensis</i>)	36	5366	2	4747
Algae (low oil content)*	30	58,700	0.2	51,927
Algae (medium oil content)*	50	97,800	0.1	86,515
Algae (high oil content)*	70	136,900	0.1	121,104

*The productivity of algae is based on experimentally demonstrated biomass productivity in photo bioreactors [6].

Since the beginning, vegetable oil has always been the feed stock to produce bio-diesel throughout the world with major feed stocks being Soyabean, Corn/Maize, Canola, Palm and Sunflower. For a country like India, whose most of the fuel demand is met by importing fuels, it becomes extremely important to find the alternative energy routes. With population more than 1.2 billion it surely cannot depend upon the food crops for energy production. Hence, it is necessary to turn our focus on some other feedstock which can easily meet the raw material requirement and doesn't compete with food crops for land.

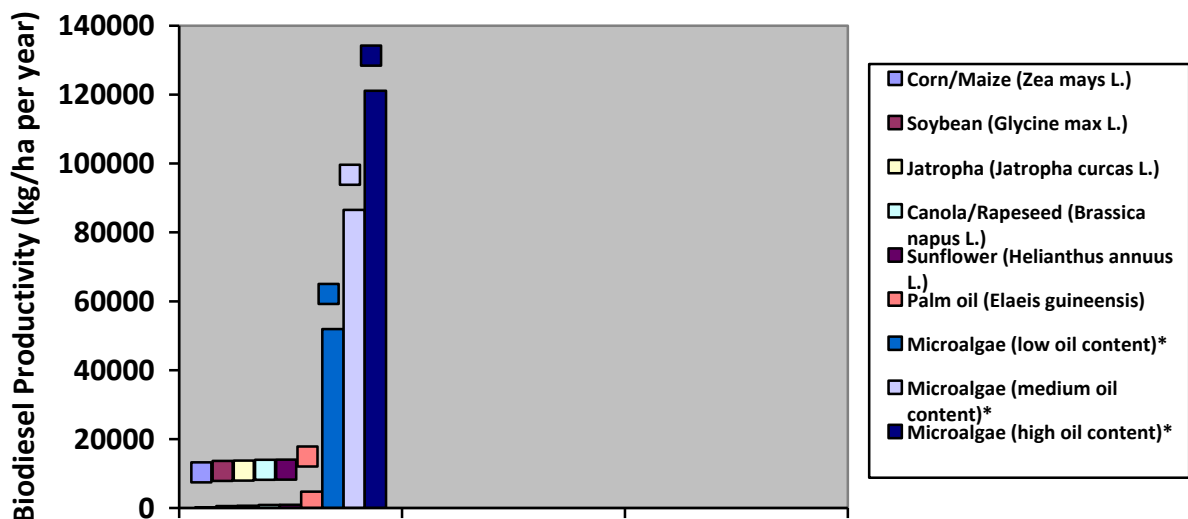


Figure 2.1: Comparison of biodiesel productivity of various oil sources based on Table 2.1

It is clear from Figure 2.1 that algae have a huge potential for producing biodiesel. Apart from high oil content, algae have high growth rate and high biomass yield [17]. Also, algae have a distinctive advantage over food crops i.e. food crops present a question of heavy competition between food supply and biodiesel production but algae can be used for the same purpose without any hesitation and it can be grown on unarable land. Algal oil yield varies between species significantly, as shown in Table 2.2.

Table 2.2: Lipid content and lipid biomass productivities of different marine and freshwater microalgae species [16] (Reproduced by permission)

	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/day)	Areal productivity of biomass (g/m ² /day)
<i>Ankistrodesmus</i> sp.	24.0–31.0	–	–	11.5–17.4
<i>Botryococcus braunii</i>	25.0–75.0	–	0.02	3.0
<i>Chaetoceros muelleri</i>	33.6	21.8	0.07	–
<i>Chaetoceros calcitrans</i>	14.6–16.4/39.8	17.6	0.04	–
<i>Chlorella emersonii</i>	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97
<i>Chlorella protothecoides</i>	14.6–57.8	1214	2.00–7.70	–
<i>Chlorella sorokiniana</i>	19.0–22.0	44.7	0.23–1.47	–
<i>Chlorella vulgaris</i>	5.0–58.0	11.2–40.0	0.02–0.20	0.57–0.95
<i>Chlorella</i> sp.	10.0–48.0	42.1	0.02–2.5	1.61–16.47/25
<i>Chlorella pyrenoidosa</i>	2.0	–	2.90–3.64	72.5/130
<i>Chlorella</i>	18.0–57.0	18.7	–	3.50–13.90
<i>Chlorococcum</i> sp.	19.3	53.7	0.28	–
<i>Dunaliella salina</i>	6.0–25.0	116.0	0.22–0.34	1.6–3.5/20–38
<i>Dunaliella primolecta</i>	23.1	–	0.09	14
<i>Dunaliella tertiolecta</i>	16.7–71.0	–	0.12	–
<i>Dunaliella</i> sp.	17.5–67.0	33.5	–	–
<i>Euglena gracilis</i>	14.0–20.0	–	7.70	–
<i>Isochrysis galbana</i>	7.0–40.0	–	0.32–1.60	–
<i>Isochrysis</i> sp.	7.1–33	37.8	0.08–0.17	–
<i>Monallanthus salina</i>	20.0–22.0	–	0.08	12
<i>Nannochloris</i> sp.	20.0–56.0	60.9–76.5	0.17–0.51	–
<i>Nannochloropsis</i>	22.7–29.7	84.0–142.0	0.37–0.48	–

<i>oculata.</i>				
<i>Nannochloropsis</i> sp.	12.0–53.0	37.6–90.0	0.17–1.43	1.9–5.3
<i>Neochloris</i> <i>oleoabundans</i>	29.0–65.0	90.0–134.0	–	–
<i>Pavlova lutheri</i>	35.5	40.2	0.14	–
<i>Phaeodactylum</i> <i>tricornutum</i>	18.0–57.0	44.8	0.003–1.9	2.4–21
<i>Porphyridium</i> <i>cruentum</i>	9.0–18.8/60.7	34.8	0.36–1.50	25
<i>Scenedesmus</i> <i>obliquus</i>	11.0–55.0	–	0.004–0.74	–
<i>Scenedesmus</i> sp.	19.6–21.1	40.8–53.9	0.03–0.26	2.43–13.52
<i>Skeletonema</i> sp.	13.3–31.8	27.3	0.09	–
<i>Skeletonema</i> <i>costatum</i>	13.5–51.3	17.4	0.08	–
<i>Spirulina</i> <i>platensis</i>	4.0–16.6	–	0.06–4.3	1.5–14.5/24–51
<i>Spirulina</i> <i>maxima</i>	4.0–9.0	–	0.21–0.25	25
<i>Thalassiosira</i> <i>pseudonana</i>	20.6	17.4	0.08	–

Oil is present in form of lipid in algae. This oil needs to be extracted before it can be converted to biodiesel. Next section deals with the various extraction methods.

2.2. Algae oil extraction

There are various methods used for extraction of oil from algal biomass out of which the most widely used methods are:

1. Mechanical methods
 - (i) Expeller press
 - (ii) Ultrasonic-assisted extraction
2. Chemical methods
 - (i) Solvent extraction method
 - (ii) Supercritical fluid extraction

2.2.1. Solvent extraction

Sathish et. al (2012) [18] used hexane as solvent in their wet lipid extraction procedure and achieved 79% extraction of tranesterifiable lipid from wet biomass.

Demirbas (2009) [19] used solvent extraction. Oil from algal paste is extracted with the help of various organic solvents such as benzene, cyclo-hexane, hexane, acetone, chloroform etc. Oil is extracted from algae by solvents by destroying algal cell wall. Since, oil has higher solubility in organic solvents than aqueous medium they are extracted from it. Distillation can be used to remove solvent from oil. Hexane was found to be the most efficient solvent because of its high extraction capability and low cost.

A.B.M. Sharif Hossain et al., (2008) [20] used grounded algae and removed moisture to dry it in an incubator at 80°C for twenty minutes. Oil was extracted by mixing equal volume mixture of hexane and ether (20ml each) with dried and grounded algae and kept for settling. It was reported that the solvent mixture was able to extract oil efficiently.

Fajardo et al., (2007) [21] used solvent extraction for lipid extraction by using a two-step process. In first step ethanol is used for extraction of the lipids and then in second step, hexane is used for purifying the extracted lipids. They have reported about 80% of lipid recovery yields by this two-stage extraction.

Xiou et al., (2005) [22] used butanol in solvent extraction instead of ethanol for lipid extraction as butanol has also been shown effective in extracting lysophospholipids. But the shortfall of this method is that butanol has high boiling and is therefore difficult to evaporate and secondly it tends to extract more impurities due to its high polarity.

Pratoomyot et al., (2005) [23] used solvent extraction and found that fatty acid content in microalgae varied between different species when extracted using chloroform: methanol (2:1, v/v) as a solvent. However, this particular method is unsuitable due to the use of environmentally destructive solvents.

2.2.2. Supercritical fluid extraction (SFE)

Grierson et al. (2011) [24], used supercritical fluid extraction and their work compares the use of organic solvent, supercritical carbon dioxide (SC-CO₂), and pyrolysis to assess their relative capacity to derive oil from the marine microalgae *Tetraselmis chui*. The SC-CO₂ technique was shown to be the least effective in natural oil extraction from *T. chui*. The results reveal that pure solvent extraction produces the most complete extraction of natural oil at just under 15% by weight.

Macias-Sanchez, (2005) [25] used supercritical fluid extraction and studied it for the extraction of lipids from microalgae. He found that SFE method is extremely time efficient because it uses high pressures and temperatures to rupture the cells.

Andrich et al., (2005) [26] used supercritical fluid extraction and his study shows the kinetics of SFE in extraction of *Nanochloropsis sp.* to produce bioactive lipid (PUFA). Even after applying different SFE parameters (temperature range between 45 and 55 °C and pressure ranging 400–700 bar), no change was observed in PUFA profile. Similar results were obtained for lipid extraction when SFE system and solvent extraction using hexane were both used.

Canela et al., (2002) [27] used supercritical fluid extraction and studied the effect of temperature and pressure. Their studies revealed that the temperature and pressure of SFE did not have any effect on yield of extracted compounds but they influence the extraction rate.

Mendes et al. and Reis et al.(1994) [28] submitted freeze-dried samples of the microalga *Chlorella vulgaris* to supercritical CO₂ at temperatures of 40 °C and 55 °C and pressures up to 35 MPa. This study was carried out on whole and crushed algae. The extraction yields of carotenoids and other lipids were low in the former case and improved significantly in the latter one. Extraction yields of carotenoids and other lipids increased with pressure. The fraction of carotenoids in the oil was also greater at higher pressures. On the other hand, supercritical CO₂ extraction of carotenoids compared favourably with hexane and acetone extractions.

2.2.3. Expeller press method

Popoola et al., (2006) [30] used expeller press method for extraction of oil from microalgae. To ensure the efficacy of this process, algae had to be dried. Press uses pressure to break cells and compress out oil. Though this method extracts almost 75% of oil and no special skill is required, this conventional method has been reported to be less effective due to relatively longer extraction time.

2.2.4. Ultrasonic assisted extraction

Yunus et al., (2011) [31] study aims at describing the characteristics of the microalgae oil extraction from *Nannochloropsis* sp. using soxhlet and ultrasonic methods with ethanol as common solvent. Various parameters were used to find the characteristics of each extraction. For SE (Soxhlet Extraction): Ethanol concentration and time; whereas for UE (Ultrasonic Extraction, Frequency = 40 kHz): Ethanol volume, time and temperature. The quality of algae oil proceeded by SE was shown by the level of FFA (Free Fatty Acid) and saponification number. In the SE study, the best combination was gained when the ethanol concentration was 70% and the given time was 200 min in which the FFA level was 9.4% and the saponification number was 286.8. While in the UE study, 51.6 min, 98% of ethanol concentration and 69.62°C were the best circumstance in which the quantity of the oil yield got its maximum. In SE, the higher solvent concentration, the higher FFA level and saponification number were gained. However, after reaching the peak at particular circumstance, the saponification number decreased gradually. Meanwhile, UE reduced the length of extraction time.

Pernet and Tremblay, (2003) [32] have reported their research work using ultrasound for complete extraction of lipids from *Chaetoceros gracilis*. Effect of storage time and treatment method on the yield of lipid extracts was studied and it was concluded that ultrasonic increased extraction rate thus affecting recovery of lipid extracts throughout the process.

Wiltshire et al., (2000) [33] with the help of ultrasound was able to gain 90% extraction of fatty acids and pigments from *Scenedesmus obliquus*. No change in products i.e. breakdown or alteration was reported to be observed during the extraction.

Solvent extraction method was chosen to extract lipids from the algae keeping in mind the availability of the equipments for the process and the reported recovery efficiency.

2.3. Catalytic upgradation

2.3.1. Historical development

In the early 1890's Rudolph Diesel developed the diesel engine which was reliable, gave high fuel economy and was a good source of power. He also experimented to pursue vegetable oils as fuel for diesel engines. But, the credit for initial experiments to produce bio-diesel has been given to two scientists E. Duffy and J. Patrick [29]. In 1853, these two chemists used vegetable oils to produce soap by transesterification. They couldn't find a solid suitable application of the by-product "Bio-diesel" until the invention of diesel engine. Post 1912, diesel engine manufacturers decided to use the lower viscous fossil fuels to power their engines, which led to modifications in engines and since the fossil fuels (petro-diesel) were cheaper, they suddenly became popular and all this led to the elimination of bio-fuels. In present conditions, bio-fuels have again popularity and significant efforts are being made to make bio-fuels efficient and cheaper.

The catalyst used in early transesterification reactions were sodium hydroxide or potassium hydroxide or sulphuric acid. All these chemicals pose various difficulties i.e. in case of base catalyst like NaOH or KOH, saponification occurs which leads to lower yields. Also, the basicity of the resulting product increases and therefore had to be washed 2-3 times to make it suitable for use. Similarly in case of acid catalysts like H₂SO₄ acidity increases and hence more purification costs. With all these disadvantages, scientist began experimenting with various heterogeneous catalysts to obtain better yield and have less difficulties in handling the products.

2.3.2. Types of catalyst

2.3.2.1. Homogeneous catalyst

2.3.2.2. Heterogeneous catalyst

Most of the commercial biodiesel that is currently produced comes from the transesterification using homogeneous base (NaOH or KOH) catalyzed processes. The base catalyzed process is less corrosive than the homogeneous acid (H₂SO₄) catalyzed one and proceeds at a much higher rate. Methanol and to lesser extent ethanol are the most frequently used alcohols in biodiesel synthesis because of their availability and low cost. Even though homogeneous catalyzed biodiesel production processes are relatively fast and show high conversions with minimal side reactions, they are still not very cost-competitive with petrodiesel ones because: (1) the catalyst cannot be recovered and must be neutralized at the end of the reaction, (2) there is limited use of continuous processing methodologies, and (3) the processes are very sensitive to the presence of water and FFAs, consequently they need a high quality feedstock (e.g., virgin or refined VOs) to avoid undesired side reactions (hydrolysis and saponification) or additional reaction steps to first convert/eliminate the free fatty acids (FFA). [34]

2.3.3. Heterogeneous catalyst

Biodiesel synthesis using solid catalysts instead of homogeneous liquid catalysts could potentially lead to cheaper production costs because of reuse of the catalyst and the possibility for carrying out both transesterification and esterification simultaneously [35].

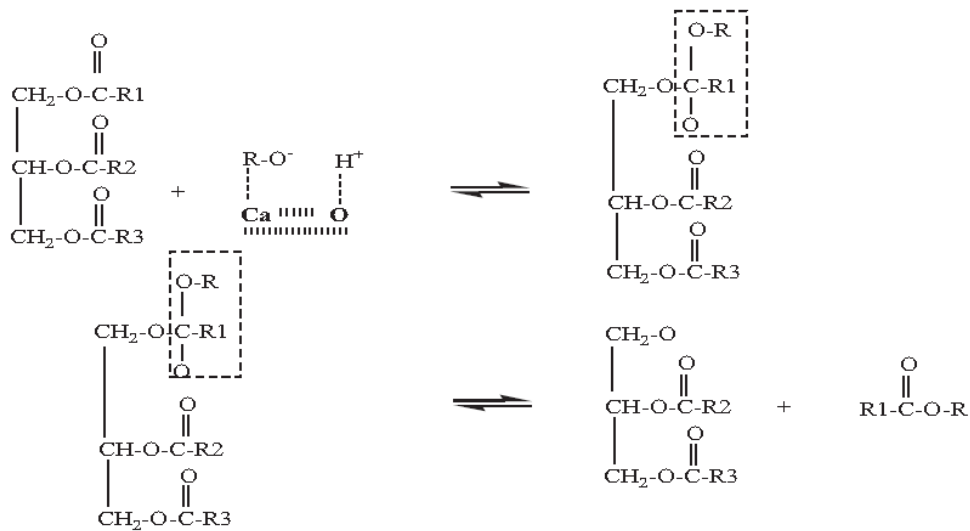
2.3.3.1. Mechanism of heterogeneous catalyst

In the first step, catalyst abstracts proton from alcohol to form alkoxide anion. Alkoxide anion attacks the carbonyl carbon atom in the triglyceride molecule, which leads to the formation of alkoxycarbonyl intermediate which then separates into two molecules: Fatty acid methyl ester and diglyceride, forming the second step of the reaction. Catalyst is regenerated in the third and final step of the reaction. Figure 2.2 shows the mechanism of transesterification reaction using heterogeneous catalyst for the production of biodiesel using soyabean oil.

Step 1:



Step 2:



Step 3:

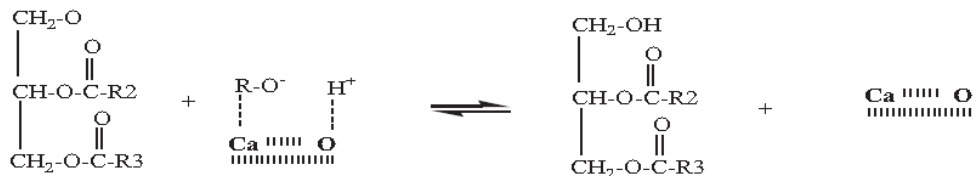


Figure 2.2: Transesterification mechanism of heterogeneous catalyst for the production of bio-diesel using soyabean oil [34] (Reproduced by permission)

2.3.3.2. Work done on heterogeneous catalyst

Many researchers have developed heterogeneous catalyst for the production of biodiesel from different feeds stocks. Table 2.3 enlist some heterogeneous catalyst developed for transesterification of various feed stocks along with the reaction conditions used in the process.

Table 2.3: Literature survey on solid catalysts for different oil sources for heterogeneous transesterification

S.No.	Substrate source	Reaction Conditions	Catalyst	Biodiesel yield (%)	Cited Literature
1	Palm oil	T = 70 °C, t = 2–3 h, R = 15:1 and c.c = 3–6 wt. %	KOH loaded on Al ₂ O ₃ and KOH loaded on NaY Zeolite	91.07	[36]

2	Unrefined oil or waste oil	T = 170-220 °C, t= 3h, R= 36:1	ZnO–La ₂ O ₃	96.0	[37]
3	Soybean frying oil	-	Mg MCM-41, Mg–Al Hydrotalcite, K ⁺ loaded Zirconia	97	[38]
4	Soybean oil	t= 3.5h, R=54:1, c.c=5 wt. %	anhydrous Na ₂ MoO ₄	95	[39]
5	Palm kernel oil and coconut oil	T=60°C, t=3h, R=65:1, c.c=10, 15-20wt. %	Al ₂ O ₃ supported various alkali and alkaline earth oxides	93	[40]
6	Rapeseed oil	T=60°C, t=3h, c.c<0.1 wt. %	CaO–Ca(OCH ₃)	90	[41]
7	Palm kernel oil	T=60°C, t=1h, R=30:1 c.c=10 wt. %	CaO-ZnO mixture	>94	[42]
8	Edible and nonedible oil	T=65°C, t=0.3h, c.c= 5 wt. %	Mg/La	>90	[43]
9	Sunflower oil	T=60°C, t=5h, c.c= 1 wt. %, R=12:1	CaO supported on SBA-15, MCM-41, and fumed silica	95	[44]
10	Palm kernel oil	T=60°C, t=3h, c.c= 10 wt. %, R=15:1	Modified dolomites – CaO catalyst	99.9	[45]
11	Soybean oil	T=65°C, t=1h c.c = 6 wt. %, R=12:1	Ba–ZnO	96	[46]
12	Cotton seed oil	T=230°C, t=3h, c.c= 2 wt. %, R=12:1	MgO, CaO, and MgO–Al ₂ O ₃	90	[47]
13	Soybean oil	-	(i) KF/CaO (ii)MgO/calcined hydrotalcite	90 >90	[48], [60]
14	Algal Oil	T=50°C, t=4h, c.c= 2 wt. %, R=30:1 T=50°C, t=4h, c.c= 2 wt. %, R=6:1	(i) CaO supported on Al ₂ O ₃ (ii) MgO supported on Al ₂ O ₃	97.5 16	[49]
15	Algal Oil	-	Mg–Zr solid base	28	[50]

We can conclude that many researchers have put forward their feet to explore new and different heterogeneous catalyst with variety of feed stocks for the production of bio-diesel. This area of research is very vast and full of new possibilities. Many catalysts have been synthesized and have produced good results. As evident from table above, the catalyst which have given good results are mostly basic and contains alkali or alkaline earth metal ions in them.

CaO is the most popular and widely used heterogeneous catalyst for the production of biodiesel [51], [34], [52], [53]. It is used in various forms such as neat CaO, loaded CaO, mixed CaO, waste CaO and supported CaO [54].

Ismadji et. al [55] used waste capiz (*Amusium cristatum*) shell as a raw material for obtaining CaO by calcination of CaCO_3 present in the shell. The waste raw shells obtained from local fish market were washed to remove the dirt and unwanted materials. Next, the moisture was removed by keeping it in oven for twenty-four hours at 100°C and thereafter pulverized before calcining in the furnace at 900°C for 2 h. The calcinated material was crushed and sieved through 170 mesh screens and was stored in dessicators for further use. Biodiesel was produced using the following process conditions: mole ratio methanol/palm oil mole ratio of 8:1, stirring speed of 700 rpm, and reaction temperature was 60°C and reaction time of four, five, and six hours. The amount of catalyst was varied at between 1-5 wt. %. The maximum yield of biodiesel was $93 \pm 2.2\%$, obtained at 6 h of reaction time and 3 wt % of amount of catalyst.

Sharma et. al [56], [57] also studied CaO for tranesterification by using Egg shell and *Mereterix mereterix*. In the first study the calcined egg shells were used and reaction conditions; methanol-oil molar ratio of 8, catalyst concentration (wt. %) of 2.5 and time 2.5 hours at boiling point of methanol gave yield of 95.0% and conversion of 97.4%. In the second study waste frying oil was used to produce biodiesel using powdered calcined clamshell. Clamshell calcined for different times differed in catalytic activity and reduced reaction time (3.5h calcined catalyst). The XRD analysis of the unused catalyst showed the peaks of CaO and few of Ca(OH)_2 , whose presence was confirmed by FTIR. Further, the optimized reaction conditions were found to be as methanol-oil ratio of 6.03, catalyst weight of 3g (about 1 wt. %) at 60°C in 3 h which gave in yield of more than 89 % and conversion of more than 97 %.

CaO is selected as the target material to be used for synthesizing heterogeneous catalyst. Recently, Wen et. al [58] used TiO₂ to improve the stability of the catalyst and obtained biodiesel yield of 79.9 % . Kawashima et. al. [59] prepared CaTiO₃ using equimolar mixture of CaO and TiO₂ and obtained 79 % yield of biodiesel using rapeseed oil. This study will investigate the alteration, if any, caused in the biodiesel yield by using CaO and TiO₂ mixtures of various molar ratios as catalysts for upgradation of oil.

CHAPTER 3

METHODS AND PROCEDURES

3.1. Collection and pre-treatment of algal biomass

The algal biomass was collected from Solani aqueduct (Figure 3.1) in different batches. Each batch contained around 5-7 kg wet algal biomass. The collected wet biomass was brought to the lab and then washed with tap water several times to remove the dirt, sand and other unwanted organic matter. The washed wet biomass was then spread on the net and kept in sunlight for 3 days to evaporate water. The dry biomass thus obtained was pulverised and sieved to obtain particles of size 100- 250 microns. The procedure was again repeated with the oversize until only a few grams of oversize biomass were left.



Figure 3.1: Photographs of algae in Solani aqueduct

3.2. Identification of algal species

Taxonomy study of the native algal sample was carried out at Central Drug Research Institute, Lucknow to identify the algal species present in the biomass.

3.3. Determination of maximum lipid content of the algal biomass. [63]

Lipid extraction was performed on the algae sample collected from Solani River, Roorkee to find the maximum amount of oil available from a given mass of dried algal biomass using Bligh and Dyer protocol. All glass equipment used in the extraction were first washed with soap and water, soaked in 1 N hydrochloric acid (HCl) and then were cleaned properly by distilled water. Then, they were left in oven at 100°C overnight. Baked glass wares were removed from the oven and left in the open to cool down to ambient temperature.

About 1 g of dry biomass was pulverized using a mortar and pestle. The powder was added into a 100 ml glass-beaker with a stir bar plus 10 ml of chloroform, 20 ml of methanol and 10 ml of distilled water. The flask (containing chloroform, methanol, water, dry biomass and stir bar) was placed on a magnetic stir plate (stirrer) for 24 hours. Sample was then filtered through whatman grade # 1 filter paper. The initial flask and the filter were rinsed with 10 ml of chloroform plus 10 ml of distilled water to collect any remaining oil (lipid). Collected solvent mixed with algal oil (lipid) was transferred in a 250 ml glass separator funnel and settled for 2 hours to allow a total separation of water/methanol (top layer) from chloroform/lipid (bottom layer).

The bottom layer was collected in a pre-weighted flask, followed by solvent evaporation using a water bath set at 40-45°C. After the total evaporation of solvent, the remainder was algal lipid or oil. It was placed in the oven overnight at 45°C to remove any remaining moisture. The lipid content (g of lipid per g of dry algae) was calculated using equation

$$\text{Lipid Content(\%)} = \frac{\text{mass of (flask + lipid)} - \text{mass of empty flask(g)}}{\text{mass of dry algal biomass(g)}} * 100$$

3.4. Extraction of oil from algal biomass using hexane

Oil from the milled biomass was extracted using hexane as a solvent in a Soxhlet apparatus as shown in the Figure 3.2.

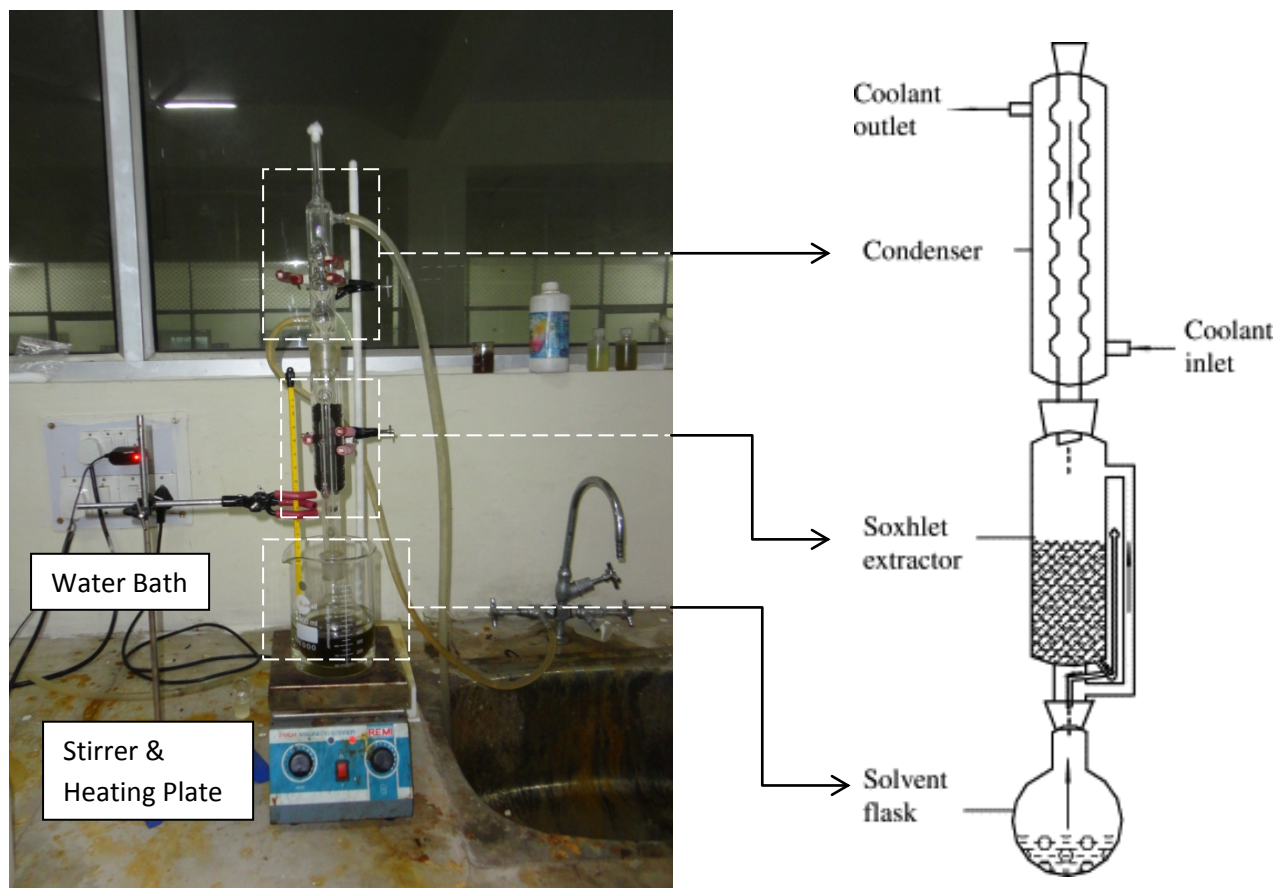


Figure 3.2: Soxhlet apparatus

For a batch, some dried biomass was kept in the extractor and 200 ml of hexane was filled in the round bottom flask. The extractor was connected to a condenser to recover hexane. Before filling the extractor with biomass, whatman grade #1 filter paper was kept in the bottom of the extractor covering the hole to prevent the passage of biomass particles in the solvent flask. The round bottom flask was heated from the bottom using water bath as shown in Figure 3.1 to evaporate hexane. The hexane vapour travels through the side pipe of the extractor and condenses in to liquid in the condenser. The liquid hexane then drops directly onto the algal biomass in the extractor and extracts lipid from it. The hexane along with the lipid passes through the biomass bed in the extractor and is then siphoned out through the siphon getting collected in the round bottom flask at the bottom of the apparatus. The hexane again evaporates and the process continues.

The procedure was conducted in batches, each for 12 hours. The left over biomass was washed with hexane to remove any leftover oil adhered to the surface of the biomass particles. All oil-hexane mixtures were mixed and hexane was recovered by evaporation and condensation.

3.5. Synthesis of catalyst for biodiesel production

Catalyst was synthesized by conventional solid state reaction [59], [61]. Calcium oxide and titanium dioxide (both obtained from Thomas Baker (Chemicals) Pvt. Ltd.) in various molar ratios were milled and mixed in an agate mortar. Ethanol was used to wet the mixture and ensure good mixing. Further, the mixtures were laid on the aluminium foil and calcined to 900°C for 8 h to enable the formation of CaTiO₃. After 8 h, the mixtures were taken out, cooled in the desiccator and milled again. The mixtures were again calcined to 650°C for 3h to remove environmental contamination, if any that could have occurred in the above step. The temperature was kept lower to avoid agglomeration of catalyst particles because subsequent milling would have again contaminated the catalyst samples. After calcination, the mixtures were cooled in the desiccator, packed in air tight sample tubes and kept in the desiccator.

The catalysts with different amount of TiO₂ and CaO were prepared as shown in Table 3.1 to determine the one with the best catalytic activity.

Table 3.1: Various catalyst compositions used in the study

S.No.	TiO ₂ (g) [Mol. Wt. 80g]	CaO(g) [Mol. Wt. 56g]	TiO ₂ :CaO (Molar Ratio)	Name
1	8	2.8	2	TiO ₂ -CaO-2
2	4	2.8	1	TiO ₂ -CaO-1
3	2	2.8	0.5	TiO ₂ -CaO-0.5
4	1	2.8	0.25	TiO ₂ -CaO-0.25
5	0	5	0*	CaO

* No TiO₂ was present in the sample.

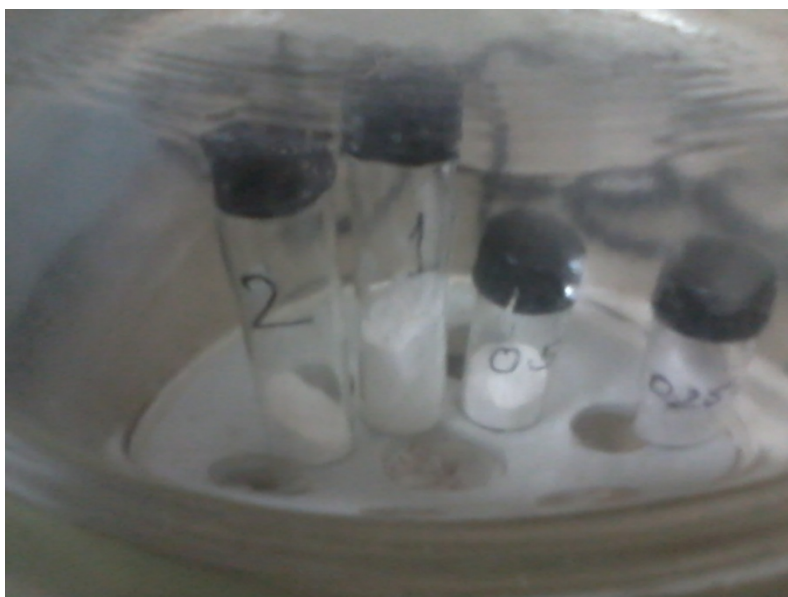


Figure 3.3: Various catalyst samples

Further, the best composition catalyst was subjected to various temperatures for calcination as shown in Table 3.2, to check for any increased/decreased catalytic activity.

Table 3.2: Various calcination temperatures used for best composition catalyst in the study

S.No.	Temperature
1	650°C
2	700°C
3	750°C

3.6. Selection of catalyst

To determine the best composition catalyst, each catalyst sample as stated in Table 3.1 was subjected to transesterification reaction. 20g of warm algal oil was mixed with 33g of methanol (methanol:oil molar ratio = 15) and 1g of catalyst (catalyst conc. = 5 wt.%) and the mixture was kept on a magnetic stirrer in conical flask fitted with a condenser under reflux at 50°C for 8 h. CaO was used as obtained.

After the reaction, excess methanol was removed by vacuum drying and the remaining mixture was centrifuged. The centrifuged mixture contained 3 layers, the bottom with the used catalyst and coloured pigment, middle with the glycerol and upper layer with oil and biodiesel. The upper layer was recovered and rest discarded. The upper layer was again centrifuged at 8000g for 10 minutes. The top layer was recovered, analysed and weighed. % biodiesel yield was defined as =

$$\frac{(\text{amount of biodiesel(g) in upper layer})}{(\text{initial amount of microalgal lipid(g)})} \times 100$$

Best composition catalyst mixtures calcined at different temperatures were subjected to transesterification in different beakers. The procedure and reaction conditions used were same as were used for the best composition catalyst. The catalyst with the highest yield was stated as best.

3.7. Catalyst characterization

X-ray powder diffraction analysis was conducted with a Bruker X-ray diffractometer (model D8 Advance), employing Cu-K α radiation from 5°-90° interfaced with Leptos and EVA computer software. Thermo-gravimetric data of the samples were acquired using a thermal analyzer (SII 6300 EXSTAR) at a scan rate of 10 °C/min from room temperature to 900 °C. SEM images were taken using LEO 435 VP.

3.8. Optimization of process conditions and modelling of the process

Optimization and modelling of the transesterification reaction was done using Design Expert software version 8.0.7.1 trial. Box-Behnken model using 3 factors (methanol to oil ratio, catalyst doze and reaction time) with 3 levels each was used to fix the experimental conditions. Various levels are methanol to oil ratio (12, 15, 18), catalyst conc. (3, 5, 7 wt. %) and reaction time (6, 8, 10 h). A set of 17 experimental conditions was obtained. Design expert software version 8.0.7.1 trial has been used to process the experimental data to develop an empirical model correlating process variables with biodiesel yield (R1). Desirability values for the data set were also generated using the software. Table 3.3 gives the design summary and Table 3.4 shows the experiment points.

Table 3.3: Design summary

Factor	Name	Units	Type	Minimum	Maximum	Coded Values		Mean	Std. Dev.
						-1	+1		
A	Methanol:Oil	Ratio	Numeric	12	18	12	18	15	2.06
B	Catalyst Conc.	Wt %	Numeric	3	7	3	7	5	1.37
C	Time	Hours	Numeric	6	10	6	10	8	1.37

Table 3.4: Design of experiment

Run	Methanol: Oil (Ratio)	Catalyst Concentration (wt %)	Time (Hours)
1	15.00	7.00	6.00
2	12.00	3.00	8.00
3	18.00	7.00	8.00
4	15.00	5.00	8.00
5	15.00	5.00	8.00
6	15.00	5.00	8.00
7	15.00	5.00	8.00
8	18.00	3.00	8.00
9	15.00	3.00	10.00
10	18.00	5.00	10.00
11	15.00	3.00	6.00
12	18.00	5.00	6.00
13	12.00	5.00	6.00
14	15.00	5.00	8.00
15	12.00	5.00	10.00
16	15.00	7.00	10.00
17	12.00	7.00	8.00

Design expert calculates the value of Std. Deviation, R-Squared, Adjusted R-Squared, Predicted R-squared and PRESS for various models i.e linear, quadratic, cubic etc. The model which exhibits low standard deviation, high adjusted R-squared value and low PRESS value is chosen. Analysis of variance is done for the response parameter i.e. % Biodiesel Yield for the selected model. The software calculates coefficients for various factors by regression and thus the final model is obtained. With the help of final model, the final optimized point is thus obtained. The predicted biodiesel yield has been compared with the experimental yield.

3.9. Characterization of bio-oil and bio-diesel

Properties like viscosity (IS: 1448 (part-25): 1970), cloud point and pour point (BIS: 1448(part-10): 1970), flash point (BIS 1460-2000), density etc. have been found out and compared with biodiesel standard IS-15607:2005.

GC-MS analysis of biodiesel was done to analyse biodiesel. GC MS Perkin-Elmer Clarus 500 model equipped with GC Column (HP-1, 30m X 0.25mm X 0.25 μ m) are used for this purpose. The temperature program used: Injector temperature - 250°C, oven temperature: 50°C and hold for 1 min; 50°C to 250°C at 10°C/min and hold for 3 min. Interface temperature was 250°C and helium was used as a carrier gas with flow rate of 1.2mL/min.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Identification of algal species

The algal species found in the Solani aquaduct is *Hydrodictyon reticulatum* (L.) Lagerheim, which belongs to the Phylum: Chlorophyta, Order: Chlorococcales, Family: Hydrodictyaceae, Genus: *Hydrodictyon* and Species: *reticulatum*. It is thallus, macroscopic and composed of cylindrical cells which are adjoined at their ends to form a cylindrical net with 5 or 6 sided meshes. Cells are coenocytic, and the chloroplast is parietal plate at first with a single pyrenoid, later becomes reticulate with numerous pyrenoids. Cells up to 200 micron in diameter as much as 1 cm long when fully enlarged forming net up to 2 dm in length. Trace amount of *ulotrichalean* strain was also found in the algal sample. The oil content of the algal biomass, determined as per Bligh and Dyer method was found = ~ 14 wt. %. However, the amount of oil extracted from the algal biomass was only 5.8 wt. % i.e. efficiency, $\eta = 41.4\%$. This is because using hexane eliminates the extraction of non-lipid content [62]. Extraction of oil (lipid) from algal biomass changes the morphology as evident from Figure 4.1 & Figure 4.2.

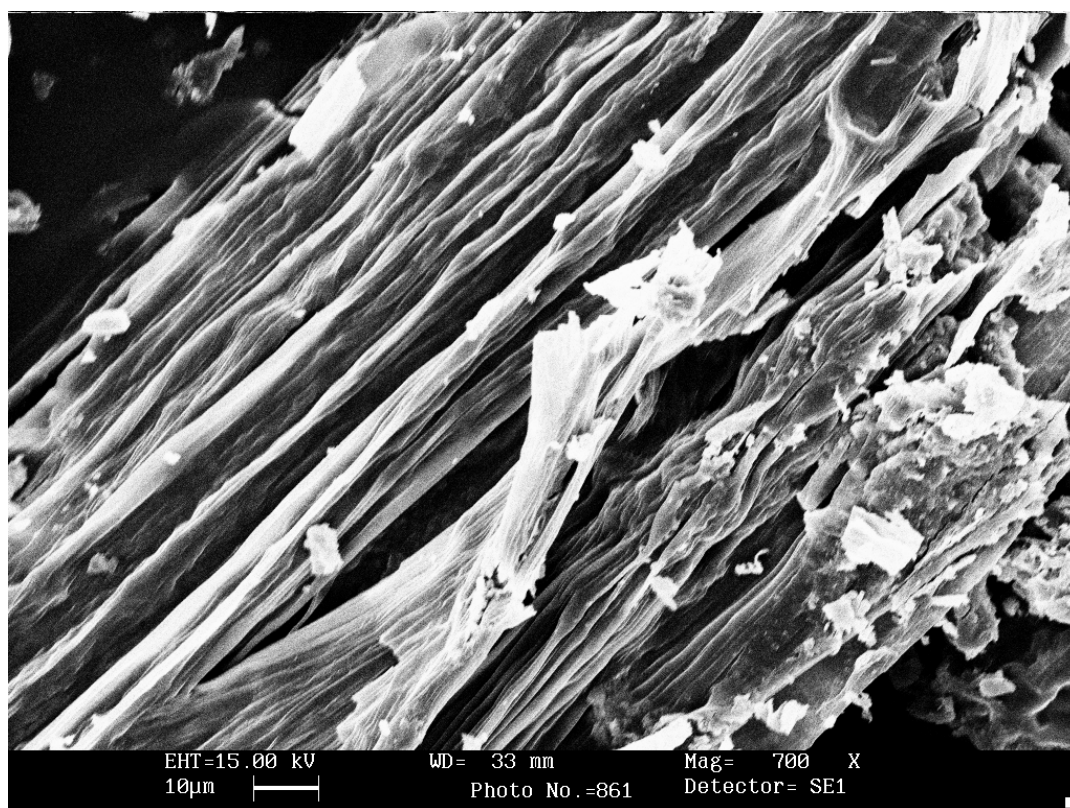


Figure 4.1: SEM image of algae surface before extraction

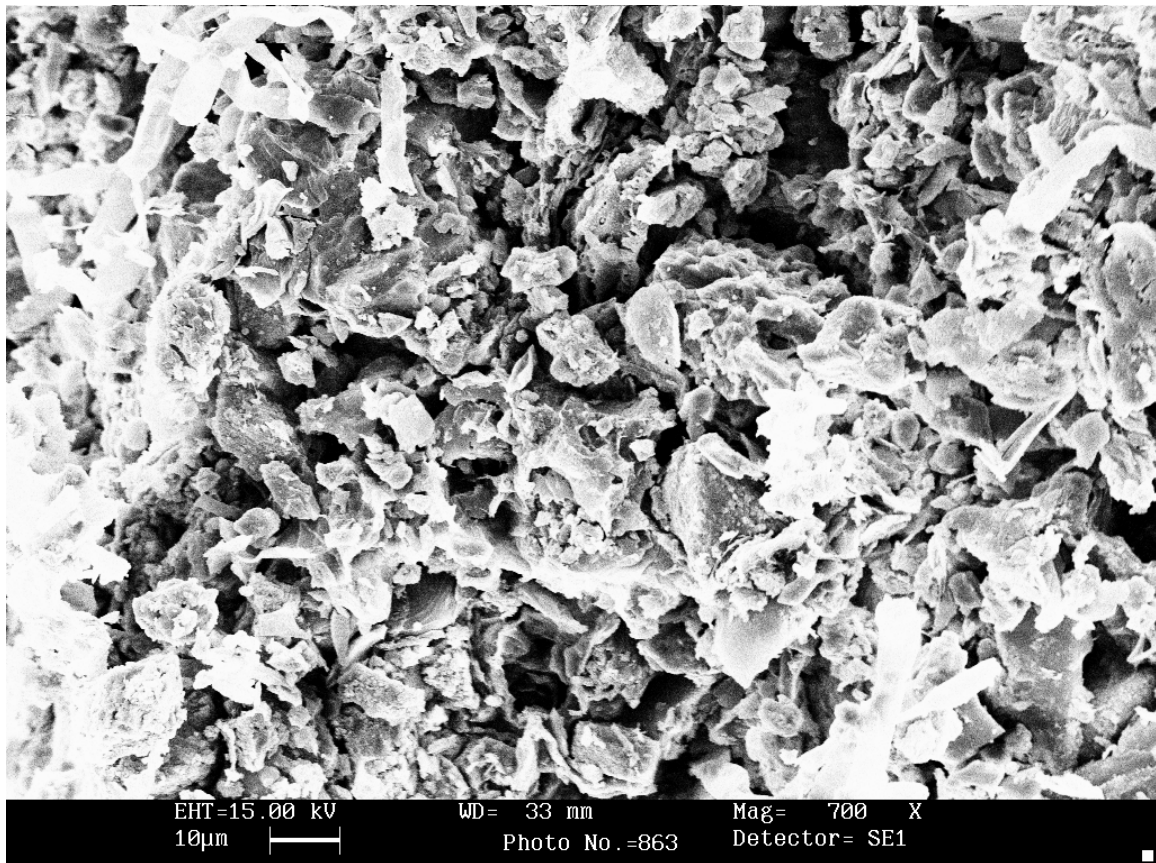


Figure 4.2: SEM image of algae surface after extraction

4.2. Selection of catalyst

The effect of catalyst type on the % biodiesel yield is shown in Figure 4.1.

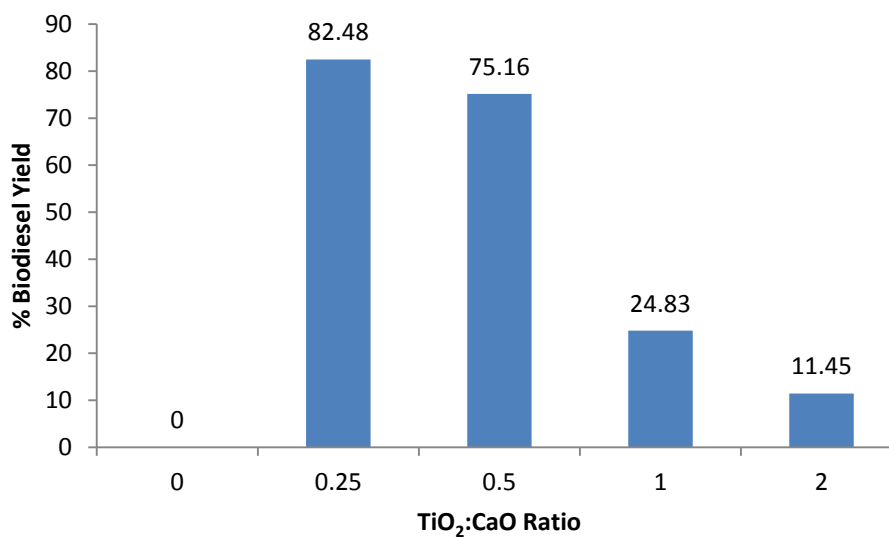


Figure 4.3: Variation of biodiesel yield with various molar composition of catalyst calcinated at 650°C
 (Reaction conditions as methanol/oil ratio of 15, catalyst concentration of 5 wt. % and reaction time of 8h under reflux)

From Figure 4.3 it is evident that with increase in $\text{TiO}_2:\text{CaO}$ ratio in the catalyst the % biodiesel yield was maximum at the $\text{TiO}_2:\text{CaO}$ ratio of 0.25 and decreases as the molar ratio increases. Since calcium is known to leach in the biodiesel [34], using mixtures containing high amount of CaO would have caused more leaching into biodiesel increasing the purification time. Hence, they were not considered in the study.

Figure 4.4 shows the effect of calcination temperature on the catalytic activity of $\text{TiO}_2\text{-CaO-0.25}$.

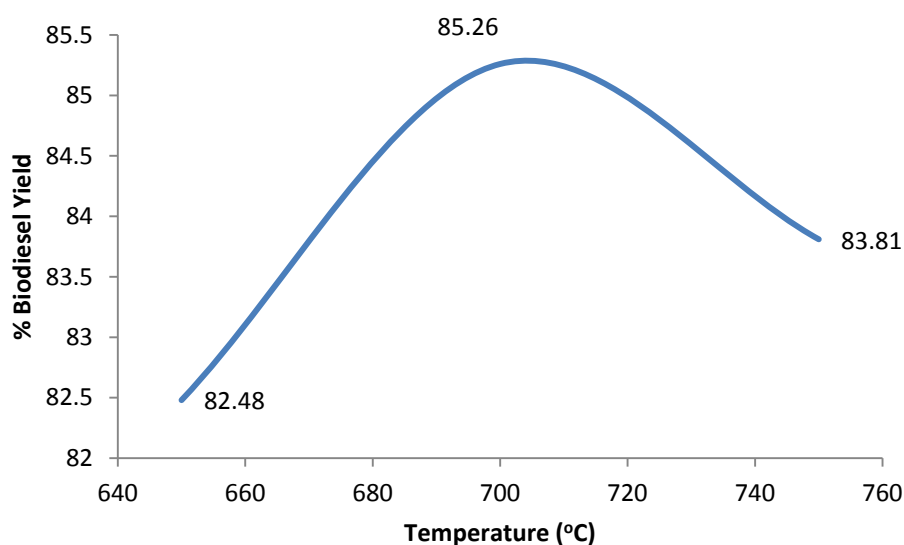


Figure 4.4: Effect of calcination temperature on the catalytic activity of $\text{TiO}_2\text{-CaO-0.25}$
(Reaction conditions as methanol/oil ratio of 15, catalyst concentration of 5 wt. % and reaction time of 8h under reflux)

From Figure 4.4, we can conclude that the catalyst calcined at temperature of 700°C gives the best yield among all the three. The catalyst is named as **$\text{TiO}_2\text{-CaO-0.25-700}$** .

4.3. Characterization of Catalyst

4.3.1. TGA/DTG/DTA Studies

Figure 4.5 to Figure 4.8 shows TG/DTG/DTA graph of various catalysts used in the study.

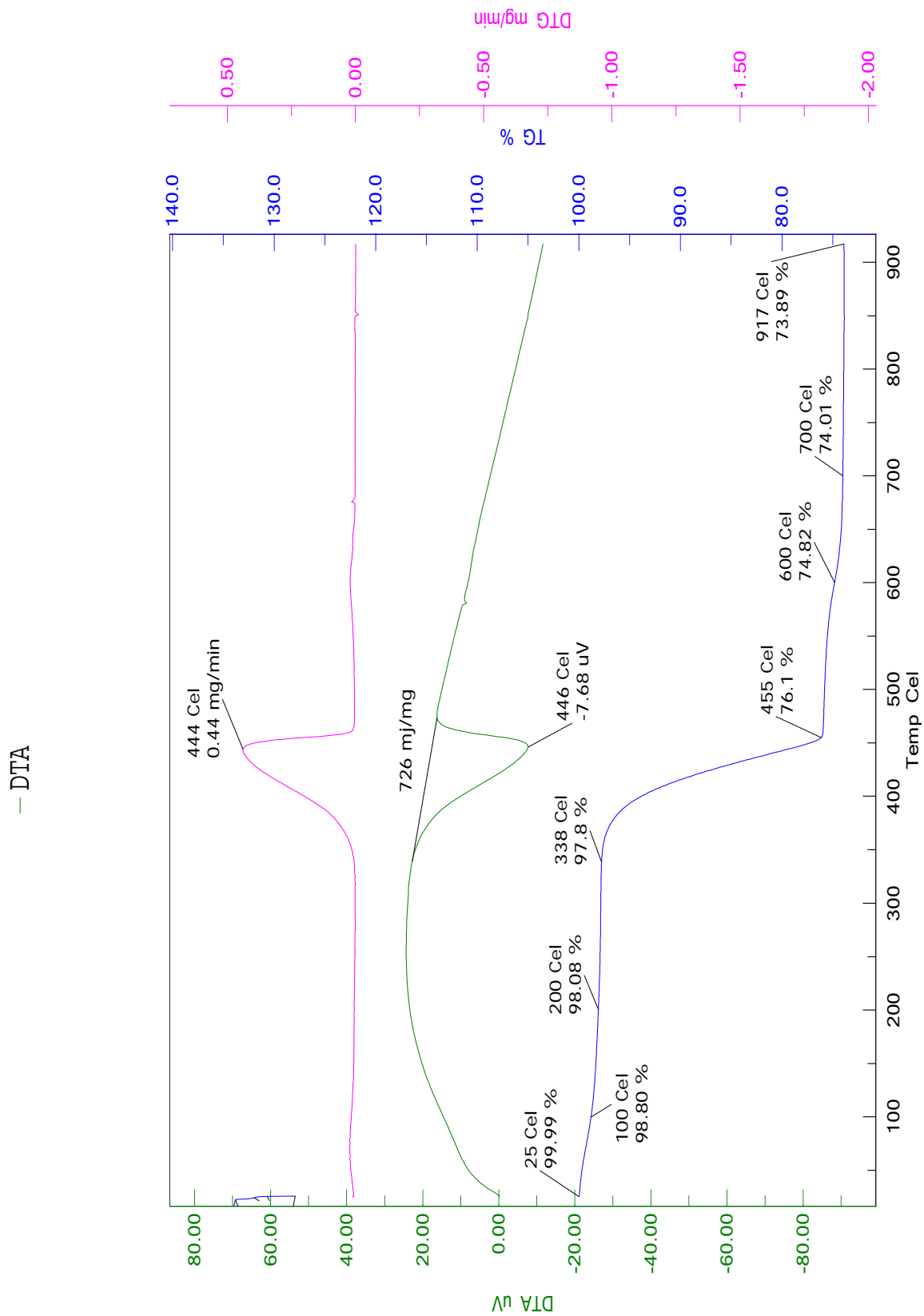


Figure 4.5: TG/DTG/DTA graph for CaO

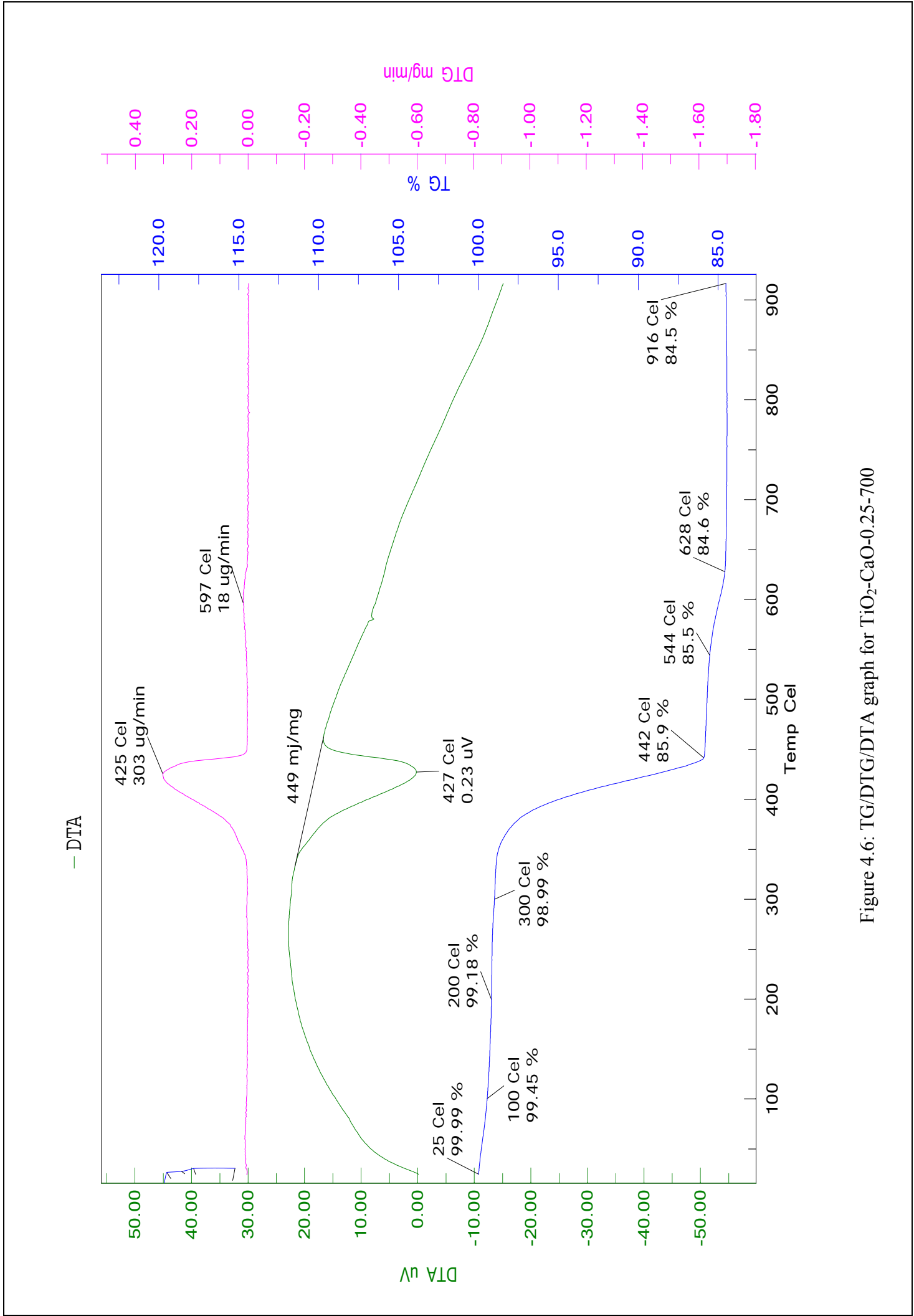


Figure 4.6: TG/DTG/DTA graph for $\text{TiO}_2\text{-CaO-0.25-700}$

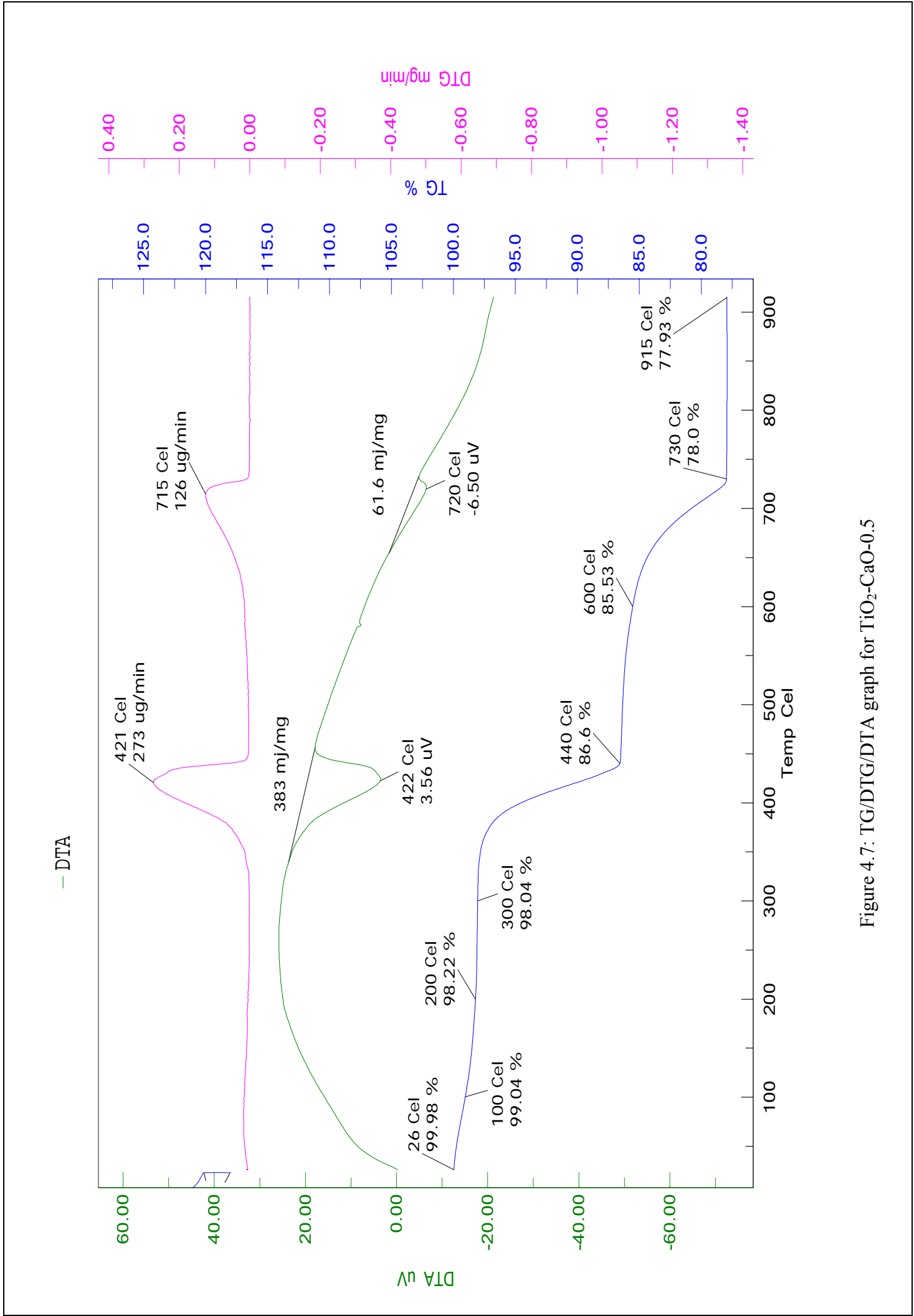


Figure 4.7: TG/DTG/DTA graph for TiO₂-CaO-0.5

— DTA

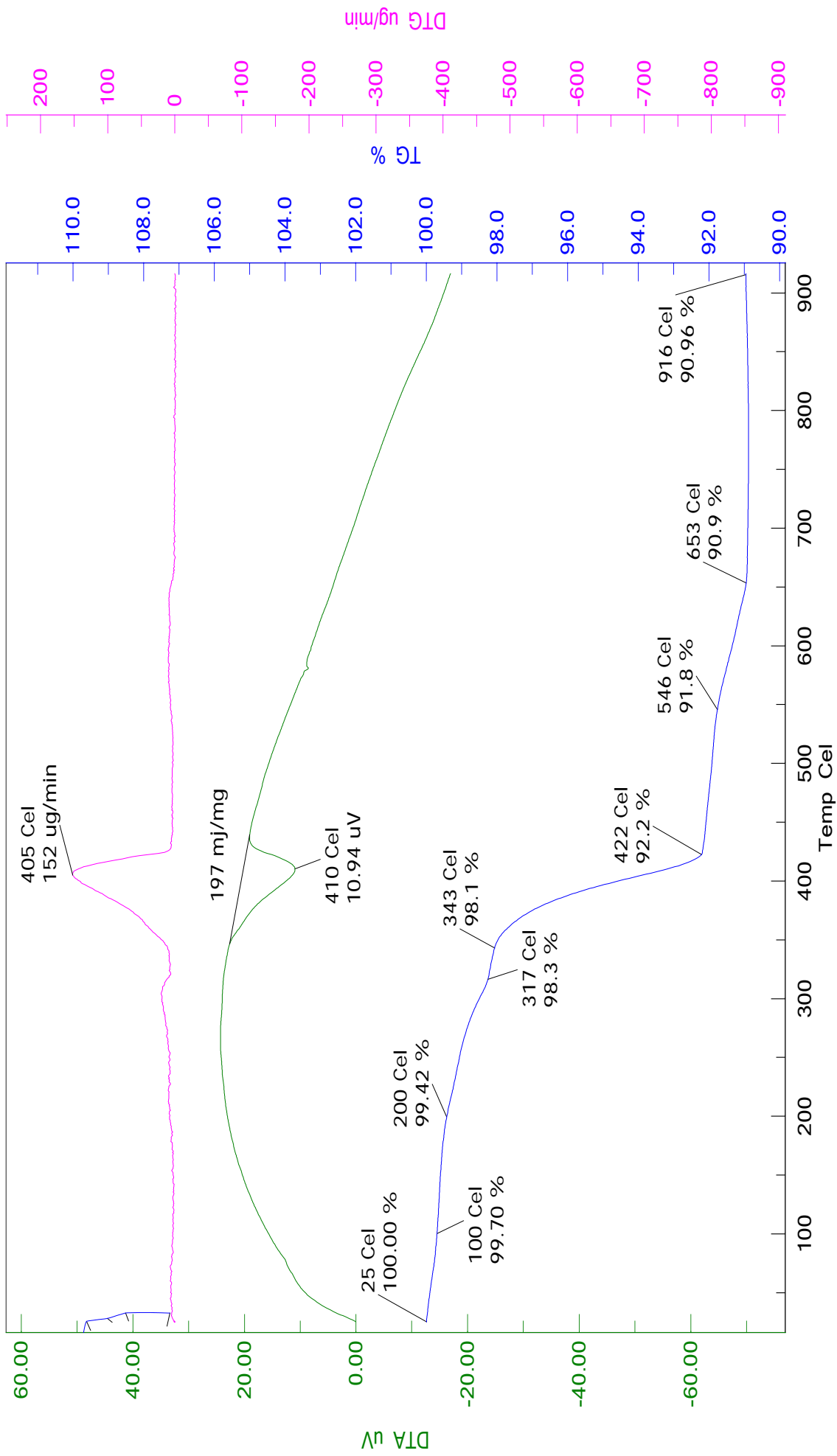


Figure 4.8: TG/DTG/DTA graph for TiO₂-CaO-1

From Figure 4.5 to Figure 4.8, it seems that all the catalyst samples contain Ca(OH)_2 in major amount, which is evident by the mass loss at temperatures between $400\text{-}500^\circ\text{C}$. The mass loss is because of evaporation of water coming from the decomposition of calcium hydroxide into calcium oxide and water. Also, from Figure 4.5 to Figure 4.8 it is evident that all the catalyst samples except $\text{TiO}_2\text{-CaO-0.5}$ contain 1-2 % CaCO_3 (mass loss between $600\text{-}700^\circ\text{C}$), whereas $\text{TiO}_2\text{-CaO-0.5}$ contained more than 8% CaCO_3 . The presence of Ca(OH)_2 and CaCO_3 is due to the exposure of samples to atmosphere before the analysis.

4.3.2. X-Ray Diffraction

Figure 4.9 to Figure 4.12 show the X-Ray diffraction pattern of various catalyst samples used in the study.

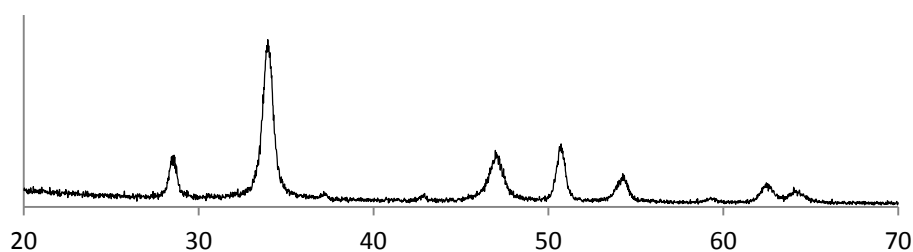


Figure 4.9: XRD pattern of CaO

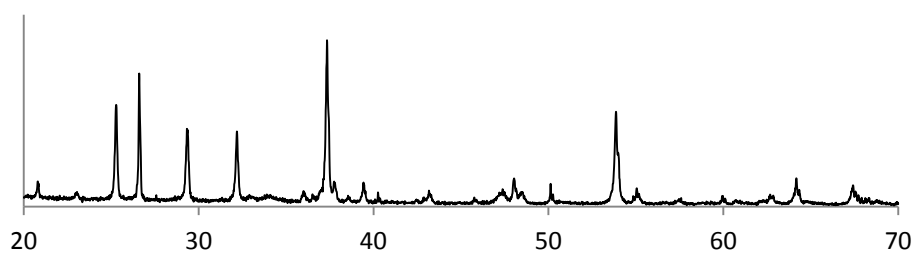


Figure 4.10: XRD pattern of $\text{TiO}_2\text{-CaO-0.25-700}$

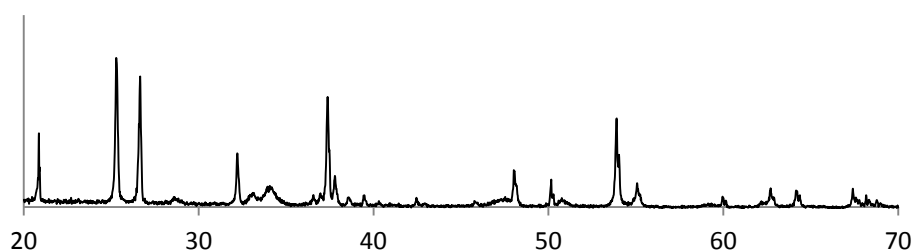


Figure 4.11: XRD pattern of $\text{TiO}_2\text{-CaO-0.5}$

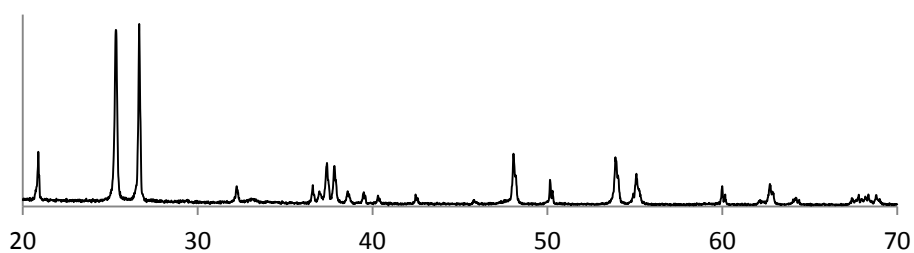


Figure 4.12: XRD pattern of TiO₂-CaO-1

The diffraction angle showed the presence of CaO and Ca(OH)₂ in Figure 4.9, which is because of conversion of some CaO into Ca(OH)₂ due to exposure to the atmosphere before and during the analysis. The diffraction peaks in the Figure 4.10 shows the presence of CaO, TiO₂, CaTiO₃ and Ca(OH)₂. Presence of Ca(OH)₂ is due to the reason stated before. Figure 4.11 shows the presence of CaO, TiO₂, CaTiO₃, Ca(OH)₂ and CaCO₃. Presence of Ca(OH)₂ and CaCO₃ is because of the reason stated above. Figure 4.12 shows the presence of CaO, TiO₂, CaTiO₃ and Ca(OH)₂. All the catalytically active mixtures contained CaTiO₃ and TiO₂ as a component. CaO with little Ca(OH)₂ was found to be catalytically inactive at the present reaction conditions as evident from Figure 4.3. From the results of XRD and thermogravimetric analysis it may be concluded that CaTiO₃ along with TiO₂ are the main catalytic species in the catalysts.

4.3.3. SEM

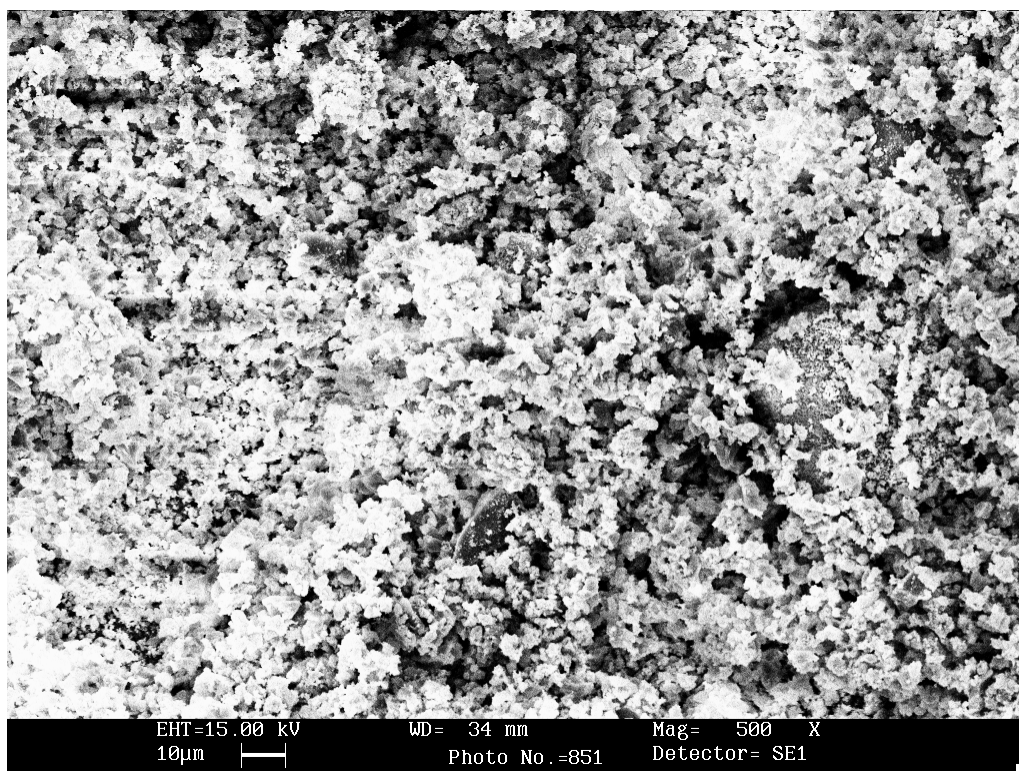


Figure 4.13: SEM photograph of TiO₂-CaO-0.25-700 before reaction

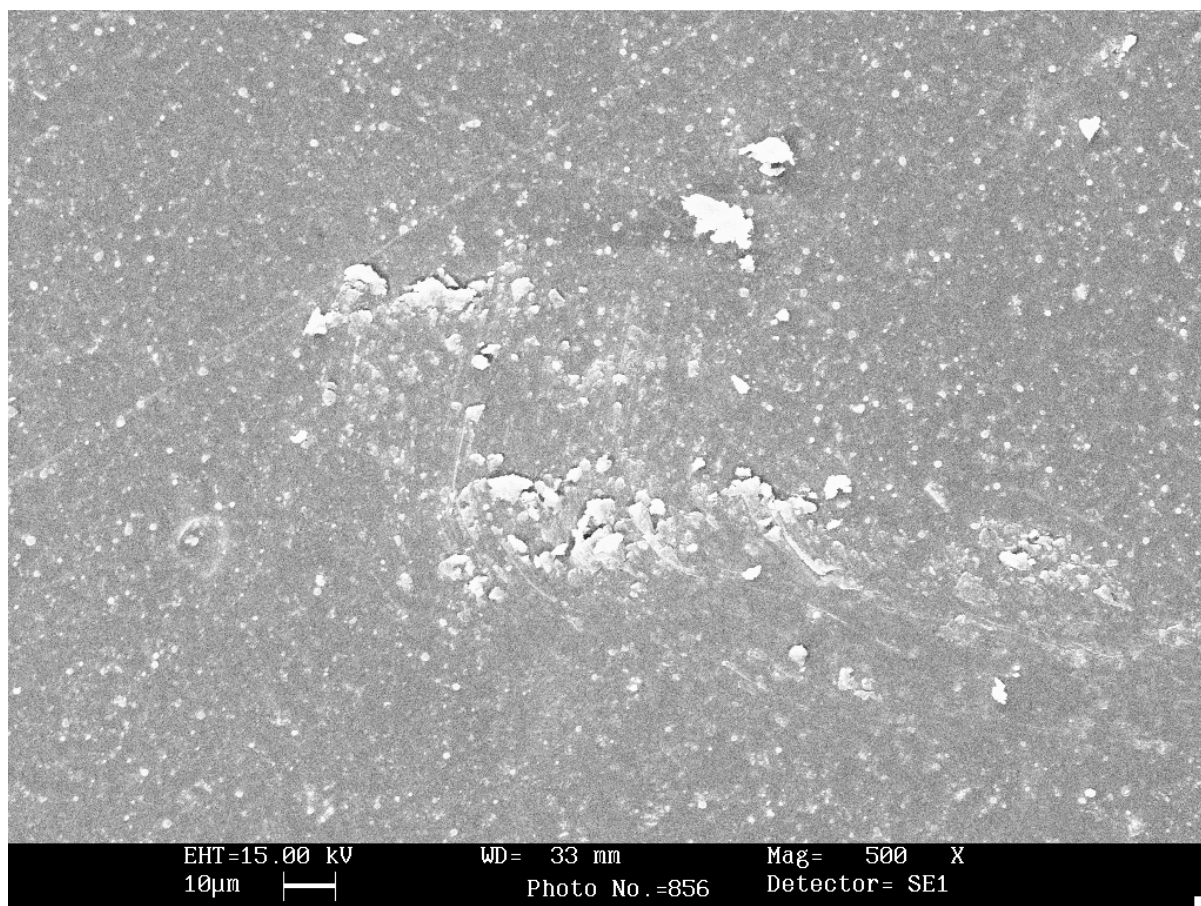


Figure 4.14: SEM photograph of $\text{TiO}_2\text{-CaO-0.25-700}$ after reaction

The spent catalyst had different morphology than the original, which shows that the structure of the catalyst could have been modified during the transesterification.

4.4. Optimization of reaction conditions and modelling of the process

For optimization, 17 experiments were conducted as per Box Behnken method as discussed in section 3.8. The results are shown in Table 4.1. Figure 4.16 shows the response surface for variation in % biodiesel yield (=R1) with methanol/oil ratio (=A), and catalyst conc. (=B). Figure 4.17 shows the response surface for variation in desirability with methanol/oil ratio (=A) and catalyst conc. (=B). Figure 4.18 shows the response surface for variation in % biodiesel yield (=R1) with methanol/oil ratio (=A) and time (=C). Figure 4.19 shows the response surface for variation in desirability with methanol/oil ratio (=A) and time (=C). Figure 4.20 shows the response surface for variation in % biodiesel yield (=R1) with catalyst conc. (=B) and time (=C). Figure 4.21 shows the response surface for variation in desirability with catalyst conc. (=B) and time (=C).

Table 4.1: Biodiesel Yield (%) for optimization of reaction conditions

Run	Methanol: Oil (Ratio)	Catalyst Concentration (wt %)	Time (Hours)	Biodiesel Yield (%)
1	15.00	7.00	6.00	72.59
2	12.00	3.00	8.00	56.22
3	18.00	7.00	8.00	69.83
4	15.00	5.00	8.00	85.26
5	15.00	5.00	8.00	85.26
6	15.00	5.00	8.00	85.26
7	15.00	5.00	8.00	85.26
8	18.00	3.00	8.00	74.05
9	15.00	3.00	10.00	73.38
10	18.00	5.00	10.00	76.92
11	15.00	3.00	6.00	60.47
12	18.00	5.00	6.00	62.58
13	12.00	5.00	6.00	57.96
14	15.00	5.00	8.00	85.26
15	12.00	5.00	10.00	62.13
16	15.00	7.00	10.00	73.14
17	12.00	7.00	8.00	68.36

Table 4.2 shows the model summary statistics, which shows the reason for choosing quadratic model over any other model. The model having the maximum value for adjusted R-squared and predicted R-squared will be our choice.

Table 4.2: Model summary statistics

Model	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-squared	
Linear	10.09	0.2156	0.0346	-0.1831	
2FI	10.92	0.2932	- 0.1309	-0.7240	
Quadratic	0.86	0.9970	0.9930	0.9512	Suggested
Cubic	0.00	1.0000	1.0000		Aliased

From Table 4.2, it is noteworthy that quadratic model has maximum adjusted R-squared value, and maximum predicted p-squared value. Also, quadratic model has predicted R-squared value of 0.9512 which shows much deviation from linear model value. So quadratic model was the chosen as it exhibits low standard deviation and high adjusted R-squared value (near to 1).

Table 4.3 gives the analysis of variance for algal oil extraction using response surface quadratic model (ANOVA).

Table 4.3: Analysis of variance for biodiesel yield (%)

Source	Sum of Squares	df	Mean Square	F value	p-value prob>F	
Model	1683.33	9	187.04	254.25	< 0.0001	significant
A-Methanol:Oil	187.31	1	187.31	254.62	< 0.0001	
B-Catalyst Concentration	49.01	1	49.01	66.61	< 0.0001	
C-Time	127.76	1	127.76	173.67	< 0.0001	
AB	66.91	1	66.91	90.96	< 0.0001	
AC	25.86	1	25.86	35.15	0.0006	
BC	38.19	1	38.19	51.92	0.0002	
A ²	563.76	1	563.76	766.35	< 0.0001	
B ²	181.95	1	181.95	247.34	< 0.0001	
C ²	325.42	1	325.42	442.35	< 0.0001	
Residual	5.15	7	0.74			
Lack of Fit	5.15	3	1.72			
Pure Error	0.000	4	0.000			
Cor Total	1688.48	16				

The Model F-value of 254.25 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. P-value less than 0.0500 indicate model terms are significant whereas values greater than 0.1000 indicate that model are insignificant. In this case A, B, C, AB, AC, BC, A², B², C² are significant model terms. Table 4.4 shows the values of various R-squared terms.

Table 4.4: Values of various R-Squared terms

Standard Deviation	0.86	R-Squared	0.9970
Mean	72.58	Adj R-Squared	0.9930
C.V.%	1.18	Pred R-Squared	0.9512
PRESS	82.39	Adeq Precision	44.919

The "Pred R-Squared" of 0.9512 is in reasonable agreement with the "Adj R-Squared" of 0.9930. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 44.919 indicates an adequate signal. This model can be used to navigate the design space. The R-squared terms are desirable when their values are near to 1, which in this case is acceptable. Table 4.5 shows the estimated values of regression coefficients for various factors.

Table 4.5: Estimated values of regression coefficients for various factors

Factor	Coefficient Estimate	df	Standard Error	95% CI		VIF
				Low	HIGH	
Intercept	85.26	1	0.38	84.35	86.17	
A-Methanol:Oil	4.84	1	0.30	4.12	5.56	1.00
B-Catalyst Concentration	2.47	1	0.30	1.76	3.19	1.00
C-Time	4.00	1	0.30	3.28	4.71	1.00
AB	-4.09	1	0.43	-5.10	-3.08	1.00
AC	2.54	1	0.43	1.53	3.56	1.00
BC	-3.09	1	0.43	-4.10	-2.08	1.00
A ²	-11.57	1	0.42	-12.56	-10.58	1.01
B ²	-6.57	1	0.42	-7.56	-5.59	1.01
C ²	-8.79	1	0.42	-9.78	-7.80	1.01

Table 4.6 shows the value of coefficients for different factors.

Table 4.6: Coefficient for different factors

Factor	Coefficient Estimate
Intercept	85.26
A	4.84
B	2.47
C	4.00
AB	-4.09
AC	2.54
BC	-3.09
A ²	-11.57
B ²	-6.57
C ²	-8.79

Final Equation in terms of coded factors and actual terms is shown below.

$$\begin{aligned}
 R1 = & 85.26 & R1 = & - 463.30844 \\
 & +4.84* A & & +40.20208 * \text{Methanol:Oil} \\
 & +2.47* B & & +34.07688 * \text{Catalyst Concentration} \\
 & +4.00* C & & +34.66938 * \text{Time} \\
 & - 4.09* A * B & & -0.68167 * \text{Methanol:Oil} * \text{Catalyst Concentration} \\
 & +2.54* A * C & & +0.42375 * \text{Methanol:Oil} * \text{Time} \\
 & -3.09* B * C & & -0.77250 * \text{Catalyst Concentration} * \text{Time} \\
 & -11.57* A^2 & & -1.28569 * (\text{Methanol:Oil})^2 \\
 & -6.57* B^2 & & -1.64344 * (\text{Catalyst Concentration})^2 \\
 & -8.79* C^2 & & -2.19781 * (\text{Time})^2
 \end{aligned}$$

Table 4.7 shows the values of % biodiesel yield calculated using the above model. The predicted values are then plotted against the actual values as shown in Figure 4.15.

Table 4.7: % Error in predicted value of %biodiesel yield obtained using the model

Run	Actual value	Predicted value	% Error [†]
1	72.59	71.4643	1.550
2	56.22	55.7119	0.903
3	69.83	70.3398	-0.730
4	85.26	85.2608	-0.001
5	85.26	85.2608	-0.001
6	85.26	85.2608	-0.001
7	85.26	85.2608	-0.001
8	74.05	73.5701	0.648
9	73.38	74.5073	-1.536
10	76.92	76.2763	0.836
11	60.47	60.3346	0.223
12	62.58	63.1986	-0.988
13	57.96	58.6054	-1.113
14	85.26	85.2608	-0.001
15	62.13	61.5131	0.992
16	73.14	73.2770	-0.187
17	68.36	68.84168	-0.704

$$^{\dagger}\% \text{ Error} = 100 * (\text{Actual value} - \text{Predicted value}) / \text{Actual value}$$

The predicted values are plotted against actual value in the Figure 4.15. Error % varies between -1.536 % to 1.550 %.

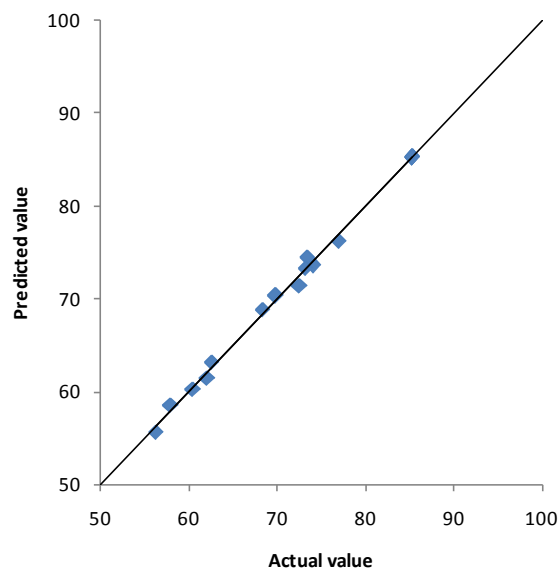


Figure 4.15: Predicted value vs. Actual value

Design-Expert® Software
Factor Coding: Actual

R1



X1 = A: Methanol:Oil
X2 = B: Catalyst Concentration
Actual Factor
C: Time = 8.50

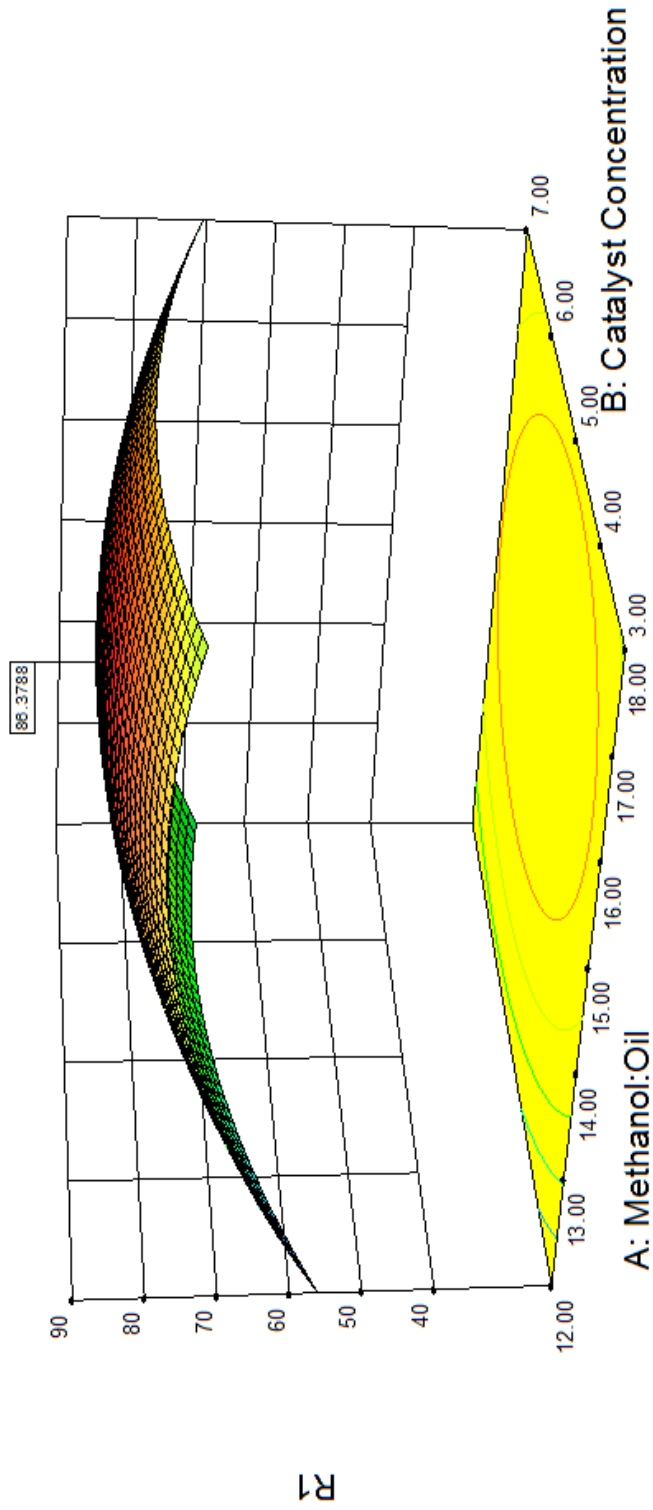


Figure 4.16: Response surface for variation in biodiesel yield (%) with respect to methanol: oil ratio and catalyst concentration (wt %) at time = 8.50 h

Design-Expert® Software
Factor Coding: Actual
Desirability
1.000
0.000

X1 = A: Methanol:Oil
X2 = B: Catalyst Concentration

Actual Factor
C: Time = 8.50

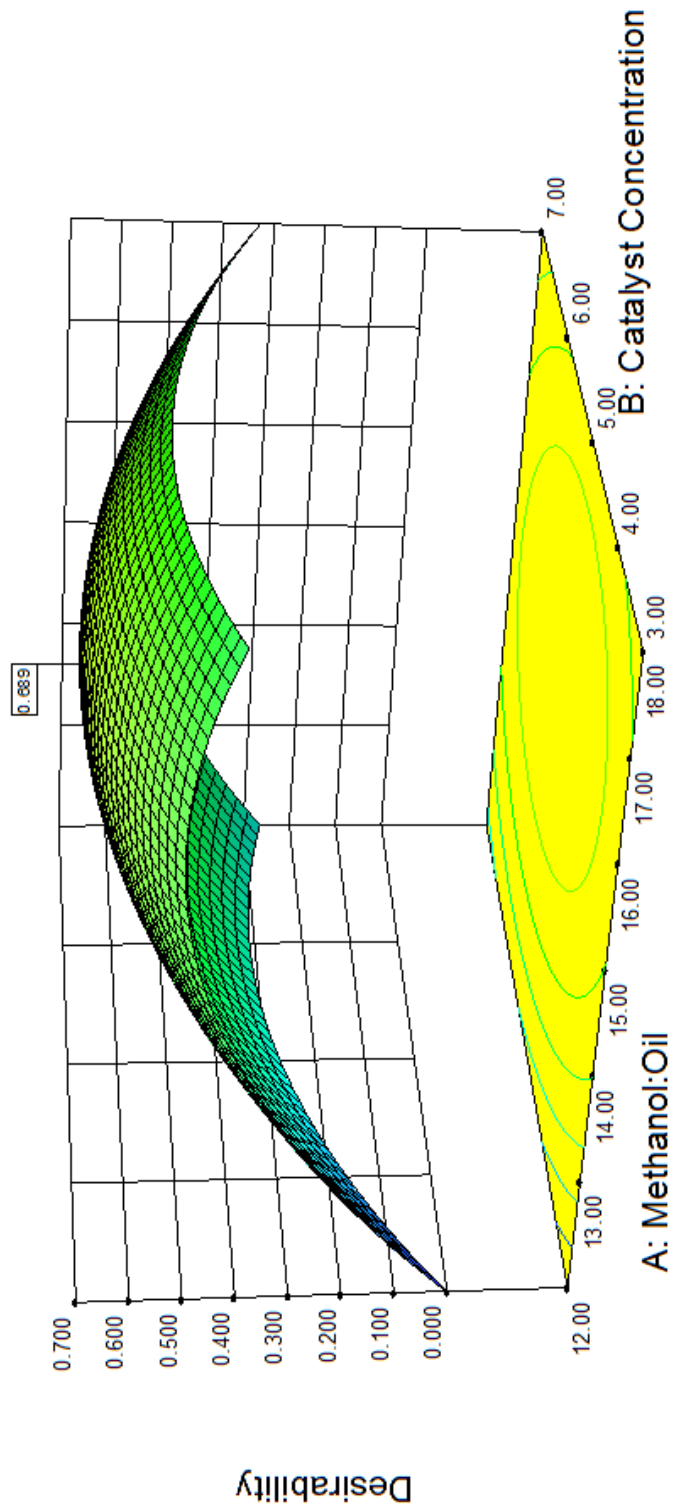


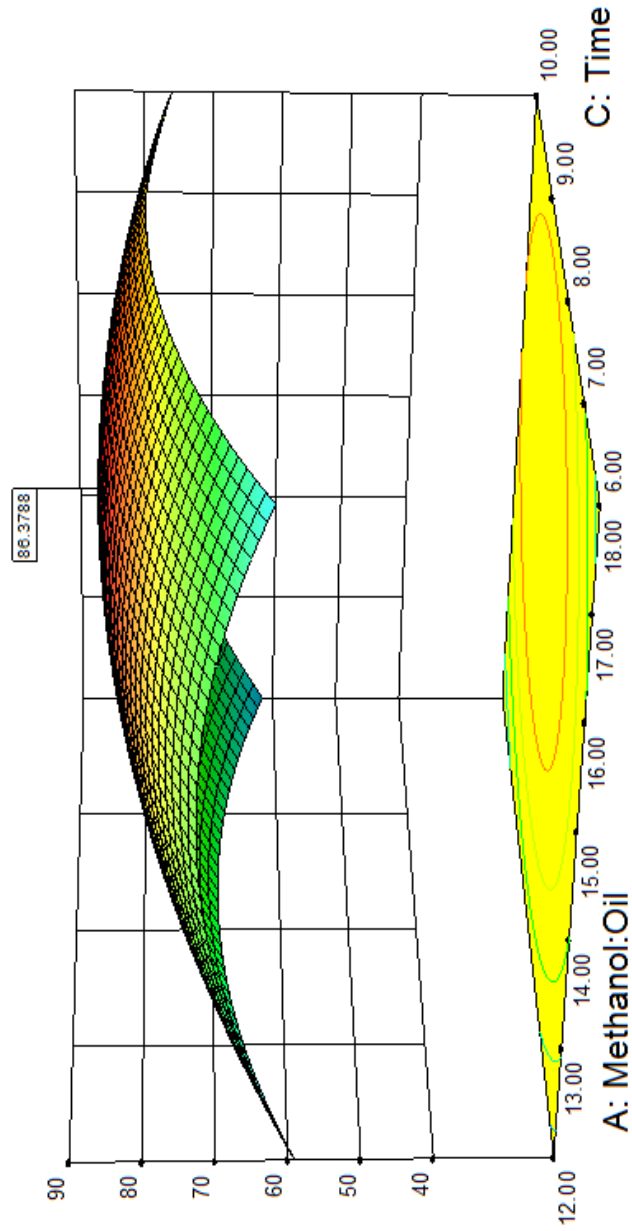
Figure 4.17: Response surface for desirability with respect to methanol:oil ratio and catalyst concentration (wt. %) keeping time of constant equal to 8.50 h

Design-Expert® Software
Factor Coding: Actual

R1
85.26
56.22

X1 = A: Methanol:Oil
X2 = C: Time

Actual Factor
B: Catalyst Concentration = 5.12



R1

Figure 4.18: Response surface for variation in biodiesel yield (%) with respect to methanol: oil ratio and time (h) at catalyst concentration (wt. %) = 5.12

Design-Expert® Software
Factor Coding: Actual
Desirability



X1 = A: Methanol:Oil
X2 = C: Time

Actual Factor
B: Catalyst Concentration = 5.12

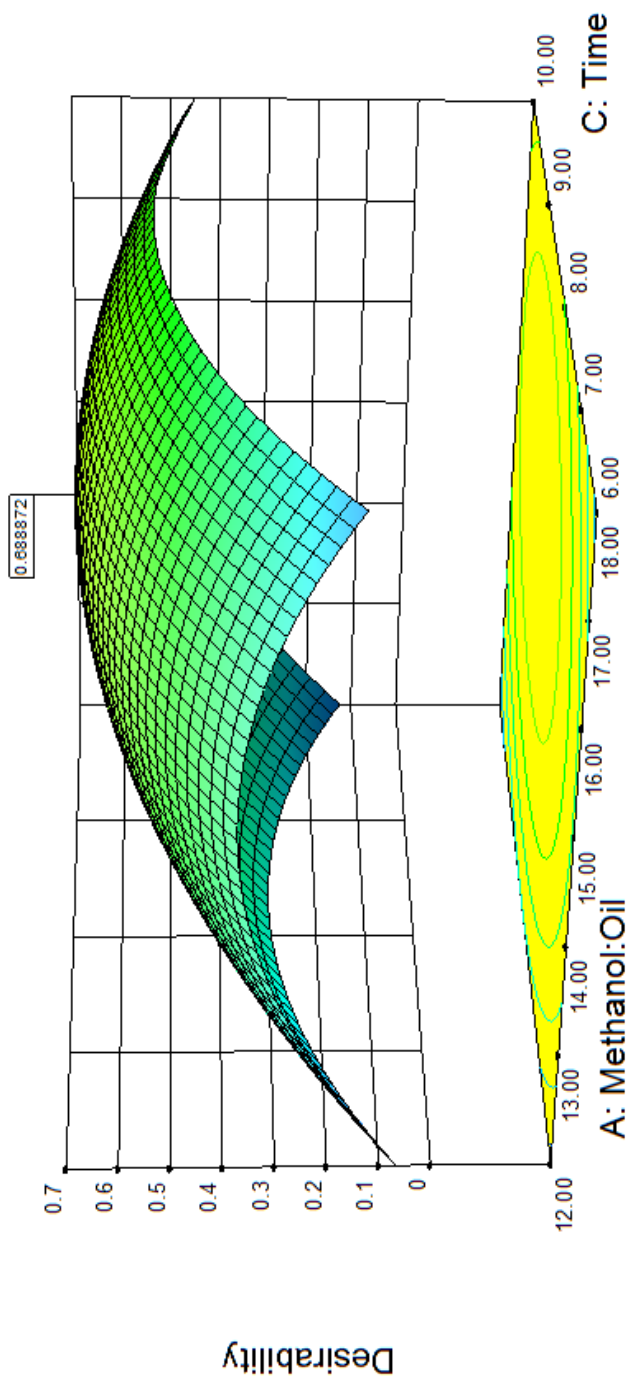


Figure 4.19: Response surface for desirability with respect to methanol:oil ratio and time (h) at constant catalyst concentration (wt. %) = 5.12

Design-Expert® Software
Factor Coding: Actual

R1
85.26
56.22

X1 = B: Catalyst Concentration
X2 = C: Time

Actual Factor
A: Methanol:Oil = 15.68

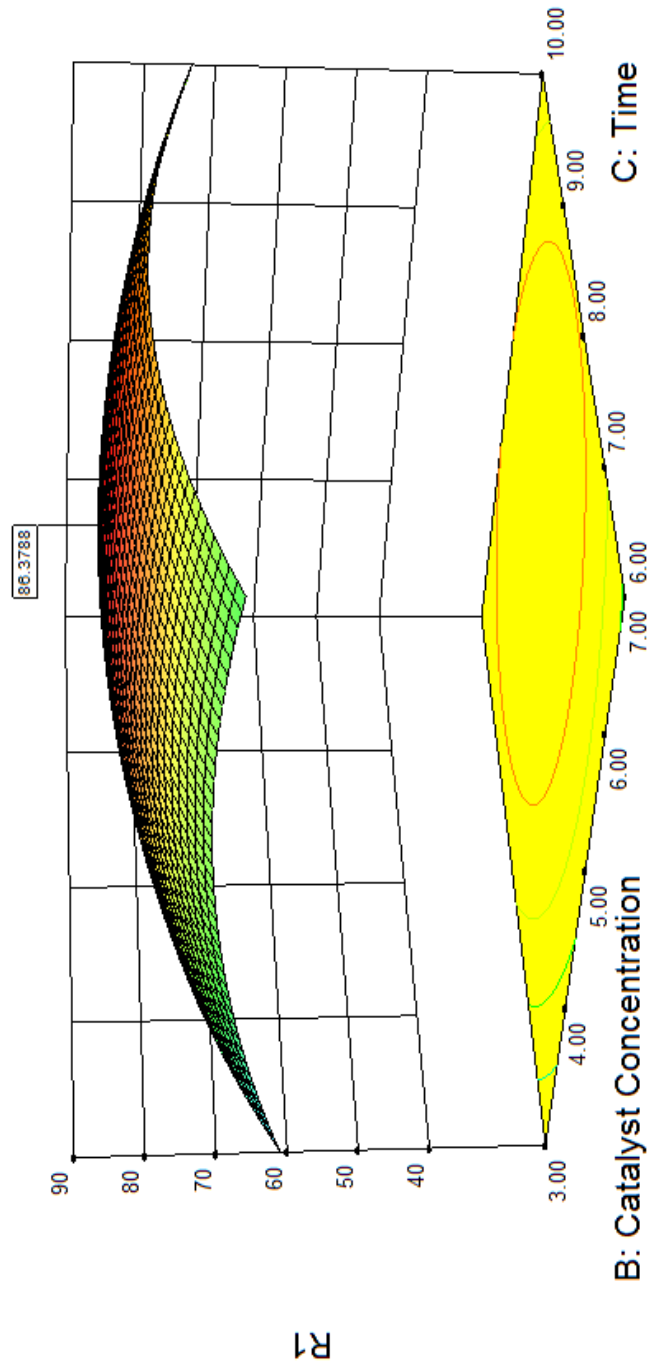


Figure 4.20: Response surface for variation in biodiesel yield (%) with respect to catalyst concentration (wt. %) and time (h) at methanol:oil ratio = 15.68

Design-Expert® Software
Factor Coding: Actual
Desirability



X1 = B: Catalyst Concentration
X2 = C: Time

Actual Factor
A: Methanol:Oil = 15.68

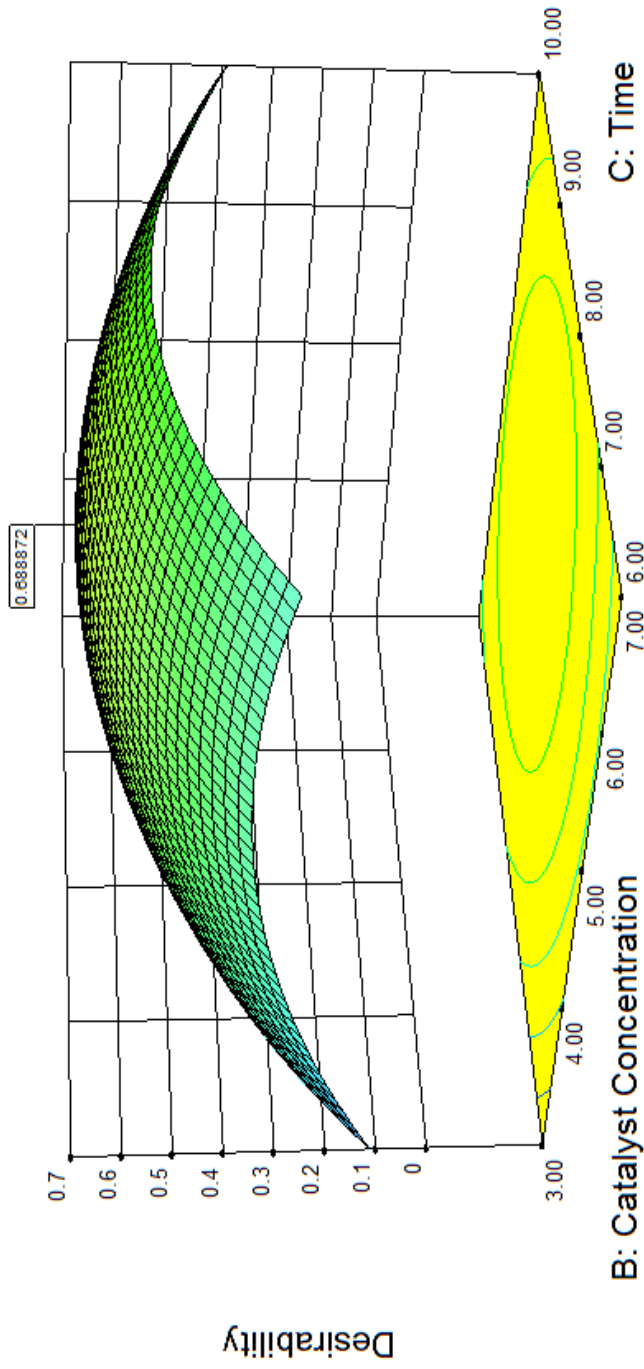


Figure 4.21: Response surface for desirability with respect to catalyst concentration (wt. %) and time (h) at constant methanol:oil ratio = 15.68

Final optimization point using design expert software is as follows “**Methanol:Oil = 15.68, Catalyst Concentration = 5.12 wt% and Time = 8.50 h**” which gives an overall biodiesel yield of “**86.3788 %**” having “**desirability of 0.689**”. Figure 4.22 shows the above optimization point.

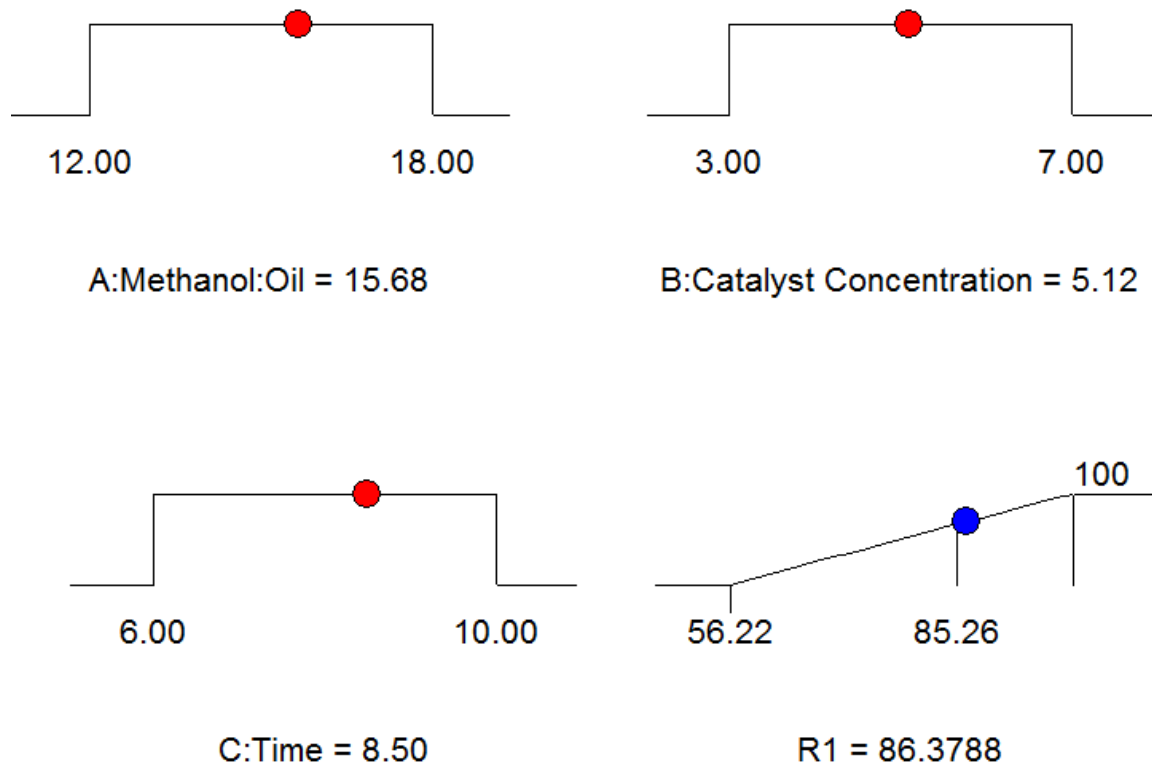


Figure 4.22: Ramps showing the range in which optimization was carried out. Dotted points show the optimization value for different factors

4.5. Characterization of Algal Oil and Biodiesel

4.5.1. Properties of algal oil and biodiesel

Table 4.8 shows various physical properties of algal oil and algal bio-diesel.

Table 4.8: Various properties and elemental composition of algal oil and algal biodiesel

Properties	Bio-Oil	Bio-Diesel	Standard Biodiesel IS-15607:2005
Viscosity (mm ² /sec)	26.5	5.3	2.5-6.0
Cloud Point (°C)	-7	-12	-
Pour Point (°C)	-8	-13	-
Flash Point (°C)	128	115	Min. 120
Density (g/ml)	0.894	0.871	860-900
Aniline Point (°C)	52	87	-
Cetane Number *	36	52	Min. 51

* Calculated on the basis of aniline point using correlation (CN = 0.72 D.I +10, where D.I = ((aniline point (°F)* °API Gravity (60 °F))/100)

Table 4.9 shows elemental composition of algal oil and algal bio-diesel.

Table 4.9: Elemental composition of algal oil and biodiesel

Element	Algal Oil	Biodiesel
C (wt. %)	76.94	68.21
H (wt. %)	17.20	19.57
N (wt. %)	1.28	0.32
S (wt. %)	0.08	0.04

The biodiesel produced from the algal oil meets the Indian standard (IS15607:2005) for B100 and hence the biodiesel produced from the oil of the native algae of solani river can be used as a blending stock for B20. Figure 4.23 shows the photograph of bio-diesel after removal of colouring pigments.

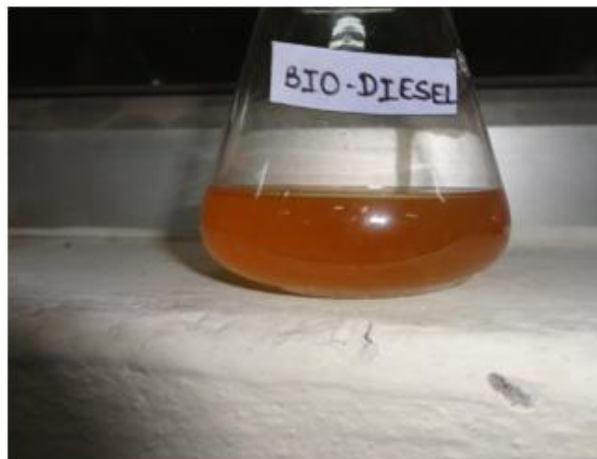


Figure 4.23: Photograph of algal oil and biodiesel

4.5.2. GC-MS analysis of biodiesel

GC-MS analysis was used to find out the constituents of bio-diesel.

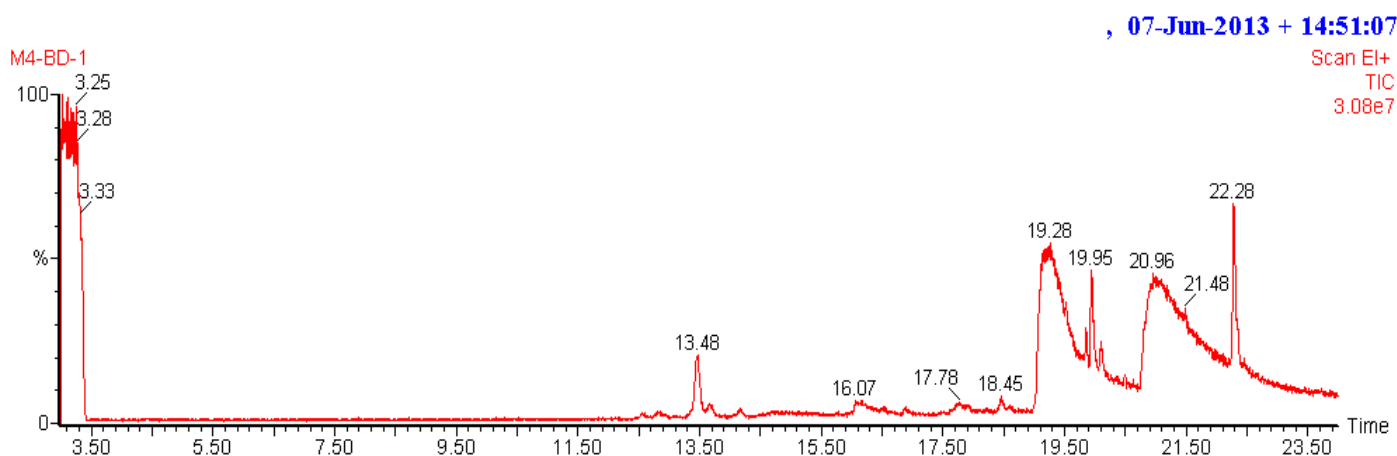


Figure 4.24: GC Chromatograph for biodiesel

GC-MS analysis revealed more than 15 compounds in biodiesel. The result is shown in Table 4.10.

Table 4.10: Compounds present in algal biodiesel

S. No.	Compound Name	Chemical Formula
1	METHYL 4,8-DIMETHYLNONANOATE	C ₁₂ H ₂₄ O ₂
2	DODECANOIC ACID, 10-METHYL-, METHYL ESTER	C ₁₄ H ₂₈ O ₂
3	TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER	C ₁₆ H ₃₂ O ₂

4	HEXADECANOIC ACID, 9-METHYL-, METHYL ESTER	C18H36O2
5	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	C18H36O2
6	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	C18H36O2
7	HEPTADECANOIC ACID, METHYL ESTER	C18H36O2
8	CYCLOPENTANETRIDECANOIC ACID, METHYL ESTER	C19H36O2
9	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	C19H38O2
10	OCTADECANOIC ACID, 11-METHYL-, METHYL ESTER	C20H40O2
11	NONADECANOIC ACID, METHYL ESTER	C20H40O2
12	EICOSANOIC ACID, METHYL ESTER	C21H42O2
13	DOCOSANOIC ACID, METHYL ESTER	C23H46O2
14	TETRACOSANOIC ACID, METHYL ESTER	C25H50O2
15	HENEICOSANOIC ACID, 18-PROPYL-, METHYL ESTER	C25H50O2
16	PENTACOSANOIC ACID, METHYL ESTER	C26H52O2
17	HEXACOSANOIC ACID, METHYL ESTER	C27H54O2
18	HEPTACOSANOIC ACID, 26-METHYL-, METHYL ESTER	C29H58O2
19	HEPTACOSANOIC ACID, 25-METHYL-, METHYL ESTER	C29H58O2

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

On the basis of above discussions the conclusion and future recommendations are listed below.

5.1. Conclusions

- Algae was successfully collected from the Solani Aqueduct, Roorkee and was found to be a mixture of different algal species having Hydrodictyon and Ulotrichalean strains of green algae as the prominent ones.
- Oil from the dried and crushed algae was successfully extracted by solvent extraction method using soxhlet apparatus with n-hexane as solvent giving recovery of 41.4 %.
- Heterogeneous catalysts consisting of various molar ratios of TiO_2 and CaO (0.25, 0.5, 1, 2) were prepared using conventional solid state reaction. Mixture with TiO_2 :CaO molar ratio of 0.25 calcinated at 700°C was found to be the most active.
- Catalyst was characterized by X-Ray diffraction, thermo gravimetric analysis method and scanning electron microscope.
- Optimization of process conditions i.e. methanol-oil ratio, catalyst concentration and reaction time was done using Box-Behnken method and response surface methodology and following conditions were obtained: methanol:oil ratio = 15.68, catalyst concentration = 5.12 wt.% and time = 8.5h giving biodiesel yield = 86.37 %.
- Proposed model was successfully able to predict the % biodiesel yield with an error of $\pm 1.5\%$.
- Various properties of algal oil and algal biodiesel were found out. Properties of biodiesel were in range as prescribed in IS 15607:2005.
- GC-MS analysis of Algal oil revealed the presence of various compounds containing carbon atoms, ranging from C14 to C29.

5.2. Recommendations

- More work is needed in the areas of algae growth, algae harvesting involving micro algae and focussing on finding suitable conditions should for obtaining high yields of algal biomass with high lipid content.
- A comparison study for bio-oil extraction involving bio-oil yield and production should be conducted for the native algae between supercritical extraction of bio-oil using CO₂, ultrasonic assisted extraction and solvent extraction in order to choose an efficient given high recovery of oil and at the same time is cost-effective.
- A study on reusability of catalyst should be conducted in order to establish the catalyst as effective and economically viable.
- A study on deactivation of catalyst due to exposure to environment can be conducted.
- A study can be carried out on management of algal biomass after oil extraction i.e. could be used for bio adsorption or fuel production via pyrolysis or gasification or for producing manure.

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