EFFECT OF PHOTOSYNTHETIC BACTERIA BASED FEEDSTOCK AND TEMPERATURE ON BIODIESEL YIELD USING MICROWAVE ASSISTED TRANSESTERIFICATION WITH AN APPLICATION OF BOX BEHNKEN SUPPORTED RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Algae based third generation biodiesel production is a recent advancement in renewable energy due to its minimal land requirements, cultivation in wastelands etc than food stock based second generation biodiesel production. This paper addresses on the study of the optimum yield of biodiesel produced from oxygenic photosynthetic bacteria-based Spirulina Platensis algae by microwave assisted transesterification. Effect of microwave irradiation time which affects the temperature on the extraction of algal oil and simultaneous in-situ transesterification was investigated for biodiesel production. The response surface methodology using Box Behnken Design was used to analyze constituent parameters like catalyst concentration, alcohol concentration and process parameters like microwave time. Results indicate that microwave assisted transesterification yields at 70.7% of biodiesel production with respect to algae dry weight at an optimum of catalyst concentration of 1.6%, with alcohol concentration of 1:9.7 and microwave irradiation time of 3.2 minutes.

Keywords: Transesterification, biodiesel, algae, photosynthetic bacteria.

1. INTRODUCTION

Global carbon emissions are growing at a rate of 1.4% per year and CO_2 levels in atmosphere is expected to reach 550 ppm by 2050, if current emissions remain unabated [1] and there is a push among all countries to reach net zero emissions by 2050. Demand of fossil fuels provide for 86% of the world's energy requirement and account for more than 75% of anthropogenic carbon emissions [2]. Biofuels extracted from feedstock like oil palm, sunflower, algae etc have lower emissions and can be blended with conventional fuels without making any significant modifications in the engine. First and second-generation biofuels are produced from food and non-food crops respectively. Third generation biofuels are produced from microbes, which are a delicacy in Asian countries [3] and won't put undue pressure on agricultural land, so the ethical question of Malthusian sustainability won't arise [4,5]. According to IEA report if net zero emissions have to be achieved by 2050, then biofuels production should grow at 14% during 2020 - 2030 and share of second and third generation biofuels has to be increased to 45% from the current 7% [6]. Table 1 provides a brief comparison of biodiesel with respect to oil yield and biodiesel productivity [7,8].

Table 1: *Comparison of biodiesel feedstock*

Crop / Feed stock	Biofuel Generation	Oil Yield (L/ha.year)	Biodiesel productivity (kg/ha.year)	Energy Efficiency Ratio (EER)
Oilpalm	First	5950	4747	2.42-3.58
Rapeseed	First	1190	862	1.44-5
Sunflower	First	952	946	3.5
Jatropha	Second	1892	656	1.85-3.4
Algae				
Microalgae[a]	Third	1,36,900	1,16,365	0.35-4.34
Microalgae[b]	Third	58,700	49,895	0.35-4.34

a – 70% oil (by weight) in dry biomass.
b – 30% oil (by weight) in dry biomass.

Recently, production of third generation biodiesel from algae is popular as inferred from Table 1, algae have high oil content with upto 80% of dry biomass weight, high oil yield and productivity, high growth rate where biomass doubles in 24 hours during exponential growth phase. Algae can also be grown in unproductive wastelands with nutrients even from industrial waste water [9–11]. Cyclotella, Dunaleilla, Nannochloropsis, Spirulina are some of the algae used for biodiesel production due to their lipid content, resilience to environment and low cost of cultivation [12,13]. Carlos J et al found that growing Spirulina in open atmosphere is preferable for large scale algae production [14]. Radmann et al performed repeated batch cultivation of Spirulina Platensis and found that under optimum conditions, cost of production can be significantly lowered [15]. Goksan et al have said that Spirulina Platensis is easy to grow having a simple harvest and drying process [16].

In common practice, biodiesel is produced through cultivation, harvesting, lipid extraction and finally trans esterification of feedstock containing high triglyceride content like vegetable oil which are reacted with a lower alcohol like methanol or ethanol in the presence of a catalyst [17,18]. The reaction produces Fatty Acid Methyl Esters (FAME) which are the major constituents of biodiesel [19,20]. Freedman B et al have said that the yield of trans esterification reaction depends on free fatty acids in oil, molar ratio between alcohol to oil, reaction time and temperature etc [21]. Vidya et al have found that moisture content, catalyst content and concentration, usage of organic co-solvents affect the quality of biodiesel produced [22]. Syahirah Yahya et al optimized biodiesel production from waste cooking oil using Response Surface Methodology (RSM) controlling the reaction time, catalyst concentration and alcohol concentration [23]. Srikanth et al used Box Behnken Design (BBD) to optimize the production of biodiesel from washed milk scum by controlling reaction time, temperature, catalyst concentration and alcohol to oil molar ratio [24]. Rahul Chamola et al optimized biodiesel yield upto 90% using RSM by considering factors like alcohol concentration, time, temperature and reaction time [25].

Further, conventional trans esterification processes require a longer time for the reaction to complete and is energy intensive. Boldor et al have proved that using microwave assisted transesterification completes both lipid extraction and transesterification as a single process within a short time, saving energy and chemical reagents [26]. Nayak et al have created a microwave assisted transesterification setup and used papaya oil to produce biodiesel. Biodiesel yield was optimized using RSM by varying alcohol, catalyst concentration, time and temperature [27]. Onumaegbo et al used microwave assisted transesterification to produce biodiesel from Scendesmus Quadricauda microalgae. They varied microwave power, time and extraction time as input factors in a Box Behnken Design (BBD) [28]. They found that a higher microwave power yielded better biodiesel yield [29]. Hasnain et al used a Central Composite Design (CCD) to optimize production of biodiesel using microwave assisted transesterification and found that Ulothrix sp gave a biodiesel yield of 88% [30,31].

Consumption of energy for the conversion of algal oil to biodiesel is measured through Energy Efficiency Ratio (EER) and is defined as Equation 1 and a higher value of EER is desirable.

$$EER = \frac{Energy\ produced\ from\ fuel}{Energy\ required\ to\ produce\ fuel} \quad .....(1)$$

Biomass drying and oil production are the most energy intensive processes in biodiesel production and so they require improvements [8].

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This paper aims to optimize the energy requirements for producing third generation biodiesel from Spirulina Platensis using a novel microwave assisted transesterification process at a laboratory model scale. A RSM using BBD is introduced to optimize the production of biodiesel by varying microwave time, alcohol concentration and catalyst concentration at three levels. The second section of the paper deals with experiments used for microwave assisted transesterification. The third section deals with experiments performed using BBD and analysis of results. The final section contains the results of the paper and its future prospects.

II. EXPERIMENTAL

This section deals with various methods and materials used for this work which highlights 1. Transesterification of algal oil, 2. Microwave Assisted Transesterification, 3. Biodiesel production, 4. Factors affecting microwave assisted transesterification and 5. Response Surface Methodology.

1. Transesterification of algal oil:

Trans esterification reaction which converts into mixture of fatty acids is shown in Figure 1 [11,32]. Algal oil is extracted from Spirulina Platensis which contains triglycerides. The algal oil is mixed homogeneously with a catalyst and alcohol which produces biodiesel with a mixture of fatty acid methyl ester and glycerol. The reaction is given by,

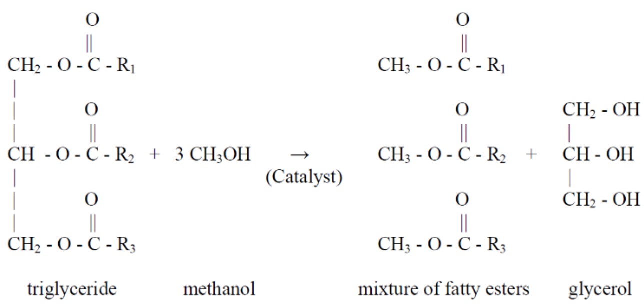


Figure 1. *Trans esterification of algal oil.*

Most of the third generation biodiesel is produced through a trans esterification reaction where a less corrosive basic catalyst like NaOH, KOH is preferred.

2. Microwave Assisted Transesterification:

Conventional mechanical and chemical oil extraction methods and direct trans esterification are energy intensive, require long extraction time and huge quantities of reagents and are costly [26,33]. Recently, microwave assisted trans esterification maybe used instead of direct trans esterification process which is working based on dipolar polarization mechanism as shown in Figure 2 [34].

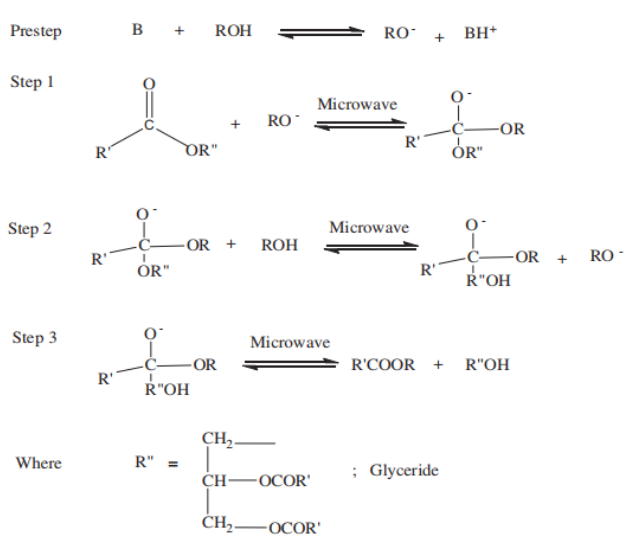


Figure 2. *Microwave assisted transesterification process.*

It can be used for algal oil extraction as the microwaves have the ability to penetrate the rigid algal cell wall structure, which cannot be easily broken down by conventional mechanical and chemical methods. When the algal cell is irradiated with microwaves, it leads to rapid heating, which increases the temperature and pressure, resulting in accelerated cell wall degradation and enhanced mass transfer rates [26,35]. The microwaves also enhance the evaporation of methanol, which is a strong microwave absorption material. When the microwaves interact with the triglycerides and methanol, it leads to a large reduction of activation energy due to increased dipolar polarization phenomenon [36].

3. Biodiesel Production:

The process involved in microwave assisted transesterification reaction to produce biodiesel is represented in Figure 3.

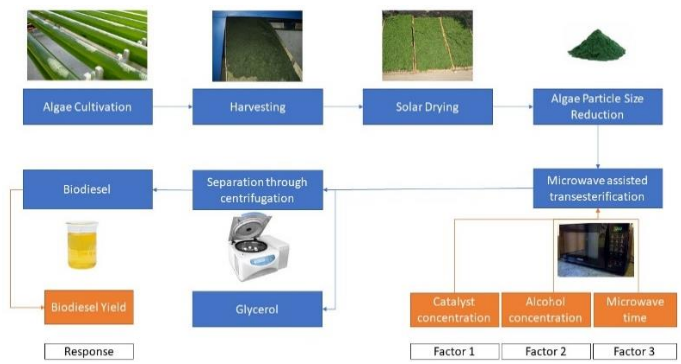


Figure 3. *Microwave assisted transesterification of biodiesel.*

Generally, Spirulina Platensis algae is cultivated in wastelands near open ponds having abundant recycled effluent water. Approximately, 600 litres of water are required for producing 1 kg of biodiesel when water is recycled [37]. The algae can be harvested after a period of 4 weeks from plantation. The harvested algae are dried under direct sunlight or in a solar dryer for 2 days at 40°C for removing moisture content. Algae typically lose 10% of their weight during drying. Particle size of the dried algae is reduced through grinding which enables to increase contact area during subsequent trans esterification reaction. The powdered algae are then mixed homogeneously with an alcohol and catalyst at the predetermined level of concentration. The mixture is then kept under a microwave oven to expedite the trans esterification reaction and produces a mixture of biodiesel and glycerol. The biodiesel may be separated from the mixture using a centrifuge.

4. Factors affecting microwave assisted transesterification:

The performance of the biodiesel production is measured by an output response called biodiesel yield. This is affected by two types of parameters namely constituent parameters and process parameters. Constituent parameters are related to the input mixture of trans esterification process and process parameters specifies the environmental factors. Constituent parameters like reagent type, catalyst concentration, alcohol concentration, molar ratio etc and process parameters like reaction temperature, microwave time etc. are considered as input factors which controls the output response. In this work, due to their vitality, catalyst concentration, alcohol concentration and microwave time are the factors considered. The effects of such factors on biodiesel yield are analyzed below [22].

Catalyst concentration

Catalyst concentration is the ratio of mass of catalyst added to the mass of dry algal powder which is expressed in percentage. Higher or lower catalyst quantities will lead to soap formation, which reduces biodiesel yield [21].

Alcohol concentration

Alcohol concentration is defined as the ratio of volume of alcohol with respect to the volume of dry algal powder added. Alcohol has to be added in excess of the stoichiometric ratio to drive the reaction towards the right. For lesser alcohol concentration, the reaction will be incomplete.

Microwave time

Microwave time is the amount of time during which the homogeneous mixture of dry algal powder, alcohol and catalyst are irradiated with microwaves to facilitate trans esterification reaction. Increase in microwave time increases biodiesel yield up to a certain point beyond which biodiesel yield will be affected as the alcohol gets decomposed. The triglycerides in oil decomposes to diglycerides which in turn turns into monoglycerides. The monoglycerides finally decompose to FAME [17]. At the initial stages of the reaction, the proportion of monoglycerides increases and then decreases at the end of the reaction.

Biodiesel Yield:

Biodiesel yield is the ratio of volume of biodiesel produced to the volume of homogeneous mixture supplied for trans esterification reaction. The biodiesel yield is measured using Equation 2.

$$Biodiesel\ Yield = \frac{Volume\ of\ product}{Volume\ of\ feed}\ x\ 100\% \qquad \text{.....(2)}$$

5. Response Surface Methodology (RSM):

Response Surface Methodology (RSM) is a mathematical and statistical technique used to identify the factors that have an influence on the response through an experimental design, so that the output response is optimized. The control variables that are independent and studied by the experimenter are called as factors. The dependent variable which has to be optimized is called as the output response. A second order polynomial mathematical model is created so that the response is plotted as a surface, which helps in identifying the minimum, maximum values and slope lines of the response. An appropriate approximation relationship between input factors and output response under optimal operating conditions for the system is investigated through experimental designs. Box Behnken Designs (BBD) and Central Composite Design (CCD) are two main experimental designs used in RSM [38]. A second order quadratic polynomial model will be generated to predict the biodiesel yield.

RSM is done as a seven-step process [39] which includes, 1. The response is selected as biodiesel yield for optimization. 2. Catalyst concentration, alcohol concentration and microwave time is studied at normalized and coded levels of -1, 0, 1 as they are suspected to have an effect on biodiesel yield. 3. A proper experimental design is chosen such that the number of experiments that have to be performed are less and at the same time the results generated should be repeatable and statistically valid. BBD and CCD are popular experimental designs [40]. The experiments are then performed according to the chosen experimental design and the response is noted. 4. Regression analysis in which the response is fitted to a second order polynomial, so that the response can be plotted as a surface graphically with respect to the factors studied. The generated model is validated through a student t test by checking the coefficient of determination (R^2) in which a value greater than 95% is desired [41].

The polynomial model is given in equation 3. 5. Development of a mathematical model for response is done so that the response can be predicted for varying factor levels for which experiments were not done. Analysis of Variance (ANOVA) is performed to quantify the effect of factors on response. A part of the variation in response cannot be attributed to any factor and is noted as error, which is due to environment or factors beyond the control of the experimenter. 6. Development of a 3D surface plot and 2D contour plot for response is done using the mathematical model developed. This shows how the response varies across factor levels graphically. 7. Analysis of optimum conditions is done using the mathematical model and the graphs so that the effect of factors on the response is studied.

$$\mu = \beta_0 + \sum_{i=1}^{3}\beta_i x_i + \sum_{i=1}^{3}\beta_i x_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}x_i x_j + error \qquad \text{.....(3)}$$

The advantage of RSM is that it saves time and resources by reducing the number of experiments that have to be performed when compared with a Full Factorial Design. The results of RSM are repeatable and it also gives a way for predicting response.

Experiment:

Spirulina Platensis samples were procured from Antenna Farms, Madurai, India. The samples were dried in a solar dryer at 40°C for two days, which was then powdered using a grinding machine. Two grams of this dry algal powder was taken in a 100 ml borosilicate glass vessel which is transparent to microwaves. A mixture of homogeneous potassium hydroxide which acts as the catalyst and methanol solution which acts as the alcohol is added to the powdered algae in the glass vessel according to the proportions defined by the experimental design. The glass vessel was then irradiated with microwaves by a Samsung domestic microwave oven whose power output is 800 W for predetermined time period so that the transesterification reaction can take place. Once the reaction is over, hexane was added and this solution was centrifuged at 3200 rpm for five minutes so that the upper organic layer which contains non polar lipids is removed. The remaining heavier components contain the Fatty Acid Methyl Esters (FAME). The experiment is then repeated according to the Box Behnken Design and the results were analyzed using Design Expert 13 in a computer having 8th gen i5 processor and 8 GB of RAM. The yield of biodiesel is computed using Equation 2. The results of the experiment are fed into the experimental design which is then analyzed using Design Expert.

III. RESULTS AND DISCUSSION

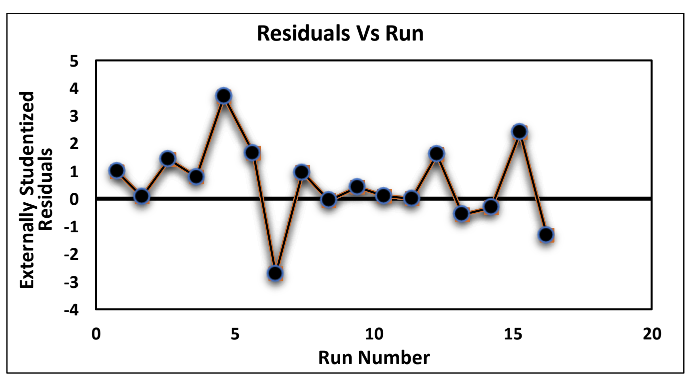
The effects of alcohol concentration, catalyst concentration and microwave time on biodiesel yield. 17 experiments were performed according to BBD as given by Design Expert. This study is expected to find the optimal conditions for biodiesel production at a large industrial scale. The factors along with their levels chosen for study are given in Table 2.

Table 2. *Input factors and their levels.*

Factor	Units	Low Level (-1)	High Level (+1)
x1 – Catalyst Concentration	%	0.5	2.5
x2 – Alcohol Concentration	-	9	15
x3 – Microwave Time	mins	3	9

A BBD model has a standard order of experiments. But there may be an element of bias introduced due to the order of experiments. So, a new run order is created by Design Expert by randomizing the standard order. The experiments were conducted based on the run order and the yield of the biodiesel got is given in Table 3. Based on the experimental results, the BBD was analyzed and a regression model was obtained for biodiesel yield. The quadratic polynomial model is given as equation 4. The co-efficient of determination, R^2 value for the model is 98%, which is greater than 90% and adequate precision value is greater than 4 [42]. Using the quadratic model for biodiesel yield, the predicted response is computed using the coded values for the input variables, which is shown in Table 3. The predicted biodiesel yield values fall in close line with the experimental results. The residual biodiesel yield and studentized residuals are within acceptable limits. The normalized residuals are also distributed randomly having a long tail. So the generated models can be considered as depicting the experimental results fairly. The residuals and expected biodiesel yields are shown in Figure 4.

$$\text{Biodiesel Yield} = 65.96 + 5.2625*x_1 - 3.05*x_2 - 6.2875*x_3 + 0.1*x_1x_2 + 3.675*x_1x_3 + 2.5*x_2x_3 - 6.9425*x_1^2 - 3.6675*x_2^2 - 3.8925*x_3^2 \qquad \text{.....(4)}$$



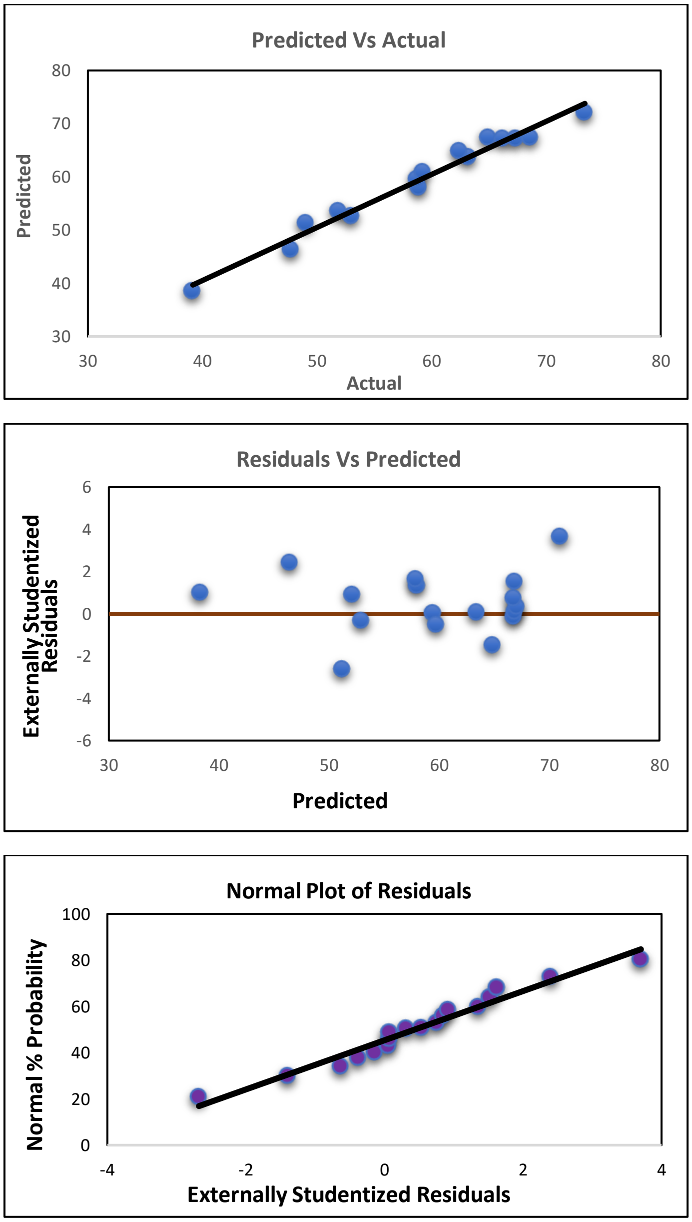


Figure 4. *Residual graph and predicted values of biodiesel yield.*

Table 3. *Box Behnken Design Result.*

Order	Run	x_1	x_2	x_3	Biodiesel Yield (%)	
					Experiment	Predicted
7	1	-1	0	1	40.3	39.9
10	2	0	1	-1	58.7	59.2
4	3	1	1	0	58.5	57.6
14	4	0	0	0	66.4	66
9	5	0	-1	-1	72.1	70.2
13	6	0	0	0	67.7	66
12	7	0	1	1	49.7	51.6
11	8	0	-1	1	53.1	52.7
15	9	0	0	0	64.9	66
16	10	0	0	0	65.7	66
6	11	1	0	-1	62.6	63
17	12	0	0	0	65.1	66
8	13	1	0	1	58.8	57.8
5	14	-1	0	-1	58.8	59.8
1	15	-1	-1	0	52.4	53.2
3	16	-1	1	0	48.4	46.9
2	17	1	-1	0	62.1	63.5

Model Evaluation:

The ANOVA results for biodiesel yield is given in Table 4. The p value for lack of fit analysis is 0.1072 which is greater than 0.05 (confidence level is 95%) and so the variations in biodiesel yield are satisfactorily explained by the factors considered. Microwave time and catalyst concentration have the most effects on biodiesel yield. The response surfaces are plotted by taking factors and interactions which are significant. Optimal conditions of alcohol, catalyst concentration and microwave irradiation time to increase biodiesel yield are found as 1:9.7, 1.6% and 3.2 minutes respectively. The optimal biodiesel yield was determined as 70.4%. The response surfaces and perturbation charts are shown in Figure 5. The effects of individual factors and their interactions are discussed below.

Table 4: Analysis of Variance

Source	SS	df	MS	F value	p value	Contribution (%)
x_1	221.55	1	221.55	75.38	< 0.0001	21.4
x_2	74.42	1	74.42	25.32	0.0015	7.2
x_3	316.26	1	316.26	107.60	< 0.0001	30.6
$x_1 * x_1$	202.94	1	202.94	69.05	< 0.0001	19.6
$x_2 * x_2$	56.63	1	56.63	19.27	0.0032	5.5
$x_3 * x_3$	63.80	1	63.80	21.71	0.0023	6.1
$x_1 * x_2$	0.0400	1	0.0400	0.0136	0.9104	0
$x_2 * x_3$	25.00	1	25.00	8.51	0.0225	2.4
$x_3 * x_1$	54.02	1	54.02	18.38	0.0036	5.2
Lack of fit	15.42	3	5.14	3.99	0.1072	1.5
Pure Error	5.15	4	1.29			0.5
Total	1068.56	16				100

Effect of process parameters on biodiesel yield:

Effect of microwave time:

Variation in microwave irradiation time contributes to more than 30% variation in biodiesel yield. Maximum biodiesel yield is obtained for an irradiation time of 3.2 minutes. Microwaves extract oil from algae through diffusive and disruptive extraction. It also heats up the alcohol and oil, expediting the trans esterification process. If irradiation time is low, then both the oil extraction process and trans esterification will be incomplete. If the microwave time is high, then the reaction mixture will be over heated which will lead to loss of solvent, production of by products and lesser biodiesel production [43].

Effect of catalyst concentration:

Catalyst concentration contributes the second highest to biodiesel yield. The optimum catalyst concentration is inferred as 1.6% at which biodiesel yield is maximum. Lesser concentration of catalyst has a lesser yield as the transesterification reaction is slow, because the catalyst interacts with a variety of compounds produced during the reaction. As catalyst concentration is increased, the biodiesel yield increases. But, high concentrations affect biodiesel yield adversely due to the formation of soap as a byproduct [44]. Potassium hydroxide forms a good homogeneous solution along with methanol and is susceptible to microwave radiation which increases biodiesel yield [11,45].

Effect of alcohol concentration:

Increase in ethanol concentration has a positive effect on biodiesel yield up to 1:9.7 and has a negative impact beyond that. This may be due to the fact that higher methanol concentration will increase the contact area with the oil/lipids and lead to better biodiesel yield. Methanol's dipole quickly reorients itself and destroys the two-tier structure that exists between oil and methanol when it is exposed to microwaves. This enables methanol to act as a solvent for the transesterification reaction. A lesser concentration of biodiesel will retard the reaction as there won't be enough methanol to act as a solvent and as a reactant. If the methanol concentration becomes too high, the ethanol will dilute the catalyst which in turn will reduce the biodiesel yield. It is also prudent to reduce the concentration of methanol as a higher alcohol concentration will increase the downstream separation cost of methanol and biodiesel [46].

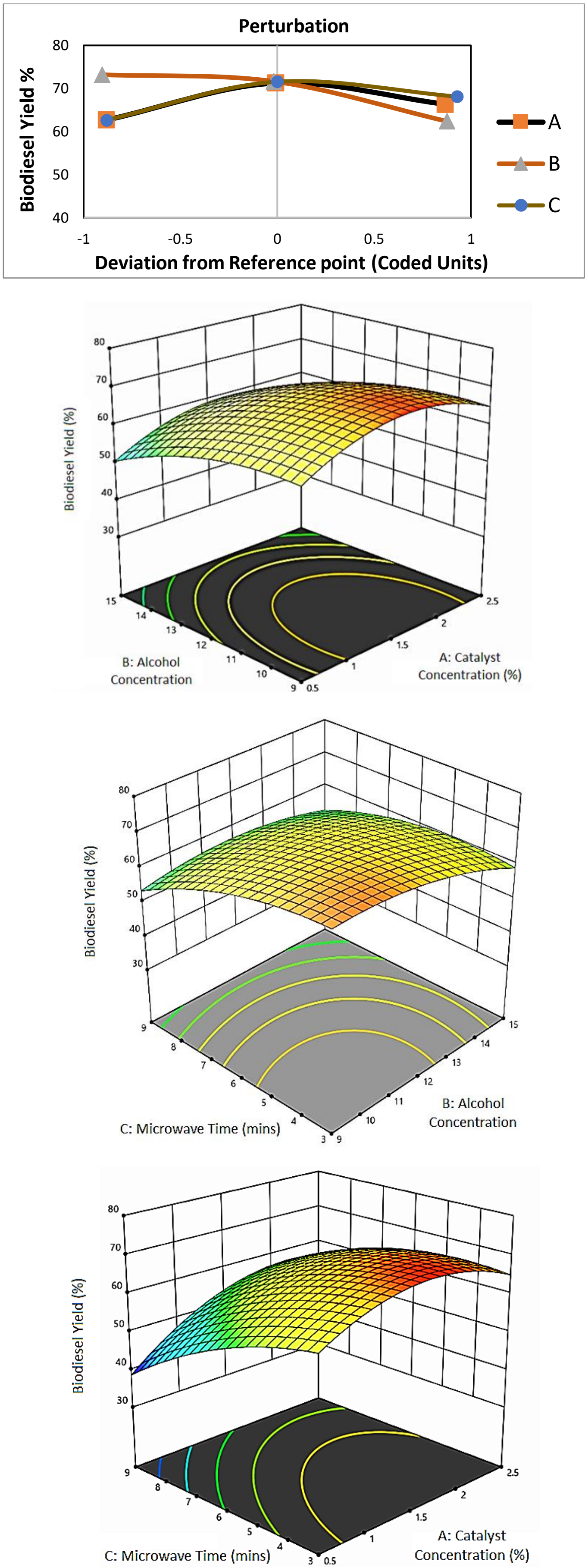


Figure 5. *Response surface and perturbation plot.*

Validation of RSM Model

The transesterification reaction was repeated at the optimum conditions of catalyst concentration, alcohol concentration and microwave time. A biodiesel yield of 70.7% was got. This experiment validates the RSM model created.

Biodiesel Product Analysis

The functional groups present in the biodiesel are given in Table 5 and Figure 6. The spectra show the presence of carbonyl groups (C=O), with a peak of 1820 cm^{-1}. The stretching vibrations due to ester groups (C-C) are confirmed with the presence of distinctive peaks at 1180, 1240 cm^{-1}. The spectra show the stretching vibration of methine group (C-H) at 2930 and 2860 cm^{-1}. The phenol group (O-H) has a peak at 3470 cm^{-1} [47] which shows the presence of O-H groups due to the presence of Free Fatty Acids and residual glycerol present in biodiesel [48].

Table 5: FTIR Analysis results

Stretching Vibration	Functional Group	Reference wave number (cm^{-1})	Experimental wave number (cm^{-1})
C-C	Ester Group	1210-1160	1180, 1240
C=O	Carbonyl Group	1750-1735	1820
O-H	Phenol group	3550-3200	3470
C-H	Methine group	2950-2850	2930, 2860
C=C	Alkene group	1700-1500	1690

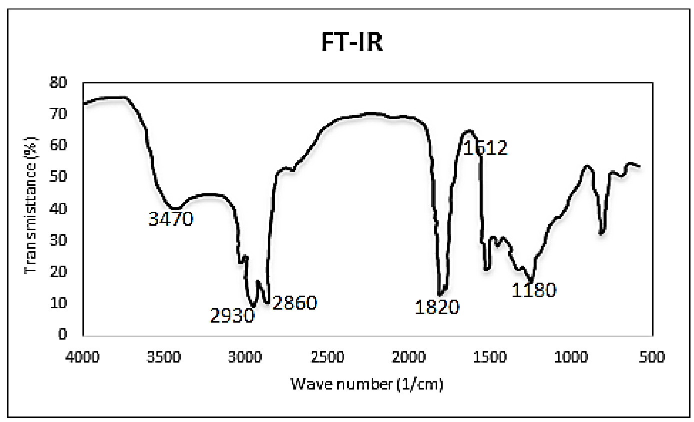


Figure 6. *FT-IR spectra of biodiesel.*

Comparison with conventional transesterification

The entire microwave assisted transesterification process along with oil extraction from algae can be completed within a short time period between 3 and 4 minutes. Conventional processes take a complete day for the process to be completed. Newer processes like super critical methanol extraction can complete the entire reaction within 30 minutes, but requires a high pressure and temperature to be applied which increases energy requirements. Microwave assisted transesterification reduces the time required for biodiesel extraction. It is also possible to optimize the yield of biodiesel by controlling the constituent and process parameters.

CONCLUSION

Microwave assisted transesterification reaction was studied using response surface methodology at a model laboratory scale. This novel process has the potential to reduce energy consumption for biodiesel production. Future studies have to be done for scaling up the biodiesel production to an industrial scale.

ACKNOWLEDGEMENTS

The authors are thankful to the management of Mepco Schlenk Engineering College, Sivakasi for providing resources to perform research.

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