Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Enzymatic Transesterification of Biodiesel Production from *Scenedesmus* sp and its Parameter Optimizing through Response Surface Methodology

Sasireka G* and Muthu Velayudham. R

Department of Chemical Engineering, Annamalai University, Annamalai Nagar.
Chidhambaram,Tamil Nadu-608 002, India. ***Corresponding Author:** E-mail: mailforreka@gmail.com

Abstract

This research work focused on increasing algal biodiesel efficiency using enzymatic transesterification method and its properties were optimized using Response surface methodology (RSM), design expert software version 8.0.7.1. The box benkenn design was used for optimizing six production parameters such as (A) Oil: Solvent molar ratio, (B) Amount of Catalyst, (C) Reaction time, (D) Reaction temperature, (E) Water and (F) Speed. Solvents of Chloroform: methanol (1: 2) mixture was used to extract the algal oil from *Scenedesmus arcuatus* (Lemmerm.) Lemmerm. Supernatant of lipase from *Aspergillus Niger* was used as a catalyst and the enzyme immobilized in sodium alginate beads used for low cost and efficient biodiesel production.

Keywords: Response surface methodology, *Scenedesmus microalgae* biodiesel, Immobilized lipase conversion reaction.

Introduction

Now a day this world facing many environmental effects likes deluge, scorching, vehicle emission, industrial pollution due to depletion of fossil fuels leads to polluting the environment by toxic gases, incomplete combustion of diesel and petrol (Klass, L. D.,1998). , Increasing population leads to create several mankind's activities, hence the replacing chemical fuels into alternative resources from the renewable biomass is the great substitute to avoid pollution caused by chemical fuels (Goldemberg, J., 2000). Lipids, triglycerides present in the seeds (soybean, palm, canola and caster), and the kernels (sunflower, rice bran) are the ability to convert biodiesel with the presence of catalytic conversion to perform in the production of esters of fatty acid methyl ester which is non toxic, renewable and alternative for diesel and petroleum fuels (Bansal, B. K. and Sharma, M. P. 2005). Under the optimized environmental factors such as pH, temperature, light, nutrient levels, salinity stress conditions which ability to consist of some of fatty acid composition like C14 tetradecanoic acid, C16 hexadecanoic acid and palmittic acid,methyl ester, C18 octadecenoic acid/ oleic acid),C20 eicosene/ arachidic acid is suitable for biodiesel properties(Thomas *et al*., 1984, Poisson L *et al*.,2002, Moheimani 2005). Cheapest sources of microalgae cultivated with suitable condition is potential for biodiesel production, but the technological design for cultivation system capturing light and other nutrient condition is required for economically viable process of small and large scale process (Teresa M. Mata et al., 2010). The catalyst plays a greater role in faster reaction appeared and transesterification process (Gerpen, J.V.,2005).Separation of crude biodiesel and soap formation is difficult in chemical catalyst and its required recycling and high energy input (Bisen PS 2010). Alkali and enzyme catalyst perform a more fatty acid composition in transesterification reaction, especially enzymatic conversion is easy separation and recycle enzyme and it reduces soap formation (Ranganathan, S.V., 2008) Algal biodiesel efficiency of 95.68% was achieved from N.Oculata using lipase immobilized in alginate beads with methyl acetate as an acyl acceptor and solvent free system(Duraiarasan Surendhiran et *al* 2015). *Aspergillus Niger* added with carbon source of olive oil showed a higher lipolytic activity compared to other bacteria and yeast substrate using enzyme stability of pH 4-7 at 30^{o}C 24 hrs of cultivation period. Enzyme can greatly affect several environmental factors such as temperature, pH, Solvent molar ratio, agitation. Olive oil , glucose and moisture content were optimized for lipase production using Box-Behnken experimental design consisted in response surface methodology (G. Falony *et al.,*2006). *C. Subvermispora* fungi used for productive and ligninolytic enzyme for decolorizing process and their media composition with immobilization, support were optimized by response RSM (Zheng H 2011). In industries the bulk medium and their product formation

interacting with several variables and parameters such as variables affecting the process and individual interaction of variables in the system were analyzed and optimized using response surface methodology, its design is helped to understand the interactions of variables and parameter affecting the process can be recovered easily by using this tool (Janja Babic *et al* 2012).

Materials and Methods

Microalgae cultivation procedure

Algal strain was collected from local pond in chidhambaram, cuddalore District. The algal samples were undergone various subculture using agar (Himedia laboratory) Bold Basal Medium (Bold 1949 Bischoff and Bold 1963) to get unialgal culture. Bold basal medium is used for cultivating *Scenedesmus arcuatus* in the lab scale level. The culture was grown in 100 ml conical flask plugged with cotton in laboratory condition. The medium pH was 7.4 and the light was maintained at 8hrs Light & 16hrs Dark ratio and Temperature of 25ºC. This microalga utilizes atmospheric carbon dioxide for their photosynthesis process. After the incubation of 15 days of cultivation the matured cultured of *Scenedesmus arcuatus* were centrifuged and the pellets was shade dried for maintaining actual characteristics of microalgae for the extraction process (Kamen F.L 2016). 5gm of dried algal biomass extracted with Hexane: methanol (2:1) mixture of different concentration 60ml,80ml,100ml of solvent mixture. Lipid of algal oil was determined by initial volume of aqueous and organic phase weighed into that of the lipids in aqueous and organic phase with evaporation of the solvent using rotary evaporator.

$$\% \text{ Algae oil extracted} = \frac{Amount\ of\ algal\ oil\ obtained}{Amount\ of\ algae\ used} X\ 100. \qquad (1)$$

Isolation and Culture Condition of Aspergillus Niger

Dig the earth's surface and collected the soil sample from local oil mill for isolation of lipase enriched species. Seven sets of test tubes with 9ml of distilled water were taken from the method of serial dilution factor for isolating fungi (Yan Li *et al* 2014). From the collected soil samples 1gram of soil sample is mixed with 10ml of distilled water solution in the 1^{st} tube which is 10^0 dilutions. From the 1^{st} tube again 1ml is transferred into the 2^{nd} tube containing 9ml which is 10^{-1} dilution and 3^{rd} tube is 10^{-2} dilution similarly the organism strain was diluted for all the 7 test tubes. Procured the potato dextrose agar from Himedia laboratory and prepared PDA agar for solid cultivation. 0.5 ml of 10^{-4} dilution was taken and spread on the spread plate method containing sterilized PDA using L rod which is incubated in 28^OC for 48hrs. Isolation of fungi other species were arrested using streptomycin. The pure spores of *Aspergillus* were taken and transferred into potato dextrose broth 2% palm oil maintained at 28^OC and pH 5 for 7 days of incubation period. The pH of the medium was adjusted to 5.0 using 100ml of 0.1M potassium hydrogen phthalate and 45.2 ml of 0.1M sodium hydroxide. The Culture was incubated at 30^OC for 7 days and the growth rate was monitored using a spectrophotometer. After incubation period cells were filtered using whatmann filter paper. Supernatant of the *Aspergillus* broth was collected and used as a crude lipase enzyme for measuring activity for further process (Amjad khan afridi).

Immobilization of lipase enzyme in alginate beads

Lipase from *Aspergillus* broth was immobilized, using method of (Zheng H 2011)

Lipase activity assay using p-nitrophenylpalmitate (pNP)

Lipase activity of the samples was determined by the pH titrimetric method (Janja Babic *et al*. 2012).

Transesterification reaction using immobilized lipase enzyme using response surface methodology

The optimization of lipase catalyzed transesterification reaction with six production parameters was done using RSM. The various factors and its interaction effects were optimized using response surface methodology, Design –Expert Software version 8.0.7.1. box behnken Design was used to optimize the parameters affecting the transesterification reaction for achieving maximum biodiesel production with minimum run numbers. Six major factors affecting immobilized enzyme of *scenedesmus* biodiesel were (A) Oil: solvent molar ratio in the range 1:1, 1:3, 1:5. (B) Enzyme concentration in the range of 1gm, 3gm, 5gm. (C) Time ranges

like 36hrs, 48 hrs, 60 hrs. (D) Temperature from 20^{O}C, 30^{O}C and 40^{O}C. (E) Water in the ranges of 3ml, 6ml and 9ml. (F) Agitation from 100 Rpm, 200 Rpm and 300 R pm. In this Study each independent variables with interaction parameter were studied at three different levels given in Table 1 low (-1), medium (0) and high (1).Total 54 runs were evaluated for optimizing the biodiesel parameters. All the experiments were carried out in triplicate to ensure the reproducibility of the data (Jang MyungGwi et al.,2012). The analysis has only ±5% error of analysis in the experimental procedure. The production mixture is biodiesel and glycerol was separated using a gravity separator. The upper layer containing fatty acid methyl ester was washed with hot water and cold water alternately to remove impurities of excess solvents present in the product. The crude biodiesel was dried and stored for further analysis.

$$\text{The yield of biodiesel} = \frac{weight\ of\ biodiesel}{weight\ of\ agal\ used} \text{ X } 100. \qquad (2)$$

The micraolagae biomass was analyzed for FTIR analysis ((Elumalai *et al*. 2011)) biodiesel obtained was analyzed by GC-MS ((Tadashi 2009).

Table.1. Independent variables and their levels used for Experimental design Coded Levels

S. No.	Factors	Symbol	-1	0	1
1	Oil :Solvent ratio(v/v)	A	1:1	1:3	1:55
2	Enzyme Concentration (gm)	B	1	3	5
3	Time(min)	C	36	48	60
4	Temperature(^{o} C)	D	20	30	40
5	Water (%)	E	3	6	9
6	Agitation Speed (RPM)	F	100	200	300

Results and Discussion:

Regression Analysis:

Table 2 shows that Box Behnken design of 54 Runs were operated using Second Order Polynomial Equation which was measured by yield response (Y), Where Y is the percentage of Biodiesel Yield,

Percentage Yield (Y) = +71.20+ 0.99* A -0.13 * B +0.27* C-1.31 * D-0.39* E-0.43 * F-0.79 * A * B+0.10 * A * C-1.07 * A * D-0.40 * A * E -0.030 * A * F +0.31 * B * C -0.60 * B * D+0.28 * B * E+0.049 * B * F+0.35 * C * D+0.54 * C * E-0.11 * C * F-0.11 * D * E -0.39 * D * F-0.87 * E * F-8.64 * A^2 -3.38 * B^2+0.046 * C^2-1.13 * D^2-0.78 * E^2-1.47 * F^2 (3)

Box behnken design of 54 experimental runs was carried to evaluate the significant values of % Yield, regression coefficient, anova table and residuals of second order polynomial equations. Where, Y is the measured response surface quadratic model for oil yield. A, B, C, D, E, F are the variable input of code levels for oil:solvent ratio, enzyme concentration, time, temperature, agitation and water respectively. Interaction terms are also evaluated for operating experiment with effect of two parameters AB, AC, AD, AE, AF, BC, BD, BE, BF, CD, CE, CF, DE, DF and EF. A^2, B^2, C^2, D^2, E^2 and F^2 are the independent variable in the squared terms. R^2 Coefficient and Quadratic polynomial equation evaluate the model being fitted with the Response Surface Plot (Chen X 2008).

Table.2. Box Behnkenn Design of Response Surface Methodology for *Scenedesmus Spp* Biodiesel Production.

Run No	O:S Ratio	Enzyme Conc	Time	Temperature	Water	Agitation	%Y Exp	%Y pred
1.	3.00	3.00	48.00	30.00	6.00	200.00	71.2	72.44
2.	5.00	3.00	48.00	20.00	9.00	200.00	63.31	62.9
3.	3.00	5.00	60.00	30.00	3.00	200.00	67	68.11
4.	1.00	3.00	60.00	30.00	6.00	100.00	60.79	61.7
5.	3.00	5.00	48.00	30.00	3.00	300.00	65.9	66.27
6.	1.00	3.00	36.00	30.00	6.00	100.00	60.2	61.36
7.	1.00	5.00	48.00	40.00	6.00	200.00	56.89	57.90
8.	1.00	3.00	36.00	30.00	6.00	300.00	59.68	60.02
9.	1.00	5.00	48.00	20.00	6.00	200.00	58.5	59.10
10.	1.00	3.00	48.00	20.00	3.00	200.00	59.99	60.38
11.	3.00	3.00	36.00	40.00	6.00	300.00	66.03	67.51
12.	5.00	3.00	48.00	20.00	3.00	200.00	64.78	65.14
13.	1.00	3.00	60.00	30.00	6.00	300.00	59.89	60.01
14.	3.00	3.00	60.00	20.00	6.00	300.00	69.8	70.67
15.	3.00	3.00	48.00	30.00	6.00	200.00	71.2	72.10
16.	3.00	5.00	36.00	30.00	3.00	200.00	66.99	67.07
17.	3.00	1.00	60.00	30.00	9.00	200.00	67.03	68.10
18.	5.00	3.00	48.00	40.00	9.00	200.00	58.1	58.56
19.	5.00	1.00	48.00	40.00	6.00	200.00	58.29	59.05
20.	5.00	3.00	48.00	40.00	3.00	200.00	60.2	60.89
21.	3.00	1.00	36.00	30.00	9.00	200.00	66.1	66.82
22.	5.00	5.00	48.00	40.00	6.00	200.00	55.29	55.90
23.	3.00	3.00	36.00	20.00	6.00	300.00	70.05	70.63
24.	5.00	5.00	48.00	20.00	6.00	200.00	61.12	61.78
25.	3.00	3.00	48.00	30.00	6.00	200.00	71.2	71.2
26.	3.00	1.00	60.00	30.00	3.00	200.00	67.3	67.8
27.	3.00	5.00	48.00	30.00	9.00	100.00	66.6	67.06
28.	3.00	3.00	60.00	40.00	6.00	300.00	66.99	67.28
29.	1.00	3.00	48.00	40.00	3.00	200.00	59.61	60.17
30.	3.00	1.00	48.00	30.00	3.00	300.00	66.71	67.07
31.	3.00	3.00	36.00	40.00	6.00	100.00	67.39	67.89
32.	3.00	3.00	60.00	40.00	6.00	100.00	68.93	69.10
33.	3.00	5.00	48.00	30.00	3.00	100.00	65.11	66.18
34.	3.00	1.00	48.00	30.00	3.00	100.00	65.92	66.01
35.	3.00	1.00	48.00	30.00	9.00	100.00	66.56	66.45
36.	1.00	3.00	48.00	40.00	9.00	200.00	59.31	59.89
37.	3.00	3.00	48.00	30.00	6.00	200.00	71.2	71.45
38.	1.00	1.00	48.00	20.00	6.00	200.00	55.96	56.01
39.	5.00	3.00	60.00	30.00	6.00	100.00	63.11	63.18
40.	3.00	5.00	36.00	30.00	9.00	200.00	65.78	65.89
41.	3.00	1.00	48.00	30.00	9.00	300.00	63.69	63.71
42.	3.00	3.00	48.00	30.00	6.00	200.00	71.2	71.39
43.	3.00	3.00	48.00	30.00	6.00	200.00	71.2	71.20
44.	3.00	5.00	48.00	30.00	9.00	300.00	64.12	64.27
45.	5.00	3.00	36.00	30.00	6.00	300.00	61.48	61.89
46.	1.00	3.00	48.00	20.00	9.00	200.00	59.94	60.11
47.	5.00	3.00	36.00	30.00	6.00	100.00	62	62.16
48.	3.00	3.00	36.00	20.00	6.00	100.00	70.03	70.25
49.	5.00	1.00	48.00	20.00	6.00	200.00	61.69	61.8
50.	5.00	3.00	60.00	30.00	6.00	300.00	61.97	62
51.	3.00	5.00	60.00	30.00	9.00	200.00	68	68.30
52.	3.00	1.00	36.00	30.00	3.00	200.00	68.5	68.91
53.	3.00	3.00	60.00	20.00	6.00	100.00	70.02	70.10
54.	1.00	1.00	48.00	40.00	6.00	200.00	56.71	56.98

Table.3. ANOVA table for Response Surface Quadratic Model

Sum of Squares	Mean	F	Source	df Square	p-value
A-Oil:Solvent	*23.74*	*1*	*23.74*	*1956.01*	*< 0.0001*
B-Enzyme Conc	*0.42*	*1*	*0.42*	*34.28*	*< 0.0001*
C-Time	*1.82*	*1*	*1.82*	*149.5*	*< 0.0001*
D-Temp	*41.21*	*1*	*41.21*	*3395.52*	*< 0.0001*
E-Water	*3.74*	*1*	*3.74*	*307.87*	*< 0.0001*
F-Agitation	*4.46*	*1*	*4.46*	*367.74*	*< 0.0001*
AB	*4.95*	*1*	*4.95*	*407.46*	*< 0.0001*
AC	*0.08*	*1*	*0.08*	*6.59*	*0.0163*
AD	*18.38*	*1*	*18.38*	*1514.55*	*< 0.0001*
AE	*1.3*	*1*	*1.3*	*106.78*	*< 0.0001*
AF	*7.20E-03*	*1*	*7.20E-03*	*0.59*	*0.4481*
BC	*0.78*	*1*	*0.78*	*64.37*	*< 0.0001*
BD	*2.87*	*1*	*2.87*	*236.3*	*< 0.0001*
BE	*1.29*	*1*	*1.29*	*106.61*	*< 0.0001*
BF	*0.019*	*1*	*0.019*	*1.57*	*0.2219*
CD	*0.95*	*1*	*0.95*	*78.45*	*< 0.0001*
CE	*2.35*	*1*	*2.35*	*193.98*	*< 0.0001*
CF	*0.21*	*1*	*0.21*	*17.06*	*0.0003*
DE	*0.097*	*1*	*0.097*	*7.98*	*0.009*
DF	*1.2*	*1*	*1.2*	*98.97*	*< 0.0001*
EF	*6*	*1*	*6*	*494.6*	*< 0.0001*
A^2	*767.85*	*1*	*767.85*	*63263.4*	*< 0.0001*
B^2	*117.4*	*1*	*117.4*	*9672.79*	*< 0.0001*
C^2	*0.021*	*1*	*0.021*	*1.77*	*0.195*
D^2	*13.02*	*1*	*13.02*	*1072.81*	*< 0.0001*
E^2	*6.25*	*1*	*6.25*	*515.22*	*< 0.0001*
F^2	*22.09*	*1*	*22.09*	*1820.18*	*< 0.0001*
Residual	0.32		26	0.012	
Lack of Fit	*0.32*		*21*	*0.015*	
Pure Error	*0*		*5*	*0*	
Cor Total	1126.46	53			

Table 3 shows that the yield coefficient 71.2% quadratic model was fitted with experimental values. Based on p-values, the regression coefficients were significant at $p < 0.0001$ were selected for the models that resulted in equations (1). Analysis Of Variance (ANOVA) was conducted to evaluate the adequacy and consistency of the models using f-statistic. The Model F-value of 3436.44 implies the model is significant. There is only 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicates model terms are significant. Values of "Prob>F" less than 0.0500 indicates model terms are significant. The "Pred R-Squared" of 0.9985 is in reasonable agreement with the "Adj R-Squared" of 0.9994."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 202.293 indicates an adequate signal. This model can be used to navigate the design space.

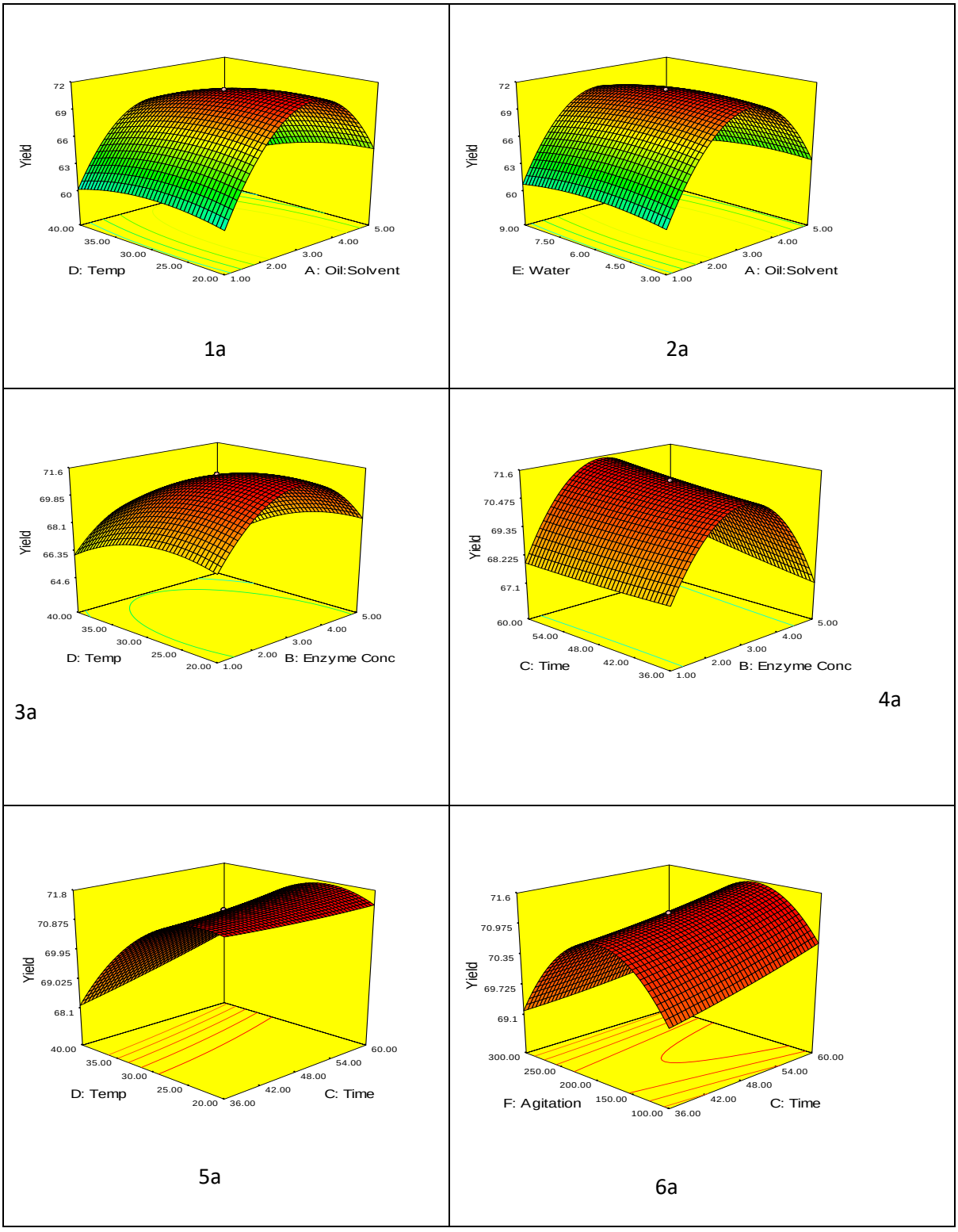

Figure.1. Response surface plots for percentage yield of biodiesel from microalgae- *scenedesmus spp* for various parameter and their interaction effect

The 3D Surface and 2D Contour plot were actually showing the interaction of variables to yield, which means optimum values were plotted in the graphs. Six surface plots and contour plots were plotted the investigation of different parameter interaction on the yield of biodiesel production. The contour and surface plots shows the elliptical curve means that the interaction between two parameters on the biodiesel yield was more significant that of independent variables. The straight line of the graph shows that the variables, interaction on yield was not significant. Figure 1a, 1b shows that the interaction of Oil:Solvent and temperature on *Scenedesmus* biodiesel production. The minimum value of 1vol Oil:solvent and 28 minutes produced less yield of 60% biodiesel after gradually increasing these two parameters yield was raised and reached 70% at 3 molar Oil:Solvent and 30 minutes, further increasing these parameters the conversiuon ratio of biodiesel was low at 68%. Figure 1 b of contour plot of results shows that interaction between Oil:solvent and temperature is significant. Figure 2a, 2b shows that the interaction of Oil:Solvent and water on *Scenedesmus* biodiesel production. The minimum value of 1vol and 3 moles of Oil:Solvent and water produced less yield of 61% biodiesel after gradually increasing these two parameters yield was raised and reached 71% at 3 molar Oil:Solvent ratio and 6 moles of water ,further increasing these parameters the biodiesel was reduced to 68% at 5 mol of Oil:Solvent ratio and 9 moles of water. Figure 2b of contour plot of results shows that interaction between Oil:solvent and water is significant. Figure 3a, 3b shows that the interaction of Enzyme concentration and temperature on *Scenedesmus* biodiesel production. The minimum value of 1wt of Enzyme concentration and $20^{O}C$ produced less yield of 63% biodiesel after gradually increasing these two parameters yield was raised and reached 69% at 3 wt of Enzyme concentration and $30^{O}C$ further increasing these parameters the conversion ratio of biodiesel was low at 66% at 5wt of B and $40^{O}C$. Figure 3b of contour plot of results shows that interaction between Enzyme concentration and temperature is significant. Figure 4a, 4b shows that the interaction of Enzyme concentration and time on *Scenedesmus* biodiesel production. The minimum value of 1wt of Enzyme concentration and 36 minutes produced low yield of 63% biodiesel after gradually increasing these two parameters yield was raised and reached 70% at 3 wt and 48 minutes further increasing these parameters the conversion ratio of biodiesel was low at 68% at 5 wt of B and 60 minutes. Figure 4b of contour plot of results shows that interaction between Enzyme concentration and time is not significant. Figure 5a shows that the interaction of time and temperature on Scenedesmus biodiesel production. The minimum value of 36 minutes and $20^{O}C$ produced 68% biodiesel after gradually increasing these two parameters as 48minutes and $30^{O}C$ yield reached 69.95% further increasing these parameters the biodiesel was reduced to 68% at 68 minutes and $40^{O}C$. Figure 5b of contour plot of results shows that interaction between time and agitation is not significant. Figure 6a, 6b shows that the interaction of time and agitation on Scenedesmus biodiesel production. The minimum value of 36 minutes and 100 rpm produced 68% biodiesel after gradually increasing these two parameters as 48minutes and 200 rpm yield reached to 70.5% further increasing these parameters the biodiesel was reduced to 69% at 68 minutes and 300 rpm . Contour plot of results shows that interaction between time and agitation is not significant. The results of statistical analysis predicted as algal oil: solvent ratio 10:70, catalyst amount 2.5 gram, reaction temperature $60^{O}C$ and reaction time 60 minutes. The R^2, adjusted R^2 and predicted values are 0.9813, 0.956 and 0.9300 respectively, which were fitted with the experimental values hence this model is significant for biodiesel production.

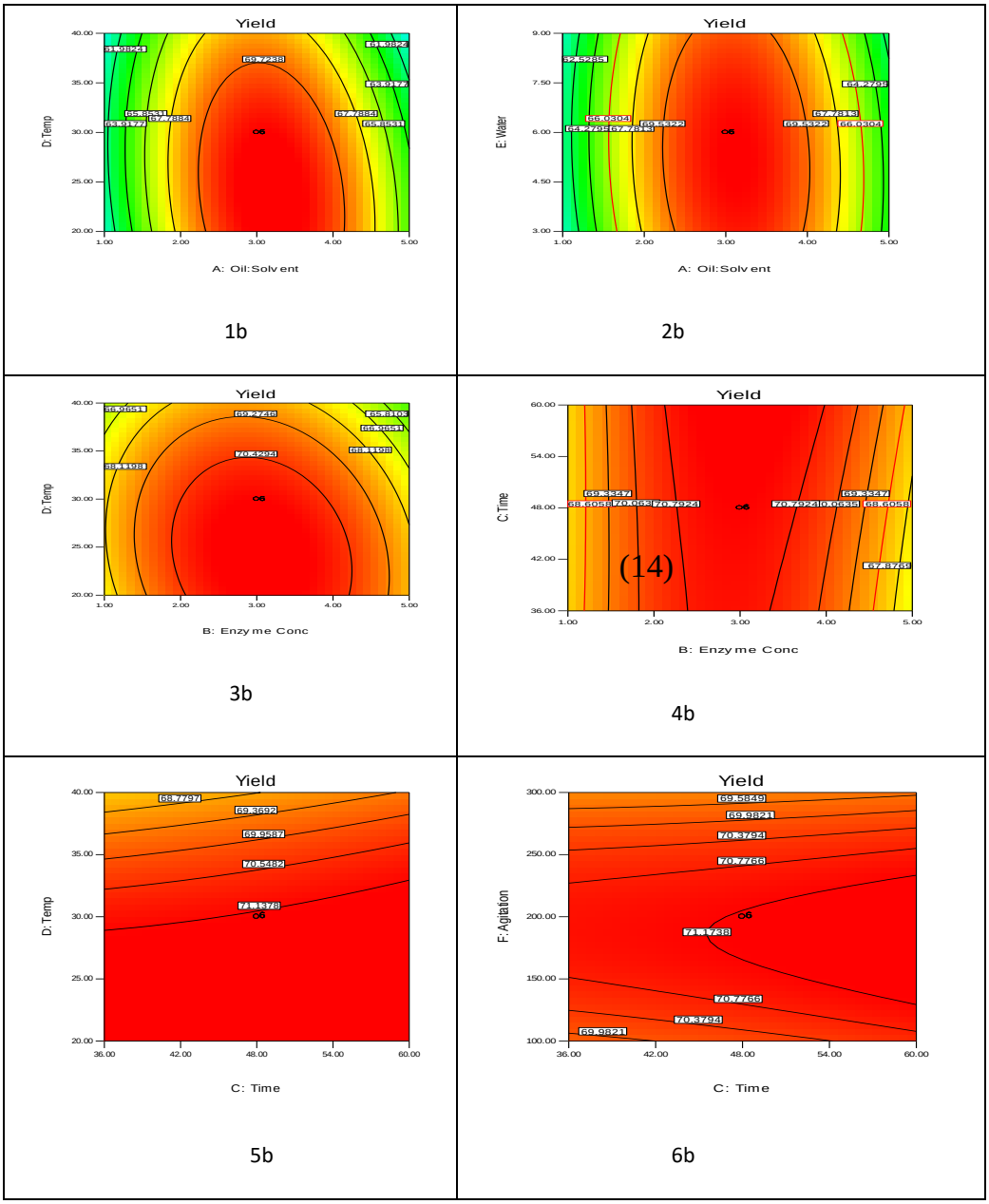

Figure.2. Contour plots for percentage yield of *Scenedesmus* biodiesel

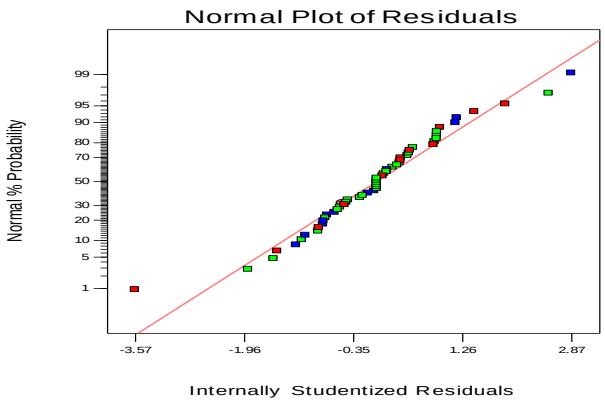

Figure.3. Normal plot for *Scenedesmus Spp* Biodiesel

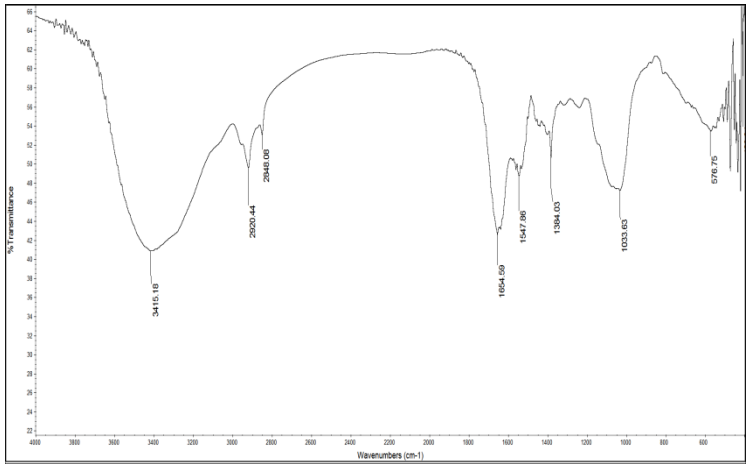

Figure .4. FTIR Spectroscopy of Bond Specification of *Scenedesmus* Biodiesel.

Figure 4 showed FTIR absorption spectrum of Infrared spectrum with the peaks showed the presence of components in the system with the particular stretching frequency. This IR spectrum used to check our compound is available in the ranges using different peaks (http://www2. ups.edu/faculty/ hanson/ Spectroscopy/ IR/IRfrequencies.html). The stretching frequency of 3415.18 cm^{-1} vibration indicates that the medium bond of the N-H amide group. The C-H alkenes compound is the strong stretching absorption bands in the region of 2920.44 cm^{-1}. The stretching band in the region of 2848.08 cm^{-1} aldehyde compound is the strong absorption band. C=O amide compound are the strong stretching absorption bands in the region of 1654.59 cm^{-1}. C=C aromatic compound is the multiple weak with multiple bands stretching absorption bands in the region of 1547.86 cm^{-1}. C-O Ester compounds are two or more bands stretching absorption bands in the region of 1033.63 cm^{-1}. C-Br alkyl halide compounds are the strong bands stretching absorption bands in the region of 576.75 cm^{-1}. The analysis of infrared spectroscopy with the absorption and adsorption band peak marked in the Figure 4 shows that the various organic, ester and aromatic components were present in the *scenedesmus* biodiesel showed a better quality of biodiesel.

All the saturated, un saturated, mono saturated and poly saturated fatty acids are presented in these microalgae was extracted using the solvent of chloroform:methanol (2:1) ratio (Yan Li *et al*.2014). Table 4 ,Figure 5 shows that synthesis of fatty acid composition, lipids and tryglycerides from *Scenedesmus* biodiesel were analyzed using gas chromatography which contains totally 33 compounds the major compounds present in the *Scenedesmus* include C12:0 Dodecanoic acid, C14:0 Tetradecanoicacid, C16:0 Hexadecanoicacid, C18:1 Octadecenoicacid/ oleic acid, C18:0 Octadecanoicacid / myristic acid.

Table.4. Fatty acid profile of *Scenedesmus Spp* Biodiesel

S.no	Lipid Number	Common Name	Chemical Name	Molecular Formula
1.	C12:0	Lauric acid	Dodecanoicacid	$C_{12}H_{24}O_2$
2.	C14:0	Myristic acid	Tetradecanoicacid	$C_{14}H_{28}O_2$
3.	C17:0	Palmitic acid	Hexadecanoicacid	$C_{17}H_{34}O_2$
5.	C18:0	Oleic Acid	trans-9-octadecenoic acid.	$C_{18}H_{34}O_2$
6.	C19:0	Stearic acid	palmitic acid	$C_{19}H_{38}O_2$

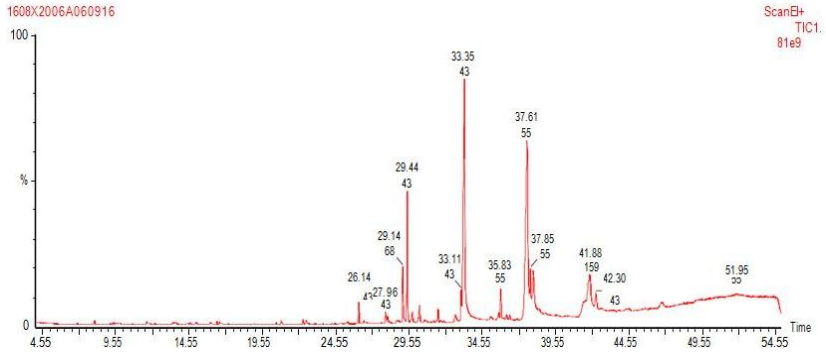

Figure.5. GC- MS chromatogram of *Scenedesmus* fatty acid production

Conclusion

Box behnken design was used for optimization of methanol/oil ratio, catalyst concentration, reaction temperature and time on the transesterification of *Scenedesmus arcuatus* algal oil. Thus, research work gave an optimal value of 71.5 % yield of biodiesel at 60 °C, in 60 minutes of reaction time with 2.5 gram of lipase enzyme and methanol/oil ratio of 10:70. Quadratic polynomial models were obtained to predict yield of biodiesel. The result showed that the predicted value was in agreement with the experimental value, which was established with additional experiments to confirm the optimized parameters. The GC result of unsaturated fatty acids showed a reasonable balance of fuel properties. In this research the chain length of fatty acids from *Scenedesmus* biodiesel in the range between C14 and C20. Therefore, fatty acids from *Scenedesmus* biodiesel were more applicable for producing a high quality of biofuel, since it contained a high content of C16 (Hexadecanoic acid) and C18 (oleic acid).Therefore production of biodiesel from algal oil using response surface methodology is a feasible process.

Acknowledgement

Authors would like to thank the University Grant Commission (UGC-Rajiv Gandhi National Fellowship), New Delhi, providing fund for the research work. UGC-grant number. F1-17.1/2015-2016/RGNF-2015-2017-SC-TAM-4524 /(SA-III/Website).

References

Amjad khan afridi. https://www.slideshare.net/amjadkhanafridi4all/isolation-of-fungi-from-soil-sample.

Anitha and Sriman Narayanan, J. 2012 Biodiesel production from chlorella vulgaris with biodiesel production from *chlorella vulgaris* with special emphasis on immobilized lipase catalyzed transesterification. *International Journal of Recent Scientific Research* **3(9)**, pp. 733 - 737, September, 2012

Bansal, B. K. and Sharma, M. P. 2005 Prospects of biodiesel production from vegetables oils in India. 363–78.

Bischoff, H.W., And Bold, H.C. 1963.Phycological Studies Iv. Some Soil Algae From Enchanted Rock And Related Algal Specie. University Of Texas, Austin, **6318**: 1 - 95.

Bisen PS, Sanodiya BS, Thakur GS, Baghel RK, Prasad GBKS , 2010. Biodiesel production with special emphasis on lipase-catalyzed transesterification. *Biotechnol Lett* **32**:1019–1030

Bold, H.C. 1949. The Morphology Of *Chlamydomonas Chlamydogama* Sp. Nov.. *Bull. Torrey Bot. Club.* **76**: 101 - 108.

Chen X, Wei D, Liu D. 2008 Response surface optimization of biocatalytic biodiesel production with acid oil. *Biochem Eng Journal* **40** : 423-429

Duraiarasan Surendhiran *et al*., 2015 An alternative method for production of microalgal biodiesel using novel Bacillus lipase. *3 Biotech* **5** :715–725.

Elumalai, S, Sakthivel, R and Ganesh Kumar, S 2011. Ultra Structural and Analytical Studies of Biodiesel Producing Microalgae (Chlorella vulgaris and Scenedesmis sp.) Collected from Tamil Nadu, India. *Current Botany*. **2(6)**: 19-25

G. Falony *et al.,* 2006 Production of Extracellular Lipase from *Aspergillus niger* by Solid-State Fermentation. *Food Technol. Biotechnol. **44*** **(2)**: 235–240.

Gerpen, J.V. (2005). Biodiesel processing and production. *Fuel Process Technology*. **86**. 1097- 1107

Goldemberg,J. 2000 World Energy Assessment,Preface.United Nations Development Programme, new York, NY, USA.

http://www2.ups.edu/faculty/hanson/Spectroscopy/IR/IRfrequencies.html

Jang MyungGwi, Kim DeogKeun, Park SoonChul, Lee JinSuk, Kim Seung Wook., 2012.Biodiesel production from crude canola oil by two-step enzymatic process. *Renew Energy* **42** :99–104.

Janja Babic *et al*., 2012 Optimization of Ligninolytic Enzyme Activity and Production Rate with *Ceriporiopsis subvermispora* for Application in Bioremediation by Varying Submerged Media Composition and Growth Immobilization Support. *Int. J. Mol. Sci. **13***: 11365-11384; doi:10.3390/ijms130911365.

Kamen F.L., 2016 Application of Response Surface Methodology In The Optimization of Biodiesel Production From Microalgae Oil. *Journal of Multidisciplinary Engineering Science and Technology* (JMEST) ISSN: 2458-9403. **3(11)**.

Klass, L. D. 1998 Biomass for Renewable Energy, Fuels, and Chemicals. Academic Press, NewYork. pp 1-2.

Moheimani NR. 2005 The culture of Coccolithophorid Algae for carbon dioxide bioremediation. PhD thesis. Murdoch University.

Poisson L, Devos M, Pencreac'h G, Ergan F. 2002 Benefits and current developments of polyunsaturated fatty acids from microalgae lipids. OCL – Oleagineux Corps Gras Lipides, **9(2-3)** :92–5.

Ranganathan, S.V, Narasimhan, S.L. and Muthukumar, K. 2008. An overview of enzymatic production of biodiesel. *Bioresource Technology*. **99**, 3975-3981.

Tadashi Matsunaga., Mitsufumi Matsumoto., Yoshiaki Maeda., Hiroshi Sugiyama., Reiko Sato. and Tsuyoshi Tanaka. 2009 Characterization of marine microalga, Scenedesmus sp. Strain JPCC GA0024 toward biofuel production. *Biotech lett* DOI 10.1007/s10529-009-0029

Teresa M. Mata *et al*. 2010 Microalgae for biodiesel production and other applications: *Renewable and Sustainable Energy Reviews*. **14(1)** : 217-232.

Thomas WH, Tornabene TG, Weissman J. 1984 screening for lipid yielding microalgae: activities for 1983. SERI/STR-231-2207.

Yan Li et al., 2014 A comparative studies: the impact of different lipid extraction methods on current microalgal lipid research. *Microb Cell Fact*. **13**: 14.

Zheng H, Yin J, Gao Z, Huang H, Ji X, Dou C.,2011 Disruption of Chlorella vulgaris cells for the release of biodiesel-producing lipids: a comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves. *Appl Biochem Biotechnol* **164**:1215–1224.