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1 Modelling and optimization of wet microalgae *Scenedesmus quadricauda* lipid extraction
2 using microwave pre-treatment method and response surface methodology

3
4 **C. Onumaegbu¹, A. Alaswad², C. Rodriguez^{1,*}, A. Olabi³**

- 5
6 1. Institute of Engineering and Energy Technologies, School of Engineering and
7 Computing, the University of the West of Scotland, Paisley PA1 2BE, UK.
8 2. School of Engineering and the Built Environment, Birmingham City University,
9 Birmingham B5 5JU, UK.
10 3. Chair in Mechanical Engineering and Design, Aston University, School of
11 Engineering and Applied Science, Aston Triangle, Birmingham, B4 7ET, UK
12

13 Abstract:

14 The process of extracting lipids from high-moisture *Scenedesmus quadricauda* microalgae
15 biomass disrupted with microwave was examined. The study showed that microwave pre-
16 treatment is effective in algae cell rupture while microwave power was found to be a
17 significant factor to enhance the degree of cell disruption. Though microwave pre-treatment
18 time had some effect, the degree of cell rupture seemed to decrease after a certain pre-
19 treatment time. The total lipid from *Scenedesmus quadricauda sp.* were extracted using a
20 mixture methanol and sulphuric acid as an organic solvent. In addition, it was discovered that
21 microwave pre-treatment enhances the disruption of microalgae cells to attain a high level of
22 lipid yields. Optimal lipid yield obtained in this study was 49% at power 600 W, heating time
23 of 8 min and extraction time of 3.5 h.

24 Keywords: microalgae, lipid extraction, microwave pre-treatment, modelling, optimization,
25 biodiesel

26 *Corresponding author. E-mail address: cristina.rodriguez@uws.ac.uk

27 **1. Introduction:**

28 Though algae biofuels are not yet commercial, their economic outlook is promising [1–4].
29 The obsolete development of lipid extraction from microalgae cells often involves the
30 consumption of a large amount of energy because of microalgae dewatering process [5].
31 Using microalgae biomass a potential substitute fuel production has increased globally [6], as
32 microalgae represent a renewable energy resource which captures atmospheric carbon
33 dioxide (CO₂) photosynthetically and produces lipids that can be converted to biodiesel [7–
34 9]. However, large-scale production of microalgae biomass and energy efficiency is yet to
35 become a sustainable reality.

36 Fundamental issues are obviously high lipid productivity, energy efficient downstream
37 processes and energy balance in the case of dry route lipid extraction is not positive.
38 According to K. Sander and G. Murthy [10], the minimum net energy input is 3982 MJ for 24
39 kg of biomass with a lipid content between 30 and 40% (w/w), necessary for the production
40 of 1000 MJ microalgae biodiesel. However, a natural gas dryer requires 3556 kJ/kg water
41 removed which represents 89% of the total energy input. Generally, life-cycle assessment
42 (LCA) studies of biodiesel from microalgae pointed out that the step which requires the most
43 energy input is the biomass drying operation [11]. If the energy input is reduced with an
44 improvement or removal of the drying operation, the net energy balance and cost would be
45 positive [12].

46 Therefore, lipid recovery by wet extraction is of interest to reduce the energy demand. While
47 Chisti et al. [13] confirm that biorefinery concepts are mainly used to valorise the whole
48 biomass as a strategy to decrease the overall cost of the production, which must not exceed
49 0.25 dollar/kg to compete for the petroleum. In addition, the energy applied during
50 microwave pre-treatment has been noted to affect microalgae solubilisation, where Dai et al.
51 [14] confirm that increasing microwave pre-treatment power from 400 to 1000 W increases
52 microalgae lipid yield. Qv et al. [15] observed that increasing microwave power from 140 to
53 560 W increases lipid extraction efficiency. However, most previous studies also reported
54 that further increase in microwave power 700 W decreases microalgae lipid yield. A study
55 conducted by Biller et al. [16] confirms that increasing the microwave power from 25 - 61
56 Wh/g resulted in increased lipid yield from *Nannochloropsis sp.* biomass from 1.6 to 10%.

57 Passos et al. [17], noted that increasing the microwave energy from 300-900 W increases
58 microalgae biomass solubilisation. The energy consumed during microwave irradiation pre-
59 treatment depends on the temperature and duration of cell disruption. Some previous studies
60 have studied the effects of energy consumed during microalgae cell disintegration on lipid
61 yield. Balasubramanian et al [18], arrive at a conclusion that 76-77% of the oil from dried
62 *Scenedesmus obliquus sp.* was achievable using microwave radiation with an energy
63 consumption of 60 Wh/g. The high moisture of microalgae growth medium of 99.9% w/w
64 has increasingly become a barrier for the entire production process [19]. Lee et al. [20]
65 confirm that disrupting 100 ml of microalgae cell suspension by microwave with an energy
66 input of 700 W for 5 min, the energy consumed is equivalent to 420 MJ kg⁻¹ of dry algal
67 mass. In addition, physical and chemical harvesting techniques such as sedimentation,
68 flocculation, freeze dry and centrifugation can only decrease the quantity of moisture close to
69 90% (w/w), where further removal of moisture can only be achieved by drying process [19].
70 The dry process is not energy efficient and cost-effective, as this increases the possibility of
71 making the entire production process not economically efficient. Also, the size of microalgae
72 strains [21], and the existence of rigid cell wall that requires being ruptured [22–24] to
73 enhance lipid extraction, still has significant challenges in microalgae production process.
74 However, the development of production processes and the conversion of algal biomass to
75 biodiesel to achieve cost efficiencies that rival petroleum-based fuels is an ongoing challenge
76 that demands an in-depth understanding of both algal biology and process engineering [25–
77 27]. Also, the high-quality of algal species is essential in determining the amount of lipid
78 produced, an efficient effective method of lipid extraction is of much importance towards
79 commercial biofuel production [28,29]. Subsequently, for lipid extraction process to be
80 successful using microalgae biomass, there is a need for an efficient cell disruption phase that
81 will enhance lipid production. Previous studies have used both mechanical and non-
82 mechanical pre-treatment for microalgae cell rupture[30]. A study conducted by Halim et al.
83 [22] used direct counting and average colony diameter methods to determine the disruption
84 efficacy of many treatments to lyse *Chlorococcum sp.*, these includes; high pressure
85 homogenizer (73.8%), sulphuric acid treatment (33.2%), bead beating (33.2%), and ultrasonic
86 (4.5%). They concluded that high-pressure homogenizer has the highest percentage of cell
87 rupture but is not energy efficient. Lee et al. [31] affirms that bead beating effectively
88 disrupts algae cell more efficiently. A study by Chisti et al. [32] evaluated the use of

89 mechanical disruption technique using bead beating, HPH with liquid shear, ultrasonic and
90 freeze press, and they concluded that cell rupture is dependent on the microorganism. The
91 outstanding problem about mechanical cell rupture is that they are not energy efficient. For
92 this reason, previous studies have demonstrated that microwave pre-treatment has been
93 effectively used in cell disruption of microalgae cell walls [18,33–35] to enhance lipid
94 production. This method has been applied in numerous areas which includes: chemical
95 synthesis, solvent extraction, and solid state reaction [36]. Other applications includes;
96 catalytic and non-catalytic transesterification processes [37], pyrolysis and hydrothermal
97 liquefaction of microalgae for biofuel production[38].

98 Other studies that applied microwave irradiation pre-treatment on different biomass material
99 to produce biogas includes [17,39–42]. In addition, Refaat et al. [43], applied microwave pre-
100 treatment using sunflower and achieved 5.96% of lipid, Chen et al. [44] uses waste cooking
101 oil and produces 38.31% of lipid and Cheng et al. [45] also applied microwave pre-treatment
102 using *Nannochloropsis Oceanica sp.* and recorded 38.46% of lipid yields. Balasubramanian
103 et al. [18] added that increasing reaction time from 10 and 20 min using microwave pre-
104 treatment on *Scenedesmus obliquus sp.* enhances lipid yield from 10% to 22%. Thus,
105 microwave energy can play an important role in microalgae cell pre-treatment to enhance
106 biofuel production. Also, microwave time plays a significant role during microalgae cell
107 disruption, which determines the recovery efficiency of the lipids present in microalgae
108 biomass [46]. Menendez et al. [47] observed the effect of increasing microwave pre-
109 treatment time from 10 -20 mins using *Nannochloropsis gaditana* and achieved a lipid yield
110 of 29-40%. Balasubramanian et al. [18] affirmed that increasing the microwave heating time
111 from 10-20 mins resulted in an increased in lipid yield from 10-22% using *Scenedesmus*
112 *obliquus* after pre-treatment. while Dai et al. [14] concluded that that increased in microwave
113 extraction time from 10 to 40 min resulted in increased microalgae lipid recovery 14 to 18%.
114 However, all the research works mentioned above used dry and different biomass material
115 for lipid production, at present no study has used microwave pre-treatment on *Scenedesmus*
116 *quadricauda* to enhance lipid extraction. Considering the energy and equipment cost related
117 to drying and dewatering microalgae cells, it would be cost-effective if wet microalgae cells
118 can be used directly for biofuel production after pre-treatment. Also, the extraction of lipids
119 from dried microalgae cells incurs a large amount of energy during dewatering process. To

120 improve this situation, some research studies has focused on an alternative approach for the
121 lipid extraction using wet microalgae, as discussed in [31,36,48–50].
122 Therefore, the objectives of the study include; (a) Modelling and optimization microwave
123 pre-treatment parameters using response surface method after lipid extraction. (b) Performing
124 numerical optimization to find the optimal combination of microwave power and time and
125 reaction time that could maximize the % of lipid yield, which is cost efficient as compared to
126 other previous works.

127

128 **2. Materials and Methods:**

129 2.1. Microalgae Cultivation

130 Microalgae strain (*Scenedemus quadricauda*) were purchase from Sciento-Manchester. 50
131 ml of each algae sample was kept in freezer at a temperature of 0 to 4°C to maintain a
132 constant growth rate. The sample was cultured within the School of Engineering, University
133 of the West Scotland (UK), in a 4-liter flask each after sterilization with distilled water at a
134 temperature of 60°C for 4 hours and 3 g of the unicellular culture medium (K10) was bought
135 from Sciento (Manchester, UK) was then added. The chemical composition of K10
136 unicellular medium includes; Sodium nitrate, Magnesium sulphate, Dipotassium hydrogen
137 orthophosphate, Calcium chloride, Ammonium chloride and Trace elements with weight (%)
138 of 62, 16, 15, 4 ,3 and <1 respectively). The flask was vigorously hand shake twice each day
139 to enhance appropriate circulation of the nutrients during cultivation period. Room
140 temperature of 15°C to 25°C was maintained throughout the culture period. A
141 spectrophotometer at a wave length of 600 nm was used to determine the initial cell
142 concentration before and at the end of culture period; which has the value of 1.815×10^8
143 cell/ml and 7.7637×10^{16} cell/ml. After 20 days, the cultivation process was completed.

144 2.2. Microwave Pre-treatment

145 500 ml sample of the standard culture were subjected to microwave pre-treatment using a
146 round bottom open glass. The samples were pre-treated at different microwave power of
147 600 W, 390 W and 180 W and time between 8, 5 and 2 minutes, until each pre-treatment
148 phase is completed. The pre-treatment was performed using a stainless-steel microwave oven

149 (Bosch BOSHMT75M451B, 800 W, 5 power levels and 60 min timer). All the experiments
150 were run in duplicate and the average results are presented in this paper.

151 2.3. Extraction Procedure:

152 Initially, 500 ml of wet algae sample were pre-treated using a conventional microwave
153 according to pre-determine microwave power and time. The two parameters were selected
154 based on previous research studies to give a distinct percentage of cell disruption [19]. A
155 500 ml of each pre-treated sample were placed in a flask by adding 500 cm^3 of methanol and
156 10 ml of sulphuric acid. Anti-bump granules were added to the flask and reflux at each
157 selected time of reaction. After the refluxing, the sample was extracted using 3 x 150 ml and
158 washed with 5% of sodium bicarbonate solution. The reflux process was repeated for 14
159 different experimental conditions with different extraction times (3, 3.5 and 4 h respectively).
160 The solvent used was evaporated using a steam bath to obtain the liquid extract.

161 2.4. Design of Experiments:

162 The experimental modelling was designed for 3 input parameters with three levels.
163 Microwave power varies from 180 to 600 W, microwave time between 2 to 8 min and
164 reaction time between 3 to 4 hrs. The output response was % of lipid recovered after each
165 extraction time. Both the process parameter and output response results are indicated in
166 Table 2. A Box-Behken Design with three factors was selected for design of experiments.
167 Fourteen experiments were determined by DOE, statistical analysis as well as the provision
168 of extensive graphs that showcase the relationship between the input parameters and the
169 output responses [51,52]. The process parameters selected was microwave power, time and
170 extraction time. The response was the % of lipid produced per each 500-ml sample produced.
171 RSM is considered by high adherence to the experimental data describing the reality of what
172 was studied [53]. Moreover, RSM methods are able to exhibit the factor contributions from
173 the coefficients in the regression model to identify the insignificant factors and thereby,
174 reduce the complexity of the problem[54]. Table 1 summarises the three levels and ranges of
175 process parameters used in the design, while Table 2 shows the experimental conditions and
176 amount of lipid recovered using Box-Behken design.

177 Table 1. Process variables and their units, levels used in the Experimental Design.

Variable	Units	Levels		
		-1	0	1
Microwave Power	W	180	390	600
Time	min	2	5	8
Extraction Time	h	3	3.5	4

178

179 Table 2. Box-Behken Design experimental design matrix showing the effects of process
 180 parameter on the output response (% recovered lipids).

Run	Input			Results
	Factor 1	Factor 2	Factor 3	Response
	A: Power	B: Heating Times	C: Extraction Time	% Recovered lipid
	W	min	h	%
1	180	5	3	14.01
2	180	2	3.5	14.44
3	180	8	3.5	10.83
4	180	5	4	18.86
5	390	2	3	18.87
6	390	8	3	18.87
7	390	5	3.5	32.43
8	390	5	3.5	11.68
9	390	5	3.5	25.46
10	390	2	4	14.44
11	390	8	4	37.84
12	600	5	3	32.43
13	600	2	3.5	18.69
14	600	8	3.5	48.65
15	600	5	4	25.45

181

182 2.5. Analysis method

183 The experimental data analysis was performed using Design Expert software version 10,
184 which predicts the optimal condition. The quadratic polynomial model used for response
185 surface regression procedure for this work is shown in Eq 1. Also, RSM consist of a group of
186 mathematical model and statistical techniques used in the development of an adequate
187 functional relationship between a response of interest, y, and several associated control or
188 input parameters denoted by $x_1, \dots, x_2, \dots, x_k$. Hence, the second order polynomial equation
189 is shown in Eq. (1), this is used to describe the true functional relationship between the input
190 parameters and the output response.

191
$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_{ii}^2 + \sum b_{ij} X_i X_j \quad (1)$$

192 Where Y is the amount of lipid produced (Output Response), b_0 is the coefficient of the
193 equation, X_i and X_j are the coded levels variables. X is the independent parameter and b_i , b_{ii}
194 and b_{ij} are the intercept, linear quadratic and interaction regression coefficients respectively.
195 The statistical significance of the model and the process parameters were assessed by analysis
196 of variance (ANOVA), while the quality of the model was determined by the determination
197 coefficient (R^2). The ANOVA table for the response surface quadratic model on % of
198 recovered lipid is shown in Table 3.

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Table 3. ANOVA for response surface quadratic model.

Source	Sum of Squares	df	Mean Square	F- Value	p-value
Model	1344.77	7	192.11	4.54	0.0320
A-mw power	562.47	1	562.47	13.28	0.0082
B-mw time	309.38	1	309.38	7.31	0.0305
C-reaction time	19.25	1	19.25	0.45	0.5218
AB	281.74	1	281.74	6.65	0.0365
AC	34.99	1	34.99	0.83	0.3936
BC	136.89	1	136.89	3.23	0.1152
A ²	0.055	1	0.055	1.3·10 ⁻³	0.9722
Residual	296.45	7	42.35		
Lack of Fit	73.44	5	14.69	0.13	0.9695
Pure Error	223.01	2	111.51		
Cor Total	1641.22	14			
R ² = 0.8194 Pred R ² = 0.4903 Adj R ² = 0.6387					

209

210 3. Results and Discussion:

211 3.1. Development of a regression model.

212 The 15-experimental results for *Seneesdemus quadricauda* are shown in Table 2. The
213 percentage of recovered lipid ranged from 14.01% to 48.65%. The final mathematical model
214 associated with the response in terms of actual factors is shown in Eq. 2, while the ANOVA
215 test is indicated in Table 3.

$$216 \% RL = 42.20 + 0.07A - 16.77B - 5.41C + 0.01AB - 0.03AC + 3.90BC + 2.75 \times 10^{-6}A^2 \quad (2)$$

217 where RL: Recovered lipids A- microwave power, B-microwave time, C-reaction time as
218 indicated in Table 3.

219 A variation less than 0.2 between adjusted-R² = 0.6387 and Predicted-R² = 0.4903, indicated
220 that the adopted model is adequate. The entire adequacy measures are less than 0.2, which are
221 in reasonable agreement and significantly shows adequate model [55,56], because the
222 statistical analysis as considered by the *Design Expert*, it indicates that any value equal less

223 than 0.2 are considered when determining the adequacy measures of adjusted- R^2 and
224 Predicted- R^2 . Where lack of Fit F-value of 0.13 implied that lack of fit was not significant
225 relative to the pure error (Table 2), this was tested to know if the Prob >F of the lack of fit
226 exceeds the level of significance as shown in table 3. Also, in Response surface methodology
227 (p-value) of lack fit if >0.05 (not significant) signifies that the model fits well and there is no
228 significant effect on parameters on output response. Hence, the term adjusted R-squared as
229 indicated in the ANOVA table 3. compares the explanatory power of regression models that
230 contain different numbers of predictors, also it is a modified version of R-squared that has
231 been adjusted for the number of predictors in the model. They increase only if the new term
232 improves the model more than would be expected by chance. While predicted R-squared
233 indicates how good a regression model predicts response for new observation, it determines
234 when the model fits the original data but less capable of providing valid predictions for new
235 observation.

236 3.2. Effects of interaction between parameters using response surface methodology plots.

237 The response surface plot (Fig 1) obtained from the model shows the effect of microwave
238 power and reaction time in the % of recovered lipids. For a fixed microwave time of 8 min
239 and extraction time 4 hrs, increasing the power from 180 to 600 W, the % of lipid-recovered
240 increases by 150% respectively. For a maximum pre-treatment conditions of 600 W and
241 8 min, the % of recovered lipids increased by 25% by increasing the reaction time from 3 to
242 4 h. The effect of pre-treatment time is shown in Fig 2, for a fixed reaction time of 3.5 hrs
243 and a microwave power of 600 W, an increase of 200% is achieved by increasing the pre-
244 treatment time from 2 to 8 min. If the microwave power is set at the lowest value of 180 W,
245 for the same variation in pre-treatment time, the increased obtained is 50%. This shows that
246 pre-treatment time has a higher effect on high microwave power. Combining high microwave
247 power and time, the highest % of recovered lipids are achieved.

248

Design-Expert® Software

Factor Coding: Actual

Recovered lipids (%)

● Design points above predicted value

○ Design points below predicted value

48.65

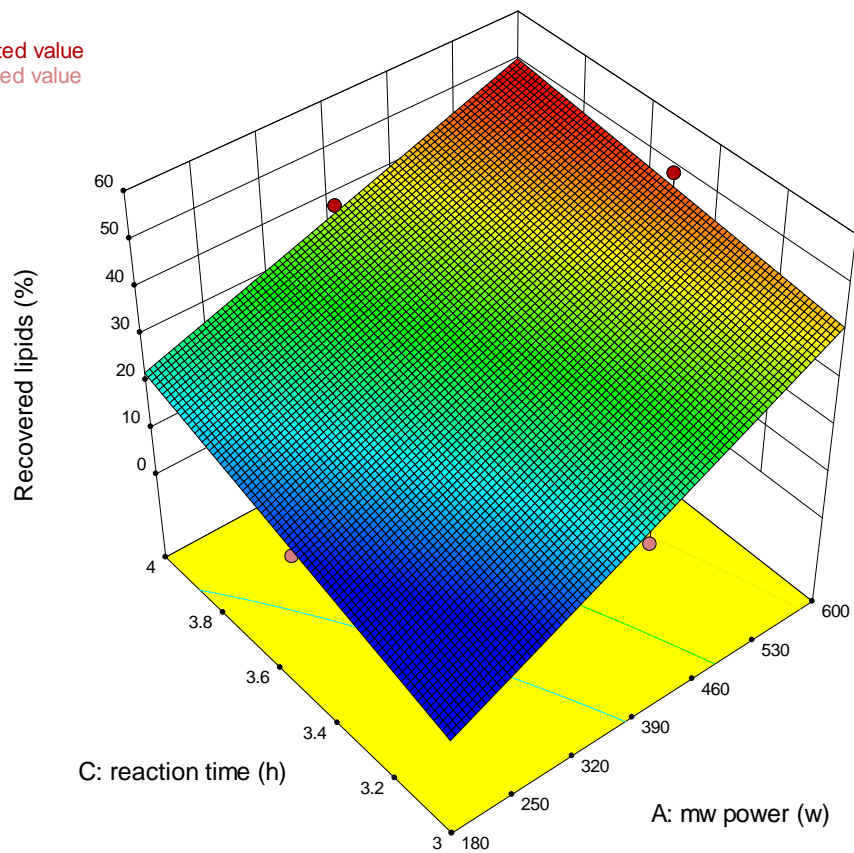
10.83

X1 = A: mw power

X2 = C: reaction time

Actual Factor

B: mw time = 8



249

250 Figure 1. 3D response surface plot for % of recovered lipid using microwave power and time.

251

Design-Expert® Software

Factor Coding: Actual

Recovered lipids (%)

● Design points above predicted value

○ Design points below predicted value

48.65

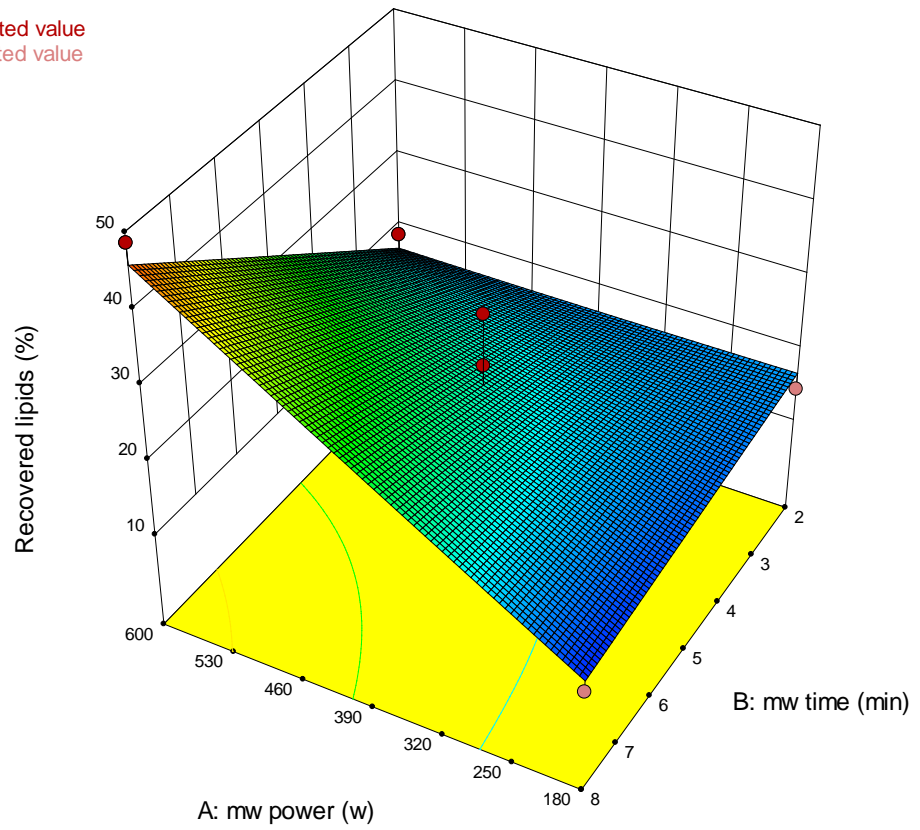
10.83

X1 = A: mw power

X2 = B: mw time

Actual Factor

C: reaction time = 3.5



252

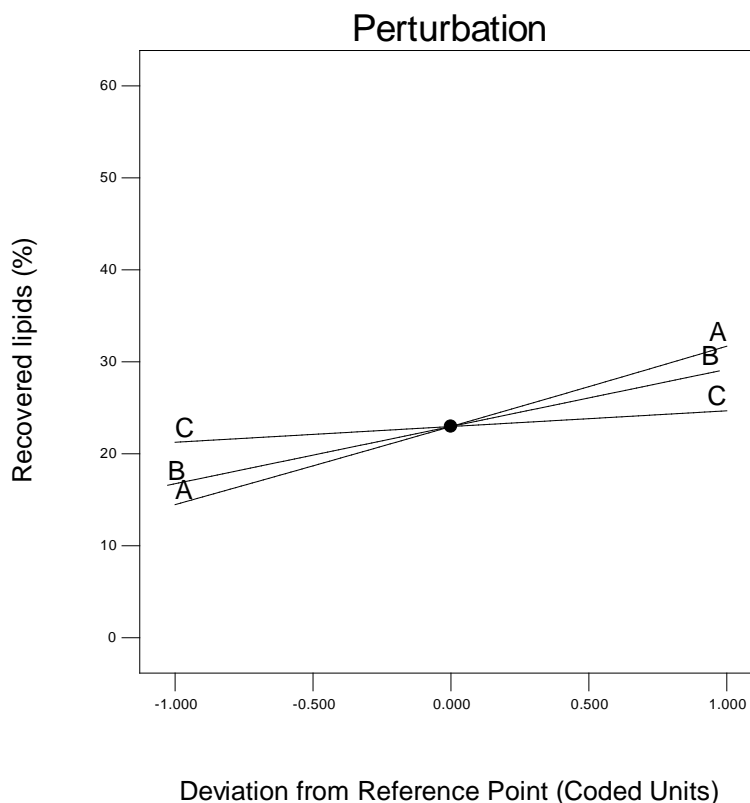
253 Figure 2. 3D response surface plot for % of lipid recovered using microwave power and
254 reaction time.

255 This proves that at a pre-treatment power of 600 W for 8 min the algae cells have been
256 disrupted to enhance the lipid extraction. This fact correlates with the study conducted by
257 [33,34,41,46,57–62] that using a high microwave power increases lipid efficiency. Though a
258 decrease in both microwave power and time reduces lipid efficiency, this may be because of
259 some of the algae cells remain undisrupted which inhibit the rate of lipid extraction. The
260 reaction time has a significant effect on the % of lipid recovered. Generally, extended pre-
261 treatment time provides an enhanced exposure of microalgae mixture to microwave effect,
262 which improves a better yield of lipid. Decreasing the exposure time seems not to provide
263 enough cell-disruption degree to achieve high % of recovered lipids. For this reason, one can
264 assume that a low pre-treatment time, the algae cell remains intact which may lead to a low
265 lipid yield (Table 2). The reaction time around 3.5 to 4 h and heating time of 8 min seems to

266 be satisfactory for complete extraction under microwave pre-treatment. Thus, the efficiency
267 of lipid extraction increased due to high cell-disruption after microwave pre-treatment. Also,
268 an import fact to note for future research is that more work should focus on the
269 effects/benefits of harvesting microalgae cells as summarized by the [63]. In addition, few
270 reviews studied the effect of microwave pre-treatment to enhance lipid extraction efficiency
271 for biofuel production. Cheng et al. [45] observed the effect of pre-treating *Nannochloropsis*
272 *Oceania sp.* using microwave irradiation at a frequency of 245 MHz and a power increase
273 from 635-1022 W for 15 mins pre-treatment. It was recorded a 38.46% of lipid yields. This
274 present shows that lipid production was achieved at a higher microwave energy and pre-
275 treatment time. This result agreed well with [14,20,64], who realized that increasing in
276 microwave power have a significant effect on the production of lipid using different
277 microalgae cells. A different study conducted by Cheng et al. [65] noticed the effect of
278 microwave pre-treatment at a frequency of 2.45 GHz, a reduction in power from 600-500 W
279 for 5-60 mins on (*Chlorella pyrenoidosa*). The author realized a 15% of lipid yield after
280 decreasing the microwave power to 500 W with an increase in pre-treatment time. A similar
281 result obtained by [14,47,66] agrees that increasing microwave pre-treatment time increases
282 lipid yield production. At the end of the microwave pre-treatments, the lipid yield was 10-
283 22%, 29-40% and 14-18% respectively. In this research work, a short microwave pre-
284 treatment time of 8 mins power of 600 W increase the lipid extraction rate to 49% using wet
285 microalgae cell (*Scenedesmus quadricauda sp.*) which is higher than all the above results as
286 discussed.

Design-Expert® Software
Factor Coding: Actual
Recovered lipids (%)

Actual Factors
A: mw power = 390
B: mw time = 5.08108
C: reaction time = 3.5



287

288

Figure 3. Perturbation plot for % of recovered lipid.

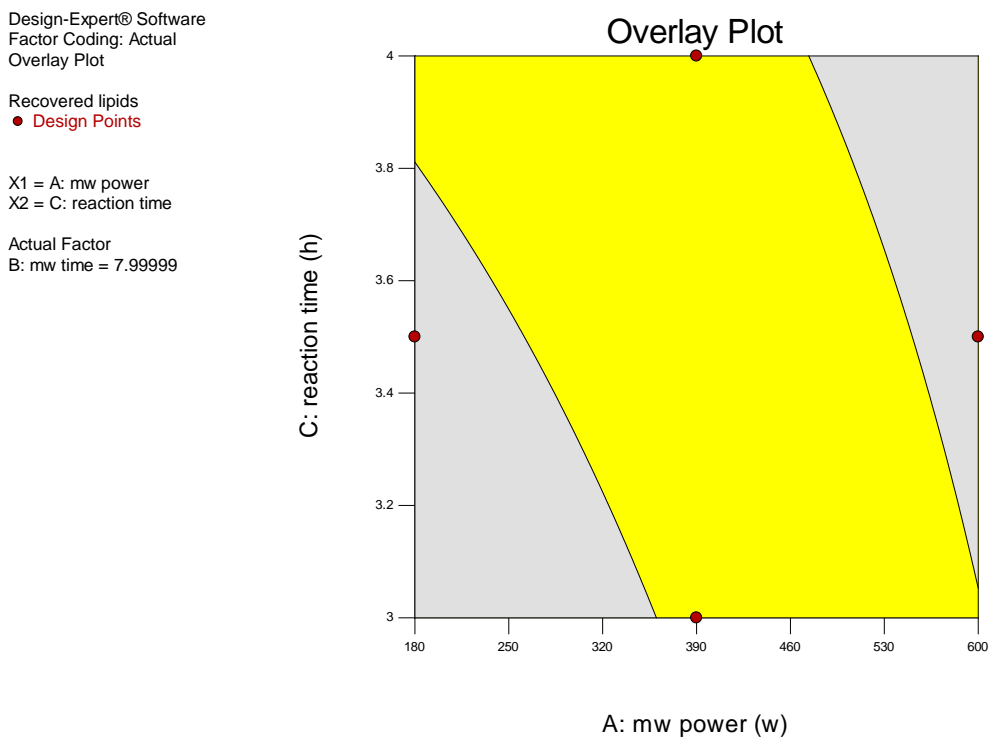
289

290 The perturbation plot in Fig. 3 clearly shows how % of recovered lipid is affected by the
291 input parameters microwave power and time and reaction time. Increasing the microwave
292 power and heating time, the % of lipid recovered will increase linearly. Reaction time has
293 little effect on lipid recovery as shown by the quasi-horizontal line C in Fig 3.

294 3.3. Optimization of lipid recovered

295 With respect to the model as represented in in Eq. 2, above, which systematically gives a
296 concise description of the effects of input parameters to the output response (% of lipid
297 recovered), optimization was performed using Design Expert software version 10. Hence,
298 optimization principle is based on the combination of final product maximization
299 (productivity). In this case, optimization simply means maximizing operational efficiency to
300 improve output efficiency. The aim of the optimization is to find the optimal combination of
301 microwave power and times that could maximize the % yield of lipid yield. The % of lipid

302 recovered was maximized with level 5 and microwave power was minimized with level 3. An
 303 optimum % of recovered lipid of 41.94 was obtained at microwave time of 8 min, microwave
 304 power of 473 W and reaction time of 4 h. The optimization plots (Fig 4 and 5) gives a concise
 305 description of the optimal process parameters by means of visual observation. The yellow
 306 region in the optimization plot signifies the values that meet the planned standards truly
 307 established by the curves agree with the standard of the optimization criteria. The plots
 308 clearly established that the optimum conditions for a maximized % of recovered lipids are
 309 above 350 W and 4 min of microwave pre-treatment.



310

311 Figure 4. Graphical optimization showing the effect of reaction time and microwave power.

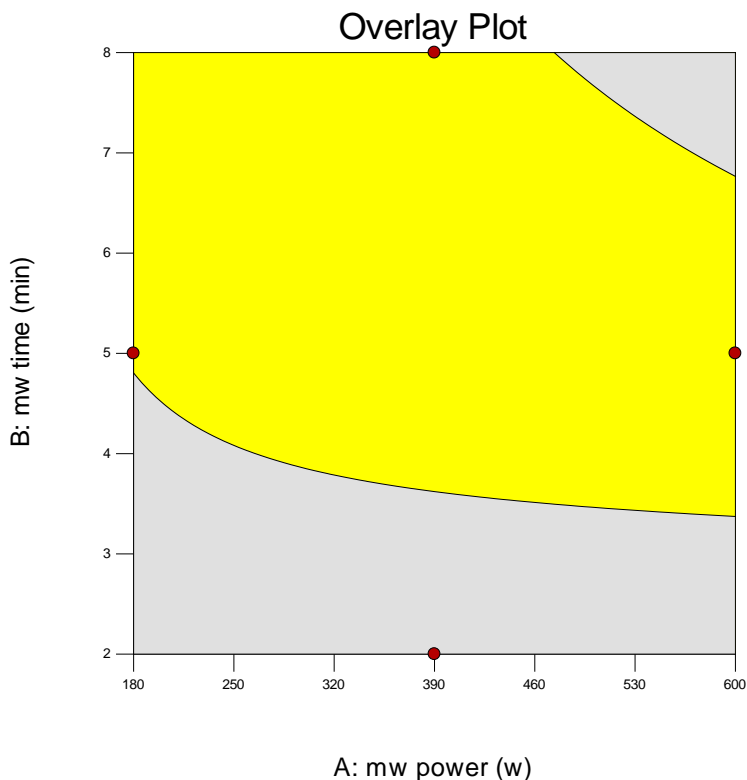
312

Design-Expert® Software
 Factor Coding: Actual
 Overlay Plot

Recovered lipids
 ● Design Points

X1 = A: mw power
 X2 = B: mw time

Actual Factor
 C: reaction time = 4



313

314 Figure 5. Graphical optimization showing the effect of microwave time and power.

315 To confirm the viability of this method, the optimum point of the RSM (section 3.3) was
 316 compared with a commercial sample of biodiesel from a petrol station (Biodiesel 80:20 mix).

Analytes	Commercial Sample (µg/ml)	Optimum sample (µg/ml)
methyl myristate - C14	0.00	154.73
methyl palmitate - C16	110.50	268.12
methyl stearate - C18	102.23	27.92
methyl linoleate - C18:2	171.00	8.38
methyl arachidate - C20	117.45	10.21
methyl eicosate - C20:1	515.20	21.22
methyl eicosadienoate - C20:2	359.45	14.46
methyl erucate - C22:1	13.11	4.91

317

318 The result clearly established the presence of individual FAME's that are required to
319 accurately identify the sample as viable for biofuel production. From the table 4, the
320 concentration of FAME sample was found to be 268.12µg/ml higher in methyl palmitate -
321 C16 as when compared with the commercial biodiesel (80:20 mix), methyl myristate - C14
322 was not present in the commercial biodiesel as it was present in FAME extract with a
323 concentration of 154.73µg/ml. It can be concluded that this method has a significant
324 contribution towards microalgae biofuel industry.

325 **4. Conclusion**

326 Pre-treating algae biomass with microwave for 600 W, from 2 to 8 min enhances the % of
327 recovered lipid to 49%. In addition, the reaction time from 3.5 to 4 hrs seems to be
328 satisfactory for complete extraction under microwave pre-treatment for lipid extraction
329 efficiency. An optimization study was accomplished to reduce the operating cost and pre-
330 treatment time to maximize the lipid production efficiency. The basic aim is to maximize the
331 % of lipid production while minimizing the microwave pre-treatment time. An optimum %
332 lipid yield of 41.94 was obtained at a microwave time 8 min, a reaction time of 4 hrs and
333 power 473 W. The highest lipid yield reported after pre-treatment as when compared with
334 results obtained from literature was reviewed in this research study. As cheng et al. [45]
335 reported a lipid extraction using a dry algae cell to achieve 38.46% lipid after pre-treatment,
336 while Menendez et al. [47] achieve 29-40% of lipids by increasing the time to 20 mins. Other
337 results as reported in the literature above has a low value of lipid yield even with a high pre-
338 treatment time as compared to this present study. This idea concludes the fact that using a wet
339 microalgae biomass shows a desirable value and lipid profile as a potential feedstock for
340 biodiesel production.

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