Global Scientific JOURNALS

GSJ: Volume 8, Issue 2, February 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

UTILIZATION OF RESPONSE SURFACE METHODOLOGY FOR THE OPTIMIZATION OF OIL EXTRACTION FROM ALGAE

LamidoSaniInuwa, AminuUbaAlhassan, Muazu Ibrahim.

Department of ChemicalEngineering, Kaduna Polytechnic, Nigeria.

KeyWords
Algae, Oil, Solvent, Extraction, Optimisation, Response Surface, Box-Behnken

ABSTRACT

In this study, algae oil extraction with n-hexane was investigated. The effects of extraction Time, Particle size, and Solvent volume on the yield were studied using Response Surface Methodology(RSM). Optimization of algae oil solvent extraction using Box-Behnken Design was used to generate 15 experimental runs in a three-factor-three level design to investigate the optimum conditions for the extraction process and the selected variables were Time (1, 2, 3 h), Particle size (0.154, 0.45, 0.90 mm) and Solvent volume (100, 125, 150 ml) and oil yield were evaluated as the response. In this result, a minimum oil yield of 6.5% and maximum of 20.1% was realized. The optimum yield (13.79%) was obtained using the polynomial model of quadratic form, at the Time of 2.7758 h, Particle size of 0.4375 mm, and Solvent volume of 156.56 ml, respectively. Analysis of Variance (ANOVA) showed R-square value of 0.99995 and adjusted R-square of 0.99964. Selected physiochemical properties [Saponification value, Acid value, Iodine value, Peroxide value, Density, pH, and Free fatty acid] of the extracted oil were determined according to American Society for Testing Materials (ASTM) Standards, to be [79.1 KOH/g, 1.79 mg, 96.2, 48.2, 0.8891 g/cm^3, 6.9, and 0.89 %] respectively.

1.0 INTRODUCTION

In recent years, microalgal species have gained prominence and attention because of their wide range of applicationfor biofuels production such as biodiesel and bioethanol. The major classes of algae are: Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae) and classification based on size as macroalgae or microalgae. Macroalgae (seaweed) are multicellular, large-size algae with leaves, roots and stem, while microalgae are microscopic single cells and may be prokaryotic, similar to cyanobacteria (Chloroxybacteria), or eukaryotic, similar to green algae (Chlorophyta) [1,2]. Microalgae are unicellular or simple multicellular structural organisms that are photosynthetic in nature [3]. They belong to Protista group and have the size measured in micrometers. Thallophytes are classes of microalgae without plant roots, stems, and leaves. They also lack sterile covering of cells around the reproductive cells [4].With ongoing researches, microalgae are becoming an economical and environmentally sustainable, renewable sources of biomass for the production of biofuels.

About 77.4% of global renewable energy supply is gotten from biomass serving as the largest renewable energy feedstock in the world [5]. An increase in global biofuel production from 4.8 billion gallons in 2000 to about 16 billion in 2007 was noticed, but this is still below the global transportation fuel demand[6]. Thus according to another source, global bioethanol production alone has vigorously increased from to about 39 billion within a few years and is expected to reach 100 billion soon [7]. Algae contain lipids/oils which could be used as raw material for biodiesel production [8, 9]. Microalgae can beuitilise in various applications ranging from biofuels, health supplements, pharmaceuticals, to cosmetics [10]. They also have applications in wastewater treatment and atmospheric CO_2 mitigation. Microalgae produce a wide range of bioproducts, including polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants [11]. They also have carbohydrates, which can be converted into bioethanol, biohydrogen and biogas. The rapid growth rate and ability to grow on wide range of waste water using atmospheric CO2 as the carbon source make algae most suitable candidate for biofuel production [12].

Besides hydro distillation, Soxhlet extraction is the most common method being used to extract and recover oil from natural products by the use of several solvents. The most widely used as solvent in this method is hexane (C_6H_{14}) [13,14]. It is the most preferable solvent for most extraction processes as it has good properties over other solvents such as: oil solubility, does not change chemical composition of product, has appropriate boiling temperature, stable under process conditions and is noncorrosive to metal [15]. Over the years, researchers have discovered that oil purity and final yield from depends on the variable process parameters of the extraction process. They also found that increased oil yield significantly affects the quality of the oil [16].

Response surface methodology is a collection of statistical and mathematical techniques useful to develop, improve and optimize processes and products. The technique is largely applied in industry, particularly in the situations where several input variables influence some process performances or quality characteristics. In the case of a chemical reaction the dependence between the response variable yield and the two inputs, process or independent variable time and temperature can be represented. It consists on experimental strategy for exploring the process space or independent variables, empirical statistical modeling to establish an adequate approximate relation between response and process variables. The method allows the determination of optimum set of experimental conditions which minimize or maximize the response and the changes in response surfaces produced by variation of independent variables [17, 18].This statistical technique has been applied in research for complex variable systems. It has advantage of limited number of experimental runs required to generate adequate information for statistically acceptable results. It is an effective tool for process optimization [18].

2.0 METHODOLOGY

Microalgae samples were collected from ABU Zaria dam, Kaduna, Nigeria. The wet algae were sieved to drain excess water out, weighed (w_1) and then placed in the oven at 50°C until constant weight (w_2) was obtained using the method [20].

2.1 Extraction Process

The extraction was done with a Soxhlet apparatus of 250ml capacity using n-hexane of analytical grade as the solvent. The extraction was done by using a prepared sample of 20g of dried algae, extraction time of 1 to 3 hour, and solvent extraction volume of 100 t0 150, and particle size of 0.154 to 0.900.]. Box Behnken Design (BBD) with three factors was chosen to design the experiment because it has the advantage of requiring fewer numbers of runs, and is rotatable. The coded and uncoded levels of the independent variables were shown in Table 1. For STATISTICA analysis, the relationship between the coded and actual (uncoded) variables can be represented by Equation (1).The experimental runs were carried out according to the experimental runs generated from STATISTICA Version 10.0. The solvent used was recovered at every experimental run throughout. This was repeated fifteen times and oil recovered was stored for further analysis.

$$X_i = \frac{Z_i - Z^*}{\Delta Z} \qquad 1$$

Where:
Xi = The coded i^{th} variable,
Zi = The actual i^{th} variable,
Z* = Center point values for the i^{th}variable,
ΔZ = Step change of Z variable, and Number of variable, i

2.2 Experimental Design

Response surface methodology was chosen to study the optimization of three selected input parameters: Time, Solvent volume and Particle size, and Yield as the output parameter using Response Surface Methodology (RSM). RSM is a mathematical tool used for designing experiments, developing polynomial models for predicting response, evaluating the significant effects of factors and optimizing the required function. [19]

Table 1: Coded Levels of Factors

Codes	Factors	Coded factor levels		
		(Low) -1	(Center) 0	(High) +1
A	Time (hr)	1	2	3
B	Particle size (mm)	0.154	0.450	0.900
C	Solvent Vol. (ml)	100	125	150

By this design, a total of 15 experimental runs were carried out. The center point was replicated three times to evaluate errors. Equation (2) is the general polynomial model of quadratic form that was used to fit the experimental data obtained during the extraction of oil.

$$\mathbf{Y(\%) = \beta_o + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_1x_1^2 + \beta_2x_2^2 + \beta_3x_3^2 + \beta_1x_1x_2 + \beta_2x_1x_3 + \beta_3x_2x_3} \qquad 2$$

Where:
X_1 = Are independent variables upon which:
Y = is dependent variables,
β_o = is the offset or constant term or center points, while
β_1 = is the ith linear coefficient
$\beta_2$and β_3 = are the quadratic and interaction coefficients respectively.

STATISTICA version 10.0 software was used for analysis of variance (ANOVA), and multiple regression analysis of the data obtained. The fit for regression model was checked by coefficient of determination R^2 and its associated probability P were used to determine the overall model significance. The respective effect of the variables and their interactions were tested using p-test, response surface plots and pareto charts. While the coefficients of the quadratic polynomial model were determined via multiple regressions and subsequent solution was carried out to evaluate the optimum operating variables.
Table 2 shows the Box-Behnken Design with 15 experimental runs at three (3) different level with their corresponding responses for the 15 – runs of the experimental Design.

Table 2: Response Surface Methodology Experimental Run and Results of Oil Yield

	1 Time (hr)	2 Particle Size (mm)	3 Sol. Vol. (ml)	4 Yield (%)	5 Var5
1	1.000000	0.154000	125.0000	11.6	
2	3.000000	0.154000	125.0000	19.5	
3	1.000000	0.900000	125.0000	9.05	
4	3.000000	0.900000	125.0000	20.1	
5	1.000000	0.450000	100.0000	6.5	
6	3.000000	0.450000	100.0000	15.05	
7	1.000000	0.450000	150.0000	10.5	
8	3.000000	0.450000	150.0000	20	
9	2.000000	0.154000	100.0000	7.5	
10	2.000000	0.900000	100.0000	10.2	
11	2.000000	0.154000	150.0000	13.6	
12	2.000000	0.900000	150.0000	10.2	
13	2.000000	0.450000	125.0000	16.05	
14	2.000000	0.450000	125.0000	16.15	
15	2.000000	0.450000	125.0000	16.22	
16					

Table 3: Summary of Effect Estimates

Effect Estimates; Var.:Yield (%); R-sqr=.99995; Adj:.99964 (algae BBD)
3 3-level factors, 1 Blocks, 15 Runs; MS Residual=.0073
DV: Yield (%)

Factor	Effect	Std.Err.	t(2)	p	-95.% Cnf.Limt	+95.% Cnf.Limt	Coeff.	Std.Err. Coeff.	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	12.78312	0.024839	514.6396	0.000004	12.67625	12.88999	12.78312	0.024839	12.67625	12.88999
(1)Time (hr)(L)	9.43338	0.063954	147.5024	0.000046	9.15821	9.70855	4.71669	0.031977	4.57910	4.85428
Time (hr)(Q)	-0.74774	0.044900	-16.6537	0.003586	-0.94093	-0.55456	-0.37387	0.022450	-0.47047	-0.27728
(2)Particle Size (mm)(L)	-0.76667	0.063683	-12.0388	0.006829	-1.04067	-0.49266	-0.38333	0.031842	-0.52034	-0.24633
Particle Size (mm)(Q)	1.78912	0.044900	39.8471	0.000629	1.59593	1.98231	0.89456	0.022450	0.79797	0.99115
(3)Sol. Vol. (ml)(L)	3.58025	0.064760	55.2852	0.000327	3.30161	3.85889	1.79013	0.032380	1.65081	1.92944
Sol. Vol. (ml)(Q)	3.93976	0.044900	87.7460	0.000130	3.74657	4.13294	1.96988	0.022450	1.87328	2.06647
1L by 2L	1.57500	0.085440	18.4340	0.002930	1.20738	1.94262	0.78750	0.042720	0.60369	0.97131
1L by 2Q	-0.06243	0.061055	-1.0226	0.414063	-0.32513	0.20027	-0.03122	0.030528	-0.16257	0.10013
1Q by 2L	0.31250	0.060415	5.1725	0.035403	0.05255	0.57245	0.15625	0.030208	0.02628	0.28622
1L by 3L	0.47500	0.085440	5.5595	0.030864	0.10738	0.84262	0.23750	0.042720	0.05369	0.42131
1Q by 3L	-0.39769	0.061055	-6.5135	0.022768	-0.66039	-0.13499	-0.19884	0.030528	-0.33019	-0.06749
2L by 3L	-3.05000	0.085440	-35.6975	0.000784	-3.41762	-2.68238	-1.52500	0.042720	-1.70881	-1.34119

Coefficient of determination $R^2 = 0.99995$

Table4: ANOVA for Polynomial Quadratic Model

ANOVA; Var.:Yield (%); R-sqr=.99995; Adj:.99964
3 3-level factors, 1 Blocks, 15 Runs; MS Residual=
DV: Yield (%)

Factor	SS	df	MS	F	p
(1)Time (hr) L+Q	160.8505	2	80.42523	11017.16	0.000091
(2)Particle Size (mm) L+Q	13.8115	2	6.90574	945.99	0.001056
(3)Sol. Vol. (ml) L+Q	78.5174	2	39.25870	5377.90	0.000186
1*2	2.7772	3	0.92573	126.81	0.007834
1*3	0.5353	2	0.26767	36.67	0.026549
2*3	9.3025	1	9.30250	1274.32	0.000784
Error	0.0146	2	0.00730		
Total SS	284.3332	14			

From Table 3, all the investigated parameters (time, particle size and solvent volume) shows significance in both linear and quadratic terms (with p-values less than 0.05) so is their interactions except that of the linear interaction between time and particle size. The significance of the linear, quadratic and interactive terms of the process variables were checked by F and p-tests. The result in Table 4 showed that Time linear and quadratic term are the most significant with highest F-value and least P-value of **11017.16** and **0.000091** respectively. The significance of the rest of the terms were checked in the same manner. Also, the ANOVA Table shows how well the experimental data fits the model equation. The high regression coefficient of determination (R^2) for the model was **0.99995** and adjusted R^2 is **0.99964** both indicating the good fitness of the model.

2.3 Response Surface Analysis and Pareto Chart

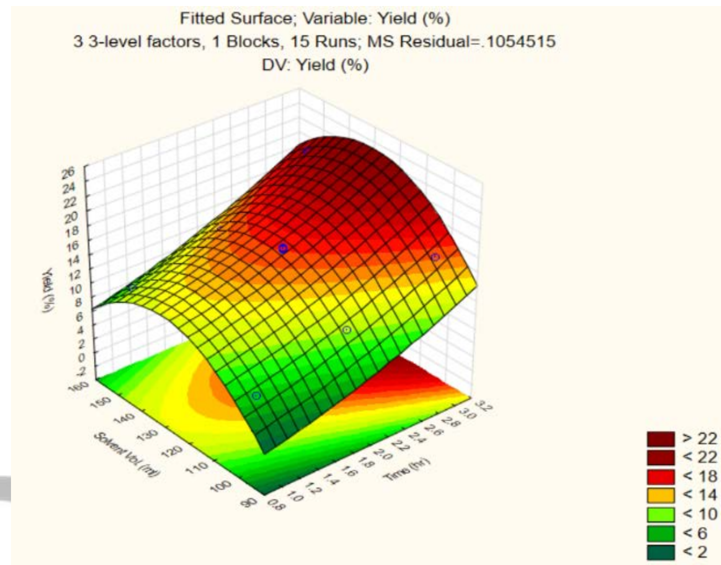
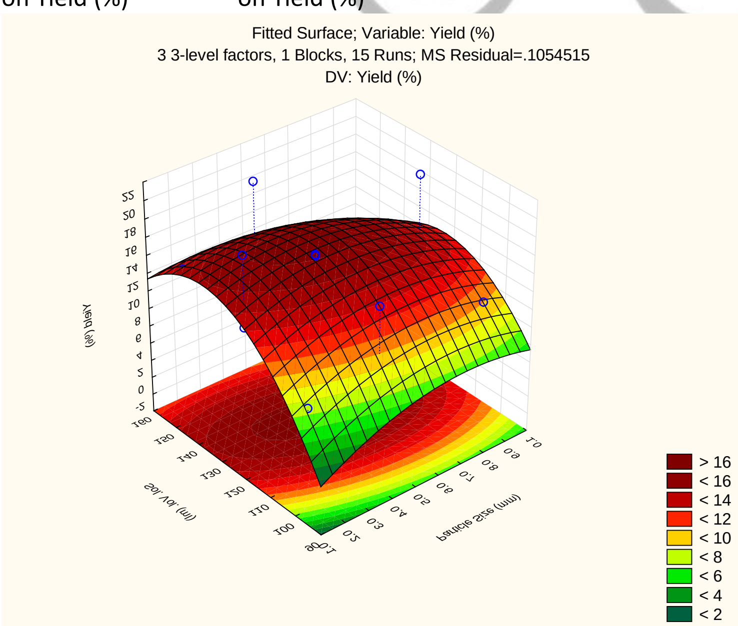


Figure 1A: Effect of Particle size (mm) and Time (hr) on Yield (%)

Figure 1B: Effect of Solvent volume (ml) and Time (hr) on Yield (%)



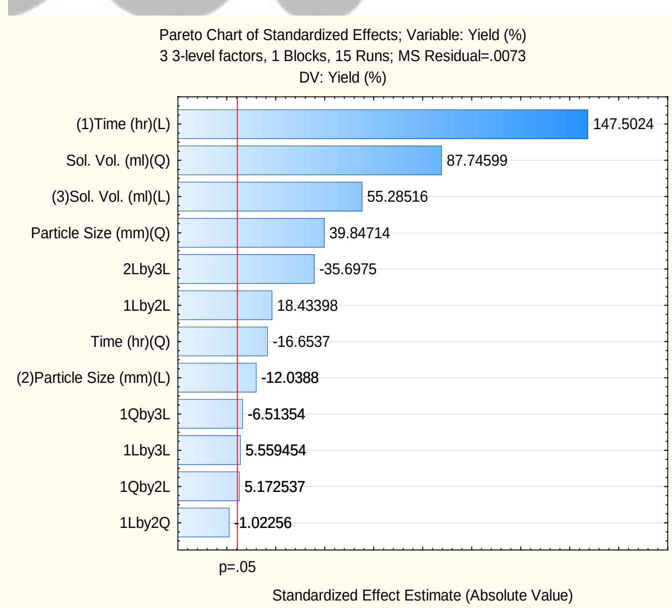


Figure 1C: Effect of Solvent volume (ml) versus Particle size (mm) on Yield (%)

Figure 2: Pareto Chart of the Standardized Effects of input variables

The effects of the process variables on the response variable can be further elaborated by visualization using response surface plots and a Pareto chart generated by the STATISTICA software as shown in Figure 1-2.Thus, in Figure 1A, increase in percentage oil yield was observe with decrease in particle size between the ranges of 0.4 to 0.7 mm. On the other hand, oil yield increases with increase in Time up to the highest time of 3 hrs as investigated in this research work. Figure 1B illustrates the effects of solvent volume and Time on oil yield, the response plot shows almost the same pattern just discussed, but with more quadratic effect of solvent volume than that of particle size indicated by a more curvature towards the peak of the surface. Figure 1C indicates the combined effects of particle size and solvent volume on the oil yield, thus the quadratic effect of both parameters are dominant with a pronounced curvature of the plot. This further explains that all the three plots are devoid of high significance of linear effects of the variables, but with overall quadratic effect being most significant. The various effects of the input variables on the response are further elaborated by Figure 2. It is obvious that the linear effect of Time at confidence level 95% is the most significant and more dominant. This is followed by the quadratic effect of Solvent volume and then that of solvent volume by linear effect. The only parameter with least significance is the combine effect of liner term of time and that of the quadratic term of particle size.

2.4. Polynomial Model Fitting

The results in Table 2 were used to run ANOVA and Multiple Regression Analysis in STATISTICA V10 software from which the optimum oil yield and the corresponding optimum variables can be predicted. STATISTICA analysis of the model was performed to evaluate the ANOVA and check the adequacy of the empirical model.From the regression analysis results the optimum input values of Time (X1), Particle size (X2), and Solvent volume (X3) in coded and uncoded terms are presented in Table 5. The uncoded variables were evaluated using Equation (1) which was used to convert the values from coded to uncoded form.

Table 5: Multiple Regression Summary of Optimum Input Parameters

Parameter	Coded	Un-coded
Time (hr)	0.7758	2.7758
Particle size (mm)	-0.0812	0.4375
Solvent volume (ml)	0.3156	156.56

The regression analysis results of model equation with yield as response (Y), while X_1 represent Time, X_2 represent Particle size, and X_3represent Solvent volume from Equation (3).

Y(%) = - 112.393 +5.699 Time + 27.545 particle size + 1.768 solvent volume – 14.369 particle size ^{2} ***– 0.006 solvent volume*** ^{2} ***+ 4.968 Time * particle size – 0.054 Time * solvent volume – 0.164 particle size * solvent volume*** (3)

Substituting for the optimum values, the optimum yield obtained is 13.79%.

Confirmatory experiment in triplicate were conducted to ascertain the optimum oil yield of **13.79 %**. The average of the tree runs at optimum conditions corresponding to solvent volume of **156.56 ml**, extraction time of **2.7758 hr**, and particle size of **0.4375 mm**givean oil yield of 14.02%. This confirms the optimum value to be correct.

2.5 Predicted value vs. Observed values of the Standardized effect for Yield Response.

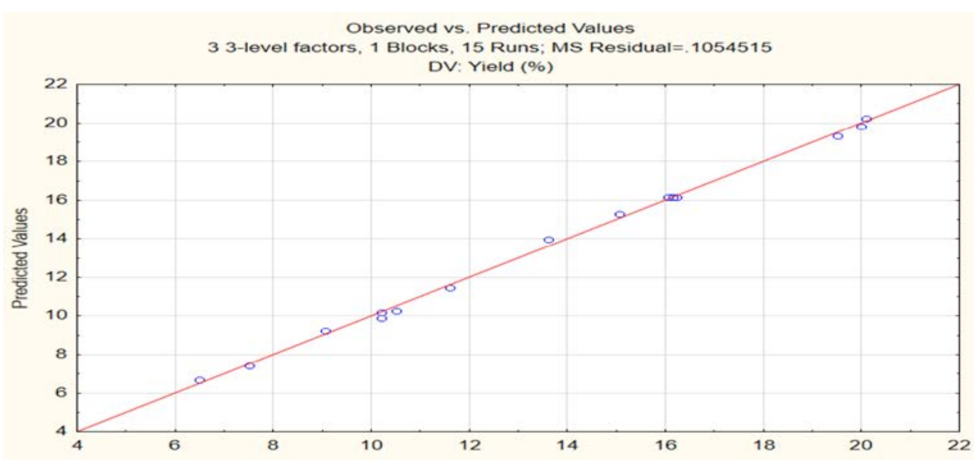


Figure 3: Predicted values vs. Observed values of the Standardized Effect for yield Response.

The predicted vs. observed values plot for oil Yield response as illustrated in Figure 3 shows the closeness of the experimental values denoted by the dotted points to the predicted model values represented by the red straight line. Thus, the predicted model with coefficient of determinant (R^2) of 0.99995 can be used to predict the percentage oil yield. This also shows that the values obtained follow the predicted values indicating that model assumptions were correct.

Table 6: Physiochemical properties and fatty acid composition of algae oil

Properties	Values
Saponification value	79.1KOH/g
Acid value	1.79mg
Iodine value	96.2
Peroxide value	48.2
Density (g/cm^3)	0.8891g/cm^3
pH	6.9
Free fatty acid	0.89%
Appearance	Greenish

Table 6 gives the physicochemical properties of the extracted algae oil. The oil is characterized by low free fatty acid (FFA) of 0.89%. This value then suggests the viability of algae oil as a prospect for biodiesel production.

Conclusion

Based on the findings, the following conclusions are made:

- Oil from Algae biomass was successfully extracted through solvent (n-hexane) extraction method. RSM was used to determine the optimal conditions of percentage oil yield. Box-Behnken design model predicted the optimal conditions for extraction of oil from algae were given as a solvent volume of 156.56 ml, time of 2.7758 hr, and particle size of 0.4375 mm, with the predicted oil yield of 13.79%.

- The experimental data and the predicted data are in agreement with a high value of R^2 = 0.99995 which shows that the polynomial model equation indicate the good fitness of the model.

- Physiochemical properties shows that algae oil has a very low Free Fatty Acid (FFA) of less than one (< 1%), which indicate a good property for biodiesel production.

Acknowledgment

The research group wishes to acknowledge the provision of research grant by Tertiary Education Trust Fund (TETFUND) under the Institution Based Research (IBR) Fund. We also want to acknowledge the Management of Kaduna Polytechnic for facilitating access to the grant.

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