Response Surface Optimization of Some Process Condition in Bioethanol Production Using Fresh Water Microalgae Biomass

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Abstract:

Background: *The increase in the price of fossil fuel, strict government regulations on exhaust emissions and future depletion of worldwide petroleum reserves trigger studies to look for alternative fuels. Bio-ethanol from different kinds of biomass is one way to reduce both consumption of crude oil and environmental pollution. Although extensive efforts have been put in place to evaluate the potential of microalgae as a biofuels feedstock during the past 4–5 decades but there is currently limited information on the state of Microalgae biomass conversion to ethanol, this study aimed at harnessing the potentials of microalgae as third generation biomass for bioethanol production.*

Materials and Methods: *The hydrolysis of Spirogyra biomass was carried out using dilute acid, amylase and combine acid and enzyme hydrolysis. Fermentation of the algal hydrolysate were done using baker's yeast (Sacchromyces cereviacea) isolated from sugarcane juice. Some important process conditions (pH, temperature and incubation time) were subjected to optimization using response surface methodology in order to assess their effect in relation to the bioethanol yield. The modeling and statistical analysis were performed using Design expert software, version 6.0.6.*

Results: *the maximal bioethanol yield of 12 % was obtained with pH of 5.2, at 37.5°C for 152hrs. So also three dimensional contour surface interactions of the parameters shows positive effect of incubation time with less effect of pH and temperature ranges used respectively. Subjecting the product to FT-IR revealed the presence of single carbon bond (2981cm^{-1}), methyl (1417cm^{-1}) and OH group (3331 cm^{-1}) and boiling of 78.73 ± 0.03^{o}C.*
Conclusion: *Base on the result of this study fresh water Spirogyra spp, could serve as low cost biomass for bioethanol production using combine acid and enzyme hydrolysis at optimized pH, temperature and incubation time.*

Key words: *Bioethanol, Spirogyra biomass, Baker's yeast (Sachromyces Cereviacea), fermentation, hydrolysis.*

i. Introduction

A development is 'sustainable' if it "meets the needs of the present without compromising the ability of the future generations to satisfy their own needs. The world population reached 7.3 billion in 2015, and projected to increase by 33% to reach 9.7 billion in 2050, and by 53% to cross 11.2 billion in 2100 (Thirumvengadathen and Thrimalai, 2017). This rapid growth of human population has led to mounting energy demands, which is projected to increase by 50% or more by 2030 (Eyasu *et al*., 2018) and

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the natural petroleum from fossils cannot meet-up the current consumption rate, which is already reported to be 105 times faster than nature can create (Shukla *et al*., 2016). Fossil fuels are non-renewable sources of energy and their global supplies are unlikely to last more than 120 years if are to be used at current rate of consumption (ICPC, 2007).

Micro Algal feed stocks are regarded as one of the most promising nonfood feed stocks for biofuels and that Algae based technologies could be a key tool for reducing greenhouse gas emission (Mamta and Rajiv, 2011). Zhenyi (2013) reported that microalgae are the dominant algae being researched for biodiesel production and can also be utilize for ethanol production by converting their storage material to fermentable sugars. The absolute absence or near absence of lignin makes the enzymatic hydrolysis of algal cellulose less costly and time saving in bioethanol production (Karunakaran *et al*., 2018). In addition, Micro algae have fast growing ability than land and require much less water than the traditional cereals, produce more biomass, can be grown in salt water or in sewage water with minimal impact on freshwater resources, easily biodegradable and relatively harmless to the environment if spilled (Shukla *et al.,* 2016). Generally, microalgae (red, brown, and green) are obtained from natural and cultivated resources (Nguyen *et al.,* 2012). The harvested microalgae are mainly used for production of different hydrocolloids, e.g., agar and alginate and small amount of these materials are also used for production of food (Yazdani *et al*., 2014).

Traditionally, the yeast *Saccharomyces cerevisiae* has been used all over the world as the major ethanol fermenting microorganism. The larger size, thicker cell wall, better growth at low pH, less stringent nutritional requirement and greater resistance to contamination give yeast advantages over bacteria for commercial fermentation (Tiwari, 2015). Several microbes, including *Clostridium* sp., have been regarded as ethanologenic microbes, but the yeast *Saccharomyces cerevisiae* and facultative bacterium *Zymomona smobilis* are better candidates for industrial alcohol production.

In general, the steps for bioethanol production from biomass include pretreatment, enzymatic hydrolysis, fermentation, and distillation (Sulfahri *et al*., 2016). Almost all kinds of macroalgae can be converted to bioethanol by degrading their polysaccharides into corresponding monosaccharide, followed by fermentation with suitable microorganisms (Jin *et al*., 2014). However, the development of microalgae conversion technology is still at an early stage, and the researches were conducted mainly on lab-scale (Jinyun *et al*, 2015).

The response surface methodology (RSM) is extensively used in bioethanol production as this model predicts experimental modifications like changes in operational conditions, various processing steps, which ultimately help in designing an experimental setup with minimum requirements and maximum yields (Demirbas, *eta al.,* 2011). RSM comprises of a group of mathematical and statistical procedure that can be used to study the optimization of culture conditions and it has already been successfully applied for optimization of media and culture conditions in many fermentation processes for production of ethanol, enzymes and amino acids (Dash *et al.* 2017).

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The objective of this study is to assess the potentials of micro-algae biomass for bioethanol production using *Saccharomyces cerevisiae and* to optimize pH, temperature and incubation time for bioethanol production by response surface optimization (RSM).

ii. MATERIALS AND METHOD

Collection of Water sample

Water sample was collected from Ajiwa Dam Katsina state. Ajiwa Dam is located at Batagarawa local government area of Katsina state and lies between latitude 12°30-13° 00 North longitude 7 ° 30-80 00 East in the sudan savannah ecological zone of Nigeria. The elevation of the site is about 518m above sea level. The Dam has catchment area of 1678km with 12 metres height and spill ways of 60 meters, lift pump of 1040km/hr capacity. The water sample was collected using a 100ml brown bottle container as described by Indabawa (2014). The samples were transported in a clean transparent robber container with ice to Department of Plant Biology, Bayero University Kano for microscopic screening of microalgae.

Isolation and Identification of microalgae

The micro algae were isolated using pipetting method (Sulfahri, 2016). Individual microalgal cell was microscopically identified using microalgae identification guide developed by (Pelmer, 1980).

Microalgae Culture

Individual cell of *Spirogyra spp.* being the most dominant specie was picked using capillary tube and inoculated in to the medium (BG-11). The Culture was allowed to grow and bloom in photo-bioreactor for three weeks in the Department of Biochemistry Bayero University Kano before harvesting for bioethanol Production.

Post-harvest Processing of the Biomass

The cultured algae were harvested and subjected to sun drying to remove moisture content for 72hrs. The dried biomass was then grounded and sieved with 1mm pore size. Fine powder of the spirogyra biomass was used for all fermentation and optimization experiments.

Isolation and preparation of yeasts inoculum

Exactly 3kg of sugarcane stalk was collected from Yan rake market in Kano state Nigeria. The stalk was squeeze using clean mortar and the resulting juice were collected in clean petri dishes. After 2hrs of exposure to air 1ml of sugarcane juice was taken

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aseptically into test tubes. The samples were then serially diluted 10-fold in sterilized distilled water. One ml of the serially diluted sediment was inoculated by streaking on plates of standard yeast extract patato dextrose agar media (YPD) (supplemented with chloramphenicol (0.05 mg/l) (Nwachukwu, 2001) and incubated at 28°C for 24 hours (Offosu appiah 2013). Cell suspension (10ml) of *Sacchromyces cereviacea* prepared from 2 days old slant culture was inoculated in to 100ml of medium and incubated at 30ºC for 48hrs on a rotary shaker. The *S. cereviacea*cells were then collected by centrifugation and inoculum concentration of 0.3 % (dry weight/volume) was utilized for the fermentation.

Hydrolysis of microalgae biomass

The biomass was subjected to combine dilute acid and enzymatic hydrolysis. The biomass was first hydrolyzed with 5% 2N HCl, at 121°C for 45 min and then neutralized to pH 4.5 with citrate buffer. The solution was then incubated with 3% amylase enzyme preparations and kept in a water bath for 12 h. Then centrifuged at 3000 rpm for 35 min and filtered to obtain clear supernatant for fermentation.

The three hydrolysates were analyzed for total reducing sugars before fermentation with *Sacchromyces cereviacea*.

Optimization of pH, temperature and incubation time for bioethanol production from microalgae *(Spirogyra spp)*

The statistical model based optimization was used to study the effect of pH, temperature and incubation time on ethanol yield using Central Composite Design (CCD). pH, (A), temperature (B) and incubation time (C) were taken as independent variables and ethanol yield was chosen as the dependent variables (Table 2). The resulted twenty runs experiment from the software CCD-based were carried out with different combinations of variables (Table 3.4). The modeling and statistical analysis were performed using Design expert software, version 6.0.6. All fermentation experiments were carried out in 250 ml Erlenmeyer flasks with working volume of 100 ml and agitation rate 200 rpm.). Multiple regression analysis of the observed responses in terms of the coded factors resulted in the quadratic model below (Equation (1)) ……

$$Y=+8.49-0.057*A-0.021*B+1.46*C-0.086*A^2-0.174*B^2-0.048*C^2-0.087*A*B-0.081A*C-0.064*B*C$$

A, B and C represents variables (coded values) of pH, temperature and harvesting time respectively (Table 1).

Table 1: Factors Of RSM Experimental Design

Factor	Indicator	Low level	High level
pH	A	4.5	6.0

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Temperature (˚C)	B	30	45
Inc. time (hrs)	C	24	120

Table 2: RSM Experimental Design for Bioethanol production from microalgae biomass.

Exp. Runs	pH	Temperature (^{o}C)	Incubation Time (hrs)
1	4.5	30	120
2	5.25	37.5	8.72
3	5.25	37.5	72
4	5.25	50	72
5	6	45	24
6	5.25	37.5	72
7	5.25	37.5	72
8	4.5	45	24
9	4.5	30	24
10	6	30	24
11	5.25	37.5	152
12	3.99	37.5	72
13	5.25	37.5	72
14	6.51	37.5	72
15	5.25	24.9	72
16	5.25	37.5	72
17	6	30	120
18	6	45	120
19	5.25	37.5	72
20	4.5	45	120

Validation of the second order polynomial model

The second order polynomial model obtained from RSM was validated by conducting a series of experiments randomly selected from the design in Table 3. The experiments were done by choosing random values of parameters within the optimized levels Table 3. Also, experiments were conducted at the optimized conditions generated by the software. The experimental output was then compared to the values predicted by the second order model obtained from CCD, to estimate the goodness of fit of the model.

Table: 3. Experimental set up for model validation of bioethanol production from microalgae biomass (*Spirogyra spp)*

Exp. Run	pH A	Temp. (°C) B	Inc. Time(hr.) C
1	5.3	37.5	152
2	5.25	37.5	72
3	4.5	30	120

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4	3.99	37.5	72
5	3.9	37.5	72
6	6.0	45	24

iii. Results and Discussion

Isolation of micro algae

Table 4. show the results of micro algae species isolated from water sample collected from Ajiwa Dam. *Spirogyra spp.*cells was found to appears 72 times from the water sample followed by *Chlorella vulgaris* with a total of 12 cells while least number of cells was recorded in *Snesdesmus spp*. *Spirogyra spp.*being the most dominant specie was subjected to culture for bioethanol production.

Table 4: Isolated micro algal specie from studied water sample

SN	Microalgal Specie	Number of cell
1	*Spirogyra spp*	72
3	*Chlorella vulgaris and*	12
3	*Snesdesmus spp*	8

Isolation of fermentative Yeast (*Saccharomyces cerevisiae)*

Three *Sacchromyces spp.* isolates were identified, On the basis of colony and cell morphology including the growth of isolates in liquid medium. All three isolates were aerobes with creamish colony and spherical shaped **(plate 1).** The isolated yeast from sugarcane juice appear as unicellular, large spherical individual cells with creamish appearance and all isolates fermented glucose, fructose and sucrose, but not lactose **Table 5.** These observations were similar to those reported by Ifosu-appiah (2014) and Elijah *et al*., (2010) who reported the isolation of *Saccharomyces cerevisiae* and other yeast from palm wine.

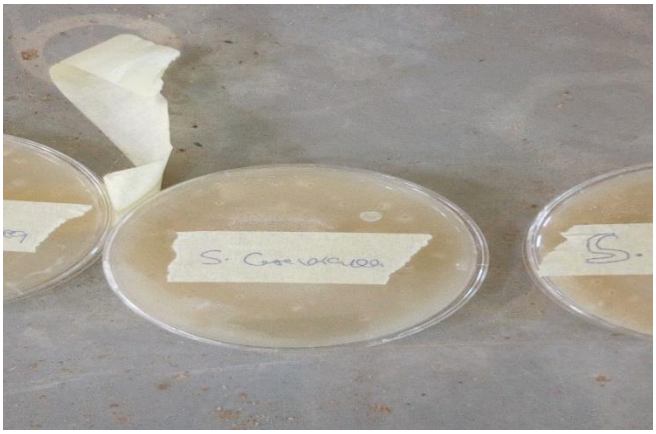

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Table 5: Fermentation of some simple sugars by yeast isolate

Isolate	Glucose	Lactose	Fructose
A	+ Gas	-	+ Gas
B	+ Gas	-	+ Gas
C	+ Gas	-	+ Gas

Optimization of bioethanol production condition

Physical factors such as pH, temperature and incubation time are considered among most important fermentation parameters due to their effect on growth of microorganism, fermentation efficiency and by-product formation. Therefore, maintenance of these parameters is therefore of great significance in fermentation for better yield. The responses obtained for each experimental run and the predicted responses were much closer to each other. It can be observed from **Table: 6** that increased in temperature and pH results to decrease in ethanol yield. However, the yield increased positively with increased in incubation time. Maximum ethanol yield of 12% was achieved at 152 h of incubation at 37.5 ^{o}C and pH 5.2. (Run 11, Table 6) while least bioethanol yield was recorded at 72hr incubation at 50 ^{o}C and pH 5.2 (Run 4, Table 6).

Table 6: Actual and predicted bioethanol yield at different condition of pH, temperature and incubation time

Exp. Runs	pH	Temperature (^{o}C)	Incubation Time (hrs)	Response Yield (%)	Predicted value (%)
1	4.5	30	120	8.7	9.43
2	5.25	37.5	8.72	5.0	4.65
3	5.25	37.5	72	9.2	8.49
4	5.25	50	72	3.1	3.19
5	6	45	24	5.5	4.96
6	5.25	37.5	72	9.2	8.49
7	5.25	37.5	72	9.2	8.49
8	4.5	45	24	5.0	4.65
9	4.5	30	24	5.1	3.63
10	6	30	24	3.2	3.10
11	5.25	37.5	152	12	10.80
12	3.99	37.5	72	5.8	7.01
13	5.25	37.5	72	7.6	8.50
14	6.51	37.5	72	6.6	5.10
15	5.25	24.9	72	4.3	3.92
16	5.25	37.5	72	8.2	8.49

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17	6	30	120	6.3	6.85
18	6	45	120	3.3	4.29
19	5.25	37.5	72	7.4	7.51
20	4.5	45	120	8.7	7.91

The decreased in bioethanol yield observed with increased in pH. This could be due to lesser enzyme activity of the fermenting organism *S. cereviacea* at pH greater than 5.5. This agrees with observation of Hwang *et al.*, (2004) who reported that the activities of bioethanol producers are slightly suppressed at pH below 4.5 and 6.0 above. Srivastava *et al*. (1997) showed that the optimum, initial pH of guava pulp medium was 5 and achieved maximum yield of 5.8 % of ethanol at that pH. Periyasamy *et al*. (2009) obtained the maximum bioethanol at pH 4.8 from sugar molasses using *S. cerevisiae.* Ado *et al.* (2009) studied bioconversion of cassava starch into ethanol and found maximum yield of ethanol at pH 5. Asli (2010) studied efficient parameters in batch fermentation of ethanol using *S. cerevisiae* in red grapes substrate, and achieved maximum concentration of bioethanol at pH 4.5.

Moreover, this study also revealed gradual increased in bioethanol concentration with increasing incubation time. This in line with the findings of Marakis and Marakis (1996) who obtained maximum ethanol concentration of 5.8 % at pH 4.5 from aqueous carob pod extract after 120 hr of incubation. Neelakandan and Usharani (2009) produced bioethanol from cashew apple juice using immobilized yeast and reported maximum bioethanol yield at 32 ^{0}C after140 hr of incubation.

The observed values of bioethanol yield were compared with the yield values as predicted by the second order models for validation. The result indicated that there was very good correlation between experimental and predicted values and in turn proves the validity of the models.

Table 7: Validation runs with observed and predicted bioethanol yield from *Spirogyra* biomass.

Exp. Run	**pH**	**Temp. (^{o}C)**	**Inc. Time(hr.)**	**Observed yield (%)**	**Predicted yield**
	A	**B**	**C**	**±0.05**	**(%)**
1	5.3	37.5	152	11.85	10.8
2	5.25	37.5	72	8.75	8.49
3	4.5	30	120	9.05	9.43
4	3.99	37.5	72	6.87	7.01
5	3.9	37.5	72	5.80	7.01
6	6.0	45	24	5.65	4.96

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Test for fitness of the experimental model

It can be deduced from **Table 8** the analysis of variance for a P-value < 0.05 indicates a significant effect on the response. Hence the Model F-value of 6.01 implies the model is significant. There is only 0.49% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case C, A^2, B^2 are significant model terms. The "Lack of Fit F-value" of 4.15 implies there is a 7.23% chance that could occur due to noise. Regression analysis produced the following second-order polynomial fit with a satisfactory coefficient of determination (R^2= 0.84402).

Ethanol. yield = -90.92112 +17.51818* pH +2.50683 * inc. temp. +0.21818 * Inc. time -1.53018 * pH^2 -0.031015 * inc. $temp.^2$ -2.06406E-005 * Inc. $Time^2$ -0.015556 * pH * Temp. -0.022569 * pH * Inc. time-1.77083E-003 * temp. * Inc. time……..Equation ii.

Where, A, B and C represents pH, temperature and incubation time respectively. AB, AC and BC are the interactions, and A^2, B^2 and C^2 are the quadratic terms.

Table: 8 Analysis of variance for the regression equation of the bioethanol yield from microalgae biomass *(Spirogyra spp).*

Source	Squares	DF	Square	Value	Prob> F	
Model	0.937977	9	0.10422	6.012338	0.0049	Significant
A	0.044032	1	0.044032	2.540142	0.1421	
B	0.006235	1	0.006235	0.359715	0.5620	
C	0.289172	1	0.289172	16.68208	0.0022	
A2	0.106767	1	0.106767	6.15927	0.0324	
B2	0.438632	1	0.438632	25.30431	0.0005	
C2	0.000326	1	0.000326	0.018802	0.8937	
AB	0.000613	1	0.000613	0.035335	0.8547	
AC	0.052813	1	0.052813	3.046705	0.1115	
BC	0.032513	1	0.032513	1.875617	0.2008	
Lack of Fit	0.13966	5	0.027932	4.146254	0.0723	not significant

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R-Squared	0.84402	Adj R^2	0.70364	Adeq Precision	8.17056

Interaction of pH, tempera[illegible]nd incubation time for optimization of ethanol yield from microalgal biomass

Response surface was gener[illegible] plotting the response (bioethanol yield) on the y-axis against any two independent variables on the x-axis, while keeping [illegible]ther independent variables at zero level. Therefore, three response surfaces were obtained by considering the possible combinations.

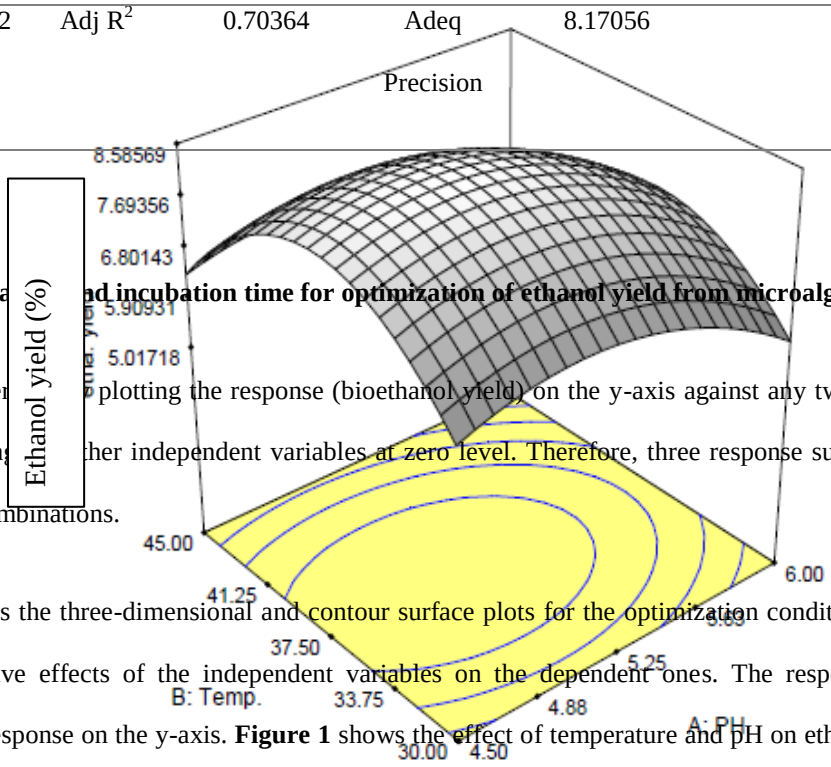

Figure 1, 2 and 3 represents the three-dimensional and contour surface plots for the optimization conditions. The plot illustrates the main and the interactive effects of the independent variables on the dependent ones. The response surface plots were generated by plotting the response on the y-axis. **Figure 1** shows the effect of temperature and pH on ethanol production keeping the other variable (incubation period) constant (120hr) level. Bioethanol yield was found to increase with the increased in temperature and pH. However, the ethanol yield was more pronounced at 41ºC and pH 5.6 but beyond these the yield declined and this decreased may be due to inhibition of fermenting organism activity at higher temperature. As shown in **Figure 2** and **3** harvesting time exert a positive effect on the bioethanol yield showing a linear increased significantly with time p value = 0.002 > 0.05.The linear graph of time shows, time is independent on pH, and temperature in relation bioethanol yield respectively.

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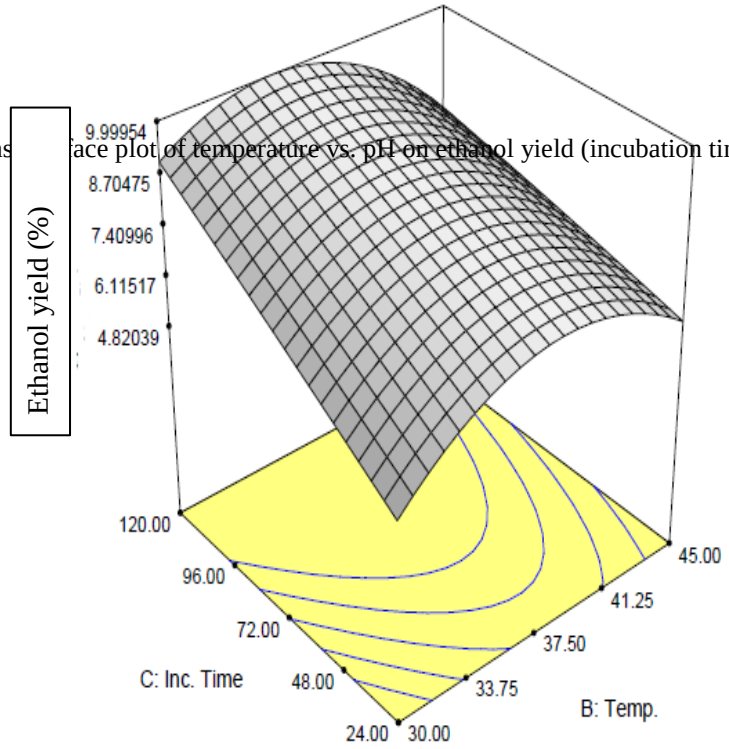

Figure 1: 3-dimensionalresponse surface plot of temperature vs. pH on ethanol yield (incubation time kept constant at 120 hr).

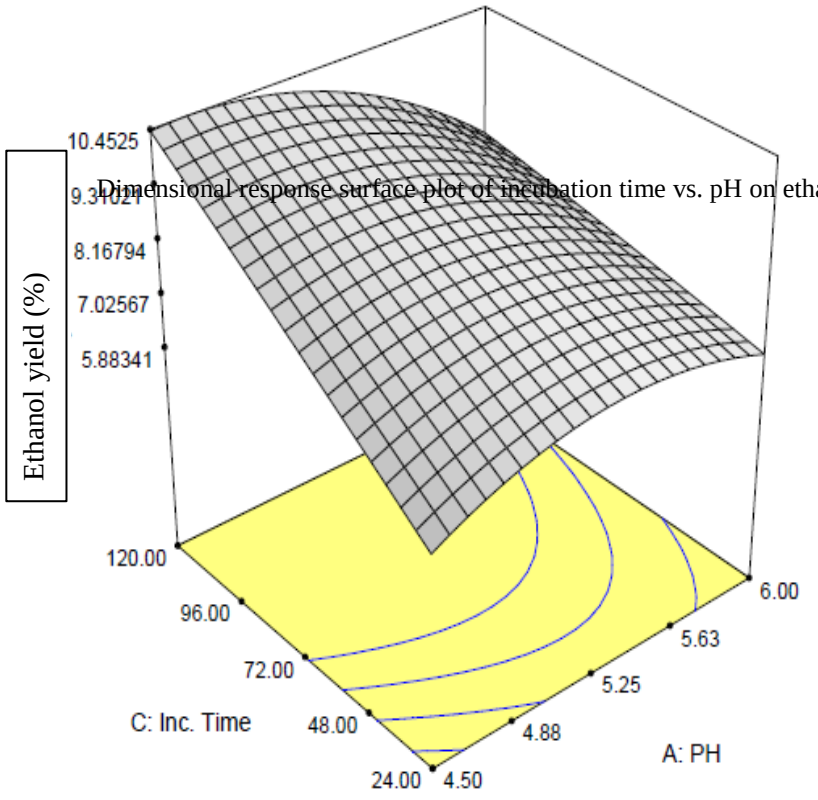

Figure 2: 3-Dimensional response surface plot of incubation time vs. pH on ethanol yield

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Figure 3: 3- Dimensional response surface plot of incubation time vs. temperature on ethanol yield

iv. Conclusion

Base on the result from this study Spirogyra biomass possess potential for bioethanol production. Both pH, temperature and incubation time were found to exert effect on bioethanol yield from microalgae spirogyra biomass. By optimizing these conditions incubation time were found to exert more positive effect on bioethanol yield compared to pH and temperature ranges used.

In conclusion by exploiting the potentials of low-cost substrate such as *spirogyra spp* as it is available abundantly in fresh water, can be easily grown on non-arable land and more importantly it has very low lignin content may open new road map for the bioethanol-production technology which is regarded to be eco-friendly and sustainable fuel.

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Conflict the interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that data presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

REFERENCES

[1]. Thiruvengadathan, Thirumalai Nambi, "Bioethanol Production Using Saccharomyces cerevisaeCultivated In Sugarcorn Juice" (2017). *Electronic Thesis and Dissertation Repository*. 4645

[2]. Eyasu S., Shubaa b., Demeke K. (2018). Microalgae to biofuels: 'Promising' alternative and renewable energy, review. *Reviews*, (81):743–755

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[3]. Shukhadar, M., Ravel, M., Arunkumar, K. and Perumal, K. (2016). Characterization, pretreatment and saccharification of spent seaweed biomass for bioethanol production using baker's yeast. *Biomass and Bioenergy*, 90:48e154.

[4]. IPCC. (2007). *Climate Change 2007: An Assessment of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland

[5]. MamtaA., and Rajiv K. S. (2011). Development of algae for the production of bioethanol, biomethane, biohydrogen and biodiesel, *Int J CurrSci*, 1: 14-23.

[6]. Zhenyi, D. (2013). *Thermochemical Conversion of Microalgae for Biofuel Production.* University of Minnesota.

[7]. Karunakaran, S., Senbagam, D., Gurusamy R., Anbarasu K. and Senthilkumar B., *(20180).* Evaluation of the Saccharification and Fermentation Process of two different Seaweeds for an ecofriendly Bioethanol Production. *J. Viet. Env.* 1(3):25-29 25.

[8]. Nguyen, Minh, T.H., Vu, and Hanh, V. (2012). Bioethanol production from marine algae biomass: prospect and troubles,*J. Viet. Env.*, 3(1):25-29.

[9]. Yazdani, Zamani P., Karimi K. and Taherzadeh M.J., (2014). Characterization of *Nizimuddinia zanardini*macroalgae biomass composition and its potential for biofuel production. *Bioresource Technology*. S0960-8524(14)01573-9.

[10]. Tiwari, K. L., Jadhav, S. K., Tiwari, S. (2015). Bioethanol production from rice bran with optimization.

[11]. Sulfahri, B., Mohamad A., Sutiman B., Sumitro, D. and MurniS., (2016). Bioethanol production from algae *Spirogyra hyalina* using *Zymomonasmobilis.* BIOFUELS 3(2):55-59.

[12]. Junying, C., Jing, B., Hongliang, L., Chun, C., and Shuqi, F. (2015).Prospects for Bioethanol Production from Macroalgae, *Tr Ren Energy*, 1(3):185-197.

[13]. Idris, M. N. and Owofu, M. O. (2016). Experimental Studies on the Production of Biofuel (Bioethanol) from *Spirogyra* Biomass. International Journal of Engineering and Modern Technology ISSN 2504-8856 2: Pp1.

[14]. Demirbas, A, Fatih., Demirbas, M. (2011). Importance of algae oil as a source of biodiesel. *Energy Conversion and Management;* 52(1):163-170.

[15]. Indabawa, I. I. (2014). Bioinstrumentation lecture note for post graduate students, Bayero university, Kano (Unpublished).

[16]. Palmer, (1989). Microalgae identification guide. ISBN 071940052X. Castle House Pub. Ltd.

[17]. Nwachukwu I., (2001). Studies on the effects of *Ceiba pentandra, Lorantus bengwelensis, citrate on species of dermatophytes.M.sc Thesis, University of Jos*, Nigeria.

[18]. Hwang S.S., Lee S.J., Kim H.K., (2008). Biodegradation and saccharification of wood chips of *Pinusstrobus*and *Liriodendron tulipifera*by white rot fungi. *J. MicrobiolBiotechnol*, 18: 1819-1825.

[19]. Periyasamy, S., Venkatachalam, S., Ramasamy, S. and Srinivasan, V. (2009). Production of bioethanol from sugar molasses using *Saccharomyces cerevesiae*. *Mod ApplSci.,* 3(8):32–37.

[20]. Ado, S. A., Olukotun, G. B., Ameh, J. B. and Yabaya, A. (2009). Bioconversion of cassava starch to ethanol in a simultaneous saccharification and fermentation process by co-cultures of *A. niger* and *S.cerevisiae. J. Sci World*, 4(1):19–22

[21]. Asli, M. S. (2010) A study on some efficient parameters in batch fermentation of ethanol using S. cerevisiae SC1 extracted from fermented pomace. *Afr J Biotech.*, 9(20):2906–291.

[22]. Marakis S.G, Marakis GS (1996) Fructose syrup and ethanol from deseeded carob pod. *J Food Sci. Technol.*, 33:108–111

[23]. Neelakandan T, Usharani G (2009) Optimization and production of bioethanol from cashew apple juice using immobilized yeast cells by S. cerevisiae. *Am Eurasian J Sci Res.*, 4(2):85–88

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