Chemical Science International Journal

30(5): 32-43, 2021; Article no.CSIJ.70325
ISSN: 2456-706X
(Past name: American Chemical Science Journal, Past ISSN: 2249-0205)

The Production and Optimization of Bioethanol from Oil Palm Fronds: A Source of Renewable Energy

I. Eiroboyi[1*] and Y. Yerima[1]

[1]Department of Chemical/ Petroleum Engineering, Igbinedion University, Okada, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CSJI/2021/v30i530232
Editor(s):
(1) Thomas P. West, Texas A&M University-Commerce, USA.
Reviewers:
(1) Kartikeyan Patma Nesan, Universiti Teknologi Petronas, Malaysia.
(2) Carlos Alfonso Sagaste Bernal, Universidad Autónoma de Baja California, México.
Complete Peer review History: http://www.sdiarticle4.com/review-history/70325

Original Research Article

Received 19 April 2021
Accepted 29 June 2021
Published 29 June 2021

ABSTRACT

Environmental issues and the desire to be less dependent on fossil fuel have intensified research efforts towards the production of biofuels since they are a safe and clean alternatives to fossil fuels. However, the cost of carbohydrate raw materials has become a limiting factor for large-scale production, hence the need to source for low cost feedstock.

This study analyzed the processes and optimization involved in the production of bioethanol from oil palm fronds from Okada, Edo State, Nigeria, as an alternative source of energy. In this study, solid-state fermentation was carried out for the production of fermentable sugars from oil palm fronds inoculated with local isolate *Aspergillus niger*, the results from this analysis show that the pretreatment of oil palm frond substrate using *Aspergillus niger* was effective, The process was effectively optimized within the confines of the following parameters; temperature X_3 (36-42^{o}C), pH, X_2 (5.1-5.7), inoculum, X_4(4-22) and fermentation time X_1 (0-36hr), an optimum Ethanol yield Y, of 110% was obtained.

Keywords: Oil palm frond; bioethanol; fermentation; optimization; pretreatment; lignocellulosic.

**Corresponding author: E-mail: ebitoh@yahoo.com;*

NOMENCLATURE

OPF : Oil Palm Frond;
RSM : Response Surface Methodology;
SEM : Scanning Electron Microscope;
SG : Specific Gravity.

1. INTRODUCTION

Biofuels are liquid or gaseous extracts from biomass that are high in sugar (such as corn and cassava) or oils (such as soybeans, coconut, sunflower, and palms). The two most commonly used biofuels are ethanol and biodiesel. Bioethanol is mainly produced by the sugar fermentation process, although it can also be produced by the chemical process of reacting ethylene with steam.

Today, converting renewable non-fossil carbon, such as organic waste and biomass consisting of all growing organic matter (plants, grasses, fruit wastes, and algae) to fuel, would assure a continual energy supply [1]. The economics of bioethanol production by fermentation is significantly influenced by the cost of the raw materials, which accounts for more than half of the production costs [2], to achieve a lower production cost, the supply of cheap raw material is thus a necessity.

Many agricultural raw materials rich in fermentable carbohydrates were tested worldwide for bioconversion from sugar to bioethanol, but the cost of carbohydrate raw materials has become a limiting factor for large-scale production by industries employing fermentation processes. Since the price of feedstock contributes more than 55% to the production cost, inexpensive feedstocks such as lignocellulosic biomass and agro-food wastes, are being considered to make bioethanol competitive in the open market [3]. However, these wastes often end up in dumps or drainage systems which impact negatively both surface water and general human health conditions. Thus, it is necessary to convert these wastes to useful end products rather than leaving them as a nuisance in the environment [4]. The production of bioethanol from a comparatively cheaper source of raw materials using efficient fermentative microorganisms is the only possible way to meet the great demand for bioethanol in the present situation of energy crisis [5,6]. A variety of ligno-cellulosic materials like agricultural residues, municipal, and industrial wastes are being investigated for bioethanol production [7,8,9].

Oil Palm frond (OPF) is a solid agro-waste that is abundantly available on oil palm plantations [10]. Currently, the disposal of the oil palm fronds is by decaying in the natural environment or by burning on site with only a small amount being composited, these practices are creating environmental problems [11], also alternative ways to utilize and/or to dispose of oil palm fronds are needed. Hence, the utilization of oil palm biomass for the production of environmentally friendly biofuels has become an attractive approach instead of creating environmental pollution problems. Oil palm frond (OPF) consists primarily of lignocellulosic components, i.e, cellulose, hemicellulose components, and lignin [12]. Only a few studies [13,14] have focused on utilizing the lignocellulosic components of oil palm fronds.

1.1 Cellulose/ Lignin Content

The proportions of cellulose and lignin in biomass are important only in biochemical conversion processes. The biodegradability of cellulose is greater than that of lignin [15]. For plants with a higher proportion of lignin, it is a determining factor when selecting biomass plant species for biochemical processing. Table 1 shows the proportions of cellulose/ hemicellulose/ lignin for some selected biomass.

Cellulose/ Lignin Content of Selected Biomass

Table 1. Comparative composition of some lignocellulosic biomass materials

Biomass	Lignin (%)	Cellulose (%)	Hemicelluloses (%)
Softwood	27–30	35–40	25–30
Hardwood	20–25	45–50	20–25
Wheatstraw	15–20	33–40	20–25
Switch grass	5–20	30–50	10–40
Oil palm frond	15.4	44.0	30.4

Adopted from [15]

1.2 Properties of Oil Palm Frond Component

Oil palm frond consists of four major components, namely, petiole, stem, rachis, and leaflet as shown in Fig. 1.

The cellulosic materials and sugar are the main components of petiole. Therefore, its contribution to nutrient (nitrogen, phosphorus, and potassium) recycling is low. The petiole only contributes 34% to OPF total nutrient content, which comparatively has a low amount of nitrogen [16]. The main contributor of nitrogen is the leaflet (Table 2).

Oxygen content was determined by the difference between the content C, H, N, and S in percentage and the total of 100%. The sulphur content was below the detection limit of 2% of the method used. [16,17] showed that cellulose is the major component of rachis stem and petiole, while the leaflet contains a higher amount of hemicellulose and lignin (Table 3). The concentration of pectin and protein of the petiole are almost the same to that of the stem, but much lower than that of the rachis. While the concentration of leaflet is above that of pectin and protein.

Oxygen content was determined by the difference between the content of C, H, N, and S in percentage and the total of 100%. The sulphur content was below the detection limit of 2% of the method used.

1.3 Response Surface Methodology

Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which the response of interest is influenced by several variables and the objective is to optimize this response [18].

This study analyzed and optimized the production of bioethanol from oil palm fronds from Okada, Edo State, Nigeria. It also examined the morphological structure using the Scanning Electron Microscope (SEM).

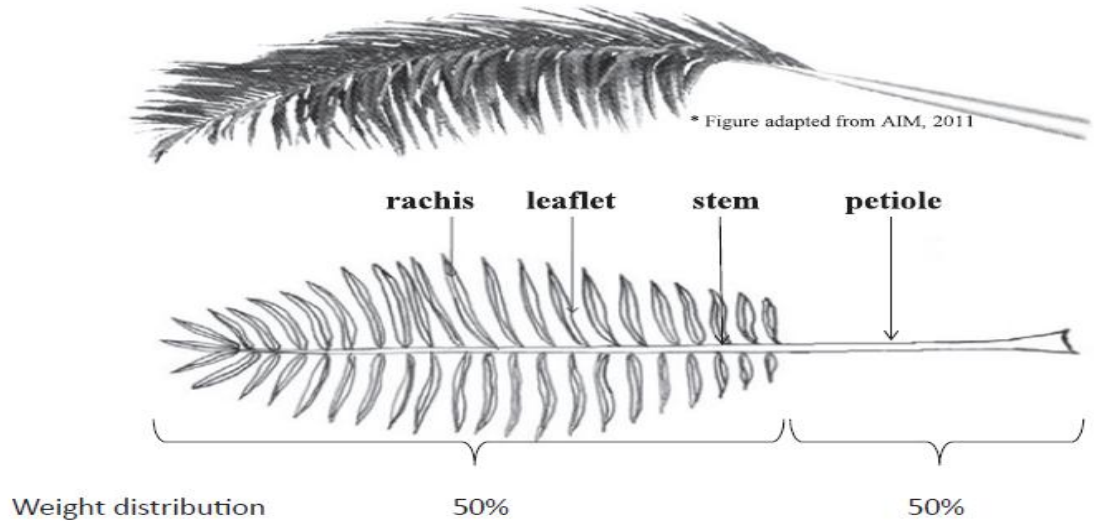

Fig. 1. Showing the various components of Oil Palm Frond (OPF)
Adopted From [16]

Table 2. CHNS analysis of OPF

Analysis	Leaflet	Rachis	Stem	Petiole	Juice
Hydrogen (%)	6.06	6.06	5.87	6.95	-
Oxygen (%)[1]	46.39	46.39	47.88	49.46	-
Carbon (%)	46.72	46.72	45.74	44.02	39.0
Nitrogen (%)	0.83	0.83	0.51	0.5	0.8
C/N ratio (%)	56.1	56.1	90.1	77.1	49.1
Sulphur (%)[2]	ND	ND	ND	ND	0.4

Data quoted from [17]

Table 3. Total composition of oil palm frond biomass

Lignocelluloses							
Sample	**Moisture**	**Lignin**	**Hemicelluloses**	**Cellulose**	**Crude protein**	**Starch**	**Pectin**
Leaflet	72.0±3.6	5.91±0.24	12.10±0.60	3.90±0.27	2.55±0.13	1.26±0.06	0.84±0.04
Rachis	60.0±2.1	1.79±0.11	13.85±0.55	19.57±0.97	1.76±0.09	0.94±0.06	0.24±0.01
Stem	75.0±5.3	2.53±0.12	7.42±0.51	11.41±0.45	0.80±0.05	1.55±0.07	1.55±0.00
Petiole	77.0±5.4	2.86±0.11	7.11±0.35	8.53±0.59	0.90±0.04	1.87±0.09	0.07±0.01
Juicel	-	-	-	-	NM[2]	-	NM[2]

Percentages									
Elemental analysis									
Sample	**Si**	**P**	**S**	**C1**	**K**	**Ca**	**Mn**	**Others**	**Total**
Leaflet	0.39±0.06	0.01±0.00	12.10±0.60	0.08±0.00	0.15±0.00	0.66±0.05	0.11±0.01	0.01±0.00	100
Rachis	0.26±0.02	0.02±0.01	0.04±0.00	0.12±0.04	0.43±0.07	0.87±0.01	0.10±0.00	0.01±0.00	100
Stem	0.08±0.02	0.01±0.00	0.02±0.00	0.08±0.00	0.49±0.03	0.55±0.00	ND	<0.00±0.00	100
Petiole	0.15±0.03	0.02±0.00	0.03±0.00	0.11±0.01	0.50±0.00	0.77±0.04	ND	0.04±0.00	100
Juice	-	0.02	0.40	2.30	2.30	2.90	2ppm[3]	-	-

Data quoted from [17]

2. Methodology

2.1 Sample Preparation

One kilogram of oil palm fronds was collected from a farm in Ovia, Northeast Okada, Edo State of Nigeria. The oil palm fronds obtained were dried at 30°C±2°C [room temperature] and the sample was chopped into small pieces before being grounded with a grinder machine and sieved to a particular size of 1 mm.

2.2 Inoculum Preparation

The organism, *Aspergillus niger* , was obtained from the Microbiology Department, University of Ilorin, Kwara State, Nigeria. The *Aspergillus niger* was re-cultured on potato Dextrose Agar (PDA) slants and incubated at 30°C for 5days until the organism sporulate. The culture was kept at 4°C until when needed.

2.3 Biological Pre-treatment Process Using Fungal Culture

The culture of *Aspergillus niger* earlier kept and was re-cultured on Potato Dextrose Agar (PDA) media in Petri-dishes. The conical flask containing the fine ground sample was diluted by adding sterile distilled water. After diluting, the sample was then autoclaved at 121°C for 15mins at 15 psi, and then the pH of the sample was adjusted to pH 5.6 which is the optimum pH for fungal growth. The sample in the conical flask was inoculated with 12 ml of fungi culture of *Aspergillus niger* of inoculum of 1×10^7 spores/ml, contents were mixed thoroughly and incubated at 30°C ± 2°C [room temperature]. The conical flasks were then wrapped with aluminum foil. The pretreatment process was carried out on the fresh sample (OPF) by mixing it in a crucible with 4% (W/V) of sodium hydroxide solution. The mixture was then heated in a muffle furnace and allowed to boil at a temperature of 101◦C and finally made to cool in a desiccator.

2.4 Scanning Electron Microscopy (SEM) Sample Preparation

Scanning electron microscopy (SEM) analysis was conducted to view and compare the internal structural changes before and after pretreatment. The pretreated biomass samples were subjected to morphological study. The residue remaining after enzymatic saccharification was also selected for microscopic observation. The sample from each pretreatment was filtered and washed using hot water prior to drying at 80°C for 24 h. The dried sample was subjected to SEM Analysis, the process adopted was in line with [13].

2.5 Fermentation

The crude fermentable sugar from the fermented materials was extracted by a simple contact method. 40 g of fermented OPF was dissolved in 1litre distilled water. The contents were mixed properly every 20 minutes for 2hours at 30°C±2°C [room temperature]. The suspension was filtered and centrifuged for 20 minutes at 4,000 rpm to obtain clear crude fermentable sugar. The crude fermentable sugar was concentrated using a rotary evaporator and used as crude fermentable sugar for the analysis. The concentrated fermentable sugar was used as a fermentation medium source for the production of bioethanol.

The pretreated samples in conical flasks were set at pH 4.5, which is the optimum pH for yeast growth and fermentation. 20g of yeast granules were added to the pretreated sample only and mixed properly. The conical flasks were covered completely to make it anaerobic and kept in the dark between 0-48 hrs for fermentation. This was carried out using the Soxhlet apparatus. The sample mixture was centrifuged to separate the solid debris from the solvent mixture. The mixture was then loaded into a distillation flask of the Soxhlet apparatus. The temperature is set to 78°C as the boiling point of ethanol.

2.6 Design of Experiment/Optimization

Optimization of process parameters in the pretreatment of palm fronds with *Aspergillus niger* was studied using CCD experiments. Fermentation period (X_1, g/l), pH (X_2), temperature (X_3, °C), and inoculum (X_4) were chosen as the independent variables and were shown in Table 2 Ethanol yield (Y, %) was used as the dependent output variable for 750 rpm.

$$x_i = \frac{x_i - x_e}{\Delta x_i} \qquad i - 1, 2.3, 4 \qquad \textbf{(1)}$$

The variables x_i were coded as x_i as per the equation (1) in which x_i is the dimensionless value of an independent variable, x_i the real value of the independent variable, x_c the real value of the independent variable at a central point and Δx_i is the step change of variable i. The true values of the variables are also given in Table 2. A 24 factorial Central Composite Experimental Design, with eight axial points and

six replications at the centre points leading to a total number of 31 experiments was employed for the optimization of parameters.

2.7 Distillation Process

This was carried out using the Soxhlet apparatus. The sample mixture was centrifuged to separate the solid debris from the solvent mixture. The mixture is then loaded into a distillation flask of the Soxhlet apparatus. The temperature is set to78°C as the boiling point of ethanol. Ethanol is volatile at this temperature and condenses in the inner vessel of the Soxhlet apparatus.

2.8 Estimation of Ethanol by Specific Gravity Method

In this process, a "Specific Gravity Bottle" was used to hold a known volume of liquid, i.e., water at a specified temperature. The bottle was weighed, filled with the ethanol whose specific gravity was to be found, and reweighed again [19].

$$Specific\ gravity\ of\ ethanol = \frac{\text{SG of bottle with ethanol} - \text{SG of bottle}}{SG\ of\ bottle\ with\ water - SG\ of\ bottle}$$

3. RESULTS AND DISCUSSION

3.1 Determination of Moisture Content of the Oil Palm Fronds before Treatment

Moisture content in the dry oil palm frond was calculated to be 8.98%, which is low, thus the low moisture content in the oil palm frond has made it suitable for use as a substrate in fermentation. The lignin content in the oil palm fronds is lower compared to other hardwood plants. The moisture content of the fermentation medium often determines the success of a SSF process.

3.2 Ash content of the OPF before Treatment

Ash content in the dry OPF was calculated to be 7.9%, which is low, thus the low ash content in the OPF has made it suitable for use as substrate in fermentation.

3.3 Morphology of the Pretreated OPF

Fig. 2a-b shows the morphological (internal structure) of the oil palm frond at different magnifications using the SEM. Findings show that the pretreatment carried out in this study was able to remove lignin, which created pores that enhanced an easy release of sugars from the cellulose, this agrees with [13].

3.4 Temperature of Cellulose Substrate of Pre Treated OPF

A linear relationship between temperature and fermentation period is evident from the graph below (Fig. 3), it shows that an increase in fermentation period brings about an increase in temperature, and this is due to activity of the enzymatic reaction with the substrate, and due to the anaerobic conditions which it is subjected to. The temperature was constant at 34°C from 4hr to 30hrs and was also constant at 36°C from 36hr to 42hr, this shows that the metabolic activity of the enzyme was active. However, the operation temperature cannot be too low since the biochemical reaction rate usually decreases with decreasing temperature.

3.5 Reducing Sugar Yield

With an increased time of fermentation, the reducing sugar was measured at 2hrs interval, from Fig. 4, the result of the study revealed that reducing sugars released from pretreated oil palm decreased linearly, which explains the fact that the pretreatment process using *Aspergillus niger* has the advantage of not only solubilizing hemicelluloses but also converting solubilized hemicelluloses to fermentable sugars.

3.6 pH of Cellulose Substrate of Pretreated OPF

The graph below Fig. 5 shows a directly proportional relationship between pH and fermentation period, this shows that the enzyme *Aspergillus niger* works maximally in an acidic medium, which explains that fermentable sugar production was favoured by acidic pH.

3.7 Estimation of Ethanol by Specific Gravity Method

From the graph below in Fig. 6, it was observed that the specific gravity of ethanol increased from 2hrs of ethanol production, it was linearly proportional to the fermentation period. This could be caused by the high rate of metabolic activity of the enzyme (the exponential phase is preceded by the lag phase) at 12hrs of fermentation time and 88% bioethanol yield.

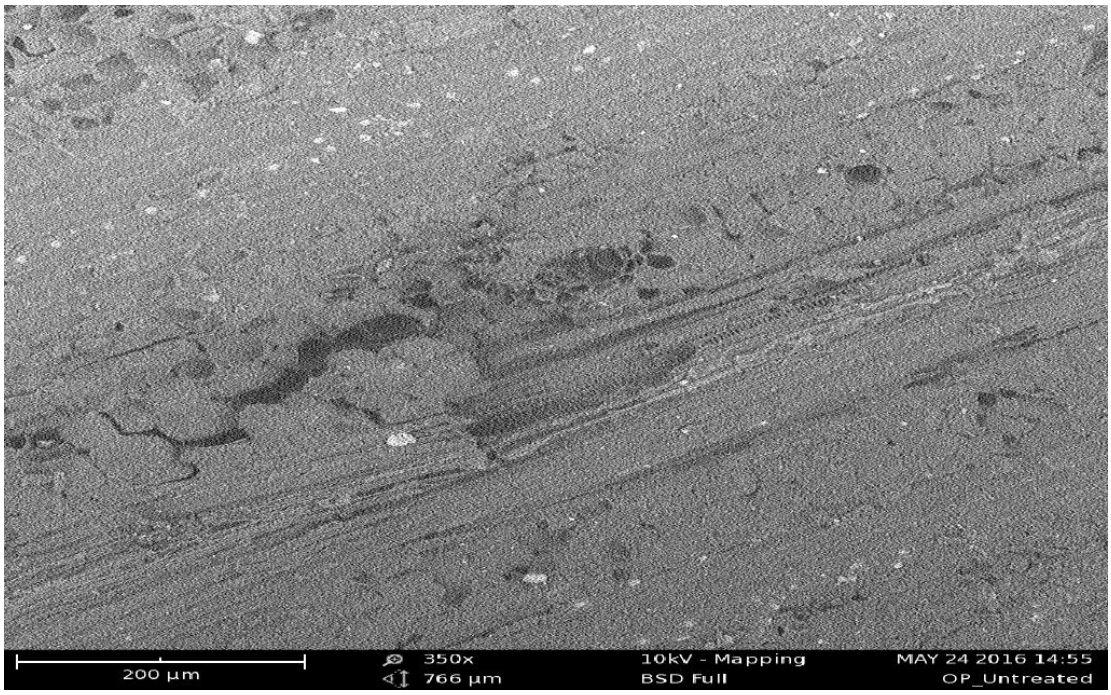

Fig. 2a. SEM of pretreated oil palm frond

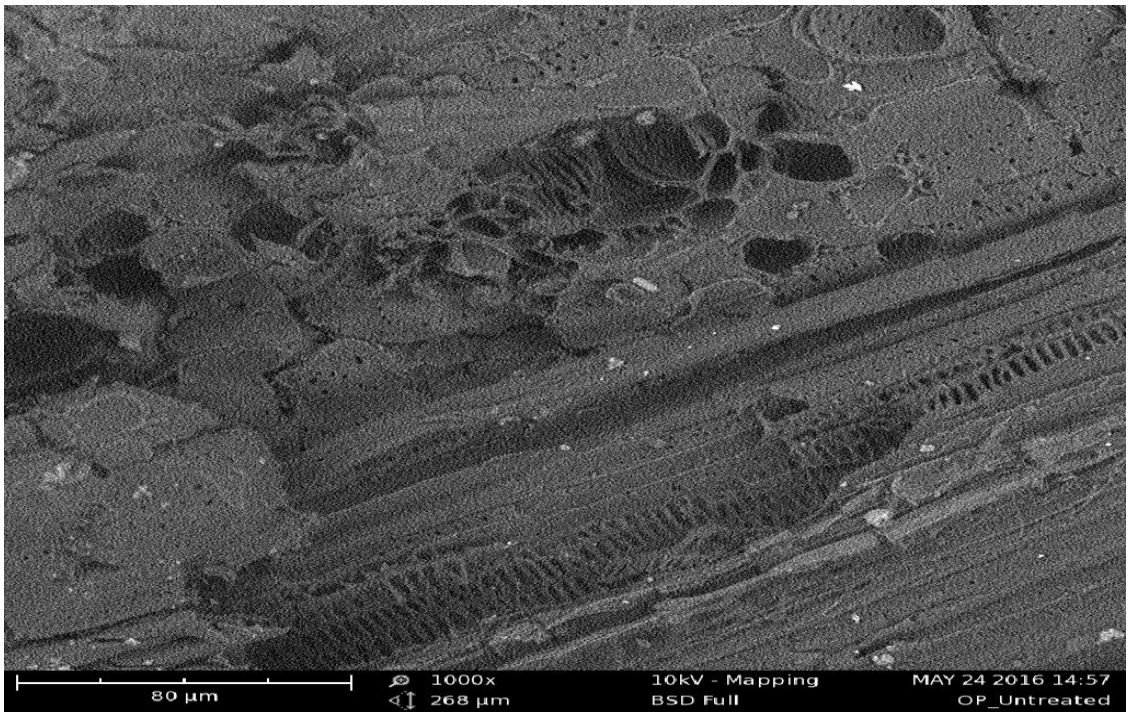

Fig. 2b. SEM of pretreated oil palm frond

The factors affecting the Simultaneous saccharification and fermentation of OPF with *Aspergillus niger* were studied using CCD experiments. The fermentation period (X_1, hrs), pH (X_2), temperature (X_3,°C), and the concentration of the inoculum (X_4) were chosen as the independent variables as shown in Table 4. Ethanol yield (Y) was chosen as the dependent output variable. Thirty-one experiments based on the CCD were carried out with different combinations of variables. The results were presented in Table 5. The data

obtained from the four-level central composite design matrix were used to develop models in which each dependent variable (Ethanol yield, Y) was obtained as the sum of the contributions of the independent variables through the second-order polynomial equation and interaction terms. The actual ethanol yield obtained in the experiments and the yields predicted by the model equation (2) are given. It showed that the regression coefficients of the linear terms and all quadratic coefficients of X_1, X_2, X_3, and X_4 were significant at < 1% level. The individual effect of the four parameters studied, quadratic effects, and interaction effects between the dependent variables were found to be significant from the response surface plots shown in Figs. 7 to 8. The clear elliptical shape of the curve shown in Figs. 7 to 8 indicates the interaction effects between the four independent variables were significant. Hence, the optimum combinations of the fermentation period, pH, pretreatment temperature, with the concentration of the inoculum play a major role to get the maximum bioconversion of OPF to ethanol.

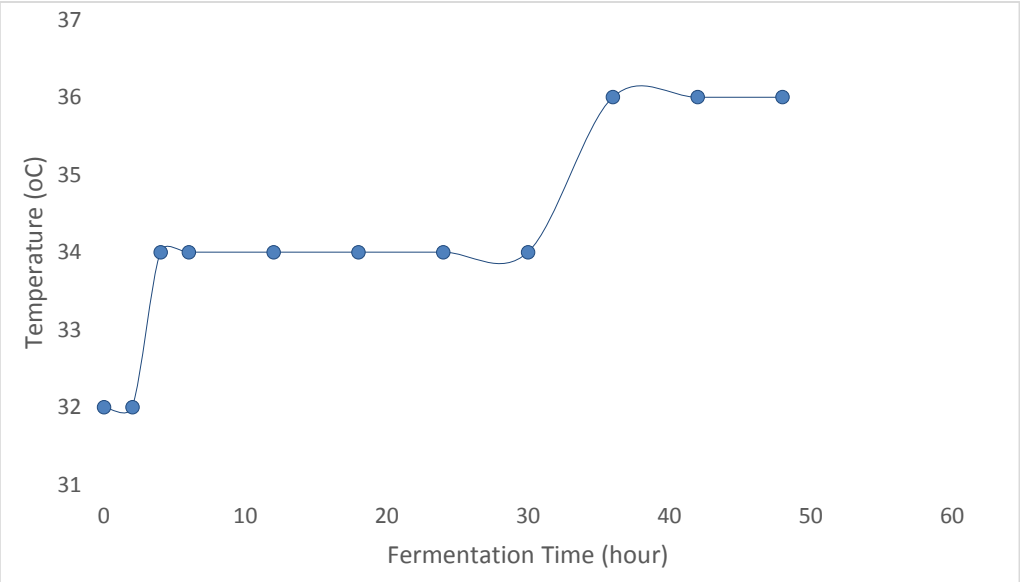

Fig. 3. Graph of cellulose substrate temperature of pretreated OPF

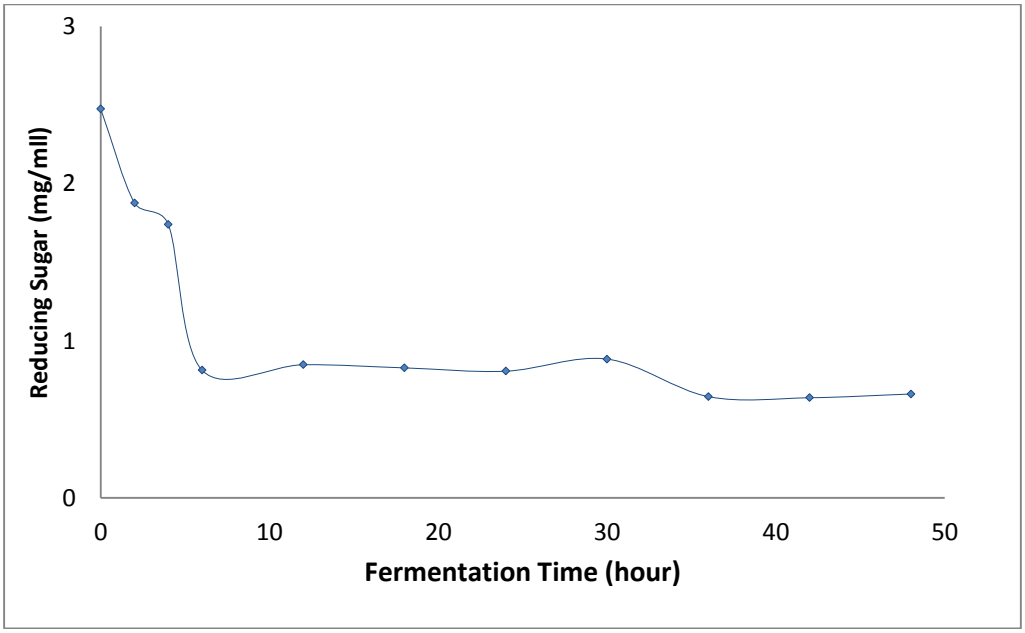

Fig. 4. Graph of reducing sugar

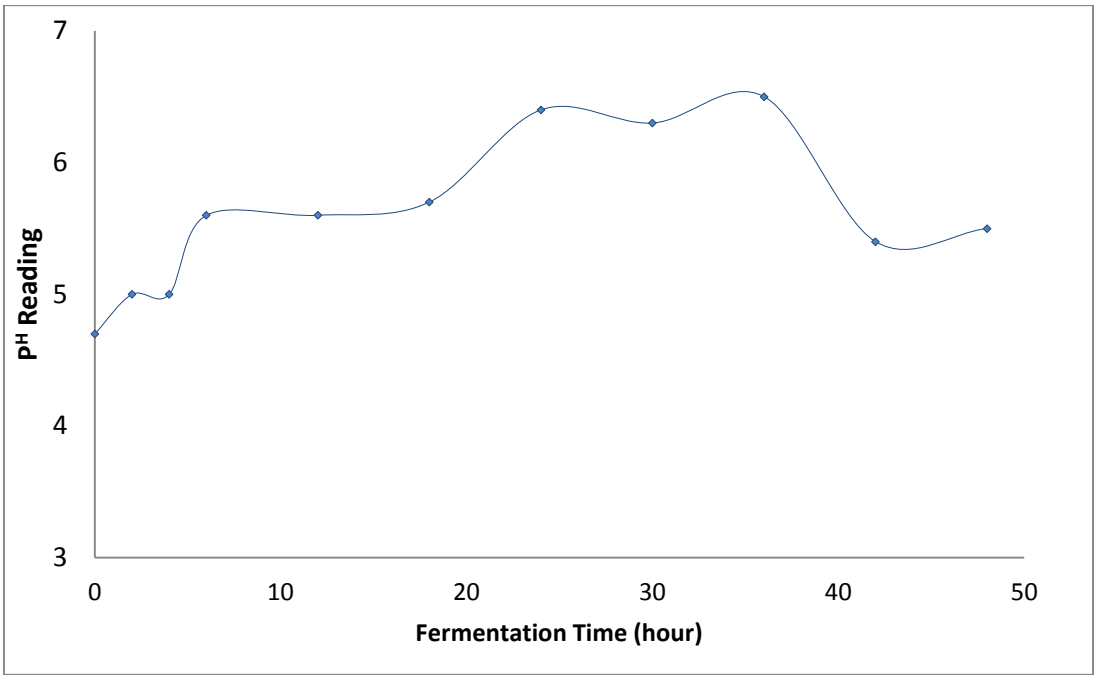

Fig. 5. Graph for pH reading of cellulose substrate of pretreated OPF

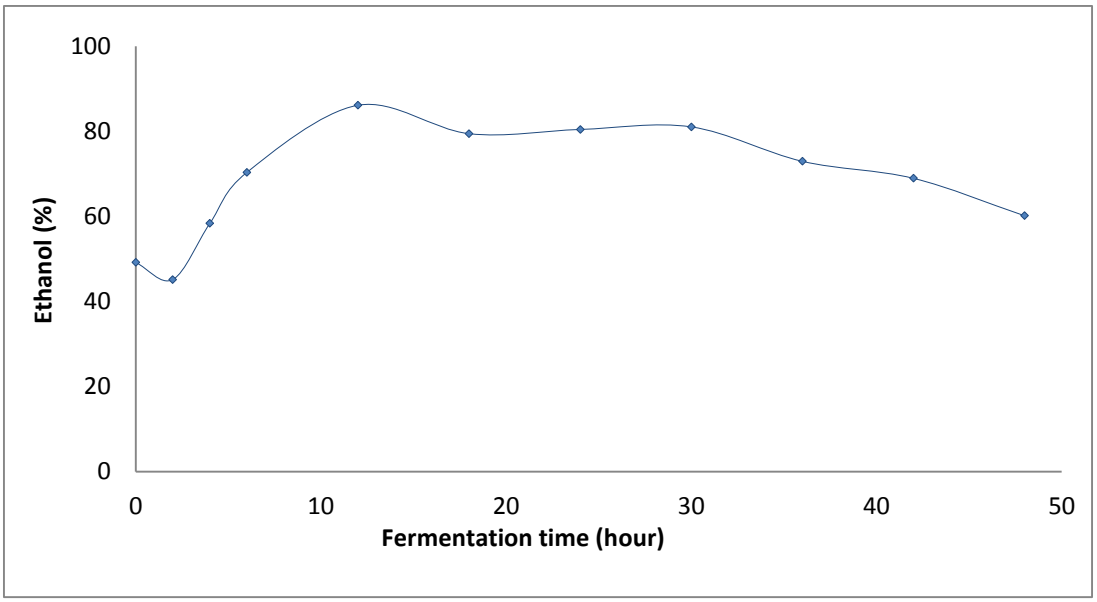

Fig. 6. Graph of Ethanol contents of OPF Optimized process variables on ethanol production

Table 4. Experimental variables and their levels

Factors	Low level	High level
Temperature, ^{o}C	36	42
pH	5.1	5.7
Inoculum	4	22
Fermentation, hr	0	36
Ethanol yield, %	90	110

Table 5. Experimental design showing the predicted and actual yield

S/N	Temperature (ºC)	pH	Innoculum	Fermentation	Actual yield/ ethanol yield	Predicted yield
1	38.99	5.49	20.94	10.77	97.49	1.000
2	39.66	5.59	10.08	1.67	99.02	1.000
3	39.05	5.61	17.26	25.30	100.79	1.000
4	38.07	5.60	13.01	15.15	98.74	1.000
5	38.58	5.13	19.07	8.40	99.98	1.000
6	37.20	5.16	16.27	19.10	99.56	1.000
7	38.37	5.41	15.44	13.32	98.66	1.000
8	40.57	5.58	12.83	0.80	98.72	1.000
9	36.25	5.45	10.81	23.73	97.10	1.000
10	39.19	5.11	5.12	21.25	102.35	1.000
11	41.97	5.24	11.52	34.38	98.99	1.000
12	37.31	5.57	15.21	9.94	98.26	1.000
13	41.78	5.62	5.63	28.12	100.42	1.000
14	39.87	5.37	16.30	32.24	103.07	1.000
15	38.75	5.14	8.02	29.50	101.26	1.000
16	37.72	5.14	19.38	21.16	99.41	1.000
17	36.84	5.46	8.68	18.86	97.98	1.000
18	38.55	5.59	9.21	17.73	99.43	1.000
19	40.80	5.39	4.27	30.88	106.04	1.000
20	41.63	5.57	17.48	19.84	97.93	1.000
21	41.66	5.23	5.00	5.69	100.61	1.000
22	40.28	5.47	9.27	15.49	100.14	1.000
23	36.18	5.11	16.64	4.43	100.32	1.000
24	40.03	5.38	14.74	20.70	101.26	1.000
25	37.82	5.36	15.85	33.33	102.58	1.000
26	38.65	5.13	20.09	13.81	99.23	1.000
27	37.67	5.58	8.79	16.25	98.62	1.000
28	39.81	5.53	13.75	18.30	99.95	1.000
29	40.99	5.68	10.89	23.87	98.39	1.000
30	40.65	5.55	7.81	10.10	98.94	1.000

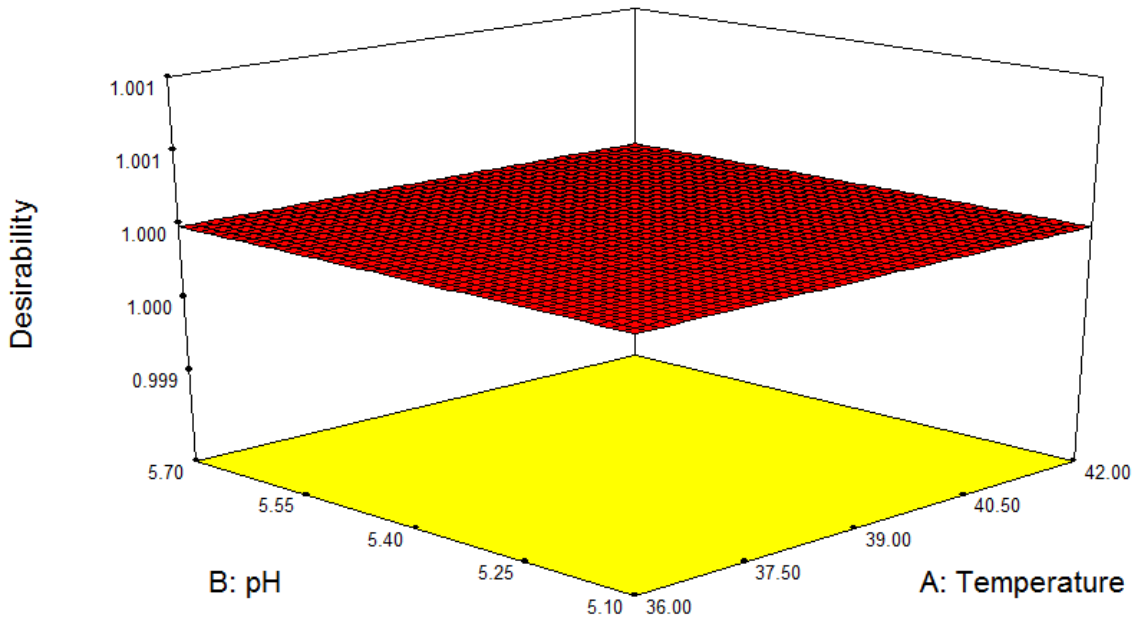

Fig. 7. Surface response of bioethanol yield with respect to pH and fermentation time

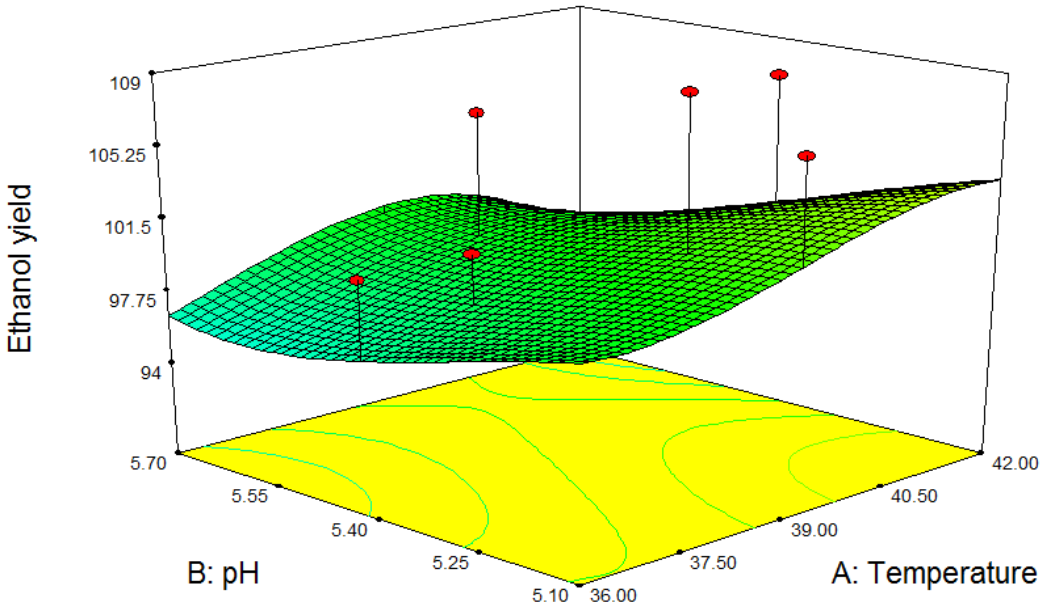

Fig. 8. Surface response of bioethanol yield with respect to pH and temperature

4. CONCLUSION

In the experiment, the pretreatment of oil palm frond substrate with *Aspergillus niger* carried out using SSF to extract, hydrolyze, and ferment the cellulose from the oil palm fronds was effective. The result showed that the bioethanol could be produced from oil palm fronds. Increasing bioethanol production from this substrate was statistically significant as the fermentation time increased, which was evident by the increase in the percentage of ethanol contents. This is a clear indication of yeast fermenting the sugar resulting in ethanol production. The pH values of the process of fermentation ranged from 4.7 to 6.5. Therefore, it can be concluded that oil palm fronds show good potential for bioethanol production and the pretreatment process was an effective method to release fermentable sugar. The process was effectively optimized within the confines of the following factors: temperature X_1 (36-42^{o}C), pH, X_2 (5.1-5.7), inoculum, X_3 (4-22), and fermentation time X_4 (0-36hr) of which an optimum yield of 110% was obtained.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lloyd TA, Wyman CE. Combined sugar yields for dilute sulfuric acid pretreatment of cornstover followed by enzymatic hydrolysis of the remaining solids. Bioresources Technology. 2005;96:1967–1977.
2. Gámez S, Ramírez JA, Garrote G, Vázquez M. Manufacture of fermentable sugar solutions from sugar cane bagasse hydrolyzed with phosphoric acid at atmospheric pressure, Journal of Agricultural and Food Chemistry. 2004; 74:4172-4177.
3. McMillan JD. Pretreatment of lignocellulosic biomass in enzymatic conversion of biomass for fuels production. American Chemical Society. 1994;292-324.
4. Osemwengie SO, Osagie EI, Onwukwe B. Optimization of bioethanol production from cassava peels. J. Appl. Sci. Environ. Manage. 2020;24(12):2077-2083. DOI: https://dx.doi.org/10.4314/jasem.v24i12.11
5. Kader J, Getha K, Wan M, Othman O. Hydrolysis of empty fruit bunch of oil palm using cellulolytic enzymes from Aspergillusterreus IMI 28243. Journal. Microbiology. Biotechnology. 1999;9:514–517.
6. Keller FA, Hamilton JE, Nguyen QA. Microbial pretreatment of biomass: Potential for reducing severity of thermochemical biomass pretreatment. Applied Biochemistry and Biotechnology. 2003;105–108:27– 41.

7. Braide W, Kanu IA, Oranusi US, Adeleye SA. Production of bioethanol from agricultural waste. J. Fundam. Appl. Sci. 2016;8(2):372-386.
8. Sarkar N, Ghosh, SK, Bannerjee S, Aikat K. Bioethanol production from agricultural wastes: An overview, Renewable Energy. 2012;37(1):19-27, Available:https://doi.org/10.1016/j.renene.2011.06.045
9. Ingale S, Joshi SK, Gupte A. Production of bioethanol using agricultural waste: Banana pseudo stem. Braz. J. Microbiol. 2014;45(3). Available:https://doi.org/10.1590/S1517-83822014000300018
10. Goh CS, Tan KT, Lee KT, Bhatia S. Bio-ethanol from lignocelluloses status, perspective and challenges in Malaysia. Bioresource Technology. 2010;101(13): 4834-4841.
11. Ariffin H, Hassan MA, UmiKalsom MS, Abdullah N, Ghazali FN, Shirai Y. Production of bacterial endoglucanase from pretreated oil palm empty fruit bunch by bacillus pumilus. EB3. Journal. Bioscience. Bioengineering. 2008;106: 231–236.
12. Dashtban M, Schraft H, Syed TA. Fungal biodegradation and enzymatic modification of lignin. International Journal of Biochemistry Mol Biol. 2010; 1:36–50.
13. Adela NB, Nasrin AB, Loh SK, Choo YM. Bioethanol Production by Fermentation of Oil Palm Empty Fruit Bunches Pretreated with Combined Chemicals. J. Appl. Environ. Biol. Sci. 2014;4(10):234-242.
14. Triwahyuni E, Hariyanti S, Dahnum D, Nurdin M, Abimanyu H. Optimisation of saccharification and Fermentation process in Bioethanol production from Oil Palm Fronds. Procedia Chemistry (Elsevier). 2015;16:141–148.
15. McKendry P. Energy production from biomass (Part 1): Overview of biomass. Bioresource Technology. 2002;83:37–46. DOI: 10.1016/S0960-8524(01)00118-3·Source: PubMed
16. Roslan AM, Zahari MA, Hassan MA, Shirai Y. Investigation of Oil Palm Frond Properties for Use as Biomaterials and Biofuels. Trop. Agr. Develop. 2014;58(1).
17. Zahari MA, KM M, Zakaria R, Ariffin H, Mokhtar MN, Salihon J, et al. Bioresource Technol. 2012;110:566-57.
18. Montgomery DC. Design and analysis of experiments, 6th Ed. New York. John Wiley and Sons, Inc; 2005.
19. Ishola F, Adelekan D, Mamudu A, Abodunrin T, Aworinde A, Olatunji O, et al. Biodiesel production from palm olein: A sustainable bioresource for Nigeria. Heliyon. 2020;6:e03725. Available:https://doi.org/10.1016/j.heliyon.2020.e03725

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/70325