Research Article / Review Article FUPRE JOURNAL 9(2):52-58(2025)

FUPRE Journal

of

Scientific and Industrial Research

ISSN: 2579-1184(Print) ISSN: 2578-1129 (Online)

http://fupre.edu.ng/journal

Evaluation and analysis of SUWAN-1-SR Escherichia coli-mediated acetone-butanol-ethanol fermentation using roasted corn cob species for biobutanol synthesis

OSSAI, J. E .[1,*], *OSHOMOGHO, F. O.*[2]

[1] *Department of Chemical Engineering, Faculty of Engineering, University of Benin, PMB1154, Benin, Edo State, Nigeria*

[2]*Department of Chemical Engineering, Faculty of Engineering, University of Benin, P.M.B. 1154, Benin, Edo State, Nigeria*

ARTICLE INFO

Received: 05/05/2025
Accepted: 24/07/2025

Keywords

Biobutanol, ABE fermentation, Escherichia coli, SUWAN-1-SR roasted corn cob, Response Surface Methodology.

ABSTRACT

The Objective of this paper was to describe and assess the SUWAN-1-SR species of roasted corn cobs for the acetone-butanol-ethanol fermentation process using Escherichia coli to produce biobutanol. The feedstock percentage content of cellulose, hemicellulose, and lignin was estimated, with values of 43.65%, 51.50%, and 4.85%, respectively. Variations in elemental composition were found when the SUWAN-1-SR Species of maize cobs were characterized; potassium had the highest weight percentage of 0.75 and a of peak 4143 cp/Ma The Response surface model optimization showed how time 27.1978 minutes, temperature of 80.6981^{0}C and a maximum concentration of 0.4623mol/dm3 were adjusted based on the model developed from each response and gave the percentage total sugar yield of 1489.88 g/l for SUWAN-1 SR species of roasted corn cobs, the adjusted R^2 value was 0.9054. HPLC analysis was also performed to determine the concentration of biobutanol produced from the ABE fermentation process.

1. INTRODUCTION

The increasing global demand for energy, coupled with the finite availability of conventional fossil fuels and rising environmental concerns, has necessitated a shift towards renewable and sustainable energy sources (Bajpai, 2016). In the twentieth century, studies were concentrated on the development of the cheap use of fossil fuels for heat, electricity, transportation fuels, and chemicals, including pharmaceuticals, detergents, synthetic fiber, plastics, pesticides, fertilizers, lubricants, to meet the growing demand of the population (Abbate *et al*, 2023). Lignocellulose biomass is an abundant, inexpensive, and readily available source of fermentable sugars (Ahmed, 2021). The major issue associated with the use of lignocellulose as an energy source is the recalcitrant nature of the material (Modenbach, 2013). Corn cob contains a sufficient amount of cellulosic material, which is the best source of fermentable sugars. In order to produce these fermentable sugars from biomass, the pretreatment is usually the initial process to improve the accessibility of the cellulose polymers (Kim, 2018). The acid pretreatment step of biobutanol production is affected by factors such as temperature, time, and acid concentration, and it is necessary to optimize these factors in order to achieve optimum yields of biobutanol (Jabbar *et al*;2018). Response surface methodology (RSM), in

*joy.ossai@uniben.edu
DIO

conjunction with experimental design techniques, has been applied for the optimization of numerous bioprocesses and has proven to be quite successful (Lenth, 2012). Therefore, this study aimed to maximize the pretreatment conditions used in the fermentation of roasted SUWAN-1-SR corn cobs species for the production of biobutanol using the Acetone-Butanol-Ethanol (ABE) method Xue *et al*, 2016).

2 MATERIALS

The corn Species SUWAN-1-SR was obtained from the Agricultural Development Program (ADP) in Benin City, Edo State, Nigeria, and was used as the feedstock in this study. The Escherichia coli used for the study as the fermenting organism was obtained from the Microbiology department of the University of Benin, Benin City, Nigeria. All chemicals and reagents that were used in this work are of analytical grade and were purchased from a material supplier in Onitsha, Anambra State, Nigeria.

2.1 Methods

2.1.1 Pretreatment

The process was carried out after the corn cobs were oven dried at a temperature of 600^0C for five hours. The cobs were chopped for easy grinding in a mechanical grinder. It was sieved through a mesh with a pore size of about 245µm. Then, 10g of the corn cobs was used for each experiment, and 100ml of dilute H_2SO_4 was added to the sample in the conical flask and heated at the stipulated temperature and set time. A pH of 7 was then achieved by adding 2 milliliters of NaOH solution to neutralize it. Exactly 1 milliliter of 3,5-dinitrosalicylic acid (DNS) was then added to the 3 milliliters of the neutralized solution, which was then transferred into the boiling tube. It was then diluted by 50% through the addition of 4 ml of distilled and deionized water.

2.1.2 Enzymatic Hydrolysis

The pretreated corn cobs were enzymatically hydrolyzed using cellulase, which was purchased in powdered form. Water was added to the cellulase and heated to about 40-50 °C. The pretreated sample was maintained at the same temperature, and cellulase was added to the solution, which was then mixed thoroughly for approximately 30 minutes while keeping the temperature constant. The solution was then left for 24 hours before fermentation.

2.1.3 Fermentation

The bacterial strain utilized in the fermentation process to synthesize the biobutanol was *E. coli*. Fermentation of the corn cob hydrolyte was performed in serum bottles for approximately 4 days to utilize the reducing sugars present in the pretreated samples. The fermentation was performed at about $40\text{-}50^0$C. The Batch Fermentation process took place under anaerobic conditions.

2.1.4 Response Surface Modeling

The central composite design was used to study the effect of temperature (40 -120^0C), concentration (0.1 – 1.0 mol/dm^3), and time (10 – 60 minutes) on the pretreatment of corn cob.

The process was optimized based on the number of runs and a second-degree polynomial shown in Equation (1) fitted to the experimental data. This was carried out to estimate the response of the dependent variables using multiple linear regressions and to determine the optimum fermentation conditions that resulted in the maximum concentration of the biobutanol.

$$\boldsymbol{Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} A_1 B_2 + \beta_{13} A_1 C_3 + \beta_{23} B_2 C_3 + \beta_{11} A_1^2 + \beta_{22} B_2^2 +}$$

$$\boldsymbol{\beta_{33} C_3^2} \qquad (1)$$

Where, y = predicted response, $A = temperature$, $B = concentration$, $C = time$

3 RESULTS AND DISCUSSION

3.1 Experimental Results for Pretreatment of Feedstock

The pretreatment of lignocellulosic materials includes fragmentation of solids, alteration of the lignocellulose structure, and increasing the contact area between the material and chemical reagents

Table 1: Results of Dilute Acid Hydrolysis of SUWAN-1-SR with CCD

No of Runs	Temp (^{0}C)	Dilute acid Conc. (mol/dm^3)	Time (minutes)	Sugar yield (g/l)
1	120.00	0.550	35.00	1649.40
2	103.78	0.282	20.13	1818.16
3	40.00	0.55	35.00	1103.41
4	56.22	0.282	49.86	1323.91
5	103.78	0.282	49.86	933.75
6	80.00	0.100	35.00	193.73
7	56.22	0.817	20.13	1485.15
8	80.00	0.550	35.00	1635.86
9	56.22	0.282	20.13	1053.78
10	80.00	0.550	35.00	1635.86
11	103.78	0.817	20.13	1212.61
12	80.00	0.550	35.00	1301.05
13	56.22	0.817	49.86	1492.37
14	80.00	0.550	10.00	1659.33
15	80	0.55	35	1506.81
16	80	1	35	815.526
17	80	0.55	35	1405.73
18	80	0.55	35	1212.61
19	80	0.55	60	1399.42
20	103.784	0.817572	49.86	1336.25

Table 1 shows the central composite design matrix for dilute acid hydrolysis of SUWAN-1-SR. A procedure known as "diluted acid hydrolysis" uses an inorganic acid, such as sulfuric acid, to hydrolyze cellulose into glucose monosaccharides. The temperature range of 40–120 °C, the concentration of 0.1–1.0 mol/dm^3, and the time interval of 10–60 minutes were used to create the table in order to determine the number of runs for the feedstock.

3.2 SUWAN-1-SR Roasted Corn Cob Regression Analysis

Regression analysis was used to fit the response. The link between temperature (A), concentration (B), time (C), and sugar yield (Y) is depicted in the proposed model. The model, as given in Equation 4.3, is expressed in terms of the coded factors.

$$y = 1486.16 + 159.22A + 201.60B - 163.38C - 264.20AB + 34.07AC + 256.98BC - 28.29A^2 - 336.51B^2 + 25.79C^2 \qquad (2)$$

Table 2: Analysis of Variance (ANOVA) for SUWAN-1-SR Roasted Corn cob

Source	Sum of Squares	Df	Mean Square	f-value	p-value
Model	4.04E+06	9	4.494E+05	21.21	< 0.0001
A-Temp	3.46E+05	1	3.462E+05	16.34	< 0.0024
B-Conc	5.55E+05	1	5.550E+05	26.19	0.0005

C-Time	3.64E+05	1	3.645E+05	17.20	0.0020
AB	5.58E+05	1	5.584E+05	26.35	0.0004
AC	9284.95	1	9284.95	0.4381	0.5230
BC	5.28E+05	1	5.283E+05	24.93	0.0005
A^2	11536.36	1	11536.36	0.5444	0.4776
B^2	1.63E+06	1	1.632E+06	77.01	< 0.0001
C^2	9584.34	1	9584.34	0.4523	0.5165
Resid-ual	2.12E+05	10	21192.16		
Lack of Fit	83446.24	5	16689.25	0.6495	0.6763
Pure Error	1.28E+05	5	25695.07		
Cor Total	4.25E+06	19			

The Model F-value of 21.21 indicates the model is significant, as indicated in Table 2. This kind of huge F-value has a 0.01% probability of being caused by noise. Model terms are considered significant when P-values are less than 0.0500. A, B, C, AB, BC, and B^2 are important model terms in this instance. The model terms are not important if the value is bigger than 0.1000. The 0.65 Lack of Fit F-value indicates that the Lack of Fit is not statistically significant in comparison to the pure error. A significant Lack of Fit F-value has a 67.63% probability of being caused by noise. Good—we want the model to fit—is a non-significant lack of fit.

Table 3: Fit Statistical Information **for SUWAN-1-SR Roasted Corn cob**

R2	0.9502
Adjusted R2	0.9054
Predicted R2	0.8077
Adequate Precision	17.1576
Std Deviation	145.58
Mean	1254.66
C. V. (%)	11.60

Table 3 shows that the difference between the predicted R^2 of 0.8077 and the Adjusted R^2 of 0.9054 is less than 0.2, indicating good agreement. Adeq Precision measures the signal-to-noise ratio. Ideally, this ratio should be above 4. With a ratio of 17.158, your signal is strong.

3.3 Optimization of SUWAN-1-SR Roasted Corn cob Hydrolysis Conditions Using Response Surface Methodology

Response surface plots for SUWAN-1-SR roasted corn cob are displayed below to help identify the ideal values of the variables that affect its acid hydrolysis:

The yield of total sugar in the SUWAN-1-SR Roasted Corncob increased with temperature and concentration.

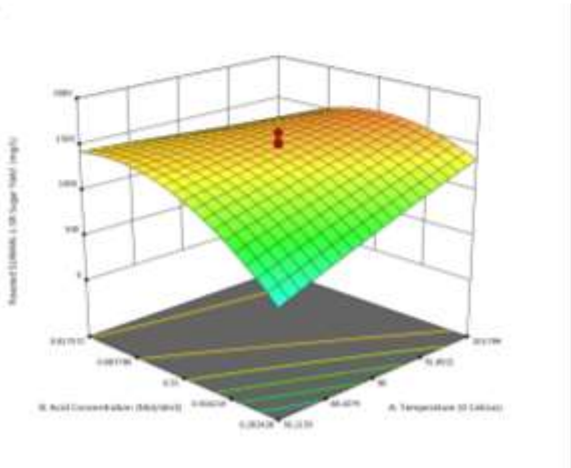

Figure 1: Response Surface Plot Showing the Effect of Acid Concentration and Temperature on Total Sugar Concentration for SUWAN-1-SR Roasted Corn Cob

In Figure 2, the response surface plot of SUWAN-1-SR Roasted Corncob as a function of two independent variables, temperature and time, is displayed. The yield of total sugar in SUWAN-1-SR Roasted Corncob increased with increasing temperature and time. This suggests that higher temperatures and longer roasting times are beneficial for maximizing the total sugar content in the corncob.

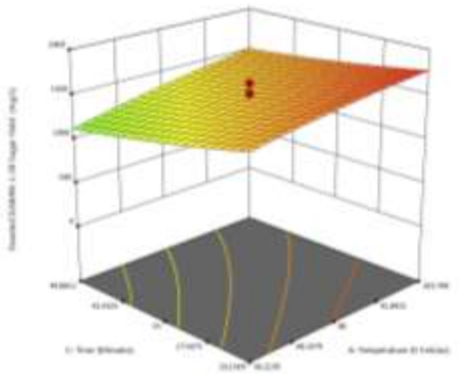

Figure 2: Response Surface Plot Showing the Effect of Temperature and Time on Total Sugar Concentration for SUWAN-1-SR Roasted Corn Cob

The response surface plot also shows that there is an optimal combination of temperature and time that can be used to achieve the highest yield of total sugar. Roasting helps to weaken the cellulose content of the sample, thereby making easy disintegration during acid pretreatment.

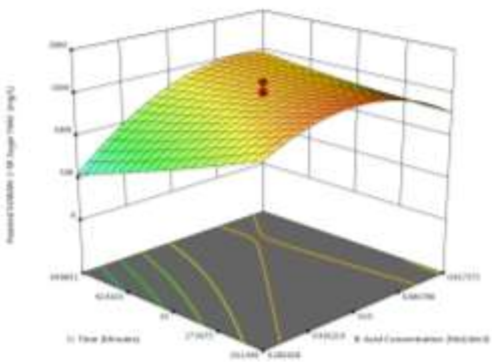

Figure 3: Response Surface Plot Showing the Effect of Acid concentration and Time on Total Sugar Concentration for SUWAN-1-SR roasted Corn Cob

Figure 3 displays the response surface plot on SUWAN-1-SR Roasted Corn cob as a function of two independent variables, concentration and time. The SUWAN-1-SR Roasted Corn cob's yield of total sugar increased with increasing time and concentration.

Table 4: Results of optimal *SUWAN-1-SR Roasted Corn Cob* pretreatment condition

Experimental Design Factor	Value
Temperature (^oC)	93.18
Acid conc (mol/dm3)	0.72
Time (minutes)	44.08
Sugar yield (g/l)	1489.88

The result of optimum acid pretreatment is presented in Table 4. A temperature of 93.18 ^oC, acid concentration of 0.72 mol/dm3, and pretreatment time of 44.08 minutes, which would generate a sugar yield of 1489.88 g/L. These conditions were found to be the most effective in breaking down the biomass and releasing the highest amount of sugars. The sugar yield of 1489.88 g/L demonstrates the efficiency of this acid pretreatment process in converting the biomass into fermentable sugars for biofuel production. This optimal yield (1489.88 g/L) was selected considering temperature and time involved. Higher yield can be obtained, but would require high energy input to generate a higher temperature for the material digestion.

4 CONCLUSIONS

The use of the design of experiment of response surface methodology (RSM) was demonstrated to be useful in determining the optimum conditions of lignocellulosic feedstock hydrolysis. Optimum sugar yield (1489.88 g/L) was obtained from Roasted corn cob using statistical analysis. Enzymatic hydrolysis using cellulase further increases the sugar yield and makes the substrate more bioavailable for microbial fermentation, which produces biobutanol from the Acetone-Butanol-Ethanol process using **Escherichia. coli**. The HPLC analysis result gave the concentration of biobutanol produced from the ABE fermentation process.

References

Abbate, S. P., Centobelli, R., Cerchione, G. and Giardino, R. P. (2023). Coming out of the egg: assessing the benefits of circular economy strategies in agri-food industry *Journal of Cleaning Production* 385, 135665.

Achinivu, E. C., Mohan, H. C., Das, L., Huang, K., Magurudeniya, H. D., Pidatala, V. R., George, A., Simmons, B. A. and Gladden J. M. (2021). A predictive toolset for the identification of effective lignocellulosic pretreatment solvents: a case study of solvents tailored for lignin extraction, 23(18), 7269-7289

Africa, W., Bensah, E. C., Kádár, Z., and Mensah, M. Y. (2015). Ethanol Production from Hydrothermally-Treated, *10*(4), 6522–6537.

Ahmed, A. (2021). Biofuel Feedstock Plantations Closure and Land Abandonment in Ghana: New Directions for Land Studies in Sub-saharan AfricaLand Use Policy, vol. 107 , 10.1016/j.landusepol.2021.105492

Amiri, H., and Karimi, K. (2016). Integration of Autohydrolysis and Organosolv Deligni fi cation for E ffi cient Acetone , Butanol , and Ethanol Production and Lignin Recovery. https://doi.org/10.1021/acs.iecr.6b00110

Anukam, A. I., Goso, B. P., Okoh, O. O., and Mamphweli, S. N. (2017). Studies on Characterization of Corn Cob for Application in a Gasification Process for Energy Production. *Journal of Chemistry*, *2017*. https://doi.org/10.1155/2017/6478389

Ave, G. D., and Ii, T. A. A. (2018). Techno-economic comparison of Acetone-Butanol-Ethanol fermentation using various extractants. *Energy Conversion and Management*, *156*(November 2017), 288–300. https://doi.org/10.1016/j.enconman.2017.11.020

Bajpai, P. (2016). Pretreatment of Lignocellulosic Biomass for Biofuel Production. https://doi.org/10.1007/978-981-10-0687-6

Baral, N. R., Slutzky, L., Shah, A., Ezeji, T. C., Cornish, K., and Christy, A. (2016). Acetone-butanol-ethanol fermentation of corn stover: Current production methods, economic viability and commercial use. *FEMS Microbiology Letters*, *363*(6), 1–11. https://doi.org/10.1093/femsle/fnw033

Borisov, I. L., Golubev, G. S., Vasilevsky, V. P., Volkov, A. V, and Volkov, V. V. (2017). crossmark. *Journal of Membrane Science*, *523*(October 2016), 291–300. https://doi.org/10.1016/j.memsci.2016.10.009

Bringezu, S., O'Brien, M., and Schütz, H. (2012). Beyond biofuels: Assessing global land use for domestic consumption of biomass. A conceptual and empirical contribution to sustainable management of global resources. *Land Use Policy*, *29*(1), 224–232. https://doi.org/10.1016/j.landusepol.2011.06.010

Ezeji, T. C., Qureshi, N., and Blaschek, H. P. (2007). Bioproduction of butanol from biomass: from genes to bioreactors. *Current Opinion in Biotechnology*, *18*(3), 220–227.

Jabbar, S., Algayyim, M., Wandel, A. P., Yusaf, T., and Hamawand, I. (2018). Production and application of ABE as a biofuel. *Renewable and Sustainable Energy Reviews*, *82*(October 2017), 1195–1214. https://doi.org/10.1016/j.rser.2017.09.082

Karimi, K., Tabatabaei, M., Horváth, I. S., and Kumar, R. (2015). Recent trends in acetone , butanol , and ethanol (ABE) production, *8*, 301–308. https://doi.org/10.18331/BRJ2015.2.4.4

Kim, D. (2018). Physico-chemical conversion of lignocellulose: Inhibitor effects and detoxification strategies: A mini review. *Molecules*, *23*(2). https://doi.org/10.3390/molecules23020309

Kuhar, S., Nair, L. M., and Kuhad, R. C. (2008). Pretreatment of lignocellulosic material with fungi capable of higher lignin degradation and lower carbohydrate degradation improves substrate acid hydrolysis and the eventual conversion to ethanol. *Canadian Journal of Microbiology*, *54*(4), 305–313. https://doi.org/10.1139/W08-003

Kumar, A., Gautam, A., and Dutt, D. (2016). Biotechnological Transformation of Lignocellulosic Biomass in to Industrial Products: An Overview. *Advances in Bioscience and Biotechnology*, *07*(03), 149–168. https://doi.org/10.4236/abb.2016.73014

Kumar, M., and Gayen, K. (2011). Developments in biobutanol production: New insights. *Applied Energy*, *88*(6), 1999–2012. https://doi.org/10.1016/j.apenergy.2010.12.055

Kumar, P., Barrett, D. M., Delwiche, M. J., Stroeve, P., Kumar, P., Barrett, D. M., … Stroeve, P. (2009). Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. https://doi.org/10.1021/ie801542g

Lenth, R. V. (2012). Response-surface methods in R , using rsm. *Journal of Statistical Software*, *32*(7), 1–17. https://doi.org/http://dx.doi.org/10.18637/jss.v032.i07

Liu, Z., Yao, X., Zhang, Q., Liu, Z., Wang, Z., and Li, F. (2017). Modulation of the Acetone / Butanol Ratio during Fermentation of Corn Stover-derived Hydrolysate by Clostridium beijerinckii NCIMB Downloaded from http://aem.asm.org/ on December 1 , 2017 by guest, (January). https://doi.org/10.1128/AEM.03386-16

Melin, K., and Lehtonen, J. (2016). Comparison of Reactive Distillation and Dual Extraction Processes for the Separation of Acetone , Butanol , and Ethanol from Fermentation Broth. https://doi.org/10.1021/acs.iecr.5b03196

Modenbach, A. (2013). Sodium hydroxide pretreatment of corn stover and subsequent enzymatic hydrolysis : An investigation of yields , kinetic modeling and glucose recovery.

Mudzanani, K. (2015). Optimization and Kinetics Study of Solvent Pretreatment of South African Corn Cob for Succinic Acid Production Proposal, (November).