FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 7 No. 6, December, 2023, pp 307 - 312
DOI: https://doi.org/10.33003/fjs-2023-0706-2132

OPTIMIZATION AND QUALITY ANALYSIS OF BIO-BUTANOL PRODUCTION FROM SUGARCANE (*Saccharum officinarum*) BAGASSE HYDROLYSATE

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ABSTRACT

Several companies are currently investigating in development for the production of Biobutanol. This research was concerned with Biobutanol production from ABE (Acetone, Butanol, and Ethanol) fermentation of sugarcane bagasse hydrolysate by *Clostridium Perfringens*. The sample was hydrolyzed with concentrated H_2SO_4 solution for 1 hour at 121^{o}C. Response surface designed by optimizing the fermentation parameters (Time, Temperature, and pH) by the used media (*C. Perfringens*) showed the highest Biobutanol yield at temperature (35^{0}C), Time 48 (Hhs), and pH 6.0 with 163 cm^3 which is exactly around 0.543(g/L) by converted to a gram per liter as mostly represented by many researchers. The produced Biobutanol from the sugarcane bagasse hydrolysate was characterized using FT-IR and GC-MS analysis. Conclusively this work revealed the importance of sugarcane bagasse as a good potential feedstock of Biobutanol.

Keywords: Biofuels, Butanol, *Clostridium Perfringens,* Hydrolysate. *Saccharum officinarum,* Biobutanol, Optimization, Sugarcane bagasse

INTRODUCTION

Sugarcane bagasse is a fibrous residue of plant material that remains of sugarcane after undergoing conventional milling. Sugarcane bagasse is a suitable substrate for solvent production; it is composed approximately of 40% cellulose, 24% hemicellulose, and 25% lignin. Its hydrolysate contains hexose sugars cellulose, celludextrins, and pentose (all of which can be utilized by solvent-producing *Clostridia* (Liao *et al*., 2018).

The ABE fermentation process suffers from low butanol yield and productivity because of the co-production of acetone, ethanol, and organic acids. Moreover, the concentration of butanol in the final fermentation liquid is limited to very low concentrations (typically < 13 g/L) due to butanol toxicity to the producing bacteria (Green et al. 2013).

Abundant and inexpensive feedstocks are desirable for bio-butanol production. Using lignocellulosic material as a substrate for butanol production is an attractive approach for introducing an economically competitive biological process (Garcia *et al*., 2020). Lignocellulosic biomass represents a feedstock with massive potential for the production of biofuels and chemicals because it is the most abundant renewable material in the world and because in many cases, it is considered a residue that does not compete with food. Numerous efforts have been made to produce butanol from lignocellulosic material, such as wheat straw (Qureshi *et al.,* 2017), corn stoves, rice husks. Hydrolysis of lignocellulosic material by concentrated mineral acid could release most sugars at high sugar yield. However, a high concentration of toxic compounds could be generated, including weak acids, furan derivatives and phenolic compounds, which could strongly inhibit cell growth and decrease solvent production (Ouyang *et al.,* 2013)

Sugarcane bagasse is a residue produced in large quantities by the sugar and alcohol industries in Brazil, India, Cuba, and China(CONAB, 2020) In general, 1 ton of sugarcane generates 280 kg of bagasse, and bagasse is primarily composed of lignin (20–30%), cellulose (40–45%), and hemicelluloses (30–35%) (Peng *et al*., 2019). Thus, technologies that would recover reducing sugars from sugarcane bagasse are needed. The annual production of sugarcane globally is about 1.6 billion tons and that generates millions of metric tons of sugarcane (Chandel *et al.*, 2012).

MATERIALS AND METHODS

Media preparation

Isolation and Identification of Bacteria (Clostridium Perfringens))

Three different samples of contaminated soil and faeces were collected and a gram of each was suspended in a nutrient broth in a test tubes and incubated anaerobically at 37^{0}C for 24 hours and subjected to a blood agar base plate and cooked meat agar and incubated again for 24 hrs. The *C. Perfringens* colony was distinguished with blood hydrolysis in the blood agar base and black color in cooked meat agar. The colony was sub-cultured to nutrient agar for 24 hourrs.

The twenty-four hours old culture of the isolation was used to fix the smear and strain with gram's staining reagent. It was also viewed in the microscope with 100x objective lent (OIE, 2000).

Biochemical Characterization

Several biochemical tests such as the motility test, indole test, methyl-red test, voges-Proskauer test, hydrogen sulphide test, gas test, and sugar fermentation test were conducted as per procedures described by OIE (2000).

Pretreatment methods

Bagasse was cut into small pieces using a pair of scissors to reduce the size and increase the surface area of the cellulose material. Microwave alkali pretreatment of sugarcane bagasse was carried out with NaOH as a pretreatment reagent. The bagasse was then pretreated with 15% NaOH (w/v) and dried in an oven at 60^{O}C for 25 min. After pretreatment and delignification, the substrate was thoroughly washed with distilled water till the pH of effluent was similar to that of distilled water and then dried. The dried solid residue was used for the analysis of its composition (Merci *et al*., 2019).

Hydrolysis of sugarcane bagasse

Fifty grams (50 g) of dried pretreated sugarcane bagasse was weighed in 1 dm^3 capacity conical flasks and 500 cm^3 of (0.4%) dilute sulfuric acid was added to each conical flask. The flask was covered with aluminum foil and heated for 1 hour and 15 minutes on a water bath and autoclave for 30 minutes at 121°C. The flasks were allowed to cooled and filtered through Whatman NO.1 filter paper. The pH was adjusted based on an experimental design from Table 1.1 (Oyeleke and Jibril, 2009).

Fermentation

Fermentation was carried out with *Clostridium* species according to the procedure reported by Owuna *et al*. (2018). The hydrolysate obtained from hydrolysis was used as the fermentation media. Hydrolysates (30 g) were transferred into 500 cm^3 capacity conical flasks. It was covered with cotton wool and aluminum foil and heated at 25^0C, 30^0C and 35^0C and then cooled to 35^0C. The pH of the fermentation medium was adjusted to 4.5, 6, and 7.5. Then 1cm^3 of prepared suspension of the organism (0.5%) was added to the hydrolysate. The fermentation was carried out in an anaerobic incubator with frequent shaking.

Distillation

Butanol produced from the fermentation process contains a significant quantity of water, which must be removed (Binod *et al*., 2012). The fermentation broth was transferred into a round-bottom flask fixed to a distillation column with running tap water through the column. A conical flask was fixed to the other end of the distillation column to collect the distillate. A heating mantle with the temperature adjusted at 117.2 °C was used to heat the round-bottomed flask containing the fermentation broth for each group. The distillate was collected in a conical flask after the condensation (Xing *et al*., 2010)

$$\text{Biobutanol yield (\%)} = \frac{\text{Volume of biobutanol produced}}{\text{Volume of sample used}} \times 100 \quad (1)$$

Optimization of Fermentation Parameters

Experimental Design

The experiment was designed using Box-Behnken, a response surface design (RSD) on minitab 17 statistical software. The effect of three quantitative variables i.e. pH, temperature, and time wad investigated. The design generated 30 runs. The optimum parameters were determined for the highest yield of biobutanol.

Table 1: Optimization parameters and their levels

Factors	unit	Low level (-)	High level (+)
Time	Hrs	24	72
Temperature	^{0}C	25	35
pH	-	4.5	7.5

Characterizing the produced biobutanol

FTIR Analysis Method

The FT-IR analysis was carried out at the Center for Advanced Science Research and Analytical Services (CASRAS) in Usumanu Danfodiyo University Sokoto. Using Agilent carry 630 FTIR Spectrometer. The analysis was in the range of 650—4000 cm^{-1} at a resolution of 4 cm^1. The generation of the sample spectra was achieved through MicroLab software.

GC-MS Analysis of Biobutanol Produced from Sugarcane Bagasse

The GC-MS analysis of produced biobutanol was carried out at Umaru Musa Yar aduwa University, Katsina with Agilent Tech. GC/MS Machine. The trremperature was initially programmed at 90^0C for 1 minute and then programmed to 280^0C at the rate of 25^0C per minute. The temperature retained to four minutes and 31 minutes for the full run time. The injection volume was 1.0ul and the inlet temperature was maintained at 250^0C. The spectrum of the separated compounds was likened with the information of the spectrum of known compounds saved in the NIST02 Reference Spectral Library.

RESULTS AND DISCUSSION

Optimization of process for Biobutanol Production

The mean yield of biobutanol obtained from the 30 runs of the experiment conducted at different levels of the three process variables investigated are presented in Table 2. The biobutanol yield varies from a minimum of 101 cm^3 (0.336g/L) at 4.5 pH, Temperature (35^0C), and Time at 48 hours to a maximum of 163 cm^3 (0.543g/L) at pH 6, and Temperature at (25^0C), and Time 24hrs. There is a clear indication that the effect of time and temperature is more pronounced. The use of media *Clostridium Perfringens* in the fermentation process after the hydrolysis of bagasse by H_2SO_4 solution gave the highest biobutanol concentration more than 0.27g/L as reported by Woranart et al. (2014).

Table 2: Optimization of process for Biobutanol Production.

StdOrder	RunOrder	PtType	Blocks	Time(hrs)	Temp(°C)	pH	Yield(cm^3)	Yield(g/L)
1	1	2	1	24	25	6.0	101.0	0.336
2	2	2	1	72	25	6.0	133.0	0.443
3	3	2	1	24	35	6.0	161.0	0.536
4	4	2	1	72	35	6.0	158.0	0.526
5	5	2	1	24	30	4.5	134.0	0.446
6	6	2	1	72	30	4.5	107.4	0.356
7	7	2	1	24	30	7.5	161.7	0.539
8	8	2	1	72	30	7.5	121.0	0.403
9	9	2	1	48	25	4.5	112.0	0.373
10	10	2	1	48	35	4.5	163.0	0.543
11	11	2	1	48	25	7.5	127.0	0.423
12	12	2	1	48	35	7.5	141.0	0.47
13	13	0	1	48	30	6.0	148.0	0.493

14	14	0	1	48	30	6.0	117.7	0.392
15	15	0	1	48	30	6.0	119.0	0.396
16	16	2	1	24	25	6.0	109.4	0.364
17	17	2	1	72	25	6.0	131.0	0.436
18	18	2	1	24	35	6.0	145.0	0.483
19	19	2	1	72	35	6.0	120.6	0.402
20	20	2	1	24	30	4.5	148.0	0.495
21	21	2	1	72	30	4.5	125.0	0.416
22	22	2	1	24	30	7.5	160	0.533
23	23	2	1	72	30	7.5	106.7	0.355
24	24	2	1	48	25	4.5	114.0	0.38
25	25	2	1	48	35	4.5	118.0	0.396
s26	26	2	1	48	25	7.5	119.7	0.399
27	27	2	1	48	35	7.5	123.4	0.411
28	28	0	1	48	30	6.0	120.0	0.4
29	29	0	1	48	30	6.0	130.0	0.433
30	30	0	1	48	30	6.0	107.0	0.356

Key; g/L= gram per liter

Factorial Regression Analysis of Biobutanol Yield

The effect of the fermentation variables was analyzed using response surface design (RSD). To determine whether or not the value of their interactions was statistically significant, the p-value utilized. It determined that the p-value higher than (0.05) is statistically significant or vise-verse. The variables fit the model, as shown the relation coefficient R^2 of the model which is 46.77%, (table 3). The variables are constant, the temperature is significant on the biobutanol yield. The variables time, pH, time*time, temperature*temperature, pH*pH, time*temperature, time*pH, temperature*pH all have P-values higher than a-value indicating that their effect on biobutanol yield are statistically insignificant.

Table 3: Results of Regression Analysis showing the estimated coefficient of the Model

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF	S
Constant		123.62	6.68	18.52	0.000		S
Time	-14.68	-7.34	4.09	-1.79	0.088	1.00	NS
Temp	22.86	11.43	4.09	2.80	0.011	1.00	S
pH	4.89	2.44	4.09	0.60	0.557	1.00	NS
Time*Time	14.47	7.24	6.02	1.20	0.243	1.01	NS
Temp*Temp	3.05	1.52	6.02	0.25	0.803	1.01	NS
pH*pH	4.25	2.12	6.02	0.35	0.728	1.01	NS
Time*Temp	-20.25	-10.13	5.78	-1.75	0.095	1.00	NS
Time*pH	-11.10	-5.55	5.78	-0.96	0.349	1.00	NS
Temp*pH	-9.33	-4.66	5.78	-0.81	0.429	1.00	NS

Key: S= statistically significant, and NS= Statistically not significant, Regression Equation Coefficient (COef), Standard error for coefficient (SE), T-Value= Statistic value, P-Value= Probability value.

Regression Equation in Uncoded Units

Yield cm3 = -100 + 1.94Time + 6.4Temp + 16.4pH + 0.0126Time*Time + 0.061Temp*Temp
+ 0.94 pH*pH - 0.0844 Time*Temp
- 0.154 Time*pH - 0.622 Temp*pH

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
16.3518	46.77%	22.82%	0.00%

Effect of Reaction Temperature (^{0}C) and Time (hrs) on Biobutanol yield

Figure 1 below shows that at a temperature between 32.5^{o}C—35.0 (^{0}C), the yield increases with time such that a yield greater than 160 cm^3 can only be reached when it is between 24—34 (hrs). In similarly vein, the temperature range of 25^{o}C—25.5 (^{0}C) and time interval of 24—67 (hrs) produced the lowest yield. Therefore, the biobutanol also grows when the temperature and time increase.

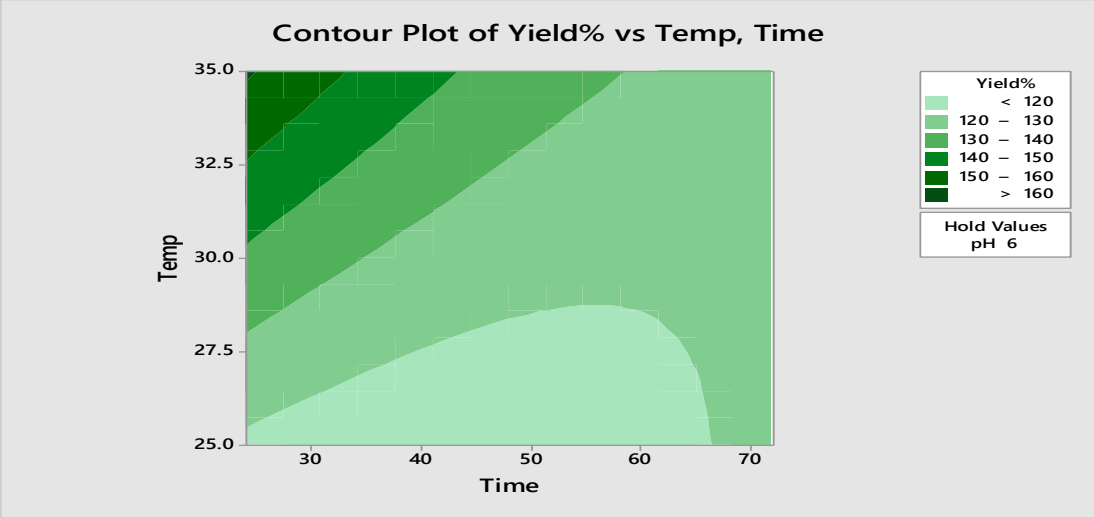

Figure 1: Contour plot of interaction of Temperature reaction and Time on Biobutanol yield.

Effect of Reaction Temperature (^{0}C) and Reaction pH on Biobutanol yield

The yield increase at temperature between 33.0^{o}C—35 (^{0}C), as shown in figure 2 below. the yield more than 140 cm^3 can only be reached at a pH is between 4.5—7.5. In a similar vain the pH range of 4.5—5.0 and temperature range of 25.0^{o}C—25.5 (^{0}C). Produced the lowest yield, thus the amount of biobutanol increases as the temperature and pH rise.

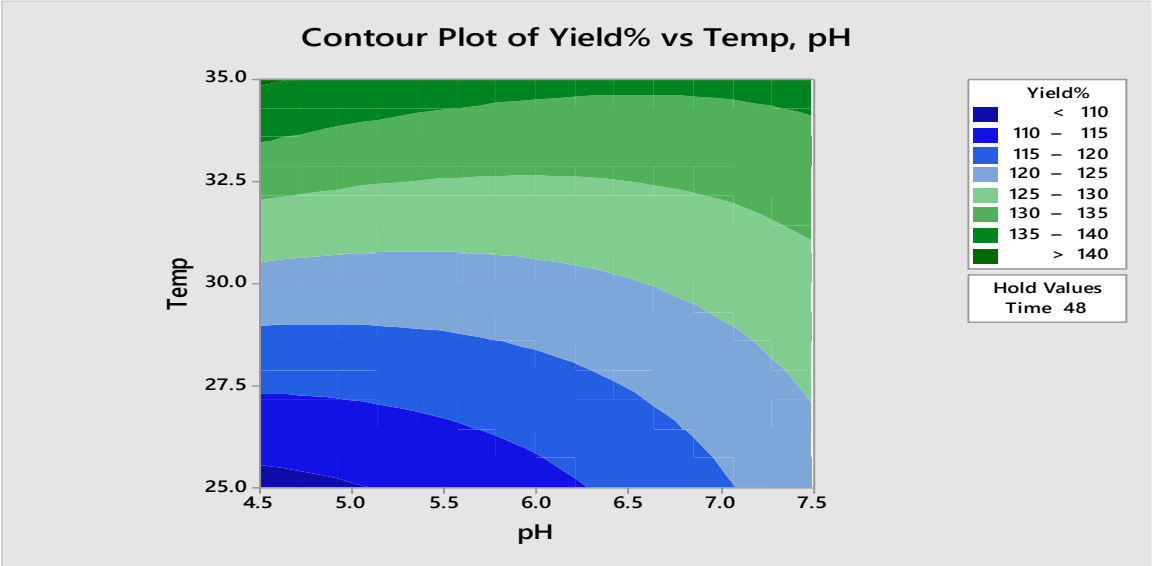

Figure 2: Contour plot of the interaction of Temperature (^{o}C) and Reaction pH on Biobutanol yield

FT-IR Analysis

The transmittance and peak in Figure 3 indicate the functional groups of the sample's produced biobutanol. The broad and powerful O-H peak in the 3622.04-3205.66 cm^{-1} region and C=O peak in the region of 1640.0-1700 cm^{-1} (Appendix IV). These findings are in consistent with the study that Sanusi et al. (2021) published.

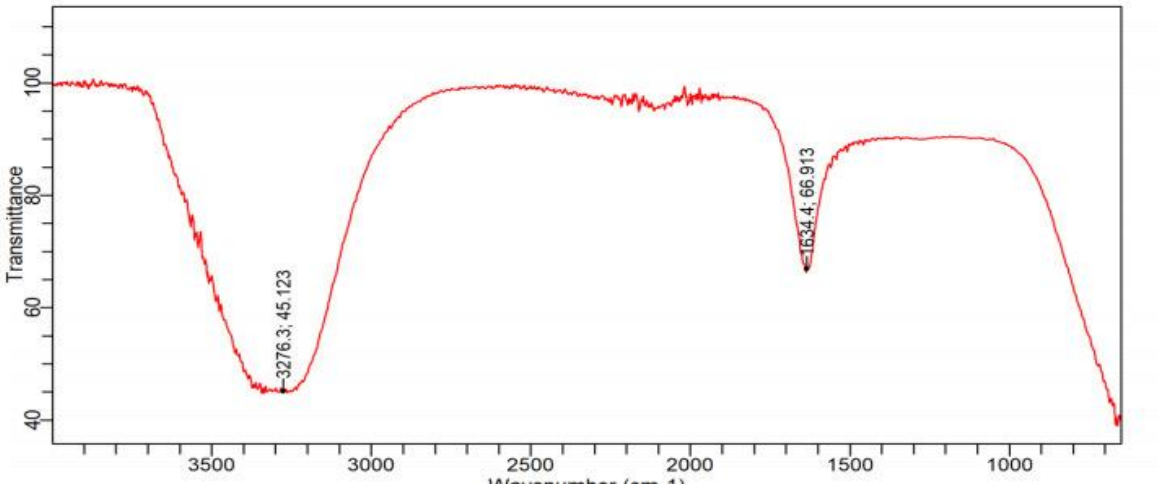

Figure 3: The FT-IR Spectrum of the biobutanol.

GC-MS Analysis

The Gas Chromatography-Mass Spectrum (GC-MS) was used to characterise the compounds that remained after fermentation and distillation. The different spectra of a 13 compounds are displayed in figure 4 below. A details analysis of the spectra from the chromatogram on mass spectrometry revealed that the compounds are mostly alcohols, fatty acids, and fatty acid aldehydes. The fermentation products contained alcohol 1-Butanol at area (4.40%) higher than work reported by Sanusi et al. (2021).

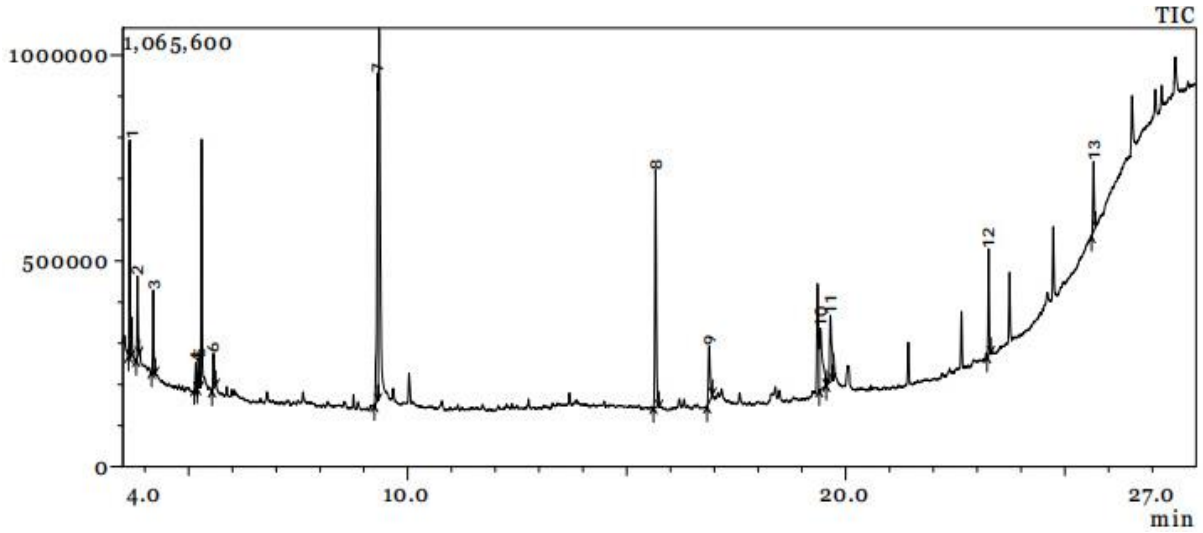

Figure 4: GC-MS Spectrum of the biobutanol

CONCLUSION

This study showed that the proximate analysis of sugarcane bagasse and the fermentation of sugarcane bagasse hydrolysates with the selected media (*Clostridium Perfringens*) achieved results that were similar to or even closer to those reported in recent literature, achieving highest yield of 163 cm^3 (0.543g/L) at 35°C and pH 6.0. The use of lignocellulosic sugars (second-generation biomass) after acid hydrolysis and the determination of reducing sugar, highlighted the potential for future development of ABE (Acetone, Butanol, and Ethanol) fermentation. The results were achieved successfully by using response surface methodology which optimize the fermentation parameters (Temperature, Time, and pH) into the lower and upper level and also analyzes the yield of the biobutanol. *Clostridium Perfringens* in this study has shown potential for biobutanol production from Bagasse, it was reported to be the most ideal and used organism for butanol production. The produced biobutanol from the sugarcane bagasse hydrolysate was characterized using FT-IR and GC-MS analysis, which confirmed the broad and intense O-H peak in the 3622.04-3205.66 cm^{-1} region and C=O peak in the region of 1640.0-1700 cm^{-1}, and chromatogram on mass spectrometry revealed that the compounds include alcohols, fatty aldehydes, and fatty acids as the major compounds also the alcohol 1-Butanol (4.40%) was found in the fermentation products respectively. Based on the findings of this research work we recommend that purification must be done after fermentation to remove some impurities for higher butanol yield, the Yield of the Biobutanol should be increased, and there is for more research should be conducted on selecting the most efficient and effective media for ABE fermentation and also hydrolysis

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