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Response Surface Optimization of Biobutanol Production from Boiled *SUWAN-1-SR* Corn Cobs

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ABSTRACT

In this study, response surface methodology (RSM) was applied to optimize the conditions during the acetone-butanol-ethanol (ABE) fermentation of hydrolyzed boiled corn cobs to produce biobutanol using Escherichia coli (E. coli). The species of corn used was SUWAN-1-SR and High-Performance Liquid Chromatography (HPLC) was used to find the proportion of biobutanol in the fermentation process of ABE. A three-variable Central Composite Design (CCD) with 20 runs was employed to develop a statistical model for the fermentation process. Analysis of variance (ANOVA) was used to analyze the experimental data, and the F-test was used to determine the significance of the model factors. Results showed that the optimal fermentation conditions, which included a maximum temperature of 80.70°C, concentration of 0.46mol/dm^3, and duration of 27.20 minutes, gave the best maximum sugar output of 1941.33g/l. A quadratic polynomial function was obtained for biobutanol production using regression analysis. ANOVA showed that both temperature and concentration were highly significant (F-value$_{(cal)}$ < F-value$_{(tab)}$ at 1% significance level) factors in the yield of biobutanol. The effect of time was nonsignificant. There were highly significant interaction effects of temperature and concentration as well as temperature and time in the yield of biobutanol. There was a highly significant F-test for the model, suggesting that the model could be used to predict the response variable accurately. These findings demonstrate that under these ideal circumstances, the E. coli used in this investigation had its maximum metabolic activity.

1. Introduction

Over the past few decades, a great deal of research has been done on renewable sources of liquid fuels to replace fossil fuels due to long-term economic and environmental concerns. Burning fossil fuels like coal and oil releases carbon dioxide (CO_2), one of the main causes of global warming (Kumar et al., 2009). Biofuel is a suitable alternative to solving the problem associated with fossil fuels since it is economical, renewable, and environmentally friendly. Due to its many advantages, the fermentation-based synthesis of biobutanol for use as biofuel is gaining a lot of attention. Among these benefits are increased energy content, less water adsorption and corrosion, improved blending capabilities, and smooth compatibility with conventional internal combustion engines. Various strains of *Clostridium* anaerobic bacteria that produce solvents can be fermented to produce biobutanol from starch or sugar-based substrates. (Bhatia et al., 2020). However, the demand for feedstock that is cheap and reliable seems to be a major concern in biofuel production. The exploitation of edible feedstock for biofuel is not recommended as this will lead to the shortage of edible materials for consumption. The production of biobutanol from lignocellulosic biomass, the most available raw material, has made the biofuel production process more economical, reliable, and a worthwhile process.

In smaller quantities, plant biomass also contains ash, protein, pectin, hemicellulose, lignin, cellulose, and hemicellulose. Depending on the species of plant, these components can have different compositions. Conditions such as age and growth stage affect the ratios of different components within a single plant. (Devi et al., 2022). These polymers have varying degrees of interaction with one another in a hetero matrix and a varied relative composition, depending on the kind, species, and even the source of the biomass. Three polymers, cellulose, hemicellulose, and lignin, make up the majority of lignocellulose biomass. Based on the type of lignocellulose biomass, these polymers are organized in complex non-uniform three-dimensional structures to different degrees and varying relative composition (Bajpai, 2016). Figure 1 shows the interior structure of a plant cell, the position of hemicellulose, lignin, and cellulose, and their chemical structures.

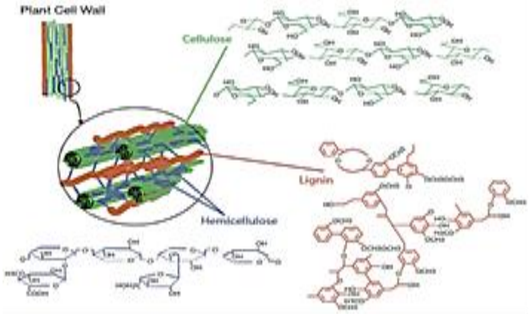

Figure 1: Position of hemicellulose, lignin, and cellulose in a plant cell and their chemical structures (Source: Sun & Cheng, 2002).

The primary constituent of lignocellulose biomass is cellulose. In contrast to glucose in other glucan polymers, the repeating cellulose in the chain is represented by the disaccharide cellobiose, whose structure is comprised of vast intra- and intermolecular hydrogen bonding networks that firmly bind the glucose units as illustrated (Isikgor & Becer, 2015).

Lignocellulose biomass is a desirable feedstock due to its high polysaccharide content and reasonable pricing. Utilizing lignocellulose resources to produce butanol and other compounds is not simple — pretreatment is necessary to change the lignocellulose structure. Consequently, larger yields and a faster hydrolysis rate can be attained. Pretreatment generally strives to reduce lignin content (Birgen et al., 2019; Jang et al., 2012; Wang et al., 2017). The crystalline structure of cellulose can be decreased by pretreatment, which is beneficial for the subsequent hydrolysis. Although the anaerobic digestion process used to make ethanol is unable to utilize the pentose sugars coming from hemicellulose degradation, it should be noted that the capacity to remove hemicellulose is typically considered a benefit in the pretreatment procedure. (Kumar et al., 2009). The pretreatment objective is the conversion of biomass to biofuels (Figure 2).

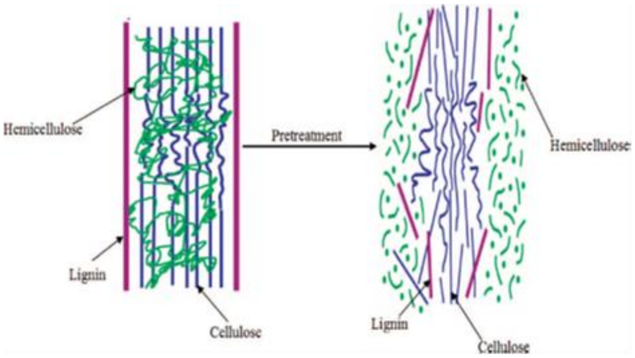

Figure 2: Schematic of the role of pretreatment in the conversion of biomass to fuel (Source: Kuhar et al., 2008).

Corn cob contains enough cellulosic material, which is the best source of fermentable sugars (Kim, 2018). Tons of corn cob are discarded annually as waste after the corn or maize grain has been processed into various food products (Chen et al., 2021). The corn cobs end up in open dumps or drainage systems where they cause environmental pollution, contaminating ground and surface water bodies. Therefore, it is crucial to turn these wastes into valuable final products. (Mudzanani, 2015). To produce fermentable sugars from biomass, there is a need for pretreatment, which is usually the initial process to improve the accessibility to the cellulose polymers (Kim, 2018). For greatest yield and production rate, a deeper understanding of the process inputs is made possible through the optimization of biofuel production processes.

Optimizing the processes involved in producing these biofuels is the main goal to increase yields (Ghosh et al., 2012). An appropriate experimental design can obtain and solve multivariate equations concurrently with the help of Response Surface Methodology (RSM), a statistical technique for modelling and optimizing numerous variables (Ferreira et al., 2009). It investigates the connections between one or more response variables and many explanatory variables. The RSM's primary objective is to identify the best possible response and find the optimum that does not maximize only one reaction that is crucial when there are multiple responses. The second objective is to ascertain how altering the design factors affects the response's direction of change. The graph makes it easier for the ridge lines, hills, valleys, and response surface shapes to be seen (Sarrai et al., 2016). The primary barrier to the synthesis of biobutanol is the high energy cost of product recovery through conventional distillation because of the low butanol titre in fermentation broth brought on by the extreme cell toxicity of butanol. (Kumar & Gayen, 2011).

2. Materials and Methods

2.1 Materials

Materials and equipment used were corn (SUWAN-1-SR species), round bottom flask, electronic balance, conc. H_2SO_4, and E. coli.

2.2 Methods

About 10g of species sample was used for each experiment, and 100ml of diluted H_2SO_4 was added to the sample in the conical flask and heated at the stipulated temperature and set time. Then 2ml of NaOH solution was added to neutralize it until a pH of 7 was obtained. Exactly 1ml of 3,5-dinitrosalicylic acid (DNS) was then added to the 3ml of the neutralized solution and poured into the boiling tube. Then 4 ml of distilled and deionized water was added to dilute it by 50%. The pretreated sample was maintained at approximately 40–50°C, cellulase was then added to the solution and stirred. Then 100ml of E. coli was introduced into the solution, which was allowed to undergo fermentation. The percentage of biobutanol produced in the fermentation broth was determined by high-performance liquid chromatography (HPLC).

2.3 RSM Model Development

In this study, concentration, temperature, and duration were selected as factors in the Central Composite Design (CCD). Their effects on the corn cob pretreatment were investigated by the CCD. The process was optimized based on the number of runs (20 runs) in Design Expert (Stat-Ease Inc., USA) and the CCD was appropriate for the quadratic RSM. Table 1 shows the factors, and their minimum and maximum values used in the CCD.

To reduce the impact of unexplained variability in response caused by outside influences, the experimental runs were randomized. Equation 1 shows a polynomial of second degree that was fitted to the experimental data.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} {x_1}^2 + \beta_{22} {x_2}^2 + \beta_{33} {x_3}^2 \qquad (1)$$

Where, Y = predicted response, x_1 = temperature, x_2 = time, x_3 = concentration, β_0 is a constant, and β_1, β_2, and β_3 are coefficients of the various factors. It should be noted that these are independent variables.

Table 1: The three factors used in the central composite design.

Factor	Property	Unit	Min.	Max.
X_1	Temperature	°C	40	120
X_2	Time	Minutes	10	60
X_3	Concentration	Mol/dm^3	10	1.0

3. Results and Discussion

3.1 Experimental Results for Pretreatment of Feedstock

As stated, dilute sulfuric acid was used to pretreat the lignocellulose biomass into glucose monosaccharide. 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 Table 2 to determine the number of runs for the feedstock.

Using regression analysis, the response was fitted to a quadratic function. Temperature (A), concentration (B), time (C), and sugar yield (Y) are related in the suggested model. The coded factors represent the regressed model of equation 2.

$$Y = 1585.60 + 468.20A + 257.37B + 43.06C + 340.45AB + 265.77AC - 17.15BC - 225.30A^2 - 320.22B^2 - 296.29C^2 \quad (2)$$

Equation 2 made it easier to ascertain how various factors, taken separately or in combination, impacted the reaction. While negative coefficients indicated negative factor interactions and their impacts on overall yield, positive efficiency values demonstrated positive factor interactions and their effects on sugar yield. Actual sugar yield (wt. %) was 7.29%.

Table 2: Central Composite Design for dilute acid hydrolysis of SUWAN-1-SR.

Run	Factors in the Central Composite Design			*SUWAN-1-SR* Response
S/No.	Temperature (°C)	Conc. (Mol/dm^3)	Time (Minutes)	Boiled corn cob (mg/l)
1	120	0.55	35	1707.16
2	103.784	0.282428	20.13	214.488
3	40	0.55	35	20.4588
4	56.2159	0.282428	49.86	27.6784
5	103.784	0.282428	49.86	1026.7
6	0.8	0.1	35	233.439
7	56.2159	0.817572	20.13	396.785
8	80	0.55	35	1373.25
9	56.2159	0.282428	20.13	796.574
10	80	0.55	35	1538.4
11	103.784	0.817572	20.13	1694.52
12	80	0.55	35	1699.03
13	56.2159	0.817572	49.86	214.488
14	80	0.55	10	554.715
15	80	0.55	35	1643.98
16	80	1	35	957.212
17	80	0.55	35	1643.98
18	80	0.55	35	1643.98
19	80	0.55	60	771.306
20	103.784	0.817572	49.86	2057.31

3.2 Statistical Analysis

Table 3 shows the ANOVA in CCD for the yield of biobutanol in the fermentation of boiled SUWAN-1-SR corn cobs. As shown in Table 3, A, B, AB, AC, A^2, B^2, and C^2 were important model terms in this study. In other words, both temperature and concentration were highly significant factors in the yield of biobutanol, while the effect of time was nonsignificant. There were highly significant interaction effects of temperature and concentration as well as temperature and time in the yield of biobutanol. However, the interaction effect of concentration and time was nonsignificant in the yield of biobutanol. A highly significant *F*-test for the model (Table 3) suggests that the model could be used to predict the response variable accurately.

Furthermore, since the computed *F*-value for Lack-of-Fit (3.39) is less than the tabular *F*-value at 1% significance level (5.64), the Lack-of-Fit is statistically nonsignificant. According to several studies, noisy data have a 10.33% chance of producing a significant Lack-of-Fit *F*-value. Thus, a nonsignificant mismatch in the model's fitness obtained in this study suggests a less noisy data.

3.3 The Fit Statistics

Table 4 is the summary statistics for the yield of biobutanol in the fermentation of boiled SUWAN-1-SR corn cobs, Table 4 shows that there is less than 0.2 discrepancy between the Adjusted r² and the Prediction r². Analyzing the signal-to-noise ratio (S/N) was done by Adequate Precision, which compares the range of the predicted values at the design points with the average prediction error. Usually, an S/N higher than 4 is preferable. Thus, an S/N of 17.307 obtained in this study is sufficient to move around the design space and utilize this model.

Table 3: Analysis of Variance (ANOVA) in Central Composite Design (CCD) for the yield of biobutanol in the fermentation of boiled SUWAN-1-SR corn cobs.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	Computed F-value	Tabular F-value	
					5%	1%
Model	9	8.337E+06	9.263E+05	30.04**	3.02	4.96
A – Temp.	1	2.994E+06	2.994E+06	97.10**	4.96	10.04
B – Conc.	1	9.046E+05	9.046E+05	29.34**	4.96	10.04
C – Time	1	25322.60	25322.60	0.8213ns	4.96	10.04
AB	1	9.273E+05	9.273E+05	30.07**	4.96	10.04
AC	1	5.651E+05	5.651E+05	18.33**	4.96	10.04
BC	1	5.651E+05	5.651E+05	0.0763ns	4.96	10.04
A^2	1	7.315E+05	7.315E+05	23.73**	4.96	10.04
B^2	1	1.478E+06	1.478E+06	47.93**	4.96	10.04
C^2	1	1.265E+06	1.265E+06	41.03**	4.96	10.04
Residual	10	3.083E+05	30833.32	-	-	-
Pure Error	5	70275.35	14055.07	-	-	-
Lack of Fit	5	2.381E+05	47611.57	3.39ns	3.33	5.64
Totals	19	8.645E+06	9.57E+05	-	-	-

** highly significant

ns nonsignificant

Table 4: Summary statistics for the yield of biobutanol in the fermentation of boiled SUWAN-1-SR corn cobs after modelling by RSM.

Indicator	Value
Standard Deviation (mg/l)	175.59
Mean (mg/l)	1,010.77
Coefficient of Variation (%)	17.37
r^2	0.9643
Adjusted r^2	0.9322
Prediction r^2	0.7551
Adequate Precision	17.3066

3.4 Graphical Representation of Regression Model for Optimization of Fermentation Conditions to Produce Biobutanol from Boiled SUWAN-1-SR Corn cobs

Response surface plots for the effects of model factors on the yield of biobutanol from boiled *SUWAN-1-SR* corn cobs are shown in Figure 3, which helped to identify the optimal values of the variables that affected the acid hydrolysis. As shown in Figure 3a, the yield of total sugar from boiled *SUWAN-1-SR* corn cob increased with temperature and concentration. Similarly, the yield of total sugar from boiled *SUWAN-1-SR* corn cob increased with increasing temperature and time (Figure 3b). Regarding the effect of concentration and time, boiled *SUWAN-1-SR* corn cob yielded more total sugar when the time and concentration were increased (Figure 3c).

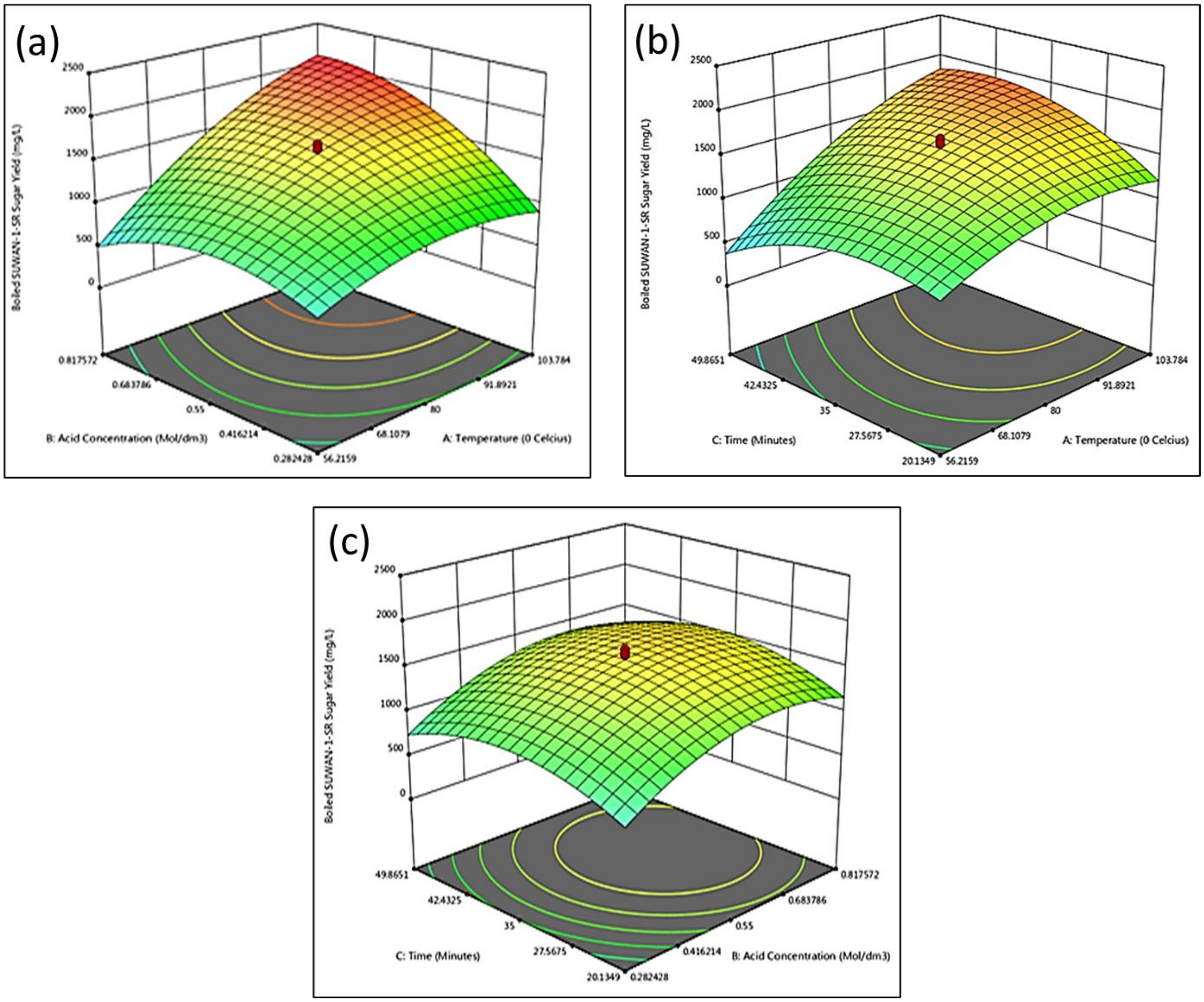

Figure 3: Response Surface Plots for the Effect of (a) Acid Concentration and Temperature, (b) Temperature and Time, and (c) Acid Concentration and Time on Total Sugar Concentration of Boiled *SUWAN-1-SR* Corn cob.

4.0 Conclusions

Biobutanol production by E. coli fermentation of total sugar obtained from pretreatment of *SUWAN-1-SR* corn cob was investigated. This was carried out as an integrated system encompassing feedstock pretreatment, enzymatic hydrolysis, microbial fermentation, and product recovery through distillation. After the optimal fermentation conditions were identified as 80.70°C maximum temperature, 0.46mol/dm^3 acid concentration, and 27.20 minutes, the best maximum sugar output of 1,941.33g/l was attained. The analysis using HPLC was done to find the proportion of biobutanol in the fermentation process of ABE. Biobutanol was found to provide a percentage concentration of 7.29%. These findings demonstrate that under these ideal circumstances, the fermenting organism, E. coli, used in this investigation had its maximum metabolic activity.

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