



Original Research Article

Optimization of Bioethanol production from Cocoa (*Theobroma cacao*) Bean Shell

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A B S T R A C T

This work produces ethanol from cocoa bean shell. The dried, sorted cocoa bean shell was milled and hydrolysed using H₂SO₄. The hydrolysis was optimized by varying the temperature (30 to 80 °C), time (50 to 150 min) and acid molar concentration (0.5 and 5 M/dm³) using Central composite design of response surface methodology. The response for the hydrolysis was the reducing sugar concentration. The optimum yield variables from the hydrolysis were used to design the fermentation process. The fermentation was optimized by varying the yeast concentration (0.01 to 0.05mg/g), fermentation time (24 to 144 h) and pH (4.5 to 7.0). Optimum reducing sugar was obtained at 0.5M/dm³ acid molar concentration, 80 °C and 150 min which produced 45.083 mg/g of reducing sugar while bioethanol optimum yield was produced at pH 8, 0.05 mg/g of yeast concentration and 144 h which produced 8.46% bioethanol. Statistical analysis showed that the model was significant ($F \leq 0.05$) while pH (A) and A² were significant ($F \leq 0.05$) model terms. The R² and R² adjusted values were 0.7706 and 0.5642 respectively. The result showed that pH had the highest effect on the cocoa bean shell bioethanol yield followed by fermentation time and the yeast concentration.

Keywords

Bioethanol,
cocoa bean
shell,
hydrolysis,
fermentation,
response
surface
methodology

Introduction

Fossil fuels are the most developed energy source, but raise many doubts regarding issues related to environmental sustainability and economy (Papini and Simeons, 2010). The increasing problems of carbon dioxide emissions in addition to some energy security concerns have strengthened the interest in alternative non-petroleum-based sources of fuel (Alvira, *et al.*, 2010). The reduction of world oil reserves is aimed at causing an increase in price (Kilian *et al.*,

2009; Saxena *et al.*, 2009; Korpela, 2006). Moreover, burning of petroleum-based fuels is the main cause of the rise in atmospheric carbon dioxide concentration, which is considered to be the main reason for climate change (Allen *et al.*, 2009; Solomon *et al.*, 2009).

The noticeable characteristics of ethanol distinguish it as a better alternative fuel. It has high octane number and rating with high

latent heat of vaporization, and low emission of toxic compounds on combustion (Mathewson, 1980). The combustion of gasoline produces water, carbon dioxide, carbon monoxide and other impurities such as oxide of sulphur and nitrogen, and heavy metals. On the other hand, pure ethanol is burned to produce carbon dioxide, water and a much lower amount of carbon monoxide (Akpan *et al.*, 2008). Interest in biogas technology is increasing around the world as a result of the requirements for renewable energy production in addition to reduction in harmful emissions and reuse of waste materials.

Since the 1970s, there has been considerable interest in developing bio-renewable alternatives to petroleum-based commodities such as transportation fuels. The most common example is bioethanol, which has emerged as a potentially important alternative transportation fuel (Bennett and Anex, 2009). Remarkable efforts have been put into studying the potential of different agricultural crops as feedstock for bio-production of fuels and chemicals. Biomass is the only suitable and renewable primary energy resource that can provide alternative transportation fuels such as bioethanol and biodiesel in the short-term (Hamelinck *et al.*, 2005; Sun and Cheng, 2002). Low cost, huge supply and easy conversion have made carbohydrate from corn and sugar cane the most popular feedstocks for bio-commodities like ethanol (Bennett and Anex, 2009). However, as feedstocks such as corn and sugarcane are basically food, their use in bioethanol production has been criticised due to rising food prices and global food shortage (Mussatto *et al.*, 2010).

Bioethanol from lignocellulosic biomass such as agricultural residue is one of the important alternatives being considered for large scale production and are expected to be major feedstocks for production of this

biofuel in the future (Awolu and Ibileke, 2011; Mussatto *et al.*, 2010). This biomass is an alternative inexpensive source of fermentable sugars (Gray *et al.*, 2006). Cocoa bean shell which was used for this study is a by-product of cocoa and the testa immediately surrounding the cocoa nib which constitutes about 10% of the cocoa seed (Ayinda *et al.*, 2010). According to Aina (1998), about 10,500 tonnes of cocoa bean shell is produced annually in Nigeria which constitute disposal problem in the cocoa milling and chocolate industry. The optimum conditions for the production of bioethanol were investigated using response surface methodology (RSM) based on a three-factor central composite design (CCD) which involved temperature ($^{\circ}\text{C}$), molar concentration of acid (mol/dm^3), yeast concentration (mg/L), pH and time (mins and hrs).

Materials and Methods

Materials

The cocoa bean shell was obtained from Coop Cocoa Processing Industry, Akure, Nigeria. Sulphuric acid, potassium hydroxide, dinitrosalicylic acid-all of analytical grade and *Saccharomyces cerevisiae* were purchased at Pascal Scientific, Akure, Nigeria.

Pretreatment

This was done using the method described by Alvira *et al* (2010). Cocoa bean shell was sorted, dried at 105°C in a hot air oven (Laboratory oven, DHG-9101.1SA by Unigold Medical, England) and comminuted using a mill (Asiko A11 double grinding mill). It was sieved using $500\mu\text{m}$ sieve size and packed in polyethylene bags to protect from moisture and stored at room temperature till further use.

Hydrolysis

The method described by Palmquist and Halm-Hagerdal (2010) was used. Molar concentrations of H_2SO_4 from 0.5 to 5.0 mol/dm³ were used alongside temperature ranging from 30-80 °C and length of time from 50 to 150 mins. 40 g of fine sample was weighed into a 500 ml beaker and added 160 ml of the particular molar of acid for each experimental run and then stirred very well. Hydrolysis was done at the required temperature in a water bath (Uniscope SM801A Laboratory water bath) and time for each run. At the expiration of each operation, the treated material was allowed to cool and then filtered through a sieve cloth to remove the undigested debris. The pH of the hydrolysate was adjusted to 5.0 with 1 M potassium hydroxide solution. 95 g of fine sample was weighed into 500 ml beaker and added 380 ml of H_2SO_4 of the molar of the factors that produced the highest reducing sugar in the trial and hydrolysed using other corresponding factors (temperature and time). Other treatments for the trial were as well given but adjusted to the required pH (from another experimental design) than 5 alone.

Fermentation

The hydrolysate was fermented using baker's yeast from *Saccharomyces cerevisiae* at concentrations from 0.01 to 0.05 mg/L, pH range from 4.5 to 7 and time from 24 to 144 hrs at ambient temperature ($28\pm2^{\circ}C$) in improvised air tight fermentation set up.

Distillation

The recovery of ethanol was done using the method described by Wyman (2005) where the separated ethanol was concentrated via a second distillation at 78 °C.

Determination of Total Reducing Sugar

Dinitrosalicylic acid (DNSA) method was used as described by Natasa *et al* (2010) with slight modification.

Percentage Ethanol Yield

The percentage ethanol yield was calculated as described by Stephen *et al* (2013).

Results and Discussion

Hydrolysis

The acid hydrolysis carried out in different concentrations ranging from 0.5 to 5M produced the highest glucose yield of 45 mg/cm³ at 0.5 M. The hydrolysis at higher concentration of acid produced lower reducing sugars which could be attributed to the fact that at higher concentration of acid, glucose can be converted to levulin and formic acid (Klinke *et al.*, 2004) which eventually leads to decrease in glucose yield. The hydrolysis of cocoa bean shell at temperature ranging from 30-80 °C using different acid concentrations showed increase in glucose yield with time (Table 1). The yield at different molar concentrations, temperature and time is an indication of how each of these factors affected the yield (Table 1). At low acid concentration, high temperature and longer time, more glucose was produced than at a higher acid concentration, lower temperature and shorter time. At low acid concentration, high temperature and longer time, more glucose was produced than at a higher acid concentration, lower temperature and shorter time.

The response surface plot shows an increase in the reducing sugar yield along the side of molar concentration of acid as the temperature increased (Fig 1). The 3D plot

shows that the interaction between the two factors (molar concentration and time) was significant. Molar concentration affected reducing sugar yield more than time (Fig 2). The reducing sugar yield increased as molar concentration increased but later declined with increase in molar concentration. The effect of temperature and time on reducing sugar concentration as shown in Fig. 3 indicated that sugar concentration declined as time increased but continued on a steady rate with increase in temperature.

Fermentation

The factors for fermentation were optimized by RSM which involved yeast concentration ranging from 0.01 to 0.05 mg/L of cocoa bean shell hydrolysate, duration of

fermentation (time) from 24-144 hrs and pH 4.5 to 7. The highest percentage of ethanol of 8.46 was produced at pH 8, 0.05 mg/L of yeasts concentration and over a period of 144 hours (Table 3). The production of highest yield at pH 8 shows great deviation from optimum pH 5 to 5.2 for baker's yeast activity. This could be attributed to over liming as a result of high level of fermentation inhibitors in the hydrolysate (Olsson and Hahn-Hagerdal, 1996; Kline *et al.*, 2004).

At pH below 4.5 and above 8, there was no yield (Table 2). At yeast concentration of 0.03 to 0.05 and pH below 6.25, the corresponding yield was lower when compared with others having the same yeast concentration but higher pH.

Table.1 Process Variable of a Three-factor Central Composite Design (CCD) for Acid Hydrolysis with corresponding Response

| Experimental run | Factor 1 | Factor 2 | Factor 3 | Abs | Response 1 |
|------------------|----------|----------|----------|-------|------------|
| | 0.50 | 80.00 | 50.00 | 1.490 | 44.106 |
| 2 | 5.00 | 80.00 | 150.00 | 0.945 | 27.974 |
| 3 | 0.50 | 30.00 | 50.00 | 1.428 | 42.271 |
| 4 | 2.75 | 94.04 | 100.00 | 1.367 | 40.465 |
| 5 | 2.75 | 55.00 | 100.00 | 1.258 | 37.239 |
| 6 | 2.75 | 55.00 | 100.00 | 1.404 | 41.561 |
| 7 | 5.00 | 30.00 | 150.00 | 0.506 | 14.978 |
| 8 | 2.75 | 55.00 | 100.00 | 1.250 | 37.002 |
| 9 | -1.03 | 55.00 | 100.00 | 0 | 0 |
| 10 | 2.75 | 55.00 | 184.09 | 1.366 | 40.436 |
| 11 | 0.50 | 80.00 | 150.00 | 1.523 | 45.083 |
| 12 | 2.75 | 55.00 | 100.00 | 1.410 | 41.738 |
| 13 | 5.00 | 30.00 | 50.00 | 1.345 | 39.814 |
| 14 | 0.50 | 30.00 | 150.00 | 1.471 | 43.544 |
| 15 | 2.75 | 55.00 | 100.00 | 1.176 | 34.811 |
| 16 | 2.75 | 12.96 | 100.00 | 1.008 | 29.838 |
| 17 | 2.75 | 55.00 | 100.00 | 1.289 | 38.156 |
| 18 | 5.00 | 80.00 | 50.00 | 1.021 | 30.223 |
| 19 | 2.75 | 55.00 | 15.91 | 1.300 | 38.482 |
| 20 | 6.53 | 55.00 | 100.00 | 0.799 | 23.652 |

Factor 1: Molar concentration (mol/dm³), Factor 2: Temperature (oC), Factor 3: Time (mins), Response 1: Reducing Sugar concentration (mg/cm³), Abs: Absorbance (nm).

Table.2 Process variables of a Three-factor Central Composite Design (CCD) used for Fermentation with corresponding Response

| Experimental Run | Factor 1 | Factor 2 | Factor 3 | Vol | Response 1 |
|------------------|----------|----------|----------|------|------------|
| 1 | 8.00 | 0.01 | 144.00 | 3.5 | 2.96 |
| 2 | 6.25 | 0.03 | 84.00 | 7.0 | 5.92 |
| 3 | 4.50 | 0.05 | 144.00 | 4.0 | 3.38 |
| 4 | 6.25 | 0.03 | 84.00 | 6.0 | 5.07 |
| 5 | 8.00 | 0.01 | 24.00 | 1.5 | 1.26 |
| 6 | 9.19 | 0.03 | 84.00 | 0 | 0 |
| 7 | 6.25 | 0.03 | 184.91 | 5.5 | 4.65 |
| 8 | 6.25 | 0.03 | 84.00 | 7.0 | 5.92 |
| 9 | 6.25 | 0.03 | 84.00 | 7.0 | 5.92 |
| 10 | 4.50 | 0.01 | 24.00 | 3.5 | 2.96 |
| 11 | 6.25 | 0.03 | 84.00 | 7.0 | 5.92 |
| 12 | 8.00 | 0.05 | 144.00 | 10.0 | 8.46 |
| 13 | 6.25 | -0.00 | 84.00 | 0 | 0 |
| 14 | 4.50 | 0.05 | 24.00 | 2.5 | 2.11 |
| 15 | 6.25 | 0.06 | 84.00 | 3.5 | 2.96 |
| 16 | 4.50 | 0.01 | 144.00 | 3.5 | 2.96 |
| 17 | 8.00 | 0.05 | 24.00 | 4.0 | 3.38 |
| 18 | 3.31 | 0.03 | 84.00 | 0 | 0 |
| 19 | 6.25 | 0.03 | -16.91 | 0 | 0 |
| 20 | 6.25 | 0.03 | 84.00 | 6.0 | 5.07 |

Fig.1 Response plots showing the interactive effect of molar concentration and temperature on reducing sugar concentration

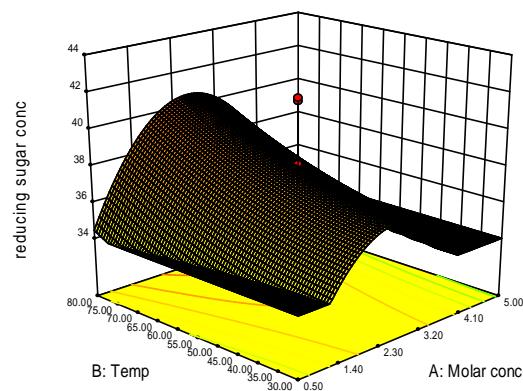


Fig.2 Surface and contour plots showing the interactive effect of molar concentration and time on reducing sugar yield

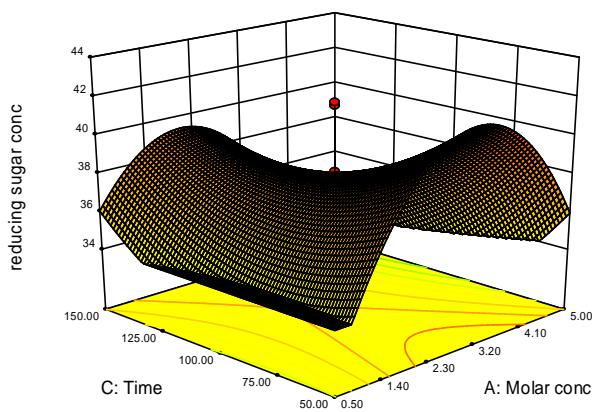


Fig.3 Response plots of the interactive effect of temperature and time on reducing sugar production

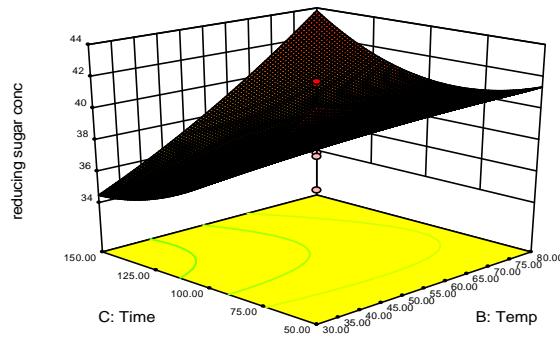


Fig.4 Response plots of the interactive effect of pH and yeast concentration on ethanol production

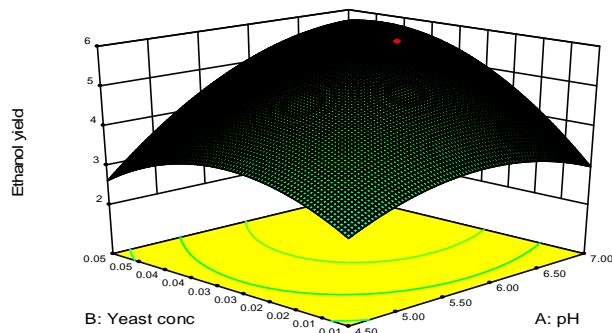


Fig.5 Surface and contour plots showing the interactive effect of pH and time on ethanol production

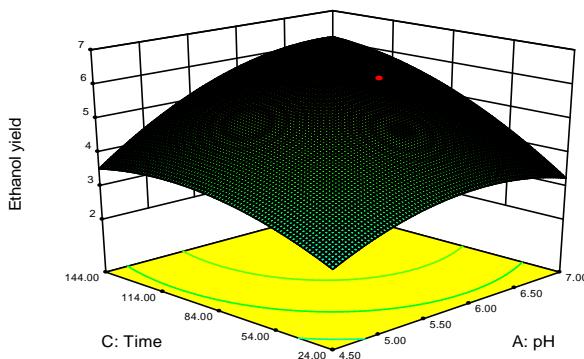
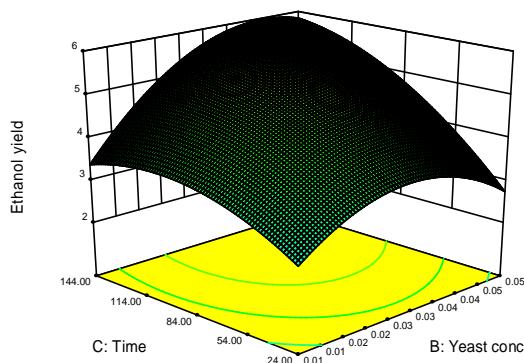


Fig.6 Surface and contour plots showing the effect of yeast concentration and time on bioethanol and yield



The analysis of variance of quadratic model indicates that model F-value of 3.73, is significant with $p<0.05$. A and A^2 are significant model terms ($p\leq 0.05$). R-squared of 0.7706 and Adjusted R-squared of 0.5642 give better prediction of the response and significance of the model.

The effect of pH and yeast concentration on bioethanol yield is shown in Fig. 4. The ethanol yield increased with increase in both pH and yeast concentration but later declined slightly towards the end of the process with further increase in the two factors. The effect of pH on the ethanol yield was more compared to that of yeast concentration.

The effect of time and pH on bioethanol yield is shown in Fig. 5. The plot showed that the interaction between the two independent variables affected the corresponding ethanol yield, indicating that both pH and time have positive effect on bioethanol yield. The effect of time was more pronounced than that of yeast concentration.

The study examined bioethanol production from cocoa bean shell using acid hydrolysis and *Saccharomyces cerevisiae*.

RSM was used as statistical tool to optimize the factors of hydrolysis and fermentation (molar concentration of acid, temperature,

time, pH and yeast concentration). The optimum hydrolysis conditions were identified to be: 0.5 M, 150 mins and 80 °C while 0.05 mg/L yeast concentration, pH 8 and 144 hours were identified for fermentation. The fermentation results indicate that RSM is a promising tool in optimisation of bioethanol production as pH high as 8 could not be thought of for yeast activity in ethanolic fermentation except in optimisation where there are values outside the ranges input into the model. As the bioethanol yield was low, therefore, further study to improve the yield and as regards the economics of bioethanol production from cocoa bean shell will be useful.

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