BIOETHANOL PRODUCTION FROM THE FERMENTATION OF CASSAVA WASTE. Annual General Meeting and International Conference of the Nigerian Society of Mechanical EngineersAt: Enugu, Nigeria

Conference Paper · February 2012

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BIOETHANOL PRODUCTION FROM THE FERMENTATION OF CASSAVA WASTE.

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ABSTRACT

This experiment has been carried out with the singular aim of converting the cassava waste to ethanol. To achieve this, the conversion of the organic waste was carried out via acid and microbial hydrolysis which yielded 66% fermentable sugar wort. This was then converted into ethanol by fermentation process using Saccharomyces cerevisae. 98% ethanol was obtained by distillation of the fermentable wort and the total volume of the ethanol produced from 105grams of cassava waste was 103.4ml. Fermentation logistic parameters where evaluated considering the percentage fermentable sugar yield from the biomass in study. It is more economical to produce ethanol from organic waste compared to gasoline from crude oil for vehicle engines. The total volume of ethanol produced was used to verify the optimization and room temperature was considered to be the optimum temperature for the fermentation of ethanol from cassava waste. 120 minutes offermentation time gave 96.76% production yield.

Keywords: Bioethanol, Cassava Wastes, Fermentation.

1.0 Introduction

Bioethanol is a form of renewable energy that can be produced from agricultural feed-stock. It can be made from common crops such as sugarcane, potato, cassava and corn. There has been debate about how useful bioethanol will be in replacing gasoline. Concerns about its production and use relate to increased food prices due to the large amount of arable land required for crops, as well as the energy and pollution balance of the whole cycle of ethanol production especially from cassava and corn**(**1).

Ethanol produced by fermentation has been found to serve considerably as transportation fuel for cars, trucks and trains. The production of ethanol is not limited but constantly replenished by growing plants and is advantageous over petroleum as a source of fuel in that petroleum source is steadily depleted with usage. Ethanol fuel has not been fully exploited because gasoline is getting narrower. Bioethanol fuel is an alternative fuel to petrol and diesel with less of negative environmental impact (2).

Bioethanol fuel is produced by the fermentation, distillation and dehydration of starch plants such as cassava, sugar plants and sometimes trees. The alcohol is then formed and can be used within cars in one of two ways. The bioethanol fuel can either be blended with petrol or used as a direct substitute for petrol. A 5% bioethanol blend can be used in all petrol engines but modifications are often required for higher blends or the sole use of this fuel. It is thus the principle fuel used as a petrol substitute for road transport vehicles (3,4).

MATERIAL AND METHODS

2.0. BIOETHANOL PRODUCTION PROCESS

The process of fermentation has been adopted for producing bioethanol from the cassava waste.

2.1 MATERIALS USED FOR YEAST CULTURE

All materials used in the culturing process were sterilized before usage and the reagents used were tested to be of analytical grade.

2.1.1 Apparatus Used

- Autoclave
- Spectrophotometer
- Conical flasks, Beakers and pipette
- Magnifying Glass

2.1.2 Reagents Used

- Sodium Hydroxide
- Sugar Solution
- Distilled Water
- Citrate-phosphate
- Yeast Extract

2.2 PROCEDURE FOR THE YEAST CULTURE

All media, chemicals and reagents used were prepared according to the manufacturersspecification. The culture media used were sterilized using the autoclave at a temperature of 121◦C for 15minutes while the glass petridishes, pipette and other glasswares were sterilized using the hot air oven at 160◦C For 1 hour.

The monoculture of two strains Saccharomyces cerevisae was purchased at Aba Chemical market, scientific line. 200g each of sugar and flour was added respectively to 250ml of water. The solution was heated to 100◦C for 45 minutes and then cooled to room temperature. 20g of dried yeast (Saccharomyces cerevisae) was added to the mixture. The yeast culture was maintained in the refrigerator at 20◦C until required.

2.3 FERMENTATION SETUP

2.3.1 Materials used for Fermentation setup

Various reagents and apparatus were used in the fermentation setup. It was ensured that all the apparatuses used for the fermentation were sterilized before use in order to avoid the contamination of our yeast cultures during the fermentation process and also to prevent the introduction unwanted microbes into the setup which would interfere with the activity of our yeast. Also, reagents used were of analytical grade and within the range of acceptable purity.

2.3.2 EQUIPMENT USED

- Sieve shaker.
- Hot plate or cooker.
- Conical flask with Tubes.
- Rotary evaporator.
- Visible Spectrophotometer.
- Automated pH meter
- Autoclave

2.3.3 POST HARVEST EQUIPMENT

- Washers
- Refrigerator
- Blender
- Hot air oven
- Sealers

2.4 PROCEDURE FOR THE PRODUCTION

Solid Cassava peel wastes were obtained from a rural farm at Ihiagwa, Imo State, Nigeria. They were sorted and then washed under running tap water to remove sand and other dirt particles. Samples of the cassava peels were dried over- night at 60◦C in a hot air oven and then milled into powder form (flour) by using an industrial blender and sieve shaker to extract the pure starch (dry milling). The moisture content was determined from the 35% weight (105g) per volume cassava peels. The mill or pure starch is mixed with water, alpha-amylase and passed through a hot plate where the starch is liquefied. Heat is necessary applied to enable the liquefaction process. A hot plate with a high temperature stage (120◦C - 150◦C) and a lower temperature holding period (90◦C) is used. The high temperature reduces bacteria level in the marsh. The marsh from the hot plate is then cooled at a room temperature pre-treated by the addition of 165g of dilute sulphuric acid and was heated to 100◦C for 6 hours to hydrolyze the starch to sucrose which is a fermentable sugar and this process is called Saccharification. The hydrolyzed material was soaked in water and drained four times. The solid residue was then de-watered and soaked 66g of sulphuric acid for 4 hours. The material was then de-watered, dried and re-mixed with66g sulphuric acid and maintained at 100◦C for 3 hours. The content was filtered to remove solid and recover the sugar/ acid solution. The sugar/acid solution was neutralized using sodium hydroxide to a pH of 5.5 for fermentation process. The starch were filtered, mixed and diluted with water to adjust the initial sugar concentration. The activated yeast culture was added together with 300ml of sugar syrup and fermentation was carried out in an air tight 100ml conical flask and the culture was incubated in a rotary shaker at 50rpm for 48 hours at 30◦C. Fermentation was done for four days at a temperature of 28◦C - 30◦C and at a pH value ranging from 5.0 – 5.5 was maintained throughout the fermentation period. At the end of the fermentation period, the ethanol was separated from the extract using simple distillation method at a 12 hour interval. The fermented mash now called ‘’beer’’ contains about 10% alcohol as well as the non-fermentable solids from the feed-stock and the yeast. 300ml of the fermented cassava peel was measured into a volumetric flask at 20◦C and was washed into the distillating flask placed on the hot plate with a thermostat and the condenser connected to a thermometer used to know when the temperature of the mixture is above the boiling point of ethanol with 50ml distilled water. The sample was distilled slowly into the distillating flask at a temperature between 78.8◦C - 80◦C and a total of 103.4ml distillate was collected after four days of fermentation.

2.5 DETERMINATION OF ETHANOL AND BIOMASS CONCENTRATIONS

The concentration of sugar in the wort was determined at a 24-hour interval (daily) using a sugar automatic analyzer (model YP-2378PI). The thin glucose plastic sample tubing from the glucose analyzer was inserted into the wort sample tube. The sugar analyzer was switched on the sample key was pushed. The sugar concentration was displayed with a minute and the value was recorded.

Every 24-hour interval (daily) of the fermented wort was distilled using the rotary evaporator. The biomass and bioethanol concentration was determined using absorption spectrophotometer (model 63 SPEC-nm). A small quantity of the sample was taken and placed in the spectrophotometer. The absorbance of the sample was taken at a wave length of 630nm using water as a blank.

2.6 TESTING THE PRODUCT FORMED

The physical properties of the product formed was determined and compared to the properties of bioethanol obtained from literature to ascertain whether the product formed was ethanol. The physical properties used include colour, specific gravity, and boiling points.

2.7 PRECAUTIONS

Due to the very sensitive nature of our experiment, adequate care was taken to avoid the contamination of our yeast and enzymes and the fermentation setup. We ensured that;

- The yeast culture was well cultured in a nutrient rich medium and was grown up to a known density before it was harvested and introduced into our fermentation setup.
- All the apparatuses used where well sterilized to prevent the introduction of unwanted microbes that could interfere with the activity of our yeast and enzyme
- The cassava samples used for the production set up where washed free of unwanted material that could affect the mass of the sample.
- The yeast culture was also thoroughly filtered to remove all forms of spore and mycelium of the growing yeast before it was introduced into the fermentation setup.

3.0 RESULT AND DISCUSSION

3.0 HAND TEMPERATURE VARIATION

Table 3.1: Temperature and pH variation with time for the fermentation.

Time(Days)	1	2	3	4
pH	5.5	5.4	5.0	5.5
Temperature(◦C)	29	28	28	30

3.1CONCENTRATION OF ETHANOL FERMENTED

Five different standards of ethanol concentration in percentagewas prepared from 99.9% ethanol stock solution. The absorbance reading of these standards where determined using a visible UV-Spectrophotometer and the graph of absorbance against the concentration of ethanol was plotted;

Table3.2: Absorption of varying concentration ethanol standard

Percentage Con. (%)	10	20	30	40	50
Conc. (mg/L)	0.01	0.03	0.05	0.07	0.09
Absorbance	0.0349	0.1047	0.01745	0.2443	0.3141

Table 3.2: Showing the Volume, concentration of ethanol fermented and % of ethanol fermented for four days.

Time (Days)	**Biomass Weight (g)**	**Volume of Ethanol fermented (ml)**	**Conc. Of Ethanol fermented (mg/ml)**	**% Conc. Of Ethanol fermented**
1	105	25.85	0.0188	24.62
2	105	30.45	0.0198	29.00
3	105	23.96	0.0185	22.82
4	105	23.14	0.0179	22.04

3.2 CONCENTRATION AND VOLUME OF SULPHURIC ACID USED FOR HYDROLYSIS

Using; $C_1V_1 = C_2V_2$

Where; C_1 = Molarity of Sulphuric acid

V_1 = Volume of concentration of acid required

C_2 = Concentration of Sulphuric acid used

V_2 = Volume of Solution.

From experiment;

C_1 = 18.02 g/mol

V_1 =?

C_2 = 0.1 g/mol

V_2 = 500ml

Therefore;

$$V_1 = \frac{0.1 \times 500}{18.02} = 2.78ml$$

3.3 THEORITICAL ETHANOL YIELD

Under anaerobic condition, glucose is converted to ethanol and carbon dioxide via glycolysis. The overall reaction produces two moles of ethanol and carbon dioxide for every mole of glucose consumed.

$C_6H_{12}O_6 2C_2H_5OH + 2CO_2$ + Energy (stored as ATP)

Every gram of glucose converted will yield 0.511 g of ethanol

3.4 MATHEMATICAL MODELINGAND OPTIMIZATION

3.4.1 Model fitting and Statistical analysis

A linear model without interaction was developed to predict the response as a function of independent variables. The linear model is described in equation 5.1 in this equation, Y is the predicted response, a_0 to a_4 are the constant coefficients[5].

$Y = a_0 + a_1 x_1 + a_2 x a_2 + a_3 x_3 + a_4 x_4$……………………….3.1

Where X_i (i = 1,2,,4,) are the independent variable

X_1 represents weight of biomass

X_2 represents ethanol concentration

X_3 represents temperature

X_4 represents fermentation time

The coefficient of the model for the linear response where estimated linear regression analysis. The mathematical model of the ethanol product yield (Y1) is presented in Equation 3.2

$$Y = 1.09757-0.004058X_1-0.0911X_2-0.00253X_3-0.000025X_4 \quad \ldots\ldots 3.2$$

The appropriateness of the model was judged from the determination coefficient R^2, which reveals a total variation of the observed values of activity about its mean. In this fitting, the regression coefficient are estimated about 0.79. The R^2 value shows no agreement between the experimental and predicted values.

3.4.2 Linear model with interaction

Because of the inappropriateness of the first model, we developed another liner model which incorporates the interactions of the independent variable. The model is expressed in equation 4.3 below.

$$Y = a_0+a_1x_1 + a_2x_2+a_3x_3 + a_4x_4+a_5x_1x_2 + a_6x_1x_3 + a_7x_1x_4 + a_8z_2x_3 + a_9x_2x_4 + a_{10}x_3x_4 \ldots\ldots 3.3$$

$a_o$to a_4 are the linear terms and a_5 to a_{10} are the interaction terms.

Where Xi (i = 1,2,3,4) are the independent variables

X1 represents weight of biomass

X2 represents ethanol concentration

X3 represents temperature

X4 represents fermentation time

The coefficient of the model for the linear with interaction response wre also estimated with linear regression analysis. The empirical mathematical model of the bioethanol product yield (Y) is presented in equation 3.4

$$Y = 94.65597+1.34722x_i - 2.16417x_2 + 0.11475x_3 + 0.06179x_4 + 19.71667x_1x_2 0.32x_1x_3 - 0.14296x_1x_4 - 0.26983x_2x_3 - 0.2272x_2x_4 + 0.00019x_3x_4 \ldots\ldots 3.4$$

The appropriateness of the model is also judged from the determination coefficient, R_2. In this model fitting, the regression coefficient are estimated about 0.97. The R^2 value shows a good agreement between the experimental and predicted values of the fitted model. It implies that 96% of the total variation in the response is justified by the model. The correlation between the experimental and the predicted values is also acceptable.

The adequacy of the model was checked with analysis variance (ANOVA). And the adequacy of the model was conformed.

3.5 OPTIMIZATION

The optimization was done with the aid of matlab optimization tool box. After optimization, the optimum conditions were discovered as follows;

- Minimum weight of biomass = 1.5
- Minimum temperature = 29◦C
- Maximum fermentation time = 120minutes
- And this condition gives a product yield of 96.76wt%.

4.6DISCUSSION

From the research experiment in which the yeast Saccharomyces cerevisae was used in the fermentation of bioethanol from Cassava waste, it was found that the alpha-amylase converted 66% sugar that was fermented to Ethanol. The volume of ethanol produced from 105g of cassava waste was 103.4ml.

About 10ml of yeast suspension was added to 300ml of cassava peels. This observation showed that the fermentation was efficiently done and the products are safe for use as ethanol. The concentration of ethanol in the distilled product is about 98.48% ethanol.

5.0CONCLUSION

The production of bioethanol from the fermentation of cassava waste has proven to be one of the most effective ways of producing fuels for road transport vehicle engines.

It can be concluded that in order to enhance the production of bioethanol through the fermentation of cassava waste in an industrial quantity, batch fermentation should be employed, the pH and temperature should be kept within a specific range.

A mathematical model was developed using the experimental results with the aid of MATLAB. Optimization tool box to obtain the condition as follows;

- Minimum weight of biomass = 1.5
- Minimum temperature = 29◦C

Maximum fermentation time = 120minutes

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