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Suitability of Groundnut Shells for Bioethanol Production using *Saccharomyces cerevisae*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Groundnut shells were milled to 100 microns and bioethanol was produced using acid hydrolysis and batch fermentation process. Acid hydrolysis was achieved using 5% sulphuric acid solution at 90^0C for 2 hours. Fermentation was proceeded after sterilization of wort in an autoclave at 115^0C for 30 minutes after which the mixture was pitched with baker's yeast (*Saccharomyces cerevisae*) and incubated for three days. A bioethanol yield of 27.5% was obtained from groundnut shells with an energy value of 14MJ/KG. This biofuel produced had a purity of 90.62%v/v which is within the range of acceptable commercial grade and proves groundnut shells could be used as a suitable feedstock for bioethanol production.

Keywords: Bioethanol; groundnut shells; hydrolysis; fermentation.

1. INTRODUCTION

Bioethanol is a biofuel, it refers to ethyl alcohol produced by microbial fermentation processes, as opposed to synthetically produced ethanol from petrochemical sources. It is obtained after distillation of the ethanolic wash emanating from fermentation of biomass-derived sugars [1]. Generally, fermentation is the breakdown of complex molecules in an organic compound

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under the influence of ferment such as yeast, bacteria, enzymes *etc*. Fermentation is a well-established and widely used technology for the conversion of grains and sugar crops into ethanol [2].

Nowadays, wastes are scattered all around. Burning them is one of the ways to eliminate them but it can only add pollution to the environment [3-5]. Unless our society shifts away from the consumption of crude oil and fossil fuels to the re-cyclical use of agro wastes such as lignocellulosic biomass, it is difficult to secure sustainability of human life [6-10]. The harmful elements that create health and environmental hazards are being gradually banned by environmental agencies [11]. The conversion of biomass into biofuels, chemicals, energy, and new materials is now vital to solving these problems [12,13,14]. The key to making bioethanol competitive as an alternative fuel is the ability to produce it from low-cost waste materials [15]. These lignocellulosic wastes not only offers the potential for being ideal feedstock for liquid biofuels (bioethanol, butanol) but has great potential in gaseous fuel production as well as value-added products [16]. Thus, biomass can play an important role in the domestic bio-based economy by producing a variation of biofuels and biochemicals that are currently derived from petroleum-based feedstock [17,18,19] and solving the problem of waste disposal [12]. Use of agricultural waste having zero economic value for bioethanol production gives a better way of efficiently utilizing agricultural land [20-23]. Sugarcane molasses, groundnut shells, rice husks, straw, corncobs, etc. are good substrates for bioethanol production.As, Groundnut shells contain high cellulose (67%), its utilization for ethanol production is studied to know the efficiency of fermentation and provide better yield [12].

2. MATERIALS AND METHODS

This experiment was carried out at biotechnology laboratory at the Federal Institute of Industrial Research, Oshodi, Nigeria. The Groundnut shells were sourced from the groundnut processing pilot plant in FIIR-Oshodi, Lagos, Nigeria. The shells were dried and milled to 100 microns to increase surface area.

2.1 Experimental Procedure

The Production of Bioethanol consists of four major stages: Pretreatment, Hydrolysis, Fermentation (of hexoses and pentoses), and Product separation/distillation.

2.2 Hydrolysis

Groundnut shells glucose (or wort) was produced from crude cellulose material using acid conversion method. 200g of Groundnut shells were mixed with 800ml of 5% sulphuric acid solution to form a slurry. The hydrolysis process was conditioned in a water bath at 90^0C for 2hrs. The hydrolysed sample was neutralized with 1N KOH solution and the slurry was filtered and washed with distilled water.

2.3 Fermentation

The fermentation medium consists (g/L) 1000ml of glucose syrup, 0.5g of magnesium sulphate ($MgS0_4.7H_2O$), 0.5g of Di-potassium Hydrogen Phosphate (K_2HPO_4), 2.65g of Ammonium Sulphite $(NH_4)SO_4$; and 1.8g of Ammonium Di-Hydrogen phosphate $(NH4)_2H_2PO_4$. The mixture was properly mixed and sterilized in an autoclave at 115^0C for 30mins. The fermentation medium was cooled to room temperature and subsequently pitched with Baker's yeast (*Saccharomyces cerevisiae*) at ($50x10^5$ cells/1000mL). The flasks containing the inoculated fermenting mashed were incubated at $25^0C \pm 2^0C$ for 3 days. Samples were withdrawn at 24hr intervals and analysed sugar brix (soluble solids), pH and total titratable acidity.The total soluble solids as sugar brix of the fermenting wort were determined using handheld refractometer (Abbe refractometer). One or two drops of sample was placed onto the refractometer surface lens and the reading was observed on the eyepiece of the hand refractometer.

Ten milliliters (10 ml) of fermenting mash was taken or measured into 50ml beaker. The pH was determined with the aid of a previously calibrated pH meter (model Hanna P^{2H}) with pH 4.0 and pH 7.0 buffers.

10 ml of sample was measured into a beaker, about 3-5 drops of phenolphthalein indicator was added to the sample. The sample was then titrated with 0.1N sodium Hydroxide (1/10 N NaOH) until the solution starts to turn pinkish to a purple colour endpoint. The amount of the sodium hydroxide solution used during the filtration was noted and recorded. The percentage total titratable acidity in the sample was calculated as follows.

$$\% \text{ TTA} = \frac{Titre\ value * 0.1\ N\ morality * milli\ equivalent\ weight\ of\ acid * 100}{volume\ of\ sample\ used * 1000}$$

2.4 Distillation

Distillation is the separation of the alcohol from other ingredients in the fermented mixture. In making fuel alcohol it is necessary to get all the alcohol and water separated. The separation of the alcohol and water by distillation was made possible by the fact that alcohol boils at about 173^0F. As the mixture of water and alcohol is boiled, vapours with a greater concentration of alcohol will be formed and liquid with a lesser concentration of alcohol will remain behind. The principle of adding heat is to boil the fermented wort and provide vapour for the distillation process.

2.5 Specific Gravity (S.G) of Alcohol

The specific gravity of the alcohol distillate or "stillage" was determined using a specific gravity bottle. With a clean grease and water free gravity bottle, empty bottle was weighed and thereafter filled with distilled water and the weight taken. The same bottle was also filled with the alcohol and then the weight taken. The specific gravity of the sample was calculated as follows;

$$\text{Specific gravity} = \frac{weight\ of\ sample}{weight\ of\ equal\ volume\ of\ water}$$

Thus, the value obtained from this calculation is looked up for its equivalent percentage by volume in official methods of Analysis.

2.6 Energy Value

The energy value of sample was measured with an IKA C5000 Bomb-calorimeter. This bomb calorimeter is a pressure-tight container of acid-resistant steel, which contains the burning material under a pressure of 30 bar of oxygen. The energy value was obtained by complete combustion of one gram bioethanol in the presence of sufficient oxygen.

3. RESULTS AND DISCUSSION

3.1 pH, Soluble Sugar and %TTA of Sample at Various Fermentation Intervals

The tables showing the pH, soluble sugar and total titratable activity of sample at various fermentation intervals are given below.

3.2 Concentration of Alcohol

The Specific gravity of sample was obtained using a 50ml specific gravity bottle at 25^0C.

$$\text{Specific Gravity} = \frac{weight\ of\ Sample}{weight\ of\ equal\ volume\ of\ water}$$

Weight of empty specific gravity bottle = 18.933g
Weight of empty specific gravity bottle + sample = 39.899g
Weight of sample = 39.899 – 18.933 = 20.966g
Weight of specific gravity bottle + water = 43.722g
Weight of water = 43.722 – 18.933 = 24.789g

$$\text{Specific gravity} = \frac{20.966g}{24.789g} = 0.84578$$

Concentration of Alcohol by weight from the Association of Official Analytical Chemist (A.O.A.C) Alcohol chart = 90.62% v/v

3.3 Percent Yield of Bioethanol

The yield of bioethanol was determined using the formula:

$$\text{Percent Yield} = \frac{Weight\ of\ obtained\ bioethanol(g)}{Weight\ of\ groundnut\ shells\ used(g)} X100\%$$

Weight of peanut shells used = 200g

To calculate the weight of obtained bioethanol (Actual yield): 200g of peanut shells per 800ml of sulphuric acid solution; 200g/800ml = 0.25g/ml

0.25g/ml X 2200ml = 55g

$$\text{Percent yield} = \frac{55}{200} * 100\% = 27.5\%$$

Figs. 1, 2 & 3 above illustrate that the pH and total soluble sugar of fermenting sample reduced significantly while %TTA increased as fermentation time increased. From Fig. 1, the decreasing rate of pH during fermentation is due to the fact that the nutrients were consumed by the microorganisms thereby excreting organic acids which are released into the medium, thus causing the decrease in pH. In Fig. 2, a decrease in sugar brix or reducing sugar of fermenting samples was due to the gradual conversion of sugar to ethanol. Fig. 3 shows an increase in total titratable acidity which is due to the carbonic acids produced by the yeast and bacteria during fermentation.

Table 1. pH of sample at various fermentation intervals

Fermentation time (hour)	pH (Run 1)	pH (Run2)	Avg. pH
0	6.16	6.12	6.140
24	5.96	5.93	5.945
48	5.54	5.51	5.525
72	5.22	5.18	5.200
96	4.96	4.93	4.945
120	4.46	4.50	4.480

Table 2. Soluble sugar of sample at various fermentation intervals

Fermentation time (hr)	Soluble Sugar Run1 (^{0}Brix)	Soluble Sugar Run 2 (^{0}Brix)	Avg. Soluble Sugar (^{0}Brix)
0h	31.5	30.5	31.00
24	20.4	20.6	20.50
48	15.7	16.1	15.90
72	10.5	10.5	10.50
96	6.2	6.0	6.10
120	3.5	3.0	3.25

Table 3. Titre value of sample at various fermentation intervals

Fermentation time (hr)	Titratable acidity Run1 (ml)	Titre value- Run 2 (ml)	Avg. Titre Value (ml)	% TTA
0h	5.06	5.04	5.050	0.30
24	6.74	6.69	6.715	0.40
48	7.27	7.33	7.300	0.44
72	7.84	7.87	7.855	0.47
96	8.56	8.64	8.600	0.52
120	9.21	9.19	9.200	0.55

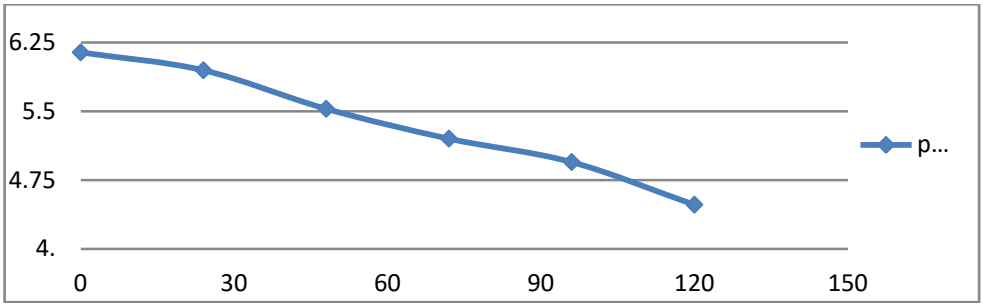

Fig. 1. Graph of pH against fermentation time

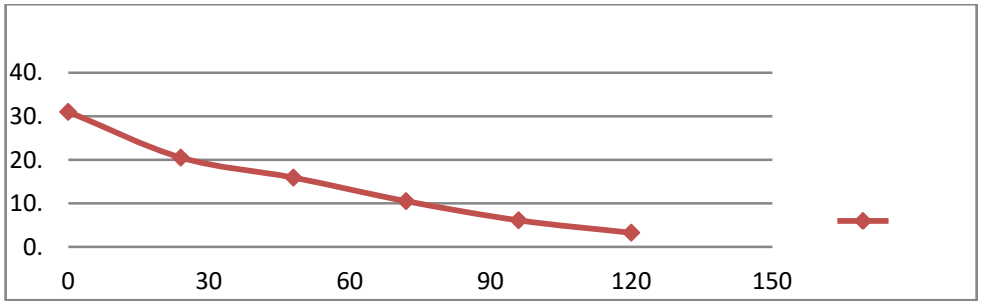

Fig. 2. Graph of Soluble sugar against fermentation time

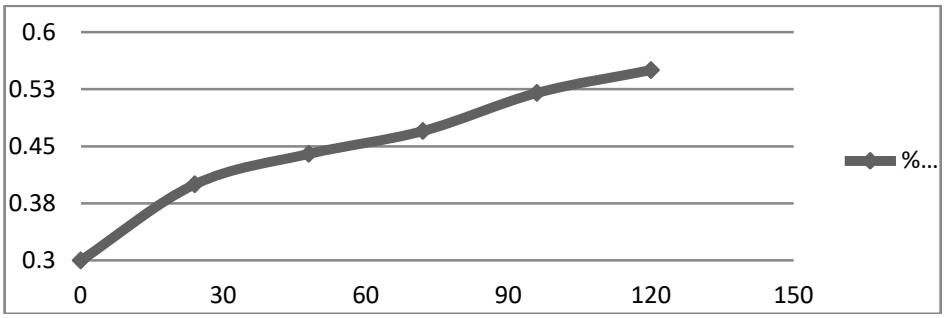

Fig. 3. Graph of TTA (%) against fermentation time

The specific gravity of sample was found to be 0.84578 which is in accordance to standards. and 90.62v/v% concentration of alcohol was obtained from the Association of Official Analytical Chemist (A.O.A.C) Alcohol chart using the specific gravity value.

4. CONCLUSION

This project was aimed at utilizing groundnut shells in bioethanol production using acid hydrolysis process and batch fermentation with *saccharomyces cerevisae*. In this process, 90.62 % v/v purity of ethanol was obtained, and yield was found to be 27.5 %. The heating value of the bioethanol is found to be 14 MJ/Kg compared to commercial grade ethanol of 19 MJ/Kg. From the experimental results obtained, it can be concluded that groundnut shells can be used as an appropriate feedstock for bioethanol production as higher yields can be obtained from its utilization.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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