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ORIGINAL ARTICLE

Bioethanol Production Using Simultaneous Saccharification and Fermentation Method

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ABSTRACT

Bioethanol, an alcohol produced by fermentation of plant biomass containing carbohydrates by micro-organisms is considered a dominant form of fuel for future. Production of this renewable fuel, especially from starchy materials such as tuber/energy crops, and cereal holds a remarkable potential to meet the future energy demand because of its potential for high productivity. However, this potential can only be realized by selecting appropriate feedstock and production system. This study focused on comparison of bioethanol productivities from tuber/energy crops such as cassava, sweet potato, yam, cocoyam and (cereal) maize using the co-cultures of Rhizopus sp and Saccharomyces cerevisiae IR2 in simultaneous saccharification and fermentation method. Gelatinized sweet potato and cocoyam flours using solid state cultures (koji) gave the highest bioethanol production of 2.29 % (v/v). The gelatinization of flours resulted in increased ethanol production compared to raw flours.

1 Introduction

Biofuels produced from starch, sugar, and vegetable oils are considered as first-generation biofuels and are currently the dominant form of biofuels with high potential to complement or replace the current fossil fuels in the future. Biofuels (produced by fermentation of grains and plant biomass containing carbohydrates using microorganisms or by transesterification of oils extracted from biomass materials) are used to power gasoline and diesel automobiles, trucks, and other vehicles. They are environmentally friendly since they do not contribute to net increase in greenhouse gases [1][2].

Energy crops (such as maize, waste straw, cassava, potato, sorghum, sweet potato) grown specifically for energy use are the main sources of feedstock to produce ethanol. Renewable fuel produced from tuber crops holds a remarkable potential to meet energy demand because of its high productivity. These crops which are mainly used for food can easily be produced in excess in Nigeria due to abundant arable land. The excess can then be used for biofuel production, thereby reducing our dependency on importation, increasing farmers' earnings and creating employment for the teeming unemployed youths [1].

There have been several reports of ethanol production from energy crops such as from cassava peels [3], sweet potato and cassava [4], corn, wheat, sugar cane and barley [1], cocoyam peels [5], and sweet potato [6] to mention a few. Bioethanol is colourless, biodegradable, reduces air pollution because it causes little environmental pollution and it is low in toxicity [2][7][8].

Ethanol blended with gasoline is mostly sold in the USA, it burns more completely and reduces pollution emissions. Ethanol and gasoline blend in the ratio of 1:9 known as E10 (10% ethanol and 90% gasoline) is the most common blend. There is no need for modification of gasoline engines to run on E10 [9].

Bioethanol, with all the advantages which made it a better alternative to fossil fuels, has some disadvantages such as low vapour pressure, low flame luminosity, miscibility with water,

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increased corrosiveness, and when compared to gasoline, it has low energy density [10]. There has been an energy crisis in both developing and developed countries that are oil dependent most especially in Nigeria with fuel price rising above ₦600/litre, there is the need for alternative fuel to cushion the effect of hike fuel price which has led to hike in the prices of essential commodities in the country.

This study was therefore designed to produce bioethanol using tuber/energy crops such as cassava, sweet potato, yam, cocoyam and (cereal) maize which are in abundance in Nigeria integrating both the starch and cellulose contents of each crop to avoid generating waste. The use of these energy crops for the production of bioethanol will lead to an increase in productivity of the crops which will create more jobs in the rural areas and improve the means of livelihood of rural dwellers.

2 Materials and Methods

2.1 Procurement And Preparation of Raw Materials

The tubers (yam, cassava, sweet potato and cocoyam) and cereal (maize) were purchased from Crop Science Department, University of Nigeria and Ogige Market both in Nsukka, Enugu State. Both the tubers and the cereal were taken to the Industrial Laboratory of Microbiology Department, University of Nigeria, Nsukka, Enugu State and were milled into flour but the tubers were washed to remove adhering soil particles, cut into small pieces, sun-dried for a week before milling. The flours were stored in polythene bags until required.

2.2 Sample Collection and Isolation of Organisms

2.2.1 Sample Collection

Yam, cassava, banana and plantain peels were collected from various places within the university community. Infected bark of a tree was collected from the Industrial Laboratory area of Microbiology Department, Faculty of Engineering and beside Awolowo Hall. Soil samples were collected from the Department of Crop Science Garden, all within the premises of University of Nigeria, Nsukka, Enugu State.

2.2.2 Isolation of Organisms

1 g sample each of the peels (yam, cassava, plantain and banana) were weighed separately and added to 10 ml distilled water. Emerson's yeast phosphate soluble starch (YPSs) agar was prepared [11]. The medium was sterilized, allowed to cool, and then 1 ml each of the samples was plated out using the pour plate method and incubated for 3 days at room temperature.

In the same way, 1 g of the soil sample was added to 10 ml distilled water and mixed to wash off the adhering cells into the water and 1 ml of the dilution was plated out on Emerson's yeast phosphate soluble starch agar. It was incubated for three days at room temperature.

2.3 Identification of Fungal Isolates

The isolates were characterized and identified based on colony morphology and microscopic examination. Among the characteristics used were colonial characteristics such as surface appearance and colour of the colonies. Microscopic examination revealed the type of hyphae i.e. septate or aseptate, and the vegetative mycelia. Slide culture method was used to identify the isolate to the generic level and appropriate references were then made [12][13]. The isolate was stored in starch agar slants until required.

2.4 Solid State Cultivation

2.4.1 Preparation of Koji

Three test tubes containing 10 ml distilled water, 20 g rice bran and a piece of white cloth were sterilized in an autoclave at 121°C for 15 min. On cooling, the sterile water from each of the tubes was emptied into 3 slants containing a 72-h old culture of the isolate. The spores were dislodged with a sterile wire loop. Cooked rice (100 g) was carefully weighed into the sterile white cloth, 20 g rice bran was added and thoroughly mixed together. It was inoculated with 30 ml fungal spore suspension harvested from the 3 slant cultures. It was thoroughly mixed together to ensure even distribution of the spores, and the white cloth was tied and incubated for 72 h at room temperature. Every 24 h during the incubation period, the cloth was untied and the contents mixed thoroughly, retied and incubated again. After the incubation, 10 g of the koji (enzyme source) was used for the fermentation.

2.5 Ethanol Fermentation

Ethanol fermentation was carried out, a mixture of the isolate and *Saccharomyces cerevisiae* IR2, using simultaneous saccharification and fermentation (SSF) method. Two sources of enzymes were employed; enzymes from suspended cell culture of the isolate and the solid state culture (koji as prepared above). The flours were used as carbon sources for ethanol production. They were either used in raw form (ungelatinized) or gelatinized before addition of the enzyme source and the yeast cells.

2.5.1 Preparation of Yeast Inoculum

Yeast cells (*Saccharomyces cerevisiae* IR2) were inoculated into already prepared nutrient and potato dextrose broths, and incubated at room temperature for 48 h.

2.5.2 Simultaneous Hydrolysis And Fermentation of Raw Flours Using Enzyme From Suspended Cultures

Five conical flasks containing 75 ml of 1 % (w/v) starch medium were prepared, and on cooling, they were inoculated with the isolate and incubated for 72 h at room temperature. The raw flour (5 g) was added to each of the five conical flasks containing the 72 h culture broth (the crude enzymes) and 25 ml yeast inoculum was added into each of the flasks. They were incubated for 120 h at room temperature and at every 24 h, samples were taken and analyzed for ethanol production.

2.5.3 Simultaneous Hydrolysis And Fermentation of Gelatinized Flours Using Enzyme From Suspended Cultures

Each of the flours (5 g) was gelatinized in 50 ml of water. After cooling, 50 ml of 72h-old culture of the isolate was added as crude enzyme. This was followed by an addition of 25 ml of yeast inoculum. They were then incubated at room temperature for 120 h and at every 24 h, samples were taken and analyzed for ethanol production.

2.5.4 Simultaneous Hydrolysis And Fermentation of Raw Flours Using Koji As The Source of Enzyme

A 5 g sample each of the raw flours (yam, cassava, cocoyam, sweet potato and maize) was suspended in 75 ml of distilled water in a 250 ml conical flask. It was followed by the addition of 10 g of koji which served as the source of the enzymes and finally 25 ml of yeast inoculum was added to each flask. The flasks were then incubated at room temperature for 120 h and at every 24 h, samples were taken and analyzed for ethanol production.

2.5.5 Simultaneous Hydrolysis And Fermentation of Gelatinized Flours Using Koji As The Source of Enzyme

A 5 g sample of the flour was gelatinized in 75 ml of water. On cooling, 10 g of koji and 25 ml of yeast cells were added to the flasks. The flasks were incubated at room temperature for 120 h, and at every 24 h, samples were taken and analyzed for ethanol production.

2.6 Analytical Methods

2.6.1 Measurement of Ethanol Production

Ethanol production in the fermentation medium was measured using acid dichromate method (www.outreach.canterbury.ac.nz). This is a method where the ethanol in acid dichromate solution is titrated against sodium thiosulphate. The titration values were used to extrapolate ethanol productions from the standard curve prepared earlier.

2.6.2 Data Analysis

All data were analyzed by Analysis of Variance (ANOVA) using GENSTAT Discovery Edition 3 and significant differences were determined by Least Significant Difference (LSD) at 5 % probability level.

3 Results and Discussions

3.1 Identification of Isolate

The isolate was morphologically and microscopically identified as *Rhizopus* sp. The results of the morphological and microscopic characteristics are as shown in Table 1.

Table 1: Microscopic Characteristics And Identification of the Isolate

Morphological characteristics	Microscopic characteristics	Suggested species
Fast growing fungus quickly filing the plate with a dense white cottony aerial mycelium.	Mycelium aseptate, with many hyphal branches connecting groups of unbranched sporangiophores.	*Rhizopus* sp

3.2 Ethanol Production from Raw Flours Using Rhizopus Enzyme from Suspended Cultures

As shown in Figure 1, sweet potato yielded the highest ethanol production of 1.2 %, followed by maize with 1 % ethanol. Ethanol production obtained with cocoyam (0.46 % v/v) was significantly lower than the values obtained with the other crops (yam 0.59 %, cassava 0.85 %, sweet potato 1.2 %, and maize 1 %). Although cocoyam had the least ethanol production (0.46 % / 5 g or 18.4 %/200 g) of all the crops tested, it is higher than the quantity of ethanol (12.9 %) by [14] from the fermentation of 200 g cocoyam by bacteria alpha-amylase and *Saccharomyces uvarum.*

However, ethanol production of 7.26 g/L (0.46 % / 5 g = 0.92 %/10 g) is lower than 34 – 43 g/L by [15] obtained from the fermentation of 10 g lignocellulo-starch biomass (peels of sweet potato, elephant foot yam, tannia, greater yam and beet root) using *Saccharomyces cerevisiae* but compares favourably with the maximum ethanol production of 0.485 % by [16] from wild cocoyam fermented by *Aspergillus* and *Saccharomyces* spp.

3.3 Ethanol Production from Gelatinized Flours Using Rhizopus Enzyme from Suspended Cultures

As shown in Figure 2, gelatinized sweet potato flour yielded the highest ethanol production of 1.43 %/5 g, followed by cassava with 0.89 %/5 g ethanol. This was lower than the alcohol content (8.5 %) by [3] from the fermentation of cassava peels grounded to flour using *Aspergillus niger* and *Saccharomyces cerevisiae* but favourably compared to the work of [17] who obtained 5.3 %/50 g ethanol production from cassava starch using *Saccharomyces cerevisiae* (Baker's yeast).

Ethanol production obtained with maize was significantly lower than the values obtained with the other crops. Figure 3 shows a comparison of ethanol production from raw and gelatinized flours. Except for maize substrate, gelatinized flours produced significantly higher ethanol production than raw flours.

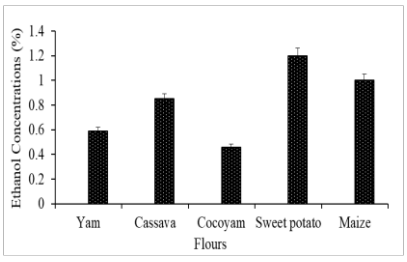

Figure 1: Ethanol production from raw flours by simultaneous hydrolysis and fermentation using enzymes from suspended culture of Rhizopus sp and fermented by Saccharomyces cerevisiae in suspended cultures

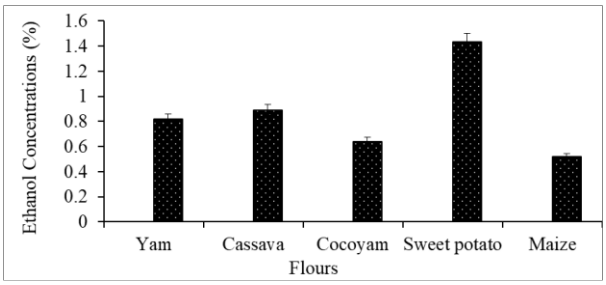

Figure 2: Ethanol production from gelatinized flours by simultaneous hydrolysis and fermentation by Rhizopus sp and Saccharomyces cerevisiae in suspended cultures

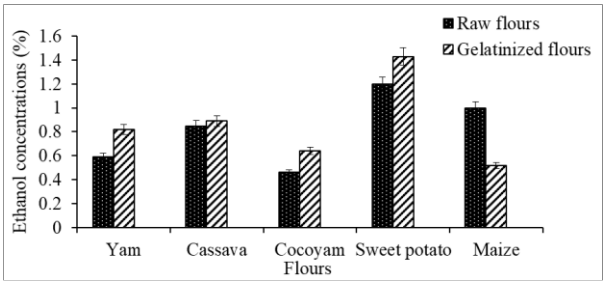

Figure 3: Comparison of ethanol production from raw and gelatinized flour by simultaneous hydrolysis and fermentation by Rhizopus sp and Saccharomyces cerevisiae in suspended culture

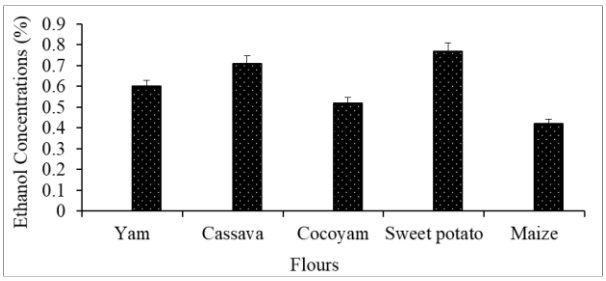

Figure 4: Ethanol production from raw flours by simultaneous hydrolysis and fermentation by solid state culture of Rhizopus sp and Saccharomyces cerevisiae

3.4 Ethanol Production from Raw Flours Using Rhizopus Koji as the Source of Enzyme

The results of ethanol production from raw flours using *Rhizopus* koji as the source of enzyme are shown in Figure 4. As in the case of crude enzyme from suspended culture, sweet potato gave the highest ethanol production. Ethanol production obtained from maize (0.42 %) was lower than those of other crops.

3.5 Ethanol Production from Gelatinized Flours Using Rhizopus Koji as the Source of Enzyme

The results of ethanol production from gelatinized flours using *Rhizopus* koji as the source of enzyme are shown in Figure 5. Sweet potato and cocoyam gave the highest ethanol production of 2.29 % each. The result of this study (2.29 % = 2.29 ml/100 ml from 5 g, it is however equal to 21.69 g/100 ml from 60 g) is higher than that of [10] who reported 5.65 g/100 ml ethanol yield from 60 g cocoyam peels powder and ethanol yield of 4.89 ml/20 g by [18] from Edeofe specie of cocoyam (9.16 ml/100 ml from 20 g reported by this study).

Komlaga [4] reported a higher ethanol production of 16.2 % with the combination of 50 g each of sweet potato and cassava which is lower than the result of this work of 22.9 % from 50 g flour. This might be as a result of high concentration of fermentable sugar present in sweet potato and starch concentration in cocoyam since the whole tuber (the peel and the flesh) was used for the fermentation.

The highest ethanol production (18.07 g/L) obtained in this work is however lower than the result of Kangor [8] who reported the following ethanol productions (g/L) for the following crops cassava (64.052 ± 0.098; 1.334), maize (66.670 ± 0.227; 1.389), sorghum (62.382 ± 2.148b; 1.300) and maerua shrub (61.988 ± 0.160, 1.291). Ethanol production from yam was the lowest. In this work, ethanol production of 2.09 % (16.5 g/L) from maize is lower compared to the report of Ebabhi [19] who obtained 26.353.80 g/L from maize cobs.

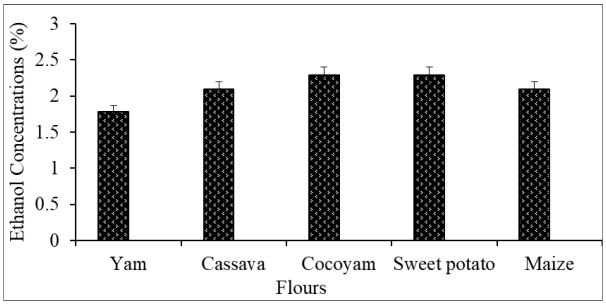

Figure 5: Ethanol production from gelatinized flours by simultaneous hydrolysis and fermentation by solid state culture of Rhizopus sp and Saccharomyces cerevisiae

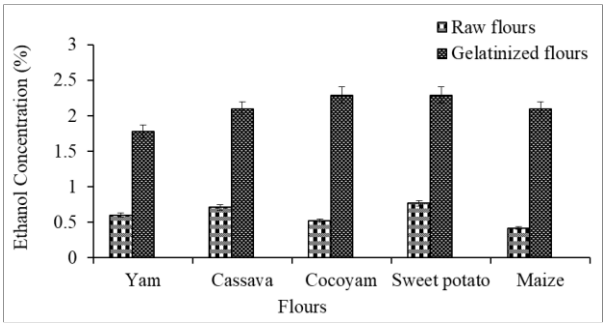

Figure 6: Comparison of ethanol production from raw and gelatinized flours by simultaneous hydrolysis and fermentation by solid state culture of Rhizopus sp and Saccharomyces cerevisiae

Statistically, there were no significant differences in ethanol production from cassava, cocoyam, sweet potatoes and maize. Figure 6 shows a comparison of ethanol production from raw and gelatinized flours while Figure 7 shows the comparison of ethanol production from suspended and solid state cultures using *Rhizopus* crude enzymes. Significantly higher ethanol

production was obtained from gelatinized flour than raw flour, regardless of the crop used. Furthermore, in all the flours used, ethanol production obtained with koji as the source of enzyme were more than two times higher than the values obtained with crude enzyme from suspended culture.

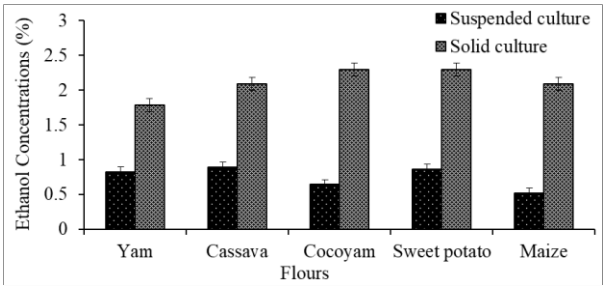

Figure 7: Comparison of the maximum ethanol production obtained from gelatinized flours in suspended and solid-state cultures of Rhizopus sp and Saccharomyces cerevisiae

4 Conclusions

The above results have shown that all the crops tested and compared were good feedstocks for ethanol production in Nigeria. Since all the crops are potential biomass for ethanol production, cassava which is much cheaper and available all the year round is thus recommended for large scale bioethanol production in Nigeria. Furthermore, gelatinization improved ethanol production. However, the process of gelatinization increases the energy consumption and thus the final production costs. It is thus very important to develop a cheap and simple method of gelatinizing flours for ethanol production; this will eventually reduce production cost. This includes exploring the use of solar heat or channelling the heat from distillation for gelatinization. The result of this work has confirmed that solid state culture (koji) is much more efficient than the suspended cell culture as the source of enzyme for ethanol production. Solid state cultures are simple, cheap and can be done even in rural areas without constant electricity supply. It is therefore recommended for large scale bioethanol production.

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Declaration of Competing Interest

The authors declare no known competing interest or personal relationship that could influence the work reported in this paper.

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