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Effect of Fermentation on Production of Bioethanol from Peels of Cocoyam Using *Aspergillus niger* and *Saccharomyces cerevisiae*

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Authors' contributions

This work was carried out in collaboration with both authors. Author DVA designed the research, managed the literature searches and wrote the first draft of the manuscript. Author DOU wrote the protocol, managed the analyses of the study and performed the statistical analysis of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Bioethanol is an alternative to fossil fuel and it's produced by fermentation of sugar components of plant materials. The effect of fermentation on production of bioethanol from peels of cocoyam using sequential mono-cultures and co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae*. Standard methods were used to carry out isolation, identification and analysis of the samples. Sixty grams of cocoyam peels was dried and ground; and was subjected to heat pretreatment. Direct fermentation of cocoyam peels to ethanol by sequential monocultures and co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae* was done for 7 days during which reducing sugar yield, amylolytic activity rate, residual starch and ethanol yield were determined. Residual starch (9.18-4.42 g/100 ml) and reducing sugar (9.86-4.21% g/100 ml) yield decreases as fermentation

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progresses. Amylolytic activity rate (31.00-0 U/ml) and ethanol yields (5.65-0.00 g/100 ml) increased several-fold in co-cultures and mono-cultures. From this study, it is concluded that the peels of cocoyam can be employed for bioethanol production.

Keywords: Fermentation; bioethanol; cocoyam peels; co-cultures; monocultures.

1. INTRODUCTION

Burning of petroleum-based fossil fuels in machines and vehicles accompanied by evolution of dangerous gases have been a major cause of increase in environmental problems. Researchers have focused interest away from petroleum-based fuel to a greener technology in production of bioethanol using various renewable materials. Apart from these global concerns, increase in prices of fuels and political instability existing in oil producing countries are part of numerous factors that led to looking up for other alternative source of energy. [1] reported that use of renewable materials as alternative source of energy can help in finding solutions to these environmental issues.

Ethanol can be produced synthetically and naturally by yeasts. About 51.3 billion liters of ethanol was produced worldwide in 2006, and the production of ethanol will increase in the future. Ethanol fermentation has been used for the production of alcoholic beverages, and for the rising of bread dough for centuries. Since 1908, fuel ethanol has found use for transportation. Bioethanol has become an attractive fuel because its production is based on renewable natural sources [2]. [3] indicated that fuel ethanol reduces the emission of carbon dioxide and aromatic compounds. Currently, fuel ethanol is already used in a pure form or in a mixture with gasoline as gasohol for transportation in Brazil and in some states of the U.S. [4,2]. [5] reported that 45% of the produced ethanol are used as potable alcohol, 40% are utilized in chemical and pharmaceutical industries and the rest is deployed in mixture with gasoline. Besides Ethyl Tertiary-Butyl Ether (ETBE) is produced from ethanol [6].

Ethanol is non-toxic, and is a non-contaminant to water sources. Compared to the other fuel additives such as methyl tertiary butyl ether (MTBE), ethanol's octane number is greater [3]. Although bioethanol has been introduced as an alternative to petroleum-derived fuels, it has some disadvantages such as increased corrosiveness, low flame luminosity, miscibility with water, low vapor pressure and low energy density compared to gasoline [2]. Aside from fuel, ethanol has other applications in various industry branches such as: Personal care products, cleaning agents, pharmaceuticals, and beverages.

A variety of raw material is used for ethanol production**;** however these materials have some disadvantages. Corn is the major source for ethanol production in the U.S. (97%), but production of this crops causes more soil erosion and requires more nitrogen fertilizer than other crops. In Brazil, sugar cane production also has environmental limitations [2].

Cocoyam (*Colocasia esculenta*) is a tropical starchy tuberous root crop. [7] reported that nutritional contents of cocoyam roots and tuber crops are known to be very high. The gap between certain dietary requirements of the body can be linked and balanced by high nutritional benefits offered my many root and tuber crops. The protein, Vitamin and Mineral contents of cocoyam is higher than the nutritional values obtained in other root crop and tuber crops such as yam and cassava. Cocoyam is a very popular staple crop in third world countries and developing countries used as weaning food while its leaves serve as vegetable in some countries. Africa is known to produce highest proportion of cocoyam while the carribean produce the least amount with the Asian countries producing the minority. Cocoyam along with other staple crops such as yam, potato and cassava is usually available throughout the year. Cocoyam peels is an agro wastes which consists of skin and thin outer cortex of their tubers that represent a major wastes during processing. It can be used to replace conventional food materials such as maize, wheat, and other crops used as source of biofuels and mineral for animal diets [8].

Fermentation is one of the oldest biotechnologies, having been used in food processing and preservation as well as beverages production for over 6,000 years [9]. The fermentation process of substrates serves as a means providing a major source of

nourishment for large rural populations, and contributes significantly to food security by increasing the range of raw materials which can be used in the production of edible products [10].

In the currently employed industrial production of ethanol from starchy materials, starch is firstly hydrolyzed by adding a liquefying enzyme, α-amylase (EC 3.2.1.1) to avoid gelatinization, and then cooked at high temperature (140~180°C). The liquefied starch is then hydrolyzed to glucose with a saccharifying enzyme, glucoamylase (EC 3.2.1.3). Finally, the glucose is converted to ethanol by yeast cells [11]. Traditional processes for bioethanol production from starch are expensive and these processes should be improved.

Achinewhu et al. [12] reported that nutrient content of foods can be improved by fermentation process which results in biosynthesis of vitamins, essential amino acids and protein improving protein quality and fibre digestibility. Micronutrient bioavailability and degradation of anti-nutritional factors can be enhanced by fermentation. The main aim of this research is to evaluate the effect of fermentation in production of bioethanol from peels of cocoyam using mono-cultures and co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae.*

2. MATERIALS AND METHODS

2.1 Sample Collection

The peels of cocoyam were collected from the Oba market and Domestic Dumping Sites in Akure. The samples were washed thoroughly; sun dried and powdered using food processor. Pretreatment of the sample was carried out by heating the powder at a temperature of 120°C with acid on a pressure pot for 30 mins. The pretreated sample was dried in an oven at 65°C [13].

2.2 Isolation of Microorganisms and Its Maintenance

Saccharomyces cerevisiae was obtained from the stock culture of Microbiology Laboratory Federal University of Technology, Akure, while *Aspergillus niger* was isolated from the peels of cocoyam. Pure culture of *Aspergillus niger* was obtained by streak plate method. The Organisms were maintained on PDA slants at 4°C.

2.3 Starch Hydrolysis Test of Isolated Strains of *Aspergillus niger*

An inoculum from a pure culture was streaked on a sterile plate of starch agar. The inoculated plate was incubated at 27°C for 5 to 7 days. Iodine reagent was then added to cover the growth. Presence of clear zone surrounding colonies confirmed the positive result and accounts for their ability to digest the starch and thus indicates presence of alpha-amylase [14].

2.4 Preparation of Inoculum

Saccharomyces cerevisiae was grown at 30°C for 24 h in medium composed of (per 100 ml): Glucose, 1 g; yeast extract, 0.2 g; malt extract, 0.1 g; $CaCl_2{\cdot}2H_2O$, 0.2 g; $(NH_4)_2SO_2$, 0.2 g; $MgSO_4{\cdot}7H_2O$, 0.2 g and KH_2PO_4, 0.002 g. In order to maintain viability, the culture was stored at 4°C while *Aspergillus niger* will be maintained on Potato dextrose agar slants at [15].

2.5 Bioethanol Production

The following steps were used for production of bioethanol: Starch hydrolysis and fermentation, centrifugation and distillation process.

Fermentation was carried out in 500 ml flasks containing 60 g powdered cocoyam and sweet potato peels in 300 ml of distilled water. The pH of the medium was adjusted to 6.0 using one normal HCl and NaOH. The flasks were sterilized by autoclaving at 121°C for 30 min. The flasks for co-culture fermentation were inoculated with 4% (v/v) freshly harvested inoculum of *Aspergillus niger* and 3% (w/v) inoculum of *Saccharomyces cerevisiae* while those for mono-culture fermentation were sequentially inoculated with only 4% (v/v) freshly harvested inoculum of *Aspergillus niger.* After four days of fermentation, the broth medium was autoclaved, cooled and inoculated with 3% (w/v) inoculum of *Saccharomyces cerevisiae*. The flasks were incubated at ambient temperatures on an orbital shaker set at 250 rpm for 7 days [16].

2.6 Centrifugation and Distillation

After fermentation, the broth was centrifuged at 6000 rpm for 10 minutes. The supernatant was collected and fed into a simple Laboratory distillation column. The boiling temperature of ethanol is 78°C hence distillation was carried out around that temperature to facilitate the evaporation of ethanol. The vapour was collected

and got condensed by means of the circulation of cold water around the column. The distillate having ethanol was recovered in a conical flask at the other end of the column.

2.7 Determination of Reducing Sugar Produced

The reducing sugar was determined by titrimetric methods. Thirty ml of fermentation broth was weighed into the burette. Ten ml of mixed Fehling's solution was pipetted into a conical flask and 4 drops of 1% methylene blue indicator was added. The solution was heated and while boiling the broth in the burette was titrated against the solution on the conical flask until the color disappeared [17]. The reducing sugar was calculated as follows:

%Reducing Sugar = 47.5 x 300/T x W

T represents the titre value

W represents the weight of the peel sample (in grams)

2.8 Determination of Residual Starch

A residual starch concentration in a 100ml sample of undiluted broth was determined by using phenol-sulphuric acid [17]. The amount of starch in the sample was calculated by using the formula of keer: glucose (grams/100 ml) x 0.9= starch (grams/100 ml)

2.9 Determination of Amylolytic Activity

The extracellular amylolytic activity was determined by measuring reducing sugar release from starch in which reduction of 3, 5-dinitrosalicylic acid to nitroaminosalicylic acid by reducing sugar in the sample was determined [18]. A standard curve for this colorimetric assay was constructed using glucose as the standard. One unit of amylolytic activity is defined as the amount of enzyme in 1 ml that liberates 1 µmol of reducing sugar from starch in 3 min.

2.10 Determination of Ethanol Concentration

Ethanol concentration was determined by comparing the density of the ethanol produced with the standard ethanol density curve. Standard ethanol curve was obtained by taking series of percentage (v/v) ethanol (10%, 20%, 30%, 40% and 50%) solution which was prepared in a 100 ml volumetric flask and the weights were measured as described by [19,20]. The density of each of the prepared ethanol solution were calculated and a standard curve of density against percentage ethanol (w/v) was plotted.

3. RESULTS AND DISCUSSION

Fig. 1 showed the pH of the cocoyam peels during the production of ethanol. From the result, the pH of the cocoyam peels generally decreased during the period of fermentation. There was a progressive decrease in pH of the samples during 6 days and then a slight increase in pH value was observed in Day 7. This is an indication that the fermentation process becomes more acidic as a result of the production of other secondary metabolites and activities of microorganisms in the fermentation medium. The changes in the pH may be attributed to the acid released by the microorganisms present in the fermentation medium. This is in accordance with findings of [21] which observed decrease in pH during fermentation of sweet potato peels.

The results in Fig. 2 showed the pattern of residual sugar during the fermentation period. The residual sugar in the fermentation media was observed to decrease with increase in fermentation time except in monocultures which shows progressive increase till day four. The reducing sugar reduced drastically following the sequential inoculation of mono-culture of *Saccharomyces cerevisiae* on the fourth day. This could be attributed to the utilization of the sugar as carbon source for the growth of the microorganisms (*S. cerevisiae and A. niger*) and subsequent ethanol production. The result was similar to findings of [15] of decline in reducing sugar in fermentation of corncobs. In co-culture, there is rapid fermentation of sugar to ethanol during the rest of the fermentation (days 1 to 7) by *S. cerevisiae* kept the sugar concentration low enough to prevent feedback inhibition of amylolytic activity observed in initial monoculture of *A. niger.*

The result of amylase activity (Fig. 3) showed progressive increase in enzyme activity from first to fourth day followed by decline from day 5 to 7. Amylase is an induced enzyme and its production increased with increase in fungal biomass over the incubation period. There is higher amylase activity in monocultures than in co-cultures and this can be attributed to increase in growth and activity of hydrolytic organism (*A. niger*) in monoculture unlike in co-culture which is limited by competition for nutrients by both *A. niger* and *S. cerevisiae* [22].

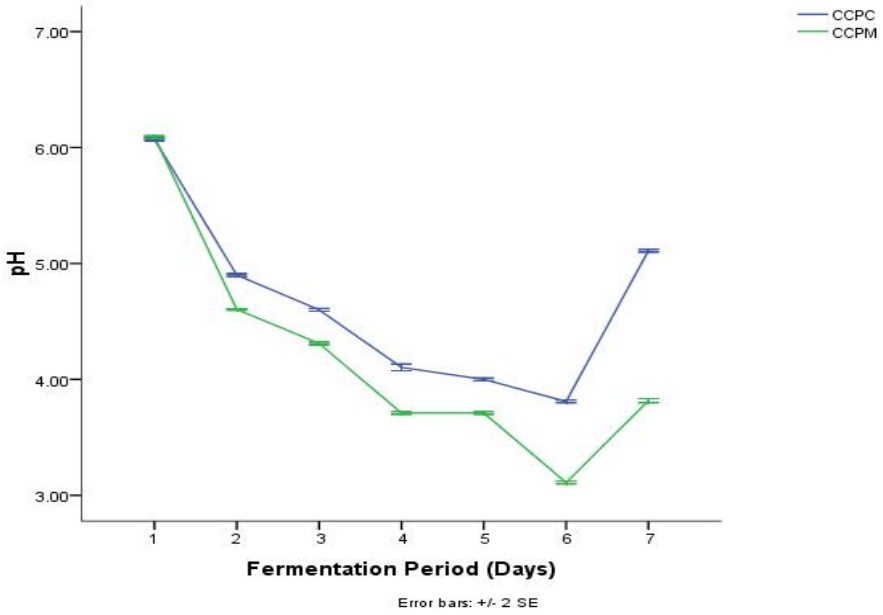

Fig. 1. pH of cocoyam peels during fermentation of the samples
Key: CCPC: Cocoyam peels co-culture, CCPM: Cocoyam peels monoculture

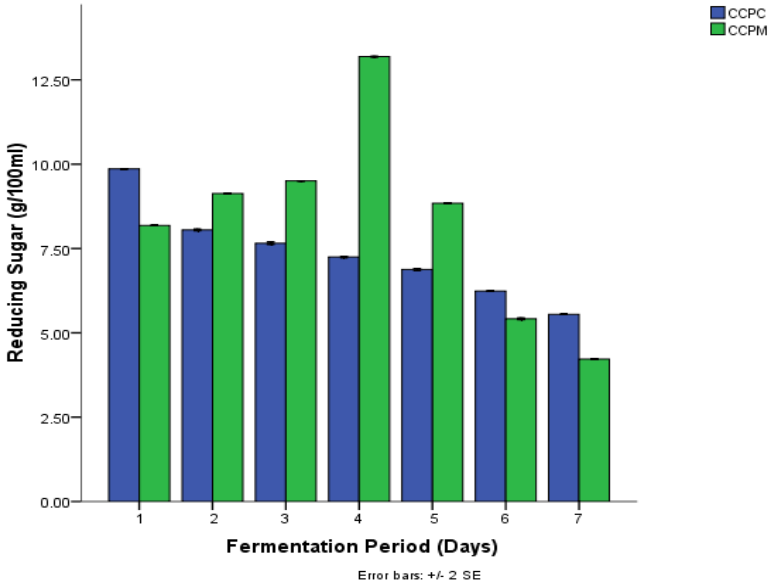

Fig. 2. Reducing sugar of cocoyam peels during fermentation of the samples
Key: CCPC: Cocoyam peels co-culture, CCPM: Cocoyam peels monoculture

The residual starch (Fig. 4) of the cocoyam peels decreased with increase in fermentation time. This observation can be as result of conversion of the cocoyam peels starch to fermentable sugar by starch hydrolyzing *A. niger*. This is in agreement with findings of [16] which reported decline in residual starch in fermentation of potato peels.

Fig. 5 revealed the results of bioethanol yield. There is progressive increase in the amount of ethanol produced as the fermentation progresses. It can be observed that monoculture fermented cocoyam peels produced the highest amount of ethanol than co-culture fermented cocoyam peels. The maximum ethanol quantity in terms of concentration (5.65 g/100 ml) was

obtained from sequential mono-culture culture of cocoyam peels. This result is lower than maximum quantity of ethanol produced (6.51 g/100 ml) by [23] from sweet potato peel using *Gleophyllum sepiarium* and *Pleurotus ostreatus* for hydrolysis and *Z. mobilis* and *S. cerevisiae* for fermentation. This might be attributed to high amount of fermentable sugar in sweet potato peels.

However, [20] reported 63.8% for *A. niger* and *Z. mobilis* when used simultaneously for millet. This result can be attributed to the high amount of carbohydrate found in sweet potato peels than

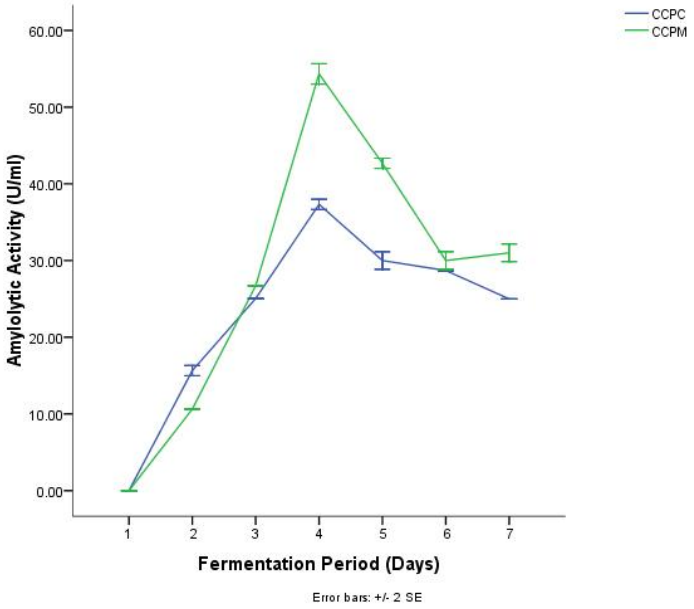

Fig. 3. Amylolytic activity of cocoyam peels during fermentation of the samples
Key: CCPC: Cocoyam peels co-culture, CCPM: Cocoyam peels monoculture

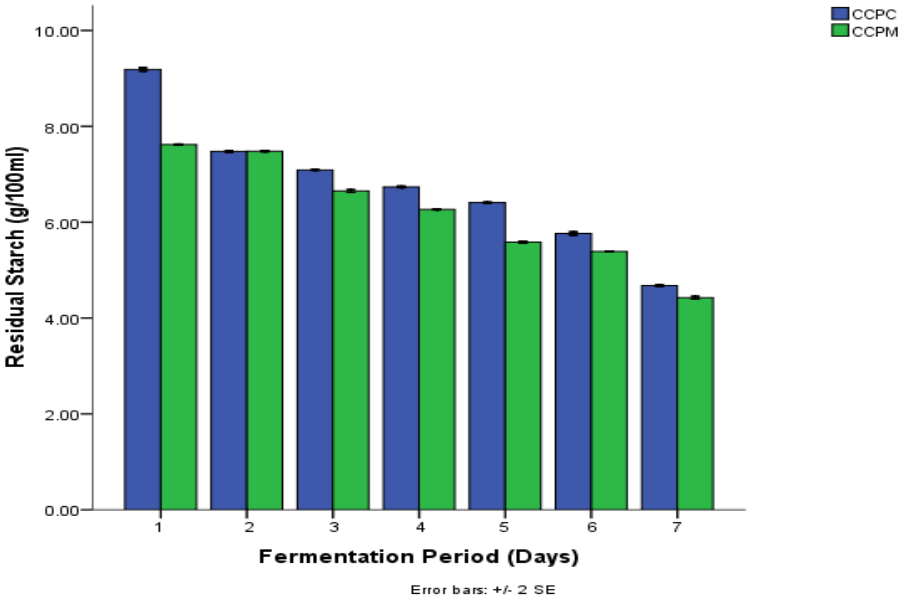

Fig. 4. Residual Starch of cocoyam peels during fermentation of the samples
Key: CCPC: Cocoyam peels co-culture, CCPM: Cocoyam peels monoculture

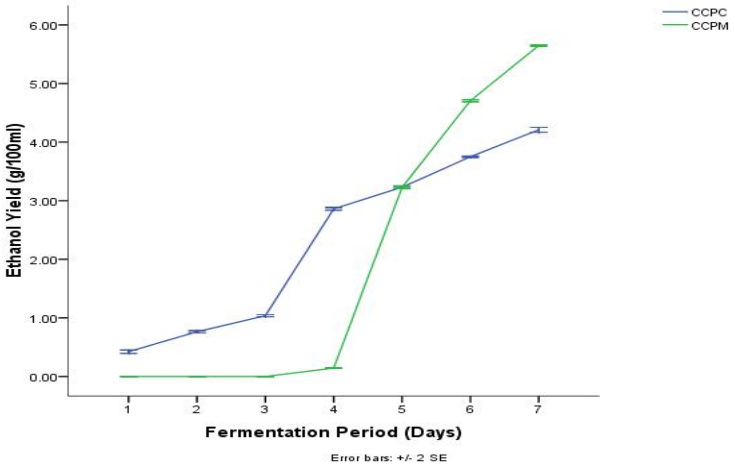

Fig. 5. Ethanol yield of cocoyam peels during fermentation of the samples
Key: CCPC: Cocoyam peels co-culture, CCPM: Cocoyam peels monoculture

cocoyam peels thereby making more starch available for conversion to ethanol. There is high amount of ethanol produced in mono-cultures fermentations than in co-culture fermentations in cocoyam peels. These findings can be as a result of presence of readily available fermentable sugar in monocultures released by starch hydrolyzing organism (*A. niger*) which are easily converted to ethanol by *S. cerevisiae.*

4. CONCLUSION

In conclusion, this study revealed that mixture of starch-hydrolyzing fungus (*Aspergillus niger)* and sugar-fermenting organism (*Saccharomyces cerevisiae*) can be employed in simultaneous and sequential fermentation of cocoyam peels to produce bioethanol. Many commercial processes of bioethanol production utilizes enzymatic starch hydrolysis which can be replaced by synergistic combination of these organisms in production of ethanol from starch substrates. This method of starch fermentation to ethanol will significantly improve the economy by reducing the cost of production of bioethanol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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