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Bioethanol production from cassava *(Manihot esculenta)* peels

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Abstract

In recent years, the production of ethanol from plentiful, low cost cellulosic biomass or agricultural wastes has grown in importance due to the hope that it could reduce the cost of ethanol production and benefit the global environment. The application of using cassava residues for ethanol production could be of great advantage to a country's economy; hence, this study was carried out to determine the possibility of bioethanol production from cassava peels as a cheaper bioethanol source. Cassava peels were collected, cleaned, chopped and fermented for 14 days by Saccharomyces cerevisiae isolated from palm wine. In this study, parameters including biomass, ethanol yield, pH, titratable acidity and reducing sugar were analyzed at two day intervals using standard methods. There was a drop in pH from 5.0 to 3.8 in the yeast ameliorated batch of fermentation with ethanol yield of 7.5 mL and about 8.1% alcohol content produced. There was a progressive increase in titratable acidity and cell biomass; and a decrease in reducing sugar during the course of fermentation of both the test and control batches. The results from this study showed that ethanol production from cassava peels could provide solution to the problems of their disposal into the environment and also serve as an alternative option to ethanol production from cheaper available raw materials.

Keywords: cassava peels; bioethanol; fermentation; *Saccharomyces cerevisiae.*

Producción de bioetanol a partir de cáscaras de yuca *(Manihot esculenta)*

Resumen

En los últimos años, la producción de etanol a partir de la abundancia de biomasa de celulosa de bajo costo o de residuos agrícolas ha crecido en importancia, debido a la esperanza de reducir el costo de la producción de etanol y beneficiar el medio ambiente global. La aplicación del uso de residuos de yuca para la producción de etanol podría ser de gran ventaja para la economía de un país; por lo tanto, este estudio se llevó a cabo para determinar la posibilidad de la producción de bioetanol a partir de cáscaras de yuca como una fuente más barata de bioetanol. Las cáscaras de yuca fueron recolectadas, limpiadas, picadas y fermentadas durante 14 días por *Saccharomyces cerevisiae* aislado del vino de Palma. En este estudio se analizaron parámetros que incluyeron biomasa, rendimiento de etanol, pH, acidez titulable y azúcar reductora. Se observó que hubo una disminución en el pH de 5,0 a 3,8 en el lote de fermentación mejorado con levaduras con rendimiento de etanol de 7,5 ml, y aproximadamente 8,1% de contenido de alcohol. Presentó un aumento progresivo de la acidez valorable y de la biomasa celular; y una disminución en la reducción del azúcar durante el curso de la fermentación de los lotes de ensayo y control. Los resultados de esta investigación, demostraron que la producción de etanol a partir de cáscaras de yuca, podría dar solución a los problemas de su eliminación en el medio ambiente y también servir como una opción alternativa a la producción de etanol, a partir de materias primas disponibles más baratas.

Palabras Clave: cáscaras de yuca; bioetanol; fermentación; *Saccharomyces cerevisiae.*

INTRODUCTION

Bioethanol is a principal fuel that can be used as petrol substitute for vehicle. It is a renewable energy source produced mainly by sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. The main sources of sugar required to produce ethanol come from fuel or energy crops like cassava and cassava products, waste straw, sawdust, etc. (1).

Yeast (*Saccharomyces cerevisiae*), have been known to humans for thousands of years as they have been used in fermentation processes like in the production of alcoholic beverages (2) and bread leavening (3). Yeasts metabolize sugar to produce ethanol and carbon dioxide. The basic carbon and energy source for yeast culture are sugars (2) (3).

Cassava (*Manihot esculenta*), also known as manioc, tapioca or yucca, is one of the most important food crops in the humid tropics, being particularly suited to conditions of low nutrients availability and is able to survive drought (4). It is the third largest source of carbohydrates for human consumption in the world, with an estimated annual world production of 208 million tonnes (5). The major harvested organ is the tuber, which is actually swollen root. The nutrient reserve of cassava is made up of starch. Cassava peels is gotten during the processing of the cassava tuber and it is an agricultural waste. Cassava peels contain starch which when treated with a varying level of H2SO4 undergoes an abrupt change in the physical structure of the glycosidic bond linking □ amylase and amylopectin. Glycosidic bond are broken to produce glucose and oligosaccharide residues (4). In Africa, especially Nigeria, which is one of the largest centre of cassava production, it is grown on 7.5 million hectares of land and produces about 60 million tonnes per year. Thus, wastes (especially cassava peels) generated from the processing of cassava into various products are littered or dumped in the environment causing pollution. There is therefore the need for revalorization of cassava peels waste into useful products. The application of using cassava peels for ethanol production could be of great advantage to a country's economy.

This study therefore determines the possibility of bioethanol production from cassava peels which could provide a cheaper bioethanol source; also, exploit the fermentative ability of Saccharomyces cerevisiae isolated from palm wine in the production of the desired bioethanol.

MATERIALS AND METHODS

Collection and processing of cassava peels

Cassava peels were obtained from a cassava milling factory in Uselu market, Benin City, Edo State, Nigeria. The peels were washed in clean water (to remove sand and cyanide content) and weighed on a laboratory scale. Thereafter, the peels was allowed to dry naturally (de-watering) for 4 hours on a clean tray, after which they were chopped into bits and transferred into a mortar where they were mashed using a pestle to attain sufficient size reduction. This was to ensure the creation of sufficient surface area of the material to aid the process of fermentation.

Isolation and identification of yeast *(Saccharomyces cerevisiae)* from palm wine

Yeast used for this experiment was isolated from fermented palm wine. One mL of the serially diluted palm wine sample was plated on sabouraud dextrose agar supplemented with streptomycin (0.05 mg/L) using pour plate method and incubated at 28oC for 48 hours. The yeast colonies that developed were isolated and purified by spread plate method on fresh sabouraud dextrose agar plates. Identification of the yeasts was by the use of standard morphological and physiological tests and identification keys described by Barnett et al. (6) (14).

Preparation of sample for fermentation

100g of the mashed cassava peel was transferred into two different 1L fermentation flasks and 1000mL of distilled water was added to each of them. The flasks were autoclaved at 121oC for 15 minutes and allowed to cool. The contents of the flasks were then filtered using a muslin cloth to obtain the desired cassava medium and again autoclaved and allowed to cool. For hydrolysis to form sugars, 5mL of 5% H2SO4 and 5mL of 5% NaOH were added to each jar and heated to about 50oC. Thereafter, 20mL suspension of the inoculum (yeast) was introduced aseptically into one of the fermentation flasks which served as the test experiment, while

the other fermentation flask had no inoculum and served as the control. The flasks were corked with a rubber stopper and left to ferment for 14 days. Certain parameters of the samples were analysed at 2 day intervals for the period of fermentation.

Biochemical parameters determined during fermentation

Samples obtained from the fermentation flasks were centrifuged and the supernatants were examined for various parameters including; pH, temperature, total reducing sugars, ethanol yield, cell biomass and titratable acidity.

Measurement of pH value

The pH values of each fermented medium was determined with the aid of a pH meter after standardization with buffer at pH 5. This was done by dipping the electrode into the different samples in the flask and readings taken before and after fermentation.

Measurement of temperature

Temperature values of each fermented medium was determined by the use of a thermometer before and after fermentation.

Titratable acidity determination

Fermented media were analysed for titratable acidity using phenolphthalein as indicator. This was carried out using the methods of Isitua and Ibeh (2); Association of Analytical Communities, AOAC (7) by titrating 10mL of sample against 0.1M NaOH using the first permanent colour change.

Assay for reducing sugar content

The total reducing sugar content in the samples were determined using 3, 5-dinitrosalicylic acid (DNS) reagent. Briefly, 3 mL of DNS reagent was added to 3 mL of each of the samples and then, test tubes were tightly caped to avoid loss of liquid due to evaporation. Test tubes contents were heated at 90oC for 5-15 minutes to develop a red/brown colour. 1 mL of a 40% potassium sodium tartrate (Rochelle salt) was added to stabilize the colour. After cooling to room temperature in cold water bath, absorbance was read in a spectrophotometer at 600 nm wavelength against reagent blank (7).

Cell biomass determination

Cell biomass of the fermented media was determined by measuring the optical density of each fermentation media at 600 nm. The aliquots (about 2.5 mL) of the different fermentation media were transferred into a 3 mL cuvette and absorbance were read against a reagent blank. The reagent blank was prepared with the uninoculated cassava medium (control) in place of the inoculated cassava medium. This analysis was to determine the turbidity in each fermentation medium during the fermentation process (7).

Determination of the amount of alcohol produced

The amount of alcohol (% v/v) produced in each of the fermentation medium after the period of fermentation was determined according to the method described by Isitua and Ibeh (2) from values of their respective specific gravities obtained by using a hydrometer and calculated as follows:

Alcohol content (% v/v) = (D1 – D2) x 105 x 1.25

Where D1 = 1.06

D2 = Mean of hydrometer readings for each fermentation sample.

To obtain the ethanol yield, the fermented cassava medium was filtered using cheese cloth to obtain liquid for distillation. Distillation involved separating ethanol from water and other residual solids after fermentation. Ethanol was boiled off from the rest of the solution in a distillation column. The condensed ethanol and water were allowed to travel through a rectifier column where the water remaining in the ethanol was finally removed. This was carried out at a temperature of about 78.5oC. Ethanol yields after distillation were measured after 10 minutes for the cassava medium containing S. cerevisiae as well as the control experiment (8).

Statistical analysis

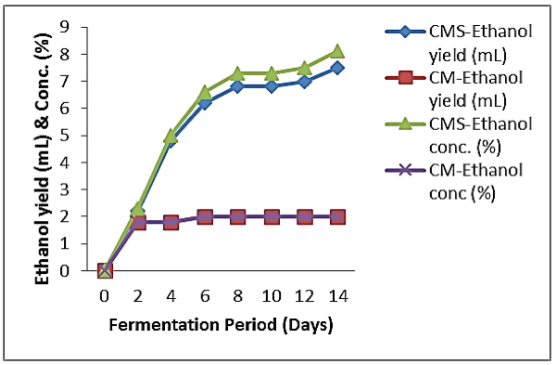
The assays were carried out in triplicate; the results were mean values ± standard deviation and expressed as mg / mL of sample. The amount of alcohol produced was expressed in % v/v of the fermented medium.

RESULTS

During the fermentation, the amount of ethanol yield, ethanol concentration, cell biomass (Figure 1), pH, temperature, reducing sugar and titratable acidity were determined and the results are shown in Figure 2 (a, b, c, d). The ethanol yield and concentration after distillation of fermented cassava peels medium ranged from 2.2 mL to 7.5 mL and

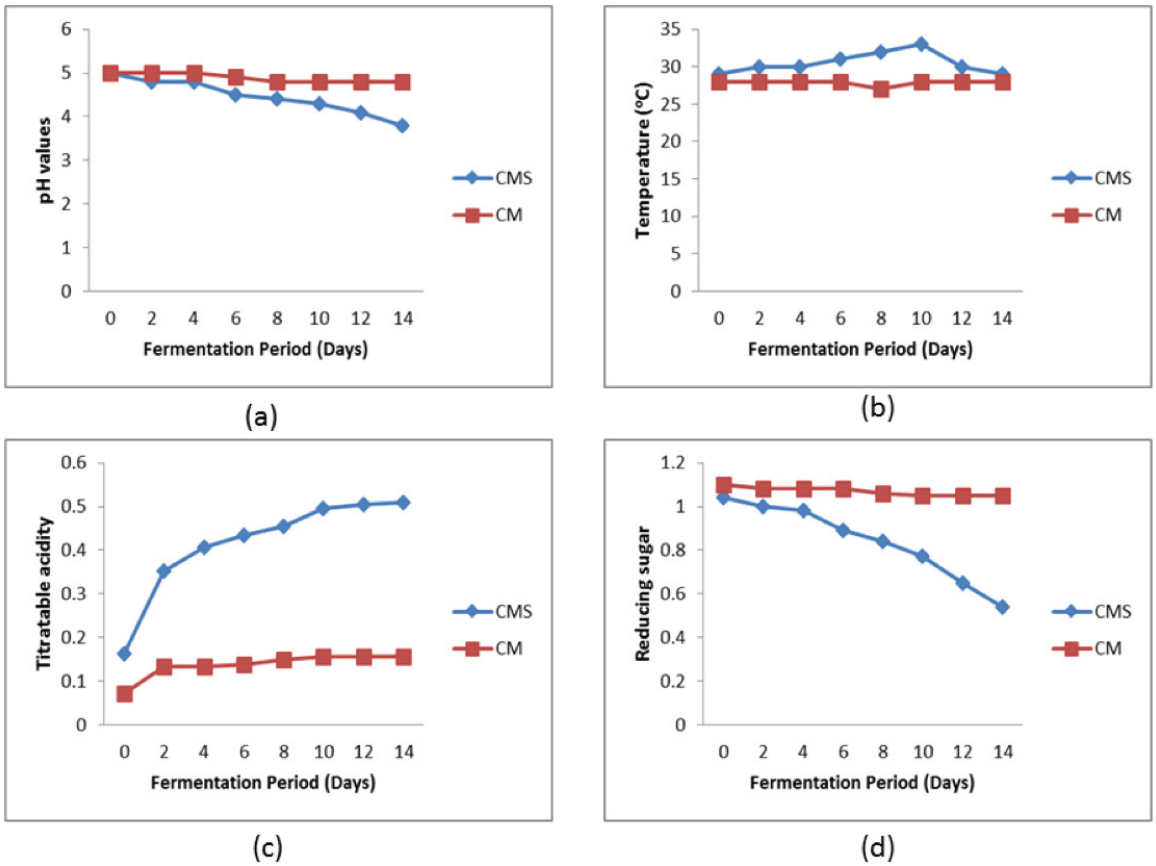
2.3% v/v to 8.1% v/v respectively for the cassava medium containing S. cerevisiae (test sample); while it was from 1.8 mL to 2.0 mL and 1.8% v/v to 2.0% v/v respectively for the control (Figure 1).

There was a decrease in pH (from 5.0 to 3.8) during the fermentation period, though within acidic range (Figure 2a), while the temperature varied throughout the fermentation period; ranging from 28.0oC to 33.0oC for test sample (Figure 2b). Titratable acidity increased all through the fermentation period (Figure 2c) with a decrease in reducing sugar (Figure 2d), as well as an increase in cell biomass (Figure 3).



CMS= Cassava medium with S. cerevisiae. CM= Cassava medium without S. cerevisiae

Figure 1. Ethanol yield and concentration during fermentation period of cassava peels



CMS= Cassava medium with S. cerevisiae. CM= Cassava medium without S. cerevisiae

Figure 2. (a) pH values; (b) temperature (oC); (c) titratable acidity; (d) reducing sugar during fermentation period of cassava peels.

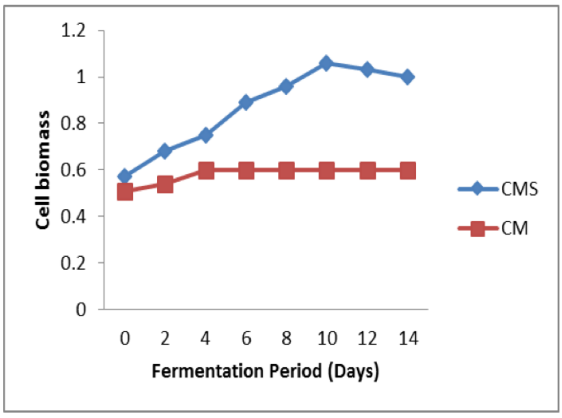


Figure 3. Cell biomass values ($O.D_{600nm}$) during fermentation period of cassava peels
CMS= Cassava medium with S. cerevisiae. CM= Cassava medium without S. cerevisiae

DISCUSSION

Over the years, the peels obtained from the processing of cassava are disposed off as agricultural wastes constituting nuisance and eyesore in the environment. Consequently, a large amount of cassava peels waste is generated annually (9), (10), (11). In developed countries, substrates such as corn, sugarcane and beets have been used in the production of ethanol. In this study, we have explored the utilization of readily available wastes as cassava peels in the production of ethanol.

Ethanol yield after distillation of fermented cassava medium containing yeast isolates during the fermentation period was higher than the ethanol yield from the control experiment (Figura 1). This high ethanol yield from the test sample could be attributed to the presence and fermentative activity of the yeast S. cerevisiae; and this findings is similar to result obtained by Srinorakutara et al. (8) who reported a range of 3.5ml to 12.0ml for ethanol yield from cassava wastes. The successful production of ethanol from cassava peels (wastes) have shown that these waste contain fermentable material which was evident from the increase in ethanol yield throughout the fermentation period (2). The ethanol produced was recorded for all experimental days except day 0, and the values recorded show that ethanol production from cassava medium with yeast had the highest value of 7.5ml as at day 14 and the least value of ethanol yield was recorded on day 2 as 2.2ml.

There was a decrease in pH during the fermentation period, though within acidic range, suggesting that the fermenting microorganism (*S. cerevisiae*) must have the inherent capacity to tolerate acidic condition. The pH values ranged from 5.0 to 3.8 in the test medium and 5.0 to 4.8 in the control medium. This decrease in pH values could be attributed to the formation of organic acids during fermentation (12). The variability in temperature throughout the fermentation period indicate that heat is released during fermentation; while the increase in titratable acidity may be due to increase in acid production in the fermentation media. The reducing sugar of the test sample declined gradually throughout the fermentation period, and this may be due to the bioconversion of the fermentable sugar present to ethanol by *S. cerevisiae* (2), (13), (14). For the test sample, the cell biomass increased till day 10 and started declining slightly until the end of the fermentation period.

CONCLUSION

The various important uses of ethanol and the importance of ridding the environment of the harmful effects due to piling up of agricultural waste products, such as cassava peels, underscore the significance of this study. The results obtained from this experiment revealed that fermentable material is present in a reasonable amount in cassava peels waste. If these peels waste are fermented under stipulated experimental conditions using S. cerevisiae, a substantial amount of ethanol which can be used as chemical feedstock will be produced. Thus, the importation of ethanol can be reduced if substantial resources are devoted to the production of ethanol from cassava wastes.

CONFLICT OF INTEREST

Authors have declared no conflict of interest.

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