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Bioethanol production from cassava peels using different microbial inoculants

Obianwa Chibuzor[1], Edak A. Uyoh[1]* and Godwin Igile[2]

[1]Department of Genetics and Biotechnology, University of Calabar, Cross River State, Nigeria.
[2]Department of Biochemistry, University of Calabar, Cross River State, Nigeria.

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The potential of bioethanol production using different microbial inoculants for the simultaneous saccharification and fermentation of cassava peels from three cassava cultivars was investigated. Peels obtained from three cassava cultivars namely TME 0505, TME 419 and TME 4779, were washed, dried in a laboratory air oven dryer at 120°C for 3 h, ground into a fine texture and sieved with 1.5 µ nylon sieve. The sieved material was cultured using the following inoculant combinations: A = *Rhizopus nigricans* + *Saccharomyces cerevisiae*; B = *Aspergillus niger* + *Saccharomyces cerevisiae*; C = *Rhizopus nigricans* + *Aspergillus niger* + *Saccharomyces cerevisiae*; D = *Rhizopus nigricans* + *Spirogyra africana* + *Saccharomyces cerevisae*; E = *Aspergillus niger* + *Spirogyra africana* + *Saccharomyces cerevisiae*. These combinations have not been tested before on cassava peels. The control was inoculated with *S. cerevisiae* only. The cultures were distilled on the 21st day and the quantity of ethanol produced in each treatment group recorded. Results obtained showed significant differences (P<0.05) in the amount of ethanol produced and in its concentration among the five inoculants. Significant differences (P<0.05) were also obtained in ethanol yield from the three cassava varieties. Cassava peels from TME 4779 gave the highest ethanol yield of 14.46 ± 2.08 g/cm^3 using *R. nigricans* + *S. africana*+ *S. cerevisiae*. Similarly, cassava peels from TME 0505 gave the second highest ethanol yield of 13.33 ± 0.67 g/cm^3 using the same combination, namely *R. nigricans* + *S. africana* + *S. cerevisiae*. Low ethanol yields of 4.82 ± 1.00, 6.43 ± 0.58 and 7.77 ± 0.88 g/cm^3 were obtained from the cassava peels of TME 419, TME 0505 and TME 4779, respectively using *S. cerevisiae* alone. The yield reported in this study competes favorably with those reported from cassava peels, potato peels and millet husks using other inoculant treatments by other workers. Inoculants used in this study thus showed great potential for bioethanol production from cassava peels.

Key words: Bioethanol, cassava peels, microbial inoculants.

INTRODUCTION

The quest by many countries for energy independence as well as the widespread awareness of the need to reduce

*Corresponding author. E-mail: gen_uyoh@yahoo.com. Tel: +2348037929022.

green-house gas emissions have heightened the search for alternative energy sources (Farrell et al., 2006). They have also served as drivers for new government initiatives to increase alternative fuel sources, principally ethanol from biological feed stocks such as cassava (*Manihot esculentum),* corn (*Zea mays)* and sweet potato (*Ipomoea batatas)*. Biofuels are expected to reduce dependence on imported petroleum with associated political and economic vulnerability, reduce greenhouse gas emissions and other pollutants, and revitalize the economy by increasing demand and prices for agricultural products (Balat 2009). There is thus an increasing demand for bioethanol as alternative source of energy and Nigeria currently depends on the importation of ethanol to meet its local demand.

In Nigeria and many developing countries, there is a growing interest in the conversion of the huge biomass of organic wastes generated by the food processing sector and other human endeavors into useful products such as ethanol. A number of studies have been carried out in an attempt to optimize the yield of ethanol from cassava peel using different organisms including *Saccharomyces cerevisiae* (Adesanya et al., 2008; Marx and Nquma, 2013), *Zymomonas mobilis* and *S. cerevisiae* (Sulfahri et al., 2011) *Gloeophyllum sepiarium* plus *Pleurotus ostreatus* for hydrolysis and *Z. mobilis* and *S. cerevisiae* for fermentation (Oyeleke et al., 2012; Adiotomre, 2015), *Aspergillus niger* for hydrolysis and *S. cerevisiae* for fermentation (Adetunji et al., 2015). The search is still ongoing. Odunfa and Olanbiwoninu (2012) recommended that cassava peels could be subjected to pretreatment with dilute sulphuric acid or methanolysis prior to fermentation for higher ethanol content. The present study was thus aimed at contributing to this ongoing effort by using new combinations of microorganisms (*A. niger, Rhizopus nigricans and Spirogyra africana*) in the combined saccharification and fermentation process to produce ethanol from the peels of cassava. To the best of the authors' knowledge, this combination has not been tried before for this purpose.

MATERIALS AND METHODS

Cassava cultivars and microorganisms

Three cultivars of cassava identified as TME 97/ 0505, TME 419 and TME 92/4779 were obtained from National Roots Crops Research Institute (NRCRI) at Umudike, Abia State, Nigeria. The microorganisms used in this study were *A. niger, R. nigricans, S. cerevisae* (bakers' yeast) and the microalgae *S. africana* obtained from Mr. U. A. Offor, a Microbiologist in the Department of Microbiology, Cross Rivers State University of Science and Technology, Calabar, Nigeria.

Preparation of broth culture of *A. niger* and *R. nigricans*

Broth cultures of *A. niger* and *R. nigricans* were prepared in 100 ml of potato dextrose broth medium using standard methods as described by Baker et al. (2001).

Preparation of peels from cassava cultivars

The three cassava cultivars were hand peeled using a table knife. The peels were washed under running tap to remove sand and other impurities, oven-dried at 120°C for 4 h in a laboratory air-oven dryer, milled into a powder (flour) using locally made milling machine and sieved with 1.5 µ nylon sieve. The flour was packed into sterile plastic containers, sealed and labeled accordingly.

Simultaneous saccharification and fermentation

Fifty grams of the sieved cassava peel flour from each of the three cultivars, was dissolved in 500 ml of distilled water in separate conical flasks. For each cultivar, this was replicated six times giving a total of 18 flasks in all. The flasks were plugged with sterile cotton wool, shaken thoroughly and autoclaved for 15 min at 120°C (Adesanya et al., 2008). The six flasks of each cultivar were inoculated with the following respectively: 1. 5 ml *A. niger* + 2 g *S. cerevisae*; 2. 5 ml *R. nigricans* + 2 g *S. cerevisae;* 3. 5 ml *A. niger* + 5 ml *R. nigricans* + 2 g *S. cerevisae*; 4. 5 ml *A. niger* + 1 g *S. Africana* + 2 g *S. cerevisae*; 5. 5 ml *R. nigricans* + 1 g *S. Africana* + 2 g *S. cerevisae*; 6. 2 g of *S. cerevisae.*
The mixture in each conical flask was sealed with aluminum foil and kept for twenty-one (21) days under anaerobic conditions and temperature of 28°C. Thereafter, the samples were filtered with Whatman No.4 filter paper and 30 ml of the filtrate was distilled at 78°C (standard temperature for ethanol distillation). This was done for each fermented sample.

Determination of quantity of ethanol produced and data analysis

The volume of the distillate collected was determined using a measuring cylinder and expressed as quantity of ethanol produced in g/cm^3 by multiplying the volume of the distillate by the density of ethanol (0.8033 g/cm) (Humphrey and Okafoagu, 2007). Means ± standard errors were obtained and subjected to analysis of variance tests. Significant tests were separated using least significant difference tests.

Determination of ethanol concentration

Ethanol concentration (v/v) was determined by extrapolation using the absorbance of ethanol obtained from the standard ethanol concentration curve. The standard ethanol curve was obtained according to the methods of Oyeleke and Jubril (2009).

RESULTS AND DISCUSSION

Analysis of variance showed a significant difference ($P<0.05$) in the yield (g/cm^3) and the percentage concentration yield obtained amongst the inoculants and varieties of cassava. Inoculum D (*R. nigricans* + *S. Africana* + *S. cerevisae*) consistently produced the highest volume yield in all the three cultivars while *S. cerevisae* (control) produced the least in all three cultivars

Table 1. Ethanol yield (g/cm^3) from the three cassava cultivars treated with different inoculant.

Inoculum	TME 419	TME 0505	TME 4779
A	$8.57^{b} \pm 0.67$	$10.18^{a} \pm 0.88$	$9.37^{b} \pm 0.88$
B	$8.57^{b} \pm 0.88$	$9.37^{a} \pm 1.46$	$10.71^{b} \pm 1.46$
C	$8.84^{b} \pm 1.46$	$11.00^{a} \pm 1.00$	$11.51^{b} \pm 1.20$
D	$12.59^{a} \pm 0.88$	$13.33^{a} \pm 0.67$	$14.46^{a} \pm 2.08$
E	$10.98^{a} \pm 1.45$	$13.00^{a} \pm 1.00$	$10.71^{b} \pm 1.46$
Control	$6.43^{c} \pm 0.58$	$4.82^{b} \pm 1.00$	$7.77^{b} \pm 0.88$

A = *Rhizopus nigricans* + *Saccharomyces cerevisae;* B = *Aspergillus niger* + *Saccharomyces cerevisae;* C = *Rhizopus nigricans* + *Aspergillus niger* + *Saccharomyces cerevisae;* D = *Rhizopus nigricans* + *Spirogyra africana* + *Saccharomyces cerevisae;* E = *Aspergillus niger* + *Spirogyra africana* + *Saccharomyces cerevisae;* control = *Saccharomyces cerevisae.* Means followed by similar case letters in each column are not significantly different (P<0.05).

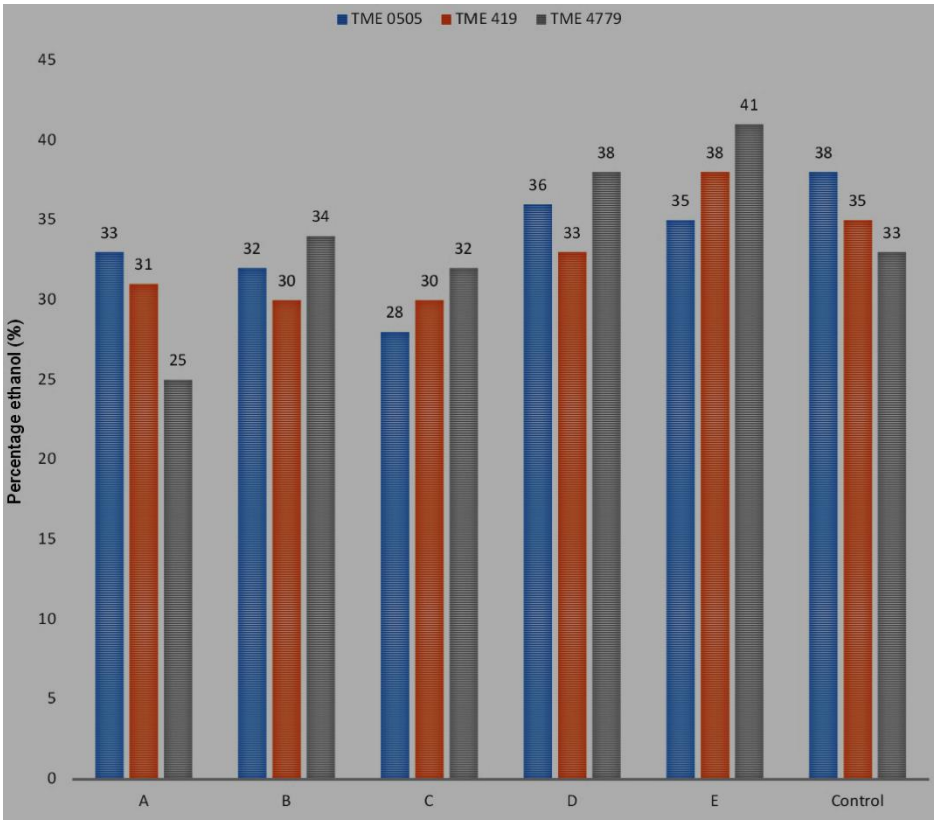

Figure 1. Percentage ethanol concentration (purity) from the three cassava cultivars treated with different inoculants. A = *Rhizopus nigricans* + *Saccharomyces cerevisae;* B = *Aspergillus niger* + *Saccharomyces cerevisae;* C = *Rhizopus nigricans* + *Aspergillus niger* + *Saccharomyces cerevisae;* D = *Rhizopus nigricans* + *Spirogyra Africana* + *Saccharomyces cerevisae;* E = *Aspergillus niger* + *Spirogyra Africana* + *Saccharomyces cerevisae;* control = *Saccharomyces cerevisae.*

as shown in Table 1. Cassava TME 4779 gave the highest concentration of 41% (v/v) when treated with inoculum E (*A. niger* + *S. africana* + *S. cerevisae*) as shown in Figure 1. Fermentation results obtained on the 21st day from the three cassava varieties using five inoculants and control are shown in Table 1.

The production of bioethanol from cassava peels using different combinations of microorganisms was examined.

The microorganisms expectedly produced different amylolytic enzymes and to different levels which acted on the peels from the three cassava cultivars.

The highest ethanol yield of 14.46 g/cm^3 was obtained from cassava cultivar TME 4779 and a concentration of 38% (v/v) when treated with *R. nigricans* + *S. africana* + *S. cereviceae.* This could be attributed to the presence of more carbohydrates from *Spirogyra* which is fermented to ethanol in the presence of the amylolytic microorganisms. *Spirogyra* generally is known to be autotrophic and its carbohydrate composition can also lead to increase in the release of sugars for fermentation. This result is in line with the work of Sulfahri et al. (2011) but gave a higher yield because of the presence of cassava peel substrate and good pH conditions. Sulfahri et al. (2011) obtained 9.70% of ethanol from *Spirogyra* with fermentation by *Zymomonas mobilis* and *S. cereviceae* after 96 h (4 days). The present result is also higher than that obtained by Asif et al. (2015), who obtained 9.3 (v/v) and 8.3% (v/v) of ethanol from sugarcane molasses using *Z. mobilis* and *S. cerevisiae,* respectively. It is comparable with the report by Adiotomre (2015) of 23% ethanol from 50 g of substrate using *Gloeophyllum sepiarium* and *Pleurotus ostreatus* for hydrolysis of the peels and *Z. mobillis* and *S. cerevisiae* for fermentation

Other microbial combinations such as *A. niger* + *S. cereviceae*, as well as *R. nigricans* + *S. cerevisiae* also gave relatively high yields of 10.71 and 10.18 g/cm^3, respectively. This is slightly higher than the report of Oyeleke et al. (2012), whose study gave 10.6 g/cm^3 when *Z. mobilis* and *S. cerevisiae* were used to ferment cassava peels. The similarities can be ascribed to the enzyme content of *A. niger* and *R. nigricans*, both organisms are known to contain enzymes such as α-amylase, glucoamylase and cellulase necessary for the breakdown of the complex cellulose composition of cassava peels (Akpan et al., 1996). The average percentage concentration of ethanol obtained in the present study is relatively high as compared to the average yield reported from spoilt mangoes by Agulejika et al. (2005). They reported an average ethanol concentration yield of 16%. This is likely to be due to the presence of more carbohydrate content in cassava peels than in spoilt mangoes. The present report is also higher than the 8.5% given by Adetunji et al. (2015) using *A. niger* and *S. cerevisiae* on cassava peel slurry. On the other hand, the percentage concentration of ethanol obtained in the present study is much lower than reports by Oyeleke and Jubrin (2009) of 67.7 and 63.8% when *A. niger* and *Z. mobilis* were used simultaneously on guinea corn husk and millet husk, respectively. It is also lower than the 83% yield reported by Sivamani and Baskar (2015) in cassava peel using a saccharification and fermentation mixture containing glucoamylase and *Z. mobilis* with optimum conditions of 69.82 g/l substrate concentration, 24.74% (v/v) α-amylase concentration and 5.22% saccharification and fermentation mixture. Sometimes, the differences in ethanol yield may be attributed to the actual amount of carbohydrate present in the peel at the start of the experiment.

S. cerevisiae also known as baker's yeast has been successfully grown on several substrates like molasses, cashew and apple juice for the production of single-cell protein and bioethanol. It is used commercially for the fermentation of glucose to ethanol and it is known for its high tolerance to ethanol, rapid fermentation rates and insensitivity to substrate concentrations (Linden and Hahn-Hagerdal, 1989). Ethanol yields as high as 65.27% have been reported from hydrolyzed pineapple peel using *S. cerevisiae TISTER 5048* (Niwaswong et al., 2014). It has, however, been reported to be a non-amylolytic microorganism, unable to hydrolyze starch (Jamai et al., 2006). Varying concentrations of ethanol ranging from 4.82 to 7.77 g/cm^3 were obtained in this study. Similarly, Ashok et al. (2014) obtained ethanol concentration of 7.95% (v/v) from sweet potato using *S. cerevisiae* MTCC-170. This shows that *S. cerevisiae* has the ability of producing ethanol from starch but at a low rate.

Conclusion

This study showed that the combination of *R. nigricans, S. africana* and *S. cereviceae* may be the most suitable for production of ethanol from cassava peels. The study also suggests that the choice of cassava cultivar also plays a role in the optimum production of ethanol with cassava cultivar TME 4779 giving the highest yield.

Conflict of Interests

The authors have not declared any conflict of interests.

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