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Comparative Study of Bioethanol Production and Reducing Sugar Yields from Cassava Peels Using Fungi

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Abstract

This study compared the yields of bioethanol from the fermentation of pretreated cassava peels using yeasts isolated from palm wine, and a pretreatment method with methanol + acid before solid-state fermentation of cassava peels using *Trichoderma reesei* for 5 days optimally yielded 1.78 g/mL of reducing sugar. The hydrolysate was fermented for bioethanol production using *Saccharomyces cerevisiae* and *Candida tropicalis*. *S. cerevisiae* performed optimally at 30°C, pH 4.5, and produced ethanol with a concentration of about 40.72 g/L, while *C. tropicalis* produced 29.90 g/L of ethanol concentration at 35°C, and pH 5. Both yeast isolates took the same fermentation time of 96 h. In conclusion, cassava peels are agricultural waste that is a degradable material suitable to produce simple reducing sugars, which can be fermented by yeast to produce bioethanol. The yield of ethanol was higher for *S. cerevisiae* than *C. tropicalis*.

Keywords: methanol, acid hydrolysis, reducing sugar, fermentation, ethanol, palm wine.

Резюме

Това проучване сравнява добивите на биоетанол от стърготини от маниока, предварително обработени по 2 различни начина (с дрожди, изолирани от палмово вино или с метанол + киселина) и последвала твърдофазова ферментация при използването на *Trichoderma reesei.* Ферментацията продължава 5 дни, а оптималният добив от редуциращи захари е 1.78 г/мл. Полученият хидрролизат се подлага на ферментация с участието *Saccharomyces cerevisiae* and *Candida tropicalis*. *S. cerevisiae* се култивира при 30°C и pH 4.5 и полученият етанол е с концентрация около 40.72 г/л. В същото време, *C. tropicalis* произвежда етанол с концентрация 29.9 г/л при температура 35°C и pH 5.0. Продължителността на ферментация и при двете дрождеви култури е 96 ч. В заключение, стърготините от маниока са селскостопански отпадък и разградим материал, подходящ за получаването на захари, които могат да бъдат подложени на ферментация от дрожди за производство на биоетанол. Добивът на етанол при използването на *S. cerevisiae* е по-висок от този при използването на *C. tropicalis*.

Introduction

Cassava (*Manihot esculenta*) is an erect perennial shrub, propagated vegetatively from hardwood stem cuttings. It is a significant crop within a wide range of tropical environments and is an important part of cropping systems (Apiwatanapiwat *et al*., 2011). It is a food crop that survives extremely harsh environmental conditions such as drought and low nutrient availability.

Cassava peels are one of the main agricultural waste in Nigeria and it is generated through the processing of cassava tubers for human and industrial use, for example, in the processing of garri. It is 1 - 4mm thick and accounts for 20-35% of the weight of the tuber (Olanbiwoninu and Odunfa, 2012). The cassava peels obtained from garri processing are usually discarded as waste and allowed to rot in the open, and this could result in health hazards that can affect humans and animals. Ethanol obtained from agricultural feedstock is known as bioethanol. Bioethanol is a form of renewable energy that is obtained from the conversion of carbon-based feedstock. It is generally investigated as a renewable

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fuel source because in many respects it is superior to gasoline fuel (Jones *et al*., 1994).

The sap of the oil palm tree (*Elaesis guinnensis*) serves as a rich and highly nutritious growth substrate for various types of microorganisms. The palm sap is tapped and allowed to undergo natural fermentation that is spontaneous, which allows the proliferation of yeast species to convert the sweet substrates into an alcoholic beverage. Several other studies have shown that the alcohol fermenting yeast *Saccharomyces cerevisiae* naturally colonizes palm sap (Nwachukwu *et al.*, 2006).

In Nigeria and many developing countries, there is an increasing interest in the conversion of the huge amount of cassava peels generated into ethanol. Several studies have been carried out to optimize the yield of ethanol from cassava peels using different organisms including *S. cerevisiae*. *Aspergillus niger* is also used for hydrolysis and *S. cerevisiae* for fermentation. Odunfa and Olanbiwoninu (2012) also recommended that cassava peels could be subjected to pretreatment with dilute sulphuric acid or to methanolysis before fermentation for higher ethanol content. The present study was thus aimed at contributing to this ongoing effort by using the combination of methanolysis and dilute sulphuric acid as pretreatments before microbial hydrolysis using *Trichoderma reesei,* and comparing the ethanol yields using *S. cerevisiae* and *Candida tropicalis* isolated from palm wine.

Materials and Methods

Sample collection and substrate preparation

Fresh cassava peels from the garri (a roasted granular hygroscopic starchy food from cassava consumed in the West African sub-region) processing site in Ajibode, University of Ibadan, Ibadan, Nigeria, were collected and washed thoroughly under running tap water to remove sand and other impurities, milled using a blender machine and dried overnight at 80°C in the laboratory air-oven dryer according to the method of Olanbiwoninu and Odunfa (2015). The palm wine samples were obtained from Ibadan metropolis (Challenge and Eleyele) in a sterile plastic container placed in an ice-pack and then transported to the Microbiology Laboratory of the Department of Microbiology, University of Ibadan.

Fungal isolates

An isolate of *T. reesei* with preceding activities of the high zone of clearance on 1% CMC agar plate (78 mm) and a high index of relative enzyme activity (2.299 U/ml)] used in this work was obtained from the Department of Microbiology, University of Ibadan, Nigeria. The isolate was maintained on potato dextrose agar (PDA) slant and kept at 4°C for further use. The yeast starters for bioethanol production used in this study were isolated from palm wine because of their high tolerance to ethanol, sugar, and salt during the screening process for selection. Yeast isolates were recovered from palm wine by aseptically withdrawing 1mL of the palm wine and diluting it using ten-fold serial dilution techniques. Pour plate method was employed by inoculating 0.1 mL of dilutions ranging from dilution factors of 10^{-2}, 10^{-4}, and 10^{-6} on a Yeast Extract Peptone Dextrose Agar supplemented with 0.002 mg/mL of Chloramphenicol to suppress bacterial growth and incubated at 30°C for 48 hours (Fawole and Osho, 2007).

Identification of the yeast isolates

The yeast isolates were characterized based on their cultural characteristics (colony shapes, pigment, elevation, edge, consistency, and surface appearance), in addition to their morphological and biochemical characterization, according to Sanni and Lonner (1998); Messa *et al*. (1999); Barnett *et al*. (2002); Ogbo (2005); Fawole and Oso (2007). The yeast isolates were cultured and maintained in yeast extract peptone dextrose agar medium.

Screening of the isolated yeasts

According to the methods of Osho (2005) and Shafkat (2013), the ethanol tolerance, thermo-tolerance, osmo-tolerance, and growth at 15% sugar concentration of both yeast isolates were tested.

Cassava peel pretreatment

Treatment with methanol + acid hydrolysis by H_2SO_4

This pretreatment was carried out according to the method conducted by Olanbiwoninu and Odunfa (2012). About 10 g of milled cassava peel was suspended in 100 ml of methanol, with 0.05 M concentrations of sodium acetate as a catalyst. After treatment, the solid residues were collected and washed under running tap water for 10 minutes, oven-dried at 55°C overnight before separate hydrolysis with acid. Acid hydrolysis of the methanol pretreated cassava peel powder was carried out according to the methods of Olanbiwoninu and Odunfa, (2012) and Zainal *et al*. (2014). Approximately 15 g of the methanol pretreated cassava peel powder was hydrolyzed in 100 ml of 0.5 M sulphuric acid at 100°C for 60 min in duplicates. The solid residues were collected and neutralized with

2 M NaOH until a neutral pH was achieved. The residue was oven-dried at 55°C overnight, and then subjected to microbial hydrolysis using *T. reesei*.

Microbial hydrolysis of pre-treated cassava peel powder

Solid-state fermentation design was used in the microbial hydrolysis in reference to Ahmed *et al*. (2010) and Agarwal *et al*. (2014). Approximately 10 g of the methanol and acid-treated cassava peel powder was placed in 250 mL Erlenmeyer flasks. The substrate was moistened with minimal salt solution (pH 5.0) containing (g/L): KH_2PO_4 1, KCl 0.5, $MgSO_4$ 0.5, $FeSO_4.7H_2O$ 0.01, $NaNO_3$ 2, stirred properly and then sterilized. The flasks were then inoculated with 1mL of 10% (v/w) inoculum containing 1×10^8 CFU/ ml *T. reesei* conidia under aseptic conditions, and then incubated at 30°C for 5 days in duplicates. The conidial suspension was prepared by flooding a 7-day-old PDA slant of *T. reesei* with 2 mL of sterile distilled water. The reducing sugar concentration was determined at 24-hour intervals. After incubation, the fermented substrate was mixed with 100 ml of the sterilized distilled water (i.e., 1:10 w/v) for 1 hour on an orbital shaker at 180 rpm. Finally, the contents of the flask were filtered through a muslin cloth, and then the filtrate centrifuged at 2500 rpm for 15 minutes to obtain the hydrolysate. Optimization of conditions for reducing sugar yield was carried out as well. The reducing sugar content was measured quantitatively using the DNS method, as recommended by Miller (1959).

Estimation of reducing sugar

The reducing sugar concentration was determined quantitatively using the dinitro-salicylic acid (DNSA) method according to Miller (1959) in duplicates. Approximately, 1 mL of dinitrosalicylic acid was added to 1 mL of each supernatant (filtrates) in test tubes labeled accordingly, and then the mixtures were heated in boiling water for 10 minutes. The test tubes were cooled rapidly in tap water and the volume was adjusted to 12 ml using distilled water. A blank containing 1 mL distilled water and 1 mL of DNSA was prepared. The optical density of the samples was measured against the blank using a spectrophotometer (JENWAY: Model 6405, UK) set at 540 nm. The concentration of the reducing sugar in the supernatant was estimated using the glucose standard curve.

Yeast cell preparation

A broth medium containing: 0.3% yeast extract, 1% peptone, 2% glucose, 1.5% agar, adjusted to pH 5.0 was prepared, sterilized, inoculated individually with 48-hour old culture of each yeast isolate, and then incubated at 30°C for 3 days in a rotary shaker. The supernatant was discarded, while the cells were washed using a buffer with pH 7.0 before the wet pellet was used for inoculation (Ana, 2013).

Yeast fermentation of the hydrolysate

Fermentation was carried out in a fermentation medium, (cassava peel hydrolysate medium) which comprised 0.2% yeast extract, 0.2% $(NH_4)NO_3$, 0.1% $MgSO_4.7H_2O$, 0.2% KH_2PO_4 in 100 ml of the hydrolysate. The fermentation medium was sterilized at 121°C for 15 min, after which it could cool. Fermentation was initiated in an Erlenmeyer flask after inoculating the media individually with 10 ml of 0.5 McFarland standard inoculum sizes (1.5×10^8 cells/mL) of *S. cerevisiae* and *C. tropicalis*, under aseptic conditions (Gupta *et al*., 2009). This was allowed to ferment and the ethanol concentration was monitored at 24-hour intervals for 5 days.

Optimization of cultural conditions during yeast fermentation

To optimize bioethanol production from the substrates, the cultural and nutritional parameters such as: fermentation time (24-120 hours), pH (4.0, 4.5, and 5.0), temperature (30, 35, and 40°C), agitation, and stationary incubation, were optimized for higher ethanol production. These were carried out in duplicates.

Determination of ethanol concentration using the dichromate test

Ethanol analyses were carried out using the method developed by Lees (1975). An aliquot of 1 mL of each sample of the fermentation broth was taken into a test tube. A volume of 1 mL of 10% $K_2Cr_2O_7$ was added, and then the tubes were transferred to an ice bath for 5 minutes, after that, 5 mL of concentrated H_2SO_4 was added to each tube, and then 3 mL of distilled water was added to each tube and then mixed well while still maintained in the ice bath. The absorbance of the mixture was measured at 600 nm using a spectrophotometer JENWAY: Model 6405, UK.

Results

Frequency of occurrence of the yeast isolates from palm wine

A total of 80 isolates were obtained from different samples such as palm wine, cassava wastewater, fruits (e.g., mango, orange, pears, apple, and

banana), rice wastewater, honey, and fermented foods (e.g., kunu and ogi). Freshly prepared cultures of these yeast isolates were subjected to screening on salt and ethanol incorporated media at different concentrations of 6% - 15% (3% intervals) for salt, and 2.5%-15 % (2.5% intervals) for ethanol, respectively. Thirty yeast isolates showed growth on the plates with the highest concentrations of salt and ethanol and were further screened by inoculating 0.5 McFarland standard inoculum size (1.5×10^8 CFU/mL) of the yeasts into Yeast Extract Peptone Dextrose broth exogenously incorporated with 15% salt concentration, 50% glucose concentration and 15% ethanol concentration in duplicates were read at 600 nm wavelength after 48 hours. Two out of these thirty yeasts showed promising abilities from the results shown in Table 1 and were used as a starter culture for bioethanol production. The percentage occurrence of the probable yeasts selected after the initial screening on salt and ethanol incorporated media at different concentrations is shown in Fig. 1.

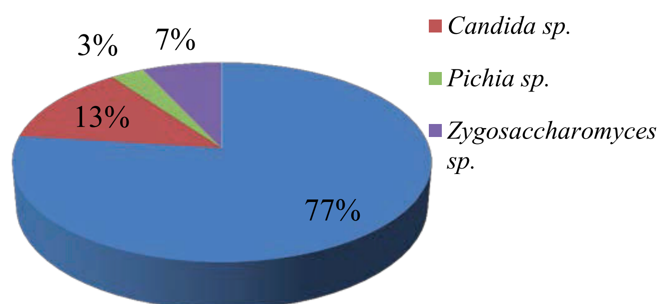

Fig. 1. Frequency of occurrence (%) of the selected yeasts obtained from different samples

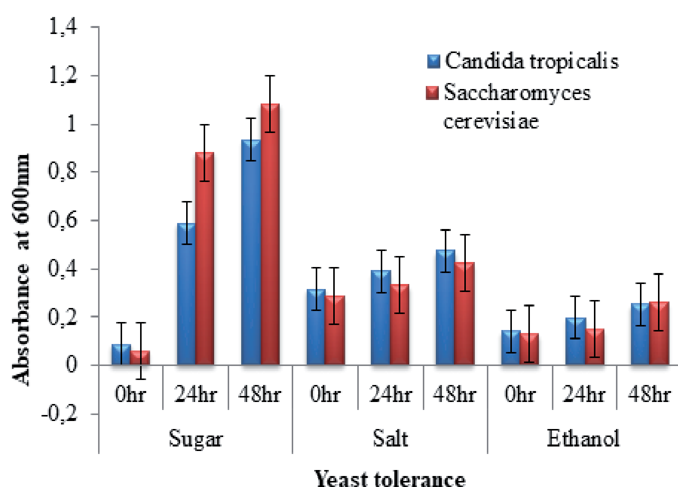

Fig. 2. Tolerance of *C. tropicalis* and *S. cerevisiae* to 15% of Glucose, 15% of NaCl, and 15% of ethanol concentration at a different time interval

Table 1. Tolerance of the screened yeasts to sugar, salt, and ethanol after 48 hours Incubation.

Probable yeast	OD at 15% salt conc.	OD at 50% glucose conc.	OD at 15% ethanol conc.
S. cerevisiae	1.772	176	0.141
Saccharomyces sp.	1.855	0.830	0.002
Saccharomyces sp.	1.438	0.939	0.012
Saccharomyces sp.	0.662	0.622	0.101
Saccharomyces sp.	0.869	1.020	0.009
Pichia sp.	1.072	0.858	0.041
S. cerevisiae	1.315	1.058	0.533
Candida sp.	1.699	0.959	0.018
S. cerevisiae	1.183	0.829	0.006
Saccharomyces sp.	0.693	0.705	0.041
Saccharomyces sp.	1.306	0.847	0.360
Saccharomyces sp.	0.070	1.095	0.074
S. cerevisiae	1.607	1.141	0.626
S. cerevisiae	1.964	0.688	0.047
S.cerevisiae	1.326	1.014	0.023
Zygosaccharomyces sp.	0.962	0.582	0.052
Candida sp.	1.484	0.619	0.048
Zygosaccharomyces sp.	1.377	1.079	0.042
Saccharomyces sp.	1.544	1.006	0.030
Candida sp.	1.385	1.130	0.013
Saccharomyces sp.	1.276	0.851	0.033
S. cerevisiae	1.688	0.956	0.018
S. cerevisiae	1.622	0.907	0.037
S. cerevisiae	1.508	0.888	0.008
S. cerevisiae	1.740	0.947	0.009
Saccharomyces sp	1.957	0.992	0.004
Saccharomyces sp.	1.320	0.690	0.020
Candida tropicalis	1.747	1.090	0.566
Saccharomyces sp.	0.380	0.698	0.025
Saccharomyces sp.	0.720	0.899	0.193
Control	0.265	0.364	0.001

OD= Optical density

Twenty-three, which represents 77%, were identified as *Saccharomyces* sp., 4 out of the 30 were *Candida* sp. representing 13%, 2 (7%) were *Zygosaccharomyces* sp., while 1 (3%) belonged to *Pichia* sp. About 7 (30%) of the *Saccharomyces* sp. were obtained from fruits, 7 (30%) from palm wine, 5 (22%) from food waste, and 4 (18%) from fermented foods. *Zygosaccharomyces* sp. was isolated from honey while *Pichia* sp. was isolated from rotten apple.

Tolerance of yeast isolates

Results of tolerance to ethanol (15% concentration), NaCl tolerance (15% concentration), sugar tolerance (15% concentration) for *S. cerevisiae* and *C. tropicalis* to show their growth pattern at 24-hour intervals are shown in Fig. 2. The absorbance was read off spectrophotometrically at 600 nm, at 0, 24, and 48 hours, respectively.

Reducing sugar concentration

The cassava peel was pretreated with dilute acid after methanolysis with sodium acetate as a catalyst. This resulted in the release of 1.26 mg/ml of reducing sugar. Moreover, the residue from the pretreatment was subjected to microbial hydrolysis using *T. reesei* under optimized conditions which yielded 1.78 mg/ml of reducing sugar. The untreated control released 0.39 mg/ ml of reducing sugar. *T. reesei* produced the highest reducing sugar concentration at the detected optimum cultural conditions of 30°C, pH 5.0, 70% moisture, using glucose and ammonium sulfate as the best choice of carbon and nitrogen source, respectively at day 3 (72 hours) of fermentation, as shown in Figures 3, 4, 5, 6, 7, and 8.

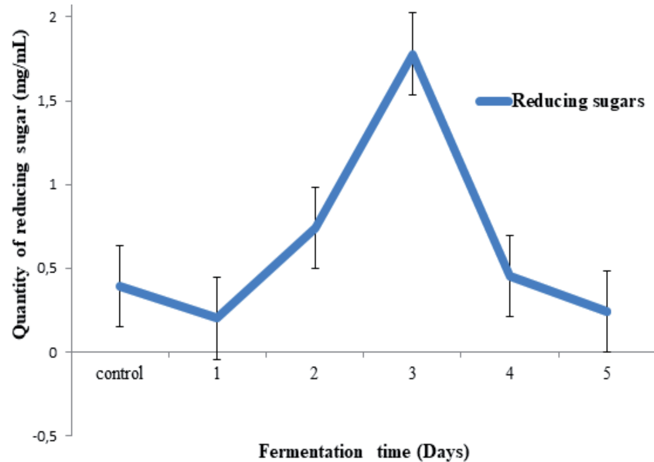

Fig. 3. Fermentation time for microbial hydrolysis of pretreated cassava peel

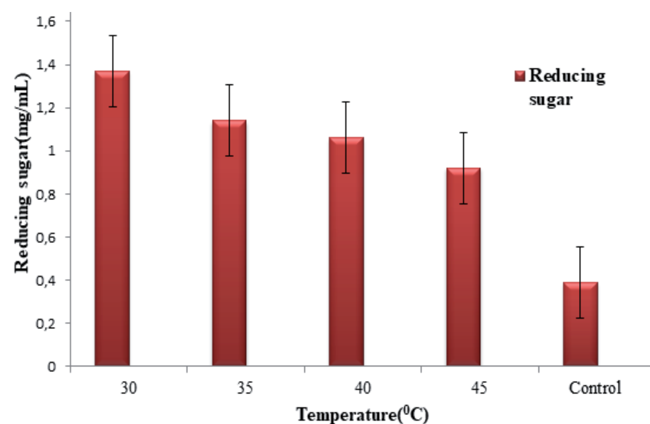

Fig. 4. Effect of temperature on microbial hydrolysis of pretreated cassava peel (M+A) by *T. reesei,* MT + Ac=Methanol treated + Acid

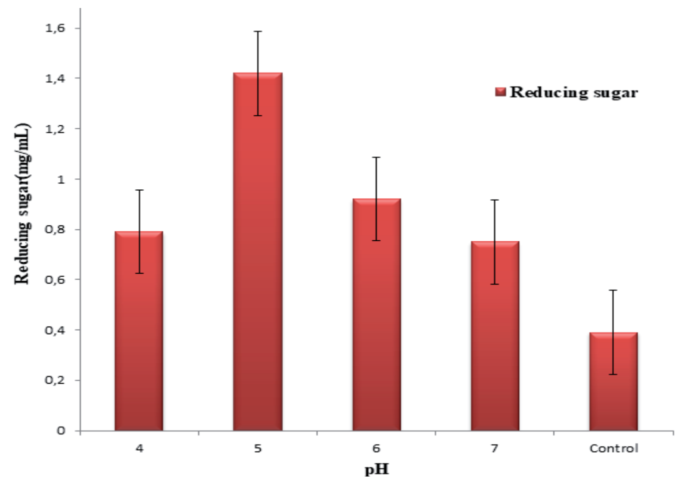

Fig. 5. Effect of pH on microbial hydrolysis of pretreated cassava peel (MT+Ac) by *T. reesei*
MT + Ac=Methanol treated + Acid

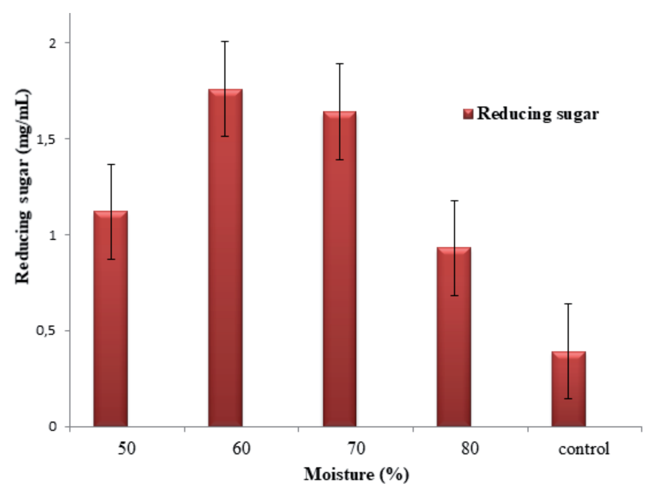

Fig. 6. Effect of moisture content on microbial hydrolysis of pretreated cassava peel (MT+Ac) by *Trichoderma reesei,* MT + Ac=Methanol treated + Acid

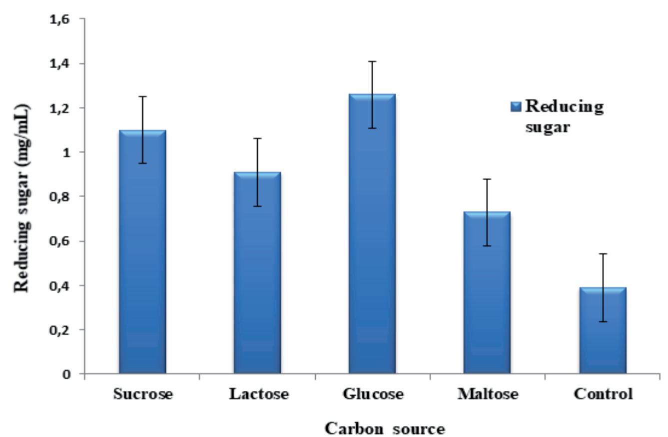

Fig. 7. Effect of carbon source on microbial hydrolysis of pretreated cassava peel (MT+Ac) by *T. reesei,* MT + Ac=Methanol treated + Acid

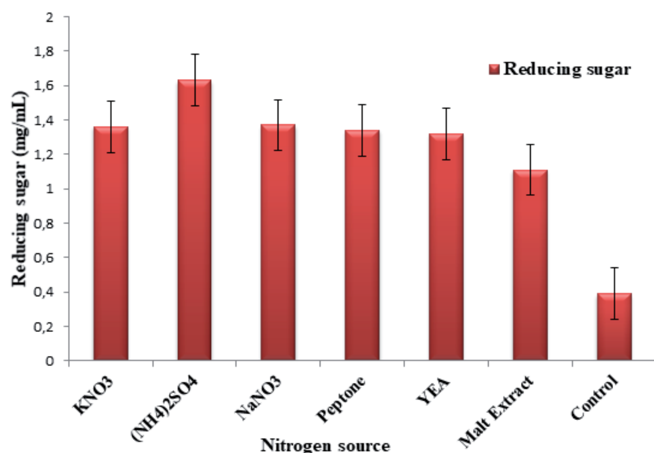

Fig. 8. Effect of nitrogen source on microbial hydrolysis of pretreated cassava peel (MT+Ac) by *T. reesei,* MT + Ac=methanol treated + Acid

Fermentation of the hydrolysate

The fermentation time for *S. cerevisiae* and *C. tropicalis* peaked at 96 hours as shown in Fig. 9, the yields of both isolates being relatively different, recording an ethanol concentration of 29.36 g/L for *S. cerevisiae*, compared to *C. tropicalis,* which had 16.41 g/L over the same period. The effect of temperature on the fermentative ability of both isolates was studied at different temperatures ranging from 30°C to 40°C at 5°C intervals.

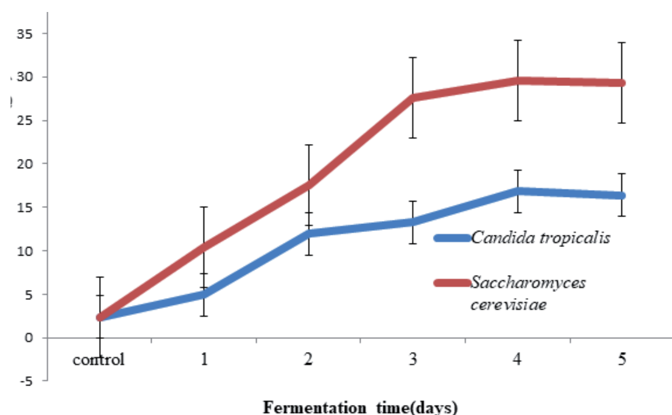

Fig. 9. Fermentation time of methanol + acid pretreated cassava peels+ microbial hydrolysis by *C. tropicalis* and *S. cerevisiae*

Figure 10 shows that *S. cerevisiae* has an optimum ethanol concentration of 30.2 g/L at 30°C, while *C. tropicalis* has optimum ethanol concentration of 18.82 g/L at 35°C. The study of the effect of pH on the fermentative ability of both yeast isolates indicated that *S. cerevisiae* thrives more at pH 4.5 with a yield of ethanol concentration of 30.41 g/L, while *C. tropicalis* has 19.40 g/L at pH 5.0, as shown in Fig. 11, the ethanol concentration is maximum during agitation compared to the stationary incubation, as shown in Fig. 12. During the agitation at revolutions per minute (rpm), *C. tropicalis* and *S. cerevisiae* gave 29.90 g/L and 40.72 g/L, while at stationary incubation it was 18.91 g/L and 28.68 g/L, respectively. The optimum conditions for ethanol production by *S. cerevisiae* are pH 4.5 and incubation temperature of 30°C, while that for *C. tropicalis* are pH 5.0 and temperature of 35°C, respectively. The ethanol concentrations obtained for *C. tropicalis* and *S. cerevisiae* during ethanolic fermentation of the hydrolysate were 29.90 g/L and 40.72 g/L, respectively.

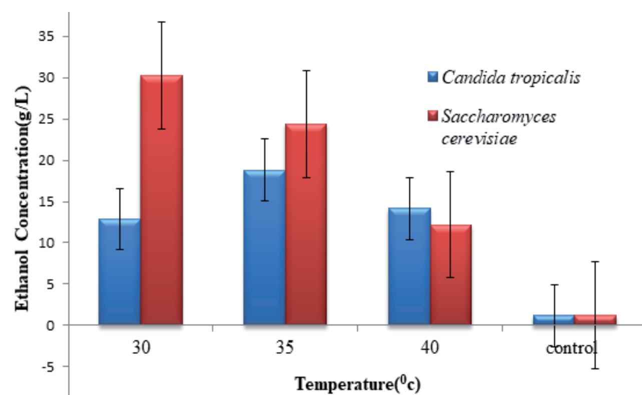

Fig. 10. Effect of temperature on fermentation of methanol + acid pretreated cassava peels + Microbial hydrolysis by *C. tropicalis* and *S. cerevisiae*

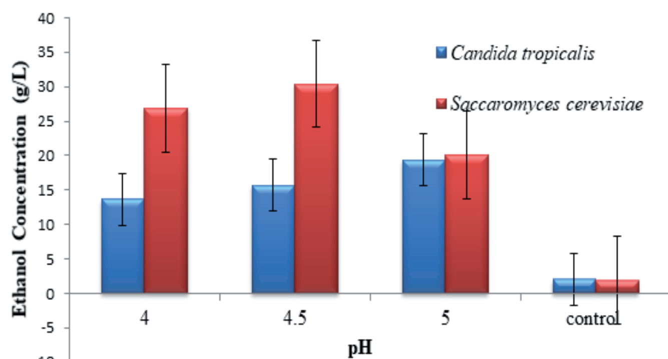

Fig. 11. Effect of pH on fermentation of methanol + acid pretreated cassava peels+ microbial hydrolysis by *C. tropicalis* and *S. cerevisiae*

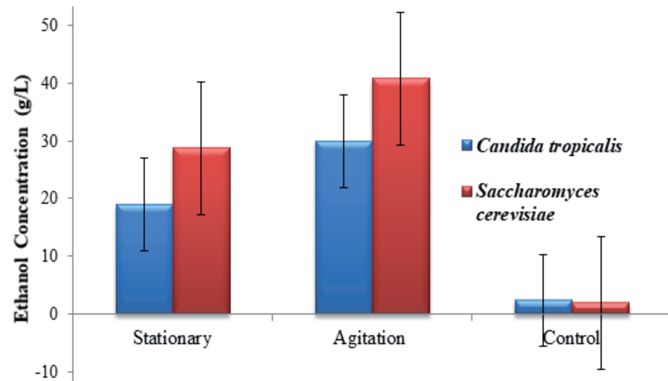

Fig. 12. Effect of agitation on fermentation of acid + methanol pretreated cassava peels + microbial hydrolysis by *C. tropicalis* and *S. cerevisiae*

Discussion

In this study, two yeasts isolated from palm wine satisfied the conditions for the selection of starter culture to initiate fermentation of pretreated cassava peels hydrolysate for bioethanol production. Based on their colony characteristics (white and creamy texture), ovoid or ellipsoidal microscopic shape, the presence of budding pattern (multipolar), and sugar fermentation, the isolates were found to be *S. cerevisiae* and *C. tropicalis,* which are unicellular ascomycetes according to Sanni and Lonner (1998); Boekhout and Kurtzman (2006). They are diverse in sugar utilization and from the sugar tolerance test it was found that both *S. cerevisiae* and *C. tropicalis* grew well in yeast extract peptone dextrose broth incorporated with 15% glucose concentration after 48 hours, with *S. cerevisiae* showing more growth within this period.

In this study, the osmo-tolerance test of *S. cerevisiae* and *C. tropicalis* in 15% concentration of NaCl in Yeast Extract Peptone Dextrose broth indicates an increase in growth after 48 hours. However, *C. tropicalis* had a higher salt tolerance level within the same period. This finding is in agreement with the work of Maria *et al*. (1997), who found that *C. tropicalis*, a vigorous respiratory yeast, in glucose media showed a better adaptation to Na^+ and Li^+ and maintained a higher intracellular Na^+: Li^+ and Na^+: K^+ ratios than *S. cerevisiae* fermentative yeast.

Ethanol concentrations are the major influencing factors during the fermentation process as they inhibit or depress the fermentative yeasts used. Both *S. cerevisiae* and *C. tropicalis* in this study were able to tolerate and grow in 15% ethanol concentration incorporated into YEPD broth after 48 hours. However, *S. cerevisiae* showed better growth within this period. This finding is in agreement with Nwachukwu *et al*. (2006), who reported that *S. cerevisiae* isolated from palm wine was able to tolerate 16% ethanol concentration. Teramoto *et al*. (2005) reported almost similar 16.5% (v/v) ethanol tolerance. The works of Ameh and Okagbue (1990) and Ezeogu and Emeruwa (1993) also support these findings.

The fermentation of the (MT + Ac) pretreated cassava peel by *T. reesei* yielded 1.78 mg/mL of reducing, sugar which is higher than 1.27 mg/mL of reducing sugar reported by Aransiola and Fagade (2015) from the microbial hydrolysis of cassava peel using *T. viride*. This could be the result of the pretreatment methods used before microbial hydrolysis and also the mold used for the fermentation.

In this study, *T. reesei* was inoculated into methanol+acid pretreated cassava peel to bring about cellulose enzymatic hydrolysis under solid-state fermentation. The maximum amount of 1.78 mg/ml of the reducing sugar was recorded on the third day. This is in agreement with the work of Olanbiwoninu and Odunfa (2012), who reported maximum hydrolysis of the methanol and acid pretreated cassava peel after the third day using *A. terreus*. Contrary to this result were the findings reported by Aransiola and Fagade (2015), who recorded maximum reducing sugar on the 14th day using *T. viride,* and Olayide *et al*. (2015), who recorded maximum reducing sugar on the 7th day using *A. niger*. The variations could be because there was no pretreatment of the cassava peels before fermentation as this enhances the release of the reducing sugar from the peels (Olanbiwoninu and Odunfa, 2015). The cultivar of the cassava peel and the cellulolytic ability of the organisms used could be the other reasons for this variation.

T. reesei was able to grow over a range of temperatures of 30-45°C with maximum reducing sugar production obtained at 30°C. This might be due to the better growth of *T. reesei* at this temperature. This result is considerably similar to what was reported by Shafique *et al*. (2009), who recorded optimum temperature for maximum cellulase and by implication maximum reducing sugar yield for *T. reesei* at 30 ± 2°C.

The optimum pH for reducing sugar yield in this study was achieved at pH 5. This result is in agreement with the work of Li *et al*. (2000), who reported that the optimum pH for cellulase production from *T. viride* was at pH 5. It is also in agreement with the work of Olufunke and Ogugua (2013). Optimum pH for fungal cellulase varies from species to species but it is usually in a medium of low acidic level for their growth and enzyme biosynthesis (Haltrich *et al*., 1996).

The optimum reducing sugar yield for the cellulose enzymatic hydrolysis by *T. reesei* was obtained at 60% moisture content. This result is supported by the work of Olufunke and Ogugua (2013), who reported an optimum reducing sugar yield at 60% moisture content for the fermentation of cassava peel by *T. viride* under SSF. From this result, the level of sugar decreases above 60% moisture content, which is in disparity with the report by Sun *et al*. (2010), who showed that cellulase production decrease above 70% moisture content. The decrease as the case may be could be due to a decrease in porosity, which changes substrate in-

teraction, reduce aeration, and promotes stickiness. This results in lowered oxygen movement and thus reduces the growth of *T. reesei*.

In the present research, the medium containing glucose induced the highest yield of reducing sugar followed by sucrose and the least for maltose. This is in agreement with Coban and Biyth (2011), who reported that glucose gave the highest yield followed by fructose, sucrose, and ethanol. In contrast, Szakacs *et al*. (2006) reported that glucose represses the production of cellulase.

The study of the effect of nitrogen source showed that ammonium sulfate induced the highest reducing sugar yield, and the malt extract the least. This is in agreement with the work of Navita *et al*. (2015), who reported that ammonium sulfate was found to be the best nitrogen source for the production of cellulase enzyme by *Aspergillus* sp. Olufunke and Ogugua (2013) also reported that ammonium sulfate was the best nitrogen source during the fermentation of cassava peels for nutritional enrichment using *T. viride*. The work of Inuwa and Fagade (2010) also support this finding. It might be due to the fact that ammonium compounds are better nitrogen compounds for protein and enzyme synthesis as compared to other nitrogen sources.

The production of bioethanol from pretreated cassava peel hydrolysates is affected by some factors such as fermentation time, temperature, pH, etc. The fermentation time in this study showed that maximum ethanol concentrations were recorded on the fourth day for both *S. cerevisiae* and *C. tropicalis*. This finding is in agreement with the works of Zainal *et al*. (2014), Armanul *et al*. (2014), and Swain *et al*. (2007). Contrary to this finding is the work reported by Jirasak and Buddhiporn (2011). They recorded maximum ethanol concentration on the second day of fermentation using *S. cerevisiae* 7532 on pretreated cassava peel. This variation in fermentation time could be as a result of the strain of yeast used, the biochemical composition of the substrate, and pretreatment methods employed as this would affect the initial concentration of reducing sugar in the fermenting medium and finally, the fermentation system put in place for the production of the bioethanol (Henk and Linden, 1996; Chen *et al*., 2007).

The specific rate of yeast growth and ethanol production are usually influenced by the pH of the fermentation medium (Tesfaw and Assefa, 2014). In the present study, the optimum pH for maximum ethanol concentration during fermentation using *S. cerevisiae* was found to be 4.5. This finding is in agreement with the work of Kanagaraj and Rajandran (2013). On the contrary, Akponah and Akpomie (2012) reported optimum pH for bioethanol production from cassava effluent using *S. cerevisiae* to be 5.5. Also, the work of Fakruddin *et al*. (2013) does not support this finding. The optimum pH for maximum ethanol concentration using *C. tropicalis* was 5.0. This is in agreement with the work of Soledad *et al*. (2015). They reported a pH of 5.0 as the optimum pH for maximum ethanol concentration during the production of ethanol from Olive pruning.

In this study, the optimum temperature for *S. cerevisiae* during the fermentation of the pretreated cassava peel was 30°C with a maximum ethanol concentration of 30.2 g/L. This is in agreement with the work of Kanagaraj and Rajandran (2013). Also, Armanul (2014) reported that maximum ethanol concentration was recorded at 30°C during the fermentation of molasses using *S. cerevisiae.* The optimum temperature for *C. tropicalis* was recorded at 35°C with the maximum ethanol concentration at 18.82 g/L.

Fermentation of the pretreated cassava peel hydrolysate under shaking condition proved to be better than the stationary or non-shaking form. In this study, the maximum ethanol concentrations using *S. cerevisiae* under shaking conditions was 29.90 g/L while in the stationary mode it was 40.72 g/L. Similarly, for *C. tropicalis*, the ethanol concentrations were 26.68 g/L and 18.91 g/L. This better yield could be due to the even distribution of the nutrients and hence better utilization by the yeast cells.

Conclusion

The results obtained from this work show that cassava peel is a source of cheap, degradable material for the production of simple reducing sugars, which can be fermented by yeast to produce ethanol, as a cheap energy source for use in our local communities. The yield of ethanol was higher for *S. cerevisiae* than *C. tropicalis*.

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