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Optimizing bioethanol production from cassava peels through agitation timing variation in separate hydrolysis and co-fermentation

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Abstract

Background: This study assessed the effect of various agitation timings on bioethanol production from cassava peels (CP) using separate hydrolysis and co-fermentation (SHCF) technique.

Methods: The milled CP was divided into three groups each of 20 g and subjected to two-stage hydrolysis carried out at 100 °C for 60 min and 50 minutes, respectively. Experimental layouts were Sample 1 (B1) [0 hours i.e., no shaking], Sample 2 (B2) [3 hours shaking with an electric shaker at 200 rev/min^{-1}], and Sample 3 (B3) [6 hours shaking at 200 rev/min^{-1}]. Fermentations were carried out at 30 °C for 72 days. Data were analyzed using descriptive statistics, one–way ANOVA, and New Duncan's multiple range test at $P=0.05$.

Results: The TSS and pH readings of the treatment groups before and after fermentation were: B1 – TSS (27.15 ± 0.15, 17.25 ± 0.07 °Bx), pH (5.50 ± 0.00, 4.53 ± 0.04); B2 – TSS (27.32 ± 0.08, 14.78 ± 0.12 °Bx), pH (5.50 ± 0.00, 4.74 ± 0.06); and B3 – TSS (27.17 ± 0.07, 10.24 ± 0.08 °Bx), pH (5.50 ± 0.00, 4.77 ± 0.05) ($P<0.05$). The mean fermentation efficiency (FE) and ethanol productivity (EP) were B1 (15.17 ± 0.07%, 0.122 ± 0.001 gL^{-1}h^{-1}), B2 (15.70 ± 0.18%, 0.126 ± 0.002 gL^{-1}h^{-1}), and B3 (18.80 ± 0.14%, 0.151 ± 0.001 gL^{-1}h^{-1}) ($P<0.05$). All treatment groups attained the maximum ethanol yields at 72 hours of fermentation ($P<0.05$). Agitation at 200 rev/min^{-1} for 6 hours gave the optimal FE (%), EP, and ethanol yield.

Conclusion: The established condition improved the Bioethanol quality and yield of CP. Thus, optimizing bioethanol production from CP would help enhance sustainable biofuel production without affecting food security.

Keywords: Ethanol, Fermentation, Hydrolysis, Saccharomyces cerevisiae, Sugars

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Introduction

The present urban sprawl, industrialization, and technological development of the 21st century have brought about interest in environmental sustainability and renewable energy. This interest has been further rekindled by the awareness of the limited global crude oil reserves and its rapid dwindling than previously predicted (1-3). Currently, fossil fuels such as coal, oil, and natural gas account for more than a third-quarter of the global energy source, nevertheless they are non-renewable and depletable in nature. Moreover, it has been predicted that by 2025, the global energy demand will rise by 50% with a larger percentage of this coming from the third-world countries (4-6). Taking into account the above-mentioned scenario, along with the current fluctuation in petrol prices and the alarming global warming, the need for a rigorous pursuit and expansion of a sustainable alternative energy program becomes inevitable (2). This alternative energy can be exploited from feedstocks such as agro-residues, forestry resources, animal waste, and municipal solid waste, which are abundant and economically viable (7).

Biofuel is a viable example of an alternative energy source and it is obtained from biomasses, which are renewable organic materials obtained from plants and animals (8,9). It is a renewable and green energy, which burns with clean air and has a net carbon emission of zero

because the carbon dioxide released during its combustion is offset by the absorption from the atmosphere (10-13). Many developed nations are expanding their biofuel industries for the transport sector, and similar keen interest has been shown by many third-world countries to modernize biomass utilization and develop greater access to clean liquid fuels (3,14). In the energy production cycle, the most promising biofuel is bioethanol (9). Ethanol is a simple alkyl alcohol that can be utilized as automobile fuel in spark ignition engines. It has a high-octane rating and can either be used partly or wholly in vehicles (13). Several feedstocks serve as precursors for bioethanol production. This includes molasses, sugarcane juice, starch-based materials such as wheat and corn but not limited to the aforementioned substrates (11). Globally, the leading players in the bioethanol industry are the United States and Brazil with many nations in Europe developed policies in support of domestic biofuel industries. The interest in biofuels has further increased in the past decades, with more than sixty countries having since launched biofuel programs and set targets for blending biofuels into their fuel pools (12,15,16). Taking into cognizance the landmark achievement attained by the aforementioned countries, it became exigent for African countries to follow suit.

However, this current production system is not sustainable and poses a serious threat to food security. To circumvent such a threat, many researchers advocate the utilization of lignocellulosic biomass in the production of bioethanol. This emerging form of bioethanol production from non-edible feedstocks tends to be promising and may be a giant stride in the bioethanol revolution (17,18). Lignocellulosic biomasses are renewable, inedible feedstocks, which are inexhaustible and non-depletable resources for alternate fuel production. They consist basically of plant fiber and have cellulose, hemicelluloses, and lignin as their constituents (18,19). Lignocelluloses have been suggested to be a foremost and promising substitute to traditional starch feedstock. It has an excellent well-to-wheel assessment due to its availability; economical and high polysaccharides yield (20,21).

Cassava (*Manihot esculenta*), a tuberous root crop, is planted as a starchy source of food for both people and animals. Cassava is one of the main staple foods in Nigeria, so a lot of CP are produced both domestically and industrially by cassava processing factories (22,23). With an annual production of about 54 million metric tons (MT), Nigeria is the world's largest producer of cassava (24). According to a study by Izah and Ohimain (25), poor management of cassava peels is practiced in Nigeria through open dumping into the environment, which leads to aesthetic nuisances like bad odor (air pollution), environmental degradation, and water body pollution near cassava processing sites. CP can be used as a lignocellulosic feedstock for the manufacturing of bioethanol, which acts as a recovery method because they include chemical components such as lignin, hemicellulose, and cellulose, which are ideal sources for the synthesis of bioethanol. This is one of the most effective methods for managing the wastes left over from processing cassava because it lessens the harm that the pollutants do to the environment (26). Many scientists believe that to commercialize the production of bioethanol from lignocellulosic materials like CP, a more thorough study on the optimization of production parameters, which influence the yield of ethanol from this class of feedstocks, is required (8,18,26,27). Additionally, research on the effects of operation parameters, such as temperature, agitation rate, substrate type, and other process parameters, such as pH, microbial growth, and sugar content, can be beneficial in elucidating its influencing factors, product production pattern, and optimization information (28-30). This study investigated the effect of various agitation timings on bioethanol production from CP using separate hydrolysis and co-fermentation (SHCF) technique.

Materials and Methods

Study design

The study is experimental and laboratory-based involving hydrolysis, biochemical characterization, microbial fermentation and optimization. The study employed the SHCF technique using CP as the substrate for bioethanol production.

Biomass source

CP was obtained from the cassava processing center at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The institute has a cassava plantation, which covers about one hectare of land and a cassava processing unit with a modernized processing machine for making Garri (a local product from cassava). Large quantities of CP are generated from this production process while the wastewater is subjected to anaerobic digestion in a sedimentation tank to produce biogas.

Collection and transport of the biomass

A considerable quantity of fresh CP was packed from the cassava processing center at IITA and transported in polythene bags or sacks to the laboratory for experimental processing.

Optimization of bioethanol production from cassava peels at laboratory scale

The bioethanol production and optimization from CP was carried out at the Microbiology Laboratory of the Institute of Agriculture Research and Training (IAR&T), Moore Plantation, Apata, Ibadan, Nigeria. The procedure adopted in this research was the SHCF technique recommended by Farone and Cuzens (31,32) and modified by other researchers (3,9,26). This acid-

based technology is a generic process that consists of six basic steps as listed below.

- Biomass pre-treatment
- Chemical hydrolysis of lignocellulose to produce sugars
- Neutralization process (to separate the sugars from acid)
- Sugars fermentation (glucose) to ethanol
- Distillation (to obtain pure ethanol)
- Analytical assay: The pH, total soluble solids (TSS), glucose yield, total reducing sugar (TRS), and bioethanol yield of the CP were analyzed.

Pre-treatment of the Cassava Peel

The CP was oven-dried at 27 °C until it lost all its moisture content. The dried cassava peel was then pounded with a mortar and pestle to powdery form and, sieved with a + 1.5 mm sieve to get a homogeneous sample. This process was to expose a large surface area of the biomass to chemical hydrolysis.

Weighing of the substrate

The powdery CP utilized in the experiment was weighed using a Toledo Mettle weighing balance (±0.001 g). Hence, 20 g of the substrate (powdery CP) was utilized for each of the experiments.

Experimental layout

The optimization phase was divided into three sample groups of experimental layouts as described by Hossain et al (33). A uniform mass of 20 g of each of the milled CP was utilized in the study. This was done to assess the effect of varying agitation timing treatments using an electrical shaker at 200 revolutions/min (200 rev/min^{-1}) on bioethanol concentration, TSS, and pH of the samples before and after fermentation at 30 °C, respectively. The experimental layout of the samples used in this study is shown in Table 1.

Hydrolysis of the biomass

Twenty grams of each of the milled CP was treated with 100 mL of 13.1M H_2SO_4 in 1:5 (w/v) in a two-stage hydrolysis using a glass jar with a cover lid. The acid and biomass mixture was steamed in a water bath to 100 °C for 60 minutes in the first hydrolysis stage. This led to the development of a thick gel, which was pressed using a sieve to extract the acid-sugar stream. The left-over residues in the first hydrolysis were again hydrolyzed with 100 ml of 13.1M H_2SO_4 at 100 °C for 50 minutes for the production of a second hydrolysate. The streams from both hydrolyses were combined and the volume of the hydrolysates was recorded. The hydrolysis reaction is depicted in Eq. (1).

Equation of the reaction:

$$\underset{Lignocellulose}{(C_6H_{10}O_5)n} \xrightarrow[Conc.H_2SO_4]{+H_20} \underset{Reducing\ sugar}{(CH_20)n} \quad (1)$$

The leftover solid, lignin, was discarded.

The glucose hydrolysis efficiency (GHE) (%), glucose productivity (gL^{-1}h^{-1}), and TRS productivity (gL^{-1}h^{-1}) were calculated by adopting the formula described by Zhu et al (34), in equations 2 to 4:

$$\text{GHE}(\%) = \frac{Glucose\,concentration\ (gL^{-1})}{Cassava\ peels\,conc.\times 51.52\%\times 1.1\ (gL^{-1})}\times 100 \quad (2)$$

$$\text{Glucose Productivity}(gL^{-1}h^{-1}) = \frac{Glucose\,concentration\ (gL^{-1})}{Hydrolysis\,time(hr)} \quad (3)$$

$$\text{TRS Productivity}(gL^{-1}h^{-1}) = \frac{TRS\,concentration\ (gL^{-1})}{Hydrolysis\,time(hr)} \quad (4)$$

Note:

Cassava peels concentration = 20% i.e. 20 grams per 100 ml acid

Total hydrolysis time = 110 min = 1.833 h

Neutralization process

The hydrolysates obtained from the acid hydrolyses were each titrated with lime water [Ca(OH)$_2$]aq as illustrated in Eq. (5). This was done to neutralize the sulfuric acid and raise the hydrolysate pH to about 5.5. The filtrate formed, a free sugar, was filtered using a Whatman No. 1 filter paper while the leftover gypsum ($CaSO_4$) was discarded. The filtrates were qualitatively assessed for reducing sugar using Fehling solution. Also, TSS, glucose yield, and TRS were quantitatively determined in the filtrate.

$$\underset{Hydrolysate}{C_6H_{12}O_6\ldots H_2SO_4} + \underset{Lime}{Ca(OH)_2} \rightarrow \underset{Free\ Sugar}{C_6H_{12}O_6} + \underset{Gypsum}{CaSO_{4(PPT)}} + \underset{Water}{2H_2O} \quad (5)$$

Sugars fermentation

Weighing of Saccharomyces cerevisiae

The weight of the dry yeast to be added to each sugar solutions was quantified using the procedure enunciated by Hossain et al (33). This involved the use of 3 g of dry yeast per liter of sugar solution. Rehydration of the dry yeast was done in a water bath at 40°C, afterward cooled to room temperature before the addition of sugar solution. The baker's yeast was weighed using a KERRO Electronic compact scale, BLC2000 series made in Taiwan with the precision of ± 0.001 g.

Table 1. Experimental layout for the optimization of bioethanol production from Cassava peel

Sample number	Mode of treatment
Bottle 1 (control)	No agitation (0 h) serves as control
Bottle 2	Three hours of agitation
Bottle 3	Six hours of agitation

Procedure

The sugar solutions were fermented using *Saccharomyces cerevisiae*. 3 g/L of yeast, *S. cerevisiae*, was added into each set of closely air-tightened bottles containing sugar solution (Table 2) and kept in an incubator at the desired temperature. Fermentation was done at a temperature of 30 °C. The effect of agitation timing by an electrical shaker on the CP fermenting broths was assessed using different agitation timings (0, 3, and 6 hours) at 200 rev/min^{-1} for the various treatment groups (bottles 1-3). Ethanol presence, ethanol concentrations, and yields were carried out by periodically taking samples from the fermenting broth every 24 hours. Also, the pH and TSS of the solutions were determined after fermentation. The fermentation reaction is illustrated in Eq. (6).

Equation of the reaction:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{yeast}} 2\underset{\text{Ethanol}}{C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO_{2\uparrow(g)}} \quad (6)$$

Confirmatory test for ethanol

A few drops of acidified $KMnO_4$ solution were mixed with 1 mL of each of the fermented broths and heated to boil. The purple color of $KMnO_4$ was decolorized and turned into a colorless liquid with an unpleasant smell of ethanal, which confirmed the presence of ethanol. The fermentation efficiency and ethanol productivity were calculated using the formula described by Zhu et al (34) in Eqs. (7 and 8).

$$\text{Ethanol Productivity}(gL^{-1}h^{-1}) = \frac{Ethanol\ concentration\ (gL^{-1})}{Fermentation\ time(hr)} \quad (7)$$

$$\text{Ethanol Productivity}(gL^{-1}h^{-1}) = \frac{Ethanol\ concentration\ (gL^{-1})}{Fermentation\ time(hr)} \quad (8)$$

Note: FE = Fermentation Efficiency

Distillation

The bioethanol solution was distilled in a 2 L distillation flask and heated to 78 °C to obtain pure ethanol. The volume of ethanol obtained from the distillation was measured and recorded. The following were also calculated:

- Actual yield of ethanol in gram i.e., the mass of ethanol present in 1 liter solution of the fermented broth.
- The theoretical yield of ethanol in gram i.e., the mass of ethanol that would be obtained using the balanced stoichiometric reaction in Eq. (9):

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{yeast}} 2\underset{\text{Ethanol}}{C_2H_5OH} + \underset{\text{Carbon dioxide}}{2CO}\uparrow(g) \quad (9)$$

Molar mass 180 g 92 g 88 g

X g Y g

(Assuming working with a total reaction volume of 1 L)

Hence, Y g (Theoretical yield of ethanol in the reaction volume of 1 L)

$$= \frac{X\ g \times 92\ g}{180\ g}$$

Where *X g* is the weight in gram of TRS per 1 L solution.

- Percentage yield of ethanol was calculated using the formula described in Eq. (10):

$$\%\ \text{Yield} = \frac{Actual\ yield}{Theoretical\ yield} \times 100\% \quad (10)$$

- Percentage concentration of Ethanol by volume (%v/v) was calculated using the formula described in Eq. (11):

$$\text{Volume}\ \% = \frac{Volume\ of\ ethanol\ recovered}{Total\ volume\ of\ solution} \times 100\% \quad (11)$$

Analytical assay

The following physicochemical and proximate analyses were carried out as highlighted below:

- Determination of pH: The pH of the solutions was assessed using a pH meter.
- TSS determination: This was measured using a refractometer following the method described by Hossain et al (33).
- Determination of glucose and TRS concentrations and yields: The methods described by the Association of Official Analytical Chemists (AOAC) methods (35) were employed to determine glucose yield and TRS. The glucose concentration was determined following the Anthrone procedure outlined by the AOAC (35) while the TRS content was determined quantitatively using the phenol-sulfuric acid method as described by Dubois et al (36). The concentrations of glucose and TRS released were colorimetrically determined using a UV spectrophotometer at a wavelength of 490 nm. A calibration curve was obtained using D-glucose as standard. The glucose and TRS concentrations and yield in the original sample were calculated by adopting the formula described in Eqs. (12 and 13):

$$Concentration\ (C_1) = \frac{Absorbance\ reading}{Gradient} \quad (12)$$

$$Yield = \frac{(C_1 \times 125) \times 1000}{D}\ mg\ /\ kg\ dry\ weight \quad (13)$$

Table 2. The mean volume of sugar solution obtained from the 13.1M acid hydrolysis and subjected to fermentation

Samples	Volume of sugar solution (mL) (Mean ± SD)
Bottle 1	153.33 ± 1.52
Bottle 2	158.00 ± 2.00
Bottle 3	170.33 ± 1.52

Where C_1 is the concentration of reducing sugars (mg/mL) read from the appropriate calibration graph. *125* is the dilution factor since 2 g of the analyte was dissolved in 250 mL of the distilled water. *D* is the dry weight of the original sample, which is 20 g. *1000* is the conversion factor, to convert the final value from mg/g to mg/kg.

- Ethanol assay: Ethanol concentration was quantitatively determined through a UV spectrophotometer using acidified potassium dichromate solution (37-39). Ethyl alcohol working standards in the range of 10 -50 µg/mL were prepared from 100 µg/mL stock ethanol standard. The absorbances of the working standards, sample extracts, and blank were read from a Cecil 2483 spectrophotometer ($\lambda = 313$ nm). The ethanol concentration and yield were calculated as described in Eqs. (14 and 15):

Calculation

%Ethanol (g/100 ml) was calculated as follows:

$$= \frac{(AbS - AbB) \times AG}{Volume\,of\ sample\,taken} \times 100 \quad (14)$$

$$\text{Ethanol Yield} = \frac{C \times V \times 50 \times 10^6}{Density\,of\ ethanol}\ ml/kg\,dry\ \ weight \quad (15)$$

Where *AbS* is the absorbance of the sample, *AbB* is the absorbance of blank, *AG* is the average gradient, *C* is the concentration of ethanol (mg/mL) [multiply with 10^{-6} to convert from mg/mL to kg/mL], *V* is volume of sugar (ml), *50* is the multiplication factor used to extrapolate the results from the original sample of 20 to kg. *The density of ethanol* is 789 kg/m^3. *10^6* is conversion factor to convert final answer from m^3/kg to cm^3/kg (mL/kg).

Data analysis

The data collected during this research were analyzed using the IBM Statistical Product and Service Solutions (IBM SPSS Statistics), version 27.0 software for descriptive and inferential statistics. Descriptive statistics was used to summarize data using bar charts, line graphs, means, and standard deviations. The results obtained from the physiochemical and proximate analyses of the laboratory studies were subjected to one-way analysis of variance (ANOVA) as described by the statistical analysis system (40) and New Duncan's multiple range test (41) for means separation at 95% level of probability.

Results

Hydrolysis results of the Cassava peels

Figure 1 illustrates the various parameters assessed in the hydrolysis stage viz: glucose concentration, GHE%, glucose productivity, TRS concentration, and TRS productivity of the CP, respectively.

The overall mean glucose and TRS concentrations obtained from the CP in the laboratory study were 19.00 ± 0.19 and 35.92 ± 1.02 gL^{-1}, respectively. Likewise, the overall mean GHE%, glucose productivity, and TRS productivity of the CP in the laboratory work were 16.77 ± 0.07%, 10.37 ± 0.04, and 19.60 ± 0.56 gL^{-1}h^{-1}, respectively. All these results imply a promising yield of sugar production from CP using the described hydrolysis method.

Optimization of agitation timings on the proximate analysis of Cassava Peel's hydrolysate in laboratory studies

The mean concentrations of bioethanol, TSS, and pH of CP hydrolysate on a laboratory scale using different agitation timings (0, 3, and 6 hours) of 200 revolutions per minute (200 rev/min^{-1}) are shown in Table 3. As shown in this table, the mean values of the TSS for the fermented biomass were significantly lesser than those before fermentation ($P < 0.05$). Also, the mean TSS in the fermented biomass across the different agitation timing regimes was significantly different ($P < 0.05$). From the mean pH values in Table 3, readings of the mean pH pre-fermentation were greater than those obtained in post-fermentation ($P < 0.05$). The pH value of Bottle 1 (control, 0 hours) was lower than those of Bottles 2 (3 hours agitation) and 3 (6 hours agitation), respectively. There were significant differences in the pH of the different bottles at $P < 0.05$.

The mean bioethanol concentration of the CP hydrolysate at varying shaking hours of 200 rev/min^{-1} was compared. The fermented CP broth in Bottle 3 produced the maximum mean bioethanol concentration of 1.09 ± 0.02% (w/v), followed by Bottle 2 (0.91 ± 0.01% w/v) while Bottle 1 only produced 0.88 ± 0.01% w/v of bioethanol concentration as shown in Table 3. It can be observed that the bioethanol concentration of the CP increases as the agitation timing increases. There was significant variation in the bioethanol concentrations obtained from the CP hydrolysate at various agitation timings ($P < 0.05$).

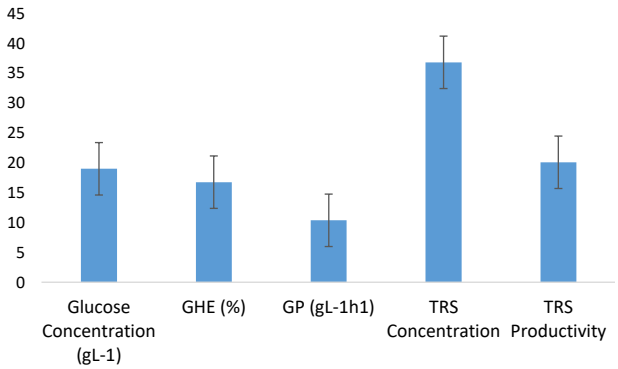

Figure 1. Glucose hydrolysis efficiency (GHE), glucose productivity (GP), and TRS productivity of the Cassava peels

Effect of different agitation timings on glucose, TRS, and bioethanol yields of CP in the laboratory studies

Table 4 shows the effect of agitation timing variations (0, 3, and 6 hours) at 200 rev/min^{-1} on the mean values of glucose, TRS, and bioethanol yields of the CP hydrolysate on a laboratory scale. There were significant differences in the mean glucose and TRS yields after fermentation ($P<0.05$) across the different treatment regimes. As shown in Figure 2, fermentation in Bottle 3 (6 hours agitation timing) gave the highest bioethanol yield while the lowest yield was obtained in Bottle 1 (control) across the different fermentation timings. These results were statistically significant ($P<0.05$).

Effect of varied agitation timings on fermentation efficiency and ethanol production in laboratory studies

The effects of different agitation timings (0, 3, and 6 hours) at 200 rev/min^{-1} on the fermentation efficiency and ethanol production of CP in the laboratory work are depicted in Figure 3. Fermentation in Bottle 3 gave the highest mean fermentation efficiency and ethanol production ($18.80 \pm 0.14\%$, 0.151 ± 0.001 gL^{-1}h^{-1}); while the least results were given by Bottle 1 (control) [$15.17 \pm 0.07\%$, 0.122 ± 0.001 gL^{-1}h^{-1}]. These differences were observed to be significant ($P<0.05$).

Distillation results for laboratory studies

Figure 4 shows the measurements of the mean volume of ethanol recovered from the various treatments (Bottles 1–3) in the laboratory studies. Bottle 3 had the highest mean volume of ethanol recovery of 53.07 ± 2.15 ml while Bottle 1 had the least mean volume recovery of 33.17 ± 1.76 ml. The variations in the mean ethanol volume recovered (ml) from the different bottles were significant at $P<0.05$. Also, the mean percentage ethanol concentration by volume (% v/v) produced by the different samples' treatments (Bottle 1–3) is presented in Figure 5. The highest mean volume of ethanol concentration (% v/v) was obtained in Bottle 3 ($37.71 \pm 0.59\%$ v/v) while the lowest value was obtained in Bottle 1 (27.78 ± 0.65) ($P<0.05$). Lastly, Figure 6 shows the mean percentage ethanol yield obtained from the various treatment regimes. Bottle 3 gave the highest percentage yield of $57.76 \pm 1.32\%$; while the lowest percentage yield of $47.20 \pm 1.47\%$ was obtained in Bottle 1.

Discussion

The most widely studied and industrially established yeasts explored in the transformation of sugars to ethanol are similar members of *Saccharomyces cerevisiae* (42,43). *S. cerevisiae* is the world's premier fermenter in the ethanol sector based on its aptness to convert hexoses to huge ethanol yield and its high tolerance to inhibitory substances (44). However, yeast's ability to convert

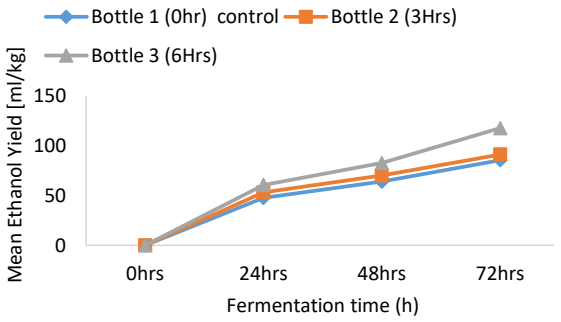

Figure 2. Comparison of the mean bioethanol yields of cassava peels at different agitation timings of 200 rev/min^{-1} in the laboratory studies

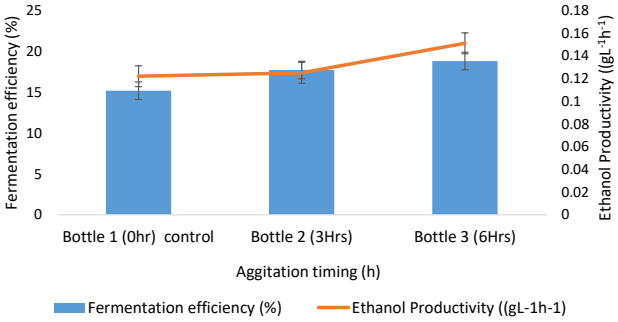

Figure 3. Effect of agitation timing variation on fermentation efficiency and ethanol production of cassava peels in the laboratory studies

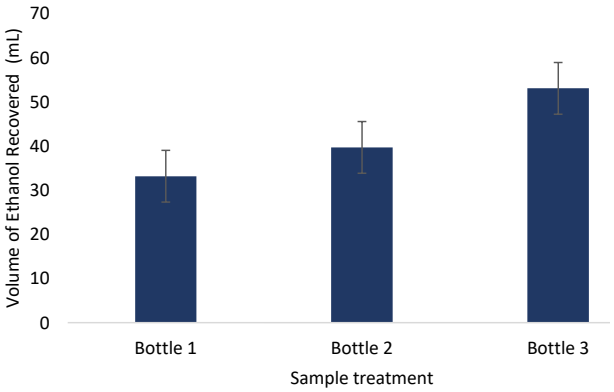

Figure 4. The mean volume of ethanol recovered (mL) from the various treatment groups

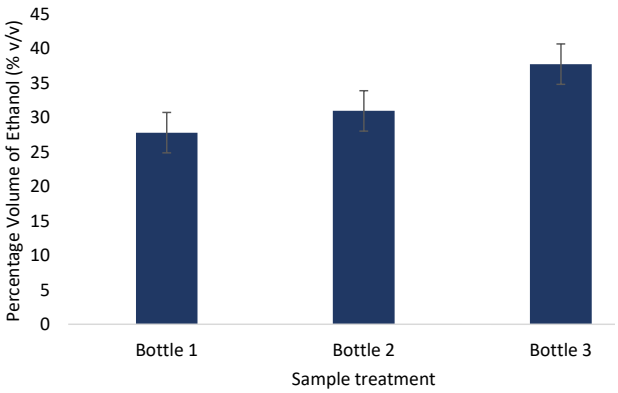

Figure 5. Mean percentage ethanol concentration by volume (%v/v) for the various treatment groups

Table 3. Effect of agitation timings at 200 rev/min^{-1} on the mean concentrations of bioethanol, total soluble solids, and pH of cassava peels (Fermentation at 30 °C) in the laboratory studies (Mean ± SD, n = 3)

Parameter (agitation timings)	TSS g/100 g (°Bx)		pH		Glucose concentration %(w/v) [g/100 mL]				Total reducing sugars concentration %(w/v) [g/100 mL]				Ethanol concentration %(w/v) [g/100 mL]			
	Initial	After	Initial	After	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Bottle 1 (0 hr) control	27.15 ± 0.15^{a}	17.25 ± 0.07^{c}	5.50 ± 0.00^{a}	4.53 ± 0.04^{a}	1.90 ± 0.01^{a}	1.10 ± 0.01^{a}	0.40 ± 0.01^{b}	0.04 ± 0.01^{b}	3.64 ± 0.13^{a}	2.67 ± 0.06^{b}	1.28 ± 0.09^{b}	0.56 ± 0.06^{b}	0.00 ± 0.00	0.49 ± 0.01^{a}	0.66 ± 0.01^{a}	0.88 ± 0.01^{a}
Bottle 2 (3 h)	27.32 ± 0.08^{a}	14.78 ± 0.12^{b}	5.50 ± 0.00^{a}	4.74 ± 0.06^{b}	1.90 ± 0.01^{a}	1.08 ± 0.02^{a}	0.33 ± 0.00^{a}	0.04 ± 0.01^{ab}	3.56 ± 0.10^{a}	2.57 ± 0.06^{b}	1.16 ± 0.11^{ab}	0.47 ± 0.04^{ab}	0.00 ± 0.00	0.53 ± 0.01^{b}	0.70 ± 0.01^{b}	0.91 ± 0.01^{b}
Bottle 3 (6 h)	27.17 ± 0.07^{a}	10.24 ± 0.08^{a}	5.50 ± 0.00^{a}	4.77 ± 0.05^{b}	1.90 ± 0.01^{a}	1.07 ± 0.01^{a}	0.31 ± 0.01^{a}	0.03 ± 0.01^{a}	3.68 ± 0.11^{a}	2.38 ± 0.10^{a}	1.05 ± 0.06^{a}	0.35 ± 0.06^{a}	0.00 ± 0.00	0.56 ± 0.01^{c}	0.77 ± 0.01^{c}	1.09 ± 0.02^{c}
F-value	2.40	4525.6	0.00	20.98	0.00	0.00	100.50	6.33	0.97	12.63	5.01	12.91	-	82.750	103.625	113.22
P value	0.171	0.000*	1.000	0.002*	1.000	0.998	0.000*	0.033*	0.430	0.007*	0.053	0.007*	-	0.000*	0.000*	0.000*

Different letters (a, b, and c) indicate significant differences along the columns.
*Significant at *P* = 0.05.

Table 4. Effect of agitation timing at 200 rev/min^{-1} on the mean values of glucose, total reducing sugars, and bioethanol yields of cassava peels (Fermentation at 30 °C) in the laboratory studies (mean ± SD, n = 3)

Parameter (Agitation timings)	Glucose yield [g/kg]				Total reducing sugars (TRS) Yield [g/kg]				Ethanol yield [mL/kg]			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Bottle 1 (0 h) Control	118.77 ± 0.55^{a}	68.47 ± 0.55^{a}	24.87 ± 0.39^{c}	2.85 ± 0.21^{b}	227.38 ± 8.13^{a}	167.09 ± 3.24^{b}	79.77 ± 5.54^{b}	34.83 ± 3.35^{b}	0.00 ± 0.00	47.45 ± 0.76^{a}	63.81 ± 1.13^{a}	85.19 ± 1.22^{a}
Bottle 2 (3 h)	118.60 ± 0.48^{a}	67.48 ± 1.10^{a}	20.46 ± 0.20^{b}	2.31 ± 0.25^{ab}	222.31 ± 6.19^{a}	160.71 ± 3.82^{b}	72.65 ± 6.63^{ab}	29.38 ± 2.45^{ab}	0.00 ± 0.00	52.84 ± 1.17^{b}	69.93 ± 1.49^{b}	90.86 ± 2.50^{b}
Bottle 3 (6 h)	118.75 ± 0.47^{a}	66.75 ± 1.30^{a}	19.17 ± 0.63^{a}	1.79 ± 0.32^{a}	230.27 ± 7.00^{a}	148.50 ± 5.81^{a}	65.94 ± 3.87^{a}	21.69 ± 3.66^{a}	0.00 ± 0.00	60.34 ± 1.14^{c}	82.42 ± 2.21^{c}	117.34 ± 1.19^{c}
F-value	0.10	2.08	136.85	8.71	0.95	13.63	4.81	12.79	-	116.04	96.38	232.82
P value	0.906	0.207	0.000*	0.017*	0.438	0.006*	0.057	0.007*	-	0.000*	0.000*	0.000*

Different letters (a, b, and c) indicate significant differences along the columns.
*Significant at *P* = 0.05.

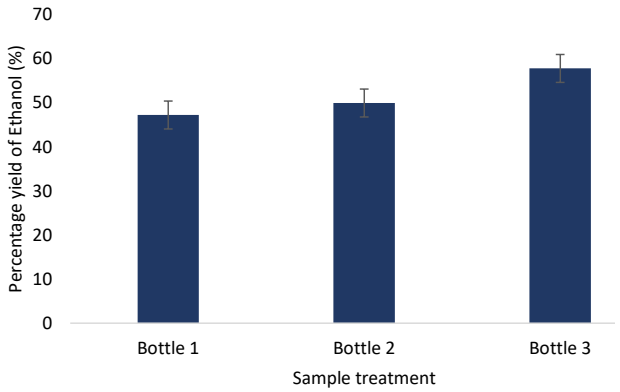

Figure 6. The mean percentage yield of ethanol (%) for the various treatment groups

sugars to ethanol depends on many parameters including growth factors, strains, and the optimum environmental conditions (45). In the present study, the effect of various agitation timings on bioethanol production by the stress-tolerant yeast (*S. cerevisiae*) was investigated.

In this study, an SHCF configuration similar to the separate hydrolysis and fermentation (SHF) process was applied, which involved both hexoses and pentoses fermentation. The glucose, and total reducing sugar concentrations and productivity of 20% CP obtained in the laboratory were assessed. As shown in Figure 1, GHE% was also investigated. The findings of the hydrolysis stage revealed that hydrolysis of 20% CP with 13.1M H_2SO_4 for 110 minutes at 100 °C using a water bath in the laboratory led to a significant increase in glucose and TRS concentrations and productions. These findings are corroborated by the reports of other authors (3,26,31). These workers reported that higher concentrations than 70% (13.1M) acid concentration will lead to charring of the biomass. Also, Zhu et al (34) reported a glucose production as high as 66.86 gL^{-1} from 20% CP using fed-batch hydrolysis for 120 hours, in which enzymatic cocktails were introduced at hydrolysis timings of 24 hours and 48 hours, respectively. The promising yield of glucose and TRS by CP can be ascribed to the high proportion of cellulose and hemicellulose contents in CP, which are polymers of numerous pentose (C_5) and hexose (C_6) sugars. Yoonan and Kongkiattikajorm (46) have also reported that CP has an appreciable quantity of cellulose (20.8%) and hemicellulose (29.0%), respectively. The GHE (%) obtained in the study was however lower than the 90% GHE reported by Kosugi et al (47) and the 74.32% GHE observed by Zhu et al (34).

The pH of the fermenting medium greatly affects the production rate of ethanol by the yeast cells. Thus, either elevated or lower pH environments would impede the metabolism of yeast, and consequently, its cell growth (48). The pH values of the CP hydrolysate before and after fermentation in this study were within the range of 4.53 - 5.50 as evident in Table 3. This finding aligns with that of Fakruddin et al (49), who opined that all the three strains of the stress-tolerant yeast - *Saccharomyces uniparous* (P), *S. cerevisiae* (C) and (T) gave the highest ethanol yields at a pH of 5.5 after 5 days except for T strain at a pH of 6.00 of the fermentation setting. From the experimental results obtained in Tables 3 and 4, the highest ethanol concentration and yield from the Cassava peel were obtained at 72 hours of fermentation for all treatment groups. It is logical to conclude, that lengthy fermentation duration has a positive impact on fermentation rates of *S. cerevisiae* since a longer time resulted in higher rates of product formation. The lengthy time helps the yeast to maximally utilize the sugars contained in the CP (50). This outcome is consistent with the results of the study by Pippo and Luengo (51), who reported a maximum ethanol yield of 8% (w/v) after 72 hours of fermentation.

It is generally believed that the production of ethanol by yeast is by anaerobic process. It has likewise been suggested that agitation could influence metabolite formation if the reaction of product formation is dependent on oxygen (52). Agitation plays a vital role in fermentation, as it helps maintain adequate contact between enzymes and the substrate, accelerate heat and mass transfer within the fermenting vessel, and improve the aeration of the fermenting broth thus increasing oxygen availability to the microbes as a nutrient. However, it has also been proven that too much mixing can lead to enzyme deactivation and decrease the transformation product, as a result of the generation of shear force by the mixer and trapping of air bubbles at the air-liquid interface of the medium (53,54). In this study, the effect of different agitation timings using an electrical shaker at 200 rev/min on ethanol production from Cassava peel was assessed at different shaking hours of 0, 3, and 6 hours in the laboratory. The agitation timing at 6 hours (Bottle 3) gave the optimal ethanol concentration and yield of 1.09 ± 0.02% (w/v) and 117.34 ± 1.19 ml/kg, respectively, as shown in Figure 2. This finding is supported by Hossain et al (33) who reported a maximum bioethanol concentration at optimum shaking time of 6 hours from decaying banana fermented by *S. cerevisiae*. This finding is also in consonance with those of several authors who reported a significant increase in ethanol production by isolated *S. cerevisiae* IFST-072011, when fermentation was performed under agitation (49,53).

The results of the fermentation stage in the present study revealed that Bottle 3 (6 hours agitation) gave the highest fermentation efficiency (FE) and ethanol productivity by the yeast (*S. cerevisiae*) as shown in Figure 3. In a study by Zhu et al (34), the maximum ethanol concentration and FE of 29.39 gL^{-1} and 68.19%, respectively, were reported using the SHF process. As shown in Figures 4 and 5, Bottle 3 (6 hours agitation) had the highest mean ethanol recovery and percentage ethanol volume concentration of 53.07 ± 2.15 mL and 37.71 ± 0.59% (v/v), respectively,

in the laboratory studies. This value is greater than that (8.5%) reported by Adetunji et al (55) utilizing both *S. cerevisiae* and *A. niger* on CP slurry; and also, greater than the average ethanol concentration of 16% reported by Agulejika et al (56) from decomposing mangoes. Also, as shown in Figure 6, the highest ethanol yield percentage of 57.76 ± 1.32% was produced by Bottle 3 (6 hours agitation). Toma et al (57) studied the impact of agitation on the efficiency of *Zymomonas mobilis* in a continuous culture. The authors noted that both yield of biomass and ethanol production were enhanced at greater stirring frequencies followed by a decline in the by-product generation. Hence, they concluded that vigorous agitation led to enhanced synergy between catabolism and anabolism. The agitation speed of fermenting broth exhibits different effects on the ethanologenic organism. This includes disruption of cell structure, changes in morphology, production formation, and growth rate variations (53,54,57). Rodmui et al (58) reported that agitation could be advantageous to the growth and efficiency of the ethanologenic organism through mass transfer properties concerning substrates, products/by-products, and oxygen supply.

Currently, yeasts are employed to produce fuel ethanol from renewable energy sources. A few yeast strains, including *Pichia stipitis* (NRRL-Y-7124), *S. cerevisiae* (RL-11), and *Kluyveromyces fragilis* (Kf1), were noted as being capable of producing ethanol from a variety of sugars (59). Due to its low cost and ease of availability, baker's yeast has long been employed as a starter culture in the manufacturing of ethanol. However, wild-type yeast contaminated industrial processes because baker's yeast and other *S. cerevisiae* strains were unable to compete with it. To expedite downstream processing, enable operation at high cell density, and increase overall productivity, flocculent yeasts were also utilized during biological fermentation for the generation of ethanol (60). The yeast's capacity to ferment pentose carbohydrates causes further issues with bioethanol production. Only a few yeast species belonging to the genera *Pichia*, *Candida*, *Schizosaccharomyces*, and *Pachysolen* can ferment pentoses to ethanol (59). Using hybrid, genetically modified, or co-cultures of two yeast strains, pentose fermentation issues can be resolved. Pentose and hexose carbohydrates are concurrently fermented to ethanol using hybrid yeast strains. The hybrid strain was created by combining *S. cerevisiae* protoplast with xylose-fermenting yeast protoplast from *Pachysolen tannophilus*, *C. shehatae*, and *P. stipitis* (61). Also, a cross between *S. paradoxus* and *S. cerevisiae* will probably produce a hybrid strain that can tolerate high temperatures as well as ethanol (62). The efficiency of ethanol production on an industrial scale will be increased using yeasts that are tolerant to inhibitors (63). The common challenges of yeasts can be overcome using ethanol-tolerant and thermotolerant yeast. Ethanol-tolerant and thermotolerant strains, which can resist stresses can be isolated from natural resources such as soil, water, plants, and animals. This is because cells adapt to their environment over time by natural selection. Ethanol fermentation at high temperatures is a beneficial process as it selects thermo-tolerant microorganisms and does not require cooling costs and cellulase (64). For example, thermotolerant yeast *Kluyveromyces marxianus* can co-ferment hexose and pentose carbohydrates and can withstand temperatures between 42 °C and 45 °C (65).

Despite the high promising yield of bioethanol production from CP through optimization of agitation timing using the SHCF technique by *S. cerevisiae*, there are still several limitations to this study. Firstly, the study only uses CP as the carbon substrate, which may limit the generalizability of the findings to other substrates. Secondly, the study fails to make a comparison on the effectiveness of agitation timings variation using SHCF, which is an acid-based technology, and other enzymatic production techniques such as simultaneous saccharification and co-fermentation process. Thirdly, the study only investigates the effects of agitation timing on fermentation efficiency and ethanol production, without considering other factors that may affect yeast growth and production efficiency. However, the study investigates the effects of agitation timing on fermentation efficiency and ethanol production by *S. cerevisiae* in a mixture of hexose and pentose sugars, and thus, enriches the existing database on biofuel production. Also, the study provides specific findings on the optimal agitation timing for maximizing ethanol yield from CP, which can be useful for industrial applications.

Conclusion

This study demonstrates the importance of the effects of agitation timing on fermentation efficiency and ethanol production by the organism *S. cerevisiae* in a mixture of hexose and pentose sugars. Ethanol productivity was significantly influenced by the different agitation timings. In this study, agitation for 6 hours was found to result in optimal fermentation efficiency and ethanol yield from CP by the ethanologenic organism. Consequently, by varying the agitation timing in the fermenting broth, the growth of the yeast can be enhanced, and therefore, the fermentation efficiency and ethanol production can be improved. In addition, there is a need for future studies to take into account the bioreactor design, type and concentration of carbon substrate, aeration, and cell mass, which are all necessary for the optimization of fermentation conditions for improving the kinetic behavior of yeast.

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Competing interests

The authors declare that they have no competing interests.

Ethical issues

The authors certify that this manuscript is the original work of the authors, and all data collected during the study are as presented in this manuscript, and no data from the study has been or will be published separately elsewhere.

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References

1. Sreekumar A, Shastri Y, Wadekar P, Patil M, Lali A. Life cycle assessment of ethanol production in a rice-straw-based biorefinery in India. Clean Technol Environ Policy. 2020;22(2):409-22. doi: 10.1007/s10098-019-01791-0.
2. Sokan-Adeaga AA. Nigeria's Perennial Energy Crisis and the Way Forward. Academia Letters. Academia Letters. 2022. Article 5098. doi: 10.20935/al5098.
3. Ana GR, Sokan-Adeaga AA. Bio-ethanol yield from selected lignocellulosic wastes. Int J Sustain Green Energy. 2015;4(4):141-9. doi: 10.11648/j.ijrse.20150404.13.
4. Belboom S, Bodson B, Léonard A. Does the production of Belgian bioethanol fit with European requirements on GHG emissions? Case of wheat. Biomass Bioenergy. 2015;74:58-65. doi: 10.1016/j.biombioe.2015.01.005.
5. Buchspies B, Kaltschmitt M. Life cycle assessment of bioethanol from wheat and sugar beet discussing environmental impacts of multiple concepts of co-product processing in the context of the European Renewable Energy Directive. Biofuels. 2016;7(2):141-53. doi: 10.1080/17597269.2015.1122472.
6. Sokan-Adeaga AA, Ana GR. A comprehensive review of biomass resources and biofuel production in Nigeria: potential and prospects. Rev Environ Health. 2015;30(3):143-62. doi: 10.1515/reveh-2015-0015.
7. Oseji ME, Ana GR, Sokan-Adeaga AA. Evaluation of biogas yield and microbial species from selected multi-biomass feedstocks in Nigeria. London Journal of Research in Science: Natural and Formal. 2017;17(1):1-20.
8. Sokan-Adeaga AA, Ana GR. Source identification and characterization of lignocellulosic wastes from selected biomass sources in Ibadan, Nigeria. J Solid Waste Technol Manag. 2015;41(3):262-9. doi: 10.5276/jswtm.2015.262.
9. Sokan-Adeaga AA, Ana GREE, Sokan-Adeaga ED. Evaluation of Bio-Ethanol Production Potentials of Selected Lignocellulosic Wastes. Germany:LAMBERT Academic Publishing (LAP); 2015. Pp. 1-173.
10. Agarwal AK. Biofuels: In wealth from waste; trends and technologies. (ed Lal and Reedy). 2nd Ed. The Energy and Resources Instituted (TERI) Press; 2005.
11. Jeswani HK, Chilvers A, Azapagic A. Environmental sustainability of biofuels: a review. Proc R Soc A Math Phys Eng Sci. 2020;476(2243):20200351. doi: 10.1098/rspa.2020.0351.
12. Renewable Energy Policy Network for the 21st Century (REN21). Renewables 2019 – Global Status Report. 2019. Available from: https://www.ren21.net/wp-content/uploads/2019/05/gsr_2019_full_report_en.pdf.
13. Morales M, Quintero J, Conejeros R, Aroca G. Life cycle assessment of lignocellulosic bioethanol: environmental impacts and energy balance. Renew Sustain Energy Rev. 2015;42:1349-61. doi: 10.1016/j.rser.2014.10.097.
14. Sokan-Adeaga AA, Ana GR, Sokan-Adeaga MA, Sokan-Adeaga ED. Resilience and sustainability of the ecosystem: an environmental health perspective. London Journal of Research in Science: Natural and Formal. 2018;18(1):7-25.
15. Elbehri A, Segerstedt A, Liu P. Biofuels and the Sustainability Challenge: A Global Assessment of Sustainability Issues, Trends and Policies for Biofuels and Related Feedstocks. Rome, Italy: Food and Agriculture Organization of the United Nations (FAO); 2013. p. 174.
16. European Commission. Directive (EU) 2018/2001 of the European Parliament and of the Council of 11 December 2018 on the Promotion of the Use of Energy from Renewable Sources. Brussels, Belgium: Official Journal of the European Union; 2018.
17. Cherubini F, Strømman AH. Principles of biorefining. In: Pandey A, Larroche C, Ricke SC, Dussap CG, Gnansounou E, eds. Biofuels: Alternative Feedstocks and Conversion Processes. Amsterdam: Academic Press; 2011. p. 3-24. doi: 10.1016/b978-0-12-385099-7.00001-2.
18. Sokan-Adeaga AA, Ana GREE, Sokan-Adeaga MA, Sokan-Adeaga ED. Lignocelluloses: an economical and ecological resource for bio-ethanol production-a review. International Journal of Natural Resource Ecology and Management. 2016;1(3):128-44. doi: 10.11648/j.ijnrem.20160103.18.
19. Manochio C, Andrade BR, Rodriguez RP, Moraes BS.

Ethanol from biomass: a comparative overview. Renew Sustain Energy Rev. 2017;80:743-55. doi: 10.1016/j.rser.2017.05.063.
20. Sokan-Adeaga AA, Ana GR, Olorunnisola AO. Temperature variations effect on bioethanol production from lignocellulosic biomass using locally developed small-scale production facility: a pilot study. In: Ramesh K, Mebarek-Oudina F, Souayeh B, eds. Mathematical Modelling of Fluid Dynamics and Nanofluids. CRC Press, Taylor and Francis Inc; 2023. p. 215-49. doi: 10.1201/9781003299608-14.
21. Sokan-Adeaga AA. Waste Quantification, Characterization and Exploration of Bioethanol Production Potentials of some selected Lignocelluloses Wastes in Ibadan, Nigeria. In 2018 ISWA-SWIS Winter School Proceedings eBook. (pp. 183-192). SWIS Publications; 2019. Available from: https://www.amazon.com/2018-ISWA-SWIS-WINTER-SCHOOL-PROCEEDINGS-ebook/dp/B07SH2P7QB.
22. Onyenwoke CA, Simonyan KJ. Cassava post-harvest processing and storage in Nigeria: a review. Afr J Agric Res. 2014;9(53):3853-63. doi: 10.5897/ajar2013.8261.
23. Kura AM. An overview of bioethanol production from cassava feedstock in Nigeria. J Biol Sci Bioconserv. 2014;6(1):59-72.
24. Food and Agriculture Organization of the United Nations (FAO). FAOSTAT Database Collections. Rome: FAO; 2013.
25. Izah SC, Ohimain EI. The challenge of biodiesel production from oil palm feedstock in Nigeria. Greener J Biol Sci. 2013;3(1):1-12. doi: 10.15580/gjbs.2013.1.010613363.
26. Bolade DO, Ana GR, Lateef SA, Sokan-Adeaga AA. Exploration of the bioethanol yield of single and multi-substrate biomass from cassava processing wastes. J Solid Waste Technol Manag. 2019;45(3):305-14. doi: 10.5276/jswtm/2019.305.
27. Anwar Z, Gulfraz M, Irshad M. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review. J Radiat Res Appl Sci. 2014;7(2):163-73. doi: 10.1016/j.jrras.2014.02.003.
28. Bayu AB, Akuma DA, Hundie KB. An integrated approach to optimization of fermentation conditions for bioethanol production from local leftover Injera waste using central composite design. Environ Health Eng Manag. 2022;9(3):281-93. doi: 10.34172/ehem.2022.29.
29. Amibo TA. Modeling and pulping variables optimization of ethanol-alkali pulping and delignification of *Grevillea robusta* in Ethiopia by response surface methodology. Eur J Mater Sci Eng. 2021;6(1):34-51. doi: 10.36868/ejmse.2021.06.01.034.
30. Ghasemian M, Taheri E, Fatehizadeh A, Amin MM. Biological hydrogen production from synthetic wastewater by an anaerobic migrating blanket reactor: artificial neural network (ANN) modeling. Environ Health Eng Manag. 2019;6(4):269-76. doi: 10.15171/ehem.2019.30.
31. Farone WA, Cuzens JE. Method of Producing Sugars Using Strong Acid Hydrolysis of Cellulosic and Hemicellulosic Materials (US Patent No. 5562777A). USA: Arkenol Inc; 1996.
32. Farone WA, Cuzens JE. Method of Separating Acids and Sugars Resulting from Strong Acid Hydrolysis (US Patent No. 5580389A). USA: Arkenol Inc; 1996.
33. Hossain AB, Ahmed SA, Alshammari AM, Adnan FM, Annuar MS, Mustafa H, et al. Bioethanol fuel production from rotten banana as an environmental waste management and sustainable energy. Afr J Microbiol Res. 2011;5(6):586-98. doi: 10.5897/ajmr10.231.
34. Zhu M, Li P, Gong X, Wang J. A comparison of the production of ethanol between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using unpretreated cassava pulp and enzyme cocktail. Biosci Biotechnol Biochem. 2012;76(4):671-8. doi: 10.1271/bbb.110750.
35. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. 18th ed. Arlington: AOAC; 2015. p. 806-14.
36. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956;28(3):350-6. doi: 10.1021/ac60111a017.
37. Association of Official Analytical Chemists (AOAC). Methods 925.10, 65.17, 974.24, 992.16. Gaithersburg, MD: AOAC; 2000.
38. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. 19th ed. Gaithersburg, MD: AOAC; 2012.
39. Sayyad SA, Chaudhari SR, Panda BP. Quantitative determination of ethanol in arishta by using UV-visible spectrophotometer. Pharm Biol Eval. 2015;2(5):204-7.
40. SAS. Statistical analysis system, user guide. Cary, North Caroline: SAS Institute; 1997.
41. Duncan DB. Multiple range and multiple F tests. Biometrics. 1955;11(1):1-42. doi: 10.2307/3001478.
42. Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S, Kong H. Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742. Biomass Bioenergy. 2012;47:395-401. doi: 10.1016/j.biombioe.2012.09.019.
43. Hossain N, Haji Zaini J, Indra Mahlia TM. A review of bioethanol production from plant-based waste biomass by yeast fermentation. Int J Technol. 2017;8(1):5-18. doi: 10.14716/ijtech.v8i1.3948.
44. Bharti, Chauhan M. Bioethanol production using *Saccharomyces cerevisiae* with different perspectives: substrates, growth variables, inhibitor reduction and immobilization. Ferment Technol. 2016;5(2):131. doi: 10.4172/2167-7972.1000131.
45. Leonald-Emeka EL, Aransiola EF, Ibrahim TH, Oyedele JO, Gidado RM, Iloh AC, et al. Comparative performance of different yeast strains in the production of bioethanol using simultaneous liquefaction saccharification and fermentation (SLSF). Afri J Biotechnol. 2020;19(12):858-67. doi: 10.5897/ajb2020.17089.
46. Yoonan K, Kongkiattikajorn J. A study of optimal conditions for reducing sugars production from cassava peels by diluted acid and enzymes. Agric Nat Resour. 2004;38(5):29-35.
47. Kosugi A, Kondo A, Ueda M, Murata Y, Vaithanomsat P, Thanapase W, et al. Production of ethanol from cassava pulp via fermentation with a surface-engineered yeast strain displaying glucoamylase. Renew Energy. 2009;34(5):1354-8. doi: 10.1016/j.renene.2008.09.002.
48. Willaert R, Nedovic VA. Primary beer fermentation by immobilised yeast—a review on flavour formation and control strategies. J Chem Technol Biotechnol. 2006;81(8):1353-67. doi: 10.1002/jctb.1582.
49. Fakruddin M, Islam MA, Ahmed MM, Chowdhury N. Process optimization of bioethanol production by stress

tolerant yeasts isolated from agro-industrial waste. Int J Renew Sustain Energy. 2013;2(4):133-9. doi: 10.11648/j.ijrse.20130204.11.
50. Saka AA, Afolabi AS, Ogochukwu MU. Production and characterization of bioethanol from sugarcane bagasse as alternative energy sources. Proceedings of the World Congress on Engineering 2015 Vol II WCE 2015, July 1–3, 2015. London, UK.
51. Pippo WA, Luengo CA. Sugarcane energy use: accounting of feedstock energy considering current agro-industrial trends and their feasibility. Int J Energy Environ Eng. 2013;4(1):10. doi: 10.1186/2251-6832-4-10.
52. Zabed H, Faruq G, Sahu JN, Azirun MS, Hashim R, Boyce AN. Bioethanol production from fermentable sugar juice. ScientificWorldJournal. 2014;2014:957102. doi: 10.1155/2014/957102.
53. Muregi MA, Abolarin MS, Okegbile OJ, Eterigho EJ. Influence of temperature and agitation speed on fermentation process during production and bioethanol fuel from cassava. Int J Eng Adv Technol Stud. 2021;9(3):40-6. doi: 10.37745/ijeats.13.
54. Hawaz E, Tafesse M, Tesfaye A, Kiros S, Beyene D, Kebede G, et al. Optimization of bioethanol production from sugarcane molasses by the response surface methodology using *Meyerozyma caribbica* isolate MJTm3. Ann Microbiol. 2023;73(1):2. doi: 10.1186/s13213-022-01706-3.
55. Adetunji RO, Youdeowei PK, Kolawole OO. Production of bioethanol from cassava peel. In: Proceedings from International Conference on Renewable Energy and Power. Vol 1. 2015; Atlanta, Georgia.
56. Agulejika EO, Olabode FI, Babatunde KA. Ethanol production from waste fruits. Int J Food Agric Res. 2005;2(2):190-4.
57. Toma MM, Kalnenieks U, Berzins A, Vigants A, Rikmanis M, Viesturs U. The effect of mixing on glucose fermentation by *Zymomonas mobilis* continuous culture. Process Biochem. 2003;38(9):1347-50. doi: 10.1016/s0032-9592(03)00004-9.
58. Rodmui A, Kongkiattikajorn J, Dandusitapun Y. Optimization of agitation conditions for maximum ethanol production by coculture. Agric Nat Resour. 2008;42(5):285-93.
59. Mussatto SI, Machado EMS, Carneiro LM, Teixeira JA. Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. Appl Energy. 2012;92:763-8. doi: 10.1016/j.apenergy.2011.08.020.
60. Choi GW, Um HJ, Kang HW, Kim Y, Kim M, Kim YH. Bioethanol production by a flocculent hybrid, CHFY0321 obtained by protoplast fusion between *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. Biomass Bioenergy. 2010;34(8):1232-42. doi: 10.1016/j.biombioe.2010.03.018.
61. Kumari R, Pramanik K. Bioethanol production from *Ipomoea carnea* biomass using a potential hybrid yeast strain. Appl Biochem Biotechnol. 2013;171(3):771-85. doi: 10.1007/s12010-013-0398-5.
62. Zhang H, Skelton A, Gardner RC, Goddard MR. Saccharomyces paradoxus and *Saccharomyces cerevisiae* reside on oak trees in New Zealand: evidence for migration from Europe and interspecies hybrids. FEMS Yeast Res. 2010;10(7):941-7. doi: 10.1111/j.1567-1364.2010.00681.x.
63. Tofighi A, Mazaheri Assadi M, Asadirad MH, Zare Karizi S. Bio-ethanol production by a novel autochthonous thermotolerant yeast isolated from wastewater. J Environ Health Sci Eng. 2014;12(1):107. doi: 10.1186/2052-336x-12-107.
64. Fonseca GG, Heinzle E, Wittmann C, Gombert AK. The yeast *Kluyveromyces marxianus* and its biotechnological potential. Appl Biochem Biotechnol. 2008;79(3):339-54. doi: 10.1007/s00253-008-1458-6.
65. Yanase S, Hasunuma T, Yamada R, Tanaka T, Ogino C, Fukuda H, et al. Direct ethanol production from cellulosic materials at high temperature using the thermotolerant yeast *Kluyveromyces marxianus* displaying cellulolytic enzymes. Appl Biochem Biotechnol. 2010;88(1):381-8. doi: 10.1007/s00253-010-2784-z.