Original Research Article

PRODUCTION OF BIOETHANOL FROM SELECTED LIGNOCELLULOSIC AGROWASTES

ABSTRACT

This study evaluated the ability of cassava peels, banana peels, orange peels and corn cobs hydrolysates to produce bioethanol. Fibre fractions analysis was carried out using standard methods. The samples were pretreated with acid and base, followed by simultaneous saccharfication and fermentation (SSF) for bioethanol production. During fermentation, pH, total titratable acidity, reducing sugar, microbial load and bioethanol yield were determined. The reducing sugar yield for *Aspergillus niger* and *Bacillus cereus* were 30.28g and 13.35g for corn cobs. The pH was observed to decrease during fermentation period with orange peels having the lowest pH of 2.6 after 240 hours of fermentation using *A. Niger* and *S. cerevisiae*, when *B. cereus* and *S. Cerevisiae* were used the pH was observed to be 4.10. Total titratable acidity showed increase in all the substrates, with corn cobs having the highest when *B. cereus* and *S. Cerevisiae* were used (1.62), followed by cassava peels when *A. niger* and *S. cerevisiae* were used (1.52). Highest ethanol yield following simultenous saccharfication and fermentation with *A. niger* and *S. cerevisiae* was obtained in corn cobs with 17.43g/100g, while orange peels gave the lowest with 8.02g/100g, the ethanol yield from each substrates as well as the combined substrates were significantly different at $p \leq 0.05$. The combined substrates (1:1:1:1) gave the

highest ethanol yield of 12.44g/100g using *A. niger* and *S. cerevisiae.* This study therefore revealed that *A. niger* had the highest bioethanol yield using corn cobs as the carbon source, therefore it could be used for mass bioethanol production.

Key words: simultaneous saccharification, bioethanol, agrowastes, titratable acidity, reducing sugar

INTRODUCTION

Agricultural wastes have become an alternative raw material for bioethanol production, to prevent competition between food security and ethanol production that the initial use of food crops for bioethanol has caused. This biomass can easily be produced compared to the food crops and holds the key to supplying society's basic needs for sustainable production of liquid transportation fuels without impacting the nation's food supply (Alexander *et al.,* 2012).

Bioethanol can be produced from several different biomasses: starchy materials such as cassava peel, and lignocellulosic biomass such as corn cob, its production is promising especially in those countries with limited lands availability. In fact, residues are often widely available and do not compete with food production in terms of land destination (Hossain *et al*., 2011). Its production is also characterized by common steps: hydrolysis of cellulose and hemicellulose to monomeric sugars, fermentation and product recovery. The main differences lie in the hydrolysis phase, which can be performed by dilute acid, concentrated acid or enzymatically (Alexander *et al*., 2012)

MATERIALS AND METHODS

Collection of samples

One thousand (1,000) grams each of fresh orange peels, cassava peels, banana peels, and corn cob were collected from FUTA farm and Oba Market in Akure South Local Government, Ondo State, Nigeria. The samples were then sundried for three days after which they were milled. The dried samples were divided into two portions; the first portion was pretreated while the second was not.

Pretreatment of Samples

A two - stage process which combines the dilute acid pre-hydrolysis (DAPH-100-121) and alkaline delignificaton using NAOH as described by Olugbenga and Ibileke (2011) was used. Dry samples were treated with dilute sulfuric acid which involved the use of 1.25% (w/v) H_2SO_4 solution in a 1: 8, g : g, solid : liquid ratio. The one step dilute acid pre-hydrolysis (DAPH-100-121) was performed in an autoclave at 121^0C for 17min, after which the solids were collected and drained. The solids were then treated with 2% (w/v) sodium hydroxide solution in a solid: liquid ratio of 1: 20, g: g, at 120^0C for 90 min. after that, the residual solid material (Cellulose pulp) separated by filtration was washed with water to remove the residual alkali, and was dried at 50 ± 5^oC for 24 hours.

Sterilization, Preparation of Culture Media and Isolation

All glass wares (Petri dishes, beakers, conical flasks) were washed thoroughly, air dried, sterilized in hot oven around 180^{o}C for 2 hours. Nutrient agar (NA) and Potato dextrose agar (PDA) were prepared according to manufacturer's specifications and autoclaved at 121^{o}C for 15 minutes and allowed to cool to 45^{o}C before pour plating.

Six fold serial dilutions was carried out on collected agro waste samples and pour plated with molten nutrient agar and the potato dextrose agar media, cooled to 45^{o}C. Nutrient agar plates were incubated at 37^{o}C for 24 hours for bacteria and 28^{o}C for 3 to 5 days for fungi on potato dextrose agar plates respectively in triplicate before examination for microbial growth. The bacterial isolates were purified by streaking on fresh sterile nutrient agar before sub culturing. Fungal isolates were also sub cultured to obtain pure isolates. The pure isolates were stored temporarily on slants and kept at 4^{o}C for further use (Fawole and Oso, 2012). Colony count was carried out on plates (in triplicates) by using colony counter and expressed as colony forming unit for bacteria and spore forming unit for fungi respectively.

Starch hydrolysis test

This test was used to detect the ability of bacterial isolates to produce starch degrading enzymes. It was performed for fungi isolates also. Nutrient agar and potato dextrose agar were both prepared with 1% soluble starch for bacteria and fungi respectively. The media was sterilized, poured into sterile petri-dishes and allowed to solidify. Bacterial isolates were inoculated onto the surface by streaking after which incubation at 37^{o}C for 24 hours, while fungi isolates were inoculated by stabbing followed by incubation at ambient temperature for 3 days. After incubation,

65 the plates were flooded with iodine; positive results were indicated by a clear zone around the colony which implies that starch was hydrolyzed,
66 while a blue black coloration indicated a negative result (Fawole and Oso, 2001).

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68 **Determination of cellulose, Hemicellulose and lignin**

69 The method of AOAC (2012) was used as described by Ververis *et al*. (2002). The substrates were analyzed for cellulose, hemicellulose
70 and acid insoluble lignin which were done before and after pretreatment. Cellulose was determined using a colorimetric method with the
71 anthrone reagent. Ground samples were treated and boiled at 100^0C with a mixture of nitric/acetic acid (1: 8, v/v) for 1 hr to remove lignin,
72 hemicelluloses and xylosans after successive cetrifugations, and diluted with 67% H_2SO_4 (v/v). Cellulose was then determined at 620nm using
73 cold anthrone reagent.

74 Hemicellulose and lignin contents of the substrates were determined as follows; the residue from above containing Hemicellulose and
75 lignin was then boiled with 5 ml of 72% (w/w) H_2SO_4 solution for 4.5 hours in order to hydrolyze the hemicellulose. The suspension remaining
76 after the above treatment was filtered through a crucible and the solid residue dried at 105^0C for 24 hours and weighed (W1). The residue was
77 then transferred to a preweighed dry porcelain crucible and heated at 600^0C for 5 hours. After cooling down, it was weighed (W2). Acid
78 insoluble lignin was then calculated by the difference (W1-W2).

79 The filtrate from the H_2SO_4 treatment that contained the sugars released from hemicellulose was thoroughly stirred and homogenized. Glucose
80 (C1) and reducing sugar (C2) concentrations in the filtrate were determined. Following these measurements, the hemicellulose content was then
81 calculated from the following equation:

$$\%\left(\frac{w}{w}\right) hemicellulose = \left\{\frac{W}{S}\right\} \times (C2 - C1) X \left\{\frac{V}{M}\right\} X\ 100$$

82 Where; W= molecular weight ratio of the polymer and monomer pentose, S= saccharification yield, C2= determined reducing sugars
83 concentration (g/L), C1= glucose concentration (g/L), V= total volume of sugar solution (L), M = dry weight of the sample (g).

84 **Microbial hydrolysis**

85 One hundred (100) grams of each pretreated substrates was weighed in duplicates into 1000ml conical flasks and made up to mark with
86 distilled water, corked and sterilized at 121^0C for 15 min. sterile distilled water was added to the flasks to final volume 1 liter and the flasks
87 plugged with sterile cotton wool. After cooling, the medium was inoculated with 50ml of 36 hours culture of *Aspergillus niger* and *Bacillus*
88 *cereus* separately; the pH of the medium was then adjusted to 5.0. Hydrolysis was carried out at room temperature for three days. A second un-
89 inoculated flask served as control. Samples were taken at the end of three days for reducing sugar determination (Abdullahi, 2013).

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91

Determination of reducing sugar

The method of Olugbenga and Ibileke (2011) was used. Two mls of the hydrolyzed sample was placed in a test- tube and 1g of activated charcoal was added. The mixture was shaken thoroughly. The mixture was then filtered with filter paper until a colorless filtrate was obtained. One ml of filtrate was placed in a test-tube and two drops of alkaline DNS reagent were added and the tube was placed in boiling water for 5 min. the mixture was allowed to cool and the absorbance was measured at 540nm. This measurement was taken after three days. A standard curve of glucose was prepared and used to calculate the percentage reducing sugar.

Physicochemical analysis

The following physicochemical properties of each fermenting substrate were measured;

Determination of pH

The pH of each fermenting substrate was measured at 24 hours interval for seven days using a digital pH meter, standardized with buffer of 7.0 the pH was then determined by inserting the electrode bulb into a sample from each fermenting substrate.

Total titratable acid

This was determined using the method of Lyumugabe et al. (2010) 10ml of the fermenting medium was transferred into a beaker, followed by the addition of 3 drops of phenolphthalein indicator. The sample was then titrated against 0.1M NaOH to an end point of a definite pink colour. The volume of NaOH used was noted and the titratable acid percentage was calculated using the following formula;

TTA (%) = V x 0.15

Where; V = Volume of NaOH.

Preparation of inoculum

Aspegillus niger, Bacillus cereus and *Saccharomyces cerevisiae* inocula were prepared by introducing slant cultures to 150ml of sterile growth media contained in 500ml conical flasks. The flasks were incubated on a rotary shaker at 30^0C for 96 hours (Ado *et al.,* 2009).

Standardization of inoculum (McFarland Turbidity standard)

Method modified by Cheesbrough (2006), was used to prepare the McFarland 0.5 turbidity standard which was used to measure the density of microbial cells. In this method, fifty milliliter (50ml) of a 1.175% (wt/vol) dehydrates Barium chloride ($BaCl_2.2H_2O$) solution was added to 99.4ml of 1% (vol/vol) sulfuric acid. McFarland standard tube was then sealed with Paraffin to prevent evaporation and stored in the

dark at room temperature. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer with a 1cm light path. The 0.5 McFarland standards were vigorously agitated before use

Fermentation

Five sets of liquid state fermentation were carried out using the pretreated hydrolyzed samples. The hydrolysates from the above were transferred into another set of conical flasks and labeled correctly, covered, autoclaved at 121^{o}C for 15 minutes and allowed to cool. The flasks were inoculated with *Saccharomyces cerevisiae* to carry out fermentation for ten days. The fermentation was then monitored from day 1, the pH of the hydrolysate containing *Saccharomyces cerevisiae* was adjusted to 5.0 and fermentation carried out at 30^{o}C in a rotary shaker. The ethanol yield was determined at 24 hours interval during fermentation. The fermentate was separated by centrifugation at 9000 rpm to separate the waste from the supernatant (Abdullahi, 2013). All procedures were carried out in triplicates.

Distillation

It was carried out using a set up distillation apparatus. The fermented liquid was transferred into round bottom flask and placed on a heating mantle fixed to a distillation column enclosed in a running tap water. Another flask was fixed to the other end of the distillation column to collect the distillate at 78^{o}C (standard temperature for ethanol production). Ethanol yield was then determined by obtaining the mass of the distillate in grams. Percentage ethanol was then determined by obtaining the specific gravity of the ethanol produced and using it to calculate the percentage (v/v) ethanol produced (Abdullahi, 2013)

Statistical analysis

Data are presented as mean ± standard error (SE). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Science) version 20 software. For all tests, the significance was determined at the level of $P \leq 0.05$.

RESULTS

Effect of acid pretreatment on cellulose, hemicellulose and lignin of the agricultural wastes.

Table 1 shows the effect of pretreatment on the cellulose; hemicellulose and lignin components of cassava peels, orange peels, banana peels and corn cobs. The result indicates that there was significant difference ($p \leq 0.05$) in the effect of acid pretreatments of the substrates. There was high increase in cellulose content of corn cobs from 39.39% to 59.21%, while cassava peels showed an increase from 12.66% to 20.66%, orange peels also showed cellulose content increment after pretreatment from 13.64% to 17.06% and banana peels which had the lowest showed an increase from 2.09% to 9.43%. Hemicellulose content on the other hand decreased after pretreatment in cassava peels from 8.28% to 3.11%, in banana peels from 11.46% to 1.33%, in orange peel from 6.29% to 4.23% and in corn cob from 43.34% to 16.95%. Lignin content of corn cobs reduced drastically from 16.3% to 6.23%, similar decrease was also recorded for the lignin content of cassava peels, banana peels and orange peels.

Reducing sugar produced by each substrates after 3 days of hydrolysis using *Aspergillus niger* and *Bacillus cereus*.

The reducing sugar produced by each substrate as well as the combinations of the substrates in ratio 1: 1: 1: 1 after three days of hydrolysis using *Aspergillus niger* is given in Figure 1. The result revealed that highest reducing sugar yield was obtained in corn cobs with 30.28g, followed by cassava peels with a yield of 26.36g, combinations of all the substrates (OCBC) gave a yield of 21.62g, and banana peels also gave a reducing sugar yield of 20.32g, while orange peels had the lowest with 16.23g.

Furthermore, figure 1 also shows the reducing sugar yield of each substrates and combinations of the substrates in ratio 1: 1: 1: 1 after three days of hydrolysis using *Bacillus cereus*. However, the yield was considerably lower than what was obtained using *Aspergillus niger*. Corn cobs gave the highest reducing sugar yield with 13.35g, followed by cassava peels with 11.14g, combinations of all the substrates (OCBC) gave a yield of 9.34g, and banana peels also gave a reducing sugar yield of 8.44g, while orange peels had the lowest with 5.88g.

Table 1: Effect of acid pretreatment on cellulose, hemicellulose and lignin of the agricultural wastes

Parameter	CpB (%)	CpA (%)	BpB (%)	BpA (%)	OpB (%)	OpA (%)	CcB (%)	CcA (%)
Lignin	9.34±0.04^{b}	4.18±0.02^{c}	12.23±0.02^{c}	2.35±0.01^{b}	2.25±0.02^{a}	1.19±.03^{a}	16.34±0.01^{d}	6.23±0.02^{d}
Hemicellulose	8.28±0.04^{b}	3.11±0.00^{b}	11.46±0.04^{c}	1.33±0.03^{a}	6.29±0.13^{a}	4.23±0.02^{c}	43.34±0.06^{d}	16.95±0.0^{d}
Cellulose	12.66±0.01^{b}	20.66±0.30^{c}	2.09±0.03^{a}	9.43±0.022^{a}	13.64±0.01^{c}	17.0600±0.03^{b}	39.39±0.08^{d}	59.21±0.02^{d}

Key: **CpB (%)** = Cassava peels before pre-treatment, **OpB (%)** = Orange peels before pre-treatment, **BpB (%)** = Banana peels before pre-treatment
CcB (%) = Corn cob before pre-treatment, **CpA (%)** = Cassava peels after pre-treatment, **OpA (%)** = Orange peels after pre-treatment, **BpA (%)** = Banana peels after pre-treatment, **CcA (%)** = Corn cob after pre-treatment.

Values are means ± Standard error of agricultural wastes. Values in the same row carrying the same superscript are not significantly different at ($p \leq 0.05$) using Duncan's New Multiple Range test.

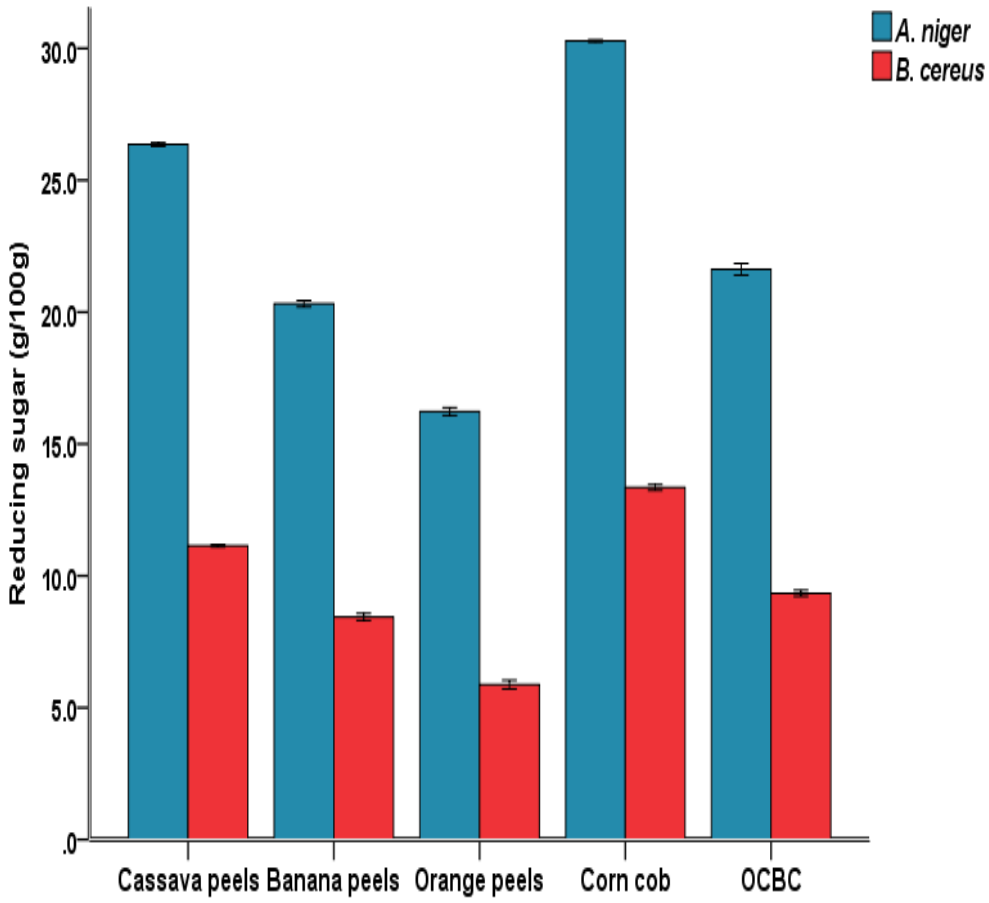

Fig 1: Reducing sugar produced by each substrates after 3 days of hydrolysis using *A. niger* and *B. cereus* respectively.

Bars represent reducing sugar (g/100g) ± standard error, significant difference were taken at ($P \leq 0.05$) according to Duncan's New Multiple Range tests.

Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob (Ratio 1:1:1:1) in grams

Changes in pH during fermentation of different agricultural wastes using *A. niger* and *S. cerevisiae.*

The changes in pH during the fermentation of cassava peels, banana peels, orange peels, corn cobs and the combinations of all the substrates in ratio 1: 1: 1: 1(OCBC) using *A. niger* and *S. cerevisiae* are represented in Figure 2. A general decrease in the pH was observed from the initial standardized pH of 5.0 as fermentation proceeded. Fermentation of orange peels showed a decrease, with a pH of 3.0 after 7 days, cassava peels with a final pH of 4.0, banana peels with a pH of 4.0 after 7 days, and corn cobs with a final pH of 3.6. The combinations of all the substrates in ratio 1:1:1:1 (OCBC) showed a decrease from the initial pH of 5.0 to 3.0 after 7 days of fermentation.

Changes in pH during fermentation of different agricultural wastes using *B. cereus* and *S. cerevisiae.*

Figure 3 showsthe changes in pH during the fermentation of cassava peels, banana peels, orange peels, corn cobs and the combinations of all the substrates in ratio 1: 1: 1: 1(OCBC) using *B. cereus* and *S. cerevisiae*. A decrease in the pH was observed from the initial standardized pH of 6.0 as fermentation proceeded. Fermentation of corn cobs showed a decrease with a final pH of 4.8 after 8 days, cassava peels recorded a decrease with a final pH of 4.2, with the combinations of all the substrates in ratio 1:1:1:1 (OCBC) having a decrease from the initial pH of 6.0 to 4.2, while orange peels had the lowest final pH of 4.0. However, a slight fluctuation was observed from day 4.

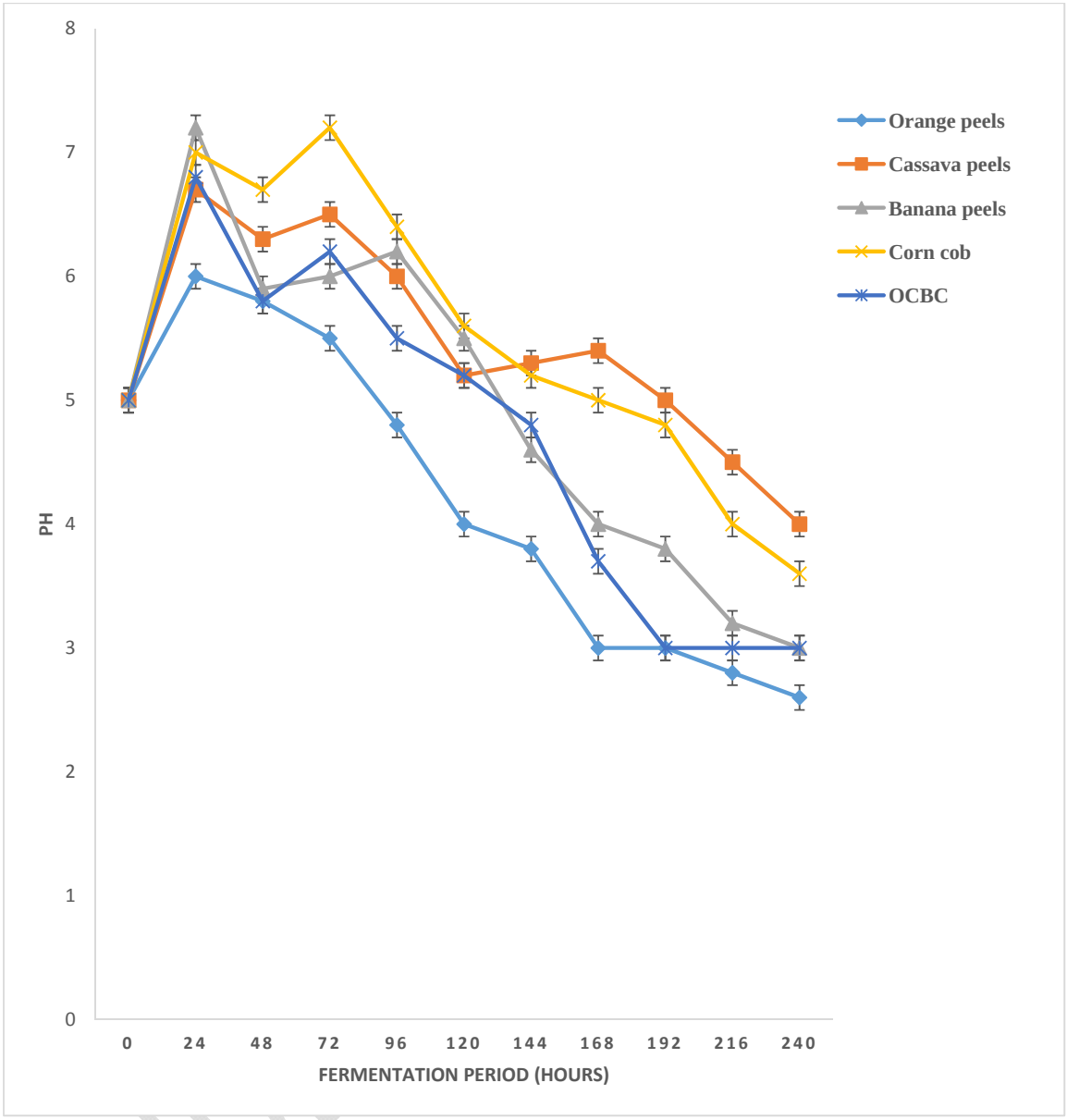

Fig 2: Changes in pH during fermentation of different agricultural wastes using *A. niger* and *S. cerevisiae.*

Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob (Ratio 1:1:1:1) in grams

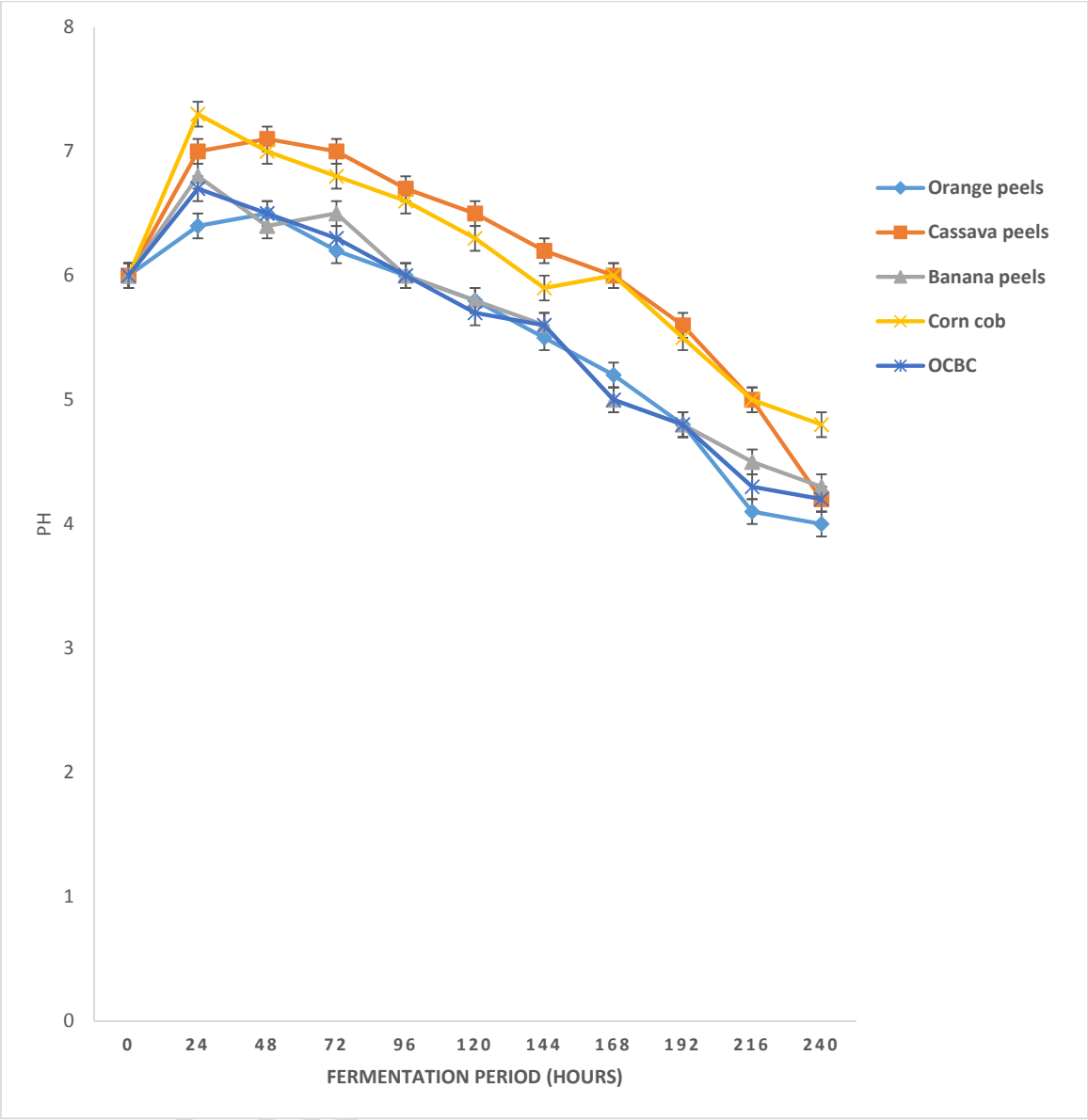

Fig 3: Changes in pH during fermentation of different agricultural wastes using *B. cereus* and *S. cerevisiae*

Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob (Ratio 1:1:1:1)in grams.

Total titratable acidity during fermentation of different agricultural wastes using *A. niger* and *S. cerevisiae*

The total titratable acidity during fermentation of each susbtrate using *A. niger* and *S. cerevisiae* is shown in Figure 4. An increase in the TTA was observed from the initial TTA as fermentation proceeded. Fermentation of corn cobs showed an increase in TTA, from an initial TTA of 0.12% to 1.27% after 168 hours; banana peels showed an increase from 0.1% initial to a final TTA of 0.9%, cassava peels also showed a very high TTA from 0.14% initial to a highest of 1.5%. The combinations of all the substrates in ratio 1:1:1:1 (OCBC) showed an increase in TTA from 0.09% to a highest of 1.23%.

Total Titratable acidity during Fermentation of different Agricultural Wastes using *B. cereus* and *S. cerevisiae*

Figure 5 shows the total titratable acidity during fermentation of different agricultural wastes using *B. cereus* and *S. cerevisiae.* The result revealed that, as fermentation proceeded from day zero to day seven, increase in the TTA was observed, corn cobs TTA was conspicuously higher than the rest from an initial TTA of 0.1% to 1.7%, followed by combinations of all the substrates in ratio 1:1:1:1 (OCBC) from initial TTA of 0.09% to 0.84%, while the lowest TTA was recorded for orange peels from 0.07% to 0.38%

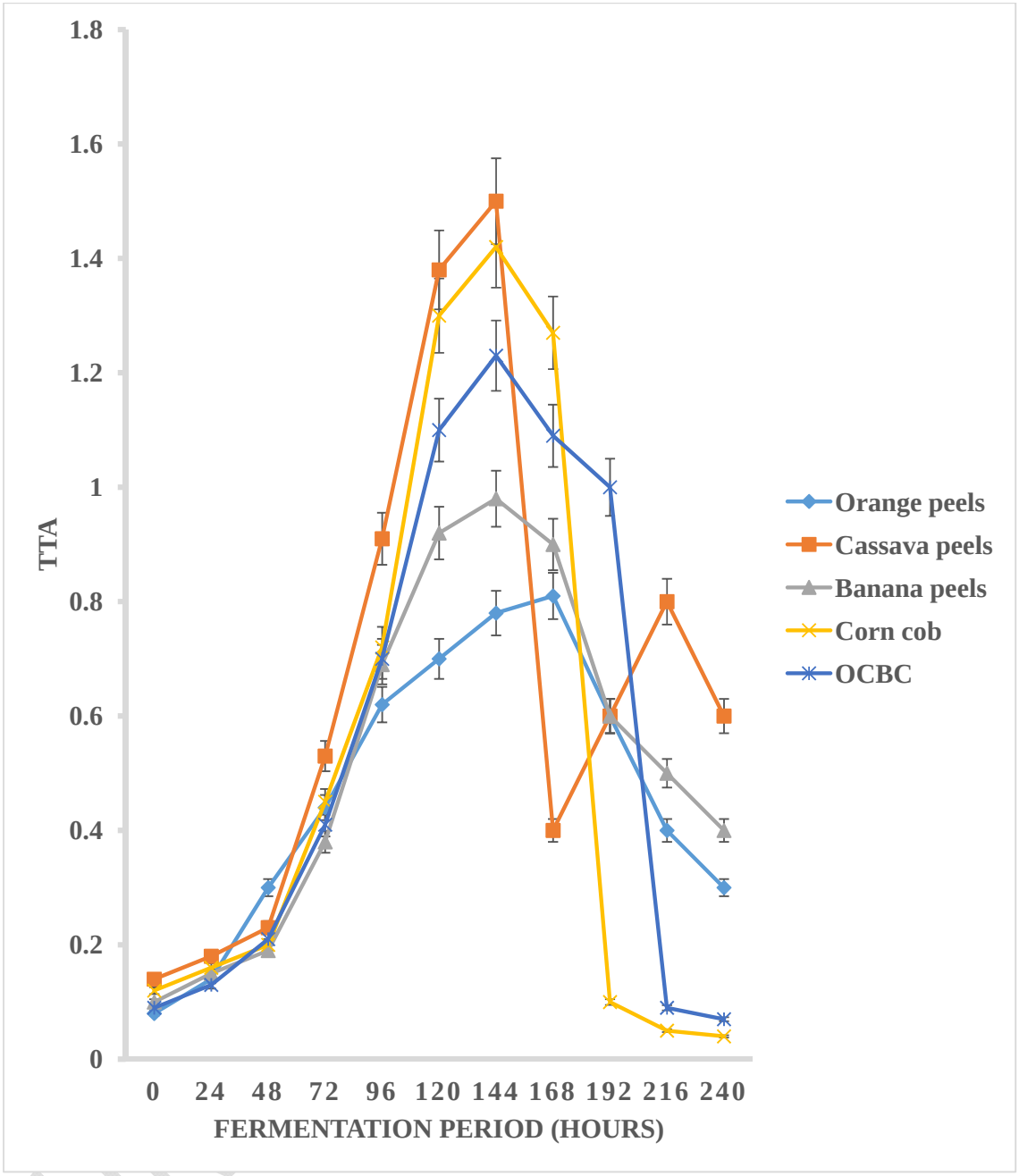

Fig 4: Total titratable acidity during fermentation of different agricultural wastes using *A. niger* and *S. cerevisiae*

Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob (Ratio 1:1:1:1) in grams

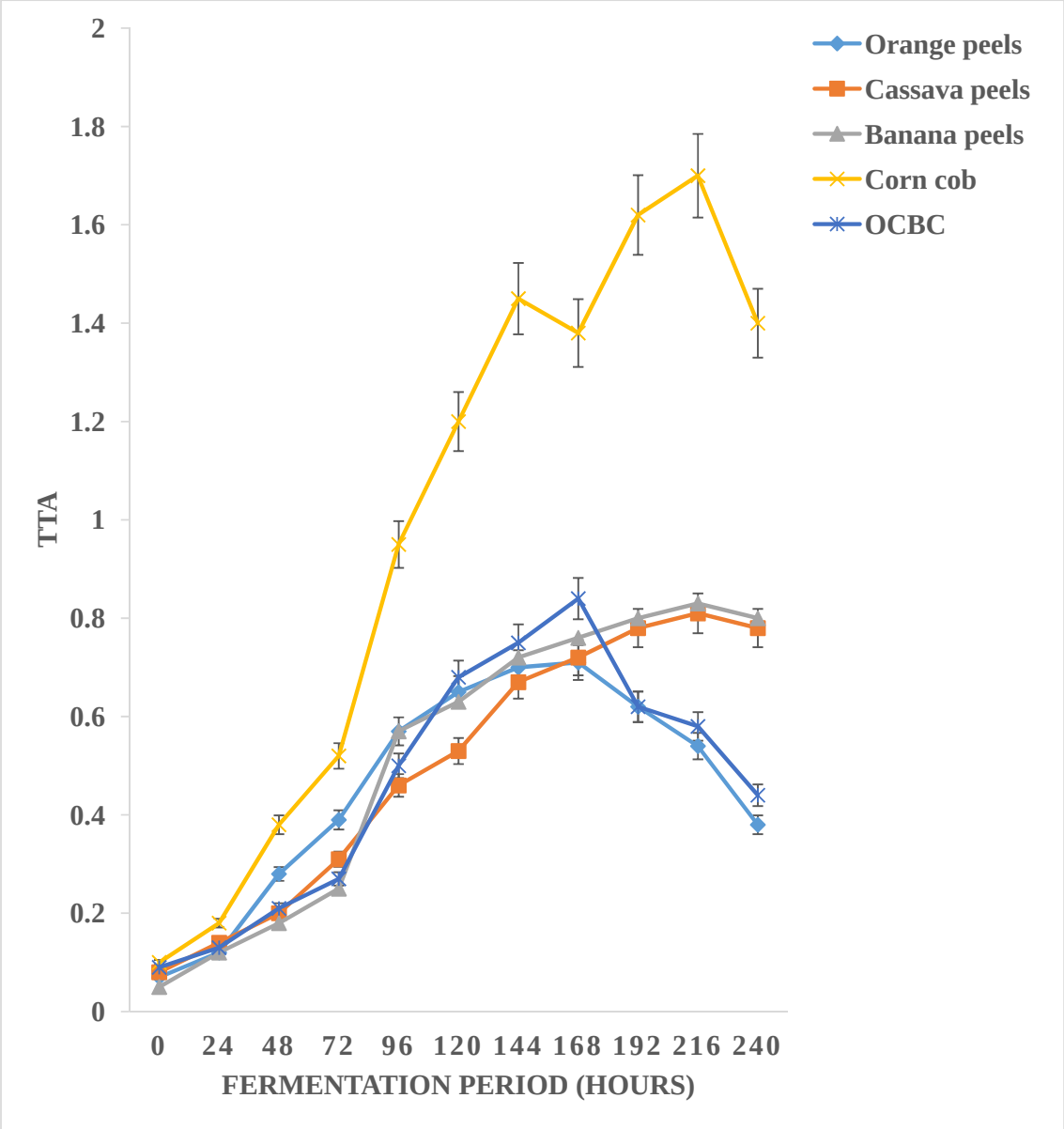

Fig 5: Total Titratable acidity during Fermentation of different agricultural wastes using *B. cereus* and *S. cerevisiae*

Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob (Ratio 1:1:1:1) in grams

Ethanol yield from different agricultural wastes using *A. niger* and *S. cerevisiae*

Figure 6 shows the ethanol yield of the various substrates and their combination during days of fermentation using *A. niger* and *S. cerevisiae*.. The ethanol yield was observed to increase as the fermentation continued. Corn cobs had the highest initial yield of 3.22g after 48 hours; followed by banana peels which had an initial yield of 2.21g, cassava peels had 2.07g, while orange peels recorded the lowest with 1.30g.The combinations of all the substrates in ratio 1:1:1:1 (OCBC) also had ethanol yield of 1.90g after 48 hours of fermentation, it was observed that corn cobs had the highest final ethanol yield of 17.43g, followed by cassava peels which gave a yield of 15.1g, while combinations of all the substrates in ratio 1:1:1:1 (OCBC) gave a yield of 12.44g. Orange peels on the other hand recorded the least ethanol yield of 8.03g

Ethanol yield from different agricultural wastes using *B. cereus* and *S. cerevisiae*

The ethanol yield of the various substrates and their combination during days of fermentation using *B. cereus* and *S. cerevisiae*are presented in Figure 7. The ethanol yield was observed to increase as the fermentation proceeded, however it can be observed that the ethanol produced was considerably lower than that produced by *A. niger* and *S. cerevisiae.* The combinations of all the substrates in ratio 1:1:1:1 (OCBC) had the highest initial yield of 2.46g after 24 hours, followed by corn cobs which had an initial yield of 2.16g. Cassava peels also had 1.91g, followed by banana peels with 1.41g, while orange peels had the lowest initial yield of 0.82g after 24 hours. After 8 days of fermentation, corn cobs were shown to have the highest final ethanol yield of 9.39g, followed by the combinations of all the substrates in ratio 1:1:1:1 (OCBC) which gave a yield of 9.14g. However, it can be observed that orange peels recorded the lowest ethanol yield of 5.50g after 7 days of fermentation.

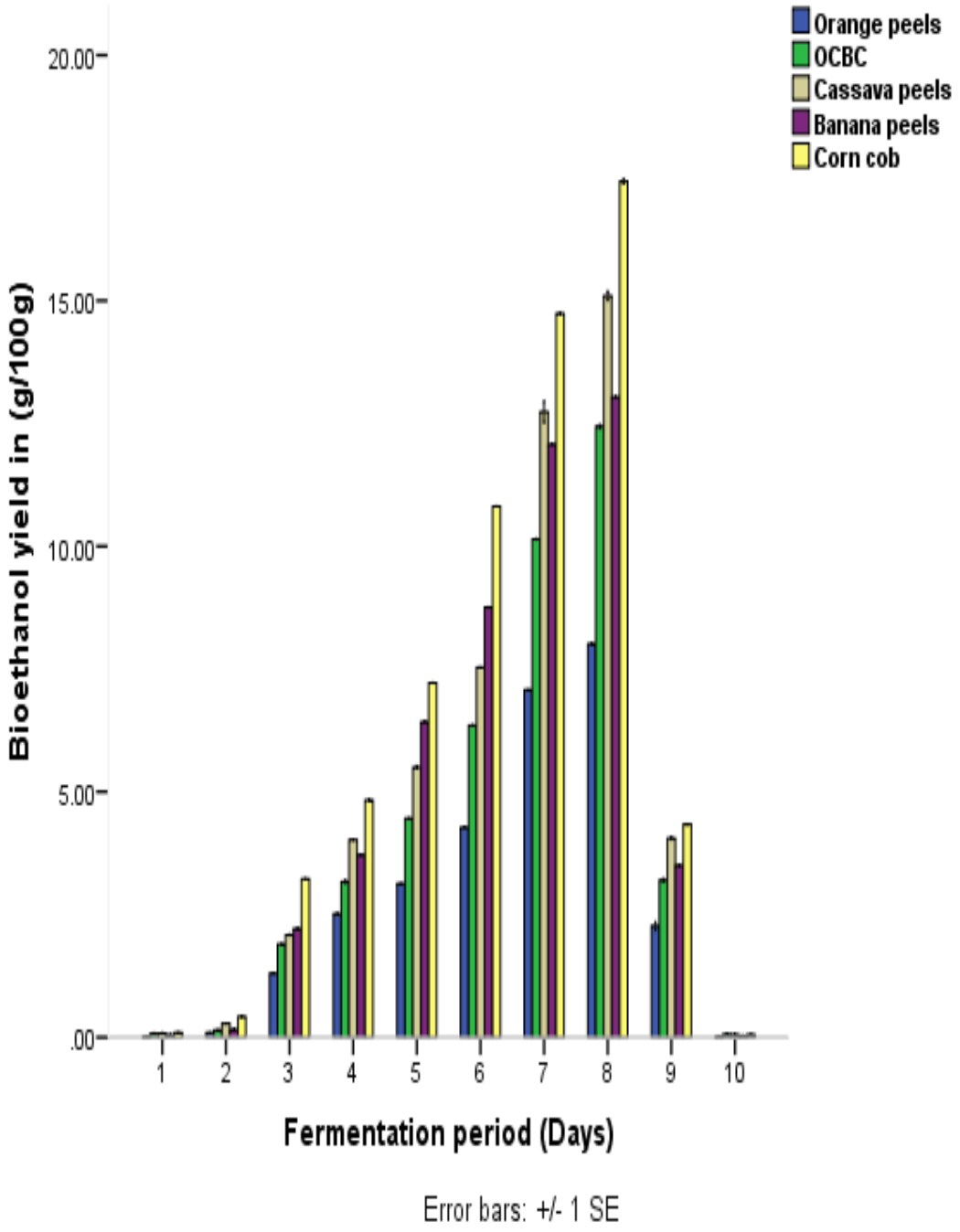

Fig 6: Ethanol yield from different agricultural wastes using *A. niger* and *S. cerevisiae*

Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob (Ratio 1:1:1:1) in grams.

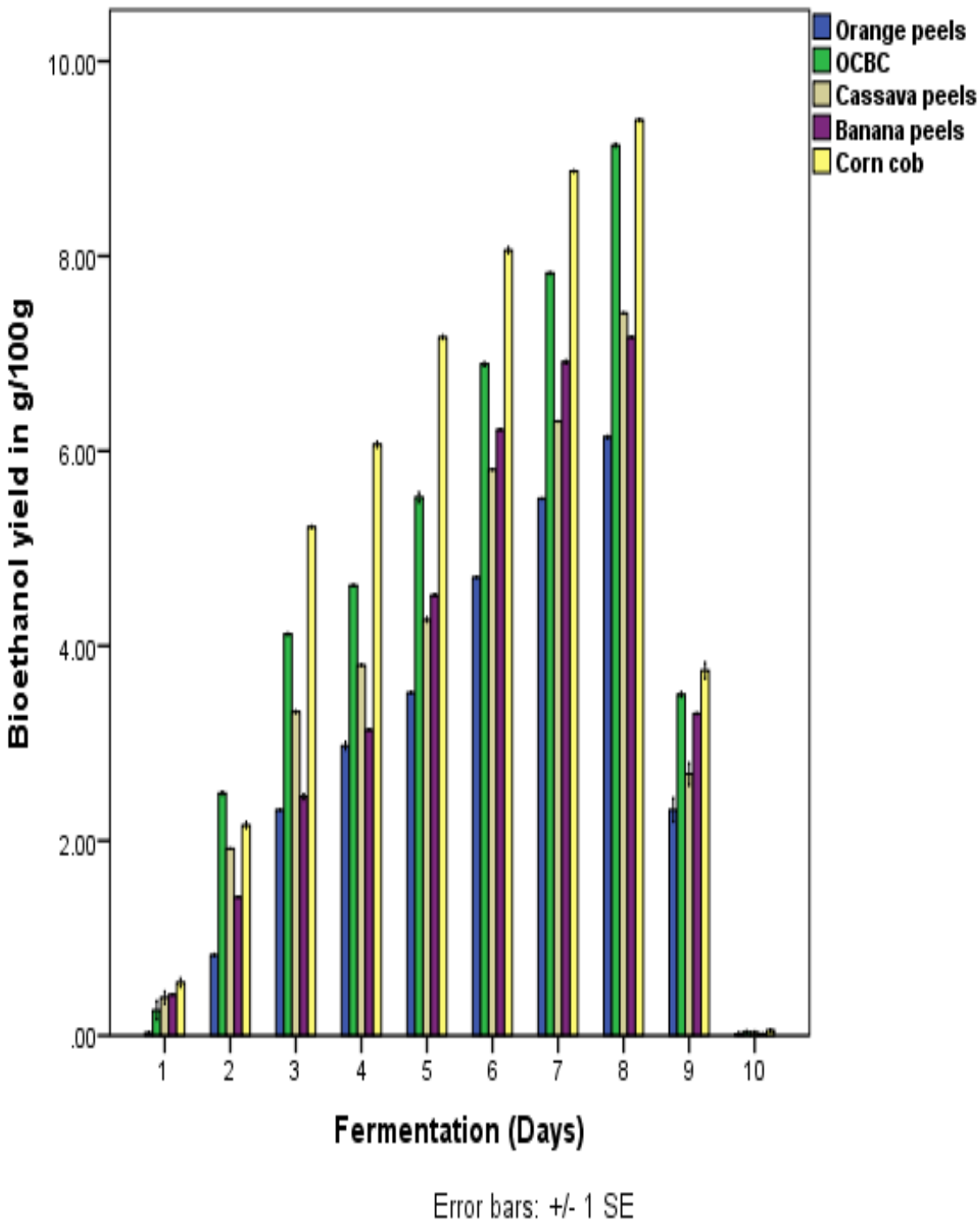

Fig 7: Ethanol yield from different agricultural wastes using *B. cereus* and *S. cerevisiae*

Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob (Ratio 1:1:1:1) in grams

334 **Bacterial counts in Cfu/mL during fermentation of the agricultural wastes**

335 The result of bacterial counts observed on nutrient agar from fermentation of orange
336 peels, cassava peels, banana peels, corn cobs and combinations of all the substrates in ratio
337 1:1:1:1(OCBC) is presented in Table 2.The results showed that cassava peels had the highest
338 initial count of 5.10 x 10^6Cfu/mL, while orange peels had the lowest of 1.8 x 10^6 Cfu/mL.
339 The combinations of all the substrates in ratio 1:1:1:1(OCBC) had the highest microbial load
340 on Nutrient agar of 56.4 x 10^6Cfu/mL after 6 days of fermentation, while orange peels was
341 observed to have the lowest with 10.08 x 10^6Cfu/mL after 9 days.

342

343 **Fungal counts in Sfu/mL during the fermentation of the agricultural wastes**

344 Table 3 shows Fungal Counts in Sfu/mL on PDA during the fermentation of the agricultural
345 wastes, the result revealed that, banana peels had the initial highest count of 6. 7 x 10^5
346 Sfu/mL, while orange peels had the lowest of 2.1 x 10^5 Sfu/mL. After seven days of
347 fermentation, the combinations of all the substrates in ratio 1:1:1:1(OCBC) had the highest
348 fungal load of 5.2 x 10^5Sfu/mL, followed by banana peel with 4.1 x 10^5 Sfu/mL, while
349 orange peels recorded the lowest overall after several days of fermentation with 1.1 x 10^5
350 Sfu/mL

351

352 **Table 2: Bacterial counts in Cfu/mL during fermentation of the agricultural wastes**

353

FERMENTATION DAYS	Orange peels Cfu/mL x 10^6	OCBC Cfu/mL x10^6	Cassava peels Cfu/mL x 10^6	Banana peelsCfu/mL x 10^6	Corn cob Cfu/mL x 10^6
0	1.8 ± 0.12^{g}	4.90 ± 0.21^{ab}	5.10 ± 0.10^{b}	3.70 ± 0.30^{c}	2.10 ± 0.10^{d}
1	2.2 ± 0.10^{bc}	5.20 ± 0.00^{f}	5.50 ± 0.10^{e}	3.90 ± 0.20^{ab}	2.70 ± 0.30^{gh}
2	2.8 ± 0.40^{ef}	5.80 ± 0.10^{c}	6.10 ± 0.00^{ab}	4.00 ± 0.30^{cd}	25.05 ± 0.20^{a}
3	3.2 ± 0.08^{a}	2.61 ± 0.30^{bc}	11.22 ± 0.06^{ef}	12.13 ± 0.10^{e}	29.02 ± 0.17^{f}
4	11.0 ± 0.00^{d}	50.8 ± 0.17^{de}	14.13 ± 0.40^{bc}	25.05 ± 0.15^{ef}	35.08 ± 0.10^{g}
5	21.2 ± 0.09^{b}	50.6 ± 0.00^{a}	27.08 ± 0.17^{f}	29.18 ± 0.10^{b}	48.17 ± 0.27^{ab}
6	26.01 ± 0.12^{e}	56.4 ± 0.00^{ef}	31.05 ± 0.14^{b}	31.10 ± 0.13^{d}	50.30 ± 0.10^{c}
7	29.12 ± 0.10^{h}	52.2 ± 0.26^{g}	34.21 ± 0.06^{a}	41.09 ± 0.27^{bc}	52.22 ± 0.17^{cd}
8	33.42 ± 0.00^{c}	48.31 ± 0.11^{d}	25.11 ± 0.26^{g}	52.03 ± 0.23^{de}	53.10 ± 0.10^{h}
9	22.15 ± 0.02^{ab}	36.12 ± 0.00^{cd}	19.06 ± 0.15^{h}	40.20 ± 0.23^{gh}	42.12 ± 0.20^{de}
10	10.08 ± 0.14^{bc}	16.10 ± 0.00^{ab}	14.10 ± 0.00^{d}	21.00 ± 0.20^{a}	12.01 ± 0.13^{b}

354

355 Values are means ± Standard error of agricultural wastes. Values in the same column carrying the same superscript are not significantly different
356 at ($p\leq 0.05$) using Duncan's New Multiple Range test.
357 Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob
358 (Ratio 1:1:1:1) in grams
359

Table 3: Fungal counts in Sfu/mL during the fermentation of the agricultural wastes

FERMENTATION DAYS	ORANGE PEELS Sfu/mL x 10^5	OCBC Sfu/mL x 10^5	CASSAVA PEELS Sfu/mL x 10^5	BANANA PEELS Sfu/mL x 10^5	CORN COB Sfu/mL x 10^5
0	2.1 ±0.14^{bc}	2.90 ±0.27^{b}	5.10 ±0.20^{h}	6.70 ±0.10^{d}	3.60 ±0.22^{e}
1	3.2 ±0.20^{c}	5.10 ±0.00^{ab}	5.40 ±0.12^{b}	7.20 ±0.23^{ef}	4.20 ±0.15^{cd}
2	3.8±0.30^{f}	5.80 ±0.10^{gh}	6.00 ±0.00^{e}	8.00 ±0.20^{cd}	4.50 ±0.30^{a}
3	4.2 ±0.16^{ab}	2.1 ±0.20^{a}	1.10 ±0.10^{ef}	1.90 ±0.10^{g}	1.20 ±0.12^{b}
4	1.10 ±0.10^{f}	3.0 ±0.15^{e}	1.50 ±0.21^{g}	2.6±0.33^{a}	1.3 ±0.16^{d}
5	2.3±0.20^{d}	4.0 ±0.30^{ef}	2.10 ±0.10^{a}	2.8±0.12^{e}	1.8 ±0.18^{c}
6	2.80 ±0.27^{g}	4.8±0.00^{d}	2.90±0.15^{cd}	3.20 ±0.00^{ab}	2.2 ±0.20^{gh}
7	2.9 ±0.37^{h}	5.2 ±0.20^{ab}	3.45 ±0.00^{de}	4.10 ±0.20^{b}	2.70±0.12^{g}
8	3.00 ±0.10^{a}	4.9±0.20^{bc}	2.7±0.28^{f}	5.30±0.23^{c}	1.72 ±0.30^{h}
9	2.1±0.12^{bc}	3.2±0.00^{ab}	2.0±0.11^{g}	4.20±0.20^{f}	1.52±0.20^{g}
10	1.10±0.14^{f}	1.8 ±0.00^{d}	1.3±0.00^{ef}	2.2 ±0.10^{ab}	1.4 ±0.00^{cd}

Values are means ± Standard error of agricultural wastes. Values in the same column carrying the same superscript are not significantly different at ($p \leq 0.05$) using Duncan's New Multiple Range test.

Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob (Ratio 1:1:1:1) in grams

Comparison of commercial ethanol and bioethanol produced from different substrates

The Comparison of conventional ethanol commercially available and bioethanol produced from different agro wastes substratesis presented in Table 4, all the ethanol produced and commercial ethanol appeared colourless, burns with blue flame and have refractive index of 1.36. Other properties such as relative density, boiling point, melting point, viscosity, and flash point showed little discrepancies.

412 **Table 4: Comparison of commercial ethanol and bioethanol produced from different substrates**

Ethanol Properties	**Bioethaol From cassava peels**	**Bioethaol From Banana peels**	**Bioethaol From Orange peels**	**Bioethaol From Corn cob**	**Bioethaol From OCBC**	**Commercial Ethanol**
Appearance	Colourless	Colourless	colourless	colourless	colourless	Colourless
Relative Density (g/cm^3)	0.756	0.773	0.777	0.782	0.774	0.789
Melting point (^0C)	-112	-114	-113	-112	-113	-114
Boiling point(^0C)	78.40	78.36	78.38	78.37	78.40	78.37
Viscosity	0.0092	0.0122	0.0119	0.0060	0.0114	0.0012 pa s at 20^0C
Burning characteristics	Burns with blue flame	Burns with blue flame	Burns with blue flame	Burns with blue flame	Burns with blue flame	Burns with blue flame
Refractive index	1.36	1.36	1.36	1.36	1.36	1.36
Flash point(^0C)	11	12	12	11	12	13-14

413 Key: **OCBC** = Combinations of Orange peels /Cassava peels /Banana peels /Corn cob (Ratio 1:1:1:1) in grams

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DISCUSSION

The result of the acid pretreatment of the substrates was highly effective after the application of NaOH. The result showed a drastic increase in the cellulose composition of the agro wastes with corn cob having the highest amount of cellulose, and a subsequent decrease in the hemicellulose and lignin content. This is a direct implication of the acid treatment that solubilized the hemi cellulosic fraction and increased the diffusion of sodium hydroxide into the lignocellulosic structure, thus enhancing soda pulping and liberating the cellulose fibers from lignin thereby causing the washing away of hemicellulose and lignin during the filtration hence obtaining a solid residue with high content (Abo- State *et al.,* 2014). The results obtained in this study are in agreement with the findings of Chen *et al.* (2010) who reported similar increase in cellulose and decrease in the hemicellulose and lignin contents of acid pretreated lignocellulosic substrates, and in contrast to that of Abo- State *et al.* (2014) who reported a decrease in all three components, probably due to simultaneous pretreatment and hydrolysis. The high cellulose content and decreased hemicellulose and lignin contents would allow for the enhancement of microbial saccharification (Jeya *et al.,* 2009).

It was observed in this study that the reducing sugar yield of *A. niger* was higher than *B. cereus* yield. This was in agreement with Elsayed (2011) who showed a great difference between the cellulase activity of *Trichodema sp* and *Bacillus sp* using rice straw residues as lignocellulosic substrate.This could be attributed to the ability of *Aspergillus niger* to produce all components of cellulase complex, endoglucanase, exoglucanase, and β- glucosidase in good proportions as well as production of other enzymes such as xylanases or laccases in comparison to other enzyme producers (Arantes and Saddler 2010). Since the main part of the reducing sugar originated from the cellulose fraction, the difference in reducing sugar yield observed for each substrate combination is invariably proportional to the initial cellulose contained by each substrate after pretreatment (Taherzadeh *et al.,* 2007). It could therefore be inferred from the findings that the amount reducing sugar generated by hydrolysis was a function of how effective the pretreatment stage was.

There was significant decrease in the pH of the fermenting media. This may be due to the release of various organic acids from the utilization of the substrates. It was observed that the combinations of all the substrates in ratio 1:1:1:1 (OCBC) showed the lowest pH in all the five fermentation sets after 7 days of fermentation. This could be the result of better nutrient composition which favoured the growth of the microorganisms and hence the production of metabolites. There was increase in total titratable acidity; this could be as a result of utilization of free sugars by yeast and *Bacillus* (Akinyele *et al.,* 2014). The result however showed no direct relationship between the pH and TTA and this can be attributed to the production of other metabolites by the microorganisms (Rajkovic *et al.,* 2007). The observed variation in both pH and TTA values for each substrate combination is a direct result of nutrient variation and hence metabolism of the microorganisms.

The fermentation of the substrates using *Saccharomyces cerevisiae* showed that the yield of ethanol is proportional to fermentation time, where the yield increased with increase in fermentation time, this correlation exist as a result of continuous utilization of the sugar by yeast, and this is in agreement with the findings of Chen *et al.*(2010). It was also revealed that the combination of *A. niger and S. cerevisiae* gave considerably gave higher ethanol yield in all the substrates as well as the substrates combination (OCBC), 100g of corn cob for instance gave an ethanol yield of 17.43g using *A. niger and S. cerevisiae,* and 9.39g using *B. cereus* and *S. cerevisiae* . Cassava peel also recorded high ethanol yield of 15.1g, this was higher than what was reported by Witantri *et al.* (2016) who produced bioethanol by utilizing cassava peels. This may be due to the efficiency of the microorganisms employed during the hydrolysis stage. However, the relatively low yield observed during the fermentation of orange peel may be as a result of antimicrobial activity of the peels that have been reported (Shetty *et al.,* 2016), which slowed down the efficiency of the microorganisms involved in hydrolysis and fermentation respectively, it could also be as a result of lignin which prevented the free access of cellulose by the microorganisms (Subramanian, 2010). The combination of all the substrates gave maximum ethanol yield of 12.44 less than 17.43 reported for corn cobs in this study, this in contrast with the work of Elsayad (2013) who stated that the ethanol yield of each substrate is

directly proportional to its cellulose content. This could be attributed to a number of factors including nutrient variation of the substrates.

Bacteria counts obtained from the fermentation of cassava peels, banana peels, orange peels and corn cobs showed that cassava peel had the highest initial count on nutrient agar, while the combinations of all the substrates in ratio 1:1:1:1(OCBC) had the highest microbial load on nutrient agar after 6 days of fermentation, this was probably due to the fact that the combined substrates may contain varieties of components, thus serving as a better source of nutrients for microbial growth than individual substrate. These findings conform to the work of Lyumugabe *et al.* (2010) and Ibeabuchi *et al.* (2014) that reported significant bacterial counts on nutrient agar for fermented products. The fungal counts of each substrate during fermentation on PDA in this study showed that banana peels had the highest initial count, while orange peel had the lowest, this could be attributed to the fact that, banana peels has been described as a mycological medium (Essien *et al.,* 2008). In addition it has the highest percentage of dietary fibres from this study, while orange peel possibly has antimicrobial property as reported by Shetty *et al.*(2016) which invariably have adverse effect on fungal growth in the fermentation medium.

The comparison between the properties of cassava peels, banana peels, orange peels, corn cob and combinations of all the substrates in ratio 1:1:1:1(OCBC) with those of the conventional ethanol showed that, the flash point of the conventional ethanol ranges between 13^0C and 14^0C, slightly higher than 12^0C noted for the correlation of both banana peels and orange peels, the properties of the alcohols shows that bioethanol derived from plant sources can serve similar purpose as their conventional counterparts.

Conclusion

This study established the efficacy of cassava peels, banana peels, orange peels, and corn cobs for bioethanol production, as well as the efficiency of selected cellulolytic microorganisms in the production process. *Aspergillus niger* was found to be more effective in cellulose hydrolysis than *Bacillus cereus,*

thereby generating higher reducing sugar in each substrate and their respective combinations. Furthermore, it was also observed that among the four substrates utilized, corn cob was found to be the most efficient substrate for bioethanol production.

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