

# Production of Bioethanol by Co-Culture of *Aspergillus niger* and *Saccharomyces cerevisiae* using Watermelon Peels as Substrate

Bashir Ismail Olawale<sup>1</sup>, Mahmud Yerima Iliyasu<sup>1</sup>, Bishir Musa<sup>2,3</sup>, Ahmad Abdulrahman<sup>1</sup>, Ahmed Faruk Umar<sup>1</sup>

<sup>1</sup> *Abubakar Tafawa Balewa University*  
PMB 0248, Bauchi, 740272, Nigeria

<sup>2</sup> *Ahmadu Bello University*  
Zaria, 810211, Nigeria

<sup>3</sup> *University of Hohenheim*  
1 Schloss Hohenheim, 70599, Stuttgart, Germany

DOI: [10.22178/pos.75-10](https://doi.org/10.22178/pos.75-10)

LCC Subject Category:  
[QH540-549.5](#)

Received 21.09.2021  
Accepted 25.10.2021  
Published online 31.10.2021

Corresponding Author:  
[microbashes@yahoo.com](mailto:microbashes@yahoo.com)

© 2021 The Authors. This article is licensed under a [Creative Commons Attribution 4.0 License](#) 

**Abstract.** Bioethanol production from lignocellulosic biomass as an alternative fuel source has received considerable attention from researchers worldwide. Bioethanol was produced from watermelon peels by fermentation in the current work, using *Aspergillus niger* and *Saccharomyces cerevisiae* co-cultures isolated from soil and local beverage (*kunun zaki*), respectively. The isolates were characterised both macroscopically and microscopically. While starch hydrolysis test was carried out for the mould isolates, thermotolerance, glucose and ethanol tolerance tests were used to identify the yeast isolates. According to standard procedures described by the Association of Official Analytical Chemists (AOAC), Proximate analyses were carried out on the watermelon peels. The ethanol yield was determined using the specific gravity method. Isolate BGD of the moulds had the highest (40 mm) zone of clearance on starch agar. The identified yeasts showed the best growth at a maximum of 35 °C while tolerating up to 50% wt/v and 18% v/v of glucose and ethanol, respectively. The yeasts also fermented all the different sugars tested but lactose. The proximate composition of the substrate revealed low protein (3.5%) and ash contents (7.38%) but a remarkably high carbohydrate content of 79.22%. At optimal fermentation conditions (30 °C, pH 6.0, 4% g/L substrate, 200rpm and five (5) days fermentation period), the maximum ethanol yield produced by the co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* was 57 g/L. However, only 13.5 g/L and eight g/L were produced by individual cultures of *A. niger* and *S. cerevisiae*, respectively. The bioethanol produced has a specific gravity of 0.994, miscible in water with a pleasant smell. This colourless liquid boils at a temperature of 78 °C with a pH of 3.02 and ignites at room temperature. It was concluded that watermelon peels are a suitable substrate for bioethanol production and was used successfully to produce an ethanol yield of 57 g/L by co-culture of *A. niger* and *S. cerevisiae*.

**Keywords:** watermelon peels; lignocellulosic biomass; co-culture; *Aspergillus niger*; *Saccharomyces cerevisiae*; bioethanol.

## INTRODUCTION

Global concern about climate change and the consequent need to diminish greenhouse gas emissions have encouraged bioethanol as a gasoline replacement or additive. Prelude to the prediction of the world energy consumption to increase by 54 % between 2001 and 2025 as a consequence of the increase in world population at

an alarming rate that is associated with such global concerns as global warming, depletion of fossil fuel reserves and augmentations in prices of petroleum products have obliged the search for alternative energy sources with lesser greenhouse gas emissions, cost-effectiveness and sustainable, carbon-neutral energy sources to meet future needs [1]. The excessive consumption of non-renewable energy has dramatically resulted

in environmental deterioration and public health problems [2]. This, in turn, has resulted in the need to find a source of renewable energy. Bioethanol produced by fermentation of plant biomass is an environmentally friendly alternative to fossil fuels and can suitably replace gasoline as a transportation fuel [3]. The cost of the raw materials significantly influences the economics of bioethanol production and reduce this cost. Cheap materials are sourced as feedstock for ethanol production [4].

Watermelon (*Citrullus lanatus*) is cultivated throughout the globe in 96 countries. There are around 1200 varieties of watermelon found in different parts of the world. The total global production of watermelon was 108.9 million tons, whereas India's production was 0.4 million tons in 2013 [5]. The leading growers were China, Turkey, Iran, Brazil and the United. Watermelon is cultivated in hot and dry climates with a mean temperature of 22–30 °C and little available water. The shape of the fruit varies from spherical to oblong. It is harvested in the summer months and liked by the consumer due to its delicate flavour and attractive colour [6]. A watermelon is nominally 60 % flesh, and about 90 % of the flesh is the juice that contains 7 to 10 % (w/v) sugars. Thus, over 50 % of a watermelon is readily fermentable liquid [7]. Fermentation is a metabolic process of converting carbohydrates to alcohol or organic acids using microorganisms, usually yeasts or bacteria, under anaerobic conditions. However, a similar process occurs in the leavening of bread and the preservation of sour foods such as sauerkraut and yoghurt with lactic acid production [8].

Nowadays, bioethanol is the most common biofuel worldwide (accounting for more than 90 % of total biofuel usage), with more than 50 billion litres (fuel and non-fuel) produced in 2006. Ethanol produced by fermentation of plant biomass is an environmentally friendly alternative to fossil fuels [9, 10]. Ethanol produced by fermentation of plant biomass is an environmentally friendly alternative to fossil fuels [9, 10]. The search for renewable biomass sources that do not serve as human food and animal fodder have focused primarily on plant biomass that possesses mainly cellulose and lignocellulosic materials. Co-products (e.g., animal feed) help reduce production costs [11]. Lignocellulosic cellulose is abundant in nature. Cellulosic biomass of forestry, agriculture and municipal origin serves best as a feedstock to produce biofuels and other products [12]. However, such characteristics of

this lignocellulosic biomass include its crystallinity, lignin and hemicellulose, inaccessible surface area, degree of cellulose polymerisation, and degree of acetylation hemicelluloses roots its resistance to enzymatic degradation. The pretreatment step is thus of paramount importance to economically convert the lignocellulosic cellulose into fermentable sugars [13]. Research studies are now being directed in two areas: the production of ethanol from cheaper raw materials and the study of new microorganisms or yeast strains efficient in ethanol production [14]. Inexpensive raw materials such as agricultural wastes, cellulosic wastes, fruit wastes, vegetable wastes, municipal and industrial wastes can be used to produce ethanol cheaply. Increased ethanol production yield by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology [15].

Microorganisms used for bioethanol production must have the ability to withstand the high level of acidity, temperature, high concentration of ethanol in fermentation, and they must be able to utilise a high concentration of sugar and ferment varieties of sugars [16]. Commercial amylases are enzymes produced by microorganisms; such enzymes hydrolyse starch molecules to glucose units. Amylases have potential application in many industrial processes such as fermentation and some pharmaceuticals industries [17]. Fruits are edible products of plants, usually soft and fleshy. However, their high moisture content made them perishable in the freshly harvested state due to spillage, physiological decay, water loss, mechanical damage during harvesting and packaging [18]. Agricultural waste generated during the processing of agricultural products is usually utilised as low-cost raw materials to produce other valuable products such as citric acid, bioethanol, and gluconic acid with the expectancy of reducing the cost of production [19]. Therefore, this research focused on producing bioethanol by co-culture *Aspergillus niger* and *Saccharomyces cerevisiae* using watermelon peels as substrate.

## METHODS

### Collection of Samples, Isolation and Characterisation of *Aspergillus niger* and *Saccharomyces cerevisiae*

While the local beverage (*kunun zaki*) used in this research for the isolation of *Saccharomyces*

*cerevisiae* was obtained from Yelwa market in Bauchi, Bauchi State, Nigeria, the soil sample used for the isolation of *Aspergillus niger* was obtained from the botanical garden and flower beds of Abubakar Tafawa Balewa University, Bauchi, Bauchi State, Nigeria.

*Aspergillus niger* was isolated from the humus garden soil. One gram of each soil sample was suspended separately into labelled test tubes containing 9 ml of distilled water and streptomycin (0.005 g) to suppress bacterial growth. The suspension was diluted serially, an aliquot of 0.1 ml from 10<sup>-5</sup> dilution was inoculated on Sabouraud Dextrose Agar (SDA) plate and then incubated at room temperature (28 °C) for five days. The mould isolates were further sub-cultured on sterile SDA plates and incubated at room temperature for another five days to obtain pure isolates [20]. The isolates were characterised by morphological [21] and microscopic (Tease-mount) examinations [22], as well as the

potential to produce amylase for starch hydrolysis [23].

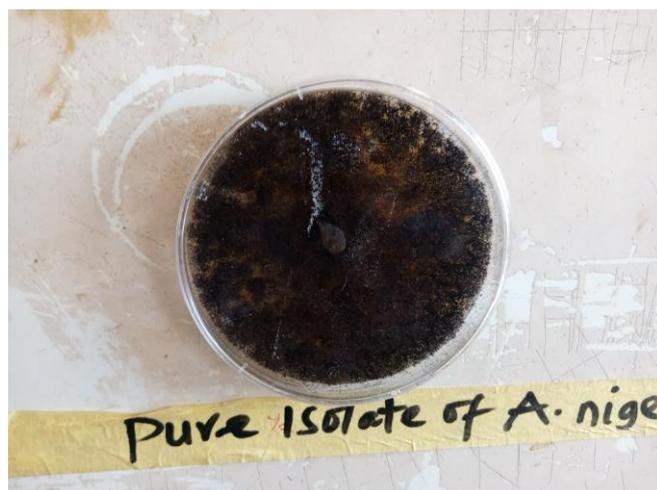


Plate 1 – Pure isolate of *Aspergillus niger* from soil garden

Table 1 – Isolation and Characterisation of *Aspergillus niger*

| Macroscopic Characteristics |                        |        |                       |            |          | Microscopic Characteristics |              |                 |
|-----------------------------|------------------------|--------|-----------------------|------------|----------|-----------------------------|--------------|-----------------|
| Isolates                    | Surface Colour         | Margin | Reverse Side          | Elevations | Growth   | Hyphae                      | Conidiophore | Conidia         |
| BG-A                        | Dark Brown to Black    | Entire | Without Colour        | Umbonate   | Rapid    | Branched Septate            | Globose      | Blackish Brown  |
| BG-B                        | Yellow / Greyish Green | Entire | Colourless to Yellow  | Umbonate   | Moderate | Branched Septate            | Globose      | Yellowish Green |
| BG-C                        | Green to Dark Green    | Entire | Colourless to Yellow  | Umbonate   | Rapid    | Branched Septate            | Dome Shaped  | Blue Green      |
| BG-D                        | Pinkish                | Entire | Pale to Bright Yellow | Umbonate   | Moderate | Branched Septate            | Globose      | Creemish        |

Notes: Botanical Garden Soil

On the other hand, *Saccharomyces cerevisiae* was isolated from a locally fermented beverage (*kunun zaki*). Also aliquot of 0.1 ml from the 10<sup>-5</sup> dilution of the beverage was spread-inoculated on sterile Malt Yeast Peptone Glucose (MYPG) agar plates and incubated at 30 °C for 48 h. Colonies suspected to be *Saccharomyces* spp. were sub-cultured on MYPG slants. A smear of the isolate was examined microscopically (100 X) after staining with lactophenol cotton blue [24]. Stress tolerance test which includes thermotolerance (25, 30, 35, 40 and 45 °C), ethanol tolerance (3, 6, 9, 12, 15 and 18% v/v) [25], as well as sugar utilization (10, 20, 30, 40 and 50% wt/v glucose) [25] and fermentation tests were carried out on the yeast isolates [26].

### Preparation of the Substrate (Watermelon Peels) and Determination of Proximate Composition

The watermelon peels obtained were washed with distilled water to remove sand and dirt before being chopped into smaller pieces of approximately 3 cm. The peels were then sun-dried for 14 days before grounding to fine powder [27], which increases the surface area and, ultimately, the speed of the metabolic reaction.

Proximate analysis of the prepared dried watermelon powder was carried out at the Abubakar Tafari Ali Polytechnic (ATAP), Department of Chemistry, Bauchi, Bauchi State. As a result, carbohydrates, moisture, ash, crude protein, crude

fat and crude fibre contents were determined by the standard procedures described by the Association of Official Analytical Chemists [28].

### **Fermentation of Watermelon Peels for Bioethanol Production by co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae***

*Preparation of the Fermentation Medium.* The fermentation medium that was used for ethanol production in the research contained (per 100 ml); Soluble Starch 1g, Glucose 1 g, Peptone 0.1 g, Malt Extract 0.1 g, Yeast Extract 0.2 g, Magnesium Chloride 0.1 g, Calcium Carbonate 0.2 g, Ammonium Phosphate 0.2 g and Ferrous Sulphate 0.001 g. One normal HCl and one standard NaOH were used to adjust the pH of the medium to 6.0 [23].

*Preparation of Inocula.* *Aspergillus niger* and *Saccharomyces cerevisiae* inocula were prepared using slant cultures to inoculate 50 ml of sterile growth medium containing 200 ml Erlenmeyer flasks. The flasks were incubated (30 °C 200 rpm) for 48 h for *Saccharomyces cerevisiae* and 72 h for *Aspergillus niger* [29]. Inoculum of *Aspergillus niger* was prepared from fully sporulated (5 days old) culture grown on SDA slant. The colonies were flooded with 10 ml of sterile distilled water containing 2.5% Tween 80 (polyoxyethylene sorbitan monooleate), added to facilitate the uniform distribution of *Aspergillus niger* spores. The spores were dislodged by vigorous shaking for 15 minutes. One millilitre of the stock suspension was diluted into 99ml sterile distilled water, 0.5ml of the diluted culture suspension was dropped on a haemocytometer and counted under the microscope using 10X and 40X objectives to know the number of spores / ml inoculated into the fermentation medium. Ten millilitres of the inoculum were transferred into the fermentation medium [29].

On the other hand, the yeast isolate was grown in yeast peptone dextrose (YPD) broth (1% Yeast extract, 2 % Peptone and 2% dextrose) containing 0.05 mg/ml of chloramphenicol. Ten millilitres of the YPD medium were inoculated with a single selected colony of the yeast isolate, using a wire loop, and then incubated overnight at 30 °C with an agitation rate of 200 rpm. After incubation, 1 ml of the yeast culture was transferred into 200 ml of the YPD medium. The yeast cells were allowed to grow at 30 °C for 24 h on a shaker-incubator. The cells were then harvested by

centrifugation at 3000 rpm for 5 minutes. The pellets formed in the centrifuge were re-suspended in 30 ml sterile distilled water and washed three times, after which it was prepared according to McFarland standard one. One millilitre of the yeast suspension was inoculated into the growth medium; ten millilitres of the inoculum was transferred into the fermentation medium [29].

*Experimental Set-Up, Fermentation and Analytics of the Bioethanol.* During the experiment, a batch of 200 ml medium in 500 ml Erlenmeyer flask with 4% wt/v of the previously prepared watermelon peels was used. The fermentation medium was sterilised by autoclaving (121 °C for 15 minutes) and allowed to cool before inoculating with  $2.5 \times 10^6$  spores/ml of *Aspergillus niger* and  $3 \times 10^8$  cells/ml of *Saccharomyces cerevisiae*. The flasks were incubated at 30 °C, 200 rpm on a rotary shaker incubator for five days as described by [23]. After 24 h interval during the fermentation, a 30 ml aliquot from the flask was taken after thorough shaking of the flask to homogenise the contents. This was used for the analysis of ethanol produced [23]. This fermentation product was transferred into a round bottom flask and placed on a heating mantle fixed to a distillation column enclosed in running tap water. Another flask was fixed to the other end of the distillation column to collect the distillate (ethanol) at 78 °C [30]. To determine the specific gravity of the bioethanol produced, a clean, dry 50 ml specific gravity bottle was weighed with its stopper before it was filled with an equal volume of distilled water (20 °C) as the distillate to be measured. The water was then emptied, and the bottle dried before reweighing it. It was then filled with the distillate at 20 °C and reweighed, and the specific gravity was calculated as:

$$\text{Specific gravity} = \text{Density of distillate} / \text{Density of equal volume of water}$$

The specific gravity values obtained were used to determine ethanol concentration from a standard curve prepared using known concentrations of ethanol (100 %) as reported by [31].

*Preparation of ethanol standard curve.* Six stocks of varying concentrations of ethanol (2, 4, 6, 8, 10 and 12% wt/v) were used. Aliquots of 5 ml of each of these concentrations were taken into the specific gravity bottle and weighed against the equal weight of distilled water. The values ob-

tained were used to plot a standard ethanol curve against the six known concentrations previously determined [31].

## RESULTS AND DISCUSSION

The growth of *Aspergillus niger* on PDA Plate produced initially white colonies that quickly became black with conidial production. The reverse is a pale yellow that produces radial fissures in the agar. The macroscopic observation of the yeast isolate showed elevated colonies that were milky in colour, entire shape with smooth surface and had alcoholic odour typical of *Saccharomyces cerevisiae* (plates 2).



Plate 2 – Colonies of *Saccharomyces cerevisiae* on Malt Yeast Peptone Glucose agar plate after 48 hr

When stained with lactophenol cotton blue, the yeast isolates were transparent and spherical with a multilateral type of budding under the microscope using a 40X objective. The *Aspergillus niger* that was used was isolated from the school botanical garden. This shows their natural diversity is found everywhere where the environmental condition is favourable, especially in plant soil and debris [32], which suggests that the soil is a natural habitat for amylase-producing organisms, especially *Aspergillus niger*. The macroscopic and microscopic identification of the fungi was based on the colour of their colony and the structure of the conidial head as classified by [21], who reported that colonies of *Aspergillus niger* are carbon black with a dark globular conidial head. *Aspergillus niger* strain isolated from soil samples was found to have good amylase pro-

duction potential, which is essential for breaking down starch. This is in agreement with the work [33]. They reported that *Aspergillus niger* could be used for the industrial production of ethanol and some other acid because of its hydrolytic capacities in amylase production and its high tolerance to acidity, thereby preventing bacterial contamination.

Table 2 – Amylase Producing Potential of the *Aspergillus niger* on Starch Agar Plates

| Isolates | Zone of Clearance (mm) |
|----------|------------------------|
| BG-A     | 37.02                  |
| BG-B     | 26.10                  |
| BG-C     | 25.06                  |
| BG-D     | 40.01                  |

Notes: Values and mean  $\pm$  Standard deviations of three replications; soil from beneath the flower beds of botanical garden soil of ATBU Bauchi



Plate 3 – Amylase production of the *Aspergillus niger* on starch agar

The result obtained after flooding *Aspergillus niger* colonies on starch agar plates with iodine solution showed a zone of clearance on the plate, indicating starch hydrolysis potential of the isolate. However, from the experiment, only one out of the four isolates BGD showed the highest clearance zone (40 mm).

In this study, the yeast isolates used in the research were isolated from local beverages *kunun zaki* bought from Yelwa Market Bauchi State. The yeasts were fermentative in the breakdown of hexose and pentose sugars to produce ethanol and carbon dioxide. Hitherto, several workers such as [35] have reported some yeast strains'

activities in bioethanol production. The microscopic and macroscopic identification was found to belong to the *Saccharomyces* type of unicellular ascomycetes, according by [36].

The isolate was able to withstand various stress conditions of temperature, ethanol concentration, high glucose concentration; it was also able to utilise various sugars of glucose, maltose, fructose, sucrose and galactose except for lactose.

This agrees with the work of [37], who reported that *Saccharomyces cerevisiae* is a yeast capable of withstanding stressful conditions and has high fermentation efficiency, effective sugar used, tolerance to high ethanol concentration, osmotolerance and thermotolerance, which are fundamental for industrial used.

Table 3 – Cultural, Microscopic and Sugar Fermentation Characteristics of the Isolates

| Isolates | Cultural Characteristics |                    |                         |           |        | Sugar Fermentation |     |     |     |     |     | Inference            |
|----------|--------------------------|--------------------|-------------------------|-----------|--------|--------------------|-----|-----|-----|-----|-----|----------------------|
|          | Colony Colour            | Colony Nature      | Appearance              | Elevation | Margin | Glu                | Mal | Lac | Suc | Gal | Fru |                      |
| K-1      | Whitish colour           | Smooth and shining | Ovoid                   | Concave   | Entire | +                  | -   | -   | +   | +   | +   | <i>S. cerevisiae</i> |
| K-2      | Whitish cream colour     | Smooth             | Yeast like              | Raised    | Wavy   | +                  | -   | -   | +   | -   | +   | <i>S. cerevisiae</i> |
| K-3      | Cream colour             | Smooth             | Yeast like              | Convex    | Entire | +                  | +   | -   | +   | +   | +   | <i>S. cerevisiae</i> |
| K-4      | Cream colour             | Smooth             | Glabrous and Yeast like | Convex    | Entire | +                  | +   | -   | +   | -   | +   | <i>S. cerevisiae</i> |

Notes: Keys: + = Presence of acid and gas; - = Absence of acid and gas; Glu=Glucose; Mal= Maltose; Lac= Lactose; Suc= Sucrose; Gal= Galactose; Fru= Fructose

The yeast isolate was unable to ferment lactose. This agrees with the literature reports by [38] that species of *Saccharomyces cerevisiae* cannot ferment lactose as they lack the enzyme lactase but were found to ferment different types of sugars such as glucose, fructose, maltose, sucrose, and galactose with the evolution of gas in the Durham tubes except lactose. The results obtained in this study shows that as the glucose concentration increases, the optical density of the organisms decrease. The highest optical density of about 2.034 was obtained at a glucose concentration of 1g, while the least of 0.468 optical density was obtained at 5 g of glucose. As the glucose concentration increases, the optical density decreases respectively, as shown in Table 4.

Table 4 – Growth of *Saccharomyces cerevisiae* in 10–50 % w/v glucose after 48 hrs incubation

| Glucose Concentration (% w/v) | Optical Density of Cells (600 nm) |
|-------------------------------|-----------------------------------|
| 10                            | 2.034+0.002                       |
| 20                            | 1.907+0.019                       |
| 30                            | 1.025+0.017                       |
| 40                            | 0.709+0.025                       |
| 50                            | 0.468+0.001                       |

Notes: Values and mean ± Standard deviations of three replications

This result agreed with [39], who reported that yeast growth is inhibited by high glucose concentration. The yeast isolate grew in a medium containing 3, 6, 9, 12, 15, and 18% v/v of ethanol. The optical density was found to decrease with increased ethanol concentration, as shown in Table 5.

Table 5 – Ethanol tolerance test of the yeast isolated produce

| Ethanol Concentration (%v/v) | Optical Density of Cells (600 nm) |
|------------------------------|-----------------------------------|
| 3                            | 1.54+0.008                        |
| 6                            | 1.42+0.012                        |
| 9                            | 1.28+0.016                        |
| 12                           | 0.98+0.001                        |
| 15                           | 0.67+0.003                        |
| 18                           | 0.21+0.002                        |

Notes: Values and mean ± Standard deviations of three replications

The effect of temperature on the growth of the yeast isolate was carried out, and the yeast have shown good growth of up to 35 °C, weak growth

at 40 °C, and there was no growth at 45 °C, as shown in Table 6.

Table 6 – Relative thermotolerance of the yeast isolate observed after 48 hrs

| Yeast (Temperature) | Level of Growth |
|---------------------|-----------------|
| 25°C                | ++              |
| 30°C                | ++              |
| 35°C                | ++              |
| 40°C                | +               |
| 45°C                | -               |

Notes: ++ = Good growth ≥ 180 colonies; + = minimal growth ≤ 100 colonies; - = no growth

As the temperature rises above 35 °C, the growth of the yeast strains is affected, while complete inhibition of growth was observed at 45 °C. This agreed with the study of [40], who reported good growth of yeast up to 37 C, and at higher temperatures, the growth was inhibited. Similar results were obtained in this study. The inability of the yeast isolates to grow at 45 °C in this study agrees with the mesophilic character of yeast, as reported by [41].

The proximate composition of the watermelon peels used in this research was found to have a high carbohydrate content of 79.22%, with a significant amount of ash content, fibre content, protein, fat, and moisture content, as shown in Table 7.

Table 7 – Proximate compositions of the watermelon peels

| Parameters    | Composition (%) |
|---------------|-----------------|
| Moisture      | 2.12            |
| Ash           | 7.38            |
| Fiber (crude) | 6.50            |
| Protein       | 3.50            |
| Crude lipid   | 1.28            |
| Carbohydrate  | 79.22           |

The watermelon peels proximate analysis shows a high concentration of readily fermentable carbohydrates. This agrees with the work of [7] that reported a high concentration of sugar in watermelon peels. The carbohydrate and protein content of the watermelon peel from this research is an indication that the waste peels can be used as an alternative substrate for the cultivation of fungi and hence to produce bioethanol since the raw materials for bioethanol production are sugary, starchy and fibrous materials [42].

From this research, the ethanol yield increased with an increase in substrate concentration which was statistically significant ( $P < 0.05$ ). Still, it was highest at 8% (4.20–11.40%) for the Co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae*, as shown in Figure 1.

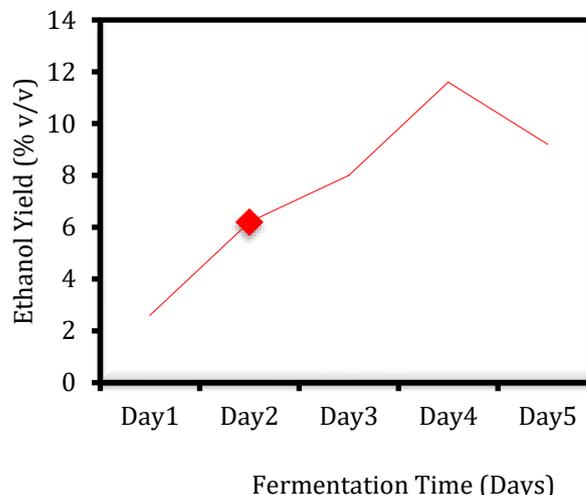


Figure 1 – Ethanol production by co-culture of *Aspergillus Niger* and *Saccharomyces cerevisiae* for five days at pH 6.0, 30°C and 8 g of the substrate

The increase in ethanol yield maybe because the yeast tends to starve at low substrate concentrations, and productivity decreases. Although, when the individual organisms were subjected to the same fermentation treatment, *Aspergillus niger* produced an ethanol yield of a maximum of 2.70%, as shown in Figure 2.

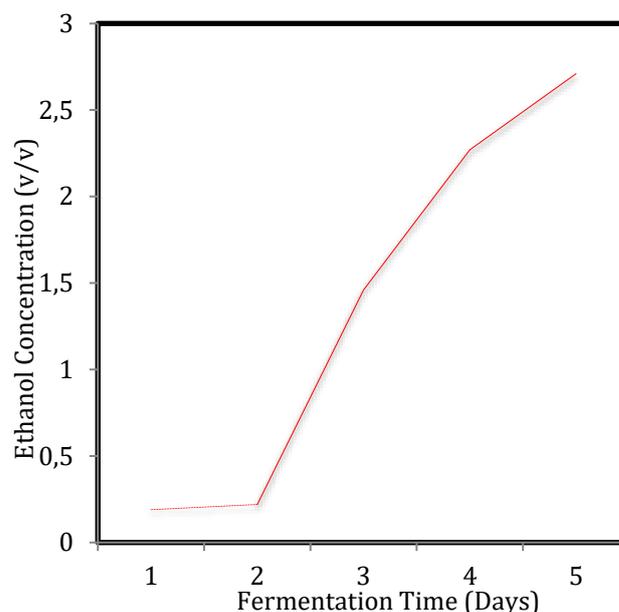


Figure 2 – Ethanol Produced by *Aspergillus niger* for Five Days at pH 6.0, 30 °C, and 8 g Substrates

In comparison, *Saccharomyces cerevisiae* produced an ethanol yield of 1.60%, as shown in Figure 3.

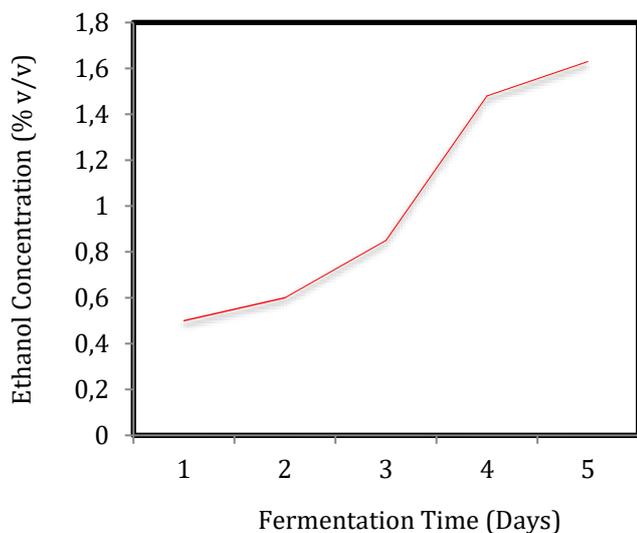


Figure 3 – Ethanol Produced by *Saccharomyces cerevisiae* for Five Days at pH 6.0, 30°C, and 8 g Substrates

Also, an increase in ethanol yield could be due to the presence of substrates that can readily be hydrolysed to sugar by the amylolytic activity of *Aspergillus niger* and subsequent sugar conversion to ethanol by the yeast cells in the medium [43]. This work agrees with [23, 44], who carried a similar work using cassava starch. However, there was a decrease in ethanol yield at 10% substrate concentration (8.37%), which was statistically significant (at  $P < 0.05$ ). At varying substrate concentrations from 2 to 10%, the ethanol yield at 30 °C, pH 6.0 and 200 rpm for five days. There was a gradual increase in ethanol yield as the substrate concentration increased, but the ethanol yield decreased at 10% substrate concentration. The maximum ethanol yield was obtained at 8% substrate concentration on the fourth day of fermentation (7.50 %v/v).

In comparison, 2% substrate concentration gave a minuscule ethanol yield with its maximum yield obtained on the fourth day (2.2 %v/v). The decrease in ethanol yields may be because high substrate concentration causes catabolite repression of the oxidative pathway, which causes metabolite repression of the yeast, leading to a decrease in ethanol yield [45]. The decrease in ethanol yield due to high substrate concentration may also be because of increase in the substrate

concentration resulted in more sugar available for hydrolysis. Since the number of cells was not proportionally added, this probably means that the sugar quantities will become excess for the cells digestive capacity, which is left inactive, thereby resulting in a low ethanol yield [45]. This work does not agree with [27, 46]. They reported that ethanol yield increased with an increase in substrate concentration, where the optimum concentration for ethanol yield was recorded to be 10 % and 12 %, respectively.

Bioethanol from lignocelluloses agro-waste is another way of developing clean and environmentally friendly energy. Its carbon dioxide emission is equal to its absorptions during crop production. The industry is endowed with incredible opportunities and would assist in rural development in Nigeria. There will be sufficient crop supply for human and industrial use. It would also create jobs, wealth, develop rural infrastructure, expand the rural market, and reduce the scourge of poverty. However, challenges may face the development of the industry in Nigeria. These problems can be overcome through careful planning and implementation strategies.

From the results obtained, the ethanol produced has a specific gravity of 0.994. It is miscible in water, has a pleasant smell, is a colourless liquid that boils at a temperature of 78 °C with a PH of 3.02 and ignites at room temperature, as shown in Table 8.

Table 8 – Physicochemical properties of the bioethanol produced

| Parameters          | Content                    |
|---------------------|----------------------------|
| Specific gravity    | 0.994                      |
| Miscibility         | Miscible                   |
| Boiling temperature | 780C                       |
| Odour               | Pleasant smell             |
| Colour              | Colourless                 |
| PH                  | 3.02                       |
| Density             | 0.969 g/cm <sup>3</sup>    |
| Flash point         | Ignite at room temperature |

## CONCLUSIONS

This research work has yielded results in bioethanol production using watermelon peels which is a waste from municipal as a fermentation substrate and several organisms such as the yeasts and fungi. This indicates that these organisms exhibited good fermentation attributes and were

successfully used for bioethanol production using the fermentation process. The utilisation of lignocellulosic biomass for bioethanol production necessitates the production technology to be cost-effective and environmentally sustainable. The available statistics show that the need for bioethanol for the transport sector could be met by using watermelon peels.

### Acknowledgement

Our profound gratitude goes to the supervisors and co-authors for dedicating so much time and energy to guiding, instructing, and colleagues that contributed in one way or the other toward the successful completion of the work.

### Conflict of interests

The authors declare no conflicting interest.

### REFERENCES

- Balat, M., Balat, H., & Öz, C. (2008). Progress in bioethanol processing. *Progress in Energy and Combustion Science*, 34(5), 551–573. doi: [10.1016/j.pecs.2007.11.001](https://doi.org/10.1016/j.pecs.2007.11.001)
- Pegkas, P. (2019). The impact of renewable and non-renewable energy consumption on economic growth: the case of Greece. *International Journal of Sustainable Energy*, 39(4), 380–395. doi: [10.1080/14786451.2019.1700261](https://doi.org/10.1080/14786451.2019.1700261)
- Itelima, J., Onwuliri, F., Onwuliri, E., Onyimba, I., & Oforji, S. (2013). Bio-Ethanol Production from Banana, Plantain and Pineapple Peels by Simultaneous Saccharification and Fermentation Process. *International Journal of Environmental Science and Development*, 213–216. doi: [10.7763/ijesd.2013.v4.337](https://doi.org/10.7763/ijesd.2013.v4.337)
- Franco-Duarte, R., Umek, L., Mendes, I., Castro, C. C., Fonseca, N., Martins, R., ... Schuller, D. (2016). New integrative computational approaches unveil the *Saccharomyces cerevisiae* phenometabolomic fermentative profile and allow strain selection for winemaking. *Food Chemistry*, 211, 509–520. doi: [10.1016/j.foodchem.2016.05.080](https://doi.org/10.1016/j.foodchem.2016.05.080)
- U. N. Food and Agriculture Organization. (2016). *FAO Production Yearbook*. Rome.
- Tressler, D., Joslyn, M. (1961). *Fruit and vegetable juice processing technology* (2nd ed.). Westport: AVI Publishing Co.
- Kim, S., Kim, W., Lee, S., & Byun, S. (1984). Alcohol fermentation of Korean watermelon juice. *Journal of the Korean Society of Agricultural Chemistry and Biotechnology*, 27, 139–145.
- McGovern, P. E., Zhang, J., Tang, J., Zhang, Z., Hall, G. R., Moreau, R. A., ... Wang, C. (2004). Fermented beverages of pre- and proto-historic China. *Proceedings of the National Academy of Sciences*, 101(51), 17593–17598. doi: [10.1073/pnas.0407921102](https://doi.org/10.1073/pnas.0407921102)
- Mabee, W. E. (n.d.). Policy Options to Support Biofuel Production. *Advances in Biochemical Engineering/Biotechnology*, 329–357. doi: [10.1007/10\\_2007\\_059](https://doi.org/10.1007/10_2007_059)
- Farrell, A. E., Plevin, R. J., Turner, B. T., Jones, A. D., O'Hare, M., & Kammen, D. M. (2006). Ethanol Can Contribute to Energy and Environmental Goals. *Science*, 311(5760), 506–508. doi: [10.1126/science.1121416](https://doi.org/10.1126/science.1121416)
- Hahn-Hägerdal, B., Karhumaa, K., Fonseca, C., Spencer-Martins, I., & Gorwa-Grauslund, M. F. (2007). Towards industrial pentose-fermenting yeast strains. *Applied Microbiology and Biotechnology*, 74(5), 937–953. doi: [10.1007/s00253-006-0827-2](https://doi.org/10.1007/s00253-006-0827-2)
- Carere, C., Sparling, R., Cicek, N., & Levin, D. (2008). Third Generation Biofuels via Direct Cellulose Fermentation. *International Journal of Molecular Sciences*, 9(7), 1342–1360. doi: [10.3390/ijms9071342](https://doi.org/10.3390/ijms9071342)
- Wyman, C. (1996). *Handbook on bioethanol: production and utilisation*. Washington: Taylor & Francis.

14. Akin-Osana, B. C., Nzelibe, H. C., & Agbaji, A. S. (2008). Ethanol Production from Carica papaya (Pawpaw) Fruit Waste. *Asian Journal of Biochemistry*, 3(3), 188–193. doi: [10.3923/ajb.2008.188.193](https://doi.org/10.3923/ajb.2008.188.193)
15. Joshi, S., Dhopeswarkar, R., Jadav, U., Jadav, R., D'Souza, L., & Dixit, J. (2001). Continuous ethanol production by fermentation of waste banana peels using flocculating yeast. *Indian Journal of Chemical Technology*, 40, 153–156.
16. Scully, S., & Orlygsson, J. (2014). Recent Advances in Second Generation Ethanol Production by Thermophilic Bacteria. *Energies*, 8(1), 1–30. doi: [10.3390/en8010001](https://doi.org/10.3390/en8010001)
17. Souza, P. M. de, & Magalhães, P. de O. e. (2010). Application of microbial  $\alpha$ -amylase in industry - A review. *Brazilian Journal of Microbiology*, 41(4), 850–861. doi: [10.1590/s1517-83822010000400004](https://doi.org/10.1590/s1517-83822010000400004)
18. Subhadra, B., & Grinson-George. (2010). Algal biorefinery-based industry: an approach to address fuel and food insecurity for a carbon-smart world. *Journal of the Science of Food and Agriculture*, 91(1), 2–13. doi: [10.1002/jsfa.4207](https://doi.org/10.1002/jsfa.4207)
19. Bhatia, L., Johri, S., & Ahmad, R. (2012). An economic and ecological perspective of ethanol production from renewable agro waste: a review. *AMB Express*, 2(1), 65. doi: [10.1186/2191-0855-2-65](https://doi.org/10.1186/2191-0855-2-65)
20. Knudsen, S., Saadbye, P., Hansen, L. H., Collier, A., Jacobsen, B. L., Schlundt, J., & Karlström, O. H. (1995). Development and testing of improved suicide functions for biological containment of bacteria. *Applied and Environmental Microbiology*, 61(3), 985–991. doi: [10.1128/aem.61.3.985-991.1995](https://doi.org/10.1128/aem.61.3.985-991.1995)
21. Verweij, P.E., Brandt, M.E., Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L., & Pfaller, M.A. (2006). *Aspergillus, Fusarium, and other opportunistic moniliaceous fungi*. Retrieved from [https://www.researchgate.net/publication/285864066\\_Aspergillus\\_Fusarium\\_and\\_other\\_opportunistic\\_moniliaceous\\_fungi](https://www.researchgate.net/publication/285864066_Aspergillus_Fusarium_and_other_opportunistic_moniliaceous_fungi)
22. Barnett, H. L., & Hunter, B. B. (2010). *Illustrated genera of imperfect fungi*. St. Paul: APS Press.
23. Ado, S., Olukotun, G., Ameh, J., & Yabaya, A. (2010). Bioconversion of cassava starch to ethanol in a simultaneous saccharification and fermentation process by co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae*. *Science World Journal*, 4(1). doi: [10.4314/swj.v4i1.51832](https://doi.org/10.4314/swj.v4i1.51832)
24. Guimarães, T. M., Moriel, D. G., Machado, I. P., Picheth, C. M. T. F., & Bonfim, T. M. B. (2006). Isolation and characterization of *Saccharomyces cerevisiae* strains of winery interest. *Revista Brasileira de Ciências Farmacêuticas*, 42(1), 119–126. doi: [10.1590/s1516-93322006000100013](https://doi.org/10.1590/s1516-93322006000100013)
25. Breisha, G. Z. (2010). Production of 16% ethanol from 35% sucrose. *Biomass and Bioenergy*, 34(8), 1243–1249. doi: [10.1016/j.biombioe.2010.03.017](https://doi.org/10.1016/j.biombioe.2010.03.017)
26. Ameh, J., Okagbue, R., Ahmad, A. (1990). Isolation and characterisation of local yeast strains for ethanol production. *Nigerian Journal of Technology Research*, 1, 47–52.
27. Ajay, K., Sanat, R., Yashab, K., Harison, M., Jyotsna, K., Jane, C., Pradeep, K., & Pankaj, S. (2014). Bio-Ethanol Production from Banana peel by Simultaneous Saccharification and Fermentation Process using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae*. *International Journal of Current Microbiology and Applied Sciences*, 3(5), 84–96.
28. Association of Official Analytical Chemists. (2010). *Official Methods of Analysis*. Arlington: The Association.
29. Byaruagaba-Bazirake, G., & Rensburg, P. (2013). Characterisation of banana wine fermented with recombinant wine yeast strains. *American Journal of Food and Nutrition*, 3(3), 105–116. doi: [10.5251/ajfn.2013.3.3.105.116](https://doi.org/10.5251/ajfn.2013.3.3.105.116)
30. Oyeleke, S., Dauda, B., Oyewole, O., Okoliegbe, I., & Ojebode, T. (2012). Production of Bioethanol from Cassava and Sweet Potato Peels. *Advances in Environmental Biology*, 6(1), 241–245.

31. Whelan, P. (1978). *Essential principle of physics*. London: John Murray.
32. Habib, M., Abdu, P., Kwanashie, C., Kabir, J., & Negedu, A. (2015). Isolation and identification of *Aspergillus* species from poultry feeds in Kaduna State, Nigeria. *Microbiology Research International*, 3(2), 27–32.
33. Omemu, A., Akpan, I., Bankole, M., & Teniola, O. (2005). Hydrolysis of raw tuber starches by amylase of *Aspergillus niger* AM07 isolated from the soil. *African Journal of Biotechnology*, 4(1), 19–25.
34. Oyeleke, S., & Jibrin, N. (2009). Production of bioethanol from guinea corn husk and millet husk. *African Journal of Microbiology Research*, 3(4), 147–152.
35. Kassim, M. A., Kheang, L. S., Bakar, N. A., Aziz, A. A., & Som, R. M. (2011). Bioethanol Production from Enzymatically Saccharified Empty Fruit Bunches Hydrolysate using *Saccharomyces cerevisiae*. *Research Journal of Environmental Sciences*, 5(6), 573–586. doi: [10.3923/rjes.2011.573.586](https://doi.org/10.3923/rjes.2011.573.586)
36. Reis, V. R., Bassi, A. P. G., Silva, J. C. G. da, & Ceccato-Antonini, S. R. (2013). Characteristics of *Saccharomyces cerevisiae* yeasts exhibiting rough colonies and pseudohyphal morphology with respect to alcoholic fermentation. *Brazilian Journal of Microbiology*, 44(4), 1121–1131. doi: [10.1590/s1517-83822014005000020](https://doi.org/10.1590/s1517-83822014005000020)
37. Andrietta, M., Andrietta, S., Steckelberg, C., & Stupiello, E. (2007). Bioethanol – Brazil, 30 years of Proalcool. *International Sugar Journal*, 109(1299), 195–200.
38. Kurtzman, C. P. (1983). Fungi-Sources of Food, Fuel, and Biochemicals. *Mycologia*, 75(2), 374–382. doi: [10.1080/00275514.1983.12021679](https://doi.org/10.1080/00275514.1983.12021679)
39. Osho, A. (2005). Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice. *African Journal of Biotechnology*, 4(7), 660–662. doi: [10.5897/ajb2005.000-3119](https://doi.org/10.5897/ajb2005.000-3119)
40. Patil, K. R., Rocha, I., Förster, J., & Nielsen, J. (2005). Evolutionary programming as a platform for in silico metabolic engineering. *BMC Bioinformatics*, 6(1). doi: [10.1186/1471-2105-6-308](https://doi.org/10.1186/1471-2105-6-308)
41. Sree, N. K., Sridhar, M., Suresh, K., Banat, I. M., & Rao, L. V. (2000). High alcohol production by repeated batch fermentation using an immobilized osmotolerant *Saccharomyces cerevisiae*. *Journal of Industrial Microbiology and Biotechnology*, 24(3), 222–226. doi: [10.1038/sj.jim.2900807](https://doi.org/10.1038/sj.jim.2900807)
42. Essien, J. P., Akpan, E. J., & Essien, E. P. (2005). Studies on mould growth and biomass production using waste banana peel. *Bioresource Technology*, 96(13), 1451–1456. doi: [10.1016/j.biortech.2004.12.004](https://doi.org/10.1016/j.biortech.2004.12.004)
43. Stenberg, K., Bollok, M., Reczey, K., Galbe, M., & Zacchi, G. (2001). Effect of substrate and cellulase concentration on simultaneous saccharification and fermentation of steam-pretreated softwood for ethanol production. *Biotechnology Bioengineering*, 68(2), 204–210.
44. Wen, Z. (2004). Hydrolysis of animal manure lignocellulosics for reducing sugar production. *Bioresource Technology*, 91(1), 31–39. doi: [10.1016/s0960-8524\(03\)00166-4](https://doi.org/10.1016/s0960-8524(03)00166-4)
45. Verma, G., Nigam, P., Singh, D., & Chaudhary, K. (2000). Bioconversion of starch to ethanol in a single-step process by coculture of amylolytic yeasts and *Saccharomyces cerevisiae* 21. *Bioresource Technology*, 72(3), 261–266. doi: [10.1016/s0960-8524\(99\)00117-0](https://doi.org/10.1016/s0960-8524(99)00117-0)
46. Jimoh, S. O., Ado, S. A., & Ameh, J. B. (2009). Effect of substrate concentration and agitation rate on ethanol production using sorghum bran as substrate. *Biological and Environmental Science Journal for the Tropics*, 6(1), 130–133.