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Research Article

Exploring Bio-Ethanol Production from Fruit Wastes Through Fermentation with *Saccharomyces Cerevisiae* and *Aspergillus Niger*

Debebe Landina Lata[1, *], Lukas Birhanu[1], Mohammed Lengichow[2], Getnet Degemu[3], Tsegaye Atnaf[4], Ayansa Kebenessa[1]

[1]Department of Biotechnology, College of Natural and Computational Science, Wolkite University, Wolkite, Ethiopia
[2]Department of Biology, College of Natural and Computational Science, Wolkite University, Wolkite, Ethiopia
[3]Department of Computer Science, College of Computing and Informatics, Wolkite University, Wolkite, Ethiopia
[4]Department of Chemistry, College of Natural and Computational Science, Wolkite University, Wolkite, Ethiopia

Abstract

Bioethanol, a sustainable alternative fuel derived from organic materials, is essential for addressing global energy demands and environmental concerns. This study aimed to produce bioethanol from banana and mango peels using co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae* through a simultaneous saccharification and fermentation (SSF) process. Fully ripened banana and mango peels, obtained from a local market, were dried, ground into fine particles, and used as substrates for bioethanol production. The fermentation process was carried out by sequentially inoculating the substrates with *Aspergillus niger* to enhance starch hydrolysis, followed by *Saccharomyces cerevisiae* to facilitate fermentation. The process lasted for 7 days under controlled conditions, with a pH range of 5.5-6.0 and a temperature of 28±2 ℃. Among the tested samples, the mixed substrate of banana and mango peels yielded the highest ethanol concentration at 79% (w/v), while mango peels alone produced 74% and banana peels produced 71%. The enhanced performance of the mixed substrate highlights the synergistic effect of combining different fruit wastes. The presence of *Aspergillus niger* played a crucial role in breaking down complex starches into simpler sugars, enabling *Saccharomyces cerevisiae* to effectively convert these sugars into ethanol. This study demonstrates the potential of fruit waste, specifically banana and mango peels, as cost-effective and sustainable raw materials for bioethanol production, providing a promising alternative to fossil fuels. Future research should focus on optimizing fermentation conditions, exploring the potential of additional fruit waste substrates, and scaling up the process for commercial viability.

Keywords

Agricultural Waste, *Aspergillus niger*, Bioethanol, Fermentation, *Saccharomyces cerevisiae*

[*]Corresponding author: landinadebe16fbwku@gmail.com (Debebe Landina Lata)

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1. Introduction

Bioethanol has proven to be a versatile bio-solvent, widely used in laboratory, pharmaceutical, cosmetic, medical, and biomedical industries. Its role as an alternative fuel for engines has also gained significant attention [1, 2]. Ethanol production through the fermentation of corn and other feed crops is a well-established process. As the demand for sustainable liquid fuel alternatives to fossil fuels continues to grow, especially in countries like the United States, it has become imperative to explore viable substitutes [3]. Given that the cost of raw materials accounts for as much as 50% of the total production expenses, current research focuses on utilizing inexpensive and readily available sources [4]. Various biomass resources such as starch-based crops, oil plants, and agricultural or municipal wastes are under consideration, but lignocellulosic materials are the most plentiful biomass resource globally [3, 5].

Ethanol derived from lignocellulose biomass is undoubtedly the most sustainable option for producing transportation fuels, offering remarkable environmental, economic, and infrastructure advantages. Lignocellulosic materials, including agricultural residues, municipal solid wastes, pulp mill refuse, switchgrass, and lawn and garden wastes, are harnessed in this process [6]. The production of bioethanol from biomass has emerged as a promising alternative to fossil fuels, suitable for use in vehicles either as a pure fuel in specially designed engines or in fuel blends [7].

The growing global interest in sustainable biofuels has led to increased research on microbial fermentation processes, particularly for ethanol production from various biomass sources. Among these, the SSF approach has shown promise for efficiently converting lignocellulosic materials into bioethanol. *Saccharomyces cerevisiae*, commonly known as Baker's yeast, is widely distributed in nature, thriving on fruits, decaying vegetation, and organic-rich soils [8]. It plays a vital role in bioethanol production due to its ability to ferment sugar solutions under low-oxygen conditions, producing ethanol and carbon dioxide [11, 12]. This yeast species, in its various forms Baker's yeast, Brewer's yeast, wine yeast, and distiller's yeast has been applied to SSF processes using substrates like lignocellulosic materials and yam peels, showcasing its effectiveness in biofuel production [13, 14]. Another critical microorganism in bioethanol production is *Aspergillus niger*, whose spores are commonly associated with organic material and soil. It is widely utilized for the production of industrial enzymes, including amylase, cellulase, and pectinase, which break down complex carbohydrates into fermentable sugars. These enzymes, produced by *Aspergillus niger*, play a crucial role in the SSF process, contributing to both saccharification and fermentation, making it an essential component of bioethanol production [6, 15].

Cellulosic materials sourced from wood, agricultural residues, municipal solid waste, and energy crops are among the most plentiful forms of biomass worldwide [16]. The diminishing reserves of fossil fuels are a significant cause for concern in many nations. Increasing environmental concerns regarding the impact of fossil fuels on global warming, combined with their declining availability, have expedited the quest for alternative, eco-friendly energy sources. In response to the growing need to reduce reliance on fossil fuels due to their depletion, rising fuel costs, a growing global population, and the worsening effects of climate change, there has been increasing interest in renewable energy sources, such as bioethanol [17]. One feasible solution to these challenges is producing bioethanol from organic matter, such as fruits and waste products.

Bioethanol can be produced through the fermentation of sugars like glucose, fructose, or sucrose using microbial organisms such as *Saccharomyces cerevisiae* and *Aspergillus niger* at ambient temperatures. Currently, bioethanol, mainly derived from sugar and starch-rich feedstocks, is a leading candidate for replacing a significant portion of liquid fuels produced from petroleum. It has also been recognized as a promising bio-based raw material for the chemical industry [18]. This study aims to produce bioethanol using readily available and non-food waste materials, specifically banana and mango peels, through a co-culture fermentation process involving *Aspergillus niger* and *Saccharomyces cerevisiae.* This approach not only provides a sustainable source of bioethanol but also addresses concerns about food security by utilizing waste materials.

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted in the Molecular Biotechnology Laboratory, Department of Biotechnology and Food Processing Engineering Laboratory, Department of Food Processing Engineering, Wolkite University, Wolkite, Ethiopia. Welkite (also transliterated Wolkite), the capital town and separate woreda in Southwestern Ethiopia, serves as the administrative center of the Gurage zone of SNNPRs. The town is located at a latitude and longitude of (8°17′N 37°47′E) and is approximately 158 km and 256.4 km away from Addis Ababa and Hawassa, respectively. The Gurage zone encompasses altitudes ranging from 1001 to 3500 meters above sea level and is classified into three agro-climatic zones: Dega (high altitude), covering 28.3% of the area and ranging between 2500-3662 meters; Weina Dega (mid-altitude), covering about 64.9% of the area and ranging between 1500-2500 meters; and Kola (lowland), covering 6.8% of the area and ranging between 100–1500 meters. The average annual minimum and maximum temperatures and rainfall in Wolkite range from 18 ℃ to 39 ℃ and 450 to 820 mm, respectively (Climate: Wolkite from: climate-data.org).

2.2. Data Collection and Plant Material Preparation

In this study, primary data was collected from a local wholesale fruit market in Gubre town and the experiment was conducted at the Molecular Biotechnology laboratory and Food Processing Engineering laboratory at Wolkite University. The study design will be a completely randomized design (CRD) based experimental/laboratory procedure. Fully ripened, unmarketable mango and banana fruit samples were sourced from a local wholesale fruit market in Gubre town, Wolkite, Ethiopia. The varieties of mango and bananas used in the study were widely grown in the region for commercial juice production. After being transported to the laboratory, the fresh fruits were washed, and the pulp was separated from the peels. The fruit pulp was homogenized using a simple wet milling technique without the addition of water, and the resulting puree was used for further experiments. A mixed pulp sample was also prepared by combining equal amounts of mango and banana (M1: B1 w/w). The peels from both the ripe bananas and mangoes were chopped into small pieces (2–3 cm) and dried in a hot air oven at 65 ℃ for 24 hours. Once dried, the materials were ground using a Wiley mill to a fine powder, which passed through a 1 mm sieve. This powdered material was stored at room temperature for further analysis, with a composite sample of both fruit peels prepared similarly. To initiate further processes, the material was inoculated with *Aspergillus niger*, followed by *Saccharomyces cerevisiae* to promote fermentation. After fermentation, the mixture was distilled to produce bioethanol.

2.3. Microbial Isolation and Culture Maintenance Techniques

Soil samples were randomly collected from three different locations, taking from the top 2 cm of the soil layer. About 20 grams of soil were gathered from each site, placed in plastic bags, and transported to the laboratory. Once there, the soil samples were air-dried at room temperature (28±2 ℃) for 24 to 48 hours. After drying, the soil was processed to eliminate stones and plant debris. From each sample, 0.1 grams of soil was placed into labeled test tubes containing five milliliters of sterile saline solution (0.9% NaCl) following the method described by Rath *et al*. [8]. The test tubes were vortexed to ensure thorough dispersion of the soil in the saline. A 100 µl portion of each suspension was then spread evenly onto potato dextrose agar (PDA, Himedia, India) plates using a sterile spreader, and the plates were incubated at 28±2 ℃. After 5-7 days, mixed colonies were observed on the plates. Pure *Aspergillus niger* cultures were isolated using the streak plate method and preserved on PDA slants at 4 ℃. Additionally, a strain of *Saccharomyces cerevisiae* (Baker's yeast) was sourced from a local market in Gubre town and similarly maintained on PDA slants at 4 ℃ for preservation [4, 9, 10].

2.4. Evaluation of Starch Degradation in Isolated *A. niger* Strains

A loopful of the pure culture was streaked onto a sterile starch agar plate, following the method outlined by Rath *et al.* [8] with slight modifications. The inoculated plate was incubated at 28±2 ℃ for 5 to 7 days. After the incubation period, an iodine reagent was applied to the plate to cover the microbial growth. The appearance of a clear zone around the colonies indicated a positive result, confirming the microorganism's ability to break down starch, which suggests the presence of alpha-amylase. Following the iodine reagent test, the *Aspergillus niger* strain was cultivated in a broth medium containing potato and dextrose extracts. A 4% (v/v) aliquot of the broth culture was then transferred to test tubes to begin the fermentation process [12, 19-21].

2.5. Preprocessing Techniques for Mango and Banana Peel Substrates

Mango and banana peel waste was obtained from local markets in Gubre town. Before processing, the ripe banana and mango peels were cleaned, cut into pieces measuring 3-5 cm, and disinfected using 70% ethanol. The peels were then sun-dried for 5 to 7 days and ground into a fine powder (Figure 1).

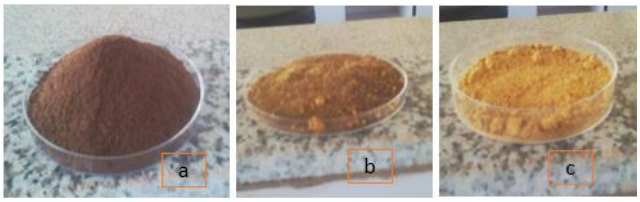

Figure 1 *Preprocessing (grinding) techniques of samples: banana sample (a), mango sample (b) and mixed sample (c).*

2.6. Concurrent Saccharification and Fermentation Processes for Mango and Banana Peel Wastes

Ethanol fermentation was conducted in separate 250 ml flasks, each containing 10 grams of powdered banana, mango, or a mixture of both, dissolved in 97 ml of distilled water as the method described by Ahamad *et al.* [20]. The flasks were sterilized by autoclaving at 121 ℃ for 15 minutes. A 4% (v/v) broth medium was inoculated with *Aspergillus niger*, and an additional 3% (w/v) inoculum of *Saccharomyces cerevisiae* was added. The flasks were then covered with aluminum foil and placed on a shaker for two days to initiate fermentation. Following this, the flasks were incubated at a controlled pH of 5.5-6 and a temperature of 28±2 ℃ for 7 days to complete the fermentation process. After fermentation, the ethanol content was measured using a distillation unit, and the resulting bioethanol was collected in a volumetric flask [11, 15].

2.7. Qualitative Evaluation of Ethanol

The quality of the produced bioethanol was assessed using the Jones reagent [$K_2Cr_2O_7$ + H_2SO_4]. A mixture containing 1 mL of 2% potassium dichromate ($K_2Cr_2O_7$), 5 mL of sulfuric acid (H_2SO_4), and 3 mL of the fermented sample was prepared. After fermentation, the ethanol in the sample was oxidized to acetic acid in the presence of an excess amount of potassium dichromate and sulfuric acid, resulting in a blue-green color [1]. This color change was compared with that of standard ethanol by adding 1 mL of Jones reagent to 3 mL of ethanol, which also produced a blue-green color, confirming the presence of ethanol [7, 13].

3. Results and Discussion

This study investigated bioethanol production from banana and mango peels using *Aspergillus niger* and *Saccharomyces cerevisiae*. Both microorganisms were isolated from natural sources and co-cultured during fermentation. The fungal isolate was identified as *Aspergillus niger* through its morphological, cultural, and physiological characteristics. *Aspergillus niger* was characterized by an initially white surface that darkened to black over time. The reverse side of the culture exhibited hues of white, gold, or brown. The organism grew rapidly, with full culture development occurring within 3 to 5 days (Figure 2), aligning with findings from Kitson-Hytey *et al.* [15].

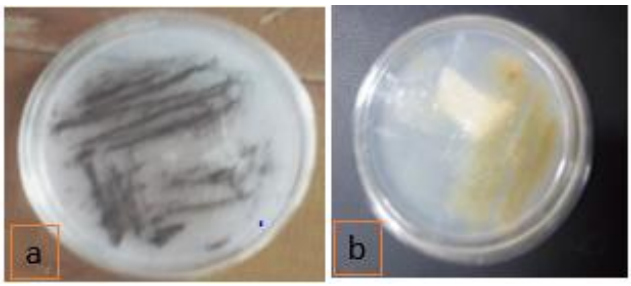

Figure 2. *Pure clones of Aspergillus niger (a, b).*

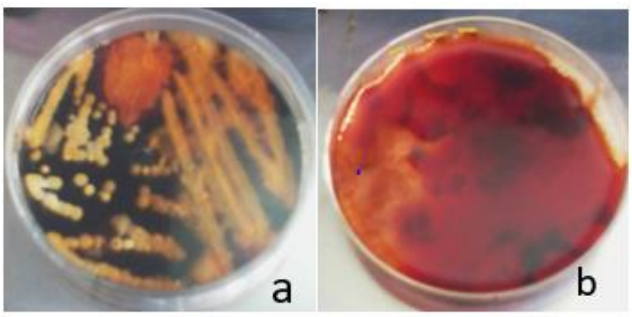

Figure 3. *Starch hydrolysis test by Aspergillus niger using iodine reagent (a, b).*

The fungi isolate demonstrated efficient production of starch hydrolyzing enzymes, as evidenced by the clear zone around the fungi on starch agar plates (Figure 3). The presence of this clear zone upon the addition of iodine reagent solution strongly suggests that the fungi hydrolyze starch to degrade amylase. These findings are consistent with prior research conducted by Afrida *et al.* [1] and Brooks [13].

Saccharomyces cerevisiae was utilized as the fermentation agent. The fermentation of banana and mango peel waste resulted in a substantial production of ethanol (Figure 4). Another study showed a gradual decrease in reducing sugar concentration across all substrates as the fermentation period progressed, leading to an increase in bioethanol yield [3]. The ethanol yield from the three substrates increased progressively from the first day to the seventh day, with pineapple peel yielding the highest at 8.34% (v/v), followed by banana peel at 7.45% (v/v), and plantain peel yielding the least at 3.98% (v/v) [14].

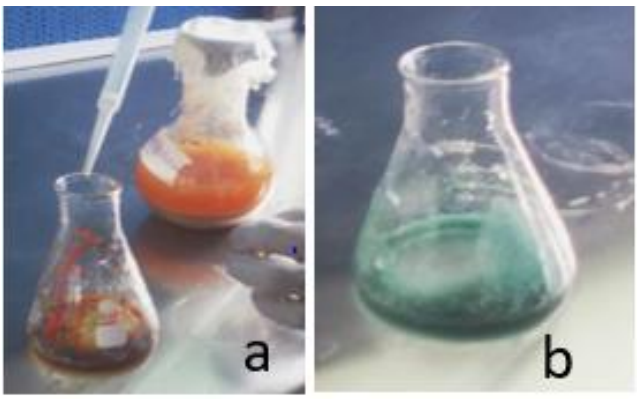

Figure 4. *Qualitative estimation of fermented media: (a), fermented sample and (b), the correct result.*

The amount of ethanol produced changed based on the differences in waste peel samples, yeast concentrations, fermentation time, and types of fungi used. *Aspergillus niger* yielded more ethanol compared to *Saccharomyces*. cerevisiae (Figure 5).

Figure 5. *Bio-ethanol production (a): Banana, mango & mixed bio-ethanol produced by Aspergillus niger and Saccharomyces cerevisiae and (b): Banana and mango bio-ethanol produced by Aspergillus niger and Saccharomyces cerevisiae.*

The bio-ethanol concentration increased progressively with the longer incubation period for both mango and banana peels. The mixed sample inoculum of both mango and banana (MBMI) peels resulted in a higher bioethanol concentration of 79% w/v compared to 74% w/v for mango mixed inoculum (MMI) and 72% w/v for banana mixed inoculum (BMI) after 7 days of fermentation. Additionally, the use of mango mixed sample inoculum led to a steady increase in bioethanol concentration, reaching 74% w/v compared to 71% w/v for banana mixed inoculum (Figure 6). The cell densities increased gradually from day 1 to day 7, indicating increased carbon utilization for ethanol production. Furthermore, the pH decreased from day 2 to day 7 of fermentation, consistent with findings by [2], who observed a decrease in pH from 4.2 to 3.5 as bioethanol production increased. Additionally, it is known that organic acids are one of the fermentation products.

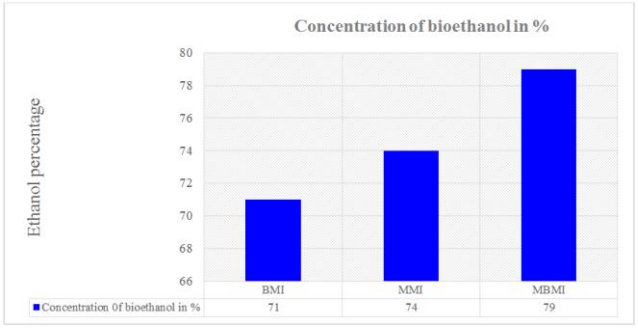

Figure 6. *Concertation of bioethanol produced from mixed sample inoculum.*

The fermentation of a mixed inoculum of mango and banana with *Aspergillus niger* (MMBAI) and *Saccharomyces cerevisiae* (MMBSI) was successful, taking 7 days to complete. Yields were 77% w/v and 65% w/v, respectively. *Aspergillus niger* exhibited a high rate of substrate utilization, using glucose and other substrate hydrolysates faster than *Saccharomyces cerevisiae*, resulting in higher fermentation efficiency (Figure 7). Simultaneous saccharification and fermentation for bioethanol production from yam peels using *S. bayanus* yielded an ethanol concentration of 10% [11], similar to the bioethanol yield from the present study. Ethanol concentration increased as the fermentation days progressed, indicating that *Aspergillus niger* is best suited for bioethanol production from agro-waste materials such as waste fruit.

In the current study by Kitson‐ Hytey *et al.* [15] bioethanol production was observed to steadily increase during the fermentation cycle, followed by a decrease in sugar concentration. This investigation utilized *Aspergillus niger* and *Saccharomyces cerevisiae*, each with its fermentation characteristics. Both species express fermentation enzymes constitutively, accounting for up to 50% of the total protein in the cells. Derman *et al.* [12] found that poor biomass production, cell proliferation, and fermentation are not correlated, resulting in less biomass and efficient ethanol production compared to *Aspergillus niger* and *Saccharomyces cerevisiae*, as reported by Oadipo *et al.* [21].

Both species demonstrated the ability to metabolize their substrates rapidly, outcompeting other microbes and enabling the production of ethanol from non-sterile substrates. This has the potential to reduce the energy costs associated with substrate sterilization. As highlighted by Mizik [7], key factors in bioethanol production include high productivity, low raw material cost, and low processing cost. This study suggests that mango and banana could serve as cost-effective alternatives for bioethanol production under suitable conditions, without the need for additional nutrients in the fermentation process. Both species were able to thrive on the substrates without the addition of extra nutrients.

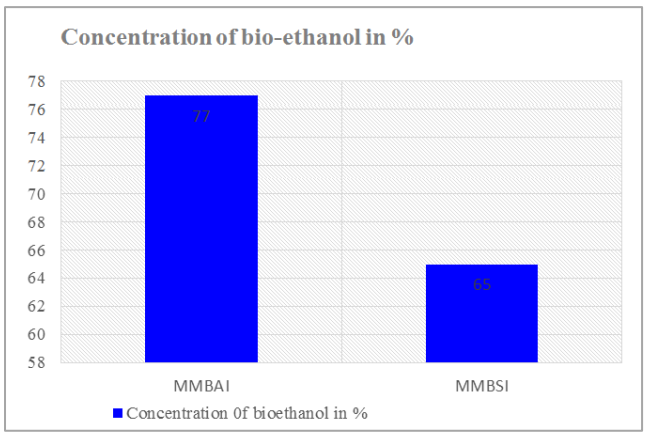

Figure 7. The concentration of bioethanol produced from two mixed fruit wastes inoculated with *Aspergillus niger* and *Saccharomyces cerevisiae.*

4. Conclusion

The study successfully demonstrated the production of bioethanol from banana and mango peels through a co-culture fermentation process involving *Aspergillus niger* and *Saccharomyces cerevisiae*. The results showed a significant increase in bioethanol yield, with the highest concentration of 79% w/v obtained from the mixed inoculum of both peels after seven days of fermentation. This indicates that utilizing fruit waste not only contributes to waste management but also serves as an efficient and sustainable alternative source of bioethanol. The presence of elevated sugar levels in banana and mango pulp has been found to increase ethanol yield. The findings highlight the efficiency of *Aspergillus niger* in breaking down complex carbohydrates into fermentable sugars, which were then converted into ethanol by *Saccharomyces cerevisiae*. The fermentation process proved effective, with a gradual increase in ethanol concentration correlating with extended fermentation time.

The use of non-food biomass for ethanol production alleviates food security concerns while promoting the utilization of readily available agricultural waste. Future research should scale up fermentation and utilize diverse agricultural waste to enhance the sustainability and economic viability of bioethanol production, supported by life cycle assessments and favorable policies.

Abbreviations

SSF	Simultaneous Saccharification and Fermentation Process
PDA	Potato Dextrose Agar
CRD	Completely Randomized Design

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Author Contributions

Debebe Landina Lata: Writing-original draft, writing-review editing, Validation, Methodology

Lukas Birhanu: Conceptualization, Data curation, facilitating

Mohammed Lengichow: Investigation, Supervision, material funding acquisition

Getnet Degemu: Validation, Resources

Tsegaye Atnaf: Validation, Resources

Ayansa Kebenessa: Conceptualization, Data curation, and facilitating

Ethics Approval

There is no ethics problem and the manuscript is original and has not been published elsewhere.

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Data Availability Statement

Data will be made available on request.

Conflicts of Interest

The authors declare no conflicts of interest.

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