

Research Article

Integrated Fermentation of Fruit Processing Wastes into Bioethanol by *Saccharomyces Cerevisiae* and *Aspergillus Niger*

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Abstract

The detrimental environmental impact of fossil fuels, primarily driven by harmful greenhouse gas emissions, necessitates the urgent development of sustainable alternative energy sources. Concurrently, effective municipal solid waste management, particularly concerning organic fractions, presents a significant challenge in urban centers like Arba Minch, Ethiopia. This research addresses both issues by investigating the valorization of locally abundant fruit peel waste specifically from bananas and mangoes—into bioethanol, a viable renewable fuel. The study employed a bioprocessing strategy utilizing microbial co-culture. Waste peels were pre-processed by chopping into 3-5 cm pieces, followed by seven days of sun-drying to facilitate milling and enhance substrate accessibility. The resulting dried powder, rich in fermentable cellulose and hemicellulose, served as the primary feedstock. Bioethanol production was achieved through a seven-day simultaneous saccharification and fermentation (SSF) process using a co-culture of *Aspergillus niger* (for enzymatic hydrolysis/saccharification) and *Saccharomyces cerevisiae* (for ethanol fermentation). The fermentation media was maintained at a pH of 5.5-6.0 and a temperature of 30 °C. An initial phase involved agitation on a dry shaker for two days to ensure mixing and oxygen transfer for microbial growth, followed by static incubation to promote anaerobic fermentation. Post-SSF, the broth underwent distillation to recover the bioethanol. Qualitative analysis confirmed ethanol production, while quantitative yield was determined spectrophotometrically. Results demonstrated that the mixed inoculum of *A. niger* and *S. cerevisiae* achieved a substantial ethanol yield of 79% from the mixed peel substrate after seven days. Notably, comparative analysis revealed that *A. niger* monoculture fermentation produced a significantly higher yield of 77% ethanol relative to *S. cerevisiae* alone. This work successfully establishes a practical method for converting problematic fruit peel waste from Arba Minch into valuable biofuel, highlighting the particular efficacy of *A. niger* in this process.

Keywords

Aspergillus Niger, Bioethanol Production, Fruit Peel Waste, Organic Waste Management, Renewable Fuel, *Saccharomyces Cerevisiae*, Simultaneous Saccharification and Fermentation (SSF), Waste Valorization

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1. Introduction

The production of ethanol from lignocelluloses biomass has received considerable attention because of the potential of producing large quantities of ethanol for use as a transportation fuel. Hemicelluloses and cellulosic components of lignocelluloses biomass are hydrolyzed to their component sugars for subsequent conversion to ethanol by a fermentative process [1]. Production of fuel ethanol from biomass seems to be an interesting alternative to traditional fossil fuel, which can be utilized as a sole fuel in cars with dedicated engines or in fuel blends. Ethanol is currently produced from sugars, starches and cellulosic materials [2].

Currently the basic energy requirements are largely met by the use of fossil fuel. Bio ethanol, which is mainly produced from sugars and starch-rich materials, is one of the leading candidates to replace a large fraction of liquid fuels produced from oil. Recently bio-ethanol has also been identified as one of the most promising bio-based raw materials for the chemical industry [3].

The aim of this research was to investigate the possibility of using and transforming fruit peel waste to something valuable, namely ethanol using the fungus *Saccharomyces cerevisiae* and *A. niger* as ethanol producing organism. The study revealed that how microorganisms are involved in production of ethanol. The study service as a source input data for the scaling up bio-ethanol production from different agricultural fruit waste. This study was focus on the bio-ethanol production from banana and mango peels and fermentation process. This increases possibility of using and transforming fruit peel waste to something valuable product, namely ethanol there by contributing towards alternative energy supply as well as creating an employment opportunity.

Fossil fuel depletion is the basic need of any country. Rising concerns with the contribution of fossil fuels to global warming coupled with their global depletion trends provide added impetus to the research for alternative fuels that are environment friendly [4]. To decrease dependence on fossil fuel as a result of depletion, increasing global fuel price, increasing population and increasing global warming, there has been increased interest in the use of renewable energy sources of which bio ethanol is one [3].

Cellulosic materials obtained from wood and agricultural residuals, municipal solid wastes and energy crops represent the most abundant global source of biomass [5]. From agro-wastes such as mango and banana peel by batch culture using *S. Cerevisiae* and *A. Niger* is one alternative to produce bio ethanol from fruits, other grown organic matter or waste [3, 6]. Bio ethanol can be obtained via the fermentation of glucose, fructose or sucrose under the influence of *Saccharomyces* and *Aspergillus niger* at room temperature.

Biofuels, encompassing alcohols, ethers, esters, and related chemicals derived from cellulosic biomass, represent renewable energy sources due to their biomass origin [1]. While combustion releases carbon dioxide (CO₂) comparable to fossil fuels, the cultivation of the biomass feedstock sequesters

atmospheric CO₂. Consequently, biofuels theoretically achieve near net-zero CO₂ emissions over their lifecycle [7].

Biodiesel, specifically, is a fatty acid alkyl ester (FAAE) produced via transesterification of triglycerides found in vegetable oils or animal fats [7]. It is commonly blended with petroleum diesel, with B20 (20% biodiesel, 80% petroleum diesel) being a prevalent formulation compatible with conventional diesel engines without significant modification [7].

The term "alcohol" originates from the Arabic *al-kuhul*, referring to a fine cosmetic powder, later adopted by alchemists for distilled substances [8]. Bioethanol (ethyl alcohol, CH₃CH₂OH) is produced through the fermentation and distillation of sugars derived from various domestic resources, including agricultural residues, forestry waste, the organic fraction of municipal solid waste (OFMSW), and dedicated energy crops [9, 10]. Its favorable physicochemical properties enable diverse applications. Global interest in bioethanol stems from concerns regarding climate change, energy security, dependency on petroleum imports, and the burden thereof [9]. Blending ethanol with gasoline enhances engine performance, reduces air pollutants, and contributes to decreased petroleum consumption, greenhouse gas (GHG) emissions, and fossil energy utilization [11].

Alcohol fuels are produced by fermentation of sugars derived from wheat, corn, sugar beets, sugar cane, molasses and any sugar or starch from which alcoholic beverages such as whiskey, can be made. The ethanol production methods used are enzyme digestion, fermentation of the sugars, distillation and drying. The distillation process requires significant energy input for heat (often unsustainable natural gas fossil fuel, but cellulosic biomass such as biogases, the waste left after sugar cane is pressed to extract its juice, can also be used more sustainably [12].

Inadequate municipal and industrial solid waste collection and disposal creates a range of environmental problems in our country. A considerable amount of waste ends up in open dumps or drainage system, threatening both surface water and ground water quality and causing flooding, which provides a breeding ground for diseases-carrying pests. Open air burning of waste, spontaneous combustion in landfills and incinerating plants that lack effective treatment for gas emissions are causing air pollution [13]. The situation is exacerbated in slums where households cannot make use of garbage collection containers. Lack of the most basic solid waste services in crowded, low income neighbors are a major contributor to the high morbidity and mortality among the urban poor.

2. Materials and Methods

2.1. Description of the Study Area

Arba Minch, a city and administrative unit in southern Ethiopia, is situated approximately 500 km south of Addis

Ababa. It lies at an elevation of 1,285 meters above sea level, within the Gamo Zone of the Southern Ethiopia Peoples' Region (SEPR), and ranks as the region's first-largest urban center. The city is positioned west of Lake Abaya and adjacent to Lake Chamo, forming part of the East African Rift Valley system. Its topography ranges from 1,400 to 2,200 meters above sea level. The region experiences an annual rainfall of 623.5–1,661 mm, with mean temperatures reaching 32 °C. Dominant vegetation includes mango (*Mangifera indica*), banana (*Musa* spp.), and acacia (*Acacia* spp.). This study focuses specifically on Arba Minch University's Abaya Campus, located on the eastern foothills of the Gamo Highlands. Research will center on agro-waste valorization from mango and banana peels sourced within this agro-ecological zone.

2.2. Data Collection and Plant Material Preparation

Primary data were generated through laboratory experiments conducted in the Microbial and Industrial Biotechnology Laboratory, Department of Biology, Arba Minch University.

Banana (*Musa* spp.) and mango (*Mangifera indica*) peels were sourced from local markets in Arba Minch, Ethiopia. Substrates were washed and surface-disinfected with 70% ethanol, chopped (3–5 cm pieces), sun-dried for 7 days, and ground to fine powder (≤ 1 mm particle size), sieved (ASTM No. 18 mesh) to ensure uniformity. Through doing experimental in Microbial and Industrial Bio technology laboratory in the department of Biology, Arba Minch University. He following general steps were taken place (Figure 1).

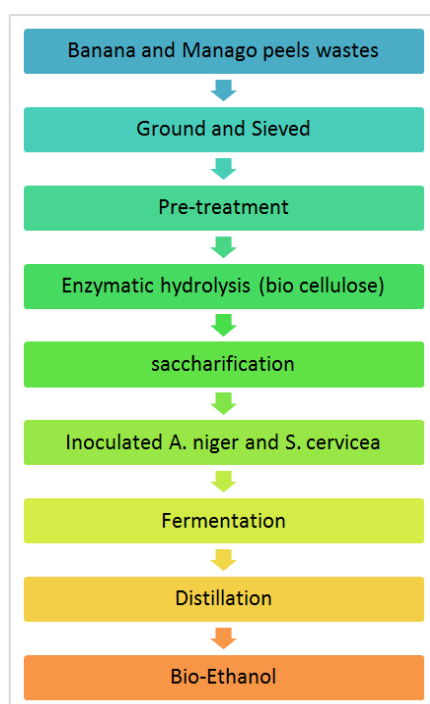


Figure 1. Flow diagram for production of bio ethanol using *S.cervicae* and *A.niger*.

2.3. Isolation of Microorganisms and Its Maintenance

Soil samples collected from three sites (library, dormitory, caf  ) at 2 cm depth of the soil profile. Approximately 50 g of soils were collected from each site and put into plastic bags and brought to the laboratory. Air-dried (27 ± 1 °C, 24–48 h), homogenized, and suspended in sterile saline (0.9% NaCl) with streptomycin (30 mg/L) to inhibit bacteria [14]. In order to suppress bacterial growth, 30 mg/l of streptomycin was added. Each of the test tubes were vortex mixed until all soil was well dispersed throughout the tube. 100 µl of each of the suspension was evenly spreaded on PDA plates with a spreader and incubated at 30 °C. Mixed colonies on the plates were observed after 3-5 days. Pure cultures obtained via streak plating (Figure 2) and maintained on PDA slants (4 °C). *Saccharomyces cerevisiae* Commercial baker's yeast (local market) maintained on PDA slants (4 °C).

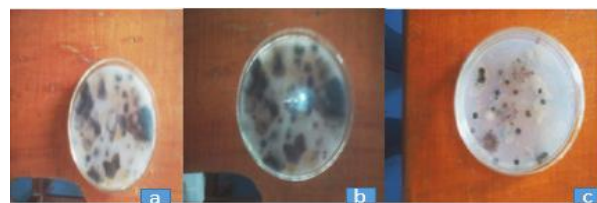


Figure 2. Mixed clones of *A.niger* a). Mixed clone from library b). Mixed clone from dorm c). Mixed clone from caf  

Pure culture of *Aspergillus niger* was obtained by streak plate method.



Figure 3. Pure clones of *A.niger* a). Pureclonefromlibrary b). Pureclonefrom dorm c). Pureclone from caf  

It was then maintained on PDA slants at 4 °C for preservation.



Figure 4. *A.niger* maintained on PDA slant at 4 °C.

Yeast strain *Saccharomyces cerevisiae* (Baker's yeast) was obtained from the local market. It was maintained on PDA slants at 4 °C.

2.4. Starch Hydrolysis Test of Isolated Strains of *Aspergillus Niger*

A loopful of pure culture was streaked on a sterile plate of starch agar medium. The inoculated plate was incubated at 27 °C for 3-5 days. After incubation, Iodine reagent was added to flood the growth. Presence of clear zone surrounding colonies confirmed the positive result & accounts for their ability to digest the starch & thus indicates presence of alpha-amylase.

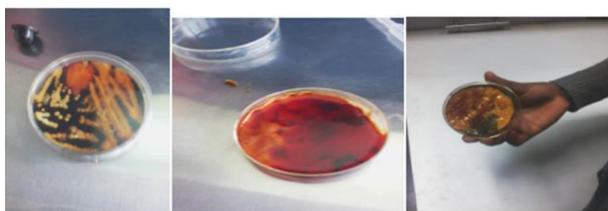


Figure 5. Hydrolysis test by iodine reagent.

After the strains of *aspergillus niger* tested by iodine reagent. Then strain of *Aspergillus niger* was cultured on broth media that contain potato extract and dextrose. 4% (v/v) of broth media cultured on test tubes for the fermentation stage. Simultaneously before conducting fermentation, we had the preparation of media for the yeast, *Saccharomyces cerevisiae*. In order to prepare the media, we should had the favorable condition for yeast growth or to supply the required amount of nutrients [15]. Mix the following nutrients in there proportion below samples. A. *niger* broth 4% (v/v) inoculum in potato dextrose broth. S. *cerevisiae* broth 3% (w/v) inoculum in medium containing dextrose (10 g/L), yeast extract (3 g/L), urea (3 g/L), and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1 g/L).

2.5. Pre-Treatments of Banana and Mango Peels Substrates



Figure 6. Grind sample a). Banana sample b). Mango sample c). Mixed sample.

Banana and mango peels wastes were procured from local market in Arba Minch, Ethiopia. Before processing ripe

wastes of banana and mango peels, it was cleaned, chopped (3-5cm) and disinfected with 70% ethanol. It was sun dried for 7 days and ground to fine powder.

2.6. Simultaneous Saccharification and Fermentation (SSF) of Banana and Mango Peels Wastes

Ethanol fermentation were carried out in different 250 ml flasks containing sample of 10g powdered banana, mango and mixed in 96 ml distilled water. The flasks were sterilized by autoclaving at 121 °C for 30 min and broth media of 4% (v/v) or 4 ml were added inoculums of *Aspergillus niger* and 3% (w/v) inoculum of *Saccharomyces cerevisiae* prepared was also added. The conical flasks were properly covered with aluminum foil. The conical flask were then placed on shaker for two days and were kept for in incubator with adjusted pH of 5.5-6 and temperature 30 °C for 7 days for fermentation process and the ethanol content was measured on distillation unit and finally bio-ethanol harvested in volumetric flask.



Figure 7. Fermentation stage and Distillation a) Fermentation stage b) Distillation stage.

2.7. Qualitative Analysis of Ethanol

Fermented broth distilled at 78.5 °C; condensate collected in volumetric flasks. The qualitative of bio-ethanol production was examined by Jones reagent [$\text{K}_2\text{C}_2\text{O}_4 + \text{H}_2\text{SO}_4$] 1mL of $\text{K}_2\text{C}_2\text{O}_7$ (2%), 5ml H_2SO_4 and 3ml of sample was added after fermentation of the sample it was observed that ethanol oxidized to acetic with an excess of potassium dichromate in the presence of sulfuric acid, giving off a blue-green color simultaneously it was compared with normal ethanol by taking one ml of Jones reagent and 3ml of ethanol was give blue green color [16].

3. Results and Discussion

3.1. Results

Aspergillus niger was isolated from soil samples (library, dormitory, caf ) and identified through morphological, bio-

chemical, and physiological analyses (Table 1). Colonies exhibited rapid growth (3 days) on PDA at 27-30 °C, with initial white surfaces transitioning to black pigmentation. Starch hydrolysis assays confirmed robust amylase activity, evidenced by clear zones (≥ 5 mm diameter) upon iodine addition (Figure 5), aligning with enzymatic efficiency reported by [16].

Isolated fungal culture was identified depending on its morphological, culture, biochemical and physiological characteristics and confirmed as *A.niger*. The growth of the isolates *A. niger* (isolated from soil) was supported on potato dextrose agar (PDA) as the carbon source.

Table 1. General characteristics of *A.niger*.

Characteristics	Descriptions
Surface	Initial white and shade of black
Reverse	White, Goldish or Brown
Growth rate	Rapid, culture with 3 days
Recommended media	Potato dextrose agar
Incubated at	27-30 °C for 2-7 days

Efficient starch hydrolyzed production by the fungi isolates was observed based on the zone of clearance around the fungi on starch agar plates. The appearance of the clear zone around the clones when the iodine reagent solution was added was strong evidence that the fungi hydrolysis the starch in order to degrade amylase [16].

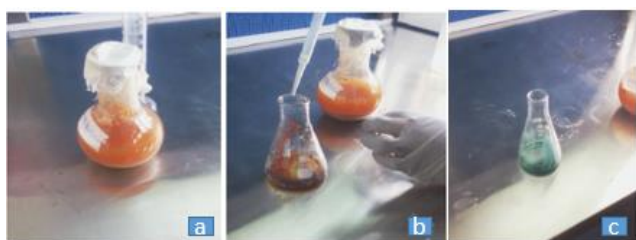


Figure 8. Qualitative estimation of fermented media a) Fermented sample b) Addition stage c) Result checked.

Saccharomyces cerevisiae was used as the source of fermentation processes. The result of the fermented banana and mango peels waste produced a significant amount of ethanol. Mango and banana peels were selected because of their commercial abundance in Arba Minch. Their usage for the production of ethanol via fermentation will promote the Agro industry.

The volumetric production of ethanol varied according to the variations in sample waste peels and at different yeast concentrations. It also varied according to fermentation time and fungal strains. More production was obtained from

A.niger when compared with *S.cervisea*.



Figure 9. Bio-ethanol produced a) Banana, mango & mixed bio-ethanol produced b) Banana and mango bio-ethanol produced.

3.2. Spectrophotometric Estimation of Ethanol

40 g of potassium dichromate was dissolved in 200 ml of distilled water and the solution is made up to 500 ml and sulfuric acid was added in to flat bottle flask. Then 3ml tri-butyl phosphate was added. From that standard solution the working standard were prepared in different 5 volumetric flasks. 100ml of solution were added in to each of which working standard. Then from first volumetric flask 1ml of solution was discarded and 1ml of ethanol was added. From the second volumetric flask 2 ml solutions also discarded. From the third volumetric flask 3 ml solutions also discarded.

From the forth volumetric flask 4 ml solutions also discarded. And from the fifth volumetric flask 5ml solution discarded respectively. After that spectrophotometer was adjusted at 595nm then from each working standard 1ml from first solution, 2ml from second solution, 3ml from third solution, 4ml from forth solution, and 5ml from fifth solution were read on the spectrophotometer and there absorbance were obtained then five unknown sample of bio ethanol were produced also read on spectrophotometer.



Figure 10. Media preparation for Spectrophotometer reading a) Preparation solution for standard b) prepared working standard.

We detailed discussed through calculating the concentration of bioethanol for each produced of mixed sample with mixed inoculum and compare and contrast each value harvested.

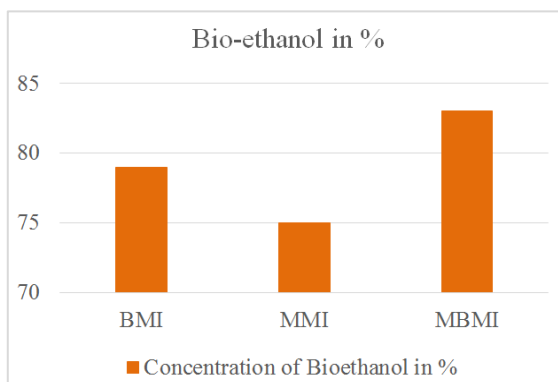


Figure 11. Concentration of bioethanol produce from mixed sample inoculum.

From the above graph we can see that large production of bioethanol harvested was banana and mango with mixed inoculums when compared with each other.

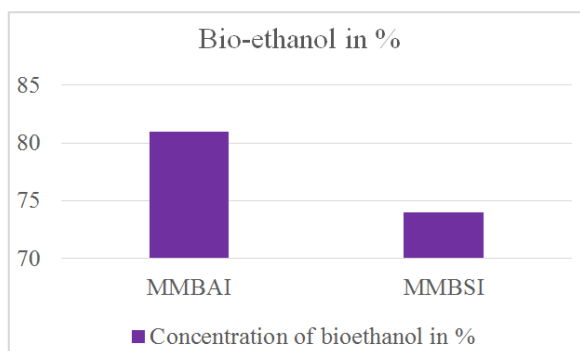


Figure 12. Concentrated of bioethanol produce from mixed sample inoculum.

From this graph large production of bioethanol collected was mixed banana and mango with *A.niger* when compared with *S.cervesiea*. So that *A.niger* the best for production of bioethanol from agro-waste material (waste fruit).

4. Conclusions

Bioethanol can be used as an alternative fuel to reduce the load on conventional fossil fuel resources. To save the future generation from the acute shortage of fuel resources, it is important to produce and use bioethanol from relatively cheaper sources like fruit wastes. In this study, we proved that the peels of banana and mango as one of the novel and potent raw material for production of bioethanol.

The amount of ethanol content increased with the increase in fermentation time. Simultaneous fermentation of starch to ethanol can be conducted efficiently by using co-cultures of the amylolytic *Aspergillus niger* and a non-amylolytic sugar fermenter, *Saccharomyces cerevisiae*. Due to higher amounts of sugars in banana and mango pulp ethanol yield is more and

addition of sucrose as additional supplement in the media produced higher amount of ethanol. Ethanol can be used as renewable energy source and it can produce from different agro-wastes successfully.

However, optimization of substrate concentration and other environmental conditions are required for an industrial application. Processing of waste using *Aspergillus niger* and *Saccharomyces cerevisiae* yeast were produced ethanol.

The study revealed that it is possible to produce bio-ethanol from agro waste materials Such as banana, and mango peels. Further studies should be conducted on. Pretreatment of other agro waste materials to release high fermented sugars to increase Ethanol yield from agro wastes. Further work is again necessary to look at the effect of inhibitor on bio-ethanol Production as a result of pretreatment. Efforts should also be made to check the bio-ethanol quality of different agro wastes by UV visible spectrometer too.

Further study is very important to describe how absolute bio-ethanol can be produced from agro wastes by using distillation unit. Most of the solid wastes including fruit peel waste in our country have no or very low conversion to different usable products and as such among the major problems of health especially for cities such as Arba Minch. Hence, that government or other investor's to recover this very valuable product as well as to contribute to the country in reducing the highly rising quantity of wastes. To conclude the recommendation, there is an urgent need for proper collection, documentation and assessment of fruit peel yields of orange, mango and banana as well as their seasonal variation in our country.

Abbreviations

BMI	Body Mass Index
UV	Ultraviolet
PDA	Potato Dextrose Agar
SSF	Simultaneous Saccharification & Fermentation Process

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Author Contributions

Musa Tuke Tufa: Conceptualization, Data curation, Formal analysis, Investigation, Methodology

Alemayehu Letebo Albejo: Project administration, Resources, Supervision, Validation

Jelalu Nesre Aerato: Software, Visualization, Writing – original draft, Writing – review & editing

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Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest

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Research Field

Musa Tuke Tufa: Industrial Biotechnology, Biotechnology, Molecular biology, Bioinformatics

Alemayehu Letebo Albejo: Medical Biotechnology, Biotechnology, Molecular Epidemiology

Jelalu Nesre Aerato: Data Science, Artificial Intelligence, Information Systems Security, Educational technology, Service innovation