

## Fermentative Conversion of Fruit and Vegetable Waste into Bioethanol through Microbial Fermentation

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### ABSTRACT-

Waste fruits and vegetables are a problem for the environment and require recycling. Waste biomass feedstock is a source of renewable energy resources and an amount of energy production potential and the 10-15 % energy required from global demands and solved from the issue of fuel scarcity, fossil fuel exhaustion, and greenhouse gas emissions. Bioethanol as a renewable bio-energy is very importance in recent years for a greener earth and global matter against world air pollution. Simple, complexity and highest Carbohydrate are content of fruits and vegetables residues, these also are sugar, which may be used as fresh material, as raw for producing of bioethanol through microbial culture. 80 % bioethanol in recent studies are obtained from the food stock sugar, starch in compared to lignocellulosic material more interest is generated. It is the purpose of the study to explain levels of the fermentation and pretreatment process, involved in fruits and vegetables biomass, and conditions which influence microbial culture and ethanol yield.

**Keywords:** Bioethanol, raw materials, *Saccharomyces cerevisiae*, pretreatment, hydrolysis,

### I. INTRODUCTION

The worldwide energy demand of all the countries is continuously increasing with the passage of time. And the new resource of the energy is needed to make it. Above all, it is essential for finding the new resources of energy. In the context of circular economy and sustainable development goals, conversion of waste to bioenergy is an auspicious technology. In this backdrop, the biofuel having wide effectiveness is bioethanol. The presence of fruit and vegetable waste in municipal solid waste is significant and the formation of a wide range of environmental challenges from methane gas emission is a matter of serious concern (Kalkan et al., 2022) These are the major sources of fermentable sugar for bioenergy production comparison. Several vital fermentation parameters, like sugar concentration, pH, and production time for bioethanol, are also being optimized in this study (Bera et al., 2022).

The procedure for increasing the using of the renewable fossil fuels causing the environmental pollution in the supplement to the reduction of the reserving materials. The research for renewable bioenergy resources and the alternatives for the sustainable biofuel energy production has been discussed in this book (Atiku et al., 2024). Vegetables and fruits waste are rich in simple sugars these all are one of the very cheaper renewable sources for the production of bioethanol. Of all these renewable biofuels are easily available in the market. Using renewable wastes as the feedstock for bioethanol production could help solve waste disposal problems while also reducing costs in the process. This review explains the production of bioethanol by using the decaying of vegetables and fruits waste. The waste materials are needed to be decaying (Meganathan et al., 2019).

The renewable bioenergy is essential for the growth of human beings. Some people, due to world-wide and global demand, cannot even think of doing the most significant work of the house. For example, storage of the food, cooking, and lighting a house in recent years. Fruits and vegetable waste refer to the decaying substances in fruits and vegetable which is generated in large quantity from different source like municipality, domestic, industry, some market (Verma et al., 2022). Different methods can convert vegetables and fruits wastes into valuable bioproducts with the help of knowledge available in the literature (Dhar et al., 2025). The use of biofuels derived from natural resources can lead to a wide range of issues. The fast rise of CO<sub>2</sub> levels in the environment due to the energy of global warming results in the negative impacts of paid biofuel energy from current petroleum-based materials. In this process, plants containing a significant amount of sugar are used as raw materials in the production of bioethanol. The most used ones are pineapple, sugarcane, and potato which results in a high yield of this renewable bioethanol. The by-product formation due to the presence of sugar cane in its production (Khandaker et al., 2020).

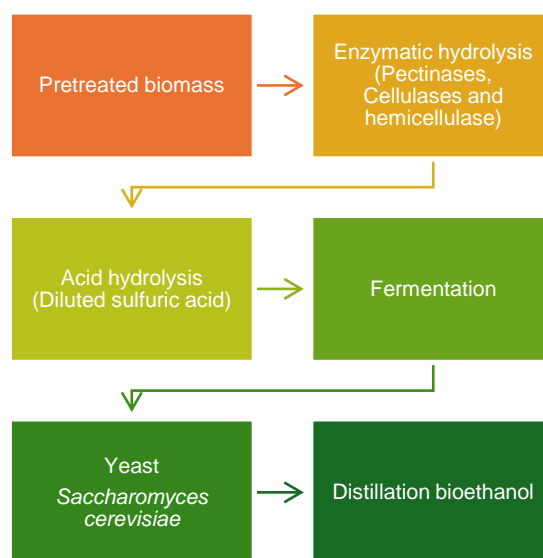
## II. MATERIALS AND METHODS

All the experiments were done in the average value and duplicates has been represented. Sample and waste raw materials was collected from the different-different raw material resources.

### 2.1 Bioethanol from tropical fruits

This tropical fruit waste can be an interesting resource for the production of bioethanol due to its exceptional diversity

in many types, such as different structures of the compound and sugar-rich compound structures. Jackfruit, pineapple, and sweet potato are three fruits that can be extensively studied for bioethanol production from their waste biomass. The general process of bioethanol production from each of their feedstocks resembles. After the process of pretreatment, the lignocellulosic biomass is subjected to the enzymatic or acidic hydrolysis. After that, bioethanol is obtained from the microbial fermentation process (Saputro et al., 2023).



**Figure 2: Bioethanol production process (Kasaeian et al., 2025)**

### 2.2 Raw materials for the fermentation

Vegetables and fruits wastes from the water such as sweet potato, jackfruit, chestnuts, and pineapple were used. Selecting the vegetables and fruits wastes were done due to their availability. Outer coverage area for the covering of water chestnuts, the leafy shoots of pine apple, skin peels of the sweet potatoes, and the waste of jackfruits all are collected from the market of fruits and from household wastes (Dhar et al., 2025). Collected fruits waste was washed with the tap water.

Obtaining materials cut into the small pieces and kept it in a tray and it allowed in the oven drying process for 24 to 48 hours to remove the moisture content. Oven drying fruits and vegetable wastes was powdering using a blender and stored it in the air tight containers (Gosavi et al., 2017).

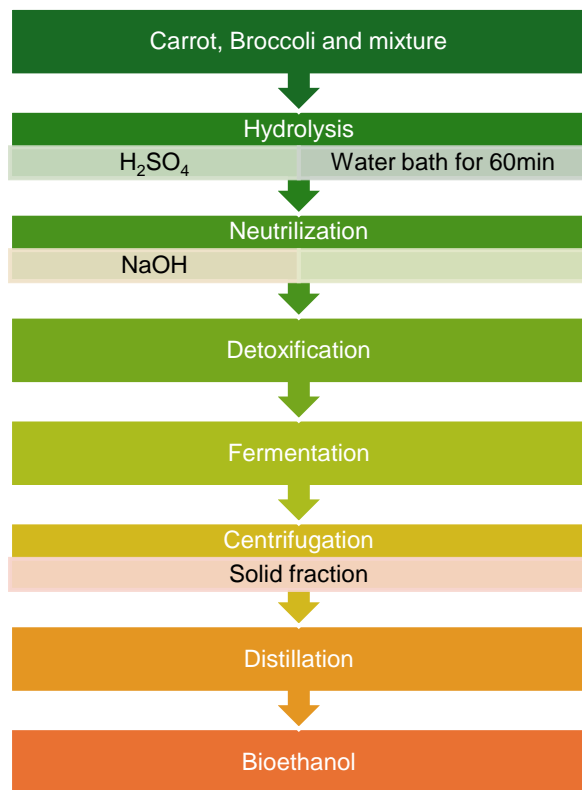
### 2.3 Composition of vegetable and fruit waste

Abundant studies and reviews explore vegetable and fruit waste maximization for renewable bioenergy or biofertilizers remainders underexplored and fractured in the literature. This study fills the gap for degradation of microbial pathways, industrial applicability, and mechanism of the extractions. These are also recognized as regulatory, technological, and economic limitations to future new ideas and framework policies. These are a lack of the comprehensive studies linked to the yield of bio-solvents efficiency and the economic feasibility and scalability of the production pathways of bio-solvent and detailing the analysis between solvent-based treatment and bioremediation for the industrial and environmental context (Dhar et al., 2025). Broccoli *brassica oleracea* and carrot *Daucus carota* wastes are rich carbohydrates, making them suitable substrates for the production of bioethanol. By using a microbial fermentation process. Broccoli is rich in cellulose, hemicellulose, and lignin, which requires an intensive pretreatment process. Carrots are very high in simple sugars like glucose and sucrose for easier conversion (Kasaeian et al., 2025). The raw materials of broccoli and carrot have moisture content of 85-95% and sugars of 5-10%, and they determine free lower sugars. In this process,

collecting the carrot peels and broccoli leaves or stems. Washed clearly to remove the dirt and cropped them into small pieces of 1-2 cm and ground them in a blender for converting into slurry (Firehiwot et al., 2024).

For pretreatment, broccoli contains lignocellulosic biomass during a strong pretreatment process. By using acid pretreatment, 1-2%  $H_2SO_4$  and heat at  $121^\circ C$  in an autoclave for 20-30 minutes were used. Breakdown of hemicellulose for releasing the simple sugars occurs with alkaline pretreatment using 1-2% of NaOH and temperature kept at  $80-100^\circ C$  to remove the lignin for increasing the enzyme accessibility. Hydrolysis for the carrot already contains sugars for the optional enzymatic activity. Amylase and pectinase are used at a temperature of  $50^\circ C$ ; pH occurs from 4.5 to 5.5 for the hydrolysis process (Cabas et al., 2020). Hydrolysis of broccoli is an essential step where cellulase enzymes are used at specific conditions such as pH 4.8, temperature  $45-50^\circ C$ , and time 24-72 hours. In the fermentation process, *Zymomonas mobilis* and *Saccharomyces cerevisiae* are used at optimum conditions such as  $30-35^\circ C$  temperature, pH 4.5 to 5.5, and time 24 to 72 hours for the fermentation. Selection the vegetables and fruits wasted was done due to their availability (Mulopo et al., 2022). Outer coverage area for the following: water chestnuts, the leafy shoots of pineapples, skin peels of the sweet potatoes, and the waste of jackfruits all are collected from the market of fruits and from household wastes. Collected fruit waste was washed with the tap water. Obtained materials were cut into the small pieces and kept in a tray and allowed in the oven-drying

process for 24 to 48 hours to remove the moisture content. Oven-drying fruits and vegetable wastes was powdering using a blender and storing them in the airtight containers (Gosavi et al., 2017).



**Figure 1: Conversion of agriculture and food waste in to bioethanol (Gonzalez et al., 2023).**

### 2.4 Pretreatment

Pineapple waste is rich in lignocellulosic biomass, a complex structure made up of cellulose, hemicellulose, and lignin. This rigid composition makes it difficult for enzymes to access and break down the material directly. Therefore, a chemical pretreatment step becomes essential. In this process, the pineapple waste is treated with a dilute solution of sulfuric acid (about 1–2%) and then heated at 121°C for approximately 30 minutes (Kalkan et al., 2026). This combination of acid and heat helps to break down the tightly bound lignocellulosic structure, loosening the

fibres and partially removing lignin. As a result, the internal carbohydrates become more exposed and accessible, allowing enzymes to act more efficiently during further hydrolysis and fermentation processes (Bera et al., 2025).

Sweet potato, on the other hand, is primarily valued for its high starch content, which typically ranges from 60–70%. In addition to starch, it naturally contains simple sugars such as glucose, fructose, and sucrose, along with small amounts of fibre, minerals, and proteins. This composition makes sweet potato an excellent raw material for producing fermentable sugars. During its treatment, the sweet potato slurry is heated to a temperature between 80–100°C. At this stage, the heat causes the starch granules to undergo gelatinization—a process where they absorb water, swell, and lose their crystalline structure. This transformation is important because it converts the starch into a form that enzymes can easily digest, thereby improving the efficiency of sugar conversion (Gonzalez et al., 2023).

Similarly, jackfruit waste represents another valuable substrate for bioconversion due to its significant carbohydrate content. Jackfruit is widely consumed in tropical regions, and its processing generates a large amount of waste, particularly peels and fibrous residues (Verma et al., 2022). These wastes contain essential components such as cellulose, lignin, and a high proportion of starch (around 60–70%). The peel, in particular, has a substantial amount of lignocellulosic biomass, which requires an effective pretreatment process. To address this, the jackfruit waste is treated with dilute sulfuric acid and subjected to

heating at temperatures ranging from 100–120°C. This treatment not only helps in breaking down the lignocellulosic matrix but also promotes starch gelatinization, making the material more suitable for enzymatic action (Kalkan et al., 2026).

## 2.5 Hydrolysis

Hydrolysis is a chemical decomposition in which water molecules break bonds in a substance. Here complex starch chains are broken down into simpler units like dextrin, isomaltose, maltose or glucose. Water alone is slow so catalysts are used to increase reactivity. Science is messy. The choices in industry are enzymes, concentrated acids, and dilute acids. Some says enzymes offer more precision, better control and lower refining costs. In fact, these biological catalysts suffer from high cost and slow activation rates (Camillo et al., 2023). That is the fact. With respect to dilute acid catalysts, the advantage of smaller volumes and shorter residence times in the batch is seen. Unfortunately, the high temperatures needed during this particular approach often decrease total sugar concentration. Engineering is the art of compromise and of risk mitigation. If you don't take longevity of equipment into account, acidic environments will eventually bite back. Ultimately, success is a matter of local conditions and availability of raw material. Choosing a way forward requires careful analysis of cost, time and potential machinery failure. In the lab, every choice is significant (A.C. Litébé et al., 2025). All successful trials are a question of balance. Knowing these physical limitations is the key to selecting the right catalyst, because there is no perfect solution with no drawbacks. Engineering is a trade-off, and measured

mitigation of risk. If you don't factor in equipment longevity, the acidic environment will eventually bite back. Concentrated acid catalysts work well at lower temperatures and still give high yields of the desired sugars. Corrosion is a still significant issue, especially when iron equipment is exposed to such aggressive chemistry. Broken machinery is a huge headache to deal with. Moreover, this method requires long reaction times, which are often in the range of two to six hours. In these arrangements, scientists often trade speed for efficiency (Gupta et al., 2025)

## 2.6 Acid hydrolysis

The fruit waste fine powder was subjected to acid hydrolysis using sulphuric acid 20ml of different concentrations i.e. 2%, 4%, 6%, 8% and 10%. The mixture was autoclaved at 121°C, 15psi for 20 min and cooled to room temperature. The hydrolysate was filtered to remove the residues. The hydrolysate was neutralized with Sodium hydroxide. The total amount of total carbohydrates, glucose and xylose in the filtrates were determined. This served to narrow down the concentration of H<sub>2</sub>SO<sub>4</sub> at which best hydrolysis was obtained. The total carbohydrates obtained from Indian water chestnut waste, sweet potato waste, pineapple waste and jackfruit waste by using different concentrations of H<sub>2</sub>SO<sub>4</sub> (Camillo et al., 2023).

## 2.7 Preparation of the fermentation medium

The neutralized hydrolysate pH 5.6 was supplemented with 1.6g of Peptone. It was autoclaved at 15 psi, 121°C for 15 mins used as fermentation medium. The glucose amount was estimated using the dinitro

salicylic acid method (Gonzalez et al., 2023).

## 2.7 Fermentation process

Fermentation is a natural yet powerful biological process that acts like a catalyst, driven by microorganisms such as bacteria, fungi, and especially yeasts. In this process, complex carbohydrates present in fruit wastes are first broken down into simpler sugars, which are then converted into useful end products like organic acids or alcohol. In the case of bioethanol production, this conversion typically results in an alcohol concentration of around 8–10%, depending on the substrate and conditions used (Atiku et al., 2024).

One of the simplest approaches to fermentation is spontaneous fermentation, where naturally occurring microorganisms present in the raw material initiate the process without external intervention. This method is attractive because it is low-cost and does not require sophisticated equipment, making it accessible for small-scale producers. However, it comes with significant challenges (Kalkan et al., 2025). The process is often slow and difficult to control, and there is always a risk of contamination by unwanted microorganisms. Such contamination can reduce yield, affect product quality, and sometimes even spoil the entire batch. In practical terms, while spontaneous fermentation is economical, it can be unpredictable and inconsistent.

To overcome these limitations, controlled or non-spontaneous fermentation is widely used in scientific and industrial applications. In this method, a specific and pure microbial culture—most commonly *Saccharomyces cerevisiae*—is introduced

into a sterile fermentation medium. This yeast species is highly preferred because of its efficiency in converting sugars into ethanol and its tolerance to alcohol. The process begins by inoculating a known quantity of the pure culture into sterilized fermentation broth under aseptic conditions. The flasks are then incubated at room temperature, either under static conditions or with continuous shaking to ensure proper mixing and oxygen distribution where required (Firechiwot et al., 2024).

Before inoculation, the cell density of the yeast suspension is carefully measured using a haemocytometer to ensure consistency and accuracy. For example, a measured volume of about 2 ml of culture, with a cell density of approximately  $0.529 \times 10^6$  cells per  $\text{mm}^3$ , is added to the fermentation medium. Once introduced, the microorganisms begin metabolizing the available sugars, producing ethanol as a primary product. Samples are collected at regular time intervals to monitor the progress of fermentation (Meganath et al., 2019).

To quantify ethanol production, the fermented broth is first distilled, and the distillate is then analysed using the dichromate method. In this technique, a standard calibration curve is prepared using known concentrations of alcohol (typically ranging from 0.2 to 1.0 mg/ml). The ethanol content in the sample is then determined by comparing it with this standard graph, ensuring accurate estimation of the final yield (Mulopo et al., 2022).

In the broader context of bioethanol production, fermentation of fruit wastes plays a crucial role. These wastes contain a

mixture of sugars derived from starch and lignocellulosic materials. To maximize ethanol yield, different processing strategies are used. Some methods focus on hydrolysis followed by fermentation, while others aim to simultaneously convert both glucose (from cellulose) and pentose sugars (from hemicellulose). Efficient utilization of both types of sugars is important for improving overall productivity (Teymouri et al., 2025).

Despite the advantages of controlled fermentation, it requires strict regulation of process parameters such as temperature, pH, and sterility. This increases operational costs due to the need for specialized equipment and maintenance. On the other hand, spontaneous fermentation reduces these costs but sacrifices reliability and efficiency. Thus, the choice between these methods depends on the scale of production, available resources, and desired product quality (Verma et al., 2022).

### 2.7 Distillation

Recovery of ethanol in downstream processing starts with several unit operations after fermentation which are performed for the recovery of bioethanol from the fermentation broth. First, liquid-solid separation is carried out to get solid fractions including residual saccharides and bioethanol from the fermentation broth. Filtration and centrifugation is definitely the best solution for liquid-solid separation. To reduce the water content of the hydrolysate, the supernatant is transferred to a rotary evaporator. Serial evaporation attains pure condensate with concentrated syrup. Then comes evaporation, then distillation. The

bioethanol condensate will be circulated to the distillation unit (Cabas et al., 2023).

Separation of ethanol from Ethanol separation from the condensate is based on the difference of the boiling points of the mixture of water 100 C and bioethanol ~78 C. For very dilute solutions of water and ethanol, multiple distillations are preferred to obtain >95% concentration of ethanol. The recovery of bioethanol by distillation achieves 99.6% efficiency for reducing the losses of the evaporated part of bioethanol. condensate is based on the differences in the boiling points of water 100C and bioethanol ~78C mixture. If water and ethanol solution is very dilute, repeated distillation is preferred to attain >95% of ethanol concentration. Bioethanol recovery using distillation attains 99.6% efficiency to minimize the losses of the evaporated portion of bioethanol (Saputro et al., 2023).

### III. CONCLUSIONS

The selection of fruit wastes such as Indian water chestnut, sweet potato, pineapple and jackfruit was based on the carbohydrate content of the fruit. *Saccharomyces cerevisiae* is selected due to its ability to convert sugar into ethanol by fermentation process. Pure culture of *Saccharomyces cerevisiae* was obtained. Monochrome staining was used to check its purity and its growth characteristics were studied. Pure cultures of *Saccharomyces cerevisiae* were kept on agar slants. Subcultures were routinely done at 1-2 weeks. Study was done on culture grown for 24- 48 hr.

The waste of vegetables and fruits could be used as raw material for bioethanol production. In terms of availability, the

number is increasing each year with the growth of the human population. Meanwhile, hemicellulose, cellulose and sugar are in relatively high amounts from the constituent components in several types of vegetable and fruit waste such as sweet potato and pineapple peel. This process stages become a challenge in the conversion of the waste to bioethanol. But, from previous research done, it is not impossible to obtain bioethanol with high purity from these raw materials. It is recommended that further research be conducted on the production of bioethanol from a mixture of fruit and vegetable waste

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