# Bioconversion of Starch Base Food Waste into Bioethanol

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Food wastes are organic wastes or biodegradables. They are generated from various sources such as restaurants and cafeterias, industrial sectors, commercial and domestic kitchens, food processing plants, and other areas where a large number of people consume food. The global demand for fuel keeps increasing daily. The massive depletion of fossil fuels and their influence on the environment as pollution is a severe problem. Meanwhile, food waste disposal is also a complex problem in solid-waste management since one-third of every food consumed is discarded as waste. The standard waste management methods, including food waste incineration and landfilling, are considered hazardous to the environment. Food waste constituents are majorly starch-based and contain various biomolecules, including sugar, lipids, proteins, vitamins, cellulose, etc. These polysaccharides can be hydrolysed into monosaccharides such as glucose, which can then be fermented using microorganisms to produce ethanol through the fermenting of sugars derived from enzymatic hydrolysis treatment of food wastes. The human food system is rich in starch, which can be a potential resource for bioethanol production.

Keywords: starch ; food waste ; bioethanol

## 1. Bioethanol Production on Starch-Based Food Wastes

Bioethanol is generated through the fermenting of simple sugars found in biomass as well as sugars derived from earlier enzymatic hydrolysis treatment of food wastes [1]. Fermentation is then carried out by microorganisms, generally yeasts. However, bacteria such as *Zymomonas mobilis* [2] have also been utilised. Co-culture of *S. cerevisiae* and *P. stipitis* leads to higher ethanol yield of 0.13 ± 0.01 g/g of food waste [3]. Following fermentation, the ethanol produced is recovered from the fermentation medium using either traditional rectification and distillation or more efficient separation techniques such as membrane filtration, pervaporation, or molecular sieves. **Figure 1** depicts a schematic of starch-based bioethanol manufacturing.

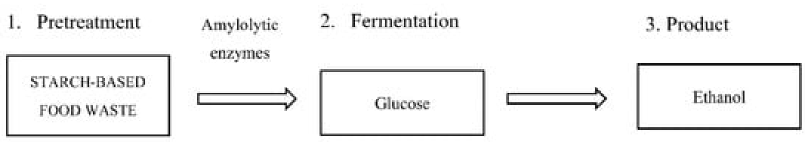


**Figure 1.** Bioethanol production on starch-based food waste.

## 2. Pretreatment

Food waste comes in a variety of forms. It can either be in raw or in cooked form. Because it is regarded as waste, it necessitates some preprocessing before it can be processed for the production of ethanol [4]. Physical, chemical, and physio-chemical pretreatments have been used in this manner. Pretreatment can be used depending on the nature of the food waste. In most circumstances, extensive pretreatment prior to enzymatic hydrolysis is not required. Various modified hydrolysis and enzymatic hydrolysis are conducted to boost ethanol output. Instead, autoclaving food wastes before fermentation is frequently required to increase the purity and yield of the product, albeit at the expense of increased energy and water usage. It should be mentioned that heat treatment might cause a partial breakdown of sugars and different biological function components and side reactions (e.g., the Maillard reaction) in which the quantity of beneficial amino acid and sugars square measure could be reduced [5].

Furthermore, recent and wet food waste appears to be far more efficient than rewetted dried food waste [6]. This is due mainly to the surface area of the dried substrate, which manifests in the substrate–enzyme reaction efficiency. Consequently, drying food waste is beneficial for high-yielding ethanol with controlled contamination by microorganisms.

Contamination by microorganisms can be avoided in acidic conditions without thermal treatment. As a result, acid-tolerant alcohol microbes such as *Zymomonas mobilis* were used for fermentation [6].

## 3. Starch Hydrolysis

Starch hydrolysis is an essential stage in starch-based food waste processing for bioethanol generation. The primary function of this process is to convert two key starch polymer constituents, branched amylopectin, a *α*-D-(1-4)-glucan with *α*-D-(1-6) linkages at the branching, and amylose, a mainly linear *α*-D-(1-4)-glucan, to simple sugars that can then be turned to alcohol by microorganisms (bacteria and yeast). Acids can be used to perform hydrolysis, an older method that has mostly been abandoned in favour of a more effective enzymatic method. Recently, some researchers have also used bacterial consortia for this purpose [7][8][9][10]. Starch-based bioethanol production has been widely popular for around 30 years; during that time, enormous advances in process cost, enzyme efficiency, time reduction, and increased hydrolysis and bioethanol production have been accomplished [11]. Current discoveries in the development of thermostable *α*-amylases, which are starch hydrolysing enzymes that catalyse the hydrolysis of internal *α*-D-(1-4)-glucosidal linkages in starch in a random fashion, and efficient glucoamylases, that are saccharifying starch enzymes that catalyse the hydrolysis of *α*-D-(1-6)- and *α*-D-(1-4)-glycosidic bonds in starch to glucose have brought about the commercial establishment of the popular two-step enzymatic cold process. The main benefits of this technique are the consumption of lesser energy and a reduced proportion of non-glucosidal contaminants, making it considerably more suitable for ethanol synthesis. Enzymatic starch hydrolysis is carried out under relatively mild operative conditions: lower temperatures (up to 100 degrees Celsius), normal pressure, and a pH of between 6–8 [12]. The quantity of endogenous enzymes used in starch hydrolysis, and the hydrolysis parameters, including temperature, process time, concentration, pH, etc., are influenced by the type of food waste, its chemical composition, the source and activity of endogenous enzymes, and the presence of native autoamylolytic potential. Additionally, primarily physical treatments, such as cooking and steaming, micronisation, grinding, ultrasound, microwave, and so on, enhance the gelatinisation process and the susceptibility of the food waste substrate to enzymes, and can strongly impact and enhance the influence of hydrolysis and subsequent fermentation of ethanol [11].

## 4. Fermentation

Efficient bioethanol production necessitates an accelerated fermentation that results in high ethanol concentrations; consequently, the microbial strain used should possess a good specific growth rate and specific ethanol production rate at high ethanol concentration and high osmotic pressure [13]. A critical problem for efficient ethanol production is optimising the fermentation phase in terms of the following key parameters: pH, temperature, the composition of the medium, aeration, mixing, elimination of infection, etc. [14].The fermentation phase is carried out under temperature range of 28–32 °C, and pH range of 4.8–5.0 [15]. Additionally, anaerobic digestion produces an acidic substrate, which could interfere with the fermentation process [15][16]. It is critical to select and develop an efficient production microorganism. As a result, much research is currently being conducted to develop a microorganism resistant to high concentrations of substrate and ethanol. A yeast strain's ability to produce a high level of alcohol is significantly dependent on the nutritional conditions and protective activities that specific nutrients can supply [17].

At 14% (*v*/*v*), the threshold for ethanol production from starch fermentation is reached [18]. Over this threshold, the growth of the microbes responsible for fermentation is inhibited, and creative approaches are applied to overcome this limitation. The immobilisation of yeast or the fermentation microorganism for bioethanol production has been extensively researched to overcome substrate and product inhibition and enhance ethanol tolerance. Among these approaches, the most studied are yeast immobilisation in/on appropriate matrices like poly-acrylamide-alumina calcium, k-carrageenan gel, alginate, orange peel, PVA gel, wooden chips, etc. [11]. Bai et al. [19] prioritised self-flocculation and simple adsorptive immobilisation techniques because these allow slow developing cells to be removed from the system. The most challenging research on the subject is obtaining a fermentation microorganism with a metabolism that would enable the utilisation of a broader sugar spectrum and thus facilitate complete substrate utilisation [11]. These are the most common applications of technologies of genetic engineering.

## 5. Methodologies for Enhanced Bioethanol Production

Optimising the substrate medium is one of the most common ways to boost ethanol production. This process can be accomplished utilising various strategies from one-factor-at-a-time to multifactor-at-a-time [20][21] as well as advanced mathematical and statistical techniques such as artificial neural networks, genetic algorithms, etc. [20][21][22][23]. The optimisation of substrate medium entails the formulation of a fermentation medium through screening different carbon and nitrogen sources and their combinations to improve the viability and growth of the ethanologenic microorganisms and, as a result, the production yield of ethanol. Adding cauliflower and/or cabbage waste to molasses increased ethanol production yield by 40.8–52.6% compared to using only molasses [24]. The optimisation of the substrate can be improved by employing the metagenomic method, whereby it offers insights into the metagenome-based bioinformatic roles of unstudied microorganisms [9].

In complimenting the efforts of medium optimisation, strain enhancement via genetic engineering approaches has been used to boost the yield of bioethanol. It should be highlighted that, during the optimisation of a fermentation medium, genetic manipulation or the search for novel ethanologens must constantly be considered. This requirement stems from each microorganism's inability to synthesise certain metabolites at the gene level [23]. The development of ethanologenic bacteria can be accomplished in three ways: (i) by replacing or introducing heterogeneous genes from a potent ethanol-producing strain; (ii) by overexpressing the native genes which are responsible for ethanol synthesis; and (iii) by eliminating native metabolic pathways, they could compete with ethanol production (e.g., hydrogen and organic acids) [25].

'Separate hydrolysis and fermentation' and 'Simultaneous saccharification and fermentation' techniques have been used in enhancing bioethanol yield from food wastes (**Table 1**). Traditional fermentation can also be combined with innovative technologies to boost bioethanol production. Electrochemistry is one of the innovative technologies which allows for regulating the metabolism of microbial fermentation [26]. Incorporating this selective technique may improve sugar assimilation efficiency, improve cell growth, and product recovery while reducing the need for pH control chemicals [26]. The use of electrodes that can operate as an electron source or act as an electron sink has been implicated with the unbalanced growth of microbial cells. These electrochemical changes have the potential to have a large selective effect on the population of microbial cells, interactions of interspecies, metabolism, and cellular regulation [26]. Joshi et al. [26] employed *Wickerhanomyces anomalous* in a cathodic chamber and *Saccharomyces cerevisiae* in an anodic chamber. When the electrochemical cell was fed externally with 4 V, the cultures yielded 19.8 and 23.7% more ethanol when compared to the controls (12.6 and 10.1 g/L, respectively). Culturing *Saccharomyces cerevisiae* in a platinum nanoparticle-coated anodic chamber and *Wickerhamomyces anomalous* in a neutral red-coated graphite cathode considerably increased the production yield of bioethanol (61.5%) from lignocellulosic biomass hydrolysate with a 3.3% reducing sugar concentration [26].

**Table 1.** Production of ethanol from food wastes with monoculture.

| Method | *Microorganism* | Enzyme Used | Process Parameters | Ethanol (g/L) | Reference |
| --- | --- | --- | --- | --- | --- |
| **Simultaneous saccharification and fermentation** | ***S. cerevisiae*—*Fusarium oxysporum*** | **on-site produced enzymes glucoamylase** | **Ratio I:FW = 1/10 *w*/*w*<br>$C_i$ = 30% *w*/*v*<br>pH = 6.0<br>T = 30 ± 1 °C<br>t = 94 h<br>Agitation = 80 rpm<br>Mode = Batch** | **30.8** | [27] |
| **Open fermentative production** | ***Zymomonas mobilis*** | | **Ratio I:FW = 10% *v*/*v*<br>$C_i$ = 200 g glucose/L<br>Initial pH = 4<br>T = 30 °C<br>t = 44–48 h<br>Agitation = 100 rpm<br>Mode = Batch** | **99.78** | [28] |
| **Separate hydrolysis and fermentation** | ***S. cerevisiae*<br>(dry baker's yeast)** | **on-site produced enzymes** | **Ratio I:FW = 15 mg/g solids<br>$C_i$ = 25 g hydrolyzed FW/100 mL<br>pH = 4.5<br>T = 30 °C<br>t = 48 h<br>Agitation = 100 rpm<br>Mode = N/A** | **19.27** | [29] |

| Method | *Microorganism* | Enzyme Used | Process Parameters | Ethanol (g/L) | Reference |
|---|---|---|---|---|---|
| **Separate hydrolysis and fermentation** | ***S. cerevisiae*** **(dry baker's yeast)** | **on-site produced enzymes** | **Ratio I:FW = 10% *v*/*v*<br>$C_i$ = 116 g/L<br>pH = 4.5<br>T = 30 °C<br>t = 72 h<br>Agitation = 100 rpm** | **58.0** | [30] |
| **Simultaneous saccharification and fermentation** | ***S. cerevisiae*** **(dry baker's yeast)** | **Cellulase** | **Ratio I:FW = 10% *v*/*v*<br>$C_i$ = 64.8 ± 1.8 g/L<br>pH = 4.5<br>T = 30 °C<br>t = 48 h<br>Agitation = 150 rpm** | **23.3** | [31] |
| **Separate hydrolysis and fermentation** | ***S. cerevisiae*** | **Glucoamylase, amylase** | **Ratio I:FW = 1 mL to 50 mL<br>$C_i$ = 5.4 mg/mL<br>pH = 6<br>T = 30 °C<br>t = 24 h<br>Agitation = 150 rpm** | **8.0** | [32] |
| **Simultaneous saccharification and fermentation** | ***S. cerevisiae*** | **Carbohydrase, glucoamylase, amylase** | **Ratio I:FW = N/A<br>$C_i$ = 30 g/L<br>pH = 4.5<br>T = 35 °C<br>t = 14 days<br>Agitation = N/A** | **44** | [33] |
| **Simultaneous saccharification** | ***S. cerevisiae*** | **Glucoamylase** | **Mode = Continuous<br>Ratio I:FW = N/A<br>$C_i$ = N/A<br>pH = 4.18<br>T = 35 °C<br>t = 67.6 h<br>Agitation = N/A<br>Mode = open batch fermentation** | **33.05** | [34] |

Note: Ci = Initial substrate concentration, Ratio I:FW = Ratio of inoculant to food waste, N/A indicates that information is not available.

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