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Review

Bioconversion of Starch Base Food Waste into Bioethanol

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Abstract: 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: bioconversion; food waste; bioethanol; starch

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1. Introduction

Lately, the widespread utilisation of fuels has resulted in a decrease in the availability of fossil fuels. Petroleum-based fossil fuels, in particular, are challenging to manage. Bioethanol has been offered as an alternative source by combining it with petrol in a proportionate ratio to meet the global need for biofuel [1]. Ethanol is utilised as a fuel because it has various advantages, including low price, a lower thermal energy content (approximately 45% less per gallon than diesel), and reduced pollutants than diesel or gasoline. Furthermore, because ethanol has a higher octane number (99) than gasoline (80–100), pre-ignition does not arise when it is utilised. As a result, ethanol is commonly utilised as a competing fuel additive with gasoline but rarely in its pure form [2].

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. Food waste disposal is a complex problem in solid-waste management since one-third of every food consumed is discarded [3]. About 1.3 billion tonnes of food are lost along the food production chain, including fruits, fresh vegetables, bakery, meat, and dairy products [4]. Due to population and economic expansion, the quantity of food waste is estimated to rise over the next 20 years. Food depletion, on the other hand, is a major concern; this means that the number of hungry people will keep increasing. A responsible bioeconomy can convert organic wastes, including food wastes, into essential resources and create incentives and innovations to assist retailers and customers in reducing food waste by half [5]. However, the usage of food waste and the bioconversion of food waste remains limited. This is owing to present limits in its quantification throughout the global food supply chain, poor data on its homogeneity and quality, and the differences in national waste regulation implementation [5]. These food wastes are mainly disposed of

by composting, incinerating, or landfilling. However, these are harmful to the ecosystem. Traditionally, food waste is burnt alongside combustible municipal waste to generate energy or heat. Other uses for food wastes include animal and livestock feed and biogas generation and valorisation.

Food waste composition is not steady. It varies significantly depending on the season, the region, and the population's dietary habits. Despite the inevitability of diversity in the content of food waste, it is undeniably rich in carbohydrates, lipids, minerals and proteins, making it an attractive raw material for the manufacture of biofuels via microbial conversion [6,7]. Generally, mixed food wastes are utilised in the production of ethanol. Food wastes contain a variety of biomaterials as polysaccharides, including carbohydrates, starch, proteins, cellulose, lipids, amino acids, and vitamins. These excellent biomaterials ought to be a promising source for the synthesis of bioethanol. However, in nature, polysaccharides cannot be directly converted to ethanol by microorganisms.

There are two main research efforts on the fermentation of food waste into bioalcohol; hydrolysis pretreatment and mixed cultures [8–11]. Both these methods involve the solubilisation of polysaccharides into monosaccharides like glucose prior to fermentation. There are three main hydrolysation methods: enzymatic hydrolysation [12], cellulose hydrolysation [13], and acid hydrolysation [14]. To break down raw material, acidic and physical pretreatment prior to hydrolysis are required. Grinding, filtration, and hydrothermal treatment are all examples of physical preparation. Acidic treatment involves using acids such as H_2SO_4, HCl, and others. When utilising food wastes, such pretreatment is usually unnecessary because hydrolysation acts as a pretreatment. A modified acid-enzymatic pretreatment was developed by Hafid et al. [14] to increase the amount of fermented sugar. Polysaccharides are transformed into monosaccharides during hydrolysis, which can then be fermented by microorganisms to produce ethanol. The enzymatic treatment produced by mixed cultures has been possible since the 19th century in the form of Amylolytic starters in the form of a thick starchy hard cookie, but it was more of a black box process as the kinetics of conversion from polysaccharides to glucose was not clearly understood. It was known that the principal amylolytic moulds are *Amylomycess rouxii*, *Rhizopus* spp. *Mucor* spp. and *Aspergillus* spp. [15]. With the advancement in technology, metagenomic sequencing has recently been used [16] to analyse fermentation processes and has been key in understanding many unculturable and unstudied microorganisms.

The utilisation of food waste to manufacture biofuels is also consistent with the United Nations' 2030 Agenda for Sustainable Development, established in 2015 [17]. Food waste contains a significant moisture level, resulting in the creation of dioxins during combustion alongside other wastes with high calorific value and low humidity [18]. Carbohydrate hydrolysis in food waste may occur in the breakdown of glycoside linkages, releasing monosaccharides and oligosaccharides that are more fermentable. Food waste's total protein and sugar compositions range from 3.9 to 21.9% and 35.5 to 69%, respectively. As a result, food waste has been employed as the only microbial feedstock for the production of a variety of value-added bio-products, including methane, ethanol, hydrogen, enzymes, biopolymers, bioplastics, and organic acid. Biofuels ($200–400/tonne biomass) have a higher value than animal feed ($70–200/tonne biomass) and electricity ($60–150/tonne biomass). Because of its inherent chemical complexity, food waste can also be used to produce high-value commodities like biodegradable polymers, organic acids, and enzymes ($1000/tonne biomass) [18].

Technically, bioethanol synthesis is a well-studied technique that has recently been reviewed [19]. Pretreatment, enzymatic hydrolysis, fermentation, and ethanol recovery are all part of the process. The pretreatment step tries to change the structural properties of the raw material so as to facilitate enzyme access and maximise the synthesis of sugar monomer. Depending on the texture and type of the food waste, pretreatment can include size reduction, heat treatment and/or drying. The structural carbohydrates starch, hemicellulose, and cellulose are targeted by enzymatic hydrolysis. Hexoses and pentoses that can be utilised in the fermentation process are freed during this step. Microorganisms digest the

readily available carbohydrates in the following fermentation step, creating ethanol, which is then extracted through distillation. Enzymatic hydrolysis is the most expensive stage in bioethanol synthesis, greatly increasing the total cost and acting as a barrier to the deployment of bioethanol production [20]. Two solutions are proposed to meet this challenge. The first is to produce the appropriate enzymes on-site rather than using enzymes that are available commercially [21]. The second is to use locally sourced multi-cultures studied and understand using metagenomic sequencing for commercial production [8,16,22–24]. The fermentation produces various enzymes, and via a metagenomic study, it may be possible to manipulate the fermentation to suit the required need to reduce product inhibitors and/or increase bioalcohol production. Few organisms can manufacture the required enzymes. This article will go over the bioconversion of starch base food waste for producing several types of biofuels such as hydrogen, ethanol, biodiesel, and methane through the synthesis of quantitative and qualitative research results from quantitative and qualitative studies. It also explained the methodologies of 'separate hydrolysis and fermentation' and 'simultaneous saccharification and fermentation' for enhanced bioethanol production.

2. 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 [25]. Fermentation is then carried out by microorganisms, generally yeasts. However, bacteria such as *Zymomonas mobilis* [26] 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 [9]. 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.

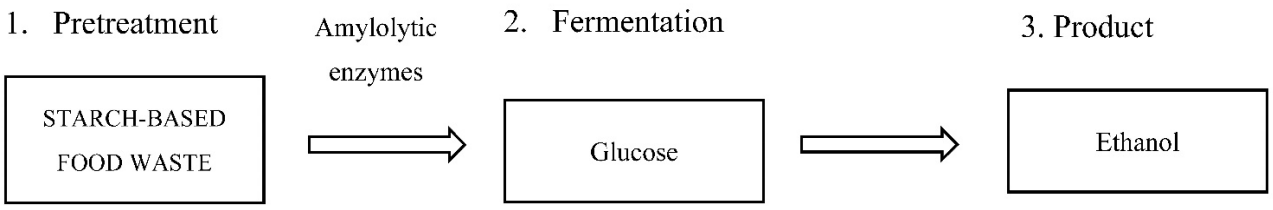

Figure 1. Bioethanol production on starch-based food waste.

3. 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 [27]. 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 [28].

Furthermore, recent and wet food waste appears to be far more efficient than rewetted dried food waste [1]. 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 [1].

4. 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 [16,22–24]. 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 [29]. 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 [30]. 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 [29].

5. Fermentation

The metabolic pathway and ethanol fermentation are illustrated in Figure 2. 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 [31]. 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. [32].The fermentation phase is carried out under temperature range of 28–32 °C, and pH range of 4.8–5.0 [33]. Additionally, anaerobic digestion produces an acidic substrate, which could interfere with the fermentation process [33,34]. 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 [35].

At 14% (v/v), the threshold for ethanol production from starch fermentation is reached [36]. 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, algi-

nate, orange peel, PVA gel, wooden chips, etc. [29]. Bai et al. [37] 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 [29]. These are the most common applications of technologies of genetic engineering.

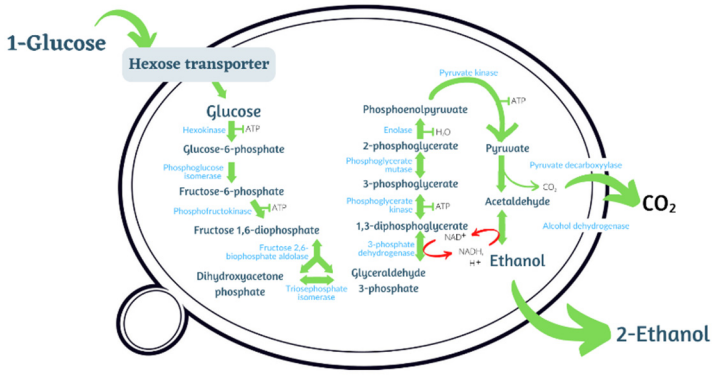

Figure 2. Metabolic pathway and ethanol fermentation [38–40].

6. 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 [41,42] as well as advanced mathematical and statistical techniques such as artificial neural networks, genetic algorithms, etc. [41–44]. 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 [45]. 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 [23].

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 [44]. 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) [46].

'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 [47]. Incorporating this selective technique may improve sugar assimilation efficiency, improve cell growth, and product recovery while reducing the need for pH control chemicals [47]. 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 [47]. Joshi et al. [47] em-

ployed *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 [47].

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* C_i = 30% *w*/*v* pH = 6.0 T = 30 ± 1 °C t = 94 h Agitation = 80 rpm Mode = Batch	30.8	[10]
Open fermentative production	*Zymomonas mobilis*		Ratio I:FW = 10% *v*/*v* C_i = 200 g glucose/L Initial pH = 4 T = 30 °C t = 44–48 h Agitation = 100 rpm Mode = Batch	99.78	[48]
Separate hydrolysis and fermentation	*S. cerevisiae* (dry baker's yeast)	on-site produced enzymes	Ratio I:FW = 15 mg/g solids C_i = 25 g hydrolyzed FW/100 mL pH = 4.5 T = 30 °C t = 48 h Agitation = 100 rpm Mode = N/A	19.27	[49]
Separate hydrolysis and fermentation	*S. cerevisiae* (dry baker's yeast)	on-site produced enzymes	Ratio I:FW = 10% *v*/*v* C_i = 116 g/L pH = 4.5 T = 30 °C t = 72 h Agitation = 100 rpm	58.0	[50]
Simultaneous saccharification and fermentation	*S. cerevisiae* (dry baker's yeast)	Cellulase	Ratio I:FW = 10% *v*/*v* C_i = 64.8 ± 1.8 g/L pH = 4.5 T = 30 °C t = 48 h Agitation = 150 rpm	23.3	[51]
Separate hydrolysis and fermentation	*S. cerevisiae*	Glucoamylase, amylase	Ratio I:FW = 1 mL to 50 mL C_i = 5.4 mg/mL pH = 6 T = 30 °C t = 24 h Agitation = 150 rpm	8.0	[52]
Simultaneous saccharification and fermentation	*S. cerevisiae*	Carbohydrase, glucoamylase, amylase	Ratio I:FW = N/A C_i = 30 g/L pH = 4.5 T = 35 °C t = 14 days Agitation = N/A	44	[53]
Simultaneous saccharification	*S. cerevisiae*	Glucoamylase	Mode = Continuous Ratio I:FW = N/A C_i = N/A pH = 4.18 T = 35 °C t = 67.6 h Agitation = N/A Mode = open batch fermentation	33.05	[54]

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

7. Separate Hydrolysis and Fermentation

Separate hydrolysis and fermentation are a technique of separately performing enzymatic hydrolysis and fermentation successively. Enzymatic saccharification of starchy

biomass is performed initially in this method at the saccharifying enzyme's optimum temperature. Following that, suitable microorganisms are introduced for the fermentation of the saccharified mixture. The temperatures of fermentation and enzymatic hydrolysis can be regulated individually in this process. Enzymatic hydrolyses require fewer saccharifying enzymes than simultaneous saccharification and fermentation since they are accomplished at an optimal temperature [55].

Furthermore, the contamination risk is lowered since saccharified liquid containing fermentable sugar can be sterilised. The separate hydrolysis and fermentation method, on the other hand, is done in two different processes, which require two independent bioreactors for the fermentation and saccharification processes; as a result, the cost of the simultaneous process is lower than that of the capital cost [55].

8. Simultaneous Saccharification and Fermentation

Simultaneous saccharification and fermentation obtain value-added products by combining enzymatic hydrolysis and fermentation in a single stage. This method relies on the utilisation of an enzymatic complex in hydrolysing cellulose to produce sugars. These sugars are then utilised by microbes to create value-added goods [56]. Apart from the utilisation of enzymatic hydrolysis, mixed culture fermentation can be used (see Table 2); several studies have been conducted using metagenomic sequencing to develop the metagenome-based bioinformatics that can assist in the optimisation of the mixed culture fermentation [16,22–24]. Although these works were not in producing bioalcohol, the technology can be applied to food waste bioconversion to bioalcohol using mixed culture. Several research works were done on mixed cultures to convert food waste into bioalcohol [8–11,57].

Table 2. Production of ethanol from food wastes with mixed culture.

Food Waste	Pretreatment Method	Fermentation Method	Mixed Culture Name	*Main Types of Microorganism*	Outcome	Reference
Food waste, South Dakota, USA		Co-culture of thermophilic microbes in serum bottles, 1 L DASGIP reactor and sequential cultivation		*G. thermoglucosidasius (ATCC 43742) and T. ethanolicus (ATCC 31938)*	*α-amylase* and *amyloglucosidase* activities higher in 1 L DASGIP than serum bottles. Sequential cultivation improved the ethanol yield to 16.1 g/L in 1 L bioreactor with 20% (*w*/*v*) of food waste. Scaling up to 40 L gave 18.4 g/L ethanol. 92% recovery of substrate and complete utilisation of sugars.	[8]
Household food waste, Halandri, Greece	1 g H_2SO_4/100 g dry food waste	Simultaneous saccharification and fermentation		*S. cerevisiae—Fusarium oxysporum*	Mixed culture increased bioethanol volumetric productivity compared to monoculture from food waste. Food waste contained 4.96% *w*/*w* dry basis of total reducing sugars. Ethanol yield was 20.6 g/L after 42 h.	[10]

Table 2. *Cont.*

Food Waste	Pretreatment Method	Fermentation Method	Mixed Culture Name	*Main Types of Microorganism*	Outcome	Reference
Coarse fibres of wet solid and dry solid sago waste	Delignification using NaOH and hydrolysis with HCl	Simultaneous saccharification and fermentation	*Tapai*		High bioethanol content (45.7021% *v*/*v*) was produced for wet sago waste with Tapai.	[11]
Kitchen food waste, Halandri, Greece	Heat drying (92 °C), shredding, and enzyme treatment (cellulolytic and amylolytic)	Simultaneous saccharification and fermentation		*Saccharomyces cerevisiae (CECT 1332) and Pichia stipites (CECT 1922)*	The co-culture produced maximum valorisation of the carbohydrates (~40 g/L) from the food waste. The maximum ethanol yield was 0.13 g/g of waste.	[9]
Sago waste, Johor, Malaysia	Microwave irradiation (with H_2SO_4), conventional heating and without pretreatment	Simultaneous saccharification and fermentation	*Ragi Tapai*	Various strains of fungus, yeast, and bacteria	Simultaneous fermentation converted the unhydrolysed starch into reducing sugar and produced 7.24 g ethanol/100 g sago waste.	[57]

When compared to separate hydrolysis and fermentation, simultaneous saccharification and fermentation have the following advantages: utilising a single reactor for saccharification and fermentation reduces residence periods and costs. Another significant advantage is the reduction of chemical inhibitors from enzymatic hydrolysis; this enhances the process's overall performance [58]. Simultaneous saccharification and fermentation have been intensively researched for manufacturing biofuels from starchy raw materials due to these advantages [59].

Simultaneous saccharification and fermentation have drawbacks that limit their application in the industry. For example, the optimum temperature for enzymatic hydrolysis is often higher than the temperature for fermentation. As a result, it is essential to discover that point of equilibrium where the process works appropriately [56].

9. Conclusions

Food waste management has become a major environmental and economic concern. According to this review, the bioconversion of food waste to bioethanol is economically feasible. Thus, producing bioethanol from food waste appears to be a potential way to meet worldwide ethanol demand and the demand for solid waste management with the production of up to 99.78 g/L of ethanol from food wastes. However, the problems involved with collecting and transporting food waste must also be considered. Nonetheless, the low or negligible cost of food waste and the environmental benefits of waste disposal would offset the initial investment costs of biorefineries. The efficiency and cost basis of bioethanol production might be improved further by conducting further research and optimisation studies on incorporating different processes of value-added product manufacturing.

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