

REVIEW

Production of Bioethanol using Waste Fruits under Acid and Alkali Catalytic Hydrolysis: A Review

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The present world highly depends on petroleum fuels to gain energy for transportation resulting in the vast side of environmental problems such as global warming and air pollution. Due to this, the price of conventional fuel escalating day by day. Accordingly, the world needs renewable, ecologically suitable, cost-effective alternate against fossil fuels. Bioethanol is one of the most usable fuel or fuel additives among the other biofuels. Ingoing qualities of bioethanol such as high-octane number, high oxygen content, and low energy content are revealed that application of bioethanol produced from different types of waste materials feedstock in the transportation and energy sector diminishes environment pollution. It provides a solution for waste management. The world releases a considerable amount of fruits as waste annually. Thereby, fruit waste is the cheapest feedstock to produce bioethanol. Fruit waste such as whole rotten fruits, fruit peels, seeds and other residues consists of cellulose, hemicellulose, lignin, starch and simple sugars. Conversion of cellulose and hemicellulose to ethanol is vital to advance pretreatment and hydrolysis techniques to obtain maximum ethanol content. The production process of bioethanol from fruit waste mainly contains pretreatment; hydrolysis, saccharification, fermentation and ethanol extracting process (distillation) steps. Yeast (*S. cerevisiae*) is primarily used in the fermentation process because of its high conversion efficiency, cost-effectiveness and feasibility of handling. Considering the optimum configuration for bioethanol production, simultaneous saccharification and fermentation (SSF) is the best commensurate method having maximum bioethanol concentration. The fermentation process could be appreciated through various factors, such as temperature (30-33 °C), pH of the medium (4-5), time of incubation, feedstock concentration, inoculum size, agitating rate, N sources in the medium to gain high bioethanol concentration.

Keywords: Bioethanol, Fermentation, Fruit wastes, Hydrolysis, Lignocellulose biomass.

INTRODUCTION

Today, almost entirely of the transportation sector is reliant on petroleum-based fuels [1]. Otherwise, due to the increasing population and industrialization, the demand for fossil fuels is increasing day by day [2]. Moreover, fossil fuel consumption had created vast side problems in how it is responsible for more than 70% of global carbon monoxide (CO) emissions and 19% of global carbon dioxide (CO₂) emission [3,4]. This inevitable consumption of fossil fuel accounts for mainly global warming by the greenhouse effect on the earth [5]. In addition, fossil fuels are associated with various energy and security problems and they are non-evenly distributed within nations and equally non-renewable. Although the rapid consumption of conventional fossil fuels and their unpredictable prices, the

world needs to find alternative renewable energy sources such as biofuels [6,7].

Diminishing the difficulties mentioned above, many researchers tend to produce ecologically sustainable bio-fuels all over the world. There are two primary forms of biofuels viz. bioethanol and biodiesel, which account for more than 90% of worldwide biofuel use. Biodiesel is produced through a chemical process named transesterification [8]. Ethanol produced from the fermentation of renewable recourses for use as fuel or fuel additives is called bioethanol has advantageous applications in the industrial, transportation and energy sectors [9]. Bioethanol production is being done by several countries such as the USA, Brazil, Ukraine, Pakistan, South Africa and some countries in Asia [10,11].

Ethanol or ethyl alcohol is a volatile, colourless, flammable, liquid biofuel with a molar mass of 46.07 g/mol, a density

of 0.789 g/mL, a melting point of -114 °C and a boiling point of 78.37 °C. Due to its favourable chemical and physical properties, ethanol is used as solvent, alcohol beverages, antiseptics, household heating and chemical intermediate for other organic solvents, especially because of its versatility [6,12,13]. Also, bioethanol has great chemical and physical qualities, which are appropriate use as fuel for the transportation sector and energy sector. While bioethanol may be used as a fuel in its pure form, it is often utilized as a fuel additive [14]. Bioethanol can be blended up to 5-20% with conventional gasoline for enhancing octane number, cleaning combustion of engines (burns up to 75% than fossil fuel), reducing the emission of CO₂ and improving the air quality [7,15]. Common ethanol blending ratios are E5 (5% ethanol and 95% gasoline), E10 (10% ethanol and 90% gasoline). This blending requires no engine modification [16]. When considering the bioethanol qualities, it has high oxygen content (35% w/w), reducing hydrocarbon and CO emission [17,18]. It has high octane number (107) and high latent heat of vaporization (0.91 MJ/kg), preventing premature ignition and cylinder knocking and occurring spontaneous ignition in the internal engine [19,20]. Moreover, it also has low energy content (21.2 MJ/dm³) by increasing compression ratio and power, decreasing burn time [21,22]. Bioethanol with gasoline improves the air quality by checking emission of E0 (standard or 100% gasoline), E5 and E10 blending using bioethanol produced from rotten mango fruits [14].

At the industrial level chemical process also involves the synthesis of ethanol *viz.* a reaction between ethylene and steam in high temperature and pressure generates various adverse effects on the environment by releasing harmful fumes. Therefore, it consolidates that a biological process must be advantageous over a chemical process in bioethanol production [23,24]. Another way of ethanol production is alcoholic fermentation of reducing sugar, which is the biological process of converting sugar into ethanol by the action of microorganisms releasing CO₂ as a byproduct [3]. There are some advantageous effects on microbial fermentation in terms of low generation time, easy to operate downstream processing, low maintenance, low cost and ease of handling [25]. Basically, the microbial fermentation includes three steps *viz.* (i) making fermentable sugars; (ii) fermentation of sugars under optimum conditions and (iii) purification of ethanol produced is usually achieved by distillation [26].

According to literature, the first generation of bioethanol production used sugarcane and starchy crops *e.g.* corn, wheat, sugar beet and sorghum as feedstock [27]. Fruit wastes, also known as the first generation of bioethanol feedstock, becoming one of the richest sources of different fermentable sugars [24]. Second-generation bioethanol obtained from lignocellulosic materials such as energy crops, forestry products and solid wastes like sugarcane bagasse. Researchers tended to give attention to lignocellulosic material due to their abundance and immense potential for conversion into sugars and fuel [28]. When comparing first and second generations of bioethanol feedstock, sugary and starchy material can easily convert to bioethanol compared with lignocellulosic materials, which requires additional physical, chemical and enzymatic pretreatment techniques.

Therefore, there are limiting applications of commercialization and industrialization in developing countries. Due to this situation, the low cost of carbohydrate feedstock is considered to make bioethanol giving competitive demand in the world market [29].

Possible feedstocks for bioethanol production: Almost any plant-based resource may be used as a feedstock for ethanol synthesis, as all plant materials contain sugars in varying amounts depending on their biological variety. These sugars can be fermented into ethanol. Another possible ethanol feedstock is starch, which is composed of glucose molecule chains. These starchy materials in plant-based feedstocks can be converted into glucose molecules by hydrolysis with water (saccharification) and some starchy materials also need the enzymatic saccharification step [30]. Arumugam & Manikandan [29] analyzed the composition of banana and mango fruits. As they sowed, the starch content of fruit pulp ranged from 0.507% to 0.632% and from 1.074% to 1.706% ranged in fruit peels. At the ripening stage, several workers indicated that the starch could be converted into free sugars due to the combined action of enzymes contained in fruits. When comparing the fruit pulp and peels, in the fruit pulp, the starch degradation into free sugar is more rapid than in fruit peels. Confirming that Lima *et al.* [31] reported starch, the main carbohydrate in the mango fruit, becomes only trace after converting them into free sugars [31-34]. Cellulosic materials can be used for ethanol production by treating special enzymatic and chemical pretreatment, which convert cellulose into simple sugars. Hemicelluloses are another abundant material, originally believed to be intermediate in the biosynthesis of cellulose. It is a heterogeneous polymer of pentose (5C), hexoses (6C) and sugar acids. Due to hemicelluloses branched structure and amorphous nature, they are relatively easy to hydrolyze using chemical and enzymatic treatments. The cheapest and easily available source for bioethanol production is fruit waste due to the content of the sugar, starch, cellulose, hemicellulose and lignin in different levels following the biological nature of fruits [6,12,14,30,35].

According to Sanchez-Orozco *et al.* [36] lignocellulosic feedstock such as orange peels, orange bagasse, banana peels and mango peels were investigated to get proximate analysis of their carbohydrates content. They showed that the fruit residues have potential in the application as low cost and easily available raw materials because of their acceptable content of cellulose and hemicellulose and low amount of lignin. Their result shows orange bagasse contained a high amount of carbohydrates (0.591 g g⁻¹) among other fruit peels following banana peels (0.487 g g⁻¹) [36]. According to Mansouri *et al.* [37] over ripe grapes contain a high level of the sugar content of around 26 g in 100 g of grape juice, which is higher than in maize, sugarcane and beetroot. They evaluated approximate composition of mango fruit pulp as 81.26% moisture, 7.96% protein, 1.48% lipid, 13.08% ash and 0.507% starch. Also, mango peel and seeds have a high level of celluloses and hemicelluloses which can be readily hydrolyzed into fermentable sugars [14,29]. In banana fruits, cellulose, hemicellulose and lignin content were reported as 28.92%, 25.23% and 10.565%, respectively [38]. The composition of sugar, starch, celluloses,

hemicelluloses and others that can convert into simple sugars can vary within different types of fruits used as feedstock for ethanol production (Fig. 1) [39]. The yield of the bioethanol production is represent in Fig. 2 [40].

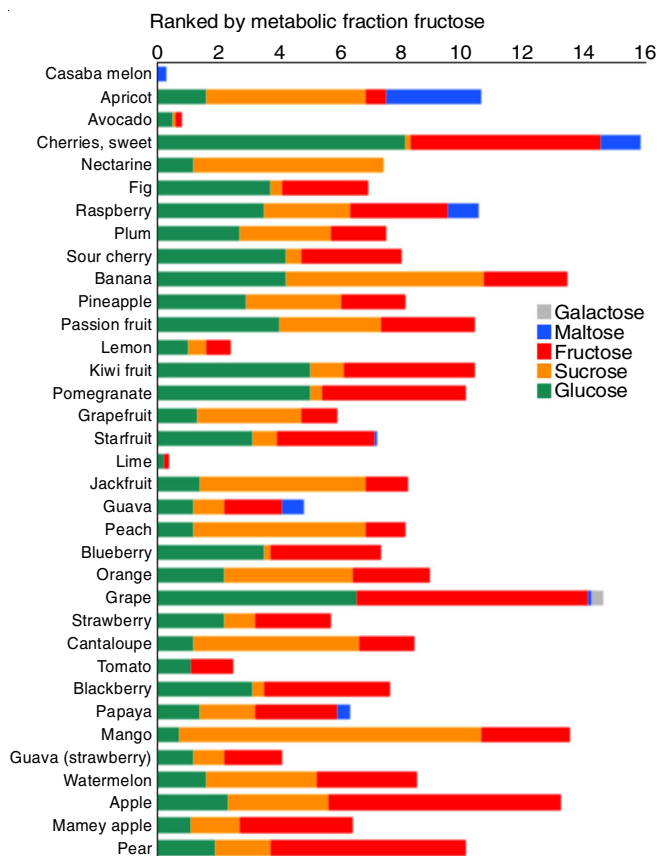


Fig. 1. Percentage of sugar composition in various fruits and vegetables [39]

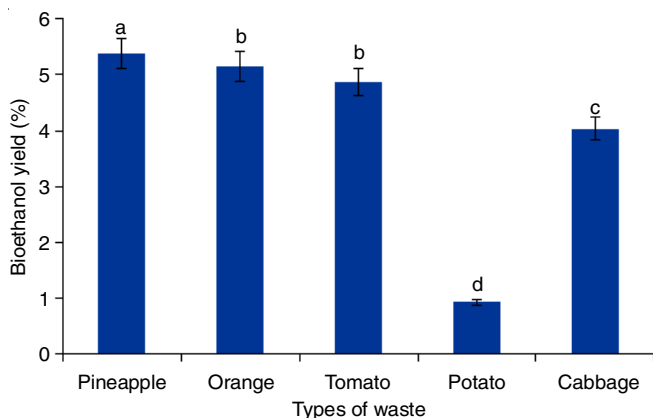


Fig. 2. Yield of bioethanol obtained from various wastes [40]

Another point is that when changes in the type of feedstock (biomass), slightly modification in bioethanol production can be observed (Fig. 3). Each process consists of common steps; pretreatment, hydrolysis, fermentation distillation and makes anhydrous ethanol. Steps before the fermentation steps involve not only sterilize the substrates but also making enzymes more accessible to improve the efficiency of bioethanol production [40].

In recent years, different types of study on fruit wastes, mostly fruits peels, produce bioethanol, proving that their abundance and containing materials can be converted into fermentable sugars [41,42]. Banana is the second large produced fruit of total world's fruit generation and their waste like banana peels contain useful reducing sugars, which can be used to produce ethanol by fermentation [24,43]. Waghmare & Arya [44] showed unripe banana peels are cost-effective lignocellulosic biomass to produce bioethanol yielding maximum ethanol concentration (35.6 g/L), productivity (1.5 g/L/h) and conversion efficiency (71.0%w/w) using *S. cerevisiae* NCIM 3095 under the optimum condition: pH (5), temperature (30 °C) and fermentation time (36 h). They used the acid hydrolysis method to get maximum ethanol yield using (1.5 %v/v) H₂SO₄. Another work used banana pseudostem as second-generation bioethanol feedstock. According to their observations, maximum ethanol (17.1 g/L) with 84% yield was achieved using *S. cerevisiae* NCIM 3570 after 72 h and they used enzymatic hydrolysis to achieve maximum ethanol yield from banana pseudostem activity of microorganisms *A. fumigatus* and *A. ellipticus* strain reported producing cellulase enzyme [45]. Vaidya *et al.* [46] have used banana peels, banana pseudostem and spoiled banana wastes in the comparative study of bioethanol production. As Matharasi *et al.* [46] reported that spoiled banana waste showed better ethanol yield 23.42 g/L followed by banana peels (17.00 g/L) and banana pseudostem (15.61 g/L) using isolated *S. cerevisiae* (KX.33585) from spoiled banana waste subsequent identification by morphological, cultural characteristics and rRNA sequencing studies. Another interesting work, where banana peels were used to produce bioethanol using a microorganism named *Enterobacter sp.* EtK3 isolated from kitchen wastewater sample and identified by partial sequencing of 16s rRNA gene. The strain *Enterobacter sp.* EtK3 directly biotransforms fruit waste to ethanol, completing the whole process in a single stage providing economic benefits. The maximum yield of ethanol was achieved 2.99 g/L after 48 h (23.6%) under optimized conditions; pH 7, incubation temperature 35 °C and substrate concentration 15.5 g/L [24]. Jahid *et al.* [7] used banana peels and pineapple peel to produce bioethanol using both acid hydrolysis and enzyme hydrolysis (cellulase and xylanase enzyme). They observed maximum yield of subsequent bioethanol processes of cellulase enzyme activity and acid hydrolysis under separate hydrolysis and fermentation (SHF) for banana peels and pineapple peels 6.3 g/L and 4.59 g/L, respectively. These maximum yields were given after 15 h incubation period of fermentation with baker's yeast (*S. cerevisiae*). Gosavi *et al.* [15] used the outer covering of Indian water chestnuts, leafy shoots of pineapple and fruit waste of jackfruits. They used acid hydrolysis to increase the total reducing sugars of substrates and baker's yeast (*S. cerevisiae*) used for fermentation. The maximum ethanol yields for pineapple (0.90 mg/mL), Indian water chestnuts and jackfruits (0.45 mg/mL) were obtained after the 5th day of fermentation and under optimum concentration (2% H₂SO₄) for acid hydrolysis. Casabar *et al.* [9] used pineapple fruit peels under alkali pretreatment to produce bioethanol using *S. cerevisiae*. After 48 h of the incubation period, the results showed that

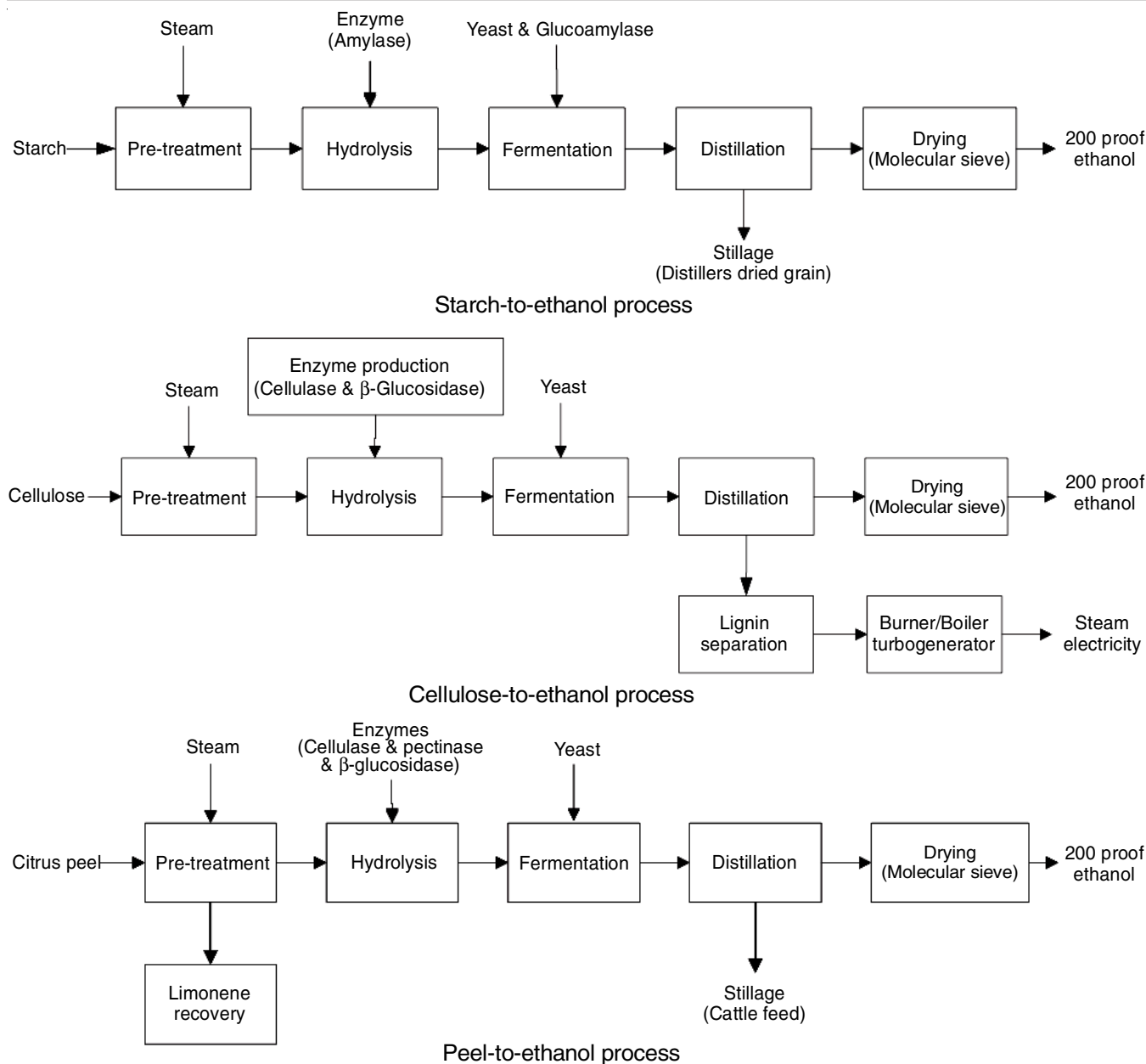


Fig. 3. Process block diagram for making ethanol from different biomass resource [40]

the maximum yield of bioethanol 5.98 g/L was produced under 10% of NaOH of alkali pretreatment, consolidating that alkali pretreatment is no suitable for efficient production of bioethanol from this kind of feedstock [9]. However, Kim [47] showed that the alkali pretreatment effectively reduced the hemicellulose and lignin in the palm fruit bunch. The results proved that yielding maximum bioethanol (33.8 g/L), productivity 1.57 g/L*h and 91.2% sugar conversion efficiency after a short period of 20 h. Similarly, Shim [48] used orange, tangerine and apple under subsequent hydrolysis of cellulose, which consists in their fruit peels by cellulase and fermentation by *S. cerevisiae*. When considering the results, apple peels had a maximum ethanol yield (around 14-15 g/L) than other fruit peels, followed by tangerine peels (around 10-11 g/L), orange peels (around 7.0 g/L) [48]. Evcan & Tri [49] produced bioethanol from apple pomace by using cocultures of *T. harzianum*, *A. sojiae* and *S.*

cerevisiae to invent renewable and low-cost alternative feedstock fuel production. The best result was obtained when three cultures were used together and maximum bioethanol concentration reached 8.75 g/L while overcoming the ethanol concentration (4.46 g/L) of only *S. cerevisiae* was inoculated [49]. Apple pomace was also studied by Magyar *et al.* [50]. The overall ethanol yield was 134 g/kg (13.4%) of dry apple pomace. When considering the mango fruit waste, recent research shows that the heights yield of ethanol (19.98%) was obtained [51]. Jahanbakshi & Salehi [52] processed watermelon waste using *S. cerevisiae* (yeast) for production of bioethanol using a bioethanol extracting device which consists of two parts: (a) hydrolysis reactor and (b) fermentation reactor. The bioethanol amount extracted from watermelon waste (35.5 g/kg of watermelon sugar syrup) indicates that watermelon waste is also a more efficient feedstock for bioethanol production.

Production of bioethanol from fruit waste biomass

Pretreatment: Comparing the bioethanol production from three major types of biomass, sugary, starchy and lignocellulosic material (LCB), which needs the most costly and complicated technique called pretreatment. Lignocellulosic biomass includes cellulose, hemicellulose and lignin as main components. These components act as a barrier in their natural condition to prevent external damages by chemicals and enzymes because of their complex structure. Therefore, the pretreatment step aims to destroy the lignin shell protecting cellulose and hemicellulose within the plant materials and reduce the degree of cellulose-hemicellulose crystallinity structure, leading to more accessibility for chemicals hydrolytic enzymes to hydrolyze them into sugars [3,6,53].

As shown in Fig. 4, several pretreatment techniques are there, depending on the type of lignocellulosic biomass. However, the selection of pretreatment process depends on the several factors as follows: (i) nature of lignocellulosic biomass, (ii) heterogeneity of lignin polymer, (iii) generation toxic inhibitors for downstream processes, (iv) higher energy requirement to yield a lower product, (v) capability of recycling chemical used, and (vi) waste management [54].

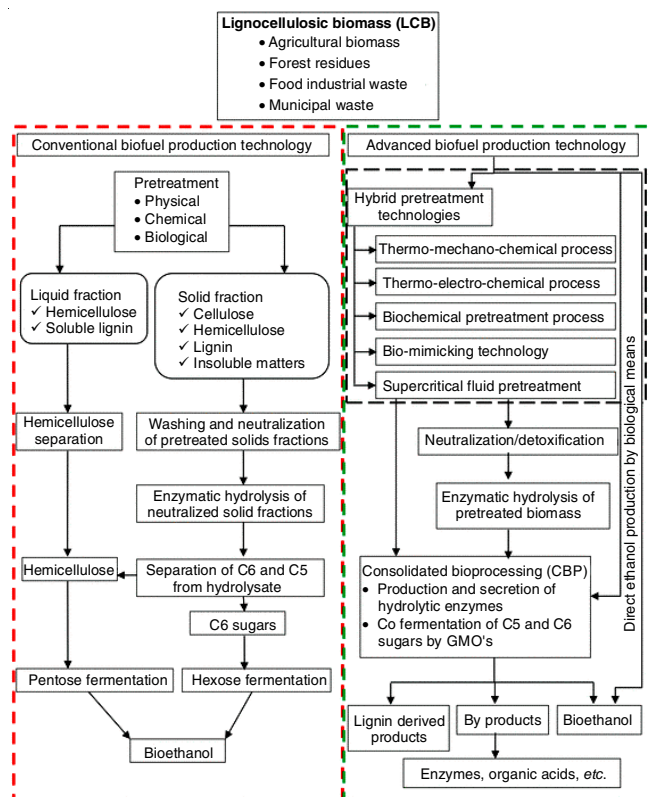


Fig. 4. Schematic diagram of advanced pretreatment technology for suitable production of second-generation bioethanol [54]

Physical pretreatment is the first step of bioethanol production. Solid wastes of fruit are cut in small pieces by milling, grinding or chipping while reducing the degree of cellulose crystallinity, increasing surface accessibility area and pore volume of cellulose, partial depolymerization of lignin. Pyrolysis is also a physical pretreatment method that uses high tempera-

tures. When lignocellulosic biomass is treated with greater than 300 °C, cellulose decomposes to produce gaseous products. Pyrolysis is done under milder acid conditions (1N H₂SO₄, 97 °C, 2.5 h) to achieve 80-85% cellulose conversion to reducing sugar [13].

Chemical pretreatment involves primarily acid; HNO₃, H₃PO₄, HCl and H₂SO₄ and alkali: major alkali is NaOH. Otherwise, ammonia, organic solvents, SO₂, CO₂ are used for hydrolysis, de-lignification and decrease of cellulose crystallinity [39].

Physio-chemical pretreatment is another technique that combination of both physical and chemical pretreatment qualities. *i.e.* ammonia fiber explosion, CO₂ explosion, steam explosion. Among these techniques, the steam explosion method is considered the most cost-effective and adequate for hardwoods than softwoods [55].

Biological pretreatment using microorganisms that can cause alternation of cellulose and lignin structure is another low energy consumable process. Microorganisms involved in biological pretreatment are white-rot fungi, brown rot fungi, soft rot fungi (these fungi especially attack cellulose and lignin structures), *Bacillus*, *Trichoderma*, *Aspergillus*, *etc.* [56,57].

Detoxification is the process of removal of toxic compounds releasing with the pretreatment process. These toxics can be inhibitors for the downstream hydrolysis and fermentation process. The presence of inhibitor per level of recovered sugar is an important consideration while evaluating the pretreatment efficiency. Common inhibitors such as hydroxymethylfurfural, phenolic compounds, glycolaldehyde have been reported as important inhibitors in lignocellulosic hydrolysates for subsequent fermentation. Detoxification methods, such as treating with the charcoal supplement, can control the inhibitory action for downstream processes [58-60]. Gosavi *et al.* [15] performed detoxification step after acid hydrolysis of fruit wastes (chestnut, pineapple, jackfruit waste) using sulfuric acid. When compared the controlled experiment without the detoxification step, the cell density of yeast (*S. cerevisiae*) was high in the detoxified samples. Therefore, the detoxification step is necessary after acid pretreatment. The detoxification step was done by heating the hydrolysate at 600 °C, then adding NaOH until pH reached 9.0-9.5. After that Ca(OH)₂ was added till pH reached 10.0 [15]. Jahid *et al.* [7] revealed that when fruit waste samples have low lignin content, biological samples can be easily recovered to fermentable sugars without using any chemical or biological process, only steamed water pretreatment. When pretreatment is done without any chemical, detoxification steps could also possibly be due to low inhibitory lignin present in fruit waste biomass [7].

Danmaliki *et al.* [6] performed a comparative study between alkali pretreatment, water pretreatment and acid pretreatment to evaluate the variation of ethanol yield using banana peels. Banana peels were subjected with 10% (w/w) NaOH, liquor to fiber ratio of 6:1 and 120 °C for 6 h to perform alkali pretreatment. For acid pretreatment, 40 g of banana peel sample mixed in 200 mL of water was treated with 5% H₂SO₄ and kept at 120 °C for 6 h. Water pretreatment was done air-dried banana peel sample was cooked at 120 °C using a water: fiber ratio of

1:10 for 6 h. The research group has taken reducing sugars yield after hydrolysis showed the highest amount of glucose concentration (1.4%) was obtained by acid pretreated banana peel sample after 48 h of incubation time followed by alkali pretreatment and water pretreatment, 1.2% after 48 h and 1.0% after 72 h, respectively. However, after the fermentation process with *S. cerevisiae*, alkali pretreatment achieved the best ethanol yield (80 ppm) following water and acid pretreatment of 40 and 60 ppm, respectively. The above results indicate that each pretreatment has its inherent advantages [6].

The high concentration (more than 2%) of sulfuric acid can produce toxic compounds that can act as inhibitors for the subsequent fermentation process [15]. Alkali pretreatment of palm fruit bunch showed that ~55.4% delignification efficiency treated with NaOH reduces hemicellulose and lignin components [47]. Consideration of economic effectiveness also, there is high potential on alkali pretreatment, which effectively remove lignin and leave carbohydrate portion relatively intact. Ingale *et al.* [45] analyzed the reducing sugar percentage, saccharification percentage and ethanol yield after treating with both alkali pretreatment and alkali pretreatment + enzymatic saccharification using banana pseudostem. The results showed that treatment with 1 N NaOH + crude enzyme saccharification had the highest value of % reducing sugar (~833 mg %), % saccharification (41%) and produce ethanol yield (~190 mg). According to Arumugam & Manikandan [26], sugar yield in banana and mango pulp after pretreatment was not significantly affected by liquid hot water and dilute acid pretreatments. However, reducing sugar content in both banana and mango peel showed that after diluting acid (0.05 N, H₂SO₄) pretreatment, reducing sugar content was higher than liquid hot water pretreatment [29].

Hydrolysis: Hydrolysis aims to open the accessible area of cellulosic and hemicellulose of lignocellulosic biomass to effectively alter macroscopic and microscopic structures. Moreover, the hydrolysis process contributes to the formation of fermentable sugars from the cellulosic and hemicellulosic feedstock. Therefore, the hydrolysis step should be cost-effective and must avoid the degradation of loss of carbohydrates in the mash and the formation of inhibitory compounds for subsequent processes. Most of the hydrolysis techniques are acid, alkali base hydrolysis and enzymatic hydrolysis [49,61].

Acid hydrolysis: Acid has been used as catalyzing for hydrolyzation of starch and cellulosic biomass. In the process, acids are operated at a temperature between 50 to 150 °C and in two basic types of concentration: concentrated acid (10-30%) and diluted acid (1%). Concentrated acid hydrolysis proceeds under low temperature, pressure and long reaction time. Thus, dilute acid hydrolysis is done under high temperature, pressure and short reaction time [62]. The dilute acid hydrolysis process limits their glucose recovery efficiency to around 50% [63]. This process involves two reaction parts; the first reaction converts the cellulosic materials to sugars and the second reaction converts sugars into other chemicals. Nevertheless, conditions for the first reaction that occurs also are the right conditions in the second reaction [64]. The dilute acid process has a fast reaction, but lower sugar yield is the biggest challenge

for economically viable industrial processes. Dilute acid hydrolysis occurs in a two-stage process to efficiently recover 5-carbon sugars and 6-carbon sugars from hemicellulose and cellulose. The first stage is conducted at low temperature (milder condition) to maximize 5-carbon sugars from hemicellulose. In contrast, the second stage is performed at high temperatures (harsher conditions) to recover the 6-carbo sugars from cellulose [63,65].

Patsalou *et al.* [66] use citrus peel waste (CPW) hydrolysate through acid hydrolysis to produce bioethanol. They revealed that suitable conditions, 0.5% sulfuric acid and 5-6% 9 w/v dry solid of CPW, temperature 116 °C for 10-13 min, are given a higher yield of ethanol (5.8 g/L). Another workers, Evcan & Tari [49] studied ethanol yield after coculture fermentation of apple pomace hydrolysate through dilute acid hydrolysis with phosphoric acid (4%), solid: liquid ratio 1:10 (w/v), 110 °C for 40 min. According to their study, the maximum reducing sugar and ethanol yield obtained 0.945 g/g and 8.748 g/L, respectively [49]. Gosavi *et al.* [15] examined the optimum acid concentration for the acid hydrolysis with sulfuric acid using water chestnut, pineapple and jackfruit waste. The results revealed that the total carbohydrate (~0.6 mg/mL) in 2% acid hydrolyzate was highest after acid hydrolysis [15]. Jahid *et al.* [7] examined how the solid load, acid concentration and reaction time affect the final yield of sugars through the acid hydrolysis process using peels of banana, pineapple, mango and papaya. Their results revealed that 0.5% of sulfuric acid for a soaking time 30 min is sufficient for hydrolysis of cellulosic and hemicellulosic content of fruit waste.

The concentrated acid hydrolysis process provides complete degradation of cellulose producing glucose and sugars while little degradation of hemicellulose in the feedstock. The acid concentration used in this process range between 10-30%, with a long reaction time [67]. The primary advantage of concentrated acid hydrolysis is the higher sugar recovery efficiency which is over 90%. Nevertheless, the process is relatively slow and a cost-effective and the acid recovery process has been difficult to develop. For the neutralization step, a large amount of calcium sulphate and a large amount of salt is produced [65,68]. Danmaliki *et al.* [6] used concentrated acid hydrolysis for the banana peel to produce bioethanol. Banana peels hydrolyzate after acid pretreatment was treated with 10% sulfuric acid at a temperature of 120 °C for 6 h and maximum glucose concentration (1.4% from total sugars) and ethanol yield (60 ppm) were observed.

The previous experiment reported that the alkali pretreatment is more suitable for removing lignin, which remarkably improved the reactivity of the remaining parts of polysaccharides. Hydrolysis of alkali is the most suitable for agricultural residue and herbaceous, not for woody biomass because of their high lignin content. In alkali treatment aligning structure is altered by glycosidic bond and ester degrading side chains of swelling and cellulose de-crystallization [69,70]. Alkali treatment with 5% plant biomass, 1N NaOH, for 18 h at room temperature is optimum for delignification [45].

Enzymatic hydrolysis: It liberates fermentable sugars from cellulose, hemicellulose and other starchy materials contained in lignocellulosic biomass. This is a quite new approach comp-

ared to concentrated acid and dilutes acid hydrolysis. Hydrolytic and oxidative enzymes which are extracted from fungi and bacteria or whole organisms involves in the hydrolytic process under their optimum conditions [46]. *T. viride*, *T.harzianum*, *A. famigatus*, *A. ellipticus* (cellulase activity) [9,45,71]. *Bacillus subtilis* (α -amylase activity), *Aspergillus niger* (glucoamylase activity) [29]. *Geobacillus stearothermophilus* that produce a cocktail of thermo-alkali-stable xylano-pectino-cellulolytic enzymes, etc. are microorganisms used for the enzymatic hydrolysis process [38]. Hossain *et al.* [72] reported mango, banana, pineapple and rambutan to produce ethanol production under only enzymatic hydrolysis. The maximum ethanol yield (9.96%v/v) was obtained by rambutan fruit pulp than other fruit skin and the mixture of skin and pulp. They mentioned that the pulp of fruit looks soft enough to penetrate enzymes that hydrolyze hard cellulosic tissues in the pulp other than parts of fruits as the reason [72]. Corroborating the main purpose of bioethanol production from fruit waste, Ingale *et al.* [45] revealed that the banana pseudostem could be used as ligno-cellulosic material under enzymatic hydrolysis with the cellulolytic enzyme produced by coculturing of *A. famigatus* and *A. ellipticus* microorganisms. The study revealed that at maximum reducing sugar (~833.0 mg) and ethanol yield 17.1 g/L achieved by the combination of alkali pretreatment and enzymatic saccharification. Isolated *Geobacillus stearothermophilus* from the natural resource, which produces a cocktail of thermo-alkali-stable xylano-pectino-cellulolytic used by Prakash *et al.* [38] for production of ethanol under optimized condition using yellow ripen banana peels. They have obtained a maximum ethanol yield of 21.1 g/L and fermentation efficiency of 76.50%. The factors affecting the enzyme activity in the hydrolysis process are medium pH, metal ions in the medium, enzyme dose and substrate concentration were revealed in that study are temperature [38]. According to study work on pineapple fruit peels which treated the subsequent enzymatic hydrolysis process (cellulase activity of *T. harzianum*) and fermentation by *S. cerevisiae*, maximum bioethanol yield 5.98 g/L and 82.42% conversion of fermentable sugar after 48 h, can be obtained. In another way, after treating with *T. harzianum* cellulase activity, the degree of the polymerization reduced to 1.38 from 1.64 of unhydrolyzed samples [9]. The kinetics of hydrolysis can improve by increasing the temperature of the reaction medium and the concentration of the enzyme also. Thermo-resistant enzymes of bacterial strains *Bacillus subtilis* produce α -amylase, *Aspergillus niger* which produces glucoamylase can be used in high temperatures [29]. However, this may result in the degradation of enzymes during the hydrolysis for a long time because of the formation of cellobiose and other inhibitory compounds, especially under cellulase and xylanase enzyme activity. As a result, it is critical to understand the mechanism of enzyme activity on the hydrolysis of LCB to improve the yield of sugar from fruit waste [73]. When fruit waste contains a low lignin content, the inhibitory effect is minimal during the enzymatic hydrolysis [7].

Enzymatic hydrolysis can be done in several ways *e.g.* separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). In SHF process,

enzymatic hydrolysis is performed separately from the fermentation step. Therefore, optimum conditions for both the enzymatic hydrolysis and fermentation process can be optimized independently. The SHF processes even achieved ethanol content 48.0 g/L after 120 h (72 h enzymatic hydrolysis, 48 h fermentation) and the productivity 0.4 g/L/h was achieved that lower to SSF's productivity [74]. However, in the SHF process, cellulase and β -glucosidase activity are inhibited by glucose released during hydrolysis. Therefore, lower solid loading and a high amount of enzyme are needed to achieve reasonable sugar yield [75]. SSF process is a promising process, providing enzymatic hydrolysis and fermentation in the same medium of a vessel simultaneously. This process reduces the risk of enzyme inhibition, the number of process steps and the cost of production. The advantageous SSF, enhanced rate of hydrolysis, needs lower enzyme loading, results in higher bioethanol yield, reduce the risk of contamination [76]. Olofsson *et al.* [76] studied the enzymatic hydrolysis and fermentation in two ways that SSF and SHF comparatively. Using cellulose degradable enzyme (Cellic® CTec2) (40 FPU), they produced 4.74% of ethanol in 72 h fermentation by SHF process and 6.05% of ethanol in 24 h by SSF process. Results revealed that the SSF process is more suitable for rapid ethanol production and high yield of ethanol production. However, Guerrero *et al.* [74] have improved the SSF process in little advance. Banana rachis hydrolysate was used to perform only enzymatic hydrolysis until 8 h, then after, culture media was inoculated with *S. cerevisiae*, incubated 350 °C in an orbital shaker (150 rpm). This process, called pre-hydrolysis and simultaneous saccharification (PSSF), achieved maximum ethanol yield and productivity 48.3 g/L and 0.67 g/L/h, respectively. Table-1 showed that comparative results from SHF, SSF and PSSF processes from the above study work.

TABLE-1
COMPARISON OF RESULTS OBTAINED IN THE FOUR CONFIGURATIONS ANALYZED FOR THE FERMENTATION PROCESS OF THE WIS FRACTION OF BANANA RACHIS [74]

Configuration	Ethanol production (g L ⁻¹)	Time of reaction (h)	Maximum attainable yield (%)	Volumetric ethanol productivity (g L ⁻¹ h ⁻¹)
SHF	48.0	120	85.9	0.40
SSF	46.2	72	82.8	0.64
PSSF (8 h)	48.3	72	86.6	0.67
PSSF (24 h)	46.1	80	84.5	0.58

Fermentation: Fermentation is a biological method, prevalent, traditional, well established natural metabolic process that lignocellulosic biomass is converted into bioethanol. This process occurs under the action of yeast, bacteria and enzymes. When fermentation is done at the large scale industrial level, using microorganisms as biocatalysts are cost-effective because there is minimal maintenance and operational cost. Yeast (*S. cerevisiae*) is the most primitively well-known organism, multi-cellular or eukaryotic microorganism, to assist industrial bioethanol production [24,77].

Fermentation process occurred by yeast: First invertase enzyme produced by yeast cells, convert sucrose into glucose

and fructose, second, zymase, another enzyme present in the yeast, convert the glucose and fructose into ethanol [78]. However, *S. cerevisiae* cannot utilize xylose, one of the main sugars in the lignocellulosic biomass like fruit peel (pomace). Otherwise, Evcan & Tari [49] revealed that various types of filamentous fungi, *Trichoderma*, *aspergillus* species, can utilize lignocellulosic biomass to produce bioethanol directly. These fungi contain two biological systems: one system produce cellulase enzyme under aerobic condition and other systems produce bioethanol under anaerobic condition [79].

Inparuban *et al.* [80] examined the optimum culture condition for baker's yeast (*S. cerevisiae*) with parameters: sucrose concentration (optimum: 50 g/L); aeration rate (optimum: 200 bubbles/min); medium volume and reactor volume ratio (medium (1 L) maintained in the 2 L flask gave the highest cell mass production); the volume of growth medium (10-20% of flask volume). Nitrogen composition is the main factor in yeast growth. A low level of assimilable nitrogen compounds (YAN) affects yeast by reducing cell multiplication and decreasing the rate of glycolysis indirectly. The growth rate of yeast shows is high when medium contains N sources such as ammonia, glutamine or asparagine than medium contains poor N sources such as urea and proline. The mixture of ammonia and amino acid delayed yeast growth when high content of YAN is present in the medium. Thus, amino acid and ammonia composition in the medium is preferable to be examined before adding excess ammonium [81,82]. Several reports have been published about the use of immobilized yeast cell medium for bioethanol production by fermentation. Neelakandan & Usharani [83] have treated yeast cells with Na-alginate, Ca-alginate and CaCl₂ to produce immobilized yeast cells in microbeads. They also examined the optimum fermentation condition with immobilizing yeast using cashew apple juice. They obtained maximum ethanol content (7.46%) after 26 h incubation period.

Factors affecting bioethanol production: The temperature during the fermentation process assists a significant role; with increasing temperature, the fermentation process is improved and thus the increase of ethanol yield, respectively. Many research works reported that the optimum temperature for the fermentation process was in the range 25-40 °C [84]. At temperature below the optimum range, the catalytic ability of the enzyme decreases and the reaction rate slow down. When temperature increases gradually, the fermentation rate increases, but at that point, enzymes begin to denature or unfold and thus become inactive. Each enzyme has its relative denaturing temperature and even one of the enzymes in the biological pathway, inactive the organism, fails to grow [85]. Thus, reducing the ethanol concentration, due to the inability to maintain the optimum temperature reduces bioethanol yield.

The pH of the medium is another factor that affects to fermentation process because the yeast in the fermentation process is pH sensitive. The optimum range for growth of *S. cerevisiae* is persuaded by pH in the 2.75-4.25 range, but during the fermentation process, optimum pH range for *S. cerevisiae* is 4.0-5.0. Therefore, at the optimum pH, fermentation achieves its maximum ethanol content with the maximum growth rate of yeast cells [86,87]. It was reported that the initial pH affected

the alcohol level that produces a specific pH for its fermentation process [14]. A high ethanol concentration was obtained at pH 5 by hydrolyzate of bagasse. Beyond the optimum pH range, the ethanol production rate going to decrease.

The carbon source concentration of feedstock affects bioethanol content during the fermentation process; with the substrate concentration increases, ethanol yield increases by increasing the fermentation rate of microorganisms [16]. At a high level of the substrate, more substrate molecules bind to the enzyme's active site. However, prolong increase of substrate concentration effect reduce the bioethanol production [87]. According to Babu *et al.* [86], the sugar levels in hydrolyzate increase gradually. The uptaking capacity of the microbial cell exceeds the fermentation rate leading to a steady state [86]. Along with increasing carbon source concentration, ethanol yield also increases and at 80% of carbon source concentration, the best outcome of ethanol is achieved.

The period of fermentation also affected the final concentration of bioethanol *via* the rate of microbial growth. High ethanol concentration in less time results in improved productivity, which directly impacts the process economically feasible [88]. When the amount of alcohol present in the medium is high, the growth rate of the microorganisms decreases *via* delaying the cell growth. However, the presence of alcohol can damage mitochondrial and impaired cellular wall permeability by disrupting sorting and signalling functions [89,90].

The fermentation medium's shaking speed or agitating speed controls the entry of nutrients into the cell and inside the cell from the fermentation medium and removes produced ethanol into the medium. The higher rate of simulation by shaking increases ethanol production and usually, agitating speed used in the experiment ranged from 150-200 rpm. Nonetheless, it is not recommended to utilize an excessive rotational rate because it decreases the metabolic activity of the cell. Therefore, it is advisable to produce ethanol smoothly [91].

Inoculation size: the substrate concentration is constant, the initial inoculation size would increase ethanol concentration because the number of microorganisms increases to produce maximum ethanol. After which, a further rise in inoculum size would decrease ethanol production due to the depletion of nutrients. However, a smaller inoculum size reduces the cost of the production of ethanol fermentation. The worker's findings revealed the inoculum size 4-12% *S. cerevisiae* gave a remarkable increase in ethanol production [46].

Conclusion

Waste fruits released from industrial-scale fruit production and the regardless management of fruit harvesting, storing and transporting, are suitable for the bioethanol production. Waste fruits are the cheapest feedstock and they have not second value after they are averted. Most waste fruits are released into the environment as landfilling or garbage can cause severe environmental pollution. Thus it is the best solution that waste fruits are considered as the feedstock of bioethanol production. The parts of each fruit contain sugars, starch, cellulose, hemicellulose and lignin in different compositions. The composition of them also varies among the variety of fruits. Bioethanol produced

from the waste of fruit has a high octane number, high oxygen content and lower emission of air pollutants than conventional fossil fuels. Therefore, the use of bioethanol as fuel or fuel additives reduce environmental pollution and global warming. The discussion shows that the principle idea of using waste fruit for bioethanol production with the cleaning of the environment is achieved. Lignocellulosic materials in the fruit parts with advanced treatment increase the ethanol yield by converting cellulose, hemicellulose into fermentable sugars. Alkali hydrolysis and acid hydrolysis tend to reduce the degree of polymerization of lignocelluloses and tend to release simple sugars with the subsequent detoxification process. The composition of lignocellulosic feedstock determines the choice of the hydrolysis process. The simultaneous saccharification and fermentation (SSF) process of bioethanol production can be considered a better process than separate hydrolysis and fermentation (SHF), providing a rapid bioethanol production process and the highest concentration of bioethanol. The optimization of fermentation, which is the critical step in ethanol production, involves gaining a higher amount of ethanol quickly. Optimum parameters of fermentation are temperature ranged 30-33 °C, pH ranged 4-5 and the fermentation medium shaking speed range in 150-200 rpm.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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