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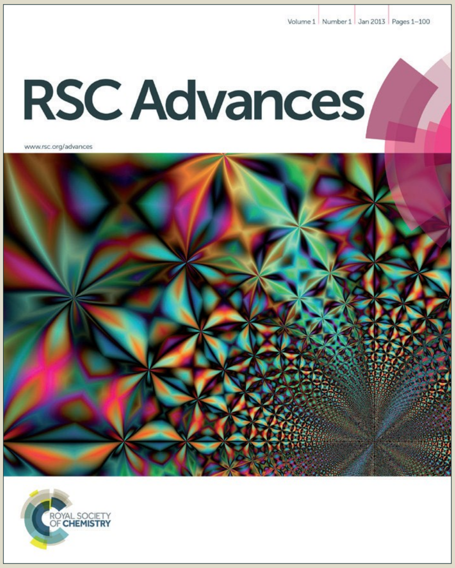

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A Prospective of Bioethanol Production from Biomass as Alternative Fuel for Spark Ignition Engine

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Abstract

The increasing fuel consumption of fossil fuels has led to the development of alternative fuel for the future. Domestic biofuel production and utilization of alternative fuel can decrease dependency on petroleum oil, reduce trade deficits, reduce air pollution and reduce carbon dioxide emission. Bioethanol is a renewable fuel produced by fermentation of sugar which derived from plants such as sugarcane or beet, maize or cassava etc. However, bioethanol consumption in an engine is approximately 51% higher than gasoline since the energy per unit volume of ethanol is 34% lower than for gasoline. Bioethanol is an oxygenated fuel that contains 35% oxygen, which can reduces particulate matters and NO_x emissions from the combustion. Therefore, bioethanol-gasoline blends can significantly reduce petroleum use and GHG emission. In addition, utilization of lignocellulosic materials in bioethanol production is the most viable pathway from environmental point of view. This paper reviews the current status and technologies involved in bioethanol production, properties and engine performance from various biomass feedstocks which recommended as the sustainable alternative fuel in the future.

Keywords: *Biomass*; *bioethanol*; *lignocellulosic*; *gasoline*; *alternative fuel*; *environmental*

1. Introduction

Energy sector plays a vital role in the world economy. Increasing fossil fuel prices, energy security issues and climate change have driven and forced the energy development towards alternative and renewable energy sources. Due to the soaring interest in developing renewable and cleaner energy sources, biofuel production caught our attention in the early 2000s.[1] Biofuels are seen as part of the drive to move beyond the dominant fossil-fuel paradigm and the production has been increasing remarkably. This is due to many countries' attempts to reduce oil import, boost rural economies and improve air quality. Brazil and USA are the largest world's ethanol producers, possess 70% of the world's ethanol production.[2] The countries were able to export in 2006 with outputting 17 and 20 billion liters from Brazil and USA respectively.[3] European stands out with sugarcane bioethanol program, and outstanding results have been gained along the entire production chain from the development, improvement in producing more sugarcane varieties, and engines manufacturing of which can run on any gasoline and bioethanol blends.[4] North America and Sweden introduced vehicles that operate on fuel blend of 85% ethanol with 15% gasoline.[5] The world's major bioethanol producers are shown in **Fig. 1**.[3] The USA leads the world's bioethanol production by almost 20 billion liters bioethanol has been produced. The second is by Brazil that produced almost 17 billion liters of bioethanol, with cheaper production cost of $0.18-0.20/liter compared with USA ($0.33-0.47/liter). EU draws high production cost for every liter of bioethanol produced, which is ranged $0.50-0.97/liter, although in comparison to China, EU produced lesser bioethanol by 3.4 billion liters, while China produced 3.85 billion liters. Expanding the bioethanol production will increase the use of renewable energy resources, thus enabling the ability to be independent from fossil fuel. Bioethanol has been receiving widespread interests at the international, national and regional levels. The research on ethanol production has been established with the potential of ethanol as a valuable replacement of gasoline in the transportation fuel market.

Ethanol can be obtained from microbial conversion of lignocellulosic biomass through fermentation.[6] The quality of bioethanol depends on the feedstock. Therefore, many researchers have reviewed the designs and implementation technologies from a simple conversion of various biomass, such as sugarcane, corn, cassava, rice straw and other agricultural wastes by fermentation, to the multi-stage conversion system of lignocellulosic biomass into bioethanol.[7, 8] The fermentation method generally comprises of three steps: (1) the formation of fermentable solution from the selected biomass, (2) the fermentation of this solution and (3) the separation and purification of the ethanol, usually by distillation.[9] Favorability of bioethanol can be seen from its properties, including density, vaporization heat, flame temperature, fuel octane, and hydrogen and carbon content which affects the compression ratio.[10] By using 10% of bioethanol in the gasoline blend benefits as: reducing CO emissions of 8-30%, reducing toxic emissions by 5-15%, slowing depletion of ozone layer and lessening pollution.[11] Currently, Many countries implemented 5–10% anhydrous ethanol blended with 95–90% gasoline (E10), and this mixture contains 3.5% oxygen compared to only 2.7% by whole gasoline.[10, 12] The higher oxygen in the blend can contribute to a better combustion and an increase in thermal efficiency.[5, 13] Furthermore, blending of bioethanol with gasoline affects fuel properties, such as viscosity, lubricity, octane number, energy content, volatility and stability.[14] However, a major drawback of bioethanol-gasoline fuel is that high blending of ethanol (40–60%) causes wear and corrosion of fuel pumps.[5] The aim of this paper is to give a thorough overview of the current status of production, properties and engine performance of bioethanol as the substitute for gasoline fuel.

[Fig. 1]

2. Feedstock for bioethanol production

The feedstock availability for bioethanol depends on region, climate conditions as well as soil properties.[15] Refer to Balat et al.[16], feedstock for bioethanol production can be categorized into three biomass types: (i) lignocellulosic biomass (e.g. straw, grasses and wood), (ii) starchy materials (e.g. corn, barley, corn, etc.) and (iii) sucrose-containing feedstock (e.g. sugarcane, sugar beet, sweet sorghum, etc.). Algae is the feedstock with the incredibly high ethanol yield, which change the game on potential feedstock availability.[4, 15, 17] The potential for bioethanol production from various biomass feedstock and their comparative production potential are given in **Table 1**.[18, 19] Diverged classification of biofuels feedstocks and processes are currently being developed to meet sustainability and fuel quality standards, as well as the need of road, aviation and marine end users. Biofuel is a challenge that can be marketed well as renewable and sustainable alternative fuel in the future. The classification of the generation for biofuel feedstock is described in **Fig. 2**.[20] It is clearly seen that the main difference of the classifications is due to their feedstock type. First generation utilizes carbohydrate-source crops, which is in general obtainable in the forms of seeds, grains and sugars. Second generation uses lignocellulosic biomass, which sourced from agricultural residues. In third generation, algae (micro and macro type) are utilized as the bioethanol production feedstock with the ability of higher reproducibility rate in lesser land required.

[Table 1]

[Fig. 2]

2.1 First generation biomass

First generation bioethanol is produced from sugar-rich biomass (consists of linkages of monosaccharides of glucose and fructose) and starch-rich (polysaccharides of glucose) crops such as grain and corn. The contained sugars can be converted to ethanol only after hydrolysis to yield fermentable sugars. The technology is well-known but high price is the undesirable side effect of this raw material. On the other hand, first generation has doubtful debate about using food products for fuel production.[21] The details of bioethanol production from various sources for first generation are discussed below.

2.1.1 Sugarcane and sugar beet

Sugarcane (*Saccharum officinarum*) is from the genus *sacchurum* belonging *poaceae* family; it contains high organic nutrients and minerals to make free sugars as an ideal raw material for bioethanol production.[22] It is the most important feedstock grown in tropical and subtropical countries that can be used as juice or molasses (by product of sugar mills) for bioethanol production.[16] The sugar produced from sugarcane has high capability to be converted into glucose (the simplest sugar monomer) and can be used as fermentation substrate under anaerobic conditions. Besides, glucose is converted to ethanol and carbon dioxide by glycolysis.[23] The typical content of fermentable sugar is 12–17%, in which 90% is sucrose and 10% accounts glucose and fructose.[22, 24] Therefore, sugarcane contain high sugar and it is the main feedstock used for ethanol production in Brazil and India..[15] In addition, the sugarcane crop is favorable since its growing season only takes 3–5 months.[25]

Sugar beet (*Beta vulgaris*), or also called as beet molasses, is the suitable feedstock for bioethanol production due to the high sugar contents that can be extracted by fermentation without any modification. Sugar beet contains 15–20% of dry matters, 85–90% of fermentable sugars and free sugars of 10–15%.[4, 22] The high free sugar content in sugar beet is usable in fermentation with adjustment of the pH, and this feedstock gives a more profitable profile for bioethanol production.[26, 27] The composition profile of sugarcane and sugar beet are listed in **Table 2**.[22, 28]

[Table 2]

2.1.2 Sweet sorghum

Sweet sorghum (*Sorghum bicolor* (L) *moench*) has been used as one of the potential bioethanol feedstock due to the high yield biomass and high concentration of readily fermentable sugars.[25, 29] Sweet sorghum has a wide range of adaptability for tropical region, is well adapted to sub-tropical and temperate regions of the world, and is water efficient.[25, 30], Moreover, sorghum has efficiency in photosynthesis utilization of soil nutrients and less water consumption and is tolerant to drought and flood, compared to sugarcane.[31, 32] Sweet sorghum has fast lifecycle ranging from 3–5 months of growing season.[25, 33] Sweet sorghum tolerates compacted subsoil at pH range of 5.0–8.5 with some degree of salinity and alkalinity and poor drainage, and its planting temperature is above 12°C.[30, 34] The productivity of sweet sorghum delivers 2000–8000 liter/ha of bioethanol, makes sweet sorghum is suitable feedstock for bioethanol production.[8, 26] The composition profile of sweet sorghum is shown in **Table 3**.[35]

[Table 3]

2.1.3 Wheat, corn, and barley grain

Wheat (*Triticum vulgare)* is a very good raw material for bioethanol production due to complete gelatinization of wheat starch at temperature of 65°C.[18] Utilization of wheat or unusable wheat leads to cost effectiveness and hence is good option for bioethanol production.[36] Moreover, 3.5 tons of wheat grain, or 3.03 tons dried wheat, can be harvested from one acre of good arable land.[37]

Corn (*Maize*) is species of *Zea* and is an annual *monoecious* flowering hybrid of *zea mays*.[38] Corn cultivars are widely adapted and can be grown from 0–55^{o} latitude, from sea level to 12,000 ft and with growing seasons of 42–400 days.[39] World's bioethanol production accounts 29% from corn grain.[40]

Barley (*Hordeum vulgare* L.) is a very attractive feedstock for bioethanol production and is being used in Europe (62%), Asia (15%) and North America (14%).[41] Barley grows well in many areas and it can become a financially cost effective bioethanol feedstock for many regions. Barley is typically utilized for brewing, animal feed, edible human food and other industrial uses.[42] Barley is harvested sooner, and it still can give good maturity and high yield.[43, 44] Furthermore, 70% of barley composition is carbohydrate, which indicates the potential in bioethanol production.[43] The composition profile of are presented in **Table 4**.[45-48]

[Table 4]

2.1.4 Cassava

Cassava (*Manihot esculenta Crantz*), with synonyms: manioc, yucca, tapioca, has diameter and length of 5–10 cm and 15–35 cm respectively.[49] It is considered as a tuberous root plant that is native to South America and cultivated around tropical and subtropical countries.[50, 51] Cassava can grow at ambient temperature of 25–30°C with optimum annual rainfall requirement of 760–1,015 mm.[25] Cassava can tolerate prolonged drought since it has the ability to utilize nutrients from the stalk and the storage roots once moisture is restored.[52] Cassava is harvested around 8–24 months after planting.[49] Cassava is one of the most important carbohydrate sources in the tropics since it holds high content of starch up to 90% dry weight.[50] The rich starch in cassava is related to the unique properties which make cassava suitable for food and non-food applications.[53] Therefore, cassava starch is one of the best fermentable substances for the production of ethanol. Cassava also contains protein 1% (fresh) and 1.41% (dry), 65% of moisture, 0.9% of ash and 0.03% of phosphorus.[54] Furthermore, cassava has high carbohydrate content of 32–35%, which is considered productive in agro economic application. The composition profile of cassava biomass is presented in **Table 5.**[49, 55]

[Table 5]

2.2 Second generation biomass (lignocellulosic biomass)

Second generation biomass is derived from lignocellulosic components of agricultural residues, such as straws, wood and other agricultural wastes.[56-59] This type of biomass is usable in second-generation biofuel (biomass to liquid) that has an improvement of CO_2 balance with the use of cheap, waste sources and do not compete with human food products.[15, 56, 60] The use of agricultural residues can provide benefit for the agricultural sector in order to provide an additional potential income stream for farmers.[61]

The composition of lignocellulosic biomass is divided into three main components: lignin (10–20% dry wt.), cellulose (30–50% dry wt.) and hemicellulose (15–35% dry wt.).[17, 62] Hemicellulose ($C_5H_8O_4$)n, is a polymer structure of hexose (D-glucose, D-mannose and D-galactose) and pentose sugars (D-xylose and L-arabinose).[63] It contains D-glucuronic, D-galacturonic and methylgalacturonic acids in sugar acids (uronic acids).[64] Hemicelluloses are available as xyloglucans or xylans depending on the types of plants.[65] Moreover, hemicelluloses are linked to cellulose as abundant carbonic in plants.[66] The dominant source of hemicelluloses biomass are woody biomass, hardwoods and softwoods.[17, 67, 68] Cellulose $(C_6H_{10}O_5)_n$ is a long-chain structural component of glucose monomers (D-glucose) linked to β-(1,4)-glycosidic bonds.[69] The cellulose molecules are also consisted in long chain of β-glucose monomers gathered into micro-fibril bundles.[70] Cellulose is insoluble in water, and hydrolysis process is required to break down the polymer to free complex sugar molecules or also known as saccharification process.[71] The product of cellulose is a six-carbon sugar from woody and agricultural biomass.[67] Lignin $C_9H_{10}O_3$ (OCH3)0.9–1.7$]_n$ is a structural compound of three types of monomers (p-coumaryl, synapyl alcohols and coniferyl) joined together by a set of linkages to create matrix.[72] The complex structures such as hydroxyl, methoxyl and carbonyl have high polarity which responsible to the hardness of the bonds and constituent of binding material which holds the cellulosic fibers.[73] Lignin is resistant to chemical and biological degradation, which affects the quality of ethanol in bioethanol production.[66] The major lignin sources are softwood barks, hardwood barks and herbaceous species such as bagasse, corncobs, peanut shells, rice hulls and straws.[7, 41, 74] The molecule structure of hemicellulose, cellulose and lignin are shown in **Fig.3**.[75, 76] The composition profile of the lignocellulosic biomass is presented in **Table 6**.[41, 69, 77-81] Furthermore, extensive chemical ash compositions are summarized in **Table 7.**[82-85]

[Fig. 3]

[Table 6]

[Table 7]

2.3 Third generation biomass

Currently, microalgae and seaweeds are gaining their popularity as third generation biofuel (TGB) feedstock since the wider expansion of bio-refineries leads to the shortage of energy crops that are titled for biofuel industries.[17] The potential of TGB feedstock depend on structure of various polysaccharides walled by cellulose-based cell walls, which can be further processed and fermented into biofuel.[6, 17] TGB does not require land as much as the conventional agriculture, has high productivity area, recycles carbon dioxide, is able to grow from different type of water source (e.g., seawater, brackish water and wastewater), and compatibles with the bio-refineries integrated bioethanol production system.[86] Besides that, TGB does not raise doubts about impact on food supply and land reservation security.[87]

2.2.1 Microalgae

Microalgae are abundant and carbon neutral renewable resource which considered as a TGB that can be used in bioethanol production.[88] There are various types of microalgae such as *C. vulgaris, Chloroccum sp., N. gaditana,* etc. as the potential feedstock for biomass conversion into bioethanol.[87, 89] Microalgae have fast grow rate with large yield of the biomass and are able to sustain hostile environment including insufficient nutrients and high salinity.[90, 91] The cell walls of microalgae are cellulose, mannans, xylans, and sulfated glycans.[92] **Table 8** summarizes the composition profile of algae biomass sources.[86, 91, 93, 94]

2.2.2 **[Table 8]***Seaweeds*

Seaweeds are considered as a third generation biomass which have carbon neutral renewable resource compared to first and second generation biomasses.[95] Seaweeds yield high rate of biomass and fast growth with good productivity in comparison to the land crops.[96] The high yield of seaweeds is supported by the lesser energy requirements coupled with their ability to absorb nutrients on throughout their surface areas. This makes seaweeds possible to save energy since there is no requirement to transport the nutrient internally.[97] The constituents of seaweeds depend on species, habitat, maturity and environmental conditions, but generally they contain fatty acids, oxylipins, non-starch polysaccharides, dietary fiber, minerals and vitamins, terpenoids, carotenoids, phlorotannins, steroids and protein.[98, 99] Seaweeds can be harvested naturally or by additional technological efforts, which both give variation in production cost and yield.[100] Seaweeds are able to use unlimited supply of water and nutrients from the ocean and resulted yield about ±90% of the ±1.6 million tons of total dry seaweed.. The growing process of seaweeds also dissolve carbon dioxide on the water surface, and the required nutrients can be also absorbed from processing effluents.[101] The composition profile of seaweeds is presented in **Table 9**.[96, 98, 99, 101-104]

[Table 9]

3. Conversion process of biomass to bioethanol

3.1 Biomass feedstock handling

The handling of biomass feedstock is the preliminary step to prepare the feedstock, involving cleaning and preliminary cutting to reduce the physical size beforehand the pretreatment process.[105] During feedstock handling, storage of biomass feedstock is equally essential to maintain the quality of the supply for bioethanol production. The large storage place is used for biomass feedstock to avoid the biomass from the excessive humidity, rodents and microbial growth. Moreover, drying under the sun and thermal or mechanical drying techniques are the common methods beforehand the storage.[106] **Fig. 4** Conversion process of biomass to bioethanol.[107]

[Fig. 4]

3.2 Pretreatment of biomass feedstock

In bioethanol production, biomass has complex structures that are required to broken down into oligomeric subunits by pretreatment.[68] The purposes of pretreatment are: to remove hemicellulose, to remove or redistributing lignin (for lignocellulosic biomass); to increase the mean pore size and to provide better access for enzymes (during enzymatic hydrolysis).[108] The pretreatment methods can be largely classified into four methods as below.

3.2.1 Physical pretreatment

Physical pretreatment reduces the particle size of the feedstock to increase surface/volume ratio, which eases the subsequent processes in the production.[109, 110] Physical pretreatment is divided into three techniques as follow:

- *Mechanical process*

Mechanical pretreatment reduces the feedstock's size by breaking the physical structure. The typical process starts by washing, size reduction by grinding, extracting the essence and solid fraction separation.[111, 112] In lignocellulosic material, size reduction is important to increase the lignocellulose exposure in hydrolysis process.[68] However, it has been reported that for particle size below ± 40 mesh is not effective for conversion bioethanol.[112] In addition, the biomass is generally grounded to about 3–8 mm particles to a compacter form with higher density such as pellets or briquettes.[113] Therefore, particle size is an important property to be optimized since it affects the power required for the equipment (e.g. knife mill, hammer mill) as well as the economic feasibility of the selected method.[114] Zheng et al.[115] stated that mechanical process such as attrition milling, ball milling, compression milling and steam treatment can be used to destruct lignin and give better access for enzymes to attack cellulose and hemicellulose in enzymatic hydrolysis.

- *Extrusion process*

Extrusion process is a combination of physical and chemical process to prepare the biomass in bioethanol production.[68] This process practices heating, mixing, shearing and screw speed to shatter the lignocellulose structure and finally increasing accessibility of carbohydrates to enzymatic attack.[116] Extrusion process can be improved in removing hemicelluloses and lignin by micro/nano fibrillation. This process yields five times higher than hot compressed water treatment, which resulting 77% delignification, and conversion of 69% and 38% of cellulose and hemicelluloses respectively into glucose, xylose and arabinose.[115, 117] Zheng et al.[115] stated that twin-screw extruder can remove 80% of xylose with soluble lignin. The process was conducted to corncobs with 100 rpm screw speed, 100 °C barrel temperature, mass flow rate of 4 kg/h, and it produced 88% of glucose. Furthermore, extrusion method was studied by employing sequential extrusion and clean fraction pretreatment, which resulted 90% glucose yield due to solvents involvement in between and it is recognized as a promising method for lignocellulosic biomass pretreatment.[118] Similarly, Karunanithy and Muthukumarappan[119] reported that mixing, shearing and heating disturb the feedstock's composition physically and chemically during the extrusion process in passage through to the extruder barrel. The effect extrusion process for various feedstocks is presented in **Table 10.**[119-124]

[Table 10]

- *Ultrasonic process*

In ultrasonic pretreatment, ultrasonic wave breaks feedstock cell by mechanical shearing and formation of shock waves.[125] In lignocellulosic sludge type biomass, ultrasonic process brings benefits including improvement in biodegradability, efficient sludge disruption, less processing time, and no additional chemical substances required.[126] Additionally, ultrasonication process improves the feedstock digestibility by affecting the chemical, biological and physical properties of the feedstock.[127] The destruction of cells sets hydrolytic enzymes free and helps to increase the hydrolysis rate of biomass, and the irradiation of cellulose by ultrasonic increases enzymatic hydrolysis rate to around 200%.[128, 129] However, this process is well-known as an expensive method and requires high energy input. In a study, Kidak et al.[130] reported the material disruption by ultrasonification gives desirable result with higher ultrasonic power. They also found that the method only requires less retention time since longer ultrasonification period gives no effectively.

3.2.2 Chemical pretreatment

Chemical pretreatment is identified as structure disruption of the biomass through chemical reactions. Likewise mechanical pretreatment, chemical pretreatment removes lignin, reduces crystallinity of cellulose and enhances biodegradability of the biomass.[131] Chemical pretreatment route is the widely used among other techniques. The common methods of chemical pretreatment are as the following:

- *Acid process*

Acid pretreatment utilizes acidic substances (typically H_2SO_4 or HCl) for lignocellulosic biomass due to its powerful ability to rupture the components into simpler form.[110, 114] The low temperature for this process makes it as a low cost pretreatment, and it can loosen the cell wall matrix through hemicellulose degradation.[68] The process does not affect lignin, but cellulose microfibrils is sufficient to produce high yield of monomeric sugars for fermentation.[26, 132] However, the utilization of acid pretreatment will increase the tendency of corrosion to the equipment, hence it is important to overcome this issue especially in a large scale production with high acid reagent .[110, 112]Therefore, many acid processes have been modified to improve all downstream processes as well the bioethanol yield and prices.[26] Tang et al.[134] performed acid pretreatment on *Eulaliopsis* binate using dilute sulfuric acid, resulting 21.02% total sugars with low inhibitors level after pretreated by 0.5% dilute sulfuric acid at 160^{o}C for 30 min and at a solid-to-liquor ratio of 1:5. Marzialetti et al.[135] performed acid hydrolysis on loblolly pine using various acids, including trifluoroacetic acid, HCl, H_2SO_4, HNO_3, and H_3PO_4. The research found that trifluoroacetic acid yielded the most sugar monomers of 70% from hemicellulose at 150◦C at pH 1.65, and this acid is claimed as the mildest acid among the rest acid employed.

- *Alkali process*

Alkali process is pretreatment process of feedstock catalyzed by alkali chemical at room temperature up to 24 h.[112, 132] This method is applied in a simple operation and gives high conversion yields with only short span of time.[110] It produces less sugar degradation, but the appearing inhibitors are expected to be eliminated in order to optimize the pretreatment conditions.[136]Therefore, this pretreatment method is claimed to be more effective in bioethanol production than acid pretreatment.[137] The suitable alkali reagents are NaOH and lime, which are employed to break intercellular bonds crosslinking hemicellulose and other component (lignin and cellulose).[133] Moreover, the alkali pretreatment can cause structure swelling, increasing porosity, decreasing polymerization degree and crystallinity, separation of lignin, hemicellulose, and cellulose and also disrupts the lignin structure.[114] Many studies on alkali process have focused on the pretreatment to improve substrate digestibility. Sun and Cheng[133] reported that alkali pretreatment is capable in lignocellulosic delignification and at the same time enhancing the carbohydrates reactivity. It is also found that by utilizing sodium hydroxide in alkali pretreatment, it improves lignocellulose digestibility in typical lignocellulosic feedstock, for instance wheat straw, thus it is appropriate for second generation biomass pretreatment.[138, 139] Chang et al.[140] reported that utilized oxidative lime pretreatment with feedstock of poplar wood. The study resulted that 78% of lignin was removed with glucose improvement of 71% at the enzymatic hydrolysis. Sun et al.[141] conducted alkali pretreatment by different alkali solutions to release hemicellulose for wheat straw. It resulted that 80% hemicellulose was released as well as 60% of lignin at 20^{o}C by 1.5% sodium hydroxide for 14 hours of pretreatment. Additionally, hemicellulose can produce high amount of xylose compared to glucose and galactose at the subsequent hydrolysis process. Park et al.[142] conducted bioethanol research by utilizing alkali pretreatment, resulting 74% ethanol after 19 hours fermentation by combination of *Saccharomyces cerevisae* and *Pichiastipitis* as the fermentation process. Alkali reagents such as KOH, NaOH, hydrazine, anhydrous ammonia and $Ca(OH)_2$, are typically featured in alkali pretreatment of biomass. Moreover, alkali reagent can cause biomass swelling that exposes internal surface area as well as decrease in crystallinity and polymerization degree.[138, 143]

- *Organosolv process*

Organosolv process utilizes organic solvent to break the internal lignin-hemicellulose bonds at high temperature (100–250^{o}C), and it provides more access to cellulose.[68, 144] The typical suitable organic solvents for this pretreatment method are namely tetrahydrofurfuryl alcohol, ethylene glycol, methanol, acetone and ethanol, and they are noted for their ability to solubilize lignin as well as treating cellulose component to be digestible for enzymatic hydrolysis.[68, 145] Organosolv pretreatment effect on enzymatic hydrolysis was studied by Mesa et al.[146]. The pretreatment of sugarcane bagasse is to produce glucose under temperature of 175^{o}C for 40-60 min yielded 22.1 g glucose/100 g biomass. Moreover, pretreatment by organosolv technique can also be combined with acid (by adding HCl, H_2SO_4, oxalic or salicylic acid) if the process is conducted with in temperature below 185–210^{o}C to break the hemicellulose bonds.[147] However, it is suggested that the mentioned combined technique should separate lignin and hemicellulose components so that xylose yield can be focused at the most desirable yield amount.[132] In addition, removal of solvents in organosolv process is generally required due to production of inhibitors which limits the activity of enzymatic hydrolysis and fermentative microorganisms.[133] The advantage of organosolv pretreatment is able to produce high digestible cellulose subtracts for all biomass with high value utilization and to remove lignin which troubles the cellulolytic enzymes for lower enzymes dosage.[68, 112] However, this technique produces high amount of soluble phenols, furfural, 5-hydroxymethyl-2-furfural (HMF), which are obtained from lignin after pretreatment.[68, 110] High cost of solvents is the disadvantage of organosolv pretreatment method, but ethanol and methanol are most commonly used solvents in this method due to their low cost .[133]*Ozonolysis process*

Ozonolysis pretreatment involves ozone as a strong oxidant with high delignification efficiency.[148] This process removes lignin, slightly attacks hemicellulose and hardly effect on the cellulose.[110] Ozonolysis is pretreatment method of feedstock which performed catalyzed at atmospheric conditions (normal pressure and room temperature) and it does not produce inhibitors that impact the hydrolysis and fermentation in bioethanol production.[136] Ozonolysis carried out environmental sustainability due to ozone is a substance that can be decomposed by high temperature or catalytic bed, hence lesser pollution is ejected to the environment.[147] Lignocellulosic materials, for instance rye straw, wheat straw and cotton straw, are seen suitable to be handled with this pretreatment method.[132] Nevertheless, further researches on ozonolysis for bioethanol production are essential to be performed more beneficial points and practicality. Since it is very known that the method is a costly process due to the high amount of required ozone for this pretreatment..[68, 149]

In addition, García-Cubero et al.[150] performed ozonolysis pretreatment to improve sugar yield from wheat and rye straws. It was found that enzymatic hydrolysis of the ozone pretreated biomass resulted better improvements of 57 and 88.6% of hydrolysis yield than by the non-ozone pretreated hydrolysis yields of 16 and 29% from rye and wheat straw respectively. The studied concluded that biomass type and moisture content are the substantial factors in ozonolysis. Ozonolysis has also been used in industrial wastewater treatment, and as the pretreatment method for wider various applications.[151] The favorableness in its high delignification efficiency, ozonolysis is also utilized in pulp bleaching of paper industry.[152, 153]

- *Ionic liquids (ILs) process*

Ionic liquids pretreatment (ILs) utilizes the organic cations and inorganic anions in salts to pretreat the biomass at room temperature. [110, 145] In lignocellulosic biomass, ILs process is purposed to dissolve lignin or cellulose, and it decreases cellulose crystallinity for a better hydrolysis process as the subsequent process after pretreatment.[145] Besides, the ILs process has good traits including non flammable, low vapor pressure, and balanced chemical and thermal stability. It also tends to sustain its liquid form in a quite wide temperature range.[68] This means that this pretreatment method supports the improvement of cellulose accessibility at room temperature with or without catalyst (acid or alkali), and it avoids the formation of inhibitors.[110, 116] Remsing et al.[154], found that in ILs method carbohydrate in the biomass is more attracted with anion than with cation. This is due to the preference of chloride ions to be interacted with hydroxyl protons of the carbohydrate sugar, and it occurs in stoichiometry ration of 1:1.

Fransisco et al.[155] reported that pretreatment of lignocellulosic biomass has gained many attention and it can dissolve carbohydrates and lignin simultaneously. The cation structure and degree of anion charge delocalization are two major factors that can affect properties of ILs such as refractive index, density, viscosity and solubility respectively. Thus, ILs method holds a strong thermal stability as well as negligible vapor pressure and these properties are subject to change depending on the user’s desire and requirement. ILs pretreatment may produce impurities, namely water, halides and other volatile substances formed from the biomass or the solvent, although they can be removed by evaporation process.[156] ILs is namely green solvents as it does not contain toxic or explosive substances, and can be recovered to reduce their usage and make more environmentally.[68, 157] However, other studies have reported the drawback of this method, including the compounds harmfulness and issues of ILs recovery.[110, 145] Furthermore, this technology is expensive and requires further investigation of ILs feasibility on large-scale application, since it holds the potential in bioethanol production industry.[68, 158]

3.2.3 Biological pretreatment

Microorganismsare employed to degrade the structure of biomass in biological pretreatment.[41, 112] The typical microorganisms employed for this technique are white, brown and soft-rot fungi.[112] Several of white-rot fungi, namely *Ceriporia lacerata*, *Phanerochaete chrysosporium*, *Pleurotus ostreaus, Cyathus stercolerus*, etc., have been evaluated and resulted in high delignification efficiency of various lignocellulosic biomasses.[68, 159] Brown rot fungi aims hemicellulose and cellulose components of the biomass, white fungi degrades cellulose and lignin components, and soft rot fungi are useful to release cellulose from the degradation of lignocellulose complex.[8, 160] Biological pretreatment is very well-known for its environmental friendliness, although it tends to work on a slow rate compared to other pretreatment methods.[161, 162] The advantages this method are low process cost and low energy, which due to less mechanical assistance, no requirements of additional chemical substances and applied pressure, does not cause corrosion to the equipment, and low production of inhibitors.[6, 68] However, the disadvantages of biological pretreatment is the supremely low degradation rate to attain high degree of lignin degradation (as long as few months). This causes the inefficiency of the subsequent processes of hydrolysis and fermentation to complete the entire bioethanol production.[159] Therefore, development of biological pretreatment is focused due to this method has been described as one of the most economic techniques to pretreat biomass for bioethanol industry.[105, 136]

There are many reviews reported in biological pretreatment and its future perspectives [163-166] . There are few studies reported that *Aspergillus terreu*, *Trichoderma* spp, *Cyathus stercoreus* and *Lentinus squarrosulus* are microbes can be degraded lignocellulosic material (lignin and holocelluloce) at 65-80% and 45-75% respectively, with the pretreatment condition of 25-35^{o}C for 3-22 days.[165-166] In a similar study, Zheng et al.[115] found that the effectiveness of white-rot fungi in decomposing lignocellulosic biomass, as well as enhancing the subsequent enzymatic hydrolysis. The study resulted that *Irpex lacteus* (the employed white-rot fungi type) succeeded to degrade lignin by 43.8%, and the subsequent enzymatic hydrolysis was found higher by 7-fold.

3.2.4 Thermochemical pretreatment

The application of direct combustion is utilized in thermochemical pretreatment as one simple biomass pretreatment, and this pretreatment generally relies on the biomass properties, including volatiles content, moisture content, fixed carbon content, impurities (S, N, Cl) concentration, and ash content, of which influence the technical and economic factors of the bioethanol production. [167, 168] The pair of low cost feedstock with thermochemical pretreatment for bioethanol production can bring out future securities in terms of energy, economic and infrastructure.[169] **Fig. 5** shows thermochemical processes and they can be used to convert to liquid, solid and gaseous fuels from lignocellulosic biomass.[170] In addition, several thermochemical pretreatment methods that are commonly applied in bioethanol production are discussed below.

[Fig. 5]

- *Steam explosion (STEX)*

Steam explosion (STEX) is widely used in bioethanol production for lignocellulosic biomass thermochemical pretreatment.[68, 133] This pretreatment used high temperature (160–260°C) and pressure (0.69-4.83 MPa) and also a sudden depressurization.[68] The mechanism used in STEX can help by natural catalyst of acetic acid, which is released by the hemicellulose acetate and organic acids for instance levulinic and formic acid. The STEX method can used through auto hydrolysis process or naturally process.[171] Some factors can influence STEX efficiency such as moisture content, residence time, temperature, catalyst concentration, and time of presoaking can improve the sugar release, as a study reported that STEX method is able to yield xylose-sugars in range of 45-65% [71,172].However, STEX can generate some toxic compounds that could affect hydrolysis and fermentation steps, such as acetic acid, furfural and hydroxymethylfurfural.[143] Therefore, inhibitors removal is required for every STEX pretreatment due to the inhibitors can disturb the sustainability of enzymes (in hydrolysis) and yeast (in fermentation), which leads to production additional cost. [171]

- *Liquid hot water (LHW)*

The working principle of LHW process is a thermochemical pretreatment of lignocellulosic biomass through subcritical pressure water with the assistance of CO_2 to enhance the hydrolysis process.[173] The LHW pretreatment is conducted with hot compressed liquid water at temperatures of 160–240 °C and pressure above 5 MPa for 15 min.[174] LHW process is able to yield high xylose recovery of 88–98%, with no additional substances as catalyst.[175, 176] The advantages of LHW process are as a simple pretreatment process with low capital and maintenance costs (reactor materials) due to not affected to corrosion problem as well it releases high sugar yields.[136, 176] Additionally, lignin and hemicellulose are soluble in LHW pretreatment since it uses high amount of water.[116] Some studies have reported the suitability of LHW pretreatment in bioethanol production from various feedstocks, such as wheat straw, corn stover and sugarcane bagasse. However, LHW process requires high water and energy consumption which makes this process is not viable to be developed at commercial scale.[164, 175] Nevertheless, LHW pretreatment method draws attention of researchers, especially in lignocellulosic biomass pretreatment. Perez et al.[177] performed LHW pretreatment of wheat straw, yielding sugar recovery of 43.6%, and the subsequent enzymatic hydrolysis yielded 79.8% of sugar recovery. Jiang et al.[178] employed LHW pretreatment on branch, stem and boll of cotton stalk. It was found that 4.34 and 4.42% of hemicellulose was removed from branch and stem respectively, while 58.09% hemicellulose was removed from boll shell. This study also results in bioethanol yield of 18.3, 16.27 and 21.08 g per 100 g of stem, branch and boll shell respectively. Imman et al.[179] added NaOH in LHW pretreatment of rice straw, and claimed that sugar yield was higher with the NaOH presence

(0.25-1.0%). Furthermore, hemicellulose and lignin were efficiently removed from solid residue and high pentose yield were obtained in the liquid phase with low formation of unwanted by-products.

- *Ammonia fiber explosion (AFEX)*

AFEX utilizes ammonia to decrease crystallinity of cellulose in solid lignocellulosic biomass, as well it disrupts carbohydrate-lignin bonds.[80] AFEX pretreatment can increases lignocellulosic digestibility and enhancing the yield from the enzymatic hydrolysis as the subsequent process.[116] Moreover, AFEX can achieve conversion of 90% from cellulose hemicellulose into fermentable sugars and this pretreatment can be employed on numerous varieties of lignocellulosic materials.[110]In fact, AFEX pretreatment can be also employed in a bioethanol production method of saccharification and cofermentation, using recombinant *S.cerevisisae* strains as the fermentation agent in obtaining high ethanol yields.[68] AFEX method brings no inhibitors for the subsequent processes in the production line, and cell walls extractives, for instance lignin phenolic fragments, remain on the surface of cellulose.[132] The disadvantage of AFEX method is to recovery ammonia due to its high volatility which boosts up the overall production cost.[68, 143] AFEX pretreatment method is also woody biomass and herbaceous crops.[116, 147] Numerous studies have been conducted to determine the optimal AFEX pretreatment for different biomas sources., Alizadeh et al.[180] pretreated switch grass with AFEX at 100^{o}C and ammonia to biomass ratio of 1:1, yielding 0.2 g ethanol/g dry biomass. Bals et al.[181] observed that enzyme formulation by AFEX pretreatment produced high sugar yields. From switch grass as the biomass, 520 g sugar/kg biomass was released after enzymatic hydrolysis with AFEX pretreatment, while 410 g sugar/kg biomass is normally released.

- *Wet oxidation (WO)*

Wet oxidation pretreatment uses combination of water, oxygen pressure, elevated temperature and alkali hydrolysis, and it is proven as an efficient pretreatment for lignocellulosic biomass to produce high yield bioethanol. [68, 137] Oxidation pretreatment operates at relatively low temperatures (170–200°C), short reactor times (5–15 min) and pressures from 10–12 bar O_2.[132] In fact, a higher oxygen at temperatures above 170°C reduces the energy demand as the result of exothermic process.[68] In wet oxidation process, lignin and hemicellulose, as well as cellulose, are solubilized and enhanced its digestibility respectively, due to the oxidative reaction and the hydrolytic acid formation.[149] Wet oxidation produces lower inhibitors (furfural and HMF) than LHW or steam explosion methods.[182] In fact, additional of alkali substance (Na_2CO_3) in this method maintains process's pH, reduces toxic compounds, and neutralizes carboxylic acid.[110, 137] However, this pretreatment method requires high oxygen and catalyst supply, thus driving up the production cost.[183] Arvaniti et al.[184] reported that wet oxidation method can pretreat the rape straw to produce bioethanol with yielding of 67% through simultaneous saccharification and fermentation (SSF).

- *Microwave process (MWP)*

Microwave process (MWP) combines thermal and non-thermal effects in watery environment. The microwave gives vibrations to the biomass molecules, which leads to intermolecular collisions that result in heat provision and acceleration of the process.[16] In fact, microwave gives higher heat effect compare to the conduction or conventional pretreatment since there is a direct interaction between the object and the electromagnetic wave in a form of supercritical heat transfer.[185] MWP is also can be performed paired with alkali catalysts, namely NaOH, $Ca(OH)_2$, Na_2CO_3, and has been utilized to pretreat lignocellulosic materials including coastal berm, switch grass, and rice straw and hulls.[186, 187] The major positive capability of this method is able to start and stop the process at instant, as well as it requires lesser energy to run.[188] However, the feasibility of microwave process for commercial scale is doubted, and hence further study is strongly suggested to bring the method more feasible in the future.[189] Chittibabu et al.[190] studied the effect of microwave pretreatment on banana pseudostem assisted with alkali (NaOH), yielding reducing sugar as much as 84% after enzymatic hydrolysis with cellulase. In a similar study, Nikolić et al.[191] employed MWP on cornmeal, and after fermented by *Saccharomyces cerevisiae* var. ellipsoideus 13.4% ethanol was yielded.

- *CO_2 explosion pretreatment*

CO_2 explosion pretreatment is employed and conditioned as a phase of supercritical fluid, which a compressed gas above the critical point temperature and forming liquid-like substance.[68, 192] CO_2 is injected to the biomass reactor in a very high pressure and is heated at high temperature up to 200°C and is maintained at the desirable period of time.[193, 194] At this high pressure, the injected CO_2 then disrupts the biomass and dissolves in water, which carbonic acid will be formed from this reaction and the acid will support the enhancement of the biomass hydrolysis. However, CO_2 explosion method does not compatible on dry biomass by nature (no moisture content.[193, 195] CO_2 explosion pretreatment also can be paired with other pretreatment method, for instance, Cha et al.[196] combined CO_2 explosion with ammonia explosion to pretreat rice straw, resulting 93.6% of glucose was yielded. Gu[197] reported that CO_2 explosion process is a nontoxic green solvent. In this method, the CO_2 used to pretreat the biomass can be taken from the CO_2 produced from fermentation process and it can emit the CO_2 emission to the atmosphere. The advantages of employing CO_2 are cheaper cost compare to ammonia in the explosion, lower production of inhibitor compounds than steam explosion, and higher yield than enzymatic hydrolysis without pretreatment.[110]

- *Sulfite process to overcome recalcitrance of lignocellulose (SPORL)*

Sulfite process recalcitrance of lignocellulose (SPORL) is a pretreatment method can be used to react with a strong rigidness of lignocellulosic character such as woody biomass or softwood. This can overcome the recalcitrance of lignocellulose in the ineffectiveness of the sugar and ethanol recovery in bioethanol production.[26, 110] In this pretreatment, sulfite or bisulfite solution is reacted with the woody biomass at temperature, pH and pretreatment period of 160–

190°C, 2–5, and 10–30 min respectively.[26] SPORL method enhances sugar yield (57-88%) compared to acid pretreatment and reduces the inhibitor compounds (up to 65%) produced.[182] Therefore, SPORL is seen as one promising pretreatment method for woody biomass as it affects the downstream processes, as well as it enhances the efficiency of the bioethanol production yield and its production cost. [110, 147] Zhang et al.[198] reported that SPROL pretreatment improves the biomass digestibility, reducing lignin hydrophobicity by lignin sulfonation, and removes hemicellulose from the biomass. The pretreatment was applied within the range of temperature (163–197°C), time (3–37 min), sulfuric acid dosage (0.8–4.2% on switchgrass) and sodium sulfite dosage (0.6–7.4% on switchgrass). The results showed that SPORL pretreatment improved the digestibility of switchgrass through sufficiently removing hemicellulose, partially dissolving lignin, and reducing hydrophobicity of lignin by sulfonation.

3.3 Hydrolysis

Hydrolysis process breaks down the complex sugar structures that are built up in the biomass, for instance, polysaccharides in starch and lignocellulose, into the simplest sugar monomers, for instance xylose, glucose, etc. In general, hydrolysis is purposed to provide the suitable fermentable sugars for the fermentation microbes in fermentation process, as the subsequent process. This effort is necessary since particular microbes are only able to convert the particular sugar monomers into ethanol.[66, 112] Commonly, hydrolysis can be carried out in the following methods:

- *Dilute acid hydrolysis*

Dilute acid is typically utilized to hydrolyze lignocellulosic biomass, since acid is capable in degrading the lignocellulosic structure into fermentable sugars. Dilute acid hydrolysis utilizes around 1% of the acid concentration to degrade the biomass, and it yields around 50% of glucose as the end product.[199, 200] Dilute acid hydrolyzes of hemicellulose and cellulose have different approaches in order to identify composition of lignocellulosic. Balat et al.[16] reported that hemicellulose can degraded by the dilute acid and releases xylose monomers (5-carbon sugar) under a mild hydrolysis environment. Meanwhile, cellulose component requires higher temperature for the dilute acid to strike the complex structure of cellulose, and this second approach yields glucose monomers (6-carbon sugar).[16] Dilute acid is a practicable hydrolysis method for a continuous-production type of bioethanol plant, but prior size reduction of the feedstock is required. Dilute acid is performed in an affordable hydrolysis period and produces a fair end-product. However, it is still a challenge for this method to produce a desirably high sugar yield.[167] Besides, inhibitory products are formed as by-products of the process, including acetate, furfural, hydroxybenzaldehyde (HBA), 5-hydroxymethyl furfural, etc., which are harmful for fermenting microorganisms.[201]

- *Concentrated acid hydrolysis*

Concentrated acid is used to hydrolyze typically lignocellulosic biomass in a fair temperature condition and it employs strong acid (concentration more than 10%). Concentrated acid hydrolysis brings out better sugar yield compared to dilute acid hydrolysis, and it is aimed for a better cost efficiency of hydrolysis process. Despite the high sugar recovery and the ability of the acid to be recovered and reconcentrated, concentrated acid hydrolysis requires extreme precautions, especially during handling the acid substance since concentrated acid is extremely hazardous to human's health and environment.[16, 202] The drawback of concentrated acid hydrolysis method is able to corrode the production equipment, which potentially bringing big loss economically to the overall bioethanol production. This method is claimed to have a shorter processing time[203], although other argued that it is done under a longer hydrolysis period compared to dilute acid hydrolysis.[199]

- *Enzymatic hydrolysis*

Enzymatic hydrolysis is common method in bioethanol productionwhich used specific type of enzyme based on type of the biomass component. For instance, cellulase is used to hydrolyze cellulose and amylase to hydrolyze amylose. This hydrolysis method is a complex degradation process, especially for lignocellulosic material, aimed to release the simplest and fermentable sugar monomers. In lignocellulosic biomass, enzymatic hydrolysis works in three degree: (i) the biomass is deformed physically and chemically, (ii) degradation of biomass's surface, releasing less-complex sugar formation in the solution, (iii) and the last is hydrolysis of the released less-complex sugars in the solution into sugar monomers.[204]

The advantage of enzymatic hydrolysis is able to be operated at only a mild hydrolysis environment (pH of around 4.8 and temperature of around 318-323 K). Enzymatic hydrolysis produce high yield and better end-product result compared to acid hydrolysis which does not promote any corrosion tendency to the processing equipment.[205]The costly manufacturing of enzyme indeed accordingly drives the enzymatic hydrolysis cost, and ultimately the overall bioethanol production cost. However, the growing advancement of technologies and rapid development of enzyme manufacturing can able to reduce enzyme price. This will affect bioethanol industry as well to become more viable and expandable.[206] The production of enzymes, for instance cellulase, can be extracted from fungi and bacteria. The recorded fungi as cellulase and hemicellulose-source microbes are including *Trichoderma reesei, Trichoderma longibrachiatum,* and *Trichoderma viride.*[207] Meanwhile, the typical cellulase-producing bacteria are including *Acetovibrio, Bacteriodes, Bacillus, Cellulomonas, Clostridium, Erwinia, Microbispora, Ruminococcus, Streptomyces* and *Thermomonospora*.[205]

By employing a commercial cellulase, Tye et al.[208] utilized kapok (*Ceiba pentandra*) fiber as the second generation biomass in an attempt to yield the most fermentable sugar. Their observed that cellulase was managed to alter the complex structure of the fiber and yielded 85.2% of reducing sugar with prior acid pretreatment. Tan and Lee[95] have performed hydrolysis on seaweed solid waste enzymatically to produce bioethanol. The authors observed that enzymatic hydrolysis produced very high glucose content of 99.8% at very mild hydrolysis conditions of

50°C and pH 4.8. The study also performed fermentation subsequently and it yielded a desirable quality of ethanol of 55.9%. However, simultaneous saccharification and fermentation (SSF) was modified in order to obtain high ethanol yield of 90% from seaweed waste. It is show that enzymatic hydrolysis is able to be enhanced with the correct additional process and paired with the suitable biomass. Simultaneous saccharification and fermentation (SSF) of seaweed waste can yield higher ethanol quality of 90.0%. This shows that enzymatic hydrolysis is able to be enhanced with the correct additional process and paired with the suitable biomass[95].

3.4 Fermentation

Fermentation is a biomass conversion process into bioethanol by microorganisms(yeast and fungi or bacteria), which by digesting fermentable sugars and producing ethyl alcohol and other byproducts.[26, 112] *Saccharomyces* and *Pichia* are the most common yeast type employed in bioethanol production as well asbacteria *Zymomonas* and *Escherichia* and *Aspergillus*.[6] The nature type of fermentation microorganisms are able to convert particular sugar monomer into ethanol, namely hexoses (C6), which usually yielded in first and second generation production, and pentoses (C5), which usually yielded in second generation production.[209] Most of the studies used *Saccharomyces cerevisiae* yeast type. Wyman et al.[210] reported that *Saccharomyces cerevisiae* is able to produce theoretically 90% of ethanol from glucose sugar. Similar study by Abbi et al.[211] stated that S. *cerevisiae* is able to ferment hexose sugar, while P. *stipitis*, P. *tannophilus* and C. *shehatae* yeast are typically used to ferment xylose into ethanol efficiently. *Kluyveromyces marxianus* yeast is able to withstand high temperature range of 45–52°C, and is capable to digest wide variety of sugar monomers, namely xylose, mannose, arabinose, and galactose.[66, 212] In order to upgrade the ability of the conversion process, microbes engineering is one alternative which resulted from the technological advancement. The benefits include higher ethanol yield, enabling more substrate to be digested, and more resistive against inhibitors.

In conventional path, fermentation is carried by last after the biomass is pretreated and completely hydrolyzed. However, as the technology advances, methods to conduct fermentation are developed as the effort to gain better ethanol quality as well as better production efficiencies. This paper gathers the varieties in fermentation methods, and each of the method is discussed as follow.

- *Simultaneous saccharification and fermentation (SSF)*

Simultaneous saccharification and fermentation (SSF) technique is a single fermentation reactor in order to minimalize the inhibitors production, combining both processes (saccharification and fermentation) at one time, and to reduce additional equipment cost.[114, 213] In a single reactor, enzymatic hydrolysis of the biomass is run, and immediately the presented fermentation agent converts the released sugar monomers into ethanol. Moreover, the SSF technique can decrease production period and enhances the efficiency of the production.. Additionally, ethanol accumulation in the reactor does not inhibit the hydrolysis activity, making SSF as one favorable method for bioethanol production.[15, 144] However, this fermentation technique is limited by the respective optimum conditions for both hydrolysis and fermentation, which quite challenging to control to meet both satisfactory conditions at the same time.[214] In common practice, hydrolysis is best to be carried out at around 50°C, but fermentation requires warm-mild temperature for the microbes to sustain at around 28-37°C, hence to meet both desired conditions requires special engineering effort. **Fig. 6** shows the flowchart of simultaneous saccharification and fermentation process.[215]

[Fig. 6]

- *Separate hydrolysis and fermentation (SHF)*

Separate hydrolysis and fermentation (SHF) technique is a fermentation process can perform under different vessels, where each carries specific task.[8] SHF allows each both processes, hydrolysis and fermentation at their most optimum conditions and yielding the desired products to the maximum potential.[15] For instance, this technique enables the hydrolysis to be performed at the optimum temperature of 45-50°C and fermentation to be performed at about 30°C without any hassle [8,15]. SHF also allows fermentation of sugar based on its type. The hydrolysate is first flowed into the first vessel to ferment the glucose content of the hydrolysate by glucose-fermenting microbes. The process is then continued by distilling the solution and flowing it to the second fermentation vessel to ferment the next sugar type by the specific fermentation microbes.[26] SHF technique also reduces the inhibition production since the removal is practically allowable [115]. SHF technique is considerably cost effective when it focuses on the substrates, substances employed and the high quality ethanol yield, although it may not be cost effective in the equipment installment.[8, 216] **Fig 7** shows the flowchart of separate hydrolysis and fermentation process.[215]

[Fig. 7]

- *Simultaneous saccharification and co-fermentation (SSCF)*

This fermentation technique utilizes the integration principle in employing mixed microbes to ferment both more than one sugar type (e.g. pentoses and hexoses) resulted from the previous processes of pretreatment and hydrolysis.[8, 10] The use of mixed microbes is limited on the respective ability of the microbes, where hexose-fermenting microbes are usually grow faster than pentose-fermenting microbes, and this leads to the higher rate of ethanol conversion from hexose.[6] SSCF holds several beneficial characters in bioethanol production, including lesser enzyme requirement, faster production rate and lesser cost projected since the process is performed in a single reactor.[15] In contrary, SSFC requires extra conscientious controlling since the employed mixed microbes may require different optimum temperature, as well as between the hydrolysis and fermentation process since they are run in the same reactor.[217] *C. shehatae, S. cerevisiae, E. coli* KO11, *E. coli* FBR5 are the type of employed microbes to be mixed in SSCF technique to produce bioethanol from varieties of biomass, namely barley hull, wheat straw, etc.[1, 43, 218, 219] **Fig. 8** describes the flowchart of SSCF process.[215]

[Fig. 8]

- *Consolidated bioprocessing (CBP)*

Consolidated bioprocessing is a technique in bioethanol production where enzymes production, enzymatic hydrolysis and biomass fermentation are carried out within the same single reactor. Hence, this technique is usually called direct microbial conversion (DMC).[132] In general, CBP employs a specific microorganism type that is capable in conducting such array of tasks without additional flowing or removal process to another reactor. In lignocellulosic biomass, for example, production of cellulase enzyme, hydrolysis of cellulose, and fermentation of the yielded sugars are performed by one type of microorganism. This method is a low cost route conversion of cellulosic biomass into bioethanol with high rate and acceptable yields. [17, 26] Although others have reported that CBP consumes long processing time and undesirable ethanol yield.[1, 26] With CBP, external cellulose enzyme is unnecessary to be added into the pretreatment and hydrolysis processes of the bioethanol production.[26, 220] The common microbial employed for this method including *Thermo anaerobacter ethanolicus*, *Clostridium thermos hydrosulfuricum*, *Thermo anaerobacter mathranii*, *Thermoanaerobium brockii*, *Clostridium thermosaccharolyticum.* These microbial have good abilities that are utilized in many inexpensive lignocellulosic biomass-to-ethanol conversion and are able to withstand extreme temperature.[8] Cardona and Sánchez [221]reported the potential yeast strain of K. *marxianus* for CBP method and it resulted a good growth display of endoglucanase and b-glucosidase on the cell surface at temperature as high as 48°C of which the ethanol was produced from the cellulosic material b-glucan with a yield of 0.47 g ethanol per gram of consumed carbohydrate. However, CBP technique is not highly recommended since the typical microorganisms employed for CBP are intolerant with high amount of ethanol yield, hence further microbial engineering is utterly suggested recommended to upgrade the applicability of this technique.[222, 223] **Fig. 9** describes the flowchart of consolidated bioprocessing process.[215]

[Fig. 9]

3.5 Distillation and dehydration

Distillation in bioethanol production is required to separate the ethanol content out of the fermented mixture by utilizing the boiling point of ethanol (78.3°C). In other words, in water-ethanol distillation, ethanol will be vaporized before water.[16, 224] In a typical bioethanol refinery, the fermentation product is flowed to the distillation column to be distillated, and the product from this process is then flowed to the rectifier and concentrated to just below the azeotropic point, as seen in **Fig. 10** that shows the water-ethanol azeotropic point.[109, 225] Meanwhile, the remaining un-distilled fermented product is flowed to stripping column to additionally remove the water content. Afterwards, the product of this process is combined with the ethanol produced from the previous process.[226] As the result, bioethanol recovery produces quality about 99.6%.[67] The solid compounds of the product are separated using centrifuge.[227] Distillation or bioethanol recovery process is an energy intensive process which requires high volumes of cooling water and produce some gallons of water used for every gallon of ethanol obtained.[108]

[Fig. 10]

Dehydration is advantageous to remove the remaining water in azeotropic phase.[107, 228] The dehydration technique is quite similar with extractive distillation due to this process utilizes additional component which lowers the heterogeneous boiling azeotrope.[229] Prior to dehydration process, the ethanol is first distilled to achieve above 96%, then is filtered through molecular sieves, which absorbs the water component from the mixture.[109] The result of this process is an upgraded ethanol product. The used molecular sieves can be recycled by heating the sieves to remove the absorbed water.[114]

Overall, the literatures for bioethanol production from biomass feedstock are tabulated in **Table 11.**[230-237] and **Table 12.**[238-243]

[Table 11]

[Table 12]

4. Fuel properties bioethanol

The quality of combustion engine fuel can be identified through the chemical and physical properties of the fuel.[244] The standardized properties for bioethanol are listed under ASTM D4806 standard, and the comparison with gasoline is given respectively in **Table 13.**[245-247] and **Table 14.**[247-249]

[Table 13]

[Table 14]

The detailed of the some properties related bioethanol and gasoline are discussed below.

4.1 Ethanol yield

Ethanol yield is the parameter to evaluate the performance of the feedstock in bioethanol production.[250] Ethanol yield can be described as theoretical and actual/experimental ethanol yield. Theoretical ethanol yield is obtained through stoichiometry calculation of the fermentable sugars, and is usually used as comparison factor. Meanwhile, actual ethanol yield is obtained from the actual production process of sugars fermentation, which derived from the employed biomass. Actual ethanol yield depends on the conditions of fermentation, especially on the employed fermentation agents.[251] Therefore, the feedstock and microorganism selection were employed in the ethanol production in order to produce high yield and good ethanol quality. The concentration of the produced ethanol can be detected by checking their corresponding refractive indexes obtained from the refractometer readings.[252] The standard of ethanol yield or content for bioethanol is ASTM D4806.[253]

4.2 Acidity or alkalinity

The acidity or alkalinity of bioethanol is tested by reacting it with phenolphthalein to test whether the solution is alkali or acid, and the acidity is expressed as a percentage by mass of acetic acid.[254] The acidity equation is given as below[254]:

$$\text{Acidity} = \frac{0.0006 \times V}{\rho} \tag{1}$$

Where:

V = the volume of the NaOH solution (in mL)

ρ = the density of the sample at 20°C (in g/mL)

0.0006 = the mass of CH_3COOH corresponding to 1 mL of NaOH (in g)

An acidity or alkalinity of bioethanol describes the concentration of acid-type compounds that are presented in the tested solution, and it is usually regarded as total acid number.[255] High acid content can cause the formation of gums and lacquers on metal surfaces, increases viscosity that impairs oil circulation, and also causes engine corrosion.[256] ASTM Standard D1613 is the standard to measure the acidity or alkalinity of bioethanol. According to this standard, acidity or alkalinity of bioethanol has maximum value of 0.007 mg/L.[257]

4.3 Water content

The stability of ethanol depends on the chemical composition of the bioethanol, water content and temperature.[258] Water content in ethanol generally regards to the two types of purities: (i) anhydrous, where water content obtained is less than 1%; (ii) hydrous, where water content possession is ranged 5−10% in the ethanol.[248] Water contamination in ethanol is possible since ethanol has hygroscopic nature, which absorbs water from the atmosphere it stored in open container.[259] The water content in the bioethanol can be measured as accordance to ASTM E203 method, where this standard describes the maximum specification of 1.000% vol. s.[246]

4.4 Denatured content

Denatured ethanol contains certain additives that can make it inconsumable and toxic, which purposed to prohibit recreational consumption. The additives also give bas taste and foul smell to the ethanol.[247] Foul-taste or toxic substances is added for about 10% of the produced ethanol after distillation to accomplish this parameter.Denatured ethanol also can be blended with gasoline to improve the octane number of gasoline.[248] The denatured ethanol is set to a minimum value of 1.960% vol. and maximum value of 4.760% vol. according to ASTM D 4806.[246]

4.5 pHe

The pHe is pH of a denatured fuel ethanol, and it is quite difficult to measure this property since high quality denatured ethanol is not an aqueous solution.[260] Typically, the pHe test is conducted after the denaturing process and corrosion inhibitors addition.[261] The ASTM D6423 method describes the pHe test, which covers rehydration specification of the probe between readings and repeatability for same operator, same apparatus and identical test material.[247] The range of pHe of bioethanol is 6.5–9.0 with 90% confidence factor and reproducibility of 0.52.[259, 260]

4.6 Octane number

A measurement of fuel resistivity towards denotation and self-ignition is described as octane number.[262] Octane number can be rated as motor octane number (MON) or research octane number (RON), which both concludes the behavior of particular fuel combustion in an engine during high or steady load condition.[105, 263] As an excellent anti-detonating additive, bioethanol can improve the octane number of gasoline-base fuel.[263, 264] The octane number of bioethanol is specified by ASTM D2700, which are MON is 92; RON is 108; and bioethanol-gasoline blend ranges of MON is 81–90 and RON is 90–100.[248, 249]

The comparison properties of bioethanol and bioethanol gasoline blends are summarized in **Table 15**[251, 253, 265-269] and **Table 16.**[253, 269-274]

[Table 15]

[Table 16]

5. Engine performance and emissions using bioethanol and the blends

As one of renewable energy resources obtained from biomass, bioethanol has been tested intensively in the internal combustion engines. Bioethanol is a viable alternative to unleaded gasoline fuel as automotive fuel, and it can be used without modification engines.[271, 275, 276] The application of bioethanol in spark-ignited engine has been studied and assessed through its combustion performance parameters, including brake thermal efficiency, brake specific fuel consumption, and engine power, which hence brings the bioethanol viability as biofuel closer to the major practice. Additionally, gasoline bioethanol blends which substitute as fuel can enhance the blend property qualities and combustion performance in engine. Ghazikhani et al.[270] analyzed the performance of two stroke SI engine using gasoline-ethanol blend, which resulted a CO emission reduction by 32% from ethanol concentration of 15% in the blend. Similarly, Schifter et al.[272] observed that by 20% ethanol blend in single cylinder engine the CO emission was reduced by 52%. In a four-stroke SI engine, Najafi et al.[271] investigated the performance of the engine fueled by gasoline-ethanol blends, and they observed that E20 enhanced the combustion efficiency by 30% and CO emitted was yielded 45.42%, compared to only gasoline fuel. Using higher ethanol ratio in the blend, Koç, et al.[277] studied a single-cylinder SI engine combustion performance powered by blends E50 and E85. The authors reported that brake specific fuel consumption (BSFC) was improved to 20.3% and 45.6%, and NO_x emission was decreased to

14.29% and 11.22% for E50 and E85 respectively. The benefit of blending fossil fuel with bioethanol is also eligible for diesel engine. As reported by Gomasta and Mahla[275], stated that engine performance efficiency was increased to 13% for ethanol-diesel blends, and hydrocarbon (HC) emissions was reduced to 35% at full load for 20% ethanol ratio in the blend compared to only diesel fuel. The summary of engine performance and emissions productions using ethanol blends are presented in **Table 17.**[270-272, 275, 277-279]

[Table 17]

6. Conclusion

Bioethanol is drawn to the surface for its sensibility to fit in the current energy crisis. Bioethanol is produced diversely according to the feedstock type, although in general it follows the full sequence of pretreatment, hydrolysis, fermentation and distillation. In first generation bioethanol production sugar-rich food crops are treated, hydrolyzed and fermented to produce ethanol. Besides that, agricultural crops residues can utilized for the second generation production. Moreover, the lack of source availability of first and second generation led the researchers to explore new feedstock in bioethanol production which by utilizing micro and macro algae due to their favorability in rapid growing rate. This is bring the advancement of technology give more available methods and techniques, for instance pretreatment process can be performed by tens of different techniques. The availabilities enable better approach in selecting the most suitable routes that match with one’s desired requirements and outcomes from the existed sources. Furthermore, the suitability of gasoline bioethanol blends as an alternative fuel in spark-ignited engine is characterized by the fairness of its fuel properties. The properties of gasoline bioethanol blends such as ethanol purity ratio of 99%, pHe of 6.5-9.0, water content of 1.0%, density of 785-809.9 were ruled according to ASTM standard. The gasoline bioethanol blends within these properties are suitable as an alternative fuel in spark-ignited engine.

This review provides overall available method in the production of bioethanol as an alternative fuel with tremendous potentials. It also displays suggestion that bioethanol production should not only be focused on the feedstock potential and the selection of production route, but also it encourages the other practical aspects when it comes to actual realization, especially the environmental impact. The feedstock exploration for bioethanol production is expandable, which means there are many rooms of improvement and large number in combinations of possible production path to be sorting out and arranged for the suitable feedstock. Bioethanol is indeed acted as the most influential variable for transportation sector, since with its benefits bioethanol will bring the independency of the non-renewable and worse pollution-emitting of fossil fuel. Finally, bioethanol is one solution for energy and environmental crisis and it is suggested to have a good synergy from both technological developments and supporting energy policies to solve all energy Acknowledgments

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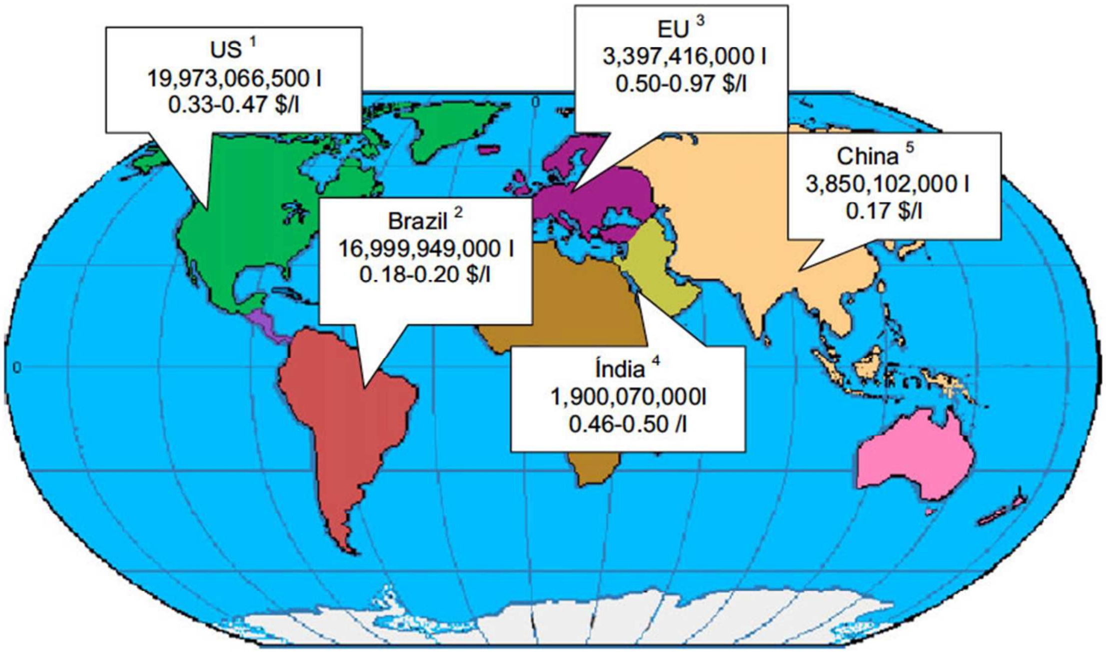

Fig. 1 The main producers and production costs of bioethanol.[3]

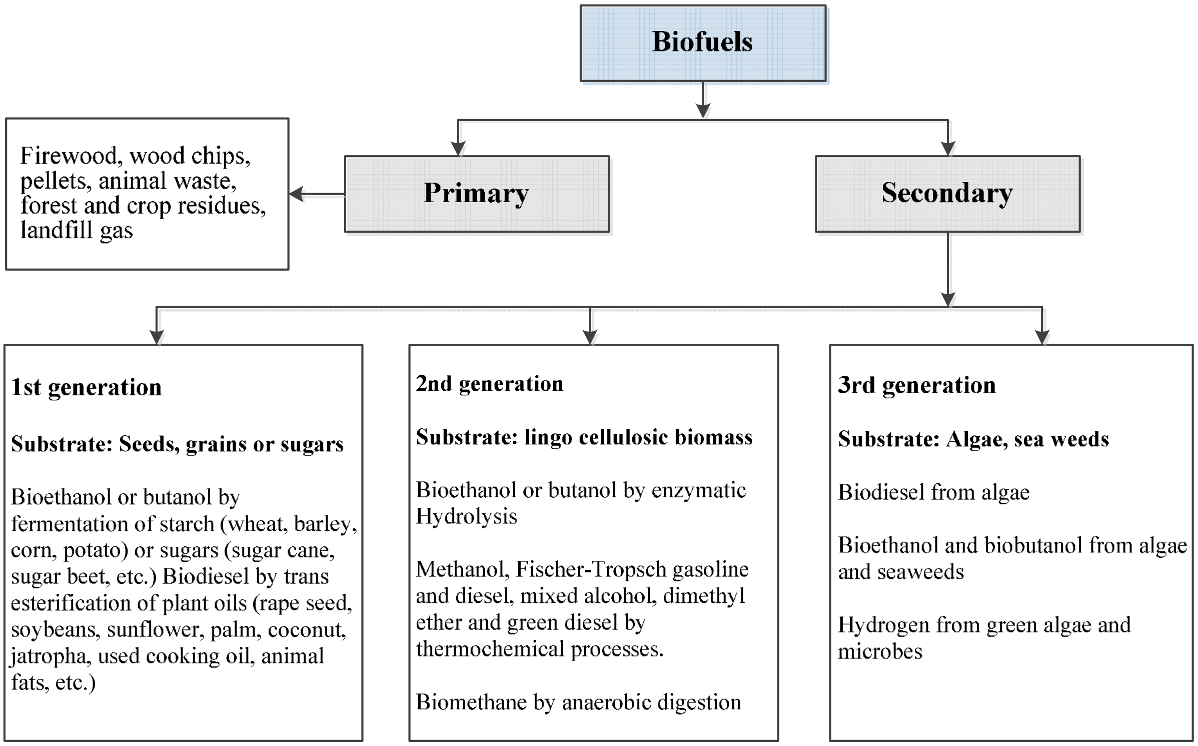

Fig. 2 The classification of the generation for biofuel feedstock.[20]

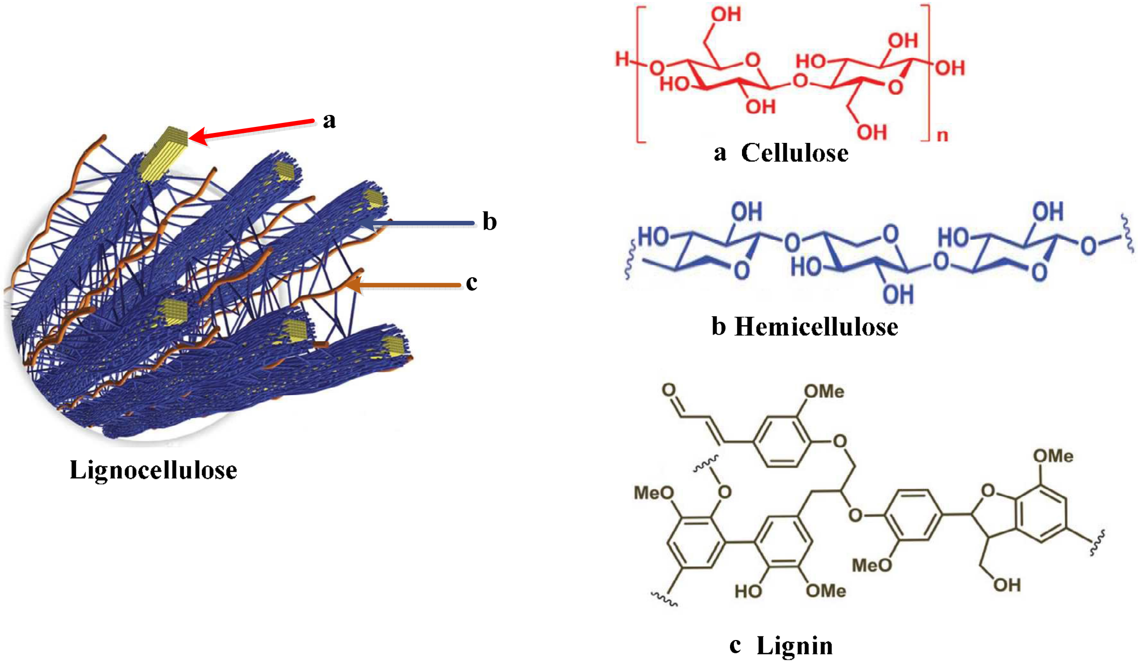

Fig. 3 Lignocellulosic biomass (a) cellulose, (b) hemicellulose and (c) lignin .[75, 76]

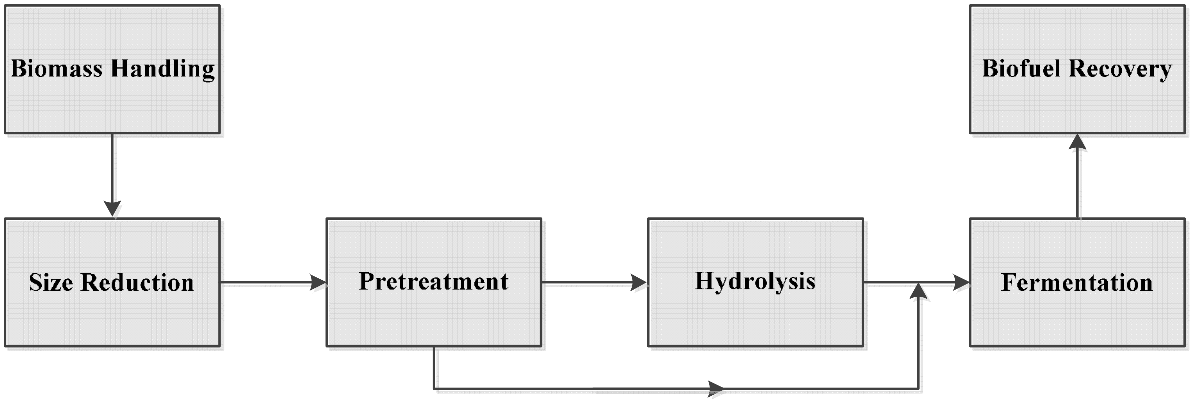

Fig. 4 Conversion process of biomass to bioethanol.[107]

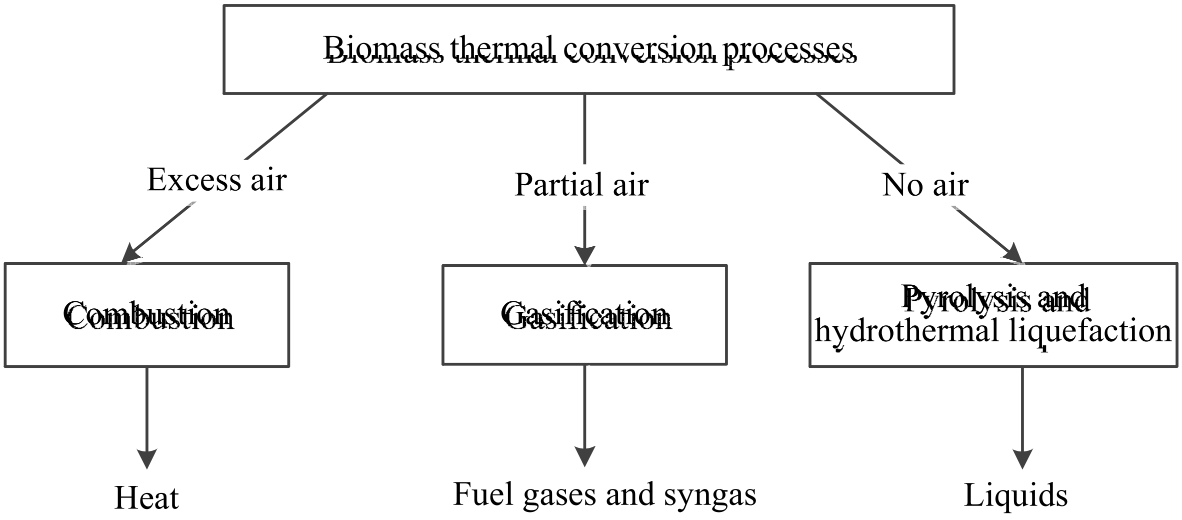

Fig. 5 Lignocellulosic biomass by thermochemical technology.[170]

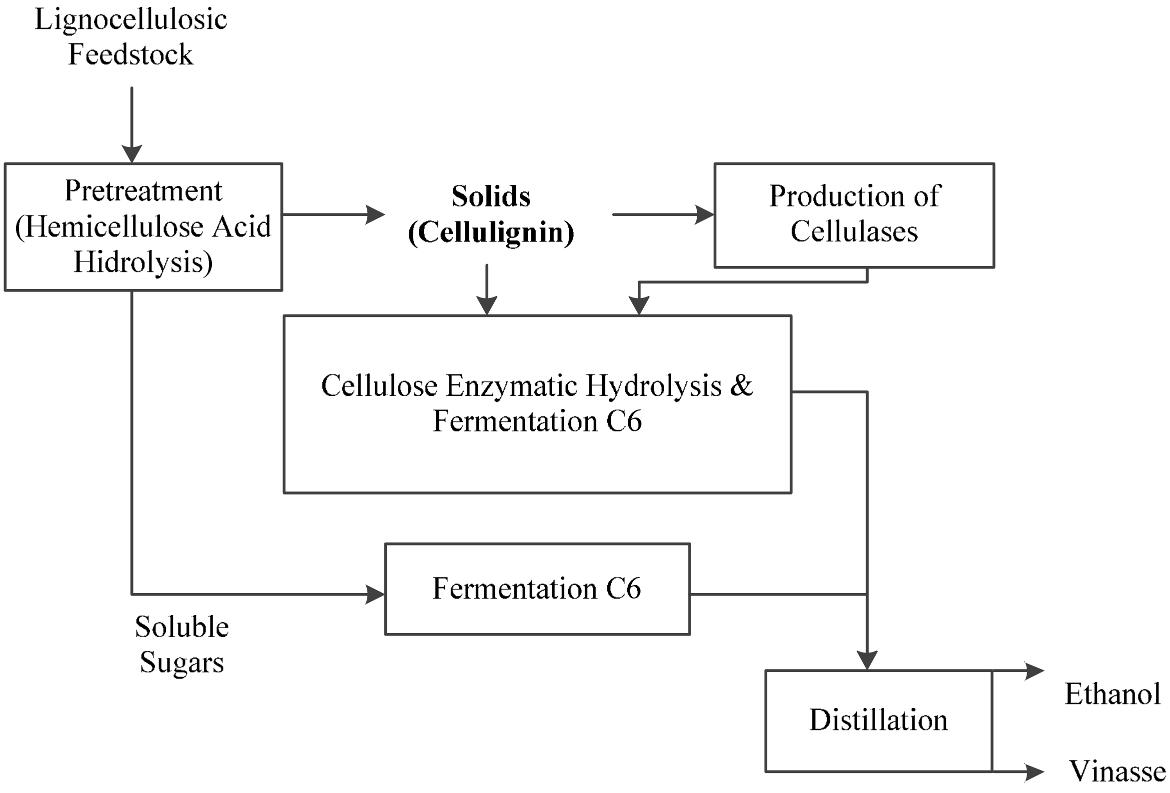

Fig. 6 The flowchart of the simultaneous saccharification and fermentation (SSF) process.[215]

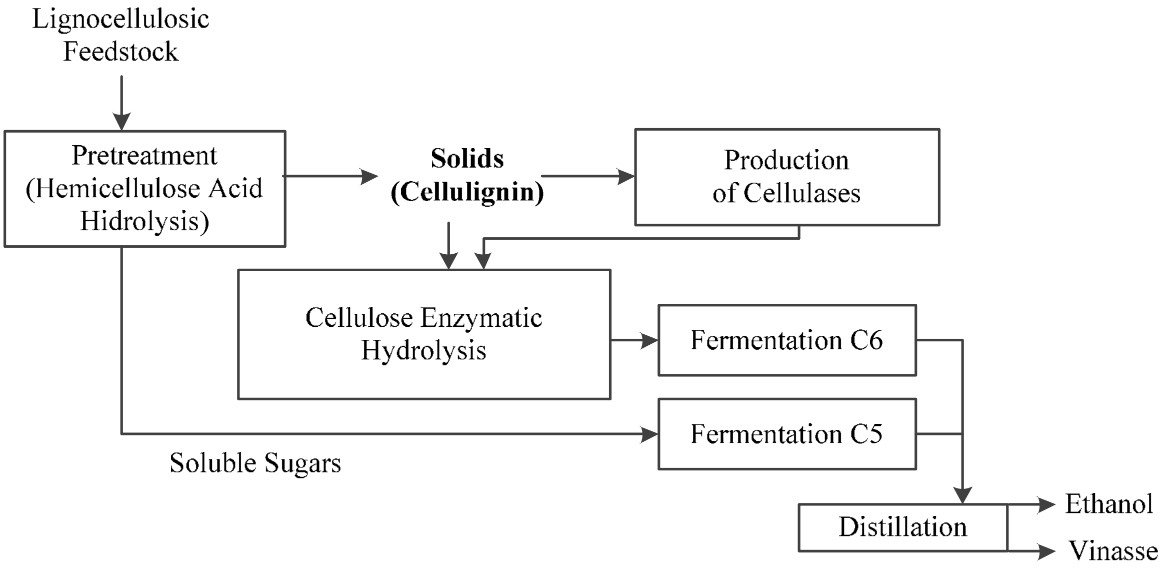

Fig. 7 The flowchart of the separate hydrolysis and fermentation (SHF) process.[215]

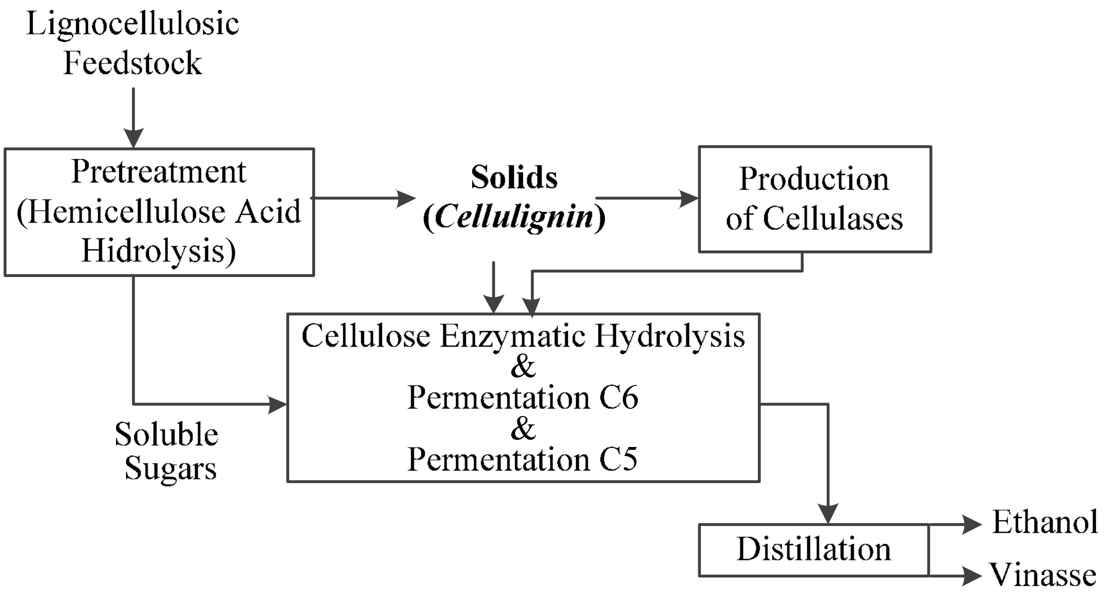

Fig. 8 The flowchart of the simultaneous saccharification and co-femermentation (SSCF) process.[215]

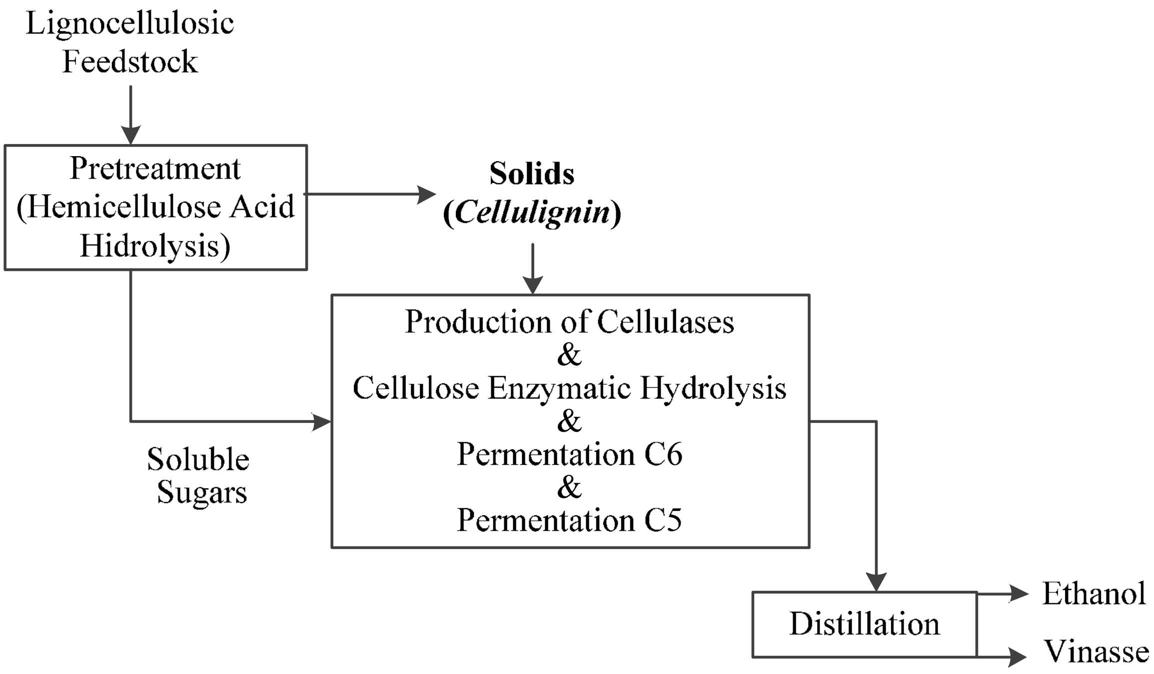

Fig. 9 The flowchart of the consolidated bioprocessing (CBP) process.[215]

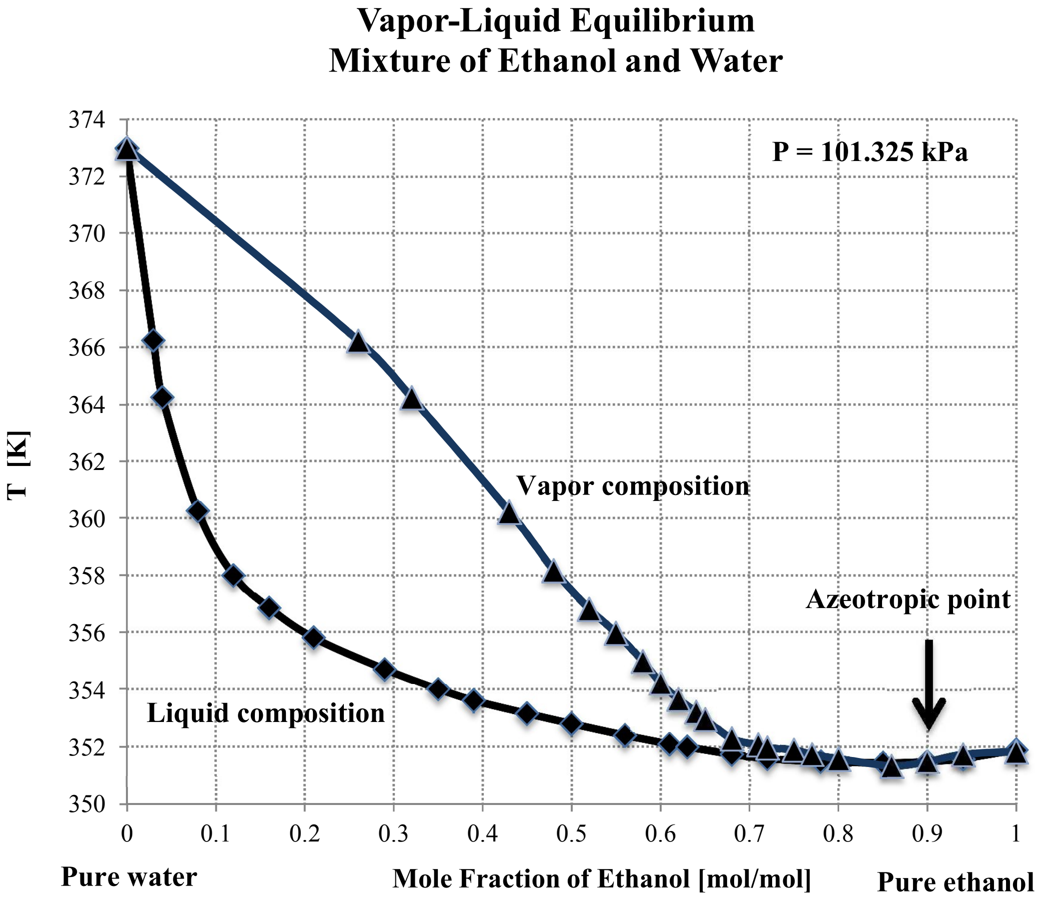

Fig. 10 Water-ethanol equilibrium mixture and azeotropic point.[109, 225]

Table 1 Potential bioethanol production for various biomass feedstock.[18, 19]

Bioethanol feedstock	Bioethanol production potential (L/ton)
Sweet sorghum	60
Sugarcane	70
Sugar beet	110
Potato	110
Sweet potato	125
Cassava	180
Barley	250
Bagasse and other cellulose biomass	280
Wheat	340
Maize	360
Rice	430

Table 2: The composition profile of sugarcane and sugar beet.[22, 28]

Components	Concentration	
	Sugarcane	Sugar beet
Solids (%)	13.7	17.3
Sugar (%)	12–17	16.5
Raffinose (%)	–	0.07
Monosaccharides (%)	0.63	0.15
Polysaccharides (%)	0.028	0.019
Lactate (%)	0.016	–
Acetate (%)	0.033	–
Sulphate (%)	0.039	0.02
Phosphate (%)	0.033	0.047
Nitrate (%)	–	0.015
Nitrite (%)	–	0.005
Aconitate(%)	0.09	–
K (%)	0	–
Na (%)	0.005	0.015
Cl (%)	–	0.003
Ca (%)	0.04	–
Mg (%)	0.028	–
Total-N (%)	–	0.105
Betaine-N (%)	–	0.046
Amino acid-N (%)	–	0.026
Ammonia-N (%)	–	0.006
Amide-N (%)	–	0.011

Table 3: The composition profile of sweet sorghum.[35]

Composition	Sweet sorghum
Juice[a] (% total)	71.9
Sucrose (% juice)	7.6
Glucose (% juice)	2.6
Fructose (% juice)	1.6
Total sugars (% juice)	11.8
Fiber (% wt.)	13.0
Cellulose (% wt.)	44.6
Hemicellulose (% wt.)	27.1
Lignin (% wt.)	20.7
Ash (%)	0.4
Brix (% juice)	11-13

[a] One kg of stalk was crushed at 2,500 psi for 1 min in a press to extract juice

Table 4: The composition profile of wheat, corn and barley grain biomass.[45-48]

Composition	Wheat	Corn	Barley
Moisture (%)	12.5	15	11.1
Calories (cal/100g)	330	361	349
Protein (%)	12.3	10.2	2.4
Fat (%)	1.8	4.3	1.0
Ash (%)	1.7	1.2	0.9
Sugar (%)	3.6	5.3	5.3

Table 5: The composition profile of cassava biomass (100 g basis).[49, 55]

Composition	Cassava
Calories (cal)	135
Peel (%)	10–20
Cork layer (%)	0.5–2.0
Edible portion (%)	80–90
Moisture (%)	62–66
Total solids (%)	38
Volatile solids (%)	99
Protein (g)	1.2
Total nitrogen (%)	0.22
Lipid (g)	0.20
Starch (g)	18–32
Fiber (g)	1.10
Carbohydrate (%)	35
Total carbon (%)	19
Ash (%)	0.9–1
Fat (mg)	0.1
Calcium (mg)	26
Phosphorus (mg)	32
Iron (mg)	1
Sodium (mg)	2
Potassium (mg)	394
Vitamin B2 (mg)	0.04
Vitamin C (mg)	34
Niacin (mg)	0.60
Cyanide (%)	–

Table 6: The composition profile of hemicellulose, cellulose and lignin from lignocellulosic biomass.

Lignocellulosic biomass	Cellulose glucan (% wt.)	Hemicellulose (% wt.)				Lignin (% wt.)		Ref
		Xylan	Arabinan	Galactan	Mannan	Acid insoluble lignin	Acid soluble lignin	
Barley straw	35.65	16.86	2.11	–	13.8	20.7	2.4	41
Corn stover	38.3	21.0	2.7	2.1	-	17.4	–	78
Wheat straw	30.2	18.7	2.8	0.8	-	17	–	79
Rice straw	31.1	18.7	3.6	-	-	13.3	–	69
Rye straw	30.9	21.5	-	-	-	22.1	3.2	69
Oat straw	39.4	27.1	–	–	–	17.5	–	77
Sunflower stalks	42.1	29.7	–	–	–	13.4	–	77
Sugarcane bagasse	43.1	31.1	–	–	–	11.4	–	69
Sweet sorghum bagasse	27.3	13.1	1.4	-	-	14.3	–	69
Olive tree pruning	25.0	11.1	2.4	1.5	0.8	16.2	2.2	81
Poplar	43.8	14.8	-	-	-	29.1	–	80
Spruce	43.8	6.3	-	-	14.5	28.3	0.53	69
Oak	45.2	20.3	-	-	4.2	21.0	3.3	69

Table 7: The chemical composition of different biomass.

Feedstock	Chemical composition (% wt.)										Ref
	SiO_2	CaO	K_2O	P_2O_5	Al_2O_3	MgO	Fe_2O_3	SO_3	Na_2O	TiO_2	
Wood and woody biomass											
Eucalyptus bark	10.04	57.74	9.29	2.35	3.1	10.91	1.12	3.47	1.86	0.12	82
Poplar bark	1.86	77.31	8.93	2.48	0.62	2.36	2.36	0.74	4.84	0.12	82
Wood residue	53.15	11.66	4.85	1.37	12.64	3.06	3.06	1.99	4.47	0.57	84
Agriculture residue											
Bamboo whole	9.92	4.46	53.38	20.33	0.67	6.57	0.67	3.68	0.31	0.01	85
Rice husks	94.48	0.97	2.29	0.54	0.21	0.19	0.22	0.92	0.16	0.02	84
Sugarcane bagasse	46.79	4.91	6.95	3.87	14.6	4.56	11.12	3.57	1.61	2.02	84
Sunflower husks	23.66	15.31	28.53	7.13	8.75	7.33	4.27	4.07	0.8	0.15	83
Other biomass residue											
Mixed waste paper	28.62	7.63	0.16	0.2	53.53	2.4	0.82	1.73	0.54	4.37	84
Sewage sludge	33.28	13.04	1.6	15.88	12.91	2.49	15.7	2.05	2.25	0.8	83
Wood yard waste	60.1	23.92	2.98	1.98	3.08	2.17	1.98	2.46	1.01	0.32	84

Table 8: Composition profile of microalgae.

Microalgea	Composition (% wt.)									Ref.
	Carbohydrates	Xylose	Mannose	Glucose	Galactose	Lipids	Proteins	Starch	Others	
G. *verrucosa*	42.67	–	–	24	–	–	–	–	8.83	86
C. *vulgaris*	44	–	–	20.52	–	35	32	12.–17	–	94
C.*infusionum*	32.52	9.54	4.87	15.22	2.89	–	–	11.32	56.16	93
T. *suecica*	27.41	–	–	–	–	14.25	58.32	–	–	94
Chloroccum sp.	32.52	9.54	4.87	15.22	2.89	–	–	11.32	56.16	93
C. *sorokiniana*	18.2	13.8	–	70.8	–	–	42.8	–	0.78	91
N. *gaditana*	11.2	28.8	–	59.0	–	–	39.5	–	0.17	91
S. *almeriensis*	14.5	33.4	–	52.2	–	–	36.7	–	0.71	91

Table 9: Composition profile of seaweeds.

Seaweeds	Composition (% wt.)						Ref.
	Carbohydrates	Fiber	Lipids	Protein	Moisture	Ash	
Laminaria spp	–	–	2	12	–	26	101
C. *lentillifera*	59.27	3.17	0.86	12.49	25.31	24.21	99
S. *horneri*	19.93	–	0.82	22.94	86.94	32	102
C. *veravelensis*	37.23	–	2.8	7.77	87.88	33.7	96
Sargassum spp	41.81	9.84	0.75	10.25	11.16	26.19	103
T. *triquetra*	45.68	–	4.83	10.12	15.83	40.34	96
S. *naozhouense*	47.73	4.83	1.06	11.2	–	35.18	98
H. *clathratus*	82.26	2.7	2.18	6.39	59.63	6.47	104
U. *reticulata*	55.77	4.84	0.75	21.06	22.51	17.58	99

Table 10: The effect of extrusion process for various feedstock.

Extrusion process	Lignocellulose	Extruder	Extrusion conditions	Sugar yield	Ref.
Physical pretreatment	Corn stover	Single screw extruder	Screw speed: 75 rpm, temperature: 125°C	Glucose : 75%, xylose : 49% combined sugar: 61%	119
	Big bluestern	Single screw extruder	Screw speed: 100 rpm, temperature: 150°C, moisture content: 20% wb, particle size: 8 mm	Glucose : 55.2%, xylose : 92.8% combined sugar: 65.4%	122
Acid pretreament	Rice straw	Twin screw extruder	Screw speed : 40 rpm, temperature: 120°C H_2SO_4 concentration: 3% wt.	Xylose: 83.7%	124
	Pine sawdust	Twin screw extruder	Screw speed: 110 rpm, temperature: 60°C, head pressure: 780 psi, H_2SO_4 concentration 70 % wt.	Glucose: 44.4%	123
Alkali pretreament	Corn stover	Twin screw extruder	Screw speed: 80 rpm, temperature: 140°C, NaOH ratio: 0.04 g/g biomass	Glucose: 86.6%, xylose: 50.5%	121
	Switchgrass	Single screw extruder	Screw speed : 155 rpm, temperature: 176°C, particle size: 8 mm, NaOH concentration: 0.02 g/g biomass	Glucose: 40.5%, xylose: 60% combined sugar: 47.9%	120

Table 11: Comparison of bioethanol production for first generation biomass feedstock.

Biomass	Fermentation process	Results (ethanol concentration)	Ref
Sugarcane (*Saccharum officinarum*)	*Escherichia coli* KO11 and *Klebsiella oxytoca* P2	39.4–42.1 g/L	230
Sweet sorghum (*Sorghum bicolor)*	*Kluyveromyces marxianus* DMKU 3-1042	7.43 % w/v at 37^{o}C	234
Wheat (*Triticum vulgare)*	*Pichia kudriavzevii*	71.95 g/L at 40 ^{o}C	233
Sugar beet (*Beta vulgaris*)	*S. cerevisiae* Ethanol Red and *S. cerevisiae safdistill* C-70.	85.0–87.0 g/L	235
	S. cerevisiae IR-2, *S. cerevisiae* ATCC 26603, *S. cerevisiae* IFO 0309 and Z. *mobilis* IFO 13756	0.44 g/g and the lowest productivity of 0.08 g/h/L by Z. *mobilis*	232
Sweet sorghum (*Sorghum bicolor*)	*S. cerevisiae* CFTR 01 and SG	9.0% w/v by Keller variety	231
	S. cerevisiae ATCC 7754 and *Z. mobilis* ATCC 29191	50.26 mL/L	237
Watermelon (*Citrullus lanatus*)	*S.cerevisiae* Ethanol Red	25.0% w/v at pH3.0 and 35.0% w/v at pH5.0	236

Table 12: Comparison of pretreatment process for second generation biomass feedstock.

Biomass	Pre-treatment	Hydrolysis	Results	Ref.
Sugarcane bagasse	Ball milling (4h)	*Acremonium* cellulase at 5 FPU/g subtrate of cellulase and 20U/g subtrate of xylanase from optimash BG at 45^{o}C, pH 5.0 for 72 h	Glucose of 89.2 ± 0.7% Xylose of 77.2 ± 0.7%	238
	H_2SO_4 of 1% vol. at 60^{o}C for 24 h	In an autoclave at 121^{o}C for 40 min after removing the excess H_2SO_4 of 1% vol.	Total sugar of 68.0 g/L	239
Wheat straw	Knife milling with 0.7–1.0 mm and washed with water and dried	H_2SO_4 of 1.85% vol. at 90^{o}C with for 18 h which 20:1 of liquid to solid ration. Suspension centrifuged and the residue is washed with hot water	Glucose of 1.70 ± 0.30 g/L Xylose of 12.80 ± 0.25 g/L	240
Rice straw	Chopped to 5–6 mm	H_2SO_4 of 4.4% vol. at 1:10 solid to liquid ration in boiling water bath for 1 h. The process was filtered and pH was adjusted to 5.5	Total sugar of 20 g/L	241
	Chopped and steam exploded at 3.5 Mpa, 275^{o}C for 2 min	Saccharification used *cytolase*, *novozyme* at 50^{o}C for 120 h	Xylose of 5–10 g/L	242
Maize straw	NaOH 2% wt. at 80^{o}C for 1h	Hydrolysis by cellulase of *trichoderma reesei* ZU-02 and cellobiose of *Aspergillus niger* ZU-07	Xylose of 23.6 g/L Glucose of 56.7 g/L Arabinose of 5.7 g/L	243

Table 13: Bioethanol properties based on ASTM D4806.[245-247]

Property	Specification	ASTM Test Method
Ethanol min, (% vol.)	92.1	D 5501
Methanol, max, (% vol.)	0.5	–
Solvent-washed gum max, (mg/100 mL)	5.0	D 381
Water content, max, (% vol.)	1.0	E 203
Denaturant content, min, (% vol.)	1.96	–
Denaturant content, max, (% vol.),	4.76	–
Inorganic Chloride content, max,(mg/L)	40	D 512
Copper content max , (mg/kg)	0.1	D1688
Acidity (as acetic acid CH_3COOH) max, (mg/L)	0.007	D1613
pHe	6.5-9.0	D 6423
Appearance -Visibly free of suspended or precipitated contaminants (clear & bright)		

Table 14: Properties of bioethanol and gasoline.[247-249]

Property	Ethanol	Gasoline
Chemical formula	C_2H_5OH	C4 to C12
Molecular weight (g/mol)	46.07	100–105
Carbon (% wt.)	52.2	85–88
Hydrogen (% wt.)	13.1	12–15
Oxygen (% wt.)	34.7	0
Specific gravity	0.794	0.7–0.78
Density @ 15°C, (kg/m^3)	785-809.9	750-765
Boiling temperature, (°C)	78	27-225
Reid vapor pressure @37.8^{o}C, (kPa)	17	53-60
Research octane no.	108	90–100
Motor octane no.	92	81–90
(R + M)/2	100	86–94
Cetane no.	–	5–20
Fuel in water, (%vol).	100	0
Water in fuel, (%vol.)	100	0
Freezing point, (°C)	–114	-40
Kinematic viscosity (mm^2/s)	1.2-1.5	0.5-0.6
Flash point, closed cup, (°C)	12	-42
Auto ignition temperature, (°C)	423	257
Lower Heating Value (MJ/kg)	26.9	44
Higher Heating Value (MJ/kg)	29.7	47.3
Vapor Flammability Limits, (%vol.)	3.5-15	0.6-8
Specific heat, liquid (kJ/kgK)	1.7	2.4
Specific heat, vapor (kJ/kgK)	1.93	2.5
Stoichiometric air/fuel ratio	9	14.5-14.7
Fuel in vaporized stoichiometric mixture, (% vol.)	6.5	2

Table 15: The comparison properties of bioethanol from various biomass feedstock.

Properties	Unit	Sugar molasses[269]	Sugarcane[266]	Farmstead[253]	Cassava tubers[265]	Cassava flours[251]	Palm juice[268]	Sweet sorghum syrup[267]
Boiling point	°C	78.5	78.4	–	78.5	–	–	78
Heat of combustion	MJ/L	23.625	–	–	–	–	–	–
Heat of vaporization	kJ/mole	33.74	–	–	–	–	–	–
Lower heating value	kJ/cm^3	–	–	–	–	–	–	21.09
Octane rating	–	106–108	–	–	–	–	–	–
Stoichiometric air/fuel ratio	–	9/1	–	–	–	–	–	–
Concentration	–	99.6–99.8	–	–	–	98	96	–
pH	–	–	–	–	5–6	6.78	3-4	5.0
Acidity	mg/L	≤ 30	–	–	–	–	≤36	–
Water content	% vol.	≤ 0.3	0.378	6.94	–	–	–	–
Viscosity at 40°C	mm^2/s	–	–	–	–	2.2	–	–
Density @ 20°C	kg/m^3	789.0	791	816.2 at 15°C	791.0	–	795.0 at 25°C	785.0
Point of humidity	–	< 6.5	–	–	–	–	–	–
Flammability limits	°C	–	–	–	–	–	–	13-42
Flash point	°C	–	13-14	24.5	–	23	–	13
Vapor pressure	kPa	–	–	14.5	–	–	–	17
Auto ignition temperature	°C	–	–	–	–	–	–	366
Distillation	% vol.	–	–	–	–	78–100	–	–

Table 16: The properties of bioethanol gasoline blends.

Property	Unit	Test method	Gasoline	Farmstead[253]		Sugar molasses[269]					Cassava[270]		
				E58.9	E60	E10	E15	E20	E25	E30	E5	E10	E15
Density @ 20°C	kg/m^3	ASTM D1298	745.4	917.0	908.0	739.6	749.5	754.1	757.1	761.3	740.8	743.9	746.9
Flash point	°C	ASTM D93	–	24.5	24.5	–	–	29.2	30.0	29.2	–	–	–
Heating value	MJ/kg	ASTM D240	42.54	–	–	–	–	–	–	–	43.45	42.51	41.58
Auto ignition temperature	°C	–	246.0	–	–	–	–	–	–	–	–	–	–
Research octane number	–	ASTM D2699	91.3	–	–	97.1	98.6	100.4	99.5	102.5	–	–	–
Motor octane number	–	ASTM D2700	84.0	–	–	–	–	–	–	–	–	–	–
Distillation		ASTM D86											
(a) Initial boiling point	% vol.		38.8	–	–	–	–	–	–	–	–	–	–
(b) 10% evaporated	% vol.		68.5	–	–	–	–	–	–	–	–	–	–
(c) 50% evaporated	% vol.		109.6	–	–	–	–	–	–	–	–	–	–
(d) 99% evaporated	% vol.		161.5	–	–	–	–	–	–	–	–	–	–

Table 16: (continue 1)

Property	Unit	Test method	Gasoline	Cassava[273]									
				E10	E20	E30	E40	E50	E60	E70	E80	E90	E100
Density @ 20°C	kg/m^3	ASTM D1298	745.4	750.8	760.5	778.2	779.2	780.5	781.2	782.3	783.4	784.0	789.0
Flash point	°C	ASTM D93	–	–40	–20	–15	–13.5	–5.0	–1.0	0.0	5.0	8.5	12.5
Heating value	MJ/kg	ASTM D240	42.54	–	–	–	–	–	–	–	–	–	–
Auto ignition temperature	°C	–	246.0	260	279	281	294	320	345	350	362	360	365
Research octane number	–	ASTM D2699	91.3	93	94	95	97	99	100	103	104	106	129
Motor octane number	–	ASTM D2700	84.0	–	–	–	–	–	–	–	–	–	–
Distillation		ASTM D86											
(a) Initial boiling point	% vol.		38.8	–	–	–	–	–	–	–	–	–	–
(b) 10% evaporated	% vol.		68.5	–	–	–	–	–	–	–	–	–	–
(c) 50% evaporated	% vol.		109.6	–	–	–	–	–	–	–	–	–	–
(d) 99% evaporated	% vol.		161.5	–	–	–	–	–	–	–	–	–	–

Table 16: (continue 2)

Property	Unit	Test method	Gasoline	Potato waste[271]				Cassava[272]				Sugarcane[274]
				E5	E10	E15	E20	E6	E10	E15	E20	E20
Density @ 20°C	kg/m^3	ASTM D1298	745.4	–	–	–	–	754	757.6	758.6	759.7	759.7
Flash point	°C	ASTM D93	–	–	–	–	–	–	–	–	–	–
Heating value	MJ/kg	ASTM D240	42.54	–	–	–	–	41.41	40.8	40.08	39.47	39.47
Auto ignition temperature	°C	–	246.0	–	–	–	–	–	–	–	–	–
Research octane number	–	ASTM D2699	91.3	89.7	92.3	94	99.4	92.8	93.0	92.4	93.0	93
Motor octane number	–	ASTM D2700	84.0	–	–	–	–	84	83.8	82.8	83.4	83.4
Distillation		ASTM D86										
(a) Initial boiling point	% vol.		38.8	40.9	38.9	44	40.8	42.6	47.1	43.2	40.5	40.5
(b) 10% evaporated	% vol.		68.5	54.3	53.1	57.2	55.4	61.3	65.1	62.7	62.4	62.4
(c) 50% evaporated	% vol.		109.6	93.5	71.9	71.4	71.6	111.4	98.3	87.6	79.2	79.2
(d) 99% evaporated	% vol.		161.5	184.1	175.1	182.4	176.6	206.1	191.6	203.6	201.4	201.4

Table 17: The summary of engine performance and emissions using ethanol blends.[a]

Engine	Fuel type	Test condition	Engine performance analysis			Emissions analysis				Ref.
			BSFC	BTE	BP	CO_2	CO	HC	NO_x	
1C, Two stroke, SI	E0, E5, E10, E15	2500–4500 rpm	BSFC ↓	–	–	6.3% ↓	32% ↓	6% ↓	38% ↓	270
1C, Four stroke, SI	E0, E6, E10, E15, E20	2000 rpm	BSFC ↑	–	–	–	52% ↓	19%↓	60%↑	272
4C, Four stroke, SI	E0, E5, E10, E15, E20	1000–5000 rpm	BSFC ↓	30% ↑	BP ↑	10.41%	45.42%	31.69%↓	45.55%↑	271
1C, Four stroke, SI	E0, E50, E85	1500–5000 rpm	Min. 20.3% and Max. 45.6% ↑	–	BP ↑	–	1%↓	24%↓	–	277
4C, Four stroke, SI	DME0, DME1, DME2	1400 rpm	–	Max 10%, ↑	–	–	–	702 ppm ↓	1054 ppm ↑	278
1C, Four stroke, CI	E0, E5, E10, E15, E20	1500 rpm	BSFC ↓	13%↓	–	–	38%↓	35%↓	–	275
4C, Four stroke, SI	E69.5 + 0.5, E64.6+ 0.4, E59.7 + 0.3, E49.8 + 0.2	2000–2800 rpm	–	31.89%↑	–	8%↓	0.08%↓	–	–	279

[a].BSFC = Brake specific fuel consumption; BTE = Brake thermal efficiency; BP = Brake power; C = cylinder; ↓= decrease; ↑= increase; E0 = 100 %vol. Gasoline; E5 = 5 vol% ethanol–95 vol% Gasoline; Gasoline; DME1 = 1 vol% Dimethyl ether–99 vol% ethanol; E69.5+0.5 = 69.5 vol% Ethanol-0.5 vol% cycloheptanol-30 gasoline;; SI = Spark Ignition; CI = Compression Ignition