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Progress in bioethanol processing

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Abstract

Production of ethanol (bioethanol) from biomass is one way to reduce both consumption of crude oil and environmental pollution. Bioethanol is appropriate for the mixed fuel in the gasoline engine because of its high octane number, and its low cetane number and high heat of vaporization impede self-ignition in the diesel engine. So, ignition improver, glow-plug, surface ignition, and pilot injection are applied to promote self-ignition by using diesel-bioethanol-blended fuel. Disadvantages of bioethanol include its lower energy density than gasoline, its corrosiveness, low flame luminosity, lower vapor pressure (making cold starts difficult), miscibility with water, and toxicity to ecosystems. Bioethanol can be produced from cellulosic feedstocks. One major problem with bioethanol production is the availability of raw materials for the production. The availability of feedstocks for bioethanol can vary considerably from season to season and depends on geographic locations. Lignocellulosic biomass is the most promising feedstock considering its great availability and low cost, but the large-scale commercial production of fuel bioethanol from lignocellulosic materials has still not been implemented. Conversion technologies for producing bioethanol from cellulosic biomass resources such as forest materials, agricultural residues and urban wastes are under development and have not yet been demonstrated commercially. For designing fuel bioethanol production processes, assessment of utilization of different feedstocks (i.e. sucrose containing, starchy materials, lignocellulosic biomass) is required considering the big share of raw materials in bioethanol costs. In this work a review of the biological and thermochemical methods that could be used to produce bioethanol is made and an analysis of its global production trends is carried out.

Keywords: Bioethanol; Fuel properties; Feedstock; Production; Bioconversion; Fermentation; Hydrolysis

Contents

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1. Introduction

Although CO_2 is the most important greenhouse gas (GHG), several studies show that it is important to consider other GHGs as well [1]. The continued use of fossil fuels to meet the majority of the world's energy demand is threatened by increasing concentrations of CO_2 in the atmosphere and concerns over global warming [2,3]. The combustion of fossil fuels is responsible for 73% of the CO_2 production [4].

The heightened awareness of the global warming issue has increased interest in the development of methods to mitigate GHG emissions [5]. Much of the current effort to control such emissions focuses on advancing technologies that: (i) reduce energy consumption, (ii) increase the efficiency of energy conversion or utilization, (iii) switch to lower carbon content fuels, (iv) enhance natural sinks for CO_2, and (v) capture and store CO_2. Reducing use of fossil fuels would considerably reduce the amount of CO_2 produced, as well as reduce the levels of pollutants [6]. As concern about global warming and dependence on fossil fuels grows, the search for renewable energy sources that reduce CO_2 emissions becomes a matter of widespread attention [7]. To reduce the net contribution of GHGs to the atmosphere, bioethanol has been recognized as a potential alternative to petroleum-derived transportation fuels [8].

It began with the use of ethanol in the internal combustion engine (ICE) invented by Nikolas Otto in 1897 [9]. Alcohols have been used as fuels since the inception of the automobile. Fuel ethanol blends are successfully used in all types of vehicles and engines that require gasoline [10]. Ethanol is made from a variety of products such as grain, molasses, fruit, cobs, and shell; its production, excluding that of beverages, has been declining since the 1930s because of the low cost [11]. With the oil crises of the 1970s, ethanol became established as an alternative fuel [10]. In 1975, only 76×10^6 l of proof industrial ethanol was produced by fermentation compared to 7.95×10^6 l by synthesis [11]. Since the 1980s, ethanol has been considered one possible alternative fuel in many countries.

2. Fuel properties of bioethanol

Bioethanol (ethyl alcohol, grain alcohol, CH_3–CH_2–OH or ETOH) is a liquid biofuel which can be produced from several different biomass feedstocks and conversion technologies. Bioethanol is an attractive alternative fuel because it is a renewable bio-based resource and it is oxygenated thereby provides the potential to reduce particulate emissions in compression–ignition engines [12]. However, for example corn ethanol production causes more soil erosion than any other crop grown and uses more nitrogen fertilizer than any other crop grown. These two environmental limitations also apply to sugar cane production in Brazil [13].

Bioethanol has a higher octane number, broader flammability limits, higher flame speeds and higher heats of vaporization than gasoline. These properties allow for a higher compression ratio, shorter burn time and leaner burn engine, which lead to theoretical efficiency advantages over gasoline in an internal combustion engine [14]. Disadvantages of bioethanol include its lower energy density than gasoline (bioethanol has 66% of the energy that gasoline has), its corrosiveness, low flame luminosity, lower vapor pressure (making cold starts difficult), miscibility with water, and toxicity to ecosystems [15]. Some properties of alcohol fuels are shown in Table 1.

Ethanol is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NO_x emissions from combustion. Ethanol has a higher octane number (108), broader flammability limits, higher flame speeds and higher heats of vaporization. These properties allow for a higher

Table 1
Some properties of alcohol fuels

Fuel property	Isoctane	Methanol	Ethanol
Cetane number	–	5	8
Octane number	100	112	107
Auto-ignition temperature (K)	530	737	606
Latent heat of vaporization (MJ/Kg)	0.26	1.18	0.91
Lower heating value (MJ/Kg)	44.4	19.9	26.7

Source: Ref. [14].

compression ratio and shorter burn time, which lead to theoretical efficiency advantages over gasoline in an ICE. Octane number is a measure of the gasoline quality and can be used for prevention of early ignition which leads to cylinder knocks. Higher octane numbers are preferred in internal combustion engines. An oxygenate fuel such as bioethanol provides a reasonable antiknock value. Also, as it contains oxygen, fuel combustion is more efficient, reducing hydrocarbons and particulates in exhaust gases. Complete combustion of a fuel requires in existence the amount of stochiometric oxygen. However, the amount of stochiometric oxygen generally is not enough for complete combustion. Oxygen content of a fuel increases its combustion efficiency. Because of this the combustion efficiency and octane number of bioethanol are higher than those of gasoline.

The presence of oxygen in bioethanol improves combustion and therefore reduces hydrocarbon, carbon monoxide, and particulate emissions; but oxygenated fuels also tend to increase nitrogen oxide emissions. Bioethanol is appropriate for the mixed fuel in the gasoline engine because of its high octane number, and its low cetane number and high heat of vaporization impede self-ignition in the diesel engine. So, ignition improver, glow-plug, surface ignition, and pilot injection are applied to promote self-ignition by using diesel–bioethanol blended fuel [16]. The most popular blend for light-duty vehicles is known as E85, and contains 85% bioethanol and 15% gasoline. In Brazil, bioethanol for fuel is derived from sugar cane and is used pure or blended with gasoline in a mixture called gasohol (24% bioethanol, 76% gasoline) [7]. In several states of the United States, a small amount of bioethanol (10% by volume) is added to gasoline, known as gasohol or E10. Blends having higher concentrations of bioethanol in gasoline are also used, e.g. in flexible-fuel vehicles that can operate on blends of up to 85% bioethanol—E85 [17]. Some countries have exercised biofuel program involving both form bioethanol–gasoline blend program, e.g. the United States (E10 and for Flexible Fuel Vehicle (FFV) E85), Canada (E10 and for FFV E85), Sweden (E5 and for FFV E85), India (E5), Australia (E10), Thailand (E10), China (E10), Columbia (E10), Peru (E10), Paraguay (E7), and Brazil (E20, E25 and FFV any blend) [18].

Methanol is produced by a variety of processes, the most common of which is the distillation of liquid products from wood and coal, natural gas, and petroleum gas. Ethanol cost is higher than that of methanol because ethanol is produced mainly from biomass bioconversion. The systematic effect of ethyl alcohol differs from that of methyl alcohol. Ethyl alcohol is rapidly oxidized in the body to carbon dioxide and water; and, in contrast to methyl alcohol, no cumulative effect occurs. Methanol is considerably easier to recover than ethanol. Ethanol forms an azeotrope with water, so it is expensive to purify ethanol during recovery. If the water is not removed, it will interfere with the reactions. Methanol recycles easily because it does not form an azeotrope.

3. Current status and potential production of bioethanol

In 2006, global production of bioethanol reached 13.5 billion gallons, up from 12.1 billion gallons in 2005 [19]. Bioethanol currently accounts for more than 94% of global biofuel production, with the majority coming from sugar cane [20]. About 60% of global bioethanol production comes from sugar cane and 40% from other crops [21]. Brazil and the United States are the world leaders, which exploit sugar cane and corn, respectively, and they together account for about 70% of the world bioethanol production. However, the US and Brazil are not oil independent countries. The top ten bioethanol producers are presented in Table 2.

Nearly all bioethanol fuel is produced by fermentation of corn glucose in the United States or sucrose in Brazil, but any country with a significant agronomic-based economy can use current technology for bioethanol fermentation. This is possible because, during the last two decades, technology for bioethanol production from nonfood-plant sources has been developed to the point at which large-scale production will be a reality in the next few years [22]. In the United States, 90% of bioethanol is derived from corn [7]. According to the Renewable Fuels Association (RFA) 2007 figures [19], bioethanol production of the United States has increased significantly, from 3.54 billion gallons in 2004 to 4.85 billion gallons in 2006. All of Brazil's bioethanol is produced from sugar cane, most is used domestically substituting 40% of Brazilian petrol consumption and approximately 20% is exported to the United States, EU and other markets [23]. The biomass produced in Brazil largely results from an ethanol fuel production program started in 1975 from sugar cane crops grown specifically for fuel use, presently occupying 2.7 million hectares of land and employing about 350 distilleries. Ethanol currently provides over 40% of the fuel consumed by cars and light trucks [24]. Approximately 4.5 billion gallons of bioethanol are made annually from sugar cane in Brazil [19].

The potential bioethanol production could replace 353 billion liters of gasoline (32% of the global gasoline

Table 2
The top ten bioethanol producers (billion gallons)

Country	2004	2005	2006
USA	3.54	4.26	4.85
Brazil	3.99	4.23	4.49
China	0.96	1.00	1.02
India	0.46	0.45	0.50
France	0.22	0.24	0.25
Germany	0.07	0.11	0.20
Russia	0.20	0.20	0.17
Canada	0.06	0.06	0.15
South Africa	0.11	0.10	0.10
Thailand	0.07	0.08	0.09

Source: Ref. [19].

consumption) when bioethanol is used in E85 fuel for a midsize passenger vehicle. Furthermore, lignin-rich fermentation residue, which is the coproduct of bioethanol made from lignocellulosic residue, can potentially generate both 458 terra-watt-hours (TWh) of electricity (about 3.6% of world electricity production) and 2.6 EJ of steam [25].

The potential demand for bioethanol as fuel for transportation in EU countries, calculated on the basis of Directive 2003/30/EC, is estimated at about 6 billion liters in 2006 and 12.7 billion liters in 2010. This is in market disproportion with the current level of EU production capacity of about 2 billion liters per year [26]. In Europe, the feedstock used for bioethanol is predominately wheat, sugar beet and waste from the wine industry. It is estimated that between 4 and 13% of total agricultural land in the EU would be needed to produce the biofuels needed to fulfill the directive from domestically produced biofuels [27]. The beet-growing area for sugar production in EU-25 was about 2.55 million ha in 1999 and 2.14 million ha (15% less) in 2005. As a result of the newly introduced reform of the EU sugar market regime, one can expect that area will reduce to 1.30 million ha in 2015 [26].

4. Feedstocks for bioethanol production

Biofuels originate from plant oils, sugar beets, cereals, organic waste and the processing of biomass. Biological feedstocks that contain appreciable amounts of sugar—or materials that can be converted into sugar, such as starch or cellulose—can be fermented to produce bioethanol to be used in gasoline engines [17]. Bioethanol feedstocks can be conveniently classified into three types: (i) sucrose-containing feedstocks (e.g. sugar beet, sweet sorghum and sugar cane), (ii) starchy materials (e.g. wheat, corn, and barley), and (iii) lignocellulosic biomass (e.g. wood, straw, and grasses). Different feedstocks that can be utilized for bioethanol production and their comparative production potential are given in Table 3 [28].

Table 3
Different feedstocks for bioethanol production and their comparative production potential

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

Source: Ref. [28].

One major problem with bioethanol production is the availability of raw materials for the production. The availability of feedstocks for bioethanol can vary considerably from season to season and depend on geographic locations. The price of the raw materials is also highly volatile, which can highly affect the production costs of bioethanol [29]. Because feedstocks typically account for greater than one-third of the production costs, maximizing bioethanol yield is imperative [30].

4.1. Sucrose-containing feedstocks

Feedstock for bioethanol is essentially comprised of sugar cane and sugar beet [31]. Two-third of world sugar production is from sugar cane and one-third is from sugar beet [28]. These two are produced in geographically distinct regions. Sugar cane is grown in tropical and subtropical countries, while sugar beet is only grown in temperate-climate countries. Since bioethanol trade is mainly from the South, feedstocks may eventually impact cane sugar trade. World cane sugar export has not increased over the period 2000–2004 (Table 4) [31].

Brazil is the largest single producer of sugar cane with about 27% of global production and a yield of 18 dry Mg/ha highest yield occurs in Peru, which produces more than 32 Mg of dry sugar cane per hectare [25]. Bioethanol production from sugar cane is very economical in Brazil because of two primary reasons. Brazil dropped support of sugar prices to support the bioethanol industry with government established mandates for the blending of bioethanol with gasoline. This drastically lowered the cost of the feedstock, sugar cane, and created a demand for and supported the price of bioethanol [32]. The center-south region of Brazil accounts for almost 80% of feedstock production [33]. The Brazilian bioethanol industry was poised for a major jump during 2006–2008 as a part of new national plan to increase sugar cane production by 40% by 2009 [34]. In Asia (India, Thailand, Philippines), sugar cane is produced on small fields owned by small farmers. For example India has around 7 million small farmers with an average of around 0.25 ha sugar cane fields [35].

In European countries, beet molasses are the most utilized sucrose-containing feedstock [36]. Sugar beet crops are grown in most of the EU-25 countries, and yield substantially more bioethanol per hectare than wheat [37]. The advantages with sugar beet are a lower cycle of crop production, higher yield, and high tolerance of a wide range of climatic variations, low water and fertilizer requirement.

Table 4
Evolution of world exports of raw cane sugar

	2000	2001	2002	2003	2004
Value ($ billion)	3.2	4.3	2.8	3.4	2.9
Quantity (million tons)	16.5	17.9	12.9	16.7	14.5

Source: Ref. [31].

Compared to sugar cane, sugar beet requires 35–40% less water and fertilizer [28]. A producer price for sugar beet as B-quota 32.42 €/ton sugar beet, and a bioethanol yield of 100 l bioethanol/ton sugar beet, are giving a final feedstock cost of 324.2 €/1000 l bioethanol [38].

Sweet sorghum (*Sorghum bicolor* L.) is one of the most drought resistant agricultural crops as it has the capability to remain dormant during the driest periods. Of the many crops being investigated for energy and industry, sweet sorghum is one of the most promising candidates, particularly for bioethanol production principally in developing countries [28].

A recent EU funded (LAMNET program) research program investigated the possibility of combining waste products of several crops for use in the processing of bioethanol. One of the studies concluded that sweet sorghum is a very useful plant, whereby the complete plant can be used without leaving any waste. It is concluded that bioethanol produced from sugar cane is an attractive proposition [35]. The cost levels and comparison of bioethanol yield produced from different energy crops is presented in Table 5 [35,39].

The conversion of carbohydrates with 5 and 6 carbons into bioethanol is easier compared to starchy materials and lignocellulosic biomass because previous hydrolysis of the feedstock is not required since this disaccharide can be broken down by the yeast cells; in addition, the conditioning of the cane juice or molasses favors the hydrolysis of sucrose [36].

4.2. Starchy materials

Another type of feedstock, which can be used for bioethanol production, is starch-based materials [29]. Starch is a biopolymer and defined as a homopolymer consisting only one monomer, D-glucose [40]. To produce bioethanol from starch it is necessary to break down the chains of this carbohydrate for obtaining glucose syrup, which can be converted into bioethanol by yeasts. This type of feedstock is the most utilized for bioethanol production in North America and Europe. Corn and wheat are mainly employed with these purposes [36].

The United States has a large corn-based bioethanol industry with a capacity of over 15 billion l per year; production capacity is anticipated to continue rising to about 28 billion l per year by 2012, as dictated by the Energy Policy Act of 2005 [41]. The bioethanol industry used more than 1.4 billion bushels (1 bushel = 56 pounds) of corn in 2005, valued at $2.9 billion. Feedstock availability is not expected to be a constraint for bioethanol production over the next decade. Corn, which is currently used to make about 90% of all US bioethanol, is expected to remain the predominant feedstock, although its share likely will decline modestly by 2015. A combination of improved corn yields and acreage shifts from other crops will enable the US corn sector to supply the bioethanol industry without significant increases in prices that would adversely affect bioethanol profitability or the livestock and poultry industry. As corn stocks are drawn down from this season's 2.4 billion bushel projected carryout, farm-level corn prices will increase, reaching $2.58 per bushel by the 2015 marketing year. The impact of this level of demand for bioethanol on stocks measured by the stocks to use ratio and farm-level corn prices is illustrated in Fig. 1 [42]. Bioethanol is being produced in Brazil and the US by subsidies. More than $6 billion per year is subsidizing ethanol production in the US. The subsidies per gallon of ethanol in the US is 60 times higher than the subsidies per gallon of gasoline.

The single greatest cost in the production of bioethanol from corn, and the cost with the greatest variability, is the cost of the corn. Corn prices vary from year to year and in the last few years have ranged from $1.94 per bushel to $3.24 per bushel [43]. The price of corn in the US is now close to $4.00 per bushel. Corn prices will also vary in different locations due to shipping distance from the field to the plant [43].

Considering that corn transportation to distilleries requires 0.63 GJ per m^3 of bioethanol produced, and that bioethanol conversion consumes 13.7 GJ of energy per m^3 of bioethanol produced in situ, the resulting energy output–input ratio for US bioethanol production is 1.1, which is significantly lower than the ratio of 3.7 for Brazilian bioethanol from sugar cane [7]. Transportation, refinery and cleanout costs were excluded for on-site systems.

Starch consists of long chains of glucose molecules and can also be converted to fermentable sugar by a method called "the hydrolysis technique". Hydrolysis is a reaction of starch with water, which is normally used to break down

Table 5
Comparison of production cost and bioethanol yield from different energy crops

Type	Yield (t/ha/year)	Conversion rate to sugar or starch (%)	Conversion rate to bioethanol (l/ton)	Bioethanol yield (kg/ha/year)	Cost[a] ($/$m^3$)
Sugar cane	70	12.5	70	4900	~160
Cassava	40	25	150	6000	700
Sweet sorghum	35	14	80	2800	200–300
Corn	5	69	410	2050	250–420
Wheat	4	66	390	1560	380–480

Source: Refs. [35[a],39].

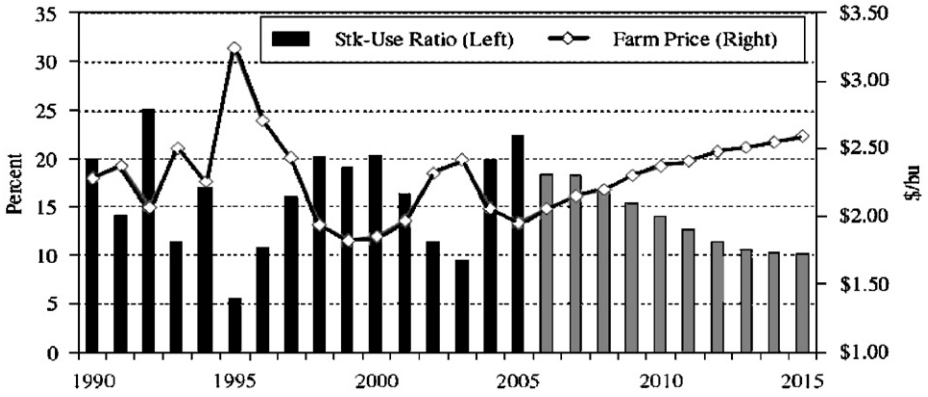

Fig. 1. US corn stocks and farm price (*source*: Ref. [42]).

the starch into fermentable sugar [29]. There are two types of hydrolysis—enzymatic hydrolysis and acid hydrolysis. The hydrolysis of starch by amylases at relatively high temperatures is a process known industrially as liquefaction. The factors that affect the enzymatic hydrolysis of starch include substrates, enzyme activity, and reaction conditions (temperature, pH, as well as other parameters) [44]. The starch-based bioethanol industry has been commercially viable for about 30 years; in that time, tremendous improvements have been made in enzyme efficiency, reducing process costs and time, and increasing bioethanol yields [41]. There are two main reasons for the present high cost: one is that, as the yeast *Saccharomyces cerevisiae* cannot utilize starchy materials, large amounts of amylolytic enzymes, namely, glucoamylase and α-amylase, need to be added; the other is that the starchy materials need to be cooked at a high temperature (413–453 K) to obtain a high bioethanol yield [45]. Recently, Mojovic et al. [46] studied the two-step enzymatic hydrolysis of corn meal by commercially available α-amylase and glucoamylase and further bioethanol fermentation of the obtained hydrolyzates by *Saccharomyces cerevisiae* yeast. They obtained a bioethanol yield of more than 80% after 4 h of reaction at a lower temperature (305 K).

4.3. Lignocellulosic biomass

Lignocellulosic biomass, such as agricultural residues (corn stover and wheat straw), wood and energy crops, is an attractive material for bioethanol fuel production since it is the most abundant reproducible resource on the Earth. Lignocellulosic biomass could produce up to 442 billion l per year of bioethanol [47]. Thus, the total potential bioethanol production from crop residues and wasted crops is 491 billion l per year, about 16 times higher than the current world bioethanol production [25]. Rice straw is one of the abundant lignocellulosic waste materials in the world. It is annually produced about 731 million tons, which is distributed in Africa (20.9 million tons), Asia (667.6 million tons), Europe (3.9 million tons), America (37.2 million tons) and Oceania (1.7 million tons). This amount of rice straw can potentially produce 205 billion l bioethanol per year, which is the largest amount from a single biomass feedstock [48].

Lignocellulosic perennial crops (e.g. short rotation coppices and inedible grasses) are promising feedstock because of high yields, low costs, good suitability for low quality land (which is more easily available for energy crops), and low environmental impacts. Table 6 presents biochemical compositions for several suitable feedstock. Pine has the highest combined sugar content, implying the highest potential bioethanol production. The lignin content for most feedstock is about 27%, but grasses contain significantly less, and may thus co-produce less electricity [49].

The basic structure of all lignocellulosic biomass consists of three basic polymers: cellulose $(C_6H_{10}O_5)_x$, hemicelluloses such as xylan $(C_5H_8O_4)_m$, and lignin $[C_9H_{10}O_3 \cdot (OCH_3)_{0.9-1.7}]_n$ in trunk, foliage, and bark [50,51].

Cellulose fibers provide wood's strength and comprise ~40–50 wt% of dry wood [52]. Cellulose is a homopolysaccharide composed of β-D-glucopyranose units linked together by (1→4)-glycosidic bonds. The cellulose molecules are linear; the β-D-glucopyranose chain units are in a chair conformation and the substituents HO−2, HO−3, and CH_2OH are oriented equatorially [53]. Glucose anhydride, which is formed via the removal of water from each glucose, is polymerized into long cellulose chains that contain 5000–10,000 glucose units. The basic repeating unit of the cellulose polymer consists of two glucose anhydride units, called a cellobiose units [52].

A second major wood chemical constituent is hemicellulose, which is also known as polyose. A variety of hemicelluloses usually account for 25–35% of the mass of dry wood, 28% in softwoods, and 35% in hardwoods. Hemicellulose is a mixture of various polymerized monosaccharides such as glucose, mannose, galactose, xylose, arabinose, 4-*O*-methyl glucuronic acid and galacturonic acid residues [52]. Xylose is the predominant pentose sugar derived from the hemicellulose of most hardwood feedstocks, but arabinose can constitute a significant amount of the pentose sugars derived from various agricultural

Table 6
Biochemical compositions for several suitable feedstock for bioethanol production

Feedstock		Hardwood			Softwood	Grass
		Black locust	Hybrid poplar	Eucalyptus	Pine	Switch grass
Cellulose		41.61	44.70	49.50	44.55	31.98
Glucan	6C	41.61	44.70	49.50	44.55	31.98
Hemicellulose		17.66	18.55	13.07	21.90	25.19
Xylan	5C	13.86	14.56	10.73	6.30	21.09
Arabinan	5C	0.94	0.82	0.31	1.60	2.84
Galactan	6C	0.93	0.97	0.76	2.56	0.95
Mannan	6C	1.92	2.20	1.27	11.43	0.30
Lignin		26.70	26.44	27.71	27.67	18.13
Ash		2.15	1.71	1.26	0.32	5.95
Acids		4.57	1.48	4.19	2.67	1.21
Extractives		7.31	7.12	4.27	2.88	17.54
Heatin value ($GJ_{HHV}/tonne_{dry}$)		19.50	19.60	19.50	19.60	18.60

Source: Ref. [49].

residues and other herbaceous crops, such as switchgrass, which are being considered for use as dedicated energy crops. Whereas arabinose makes only 2–4% of the total pentoses in hardwoods, arabinose represents 10–20% of the total pentoses in many herbaceous crops. Arabinose contents can be as high as 30–40% of the total pentoses in corn fiber, a by-product of corn processing [54].

The lignins are highly branched, substituted, mononuclear aromatic polymers in the cell walls of certain biomass, especially woody species, and are often bound to adjacent cellulose fibers to form a lignocellulosic complex. This complex and the lignins alone are often quite resistant to conversion by microbial systems and many chemical agents. The lignin contents on a dry basis in both softwoods and hardwoods generally range from 20% to 40% by weight and from 10% to 40% by weight in various herbaceous species, such as bagasse, corncobs, peanut shells, rice hulls and straws [55].

The cost of bioethanol production from lignocellulosic materials is relatively high when based on current technologies, and the main challenges are the low yield and high cost of the hydrolysis process [56]. Because the feedstock can represent >40% of all process costs, an economical biomass-to-bioethanol process critically depends on the rapid and efficient conversion of all of the sugars present in both its cellulose and hemicellulose fractions [8,54,56].

5. Processing of lignocellulosics to bioethanol

The bioconversion of cellulose and hemicellulose to monomeric sugars for example carbohydrates with 5 and 6 carbons is harder to accomplish than the conversion of starch, presently used for bioethanol production [57]. There are several options for a lignocellulose-to-bioethanol process, but regardless of which is chosen, the following features must be assessed in comparison with established sugar- or starch-based bioethanol production [58].

- Efficient de-polymerization of cellulose and hemicellulose to soluble sugars.
- Efficient fermentation of a mixed-sugar hydrolysate containing six-carbon (hexoses) and five-carbon (pentoses) sugars as well as fermentation inhibitory compounds.
- Advanced process integration to minimize process energy demand.
- Lower lignin content of feedstock decreases of the cost of bioethanol.

One of the advantages of bioconversion with lignocellulosics is the opportunity to create a biorefinery, producing value-added co-products plus fuel bioethanol. For instance, sugars may be subjected to bacterial fermentation under aerobic and anaerobic conditions, producing a variety of other products including lactic acid, which in turn may be processed into plastics and other products. The non-carbohydrate components of lignin also have potential for use in value-added applications [41]. Processing of lignocellulosics to bioethanol consists of four major unit operations: pre-treatment, hydrolysis, fermentation and product separation/distillation. Schematic flowsheet for the bioconversion of biomass to bioethanol is shown in Fig. 2.

5.1. Pre-treatment

The first step in bioconversion of lignocellosics to bioethanol is size reduction and pre-treatment [59]. The goal of any pre-treatment technology is to alter or remove structural and compositional impediments to hydrolysis in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicellulose [60]. Pre-treatment is an important tool for practical cellulose conversion processes. Pre-treatment is

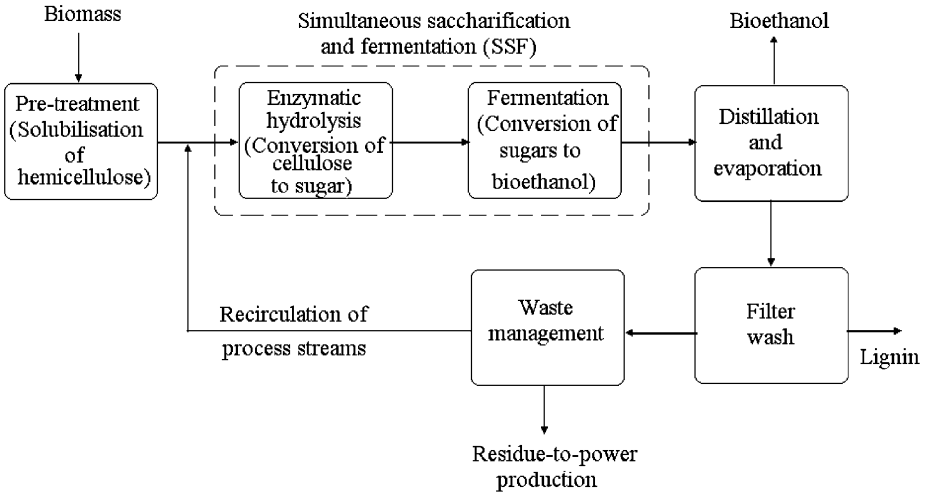

Fig. 2. Schematic flowsheet for the bioconversion of biomass to bioethanol (*source*: Ref. [58]).

required to alter the structure of cellulosic biomass to make more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars and to cellulase producing microorganisms [61]. A successful pre-treatment must meet the following requirements [62]: (i) improve formation of sugars or the ability to subsequently form sugars by hydrolysis, (ii) avoid degradation or loss of carbohydrate, (iii) avoid formation of byproducts inhibitory to subsequent hydrolysis and fermentation processes, and (iv) be cost effective.

The pre-treatment stage promotes the physical disruption of the lignocellulosic matrix in order to facilitate acid- or enzyme-catalyzed hydrolysis. Pre-treatments can have significant implications on the configuration and efficiency of the rest of the process and, ultimately, also the economics [41]. To assess the cost and performance of pre-treatment technologies, techno-economic analyses have been performed recently [49,63,64]. Studies have shown that pre-treatment is the most significant determinant of success of the cellulosic bioethanol technology because it defines the extent to and cost at which the carbohydrates of cellulose and hemicellulose can be converted to bioethanol. There is a huge scope in lowering the cost of pre-treatment process through extensive research and development (R&D) approaches [65]. Cost-effective pre-treatment of cellulosic biomass is a major challenge of cellulose-bioethanol technology research and development [49].

Pre-treatment can be carried out in different ways such as mechanical pre-treatment [66], steam explosion [67,68], ammonia fiber explosion [69–72], supercritical CO_2 treatment [73], alkali or acid pre-treatment [74–76], ozone pre-treatment [72], and biological pre-treatment [61]. Comparison of various pre-treatment options is given in Table 7 [49,77].

5.1.1. Steam explosion (autohydrolysis)

Steam explosion is one of the biomass fractionation processes. Processes include steam explosion, aqueous separation, and hot-water systems. Commercial products of biomass fractionation include levulinic acid, xylitol, and alcohols.

Main fractionation chemicals from biomass ingredients are:

1. Dissociation of cell components → Lignin fragment + Oligosaccharides + Cellulose.
2. Hydrolysis of cellulose (Saccharification) → Glucose.
3. Conversion of glucose (Fermentation) → Ethanol + Lactic acid.
4. Chemical degradation of cellulose → Levulinic acid + Xylitol.
5. Chemical degradation of lignin → Phenolic products.

Steam explosion is being developed by Stake Technology Ltd., Canada which involves extrusion of the biomass at a high temperature and pressure, while peroxide extrusion (being developed by Xylan Inc., USA) uses a chemical pre-treatment along with extrusion to accomplish the same goal of breaking down the internal structure of the biomass fibers [78]. In this process, high-pressure, high-temperature steam is introduced into a sealed chamber containing woody lignocellulosic material in the form of chips or agricultural residues. After 1–5 min, the pressure is released, causing the steam to expand within the lignocellulosic matrix, separating individual fibers with minimal loss of material [79].

Uncatalyzed steam explosion refers to a pre-treatment technique in which lignocellulosic biomass is rapidly heated by high-pressure steam without addition of any chemicals. The biomass/steam mixture is held for a period of time to promote hemicellulose hydrolysis, and terminated by an explosive decompression [60].

Hemicellulose is thought to be hydrolyzed by the acetic and other acids released during steam explosion pre-treatment. Steam explosion involves chemical effects and

a reaction sequence of the type shown in Fig. 3 since acetic acid is generated from hydrolysis of acetyl groups associated with the hemicellulose and may further catalyze hydrolysis and glucose or xylose degradation. Water, itself, also acts as an acid at high temperatures [60]. For softwood, steam pre-treatment with the addition of an acid catalyst such as H_2SO_4 or SO_2 is a prerequisite to reach high sugar yields. Acid increases the recovery of hemicellulose sugars and it improves the enzymatic hydrolysis of the solid fraction; the acid catalyst in steam pre-treatment functions similar to acid pulp cooking but with less liquid [58]. H_2SO_4 is a strong catalyst that highly improves the hemicellulose removal, but also easily yields inhibitory substances. SO_2, on the other hand, is generally a milder catalyst, giving less inhibitors but also a less extended hemicellulose hydrolysis [80]. Some optimized pre-treatment conditions are listed in Table 8.

Pre-treatment by water and steam alone in a steam explosion process relies on release of natural acids from hemicellulose to break down the hemicellulose, followed by rapid pressure release to quench the reaction and disrupt the fibrous structure. Although conceptually simple, the yields of sugars from hemicellulose are low at $<65\%$ for these so-called batch steam explosion techniques, and such yields are too low to be attractive [81].

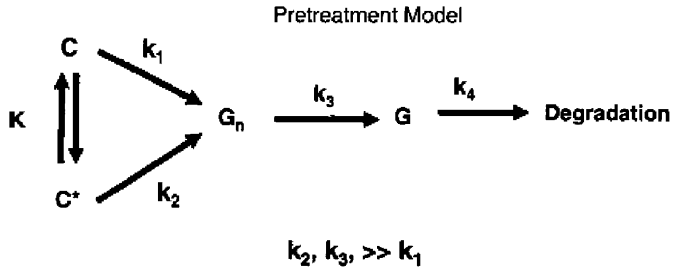

Fig. 3. Schematic representation of pretreatment steps. Transformation between crystalline (C) amorphous cellulose (C*) is reversible. Both forms may yield oligosaccharides, which in turn form glucose. Glucose (G) degradation can then occur to form fermentation inhibitors. Where K is equilibrium constant and k is rate constant (*source*: Ref. [60]).

To summarize the effects of steam explosion treatment on lignocellulosics reported in literature [82]: (i) steam explosion treatment increases crystallinity of cellulose by promoting crystallization of the amorphous portions, (ii) hemicellulose is easily hydrolyzed by steam explosion treatment, and (iii) there is evidence that steam explosion promotes delignification.

The advantages of steam explosion are low energy requirement compared to mechanical comminution (70% more energy required) and involves no recycling or environmental cost. It is considered the most cost-effective option for hard wood and agriculture residues, but is less effective for soft wood [83].

5.1.2. *Ammonia fiber/freeze explosion*

Ammonia fiber/freeze explosion (AFEX) pre-treatment involves liquid ammonia and steam explosion [49]. The AFEX is a process in which ground, pre-wetted lignocellulosic material at a moisture content of 15–30% is placed in a pressure vessel with liquid ammonia (NH_3) at a loading of about 1–2 kg NH_3/kg dry biomass. Pressures exceeding 12 atm are required for operation at ambient temperature [62]. It is simple and has a short process time. It is effective for the treatment of corn stover. However, against aspen chips, which contain higher lignin content than sugar cane bagasse, the AFEX process is less effective [62]. This system does not directly liberate any sugars, but allows the

Table 8
Acid catalyzed steam pre-treatment conditions

Two-step pretreatments		One-step pretreatments
First step	Second step	
453 K, 10 min, H_2SO_4 (0.5%)	473 K, 2 min, H_2SO_4 (2%)	498 K, 5 min, H_2SO_4 (0.5%)
463 K, 2 min, SO_2 (3%)	493 K, 5 min, SO_2 (3%)	483 K, 5.5 min, SO_2 (3.5%)

Source: Ref. [80].

Table 7
Comparison of various pre-treatment (lignin removal and hemicellulose hydrolysis) options

Pre-treatment method	Chemicals	Temperature/ pressure	Reaction time (min)	Xylose yield (%)	Downstream enzymatic effect	Costs	Available
Dilute acid hydrolysis	Acid	>433 K	2–10	75–90	<85%	+	Now
Alkaline hydrolysis	Base			60–75	55%	++	Now
Uncatalyzed steam explosion	–	433–533 K	2	45–65	90%	–	2–5 year
Acid catalyzed steam explosion	Acid	433–493 K			88% (2 steps)	–	2–5 year
Ammonia fiber explosion	Ammonia	363 K	30		50–90% (2 steps)		
CO_2 explosion	CO_2	56.2 bar			75% (2 steps)		

Source: Refs. [49,77].

polymers (hemicellulose and cellulose) to be attacked enzymatically and it reduces to sugars [78].

The AFEX pre-treatment yields optimal hydrolysis rates for pretreated lignocellulosics with close to theoretical yields at low enzyme loadings (<5 FPU per gram of biomass or 20 FPU/g cellulose) [60]. The AFEX process requires efficient ammonia recovery to be economical due to the high cost of ammonia. A possible approach is to recover the ammonia after the pre-treatment by evaporation [84].

5.1.3. Acid pre-treatment

Acid pre-treatments normally aim for high yields of sugars from lignocellulosic biomass [84]. There are many types of acid pre-treatmenst including use of sulfuric acid [85], hydrochloric acid [86], peracetic acid [87], nitric acid [88], or phosphoric acid [89]. This process soon found its way to the United States, culminating in two commercial plants operating in the southeast during World War I. These plants used what was called "the American Process"—a one-stage dilute sulfuric acid hydrolysis [90]. Acid pre-treatment can utilize either dilute or concentrated acids to improve cellulose hydrolysis [62]. Among all the pre-treatment methods, dilute acid pre-treatment was one of the most studied and widely used [48,78,91–94].

Dilute acid pre-treatments at moderate temperatures using either sulfuric or phosphoric acid were used for converting lignocellulosic biomass, including the hemicellulose fraction, to soluble sugars, followed by enzyme-catalyzed hydrolysis of the cellulosic fraction to glucose [95]. There are primarily two types of dilute acid pre-treatment processes: low solids loading (5–10% [w/w]), high-temperature ($T>433$ K), continuous-flow processes and high solids loading (10–40% [w/w], lower temperature ($T<433$ K), batch processes [62]. In general, higher pre-treatment temperatures and shorter reactor residence times result in higher soluble xylose recovery yields and enzymatic cellulose digestibility. Higher-temperature dilute acid pre-treatment has been shown to increase cellulose digestibility of pretreated residues [91]. Depending on the substrate and the conditions used, between 80 and 95% of the hemicellulosic sugars can be recovered by dilute acid pre-treatment from the lignocellulosic feedstock [48,96,97]. Corn fiber can be enzymatically saccharified to fermentable sugars with a yield of 85–100% after pre-treatment with dilute acid at a moderate temperature [98].

In recent years, treatment of lignocellulosic biomass with dilute sulfuric acid has been primarily used as a means of hemicellulose hydrolysis and pre-treatment for enzymatic hydrolysis of cellulose [99]. Dilute sulfuric acid is mixed with biomass to hydrolyze hemicellulose to xylose and other sugars, and then continue to break xylose down to form furfural. The furfural is recovered by distillation. The volatile fraction contains the furfural, which is purified and sold. The acid is mixed or contacted with the biomass, and the mixture is held at temperatures 433–493 K for periods ranging from minutes to seconds [60].

The hot-wash process, a variation of the dilute acid pre-treatment, involves high-temperature separation and washing of the pre-treated solids, which is thought to prevent re-precipitation of lignin and/or xylan that may have been solubilized under pre-treatment conditions. Re-precipitation of lignin can negatively affect the subsequent enzymatic hydrolysis of the pre-treated solids [100].

The high-temperature countercurrent continuous approach results in a higher product yield, but generates a much more dilute sugar stream. With batchwise dilute-acid hydrolysis, only about 50–55% of the cellulose in wood can be converted to sugar. The balance of the material is either left as residual cellulose or is degraded. Therefore, while the technology is inexpensive, it is not sufficiently effective for commercial development unless the feedstock is very cheap [96].

5.1.4. Alkaline pre-treatment

Alkali pre-treatment processes utilize lower temperatures and pressures compared to other pre-treatment technologies. Alkali pre-treatment may be carried out at ambient conditions, but pre-treatment time is measured in terms of hours or days rather than minutes or seconds. Unlike acid-catalyzed pre-treatments, a limitation occurs because some of the alkali is converted to irrecoverable salts or incorporated as salts into the biomass by the pre-treatment reactions [62]. The characteristic of alkaline pre-treatment is that it can remove the lignin without having big effects on other components [101]. NaOH treatment causes lignocellulosic biomass to swell, leading to an increase in the internal surface area, a decrease in the degree of crystallinity, and disruption of the lignin structure [102]. After treatment with the acid, an alkaline for example lime or soda treatment is needed to stop the acid activity.

Alkali pre-treatment reduces the lignin and hemicellulose content in biomass, increases the surface area, allowing penetration of water molecules to the inner layers, and breaks the bonds between hemicellulose and lignin-carbohydrate. Dilute NaOH is usually used for alkali pre-treatment [84]. Considering economic and environmental aspects, dilute NaOH treatment would be much more suitable than the concentrated NaOH pre-treatment. Combination of dilute NaOH treatment and other treatments seems more efficient. For example, corn stover pre-treatment of dilute NaOH (2%) combined with irradiation (500 kGy) caused the glucose yield to increase from just 20% for NaOH pre-treatment to 43% [102].

Lime (calcium hydroxide) has been used to pre-treat wheat straw (358 K for 3 h), poplar wood (423 K for 6 h with 14-atm oxygen), switchgrass (373 K for 2 h), and corn stover (373 K for 13 h) [60]. Calcium hydroxide, water, and an oxidizing agent (air or O_2) are mixed with the biomass at temperatures ranging from 313 to 426 K for a period ranging from hours to weeks. The major effect is the removal of lignin from the biomass, thus improving the reactivity of the remaining polysaccharides. In addition, this pre-treatment removes acetyl and the various uronic

acid substitutions on hemicellulose that lower the accessibility of the enzyme to the hemicellulose and cellulose surface [103].

5.1.5. Biological pre-treatment

Biological pre-treatments use fungi to solubilize the lignin. Biodelignification is the biological degradation of lignin by microorganisms. It is mentioned in 1984 as possibly useful in the future, although at that time it was inadequate and expensive, required a long process time and the microorganisms were poisoned by lignin derivatives [49,77]. These technologies could greatly simplify pre-treatment, but the rates are slow, yields are low, and little experience with these approaches exists [81].

5.2. Hydrolysis

As the pre-treatment is finished, the cellulose is prepared for hydrolysis, meaning the cleaving of a molecule by adding a water molecule [83]:

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6. \quad (1)$$

This reaction is catalysed by dilute acid, concentrated acid or enzymes (cellulase) and the latter has many advantages as the very mild conditions (pH = 4.8 and temperature 318–323 K) give high yields and the maintenance costs are low compared to alkaline and acid hydrolysis due to no corrosion problems [83]. Hydrolysis without preceding pre-treatment yields typically <20%, whereas yields after pre-treatment often exceed 90% [49].

A number of processes for hydrolyzing cellulose into glucose have been developed over the years. The vast majority of processing schemes utilizes either cellulolytic enzymes or sulfuric acid of varying concentrations. Historically, enzymes have been too expensive for economical production of fuel ethanol from biomass. Sulfuric acid, itself, is less expensive than cellulolytic enzymes, although disposal costs associated with the use of sulfuric acid significantly increase its cost. However, the single largest drawback to using sulfuric acid is that it also readily degrades glucose at the high temperatures required for cellulose hydrolysis [104].

Lignocellulose biomass may be hydrolyzed by gamma-ray or electron-beam irradiation, or microwave irradiation [105,106]. Hydrolysis of lignocellulosic biomass is more complicated than that of pure cellulose due to the presence of nonglucan components such as lignin and hemicellulose [107].

5.2.1. Acid hydrolysis

From the research studies it was revealed that under controlled treatment conditions, acid hydrolysis of lignocellulosic biomass mainly produced xylose from xylan with the cellulosic and lignin fractions remaining unaltered. Xylan is more susceptible to hydrolysis by mild acid treatment due to its amorphous structure compared to cellulose, which needs severe treatment conditions for its crystalline nature [108]. The acid hydrolysate from sugar cane bagasse contains xylose as the main component. During acid hydrolysis, xylose is degraded rapidly to furfural and other condensation byproducts. These degradation products are inhibitory to microorganisms. The inhibitory effect of different compounds like furfural, 5-hydroxymethyl furfural (HMF), acetate, hydroxybenzaldehyde (HBA), siringaldedyde (SGA) and vanillin on yeast growth is well documented [109].

Acid-catalyzed cellulose hydrolysis is a complex heterogeneous reaction. It involves physical factors as well as the hydrolytic chemical reaction. The molecular mechanism of acid-catalyzed hydrolysis of cellulose (cleavage of β-1-4-glycosidic bond) follows the pattern outlined in Fig. 4 [110]. Monosaccharide products can be further degraded into undesirable chemicals. The number of possible side reactions depends upon, among other things, the permeate composition. As such, evaluation of acid hydrolysis as a means to generate monosaccharides from lactose in whey permeate must be carried out within the context of the intended use of the hydrolysis products [111]. The acid hydrolyzed substrates were then subjected to enzyme hydrolysis to give vastly improved yields as high as 100% for corn stover and 90% for oak wood [82]. There are two basic types of acid hydrolysis processes commonly used: dilute acid and concentrated acid.

5.2.1.1. Dilute acid hydrolysis. This is the oldest technology for converting cellulose biomass to bioethanol [59]. In dilute acid hydrolysis, the hemicellulose fraction is depolymerized at lower temperature than the cellulosic fraction. Dilute sulfuric acid is mixed with biomass to hydrolyze hemicellulose to xylose and other sugars [65]. The dilute acid process involves a solution of about 1% sulfuric acid concentration in a continuous-flow reactor at a high temperature (about 488 K) [59]. Most dilute acid processes are limited to a sugar recovery efficiency of around 50% [112]. The primary challenge for dilute acid hydrolysis processes is how to raise glucose yields higher than 70% in an economically viable industrial process while maintaining a high cellulose hydrolysis rate and minimizing glucose decomposition. Percolation reactors have been used in most of the wood sugar processes [113]. Strong acids can reduce the crystalline region but they degrade glucose [84].

Dilute acid hydrolysis occurs in two stages to take advantage of the differences between hemicellulose and cellulose. The first stage is performed at low temperature to maximize the yield from the hemicellulose; and the second, higher-temperature stage is optimized for hydrolysis of the cellulose portion of the feedstock [114]. The first stage is conducted under mild process conditions to recover the 5-carbon sugars while the second stage is conducted under harsher conditions to recover the 6-carbon sugars [115,116]. Schematic flowsheet for dilute acid hydrolysis is given in Fig. 5.

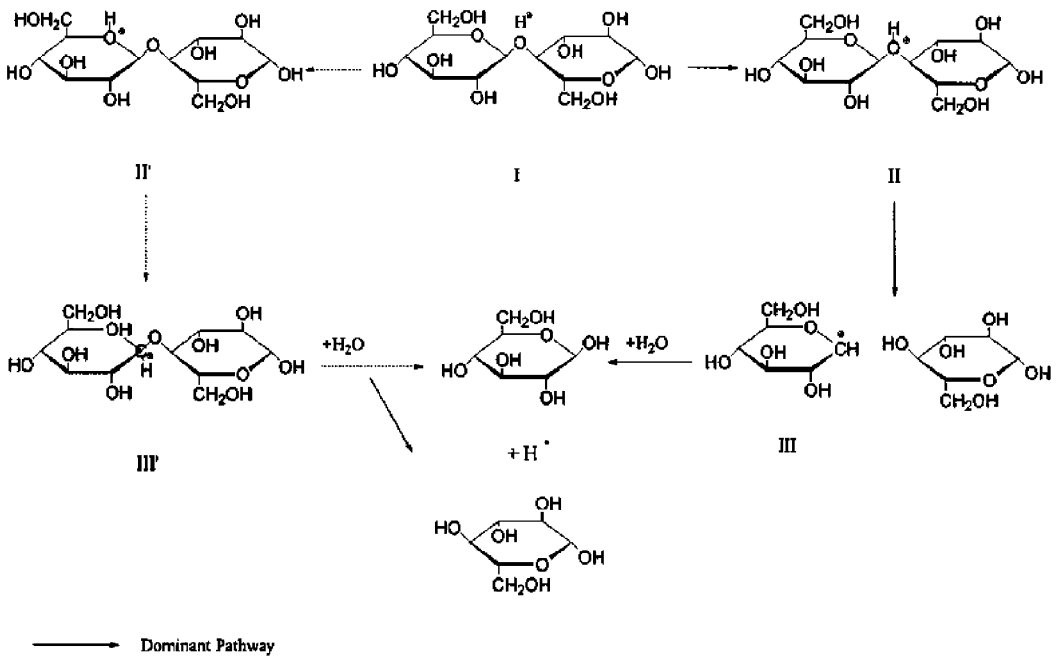

Fig. 4. Mechanism of acid catalyzed hydrolysis of β-1-4 glucan. The legends of I, II, II′, III and III′ are anhydro glucose unit including H$^{\bullet}$ radical, anhydro glucose intermediate including O$^{\bullet}$ radical (with high energy), anhydro glucose intermediate including O$^{\bullet}$ radical (without high energy), fragment from anhydro glucose unit includes C$^{\bullet}$ radical and anhydro glucose intermediate includes C$^{\bullet}$ radical, respectively (*source*: Ref. [110]).

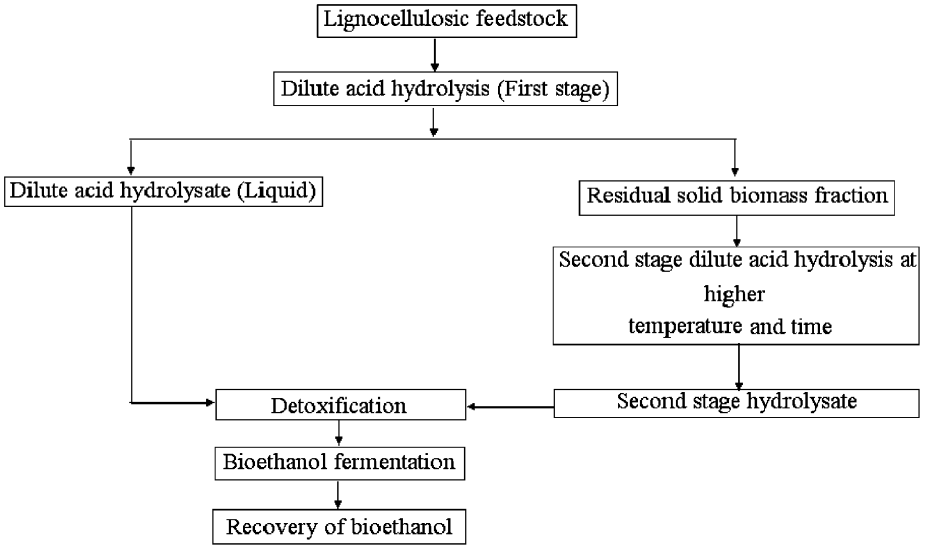

Fig. 5. Dilute acid hydrolysis (first-stage and two-stages) and separate fermentation of pentose and hexose sugars (*source*: Ref. [65]).

The biggest advantage of dilute acid processes is their fast rate of reaction, which facilitates continuous processing. Their biggest disadvantage is their low sugar yield. For rapid continuous processes, in order to allow adequate acid penetration, feedstocks must also be reduced in size so that the maximum particle dimension is in the range of a few millimeters [112].

5.2.1.2. Concentrated acid hydrolysis. Concentrated acid process provides a complete and rapid conversion of cellulose to glucose and hemicelluloses to 5-carbon sugars with little degradation. The critical factors needed to make this process economically viable are to optimize sugar recovery and cost effectively recovers the acid for recycling [105,116]. The concentrated acid process uses relatively mild temperatures, and the only pressures involved are those created by pumping materials from vessel to vessel. Reaction times are typically much longer than for dilute acid process [59]. The concentrated acid process uses 70% sulfuric acid at 313–323 K for 2–4 h in a reactor. The low

temperatures and pressure will lead to minimization of the sugar degradation. The hydrolyzed material is then washed to recover the sugars. In the next step, the cellulosic fraction has to be deploymerized. The solid residue from first stage is de-watered and soaked in 30–40% sulfuric acid for 50 min. at 373 K for further cellulose hydrolysis [65].

The primary advantage of the concentrated acid process is the potential for high sugar recovery efficiency [106]. Table 9 shows the yields of bioethanol by concentrated sulfuric acid hydrolysis from cornstalks. The concentrated acid process offers more potential for cost reductions than the dilute sulfuric acid process [114]. Concentrated sulfuric or hydrochloric acid is difficult to work with, and essentially all of the acid must be recovered and reconcentrated in order for the process to be economical [96].

Table 9
Yields of bioethanol by concentrated sulfuric acid hydrolysis from cornstalks

Amount of cornstalk (kg)	1000
Cellulose content (kg)	430
Cellulose conversion and recovery efficiency (% dry weight)	76
Bioethanol stoichiometric yield (% dry weight)	51
Glucose fermentation efficiency (% dry weight)	75
Bioethanol yield from glucose (kg)	130
Amount of cornstalk (kg)	1000
Hemicelluloses content (kg)	290
Hemicelluloses conversion and recovery efficiency (% dry weight)	90
Bioethanol stoichiometric yield (% dry weight)	51
Xylose fermentation efficiency (% dry weight)	50
Bioethanol yield from xylose (kg)	66
Total bioethanol yield from 1000 kg of cornstalks	196 kg (225.7 L = 59 gallons)

Source: Ref. [106].

5.2.2. Enzymatic hydrolysis

Another basic method of hydrolysis is enzymatic hydrolysis. Enzymes are naturally occurring plant proteins that cause certain chemical reactions to occur. There are two technological developments: enzymatic and direct microbial conversion methods [106]. The enzymes are very costly in the US.

Enzymatic hydrolysis of natural lignocellulosic materials is a very slow process because cellulose hydrolysis is hindered by structural parameters of the substrate, such as lignin and hemicellulose content, surface area, and cellulose crystallinity [117]. Since enzymatic hydrolysis of native lignocellulose usually results in solubilization of V20% of the originally present glucan, some form of pre-treatment to increase amenability to enzymatic hydrolysis is included in most process concepts for biological conversion of lignocellulose. Pre-treatment, under appropriate conditions, retains nearly all of the cellulose present in the original material and allows close to theoretical yields upon enzymatic hydrolysis [107]. The enzymatic hydrolysability (cellulose-to-glucose conversion yield) of different substrates is shown in Fig. 6.

Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 318–323 K) and does not have a corrosion problem [118]. Enzymatic hydrolysis is attractive because it produces better yields than acid-catalyzed hydrolysis and enzyme manufacturers have recently reduced costs substantially using modern biotechnology [119].

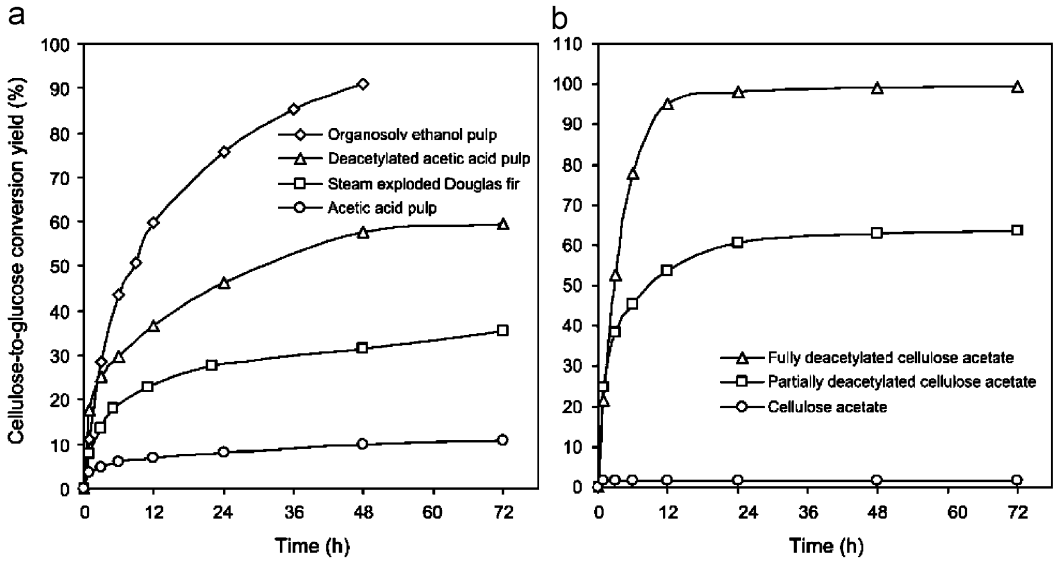

Fig. 6. Enzymatic hydrolysis of different substrates. (a) Acetic acid pulp from Douglas fir (AcP), deacetylated AcP (DAcP), steamexploded Douglas fir (SEDF) and organosolv ethanol pulp from mixed softwood (EP). (b) Cellulose acetate (CA), partially deacetylated CA (PDCA) and completely deacetylated CA (DCA). Enzymatic hydrolysis conditions: cellulase loading of 20 filter paper units gy1 cellulose, b-glucosidase loading of 40 IU gyl cellulose, 2% (w/v) consistency of cellulose in 50 mM acetate buffer, pH 4.8, 318 K, and shaker speed, 150 rpm. *Source*: Ref. [117].

During the enzymatic hydrolysis of cellulosic substrates, several factors restrict the sustained catalytic activity of the cellulase mixture. It has been suggested that these limitations are owing to both substrate- and enzyme-related factors [120,121]. It has been difficult to evaluate the reuse and/or recycle of cellulases, primarily because our current knowledge of the characteristics of cellulase adsorption onto lignocellulosic substrates is insufficient [120]. The enzymatic degradation of solid cellulose is a complicated process that takes place at a solid–liquid phase boundary, where the enzymes are the mobile components [122]. When cellulase enzyme systems act in vitro on insoluble cellulosic substrates, three processes occur simultaneously [104]: (i) chemical and physical changes in the residual (not yet solubilized) solid-phase cellulose, (ii) primary hydrolysis, involving the release of soluble intermediates from the surface of reacting cellulose molecules, and (iii) secondary hydrolysis, involving hydrolysis of soluble intermediates to lower molecular weight intermediates, and ultimately to glucose.

The rate of enzymatic hydrolysis of the cellulosic materials always decreases rather quickly. Generally, enzymatic cellulose degradation is characterized by a rapid initial phase followed by a slow secondary phase that may last until all substrate is consumed. This has been explained most often by the rapid hydrolysis of the readily accessible fraction of cellulose, strong product inhibition, and slow inactivation of absorbed enzyme molecules [122].

Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic materials. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria belonging to *Clostridium, Cellulomonas, Bacillus, Thermomonospora, Ruminococcus, Bacteriodes, Erwinia, Acetovibrio, Microbispora*, and *Streptomyces* can produce cellulases [118].

The widely accepted mechanism for enzymatic cellulose hydrolysis involves synergistic actions by endoglucanses or endo-1,4-β-glucanases (EG), exoglucanases or cellobiohydrolases (CBH), and β-glucosidases (BGL) [107,123,124]. EG play an important role in the cellulose hydrolysis by cleaving cellulose chains randomly and thus encouraging strong degradation [125]. EG hydrolyze accessible intramolecular β-1,4-glucosidic bonds of cellulose chains randomly to produce new chain ends; exoglucanases processively cleave cellulose chains at the ends to release soluble cellobiose or glucose; and BGL hydrolyze cellobiose to glucose in order to eliminate cellobiose inhibition [123]. BGL complete the hydrolysis process by catalyzing the hydrolysis of cellobiose to glucose [126].

Filamentous fungi are the major source of cellulases and hemicellulases [127]. Wild type and mutant strains of *Trichoderma* sp. (*T. viride, T. reesei, T. longibrachiatum*) have long been considered to be the most productive and powerful destroyers of crystalline cellulose. CBH I and CBH II are the major *T. reesei* enzymes, the content of CBH I comprises up to 60% of the total cellulolytic protein; whereas, the content of CBH II is about 20% [128]. Similarly, EG I and EG II are the dominant endoglucanases in *T. reesei*, and presumably act as important partners to CBH I in nature [129]. Such protein yields are comparable or exceed the respective parameters for the best *Trichoderma* sp. strains (35–40 g/L) [127].

5.2.2.1. Separate hydrolysis and fermentation (SHF). Enzymatic hydrolysis performed separately from fermentation step is known as SHF [65]. In the SHF configuration, the joint liquid flow from both hydrolysis reactors first enters the glucose fermentation reactor. The mixture is then distilled to remove the bioethanol leaving the unconverted xylose behind. In a second reactor, xylose is fermented to bioethanol, and the bioethanol is again distilled [49,77]. The SHF with separate pentose and hexose sugars and combined sugar fermentation are shown in Fig. 7. Compared to SHF the final bioethanol yield is higher, less energy is required and production costs are minimized [130]. The primary advantage of SHF is that hydrolysis and fermentation occur at optimum conditions; the disadvantage is that cellulolytic enzymes are end-product inhibited so that the rate of hydrolysis is progressively reduced when glucose and cellobiose accumulate [58]. Iogen Corporation, a major manufacturer of industrial enzymes in Canada, developed an SHF process comprising a dilute-acid-catalyzed steam explosion and the removal of the major part of the acetic acid released during the pre-treatment, the use of *Saccharomyces cerevisiae* as a fermenting organism, distillation of broth, bioethanol dehydration and disposal of stillage in landfill [36].

5.2.2.2. Simultaneous saccharification and fermentation (SSF). The sugars from the pre-treatment and enzymatic hydrolysis steps are fermented by bacteria, yeast or filamentous fungi, although the enzymatic hydrolysis and fermentation can also be performed in a combined step—the so-called simultaneous SSF [58]. It is often effective when

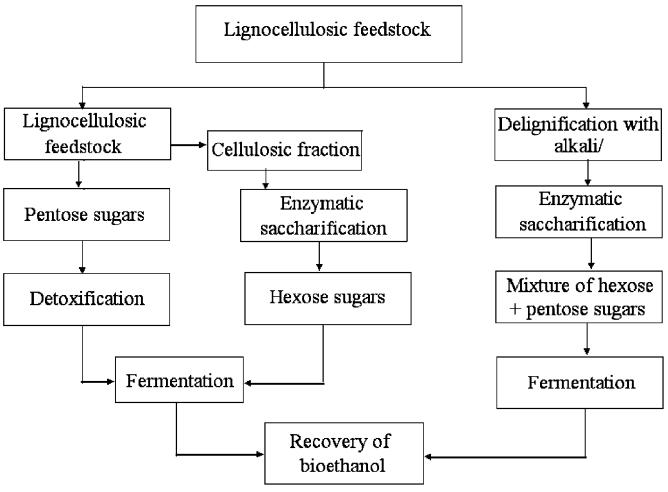

Fig. 7. Separate hydrolysis and fermentation (SHF) with separate pentose and hexose sugars and combined sugar fermentation (*source*: Ref. [65]).

combined with dilute-acid or high-temperature hot-water pre-treatment. In SSF, cellulases and xylanases convert the carbohydrate polymers to fermentable sugars. These enzymes are notoriously susceptible to feedback inhibition by the products—glucose, xylose, cellobiose, and other oligosaccharides [96]. Fig. 8 shows SSF with combined sugars (pentoses and hexoses) fermentation.

SSF gives higher reported bioethanol yields and requires lower amounts of enzyme because end-product inhibition from cellobiose and glucose formed during enzymatic hydrolysis is relieved by the yeast fermentation [30,65]. The efficiency of product formation increases with increasing bioethanol concentration up to about 5% on a w/w basis, so fermentation at high temperatures (>313 K) and at or above 5% bioethanol are priorities for commercialization of this technology [96]. SSF is a batch process using natural heterogeneous materials containing complex polymers like lignin, pectin and lignocelluloses [131]. Karimi et al. [48] studied SSF of dilute-acid pre-treated rice straw with Saccharomyces cerevisiae and Mucor indicus under aerobic or anaerobic conditions. They also claimed to obtain highest bioethanol and glycerol yields on anaerobic SSF of the pre-treated rice straw with *M. indicus*. Results of this study are shown in Table 10.

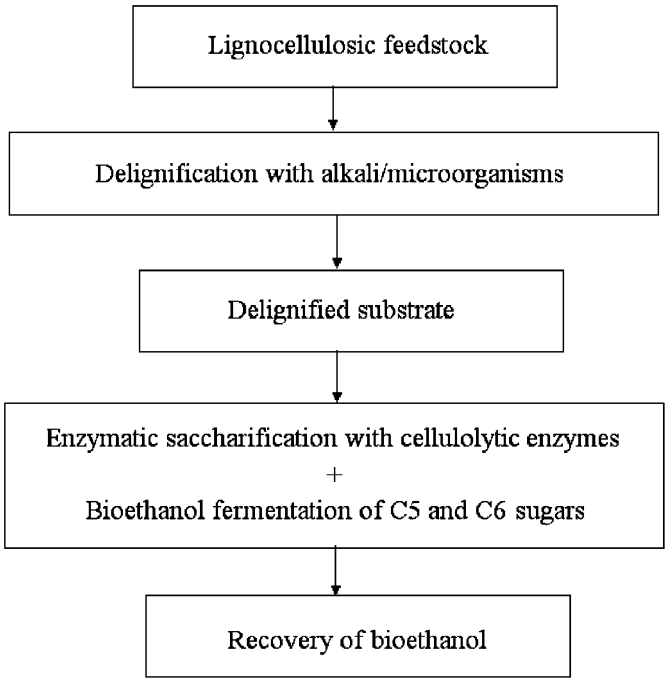

Fig. 8. Simultaneous saccharification and fermentation (SSF) with combined sugars fermentation (*source*: Ref. [65]).

Major advantages of SSF as described by Sun and Cheng [56], include: (i) increase of hydrolysis rate by conversion of sugars that inhibit the cellulase activity, (ii) lower enzyme requirement, (iii) higher product yields, (iv) lower requirements for sterile conditions since glucose is removed immediately and bioethanol is produced, (v) shorter process time; and (vi) less reactor volume. SSF process has also some disadvantages. The main disadvantage of SSF lies in different temperature optima for saccharification and fermentation [132]. In many cases, the low pH, e.g., less than 5, and high temperature, e.g., >313 K, may be favorable for enzymatic hydrolysis, whereas the low pH can surely inhibit the lactic acid production and the high temperature may affect adversely the fungal cell growth [133]. *Trichoderma reesei* cellulases, which constitute the most active preparations, have optimal activity at pH 4.5 and 328 K. For *Saccharomyces* cultures SSF are typically controlled at pH 4.5 and 310 K [30].

More recently, the SSF technology has proved advantageous for the simultaneous fermentation of hexose and pentose which is so-called simultaneous saccharification and co-fermentation (SSCF). In SSCF, the enzymatic hydrolysis continuously releases hexose sugars, which increases the rate of glycolysis such that the pentose sugars are fermented faster and with higher yield [58]. SSF and SSCF are preferred since both unit operations can be done in the same tank, resulting in lower costs [60].

5.2.3. Fermentation

Lignocellulose is often hydrolyzed by acid treatment; the hydrolysate obtained is then used for bioethanol fermentation by microorganisms such as yeast. Because such lignocellulose hydrolysate contains not only glucose, but also various monosaccharides, such as xylose, mannose, galactose, arabinose, and oligosaccharides, microorganisms should be required to efficiently ferment these sugars for the successful industrial production of bioethanol [134]. According to the reactions, the theoretical maximum yield is 0.51 kg bioethanol and 0.49 kg carbon dioxide per kg of

Table 10
The yield of bioethanol and byproducts and bioethanol concentration in SSF of dilute-acid pretreated rice straw with *Saccharomyces cerevisiae* and *Mucor indicus*

Strain	Enzyme (FPU/g DM)	Condition	Max bioethanol concentration (g/l)	Max theoretical bioethanol yield (%)	Max glycerol yield (mg/g)
S. cerevisiae	15	Aerobic	6.83±0.25	40.69±1.49	83.9
S. cerevisiae	15	Anaerobic	10.20±0.37	60.77±2.20	89.6
M. indicus	15	Aerobic	7.79±0.27	46.41±1.61	48.0
M. indicus	15	Anaerobic	11.35±0.40	67.62±2.38	117.3

Source: Ref. [48].

xylose and glucose [49,77,83]:

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2, \quad (2)$$

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2. \quad (3)$$

Fermentation involves microorganisms that use the fermentable sugars for food and in the process produces ethyl alcohol and other byproducts. These microorganisms can typically use the 6-carbon sugars, one of the most common being glucose. Therefore, cellulosic biomass materials containing high levels of glucose or precursors to glucose are the easiest to convert to bioethanol. Microorganisms, termed ethanologens, presently convert an inadequate portion of the sugars from biomass to bioethanol [106]. There are a number of microorganisms that produce significant (greater than 1% w/v) quantities of bioethanol [135].

Xylose-fermenting microorganisms are found among bacteria, yeast and filamentous fungi [58]. Today, xylose-fermenting bacteria include both native and genetically engineered organisms, and many have characteristics useful for simultaneous saccharification and fermentation (Table 11) [96]. One of the most effective bioethanol-producing yeasts, *Saccharomyces cerevisiae*, has several advantages owing to its high bioethanol production from hexoses and high tolerance to bioethanol and other inhibitory compounds in the acid hydrolysates of lignocellulosic biomass. However, because wild-type strains of this yeast cannot utilize pentoses, such as xylose and arabinose, and celloligosaccharides, bioethanol production from a lignocellulose hydrolysate is inadequate [134]. For xylose-using *S. cerevisiae*, high bioethanol yields from xylose also require metabolic engineering strategies to enhance the xylose flux [58].

Table 11
Native and engineered bacterial species capable of fermenting xylose to bioethanol

Species	Characteristics
Clostridium acetobutilicum	Useful in fermentation of xylose to acetone and butanol; bieethanol produced in low yield
Clostridium thermocellum	Capable of converting cellulose directly to ethanol and acetic acid: bioethanol concentrations are generally less than 5 g/l
Escherichia coli	Native strains ferment xylose to a mixture of bioethanol, succininc, and acetic acids but lack ethanol tolerance; genetically engineered strains predominantly produce bioethanol
Klebsiella oxytoca	Native strains rapidly ferment xylose and cellobiose; engineered to ferment cellulose and produce bioethanol predominantly
Lactobacillus pentoaceticus	Consumes xylose and arabinose. Slowly uses glucose and cellobiose. Acetic acid is produced along with lactic in 1:1 ratio
Lactobacillus casei	Ferments lactose very well; particularly useful for bioconversion of whey
Lactobacillus xylosus	Uses cellobiose if nutrients are supplied: uses *n*-glucose, D-xylose, and L-arabinose
Lactobacillus pentosus	Homolactic fermentation. Some strains produce lactic acid from sulfite waste liquors
Lactobacillus plantarum	Consumes cellobiose more rapidly than glucose, xylose, or arabinose. Appears to depolymerize pectins; produces lactic acid from agricultural residues
Zymomonas mobilis	Normally ferments glucose and fructose; engineered to ferment xylose

Source: Ref. [96].

The ethanologenic bacteria that currently show the most promise for industrial exploitation are *Escherichia coli*, *Klebsiella oxytoca* and *Zymomonas mobilis* [30]. *Zymomonas* is well recognized for its ability to produce bioethanol rapidly and efficiently from glucose-based feedstocks, and comparative performance trials have shown that *Z. mobilis* can achieve 5% higher yields and up to five-fold higher volumetric productivity when compared with traditional yeast fermentations. *Z. mobilis* has demonstrated bioethanol yields up to 97% of theoretical and bioethanol concentrations up to 12% (w/v) in glucose fermentations [54]. A generalized flow diagram for the conversion of lignocellulosics to bioethanol based on recombinent *Z. mobilis* is shown in Fig. 9 [136]. *Z. mobilis* also efficiently produces bioethanol from the hexose sugars glucose and fructose but not from pentose sugars, although a xylose-fermenting *Z. mobilis* was generated by introducing a xylose-metabolizing pathway from *E. coli* [58]. Despite its advantages as an ethanologen, *Z. mobilis* is not well suited for biomass conversion because it ferments only glucose, fructose and sucrose. However, over the last decade, researchers at the National Renewable Resources Laboratory (Department of Energy, United States) have successfully engineered strains capable of fermenting xylose and arabinose [30]. *E. coli* and *K. oxytoca* naturally

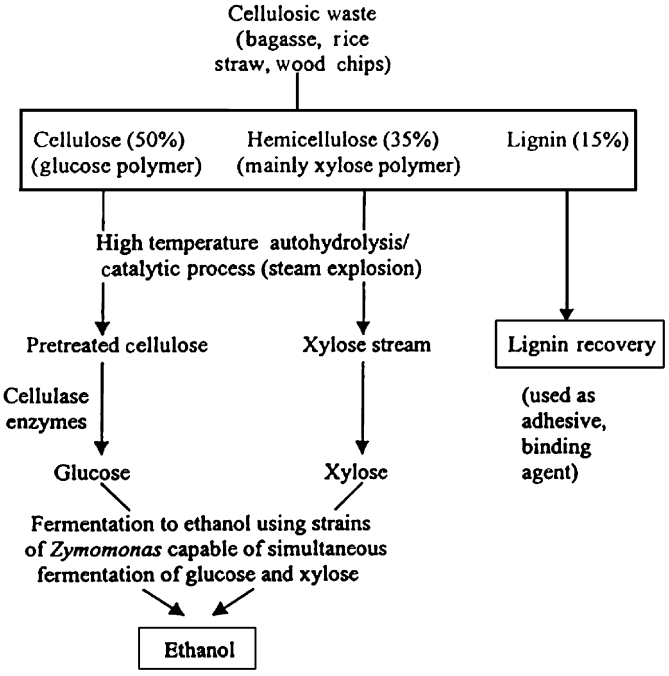

Fig. 9. *Zymomonas* based process for conversion of lignocellulosic hydrolysates to ethanol (*source*: Ref. [136]).

metabolize arabinose, such that the ethanologenic strains ferment all lignocellulose-derived sugars [58]. Under aerobic conditions, succinate is not produced as a by-product in *E. coli* and acetate is the main by-product. Numerous metabolic engineering strategies to enhance succinate production in *E. coli* have met with success [137]. *K. oxytoca* is an enteric bacterium found growing in paper and pulp streams as well as around other sources of wood. The microorganism is capable of growing at a pH at least as low as 5.0 and temperatures as warm as 308 K. *K. oxytoca* will grow on a wide variety of sugars including hexoses and pentoses, as well as on cellobiose and cellotriose. Culture characteristics of each of the strains discussed are compared in Table 12. *E. coli* and *K. oxytoca* have wider substrate ranges than *Z. mobilis* (Table 12) [30].

Natural xylose-fermenting yeasts, such as *Pichia stipitis*, *Candida shehatae*, and *Candida parapsilosis*, can metabolize xylose via the action of xylose reductase (XR) to convert xylose to xylitol, and of xylitol dehydrogenase (XDH) to convert xylitol to xylulose. Therefore, bioethanol fermentation from xylose can be successfully performed by recombinant *S. cerevisiae* carrying heterologous XR and XDH from *P. stipitis*, and xylulokinase (XK) from *S. cerevisiae* [134].

Microorganisms for bioethanol fermentation can best be described in terms of their performance parameters and other requirements such as compatibility with existing products, processes and equipment. The performance parameters of fermentation are: temperature range, pH range, alcohol tolerance, growth rate, productivity, osmotic tolerance, specificity, yield, genetic stability, and inhibitor tolerance [105]. All the recombinant strains are mesophilic organisms and function best between 303 and 311 K [138]. An organism must maintain a fairly constant balance of pH to survive. Most bacteria grow best in a narrow range of pH from 6.5 to 7.5 [139]. Yeast and fungi tolerate a range of pH 3.5–5.0. The ability to lower pH below 4.0 offers a method for present operators using yeast in less than aseptic equipment to minimize loss due to bacterial contaminants. The majority of organisms cannot tolerate bioethanol concentrations above 10–15% (w/v) [138].

Fermentation can be performed as a batch, fed batch or continuous process. The choice of most suitable process will depend upon the kinetic properties of microorganisms and type of lignocellulosic hydrolysate in addition to process economics aspects [65]. Fed-batch reactors are widely used in industrial applications because they combine the advantages from both batch and continuous processes [140]. The major advantage of fed-batch, comparing to batch, is the ability to increase maximum viable cell concentration, prolong culture lifetime, and allow product accumulation to a higher concentration [141]. A typical fed-batch fermentation process consists of three technological stages: batch–feeding–batch. Optimization problem is to determine the feed start and finish time points and the feed-rate time profile during the feeding time interval. An optimal feed-rate time profile is usually close to exponential, however, the simplified time profiles such as a constant rate or a ramp shape profiles can give process optimization results close to optimal [142]. This process allows for the maintenance of critical process variables (e.g., temperature, pH, and dissolved oxygen) at specific levels through feedback control [143].

Table 12
Culture characteristics of host strains used for ethanol production

Host	Ara	Gal	Glc	Man	Xyl	T (K)[a]	pH[a]
Escherichia coli	+	+	+	+	+	308	6.5
Klebsiella oxytoca	+	+	+	+	+	303	5.5
Zymomonas mobilis	+	–	+	–	+	303	5.5

Ara arabinose, *Gal* galactose, *Glc* glucose, *Man* mannose, *Xyl* xylose.
Source: Ref. [30].
[a]Typical culture conditions for single-sugar fermenting cultures; conditions are varied for simultaneous saccharification and fermentation.

5.2.4. Product and solids recovery

As biomass hydrolysis and fermentation technologies approach commercial viability, advancements in product recovery technologies will be required. For cases in which fermentation products are more volatile than water, recovery by distillation is often the technology of choice. Distillation technologies that will allow the economic recovery of dilute volatile products from streams containing a variety of impurities have been developed and commercially demonstrated [144]. A distillation system separates the bioethanol from water in the liquid mixture. Water content of virgin bioethanol is generally higher than 80%. Large quantities of energy are required to concentrate the ethanol to 95.6% (azeotrope mixture of ethanol with water). The beer column separates most of the bioethanol from water (and solids, if any) and produces a top stream rich in bioethanol, and a bottom stream rich in water [145]. In this flow, bioethanol from cellulosic biomass has likely lower product concentrations ($\leqslant 5$ wt%) than in bioethanol from corn. The maximum concentration of bioethanol tolerated by the microorganisms is about 10 wt% at 303 K but decreases with increasing temperature. To maximize cellulase activity, the operation is rather at maximum temperature (310 K), since the cost impact of cellulase production is high relative to distillation [49,77,146].

The first step is to recover the bioethanol in a distillation or beer column, where most of the water remains with the solids part. The product (37% bioethanol) is then concentrated in a rectifying column to a concentration just below the azeotrope (95%) [49]. The remaining bottom product is fed to the stripping column to remove additional water, with the bioethanol distillate from stripping being recombined with the feed to the rectifier [147]. The recovery of bioethanol in the distillation columns in the plant is fixed to be 99.6% to reduce bioethanol losses [145].

After the first effect, solids are separated using a centrifuge and dried in a rotary dryer. A portion (25%) of the centrifuge effluent is recycled to fermentation and the rest is sent to the second and third evaporator effects. Most of the evaporator condensate is returned to the process as fairly clean condensate (a small portion, 10%, is split off to waste water treatment to prevent build-up of low-boiling compounds) and the concentrated syrup contains 15–20% by weight total solids [148].

6. Thermochemical bioethanol production processes

There are two bioethanol production processes that currently employ thermochemical reactions in their processes. The first system is actually a hybrid thermochemical and biological system. Cellulosic biomass materials are first thermochemically gasified and the synthesis gas (a mixture of hydrogen and carbon monoxide) bubbled through specially designed fermenters [106,112]. A genetically engineered microorganism that is capable of converting the synthesis gas is introduced into the fermentation vats under specific process conditions allowing bioethanol to ferment [149].

The second thermochemical bioethanol production process does not use any microorganisms. In this process, biomass materials are first thermochemically gasified and the synthesis gas passed through a reactor containing catalysts, which cause the gas to be converted into bioethanol. Numerous efforts have been made since then to develop commercially viable thermochemical-to-bioethanol processes. Bioethanol yields up to 50% have been obtained using synthesis gas-to-bioethanol processes. Some processes that first produce methanol and then use catalytic shifts to produce bioethanol have obtained bioethanol yields in the range of 80%. Unfortunately, like the other processes, finding a cost-effective all-thermochemical process has been difficult [106,112,149]. Thermochemical processing options appear more promising than biological options for the conversion of the lignin fraction of cellulosic biomass, which can have a detrimental effect on enzymatic hydrolysis but also serves as a source of process energy and potential co-products that have important benefits in a life-cycle context [150].

7. Bioethanol economy

Lignocellulosic biomass (a complex comprised of several polysaccharides) is the most promising feedstock considering its great availability and low cost, but the large-scale commercial production of fuel bioethanol from lignocellulosic materials has still not been implemented. For designing fuel bioethanol production processes, the assessment of the utilization of different feedstocks (i.e. sucrose containing, starchy materials, lignocellulosic biomass) is required considering the big share of raw materials in bioethanol costs [36].

Today the production cost of bioethanol from lignocellulose is still too high, which is the major reason why bioethanol has not made its breakthrough yet. When producing bioethanol from maize or sugar cane the raw material constitutes about 40–70% of the production cost. By using cheaper waste products from forestry, agriculture and industry, the costs may be lowered. However, it is obvious that we have to make use of the feedstock as efficient as possible, e.g. have to improve the production process and as a result achieve higher bioethanol yields [151]. Approximately 60% of the production cost of bioethanol comes from raw materials [136]. The cost of raw material, which varies considerably between different studies (US$22–US$61 per metric ton dry matter), and the capital costs, which makes the total cost dependent on plant capacity, contribute most to the total production cost [58]. With these relatively high raw material costs (which includes enzyme pre-treatment when starch-based crops are used), such fermentation products are currently more expensive to produce than fuels or chemicals produced from lower cost hydrocarbons [136]. Pre-treatment has been viewed as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion with costs as high as US$0.3/gallon bioethanol produced [60]. Enzyme price is assumed to be such that the total contribution of enzymes to production costs is about US$0.15/gallon of bioethanol with some variation depending upon actual bioethanol yields resulting from the particular pre-treatment approach [63].

The costs of producing bioethanol were estimated for a 50 million gallons per year dry mill bioethanol plant using current data for corn, distillers dried grains (DDG), natural gas, enzymes, yeast and chemicals, electricity, and wage rates. The bioethanol plant of this size will produce 51.5 million gallons of denatured bioethanol annually from 18.1 million bushels of corn. In addition to bioethanol, the plant will produce 154,500 tons of DDG. The cost of producing bioethanol in a dry mill plant currently totals US$1.65/gallon, as shown in Table 13. Corn accounts for 66% of operating costs while energy (electricity and natural gas) to fuel boilers and dry DDG represents nearly 20% of operating costs [152].

Until recently, Brazil had been the largest producer of bioethanol in the world. Brazil used sugar cane to produce bioethanol and sugar cane is a more efficient feedstock for bioethanol production than corn grain [153]. The costs of producing bioethanol in Brazil are the world's lowest. Production cost for bioethanol in Brazil is in the range US$0.68–US$0.95 per gallon range [32]. Factors contributing to Brazil's competitiveness include favorable climate conditions, low labor costs, and mature infrastructure built over at least three decades [154].

Estimates show that bioethanol in the EU becomes competitive when the oil price reaches US$70 a barrel while in the United States it becomes competitive at US$50–60 a barrel. For Brazil the threshold is much lower—between US$25 and US$30 a barrel. Other efficient sugar producing

Table 13
Operating costs 50 million gallons per year Dry Mill Bioethanol plant in 2006

Operating costs	Units/gallon	Unit price	Cost	
			million $/yr	$/gallon
Raw materials				
Corn (bu)	0.364	$3.01	$54.73	$1.09
Enzymes (lb)	0.035	$1.02	$1.79	$0.04
Yeast & chemicals (lb)	1.126	$0.02	$0.84	$0.02
Denaturant (gal)	0.030	$1.60	$2.40	$0.05
Electricity ($/KWh)	0.800	$0.06	$2.31	$0.05
Natural gas ($/MCF)	0.036	$7.78	$14.00	$0.28
Water (thou gal/bu)	0.010	$0.37	$0.18	$0.00
Waste water (thou gal/bu)	0.008	$0.50	$0.19	$0.00
Direct labor & benefits ($.032/gal)			$1.600	$0.03
Maintenance & repairs ($.026/gal)			$1.300	$0.03
GS&A ($.06/gal)*			$3.000	$0.06
Total costs			$82.347	$1.65

*GS&A: Overhead and marketing expenditures (*source*: Ref. [152]).

countries such as Pakistan, Swaziland and Zimbabwe have production costs similar to Brazil's [14,21].

8. Conclusion

Bioethanol is a fuel derived from biomass sources of feedstock; typically plants such as wheat, sugar beet, corn, straw, and wood. Production of bioethanol from biomass is one way to reduce both the consumption of crude oil and environmental pollution. Large amounts of CO_2 are released during corn bioethanol production contributing to the global warming problem. Using bioethanol-blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission. Bioethanol is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NO_x emissions from combustion. Ethanol has a higher octane number (108), broader flammability limits, higher flame speeds and higher heats of vaporization. These properties allow for a higher compression ratio and shorter burn time, which lead to theoretical efficiency advantages over gasoline in an ICE. Bioethanol is blended with gasoline to form an E10 blend (10% bioethanol and 90% gasoline), but it can be used in higher concentrations such as E85 or E95.

Bioethanol is currently made by large-scale yeast fermentation of sugars that are extracted or prepared from crops followed by separation of the bioethanol by distillation. Yeast and fungi tolerate a range of pH 3.5–5.0. The ability to lower pH below 4.0 offers a method for present operators using yeast in less than aseptic equipment to minimize loss due to bacterial contaminants. The majority of organisms cannot tolerate bioethanol concentrations above 10–15% (w/v).

Fermentation involves microorganisms that use the fermentable sugars for food and in the process produces ethyl alcohol and other byproducts. These microorganisms can typically use the 6-carbon sugars, one of the most common being glucose. Therefore, cellulosic biomass materials containing high levels of glucose or precursors to glucose are the easiest to convert to bioethanol. Microorganisms, termed ethanologens, presently convert an inadequate portion of the sugars from biomass to bioethanol. There are a number of microorganisms that produce significant ($>1\%$ w/v) quantities of bioethanol.

One major problem with bioethanol production is the availability of raw materials for the production. The availability of feedstocks for bioethanol can vary considerably from season to season and depend on geographic locations. The price of the raw materials is also highly volatile, which can highly affect the production costs of the bioethanol. Because feedstocks typically account for greater than one-third of the production costs, maximizing bioethanol yield is imperative.

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