



Different pretreatment technologies of lignocellulosic biomass for bioethanol production: An overview

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ABSTRACT

Regarding the supply of fossil fuels, greenhouse gasses emission, global warming, and increasing fuel price; there is a need to find alternative energy resources, which are renewable, environmentally sustainable, and economically viable. Agro-industrial biomass such as sugarcane bagasse, rice and wheat straw, corn stover, and switchgrass, named as lignocellulosic biomass (LCB) which is inexpensive, abundant, renewable, and provides a unique natural resource for large-scale and cost-effective bio-energy production. During the production of biofuels, pretreatment is a necessary step due to the recalcitrant structure of LCB. Various pretreatment methods were employed with a different mechanism of action, feasibility, and practicability to produce high yield biofuels. Since many years ago, the development of an effective pretreatment method has been challenging due to the existence of barriers and obstacles. Besides, each pretreatment method has its advantage and disadvantage, which should be investigated to obtain a higher yield of bioethanol. In this paper, the recent findings regarding the application of various pretreatment techniques such as chemical, physical and biological methods for bioethanol production from LCBs have been reviewed. The limitations of the mentioned methods based on the existing barriers were explained, and the future recommendations were discussed.

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1. Introduction

Energy plays a crucial role in economic development, human, and social improvements [1,2]. However, concerns about the depletion of fossil fuels and the increasing rate of energy consumption and industrialization, have encouraged the researchers to find alternative energy sources [3,4]. Owing to a reliable supply of required fuels for transportation and environmental pollution,

cellulose-based fuel is an answer to the current issues [5]. Besides, the generated agricultural waste, through or after agricultural crop processing is a kind of renewable and lignocellulose-rich biomass resource [6]. It should be noted that agricultural wastes do not need land, water, and energy requirements and are not directly associated with food security for conversion to energy [7]. Lignocellulosic waste is a kind of agro-industrial biomass, which is available at a reasonable price categorized as a renewable source. Generally, lignocellulose biomass (LCB) can be categorized into four main sources: agricultural and forest residues, energy crops, and cellulosic wastes. As a wide category of biomass, agro-industrial biomass incorporates both the food-based and the non-food-based portions of crops [8]. Globally, wheat, maize, rice, and sugarcane are the major cultivated crops which use as feedstock to produce the LCBs

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and yield more than 5300 million tons of dry biomass per year [9,10].

Since many decades ago, the conversion of LCB to different types of fuel such as biohydrogen, bioethanol, biodiesel, biogas, bio-butanol, and bio-methane has drawn the attention of researchers [11–16]. The produced biofuels from lignocellulosic feedstock by microbial fermentation and saccharification is called the second-generation of biofuels [17]. Based on the literature, bioethanol is one of the most useful biofuels to be substituted by fossil fuels [18]. The potential of different types of LCBs such as solid starchy wastes [19], softwoods [20], cassava starch [21], municipal solid waste like paper and paperboard, timber, wood, and garden waste [22], sweet sorghum bagasse [23], and sugarcane bagasse [24] have been widely investigated. In countries with a high rate of agricultural activities, agricultural wastes have shown good potential to produce biofuels [25]. The main advantages are their accessibility, affordability (low-cost), abundance, and high carbohydrate content [26]. Ensuring the round year production of LCBs requires utilizing different biomass sources as feedstock, which derived from seasonal nature and annual variability of LCBs [27].

It should be considered that cellulose-based fuels are not produced at the competitive price compared to ethanol produced from sugar-based and starch-based feedstocks. Therefore, adopting the cost reduction strategy should be on the top priority compared to petroleum-based transportation fuels [28]. The two main steps in fuel production are pretreatment and hydrolysis steps which are based on the conversion of the biomass into different sugars [18]. In this regard, many studies have been conducted to increase fermentable carbohydrate recovery from sugar degradation during the pretreatment process. Therefore, the investigation of the pretreatment condition is the main goal of scientists to produce high-yield bioenergy in a cost-effective way.

1.1. LCBs structure

Lignocellulose is an inevitable part of the plant cell wall. It is a natural and complicated compound and composed of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose, as two major polysaccharides in LCB, are strongly associated with lignin and make a complex lignocellulosic network. This network is highly robust and rebellious to depolymerization. Consequently, producing biofuel from LCB needs a complicated conversion process, which has made it commercially difficult [29,30].

Cellulose is a polymer of glucose and its structure aid to have tightly packed polymer chains, resistant to depolymerization, and highly crystalline structure [31]. Hemicellulose is another carbohydrate component that has an amorphous, branched, and the random structure that includes five or six carbon sugars. Since hemicellulose and cellulose are polymers of sugars, they can be considered as a potential sources to produce fermentable sugars [32]. More than 75% of carbohydrates must be converted to the monosaccharide, the conversion of LCBs to biofuel may not be in a feasible way due to some barriers [33]. Lignin is the second most abundant natural polymer of LCBs has a three-dimensional and highly cross-linked molecular structure and its stable nature and in-solvability in water act as “glue” for connection of cellulose and hemicellulose. Therefore, the existence of lignin is the most important barrier in producing biofuels [34]. Strong interrelationship among lignin, hemicellulose, and cellulose may limit access to the carbohydrates. However, several chemical, physical, and biological pretreatment methods have been developed to open up their structure and remove lignin [35,36].

1.2. Biofuel production steps from LCBs

Three main steps in the biofuel production from LCBs are pretreatment, hydrolysis, and fermentation and the pretreatment step is the most costly and limiting step [37]. Appropriate pretreatment methods can improve the efficiency of the whole process by increasing concentrations of fermentable sugars after enzymatic saccharification [38]. There are some types of reducing sugars like arabinose, galactose, fructose, mannose, which are released from LCBs during the pretreatment step. Fermentation is based on the activity of microorganisms, which uses the sugars as a food source to produce bioenergy. Since many years ago, *Saccharomyces cerevisiae* has preferred microorganism for fermentation of sugars to ethanol [39,40]. The overall process of biofuel production from LCB is shown in Fig. 1.

1.3. Delignification of LCB

Lignin–carbohydrate complexes are recalcitrant parts in LCB structure and do not incorporate in the fermentation process of bioethanol production [41]. Delignification is defined as removing lignin from LCB by employing different natural enzymatic or chemical methods [42]. For instance, the lignin fraction of sugarcane bagasse contains 17–32% of the biomass, which is the highest amount than several non-wood biomass wastes [43]. The size of surface area is an important parameter for an effective delignification [44]. The fraction of LCB to bioethanol is shown in Fig. 2. After removing recalcitrant compounds from the biomass, the LCB can be degradable either chemically or biologically [45]. Furthermore, the most important parameters of an ideal delignification are: producing a limited amount of sugar and residual lignin; providing high degradable cellulose, and high potential to recover approximately all carbohydrates [46].

The structure of lignin may be modified and not destroyed completely as all pretreatments are not led to substantial changes during delignification. However, the raw biomass in comparison with the pretreated biomass is more digestible, even it may have almost the same lignin content as non-pretreated [47].

2. Pretreatment

Pretreatment is one of the most important steps in LCB conversion to biofuels from the point of economic view. However, rebellious nature and the complicated hierarchical structure of LCB, has presented pretreatment as the most critical step during biomass conversion to biofuels [48]. It should be noted that the efficiency of pretreatment is an important issue to obtain a higher yield of fuel. Expanding the surface area of biomass, dissolving hemicellulose and/or lignin and reducing the particle sizes of biomass are the most important objectives of the pretreatment step. This can be done by chemical or physical modification of the LCBs structure. Subsequently, the hydrolysis of cellulose improves by increasing the accessibilities of acids or enzymes to the surface of cellulose [49]. The physical, chemical, biological, and combined pretreatments are the most used methods in the previous studies. For these methods, different types of materials have been used as shown in Fig. 3.

As many papers have been published regarding applying different pretreatment methods, there is a need to compile recent studies to evaluate the most efficient methods. Besides, over the last three years, many review papers have been published regarding pretreatment methods [50], emerging technologies for the pretreatment of LCB [51], pretreatment of LCB [27], pretreatment of lignocellulosic feedstocks to produce bioenergy [52], acid pretreatment of LCB [53], biological pretreatment of LCB [54–56],

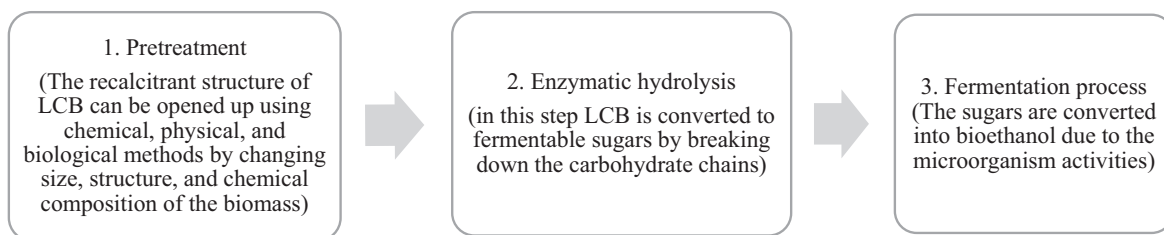


Fig. 1. Conversion of the LCB to bioethanol.

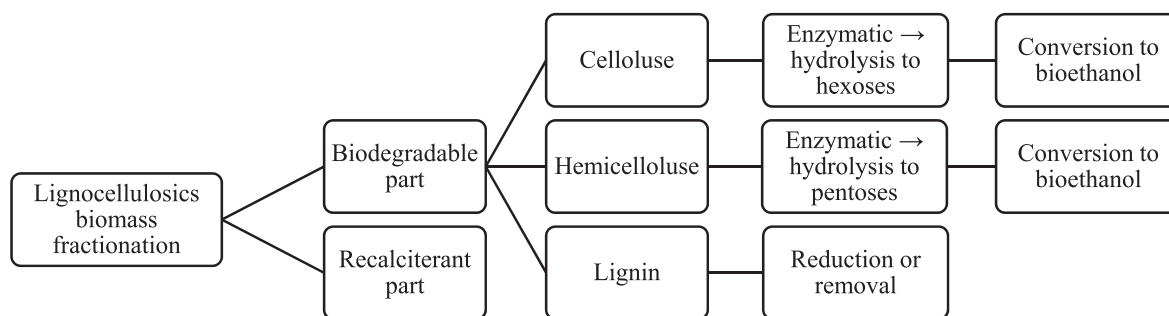


Fig. 2. Fractionation of LCB to bioethanol.

alkaline hydrogen peroxide pretreatment [57], combined pretreatment methods [58], effect of pretreatment on lignin structure [59], ionic liquids pretreatment [60]. These studies mostly focused on the single pretreatment in detail while there is no review regarding the compilation of the most important and practical methods in a review. Therefore, the most recent published studies of LCB pretreatment in the main categories of chemical, physical, and biological, have been reviewed in this paper. The methods were alkaline, acid, ionic liquid (IL), microwave-assisted, steam explosion, hydrothermal, organic solvent, fungal and enzymatic pretreatment. The future recommendation is given based on the existing barriers and limitations in different methods of pretreatments.

3. Chemical methods

This method is based on the utilization of chemicals for pretreatment which are divided into four main methods LCBs as follows:

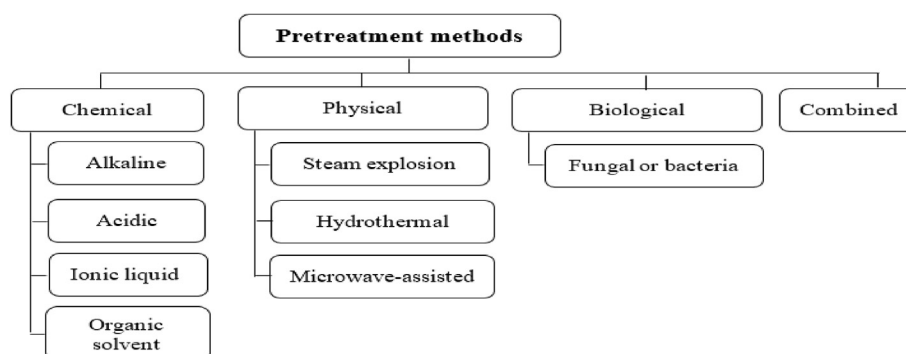


Fig. 3. Different pretreatment methods (Adopted from Ref. [49]).

3.1. Alkaline pretreatment

This method is one of the most reliable pretreatments due to its strong effect and the relatively simple process. Selective removal of lignin without losing reducing sugar and carbohydrates, enhancing porosity and surface area of biomass, and therefore improving enzymatic hydrolysis can be considered as the most important advantages of the alkaline method [61]. However, the main disadvantage of this method compared to the other pretreatments methods is longer reaction times (several hours up to one day) [62]. The major role of this method is improving the enzymatic digestibility through delignification of biomass. Alkyl-aryl linkages in lignin are easily broken under alkaline circumstances [63]. Different types of alkaline solutions have been used such as sulfite, sodium hydroxide, ammonium hydroxide, and lime. Based on the literature, sodium hydroxide is the most used alkaline solution as it is effective for delignification and works in various conditions [64]. Table 1 shows recent studies of bioethanol production from LCB using different types of alkaline pretreatment.

Table 1
Recent studies of alkaline pretreatment of LCB for bioethanol production.

Raw material	Alkaline type	Pretreatment condition	Reducing sugars	Delignification rate (%)	Fermentation condition	Bioethanol yield (g/L)	Reference
Sugarcane bagasse	Na ₂ CO ₃ (5%)	140 °C; 1 h	Glucose: 97.6%	40–59	37 °C; 72 h	7.27	[65]
Sugarcane bagasse	NaOH (15%)	175 °C; 1.5 h	5.29 g/L	22–90	37 °C; 10 h	8.8	[66]
Eucalyptus	NaOH (10%)		2.70 g/L	11–51		3.4	
Sugar cane straw	NaOH (5%)		2.66 g/L	60–99		6.4	
Rice straw	NaOH	50–90 °C	Glucose: 81%	64.51	37 °C; 72 h	0.032 g/g	[67]
Bagasse	NaOH	50 °C; 1 h	NA ^a	NA	30 °C; 72 h	7.05	[68]
Wheat straw	Na ₂ CO ₃ (11%)	75 °C; 10–85 min	Xylose: 85.7%	70.4	30 °C; 96 h	65	[69]
Rice straw	Na ₂ CO ₃	93 °C; 3–10 h	77.4 g/L	54.5–62.7	37 °C; 120 h	83.1	[70]
Rice straw	NaOH (1%)	50 °C; 72 h	94 g/L	NA	35 °C; 144 h	13.8	[71]
Wheat straw	NaOH/H ₂ O ₂	50 °C; 3–15 h	61.9 g/L	60	37 °C; 96 h	31.1	[72]
Sugarcane bagasse	NaOH (0.5%)	60 °C; 20 min	269.30 g/kg	48.31	30 °C; 96 h	17.26	[73]

^a NA: not available.

Table 2
Recent studies of acid pretreatment of LCB for bioethanol production.

Raw material	Acid type	Pretreatment condition	Reducing sugars	Delignification rate (%)	Fermentation condition	Bioethanol yield (g/L)	Reference
Wheat straw	H ₂ SO ₄ (2%)	180 °C; 10 min	43 g/L	NA	30 °C; 72 h	0.44	[80]
Rice straw	H ₂ SO ₄	100 °C; 2 h	14 g/L	92	30 °C; 72 h	40.6	[81]
Corn and corn Stover	H ₂ SO ₄	160 °C; 10 min	184.4 g/L	NA	30 °C; 96 h	99.3	[82]
Wheat straw	H ₂ SO ₄ and Na ₂ SO ₃	180 °C; 30 min	NA	21	48 h	17.25 g/g	[83]
Rice straw	H ₂ SO ₄	200 °C; 1 min	NA	NA	30 °C; 48 h	2.3	[84]
Rice straw	H ₂ SO ₄	120 °C; 30 min	NA	NA	45 °C; 24 h	11.77	[85]
Rye	H ₂ SO ₄	121 °C; 60 min	47.2 mg/g	NA	35 °C; 72 h	20.63	[86]
Wheat		121 °C; 60 min	42.8a mg/g	NA	35 °C; 72 h	17.86	
maize		131 °C; 60 min	134.4 mg/g	NA	35 °C; 72 h	35.44	
Rapeseed straw	H ₂ SO ₄	180 °C; 10 min	66–80%	NA	30 °C; 48 h	88–90%	[87]

3.2. Acid pretreatment

Acid pretreatment is a well-known method for producing bioethanol from LCBs. Using acid to pretreat the LCB results in increasing the degradation of hemicellulose. Phosphoric acid, acetic acid, and sulfuric acid are the most common types of acids [74]. The main effective parameters for acid pretreatment include solids loading, acid concentration, temperature, and residence time [53]. Besides, acid is able to break all polysaccharide-lignin linkages which help to the recovery of the majority of monomeric sugars [75]. During this method, hemicelluloses converted into soluble sugar by changing the structure of LCB. Increasing the temperature can enhance the speed of the conversion of hemicelluloses in dilute acid pretreatment [76]. The advantages of the acid pretreatment method rather than other methods could be attributed to the lignocellulosic matrix disruption and amorphous cellulose conversion. However, this method has some disadvantages such as the high cost of acid recovery, and the formation of inhibitors. It is worth to know that the economic and environmental aspects of the acid pretreatment process have been improved over time [53].

Dilute acid pretreatment is one of the most common methods for LCB pretreatment, which makes a harsh condition to recover monomeric sugars from the biomass [77]. As compared to a concentrated acid, dilute acid hydrolysis has an advantage of lower acid consumption while the higher temperature is required to achieve a reasonable yield of glucose from crystalline cellulose [78]. For instance, the pretreatment using sulfuric acid was applied to enhance the digestibility of the conversion of cellulosic biomass into ethanol. The structure of biomass was disrupted under acid pretreatment and the crystallinity decreased due to cleavage of lignin structure [79]. Table 2 shows recent studies of bioethanol production from LCB using acid pretreatment.

3.3. IL pretreatment

Using ILs for the pretreatment of LCB has gained more attention due to the high solubility of biomass in ILs resulted in a high yield of sugars and carbohydrates [88,89]. ILs pretreatment enhances enzymatic delignification by maintaining *cellulase* activity and stability. Moreover, temperature and biomass loading affect the hydrolysis rate during IL pretreatment [90]. Numerous types of ILs, including imidazolium-based ($[(C_3N_2)X_n]^+$), pyridinium-based ($[(C_5N)X_n]^+$), pyrrolidinium-based ($[(C_4N)X_n]^+$), ammonium-based ($[NX_4]^+$), phosphonium-based ($[PX_4]^+$), and sulfonium-based ($[SX_3]^+$) ILs, have been used for the pretreatment of LCB [91]. Acidic ionic liquids (AILs) as a type of ILs, have a high potential for pretreatment of LCB by breaking the ether linkage to extract lignin and have a remarkable effect on the de-polymerization of lignin [92]. The disadvantages of IL method are high energy requirement to recycle pure IL, high cost and high waste generation with difficult recovery [93]. Employing the ILs at high water content as pretreatment solvents for LCB is a significant way to reduce the chemical costs. However, when water contents exceed the limits, the dissolving capacity of the ILs decreases notably [94,95]. Despite the advantages of ILs, the development of efficient recycling and reuse methods of degraded ILs, is vital for better understanding of the behavior of IL–biomass mixtures [96].

Many papers has been published regarding using IL solutions for the pretreatment of different types of LCBs. For instance, 1-ethyl-3-methylimidazolium acetate [EMIMAcO] was used for the pretreatment of barley straw. Based on the results, a yield of total reducing sugars was 80% which is much higher than in non-pretreated samples [97]. In another study, pretreatment with 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) significantly improved the enzymatic saccharification of the cellulose content of bamboo. In addition, the enzymatic hydrolysis rate for IL pretreated biomass was 4.7 times higher than dilute sulfuric acid pretreated

Table 3
Recent studies of IL pretreatment of LCB for bioethanol production.

Raw material	IL type	Pretreatment condition	Reducing sugars	Delignification rate (%)	Fermentation condition	Bioethanol yield	Reference
Sugarcane bagasse	BMIMCl; PEG	154.6 °C; 60 min and 5 (%w/w) of PEG	62%	NA	30 °C; 15h	84%	[99]
Cotton stalks	HBIMHSO ₄	120 °C, 4 h	NA	35	50 °C; 72h	NA	[100]
Corn straw	([Emim]Ac); NaOH	[Emim]Ac: 55.5% NaOH: 0.444% 105 °C	NA	81.73	NA	NA	[101]
Corn stalks	([Bmim]BF ₄)	150 °C; 5h	NA	31.84	50 °C; 48h. hydrolysis 37 °C; 96 h	NA	[102]
Triticale straw	1-ethyl-3-methylimidazolium acetate	120 C; 2h	81%	NA		10.64 g/dm ³	[103]
Sugarcane bagasse	1-butyl-3-methylimidazolium chloride [Bmim]Cl); surfactant (PEG-8000)	90 C; 2h	254 mg/g	NA	30 °C; 72 h	0.117 g/g	[104]

Table 4
Recent studies of organic solvent pretreatment of LCB for bioethanol production.

Raw material	Pretreatment type	Pretreatment condition	Reducing sugars (%)	Delignification rate (%)	Fermentation condition	Bioethanol yield	Reference
Rice straw	Choline chloride-based solvent	60–121 °C 30 min–12 h	Xylose: 88	57.273.6	37 °C; 72 h	36.7 g/L	[109]
Wheat straw	Organosolv	180 °C, 40 min	89.2	76.4	35 °C; 8 h	67%	[110]
Sugarcane bagasse	FeCl ₃ -catalyzed organosolv	160 °C, 72 h	Glucose: 93	62.7	50 °C; 72 h.	NA	[111]
Wheat straw	Organosolv	190 °C, 1 h	Glucose: 99	84.5	50 °C; 72 h.	NA	[112]
Corn stover	Solvent magnesium oxide-ethanol	190 °C; 40 min	Glucose: 71 Xylose: 39	60	50 °C; 72 h	NA	[113]
Cotton Stalks	Organosolve	80 °C, 1 h	NA	81	30 h.	52%	[114]
Sorghum bagasse	Organosolve	200 °C, 1 h	Glucose: 90 Xylose: 70	15.3	35 °C; 72 h	61.9 g/L	[115]
wheat straw	Eutectic solvents	70 °C, 9 h	Glucose: 79.7	71.4	50 °C; 72h	89.8%	[116]

biomass [98]. Table 3 shows recent studies of bioethanol production from LCB using ILs pretreatment.

3.4. Organic solvent pretreatment

Organic solvent pretreatment has some advantages such as the ability to fractionate LCB into cellulose, hemicellulose and lignin with high purity, easy solvent recovery, and solvent reuse. Many studies investigated the use of organic solvents for the pretreatment of LCB with significant improvement in the conversion of biomass into sugars [105]. A wide range of organic solvents, such as alcohol, phenol, esters, propionic acid, acetone, formaldehyde dioxane and amines with and without catalyst, have been used for the pretreatment of LCBs [106]. Ethanol and methanol as alcohols with low boiling points are preferred due to their low cost and ease of recovery [107]. The organosolv is one type of organic pretreatment that utilizes organic or aqueous-organic solvent at temperatures ranging from 100 to 250 °C. The two advantages of this method are the separation of high purity cellulose with only minor degradation and hemicellulose fractionation with high efficiency. The high-purity lignin and lignin derivative chemicals as well as a high-value co-products from organic pretreatment are major economic concerns of this method rather than dilute acid pretreatment [108]. Table 4 shows recent studies of bioethanol production from LCB using organic solvent pretreatment.

4. Physical methods

Physical/mechanical pretreatment is able to open up the structure of LCBs by disrupting their surface structure and reducing the size to 10–30 mm using shear or compression forces [117]. The

grinding process contributes to a high fraction of the total operating cost in LCB pretreatment [17,118]. Biomass characteristics and final particle size are the most influential factors for physical pretreatment. Meanwhile, the high power consumption and usually low efficiency are the main drawbacks of this method [30]. There are various types of physical pretreatments such as milling, high-pressure homogenization [119], electron beam irradiation [120], hot compression [121] and photocatalysis [122] which have been used rarely. The milling method increases enzymatic hydrolysis and digestibility and includes hammer milling, disk milling, ball milling, and vibratory milling [123]. In a recent study, three physical methods including air classifier mill, continuous ball mill, and high-speed mill applied for the pretreatment of corn stover. All these methods reduced the size of biomass in the range of 100–230 μm resulted in an increase in the surface area for the action of the enzyme. In addition, the size reduction of biomass can increase the glucose in the range of 29–44% which showed that the high-speed mill and air classifier mill had a high pretreatment efficiency [124].

The effectiveness of vibratory ball milling in reducing cellulose crystallinity is higher than the ball milling method [44]. Additionally, disk milling produces fibers, while the hammer milling produces finer particles [125]. Although milling of LCB enhances biofuel yield due to its high energy consumption in large scale production, it is not cost-effective. In a recent study, mechanical pretreatment enhanced the breakdown of structural materials in two different phases by reduction of the lagging time during anaerobic digestion (AD) and an increase in biofuel yield up to 22% [126].

Thermal pretreatment is considered as a type of physical pretreatment which is based on heating LCB at a certain temperature and pressure in the range of 50–240 °C. For example, wheat straw

Table 5
Recent studies of microwave-assisted pretreatment of LCB for bioethanol production.

Raw material	Pretreatment type	Pretreatment condition	Reducing sugars (mg/g)	Delignification rate (%)	Fermentation condition	Bioethanol yield (g/L)	Reference
Wheat straw	Microwave associated NaOH	160 °C; 15min	718	69.48	30 °C; 108h	6.82	[138]
Wheat straw	Microwave assisted alkali	80 °C; 20min	610.35	11.2	72h	6.84	[139]
Rice straw	Microwave assisted alkali	60 °C; 25 min	NA	NA	30 °C; 24h	1.383	[140]
maize	Microwave-assisted H ₂ SO ₄	50 °C; 20min	104	NA	50 °C; 24h	0.511	[133]
Eucalyptus sawdust	Ultrasound-assisted ionic liquid	50 °C; 48 h	426.6	26.3	50 °C; 48h.	NA	[141]
eucalyptus sawdust	Microwave assisted	180 °C; 17 min	104.4	112.1	50 °C; 48h	NA	[142]
Cotton Stalks	Microwave-assisted acid	210 °C; 10 min,	NA	81	210 °C; 10 min	15.9	[114]
Sugarcane bagasse	Ultrasound	120 °C; 30 min	NA	NA	49 °C; 12 h	6.38	[143]
Corn	Microwave	160–200 °C; 10–15 min	NA	NA	35 °C; 24 h	8.73	[144]

pretreated at 180 °C showed 53% higher yield as compared to untreated with a substantial change in chemical bonds of the lignocellulosic structure of wheat straw [127]. This pretreatment can be used in combination with other pretreatments [128]. Recently, Shahabazuddin et al. [129], used thermal assisted alkaline pretreatment for rice husk biomass. The physico-chemical properties of biomass after pretreatment showed the lignin significantly removed with minimal loss of hemicellulose and cellulose content. In the next section, the most common physical pretreatment of LCBs includes steam explosion, liquid hot water, and organosolv were discussed.

4.1. Microwave-assisted pretreatment

Microwave-assisted pretreatment is one of the most used methods for LCBs and works based on irradiation. Researchers have gained a remarkable bioethanol yield with this method over 30 years, which has been implemented gradually from laboratory scale to pilot one [130]. Some advantages such as the lower reaction activation energy and the shorter reaction made microwave as a suitable pretreatment method [131]. By using microwave heating, the disruption of recalcitrant structures in LCB occurred by penetrating irradiation into the rigid structure of LCB [132,133]. Many studies have been conducted to promote the usage of microwave pretreatments such as designing particular microwave vessels for loading biomass and new microwave reactors [130,134].

According to Zheng et al. [135], microwave pretreatment in glycerol can be considered as an effective method to increase the sugar yield of corncob due to the removal of alkali and alkaline metals during microwave pretreatment. In another study, CaCl₂-assisted microwave pretreatment increased hemicellulose degradation to 85.90% and the specific surface area to 168.93%, compared to untreated corn stover [136]. It was found that with decreasing particle size of corn stover, the yield of microwave pyrolysis increased due to promote the activity meanwhile acid pretreatment substantially decreased the yield [137]. Table 5 shows recent studies of bioethanol production from LCB using microwave-assisted pretreatment.

4.2. Steam explosion pretreatment

This method is a thermophysical-chemical process, which provides mechanical deconstruction of LCBs by combining vapor cracking and explosive decompression steps [145]. In this process, the biomass is subjected to high-pressure saturated steam in the short run, which is measured in minutes. Then, the pressure is

suddenly released, causing a disruption in the cell wall structure and solubilizing mainly the hemicellulose and lignin fractions [146]. The steam explosion is one of the most commonly used methods with high potential in removing hemicellulose and lignin from biomass structure. The steam explosion can affect the physicochemical properties of LCBs by increasing the crystallinity of the cellulose and changes in lignin structure [147]. Breaking down the lignocellulosic structure, defibrating, depolymerizing lignin components, hydrolyzing the hemicellulosic fraction, low environmental impact and high energy efficiency are the most advantages of steam pretreatment [74,148,149]. However, incomplete lignin removal and producing some toxic chemicals during the process are the disadvantages of this method [150].

The Ammonia Fiber Explosion (AFEX) pretreatment method is approximately similar to the steam explosion method. The mechanism of AFEX pretreatment is the swelling of biomass, which increases the available surface area, disruption of biomass fibers, decrystallization of cellulose and breaking down of lignin-carbohydrate linkage [151,152]. During AFEX, hemicellulose is degraded into oligomeric sugars and is deacetylated, while lignin reacts with aqueous ammonia, causing depolymerization of the lignin and cleavage of the lignin-carbohydrate linkages. For instance, the combination of dilute acid and AFEX pretreatment of corn stover resulted in significant cellulase accessibility due to the high efficiency of AFEX. The dilute acid mainly removed hemicellulosic fraction of biomass (xylan) while, AFEX confirmed no loss of hemicellulose or lignin. Although AFEX treated corn Stover had a better enzymatic activity, economics of ammonia pretreatment and its recovery is an issue for commercial-scale applications [153]. Table 6 shows recent studies of bioethanol production from LCB using steam explosion pretreatment.

4.3. Hydrothermal (liquid hot water) pretreatment

Hydrothermal or liquid hot water (LHW) method works based on using of water in high temperature for pretreatment of LCB for bioethanol production [162]. In this method, hot water applied at high pressure to maintain its liquid form to increase the lignocellulosic matrix degradation at a temperature between 160 °C and 240 °C [74]. It is considered as a physicochemical pretreatment and is widely accepted as a green technology which does not require the use of chemical reagents. This is a remarkable advantage in terms of costs in the whole production process while the disadvantage of this method is high energy and water consumption, low concentrations of sugars, as well as fermentation inhibitors [151,163]. Besides, the efficiency of hydrothermal pretreatment

Table 6
Recent studies of steam explosion pretreatment of LCB for bioethanol production.

Raw material	Pretreatment condition	Reducing sugars (%)	Delignification rate (%)	Fermentation condition	Bioethanol yield	Reference
Triticale straw	200 °C; 5–10 min	NA	NA	37 °C, 96 h	84.7%	[154]
Barley straw	180 °C; 3.5 min	NA	85	30 °C, 16 h	0.34 g/g	[155]
Corn Stover	200 °C; 10 min	84.7%	NA	35 °C, 16 h	0.40 g/g	
Switchgrass	200 °C; 10 min	Glucose: 50.9 Xylose: 27.9	50.6	50 °C, 72 h	78.3%	[156]
Sweet sorghum	25 °C; 16 h	NA	NA	50 °C, 96 h	88.3%	[146]
Wheat straw	165 °C; 10 min	Glucose: 38.7 Xylose: 24.1	36.0	30 °C, 96 h	90%	[157]
Sweet sorghum	215 °C; 2 min	Fructose: 11 Glucose: 10.5	11.2 ± 0.7	50 °C, 48 h	NA	[158]
Corn Stover	48 h	NA	8.09	25 °C, 15 h	37.3%	[159]
Wheat straw	151 °C; 16 min	Glucose: 59.3 Xylose: 55.7	20.8	39 °C, 72 h	NA	[160]
				33 °C, 96 h	55 g/L	[161]

Table 7
Recent studies of hydrothermal pretreatment of LCB for bioethanol production.

Raw material	Pretreatment condition	Reducing sugars (%)	Delignification rate (%)	Fermentation/hydrolysis condition	Hydrolysis yield (%)	Reference
Corn stover	180 °C; 4 min	Glucose: 89 Xylose: 134	52.6	62 °C; 72 h	NA	[172]
Rice straw	40–180 °C; 5–20 min	71.8	NA	50 °C; 72 h	NA	[173]
Switchgrass	180 °C; 60 min	Glucose 90 Xylose 100	50.1	50 °C; 72 h	92	[174]
Corn cob	190 °C; 30 min	NA	54.3	30 °C; 1 h	76	[175]
Corn husk						
Wheat straw						
Eucalyptus and aspen hard- wood	170 °C; 45–90 min	NA	17.8	100 °C; 10 min	NA	[171]
Corn Stover	140–200 °C; 0–90 min	Xylose: 74.95 Glucose: 83.38	30.7	50 °C; 72 h	90.8	[176]
Palm-oil	185 °C; 30 min	Xylose: 24.0 mg/g Glucose: 3.4 mg/g Arabinose: 9.6 mg/g	NA	37 °C; 48 h	NA	[177]
wheat straw	180 °C; 40 min	Cellulose: 84.15	23.52	50 °C; 48 h.	84.15	[178]
Sugarcane bagasse	180 °C; 20 min	Glucose: 69	77.0	50 °C; 72 h.	60.8	[179]
Bamboo	170 °C; 2h	70	NA	50 °C; 72 h	NA	[169]

influenced by the presence of cross-linking between cellulose and lignin in the original biomass [164]. The severity is an important factor as the hemicellulose removal was effective (up to 97%) in a higher value of severity factor [165].

The mechanism of this method is the removal of the majority of hemicellulose and part of lignin by degrading them into soluble fractions, resulted in disruption of cell wall recalcitrant structure [166]. This happens by transferring the structural components from the water-insoluble phase to the soluble phase. However, the formation of inhibitors may occur to the microorganisms during AD due to the toxicity of the degraded products [167]. For instance, the hydrothermal pretreatment was used for safflower straw at three different temperatures 120, 150, and 180 °C As a result, cellulose and hemicellulosic monomers were obtained as main solid and liquid polymers, respectively. The highest biofuel yield was 191.4 NmL/g VS at the least severe conditions 120 °C for 1 h. They concluded that hydrothermal pretreatment improves biogas yield at low severity pretreatment conditions [168].

Alkaline LHW was used for the pretreatment of recalcitrant structures of bamboo. The results indicated that pretreatments removed lignin with the change of cell wall structure. In addition, the surface area increased after LHW pretreatment but further removal of lignin and hemicellulosic fractions was decreased [169]. The LHW increased the hemicellulose conversion and destroyed the rigid structure of sugarcane bagasse for the higher yields of

enzymatic hydrolysate with total sugars yield of 53.65 g/100 g of raw material, equal to 90.13% of glucose [170]. LHW followed by mechanical extrusion is a promising chemical-free pretreatment for cellulosic ethanol production from rigid hardwood. Extending LHW extraction time from 45 to 90 min, resulting in the subsequent mechanical deconstruction of woody biomass significantly facilitated without loss of the carbohydrates [171]. After LHW, the cell wall structure changed and surface area increased but further removal of lignin and hemicellulosic fractions was decreased [169]. Table 7 shows recent studies of bioethanol production from LCB using LHW pretreatment.

5. Biological methods

Biological pretreatment (BP) is one of the most significant methods for the treatment of LCB for bioethanol production. This method mostly utilizes fungal and bacterial strains or their enzymes. This method is drawing more attention due to its ability to operate in the relatively short reaction time and needs the low nutrition requirement for the enzymatic reactions [180–182]. Some bacteria (such as *Clostridium* sp., *Cellulomonas* sp., *Bacillus* sp., *Thermomonospora* sp., *Streptomyces* sp. etc.) and several fungi (such as *Phanerochaete chrysosporium*, *Trichoderma reesei*, *Trichoderma viride*, *Aspergillus niger* etc.) have been widely used in biological pretreatment process [54,104]. The main objectives of biological

Table 8
Recent studies in BP of LCB for bioethanol production.

Raw material	Microorganism/ enzyme	Pretreatment condition	Reducing sugars	Delignification rate (%)	Fermentation/ hydrolysis condition	Bioethanol yield (g/L)	Reference
Rice straw	<i>T. reesei</i> Aq-5b and <i>T. viride</i> NSW-XM	28 °C; 2–4 days	22.74 g/l	NA	30 °C; 48 h	2.17	[195]
Rice straw	<i>Saccharomyces cerevisiae</i>	25 °C; 30 min	NA	NA	30 °C; 24 h	0.24	[196]
Wheat straw	White-rot fungus <i>Irpex lacteus</i>	121 °C; 20 min	11.5%	Straw 45.8	50 °C; 94 h	Straw:12.5	[197]
Corn Stover				Corn 42.3		Corn: 13.5	
Rice straw	Alkalophilic fungus MVI.2011	27 °C; 14 days	24.95%	25	50 °C; 8 h	NA	[198]
Paddy straw	<i>Trametes hirsuta</i> MTCC 136	30 °C	52.91 g/l	71.34	50 °C; 24 h	0.86	[199]

pretreatment are creating the more susceptible structure of cellulose to an enzymatic or microbial attack through disrupting its crystalline structure, breaking and removing the lignin seal, and minimizing the loss of carbohydrates. Therefore, by using cellulolytic and hemicellulolytic microorganisms, the cellulose and hemicellulose are usually hydrolyzed into monomeric sugars [55]. The most important benefits of biological methods include no chemical recycling after pretreatment, low downstream processing costs, minimum inhibitor formation, simple operating and low energy consumption [183,184]. However, the extremely low rate of hydrolysis is the main obstacle in developing BP methods [38].

Besides, bacteria and fungi can generate oxidative and hydrolytic enzymes which can breakdown the rigid structure of LCBs (enzymatic method). *Cellulase* is the most commonly applied enzyme followed by β -glucosidase and xylanase. Hemicellulose and cellulose can be degraded by anaerobes ((*Bacteroidetes* and *Firmicutes* (especially *Clostridia*)) [185]. Based on the previous studies, enzymatic pretreatment improved the methane yield to more than 100%, hence, the effectiveness of the enzymatic pretreatment is determined by different factors [186,187].

Pretreatment with fungi requires long incubation time (from weeks to months), while bacterial and enzymatic pretreatments need a few hours to be completed. Fungal pretreatment, especially with white-rot fungi (WRF) decreases the recalcitrance structure of LCBs and improve the enzymatic digestibility at low temperatures [56,188,189]. WRF with a high selective lignin degradation over cellulose loss plays a crucial role in fungal pretreatment [190]. As softwood is more recalcitrant than hardwood, the fungal pretreatment was more effective. For example, WRF species (*Trametes versicolor*) was used for BP of some cereal crops such as wheat, rye, and barley before anaerobic co-digestion of cow manure which improved the cellulose degradation up to 80% [191]. In the recent study, fungal pretreatment improved the enzymatic digestibility of corn Stover, hardwood, softwood, and *miscanthus* by increasing the glucose yield and the xylose yield [192]. In another study, pretreatment of sugarcane bagasse with different WRF species increased the lignin degradation and the extracted *cellulases* from brown-rot fungus were effective in biomass hydrolysis into reducing sugars [193]. By using *Ceriporiopsis subvermispora* strain, the biomass digestibility and sugar yield of wheat straw increased up to 60% and 44% after ten weeks pretreatment, respectively [194]. Table 8 shows recent studies of bioethanol production from LCB using BP.

6. Combined methods

Based on the drawbacks of single pretreatment methods, researchers have been trying to combine these methods to overcome the problems and increase efficiency. Since many years ago, many studies have been carried out by a combination of various pretreatment methods. For example, the main disadvantage of fungal pretreatment is a long operation time. In this regard, combining the

fungal pretreatment with other physical and chemical methods is a way to overcome this drawback [58]. For instance, the olive tree was treated by fungal and dilute acid pretreatment resulted in increases in sugars recovery to 51% and the enzymatic hydrolysis yield enhanced 34% compared to the acid pretreatment alone [200].

In another study, the yield of xylose and glucose yield increased by 34% and 12% respectively when hydrogen peroxide combined with the steam-pretreatment. The yield of arabinose and mannose has not been increased significantly. Hence, the hydrogen peroxide not improved the formation of lignocellulose-derived by-products throughout the pretreatment process [201]. Recently, Zhang et al., [218] used microbial combined dilute acid pretreatment and microbial combined dilute alkaline pretreatment for water hyacinth biomass to increase the ethanol production yield. They obtained 430.66 mg/g reducing sugars and 1.40 g/L bioethanol which had increased by 9.2% and 8.6% in comparison with each method alone. It can be explained as microbial pretreatment was effective in delignification and then, the dilute acid could breakdown the hemicellulose part to increase the sugar and ethanol production rate [202].

Bacteria-enhanced dilute acid (BE-DAP) pretreatment using *Cupriavidus basilensis* B-8 and dilute acid pretreatment (DAP) using H_2SO_4 was studied for pretreatment of rice straw. The results showed that DAP increased rice straw surface area and the structure became penetrable to facilitate access of bacteria to inner cellulose. Therefore, enzymatic hydrolysis and digestibility of biomass increased by 70% using BE-DAP compared to DAP alone [203]. In the recent study, alkaline hydrogen peroxide is used due to its effectiveness for a wide range of LCBs with high efficiency of enzymatic hydrolysis when used alone or combined [204]. Table 9 shows recent studies of bioethanol production from LCB using combined methods.

7. Pretreatment limitations

Various pretreatment methods are conducted to overcome the strong natural recalcitrance of LCBs by identifying the restrictive factors on their enzymatic hydrolysis. The most significant criteria for effective pretreatment have been listed as follows [55]:

- Preserving a high amount of sugar storage;
- Avoiding the formation of inhibitory;
- Requiring low energy;
- Cost-effectiveness process;
- An eco-friendly process as it uses a minimum amount of chemicals and therefore produces less harmful by-products;
- Producing a high amount of carbohydrates and less lignin;
- Keeping the original biomass size.

Although various methods have been developed for the LCB pretreatment such as microwaves, acid, alkaline, IL, organic solvent, steam explosion, hydrothermal and biological, application of these

Table 9
Recent studies using combined pretreatments of LCB for bioethanol production.

Raw material	Combined pretreatment	Pretreatment condition	Reducing sugars	Delignification rate (%)	Fermentation condition	Bioethanol yield	Reference
Rice straw	Microwave-alkali-acid	28 °C; 14 days	8.11 g/L	50.65	30 °C; 24 h	0.38 g/g	[205]
Rice straw	<i>M. indicus</i> fungus + NaOH	93 °C; 10 h	Glucose: 55 g/L Xylose: 48.6 g/L	NA	32 °C; 24 h.	67.3%	[206]
Sugarcane bagasse	HC-assisted alkaline hydrogen peroxide	60 °C; hydrogen peroxide concentration (0.2–1.0%v/v)	Xylose: 38 g/L Glucose: 80 g/L	63.3	70 °C; 20 min	0.49 g/g	[207]
Corn Stover <i>Cynara cardunculus</i> wheat straw	Ethanol-water + diluted sulfuric acid	130–170 °C; 60–90 min	50–60%	30–66	50 °C; 72 h.	NA	[208]
Wheat straw	Phosphoric acid + hydrogen peroxide (PHP)	40.2 °C; 2.9 h H ₃ PO ₄ (85%, w/w) H ₂ O ₂ (30%, w/w)	NA	70.8	38 °C; 96 h	15.5 g	[209]
Wheat straw	Alkaline + steam explosion	151 °C; 16 min	Glucose:59.3% Xylose:55.7%	20.8	50 °C; 72 h	54.5 g/L	[161]
Corn Stover	Alkaline organosolv	20–100 °C; 0.5–3.0 h	29.5 g/L	NA	NA	0.5 g/g	[210]
Sugarcane bagasse	Liquid hot water + disk milling	121 °C; 1 h	Glucose: 0.392 g/g Xylose: 0.132 g/g	47	50 °C; 72 h	79.6%	[211]
Wheat straw	Alkaline + alkaline peroxide	50 °C; 7 h	NA	0.5–3.4	30 °C; 24 h	31.1 g/L	[72]
Wheat straw	autohydrolysis pretreatment + alkaline post-extraction	140 °C; 40 min	NA	21.6	50 °C; 72 h hydrolysis 83.7%)	NA	[212]
Bamboo	Alkaline pre-extraction + alkaline hydrogen peroxide	100 °C; 30–180 min	Glucose: 17.6 g/L Xylose 8.5 g/L	62.9	30 °C; 24 h	4.6% w/v	[213]
Sunflower stalk	IL and alkali	90 C; 2 h	Xylose: 16.1%	NA	30 °C; 72 h	0.125 g/g	[214]
Olive tree	Fungal and dilute acid	120 °C; 15 min	Glucose: 9.9%	10.3	50 °C; 72 h	NA	[200]

Table 10
Limitation and suggestion for different pretreatments methods.

Pretreatment type	limitations	Recommendation	Reference
Organic solvent	Low biomass recovery rate	Optimizing the pretreatment conditions such as pretreatment temperature, and time, solvent concentration, and catalysts type	[215]
Biological (Fungal)	Increasing costs and contamination due to very long pretreatment time Low downstream yields, loss of carbohydrates	Combined fungal pretreatment can improve enzymatic digestibility and shorten the pretreatment time.	[190,216,217]
Microwave irradiation (MWI)	High cost of pretreatment, Lack of large-scale equipment High energy demand Unknown effect of MWI on different biomass structure	Design the more energy-efficiency microwave systems Investigation the interaction and relationship between MWI and biomass structure The microwave pretreatment reactors should be scaled up	[130,218]
Ionic liquid (IL)	Lack of economical solutions to ILs recycling	Conduct pilot scale research rather than only lab scales Usage of suitable IL in terms of interaction with biomass It is necessary to find the new and innovative methodologies for reusing and recycling ILs	[60,90]
Acid and Alkaline	High cost Harmful by-products Requirement of intensive energy	Elucidation of structure and other characteristics of biomass	[26]

methods at an industrial scale requires overcoming many barriers. From the literature, each method has some limitations and then recommendations are suggested as shown in Table 10.

8. Existing gaps

Based on the review of the literature, all these methods have their own advantages and drawbacks; therefore, one method is not suitable for all types of biomass. It shows that still there is a lack between novel findings and practical applications of different pretreatment methods. Hence, there is a need to evaluate the efficiency of various pretreatment processes, different biomass structure, composition and the relationship between the composition of LCB and the suitable pretreatment according to their different composition. In addition, there is a lack of fundamental studies to increase understanding the effect of each pretreatment

on a structural and molecular level. It should be noted that the investigation of interacts with the subsequent hydrolysis and fermentation for different LCBs needs more research in the field of interdisciplinary to alter the structure of LCB fundamentally.

The process modeling and simulation by using computational tools can develop economic aspects with the help of process optimization. To establish reliable pretreatment, it is necessary to investigate the physical and chemical reaction mechanisms in more detail. Meanwhile, new pretreatment methods cannot be implemented in a large scale yet. The efficiency of biological pretreatment as a potential method for the improvement of the digestibility is still unclear due to complicated microorganism structure. In the end, there is a lack of research to explore the application of different substrates in full-scale and possible by-product applications.

9. Conclusion and future recommendations

In this paper, the most applicable pretreatment technologies on LCBs such as chemical, physical, biological and combined methods for bioenergy production have been reviewed. Based on the finding, even every single method has its advantage and disadvantages, but more research is needed to find an efficient combination of the existing methods. In addition, the pretreatment efficiency can be enhanced by doing more research on either the mechanism or beneficial application of generated by-products. Although, the pretreatment procedure should be optimized in terms of energy consumption efficiency and eco-friendly process.

Therefore, the trend of future research should be directed to address some issues including the increase in the commercialization on a large scale by analysis of economic aspects and application of a suitable method based on the different LCB structures. Finally, the design of suitable research in order to find an efficient combination of the existing methods is recommended.

Declaration of competing interest

All the authors confirmed that there is no conflict of the interest.

CRediT authorship contribution statement

Shahabaldin Rezania: Writing - original draft, Writing - review & editing, Data curation. **Bahareh Oryani:** Writing - review & editing. **Jinwoo Cho:** Conceptualization, Supervision, Validation. **Amirreza Talaiekhazani:** Writing - review & editing. **Farzaneh Sabbagh:** Data curation, Writing - original draft. **Beshare Hashemi:** Software, Data curation. **Parveen Fatemeh Rupani:** Writing - review & editing. **Ali Akbar Mohammadi:** Formal analysis.

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