Egypt. J. Chem. Vol. 67, SI: M. R. Mahran, pp. **2053 - 2072** (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

A comprehensive overview of lignocellulosic biomass exploiting for sustainable bioethanol production: recent advances and emerging challenges towards commercial implementation

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Abstract

The expanding global population and industrial activities are driving an increasing demand for fossil fuel-based energy sources, threatening global energy reserves. This necessitates an urgent shift towards reliable energy alternatives that meet rising needs sustainably. Biofuels have emerged as a promising option, meeting environmental standards with zero carbon dioxide emissions. Bioethanol, an extensively studied biofuel, is already integrated into the energy market sector, primarily produced through fermenting edible crops like sugarcane and molasses, marking the first generation of bioethanol production. Economic and food security concerns are now prompting a shift towards second-generation bioethanol production, utilizing raw lignocellulosic materials. This transition offers a dual benefit by addressing waste materials production costs and environmental impacts. However, significant challenges hinder the widespread commercialization of bioethanol from lignocellulosic feedstocks. Overcoming these obstacles requires holistic approaches that comprehensively address key challenges at every stage of bioethanol production, incorporating recent advancements in the field, as detailed in this review.

***Keywords*:** lignocellulosic biomass, bioethanol, waste pretreatment, yeast, and fermentation**.**

1. Introduction

The increasing presence of pharmaceutical contaminants, such as antibiotics, in water sources presents a significant environmental and public health challenge[1, 2]. Fuel deprivation is currently considered one of the main challenges facing humanity. Over the past few decades, the continued reliance on non-renewable fossil fuels has drastically increased their depletion rate to a very critical level. It was estimated that the global reserve of fossil fuels (oil, coal, and gas) has been significantly affected. In 2009, Shafiee and Topal expected a complete depletion of fossil fuels after 35, 107, and 37 years for the three types (oil, coal, and gas), respectively [1]. This alarming fuel shortage crisis asserted the urgent need for new energy sources. On the other hand, this intensive application of fossil fuels increased carbon dioxide (CO_2) emissions, the main greenhouse gas, adversely impacting the environment through climate change [2]. Hence, extensive research was directed toward overcoming this challenge by exploring several fuel alternatives [3–5]. The new energy source exploration is generally underlined through three main criteria: efficiency or energy equivalence, which expresses the power capacity concerning the production cost. The other two factors include the sustainability and eco-friendly nature of the newly developed energy sources, which target continuous production with little to zero CO_2 gas emissions [6,7]. Several energy alternatives represent promising candidates with growing interest and share in the energy market, including solar, wind, geothermal, and biofuel energy sources [3,8,9]. Among others, biofuel (fuel produced through biomass) largely fulfills the efficiency criterion of the required energy alternative with lower dependence on the large capital investment for their production [7]. Several biofuel sources are currently available, including bio-hydrogen and biodiesel, whereas bioethanol is the most extensively studied and applicable among all [10,11].

Bioethanol (C_2H_5OH) production has been around for centuries, originally for human consumption. However, in recent decades, the focus has shifted towards using edible grains for energy applications, known as the first generation of bioethanol production, pioneered by the United States of America (USA) and Brazil [12]. Due to economic and environmental reasons, bioethanol production has been revolutionized through the worldwide availability of LCB [13]. This shift in bioethanol production (second generation) greatly abridged the production cost and environmental

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Receive Date: 27 August 2024, Revise Date: 16 September 2024, Accept Date: 01 October 2024
DOI: 10.21608/ejchem.2024.312880.10268

impacts of the accumulated LCB [3]. The annual output of agricultural LCB was estimated to be 180-200 billion tons, mainly accumulated in the environment [14]. A minor share of those wastes is usually recycled, mainly in paper manufacturing and animal stock feeding (8 billion tons). At the same time, a large portion is directly burned, resulting in huge amounts of CO_2 emissions that severely impact the environment with a direct share of greenhouse gas accumulation [11,15]. Hence, the widespread availability of LCB presents an opportunity for cost-effective production of various value-added products, particularly in biofuel production [11,13].

The choice of fermentation organism directly affects bioethanol production. Yeasts, especially *Saccharomyces cerevisiae*, are commonly used due to their high yield and tolerance to ethanol accumulation [3,16]. Other organisms are also involved in bioethanol production, including *Mucor indicus*, *Zymomonas mobilis*, and *Escherichia coli* [17,18]. On the other hand, the bioethanol recovery processes also affected the final yield and cost. Ethanol is usually recovered through distillation in a separate batch strategy, as generally described in **Fig. 1** [19], as described in **Fig. 1.** However, other approaches were recently developed based on membrane separation, including pervaporation and adsorption, due to the inability of conventional distillation method to fulfill continuous and simultaneous extraction requirements [18].

Accordingly, many factors interfere with the bioethanol production process, including the waste treatment process, type of cells, fermentation strategy, and applied recovery approach. These factors ultimately impact the final bioethanol yield. Therefore, the current review is dedicated to exploring the main factors affecting the whole bioethanol production process, referring to the advances in each field to fully comprehend the integration of the production process for better and maximum bioethanol production.

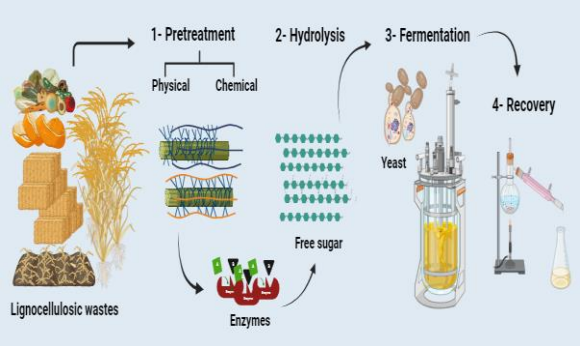

Figure 1. A general scheme for the four steps of bioethanol production from LCB includes pretreatment, waste hydrolysis, fermentation, and bioethanol separation or recovery.

1. Lignocellulosic Biomass (LCB)

Biomass wastes were utilized as an alternate and sustainable substrate for bioethanol production, aiming to reduce bioethanol production costs, environmental impact, and greenhouse gas emissions. Biomass, a non-fossil organic material derived from different sources such as agricultural, industrial, and food wastes with intrinsic chemical energy, could be a feasible energy alternative [20,21]. Biomass encompasses all living matter and is classified into two categories: LCB and non-lignocellulosic biomass [22]. LCB is a renewable and most abundantly underutilized biomass feedstock globally assessed at 200 billion tons per year, of which only 3- 5% has been used in pulp and animal breeding fields (wood, logging residues, and trees), waste materials (agricultural wastes, crop residues, wood wastes, urban waste, etc.), and aquatic biomass (algae, water weed, water hyacinth). Currently, LCB supports a sustainable production base for diverse value-added products such as enzymes and other platform chemicals [23]. There are six major groupings of LCB: crop residues (corn stover, corn cobs, rice husks, barley husks, rye straw, oat straw, rice straw, wheat straw, corn stalks, cotton stalks, soya stalks, sugarcane bagasse), hardwoods (eucalyptus, acacia, poplar, black locust), softwoods (salix, spruce, pine), cellulose wastes (newspaper, waste office paper, recycled paper sludge), herbaceous biomass (alfalfa stems, switch grass), and municipal solid wastes (food wastes and kraft paper) as shown in **Fig. 2** [24,25].

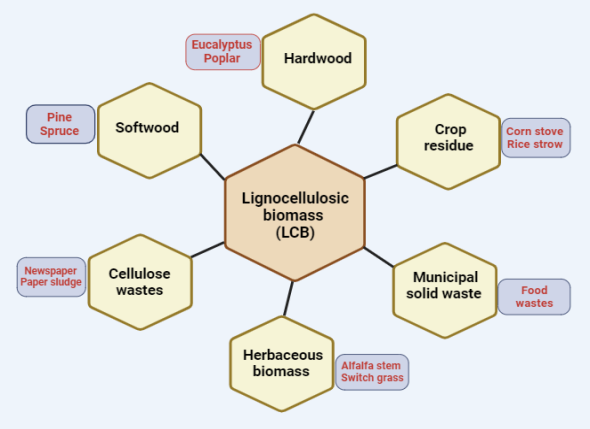

Figure 2. Common types of LCB from diverse agriculture and industrial wastes [26].

The LCB structure is comprised of complex polysaccharides, including cellulose (a crystalline polymer made up of glucose), hemicelluloses (a complex amorphous polymer with an essential component as a xylose monomer units), and lignin (a large polyaromatic compound). The three polysaccharide types are tightly connected through covalent and hydrogen bonds, resulting in a tough cell wall structure that is highly resistant to hydrolysis as showed in **Fig. 3**. Typically, LCB is composed of approximately 40-50% cellulose, 25- 35% hemicelluloses, and 15- 20% lignin, with small quantities of other substances like ash, proteins, and pectin. The composition of these major components of LCB varies based on biomass type and geographical distribution [27,28].

2.1. Cellulose

Cellulose is the most common, renewable, and biocompatible natural polymer on the planet and generally originated as the primary component of plant cell walls. As represented in **Fig. 3**, it is a linear polymer formed via β-(1 → 4) glycosidic bonds of D-glucose subunits [29] with a general chemical formula of $[(C_6H_{10}O_5)n]$. It is extensively available and can be obtained from various sources, such as wood, bacteria, and algae. There are numerous varieties and configurations of celluloses whose chemical and physical characteristics are greatly dependent on the biosynthesis route and the extraction process [30], such as cellulose

nanocrystals [31], cellulose nanofibers [32], bacterial nanocellulose [33], and microfibrillated cellulose [34].

2.2. Hemicellulose

Hemicellulose is a wide family of polysaccharides originating in the primary and secondary cell walls of terrestrial plants, freshwater plants, and some seaweeds. It is a comparatively complicated structure of dissimilar polysaccharides, including xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan, with the chemical formula [$(C_5H_{10}O_5)n$] as shown in **Fig. 3**. It is the second-largest renewable part of LCB, following cellulose, with an annual global production of approximately sixty billion tons [35,36]. It is used in the production of various materials (emulsifiers, films, hydrogels, etc.) and fine chemicals (xylitol, bioethanol, and furfural, etc.) for food, medical, and energy storage applications [37,38].

2.3. Lignin

Lignin is a complex aromatic polymer located mainly in the secondary cell walls of plants and trees that constitutes the majority of biomass, together with cellulose and hemicellulose. It constitutes the most abundant non-carbohydrate fraction in lignocellulose [39]. It consists of monolignol units **(Fig. 3)** created when phenylalanine reacts via the phenylpropanoid pathway. During this reaction, three distinct monolignols are produced, namely p-hydroxyphenyl, guaiacyl (4-hydroxy-3-methoxyphenyl), and syringyl (3,5-dimethoxy-4-hydroxyphenyl). These aromatic alcohols are polymerized by a free radical mechanism, forming different types of ether bonds (C − O − C) and carbon-carbon (C − C) bonds [40].

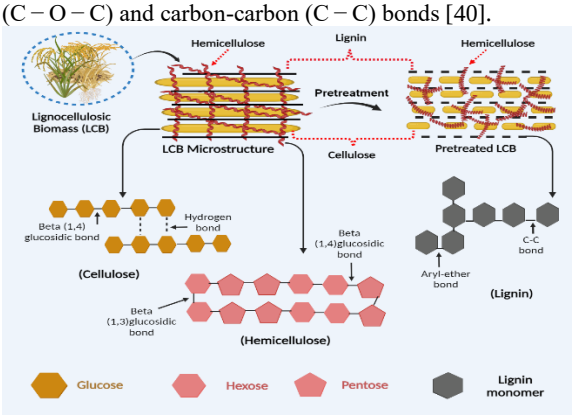

Figure 3. A simplified illustration of the chemical structure of LCB, showing cellulose, hemicellulose, and lignin [28].

2. Valorization of LCB for bioethanol production

Exploitation of LCB as a sustainable source for bioethanol production relies upon four main steps**,** including pretreatment, hydrolysis, fermentation, and recovery as represented in Table 1. Each step comprises possibilities for second-generation bioethanol production [41], impacts the final bioethanol yield. A comprehensive exploration of each production step's nature, significance, and limitations would improve the production process for enhanced bioethanol yield.

3.1. LCB pretreatment approaches

From an economic perspective, pretreatment is one of the most important steps in LCB conversion to second-generation bioethanol. The main objectives of pretreatment are to decrease the crystallinity of cellulose, increase the biomass surface area, and break down the lignin seal in LCB (**Fig. 1**), thus optimizing the conditions for efficient hydrolysis of pretreated LCB [42]. Appropriate pretreatment techniques ensure efficient enzymatic saccharification, improving overall production efficiency [43]. Consequently, pretreatment should fulfill the following requirements: (1) improve sugar yield or the ability to form sugars through enzymatic hydrolysis; (2) eliminate the degradation of carbohydrates; and (3) ensure economic feasibility [27,41].

3.1.1. Chemical pretreatment approach

Chemical pretreatment of LCB is essential for effective biological conversion in subsequent steps to produce bioethanol. During this process, lignin is effectively solubilized, and hemicellulose is effectively dissolved [44,45]. Chemical pretreatments typically aim to alter the crystal structure of cellulose and eliminate the hemicellulose and lignin fractions from LCB using various chemical agents such as acid, alkali, solvents, and other oxidizing compounds as illustrated in Table 1 [44].

3.1.2. Physical pretreatment approach

The physical pretreatment methods reduce the particle size of LCB feedstock, increasing surface area and leading to higher LCB degradation than chemical pretreatment. Physical methods commonly used for industrial-scale LCB pretreatment include milling, microwave pretreatment, and ultrasonication [46]. Physical pretreatment reduces cellulose crystallinity and polymerization level while increasing surface area, mass transfer capacity, and biomass hydrolysis productivity [47].

3.1.3. Physico-chemical pretreatment approach

One of the most prevalent pretreatment methods is physico-chemical, which employs chemical and physical procedures for efficient lignin removal and cellulose crystallinity reduction in treated LCB [48]. This approach not only ensures effectiveness, but also solubilizes the LCB structure without creating fermentation inhibitors [49]. The significant simplicity and efficiency of physical and chemical approaches encourage their commercial implementation in the industrial-scale level for LCB treatment. However, the two methods' application has several environmental and economic drawbacks. While effective in reducing particle size and increasing biomass surface area, physical methods can be energy-intensive and costly, potentially raising operational expenses. Chemical pretreatments, though efficient in solubilizing lignin and hemicellulose, often involve hazardous chemicals that pose environmental risks and require careful disposal. Both methods can lead to high operational costs and extended processing times, impacting overall feasibility and sustainability in lignocellulose conversion to bioethanol [50].

Green pretreatment approach

Green pretreatment is an eco-friendly approach with little hazardous chemical use and release for enhanced sugar yields and lignin degradation. These methods include environmentally friendly and energy-efficient processes such as ozonolysis, ionic liquids, deep eutectic solvents, organosolv, and steam explosion [51]. The delignification efficacy of this approach depends on the method applied and the nature of LCB. Ozonolysis pretreatment supported 96% delignification of oil palm biomass [52], whereas deep eutectic solvents and organosolv reported 88% and 67.2%, respectively [53,54].

3.1.4. Biological pretreatment approach

Biological pretreatment is an environmentally friendly and promising method for treating LCB during bioethanol production. It mostly involves using fungal, actinomycetes,

and/or bacterial strains or their enzymes (**Table 1**). Fungal or actinomycete pretreatment of LCB requires a long incubation time (weeks to months), while bacterial pretreatment requires a few hours to days [55]. In a typical biological pretreatment process, the microorganisms can grow on LCB either by submerged culture or solid-state cultivation techniques, which then multiply and carry out their metabolism [56,57].

Table 1. Comparative study on advantages and disadvantages of different LCB pretreatment strategies, including chemical, physical, green, and biological approaches, with some examples.

Method	Example	Advantage	Disadvantage	Reference
Chemical	Sodium hydroxide (NaOH)	- Delignification - Decrease of the cellulose crystallinity and polymerization - Partial or complete solubilization of hemicelluloses.	- High cost of chemical catalysts and corrosive-resistant- equipment - Alteration of lignin and inhibitor formation	[58]
	Ammonia (NH_3)			[48]
	Hydrochloric acid (HCl)			[59]
	Sulfuric acid (H_2SO_4) and Phosphoric acid (H_3PO_4)			
Physical	Ball-milling	- Decrease of the cellulose crystallinity and polymerization - Partial hydrolysis of hemicelluloses and lignin - No toxic materials generated	- High energy costs - Low efficiency for lignin removal	[60]
	High pressure steaming			[61]
	Pyrolysis			[62]
	Microwave irradiation			[63]
Physico-chemical	Steam explosion	- Increases accessible surface area - De-crystallizes cellulose - Removes hemicellulose and lignin	- High energy/water input - Toxic compound generation	[64]
	Liquid Hot Water (H_2O)			[65]
	Ammonia Fiber Explosion			[66]
Green	Ozonolysis	- Efficient method - Assist delignification - No hazardous chemicals or energy consumption	- High cost - Need more processing for solvent recovery	[67]
	Ionic liquids			[68]
Biological through microorganisms.	*Aspergillus*, *Penicillium*, *Trichoderma,*	- Reduction of hemicellulose and cellulose polymerization of - Low energy requirements - No generation of toxic compounds	- Low efficiency for lignin removal - Time consumed - Required large space to perform	[69]
	Streptomyces sp, *Micromonospora* sp, *Pseudomonas*			[70,71]

3.2. Trending approaches for LCB pretreatment

As previously mentioned, the pretreatment step is crucial for converting LCB into fermentable sugars for bioethanol production. Conventional pretreatment approaches, such as physical, chemical, physicochemical, and biological pretreatment, greatly impact the cost, constituting approximately 40% of the total processing cost. A novel, efficient, cost-effective, and environment-friendly approach has been developed to address this issue. Therefore, trending pretreatment methods have been listed in this context as nanomaterials, high-pressure homogenization, plasma technology, deep eutectic solvents, and microbial fuel cells.

3.2.1. Nanomaterials

The extensive application of nanomaterials in biofuel production has attracted much attention. Several studies have reported that nanoparticles play an important role in the enzymatic hydrolysis of LCB through the immobilization of various enzymes, such as cellulases, hemicellulases, and laccases, on different nanomaterials [72,73]. The small size of metal nanoparticles is believed to contribute to their enhanced efficiency in penetrating the cell wall of LCB, thus facilitating easy interaction with LCB components and promoting the release of carbohydrates while minimizing the production of cell-wall-derived inhibitors [74]. Magnetic nano-biocatalysts are used as a promising nanomaterial for the immobilization of enzymes and the functionalization of acids. The nano-biocatalysts immobilize enzymes, enabling them to be utilized multiple times for hydrolysis reactions. This repeated hydrolysis contributes to economic efficiency and enhances hydrolysis and sugar recovery by allowing the immobilized enzyme to be reused numerous times [75]. Acid-functionalized magnetic nanoparticles are robust nano-catalysts with strong acid properties, such as nanoscale magnetic particles. These nanoparticles exhibit acid-like behavior, such as the decomposition of LB during pretreatment [74]. Sugarcane bagasse underwent pretreatment using two acid-functionalized magnetic nanoparticles, i.e., alkyl sulfonic acid and butyl carboxylic acid. This pretreatment released significant amounts of sugar, about 18.83 g/L and 18.67 g/L, respectively. It was comparatively high compared to the sugar yield of non-treated biomass [76].

3.2.2. High-pressure homogenization

High-pressure homogenization is a mechanical technique with extensive applications in the food, pharmaceutical, and dairy sectors. It is primarily employed to reduce particle size and enhance the stability of emulsions. The primary impacts

on LCB are the removal of lignin, the decrease in the degree of polymerization, and the reduction in the crystallinity of cellulose [77]. Several researchers have explored high-pressure homogenization as a potential method for pretreating LCB. The household kitchen was homogenized and shredded, producing the highest bioethanol concentrations of 20 g/L [78]. High-pressure homogenization offers numerous benefits, such as the absence of any need for chemicals, the ability to operate at room temperature, its environmentally friendly nature, a shorter processing time, and the lack of inhibitors. In contrast, there are drawbacks to using high-pressure homogenization, which include the high energy requirements associated with high input pressure and the necessity for biomass size reduction [77].

3.2.3. Plasma technology

Plasma is generated by the application of electricity to a gas. Consequently, this ionized gas becomes stimulated, ionized, and dissociated, creating a dynamic setting conducive to forming diverse types of ions, radicals, excited atoms, and molecules, in addition to neutral ground-state molecules [79,80]. Plasma is usually classified as thermal plasma or non-thermal plasma [81,82]. Thermal plasma is characterized by a high energy density and temperature, thereby enabling the coexistence of thermal equilibrium among all neutral and electric particles [83]. Non-thermal plasma detoxified treated wheat and rice straws to reduce inhibitory compounds and acids. This method could reduce the amounts of acetic acid, formic acid, and furfural by 73, 83, and 68% in the resulting hydrolysate [84]. Another investigation reports that the atmospheric cold plasma was adopted to degrade the toxic compounds within H_2SO_4-hydrolyzed sugarcane bagasse. After atmospheric cold plasma treatment, there were significant decreases in the fermentation's inhibitors (31% of the formic acid, 45% of the acetic acid, 80% of the hydroxyl methyl furfural, and 100% of the furfural), which improved the bioethanol productivity from 0.25 to 0.65 g/L/h [85]. The dielectric barrier discharge plasma was used to pretreatment walnut shells to extract micro- and nano-cellulose fibers. The results showed that the plasma application reduced the cellulose extraction efficiency from about 26% to 22%, with a decrease in the C-C/C-H and C-OH/C-O-C bonds [86]. The advantages of plasma technology are short process duration, non-polluting, use of dry gases, mild process conditions, recovery of chemicals and waste treatment that is not required, and no generation of fermentation inhibitors [77].

3.2.4. Deep eutectic solvents

Deep eutectic solvents have been used in biomass pretreatment as delignification agents [87] and for the separation of bioethanol during the fermentation process [88]. Deep eutectic solvents are mixtures of at least two components with high melting points that, when mixed at a specific ratio, become liquid and stable at room temperature. The components of the deep eutectic solvents are a hydrogen bond acceptor, which usually consists of quaternary ammonium, phosphonium, or sulfonium cation, or a metal chloride, and a hydrogen bond donor, which can be a carboxylic acid, alcohol, amide, or sugar [89]. In recent years, deep eutectic solvents have been used to enhance the LCB conversion into bioethanol. Pérez-Pérez and his colleagues reported using an eco-friendly method based on microwave-assisted autohydrolysis and deep eutectic solvents to convert *Robinia pseudoacacia* wood to bioethanol. Afterward, different conditions were assessed for the optimal delignification of *Robinia pseudoacacia* wood with the deep eutectic solvents (choline chloride combined with lactic acid), reaching delignification ratios up to 86% to enhance the bioethanol yield to 83% [90]. Another study applied a deep eutectic solvent cocktail (betaine-glycerol and choline chloride-oxalic acid (or choline chloride-acetamide)) for the pretreatment of corn stover to degrade 89.51% glucan and 75.43% xylan, and the bioethanol yield reached 73.35% [91]. Another study reported that approximately fifteen different hydrophobic deep eutectic solvents have been screened by employing a conductor-like screening model of real solvents, with only three deep eutectic solvents (1,2-Decanediol: Thymol, Atropine: Thymol, and Lauric acid: Lidocaine) showing the best separation performance for ethanol [88].

3.2.5. Microbial fuel cell

Microbial bioprocessing of LCB to produce bioethanol is considered a sustainable approach for reducing the depletion of energy reserves and shrinking the carbon footprint**.** Microbial electrolysis cell is a technology that combines biological fermentation and electrochemical technologies. This approach has shown promising applications in waste treatment and has been extensively used in the treatment and preparation of solvents and alcohols [92–94]. Microbial cell technology can be used to enhance the resistance of bacterial strains to inhibitors, which promotes the ethanol fermentation capacities of applied strains. Accordingly, reducing raw material processing costs, improving ethanol production efficiency, and facilitating the one-step fermentation of lignocellulose are promising characteristics of microbial cell technology [95,96]. Wang et al. constructed a microbial electrolysis cell system for bio-oil detoxification and efficient ethanol production using evolved *Escherichia coli* to overcome the bioethanol production and utilization challenges highlighted in previous studies. The *E. coli-H* strain exhibited significantly higher levoglucosan consumption and ethanol production capacities in electrically treated bio-oil media than the control. In non-detoxified bio-oil media containing 1.0% (w/v) levoglucosan, *E. coli*-H produced 0.54 g ethanol/g levoglucosan, reaching 94% of the theoretical yield [95]. Another study reported a co-generation system based on microbial fuel cells and charging batteries. The results demonstrated that the maximum ethanol reached 0.14% (w/v) along with battery charging from 1.09 to 1.17 V [97]. Therefore, new innovations in LCB conversion involve diverse nonconventional LCB pretreatment methods, including nanomaterials, high-pressure homogenization, plasma technology, deep eutectic solvents, and microbial fuel cells. Nanomaterials enhance enzymatic hydrolysis by supporting enzyme immobilization and increasing the interaction of lignocellulose components with enzymes. High-pressure homogenization reduces particle size and crystallinity in cellulose without chemicals, making it eco-friendly. Plasma technology is very effective in removing inhibitors of fermentation and detoxification of hydrolysates, hence assuring maximum bioethanol yield. Deep eutectic solvents are environmentally friendly delignification media that ensure high efficiency and low environmental impact. Hence, as compared to conventional methods, trending pretreatment methods offer significant advancements by enhancing efficiency, reducing environmental impact, and lowering costs. These innovative

approaches surpass traditional methods by optimizing enzyme interactions, minimizing chemical use, and improving overall bioethanol yields [98].

4. LCB hydrolysis (saccharification) process

Hydrolysis of LCB targeted conversion of cellulose and hemicellulose portions into fermentable simple sugars. This process is usually carried out through microbial enzymes, including cellulases, xylanases, β-glucosidase, and other accessory enzymes such as polysaccharides monooxygenases [99,100]. Though acids hydrolysis is also applied for LCB hydrolysis (usually through HCl and H_2SO_4), their adverse effects on the subsequent fermentation process with the corrosive nature of most acids restricted its wide commercial applications and privileged the enzyme hydrolysis approach [101]. Unlikely, the enzymatic hydrolysis process is challenged by several factors including enzyme inhibitors accumulated in the treatment process or during the hydrolysis process from LCB. Polyphenolic compounds and lignin derivatives are common examples of such compounds [102,103]. Accumulated simple sugar also inhibits and reduces activity in most cellulose-degrading enzymes (feedback inhibition). Additionally, the necessity for a diverse enzymatic system (enzyme cocktail) toward complete LCB saccharification imposes a challenge for synergistic activity and conditions prerequisites in applied enzymes [99]. Other physical and technical factors interfering with the hydrolysis results include LCB type, particle size, surface area, and load, which should be optimized for each LCB for maximum saccharification. The high cost of enzymes (about 20% of the total production cost of bioethanol) also interfered with the total bioethanol source, which forced cost-effective enzyme production using the LCB [104]. Therefore, several studies adopted the application of LCB for different cellulose-degrading enzymes. In our group, paper sludge, a waste from the paper manufacturing industry, was applied for economic cellulase production from *Streptomyces rochei* local isolate [33], which was used for bacterial cellulose production from the same LCB (paper sludge). Currently, the one-site production and application is an expression adopted for cellulase production and application for bioethanol production from the same LCB [105]. This strategy improved enzyme production cost and enzyme efficiency for LCB hydrolysis [105].

Additionally, enzyme recycling and immobilization were proposed for the efficient and cost-effective application of applied enzymes [106,107]. Immobilized enzymes could be reused for numerous hydrolysis runs, which is attributed to higher stability and retrieval simplicity compared to free enzymes [107,108]. The enzyme immobilization strategy and applied matrix significantly affected the final enzyme efficiency and the bioethanol process. Among others, immobilization on nanoparticles (NPs) revealed significant applicability regarding enzyme stability and recovery [108,109]. For durable LCB hydrolysis, a cellulosic-hydrolysis enzyme cocktail (Cellic CTec2) was immobilized on magnetic graphene oxide-NPs (GO-MNP-EnZ) and proved successful application for sugarcane bagasse hydrolysis cycle with efficiency of about 80% after several application cycles [110]. However, the free enzymes (cellulases and xylanases) reported a higher conversion rate for sugarcane bagasse in the previous study, and the half-life of immobilized enzymes (GO-MNP-EnZ) was higher. The same enzyme cocktails (Cellic CTec2) proved high reusability potential when immobilized with IOMNP@SiO_2-NH_2 magnetic NPs up to 68.21% and 52.6% conversion rates in the second and third hydrolysis cycles, respectively. In this study, the immobilized enzymes (20 FPU/gm.) revealed a conversion rate of sugarcane bagasse up to 74.19% in the first cycle, which is comparable to free enzyme cocktails (70.71%) at an enzyme dose of 10 FPU/gm. [111].

Likewise, enzyme improvement was a strategic approach for efficient hydrolysis. Wang and his colleagues reported an engineered xylanase/feruloyl esterase (XynII-Fae) with efficient synergistic characteristics when used with commercial cellulase. Applying an enzyme cocktail of XynII-Fae and cellulase (40: 60%), increased reducing sugar production by 65% when compared to individual commercial cellulase [112].

5. Bioethanol fermentation strategies: conventional and nonconventional approaches.

Bioethanol fermentation is a microbial process in which sugar contents (accumulated biomass hydrolysate) are converted into ethanol through certain microorganisms, mainly unicellular fungi (yeasts). This sugar conversion could be mediated in an aqueous environment, known as submerged fermentation (SMF), or in a solid environment, known as solid-state fermentation (SSF).

5.1. SMF *vs.* SSF in bioethanol production

SMF is the conventional technology for bioethanol production [113]. Sugar conversion is mediated in an aqueous environment in one mode, including batch, fed-batch, or continuous fermentation. In the batch mode, the total sugar in hydrolysate is fermented by organisms as one lot. However, the simplicity of batch mode and the initial high sugar concentration significantly increase the osmotic streets upon the applied organism and influence bioethanol production [114]. Additionally, the time and effort wasted in process initiation and closing increased the production process irregularity and cost [115]. Fed-batch and continuous fermentation modes were proposed to overcome this limitation through substrate feeding at appropriate concentrations for maximum bioethanol production. Several studies reported the application of two fermentation modes of enhanced bioethanol production [3,116,117].

On the other hand, the absence of free water is the main characteristic of SSF. Bioethanol-producing organisms are cultivated directly on the wet LCB, eliminating the prerequisite for separate saccharification, wastewater production, and risk of process contamination [113,118]. Furthermore, bioethanol recovery is much easier due to low water content. Prasoulas and his colleagues reported the efficient food waste conversion into bioethanol, about 20.6 g/L, by a co-culture of *Fusarium oxysporum* F3 and *S. cerevisiae* under SSF [119]. Difficulties in production scalability and overall process controls (pH, heat accumulation, and mass transfer) are the main challenges facing the widespread commercial SSF technology [118,120].

5.2. Common strategies for bioethanol fermentation

Two main strategies are currently involved in commercial bioethanol production processes: the conventional separate hydrolysis and fermentation approaches and the

simultaneous hydrolysis and co-fermentation approaches. The two methods are currently applied in the commercial production of bioethanol.

The first strategy **(Fig. 4)** is a conventional and widely applied commercial process based upon the separate fermentation of sugar hydrolysate derived from individual pretreatment and hydrolysis processes [121]. The strategy is simple and offers full control of the production process. However, the high energy implemented and the time wasted between each separate process greatly influenced the final bioethanol cost, forcing the development of other efficient strategies [3].

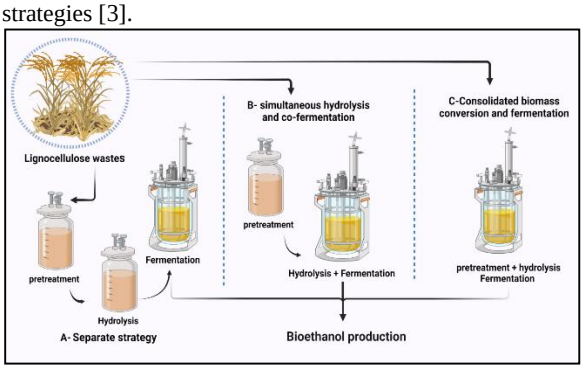

Figure 4. The three strategies involved in bioethanol production are conventional separate approach, simultaneous hydrolysis and co-fermentation approach, and consolidated methodology.

The second strategy involved a simultaneous lignocellulose saccharification and fermentation approach **(Fig. 4)**, which recently arose as a more efficient and less time-dependent method [122]. This strategy aimed to reduce the capital investment and energy consumption for separate lignocellulose hydrolysis as represented in **Table 2**. Additionally, the companion co-fermentation directly enhanced lignocellulosic hydrolysis, sugar fermentation, and ethanol production. Attributed to feedback inhibition of most hydrolytic enzymes involved in cellulose hydrolysis by high sugar concentration, continuous removal of the resulting sugar will maintain the enzymes' activity. Furthermore, the steady supply of liberated sugar will alleviate the osmotic stress of high sugar concentration on the yeast cell and improve the sugar conversion rate [123]. Several studies reported the high bioethanol yield related to a simultaneous strategy compared to a separate one [124,125]. This strategy was applied in fed-batch mode upon pretreated sugarcane bagasse using *Kluyveromyces marxianus* JKH5 C60 and proved an efficient bioethanol production approach. The maximum ethanol titer (73.4±1.2 g/L) was achieved at 24°C using a total solid LCB of 20% and enzyme dose of 20 FPU/g, whereas the bioethanol production efficiency and productivities were 78 % and 3.0, respectively [126]. The optimum temperature difference between hydrolysis enzymes and yeast growth technically challenges the strategy. Generally, lignocellulose hydrolysis enzymes work better at higher temperatures (50-60 °C), which could inhibit the most bioethanol-producing microorganisms [127]. Hence, Panda and Maiti applied the cyclic temperature-shifting strategy to overcome this limitation in their study. They adopted simultaneous hydrolysis of treated rice straw at 30°C for 2 h and fermentation at 40°C for 2 h, which increased the bioethanol production titer to 5.1-fold compared to the conventional separate approaches [128]. A different study reported implementing a temperature-tolerant yeast strain (*S. cerevisiae PE-2* in a bubble column bioreactor for simultaneous saccharification and fermentation process. The applied yeast efficiently grows at 40°C with maximum bioethanol production of 9.31 g/L using the sugar released from hydrothermally pretreated wheat straw (10% waste) hydrolyzed with 15 FPU/g cellulase [129]

A third strategy was proposed for consolidated biomass conversion and fermentation **(Fig. 4).** This process guarantees a lower capital investment and efficient bioethanol production in one fermentation tank (**Table 2**). This strategy is minted to improve every aspect of the process by diminishing labor effort, capital investment, and energy consumption [3,130]. However, despite the promising traits of this strategy and the increasing research dedicated to its implementation, the strategy is still in the research and development stage with no commercial application adoption [3]. In this direction, several improvements should be made to an efficient catalytic system for efficient lignocellulose hydrolysis at low temperatures. Furthermore, strain development for stress tolerance and complete and efficient sugar conversation is mandatory for consolidated strategy applicability [3].

Table 2. A general comparison of three ethanol production strategies illustrates each approach's pros and cons.

Criteria	Separate	Simultaneous	Consolidated
Base	Distinct production step	Combined saccharification and fermentation or/and purification	All production steps in one tank
Level	Commercial	Commercial	R&D level
Prose	- Simple approach - Process full control applicability	- Cost and effort reduction - Higher biomass conversion rate - Higher bioethanol yield	- Proposed Cost and effort reduction to a minimum - Higher bioethanol productivity
Cones	- High capital investment - Tedious labor work	- Difficulties in process control and optimization - Need for more developed enzymes and separation membranes	- Still in the R&D level with mutable challenges

5.3. Strain development for enhanced bioethanol production

Unicellular fungi are the main players in commercial bioethanol production, especially those of the species *S. cerevisiae* [131]. Though other wild and genetically modified strains were also implemented, their low ethanol productivity and fastidious cultivation nature challenged their production applicability [132]. The absence of cellulolytic activity in *S. cerevisiae* inspires the application of genetic engineering technology toward an efficient bioethanol-producing strain. Using CRISPR-Cas9 genetic editing tools, three cellulases/xylanases genes from *Ampullaria gigas Spix* were successfully integrated into the *S. cerevisiae* genome, which enhanced bioethanol production in a simultaneous saccharification and fermentation process without the need for external enzyme addition [133]. Likewise, pentose assimilation and internal ethanol accumulation are two main obstacles facing the *S. cerevisiae* bioethanol production process [134]. The inability of wild-type *S. cerevisiae* to assimilate pentoses from fermentation media restricts the efficient and complete conversion of released sugars into bioethanol. Despite the high tolerance of normal yeast to ethanol, the internal accumulation of produced ethanol in the yeast cells limits their growth and finally leads to cell death. Several attempts were made to explore new yeast cells with better pentose assimilation, like *Pichia kudriavzevii* and *Candida tropicalis* [135,136]. Likewise, another direction was adopted by developing new and more efficient strains through genetic engineering for enhanced pentose assimilation and overcoming the final fed-back inhibition through bioethanol accumulation in the fermentation media [137]. In this regard, *S. cerevisiae* (SF7-Ft3 strain) with xylose consumption ability was constructed by duple insertion of xylose genes (*XYL2*) from *Candida tropicalis* into wild *S. cerevisiae,* which enhanced the bioethanol production from 11- 42% from three LCB compared to the wild strain [138]. In the same direction, six novel stress-tolerance genes were identified in *S. cerevisiae by* Wang and his colleagues [139]. Among the six genes, the overexpression of gene-coded ENA5 greatly enhanced the fermentation performance of *S. cerevisiae* toward cultivation stress, including elevated temperature, high sugar concentration, and ethanol accumulation, which finally enhanced bioethanol production.

On the other hand, a co-culture strategy was proposed as a novel approach for enhanced bioethanol production [140]. In this approach, two or more yeasts were applied to the fermentation medium to overcome the limitation of single-applied strains. This approach improved the bioethanol production from tea industry waste to 21.9 g/L by using a consortium of *Candida boidinii* and *S. cerevisiae,* compared to 12.1 and 14.1 g/L, respectively, when used as single organisms under the same cultivation conditions [140]. Mishra and Ghosh applied *Candida shehatae* and *Zymomonas mobilis* for efficient assimilation of hexose and pentose released from three lignocellulosic was LCB tes (sugarcane bagasse, Kanas grass, and wheat straw) upon fractional hydrolysis. Their results proved more than 90% assimilation for available sugars, with about 82% bioethanol production from the theoretical yield [141]. To sum it all up, the following Table 3 summarizes the recently published data concerning bioethanol production from different LBCs, where we can survey the applied LCB types and their applicability for bioethanol high-yield production.

6. The bioethanol recovery processes and their impacts on final bioethanol yield

Bioethanol production is usually carried out in an aqueous environment, necessitating bioethanol separation as a prerequisite for application. There are three ethanol separation types including distillation, dehydration, and purification are the conventional general steps for commercial downstream of fuel-grade bioethanol as represented in Table 4 [161]. It was estimated that azeotropic ethanol (with 5- 10% water content) from the distillation process consumed about 80% of the total bioethanol downstream process [162]. Normally, dehydration and purification are two additional purification steps required to attain fuel-grade ethanol (anhydrous ethanol) with water content below 1% [161]. The conventional distillation process is based upon the volatility differences between ethanol and water attributed to different pooling boing [163]. The ethanol boiling point is around 78.2°C, which facilitates its vaporization from water. The process is widely applied commercially and accounts for the most energy consumption during ethanol production [161]. Numerous advances were developed toward an efficient and cost-affordable distillation process, including external additives (known as entrainers) to alter the ethanol/water volatility ratio as ethylene glycol, toluene, and diethyl ether, which facilitate the ethanol separation process at lower temperatures [161]. Though different studies reported advanced distillation strategies toward efficient ethanol extraction, including sidestreams, pump-assisted, and multi-effect distillation, energy consumption is still challenging [164,165]. Due to the high cost and time involved in the distillation process, intensive research was directed toward cost-effective and more reliable approaches. Several non-conventional strategies have been reported for bioethanol recovery, including pervaporation and adsorption [161]. The two approaches rely on selective permeability for specific semi-permeable barriers (**Table 4**).

In the pervaporation approach, volatile molecules usually permeate through one side of the selective membrane (upstream side) or liquid feeding side to the other (downstream side) or permeate side. Pressure changing on one side of the applied membrane is necessary, which could be achieved through purging gas at the membrane's upstream side or vacuum force at the downstream side [166]. One of the critical points in the pervaporation process is the membrane material, which directly influences the extraction efficiency and required energy [167]. Several organic and inorganic polymers were recently proposed for efficient bioethanol extraction, including polyvinyl alcohol/graphene oxide membrane [167], polyacrylic acid-grafted carbon nitride, and polyvinyl alcohol [168]. In our research group, a membrane of sulfonated polyvinyl chloride with poly (2-acrylamido-2-methyl-1-propanesulfonic acid) proved an efficient capability for bioethanol separation, whereas the bioethanol permeate flow was 253.06 g/m^2 h, which was around that of laboratory ethanol (289.54 g/m^2 h) used as a control [18].

Table 3. Summary of different LCB applied for bioethanol production regarding geographical location, treatment approach, and fermentation strain with optimized maximum bioethanol yield.

No.	LCB	Location	Pretreatment	Hydrolysis	Strain	Maximum yield	Reference
1	Rice Straw	Egypt	Hot water, dilute H_2SO_4, and acid (H_2SO_4)/alkali (NaOH)	Enzymatic by Cellulase (89.4 FPU/ml, Sigma)	*Pichia occidentalis* AS.2	23.7 g/L	[142]
2	Sugarcane Bagasse	Egypt				21.4 g/L	
3	Rice Straw	Republic of Korea	Popping pretreatment (direct heat and direct open at high temp.	Enzymatic by cellulase and xylanase	*S. cerevisiae*	0.177 g/g	[143]
4	Pine Chips	China	Hydrotropic p–toluenesulfonic acid mixed with pentanol	Enzymatic by Cellic® CTEC 2 (15 FPU/g-glucan)	*S. cerevisiae*	225 g kg^{-1}	[15]
5	Palm Fronds	Tunisia	Chemical (dilute H_2SO_4) and hydrothermal (Freezing/Thawing)	Novozyme: Cellic H-Tech 2 (2 mg protein/mL)	*S. cerevisiae*	41.29%	[144]
6	Eucalyptus Chips					26.61%	
8	Almond Shells					16.54%	
9	Aleppo Pinecones					18.1%	
10	Corn Stover	Egypt	Chemical (maleic acid ($C_4H_4O_4$) and citric acid ($C_6H_8O_7$))	Cellulase (20 FPU/g)	*S. cerevisiae*	10 g/L	[145]
11	Corn Stover	China	Physico-chemical (Steam explosion)	Novozyme: cellulase (145±5 FPU/mL)	*S. cerevisiae*	51.12 g/L	[146]
12	Wheat Straw	China	Untreated	Xylanase and cellulase-producing Streptomyces strain	*S. cerevisiae*	10.8 g/L	[147]
13	Wheat Straw	Iran	Robust pretreatment (Ultrasound irradiation and ionic liquid)	Cellulase (56 FPU/mL) (CelluMax GFL-)	*S. cerevisiae* PTCC 5052	42.0 g/L	[148]
14	Sugarcane Bagasse	Taiwan	Chemical (dilute H_2SO_4 and HCl)	Acid hydrolysis by 1.84 N H_2SO_4	*Kluyveromyces marxianus* K21	18.01 g/L	[59]
15	Corn Cops	Indonesia	Biological by fungi (*Dekkera Bruxellensis*)	Acid hydrolysis by 2% H_2SO_4	*S. cerevisiae*	2.95 %	[149]
16	Newspaper	Indonesia	Soaked in H_2O	Acid hydrolysis by 0.1 N HCl	*S. cerevisiae*	9.47 %	[150]
17	Cassava Peels		Grinding				
18	Rice Husk	Nigeria	Hot water and dilute (HCl, NaOH, and $FeCl_3$)	Cellulase obtained from *Trichoderma reesei* ATCC 26921	*S. cerevisiae*	3.80 %	[58]
19	Rice Bran	Brazil	Grinding	Enzymatic by protease, α-amylase, and amyloglucosidase	*S. cerevisiae*	2.42 g/L/h	[151]
20	Hardwood	Republic of Korea	Chemical by hydrogen peroxide (H_2O_2)–acetic acid (CH_3COOH)	Cellulase and xylanase from *Trichoderma longibrachiatum*,	*S. cerevisiae* and *Pichia stipitis*	0.32 g/L/h	[152]
21	Softwood	Canada	Chemical by sodium sulfite (Na_2SO_3) and sulfur dioxide (SO_2)	Cellulase (Cellic Ctec-3-Novozyme) (171.7 FPU/ml)	*S. cerevisiae T2*	80 g/L	[153]
22	Sweet Sorghum	Ethiopia	Mechanically extracted from Sweet Sorghum stalks/stems	ND	*S. cerevisiae*	18.76 %	[154]
23	Nut Shells	Morocco	Physical by Ultra Turrax homogenizer	Cocktail from cellulase, hemicellulase, arabinase, β-glucanase, and xylanase)	*S. cerevisiae* ATCC 7754	45.25%	[155]
24	Grasses	South Africa	Chemical by acid mine drainage and H_2SO_4 acid	Celluclast 1.5 L cellulase enzyme (Novozyme)	*S. cerevisiae*	80 %	[156]
25	Banana waste (pseudostem and rachis)	Spain	Treated by acid (H_2SO_4)-catalyzed steam explosion	Novozyme (16.0 and 14.9 FPU.g^{-1})	*S. cerevisiae*	112 L/T and 103 L/T	[157]
26	Banana Peels	Mexico	Grinding	Cellulolytic complex Celluclast 1.5 L (15 FPU/g) (Novozyme)	*S. cerevisiae* and *Kluyveromyces marxianus*	32.6 g/L	[158]
27	Wheat Bran	Germany	Biological by *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, *Trichoderma* and fungal consortium	Microbial hydrolysis by *Aspergillus niger, Penicillium chrysogenum*, *Trichoderma viride*	*S. cerevisiae, Kluyveromyces marxianus* and *Zymomonas mobilis*	7.6 %	[69]
28	Peanut Shells	India	Chemical by (NaOH, HCl, H_2SO_4), steam explosion, and alkali steam-assisted pre-treatment	Microbial hydrolysis by *Aspergillus niger*	*S. cerevisiae* and *Zymomonas mobilis*	1.96 and 1.79 g/g	[159]
29	Orange Peel	Egypt	Grinding and heating	Dilute HCl (10%)	*Bacillus subtilis* and *Pseudomonas aeruginosa*	92.25 and 82.70 g/L	[160]

Additionally, membranes based on Polydimethylsiloxane are gaining increased interest in efficient alcohol separation [169]. The pervaporation membrane of Polydimethylsiloxane/polyamide (PDMS) was recently coupled with bioreactor fermentation of cassava hydrolysate. The sugar hydrolysate was supplied in a fed-batch mode to overcome the volume expansion of the fermentation broth. The ethanol produced was separated by a coupled membrane, reporting separation factors and an average membrane flux of about 5.3 and 645 g^2/h. This approach revealed more than 96.6 % concentration for the permeated ethanol, followed by the fractional condensation approach [170].

The adsorption approach uses a molecular sieving process based on the size difference between ethanol and water molecules. Fortunately, water molecules (2.5 Å) are smaller than ethanol ones (4 Å), which could be used as a base for adsorption using sieves of about 3 Å. One of the common ethanol-applied sieves is Zeolite, which was applied by Mekala and his colleagues in a mixture with silica gel for a concentration of 95% ethanol and achieved around 99.95% concentration at 30°C [171]. Additionally, molecular-sieving carbon (MSC) was effectively applied for small-scale bioethanol concentration (98.5%) in a single step from fermentation broth at mild extraction temperature (45°C) [172]. Despite the energy-saving and eco-friendly nature of membrane separation technology (**Table 4**), its commercial applicability is largely challenged by the short lifetime of most applied materials [173].

7. Worldwide growth in bioethanol production and application, especially referring to African and Middle Eastern countries

In light of the worldwide direction toward sustainable energy dependence, satisfying the growing energy need and diminishing the market dependence upon nonrenewable fossil fuels, bioethanol application was intense, with a considerable share in the fuel market economy. This intensification in bioethanol application magnifies the annual bioethanol production from 18 to 110 billion liters in less than twenty years (from 2000 to 2019) and is expected to achieve 132 billion liters by 2030 [174,175]. Currently, the USA is the main player in the bioethanol production and application field, representing around 55% of the total bioethanol production; Brazil, the European Union, and China come next with 28%, 6%, and 4% of the bioethanol global share, respectively [131,175]. Though bioethanol production for fuel application was pioneered in Brazil, the sugarcane feedstock shortage (main production feedstock) decreased bioethanol production from 37.38 billion liters in 2019 to 31.35% in 2022, under the government policies for dedicating available sugarcane for sugar production [176].

In light of its low share of global bioethanol production compared to the USA and Brazil, the Chinese government adopted several policies to force the bioethanol production rate to meet the growing commercial demands. To overcome bioethanol production as a building block for industrial chemical synthesis, the government subsidizes corn-based bioethanol production for the fuel sector through blending with gasoline [176]. As previously stated, this direction elevated the annual bioethanol production rate by 7.1% between 2005 and 2018 based on corn feedstock [177]. Furthermore, several governmental policies directed the application of Cassava and sweet sorghum to fill any gap in bioethanol production feedstock [178]. Maize and sugarcane are the main sources of fermentable sugar and share about 60% and 25% of the total bioethanol produced, respectively. Other grains and plant-derived materials such as molasses and cassava cover the remaining 14% of bioethanol production [131]. The bioethanol production from LCB and algal biomass share in the current fuel economy is insignificant and in the developing stage.

Among African countries, South Africa established its first step in bioethanol production for electricity generation, depending on sugarcane and maize [179]. The bioenergy production project was launched in Early 2007 toward a sustainable economy and less dependence on fossil fuels for electricity generation. Whereas in 2015, bioethanol was applied in vehicle fuels through mixing with gasoline [180]. Some other African countries, such as Nigeria and Zambia, adopted a national biofuel production program in 2007 based on Cassava and sugarcane with no significant impact on the national fuel market [181].

Similarly, bioethanol production in most Arabian and Middle Eastern countries doesn't exceed the initial steps or even the research zone, with no significant share in their fuel market [182–184]. This production lag could be attributed to land and water scarcity, especially in most MENA countries [182,185]. Hence, food security reasons greatly challenge dependence on the first generation of bioethanol production. Additionally, the nature of climate favors wind and solar energy generation, considering the availability of conventional fossil energy sources [185].

In the Turkish situation, E10 (gasoline with 10% bioethanol) is a national target by 2023, which necessitates the production of 0.3 billion liters of bioethanol to cover this governmental task [186], whereas E100 was estimated in 2014 to be achieved by bioethanol production rate of 4.65 billion liters [187]. In their study in 2019, Melikoglu and Turkmen reported the applicability of achieving 0.335 and 0.555 billion liters of bioethanol through wasted crops and cereals (wheat, rice, maize, and potato) to accomplish the E10 goal of 2030 in a sustainable and economically feasible approach [188].

8. Current challenges and future prospects of bioethanol production

Considering the economic and environmental issues, intensive research was directed toward implementing LCB for bioethanol production. However, several limitations restrict commercial reliance on this direction, reflected in the market share of lignocellulosic bioethanol by about 1% of the global bioethanol market [189]. Three main categories could underline the challenges for the bioethanol production process from LCB: process efficiency and process integration

Table 4. A common assessment of three ethanol separation types, including separation base, current applicability, advances, and disadvantages of each type.

Criteria	Distillation	Pervaporation	Adsorption
Separation base	Volatility	Selective permeability	Molecular sieving
Applicability	Commercial strategy	Pilot plant scale	Commercial to pilot plant scale
Advantages	- High efficiency under low ethanol conc.	- Energy saving - Cost-effectiveness - Ecofriendly nature - Ability to process integration	- Energy saving - Ecofriendly nature - Ability to process integration
Disadvantages	- High energy required - Not applicable on small scale	- Membrane blocking and lower durability of membranes	- High cost of currently available adsorbents

8.1. Process efficiency: the first challenge category comes from the complex structure of LCB, which necessitates several pretreatment and hydrolysis processes before the fermentation step. Additionally, the waste structure is heavily loaded with microbial toxins (furfural derivatives) and growth inhibitors (polyphenolic compounds and aliphatic acids) that usually accumulate in the resulting hydrolysate [190]. Therefore, intensive research should be directed toward developing a cost-effective and reliable approach for waste pretreatment and hydrolysis with simultaneous detoxification of the resulting hydrolysate. In this regard, two points should be highlighted: the first concerns the enhancement of the enzymatic system involved in the lignocellulosic material hydrolysis through immobilization and directed evolution to increase the hydrolysis efficiency while diminishing the inhibition from the pretreatment process. Also, novel catalytic systems should be explored, depending on the catalytic properties of the many developed nanoparticles. The second related to integrating membrane separation technology for detoxification and sugar concentration simulations for enhanced pretreatment and hydrolysis efficiency.

8.2. Process integration: the second challenge category could be addressed among the most important challenges in bioethanol production [166,191]. The need for several independent steps (4 steps) in conventional bioethanol production greatly challenged production technicality and economic feasibility [192]. Hence, intensive research should be directed at this point to minimize the production process steps, which will directly affect the production cost and efficiency. The integration between pretreatment and hydrolysis steps was widely reported in this regard. Results reported by Dhiman et al, revealed the successful integration between soaking pretreatment and enzymatic hydrolysis for efficient saccharification of rice straw and willow in one step [193]. Additionally, Mahboubi et al. applied double-staged immersed membrane bioreactors for continuous bioethanol production from wheat straw in an integrated process depending on membrane separation [192]. Their results asserted that at the optimum system flow rate (0.3 L/h), bioethanol production continued integrally at minimum process downtime. The ethanol recovery approaches should be directed toward separation techniques that could be implemented and integrated directly into the bioethanol production environment, considering the fermentor (organism) growth conditions, self-cleaning, durability, and sterilization ability.

8.3. Process recycling: toward sustainable LCB application for bioethanol production, overall process recycling is mandatory, in terms of applied enzymes, additives, black liquor, resulting slurry, and yeast cells. The process recycling principle reduces the final production cost through sustainable production practices. Aside from the resulting slurry, applied water is critical in recycling. Water recycling will facilitate lower chemical consumption and accumulation in the environment and the total water consumption in the production process. To increase economic and environmental efficiency, a lot of work has gone into developing recycling techniques, such as recycling wastes to reduce secondary pollution, cellulases and yeasts to reduce costs, and recycling residues to produce byproducts. Based on recycling processes, LCB can be converted into various byproducts, including xylose, xylitol, lignin, fertilizer, biomethane, and energy. Furthermore, LCB is inexpensive, but the recycling-related additives, including cellulase and yeast, are costly [106]. High-value additives must be used multiple times, and the suggested recycling technique offers some tactics to help with this. Even with full utilization of high-value additives and LCB, ultimate wastes cannot be avoided, leading to a considerable reduction in environmental and economic performance. Therefore, all wastes and feedstock are considered in the recommended recycling process.

8.4. Byproduct formation: during the pretreatment process, various inhibitory compounds are formed as byproducts, including formic (CH_2O_2), CH_3COOH, and levulinic ($C_5H_8O_3$) acids, which are considered a major drawback of all pretreatment approaches. These weak acids can impact cellular development and ethanol yield by diffusing across the plasma membrane and changing the cytosolic pH [194]. The consequent rise in intracellular causes DNA damage [H+] radical concentration, which also influences cellular ATP levels. Additionally, it has been shown that the yield and production of bioethanol are significantly influenced by the formed phenolic compounds as byproducts after the degradation of lignin. These formed byproducts and the primary fermentable sugar loss in the hydrolysate adversely affected the microbial system efficiency during the bioethanol fermentation process. Physicochemical techniques (such as solvent extraction, evaporation, overliming, and ion exchange activated charcoal adsorption) and biological techniques (such as microbial or enzymatic conversion of inhibitors into less toxic compounds) are the conventional methods used to remove inhibitors from lignocellulosic hydrolysates [195]. In addition to retaining potent inhibitors, most of these detoxification approaches have other drawbacks, like the production of waste products,

fermentable sugar loss, and a costly, labor-intensive, and time-consuming operational procedure.

9. Conclusion and recommendations

The rapid escalation of global urbanization and industrialization has led to a substantial increase in fossil fuel consumption, exacerbating the complexities of global warming and posing a significant threat to the global economy by depleting nonrenewable fuel reservoirs. Bioethanol has emerged as a promising alternative, demonstrating economic viability in both the transportation and electricity generation sectors. However, the initial reliance on food crops for bioethanol production raised concerns regarding food security. In response, efforts have been redirected towards utilizing LCB s for bioethanol production, aiming to mitigate environmental impacts and alleviate fluctuations in grain prices. Nevertheless, this approach encounters numerous challenges due to the intricate structure of lignocellulose, which complicates its hydrolysis into fermentable sugars. Moreover, the current production strategies pose additional process complexities. Addressing these challenges requires efficient waste pretreatment methods, improved catalytic activity, and advancements in membrane concentration technology to facilitate simultaneous sugar liberation from diverse lignocellulosic materials.

Furthermore, enhancing yeast strains through genetic engineering and implementing membrane separation techniques for continuous bioethanol separation could streamline production and enhance commercial viability. Emphasizing process integration and recycling across all production steps promises to reduce bioethanol costs and ensures sustainable production from LCB. The imperative for clean energy sources to mitigate gas emissions has accelerated the adoption of renewable energy, with bioethanol emerging as a frontrunner. However, realizing bioethanol's potential relies heavily on efficient LCB treatment strategies and ethanol separation processes. Recommendations include developing cost-effective LCB treatment methods and affordable polymers based on nanofibers for efficient bioethanol separation. Encouragingly, increased investment in research and development, supported by global funding agencies, can drive innovation and scale up bioethanol production to commercial levels, thereby fostering a sustainable energy future.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA, for funding this research work through the project number "NBU-FFR-2024-337-07".

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Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data presented in this study are contained within the article.

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