

Importance of Lignocellulose in High-Value Product Production

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Lignocellulose consists of cellulose, hemicellulose, and lignin and is a sustainable feedstock for a biorefinery to generate marketable biomaterials like biofuels and platform chemicals. Enormous tons of lignocellulose are obtained from agricultural waste, but a few tons are utilized due to a lack of awareness of the biotechnological importance of lignocellulose. Underutilizing lignocellulose could also be linked to the incomplete use of cellulose and hemicellulose in biotransformation into new products. Utilizing lignocellulose in producing value-added products alleviates agricultural waste disposal management challenges. It also reduces the emission of toxic substances into the environment, which promotes a sustainable development goal and contributes to circular economy development and economic growth.

Keywords: lignocellulose ; cellulose ; hemicellulose ; lignin ; value-added products

1. Introduction

Lignocellulose is a plant biomass available in large amounts, and it is a renewable resource. It is a complex structure primarily composed of the polymers cellulose and hemicellulose (polysaccharides), lignin (a phenolic macromolecule), and other components, such as proteins, lipids, and inorganic compounds ^{[1][2][3]}. These polymers contain cellulose ranging from 35 to 55%, hemicellulose from 20 to 40%, lignin (10–25%) by mass, and other polar and non-polar compounds ^[4]. The elemental compositions of most lignocellulosic biomass are classified as major elements (e.g., C, H, O, N, K, and Ca), minor elements (Mg, Al, Si, P, Cl, Na, S, and Fe), and trace elements (e.g., Mn and Ti) ^{[5][6]}.

The cellulose in lignocellulose is a homopolysaccharide with chains of D-glucose monomers linked together via β -1-4 glycosyl units, stabilized by hydrogen bonds and van der Waals forces ^[1]. Cellulose comprises the repetitive structural unit called cellobiose (D-glucopyranosyl- β -1,4-D-glucopyranose) ^[7], and it is linked to lignin by hemicellulose via hydrogen and covalent bonds ^[8]. Hemicellulose is a branched heteropolysaccharide with two or more free monosaccharides, such as xylose and arabinose (five-carbon sugars), mannose, glucose, galactose (six-carbon sugars), and carboxylic acids (e.g., mannuronic acid and galacturonic acid) ^{[8][9]}. Moreover, hemicellulose bridges the deposition of lignin monomers in the secondary cell wall ^{[9][10]}. Meanwhile, lignin is a complex amorphous polymer that contains various monolignols, such as p-coumaryl alcohol, sinapyl alcohol, and coniferyl, and it is a crosslinked macromolecule formed via the polymerization of phenylpropanoid monomers (p-coumaryl alcohol, sinapyl alcohol, and coniferyl) ^{[8][10]}. Lignin is hydrophobic and highly resistant to hydrolysis; it binds hemicellulose to cellulose in the cell wall and acts as a barrier that limits cellulose accessibility ^[11].

Extensive studies on the physicochemical properties of lignocellulose have yet to be conducted; however, literature searches have indicated that lignocellulose physicochemical properties are assessed based on its particle size, density, flowability, moisture sorption, grindability, and thermal properties (physical properties), along with ash, volatile matter, moisture, and fixed carbon (chemical properties) ^[12].

2. Lignocellulosic Biomass Sources

Lignocellulosic biomass resources are widely available, and they are agricultural and forestry residues from plant wastes ^[13]. Industrial and food wastes are also sources of lignocellulose ^[14]. Several harvests from a single planting that reduce the average annual cost of managing energy crops compared to conventional crops make lignocellulosic biomass resources the most promising future resources to generate value-added products ^[15]. Rice, wheat, sugarcane, and maize are the major crops that generate a large amount of lignocellulosic biomass. The world's first most important cereal crop is corn. In 2022/2023, around 1.2 billion metric tons of corn and nearly 783.8 million metric tons of wheat were produced, followed by 510 million metric tons of milled rice (the second-most important cereal crop) ^[16]. Approximately 177.3 million metric tons of sugarcane were also produced in 2022/2023 ^[16]. China and the United States of America account for more

than half of worldwide corn production, while China is the world's leading rice producer, followed by India and Bangladesh [16]. Similarly, China, followed by India, Russia, and the United States of America are the four largest wheat producers in the world, while India and Brazil are the world's top two sugar producers [17].

Vast waste, such as rice straw, wheat straw, sugarcane bagasse, corn stover, etc., is generated annually via agricultural crop production. Rice straw (stems, leaf blades, and sheets) is generated from the rice harvest [18][19], and wheat straw is the waste obtained from wheat grain production [15]. Sugarcane waste or bagasse is obtained after sugarcane stalks are crushed for sugar [20], while corn stover (consisting of leaves, cobs, husks, and stalks) is the waste product obtained from corn kernel processing [21] from the maize plants. These wastes constitute a major portion of lignocellulosic biomass. Other agricultural wastes that contribute to a small amount of the total agricultural waste production include barley straw, cotton stalks, sweet sorghum straws, potato haulms (the tops, stems, and foliage of potato plants), and others [15].

3. Conversion of Lignocellulosic Biomass into Value-Added Products

3.1. Pretreatment Methods of Lignocellulose

The pretreatment of lignocellulose is a delignification process that makes lignocellulosic materials accessible to generate sugars. In many cases, lignocellulose was reported to be recalcitrant due to the complexity of the cell wall, lignin components, and crystalline structure of cellulose [13]. As such, selecting a suitable pretreatment method to generate sugars for the downstream application is essential. Lignocellulose can be pretreated using physical, chemical, physicochemical, biological, or nanotechnology methods [22][23][24].

Different processes used in chemical pretreatment methods include dilute acid, alkaline, ionic liquid, organosolv process, ozonolysis, and deep eutectic solvents [25]. In the dilute acid pretreatments, inorganic or organic acids, such as HCl, H₂SO₄, HNO₃, and formic acid, break down the hydrogen and glycosidic bonds in cellulose/hemicellulose [26][27]. Bases such as NaOH, NH₄OH, Ca(OH)₂, and KOH are always used in alkaline pretreatment methods to solubilize lignin [3].

Physicochemical pretreatment methods such as ammonia fiber explosion (AFEX) or carbon dioxide explosion are employed where the milled or ground lignocellulose is treated with ammonia under a high temperature (e.g., 90 °C) or carbon dioxide pressure is released to disrupt the structure of cellulose [28][29]. The disruption of the structure of cellulose reduces cellulose crystallinity, enhances cellulose permeability, and increases its surface area, thereby increasing the accessibility of enzymes [30].

Biological pretreatments also offer capable commercially available microbial enzymes or crude enzymes in the delignification of lignocellulose. The different enzymes used in biological pretreatment include ligninolytic enzymes such as phenol oxidase (e.g., laccase) and heme peroxidase (e.g., lignin peroxidase). Additionally, fungi, such as white rot (e.g., *Irpex lacteus*, *Ceriporiopsis subvermispura*, and *Lentinus edodes*) [31][32][33], red rot (e.g., *Fomitopsis annosa*) [34], and brown rot (e.g., *Neolentinus lepideus* and *Gloeophyllum trabeum*) [35], have been used to attack lignin, hemicellulose and cellulose, lignin and hemicellulose, or cellulose and hemicellulose directly due to the lignolytic enzymes they produce [32][36].

Previous studies that had used white rot fungi in lignocellulose pretreatment established that: (i) two strains of *Ceriporiopsis subvermispura* used to pretreat wheat straw for seven weeks revealed that *Ceriporiopsis subvermispura* (CS), mostly CS1, showed a higher selectivity in lignin degradation than CS2, with higher laccase activity but lower manganese peroxide than C2 [37]; (ii) there was a selective degradation of lignin wheat straw and lignin oak wood chips when incubated with *Ceriporiopsis subvermispura* and *Lentinus edodes*, and alkylitaconic acids for delignification were produced by *Ceriporiopsis subvermispura* and *Lentinus edodes* [33]; (iii) there was degradation of 265 g·kg⁻¹ of lignin and 320 g·kg⁻¹ of neutral detergent soluble when eight different cultivars of wheat straw were incubated with *Irpex lacteus* for 56 days at 28 °C [38]; and (iv) the lignin content of wheat straw pretreated with *Ceriporiopsis subvermispura*, CS1 (CBS 347.63), at 24 °C reduced by 48.5% [39].

Lastly, the pretreatment method based on nanotechnology employs the ability of nanoparticles to penetrate the cell membrane of lignocellulose [23]. Recycling and reusing magnetic nanoparticles for subsequent cycles in lignocellulose pretreatment reduces the overall processing cost [13]. Examples of nanotechnology pretreatments are acid-functionalized magnetic nanoparticles and nano-scale shear hybrid alkaline methods. Acid-functionalized magnetic nanoparticles are strong acid nanocatalysts that effectively degrade lignocellulose [40]. In the nano-scale shear hybrid alkaline method, lignocellulose is degraded by combining chemical catalysts and the high-speed shear force [41].

Meanwhile, each pretreatment method has its pros and cons. For instance, physical methods do not generate inhibitory compounds. They can offer green pretreatments, in which the product (hydrolyzate) can be directly utilized to generate sugars. Still, physical methods, such as mechanical comminution and pyrolysis, have been considered to be too expensive for a full-scale process due to their high energy consumption; however, the main disadvantage of physical pretreatment methods is their inability to degrade the structure of lignin [24][42].

In chemical pretreatments, the hydrolysis of lignocellulose by acid alters the structure of lignin, thus resulting in high glucose yields and solubilizing hemicellulose to xylose and other sugars. The drawbacks of acid hydrolysis include the high cost of corrosive-resistant equipment and the generation of inhibitors, such as levulinic, formic, and acetic acids. Low inhibitors are produced under alkaline hydrolysis, but this process requires a long residence time and a high cost of alkaline catalysts [43].

Nanotechnology pretreatment methods have been considered the best option for delignification, as these pretreatment methods are cost-effective because the immobilized enzymes are easily retrievable and reusable [44][45]. Depending on the type of nanomaterial used, a few drawbacks of nanotechnology pretreatment methods include their potential poor dispersion abilities of some nanoparticles (due to the difficulty of dispersing in the aqueous solution, where hydronium ions are not effective) [46], and biocatalyst desorption could arise due to the weak bonds [47].

3.2. Hydrolysis of Lignocellulose

The pretreated lignocellulose is then subjected to hydrolysis. Hydrolysis is the process that liberates monomeric sugar molecules, viz. glucose, mannose, galactose, xylose, or arabinose, from structural polysaccharides, such as cellulose and hemicellulose in lignocellulose [48][49]. Cellulose hydrolysis using acids or enzymes has been reported. The first acid hydrolysis technology was developed in 1923, when a sulfuric acid solution was used to hydrolyze white spruce wood; the sugars obtained were glucose, mannose, galactose, xylose, and arabinose [50]. Inorganic acids (e.g., hydrochloric acid and hydrogen fluoride) and organic acids (e.g., citric, oxalic, and maleic acids) were also used in cellulose hydrolysis [51][52][53].

The hydrolysis of cellulose under room temperature using ca. 12 mol·L⁻¹ of hydrochloric acid yielded approximately 32 percent of volume-reducing sugar [54], and cellulose hydrolysis with 6–7 mol·L⁻¹ of hydrochloric acid at 90 °C in the presence of CaCl₂ and LiCl as additives resulted in an 85% glucose yield [55]. When cellulose was hydrolyzed using hydrogen fluoride, the sugar yield was approximately 45% at 0 °C [53]. The most notable drawbacks of acid hydrolysis include problems in product/catalyst separation, catalyst recycling, corrosion of reactors, and waste effluent treatment that makes it environmentally unfriendly.

3.3. Fermentation of Sugars

Fermentation is an enzyme-catalyzed biochemical process in which capable microorganisms convert sugars into new products [56], especially value-added products. Several fermentation products include biofuels like alcohol (e.g., ethanol), gases (such as methane and biogas), and organic acids (e.g., lactic, citric, succinic, and acetic acids) [15][57][58][59][60][61][62]. Producing these new products depends on the selected microorganisms and fermentation conditions. Different fermentation modes and methods have been employed in producing value-added products. These modes include batch fermentation, fed-batch fermentation, repeated-batch fermentation, and continuous fermentation [63].

Importantly, the systems are closed in batch mode, and all the required ingredients and microorganisms are added prior to fermentation. The pH is usually regulated during fermentation via an attached acid or alkaline system [63]. The fed-batch system contains the same required components as in the batch system, but during the fed-batch fermentation process, the depleted required components (e.g., carbon and nitrogen) are sequentially added at regular intervals to actively control microbial growth [64]. In repeated-batch fermentation, microbial cells are increased through repeated re-inoculation of microbial cells from one batch fermentation into the next batch [59][65].

Fermentation methods, such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), separate hydrolysis and co-fermentation (SHcoF), simultaneous saccharification and co-fermentation (SScoF), and consolidated bioprocessing (CBP), have been described in the literature [14][66] as methods that can be used during fermentation tasks. In SHF, lignocellulose is first pretreated, and following the degradation of lignin, the pretreated lignocellulose (hydrolyzate) is subjected to saccharification, followed by fermentation of the simple sugar. In SSF, the pretreated lignocellulose (hydrolyzate) is subjected to simultaneous saccharification and fermentation [14]. Separate hydrolysis and co-fermentation (SHcoF) is similar to SHF; the difference is the presence of at least two sugars for fermentation in SHcoF [67].

3.4. Purification of Value-Added Product

Purification is one of the most essential stages in value-added product production. Factors that elevate the difficulties of product recovery include, but are not limited to, the low concentration of the product, the presence of impurities, the product produced intracellularly, and heat-labile products. The extraction and purification of fermentation products depend on the specific product. The choice of purification process is based on the concentration of the product, intracellular or extracellular location of the product, physicochemical properties of the product, the impurities in the fermentation broth, acceptable standard of purity, and the product's intended use.

The stages in the recovery/purification of products, such as organic acids (e.g., citric, lactic, and succinic acids) from fermentation broth (extracellular product), involve the removal of solid particles and microbial cells using filtration and centrifugation followed by broth extraction into different fractions [68]. Ultrafiltration, adsorption, precipitation, distillation, liquid–liquid extraction, supercritical fluid extraction, ion exchange, dialysis, electrodialysis, or membrane separation can be employed for broth extraction [68][69][70][71][72].

Moreover, biofuels can be recovered/purified using different methods. For instance, the conventional distillation process is the first step of ethanol recovery, followed by dehydration using azeotropic distillation, adsorption, pervaporation, or membrane processes [73]. Different methods like equilibrium-based separation (e.g., distillation, liquid–liquid extraction, and supercritical fluid extraction), affinity-based separation (adsorption and ion exchange), solid–liquid separation, and membrane-based separation have been employed in biodiesel purification [74]. Generally, the biodiesel purification process is wet washing using water and dry washing using adsorption, ion exchange, and membrane separation [75].

4. Value-Added Products from Lignocellulosic Biomass

4.1. Biofuels

Biofuels are an inexhaustible and biodegradable class of renewable energy obtained from living materials [76]. Biofuels are primarily used as transportation fuels and can be used to generate electricity and heat [77]. The three different generations of biofuel are: (i) first-generation biofuels (produced from edible crops); (ii) second-generation biofuels (produced from lignocellulose); and (iii) third-generation biofuels (produced from algae and microorganisms) [78]. In 2021, the United States of America produced 643,000 barrels of oil equivalent per day, followed by Brazil and Indonesia (which produced 376,000 and 140,000 barrels of oil equivalent per day, respectively) [79][80]. Additionally, biofuels are eco-friendly and capable of eliminating the emission of hazardous gases such as sulfur oxide and carbon monoxide, thereby maintaining a cleaner environment [81]. The most common biofuels include alcohols, biodiesel, biohydrogen, and biogas [15].

4.1.1. Alcohols

Alcohol is often used to denote ethanol or methanol. Since the development of the internal combustion engine, ethanol has been used as a motor fuel [82]. Bioethanol is produced from lignocellulose via pretreatment to break the recalcitrant structure of lignocellulose, followed by the enzymatic saccharification of cellulose and hemicellulose into simple sugars, and, lastly, fermentation of the generated simple sugars by microorganisms such as *Saccharomyces cerevisiae*, *Zymomonas mobilis*, and several genetically engineered microorganisms [83][84][85]. Notably, several recombinant microorganisms were developed to ferment hexose and pentose into ethanol [86][87][88]. The concentration and productivity of bioethanol depend on the lignocellulose source, the selected pretreatment method, and the microorganism(s) used in fermentation [89].

4.1.2. Biodiesel Production

Biodiesel or fatty acid methyl ester (FAME) with lower alkyl esters and long-chain fatty acids [89]. Biodiesel is a clean-burning, renewable substitute for petroleum diesel, and like petroleum diesel, it is used in diesel engines (e.g., generators and vehicles) and heating oil [90][91][92]. Pure biodiesel is called B100, and the most common blend is B20, which contains 20% biodiesel and 80% petroleum diesel [91]. Biodiesel increases energy security and improves air quality [90]. It was reported that a gallon of biodiesel (B100) produces 74% less carbon dioxide than petroleum diesel [91].

Biodiesel can be produced from second-generation biological materials, such as vegetable waste oil, non-edible vegetables, oleaginous microbes, and jatropha [93][94]. In biodiesel synthesis using lignocellulose, the pretreated hydrolyzate is saccharified, and oleaginous microbes such as *Rhodospiridium toruloides*, *Gordonia* sp., *Yarrowia* sp., *Rhodotorula* sp., etc., convert the generated simple sugar into pyruvate that will be further converted to lipids in the microbes [94][95]. The lipids are extracted via cell disruption using various methods that have been described by Khot (2020) [95]. The extracted lipids are then converted into biodiesel via transesterification, in which the lipid reacts with short-

chain alcohols, such as methanol and ethanol, in the presence of a catalyst [89][94]. Different biodiesel production processes have been described [89][94]. Still, supercritical non-catalytic and enzymatic biodiesel production technologies are the best, as these technologies can process low-quality feedstock without pretreatment [96]. The production of biodiesel using second-generation feedstock is underdeveloped; as such, limited information on biodiesel production from lignocellulose is available.

4.1.3. Biohydrogen Production

Biohydrogen is an elementary substrate for ammonia, methane, methanol, synthesis gas, and olefin hydrogenation synthesis [97]. Commercial-scale biohydrogen production technologies are yet to be established; as such, more research focus should be directed towards biohydrogen production. Generally, biohydrogen can be produced using thermochemical, photoelectrochemical, electrolysis, and biological technologies, among which the biological method (dark fermentation) is eco-friendly and sustainable [98].

4.1.4. Biogas Production

Biogas (or biomethane) is a renewable pure energy source generated through biodigestion. Biogas has various applications in cooking, drying, cooling, and generating heat and electricity [99]. Biogas is produced through anaerobic digestion under a naturally occurring biological process that involves five steps. The steps are: (i) pretreatment of lignocellulose for easy accessibility to cellulose and hemicellulose to produce hydrolyzates; (ii) saccharification of hydrolyzate, resulting in monomers such as sugars, amino acids, and fatty acids; (iii) conversion of these monomers by acidogens into short-chain volatile fatty acids (acidogenesis); (iv) conversion of volatile fatty acids by acetogens into acetate, carbon dioxide, and hydrogen (acetogenesis); and (v) acetate, carbon dioxide, and hydrogen are converted into biomethane by methanogens (methanogenesis) [100].

4.2. Platform Chemicals

4.2.1. Fermentative Production of the Platform Chemicals from Lignocellulose

The traditional fermentative production of lactic, succinic, citric, and acetic acids from lignocellulose is completed through sequential pretreatment steps (to make cellulose and hemicellulose accessible to subsequent enzymatic hydrolysis), followed by enzymatic saccharification or hydrolysis (for the generation of simple sugars), and, finally, fermentation of the simple sugars by capable microorganisms. The microorganisms involved in simple sugar (hexose and pentose) fermentation to lactic acid include bacteria (LAB), *Enterococcus faecalis*, and *Rhizopus* sp. (for lactic acid production) [59][60][101]. *Actinobacillus succinogenes*, *Saccharomyces cerevisiae*, and other engineered microorganisms were used to produce succinic acid [76][102].

Fermentable sugars (hexose and pentose) are metabolized to lactic, succinic, citric, and acetic acids through various microbial metabolic pathways. For instance, lactic acid can be produced via the (i) glycolytic, (ii) phosphoketolase, and (iii) pentose phosphate pathways [103]. In the glycolytic pathway, lactic acid bacteria, under anaerobic conditions, use glucose (a carbon source) to produce pyruvate, and lactate dehydrogenase catalyzes the conversion of pyruvate into lactate [103]. In the phosphoketolase pathway, glucose is converted into lactate, ethanol, and carbon dioxide, while bacteria, such as *Leuconostoc* sp., metabolize pentose to form lactate and acetate [103][104].

Succinic acid is biosynthesized from simple sugars via (i) reductive tricarboxylic acid (rTCA), (ii) the tricarboxylic acid (TCA) oxidation cycle; or (iii) glyoxylic pathways [105]. The rTCA pathway (the main succinic acid production pathway under anaerobic conditions) occurs by converting the simple sugar (e.g., glucose) into phosphoenolpyruvic (PEP) acid and PEP to oxaloacetic acid by PEP carboxykinase. Oxaloacetic acid is then reduced to succinic acid by malate dehydrogenase, fumarase, and fumarate reductase [57][58]. In the TCA cycle, glucose is converted into acetyl-CoA, citrate, isocitrate, and succinate by succinate dehydrogenase under aerobic conditions. The theoretical succinic acid yield of 1 mol mol⁻¹ glucose with the release of 2 mol carbon dioxide is obtained in the TCA cycle, while in the glyoxylic pathway, the succinic acid yield is 1.71 mol mol⁻¹ glucose due to carbon loss during the oxidative carboxylation reaction [57][58].

Citrate is produced from the aldol condensation of oxaloacetate and acetyl CoA in the Krebs cycle (known as the TCA cycle) by citrate synthase. Acetyl CoA may be derived from oxidative decarboxylation of pyruvate from glycolysis (where there is β -oxidation of fatty acids in the mitochondrial matrix) or by oxidative degradation of certain amino acids (e.g., leucine, isoleucine and threonine) [106].

4.2.2. Global Production and Market Values of the Platform Chemicals

The commercial production of lactic, succinic, citric, and acetic acids from lignocellulose has gained enormous attention. The demand for lactic acid (LA) in the past years has mainly increased due to polylactic acid (PLA) production, as LA serves as a building block for PLA production. Polylactic acid is used for drug delivery systems, prostheses, biodegradable packaging materials, and surgical suture production ^[107]. Furthermore, lactic acid is also used in the cosmetics, food, pharmaceutical, and chemical industries. For instance, LA is used in producing: (i) parenteral dialysis solutions, (ii) moisturizing and anti-acne creams, (iii) flavoring and preservatives, and (iv) acidulants and pH regulators ^[107]. In 2022, the lactic acid market volume was approximately 1.5 million metric tons, and its market value reached about USD 1.46 billion ^[108].

The demand for succinic acid (a dicarboxylic acid) is due to the global movement towards sustainability. In the organic and natural food industry, succinic acid is frequently used as a taste enhancer and food additive due to its ability to increase flavor and improve shelf life ^[109]. Succinic acid is also extensively used in the pharmaceutical industry as an excipient medicine formulation, and it is a precursor in the chemical industry to produce resins, polymers, solvents, plastics, fumaric acids, and glyoxylic acids ^{[109][110][111]}.

Acetate is an anion form of acetic acid, and salts are formed by combining acetic acid with alkaline or other bases. Acetate is a vital building block in various industry applications ^[112]. Acetate is a coating solvent for paints and varnishes, printing inks, and nail polish ^[113]. It is used in the food industries as a food preservative (e.g., sodium acetate and potassium acetate) and a synthetic flavor enhancer (e.g., ethyl acetate, the ester of ethanol and acetic acid) ^[112]. Ethyl acetate is also used as a solvent for stains, fat, and dry cleaning ^{[113][114]}, while vinyl acetate monomer (produced from the combination of acetic acid and ethene in the presence of oxygen), a building block of polyvinyl alcohol and polyvinyl acetate, is used to make packaging materials ^[115]. The increase in acetic acid demand results from its end-use applications, including vinyl acetate monomer and ethyl acetate. Vinyl acetate monomer accounts for 35% of global acetic acid consumption, and polyvinyl alcohol, polyvinyl acetate, and ethene vinyl acetate are the main downstream markets for vinyl acetate monomer ^[114].

5. Challenges and Alleviation Strategies in Upcycling Lignocellulose

Several difficulties must be mitigated to be able to fully utilize lignocellulose for economically feasible value-added product production. These difficulties include the high cost of pretreatment technology, production of inhibitors after delignification (which adversely affects the quality of the hydrolyzed sugars for fermentation), feedback inhibition, substrate inhibition, end-product inhibition, the high cost of hydrolytic enzymes, and challenges in developing efficient enzyme cocktails for the effective hydrolysis of cellulose and hemicellulose ^{[63][116]}.

By-products (inhibitors) such as coumaric acid, acetic acid, formic acid, furfural, levulinic acid, and aldehyde formed during the chemical pretreatment of lignocellulose have been reported to affect microbial growth, substrate utilization, and fermentation adversely ^{[117][118][119]}. In saccharification of lignocellulose hydrolyzates, increased cellobiose and glucose concentrations could inhibit cellulase in breaking cellulose to cellobiose and glucose, thereby resulting in feedback inhibition ^[14]. In fermentation, challenges of substrate and end-product inhibition could occur. Substrate inhibition occurs when the fermentative microorganisms' growth is inhibited due to a high feedstock concentration (glucose or pentose). Growth inhibition occurs due to low water activity, high osmotic pressure, and cell lysis ^[14].

The cost-effective operation of lignocellulose biorefinery will incline if these challenges are abated and all three constituents (cellulose, hemicellulose, and lignin) of lignocellulose are efficiently converted into value-added products. Nanotechnology, where enzyme immobilization is used in delignification, could be the best pretreatment technology for solving the bottlenecks of by-product inhibition ^[47]. Reports have shown that feedback inhibition can be minimized by removing sugars during saccharification using electrodialysis, avoiding cellobiose accumulation, and optimizing enzymatic activities during hydrolysis ^{[120][121]}.

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