Pretreatment Technologies for Lignocellulosic Biomass: Research Progress, Mechanisms, and Prospects

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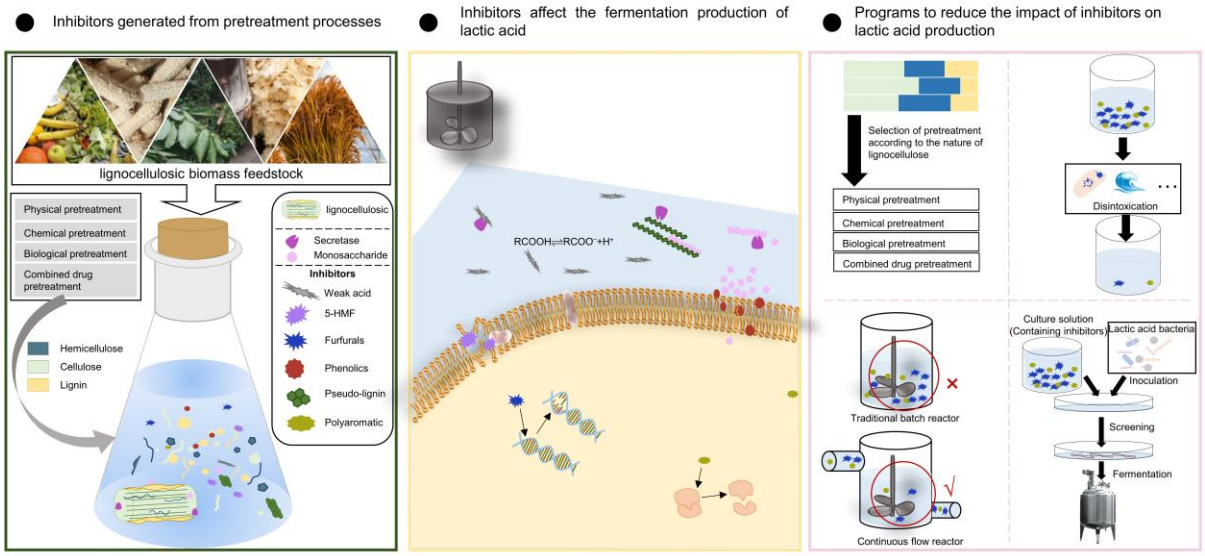
DOI: 10.15376/biores.20.2.Fan

GRAPHICAL ABSTRACT



Pretreatment Technologies for Lignocellulosic Biomass: Research Progress, Mechanisms, and Prospects

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Lignocellulose, which consists of cellulose, hemicellulose, and lignin, has very stable properties. Among them, cellulose makes up 30% to 50% of the content, and hemicellulose makes up 20% to 43%. Cellulose and hemicellulose can be converted into fermentable sugar through saccharification, and then into bioresources through fermentation. Pretreatment methods such as high temperature and high pressure, acid and alkali cooking, enzymatic digestion can effectively decompose the lignocellulose structure, remove lignin, increase the porosity of lignocellulose, specific surface area, *etc.*, increase the efficiency of saccharification, and improve the utilization of lignocellulose. Pretreatment is a key stage in the production process of bioresources. However, the pretreatment process produces by-products known as inhibitors such as acetic acid, furfural, and phenols. These inhibitors tend to inhibit the activity of biological enzymes, impede the saccharification of cellulose and hemicellulose, disrupt the integrity of the cell membrane of the fermenting bacteria, lead to mutation of the fermenting bacteria, and result in a decrease in the yield of the bioresource. This paper reviews recent advances in pretreatment methods, analyzes the reasons for the emergence of inhibitors, and summarizes methods to reduce the effects of inhibitors.

DOI: 10.15376/biores.20.2.Fan

Keywords: Fermentation; Lignocellulose; Structure; Pretreatment; Inhibitors

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INTRODUCTION

With the increasing consumption of fossil energy and the worsening energy crisis, the production of bioresources through biotechnology to replace fossil energy have the potential to alleviate the energy crisis and reduce pollution emissions (Wang *et al*. 2024). Among the many raw materials used in the production of bioresources, lignocellulose is favored by researchers because of its large global stock, cheap price, easy availability, and renewability (Oliveira *et al*. 2018).

Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin, of which cellulose and hemicellulose are the main components utilized in biotechnology for the making such products as ethanol. There is great interest in the saccharification of

cellulose and hemicellulose into fermentable sugars such as glucose and xylose, and then microorganisms ferment the fermentable sugars into bioresources through metabolic activities (Wang *et al*. 2015). However, the utilization of hemicellulose and cellulose is hindered by the complex polymer compound lignin, which consists mainly of guaiacyl (G), p-hydroxyphenyl (H), and butyryl (S) units (Ekielski and Mishra 2020). Scholars have found through research that a variety of methods such as crushing and crushing, high temperature and pressure cooking, acid and alkali soaking, and microbial degradation are effective in separating and removing lignin, thus exposing cellulose and hemicellulose, a step known as pretreatment (Ojo and de Smidt 2023). Therefore, the production of bioresources from lignocellulose requires pretreatment, saccharification, and fermentation steps. Currently, pretreatment technologies are divided into four main categories, physical pretreatment, chemical pretreatment, biological pretreatment and combined pretreatment. With the improvement of science and technology, the diversity of radiation pretreatment in physical pretreatment has increased, ultrasound and gamma irradiation are applied to the pretreatment of lignocellulose (Chen *et al*. 2022, Kucharska *et al*. 2018), and in the category of chemical pretreatment, the application of deep crystalline solvents and ionic solutions to the pretreatment test of lignocellulose has also increased (Liu *et al*. 2012; 2021).

After the pretreatment step, the structure of lignocellulose becomes fluffy and porous, the specific surface area is greatly increased, and the previously smooth planes become rough. These changes provide more attachment points for enzymes or cells in the saccharification step, which promotes the yield of fermentable sugars (Wang *et al.* 2024). Pretreatment can increase the utilization of lignocellulose, but some by-products are produced during pretreatment, such as acetyl shedding of hemicellulose to form acetic acid (AA) in high-temperature pretreatment, formation of aldehydes from monosaccharides in high-temperature acidic environment of inorganic acid pretreatment, and formation of phenolic compounds from lignin in high-temperature alkaline or acidic environments. These substances alter the pH environment, reduce enzyme activity, and disrupt cellular integrity in ways that impede the hydrolysis and fermentation of lignocellulosic biomass and reduce renewable energy production, and are referred to herein as inhibitors. (Klinke *et al*. 2004; Yee *et al*. 2018).

This paper summarizes and classifies the existing pretreatment methods from the structure of lignocellulose and discusses the principle of inhibitor generation and the mechanism of inhibitors affecting renewable energy production. Finally, taking the production fermentation of lactic acid as an example, it summarizes the feasible methods to reduce the impact of inhibitors on renewable energy production.

LIGNOCELLULOSE COMPOSITION AND STRUCTURE

Lignocellulosic biomass is the most widespread source of renewable organic compounds on earth. The global production of lignocellulose, including agricultural waste, garden waste, and part of municipal organic solid waste, is over 220 billion tons per year (Zagrodnik *et al*. 2021). Using lignocellulose as a raw material to produce bioresources can reduce production costs and improve inedible biomass utilization. Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin (Fig. 1).

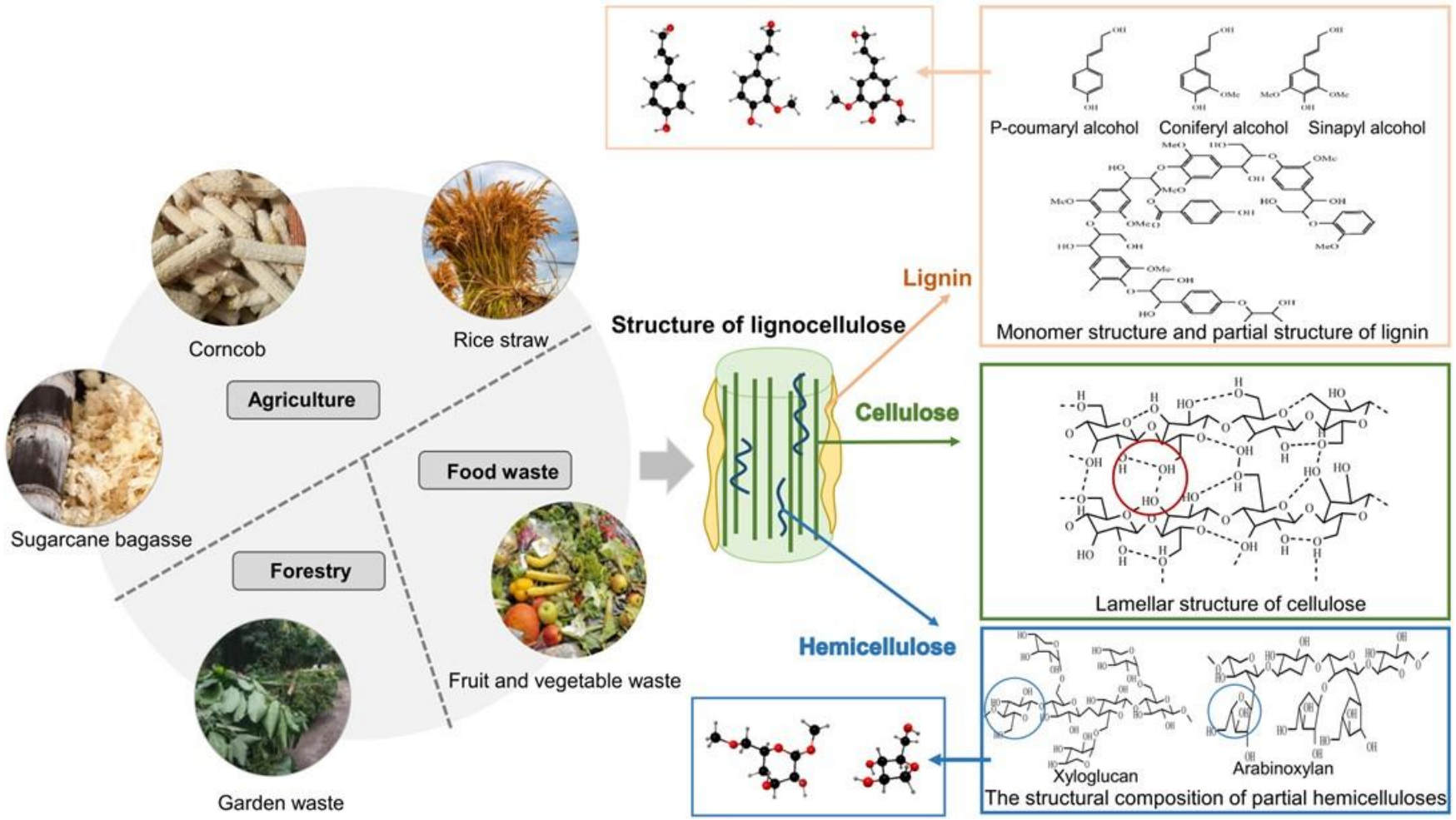

Fig. 1. Basic structure and composition of lignocellulose and the unique characteristics of the three basic structures

Cellulose

Cellulose is a renewable carbohydrate composed of 10000 to 15000 glucose monomers. The cellulose content in lignocellulose in general lies between 30.0% and 50.0%. For wheat straw it has been reported to be 30.0%, for corncob 45.0%, and for corn straw 37.5%. Two glucose molecules are linked by a β-1,4-glycosidic bond in which one glucose molecule is rotated 180° relative to the other; this arrangement produces the smallest structural unit of cellulose, called cellobiose (Sun *et al*. 2002). Subsequently, the glucose chains are linked *via* hydrogen bonds to form a layer, then the layers are linked to each other by hydrogen bonding into a three-dimensional structure (Fig. 1). The ordered crystalline and disordered amorphous regions resulted from the layers being held together by hydrogen bonds and van der Waals forces. Cellulose consists of crystalline and amorphous regions, making it insoluble in water and conventional organic solvents but soluble under highly alkaline or high-temperature conditions (Monte *et al*. 2017).

Hemicellulose

Hemicellulose is the second component of lignocellulose after cellulose. The hemicellulose content is usually between 20.0% and 43.0%. It consists of a variety of secondary structures, including O-acetyl-4-O-methylglucuronoxylan, arabinoxylan, xyloglucan, and arabinogalactan (Fig. 1). Unlike cellulose, hemicellulose chains have side groups. They also lack crystalline domains. The molecular chains of hemicellulose are shorter than those of cellulose. Certain hemicellulose chains are readily soluble in water or in a variety of solvents, including acids, alkalis, and organic solvents. Hemicellulose can be divided into two categories: water soluble and alkali soluble (Zhou *et al*. 2021). Notably, the furanose and pyranose sugar units composed of hemicellulose undergo dehydration upon dissolution, resulting in the formation of furfural and 5-hydroxymethyfurfural (HMF); they also shed the acetyl groups of the branched chains, forming AA, which is a common inhibitor during fermentation and affects the hydrolysis efficiency of enzymes and microbial activity (Feng *et al*. 2022).

Lignin

Lignin is the primary structural support in plant cells and is mainly composed of G, H, and S units, in which the G, H, and S are formed by the dehydrogenation of coniferyl alcohol, coumaric alcohol, and sinapyl alcohol (Fig. 1). G, H, and S are linked by various chemical bonds such as β-β, β-5, β-O-4, β-1, C-C, and 5-5 to create lignin units with a molecular weight exceeding 10,000 (Rajesh *et al*. 2019). Lignin acts as the "glue" to connected to carbohydrates via phenyl glycoside bonds, benzyl ether bonds, and gamma esters bonds, making lignocellulose a tightly integrated whole that increases the firmness of plant cells, reduces the adsorption of cellulose and hemicellulose on enzymes, and reduces the utilization rate of lignocellulose.

PRETREATMENT TECHNOLOGY

The purpose of pretreatment of lignocellulose generally is to make the cellulose component accessible to the action of cellulase enzymes. In some cases, the goal of pretreatment is to separate cellulose and hemicellulose and remove lignin, thus increasing the accessible surface area and pore structure of hemicellulose and cellulose enzymes, effectively promoting enzymatic hydrolysis, and obtaining more fermentable sugars.

Compared with lignocellulose without pretreatment, the recovery rate of cellulose and hemicellulose from lignocellulose after pretreatment can be improved, and the pretreatment process can also effectively remove poorly used lignin (Fig. 2). Pretreatment methods include physical (thermal pretreatment, mechanical pretreatment, irradiation pretreatment), chemical (acid pretreatment, alkali pretreatment, inorganic salt pretreatment, organic solvents pretreatment, deep eutectic solvents (DES) pretreatment, ionic liquids (ILs) pretreatment), biological (fungal pretreatment, bacterial pretreatment, and enzymatic pretreatment), and comprehensive treatment methods (combination of multiple pretreatment methods for better pretreatment results) (Fig. 3).

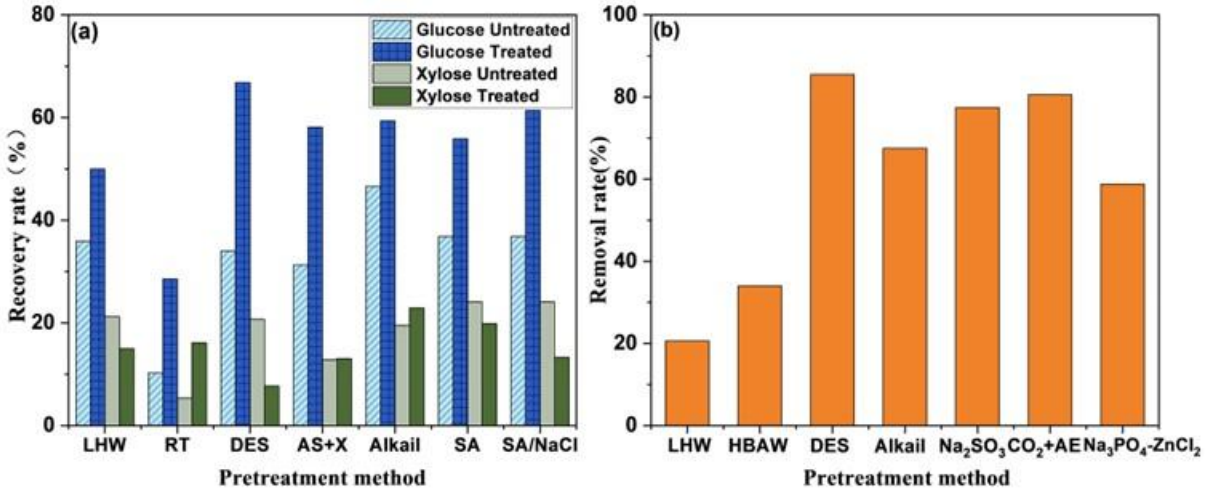

Fig. 2. Cellulose and hemicellulose recovery and lignin removal of lignocellulose in reported researches by various pretreatment methods.. (a) cellulose and hemicellulose recovery rate of lignocellulose before and after pretreatment (LHW, liquid hot water (Gunes *et al*. 2022); RT, rolling thread (Deng and Li 2021); DES, deep eutectic solvents (Huang *et al*. 2020); AS+X (ammonium sulfite+xylanase), ammonium sulfite + xylanase (Yu *et al*. 2020); Alkail (Wang *et al*. 2017); SA, sulfamic acid (Song *et al*. 2022);SA (sulfamic acid)/NaCl, sulfamic acid/sodium chloride solutions (Song *et al*. 2022)); (b) lignin removal rate of lignocellulose before and after pretreatment (LHW, liquid hot water (Gunes *et al*. 2022); HBAW, high-boiling point alcohol/water (Liu *et al*. 2017); DES, deep eutectic solvents (Huang *et al*. 2020); Alkail (Wang *et al*. 2017); Na_2SO_3, sodium sulfite (Chen *et al*. 2019); CO_2+AE, CO_2+Alkail explosion (Triwahyuni *et al*. 2023); $Na_3PO_4.12H_2O$ + $ZnCl_2$ (Hassan *et al*. 2020)). The data were cited from the related values in published papers.

Physical Pretreatment

Thermal pretreatment includes liquid hot water (LHW), high-pressure steam, and steam explosions (Hendriks and Zeeman 2009). Heat pretreatment separates hemicellulose as a result of the acetyl groups shed from the molecular chains of hemicellulose, which acidifies the environment and promotes hemicellulose dissolution. With the dissolution of hemicellulose, the lignocellulose structure becomes fluffy and disordered, and the cellulose is exposed to the solution (Fan *et al*. 2013). The steam explosion equipment consists of a steam generator and a pressurizer, which first creates a high-pressure saturated steam environment and then releases steam. The sudden decrease in pressure results in an explosion that fractures the lignocellulose into uneven fragments. High temperatures and pressures open the aromatic rings of lignin, leading to the separation of lignin from lignocellulose. Additionally, steam explosions disrupt the relationship between crystalline cellulose and disordered regions. Gunes *et al*. (2022) studied the subsequent effect of pretreatment of *Miscanthus* × *giganteus* biomass with LHW, enzymatic hydrolysis was

improved, and the concentration of fermentative sugar was increased. The primary objective of the thermal pretreatment is to eliminate hemicellulose and enhance its solubility, which is advantageous for subsequent enzymatic hydrolysis. Nevertheless, the removal of lignin is not efficient, and the thermal pretreatment process results in the production of inhibitors, such as furan and AA (Ko *et al*. 2015).

Mechanical pretreatment includes ball milling, rolling treat, shearing, and ultrasonic, *etc.* (Ouajai and Shanks 2006; Hendriks and Zeeman 2009). The lignocellulose can be cut into small pieces *via* the force of ball milling, shearing, and thread rolling, then increasing the specific surface area of the lignocellulose of enzymatic hydrolysis (Cao *et al*. 2023). After ball milling, the crystallinity index of pretreated hemp fibres decreased. After thread rolling, the cell wall of pretreated corn stalks was torn. Then the torn fibres could be converted into a fluffy structure, which was conducive to the contact of the enzyme with the cellulose (Ouajai and Shanks 2006). Ultrasonic pretreatment is a more appealing technology that disintegrates long chain organic compounds thanks to the vibration and the high-pressure environment generated inside the ultrasonic bath (Kucharska *et al*. 2018).

Irradiation pretreatment includes electron-beam irradiation (EBI), microwave irradiation, and gamma irradiation. Irradiation pretreatment transfers energy to the irradiated lignocellulose in the form of electron lines or radioisotopes generated by an electron accelerator. Ionizing radiation promotes ionization and excitation inside lignocellulose, releases orbital electrons, forms free radicals, and realizes the fission of the internal structure of lignocellulose (Guo *et al*. 2020). EBI pretreatment causes lignocellulose to split and layer rapidly, and the structure becomes coarse and porous (Fei *et al*. 2019). Microwave radiation pretreatment can be used to heat lignocellulose evenly in a short time, thereby promoting its hydrolysis and avoiding a large loss of cellulose (Ahorsu *et al*. 2019). Gamma irradiation pretreatment can effectively induce lignocellulosic cell disruption and lysis, thereby increasing the concentration of fermentation sugars in the solution (Chen *et al*. 2022). Irradiation pretreatment requires attention to the dose of irradiation used, as excessive irradiation can lead to the production of acetic and formic acids, and gamma pretreatment requires attention to the safety of its operation.

Physical pretreatment is environmentally friendly and easy to operate. Since it does not require a large amount of chemical reagents, it reduces chemical pollution. However, mechanical pretreatment, as part of physical pretreatment, has limitations. The equipment costs are high, and energy consumption is substantial. Some methods such as crushing and cutting only change the macroscopic structure of lignocellulose. It's difficult to break down lignocellulose at the molecular level. Therefore, it often needs to be combined with chemical and biological pretreatment methods. Thermal pretreatment and irradiation pretreatment can generate formic acid, acetic acid, and furan - based organic compounds during processing. These substances can inhibit subsequent enzymatic hydrolysis and fermentation reactions, which influence product yield. The generation of inhibitors is related to the composition of raw materials. Different biomasses vary in the content and structure of cellulose, hemicellulose, and lignin, and thus the types of inhibitors produced also differ. It is necessary to rationally select processing temperatures, time, irradiation doses, *etc.*, according to the composition of raw materials to minimize the generation of inhibitors.

Chemical Pretreatment

Inorganic solvent pretreatment includes acid, alkali, and inorganic salt pretreatments (Ojo and de Smidt 2023). Acids are widely used in pretreatment, including sulfuric, hydrochloric, aminosulfonic, and nitric acids. Because the hemicellulose molecular chain contains many hydroxyl groups, the acidic environment is enriched with hydrogen ions, and the hydrated hydrogen ions protonate the hemicellulose molecular chain glycosidic bond of oxygen atoms, thereby breaking the glycosidic bond in the molecular chain. Acid pretreatment can dissolve hemicellulose in the liquid and catalyze the substitution reaction of the lignin aromatic ring and the dehydration-condensation reaction of the sugar, removing the lignin, thereby exposing the cellulose, promoting enzymatic digestion, and increasing lactic acid production (Kucharska *et al*. 2018). As one of the classical pretreatment methods, acids are widely used in pretreatment. When sugarcane leaves were pretreated with acid, hemicellulose was completely removed, and the glucose yield was greater than 80% (Martins *et al*. 2022). With formic acid pretreatment, approximately 90.1% of cellulose and 87.1% of lignin were removed from corncobs (Qiao *et al*. 2021). Furthermore, the temperature must be controlled when acid is used as pretreatment, and cellulose can be dissolved when the temperature is higher than 160°C, decreasing the yield of fermentative sugar. It is also important to highlight that acids facilitate the condensation reaction of polysaccharides on the aromatic ring of lignin, resulting in the generation of pseudo-lignin, which subsequently inhibits enzymatic hydrolysis reaction (Martins *et al*. 2022).

Alkaline pretreatment includes the use of sodium hydroxide, calcium hydroxide, and ammonia (Guo *et al*. 2011; Ojo and de Smidt 2023). Alkaline pretreatment can break the ester bonds among lignin, cellulose, and hemicellulose and separate them, thus increasing the availability of fermentative sugars. Triwahyuni *et al*. (2023) used carbon dioxide and caustic soda for the pretreatment of empty oil palm fruit bunches; the rate of delignification reached an impressive 80.5%, and the glucose yield was 99.3%. The lignin in the straw consisted of G and S units with a minute amount of H. The G unit in lignin reacts with ammonia or hydroxide, which separates the lignin and facilitates the hydrolysis of hemicellulose and cellulose. Consequently, alkaline pretreatment is commonly used to preprocess lignocellulose from agricultural straw (Guo *et al*. 2011). According to Wang *et al*. (2017), the removal of lignin reached 67.5% and the hydrolysis efficiency increased by 2.12 times after the alkali pretreatment of *Sophora flavescens* with sodium hydroxide, and the efficiency of alkaline pretreatment was 3% higher than that without pretreatment. An unfavorable aspect of alkali pretreatment is that the pretreated biomass needs to be washed and sewage is produced, which increases production costs.

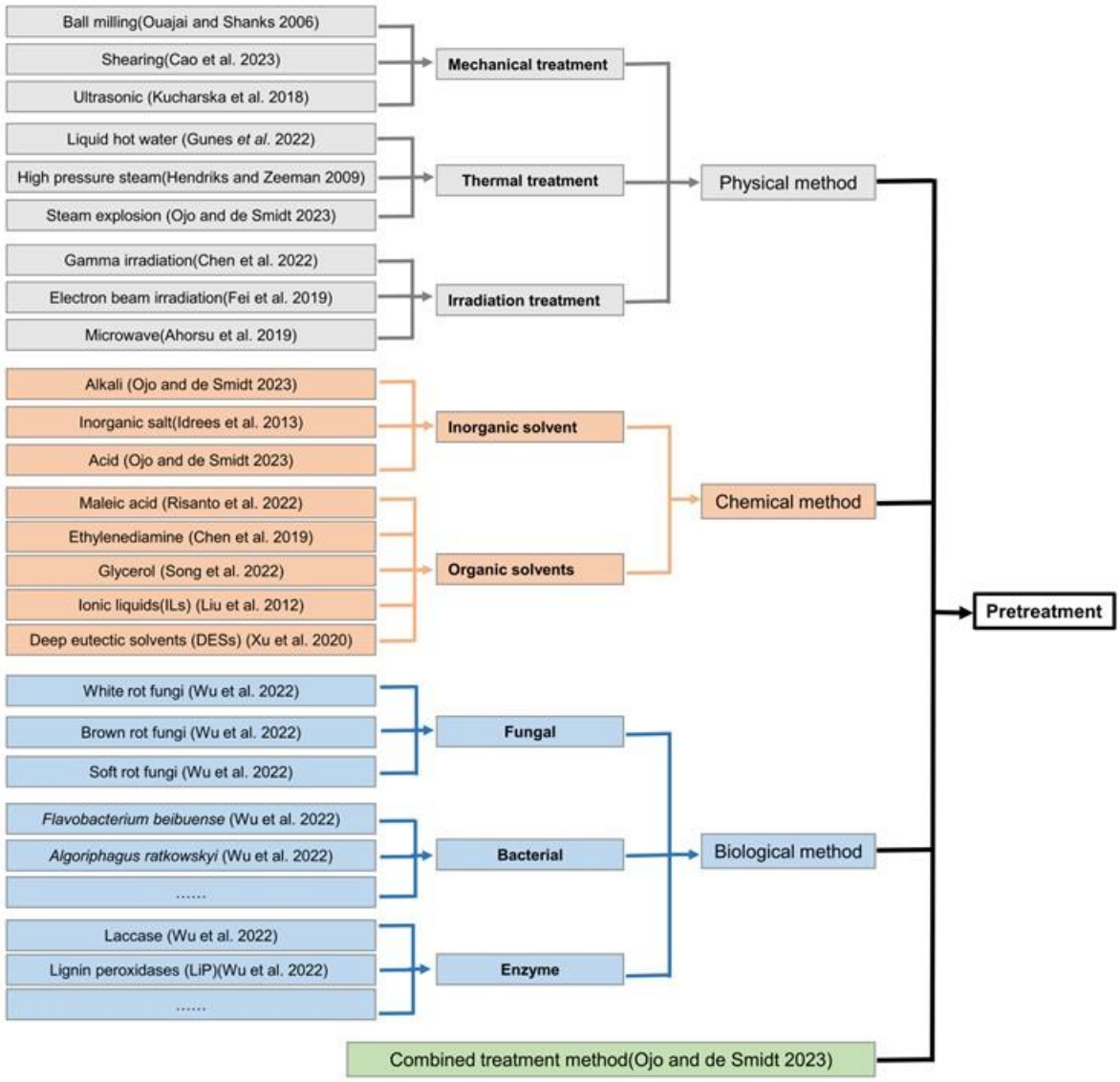

Fig. 3. Classification of lignocellulosic pretreatment technologies

Inorganic salt pretreatment involves the use of sulfate, sulfite, ferric chloride, and ferrous chloride (Wei *et al*. 2019). Metal ions primarily affect the separation of lignin from hemicellulose. The lower the lignin content in the biomass, the more noticeable the impact, and the higher the valence metal ions, the higher the removal of hemicellulose (Idrees *et al*. 2013). In the pretreatment of sulfate and sulfite, sulfate reacts with lignin to form sulfonated lignin, increasing the hydrophilicity of lignin and promoting the exposure of cellulose. When water hyacinth and bagasse were treated with Na_2S, lignin was almost completely removed (Sewsynker-Sukai *et al*. 2018). After pretreatment with $Na_3PO_4.12H_2O$-$ZnCl_2$, the silica on the surface of the oil palm empty fruit bunch (OPEFB) was removed, the internal lignin content was greatly reduced, and the crystal strength of the material was also reduced (Hassan *et al*. 2020). However, the residual metal ions after pretreatment are a major drawback, and metal ions can affect the protein activity of microorganisms, inhibiting the activity of the strain and reduces the yield (Huo *et al*. 2018).

The organic solvents used for lignocellulose pretreatment include glycerol, ethylenediamine (EDA), maleic acid, n-propylamine, isobutanol, acetone, and

tetrahydrofuran (Tang *et al*. 2017; Karnaouri *et al*. 2021; Chen *et al*. 2019; Risanto *et al*. 2022). Organic solvent pretreatment can be performed under mild conditions, and the solvent recovery efficiency after the treatment is very high. Two-step organosolv pretreatment allowed 86% glycerol and 92% ethanol recovery with 81.5% lignin removal (Song *et al*. 2022). Different organic solvents have varying effects on lignocellulose. Lignocellulose can disintegrate because of the strong affinity of EDA for the hydrogen-oxygen bonds between hemicellulose and cellulose. With pretreatment with EDA, the lignin and hemicellulose were removed, the surface features of the rice straw became rougher, and the pore volume and external surface of lignocellulose also increased, achieving a higher lactic acid concentration of 92.5 g/L (Chen *et al*. 2019). When sugarcane leaf was pretreated with maleic acid, the amorphous cellulose of the sugarcane matrix was affected, the surface of the matrix became rough, and the voids increased and were clearly visible (Risanto *et al*. 2022).

Organic solvent pretreatment has a certain selection effect on the lignocellulose components. After methanol pretreatment, pine and beech almost completely dissolved the hemicellulose. The main function of organic peroxide acids is to remove lignin, which can be tested at room temperature to prevent the formation of furfural and HMF at high temperatures. Glycerol solvent pretreatment can effectively extract sulfur-free lignin and promote lignin recycling. Nevertheless, organic solvent pretreatment has various disadvantages: methanol is toxic, has a low boiling point, and can easily form toxic vapors, and organic peroxide acids are expensive and increase production costs (Zhao *et al*. 2009).

Deep eutectic solvents are homogeneous mixtures of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). The melting points of the mixture are lower than those of single compounds (Liu *et al*. 2021). DESs are simple to prepare, highly stable, easily degradable, and exhibit good biocompatibility. The HBDs of a DES generally include glycerol, ethylene glycol, 4-hydroxybenzoic acid, acetic acid, and other organic acids. The HBA are generally betaine (Ba), acetamide (Am), and acetylcholine (ChCl). DESs have a highly selective extraction ability for lignin because they can form hydrogen bonds with lignin *via* the proton supply and electron acceptance ability of DES, break ether bonds and hydrogen bonds in lignin and deconstruct lignocellulose. In a study by Xu *et al*. (2020), corncob pretreatment by DES was analyzed using scanning electron microscopy (SEM) and X-ray diffraction (XRD), which revealed that the surface of the corncob became rough and loose, with obvious layered fractures, and the lignin and hemicellulose that covered the cellulose were destroyed. The higher crystallinity index values shown by XRD also indicate that the pretreatment removed lignin and hemicellulose. In a report by Liu *et al*. (2019), the wheat straw was pretreated *via* the DES, which is composed of benzyltriethyl ammonium chloride (TEBAC)/LA, and the removal rate of lignin reached 79.73 ± 0.93. Numerous studies have demonstrated that DES can be effectively used to pretreat lignocellulose. Despite the presence of DES in the pretreated liquid, cellulase and xylanase maintained their stability.

Ionic liquids are organic salts composed of organic cations, organic anions, or inorganic anions with a melting point near ambient temperature (Liu *et al*. 2012). ILs can be divided into four categories according to the type of organic cation used: quaternary ammonium, N-alkylpyridine, N-alkyl isoquinoline, and 1-alkyl-3-methylimidazole. Cellulose can be dissolved in certain ILs and recovered using water or ethanol. Wheat straw was heated with 1-ethyl-3-methylimidazolium acetate [EMIM]. Lignin was removed in large quantities, and the internal crystal structure of cellulose was significantly destroyed. After enzymatic hydrolysis, it was fermented by *Lactobacillus breve*, and the lactic acid

yield reached 0.49 g/g (Grewal and Khare 2018). Dadi *et al*. (2006) treated lignocellulose with 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), which increased enzymatic hydrolysis efficiency by a factor of 50. ILs can be recycled after lignocellulosic pretreatment without consuming significant amounts of energy. This is a green, environmentally friendly, and promising pretreatment method. The disadvantages of IL pretreatment are that the presence of IL inhibits microbial activity, is toxic to cells, and disrupts cell membranes.

After chemical pretreatment of lignocellulose, three major groups of inhibitors are produced: aldehydes, which are produced by dehydration of sugars under high-temperature acidic pretreatment conditions; weak acids, which are mainly derived from hydrolysis of acetyl groups in hemicellulose; and phenols, which are mainly produced during the degradation of lignin. In addition, residual inorganic ions are part of the inhibitors during pretreatment with inorganic salt solutions. These inhibitors interfere with the protein structure of the enzyme and affect the saccharification process. In order to reduce the production of inhibitors, one can choose the appropriate treatment according to the compositional characteristics of the lignocellulosic material. For example, when the content of hemicellulose in lignocellulose is high (*e.g.* from 20.0% to 43.0%.), one can choose organic solvents to treat hemicellulose relatively gently to reduce the production of acetic acid. When the cellulose content in lignocellulose is very high (*e.g.* between 30.0% and 50.0%), it is necessary to pay attention to the pretreatment temperature, the concentration of the acid solution and the length of pretreatment, to reduce the production of aldehyde inhibitors.

Biological Pretreatment

Biological pretreatment includes fungal, bacterial, and enzymatic pretreatment (Fig. 4). Fungal pretreatment includes white rot, brown rot, and soft rot fungi (Tian *et al*. 2018). Fungi secrete a series of extracellular enzymes to corrode and digest lignocellulose, transforming it from macromolecular to small molecular substances. *Phanerochaete chrysosporium* secretes lignin peroxidase (LiP), manganese peroxidase (MnP), and laccases. Laccase is a polyphenol oxidase containing four copper atoms that can react with lignin (Wu *et al*. 2022). The presence of laccase and MnP enables white-rot fungi to degrade lignin selectively. When white-rot fungi are used in the pretreatment of lignocellulose, hemicellulose and cellulose are retained as much as possible, and lignin is removed to the greatest extent. With the use of lignin and hemicellulose, cellulose is also catalyzed and utilized by fungi. Corn stover was pretreated with the white rot strain Blood Red Radish NRRL-FP-103506-Sp at 28 °C and 74.0% humidity for 30 days, and it was found that the loss of lignin reached 51.0 ± 1.2%, and the loss of hemicellulose and cellulose were 50.7 ± 2.1% and 25.4 ± 0.3%, respectively (Saha *et al*. 2016). Therefore, fungal pretreatment should focus on the processing time (Bao *et al*. 2022).

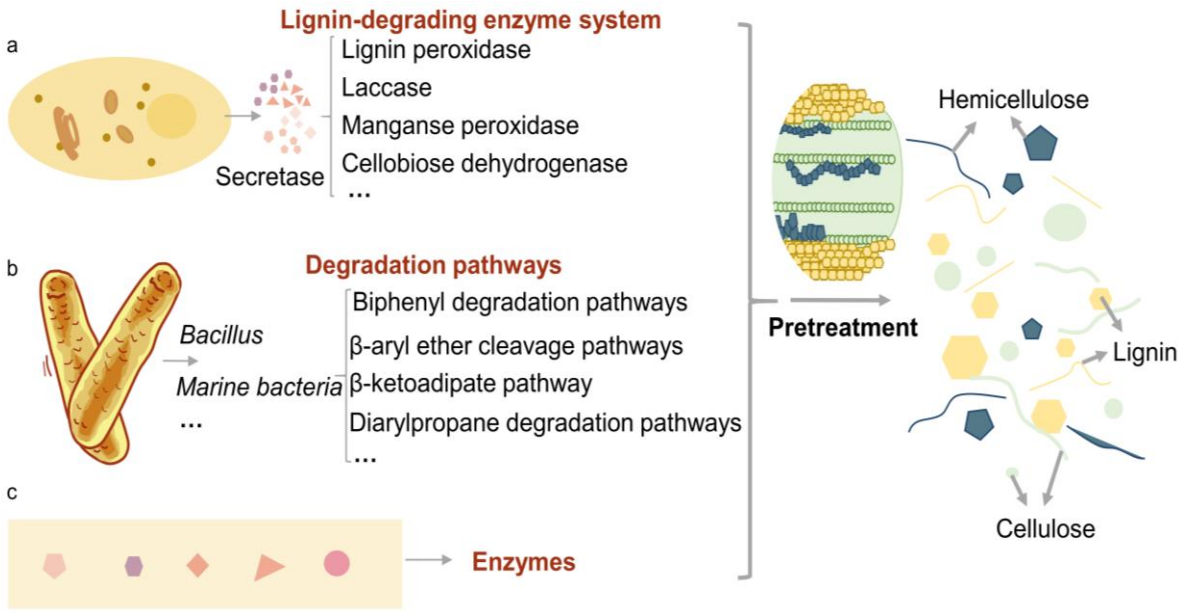

Fig. 4. Three ways of lignocellulose biological pretreatment. (a) fungal pretreatment; (b) bacterial pretreatment; (c) enzyme pretreatment.

Many types of bacteria are used for biological pretreatment. The most common way to break down the lignocellulose of bacteria is the β-ketoadipic acid pathway. Sodré *et al*. (2021) identified 10 species of bacteria that can break down the lignin *via* a β-ketoadipic acid pathway. *Bacillus lignophilus* L1 breaks down lignin *via* the gentianic acid pathway, benzoic acid pathway, and β-adipic acid ketone pathway. At 50 °C, lignin was the only carbon source, and *B. lignophilus* L1 degraded 38.9% of lignin within 7 days. The living environments and directional screening of bacteria are more extensive than those of fungi. *Flavobacterium beibuense*, *Algoriphagus ratkowskyi*, *Pseudomonas putida*, and *Halomonas meridiana* decomposed lignocellulose in high-salt environments. *Arthrobacter sp.* C2 can decompose lignin at a low temperature of 15 °C and has the activity of decomposing lignin in the range of pH 3 to 10 (Wu *et al*. 2022).

Biological enzymes play an important role in biological pretreatment. Laccase can degrade lignin through cleavage of the lignin side chain and a demethylation reaction and can use H_2O_2 as an oxidant to convert Mn^{2+} into a smaller chelating agent, Mn^{3+}, and then it can penetrate the dense structure. The β-ether enzyme also can degrade lignin without the cofactors (Tian *et al*. 2018). The purpose of lignocellulose pretreatment to promote the lactic acid yield can be achieved by adding enzymes, thereby eliminating the consumption of nutrients during strain growth (Fig. 4). As an indispensable enzyme in lignocellulosic bio-pretreatment, laccase has been genetically engineered to achieve rapid mass production in *Escherichia coli* with good heat resistance and thermal stability. In addition, the use of composite enzymes for lignocellulose treatment is superior to the use of single enzymes because of the synergistic effect between the composite enzymes (Wu *et al*. 2022).

Biological pretreatment can be carried out at an ambient temperature ranging from 25 to 50 °C. This not only significantly saves energy and reduces costs but also ensures the safety of the treatment process. Fungi have a stronger ability to decompose lignin compared to bacteria, and the pretreatment cost of fungi is lower than that of enzyme treatment (Saini and Sharma 2021). However, fungi require time to grow, which will extend the pretreatment cycle. Bacterial pretreatment has low energy consumption, does not require

chemical reagents, and has mild conditions. Nevertheless, many bacteria are difficult to cultivate successfully in the laboratory, and their performance cannot be stably inherited over successive generations, resulting in unstable pretreatment effects. Biological enzymes can efficiently hydrolyze biomass raw materials and improve the treatment efficiency. However, biological enzymes cannot regenerate themselves and need to be supplemented at an additional cost, which increases the overall treatment cost.

Combined Pretreatment Method

To improve the effectiveness of pretreatment in increasing bioresources yield, researchers have proposed comprehensive pretreatment; the combination of any two or even three of the aforementioned pretreatment methods can be primarily classified as a combination of physical and biological pretreatment. Expansion combined with *Irpex lacteus* fungal treatment to degrade wheat straw reduced the crystallinity of cellulose and destroyed lignin chemical bonds, which reduced the structural resistance of subsequent *I. lacteus* treatments and increased the enzyme activity of *I. lacteus*. Compared with *I. lacteus*, the pretreatment cycle was greatly reduced during co-processing (Cao *et al*. 2023). Pre-fermentation combined with acid pretreatment easily converted the water-soluble carbohydrates (WSCs) contained in bagasse into inhibitors during the pretreatment process. Pre-fermentation converted 98% of WSC into lactic acid, increasing total lactic acid production by 180% (Qiu *et al*. 2022). For microwave-assisted alkali pretreatment, a mixed solution of vinasse and NaOH was placed in a microwave, and after the pretreatment, lignin was removed and certain amounts of hemicellulose and cellulose were increased (Cao *et al*. 2019). Organic amine and organosolv synergistically pretreated corn stover using n-propylamine as a catalyst and aqueous ethanol as a solvent resulted in a delignification of 81.7%, and the total sugar yield was increased to 83.2% (Tang *et al*. 2017). The defining characteristic of joint preprocessing is that the preceding processing can offer certain advantages to subsequent processing; consequently, it is not possible to categorize and summarize this process in a straightforward manner. The combined pretreatment methods that have been practiced are listed in Table 1.

The pretreatment process usually causes lignocellulose to produce three types of inhibitors: acetates, furfural, and phenols. Hemicellulose contains a large number of acetyl groups in its structure, which are shed from the molecular chain of hemicellulose to form acetic acid under high temperature or acidic treatment conditions. Cellulose and hemicellulose are partially decomposed into monosaccharides such as hexose and pentose under pretreatment conditions, and under high temperatures and acidic conditions, pentose removes three molecules of water and undergoes molecular rearrangement to form furfural, and hexose removes three molecules of water and then undergoes molecular rearrangement under acidic conditions to form HMF. The main purpose of pretreatment technology is to remove lignin, and a variety of high temperatures, acids and alkalis, and strong oxidizing conditions will promote the connection of the structure of lignin bond breakage. Various high temperatures, acidic and alkaline conditions, and strong oxidizing conditions will cause the linkages in the structure of lignin to break, such as the main linkage of lignin, β-O-4, the common linkage β-O-4, and C-C, *etc*., and their breakage will lead to the production of phenolic inhibitors. Demethylation of the methoxy functional group in lignin or in alkaline environments generates phenolic inhibitors (Chandel *et al*. 2011).

EFFECT OF PRETREATMENT ON HYDROLYSIS AND FERMENTATION

Enzymatic hydrolysis of Cellulose and Hemicellulose

After pretreatment, the cellulose and hemicellulose in lignocellulose are initially broken down into fragmented structures of short sugar chains. The saccharification step breaks down polysaccharides into fermentable sugars such as glucose, xylose, arabinose, *etc*., and then microorganisms convert the sugars into bioresources through metabolic activity (Zhou *et al*. 2021). The cellulase system consists of endoglucanase, exoglucanase, and β - glucosidase. Endoglucanase randomly cleaves the β - 1,4 - glycosidic bonds within cellulose, breaking the long-chain cellulose. Exoglucanase cleaves cellobiose units from the non-reducing end of the cellulose chain, ultimately producing a large amount of cellobiose. Subsequently, β-glucosidase hydrolyzes cellobiose into glucose. The hemicellulase system includes xylanase, mannanase, arabinofuranosidase, *etc*. Different enzymes act on different glycosidic bonds of hemicellulose. Xylanase acts on the β-1,4-glycosidic bonds of the xylan main chain, degrading xylan into oligosaccharides and xylose. Mannanase hydrolyzes the β-1,4-glycosidic bonds in the mannan main chain. Arabinofuranosidase hydrolyzes the arabinofuranosidic bonds on the side chains of hemicellulose, causing the side chains to detach from the main chain, which facilitates the enzymatic hydrolysis of the main chain (Wang *et al*. 2015; Li *et al*. 2024). Other enzymes, such as galactosidase and glucuronidase, act on the corresponding glycosidic bonds (Ojo and de Smidt 2023). Pretreatment opens the structure of lignocellulose, separates lignin, cellulose and hemicellulose, and increases the contact sites for enzymes, promoting saccharification efficiency. However, the various inhibitors produced in the pretreatment can also have an effect on the saccharification step (Yang *et al*. 2024).

Saccharification strategies can be categorized into two types based on the source of enzymes: off-site and on-site. Separate hydrolytic fermentation (SHF) and simultaneous saccharification and fermentation (SSF), as well as separate hydrolytic co-fermentation (SHCF) and simultaneous saccharification co-fermentation (SSCF), which are derived from the combination of these two processes and the downstream fermentation process, are off-site saccharification strategies, where individually produced enzymes are added for saccharification to produce fermentable sugars. The two production processes of consolidated bioprocessing (CBP) and consolidated bio-saccharification (CBS), on the other hand, belong to the on-site method saccharification process, *i.e*., the enzyme production and saccharification process are concentrated in one system, and this saccharification strategy reduces the cost of enzyme production and isolation. Regardless of the saccharification strategy, the enzymes in the saccharification system are affected by inhibitors (Wang *et al.* 2024).

The inhibition of the enzyme in the saccharification step is simply divided into two types. One is the effect of inhibitors produced in the pretreatment step on the enzyme. Phenolic compounds will change the spatial structure of the enzyme, so that the center of the enzyme activity of the conformation of the enzyme activity is changed to reduce the activity of the enzyme. The reaction of the enzyme proteins weakens the stability of the enzyme. Phenolic inhibitors also adsorb on the surface of the substrate, hindering the contact between the enzyme and the substrate. Acid inhibitors affect the pH of the glycation system, inhibit secretion of enzyme synthesis, and inactivate the enzyme by altering its three-dimensional structure. Furfural inhibitors react with the functional groups of amino acids, thereby altering the chemical structure and properties of the enzyme and reducing its affinity for the substrate. On the other hand, as saccharification proceeds, cellobiose,

glucose, and xylose also inhibit the saccharification process. There is mainly glucose inhibition of β-glucosidase, xylan inhibition of cellulase, and cellobiose inhibition of cellobiose hydrolase. High concentrations of xylose also inhibit the binding of cellobiose to the enzyme's active site. When the saccharification step is in the same environment as the fermentation step, cellulase activity is also inhibited as the concentration of fermentation products increases (Wang *et al*. 2024).

The positive effect of pretreatment on fermentation

Good reaction conditions for enzymatic hydrolysis can be achieved by pretreatment. Scanning electron microscope (SEM) analysis in many pretreatment studies has shown that pretreatment would destroy the dense and ordered structure of the lignocellulose surface and expand the internal space of the material. After DES treatment, the biomass surface of aloe vera leaves became rough and disordered, and the epidermal structure of aloe vera leaves were destroyed (Rajeswari and Jacob 2022); after high boiling alcohol/water (HBAW) pretreatment, bamboo chips were disrupted and fragmented, after LHW pretreatment, large substrate pore volume was developed (Liu *et al*. 2017). In the rolling pretreatment, the fiber of corn stalk was separated and the top of the pile was cracked, the internal structure of the material was fluffy, and the specific surface area and pore size were significantly improved (Deng and Li 2021). After ball milling, the specific surface area of hemp doubled, and the pore volume expanded by 5.6 times (Ouajai and Shanks 2006). Fourier transform infrared (FTIR) analysis of many pretreatment studies has shown that various chemical bonds in lignocellulose, especially in hemicellulose and lignin, are broken under the action of temperature and chemical reagents. In one study, under the action of chemical reagents (or aqueous solution), lignocellulose was dissolved and generated sulfonated lignin, cellulose was exposed thereby promoting enzymatic hydrolysis, which showed that the peak intensity at 1734 cm^{-1} in the FTIR spectra of wheat straw pretreated by EBI was significantly reduced (Guo *et al.* 2020). Compared with the FTIR spectra of natural OPEFB, there were no bands at 1735 and 1740 cm^{-1} in the FTIR spectra of maleic acid pretreated OPEFB, indicating that pretreatment can effectively break down lignocellulose (Risanto *et al*. 2022).

Here, the fermentation production of lactic acid is used as an example to analyze the influence of the pretreatment step. Pretreatment increased lactic acid production (Table 2) by increasing the concentration of fermented sugars. The fermentable sugar concentration of bagasse was increased to 80.0 g/L after alkali pretreatment, and the fermentative sugar yield of olefinic acid pretreated bagasse increased from 0.13 to 0.65 g/g (Katepogu *et al*. 2022). Owing to the chemical properties of lignocellulose itself, especially those of hemicellulose, which is easily soluble at high temperatures, most of the hemicellulose is lost during pretreatment (Chang *et al*. 2012). Qiao *et al*. (2021) removed approximately 90.10% of xylan *via* formic acid cooking pretreatment. Similarly, alkaline pretreatment can remove a significant amount of lignin (43.0%) and hemicellulose from eucalyptus sawdust (Camesasca *et al*. 2021; Qiao *et al.* 2021). In contrast to previous knowledge, hemicellulose can also be converted to lactic acid by microorganisms when hydrolyzed to xylose, arabinose, and other pentoses. Therefore, appropriate pretreatment methods can be selected according to the characteristics of the fermentation strains. *Lactobacillus delbrueckii* sp. *bulgaricus* can make good use of cellulose to produce lactic acid, but the limitation is that the strain cannot use pentose; therefore, when selecting the pretreatment method, one needs only to consider the retention rate of cellulose (Karnaouri *et al*. 2020; Zhang *et al*. 2022). Pentose and hexose can be converted to lactic acid by some

strains, such as *Lactobacillus pentosus* CECT4023T and *Enterococcus mundtii* QU 25, and the pretreatment method with a higher hemicellulose retention rate is more suitable for use; for example, pretreatment with $Na_3PO_4.12H_2O$ resulted in a hemicellulose removal of 9.8% (Wang *et al*. 2014; Hassan *et al*. 2020).

Negative effects of inhibitors on fermentation

As shown in Table 2, pretreatment can enhance enzymatic hydrolysis and increase yield, but it may also introduce certain inhibitors. Pretreatment generally causes lignocellulose to produce three types of inhibitors: furans (HMF and 2-furfural), organic acids (AA, formic acid, and levulinic acid), and phenols (vanillin, syringaldehyde, 4-hydroxybenzaldehyde, pinealdehyde, ferulic acid and cinnamic acid) (Klinke *et al*. 2004; Yee *et al*. 2018). Inhibitors can enter the microbial cells, change the permeability of the cell membrane, destroy the integrity of the cell membrane, inhibit the growth and metabolism of microorganisms, reacting with nucleic acids and causing DNA damage, react with proteins, change the spatial structure of enzymes, interfere with enzyme activity, and change the original metabolic pathway. Acetic acid will also change the pH of the fermentation broth, bringing acid-base stress to microorganisms from the outside, increasing the energy burden of microorganisms, acetic acid can also enter the cell, reducing intracellular pH, affecting the survival of cells. Phenolic inhibitors have a certain degree of fat solubility, will destroy the cell's phospholipid bilayer, interfering with the normal physiological function of the cell. In addition, the condensation reaction between furfural and phenolic substances will form pseudo-lignin, which will be attached to the substrate and hinder the metabolic utilization of sugar by microorganisms (Chandel *et al*. 2013, Kucharska *et al*. 2018). Some solvents used in the pretreatment process, if not completely removed, will also become a class of substances that inhibit the metabolic activities of microorganisms, such as ILS, and ILS remaining in the pretreatment solution will lead to microbial poisoning (Wahlström and Suurnäkki 2015). During EBI pretreatment, cellulose reacts with the amino compounds produced by EBI to form copolymers, thereby inhibiting the catalytic activity of the enzyme (Fei *et al*. 2019). After pretreatment with EDA, the residual EDA did not affect the activity of the hydrolysis enzyme, but it inhibited the production and metabolism of lactic acid bacteria (Chen *et al*. 2019). In summary, inhibitors can affect the conversion and utilization of sugar by microorganisms and reduce product yield (Fig. 5).

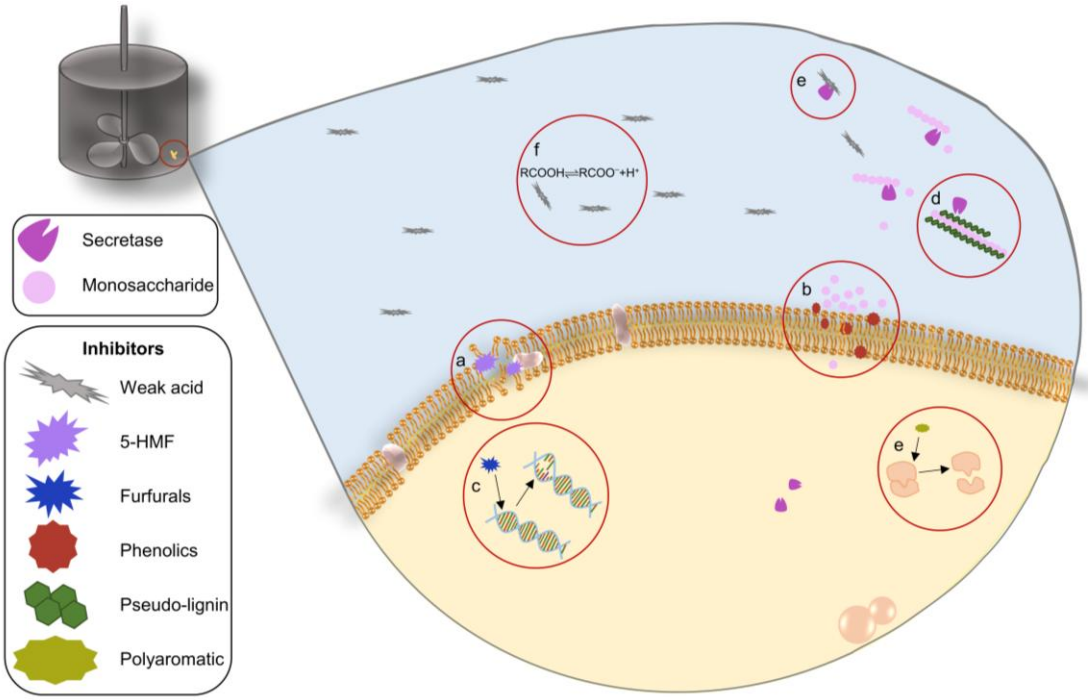

Fig. 5. Effect of inhibitors on Lactic acid production. (a) inhibitors interfere with the biofilm, causing its integrity to be lost; (b) Inhibitors interfere with the cell membrane and hinder the absorption and utilization of sugar by bacteria; (c) Inhibitor-induced gene mutation; (d) Pseudolignin deposition, hinder the enzymatic hydrolysis of sugar; (e) inhibitors lead to a decrease in enzyme activity; (f) The hydrolysis of weak acid will affect the pH value of fermentation broth.

Strategies to Overcome the Adverse Effects of Inhibitors

The presence and influence of inhibitors can be reduced in four aspects.

I: Appropriate pretreatment method selection to reduce the production of inhibitors. Qiu *et al*. (2023) reported that pre-fermentation and pretreatment can decrease the generation of inhibitors. The WSCs in bagasse are converted to furan inhibitors during acid pretreatment, and the pre-fermentation step converts 98.00% of the soluble carbohydrates into lactic acid, which reduces HMF formation.

II: A detoxification step is performed after pretreatment to reduce the inhibitors. The produced inhibitors can be removed or reduced in concentration by washing and cleaning the pretreated substrate with organic solvents. Chen *et al*. (2019) reported that the concentration of EDA can be decreased from 5.61 wt.% to 0.70 wt.% via washing in dilute EDA pretreated rice straw.

Table 1. Studies of Lignocellulosic Pretreatment in Recent Years

Treatment	Methods and Conditions	Lignocellulosic Biomass Materials	Pretreatment Effect	Reference
Physical	Steam explosion	Corn stalk	62.1% hemicellulose was removed	(Guo *et al*. 2011)
	Electron beam irradiation (100 kGy)	Wheat straw	The yield of lactic acid increased by 49.2%	(Guo *et al*. 2019)
	Microwave processes (190 °C)	Walnut shell	The conversion of hemicellulose was 96.4%	(Ahorsu *et al*. 2019)
	Ball-milled	Hemp fibre	Fibre bundles were being crushed, crystalline cellulose was destroyed	(Ouajai and Shanks 2006)
	Pressurized hot water	The plum orchard biomass	High cellulose content (48.5 g/100 g)	(Senila *et al*. 2023)
	Liquid hot water (200 °C)	Bamboo	The removal of hemicelluloses was 86.6%	(Liu *et al*. 2017)
	High temperature autohydrolysis (226 °C)	Forest waste	Yielding a glucan recovery of 93.0%	(Pontes *et al*. 2021)
	Thread rolling	Cane bagasse, corn stalks	The enzymatic hydrolysis effect was improved by 33.1%	(Deng and Li 2021)
Chemical	1.5% H_2SO_4	Wheat straw	The LA yield reaches 70.9% of the theoretical value	(Ouyang *et al*. 2020)
	1.0% H_2SO_4	Wheat straw	The concentration of reducing sugar increased by 30 fold	(Chawla and Goyal 2022)
	12.0% sodium sulfite	Corncob residue	The cellulose content in the pretreated residue was 85.2%	(Chen *et al*. 2019)
	Metal chlorides (0.3 mol/L Cl^-)	Eucalypt chips	Promote the efficient conversion of cellulose (96.3%)	(Huo *et al*. 2018)
	Formic acid (88%)	Corn cob	90.1% xylan and 87.1% lignin were removed	(Ouyang 2021)
	6.0% EDA[a]	Rice straw	LAyield 0.58 g/g	(Chen *et al*. 2019)
	15 % NaOH	Eucalyptus sawdust	Removal of 43% lignin	(Camesasca *et al*. 2021)
	2.5% maleic acid	Sugarcane trash	11.2 g/L of xylose was produced in sugarcane trash	(Oktaviani *et al*. 2019)
	DES (ChCl:Glyc,1:2) [b]	Fresh aloe vera	Reduce the fermentative inhibitors by 2.36-folds	(Rajeswari and Jacob 2022)
	DES (TEBAC:LA,1:9) [b]	Cane bagasse	Cellulose digestibility increased by 228.0%	(Liu *et al*. 2021)
	DES (ChCl:LA,1:3) [b]	Rice straw	Total sugar yield 75.0 g/L	(Huang *et al*. 2020)
Biological	*Irpex lacteus* incubate for 56 days at 28 °C	Wheat straw	Irpex lacteus degraded the lignin of wheat straws by more than 50.0%	(Niu *et al*. 2020)
	Coriolus versicolor	Sorghum bagasse	43.0% lignin degradation	(Mishra and Jana 2019)
	Enzymatic pretreatment	Corncob and vine trimming	Lignin removal up to 80%	(Perez-Rodriguez *et al*. 2016)
Combined	Ammonium sulfite + xylanase	Wheat straw	The total sugar recovery was 80.6%	(Yu *et al*. 2020)

	Oxidative+ tetrahydrofuran	Beechwood	Production of 62.0 g/L lactic acid	(Karnaouri *et al*. 2020)
	4.0% sulfamic acid and 3% sodium chloride	Corn stover	The cellulose transformation rate was 97.6%	(Song *et al*. 2020)
	CO_2 impregnation+ alkali explosion	Oil palm empty fruit bunch	Obtained 73.7% of delignification	(Triwahyuni *et al*. 2023)
	Fungus (*Irpex lacteus*)+ expansion	Buckwheat straw	Under the same degree of degradation, the time is shortened by half	(Cao *et al*. 2023)
	Ammonia+mechanical	Wheat Straw	Delignification of 62.5% and total sugar yield of 89.4%	(Cao *et al*. 2023)
	Alkaline+mechanical	Rice straw biopowder	The volume deformation increased by 110.0%, getting more sugar	(Chuetor *et al*. 2021)
a: EDA, ethylenediamine b: DES, Deep eutectic solvents (ChCl , acetylcholine; Glyc, glycerol; TEBAC, benzyltriethyl ammonium chloride; LA, lactic acid)				

Table 2. The Inhibitor Content in the Liquid after Pretreatment of Lignocellulosic Biomass, the Sugar Content in the Hydrolysate and the Lactic Acid Yield

Method and Operation	Lignocellulosic Biomass	Sugar Concentration (g/L)	Inhibitor Concentration	Fermentation Strains	Lactic Acid Yield (g/g)	Reference
1.50% H_2SO_4	Wheat straw	Xylose, 16.38; glucose, 2.42	Total inhibitor, 0.85 g/L	*Bacillus coagulans* CC17A	0.44	(Ouyang *et al*. 2020)
Pre-fermentation +2.50% H_2SO_4	Cane bagasse	Total sugar, 31.3	Acetate,2.7 mg/g; Furfural,20.7; HMF [b], 45.9 mg/L	*Pediococcus acidilactici* XH11	0.58	(Qiu *et al*. 2023)
6.00% EDA [a]	Rice straw	Total sugar, 22.66	Residual EDA	*Bacillus coagulans* LA-15-2	0.58	(Chen *et al*. 2019)
1.00% H_2SO_4 (120°C)	Wheat straw	Reducing sugar, 57.60	Furfural,0.47 g/L; HMF [b], 0.16 g/L	*Bacillus sonorenesis* DGS15	0.97	(Chawla and Goyal 2022)
1.00% H_2SO_4 (1:7) high solid loading	Garden garbage	Glucose, 40.90; Xylose, 20.40	Acetic acid, 4.11; Ethanol, 0.88 (g/L)	*Enterococcus mundtii* CGMCC 22227	0.62	(Zhu *et al*. 2023)
Aqueous extraction + H_2SO_4 catalyzed +steam explosion	Gardening residues	Total reducing sugar 30.95	Furfural, 0.20; HMF[b], 0.21; acetic acid, 1.15; formic acid, 0.23 g/L	*Lactobacillus pentosus* CECT4023T	0.70	(Cubas-Cano *et al*. 2020)
High solid loading+0.50 M sodium hydroxide	Sugarcane bagasse	Glucose, 63.80; Xylose, 16.18	Pseudo-lignin	*Thermophilic Bacillus coagulans* NCIM 5648	0.81	(Nalawade *et al*. 2020)
a: EDA, ethylenediamine b: HMF, 5-hydroxymethyfurfural						

Atmospheric glycerol solvent (AGO) can remove lignin components in lignocellulose well, but a large amount of residual AGO will adhere to the pretreatment substrate, which seriously affects the efficiency of enzymatic hydrolysis. Washing AGO with ethanol solution, xylose concentration increased from 8.50% to 62.0% (Song *et al*. 2022). Suitable strains can be selected for biological detoxification of the culture medium during fermentation. In the experiment of dry acid pretreatment of wheat straw, fungus was used to successfully decompose the inhibitor with a total concentration of 29.30 mg/g dry matter to a concentration of 2.20 mg/g, and the lactic acid yield was as high as 130.00 g/L after detoxification (Sodré*et al*. 2021; He *et al*. 2023).

III: An advanced fermentation production process was selected to reduce the concentration of inhibitors. Before fermentation, nanofiltration (NF) and reverse osmosis membranes were used to detoxify the inhibitors and concentrate the fermentable sugars. Under a certain pressure range, the NF membrane had good conductivity for inhibitors; formic acid was 90.30%, AA was 88.30%, furfural was 98.10%, HMF was 95.50%, and vanillin was 88.50%. Therefore, the NF membrane had excellent detoxification performance. Pan *et al*. using the batch feeding strategy for detoxification. A high inhibitor concentration of 13.50 g/L was achieved during the cork sequential pretreatment of sulfuric acid and steam (Pan *et al*. 2019). The use of and fed-batch fermentation process batch via *Pediococcus acidilactici* TY112 can convert inhibitors, with the lactic acid yield reaching 0.86 g/g (Campos *et al*. 2022).

IV: A reasonable selection of fermentation strains can reduce the effects of inhibitors to a certain extent. *Pediococcus pentosaceus* HLV1 can produce lactate under the stress of inhibitors (Katepogu *et al*. 2022), Ouyang *et al*. (2020) tested *Bacillus coagulans* CC17A, which can digest multiple inhibitors in the hydrolysate and 35.5 g lactic acid was produced from 80 g wheat straw, and the combination of *Pseudomonas putida* KT2440 and *B. coagulans* NL01 could directly produce lactic acid in highly toxic hydrolysates. *P. acidilactici* TY112 is a genetically engineered bacterium that cannot metabolize the fermentable sugars in the culture medium, but it can convert 100.0% of the organic acids and furan inhibitors produced by pretreatment, and the removal rate of most monoaromatic compounds is 90.0% (Aulitto *et al*. 2019). After the engineered bacteria are converted to remove most of the inhibitors, the yield of LA synthesized by *B. coagulans* NL01 using the hydrolysate was 0.80 g/g (Zou *et al*. 2021). The microbial community DUT47 was found to be robust to inhibitor compounds and could produce lactic acid 0.50 g/g in a sulfuric acid-pretreated sugarcane solution without detoxification treatment (Sun *et al*. 2021).

CONCLUSIONS AND PERSPECTIVES

Lignocellulose represents a significant raw material for bioresources production. The composition of lignocellulose includes cellulose, hemicellulose, and lignin. These polymers are held together by a multitude of chemical bonds, resulting in a highly stable and complex structure. Pretreatment represents an effective method for disrupting the overall structure of lignocellulose, facilitating the separation of lignin, the exposure of cellulose and hemicellulose, and enhancing the utilization of lignocellulose.

The primary objective of physical pretreatment is to destroy the physical properties of lignocellulose and disrupt its crystal structure. This process is relatively straightforward

and can be easily operation. Chemical pretreatment can select the appropriate treatment solution according to the specific type of lignocellulose components present, this method can effectively remove lignin while retaining hemicellulose and cellulose content. Biological pretreatment can be conducted under mild conditions and is an energy-efficient and environmentally friendly process. Nevertheless, it should be noted that the application of pretreatment techniques may result in the formation of a range of inhibitory compounds, including furfural, acetic acid, and phenolics. Inhibitors can be classified into three primary categories: 1) those that result from the further deoxygenation of monosaccharide substances during the pretreatment process, leading to the formation of furfural inhibitors. 2) acetic acid, which is formed by the deacetylation of sugar-rich acetyl groups, is the primary source of acid inhibitors. 3) various phenolic inhibitors are produced by lignin groups during the pretreatment process. Inhibitors affect both the saccharification step and the fermentation step. Inhibitors bind to the active site of the enzyme, preventing the enzyme from binding to polysaccharides, inhibitors react with groups of amino acids, altering the structure of the enzyme and affecting enzyme stability, inhibitors react with the enzyme, altering the enzyme's three-dimensional structure and rendering it catalytically incapable, and in the on-site saccharification strategy. Inhibitors also affect the activity of the cell, and the acidic environment created by acidic inhibitors exhausts the cell to resist the acidic environment, decreasing enzyme production. These effects are specific to the effect of inhibitors on the saccharification step. The specific effects of inhibitors on the fermentation step are reflected in the effects of various substances on cell integrity and enzyme activity. Inhibitors can enter the microbial cell, change the permeability of the cell membrane, damage the integrity of the cell membrane, damage the cell DNA, inhibit the growth and metabolism of microorganisms, interfere with the activity of enzymes, and change the metabolic pathway. Acetic acid also alters the pH of the fermentation broth, bringing acid-base stress to the microorganisms and increasing the energy burden of the microorganisms.

By analyzing the lignocellulosic pretreatment test and LA fermentation test in recent years, several feasible solutions to reduce the impact of inhibitors on LA production were summarized: improving pretreatment methods, detoxifying pretreatment solutions, optimizing fermentation production methods, and cultivating resistant strains. Taking this as a reference, it provides some new ideas for realizing more efficient bioresources production, alleviating energy crisis and creating environmentally friendly and green industrial products in the future.

ACKNOWLEDGMENTS

This work was supported by the National Key R&D Program of China (Grant NO. 2022YFE0118800), the National Natural Science Foundation of China (Grant NO. 22276012), the Ecological Environment Science & Technology Project of Anhui Province (Grant NO. 2023hb0015), the GDAS' Project of Science and Technology Development (Grant NO. 2019GDASYL-0102005), and the Guangdong Foundation for Program of Science and Technology Research (Grant NO. 2023B1212060044).

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Article submitted: November 14, 2024; Peer review completed: December 20, 2024; Revised version received: February 16, 2025; Further revision received and accepted: March 13, 2025; Published: March 24, 2025.
DOI: 10.15376/biores.20.2.Fan