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Agro-residue Pretreatment Tools and Techniques: A Detailed Review

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Abstract

The present review examined the significance of biomass pretreatment in facilitating efficient biofuel production from lignocellulosic materials. Different methods of pretreatment, such as acid, alkaline, liquid hot water, ionic liquids, organosolv, ozonolysis, wet oxidation, steam explosion, ammonia fiber explosion, carbon dioxide explosion, and biological pretreatment, were examined in terms of their underlying principles, benefits, and drawbacks. Acid pretreatment effectively hydrolyzes hemicellulose but suffers from equipment corrosion and high operational costs, while alkaline pretreatment removes lignin but requires long residence times and results in irrecoverable salts. Physicochemical methods such as steam explosion and ammonia fiber explosion offer economical options with varying effectiveness in degrading lignin and hemicellulose. Biological pretreatments are energy-efficient but have a slower hydrolysis rate. Overall, the present review provided insights into the diverse array of biomass pretreatment methods, guiding researchers and practitioners in selecting the most suitable approach for efficient biofuel production. It emphasized the importance of disrupting the complex lignocellulosic structure while considering factors such as cost-effectiveness, environmental impact, and process efficiency. By understanding the advantages and limitations of each method, stakeholders can make informed decisions to advance sustainable biofuel production from lignocellulosic biomass. This comprehensive review underscored the critical role of biomass pretreatment in overcoming structural barriers to bio-conversion processes. By evaluating various methods against criteria such as carbohydrate degradation, inhibitor formation, and cost-effectiveness, it offered a nuanced understanding of the complexities involved. This knowledge is essential for optimizing biofuel production while minimizing environmental impact. Drawing from botanical insights, the review delves into the intricate composition of lignocellulosic materials, highlighting the significance of disrupting the synergistic network of cellulose, hemicellulose, and lignin for efficient bio-conversion.

Keywords: biomass, hemicellulose, cellulose, lignin, treatment

INTRODUCTION

Biofuels can be produced from various lignocellulosic materials, such as wood, agricultural waste or forest [1]. The complex structural organization of lignocellulosic biomass often hinders with the bio-conversion processes. Therefore, biomass conditioning or biomass pre-treatment is necessary before using any of these technologies for disrupting the basic structure to ensure success of the process [2].

Lignocellulosic biomass, derived from various sources such as wood, agricultural waste, or forest residues, represents a promising avenue for biofuel production [3, 4]. However, the inherent complexity of its structure, comprising primarily of hemicellulose, cellulose, and lignin, poses significant

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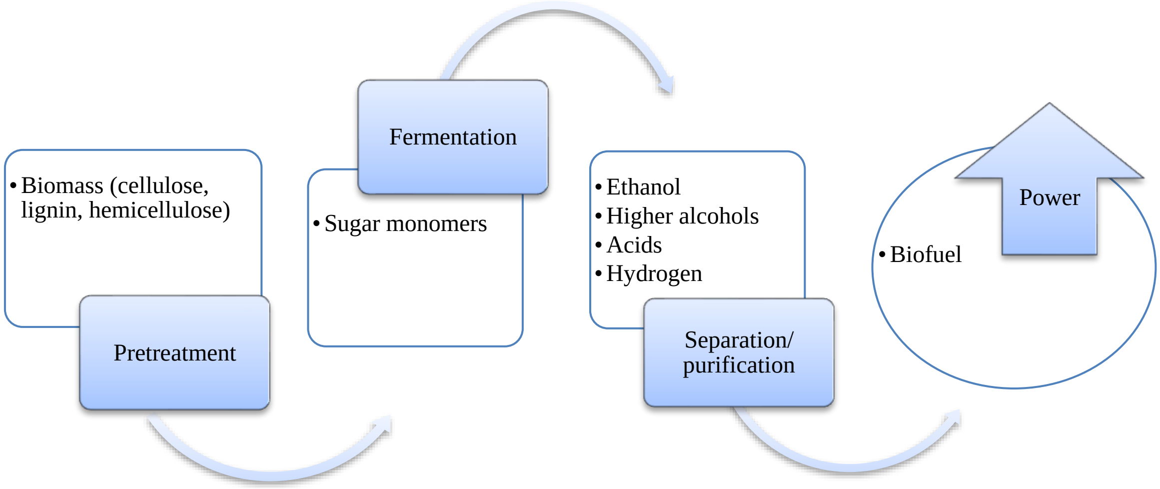


Figure 1. Significance of biomass pretreatment.

challenges to efficient bio-conversion processes [5]. Cellulose—a major component—is organized into macrofibrils, further composed of microfibrils, which, along with lignin and hemicellulose, impede enzymatic breakdown crucial for the conversion of biomass into fermentable sugars [6]. To overcome this barrier, biomass pretreatment becomes indispensable. Pretreatment methods (Figure 1) aim to disrupt the intricate architecture of lignocellulosic biomass, thereby liberating the sugar molecules necessary for subsequent fermentation into alcohols. Delving deeper into the botanical realm, cellulose—the structural backbone of plant cell walls, provides rigidity and support, while hemicellulose acts as a cementing material, contributing to cell wall integrity. Lignin, on the other hand, provides impermeability and resistance to microbial degradation, thus fortifying the plant against environmental stresses [7]. Understanding the intricate interplay of these components underscores the importance of tailored pretreatment strategies to unlock the biofuel potential inherent in lignocellulosic biomass, fostering sustainable energy solutions while leveraging botanical knowledge to drive innovation in bioenergy research. Biomass conditioning or fuel pretreatment includes all the steps necessary to produce an upgraded biomass fuel from a harvested biomass resource. An effective pre-treatment should meet the following requirements:

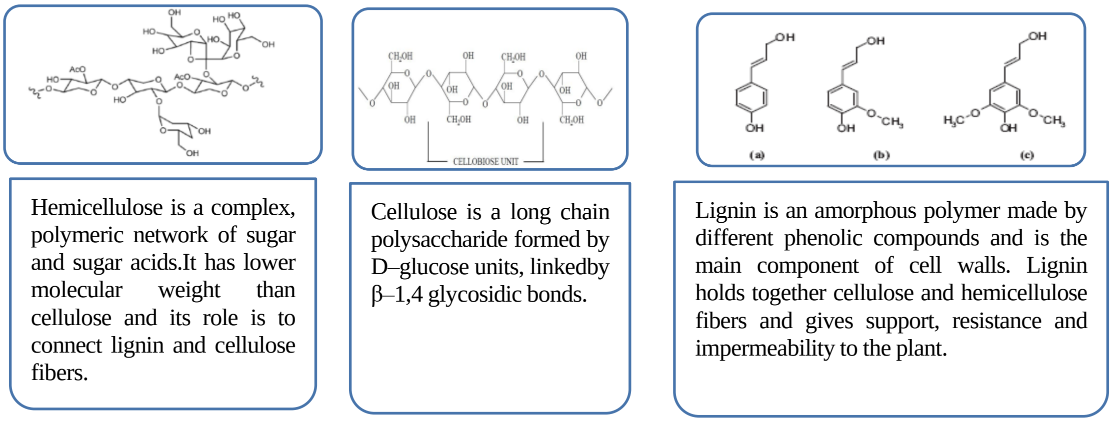
1. Disrupt the three-dimensional structure of lignocellulose, cellulose, and hemicellulose.
2. Facilitate high yields of sugars or chemicals.
3. Check carbohydrate degradation.
4. Check the formation of inhibitory toxic byproducts.
5. Allow lignin recovery to give valuable coproducts.
6. Be cost-effective, involving reasonable size reactors, low wastes amount and low energetic requirements [8].

BASIC PRINCIPLE OF PRE-TREATMENT

Before studying existing biomass pretreatment, let us have a look at the basic components of lignocellulosic biomass (Figure 2):

1. Hemicellulose
2. Cellulose
3. Lignin

Besides this, cellulose is made up of macrofibrils, which in turn are made up of microfibrils and these structures along with lignin and hemicellulose hinder the enzymatic actions necessary for breakdown of biomass into sugars that can be fermented to alcohols. Thus, the role of pre-treatment methods is to disrupt complex structure of lignocellulosic biomass and free the sugar molecules [9]. Chemical composition of major agro-residues in India are depicted in Table 1 and some concept behind pretreatment are depicted in Figure 3.



Hemicellulose is a complex, polymeric network of sugar and sugar acids.It has lower molecular weight than cellulose and its role is to connect lignin and cellulose fibers.

Cellulose is a long chain polysaccharide formed by D–glucose units, linkedby β–1,4 glycosidic bonds.

Lignin is an amorphous polymer made by different phenolic compounds and is the main component of cell walls. Lignin holds together cellulose and hemicellulose fibers and gives support, resistance and impermeability to the plant.

Figure 2. Building blocks of lignocellulosic biomass.

Table 1. Chemical composition of major agro-residues in India (Percentage dry weight basis).

Biomass	**Cellulose**	**Hemicellulose**	**Lignin**
Rice	36–47	19–25	10–24
Wheat	33–50	24–36	9–17
Maize	35–40	17–35	7–18

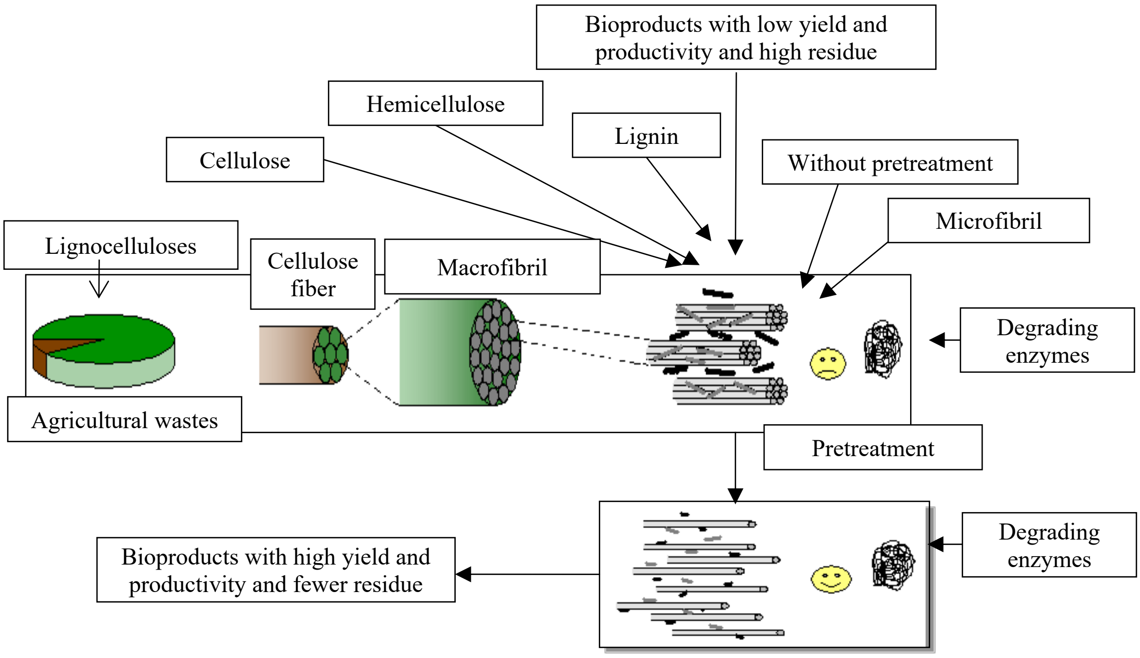


Figure 3. Concept behind pretreatment.

Different Types of Available Pretreatment Methods

Today many types of pretreatment methods are available and choice of the method depends upon the type of conversion process, desired end product, and effective cost of the process (Figure 4) [10].

Physical Pretreatment

Physical pretreatment methods (Table 2) aim at changing physical properties of the biomass to facilitate its conversion. The most commonly used physical treatments include mechanical chunking, milling or grinding, and extrusion [11, 12]. Figures 5 and 6 are Hammer Mill and twin-screw extruder which is used in the physical pre-treatment.

Physical methods	**Chemical and physio-chemical**	**Physico-chemical methods**	**Biological methods**
• Mechanical • Irradiation: • Gamma-ray irradiation • Electron-beam irradiation • Microwave irradiation • Extrusion	• Alkaline • Acid • Wet oxidation • Ozonolysis • Organosolv • Ionic liquids • Hot liquid water	• Steam explosion • Ammonia fiber explosion • Carbon dioxide explosion	• Bacterial enzymes • Fungal enzymes

Figure 4. Different pretreatment methods available.

Table 2. Physical pretreatment.

Mechanical	The objective of the mechanical pretreatment is reduction of particle size and to increase the specific. This procedure involves a combination of chipping, grinding, or milling, which varies depending on the desired particle size of the material (ranging from 10 to 30 mm after chipping to 0.2–2 mm after milling or grinding). Different type of milling processes such as ball milling, two-roll milling, hammer milling, etc. can be used to improve the biodegradability of waste materials. *Advantages:* Reduces cellulose crystallinity [13]. *Limitations:* High power consumption by the machines make it uneconomical.
Extrusion	The lignocellulosic structure is affected by the screw speed and barrel temperature causing heating, mixing and shearing of materials resulting in physical and chemical modifications, defibrillation, fibrillation, and shortening of the fibers which increases accessibility to enzymatic attack. *Advantages:* Completely breaks the 3-D structure *Limitations:* High power consumption [14].

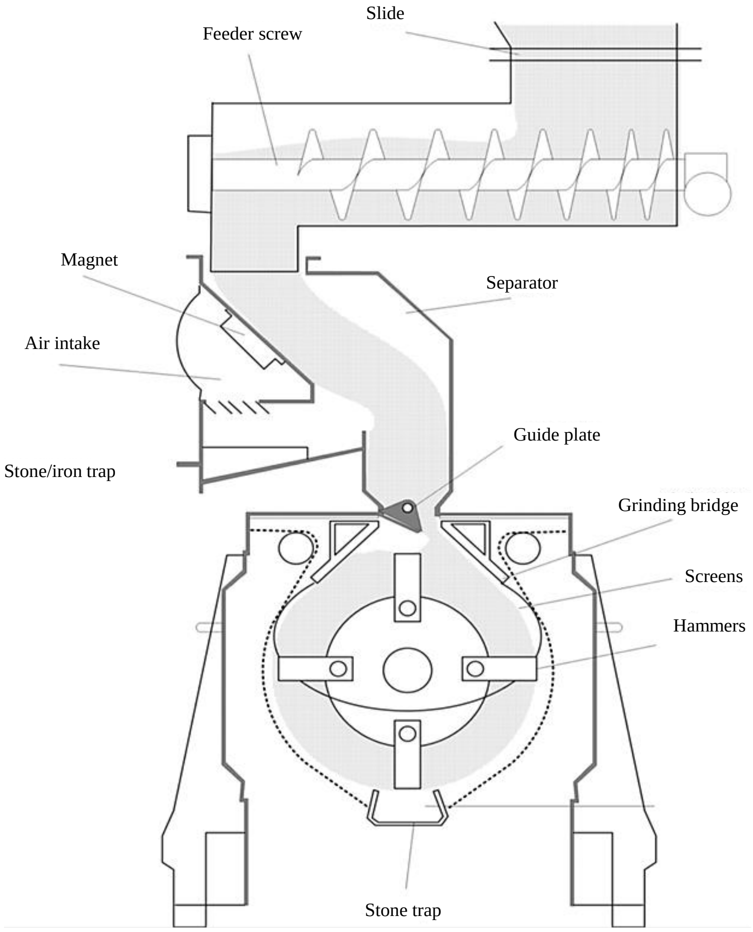


Figure 5. Hammer Mill.

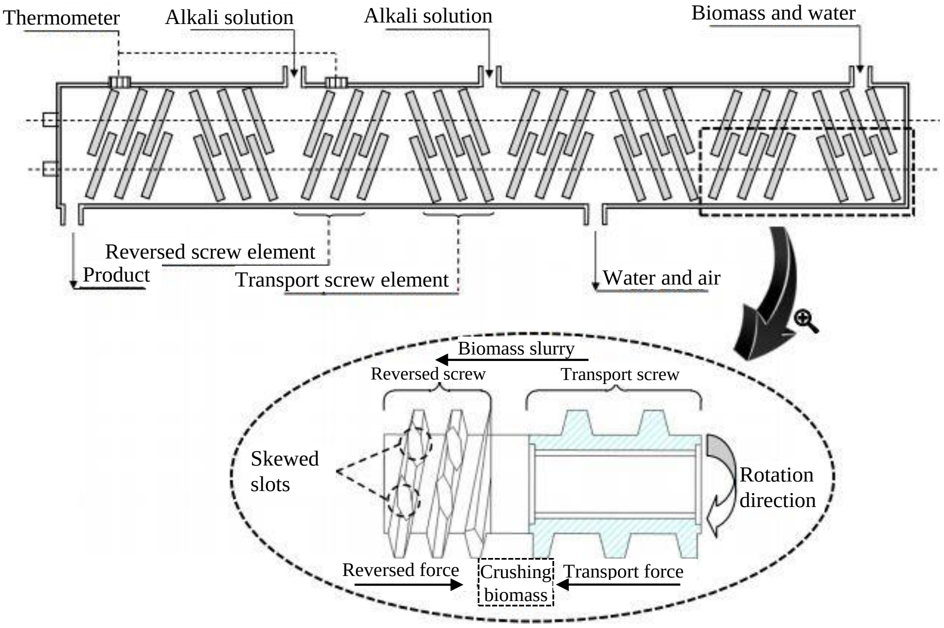


Figure 6. Schematic diagram of the specially designed twin-screw extruder.

Chemical Pretreatment

This type of pretreatment has the capability of removal of lignin and thus decreasing the degree of complexity of the structure. Some of the commonly used methods are (Table 3) [15].

Table 3. Chemical pretreatment.

Acid pretreatment	Hydrolysis of hemicellulose into xylose, mannose, acetic acid, galactose, and glucose occurs during acid pretreatment. Both dilute and concentrated acids can be used for it. Sulfuric acid is the most used acid, while other acids, such as nitric acid, hydrochloric acid, phosphoric acid have also been used. *Advantage:* The use of acids is very effective in pretreatment of the lignocellulosic feedstock especially for bio-alcohol production. *Limitations:* It is less attractive due to the formation of inhibiting compounds, equipment corrosion, toxic nature, and high operational and maintenance costs.
Alkaline pretreatment	The primary impact of alkaline pretreatment is the elimination of lignin from the biomass, leading to enhanced reactivity of the remaining polysaccharides. Furthermore, alkali treatments remove diverse functional groups linked to hemicellulose, which otherwise hinder enzyme access to the surfaces of hemicellulose and cellulose [16]. *Advantage:* Removes hemicelluloses and lignin; increases surface area. *Limitations*: Long residence times is needed. Salts that are formed and become integrated into the biomass cannot be recovered.
Liquid Hot Water (LHW)	LHW pretreatment is similar to steam explosion but uses water in the liquid state at elevated temperatures (160–240°C) instead of steam. *Advantage:* Results in hemicelluloses hydrolysis and removal of lignin, rendering cellulose in the biomass more accessible while avoiding the formation of fermentation inhibitors that occur at higher temperatures [17]. *Limitation:* High power and water consumption.
Ionic liquids (ILs)	The hydrogen bond breaks between molecular chains of the cellulose on interaction between cellulose-OH and ILs and results in dissolution of cellulose. Most ILs used in the biomass fractionation are imidazolium salts such as N-methylmorpholine-N-oxide monohydrate (NMMO), 1-n-butyl-3-methylimidazolium chloride (BMIMCl), 1-allyl-3-methylimidazolium chloride (AMIMCl), 3-methyl-N-bytylpyridinium chloride (MBPCl). *Advantage:* Low toxicity; high selective breakdown of biomass components. *Limitation:* High cost for plant and ILs; high viscosity.
Organosolv Process	In this process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H_2SO_4) is used to break the internal lignin and hemicellulose bonds. Organic acids such as oxalic, salicylic and acetylsalicylic acids can also be used as a catalyst in the organosolvation process.

	The solvents commonly used in the process are methanol, ethanol, acetone, ethylene glycol, triethylene glycol, glycerol, aquous phenol. The usual operation temperature of organosolv falls in the range of 150–200°C [18]. *Advantages:* Hydrolyzes lignin and hemicellulose. *Limitations:* Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost.
Ozonolysis	Ozone has the capability to breakdown lignin and hemicellulose in numerous types of lignocellulosic materials, including wheat straw, rye straw, bagasse, green hay, peanuts, pine, and cotton straw. *Benefit:* This method is conducted under standard room temperature and pressure conditions, avoiding the generation of inhibitory compounds. In contrast to alternative chemical treatments, it does not yield any harmful residues. *Drawbacks:* The process can become costly due to the substantial quantity of ozone needed.
Wet Oxidation	Wet oxidation is an oxidative pretreatment technique utilizing oxygen or air as a catalyst at temperatures exceeding 120°C. Key parameters such as temperature, reaction duration, and oxygen pressure play pivotal roles in wet oxidation. *Benefit:* Adding a base to wet oxidation effectively oxidizes lignin in wheat straw, rendering the polysaccharides more susceptible to enzymatic breakdown [19]. *Drawbacks:* Close supervision of temperature and pressure is essential, and the process requires a lengthy reaction time.

Physicochemical Pretreatment

Such treatment methods use a combination of both physical and chemical action for breaking the complex structure (Table 4).

Table 4. Physicochemical pretreatment.

Steam explosion	In this method, the material is treated with steam at a high temperature for few minutes to facilitate subsequent enzymatic hydrolysis of cellulose and hemicellulose to monomeric hexose and pentose sugars. The material is also impregnated with an acid prior to steam pretreatment to increase the overall sugar yield. Steam explosion is conducted at a temperature of 160–260°C and 0.69–4.83 MPa pressure for several seconds to few minutes before the material is exposed to atmospheric pressure. *Advantage:* Causes hemicellulose degradation and lignin transformation and is an economical process. *Limitations:* Incomplete disruption of the lignin–carbohydrate matrix; generation of inhibitory compounds [20].
Ammonia fiber explosion (AFEX)	In the AFEX method, biomass undergoes treatment with liquid ammonia under elevated temperature and pressure. Shortly thereafter, the pressure rapidly decreased. A typical AFEX procedure involves using 1–2 kg of ammonia per kg of dry biomass at 90°C for 30 min. During the pretreatment, only a small amount of solid material solubilizes; that is, almost no hemicellulose or lignin is lost. The structure of the material is changed, resulting in increased water holding capacity and higher digestibility. *Advantages:* Enhances surface area accessibility, partially eliminates lignin and hemicellulose, and does not generate inhibitors for subsequent processes. *Limitations:* Not efficient for biomass with high lignin content.
Carbon dioxide explosion	This method utilizes CO_2 as a supercritical fluid at elevated pressure, effectively solubilizing lignin. CO_2 permeates the biomass, leading to enhanced digestibility under high pressure. The theory suggests that when CO_2 mixes with water, it forms carbonic acid, which aids in the hydrolysis of hemicellulose. *Advantage:* Increase surface area; cost-effective; does not cause formation of inhibitory compounds. *Limitation:* Does not modify lignin or hemicelluloses.

Biological Pretreatment

In this group of pretreatments, microorganisms such as bacteria and fungi are employed to degrade cellulose, hemicellulose and lignin. The rate of hydrolysis and energy requirements is very low [21] (Table 5). Figures 7 and 8 show cellulases and xylanases.

Table 5. Biological pretreatment.

Cellulases	Cellulose is enzymatically degraded to glucose by the synergistic action of cellulases. Commercial sources of cellulases are *Aspergillus niger*, *Trichoderma reesei*, *Bacillus* sp., etc.
Xylanases	Hemicellulosic fraction of lignocellulosic biomass mainly consists of xylan which can be degraded by a group of enzyme called xylanases. Commercial sources of cellulases and xylanases are *A. niger*, *T. reesei*, *Bacillus* sp., etc.
Peroxidases	The breakdown of lignin is facilitated by a specific category of enzymes called peroxidases. True ligninases, known for their elevated redox potential, were initially identified in *Phanerochaete chrysosporium*.

Laccases	The breakdown of lignin is facilitated by laccases, which are enzymes containing copper. Laccases are also referred to as blue copper proteins or blue copper oxidases.
Other helper proteins	The helper proteins work synergistically with the hydrolytic enzymes such as cellulases to increase the accessibility of the substrate to the enzymes. *T. reesei* is known to secrete a helper protein known as swollenin which disrupts the structure of cotton fibers.

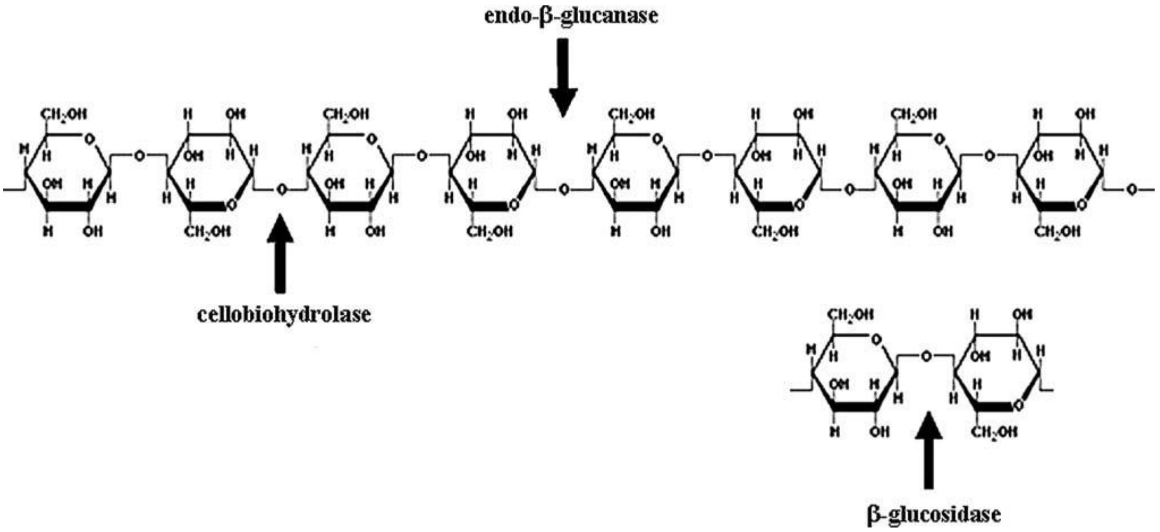


Figure 7. Cellulases.

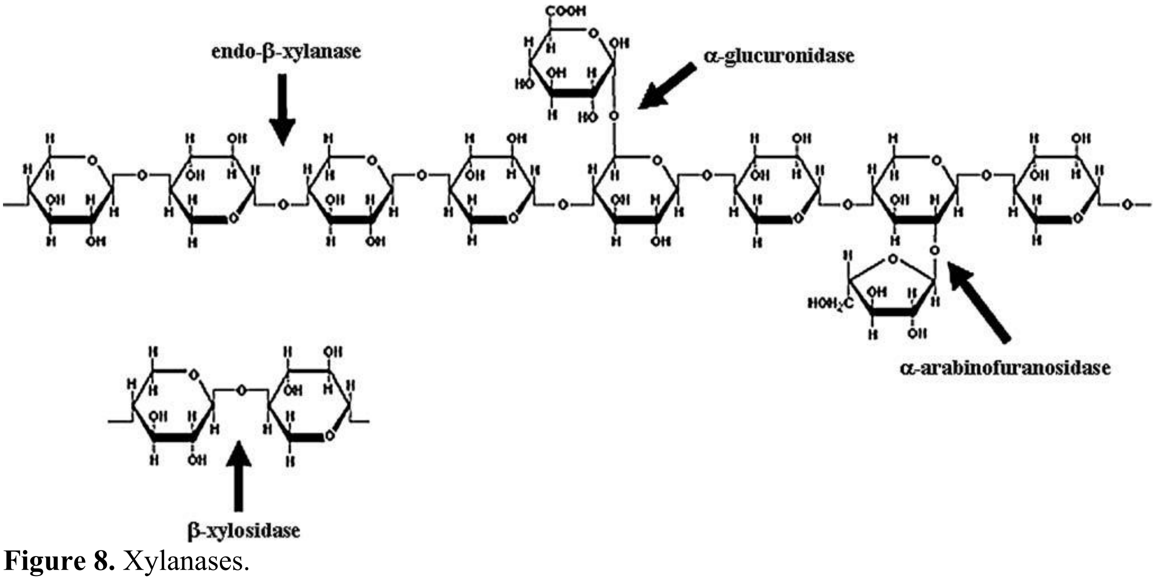


Figure 8. Xylanases.

Separation Techniques

During hydrolysis, at high temperature and pressures, glucose and xylose degrade into compounds such as formic acid, acetic acid, and phenol derivatives etc. which have inhibitory effect on enzymes secreted by the yeast *Saccharomyces cerevisiae* that brings about fermentation of these sugars into alcohols. Therefore, these inhibitors are required to be removed prior to fermentation to improve the efficiency of the process. Various detoxification techniques have been employed to transform inhibitory compounds into inert substances or to decrease their concentration [22, 23]. Summary of various pretreatment methods is depicted in Table 6.

- *Physical methods:* Evaporation removes volatile compounds such as acetic acid.
- *Chemical methods:* Neutralization, calcium hydroxide overliming, use of ion exchange resins, activated charcoal.
- *Biological methods:* Enzymatic detoxification such as peroxidase and laccase.

Table 6. Summary of various pretreatment methods.

S.N.	Pretreatment method	Feed-stock	Pretreatment conditions	Yield
1.	Physical pre-treatment using singe screw extruder	Switchgrass	Barrel temperature 176°C, screw speed 155 rpm, moisture content 20% wb, particle size 8 mm, Cellulase 15 FPU/g and β-glucosidase 60 CBU/g of dry matter.	Glucose 41.4%, xylose 62.2%
1.	Acid pre-treatment	Sugarcane bagasse	2–6% H_2SO_4, 100–128°C temperature, 0–300 min	21.6 g/l xylose, 3 g/l glucose, 0.5 g/l furfural and 3.65 g/l acetic acid in 24 min at 122°C and 2% H_2SO_4
2.	Acid pre-treatment	Cellulose and grass	1–10% H_3PO_4, 150–200°C temperature, 0–15 min	6.7% (w/w) xylose, 2.5% (w/w) arabinose and 6.1% (w/w) glucose of dry grass in 15 min at 170°C and 2.5% H_3PO_4
3.	Acid pre-treatment	Sugarcane, depithed bagasse and pith bagasse	1.2% (v/v) HCl, 121°C temperature for 4 h	Reducing sugar yield 37.21% for sugarcane depithed bagasse and 35.37% for sugarcane pith bagasse
4.	Alkaline pre-treatment	Wheat straw	2.15% H_2O_2 (v/v), pH 11.5, 35°C temperature for 24 h	8.6% (w/v) monomeric sugars
5.	Alkaline pre-treatment	Sorghum bicolor straw	2% NaOH, 60°C temperature for 60–90 min	4.3-fold increase in total sugar with 2% NaOH at 60°C for 90 min
6.	Alkaline pre-treatment	Corn stover	0.5 g Ca $(OH)_2$, 55°C temperature for 4 weeks, enzyme 15 FPU/g cellulose	Yield of glucose 93.2% and xylose 79.5%
7.	Steam explosion	Olive-tree pruning	190–240°C temperature with impregnation by water or H_2SO_4 solution	Ethanol yield (7.2 g of ethanol/100 g of raw material) is obtained with water impregnated at 240°C
8.	LHW	Wheat straw	Temperature (170°C and 200°C), residence time (0 and 40 min), solid concentration (5% and 10% (w/v)), pressure in reactor (30 bar), enzymatic hydrolysis using commercial cellulases	Sugar recovery (53% of content in raw material) and enzymatic hydrolysis yield (96% of theoretical)
9.	AFEX	Corn stover	Aqueous ammonia for soaking, period of 10–60 days at room temperature and atmospheric pressure, Spezyme CP enzyme, simultaneous saccharification and fermentation with *S. cerevisiae* (D5A)	About 55–74% lignin removed but retained nearly 100% of the glucan and 85% of xylan. About 77% of ethanol yield based on glucan and xylan content.
10.	CO_2 explosion	Aspen (hardwood), southern yellow pine (softwood)	Pretreatment with supercritical CO_2 (SC-CO_2) at 3100 and 4000 psi, temperature 112–165°C for 10–60 min, moisture content 0–73% (w/w), enzymatic digestibility with commercial cellulose	Yield of 84.7 ± 2.6 and 27.3 ± 3.8% sugar at 3100 psi and 165°C for 30 min. SC-CO_2 pretreatment with moisture content of 40%, 57%, and 73% showed higher final sugar yields as compared to without SC-CO_2.
11.	Ionic liquids	Wheat straw	Using ionic liquid 1-ethyl-3-methyl-imidazolium diethyl phosphate, temperature 30°C for 30 min fermentation with *S.cerevisiae*	Yield of reducing sugar reached at 54.8% after being enzymatically hydrolyzed for 12 h, ethanol production was 0.43 g/g glucose within 26 h.
12.	Organosolv process	Hybrid poplar chips	Temperature, time, catalyst dose and ethanol concentration using a composite (180°C, 60 min, 1.25% H_2SO_4 and 60% ethanol) enzyme loading (20 filter units of cellulose/g cellulose).	About 82% of cellulose was recovered as monomeric glucose for 24 h, ~85% was recovered after 48 h hydrolysis.
13.	Ozonolysis	Wheat and rye straw	Ozonated wheat and rye straw under room condition.	Yields of upto 88.6% and 57% as compared to 29% and 16% in non-ionated wheat and rye straw.
14.	Wet oxidation	Softwood (*Picea abies*)	Temperature 200°C for 10 min at neutral pH.	Highest yield of about 79% in seven hours.

CONCLUSION

Biomass pretreatment plays a pivotal role in unlocking the potential of lignocellulosic materials for biofuel production. Despite diverse challenges and considerations, ranging from structural complexities to economic viability, a nuanced understanding of pretreatment methods empowers stakeholders to navigate towards sustainable and efficient bioconversion processes. By balancing technological innovation with environmental stewardship, the path towards a greener energy future becomes increasingly attainable.

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