

Agro- residue Pretreatment Tools and Techniques: A Detailed review

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Abstract:

This review examines the significance of biomass pre-treatment in facilitating efficient biofuel production from lignocellulosic materials. Different methods of pre-treatment, such as acid, alkaline, liquid hot water, ionic liquids, organosolv, ozonolysis, wet oxidation, steam explosion, ammonia fiber explosion (AFEX), carbon dioxide explosion, and biological pretreatment, are 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 AFEX offer economical options with varying effectiveness in degrading lignin and hemicellulose. Biological pretreatments are energy-efficient but have a slower hydrolysis rate.

Overall, this review provides insights into the diverse array of biomass pre-treatment methods, guiding researchers and practitioners in selecting the most suitable approach for efficient biofuel production. It emphasizes 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 underscores the critical role of biomass pre-treatment in overcoming structural barriers to bio-conversion processes. By evaluating various methods against criteria such as carbohydrate degradation, inhibitor formation, and cost-effectiveness, it offers a nuanced understanding of the complexities involved. This knowledge is essential for optimizing biofuel production while minimizing environmental impact. This comprehensive review underscores the critical role of biomass pre-treatment in overcoming structural barriers to bio-conversion processes. By evaluating various methods against criteria such as carbohydrate degradation, inhibitor formation, and cost-effectiveness, it offers 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.

We know Biofuels can be produced from various Ligno-cellulosic materials, such as wood, agricultural waste or forest[1]. The complex structural organisation of lingo cellulosic biomass often hinders with the bio-conversion processes. Therefore, before using any of these technologies Biomass Conditioning or Biomass Pre-treatment is necessary for disrupting the basic structure to ensure success of the process[2].

Ligno-cellulosic 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 challenges to efficient bio-conversion processes[5]. Cellulose, a major component, is organized into microfibrils, 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 pre-treatment becomes indispensable. Pre-treatment methods (Figure 1) aim to disrupt the intricate architecture of ligno-cellulosic 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 pre-treatment strategies to unlock the biofuel potential inherent in ligno-cellulosic biomass, fostering sustainable energy solutions while leveraging botanical knowledge to drive innovation in bioenergy research.

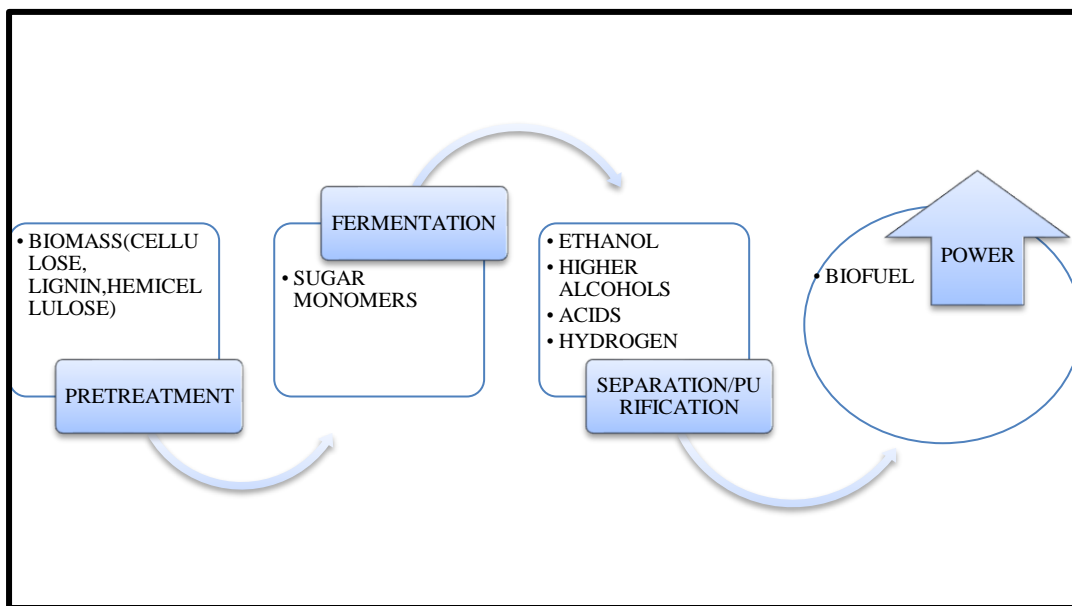


Figure 1: Significance of Biomass Pre-treatment

Biomass Conditioning or fuel pre-treatment 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 by-products

- 5) Allow lignin recovery to give valuable co-products
- 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 pre-treatment, let us have look at the basic components of Ligno-cellulosic biomass (Figure 2):

1. Hemicellulose
2. Cellulose
3. Lignin

Besides it, Cellulose is made up of microfibrils, 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 Ligno-cellulosic biomass and free the sugar molecules[9]. Chemical composition of major agro-residues in India depicts in table 1 & some concept behind pre- treatment depicts on the figure 3.

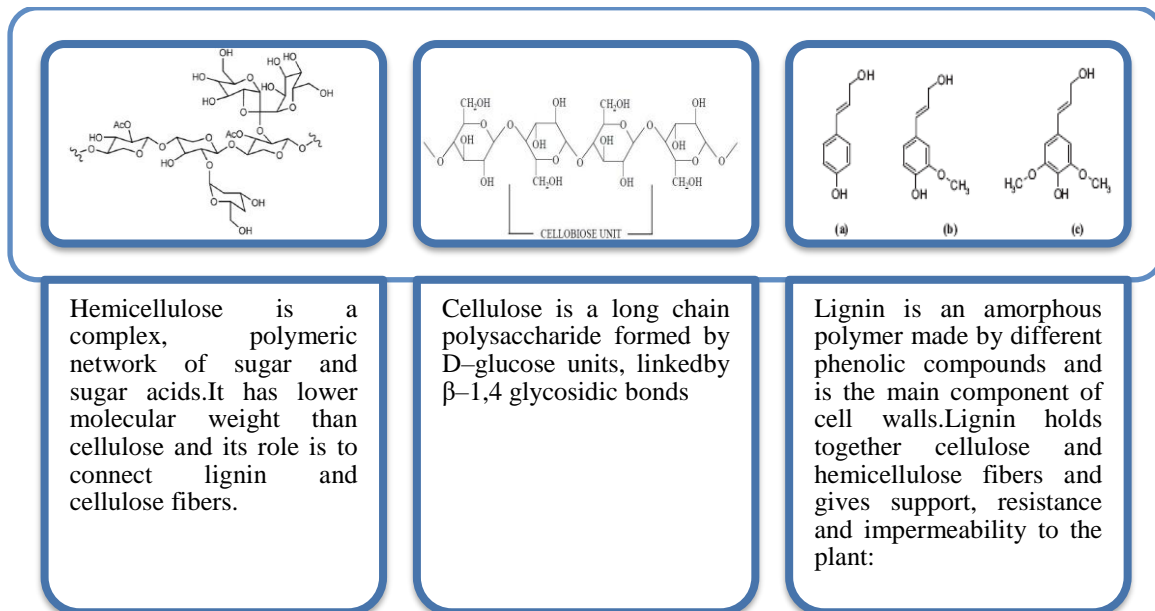


Figure 2. Building Blocks of Ligno-cellulosic Biomass

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 |

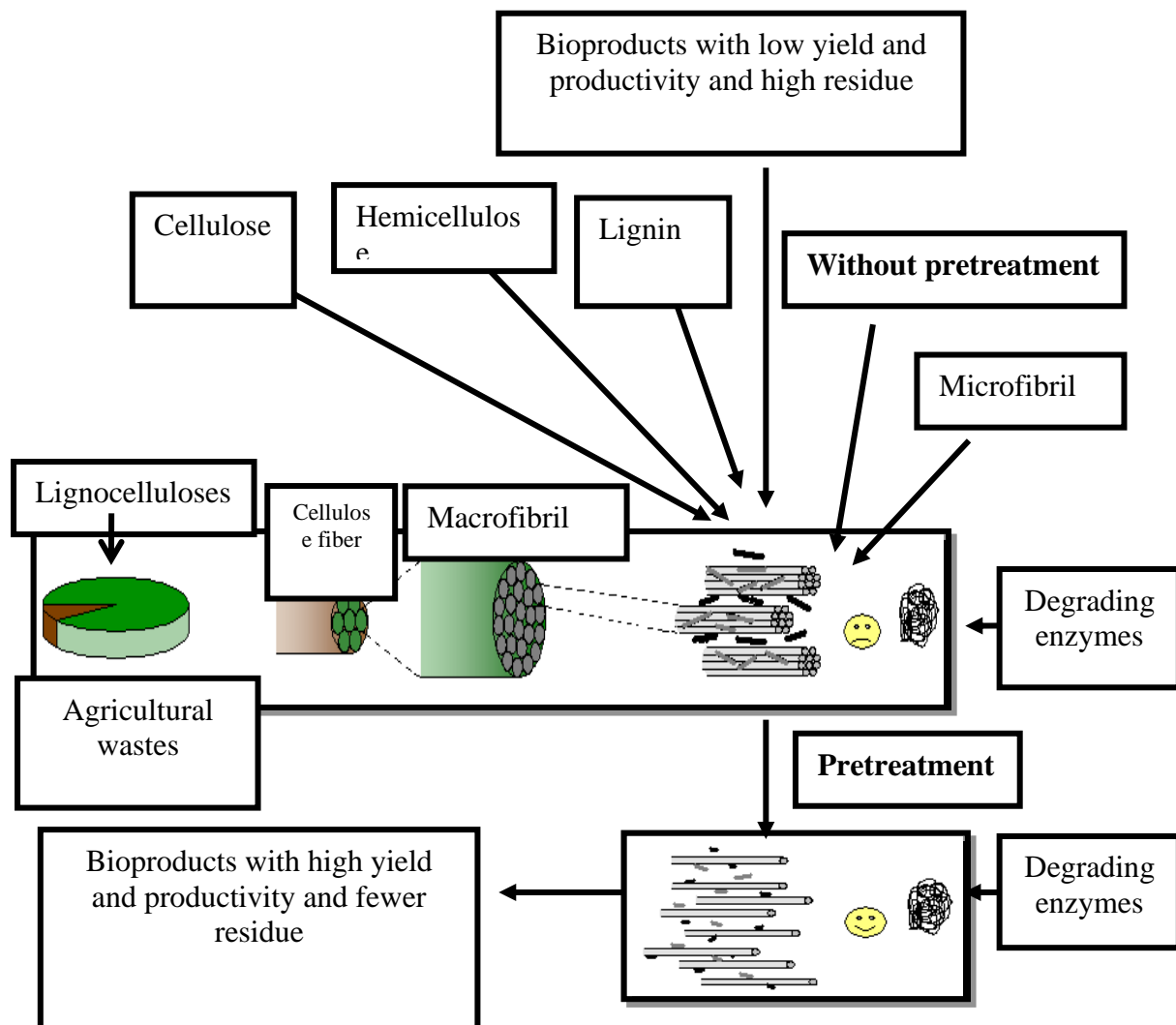


Figure 3: Concept behind Pre-treatment

Different Types of Available Pre-Treatment Methods:

Today many types of Pre-treatment 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 Methods | Chemical and Physio-chemical | Physico-chemical Methods | Biological Methods |
|---|---|--|---|
| <ul style="list-style-type: none"> • Mechanical • Irradiation: <ul style="list-style-type: none"> • - Gamma-ray irradiation • - Electron-beam irradiation • - Microwave irradiation • Extrusion | <ul style="list-style-type: none"> • Alkaline • Acid • Wet oxidation • Ozonolysis • Organosolv • Ionic liquids • Hot Liquid Water | <ul style="list-style-type: none"> • Steam Explosion • Ammonia Fiber Explosion • CarbonDioxide Explosion | <ul style="list-style-type: none"> • Bacterial Enzymes • Fungal Enzymes |

Figure 4. Different of Pre-treatment methods are available

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].

Table 2: Physical pretreatment

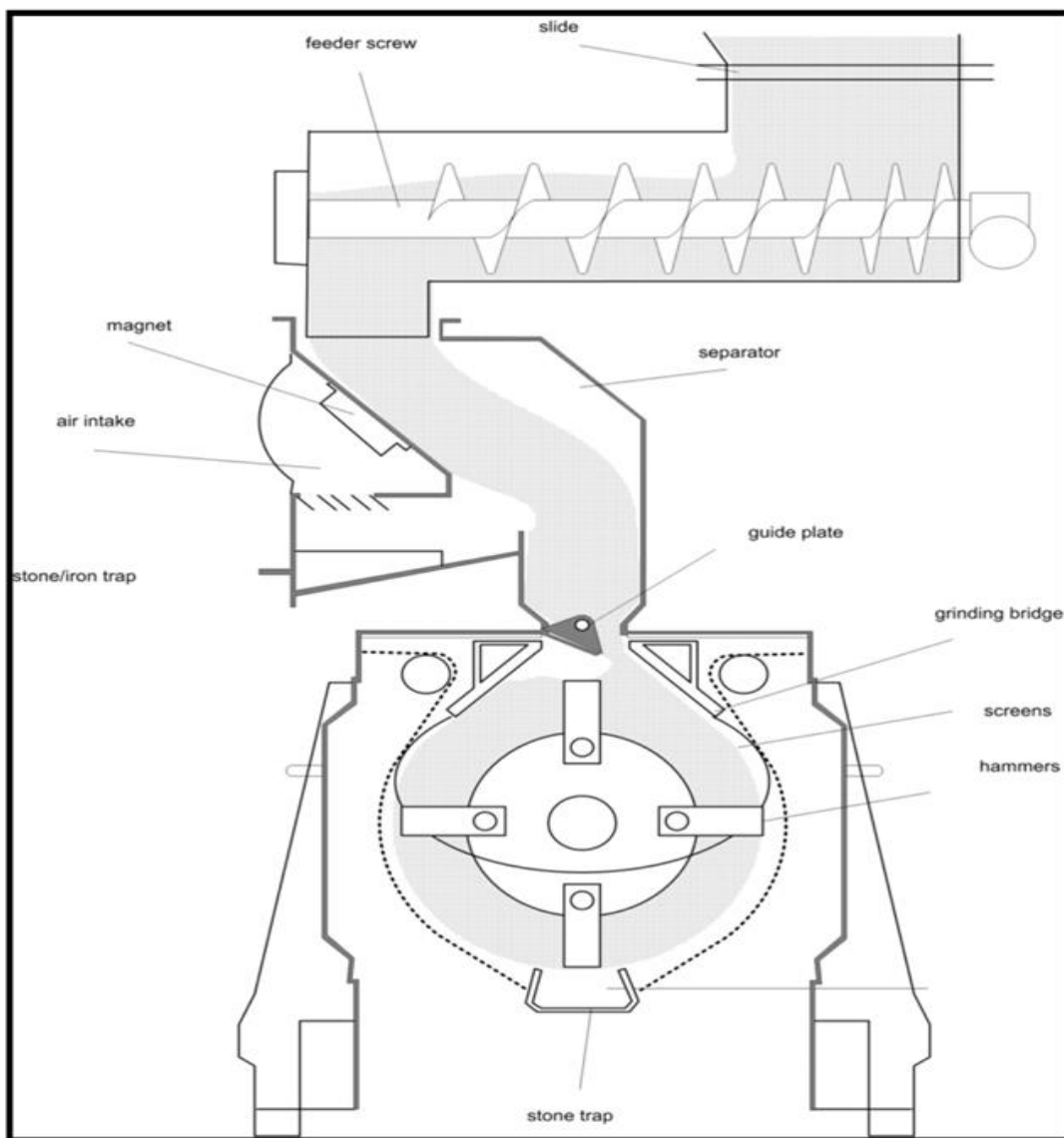
| | |
|-------------------|---|
| Mechanical | <p>The objective of the mechanical pretreatment is reduction of particle size and to increase the specific .</p> <p>This procedure involves a combination of chipping, grinding, or milling, which varies depending on the desired particle size of the material (ranging from 10-30 mm after chipping to 0.2-2 mm after milling or grinding).</p> <p>Different type of milling processes such as ball milling, two-roll milling, hammer milling etc (Figure 5). can be used to improve the biodegradability of waste materials.</p> <p>Advantages:Reduce cellulose crystallinity[13]</p> <p>Limitations:High power consumption by the machines make it uneconomical.</p> |
|-------------------|---|

Extrusion

The lingo-cellulosic 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 break the 3-D structure

Limitations: High power consumption[14]



Figure

5:

Hammer

Mill

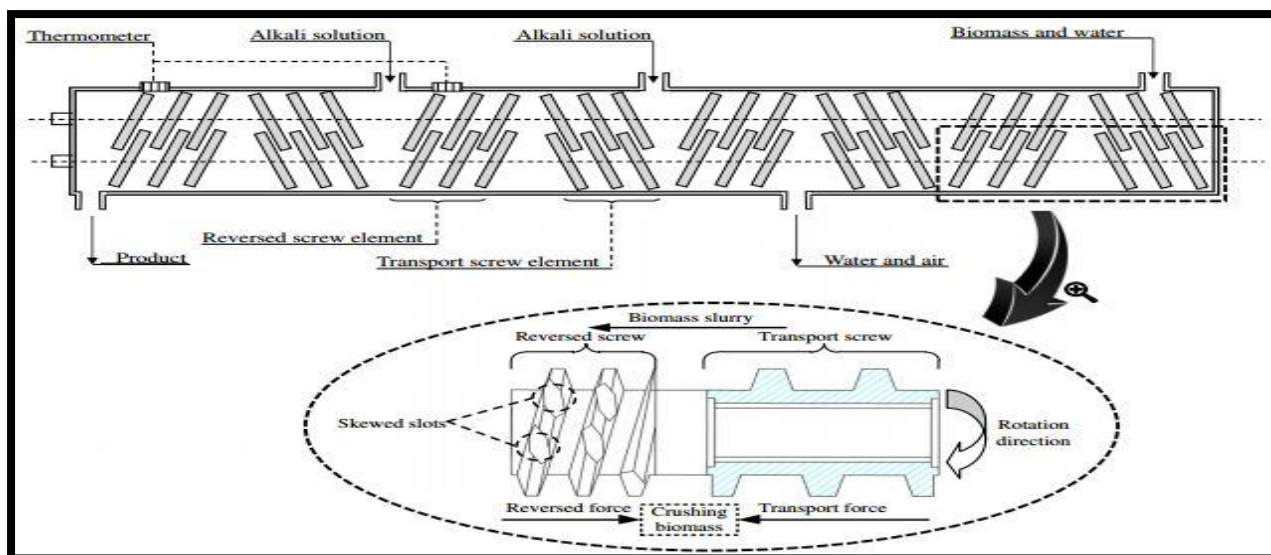


Figure 6. Schematic diagram of the specially designed twin-screw extruder

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

| | |
|-------------------------------------|---|
| <p>Acid Pretreatment</p> | <p>Hydrolysis of hemicellulose into xylose, mannose, acetic acid, galactose, and glucose occurs during acid pretreatment.</p> <p>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.</p> <p>Advantage: The use of acids is very effective in pretreatment of the lingo-cellulosic feedstock especially for Bio-alcohol production.</p> <p>Limitations: It is less attractive due to the formation of inhibiting compounds, equipment corrosion, toxic nature, and high operational and maintenance costs.</p> |
| <p>Alkaline Pretreatment</p> | <p>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</p> |

| | |
|-------------------------------|--|
| | <p>diverse functional groups linked to hemicellulose, which otherwise hinder enzyme access to the surfaces of hemicellulose and cellulose. hemicelluloses and other components such as lignin. Various bases like sodium hydroxide, calcium hydroxide (lime), aqueous ammonia, ammonium hydroxide, hydrogen peroxide or combination of two can be used for purpose[16].</p> <p>Advantage:removes hemicelluloses and lignin; increase surface area</p> <p>Limitations:long residence times is needed; Salts that are formed and become integrated into the biomass cannot be recovered.</p> |
| Liquid Hot Water (LHW) | <p>LHW pretreatment is similar to steam explosion but uses water in the liquid state at elevated temperatures (160-240 °C) instead of steam.</p> <p>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]</p> <p>Limitation:High power and water consumption</p> |
| Ionic Liquids (ILs) | <p>The hydrogen bond breaks between molecular chains of the cellulose on interaction of cellulose-OH and ILs and results the dissolution of cellulose.</p> <p>Most ILs used in the biomass fractionation are imidazonium salts like N-methylmorpholine-N-oxide monohydrate (NMMO), 1-n-butyl-3-methylimidazolium chloride (BMIMCl), 1-allyl-3-methylimidazolium chloride (AMIMCl), 3-methyl-N-butylpyridinium chloride (MBPCl).</p> <p>Advantage:Low toxicity; high selective breakdown of biomass components</p> <p>Limitation:High cost for plant and ILs; high viscosity.</p> |
| Organosolv Process | <p>In this process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCl or H₂SO₄) is used to break the internal lignin and hemicellulose bonds.</p> <p>Organic acids such as oxalic, salicylic and acetylsalicylic acids can also be used as a catalyst in the organosolvation process.</p> <p>The solvents commonly used in the process are methanol, ethanol,</p> |

| | |
|----------------------|---|
| | <p>acetone, ethylene glycol, triethylene glycol, glycerol, aqueous phenol.</p> <p>The usual operation temperature of organosolv falls in the range of 150-200 °C[18].</p> <p>Advantages:hydrolyzes lignin and hemicellulose</p> <p>Limitations: solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost.</p> |
| Ozonolysis | <p>Ozone has the capability to break down lignin and hemicellulose in numerous types of lignocellulosic materials, including wheat straw, rye straw, bagasse, green hay, peanuts, pine, and cotton straw.</p> <p>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.</p> <p>Drawbacks: The process can become costly due to the substantial quantity of ozone needed.</p> |
| Wet Oxidation | <p>Wet oxidation is an oxidative pre-treatment 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.</p> <p>Benefit: Adding a base to wet oxidation effectively oxidizes lignin in wheat straw, rendering the polysaccharides more susceptible to enzymatic breakdown[19].</p> <p>Drawbacks: Close supervision of temperature and pressure is essential, and the process requires a lengthy reaction time.</p> |

Physicochemical pretreatment

Such treatment methods use a combination of both physical and chemical action for breaking the complex structure (Table 3).

Table 3: Physicochemical pretreatment

| | |
|------------------------|--|
| Steam Explosion | <p>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.</p> |
|------------------------|--|

| | |
|---------------------------------------|---|
| | <p>The material is also impregnated with an acid prior to steam pretreatment to increase the overall sugar yield. Steam explosion is carried out 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.</p> <p>Advantage: causes hemicellulose degradation and lignin transformation and is an economical process.</p> <p>Limitations: incomplete disruption of the lignin-carbohydrate matrix; generation of inhibitory compounds[30].</p> |
| Ammonia Fiber Explosion (AFEX) | <p>In the AFEX method, biomass undergoes treatment with liquid ammonia under elevated temperature and pressure. Shortly thereafter, the pressure is rapidly decreased. A typical AFEX procedure involves using 1-2 kg of ammonia per kg of dry biomass at 90°C for 30 minutes. 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.</p> <p>Advantages: Enhances surface area accessibility, partially eliminates lignin and hemicellulose, and does not generate inhibitors for subsequent processes.</p> <p>Limitations:not efficient for biomass with high ligninContent</p> |
| Carbon Dioxide Explosion | <p>This method utilizes CO₂ as a supercritical fluid at elevated pressure, effectively solubilizing lignin. CO₂ permeates the biomass, leading to enhanced digestibility under high pressure.</p> <p>The theory suggests that when CO₂ mixes with water, it forms carbonic acid, which aids in the hydrolysis of hemicellulose.</p> <p>Advantage:increase surface area;cost-effective; does not cause formation of inhibitory compounds</p> <p>Limitation:does not modify lignin or hemicelluloses</p> |

Biological pretreatment

In this group of pretreatments, microorganisms such as bacteria and fungi are employed to degrade cellulose, hemicellulose and lignin degrades lignin and hemicelluloses; low energy requirements rate of hydrolysis is very low[31](Table 4).

Table 4: Biological pretreatment

| | |
|------------------------------|---|
| Cellulases | Cellulose is enzymatically degraded to glucose by the synergistic action of cellulases. Commercial sources of cellulases are <i>Aspergillus niger</i> , <i>Trichoderma reesei</i> , <i>Bacillus</i> sp., etc (Figure 7). |
| 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 <i>Aspergillus niger</i> , <i>Trichoderma reesei</i> , <i>Bacillus</i> sp.,etc. (Figure 8). |
| 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 <i>Phanerochaete chrysosporium</i> . |
| 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 like cellulases to increase the accessibility of the substrate to the enzymes. <i>Trichoderma reesei</i> is known to secrete a helper protein known as swollenin which disrupts the structure of cotton fibers. |

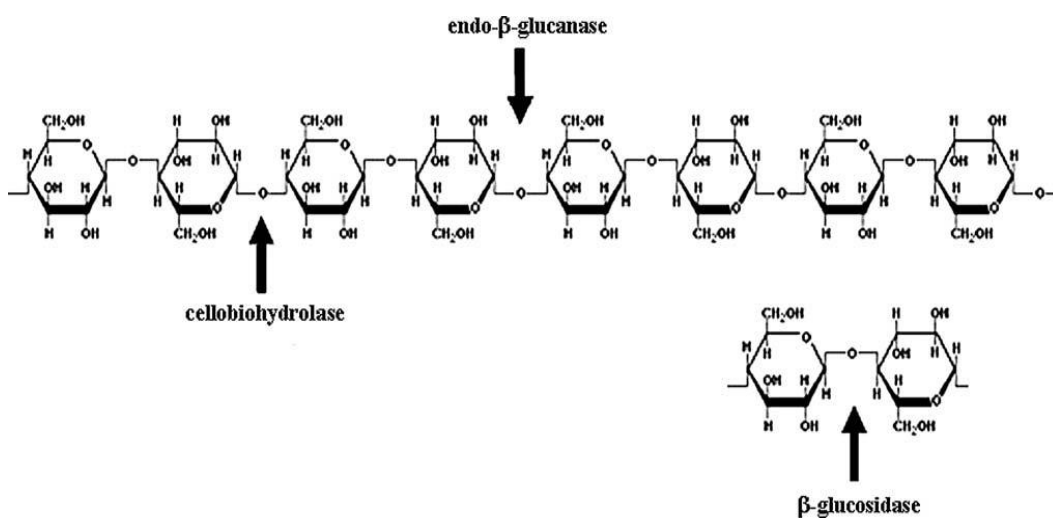


Figure 7: Cellulases

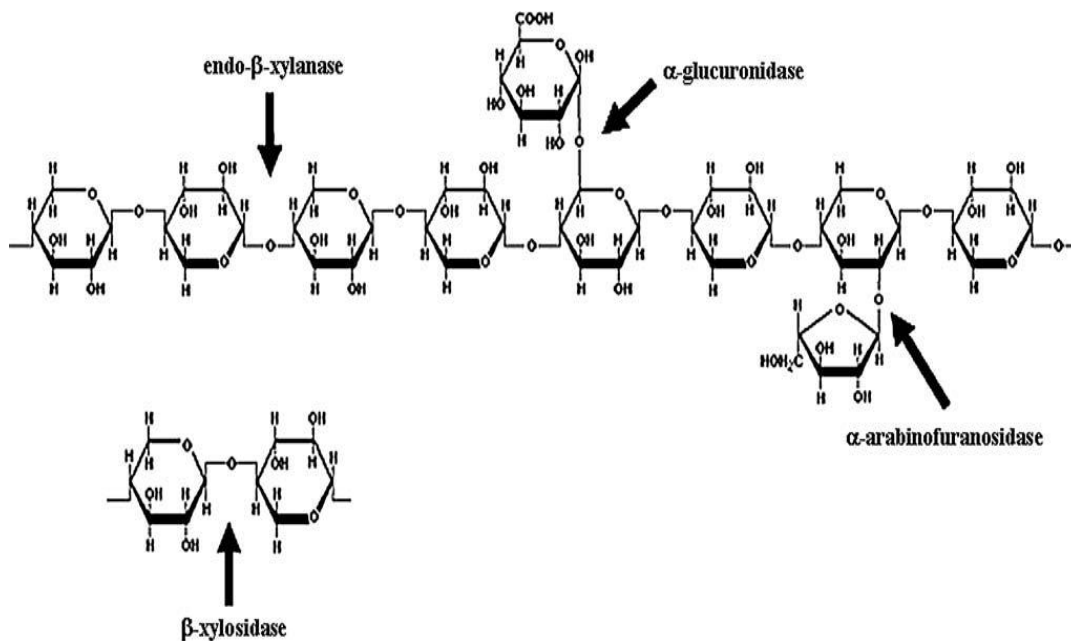


Figure 8: Xylanases

Separation Techniques

During hydrolysis, at the high temperature and pressures, glucose and xylose degrade into compounds like formic acid, acetic acid and phenol derivatives etc. which have the inhibitory effect on enzymes secreted by the yeast *S. 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 [32].

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.

Summary of Various Pre-treatment Methods depicts in the table 5.

Table 5: Summary of Various Pre-treatment Methods

| S. No. | Pre-treatment method | Feed-stock | Pre-treatment conditions | Yield |
|--------|----------------------|------------|--------------------------|-------|
| | | | | |

| | | | | |
|----|---|--|--|--|
| 1. | Physical pre-treatment using single screw extruder (Figure 6) | 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 temp, 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 temp, 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 temp 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 temp. for 24 h | 8.6% (w/v) monomeric sugars |
| 5. | Alkaline pre-treatment | Sorghum bicolor straw | 2% NaOH, 60 °C temp. 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 temp. 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 temp. with impregnation by water or H ₂ SO 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 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 (EH) 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 <i>S. cerevisiae</i> (D5A) | About 55-74% lignin removed, but retained nearly 100% of the glucan and 85% of xylan. 77% of ethanol yield based on glucan and xylan content |
| 10. | CO ₂ explosion | Aspen (hardwood) southern yellow pine (softwood) | Pre-treatment 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 pre-treatment with moisture content of 40,57, and 73% showed higher final sugar yields 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 <i>S.cerevisiae</i> | 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 ₂ SO 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% compared to 29 and 16% in non-ionated wheat and rye straw |
| 14. | Wet oxidation | Softwood (<i>Picea abies</i>) | Temperature 200 °C for 10 min at neutral pH | Highest yield of about 79% in seven hours. |

Conclusion

Biomass pre-treatment 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 pre-treatment methods empowers stakeholders to navigate towards sustainable and efficient bio-conversion processes. By balancing technological innovation with environmental stewardship, the path towards a greener energy future becomes increasingly attainable.

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