

LECTURE:18-19 (Pretreatment)

➤ INTRODUCTION

- The past three decades have witnessed significant research & development on the front of production of biofuels and many other chemicals using renewable sources, such as lignocellulosic biomass (LCB).
- LCBs are evenly distributed on the globe and abundantly available in most of the countries.
- Further they are carbon neutral too, i.e. the CO₂ released by the products of LCBs, specially the biofuels is balanced by CO₂ absorption by the plants somewhere else. As a result the carbon footprint is minimized (A carbon footprint is the amount of greenhouse gases, particularly CO₂ produced by human activities).
- The majority of LCB is composed of biological polymers such as cellulose, hemicellulose, and lignin, which are strongly bound together by covalent and hydrogen bonds making the structure highly recalcitrant.
- The presence of lignin renders the bio-polymeric structure highly resistant to solubilization thereby inhibiting the hydrolysis of cellulose and hemicellulose which presents a significant challenge for the isolation of the respective bio-polymeric components.
- This has led to extensive research in the development of pretreatment techniques utilizing various physical, chemical, physicochemical, and biological approaches which are specifically tailored towards the source biomaterial and its application.
- The success of biofuel and chemical production strongly depends on the pretreatment methods used.
- Overall, pretreatment is the major step in the successful production of valuable products from LCBs.
- With the help of pretreatment, the bonds are broken and association amongst cellulose, hemicellulose and lignin is weakened for further application.
- In the present class note, a comprehensive discussion on various aspects of the pretreatment techniques, such as physical, chemical, thermophysical, thermochemical and biological methods have been made.
- For proper understanding of the topic, basics of hydrolysis has also been discussed.

➤ HYDROLYSIS

Basics

- In chemistry and physiology, hydrolysis is defined as a double decomposition reaction with water as one of the reactants.
- Thus, if a compound is represented by the formula AB in which A and B are atoms or groups and water is represented by the formula HOH , the hydrolysis reaction may be represented by the reversible chemical equation $AB + HOH \rightleftharpoons AH + BOH$.
- The reactants other than water, and the products of hydrolysis, may be neutral molecules—as in most hydrolyses involving organic compounds—or ionic molecules, as in hydrolyses of salts, acids, and bases.
- In other simple words, hydrolysis may be defined as a chemical reaction in which a substance reacts with water so as to be changed into one or more other substances, as

a starch into glucose, natural fats into glycerol and fatty acids, or a salt into a weak acid or a weak base.

- Here water is used to break down a compound; this is achieved by breaking a covalent bond in the compound by inserting a water molecule across the bond.
- The opposite of this is a dehydration-condensation reaction.
- There are commonly three types of hydrolysis reactions as depicted in **Figure-1**.

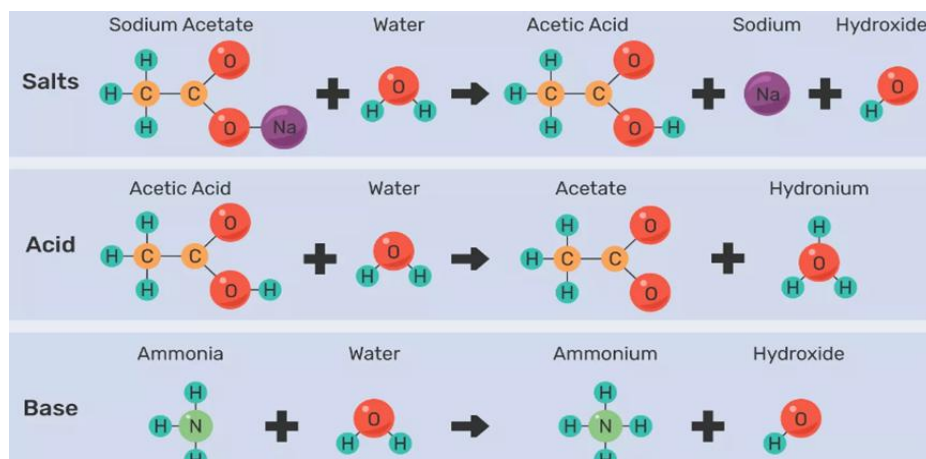
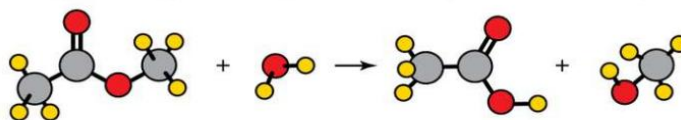
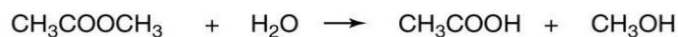


Figure-1: Three common types of hydrolysis reaction.

- Another example is the hydrolysis of ester as shown in the **Figure-2**.

Hydrolysis of ester



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Figure-2: Hydrolysis of methyl acetate to form acetic acid and methyl alcohol.

- There may be thousand of examples where a substance (compounds like starch, proteins, cellulose, hemicellulose, etc.) reacts with water to form or to give simple compounds in order to further the chemical process for achievement of the final objective.

➤ **HYDROLYSIS IN BIORESOURCE TECHNOLOGY**

- Bioresource technology is an interdisciplinary area which integrates the engineering design and the biological sciences in order to make proper utilization of the bioresources (non-fossil, biogenic origin) to meet the societal needs, such as renewable energy, clean environment and chemicals.
- Amongst the bioresources, biomass is the most prominent source which is composed of the major components: cellulose, hemicellulose and lignin.
- These natural polymeric substances cannot be further converted into usable products, unless or until they are simplified to or converted into simple sugars like glucose or pentose.
- Similarly, starch needs to be converted into sugar, natural fats into fatty acids and glycerol for further applications.

- Here, hydrolysis applied for conversion of starch, natural fats and oils and lignocellulosic materials into simple compounds for further utilization.
- Specially in fermentation, sugars obtained by hydrolysis of starch and lignocellulosic materials are easily converted by the microorganisms into useful products.
- For example: in production of acetone-butanol-ethyl alcohol, using lignocellulosic materials as feedstocks, pretreatment is given through hydrolysis where enzyme, acid, alkali hydrolyses are commonly used.
- Similarly, hydrolysis is commonly used in the production of bio-ATF, bioethanol and other biofuels.
- Hydrolysis may be divided into: physical, chemical, thermochemical, thermophysical, Biological .

➤ PRETREATMENT METHODS FOR LCBs

○ Summary

- ✓ In the past three decades, many studies on the production of biofuels and other chemicals have been conducted using renewable sources such as lignocellulosic biomass.
- ✓ Lignocellulosic biomasses are abundantly available in most countries and furthermore they are carbon neutral.
- ✓ However, the main problem in utilizing lignocellulosic materials lies in the recalcitrance of its bonding.
- ✓ In this lecture, a comprehensive overview and a brief discussion on producing biofuel and valuable chemicals from lignocellulose biomass have been taken up.
- ✓ Various aspects of the physical, chemical, thermophysical, thermochemical and biological pretreatment of lignocellulosic materials have been discussed.
- ✓ The success in biofuel and chemical production strongly depends on the pretreatment method used.
- ✓ Overall, pretreatment is the major step in the successful production of valuable products from lignocellulosic biomass.
- ✓ The various pretreatment methods, the conversion technologies which may be applied after pretreatment, and the primary objectives have been summarized by **Figure-3** and **Figure-4** shown on the next page.

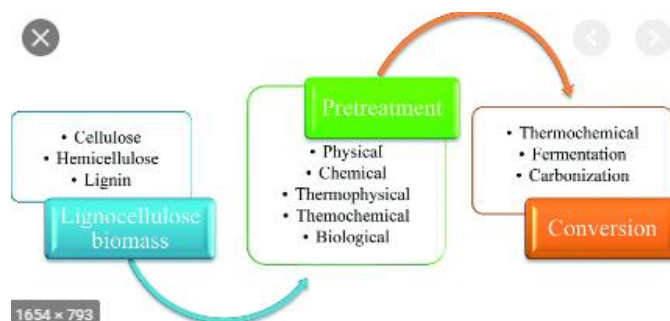


Figure-3: Variou pretreatment methods and the technologies used after treatmeny.

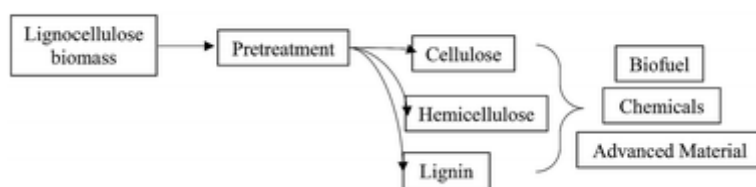


Figure-4: Primary objectives of the pretreatment.

o Introduction

- ✓ In the past two decades, lignocellulosic biomass (LCB) such as wheat straw, rice straw, sugarcane bagasse, barley, and timothy grass, woody raw materials, forest residues, softwoods and paper pulps have been extensively researched for biofuel.
- ✓ Lignocelluloses are composed of cellulose (40–50%), hemicelluloses (25–35%) and lignin (15– 20%) in an intricate structure where the components are rigidly associated through non-covalent bonds and covalent crosslinkages as shown in **Figure-5(a)**.

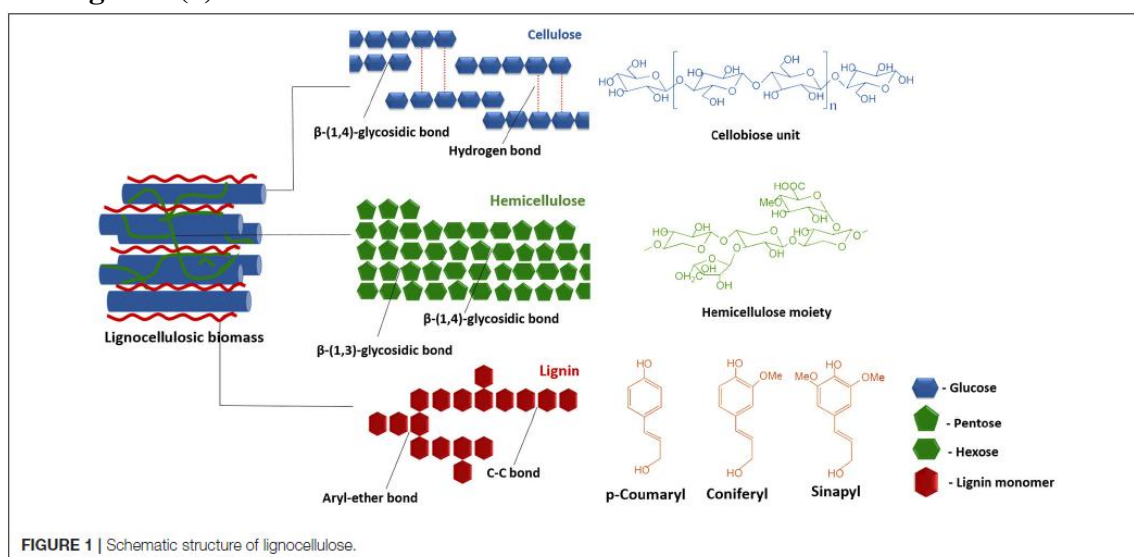


Figure-5 (a): Schematic structure of lignocellulose.

- ✓ Crystallized cellulose and hemicellulose polymer matrix are encrusted by the highly polymerized phenolic lignin that leads to the difficulties in the conversion processes.
- ✓ The complex, hierarchical and recalcitrant nature of the LCB is the primary bottleneck in the utilizing of these resources for bioenergy production or for conversion to any other value added products.
- ✓ This problem has so far been addressed by increasing the digestibility and availability of cellulosic and hemicellulosic fractions, together with the removal of the lignin fractions, through a series of targeted pretreatments
- ✓ The pretreatment techniques currently in use, may be broadly classified as physical, chemical, physicochemical, and biological processes.
- ✓ It is worthwhile to mention that the pretreatment phase contributes to a minimum of 20% of the total cost of conversion for different products and is one area where the energy inputs can be significantly lowered.

- ✓ This urges further study on the understanding of the different techniques and is imperative for the improvement upon the pretreatment phase so as to make the process of conversion of biomass to biofuels and value-added chemicals economical and adaptable to sustainable biorefinery based approaches.
- ✓ In this lecture note, the currently pretreatment techniques for production of biofuels and value-added chemicals, have been discussed with their possible pros and cons.
- ✓ Their recent advancements and effect on different LCBs have also been focussed.
- **Structure of LCB and its components**
 - ✓ LCB is mainly composed of three polymers: cellulose ($C_6H_{10}O_5$)_n, hemicellulose ($C_5H_8O_4$)_m, and lignin [$C_9H_{10}O_3(OCH_3)_{0.9-1.7}$]_x along with minor amounts of other compounds such as proteins, ash, and pectin.
 - ✓ In general, the cellulose, hemicellulose and lignin contents in a typical LCB fall within the range of 30–60, 20–40, and 15–25%, respectively .
 - ✓ However, the composition of these major components varies depending on the source as depicted in **Table-1**.

Table-1: Composition of Various LCBs

lignocellulosic material	cellulose (%)	hemicellulose (%)	lignin (%)
hardwood stems	40–55	24–40	18–25
softwood stems	45–50	25–35	25–35
nut shells	25–30	25–30	30–40
corn cobs	45	35	15
grasses	25–40	35–50	10–30
paper	85–99	0	0–15
wheat straw	30	50	15
sorted refuse	60	20	20
leaves	15–20	80–85	0
cotton seed hairs	80–95	5–20	0
newspaper	40–55	25–40	18–30
waste papers from chemical pulps	60–70	10–20	5–10
primary wastewater solids	8–15		
solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
coastal bermudagrass	25	35.7	6.4
switchgrass	45	31.4	12
swine waste	6.0	28	na

- ✓ Cellulose is a main structural and integral part of LCB which is a linear polysaccharide and consists of D-glucose subunits linked by β -(1,4)-glycosidic bonds.
- ✓ This polymer is insoluble in water unless at extremely low or high pH levels.
- ✓ However, it is soluble in solvents like ionic liquids (ILs) and N-methylmorpholine-N-oxide (NMMO).
- ✓ Cellulose possesses the advantageous properties such as biocompatibility, stereoregularity, hydrophilicity, and reactive hydroxyl groups and serves as a versatile resource for derivatized materials such as fibers, films, composites as well as fuels and chemicals.
- ✓ Hemicellulose is a second major component of LCB that consists of short chains of different polysaccharides such as xylan, galactomannan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan that are held together by β -(1,4)- and/or β -(1,3)-glycosidic bonds.

- ✓ In contrast to cellulose, hemicellulose is readily degradable into monosaccharides due to low degree of polymerization and non-crystalline nature and thereby widely used in industrial applications such as drug carriers, hydrogels, and cosmetics.
- ✓ Lignin forms a protecting boundary by covalently linking to the cellulose and hemicellulose which enhances the recalcitrance of the lignocellulose.
- ✓ It is a complex, three-dimensional cross-linked polymer that consists of phenyl propane structural units and vary depending on the substitute of the methoxyl groups present in the aromatic rings and are linked to each other by aryl ether linkages e.g., β -O-4, α -O-4 and carbon-carbon bonds e.g., 5-5, β - β .
- ✓ Three basic units that constitute the lignin polymer are p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S).
- ✓ Further, the molecular structure and lignocellulosic composition (cellulose, hemicellulose, and lignin) have been shown in Figure-5 (b).

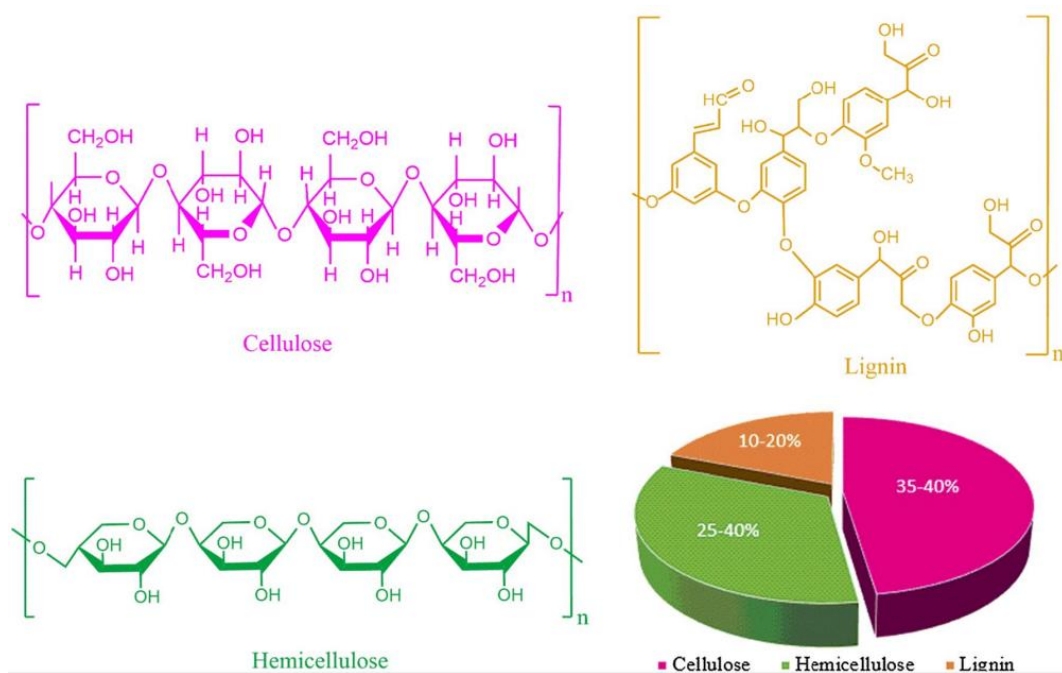


Figure-5 (b): Composition and structure of monomers of cellulose, hemicellulose and lignin.

○ Pretreatment of LCB: Necessity

- ✓ LCBs are resistant to chemical and biological breakdown, known as biomass recalcitrance.
- ✓ Several factors such as the crystalline structure of cellulose, the degree of lignification and the structural heterogeneity and complexity of cell-wall constituents are responsible for the biomass recalcitrance that must be overcome for valuable utilization of lignocellulosic feedstocks.
- ✓ In this context, the pretreatment is a significant step in the biorefinery process.
- ✓ Throughout the pretreatment process, the recalcitrant structure of lignocellulose is disrupted resulting in breakage of lignin sheath, degradation of hemicellulose and reduction in crystallinity and degree of polymerization of cellulose.
- ✓ A multitude of pretreatment techniques have been developed in the last few decades to improve the deconstruction of LCBs and can be categorized as shown in **Figure-6**.

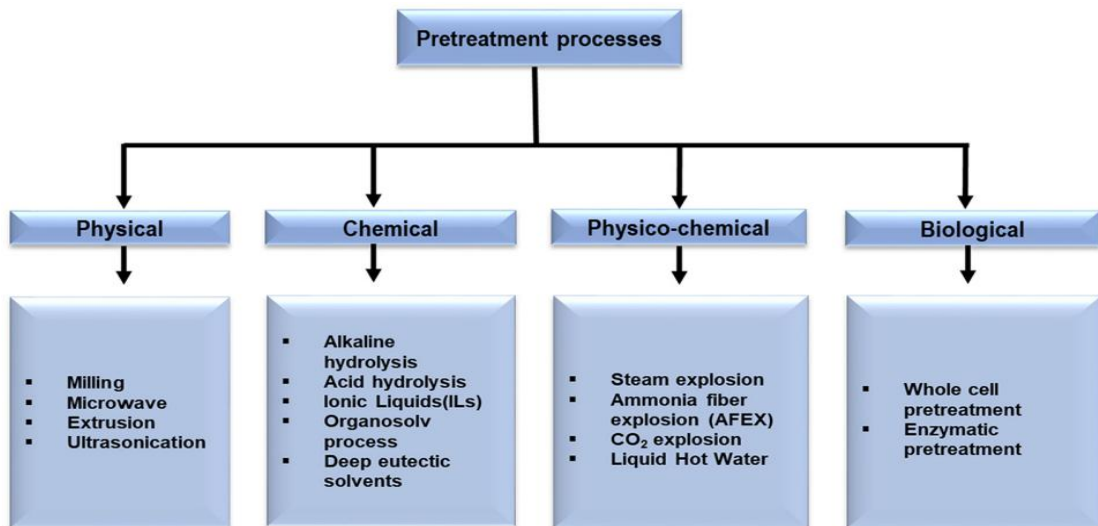


Figure-6: Flow chart diagram of pretreatment processes.

- ✓ Among the several pretreatment processes the preference of the convenient one depends on the type of LCBs used as the composition of cellulose, hemicellulose and lignin vary.
- ✓ In the following sections, the major pretreatment techniques and their effects on the separation of the complex components of various lignocellulosic sources are discussed.
- ✓ Roll of pretreatment has been shown by the **Figure-7**.

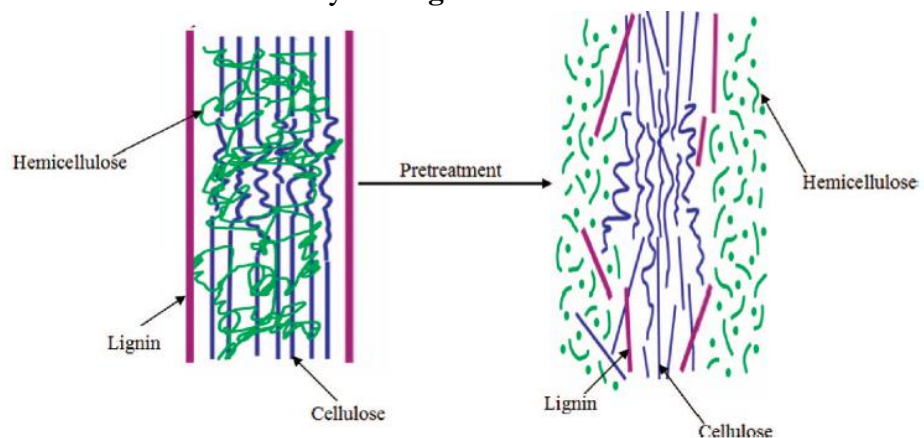


Figure-7: Schematic of the role of pretreatment in the conversion of biomass.

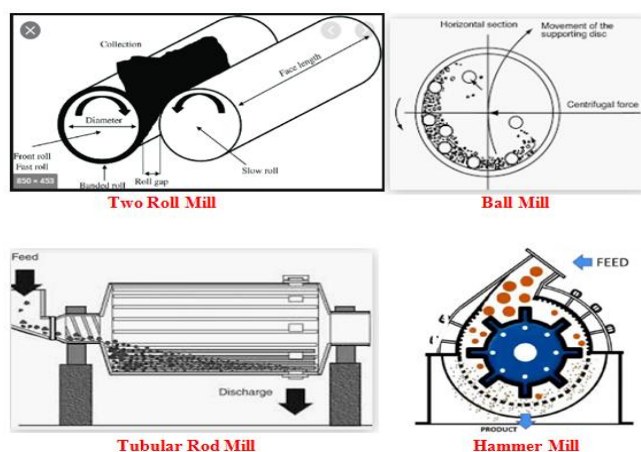
○ **Physical Pretreatment**

- ✓ Physical pretreatment of LCB is a prerequisite prior to any other pretreatment methods.
- ✓ It is primarily carried out to reduce the particle size that results in the increase in surface area, and decrease in degree of polymerization and crystallinity.
- ✓ Consequently, the subsequent processes become more effective and easier.
- ✓ These methods are eco-friendly and seldom produce any toxic material.
- ✓ However, one major disadvantage of physical pretreatment is its high energy consumption.
- ✓ Generally, energy consumption depends on the type of LCB used.

- ✓ It was reported that size reduction of softwoods such as corn stover and switchgrass requires 11.0 and 27.6 kWh/metric ton, respectively, while hardwoods such as pine and poplar chips require 85.4 and 118.5 kWh/metric ton, respectively.
- ✓ The commonly prevalent physical pretreatment methods include milling, extrusion, microwave treatment, and ultrasonication.
- ✓ These approaches are described in detail in the following sections.

Milling

- Milling is employed to reduce the crystallinity and particle size of LCB.
- Milling can reduce the particle size upto 0.2mm.
- However, Chang et al. (1997) unveiled that biomass particle of size <0.4mm has no remarkable effect on the rate and yield of hydrolysis.
- Depending upon the type of motorized equipment operated the different milling methods are two-roll milling, ball milling, rod milling, hammer milling, vibratory milling, colloid milling, and wet disk milling (**Figures 8 & 9, below**).



Figures-8: Two roll mill, ball mill, tubular rod mill and hammer mill.

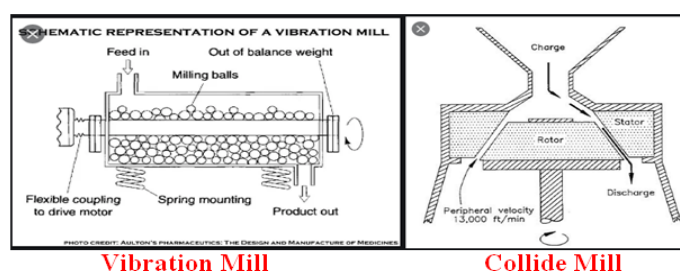


Figure-9: Vibration mill and collide mill.

- The reduction in particle size and crystallinity is determined by the type of milling method adopted, processing time and also the type of biomass used.
- Bai et al. (2018) studied the efficiency of pyrolysis using rod-milling and hammer-milling pretreatment in wheat straw wherein they found significant size reduction and a decrease in crystallinity using rod-milling at an optimum duration of 60 min.
- The effective size reduction and the decrease in crystallinity lead to high surface contact and pore volume of wheat straw.

- Also, the kinetic analysis showed that wheat straw pretreated through rod milling has lower thermal degradation temperature as compared to the hammer-milled wheat straw that enhanced the pyrolysis efficiency.
- However, one of the biggest shortcomings of milling pretreatment is its high energy requirement and the capital cost of mechanical equipment.
- Guet al. (2018) studied the effect of planetary ball milling on premilled wood fiber and found an improved energy consumption efficiency of 0.50–2.15 kWh/kg for 7–30 min of milling at 270 rpm.
- The process produced high glucose and xylose in the range of 24.45–59.67 % and 11.92–23.82 %, respectively through enzymatic hydrolysis.
- Also, mild acid hydrolysis of pretreated cellulose paper and cellulose powder using ball milling resulted in a high yield of nanocellulose with high crystallinity and high thermal stability.
- Milling pretreatment of raw LCB for solid-state anaerobic digestion process showed that reduction in particle size greatly increases the substrate solubility thereby increasing the reaction kinetics.
- Wet diskmilling has been a well-known physical pretreatment owing to its low energy consumption. A study of combined pretreatment using hydrothermal and wet disk milling on oil palm mesocarp fiber (OPMF) demonstrated that hydrothermal pretreatment of OPMF followed by wet disk milling reduces the power consumption upto 9.6 MJ/kg with more than 98% glucose yield.
- Another combined pretreatment of hot compressed water (HCW) and wet disk milling on oil palm biomass produced 85.5 and 100% of total sugar yields from oil palm empty fruit branch and oil palm frond fiber, respectively.
- Kim et al. (2013) compared three different methods of milling; ball, attrition and planetary milling.
- It was found that attrition and planetary milling effectively reduced biomass particle size as compared to ball milling and the highest yield of glucose and galactose was obtained by planetary milling.
- Since milling pretreatment does not result in any toxic or inhibitory compounds it is a preferred preliminary pretreatment method for a wide variety of lignocellulosic feedstocks.

Microwave Assisted Pretreatment

- Microwave irradiation is a non-conventional heating method that has long been used for the pretreatment of LCB under an applied electromagnetic field.
- The first study of microwave irradiation pretreatment was carried out by Ooshima et al.(1984) and since then this method has been retained a convenient one owing to its several advantages including easy operation, energy efficient, minimum inhibitors formation and high heating capacity in short period of time.
- In this method, the dielectric polarization causes molecular collisions and generates thermal energy that results in the disruption of the complex lignocellulosic structure.
- Microwave irradiation was categorized into atmospheric and high-pressure treatment.
- High-pressure microwave pretreatments are operated in closed reactors within the temperature range from 150 to 250°C.

- A study on the microwave pretreatment of *Panicum spp.* and *Miscanthus spp.* showed 7–10% higher solubility of the materials in subcritical water as compared to the untreated materials.
- The samples were pretreated at different temperatures and the optimum conditions obtained are 60 °C and 120 °C for *Miscanthus spp.* and *Panicum spp.*, respectively at 1,600W treatment power.
- In another study, the microwave pretreatment of *Hyacinthus spp.* was examined to enhance the methane production from anaerobic digestion and obtained the highest methane yield of 221 mL/ g-sub which was 38.3% higher than the substrate pretreated with waterheating.
- Nowadays, microwave pretreatment is often added up with other treatment methods as an upgraded attempt.
- Liu et al. (2018) studied the effect of microwave irradiation during alkaline treatment for the separation of cellulose and hemicellulose from a delignified hardwood kraft pulp.
- They found that under microwave treatment the complex fiber structure fractured effectively and the alkaline solution penetrated into the inner fiber structure that resulted in the significant removal of hemicelluloses and high cellulose yield of 93.05%.
- Microwave-assisted ionic liquid treatment of *Crotalaria juncea* fibers produced 78.7 % glucose at 160 °C in only 46 min processing time.
- The studies of the microwave-assisted acid pretreatment of lignin and moso bamboo sawdust have been reported that microwave temperature is the most significant factor in determining the structure of the pretreated samples.
- The microwave temperature facilitates decarboxylation and dehydration during the process.
- Different analysis showed that the pretreated samples have lower O, H, and ash contents and higher C contents, while in raw bamboo sawdust the C, H, and O are predominant.
- Likewise, microwave-assisted acid hydrolysis of jabon kraft pulp produced 49.2 % reducing sugars with 0 % lignin content at 190 °C.
- The lignin removal promotes the production of high sugar yield.

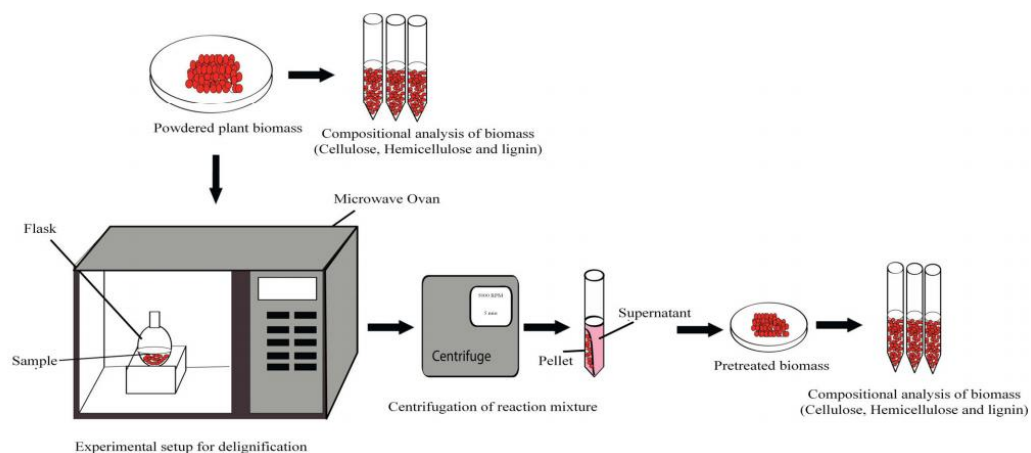


Figure-10: Pictorial representation of experimental setup and process flow of microwave assisted delignification of *Prosopis juliflora* biomass.

- Similarly, Germec et al. (2017) have studied extensively the microwave-assisted dilute acid pretreatment of different agricultural bioresources viz. barley husk, oat husk, wheat bran, and rye bran. The results established the microwave system as an assuring technique for fermentable sugar production from lignocellulosic materials.
- Microwave assisted delignification of *Prosopis juliflora* biomass has been shown in **Figure-10**, below.
- Steam or microwave-assisted sequential salt-alkali pretreatment for lignocellulosic waste (sugarcane leaf waste, SLW) has been shown in **Figure-11**.

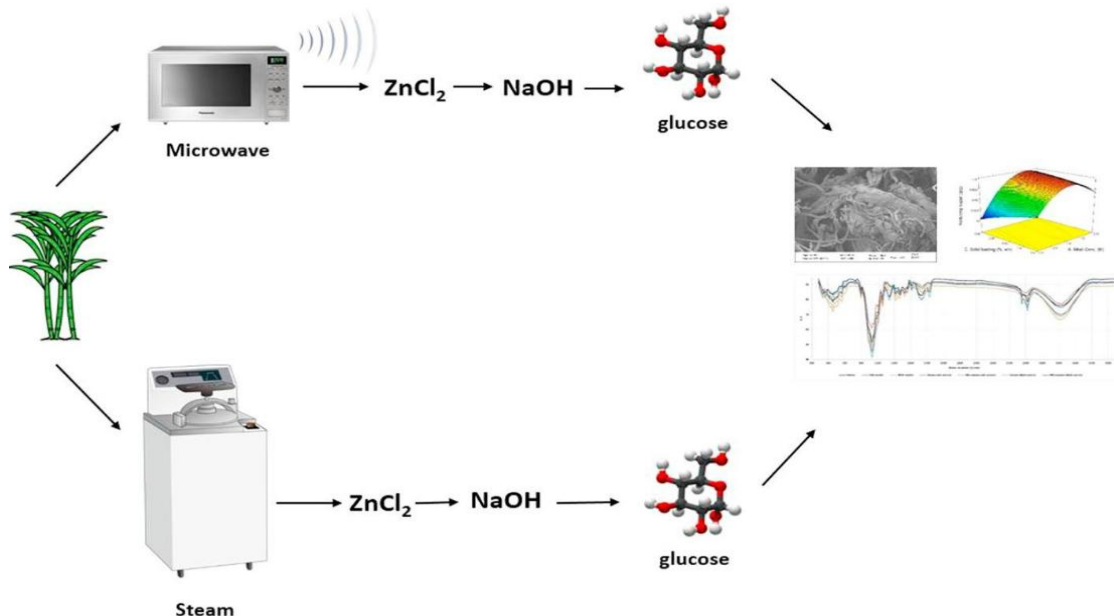


Figure-11: Pictorial representation for microwave assisted delignification of SLW.

Extrusion

- Extrusion is one of the most commonly used physical pretreatment method applied to LCBs.

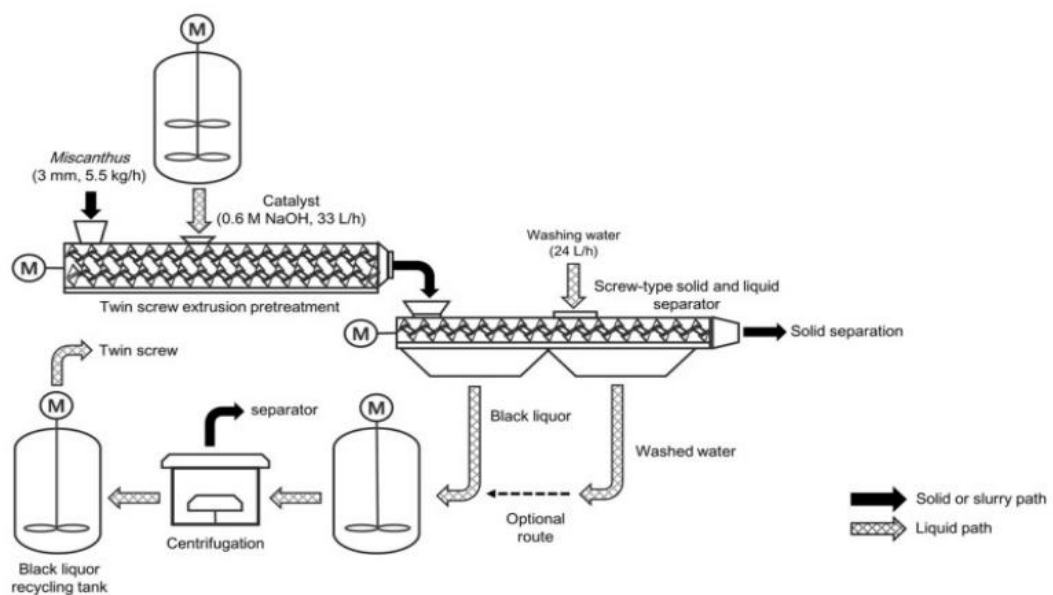


Figure-12: Schematic diagram of the twin crew extrusion pretreatment process for black liquor (BL) recycling.

- The action of one or two screws that spin into a tight barrel, which is furnished with temperature control forms the basis of this method.
- Alkaline twin-screw extrusion pretreatment of *Miscanthus* with recycled black liquor at the pilot scale has been depicted by **Figure-12**.
- The raw materials are passed through the barrel under high temperature ($>300\text{ }^{\circ}\text{C}$) where the recalcitrant structure of the lignocellulose disrupts due to the combined effects of high temperature and the shear forces caused by the rotating screw blades in the barrel.
- Extrusion machines are mainly classified into single screw extruders (made of one single solid piece) and twin screw extruders (made of small pieces called screw elements arranged cylindrically).
- The screw configuration is an important factor affecting the decomposition of LCB.
- Wahid et al. (2015) carried a study on the effect of different screw configurations during extrusion pretreatment on wheat straw and deep litter to enhance biogas production.
- They analyzed pretreatment using five screw configurations namely, mild kneading, long kneading, reverse, kneading, and reverse and kneading with reverse.
- The results showed effective sugar yield with each configurations that consequently led to significant methane production.
- Various parameters such as screw design, screw speed and barrel temperature also control the extrusion pretreatment.
- Moro et al. (2017) studied the pretreatment of sugarcane bagasse and straw using twin-screw extruder and optimized the variables viz. type of additives, biomass:additive ratio, number of extrusion passes, barrel temperature, screw speed, and screw configurations.
- The pretreatment was carried out using different additives such as water, glycerol, ethylene glycol with different loading amounts.
- Experiments showed glycerol as the suitable additive when pretreatment was conducted using a bagasse:glycerol and straw:glycerol ratios of 1:0.75 and 1:0.5 ratios, respectively.

Ultrasonication

- Ultrasonication pretreatment is based on the principle of cavitation through the employment of ultrasonic radiation.
- The cavitation generates shear forces that cleaves the complex network structure of LCB and promotes the extraction of desired compounds such as, cellulose, hemicellulose, and/or lignin.
- A study on the effects of ultrasound pretreatment on the structural changes of eucalyptus wood reveals that the crystallinity of the pretreated wood increased from 34.7 to 35.3% in aqueous soda solution, 32.6– 35.5% in distilled water and 33.4–35.5% in aqueous acetic acid solvent.
- The increased crystallinity is due to the effective removal of the amorphous hemicellulose and lignin fractions that was established by the FTIR analysis.

- It has been seen that the choice of solvents (dilute aqueous solutions of inorganic acids or alkalis, organic solvents or ionic liquids) is critical in determining the optimum conditions for ultrasonication pretreatments.
- Several factors influencing the sonication treatment includes ultrasound frequency, sonication duration, sonication power and temperature.
- Liyakathali et al. (2016) found that the enzymatic digestibility of energy cane bagasse increases with increase in the sonication time and temperature while ultrasonic frequencies had no effect on enzymatic digestibility.
- Cherpozat et al. (2017) studied the use of ultrasonic pretreatment on wood chips for bio-oil production.
- They found the combination of 170 kHz for 0.5 h and 40 kHz for 1.5 h and a power of 1,000W as adequate, resulting 12% increased yield of bio-oil as compared to untreated wood.
- However, ultrasonication for a prolonged period might cause adverse effect due to collision and aggregation between the particles.
- Likewise, high sonication power leads to cavitation near the ultrasound transducer tip that prohibits the energy transfer to the liquid medium.
- Ultrasonication is a viable pretreatment technique owing to its potential to facilitate the disruption of various lignocellulosic materials.
- In fact, the use of ultrasound can scale down the hydrolysis time of biomass up to 80%.

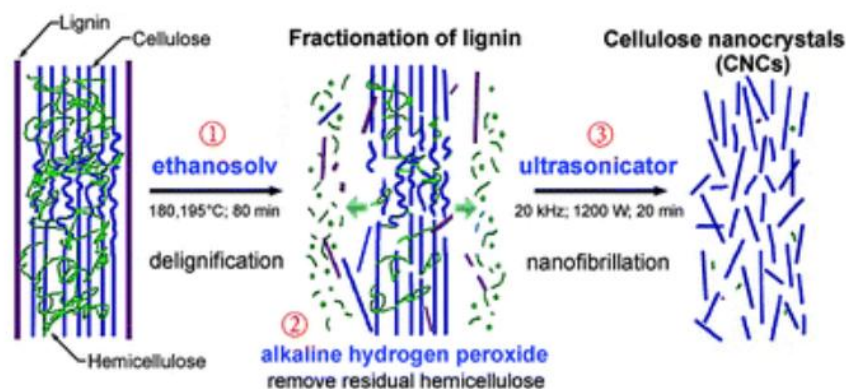


Figure-13: Ultrasonic disintegration of wood flour in two step process.

- However, the process is energy intensive and detailed investigations are necessary to optimize the process parameters for high-scale applications.
 - Cellulose nanocrystals (CNCs) were successfully extracted from wood flour by a two-step process that comprised ethanol and peroxide solvothermal pretreatment and an ultrasonic disintegration process as shown in the following **Figure-13**.
- **Chemical Pretreatment**
- Chemical pretreatment methods are used more often than biological or physical pretreatment methods because they are more effective and enhance the biodegradation of complex materials. Common chemicals used in chemical pretreatment methods for improving the AD performance of agricultural residues are sulfuric acid (H₂SO₄), hydrochloric acid (HCl), acetic acid (CH₃COOH), sodium hydroxide (NaOH),

potassium hydroxide (KOH), lime ($\text{Ca}(\text{OH})_2$), aqueous ammonia ($\text{NH}_3\cdot\text{H}_2\text{O}$), and hydrogen peroxide (H_2O_2).

Alkali Pretreatment

- Alkali pretreatment is a widely studied chemical pretreatment method which is based on the solubilization of lignin in the alkali solution.
- The various alkaline reagents used commonly for alkali pretreatment are the hydroxides of sodium, potassium, calcium and ammonium.
- Among these, sodium hydroxide is the most effective alkali pretreatment.
- A saponification reaction takes place throughout the alkali pretreatment process which causes cleavage of the intermolecular ester linkages between hemicelluloses and lignin.
- This results in solubilisation of lignin and hemicellulose fragments in the alkali solution and brings the cellulose in the interaction of enzymes.
- Also, alkali pretreatment changes the lignocellulosic structure via cellulose swelling that leads to reduction in crystallinity and degree of polymerization thereby increasing internal surface area.
- In addition, removal of acetyl groups and uronic acid substitutions in hemicelluloses during alkali pretreatment also increases the accessibility of the carbohydrates to enzymatic hydrolysis.
- Various studies reflect many advantages of the alkali pretreatment technique.
- A study on the alkaline pretreatment of rice straw for biomethane production reported that 1% NaOH at room temperature for 3 h significantly reduces the hemicellulose and lignin contents while the cellulose content remains unaltered.
- This led to an increase of methane yield by more than 34% compared to untreated rice straw.
- Alkali pretreatment is an effective technique in removing lignin and makes carbohydrates more exposed to use for the downstream processes. However, a major disadvantage of the technique is the recovery of the added alkalis which requires further investigations.

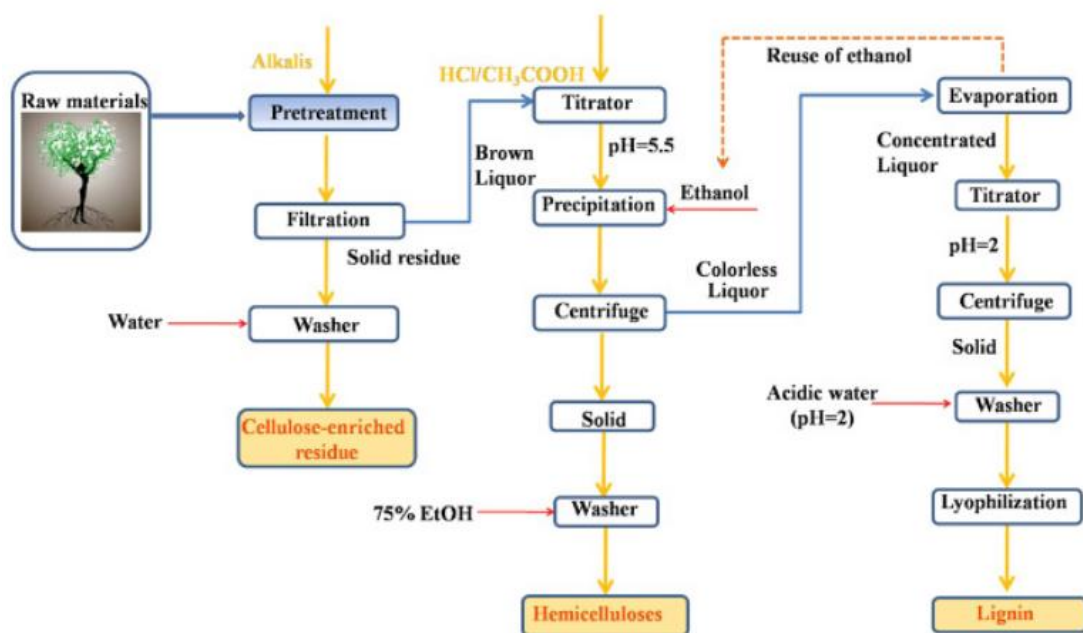


Figure-14: The conventional flow diagram of the pretreatment and fractionation of lignocellulosic biomass by an alkali-based process.

- Furthermore, alkali pretreatment is more favorable for low lignin content biomass such as herbaceous crops and agricultural residues and less productive for hardwoods.
- Selected precipitation approaches of hemicelluloses and lignin produced by alkaline pretreatment of biomass are summarized in **Figure-14**.
- Alkali pretreatment can be performed at room temperature for a few seconds up to several days. Sodium, potassium, calcium, and ammonium hydroxides are frequently used reagents for alkaline pretreatment and can improve the lignin removal efficiency.
- Pretreatment with sodium hydroxide (NaOH) is one of the most common methods and has been extensively studied in the bioconversion of lignocelluloses.
- NaOH treatment is very effective in increasing the digestibility of hardwood and agricultural residues with a low lignin content.
- Usually, alkaline pretreatment is carried out at low temperature and pressure, and it may be completed even at ambient conditions.
- The main drawback of this process is time; it might take hours or even days to complete the reaction.
- Temperature is an important factor that influences the performance of alkaline pretreatment.
- The reaction time could be saved through the increment of temperature.
- During lime [$\text{Ca}(\text{OH})_2$] pretreatment, some calcium is tainted into nonrecoverable salts or included in the biomass.
- Other alkaline pretreatment methods include calcium, potassium, sodium, barium, lithium, and ammonium hydroxides as reactants based on biomass category.
- Among these reactants, sodium hydroxide receives the most attention followed by lime, due to its advantage of being inexpensive and secure to use as well as being easily recoverable from water as an insoluble calcium carbonate by reaction with carbon dioxide.
- Further delignification of feedstocks can be enhanced by supplying surplus air or oxygen.
- We can compare alkaline pretreatment of feedstocks to Kraft pulping, where lignin was removed efficiently, thus improving the reactivity of the polysaccharides.
- Alkaline hydrolysis also effectively removes acetyl groups and uronic substitutions from hemicelluloses; thus the surface of hemicelluloses becomes more accessible to the hydrolytic enzymes.
- Furthermore the general results of alkaline pretreatment should be weighed against their influence on the ease of operation, cost of the downstream processes, and the trade-off between several costs, including operational, capital, and biomass prices.
- Hence, the cost-effective pretreatment of lignocellulosic biomass is a predominate technical bottleneck of cellulose conversion and multicomponent recovery'

Acid Pretreatment

- The primary objective through acid pretreatment is to solubilize the hemicellulosic portion of the LCB.

- Treatment can occur either at low-acid concentration and high temperature (dilute acid pretreatment) or high-acid concentration and low temperatures (concentrated acid pretreatment).
- While both dilute or concentrated acids could be used for pretreatment, using concentrated acids is less desirable because it produces more inhibiting compounds, such as HMF and furfural, than diluted acids.
- It also can cause greater corrosion and acid recovery problems compared to diluted acid, which increase the cost on a commercial scale.
- Diluted acid pretreatments have been studied for a variety of reactor types.
- The pretreatment can occur at high temperature (180 °C) for a short period of time, or a lower temperature (120°C) for a longer time period.
- CH₃COOH, HCl and H₂SO₄ pretreatments have been employed for improving the anaerobic digestion of lignocellulosic materials.
- Pretreatment with acid hydrolysis (HCl, H₂SO₄), can result in improvement of enzymatic hydrolysis of lignocellulosic biomass, to release fermentable sugars.
- Acid pretreatment results in the disruption of the van der Waals forces, hydrogen bonds and covalent bonds that hold together the biomass components, which consequently causes the solubilization of hemicellulose and the reduction of cellulose.
- The main reaction that occurs during acid pretreatment is the hydrolysis of hemicellulose, especially xylan, as glucomannan is more stable.
- Under such conditions, furfural and HMF generation can occur, because of dehydration of xylose galactose, mannose and glucose.
- Dilute acid hydrolysis pretreatment on the other hand can achieve high reaction rates and significantly improve cellulose hydrolysis.
- Lignin is hardly dissolved in most cases, but is disrupted to a high degree, thus leading to increased susceptibility of the cellulose to the enzymes.
- These pretreatments are more successful with usual concentration less than 4 wt%.
- Acid reagents, such as H₂SO₄, HCl, and CH₃COOH, at concentrations of 1, 2, and 4% (w/w) have been used for pretreatment.
- The most studied acid for pretreatment is diluted H₂SO₄ , which may in fact be the most common chemical pretreatment method.
- Experiments have shown the ability to remove nearly 100% of the hemicellulose with dilute sulfuric acid although it is not effective at dissolving lignin.
- It does disrupt lignin as well as increase the cellulose susceptibility for enzymatic hydrolysis.
- The conditions that are optimal for hemicellulose removal may not be optimal for the pretreatment for enzymatic hydrolysis.
- Studies testing various parameters of the pretreatment of olive tree biomass showed different process conditions for highest overall sugar yield, highest hemicellulose removal, and highest enzymatic hydrolysis yield.
- The major drawback of this method is formation of inhibitors. Compounds such as carboxylic acid, furfural, and phenolic compounds were formed, which may affect enzymatic hydrolysis and have a negative impact on microbial growth.

- Additional acids used for dilute acid pretreatment are the organic acids, such as fumaric or maleic acids. Initial studies show that less furfural was formed when using these organic acids, as opposed to sulfuric acid.
- However, as fewer studies have been performed with organic acids, it is unknown if they can achieve the same results as dilute sulfuric acid.
- A schematic diagram proposed for dilute acid pretreatment of biomass, Switchgrass (*Panicum Virgatum*) has been depicted in **Figure-15**.

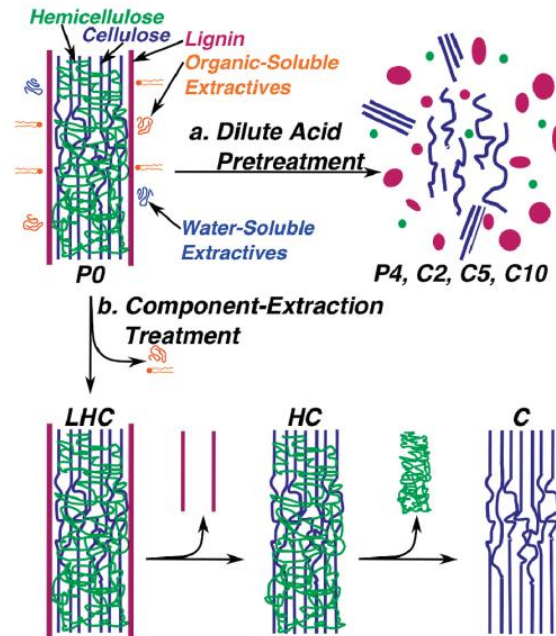


Figure-15: Schematic of (a) dilute acid pretreatment and (b) component extraction processes.

Ionic Liquids

- Ionic liquids (ILs) as cellulose solvent and ever since pretreatment of LCB by ILs has become a promising prospect.
- ILs are relatively new class of solvents with melting point $<100^{\circ}\text{C}$ which are comprised of cations and anions.
- The cations, in general, are organic viz. imidazolium, pyridinium, aliphatic ammonium, alkylated phosphonium, and sulfonium ions, while the anions include both organic and inorganic ions.
- During the pretreatment process both the cations and anions play a significant role in solubilizing the cellulose and lignin. **Figure-16** represents the interruption of the intra- and intermolecular hydrogen bonding in cellulose by IL ions.
- Also, the cellulose dissolution increases in presence of electron-withdrawing groups in the alkyl chains of IL cations.

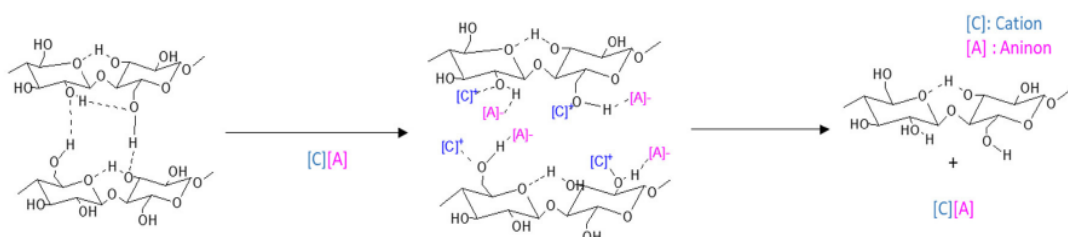


Figure-16: Interruption of intra- and intermolecular H-bonding in cellulose by ILs.

- Most of the ILs are recoverable and reusable.
- They possess the striking advantages of negligible vapor pressure, non-volatility, non-toxicity, large thermal, and chemical stability and most importantly the adjustable nature of their cations and anions on which the properties of the ILs depend .
- These are the reasons why ILs have often been described as green solvents.
- Several types of ILs include imidazolium-based ($[(C_3N_2)X_n]^+$), pyridiniumbased ($[(C_5N)X_n]^+$), pyrrolidiniumbased ($[(C_4N)X_n]^+$), ammonium-based $[NX_4]^+$, phosphoniumbased $[PX_4]^+$, sulfonium-based $[SO^3]^+$, and others such as cholin.
- Among these, imidazolium salts are the most commonly used ILs.
- Regardless of their distinctive chemical properties, ILs present the major shortcomings of being expensive and toxic to microorganisms and enzymes.
- Brandt-Talbot et al. (2017) investigated for the first time the application of the low-cost IL triethylammonium hydrogen sulfate for the pretreatment of *Miscanthus x giganteus* grass.
- They found up to 75% lignin and 100% hemicellulose solubilization in the IL solution and a yield of 77% glucose by enzymatic saccharification.
- Also the IL was reused four times with 99% recovery at each time.
- Nevertheless, further studies on these aspects with low-cost recovery technology and its toxicity to enzymes are still needed for economically-viable large-scale applications.

Organosolv Process

- In this process, LCBs are pretreated with organic solvents or their aqueous solutions that causes break down of the internal bonds between lignin and hemicellulose thereby leaving a relatively pure cellulose residue.
- During the process delignification and solubilization of hemicellulose lead to increase of pore volume and surface area of cellulose and enhances the accessibility of enzymatic hydrolysis and saccharification.
- A wide range of organic solvents such as, ethanol, methanol, acetone, organic acid, organic peracid, and ethylene glycol or their mixture with water have been employed to pretreat various LCBs.
- The process is often accompanied by the addition of a catalyst to either lower the pretreatment temperature or improve the delignification rate.
- Usually mineral acids (hydrochloric acid, sulfuric acid, phosphoric acid), bases (lime, sodium hydroxide, ammonia) and some salts are used as catalysts.
- Organosolv presents itself as an emerging pretreatment process owing to its inherent advantages such as, easy recovery of the solvents by distillation, recycles the solvents back to pretreatment and utilization of high-quality lignin isolated from this process as value-added byproducts for industrial applications.
- However, there also lies a few major disadvantages to the organosolv pretreatment.
- Most of the organic solvents are too expensive and need to be recovered as much as possible which is an energy-intensive process.
- In addition, high flammability and volatility of the organic solvents make the pretreatment to be carried out under especially controlled conditions.

- The organosolv pretreatment process for various feedstocks has been summarized in **Table-2**.

Table-2: Effect of organosolv pretreatment on different lignocellulosic substrates.

Raw material	Solvent	Catalyst	Condition	Results	References
Sorghum bagasse	25% Butanol	0.5% H ₂ SO ₄	200°C, 60 min	84.9% cellulose	Teramura et al., 2017
Barley straw	50% Acetone	0.5% H ₂ SO ₄	140°C, 20 min	83.9% glucose	Salapa et al., 2018
Sugarcane bagasse	60% Ethanol	0.025% FeCl ₃	160°C, 72 h	93.8% glucose	Zhang et al., 2018
Sugarcane bagasse	70% Glycerol	-	220°C, 120 min	94% cellulose	Sun F. F. et al., 2016
Wheat straw	25% Ethanol	1% H ₂ SO ₄	190°C, 60 min	80% glucose	Vergara et al., 2018
Wheat straw	50% Ethanol	0.35% H ₂ SO ₄	180°C, 40 min	89% cellulose	Salapa et al., 2017
Wheat straw	70% Glycerol	-	220°C, 180 min	70% cellulose	Sun F. F. et al., 2015
Corn stover	60% Ethanol	n-propylamine	140°C, 40 min	87.1% glucose	Tang et al., 2017b
Corn stalk	60% Ethanol	4% NaOH	110°C, 90 min	85% cellulose	Tang et al., 2017a
Rice straw	45% Ethanol	1% H ₂ SO ₄	180°C, 30 min	58.44% glucan	Asadi and Zilouei, 2017
Rice straw	65% Ethanol	1.1% H ₂ SO ₄	170°C, 60 min	70% cellulose	Sannigrahi et al., 2010
Bamboo	60% Ethanol	-	160°C, 60 min	67.2% glucose	Mou and Wu, 2017
Eucalyptus wood	56% Glycerol	-	200°C, 69 min	99% cellulose	Romani et al., 2016
Sweet sorghum	50% Ethanol	1% H ₂ SO ₄	140°C, 30 min	77% total sugar	Ostovareh et al., 2015

Deep Eutectic Solvents

- Pretreatment of LCB using deep eutectic solvents (DES) has attracted much interest in recent years.
- DESs are a new generation of ionic fluids comprising of two or three components, often interlinked through hydrogen bonding and form a eutectic mixture with a lower melting point than each individual components.
- They are usually liquids at temperature <100°C.
- DESs and ILs are much alike in terms of their physicochemical properties but their low-cost synthetic technology and biodegradability make them versatile alternatives to ILs .
- DESs are mostly derived by mixing a quaternary ammonium salt with a metal salt or hydrogen bond donor (HBD) which is capable of forming a complex with the halide ion of the quaternary ammonium salt.
- The decrease in melting point of the eutectic mixture is ascribed to the charge delocalization occurring between the halide ion and the hydrogen-donor moiety.
- DESs can be represented by the general formula



where Cat^+ is basically any ammonium, phosphonium or sulfonium cation, X^- is a Lewis base, commonly a halide anion, Y is Lewis or Brønsted acid, and z is the number of Y molecules that interact with the anion.

- The complexation between X^- and Y forms different anionic species.
- Being a biodegradable, non-toxic and cheap organic salt, cholin chloride (ChCl) are used in most of the DESs in combination with the low-risk HBDs like, urea, glycerol, carboxylic acids, and polyols.
- A study on the corn stover pretreatment by different DESs having the same halide salt i.e., ChCl found ChCl:Formic acid to be the ideal mixture for butanol fermentation.
- The findings implied that DES with acidic hydrogen donors could enhance the removal of lignin and hemicellulose more efficiently and are superior to ILs.
- The whole outline of DESs for biomass pretreatment and conversion has been depicted in the scheme demonstrated by **Figure-17**.

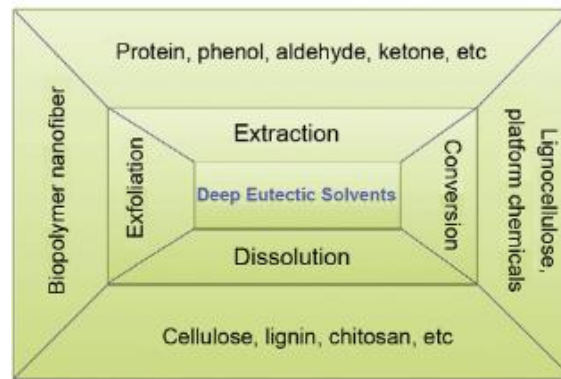


Figure-17: The whole outline of DESs for biomass pretreatment and conversion.

- Treatment of lignin with ILs required a high cost and large amount of water.
- One-pot synthesis of biofuel production using ILs also needed the adjustment of pH and the dilution before scarification/fermentation or after pretreatment.
- However, it was shown that one-pot production bio-ethanol can be achieved by using DESs via the couple of pretreatment, scarification and fermentation as shown in the **Figure-18**.

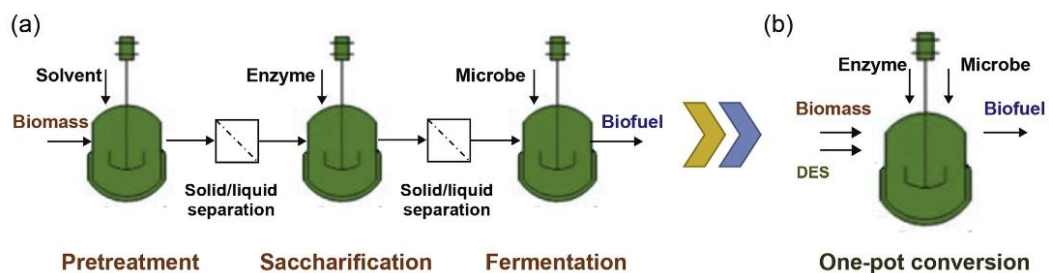


Figure-18: One-pot bioethanol production with biocompatible DESs.

○ **Physicochemical (Thermochemical) Pretreatments**

Steam Explosion

- ❖ Steam explosion is the most commonly employed and effective pretreatment method, which is typically a combination of both mechanical forces and chemical effects applied to LCBs.
- ❖ In this technique, the biomass is subjected to high-pressure saturated steam (0.69–4.83 MPa) at a temperature of 160–260 °C to let water molecules penetrate the substrate structure.
- ❖ The pressure is then suddenly reduced to let the water molecules escape in an explosive way.
- ❖ This rapid release of pressure causes explosion of the bulk LCB into splitted fibers.
- ❖ Besides, the high temperature and pressure enhance the breakdown of the glycosidic bonds in cellulose and hemicellulose and cleavage of hemicellulose-lignin bonds.
- ❖ During this treatment, the hydrolysis of hemicellulose into glucose and xylose monomers liberate acetic acid which catalyze the further hydrolysis of hemicelluloses, and; hence the process is also termed as autohydrolysis.
- ❖ Steam explosion has several advantages such as low environmental effect, limited chemicals use, high energy efficiency, no recycling costs and total sugar recovery compared with other pretreatment methods.

- ❖ It was found that almost 70% more energy is needed by the conventional mechanical methods than steam explosion to attain the same particle size reduction.
- ❖ This pretreatment is affected by several factors such as steam temperature, residence time, the size of biomass and moisture content.
- ❖ Steam explosion can be employed directly to milled LCB without employing any chemicals.

Ammonia Fiber Explosion (AFEX)

- ❖ In AFEX process, the LCB is heated with liquid ammonia (in 1:1 ratio) in a closed vessel at temperature around 60–100 °C under high pressure for 5–30 min, and then the pressure is suddenly released .
- ❖ The high pressure and given temperature causes swelling of lignocellulose and the rapid release of pressure disrupts the fibrous structure of biomass, reduces the crystallinity of cellulose and thereby improves the accessibility of enzyme.
- ❖ Optimization of AFEX pretreatment can be done by varying the four parameters including temperature, blowdown pressure, water loading and ammonia loading.
- ❖ Silvergrass (*Miscanthus spp.*) when presoaked in water prior to AFEX pretreatment showed up to 10% increase in glucan conversion indicating that moisture content plays a major role in AFEX.
- ❖ AFEX pretreatment partly removes the lignin and hemicellulose from lignocellulosic materials, but shows better enzymatic hydrolysis results at low enzyme loadings compared to other pretreatment processes.
- ❖ When oil palm empty fruit bunch fiber were pretreated with AFEX at 135 °C for 45min, the lignin-carbohydrate linkages changed along with some relocalization of lignin and resulted in 90% glucan conversion at lower enzyme loading of 13.8 FPUg⁻¹ glucan as compared to 25.5% conversion for untreated biomass.
- ❖ Therefore, AFEX pretreatment is more suitable for low lignin-content LCB such as agricultural wastes and herbaceous plants like switch grass, rice straw, corn stover etc.
- ❖ The main advantage of AFEX lies in the negligible formation of inhibitors as compared to other pretreatment methods.
- ❖ Nonetheless, ammonia should be recovered and recycled due to its high cost and volatility to reduce the overall operating cost and minimize environmental damage.
- ❖ The following **Figure-19** shows the fundamentals of ammonia fibre explosion:

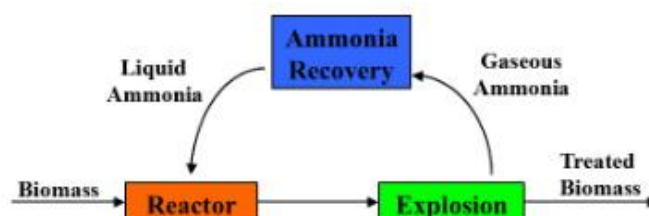


Figure-19: Ammonia fibre explosion.

CO₂ Explosion Pre-treatment

- ❖ A key drawback in the application of steam explosion pretreatment lies in its use of high thermal energy which is essential for the decomposition of the LCB.
- ❖ The AFEX pretreatments on the other hand use ammonia, which is highly corrosive with detrimental environmental effects, thereby limiting its scope.
- ❖ In this context, supercritical CO₂ explosion appears to be a viable alternative owing to its lower energy requirements and use of the greener alternative compared to ammonia.
- ❖ Because of its characteristics of mass transfer of a “gaslike” with a “liquidlike” solvating power, supercritical CO₂ can diffuse through interspaces like gas and dissolve materials like liquid.
- ❖ Under high pressure the CO₂ molecules penetrate into the biomass and shattered the higher level of structures comprising hemicellulose and lignin.
- ❖ Once dissolved in water, CO₂ forms carbonic acid, which catalyzes the hydrolysis of hemicellulose.
- ❖ This is the reason why this pretreatment process is not satisfactory to biomass with no moisture content.
- ❖ On the other hand, when the pressurized gas is released, it breaks the compact matrix structure of biomass thereby improving the accessibility of cellulose fibers.
- ❖ The penetrating rate of the CO₂ molecules into the cellulosic pores increases with pressure and leads to high glucose yield.
- ❖ Also, it was reported that addition of water-ethanol as co-solvents during supercritical CO₂ explosion, pretreatment can significantly remove the lignin and enhance enzymatic hydrolysis of corn stover.
- ❖ Benazzi et al. (2013) reported a single-step process for the hydrolysis of sugarcane bagasse using supercritical CO₂ and obtained 60% yield of fermentable sugars.
- ❖ Soybean hull pretreated with supercritical CO₂ showed a high glucose yield of 97% after enzymatic hydrolysis.
- ❖ This high glucose yield was obtained under optimal conditions of 8 MPa pretreatment pressure and 130 °C temperature for 30 min.
- ❖ Thus the pretreatment pressure and temperature also plays a significant role in supercritical-CO₂ treatment.

Liquid Hot Water (LHW)

- ❖ LHW pretreatment is very much alike to steam explosion but as the name suggests, LHW uses water at elevated temperature (170–230 °C) and pressure (up to 5MPa) in place of steam.
- ❖ Unlike steam explosion rapid release of pressure is not required in LHW and the application of pressure is only to prevent evaporation of water.
- ❖ LHW hydrolyzes hemicellulose by liberating its acetyl groups and removes lignin thereby making the cellulose fibers more exposable.
- ❖ The detached hemicellulose remains in the liquid fraction of the pretreated slurry and formation of monomeric sugars during the process is minimal.
- ❖ However, in order to avoid the sugar degradation and inhibitors formation, the LHW pretreatment is carried out at controlled pH between 4 and 7.
- ❖ Furthermore, biomass size has no influence since the particles are broken down during the treatment which makes the process more striking for large scale.

- ❖ However, the process is very energy intensive due to a large amount of water involved.

○ **Biological Pre-treatment**

Biological pretreatment is a low cost and eco-friendly technique to treat LCB prior to enzymatic saccharification. This technique is promising as there is no inhibitor formation during the process, requires lesser energy and is eco-friendly. Through this method, lignin degrading bacteria or fungi, as whole cell or enzymes, are used to pretreat LCB. The enzymes used in degradation of lignin are laccases, lignin peroxidase, manganese peroxidase, and versatile peroxidase. Fungi are the best suited for such applications as they are capable of degrading cellulose, hemicelluloses, and lignin. Biological pretreatment is not only used for lignin removal, but also for removal of specific components such as antimicrobial substances. White-rot, brown-rot and soft-rot fungi are used for degradation of lignin and hemicelluloses present in LCB. However, mostly white-rot fungi are involved in biological pretreatment due to high sugar yield associated with enzymatic saccharification. Some white-rot fungi can simultaneously degrade lignin and polysaccharides, resulting in the loss of carbohydrates, while other white-rot fungi can selectively degrade lignin.

There are two extracellular enzymatic systems involved in microorganisms, one hydrolytic and another ligninolytic system. Hydrolytic system is responsible for degradation of cellulose and hemicelluloses, while ligninolytic system depolymerizes the lignin. Lignin can be degraded by enzymes produced by various organisms, among which white-rot fungus has been found the most effective. Fungi are usually isolated from the soil, living plants, or agricultural waste materials.

Whole Cell Pre-treatment

- White-rot fungi are generally used as whole cell microorganism since it is less potent for the degradation of the cellulosic fraction of the LCB.
- The ligninolytic system in these fungi secretes one or more extracellular enzymes which are responsible for the degradation of lignin (aromatic polymer) and aliphatic fragments.
- White-rot fungi commonly employed for ligninolytic pretreatment are *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, *Ceriporia lacerata*, *Cyathus stercoleris*, *Pycnoporus cinnabarinus*, *Pleurotus ostreatus*, *Phlebia subserialis*, *Pleurotus streatus*, *Postia placenta*, *Gloeophyllum trabeum*, and *Echindodontium taxodii*.
- The above fungi are capable of efficiently metabolizing lignin in a variety of LCBs.
- Among these, *Phanerochaete chrysosporium* is a model organism for lignin degradation.
- However, white-rot fungi may face challenges in lignin degradation due to presence of carbon-carbon bonds within the large lignin polymers.
- **Table-3** summarizes the different biological pretreatment conditions that have been used in the recent past for treatment of various biomass feedstocks using whole cell biocatalyst.
- The major disadvantages of biological pretreatment methods are low efficiency and long residence periods, when used in isolation.

Table-3: Biological Pretreatment Conditions for Various Feed stocks.

Feedstock	Microorganism	Process condition	Effect on biomass	References
Sugarcane bagasse	<i>Ceriporiopsis submenispora</i>	27 ± 2°C for 60 days	47% sugar was recovered as sugar-rich syrup	Machado and Ferraz, 2017
Sawdust	<i>Pleurotus pulmonarius</i>	28°C for 30 days	Sugar concentration increased from 2.5l mol/mL to 48.0l mol/mL.	Castoldi et al., 2014
Wheat straw	<i>Ceriporiopsis subvermispora</i>	7 weeks of solid state fermentation incubated at 24°C	<i>in-vitro</i> gas production of 297.0ml g ⁻¹	Nayan et al., 2018
Paddy straw	<i>Pleurotus florida</i>	25–29°C for 28 days.	Maximum saccharification efficiency up to 75.3%	Manickam et al., 2018
Straw	Fungal consortium	Incubated at 30 and 55°C for 6 days	7-fold increase in hydrolysis	Taha et al., 2015
Corn stover	Fungal consortium	Pretreatment for 42 days with fungi	43.8% lignin removal and 7-fold increase in hydrolysis	Song et al., 2013
Corn stalks	<i>Irpex lacteus</i>	28 days incubation	82% hydrolysis yield was observed	Du et al., 2011
Bamboo clums	<i>Punctularia sp. TUFC20056</i>		50% of lignin removed	Suhara et al., 2012
Corn stover	<i>Ceriporiopsis subvermispora</i>	Solid state fermentation at 28°C for 42 days	57–67% glucose yield increase	Wan and Li, 2010
Rice straw	<i>Pholiota adiposa</i>	Saccharification for 48 h	Releases 716 mg-sugar g-substrate ⁻¹	Jagtap et al., 2013a
Aspen biomass	<i>Armillaria gemina SKU2114</i>	48 h of hydrolysis	Maximum saccharification yield of 62%	Jagtap et al., 2013b

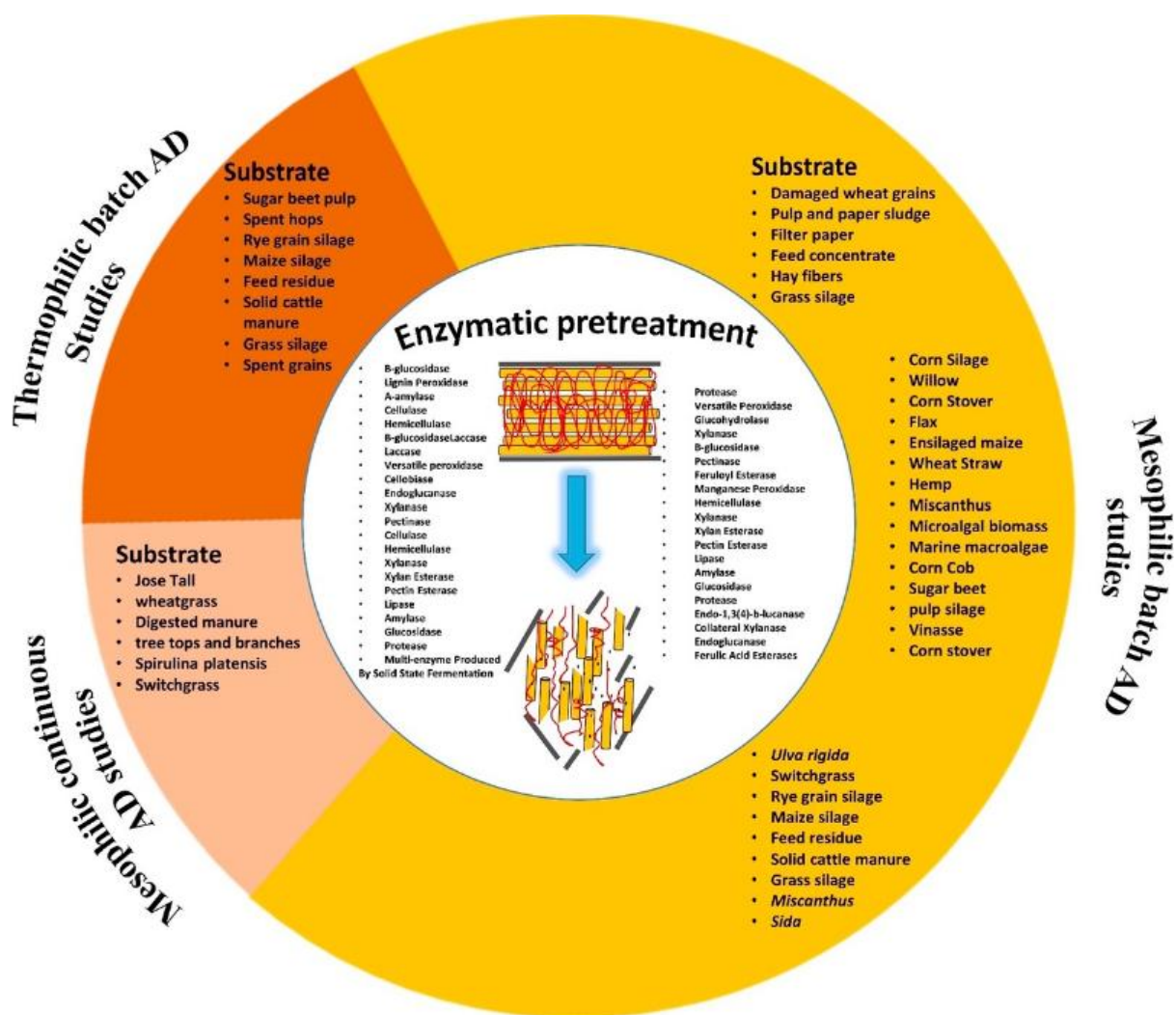
Enzymatic Pretreatment

- Lignin is the most abundant aromatic polymer consisting of phenolic and non-phenolic compounds.
- Some fungi, bacteria, and insects are capable of producing enzymes which digest lignin.
- There are two families of ligninolytic enzymes which play an important role in enzymatic degradation: phenol oxidase (laccase) (Lac) and peroxidases (lignin peroxidase (LiP), versatile peroxidase (VP), and manganese peroxidase (MnP)).
- These are heme-containing glycoproteins which require hydrogen peroxide (H₂O₂) as oxidant.
- The role of other enzymes has not yet been fully elucidated including glyoxal oxidase (GLOX; EC 1.2.3.5), glucose oxidase (EC 1.1.3.4), cellobiose dehydrogenase (CDH; EC 1.1.99.18) oxidoreductase and methanol.
- There are different microorganisms i.e. bacteria and fungi, which produce cellulolytic, hemicellulolytic and ligninolytic enzymes.
- Lignin degrading enzymes are directly employed to pretreat the biomass. These are employed in a group of one or more.
- **Table-4** shows the effect of enzymatic pretreatment on different lignocellulosic feedstocks.

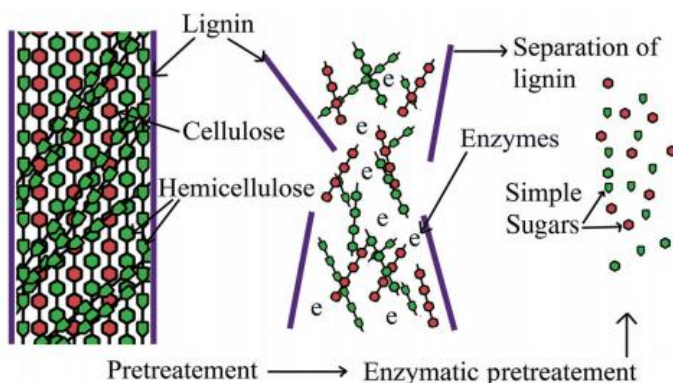
Table-4: Effect Of Enzymatic Pretreatment On Different Lignocelluloses

Enzyme	Source strain	Substrate	Conditions	Effect of biomass	References
Laccase and peroxidase	<i>Polyporus brumalis_BRFM985</i>	Wheat straw	21 days old fungal pretreated wheat straw	39% loss of lignin	Zhou X. et al., 2017
Laccase	<i>Tyromyces chioneus</i>	Rice straw and willow	Simultaneous pretreatment and saccharification (SPS)	Removal of 49.8% and 32.6% of phenolic contents from the soaked rice straw and willow, respectively and saccharification yield of up to 74.2% and 63.6% for rice straw and willow, respectively	Dhiman et al., 2014
Laccase	<i>Trametes versicolor</i>	Wheat straw	Treated with dilute sulphuric acid	Released higher glucose concentration of up to 2.3 g L ⁻¹ as compared to control	Heap et al., 2014
Laccase	<i>Tyromyces chioneus</i>	Rice straw	in a rotary shaker 150 rpm	Saccharification yield was observed up to 74.2%	Dhiman et al., 2014
Laccase	<i>Myceliophthora thermophila</i>	Wood	45°C for 72 h in thermostate shaker 170 rpm	50% of lignin removed	Rico et al., 2014

- The following **Figure-20** (a) and (b) gives basic understanding of the enzyme pretreatment.



(a)



(b)

Figure-20: Enzyme pretreatment LCB.

- **Lignin Peroxidase (LiP)**
 - ❖ Lignin peroxidase (LiP) (EC 1.11.1.4) was first discovered in *Phanerochaete chrysosporium* grown in nitrogen-limited medium.
 - ❖ LiP is H₂O₂ dependent glycoprotein, which contains heme and has a molecular mass ranges from 35 to 40 kDa.
 - ❖ LiP has been reported to produce by white-rot fungi like *Phlebia flavido-alba*, *Bjerkandera sp.* strains BOS55, *Trametes trogii*, *Phlebia tremesllosa*, *Gloeophyllum trabeum*, *Trametes versicolor*, *Phanerochaete chrysosporium*, etc.
- **Laccases (Lac)**
 - ❖ Laccases (Lac) (EC 1.10.3.2) or benzenediol oxygen oxidoreductase is a multicopper enzyme that belongs to a group of blue copper containing oxidase.
 - ❖ White-rot fungi produced laccase, catalyzes the oxidation of aromatic amines and phenolic compounds such as phenolic substructure of lignin.
 - ❖ Lac is glycoprotein and its molecular weight ranges between 60 and 80 kDa.
 - ❖ It is also able to oxidize non-phenolic substructure of lignin in the presence of low molecular weight compounds hydroxyl benzotriazole.
 - ❖ Laccase producing microorganisms are both fungi (*Trametes versicolor*, *Tremetes trogii*, *Phlebia floridensis*), and bacteria (*Citrobacter spp.*, *Straphylococcus saprophlticus*, *Bacillus subtilis*).
 - ❖ Lac enzyme plays an important role in lignin degradation and modification processes which increase the yield of both hydrolysis and fermentation process.
 - ❖ Laccase is an important enzyme since it oxidizes both toxic and non-toxic substrates.
 - ❖ It is used in textile, food processing, wood processing, pharmaceutical, and chemical industries.
 - ❖ Laccase enzyme is very specific, ecologically sustainable and a proficient catalyst.
 - ❖ Several bacterial and fungal laccases have been used for detoxification of various agro-waste (pretreated and unpretreated) feedstocks in the presence of mediator system.
- **Manganese Peroxidase (MnP)**
 - ❖ Manganese peroxidase (MnP) (EC 1.11.1.13), Mn (II) hydrogen peroxide oxidoreductase, catalyzes Mn dependent reactions.
 - ❖ Similar to LiP, MnP is also heme glycoprotein.
 - ❖ Major difference in MnP and LiP is: LiP generally oxidizes non-phenolic lignin, while MnP oxidizes phenolic ring of lignin and plays an important role in the initial stage of lignin degradation.
 - ❖ Manganese peroxidase oxidizes phenolic and non-phenolic lignin units through lipid peroxidation reactions and forms various phenolic compounds i.e., 3-ethylthiazoline-6-sulfonate, 2, 6-dimethyloxyphenol syringol, guaiacol, and non-phenol compound i.e., alcohol.
 - ❖ It oxidizes Mn²⁺ to Mn³⁺ which further oxidizes phenol rings to phenoxy radicals leading to decomposition of compounds.
- **Versatile Peroxidase (VP)**
 - ❖ Versatile peroxidase (VP) (EC 1.11.1.16) oxidizes phenolic and non-phenolic aromatic compounds.

- ❖ VP enzymes have the catalytic activities of both MnP and LiP, and are able to oxidize Mn^{2+} like MnP as well as high-redox potential non-phenolic compounds like LiP.
- ❖ VP is employed together with MnP, LiP and other microbial peroxidases in degradation of non-phenolic aromatic compounds such as veratrylglycerol β -guaiacyl ether to veratraldehyde. VP also oxidize Mn^{2+} to Mn^{3+} , veratyl alcohol to veratraldehyde and p-dimethoxybenzene to p-benzoquinone.
- ❖ VP comprises the ligninolytic heme peroxidase gene family of *Pleurotus ostreatus*.
- ❖ It is found in various *Bjerkandera species* and *Pleurotus species*.
- ❖ It catalyzes oxidation of wide range of substrates from plant peroxidase hydroquinones, substituted phenols to bulky recalcitrant lignin directly, without redox mediators.
- ❖ It is used in textile, bleaching, paper and pulp industries, production of biofuels, bioremediation of xenobiotic compounds, degradation of endocrine disrupting chemicals.

➤ ENZYME (BIOLOGICAL) HYDROLYSIS

- ✓ Enzymatic hydrolysis is the process in which cellulases are added to hydrolyze pretreated lignocellulosic biomass into fermentable sugars.
- ✓ Enzymatic hydrolysis is the breakdown of a compound in presence of enzymes following its reaction with water.
- ✓ It has been extensively used in food and power industries.
- ✓ Power industries use enzyme hydrolysis to help provide renewable energy, as with cellulosic biodiesel, ethanol, bioethanol etc.
- ✓ The rate of enzymatic hydrolysis is also influenced by enzyme sources and the proportion of different enzyme components.
- ✓ **Ionic Liquid Enzyme Hydrolysis**
 - Biofuels and -chemicals can be produced from carbohydrates in lignocellulosic biomass.
 - For an efficient total enzymatic hydrolysis of the plant cell wall polysaccharides, a pretreatment step is required.
 - Ionic liquids (ILs) such as [BMIM]Cl, [BMIM]BF₄ have recently gained considerable interest as solvents for cellulose and lignocellulosic biomass and pretreatment of lignocellulose with ILs is currently an extensively studied concept.
 - However, the applicability of ILs in an integrated process, in which enzymatic hydrolysis is done in the same vessel as the IL pretreatment without IL removal and substrate washing between the process steps, suffers from the fact that cellulose-dissolving ILs severely inactivate the cellulases used to catalyse the polysaccharide hydrolysis.
 - Impressive advances have recently been made in discovering and developing cellulases and other glycosyl hydrolases with increased IL-tolerance.
 - Different cellulase stabilisation techniques and the design of enzyme-friendly cellulose-dissolving ILs are also available.

- In light of the recent developments, the integrated enzymatic hydrolysis of polysaccharides in the presence of ILs may well prove to be a potential route for utilizing lignocellulosic biomass as feedstock in biofuel and -chemical production.
- The following **Figure-3** briefly explains the process of hydrolysis.

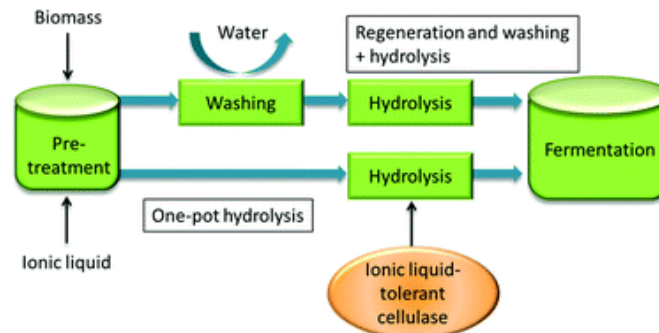


Figure-3: Ionic Liquids (ILs) enzyme hydrolysis of lignocellulosics.

- The main components of lignocellulosics are cellulose, hemicelluloses and lignin, in various ratios depending on the source of biomass.
- Together these polymers form a complex matrix, which is highly recalcitrant towards depolymerization.

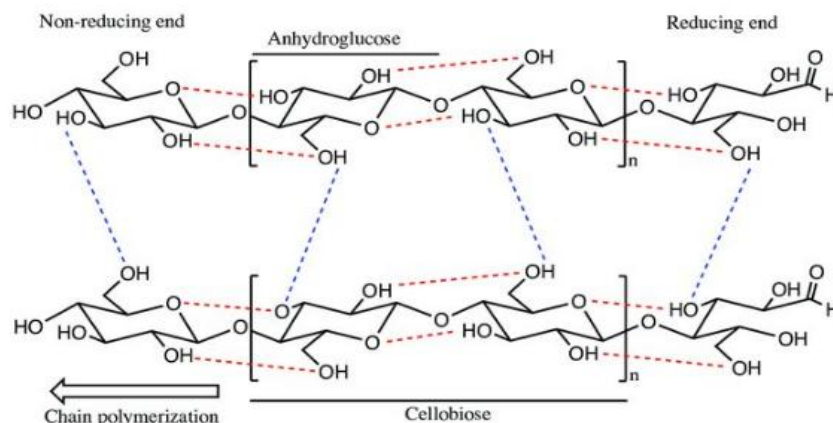


Figure-5: Cellulose structure with the intra--and inter--chain hydrogen bonds.

- Cellulose and hemicelluloses are polysaccharides.
- Cellulose is a linear homopolymer consisting of anhydroglucose units linked together by 1,4-β-glycosidic bonds.
- The anhydroglucose units are distorted 180° to each other so that the smallest repeating unit in the cellulose chain is the anhydrocellulose unit (**Figure-4**).