LIGNOCELLULOSE BIOTECNOLOGY

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- **Definition:**
- **Lignocellulose** refers to plant dry matter **(biomass)**, so called **lignocellulosic biomass**. It is the most abundantly available raw material on the Earth for the production of biofuels, mainly bio-ethanol.

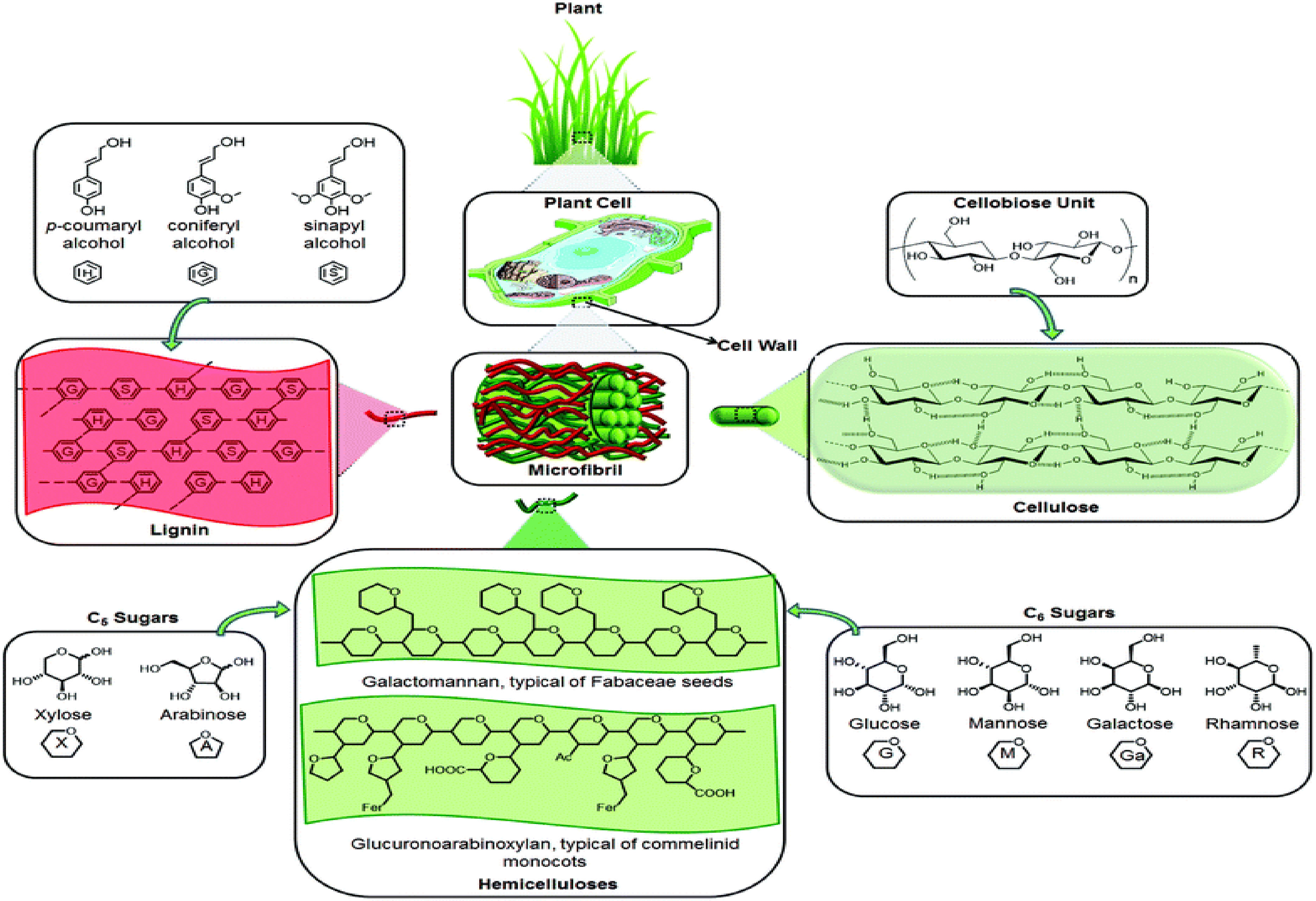
It is chiefly made up of

- Carbohydrate polymer (Cellulose, hemicellulose)
- aromatic polymer (lignin) are also predominate biopolymers of plant cell wall. Together, they are termed as Lignocellulose.

Structure and composition

- Cell walls of plants consist mainly of three organic compounds: cellulose, hemicellulose and lignin with small amounts of acetyl group, minerals and phenolic substituents.
- Lignocellulose has evolved to resist degradation and this robustness or recalcitrance of lignocellulose stems from the crystallinity of cellulose, hydrophobicity of lignin, and encapsulation of cellulose by the lignin–hemicellulose matrials major components of natural lignocellulosic materials.
- The major component of lignocellulosic biomass is cellulose. Unlike glucose in other glucan polymers, the repeating unit of the cellulose chain is the disaccharide cellobiose.
- Since about half of the organic carbon in the biosphere is present in the form of cellulose, the conversion of cellulose into fuels and valuable chemicals has paramount importance.

- Hemicellulose is the second most abundant polymer. Unlike cellulose, hemicellulose has a random and amorphous structure, which is composed of several heteropolymers including xylan, galactomannan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan.
- Hemicelluloses are imbedded in the plant cell walls to form a complex network of bonds that provide structural strength by linking cellulose fibres into microfibrils and cross-linking with lignin.
- Finally, lignin is a three-dimensional polymer of phenylpropanoid units. It functions as the cellular glue which provides compressive strength to the plant tissue and the individual fibres, stiffness to the cell wall and resistance against insects and
- Cellulose, hemicellulose and lignin are not uniformly distributed within the cell walls. The structure and the quantity of these plant cell wall components vary according to species, tissues and maturity of the plant cell wall. Generally, lignocellulosic biomass consists of 35–50% cellulose, 20–35% hemicellulose, and 10–25% lignin. Proteins, oils, and ash make up the remaining fraction.



main components and structure of lignocellulose

- Lignocellulose is the most abundant biomass available on Earth. It has attracted considerable attention as an alternate feed stock and energy resource because of the large quantities available and its renewable nature. The potential uses of lignocelluloses are in pulp and paper industries, production of fuel alcohol and chemicals, protein for food, and feed using biotechnological means.
- The current industrial activity of lignocellulosic biomass fermentation is limited mainly because of the difficulty in economic bioconversion of these materials to value-added products. Considerable improvement in many processes related to lignocellulose biotechnology appeared during the last decade.

Role of pre-treatment

- Pre-treatment is an important step for the recovery of cellulosic content from lignin based biomass as compared to the starchy materials and is also required to break down the lignin barrier to recover cellulose which is further subjected to enzymatic hydrolysis to convert into fermentable sugars. During the past few decades, several pre-treatment approaches have been developed for generating cost-effective fermentable sugar from most of the agricultural cellulose and hemicellulose containing lignocellulosic materials.

Chemical pre-treatments

- To date chemical pre-treatment is the most studied technique among various pre-treatment categories used for delignification of cellulosic materials. Chemical hydrolysis is an important treatment method for recovery of sugar monomers from cellulose and hemicellulose polymers from lignocellulosic biomass by optimizing chemical reagents. The most commonly used chemical pre-treatments include:

Acid based hydrolysis

- Chemical treatment of cellulosic biomass with concentrated hydrochloric acid or sulphuric acid is conventional procedure. The entire process of pre-treatment can be operating at very low temperature as compared to dilute-acid pre-treatment. One of the possible drawbacks of this process is that it's required in higher concentration (30–70%), therefore cause high level of corrosive reaction.

Alkali based hydrolysis

- Alkali based pre-treatment involves the use of bases such as sodium and ammonium hydroxide for the pre-treatment of agricultural lignocellulosic feed-stocks. Alkaline hydrolysis causes various structural alterations inside the lignocellulosic material during treatment process such as the depletion of lignin barrier, cellulose swelling and partial decrystallization and solvation of cellulose and hemicelluloses, respectively.
- Lignocellulosic feed-stocks that have been shown to benefit from the method of alkaline pre-treatment are corn stover, switch-grass, bagasse, wheat, and rice straw. sodium hydroxide is effectively used in pre-treatment for hardwoods, wheat straw, switch-grass, and soft-woods with less than 26% lignin content.

Biological pre-treatment

- Biological pre-treatment employs wood degrading microorganisms
- white rot fungi (WRF), brown or soft-rot fungi
- Bacteria to modify the composition and/or structure of the lignocellulosic biomass. Bio-delignification is useful for pre-treatment purposes because it replaces or supplements the chemical-based pre-treatments which include mechanical treatment with acid, alkali, and steam explosion . Biological pre-treatments are more effective, economical, eco-friendly and less health hazardous as compared other pre-treatment approaches.
- Bio-conversions of lignocellulosic materials to useful products normally require multi-step processes that include **pre-treatment, enzymatic hydrolysis and fermentation** so that the modified or pre-treated biomass is more amenable to enzyme digestion. Increasing understanding of termites and fungal systems has provided insights for developing more effective pre-treatment technologies to realize the above mentioned advantages or benefits of biological pre-treatment over some others.
- **Drawbacks**
- Bological pre-treatment is a very slow process that also requires careful control of growth conditions and large amount of space to perform treatment. In addition to this most of the lignolytic microorganisms solubilize/consume not only lignin but also hemicellulose and cellulose. Because of these drawbacks/limitations the biological pre-treatment faces techno-economic barriers and therefore is less attractive commercially.
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Enzymatic hydrolysis

- Enzymatic hydrolysis is an effective and economical method to achieve fermentable sugars under mild and eco-friendly reaction conditions from the pre-treated cellulosic biomass. The entire process of enzymatic hydrolysis critically depends on variety of factors viz., pH, time, temperature substrates and enzyme activities, etc.
- Enzymatic saccharification is done separately from fermentation known as separate hydrolysis and fermentation (SHF). When cellulose hydrolysis and fermentation are carried out simultaneously the phenomenon is known as simultaneous saccharification and fermentation (SSF). Now a days this process of simultaneous saccharification of both cellulose and hemicellulose is achieved by co-fermentation of both hexoses and pentoses sugars (SSCF) with the help of genetically engineered microbes that ferment xylose and glucose in the same medium where both enzymes for cellulose and hemicelluloses are available.

Advantage

- SSF and SSCF can be performed in the same tank which makes the entire process cheap, feasible and cost-effective (reduce the capital and operational investment). Biological, physical and chemical methods have been employed for detoxification (removal of inhibitory compounds in fermentation) of lignocellulosic hydrolyzates .

Lignocellulosic materials have different degree of inhibition and tolerance levels vary according to different microbial strains. Degree of tolerance varies with different strains of *Saccharomyces cerevisiae*, so inhibitory compounds are detoxified by changing the substrate concentration and altering the pH of media

Fermentation strategy

- Ethanol production from biomass is mainly categorized into three steps process (1) achieve a fermentable sugars (2) conversion of fermentable sugars into ethanol and (3) ethanol separation and purification through distillation. Difference between lignocelluloses or starch ethanol production is the step for obtaining sugars before fermentation. Sugar crops or starchy crops need milling and grinding for recovery of sugars by extraction and fermentation becoming a relatively simple process that requires no hydrolysis or pre-treatment steps for obtaining sugars and transformation into ethanol . Bio-ethanol production is mainly done by fed-batch process and low ethanol produce by multi-stage continuous fermentation.
- Basic steps for the conversion of lignocellulosic biomass are:

(1) pre-treatment process which can reduce the lignin content and render cellulose and hemicellulose content for enzymatic hydrolysis

(2) steps to convert enzymatic hydrolysis to break down polysaccharide to simple sugars

(3) conversions of sugars (hexoses and pentoses) for ethanol production through microorganisms

(4) production of ethanol from pentose sugars.

- Several fungal species belonging with genera *Fusarium*, *Rhizopus*, *Monilia*, *Neurospora* and *Paecilomyces* have been found potential for fermenting glucose as well as xylose. Production of bio-ethanol from cellulose is mostly conducted by using fermentative organism, but the conversion rate is very low due to byproducts formation. Filamentous fungus *Fusarium oxysporum* is also known for the production of bio-ethanol through SSF by direct utilizing the cellulose, but their conversion rate is low due to production of acetic acid as a byproduct.

Enzymes production

- To date, the production of various ligninolytic enzymes including Lignin Peroxidase, Manganese- dependent peroxidase
- versatile peroxidise and laccases and other lignocellulolytic mainly endoglucanases , cellobiohydrolases and β-glucosidases have been widely studied in submerged and solid culture processes in the laboratory ranging from flask shake to large scale.
- There are large numbers of microorganisms capable for degrading cellulose. *Trichoderma*, *Aspergillus*, *Penicillium* and *Fusarium* species are commonly used for cellulases production.
- Selections of desired fungal strains depend on several factors and selection of substrate for optimizing the cellulase producing conditions.

Applications

- Ligninolytic, cellulases and hemicellulases are important industrial enzymes having numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture.