Chapter 1

General Introduction and Review

1.1. General Introduction

Plant lignocellulose biomass has been long recognised as an important source of fermentable sugars for the production of biofuels and other value-added products (Awasthi *et al.,* 2020). Lignocellulosic biomass is composed mainly of cellulose (40-60%); hemicellulose (20-40%) and lignin (10-25%)(Ragauskas *et al.,* 2006). Cellulose contains micro fibrils, made up of upto 36 glucan chains which have glucose residues linked by β-1,4-glycosidic bonds and provides mechanical strength to the plant cell wall (Mohanram *et al.,* 2013). Hemicelluloses include xylan, β-glucan, arabinoxylan, xyloglucan, galactomannan, mannan, and arabinan which are built up of pentose sugars (D-xylose,), hexoses (D-galactose, D-mannose, D-glucose) and sugar acids (Girio *et al.,* 2010). Both cellulose and hemicellulose are good sources of simple sugars, which can be converted by fermentation and biocatalytic processes to value-added products such as biofuels, biopolymers and chemicals (Hahn *et al*., 2006). Initially attempts were made to use dilute acid for hydrolysis, however this process was inferior to the process of enzymatic hydrolysis as the chemicals generated undesirable by-products (Kucharska *et al*., 2018). Enzymatic hydrolysis is also cheaper as compared to acid or alkaline hydrolysis because enzyme hydrolysis does not have corrosion problem (Kumar *et al*., 2008). Glycoside hydrolases (GHs) act on glycosidic bonds of polysaccharides to produce sugars. GHs, such as endoglucanase, exoglucanase, endoxylanase, β-xylosidase, α-L-arabinofuranosidase, acetyl xylanesterase, β-glucosidase and cellobiohydrolase are being used to produce simple sugars from polymeric cellulose or hemicellulose which can then be converted into value added products (Zhu *et al.,* 2016). The classical model of cellulose deconstruction involves endo-1, 4-β-D-glucanglucanohydrolase (E.C.3.2.1.4), 1, 4-β-D-glucancellobiohydrolase (E.C.3.2.1.91) and β-glucosidase (E.C. 3.2.1.21). Endocellulases hydrolyze internal glycosidic linkages in a random fashion, which results in a rapid decrease in polymer length and a gradual increase in the reducing sugar concentration. Endocellulases and exocellulases act on cellulose to produce cellooligosaccharides and cellobiose, which are then cleaved by β-glucosidases to glucose (Kumar *et al*., 2008). Hydrolysis of hemicelluloses involves endo-1, 4-β-D-xylanase (E.C.3.2.1.8), xylan 1, 4-β-xylosidase (E.C. 3.2.1.37), α-L-arabinofuranosidase (E.C. 3.2.1.55) and acetyl (xylan) esterase (E.C. 3.1.1.72) (Hu *et al*., 2018). The crystalline recalcitrance of cellulose and the limited number of cellulases are the major rate-limiting steps in the bioconversion of lignocelluloses (Sun *et al*., 2002).

Therefore, better cellulase preparations with properties like high catalytic efficiency on insoluble substrate, increased stability at higher temperature, pH and higher tolerance to end product inhibition are highly desirable (Mohanram *et al*., 2013). Improving conversions of biomass to sugars by increasing hydrolysis yields, reducing enzyme loadings, and reducing pretreatment are major targets to make the process more cost effective (Wilson, 2009). Moreover using a mixture of different cellulolytic and accessory enzymes for efficient hydrolysis of cellulose and hemicelluloses to fermentable sugars would be a good approach (Kumar *et al*., 2008).

The degradation of lignocellulosic biomass in natural habitat occurs with the cooperation of many microorganisms which produce a variety of cellulolytic and hemicellulolytic enzymes (Sun and Cheng, 2002). Therefore, the use of microbial co-cultures or complex communities in the biodegradation of cellulosic biomass can be an efficient approach since it avoids the problems of feedback regulation and metabolite repression experienced by isolated single strains (Haruta *et al.,* 2002). Lignocellulose deconstructing microbial consortia and their mixed enzyme systems could reveal the complex interactions involved in lignocellulose degradation in nature (Lynd *et al*., 2005).

Moreover, if such enzyme cocktails are tailored to function optimally under the diverse conditions of pretreatment such as high temperature and extreme pH, then additional expenses required for alleviating such extreme conditions in industries prior to enzyme addition could be saved (Wongwilaiwalina *et al.,* 2010). Hence, such studies on Lignocellulolytic microbial consortium could act as a platform for potential biotechnological application in valorization of lignocellulosic biomass (Gladden *et al*., 2011).

1.2. Objectives

1. To develop thermophilic lignocellulose depolymerising microbial consortium from natural lignocellulose deconstruction environments.

2. To analyze the consortium for thermophilic glycoside hydrolases activity by in vitro and in-gel assay.

3. To study the microbial community profile and diversity of the consortia under thermophilic and mesophilic conditions by metagenome analysis.

4. To study the expression profile of CAZymes from the consortia under thermophilic and mesophilic conditions by metatranscriptomic analysis.

5. To check the efficiency of enzyme cocktail in the saccharification of agroindustrail wastes as compared with commercial enzymes at elevated temperature(s) and analysis of saccharification products.

1.3. Review of Literature

1.3.1. Agricultural wastes and Agroresidues

Agricultural wastes are carbon-based materials generated as by-products during the harvesting and processing of agricultural crops. They have fibrous structure, are low in nitrogen and vary in composition with geographic location (Adejumo *et al.,* 2021). These field residues are occasionally utilized as fertilizer, for erosion control and as fodder for livestock but almost half of these are combusted in the farm owing to their low digestibility and low nutritional content (Sadh *et al.,* 2018).

Agricultural wastes can be categorised into three types (Figure 1.1):

- **Postharvest agricultural waste:** This type of waste is classified as the primary agricultural residue. It primarily consists of straw, husk, and stalks of cultivated crops that remain after harvest (Adhikari *et al*., 2018).
- **Agro-industrial residues:** These are secondary agricultural residues that remain after the processing of agricultural crops into various bio-product forms. These include husks, molasses, bagasse, peels, hulls, etc. (Adhikari *et al.,* 2018).
- **Livestock manure:** Agricultural land, in addition to being used for cultivation is also devoted to rearing of farm animals, such as cattle, pigs, and poultry. These animal sectors generate around 120 million tonnes of manure every year which is considered as a type of agricultural waste (Chukwuma *et al.,* 2020).
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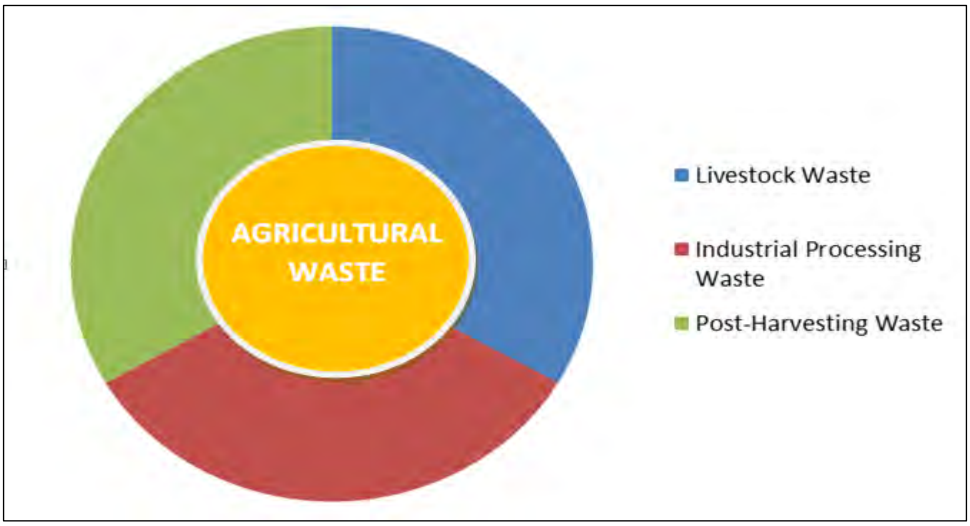

Figure 1.1: Types of Agricultural wastes.

Agricultural residues, also known as agroresidues, are the post harvesting lignocellulosic waste materials (LCW), and include a variety of materials, such as bagasse, husks, cobs, fruit peels, straws and stems from cereal grains such as rice, wheat, barley, and maize etc. that can replace woody lignocellulosic biomass in industries (Tolessa *et al.,* 2022). These agroresidues have a complex chemical composition that include lignocellulosic and polyphenolic compounds that need complicated bioconversion procedures for obtaining different value added products (Awasthi *et al.,* 2020). To ensure sustainable and cleaner production methods of value-added products, effective utilization of agricultural wastes is desirable which can be done through a variety of processes (physical, chemical, and biochemical) into wide range of chemical-based high-value products, such as fuels (ethanol, biodiesel, etc.), energy (heat, electricity, etc.), materials (fiber-based products) etc. (Sadh *et al.,* 2018).

With a worldwide production of such wastes**,** management methods require a thorough understanding of the availability of agricultural wastes worldwide. Figure 1.2 provides a rough estimate of the potential availability of some crops residues available annually across India.

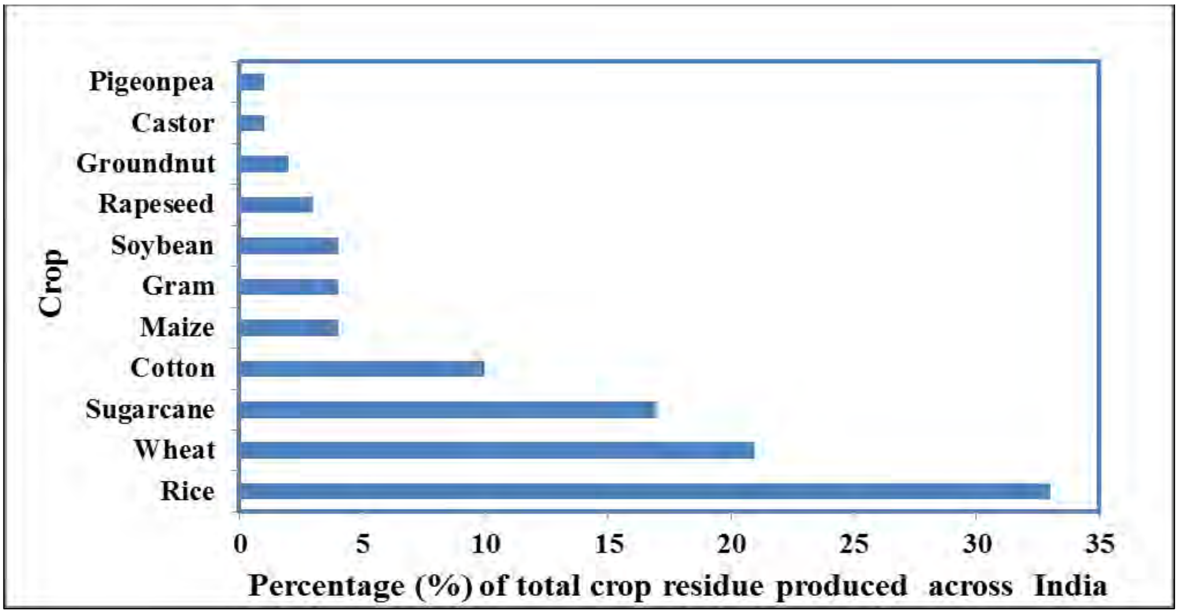

Figure 1.2: Categories of total crop residue produced across India.

1.3.2. Composition of agro-residues

Agro-residues mostly comprise of lignocellulose that is a compact structure consisting of (Chandra and Madakka, 2019) :

1. Partly crystalline and linear polysaccharide - cellulose
2. Branched non-cellulosic and non-crystalline heteropolysaccharides -hemicellulose
3. Branched (non-crystalline) lignin

Lignocellulose is made up of microfibrils of cellulose embedded in a matrix of lignin and hemicellulose (Figure 1.3). The average composition of lignocellulose is 45% cellulose, 30% hemicellulose and 25% lignin (Wang *et al.,* 2020). Table 1.1 gives an estimate of the percentage (%) chemical composition of various lignocellulosic biomasses as per recent reports (Kaloudas *et al*., 2021).

❖ Cellulose

Cellulose consists of linear chains of approximately 8000 to 12000 residues of β-D-glucopyranose units joined through β-1, 4-glycosydic linkage (Broda *et al.,* 2022). One glucose residue is the monomeric unit of cellulose (French *et al.,* 2017) and cellulosic polymers made up of 30 to 300 chains are aligned collaterally and non-collaterally to form a highly crystalline structure called cellulose microfibril (Mafa *et al.,* 2021). These microfibrils contribute to the insolubility and low reactivity of cellulose and also make it more resistant to acid hydrolysis and water entrance (Kubicki *et al*., 2018). Cellulose also has some amorphous regions with weaker organization, making the regions more accessible to enzymes and susceptible to hydrolysis (Verma *et al*., 2021). The strands of cellulose remain integrated to the lignin-hemicellulose component of lignocellulose and cellulose is known to partly resist its degradation by enzymes due to its high tensile strength, and insolubility in most solvents (Chen *et al.,* 2021). The degree of polymerization (DP) i.e., the number of glucose units in the polymer also plays an important role in facilitating the enzymatic accessibility (French *et al*., 2017). Moreover, the hydrophobic surface of cellulose results in formation of a dense layer of water which hinders the diffusion of enzymes and degradation products near the cellulose surface (Wei *et al.,* 2020).

❖ Hemicellulose

Hemicellulose is heterogeneously branched, non-crystalline heteropolyssacharide molecule present in polyssacharide in plant cell walls (Machmudah *et al.,* 2017). It contains pentoses (xylose, arabinose), hexoses (mannose, glucose, and galactose) and/or uronic acids (glucuronic, methylgalacturonic and galacturonic acids). Other sugars such as rhamnose, fucose are also present and acetyl groups may replace the hydroxyl groups of sugars at some positions (Girio *et al.,* 2010).Hemicelluloses are classified according composition of the sugars in their backbone i.e. xylan (β-1, 4-linked xylose) or mannan (β-1, 4-linked mannose). The most abundant building block of hemicellulose is xylan (Moreira *et al.,* 2016). This complex network of sugars link cellulose fibers into micro fibrils and cross-link with lignin providing structural strength to plant cell wall. Two major hemicelluloses in plant cell wall are galacto(gluco)mannans and xyloglucans. Hemicellulose is widely used in industrial applications because it is more prone to hydrolysis because of its amorphous and highly branched nature (Baruah *et al.,* 2018).

❖ Lignin:

Lignin, most abundant non-polysaccharide fraction in lignocellulose is a complex and recalcitrant aromatic polymer without any defined repetitive units. Three monolignols (p-coumaryl, coniferyl and sinapyl) and their acylated forms are the precursors of lignin (Muthukumaran *et al.,* 2021). These precursors are phenolic compounds, but a variety of ether and carbon–carbon inter-unit linkages formed during polymer synthesis makes the polymer non-phenolic (Basafa *et al*., 2023). Lignin is closely bound to cellulose and hemicellulose, providing rigidity and cohesion to plant cell wall. Due to its molecular configuration, lignin is highly resistant to chemical and enzymatic degradation (Khan *et al*., 2017). The association of lignin and hemicelluloses form a dense and organized network that surrounds the cellulose through extensive hydrogen bonding to form the lignin-carbohydrate complexes (LCC) (Bergenstrahle-Wohlert *et al*., 2022). In such LCCs lignin hinders enzyme-mediated hydrolysis of carbohydrates, since it acts as a physical barrier, restricting enzyme access to carbohydrate. Moreover in some cases lignin also interacts with cellulolytic enzymes possibly leading to non-productive binding (Huang *et al*., 2022).

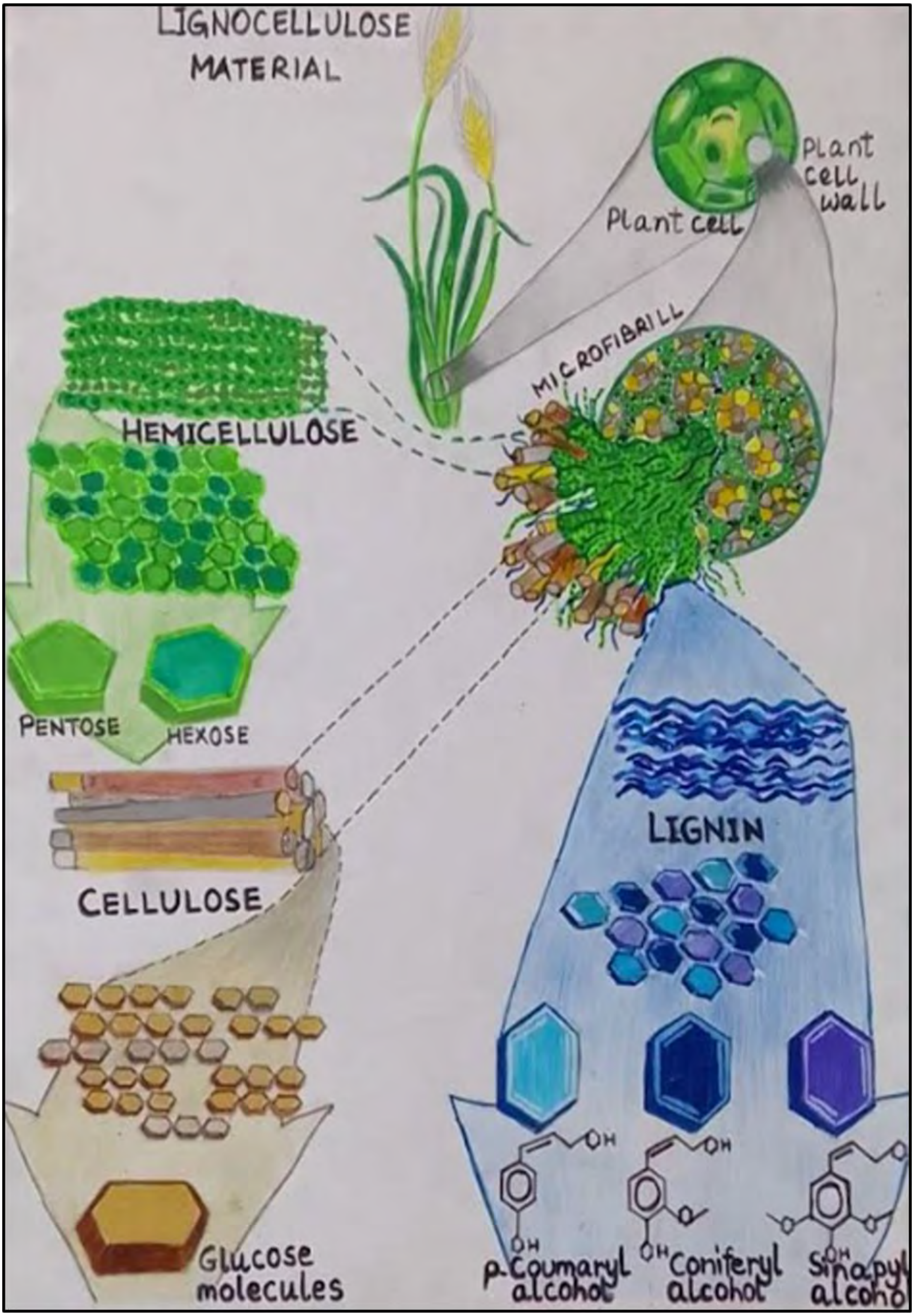

Figure 1.3: Structure of lignocellulosic biomass comprising of cellulose, hemicellulose and lignin.

Table 1.1: Percentage (%) composition of various agroresidues

Sl. No.	Agroresidues	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
1	**Barley straw**	31-45	27-38	14-19	(Saini *et al.,* 2015)
2	**Corn cob**	45	35	15	(Prasad *et al.*, 2007)
3	**Corn leaves**	26.93	13.27	15.18	(Rabemanolontsoa and Saka, 2013)
4	**Corn stover**	38	23	20	(Wan and Li, 2010)
5	**Oat straw**	31-37	27-38	16-19	(Sanchez, 2009)
6	**Rice husk**	37.1	29.4	24.1	(Kalita *et al.*, 2015)
7	**Rice straw**	29.2-34.7	23-25.9	17-19	(Isikgor *et al.*, 2015)
8	**Rye straw**	33-35	27-30	16-19	(Sanchez, 2009)
9	**Sorghum straw**	32-35	24-27	15-21	(Isikgor *et al.*, 2015)
10	**Soybean straw**	34	16	22	(Wan *et al.*, 2011)
11	**Sugarcane bagasse**	40-45	30-35	20-30	(Cardona *et al.,* 2010)
12	**Wheat Straw**	33-40	20-25	15-20	(Talebnia *et al.*, 2010)

1.3.3. Enzymatic deconstruction of lignocellulose

A number of lignocellulolytic enzymes falling under the category of Carbohydrate Active Enzymes (CAZymes) are involved in the enzymatic breakdown of lignocellulose into fermentable sugars including glucose and other sugars. These CAZymes, are classified into six classes based on their catalytic activities and amino acid sequence similarity: glycoside hydrolases (GHs), carbohydrate esterases (CEs), polysaccharide lyases (PLs), glycosyl transferases (GTs), auxiliary activities (AA), and carbohydrate-binding module (CBM) (Chettri *et al.,* 2020). Among these, cellulolytic GHs, like cellulases and hemicellulases, are crucial for cellulose depolymerization; CBMs are necessary for the binding of cellulolytic enzymes to their substrates; AAs are linked to the breakdown of lignin polymers; and CEs are essential for efficient hemicellulose activity (Zhu *et al.,* 2016). These enzymes are a large group of extracellular proteins that can be characterized into hydrolytic enzymes like cellulases, hemicellulases, esterases, mannanases, etc. and ligninolytic enzymes such as peroxidases and lacasses (Chukwuma *et al.,* 2020).

1.3.3.1. Cellulolytic enzymes

Cellulases (EC 3.2.1.4) hydrolyze the β-1, 4-glycosidic linkages of cellulose to produce glucose, cellobiose, and cello-oligosaccharides primarily. Most cellulases have a carbohydrate–binding module (CBM) which helps in the binding of the enzyme to crystalline cellulose. Cellulases produced by bacteria and fungi often have two or more functional and structural domains linked together by peptides (Ejaz *et al.,* 2018). A short polylinker region connects the cellulose binding module (CBM) of fungal cellulases to the catalytic domain (CD) (Srivastava *et al.,* 2019).

Bacterial cellulases are spread across approximately fourteen Glycoside Hydrolase (GH) families according to the International Union of Biochemistry and Molecular Biology (IUBMB) enzyme nomenclature (Panchapakesan *et al.,* 2016). Extracellular cellulases produced by microorganisms may be either cell associated or free, and on the basis of extensive evaluation of the biochemical analysis of cellulose systems from aerobic and anaerobic bacteria and fungi the following cellular system components have been categorized according to their mode of action (Elkins *et al*., 2010).

Endoglucanases or Endo-1, 4-β-D-glucan glucanohydrolases (EC 3.2.1.4)

Endoglucanases act randomly at amorphous sites in the polysaccharide chain, resulting in oligosaccharides of different lengths and new chains. It is effective against cellooligosaccharides, soluble cellulose derivatives like CMC, and acid swollen amorphous cellulose. Endoglucanases (EGs) cleave the cellulose polymer to release more free ends. They also function processively on crystalline cellulose (Guo *et al.,* 2023).

Exoglucanase or 1, 4-β-D-glucan cellobiohydrolases (EC 3.2.1.91)

Exoglucanases break down cellulose by hydrolyzing the 1, 4-β-D-glycosidic linkages and have a specific action on the reducing or non-reducing extremities of cellulose polysaccharide chains, releasing either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as primary products. These enzymes are effective against cellooligosaccharides, amorphous celluloses, and crystalline substrates like Avicel. They are ineffective, against cellobiose or substituted soluble celluloses like carboxymethylcellulose (CMC) (Annamalai *et al*., 2016).

Cellobiohydrolase (CBH) is an exocellulase that cleaves two to four units off the ends of cellulose. CBH comes in two different types, CBHI cleaves progressively from the reducing end and CBHII cleaves gradually from the non-reducing end of cellulose (Teter *et al.,* 2014)

β- glucosidases or β-D-glucoside glucohydrolases (EC 3.2.1.21)

β-glucosidase is an universal enzyme produced by bacteria, fungi, plants, animals and also by non-cellulolytic species such as human (Naraian *et al*., 2017). β-glucosidases hydrolyzes β-Dglucosidic bonds of a variety of compounds, including cyanogenic glucosides, aryl-β-D-glucosides, alkyl-β-D-glucosides, disaccharides, and short chain oligosaccharides, releasing glucose from their non-reducing end (Deng *et al.,* 2020). Crystalline or amorphous cellulose are not hydrolysed by the enzyme but β-glucosidase can also catalyze the synthesis of oligosaccharides/glycosides under certain circumstances (Shim *et al.,* 2022). Low levels of β-glucosidases are produced by microorganisms which are effectively inhibited by their end product i.e. glucose. As a result, cellobiose accumulates during cellulolysis, which in turn inhibits both endo- and exo-glucanase activities (Ahmed *et al.,* 2017).

1.3.3.2. Hemicellulolytic enzymes

Breakdown of hemicellulose is done by a combination of several enzymes that act on different sites of the hemicellulose matrix. Hemicellulases can be grouped on the basis of backbones present in the hemicellulose matrix, namely xylan, xyloglucan and mannan (Bharathiraja *et al*., 2017). The heterogeneity of xylan, demands the action of a complex enzyme system for its deconstruction. This is usually composed of β-1, 4-endoxylanase, β-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, acetylxylan esterase, and phenolic acid esterases. Among these enzymes endoxylanase and β-xylosidase have the most important role in involved in xylan hydrolysis. Side-chain cleaving enzymes, like α-arabinofuranosidase, α-glucuronidase, and acetylxylan esterase are required for the removal of side substituents of heteroxylans (Moreira *et al.,* 2016). The presence of multifunctional xylanolytic enzymes involved in synergism between the different components of the xylanolytic enzyme system has been reported from many bacterial and fungal species (Vuong *et al.,* 2022).

Endo-1, 4-β-xylanases (EC 3.2.1.8)

Endo-1, 4-β-xylanases (1, 4-β-D-xylan-xylanohydrolase; EC 3.2.1.8) catalyse the deconstruction of xylan (Peng *et al*., 2019). Endoxylanases can be demarcated on the basis of final products released from the hydrolysis of the xylan rachis (e.g., xylose, xylobiose and xylotriose, arabinose). Among all xylanases, endoxylanases are the most important due to their direct involvement in cleaving the glycosidic bonds and in liberating short xylooligosaccharides (Verma and Satyanarayana, 2012).

Xylosidases (EC 3.2.1.37)

β-xylosidases are hemicellulases that hydrolyze β-1,4-glycosidic bonds of xylobiose and short xylooligoscharides released by endoxylanases. Amongst the xylosidases, GH43-xylosidases are a very attractive source of high-performing enzymes for deconstruction of plant biomass (Rohman *et al*., 2019). β-D-xylosidases have been classified based on their affinities for xylobiose and high molecular weight xylooligosaccharides. They may be monomeric, dimeric or tetrameric with molecular weights ranging from 26 to 360 kDa and are secreted by different types of bacteria and fungi (Bretagne *et al*., 2021). Purified β-xylosidases generally do not hydrolyse xylan, their best substrate is xylobiose and their affinity for xylooligosaccharides is inversely proportional to its degree of polymerization. They are, however, capable of cleaving synthetic substrates such as p-nitrophenyl- and o-nitrophenyl-β-D-xylopyranoside (Naraian, 2018).

Arabinofuranosidases (EC 3.2.1.55)

Arabinofuranosidase hydrolyzes non reducing α-L-arabinofuranosyl groups of arabinans, arabinoxylans, and arabinogalactans. These are of two types: Exo-acting α-L-arabinofuranosidases (EC 3.2.1.55) and Endo-1, 5-α-L-arabinofuranosidases (EC 3.2.1.99). Exo-acting α-L-arabinofuranosidases are active against p-nitrophenyl-α-L-arabinofuranoside and on branched arabinans. Arabinofuranosidases usually exist as monomers, but other forms such as dimeric, tetrameric, and octameric forms have also been reported so far (Bajpai, 2014).

Acetylxylan esterase (EC 3.1.1.6)

Acetylxylan esterase removes the O-acetyl groups from positions 2 and/or 3 on the β-D-xylopyranosyl residues of acetyl xylan (Embaby *et al.,* 2022). It plays an important role as the acetyl side-groups pose steric hindrance and reduce the efficiency of enzymatic degradation.

Mannan and xyloglucan degradation

Mannans consist of a backbone of β-1, 4-linked D-mannose and D-glucose (glucomannans) having D-galactose side chains. Hydrolysis of mannans is carried out by the following enzymes:

- Endo-β-1, 4-mannanase (EC 3.2.1.78) which are endohydrolases that act on the mannan fibres by cleaving the internal β- 1, 4 bonds in mannan. This action of β-mannanases leads to formation of mannooligosaccharides.
- Exo-β-1, 4-mannosidases (EC 3.2.1.25) then act on the terminal, non-reducing ends of mannooligosaccharides and cleave them to release mannose (McGregor *et al*., 2022).

Hydrolysis of xyloglucan is carrried out by the combined action of endoglucanases (xyloglucanases) and β-glucosidases. The xyloglucan-active endoglucanases have specific modes of action (Matsuzawa *et al.,* 2021).

1.3.3.3. Ligninolytic enzymes

Lignin degrading enzymes may be basically divided into two main categories:

- ***Lignin-degrading auxiliary (LDA) enzymes:*** LDA enzymes enable the breakdown of lignin through the sequential action of many enzymes which includes hydrogen peroxide production. This group includes glyoxal oxidase (EC 1.2.3.5), aryl alcohol oxidases (EC 1.1.3.7), pyranose-2-oxidase (EC 1.1.3.10), cellobiose dehydrogenase (EC 1.1.99.18) and glucose oxidase (EC 1.1.3.4) (Kumar *et al.,* 2020).

- ***Lignin-modifying enzymes (LME):*** These are required to complete the lignin degradation process since LDA enzymes cannot breakdown lignin on their own. LME are produced by different microorganisms and this group includes lignin peroxidase (LiP, EC 1.11.1.14), Manganese-dependent peroxidase (MnP, EC 1.11.1.13), Versatile peroxidase (VP, EC 1.11.1.16), Dye-decolorizing peroxidase (DyP, EC 1.11.1.19) and Laccases (Lac, EC 1.10.3.2) (Janusz *et al*., 2020).

Table 1.2: Enzymes involved in Lignocellulose deconstruction.

LIGNOCELLULOLYTIC ENZYMES			
Holocellulolytic		**Ligninolytic**	
Cellulolytic	**Hemicellulolytic**	**Lignin Modifying Enzymes**	**Lignin Degrading Auxillary Enzymes**
1. Endoglucanase 2. Exoglucanase 3. β-D-glucosidase 4. Cellobiose dehydrogenase	1. Endoxylanase 2. β-D-xylosidase 3. Arabinofuranosidase 4. Acetyl xylan esterase 5. α-glucuronidase	1. Laccase 2. Lignin peroxidase 3.Manganese peroxidase 4.Versatile peroxidase	1. Glyoxal oxidase 2. Aryl alcohol oxidases 3. Pyranose-2-oxidase 4. Glucose oxidase

1.3.4. Natural lignocellulolytic environment

Natural lignocellulolytic environments, like forest soil, marine environment, decaying wood, marshes and swamps, are a potential source of lignocellulolytic microorganisms for efficient degradation and depolymerisation of agro-residues to reducing sugars (Bajpai, 2014). These microbes help in maintaining the organic carbon and nitrogen recycling equilibrium in nature (Khatoon *et al*., 2017). Bacteria and fungi present in lignocellulose rich habitats such as digestive tract of ruminants, guts of wood degrading termites and worms, sewage sludge, decaying vegetables, composting units use lignocellulosic biomass as an energy source and in the process; secrete various CAZymes that help to breakdown the complex biomass (Chadha *et al.,* 2019). Since it is not feasible for a single microorganism to produce all the enzymes required for efficient degradation of lignocellulose, the use of microbial consortia developed from such environments is attractive.

1.3.4.1. Insects/termite guts

Several invertebrates, like wood borers, termites, beetles, etc, feed on woody plant materials and their digestive tracts contain a very rich and diverse microbial community (bacteria, archaea and protists) that have adapted to their diverse niche and food sources by evolving various strategies that helps in effective lignocellulosic biomass degradation (Muthukrishnan *et al*., 2022). Termite gut is divided into three parts such as foregut, midgut and the hindgut. The degradation of lignocellulose occurs in the hindgut of termites is facilitated by cellulose degrading bacteria present in the hindgut (Thapa *et al.,* 2020). Various microorganisms are present in termite gut which includes gram positive bacteria like *Bacillus*, *Streptomyces*, *Paenibacillus*, *Actinobacteria* group and gram-negative bacteria like *Acinetobacter*, *Pseudomonas*. Various facultative microbes such as *Enterrobacter aerogens*, *Citrobacter farmer* and *Serratia marcescens* are also present (Mohammadipour *et al.,* 2021). Within termite's gut, cellulose is degraded by various cellulolytic species which then is subsequently metabolized into other forms (Thapa *et al.,* 2020).

1.3.4.2. Rumen microbiota

Ruminants have a compartmentalized stomach with four chambers called rumen, reticulum, omasum and abomasum. The microorganisms living within the rumen helps in various functions, such as generating energy through digestion of nutrient, increasing utilization of feed efficiency, better immune response and also helps in prevention from invasive pathogens (Mc Grosky *et al.*, 2019; Trotta *et al.,* 2021). This ruminal microbiome produces various CAZymes such as glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), and carbohydrate esterases (CEs). The synergistic action of these CAZymes help in digestion of cellulose, hemicelluloses, pectin and lignin present in the feedstock (Mizrahi *et al.,* 2021). The predominant cellulolytic bacteria present in rumen includes *Bacillus*, *Pseudomonas*, *Clostridium*, *Paenibacillus*, *Ruminococcus*, *Rhodobacter*, *Erwinia*, *Gluconacetobacter*, *Fibrobacter*, *Methanobrevibacter*, *Acetivibrio*, *Bacteroides, Thermomonospora* (Mohammadipour *et al*., 2021). Microbes producing hydrolytic enzymes also help to catalyse the hydrolysis of polysaccharides, lipids and proteins into monomeric units, which can be metabolized into organic acid via acidogenesis (Thapa *et al.,* 2020).

1.3.4.3. Soil microbiota

Soil is a source of cellulolytic microorganisms and the diversity and functions of such microbes are governed by the type of soil, its structure and composition. For example, forest soil has comparatively higher amount of cellulolytic microbes than arid, agricultural, or garden soil (Yang *et al.,* 2014). Bacteria belonging to genera *Bacillus* (*B. cereus, B. subtilis, B. licheniformis*), *Pseudomonas* and *Serratia* have also widely been reported to show cellulase activity. Other important group of cellulosic soil microbiota are *Actinobacteria*. *Cellulomonas, such as C. fimi, C. iranensis, C. terrae, C. biazotea, C. uda, C. pachnodae, C. persica, C. aurantiaca* and various species of *Steptomyces* such as *S. lividans, S. flavogriseus, S. olivochromogenes* (Spertino *et al.,* 2018; Ventorino *et al.,* 2016).

1.3.4.4. Gastropod molluscs

Slugs and snails e.g., *Achatina fulica*, *Achatina achatina* and *Archachatina marginnata,* are the most dominant species belonging to the class gastropoda. Over time, these species have evolved successfully to adapt to their environment and feed on variety of organic matter including lignocellulosic biomass with the help of the microbiota present in their gut as symbiont (Lima *et al*., 2020). Their gut microbiota comprises of various bacteria such as *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Fibrobacter succinnogenes* and some fungi that are capable of degrading cellulose and hemicellulose (Cann *et al.,* 2016). The metagenomic analysis of the gut microbiota present in *Achatina fulica* revealed the presence of coding regions for enzymes like cellulase, hemicellulase and others that are capable of degrading oligosaccharides (Pawar *et al*., 2015).

1.3.4.5. Marine microbiome

Marine environment consists of a very diverse population of microorganisms that grow under extreme conditions of pressure, temperature, and geochemical conditions and these organisms have gained enormous importance because of their role in biogeochemical cycling and also for their potential to generate various bio-chemical catalysts (Thapa *et al.,* 2020). A wide range of microorganisms including bacteria, fungi, archaea and protists having cellulolytic and xylanolytic activity have been isolated and characterized such as *Bacillus* sp. SR22 isolated from Cabo Branco coral reefs (Don Santos *et al.,* 2018), *Pseudoaltermonas haloplanktis* isolated from Antarctic Sea water (Jadhav *et al.,* 2022) and *Pseudoaltermonas* sp. DY3 isolated from Deep Sea sediment (Zhang *et al.,* 2016). *Pseudoaltermonas haloplanktis* and *Cladosporium* sp. isolated from Antarctic Sea water and Antarctic marine sponges, respectively, were reported to exhibit xylanase activity (Bruno *et al.,* 2019). The research work of Chen *et al.* (2017) showed successful implication of D-xylose isomerase isolated from sea mud at Ise Bay, Japan, in the saccharification of β-1, 3-xylan into D-xylose.

1.3.4.6. Decaying wood

Wood is mainly composed of three main components lignin, cellulose and hemicelluloses (Uraki *et al.,* 2015). Although wood has a very strong structure, they are degraded by diverse microorganisms such as *Basidiomycetes* which are fungi along with some lignocellulolytic bacteria (Singh *et al.,* 2016). Lignocellulosic degradation in the ecosystem depends on the synergistic action of oxidative (laccase) and hydrolytic enzymes (cellulase and xylanase) that break the complex molecules into simpler sugar molecules (Kumar *et al.,* 2020). Wood degrading fungi are widely studied as diverse groups of fungi that tend to show variations in wood degradation, for example the white rot fungi tend to degrade lignin more quickly than other micro-organisms (Poszytek *et al.,* 2016). Brown rot fungi degrade cellulose and hemicelluloses in wood very capably however; soft rot fungi only incompletely remove lignin by demethylation (Guo *et al.,* 2020).

1.3.4.7. Microbial consortia-based approach for production of glycoside hydrolases

The degradation of cellullosic biomass by complex microbial communities can be an effective approach, since feedback regulation and metabolite repression can be avoided in such communities (Srivastava *et al.,* 2019).Various microbial communities in natural ecosystem, such as compost, soil environment, animal rumen etc. produce cellulolytic enzymes that function synergistically to decompose plant biomass (Thapa *et al.,* 2020). However, natural ecosystems are often very diverse for direct identification of microorganisms and enzymes involved in lignocellulose deconstruction. Enrichment culture with lignocellulosic residues as sole carbon to reduce the complexity of microbial communities will be a better approach for such studies (Gladden *et al.,* 2011).

In a recent research by Lekakarn *et al.* (2021) a lignocellulose degrading thermophilic microbial consortium was developed from sugarcane bagasse compost by successive sub-cultivation. The consortium was structurally stable with the co-existence of eight major microbes (as tracked by Denaturing Gradient Gel Electrophoresis-DGGE), comprising anaerobic bacterial genera *Clostridium* and *Thermoanaerobacterium* along with an aerobic/facultative anaerobic *Rhodocyclaceae* bacterium, *Bacilli*, and uncultured bacteria and majority of the lignocellullolytic activities, including endo-glucanase, xylanase and β-glucanase were detected in the crude culture supernatant. In another study by Mhuanthong *et al.* (2015), comparative analysis of sugarcane bagasse metagenome revealed unique and conserved biomass-degrading enzymes among lignocellulolytic microbial communities. Similar studies on the community structure and enzyme systems of a lignocellulolytic microbial consortium bred on corn stover by Zhu *et al.* (2016), revealed the consortium to be primarily composed of members from the phyla *Proteobacteria, Firmicutes* and *Bacteroidetes.*

1.3.4.8. Thermophiles and thermoactive GHs as potent tools in valorization of agroresidues

Thermophiles are microorganisms that grow at high temperature of 55°C or more (minimum 45°C, optimum between 55-65°C, maximum 80°C) whereas some micro-organisms grow even at higher temperature, the optimum between 80 and 113°C, and are called hyper-thermophiles (Koul *et al.,* 2021). *Thermus aquaticus, Thermoplasma acidophilum, Bacillus stearothermophilus,* etc. are examples of thermophiles and *Pyrococcus abyssi* and *Pyrodictium occultum* exemplify hyper-thermophiles (Kanekar *et al.,* 2022).

Thermophiles typically possess lipids rich in saturated fatty acids in their cytoplasmic membranes that form a stronger hydrophobic environment thus allowing the membranes to remain stable and functional at high temperatures (Canganella, 2012). Enzymes and other proteins of thermophiles often differ in amino acid sequence from their counterparts in mesophiles. Critical amino acid substitution in only a few locations in the enzyme of thermophiles allows it to fold in such a way that makes them much more heat stable and hence they function optimally at high temperatures (Mangrola *et al.,* 2022). Additional salt bridges, hydrophobic interactions, H-bonds, increased proline content, reduced asparagine content are some of the factors responsible for the protein stability at higher temperature (Ferenczy *et al.,* 2022). Enzymes from thermophiles often show similar structural features such as amino acid sequence in and around the active sites, as their mesophilic counterpart, however, thermostable proteins have highly hydrophobic cores, which probably increase internal 'sticking' (Singh *et al*., 2021). Therefore, minor changes in amino acid sequence are apparently sufficient to render heat stability to an otherwise heat-labile protein. Hyperthermophiles also produce protein-folding proteins, called chaperonins that act on partially denatured proteins to restore the conformity of the active sites of the enzymes. One of such chaperonins, thermosomes, was found in very high concentration (80%) in the cells of the thermophiles, *Pyrodictium* when grown at 108^{o}C (Mohammad *et al.,* 2017).

With better knowledge and purification of enzymes the applications of enzymes in industries has increased many folds, and with the availability of thermostable enzymes a number of new possibilities for industrial processes have emerged (Haki *et al*., 2003). Carrying biotechnological processes at elevated temperature is advantageous as there is reduced risk of contamination by common mesophiles. Reactions at higher temperatures has added benefits of increased bioavailability and solubility of organic compounds and decreased viscosity leading to the efficient bioconversion (Sharma *et. al.,* 2019).

Thermophilic xylanases, has application in the pulp and paper industry where the wood used for the production of the pulp is treated at high temperature and basic pH. Most commercially available xylanases have optimum temperature for the activity around 50–60° C and a half-life of about 60 min. at 55°C (Fernandez-Sanroman *et al.,* 2022). Members of *Bacillus*, *Streptomyces*, *Thermoascus aurantiacus* and *Fusarium proliferatum* produce thermoactive xylanases active between 50 and 80°C (Haki *et al*., 2003). Alkalphilic and cellulase-free xylanases with an optimum temperature of 65°C from *Thermoactinomyces thalophilus* (Li *et al.,* 2020) and cellulase-free xylanases from *Clostridium abusonum* (Thomas *et al.,* 2014) are also reported .

Cellulolytic enzymes play significant role in the colour extractions of juices, in detergents, in the bio-stoning of jeans and in the pre-treatment of industrial wastes (Haki *et al.,* 2003). In order to attack the native crystalline cellulose that is water insoluble and occurs as fibers of densely packed structures, thermostable cellulases active at high temperature and high pH are required. Thermostable cellulases of archaeal origin include those isolated from *Pyrococcus furiousus* (Patel *et al.,* 2019) and *Pyrococcus horikoshi* (Lewin *et al.,* 2017). From *Thermotoga maritema* optimally active cellulase acting at 95°C and between pH 6.0 and 7.0 was reported (Satyanarayana *et al.,* 2013). Other endocellulases (CelA and CelB) from *Thermotoga neapolitana,* were purified and characterized (Basit *et al*., 2019). Cellulase with the potential to hydrolyze microcrystalline cellulose has been isolated from the extremely thermophilic bacterium *Anaerocellum thermophilum* (Zhang *et al.,* 2022) and maximal activity of this enzyme was observed at pH 5.0–6.0 and 85–95°C. A highly thermostable cellobiose (115°C at pH 6.8–7.8) was also produced from *Thermotoga* sp. (Bala *et al.,* 2018).

1.3.5. Pretreatment and saccharification of agroresidues

The intrinsic properties of lignocellulose make it difficult to degrade without any pretreatment techniques which disrupt the lignin shield of biomass and open up the carbohydrate components so that they are accessible to microbial and/or enzymatic attacks (Zheng *et al*., 2014). While the two main components of lignocellulosic biomass (cellulose and hemicellulose) contain polysaccharide thereby making it a source for the production of bioenergy, lignin has the most complex structure and has the most recalcitrant component thereby is the major barrier preventing ligocellulosic biomass to be an economically viable energy feedstock (Den *et al.,* 2018). There are various possible reasons for which pretreatment of lignocellulosic residues are essential prior to saccharification (Zhang *et al*., 2020): -

- Cellulose has different levels of crystallinity with some regions having low crystallinity (amorphous) and some regions having high crystallinity. Pretreatment helps to reduce crystallinity that can help in increasing rate of hydrolysis.
- Lignin acts as physical barrier in hydrolysis, and therefore lignin structure gets restructured or disrupted using pretreatment.

- Pretreatment is able to reduce the size of biomass and make it more porous so that the microbes and their enzymes can be made more accessible to the substrates by various pretreatment methods.

In addition, there are various factors that affect the saccharification and fermentation process, such as porosity of biomass (limited accessible surface area), crystallinity of cellulose and the amount of lignin and hemicellulose. The pore size of the substrate to the size of the enzyme also limits the enzymatic hydrolysis of lignocellulosic biomass (Park *et al.,* 2015). The presence of hemicellulose and lignin makes the cellulase enzymes less accessible to cellulose thereby reducing the productivity (Zhang *et al.,* 2015). The degree of hemicellulose acetylation and hemicellulose sheathing also affects lignocellulosic deconstruction by microorganisms or enzymes (Martins *et al.,* 2022).

The idea behind pretreatment is the removal of hemicelluloses and lignin, increasing the surface area and decreasing the crystallinity of cellulose and thus disrupting the recalcitrant structure of these rigid biopolymers (Sindhu *et al.,* 2016).The various methods used for pretreatment can be grouped as physical, physicochemical, chemical and biological, or sometimes a combination of these.

Table 1.3: Different types of pre-treatment techniques used on lignocellulose

PRETREATMENT TECHNIQUES			
Physical	**Chemical**	**Physicochemical**	**Biological**
1.Comminution	1. Dilute acid	1. Steam explosion	1. Fungi
2.Microwave treatment	2.Mild alkali	2.Liquid hot water	2.Bacterial
3.Extrusion	3.Organosolvents	3. Ammonia Fibre Expansion	3. Archeal
4.Ultrasound	4.Ionic liquids		
5. Pyrolysis	5. Deep eutectic solvents		

1.3.5.1. Physical pretreatment

Physical pretreatment involves application of high temperature or pressure to bring structural changes in the lignocellulosic biomass thereby making it less recalcitrant. It generally involves the breakdown or reduction in size and crystallinity hence making it prone to enzymatic degradation or hydrolysis, by applying methods like grinding or milling (Solomon*,* 2010). It does not involve chemicals so there is less production of inhibitory substances or waste products. Milling, crushing, microwave treatment, ultrasonic treatment, and high-energy electron radiation method are some examples of physical pretreatment method (Chen *et al.,* 2017).

Comminution

Comminution is the decrease in the size of biomass and is done by using various mechanical pretreatment methods such as grinding, milling, shredding, chipping etc. Grinding or milling are used when very small biomass particle of about 0.2-2 mm is required while for slightly bigger particles of about 10-30 mm chipping is best suitable option (Mankar *et al.,* 2021). It is an essential step for the successful conversion of biomass to biofuel. It alters the structure of lignocellulose residues by improving the availability of surface area, reducing cellulose polymerization and crystallinity (Kamarludin *et al.,* 2014). The limitation of this techniques is that it is not cost effective since it requires very high energy and this method not help in removing lignin which thereby prevents full accessibility of the enzymes to cellulose and hemicelluloses (Mankar *et al.,* 2021).

Microwave treatment

In microwave treatment, the electric field transfers its energy to molecules leading to the generation of thermal energy (Jedrzejczyk *et al.,* 2019). Microwave irradiation is a very extensively used technique for pretreatment of lignocellulosic feedstock because of less energy requirement, easy operation, high heating capacity and negligible generation of inhibitors. It also degrades cellulose fragment (Kumar *et al.,* 2017). Hoang *et al.* (2021) reported advantages of microwave application in biomethanation of organic matter that increased biogas yield. Moreover, lignin solubility, release of soluble compounds and hydrolysis rates are increased by the heat generated during microwave treatment.

Ultrasound pretreatment

Ultrasound wave produces effects which leads to the formation of small cavitation bubbles that ruptures cellulose thereby increasing `cellulose accessibility' to GHs for its efficient conversion into reducing sugars (Kumar *et al.,* 2017). Ultrasonic pretreatment method involves shorter reaction time, lower operation temperature and lower amount of chemicals used during further valorization. The effect of ultrasound pretreatment is determined by the type of lignocellulosic biomass being treated and also on the duration of sonification, however, extending sonification beyond a limit has no beneficial effect (Subhedar *et al.,* 2018). Asghar et al. (2015) reported that sonification of alkaline pretreated wheat straw for 15-35 minutes which resulted in increased delignification of 7.6-8.4%.

Extrusion

In extrusion the biomass is broken by passing it into an extruder kept under temperature control. There are rotating screws in the extruder that spin into a tight barrel, and when the contents pass through the barrel under high temperature the biomass contents are depolymerized. This process reduces the particle size, increase surface area, promotes the hydrolysis rate, and improves the anaerobic digestion process (Banu *et al.,* 2021).

1.3.5.2. Chemical pretreatment

Chemical methods of pretreatment involve chemical reactions that lead to the breakdown of lignocelluloses into simpler compounds (Kucharska *et al*., 2018). It successfully removes considerable amount of lignin and hemicelluloses and decreases cellulose crystallinity and degree of polymerization thereby increasing the biodegradation of cellulose and hence, is commonly used in paper and pulp industry for delignification of various lignocellulosic biomass for the enhancement of their enzymatic digestion (Behera *et al.,* 2014).

Alkali pretreatment

The use of alkali solutions lead to alteration in the structure and composition of lignocellulosic biomass. Alkali such as sodium hydroxide, calcium hydroxide (lime), ammonium hydroxide, potassium hydroxide, aqueous ammonia, hydrogen peroxide or a combination of these reagents are used for pretreatment (Behera *et al.,* 2014).

Alkali pretreatment can degrade or modify the structure of lignin and hemicellulose and also make the lignocellulose biomass more porous. It involves a series of different reactions in which dissolution, hydrolysis and decomposition of polysaccharide occurs (Kim *et al.,* 2016). In alkali pretreatment the chemicals help in degradation or removal of lignin which thereby increases the swelling capacity of lignocellulosic biomass. The pretreatment also helps in increasing the size of the channel and intra particle porosity (Chakraborty *et al*., 2019). It also causes reduction in the cellulose degree of polymerisation (Funahashi *et al.,* 2018) and causes saponification and salvation reactions occurs that leads to cellulose swelling and decrystallization (Behera *et al.,* 2014; Cheah *et al*., 2020; Mankar *et al.,* 2021).

This treatment degrades lignin by breaking ester and glycosidic bonds and acetyl groups and other uronic acid substitutions are also removed, thereby increasing the digestibility by hydrolytic enzymes (Awoyale *et al*., 2021). Hydrolysis of polysaccharide leads to the production of easily degradable reducing end groups and formation of alkali stable end groups (Sakamoto *et al.,* 2020).

In alkali pretreatment using NaOH the polyionic nature of biomass is enhanced thereby causing the diffusion of sodium ions into the lignocellulose. These sodium ions then remain within lignocellulose and serve as a counter charge to carboxylate ions. This polyioic nature of pretreated biomass results in swelling (Zheng *et al.,* 2018). Alkali treatment results in various changes in the structure of cellulose such as swelling, decrystallisation and depolymerisation (Hu *et al*., 2021). The extent till which the swelling of cellulose takes place depends on various parameters, such as temperature, concentration of alkali and retention time of pretreatment (Olejnik *et al*., 2017). LiOH and KOH caused swelling upto about 250% where as 300% cellulose swelling was observed in NaOH pretreatment in the form of $C_6H_{10}O_5$ (Karimi *et al*., 2013).Alkaline pretreatment methods are widely practiced mainly because it does not involve the use of any expensive materials and complicated methods. Kim *et al*., (2016) reported that alkali pretreatment is done by simply soaking the biomass in sodium hydroxide or ammonium hydroxide solutions at ambient temperature.

Acid pretreatment

This method mainly aims to solubilize and remove hemicellulose, but some portion of lignin is also solubilised resulting in improved porosity and hence increasing accesibility to microbial and enzyme degradation (Woiciechowski *et al.,* 2020). Some common acids used for pretreatment of biomass include sulphuric acid, phosphoric acid and acetic acid (Rezania *et al.,* 2020). High acidity may lead to the formation of products, such as furfural and 5-hydroxymethyfurfural which inhibits the fermentation process. These inhibitors adversely affect the microorganisms used for the fermentation by reducing the synthesis of RNA or degrading DNA, thereby decreasing the enzymatic activity. However, higher concentration of acids may also involve the risk of corrosion (Mankar *et al.,* 2021).

Organosolvents

Aqueous organic solvents such as acetone, glycol, ethanol, ethylene, methanol, etc. may be used for the degradation under specified conditions of pressure and temperature (Xiao *et al.,* 2023). This process is usually requires a catalyst (acid, base or salt) (Nauman *et al.,* 2019). Depending on the type of biomass and catalyst involved during organosolv pretreatment, the temperature may vary and may even reach up to 200^{o}C. Organosolv pretreatment method is useful in lignin extraction and cellulose fractionation leading to production of hemicellulose syrup of C5 and C6 sugars. The removal of lignin from the biomass leads to the exposure of cellulose fibres thereby increasing its susceptibility to enzymatic and microbial digestion (Zhan *et al.,* 2023). However, solvents should be removed as it may have a negative effect on the growth of microorganisms, hydrolysis by enzymes and fermentation process (Dyrda *et al.,* 2019). Another limitation of this method is the high cost of solvents, but recovering solvents through condensation and evaporation can minimise this limitation.

1.3.5.3. Physio-chemical methods

Steam-explosion

Steam explosion involves both physical and chemical techniques for the structural breakdown of lignocellulose (Duque *et al.,* 2016). This method involves exposure to very high pressure (0.7-4.8) and temperature (160-260^{o}C) for a short period of time (few seconds to minutes), that leads to the hydrolysis and release of hemicelluloses (Niju *et al.,* 2019). The steam expands the walls of the fibers, causing partial hydrolysis and increases accessibility to enzymes.

Ammonia Fibre Expansion (AFEX)

AFEX treatment involves treatment with liquid ammonia (1:1 ratio) at a moderately high temperature (90-100°C) and high pressure for 5-10 mins. The pressure is released suddenly which helps to disrupt biomass structure and reduce crystallinity of cellulose. The ammonia used can be recovered again and the major advantage of AFEX with repect to other treatments, is that few or no inhibitors are released during AFEX pretreatment (Zhang *et al.,* 2021)

Liquid Hot Water (LHW)

This hydrothermal process uses water at high temperature and pressure to disrupt the layer of the lignin, and to degrade the hemicellulose. The pH range is kept at 4-7 to avoid the release of inhibitors .Unlike steam explosion, rapid release of pressure is not required in LHW; pressure is applied only for preventing evaporation of water (Banu *et al.,* 2021).

1.3.5.4. Biological pretreatment

Biological pretreatments are cost effective and environment friendly processes that incorporate live microorganisms to treat the lignocellulosic biomass to make the biomass easily accessible for enzymatic saccharification. It does not involve formation of inhibitor compounds and is carried out by microbial enzymes for lignocellulose deconstruction (Baruah *et al.,* 2018; Den *et al.,* 2018).

The extracellular enzymatic system comprises of the hydrolytic system and the ligninolytic system (Wagner *et al.,* 2018). Hydrolytic enzymes act on cellulosic components and break down the cellulose and hemicellulose while ligninolytic enzymes include lignin degrading enzymes used for the pretreatment of the biomass (Zhang *et al*., 2020). Among the microorganisms, fungi are mostly employed for biological pretreatment because they can secrete more than one extracellular enzyme that degrade lignin and allow efficient enzymatic hydrolysis (Millati *et al.,* 2011). The main fungal enzymes are the peroxidases: Lignin peroxidases (LiPs; EC 1.11.1.14), manganese peroxidases (MnPs; EC 1.11.1.13), versatile peroxidases (VPs; EC 1.11.1.16) that catalyzes variety of oxidative reactions dependent on H_2O_2, and lacasses (EC 1.10.3.2) that oxidize phenolic compounds of lignin reducing molecular O_2 to H_2O (Huang *et al.,* 2022). Some or all of these enzymes are produced by several white-rot, brown-rot, and soft-rot fungi. Brown rot fungi primarily attack the cellulose component whereas soft rot and white rot fungi attack both cellulose and lignin structures (Qi *et al.,* 2022). The most promising of these are the soft rot fungi belonging to class *Basidiomycetes*. The commonly used white rots for biological treatment are *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora, Ceriporia lacerate, Cyathus stercolerus, Pycnoporus cinnarbarinus, Pleurotus ostreaus,* and *Cyathus cinnabarinus* (Kumar *et al.,* 2017). Their mode of action is through peroxidases and lacasses. The white rot fungus *Phenarocheate chrysosporium* is one of the model organisms used for fungal pretreatment (Nayan *et al.,* 2018) which is capable of producing a combination of enzymes lignin peroxidases (LiPs) and Manganese peroxidases (MnPs) during its secondary metabolism when the carbon and nitrogen sources are limited (Odwa *et al.,* 2020).

Fungal pretreatment is a promising avenue, however, fungal growth is slow, and rate of delignification is low (Liu *et al.,* 2013). Moreover, fungi lack the ability to selectively degrade lignin and holocellulose also breaking down carbohydrates, resulting in the loss of cellulose and hence decreased sugar yield (Wan *et al.,* 2012).

1.3.6. Microbial enzymes based saccharification of lignocellulose

Despite the fact that different biomass pre-treatment strategies and the optimization of enzyme production processes have resulted in a several-fold reduction in enzyme loading for lignocellulose hydrolysis, the efficient enzymatic conversion of cellulose and hemicellulose polymers remains the major bottleneck in the utilisation of lignocellulosic residues for bioethanol production (Ostby *et al*., 2020).

When compared to acid hydrolysis, the cost of enzymatic hydrolysis has decreased due to the use of alternative and affordable substrates such as lignocellulosic residues or the use of current technologies for production optimization such as cloning and heterologous super expression (Rodrigues *et al*., 2012).

However, the disadvantage of enzymatic hydrolysis of lignocellulosic material is feed-back inhibition by the products of carbohydrate hydrolysis, especially enzymes of the cellulolytic complex, where cellobiose can actively inhibit the function of exo- and endo-glucanases. The addition of a surplus of β-glucosidase activity is an alternative to overcome the competitive product inhibition by cellobiose (Gutierrez-Gutierrez *et al.,* 2022). The removal of sugars during hydrolysis by ultra-filtration or by employing the simultaneous saccharification and fermentation process, where the sugars produced during enzymatic hydrolysis are simultaneously fermented to ethanol, have also been reported as alternatives to overcome the problem of enzyme inhibition by the final products of carbohydrate degradation (Santos *et al*., 2022).

The addition of fresh substrate could recover free cellulases in bulk solution by adsorption, due to the high affinity of these enzymes for cellulose (Tramontina *et al*., 2020). Since β- glucosidase does not typically bind to the cellulosic substrate it cannot be reused and supplementation with this enzyme is required at the beginning of each round of hydrolysis in order to avoid the build-up of cellobiose and the subsequent end-product inhibition of cellulase (Kao *et al*., 2021). Because of these short comings, there are increased demands of efficient and robust enzymes for application in industries which have led to research in the field of designing of new enzymatic cocktails.

Use of lignocellulosic biomass to grow micro-organisms for production of such cocktails is cost effective and leads to the reuse and addition of value to waste, and also reduces environmental pollution (Dahmen *et al.,* 2019). The optimized enzyme cocktail comprising 50% of the crude extract from *P. funiculosum*, and 35 and 15% of the crude extracts from *A. niger* and *T. harzianum*, respectively, showed a hydrolysis yield of sugarcane bagasse of 91% (Frassatto *et al*., 2021). Another study demonstrated that an optimized enzymatic cocktail composed of both fungal cellulases and bacterial hemicellulases was able to hydrolyse ammonium fibre expansion (AFEX) corn stover with yields >80 and 70% of glucose and xylose, respectively, at a protein load of ~20 mg g^{-1} glucan (Juturu *et al.,* 2014).

Another strategy consists of the supplementation of cellulosic cocktails with hemicellulases, which provides improved hydrolysis. The addition of a GH11 xylanase in Celluclast - a commercial cellulase, allowed reduction of the cellulase loading by a factor of 7, because xylan hydrolysis increased cellulose accessibility by enhancing fibre porosity and swelling (Hu *et al*., 2018).

1.3.7. Application of lignocellulolytic microbes in valorisation of agro residues.

Biofuel production

Biofuels continue to be important since they are utilised as an alternative for fossil fuels. Research shows that biofuels can be produced from favourable agro-industrial leftovers such as, sugarcane bagasse, rice straw, sweet potato waste, potato waste, sawdust, sugar beet waste, and maize stalks (Duhan *et al.,* 2013; Kumar *et al.,* 2018). Saini *et al.* (2015) examined the use of diverse agricultural wastes in the production of bioethanol for second-generation applications and demonstrated that the lignocellulosic-derived biofuels have potential to be used as alternative source of energy in future. Since banana pseudo stems are an abundant waste in India, using it for bioethanol production is a viable alternative. Maiti *et al.* (2016) produced butanol from agro-industrial waste using *Clostridium beijerinckii.* As a result, using low-cost and environmentally friendly agricultural waste to produce valuable biofuels is a superior solution to meet energy demands with limited resources.

Antibiotic production

Antibiotics are secondary metabolites that selectively inhibit the growth or kill other microorganisms at low concentrations (Kumar *et al.,* 2018). Various research involving agro-industrial wastes for antibiotics production have been conducted so far. Khan *et al.* (2021) produced oxytetracycline with SSF using groundnut shell as a raw material with *Streptomyces rimosus*. Kashif *et al.* (2022) showed that by using these low-cost carbon sources, the production cost of antibiotics was drastically reduced. Khan *et al.* (2021) used oil pressed cake as a raw material for the production of extra cellular rifamycin B by using solid state using *Amycolatopsis mediterranean*.

Enzyme production

Agro-industrial wastes have a diverse composition that encourages the growth of different types of microorganisms (Sharma *et al*., 2022). Fermentation produces a variety of useful enzymes. The usage of these substrates increases the growth rate of fungi, resulting in the conversion of lignocellulosic substrate into less difficult substrates through the degrading activity of many enzymes. Guillaume *et al.* (2019) investigated the use of solid-state culture for production of enzymes such as endoglucanase and β-glucosidase from diverse agricultural wastes. Bucic-Kojic *et al.* (2017) employed corn cobs for phenolic synthesis, combining solid state fermentation with enzymatic treatment. They also studied enzymatic production of cinnamoyl esterase and xylanase. Fruit peels, seed, oil cakes, rice bran and wheat bran have also been reported to be employed in the synthesis of amylase and glucoamylase by *A. awamori* in solid state fermentation (Tiwari *et al.,* 2021). Likewise, production of amylase by *Aspergillus niger* MTCC 104 employing solid state fermentation has also been reported (Muralikandhan *et al.,* 2020).

Mushroom production

The production of mushrooms which is a good source of human nutrition from agrowastes is a noticeable method of biotechnology for valorisation. Mushroom production has ecological as well as economical benefits as it transforms agro-based residues into human food (Khan *et al*., 2021). Mushroom production is a good example of recovery of food proteins that can be carries out in small or large scale (Thakur *et al*., 2020). The commonly used agroresidues are rice straw, wheat straw etc. but cultivation of oyster mushroom with the use of coffee husks as a substrate has also been reported (Dissasa, 2022). Afify *et al.,* (2022) demonstrated increased amount of protein, lipid, carbohydrate, etc. with the use of paddy straw as a substrate during cultivation of *Pleurotus* mushroom, i.e *Pleurotus ostreatus* and *Pleurotus platypus.* They found. So, the use of paddy straw as a substrate for the cultivation of edible oyster mushroom with high protein content is suggested (Porselvi *et al.,* 2020).