REVIEW ARTICLE

Current Scenario on Thermozymes for Plant Biomass Deconstruction and Derived Commodity Chemicals

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ABSTRACT

In the hunt for alternative energy sources, lignocellulosic biomass (LCB), such as forestry and agricultural residues, appears to be a potential raw material for transformation into useful bio-products in so-called biorefineries, as it is abundant at low/no cost. The electricity generation capacity is expected to expand from 183 GW to 800 GW by 2031-32. In contrast to demand, India's indigenous energy sources are insufficient, leaving it reliant on crude oil imports (>80%). Alternative 2G renewable energy solutions have become important due to oil geopolitics and environmental concerns. As an agrarian tropical nation, crops produce significant volumes of residues, resulting in both resource waste and a missed opportunity to increase farmer revenue. As a result, forestry and agriculture leftovers on and off the farm can be used to generate bio-energy and other platform chemicals. The recalcitrance and intricacy of cellulose fibrils intertwined with hemicellulose and lignin render lignocellulosic biomass (LCB) generally inaccessible to cellulolytic enzymes in the native state, despite being renewable and inexpensive. Bio delignification/ depolymerization with ligninases can break down such complicated materials. Further hydrolysis of LCB to convert cellulosic and hemicellulosic fractions into monomeric sugars is dependent on the costs and robust enzymes such as glycosyl hydrolases (GHs), which have multiple substrates, are more stable at high temperatures and a wide pH range, and have improved catalytic efficiency. Thermozymes, enzymes obtained from thermophilic microbes possess unique characteristics such as temperature, chemical, and pH stability. They can certainly be used in several industrial processes by replacing mesophilic enzymes. Because the process works at slightly elevated temperatures, thermostable ligninases and GHs are of special importance. The biocatalyst's stability and reusability have always been important obstacles in creating biocatalytic reactions. The challenges and potential of employing thermophiles and their derived enzymes (thermozymes) in various stages of biomass conversion into a variety of commercial chemicals are discussed in this review.

Keywords: *Themophiles; Enzymes; Glycosyl hydrolases; Laccase; Lignin; Aromatic chemicals*

Background

Thermozymes are effective and functional enzymes under high and extremely high temperatures, different pH levels, substrate concentrations, and pressure and are resilient to denaturants and organic solvents (Murthy *et al.*, 2022). These microbial-derived thermozymes have been effectively used in waste management, biofuel, food, paper, detergent, medicinal and pharmaceutical industries (Kumar *et al.*, 2019). The use of thermozymes for the biological valorization of lignocellulosic biomass has gained popularity in recent years due to its low cost, environmental friendliness, sustainability, and low carbon footprint.

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The most abundant and renewable resource obtained from plants and trees is lignocellulosic biomass (LCB). The global production of LCB is predicted to be 181.5 billion tonnes per year. Currently, 8.2 billion tonnes of materials are consumed, with 1.2 billion tonnes coming entirely from agricultural waste (Dahmen *et al*., 2019). LCB is a heterogeneous mixture of three principal constituents: cellulose (35-50%), hemicellulose (20-35%), and lignin (10-25%), all of which are rich sources of value-added commodity chemicals. It also contains tiny amounts of starch, proteins, and oils (Banu *et al*., 2021; Garlapati *et al*., 2020). Various processes, such as thermochemical, enzymatic hydrolysis, and microbial fermentation, will be used to extract more than 200 high-value commodity compounds from these biomasses (Karp *et al*., 2021; Chandel *et al*., 2020). Various enzymes are required for bioconversion of LCB fractions, promoting bioenergy conversion cost-effectively and efficiently (Sankaran *et al*., 2021).

The investigation and usage of thermozymes in biological valorization has recently increased due to their ability to endure high temperatures, as temperature rises during biomass depolymerization, and chemical inhibitors generated during valorization. Finore *et al.* (2016) found that the thermozymes, xylanases, and β-xylosidase from *Geobacillus thermantarcticus* produced xylose (62.6%) and xyloglucurono-oligosaccharides (32%), respectively, from the hemicellulose fraction of (*Cynara cardunculus)*, the artichoke thistle. The benefits of thermozymes in LCB valorization are growing; therefore, this review concentrates on the various thermozymes, such as laccases, glycosyl hydrolases, and lignin peroxidases. Also included is a fish tale about LCB-derived compounds and their possible use.

Biomass Feedstock for commodity chemicals production

The most sought-after renewable biomass for generating fuels and biorefinery chemicals is lignocellulosic biomass. Understanding their structural complexity and feedstock type is critical when deciding on a pretreatment method and manufacturing pathway for commodity chemicals. The USA uses corn stover extensively to produce ethanol and other chemicals; besides, they also explore *miscanthus*, switchgrass and poplar as their major biomass feedstock for commodity chemical production (Takkellapati and Li., 2018)

India and China use a variety of biomass feedstock, mainly from agricultural production and processing. In India, the crop residues generated during 2018 is 683 million tonnes. Much of the diverse biomass produced in the European Union is diverted towards the production of bio-based and commodity chemicals (Kohli *et al.,* 2019).

First-generation biomass feedstock (Sugar crops) was successfully employed for biofuel applications. Second-generation biomass feed stock's are obtained from agricultural residues, forestry biomass and fisheries wastes are termed as LCB that can be simultaneously used to produce biofuel production and the waste after it can be valorized to generate commodity chemicals as per the recently hyped circular bioeconomy concept. By following the circular economy, the overall economy of the process can be enhanced due to the plethora of products generation via biorefinery way. However, third-generation biomass feedstock, namely microalgae, is attractive due to their simpler growth requirements and higher value chemical accumulation. Although various countries use different biomass feedstock, an evaluation by the S2Biom project on biomass availability across the EU from 2013 to 2016 to produce bio-energy reported lignocelluloses biomass to be a sustainable source of feedstock to support bio-refineries in the years to come (Panoutsou *et al*., 2016).

Structural Complexity and Challenges in Biomass deconstruction

Success in biorefinery and commodity chemical generation, besides fulfilling the country's ambitious target of biofuel production, requires understanding the structural complexity of LCB. In addition, the selection of the suitable pretreatment strategy depends on how the macro and microstructure of lignocellulosic biomass reacts and various by-products (inhibitors) generated.

The plant cell wall is composed of 35 different parts, of which thick cell wall (up to 10 µm) gives rigidity to plants and preventing attack by pathogens. The primary cell wall has composition of cellulose (20-50%), hemicellulose (15-35%), and lignin (10-30%). The minor component of the cell wall includes proteins, lipids, minerals and soluble sugars (up to 10%) (ATW and Zeeman., 2009).

Cellulose structure, complexity and deconstruction

Cellulose is a homo polysaccharide made of β 1-4 glucan chains that are tightly arranged into microfibrils of 5 nm size bonded with hydrogen and further strengthened by Vander walls forces. These binding of glucans results as the stacking of pyranose, yielding a crystalline structure that has degree of polymerization between 100 to 10,000. Native cellulose can be depolymerized up to 150 nm without much pretreatment; eventually needs severe chemical, physical and enzymatic treatments due to its crystalline structure to yield glucose units to be fermented for various products. The most predominant one being the Iβ structure, can be converted into other polymorphs, namely cellulose II, III and IV, by various thermochemical treatments. Thermo-stable cellulases are now viable for better hydrolysis and cellulase with tolerance to solvents and ionic liquids manufactured for cellulose's hydrolysis (Portillo and Saadeddin,2015).

Hemicellulose structure complexity and deconstruction

Xylose, arabinose, uronic acid and acetyl chains make up the hetero polysaccharide hemicelluloses. Alkaline solutions generally hydrolyze the hemicelluloses. Pectin, another major component in middle lamella hinders the hydrolysis of hemicelluloses. However, a gentle hot water treatment can remove pectin. Unlike cellulose, hemicelluloses can vary with species and in dicot plants, galactoglucomannons are predominant. Xylanase, an enzyme, efficiently hydrolyses the hemicelluloses and yields more than 70% monomers. Besides chemical treatments, solid catalysts were explored to yield 80-90% of conversion efficiency (loelovich and Morag., 2012). Chandra *et al*. (2020) reported that the xylanase producing *Bacillus flexus* PSX1 from earthworm casts efficiently converted the hemicellulose portion of paddy straw.

Lignin structure complexity and deconstruction

Lignin is an aromatic heteropolymer synthesized from enzymatic dehydrogenation of three monolignols: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Dehydrogenation produces phenol radicals, which combine to form a three-dimensional lignin network. Molecular weight of lignin ranges from 6700 to 23500 Da according to different structures. Lignin deposition in plant cell walls begins in the middle lamella and progresses through the primary and secondary cell walls, eventually settling in the S2 and S3 layers, needing harsh treatments to remove. Milled wood lignin, cellulolytic enzyme lignin, kraft lignin and soda lignin, and organosolv lignin are the most common ways for isolating lignin in its purest form. Several techniques employed to identify the inter-unit bonding of lignin among them the Aryl ether (β-O-4), Resinol (β-β), Phenyl coumarin (β-5), and Bi phenyl (5-5') units are found to be the main structures. Apart from these major groups the functional groups namely phenolic and aliphatic hydroxyl, benzyl ether, carbonyl is attached to the main structure. The recent quest on thermostable cellulases from thermophiles and hot springs increases the possibility of improving the saccharification efficiencies of lignocellulosics (Thankappan *et al*., 2018, 2017; Ganesan *et al.* 2020; Joshi *et al*., 2020).

Although several pretreatment methods are available, Ammonia fiber expansion (AFEX), Organosolv, and laccase can obtain efficient lignin removal combined with different solvents. Our group has extensively worked on isolating lignin-degrading *Streptomyces* sp. (Sivakumar, 1991; Sivakumar *et al.*, 1995; 2001a; 2001b) and advanced protoplast fusion techniques to enhance enzyme degradation (Sivakumar *et al*., 2004). Besides, these laccase combined with lignin peroxidase with the inclusion of mediators can remove lignin up to 80%. The authors also identified hyper laccase-producing fungi *Hexagonia hirta* MSF2 and used it for delignification (Kandasamy *et al*., 2016). Laccase with various solid catalysis in combination proved to be efficient in lignin removal ability. Besides delignification, our lab also aimed to valorize lignin into commodity chemicals generation. In this context, a green process has been developed recently with simultaneous lignin removal ability and high-value product generation. Transition-metal catalysed valorization of lignin: the key to a sustainable, carbon-neutral future (Sekar *et al*., 2018; Thangavelu *et al.*, 2018).

Thermozymes

I. Laccase

Laccases (para-diphenol: dioxygen oxidoreductases, EC 1.10.3.2) are a diverse group of multicopper oxidases (MCOs) that couple the oxidation of phenolic polymers to the reduction of molecular oxygen. They catalyze the oxidation of a wide range of inorganic and aromatic compounds, particularly phenols such as lignin. Since laccases

are implicated in numerous biological activities and oxidize a broad spectrum of substrates, these enzymes are of interest for use in pulp delignification, textile dye bleaching, and bioremediation (Piscitelli *et al*., 2010; Lallawmsanga *et al*., 2019). The search for new, efficient, and environmentally safe processes for industries has increased interest in laccases, which work essentially as 'green' catalysts that use air and produce water as the only by-product, making them more generally available to the scientific community (Piscitelli *et al.*, 2010).

Laccases are produced by many filamentous fungi (e.g., *Coprinopsis cinerea* and *Neurospora crassa*), plants (e.g., *Rhus vernicifera*), a few insects (e.g., *Bombyx* species), some bacteria (e.g., *Azospirillum lipoferum*) (Madzak *et al*., 2005) and actinobacteria (eg., *Streptomyces bikinienisis* CSC12 (Devi *et al*., 2016). Distinctive functions of bacterial laccases were reported in morphogenesis and sporulation, pigment production, and resistance to copper and phenolic compounds (Claus, 2003). The bacterial CotA (a laccase from *Bacillus subtilis*) and McoA (a metallo-oxidase from *Aquifex aeolicus*) gained much importance owing to their potential for biotechnological application (Fernandes *et al.*, 2009). While laccases from eukaryotes and bacteria have been extensively studied, archaeal multicopper oxidase homologs are less understood. Only one archaeal laccase (*LccA* of the haloarchaeon *Haloferax volcanii*) that oxidizes phenolics has been characterized at the protein level so far (Uthandi *et al.*, 2010). Later, *LccA* was engineered to produce laccase in the soluble form at a higher level (Uthandi *et al*., 2012). A related multicopper oxidase (McoP) from the hyperthermophilic archaeon *Pyrobaculum aerophilum* has been shown to have nitrous oxide reductase activity (Fernandes *et al.*, 2010). Enzymes from extremophilic archaea, such as the *H. volcanii LccA*, are promising for industrial applications as they have high intrinsic thermal and chemical stability (Uthandi *et al*., 2010). The best-known application has been using laccase from the lacquer tree *Rhus vernicifera* in paint and adhesives for more than 6,000 years in East Asia (Huttermann *et al.*, 2001). Laccases have also been used in the delignification of pulp, bleaching of textiles and carcinogenic dyes, detoxification of water and soils, removal of phenolics from wines, improving adhesive properties of lignocellulosic products, determination of bilirubin levels in serum, and transformation of antibiotics and steroids (Dougherty *et al*., 2012; Sakurai and Kataoka, 2007). Furthermore, tyrosinase and laccase-producing *Bacillus aryabhattai* TFG5 known to enhance the synthesis of humic substances from coir pith waste (Muniraj *et al.*, 2021a; 2021b). In addition, laccases have demonstrated potential for use in biosensors, bioreactors, and biofuel cells (Shleev *et al*., 2005). Thermostable laccases, stable at 80 °C and pH 3 obtained from *Trichoderma asperellum* BPLMBT1 delignified sweet sorghum stover by 76.97%, and increased the production of biohydrogen by 3.26-fold (Shanmugam *et al.*, 2018). Kandasamy *et al.,* (2016) also reported that the laccase from *Hexagonia hirta* MSF2 has an optimal temperature of 40 °C delignified corncob to 16.5%.

II. Glycosyl hydrolases

Cellulose is known for its robust structural conformation with high degree of polymerization, which involves glucose monomers. The complex H-bonding within and between the glucan chains make limited access of the glycolytic linkages to hydrolytic cleavage (Jeoh *et al.* 2007). Cellulosic biomass is valorized into simple sugars and corresponding monomers by hydrolytic enzymes, which is highly crucial in LCB-based biorefineries. These hydrolytic enzymes are otherwise termed Carbohydrate active enzymes (CAZymes). CAZymes are grouped into different families such as glycoside hydrolases (GHs), glycosyl transferases (GT), carbohydrate-binding modules (CBM), carbohydrate esterases (CE), auxiliary activities (AA) and polysaccharide lyase (PL) (Cantarel *et al.* 2009; Dougherty, *et al.* 2012).

GHs are classified based on their amino acid sequence similarities, the structural and functional relationships, the mechanistic flow of information, and substrate specificities (Henrissat *et al.*, 1997; Lombard *et al*. 2014). Among the GHs, cellulases and hemicellulases are involved in biomass deconstruction. CBMs are associated with substrate binding of cellulases, whereas auxillary enzymes are required for lignin degradation. Carbohydrate esterases are typical hemicellulases (Tsegaye, *et al.* 2019). Cellulases are cellulose-degrading GHs that catalyze the hydrolysis of β-1-4 glycosidic bonds of cellulose to glucose. The major groups of cellulases are endoglucanases (EG), exoglucanases/ cellobiohydrolases (CBH) and β-glucosidases. The subfamilies of β-glucosidases include endo-β-(1-4) glucanases, exo- β-(1-4) glucanases and exo-β-(1-4) glucosidase.

Distinct mechanisms of GHs

Glycosyl hydrolase catalysis of two amino acid residues is classified into a) inverting mechanism and b) retaining mechanism (McCarter and Withers., 1994). In the first mechanism, an anomeric position is shifted from β to α position through a single-displacement whereas, in the retaining mechanism, anomeric carbon remains in the same position due to double-displacement (Thuan *et al.,* 2013). The proton-donor position of both mechanisms remains the same and forms hydrogen bonding. However, in inverting mechanism, the catalytic base is distantly placed from the anomeric position to accommodate the water molecule between the sugar molecule and the base (Henrissat and Davies., 1997). Further, epoxides and oxacarbenium ion-like intermediate states are observed in both mechanisms (Sobala *et al*., 2020). So far, seven GHs families are reported to exhibit an inverting mechanism of catalysis (6, 8, 9, 45, 48, 74, and 124), and eleven families act through retaining mechanisms (1–3, 5, 7, 12, 30, 39, 44, 51, and 116) (Pabbathi *et al.*, 2021).

Diversity of GHs

GHs are diversified across the prokaryotic, eukaryotic, and archaeal kingdoms. To date, a total of 173 GHs families have been documented in Cazypedia based on amino-acid sequence similarity and mode of action (http://www.cazy.org/Glycoside-Hydrolases.html). GHs are further classified based on the effect of protein folding into suitable families and sub-families. The structural analysis of protein represents the similarity between the members of different GHases. For example, endoglucanase of GH-9 from *Nasutitermes takasagoensis* (a termite) encompasses 3 conserved catalytic such as two aspartic acids and one glutamic acid. Both the aspartic acid residues de-protonate an H_2O molecule (base) and create a nucleophile, which attacks the carbon at the anomeric position, breaking up the glycosidic bonds and an inversion at its anomeric position. Likewise, the glutamate residue acts as a proton donor/acid and protonates the sessile O_2 in the glycosidic linkage (Linton *et al.,* 2020). Likewise, endoglucanases belonging to GH-5 possess a conserved dyad of glutamic acid residues. Cel-1, possessed a conserved dyad of glutamic acid residues (E314 and E179) in its active site (Dadheech *et al*., 2018). Uthandi and his coworkers identified multifunctional thermophilic endoglucanase belonging to GH 15 of fungal origin *Chaetomium thermophilum* EDWF5, possessing both cellulolytic and amylolytic activities (Saranya *et al*. 2019). It is interesting to note that several families of GHases lack a catalytic proton donor/acceptor and/or nucleophile (Dennis *et al*., 2006; Hidaka *et al.*, 2006). The proposed alternative catalytic mechanisms are proton transferring network, substrate-assisted catalysis, non-carboxylate residues, and exogenous base/nucleophile (Vuong and Wilson, 2010).

The synergistic action of multiple enzymes carries out effective depolymerization from taxonomically distinct microbes that prevent metabolic repression (Stern, *et al*. 2017) Multifunctional, thermotolerant robust cellulases are bioprospected from many habitats like hot springs (Thankappan *et al*., 2017,2018), compost and animal dung (Saranya *et al.* 2019), vermicompost (Gavande *et al.*, 2021) and perennial grass species (Vegnesh *et al.,* 2019). Different GH families, along with their functions, are given in Table.1

Table 1. Cellulase groups, functions and their corresponding GH famililies

Enzyme	EC number	Functions	GH family	Reference
Endo-β-(1,4)-glucanases (1,4)-β-D-glucan-4-glucanohydrolase)	EC 3.2.1.4	Randomly cleaves β-(1,4)-glycosidic bonds and exposes the reducing and non-reducing ends	GH5, GH9, GH12, GH44, GH45, GH48, GH51, GH74, GH124	Xiros *et al.,* 2019
Exo- β -(1,4)-glucosidase (1,4)- β -D glucan glucohydrolase)	EC 3.2.1.74	Cleaves glucose from the non-reducing ends of the glucan chain liberating glucose units (prefers substrates of longer chain length) and gives inverted products	GH3, GH5, GH6, GH7, GH9, GH48	Xiros *et al.,* 2019

Exo- β -(1,4)-glucanase (1,4)- β -D glucan cellobiohydrolase)	EC 3.2.1.91	Acts at the reducing ends of - β -(1,4)-glucans, produced by endoglucanases, and cleaves cellobiose as well as cellooligosaccharides	GH5, GH6, GH7, GH9, GH48	Xiros *et al.*, 2019
β -glucosidase (β -D-glucoside glucohydrolase)	EC 3.2.1 21	Hydrolysis of cellobiose and very short chain β -D-oligosaccharides to form glucose;unlike exoglucosidases, the rate of hydrolysis decreases markedly as the degree of polymerization of the substrate increases.	GH1, GH3, GH5, GH9, GH30, GH116	Xiros *et al.*, 2019

Major GH families in lignocellulose deconstruction

i) GH 3

GH3 is one of the most abundant groups in the CAZy database, originating from plants, fungi, and bacteria that use the retaining glycosidase mechanism. The enzymes are exo β-D-glucosidases, α-L-arabinofuranosidases, β-D-xyloparanosidases and N-acetyl-β-D-glucosaminidases (Strohmeier *et al.* 2004). Few GH3 enzymes catalyse glycosidic bond formation either through reverse hydrolysis or controlled transglycosylation (Lee *et al.,* 2007). The functions attributed by GH3 family enzymes are cellulosic biomass valorization, remodelling of plant and bacterial cell walls, decoys pathogen, synthesis of functional glycoside, and metabolism. The active site of GH3 has two glucosyl-binding subsites, and the acid/base residues are found in the junction of these two subsites (Betts *et al*.,2017). GH3 enzymes are more substrate-specific which is influenced by the linkage position and chain length of the substrate. The kinetic and mechanistic analyses of NA–glucosominadases, β-D glucanglucohydrolases, β-glucosidases from *Thermotoga, Paenibacillus, Alcaligenes, Bacillus subtilis, B. tequilensis, B.licheniformis*, and fungal species *Aspergillus wentii, Flavobacterium meningosepticum, Aspergillus niger* were comprehended (Thankappan *et al*., 2018).

ii) GH 5

GH5, previously known as cellulase family A, is also a huge GH family belonging to Clan GH-A specific to only prokaryotes, eukaryotes, and viruses. GH5 enzymes consist of an amino-acid chain that forms a (β/β)8 fold that creates an open groove surrounding a conserved active site. The catalytic nucleophile Glu and general acid/base Glu at the C-terminus were harbored in the β-strands 7 and 4, respectively. The carbohydrate binds to the substrate-binding site during catalysis from the non-reducing end (+subsites) to the reducing end (-subsites). Glutamic acid is the typical catalytic residue of GH5 enzymes (Brunecky *et al.,* 2017).

iii) GH 9

The family GH9 occupies the second largest group encompassing endoglucanases and a small proportion of processive endoglucanases that includes CBM of the 3C family attached to the C-terminus of the catalytic domain (Brunecky *et al.*, 2017). GH family 9 cellulases consist of two major sub-groups EI and EII. The EI comprises only bacterial cellulases (aerobes and anaerobes), while the latter contains bacterial and non-bacterial cellulases (Sakon *et al.*, 1997). GH9 endoglucanases act on soluble cellulose derivatives such as carboxy methyl cellulose, plant polysaccharides, and phosphoric acid swollen non-crystalline cellulose, but little or no activity on crystalline cellulose (Sathya *et al*.,2014).

Further, Uthandi and coworkers identified thermophilic *B. licheniformis* VCB4 harboring multi GH families such as GH10, GH5, and GH48,especially β-1,4-endoxylanase (Joshi *et al.*, 2016).The recent next-generation sequencing technologies allow massive parallel sequencing, which provides novel data on phylogeny, metabolism and genetic diversity of targeted GHases in the microbiome. After unleashing the potential of metagenomic 16S rDNA, the phylogenetic analysis, complete details of bacterial species surviving in the habitat, and their putative genes are being explored (Fig 1).

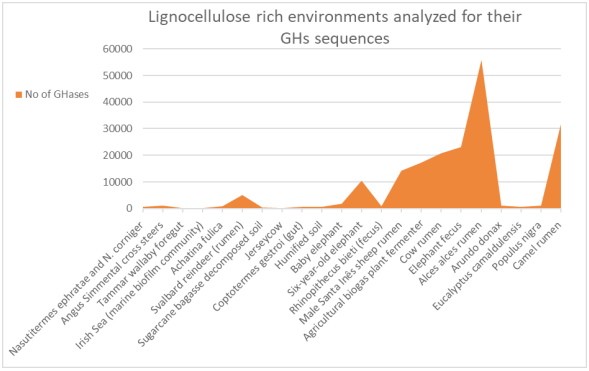

Fig 1. GHs explored from various lignocellulosic sources (Data source: Pabbathi *et al.*, 2020)

III. Lytic polysaccharide monooxygenase (LPMO)

Certain non-hydrolytic auxiliary enzymes (AA) like lytic polysaccharide monooxygenases (LPMOs) are involved in the solubilization of crystalline cellulose by oxidative cleavage of glycosidic bonds. In spite of endo–exo synergism, the LPMOs (CBM33 and GH61) improve the hydrolysis efficiency by adding new sites for the action of cellobiohydrolases and β-glucosidase. Reports on GH61 family auxiliary enzymes (AA1, AA3, and AA9) from sugarcane bagasse and Canadian soil metagenome provides more insight on auxillary enzymes (Mhuantong *et al*. 2015). LPMOs are copper-dependent enzymes that require molecular oxygen, and an extracellular electron source from cellobiose dehydrogenase and reductants in the LCB (Kont *et al.*, 2020). The proposed mechanism of action involves the insertion of oxygen at C1 and/or C4 position with subsequent formation of lactone, which is hydrolyzed to aldonic acid and ketoaldose, respectively (LPMO -26). Unlike the tunnel or cleft active sites of cellulases, LPMOs exhibits a highly conserved flat surface (Vaaje-Kolstad *et al*., 2005).

As commercial significance, LPMOs in synergy with GHs boost the saccharification of LCB, and they are incorporated in recent enzyme cocktails (Johansen *et al*., 2016; Valenzuela *et al*., 2019). Villares *et al.* (2017) demonstrated that LPMOs disrupt cellulose fibers by creating nicks that weaken the fiber cohesion. Based on sequence similarity, LPMOs are categorized in CAZy families AA9-11 and AA13-16, and are active on cellulose, various types of hemicelluloses, chitin, starch and/or oligosaccharides (Chylenski *et al.*, 2019).

Derived commodity chemicals

The LCB has been potentially used to produce value-added chemicals to satisfy the need for fine chemicals (Misra *et al*., 2013; Gromov *et al*., 2016; Sorokina *et al.*, 2017a; 2017b). Lignin gets breakdown to produce chemical compounds like methanol, dimethyl sulfoxide (DMSO), guaiacol, vanillic acid, acetic acid, benzene (Gandini, 2011). Phenolic compounds are produced from lignin during biomass decomposition and are commercially used in food, chemical, pharmaceutical, and perfumery industries (Agrawal *et al.*, 2014). The hemicellulose portion of biomass (corn stover, eucalyptus, rice straw, sugarcane bagasse, spent grain and corn cob) is used to produce a variety of industrial biochemicals like xylitol, phenols, eugenol, vanillin, vanillic acid, syringaldehyde, benzene, toluene, xylene, styrene, cyclohexane etc. (Ji *et al*., 2011; Varanasi *et al*., 2013; Ariyan and Uthandi 2019; Yamunasri and Uthandi, 2021).

I. Cellulose and hemicellulose derived compounds

The products like xylose, mannose, galactose and acetic acid from hemicellulose and glucose are derived from cellulose and hemicellulose. Hemicellulose gets hydrolyzed into xylose by the action of hemicellulolytic enzymes. At hydrolysis, hemicellulose and cellulose produce glucose (main product) and can be converted into fermentable products like ethanol, lactic acid, succinic acid, propanol, and acetone (De Bhowmick *et al.*, 2018). Hemicellulose sugars like xylose and arabinose produce ethanol and butanol through fermentation in the presence of thermophilic organisms. Xylose which looks like crystalline powder in nature, being used as natural sweetener in food production and as additives in detergents. Xylitol is derived from xylose chemically by hydrogenation or biologically (bacteria, yeast and fungi) through fermentation processes (Romaní *et al.*, 2020). Xylitol has wide applications in dental caries treatment, consumed by diabetic patients as an alternative for sugar, used in various products like nasal spray, mouth wash, cosmetics and pharmaceuticals (Baptista *et al.*, 2020).

The enzymatic hydrolysis of hemicellulose yields xylose, which is then converted into xylitol by several microorganisms *viz., Candida guilliermondii* recombinant *S. cerevisiae* (Carvalho *et al*., 2005 and Mussatto *et al.*, 2005), *E. coli* (Cirino *et al.*, 2006), recombinant *Corynebacterium glutamicum* (Sasaki *et al*., 2010) and *Candida tropicalis* (Ping *et al*., 2013). Manikandan and Sivakumar, 2019 reported that the construction of recombinant *E. coli* strain

M15 (pQE30XaCtXr) helps in the overexpression of XR protein. Harnessing the metabolic pathway and overexpression of XR protein using lignocellulose as a substrate increases the xylitol production. The recombinant Xr produced from the engineered *P. pastoris* (USYI02) efficiently converted xylose into xylitol (Yamunasri *et al*., 2021).

Sorbitol, a six-carbon sugar derived from glucose through biomass hydrolysis, issued as additives in many end products, food, pharmaceutical and cosmetic industries (Gandini, 2011). Ethanol, one of the derived commodities, is being used as an alternative for methanol and ethylene glycol poisoning, as fuels, antiseptics. At the same time, butanol has been widely used as a biofuel, base material for perfume, and paint thinner (Periyasamy *et al*., 2022). Packiam *et al*., (2018, 2017) reported that the alkali pretreatment of pearl millet yielded more fermentable sugars that can be used for bio-ethanol production. The hemicellulolytic sugars produce several organic acids like 3-hydroxy propionic acid, aspartic acid, glutamic acid, itaconic acid, fumaric acid, malic acid, 2,5 furan dicarboxylic acid, citric acid, succinic acid, lactic acid, levulinic acid through fermentation and this can be utilized in pharmaceuticals, food preservation techniques and animal feed (Mazzoli, 2021). (Hasunuma and Kondo, 2012) reported that the thermophilic yeast at a temperature above 40 - yields ethanol during fermentation of glucose. Enzymatic hydrolysis and syngas fermentation of *Clostridium sp*. can produce bioethanol (Limayem *et al.,* 2012).

In addition to biofuels and sweeteners mentioned above, biomass sugars can be converted to other industrially significant products such as furfural and organic acids. Furfural, a heterocyclic aldehyde formed through hydrolysis and dehydration of hemicellulose. The compounds derived from furfural like furans, furfural alcohol, 2-methylfuran, methyletrahydrofuran, furonic acid and furfurylamine have widespread applications in pharmaceuticals, agricultural and automotive industries. Hydroxymethylfurfural (HMF) is produced from cellulose and hemicellulose fractions and utilized as solvents, lubricants, and polymers (Yogalakshmi *et al*., 2022).

Zech *et al*., (2012) conducted an experiment on litter decomposition, identified the chemicals derived from hemicellulose degradation using GC-MS, and reported that hemicellulose-derived sugars include arabinose, fucose, xylose and rhamnose. At the same time, arabinose and xylose are by far more abundant than fucose and rhamnose in the litter. Gao *et al.* (2012) experimented on identifying the compounds derived from hydrothermal treatment of cellulose at a temperature ranging from (200 ◦C – 400 ◦C). Upon hydrothermal treatment, cellulose is degraded into two phases - gaseous phase and solid-liquid mixtures. This mixture by filtration provides solid product (Heavy oil) and an aqueous phase. The heavy oil products contained compounds such as furans (2-Acetyl-5-methyl furan, 2-Methyl-5-hydroxybenzofuran), phenols (phenol, 3-methyl, phenol, 2,4-dimethyl), carboxylic acids (acetic acid, levulinic acid, n-Hexadecanoic acid), aldehydes (2-Furancarboxaldehyde, benzaldehyde, benzodioxan-6-carboxaldehyde), esters (dioctyl ester, Di-n-octyl phthalate, mono 2-Ethylhexyl ester), ketones(Pentanone, Indenone, 2-Cyclopentanedione) and other compounds (pyrazole, naphthalenol, tetrahydroquinoxaline, 1-Acetyl-5-aminoindoline); the aqueous phase contained aldehydes (furancarboxaldehyde, benzodioxan-6-carboxaldehyde) phenols, ketones (hydroquinone, butanone, ethanone), acid (pentanoic acid) groups and sugars, which have wide applications in various industrial sectors.

II. Lignin derived compounds

Relative to hemicellulose and cellulose, lignin is the most complex, heterogeneous, energy-rich, and source of various value-added compounds, yet it is the most under-utilized biomass fraction. In recent years, lignin has been the most studied and up-and-coming raw material for high-value commodity aromatic chemicals because of its properties being the presence of diverse functional groups (primarily phenolic, aliphatic, hydroxyl, methoxyl, carbonyl, and benzene groups), biodegradability, and high antioxidant properties (Katahira *et al*., 2018; Zhou *et al.*, 2022). Some of the value-added chemicals derived from lignin include: (i) simple phenols such as vanillin, eugenol, catechol, and quinones; (ii) hydrocarbon chemicals like BTX (Benzene, toluene, and xylene), (iii) polymeric macromolecules like carbon fibers (Xu and Ferdosian, 2017), and lignin-based nanoparticles (Zhou *et al*., 2019). Some of the major value-added and low molecular weight chemicals commonly produced industrially include vanillin, syringaldehyde, and ferulic acid (Li *et al.*, 2018; Zhou *et al.*, 2022). The structure of commonly used low molecular weight lignin-derived aromatic compound structures is given in Figure 2.

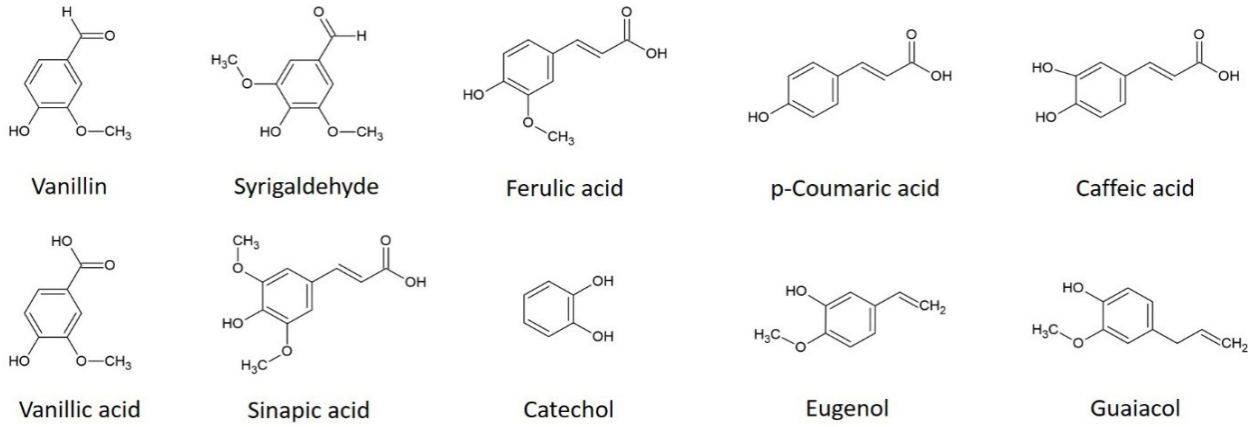

Fig 2. Structure of some commonly derived high commodity chemicals from lignin. (The structures are drawn using ACD ChemSketch software)

Vanillin is the first commercialized aromatic monomer derived from lignocellulosic biomass. Lignin contributes around 15% of synthetic or naturally identical vanillin production industrially (Fache *et al.*, 2016; Agrawal *et al.*, 2014). This industrially produced vanillin has several uses in various fields such as food, animal feed, pharmaceutical, and fragrance industry. Vanillin is used as a sweetener in ice cream, cookies, and confectioneries in the food industry. Vanillin is also used as a food preservative due to its anti-microbial and antioxidant properties (Banerjee and Chattopadhyay, 2019). Flavoring chemicals like zingiberene and jasmine oil are also being produced using lignin as a substrate in the fragrance industry (Banerjee and Chattopadhyay, 2019). In the pharmaceutical industry, vanillin is used as a masking agent. It acts as a precursor for the synthesis of certain pharmaceutical medicines such as dopamine (anti-hypotensive), papaverine (vasodilator), cyclovalone (digestant), methylodopa (anti-hypertensive), and ftivazide (anti-tuberculosis) (Bjørsvik and Liguori, 2002; Tarabanko and Tarabanko, 2017).

Syringaldehyde is less commercialized than vanillin. Like vanillin, syringaldehyde is used in the fragrance and pharmaceutical industries. In the pharmaceutical industry, syringaldehyde serves as a precursor for synthesizing pharma drugs, trimethoprim (anti-bacterial) replacing vanillin as syringaldehyde has two methoxyl groups (Mota *et al*., 2016; Tarabanko and Tarabanko, 2017; Fillat *et al*., 2012).

Ferulic acid is a scavenger of reactive oxygen species (ROS) because of functional groups such as hydroxyl, methoxyl, carboxylic acid groups (Mancuso and Santangelo, 2014). FA is also used in the food industry (food preservation and prevent food discoloration), cosmetic industry (UV protectant), and pharmaceutical industry (anti-cancer, anti-diabetic, neuro-protective, and treat cardiovascular ailments) (Kumar and Pruthi, 2014; Parmar *et al.*, 2015; Bumrungpert *et al*., 2018; Alam, 2019).

One of the properties that delimitate lignin scalability is its poor solubility. Recently scientists have explored a new way in lignin nanoparticles (LNP), reducing the raw lignin into uniform shape and sized nanoparticles (Chen *et al.*, 2018). These LNP has been synthesized by various approaches, including sonication, solvent exchange, and anti-solvent precipitation, but not biological/enzymatic methods (Figueiredo *et al.*, 2018). Synthesis of LNP using biological approaches will be the future research as it will be the most cost-effective of all the other approaches. The use of lignin is not limited to these chemicals described, but the commercialization of lignin-derived chemicals has to be emphasized.

Conclusion and future direction

The recent trends and patterns within the energy sector predict a remarkable increase in overall energy demand (over 60%) by 2030. There exists a daunting panorama of challenges with food availability and security as well as allocation of water and other natural resources. In this context, biomass has emerged as a highly attractive renewable source of chemicals, materials and fuels, addressing the fact 'Wealth from wastes'. Compared to chemical processes (acid/alkali), which liberate lots of inhibitors/reduces sugar recovery, enzymatic processes of delignification and

saccharification would be more specific, catalytic, use fewer chemicals, would be less likely to damage the fibre than chemical methods, and allow higher hydrolysis yields under less severe conditions. Enzymatic catalysis develops novel routes to biomass-derived commodity chemicals from sugars, including cellulose and hemicellulose and high-value aromatic chemicals (vanillin, phenol, styrene, etc.) from lignin would be of considerable interest to industries of plastic manufacture, food industry, personal care, etc. In this light, the newer oxidative and hydrolytic enzymes for biotransformation of both the sugar and lignin-rich biomass would be the future biobased economy for tropical countries where biomass generation is abundant. Regardless of the huge demand and scope for biomass-derived feedstock chemicals, there exist several challenges; technological validation of biomass conversion, up scaling of biomass conversion technology, biocatalysts production and formulation for the crude applications, feedstock pre-processing to prepare them for proper conversion, strategies of co-or mixed fermentation or one-pot hydrolysis cum fermentation.

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References

Agrawal, A., Kaushik, N. and S. Biswas. 2014. Derivatives and applications of lignin–an *insight. Sci.Tech. J.,*1(07): 30-36.

Alam, M. 2019. Anti-hypertensive effect of cereal antioxidant ferulic acid and its mechanism of action.*Front. nutr.,* 6121.

ATW, M. H. and G. Zeeman. 2009. Pretreatments to enhance the digestibility of lignocellulosie biomass. *Bioresour. Technol.,* 100: 10-18.

Banerjee, G. and P. Chattopadhyay. 2019. Vanillin biotechnology: the perspectives and future. *J. Sci. Food Agric.*,99(2): 499-506.

Banu, J. R., Kavitha, S., Tyagi, V. K., Gunasekaran, M., Karthikeyan, O. P., and G. Kumar. 2021. Lignocellulosic biomass based biorefinery: A successful platform towards circular bioeconomy. *Fuel*.,302: 121086.

Baptista, S.L., Carvalho, L.C., Romaní, A. and L.J. Domingues. 2020. Development of a sustainable bioprocess based on green technologies for xylitol production from corn cob. *Ind. Crops Prod.*, 156:112867.

Beslin Joshi.J and Sivakumar Uthandi. 2016. Xylanase producing thermophillic biocatalysts: growth, enzyme production and gene (*xlnB*) isolation. Extended abstract submitted on Sustainable Utilization of Tropical Plant Biomass: Bioproducts, Biocatalysts & Biorefinery (SutB).,pg no: 100-104.

Beslin Joshi, J., Priyadharshini R and Sivakumar Uthandi. 2020. Endo-glucanase producing thermophilic *Bacillus subtilis*: Gene isolation and structure-function prediction. Madras Agric. J., 107(Spl.):1-5. doi.10.29321/MAJ.S.000451

Betts, N. S., Wilkinson, L. G., Khor, S. F., Shirley, N. J., Lok, F., Skadhauge, B. and H.M. Collins. 2017. Morphology, carbohydrate distribution, gene expression, and enzymatic activities related to cell wall hydrolysis in four barley varieties during simulated malting. *Front Plant Sci*., 8: 1872.

Bjørsvik, H.R., and L. Liguori. 2002. Organic processes to pharmaceutical chemicals based on fine chemicals from lignosulfonates. O*rg. Process Res. Dev.*, 6(3): 279-290.

Brunecky, R., Alahuhta, M., Sammond, D. W., Xu, Q., Chen, M., Wilson, D. B. and V.V. Lunin. 2017. Natural diversity of glycoside hydrolase family 48 exoglucanases: insights from structure. *Biotechnol. Biofuels.*, 10(1):1-9.

Bumrungpert, A., Lilitchan, S., Tuntipopipat, S., Tirawanchai, N. and S. Komindr. 2018. Ferulic acid supplementation improves lipid profiles, oxidative stress, and inflammatory status in hyperlipidemic subjects: A randomized, double-blind, placebo-controlled clinical trial. *Nutrients*. 10(6):713.

Cantarel, L, B., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V. and B. Henrissat. 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. N*ucleic acids research*.,37(1), D233-D238.

Carvalho, W., Santos, J., Canilha, L., Silva, S., Perego, L. and E.J. Converti. 2005. Xylitol production from sugarcane bagasse hydrolysate: Metabolic behaviour of *Candida guilliermondii* cells entrapped in Ca-alginate. *Biochem*. Eng. J.,25 (1):25-31.

Chandel, A. K., Garlapati, V. K., Jeevan Kumar, S. P., Hans, M., Singh, A. K. and S. Kumar. 2020. The role of renewable chemicals and biofuels in building a bioeconomy. *Biofuels Bioprod. Biorefining*.,14(4): 830-844.

Chen, L., Zhou, X., Shi, Y., Gao, B., Wu, J., Kirk, T. B. W. Xue. 2018. Green synthesis of lignin nanoparticle in aqueous hydrotropic solution toward broadening the window for its processing and application. *Chem. Eng. J.*,346: 217-225.

Chylenski, P., Bissaro, B., Sørlie, M., Røhr, Å. K., Varnai, A., Horn, S. J. and V.G. Eijsink. 2019. Lytic polysaccharide monooxygenases in enzymatic processing of lignocellulosic biomass. *ACS Catal*., 9(6): 4970-4991.

Cirino, P.C., Chin, J.W., L.O. Ingram. 2006. Engineering *Escherichia coli* for xylitol production from glucose-xylose mixtures. *Biotechnol.Bioeng*., 95(6):1167-1176.

Claus, H. 2003. Laccases and their occurrence in prokaryotes. *Arch. Microbiol.*, 179:145–150.

Dahmen, N., Lewandowski, I., Zibek, S. and Weidtmann, A. 2019. Integrated lignocellulosic value chains in a growing bioeconomy: Status quo and perspectives. *Gcb Bioenergy.,* 11(1): 107-117.

Daphy Meurial, C., and U. Sivakumar. 2020. Xylanolytic *Bacillus flexus* PSX1isolated from earthworm casts for xylanase production and biomass conversion. *Madras Agric. J*., 107(Spl.):1-4. doi: 10.29321/MAJ.S.000447

De Bhowmick, G., Sarmah, A.K. and R.J. Sen. 2018. Lignocellulosic biorefinery as a model for sustainable development of biofuels and value added products. *Bioresour. Technol.,* 247:1144-1154.

Dennis, R. J., Taylor, E. J., Macauley, M. S., Stubbs, K. A., Turkenburg, J. P., Hart, S. J. and G.J. Davies. 2006. Structure and mechanism of a bacterial β-glucosaminidase having O-GlcNAcase activity. *Nat. Struct. Mol. Biol.,* 13(4): 365-371.

Devi, P., Kandasamy, S., Chendrayan, K., and Uthandi, S. 2016. Laccase producing *Streptomyces bikiniensis* CSC12 isolated from composts. *J Microbiol Biotech Food Sci.,* 6(2): 794-798. DOI: 10.15414/jmbfs.2016.6.2.794-798

Dougherty, M. J., Dhaeseleer, P., Hazen, T. C., Simmons, B. A., Adams, P. D. and M.Z. Hadi. 2012. Glycoside hydrolases from a targeted compost metagenome, activity-screening and functional characterization. *BMC Biotechnol.,* 12(1): 1-9.

Fache, M., Boutevin, B. and S. Caillol. 2016. Vanillin production from lignin and its use as a renewable chemical. *ACS Sustain. Chem. Eng.*,4(1): 35-46.

Fernandes, A.T., Damas, J.M., Todorovic, S., Huber, R., Baratto, M.C., Pogni, R., Soares, C.M. and L.O. Martins. 2010. The multicopper oxidase from the archaeon *Pyrobaculum aerophilum* shows nitrous oxide reductase activity. FEBS J., 277:3176–3189.

Fernandes, A.T., L.O. Martins. and E.P. Melo. 2009. The hyperthermophilic nature of the metallo-oxidase from *Aquifex aeolicus. Biochim Biophys Acta.*,1794:75–83.

Figueiredo, P., Lintinen, K., Hirvonen, J. T., Kostiainen, M. A. and H.A. Santos. 2018. Properties and chemical modifications of lignin: Towards lignin-based nanomaterials for biomedical applications. *Prog. Mater. Sci.*,93: 233-269.

Fillat, A., Gallardo, O., Vidal, T., Pastor, F. I. J., Díaz, P. and M.B. Roncero. 2012. Enzymatic grafting of natural phenols to flax fibres: Development of antimicrobial properties. *Carbohydr.Polym.,* 87(1): 146-152.

Finore, I., Poli, A., Di Donato, P., Lama, L., Trincone, A., Fagnano, M. and A. Tramice. 2016. The hemicellulose extract from *Cynara cardunculus*: a source of value-added biomolecules produced by xylanolytic thermozymes. *Green Chem*.,18(8): 2460-2472.

Gandini, A.J.G.C. 2011. The irruption of polymers from renewable resources on the scene of macromolecular science and technology. *Green Chem*.,13(5):1061-1083.

Gao, Y., Wang, X.H., Yang, H.P. and J.E. Chen. 2012. Characterization of products from hydrothermal treatments of cellulose. *Energy*., 42(1):457-465.

Garlapati, V. K., Chandel, A. K., Kumar, S. J., Sharma, S., Sevda, S., Ingle, A. P. and D. Pant. 2020. Circular economy aspects of lignin: towards a lignocellulose biorefinery. *Renew. Sustain. Energy Rev.*, 130: 109977.

Gavande, P. V., Basak, A., Sen, S., Lepcha, K., Murmu, N., Rai, V. and S. Ghosh. 2021. Functional characterization of thermotolerant microbial consortium for lignocellulolytic enzymes with central role of Firmicutes in rice straw depolymerization. *Sci. Rep*., 11(1): 1-13.

Gromov, N.V., Taran., O.P., Sorokina., K.N.,Mishchenko, T.I.,Uthandi, S., V.N.Parmon. 2016. New methods for the one-pot processing of polysaccharide components (cellulose and hemicelluloses) of lignocellulose biomass into valuable products. Part 1: Methods for biomass activation. *Catal. Ind.*,8(2): 176-186.doi: 10.1134/S2070050416020057

Hasunuma, T., and A.J.P.B. Kondo. 2012. Consolidated bioprocessing and simultaneous saccharification and fermentation of lignocellulose to ethanol with thermotolerant yeast strains. *Process Biochem*., 47(9):1287-1294.

Henrissat, B. and G. Davies. 1997. Structural and sequence-based classification of glycoside hydrolases. *Curr. Opin. Struct. Biol*., 7(5): 637-644.

Hidaka, M., Kitaoka, M., Hayashi, K., Wakagi, T., Shoun, H. and S. Fushinobu.2006. Structural dissection of the reaction mechanism of cellobiose phosphorylase. *Biochem. J*., 398(1): 37-43.

Huttermann, A., Mai, C. and A. Kharazipour. 2001. Modification of lignin for the production of new compounded materials. *Appl. Microbiol. Biotechnol*.,55:387–394.

Ioelovich, M. and E. Morag. 2012. Study of enzymatic hydrolysis of mild pretreated lignocellulosic biomasses. *BioResources.,* 7(1): 1040-1052.

Jeoh, T., Ishizawa, C. I., Davis, M. F., Himmel, M. E., Adney, W. S. and D.K. Johnson. 2007. Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. *Biotechnol Bioeng.*, 98(1): 112-122.

Ji, X.J., Huang, H., Nie, Z.K., Qu, L., Xu, Q. and G. Tsao. 2011. Fuels and chemicals from hemicellulose sugars. *Adv. Biochem. Eng. Biotechnol*.,128: 199-224.

Johansen, K. S. 2016. Lytic polysaccharide monooxygenases: the microbial power tool for lignocellulose degradation. *Trends Plant Sci.,* 21(11): 926-936.

Kandhasamy, S., Muniraj, I., Puroshothaman, N., Sekar, A., Sharmila, J.S., Kumarasamy, R. and S. Uthandi. 2016. High level secretion of laccase (LccH) from a newly isolated white rot basidiomycete, *Hexagonia hirta* MSF2. *Frontiers in Microbiol*.,7:707. https://doi.org/ 10.3389/fmicb.2016.00707.

Karp, E. M., Saboe, P., Tomashek, E., Tan, E., Lin, Y. and N. Sun. 2021. Separations consortium: Lignin rich stream fractionation and purification (No. NREL/PR-2800-79471). National Renewable Energy Lab (NREL), Golden, CO (United States).

Katahira, R., Elder, T. J. and G.T. Beckham. 2018. Chapter 1. A brief introduction to lignin structure. In Lignin Valorization: Emerging Approaches, *Energy and Environment Series* No. 19; The Royal Society of Chemistry. P. 1–20. DOI: 10.1039/9781788010351-00001.

Sorokina, K.N.,Samoylova, Yu. V., Piligaev, A.V.,Uthandi, S.,V.N.Parman. 2017. New methods for the one-pot processing of polysaccharide components (cellulose and hemicelluloses) of lignocellulose biomass into valuable products. Part 2: Biotechnological approaches to the conversion of polysaccharides and monosaccharides into the valuable industrial chemicals. *Catal. Ind*., 9(3): 264-269. doi:10.1134/S2070050417030126.

Sorokina, K.N., Samoylova, Y.V., Piligaev, A.V., Uthandi, S., V.N.Parmon. 2017. New methods for the one-pot processing of polysaccharide components (cellulose and hemicelluloses) of lignocellulose biomass into valuable products. Part 3: Products synthesized via the biotechnological conversion of poly- and monosaccharides of biomass. *Catal. Ind.*,9(3), 270-276.doi:10.1134/S2070050417030138.

Kohli, K., Prajapati, R. and B.K. Sharma.2019. Bio-based chemicals from renewable biomass for integrated biorefineries. *Energies*.,12(2): 233.

Kont, R., Bissaro, B., Eijsink, V. G., P. Valjamae 2020. Kinetic insights into the peroxygenase activity of cellulose-active lytic polysaccharide monooxygenases (LPMOs). *Nat. commun*., 11(1): 1-10.

Kumar, N. and V. Pruthi. 2014. Potential applications of ferulic acid from natural sources. *Biotechnol. Rep*.,4: 86-93.

Kumar, S., Dangi, A.K., Shukla, P., Baishya, D. and S.K. Khare. 2019. Thermozymes: adaptive strategies and tools for their biotechnological applications. *Bioresour. Technol.,* 278: 372-382.

Lallawmsanga, Leo,V.V., Passari, A.K., Muniraj, I.K.,Uthandi,S., Hashem, A., Abd Allah, E.F., Alqarawi, A.A., B.P.Singh. 2019. Elevated levels of laccase synthesis by *Pleurotus pulmonarius* BPSM10 and its potentialas a dye decolorizing agent. *Saudi J. Biol. Sci.,* 26(3), 464-468. DOI:10.1016/j.sjbs.2018.10.006.

Lee, E.J., Matsumura, Y., Soga, K., Hoson, T. and N. Koizumi. 2007. Glycosyl hydrolases of cell wall are induced by sugar starvation in Arabidopsis. *Plant Cell Physiol.*, 48(3): 405-413.

Li, T. and S. Takkellapati. 2018. The current and emerging sources of technical lignins and their applications. *Biofuels, Bioprod. Bioref*.,12(5): 756–787. doi:10.1002/bbb.1913.

Limayem, A. and S.C. Ricke. 2012. Lignocellulosic biomass for bioethanol production: current

perspectives, potential issues and future prospects. *Prog. Energy Combust. Sci*., 38(4):449-467.

Linton, S. M. 2020. The structure and function of cellulase (endo-β-1, 4-glucanase) and hemicellulase (β-1, 3-glucanase and endo-β-1, 4-mannase) enzymes in invertebrates that consume materials ranging from microbes, algae to leaf litter. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol*., 240: 110354.

Lombard, V., Golaconda R.H., Drula, E., Coutinho, P. M. and B. Henrissat. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. *Nuc. Acids Res*., 42(D1): D490-D495.

Madzak, C., Otterbein, L., Chamkha, M., Moukha, S., Asther, M., Gaillardin, C. and J.M. Beckerich. 2005. Heterologous production of a laccase from the *basidiomycete Pycnoporus cinnabarinus* in the dimorphic yeast *Yarrowia lipolytica.* FEMS Yeast Res., 5:635–646.

Packiam, M., Subburamu, K., Desikan,R.,Uthandi, S.,Marimuthu S., K.Soundarapandian. 2018. Suitability of pearl millet as an alternate lignocellulosic feedstock for biofuel production in India.*Appl. Environ. Microbiol*.,6(2):51-58.doi: 10.12691/jaem-6-2-4 (IF:)

Mancuso, C., and R. Santangelo. 2014. Ferulic acid: pharmacological and toxicological aspects. Food Chem. *Toxicol*., 65: 185-195.

Manikandan, A. and S. Uthandi. 2019. Xylitol production by xylose reductase over producing recombinant Escherichia coli M15. *Madras Agric.J*.,106 (Special):205-209.

Mazzoli, R.J.F. 2021. Current progress in production of building block organic acids by consolidated bioprocessing of lignocellulose. *Fermentation*., 7(4):248.

McCarter, J. D. and G.S. Withers. 1994. Mechanisms of enzymatic glycoside hydrolysis. *Curr. Opin. Struct. Biol*., 4(6): 885-892.

Ganesan,M., Remitha, M. V., Thankappan, S., Muniraj, I., and S. Uthandi. 2020. Thermotolerant glycosyl hydrolases-producing *Bacillus aerius* CMCPS1 and its saccharification efficiency on HCR-laccase (LccH)-pretreated corncob *biomass. Biotechnol Biofuels.,*13:124. https://doi.org/10.1186/s13068-020-01764-2 (IF:5.62).

Mhuantong, W., Charoensawan, V., Kanokratana, P., Tangphatsornruang, S. and V. Champreda. 2015. Comparative analysis of sugarcane bagasse metagenome reveals unique and conserved biomass-degrading enzymes among lignocellulolytic microbial communities. *Biotechnol. Biofuels.*, 8(1):1-17.

Swati, M.,Raghuwanshi, S. and R. K. Saxena. 2013. Evaluation of corncob hemicellulosic hydrolysate for xylitol production by adapted strain of *Candida tropicalis. Carbohydr. Polym.*, 92.2: 1596-1601.

Mota, M. I. F., Rodrigues Pinto, P. C., Loureiro, J. M., and A. E. Rodrigues. 2016. Recovery of vanillin and syringaldehyde from lignin oxidation: A review of separation and purification processes. *Sep. Purif. Rev.*,45(3): 227-259.

Muniraj, I., Shameer, S.,Ramachandran, P. and S.Uthandi. 2021a. Tyrosinase and laccase-producing Bacillus aryabhattai TFG5 and its role in the polymerization of phenols. *BMC Microbiol*.,21(187): 1-9. https://doi.org/10.1186/s12934-021-01538-x.

Muniraj, I., Shameer, S.,Ramachandran, P. and S.Uthandi. 2021b. Bacillus aryabhattai TFG5-mediated synthesis of humic substances from coir pith wastes. *Microb. Cell Factories*.,20(1):1-9. https://doi.org/10.1186/s12934-021-01538-x

Murthy, P. S., Vedashree, M., Sneha, H. P. and I. Prakash. 2022. Extremophiles as a source of biotechnological products. In *Physiology, Genomics, and Biotechnological Applications of Extremophiles,* (pp. 308-333). IGI Global.

Mussatto, S. I., Dragone, G. and I. C. Roberto. 2005. Influence of the toxic compounds present in brewer's spent grain hemicellulosic hydrolysate on xylose-to-xylitol bioconversion by *Candida guilliermondii. Process Biochem.*, 40(12):3801-3806.

Pabbathi, N. P. P., Velidandi, A., Tavarna, T., Gupta, S., Raj, R. S., Gandam, P. K., and R. R. Baadhe. 2021. Role of metagenomics in prospecting novel endoglucanases, accentuating functional metagenomics approach in second-generation biofuel production: a review. *Biomass Convers. Biorefin*., 7:1-28.

Packiam, M., Subburamu, K., Desikan, R., Uthandi, S., Subramanian, M., and S. Kamaraj. 2017. Comparison of chemical pretreatment for recovery of fermentable sugars and enzymatic saccharification. *Madras Agric. J.*, 104(7-9): 273

Panoutsou, C., Langeveld, H., Vis, M., Lammens, T. M., Askew, M., Carrez, D., and E. Alakangas. 2016. D8. 2 Vision for 1 billion dry tonnes lignocellulosic

biomass as a contribution to biobased economy by 2030 in Europe. S2Biom.

Parmar, I., Bhullar, K. S., and H. P. V. Rupasinghe. 2015. Anti-diabetic effect of ferulic acid and derivatives: An update, in Ferulic Acid: Antioxidant properties, uses and potential health benefits. Editor B. Warren (Hauppauge, NY, USA: Nova Science Publishers, Inc.), 93–116. 978-1-63463-299-7.

Periyasamy, S., Karthik, V., Senthil Kumar, P., Isabel, J. B., Temesgen, T., Hunegnaw, B. M. and D. V. N. Vo. 2022.Chemical, physical and biological methods to convert lignocellulosic waste into value-added products. A review. *Environ. Chem. Lett*., 20: 1129–1152.

Ping, Y., Ling, H. Z., Song, G., and J. P. Ge. 2013. Xylitol production from non-detoxified corncob hemicellulose acid hydrolysate by *Candida tropicalis. Biochem. Eng. J.*,75: 86-91.

Piscitelli, A., Pezzella, C., Giardina, P., Faraco, V., and G. Sannia. 2010. Heterologous laccase production and its role in industrial applications. *Bioeng. Bug*s.,1(4): 254-264.

Portillo, M. D. C.and A. Saadeddin. 2015. Recent trends in ionic liquid (IL) tolerant enzymes and microorganisms for biomass conversion. *Crit. Rev. Biotechnol.*, 35(3): 294-301.

Romaní, A., Morais, E. S., Soares, P. O., Freire, M. G., Freire, C. S., Silvestre, A. J., and L. Domingues. 2020. Aqueous solutions of deep eutectic systems as reaction media for the saccharification and fermentation of hardwood xylan into xylitol. *Bioresour. Technol*., 311: 123524.

Sakon, J., Irwin, D., Wilson, D. B., and P. A. Karplus. 1997. Structure and mechanism of endo/ exocellulase E4 from *Thermomonosporafusca. Nat. struct. Biol.,*4(10): 810-818.

Sakurai, T., and K. Kataoka. 2007. Basic and applied features of multicopper oxidases, CueO, bilirubin oxidase, and laccase. *Chem. Rec.,* 7:220–229.

Sankaran, R., Markandan, K., Khoo, K. S., Cheng, C., Leroy, E., and P. L. Show. 2021. The expansion of lignocellulose biomass conversion into bioenergy via nanobiotechnology. *Front. Nanosci*.,3:793528.

Saranya, S. and S.Uthandi. 2019. Thermophilic cellulolytic fungi: cellulose production, characterization and biomass conversion. Thesis submitted to Tamil Nadu Agricultural University.

Sasaki, M., Jojima, T., Inui, M., and H. Yukawa. 2010. Xylitol production by recombinant *Corynebacterium glutamicum* under oxygen deprivation. *Appl. Microbiol. Biotech.,* 86(4): 1057-1066.

Sathya, T., and M. Khan. 2014. Diversity of glycosyl hydrolase enzymes from metagenome and their application in food industry. *J. Food Sci.*,79(11): R2149-R2156.

Sekar, N., Andrey, C., and S. Uthandi, S. 2018. A Two-Step catalytic depolymerization of alkali treated *Pennisetum glaucum* L. and *Melia dubia* cav. into low molecular weight (LMW) aromatics. *Madras Agric. J*.,105(1-3): 120-126.

Shanmugam, S., Hari, A., Ulaganathan, P., Yang, F., Krishnaswamy, S., and Y. R. Wu. 2018. Potential of biohydrogen generation using the delignified lignocellulosic biomass by a newly identified thermostable laccase from *Trichoderma asperellum* strain BPLMBT1. *Int. J. Hydrog. Energy.,*43(7): 3618-3628.

Shleev, S., Tkac, J., Christenson, A.,Ruzgas, T., Yaropolov, A. I., Whittaker, J. W.,and L. Gorton. 2005. Direct electron transfer between copper-containing proteins and electrodes. *Biosens. Bioelectron*.,20:2517–2554.

Sivakumar, U. 1991.Lignin degradation; genetic manipulation of *Streptomyces* sp. for hyper ligninolysis. Thesis Submitted to Tamil Nadu Agricultural University, Coimbatore..

Sivakumar, U., Kalaichelvan G. and K.Ramasamy.2001a. Isolation and regeneration of protoplast of ligninolytic *Streptomyces. Madras Agric. J.*,88(4-6): 339-341.

Sivakumar, U., Kalaichelvan G. and K.Ramasamy. 2001b. Induced mutation in *Streptomyces* RK -1 for hyperligninolysis. *Madras Agric. J*., 88(4-6):342-344.

Sivakumar ,U., Karthikeyan, S., Kalaichelvan G., and K. Ramasamy 1995. Detection of plasmid in ligninolytic *Streptomyces. Madras Agric. J.*, 82 (6,7,8):413-502.

Sivakumar, U.,Kalaichelvan G., and K.Ramasamy. 2004. Protoplast fusion in Streptomyces for hyper production of laccase and associated ligninolytic enzymes. *World J. Microbiol. and Biotechnol.,* 20(6):563-568.

Sobala, L. F., Speciale, G., Zhu, S., Raich, L., Sannikova, N., Thompson, A. J. and S. J. Williams. 2020. An epoxide intermediate in glycosidase catalysis. *ACS Cent. Sci.,* 6(5): 760-770.

Stern, J., Artzi, L., Moraïs, S., Fontes, C. M., and E. A. Bayer. 2017. Carbohydrate depolymerization

by intricate cellulosomal systems. In *Protein-Carbohydrate Interactions* (pp. 93-116).Humana Press, New York, N.

Strohmeier, M., Hrmova, M., Fischer, M., Harvey, A. J., Fincher, G. B. and J. Pleiss. 2004. Molecular modeling of family GH16 glycoside hydrolases: potential roles for xyloglucan transglucosylases/ hydrolases in cell wall modification in the *poaceae*. Protein Sci., 13(12): 3200-3213.

Thankappan, S., Kandasamy, S., and S. Uthandi. 2017. Deciphering thermostable xylanases from hot springs: the heritage of Himachal Pradesh for efficient biomass deconstruction. *Madras Agric. J.*,104(7-9): 282-287

Thankappan, S.,Kandasamy, S., Joshi, B.,Sorokina, X.,Taran, O.P., and S. Uthandi.2018. Bioprospecting thermophilic glycosyl hydrolases, from hot springs of Himachal Pradesh, for biomass valorization. *AMB Express*.,8(1): 1-15.

Tarabanko, V. E., and N. Tarabanko 2017. Catalytic oxidation of lignins into the aromatic aldehydes: general process trends and development prospects.*Int. J. Mol. Sci.,* 18(11): 2421.

Thangavelu, K., Desigan, R., Taran, O.P. and S. Uthandi. 2018. Delignification of corncob via combined hydrodynamic cavitation and enzymatic (HCE) pretreatment: process optimization by response surface methodology. *Biotechnol.Biofuels*., 11:203 doi: 10.1186/s13068-018-1204.

Thuan, N. H., Park, J. W. and J. K. Sohng. 2013. Toward the production of flavone-7-O-β-D-glucopyranosides using Arabidopsis glycosyltransferase in *Escherichia coli. Process Biochem.*, 48(11):1744-1748.

Tsegaye, B., Balomajumder, C. and P. Roy. 2019. Microbial delignification and hydrolysis of lignocellulosic biomass to enhance biofuel production: an overview and future prospect. *Bull Natl Res Cent*.,43(1):1-16.

Uthandi, S., Prunetti, L., De Vera, I. M. S., Fanucci, G. E., Angerhofer, A., and J. A. Maupin-Furlow. 2012. Enhanced archaeal laccase production in recombinant *Escherichia coli* by modification of N-terminal propeptide and twin arginine translocation motifs. *J. Ind. Microbiol. Biotech*.,39(10): 1523-1532.

Uthandi, S., Saad, B., Humbard, M. A., and J. A. Maupin-Furlow. 2010. *LccA*, an archaeal laccase secreted as a highly stable glycoprotein into the extracellular medium by *Haloferax volcanii. Appl. Environ. Microbiol.,*76(3): 733-743.

Vaaje-Kolstad, G., Horn, S. J., van Aalten, D. M., Synstad, B. and V. G. Eijsink. 2005. The non-catalytic chitin-binding protein CBP21 from *Serratia marcescens* is essential for chitin degradation. *J. Biol. Chem*., 280(31):28492-28497.

Valenzuela, S. V., Valls, C., Schink, V., Sánchez, D., Roncero, M. B., Diaz, P., and F. J. 2019. Differential activity of lytic polysaccharide monooxygenases on celluloses of different crystallinity. Effectiveness in the sustainable production of cellulose nanofibrils. *Carbohydr. Polym.,*207: 59-67.

Varanasi, P., Singh, P., Auer, M., Adams, P. D., Simmons, B.A and S. Singh. 2013. Survey of renewable chemicals produced from lignocellulosic biomass during ionic liquid pretreatment. *Biotechnol. Biofuels*.,6(1): 1-9.

Vegnesh, R., Thankappan, S., Singh, B., Kennedy, Z., Saikia, R., and S. Uthandi. 2019. Glycosyl hydrolases producing bacterial endophytes from perennial grass species (*Neyraudiarey naudiana* L.) for biomass deconstruction. Madras Agric. J., 106(7-9): 444-450.

Villares, A., Moreau, C., Bennati-Granier, C., Garajova, S., Foucat, L., Falourd, X., and B. Cathala. 2017. Lytic polysaccharide monooxygenases disrupt the cellulose fibers structure. *Sci. Rep*., 7(1): 1-9.

Vuong, T. V. and D. B. Wilson. 2010. Glycoside hydrolases: catalytic base/nucleophile diversity. *Biotechnol. Bioeng*.,107(2): 195-205.

Xiros, C., Shahab, R. L. and M. H. P. Studer. 2019. A cellulolytic fungal biofilm enhances the consolidated bioconversion of cellulose to short chain fatty acids by the rumen microbiome. *Appl. Microbiol. Biotechnol.*,103(8): 3355-3365.

Xu, C. and F. Ferdosian. 2017. Conversion of lignin into bio-based chemicals and materials (pp. 133-156). New York, NY, USA: Springer.

Yamunasri, P., Priyadharshini, R. and S. Uthandi. 2021. Evaluation of efficient transformation method for xylose reductase gene integration in Pichiapastoris GS115. *Madras Agric. J.*, 107 (december (10-12):1.

Yogalakshmi, K., Sivashanmugam, P.,Kavitha, S.,Kannah, Y., Varjani, S., AdishKumar, S. and G.J.C. Kumar. 2022. Lignocellulosic biomass-based pyrolysis: A comprehensive review. *Chemosphere*.,286:131824.

Zech, M., Werner, R. A., Juchelka, D., Kalbitz, K., Buggle, B. and B. Glaser. 2012. Absence of oxygen isotope fractionation/exchange of (hemi-) cellulose derived sugars during litter decomposition. *Org. Geochem*., 42(12): 1470-1475.

Zhou, N., Thilakarathna, W.P.D.W., He, Q.S. and H.P.V Rupasinghe. 2022. A Review: Depolymerization of lignin to generate high-value bio-products: Opportunities, challenges, and prospects. F*ront. Energy Res*., 9:758744.

Zhou, Y., Han, Y., Li, G., Yang, S. and F. Chu. 2019. Lignin-based hollow nanoparticles for controlled drug delivery: grafting preparation using β-cyclodextrin/enzymatic-hydrolysis lignin. *J. Nanomater.*, 9(7): 997.