



Myceliophthora thermophila: A nature-borne biotechnologist converting agricultural biomass into renewable energy and bioproducts

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ABSTRACT

Agricultural residues, rich source of cellulose and hemicellulose, are generated in huge amount during harvesting of crops. Bioconversion of this solid lignocellulosic biomass to value-added products with the help of microbes may provide an alternate source of renewable energy and other products. Among microorganisms, thermophilic fungi are most promising because they have to adapt to their natural habitat by developing a complex system. *Myceliophthora thermophila* is a remarkably powerful lignocellulolytic fungus that generates an array of enzymes implicated in the break-down of this biomass. The biochemical, genomic, and secretome analyses of this fungus have revealed an elaborate enzymatic repertoire that includes hemicellulases, cellulases, laccases, and other enzymes having auxiliary activities, thereby including almost all the known CAZy families. These enzymes are useful in complete deconstruction of the lignocellulosic biomass. The mould is amenable to grow efficiently in submerged and solid-state fermentations using agro-residues and is efficient in biomass degradation at moderate as well as at high temperatures because of high thermal stability of these enzymes. Thermostable enzymes of *M. thermophila* require lesser time for saccharification of various plant-based polysaccharides in comparison to hydrolytic enzymes from mesophilic fungi. Also, the fungus secretes various important biomolecules of multifarious biotechnological applications using lignocellulose as substrate.

1. Introduction

Plant biomass feed-stocks are ample, renewable, and cost-effective waste materials that are utilized growingly by various bio-based manufacturing units to generate a huge array of value-added products. Example of products generated from the lignocellulosic (LC)-rich biomass feedstocks includes bioethanol, biodiesel, biohydrogen, biobutanol (second-generation biofuels), various organic acids, and other important biotechnological products. These LC feedstocks include various types of agricultural residues, like byproducts of crops and forestry waste, such as sawdust and wood chips [1,2]. Lignocellulose comprises of polysaccharides and lignin, and is a recalcitrant and heterogeneous substrate [3]. Its degradation is a costly process as first a

thermochemical pre-treatment is needed, which is accompanied by the enzyme mediated hydrolysis to break polysaccharides into readily fermentable simple sugars for the synthesis of bio-ethanol and other biochemicals [4] (Fig. 1). Currently, there exists a requirement to improve the available fungal and bacterial enzymes [5]. To make the process of hydrolysis economical, there exists a requirement of enzymes and enzymatic combinations, which solubilize LC material more effectively and completely, thereby leading to enhanced yields of fermentable simple sugars. Due to renewable nature, LC biomass is a possible source of various value-added products; thus, ethanol generation from LC biomass that majorly composed of hemicellulose and cellulose, is a favorable technology for sustainable development [6,7], which occurs by the fermentation of monosaccharides, such as glucose from cellulose

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and galactose, mannose, xylose, and arabinose from hemicellulose. Cellulose nanofibers assembly accounts for the density of the amorphous/non-crystalline cellulose chains as the initial points for enzymatic hydrolysis [8,9]. The cellulose microfibrils of fiber crops are hard to be digested, mainly due to relatively less amorphous cellulose chains accounting for low density of cellulose nanofiber assembly [8,9]. For degrading these fractions, the physical and chemical barriers need to be overcome that otherwise will hinder the hydrolysis of hemicellulose and cellulose into easily fermentable sugars. Thus, fungi are attractive organisms for biomass degradation, and enzymes secreted by them are of industrial importance [10].

The application of thermostable enzymes in saccharification of LC feedstocks is attaining extensive industrial interest [11] because they offer various benefits over mesophilic enzymes, like higher thermal stability, which reduces the enzyme dosage by enabling the longer hydrolysis time [12]. Also, better substrate solubility is achieved by using higher temperatures of 50–60 °C during saccharification. Further, elevated mass-transfer rates, lower viscosity, and a reduced contamination risks are additional benefits of thermostable enzymes [13], which reduces the overall cost of the production. Among various microorganisms, fungi synthesize an array of ligno-cellulolytic enzymes and adapt to a broad range of substrates [14]. Among fungi, thermophilic fungi have additionally active and stable enzymes towards LC biomasses compared to those acquired from mesophilic fungi, that's why thermophilic fungi are more promising for the industrial applications [15,16].

Myceliophthora thermophila (syn. *Sporotrichum thermophile*) is an

ascomycete fungus, which occurs in soil and self-heated composts. It is a thermophilic fungal species, which grows at 45–50 °C [16] and secretes a variety of enzymes, such as glycosyl hydrolases (GHs) and auxiliary oxidation and ligninolytic enzymes [17]. Further, the exo-proteome analysis of *M. thermophila* revealed that it consists of about 42 % CAZymes, out of which 80 % are GHs across 24 different families [18], which are implicated in LC biomass degradation. Its genome has been sequenced and annotated. Transcriptome analysis shows that the anticipated endoglucanases are a part of GH 45, 12, 7, and 5 [16,19,20]. A whole set of cellulolytic enzymes, like cellulases, hemicellulases, pectinases, phytases, lipases etc. are produced by *M. thermophila* that act synergistically when grown on lignocellulosic biomass [21–23]. All enzymes are thermostable having optimal activity between 40 and 85 °C and several enzymes display even higher thermostability [19,24–26]. In current scenario, thermostable enzymes have gained importance [15] because as discussed previously under extreme conditions, these enzymes from thermophilic fungi might be more stable and active. Further, enzymes with thermostability have advantages in production of biofuels and chemicals. Thus, *M. thermophila* is a promising source of thermostable enzymes, producing all major polysaccharide hydrolysing enzymes. Further, genetic modification of this mould for synthesis of bio-chemicals is possible because of the availability of its genome sequence [16] and well-developed genetic tools [27–29]. Currently, fumaric acid is produced from glucose by using engineered *M. thermophila* [30] and C4 dicarboxylic acids were produced from corn cob and cellulose [31], which indicates that it is an excellent system for

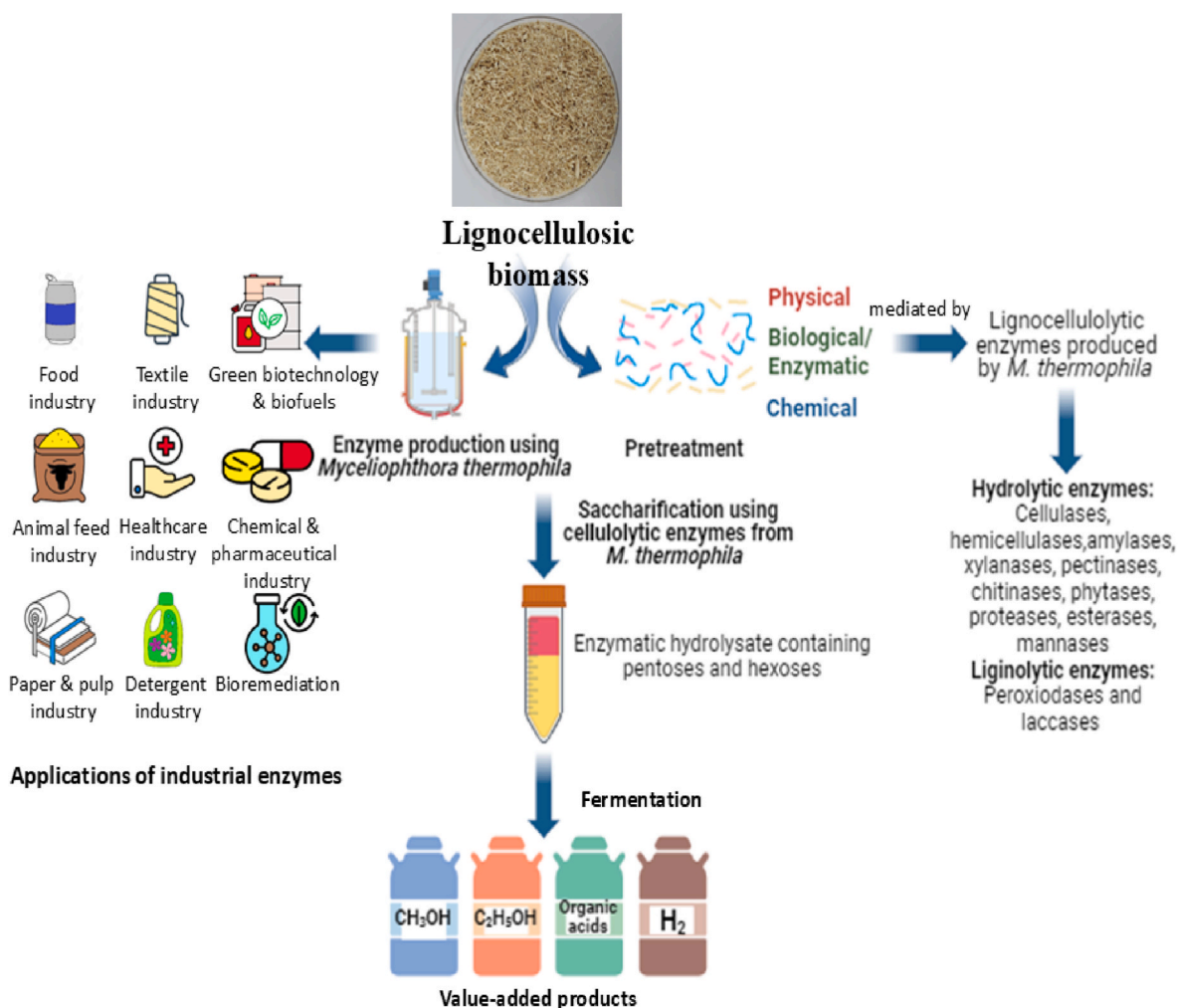


Fig. 1. Schematic representation of overall process of production of enzymes and other value-added products by *Myceliophthora thermophila* from lignocellulosic biomass along with the applications of industrial enzymes.

biofuel and chemical production.

2. Lignocellulosic biomass, fossil fuels and biofuels

There has been an increasing interest towards renewable and clean energy sources, like biofuels and in particular, the second generation ethanol (2 GE) because of increasing global warming caused by the continuous use of fossil fuels. Replacing petrochemical-based fuels i.e., chemicals and fossil fuels with truly renewable and sustainable alternatives needs bioconversion of agricultural residues into fermentable sugars [32]. The major component of the plant wall is cellulose that is a homo-polysaccharide formed of D-glucopyranose monomers linked by β -1, 4 linkages. Hemicellulose is the second largest found poly-saccharide present in the cell wall of plants. It is a branched hetero-polysaccharide that is made up of different monomers linked together, which include pentoses (arabinofuranose, arabinopyranose, xylose), hexoses (galactose, mannose, and glucose), uronic acids (galacturonic acid, D-4-O-methyl-glycuronic acid, and glucuronic acid), and deoxy-hexoses (L-fucose and L-rhamnose) [33–35].

Use of various enzymes which can degrade LC complex has been accepted to disintegrate the plant cell wall components by releasing carbohydrate monomers, which are used by yeasts and fermented into value-added products and chemicals, such as xylitol, succinate, butanol, isobutanol, ethylene glycol, glycolic acid, xylonic acid, cinnamic acid, ethanol, and acetone [36]. This has increased the interest for search of resistant and efficient enzymes generated by microbes that are capable of degrading LC biomass. Four stages i.e., pretreatment, hydrolysis, fermentation, and distillation are required during LC based bioethanol production. Major challenge for biofuel production is the efficient enzymatic hydrolysis of LC biomass. It requires a cocktail of hydrolytic enzymes for degrading the hemicellulose, cellulose, and other polysaccharides into readily fermentable sugars. Attempts have been done towards the minimization of enzyme cost, which will lower the production cost of fermentable sugars and; hence, the production of biofuels, thereby leading towards commercialization [37]. The usage of thermotolerant enzymes in hydrolysis allows high reaction temperature. As discussed previously, enzymes derived from thermophilic fungi offers advantages over those derived from mesophilic fungi. They can tolerate organic solvents, and do not lose their activity easily during processing at higher temperatures, which is required during the pretreatment of raw substrates [16].

3. *Myceliophthora thermophila*

Myceliophthora thermophila is a thermophilic filamentous fungus, which is profuse in variety of genes encoding hemicellulose and cellulose degrading enzymes [19]. This fungus is majorly observed in the self-heated soil masses of organic matter, where it decomposes plant cell wall polysaccharides ([38]; 2008 [23,39,40]). It grows between 25 and 55 °C but optimum growth occurs at 45 °C of temperature [41]. The exponential growth of *M. thermophila* on glucose (0.10 h⁻¹) and cellulose (0.09–0.16 h⁻¹) is almost similar, which reveals that it can use cellulose as effectively as glucose [15].

This is a very potent lignocellulolytic fungus that secretes an array of carbohydrate hydrolases to break cellulose and hemicellulose along with oxidoreductases that are involved in lignin degradation. It was revealed by genome analysis of *M. thermophila* that it has 30 cellulases-encoding genes, which are grouped into 10 GH families, 66 hemicellulases-encoding genes that are grouped into 10 GHs and 9 CEs, and 35 auxiliary enzymes-encoding genes. Latter includes CDHs of AA3/AA8 family, multi-copper oxidases (MCOs; laccases and laccase-like enzymes), and LPMOs of AA9 family. Out of whole set of *M. thermophila*, 80.2 % protein-encoding sequences with putative LC activity are expected to have a secretory signal peptide. 76 % hemicellulases and cellulases and 88 % accessory redox enzymes are secreted, while just a little number of enzymes remain intracellular or membrane-

bound [19].

This mould is a potential producer of many hydrolytic enzymes, which are involved in efficient biomass degradation. Enzymes generated by *M. thermophila* are more effective in biomass degradation than mesophilic fungi despite low enzyme titers [42]. Enzymes generated by mould are cellulases [22,23,43–45], xylanases [45–47, 347], feruloyl esterases [48], aldono-lactonases [49], pectinases [45], phytases [50–53], α -galactosidases [54], keratinases [55], malate dehydrogenases [56], mannanases [57,58], laccases [21,59], amylases [60], lipases [61], and glutathione-S-transferases [62] (Fig. 2). Further, genome analysis of *M. thermophila* revealed many genes putatively coding industrially relevant enzymes, like CAZy, oxidoreductases, proteases, and lipases, while for plant cell-wall degrading enzymes >200 sequences have been recognized exclusively. These sequences encode enzymes that covers most of the recognized families, like GHs and polysaccharide lyases (PLs) [19]. Specifically, secretomes of fungus cultivated on purified cellulose polymer or natural bio-mass comprised of aldose epimerase, aldolactonase, β -glucosidases, β -1, 3 glucanase, cellobiohydrolases (CBHs), cellobiose dehydrogenase, galactosidase, GH74 xyloglucanase, glyoxal oxidase, and polysaccharide mono-oxygenases while fungus secreted mixed β -1, 3 glucanase, xylosidase, β -glucosidases, β -1, 3 glucanases, β -mannosidase, polysaccharide lyase, galactosidase, endo- β -1,6-galactanase, chitinases, and aldose 1-epimerase when grown on a mixture of purified hemicellulose fractions (xylans, arabinans, and arabinoxylans). Thus, a whole group of enzymes participating in oxidation and hydrolysis of hemicellulose/pectin, cellulose, and lignin was displayed by the secretomes produced on natural biomass [63].

Specifically, *M. thermophila* is an extraordinarily good cellulolytic microorganism as it produces a whole set of cellulolytic enzymes for degrading plant cell-wall (Bhat and Maheswari, 1987). Its genome has been sequenced and annotated such that >200 sequences have been identified for cellulolytic enzymes [64]. In a similar way, fungal strains grouped in genera *Aspergillus* and *Trichoderma* are also common producers of the enzyme mixtures for degrading agricultural-based polysaccharides. But to produce biofuels, their enzyme mixtures are not adequate for economically feasible production due to lack of thermostability [32]. In an earlier work, both fungi *M. thermophila* and *Trichoderma reesei* broke down the cellulosic substrate entirely, but they differ in their cellulolytic behaviour in many ways. *M. thermophila* showed higher cell density and faster growth rate than *T. reesei*. Moreover, in *M. thermophila*, the time of complete cellulose conversion and maximal growth coincided but in case of *T. reesei* they did not [23]. Additionally, *M. thermophila* has distinguishing features from *T. reesei* and *Aspergillus niger*, like large number of enzymes capable of degrading arabinoxylan [65]. 11 xylanases were reported in *M. thermophila* belonging to GH 10 and GH 11 families compared to only 5 in both *T. reesei* and *A. niger*, while 14 arabinofuranosidase were found in *M. thermophila* from GH 62, 51, and 43 families in comparison to 3 in *T. reesei* and 13 in *A. niger* [16]. Further, Kadowaki et al. [17] revealed that a cellobiohydrolase (MtCel7A) secreted by *M. thermophila* M77 has higher thermostability and catalytic efficiency as well as lower processivity compared to a cellobiohydrolase (TrCel7A) from *T. reesei*. However, in terms of exo- and endo-glucanase activities and extracellular protein, *T. reesei* was found superior to *M. thermophila*. Further, *M. thermophila* attained a total transformation of the cellulose-based substrate at a much rapid rate in comparison to *T. reesei*, despite of its lower titre of cellulase in culture broth. Similar behaviour was also shown by an anaerobic bacteria *Clostridium thermocellum*. The substrate utilization is 2.5- fold higher in *M. thermophila* than *C. thermocellum* [23]. In addition, *T. reesei* has high endo-glucanase and exo-glucanase activities but low β -glucosidase activity; hence, it has little utility in cellulose hydrolysis [45]. In another previous work, 3 thermophilic cellulolytic fungal species (*Chaetomium thermophile*, *M. thermophila*, and *Thermoascus aurantiacus*) were compared based on growth rates, optimum temperature for growth, and conditions for high rate of cellulose

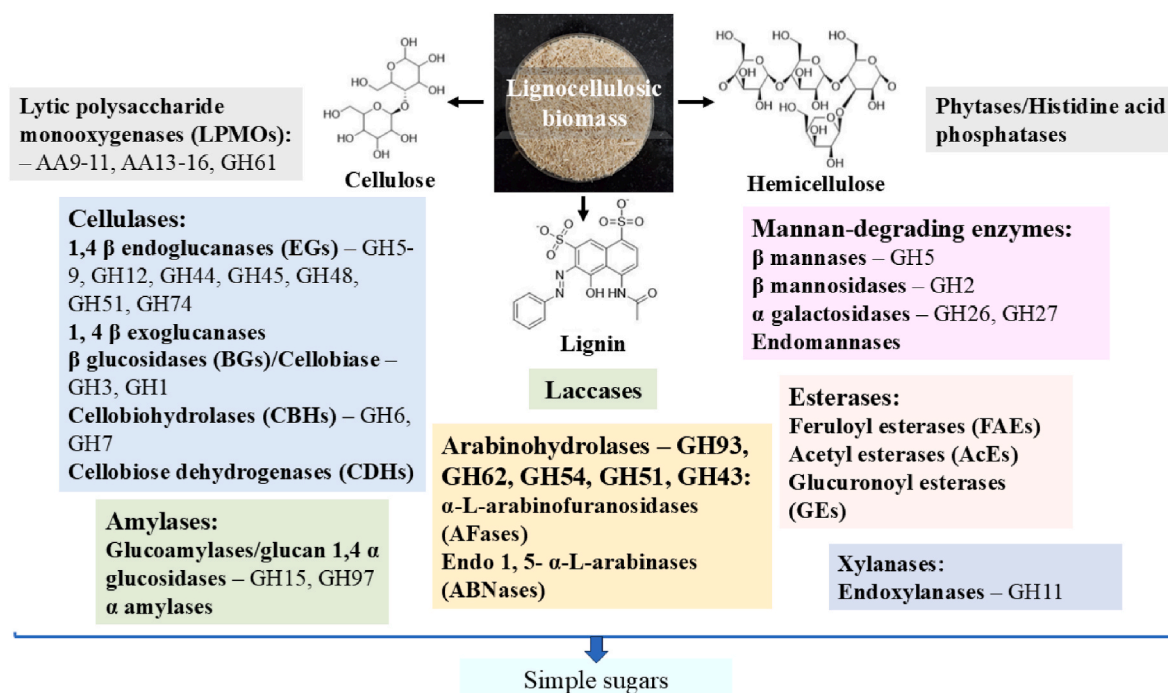


Fig. 2. Lignocellulolytic enzymes produced by *Myceliophthora thermophila* during the lignocellulosic (LC) biomass degradation.

degradation. Results obtained revealed that *T. aurantiacus* depicted the highest range of temperature for optimum growth i.e., 46–51 °C while *M. thermophila* flourished heavily over broader range i.e., 36–43 °C whereas, *C. thermophila* grew over limited range of 40–43 °C. Highest cellulose degradation was carried out by *M. thermophila* (22.4 %), followed by *C. thermophila* (13.2 %), and least by *T. aurantiacus* (12.3 %) [66].

Lastly, newly discovered *M. thermophila* C1 wild-type strain has been exploited as an ample source of industrial enzymes. However, it has also been developed as an expression platform for high-yield recombinant protein production. It has been successfully utilized in cellulase production due to low viscosity morphology, which makes it different from other filamentous fungal strains. Further, it also possesses a high capability for pharmaceutical applications by attaining g/L of antibody yields. However, same as most of filamentous fungi, it is not able to generate the human-like glycosylation. If advancements happen in this area, these could uplift C-1 to the fore-front of the bio-pharmaceutical protein production [67]. Specifically, C1 HCprt strain is an efficient host for heterologous protein expression. An IgG antibody was expressed against tumor necrosis factor- α (TNF- α) by expressing the light- and heavy-chain encoding genes by utilizing *cbh1* promoter such that the expressed gene products were released in growth medium. Further, the antibody was reported to be biologically-active despite being not hyperglycosylated [64]. In another recent study, C1 was effectively utilized as an efficient and cost-effective host for producing immunogens of vaccine quality at large scale. C1 system was utilized to express hemagglutinin (HA) and its fusion protein from various strains of influenza A/H1N1 virus, such that the mice vaccinated by C1-obtained HA products evoked similar or more vigorous immune responses than the mice vaccinated with HA proteins obtained from the prototype expression systems, thereby protecting the mice against the aggressive viral attack [68].

4. Lignocellulolytic enzymes produced by *Myceliophthora thermophila*

As discussed earlier, plant-based biomasses are efficient, cheap, and easily available feedstocks used by industries for producing an array of

value-added products [1,2]. However, lignocellulose is recalcitrant in nature, consisting majorly of polysaccharides and lignin [3], whose degradation into sugars requires costly process, such as thermochemical pretreatment and enzymatic hydrolysis. To make the process economical, there lies a need for enzymes that can efficiently and thoroughly solubilize the biomass resulting in higher release of sugars, over 60 % of which is often reached currently [11].

At industrial scale, the filamentous fungi are regarded as the highest generator of cellulolytic enzymes by utilizing LC biomass. In comparison to the bacterial cellulases, fungal cellulases are usually adopted commercially [69]. Specifically, *M. thermophila* degrades major polysaccharides of LC biomass by producing industrially significant enzymes, like xylanases and cellulases [70,71]. For instance, 38 % removal of lignin is seen after the biological pretreatment of rice straw using *M. thermophila*. The cellulose content was increased from 40 % to 49 % and hemicellulose content was decreased from 38 % to 33.2 %. These findings showed that *M. thermophila* is efficient in degrading hemicellulose and lignin [72]. Further, according to a previous work, *M. thermophila* BJAMDU5 produced 13.34, 478.23, 515.23, and 2218.12 U/g dry moldy residue (DMR) of β -glucosidase, FPase, CMCcase, and xylanase, respectively in SSF conditions at 45 °C and pH 5. The secretome analysis revealed majority of CAZymes including CEs, GHs, PLs, and LPMOs. Moreover, the enzyme cocktail produced from *M. thermophila* was utilized in hydrolysing steam pre-treated rice straw. This led to release of 184.59 mg/g of reducing sugars after 1 day [18], which was used by yeast *S. cerevisiae* to produce bioethanol. Similarly, hydrolysis by using xylanolytic and cellulolytic enzymes led to increased release of reducing sugars i.e., 356.34 mg/g from ammonia pre-treated rice straw [73]. Thus, the major enzymes generated using *M. thermophila* have discussed below.

4.1. Cellulases

Cellulose is made up of β -D-glucose units connected by 1, 4-glycosidic bonds that is broken down by the synergistic action of 3 enzymes, which are 1,4- β -endoglucanases (EGs), 1,4- β -exoglucanases, and β glucosidase (BGs) or cellobiase [74]. Majorly found enzymes in the secretome of fungi are cellobiohydrolases (CBHs) [75,76]. GH family 7 (CBH I) that attacks from reducing ends and GH family 6 (CBH II) which

attacks from nonreducing ends are main representatives [77]. Among various GH families, GH 5–9, 12, 44, 45, 48, 51, and 74 endoglucanases are widespread. Optimal activity of them were shown at acidic or neutral pH and under 50 °C [78]. Enzymes of GH3 and GH1 families constitute of BGs that hydrolyse short cello-oligosaccharides and cellobiose to glucose, which is further fermented to ethanol. Cellobiose removal is a crucial step of enzymatic hydrolysis, as it lowers the inhibitory action of cellobiose on EGs and CBHs. During the enzymatic breakdown of cellulose, the activity of BGs has often found to be rate limiting and due to this reason commercial preparations of cellulase are often complemented with BG activity [79,80]. 8 nucleotide sequences through the genome of *M. thermophila* codes for EG activity, distributed to 5, 7, 12, and 45 GHs families, all predicted to be released outside of the cell and have many O- and N- glycosylation sites [19].

Thus far, solely hydrolytic enzymes are known to have function in the break-down of hemicellulose and into cellulose simpler sugars. However, earlier studies revealed that enzymes from GH61 family exhibit lytic polysaccharide mono-oxygenase (LPMO) activity [81]. Further, an enzymatic system having the ability of oxidative cellulose cleavage is established together with a cellobiose dehydrogenase (CDH), which enhances bioconversion of lignocelluloses. The enzymes of GH61 family are accessory enzymes, which enhances the degradation of cellulose. Thus, they were referred as “cellulose enhancing factors” [82] and they are now recategorized to AA9 family [83]. Further, cellulase induction with different carbon sources from *M. thermophila* was studied by Sen et al. [84], where lactose served as the best carbon source.

According to an earlier study, *M. thermophila* is the highest producer of β -glucosidase i.e., 45.42 U/g and endoglucanase i.e., 357.51 U/g [85]. *M. thermophila* produced 85.10 U/g FPase, 96.89 U/g CMCase, and 91.92 U/g β -glucosidase after pre-treatment of rice straw for 9 days [72]. Similarly, *M. thermophila* SH1 produced 35.32 U/g cellulase and 203.20 U/g xylanase after NaOH + H₂O₂ pretreatment of *Populus deltoides* wood [86]. Bajaj and co-workers studied cellulase production by *M. thermophila* using inexpensive agricultural residues (rice husk, maize bran, and wheat bran). Maximum cellulase was obtained by using wheat bran i.e., 2000 U/l, succeeded by rice husk at 1600 U/l and maize bran at 1800 U/l [87]. Addition of peptone, soyabean meal, and mustard cake enhanced enzyme production. Highest cellulase was produced by *M. thermophila* post 2–4 days at 45 °C with 1.0 % Solka-Floc and urea [43]. Moretti and co-workers [88] showed that *M. thermophila* produced 54 U/g CMCase using wheat bran and sugarcane bagasse. Further, *M. thermophila* produced highest FPase i.e., 10.6 U/gdm by using soyabean bran and sugarcane bagasse [89]. Earlier, Moretti and co-workers conducted an experiment to study the effect of low-cost nitrogen and carbon sources on the production of enzyme by *M. thermophila* by using solid-state fermentation (SSF). A mixture containing wheat bran and corn straw at (1:1, w/w) as a source of carbon and ammonium sulfate as a source of nitrogen yielded highest activities of xylanase i.e., 446.9 U/ml, endoglucanase i.e., 94.7 U/ml, and β -glucosidase i.e., 2.8 U/ml after an incubation for 96 h at 40 °C [90]. Using cane molasses medium, in sub-merged fermentation (SmF), the economical production of 3 cellulases i.e., exo-beta-1, 4-glucanase (FPase), endo-beta-1, 4-glucanase (CMCase), and beta-glucosidase (BGL) was carried out by Bala and Singh [91]. Many CAZymes are also produced by *M. thermophila* that are involved in LC biomass degradation. The fungus produced high titres of xylanolytic and cellulolytic enzymes i.e., 515.23, 478.23, 13.34, 2218.12 U/g DMR CMCase, FPase, BGL, and xylanase, respectively when cultured on rice straw in SSF at pH 5.0 and 45 °C. Further, steam pre-treated rice straw was hydrolysed using the enzyme cocktail of the mould and was observed to produce reducing sugars at 184.59 mg/g substrate post incubation for 24 h that was further used for bio-ethanol production [18]. A β -glucosidase (50 kDa) was produced by *M. thermophila* in SSF by using a 1:1 mixture of wheat bran and sugarcane bagasse [92].

Earlier, Anu et al. [93] optimized the generation of cellulase in SSF using *M. thermophila*. Under optimized conditions, maximum cellulase

yield of 45.81 U/g DMR was obtained. Production of 316.48, 243.19, and 98.81 U/g DMR of β -glucosidase, CMCase, and FPase, respectively was obtained after optimization [93]. Matsakas and co-workers conducted a study to optimize growth conditions of *M. thermophila* to achieve maximum extracellular enzyme. Brewer's spent grain at (7 %, w/v) as a source of carbon and ammonium sulfate at (0.4 % w/v) as a source of nitrogen were found to be the optimal conditions. Optimum pH and temperature for the activity of cellulase was 5.5 and 65 °C, respectively [37]. Cellulase production by the fungus using rice straw in SSF was studied and optimized such that 4.23-, 1.28-, and 1.25-fold increase in FPase, β -glucosidase, and endoglucanase activities was noted, respectively [94]. Previously, Singh and co-workers carried the optimization of production of cellulase in SSF conditions by *M. thermophila* BJTLRMDU3, 45.81 U/g DMR cellulase was produced at 45 °C. Further enhancement in the cellulase production at 56.06 U/g DMR was observed after addition of Tween-20 (0.5 %) and K₂HPO₄ (0.25 %). Maximum reducing sugars were liberated from sodium carbonate pre-treated rice straw in comparison to the un-treated biomass [95]. Using cotton oil cake and wheat straw at 1:1, 879.26, 976.28, and 35.10 U/g DMR of CMCase, FPase, and β -glucosidase, respectively was produced by *M. thermophila* in SSF [96]. Optimal conditions led to the production of 36,420 U/l CMCase, 32,420 U/l FPase, and 5180 U/l β -glucosidase with optimal activity at pH 5.0 and 60 °C. The effectiveness of enzyme cocktail consisting of cellulases and xylanase, used in the hydrolysis of waste tea-cup paper and rice straw revealed highest sugar yield of 421.79 mg/g substrate for rice straw and 578.12 mg/g substrate for waste tea cup post-incubation for 24 h (Bala et al., 2016).

Roy and co-workers [97] purified a β -glucosidase from *M. thermophila* by using precipitation by the addition of ammonium sulfate and via column chromatography, the purified enzyme exhibited optimal activity at 60 °C and pH 4.8 and had a molecular mass of 120 kDa. A 240 kDa β -glucosidase was purified by Bhat et al. [22] from *M. thermophila* by using precipitation by adding ammonium sulfate and via ion-exchange chromatography. Roy and co-workers [98] immobilized the partially purified β -glucosidase from *M. thermophila* by using CNBr-activated sepharose to increase the conversion rate of cellobiose to glucose. The enzyme showed an improvement in storage stability and possessed maximum activity at pH 4.80 and temperature 70 °C [98].

4.2. Xylanases

Xylan is the main member of hemicellulose that is formed by beta-1, 4 linked D-xylosyl residues back-bone that is branched with uronic acids, other hexoses, and pentoses [47]. Xylanases break beta-1, 4 backbones of xylan randomly. Xylanases have various applications in industries, like hydrolysis of LC biomass, clarification of juices and beer, and bread making. Their wide use in industries have attracted considerable research interest [47].

Yadav and Jaitly [99] studied the production of xylanase with 1 % xylan at pH 6.0 by *M. thermophila*. Highest activity by the mould was observed at 2 % salt level. Katapodis and co-workers optimized the production of xylanase by using *M. thermophila* in submerged cultures such that highest xylanase production was supported by 2.7 % corn cobs and 0.7 % ammonium phosphate [47]. Further, *M. thermophila* produced high titres of xylanase (1900 U/g DMB) by using wheat bran and citrus pectin as substrates at a ratio of 1:1 in SSF post-incubation for 4 days at 45 °C and pH 7.0 [45]. Further, Singh and Satyanarayana [100] observed that maximum xylanase production was attained in SSF together with phytase by utilizing sesame oil cake by fungus *M. thermophila* after 4 days at 45 °C. Xylanase production in soluble (cellobiose, glucose, and xylan) and insoluble (wheat bran and kallar grass) substrates by *M. thermophila* was studied by Abdelrahim and co-workers. Xylanase activity was increased by 1.2-fold when kallar grass (an insoluble substrate) was complemented with 0.5 % of xylan. Optimal activity of xylanase was displayed at 70 °C and pH 6.0 [101]. In another study, neutral and acidic oligosaccharides were generated by

the birch wood xylan using an endoxylanase of the fungus *M. thermophila* [102]. Earlier, production of xylanase by *M. thermophila* by using rice straw in SSF was studied and optimized such that 2.5-fold rise in xylanase activity was observed [94]. Moreover, high-titres of endoxylanase (890.55 U/g DR) were obtained by fungus *M. thermophila* BJTLRMDU3 using rice straw in SSF and after optimizing culture conditions, a substantial rise of 4.35-fold in production of endoxylanase was attained [103]. Moreover, Gool and co-workers purified 2 novel GH11 endoxylanases (Xyl8 and Xyl7) from *M. thermophila* strain C1. Further, they studied the effect of molecular structure and solubility of substrates on the efficacy of these 2 xylanases such that disparity in degradation of low- and high-substituted substrates was shown by the xylanases. Different products from the xylan were released by the two GH11 xylanases due to the presence of a particular residue (tyrosine (Y) in Xyl8 and valine (V) in Xyl7) at a specific position i.e., 163 in their amino acid sequences of xylanase enzyme [104,105]. Furthermore, 130 nkat/ml production of xylanase by *M. thermophila* was reported by Ahirwar et al. [106] and 3455 U/g DMR production of xylanase by *M. thermophila* (in SSF) by using wheat straw and cotton oil cake was reported by Bala and Singh [96]. Earlier, Roy and co-workers [107] purified an endoxylanase from *M. thermophila* by precipitation (ammonium sulfate) and chromatography (ion-exchange). Purified enzyme of 100 kDa was most active at 65 °C and pH 4.5. Moreover, Boonrung and co-workers purified and characterized a thermo-alkali stable xylanase from *M. thermophila* strain BF1-7. At pH 12.0, xylanase was most active and 55 % of activity was maintained in wider range of pH (9–12) after an incubation of 24 h at 4 °C. Purified xylanase exhibited activity only on xylan and beech-wood xylan to release xylobiose and xylotetraose as the end-products that suggested that it was an endo-xylanase [108]. In another study, saccharification of corn cob and rice straw using endoxylanase of *M. thermophila* resulted in improved liberation of reducing sugars [103].

4.3. Laccases

Laccases are multicopper oxido-reductases (MCOs) with wide range of bio-technological applications [109]. Numerous studies have shown the ability of laccase to delignify various pretreated LC materials, thereby showing an improvement in the successive process of enzyme-based hydrolysis. For instance, the treatment of milled eucalypt wood with *M. thermophila* laccase followed by alkaline-peroxide extraction in multi-stage (4 cycles of enzyme alkaline extraction) sequence caused a 20 % reduction in lignin content and 9 % increment in glucose liberation. However, with utilization of methyl syringate (a natural mediator of laccase), lignin content further reduced to about 50 % and glucose yield increased by 30 % [110]. The treatment of steam exploded *Eucalyptus* wood chips with laccase from *M. thermophila* in combination with 1-hydroxybenzotriazole (HMT) mediator increased glucose yield to 27.1 % from 24.7 % [111]. In another recent report, steam pretreatment of olive tree pruning produced a cellulose-rich water-insoluble fraction that was used for saccharification and fermentation by *Saccharomyces cerevisiae* strains (Ethanol Red and F12) and a liquid fraction containing glucose, xylose, and inhibitory byproducts, like phenols, furan derivatives, and weak acids that limits its use for ethanol production. However, the utilization of liquid fraction is required for the economic feasibility of the process, for which a laccase obtained from *M. thermophila* was utilized to detoxify the liquid fraction by overcoming the inhibitory effects of byproducts. Laccase treatment did not affect the weak acid and furan derivatives concentrations but decreased the total phenol content by 87 %. Further, ethanol yields increased due to improved fermentative behaviour of *S. cerevisiae* strains [112].

M. thermophila genome encodes 8 putative enzymes with multicopper oxidase activity. Out of those, 4 have been elucidated and 1 i.e., MYCTH_51627 encodes a protein (LCC1-type laccase) that corresponds to *lcc1* gene product, which encodes for an extra-cellular laccase [21]. Later, *lcc1* from *M. thermophila* genome was heterologously

expressed in another fungus *A. oryzae*. An 82 kDa enzyme produced was identified as a thermo-stable laccase exhibiting high reactivity at neutral pH and room-temperature [113]. Babot and co-workers investigated the capability of two natural phenols i.e., methyl syringate and syringaldehyde to function as mediators of a recombinant laccase i.e., MtL from *M. thermophila* in delignification of eucalypt-pulp. After enzymatic treatment with MtL alone, the brightness of pulp enhanced by 3.1 points, but after MtL treatment with methyl syringate (8.3 points) and syringaldehyde (4.7 points) as mediators, higher improvements were achieved [59]. Further, the ability of laccase to enhance the oxidative-polymerization of kraft lignin (KL) was studied at various temperature, pH, treatment time, and enzyme dosage such that after incubation with laccases notable lignin modifications were resulted [114]. In an earlier study, laccase was immobilized on epoxy-functionalized silica particles and was studied for removing phenolic compounds. Catechol is removed by immobilized enzyme with 95 % efficiency after 2 h [115]. Huber and co-workers successfully immobilized a laccase obtained from *M. thermophila* onto Accurel MP 1000® beads and carried out the bio-catalyzed oxidation of liginosulfonate (LS) and KL. After laccase-mediated oxidation, 8.5-fold reduction in the fluorescence intensity of LS samples was observed and a 12-fold rise in average molecular weight was achieved [116].

4.4. Esterases

In breakdown of LC materials, the role of esterases is intricate and includes the bond breakage between the hemicellulosic part and various kind of side chains. These enzymes are grouped under 9 families and involved in hydrolysis of the acetyl and feruloyl ester bonds. Among them, feruloyl esterases (FAEs) are responsible for the cleavage of ester-bond between monomeric or dimeric ferulates and poly-saccharide chain of xylans. They are divided into 4 groups, A-D, and they work together with xylanase to liberate ferulic acid from the cell wall. The dissimilarity between D and A is their substrate specificities and their ability to liberate diferuloyl bridge [117]. The very first FAEs were produced under SSF from *M. thermophila* [118]. From *M. thermophila* extra-cellular culture supernatants, two other FAEs i.e., StFaeC of 46 kDa with 23 kDa homo-dimers [119] and StFaeB (66 kDa with 33 kDa homo-dimers) [120] were purified. Specifically, among all genome portal registered sequences, there are 4 sequences that encode proteins with FAE catalytic activity. Among them, two (MYCTH_39279 and MYCTH_48379) appeared to be analogous with already known FAEs produced from C1 strain of *M. thermophila* [121]. Heterologous expression of a sequence i.e., JDI ID - 96478 has been done in methylotrophic yeast *Pichia pastoris* X-33 that encoded a protein of 39 kDa (MtFae1a; *fae1A*) that had activity toward p-coumarate and methyl caffeate, and an inclination for hydrolysing iso-butyl and n-butyl ferulate [122]. In the study conducted by Varriale and co-workers, a methodology for the construction of evolved variants library of MtFae1a of 30,000 mutants was developed and the automated screening was performed to select the variants with enzyme activities greater than the wild type [123].

In hardwood xylans, at C-2 and/or C-3 positions, around 60–70 % of xylose residues are chemically modified via the addition of acetyl group [124]. The action of acetyl esterases (AcEs) is required for the total degradation of acetylated xylans by microorganisms, which cleaves the acetyl side groups out of heteroxylan back-bone, and shows synergism with other hemicellulases [125]. In genome of *M. thermophila*, 8 sequences that encode proteins having AcE activity were found and they exhibited the identity with characterized enzymes [126]. Further, one active esterase was recognized for their activity towards xanthan after screening of 8 carbohydrate acetyl esterases. AXE3 (acetyl xylan esterase of carbohydrate esterase (CE) family 1) obtained from *M. thermophila* C1, detached 31 % of acetyl side-groups found in xanthan after 48 h of incubation [127]. In addition, recently discovered enzymes, glucuronoyl esterases (GEs) are shown to have a role in the detachment of cellulose and hemicellulose from lignin by breaking the ester-bonds between

carboxyl groups of 4-O-methyl-d-glucuronic acid (type of uronic acid) residues and aromatic alcohols of lignin in glucuronoxylan [128]. Later, a novel and conserved sequence of amino acids (G-C-S-R-X-G) is revealed by the alignment studies of these esterases. This sequence includes a serine (S) residue, which is likely involved in the enzymatic activity of these enzymes [129]. Two genes, which encode proteins with 4-O-methyl-glucuronoyl esterase (classified to CE15 family) activity were found in *M. thermophila* genome [130].

4.5. Mannan-hydrolysing enzymes

Mannan is a crucial constituent of the hemicellulose. Thus, a whole set of genes encoding the mannan-deconstructing enzymes are present in LC degrading toolbox of *M. thermophila*. Mannan polymer comprises of a primary backbone structure, and is made up of beta-1,4-linked either mannose residues or a mixture of mannose and glucose. It can be disintegrated into monomeric units by the combined activity of beta-mannanases, β -mannosidases, α -galactosidases, and acetylmannan esterases [346]. In an earlier work, *M. thermophila* genes encoding galactomannan-degrading β -mannanase (GH 5 family, 48 kDa), β -mannosidase (GH 2 family, 97 kDa), and α -galactosidase (GH 27 family, 60 kDa) were cloned such that the recombinant products obtained were characterized [58]. In another study, the efficiency of fungal culture supernatant against hydrolysis of mannan was boosted by two GH 27 and one GH 26 α -galactosidases [131]. An endomannanase encoding gene i.e., *em26a* belonging to GH family 26, from *M. thermophila*, was expressed in *P. pastoris*. Purified putative endomannanase (MtMan26A; 60 kDa) exhibited thermal stability (at 60 °C) with 14.4 h of half-life. Increased enzymatic hydrolysis of pre-treated beechwood sawdust was attained by adding MtMan26A to a commercial enzyme mixture (Novozyme® 188 and Celluclast® 1.5 L) with more release of glucose and total reducing sugars by 12 % and 13 %, respectively [24].

4.6. Arabinohydrolases

l-arabinose is majorly found in hemicellulose-based biomass constituents, like in arabinoxylan, where beta-D-(1,4)-bound xylopyranosyl back-bone is substituted with the arabinose residues [132]. Arabinan comprises of a back-bone of alpha-(1, 5)-bound l-arabino-furanosyl residues, few of them are substituted with alpha-(1, 3)- or alpha-(1, 2)-linked arabinofuranosides [133]. On other hand, α -l-arabinofuranosidases (AFases) liberate arabinofuranose residues that are substituted at O-3 or O-2 position of di- or mono-substituted xylose residues [132]. For the decomposition of arabinan to arabinose sugars, AFases function with other arabinohydrolases [134]. AFases catalyze the hydrolysis of non-reducing, terminal l-arabinofuranosyl residues out of arabinan, while the ABNases effectively hydrolyse the resulted debranched backbone; thereby, producing various arabinooligosaccharides in an inverting manner [135]. The genome of *M. thermophila* encodes fourteen enzymes, which putatively hydrolyse arabinose oligomers or arabinose out of arabinan. Specifically, arabinohydrolases are included in GH families 93, 62, 54, 51, and 43 [65].

4.7. Lytic polysaccharide mono-oxygenases (LPMOs)

Cellulose is the most occurring natural polysaccharide having potential to synthesize biofuels and other value-added chemicals. Over last decades, several exo- and endo-glycosidases have been characterized for degradation of recalcitrant cellulose through enzymatic hydrolysis [136]. But the low depolymerization efficiency of cellulose because of highly recalcitrant nature of cellulose has significantly compromised the utilization of cellulases [3] until the determination of LPMOs [137]. LPMOs are a set of mono-copper redox enzymes that cleave the crystalline region of cellulose by breaking glycosidic bonds through oxidative mechanism [138]; thus, enhancing the efficiency of cellulases in cellulose hydrolysis [82,139,140]. From their discovery in 2010, a

variety of LPMOs from diverse range of microorganisms have been identified, having broad-range of substrate specificity including cellulose, hemicellulose, starch, chitin, and xylan [141–145]. According to sequence of amino acids, LPMOs has been categorized in 7 families of auxiliary activity (AA) enzymes in CAZymes database (AA9-11 and AA13-16) [142,145–150]. Further, based on product specificity and regioselectivity, LPMOs that acts upon beta-(1,4)-glycosidic bonds were grouped among 3 types i.e., C-4 oxidizing, C-1 oxidizing, and both C-4 and C-1 oxidizing [151,152].

LPMO activity has been demonstrated on cellulose, starch, or chitin [153]. Additionally, it is also used to degrade LC substrates as an enzyme cocktail [82,154]. As LPMOs play a crucial role in recalcitrant biomass degradation; thus, they are currently receiving more attention [137, 138]. One of the commonest LPMO from fungi is AA9, which plays a crucial role in cellulose transformation along with degradation as per the oxidative mechanism (Monclaro et al., 2017; [82]). The active centre of AA9 has a copper (Cu^{2+}) ion coordinated histidine brace as revealed by its crystal structures [82,138,155]. To degrade polysaccharide substrates, the Cu^{2+} ion at the active site must first receive an electron. Some reducing agents are needed by the enzymes during each catalytic cycle as a source of electron [156–158]. The oxidized carbon is situated at C-4, C-1, or both C-4 and C-1 [159–161], resulting in the successive hydrolysis of glycosidic bond. Hence, the crystalline structure of cellulose is distorted that makes substrate better accessible by other hydrolytic enzymes. To increase the degradation of crystalline cellulose more efficiently, AA9 is utilized as a portion of enzyme cocktail; therefore, making it simpler to get much more fermentable sugars that can be transformed into bio-ethanol and other bio-fuels [150,153,162,163]. In this way, AA9 would open a new possibility to produce bioethanol from the sustainable feedstocks [164].

The genome of *M. thermophila* had been predicted to contain 22 AA9-family LPMOs, out of which 6 have been recognized and characterized previously by distinct substrate preferences and C-1/C-4 regioselectivity, where MtLPMO9A and MtLPMO9I are C-1- and C-4-oxidizing LPMOs [17,165,166]. MtLPMO9C and MtLPMO9E are C-4-oxidizing LPMOs that work on both xyloglucan as well as cellulose [17,167]. MtLPMO9B and MtLPMO9D acts only on cellulose and produce C-1-oxidized cellobiosaccharides ([167] and 2018 [39,40]). MtLPMO9F and MtLPMO9H release C-4-oxidized and C-4/C-6-double oxidized xylo-glucooligosaccharides [170]. From *M. thermophila* C1, an AA9 i.e., MtC1LPMO was reported [165,167–169], which in presence of ascorbic acid degrades regenerated amorphous cellulose by oxidations at C-1 and C-4 positions. Additionally, the synergistic activity of MtC1LPMO with endoglucanase I was noticed. It was reported that the oligosaccharides content released by both endoglucanase I and MtC1LPMO together was 16 times greater than oligosaccharides content released by endoglucanase I alone, when incubated with regenerated amorphous cellulose [165]. Further, in a study done by Zhou and co-workers a new member of AA9 LPMO family i.e., MtLPMO9L was identified from *M. thermophila* strain ATCC 42464, which shares 62 and 61 % sequence similarity with NcLPMO9F and MtLPMO9A, respectively. MtLPMO9L can only break down cellulose by C-1 oxidation. Synergistic effects of MtLPMO9L with CBHs were also assessed in this study and the results showed that it has completely distinct effects with 2 CBHs i.e., it inhibits CBH I whereas boosts CBH II [171].

4.8. Amylases

Glucoamylases or glucan 1, 4- α -glucosidases catalyze the successive release of 1, 4-bound alpha-d-glucose units from the nonreducing ends of poly-meric maltooligosaccharides or alpha-d-glucans with the liberation of beta-d-glucose through inverting procedure. 1, 6- α -d-glucosidic bonds can also be hydrolysed by some glucoamylases when the subsequent bond in the sequence is 1, 4-alpha-D-glucosidic. Natural substrates for glucoamylases are amylose, amylopectin, malto-oligosaccharides, and starch. GH 15 consists of most of glucoamylases. GH 97 consists

of fewer glucoamylases from archaea or bacteria. Glucoamylases are abundantly present in nature, and majority of them are produced from microbes. In an earlier study, comparison between the hydrolytic performances of two glucoamylases purified from *Penicillium verrucosum* i.e., PvGla and *M. thermophila* i.e., MtGla in breakdown of various natural poly-meric substrates was done. 2- to 5-fold increased specific activities against amylose, amylopectin, and soluble starch were shown by MtGla than PvGla. Also, during the extended hydrolysis of poly-meric substrates, MtGla provided higher glucose yields [172].

Previously, the production of amylolytic enzymes by *M. thermophila* D14 was studied by batch cultivation at 45 °C in Czapek-Dox medium. For amylase production, NaNO₃ and KNO₃ were reported to be best among several nitrogenous compounds used. The synthesis of amylase was induced by starch and cellobiose, and it was reliant on growth and obtained pre-dominantly in mid-stationary phase [173]. The production of alpha-amylase was higher when *M. thermophila* D14 was inoculated in a medium supplemented with starch or various other carbohydrates having maltose units. More specifically, 1 % of starch resulted in maximum enzyme production followed by a gradual reduction with elevating concentration. With the inclusion of glucose to culture medium a marked decrease in the synthesis of α -amylase occurred and this decreased activity was correlated to glucose concentration. When the concentration of glucose drops below a critical amount, the enzyme synthesis was resumed. The repressive activity of glucose was not eliminated by the addition of cAMP. From these findings, it was revealed that extra-cellular α -amylase synthesis in *M. thermophila* D14 is inducible and regulated by the catabolite repression [60]. Furthermore, viral 2A peptide strategy adapted for *M. thermophila* in combination with efficient CRISPR/Cas9 system produced a quintuple mutant, in which the amylase activity increased by 8.2-fold, thereby establishing the hypersecretion platforms for biotechnological applications [174]. In another study by Zhu and co-workers, deletion of 4 major factors implicated in glucoamylase expression and secretion in *M. thermophila* using AsCas12a variants led to 1.85-fold higher glucoamylases activity. Further, generation of glucoamylase hyperproduction strain of *M. thermophila* i.e., MtGM12 led to 51.9–55.5-fold higher activities of amylolytic enzymes [175].

4.9. Phytases

The phytases isolated from thermophilic fungi are histidine acid phosphatases, they catalyze the phytic acid hydrolysis to inorganic and myo-inositol phosphate by an array of myo-inositol phosphate intermediates [176]. In plants, in legumes and oil plant seeds, phytic acid is a main form of storage. It has high capacity to attach to amino acids, sugars, and proteins, and to chelate minerals (Ca, Mg, Fe) and to hinder digestive enzymes. Thus, phytic acid is an anti-nutritional component which need to be hydrolysed by phytases before its inclusion in animal diets. Thus, the incorporation of phytases into animal feeds increase digestibility and promotes growth because the nutritional quality of the feed is increased by releasing phosphorus, minerals, and proteins [53, 177]. Additionally, phytases from *M. thermophila* release increased quantity of reducing sugars, inorganic phosphate, and soluble proteins from dough in comparison to bread made from industrial enzymes [178], thereby improving bread-making process [176]. In an earlier study, improvement in nutritional qualities of wheat and rice flours was observed by adding *M. thermophila* xylanase at 200 U/g of flour alone or along with *Aspergillus oryzae* SBS50 phytase at 15 U/g flour [179]. Later, Dahiya et al. [180] carried out the endoxylanase production from *M. thermophila* BJTLRMDU3 under SSF conditions using rice straw and an enhancement of 2.53-fold after optimization was observed. Higher amounts of reducing sugars at 58.58 mg/feed, inorganic phosphate at 28.34 mg/feed, and soluble proteins at 42.48 mg/g feed were released by synergistic action of phytase at 15 U/g and endoxylanase at 100 U/g on poultry feed in comparison to control with 23.55 mg/feed of reducing sugars, 10.46 mg/feed of inorganic phosphate, and 16.98 mg/feed of

soluble proteins, which were released by the action of endoxylanase only at 60 °C [180].

Earlier, Hassouni and co-workers evaluated phytase production from thermophilic fungi in SSF using sugarcane bagasse. Overall, 20 strains belonging to *M. thermophila*, *Rhizomucor* spp., *Thermoascus aurantiacus*, and *Paecilomyces variotii* were screened for phytase production. Also, parameters of SSF (initial pH, initial moisture, and aeration rate) were studied. Among them, higher phytase activity was shown by *M. thermophila* after 36 h, when cultivated on a synthetic culture medium having 10.0 g/L glucose and 2.0 g/L phytic acid, which was 2.5 times higher in comparison with mesophilic *Aspergillus ficuum* [53]. Kumari et al. [181] showed that highest phytase production was 1881.26 U/g DMR by *M. thermophila* under optimal conditions and a 11.6-fold increase in phytase production was observed using mixed substrate. In another study, Singh and co-workers isolated 138 thermotolerant/thermophilic molds from compost samples and soil from several regions of India and screened them for phytase secretion. *Sporotrichum thermophile* BJTLR50 generated high enzyme titre and after optimization 2-fold rise in production of phytase was attained [182].

5. Genome and secretome evidences of lignocellulose degradation by *M. thermophila*

M. thermophila is the first filamentous mould with a finished genome. Genome analysis of *M. thermophila* revealed that it consists of a repertoire of lignocellulolytic genes [16], thereby it degrades the LC biomass naturally very well [63]. Studying the *M. thermophila* secretome after growth for 30 h on barley and alfalfa, it was revealed that the fungus secreted 683 proteins, out of which 230 were with unknown function. Based on transcriptome analysis, several secreted accessory enzymes, proteins with unknown function, and hypothetical proteins were up-regulated, when *M. thermophila* was grown on complex substrates, like agricultural biomass in comparison to glucose, suggesting their potential role in the degradation of LC biomass [16]. Thus, it was demonstrated by the genomic potential of the fungus, that it consists of a strong enzymatic tool-box with hydrolytic, accessory, and oxidative activities that may increase its ability to degrade the LC biomass [19]. In another work, *M. thermophila* was grown on distinct types of LC biomass to evaluate how the enzymatic secretion differs with different substrates. For analysis of the transcriptome, monocots (oat, triticale, and barley) and dicots (canola, flax, and alfalfa) were used; whereas, for proteome analysis corn stover and wood pulps were used. 59 genes were up-regulated when grown on monocots and dicots, which are involved in hydrolysis of these substrates [20]. Xu and co-workers did the transcriptomic analysis for *M. thermophila* on starch, a set of 342 genes was recognized as a “starch regulon”, including majority of amylolytic enzymes. Overexpression of this led to the increased amylase activity by 35 % on starch [183].

Bala et al. [18] performed the secretome analysis of *M. thermophila* BJAMDU5 grown on rice straw using mass spectroscopy, the results described 124 distinct proteins with most of CAZymes, including GHs, LPMOs, CEs, and PLs. It was revealed by exoproteome analysis that *M. thermophila* consists about 42 % of CAZymes, out of which 80 % were GHs from 24 families, which are implicated in LC biomass degradation, including cellulases, xylanases, and pectinases [18]. In a previous study, enzyme production with lignocellulolytic potential, saccharification potential of extracts, and secretome profile of *M. thermophila* was evaluated by using soy straw, wheat bran, and rice straw in SSF. The analysis of enzymatic activities and 66 proteins recognized in secretome revealed the hydrolytic as well as oxidative properties of various lignocellulolytic enzymes generated by the fungus [184]. In another study, *M. thermophila* was grown on pre-treated sugarcane bagasse to evaluate the secretome, which revealed that CAZymes belonging to GH families and LPMOs were secreted by the fungus [185]. Moreover, when the fungus was grown on natural biomass and purified cellulose, the secretome consists of cellobiohydrolases, β -glucosidases, β -1, 3

glucanase, cellobiose dehydrogenase, glyoxal oxidase, aldose epimerase, xyloglucanase, aldolactonase, galactosidase, and LPMOs while when the fungus was grown on purified fraction of hemicellulose, like xylose, arabinans, and arabinoxylans, it has produced enzymes, including xylosidase, beta-glucosidases, beta-1, 3 glucanases, mixed β -1, 3(4) glucanase, β -mannosidase, galactosidase, endo beta-1, 6 galactanase, chitinases, polysaccharide lyase and aldose epimerase. Thus, the secretome developed on the natural biomass consisted of enzymes for hydrolysis of hemicellulose, cellulose, and lignin [63].

6. Genetic engineering studies for improving degradation potential of *M. thermophila*

Myceliophthora thermophila is a desirable system for the generation of enzymes and other chemicals by utilizing plant biomass [27]. Usually, filamentous molds are the pre-dominant microbes used to develop several industrially significant products, such as organic acids, enzymes etc. Among them, *M. thermophila* is a well-studied thermophilic fungus that has profound ability of biomass degradation between 45 and 55 °C. It is well-known to produce a collection of biomass hydrolysing enzymes that finds several applications in the field of biotechnology, especially due to their thermostability and improved catalytic rates at higher temperatures through the process. Now-a-days, this thermophilic fungus has been upgraded by genetic engineering (Fig. 3) [186]. Its broad range of industrial importance has motivated researchers to generate tools for genome-editing to increase the genetic engineering of this mould. In 2017, the first-generation CRISPR/Cas-9 technology was emerged and, thereafter, little progress has been carried out in the genetic engineering of this thermophilic fungus, but a few limitations remain [27].

Previously, Liu and co-workers developed a CRISPR/Cas-9 system for the efficient multiplex genome engineering in 2 thermophilic fungal species i.e., *M. thermophila* and *Myceliophthora heterothallica* that via NHEJ-mediated events can efficiently mutate an imported *amdS* gene. Genes of cellulase enzyme production pathway, such as *alp-1*, *ghl1-1*, *res-1*, and *cre-1* were selected as targets for editing. Thus, by using this

approach, various strains with hyper-cellulase production were produced, in which lignocellulase enzyme activities and extracellular secreted proteins were significantly increased [29]. Later they, through a single-array strategy in *M. thermophila* generated a CRISPR/Cas-12a system from *Acidaminococcus* sp. for the multi-plex genome editing. They also developed a strategy for marker-recycling, which depends on CRISPR/Cas-12a system cleavage activity to generate DNA breaks in the selectable markers. Its potential was determined by targeting 9 genes of production pathway of cellulase in *M. thermophila* through 3 rounds of transformation, using 2 selectable markers *bar* and *neo* such that nonuple mutant M9 showed 18.5- and 9-fold higher lignocellulase activity and protein productivity, respectively [27]. Further, in genome of *M. thermophila*, systematic transcriptomic analysis was carried out on the expression of genes of proteases, and for mutation analysis 5 highly expressed genes encoding extra-cellular proteases were chosen. Using CRISPR-Cas9 technique, an array of single- and multiple-gene mutants of chosen genes was created such that Δ *Mtalp1* and the quintuple mutant showed minimum of double increase in cellulase production, when compared with wild type [174]. Moreover, as stated earlier, Li et al. [187] created a novel thermophilic platform to produce glucoamylase by using *M. thermophila* as a host through 2A peptide approach combined with CRISPR/Cas-9 system. rMhGlaA exhibited stability in a wider range of pH (3–9) and thermostability at 65 °C, which suggests its probable industrial applications. To ameliorate the glucoamylase secretion, genetic engineering was performed with the existing strain OE-MhglA-gfp via potent CRISPR/Cas-9 system by which a quintuple mutant i.e., OE-MhglA-gfpOE-amyR Δ alp-1 Δ res-1 Δ cre-1 was obtained that displayed enhanced amylase activity and protein productivity by 8.2- and 12-fold, compared with wild-type [187]. Furthermore, Xu and co-workers conducted a study to determine transcriptomic profile of the mould *M. thermophila* on soluble starch such that a gene set of 342 genes was recognized as “starch regulon”, involving a main amylolytic enzyme i.e., Myc72393. 35 % increased amylase activities on starch were observed due to over-expression of this gene. By CRISPR-Cas9 system, *amyR* was deleted, which caused the release of carbon catabolite

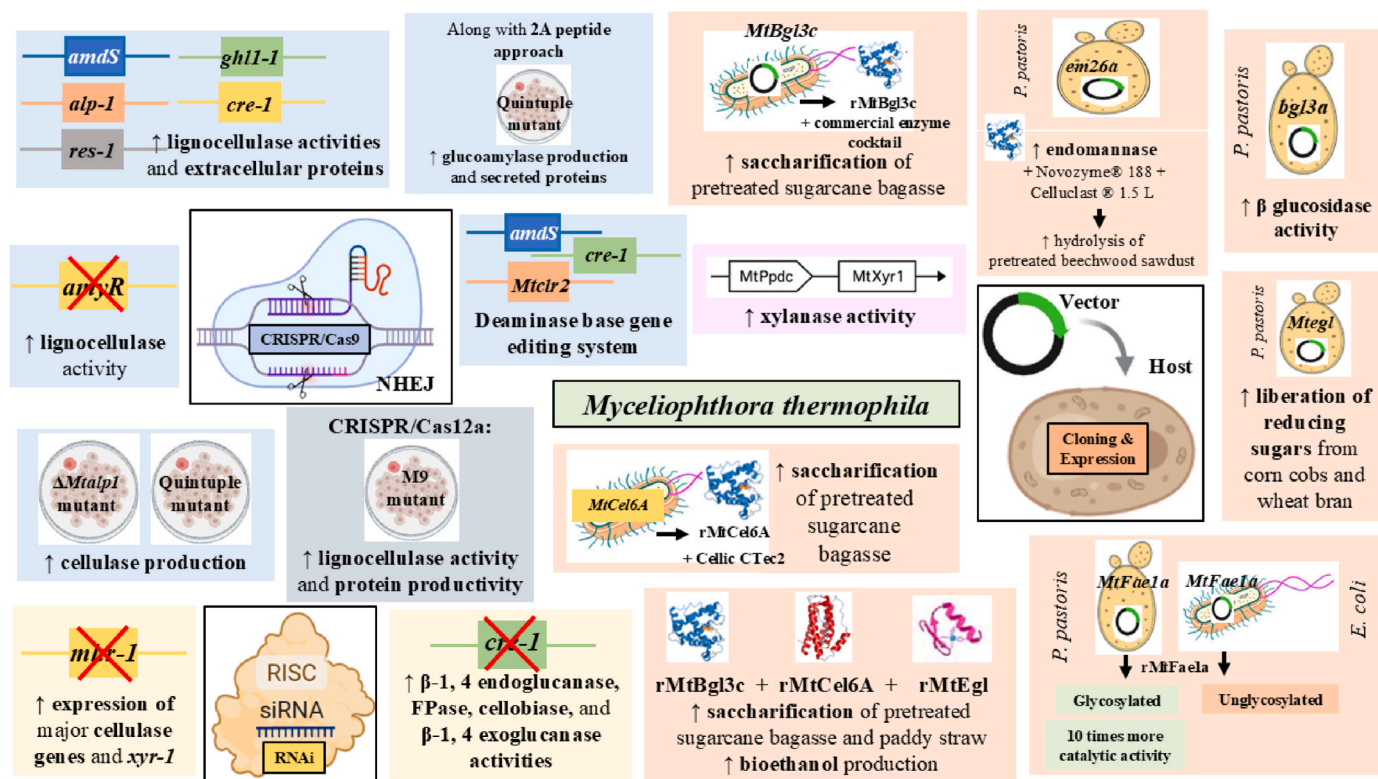


Fig. 3. Overview of genetic engineering strategies employed to enhance the lignocellulose-degrading potential of *Myceliophthora thermophila*.

repression (CCR) and three-fold increase in cellulose degradation [183].

Using RNA interference (RNAi), the role of CRE1 in *M. thermophila* ATCC42464 was investigated by Yang and co-workers (2015). The β -1, 4-endoglucanase and FPase activity in *cre1*-silenced strain C88 were increased by 1.31- and 3.76-fold, respectively, when compared to wild-type strain. Similarly, the activities of cellobiase and β -1, 4-exoglucanase were increased by 5.59- and 2.64-fold, respectively, in comparison to parental strain [188]. Further, the role of a putative transcriptional regulator (namely MHR1) of the cellulolytic enzymes in mould *M. thermophila* was established via RNA Seq-based gene-expression profiling. To determine the function of MHR1, RNAi was used. A recombinant plasmid having RNAi sequence specific to *mhr1* gene was created and transformed in *M. thermophila*. For further study, transformants (i.e., Mtr5) with greatest RNAi efficiency was utilized. The silencing of *mhr1* resulted in increased expression of major cellulase genes as well as of positive regulatory gene *xyl1*. Therefore, MHR1 of mould *M. thermophila* can suppress enzyme (xylanase and cellulase) activities [189]. Similarly, in a work, *Mtxr1* gene was deleted from *M. thermophila* and resultant strain was compared with wild type by transcriptomics and growth profiling. The generated deletion strain was not able to grow on D-xylose and xylan, and grew like the wild-type on cellulose and Avicel but depicted only a little growth decrement on the L-arabinose. Transcriptome analyses suggested reduction of xylanolytic genes implicated in xylan degradation, and genes implicated in catabolism and pentose transport. However, no or minimal effect was observed on cellulolytic genes [190]. Further, in a work by Xu and co-workers (2015), a high efficiency transformation and targeted gene disruption system for *M. thermophila* (ATCC 42464) was mediated by *Agrobacterium tumefaciens*. Using this technique, a *ku70* deletion strain was developed in *M. thermophila* background population, which has accelerated the process of rapid degradation of cellulose [28].

In a previous study, overexpression of xylanase regulator 1 protein (*MtXyr1*) was performed under the control of a strong promoter i.e., pyruvate decarboxylase promoter (*MtPpdc*) in *M. thermophila*. Activities of extracellular xylanase of transformant culture were 9.04- and 25.19-fold higher in inducing and non-inducing media after 120 h, respectively, when compared to the wild-type [191]. From *M. thermophila*, a β -glucosidase gene i.e., *bgl3a* was cloned and expressed in yeast *P. pastoris*. The recombinant yeast generated high level of β -glucosidase in the medium with enzymatic activity of 41 U/ml under methanol induction, after 192 h of growth [192]. Similarly, from *M. thermophila*, heterologous-expression of an EG and its synergistic activity with a LPMO was described by Karnaouri et al. [166]. Expression of EG gene was done in *P. pastoris*. Remarkable thermostability and high specific activity on micro-crystalline cellulose was shown by the produced MtEG5A of 75 kDa. High amount of cellobiose was released by the enzyme from spruce, wheat straw, and birch biomass [166]. In another study, a vector consisting of a coding region of laccase of *M. thermophila*, under the transcriptional regulation of an α -amylase gene terminator and promoter of *A. oryzae* was created for heterologous expression in the host *A. oryzae*. MtL (85 kDa) depicted optimal activity at 6.5 pH and retained almost all activity post-incubation for 20 min at 60 °C [21]. As stated earlier, an endomannanase gene (*em26a*) from *M. thermophila*, of GH family 26, was functionally expressed in host *P. pastoris*. rMtMan26A (~60 kDa) showed optimum activity at pH 6 and 60 °C whose addition to the commercial enzyme combination i.e., Novozyme® 188 and Celluclast® 1.5 L gave better results in increased enzymatic hydrolysis of the pretreated beechwood sawdust [24].

In study by Phadtare and co-workers, codon adaptation index (CAI) of an endoglucanase gene of *M. thermophila* BJA has been improved by *in vitro* gene synthesis. After, codon optimization *Mt-egl* gene was expressed in the host *P. pastoris* in regulation of GAP promoter. The recombinant endoglucanase (rMtEgl, ~47 kDa) showed optimal activity at 50 °C and pH 10. rMt-egl liberated high amount of reducing sugars from corn cobs and wheat bran, thereby making it suitable biocatalyst to produce bio-ethanol and other fine chemicals from various agro-residues

[193]. Dadwal et al. [194] conducted a work, in which codon optimized cellobiohydrolase encoding synthetic gene (*MtCel6A*) from thermophilic mould (*M. thermophila*) was cloned and heterologously expressed in *Escherichia coli* such that the recombinant rMtCel6A (~45 kDa) showed optimal activity at 60 °C and pH 10. A 1.9-fold rise in saccharification of pretreated sugarcane bagasse was resulted after adding rMtCel6A to the commercially available cellulase mix i.e., Cellic CTec2 [194]. Later, these authors cloned and heterologously expressed GH3 β -glucosidase gene of *M. thermophila* (*MtBgl3c*) in *E. coli*. The recombinant rMtBgl3c (~100 kDa) depicted optimal activity at 55 °C and pH 5, which showed synergistic effect with a commercial enzyme cock-tail in saccharification process of the pretreated sugarcane bagasse, thereby demonstrating its use in the bioconversion of cellulose [195]. Further, these authors developed an enzyme cock-tail of recombinant thermo-stable cellulases of *M. thermophila* (rMtBgl3c, rMtCel6A, and rMtEgl) for converting agro-residues into ethanol. The developed enzyme cocktail resulted in 80–90 % saccharification process of the pretreated sugarcane bagasse and paddy straw that is two-fold greater than that attained by using commercial enzyme mixture individually. Further, 37.0 and 55.8 g/L of ethanol was produced with fermentation efficiencies of 76 and 80 % from pre-treated sugarcane bagasse and paddy straw hydrolysates, respectively. They also optimized the most effective pre-treatment method. Pre-treatment of sugarcane bagasse by acetic acid and sodium chlorite led to 5.5-fold rise in the liberation of total reducing sugars. Similarly, pre-treatment of paddy straw with microwaves and NaOH resulted in 9-fold rise in the release of total reducing sugars. Thus, a careful blend of every enzyme component and pre-treatment method customized for a particular biomass is important for the effective production of bioethanol [196].

Using *in silico* approaches, a β -glucosidase of *M. thermophila* i.e., MtBgl3c was evaluated for its structural characterization. As the protein structure of it is not known; thus, an effort to model its 3D structure was done by using Modeller 9.23. Ramachandran plot was constructed that estimated the accuracy of 3-D model. 3D model generated was docked with the substrate cellobiose. Comparing the dock scores, it was found that cellobiose score with MtBgl3c is lesser than that of glucose, which suggests its high affinity for cellobiose [197]. In another work by Zhang and co-workers, 3 cytosine base editors (CBEs) were constructed in *M. thermophila*, where an efficient inactivation of genes by precise conversion of 3 codons (CGA, CAG, and CAA) into stop codons (UGA, UAG, and UAA) without double-strand break formation was achieved. Deaminase cytosine base editing system effectively edited 3 target genes (*Mtclr-2*, *amdS*, and *cre-1*) in *M. thermophila*. Numerous point mutations generated in the target loci of the DNA-binding domain and fungal-specific motif of MtCLR-2 revealed that former domain is crucial for fungal response to cellulose conditions and latter domain is involved in the fungal growth. Thus, base editor is an efficient tool for decoding the functions of the target genes motifs in the filamentous fungi [198]. Further, Bonzom and co-authors studied the glycosylation of a feruloyl esterase i.e., MtFae1a from *M. thermophila* expressed in *E. coli* and *P. pastoris*. *M. thermophila* and *P. pastoris* produced the glycosylated enzyme while *E. coli* produced the non-glycosylated enzyme. Largest differences were seen between glycosylated and non-glycosylated enzymes; however, significant dissimilarities were also seen between glycosylated enzymes from different hosts. Mass spectrometric analysis showed variations in the glycan chains lengths of the glycosylated enzymes produced in *P. pastoris* and *M. thermophila*. Further, glycosylated enzymes have 10 times more catalytic efficiency than non-glycosylated enzymes. Therefore, bio-technological value of an enzyme for a particular application can be optimized by precisely selecting the production host [199]. Lastly, in a recent study, the authors reprogrammed *M. thermophila* by optimizing synthetic pathway, increasing glycolytic rate, limiting NADH shuttles across mitochondrial membranes, and knocking out the ethanol (alcohol) consumption pathway to instantly convert cellulose into ethanol just like the aerobic ethanol fermentation in yeast. The final engineered strain of *M. thermophila* yielded 39.8 and

52.8 g/L bioethanol from corn-cob and cellulose, respectively without the requirement for addition of cellulase [200]. Table 1 summarizes the heterologous expression of hydrolytic enzymes encoding genes from *M. thermophila*, while Table 2 enlists various genetic engineering strategies utilized to increase the generation of biomass-degrading enzymes from *M. thermophila*.

7. Biotechnological products derived from lignocellulosic biomass degradation using the enzymatic machinery of *M. thermophila*

A major constituent of biomass is lignocellulose, it consists of about half of the plant-matter generated via photosynthesis, and is the most-abundant organic renewable resource present in soil. It is made up of lignin, cellulose, and hemicelluloses, which are connected chemically and strongly by non-covalent and covalent linkages [201]. LC materials are generated in huge amounts through agricultural and forestry practices, and paper and pulp-, agro-, and timber-industries. They create an environmental pollution concern as large amount of them are disposed off by burning [202,203]. However, the large fraction of residuary plant biomass that is generally regarded as “waste” can be potentially transformed into several value-added products, like bioethanol and other products of biotechnological importance. Further, LC wastes can also serve as energy source for microorganisms to produce various lignocellulolytic enzymes by fermentation [202].

LC biomass generated from municipal solid-waste, forestry waste, and crop residues are present in huge amount in nature and they possess the potential for bio-conversion into different value added chemical and biological products. Buildup of this LC biomass in huge amounts not only creates a problem of proper disposal but also cause wastage of valuable materials. Thus, it can be utilized to generate valuable products [201]. These LC biomasses can be utilized to produce various organic chemicals, like ethanol [204,205], butanol [206,207], acetone [206, 208], bio-methane [209–211], bio-hydrogen [212,213], citric acid ([214]; Khosravi-Darani et al., 2008), acetic acid [215,216], fumaric acid [217], lactic acid [218], xylitol [219] etc. Specifically, lignin can be used to produce aromatic compounds; whereas, ethanol, which is produced by the fermentation of sugars (generated from biomass saccharification) is used to produce low-molecular weight aliphatic compounds [202]. Thus, biomass conversion into different industrial products is an environment sustainable and cost-effective method.

7.1. Bioenergy

According to data of U.S. Energy Information Administration (EIA), the requirement of world energy continues to increase and a 56 % increase in the energy consumption will be seen between 2010 and 2040 throughout the world [220]. It is very difficult to achieve the demand of world energy consumption of 154–240 quadrillion Watt-hour from diminishing fossil fuel reserves. Thus, conversion of LC biomass to bio-fuels is a sustainable alternative to fossil fuels [221]. In conclusion, it is abundantly available, renewable resource to produce second-generation bio-energies, like bio-ethanol, bio-butanol, bio-hydrogen, and bio-methane. Due to renewable nature of LC biomass, it has gained researchers interest and is regarded as crucial, inexhaustible, and economical renewable resources found in nature [70]. Demand of renewable fuels is increasing tremendously and it is anticipated to rise by 3.4 times by 2035 [222]. A major proportion of petroleum-based fuels in transportation (~30 %) would be replaced by renewable fuels by 2025 as per the reports of US Department of Energy (DOE) [223].

7.1.1. Bioethanol

Presently, in comparison to others, ethanol is the most promising bio-fuel due to high heat of vapourization and energy density, also it has low emission of carbon dioxide. The production of bio-ethanol from LC biomass is an environmentally economic process [224]. Bioethanol production is increasing rapidly and it is replacing the fossil fuels. For bioethanol production, LC feedstocks, like agricultural, forest residuals, and industrial wastes are used widely as they are cost-effective and available abundantly [224,225]. Presently, ethanol has the vast market because of its utilization as a chemical feed-stock or primary fuel or as a fuel additive [226]. The conversion of LC biomass into bio-ethanol mainly consists of 3 steps. In the very first step, pre-treatment of biomass is done to make hemicellulose and cellulose easily accessible for hydrolysis by breaking the rigid LC structure [225]. Several chemical, biological, and physical methods of pretreatment are there for the disintegration of LC biomass [227]. The middle step is performed by the action of lignocellulolytic enzymes to hydrolyse holocellulose into easily fermentable sugars. In last step, fermentable sugars obtained after the enzymatic hydrolysis are converted into ethanol [228].

Key step in ethanol production is enzymatic hydrolysis. For efficient hydrolysis, there is a need of cocktail of enzymes which can degrade cellulose and hemicellulose into fermentable sugars. The promising candidate for thermostable cellulolytic enzyme production is *M. thermophila* [37]. Fermentation of sugars is done by various

Table 1
Heterologous expression of *M. thermophila* genes encoding hydrolytic enzymes.

Enzyme	Heterologous host	Vector	Molecular mass (kDa)	Optimal activity at pH	Optimal temperature (°C)	Enzyme production	References
β-glucosidase	<i>E. coli</i>	–	100	5.0	55	–	[195]
Cellobiohydrolase	<i>E. coli</i>	–	45	10.0	60	–	[194]
NAD ⁺ -dependent formate dehydrogenase(s)	<i>Escherichia coli</i>	pET45b(+)	42	10.5	48	–	[340]
Endoglucanase	<i>P. pastoris</i>	–	47	10.0	50	–	[193]
Endoglucanase	<i>P. pastoris</i>	pPICZαC	75	5.0–6.0	70	53 U/ml	[166]
Endoglucanase	<i>P. pastoris</i> X-33	pPICZαC	65	5.0	60	468 U/ml	[19]
Xyloglucanase	<i>P. pastoris</i>	pPH93-mt74syn	–	6.5	70–75	118 U/ml	[341]
Endomannanase	<i>P. pastoris</i>	–	60	6.0	60	–	[24]
β-glucosidase	<i>P. pastoris</i>	pPIC9	130	5.0	60	15 U/ml	[342]
Peroxidase	<i>P. pastoris</i> X-33	pPICZαC	50	4.0	60	0.41 U/ml	[343]
β-glucosidase	<i>Pichia pastoris</i>	pPICZαC	90	5.0	70	41 U/ml	[192]
Feruloyl esterase	<i>P. pastoris</i> X-33	pPICZαC	39	7.0	50	0.93 U/ml	[122]
β-mannosidase	–	–	97	5.3	40	–	[58]
β-mannanase	–	–	48	5.2	69	–	[58]
α-galactosidase	–	–	60	5.0	60	–	[58]
Glucouronoyl esterase	<i>P. pastoris</i> X-33	pPICZαC	43	7.0	55	0.0052 U/ml	[129]
Laccase	<i>Aspergillus oryzae</i>	pRaMB5	85	6.5	–	11–19 mg/l	[21]

Table 2

Genetic engineering strategies to enhance the production of biomass degrading enzymes from *M. thermophila*.

Strategy used for genetic engineering of <i>M. thermophila</i>	Outcomes of improved strain	Reference
Deletion of 4 major factors involved in glucoamylase expression and secretion in <i>M. thermophila</i> by using AsCas12a variants	Glucoamylases activity increased by 1.85-fold	[175]
3 cytosine base editors (CBEs) were constructed in <i>M. thermophila</i> . Mtevo-CDA1 editor was used to investigate the function of each motif of the cellulase transcription factor <i>M. thermophila</i> CLR-2 (MtCLR-2)	Fungal-specific motif of MtCLR-2 was found to be strongly involved in cellulase secretion, conidium formation, hyphal branching, and colony formation	[198]
Using CRISPR-Cas9, a series of single- and multi-gene mutants of 5 selected protease genes were constructed	Δ Mtalp1 and quintuple mutant showed significantly lower protease activity, decreased by 52.7 % and 58.4 %, respectively and at least double enhanced cellulase production, compared with wild type	[174]
Metabolic engineering of <i>M. thermophila</i> for ethanol production by introduction of ScADH1 into wild-type strain of <i>M. thermophila</i> , overexpression of glucose transporter (GLT-1) or cellobiosyl transferase (CDT-1/CDT-2) from <i>Neurospora crassa</i> and turned down expression of <i>pyc</i> gene	Ethanol production was increased by 57 % from glucose but not cellobiose after introduction of ScADH1, by 131 % from glucose or by 200 % from cellobiose, respectively after overexpression of glucose and cellobiosyl transferases, and by 23 % in final engineered strain	[344]
Bicistronic vector was constructed for co-expressing two heterologous genes <i>MhglA</i> and <i>egfp</i> . Obtained strain OE- <i>MhglA-egfp</i> was genetically engineered by CRISPR/Cas9 to improve glucoamylase activity	Protein productivity and amylase activity were increased by ~12- and 8.2-fold, respectively, compared with wild type	[187]
CRISPR/Cas9 tool and medium optimization were used to improve <i>M. thermophila</i> trehalase (MthT) production in <i>Aspergillus niger</i>	1698.83 U/mL production of trehalase was obtained with optimum activity of recombinant trehalase at pH 5.5 and 60 °C	[345]
Metabolic engineering of <i>M. thermophila</i> for fumarate production using CRISPR/Cas9 system	A 3-fold increase in titres of fumaric acid is observed after introducing <i>Candida krusei</i> fumarase into <i>M. thermophila</i>	[30]
A recombinant plasmid containing RNAi sequence for <i>mhr1</i> , which is pUC19-P _{ppdc} - <i>mhr1</i> -T _{ppdc} was constructed and transformed into <i>M. thermophila</i>	Cellulase and xylanase activities were repressed by the transcription factor MHR1 of <i>M. thermophila</i> . Silencing of <i>mhr1</i> resulted in increased expression of main cellulase genes and positive regulatory gene <i>xyl1</i>	[189]
Genes of cellulase production pathway (<i>cre-1</i> , <i>res-1</i> , <i>gh1-1</i> , and <i>alp-1</i>) were selected as targets for editing. Using CRISPR/Cas9 system, simultaneous multigene disruptions were accomplished with neomycin selection marker integration	Lignocellulase activities and extracellular secreted protein were significantly increased (compared with parental strain) by 13- and 5-fold, respectively	[29]
CRE1 silencing using RNA interference (RNAi)	FPase and β -1, 4-endoglucanase activity in <i>cre1</i> -silenced strain C88 were 3.76- and 1.31-fold higher, respectively	[188]
Overexpression of <i>MtXyl1</i> under the control of <i>MtPpdc</i> (pyruvate decarboxylase) promoter	Extracellular xylanase activities of transformant cultured were 25.19- and 9.04-fold higher in non-inducing and inducing	[191]

Table 2 (continued)

Strategy used for genetic engineering of <i>M. thermophila</i>	Outcomes of improved strain	Reference
Using high efficiency transformation and targeted gene disruption system for <i>M. thermophila</i> ATCC 42464, a <i>ku70</i> deletion strain was constructed using <i>Agrobacterium tumefaciens</i>	media, respectively, compared to wild type Accelerated mechanism of cellulose degradation	[28]

microorganisms, like *S. cerevisiae*, *Candida shehate*, *Candida glabrata*, *Kluyveromyces marxianus*, *Pichia stipitis*, *E. coli*, *Zymomonas mobilis* etc. [229].

Earlier, fermentation of ammonia pre-treated sugarcane bagasse hydrolysate was carried out by *P. stipitis* and *S. cerevisiae* individually and in combination, which followed in 23.56, 24.56, and 31.56 g/L bio-ethanol, respectively at 150 rpm and 30 °C after 72 h [230]. Moreover, 28.88 g/L and 27.18 g/L of bio-ethanol was generated by *S. cerevisiae* and *P. stipitis* in separate hydrolysis and fermentation (SHF) process from ammonia pretreated rice straw [95]. In another study, sulfuric acid pre-treatment of sugarcane bagasse pith led to 3.7 g/L bioethanol in simultaneous saccharification and fermentation [231] and 18.07 g/L bioethanol was generated from the hydrolysate obtained from sodium carbonate pre-treated rice straw followed by saccharification using *M. thermophila* [232]. In a study by Bala and coworkers, enzymatic cocktail of *M. thermophila* was used for the steam-pretreated rice straw hydrolysis, which resulted in 184.59 mg/g of reducing sugars after 1 day that is further used to produce bio-ethanol [18]. Further, recombinant cellobiohydrolases (rMtCel6A) from *M. thermophila* finds utility in bio-ethanol production [194]. Lastly, in a work by Pereira and co-workers, the saccharification of ozone pretreated sugarcane bagasse using enzymes from *M. thermophila* JCP1-4 resulted in remarkable glucose (4.99 g/L) release at 60 °C after 8 h of hydrolysis by 10 FPU enzyme load. Further, fermentation of hydrolysates using *S. cerevisiae* gave ~63 % of glucose-to-ethanol conversions [233].

7.1.2. Biobutanol

Biobutanol has the potential to replace gasoline; therefore, it is considered as future biofuel. In terms of energy density, safety, and engine compatibility, 1-butanol is advantageous over ethanol [234, 235]. It is also a vital pre-cursor for polymers, plastics, and paints. In comparison to ethanol, it has high energy, so now-a-days it is preferred in transport industry. Recently, efforts to synthesize butanol from LC biomass have been considered at industrial scale [236]. The generation of bio-butanol by acetone-butanol-ethanol (ABE) fermentation is done by distinct members of *Clostridium* genus: *C. acetobutylicum*, *C. perfringens*, *C. beijerinckii*, *C. sporogenes*, *C. aurantibutylicum*, *C. butylicum* etc. [229]. However, recent development of efficient gene manipulation tools for *M. thermophila* makes this fungus a potent candidate for the heterologous production of butanol [237]. The generation of butanol using several agricultural substrates, like molasses, cassava, whey permeates, potato, and corn etc. have been carried out traditionally. But these biomasses compete with food supply and they are costly; therefore, LC biomass is the potential alternative source for sustainable and economical generation of bio-butanol [235,238]. Various LC substrates, like palm kernel cake, wheat bran, corn stover, wood chips, wheat straw, rice straw, and rice bran have been used to produce biobutanol.

Specifically, 6.86 g/L butanol was shown by Su et al. [239] in simultaneous saccharification and fermentation using combinatorial pre-treatment (microwave, milling, and liquid hot water (LHW)) by *C. beijerinckii* NCIMB 8052. 4.62 g/L of butanol was reported by oxidative ammonolysis pretreated sugarcane bagasse by *C. acetobutylicum* ATCC 824 [240]. In a work by Pang and coworkers, almost all cellulose and hemicellulose in alkali-pre-treated sugarcane bagasse were hydrolysed using enzymes obtained from *Thermoascus*

aurantiacus strain QS 7-2-4. 14.17 g L⁻¹ of butanol was produced after 60 h when the hydrolysate was subjected to fed-batch fermentation carried out by the *Clostridium acetobutylicum* GX01 [241].

7.1.3. Biomethane

Biomethane is a potential fuel because it yields more energy in comparison to any other biofuel, such as bio-ethanol and bio-diesel. Organic materials are converted into biogas in the absence of oxygen by a microbial process called as biomethanation or anaerobic digestion. Mainly, biogas contains carbon dioxide and methane, which is used for the heat and electricity production for local needs [242,243]. Bio-gas can be utilized as a fuel for vehicles, for this, bio-gas is refined and up-graded to bio-methane for combustion in internal engine [244]. Various studies reported that the production of bio-methane can be done from LC biomass, like rice straw, paper and pulp sludge, wheat straw, soft-wood spruce, sunflower stalk, corn stover, and fallen leaves [229]. Further, wood-to-methane 3 + 2 (W2M3+2) industrial approach involves fermentation using microbes or a thermophilic anaerobic digestion of wood waste to biomethane along with other products, like nitrogen and potassium biofertilizers etc. The process show similarity with analogous process happening in lower termites. The major difference between two processes is that the former operates at thermophilic temperatures (55–70 °C); thus, it depends on thermophilic fungi with other thermophilic microbes, like archaea and bacteria. Thermophilic cellulolytic fungi *Thermoascus aurantiacus*, *Sporotrichum thermophile*, and *Chaetomium thermophile* var. *coprophile* along with other thermophilic fungi are engaged industrially to convert wood waste into biomethane through W2M3+2 process [245].

7.1.4. Biohydrogen

For future, biohydrogen is considered as a possible energy alternative because it is renewable and clean source of energy, as combustion of biohydrogen generates water as the only product. Also, it possesses higher energy in comparison to fossil and hydrocarbon fuels [246,247]. It is a carbonless fuel that produces water as a byproduct after combustion. Also, when compared to hydrocarbon fuels, hydrogen has three-fold high energy density [248] and has been observed to be involved in future energy needs. Because of its distinctive properties, such as less toxicity, colourless, odourless etc. hydrogen has gained interest of researcher's. To satisfy demands of hydrogen in future, it can be produced from the biomass having carbohydrates by bacterial hydrogen fermentation [246]. Currently, the generation of hydrogen from renewable and abundant agriculture residues are generally favoured.

Earlier, Ratti et al. [249] described 1.2 mol H₂/g of substrate by using alkaline delignification pre-treated sugarcane bagasse and steam explosion by using thermophilic microbial consortium in a batch reactor at 55 °C. Several mesophilic, thermophilic, and extremely thermophilic microbes, like *C. butyricum*, *Clostridium tyrobutyricum*, *Enterobacter aerogenes*, *Thermotoga neapolitana*, *Thermoanaerobacterium thermosaccharolyticum*, *Ethanoligenens*, *Thermotoga elfii* etc. are used for hydrogen production. Interestingly, both pure and mix-cultures are utilized for LC biomass conversion into hydrogen. Several LC biomasses, like hemp, rice straw, barley straw, corn cob, corn stalk, wheat straw, wheat bran, sweet sorghum bagasse, sugarcane bagasse etc. have been used for hydrogen production [229]. Specifically, a thermophilic bacterium *Caldicellulosiruptor saccharolyticus* have been found as a potential candidate to produce biohydrogen as it produces nearly nil reduced end products, thereby leading to higher hydrogen production. It is known to produce thermostable xylanolytic and cellulolytic enzymes and grow optimally on diverse LC biomass at 70 °C. Also, the use of thermophilic enzymes and microbes provide advantages over existing methods, such as lower costs, increased efficiency, and less contamination if un-treated lignocellulose enzyme-based hydrolysis, saccharification process, fermentation, and product separation can be accomplished under same conditions [250].

7.2. Organic acids

Organic acids are the intermediate components or natural products of the microbial metabolic pathways. These are useful products for synthesis of other valuable chemicals due to the presence of functional groups that makes them a suitable substrate [251]. They are generated by sugars, like glucose, sucrose, and xylose, and enzymatic hydrolysate of LC biomass. Various organic acids are generated by fermenting lignocellulose hydrolysate as substrate [251–253].

Gu and co-workers metabolically engineered *M. thermophila* using CRISPR-Cas9 system to produce fumaric acid. Introduction of *Candida krusei* fumarase into *M. thermophila* led to 3-fold rise in fumaric acid titre. Further, increase in fumarate production was achieved by disrupting intra-cellular fumarate digestion pathway such that the deletion of mitochondrial fumarase and two fumarate reductase genes led to 2.33-fold rise in fumarate titre. Lastly, impedance of the malate aspartate shuttle further raised the extracellular fumaric acid titre by 42 %. Thus, the strategic metabolic engineering led to generation of 17.0 g/L fumaric acid from glucose during fed-batch fermentation [30]. Recently, these authors carried out various metabolic rewiring strategies in *M. thermophila* to enhance malate production and eliminate succinic acid accumulation such that a titre of 182.7 and 115.8 g/L of malic acid was achieved in a 5-litre fermenter using glucose and raw corncob, respectively without accumulation of any by-product [254]. Further according to a study performed by Li and co-workers, for the very initial time, bulk chemicals were generated with titre of more than 100 g/L during fermentation of raw LC biomass in absence of any hydrolytic enzymes [31]. Later, these authors designed a novel biorefinery named as the direct microbial conversion of biomass with CO₂ fixation (DMCC), which can generate 1 ton of malic acid by 1.89 ton of raw plant biomass and fix 0.140 ton of CO₂ [255]. Earlier, these authors reported significant generation of malic acid using metabolically engineered *M. thermophila*. About 180 and 105 g/L was obtained by fermentation of crystalline cellulose and corn cob, respectively [256].

7.2.1. Citric acid

With ~1.70 million tons of citric acid production annually, it is another major fermentation product following ethanol across the world. It is a useful chemical with applications in nanotechnology, bioremediation of heavy metals, bio-medicine [252,257], food and beverage industries [258]. But excessive requirement of citric acid needs a low-cost LC biomass to enhance the microbial efficiency [259]. The use of easily available and low-cost LC agro-residues as suitable substrates to produce citric acid is shown by several reports [252]. Various LC biomass are used for the production of citric acid, like sugarcane bagasse [214,260], apple pomace [206,252], oil palm empty fruit bunch [261], cotton waste [262], cassava bagasse [253], corn husk [263], corn cobs [264], pineapple waste [265], grapes, and pomace [266] etc.

Specifically, *A. niger* is an industrially important species due to being majorly utilized for the generation of citric acid. It has many desirable features, like low pH tolerance, ability to use renewable and cheap carbon sources, high sugar to organic acids conversion efficiency, and a well-established genetic background. With regard to citric acid generation, RG0095 and brIA^{xyIP} produced 21 and 27.50 g/L citric acid, respectively [267]. High citric acid generation (17.50 g/kg of substrate) was reported by Bastos et al. [268] by *A. niger* using vinasse and sugarcane bagasse after 72 h. Various other citric acid producers are *Absidia* sp., *Aspergillus flavus*, *A. awamori*, *A. nidulans*, *A. phoenicus*, *A. wenti*, *Acremonium* sp., *Botrytis* sp., *Penicillium citrinum*, *P. luteum*, *P. janthinellu*, *P. restrictum*, *Trichoderma viride*, and *Talaromyces* sp [226,252,253,257, 269,270].

7.2.2. Acetic acid

Acid-catalyzed conversion of LC biomass leads to production of levulinic acid, formic acid, and acetic acid. Hydrolysis of the acetyl groups found on hemicellulose leads to the production of acetic acid.

Degradation of 5-hydroxymethylfurfural (HMF) and furfural results in formic acid while degradation of HMF give rise to levulinic acid. Furfural is formed by pentose sugars (arabinose and xylose) while HMF is made up of hexoses (glucose, galactose, and mannose [216,271]. Levulinic acid is a base chemical, which is used to produce various chemicals, like succinic acid, γ -valerolactone, pharmaceuticals, herbicides, resin, flavouring agents, and polymers [272]. In a previous study, simultaneous saccharification and fermentation by using *T. reesei* (soft-rot), *Gloeophyllum trabeum* (brown-rot), and *Phanerochaete chrysosporium* (white-rot) produced 1.7, 1.0, and 11.30 g of acetic acid, respectively per 0.1 kg of corn fiber [273].

7.2.3. Lactic acid

It is one the significant organic acid having a wide array of applications in textiles, pharmaceutical, food, and chemical industries [274]. Demand of lactic acid has been increasing worldwide because of its utilization in synthesis of polylactic acid and lactate solvents [275]. Plastics derived from petrochemicals can be replaced by polylactic acid because it is a bio-degradable polymer [276,277]. Lactic acid is produced by microbial fermentation or synthesized chemically. Currently, majority of the lactic acid across world is generated by microbial fermentation because it offers numerous advantages, like generation of optically pure L- or D-lactic acid and low energy consumption. But the production cost is high due to utilization of traditional substrates, like refined sugars and starch. Therefore, the use of LC biomass offers an economical and food non-competitive material to produce lactic acid [276–278]. For microbe associated fermentation of lactic acid, high sugar-rich sources/crops (starch rich feed, cane molasses) have been usually selected [279]. Various LC biomass, like rice bran, sugarcane bagasse, corn cob, wheat bran, paper sludge etc. are utilized for lactic acid production by microorganisms, like *Lactobacillus coryniformis* [280], *L. brevis* [281], *L. casei* [282], *Rhizopus oryzae* [283], and *Streptococcus thermophilus* [282,284].

Acid and alkaline pretreated sugarcane bagasse was utilized by Wischral et al. [285] and 65 g/l of lactic acid was reported by *Lactobacillus pentosus* in batch-fermentation. Similarly, 72.70 g/l of lactic acid by using *L. pentosus* was reported by Unrean [286] in fed-batch fermentation by using sugarcane bagasse. Further, 185 g/l lactic acid by *Bacillus* sp. P38 in fed-batch fermentation in sugarcane bagasse hydrolysate was revealed by Peng et al. [287]. In another earlier work, 22 and 27.3 g/l of lactic acid was obtained from sorghum stalks and corn stover, respectively by using a genetically modified *Lactobacillus plantarum* through simultaneous saccharification and cofermentation process [288]. Karnaouri and co-workers reported 62 and 36.4 g/l d-lactic acid production by *Lactobacillus delbrueckii* subsp. *bulgaricus* (ATCC® 11842) from organosolv pre-treated beechwood and pine via simultaneous saccharification and fermentation process [289]. Further, in a recent work, 210.10 g/l of lactic acid was reported from 40 % of solid-loading corn-stover hydrolysate through simultaneous saccharification cofermentation in 100 mL flasks. However, in 3-litre bio-reactors, 157.40 g/L of lactic acid was generated from 35 % of solid-loading corn-stover hydrolysate. These are the highest lactic acid titres reported till now when LC biomasses were used as substrates [290].

7.2.4. Succinic acid

Succinic acid is a four carbon (C-4) containing di-carboxylic acid. It is a widely utilized chemical in food, pharmaceutical, and agricultural industries. It is a base chemical to produce various upgraded derivatives, such as 1, 4 butanediol, 1, 3-butadiene, adipic acid, and ethylene diamine disuccinate [291–293]. Succinic acid is currently produced commercially by the petroleum based chemical processes. In recent years, succinic acid production through cost-effective LC biomass has attracted interest due to rising oil prices, limited reserves, and environmental pollution [292–294]. Different LC biomasses utilized for succinic acid production till date are sugarcane bagasse hydrolysate [291,295], corn stover [293,296], wood hydrolysate [297], corn stalk

hydrolysate [292,294] and *Cannabis sativa* [298]. By genetic engineering, thermophile *M. thermophila* was constructed into a platform to convert LC biomass into succinic acid [31].

In a previous study, 27.8 g/L succinic acid was produced by *Actinobacillus succinogenes* from corn fiber hydrolysate achieved post liquid hot-water pretreatment and enzyme-based hydrolysis of the corn fiber [299]. Further, Akhtar and Idris [300] evaluated the use of oil-palm empty fruit bunches in succinic acid production through simultaneous saccharification and fermentation by *A. succinogenes* ATCC55618. The substrate was pre-treated using alkali/autoclave and sequential dilute-acid/microwave-alkali and hydrolysed enzymatically, such that best glucose yield was obtained at 7:1 ratio of cellulase and cellobiase. 33.4 g/L succinic acid was produced, thereby suggesting the capability of agricultural waste as an alternate substrate for the efficient and economic generation of the succinic acid [300]. In another work, 17.80 g/L of succinic acid was generated from concentrated phosphoric acid pre-treated sweet sorghum bagasse for 43 min at 50 °C and biomass concentration of 130 g/L on dry-basis by using *A. succinogenes* 130Z and enzymatic hydrolysis with commercial cellulase [301].

7.3. Enhancing nutritional quality of food and feed

For enhancing the properties of vegetables and fruits, pectinases, xylanases, and cellulases are used [302]. The effect of enzyme mixture containing cellulase, pectinase, and xylanase from *M. thermophila* was studied on the yield of fruit juices of apple, grapes, and banana and observed that the yield of juices raised in comparison when pectinase was employed exclusively [45]. Properties of citrus fruits, like flavour, aroma, and texture were also enhanced by enzymes [303]. The quality of dough in bread making was increased by mixing the phytase from *M. thermophila* because it releases higher amounts of inorganic phosphate, reducing sugars, and soluble proteins [178]. The xylanases from *M. thermophila* when mixed with wheat flour in bread making process increased the overall nutritional value as compared with control [96]. Recombinant laccase from *M. thermophila* and chlorogenic acid was used to manage the mal-odour of cysteine. Their results depicted that the enzyme-treated cysteine has a faint odour while the non-treated cysteine has a powerful peculiar H₂S odour. More than 50 % reduction in cysteine was observed in HPLC analysis [304]. *M. thermophila* laccase is used to treat cork stoppers for the wine bottles. Phenols are oxidized by laccases and the liberated phenoxyl radicals go through non-enzymatic homopolymerization in this process; thus, avoiding the production of 2, 4, 6-trichloroanisole, which imparts the cork taste [305].

Laccases from *M. thermophila* was used as a dough conditioner [306]. Redox potential of laccase from *M. thermophila* is ~450 mV; therefore, laccases are used extensively in food industry [307]. Laccases from *M. thermophila* are also used for the colour enhancement in tea [308]. Laccase gene from the fungus *M. thermophila* was heterologously expressed in the host *A. oryzae* and the generated recombinant enzyme was marketed under the trade name Flavourstar, which is used for beer brewing to stop the generation of off-flavoured compounds (trans-2-nonenal) [309]. Further, as stated earlier, malic acid is produced from lignocellulose hydrolysate as substrate by *M. thermophila*, which is widely utilized as an acidulant and flavour-enhancer in food industry [254]. Similarly, succinic acid can be used as flavour enhancer in beverages, as catalyst for food seasoning preparation, and as bread-softening agent [310].

Hemicellulases and cellulases have potential applications in feed industry, where they enhance the nutritional efficacy of the animal feed [311] because they are responsible for greater emulsification of the feed materials [312] and they eliminate the anti-nutritional factors from feed [311]. Further, digestibility of animals is improved after the proper pretreatment of agricultural residues that are utilized as animal feed [313]. Cellulases enhance the silage production and increases the digestibility of the grasses. Also, the nutritional quality of forages is improved by hemicellulases and pectinases [314]. Beside these

enzymes, xylanases and phytases are also widely utilized to enhance the digestibility of the animal feed. In a previous study, wheat-based feed (also having barley and soyabean) for turkeys and broilers, when synergistically supplemented with both phytase and xylanase manifested better hydrolysis of feed with improved digestibility of phosphorous and enhanced body weight of turkeys and broilers [315]. In another study, phytase, β -glucanase, and xylanase together on wheat grain enhanced the degradation of the non-starch polysaccharides and improved the re-distribution of the poly-valent metal ions [316].

7.4. Bioremediation

Bioremediation is the process of removal of drugs, cosmetics, and pharmaceuticals by using microbes and microbial enzymes. Laccases from *M. thermophila* are used to modify a range of xenobiotics [308,317,318], while proteases are used in bioremediation [319]. Possible use of laccase from *M. thermophila* heterologously expressed in *A. oryzae* and immobilized on amino modified nonporous fumed nanosilica (AFNS) in the lindane removal was studied by Bebić et al. [320]. Maximum specific activity of 13.1 IU/mg was attained at 160 mg/g AFNS. In less than 24 h, the lindane concentration was decreased to 56.80 % of starting concentration and following 7 repeated re-uses, the enzyme maintained 70 % of its initial activity [320]. In another study by Vera and co-workers, *M. thermophila* laccase was immobilized on mono-disperse microspheres of poly-(glycidyl methacrylate) or PGMA and utilized in degradation of azinphos-methyl. By using immobilized laccase, the total conversion of azinphos-methyl was attained after 1 h [321]. Further, *M. thermophila* laccase was used in the bioremediation of poly-cyclic aromatic hydrocarbons or PAHs [322]. Laccases obtained from *M. thermophila* and *Trametes versicolor* were used in biodegradation of various steroid hormones, like estriol, 17 α -ethinyl estradiol, and 17 β -estradiol [323] such that laccase from *M. thermophila* is ~10 % more effective than laccase from *T. versicolor* [324]. Moreover, in another study, when laccase (high-redox potential) of *T. versicolor* and *M. thermophila* (low-redox potential but cost-effective) were co-immobilized on fumed silica nano-particles, both laccases together removed over 99 % of bisphenol A (BPA) from secondary effluents of a waste-water treatment plant [325].

Fungal enzymes have significant role in increasing textile processing and valorizing the waste created from the streams of clothing, fibers, and textile-manufacturing procedures to the valuable products. Xylanases are the most used enzymes in textile processing at various stages, such as bio-scouring, desizing, bio-finishing, and bio-bleaching of the cellulosic fabrics to enhance the quality of textiles [326]. Cellulases are mainly used in finishing of cellulose-based clothing textiles, with an objective of better-quality products [327,328]. The thermophilic cellulases are used normally in bio-polishing of cotton and bio-stoning of denims [329]. Further, cellulases are also used in detergents. Due to repeated washing of cotton-blended clothes, they are likely to be less attractive. Cellulases of detergents will protect the microfibrils of these clothes. In addition to cellulases, lipases [330] and proteases [319] are also used in detergents. Specifically, a recombinant cellobiohydrolases (rMtCel6A) from *M. thermophila* finds utility in textile industry [194] and a serine and alkaline protease from *M. thermophila* is utilized in detergent and leather industry [331].

Su et al. [332] coated textile fabrics with poly(p-phenylenediamine) and poly(catechol) by using *M. thermophila* laccase, such that the coated fabrics showed antioxidant character and antimicrobial activity against gram-positive as well as gram-negative bacteria, indicating it as a potential approach to produce-colored antimicrobial textiles with vast applications. Similarly, *M. thermophila* laccase was utilized by Kim et al. [333] for catechol oxidation and polymerization, which resulted in high-molecular weight poly-catechol for flax-fabrics dyeing. Better color fixation was observed compared to other laccase-mediator systems (e.g., laccase with HBT) or enzyme solely. In contrast, laccases from *M. thermophila* were also found to decolorize synthetic dyes [334]. For

instance, laccase from *M. thermophila* was im-mobilized with epoxy-functionalized silica and used in dye decolorization of dyes from textile industry [335]. Similarly, purified (recombinant) laccases from *M. thermophila* i.e., MtL was utilized for the decolorization of textile waste-water and commercial dyes samples [109]. Further, laccases from *M. thermophila* were checked for de-colourization of 4 flexo-graphic inks along with the synthetic and natural mediators [336]. Also, laccases are used for denim bleaching and finishing [308].

Further, laccases from *M. thermophila* are also used in detoxification of pretreated materials. The phenolic compounds derived from the lignin degradation are oxidized by them. They are also used in pulp and paper industry [337]. In addition to this, a recombinant cellobiohydrolase (rMtCel6A) from *M. thermophila* is also utilized in pulp and paper industry [194]. Additionally, MtCUT (a chitinase) from *M. thermophila* could degrade polyvinyl acetate, which is commonly used in paper-making and in synthetic ink or toner. The ink removal efficiency of 81.3 % and 78.4 % on newspaper and laser-printed paper, respectively was achieved at 30 °C by using MtCUT [338]. Moreover, microbial cellulases are used in the fermentation processes to make beer and wines [339] and proteases from *M. thermophila* are used in leather industry [319].

8. Conclusion and future prospects

The production of liquid bio-fuels from solid cellulosic biomass by microbial conversion provides a renewable energy source. In this regard, cellulolytic fungi are a potential group producing a variety of cellulolytic enzymes. Specifically, *M. thermophila* is a promising filamentous fungus, which produces a complete set of plant cell-wall degrading enzymes. Genome of it has been sequenced and annotated recently, for systematic examination of LC biomass degrading enzymes. It is used as potential source of thermophilic enzyme production in industries. The cellulolytic activity of *M. thermophila* is many times higher than most of the mesophilic fungi counterparts. Also, enzymes from thermophilic mould tends to be much more stable at increased temperatures than mesophilic counterparts. In conclusion, *M. thermophila* is an effective platform for industrial production of enzymes and value-added products of the biotechnological importance and due to the thermostability, its enzymes can be effectively used at elevated temperatures.

Myceliophthora thermophila possess a distinguishing feature of rapid depolymerization of LC material. However, the underlying genetics and precise biochemical mechanisms of procedure are not fully realized. Systematic examination of genome of *M. thermophila* depicted that fungus consists of a distinctive enzymatic system consisting of a peculiar repertoire of the enzymes, specifically those involved in the biomass degradation. The present review represents the lignocellulolytic potential of *M. thermophila*. Genomic data of this fungus suggests that it consists of 66 hemicellulases, 30 cellulases, and 35 proteins as auxiliary enzymes, consisting of almost all recognized CAZy families. Further, genetic-engineering studies have enhanced the generation of enzyme for industrial applications.

CRediT authorship contribution statement

Bijender Singh: Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shruti Ahlawat:** Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Partiksha Yadav:** Writing – review & editing, Data curation, Conceptualization. **Anu:** Writing – review & editing, Software, Formal analysis, Data curation. **Astha Chhillar:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Davender Singh:** Writing – review & editing, Software, Methodology, Data curation, Conceptualization.

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