The effect of a lytic polysaccharide monooxygenase and a xylanase from *Gloeophyllum trabeum* on the enzymatic hydrolysis of lignocellulosic residues using a commercial cellulase

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### Abstract

Hydrolysis of lignocellulosic biomass depends on the concerted actions of cellulases and accessory proteins. In this work we examined the combined action of two auxiliary proteins from the brown rot fungus *Gloeophyllum trabeum*: a family A9 lytic polysaccharide monooxygenase (GtLPMO) and a GH10 xylanase (GtXyn10A). The enzymes were produced in the heterologous host *Pichia pastoris.* In the presence of an electron source, GtLPMO increased the activity of a commercial cellulase on filter paper, and the xylanase activity of GtXyn10A on beechwood xylan. Mixtures of GtLPMO, GtXyn10A and Celluclast 1.5 L were used for hydrolysis of pretreated wheat straw. Results showed that a mixture of 60% Celluclast 1.5 L, 20% GtXyn10A and 20% GtLPMO increased total reducing sugar production by 54%, while the conversions of glucon to glucose and xylan to xylose were increased by 40 and 57%, respectively. This suggests that GtLPMO can contribute to lignocellulose hydrolysis, not only by oxidative activity on glycosidic bonds, but also to hemicellulose through the oxidation of xylosyl bonds in xylan. The concerted action of these auxiliary enzymes may significantly improve large-scale recovery of sugars from lignocellulose.

### 1. Introduction

Utilization of lignocellulosic residues provides an opportunity to advance sustainable human development. The complex structure and recalcitrance of lignocellulose makes the derivation of bio-products from these residues technically challenging. Despite important manufacturing advances [1], it is still necessary to identify efficient enzyme complexes that will increase the speed of biomass degradation.

Wheat straw is one of the most abundant agricultural residues available, Chile alone generates 1.5 million tons every year [2]. Currently, various groups are investigating alternative uses of this raw material which is composed of 33–40% cellulose, 20–25% hemicellulose and 15–20% lignin [3]. The relatively low level of lignin in wheat straw, compared to that in woody biomasses, suggests that its degradation should be a simpler process. Furthermore, the composition of the hemicellulosic fraction also differs from that of woody biomasses, primarily consisting of xylans in hardwoods and straw as opposed to galactomannans in softwoods [4]. Xylans from different sources differ in composition as well. Hardwood xylans contain primarily xylose and glucuronic acid, whereas grass xylans contain up to 33.5% (w/w) arabinose [5]. The nature of the acetylated polymer and the extent of acetylation also differs between plant species. About half of the β-o-xylopyranosyl units in hardwood hemicellulose are acetylated moieties [6], while only 13% and 1.7% (w/w) in cottonwood and wheat straw, respectively [7]. These differences are relevant because acetyl-substituents inhibit the enzymatic degradation of wall polymers and the released acetate can be a potent inhibitor in microbial fermentation.

The ascomycete *Trichoderma reesei* (teleomorph *Hypocrea jecorina*) is a widely used industrial fungi due to its capacity to produce cellulosytic enzymes [8]. The *T. reesei* cellulosytic system has an abundance of endo-acting cellulase (EC 3.2.1.4, endoglucanase) and exo-acting cellulase (EC 3.2.1.91 and EC 3.2.1.176, cellobiohydrolase). Despite this, the *T. reesei* genome has a relatively low number and diversity of carbohydrate-degrading enzymes [9]. For example, *T. reesei* must be supplemented with β-glucosidase in order to yield glucose [10,11]. Accessory enzymes help remove barriers to cellulose hydrolysis, assisting in plant biomass degradation [12,13]. This group includes hemicellulases, lytic polysaccharide monooxigenases, expansin-like proteins, ligininas and oxidases, among others. Endoxylanases from glycoside hydrolase family 10 (GH10) [14] are the most used due to their ability to hydrolyze...
highly substituted xylose linkages [15]. Several reports highlight the synergy between cellulases and xylanases [16,17] as an essential tool for complete hemicellulose removal and efficient carbohydrate conversion.

Lytic polysaccharide monoxygenases (LPMOs, former GH61s) contribute to cellulose deconstruction by completely different mechanisms than glycoside hydrolases [18–20]. LPMOs are metal ion dependent monoxygenases that use the ion metal copper (II) for enzyme activation [21–23]. The discovery that GH61s are copper-dependent polysaccharide monoxygenases ultimately led to the reclassification of this family to an Auxiliary Activity family number 9 (AA9) in the Carbohydrate-Active Enzymes Database (CAZY) [24]. AA9 enzymes depend on reducing cofactors for activity [22], accomplished by ascorbic acid and other reductants for in vitro reactions and by cellobiose dehydrogenase (CDH) for in vivo reactions [25]. LPMOs are interesting from both fundamental and practical points of view as they increase the activity of cellulases on pure crystalline cellulotic substrates [22,25–29] as well as on real lignocellulosic substrates [21,30–32].

Unlike T. reesi, brown rot fungi such as Gloeophyllum trabeum have many genes encoding proteins with accessory activity [33]. They metabolize the hemicellulose and cellulose in wood by a two-step mechanism comprising non-enzymatic and enzymatic reactions, finally producing a brown lignin residue [34]. The non-enzymatic reactions involve wood cell wall decomposition by oxidative mechanisms, in which hydroxyl radicals selectively depolymerize cellulose [35]. Subsequently, cellulases and hemicellulases complete the breakdown of the carbohydrates [36]. For this purpose, G. trabeum secretes a battery of enzymes, several of which have been produced by recombinant methods. For example, the endoglucanase Cel5A is unusually active on microcrystalline cellulose (Avicel) [37], which probably compensates for the absence of cellobiohydrolases in this fungus. Endoxylanases GtXyn10A and GtXyn10B are thermostable enzymes [38], a property that makes them very useful in the hemicellulose processing industry. Furthermore, β-Glucosidase [39], alcohol oxidase [40,41] and two polysaccharide monoxygenases, one of which is active on xyloglucan [31,42], have been also identified. Despite their potential, G. trabeum enzymes are not widely used as cellulase cocktail additives to increase saccharification efficiency. In our laboratory, we have identified protein fractions from G. trabeum cultures that improve the hydrolysis of wheat straw by cellulases. The most abundant proteins in these extracts were the xylanase GtXyn10A and the polysaccharide monoxygenase GtLPMO (unpublished results). This suggests that these enzymes are important contributors towards the efficiency of G. trabeum’s cellulolytic system.

The objective of this study was to examine the auxiliary effects of GtXyn10A and GtLPMO, mixed with commercial cellulase, in the degradation of wheat straw. The effect of different proportions of these enzymes was examined, finding that the efficiency of the hydrolysis can significantly increase at optimal concentrations. Additionally, these experiments suggest that G. trabeum GtLPMO not only cleaves cellulose as previously reported, but also degrades xylose by a mechanism that is dependent on the presence of a reducing factor. GtLPMO activity on xylan can therefore be used to increase the efficiency of sugar recovery from lignocellulosic biomasses.

2. Material and methods

2.1. Fungus cultivation and maintenance

The fungus Gloeophyllum trabeum isolate CTB (925-B) was isolated from a native Chilean forest by members of the Laboratory of Biodegradation and Wood Preservation at the Faculty of Forest Sciences of the University of Chile. The fungus was maintained in Potato Dextrose Agar (PDA) plates at 4 °C and transferred onto fresh plates every month. For cellulolytic enzyme production, the fungus was cultured at 28 °C in liquid medium as reported by Chouiter et al. [43], except that 0.005 g/L MgSO₄ was used. The medium contained 1% (w/v) milled wheat straw as the only carbon source.

2.2. Wheat straw pretreatment

Wheat (Triticum aestivum) straw was obtained from a local farm in the Maule district of Chile. The biomass was physically processed using a SM2000 cutting mill. Pretreatment was conducted in 10% (w/v) NaOH for two hours at 80 °C [44]. After extensive rinsing with distilled water, the biomass was dried overnight at 50 °C and sieved through a 0.5 mm mesh. The composition of the pretreated wheat straw was determined following the National Renewable Energy Laboratory (NREL) protocols [45]. Glucose and xylose quantification was carried out by high performance liquid chromatography in a Shimadzu Prominence chromatograph, model 20A, equipped with a Refraction Index Detector and a Biorad Aminex HPX-87P column.

2.3. G. trabeum RNA isolation and cDNA synthesis

The fungus was grown on 1.5% (w/v) agar plates containing the medium previously described [43] except that 3% (w/v) milled wheat straw was used as the only carbon source. The plates with the solid medium were covered by a sterile nitrocellulose membrane and inoculated with one square centimeter agar of fresh fungal mycelia previously grown in a PDA medium [46]. After 8 days at 28 °C, the mycelium covering the entire plate was scraped with a scalpel and used as the starting material for RNA extraction. The RNA extraction was carried out using the Ambion® TRIZOL® Reagent following the manufacturer’s instructions. cDNA synthesis was prepared using Superscript III Reverse Transcriptase (Invitrogen) following the manufacturer’s instructions.

2.4. Isolation and cloning of the GtLPMO and GtXyn10A genes

The sequences encoding for GtLPMO and GtXyn10A were identified from the G. trabeum genome sequence available in the JGI database (http://genome.jgi.doe.gov/Glotr1_1/Glotr1_1.home.html) [33,47]. For cloning the GtLPMO gene, the encoding sequence was isolated from the G. trabeum cDNA by a two-step PCR. In the first step, specific

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Table 1

<table>
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<th>Primer name</th>
<th>Sequence (5’–3’)</th>
<th>Purpose</th>
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<td>GAXylFfor</td>
<td>GAAACGTATTTCTAAGACCTTCACCG</td>
<td>Cloning GtXyn10A in pGemTeasy</td>
</tr>
<tr>
<td>GAXylRrev</td>
<td>TTTCCACTGCGCCACCGAC</td>
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<tr>
<td>XGT1FwEcoRI</td>
<td>GAAATTCGACCCTCTCTCCGCCGC</td>
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<tr>
<td>XGT1Rev AvrRI</td>
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<tr>
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<td>Gt61R2</td>
<td>CGCCGGCGTCAATGATGATGATGATG</td>
<td>Cloning GtPMO in pPIC9k</td>
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primers GH61F1 and GH61R1 (Table 1) were used to amplify the part of the gene corresponding to the predicted mature enzyme (i.e. without secretion signal). The amplified fragment was ligated into the pGEM-T Easy vector (Promega, Madison, WI, USA) and transformed into E. coli strain Top10. This construct was used as a template in a second PCR with primers GH61F2 and GH61R2 (Table 1), which contained restriction sites for EcoRI and NotI, respectively. The presence of the expected sequence elements was confirmed by sequencing by Macrogen (Korea). The recombinant fragment was recovered from the pGEM-T Easy vector by EcoRI and NotI restriction and cloned into pPIC9 K using standard DNA cloning procedures. All the PCRs were performed using Phusion High-Fidelity DNA polymerase (New Englands Biolabs), according to the provider’s guidelines.

For cloning the GTXyn10A gene, primers GAXyl1For and GAXyl1Rev were used in the amplification of the entire gene from the cDNA (Table 1). The fragment was ligated to the pGEM-T Easy vector and transformed into E. coli Top10. The recombinant plasmid produced was used as template for a PCR with primers XGT1FwEcoRI and XGT1RevAvrII in order to amplify the sequence encoding for the mature enzyme. The PCR product was restricted with EcoRI and AvrII and ligated to pPIC9 K vector digested with the same restriction enzymes. DNA sequencing (Macrogen, Korea) confirmed the integrity of all the recombinant constructs.

2.5. Recombinant expression and purification of GtLPMO and GtXyn10A

Production of recombinants was carried out using the Multicopy Pichia Expression Kit (Life Technologies), according to the supplier’s directions. Sall-digested recombinant pPIC9 K plasmids were introduced into Pichia pastoris KM71 by electroporation. Transformants were selected in RDB agar plates and then in YPD plates supplemented with 0.25–2.0 mg/mL geneticin. Clones exhibiting the highest resistance to geneticin were preserved at –80 °C as 20% glycerol stocks. For protein production, the stocks were spread on YPD agar plates and isolated colonies were cultured in BMGY (Buffered Complex Medium containing Glycerol) until OD600 = 6. For the induction phase, cells were transferred to BMMY medium (Buffered complex Medium with 0.5% (v/v) methanol) and cultured for 4 days at 25 °C at 300 rpm. Proteins in the supernatant were concentrated by ammonium sulfate precipitation. Reducing sugars were measured with the DNS method described previously. The values of absorbance at 550 nm were converted to xylose reducing equivalents using a calibration curve made with xylose standard.

All the activity assays were carried out using purified GtLPMO and Gtxyn10A. All the experiments were conducted at least three times.

2.7. Wheat straw hydrolysis

Gtxyn10A (10–40 μg/mL) and GtLPMO (4–20 μg/mL) were combined with Celluclast 1.5 L (Sigma; 0.454 FPU per mg protein) in hydrolysis reactions containing 2% (w/v) dry matter (DM) of pretreated wheat straw. The hydrolysis reactions were carried out in 50 mM sodium acetate buffer pH 5.0, 5 g/L Tween 20, with orbital agitation (300 rpm) at 50 °C for up to 48 h. Celluclast 1.5 L and the auxiliary enzymes were included in concentrations that varied depending on the particular experiment. β-Glucosidase from Aspergillus niger (Sigma) was maintained at 30% of the Celluclast 1.5 L concentration to ensure rapid and complete conversion of cellobiose to glucose. All of the mixtures were supplemented with bovine serum albumin (BSA) to complete 2 mg protein per gram of DM. Reducing sugars were measured using the DNS method. Glucose and xylose were determined enzymatically with kits obtained from Randox Laboratories and Megazyme, respectively, following the providers’ directions. Relative increase in the production of each sugar was calculated as the experimental value, divided by the value obtained in an experiment with Celluclast 1.5 L alone, at the same concentration and performed in parallel.

2.8. Statistical analysis

The results were subjected to Student’s t-test analyzes using Minitab 17, and values of P < 0.05 were considered statistically significant.

3. Results and discussion

3.1. Recombinant expression of GtLPMO and GtXyn10A in Pichia pastoris

The gene sequences of Gloeophyllum trabeum GtLPMO and GtXyn10A were isolated from the G. trabeum cDNA. In each case, the gene sequence encoding for the mature part of the enzyme was fused to the alpha factor signal peptide present on the expression vector pPIC9K. The recombinant plasmid was transferred into Pichia pastoris and the protein was expressed in the culture’s extracellular fraction after methanol induction. The separation process produced highly purified proteins (Fig. 1). Gtxyn10A was produced at 22 mg per liter of culture, while GtLPMO was produced at 1 mg per liter. Gtxyn10A migrated close to 38 kDa in SDS PAGE as was predicted from the sequence. On the other hand, GtLPMO produced a 50 kDa protein, almost twice the size predicted from the sequence; however, after deglycosylation with Rapid™PNGase F, the protein migrated close to the expected 27 kDa (see lanes 2 and 3 in Fig. 1). This result shows that GTLPMO produced in P. pastoris is glycosylated, mainly modified with N-linked oligosaccharides.

3.2. GtLPMO auxiliary activity in model substrates

The auxiliary activity of the recombinant GtLPMO was evaluated by measuring its effect on the hydrolysis of filter paper (FP) by cellulase. Celluclast 1.5 L, a LPMO-free commercial mixture, was used as source of cellulase activity. Results in Fig. 2 indicate that GtLPMO improves the FP hydrolysis in a dose-dependent manner, reaching 60% improvement with 3.2 μg/mL, which corresponds to 20% of the total protein load. The improvement was observed only in presence of 1 mM ascorbic acid. In the absence of Celluclast 1.5 L, virtually no production of reducing sugars was observed. Similar experiments were carried out on carboxymethyl cellulose, resulting in no effect of GtLPMO on the Celluclast 1.5 L activity, whether in presence or absence of ascorbic
demonstrated that when ascorbic acid is included in the reaction, a small but significant amount of reducing sugar is produced (Fig. 3B). The magnitude of reducing sugars production depends on the GlLPMO concentration in the reaction. In the absence of ascorbic acid (Fig. 3C), there was no production of reducing sugars, even after 18 h incubation. This suggests that the reducing sugars in Fig. 3A and B are not the result of residual hydrolytic contamination with an endoxylanase activity in the GlLPMO preparation.

Based on the sequence similarity and the product specificity, LPMOs can be classified into at least three types [49]. In a similar comparison we included GlLPMO in the amino acid sequence alignment and found that it shares several structural features with type 3 LPMOs (Supplementary Material S1). Type 1 and type 2 LPMOs hydroxylate C1 or C4 respectively, while type 3 LPMOs hydroxylate both C1 and C4. Based on the proposed cleavage mechanism of gluco-oligosaccharides in C4 [23,50,51], oxidation of carbon 4 occurs simultaneously with the production of a reducing end at C1 [52,53]. This may explain the dose-dependent production of reducing sugars with GlLPMO exclusively in presence of ascorbic acid, as seen in Fig. 3B and C. Detailed characterization of the xylan cleavage reaction products is needed in order to complete our understanding of current results.

3.3. GlLPMO auxiliary activity on wheat straw

Firstly, the sugar composition of the wheat straw was determined. The alkali-pretreated biomass was completely hydrolyzed with sulfuric acid and the main monomeric sugars and lignin were quantified. Results showed that the after pretreatment with 10% NaOH the wheat straw was 83.3% (w/w) glucose, 6.5% (w/w) xylose, 0.57% (w/w) arabinose and 10% (w/w) total lignin (Table S1 Supplementary Material). This substrate was then used to investigate the effect of GlLPMO on wheat straw hydrolysis. Fig. 4A shows that when 50%, 25% and 10% Celluclast 1.5 L (in protein base) is substituted with GlLPMO, reducing sugar production is enhanced by 10%, 32% and 35% respectively, compared to hydrolysis with Celluclast 1.5 L with the same total protein loading. Results showed that substituting 10% of Celluclast 1.5 L returns the highest reducing sugar production, c.a. 17% higher than by Celluclast 1.5 L alone. This effect was also observed with higher protein loads (Fig. 4B), although the degree of enhancement diminished as the protein loading increased. These results show that GlLPMO produced in P. pastoris in this work is active as an accessory protein on pretreated wheat straw degradation. The increments in hydrolysis are similar to those reported by Jung et al. [31], who studied GlLPMO combined with Gloeophyllum trabeum Xyl10G (a different xylanase from G. trabeum) and the endoglucanase GtCel5B on pretreated oat and kenaf.

The synergy observed between Celluclast 1.5 L and GlLPMO on wheat straw probably results from the complementary functions of the monoxoxygenase and endoglucanase activity present in the commercial mixture. Endoglucanases in cellulosytic mixtures create a synergistic interaction with LPMO, because the endoglucanases prefer the amorphous regions in cellulose and show very low activity on crystalline zones.

3.4. GtXyn10A activity on wheat straw hydrolysis

To study the ability of GtXyn10A to complement a cellulase mixture in wheat straw degradation, experiments were carried out where the total enzyme loading was maintained constant while different amounts of Celluclast 1.5 L were substituted with GtXyn10A. Results showed that when replacing 25% of Celluclast 1.5 L with GtXyn10A, production of reducing sugars was maintained without significant loss in glucose production and a 17% increase in xylose production (Fig. 5). An increase in xylose was observed even after decreasing Celluclast 1.5 L

![Fig. 1. SDS-PAGE of purified GtXyn10A (A) and GlLPMO (B). A) Lane 1, GtXyn10A; B) Lane 2, GlLPMO; Lane 3, GlLPMO after treatment with Rapid PNGase, lane 4, Rapid PNGase F. M: Molecular mass ladder.](image)

![Fig. 2. Effect of GlLPMO on cellulose hydrolysis by Celluclast 1.5 L. Filter paper hydrolysis was carried out with 15 μg/mL Celluclast 1.5 L and supplemented with GlLPMO, without ascorbic acid (gray bars) or with 1 mM ascorbic acid (black bars). In “GlLPMO only” the GlLPMO concentration was 3.5 μg/mL. Error bars represent standard deviation of experiments ran in triplicate. For each condition, different numbers of asterisks (*) indicate a significant difference between GlLPMO concentrations by one-way ANOVA followed by Student’s t-test (P < 0.05).](image)
concentration by 50%, although that also resulted in a 20% reduction in glucose produced.

Removal of the hemicellulose covering the cellulose microfibrils by xylanase probably facilitated access of Celluclast 1.5L to the cellulose by increasing fiber porosity [13]. Processive cellulbiohydrolases seemed to be the most affected by the blocking effect of the hemicellulose. Selig et al. showed that the addition of xylanolytic enzymes enhances the performance of cellulbiohydrolases on pretreated corn stover [54], an effect attributed to the removal of non-cellulosic polysaccharides that coat cellulose fibers. The synergy between Celluclast 1.5 L and GtXyn10A in glucan conversion is probably a consequence of the cooperation between the xylanase and cellbiohydrolase components in Celluclast 1.5 L. GtXyn10A may also affect wheat straw hydrolysis by removing xylooligosaccharides that are accumulated due to insufficient xylanase activity in Celluclast 1.5 L. Qing et al. demonstrated that xylooligosaccharides, xylan, and xylose to a lesser degree, are strong inhibitors of cellulose hydrolysis [55].

3.5. Effect of combinations of GtXyn10A and GtLPmo on wheat straw hydrolysis

The effect of double and triple combinations of Celluclast 1.5 L, GtLPmo and GtXyn10A on wheat straw hydrolysis were examined. The wheat straw hydrolysis was carried out with 1.5 mg Celluclast 1.5 L per g DM. GtLPmo was added during loadings at 0.2 or 0.5 mg per gDM, corresponding to approximately 10% and 25% of the total protein loading respectively. Additional reactions were carried out that included 0.5 mg GtXyn10A per g DM (equivalent to 25% of total protein loading). Hydrolysis was evaluated as the production of reducing sugars, i.e. the glucan to glucose conversion and the xylan to xylose conversion (Fig. 6). In the absence of GtXyn10A, 0.2 mg GtLPmo per g DM enhanced production of reducing sugars by 20%; however when 0.5 mg/g DM GtLPmo was used, production of reducing sugars was
produces superoxide ions, which spontaneously transform into H2O2. Non-available substrate concentrations, the LPMO’s catalytic cycle including GtLPMO9A-2, which is a second GtLPMO. Cellobionic acid is a poor substrate for dextrins, cellobionic acid and gluconic acid. It has been reported that inhibition by products of the LPMO reaction, such as oxidized cello-

Fig. 6. Wheat straw hydrolysis by Celluclast 1.5 L, GtXyn10A and GtLPMO in double and triple combinations. Hydrolysis was carried out with 1.5 mg Celluclast 1.5 L/g dry matter in mixture with GtLPMO (white columns) or with GtLPMO plus 0.5 mg GtXyn10A/g dry matter (black columns). (A) Reducing sugars. (B) Glucan to glucose conversion and (C) Xylan to xylose conversion. Error bars represent standard deviation of experiments run by triplicate. Differences between binary (Celluclast 1.5 L plus GtLPMO) and tertiary mixtures (Celluclast 1.5 L plus GtLPMO and GtXyn10A) were all statistically significant as evaluated by a Student’s t-test (P < 0.05).

reduced by 10%. When Celluclast 1.5 L was combined with GtXyn10A, there was no hydrolysis inhibition due to increased GtLPMO concentrations, producing a synergistic increase of 54% in reducing sugars production, an additive increase of 40% in glucose production and a synergistic increase of 57% in the of xylan to xylose conversion.

The inhibition observed in hydrolysis reactions supplemented with 0.5 mg/g DM GtLPMO may result from oxidative inactivation of Celluclast 1.5 L caused by hydrogen peroxide [56,57]. Under limiting or non-available substrate concentrations, the LPMO’s catalytic cycle produces superoxide ions, which spontaneously transform into H2O2 and react with transition metals to form highly toxic hydroxyl radicals and other reactive oxygen species [56]. Also, Celluclast 1.5 L could be inhibited by products of the LPMO reaction, such as oxidized cello-
dextrins, cellobionic acid and gluconic acid. It has been reported that cellobionic acid is a poor substrate for β-glucosidase, therefore it accumulates and competitively inhibits cellobiohydrolases and β-gluco-
sidases [30].

As shown in Fig. 6, the negative impact of GtLPMO on the Celluclast 1.5 L activity was not observed when GtXyn10A was present. The presence of GtXyn10A and GtLPMO during hydrolysis produced additive increments in the glucan to glucose conversion, but synergistic enhancement of the xylan to xylose conversion. These results, together with the finding of GtLPMO being active in xylan (Fig. 3), allow us to infer that GtLPMO and GtXyn10A functionally cooperate to degrade xylan. The mechanism and the molecular bases for this cooperation are uncertain and must still be investigated.

Our results suggest that GtLPMO is able to act on xylose polysaccharides. Beechwood xylan is a polysaccharide with a low substitution degree, composed of O-acetyl-(1-4)-methylglucurono) xylans and containing approximately one 4-O-methylglucuronic acid sub-
stitute for every 15 α-xylose residues [58]. To date, most of LPMOs that are active on hemicellulose use xyloglucan as a substrate [52,53] including GtLPMO9A-2, which is a second G. trabeum LPMO that shares 75% if its identity with GtLPMO [59]. Recently, Frommham et al. [60] described a Myceliophthora thermophila LPMO (MtLPMO9A) that cleaves β-(1 → 4)-xylosyl bonds in xylan, forming oxidized xylo-oligosaccharides, while simultaneously cleaving β-(1 → 4)-glucosyl bonds in cellulose, forming oxidized gluco-oligosaccharides. However, although MtLPMO9A was shown to be active on several commercial xylans, this effect occurs only if xylan is mixed with cellulose. Recently, Kim et al. [32] reported that a AA9 LPMO from Chaetomium globosum is active on cellulose and beechwood xylan. To our knowledge, this enzyme and GtLPMO are the only LPMOs that are active on both cellulose and linear xylan described to date. We have focused our efforts on optimizing reaction conditions, in order to take better advantage of the bi-functional characters of these enzymes in hydrolysis. They represent a valuable tool for improving high-scale recovery of simple sugars from lignocellulose.

4. Concluding remarks

Experimental results have confirmed the auxiliary role of GtLPMO and GtXyn10A on the hydrolytic activity of a commercial cellulase on pretreated wheat straw. GtLPMO improves the hydrolysis of model cellulose substrates by cellulase and xylan substrate by xylanase. At low concentrations GtLPMO boosts the hydrolysis of pretreated wheat straw by Celluclast 1.5 L and at high concentrations GtLPMO exerts an inhibitory effect on the hydrolytic activity. However, this inhibition is counteracted when GtXyn10A is included in the mixture. We show that, the combination of 20% GtLPMO, 20% GtXyn10A and 60% Celluclast 1.5 L resulted in significant improvements in total wheat straw hydrolysis, glucan conversion and xylan conversion, at 54%, 40% and 57% respectively. Our results suggest that, not only does GtLPMO plays an auxiliary role in lignocellulose hydrolysis by oxidative activity on glycosidic bonds, but also in hemicellulose hydrolysis by the oxidation of xylosyl bonds in xylan.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.enzmictec.2017.11.007.

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