Demonstration-scale enzymatic saccharification of sulfite-pulped spruce with addition of hydrogen peroxide for LPMO activation

Thales H. F. Costa, Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), Aas, Norway

Adnan Kadić , Department of Chemical Engineering, Lund University, Lund, Sweden

Piotr Chylenski, Anikó Várnai, Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), Aas, Norway

Oskar Bengtsson, Borregaard AS, Sarpsborg, Norway

Gunnar Lidén, Department of Chemical Engineering, Lund University, Lund, Sweden

Vincent G.H. Eijsink , Svein Jarle Horn , Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), Aas, Norway

Abstract: The saccharification of lignocellulosic materials like Norway spruce is challenging due to the recalcitrant nature of the biomass, and it requires optimized and efficient pretreatment and enzymatic hydrolysis processes to make it industrially feasible. In this study, we report successful enzymatic saccharification of sulfite-pulped spruce (Borregaard's BALI™ process) at demonstration scale, achieved through the controlled delivery of hydrogen peroxide (H_2O_2) for the activation of lytic polysaccharide monooxygenases (LPMOs) present in the cellulolytic enzyme preparation. We achieved 85% saccharification yield in 4 days using industrially relevant conditions – that is, an enzyme dose of 4% (w/w dry matter of substrate) of the commercial cellulase cocktail Cellic CTec3 and a substrate loading of 12% (w/w). Addition of H_2O_2 and the resulting controlled and high LPMO activity had a positive effect on the rate of saccharification and the final sugar titer. Clearly, the high LPMO activity was dependent on feeding the reactors with the LPMO co-substrate H_2O_2, as *in situ* generation of H_2O_2 from molecular oxygen was limited. These demonstration-scale experiments provide a solid basis for the use of H_2O_2 to improve enzymatic saccharification of lignocellulosic biomass at large industrial scale. © 2020 The Authors. *Biofuels, Bioproducts, and Biorefining* published by Society of Chemical Industry and John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Key words: biorefining; cellulases; cellulose; lytic polysaccharide monooxygenase; LPMO; hydrogen peroxide; Norway spruce

Correspondence to: Svein Jarle Horn, Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), P.O. Box 5003, N-1432 Aas, Norway. E-mail: svein.horn@nmbu.no

Background

Enzymatic saccharification is a key step in the transformation of lignocellulosic materials into fermentable sugars. It is an essential process in most biorefineries but enzyme consumption must be minimized as commercial enzymes are costly. Effective pretreatment of the feedstock and highly efficient enzymatic processes are therefore required to realize industrial-scale commercialization.

Lignocellulosic biomass mainly consists of cellulose, hemicellulose, and lignin, which are assembled in a complex matrix. This organization makes lignocellulosic materials recalcitrant to degradation, and their enzymatic hydrolysis is not efficient without some form of pretreatment. Such pretreatment may include chemical, mechanical, and physical treatments that disrupt the co-polymeric lignocellulose matrix and make polysaccharide fibers more accessible to enzymes.[1] The required severity of the pretreatment is feedstock dependent; for example, wood needs harsher pretreatment than agricultural biomass.[2,3] Pretreatment may also include separation processes in which fractions of the feedstock, for example lignin, are removed prior to the enzymatic process.

Cellulolytic enzymes include cellulases, which are hydrolytic enzymes that can act on cellulose fibrils and release cellooligosaccharides, cellobiose, or glucose as their products.[4] Other hydrolases include hemicellulases that work as auxiliary enzymes to cellulases by breaking down hemicellulose. Lytic polysaccharide monooxygenases (LPMOs), discovered only in 2010,[5] comprise a powerful additional tool, as these enzymes can cleave glycosidic bonds in the most recalcitrant regions of polysaccharides using an oxidative mechanism.[6-11] They cleave internal bonds in cellulose chains, thus generating new access points for hydrolytic cellulases. Since their discovery,[5] O_2 has been considered the main co-substrate of LPMOs, but recent findings have shown that LPMOs can utilize hydrogen peroxide (H_2O_2) and that this co-substrate is more effective in driving LPMO reactions.[12,13] While there is some discussion about the nature of the natural co-substrate of LPMOs,[8,14-16] it is clear that the use of H_2O_2 can accelerate LPMO reactions and enables the use of these enzymes under anaerobic conditions.[12,13,17] Importantly, LPMO action needs reducing power, either to 'prime' the enzyme in H_2O_2-driven reactions or to deliver two electrons per catalytic cycle in O_2-driven reactions.[8,12] It should be noted that, under aerobic conditions and in the presence of a reductant, H_2O_2 will be formed *in situ* from O_2.[15,18] The LPMO reaction involving H_2O_2 as a co-substrate can be described as a peroxygenase reaction where H_2O_2 will react with the reduced copper atom in the LPMO active site and form a copper oxyl [CuO]$^+$ species. This copper oxyl abstracts a hydrogen atom from either the C1 or C4 carbon of the glycosidic bond in cellulose. This is followed by a hydroxylation of C1 or C4 and a subsequent spontaneous elimination reaction,[19] which leads to cleavage of the glycosidic bond.[20]

The BALI™ (Borregaard Advanced Lignin) process, developed by the Norwegian company Borregaard AS, fractionates lignocellulosic biomass into lignin and sugar streams that can be processed to value-added chemicals.[21] This process shows unusual versatility in that it can be applied to a multitude of raw materials, which include bagasse, hardwoods, and softwoods. Next to generating commercially attractive lignin-rich streams through a sulfite cooking step, the BALI™ pretreatment process generates cellulose pulps that have a low lignin content and are highly accessible to enzymatic hydrolysis.[22,23] It is notable that lignosulfonates generated during the pretreatment, and present in what is referred to as spent sulfite liquor (SSL), may be beneficial for the subsequent enzymatic saccharification because they represent reducing power, which is required to drive LPMO reactions.[21,24] Indeed, lignosulfonates have been shown to work as reductants for LPMOs and to increase the efficiency of enzymatic saccharification of BALI™-pretreated spruce.[22]

After the discovery of the potential of using H_2O_2 to drive LPMO reactions,[12] we demonstrated that controlled addition of H_2O_2 may lead to more efficient enzymatic biomass processing at laboratory scale.[13] In a recent study, we showed that electrode measurements of oxidation–reduction potential (ORP) can be used to determine if LPMOs are active or inactive in enzymatic saccharification reactions with the controlled addition of H_2O_2.[25] The results from these studies suggest that biomass conversion with controlled addition of H_2O_2 is an attractive strategy that is likely to be viable in biorefining, but they also show that more work is needed to optimize such processes.

When developing biorefining processes, scale-up experiments are crucial as they test real industrial conditions that are not met in the laboratory. Furthermore, they allow upscaled reproducibility assessment and may reveal scale-up related process issues. Formally, processes at large laboratory scale, or pilot scale, are at technology readiness level (TRL) 4–5. Processes at demonstration scale are at TRL 6–7, and this usually involves using reactors at the m^3 scale.[26] Full-scale industrial plants relying on enzymatic saccharification of various biomasses for the production of second-generation ethanol have been built around the world.[27] Many of these plants, however, struggle with making cellulosic ethanol production economically viable.[28] An approach

to improve process feasibility could be an improvement in saccharification efficiency. So far, only a few studies have been published on scaling up novel technologies that could improve process feasibility. These studies[27,29,30] mostly concern combination of the saccharification and fermentation steps but none of them has been concerned specifically with controlling LPMO activity.

In this study, we assessed the enzymatic saccharification of sulfite-pulped spruce in the presence of SSL as reductant at demonstration scale (2000 L), under aerobic and anaerobic headspaces. Importantly, we evaluated the effect of continuous feeding of H_2O_2 on both LPMO activity and final glucose yields.

Methodology

Feedstock and enzymes

Sulfite-pulped Norway spruce (*Picea abies*) was sourced from the Borregaard production plant in Sarpsborg, Norway, and utilized in laboratory and demonstration-scale experiments. The pulp was produced according to a proprietary process (BALI™) and stored at 4 °C until it was used for laboratory-scale reactions and was freshly produced for the demonstration-scale experiments. Spent sulfite liquor was also sourced from the Borregaard production plant and stored at 4 °C until later use in the laboratory, or was freshly used in demonstration-scale trials.

The chemical composition of the pulp was determined according to the National Renewable Energy Laboratory standardized protocol (NREL/TP-510-42 618). The pulp had a glucan content around 90% and it had a low xylose and mannose content (Table 1), as most of the hemicellulose was removed in a washing step.

The enzyme preparation Cellic® CTec3 was kindly provided by Novozymes A/S (Bagsværd, Denmark).

Laboratory saccharification

Controlled saccharification with continuous feeding of H_2O_2 was conducted in 3 L glass bioreactors (23 cm high × 13 cm diameter; Applikon, Schiedam, Netherlands) for 144 h with 1200 mL working volume (9.0 cm height × 12 cm diameter), using 12% (w/w) loading of pulp and Cellic CTec3 at 4%

(w liquid / w substrate DM). Spent sulfite liquor was added to reach a concentration of 10 g DMkg^{-1} reaction slurry. Deionized water was used, and the pH was kept at 5.0 by automatic addition of 1 M NaOH. Hydrogen peroxide addition started after 20 hours using a feeding rate of 200 µM h^{-1} (6.8 mg H_2O_2/kg reaction slurry/h). The reaction was run at 50 °C and stirred by a steel impeller with three paddles (5.2 cm diameter) at 400 rpm. Aliquots of 2 mL were sampled at different time points, and enzymes in the samples were inactivated by incubating at 100 °C for 15 minutes. The boiled samples were centrifuged (at 3800×*g* and 4 °C), and the supernatant was appropriately diluted in deionized water prior to analysis. This experiment was run in duplicate.

Demonstration-scale saccharification

Demonstration-scale saccharifications were carried out in the Borregaard Biorefinery Demo plant (Sarpsborg, Norway; Figure S5). The facility for enzymatic hydrolysis consisted of three bioreactors each with a total volume of 4.5 m^3 and a working volume of 2–4 m^3 each. The double-jacketed reactors had an internal diameter of 1.6 m and were equipped with automated systems for temperature and pH control. The reactors were equipped with differential pressure cells for monitoring the liquid level inside the reactors, which were verified by measuring the height of the reactor headspace using a laser distance meter. One reactor (Reactor 1) was fed with solid substrate utilizing a screw press. This reactor was equipped with three two-bladed impellers with a diameter of 700 mm, pumping upwards. The other two reactors (Reactors A and N) were equipped with two two-bladed 700 mm impellers, pumping downwards.

The saccharification reactions were started in fed-batch using Reactor 1. Substrate with approximately 30% (w/w) DM was gradually fed into the reactor containing enzymes and SSL, to a targeted final working weight of approximately 4000 kg, substrate loading of 12% (w/w) DM pulp and enzyme loading of 4% (w liquid/w DM of substrate). Reactor stirring was at 75 rpm during the fed-batch phase. The temperature was 50 °C and the pH was kept at 5.0 by controlled addition of 3 M NaOH in all trials.

After the initial fed-batch phase, which lasted for 10–20 hours, the reaction was split into the two identical reactors A and N, creating two sub-batches with a working

Table 1. Chemical composition of the sulfite-pulped spruce.						
	Arabinose	Galactose	Glucose	Xylose	Mannose	Lignin[*]
Sulfite-pulped spruce[**]	n.d.	n.d.	87.4	2.7	5.2	3.3

*Lignin refers to Klason lignin.
**Values show weight percentage as percentage of dry matter; for sugars, anhydro monomer content is reported; n.d., not detected.

weight of approximately 2000 kg each. Reactions were stirred at 100 rpm following the split; temperature and pH control were as above. Headspaces in these two reactors were filled with N_2 (Reactor N) or kept with air (Reactor A), and addition of H_2O_2 was started right after the split, as described in the experimental plan (Table 2). Reactions with anaerobic headspaces had their atmosphere changed by pressurizing the headspace with N_2 to 2 bars and then releasing the overpressure in a total of three cycles. The N_2/air atmosphere was maintained throughout the reactions by addition of $50 L h^{-1}$ gas into the headspace. Hydrogen peroxide was added at a rate of approximately $200 \mu M h^{-1}$ from a 0.6% (w/w) solution tank to the designated batches. The weight of the H_2O_2 feed tank was monitored to ensure correct feeding rate. Sampling was done throughout the hydrolysis at different timepoints.

Analysis of sugars and oxidized LPMO products

Glucose and cellobiose levels in samples from the laboratory-scale experiment were quantified by high-performance liquid chromatography (HPLC) using a Dionex Ultimate 3000 system (Sunnyvale, CA, USA) coupled to a refractive index detector and equipped with a Rezex ROA-Organic Acid H$^+$ column (Phenomenex, Torrance, CA, USA) and a SecureGuard Carbo-H$^+$ guard column (Phenomenex), operated at 65 °C as described by Müller *et al.* (2018).[13] The mobile phase was 5 mM H_2SO_4 with a flow rate of 0.6 mL min^{-1}.

Concentrations of glucose, xylose, mannose, arabinose, galactose and fructose in the demonstration-scale hydrolysates were determined by HPLC using an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with an Aminex HPX-87P ion exchange

column (Bio-Rad, Hercules, CA, USA) operated at 80 °C and a refractive index detector (Agilent 1100 series). The mobile phase was deionized H_2O with a flow rate of 0.6 mL min^{-1}.

Oxidized sugars from both laboratory- and demonstration-scale experiments were analyzed by high-performance anion exchange chromatography with pulsed amperometric detection using a Dionex ICS 3000 system (Sunnyvale, CA, USA). C4-oxidized product standards (Glc4gemGlc) were produced as described by Müller *et al.* (2015).[31] The HPAEC-PAD was equipped with a CarboPac PA1 column, operated at 30 °C, and the mobile phase was a gradient with increasing concentration of sodium acetate as described by Westereng *et al.*[32]

Possible bacterial contamination in the demonstration-scale trials was assessed by quantifying organic acids throughout the reactions. Concentrations of lactate, propionate, acetate, formate, ethanol, and glycerol were determined by HPLC using an Agilent 1100 series system (Agilent Technologies) equipped with an Aminex HPX-87H ion exchange column (Bio-Rad) operated at 60 °C and a refractive index detector (Agilent 1260 Infinity). The mobile phase was 17.5 mM H_2SO_4 with a flow rate of 0.6 mL min^{-1}.

Measurement of ORP

The dependence of ORP on dissolved oxygen (DO) was verified in laboratory experiments (Figure S2). The ORP measurements were conducted in a Biostat A Plus bioreactor (Sartorius, Melsungen, Germany) containing 1 L of SSL solution (3, 12 and 21 g DM per kg reaction slurry), with pH adjusted to 5.0 with NaOH. The impeller speed was 200 rpm and the temperature 50 °C. The ORP was measured with a combination redox electrode

Table 2. Conditions applied in the different demonstration-scale saccharification reactions of sulfite-pulped spruce.

Batch[*]	Split time	Reaction[*]	SSL (g DM/kg)[*]	Head-space[*]	H_2O_2 ($\mu M h^{-1}$)	Redox electrode	Replicate[***]
H271	9 h	H271-A	21	Air	0	No	No
		H271-N	21	N_2	0	Yes	
H274	9 h	H274-A	12	Air	0	Yes	Yes - H274rep
		H274-N	12	N_2	0	No	
H281	13 h	H281-A	13	Air	204	No	Yes - H281rep
		H281-N	13	N_2	195	No	
H282	22 h	H282-A	3	Air	197	No	No
		H282-N	3	N_2	192	No	

*Two reactions (A and N pairs) were started in one reactor operated in fed-batch mode with gradual addition of substrate, and then split into two reactors with either air (A) or nitrogen (N) in the headspace. During the fed-batch phase, the headspace was aerobic. Spent sulfite liquor was added in g DM per kg reaction liquid.
**Feeding of H_2O_2 was started immediately after splitting the reactions, as indicated in the figures.
***Figures S3 and S4 show both the experiments described in the main text and the replicate experiments for H274 and H281, respectively.

(Pt4805-DPAS-SC-K8S/200, Mettler-Toledo, Greifensee, Switzerland), and the DO was measured with a dissolved oxygen sensor (Oxyferm FDA 225, Hamilton, Bonaduz, Switzerland). Before starting the measurement, the solution was sparged with 1 Lmin^{-1} nitrogen gas until the DO reached 0%. The measurement was performed by flushing the headspace with 1 Lmin^{-1} air and recording the ORP and DO, while the DO gradually increased from 0 to 100%. For some of the demonstration-scale experiments (see Table 2), a combined pH/ORP sensor Memosens CPS16D (Endress+Hauser, Switzerland) connected to a Stratos Pro transmitter (Knick Elektronische Messgeräte GmbH & Co, Berlin, Germany) was utilized.

Results and Discussion

Laboratory scale saccharification of sulfite-pulped spruce

Initially, a laboratory-scale (1.2 L) saccharification of the glucan-rich substrate (see Table 1) was run using conditions similar to those used in the subsequent demonstration-scale trials. Reducing power, which needs to be added for this type of lignin-poor substrate[22] in order to prime LPMOs, was provided in the form of SSL. The experiment was initiated by adding the enzyme cocktail Cellic CTec3, while continuous addition of H$_2$O$_2$ was started after 20 hours, when the substrate had been partly liquefied. Glucan conversion reached a maximum of around 80% after 74 hours and stayed at this level for the remaining experimental period, which ended at 144 hours (Figure 1A).

Due to the cellulase and β-glucosidase activities present in the Cellic CTec preparations, the main glucan-derived product in the hydrolysates is glucose. During saccharification, C1- and C4-oxidized LPMO products are converted to D-gluconic acid and 4-keto-cellobiose, respectively, the latter in equilibrium with its gemdiol form Glc4gemGlc.[31] The major oxidized product detected was Glc4gemGlc (Table 3), which is in line with previous works that have shown that Glc4gemGlc is the predominant oxidized product when degrading cellulose with Cellic CTec2[13] and Cellic CTec3.[22] This product is unstable, and its accumulated levels tend to start decreasing when LPMO activity ceases,[13] which we also observed in the later phase of the reaction (Figure 1B). In the initial phase of the reaction (up to 48 hours), the LPMO activity was clearly H$_2$O$_2$ limited as the production of Glc4gemGlc increased rapidly from 20 hours, when H$_2$O$_2$ pumping started. Before inactivation of the LPMOs became apparent, here referred to as the 'turning point', 66% of the H$_2$O$_2$ added
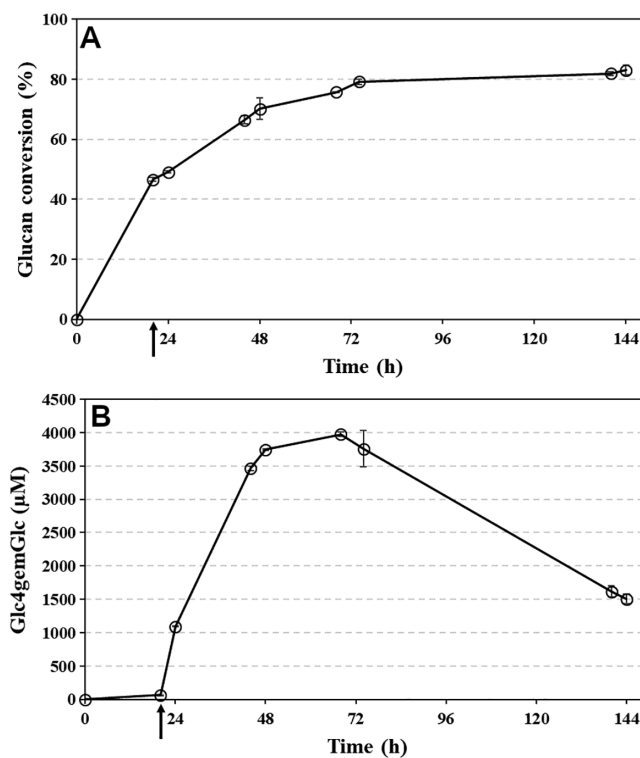

Figure 1. Laboratory-scale enzymatic saccharification of sulfite-pulped spruce. Reaction mixtures contained 12% (w/w) of substrate, 10 g DM SSL per kg reaction liquid, 4% (w liquid per w DM substrate) Cellic CTec3 and the headspace was aerobic. Pumping of H$_2$O$_2$, at a constant rate of 200 μMh^{-1}, was initiated after 20 h (indicated by the arrow) and continued for the rest of the reaction. Panel A shows glucan conversion (based on soluble glucose and cellobiose) and panel B, shows the level of C4-oxidized LPMO products (Glc4gemGlc). Note that the latter products are unstable, which explains why levels become lower as the reaction proceeds and no more LPMO products are generated in the later phase of the reaction. The data shown are the average of two independent experiments; error bars indicate the average deviation.

to the reactor was used to produce Glc4gemGlc when assuming 1:1 stoichiometry (Table 3). Interestingly, the decrease in Glc4gemGlc concentration coincided with the time at which the glucan conversion reached its maximum (around 74 hours; Figure 1). The decrease in LPMO activity results from a combination of substrate depletion and gradual inactivation of the enzymes,[12] leading to H$_2$O$_2$ accumulation and a net degradation of Glc4gemGlc. A previous study using sulfite-pulped spruce, Cellic CTec2, 1 mM ascorbic acid as reductant, and otherwise similar conditions, have shown similar results: 80% glucan yield after 48 h, a maximum Glc4gemGlc level of 3800 μM within

Table 3. Conversion of H_2O_2 to oxidized products (Glc4gemGlc) by LPMOs.

Experiment[*]		Turning point (h)[**]	H_2O_2 added (µM)	Glc4gemGlc (µM)[***]	Conversion of H_2O_2 to Glc4gemGlc (%)
Laboratory-scale		48	5600	3678	66
H281	A (air-SSL13)	47	6936	3051	44
	N (N_2-SSL13)	71	11310	3977	35
H282	A (air-SSL3)	60	7486	2657	35
	N (N_2-SSL3)	52	5760	1961	34

[*]The composition of the headspace (Air/N_2) and the loading of SSL (g DM SSL per kg reaction liquid) is indicated in brackets.
[**]Note the turning point for the end of fast initial LPMO activity varies due to different incubation times in the initial fed-batch phase; see Figures 1 and 3.
[***]Glc4gemGlc production due to *in situ* generated H_2O_2 in the fed-batch phase, before starting the addition of external H_2O_2, was subtracted.

30 h, and a decrease in Glc4gemGlc concentration in the later stages of the reaction.[13]

The sulfite-pretreated pulp is low in lignin (Table 1) and thus offers poor reducing power by itself. The presence of oxidized products at levels similar to those obtained previously in reactions with ascorbic acid as reductant[13] indicates that SSL can work as a reductant for LPMOs. Indeed, SSL contains lignosulfonates,[24,33] which have been shown to act as reducing agents for LPMOs.[22] The fact that SSL can be used as a reducing agent is especially important from the process economics point of view; there is no need to purchase external, and usually expensive, reducing agents. At the turning point (Table 3), about 400 µmol of Glc4gemGlc had been produced per gram of added SSL, which suggests that lower SSL levels may also be sufficient to reach maximum conversion.

Demonstration-scale saccharification utilizing SSL as reducing agent

The demonstration-scale experiments comprised four runs of two parallel reactors – eight experiments in total. Three process parameters were varied: the use of nitrogen or air in the headspace, the concentration of SSL, and the supply of H_2O_2 (see Table 2). Reactions with the same trial number were started as one fed-batch reaction with a final working volume of 4000 L (air in headspace), which was split into two reactors with approximately 2000 L working volume in each. Monitoring of organic acids and alcohols in the hydrolysates of the demonstration-scale trials indicated no issues, or very limited issues, with microbial contamination (data not shown).

Four different demonstration-scale saccharification trials without addition of H_2O_2 were carried out with SSL addition at either 12 or 21 g DMkg^{-1} of reaction liquid, and either nitrogen or air in the headspace. Figure 2
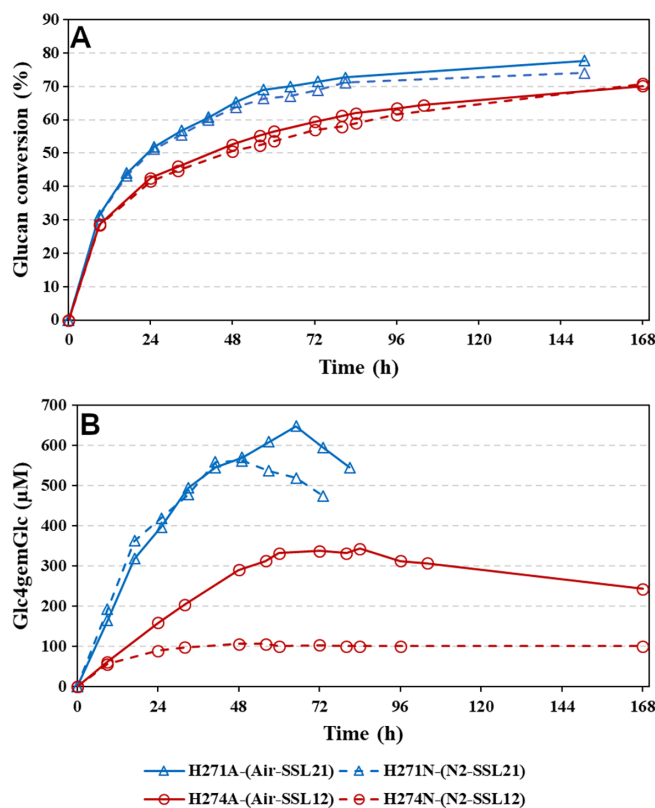

Figure 2. Demonstration-scale enzymatic saccharification of sulfite-pulped spruce, without the addition of H_2O_2, at different SSL concentrations. Reaction mixtures contained 12% (w/w) of substrate, 12 or 21 g DM SSL per kg reaction liquid, 4% (w liquid per w DM substrate) Cellic CTec3. Reactions were started as one fed-batch phase and, after approx. 9 h, were split into two with either air (solid lines) or nitrogen (dashed lines) in the headspace. Hence, for the fed-batch phase, both reactions were aerobic. Panel a shows glucan conversion and panel B shows the concentration of Glc4gemGlc.

shows the glucan conversion and production of oxidized sugars for the four runs. The rates of glucose release were clearly higher in the reactions with the highest SSL concentration, but final glucan conversions were rather similar for all reactions (71–78%). Higher initial glucan conversion rates also correlated with higher LPMO activity – that is higher concentrations of Glc4gemGlc (Figure 2B). The effects of headspace composition on LPMO activity showed considerable variation, which is likely due to variation in the actual extent of anaerobicity during the various trials (note that, due to the initial fed-batch set up, there were considerable amounts of oxygen available in each trial; see below). The observation that the presence of both air (i.e., oxygen) and SSL affected the reaction rate is understandable because *in situ* H_2O_2 generation is a function of the availability of both oxygen and reducing agent. This has been observed before, in the degradation of Avicel, where LPMO activity increased both with headspace oxygen levels and concentration of ascorbic acid.[13] The reducing power of SSL was most likely related to the content of solubilized lignin in the form of lignosulfonates, as observed previously in a study with freeze-dried SSL.[22] The first step in *in situ* H_2O_2 generation is a reduction of oxygen to superoxide radical anions. Superoxide will then spontaneously be converted to H_2O_2 by a dismutation reaction. The net reaction is: $O_2 + 2e- + 2 H^+ \rightarrow H_2O_2$, where electrons are provided by the reducing agent.[34,35]

Interestingly, the maximum levels of LPMO products shown in Figure 2 (where H_2O_2 was not added) are much lower than what was achieved by direct addition of H_2O_2 in the laboratory-scale experiment (see Figure 1). This indicates that *in situ* production of H_2O_2 was relatively low and thus a limiting factor for LPMO activity in the set of experiments shown in Figure 2.

The formation of Glc4gemGlc also with nitrogen in the headspace deserves a comment. At 50 °C, the concentration of dissolved oxygen in pure water in equilibrium with air at atmospheric pressure is 5.6 mg/L or 177 μM, which theoretically could yield 177 μM H_2O_2. Assuming that the liquid phase initially holds this oxygen concentration, this should be the maximum achievable H_2O_2 level for the reactions with N_2 in the headspace. However, the anaerobic reaction with 21 g DM SSL/kg reaction slurry reached a Glc4gemGlc concentration of 561 μM, with a net production in the N_2 phase (the initial 9 hours were aerobic) of 368 μM. This indicates that the headspace in 'anaerobic' reactions was not anaerobic but contained some oxygen that was gradually transferred into the liquid phase. The latter is confirmed by ORP measurements that are discussed below.

Demonstration-scale saccharification using H_2O_2 feeding

Four additional demonstration-scale saccharification trials were run with continuous addition of H_2O_2 and at SSL levels of 3 (H282) or 13 (H281) g $DMkg^{-1}$ reaction slurry. As before, parallel reactions with aerobic or anaerobic headspace were set up (Figure 3). The addition of H_2O_2 started after the initial fed-batch phase, which lasted 22 hours for H282 and 13 hours
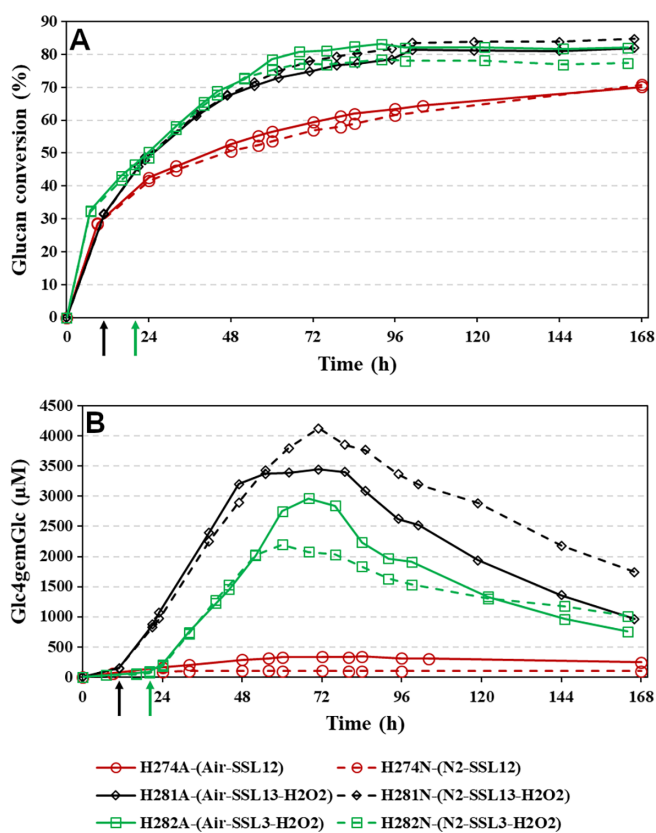

Figure 3. Demonstration-scale enzymatic saccharification of sulfite-pulped spruce with different SSL concentrations and continuous addition of H_2O_2. Reaction mixtures contained 12% (w/w) of substrate, around 13 or 3 g DM SSL per kg reaction liquid, and 4% (w liquid per w DM substrate) Cellic CTec3. Reactions were started as one fed-batch phase and were then split into two with either air (solid lines) or nitrogen (dashed lines) in the headspace at 9 h for H274, 13 h for H281, or 22 h for H282. Hence, for the fed-batch phase, all reactions were aerobic. Pumping of H_2O_2, at a constant rate of approximately 200 μ Mh^{-1} (see Table 2), was started right after splitting and is indicated by arrows for trials H281 and H282. No H_2O_2 was added in trial H274 (same as in Figure 2). Panel A shows glucan conversion and panel B shows the concentration of Glc4gemGlc.

for H281, and the division of the reaction mixture into two tanks with air or N_2 in the headspace.

All reactions with H_2O_2 supplementation stood out as glucan conversion was much faster and reached higher final levels (77–85%) than the reactions without H_2O_2 addition (71%, H274), regardless of the presence of oxygen in the headspace (Figure 3A). For example, at 60 hours, the aerobic reaction with H_2O_2 addition and 13 g DM SSL/kg reaction slurry showed 33% higher glucan conversion than the similar reaction without added H_2O_2. Moreover, in three of the four reactions with H_2O_2 addition, maximum conversion levels, amounting to 85% conversion and a final glucose concentration of 105 g L^{-1}, were reached after approximately 100 hours. Figure 2A shows that such high conversion levels were not achieved without H_2O_2 supply, even after incubation for 165 hours. Thus, continuous addition of H_2O_2 enabled much faster glucan conversion. Comparison of these results with previous studies on enzymatic hydrolysis of pretreated spruce is difficult to make due to lack of published data for demo-scale experiments. Furthermore, laboratory-scale studies have usually been carried out at lower substrate concentrations and higher enzyme loadings. In laboratory-scale experiments a glucan yield of 74% has been achieved for a similar substrate at a 5% solid loading.[22,36] In another recent study a glucan hydrolysis yield of 67% after 96 hours for SO_2 pretreated softwood at 10% solid loading was reported.[36] To the best of our knowledge, for industrially relevant conditions with high solid loading and relatively low enzyme loading, the enzymatic glucan conversion yield of 85% achieved in this study is the highest ever reported for pretreated softwood.

Figure 3 also shows that only low levels of SSL were needed when H_2O_2 was added to the reaction, because the trial with 3 g DM SSLkg^{-1} reaction slurry gave high LPMO activity and similar glucan yields to the trial with 13 g DM SSL/kg reaction slurry. This was very different from the reactions that depended on *in situ* generation of H_2O_2 (Figure 2); these reactions were highly sensitive to the concentration of reductant.

The increase in glucan conversion rate with H_2O_2 addition was associated with much higher LPMO activity than the reaction without external H_2O_2 addition (Figure 3B). Lytic polysaccharide monooxygenases activity was clearly limited by H_2O_2 supply as accumulation of Glc4gemGlc increased rapidly immediately after initiation of H_2O_2 addition (arrows in Figure 3B) in all four reactions, regardless of headspace composition. Before H_2O_2 addition, LPMO activity was limited by the *in situ* generation rate of H_2O_2, which is apparently much lower than the H_2O_2 feeding rate used in the experiments.

Although a high increase in the Glc4gemGlc release rate was achieved by H_2O_2 addition (compare trials H281 and H274; Figure 3), only 35–44% of the supplied H_2O_2 was converted into Glc4gemGlc in the first phase of the reaction (Table 3), which is less than the 66% calculated for the laboratory-scale experiment. Figure 3B further shows that the trials with less SSL (3 g DMkg^{-1} reaction slurry) produced less Glc4gemGlc than reactions with 13 g DM SSLkg^{-1} reaction slurry, which is in line with the demonstration-scale reactions without H_2O_2 (Figure 2). It is conceivable that at the lower SSL concentrations, reducing power becomes limiting, even though in H_2O_2-driven reactions reducing power is only needed to prime the LPMO and is not consumed stoichiometrically. In this regard, it should be noted that reactions between (reducing) SSL components and the added H_2O_2 will gradually deplete reducing power. Such side-reactions will also consume H_2O_2, and different reactivity (freshness) of the used SSL could explain differences seen in Table 3 between laboratory- and demonstration-scale experiments.

The initial rate of Glc4gemGlc production during the experiments with added H_2O_2 was somewhat affected by the amount of SSL (green versus black curves in Figure 3) but appeared independent of the contents of the headspace (solid lines versus dashed lines in Figure 3). However, during the later phase of the reaction, near the turning point, differences became visible. For example, in trials with 13 g DM SSLkg^{-1} reaction slurry, the highest level of oxidized products was reached in the anaerobic reaction, whereas in reactions with 3 g DMkg^{-1} SSL the level was highest in the aerobic reaction. These differences are likely to be the result of a multitude of interconnected processes such as LPMO inactivation, product instability, and a range of possible reactions involving reducing compounds, H_2O_2, and O_2 (see Eijsink *et al.,* 2019,[37] for further discussions).

Interestingly, the differences in the levels of oxidized sugars between the trials with different SSL contents and with added H_2O_2 (Figure 3B) were barely reflected in the glucan conversion curves (Figure 3A). The similarity of glucan conversion yields achieved with H_2O_2 addition despite differences in Glc4gemGlc formation indicates that fine tuning of LPMO activity is not needed for optimal glucan conversion. Apparently, while a lack of LPMO activity limited the overall efficiency of the saccharification process in the trials without added H_2O_2 (reactions H271 and H274; Figure 2), LPMO activity was sufficiently high not to limit the overall process in all trials with added H_2O_2.

The high glucan conversion rate and high final yield in the aerobic reaction with 13 g DMkg^{-1} SSL (black line; Figure 3A) at demonstration scale are in agreement with what was

observed in the corresponding laboratory-scale experiment (Figure 1A). Direct comparison of the progress curves for glucose release and accumulation of Glc4gemGlc (Figure S1) shows that the curves almost overlap. In the demonstration-scale experiment, H_2O_2 pumping started at 13 hours, compared with 20 hours in the laboratory-scale reaction, and this is reflected in an earlier onset of Glc4gemGlc generation (Figure S1B). The demonstration-scale experiments gave slightly higher glucose yields, and further work is needed to assess the causes of this difference. In any case, the high similarity between the laboratory- and demonstration-scale experiments confirms the scalability of enzymatic saccharification with external H_2O_2 addition for LPMO activation.

Redox monitoring during saccharification

The ORP (Figure 4) was monitored in some of the demonstration-scale trials. The ORP values are affected by dissolved oxygen concentration. The initial high ORP values in the trial where the headspace was filled with air (H274A) ware as expected, and in agreement with lab-scale experiments,[25] because oxygen in the head space will be transferred to the liquid. However, in the trial with nitrogen in the headspace (H271N), a high ORP values were measured for quite a long time. The LPMOs were also active in both
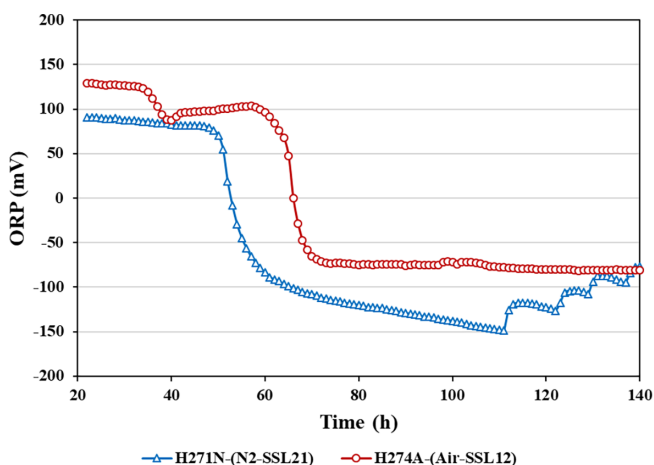

Figure 4. Oxidation–reduction potential (ORP) during demonstration-scale enzymatic saccharification of sulfite-pulped spruce. Reactions containing 12% (w/w) of substrate and 4% (w liquid per w DM substrate) Cellic CTec3 were started by a fed-batch phase with air in the headspace. The ORP was measured during the subsequent batch phase where the headspace was either air or nitrogen. The figure shows data for experiments with different SSL concentrations (12 and 21 g DM per kg reaction liquid) and without addition of H_2O_2 (see Table 2).

reactions (Figure 2B). This can only be explained by the presence of some oxygen in the liquid in the reactions with nitrogen in the headspace, which we attribute to oxygen carried over from the start-up phase.

Following the initial high ORP values, both experiments without H_2O_2 feeding underwent a rapid decrease in ORP after 50–60 hours (H271N and H274A; Figure 4). This decrease in ORP was probably caused by decreasing concentrations of dissolved oxygen (DO) as previously reported by Schuldiner *et al.*,[38] and confirmed by measurements of the effect of DO on ORP at different concentrations of SSL (Figure S2). The absence of LPMO activity during the late phase of the reactions without H_2O_2 feeding (Figure 2B; no LPMO product accumulation after 49 hours in H271N and after 72 hours in H274A) may in part be due to a lack of oxygen caused by slow gas-to-liquid mass transfer after the consumption of the oxygen that was initially dissolved. The ORP measurements for cases with H_2O_2 feeding (not shown here) were more complex due to the dual effects of H_2O_2 feeding and the presence of dissolved oxygen.

Using a correlation for the liquid phase mass transfer coefficient[39] with zero gas hold up, as there was no gas sparging, it is possible to estimate a maximum oxygen transfer rate (OTR_{max}) of approx. 20 μM h^{-1} in pilot scale (for more details see supplementary calculations 1). An LPMO oxygen consumption rate of 13 μM h^{-1} can be estimated for the experiment with air in the headspace and no addition of H_2O_2 (see Figure 2, H271A). As sufficiently high OTRs are difficult to achieve in large reactors without gas sparging, and gas sparging of large reactors filled with viscous biomass slurry is difficult and expensive, a low OTR may be a strong argument for driving the LPMO reactions with continuous addition of H_2O_2.

Reproducibility of demonstration-scale saccharifications

Two of the demonstration-scale experiments were repeated, using highly similar conditions but with different sampling points. Figures S3 and S4 show progress curves for the experiments H274 and H281 shown in Figures 2 and 3, and for the replicate experiments. For the trial without added H_2O_2 (H274), the replicates showed considerable differences in the levels of detected LPMO products (Figure S3B), but only minor differences in final glucan levels. Overall, the replicate experiments showed all the trends discussed above for H271 and H274, such as the dependency on SSL and air, and the correlation between LPMO activity and glucan yield. The considerable variation between experiments without added H_2O_2 could be due in part to variation in reducing power – that is the freshness of the SSL – because

SSL will react with oxygen during storage. As shown above, in experiments without added H_2O_2, the amount of reducing power has a very strong effect on LPMO activity, because *in situ* generation of H_2O_2 from oxygen requires two externally delivered electrons per cycle.

Interestingly, reproducibility was better for the trials with H_2O_2 addition (H281; Figure S4). Comparison of the replicate trials showed minor variations in the levels of Glc4gemGlc (Figure S4B), but the (fast) kinetics and (high) yields of glucose release were virtually identical (Figure S4A). Thus, the present observations indicate that use of H_2O_2 in saccharification makes this process more efficient and more reproducible.

Concluding Remarks

The present data show efficient saccharification of sulfite-pretreated spruce pulp using H_2O_2 feeding to drive LPMO activity in the commercial enzyme preparation of Cellic CTec3 at demonstration scale. The average glucose conversion of batches with H_2O_2 addition was $82 \pm 3\%$ after 96 ± 2 hours of hydrolysis, while the average glucose conversion of batches without H_2O_2 addition was $71 \pm 4\%$ after 162 ± 9 hours of hydrolysis. Activation of LPMO by the addition of H_2O_2 thus not only improved substrate utilization substantially but also gave a significant reduction in the required retention time. Thus, aiding biomass saccharification with H_2O_2 has a potential to decrease the operating costs and increase product yield without extra investment in new equipment and extensive remodeling of the overall process in current commercial-scale lignocellulosic ethanol plants. Harnessing the LPMO activity was best achieved by feeding the reactors with the LPMO co-substrate H_2O_2, rather than by depending on a system for *in situ* generation of H_2O_2 from molecular oxygen. Generally, the composition of the headspace (air versus nitrogen) had very little effect on the glucan conversion.

While Cellic CTec3 was efficient, and glucan saccharification yields up to 85% were achieved, our work highlights a potential for further optimization of this enzyme cocktail. Our study indicates that the LPMO content of this enzyme preparation may be unnecessarily high if the LPMO co-substrate, H_2O_2, is added to the saccharification, but also that LPMO activity is lost over time. Reducing the ratio of LPMOs to canonical cellulases and including more stable LPMOs in the enzyme cocktail may thus reduce enzyme load and, consequently, enzyme costs, contributing to a better process economy.

For types of pretreated biomass that are low in lignin, it is necessary to supply a reducing agent to activate LPMOs during the saccharification step. Here we showed that, in the absence of lignin, SSL was able to supply sufficient amounts of reducing equivalents needed to drive the LPMO reaction in demonstration-scale saccharifications of sulfite-pretreated spruce pulp. As it is a by-product of sulfite pulping and, hence, is available in large quantities, SSL is a potential reducing agent for industrial-scale biomass saccharification.

Overall, this study has shown efficient saccharification of spruce biomass at demonstration scale, which lays the foundation for further upscaling and the establishment of commercial-scale second-generation biorefinery plants for the production of fuels, chemicals[40] or microbial biomass using spruce as feedstock.[41]

Acknowledgements

References

1. Chen H, Liu J, Chang X, Chen D, Xue Y, Liu P *et al.*, A review on the pretreatment of lignocellulose for high-value chemicals. *Fuel Process Technol* **160**:196–206 (2017).
2. Kumar AK and Sharma S, Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. *Bioresour Bioprocess* **4**:7 (2017).
3. Zhu JY and Pan XJ, Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. *Bioresour Technol* **101**:4992–5002 (2010).
4. Cragg SM, Beckham GT, Bruce NC, Bugg TDH, Distel DL, Dupree P *et al.*, Lignocellulose degradation mechanisms across the tree of life. *Curr Opin Chem Biol* **29**:108–119 (2015).
5. Vaaje-Kolstad G, Westereng B, Horn SJ, Liu Z, Zhai H, Sørlie M *et al.*, An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. *Science* **330**:219–222 (2010).
6. Horn SJ, Vaaje-Kolstad G, Westereng B and Eijsink VGH, Novel enzymes for the degradation of cellulose. *Biotechnol Biofuels* **5**:45 (2012).
7. Eibinger M, Sattelkow J, Ganner T, Plank H and Nidetzky B, Single-molecule study of oxidative enzymatic deconstruction of cellulose. *Nat Commun* **8**:894 (2017).
8. Chylenski P, Bissaro B, Sørlie M, Røhr ÅK, Várnai A, Horn SJ *et al.*, Lytic polysaccharide monooxygenases in enzymatic processing of lignocellulosic biomass. *ACS Catal* **9**:4970–4991 (2019).
9. Forsberg Z, Sørlie M, Petrović D, Courtade G, Aachmann FL, Vaaje-Kolstad G *et al.*, Polysaccharide degradation by lytic polysaccharide monooxygenases. *Curr Opin Struct Biol* **59**:54–64 (2019).
10. Johansen KS, Lytic polysaccharide monooxygenases: the microbial power tool for lignocellulose degradation. *Trends Plant Sci* **21**:926–936 (2016).
11. Hemsworth GR, Johnston EM, Davies GJ and Walton PH, Lytic polysaccharide monooxygenases in biomass conversion. *Trends Biotechnol* **33**:747–761 (2015).

12. Bissaro B, Røhr ÅK, Müller G *et al*., Oxidative cleavage of polysaccharides by monocopper enzymes depends on H2O2. *Nat Chem Biol* **13**:1123–1128 (2017).

13. Müller G, Chylenski P, Bissaro B, Eijsink VGH and Horn SJ, The impact of hydrogen peroxide supply on LPMO activity and overall saccharification efficiency of a commercial cellulase cocktail. *Biotechnol Biofuels* **11**:209 (2018).

14. Hangasky JA, Iavarone AT and Marletta MA, Reactivity of O2 versus H2O2 with polysaccharide monooxygenases. *Proc Natl Acad Sci U S A* **115**:4915–4920 (2018).

15. Bissaro B, Várnai A, Røhr ÅK and Eijsink VGH, Oxidoreductases and reactive oxygen species in conversion of lignocellulosic biomass. *Microbiol Mol Biol Rev* **82**:4 (2018).

16. Wang B, Walton PH and Rovira C, Molecular mechanisms of oxygen activation and hydrogen peroxide formation in lytic polysaccharide monooxygenases. *ACS Catal* **9**:4958–4969 (2019).

17. Kuusk S, Bissaro B, Kuusk P, Forsberg Z, Eijsink VGH, Sørlie M *et al*., Kinetics of H2O2-driven degradation of chitin by a bacterial lytic polysaccharide monooxygenase. *J Biol Chem* **293**:523–531 (2018).

18. Kittl R, Kracher D, Burgstaller D, Haltrich D and Ludwig R, Production of four Neurospora crassa lytic polysaccharide monooxygenases in Pichia pastoris monitored by a fluorimetric assay. *Biotechnol Biofuels* **5**:79 (2012).

19. Phillips CM, Beeson WT, Cate JH *et al*., Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by Neurospora crassa. *ACS Chem Biol* **6**:1399–1406 (2011).

20. Bissaro B, Streit B, Isaksen I, Eijsink VGH, Beckham GT, DuBois JL *et al*., Molecular mechanism of the chitinolytic peroxygenase reaction. *Proc Natl Acad Sci* **117**:1504–1513 (2020).

21. Rødsrud G, Lersch M and Sjöde A, History and future of world's most advanced biorefinery in operation. *Biomass Bioenergy* **46**:46–59 (2012).

22. Chylenski P, Petrović DM, Müller G, Dahlström M, Bengtsson O, Lersch M *et al*., Enzymatic degradation of sulfite-pulped softwoods and the role of LPMOs. *Biotechnol Biofuels* **10**:177 (2017).

23. Chylenski P, Forsberg Z, Ståhlberg J, Várnai A, Lersch M, Bengtsson O *et al*., Development of minimal enzyme cocktails for hydrolysis of sulfite-pulped lignocellulosic biomass. *J Biotechnol* **246**:16–23 (2017).

24. Rueda C, Calvo PA, Moncalián G, Ruiz G and Coz A, Biorefinery options to valorize the spent liquor from sulfite pulping. *J Chem Technol Biotechnol* **90**:2218–2226 (2015).

25. Kadić A, Chylenski P, Hansen MAT, Bengtsson O, Eijsink VGH and Lidén G, Oxidation-reduction potential (ORP) as a tool for process monitoring of H2O2/LPMO assisted enzymatic hydrolysis of cellulose. *Process Biochem* **86**:89–97 (2019).

26. Héder M, From NASA to EU: the evolution of the TRL scale in public sector innovation. *Innov J* **22**:1–23 (2017).

27. Balan V, Chiaramonti D and Kumar S, Review of US and EU initiatives toward development, demonstration, and commercialization of lignocellulosic biofuels. *Biofuels Bioprod Biorefin* **7**:732–759 (2013).

28. Padella M, O'Connell A and Prussi M, What is still limiting the deployment of cellulosic ethanol? Analysis of the current status of the sector. *Appl Sci* **9**:21 (2019).

29. Palmqvist B, Kadić A, Hägglund K, Petersson A and Lidén G, Scale-up of high-solid enzymatic hydrolysis of steam-pretreated softwood: the effects of reactor flow conditions. *Biomass Convers Bior* **6**:173–180 (2016).

30. Koppram R, Nielsen F, Albers E, Lambert A, Wännström S, Welin L *et al*., Simultaneous saccharification and co-fermentation for bioethanol production using corncobs at lab PDU and demo scales. *Biotechnol Biofuels* **6**:2 (2013).

31. Müller G, Várnai A, Johansen KS, Eijsink VGH and Horn SJ, Harnessing the potential of LPMO-containing cellulase cocktails poses new demands on processing conditions. *Biotechnol Biofuels* **8**:187 (2015).

32. Westereng B, Agger JW, Horn SJ, Vaaje-Kolstad G, Aachmann FL, Stenstrøm YH *et al*., Efficient separation of oxidized cello-oligosaccharides generated by cellulose degrading lytic polysaccharide monooxygenases. *J Chromatogr* **1271**:144–152 (2013).

33. Zhu JY, Pan XJ, Wang GS and Gleisner R, Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour Technol* **100**:2411–2418 (2009).

34. Ma R, Xu Y and Zhang X, Catalytic oxidation of biorefinery lignin to value-added chemicals to support sustainable biofuel production. *ChemSusChem* **8**:24–51 (2015).

35. Boatright WL, Oxygen dependency of one-electron reactions generating ascorbate radicals and hydrogen peroxide from ascorbic acid. *Food Chem* **196**:1361–1367 (2016).

36. Frankó B, Jovanovic H, Galbe M and Wallberg O, The effect of blending spruce and poplar on acid-catalyzed steam pretreatment and enzymatic hydrolysis. *Bioresour Technol Rep* **7**:100241 (2019).

37. Eijsink VG, Petrovic D, Forsberg Z, Mekasha S, Røhr ÅK, Várnai A *et al*., On the functional characterization of lytic polysaccharide monooxygenases (LPMOs). *Biotechnol Biofuels* **12**:58 (2019).

38. Schuldiner KS, Piersma BJ and Warner TB, Potential of a platinum electrode at low partial pressures of hydrogen and oxygen 2. An improved gas-tight system with a negligible oxygen leak. *J Electrochem Soc* **113**:573–577 (1966).

39. Calderbank PH and Mooyoung MB, The continuous phase heat and mass-transfer properties of dispersions. *Chem Eng Sci* **16**:39–54 (1961).

40. Rosales-Calderon O and Arantes V, A review on commercial-scale high-value products that can be produced alongside cellulosic ethanol. *Biotechnol Biofuels* **12**:240 (2019).

41. Sharma S, Hansen LD, Hansen JØ, Mydland LT, Horn SJ, Øverland M *et al*., Microbial protein produced from brown seaweed and spruce wood as a feed ingredient. *J Agric Food Chem* **66**:8328–8335 (2018).

Thales H. F. Costa

Dr Costa is a postdoctoral researcher in the Faculty of Chemistry, Biotechnology and Food Science at the Norwegian University of Life Sciences (NMBU). His research is focused on bioprocessing of lignocellulosic biomass into fuels and valuable chemicals.

Adnan Kadić

Dr Kadić is a postdoctoral researcher in the Department of Chemical Engineering at Lund University in Sweden. His research is focused on the development of lignocellulosic biorefineries, with a special focus on rheology, mixing, and scale-up.

Piotr Chylenski

Piotr Chylenski is currently working as a project manager at the company SERO AS. Previously he was a postdoctoral researcher at the Norwegian University of Life Sciences, working on industrial applications of LPMO-containing commercial cellulase cocktails.

Anikó Várnai

Anikó Várnai is a researcher at the Norwegian University of Life Sciences. Her research focuses on understanding how fungal biomass-degrading enzymes work in nature and in industrial biomass conversion processes, and on applying that knowledge for process optimization to maximize biomass saccharification yields.

Oskar Bengtsson

Oskar Bengtsson is currently the section manager for the business development R&D group at Borregaard AS. He has a PhD in applied microbiology from Lund University in Sweden, specializing in microbial physiology, genetic engineering, and metabolic engineering. Bengtsson started as research scientist at Borregaard AS in 2013 and has been involved primarily in projects related to enzymatic hydrolysis and fermentation.

Gunnar Lidén

Gunnar Lidén is professor in chemical engineering at Lund University in Sweden. His research field is biochemical engineering, and he has studied lignocellulose conversion into fermentation products such as ethanol and carboxylic acids. His most recent research concerns lignin valorization. He obtained his PhD degree from Chalmers University of Technology, Gothenburg, Sweden.

Vincent G.H. Eijsink

Vincent Eijsink is a professor at the the Norwegian University of Life Sciences. His work is focused on enzymes involved in processing of chitin and lignocellulosic biomass, and includes the discovery of LPMO activity in 2010. He obtained his PhD degree from the University of Groningen, the Netherlands.

Svein Jarle Horn

Horn is a professor of bioprocess technology at the Norwegian University of Life Sciences. His research has focused on biorefining, applied enzymology, fermentation, and biogas processes. He has a PhD from the Norwegian University of Science and Technology on the topic of seaweed processing.