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Virginie Steinmetz, Maud Villain-Gambier, Armand Klem, Isabelle Ziegler, Stephane Dumarcay, et al..
In-situ extraction of depolymerization products by membrane filtration against lignin condensation.
Bioresource Technology, 2020, 311, pp.123530. 10.1016/j.biortech.2020.123530 . hal-02882142

HAL Id: hal-02882142

<https://hal.science/hal-02882142>

Submitted on 11 Sep 2020

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In-situ extraction of depolymerization products by membrane filtration against lignin condensation

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Abstract: Catalytic depolymerization of lignin is a challenging process due to competitive repolymerization reactions. In this paper, the oxidative depolymerization of lignin was catalyzed by a commercial laccase both in a batch experiment and in a membrane bioreactor using the same catalytic conditions. The membrane bioreactor was previously optimized to reach high permeation flux ($25 \text{ L}\cdot\text{h}^{-1}\cdot\text{m}^{-2}$) during lignin diafiltration. While the lignin was exclusively condensed in the batch experiment leading to high molecular weight macromolecules (from 9 to 16 kDa), its depolymerization was effective in the bioreactor producing fragments of less than 1 kDa thanks to the *in-situ* extraction of the reaction products. This paper demonstrates that the reactor configuration is playing an essential role in triggering or preventing lignin condensation. It also reports the first proof-of-concept demonstrating that *in-situ* membrane extraction of the reactive fragments of lignin from the bulk medium can be useful against detrimental repolymerization reactions.

Keywords: lignin depolymerization, membrane filtration, bioreactor, condensation, laccase.

1. Introduction

The development of a sustainable and renewable economic model is one of the most important challenges of our present society due to the limited stock of fossil resources, the increasing demand for fuels and chemicals and the harmful environmental impact of various current processes. For this very purpose, the lignocellulosic biomass, massively produced by the forestry and agricultural activities, can be used as a carbon neutral and non-edible renewable entry material (Sun et al., 2018). The deconstruction of this biomass gives access to several chemical streams mainly arising from its main constituents: cellulose, hemicelluloses and lignins. Different studies underlined that the profitability of future lignocellulosic biorefineries relies in the complete valorization of these three constituents to overcome the cost associated to processing and pretreatments (Treasure et al., 2014; Zakzeski et al., 2010).

In the last decades, significant advances have been achieved in the development of sustainable processes targeting either the fractionation of the lignocellulosic biomass or the conversion of its constituents into valuable fuels and chemicals (De Bhowmick et al., 2018; Yiin et al., 2018; Yoo et al., 2020). As the most abundant aromatic macromolecules on earth, lignins can be valorized for the production of dispersants, adhesives, resins, composite biomaterials or even carbon fibers in replacement to the petroleum-derived products currently on the market (Ragauskas et al., 2014; Rajesh Banu et al., 2019). Further upgrading of lignins into higher added-value chemicals can also be achieved by catalytic depolymerization leading to the production of aromatic platform molecules or functionalized ingredients for food, pharmaceuticals, cosmetics and other commodity products (Isikgor and Becer, 2015; Sun et al., 2018). However, up to date and despite the extensive worldwide researches, such lignin valorizations remain extremely limited at industrial scale, mainly due to its heterogeneous structure and resistance to conversion treatments under mild conditions.

Various lab-scale protocols have been developed for the depolymerization of lignin including thermal (pyrolysis, micro-waves or photo-induced methods), chemical (oxidation, reduction, solvolysis) and biological (enzymatic) catalytic processes (Rahimi et al., 2014; Liu et al., 2019; Raikwar et al., 2019; Picart et al., 2017; Bugg and Rahmanpour, 2015). All these methods mainly target the cleavage of aryl ether bonds (β -O-4) which represent 55 to 60 % of the total interunit linkages in native lignin. However, in practice, technical lignins possess lower aryl ether content due to their fractionation processes and their depolymerization is further impaired by repolymerization reactions leading to low monomer yields (Shuai and Saha, 2017). The repolymerization of lignin decomposition products through C-C linkages is also known as lignin condensation since the resulting lignin structure is highly recalcitrant to further degradation. Lignin condensation is one of the main impediments limiting the development of high-yield depolymerization processes (Wang et al., 2019; Yu et al., 2019).

Different mechanisms are responsible for repolymerization reactions depending on the catalytic process used. But, in every catalytic process, lignin depolymerization and condensation occur as two competing reactions. Achieving high-yield lignin depolymerization thus relies in finding the appropriate catalytic conditions favoring the depolymerization reactions against the repolymerization ones. The use of specific solvent or additives such as ethylene glycol and dimethyl carbonate have been reported to prevent lignin condensation by in-situ derivatization of the depolymerization products (Dabral et al., 2018; Deuss et al., 2017). The chemical functions involved in the formation of the reactive intermediates can also be trapped by capping agents. Shuai *et al.* reported the positive effect of protecting the α -hydroxyls groups on lignin condensation by forming 1,3-dioxane structures with formaldehyde (Shuai et al., 2016). Kim *et al.* succeeded in limiting char formation during thermal degradation of lignin by a selective methylation of the phenolic hydroxyl groups before depolymerisation (Kim et al., 2017). In recent years, a strategy, which involved the use of pre-oxidation method to activate lignin β -O-4 linkages, has been employed to promote lignin depolymerization under mild conditions (Guo et al., 2019; Zhang et al., 2017). Near theoretical yields of lignin monomers could be obtained by this method (Rahimi et al., 2014). Another original approach was proposed by Li *et al.* who catalyzed the lignin depolymerization on solid state (Li et al., 2018). This way, the mobility of the reactive intermediates was limited thus avoiding their direct interaction and linkage. This physical approach is interesting as it does not require further chemical addition or pre-oxidation step. However such process cannot be applied to technical lignin streams already isolated from the lignocellulosic biomass by conventional pretreatments.

In this work, a commercial laccase was used to catalyze the depolymerization of a lignin stream recovered from the process water of a thermomechanical pulping company. The enzymatic catalysis was selected as a sustainable way to depolymerize this lignin under mild

conditions (Liu et al., 2020). Laccases (EC 1.10.3.2) are multi-copper oxidases, of 50 to 110 kDa, known for their role both in the biosynthesis and biodegradation of lignin (Wong, 2009). They are able to perform hydrogen abstraction at the phenolic moieties in lignin leading to the formation of phenoxy radicals which are responsible for the cleavage of aryl ether linkages (Higuchi, 1989). However, these radicals can also undergo free radical coupling leading to lignin condensation in aqueous media (Longe et al., 2018). To prevent the repolymerization reactions without any solvent or chemical addition, an original approach was employed by developing a membrane bioreactor. In this reactor, the *in-situ* extraction of the lignin fragments by membrane filtration was used to shift the equilibriums in favor of lignin depolymerization. The extraction of the radicals from the bulk medium was also expected to prevent lignin condensation by physically isolating the reactive fragments to limit their repolymerization. The use of membrane filtration as a tool to mimic the natural compartmentalization of lignin and its decomposition products has already been mentioned in the literature and supposed to be helpful against lignin condensation (Roth and Spiess, 2015). However, this claim has never been demonstrated and applied to the enzymatic depolymerisation of a technical lignin. This paper thus provides the first proof-of-concept of the interest of membrane filtration in this field.

To be efficient, the bioreactor was conceived to maximize the transfer of the radical fragments throughout the membrane and reach high productivity, meaning high permeation flux. In membrane filtration, the productivity is directly and mainly linked to the transmembrane pressure (TMP): the highest the TMP, the highest the productivity. However, membrane fouling can be induced and exacerbated by high TMP (Chen et al., 2011). To limit this phenomenon, different membrane materials and cross flow velocities were screened in this paper as these parameters were reported to play a critical role in membrane fouling (Puro et al., 2010). The compatibility between the membrane and the commercial laccase was also

studied. Finally, the lignin was treated by the enzyme both in batch and in the optimized membrane bioreactor to evaluate the impact of the *in-situ* membrane extraction on lignin depolymerization and condensation.

2. Material and methods

2.1. Material and equipment

The lignin used in this work has been recovered from the process water of a thermomechanical pulping company using a membrane filtration process as previously published (Steinmetz et al., 2019). The commercial laccase from *Aspergillus sp.* has been purchased from Sigma Aldrich (Novozym, 51003) as a liquid stock solution and stored at 4°C. The 5 kDa polyethersulfone (PES) disc membranes came from Sartorius (SART14629-63-D) and the regenerated cellulose (RC) ones from Merck Millipore (PLCC07610). All membranes had a diameter of 76 mm and a surface area of 41.8 cm². An ultrafiltration stirred cell of 400 mL was used as membrane bioreactor (Amicon 5124).

2.2. Membrane adsorption tests

Two types of membrane materials (PES and RC) were assessed for their compatibility with the lignin substrate. The deionized water flux of each membrane was measured before and after static adsorption of the lignin solution at 500 mg.L⁻¹ for 24 h at 25 °C. Membrane permeability to deionized water (DWP) was calculated as the slope of the linear curve correlating deionized water flux (L.h⁻¹.m⁻²) to transmembrane pressure (TMP, bar). The permeability loss caused by adsorption phenomena was then calculated as:

$$\text{Permeability loss (\%)} = 100 - \frac{\text{DWP}_{\text{after}} \times 100}{\text{DWP}_{\text{before}}} \quad (1)$$

2.3. Laccase resistance to stirring

The ultrafiltration stirred cell was equipped with a 5 kDa RC membrane and filled with a laccase solution at 0.08 U.mL^{-1} . The cell was assembled on top of a magnetic stirrer. After 1 h of equilibration in static mode, the stirring was set to 50 revolutions per minute (rpm) and the enzymatic solution left under agitation for 4 h. Samples of enzymatic solution were taken every hour for laccase activity measurements. The laccase activity was quantified by following the oxidation of ABTS at 420 nm for 3 min. One unit (1 U) of laccase was defined as the amount of enzyme that oxidizes one micromole ($1 \mu\text{mol}$) of ABTS per minute. In brief, $600 \mu\text{L}$ of laccase solution were transferred in a micro cuvette with $300 \mu\text{L}$ of phosphate citrate buffer pH 4.5 and $100 \mu\text{L}$ of 5 mM ABTS solution. An extinction coefficient of $36000 \text{ L.mol}^{-1}.\text{cm}^{-1}$ was used for calculations and each sample was assayed in triplicate. The overall protocol was repeated two times with a stirring speed increased at 100 and 200 rpm. A new solution of laccase at 0.08 U.mL^{-1} was prepared for each experiment. The static stability of laccase was also evaluated during 4 h. The cross-flow velocity (CFV) associated to each stirring speed was calculated as previously described by Al Rudainy *et al* (Al-Rudainy et al., 2017). The resulting estimated CVF corresponding to 50; 100 and 200 rpm are 0.5; 1 and 2 m.s^{-1} respectively.

2.4. Laccase and RC membrane compatibility

The ultrafiltration stirred cell was equipped with a 5 kDa RC membrane and filled with deionized water to determine its initial DWP as mentioned before. The cell was then filled with a laccase solution at 0.08 U.mL^{-1} and left at room temperature without agitation. After 36 h, the laccase solution was filtered through the 5 kDa RC membrane in concentration mode until reaching a volume reduction factor of 8. Permeate was continuously collected by fractions of 20 mL and each fraction was assayed for its laccase activity by using the ABTS oxidation test. At the end of the filtration, the DWP of the membrane was again calculated and compared to its initial value.

2.5. Lignin depolymerization in batch experiment

The lignin depolymerization in batch was carried out in duplicate. The working volumes were set to 20 mL and the experiments conducted in 50 mL centrifugal plastic tubes. Each tube contained 12 mg of lignin, 0.08 U of laccase (filtered at 0.2 μm) and 10 mL of phosphate citrate buffer pH 4.5. The tubes were filled up with deionized water and incubated at 25 °C on an orbital shaker set up at 200 rpm for 5 days. Samples were taken after 30 min, 3 h, 6 h, 24 h and 120 h. They were immediately neutralized to pH 12 by addition of 10 M sodium hydroxide solution (NaOH) and frozen until analysis.

2.6. Lignin depolymerization in membrane bioreactor

The ultrafiltration stirred cell was equipped with a 5 kDa RC membrane and filled with 100 mL of working solution. This solution contained 60 mg of lignin dissolved in 90 mL of phosphate citrate buffer pH 4.5 and filled up with deionized water in the control condition or 60 mg of lignin and 0.4 U of laccase dissolved in 50 mL of phosphate citrate buffer pH 4.5 and 50 mL of deionized water in the biodegradation experiment. The cell was assembled on top of a magnetic hotplate stirrer and connected to a compressed air stream set up at 4 bars. The working solution was maintained at 28 °C and stirred at 100 rpm ($\text{CFV} = 1 \text{ m}\cdot\text{s}^{-1}$). The reactor was assimilated to a perfectly stirred reactor. The filtration was done on a diafiltration mode by maintaining the working volume at +/- 15 % of its initial value by regular addition of phosphate citrate buffer. The diafiltration was performed during 4 hours and 30 minutes corresponding to 5 hours and 15 minutes of laccase treatment due to the regular interruptions for refill. Permeate was continuously collected and the collection tubes were renewed every 30 min. The Figure 1 illustrates the bioreactor installation and specifies the assignments used in this paper for all the different fractions generated during the process. At the end of the filtration, the pH of all fractions was measured and the permeate flow was assessed by weighting the samples P1 to P9. The retentate was collected and precisely adjusted to 100 mL

with buffer before analysis. The repartition of the initial lignin content between all the different fractions was followed by spectrophotometric measurement at 240 nm. An extinction coefficient of $12 \text{ L}\cdot\text{g}^{-1}\cdot\text{cm}^{-1}$ was used for lignin quantification (Sluiter et al., 2008).

2.7. Size exclusion chromatography (SEC)

Molecular weight analyses were performed on a Prominent Shimadzu HPLC equipped with a guard column Phenomenex Polysep GFC-P (7.8 x 35 mm) and two analytical columns Phenomenex Polysep P3000 (7.8 x 300 mm) and P2000 (7.8 x 300 mm). An aqueous method was used with a solution of 10 mM NaOH as mobile phase. The samples were filtered through $0.45 \mu\text{m}$ PTFE membranes and $20 \mu\text{L}$ were injected in the system. The separation was performed at $30 \text{ }^\circ\text{C}$ with a flow rate of $0.5 \text{ mL}\cdot\text{min}^{-1}$ during 45 min. An ultraviolet (UV) spectroscopy detector (Shimadzu SPD-20A) was used and set at 280 nm. Data were treated using LabSolutions software. SEC was employed to monitor the evolution of the lignin molecular weight (M_w) and polydispersity index (PI) over time during the biocatalysis assays. The M_w in Daltons were extracted for each lignin population from the chromatograms and expressed as relative M_w in percent using the following equation:

$$\text{Relative } M_w (\%) = \frac{M_w(\text{Da})_t \times 100}{M_w(\text{Da})_i} \quad (2)$$

$M_w(\text{Da})_i$ corresponds to the initial value of lignin M_w in Daltons before the addition of the laccase. $M_w(\text{Da})_t$ represents the lignin M_w in Daltons in the sample taken after t hours of biocatalysis. The PI of all samples was calculated for the entire weight distribution (single peak integration). By definition, PI reflects the diversity of M_w in the sample and increases with the width of the distribution.

3. Results and discussion

3.1. Development of the membrane bioreactor

All the parameters of the bioreactor have been selected to maximize its productivity and ensure a high transfer rate of the reactive fragments of lignin through the membrane. The working solution volume was deliberately maintained low in order to minimize the hydraulic residence time of the reactive fragments to prevent their repolymerization. The TMP was set at 4 bars corresponding to the maximum pressure recommended by the cell constructor in diafiltration mode. To avoid membrane fouling in these conditions, the membrane material was selected after adsorption tests with the lignin substrate and the cross-flow velocity selected in regards to its impact on the laccase activity. The compatibility between the selected membrane and the commercial laccase was also checked by evaluating the laccase retention and permeation flux during filtration.

3.2. Adsorption study

Fouling is a complex phenomenon inherent to membrane filtration processes. It arises from the accumulation and concentration of molecules at the surface of the membrane and results in a great loss of the process productivity. If the molecules to be filtered present an affinity for the membrane material, adsorption interactions may be the cause of an important membrane fouling. Several studies about lignin filtration reported that such interactions were causing pore blocking and non-reversible fouling on hydrophobic membranes and that hydrophilic membranes were mandatory in achieving high permeation flux (Al-Rudainy et al., 2019; Puro et al., 2010; Servaes et al., 2017). In this work, two hydrophilic membranes (i.e. PES and RC) were studied for their compatibility with the lignin substrate. PES was selected as this material is recommended for high permeation flux in aqueous filtration and RC for its low adsorption property. The results of the adsorption tests conducted with these two membranes are presented in Fig. 2.

Adsorption interactions occurred between the lignin and the PES membrane as can be seen in Fig. 2A. The PES membrane lost 52 % of its DWP after the static adsorption step and a yellow coating layer appeared on the surface of the membrane. In contrast, the permeability of the RC membrane was not affected at all by the lignin (Fig. 2B). These results are really close to the ones obtained by Li et al. during their adsorption tests of kraft lignin on RC and PS membranes (Li et al., 1996). The PES membrane displays the same behavior as reported for the PS membrane in their study. The present results are also in agreement with those of Puro et al. who compared the performances of RC and PES membranes for the filtration of two process waters from a chemithermomechanical pulp mill (Puro et al., 2010). In their experiments, the PES membrane presented higher fouling than the RC membrane. They explained this result by the difference in membrane hydrophilicity, which is higher for the RC membrane. Possibly, the aromatic structures of PES and PS membranes may also be incriminated for their specific interactions with lignin. Anyhow, these membranes are not suitable for lignin filtration and the adsorption interactions seem to be independent from the lignin origin. Based on these results, the RC membrane was selected for further development of the bioreactor.

3.3. Cross flow velocity and laccase stability

Other forms of fouling are due to the concentration polarization phenomenon or cake layers formation at the surface of the membrane. These layers are caused by the high concentration of molecules around the membrane and are responsible for a great part of productivity loss by provoking unproductive osmotic pressure, gel formation or even precipitation at the filtration area. While working under high TMP, the cross flow circulation generated by the stirring plays a critical role in preventing such phenomena by maintaining a physical perturbation on top of the membrane (Al-Rudainy et al., 2019; Bhattacharjee and

Bhattacharya, 2006). This perturbation is even more important using a stirred cell as this type of reactor does not present any back pulse system and is equipped with flat membrane discs which are more prone to fouling than the membranes in tubular configurations. However, biological catalysts such as enzymes can be particularly sensitive to shear stress leading to their inactivation (Nguyen et al., 2013). In this sense, the resistance of the commercial laccase to different stirring speeds was evaluated in the reactor. The results are presented in Fig. 3A.

The laccase activity remained stable whatever the stirring speed tested in this experiment. This result underlines a good resistance of this enzyme to physical shear stress and, as a consequence, the highest speed tested (i.e. 200 rpm) could be selected to minimize the membrane fouling without affecting the biocatalysis yield. However, it was observed during a preliminary diafiltration test that such high speed was not compatible with the bioreactor design as air started to pass through the membrane as soon as the permeation started to reduce the working solution volume. Instead of increasing the initial filling of the reactor, a stirring speed of 100 rpm was finally chosen for lignin depolymerization. It is still worth noting that highest cross flow velocity, at least up to 2 m.s^{-1} , can be considered if using this enzyme in other bioreactor configurations.

3.4. Laccase and RC membrane compatibility

The effect of a laccase treatment on the RC membrane was evaluated by measuring its DWP after 36 h of incubation and filtration of the enzyme solution. The result, presented in Fig. 3B, indicates that the integrity and filtration capacity of the RC membrane were not affected by this enzymatic treatment as there was no evolution of its DWP after incubation. In this sense, this result underlines that neither the laccase, nor other proteins and organic compounds which may be present in the enzymatic stock solution, led to pore blocking,

adsorption phenomenon or membrane degradation. The laccase solution selected for this work is thus compatible with the RC membrane.

The permeate fractions collected during the filtration of the enzyme solution were assayed for their laccase activity and any activity was depicted in any tube. This result demonstrates the total retention of the laccase by the 5 kDa membrane, as expected with this low molecular weight cut-off.

3.5. Lignin catalytic depolymerization by laccase

The lignin recovered from the process water has been recently thoroughly characterized and reported as one of the most native lignin sourcing derived from the pulp and paper industry (Steinmetz et al., 2019). This lignin presents a low condensation degree and high aryl ether content of 27 β -O-4 linkages per 100 aromatic rings. Its structural composition, similar to Björkman milled wood lignin, thus presents a real advantage when addressing lignin depolymerization (Bouxin et al., 2015). SEC, coupled to a UV detector at 280 nm, was employed to monitor its depolymerization by the commercial laccase both in a batch experiment and in the previously developed bioreactor.

As proteins absorb at 280nm, the chromatogram of the laccase stock solution, injected at the same concentration as used in the depolymerization experiments ($0.08 \text{ U}\cdot\text{mL}^{-1}$), was recorded as a reference. Almost no signal was depicted at 280 nm for this concentration ensuring that there is no interference between the proteins and the lignin signals in the SEC analyses (data not shown). The molecular weight distribution of the initial lignin solution (IL) is presented in Figure 4A. This distribution presents a bimodal shape with two lignin populations: one presenting a higher molecular weight (Mw) of 9 kDa, referred to as HML, and the other characterized by a lowest Mw of 1 kDa and referred to as LML.

3.5.1. Lignin depolymerization in batch experiment

The evolution of the relative Mw of these two populations during the laccase biocatalysis in batch are presented in Fig. 4B and 4C. On one hand, in Fig. 4B, a progressive increase of the relative Mw of HML was observed over the 5 days of this experiment from 100 to 180 % (9 to 16 kDa). Adsorption interactions between laccases and lignin have previously been reported in the literature and could be suspected to cause this Mw increase (Saarinen et al., 2009). However, the average Mw of fungal laccases usually range between 60 and 70 kDa and thus, such interactions would have led to a much more important Mw increase, with molecules around 70-80 kDa at least (Giardina et al., 2010). Therefore, the Mw increase of the HML population is attributed to lignin condensation in this work. On the other hand, in Fig. 4C, the steadiness of the relative Mw of LML underlines that neither phenolic monomers nor lignin fragments with smaller Mw than 1 kDa were produced during this batch experiment. Ultimately, it can be concluded that the operating conditions employed in this batch experiment favored lignin condensation over lignin depolymerisation.

As can be seen on Fig. 4D, the lignin condensation started quite early during the biocatalytic process. After only 6 hours of laccase treatment, the majority of the lignin molecules were already condensed into higher molecular weight structures as pointed out by the important shift and intensity of the HML population. The condensation reactions involved both the HML and LML populations as depicted by the continuous decrease of the LML signal intensity. In the literature, polymerization triggered by laccases has been reported to predominantly involve the free phenolic functions initially present in the structure of the technical lignins (Roth and Spiess, 2015). However, new free phenolic moieties are also released during lignin depolymerization by laccases following aryl ether bond cleavages. Thus the polymerization activity of laccases can counter its depolymerization one by repolymerizing the lignin fragments. In this work, the free phenolic content of the technical

lignin used has not been previously quantified. Nevertheless, its high aryl ether content suggests a low free phenolic content as in native lignin (Cañas and Camarero, 2010). Consequently, the main mechanism suspected to cause this lignin condensation is the repolymerization of the phenolic fragments. This experiment was thus reproduced in the previously developed bioreactor with an *in-situ* extraction of the low molecular weight phenolic compounds in an attempt to avoid their repolymerization.

3.5.2. Lignin depolymerization in membrane bioreactor

The lignin depolymerization was repeated in the optimized bioreactor using the exact same catalytic conditions as in the batch experiment. A control set of data was also generated by filtering the initial lignin solution in the reactor without laccase. The process was conducted in a diafiltration mode in order to maintain a stable enzyme concentration in the bulk as in the batch experiment.

The figure 5A presents the hydraulic behaviour of the reactor during the control and biocatalysis assays. As expected, the productivity of the reactor decreased when replacing the deionized water by the lignin solution (from 33 to 25 L.h⁻¹.m⁻²). However, the permeation flux then remained perfectly stable both in the control and biocatalysis assays. In this sense, no membrane fouling was observed during the time of the experiment either due to the lignin filtration or to the presence and activity of the laccase.

Thanks to this great performance, the reactor could have been maintained for at least a few more hours. But, as can be noted in Fig. 5B, 80 % of the lignin were already filtered out of the reactor during the control assay after 4 h 30 of diafiltration so the experiment was stopped. This observation points out that the selectivity of the 5 kDa membrane does not allow a good retention of the initial substrate in the reactor. However, in this proof-of-concept study, high productivity was intentionally preferred over high selectivity to maximize the

effect of the *in-situ* extraction. This figure also illustrates the constant dilution of the lignin in the permeate fractions due to the diafiltration mode. When comparing the control and laccase assays, a difference in lignin retention can be observed as 28 % of lignin remained in the retentate at the end of the experiment with laccase against 20 % for the control. This difference implies that the lignin was modified during the biocatalysis.

The evolution of the polydispersity index (PI) of lignin in the permeate fractions comforts this result (Fig. 5C). The lignin presents higher PI indexes in the laccase experiment meaning that new molecular weights of lignin molecules were produced by the enzyme. The laccase activity on lignin can be depicted as fast as half an hour after the beginning of the biocatalysis. Whether those new molecules were associated to condensation or depolymerization reactions was determined by following the evolution of the highest and lowest molecular weight of lignin in all the different fractions generated by the process.

As can be seen in Fig. 6A and 6B, the relative Mw of the HML population was higher in the retentate and in the final permeates fractions (P7, P8, P9) than in the initial lignin solution (IL) while using the laccase. However, the same Mw increase was also observed during the control filtration without enzyme. Therefore, this evolution is attributed to the enrichment of these fractions in high molecular weight lignin molecules due to the filtration of the smallest one and cannot be associated to lignin condensation. The evolution of the relative Mw of HML with laccase perfectly fits with the control curve both in the retentate and permeates fractions, underlying that no condensation reactions occurred during the bioreactor experiment.

In this sense, the increase of polydispersity pointed out in Fig. 5C is associated to lignin depolymerization. Indeed, the evolution of the LML population significantly differs from the control condition while using the laccase (Fig. 6C and 6D). In the control condition the average MW of the LML population slightly decreased (25 %) in the retentate and

remained stable in the permeate fractions. In comparison, the enzyme induced a higher reduction of the Mw in the retentate (61 %) and also led to an important reduction of the Mw in the permeate fractions reaching 46 % in P9. As the reduction of the Mw was observed in all the permeate fractions (from P1 to P9), this result indicates that the lignin fragments were produced during all the 5 h 15 of biocatalysis. The average Mw of the LML population decreased from 1432 Da in IL to 777 Da in P9 and 563 Da in the retentate under the effect of the laccase.

By studying the evolution of the HML and LML populations during the control filtration, it is possible to get deeper information about the membrane selectivity. What is worth noting is the evolution of the highest Mw of lignin in the permeate (Fig. 6B). Initially, the first fraction (P1) presented a lower Mw than the IL. This result can be easily explained by the retention of the largest lignin molecules by the membrane. However, there was a progressive and constant enrichment in largest molecules in the permeate over time as underlined by the increase of the HML average Mw from P1 to P9. The membrane thus exhibited an interesting selectivity during the lignin filtration with a preferential filtration of the smallest molecules first and ultimately the filtration of the highest Mw molecules. As a consequence, the continuous production of small lignin fragments by laccase combined with this particular behaviour of the membrane must have delayed the filtration of some large lignin molecules during the laccase assay and explain the highest retention observed in Fig. 5B. An eventual steric hindrance caused by the proteins (enzymes) at the surface of the membrane may also be part of the explanation.

In the end, the oxidative depolymerization of lignin was effectively catalyzed by the commercial laccase in this bioreactor where the same catalytic conditions were used as in the batch experiment. This work underlines that, even if finding performant catalysts for lignin depolymerization is of high interest, process engineering aspects should also be considered at

an early stage. Of course, this work only provides the first proof of membrane filtration interest in enzymatic lignin depolymerization. Further studies will now follow and they will focus on the monomer yields and characterization as well as on the robustness of the process with different lignin sourcing.

4. Conclusions

This paper demonstrates that *in-situ* membrane filtration of lignin depolymerization products allows avoiding the condensation reactions occurring in batch experiment during the enzymatic depolymerization of a technical lignin. The semi-continuous depolymerization process proposed in this paper is easy to implement, without any chemical addition and operates under really mild conditions (aqueous media at nearly room temperature). A new way of designing sustainable lignin depolymerization processes is thus reported. This work is highly timely in the area of lignin catabolism and the process should help enhance the knowledge of working with biological catalyst for the production of high value aromatic compounds.

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FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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FIGURE CAPTIONS

Figure 1. Schematic representation of the membrane bioreactor installation and the different fractions generated by the diafiltration process.

Figure 2. DWP of the (A-) PES membrane and (B-) RC membrane before (×) and after (●) their incubation in a lignin solution for 24 h.

Figure 3. (A-) Impact of different stirring speeds on the laccase activity; (B-) DWP of the RC membrane before (×) and after (●) its incubation with the commercial laccase at 0.08 U.mL^{-1} for 36 h.

Figure 4. SEC results of lignin depolymerization in batch experiment: (A-) molecular weight distribution of the initial lignin solution (IL); evolution of the relative molecular weight of HML (B-) and LML (C-) over time; and (D-) evolution of the molecular weight distribution of lignin during laccase treatment.

Figure 5. (A-) Evolution of the permeation flux, (B-) lignin distribution between the different fractions generated by the reactor and (C-) polydispersity index of the permeate fractions during the control filtration and the bioconversion assay with laccase.

Figure 6. Evolution of the relative molecular weight of HML and LML populations in the retentate (A and C respectively) and the permeate fractions (B and D).

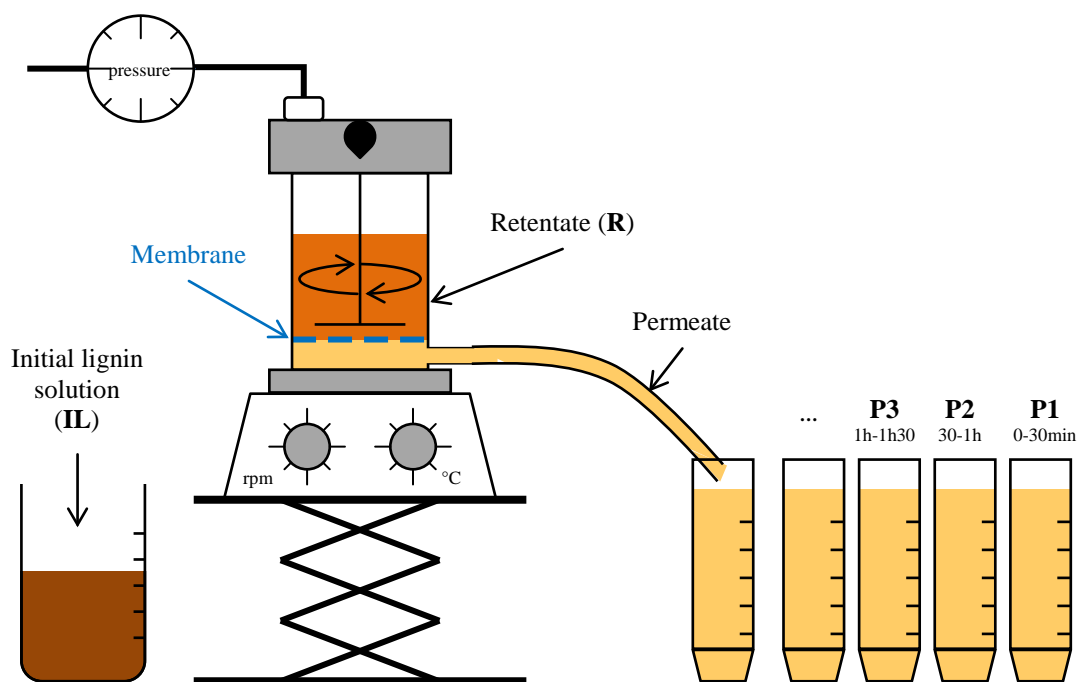


Fig. 1 double column (color online only)

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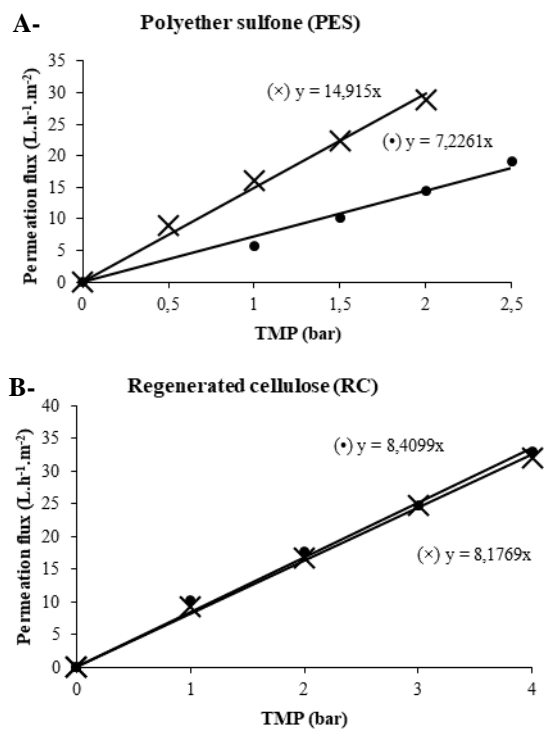


Fig. 2 single column

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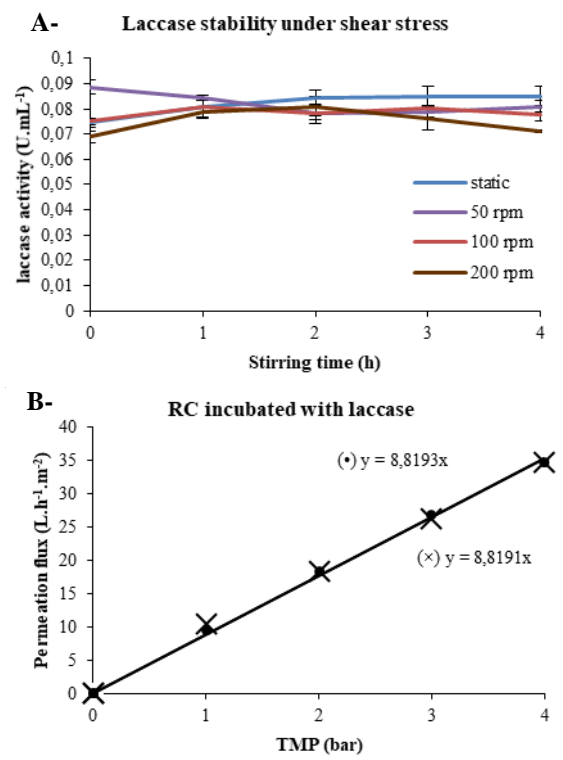


Fig. 3 single column (color online only)

10.3 × 7.5 cm

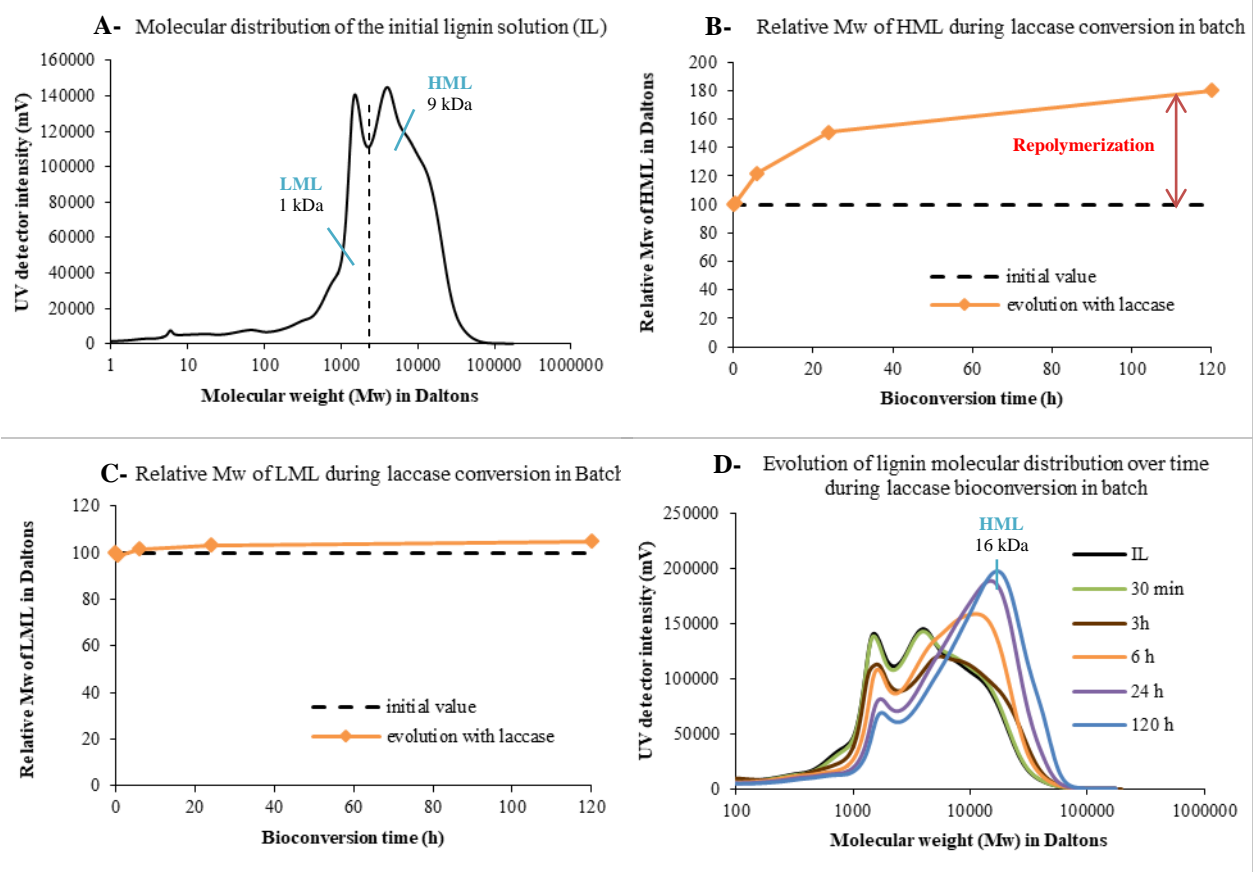


Fig. 4 double column (color online only)

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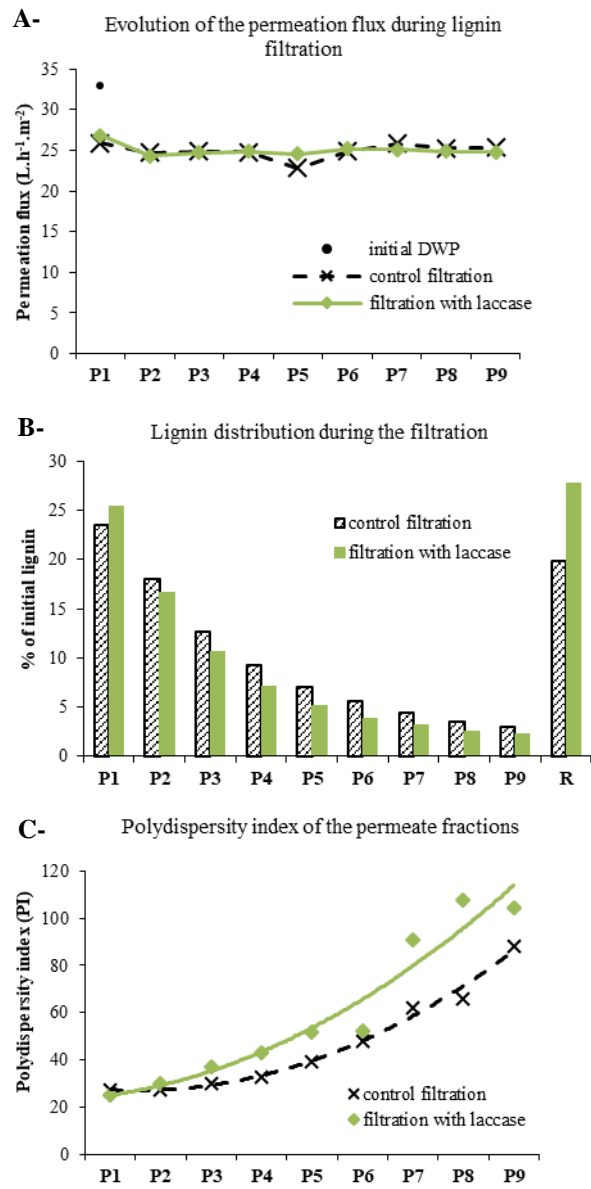


Fig. 5 single column (color online only)

16 × 8.5 cm

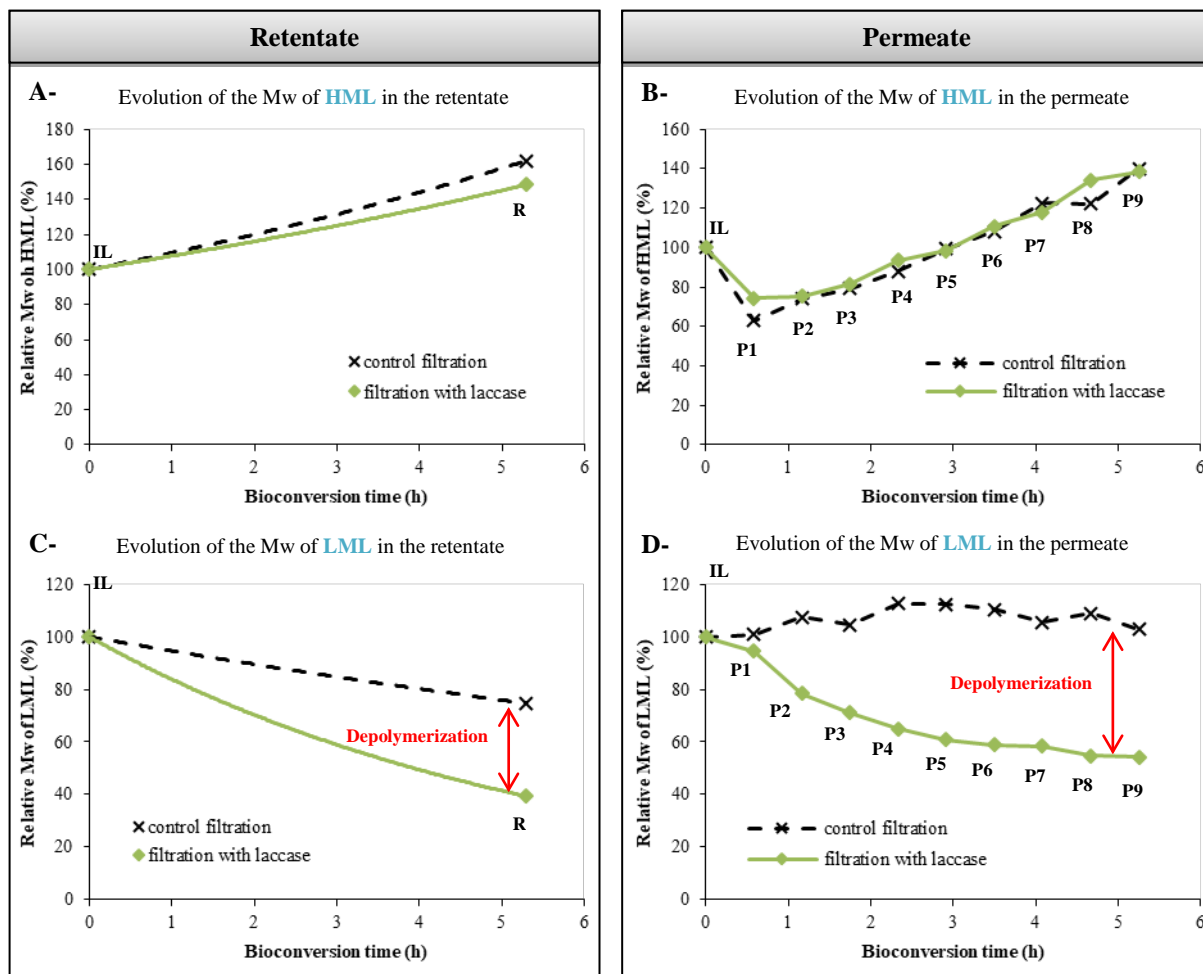


Fig. 6 double column (color online only)

13 × 16.5 cm