Mathieu, Y.; Vidal, JD.; Arribas Martínez, L.; Abad Fernández, N.; Iborra Chornet, S.; Corma Canós, A. (2020). Molecular Oxygen Lignin Depolymerization: An Insight into the Stability of Phenolic Monomers. ChemSusChem. 13(17):4743-4758. https://doi.org/10.1002/cssc.202001295

Molecular oxygen lignin depolymerization: An insight within phenolic monomers stability

PROPOSED AUTHORS LIST:

Dr. Yannick Mathieu[1], Dr. Juan D. Vidal[1], Dr. Lourdes Arribas Martínez[2], Dr. Nerea Abad Fernández[2], Prof. Sara Iborra[1] and Prof. Avelino Corma[1*].

[1] Instituto de Tecnología Química, Universitat Politècnica de València - Consejo Superior de Investigaciones Científicas, Avenida de los Naranjos s/n, 46022 Valencia, Spain.

[2] Técnicas Reunidas, José Lladó Technology Centre, Proprietary Technology Development Division, C/Sierra Nevada nº16, 28830 San Fernando de Henares, Spain.

[*] acorma@itq.upv.es

Yannick Mathieu ORCID No: 0000-0001-5747-0298, Juan D. Vidal ORCID No: 0000-0002-6862-6443, Sara Iborra ORCID No: 0000-0003-1758-5095 and Avelino Corma ORCID No: 0000-0002-2232-3527.

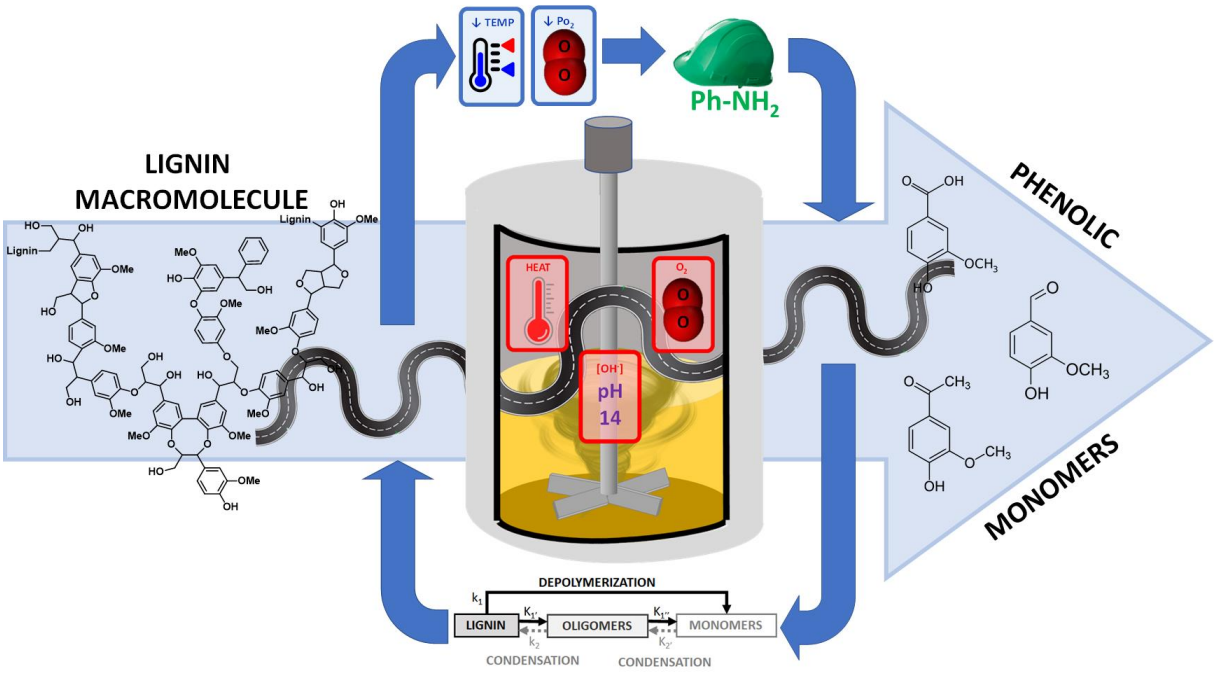
ABSTRACT

It is well known that during oxidative depolymerization of lignin in aqueous alkaline medium using molecular oxygen as oxidant, the highly functionalized primary phenolic monomers are not stable products due to various and not fully identifying secondary reaction mechanisms. However, a better understanding of the mechanisms responsible for instability of the main part of the products of interest derived from lignin is of much interest. The evaluation of their individual reactivity under oxidative conditions should significantly help to find a better way to valorise the lignin polymer and to maximize the yields of target value-added products. Consequently, the main objective of the present work was to study the individual stability of some selected lignin-based phenolic compounds such as vanillin, vanillic acid and acetovanillone, together with some other pure chemical compounds such as phenol and anisole to give an insight on the mechanisms responsible for the simultaneous formation and repolymerization of those products and the influence of the oxidation conditions. Various complementary strategies of stabilization are proposed, discussed, and applied for the oxidative depolymerization reactions of a technical lignin extracted from Pinewood with a high content of β-O-4 interconnecting bonds to try to obtain enhanced yields of value-added products.

KEYWORDS

Lignocellulosic biomass, lignin, oxidative depolymerization, alkaline medium, molecular oxygen, batch reactor, phenolic monomers, condensation reactions, stabilization strategies, chemical reversible stabilization, parametric study.

GRAPHICAL ABSTRACT



INTRODUCTION

Prosperity and sustained economic growth of the modern society strongly depends on the use of fossil resources such as crude oil, coal, and natural gas.[1] Nevertheless, by definition such resources are non-renewable and the forecasted petroleum shortage together with the increasing environmental concerns,[2] has incentivated the search for cleaner and more eco-friendly alternatives to automotive fuels and chemicals than the ones derived from the petroleum industry.[3, 4, 5, 6, 7, 8] In this context, biomass appears to be a good candidate to produce renewable fuels and chemicals. Early developments primarily concentrate on starch, sugar-rich components, and vegetable oils to produce chemicals and the so-called first-generation biofuels. However, even if this approach has demonstrated its technical feasibility,[3] and a relatively economical sustainability at industrial scale,[9] ethical concerns on the use of edible natural resources for the production of chemical commodities and automotive fuels, guided the scientific community to focus on the use of biomass raw materials which not compete directly with the human and/or animal food supply chain.[3,9] Thus, second generation bio-based technologies are currently under investigation to produce biofuels and chemicals from lignocellulosic biomass which has the advantage to be the most abundant available biomass polymer on the Earth[10] and to be often relatively inexpensive as raw material.[11]

Lignocellulosic biomass refers to a blend of three main constitutive polymers, i.e. the cellulose, hemicellulose and lignin, which are tightly bound and cross-linked to each other and is naturally present in the cell walls of the wood, plants and straws, making this raw material abundantly available from different sources such as agri-residues (i.e. corn stover, wheat and barley straws), agri-processing by-products (i.e. corn fiber, sugarcane bagasse, seed cake, …), woody biomass (hardwood and softwood) and energy crops (i.e. switch grass, poplar, banagrass, miscanthus, ...).[12] Depending on the exact nature of the biomass, different ratios of these three constitutive polymers are found and, for instance, if hardwoods usually contain around 40–55% of cellulose, 24–40% of hemicellulose and 18–25% of lignin, softwoods generally include a higher content of lignin (i.e. 25–35%) and about 45–50% of cellulose and 25–35% of hemicellulose.[13] Even if the most advanced modern biorefinery concepts have for main purpose to integrate processes and technologies for the conversion of the whole constituents of the lignocellulosic biomass to different classes of biofuels and biochemicals, most of them are focused, up to date, on utilizing easily convertible fractions such as the cellulose and hemicellulose which can be hydrolysed to sugars and then fermented to ethanol. Lignin, on the other hand, remains relatively underutilized to its real potential and is almost considered as waste.[14] In fact, due to the low reactivity, small added value, and quite heterogeneous nature of the industrially available lignins,[6] large amounts of this raw material could be left as a by-product of the biorefineries and in the pulping industry. Indeed, very few of the almost 1 million tons per year of the produced

lignosulfonates (i.e. black liquor containing a huge proportion of sulfonated lignin)[15] is valorised, and the main part is utilized as a low-grade boiler fuel to provide heat to the Kraft process.[3] However, the chemical structure of the lignin clearly indicates that it may be a good source of valuable chemicals if it could be depolymerized and converted into aromatics and/or phenolic monomers.[6,11]

The complex cross-linked structure of the native lignin is still not totally elucidated and varies depending on the exact nature of the biomass source.[16] Indeed, the native structural complexity and relatively high stability with intramolecular bonds of the lignin copolymer, mainly comes from the enzymatic activity in the plant which produces free radicals from three main phenylpropane monomer precursors (i.e. monolignols): p-coumaryl alcohol (H-unit), coniferyl alcohol (G-unit) and sinapyl alcohol (S-unit) by dehydrogenation of phenolic OH groups, allowing the initiation of a radical chain-growth polymerization and the propagation up to the chain termination leading to the complex lignin branched polymer.[19-20] The diversity in terms of percentages of these three constitutive precursors used during this natural polymerization process, is at the origin of the large variety of the lignin chemical structure from species to species. For instance, the lignin from softwood mainly contains G-units (i.e. around 90–95%), while the lignin from hardwood usually includes a mix of G- and S-units (i.e. around 25–50% and 50–75% respectively), and the lignin from grass typically is made of a blend of all three monolignols.[18, 21, 22] In addition, it is thought that this structure is significantly modified during its isolation for the obtention of technical lignins or during the Kraft process.[17,18] This leads to an extreme heterogeneity of the lignins potentially used as raw material, which has a direct effect over its reactivity and chemical properties. Another implication of the diversity in terms of percentages of constitutive monomers in different sources of lignin is the linkage irregularity connecting the alkyl-phenolic units.[21] Thus, the proportion of the major linkages found in lignin, such as β-5-, 5-5-, β-1- and β-β- linkages varies significantly according to the type of lignocellulosic biomass. This is so even if the main linkages in the lignin polymer are ether linkages, such as α-O-4-, 4-O-5- and β-O-4- that represent more than two-thirds of the total linkages, being the β-aryl ether (β-O-4) the most common with around 50 % to 60 % of total linkages in softwood and hardwood native lignin.[21, 23, 24] The predominance of the previously reported aryl ether linkages facilitates, from a chemical point of view, the depolymerization of the lignin,[25] making lignin a good candidate for the oxidation or oxidative cracking and the production of low molecular weight phenolic and aromatic chemicals.[8, 26]

Numerous possible routes to valorise lignin has been proposed and are currently thoroughly studied at laboratory scale, including cracking processes (pyrolysis, fast thermolysis, hydrogenation, etc.), hydrolysis, reduction, or oxidation reactions through homogeneous, heterogeneous, or enzymatic catalysis.[27] Among the previously mentioned upgrading strategies, oxidation reactions present interesting advantages such as the use of mild conditions, the production of high-added value

functionalized aromatic compounds and the relatively easy product separation.[28] In such context, ether-type bonds cleavage by oxidation in alkaline medium using mild oxidants such as nitrobenzene, metal oxides and molecular oxygen, caught the attention of scientists and industrials.[4, 5, 8, 29, 30] In that case, the lignin aromatic rings are preserved and aromatic aldehydes, ketones and carboxylic acids monomers such as vanillin, acetovanillone and vanillic acid can be obtained from the G-units, and syringaldehyde, acetosyringone and syringic acid from the S-units of the lignin.[31] By itself, these compounds are high added value chemicals which have numerous applications in the flavouring, fragrance, cosmetic, food and pharmaceuticals industries, and can be used as precursors for the synthesis of other fine chemicals of industrial interest.[8, 32, 33, 34, 35, 36, 37] Among the former mentioned mild oxidants, it is well accepted that nitrobenzene gives the highest yield of aldehydes and/or phenolic monomers from lignin. However, this oxidant present two limitations: it is recognised as a carcinogenic chemical, and its reduction products are difficult to separate from the reaction medium, making its use at industrial scale almost impossible.[31] However, nitrobenzene is utilized at laboratory scale as a reference reaction for the oxidative lignin depolymerization to estimate the potential of each type of lignin. On the other hand, even if air is harmless, inexpensive and, therefore, preferable from a point of view of sustainability and process economy, it gives lower conversions and selectivities than nitrobenzene to produce phenolic monomers. Then, intensive researches are being carried out to try to enhance the yields and selectivities using molecular oxygen during the oxidative depolymerization of lignin by means of heterogeneous catalysts and/or adapting the reaction conditions.[19, 23, 29, 30]

Non-catalytic and catalytic wet air oxidation (WAO) of lignin using homogeneous metal ions or heterogeneous metal oxides or supported precious metals as catalysts has been extensively studied.[38, 39] Good catalytic performances to produce aromatic aldehydes from lignin using copper-based catalysts, including homogeneous copper sulphate[40, 41, 42] and heterogeneous copper oxide,[42, 43] have been reported. Preparation of aromatic aldehydes using heterogeneous perovskite-type oxide catalysts has been also described as efficient catalysts for such purpose.[44, 45, 46, 47, 48, 49] Nonetheless, during WAO lignin upgrading, radical mechanisms take place and highly functionalized primary phenolic monomers undergo repolymerization reactions with each other to create products that are not desirable, avoiding to fully maximize the yield of desired monomers.[50] Thus, if these effects could be greatly minimized, oxidation appears to be a pathway of choice to convert lignin into value-added products, such as phenolic monomers. Then, in the present work, we have attempted to give an insight on the stability of the phenolic monomers during the alkaline oxidative depolymerization of lignin in presence of molecular oxygen to better understand the reaction network and, in particular, the reactions and mechanisms responsible for the repolymerization of the primary products. On the base

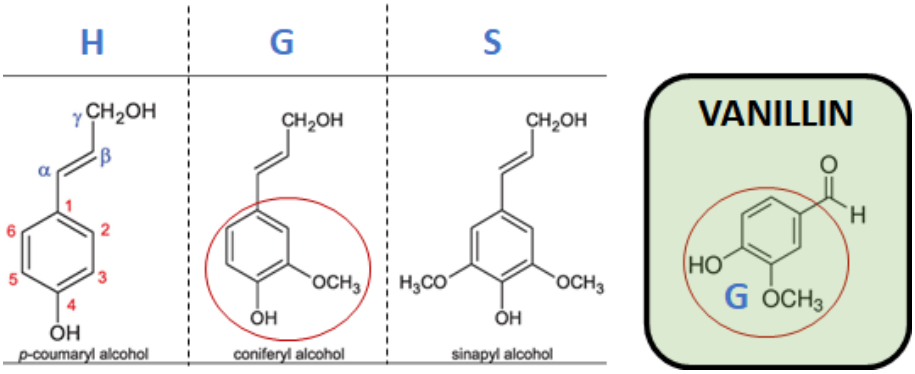
of such knowledge, some mitigation strategies are proposed and tested to maximize the production of phenolic monomers from lignin during oxidative depolymerization operations.

RESULTS AND DISCUSSION

Analysis of the stability of primary phenolic monomers products during alkaline oxidative depolymerization reactions of technical lignin in batch reactors using molecular oxygen as oxidant.

In order to clearly highlight the behavior of the main phenolic products such as aromatic aldehydes, ketones and carboxylic acids monomers during the alkaline batch oxidative depolymerization of lignin, a preliminary comparative study has been carried out using molecular oxygen or nitrobenzene as oxidative agent. In this series of experiments, batch oxidative depolymerization were performed according to the procedure presented in the experimental section. Main operative conditions were as follow: an alkaline aqueous medium with a constant base concentration of 80 g/L (i.e. NaOH), different oxygen partial pressures (PO_2) of respectively 0.5, 3.0 and 6.0 bar keeping constant the total pressure at 10 bar through the use of nitrogen, two different reaction temperatures of 133 and 163°C, a mechanical stirring of 1100 rpm and an initial lignin concentration of 5 wt.% inside the alkaline medium. Reference reactions were executed using 15 wt.% of nitrobenzene in the whole reaction medium and without oxygen but with a total pressure of 10 bar of nitrogen.

Figure 1. Chemical similarity between the coniferyl alcohol monolignol (i.e. G-unit) constitutive of the proposed technical lignin and the desired phenolic aldehyde product: the 4-Hydroxy-3-methoxybenzaldehyde or vanillin.



The processed raw material corresponds to a technical lignin isolated through acidolysis using as solvent a blend of dioxane/water 9/1 v/v containing 0.2 M of hydrochloric acid (HCl) following an adapted procedure proposed by Pepper et. Al.[51] Pinus Radiata woodchips were used as raw material and after the isolation step and purifications, the obtained technical lignin was freeze-dried to obtain a pure and dry product suitable for its further valorization through oxidative depolymerization. Detailed extraction procedure can be found in the experimental section. The choice of this softwood

raw material for the obtention of the technical lignin was motivated by: a) the high availability of such biomass, representing one the biggest wood production, notably in Spain where more than 10 % of the total wood production corresponds to Pinewood[52] and b) the interest in obtaining the highest possible yield of vanillin as high added value chemical. Indeed, as already mentioned, softwood has the advantage to be a fast-growing lignocellulosic biomass which is made up of about one-third of lignin, compared to hardwood which only has an estimated lignin content of less than one-fourth. Moreover, the intrinsic nature of the softwood lignin, almost exclusively derived from the coniferyl alcohol monolignol (i.e. G-unit), coupled with the oxidative depolymerization using mild oxidants, allow to preserve the aromaticity of the lignin and to produce high yields of aldehyde monomers and, hopefully of 4-Hydroxy-3-methoxybenzaldehyde also named vanillin (see Figure 1). Another criterion of choice has been the simplification of the analytical task as almost only G-unit derivatives such as vanillin, vanillic acid and acetovanillone are produced and only traces of S-unit derivatives such as syringaldehyde, syringic acid and acetosyringone are obtained.

Various batches of pinewood technical lignins have been prepared and the purity has been estimated in terms of Klason lignin content. For doing this, an adapted protocol based on the TP-510-42618 normative of the National Renewable Energy Laboratory (NREL) for the determination of structural carbohydrates and lignin in biomass by acid hydrolysis has been applied (see detailed procedure in the experimental section).[53] Results have shown that all the extracted technical lignins have high purity with a Klason lignin content between 90 to 95 wt.%, meaning that very few polysaccharides from the cellulose and/or hemicellulose have been extracted together with the lignin during the isolation step. On the other hand, the structural characterization of the isolated technical lignin has been done through two-dimensional ^{13}C-^{1}H Heteronuclear Single Quantum Coherence Nuclear Magnetic Resonance (2D HSQC NMR) analysis (see Figure 2), allowing the determination of the S/G/H ratio within the corresponding lignins and the quantification of the abundance of the aliphatic interconnecting bonds per 100 C9 monomers units. The signals for the different lignin linkages and the aromatic signals were assigned by comparison with the literature[54,55] and the quantification was performed following already reported methods.[56, 57, 58] The ratio of S/G/H was determined by comparing the aliphatic and aromatic signal intensities and using the integrals of the signal of the G_2 protons of the G-units, $S_{2/6}$ protons of the S-units and $H_{2/6}$ protons of the H-units, the integral values for $S_{2/6}$ and $H_{2/6}$ were divided by 2 to enable a good comparison with the G_2 value. The previously mentioned signals were also used as internal standard for determining the quantity of aliphatic interconnecting bonds of the various linkages (β-O-4, β-β, β-5) using the integrals corresponding to their α-protons and maintaining the same contour level (see detailed procedure in the experimental section). According to the 2D-HSQC NMR results, very similar chemical structures for all the different lignin batches are found and logically only traces of H- and S-units are detected confirming the almost complete G-units content of the isolated softwood lignins. At the same time, all the analyzed technical lignins are rich in β-

aryl ether units (β-O-4 linkage) with more modest amounts of phenylcoumaran (β-5 linkage) and resinol (β-β linkage). Indeed, an estimated content of around 27-30 % of β-O-4 linkages is appreciated while β-5 and β-β linkages account for only 7-8 % and less than 1 % of the interconnecting bonds, respectively. It is interesting to notice that both, the α-OH- and the γ-OH-groups of the β-O-4 linkages are completely uncapped, meaning that the lignin isolation procedure has not been too invasive and that, in particular, the primary alcohol in the β-O-4 linkage has not been esterified by the acid during the process of obtention of the technical lignins. Capping of the γ-OH-group of the β-O-4 linkage could be very detrimental for the further oxidative depolymerization as this one inhibits some of the possible cleavage mechanisms and particularly the retro-aldol reaction because the γ-OH-group cannot be oxidized, something that will subsequently restrict the C-C bond cleavage.[59,60]

Figure 2. (A) Example of 2D $^{1}H–^{13}C$ HSQC NMR spectra of the technical lignin extracted from pinewood by acidolysis using a dioxane/water blend (90/10 v/v) and 0.2 M of HCl, and (B) Identification of main aliphatic interconnecting linkages natively present in the lignin.

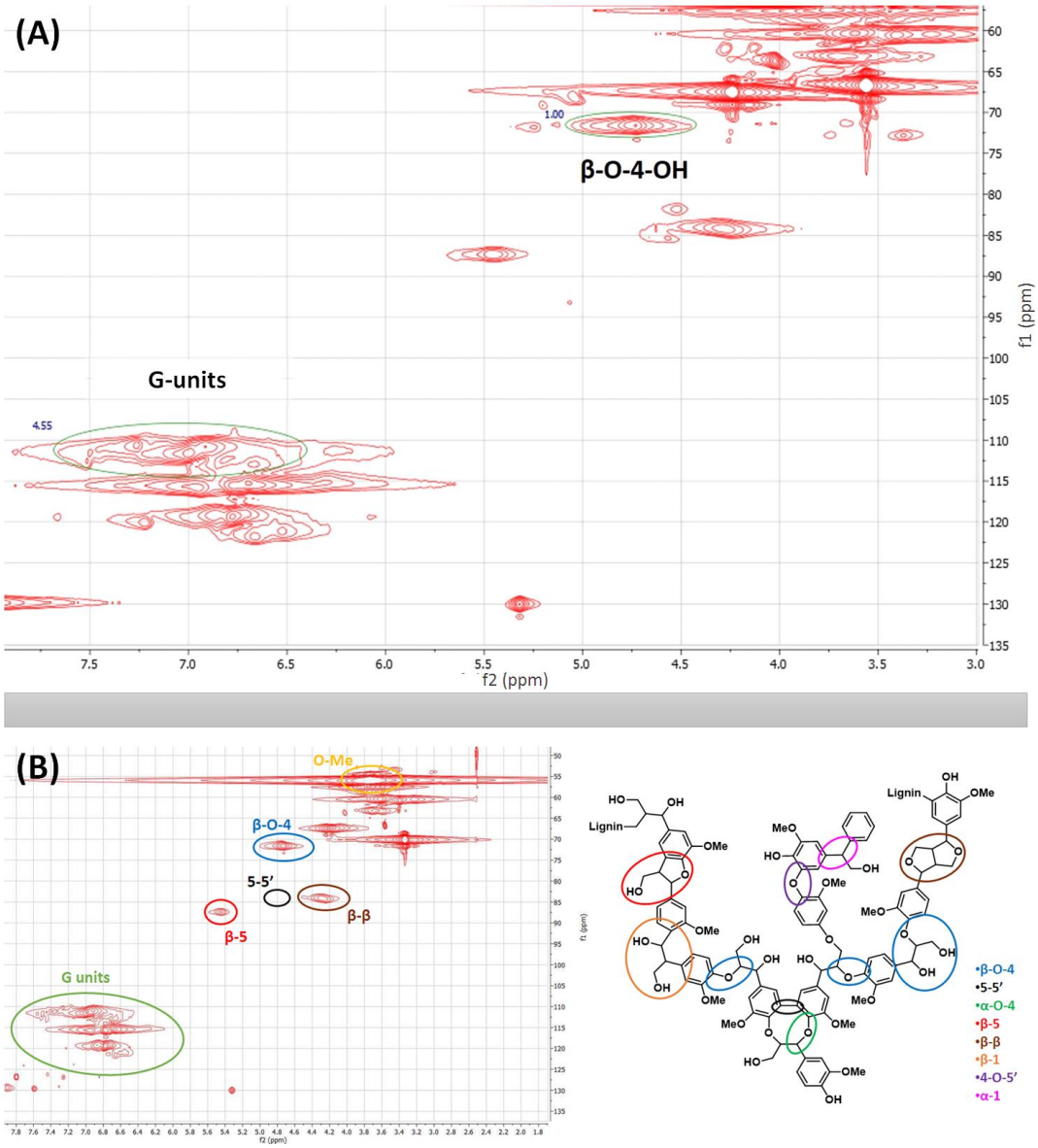


Figure 3. Individual and total yields versus reaction time of main phenolic monomers coming from the cleavage of the β-O-4 interconnecting bonds during batch oxidative depolymerization of a technical pinewood lignin using different reaction temperatures (i.e. 133 and 163°C) and oxidative conditions (i.e. Nitrobenzene, 0.5, 3 and 6 bar of oxygen partial pressure).

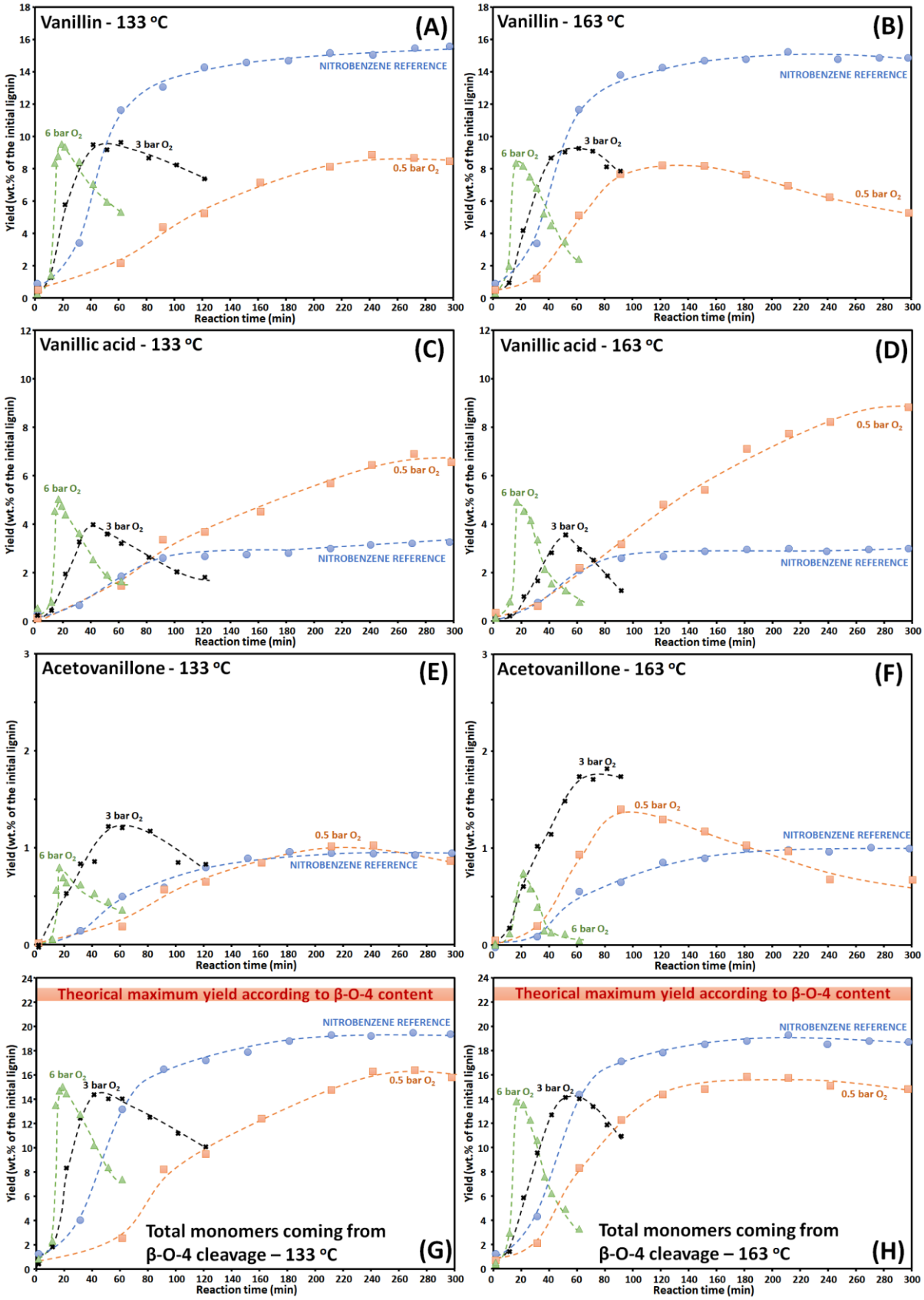
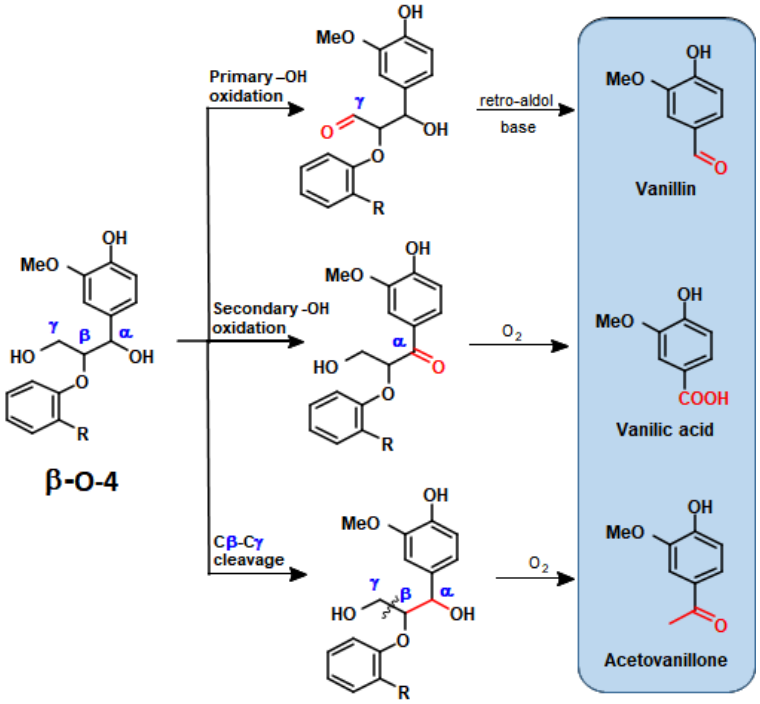


Figure 3 shows the evolution of the individual and total yields of the main phenolic monomers coming from the cleavage of the β-O-4 interconnecting bonds (i.e. vanillin, vanillic acid and acetovanillone) as a function of the reaction time during batch oxidative depolymerization reactions of the previously described technical pinewood lignin, working at different reaction temperatures (i.e. 133 and 163°C) and oxidative conditions (i.e. Nitrobenzene, 0.5, 3.0 and 6.0 bar of oxygen partial pressure). As it can be first noticed, the use of molecular oxygen as oxidant brings an instability of the phenolic products independently of the reaction temperature and oxygen partial pressure, although the use of nitrobenzene as oxidant allows to obtain stable products. Indeed, when molecular oxygen is used, all product yields evolve very similarly with an initial increase of the vanillin, vanillic acid and acetovanillone yields which are apparently primary products of the lignin depolymerization. Then, they reach a maximum before to start to decrease due to secondary reaction mechanisms. Therefore, molecular oxygen is very likely involved and has a similar importance in both mechanisms, i.e. the primary β-O-4 interconnecting bonds cleavage and the secondary monomeric instability mechanisms. Then, the higher is the oxygen partial pressure the faster is the initial increase of primary products and their subsequent reactivity, leading to similar maximum yields independently of the oxygen partial pressure and temperature applied (see Figures 3-G and H). It is nevertheless interesting to remark that for the lowest oxygen partial pressure (i.e. 0.5 bar), the stability of the phenolic monomers is greatly improved (see Figures 3-G and H), even if a significant higher maximum yield of vanillic acid is observed at the expense mainly of the vanillin yield (see Figures 3-A, B, C and D). This tendency could be easily explained by the fact that under these mild oxidative conditions, due to the fact that the primary vanillin is much more stable, a higher concentration of this molecule in the reaction medium during a longer period of time can occur. Thus, another consecutive reaction corresponding to the overoxidation of the vanillin to vanillic acid can be much more easily observed, leading to a higher vanillic acid yield which corresponds to the sum of the primary vanillic acid coming directly from the lignin depolymerization and the secondary vanillic acid coming from the vanillin overoxidation.

From the obtained results, it is also interesting to comment that, in a general way, the use of molecular oxygen instead of nitrobenzene as mild oxidant implies the obtention of significantly lower yields of vanillin and higher yields of vanillic acid and acetovanillone. Even if a part of the lower yield of vanillin could be explained by the competition with the secondary reaction and/or overoxidation mechanisms, it is though that the higher yields of the presumably primary vanillic acid and acetovanillone indicate that a representative part of the observed tendencies could be also due to intrinsic changes of the mode of action of the oxidant leading to modifications of selectivities. Indeed, as it can be appreciated in Figure 4, the obtention of vanillin as primary product from lignin involves the previous selective oxidation of the primary hydroxyl group of the β-O-4 interconnecting bonds,

while the non-selective previous secondary hydroxyl group oxidation leads to the production of vanillic acid as primary product and the direct Cβ-Cγ cleavage to acetovanillone.[62,63] Following this reasoning, it could be deduced that the use of molecular oxygen as oxidant implies a lower selectivity for the oxidation of the primary hydroxyl group and therefore for the selectivity of vanillin, for the benefit of the secondary hydroxyl group oxidation and the selectivity of the primary vanillic acid. Meanwhile, the Cβ-Cγ cleavage mechanism seems to be also slightly enhanced by the molecular oxygen. This assumption is corroborated by other studies[62, 64] and, therefore, the use of specific catalysts favoring the oxidation of the primary hydroxyl group[62] or novels strategies implying the selective pre-oxidation of lignins[65] could help to obtain enhanced yields of phenolic aldehydes from lignin when molecular oxygen is used as oxidant.

Figure 4. Main mechanisms of β-O-4 interconnecting bonds cleavage of lignin under alkaline oxidative conditions and subsequent primary phenolic monomers products.



In addition, when comparing the maximum total yield of the main phenolic monomers coming from the cleavage of the β-O-4 interconnecting bonds achieved when working with molecular oxygen, with the yields obtained during nitrobenzene oxidative depolymerization reactions (see Figures 3-G and H), it appears that the use of oxygen as oxidant does not allow to take advantage of the whole potential of the lignin to produce phenolic compounds derived from the breakage of the β-O-4 bonds (see Figures 3-G and H). This low efficiency is even more marked if compared with the estimated theorical maximum yield of total phenolic monomers that could be achieved from the cleavage of the β-O-4 interconnecting bonds assuming that the whole β-O-4 bonds are individually broken to yield phenolic monomers as described in Figure 4. In this case, it is estimated that according to the initial β-

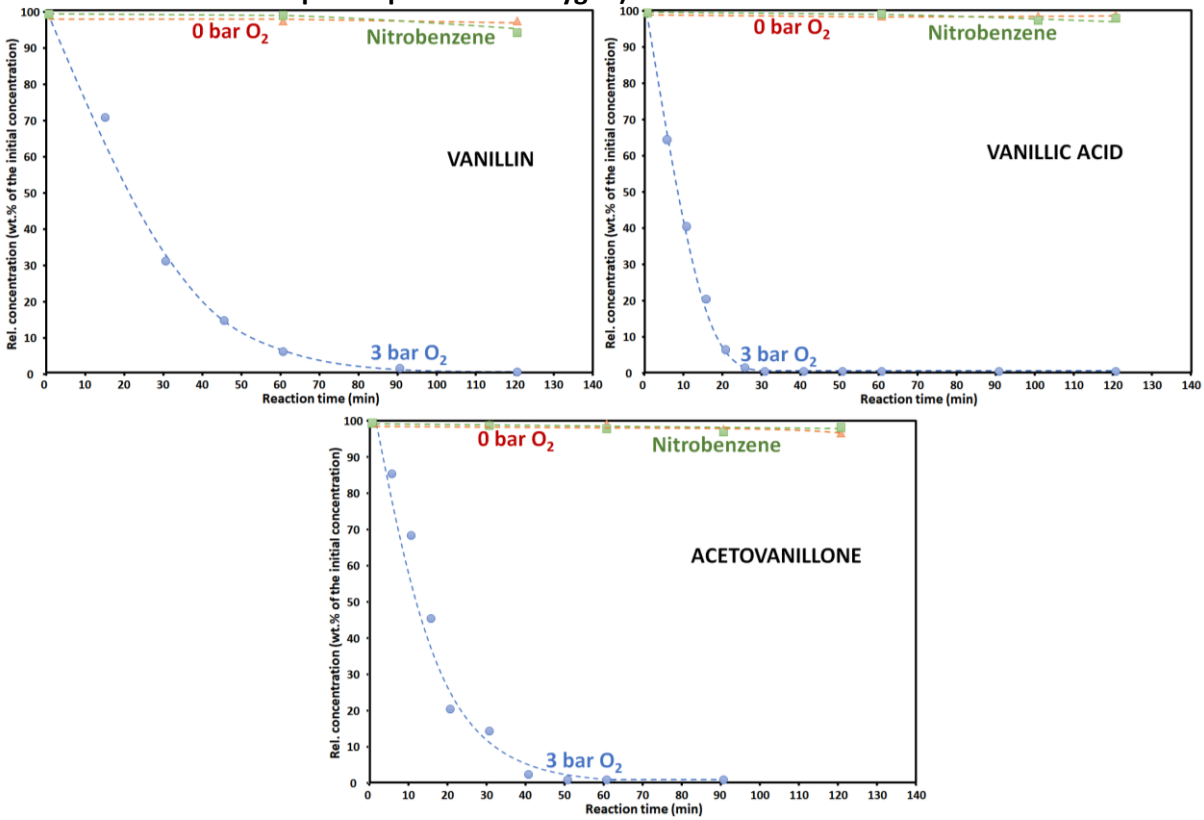
O-4 content (i.e. 27-30 %) of the used technical lignin determined through 2D-HSQC NMR analysis, a maximum yield of about 22-23 wt.% of phenolic monomers should be obtained, and only 60-65 wt.% of this potential is reached when using molecular oxygen as oxidant for the batch oxidative depolymerization in alkaline medium Meanwhile, nitrobenzene allows to attain a recovery rate of about 80-85 wt.%. By additional 2D-HSQC NMR analysis we have checked that no more protected and unprotected β-O-4 bonds are present in the residual lignin recovered after the different batch oxidative depolymerizations. Thus, even if a part of this deviation could be due to the intrinsic highly branched nature of the lignin polymer and, in particular, to the presence of other types of interconnecting bonds which are not fully cleaved and avoid the subsequent release of the phenolic monomers units, it is though that a significant part of this low efficiency could be also directly explained by the instability of the highly functionalized aromatic aldehydes, ketones and carboxylic acids monomers. If this is so, then a deeper understanding of the mechanisms responsible for these secondary reactions is of crucial importance to be able to enhance the reaction yields of phenolics from lignin oxidation.

Study of the stability of pure phenolic monomers under alkaline oxidative depolymerization conditions and strategies for stabilization.

A deeper study of the stability of pure phenolic monomers has been first performed to better understand the complex reaction network taking place during the oxidative depolymerization of lignin when molecular oxygen is used as oxidant in a batch reactor. This knowledge should help to find a way to mitigate the secondary repolymerization mechanisms that could limit the yield to monomers of interest. To gather representative information, the individual reactivity of the different phenolic monomers has been tested in the same batch reactor experimental system than the one already reported. Realistic operative conditions have been applied using an initial concentration of pure phenolic compounds of 1 wt.% inside the reaction medium. Figure 5 presents the observed individual relative concentrations of the three different pure phenolic monomers tested (i.e. vanillin, vanillic acid and acetovanillone) as a function of reaction time. As it can be seen, no significative reactivity can be detected within the reaction system under typical conditions of lignin depolymerization when no oxidant is applied. Moreover, same tendencies are observed when nitrobenzene is used as oxidant, while the presence of molecular oxygen causes a clear and fast instability of all three pure phenolic monomers, being the vanillic acid the more unstable monomer, followed by acetovanillone and finally vanillin. An almost complete disappearance of the initial monomers is obtained after only 25-30, 40-50 and 80-90 minutes of processing, respectively. These results are in agreement with the ones reported by Casimiro et. Al in a recent study[64] and confirms without any possible ambiguity the

fundamental role of the molecular oxygen in the mechanisms bringing the instability of the phenolic monomers during the oxidative depolymerization of lignin.

Figure 5. Relative reactivity of different pure phenolic monomers as a function of reaction time under realistic operative conditions of batch lignin oxidative depolymerization (i.e. 163°C, 2N NaOH, 1 wt.% of initial concentration) and different oxidative conditions (i.e. without oxidant and using nitrobenzene or 3 bar of partial pressure of oxygen).



It is important to comment that it has been impossible to detect representative contents of any other phenolic monomers such as phenol, guaiacol or even pure aromatics by GC-MS or HPLC analysis. The presence of CO and CO_2 in the gaseous phase at the end of the different experiments has been checked and no traces of such products has been detected, indicating that no decarbonylation and/or decarboxylation reactions occur under the used operative conditions. On the other hand, typical ring-opening products, including aliphatic carboxylic acids, have not been detected neither. Such results look consistent with the fact that ring cleavage generally is greatly promoted when strong oxidants such as peroxides and peracids and/or metal-based catalysts, including heterogeneous catalysts or porphyrin, are employed.[66] Moreover, it is suspected by some authors that the extent of the ring opening during oxidative lignin depolymerization reactions is greatly dependent to the aromatic ring structures and that guaiacyl groups are much less prone to undergo such reaction mechanisms than syringyl structures.[67] According to the previously reported and arguing the non-catalytic and mild oxidative conditions, together with the G-unit based lignin (i.e. extracted from softwood) used in the

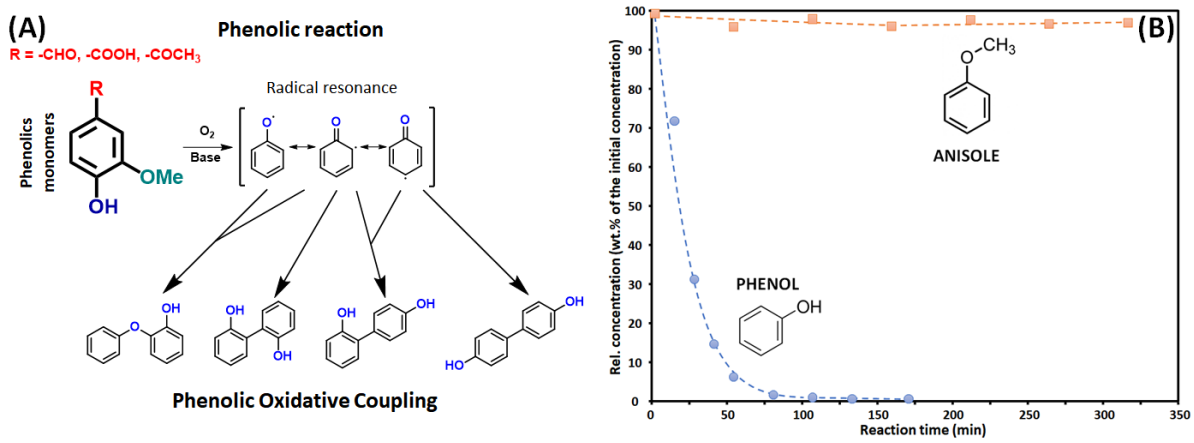
present study, it is thus not so surprising that the degradation of the aromatic ring is not a major issue in our case.

Therefore, it looks that the side reactions at the origin of the instability of the phenolic monomers are very likely due to coupling and/or condensation reactions leading to the creation of new chemical bonds and the production of dimers/oligomers, very probably through free-radical polymerization mechanisms. Even if, in the present study, the direct detection of the presence of such compounds has not been carried out due to experimental procedure limitations, such repolymerization is consistent with results previously reported by other authors and our group.[68, 69, 70] Indeed, in the framework of the present study, the routine aqueous sample post-treatment (see the experimental section for more details) implies an acidification/filtration step where repolymerized products are very likely removed at the same time than the residual or unreacted lignin, leading to the impossibility to detect them through post HPLC or GC analysis. In all cases, repolymerization mechanisms lead to products that are more resilient than the initial lignin due to C–C bond formation, limiting therefore greatly the yield of phenolic monomers during the alkaline oxidative depolymerization of lignin. Finally, it is interesting to mention that in the case of vanillin, no more than 5 wt.% of vanillic acid was detected inside the reactor during the whole experiment, confirming that part of the vanillin instability is due to the overoxidation mechanism. However, with the data obtained up to now and considering the very fast disappearance rate of the vanillic acid, it is impossible to know if the vanillin by itself undergoes reactions of coupling or if it is necessary to previously oxidize the vanillin to vanillic acid to start the secondary reactions. This point will be discussed more in detail hereafter.

Going further and focusing on the constitutive functional groups of the different phenolic monomers of interest which can activate mechanisms of coupling, it is though that the methoxy group should be quite unreactive under the reaction conditions of oxidative lignin depolymerization. On the other hand, the hydroxy group can very likely be at the origin of mechanisms such as the phenolic oxidative coupling. Indeed, in the presence of a variety of chemical oxidants, phenolic molecules can combine to form many different products arising through carbon-carbon or carbon-oxygen coupling. Simple phenols, for instance, are linked at positions ortho and/or para to the hydroxy group by resonance of the intermediate radicals to yield several possible dimers (see Figure 6-A). The further consecutive oxidization of dimers can produce trimers, polymers, and quinoid-type structures.[71] The previously mentioned assumptions were tested checking the stability of pure anisole and phenol under realistic operative conditions of lignin depolymerization using molecular oxygen as oxidant, and the results are presented in Figure 6-B. It has been found that, indeed, anisole is quite stable without any significant observed reactivity during more than 300 minutes of reaction time, confirming that the methoxy function does not bring any instability under realistic operative conditions of lignin

depolymerization using molecular oxygen as oxidant. This is not the case for the hydroxy function which is responsible for a quite fast transformation of the phenol molecule to higher molecular weight products, very probably following the phenolic oxidative coupling mechanism, observing a similar conversion than the one already detected for the vanillin molecule.

Figure 6. (A) Phenolic oxidative coupling reaction and (B) Relative individual reactivity of pure anisole and phenol as a function of reaction time under realistic operative conditions of batch lignin oxidative depolymerization (i.e. 163 °C, 2N NaOH, 1 wt.% of initial concentration and 3 bar of molecular oxygen partial pressure).



The possibility to mitigate the previously highlighted undesired condensation mechanism taking place through the hydroxy function of the phenolic monomers will be of much interest to optimize the production of desired products. Chemical protection using a capping approach, for instance through etherification, does not seems a viable route. Indeed, that strategy will very likely imply a concomitant capping of the hydroxyl groups of the β-O-4 interconnecting bonds of the lignin and, in particular, the γ-OH-group. This will subsequently inhibit some of the possible cleavage mechanisms such as the retro-aldol reaction, because the γ-OH-groups cannot be oxidized and subsequently the lignin depolymerization will be perturbated.[72, 73, 74] Another strategy to limit phenolic oxidative coupling mechanisms would be thus to find softer operating conditions that do not exceed the activation energy necessary for such mechanisms to take place. In order to investigate this possibility, the influence of the operative conditions and in particular of the temperature and partial pressure of oxygen over the pure phenol stability has been studied, and the most interesting results obtained are given in Figure 7. As it can be seen, by decreasing the severity of the operating conditions, it has been possible to drastically control the reactivity of the phenol molecule. An almost complete stability is obtained when a temperature of 103°C and an oxygen partial pressure of 0.5 bar were applied. It can be thus concluded that under these conditions the phenolic oxidative coupling reaction is almost completely inhibited. It is estimated that under these mild conditions, less than 5 wt.% of phenol was degraded after around 22 hours of processing.

Figure 7. Parametric study of the relative reactivity of pure phenol as a function of reaction time under realistic operative conditions of batch lignin oxidative depolymerization (i.e. 2N NaOH, 1 wt.% of initial concentration).

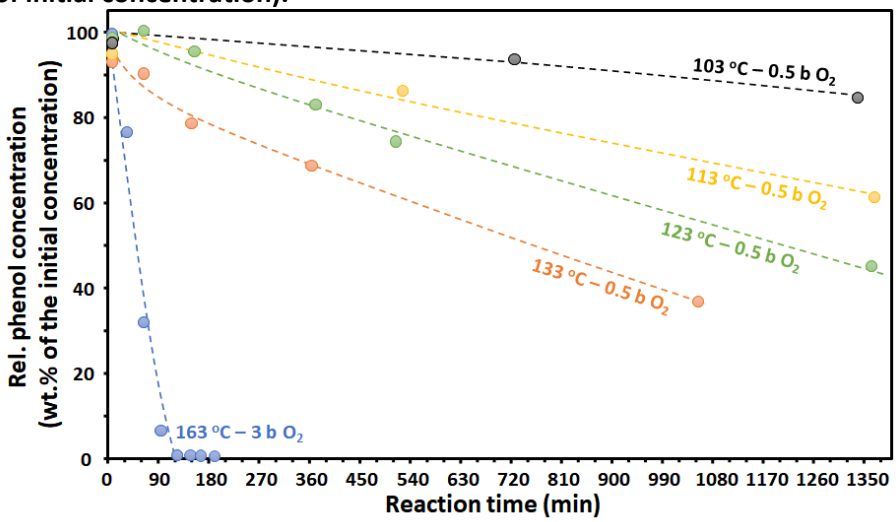
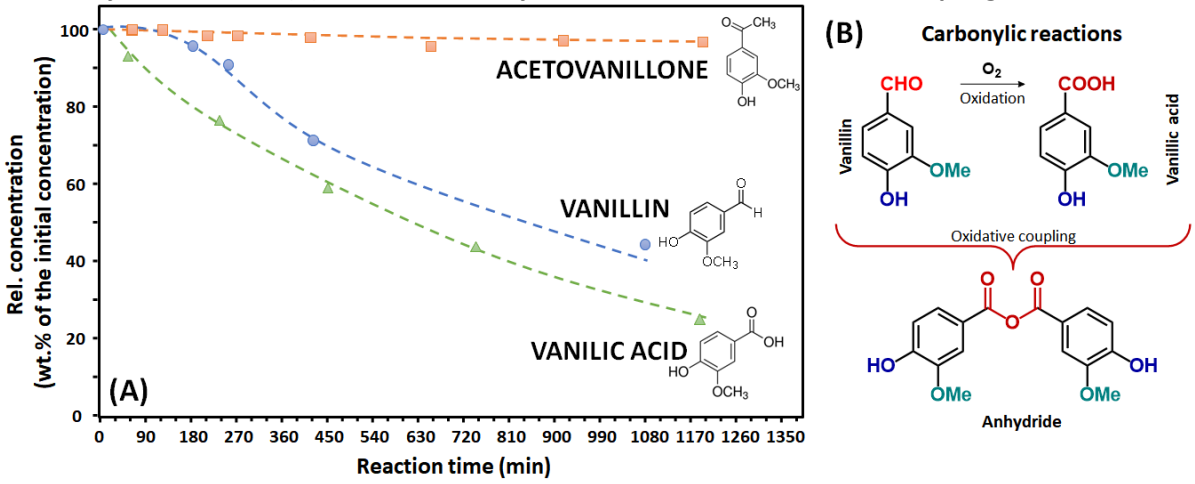


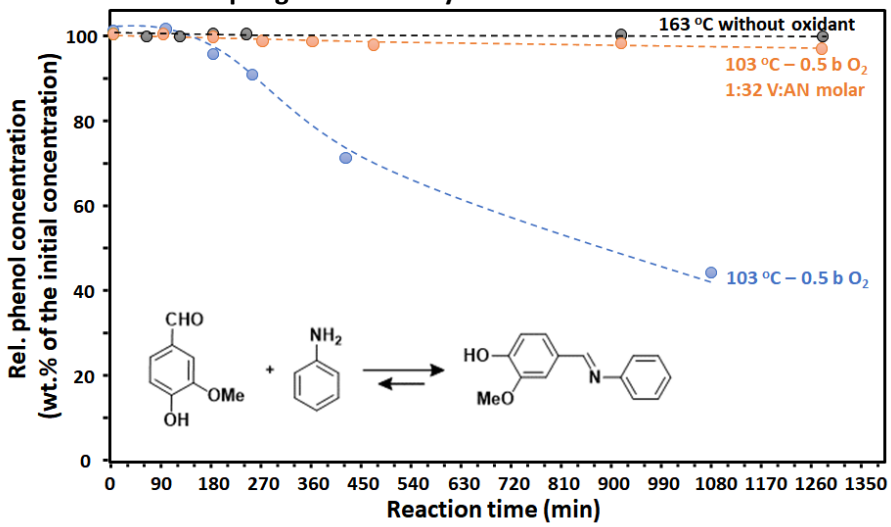
Figure 8. (A) Relative individual reactivity of pure vanillin, vanillic acid and acetovanillone as a function of reaction time under optimized operative conditions of batch lignin oxidative depolymerization (i.e. 103°C, 2N NaOH, 1 wt.% of initial concentration and 0.5 bar of molecular oxygen partial pressure) which almost completely inhibit the undesired phenolic oxidative coupling secondary reaction and (B) Possible carbonylic oxidative hetero- or homo-coupling reactions.



On the base of this promising result, it was decided to contemplate the effect of the presence of the other functional groups of the targeted phenolic monomers such as the aldehyde, carboxylic acid and ketones functions, and to verify the stability of the vanillin, vanillic acid and acetovanillone pure compounds under these optimized operative conditions (see Figure 8-A). According to our results, under conditions that almost completely inhibit the phenolic oxidative coupling reaction, vanillin and vanillic acid still present a significant instability within the reaction medium, being, again, vanillic acid less stable than vanillin, with an estimated conversion of around 50 and 60 wt.% respectively after 900 minutes of reaction. This continuous instability clearly indicates that other mechanisms take place through the aldehyde and carboxylic acid functions. As already mentioned, vanillin can be over-

oxidized into vanillic acid, but it is believed that the main part the observed instability is due to undesired reactions which consist in the oxidative homo- and/or hetero-coupling of the aldehyde and carboxylic group to form an anhydride of higher molecular weight, as presented in figure 8-B.[75] On the other hand, it is interesting to highlight that under these operative conditions, acetovanillone is very stable over a very long period of time, meaning that, very likely, the main part of its instability during the oxidative depolymerization of lignin is due to the phenolic oxidative coupling. Moreover, the possibility of some hetero-condensation through the Claisen-Schmidt mechanism where an aldehyde or ketone having an α-hydrogen reacts with an aromatic carbonyl compound lacking an α-hydrogen to form a condensed product, has been checked (results not shown). Then vanillin and acetovanillone were introduced simultaneously in the alkaline reaction medium under realistic conditions but in absence of molecular oxygen and we observed that this condensation mechanism does not occur extensively.

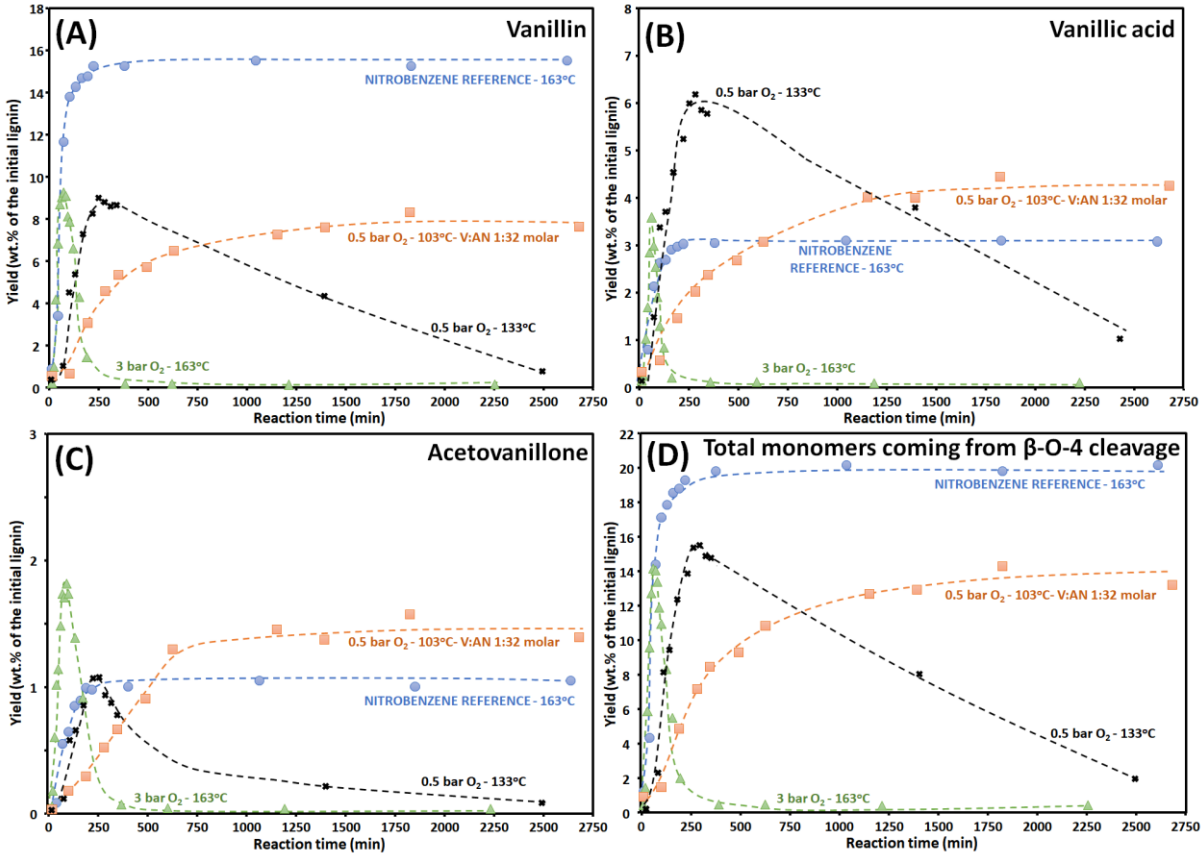
Figure 9. Scheme of reversible protection of the aldehyde group of the vanillin molecule by formation of an imine using aniline as capping agent and relative reactivity of pure vanillin as a function of reaction time under optimized operative conditions of batch lignin oxidative depolymerization (i.e. 103°C, 2N NaOH, 1 wt.% of initial concentration and 0.5 bar of molecular oxygen partial pressure) and applying an additional reversible derivatization strategy to avoid the oxidative homo-coupling of the aldehyde function.



In view of the above results, it looks very difficult to stabilize the vanillic acid within the reaction medium during lignin oxidative depolymerization reactions. Nevertheless, concerning the vanillin, where the aldehyde group is present, full stabilization could be achieved preventing the condensation side reactions that occur through the carbonyl group thank to the use of an additional reversible derivatization strategy allowing the formation of an imine (amination) using aniline as capping agent (see figure 9). One of the main advantages of this protection mechanism is that the amination reaction is highly favoured in the alkaline medium of depolymerization reactions, and in turn is completely reversible in acidic medium already necessary to precipitate the residual lignin at the end of the batch

reaction and to transform the vanillin which is under the form of vanillate ion under basic conditions. After several proofs of concept, various experiments were carried out varying the vanillin to aniline molar ratio, finally obtaining an almost total stability under the contemplated conditions using a 1:32 vanillin to anillin molar ratio as it can be clearly observed in figure 9. Moreover, the additional reversible derivatization strategy allows not only to fully protect the vanillin against the oxidative coupling of the aldehyde function but also against the mechanism of over-oxidation producing vanillic acid from vanillin.

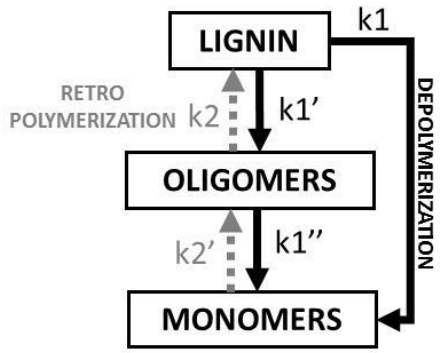
Figure 10. Individual and total yields versus reaction time of main phenolic monomers coming from the cleavage of the β-O-4 interconnecting bonds during batch oxidative depolymerization of a technical pinewood lignin using different reaction temperatures (i.e. 103, 133 and 163°C) and oxidative conditions (i.e. Nitrobenzene, 0.5 and 3 bar of oxygen partial pressure) in the presence or absence of anillin as capping agent.



At this point, it has been possible to stabilize in the reaction medium at least two of the three more important phenolic compounds coming from the β-O-4 interconnecting bonds cleavage generally obtained during mild oxidative depolymerization of lignin coming from softwood, and to greatly limit the extension of repolymerization of the third monomer (i.e. vanillic acid). To check if the direct application of the knowledge gathered during the detailed reactivity studies of pure compounds and the application of the different proposed strategies of stabilization can help in obtaining stable and higher yields during representative oxidative depolymerization reactions of lignin, reactions have been

carried out with the already presented technical lignin extracted from softwood. Comparisons have been done between the results obtained with nitrobenzene as reference, using classical molecular oxygen lignin depolymerization conditions (specially a temperature of 163 or 133°C, 3 or 0.5 bar of oxygen partial pressure, keeping constant the total pressure at 10 bar using nitrogen), and finally applying the optimized operative conditions (i.e. 103 °C and 0.5 bar of oxygen and 10 bar total) in presence of a 1:32 vanillin to anillin molar ratio which normally should guarantee the stability of the produced monomers inside the reaction medium. It is important to comment that in this case, the 1:32 vanillin to anillin molar ratio has been calculated taking into account the initial β-O-4 content of the processed lignin allowing to estimate a theorical maximum yield of monomers coming from the cleavage of these latter and supposing that the reaction will be fully selective to vanillin. It is though that, by this way, the chemical capping of the whole vanillin potentially produced and released into the reaction medium will be derivatized bringing a complete protection against the already highlighted secondary reactions. Moreover, as it is expected that the reactions will be very slow due to the very low severity conditions applied in the case of the optimized conditions, extended reactions in terms of reaction time (i.e. more than 40 hours) have been carried out in order to ensure the completion of the reactions and suitable comparisons. Main results are presented in Figure 10.

Figure 11. Possible simplified reaction scheme of lignin depolymerization under alkaline and oxidative conditions using molecular oxygen as oxidant.



As stated by results, it appears that the proposed strategies of stabilization allowed to minimize the secondary reactions such as the oxidative coupling of phenolic monomers and/or over-oxidation of vanillin normally observed when molecular oxygen is used as oxidant. In this way, vanillin, vanillic acid and acetovanillone remain stable inside the reaction medium during a period of more than 40 hours under realistic conditions for lignin depolymerization. However, it was disappointing to find that, in spite of this apparent stability, it has been impossible to reach higher yields of total monomers than the ones obtained when molecular oxygen is used under more severe operative conditions (i.e. 133 or 163°C and 0.5 or 3 bar of oxygen). Thus, also still far from the results obtained with the reference reaction using nitrobenzene (see Figure 10-D). According to us, two different possible reasons could explain these quite unsatisfactory results; a) it is very complicated to decrease the rate of the

secondary reactions responsible for the instability of the primary products in presence of molecular oxygen without affecting significantly at the same time the kinetics of the oxidative depolymerization reaction, following the simplified reaction scheme presented in Figure 11. b) the molecular oxygen as oxidant has, by itself, limitations in terms of cleavage of a part of the intramolecular bonds found in lignin avoiding the release of the whole phenolic monomers even if the totality of the β-O-4 interconnecting bonds have been cleaved.

Indeed, in the case of the first assumption, it is very probable that the softening of the operative conditions and the presence of anillin have an effect over the progress of the lignin depolymerization leading, surprisingly, to almost compensated effects between the beneficial impact of the monomers stabilization and the detrimental influence over the lignin depolymerisation. This assumption can also explain the very similar maximum yields of the desired products attained. Nevertheless, additional test reactions at 163^{o}C and 3 bar of partial pressure of oxygen have been carried out in presence of aniline (results not shown), and very similar kinetic data and maximum yields of total monomers than in absence of anillin were obtained. This indicates that the presence of aniline, by itself, does no perturbate the kinetic of the oxidative depolymerization of the lignin. In addition, 2D-HSQC-NMR analyses of the residual lignins recovered after the reactions done under low severity conditions in presence of anillin have also demonstrated that no more common interconnecting bonds and, specially, β-O-4 bonds remain in the lignin structure at the end of the reaction. Therefore, if the use of soft operative conditions implies limitations of lignin breakage, it is not related with the cleavage of the β-O-4 interconnecting bonds but to other types of interconnecting bonds present in the tested lignin. This fact could be in accordance with the second assumption and, perhaps, even under higher severity conditions, the molecular oxygen is still unable to carry out such bonds cleavages, contrary to nitrobenzene, to release the phenolic monomers from the lignin macromolecule. Therefore, if these hypotheses are true, the main point to obtain enhanced phenolic monomers yields from lignin using molecular oxygen as oxidant is not exclusively a question of β-O-4 bonds cleavage, enhancement of the γ-OH-groups oxidation and/or stabilization of the produced monomers, but also to identify other specific recalcitrant intramolecular bond(s) of the lignin and to find a suitable catalytic strategy that facilitate the cleavage of the more difficult to break intramolecular bonds when molecular oxygen is used as oxidant.

CONCLUSIONS

The thorough testing and interpretation of the stability of different selected lignin-based monomer phenolic compounds such as vanillin, vanillic acid and acetovanillone together with some other pure chemical compounds such as phenol and anisole have allowed to identify, at least, three

different side reaction mechanisms when molecular oxygen is used as oxidant; (1) the phenolic oxidative coupling, (2) the carbonylic oxidative coupling and (3) the aldehyde over-oxidation of vanillin to vanillic acid mechanisms. Based on this knowledge, possible mitigation strategies have been proposed such as the fine tuning of the operative conditions allowing to almost inhibit the phenolic oxidative coupling mechanism completely. The simultaneous use of an in-situ and reversible capping approach to limit the reactivity of the aldehyde function appeared to be an efficient strategy to inhibit the carbonylic oxidative coupling and the aldehyde over-oxidation mechanisms. Nevertheless, we were not able to find a viable way to fully stabilize the carboxylic acid function and therefore the vanillic acid monomer inside the reaction medium when molecular oxygen is used as oxidant, though softening of the operative conditions greatly mitigate the extension of its unstable behaviour.

Owing to the very promising results, realistic reactions (i.e. 2N NaOH, 5 wt.% lignin, 103°C, 0.5 bar O_2 and 10 bar of total pressure) have been applied to process a high β-O-4 content technical lignin and applying the proposed strategies of mitigation of secondary reactions. Unfortunately, despite the obtention of very stable primary monomer products, confirming the high efficiency of the proposed strategies, it has been impossible to obtain higher yields of phenolic monomers. In fact, the yields obtained are still quite far from the ones obtained when using nitrobenzene and the estimated potential of the processed technical lignin. A possible hypothesis claiming that molecular oxygen as oxidant has, by itself, limitations in terms of cleavage of a part of other intramolecular bonds found in lignin avoiding the release of the whole phenolic monomers even if the totality of the β-O-4 interconnecting bonds are cleaved has been thus proposed. If it is true, current researches about the catalytic depolymerization of lignin using molecular oxygen as oxidant, should not be only focused to find a catalyst able to selectively oxidize the primary hydroxyl group of the β-O-4 interconnecting linkages but also to cleave these recalcitrant interconnecting bonds which have to be still clearly identified.

EXPERIMENTAL SECTION

Materials and chemicals.

Lignin raw material was extracted from Pinus Radiata woodchips obtained from a local sawmill using anhydrous 1,4-Dioxane (99.8 %), anhydrous toluene (99.8 %), sodium bicarbonate (ACS reagent ≥99.7 %) and hydrochloric acid solution (36 % in water) purchased from Sigma-Aldrich (Germany) while pure absolute ethanol was purchased from VidraFOC (Spain). High purity deionized water was obtained through a Merck Milli-Q water purification system with a resistivity of 18.2 MΩ at 25°C and a TOC content lower than 5 ppb. Klason lignin content determination was done using high purity sulfuric acid (98.0 %) obtained from Sigma-Aldrich and high purity deionized water coming also from a

Merck Milli-Q water purification system. 2D $^{13}C–^{1}H$ HSQC NMR lignin samples were prepared using dimethyl sulfoxide-d_6 as deuterated solvent (99.96 atom % D) obtained from Sigma-Aldrich. Products were used as received, without further purification.

Oxidative depolymerization reactions were carried out using NaOH (98 %) purchased from VidraFOC and high purity deionized water coming from a Merck Milli-Q water purification system. High purity 5.0 oxygen, nitrogen and synthetic air were provided by Linde España (Spain). Pure phenolic monomers, i.e. phenol (puriss p.a. 99 %), vanillin (99 %), vanillic acid (97 %), acetovanillone (>98 %) and anisole (99.7 %) together with aniline used as capping agent (ACS reagent grade >99.5 %) necessary for the stability study have been all supplied by Sigma-Aldrich. HPLC mobile phase solvents were made up of HPLC grade acetonitrile purchased from Sigma-Aldrich as organic phase and a protonated aqueous phase prepared using high purity deionized water coming from a Merck Milli-Q water purification system and acetic acid (99.7%) from ABCR (Germany). During HPLC analyses, HPLC grade coumarin (>99 %) provided by Sigma-Aldrich was used as internal standard. In this case also, products were used as received, without further purification.

Lignin isolation.

Lignin isolation from Pinewood (Pinus Radiata) woodchips has been carried out through a batch acidolysis method following an adapted procedure proposed by Pepper et. Al.[51] Raw Pinewood woodchips were previously air dried overnight at 80°C before to be grounded using a rotating knife mill and to be sieved using a 0.4-0.5 mm sieve. The obtained raw material was subsequently washed three times using respectively a 2/1 v/v toluene/ethanol, pure ethanol and finally high purity deionized water liquid solution under vigorous stirring during 1 hour at room temperature and using around 1 ml of solvent for each 100 mg of processed wood. Between each washing step, the solid woodchips were separated from the solvent by filtration using a Büchner funnel and the last washing with water was repeated up to the obtention of a clear filtrate. The washed biomass was thus thoroughly dried during 24 hours in a static oven at a controlled temperature of 105°C to obtain a clean and dry parietal residue.

20 grams of this parietal residue was furthermore suspended at room temperature in a 1 liter round-bottom flask using a magnetic stirrer and 250 ml of a dioxane/water mixture 9/1 v/v containing 0.2 M of hydrochloric acid (HCl) before to add another 250 ml of the previously mentioned blend and to put the system under nitrogen bubbling during at least 10 minutes. The use of an inert atmosphere is mandatory to avoid the formation of peroxide species from dioxane when this latter is heated in presence of molecular oxygen. The prepared blend is therefore heated up to a temperature of 90-92°C using a hot plate and a reflux system letting the acidolysis reaction during 1 hour. This reaction time

was optimized to avoid excessive degradation of the extracted lignin during the acidolysis reaction and to ensure the obtention of a high quality technical lignin having the higher integrity grade as possible compared to the native lignin originally present in the lignocellulosic biomass and, in particular, a high content of β-O-4 interconnecting bonds. When the reaction time is reached, the whole system is cooled-down at room temperature still under nitrogen atmosphere and the solution is rapidly filtered in a Büchner funnel and washed with an additional 100 ml of fresh dioxane/water 9/1 v/v containing 0.2 M of hydrochloric acid (HCl) solution. The filtrate is thus neutralized using sodium bicarbonate ($NaHCO_3$) before to remove the excess of salt using another time a Büchner funnel system and to concentrate the obtained solution using a rotary evaporator at low temperature (50-60°C) under vacuum until the visual start of the lignin precipitation. The addition of some fresh dioxane/water 9/1 v/v solution without acid allows to get a clear solution with a final volume of around 20-25 ml. This clear solution is afterward injected through a laboratory syringe pump in a vigorously stirred volume of distilled water in order to adjust the dioxane/water ratio from 9/1 to at least 0.5/9.5 taking care that the continuous injection is done through a fine stream in the direction of the flow along the beaker wall, allowing the precipitation of the extracted lignin. The obtained precipitated lignin in suspension is thus filtrated using a 0.25 µm nylon filter and repeatedly washed with at least 1 liter of fresh distilled water before to be freeze-dried to obtain the pure and dry technical lignin.

Various batches have been prepared following the previously reported protocol and after checking that all the batches were quite homogeneous in terms of purity (Klason lignin content) and chemical structure (2D ^{13}C-^{1}H- HSQC NMR analysis), these ones have been blended to obtain around 15 grams of technical lignin suitable for its further processing in oxidative depolymerization reactions.

Lignin purity grade determination.

The purity of the technical lignins extracted following the procedure previously reported has been determined through an adapted procedure based on the TP-510-42618 Standard proposed by the US National Renewable Energy Laboratory (NREL) allowing to determine the content of Klason lignin inside woody lignocellulosic raw materials.[53] In this case, as the isolated lignins are already fine, clean and dry powders, no additional pretreatment procedures, as proposed in the NREL standard, has been applied and, in particular, the washing using organic solvents has been avoided as it is well known that the isolated lignin is totally and/or partially soluble in such solvents, leading to possible important deviation of the obtained results. Briefly, around 0.2 g of dry lignin was precisely weighed using an analytical balance and well mixed with 2 ml of a 72 % sulfuric acid (H_2SO_4) aqueous solution inside a clean and dry laboratory test tube. The prepared blend was left during 1 hour at 30 ± 2 °C under mild stirring. The test tube content was afterward transferred into a 100 ml Teflon lined autoclave adding 56 ml of deionized water to adjust the H_2SO_4 concentration from 72 to 3 %. A special care has been

taken to recover the whole content inside the test tube by repeated washing with the dilution water to avoid that some small lignin particles were not transferred inside the autoclave. After the hermetic sealing of the autoclave, this one was placed in an oven at 120 °C for an additional hour before being removed and cooled down at room temperature. Thus, the autoclave was thus opened and its content filtered using a filter crucible with porous bottom and a 1.0 µm pore size glass fiber filter previously washed, oven dried at 105°C overnight and cooled-down at room temperature inside a desiccator cabinet before to be precisely weighed. The filtered solid was thoroughly washed with abundant hot deionized water until a neutral pH of the washing water. In this case also, a huge care has been taken to recover the totality of the content of the autoclave by repeated washes using hot water. The whole system was placed in an oven at 105 °C initially for 12 hours and precisely weighed, repeated 4 hours drying and weighting up to the obtention of a stable weight allowed to ensure the more precise as possible Klason lignin content determination. This procedure should allow the complete acidic digestion and dissolution of the carbohydrates coming from the cellulose and hemicellulose fractions, keeping as solid residue only the lignin and therefore the Klason lignin content was determined comparing the residual weight of solid obtained after the whole protocol with the one at the beginning of the procedure. Additional adjustments of the results considering the real water content of the tested materials determined through anhydrous methanol extraction and Karl-Fischer titration have been done and it has been previously checked by Temperature Programmed Oxidation (TPO) analysis that the ash content of the technical lignins was insignificant.

2D ^{13}C–^{1}H HSQC NMR structural analysis of extracted lignins.

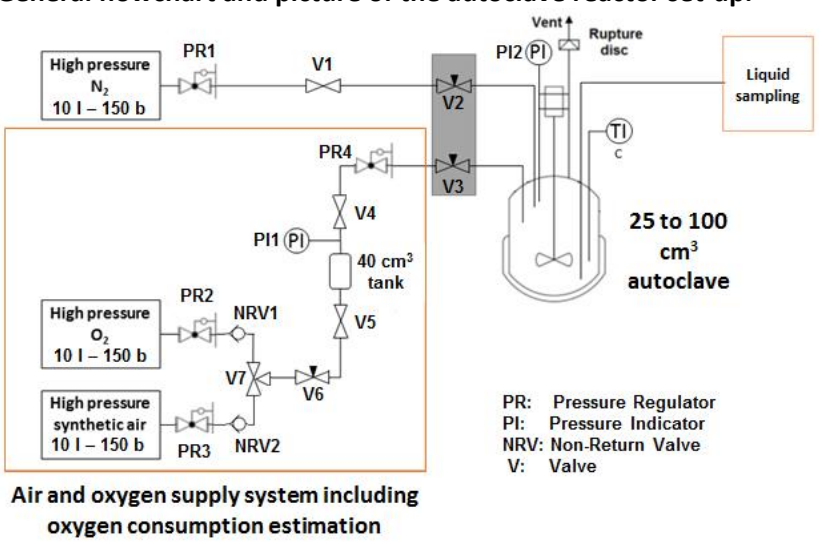
The structural analysis of the previously extracted technical lignins was carried out through 2D ^{13}C–^{1}H HSQC NMR analysis. Samples were prepared in NMR tubes dissolving 80-100 mg of lignin with 0.6 ml of DMSO-d_6. The 2D-HSQC NMR were recorded on a Bruker BioSpin 600 MHz Spectrometer equipped with a TCI cryoprobe at 25 ^{o}C in DMSO-d_6 using hsqcetgpsisp2.2 as a pulse sequence and the following measuring parameters: 5 hours, ns = 56, sw ^{1}H = 12 ppm, o1 ^{1}H = 6 ppm, sw ^{13}C = 180 ppm, o2 ^{13}C = 80 ppm, td in the indirect dimension = 256. The signals for the different lignin linkages and the aromatic signals were assigned by comparison with the literature[54,55] and the quantification was performed following already reported methods.[56, 57, 58] The ratio of S/G/H was determined by comparing the aliphatic and aromatic signal intensities and using the integrals of the signal of the G_2 protons of the G-units, $S_{2/6}$ protons of the S-units and $H_{2/6}$ protons of the H-units, the integral values for $S_{2/6}$ and $H_{2/6}$ were divided by 2 to enable a good comparison with the G_2 value. The previously mentioned signals were also used as internal standard for determining the quantity of aliphatic interconnecting bonds of the various linkages (β-O-4, β-β, β-5) using the integrals corresponding to their α-protons and maintaining the same contour level. S/G/H ratio calculation were performed in

the following manner: S content (% per 100 C9 units) = ($IS_{2/6}$/2) / (IG_2 + ($IS_{2/6}$/2) + ($IH_{2/6}$/2)) * 100, G content (% per 100 C9 units) = (IG_2) / (IG_2 + ($IS_{2/6}$/2) + ($IH_{2/6}$/2)) * 100 and H content (% per 100 C9 units) = ($IH_{2/6}$/2) / (IG_2 + ($IS_{2/6}$/2) + ($IH_{2/6}$/2)) * 100 with $IS_{2/6}$ ➔ Integral value of $S_{2,6}$ peaks including S and S', IG_2 ➔ Integral value of G_2 peak including G and G' and $IH_{2/6}$ ➔ Integral value of $H_{2,6}$ peak. While β-O-4 content (% per 100 C9 units) = (Iβ-O-4) / (IG_2 + ($IS_{2/6}$ /2) + ($IH_{2/6}$ /2)) * 100, β-5 content (% per 100 C9 units) = (Iβ-5) / (IG_2 + ($IS_{2/6}$ /2) + ($IH_{2/6}$/2)) * 100 and β-β content (% per 100 C9 units) = (Iβ-β/2) / (IG_2 + ($IS_{2/6}$/2) + ($IH_{2/6}$/2)) * 100 with $IS_{2/6}$ ➔ Integral value of $S_{2,6}$ peaks including S and S', IG_2 ➔ Integral value of G_2 peak including G and G' and $IH_{2/6}$ ➔ Integral value of $H_{2,6}$ peak, Iβ-O-4 ➔ Integral value of the α-proton of the β-O-4 linkage, Iβ-5 ➔ Integral value of the α-protons of the β-5 linkage and Iβ-β ➔ Integral value of the α-protons of the β-β linkage.

Oxidative lignin depolymerization and pure phenolic monomers batch reactor experimental setup.

The oxidative lignin depolymerization reactions and the stability study of pure phenolic monomers were performed using a discontinuous high pressure reaction system (Autoclave Engineers, USA) corresponding to a laboratory scale batch reactor electrically heated able to work up to 250 bar and 250 °C. The reactor setup is equipped with a 30 cm^3 stainless steel autoclave and a controller unit provided by Iberfluid instruments (Spain) allowing to control the mechanical stirring and the internal temperature. The starting of the heating ramp is controlled externally in order to avoid uncontrolled rises of the reaction medium temperature while when the internal temperature reaches values close to the desired temperature the control switch from external to internal control to adjust finely the reaction medium temperature to the reaction temperature. Additional gas vent and entrances of oxygen, air and nitrogen have been in-house implemented together with a sample recovery system (see Figure below). The airtightness of the reactor was ensured by a suitable Teflon O-ring seal.

General flowchart and picture of the autoclave reactor set-up.



Air and oxygen supply system including oxygen consumption estimation

In a typical reaction, 1 gram of technical lignin or the desired quantity of pure phenolic monomer is precisely weighted and solubilized in 20 mL of a 2M NaOH dissolution (5 wt.% of lignin) before to be charged into the autoclave reactor. Optional quantity of other chemicals such as nitrobenzene or aniline used respectively as oxidant and capping agent can be also added to the reaction medium. A new Teflon O-ring seal is positioned, and the reactor is mounted in the clean and dry reactor structure. The airtightness of the autoclave is verified pressuring with nitrogen at a pressure of more than 20 bar and checking that no pressure drop is observed during at least 30 minutes. If the leak test is positive, 3 nitrogen flushes at 30 bar of the autoclave atmosphere are carried out and the system is let at atmospheric pressure. The mechanical stirring system is started at 1100 rpm of velocity and the heating ramp is initiated. Classically, between 10 and 15 minutes are necessary to reach and stabilize the internal temperature to the desired reaction temperature (i.e. between 103 to 163°C). At this point, the autoclave is slightly pressured due to the water partial pressure and generally between 1 to 4 bar of pressure is already observed. Depending on the reaction temperature, this pressure is adjusted with nitrogen in function of the desired partial pressure of oxygen knowing that the total pressure is fixed at 10 bar. For instance, if the desired oxygen partial pressure is 3 bar, the autoclave pressure is adjusted with nitrogen up to 7 bar and, in the case of the nitrobenzene reference reactions, the autoclave is directly charged up to 10 bar with pure nitrogen. An initial sample is recovered corresponding to the start of the reaction and if needed the molecular oxygen is quickly introduced inside the autoclave to reach the total pressure of 10 bar. It is important to notice that the oxygen pressure regulator stays open during the whole reaction allowing to compensate the oxygen consumption during the reaction and ensuring a constant total system pressure of 10 bar. 100-150 mg liquid samples are recovered at different time intervals of the reaction time course adjusted for each reaction operative conditions as the reaction kinetics are very variable leading also to the necessity to adjust the total reaction time. The recovered samples are precisely weighted using an analytical balance before to be post-treated and analysed through an HPLC system described hereafter. Finally, it is important to mention that a special care has been taken to not remove more than 15-20 wt.% of the initial reaction medium during the sampling operations in order to avoid possible results deviations due to the displacement of the chemical equilibrium even if in this case no solid catalysts are used.

Samples post-treatment and HPLC phenolic monomers quantification.

Homogeneous recovered samples during the time course of the oxidative lignin depolymerization and stability study of pure phenolic monomers reactions were first acidified using 1 ml of a 2N HCl dissolution, allowing the precipitation of the residual lignin, and were vigorously shaken during at least 1 minute using a laboratory vortex shaker, before to be filtered using a 0.2 µm nylon syringe filter. An additional 1 ml of 2N HCl dissolution was added to the sample recovery vial and after another vigorous

shaking, the obtained second fraction was filtered using the same syringe filter than previously. This protocol ensures that the most part of the sample is recovered from the sampling vial and, at the same time, that soluble low molecular weight products are fully released from the residual lignin retained in the syringe filter. The whole obtained aqueous dissolution was precisely weighted using an analytical balance and about 0.8 gram of the latter was blended with 0.2 gram of an aqueous coumarin dissolution at 1000 ppm and precisely weighted. The aqueous prepared samples were thus directly analysed through High-Performance Liquid Chromatography (HPLC) using coumarin as internal standard. The HPLC quantitative analysis of monomeric phenols were carried out using a Shimadzu system incorporating a LC-20ADXR HPLC pump, SIL-20A auto-sampler, CTO-20A column oven, DGU-20A5R degassing unit and a SPD-M20A Diode Array Detector. An Agilent Zorbax Eclipse Plus C-18 column (4.6 mm × 150 mm, pore size 5.0 µm) was used at a controlled temperature of 30 ^{o}C and the mobile phase including HPLC grade acetonitrile (ACN) and 1.5 Vol.% of acetic acid in highly purified water coming from a Milli Q water purification system. Total analysis time was 25 minutes and gradient elution mode was used in the following manner: at 0 min 5% ACN, at 14 min 65% ACN, at 16 min 90% ACN, at 18 min 90% ACN, at 18.01 min 5% ACN, at 23 min 5% ACN and stop at 25 min. A flow rate of 1.0 ml/min was applied injecting 3 µl of sample with 6 s of needle wash. Identification and response factors determination for each phenolic monomer compounds of interest have been done analysing previously prepared standard dissolutions at different concentrations in the expected range of concentration of the final post-treated samples. Peaks integrations have been done at 280/4 nm and all chromatograms has been processed using the LabSolution software (Shimadzu). HPLC results allowed the precise individual determination of the phenolic monomers concentrations inside the analysed samples and taking into account the exact weight of reaction medium inside the reactor at the time of the sampling, the weight of the sample recovered and the dilution due to the acidification step and internal standard incorporation, it has been possible to estimate the yields of each phenolic monomers of interest in terms of wt.% of initial lignin in the case of oxidative lignin depolymerization reactions or the exact concentration inside the reactor of the pure phenolic compounds during the stability studies.

ACKNOWLEDGEMENTS

The authors thank Técnicas Reunidas for material and financial. Spanish Ministry of Science, Innovation and Universities for the funding through the “Severo Ochoa” Excellence Program (SEV 2016-0683) and the LIGNOPRIZED project form the Spanish Centre for the Development of Industrial Technology (CDTI) in the framework of the Strategic Program of National Business Research Consortia (CIEN-2016) are also acknowledged. A special and kindly thank is also given to Drs. Dalgi Sunith Barbosa Trillos and Jakob Mottweiler for their priceless help during the elaboration of the present work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. BP energy outlook, **2019,** https://www.bp.com/content/dam/bp/business-sites/en/global/corporate/pdfs/energy-economics/energy-outlook/bp-energy-outlook-2019.pdf.
2. J. Bluestein, J. Rackley, *ICF International technical report*, **2010**, Coverage of Petroleum Sector Greenhouse Gas Emissions under Climate Policy.
3. G. W. Huber, S. Iborra, A. Corma, *Chem. Rev.*, **2006**, *106*, 4044.
4. M. Fache, B. Boutevin, S. Caillol, *ACS Sustainable Chem. Eng.*, **2016**, *4*, 35-46.
5. I. Volf, V. I. Popa, Biomass as Renewable Raw Material to Obtain Bioproducts of High-Tech Value, Elsevier, **2018**, 113-160.
6. C. Chio, M. Sain, W. Qin, *Renewable Sustainable Energy Rev.*, **2019**, *107*, 232–249.
7. H. Wang, Y. Pu, A. Ragauskasd, B. Yang, *Bioresour. Technol.*, **2019**, *271*, 449–461.
8. A. Corma, S. Iborra, A. Velty, *Chem. Rev.*, **2007**, *107*, 2411-2502.
9. M. Vohra, J. Manwar, R. Manmode, S. Padgilwar, S. Patila, *J. Environ. Chem. Eng.*, **2014**, *2(1)*, 573-584.
10. P. Claassen, J. van Lier, A. Lopez Contreras, E. W. J. van Niel, L. Sijtsma, A. J. M. Stams, S. S. de Vries, R. A. Weusthuis, *Appl. Microbiol. Biotechnol.*, **1999**, *52*, 741–755.
11. B. Kamm, M. Kamm, *Appl. Microbiol. Biotechnol*, **2004**, *64,* 137-145.
12. S. Banerjee, S. Mudliar, R. Sen, L. Giri, D. Satpute, T. Chakrabarti, R.A. Pandey, *Biofuels, Bioprod. Biorefin.*, **2010**, *4*, 77-93.
13. R. Howard, E. Abotsi, E.J. Van Rensburg, S. Howard, *Afr. J. Biotechnol.*, **2003**, *2*, 602-619.
14. M. Kleinert, T. Barth, *Energy Fuels*, **2008**, *22(2)*, 1371-1379.
15. P. Bajpai, in Biermann's Handbook of Pulp and Paper (Third Edition), Elsevier, **2018**, 233-247.
16. J. Zakzeski, P. C. A. Bruijnincx, A. L. Jongerius, B. M. Wec-khuysen, *Chem. Rev.*, **2010**, *110*, 3552-3599.
17. R. J. Evans, T. A. Milne, M. N. Soltys, *J. Anal. Appl. Pyrolysis*, **1986**, *9*, 207-236.
18. E. Sjöström, Wood Chemistry – Fundamentals and Applications, 1st ed., Academic Press, Millbrae, **1981**.
19. C. A. Chen, H. Pakdel, C. Roy, *Bioresour. Technol.*, **2001**, *79*, 277-299.
20. S. Reale, A. D. Tullio, N. Spreti, F. D. Angelis, *Mass Spectrom. Rev.*, **2004**, *23*, 87-120.
21. E. Dorrestijn, L.J.J. Laarhoven, I.W.C.E. Arends, P. Mulder, *J. Anal. Appl. Pyrol.*, **2000**, *54*, 153-192.
22. G. Brunow, in Biorefineries - Industrial Processes and Products (Eds: B. Kamm, P. R. Gruber, M. Kamm), Vol. 2, Willey-VCH Verlag, Weinheim, **2006**.
23. Z. Chen, C. Wan, *Renew. Sustain. Energy. Rev.*, **2017**, *73*, 610-621.
24. Y. Pu, D. Zhang, P.M. Singh, A.J. Ragauskas, *Biofuels, Bioprod. Biorefin.*, **2008**, *2*, 58-73.
25. Z. Yuan, S. Cheng, M. Leitch, C.C. Xu, *Bioresour. Technol.*, **2010**, *101*, 9308-9313.
26. J. Reiter, H. Strittmatter, L.O. Wiemann, D. Schieder, V. Sieber, *Green Chem.*, **2013**, *15*, 1373-1381.
27. R. Behling , S. Valange, G. Chatel, *Green Chem.*, **2016**, *18*, 1839-1854.
28. S. R. Collinson, W. Thielemans, *Coord. Chem. Rev.*, **2010**, *254(15-16)*, 1854-1870.
29. M. P. Pandey, C. S. Kim, *Chem. Eng. Technol.*, **2011**, *34*, 29−41.
30. A. L. Mathias, A. E. Rodrigues, *Holzforschung*, **1995**, *49*, 273−278.
31. J. C. Villar, A. Caperos, F. Garcia-Ochoa, *Wood Sci. Technol.*, **2001**, *35*, 245-255.
32. F. G. Calvo-Flores, J. A. Dobado, *ChemSusChem*, **2010**, *3*, 1227−1235.
33. B. A. Hart, J. M. Simons, K. S. Shoshan, N. P. M. Bakker, R. P. Labadie, *Free Radical Biol. Med.*, **1990**, *9*, 127−131.
34. J. Stefanska, A. Sarniak, A. Wlodarczyk, M. Sokolowska, E. Pniewska, Z. Doniec, D. Nowak, R. Pawliczak, *Exp. Lung Res.*, **2012**, *38*, 90−99.
35. D. Yancheva, E. Velcheva, Z. Glavcheva, B. Stamboliyska, A. Smelcerovic, *J. Mol. Struct.*, **2016**, *1108*, 552−559.

36. C. Srinivasulu, M. Ramgopal, G. Ramanjaneyulu, C. M. Anuradha, C. Suresh Kumar, *Biomed. Pharmacother.*, **2018**, *108*, 547−557.
37. C. J. Baker, N. M. Mock, B. D. Whitaker, D. P. Roberts, C. P. Rice, K. L. Deahl, A. A. Averyanov, *Biochem. Biophys. Res. Commun.*, **2005**, *328*, 130−136.
38. C. Liu, S. Wu, H. Zhang, R. Xiao, *Fuel Process. Technol.*, **2019**, *191*, 181-201.
39. J. Levec, A. Pintar, *Catal. Today*, **2007**, *124*, 172-184.
40. Q. Xiang, Y. Y. Lee, *Appl. Biochem. Biotechnol.*, **2001**, *91-93*, 71-80.
41. S. G. Santos, A. P. Marques, D. L. D. Lima, D. V. Evtuguin, V. I. Esteves, *Ind. Eng. Chem. Res.*, **2011**, *50*, 291-298.
42. G. X. Wu, M. Heitz, E. Chornet, *Ind. Eng. Chem. Res.*, **1994**, *33*, 718-723.
43. S. Bhargava, H. Jani, J. Tardio, D. Akolekar, M. Hoang, *Ind. Eng. Chem. Res.*, **2007**, *46*, 8652-8656.
44. H. B. Deng, L. Lin, Y. Sun, C. S. Pang, J. P. Zhuang, P. K. Ouyang, J. J. Li, S. J. Liu, *Energ. Fuel*, **2009**, *23*, 19-24.
45. H. B. Deng, L. Lin, Y. Sun, C. S. Pang, J. P. Zhuang, P. K. Ouyang, Z. Li, S. J. Liu, *Catal. Lett.*, **2008**, *126*, 106-111.
46. S. Ansaloni, N. Russo, R. Pirone, *Waste Biomass Valorization*, **2017**, *9*, 2165-2179.
47. H. Deng, L. Lin, S. Liu, *Energ. Fuel*, **2010**, *24*, 4797-4802.
48. J. Zhang, H. Deng, L. Lin, *Molecules*, **2009**, *14*, 2747-2757.
49. P. Gao, C. Li, H. Wang, X. Wang, A. Wang, *Chinese J. Catal.*, **2013**, *34*, 1811-1815.
50. M. Gale, C. M. Cai, K. L. Gilliard-Abdul-Aziz, *ChemSusChem*, **2020**, *13(8)*, 1947-1966.
51. J. M. Pepper, P. E. T. Baylis, E. Adler, *Can. J. Chem*., **1959**, *37*, 1241-1248.
52. J. Romanyà, R. Vallejo, *J. For. Sci.*, **1996**, *42(2)*, 192–197.
53. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker in Determination of Structural Carbohydrates and Lignin in Biomass, Laboratory Analytical Procedure (LAP), Technical Report, NREL/TP-510-42618, Revised August **2012**.
54. T.-Q. Yuan, S.-N. Sun, F. Xu, R.-C. Sun, *J. Agric. Food Chem.*, **2011**, *59*, 10604–10614.
55. S. Bauer, H. Sorek, V. D. Mitchell, A. B. Ibáñez, D. E. Wemmer, *J. Agric. Food Chem.*, **2012**, *60*, 8203−8212.
56. J.-L. Wen, S.-L. Sun, B.-L. Xue, R.-C. Sun, *Materials*, **2013**, *6*, 359–391.
57. D. J. Peterson, N. M. Loening, *Magn. Reson. Chem.*, **2007**, *45*, 937–941.
58. M. Sette, R. Wechselberger, C. Crestini, *Chem. Eur. J.*, **2011**, *17*, 9529–9535.
59. V. E. Tarabanko, D. V. Petukhov, G. E. Selyutin, *Kinet. Catal.*, **2004**, *45*, 569–577.
60. T. Rinesch, J. Mottweiler, M. Puche, P. Concepción, A. Corma, C. Bolm, *ACS Sustain. Chem. Eng.*, **2017**, *5*, 9818–9825.
61. M. Sette, H. Lange, C. Crestinia, *Comput. Struct. Biotechnol. J.*, **2013**, 6(7), 1-7.
62. C. Liu, S. Wu, H. Zhang, R. Xiao, *Fuel Process. Technol.*, **2019**, *191*, 181-201.
63. B. Bujanovic, S. A. Ralph, R. S. Reiner, K. Hirth, R. H. Atalla, *Materials*, **2010**, *3*, 1888-1903.
64. F. M. Casimiro, C. A. E. Costa, C. M. Botelho, M. F. Barreiro, A. E. Rodrigues, *Ind. Eng. Chem. Res.*, **2019**, *58*, 16442-16449.
65. S. Dabral, J. G. Hernández, P. C. J. Kamer, C. Bolm, *ChemSusChem*, **2017**, *10(13)*, 2707-2713.
66. W. Schutyser, T. Renders, S. Van den Bosch, S. F. Koelewijn, G. T. Beckham, B. F. Sels, *Chem. Soc. Rev.*, **2018**, *47*, 852-908.
67. B. Rößiger, G. Unkelbach, D. Pufky-Heinrich, in Base-Catalyzed Depolymerization of Lignin: History, Challenges and Perspectives, Lignin - Trends and Applications, Matheus Poletto, IntechOpen, **2018**, Chap. 4, 99-120.
68. E. Subbotina, A. Velty, J. S. M. Samec, A. Corma, *ChemSusChem*, **2020**, *13*, 1-10.
69. C. Mattsson, S.-I. Andersson, T. Belkheiri, L.-E. Åmand, L. Olausson, L. Vamling, H. Theliander, *Biomass Bioenergy*, **2016**, *95*, 364-377.
70. R. Rinaldi, R. Jastrzebski, M. T. Clough, J. Ralph, M. Kennema, P. C. A. Bruijnincx, B. M. Weckhuysen, *Angew. Chem. Int. Ed. Engl.*, **2016**, *55(29)*, 8164-8215.
71. P. D. McDonald, G. A. Hamilton, in Organic Chemistry; Mechanisms of Phenolic Oxidative Coupling Reactions in Oxidation, **1973**, 97–134.

72. V. E. Tarabanko, N. A. Fomova, B. N. Kuznetsov, N. M. Ivanchenko, A. V. Kudryashev, *React. Kinet. Catal. Lett.*, **1995**, *55*, 161–170.
73. A. D. Venica, C.-L. Chen, J. S. Gratzl, *Holzforschung*, **2008**, *62*, 627–636.
74. T. Rinesch, J. Mottweiler, M. Puche, P. Concepción, A. Corma, C. Bolm, *ACS Sustain. Chem. Eng.*, **2017**, *5*, 9818–9825.
75. S. Gaspa, I. Amura, A. Porcheddu, L. De Luca, *New J. Chem.*, **2017**, *41*, 931-939.