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ADVANCED LIGNIN OXIDATIVE STRATEGIES
Catalysis, Biocatalysis and Biomimetic Systems

Among the fundamental aspects of lignin chemistry a special focus is toward the design and development of new selective oxidative degradation processes. Pulp and paper industry requires environmentally friendly lignin oxidation procedures that can give high yield pulps thus preserving cellulose. This aim can be reached developing oxidative strategies that do not involve the formation of hydroxyl radical species mainly responsible for cellulose degradation. An example of the design of deligninfication strategies is outlined.

Lignin is the second most abundant biopolymer on earth and as such represents an invaluable renewable resource of raw chemicals. Despite the high abundance its industrial exploitation is to date not relevant. Lignin is a polymer with a complex tridimensional structure lacking of repetetive units or bondings [1]. It consists in partially oxygenated phenyl-propanoic units (Fig. 1). Its structural complexity induced a long time based continuous effort of fundamental research in the development of suitable techniques for lignin chemical characterization. Thus the main reason for the lack to date of lignin extensive industrial exploitation lies in its structural complexity and heterogeneity. In Nature lignin degradation is selectively accomplished by white-rot basidiomycetes fungi that produce a pool of ligninolytic enzymes. Among them, laccase, lignin and manganese peroxidases are the most reactive species.

Laccase, benzenediol:oxygen oxidoreductase 1.10.3.2 is a multi-copper oxidase which performs the reduction of oxygen to water [2]. The typical substrates for laccase are phenolic systems. Their oxidation proceeds *via* an outer sphere electron transfer and proton abstraction that generate a phenoxyl radical [3]. This enzyme shows

a high thermal resistance (stable at 60 °C) and low substrate specificity by being able to oxidize a number of different aromatic substrates. Its lack of substrate inhibition coupled with its high oxidation rates (10-100 fold higher than those of Lignin peroxidase or Manganese peroxidase) make laccase useful in specific industrial applications. However laccases alone are not able to efficiently degrade lignin. It has been reported in 1992 that in the presence of suitable oxidation mediators laccase can effectively oxidize lignins [4]. Since than several studies aimed at clarifiing the reactivity of the laccase-mediator system (LMS) appeared in the literature hypothesizing for the mediator a role of either oxidizing agent for non-phenolic lignin subunits or alternatively, the capability to act as a readily diffusible oxidizing intermediate. The possibility to improve the process is tightly bound to the exhaustive knowledge of the reactions occurring. For this reason we performed a study aiming at clarify the role of the mediator in the LMS. We selected an array of phenolic and non-phenolic lignin model compounds and submitted them to laccase and laccase-mediator oxidation using 1-hydroxybenzotriazole (HBT) as mediator. On the base of the distribution of the oxidation products obtained under the different experimental conditions, and of computational studies about the bond dissocia-
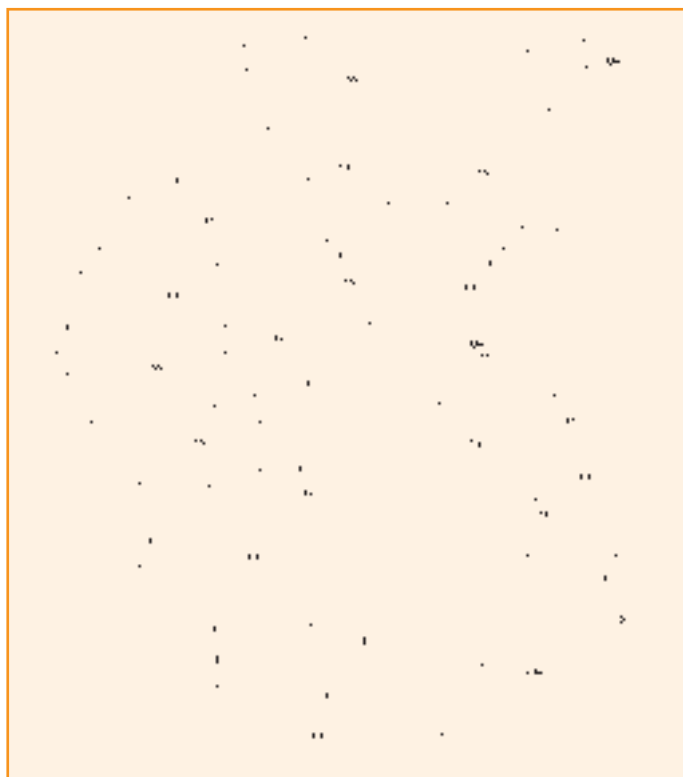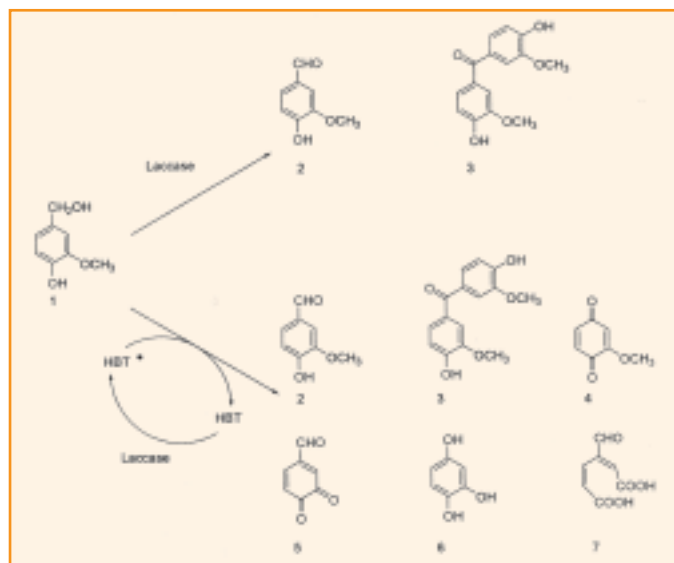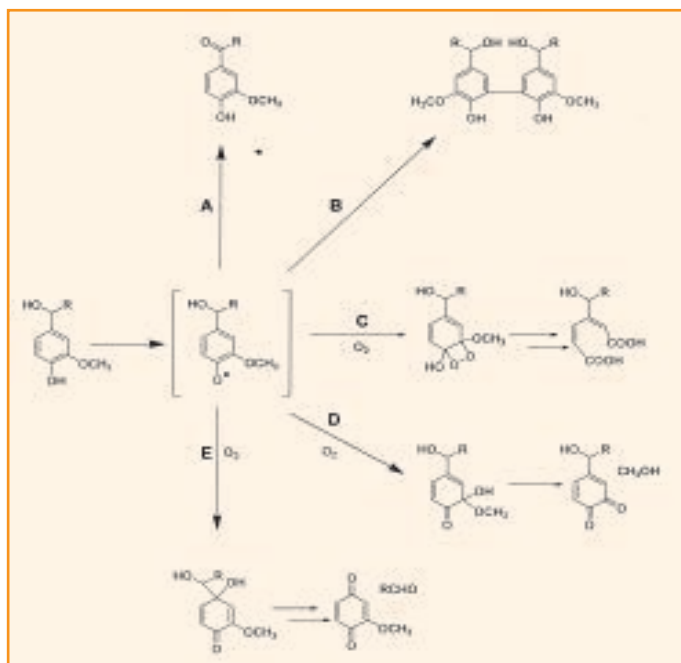

Scheme 1

tion energies for the possible hydrogen abstraction reactions, we elaborated a mechanism hypothesis for the LMS [5].

As an example, experiments with vanillyl alcohol **1** (Scheme 1) showed that in the presence of laccase only side-chain oxidation and oxidative coupling reactions occur. However, in the presence of the LMS the formation of *o*- and *p*-quinones, demethylation, aromatic ring cleavage and oxidative coupling reactions were operative. It is assumed that laccase catalyzes the reduction of oxygen to water with the generation of a phenoxyl radical by a one electron oxidation process and a proton loss. Phenoxyl radicals are long lived species with half life times of hours. The phenoxyl radicals generated by the laccase mediated oxidation of phenolic lignin subunits, in its early steps, can in principle undergo several different reactions. More specifically, disproportionation reactions yield side-chain oxidation products (Scheme 2A); radical coupling reactions yield condensed products (Scheme 2B). Further oxidation/oxygen addition in the C-3 position yields *o*-quinones and methanol (Scheme 2D) with the eventual aromatic ring cleavage via dioxetane intermediates (Scheme 2C). Oxidation in C-1 yields alkyl-aryl cleavage with *p*-quinone formation (Scheme 2E). However, since phenoxyl radicals do not react with molecular oxygen to a noticeable extent [6] ($k<10^{-2}$ $M^{-1}sec^{-1}$) reaction pathways C, D and E are not active processes. In the presence of laccase and HBT it is assumed that the HBT radical is generated. One may hypothesize, by analogy to the oxidation mechanism of non-phenolic models, that HBT can react with phenolic lignin subunits *via* hydrogen abstraction regenerating HBT.

Estimates of the bond dissociation energies for the hydrogen

Fig. 1 - Lignin structure proposed by Brunow *et al*. in 1996 [1]

Scheme 2

abstraction from vanillyl alcohol at the phenolic hydroxyl or at the benzylic carbon were found to be thermodynamically equivalent [7]. Thus in principle, hydrogen abstraction can occur either at the phenolic or at the benzylic position. In the former case a phenoxyl radical would be formed. As such HBT˙ would simply act as a diffusible radical mediator. If hydrogen abstraction occurs at the benzylic position a benzylic radical would be formed. This latter intermediate is likely to undergo rapid oxygen addition and after loss of superoxide radical anion, to yield the side-chain oxidation products shown in Scheme 3. It has been shown that benzylic radicals are reactive toward oxygen addition at rates of 10^{10} $M^{-1}sec^{-1}$. The superoxide radicals radical anions formed during this step would in turn readily react with phenoxyl radicals allowing the formation of further oxidation products (Scheme 3).

Attack at the C3 position would cause the formation of *o*-quinones and methanol, while addition at the C4 ultimately would produce the formation of aromatic ring cleavage products (muconic acids). Finally addition at C1, followed by aryl-alkyl cleavage, would result in the formation of *p*-quinones (Scheme 3). This reaction mechanism could explain the formation of products **3**-**6** (Scheme 1) from the LM system on vanillyl alcohol.

Oxidations carried out on residual kraft lignin showed that the LMS is not operating when the phenolic OH groups are protected. Extensive experiments showed the occurrence of coupling reactions during laccase oxidation [8]. In the presence of the LMS,

however, the reaction pathway was driven toward side-chain oxidation and oxygen addition products, while the formation of condensed structures was depressed. From these data a clear trend becomes apparent: the laccase and the laccase-mediator oxidation of residual kraft lignin (RKL) proceed through different reaction pathways. In the presence of HBT side-chain oxidation reactions are favored as opposed to radical coupling and condensation reactions. When the treatment was carried out in the absence of HBT, the formation of condensed units occurred.

We can conclude that HBT is able to promote the oxidative reactivity of laccase by readily diffuse into the lignin's inner structure. HBT radicals generated by laccase oxidation drive the overall reaction process toward the Ca oxidation pathway rather than the coupling process occurring with laccase alone. This is accomplished by hydrogen atom abstraction reactions at the benzylic rather than phenolic position. The superoxide radical anions formed upon oxygen addition to the benzyl radical intermediate yield in turn quinones and aromatic ring cleavage products.

Design and development of an advanced biomimetic model of lignin peroxidase

Lignin peroxidase (LiP) activates hydrogen peroxide to the oxidation of non-phenolic lignin subunits. To date any industrial application is prevented by its low stability and complete inactivation in the presence of an excess of oxidant. Hence the development of new transition metal catalysts for the activation of hydrogen peroxide toward heterolytic oxygen transfer represents the most studied chemical approach in the design of new delignification processes. Generally speaking, such catalytic systems can be defined as functional models when the synthetic catalyst participates at the same chemical
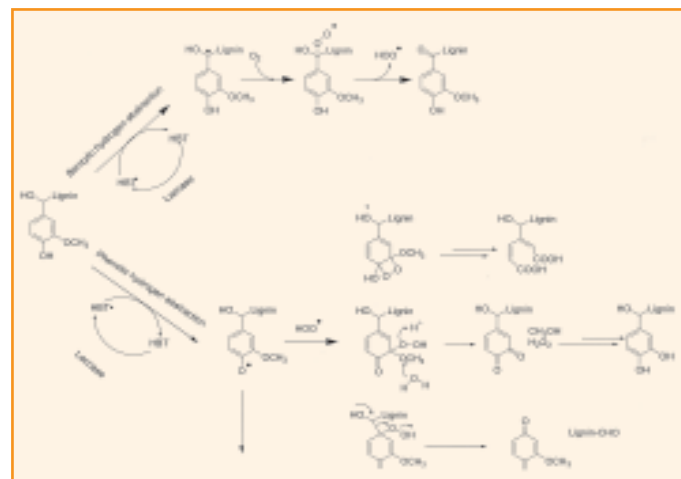
Scheme 3

processes of the biomolecule, while structural models reproduce the coordination geometry of the metallic center. The best models are those that manage to combine the characteristics of the structural and functional analogues. An interesting example of an advanced biomimetic model of lignin peroxidase is the porphyrin-mediator system (PMS) that we recently developed.

In the presence of hydrogen peroxide, the active center of LiP performs a one-electron oxidation of the lignin aromatic moieties. The catalytic cycle consists in a two electron oxidation of Fe(III) proto-porphyrin IX (high spin) to give a highly reactive oxo-iron(IV) proto-porphyrin IX p-cation radical, the LiP I complex (LiP compound I) [9]. The LiP compound I is then reduced to the initial state by two different one-electron reductions by the substrates [10] (Fig. 2).

The polypeptidic envelope of the enzyme protects the active site from side reactions, and activates it. The heterolytic cleavage of the peroxidic bond is subject to acid catalysis. His residue activates the complex to heterolytic cleavage by enhancing the electrophilic character of the oxygen atom, and reducing the strength of the metal-oxygen bond.

The ligninolitic activity of LiP is enhanced by the presence of veratryl alcohol (VA), which acts as a diffusible redox mediator in lignin oxidation [9]. Thus, the oxidative potential can be transferred from the enzyme to the bulk of the polymer avoiding a kinetic barrier for the heterogeneous reaction.

An efficient biomimetic system for lignin peroxidase is supposed to mimick the three levels of natural catalysis, namely the functions of the active site, of the polypeptidic envelope and of the oxidation mediator. Protoporphyrin IX suffers from the major disadvantage of being unstable in the presence of excess oxidants. Its lability is due either to self-destruction or to the formation of inactive μ-oxo complexes [11]. The study of biomimetic systems has thus focused toward the development of synthetic metalloporphyrins more resistant to degradative oxidation.

When synthetic metalloporphyrins are used as biomimetic catalysts in the presence of hydrogen peroxide, several side reactions can occur. The peroxidic bond can undergo homolytic scission to yield Fe(IV)-OH and hydroxyl radical in a Fenton like fashion. A second molecule of peroxide may react with the metal oxo complex in a catalase like fashion to yield the formation of H_2O and O_2, and ultimately the degradation of the active oxidant species. The metallo-oxo complex may further react to yield inactive μ-oxo dimers. The choice of the catalytic center of our biomimetic system was carried out on the basis of the comparison of several synthetic iron and manganese porphyrins [12]. The manganese porphyrins are not strictly biomimetic systems of LiP, since the natural enzyme

active site is an iron complex. However, the use of manganese complexes could override many reactivity problems. More specifically, manganese porphyrins form single adducts with nitrogen bases and in this way can be easily activated. Moreover, manganese shows a smaller tendency than iron to undergo homolytic cleavage of the
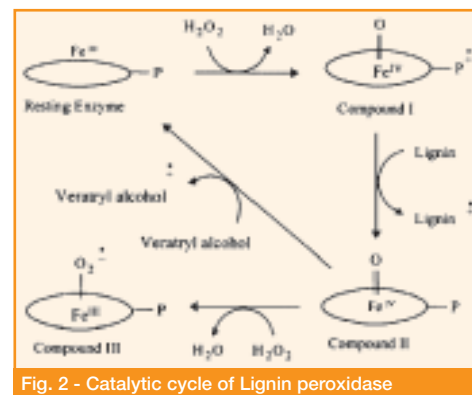

Fig. 2 - Catalytic cycle of Lignin peroxidase

peroxidic bond. The cationic manganese *meso*-tetrakis(tetramethylpyridinio) porphyrin pentaacetate was thus selected as reagent of choice of our biomimetic system [12].

The second step in the development of the Lignin peroxidase model consisted in the mimicking of the polypeptidic envelope. This stage has a relevant industrial aspect since the potential use of metalloporphyrins in lignin oxidation is bound to the possibility of a further increase of their stability toward hydrogen peroxide, and of a possible recovery and recycle of the catalyst after its use. These two aims could be reached by immobilization of the catalyst onto a suitable support [13].

Smectite clay minerals such as montmorillonite have layer lattice structures in which two-dimensional oxyanions are separated by layers of hydrated cations. The hydrated cations on the interlamellar surfaces of
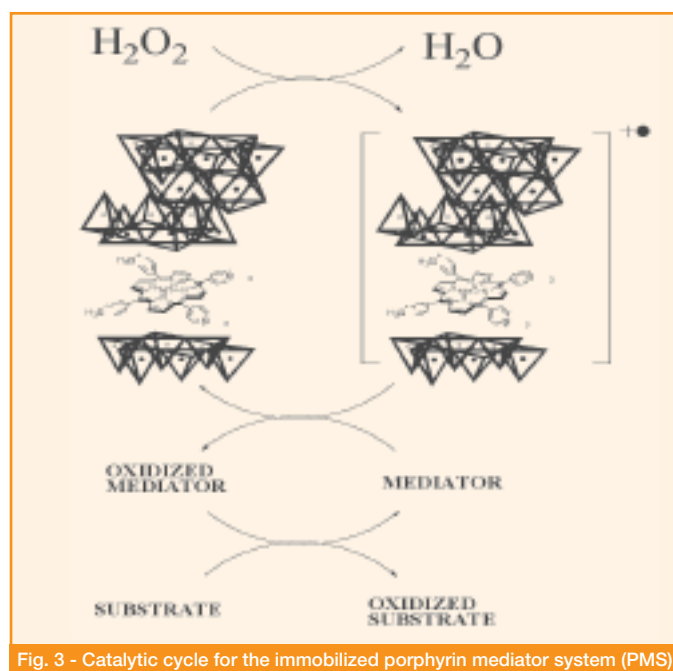

Fig. 3 - Catalytic cycle for the immobilized porphyrin mediator system (PMS)
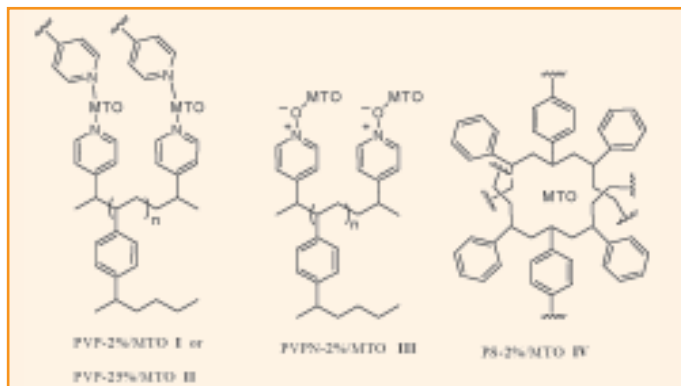
Fig. 4 - Structure of poly(4-vinylpyridine)/MTO and polystyrene/MTO catalysts I-IV

the native minerals can be replaced with almost any desired cation by utilizing simple ion exchange methods. Thus, immobilization onto clays would prevent from the formation of µ-oxo dimers. In this microenvironment a cationic metalloporphyrin can be immobilized by axial ligation of the metal center with the oxyanions layers, electrostatic interaction, and by physical encapsulation.

The cationic manganese *meso*-tetrakis(tetramethylpyridinio) porphyrin pentaacetate supported on montmorillonite Mn(TMePyP) clay revealed to be an efficient catalyst in the hydrogen peroxide oxidation of monomeric and dimeric lignin model compounds. Nevertheless, it is not suitable for lignin oxidation mainly due the presence of a kinetic barrier to the approach of the polymeric substrate [14].

A third step mimiking the role of the natural oxidation mediator was thus necessary. The mediator concept requires the choice of a low molecular weight easily diffusible molecule with such a redox potential to be easily oxidized by the catalyst to yield a radical species stable enough to diffuse to lignin and reactive enough to oxidize it. We chose for our study 1-hydroxy benzotriazole, a species already employed as a mediator in biocatalytic processes. When phenolic and non-phenolic monomeric and β-*O*-4 lignin model compounds resembling the fundamental bonding pattern in lignin were treated with the clay/porphyrin catalyst in the presence of HBT - a clay/porphyrin mediator system (clay-PMS) - they were efficiently oxidized with the occurrence of side-chain and aromatic ring oxidation reactions.

Oxidation of milled wood and technical softwood lignins, carefully studied by ^{31}P-NMR spectroscopy after phosphitylation of the oxidized samples [15], was also effective and revealed the occurrence of efficient delignification processes and absence of significant coupling reactions.

Thus an advanced biomimetic system for lignin peroxidase

has been designed and realized (Fig. 3). When using such systems, the application of the "mediator" concept avoids kinetic problems due to the solid nature of the substrate and of the heterogeneous catalyst. The porphyrin mediator systems represent the last step in the development of a "synthetic enzyme" for the pulp and paper transformations.

## Development of novel catalytic systems for delignification processes

Oxidative delignification processes avoiding radical species needed to be developed possibly by oxygen atom transfer acting by concerted mechanisms.

A novel catalyst potentially useful for this purpose is methyltrioxorhenium(VII) (MeReO$_3$, MTO) [16]. MTO, in combination with H$_2$O$_2$, has became in recent years an important catalyst for a variety of synthetic transformations, such as oxidation of olefins, alkynes, sulfur compounds, phosphines, Baeyer-Villiger rearrangement and oxygen insertion into C-H bonds. Accordingly with this high reactivity, MTO is able to catalyze the oxidation of aromatic derivatives [17]. In this latter case the reaction proceeds through the formation of reactive arene epoxide intermediates that further rearrange and are oxidized to corresponding benzoquinones.

para-Benzoquinones are usually obtained by oxidation of alkyl phenols that lack of substituents on the C-4 position of the aromatic ring. In the presence of bulky substituents, as in the case of cardanols (3-*n*-pentadecyl phenol derivatives), ortho-benzoquinones can be obtained in high yields because of steric hindrance to the approach of the catalyst to the aromatic ring [18]. Alkoxy- substituted benzenes are less reactive than the corresponding phenolic derivatives towards MTO [19].

To date, a concerted mechanism for the oxygen transfer from MTO monoperoxo and diperoxo intermediates by a butterfly transition state is the accepted reaction pathway for MTO, while radical processes have not been reported.

With the aim to design new catalytic systems for environmental friendly pulping and bleaching processes, we started to study the use of
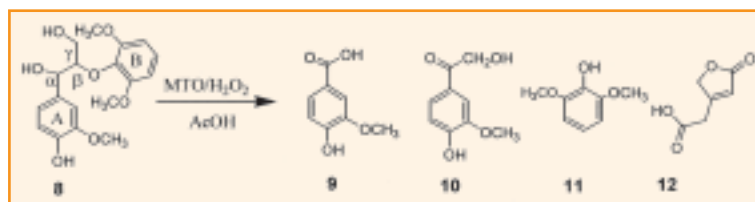
Scheme 4

MTO in the oxidative degradation of lignin and lignin model compounds with H_2O_2 as primary oxidant. We selected an array of lignin model compounds resembling the main bonding patterns in native and technical lignins and studied their reactivity with MTO [20]. Our attention was next turned to more complex lignin polymers, hydrolytic sugar cane lignin (SCL), red spruce kraft lignin (RSL) and hardwood organosolvent lignin (OSL), that are representative examples of widely diffused *para*-hydroxyphenyl-guaiacyl, guaiacyl and guaiacyl-syringyl lignins.

Their oxidation was studied by means of advanced ^{31}P NMR techniques that allow the quantitative determination of all labile OH groups on the polymer - that is, aliphatic, different phenolic OH groups, carboxylic acids - after phosphitylation of the sample [15].

MTO showed to be a powerful and efficient catalyst for the oxidation of phenolic and non-phenolic lignin model compounds representative of the main bonding pattern in native lignins, affording both side chain oxidations and aromatic ring cleavage reactions. Diphenylmethane models, that are usually recalcitrant to oxidation, were also found extensively degraded mainly by cleavage of the interunit aromatic linkages. MTO was also able to catalyse the extensive degradation of selected technical lignins, increasing their degree of solubility and reducing their content in condensed subunits.

In an effort to develop more versatile and environmental friendly heterogeneous catalysts, we described the preparation of novel rhenium compounds of general formula $(polymer)_f/(MTO)_g$ (the f/g quotient express the ratio by weight of the two components) by heterogenization of MTO on easily available and low cost polymeric support, poly(4-vinylpyridine) or polystyrene [21], applying the "mediator" concept [22] and the microencapsulation technique [23].

The structures of poly(4-vinylpyridine)/MTO and polystyrene/MTO catalysts **I**-**IV** employed, namely poly(4-vinylpyridine) 2% and 25%

Oxidation of red spruce kraft lignin (RSL) with H_2O_2 and catalysts I-IV

Lignin/treatment	Aliphatic OH (mmol/g)	Condensed OH (mmol/g)	Guaiacyl OH (mmol/g)	COOH (mmol/g)
RSL	1.59	0.71	0.28	0.88
RSL/MTO/H_2O_2	0.53	0.29	0.17	1.50
RSL/**I**/H_2O_2	1.05	0.88	0.44	1.18
RSL/**II**/H_2O_2	1.08	0.62	0.31	1.51
RSL/**III**/H_2O_2	1.13	0.81	0.41	1.22
RSL/**IV**/H_2O_2	0.91	0.48	0.25	0.88

cross-linked (with divinylbenzene)/MTO (PVP-2%/MTO **I** and PVP-25%/MTO **II**, respectively), poly(4-vinylpyridine-N-oxide) 2% cross-linked/MTO (PVPN-2%/MTO **III**) and microencapsulated polystyrene 2% cross-linked/MTO (PS-2%/MTO **IV**), are schematically represented in Figure 4.

We selected significative monomeric and dimeric lignin model compounds and studied their reactivity with supported MTO catalysts by characterisation of the main oxidation products in the presence of H_2O_2. As an example in Scheme 4 the oxidation of a phenolic β-O-4 model compound **8** is reported. In the presence of MTO and catalysts **I**-**IV**/H_2O_2 systems compound **8** was found always extensively degraded with a quantitative conversionto yield products of side-chain oxidation and fragmentation and aromatic ring cleavage (Scheme 4). The products isolated showed that MTO and catalysts **I**-**IV** are efficient for the oxidation of lignin subunits at the side-chain bondings and for the aromatic ring cleavage reactions and therefore are good candidates for the development of delignification processes.

Our attention was next turned to more complex lignin polymer, red spruce kraft lignin (RSL), that is a representative example of widely diffused guaiacyl lignins. Its oxidation was analysed by means of advanced ^{31}P-NMR techniques [15]. The Table shows the content in aliphatic OH, condensed and guaiacyl phenolic OH and car-

Strategie avanzate di ossidazione della lignina. Sistemi catalitici, biocatalitici e biomimetici

ABSTRACT

La lignina costituisce il secondo biopolimero disponibile sulla terra e come tale rappresenta una fonte rinnovabile di prodotti chimici. Nonostante la grande abbondanza, il suo sfruttamento industriale non è ad oggi un processo significativo, a causa della complessità della sua struttura.
Tra gli aspetti fondamentali della chimica della lignina un interesse particolare è rivolto allo sviluppo di nuovi processi selettivi di degradazione ossidativa. L'industria cartaria richiede infatti nuovi processi sostenibili di ossidazione della lignina che possano dare paste di cellulosa con rese elevate. Questo scopo può essere perseguito mediante lo sviluppo di strategie ossidative che non portino alla formazione di specie radicaliche, ed in particolare di radicali ossidrilici, in quanto queste specie sono le principali responsabili dei processi di degradazione ossidativa della cellulosa. Nell'articolo vengono riportati alcuni esempi significativi di pianificazione e sviluppo di queste strategie di delignificazione.

boxylic groups contents in red spruce kraft lignin before and after hydrogen peroxide oxidation with MTO or catalysts **I**-**IV**.

As a general trend the oxidation of RSL with H_2O_2 and catalysts **I-IV** gave an appreciable decrease in the content of aliphatic OH groups thus indicating the occurrence of side-chain oxidation reactions. Moreover all the lignin samples showed an increase of COOH units after the treatments in accordance with MTO reactivity (Table). Even if the efficiency of MTO in the oxidation of SCL and RSL was found appreciably higher than catalysts **I-IV**, all the supported catalysts were found reactive toward lignins. This implies that there is not a prohibitive kinetic barrier to the approach of the heterogeneous catalyst to the polymeric substrates. On the other hand the possibility that small oxidized fragments may work as oxidation carriers cannot be ruled out. The content of guaiacyl units in lignis treated with MTO and catalysts **I-IV** allowed to evidenciate a different selectivity between the soluble and immobilised catalysts. In fact, the guaiacyl (G) units were found decreased upon H_2O_2/MTO treatments; on the contrary in the presence of the catalysts **I-IV** an increase of the G units was evident. The increase of G units in treated lignin may be explained by the occurrence of aliphatic lignin side-chain oxidation processes with C-H insertion reactions and Dakin like reactions on α-carbonyl units, rather than aromatic ring oxidations. These reactions occurring preferentially on the aliphatic lignin interunit bondings would lead to residual lignins with a higher phenolic content. Our experimental results on the lignins oxidations indicate clearly the occurrence of different reaction pathways between the soluble and immobilised catalysts. These data are specifically relevant since they address the issue of selectivity in the chemical modification of lignins. The selective degradation of interunit bondings with the final production of more soluble easily oxidizable products represents a relevant target in the development of lignin bleaching and modification processes.

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