Iron and manganese surface complex formation with extracted lignin. Part 1: Adsorption isotherm experiments and EPR spectroscopy analysis

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Received (in London, UK) 1st July 2002, Accepted 16th August 2002
First published as an Advance Article on the web 18th September 2002

The adsorption of iron(III) and manganese(II) onto lignin extracted from wheat straw was investigated at 20 °C using batch adsorption experiments. Two solid substrates were studied : the Ligno-cellulosic Substrate (LS), which resulted from successive acido-basic treatments, and the Cell Wall Residue substrate (CWR), which was obtained by solvent extraction. Comparisons between these two lignin-containing substrates were made. Experiments were conducted as a function of the pH for a wide range of metal concentrations. The percentage of metal adsorbed increased with the pH and the results indicate that Fe(III) was strongly adsorbed at low pH (<3) while Mn(II) formed far less stable surface complexes from pH = 8.0. Moreover, in contrast to Fe(III), Mn(II) adsorption was significantly affected by the presence of calcium and carbonate, which are abundant ions in the soils of the Champagne-Ardenne region. The presence of calcium ions decreased the manganese adsorption by 25% to 40%. EPR spectroscopy was used to investigate the geometrical environment of the metal in the surface complexes. As the metal was adsorbed, redox processes took place, which could be observed by the change in the amount of quinonic species present in the lignin surface. Then, the evolution of quinonic species amount as a function of the metal concentration was examined by EPR measurements considering the signal centred at $g = 2.0025$. Mechanisms involved in the electronic transfer between the metal ion and the surface organic radicals are discussed.

Introduction

Adsorption onto soil organic matter such as lignin is an important process which influences the fate, transport and toxicity of metals in natural systems.[1,2] Moreover, lignins influence the micro-nutrient availability through their ability to complex inorganic cations and their potential involvement in redox reactions. Lignins exist as soluble and insoluble fractions in soil and the understanding of the factors that influence the partitioning of elements between solid and soluble phases is, therefore, an essential prerequisite for any attempt at predicting the contamination level of the environment (groundwater and soil pollution).

Most of the studies dealing with adsorption properties of lignin give quantitative results for some metal ions such as cadmium, copper, zinc, or lead.[3,4] Numerous studies have been published about the retention capacity of lignin-containing substrates (such as moss, wheat straw, soil samples, maize, *etc.*) or lignin-derived substrates (such as fulvic and humic acids).[5,6] Few have used spectroscopic tools such as EPR to describe the surface complexes formed. Among these studies, some have been performed using EPR and EXAFS techniques for humic substances after sorption of some metal cations, notably iron and manganese.[7,8] They show that Mn(II) ions are surrounded by 6 oxygen atoms with a mean distance between the metal and the oxygen atom corresponding to $Mn(H_2O)_6^{2+}$. Then, the complexes formed with manganese ions seem to be "outer-sphere" complexes, *i.e.* the metal is linked to the surface keeping its hydration sphere, through electrostatic interactions. In contrast, Fe(III) ions form "inner-sphere" complexes with humic acids and are in tetrahedral or octahedral sites with rhombic symmetry.

Extracted lignin, like various natural organic matters, is rich in carboxylic and phenolic functional groups.[9] These acidic moieties constitute potential complexation sites for metal ions through cation exchange mechanisms. In this study, the nature of the surface complexes formed with Fe(III) and Mn(II) ions is discussed. These two metallic elements are of great interest because they are very abundant in soils and are essential for plant growth. Lignin is the most resistant biopolymer to biodegradation. Consequently, it is very abundant in soils and the purpose of our study is to understand the impact of iron(III) and manganese(II) ions on the biodegradation of lignin by investigating the surface complexes formed.

First, the main factors governing the sorption properties of lignin are discussed, and some quantitative sorption results are given. Moreover, in order to understand the processes involved specifically in calcareous soils such as Champagne-Ardenne ones, the influence of calcium carbonate is also shown in this study.

Second, the surface complexes formed with iron(III) and manganese(II) were studied by EPR spectroscopy in order to determine the environment of the metal centre. Further investigations were conducted to precisely determine the interactions between the metal ion and the surface acid sites. Indeed, radical mechanisms, responsible for the degradation of lignins, are initiated not only by micro-organisms, oxygen-containing radicals (superoxides, hydroxyl radicals) but also by metal cations.[10] The radicals existing on the polymer surface are the starting point for degradative reactions (depolymerisation) and modifications of the polymer (repolymerisation, functionalisation). The amount of radicals is strongly influenced by the presence of metallic cations whose implication in biodegradation of lignins is scarcely known.

 DOI: 10.1039/b206352b

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These cations are certainly involved in the degradation processes as a result of synergetic actions with lignolytic enzymes and microorganisms.[11–13] As lignin exhibits a radical signal corresponding to semiquinonic species, EPR spectroscopy was an appropriate tool to study both paramagnetic metals and organic radicals. This study constitutes a continuation in the effort to better understand these interactions between these two paramagnetic species and to describe the surface complexes.[14,15]

Experimental

Potassium nitrate KNO_3, ferric nitrate $Fe(NO_3)_3{\cdot}9H_2O$ and manganese nitrate $Mn(NO_3)_2{\cdot}4H_2O$ were purchased from Fluka. All chemicals from commercial sources were of the highest available purity and have been used without further purification.

Substrate preparation

The LS (Ligno-cellulosic Substrate) powder was obtained after successive acido-basic treatments of wheat straw, in order to eliminate sugars, hemicellulose and soluble low molecular weight lignin molecules.[15] As a consequence, the resulting powder is quasi-insoluble in the pH range from 2 to 10 (DOC values $\ll$1%). During the treatments, undesirable structural changes may occur such as oxidation of some functional groups of lignin (*i.e.* phenolic moieties converted into carboxylic groups). Specific cleavages of ether linkages lead to a polydispersed polymer, more condensed than the native one, *i.e.* with more carbone–carbone linkages without affecting the solubility. But, the advantage of the LS substrate is its simple composition (25% lignin and almost 75% cellulose) which allows the study of the specific lignin properties (assuming, after examination, that the cellulose interaction with metal ions is negligible compared with that of the lignin).

For comparison, we chose to study another substrate, called CWR (Cell Wall Residue), extracted from the same sample of wheat straw, but obtained by a less destructive method, based on solvent (water, toluene, and ethanol) extraction with the Soxhlet apparatus.[16] Although there is no procedure that allows lignin to be obtained in its native structure, the Soxhlet extraction was proved to be less destructive than other methods such as acid hydrolysis or sulfonate treatment.[9] The CWR obtained was dried by lyophilisation and the LS was air-dried. The powders were both ground to pass through a 100 μm sieve.

Adsorption experiments

Experiments were conducted at 20 °C, at the ionic strength of 0.1 mol L^{-1} (KNO_3). Batches of 25 mL of 0.1 mol L^{-1} KNO_3 electrolyte solution with 50 mg of powder were prepared in polystyrene vessels (2 g L^{-1}). Prior to experiments, suspensions consisting of 50 mg of CWR and LS in 15 mL KNO_3 were shaken for 21 days and 1 day, respectively, to reach hydration equilibrium. The hydration time was determined as previously described by potentiometric titrations.[17]

For isotherms carried out as a function of the pH, metal (as nitrate salt) was added at the required concentration and the pH was then fixed incrementally with various amounts of 0.1 mol L^{-1} KOH or 0.1 mol L^{-1} HNO_3. Suspension pH was initially adjusted to 2.0 before adding $Fe(NO_3)_3$ solution in order to avoid iron hydrolysis. The final pH of each sample was measured after one night to ensure that the sorption equilibrium was reached. The suspension was filtered through a 0.2 μm acetate cellulose membrane filter.

For isotherms achieved as a function of the metal concentration, a 10^{-2} mol L^{-1} metal solution was added to obtain different ranges of concentrations from 6 to 500 μmol L^{-1}. The pH was adjusted to 3.3 and 8.5 respectively for Fe and Mn adsorption experiments in order to limit hydrolysis reactions. The suspensions were shaken until the equilibrium was reached and then were filtered.

Free metal ions in the filtrate were analysed using a Varian Liberty ICP/AES spectrometer. The amount of metal adsorbed was calculated from the initial concentration. Relative error lines were omitted for simplification of the graphics and the standard error deviation was found to be equal to 2% and 3% for iron and manganese measurements, respectively.

The effect of calcium and hydrogencarbonate ions on the iron and manganese sorption was evaluated using the procedure described above, except the addition of $Ca(NO_3)_2$ or $KHCO_3$, which was performed to obtain the final concentration of 5×10^{-3} mol L^{-1}. The potassium hydrogencarbonate solution was adjusted to pH 8.5 before its addition to the suspensions containing manganese ions, in order to avoid a pH increase and subsequent metal hydrolysis. The influence of hydrogencarbonate ions on iron sorption was not studied because of the acidic pH (3.3) chosen for the isotherm experiments, which cannot allow the presence of hydrogencarbonate ions in solution.

Precipitation curves as a function of the pH were obtained in the presence of the filtrate in order to take into account the presence of some potential soluble molecules from the LS and CWR substrates. Indeed, the complexing ability of these molecules in solution would lead to a higher pH of metal precipitation. The experimental procedure consisted of the filtration of the suspension after hydration time and the metal was added to the filtrate after pH adjustment. After one day of equilibration and filtration, the remaining free metal ion concentration in solution was analysed by ICP/AES.

EPR experiments

Solid state EPR spectra were obtained with a Brüker ELEXYS 500 spectrometer operating at X-band frequency with a 100 kHz modulation frequency. The spectra were recorded in the following conditions : microwave power : 15 mW, modulation amplitude : 4 G. The spectra were carried out at 4 K and 293 K for the iron and manganese complexes, respectively.

Results and discussion

Surface acidity constants have been previously determined,[14,17] and were equal to 4.0 and 8.8 for carboxylic and phenolic type sites respectively. Kinetic studies were carried out in optimal conditions of pH and metal concentration determined beforehand from adsorption curves. Thus, the optimal pH chosen for sorption experiments is equal to 3.3 for iron(III) (1×10^{-5} mol g^{-1} and 5×10^{-5} mol g^{-1} for LS and CWR respectively) and 8.5 for manganese(II) ions (1×10^{-4} mol g^{-1}). The iron(III) concentration is lower in the case of LS in order to avoid metal precipitation. The results show that the sorption equilibrium time for Mn(II) on CWR is far longer (2 h) than that on LS (10 min). This could be the result of a longer exchange time between the surface proton and the metal ion, due to a slow diffusion of the metal through the matrix. Only 30 minutes are necessary to reach the equilibrium for iron sorption. The shorter equilibrium time can be explained by the higher charge density of iron compared to that of manganese, which is strongly attracted by the negative charged surface.

Iron(III) sorption experiments

Iron(III) adsorption on LS and CWR was determined as a function of the pH (Fig. 1), taking into account the reaction time determined previously. Our results reveal high adsorption

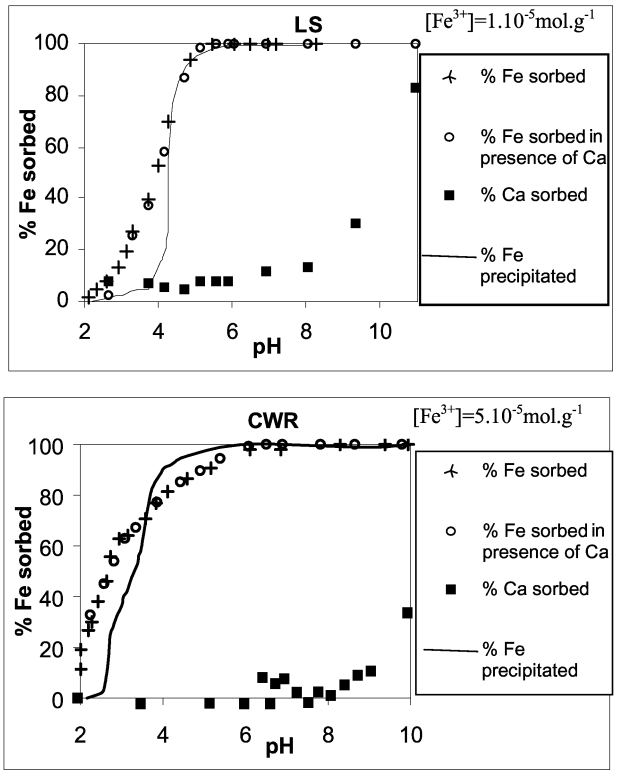

Fig. 1 Percentage of Fe^{3+} sorbed by LS and CWR as a function of the pH. Influence of Ca^{2+} ions.

of iron(III) by the two lignin-containing substrates. As shown in Fig. 1, iron sorption increases from zero at pH 2.0 to 100% at pH 5.0 while the sorption on the CWR attained 20% at pH = 2.0 and 100% at pH = 6.0. Iron precipitation at this concentration (1×10^{-5} mol g^{-1} and 5×10^{-5} mol g^{-1} for LS and CWR respectively) occurred at pH = 4.0 (Fig. 1). According to the solubility product, in the absence of soluble molecules, the precipitation pH should have been equal to 2.7 and 2.5 for iron concentrations of 2×10^{-5} mol L^{-1} and 1×10^{-4} mol L^{-1}, respectively. Fig. 1 shows clearly that metal sorption takes place before precipitation as shown by the shifting of the two curves. As expected, complexation in solution induces a displacement of the precipitation pH towards higher values. So, through sorption processes onto the organic matter, metal hydrolysis is avoided. However, in the case of CWR the sorption seems to be retarded compared to the precipitation curve in the pH range 4–6. This probably due to a coprecipitation process and complexation of iron(III) with the small quantity of soluble molecules. From these curves, we can choose pH = 3.3 for sorption isotherm experiments as a function of the metal concentration, in order to limit metal hydrolysis processes at the surface.

The different shapes of the sorption curves onto LS and CWR suggest different binding mechanisms. In the two cases, cation exchange between proton from acidic surface sites and metal ions is likely to be the main mechanism involved in complexation processes. The difference can be explained by the formation of more stable surface complexes between the metal and CWR. Indeed, complexation starts at a more acidic pH in the case of CWR: 20% of iron is sorbed at pH 2.0 for CWR and at pH 3.1 for LS.

The equilibrium at the interface solid/solution is determinant for the partitioning of iron between solution and solid phases. The competing soluble molecules, which are mainly some low molecular weight lignin molecules, monosaccharides or phenolic species present some complexing ability for iron(III). Indeed, potentiometric titrations have been carried out on the filtrate in the presence of metal at the concentration of 1×10^{-4} mol g^{-1} (data not shown) and have indicated the complexation of the total amount of metal introduced. However, we can note that, in the sorption experiments, 100% of the metal introduced is sorbed, which indicates that iron(III) is preferentially complexed at the surface of the substrate than to the soluble molecules. In the natural environment, this result seems to indicate that surface complexation of iron onto lignin surface is favoured relatively to solution complexation. Consequently, this micronutrient would be present in a form not absorbable directly by plants.

The presence of calcium ions at the concentration of 5×10^{-3} mol L^{-1} (in order to mimic calcareous soils) induces no change in the sorption pH or in the amount of metal sorbed (Fig. 1). Iron complexes are far more stable than calcium ones which are known to form outer-sphere complexes.[18,19] Our results are in good agreement with the study of Dupuis *et al.*[20] concerning fulvic acids. Consequently, in calcareous soils, it is plausible that calcium ions, even at a great concentration, do not affect iron(III) sorption onto lignin.

Fig. 2 illustrates iron adsorption isotherms for LS and CWR at pH 3.3 with metal concentrations ranging from 3×10^{-6} mol g^{-1} to 5×10^{-5} mol g^{-1} for LS and from 3×10^{-6} mol g^{-1} to 5×10^{-4} mol g^{-1} for CWR. A range of lower concentrations was chosen for LS because metal hydrolysis becomes predominant up to 5×10^{-5} mol g^{-1}. Adsorption isotherm curves have to be completed with precipitation curves, in the same conditions. Thus, we can determine the proportion of metal hydrolysis for each concentration. In order to assure that retention properties of our substrates are due mainly to the presence of lignin, the same sorption procedure was conducted with pure cellulose. The curve obtained is the same as the precipitation one, indicating that cellulose exhibits poor retention capacity for the metal cations.

Adsorption isotherm curves do not show a plateau, which indicates that the maximum iron sorption capacity is not reached, or that the metal precipitation partly occurs. Taking into account the precipitation curve, we can evaluate the

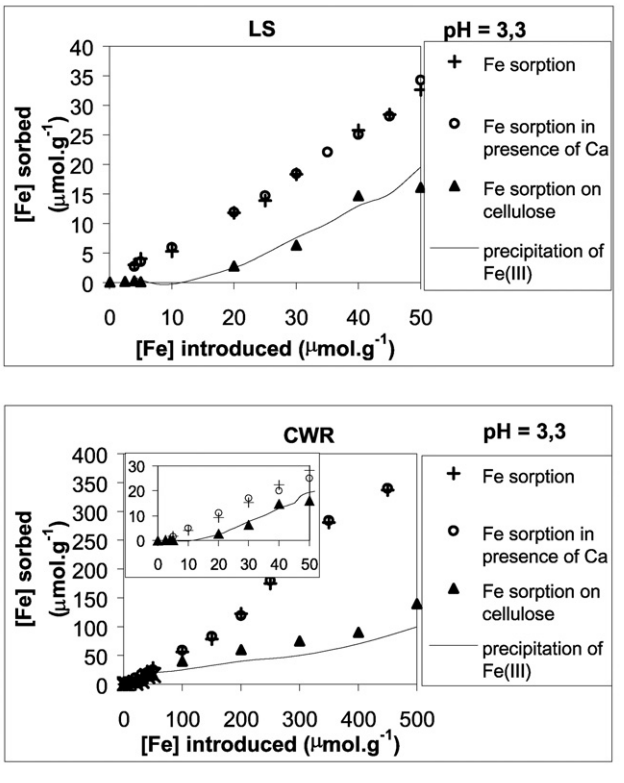

Fig. 2 Amount of Fe^{3+} sorbed by LS and CWR as a function of the metal introduced. Comparison with binding capacity of cellulose. Influence of Ca^{2+} ions.

quantity of iron sorbed onto the substrates: greater than 14 μmol g^{-1} and 10 μmol g^{-1} for the LS and CWR respectively when the concentration of iron introduced is 50 μmol g^{-1}. These values are underestimated because of the impossibility to know the exact quantity of metal precipitated at the surface of the lignin during batch experiments. Indeed, the precipitation curve cannot take into account the presence of solid in suspension, which influences the precipitation pH of the metal. In order to extend the previous results, sorption experiments have been conducted at pH 3.3 in the presence of calcium ions at the concentration of 25×10^{-4} mol g^{-1}. The isotherm curves achieved with or without calcium are superimposed (Fig. 2). Therefore, despite their great concentration, calcium ions have no effect on the iron sorption whatever the metal concentration may be, which confirms the high strength of the iron complexation.

The study of iron sorption presents some constraints due to its low pH hydrolysis: the study has to be conducted at both low concentration and low pH. In calcareous soils, pH is basic (~8–9) and iron must be present as various charged mixed oxo-hydroxylated species that can also have some complexing ability. The strong complexes formed with lignin will limit iron availability for direct plant nutrition.

Manganese(II) sorption experiments

Manganese(II) adsorption experiments onto LS and CWR substrates have been reported in Fig. 3 and show the percentage of manganese(II) sorbed as a function of the pH, with a concentration of 1×10^{-4} mol g^{-1} initially introduced in the suspension. The metal is sorbed at a basic pH equal to 8.5. In contrast to iron, the manganese precipitation and sorption curves are very similar, which does not allow a clear distinction of the two processes and suggests that they take place simultaneously. According to the ion exchange theory, the basic pH of Mn(II) sorption is consistent with the low stability of the surface complexes formed.

However, the adsorption isotherms (Fig. 4) carried out at pH 8.5 and as a function of the metal concentration clearly show that hydrolysis is not the predominant process. Moreover, sorption experiments onto cellulose allow us to conclude that metal ions have no affinity for this surface.

In the same way as iron, we can evaluate the minimum amount of metal sorbed as Mn^{2+} ions, taking into account the precipitation curve: 39 and 85 μmol g^{-1} respectively for LS and CWR with the concentration of metal introduced equal to 100 μmol g^{-1}. The nature of the wheat straw treatment is determinant for the binding capacity of the substrate. Althought manganese ions are implicated in less stable complexes, they are sorbed in higher amounts compared to iron(III). This can be explained by the more basic pH chosen for manganese sorption experiments. Moreover, the amount of metal sorbed increased with the metal concentration introduced: for example, 120 μmol g^{-1} of Mn(II) are sorbed by CWR for an initial concentration of 400 μmol g^{-1}. Consequently, and like in the iron case, the lack of plateau in the adsorption isotherm clearly indicates that the maximum binding capacities of LS and CWR powders are not reached or that the hydrolysis processes occurred at the same time. The amount of metal sorbed increased not only with the metal initial concentration but also with the pH. It is likely that the higher the pH, the stronger the manganese binding becomes.

Fig. 3 and 4 show that adsorption of Mn(II) ions is significantly lower in the presence of calcium ions present at the concentration of 5×10^{-3} mol L^{-1}. Calcium ions are sorbed onto the substrate in basic pH conditions (> 8.0) but only a part of the calcium introduced is retained at the surface: only 20% of calcium introduced at 5×10^{-3} mol.L^{-1} is sorbed at pH 8.5. The extent of calcium effect on manganese sorption was largest for the systems with the lowest Mn/Ca ratio. In contrast to Fe(III), the presence of Ca^{2+} ions decreased the Mn(II) adsorption from 25% to 40% depending on the metal concentration.

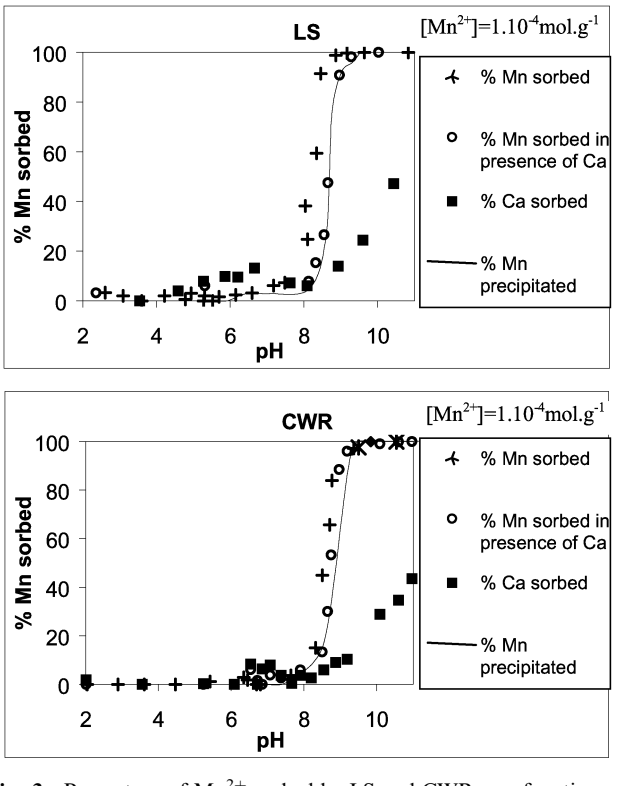

Fig. 3 Percentage of Mn^{2+} sorbed by LS and CWR as a function of the pH. Influence of Ca^{2+} ions.

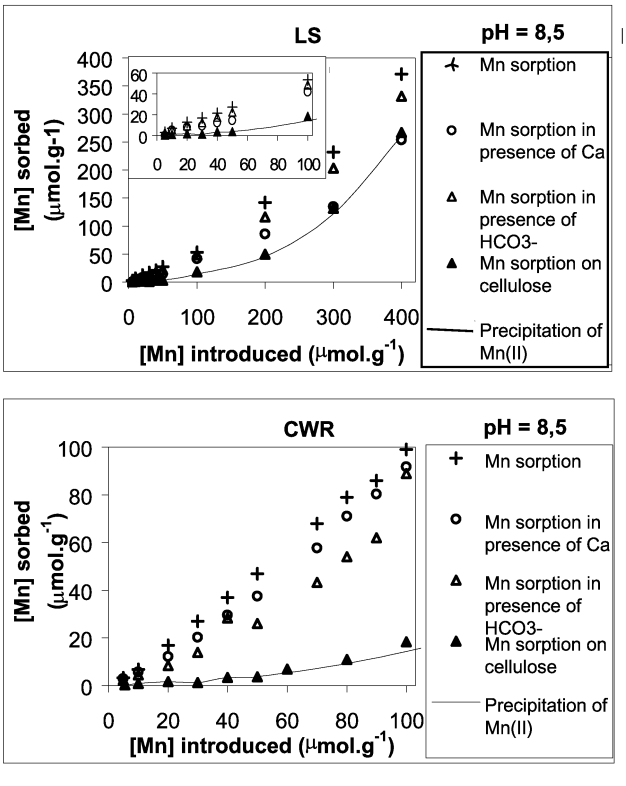

Fig. 4 Amount of Mn^{2+} sorbed by LS and CWR as a function of the metal introduced. Comparison with binding capacity of cellulose. Influence of Ca^{2+} ions.

The precipitation and the sorption curves (Fig. 4) are not superimposed, which indicates that Mn(II) ions are retained at the surface to form surface complexes.

These observations are in agreement with previous works where Mn(II) and Ca(II) ions form preferentially "outer-sphere" complexes (*i.e.* Mn(II) is complexed with its hydration sphere) with similar low stability constants compared to transition metal ones such as Cu(II) or Fe(III).[18–21] These results are confirmed by sorption experiments (Fig. 5) carried out in aqueous and potassium nitrate medium (0.1 mol L^{-1}) for comparison and with or without calcium ions (25×10^{-4} mol g^{-1}). Suspensions prepared in distilled water exhibit higher amounts of metal sorbed (Fig. 5). This observation shows that ionic strength is an important factor influencing Mn(II) sorption, due to the competing effect of potassium ions for the binding sites. Calcium and potassium ions are competitive for manganese sorption onto surface acid sites, and they have additional effects on manganese sorption. As a result, the binding capacity of lignin depends on various factors such as its extraction procedure, the pH, the electrolyte composition and the ionic strength.

Mn(II) ions are displaced not only by calcium ions but also by hydrogenocarbonate ions (at the same concentration as calcium). These ions could act as a negative charged screen of a negative surface (carboxylate and phenolate moieties), leading to more difficult access for the metal cations to the negative charged surface. Moreover, there is competitive complexation between the Mn^{2+} ions and the HCO_3^- or CO_3^{2-} ions. Consequently, in calcareous soils, Mn(II) sorption by lignin might be expected to be low due to the low stability of the complexes formed: Mn(II) ions are significantly displaced by calcium and carbonate ions and their sorption is influenced by electrolyte composition.

We can conclude from our results that lignin has a significant selectivity. The iron sorption conditions and processes are very different compared to alkali earth elements such as calcium. The surface complex stability is far higher for iron. Moreover, CWR has a higher metal binding ability than LS, probably because of the presence of monosaccharides[22] (0.755 μg mg^{-1}) which are known to complex metal ions. In calcareous soils, iron is bound to lignin by displacement of abundant endogenous metals such as calcium or potassium. Iron is strongly bound to lignin; however, it is difficult to saturate surface complexation sites by adding increasing amounts of metal because of hydrolysis processes. The functional groups of lignin responsible for the complexation are carboxylic and phenolic moieties.[23] The latter are three times as numerous as the former.[14] It is likely that LS and CWR substrates form salicylate or catecholate type bindings with metal cations. The more realistic reaction during metal complexation at acidic pH is the displacement of protons from acidic functional groups while, at more basic pH, protons from the hydration sphere of metal ions are dissociated to form hydroxo type complexes. Thus, it seems that iron or manganese ions are bound not only as M^{n+} species but also as $M(OH)_m^{(n-m)+}$.

In natural conditions, systems are more complicated because of other parameters governing the sorption: the presence of other compounds such as apatite, goethite, silica, or clay leads to the formation of ternary complexes. But, our experiments give some useful pieces of information on the specific interactions between lignin and metal ions. This can be the starting point for a more complex study introducing other components.

EPR study

To continue the investigation of metal complexes formed with lignin, EPR spectroscopy was used to describe the geometrical environment of the metal centre in the surface complexes. Moreover, it remains essential to identify the interactions between the metal and the organic semiquinonic species present at the surface of lignin.[7,24,25] A better understanding of the role of the metal in the lignin degradation is needed. The following part of this work focuses on the effect of iron and manganese concentrations on semiquinonic radical formation.

EPR spectra of LS and CWR before metal sorption show a single narrow resonance at $g = 2.0025$ (3300 G) that corresponds to a phenoxyl radical as previously described for other natural organic compounds.[26,27] The radicals that exist on the lignin surface can be classified in two categories: the native ones and the transient ones. Natural semiquinone moieties exist in native lignin and are stable in the polymer. According to its polyphenolic structure, lignin is known to generate phenoxyl radicals during oxidation processes, which are the result of the one-electron oxidation pathway of lignin. These paramagnetic species have been defined and quantified by a pulsed EPR study in a previous work.[17] In other respects, some authors reported that phenoxyl radicals produced under oxidative conditions, after UV irradiation or in basic pH conditions are transient.[28]

Before metal complex analysis, we followed the evolution of the radical level as a function of the pH in order to evaluate its effect, since metal surface complexes are prepared at two different pH values: 3.3 for iron and 8.5 for manganese. Fig. 6 presents the semiquinone signal intensity as a function of the pH in the range 2 to 10. It clearly shows a significant increase of radical concentration with pH for the two substrates. For example, the LS radical level at pH = 8.5 is more than twice as high as it is at pH = 3.3. Phenolic moieties are responsible for such a phenomenon leading to quinonic species and phenoxy radicals. Moreover, at basic pH, the lignin degradation is important and consistent with homolytic cleavages of ether linkages.

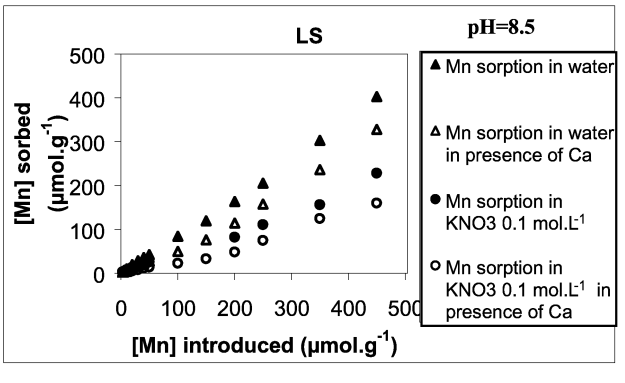

Fig. 5 Influence of the electrolyte and Ca^{2+} concentration on Mn^{2+} sorption by LS.

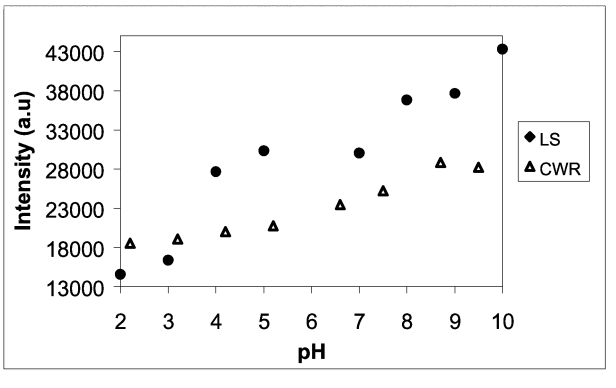

Fig. 6 Evolution of the semiquinone signal intensity ($g = 2.0025$) as a function of the pH.

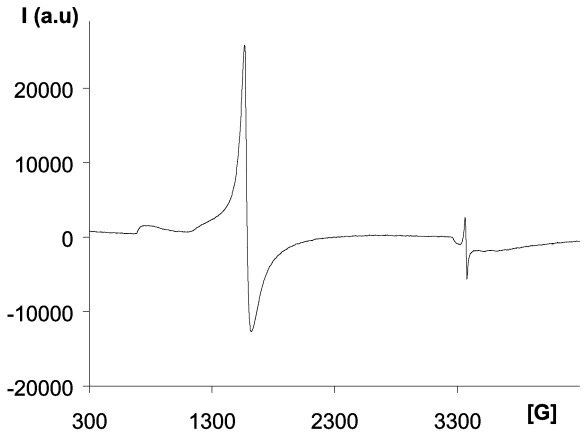

Fig. 7 EPR spectrum of Fe-CWR system ([Fe] = 50 μmol g^{-1}) at 4 K.

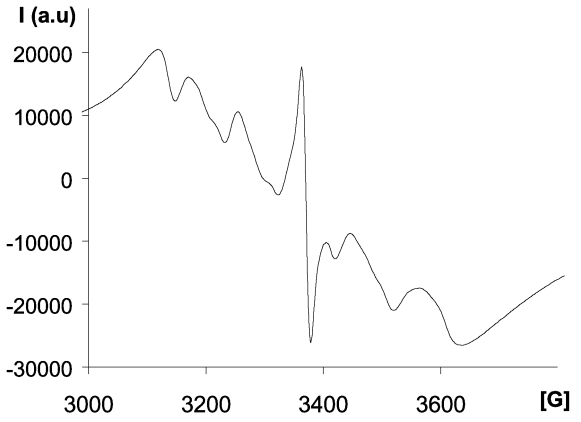

Fig. 9 EPR spectrum of Mn-LS system ([Mn] = 10 μmol.g^{-1}) at 298K showing different superimposed parallel components.

The EPR spectrum of the Fe-CWR system, collected at the X-band frequency and at 4 K, is shown in Fig. 7. An identical spectrum was obtained for the Fe-LS system. It consists of a narrow band at $g = 2.0025$ (3300 G), a strong one at $g = 4.2$ (1500 G) and an additional weak signal at $g = 9.2$ (800 G). The first resonance ($g = 2.0025$) is assigned to semiquinonic species of lignin while the two other ones at lower field are characteristic of the metal in a high-spin state, in octahedral sites. The symmetry is given by D and E terms which correspond to the tetragonal and rhombic distortion respectively. The values of the ratio E/D were found to be 1/3 in the two systems and are consistent with a rhombic symmetry. The spectrum feature is typical of a FeO_6 chromophore but it is unlikely that 6 organic ligands (carboxylic and/or phenolic moieties of LS and CWR solids) are coordinated to the metal because of the steric hindrance. Some water molecules and/or hydroxyl groups must be involved too.

The EPR spectrum (Fig. 8) performed as a function of the temperature shows a decrease of the signal intensity at $g = 4.2$ as temperature increases, according to the Curie–Weiss law.

The six-line feature of the Mn-LS system spectrum (Fig. 9) (identical to Mn-CWR one) indicates that Mn(II) is six-coordinated and is bound in octahedral sites. The spectra are similar to that of the $[Mn(H_2O)_6]^{2+}$ compound and show that the Mn(II) ion is bound to the surface acid sites by Van der Waals interactions, *i.e.* with its hydration sphere. Our results are in agreement with Davies *et al.*[8] who reported, by means of X-ray absorption spectroscopy, that Mn(II) was coordinated to 6 oxygen atoms with a distance of 2.20 Å in a complex formed with humic acid. Such a long distance corresponds to $Mn(H_2O)_6^{2+}$, which confirms the existence of an outer sphere complex.

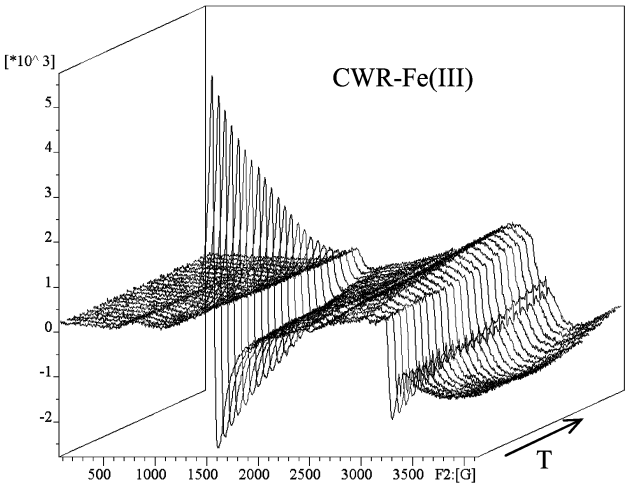

Fig. 8 Fe-CWR complex spectrum as a function of the temperature.

An accurate analysis of the Mn-LS spectrum (similar to the Mn-CWR one) in Fig. 9 shows the superimposition of several parallel components and indicates various complexation sites for manganese(II) ions, with similar symmetries. Hyperfine coupling constants of Mn(II) are particularly high (≈92 G) compared to $Mn(H_2O)_6^{2+}$ ones (22 G). Indeed, Mn(II) ions, in outer-sphere complexes, induce a distortion in the local electric field symmetry of $Mn(H_2O)_6^{2+}$. This relatively high value is a consequence of the interaction of $Mn(H_2O)_6^{2+}$ ions with the lignin surface and can also be explained by the formation of hydroxo species such as $Mn(OH)^+$ that can bind to the surface sites.

The metal concentration effect on the phenoxyl radical signal was studied and performed from 0.3 μmol g^{-1} (0.6 μmol L^{-1}) to 140 μmol g^{-1} (280 μmol L^{-1}). In Fig. 10 and 11 we plotted the metal concentration introduced in the suspensions as a function of semiquinonic and metal signal intensities. In the presence of increasing metal concentrations, the amount of phenoxyl radicals greatly increased, suggesting redox processes and electron transfer between the organic radical and the metal centre. A plateau was obtained for the semiquinonic signal at a concentration of about 20 μmol g^{-1} and 50 μmol g^{-1} for Fe(III) and Mn(II) ions respectively. A plateau is reached not only for the semiquinonic signal but also for the metal signals: about 45 μmol g^{-1} and 80 μmol g^{-1} for Fe(III) and Mn(II) respectively (Fig. 10 and 11). All measurements have been performed with no saturating conditions in order to allow comparisons of signal intensity. Instrumental conditions have therefore been optimised before. Mn(II) signal intensity was measured from the low field resonance (3100 G, $g = 2.16$) at the six-line feature because this line is not affected by the presence of the semiquinone signal. A plateau is obtained by this method in both Mn(II) and Fe(III) systems and can be explained by two hypotheses. In contrast to the isotherm in Fig. 5, the plateau (at $g = 4.2$ and 2.16) could correspond to the maximum of metal sorbed at the surface: 45 μmol g^{-1} for Fe(III) and 80 μmol g^{-1} for Mn(II) in LS and CWR. It is interesting to note that the plateau of the semiquinonic signal is obtained before that of the metal signal. This observation tends to show that all of the semiquinone present at the surface has been oxidized by the metal ions. We reached the maximum level of radical content in our conditions of pH and ionic strengh. However, it is possible that the plateau is a result of

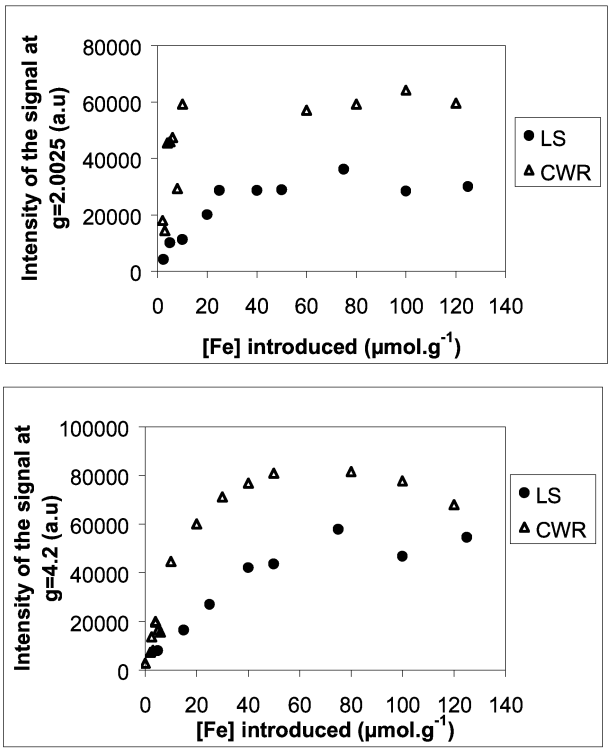

Fig. 10 Evolution of semiquinonic ($g = 2.0025$) and iron ($g = 4.2$) signal intensities in LS and CWR as a function of the iron concentration.

coprecipitation processes. The metal hydroxides formed are deposited at the surface during filtration and do not induce interactions with lignin quinone species due to a too high distance of the metal center from the surface groups. This last hypothesis could be suitable for the iron case (confirmed by sorption experiments) while it is not possible by EPR technique to exactly know the processes involved for the Mn(II) case.

We can also observe that metal introduced at low concentration decreased the radical level compared to that of the substrate alone. At high concentration, the radical concentration is increased compared to that in the powder alone. As a result, we can explain the processes that take place in the lignin surface considering two mechanisms: 1) at low metal concentration, the lignin surface is oxidised through its endogenous semiquinone species, which implies a decrease in the radical level compared to the substrate before metal addition; 2) at higher metal concentration, metal ions have oxidised all the natural radical species and generate new organic radicals, produced by phenolic oxidation. Consequently, the radical level increases. The transformation of phenoxyl radical into quinonic moieties leads to an acceleration of the organic matter degradation. The formation of complexes between metal and lignin stabilises radicals through macromolecular associations, which allow electronic transfers. Metal species are detected in the oxidation states Fe(III) and Mn(II), which suggests a rapid regeneration of the metal ions through electron transfer mechanisms between surface moieties and metal. The presence of oxygen could also be implicated in the re-oxidation reactions of the metal ions.

In fact, the mechanisms are certainly more complicated if we consider kinetic parameters. In the case of the two metals and two substrates, it is likely that reduced radical sites are regenerated with time by electron acceptor moieties present in the lignin matrix (quinhydrone species). But, the mechanisms involved in the redox processes between the metal centre and the organic radical are far more complicated and remain up to now uncertain. It seems necessary to continue the study performing kinetic experiments in order to follow the evolution of the radical signal intensity with time.

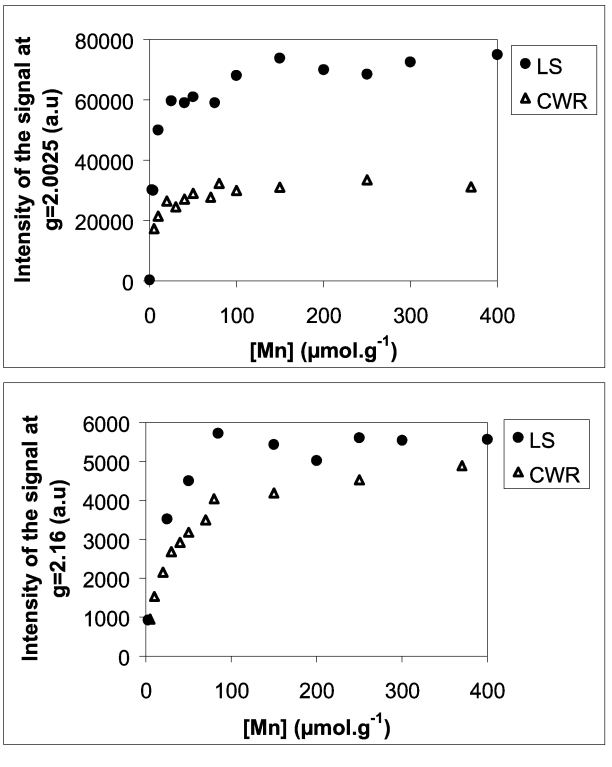

Fig. 11 Evolution of semiquinonic ($g = 2.0025$) and manganese ($g = 2.16$) signal intensities in LS and CWR as a function of the manganese concentration.

Conclusion

The values of metal sorbed evaluated indicate that the extraction procedure is a determining factor for sorption capacity of transition metal ions. Moreover, this study showed the great stability of iron surface complexes formed with lignin. Iron(III) sorption onto lignin is not affected by calcium ions in spite of their high concentration. In contrast to iron(III), manganese(II) ions are included in less stable complexes and are partly displaced by calcium and hydrogencarbonate ions. These results tend to demonstrate that, in calcareous soils, lignin can act as an effective biosorbent for iron ions, which then are not directly available and absorbed by plants. Conversely, manganese is poorly retained by lignin and mainly exists as hydroxo complexes at the basic pH of calcareous soil. We can predict the transport of these ions in soils, with a probability of reaching the ground waters.

Simultaneously to sorption processes, the degradation of lignin is accelerated by metal ions. EPR spectroscopy allowed the study of the impact of the metal on the organic radical, whose level is indicative of its degradation state. Thus, the parameters that control the production of hydroxyl radicals include pH, nature and concentration of the metal, metal chelation, nature of the organic substrate and electrolyte composition. This study could be relevant in predicting the impact of metals on lignin degradation in soils. Our results clearly demonstrate that the oxidation of lignin is accelerated by the presence of iron and manganese, as well as basic conditions. Moreover, our results provide evidence that, despite the production of phenoxyl radicals, lignin possessed an antioxidant activity. The identity of these semiquinonic species and the description of the mechanisms implicated into the oxidation processes are to be defined, by means of spin trapping experiments for example.

Finally, our results showed that the LS substrate has similar behaviour towards metal ions as the CWR substrate. Therefore, the common part of these two substrates must be responsible for their interactions with metal ions, that is to say, the most condensed part of lignin (the one that contains the more carbone–carbone linkages) is the most reactive. In spite of its more degraded state, LS is a good model of lignin.

Acknowledgements

We are grateful to the "Région Champagne-Ardenne" for financial support and to the "Département de la Marne" for a grant to P.M. We thank Y. M. Frapart (Université René Descartes, Paris V, UMR 8601, France) for EPR experiments.

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