



NOTE

Does ultrasonic dispersion and homogenization by ball milling change the chemical structure of organic matter in geochemical samples?—a CPMAS ^{13}C NMR study with ligninMICHAEL W. I. SCHMIDT¹, HEIKE KNICKER^{1,2}, PATRICK G. HATCHER² and INGRID KÖGEL-KNABNER^{1*}¹Department of Soil Science, Technical University of Munich, D-85350 Freising-Weihenstephan, Germany and ²Energy and Fuels Research Center, 209 Academic Projects Building, The Pennsylvania State University, University Park, 16802 PA, U.S.A.*(Received February 1997; accepted 12 June 1997)*

Abstract—Ultrasonic dispersion of geochemical samples suspended in water and subsequent homogenization by ball milling is widely used for fractionation of organic matter. The effect of these treatments on organic matter is investigated with lignin as a model compound. Structural alterations detectable by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy were examined. Comparison of the solid-state ^{13}C NMR spectra of untreated lignin and lignin mixed with quartz or soil did not reveal evidence for structural changes in the organic matter composition after ultrasonic dispersion and subsequent ball-milling. The chemical structure of organic matter in geochemical samples is not affected by these treatments as far as such structural alterations can be detected by solid-state ^{13}C NMR spectroscopy. © 1997 Elsevier Science Ltd

Key words—ultrasonic dispersion, soil, sediment, organic matter, lignin, CPMAS ^{13}C NMR

INTRODUCTION

The organic matter in soils and sediments can be investigated in physical fractions according to density or particle size (Castro Reis and Canto Machado, 1992; Turchenek and Oades, 1979; Christensen, 1992, 1996). Prior to fractionation samples suspended in water are treated with ultrasound to obtain complete dispersion of the different fractions. Alterations of the chemical structure of organic material due to ultrasonic dispersion cannot be excluded (Christensen, 1992; Kögel-Knabner, 1995). Although the bulk of the suspension treated with ultrasound remains at ambient temperatures, local hot spots associated with collapsing cavities may be affected by high temperatures (2100 to 5500°C) and pressures (500×10^5 Pa). Hydrodynamic shear forces created by acoustic microstreaming can cleave very large macromolecules with molecular weight $> 10^7$ and are strong enough to damage cells of organisms (Suslick, 1988; Frizzell, 1988). Due to heat and pressure, water in contact with the cavities can be decomposed to reactive hydrogen (H^+) and hydroxyl ions (OH^-) subsequently forming hydrogen peroxide (H_2O_2)

and molecular hydrogen (H_2). This can lead to secondary reactions such as oxidation, reduction, cracking and recondensation of organic and inorganic compounds (Suslick, 1989). After dispersion dried samples are homogenized by ball milling for further investigations. Structural alterations of the organic matter due to shear forces and heat development might occur.

The focus of this study is examination of the effects of ultrasonic dispersion and subsequent ball-milling on the chemical structure of organic matter (lignin) using solid-state ^{13}C NMR. Mixtures of lignin with (i) pure quartz sand and with (ii) soil material are investigated by means of solid-state ^{13}C NMR spectroscopy before and after ultrasonic dispersion and subsequent ball-milling. These spectra are compared to that obtained from untreated lignin.

MATERIAL AND METHODS

Sample material

The lignin used in this study, derives from a modern peatified angiospermous wood and was previously studied as sample CC 86-Z1 by Cameron *et al.* (1989) and Hatcher *et al.* (1989). The lignin was gently crushed (mortar and pestle) to a powder with

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particle size less than about 200 μm . For the lignin/quartz mixture 1.5 g of lignin was mixed with 28.5 g of HCl-washed quartz sand (Merck, Germany). Additionally, a lignin/soil mixture was prepared by adding 2.56 g of lignin to 27.44 g of soil derived from the eluvial A horizon from a Typic Hapludalf (Soil Survey Staff, 1994) common in Northern Germany. The soil contains 41.2% sand-(630 to 2000 μm), 34.0% silt-(2 to 63 μm) and 24.7% clay-size material (<2 μm) and 9.5 g/kg dithionite extractable iron. Prior to mixing, soil organic matter was removed with hydrogen peroxide (H_2O_2 10%) to obtain the mineral fraction only. Residual organic C (0.7 g C/kg) is a negligible fraction (1.5%) of the total C after addition of lignin. Carbon- and nitrogen-contents were determined in duplicate with an Elementar Vario EL.

Ultrasonic dispersion and homogenization by ball milling

Suspensions of the lignin/quartz mixture and the lignin/soil mixture (30 g mixture/150 ml water) were prepared in a 250 ml glass beaker and allowed to set for at least 3 h. The ultrasonic energy was applied with an ultrasonic probe (Labsonic U, Braun Melsungen, FRG) using a 19 mm diameter titanium probe immersed 15 mm into the suspension. The applied energy was calibrated calorimetrically following the method described by North (1976) resulting in a measured energy output of 75 W. This energy was applied for 15 minutes producing an energy of 440 J/ml (75 W \times sonication time(s)/162 ml suspension = applied ultrasonic energy (J/ml)). To calculate the amount of soil suspension a particle density of 2.65 Mg/m³ was used. The amount of 440 J/ml applied energy is in the range used in previous studies of particle size separates from soils (Preston *et al.*, 1994; Schmidt *et al.*, 1997; Amelung *et al.*, 1997). The temperature of the suspension was kept below 35°C by a cooling jacket. The mixtures were recovered from the suspensions by filtration with prewashed cellulose nitrate filters (0.45 μm) using steel cylinders and a pressure of approximately 5×10^6 Pa. The filter pellets could be easily removed from the filter and were freeze-dried. Subsamples of 2 g were treated in a ball mill (Retsch, Germany) for 10 minutes at medium intensity.

To increase the organic matter content of the sample and therefore its sensitivity for NMR measurement, after ultrasonic dispersion most of the quartz-particles of the lignin/quartz mixture were removed by decanting the aqueous lignin suspension. For all other samples and treatments this procedure could not be used due to the presence of fine mineral particles in the suspension.

Table 1. Chemical shift assignment of peaks in the solid-state ¹³C NMR spectra (referenced to tetramethylsilane = 0 ppm) (Lüdemann and Nimz, 1974; Wilson, 1987; Knicker, 1993)

Chemical shift range (ppm)	Assignment
220 to 160	Carboxyl/carbonyl/amide carbons
160 to 140	Aromatic COR or CNR groups
140 to 120	Aromatic C-H carbons, guaiacyl C-2, C-6 in lignin, olefinic carbons
120 to 100	Anomeric carbon of carbohydrates, C-2, C-6 of syringyl units in lignin
100 to 60	Carbohydrate derived structures (C-2 to C-5) in hexoses, C- α of some amino acids, higher alcohols
60 to 45	Methoxyl groups and C-6 of carbohydrates and sugars, C- α of most amino acids
45 to -10	Methylene groups in aliphatic rings and chains, terminal methyl groups

NMR spectroscopy

The solid-state ¹³C NMR spectra were run on a Chemagnetics, Inc., M-100 (25.035 MHz) spectrometer applying the cross polarization magic angle spinning technique (CPMAS) (Schaefer and Stejskal, 1976). Solid-state CPMAS ¹³C NMR experiments were performed with a contact time of 1.0 ms, a 90° ¹H-pulse width of 6.6 ms and a pulse delay of 600 ms. ¹³C chemical shifts are reported relative to tetramethylsilane. Chemical shift assignments are given in Table 1. Approximately 30 000 to 90 000 scans were accumulated (Table 2). For the relative intensity distribution of the solid-state CPMAS ¹³C NMR spectra of lignin, before and after treatment, the precision is, in general, 10% of the signal intensity except for the regions 220 to 160 ppm and 45 to -10 ppm. In these regions the relative standard deviation may increase to 20% due to different manual phase- and baseline correction (Knicker, 1993). Additionally one must consider, that the relative standard deviation increases with decreasing signal to noise ratio of the NMR spectrum. The solid-state CPMAS ¹³C NMR spectrum of untreated lignin is shown with line broadening of 0 Hz (Fig. 1a). For most samples dispersed with ultrasound or homogenized by ball milling a line broadening of 20 Hz is used (Fig. 1 c-e). Only for the lignin/quartz mixture after ultrasonic dispersion (Fig. 1b) is a line broadening of 0 Hz sufficient.

RESULTS AND DISCUSSION

Structure of lignin prior to treatment

The solid-state ¹³C NMR spectrum of lignin (Fig. 1a) reveals a pattern comparable to those obtained in other studies of angiosperm woods (Bartuska *et al.*, 1980; Hatfield *et al.*, 1987). The spectrum shows resonance lines for syringyl and

Table 2. Relative intensity distribution of the solid-state CPMAS ^{13}C NMR spectra of lignin, a lignin/quartz mixture and a lignin/soil mixture after ultrasonic dispersion and subsequent homogenization by ball milling

Sample	C-content (g/kg)	No. of scans (10^3)	Line broadening (Hz)	Chemical shift region							Number of methoxyl groups per aromatic ring [‡]
				δ (ppm)*							
				220 to 160	160 to 140	140 to 120	120 to 100	100 to 60	60 to 45	45 to -10	
<i>lignin</i>	539.0	31	0	8.1	17.9	15.5	13.9	24.5	13.0	7.2	1.6 ± 0.4
<i>lignin/ quartz mixture after ultrasonic dispersion after milling</i>	26.3 [§]	35	0	6.5	16.2	14.7	14.8	22.9	15.5	9.4	2.0
	26.3	Δ^+ 79	20	-20 13.2	-9 18.1	-5 15.8	+7 12.4	-7 23.9	+20 9.7	+31 6.9	1.3
		Δ^+		+63	+1	+2	-11	-3	-25	-4	
<i>lignin/soil mixture after ultrasonic dispersion after milling</i>	46.7	59	20	11.0	19.3	16.5	12.2	23.3	9.6	8.1	1.2
	46.7	Δ^+ 92	20	+36 10.6	+8 18.7	+7 16.7	-12 12.1	-5 23.9	-26 9.7	+13 8.5	1.2
		Δ^+		+31	+5	+8	-13	-3	-25	+18	

*in % of total signal intensity.

[§]Difference expressed as % of the peak area of untreated lignin.[‡] Calculated from equation 1 as given in the text using a precision of $\pm 10\%$ for the integrated areas.

guaiacyl units illustrated in Fig. 2. Signals in the chemical shift region from 60 to 100 ppm most probably originate from alkyl side chain carbon atoms of the lignin but could also arise from carbohydrate residues. The peak at 106 ppm derives mostly from C-2 and C-6 of syringyl units, but may be overlapped by the 115–120 ppm resonances of the C-2 and C-6 in guaiacyl units and/or the anomeric carbon of carbohydrate. The resonance at 148 ppm can be related to C-3 carbon in guaiacyl units. The resonance at 135 ppm has contributions from C-1 and C-4 carbons in syringyl and C-1 in guaiacyl units. The peak at 153 ppm is due to C-3 and C-5 aryl-O carbons of syringyl units and to the C-4 carbons of guaiacyl units. The high intensity of the peak at 153 ppm suggests that the syringyl units are mostly linked through ether β -O-4 bonds (Nimz *et al.*, 1981).

To estimate the distribution of syringyl and guaiacyl units in the lignin one can determine the average number of methoxyl groups per aromatic ring. This number M is calculated from the following equation.

$$M = I_{\text{methoxyl C}} / I_{\text{aromatic C}} \times 6 \quad (1)$$

Here, $I_{\text{methoxyl C}}$ represents the intensity of the methoxyl C region (60 to 45 ppm), $I_{\text{aromatic C}}$ is the intensity of aromatic C region (160 to 100 ppm) multiplied by the number of ring carbons. For the investigated lignin 1.6 methoxyl groups per aromatic ring are calculated (Table 2). This is consistent with previous studies of this sample (Hatcher *et*

al., 1989) and provides evidence that the lignin is composed of approximately equal proportions of guaiacyl and syringyl units.

A possible change in the chemical structure of lignin due to thermal, chemical or mechanical degradation is the cleavage of ether-linkages (β -O-4 bonds) in guaiacyl and syringyl structures (Fig. 2). This cleavage creates phenolic carbon at C-4 and induces a shift of the signal at 153 ppm to approximately 148 ppm for C-3 and C-5 aryl-O carbons in syringyl units. In guaiacyl units a cleavage of β -O-4 bonds shifts the resonance line of the C-4 from 152 to 146 ppm (Haw *et al.*, 1984).

A second possible alteration is a demethoxylation of lignin. Hydrogen peroxide created by ultrasonic treatment could oxidize methoxyl groups in lignin ($\text{Ar-OC}^{(+1)}\text{H}_3$) to ketone ($\text{R-C}^{(+2)}\text{OR}$) or carboxyl-groups ($\text{R-C}^{(+3)}\text{OOH}$). Demethoxylation was observed previously as an effect of coalification of angiospermous wood (Hatcher *et al.*, 1989). Demethoxylation decreases the signal intensity of the peak at 56 ppm resulting in a lower calculated number of methoxyl groups per aromatic ring.

Structure of lignin after treatment

Lignin/quartz mixture. Figure 1b shows the solid-state CPMAS ^{13}C NMR spectrum of the lignin/quartz mixture after ultrasonic dispersion. Its relative intensity distribution is given in Table 2. The solid-state ^{13}C NMR spectrum of the lignin/quartz mixture, after ultrasonic dispersion, shows a pattern almost identical to that obtained for the untreated

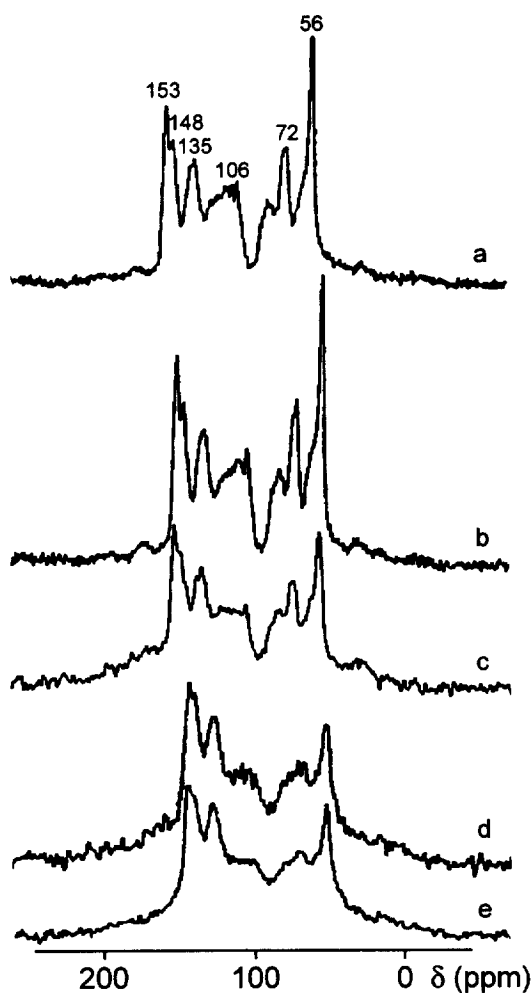


Fig. 1. Solid-state CPMAS ^{13}C NMR spectra of (a) untreated lignin, the lignin/quartz mixture after (b) ultrasonic treatment and (c) after ball milling, the lignin/soil mixture after (d) ultrasonic treatment and (e) ball milling.

lignin sample. Differences in the intensity distribution of both spectra vary in the range of experimental error ($\pm 20\%$). However, after ultrasonic dispersion, an increase in the relative signal intensity from 7.2% to 9.4% in the chemical shift region of alkyl-C (45 to -10 ppm) can be observed. Considering the low signal to noise ratio in this chemical shift region such a high experimental error can be expected (Knicker, 1993). Therefore, this increase of relative intensity does not necessarily provide evidence for chemical alterations after ultrasonic dispersion. Also the difference in the number of methoxyl groups per aromatic ring (Table 2) varies between untreated and treated sample within experimental error.

The spectrum of the homogenized sample (Fig. 1c) shows less resolution than the untreated lignin (Fig. 1a) and the same sample before homogenization (Fig. 1b). This can be explained by its lower C-content after homogenization (Table 2).

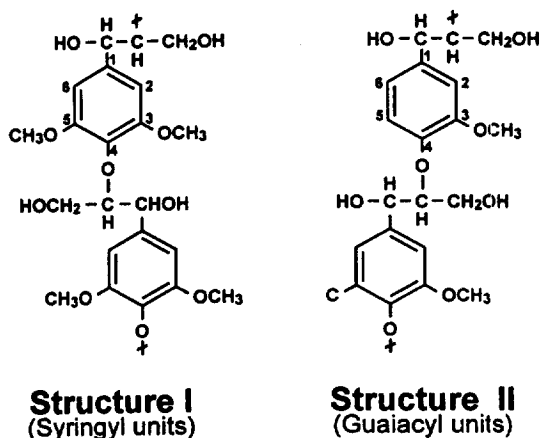


Fig. 2. Lignin units.

Due to the presence of fine particles after ball-milling the mineral matter could not be removed by decanting without losing organic material. Comparison of the intensity distribution of both spectra reveals similar relative intensities in the chemical shift regions of aromatic C (160 to 110 ppm), O-alkyl C (100 to 60 ppm) and alkyl C (45 to 0 ppm). In the methoxyl region (60 to 45 ppm) the relative intensity decreases (13.0 to 9.7%), whereas that in the carboxyl region (220 to 160 ppm) increases (8.1 to 13.2%). However, the difference in the number of methoxyl groups per aromatic ring (Table 2) varies within experimental error providing no evidence for demethoxylation. The decrease of signal intensity in the chemical shift region of methoxyl C may result from loss of signal intensity to neighboring chemical shift regions due to line broadening. Such line broadening may be caused by decreasing physical order of the lignin structure as an effect of homogenization by ball milling. The shape of the signals in the chemical shift region between 160 and 140 ppm does not display differences in peak intensities at 153 and 148 ppm suggesting that cleavage of ether-linkages did not occur to a greater extent.

Lignin/soil mixture. Compared to the solid-state CPMAS ^{13}C NMR spectra of the lignin/quartz mixture (Fig. 1b, c) the spectra of the lignin/soil mixture (Fig. 1d, e) show lower signal to noise ratios and a decrease in resolution although the number of accumulated scans is higher (Table 2) and the samples contain more organic C (Table 2). This can be explained by the presence of considerable amounts of Fe (9.5 g/kg) not present in the lignin/quartz mixture. Such paramagnetic material may decrease the amount of C detectable by NMR and can broaden the resonance lines (Preston *et al.*, 1994).

The relative intensity of the methoxyl signal (60 to 45 ppm) in the solid-state CPMAS ^{13}C NMR spectrum of the pure lignin decreases from 13.0%

to 9.6% in the lignin/soil mixture. This may be explained by an increasing experimental error caused by the decreasing signal to noise ratio. The difference between untreated and treated lignin, in the number of calculated methoxyl groups per aromatic ring (Table 2) varies within experimental error, indicating that no major demethoxylation occurred. The increase of signal intensity in the carboxyl region (220 to 160 ppm) from 8.1% to 11.0% can also result from line broadening due to paramagnetic sample material. After subsequent homogenization by ball milling no further alterations of the shape or intensity distribution of the spectrum can be observed.

CONCLUSIONS

Cleavage of the ether-linkage in syringyl and guaiacyl structures induces a shift of the signals from 153 and 152 ppm to approximately 148 and 146 ppm, respectively. Since such alterations are not visible in the spectra we conclude that cleavage of the ether linkage does not occur to a great extent.

Applying CPMAS ^{13}C NMR we observe no significant change in methoxyl content in treated samples. Demethoxylation of lignin usually decreases the signal intensity of the peak at 56 ppm resulting in a lower calculated number of methoxyl groups per aromatic ring. In the experiments conducted here, some decrease of peak height and signal intensity in the methoxyl region (60 to 45 ppm) can be observed for the spectra of the lignin/quartz mixture and lignin/soil mixture after ultrasonic dispersion and subsequent homogenization. However, for untreated lignin and treated samples the calculated number of methoxyl groups per aromatic ring varies within experimental error providing no evidence for demethoxylation. The loss of signal intensity in the chemical shift region of methoxyl groups in the spectra of the treated mixtures may best be explained by a decrease in the signal to noise ratio.

Taking lignin as a model for organic matter in sediments and soils, no major structural alterations caused by ultrasonication and subsequent homogenization by ball milling are visible with CPMAS ^{13}C NMR. Since lignin is a precursor of natural organic matter this also suggests that the chemical structure of organic matter from natural soils and sediments is not affected by these pretreatments.

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