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Demineralization of marine and freshwater sediments for CP/MAS ^{13}C NMR analysis

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Abstract

A method was developed to demineralize sediment trap material and marine sediments containing labile organic matter (OM), in preparation for cross polarization and magic angle spinning (CP/MAS) solid-state ^{13}C NMR analysis. Carbonate and silicate minerals were dissolved with HCl and a mixture of dilute HCl/HF, respectively. Demineralization kinetics were assessed for a range of freshwater and marine sediments, as well as pure mineral and organic samples. For samples with a very low organic carbon (OC) concentration (<1 wt.%) and samples containing a large fraction of acid-soluble OC, the organic molecules solubilized during the dissolution of the mineral fraction were recovered by freeze-drying the supernatants following (i) removal of dissolved calcium and residual HF by CaF_2 precipitation, (ii) removal of the dissolved paramagnetic metals by sulfide precipitation, and (iii) desalting using ion retardation chromatography. When applied to a wide range of freshwater and marine particles, demineralization resulted in OC enrichment factors that varied between 2.2 and 20.8, with losses representing less than 20.5% of the initial OC content. X-ray diffraction and fluorescence analysis of the natural and demineralized samples showed that minerals and paramagnetic metals were effectively removed. While molecular fractionation might be substantial when the mineral constituents were dissolved with HCl and HF, the small changes ($<11\%$) in the $(C/N)_a$ and $(H/C)_a$ ratios when the acid-soluble organic matter was recovered suggest that the molecular composition of the organic fraction was not appreciably altered. In combination with CP/MAS ^{13}C NMR spectroscopy, this demineralization method allows comprehensive elucidation of the chemical structure of total OC, especially in samples with very low OC concentrations and/or with a significant fraction of chemically labile organic compounds.

Keywords: Demineralization; Sedimentary organic matter; Sediment; CP/MAS ^{13}C NMR

1. Introduction

OM buried in continental margin sediments directly links the global biogeochemical cycles of oxygen, carbon and sulfur, provides a molecular record of the history of life and is a progenitor of economically important coals and petroleum (Berner, 1982, 1989; Engel and Macko, 1993; Hunt, 1996). Over the years, the study of factors that control OM burial and preservation in marine sediments has led to the development of a wealth of techniques to characterize bulk sedimentary OM (Keil et al., 1994a; Wakeham et al., 1997; Hedges et al., 1999). Despite these efforts, biochemicals that can be measured at the molecular level in marine sediments generally represent $<20\%$ of the total OC, down from about 60–80% for plankton (Cowie and Hedges, 1994; Wakeham et al., 1997). Quantifying and structurally elucidating the uncharacterized OM pool is an important prerequisite for understanding the mechanisms controlling OM preservation over geologic time and remains one of the greatest challenges in marine organic geochemistry today.

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Solid-state CP/MAS ^{13}C NMR is capable of comprehensively characterizing the chemical nature of OC found in soil and sediment (Hatcher et al., 1983; Almendros et al., 1991; Baldock et al., 1992, 1997; Preston et al., 1994; Skjemstad et al., 1994; Knicker et al., 1995; Preston, 1996). The technique is non-destructive and provides information on the chemical environment surrounding individual carbon atoms, from which molecular structures are inferred. The information provided by solid-state ^{13}C NMR may be quantitative, provided that additional more detailed ^{13}C NMR experiments (e.g. inversion recovery, variable contact time and Bloch decay pulse sequences) are performed to confirm the quantitative nature of the results (Kinchesh et al., 1995; Conte et al., 1997).

However, the low OC concentration and the potentially high concentration of paramagnetic metals in mineral soils and sediments can lead to poor quality NMR spectra (Preston et al., 1989; Baldock et al., 1992; Skjemstad et al., 1994; Schmidt et al., 1997). Paramagnetic materials introduce both localized and general interference effects that decrease sensitivity for specific functional groups and across the whole range of carbon species present in the sample. Therefore the presence of paramagnetic materials can limit the practical range of whole soils or sediments that can be usefully analyzed in a reasonable timeframe (24 h) to samples with $>1\%$ OC and a OC:Fe weight ratio >1 (Arshad et al., 1988; Skjemstad et al., 1994; Schmidt et al., 1997; Smernik and Oades, 1999). Because of these limitations, there is a dearth of information currently available on the chemical composition of bulk sedimentary OM determined by solid-state ^{13}C NMR, which prevents broad and systematic surveys to compare OM compositions in different depositional settings.

Several approaches have been developed to increase the quality of spectra obtained for OM-poor samples with CP/MAS ^{13}C NMR. Physical methods (i.e. particle size and density fractionation, hand picking of pure organic fractions) were used to increase the concentration of OC and facilitate the acquisition of acceptable ^{13}C NMR spectra of whole mineral soils (Skjemstad et al., 1986; Oades et al., 1987; Baldock et al., 1992). Physical methods do not alter the chemical nature of OC and can facilitate the acquisition of solid-state ^{13}C NMR spectra by concentrating OC into environmentally informative fractions. However, significant amounts of the soil OC may remain in fractions with high ash contents and/or high concentrations of paramagnetic species, enhancing the potential of acquiring results that are not representative of the OC found in the unfractionated sample. Methods based on selective removal of paramagnetic elements from soils by chemical extraction or on reduction of paramagnetic elements to their non-paramagnetic analogues have proven useful to increase NMR signal-to-noise ratios for samples rich in iron (Oades et al., 1987; Arshad et al., 1988; Schmidt et al., 1997). Unfortunately, because chemical methods that only reduce the concentration of paramagnetic elements do not remove the bulk of the inorganic constituents, they have proven largely unsuccessful for samples with very low OC concentration.

The ideal pretreatment for samples with low concentrations of OC and high concentrations of paramagnetic elements would be one that is capable of dissolving a significant proportion of the mineral matrix and also extracting paramagnetic elements without significantly altering the chemical structure of the OC. Chemical maceration methods have been used extensively as a de-ashing technique for refractory organic material such as coal or kerogen (Saxby, 1970; Durand and Nicaise, 1980; Robl and Davis, 1993). The most successful approaches involve hydrofluoric acid (HF), which reacts with silica to form soluble fluoride complexes. In these methods, hydrochloric acid (HCl) pretreatments are used to remove carbonates and avoid the subsequent formation of insoluble fluorite (CaF_2). However, concentrated HCl and HF have the potential to induce chemical alteration of the OM, as well as OC loss through hydrolysis and solubilization of chemically labile biomolecules. The potential for alteration and losses is particularly significant for 'immature' OM in aquatic environments such as that found in surface sediments. A large fraction of the OC in freshwater and marine samples can be composed of chemically or biologically labile biochemicals that are protected from degradation through close association with the mineral matrix (Mayer, 1994; Keil et al., 1994b). Dissolution of the inorganic matrix eliminates their protective support and may release OC into solution. OC losses in excess of 50% have been reported for the HCl/HF demineralization of recently deposited marine sediments (Durand and Nicaise, 1980).

Thus, the harsh HCl/HF demineralization procedures developed for de-ashing coal or kerogen must be adapted for recently deposited aquatic sediments, as done for soils in recent years (Preston and Newman, 1992; Skjemstad et al., 1994; Schmidt et al., 1997). In addition, to routinely obtaining representative NMR spectra of the whole organic component for samples containing varying concentrations of chemically labile OC, approaches must be developed to recover OC solubilized to varying extents during the removal of the mineral fraction. No general method succeeds in completely separating the unchanged organic material from the whole range of complex sedimentary mixtures found in freshwater and marine environments. Optimal approaches thus represent a compromise between OC enrichment and removal of paramagnetic compounds while minimizing OC losses.

This paper describes a method for the demineralization of particulate matter and recently deposited sediments

using HCl and HF, and the recovery of labile organic molecules dissolved in aqueous solutions. The protocol was tested on samples representing a range of sediments encountered in freshwater and marine environments and varying OC concentrations (0.2 to 8 wt.%). Demineralization kinetics were studied for different samples, and pure organic and mineral phases. Overall OC losses and alteration were assessed using an OC mass balance, atomic C/N ratio measurements and CP/MAS ^{13}C NMR analyses.

2. Methodology

2.1. Samples

Two groups of samples were used in this study: (1) a series of sediments was used to develop and test the demineralization methodology; and (2) particulate samples collected in sediment traps suspended in marine water columns and marine surficial sediments. The first series of samples included two standard reference materials purchased from the National Institute of Standards and Technology (NIST Buffalo River Sediment, SRM2704 and Estuarine Sediment SRM1646a; NIST, Gaithersburg, MD), a lacustrine sediment collected in Lake Washington, WA (0–20 cm), as well as Mexican (0–0.5 cm) and Washington coast (0–0.5 cm) sediments. The Washington coast sediment was collected during Cruise 94-07B of the R/V *Wecoma* (Lambourn et al., 1996), while the Mexican coast sediment was acquired during Cruise NH96 of the R/V *New Horizon* to the continental margin of Northwestern Mexico. The second series of marine samples included surficial sediments and sediment trap material from the central equatorial Pacific (0°N and 9°N) that were collected along a north-south transect during the US JGOFS EqPac program (Hernes et al., 1996), plankton tow material (64–300 μm) sampled August 1981 from Dabob Bay in Puget Sound (WA), as well as Arabian Sea trap material collected at mooring Station 3 (17°41 N and 59°61 E) during the summer monsoon of 1995 at water depths of 498 and 2896 m. In addition, a series of samples was used to assess demineralization kinetics. The clay mineral standards, Kaolinite no. 17 and Montmorillonite no. 26, were obtained from Ward's Natural Science Establishment (Rochester, NY). Ferric oxide (no. 12375) and black carbon (acetylene, no. 39723) were purchased from Alfa (Ward Hill, MA), and the NIST standard reference materials Peach Leaves, (NIST, SRM1547) and Dogfish Liver (National Research Council of Canada, DOLT-1), were used as biological samples.

2.2. Analysis

Elemental compositions were measured with a Carlo Erba model 1106 CHN analyzer (Hedges and Stern, 1984). Weight percentages of organic carbon (wt.% OC) were determined following vapor phase acidification (relative precision ±2%). Weight percentages of total nitrogen (wt.% TN) and total carbon were determined similarly, but without acidification. Weight percentages of inorganic carbon (wt.% IC) were calculated as the difference between total carbon and organic carbon. Dissolved organic carbon (DOC) was measured with a MQ 1001 high-temperature combustion total organic carbon analyzer (Qian and Mopper, 1996) fitted with a high efficiency home-made combustion column (to be published). Solutions containing residual HF were analyzed for DOC following complexation with 5% H_3BO_3 (1:1 dilution). Reagent blanks were < 5% of the measured signal and were subtracted for all procedures.

2.3. HCl/HF demineralization

The water used throughout this project for rinsing and dilution was organic-free UV-oxidized water dispensed by a Milli-Q Gradient A10 instrument from (Millipore, Bedford, MA). All glassware was cleaned by soaking in a 10% HCl bath for 24 h, rinsed thoroughly with water and muffled at 500°C for 4 h. A schematic representation of the demineralization method is shown in Fig. 1. Samples containing 10–50 mg of OC (between 0.5 and 2.5 g of dry sample) were weighed directly into 50-ml polystyrene centrifuge tubes with screw caps. To dissolve salt, carbonates and sesquioxide coatings, 30 ml of 1 N HCl were added and the samples were agitated for 60 min at room temperature (step 1 in Fig. 1). For samples with a high carbonate content (IC > 2 wt.%), 27.5 ml of UV-water was added to the centrifuge tube containing the sample and 2.5 ml of concentrated HCl was slowly added in small aliquots (100–200 μl) to avoid over-spilling caused by effervescence. Residues were recovered by centrifugation (15 min at 1000 *g*) and rinsed three times with 6.67 ml of water. The supernatant from the HCl treatment and the rinsing waters were pooled and adjusted to a final volume of 50-ml with UV-water. For some samples (see results), the OC solubilized during the HCl treatment had to be recovered to reduce losses. The procedure used to recover solubilized OC is described in Section 2.4.

The mineral fraction remaining after HCl treatment was treated with a 1 N HCl and 10% (v/v) HF solution. A 20-ml aliquot of the HCl/HF solution was added to the residue and the mixture shaken for 12 h at room temperature. The supernatant was removed by centrifugation (15 min at 1000 *g*) and the same treatment was repeated a second time. After removing the second supernatant, the residue was washed three times with 3.3 ml of UV-water (step 2 in Fig. 1). All supernatants from the HCl/HF treatments and the subsequent water rinses were retained and pooled to give a single HCl/HF supernatant. For samples with low organic carbon contents

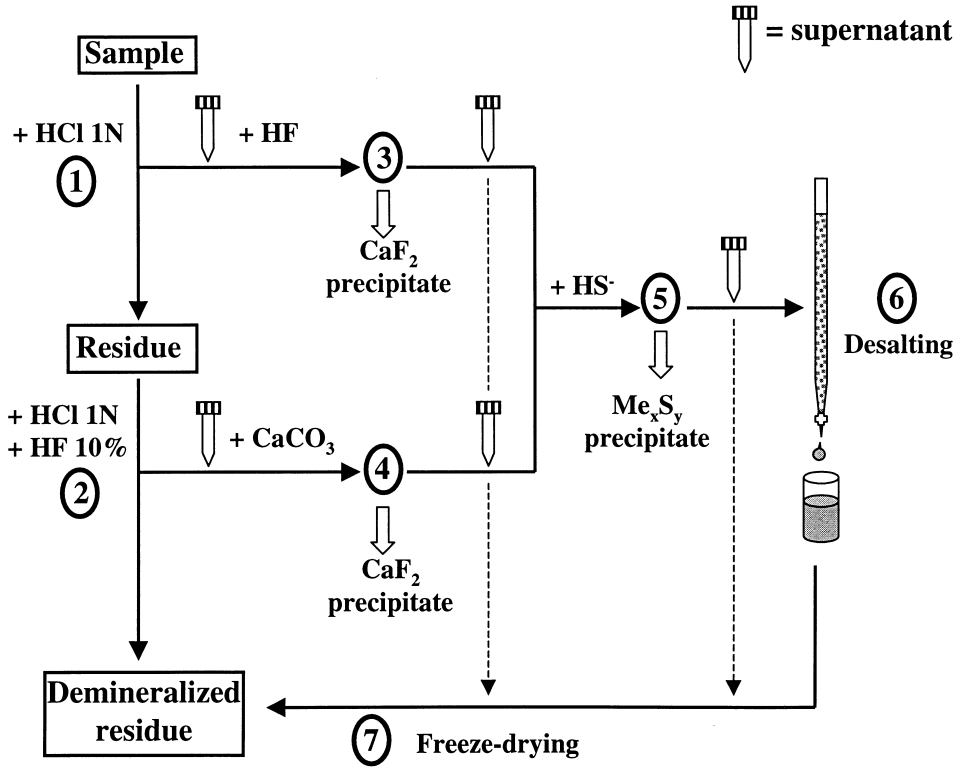

Fig. 1. Schematic of the multi-step demineralization method. The circled numbers refer to explanations in the text. Samples with relative OC losses above 20% during steps 1 and 2 were treated as in all (or some) of steps 2 to 7 to recover the acid-soluble OC fraction.

and high concentrations of hard oxide minerals, the HCl/HF treatment may have to be repeated a third time to sufficiently increase the OC enrichment factor (only two HCl/HF treatments were used in this work). The OC solubilized during the HCl/HF treatment also had to be recovered for some samples to reduce OC losses (see results, Section 2.4). Small aliquots were subsampled from the HCl and HCl/HF supernatants for DOC analysis. The final residue was freeze-dried and analyzed for wt.% TN and wt.% OC. Mass balances for OC were closely monitored throughout all procedures.

2.4. Recovery of the acid-soluble OC

To decrease the amount of inorganic material contained in the supernatant obtained from the HCl treatment, dissolved Ca^{2+} ions were removed by formation of a CaF_2 precipitate (step 3, Fig. 1). Small aliquots (100–200 μl) of concentrated HF were added to the supernatant until no more CaF_2 precipitation was evident. The supernatant and the CaF_2 precipitate were separated by centrifugation (15 min at 1000 *g*) followed by two 3-ml water rinses that were pooled with the supernatant. The solution was then adjusted to a final pH of ~7 with dilute NaOH.

In a similar manner, OC solubilized during the HCl/HF treatment was recovered for some samples. OC-free calcium carbonate (Sigma Chemicals, St. Louis, MO) was added in small aliquots to the HCl/HF supernatant to precipitate residual fluoride ions as CaF_2 (step 4, Fig. 1). The pH of the solution was kept below 2 to eliminate inorganic carbon as CO_2. The CaF_2 precipitate was separated from the supernatant by centrifugation, rinsed twice with 2 ml of water before adjusting the final pH of the solution to ~7 with dilute NaOH.

Depending on their inorganic salt and transition metal concentrations, the HCl and HCl/HF treatment supernatants were then either freeze-dried directly (for low salt and low metal concentrations) or further treated as follows prior to freeze drying: (i) addition of NaHS (step 5; for low salt concentration and high metal concentration); (ii) desalting (step 6, for high salt concentration and low metal concentrations); or (iii) addition of NaSH and desalting (steps 5 and 6; for high salt and high metal concentrations). Elemental carbon and nitrogen analysis was carried out on the freeze-dried residues before pooling them with the fraction of the sample that was not solubilized during the initial HCl and HCl/HF treatments.

It is important to note that not all treatments were necessary to suitably demineralize every sample. Two factors were considered when deciding whether to recover the organic material solubilized during the HCl and HCl/HF treatments: representativity of the demineralized sample and quantitative detection limits for spectroscopic NMR analyses. For this work, if $\geqslant 20\%$ of the initial OC was solubilized during the HCl and HCl/HF treatments, then the OC contained in the supernatants was recovered and added to the demineralization residue to ensure that the OC present in the residue was representative of that in the original sample. The NMR analysis required that $\geqslant 5$ mg C was present in the sample in order to clearly differentiate between sample and background signal intensity. Because of the low OC content of some samples, and the difficulty of obtaining a large quantity of material, the initial quantity of OC available was sometimes limited. Therefore, supernatants were also recovered when the amount of OC in the demineralized residues was $\leqslant 5$ mg OC.

2.5. Removal of dissolved metals from supernatants

For some samples, the high concentration of dissolved metals in the HCl and HCl/HF supernatants decreased OC concentration efficiency by increasing the amount of inorganic material following the freeze-drying step. Paramagnetic metals (especially Fe, Mn and Cu) also significantly decrease the quality of NMR spectra (Arshad et al., 1988; Skjemstad et al., 1994; Smernik and Oades, 1999). Metals with a low sulfide solubility product were thus precipitated as metal sulfides and separated from the supernatant by centrifugation (step 5 in Fig. 1; Rickard, 1989; Wei and Osseo-Asare, 1997). Working in a glove box filled with N_2, small aliquots of degassed 0.1 M sodium hydrosulfide ($NaHS{\cdot}9H_2O$, adjusted to pH 8) were added to the centrifugation tube containing the degassed supernatants until no more black precipitate was seen to form. This precipitate formed instantaneously upon HS^- addition and was most likely composed of amorphous FeS mixed with other minor metal sulfide phases. Note that a precipitate appeared in some HCl and HCl/HF supernatants before pH could be raised to $\sim$7, most likely because the solution reached supersaturation with respect to fluoride complexes formed during the HCl/HF treatment, or due to mineral constituents such as aluminum or iron (hydr)oxides. In these cases, the sulfide precipitation reaction was carried out at about 1 pH unit lower than the supersaturation point. The precipitate was likely composed of metal sulfide and (hydr)oxide particles.

Since most transition metals have a lower sulfide solubility product than iron (Morel and Hering, 1993), it was assumed that the solution was supersaturated with respect to most metal sulfide phases. The supernatants were left to age for 6 h in the glove box at room temperature. The tubes were then sealed and centrifuged (15 min at 1000 *g*) to separate the precipitate. They were then returned to the glove box, where the supernatant was transferred to a second tube using a glass pipet and the precipitate was rinsed with 5 ml of degassed UV-water. The rinsing water was separated by centrifugation in the same way and pooled with the supernatant.

2.6. Desalting the supernatants

Because of the natural salt content of the starting material, and the need to adjust pH at different steps of the demineralization procedure, the freeze-dried residues and supernatants from some samples also had to be desalted to further concentrate OC (step 6 in Fig. 1). To minimize losses of small water-soluble organic molecules, desalting was done using ion retardation chromatography (Bronk and Glibert, 1991; Hu and Smith, 1998). The supernatants were freeze-dried and re-suspended in 15 ml of UV-water and agitated to dissolve the inorganic salts. A 1-ml subsample was kept for DOC analysis and the remaining 14 ml were passed through a 45×1 cm column containing a mixed bed resin (BioRad BTAG11A8, 50–100 mesh) that retarded the passage of charged ions. UV-water (flow rate of 0.2 ml min^{-1}) was used to separate inorganic ions from the earlier eluting small organic molecules. A total of 60 ml of eluate was collected in 10-ml aliquots in clean glass scintillation vials. The relative salt concentration was estimated in each aliquot with a conductivity meter to select aliquots to be pooled. DOC was measured in each aliquot during the optimization of the procedure, but to minimize OC losses was only measured in the pooled aliquots when the procedure was applied routinely to samples. The pooled aliquots were then freeze-dried and added to the initial demineralized residue. The ion retardation resin was regenerated after each sample with 30 ml of 1 M NaOH, followed by one liter of UV-water.

2.7. Solid-state CP/MAS ^{13}C NMR spectroscopy

Solid-state ^{13}C NMR spectra were acquired using a Varian Unity 200 spectrometer operating at a ^{13}C frequency of 50.3 MHz. A measured mass of each sample (12–560 mg) was packed into a 7-mm diameter cylindrical zirconia rotor with Kel-F end-caps and spun at 5000 ± 100 Hz in a Doty Scientific magic angle spinning (MAS) probe. Each free induction decay (FID) was acquired using a sweep width of 40 kHz. Over an acquisition time of 15 ms, 1216 data points were collected. All spectra were zero filled to 8192 data points and processed with a 50 Hz Lorentzian line broadening and a 0.005 s Gaussian broadening. Chemical shifts were externally referenced to the methyl resonance of hexamethylbenzene at 17.36 ppm.

A range of solid-state ^{13}C NMR analyses were completed on each sample to define the NMR conditions required to maximize signal intensity and to quantify the fraction of sample OC observed. The percentage of each type of OC present in the samples was determined using a standard cross polarization pulse sequence (Wilson, 1987). A total of 20,000–262,000 transients were collected in the cross polarization experiments. The length of the recycle delay (0.3–1.0 s) used in the cross polarization pulse sequence was set at $\geqslant 7$ times the average T_1H (proton spin lattice relaxation) value derived from an inversion recovery pulse sequence (Wilson, 1987). A variable contact time experiment (Wilson, 1987) was used to define the optimal contact time at which signal intensity was maximized and to calculate an average value of $T_1\rho H$ (spin lattice relaxation time in the rotating frame) for each sample. An optimal contact time of 1 ms was used in all cases. Values of $T_1\rho H$ were calculated as the inverse slope (1/m) over the linear decay phase of the relationship between ln (total signal intensity) versus contact time for the spectra acquired in the variable contact time experiments. A cross polarization analysis was also completed for an empty sample rotor to quantify the background signal intensity derived from the Kel-F rotor end caps.

All spectra were phased and baseline corrected between −100 ppm and 300 ppm after Fourier transformation of the acquired FID. The spectra were divided into the chemical shift regions identified in Table 1, and the signal intensity associated with each region determined by integration using the instrument software. The chemical shift limits selected, and the inclusion of spectral regions with chemical shift limits >215 ppm, were used to account for signal intensity in spinning side bands. Under the NMR conditions used in this study, spinning side bands were located approximately 100 ppm on either side of their parent signals. The calculations presented in Table 1 were used to account for the signal intensity found in spinning side bands. In summary, the signal intensities in the high field side bands were doubled and added to that of the parent signal and were also subtracted from the appropriate low field spectral regions. This procedure assumed that the two side bands associated with a given parent signal were of equal intensity.

The fraction of OC in the samples that was NMR observable (C_{obs}) was assessed for all CP analyses using spin counting with glycine as an external standard and NMR conditions optimized separately for glycine and the samples. The cross polarization and variable contact time experiments were performed on glycine and the acquired data used with that of the samples to calculate C_{obs} values according to Eqs. (1) and (2).

$$T_1\rho H \text{ corrected signal intensity} = \left(\frac{\text{Aquired signal intensity}}{\exp(-\text{contact time}/T_1\rho H)}\right) \quad (1)$$

$$C_{obs} = \left(\frac{\text{Sample } T_1\rho H \text{ corrected signal intensity}}{\text{Mass of sample organic C analyzed (mg)}}\right) \times \left(\frac{\text{Glycine } T_1\rho H \text{ corrected signal intensity}}{\text{Mass of glycine C analyzed (mg)}}\right)^{-1} \quad (2)$$

2.8. X-ray diffraction and X-ray fluorescence

The demineralized samples were ground in an agate mortar and pestle and pressed into aluminum sample

Table 1
Chemical shift regions into which the acquired total ^{13}C NMR signal intensity was apportioned, and the proposed major sources of carbon from which the intensity in each region was derived

Chemical shift limits (ppm)	Proposed dominant sources of OC	Calculations used to quantify the amount of each type of carbon
300–290		
290–265	Carbonyl and amide spinning side band	
265–245	Phenolic spinning side band	
245–215	Aromatic spinning side band	
215–190	Ketone C	
190–165	Carbonyl and amide C	(190–165 ppm) + 2 × (290–265 ppm)
165–145	Phenolic C	(165–145 ppm) + 2 × (265–245 ppm)
145–110	Aromatic and unsaturated C	(145–110 ppm) + 2 × (245–215 ppm)
110–90	Di-O-alkyl C	(110–90 ppm)
90–65	O-alkyl C	(90–65 ppm)–(290–265 ppm)
65–45	Amine and methoxyl C	(65–45 ppm)–(265–245 ppm)
45–0	Alkyl C	(0–45 ppm)–(215–245 ppm)
0–50		

holders for X-ray diffraction (XRD) analysis. The obtained patterns were recorded with a Philips PW1800 microprocessor-controlled diffractometer using CoK_α radiation, variable divergence slit, and graphite monochromator. Diffraction patterns were recorded in steps of 0.05° 2θ with a 1.0 s counting time per step. X-ray fluorescence was used to quantify elemental concentrations in the standard samples before and after demineralization. The methodology of Norrish and Hutton (1969) was used with the exception that the NH flux was substituted with 12/22 Sigma X-ray flux (Sigma Chemicals Pty Ltd, Balcatta, Western Australia, Australia).

3. Results and discussion

3.1. Development of a HF demineralization procedure

The dilute HF treatment following the removal of carbonates with HCl is considered the most promising approach to demineralize soil and sediment samples. Traditionally, coal and kerogen have been demineralized with concentrated HCl and HF because these biomaterials are resistant to chemical alteration and hydrolysis (Durand and Nicaise, 1980). However, lower HF concentrations (between 0.5 and 10%) had to be used for soils to minimize OC hydrolysis and losses, with a mineral removal efficiency that increased with increasing HF concentrations (Preston et al., 1989; Skjemstad et al., 1994; Schmidt et al., 1997). For example, Skjemstad et al. (1994) showed that using a 2% instead of a 1% HF solution significantly increased the OC/Fe ratio in demineralized soil residues with no concomitant increases in OC losses. Twelve sequential HF extractions were needed to completely dissolve the mineral fraction and to avoid supersaturation of the extraction solution with respect to neoformed fluoride minerals such as ralstonite ($NaAlMgF_6 \cdot H_2O$) and hieratite (K_2SiF_6). However, because of the large volume of supernatant generated by 12 successive extractions, the recovery of solubilized OC would be tedious and the ensuing OC losses very high. Schmidt et al. (1997) found that two 12-h treatments with 10% HF induced no significant change in soil OM composition except for a possible loss of *O*-alkyl carbon (attributed to carbohydrates) as determined by CP/MAS ^{13}C NMR. However, this method was not applied to whole sediments containing a significant fraction of labile biochemicals. The number of treatments needed to remove minerals can be reduced by using a higher HF concentration, but a higher HF concentration can also increase OC losses and alteration. The effect of varying the concentration of HF between 2.5 and 15% was thus assessed for typical riverine, estuarine and coastal sediments.

Following two 12-h HCl/HF treatments using different HF concentrations, the residual dry mass decreased with increasing HF concentration for all samples (Fig. 2). OC losses increased greatly when a HF concentration of 15% was used instead of 10%, particularly for the Washington Coast sediment for which OC losses increased from 12.5 to 27.9% at the higher HF concentration (Fig. 2). OC losses were significantly lower for a HF concentration of 2.5%, but only an average of ~40% of the initial sample mass was removed. A larger fraction of the mineral mass can be removed by increasing the number of treatments, as done by Preston and Newman (1992) and Skjemstad et al. (1994). However, in this study, OC losses also increased with the fraction of the mineral mass that was dissolved when additional 2.5% HF demineralization treatments were applied. Increased OC losses with greater mineral destruction may result as labile OM originally associated with the mineral matrix becomes more available to solubilization. A HF concentration of 10% offered a reasonable compromise between maximal mineral dissolution efficiency, minimal OC losses for 2×12-h treatments and manageable volume of supernatant generated.

3.2. Dissolution kinetics

The dissolution kinetics for the mineral fraction of five sediment samples are presented in Fig. 3A, along with the OC concentration in the residual mass [Fig. 3(B)] and OC losses [Fig. 3(C)]. For most samples, mineral dissolution followed first-order kinetics, sometimes with a small offset upon the addition of the second HF solution at $t = 12$ h [Fig. 3(A)]. The small amplitude of this offset suggested that dissolution was not slowed down by a lack of fluorine ions, but rather by the slow reaction kinetics of HF with more resistant minerals. Additional tests with pure mineral phases showed that the dissolution of haematite, a dense Fe^{3+} oxide mineral, followed similar first-order kinetics when using the same experimental conditions (Fig. 4). Dissolution of expandable (montmorillonite) and non-expandable (kaolinite) sheet-silicates was complete within the first 60 min, while biogenic silica dissolved immediately (Fig. 4). These results likely explain the rapid decrease in residual mass found for the Lake Washington and the Mexican Coast sediments for which ~90% of the total mass loss occurred within the first hour of reaction, most likely because of the rapid dissolution of biogenic silica or detrital aluminosilicates [Fig. 3(A)].

As a result of the rapid dissolution of the mineral fraction, the OC concentration in the residue increased rapidly within the first hour of HF treatment [Fig. 3(B)]. Further increases in OC content with increasing HF treatment time were small for the Lake Washington and the Mexican Coast sediments, while the three other samples had more sustained OC increases because of the continual decrease in residual mass. The final OC concentration in the residues following both extractions was

between 1.5 and 32%, corresponding to OC enrichment factors (i.e. EF = final OC concentration/initial OC concentration on a dry weight basis) between 3.1 and 6.6 (Table 2).

More than 90% of the OC losses from all samples occurred during the HCl treatment and the first hour of the HCl/HF treament [Fig. 3(C)]. This pattern suggests that a large fraction of the solubilized OC was either composed of chemically labile hydrophilic compounds or molecules associated with the mineral matrix (Keil et al., 1994b). Schmidt et al. (1997) also concluded that an important fraction of the OC losses could be attributed to the removal of OM soluble in acidic aqueous solutions and to sample handling. Indeed, the mass of highly organic samples (Plankton Tow, Peach Leaves and Dogfish Liver) treated in the same way also decreased dramatically within the first 30 min with very small additional dissolution afterwards (Fig. 4). Most of the mass loss was due to dissolution of acid-soluble OC and as a consequence, more than 95% of the OC losses for these samples also occurred within the first 30 min. The OC losses corresponded to between 35 and 50% of the initial OC content (results not shown). Very little additional OC losses occurred after the first hour for the Buffalo River, Estuarine and Washington Coast sediments, despite substantial further mineral dissolution [Fig. 3(C)]. Total OC losses varied between 10 and 20% of the initial OC content, which is similar to the range of 8 to 23% obtained for the demineralization of soil samples (Skjemstad et al., 1994; Schmidt et al., 1997). Independent OC mass balance calculations using dissolved organic carbon measurements (supernatants) and elemental analysis (demineralized residues) generally agreed within 5%.

3.3. OC enrichment relative to paramagnetic metals and residual minerals

The natural and demineralized samples were analyzed for residual paramagnetic metal and mineral content by X-ray fluorescence and X-ray diffraction analysis, respectively. Paramagnetic metals broaden CP/MAS ^{13}C NMR resonances, decrease the signal to noise ratio and cause paramagnetic resonance shifts (Arshad et al., 1988; Skjemstad et al., 1994; Schmidt et al., 1997; Smernik and Oades, 1999). OC:paramagnetic metal ratios were thus calculated in the natural and demineralized sediments to assess OC enrichment relative to paramagnetic metals upon demineralization (Table 3).

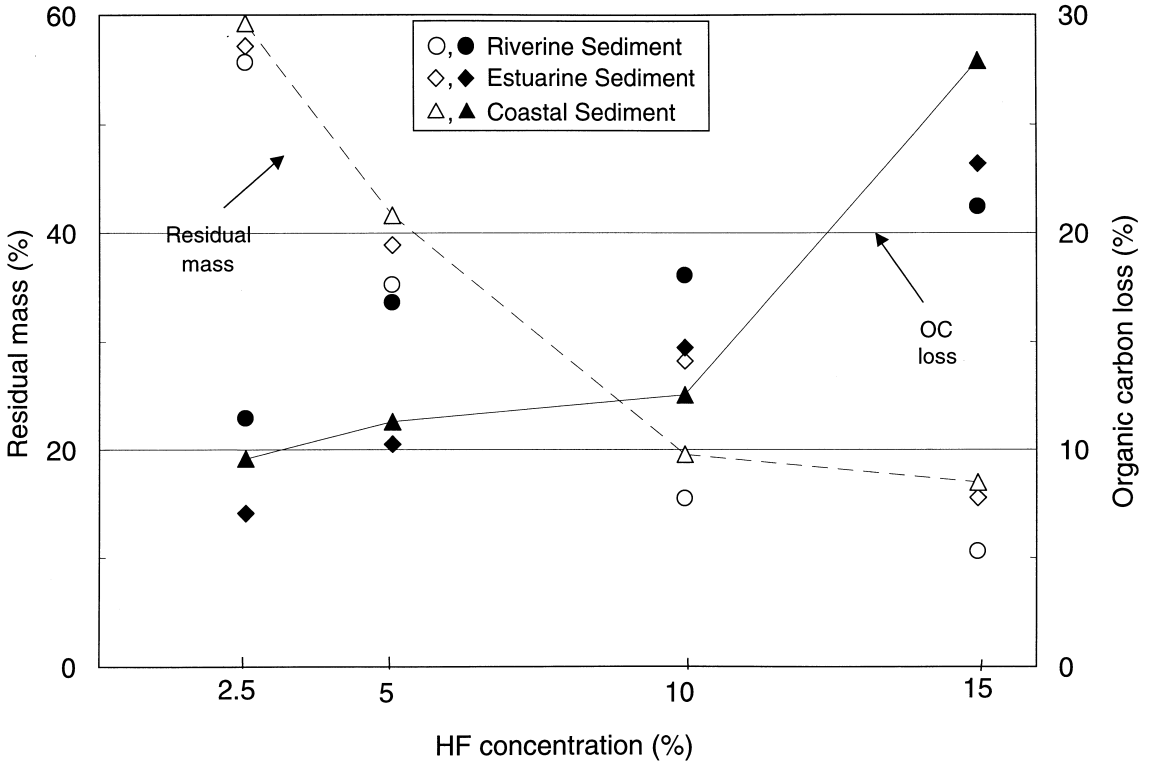

Fig. 2. Influence of HF concentration on residual mass (open symbols) and organic carbon (filled symbols) losses. The residual mass is the total dry mass (as a % of the initial dry mass) recovered following the removal of carbonates with 1 N HCl and two 12-h treatments with HCl (1 N) and HF (at different concentrations). OC losses during the same treatments are expressed as a % of the initial organic carbon content on a dry weight basis.

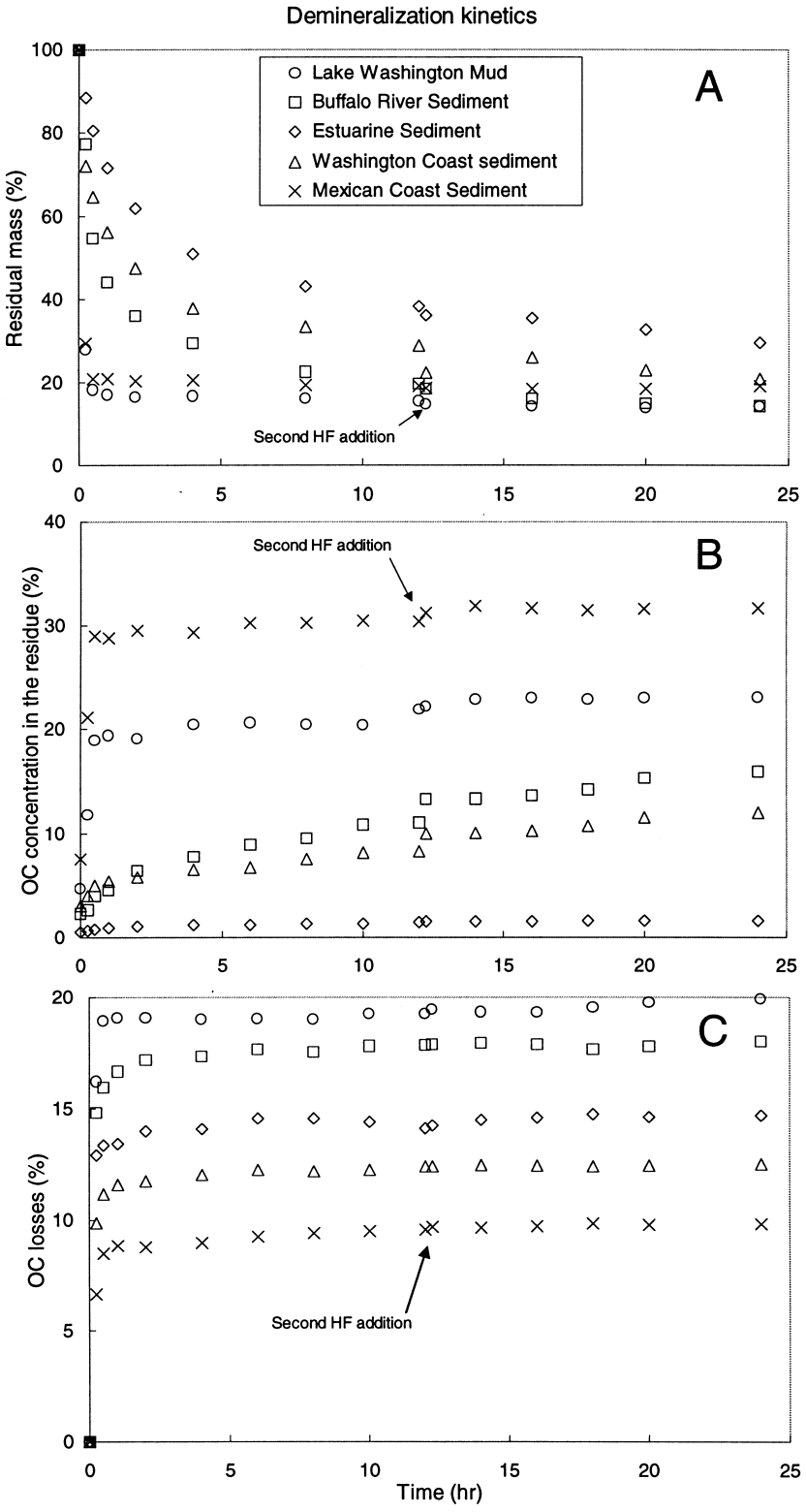

Fig. 3. Demineralization kinetics for different sediment samples. (A) Total residual mass as a % of the initial mass; (B) OC concentration in the residue as a % of the residual mass; (C) OC losses as a % of the initial OC content. All concentrations are on a dry weight basis.

Table 2
Data obtained for different samples using the demineralization method presented in Fig. 1

		HCl + HCl–HF treatments				Supernatant recovery			Removal of metals			Desalting			Final results[d]		
	Initial OC (%)	Final OC[a] (%)	Final mass[b] (%)	EF[c]	OC losses (%)	Final OC[a] (%)	Final mass[b] (%)	OC losses (%)	Final OC[a] (%)	Final mass[b] (%)	OC losses (%)	Final OC[a] (%)	Final mass[b] (%)	OC losses (%)	EF[c]	OC losses (%)	(C/N)[a] change (%)
Lake Washington mud	4.69	23.0	14.3	4.9	19.9	–[e]	–	–	–	–	–	–	–	–	4.9	19.9	19.2
Buffalo River sediment	2.43	15.9	14.3	6.6	18.0	–	–	–	–	–	–	–	–	–	6.6	18.0	23.5
Estuarine sediment	0.51	1.59	29.6	3.1	14.7	–	–	–	–	–	–	–	–	–	3.1	14.7	15.0
Washington Coast sediment	3.06	12.0	20.8	3.9	12.5	–	–	–	–	–	–	–	–	–	3.9	12.5	11.0
Mexican Coast sediment	7.21	31.6	19.1	4.4	9.8	–	–	–	–	–	–	–	–	–	4.4	9.8	2.9
Dabob Bay plankton	7.99	44.5	5.2	5.6	50.5	9.9	44.2	2.1	1.71	245.4	2.2	29.5	88.7	0.6	3.7	4.9[f]	0.2
Arabian Sea trap 498 m	8.55	39.8	17.3	4.6	6.4	21.5	28.5	1.1	2.58	229.9	2.3	19.0	25.8	11.9	2.2	15.2[f]	3.9
Arabian Sea trap 2698 m	5.71	40.1	11.5	7.0	7.5	15.7	25.7	1.0	2.95	132.1	2.3	20.0	20.3	13.0	3.5	16.3[f]	5.7
Arabian Sea sediment	1.35	8.78	11.1	6.5	26.3	8.2	12.0	1.4	1.13	67.0	1.9	11.0	4.7	10.2	8.1	13.5[f]	−3.1
EQPAC 0 N sediment	0.24	9.33	1.8	38.9	48.9	16.6	7.3	9.5	0.75	14.4	4.1	5.0	1.9	3.0	20.8	16.5[f]	−3.9
EQPAC 9 N sediment	0.68	2.27	12.5	3.3	54.6	1.3	41.8	3.2	0.79	49.1	8.2	1.6	17.9	5.9	2.4	17.3[f]	7.9
Southern Ocean sediment	0.31	19.8	0.4	63.8	68.1	1.1	18.1	4.4	0.50	29.3	10.1	2.8	7.3	6.0	9.0	20.5[f]	10.6

[a] Organic carbon (OC) concentration in the residue following the specified treatment.
[b] Residual mass as a % of the initial mass following the specified treatment.
[c] Enrichment factor = final OC concentration/initial OC concentration.
[d] Results for the residue obtained following all treatments.
[e] Supernatants not recovered.
[f] Cumulative OC losses for all treatments.

Table 3
Organic carbon enrichment relative to paramagnetic metals and CP/MAS ^{13}C NMR chemical shift integrated intensities

	OC (%)	OC:Fe (wt. ratio)	OC:Mn (wt. ratio)	OC:Cu (wt. ratio)	Observability (% of OC)	0–45[b] (area %)	45–60 (area %)	60–95 (area %)	95–110 (area %)	110–145 (area %)	145–165 (area %)	165–190 (area %)	190–215 (area %)
Lake Washington mud													
Natural	4.69	1.2	24	1340	9.5	31.8	8.7	17.3	4.1	18.6	7.4	9.2	2.9
Demineralized	23.0	53.0	928	23000	56.0	36.2	8.7	16.9	3.6	17.7	5.7	9.5	1.7
Buffalo River sediment													
Natural	2.43	0.6	41	243	8.9	13.1	7.1	16.2	6.0	36.4	10.3	6.8	4.1
Demineralized	15.9	7.0	1030	1590	36.5	27.2	7.2	14.1	3.5	28.0	8.6	9.2	2.1
Estuarine sediment													
Natural	0.51	0.2	20	34	7.2	0.0[c]	0.0[c]	5.0[c]	20.5[c]	37.9[c]	6.6[c]	14.7[c]	15.2[c]
Demineralized	1.59	1.7	158	–[a]	37.4	34.4	9.3	12.9	2.0	20.3	6.4	12.1	2.6
Washington Coast sediment													
Natural	1.47	0.7	73	271	10.5	9.3	4.1	20.7	7.5	28.7	11.8	13.1	4.8
Demineralized	6.59	17.8	860	2350	43.4	31.8	9.8	16.4	3.9	20.0	6.7	10.4	1.2
Mexican Coast sediment													
Natural	7.21	2.6	259	1200	32.7	33.2	9.5	17.8	4.1	18.2	4.6	11.2	1.5
Demineralized	31.6	67.4	5830	5360	48.7	38.7	9.3	16.5	4.0	17.8	4.5	8.6	0.6
Dabob Bay plankton													
Natural	7.99	n.a.	n.a.	n.a.	19.8	49.1	10.7	12.7	1.4	11.0	2.3	12.8	0.0
Demineralized	29.5	n.a.	n.a.	n.a.	35.9	44.5	13.4	12.7	1.9	11.3	2.2	14.0	0.0

[a] Undetectable metal concentrations in the demineralized residue.
[b] Integrated intensity between 0 and 45 ppm (parts per million) in % of the total signal.
[c] Signal intensities too small: most of the signal derived from rotor background.

OC:Fe weight ratios were low in all five natural sediments, with three of them below the OC:Fe threshold ratio of 1. The high concentration of iron relative to OC made these three natural samples very poor candidates for direct CP/MAS ^{13}C NMR analysis and the spectra acquired before demineralization were barely distinguishable from background noise. Manganese and copper were much less of a problem because of their lower abundance in this set of samples. Because demineralization selectively removed inorganic materials, the OC:Fe ratios were at least 7-fold higher in the demineralized samples, and reached a value of 67.4 in the demineralized Mexican Coast sediment. The OC:Mn and OC:Cu ratios were similarly increased by at least 1 order of magnitude in most samples. The relatively low OC:Fe value measured for the demineralized estuarine sediment sample was due to the low OC concentration in the natural sample (0.51 wt.%), and to the presence of trace amounts of HF-resistant pyrite and haematite in the demineralized residue (Table 4). For such samples, higher OC:Fe ratios and OC enrichment factors can be obtained by adding a third 12-h HCl/HF treatment.

While quartz and clay minerals constituted the dominant mineral fraction in most of the natural sediments (Table 4), ralstonite ($NaAlMgF_6 \cdot H_2O$) and hieratite (K_2SiF_6), two newly synthesized fluoride minerals, were the major inorganic components in most of the demineralized residues. The presence of fluoride minerals indicates that supersaturation relative to these mineral phases was reached during the HCl/HF treatments. Precipitation of fluoride minerals could have been avoided by reducing HF concentration and reaction time and increasing the number of HCl/HF treatments. Unfortunately, such an approach would not have been practical in this study because of the large volumes of supernatant that would have been generated. Alternatively, when the acid-soluble OC present in the HCl/HF supernatants does not have to be recovered, fluoride minerals could be dissolved using BF_3. This water-soluble gas, formed by the reaction of H_3BO_3 with HF, reacts with fluoride minerals to form water soluble fluoroborates (Robl and Davis, 1993). However, because ralstonite and hieratite do not contain paramagnetic metals and are formed in amounts corresponding to less than 10% of the initial mineral mass, their presence in demineralized samples induces only minor reductions in CP/MAS ^{13}C NMR sensitivity.

3.4. Dissolution of mineral matrices and OC in difficult marine samples

3.4.1. Recovery of the acid-soluble OM

The major mineral constituents of sediments and particulate matter vary widely in the ocean and OC concentrations range from $<0.2\%$ in deep-ocean oozes and clays, to $>10\%$ in sediments underlying oxygen-deficient waters of the Eastern Pacific and Indian margins (Premuzic et al., 1982). Sediments accumulating under the corrosive deep waters of the open ocean in carbonate oozes (EQPAC 0°N; Table 2), opal oozes (Southern Ocean), or red clays deposits (EQPAC 9°N) generally exhibit minimal OC concentrations and are considered among the most difficult to demineralize. Indeed, about half of the initial OC in these samples was lost upon dissolution of the mineral fraction (Table 2). The solubilized OC was thus recovered by freeze-drying

Table 4
Mineral composition of the natural and demineralized sediments

	Dominant (>60 wt.%)	Minor (5–20 wt.%)	Trace (<5 wt.%)
Lake Washington mud			
Natural	Amorphous material	Quartz, albite	Mica, orthoclase, smectite, amphibole
Demineralized	Ralstonite	Amorphous material	Quartz, anatase, rutile
Buffalo River sediment			
Natural	Quartz	Mica, chlorite, albite, orthoclase	Calcite, dolomite, pyrite, anatase
Demineralized	Hieratite, ralstonite	Mica	Quartz, pyrite, anatase, rutile
Estuarine sediment			
Natural	Quartz	Mica, albite, orthoclase	Pyrite, anatase, chlorite, halite, calcite
Demineralized	Quartz	Hieratite	Rutile, pyrite, haematite, anatase, mica
Washington Coast sediment			
Natural	Quartz, albite, (smectite)[a]	Orthoclase, mica	Pyrite, calcite, halite, chlorite, amphibole
Demineralized	Ralstonite, (heiratite)[a]	Mica	Quartz, pyrite, anatase, rutile
Mexican Coast sediment			
Natural	Smectite	Quartz, albite, orthoclase	Calcite, halite, pyrite, mica, kaolin
Demineralized	Ralstonite		Pyrite, anatase, hieratite, rutile

[a] Mineral in parentheses is sub-dominant, i.e. 20–60 wt.%.

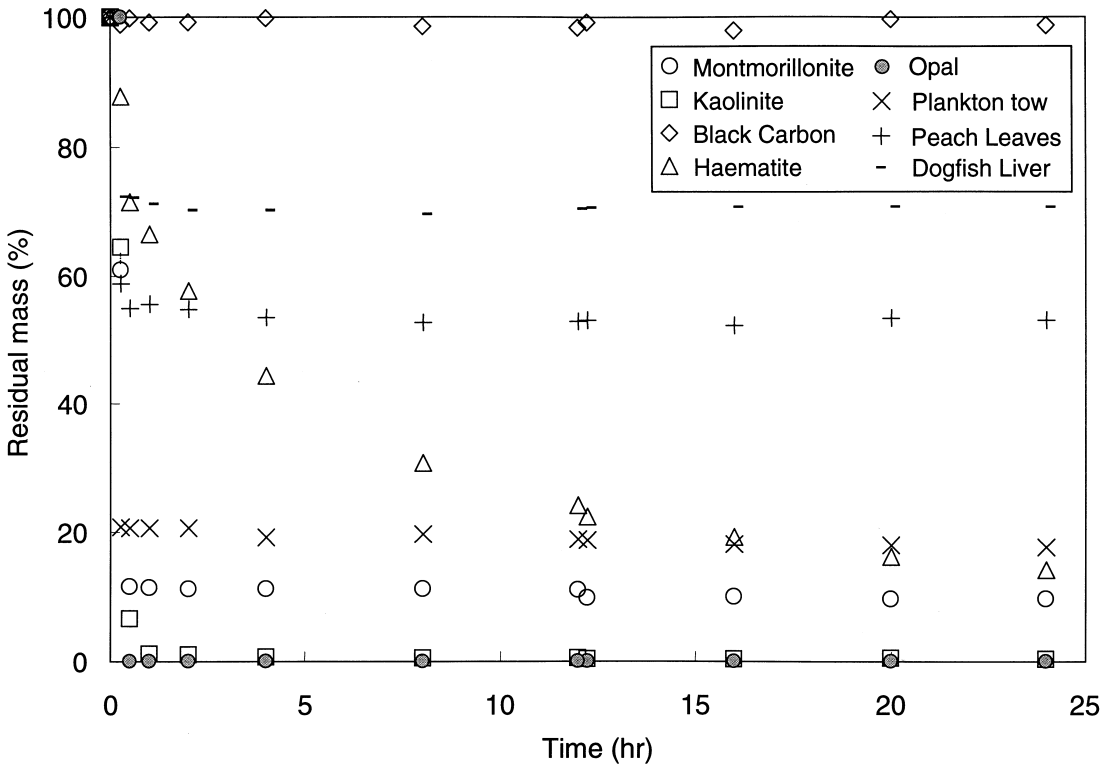

Fig. 4. Dissolution kinetics for pure mineral and biological samples using the demineralization method presented in Fig. 1.

the supernatants following acid neutralization. As shown in Table 2, most of the HF-solubilized OC was recovered in the freeze-dried residue, with the only losses being attributed to coprecipitation of a small fraction (< 5% of the solubilized OC) with CaF_2. The relative losses of OC due to coprecipitation were higher for carbonate-rich and opal-rich samples, mostly because of their low initial OC concentration and the large mass of precipitate formed upon addition of HF in the HCl supernatant or $CaCO_3$ in the HF supernatant. Despite an absolute recovery of more than 90% of the initial OC (losses of 9.5% or less, Table 2), the OC concentration in the 'freeze-dried + demineralized residue' fraction was lower than in the demineralized residue for most samples because of the concomitant recovery of the salts from both supernatants (Table 2).

3.4.2. Removal of dissolved metals and salts

For most supernatants, salts and paramagnetic metals had to be separated from the organic fraction before pooling with the demineralized residues to reduce the amount of inorganic material and interfering paramagnetic compounds. Metals were first removed by sulfide precipitation (Table 2). At a pH of ~7, dissolved iron and HS^- can coexist at a maximum of only ~12 μM Fe (Canfield and Raiswell, 1991). Amorphous metal sulfide precipitates have a large surface area and show amphoteric surface behavior (Morel and Hering, 1993; Bebie et al., 1998), but are generally assumed to be less surface-reactive than amorphous metal (hydr)oxides. However, the relative OC losses following addition of sulfide were directly related to the mass of the sulfide precipitate ($r^2 = 0.63$, data not shown), suggesting non-specific metal sulfide–OC interactions. OC losses by coprecipitation amounted to between ~5 and 44% of the OC solubilized during the HCl and HCl/HF treatments, which corresponded to a range of 1.9 to 10.1% of the initial OC (Table 2). Relative OC losses were highest for metal-rich and/or organic-poor samples. Coprecipitation may have been due to several processes, including flocculation induced by the formation of (poly)sulfide bridges between organic molecules, direct reaction with the metal sulfide surfaces, or precipitation of soluble metal–organic complexes upon addition of NaHS.

Although most of the acid-soluble OC was recovered from the supernatants at this stage, an additional chromatographic step had to be included to remove the residual salt. While OC losses by sulfide coprecipitation were low, the relative increase in the mass of the inorganic fraction through addition of NaHS to the supernatants and pH adjustment with dilute HCl and NaOH was high. The mass of the residue plus supernatants following demineralization, supernatant recovery and metal removal was sometimes higher than the initial mass, in

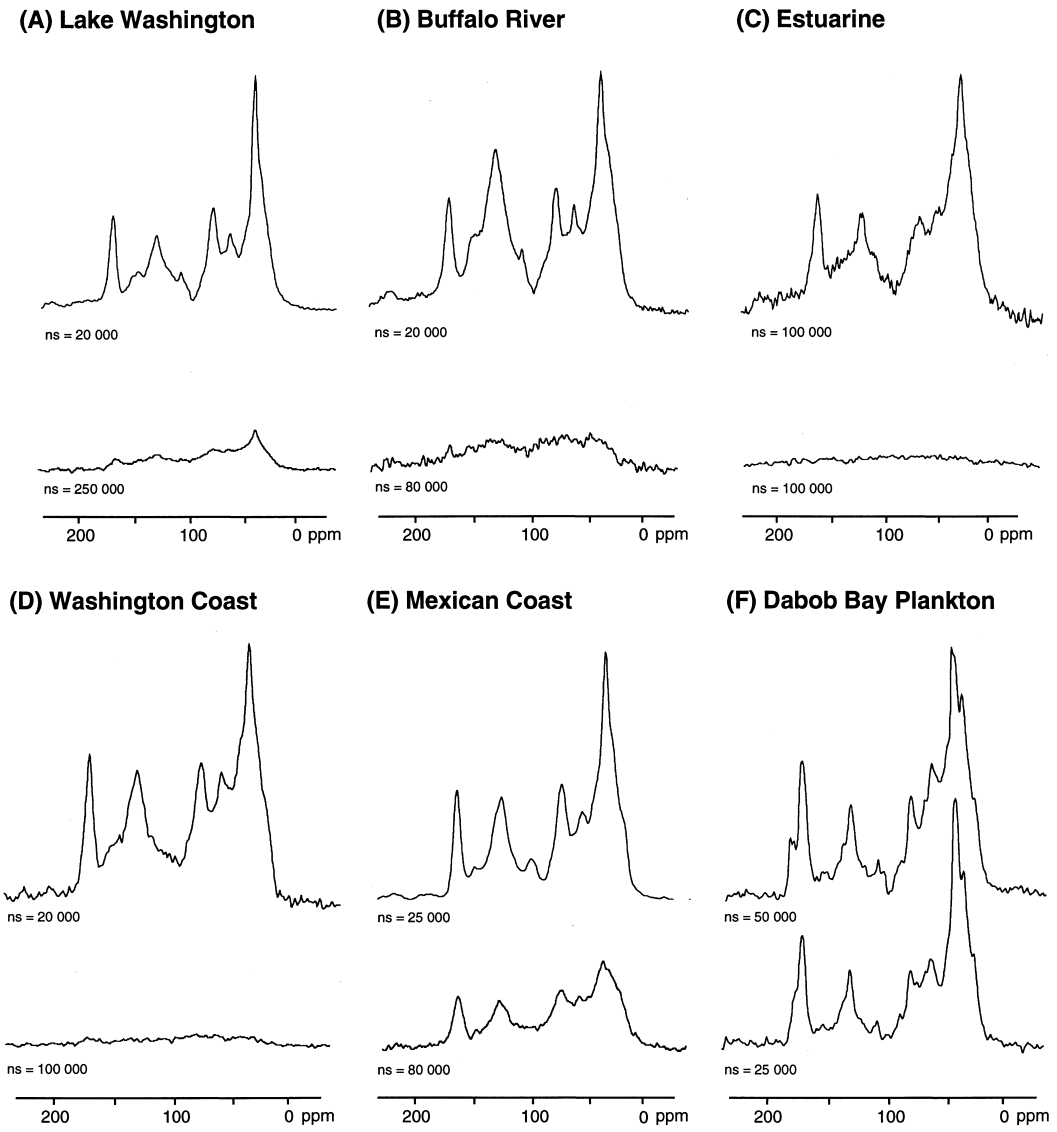

Fig. 5. CP/MAS ^{13}C NMR spectra of the demineralized (top) and natural (bottom) samples. (A) Lake Washington sediment; (B) Buffalo River sediment; (C) Estuarine sediment; (D) Washington Coast sediment; (E) Mexican Coast sediment; and (F) Dabob Bay Plankton. Vertical scales between demineralized and natural spectra are equivalent for each sample but vary between samples. ns, Number of scans acquired for each spectrum.

particular for organic-rich samples (between 14.4 and 245%, Table 2). Thus, salts added to the supernatant or originating from the dissolved mineral constituents were removed to concentrate the organic residue. This was done using a desalting chromatographic method designed to retard the passage of small inorganic ions versus hydrophilic organic molecules through a mixed-bed resin column. Bronk and Glibert (1991) reported efficient salt removal with this method. However, OC losses varied between 0.6 and 13% of the original OC present in the sample despite the low flow rate (~0.2 ml min^{-1}) and long column (45 × 1 cm) used during desalting. The lost OM was likely composed of low molecular weight organic acids that are not efficiently separated from inorganic ions by ion retardation chromatography. Better OC recoveries may have been achieved with the collection of smaller fractions (e.g. 5 ml instead of 10 ml). However, as illustrated by the significantly higher residual mass following desalting when compared to the residual mass following the HCl and HCl/HF treatments (Table 2), the complete separation of organic and inorganic constituents might not be possible. OC losses of 13% and less during desalting were thus judged acceptable.

3.5. Degree of chemical alteration of organic matter by the demineralization process

3.5.1. Elemental contents and ratios

While OC enrichment factors varied between 2.2 and 20.8 using different combinations of the demineralization method (Table 2), OC losses amounted to between 5 and 20.5% of the initial OC. Some samples preferentially lost a small fraction of N-containing material upon demineralization, as shown by the increase in the atomic C/N ratio in the demineralized residue (Table 2). This result was different from those obtained for soil samples for which carbohydrates constitute the bulk of the OC losses upon demineralization (Schmidt et al., 1997). However, the fractionation of N-containing material, which was more pronounced for freshwater sediments, can be eliminated by recovering the solubilized OC as done for the difficult samples (bottom of Table 2). Despite the many steps involved in the recovery of OC from the supernatant, removal of paramagnetic compounds and desalting procedure, OC losses for the difficult samples were in the same range as those for regular samples for which the supernatant was not recovered. More importantly, the small changes in the $(C/H)_a$ and $(C/N)_a$ ratios measured for the residues following recovery of the acid-soluble OC suggest little preferential OC fractionation or chemical alteration although more detailed analyses should be done to precisely assess the degree of OM alteration at the molecular-level, the expected small changes in OC chemical structure probably would not be detectable using solid-state ^{13}C NMR (see next section). Recovering the acid-soluble organic fraction was thus an efficient way to circumvent OM fractionation based on molecular characteristics during the dissolution of the mineral fraction.

3.5.2. Solid-state ^{13}C NMR analyses

The demineralization procedure (Fig. 1) was applied to 18 particulate samples of aquatic origin varying in both OC and paramagnetic metal concentrations. CP/MAS ^{13}C NMR spectra with adequate signal to noise ratios were acquired for all demineralized samples (results to be published). The improvement in spectral quality gained upon demineralization is illustrated in Fig. 5 for the five standard samples used for method development, as well as for the organic-rich Dabob Bay plankton material. While all spectra for the demineralized samples were less noisy than those for their natural counterpart, the increase in signal-to-noise ratio was particularly evident for the Buffalo River [Fig. 5(B)], Estuarine [Fig. 5(C)] and Washington Coast [Fig. 5(D)] sediments that all yielded unusable spectra in untreated form. A major fraction of the total signal for these three iron- and mineral-rich samples was derived from the background noise of the Kel-F rotor end caps. The increased OC concentration in the demineralized samples considerably facilitated CP/MAS ^{13}C NMR signal acquisition by reducing by a factor of 2 to 10 the number of scans required to obtain good quality spectra (from ~250,000 scans for natural samples to between 20,000 and 100,000 for demineralized samples). The acquisition of a lower number of scans also meant that each sample could be analyzed in a shorter time-frame and led to considerable savings in NMR utilization time.

As a result of the removal of the diluting mineral fraction and the interfering paramagnetic metals, the OC observability relative to a glycine standard was also significantly increased, reaching values between 36 and 56% for the demineralized samples compared to a range of 7 to 33% for the natural counterparts (Table 3). The range of observability values obtained for the demineralized sediments was about twice that of ~10 to 30% measured for demineralized soil samples by Skemjstad et al. (1994). The higher OC observability obtained in our spectra was likely due to more efficient removal of paramagnetic species by the higher HF concentration used for demineralization (i.e. 10% instead of 2% for Skjemstad et al.). Although on average about half of the OC in the demineralized samples remained invisible using ^{1}H-^{13}C cross polarization, a series of Bloch decay experiments, in which the ^{13}C atom was directly polarized, showed that the missing OC was structurally similar to the visible OC (differences of $< 5\%$ between integrated chemical shift intensities for cross polarization and Bloch decay spectra, results to be published). Observabilities of OC in the Bloch decay analyses typically ranged between 70 and 90% for the samples analyzed in this study.

The increase in signal to noise ratio and OC observability allows a more reliable quantification of the signal measured for the different classes of organic carbon atoms. Integrated intensities for the eight different chemical shift regions from the 12 spectra (shown in Fig. 5) are listed in Table 3. Within this set of samples, the organic-rich Lake Washington sediment (4.69 wt.% OC), Mexican Coast sediment (7.21 wt.% OC) and Dabob Bay Plankton (7.99 wt.% OC) gave sufficiently detailed spectra in untreated form to assess the potential for chemical alteration or fractionation during demineralization. Organic-rich samples such as these can normally be analyzed by CP/MAS ^{13}C NMR without demineralization. Differences in integrated intensities between the natural (untreated) and demineralized samples (as a % of the total signal) were less than 2.7%, except for alkyl carbon (0–45 ppm) with differences ranging between 4.4 and 5.5% (Table 3). The similar contribution from the different OC types in the natural and demineralized samples suggest little chemical alteration or specific fractionation. It should be noted that, because ^{13}C NMR reflects the chemical environment surrounding the different carbon atoms and not

directly at the chemical structure of organic molecules, very subtle chemical alterations or fractionations could have been missed. However, given the small changes in the relative chemical shift intensity upon demineralization and in atomic C/N ratios (Table 2), it is unlikely that the general structure of the organic material was modified appreciably. The improvement in spectral quality and 'usability' for mineral-rich samples far overshadows any small alteration in the component resonances. Further, the similarity between the natural and demineralized spectra for the organic-rich samples also suggests that the increase in OC observability was evenly distributed across the entire frequency range and that the presence of paramagnetic metals in the natural samples did not cause important chemical shift changes for specific OC types. The absence of such 'paramagnetic' shifts was also reported for complex OM from demineralized soil samples amended with different paramagnetic metal ions including iron, manganese and copper, as well as lanthanide shift reagents (Smernik and Oades, 1999).

The differences in chemical shift intensities between the natural and demineralized samples were much higher and much more variable for natural samples with an OC:Fe ratio <1 (Buffalo River, Estuarine and Washington Coast sediments, Table 3). These large differences were mostly due to the fact that the total background-corrected intensity of the signal acquired for these samples was similar to (Buffalo River sediment) or lower (Estuarine and Washington Coast sediments) than the total intensity of the rotor background signal. Peaks corresponding to the different OC types were thus hardly distinguishable from background noise, most likely because of low OC concentration, and also because of signal broadening across the entire frequency range and signal loss caused by a shortening of relaxation rate constants induced by paramagnetic metals (Smernik and Oades, 1999). Although OC:Fe ratio >1 does not necessarily indicate that reasonable spectra will be obtained (Skjemstad et al., 1994), this result confirms that when the OC:Fe ratio is <1, signal loss and broadening are too severe to obtain useful spectra.

4. Conclusion

Although labor intensive, this demineralization method results in a significant enrichment of the OM from recently deposited marine sediments with limited structural alteration and molecular fractionation. In combination with CP/MAS ^{13}C NMR spectroscopy, it allows the elucidation of the chemical structure of organic matter in samples with very low OC concentrations and/or with a significant fraction of labile, acid-soluble organic components. These approaches, combined with pyrolysis and chemolysis characterizations, should provide structural information useful for elucidating organic matter sources, forms and diagenetic trends in a wide range of depositional environments. Demineralization can help reduce the troublesome mineral effects that limit the accuracy and precision of organic hydrogen and oxygen concentration measurements, which in turn would allow the construction of property/property plots in the same way that van Krevelen plots are used for the characterization of kerogen (Tissot et al., 1974) and modern biomacromolecules (Perdue and Reuter, 1984). By dissolving the mineral matrix to release biochemicals entrapped within biogenic mineral matrices, this procedure also has the potential to dramatically reduce mineral-induced artifacts during thermal and chemical characterization of black carbon (results to be published) and major biochemicals (Hedges et al., 2001). Finally, demineralization can dramatically facilitate solid-state ^{15}N NMR analysis of mineral sediments.

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