Organic matter removal for the analysis of carbon and oxygen isotope compositions of siderite

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**Abstract**

The measurement of stable carbon (C) and oxygen (O) isotope compositions in siderite from sediments and soils can be useful to constrain carbonate genesis processes and/or to reconstruct paleoclimates. In order to evaluate our ability to determine C and O isotope compositions of siderite in modern sediments and soils, we prepared and analyzed synthetic samples made of variable proportions of pure siderite and yeast (selected for representing an immature organic matter because of its potential high reactivity to H3PO4 digestion). Replicate analyses of CO2 produced by phosphoric acid (H3PO4) digestion of our pure siderite standard at 130 °C provided δ13C and δ18O values of −12.3 ± 0.1‰ and −16.2 ± 0.3‰ (2σ) relative to PDB. Analysis of the synthetic sample mixtures shows δ13C and δ18O values undistinguishable from those of pure siderite within uncertainties, for proportion of yeast in the mixture equal to or lower than 10 wt.% (corresponding to ~5 wt.% of total organic carbon). In contrast, samples with proportions of yeast higher than 10 wt.% were progressively shifted to more negative values (down to −21.8‰ for C and −19.6‰ for O) with increasing proportion of yeast, as a result of an increasing contribution of CO2 produced by the reaction of yeast with H3PO4 at 130 °C. Although in the case of natural samples this organic matter CO2 contribution probably strongly depends on both organic matter source organisms and diagenetic history, our data indicate that the removal of organic matter prior to siderite analysis may be often required. We thus tested three different methods for organic matter removal on our synthetic samples, using either oxidation in solution with NaOCl or H2O2, or oxidation in low-temperature oxygen-plasma ashing system. For both methods based on oxidation in aqueous solution, we show that the determination of δ13C and δ18O is improved for concentration of yeast lower than 75 wt.% in the “siderite–yeast” mixture but is still shifted to lower values by 4.1 and 0.7‰ for C and O, respectively, for a yeast proportion higher than 75 wt.%. Moreover, these two methods induce partial siderite dissolution, preventing determination of siderite content in the samples. The best method of yeast removal for coupled C and O isotope analysis is the oxidation by low-temperature oxygen-plasma ashing, which strongly improves the accuracy of the δ13C and δ18O measurements and perfectly preserves siderite. In conclusion, any study of siderite isotope composition in organic-rich samples should include an evaluation of the need for organic matter removal using low-temperature oxygen-plasma ashing, the only method shown here to be efficient while preserving the siderite.

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1. Introduction

Siderite (FeCO3) in sediments and soils is an authigenic carbonate, formed under reducing conditions from ferrous iron (Fe2+) and carbonates dissolved in porewaters. Dissolved Fe2+ can accumulate to significant level only in the absence of molecular oxygen (O2) and hydrogen sulfide (H2S) because Fe2+ is oxidized to Fe3+ and forms iron oxyhydroxides in the presence of O2, and reacts to produce iron sulfate in the presence of H2S (see review in Coleman, 1985). Siderite is thus mostly found in settings characterized by high organic matter burial rates (driving O2 exhaustion and iron oxides reduction) and low sulfate (SO4) content (limiting H2S production by sulfate reduction).

These conditions are mostly encountered in modern continental depositional settings such as soils (Ludwigson et al., 1998; McMillan and Schwertmann, 1998; Ratering and Schnell, 2000; Rakshit et al., 2008; Driese et al., 2010), bogs (Postma, 1981) and lakes (Emerson, 1976; Bahrig, 1989; Sapota et al., 2006; Schettler et al., 2007). They can also be found in marine sediments, in which O2 and SO4 diffusion from seawater is limited by the sediments low permeability, leaving iron reduction and methanogenesis as the main mechanisms for organic matter degradation (Matsumoto, 1989; Chow et al. 2000; Pierre et al., 2000). In ancient Fe-rich sedimentary deposits such as Banded Iron Formations, siderite can be a major phase and is usually interpreted as resulting from organic matter degradation by iron reduction in sediments overlain by sulfate-poor and ferruginous water column (James, 1954; Perry and Tan, 1972; Hangari et al., 1980; Maynard, 1982; Thyne and Gwinn, 1994; Fisher et al., 1998; Busigny et al., 2013).

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Carbon and oxygen isotope compositions of sedimentary siderite can potentially be used to decipher its conditions of formation, and to test whether it involved biotic or abiotic processes. The carbon isotope composition of siderite records the signature of dissolved inorganic carbon from which siderite precipitates, similarly to other carbonates (Zhang et al., 2001). It therefore allows identification of the major biogeochemical zone in which siderite has precipitated and the involvement of specific metabolisms (anaerobic respiration or methanogenesis) in the establishment of favorable conditions for its formation (Irwin et al., 1977; Curtis et al., 1986; Moore et al., 1992; Mozley and Burns, 1993; Pierre et al., 2000). The O isotope composition of siderite primarily depends on the precipitation temperature and the porewater δ18O value (Zhang et al., 2001), thus providing information on the diageneric temperature and the origin of diageneric fluids (Mozley and Burns, 1993; Wilkinson et al., 2000). However, microbially-induced precipitation of siderite may significantly modify O isotope fractionation compared to abiotic processes (e.g. Mortimer and Coleman, 1997), complicating the use of δ18O as a tracer of diageneric temperature and fluid. Studies of siderite formation in modern systems, for which temperature, porewater δ18O and microbial activity can be characterized, are thus required to unambiguously constrain the processes controlling siderite δ18O. However, before performing such studies, it is necessary to develop a robust method for measuring C and O isotope compositions of siderite present in minor amounts in sediments rich in immature organic matter. While several authors focused on the analysis of organic C isotope composition in carbonate-rich samples (France-Lanord and Derry, 1994, 1997; Galy et al., 2007; Brodie et al., 2011a,b), the C and O isotope analyses of carbonates in organic-rich matrix remains poorly constrained, particularly for siderite. Yet, the presence of organic matter associated with carbonates has long been suspected to offset the measurement of C and O isotope compositions of calcite and aragonite, so that various treatments aiming at removing organic matter have been tested (e.g. Wierzbowsky, 2007; Fallet et al., 2009). The interference of organic matter on carbonate analysis is generally interpreted as due to CO2 and/or other molecular gaseous species production during the reaction of organic matter with phosphoric acid (H3PO4) used for carbonate dissolution (Epstein et al., 1951, 1953; Bowen, 1966; Weber et al., 1976) and incomplete separation of these gases during vacuum purification. To our knowledge, no treatments have been established for non-traditional carbonates like siderite (FeCO3) or magnetite (MgCO3). Yet, the effects associated with the presence of organic matter in these samples are expected to be even more important because of the high reaction temperature required for siderite dissolution using H3PO4 (~130 °C; Rosenbaun and Sheppard, 1986). Moreover most of the treatments have been tested for off-line CO2 extraction and purification in a vacuum line while most laboratories now use on-line methods with a gas chromatographic column separation, which may be more efficient to separate CO2 from other contaminant gaseous species. In this paper we fill this gap for siderite analysis using a GC–IRMS technique, separating contaminant gases from CO2 before C and O isotope measurements in the mass spectrometer.

We tested several procedures to remove organic matter from a synthetic siderite–organic mixture before siderite analysis. Synthetic samples were prepared by mixing pure siderite with various proportions of yeast, a living microorganism taken here as a model for the most immature organic material. We expected high reactivity to H3PO4 digestion at 130 °C, with potentially extreme C and O isotope effects, thus placing an upper limit for C and O isotope offsets expected in natural samples. Indeed, carbon and oxygen isotope analyses of the siderite using H3PO4 digestion (McCrea, 1950) and GC–IRMS isotope analyses (Assayag et al., 2006) are shown to be affected by the presence of yeast in the sample.

In order to improve siderite C and O isotope analysis, three organic matter oxidation treatments were tested: two involved wet chemistry (using NaOCl or H2O2) and one low-temperature oxygen-plasma ashing (Goreau, 1977). Roasting in vacuum or helium at 300 to 400 °C, a commonly used treatment to remove organic matter from carbonates (e.g., Epstein et al., 1951, 1953; Wierzbowski, 2007), was not considered here because siderite destabilization starts at ~300 °C (Froelich, 1980).

2. Experimental technique

2.1. Preparation of the experimental material

In the present work, pure yeast (Saccharomices cerevisiae) was selected to represent an organic matter because it is highly immature and probably most reactive to H3PO4. Additionally, yeast presents two main advantages: (1) it can be easily purchased and is inexpensive, and (2) it can be well preserved under dried and cooled conditions without any evolution of its C isotope composition. The yeast was purchased at “L’atelier de la patisserie” (labeled LEVURE-SECHE) in 500 g box as dry balls of 1 mm of diameter. It was ground, sieved to fraction of ~150 μm and homogenized.

After examination under binocular microscope, Raman spectrometry and electron microprobe, siderite from the deposit of La Mûre (France) was selected for our experiments. This siderite contains mostly Fe (90.0 ± 2.0%), with minor amount of Mg (5.0 ± 1.0%), Mn (2.4 ± 0.3%) and Ca (1.1 ± 0.2%) and does not show evidence of alteration to Fe-oxides. Siderite crystals were crushed to small fragments (~500 μm), before being carefully selected under a binocular microscope. Suspicious “colored” fragments were discarded to avoid any potential contamination with other minerals (e.g. traces of alteration, Fe oxides, or mineral inclusions). The pure fraction was then ground to ~100 μm using an agate mortar and homogenized. All grains smaller than 100 μm were used, including very fine-grained material. This siderite powder was labeled SID.

Finally, nine synthetic samples were prepared with variable proportions of yeast and siderite ranging from 0 to 100 wt.% yeast (see Table 1), and were homogenized by shaking.

2.2. Methods for organic matter removal

For the chemical treatments, about 150 mg of the nine synthetic samples were weighed and introduced into clean glass beakers. A large excess (30 to 40 ml) of oxidizing solution, either NaOCl 3.5% or H2O2 30%, was added to the powders and left at room temperature (~22 °C) during 3 days with regular shaking of the solution (adapted from Wierzbowski, 2007). The powders were then rinsed several times (n = 6) with deionized water and dried in an oven at 50 °C during 3 days.

The third method, tested in the present work, was low-temperature oxygen-plasma ashing in a POLARON PT7160 RF system. For the experiment, 35 to 320 mg of the samples were weighted and loaded in a petri dish. The petri dish was covered with riddled alumina foil and introduced in the vacuum chamber of the plasma ashing system. After evacuation of the air contained in the chamber, oxygen was injected and an electric discharge lit the plasma. The oxygen plasma is made of free radicals, which oxidize the organic matter to CO2. The reaction time was comprised between 300 and 1860 min depending on the amount of yeast in the sample.

2.3. Carbon and oxygen isotope analyses

Carbon and oxygen isotope compositions of our pure siderite and synthetic mixture samples were determined using a modified McCrea method (McCrea, 1950) and a He continuous flow mass spectrometer (AP2003, Analytical Precision, UK). The procedure can be described as follow. Two to 50 mg of powders was loaded in exetainer tubes. The air contained in the tubes was flushed with a He flow at 2.5 bar via a Gilson autosampler and 400 µl of phosphoric acid were injected manually through the septa with a 10 ml syringe. The temperature used for siderite reaction with phosphoric acid (H3PO4) was determined from...
than 130 °C, the proportion of CO2 released from the sample is negligi-

δ 13C values for siderite.

Table 1

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<th>% siderite</th>
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<th>Δ 13C (‰)</th>
<th>δ 18O (‰)</th>
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nm: not measured.

a Proportion of mass lost by the various chemical treatments used for yeast removal (i.e. NaOCl, H2O2 and oxygen-plasma).
b Time of sample treatment with low-T oxygen plasma ashing.

results of a previous study (Rosenbaum and Sheppard, 1986) combined
with those from a step-heating experiment performed in the present
work. For the step-heating experiment, 16.7 mg of powder of the sider-

tite “SID” were introduced into a Pyrex reaction vessel together with
10 ml of H3PO4. The reaction vessel was placed on a vacuum line and
evacuated to <6 × 10−4 mbar. The sample was then isolated from the
vacuum and heated progressively, in 10 °C temperature increments,
from 100 °C to a maximum of 140 °C. For each step, the duration was
120 min and the extracted CO2 was purified cryogenically and quanti-
tied manometrically on the vacuum line. The results of the step-

heating experiment are shown in Fig. 1. It clearly illustrates that siderite
dissolution and associated CO2 release pattern start at a temperature as
low as 100 °C, and are significant up to 130 °C. For temperatures higher
than 130 °C, the proportion of CO2 released from the sample is negligi-

ble. A one-step siderite digestion with H3PO4 at 130 °C during 2 h also
provides a full extraction of the CO2. The samples analyzed here were
thus reacted with H3PO4 at 130 °C during 2 h while “in-house” labora-
tory calcite (CaCO3) standards were reacted at 25 °C during 4 h. These
treatments resulted in siderite and calcite dissolution associated with
CO2 release in the headspace of the exetainer tubes. The CO2 was

Fig. 1. Results of the step-heating experiment, illustrating the CO2 release pattern for a sin-
gle siderite powder (SID) reacted with phosphoric acid. Total mass of the sample was
16.7 mg.
purified by chromatography and analyzed online on He continuous flow mass spectrometer as described in Assayag et al. (2006). The isotopic data are reported in the conventional δ unit, in permil, and referred to the PDB standard. For each sample, the δ¹³C and δ¹⁸O values reported herein correspond to the mean value of eight replicate measurements (four replicates on a single extainer tube, and two tubes per samples). Potential effects of linearity on the sample analysis were determined and corrected for, using the δ¹³C and δ¹⁸O results obtained on one of our “in-house” standards weighted at different masses. Afterward, three isotopically different calcite standards with known C and O isotope compositions were used for calibration (see details in Assayag et al., 2006), allowing the determination of the true isotopic values of the samples. The δ¹⁸O value of siderite was corrected for the O isotope fractionation induced by the reaction with H₃PO₄ (Sharp, 2006). The fractionation factor α depends on the reaction temperature and the type of carbonate involved in the reaction. The δ¹⁸O value of our siderite samples were calculated using the temperature-dependent fractionation factors calibrated by Rosenbaum and Sheppard (1986) for siderite at 130 °C and by Das Sharma et al. (2002) for calcite standards at 25 °C. Analytical precisions on δ¹³C and δ¹⁸O values were estimated from multiple measurements on calcite standards, providing an external reproducibility better than 0.11 and 0.15‰ (2σ) for C and O respectively. Siderite content in samples was estimated from the ion intensity of the CO₂ peak in the mass spectrometer with a precision of ±10% (2σ).

The yeast analysis was performed by classic Dumas combustion (for a detailed description of the method, see Adler et al., 1998). Yeast was loaded with copper oxide (CuO) in quartz tubes sealed under vacuum. It was transferred to CO₂ by combustion at 950 °C during 6 h. The CO₂ was cryogenically purified from other gases on a vacuum line and analyzed on a Delta + XP mass spectrometer in Dual Inlet mode. Carbon isotope composition of yeast was expressed using the δ¹³C notation relative to PDB standard.

3. Results and discussion

3.1. Isotope composition of untreated samples

The pure siderite SID yielded δ¹³C SID and δ¹⁸O SID values of −12.3 ± 0.1‰ and −16.2 ± 0.3‰ respectively (n = 8, 2σ). Carbon and oxygen isotope compositions of the CO₂ released from untreated synthetic samples are reported in Table 1. To assess the effect of yeast on the determination of C and O isotope compositions of siderite, we defined Δ¹³C and Δ¹⁸O as the offsets between δ¹³C or δ¹⁸O values of pure siderite and those measured in the mixtures (i.e. Δ¹³C = δ¹³C untreated siderite − δ¹³C mixture and Δ¹⁸O = δ¹⁸O untreated siderite − δ¹⁸O mixture). For proportions of yeast equal to or lower than 10%, δ¹³C and δ¹⁸O values are similar to the expected value for pure siderite (Δ¹³C < 0.1‰ and Δ¹⁸O < 0.3‰). δ¹³C and δ¹⁸O values of the untreated samples with proportion of yeast higher than 10% are progressively shifted to more negative values (down to −21.8‰ for C and −19.6‰ for O) with increasing proportion of yeast (Table 1, Fig. 2A and B). The Δ¹³C and the Δ¹⁸O increase from 0.4 to 9.5‰ and from 0.3 to 3.4‰ respectively, with increasing yeast proportion in the sample (25 to 95%, Table 1). It is interesting to note that pure yeast reacted with H₃PO₄ at 130 °C and produced 0.68 µmol/mg of CO₂, with δ¹³C and δ¹⁸O values at −27.3‰ and −22.0‰ respectively. The analysis of pure yeast by Dumas combustion shows a C isotope composition of −26.6 ± 0.05‰ (n = 3, 1σ) close to the value measured with H₃PO₄ extraction. We conclude that the shifts in δ¹³C and δ¹⁸O values, observed for untreated samples, result from the mixing of CO₂ produced by both siderite and yeast. These data obtained on untreated samples (pure yeast and mixtures) illustrate that the determination of C and O isotope compositions of siderite requires organic matter removal prior to H₃PO₄ acidification and CO₂ isotope analysis, particularly for weight ratios of yeast/siderite higher than 10 wt.% (i.e. corresponding to ∼5 wt.% of total organic carbon, TOC). In the present experiments,

![Fig. 2. Carbon (A) and oxygen (B) isotope compositions of the synthetic samples (made of yeast and siderite) as a function of the weight proportion of yeast (%) in the mixture. Error bars are smaller than dots size. White squares correspond to untreated samples. Dark-gray triangles, light-gray diamonds and black circles represent the samples treated with NaOCl, H₂O₂ and oxygen plasma ashing respectively.](image-url)
siderite used in the synthetic mixtures has a negative $\delta^{13}C$ value (−12.3‰), which is only 15% higher than that of yeast (−27.3‰). However, if the $\delta^{13}C$ value of siderite was markedly different from that of the organic matter (for instance, if siderite precipitated from CO₂ produced by methanogenesis with a positive $\delta^{13}C$ value, such as +10‰), the shift in $\delta^{13}C$ due to mixing effect would be significant even for TOC < 5 wt.% and a systematic removal of organic matter would be crucial for accurate measurement. In pioneer studies focused on oxygen and carbon isotope composition of calcite digested at 25 °C, the authors observed an enrichment in heavy isotopes of the carbonate when organic matter was present in the sample (Epstein et al., 1951, 1953; Weber et al., 1976). This finding is opposite to the results of the present work on siderite, for which the $\delta^{13}C$ and $\delta^{18}O$ are shifted toward more negative values (corresponding to enrichments in light C and O isotopes).

The major differences between our study and these previous works are the $H_3PO_4$ digestion temperatures (25 °C versus 130 °C) and the CO₂ purification methods (cryogenic versus gas chromatography). The more positive $\delta^{18}O$ and $\delta^{13}C$ values in pioneering work may have resulted from gaseous contamination (efficiently removed in our study by gas chromatography), while the more negative $\delta^{18}O$ and $\delta^{13}C$ evidenced in the present work would be due to CO₂ released by organic matter during higher temperature $H_3PO_4$ digestion. An alternative hypothesis could be that CO₂ is produced by organic matter at all temperatures but with a temperature dependent isotope fractionation, with strong enrichments in heavy isotope at low temperature and a limited one at high temperatures.

3.2. Aqueous oxidation of organic matter

3.2.1. Chemical treatment with NaOCl

The efficiency of yeast removal using NaOCl can be estimated by plotting the mass loss (after treatment) as a function of the initial yeast proportion in the mixture (Fig. 3). For a proportion of yeast higher than 75 wt.% in the sample, the mass loss after NaOCl treatment is lower than the mass of yeast initially introduced in the sample (Fig. 3), indicating that yeast was not completely removed. However, pure yeast (156 mg) was completely oxidized by reaction with NaOCl and did not leave any residual powder for further estimation of its C content and isotope composition. Pure siderite powder reacted with NaOCl and was partly dissolved, as shown by a production of bubbles in the solution, likely corresponding to CO₂ release. The dissolution of siderite is also illustrated in Fig. 3, for proportions of yeast lower than 75 wt.%, where the mass loss after NaOCl treatment is higher than the mass of yeast initially introduced in the sample. It probably reflects partial oxidation of Fe²⁺ (initially present as FeCO₃) to Fe³⁺. Since siderite is not stable under such oxidizing conditions, partial dissolution is expected for all of the samples treated with NaOCl aqueous solution.

The effects of NaOCl treatment on siderite C and O isotope compositions are illustrated in Table 1 and Fig. 2. Table 1 shows the offsets between pure untreated siderite and measured $\delta^{13}C$ and $\delta^{18}O$ values of the samples expressed as $\Delta^{13}C$ and $\Delta^{18}O$ respectively. In all but one sample, $\Delta^{13}C$ and $\Delta^{18}O$ values are lower than or equal to the uncertainty of the measurement, indicating that $\delta^{13}C$ and $\delta^{18}O$ of the siderite after chemical treatment are not significantly different from the expected values, and that organic matter removal was efficient. The sample containing 95 wt.% yeast was the only one presenting $\delta^{13}C$ and $\delta^{18}O$ significantly different from the expected values, with offsets $\Delta^{13}C$ of 4.1‰ and $\Delta^{18}O$ of 0.7‰.

3.2.2. Chemical treatment with H₂O₂

Results of the chemical treatment with H₂O₂ are presented in Fig. 3 and Table 1. Similar to NaOCl, the addition of H₂O₂ solution to the samples did not remove all of the yeast, particularly for samples with more than 75 wt.% of yeast (Fig. 3). Pure yeast was not completely dissolved and its residue was then analyzed by mass spectrometry in order to determine if it was still reactive to $H_3PO_4$ treatment. Pure siderite powder suffered partial dissolution, evidenced by the formation of CO₂ bubbles. Sample mixtures also display siderite dissolution as illustrated by the mass loss higher than the mass of yeast for samples containing less than 75 wt.% of yeast. These two effects (i.e. incomplete yeast removal and partial siderite dissolution) likely influence all samples but their impacts on bulk sample analyses are strongly dependent on the proportions of yeast and siderite in the mixtures. For instance, siderite loss will be more easily demonstrated in mixtures with low yeast proportions (high siderite proportion) while incomplete yeast removal will be better evidenced in high yeast content mixtures.

Samples treated with H₂O₂ show $\delta^{13}C$ and $\delta^{18}O$ values ranging from −12.1 to −15.4‰ and −16.0 to −16.6‰ respectively (Table 1, Fig. 2), the lowest values corresponding to the greatest amount of yeast. For proportions of yeast lower than or equal to 75%, the $\Delta^{13}C$ offset is low and close to the uncertainty on the measurement (±0.1‰), indicating that these samples treated with H₂O₂ have $\delta^{13}C$ values similar to those of pure siderite. In contrast, there is a considerable shift in the C isotope composition ($\Delta^{13}C$ up to 3.1‰) when the ratio yeast/siderite is high (90 to 95 wt.% of yeast) even after chemical treatment of the samples. All samples show a limited shift in O isotope composition ($\Delta^{18}O < 0.4‰$, Table 1), with $\delta^{18}O$ values close to those of pure untreated siderite. It is worth noting that the “apparent efficiency” of H₂O₂ treatment for O isotope measurement may results from a similarity between the CO₂ produced from residual yeast after H₂O₂ treatment and that of pure siderite (Table 1).

The residue of pure yeast recovered after H₂O₂ treatment was subsequently digested with $H_3PO_4$ and produced CO₂ (about 1.2 μmol/mg) with $\delta^{13}C$ and $\delta^{18}O$ values of −15.8 and −17.3‰ respectively (Table 1). This is significantly different from the CO₂ production (0.68 μmol/mg) and isotope values (−27.3 and −22.0‰) measured for untreated pure yeast. Unfortunately those values are very close to those expected for pure siderite ($\delta^{13}C_{sid}$ and $\delta^{18}O_{sid}$ of −12.3‰ and −16.2‰ respectively) so that any contribution of yeast-derived CO₂ will be difficult to detect in our experiments. The origin of the differences between $\delta^{13}C$ and $\delta^{18}O$ values of the CO₂ released by $H_3PO_4$ digestion of pure yeast and yeast treated with H₂O₂ is unclear but may result from preferential removal of light C and O isotopes during H₂O₂ oxidation, possibly due to a kinetic effect.

3.2.3. Synthesis of results from aqueous oxidation experiments

The two methods based on aqueous oxidation of yeast using either $H_2O_2$ or NaOCl improved the measurement of $\delta^{13}C$ value for proportions of yeast lower than 75 wt.% . However, the C isotope offset is still important for proportions of yeast higher than 75 wt.% . Oxygen isotope compositions measured after $H_2O_2$ treatments are close to the expected
values for siderite for all synthetic sample mixtures, whereas NaOCl induces significant O isotope shift for high proportion of yeast in the samples (i.e. 95 wt.%). The samples treated with H2O2 or NaOCl may be subject to three competing and, in some cases, opposite effects, which could alter the measured isotopic values: (1) kinetic isotope fractionation related to carbonate particle size (Fritz and Fontes, 1966), (2) isotopic interference by trace amounts of organic compounds on masses 45 and 46 (e.g., Epstein et al., 1951), and (3) partial organic matter removal and reaction of residual yeast to produce CO2.

For the first effect, it was shown that the δ18O value measured in calcite is the result of a kinetic isotope fractionation associated with H3PO4 digestion, and depends on the grain size of the sample powder (Fritz and Fontes, 1966). Our siderite standard is a powder ground to less than 100 μm, probably characterized by a distribution of particle size from coarse to very fine grains. Any aqueous pre-treatment with NaOCl or H2O2 may have removed some of the finest siderite particles, either by preferential dissolution or when the solid was separated from the treatment solution (after centrifugation). Yet, the identical δ13C and δ18O values measured in our pure siderite standard before and after NaOCl or H2O2 treatments (i.e. 100% siderite in Table 1) suggests that any kinetic effect related to particle size change (if any) is negligible.

The second effect that may have impacted the measured C and O isotope compositions is a contribution by trace amounts of organic compounds at masses m/z 45 and 46 (Epstein et al., 1951). Although this can be significant for classical analyses of C and O isotope composition on vacuum lines and cryogenic separation, our protocol based on chromatographic purification of CO2 is expected to avoid most interferences from other molecular compounds. Additionally, such a contribution in the mass spectrometer is thought to produce an increase of the measured δ13C and δ18O values (Epstein et al., 1951, 1953), which is opposite to the observed deviations (Table 1, Fig. 2). We thus conclude that the modifications of δ13C and δ18O values toward negative values, as observed for the samples containing more than 75% of yeast and treated with NaOCl or H2O2, most likely reflect incomplete removal of organic matter. This is also supported by the lower mass loss than expected if all the yeast was oxidized (Fig. 3). Finally, a major drawback of the two aqueous oxidation methods is that siderite content cannot be quantified because of partial siderite dissolution and incomplete removal of organic matter during the chemical treatments, which then contributes to the CO2 released by the samples.

3.3. Low temperature oxygen plasma ashing

3.3.1. Determination of the reaction conditions

Before treating the samples with low-temperature oxygen-plasma ashing, we assessed the best conditions of reaction needed for successful yeast removal. Various masses of pure yeast were reacted under variable oxygen flows and weighted at regular time intervals to monitor the oxidation rate of yeast. Fig. 4 displays the decrease of yeast mass as a function of time for various analytical conditions, considering high (~800 mg) and low (~100 mg) initial masses of pure yeast and oxygen flows of 0.4 and 0.6 ml/min. The decrease of initial mass lowers the combustion rate from 1.9 to 0.5 mg/min and from 2.4 to 0.5 mg/min for oxygen flow of 0.6 ml/min and 0.4 ml/min, respectively. Fig. 4 shows that the rate of yeast oxidation is more dependent on the initial mass introduced in the system than on the oxygen flow. However, the influence of the oxygen flow cannot be neglected. For high initial mass of sample (800 mg), the rate of yeast removal was more efficient with an oxygen flow of 0.4 ml/min. In the case of low initial sample mass (100 mg), the rates of yeast removal were similar for oxygen flows of 0.4 and 0.6 ml/min (Fig. 4B). By considering exclusively these results, an oxygen flow of 0.4 ml/min might be used. However, we observed that sample powders treated with an oxygen flow of 0.4 ml/min were covered with a dark coating, suggesting that organic matter was carbonized rather than combusted at low temperature. This color change was not observed in the experiment with an oxygen flow of 0.6 ml/min.

The experiments on “siderite–yeast” mixtures were thus conducted with an oxygen flow of 0.6 ml/min. The reaction was allowed to continue until the sample mass loss was similar to the mass of yeast initially present in the sample (see Table 1). The preservation of siderite under the conditions of low-temperature oxygen-plasma ashing was tested by loading 35 mg of pure siderite in the system during one of the maximum time of reaction used in this study (1140 min). The siderite was perfectly preserved during the reaction with no detectable mass loss and δ13C and δ18O values similar to the expected values within uncertainties (Table 1).

3.3.2. Results on synthetic sample mixtures

The treatment of the synthetic sample powders using low-temperature oxygen-plasma ashing was the best technique to preserve siderite content while removing most, if not all, of the yeast. Indeed, the proportion of mass loss during the oxidation reaction was similar to the proportion of yeast in the mixture for all samples (Fig. 3). Carbon and oxygen isotope compositions of samples treated with low-temperature oxygen plasma ashing ranges from −12.5 to −12.3% and −16.5 to −16.1% respectively (Table 1, Fig. 2). When considering all the samples, the isotopic offsets Δ13C and Δ18O are always lower than 0.2 and 0.3% respectively, indicating δ13C and δ18O values close to our pure siderite standard (SID) albeit with slightly larger offset values than the typical analytical uncertainties. Samples containing a proportion of yeast lower than or equal to 75 wt.% show even lower isotopic offsets with Δ13C and Δ18O maximum values of 0.1 and 0.2%, respectively, pointing to δ13C and δ18O values indistinguishable from those of pure siderite. Contrasting with the other techniques of yeast removal (i.e. chemical treatments with NaOCl and H2O2), low-temperature oxygen plasma ashing thus provides more accurate C and O isotope
measurements, particularly for high proportions of yeast in the mixture, such as 90 or 95 wt.% (Table 1, Fig. 2). Another advantage of the oxygen-plasma is the preservation of siderite, thus offering a possibility to estimate siderite content within a natural sample.

4. Conclusion

The present paper tests several methods for the analysis of C and O isotope compositions of siderite associated with yeast, considered as a model of highly-reactive organic material. We show that when yeast is present, the δ13C and δ18O values measured in siderite by H2PO4 acid digestion are shifted toward more negative values. The shift in δ13C and δ18O values are clearly related to the presence of CO2 released from yeast during H2PO4 digestion at 130 °C. The extent of CO2 contamination will obviously depend on the type of organic matter. Nonetheless the present study illustrates that organic matter most likely need to be removed from any sample prior siderite analysis.

The chemical oxidation methods based on NaOCl and H2O2 improves the measurement of δ13C and δ18O values for proportion of yeast lower than 75 wt.%. However, they do not remove efficiently organic matter for high organic matter/siderite ratio. Additionally, partial oxidation of siderite by NaOCl and H2O2 treatments for organic matter removal prevents any accurate assessment of siderite content. The low temperature oxygen plasma ashing is the best method for the preservation of siderite, with no observed oxidation during the treatment. The δ13C and δ18O values of the samples treated with this method are similar to the expected value for any proportion of yeast in the mixtures, although minor offsets are found for high organic matter/siderite ratio. It is likely that organic materials with different origin and diagenetic history will show a variable reactivity to H2PO4 digestion at high temperature (130 °C). Thus future studies of siderite in any type of organic-rich natural samples will require some tests prior to isotopic analysis.

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