






RESEARCH ARTICLE

# Biogenic carbonate samples: Preliminary tests on chemical protocols for radiocarbon analysis

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## Abstract

Most of the carbonate samples have a basic well-defined pretreatment protocol for <sup>14</sup>C-AMS dating, but particularities of specific organisms have to be treated with care. This is the case of stromatolite samples, in which carbonate is formed by biogenesis and also has a porous structure that could contain recent organic material as a contaminant. In this work, we analyzed the differences in the radiocarbon content by using organic matter removals before chemical treatment with HCl: sodium hypochlorite (NaOCl) a 0.7M solution with pH ~11, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) an 8.8M solution with pH ~5. These treatments were chosen because they are the most used in stromatolite samples for geochemical analysis. To compare the impact of the organic matter removal treatments in stromatolite samples we also processed them as regular carbonate samples for radiocarbon analysis, with no organic matter removal (control samples). X-ray diffraction and X-ray fluorescence have been used to obtain mineral and elemental characterization, respectively. H<sub>2</sub>O<sub>2</sub> could not influence the results of Mg-calcite concentrate samples. The use of NaOCl appears to have been effective in preserving more material than H<sub>2</sub>O<sub>2</sub> independent of the mineralogical composition of the stromatolite layers. The F<sup>14</sup>C results after HCl etching for Mg-calcite concentrated samples were similar to those without etching suggesting that the HCl etching does not impact the results in this case. The organic matter removal is more important than the etching procedure for stromatolite samples. NaOCl is more indicated to be used as chemical pretreatment for radiocarbon analysis purposes independent of the mineral matrix of samples.

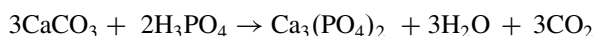
## Introduction

Stromatolites are biologically induced organomineral layered sedimentary formations and their metabolic activities induce conditions for precipitation (Dupraz et al. 2009). Its structure can host diverse microbial communities (Baumgartner et al. 2009), including bacteria, algae, and archaea (Lepot et al. 2008). Some bacteria within stromatolites may perform processes that can affect radiocarbon analyses (Barker and Fritz 1981). For example, methane-producing bacteria can introduce carbon from different sources, while sulfate-reducing bacteria can influence carbonate precipitation (Andres et al. 2006; Visscher et al. 2000). These microbial processes are intimately linked with mineral formation (Reid et al. 2003) but are also influenced by interconnected local factors such as light, pH, and temperature (Bowlin et al. 2012). Therefore, understanding the microbial community and their metabolic activities provides insights into mineralogical properties and potential variations in <sup>14</sup>C content.

The reservoir effect on stromatolites is particularly important in calcareous regions rich in ancient carbonates. This effect is well-documented such as in Carreira et al. (2008), where regions high in limestone are largely devoid of radiocarbon due to prolonged isolation from the atmospheric carbon cycle, hence, adding carbon with minimal  $^{14}\text{C}$  into surrounding waters. Stromatolites may assimilate this carbon during their growth, causing an overestimated age during radiocarbon dating due to the inclusion of older carbon sources that lack radiocarbon (Brook et al. 2013; Jull et al. 2013). As seen, organisms residing in areas with low carbonate influence are not subject to this effect (Macario et al. 2016).

The study of the formation of these structures is very important because it is believed that stromatolites have dominated 80% of all geological formation on planet Earth and are the evidence of the earliest life found, dating to around 3.5 billion years (Grotzinger and Knoll 1999; Hofmann 1969, 1973; Riding and Awramik 2000; Vologdin 1962; Walter 1976). Several paleoclimate reconstruction studies have been performed using the geochemistry proxies stored within the carbonate matrix over the last years (Bahniuk 2013; Birgel et al. 2015; Carvalho et al. 2017; Iespa et al. 2012; Silva e Silva and Senra 2000; Vasconcelos and McKenzie 1997; Vasconcelos et al. 2006). Radiocarbon dating is an important tool for a better understanding of these records. However, stromatolite has no well-defined pretreatment for being dated by the  $^{14}\text{C}$ -AMS technique.

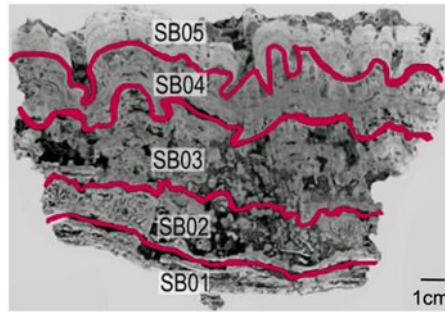
In general, the  $^{14}\text{C}$ -AMS dating protocol for carbonate samples is based on a simple removal of the outer layer with a sandblaster or a chemical etching pretreatment using hydrochloric acid (HCl), to remove possible external contamination. Then, hydrolysis is performed by using orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ), to convert the carbonate into carbon dioxide, by the following equation (Burman et al. 2005):



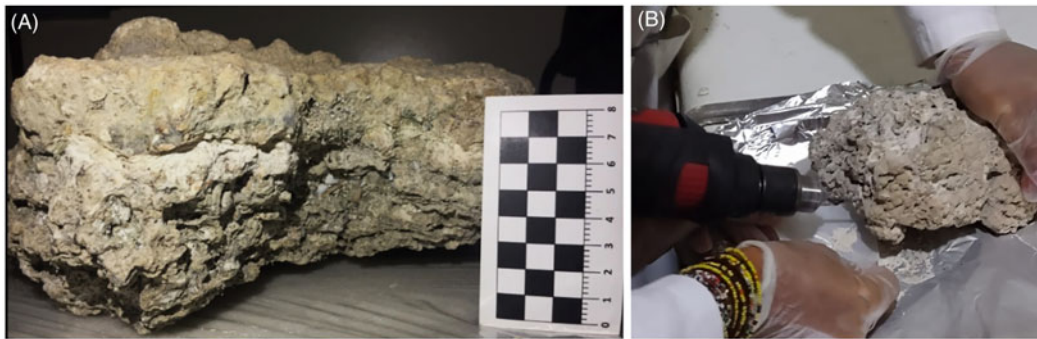
This acid does not convert organic matter to carbon dioxide, but some authors affirm that through this reaction it is possible to produce this gas and/or other molecular gaseous species (Bowen 1966, 1991; Epstein et al. 1951, 1953; Falster et al. 2018; Oehlerich et al. 2013; Weber et al. 1976). Under certain conditions,  $\text{CO}_2$  can be produced from organic matter in the acid environment if the organo-mineral interaction bonds are broken and the organic matter is more accessible to hydrolysis reactions which is not the case because  $\text{H}_3\text{PO}_4$  is a weak acid that is widely used to obtain carbon dioxide from carbonate samples for radiocarbon purposes (Wacker et al. 2013a, 2013b). Concerning stromatolites structure and composition it is necessary to consider the question: what is the impact of performing or not performing a chemical pretreatment to remove organic matter from the samples? Chaduteau et al. (2021) realized a similar test in  $\delta^{13}\text{C}$  analysis of carbonate samples and proved that it makes a difference in the results. Key et al. (2020) also assess the impact of the most used pretreatment methods of organic matter removal on  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  ratios for carbonate samples from taxonomic groups with complex mineralogies. The main purpose of this work is to attempt to answer this question by presenting  $\text{F}^{14}\text{C}$  results for  $^{14}\text{C}$ -AMS analysis, testing the extraction of organic matter and acid etching on subfacies of a stromatolite specimen which can have different mineral compositions in its growth layers.

## Materials and methods

The stromatolite specimen used in this work was collected in the Salgada Lagoon ( $21^\circ54'10''\text{S}$  e  $41^\circ00'30''\text{W}$ ) in January 2016. This lagoon is located in the coastal area of Rio de Janeiro State, Brazil, being part of the Paraíba do Sul deltaic river complex (Lamego 1955). This is one of the few hypersaline lagoons that still have stromatolite formation nowadays. Initially, X-ray microtomography (micro-CT) was performed on the raw material to identify five stromatolite subfacies the analysis was performed at the Nuclear Instrumentation Laboratory at Rio de Janeiro State University (LIN-COPPE/UFRJ). This is a non-destructive technique that allows the inspection of the internal structure of the sample and the Phoenix Vtomex|m GE micro-CT system was used. Reconstruction of the sample's volume was possible through software datos x (Version 2.5.0) and CTAn software (Version 1.18.4.0) was used to



**Figure 1.** Stromatolite Subfacies limits from the bottom (SB01) to the top (SB05) obtained through micro-CT analysis.

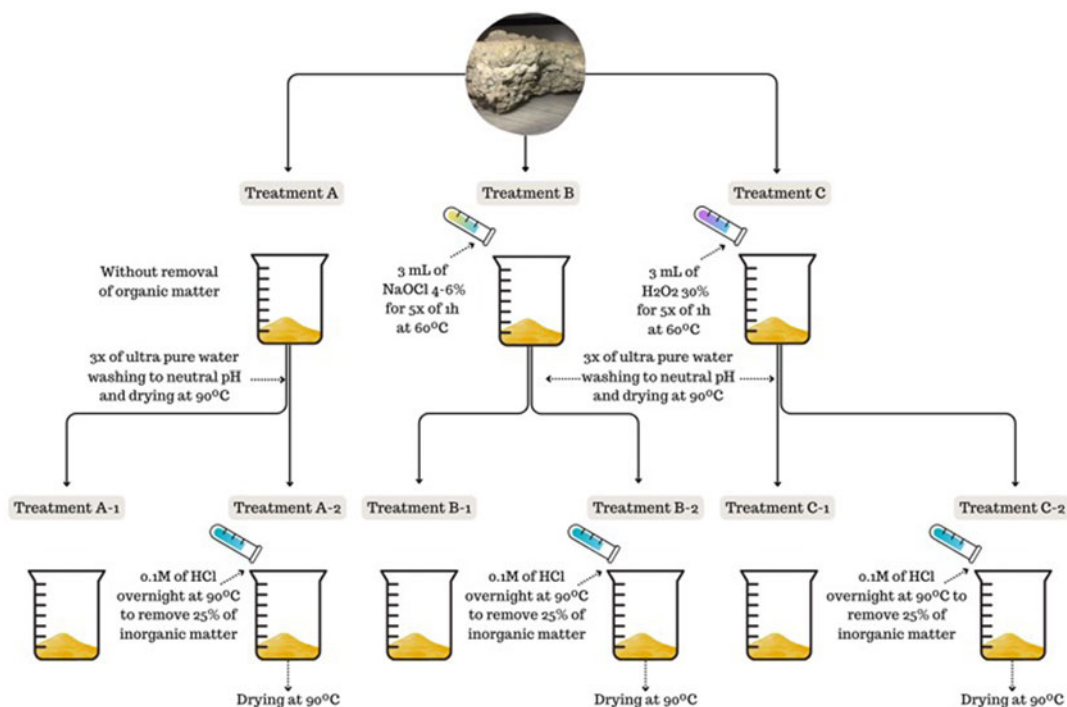


**Figure 2.** (A) stromatolite sample of Salgada Lagoon; (B) sample extraction.

perform the image segmentation and quantitative and qualitative analyses. The segmentation allowed the division of the image, which was in different gray levels, into just black and white. The micro-CT examination of the raw material allowed the detailed identification of five subfacies as shown in Figure 1. SB01 layer is the oldest one, which corresponds to the lagoon floor, and SB05 is the most recent, on the top of the stromatolite. The subfacies structure showed different degrees of porosity, as follows: SB01=18.98%, SB02=40.73%, SB03=35.74%, SB04=11.85%, and SB05= 8.99%.

The elemental and mineral composition were analyzed by X-ray fluorescence (XRF) and X-ray diffraction (XRD), respectively, before starting treatments to verify whether any differences could impact the radiocarbon results. For  $^{14}\text{C}$  pretreatment, the three intermediary samples were submitted to different organic matter treatments: hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), sodium hypochlorite ( $\text{NaOCl}$ ), and control, without organic matter removal. The latter is a common carbonate protocol for radiocarbon dating. Then, the samples were subdivided into two groups: with and without etching using hydrochloric acid ( $\text{HCl}$ ) and proceeded to the acid hydrolysis and graphitization protocol for  $^{14}\text{C}$ -AMS dating at the Radiocarbon Laboratory of the Fluminense Federal University (LAC-UFF). From the results obtained in the present work, we expect to better understand the stromatolite sample handling to improve the protocols for radiocarbon dating of biogenic carbonate samples by eliminating possible organic contamination. The stromatolite analyzed in this work was 10 cm high and 30 cm in diameter (Figure 2A).

Five subsamples were extracted using a 7 mm wall drill (Figure 2B) and crushed in an agate mortar to be homogenized at LAC-UFF to perform XRF and XRD composition analysis.



**Figure 3.** Steps of the treatment tests performed.

XRF analysis was used to determine the elemental composition of subsamples. The sample preparation and analysis were made at Laboratório de Oceanografia Operacional e Paleoceanografia at Fluminense Federal University (LOOP-UFF). A benchtop Epsilon 3X energy-dispersive X-ray fluorescence spectrophotometer (EDXRF) from PANalytical was used. It has an X-ray tube with a silver anode and a 50  $\mu\text{m}$  beryllium window, a power of 9 W, a current of 1 mA, and a voltage of 50 kV, using air and helium as carrier gas. The detector is a high-resolution Silicon Drift detector, typical of 135 eV, and a thin window of 8  $\mu\text{m}$  (Be).

XRD analysis was used to investigate the crystalline structure of the powdered samples. The sample preparation and analysis were undertaken at the Geochemistry Department at Fluminense Federal University. A BrukerD2 Phaser (Cu K $\alpha$  radiation) model was operated in a Bragg–Brentano  $\theta/\theta$  configuration, with the diffraction patterns being collected in a flat geometry with a range between 3 and 100 degrees, steps of 0.02 degrees, and accumulation time of 3.0 s per step. Phase identification was done with EVA<sup>®</sup> software. The XRD data were refined following the Rietveld method using the DIFFRAC.SUITE TOPAS<sup>®</sup> software (McCusker et al. 1999; Toby 2006).

Afterwards, the three intermediary subsamples were selected for the organic matter removal pretreatment test for <sup>14</sup>C dating. For the test, all samples were prepared and analyzed at LAC-UFF. Each subsample was subdivided to perform the organic matter removal pretreatment test as follows: [T-A]: Samples without organic matter removal; [T-B]: organic matter removal using a solution of 0.7 M of sodium hypochlorite that has pH  $\sim$ 11 (NaOCl, Isofar, Brazil, 4–6% purum p.a., CAS 7722-84-1, Lot. n. 245/2015), and [T-C]: a solution of 8.8 M of hydrogen peroxide that has pH  $\sim$ 5 (H<sub>2</sub>O<sub>2</sub>, Isofar, Brazil, 30% purum p.a., CAS 7681-52-9). NaOCl and H<sub>2</sub>O<sub>2</sub> were selected because they are powerful oxidation agents and are the most used in organic matter treatments for stromatolite samples in geochemical analysis. A treatment diagram is presented in Figure 3. The treatment T-A-1 can be referred to as a “control” treatment not in the classical sense in terms of expected values. It can be referred to as a

**Table 1.** XRF analysis results from stromatolite samples

Sample	Ca (%)	Mg (%)	Cl (%)	Fe (%)	Mn (%)
SB05	68.0	13.8	1.4	1.3	0.2
SB05-SB04	84.3	8.4	0.4	0.6	0.2
SB04-SB03	76.3	10.7	1.1	1.3	0.1
SB03-SB02	80.7	10.9	0.7	1.4	0.1
SB02-SB01	75.0	7.9	1.4	3.7	0.1
SB01	76.9	11.6	1.2	2.1	0.1

control treatment compared to the others since the sample was untreated. The T-A-2 treatment consists of etching the sample without organic matter removal. For both organic matter removal treatments, 3 mL of NaOCl or H<sub>2</sub>O<sub>2</sub> were added to approximately 100 mg of dry sample and left to react for 1 h at 60 °C in the dry bath. Each process was repeated 5 times and at the end, samples were washed 3 times with ultrapure water and dried at 90 °C to determine the mass loss percentage.

After the organic matter removal samples were split in two aliquots to proceed with the carbonate etching, as shown in Figure 3. An aliquot of the samples went straight to CO<sub>2</sub> conversion without etching addition (T-B-1 and T-C-1), and another aliquot, with approximately 40 mg, was chemically treated with 1.7 mL of 0.1M HCl and stayed overnight at 90 °C in the dry bath (T-A-2, T-B-2, and T-C-2) for a 25% etching, following the LAC-UFF etching standard protocol. At the end of the process, samples were dried at 90 °C and proceeded to CO<sub>2</sub> conversion.

The CO<sub>2</sub> was obtained by acid hydrolysis using the orthophosphoric acid (H<sub>3</sub>PO<sub>4</sub>, 85%) protocol. After CO<sub>2</sub> purification using temperature traps in the vacuum line, graphitization took place at 550 °C (7h) with TiH<sub>2</sub>, Zn and iron used as catalysts in an inner tube (Macario et al. 2017a, 2017b). Graphitized samples were measured in a NEC 250 kV single stage (SSAMS) system (Macario et al. 2013, 2015). Typical currents were 50 μA <sup>12</sup>C<sup>-1</sup> (measured at the low energy Faraday cup). Graphite standard and calcite blanks yielded average <sup>14</sup>C/<sup>13</sup>C ratios of 6×10<sup>-13</sup> and 7×10<sup>-13</sup>, respectively. The average machine background was around 50 kyr for the unprocessed graphite, while the average precision ranged from 0.3 to 0.5%. Data analyses were carried out on LACAMS software (Castro et al. 2015).

## Results and discussion

### XRF analysis

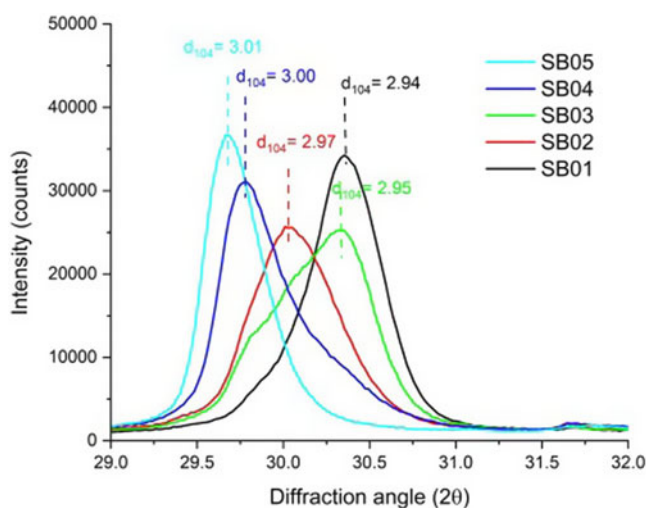
From XRF analysis the elemental composition of subsamples was determined showing a low concentration of Fe and Mn in the stromatolite chemical composition when compared to Mg concentration was observed in XRF analysis results (Table 1). For more detailed information and to obtain the mineralogical composition we conducted XRD analysis.

### XRD analysis

As shown by Moreira et al. (2020), fossils of marine organisms are likely to undergo dissolution and recrystallization. During this process, carbonate exchanges may occur, which leads to the entry of exogenous carbon, thus altering the radiocarbon concentration. The same could occur to stromatolite material during the formation of layers. Therefore, we carried out an XRD analysis to verify the crystalline composition of our samples. The results have shown that the subsamples presented are mainly calcite composition but with three types according to the d<sub>104</sub> typical peak. There is a mixture of calcite and Mg-calcite with different proportions. Table 2 presents d<sub>104</sub> (Å) results and MgCO<sub>3</sub> (%) concentrations, where SB05 and SB04 presented low MgCO<sub>3</sub> concentrations, 12% and 16%, respectively. SB02 presented 24% of MgCO<sub>3</sub> and SB03 and SB01 presented high and close

**Table 2.** *Stromatolite subfacies  $d_{104}$  (Å) results and  $MgCO_3$  (%) concentrations, where  $d_{104}$  (Å) higher than 2.99 are related to low  $MgCO_3$  (%) concentrations (SB04 and SB05) and  $d_{104}$  (Å) lower than 2.99 have high  $MgCO_3$  (%) concentrations (SB03, SB02 and SB01)*

Sample	$d_{104}$ (Å)	$MgCO_3$ (%)
SB05	3.01	12
SB04	3.00	16
SB03	2.95	42
SB02	2.97	24
SB01	2.94	41



**Figure 4.** *Comparison of diffractograms presenting the  $d_{104}$  calcite characteristic peak in Angstrom (Å).*

concentrations, 42% and 41%, respectively. It is important to mention that the balance between precipitation and dissolution of  $MgCO_3$  is a process that is influenced by the metabolic activity of the microbial community of the stromatolite during its growth, as well as by the physicochemical properties of the surrounding water, such as pH, temperature, saturation state, and the concentration of  $Mg^{2+}$  and carbonate ions in the environment. The concentration of  $MgCO_3$  in the growth layers can give information on the sedimentation environment in the past, but that is not the main goal of this work and will not be discussed in the present paper. Figure 4 shows the detailed  $d_{104}$  peaks from XRD analysis. Samples SB04 and SB05 have more calcite (the two blue peaks on the left of Figure 4). Otherwise, samples SB01, SB02, and SB03 are more Mg-calcite enriched. Samples SB01 and SB03 stand out as very Mg-enriched calcite samples (the black and green peaks on the right of Figure 4). The  $d_{104}$  values below 2.99 are typical Mg-enriched calcite samples. This appears to be related to a change in the crystallographic unit cell from the Mg-calcite to be like the one from the mineral kutnohorite ( $Ca(Mn, Fe, Mn)(CO_3)_2$ ) a rare mineral from the dolomite group and not present here (Graff 1961). Some authors also had a large shift in diffractogram peak (from 2.99Å to 2.94Å related to Mg-calcite) as a result of a very high-magnesian calcite (VHMC) or a disordered dolomite, as pointed out by Zhang et al. (2010).

As can be observed in Table 3, subfacie SB04 had the lowest concentration of Mg-calcite (36%) and the highest concentration of calcite (57%). The highest concentration of Mg-calcite and lowest concentration of calcite were found in SB01 85% and 5% respectively. SB02 and SB03 have similar mineralogical values, although SB03 has a higher Mg-calcite concentration. Carbonate precipitation and recrystallization in stromatolites can manifest in various mineral forms, such as calcite, aragonite, or

**Table 3.** Mineralogical pattern of Salgada lagoon stromatolite layers. ND: not detected; trace: value below 1%

Sample	Mg-Calcite [Ca(Mg)(CO <sub>3</sub> ) <sub>2</sub> ] (%)	Calcite [CaCO <sub>3</sub> ] (%)	Aragonite [CaCO <sub>3</sub> ] (%)	Halite [NaCl] (%)	Quartz [SiO <sub>2</sub> ] (%)
SB05	52	33	5	ND	8
SB04	36	57	3	Trace	3
SB03	69	17	9	Trace	5
SB02	64	13	12	Trace	11
SB01	85	5	4	Trace	6

dolomite, and are influenced by physicochemical, biological, and environmental conditions. The subfacie SB02, which presented 12% of aragonite in its composition, was probably subjected to different conditions during the layer growth. If occurs, the recrystallization in the same layer while studying stromatolite well-defined sequential growth layers seems to not impact the radiocarbon dating discussion.

### Chemical treatments

After the previous characterization, three inner layers were selected to move forward to the organic matter removal test: SB02 and SB03 which presented similar composition, and SB04 which presented lower Mg-calcite and higher calcite concentrations. The percentage of mass loss after the process is indicated in Table 4. Concerning weight loss during the organic matter removal, NaOCl bleaching presented similar results for all subfacies and a smaller weight loss than H<sub>2</sub>O<sub>2</sub>. This may be caused by the oxidizing strength of H<sub>2</sub>O<sub>2</sub> compared to NaOCl, which changes the inorganic matrix of the samples beyond the consumption of the organic matter. The same results were found by Chaduteau et al. (2021), in which H<sub>2</sub>O<sub>2</sub> altered the preservation of the carbonate, showing inorganic matrix dissolution, which did not occur while using NaOCl. The weight loss during the H<sub>2</sub>O<sub>2</sub> use suggests dissolution in all samples. As pointed out by Key et al. (2020), many researchers reported that H<sub>2</sub>O<sub>2</sub> is strongly corrosive to CaCO<sub>3</sub> and that H<sub>2</sub>O<sub>2</sub> at ~ 30% has a pH of ~5 which can promote the dissolution of carbonates. The highest weight loss (57%) was observed after the H<sub>2</sub>O<sub>2</sub> treatment on SB04 which presented the highest concentration of calcite (57%). Some other factors can increase the susceptibility of dissolution as the Mg content and the duration of the treatment but our study did not evidentiate this.

Following Reimer et al. (2004), radiocarbon results are reported by F<sup>14</sup>C values determined after the treatments and are shown in Table 5. It was not possible to determine the results of SB02 after T-C-1 due to probable loss during CO<sub>2</sub> purification in the vacuum line. As a result, we would expect a reduction in F<sup>14</sup>C values for all samples after the organic matter removal step since it should remove any recent carbon that could have been incorporated into the original sample matrix. Comparing the results observed for group 1 (organic matter removal without etching) it is possible to observe this behavior when comparing T-A-1 (Control) and T-B-1 (NaOCl) results for all layers. When comparing T-C-1 (H<sub>2</sub>O<sub>2</sub>) with the control T-A-1 group the layer SB03, which has more Mg-calcite, presented a reduction in F<sup>14</sup>C values. On the other hand, layer SB04 presented a higher F<sup>14</sup>C value.

The F<sup>14</sup>C results after HCl etching (group 2) for Mg-calcite concentrated samples (SB02 and SB03) were similar to those without etching. The CaCO<sub>3</sub> concentrated (SB04) presented F<sup>14</sup>C results higher than the control (T-A-2) in both combined tests: NaOCl + etching (T-B-2) and H<sub>2</sub>O<sub>2</sub> + etching (T-C-2).

In the graph (Figure 5) the results represent the F<sup>14</sup>C levels for each treatment [T-A, T-B, and T-C], grouped by subfacie. The black triangles represent results after treatments with no etching and the gray circles represent results after etching. The F<sup>14</sup>C results for SB02 and SB03 are quite similar with low

**Table 4.** Mass loss during organic matter chemical pretreatment, where [T-B] is the organic matter removal treatment using sodium hypochlorite (NaOCl) and [T-C] using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)

Sample	Treatment	Initial weight (mg)	Final weight (mg)	weight loss
SB04	NaOCl (T-B)	112	104	7 %
	H <sub>2</sub> O <sub>2</sub> (T-C)	119	51	57 %
SB03	NaOCl (T-B)	119	113	5 %
	H <sub>2</sub> O <sub>2</sub> (T-C)	121	83	32 %
SB02	NaOCl (T-B)	94	88	6 %
	H <sub>2</sub> O <sub>2</sub> (T-C)	106	68	36 %

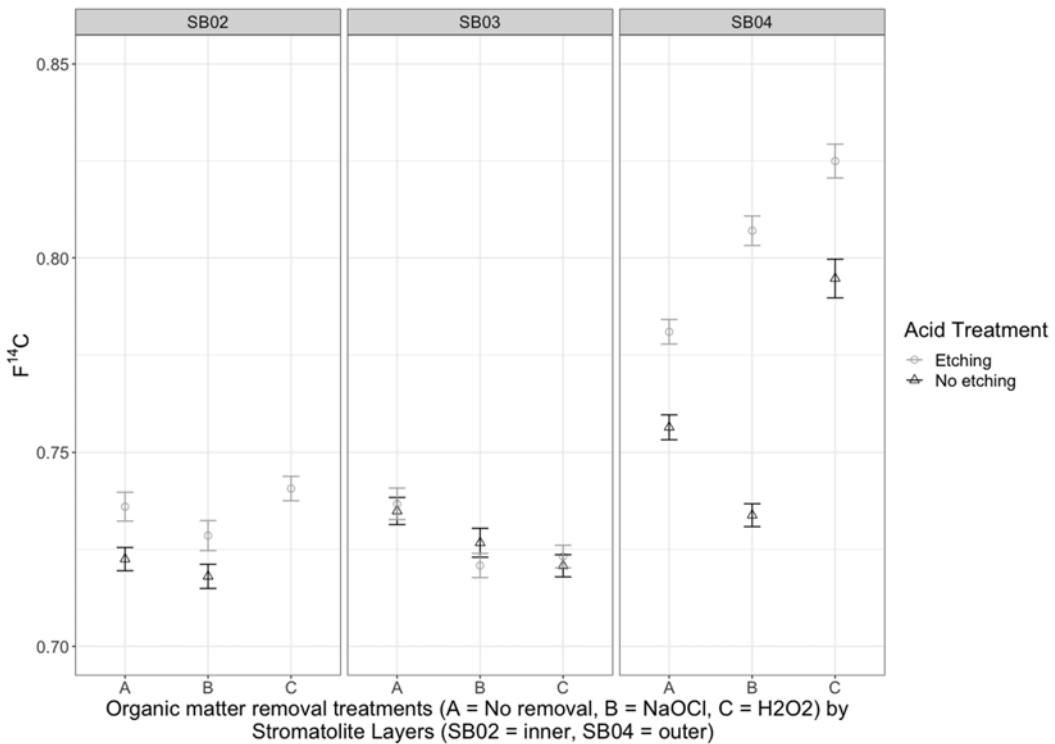
**Table 5.** Radiocarbon results (F<sup>14</sup>C) and its uncertainty after pretreatment tests. [T-A]: Control samples without organic matter removal; [T-B]: organic matter removal using sodium hypochlorite (NaOCl) and [T-C]: hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The numbers 1 and 2 represent results for samples without and with HCl etching, respectively

Sample/Treatment		Control (T-A) F <sup>14</sup> C	NaOCl (T-B) F <sup>14</sup> C	H <sub>2</sub> O <sub>2</sub> (T-C) F <sup>14</sup> C
SB04	1	0.756 ± 0.003	0.734 ± 0.003	0.795 ± 0.005
SB03		0.735 ± 0.003	0.727 ± 0.004	0.721 ± 0.003
SB02		0.723 ± 0.003	0.718 ± 0.003	—
SB04	2	0.781 ± 0.003	0.807 ± 0.004	0.825 ± 0.004
SB03		0.737 ± 0.004	0.721 ± 0.003	0.723 ± 0.003
SB02		0.736 ± 0.004	0.729 ± 0.004	0.741 ± 0.003

discrepancy. SB04 had a higher discrepancy, which seems to be an effect of its mineral composition containing higher calcite and low Mg-calcite. The results with etching (gray) for SB02 and SB04 are higher after all treatments, the same behavior is not observed for SB03 which has the highest Mg-calcite concentration.

Many works discussed the effects of previous treatments on stable isotope ratios for biogenic and inorganic carbonate samples to remove organic matter showing its dependence on the mineralogical matrices, duration of the treatment, and the importance of avoiding isotopic fractionation or isotopic exchange during the treatments (Key et al. 2020; Zhang et al. 2020). Since the selection of chemical pretreatment to be used depends on the mineral composition, wrong choices can also affect radiocarbon results, especially for stromatolite samples that could have a complex mineralogy. Carvalho et al. (2017) dated Mg-calcite-rich stromatolite samples by applying H<sub>2</sub>O<sub>2</sub>, which was the most used treatment for this kind of sample, and afterward followed the radiocarbon carbonate etching protocol. From the present study, it is possible to say that it was a good choice to apply the H<sub>2</sub>O<sub>2</sub> protocol to Mg-calcite-rich samples as it doesn't seem to impact results in the case of high Mg-calcite composition. Additionally, comparing the present work with Chaduteau et al. (2021) who also observed shifted δ<sup>13</sup>C values for calcite-rich samples after being treated with H<sub>2</sub>O<sub>2</sub>, showing it is less efficient for removing organic matter than NaOCl, it's possible to say that we have observed the same for SB04 subfacie in the present work. Furthermore, H<sub>2</sub>O<sub>2</sub> pretreatment can make the remaining organic matter more reactive to H<sub>3</sub>PO<sub>4</sub>, which is used to convert carbonate into CO<sub>2</sub> for radiocarbon analysis and could influence F<sup>14</sup>C results. On the other hand, NaOCl pretreatment presented good results for any stromatolite mineral matrix. The F<sup>14</sup>C results after etching seem to be dependent on the mineral matrix but were not significantly different for the Mg-calcite-rich layer.





**Figure 5.**  $F^{14}C$  results for each treatment [T-A, T-B, and T-C], grouped by subfacie. The black triangles represent results with no etching treatment and the gray circles represent results after etching.

## Conclusions

Before radiocarbon dating stromatolite samples it is important to identify the mineralogical composition before organic matter removal. Even though  $H_2O_2$  could not influence the results of Mg-calcite concentrate samples, it can promote the dissolution of the carbonate matrix showing an expressive weight loss for the calcite concentrated layer. The use of NaOCl appears to have been effective in preserving more material than  $H_2O_2$  independent of the mineralogical composition of the stromatolite layers. The  $F^{14}C$  results after HCl etching for Mg-calcite concentrated samples were similar to those without etching suggesting that the HCl etching does not impact the results in this case. Since stromatolites are biogenic carbonate samples with some porosity, the organic matter removal has shown to be more important than the etching procedure. The  $CaCO_3$  concentrated sample presented  $F^{14}C$  results higher than the control in  $H_2O_2$  pretreatment and both combined tests: NaOCl + etching and  $H_2O_2$  + etching. In this case, NaOCl is more indicated to be used as chemical pretreatment for radiocarbon analysis purposes independent of the mineral matrix of samples.

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