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1 **EXPERIMENTAL INVESTIGATIONS OF THE**
2 **PRESERVATION/DEGRADATION OF MICROBIAL SIGNATURES**
3 **IN THE PRESENCE OF CLAY MINERALS UNDER MARTIAN**
4 **SUBSURFACE CONDITIONS**

5
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16
17
18 **ABSTRACT**

19
20 The astrobiological exploration of Mars is ongoing. Ancient Martian terrains covered by clay
21 minerals are seen as the most promising targets to search for biosignatures as clay minerals are
22 believed to have a high potential for biopreservation. Here, we experimentally investigate the
23 biopreservation potential of saponite, a Mg-rich smectite, relying on series of experiments
24 conducted with mixtures of *E. coli* cells and saponite exposed to thermal conditions (up to
25 200°C) for different durations (up to 100 days) under a Martian atmosphere (CO₂) in the
26 presence of water, thereby simulating episodes of fluid circulation typical of those periodically
27 occurring on Mars. Residues were characterized using elemental analyses, mid-infrared (Mid-
28 IR) spectroscopy and X-ray diffraction (XRD) to document the chemistry of the residual
29 organic materials as well as the nature and crystallinity of the residual mineral materials. Results
30 show that saponite may delay the chemical degradation of some organic materials by selectively
31 trapping N-rich organic compounds within its interlayer space. However, such trapping of N-
32 rich organic compounds does not completely protect them from degradation with increasing
33 temperature and experimental duration, as shown by the N/C ratio, $\delta^{13}\text{C}$ values, Mid-IR spectra
34 and XRD patterns of the residues of experiments conducted at 200°C or at 150°C for 100 days.
35 This suggests that saponite is not that efficient in protecting biogenic organic compounds from
36 thermal degradation over long periods of time. Altogether, the present study provides
37 information on what can be found (and thus pinpointing what should be searched for) in the
38 ancient Martian geological record.

39
40 **KEYWORDS**

41 Biosignatures, Mars, Clay minerals, Smectites, Laboratory experiments

42

1. INTRODUCTION

All cases made for Martian biosignatures have been challenged so far (Sinton, 1957, 1959; Levin and Straat, 1977; McKay et al., 1996; McSween, 2019), but the consensus is that Life may have existed on Mars. Today, the surface of Mars is globally not habitable: it is cold, dry, and continuously exposed to biologically harmful radiation. Any organic material existing at the very surface of Mars (*i.e.* the first mm) would rapidly be destroyed by intense non-ionizing and ionizing radiation (*i.e.* UVs, galactic cosmic rays, solar energetic particles - Hassler et al., 2014; Fornaro et al., 2018; Fox et al., 2019; Megevand et al., 2021). But things were different during the Noachian (4.1 to 3.7 Ga): environments likely existed with liquid water and metabolic energy sources available for the development of life, possibly resembling life as we know it (Westall et al., 2013; Grotzinger et al., 2014; Kral et al., 2014; Hurowitz et al., 2017). Traces of this life may have been entombed in rocks, and because Mars lacks global plate tectonics, most ancient rocks have stayed at the surface without undergoing metamorphism, unlike the ancient rocks on Earth. Space agencies worldwide are thus sending rovers to Mars to search for ancient biosignatures in the ancient Martian geological record, anticipating their identification directly *in situ* or, more likely, within samples which will have returned to Earth (Goetz et al., 2016; Vago et al., 2017; Farley et al., 2020; Bosak et al., 2021).

Ancient Martian terrains (> 3.7 Ga) covered by clay minerals are the main targets for the astrobiological exploration of Mars (Pajola et al., 2017; Salvatore et al., 2018; Quantin-Nataf et al., 2021). In addition to being at the heart of the “clay life” hypothesis, which posits that life began as self-replicating minerals (Cairns-Smith, 1966; Joshi et al., 2015; Brucato and Fornaro, 2019), clay minerals have strong absorption capacities, conferring them a high ‘potential of biopreservation’ (Hedges and Keil, 1995; Kennedy et al., 2002; Ehlmann et al., 2008; Westall and Cockell, 2016). The presence of these minerals at landing sites of robotic missions is thus believed to maximize the chances of detecting diagnostic organic molecules, thereby eventually evidencing the past existence of Martian Life (Summons et al., 2008, 2011; McMahan et al., 2018; Bosak et al., 2021). Odds to find traces of life in ancient Martian rocks rich in clay minerals are seen as very high given that the loss of the atmosphere during the Hesperian (3.7 to 3.2 Ga) and the Amazonian (3.2 Ga to present) resulted in a drastic decrease of surface temperatures, transmuting Mars subsurface into a giant freezer (Clifford et al., 2010).

However, the subsurface of Mars may not be that suitable for the preservation of organic biosignatures. In addition to the detrimental effect of the pervasive presence of highly oxidizing Cl-rich species such as perchlorates (Hecht et al., 2009; Quinn et al., 2013; Leshin et al., 2013; Lasne et al., 2016), orbital and in-situ exploration have revealed that most Noachian clay-rich sediments are intruded by veins of (Ca, Fe, Mg, Al)-sulfates, attesting to the episodic circulation of (more or less acidic) brines at low to relatively high temperatures (Zolotov and Mironenko, 2016; Schwenger et al., 2016; Kronyak et al., 2019; Bristow et al., 2021). Such fluid circulation events may have been triggered by volcanism or impacts. In fact, any impactor, even a small one, will deliver enough energy to melt and mobilize the water ice trapped in the Martian subsurface (Boynton et al., 2002; Bramson et al., 2015; Dundas et al., 2018; Piqueux et al., 2019), initiating a long lasting circulation of fluids over distances possibly considerable (*i.e.*

87 several hundreds of kilometers - Rathbun and Squyres, 2002; Segura et al., 2002; Barnhart et
88 al., 2010; Ivanov and Pierazzo, 2011; Osinski et al., 2013). Because liquid water enhances the
89 reactivity of organic compounds (McCollom et al., 2001; Lewan and Roy, 2011; Foustoukos
90 and Stern, 2012), such episodes of fluid circulation can only be detrimental to the preservation
91 of organic biosignatures, thus complicating their recognition. To date, we do not know which
92 (nor in what state) organic biosignatures can be preserved within clay-rich Martian rocks.

93
94 A few experimental studies have dealt with the preservation/degradation of organic-clay
95 mixtures under simulated hydrothermal Martian conditions. Yet, these hydrous pyrolysis
96 experiments have been conducted either using single molecules, such as RNA (Viennet et al.,
97 2019) or glycine (Dalai et al., 2017; Gil-Lozano et al., 2020), or using complex natural samples
98 (Tan et al., 2021), rather than using model microorganisms experimentally mixed within a pure
99 synthetic mineral matrix, as previously done by some authors to document the effect of
100 diagenesis under terrestrial conditions (Oehler and Schopf, 1971; Li et al., 2014; Picard et al.,
101 2015; Alleon et al., 2016; Miot et al., 2017; Igisu et al., 2018). Here, we report results of
102 laboratory experiments conducted using cells of a model microorganism (i.e., *Escherichia coli*
103 cells) and pure saponite (a Mg-rich trioctahedral smectite) synthesized in the lab. We exposed
104 mixtures of *E. coli* cells and saponite to thermal treatments under a Martian atmosphere (CO₂)
105 in the presence of water, thereby simulating episodes of fluid circulation. Of note, rather than
106 exactly replicating the processes that may have taken place on Mars, the present experiments
107 have been designed to constrain the impact of temperature conditions on the chemical
108 degradation of microbial signatures in the presence of clay minerals and water, thereby
109 providing information on what can be found (and thus pinpointing what should be searched for)
110 in the ancient Martian geological record. Here, we used elemental analyses, infrared (IR)
111 spectroscopy and X-ray diffraction (XRD) to document the chemistry of the organic materials
112 composing the residues as well as the nature and crystallinity of the associated mineral
113 assemblage. The presence of organic compounds within the interlayer spaces of clays is
114 discussed based on additional XRD measurements conducted under vacuum.

115 116 **2. MATERIALS & METHODS**

117 118 **2.1. Starting Materials**

119 **2.1.1. *Escherichia coli***

120
121 Although extremophilic microorganisms are often used in astrobiological studies dealing with
122 survivability, *Escherichia coli* was chosen as a model microorganism for the experiments
123 reported here because it is a simple, well-known bacterium, used as a model microorganism in
124 many biological studies (Taj et al., 2014; Blount, 2015; Samie, 2017; Ruiz and Silhavy, 2022).
125 *E. coli* cells were grown at IMPMC in Paris: 10 mL suspension of precultured *E. coli* cells (C41
126 ccya B strain) was transferred into 1 liter of LB Lennox broth medium (20 g/L) (Sigma-Aldrich)
127 containing kanamycin (30 µg.mL⁻¹) to prevent growth of potential contaminants. Cells were
128 allowed to multiply overnight before being harvested by centrifugation 4 times at 5000 rpm
129 during 30 min and rinsed with distilled water at 6500 rpm for 10 min. Cells were then dried
130 overnight at 50°C.

131

132 **2.1.2. Saponite synthesis**

133

134 Martian orbital and *in situ* observations have revealed that clay minerals are widespread in
135 Noachian terrains (*i.e.* terrains older than 3.7 Ga). Among these minerals, smectites with
136 compositions ranging from nontronite (Fe) to saponite (Mg) are the most abundant (Ehlmann
137 et al., 2011; Carter et al., 2013). In order to investigate the biopreservation potential of smectites
138 on Mars while avoiding the potential additional effect of iron, we have chosen a Mg-rich
139 smectite (*i.e.* a saponite) to conduct our experiments. This smectite was synthesized in the lab
140 following the protocol described in Criouet et al. (in press): diluted solutions (0.2M) of Na₂SiO₃
141 5H₂O (Sigma Aldrich, >95%), AlCl₃ 6H₂O (Sigma Aldrich, 99%), MgCl₂ 6H₂O (Sigma
142 Aldrich, >99%) were mixed to produce a gel stoichiometrically identical to a pure saponite of
143 composition Na_{0.4}(Si_{3.6}Al_{0.4})Mg₃O₁₀(OH)₂. The gel was then immersed into pure water (milliQ
144 – 18.2 MΩ.cm) in a PTFE reactor for crystallization at 230 °C (~28 bars) for 10 days. The
145 produced saponite was then filtered and dried at 50°C for 24h.

146

147 **2.2. Experimental procedure**

148

149 Mixtures of 100 mg of dried synthetic saponite and 42 mg of dried *E. coli* cells (*i.e.*
150 corresponding to a total of 15 wt.% of carbon in the starting material) were loaded into Ti-
151 reactors (Fig. S1) in which was added 1 mL of milliQ water (18.2 MΩ.cm) for a water-to-rock
152 ratio (W:R) of 7:1. Because the gas phase (*i.e.* the atmosphere) can strongly influence the final
153 organo-mineral assemblages (Schiffbauer et al., 2012; Viennet et al., 2020), reactors were
154 closed under a pure CO₂ atmosphere (1 bar) before being placed into ovens for the experiments.
155 A first series of experiments was conducted at 100, 150 and 200 °C for 10 days, and a second
156 series of experiments was conducted at 150°C for 1, 10 and 100 days. Duplicate experiments
157 were conducted at 150°C for 10 days to ensure reproducibility. Control experiments were
158 conducted at the same temperatures (100, 150 and 200°C) and for the same durations (1, 10 and
159 100 days) with only saponite (*i.e.* in the absence of *E. coli* cells) and with only *E. coli* cells (*i.e.*
160 in the absence of saponite). At the end of the experiments, the water-soluble fractions were
161 extracted from the solid residues by rinsing and centrifuging until the water was clear. Only the
162 solid residues were analyzed, after being dried at 50°C overnight. Residues of experiments
163 conducted at 150°C for different durations were also dried on glass slides, at room temperature,
164 to obtain oriented preparations for XRD.

165

166 **2.3. Characterization techniques**

167

168 The total organic carbon (TOC) and total nitrogen (TN) contents as well as the carbon isotope
169 composition ($\delta^{13}\text{C}$) of the residues were measured using a Flash 2000 Thermo CHNSO
170 elemental analyzer (EA) coupled to a Thermo Scientific DeltaV Advantage irMS operated at
171 the SSMIM facility at MNHN in Paris. A mass of 1.2 mg of each residue was loaded in Sn
172 capsules and combusted under oxygen/helium flux at 1020°C. The N₂ and CO₂ released by
173 combustion were separated by a chromatography column and quantified using a thermal
174 conductivity detector. The calibration was performed with about 0.3 mg of 4 to 9 alanine

175 standards. Mid-infrared (Mid-IR) reflectance spectroscopy measurements were conducted
176 using a Fourier transform spectrometer Nicolet 6700 FTIR operating at IMPMC (Paris, France)
177 equipped with a KBr beamsplitter and a DTGS KBr detector. Spectra were collected over the
178 400-4000 cm^{-1} range with a 4 cm^{-1} resolution in attenuated total reflection mode (ATR) using
179 a Specac Quest ATR device fitted with a diamond internal reflection element. The X-ray
180 diffraction (XRD) patterns were collected on powder (placed on Si zero diffraction plates) at
181 room temperature, with a step size of $0.033^\circ 2\theta$ over the $3-75^\circ 2\theta$ $\text{Cok}\alpha_{1,2}$ angular range with a
182 counting time of 300 ms per step, using an X'Pert Pro instrument from PANalytical, equipped
183 with a cobalt source ($\text{Cok}\alpha$ 40 mA) and operating at IMPMC (Paris, France). The XRD patterns
184 of the residues of experiments conducted at 150°C for different durations were also measured
185 on oriented preparations, at room temperature, using the same instrument, over the $4-12^\circ 2\theta$
186 $\text{Cok}\alpha_{1,2}$ angular range (step size of $0.033^\circ 2\theta$, counting time of 300 ms per step), at both
187 atmospheric pressure and under vacuum (3.10^{-4} atmosphere) using an Anton Parr HTK 1200
188 oven coupled to an EDWARDS RV3 pump, in order to determine if organic molecules were
189 trapped within the interlayer spaces of the smectites.

190

191 3. RESULTS

192

193 3.1. EA-irMS (Elemental Analysis and isotope ratio Mass Spectrometry)

194

195 Control experiments conducted in the absence of saponite (*i.e.* with only *E. coli* cells) reveal
196 that the TOC (total organic carbon) of the residues has increased with increasing temperature
197 and with increasing duration at 150°C (Table 1, Fig. 1): from about 45 wt. % for the pristine *E.*
198 *coli* cells to almost 70 wt. % for the residues of the experiments conducted at 200°C for 10 days
199 and at 150°C for 100 days. In contrast, the TN (total nitrogen) content of the residues has
200 decreased, leading to a net decrease of the atomic N/C ratio. Last, $\delta^{13}\text{C}$ values have decreased
201 with increasing temperature and duration (the residues are richer in ^{12}C than the starting material
202 - cf Table 1).

203

204 Things are different for residues of experiments conducted in the presence of saponite. In fact,
205 instead of having increased, the TOC of the residues has decreased with increasing temperature
206 and with increasing duration at 150°C , dropping from about 14 wt. % for the pristine mixture
207 of *E. coli* cells and saponite to less than 6 wt. % for the residues from experiments conducted
208 at 150°C for 10 and 100 days (Table 1, Fig.1). Similarly, the TN (total nitrogen) content of the
209 residues has decreased. However, the N/C atomic ratio has not drastically changed during the
210 experiments, as is the case for the carbon isotope composition, which remains rather similar to
211 that of the starting material. Of note, the fact that the experiment conducted at 200°C for 10
212 days exhibits a higher TOC than those conducted at 150°C for 10 days could be due to the
213 insolubilization of organic molecules present in the soluble fraction at lower temperatures (*i.e.*
214 $< 200^\circ\text{C}$)

215

216

217

218

3.2. Mid-IR (Mid-infrared spectroscopy)

Pristine *E. coli* cells exhibit a Mid-IR spectrum very similar to the spectra of bacteria reported in the literature (Fig. 2 – Legal et al., 1991; Nadtochenko et al., 2005; Erukhimovitch et al., 2005; Garip et al., 2007; Filip et al., 2008; Nyarko et al., 2014; Li et al., 2014; Faghihzadeh et al., 2016). The four major peaks at 1651-1626, 1528, and 1515 cm^{-1} can be attributed to amide I ($\nu\text{C}=\text{O}$), amide II ($\delta\text{N-H}$ and $\nu\text{C-N}$) and aromatic bands of proteins, respectively. Noticeably, features at 1468 and 1451 cm^{-1} corresponding to C-H deformations (or R-NH_3^+ for the band at $\sim 1450 \text{ cm}^{-1}$ – Viennet et al., 2022) and at $\sim 1395 \text{ cm}^{-1}$ corresponding to $\nu_s\text{C}=\text{O}$ vibrations (Erukhimovitch et al., 2005; Garip et al., 2007; Faghihzadeh et al., 2016) or C-H bending / νCH_3 in fatty acids (Filip et al., 2008; Li et al., 2014) also exist in this spectral region (Legal et al., 1991). The band at 1227 cm^{-1} may be attributed to $\nu_{\text{as}}\text{P}=\text{O}$ (Legal et al., 1991; Garip et al., 2007) or amide III vibrations (Filip et al., 2008; Li et al., 2014), while bands at 1170, 1153 and 1118 cm^{-1} correspond to $\nu\text{C-O}$, or $\nu\text{C-N}$ vibrations. In addition, the broad band near 1060 cm^{-1} may result from the overlap of several bands corresponding to carbohydrates (Legal et al., 1991; Nadtochenko et al., 2005; Erukhimovitch et al., 2005; Nyarko et al., 2014). Bands at 2958, 2923, 2873 and 2853 cm^{-1} are related to νCH_3 and νCH_2 vibrations, and bands at 3277 cm^{-1} and 3067 cm^{-1} correspond to $\nu\text{N-H}$ vibrations (Legal et al., 1991; Filip et al., 2008; Faghihzadeh et al., 2016). Of note, δOH vibrations from water may also contribute to the absorption band at about 1630 cm^{-1} .

In the absence of saponite, the IR spectral signature of *E. coli* cells has gradually changed with increasing temperature and experimental duration, with a strong decrease of intensity of most of the main bands corresponding to C-N, C-O and P=O bonds (i.e., bands in the 1300-600 cm^{-1} range – Fig. 2). Absent from the spectrum of the pristine *E. coli* cells, an additional band at 1695 cm^{-1} (attributed to $\nu\text{C}=\text{O}$ vibrations) is observed in the spectra of residues produced at 150°C and above, which may indicate a degradation of the cellular membrane (Faghihzadeh et al., 2016). The increase of this band with increasing temperature and experimental duration is concomitant to a decrease of the shoulder at 1651 cm^{-1} . Besides C-H bands, most of the bands present in the spectrum of the pristine *E. coli* cells are less intense in the spectra of residues, including the band corresponding to OH groups. Bands at 1227 and 1118 cm^{-1} are no longer present in the spectrum of the residue of the experiment conducted at 200°C, and those corresponding to $\nu\text{N-H}$, $\nu\text{C}=\text{O}$, amide I and amide II are respectively shifted to 3324, 3060, 1700, 1636 and 1538 cm^{-1} .

The presence of saponite modifies the IR signal of the starting material, with saponite peaks dominating the 400-1200 cm^{-1} and the 3000-4000 cm^{-1} spectral regions (Fig. 3 & S2). Bands corresponding to *E. coli* cells are similar in the 1200-1800 cm^{-1} and 2800-4000 cm^{-1} spectral regions, but the bands near 1530 cm^{-1} (amides II), 1515 cm^{-1} (aromatic $\nu\text{C}=\text{C}$), 1468 cm^{-1} (C-H), 1450 cm^{-1} (C-H or R-NH_3^+), 1405 / 1390 cm^{-1} ($\nu\text{C}=\text{O}$ symmetric vibrations of COO^- group or C-H) and 1227 cm^{-1} (amide III or $\nu_{\text{as}}\text{P}=\text{O}$) are less intense. Besides C-H bands (at 2800-3000 cm^{-1} and at 1470-1456 cm^{-1}), the intensity of most bands decreases with increasing temperature and experimental duration, in a more drastic way than in the absence of saponite. For instance, the band attributed to amide III or phosphate groups (at 1227 cm^{-1}) is no longer present in the

263 spectra of the residues of experiments conducted above 100°C, while it is still present in the
264 spectra of residues of experiments conducted at 150°C in the absence of saponite. As in the
265 absence of saponite, the intensity of the band near 1650 cm⁻¹ decreases with increasing
266 temperature and experimental duration, and its position has shifted to 1628 cm⁻¹ after 10 days
267 at 150°C. A shoulder at 1695 cm⁻¹, attributed to νC=O, is observed in most residues of
268 experiments conducted in the presence of saponite. As in the absence of saponite, besides C-H
269 bands, weak bands are still present near 3277, 3067, 1695, 1633, 1564, 1515 and 1378 cm⁻¹ in
270 the spectrum of the residue of the experiments conducted at 200°C, and a band corresponding
271 to amide I (at 1628 cm⁻¹) can still be observed in the spectrum of the residue of the experiments
272 conducted at 150°C for 100 days. Of note, the band at 530 cm⁻¹ corresponding to Si-O-Mg
273 vibrations (Madejová et al., 2017; Besselink et al., 2020; J. T. Kloprogge and Ponce, 2021) is
274 no longer present in the spectra of the residues of experiments (Fig. 3), while the band at 750
275 cm⁻¹, absent in the spectrum of the starting material, possibly corresponds to Si-O-^{IV}Al
276 vibrations (Madejová et al., 2017), Al-OH vibrations (Kloprogge and Frost, 2000) or Mg₂-Al-
277 OH deformations (Kloprogge and Ponce, 2021). This band is also present in the spectra of the
278 control experiments conducted in the absence of *E. coli* cells (see Fig. S2).

280 3.3. XRD

281
282 The XRD patterns of the residues of experiments conducted in the absence of *E. coli* cells reveal
283 that saponite is stable at all the temperatures investigated (*i.e.*, saponite is the only crystalline
284 phase – Fig. S2), with a (001) reflection indicating an interlayer space of about 12 to 12.6 Å.
285 Of note, a slight effect of recrystallization may have occurred for the experiments conducted at
286 150°C for 100 days and at 200°C for 10 days, as suggested by the shift of their 02.11 and 06.33
287 reflections from 4.54 to 4.55 Å and from 1.525 to 1.526 Å, respectively (Fig. S2), but these
288 shifts may be due to slight variations in hydration. The same is true in the presence of *E. coli*
289 cells (Fig. 4). The 02.11 and 06.33 reflections are shifted from 4.55 to 4.56 Å and 1.526 to 1.527
290 Å, but the position of the (001) reflection indicates an interlayer space of about 13.5-15 Å (*vs.*
291 12-12.6 Å for the experiments conducted without *E. coli* cells), either due to mixed-layer
292 stacking and/or trapping of organic compounds (Viennet et al., 2019, 2020, 2022).

293
294 Under vacuum, the interlayer space of the saponite of the residues produced in the presence of
295 *E. coli* cells does not collapse, as indicated by the absence of shift of the 001 reflection in the
296 XRD patterns, in contrast to that of the pristine crystalline saponite never exposed to *E. coli*
297 cells which collapses from 12 to 10.45 Å (Fig. 5). This attests to the presence of organic
298 compounds locking the interlayer spaces of saponite (Viennet et al., 2019, 2020, 2022). Of note,
299 the interlayer space of the saponite in the residues produced at 150°C in the presence of *E. coli*
300 cells decreases as the experimental duration increases (from 1 to 100 days – Fig. 5), suggesting
301 a reduction of the amount and/or the size of the organic compounds trapped within interlayer
302 spaces (Lanson et al., 2022). This trend is consistent with EA-irMS results, indicating a
303 decreasing TOC and TN content with increasing experimental duration (Table 1, Fig. 1).

304
305
306

4. DISCUSSION

Temperature has a significant detrimental effect on microbial signatures, as shown by the results of the control experiments conducted here with *E. coli* cells (*i.e.* in the absence of saponite), confirming previous results reported in the literature (Oehler and Schopf, 1971; Li et al., 2014; Picard et al., 2015; Alleon et al., 2016; Miot et al., 2017; Igisu et al., 2018). The increase of temperature conditions leads to significant denitrification, deoxygenation and dehydrogenation, thereby producing residues exhibiting high TOC, low N/C ratio and spectral signatures drastically different from that of the starting material (Table 1, Fig. 1 & 2). At 150°C, the N/C ratio of the residues of the control experiments conducted with *E. coli* cells (*i.e.* in the absence of saponite) exhibits a log-linear relationship with experimental duration (Fig. 1). Such behavior cannot be related to any reaction mechanism commonly operating in solid/solid organic reactions (Rothenberg et al., 2001). However, similar log-linear behaviors have been reported for parameters describing the maturation/degradation of carbon materials as a function of the duration of thermal treatments. This suggests that the degradation of *E. coli* cells mainly occurs through a series of kinetically controlled reactions such as chain scissions coupled to polymerization/condensation reactions (Rothenberg et al., 2001; Vandenbroucke and Largeau, 2007; Sánchez-Jiménez et al., 2010; Pérez-Maqueda et al., 2014). The results of the present experiments suggest that a likely metastable equilibrium is achieved in only a few days (no significant evolution of the N/C ratio occurs between 10 and 100 days), which is consistent with previous experimental results (Alleon et al., 2016, 2017). Presumably, this equilibrium could last for much longer durations, suggesting that no significant additional chemical degradation would occur during geological times if conditions remain the same, *i.e.* in the absence of mineral phases.

Obviously, experiments investigating the degradation/preservation of traces of life have to be conducted in the presence of mineral phases to provide information about processes occurring in natural settings. As an expandable clay mineral, saponite is believed to have a high potential for biopreservation (Hedges and Keil, 1995; Kennedy et al., 2002; Ehlmann et al., 2008; Westall and Cockell, 2016). In fact, in addition to adsorption/complexation of organic compounds at edge sites (Kleber et al., 2021), clay minerals may trap organic compounds within their interlayer spaces (Laird et al., 1989; Johnston et al., 2001; Viennet et al., 2019, 2022). Interlayer water molecules of smectites can be replaced by a variety of neutral organic molecules, such as carbohydrates or hydroxyl compounds, but the intercalation of protonated species (*i.e.*, cationized organic molecules) via H₂O bridges or cation exchange is much more effective (Lagaly, 1984; Lagaly et al., 2006). Organic cations may even intercalate beyond the cation exchange capacity if van der Waals forces play a major role in binding molecules to surfaces (Lagaly et al., 2006; Bergaya and Lagaly, 2011). Trapping of organic compounds by smectites has been previously reported in natural systems as well as in laboratory experiments (Aufdenkampe et al., 2001; Kopittke et al., 2018; Viennet et al., 2019, 2020). Consistently, the present experiments evidence that clay minerals have adsorbed/trapped organic compounds within their interlayer spaces as indicated by XRD (Fig. 4 & 5), thereby delaying their chemical degradation. Here, the evolution of the N/C ratio of the experimental residues pinpoints a preferential trapping of N-rich compounds by saponite. In fact, except for the experiments

351 conducted at 200°C and at 150°C for 100 days, the N/C ratio of the residues of experiments
352 conducted in the presence of saponite and *E. coli* cells are higher than those of pristine *E. coli*
353 cells (Table 1). Such a preferential trapping may be explained by the fact that smectites are
354 more likely to trap N-rich organic molecules as they are more readily cationized (e.g.,
355 protonated) than N-poor compounds (e.g., van Santen and Liu, 2018; Kleber et al., 2021).

356
357 The preferential trapping of N-rich compounds may also explain the evolution of the $\delta^{13}\text{C}$
358 values with increasing time and temperature (Table 1). Indeed, the different biochemical
359 components of living organisms have different carbon isotopic composition (Galimov, 2012),
360 the $\delta^{13}\text{C}$ values of nucleic acids having roughly the same $\delta^{13}\text{C}$ values as the cells, while proteins
361 are slightly depleted in ^{13}C , lipids even more depleted, and carbohydrates slightly enriched in
362 ^{13}C (Spiker and Hatcher, 1984; Blair et al., 1985; Hayes, 2001; Galimov, 2012). In the absence
363 of saponite, the degradation of *E. coli* cells with increasing temperature or experimental
364 duration leads to a relative enrichment in lipids (which degrade at higher temperature than the
365 other components - Chen et al., 2005), resulting in a decrease of the $\delta^{13}\text{C}$ values of the residues.
366 In the presence of saponite, the preferential trapping of N-rich compounds (i.e. proteins or
367 nucleic acids) limits this decrease since the compounds trapped within their interlayer spaces
368 of smectites are initially richer in ^{13}C than lipids (Macko et al., 1987; Galimov, 2012).

369
370 However, such trapping of organic compounds does not completely preserve them from
371 degradation with increasing temperature and experimental duration, as shown by the N/C and
372 $\delta^{13}\text{C}$ values, Mid-IR spectra and XRD patterns of the residues of experiments conducted at
373 200°C or at 150°C for 100 days (Table 1, Fig. 1, 2 & 5). This suggests that saponite is not that
374 efficient in protecting biogenic organic compounds from thermal degradation over long periods
375 of time, in contrast to silica (Alleon et al., 2016; Igisu et al., 2018) or biominerals such as Ca-
376 phosphates (Li et al., 2014) or Fe-oxyhydroxides (Picard et al., 2015). Plus, smectites have long
377 been known to promote organic compound maturation and thermal cracking (e.g., Johns, 1979;
378 Horsfield and Douglas, 1980; Goldstein, 1983; Espitalié et al., 1984; Nikalje et al., 2000;
379 Rahman et al., 2018). The effectiveness of smectites in promoting organic reactions involved
380 in hydrocarbon generation has been demonstrated in many experimental studies: Lewis-acid
381 sites, characterized by Al^{3+} or Fe^{3+} at the edges of smectites, and Brønsted-acid sites, located in
382 the interlayer spaces, are effective in promoting decarboxylation and hydrocarbons cracking
383 (Yuan et al., 2013; Bu et al., 2017; Du et al., 2021; Cai et al., 2022). Although saponite is less
384 efficient than montmorillonite in promoting such organic reactions (Eberl et al., 1978; Whitney,
385 1983; Pusch and Karnland, 1988), its high Mg content may be detrimental to the preservation
386 of organic compounds. In fact, although life requires divalent cations (Adamala et al., 2016;
387 Dalai et al., 2018), relatively low concentrations of Mg^{2+} will disrupt vesicles made of fatty
388 acids by promoting their precipitation (Monnard et al., 2002; Deamer, 2017; Dalai et al., 2018;
389 Hong et al., 2018), and the membrane of *E. coli* cells exhibits a rather high concentration of fatty
390 acids (Lugtenberg, 1981; Nikaido, 2003; Sohlenkamp and Geiger, 2016; Wang et al., 2021). In
391 any case, in contrast to the general belief, the present experiments demonstrate that saponite
392 may not have a high potential for chemical biopreservation.

393

394 The present results have strong implications for the search for traces of life in the ancient clay-
395 rich rocks lying at the surface of Mars. Determining what could be found in Martian rocks is
396 critical for the ongoing astrobiological exploration of Mars (Summons et al., 2011; Westall et
397 al., 2015; Hays et al., 2017; McMahon et al., 2018; Bosak et al., 2021). To date, a number of
398 studies have experimentally investigated the thermal degradation of microorganisms in the
399 presence of various mineral phases (Oehler and Schopf, 1971; Li et al., 2014; Picard et al.,
400 2015; Alleon et al., 2016; Miot et al., 2017; Igisu et al., 2018), while some have investigated
401 the influence of the presence of clay minerals on the thermal degradation of specific organic
402 compounds (Viennet et al., 2019, 2020, 2022; Jacquemot et al., 2019; Vinogradoff et al., 2020a,
403 2020b), but none have investigated what would become a mixture of smectites and microbial
404 cells during an episode of fluid circulation under (rather realistic) Martian conditions as done
405 in the present study.

406
407 In contrast to the general belief, the present study illustrates that we should not expect to detect
408 pristine biogenic organic compounds on Mars, but rather by-products of their degradation.
409 Although $\delta^{13}\text{C}$ values can be preserved to some extent, depending on the temperature and the
410 duration of the experiments, it remains of limited interest for the search for traces of life on
411 Mars given that a number of abiotic pathways may lead to the production of organic compounds
412 exhibiting $\delta^{13}\text{C}$ values similar to “typical biogenic” values (Craig, 1954; Horita, 2005;
413 McCollom and Seewald, 2006). Plus, as on Earth, the $\delta^{13}\text{C}$ values of Martian microbial organic
414 compounds would depend on the values of the carbon sources and on the pathways of
415 production, i.e. on metabolisms (Hayes, 1993; Zhang, 2002; Adrian and Marco-Urrea, 2016),
416 both remaining to be identified. Still, the present experiments demonstrate that smectites
417 selectively and efficiently trap N-rich organic compounds within their interlayer spaces.
418 Although thermal degradation processes may significantly alter their initial chemical and
419 molecular structure, the remaining N-rich compounds may be considered as molecular fossils
420 to search for in the ancient Martian geological record.

421
422 Obviously, the presence of N-rich organic materials within smectites should not be the only
423 ‘biosignature’ to consider when searching for traces of life in Martian rocks: some information
424 may also be derived from the mineral assemblage itself, as previously suggested (Viennet et al.,
425 2019; Jacquemot et al., 2019). For instance, a quite uncommon mineral assemblage comprising
426 clay-organic complexes intimately associated with (poorly crystallized) nanoscale phosphates
427 and carbonates could possibly be used to attest to the past presence of microbial life (Viennet
428 et al., 2019; Jacquemot et al., 2019). Determining if such an assemblage is present in the
429 experimental residues of the present study would require characterizing them using a
430 combination of the most advanced microscopic and spectroscopic tools available in laboratories
431 worldwide, as it will be the case for the future samples which will be returned from Mars. In
432 the same way, additional characterization of experimental residues could be carried out using
433 advanced mass spectrometric techniques, such as gas or liquid chromatography (GC or LC),
434 matrix-assisted laser desorption/ionization (MALDI) or Fourier-transform ion cyclotron
435 resonance (FT-ICR) coupled to high resolution mass spectroscopy, to document the diversity
436 of organic molecules in presence after a simulated episode of fluid circulation.

437

438 Altogether, the present work can be seen as a first step towards a better understanding of the
439 taphonomic processes having possibly impacted Martian fossil biosignatures, but going further
440 will require additional experiments. Here, we used *E. coli* as a model microorganism, but
441 similar experiments should be conducted with other microorganisms exhibiting different
442 protein/lipid/sugar ratios. Also, we conducted experiments with Mg-smectites, but smectites
443 containing other cations, such as Al or Fe, may exert a different influence on the thermal
444 degradation of organic compounds. Although the effect of octahedrally charged smectites (i.e.
445 montmorillonites) is already well known (Yuan et al., 2013; Bu et al., 2017; Du et al., 2021;
446 Cai et al., 2022), the one of tetrahedrally charged dioctahedral Fe and Al-rich smectites (i.e.
447 nontronites, beidellites) remains to be determined. In the same way, because the fluids
448 penetrating the clay-rich units on Mars are likely to be richer in sulfates than pure water,
449 additional experiments should be conducted with fluids exhibiting variable concentrations in
450 sulfates, the chemistry of the fluid being likely to control organic reactions and interactions
451 with clay minerals. Finally, fluid-flow experiments, i.e. experiments under open system
452 conditions, should also be conducted, since natural settings are rarely behaving as closed
453 systems. Although a lot has still to be achieved, there is no doubt that laboratory experiments
454 will contribute to the development of a robust framework to support the ongoing astrobiological
455 exploration of Mars and the future astrobiological exploration of the Solar System.

456

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466

467 **DATA AVAILABILITY**

468 All data are available at

469 [https://drive.google.com/drive/folders/1P903A46df6onvXIUtSz8ECWb9XU7vDuK?usp=driv](https://drive.google.com/drive/folders/1P903A46df6onvXIUtSz8ECWb9XU7vDuK?usp=drive_link)
470 [e_link](https://drive.google.com/drive/folders/1P903A46df6onvXIUtSz8ECWb9XU7vDuK?usp=drive_link)

471

472 **AUTHORS' CONTRIBUTION**

473 IC, JCV and SB designed the present study. FSP cultured *E. coli*. IC conducted the syntheses
474 of saponite and the fossilization experiments. IC and JCV performed the FTIR and the XRD
475 analyses, with the help of LD and MG. All authors contributed to the interpretation of the data
476 and discussed their implications. IC, JCV and SB wrote the present manuscript, with critical
477 inputs from EB, AB, FB and LR.

478

479

480

481 **APPENDIX A. SUPPLEMENTARY MATERIAL**

482 Figure S1. Picture of the reactors used for the experiments.

483 Figure S2. Mid-IR spectra and XRD patterns of the saponite starting material (in grey) and the
484 residues of control experiments conducted in the absence of *E. coli* cells (i.e., pure saponite at
485 150°C for 100 days (in brown), and pure saponite at 200°C for 10 days (in dark grey)).

486

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1036 **TABLE**

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1038 *Table 1. EA-irMS results. Note that the values of the starting materials and of the residues of experiments conducted at 150°C*
 1039 *for 10 days are shown twice to facilitate the comparison. Standard deviations are derived from results obtained on alanine*
 1040 *standards.*

	%C ± SD	%N ± SD	N/C ± SD	δ ¹³ C (‰) ± SD
<i>E. coli</i> (Starting Material)	46.66 ± 0.23	13.60 ± 0.05	0.25 ± 0.001	-24.74 ± 0.11
<i>E. coli</i> + H ₂ O + CO ₂ - 100°C 10d	54.88 ± 0.27	13.19 ± 0.05	0.21 ± 0.001	-25.48 ± 0.11
<i>E. coli</i> + H ₂ O + CO ₂ - 150°C 10d	70.27 ± 0.35	6.51 ± 0.03	0.08 ± 0.001	-27.90 ± 0.12
<i>E. coli</i> + H ₂ O + CO ₂ - 200°C 10d	66.49 ± 0.33	5.30 ± 0.02	0.07 ± 0.0002	-27.77 ± 0.12
<i>E. coli</i> (Starting Material)	46.66 ± 0.23	13.60 ± 0.05	0.25 ± 0.001	-24.74 ± 0.11
<i>E. coli</i> + H ₂ O + CO ₂ - 150°C 1d	63.10 ± 0.31	10.18 ± 0.04	0.14 ± 0.0005	-26.93 ± 0.12
<i>E. coli</i> + H ₂ O + CO ₂ - 150°C 10d	70.27 ± 0.35	6.51 ± 0.03	0.08 ± 0.001	-27.90 ± 0.12
<i>E. coli</i> + H ₂ O + CO ₂ - 150°C 100d	68.27 ± 0.53	5.95 ± 0.02	0.07 ± 0.0004	-27.83 ± 0.13
<i>E. coli</i> + Saponite (Starting Material)	12.20 ± 0.06	3.50 ± 0.03	0.25 ± 0.001	-24.96 ± 0.13
<i>E. coli</i> + Saponite + H ₂ O + CO ₂ - 100°C 10d	9.54 ± 0.05	2.82 ± 0.01	0.25 ± 0.001	-24.95 ± 0.11
<i>E. coli</i> + Saponite + H ₂ O + CO ₂ - 150°C 10d	5.87 ± 0.03	1.99 ± 0.01	0.29 ± 0.001	-24.65 ± 0.11
<i>E. coli</i> + Saponite + H ₂ O + CO ₂ - 200°C 10d	9.13 ± 0.10	1.30 ± 0.10	0.12 ± 0.01	-26.55 ± 0.08
<i>E. coli</i> + Saponite (Starting Material)	12.20 ± 0.06	3.50 ± 0.03	0.25 ± 0.001	-24.96 ± 0.13
<i>E. coli</i> + Saponite + H ₂ O + CO ₂ - 150°C 1d	9.15 ± 0.05	2.83 ± 0.01	0.27 ± 0.001	-25.47 ± 0.11
<i>E. coli</i> + Saponite + H ₂ O + CO ₂ - 150°C 10d	5.87 ± 0.03	1.99 ± 0.01	0.29 ± 0.001	-24.65 ± 0.11
<i>E. coli</i> + Saponite + H ₂ O + CO ₂ - 150°C 100d	5.65 ± 0.04	1.32 ± 0.005	0.20 ± 0.001	-25.47 ± 0.12

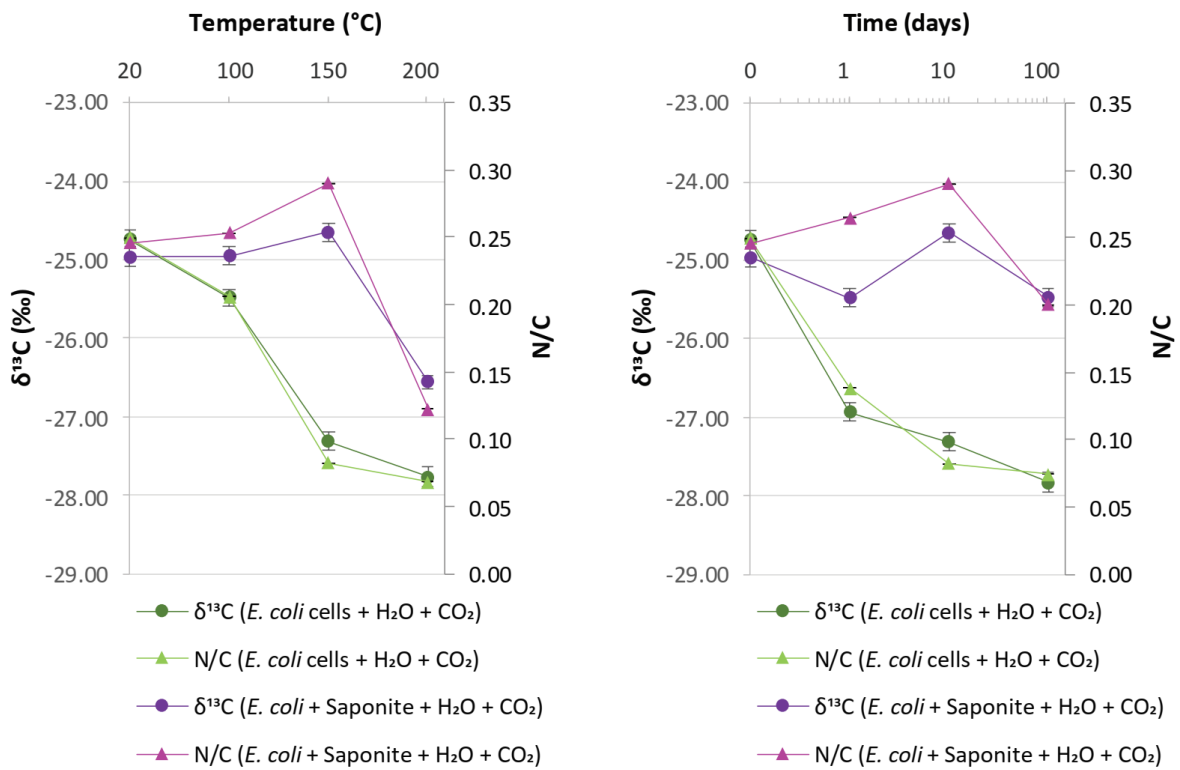
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1043 **FIGURE CAPTIONS**

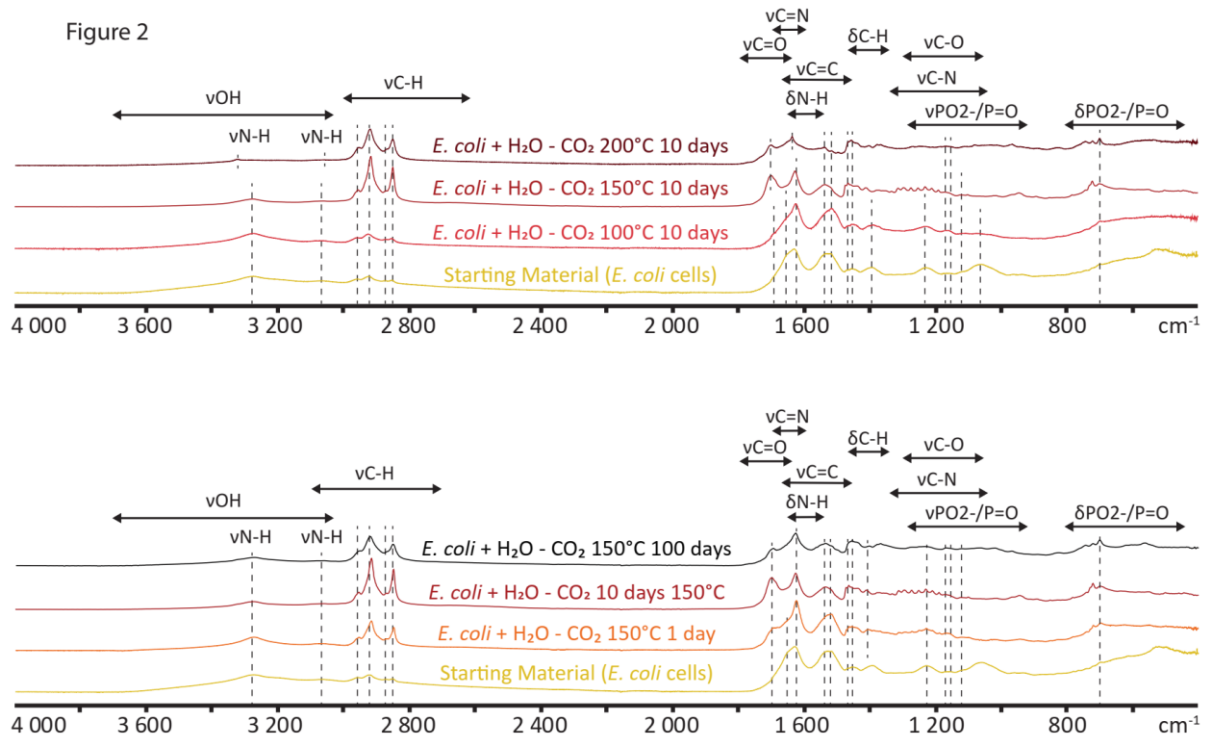
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Figure 1



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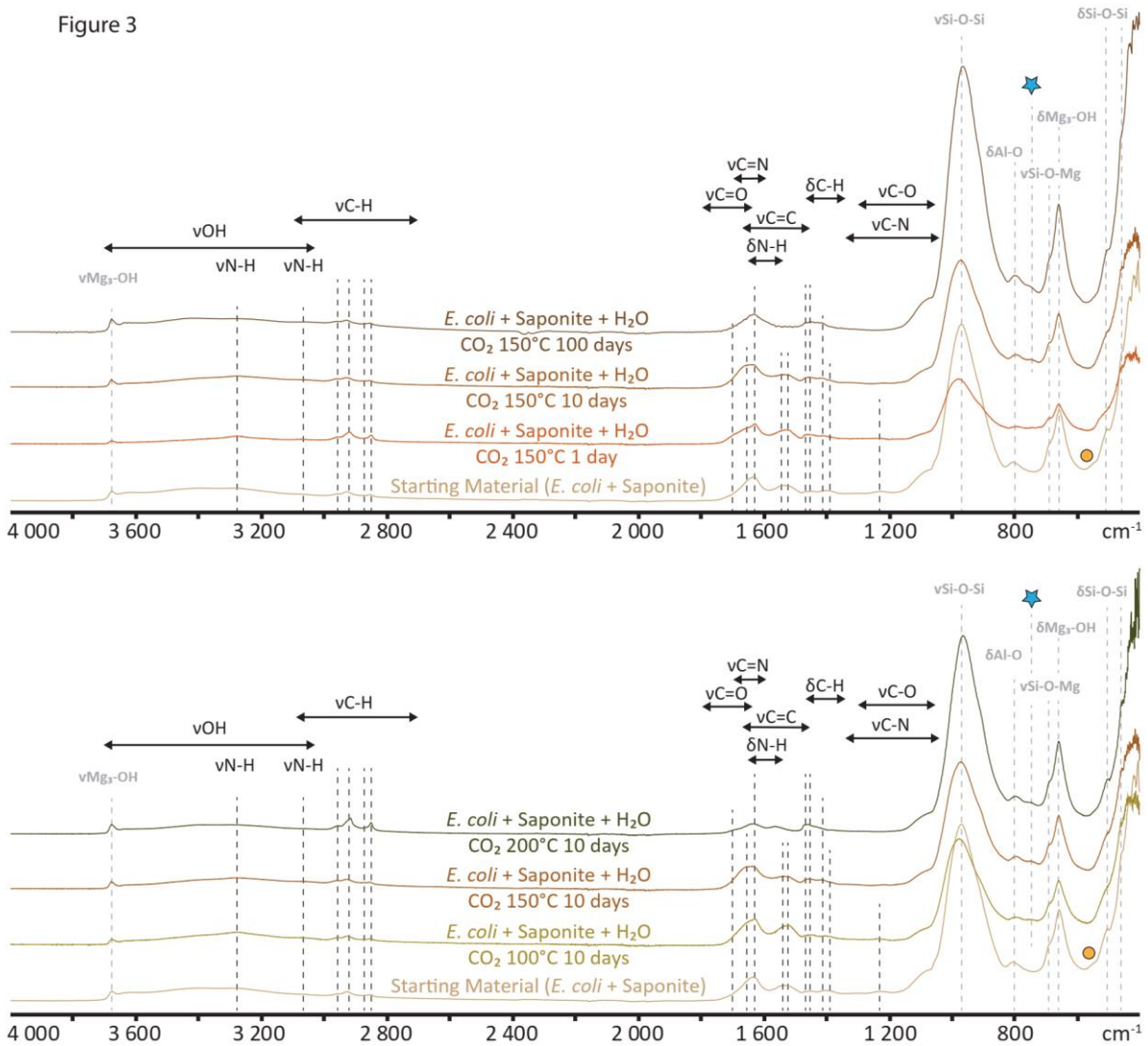
1046 Figure 2. Evolution of the N/C and $\delta^{13}\text{C}$ values with experimental duration (left) and with
 1047 experimental temperatures (right).
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Figure 2. Mid-IR spectra of residues of experiments conducted in the absence of saponite. Note that the spectra of the starting materials (pristine *E. coli* cells) and of the residue of the experiment conducted at 150°C for 10 days are shown twice to facilitate the comparison. All spectra are normalized to the CH band at 1465 cm^{-1} .

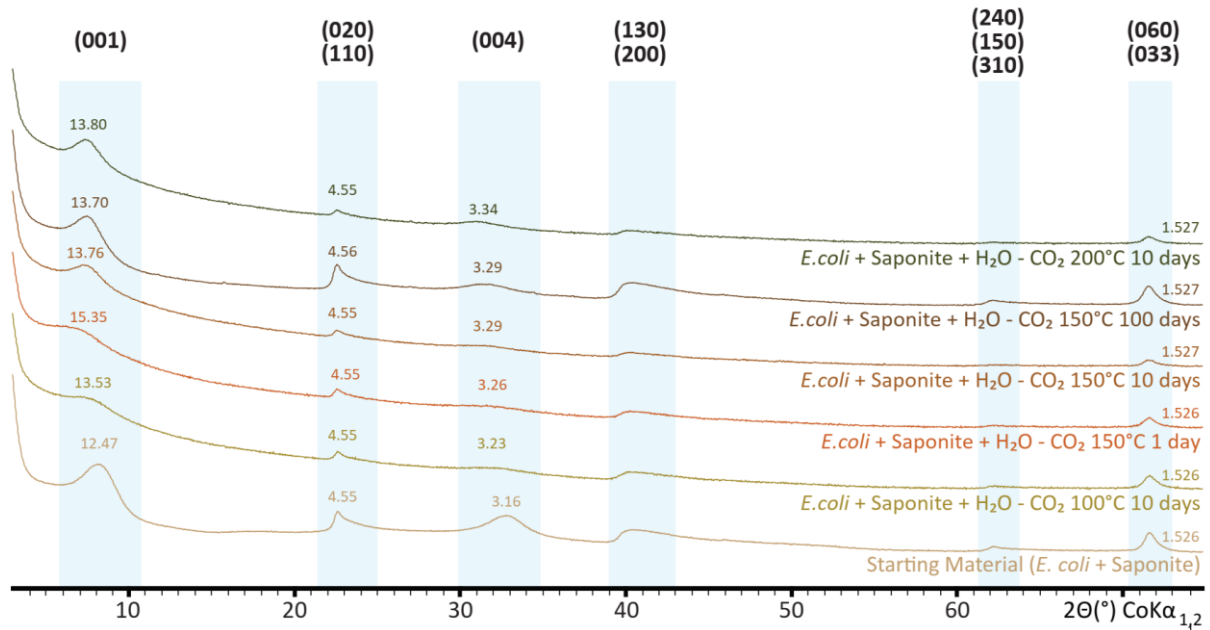
Figure 3



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Figure 3. Mid-IR spectra of residues of experiments conducted in the presence of *E. coli* + saponite. The blue star at 750 cm^{-1} and the yellow circle at 530 cm^{-1} correspond to Si-O-Al or Al-OH or Mg-Al-OH vibrations and to Si-O-Mg vibrations, respectively. Note that the spectra of the starting materials (*E. coli* cells + pure saponite) and of the residue of the experiment conducted at 150°C for 10 days are shown twice to facilitate the comparison. All spectra are normalized to the CH band at 1465 cm^{-1} .

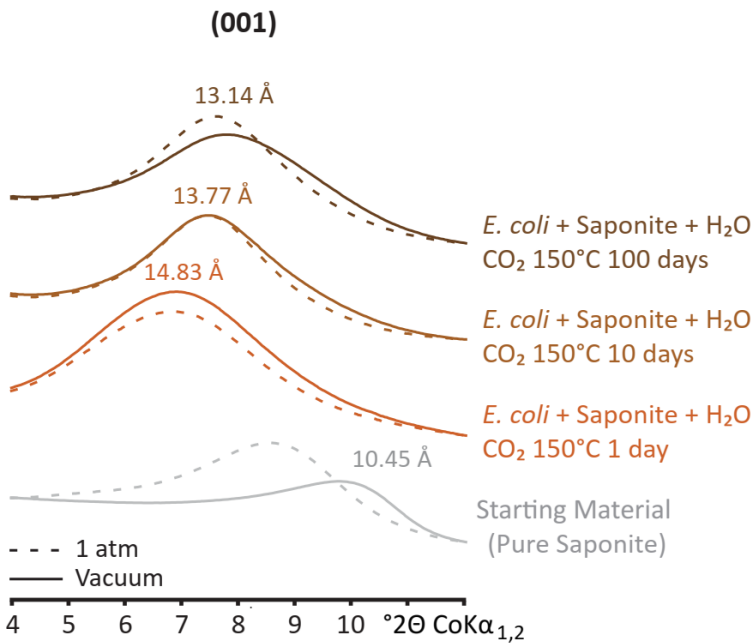
Figure 4



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Figure 4. Powder XRD patterns of residues of experiments conducted in the presence and absence of *E. coli* cells.

Figure 5



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Figure 5. XRD patterns collected at 1 atmosphere and under vacuum on oriented preparations of the residues of experiments conducted with *E. coli* cells in the presence of saponite at 150°C for different durations. The XRD patterns of the starting material (pure saponite) is shown for comparison.