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1           **Does carbon dioxide storage by cyanobacteria induce**  
2           **biomineralization in presence of basaltic glass?**

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4  
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24

25 **Abstract**

26 Cyanobacteria induced biomineralization of atmospheric CO<sub>2</sub> is a natural process leading to the  
27 formation of carbonates by spontaneous precipitation or through the presence of nucleation  
28 sites, under supersaturated conditions. As importance of basaltic rocks in the carbon cycle has  
29 already been highlighted, basaltic glass was chosen to test its ability to release cations needed  
30 for carbonate formation in presence of *Synechococcus* sp. cyanobacteria. Active cyanobacteria  
31 were expected to generate a local alkaline environment through photosynthetic metabolism.  
32 This process produces oxygen and hydroxide ions as waste products, raising the pH of the  
33 immediate cell surface vicinity and indirectly enhancing the carbonate CO<sub>3</sub><sup>2-</sup> concentration and  
34 providing the a degree of saturation that can lead to the formation of calcite CaCO<sub>3</sub> or magnesite  
35 MgCO<sub>3</sub>. In the presence of active cells, the saturation index (SI) increased from -10.56 to -9.48  
36 for calcite and from -13.6 to -12.5 for magnesite, however they remained negative due to the  
37 low Ca<sup>2+</sup> and Mg<sup>2+</sup> activities. Dead cells were expected to act as nucleation sites by the stepwise  
38 binding of carbonate with Ca<sup>2+</sup> and Mg<sup>2+</sup> on their surface. In the presence of inactive cells, SI  
39 values were closer to 0 but still negative due to the low pH and cation concentrations. Our  
40 results highlight that our current understanding of the carbon cycle suggests that Earth's climate  
41 is stabilized by a negative feedback involving CO<sub>2</sub> consumption and especially during chemical  
42 weathering of silicate minerals.

43

44 **Keywords:** biomineralization, cyanobacteria, basaltic glass, carbonate, saturation index,  
45 critical zone

46 **1. Introduction**

47

48 The rise of CO<sub>2</sub> concentrations in the atmosphere is one of the most important  
49 environmental problems on Earth today arising from anthropic activity (Gerlach, 2011). Carbon  
50 storage methods, or carbon concentration mechanisms (CCM), need to be developed urgently  
51 in order to reduce the CO<sub>2</sub> impact on Earth's terrestrial and aquatic environments. The ability  
52 of cyanobacteria to induce the biomineralization of CO<sub>2</sub> is an option which is still in its infancy  
53 and could possibly be used in the near-future to decrease atmospheric CO<sub>2</sub> levels (Martinez *et*  
54 *al.*, 2016). Previous studies have proven the link between cyanobacteria photosynthesis and the  
55 presence of metallic cations could lead to carbonate precipitation in the presence of atmospheric  
56 CO<sub>2</sub> and under suitable solution conditions (Jansson and Northen, 2010; Martinez *et al.*, 2016;  
57 Obst *et al.*, 2009).

58

59 Photosynthesis and CCM are the first steps to biomineralization of CO<sub>2</sub>. Cyanobacteria  
60 are autophototrophic microorganisms. They are primary producers, which generate their own  
61 nutrients by carrying out the oxygenic photosynthesis (Martinez *et al.*, 2016). This metabolism  
62 needs carbon as a source of inorganic carbon in the form of dissolved CO<sub>2</sub>, natural light as  
63 source of energy and water to provide electrons needed in the redox transformation of carbon  
64 (Schubert *et al.*, 1997). The Calvin-Benson cycle permits the assimilation of the inorganic  
65 carbon thanks to the initial CO<sub>2</sub>-fixing-enzyme of photosynthesis: RuBisCO (Ribulose  
66 Biphosphate Carbolxylase Oxygenase) (Fridlyand and Scheibe, 1999; Spreitzer and Salvucci,  
67 2002). Chemical and photochemical reactions take place in photosystems located on the  
68 thylakoid membrane of the cyanobacteria cell. ATP (Adenosine triphosphate) and NADPH  
69 (Nicotinamide adenine dinucleotide phosphate) are chemical substances which permit CO<sub>2</sub>  
70 assimilation. A constant supply of inorganic carbon (IC) is required to carry out photosynthesis.

71 Dissolved CO<sub>2</sub>, in the form of bicarbonate HCO<sub>3</sub><sup>-</sup> is the inorganic form of carbon used by  
72 cyanobacteria as an IC source. A metabolic system is used to attain inorganic carbon from the  
73 aqueous environment. The CCM corresponds to an environmental adaptation of cyanobacteria,  
74 it allows IC incorporation inside the cell in the form of HCO<sub>3</sub><sup>-</sup> and convert it to CO<sub>2</sub> (Price *et*  
75 *al.*, 2008). Molecules are attracted and stocked inside the cell with the help of IC transporters  
76 located in both plasma and outer membranes. HCO<sub>3</sub><sup>-</sup> transport is enhanced by the extra-ATP  
77 generated during photosynthesis. The inorganic carbon is transported into the bacterial micro-  
78 compartment where the bicarbonate molecules are transformed into CO<sub>2</sub> by the enzyme  
79 carbonic anhydrase. The remaining CO<sub>2</sub> is then assimilated by the enzyme RuBisCO for  
80 photosynthesis (Figure 1). A proton H<sup>+</sup> is consumed by each of these reactions, leading to the  
81 release of a hydroxide ion (OH<sup>-</sup>) outside the cell as a waste product along with O<sub>2</sub>. This allows  
82 the generation of an alkaline pH within the micro-environment around the cyanobacteria cell.  
83 This alkaline pH permits the deprotonation of the external bicarbonate HCO<sub>3</sub><sup>-</sup> to carbonate ions  
84 CO<sub>3</sub><sup>2-</sup>. The presence of both metal cations (e.g. Ca<sup>2+</sup>/Mg<sup>2+</sup>) and carbonate (CO<sub>3</sub><sup>2-</sup>) allows the  
85 nucleation of carbonates in the form of calcite (CaCO<sub>3</sub>) and magnesite (MgCO<sub>3</sub>) (Martinez *et*  
86 *al.*, 2016; Riding, 2006). However, the amount of metal cations and carbonate must be high  
87 enough to reach supersaturation with respect to these carbonate minerals.

88 In this study, basaltic glass was chosen as a source of metallic cations due to its  
89 composition and abundance on the Earth's surface. Its importance in the carbon cycle has been  
90 proven on several previous studies (Dessert *et al.*, 2003; Grimm *et al.*, 2019; Schlesinger and  
91 Bernhardt, 2013; Schott *et al.*, 2012). A high rate of dissolution of basaltic glass would be  
92 required to release significant metallic cation concentrations to achieve supersaturation with  
93 respect to carbonate phases. Varying an electrolyte NaNO<sub>3</sub> background concentration has been  
94 shown to affect the process of simple silicate mineral dissolution and the rate of release of  
95 metallic cations as well as their activity (Martinez *et al.*, 2014; Martinez *et al.*, 2016). An

96 optimal concentration is needed in order to reach  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  activities, which together with  
97 carbonate ion activities would lead to precipitation of carbonates. The *Synechococcus*  
98 cyanobacteria species chosen for this study is representative of seawater cyanobacteria as along  
99 with *Prochlorococcus* sp., it is responsible for approximately 30% of the photosynthesis on  
100 Earth. *Synechococcus* sp. is a unicellular coccoid cyanobacteria containing outermost surface  
101 layers but no cell covering sheath (Martinez *et al.*, 2010; Martinez *et al.*, 2008). The goal of this  
102 study is to determine whether basaltic glass is an efficient source of metal cations needed for  
103  $\text{CO}_2$  mineralization and then to quantify the rates of carbonate precipitation in presence of this  
104 primary silicate and *Synechococcus* cyanobacteria species so that optimal conditions in terms  
105 of basaltic glass dissolution and cyanobacteria biomass can be found.

106

## 107 **2. Material and methods**

108

109 A 20 mL aliquot of active *Synechococcus* sp. cyanobacteria stock at the stationary phase was  
110 placed in a 5 L Schott® bottle. *Synechococcus* sp. cultures were grown in Cyanobacteria BG-  
111 11 Freshwater Medium (Sigma-Aldrich C3061) for three weeks to stationary growth phase  
112 (Martinez *et al.*, 2010). Active cyanobacteria stocks were prepared in order to achieve  
113 photosynthesis in presence of basaltic glass (see bulk composition in Table 1), in order to  
114 quantify the influence of cyanobacteria on the dissolution of the mineral could be observed and  
115 the supersaturation conditions reached (see discussion on choice of experimental conditions in  
116 Martinez *et al.*, 2010). Stock cultures of cyanobacteria were kept at room temperature ( $23\pm 1^\circ\text{C}$ )  
117 under constant cool white fluorescent light illumination (5000 lx) and a constant filtered air  
118 injection (3 L/min at around 400 ppm) in order to provide cyanobacteria with needed inorganic  
119 carbon. The air inflow was used to keep the culture in a constant movement needed for optimal  
120 growth. For each experiment, 5 L of 1/500 diluted cyanobacteria growth medium inoculated

121 with a 20 mL aliquot of regular 1/50 BG-11 (Table 2) cyanobacteria stock was placed on a  
122 rotary shaker at 250 rpm (Martinez *et al.*, 2008). Dead cyanobacteria were prepared by  
123 autoclaving an aliquot of active stock at 121°C for 20 min. To measure the exact cyanobacteria  
124 mass, a 25 mL sample was collected. Vacuum filtration was used to filter the sample through a  
125 0.2 µm pore size cellulose nitrate filter (Whatman®). The filtered sample was placed in an oven  
126 at 60°C for 30 min and then weighed.

127

## 128 **2.1. Flow through open reactors system experimental design**

129

130 Basaltic glass dissolution experiments were performed at 23±1°C by mixing two different stock  
131 solutions in flow through reactors with a peristaltic pump with a fixed inflow rate of 1.4 µL/min.  
132 The active cyanobacteria and inorganic stocks solutions used are described in **Tables 2 and 3**.  
133 To quantify the rates of basaltic glass and carbonate precipitation in presence of *Synechococcus*  
134 *sp.* cyanobacteria, duplicate flow through reactors were used, each connected to the inorganic  
135 and active cyanobacteria stocks by a peristaltic pump (Active cyanobacteria Flow Through open  
136 reactor, AFT; **Table 2 and Figure 2**).

137 To assess the role of bio-mineralization by dead cells, a calcium chloride stock solution was  
138 used instead of basaltic glass as the source of metal cations (e.g. Ca<sup>2+</sup>). For these experiments,  
139 a flow through reactor was connected through the peristaltic pump to both the calcium chloride  
140 and dead cyanobacteria stocks previously washed by centrifuging at 10,000 rpm for 20 min  
141 (Calcium Flow Through reactor, CFT; **Table 3 and Figure 3**). Preliminary results and tests  
142 are available in Ferrini and Grandjouan (2015). These dead cells act as nucleation sites. They

143 decrease the activation energy for precipitation, but saturation index (SI) values are not  
144 influenced by the existence of nucleation sites (Martinez *et al.*, 2008).

145 For all experiments, Tygon R 3603 tubes were used to link both stocks to 200 mL flow through  
146 reactors with the help of a 4 channel peristaltic pump with a low flow rate of 1.4  $\mu\text{L}/\text{min}$ .

147

## 148 **2.2. Sample analysis**

149

150 In order to monitor the chemical evolution and the cyanobacteria biomass concentration of the  
151 solution inside the flow through reactor, the pH, conductivity, alkalinity, optical density, and  
152  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations were measured from samples collected in 50 mL polypropylene  
153 Falcon® tubes from the output of the flow through reactors, once daily. Time zero was taken  
154 to be the time of this first sampling of the flow through reactor solution (Martinez *et al.*, 2016).  
155 Reactors were placed in a water bath at 22°C in order to keep their contents solutions at a  
156 constant temperature. A 20 mL sample was taken from each reactor for all experiments. An  
157 aliquot of 7 mL of sample was used to measure the optical density of the solution at 750 nm  
158 wavelength, to approximate the concentration of cyanobacteria biomass over time. The  
159 remaining 13 mL were used to measure pH, total alkalinity and conductivity. The pH and  
160 alkalinity were measured with an automatic titration system (Metrohm® 719 Titrino automatic  
161 titrator). In this study, dominated by the presence of dissolved  $\text{CO}_2$ , the total alkalinity (TA)  
162 provides an estimate of the concentrations of bicarbonate and carbonate species in solution. The  
163 TA is determined through an acid titration (alkalinity titration) where the final concentration of  
164 the acid, at the second end-point of the carbonate system,  $\text{pH} = 4.3$ , represents the total alkalinity  
165 of the solution. Carbonate alkalinity (CA) is determined by driving the alkalinity titration to the



166 first end-point for the carbonate system at pH = 8.3. From the carbonate alkalinity, the CO<sub>3</sub><sup>2-</sup>  
167 concentration in solution, which is part of total alkalinity, can be determined:

$$168 \quad TA = [HCO_3^-] + 2 [CO_3^{2-}] + [OH^-] - [H^+] \quad (1a)$$

$$169 \quad CA = 2 [CO_3^{2-}] + [OH^-] \quad (1b)$$

170 As [CO<sub>3</sub><sup>2-</sup>], [H<sup>+</sup>] and [OH<sup>-</sup>] are negligible for neutral to alkaline pH conditions (between 6 and  
171 11), total alkalinity can be re-defined to:

172

$$173 \quad TA = [HCO_3^-] \quad (2)$$

174

175 Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations were measured by flame atomic adsorption spectroscopy (F-  
176 AAS) with an Analytik Jena Vario 6 flame atomic adsorption spectrometer. The conductivity  
177 of sample aliquots was measured using a WTW LF 325 conductivity meter where conductivity  
178 is measured in mS/cm (Harris, 2010). Conductivity measurements for a particular solution  
179 allow calculation of the ionic strength (IS). The IS is proportional to the concentration of all  
180 dissolved salts in a solution. The ionic strength is required to calculate cation activity (ac) as  
181 shown below:

$$182 \quad ac = [C]\gamma_C \quad (3)$$

183 where [C] is the species concentration and  $\gamma_C$  is the activity coefficient. The log<sub>10</sub> of  $\gamma_C$  can be  
184 calculated as per the Debye-Hückel equation Eq. 4 as a function of the solution IS determined  
185 from conductivity measurements:

$$186 \quad \log \gamma_C = (-0.51 * z^2 * \sqrt{IS}) / (1 + (\alpha * \sqrt{IS} / 305)) \quad (4)$$

187 where z represents the charge of the ion (e.g. for Mg<sup>2+</sup>, z=2),  $\alpha$  the radius of the ion in picometers  
188 (e.g. for Ca<sup>2+</sup>,  $\alpha_{Ca^{2+}} = 600$  pm) (Harris, 2010; Stumm and Morgan, 1996).

189 To predict the formation of carbonate phases in this study, it is first necessary to quantify the  
190 degree of solution supersaturation with respect to Ca or Mg carbonates. This requires  
191 knowledge of the saturation index for each of these mineral phases. However, calculation of  
192 saturation state is first necessary. The saturation state ( $\Omega$ ) represents the degree of saturation of  
193 a solution with respect to a particular mineral phase (Langdon *et al.*, 2000) and is defined as  
194 follows:

$$195 \quad \Omega = (aCa^{2+} * aCO_3^{2-}) / K_{sp} \quad (5)$$

196 Where  $K_{sp}$  is the solubility product of the mineral phase that can potentially form in solution  
197 (e.g. for calcite,  $K_{sp}=10^{-8.48}$ ) and,  $aCa^{2+}$  and  $aCO_3^{2-}$  are the activities of  $Ca^{2+}$  and  $CO_3^{2-}$   
198 respectively. The SI is then defined as:

$$199 \quad SI = \log_{10}(\Omega) \quad (6)$$

200 A positive value of SI indicates supersaturation with respect to a mineral phase and suggests  
201 that precipitation of the mineral would occur. However this depends further on solution  
202 conditions (e.g. mineral nucleation, spontaneous nucleation/precipitation) (Langdon *et al.*,  
203 2000).

204

## 205 **3. Results**

206

207 Temporal evolution of the pH, the total alkalinity and the cations concentrations has been  
208 measured. These values were used to calculate saturation index with respect to calcite and  
209 magnesite. Related values for AFT and CFT are presented in Figures 4 and 5 and Figures 6 and  
210 7, respectively. For all conditions, experiments were carried out in duplicates.

211

### 212 **3.1. Active cyanobacteria Flow Through open reactor results**

213

214 In this experiments, the pH remained alkaline, starting at  $10.9 \pm 1$  and declining until a pH of  
215 10.1 at 150 h (Figure 4). Total alkalinity increased from 1 mmol/L to 1.8 mmol/L in 242 h for  
216 both reactors (Figure 4).  $[Ca^{2+}]$  and  $[Mg^{2+}]$  remained constant for the duration of the  
217 experiments at an average of  $3.37 \pm 1.00$  mg/L for  $[Ca^{2+}]$  and  $3.28 \pm 0.44$  mg/L for  $[Mg^{2+}]$ .  
218 A slight decrease in these concentrations was observed for the experiment in the first reactor.  
219 SI values for both calcite and magnesite remained negative for the time of the experiment. The  
220  $SI_{(calcite)}$  increased from -10.6 to -9.4 over a period of 145 h whereas the  $SI_{(magnesite)}$  raised from  
221 -13.6 to -12.5 (Figure 5). Both SI curves reached a constant threshold at 150 h.

222

### 223 **3.2. Calcium Flow Through reactor results**

224

225 The pH and the total alkalinity increased through the 300 h of the experiments. A total alkalinity  
226 of 0.2 mmol/L was recorded at the start of experiments to reach a final value of 0.75 mmol/L  
227 (Figure 6). The pH was for the most part neutral but raised from 6.3 to a last value of 6.75

228 (Figure 6). The calcium chloride used permitted to obtain a rather constant  $[Ca^{2+}]$  concentration  
229 at an average of 280 mg/L. The calculated  $SI_{(calcite)}$  is negative but close to the solution  
230 supersaturation with respect to calcite. These values are remained within the range of -4.6 to -  
231 4.9 for the duration of the experiment (Figure 7).

232

#### 233 4. Discussion

234

235 None of both experiments could achieve supersaturation state with respect to calcite or  
236 magnesite. The SI remain negative but fluctuate as a function of the pH, the cation concentration  
237 and the total alkalinity. This confirms that saturation index depends on metal cations and  $\text{CO}_3^{2-}$   
238 activities and further suggests the inability of basaltic glass dissolution to provide the necessary  
239 amount of cations required for solution supersaturation with respect to carbonates. The AFT  
240 experiment shows constant  $[\text{Ca}^{2+}]$  and  $[\text{Mg}^{2+}]$  concentrations (see details in Ferrini and  
241 Grandjouan (2015) ). In this case an increase in the  $\text{CO}_3^{2-}$  activity would be required to achieve  
242 supersaturated conditions with respect to carbonate, this would be reflected in a positive value  
243 of the SI for these mineral phases. As the concentration of cations remained constant, a higher  
244 concentration of carbonate or a higher degree of bicarbonate deprotonation from a higher pH  
245 would be required. This means an enhanced deprotonation of bicarbonate  $\text{HCO}_3^-$  and the  
246 consumption of hydroxide ion ( $\text{OH}^-$ ). This then explains the decreasing pH and the increasing  
247 total alkalinity as per eq.1. According to the previously mentioned definition of the carbonate  
248 alkalinity a pH higher than 8.3 would be required in order to induce bicarbonate ion  
249 deprotonation for production of  $\text{CO}_3^{2-}$  in significant concentrations (Stumm and Morgan, 1996).  
250 A higher pH could be reached by active cyanobacteria. Indeed, cyanobacteria consume  
251 bicarbonate ions by photosynthesis which leads to the higher pH values, the formation of  
252 carbonate ( $\text{CO}_3^{2-}$ ) and a raising SI in the presence of sufficient metal cations for supersaturation  
253 In the CFT case, increasing pH rates can be explained by the presence of dead cyanobacteria  
254 mass and their interaction of their deprotonated cell surface groups with dissolving  $\text{CO}_2$  from  
255 air injection (Weber and Martinez, 2017). However, SI remains negative because of the near-  
256 neutral pH which prevents calcite precipitation as bio-mineralization needs a pH higher than  
257 8.3 to commence the generation of carbonate ions.

258 It has been shown that active cyanobacteria have a protective mechanism against carbonate  
259 precipitation on their surface, which is missing for dead cyanobacteria implying that this is the  
260 consequence of the metabolic activity of these primary producers (Martinez *et al.*, 2010;  
261 Martinez *et al.*, 2008). Dead cyanobacteria cells or cellular debris may act as nucleation sites  
262 by binding  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  cations on their surface. This could explain why the SI results become  
263 less negative in the CFT experiment in presence of dead cells. There are two mechanisms of  
264 mineral precipitation which are favored depending on the degree of solution supersaturation  
265 with respect to a particular mineral. In our study, we did not observe supersaturation with  
266 respect to carbonate minerals, nor the conditions for spontaneous nucleation of these minerals,  
267 a process that takes place saturation state values equal or in excess of 3 to 4. However, the  
268 existence of nucleation sites, does decrease the activation energy for precipitation, therefore  
269 affecting the saturation index for a particular mineral, not in the bulk solution, but in the  
270 microenvironment of dead cell surfaces, for example, where the value of the SI for a specific  
271 carbonate mineral may be enhanced due to the higher concentration of metal cations and  
272 carbonate, near the negatively charged reactive solid surface. Therefore, the presence of both  
273 active and dead cyanobacteria, as in the natural environment, contributes to controlling the  
274 mode of carbonate mineral precipitation, through the generation of an alkaline pH or due to the  
275 efficient sorption of metal cations leading to the nucleation of mineral phases (Martinez *et al.*,  
276 2010; Martinez *et al.*, 2016; Weber and Martinez, 2017).

277 Another important factor which is needed for supersaturation is the metal cations  
278 concentrations. CFT results proved that high metal cation concentrations from an efficiently  
279 dissolving source, such as calcium chloride, lead to SI values which are closer to solution  
280 supersaturation with respect to carbonates. AFT [ $\text{Ca}^{2+}$ ] and [ $\text{Mg}^{2+}$ ] concentrations from basaltic  
281 glass dissolution were insufficient to achieve carbonate precipitation. Indeed, a solution needs  
282 to be supersaturated with respect to a particular mineral in order for it to precipitate. Therefore,

283 the precipitation of carbonate minerals will depend not only on the metal cation activity but  
284 also on the carbonate activity. In our results the pH of the solutions was low, therefore we  
285 observed a low carbonate activity and a slight release of metal cations from the basaltic glass.  
286 Therefore, both of these aspects did not allow the ion activity product of metal cation and  
287 carbonate in solution, to exceed the solubility product for carbonate minerals. This implies that  
288 the use of basaltic glass for carbonation and CO<sub>2</sub> mineralization would be inefficient if  
289 employed at large scale, requiring the use of faster dissolving simple silicate minerals such as  
290 olivine, or finding a way to enhance the release of metal cations from simple and more  
291 complicated alumino-silicate minerals. The slight decrease in metal cation concentrations in  
292 these experiments, occasionally observed, can be explained by the need for more testing and  
293 development of the flow through reactor used, where a fast flow rate would cause dilution of  
294 metal cations. A slower pump rate would have to be used. Moreover, higher metal cation  
295 dissolution rates would have to be reached, however, another silicate mineral would need to be  
296 tested which can release metal cations at a faster rate. The background electrolyte concentration  
297 of 0.04 mol/L NaNO<sub>3</sub> could be modified in order to find the optimal concentration and optimize  
298 the dissolution rate and the metal cation activities. Smaller basaltic glass particles can be used,  
299 this would produce and increase mineral surface area which would lead to an increase in the  
300 basaltic glass dissolution rate.

301 As proposed on Figure 1, parameters which could contribute to achieving supersaturation state  
302 with respect to carbonates and subsequent precipitation in the presence of cyanobacteria are  
303 highlighted. As evidenced by our study, several points have to be improved in order to achieve  
304 carbonate nucleation and precipitation. *Synechococcus* cyanobacteria have to be grown in  
305 proper conditions with enough space and time to get to cell population saturation (at least 2  
306 weeks). Active cells lead to an alkaline (high pH) environment by the photosynthesis process,  
307 and indirectly to the formation of carbonate CO<sub>3</sub><sup>2-</sup>. The “flow through reactor” open system

308 permits a constant CO<sub>2</sub> supply and other nutrients required for photosynthesis and cell growth.  
309 On another hand, basaltic glass has to be dissolved in optimal conditions so as to reach high  
310 aqueous [Ca<sup>2+</sup>] and [Mg<sup>2+</sup>] concentrations. Providing all these conditions, calcite or magnesite  
311 precipitation could be expected in the flow through reactors. Using the rate model in Eq.11 an  
312 approximation of the total carbonate precipitation amount could be calculated for potential  
313 implementation of these systems at the larger scale. Scanning electron microscopy and energy-  
314 dispersive X-ray spectroscopy analyses could then help to the analysis and identification of  
315 carbonates to confirm the presence of calcite or aragonite polymorphs or that of magnesite. By  
316 finding optimal conditions for cyanobacteria induced bio-mineralization, this method could be  
317 used to stock significant quantities of CO<sub>2</sub> at a large scale.

318 Overall, our results highlight that our current understanding of the long-term carbon cycle  
319 suggests that Earth's climate is stabilized by a negative feedback involving CO<sub>2</sub> consumption  
320 and especially during chemical weathering of silicate minerals (Penman *et al.*, 2020).

321

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331



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387 **Table 1** Bulk composition of basaltic glass

Element	Unit	Concentration
SiO <sub>2</sub>	%	47.49
Al <sub>2</sub> O <sub>3</sub>	%	14.06
Fe <sub>2</sub> O <sub>3</sub>	%	13.51
MgO	%	9.38
CaO	%	11.62
Na <sub>2</sub> O	%	1.85
K <sub>2</sub> O	%	0.28
TiO <sub>2</sub>	%	1.53
P <sub>2</sub> O <sub>5</sub>	%	0.17
MnO	%	0.2
Cr <sub>2</sub> O <sub>3</sub>	%	0.086

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392 **Table 2** Composition of flow through reactors initial solution for AFT experiment.

<b>5L of active cyanobacteria stock</b>	
Reagent	Quantity
aliquot BG-11	10 mL
<i>Synechococcus</i> sp. at the stationary phase	20 mL
NaNO <sub>3</sub>	0.04 mol/L
MgSO <sub>4</sub>	0.003 mol/L
<b>"Inorganic" stock</b>	
Basaltic glass	20 g
NaNO <sub>3</sub>	0.04 mol/L
MgSO <sub>4</sub>	0.003 mol/L

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395 **Table 3** Composition of flow through reactors initial solution for CFT experiment.

<b>5 L dead cyanobacteria "autoclaved" stock</b>	
Reagent	Quantity
Active cyanobacteria "autoclaved"	
(20 min at 121°C)	60 mL
NaNO <sub>3</sub>	0.04 mol/L
MgSO <sub>4</sub>	0.003 mol/L
<b>Calcium chloride stock</b>	
CaCl	0.09 mol/L
NaNO <sub>3</sub>	0.04 mol/L
MgSO <sub>4</sub>	0.003 mol/L

## **Figure captions**

**Figure 1** Schematic interpretation of optimal conditions leading to cyanobacteria induced biomineralization in presence of basaltic glass.

**Figure 2** Active cyanobacteria Flow Through open reactor (AFT) experimental set up.

**Figure 3** Calcium Flow Through reactor (CFT) experimental set up.

**Figure 4** Evolution of pH and total alkalinity through time experiment (0.04 mol/L NaNO<sub>3</sub>; AFT experiment 2). Experiment was duplicated between 0 and 120 h.

**Figure 5** Evolution of saturation index through time experiment (0.04 mol/L NaNO<sub>3</sub>; AFT experiment 2).

**Figure 6** Evolution of pH and total alkalinity through time experiment (0.04 mol/L NaNO<sub>3</sub>; CFT experiment).

**Figure 7** Evolution of Saturation index through time experiment (0.04 mol/L NaNO<sub>3</sub>; CFT experiment).

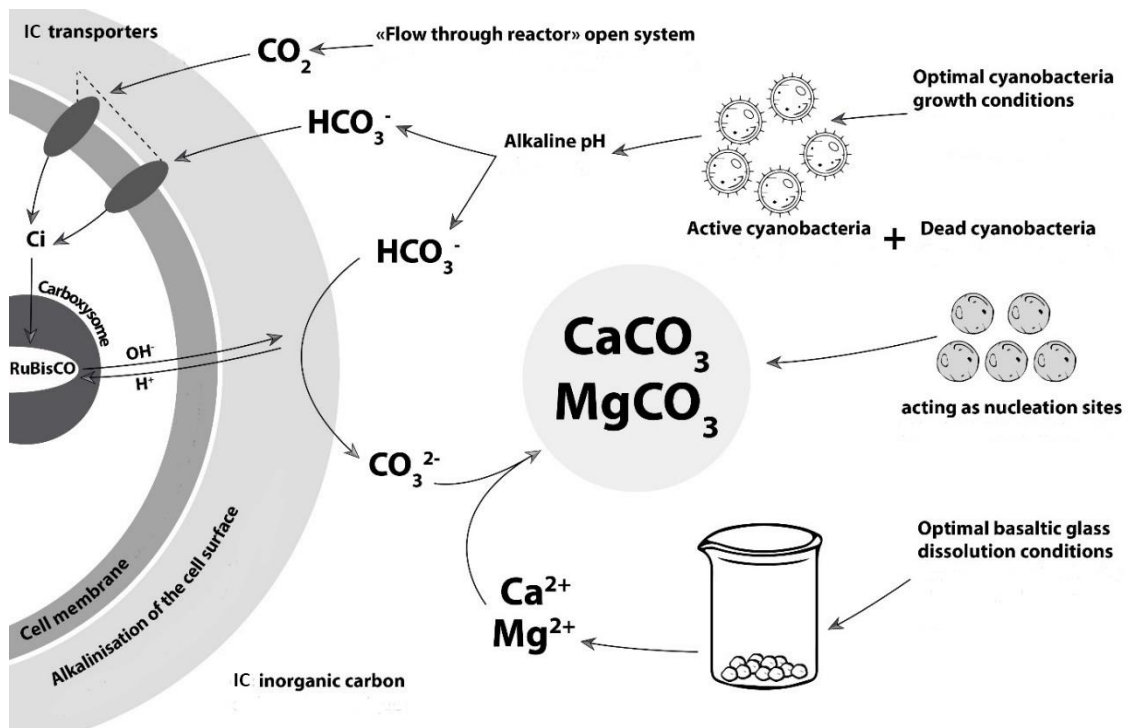
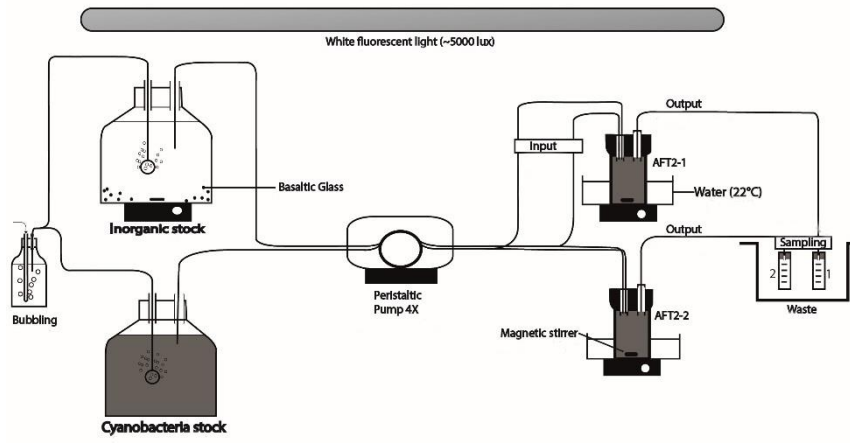


Figure 1



**Figure 2**



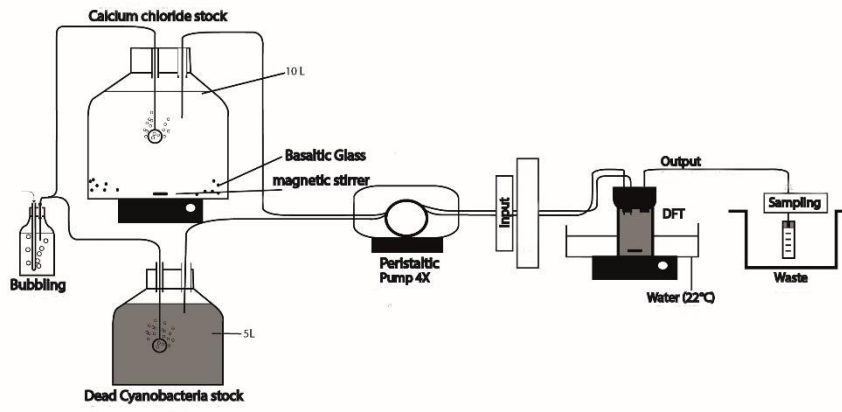
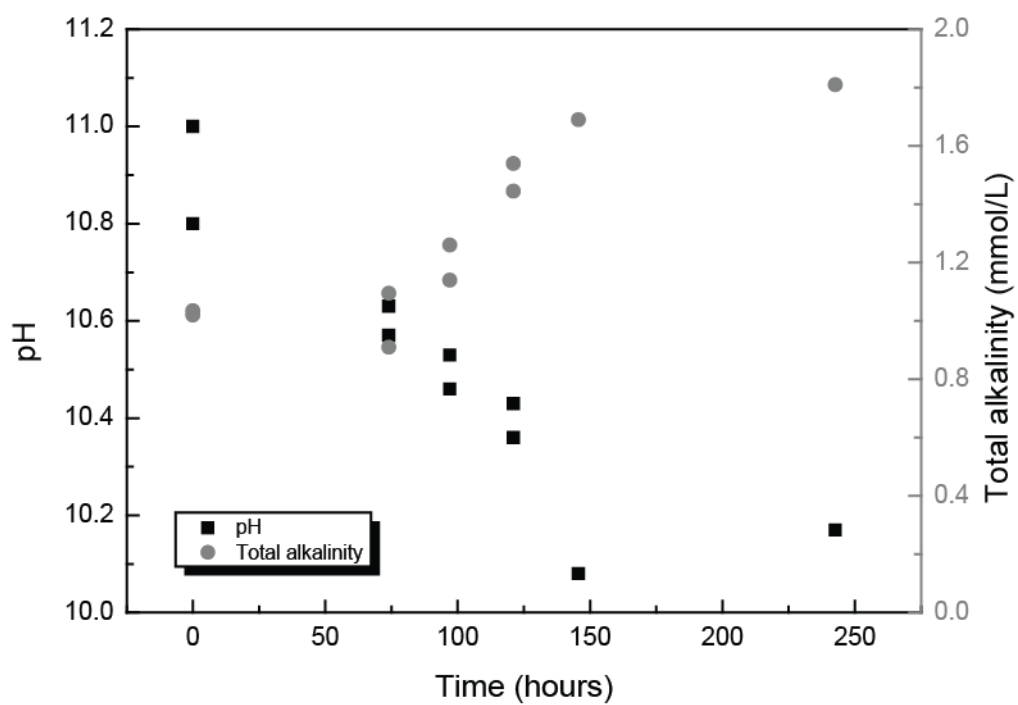
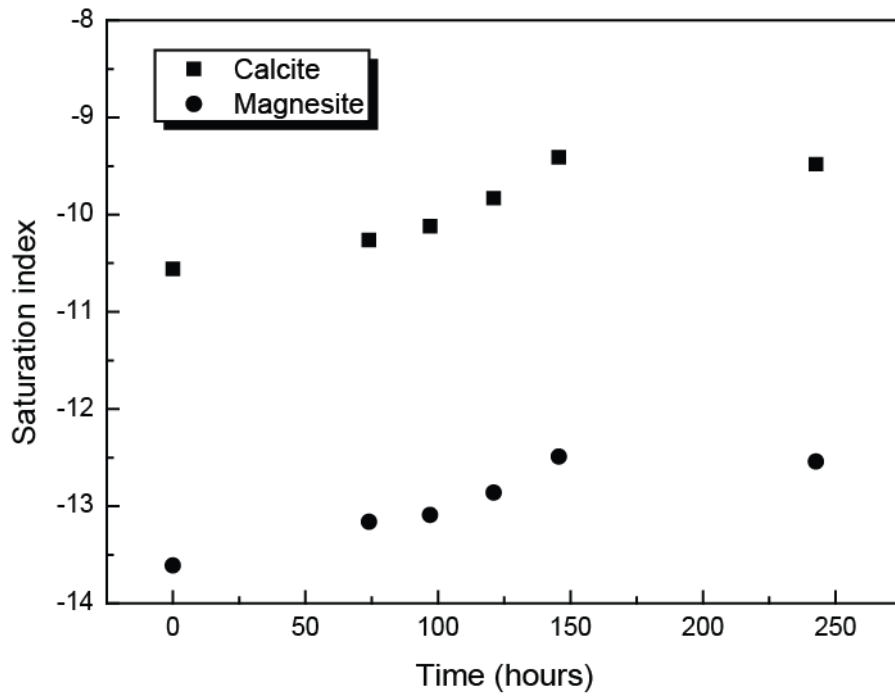


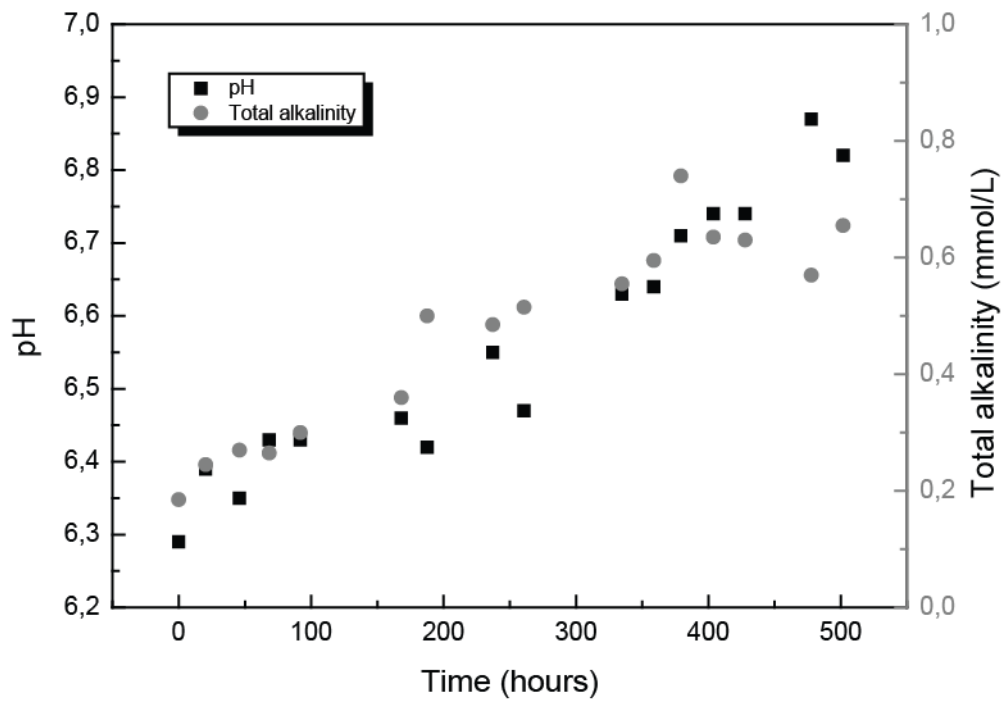
Figure 3



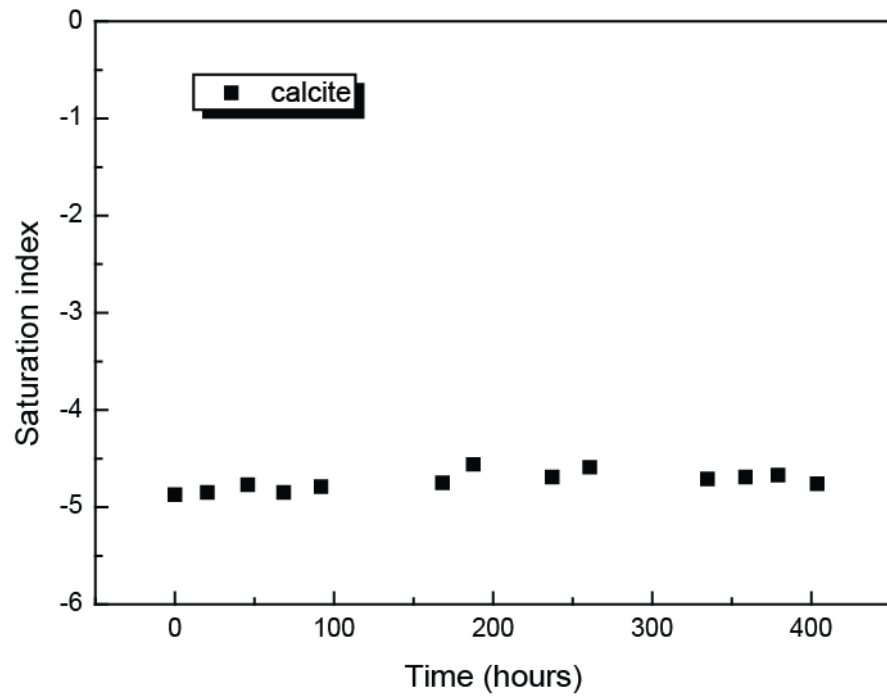
**Figure 4**



**Figure 5**



**Figure 6**



**Figure 7**