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### **Title**

Calcifying Cyanobacteria - The potential of biomineralization for Carbon Capture and Storage

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## 2 **Calcifying Cyanobacteria -**

3 The potential of biomineralization for Carbon Capture and Storage

4

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### 9 **Summary**

10 Employment of cyanobacteria in biomineralization of carbon dioxide by calcium  
11 carbonate precipitation offers novel and self-sustaining strategies for point-source carbon  
12 capture and sequestration. Although details of this process remain to be elucidated, a  
13 carbon-concentrating mechanism, and chemical reactions in exopolysaccharide or  
14 proteinaceous surface layers are assumed to be of crucial importance. Cyanobacteria can  
15 utilize solar energy through photosynthesis to convert carbon dioxide to recalcitrant  
16 calcium carbonate. Calcium can be derived from sources such as gypsum or industrial  
17 brine. A better understanding of the biochemical and genetic mechanisms that carry out  
18 and regulate cyanobacterial biomineralization should put us in a position where we can  
19 further optimize these steps by exploiting the powerful techniques of genetic engineering,  
20 directed evolution, and biomimetics.

### 21 **Introduction**

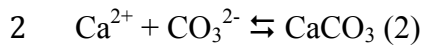
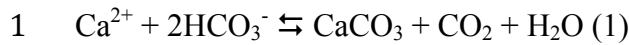
22 Strategies to reduce emissions of carbon dioxide (CO<sub>2</sub>) from fossil fuels, and hence  
23 mitigate climate change, include energy savings, development of renewable biofuels, and  
24 carbon capture and storage (CCS). For CCS, several scenarios are being considered. One  
25 approach is capture of point-source CO<sub>2</sub> from power plants or other industrial sources  
26 and subsequent injection of the concentrated CO<sub>2</sub> underground or into the ocean [1]. An  
27 alternative to this point-source CCS method is expansion of biological carbon  
28 sequestration of atmospheric CO<sub>2</sub> by measures such as reforestation, changes in land use  
29 practices, increased carbon allocation to underground biomass, production of biochar,  
30 and enhanced biomineralization [2]. In addition to geological or oceanic CO<sub>2</sub> injection,  
31 novel models for point-source CCS based on accelerated weathering and  
32 biomineralization are emerging, utilizing either abiotic [3-5] or biotic [4,6,7] processes.

33 Biomineralization of CO<sub>2</sub> by calcium carbonate (CaCO<sub>3</sub>) precipitation is a common  
34 phenomenon in marine, freshwater, and terrestrial ecosystems and is a fundamental  
35 process in the global carbon cycle [8].

36 Precipitation of CaCO<sub>3</sub> can proceed by either or both the following reactions:

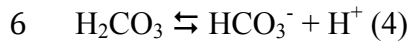
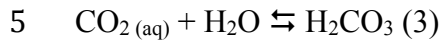
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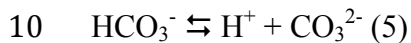
3 with reaction 2 being the principal path, at least in seawater [9,10].

4 Bicarbonate ( $\text{HCO}_3^-$ ) is ubiquitous in water and is formed via dissolution of gaseous  $\text{CO}_2$ :



7 The concentration of carbonic acid ( $\text{H}_2\text{CO}_3$ ) is small so the dissolved  $\text{CO}_2$  from reactions  
8 3 and 4 occurs predominantly as  $\text{HCO}_3^-$ .

9 A fraction of  $\text{HCO}_3^-$  dissociates to form carbonate ( $\text{CO}_3^{2-}$ ):

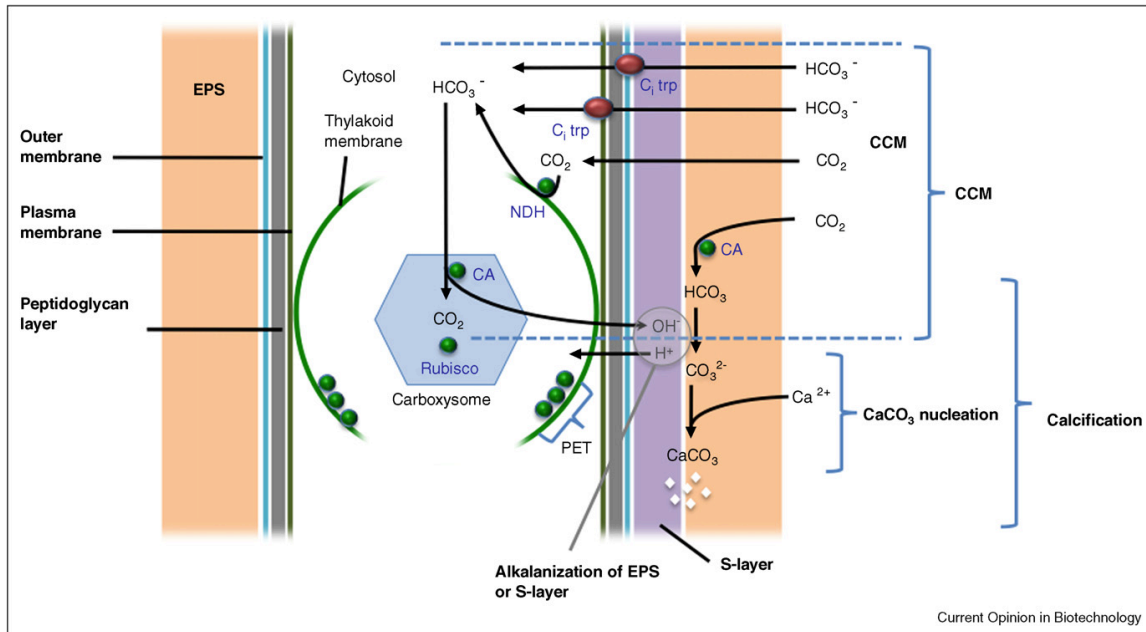


11 The lion's share of global calcification takes place through biotic processes in the oceans.  
12 Although the oceans are supersaturated with  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$ , spontaneous precipitation of  
13  $\text{CaCO}_3$  in the absence of calcifying (micro)organisms is rare owing to various kinetic  
14 barriers [11]. The contribution of microorganisms, particularly cyanobacteria, in  $\text{CaCO}_3$   
15 precipitation and sedimentation is substantial and it has played a major role in geological  
16 formations since the Archaean Era [12]. Although studies of microbially mediated  
17 biomineralization through  $\text{CaCO}_3$  precipitation have a long history, the mechanistic  
18 details of the different steps are only poorly understood [13]. In this review we discuss  
19 the potential for microorganisms, specifically cyanobacteria, in calcification, that is  
20 conversion of  $\text{CO}_2$  to recalcitrant calcium  $\text{CaCO}_3$ .

21 We begin our discussion on cyanobacterial calcification and its potential in CCS by a  
22 brief description of the general features of cyanobacteria where we elaborate on the  
23 carbon concentrating mechanism (CCM) that allows cyanobacteria to actively take up  
24 inorganic carbon ( $\text{C}_i$ ) from the external medium and perform efficient photosynthesis in  
25 aqueous environments. We then give an account on microbial biomineralization,  
26 specifically as it occurs in cyanobacteria. In this context we return to the CCM and point  
27 out the intimate association between CCM and the calcification process. Finally, we ask  
28 how biomineralization by calcifying cyanobacteria can contribute to CCS, and we point  
29 out research areas that should be prioritized to tackle some of the challenges ahead.

### 30 **Cyanobacteria**

31 Cyanobacteria are photosynthetic Gram-negative bacteria that carry out oxygenic  
32 photosynthesis and are thought to be the origin of chloroplasts of plants and eukaryotic  
33 algae via endosymbiotic events in the late Proterozoic or early Cambrian period.  
34 Cyanobacteria occupy a wide array of terrestrial, marine, and freshwater habitats,  
35 including extreme environments such as hot springs, deserts, bare rocks, and permafrost  
36 zones. In their natural environments, some cyanobacteria are often exposed to the highest  
37 rates of UV irradiance known on our globe. Cyanobacteria also have an extensive fossil  
38 record. Indeed, the oldest known fossils are of cyanobacteria from Archaean rocks of  
39 western Australia, dated 3.5 billion years old. Through their photosynthetic capacity  
40 cyanobacteria have been tremendously important in shaping the course of evolution and



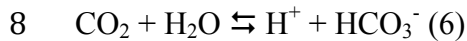
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2 **Fig. 1.** Model of the carbon-concentrating mechanism (CCM) and calcification in a  
 3 cyanobacterial cell.  $\text{CO}_2$  enters the cells mainly via active transport of  $\text{HCO}_3^-$  and also through  
 4 diffusion of  $\text{CO}_2$ , which is converted to  $\text{HCO}_3^-$  during the uptake. Cytosolic  $\text{HCO}_3^-$  is  
 5 subsequently imported to the carboxysome. CA, carbonic anhydrase;  $\text{C}_i$ , inorganic carbon; EPS,  
 6 exopolysaccharide sheath; NDH, NADPH dehydrogenase; and PET photosynthetic electron  
 7 transport. Modified from Riding (2006) [47].

8 ecological change throughout Earth's history, and they continue to contribute to a large  
 9 share of the total photosynthetic harnessing of solar energy and assimilation of  $\text{CO}_2$  to  
 10 organic compounds. For example half of global photosynthesis is carried out by  
 11 phytoplankton, which mostly consist of cyanobacteria [14]. Indeed, 25% of global  
 12 photosynthesis can be accounted for by the two marine cyanobacterial genera,  
 13 *Synechococcus* and *Prochlorococcus* [15]. Our oxygenic atmosphere was originally  
 14 generated by numerous cyanobacteria during the Archaean and Proterozoic Eras.  
 15 Cyanobacteria generally thrive in high  $\text{CO}_2$  levels and are considered as attractive  
 16 systems for  $\text{CO}_2$  capture from flue gas [16]. Many cyanobacteria are halophilic and,  
 17 therefore, cyanobacteria for biofuel production or CCS can be cultured in marine waters,  
 18 saline drainage water, or brine from petroleum refining industry or  $\text{CO}_2$  injection sites,  
 19 thereby sparing freshwater supplies. A large number of strains are thermophilic and thus  
 20 tolerate high temperatures characteristic of flue gas. Also, being bacteria, cyanobacteria  
 21 are amenable to homologous recombination, which allows rapid site-directed  
 22 mutagenesis, gene insertions, replacements and deletions in a precise targeted and  
 23 predictable manner.

24 Cyanobacteria and eukaryotic microalgae exhibit a CCM, a metabolic system that allows  
 25 the cells to enrich the amount of  $\text{CO}_2$  at the site of Rubisco (the first enzyme in the Calvin  
 26 cycle that assimilates  $\text{CO}_2$  into organic carbon compounds) up to 1000-fold over that in  
 27 the surrounding medium [17-19]. The salient features of the CCM in cyanobacteria are  
 28 shown in Fig. 1. Details differ between cyanobacteria, and the mechanisms are  
 29 incompletely understood but the general arrangement consists of transport of  $\text{HCO}_3^-$ , the

1 major uptake form of C<sub>i</sub> in cyanobacteria, across the outer membrane and the plasma  
2 membrane, through HCO<sub>3</sub><sup>-</sup>/Na<sup>+</sup> symports or ATP-driven uniports, as well as diffusion of  
3 CO<sub>2</sub>, into the cytosol. Conversion of cytosolic CO<sub>2</sub> is carried out by NADPH  
4 dehydrogenase (NDH) complexes on the thylakoid and plasma membranes. HCO<sub>3</sub><sup>-</sup> then  
5 enters the carboxysome, the protein-enclosed compartment that houses most of the  
6 Rubisco population, where it is converted to CO<sub>2</sub> in a reaction catalyzed by carbonic  
7 anhydrase (CA)



9 The conversion of CO<sub>2</sub> to HCO<sub>3</sub><sup>-</sup> via the NDH complexes relies on CA-like activities in  
10 associated proteins [18,20]. The active transport of HCO<sub>3</sub><sup>-</sup> is dependent on extra ATP  
11 generated by cyclic electron transport around Photosystem I (PSI) in the photosynthetic  
12 electron transport chain (PET) [21-23]. The C<sub>i</sub> transporters and the NDH complexes  
13 together constitute the combination of constitutive and inducible HCO<sub>3</sub><sup>-</sup> uptake systems  
14 of the cyanobacterial CCM. When cells are exposed to CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> limitation (<50 ppm  
15 CO<sub>2</sub>), the inducible transport systems are activated, accompanied with increases in  
16 Rubisco activity and carboxysome content [20].

17 Interestingly, the explanation to why many cyanobacteria and eukaryotic microalgae have  
18 the ability to tolerate very high CO<sub>2</sub> concentrations, in some cases well above 50% CO<sub>2</sub>  
19 [21,24,25] might be found in the CCM. Inhibition of Rubisco through acidification under  
20 high CO<sub>2</sub> conditions is prevented by the CA reaction and by state II transition of PET  
21 (rearrangement of the phycobilisomes to favor light absorption by PSI) [21].

22 The idea of capitalizing on the high-CO<sub>2</sub> tolerance of cyanobacteria and microalgae for  
23 mitigation of CO<sub>2</sub> emissions in flue gas in connection with biofuel production was  
24 spawned already three decades ago [26,27] (and refs. therein). Since then, a large number  
25 of studies have been published where the potential for cyanobacterial and microalgal  
26 biofuels and beneficial CO<sub>2</sub> recycling is described and discussed [16,24,28-31]. Biomass  
27 production and CO<sub>2</sub> uptake in cyanobacteria and microalgae exposed to elevated CO<sub>2</sub>  
28 levels from flue gas or other streams have been followed for a variety of strains  
29 [16,29,31-36]. The overall conclusions from a large body of experiments are that: (1)  
30 cyanobacteria and microalgae can successfully assimilate significant amounts of CO<sub>2</sub>  
31 from sources such as flue gas; (2) many species are unaffected by the NO<sub>x</sub> and SO<sub>x</sub>  
32 present in flue gas; (3) thermophiles can be employed so as to minimize the cost of  
33 cooling the flue gas; (4) nutrients can be supplied via municipal wastewater to further  
34 reduce operation costs; and (5) both freshwater and marine species can be used.

### 35 **Biomining by calcifying cyanobacteria**

36 The occurrence and distribution of calcifying microorganisms are widespread [37-39]. A  
37 number of microbial strains capable of calcification have been reported, e.g. various  
38 cyanobacteria, eukaryotic microalgae, *Bacillus*, *Pseudomonas*, *Vibrio*, and sulfate-  
39 reducing bacteria. Although the phenomenon of microbial calcification has long been  
40 recognized, its physiological function is unknown. It might confer a selective advantage  
41 in providing a protective shield against high-light exposure [40], by offering a means for  
42 excretion of toxic levels of intracellular calcium [41], by enhancing nutrient uptake  
43 [40,42], or by serving as a buffer against pH rise in an alkaline environment [40], or

1 increasing the uptake of  $\text{CO}_2$  [43]. Since calcium is an important second messenger in  
2 cellular signaling, it is crucial that cells can control the flux of calcium in and out of cells,  
3 and calcification may be part of that regulatory process.

4 Calcification is particularly obvious in cyanobacterial species [40,44]. The geological and  
5 ecological significance of cyanobacterial calcification is immense [12,44-52]; spectacular  
6 examples of cyanobacterial calcification are stromatolites [53-55] and whittings, very fast,  
7 large-scale precipitations of fine-grained  $\text{CaCO}_3$  together with organic compounds that  
8 can turn entire water bodies such as Lake Michigan and the Great Bahama Bank into a  
9 milky state [56-58]. Although our understanding of the molecular processes that trigger  
10 and control cyanobacterial calcification is hazy, and many of the mechanistic details of  
11 proposed models remain controversial, the general process is outlined in Fig. 1.

12 Cyanobacterial calcification is a non-obligate process that depends on photosynthetic  
13 activities, the CCM, extracellular surface properties, and environmental conditions  
14 [47,59]. Calcification might even be considered an integral part of the CCM.  
15 Calcification in cyanobacteria is an extracellular process and occurs on in the  
16 exopolysaccharide sheath (EPS) or proteinaceous surface layer (S-layer) that surrounds  
17 the cells [40,58,60-62]. Microenvironments of alkaline pH are generated at the EPS or S-  
18 layer owing to the CA activity in the carboxysome (reaction 6), which consumes  $\text{H}^+$  (or  
19 produces  $\text{OH}^-$ ) [63]. Other reactions that might contribute to local alkalization of the  
20 EPS or S-layer are the PET (Fig. 1) and the plasma membrane-located  $\text{Ca}^{2+}/\text{H}^+$  antiport,  
21 which transports  $\text{Ca}^{2+}$  out (and  $\text{H}^+$  in) in an effort to maintain an optimal  $\text{Ca}^{2+}$   
22 concentration in the cell [57]. The alkaline pH at the EPS or S-layer shifts the equilibria  
23 of the bicarbonate buffer system (reactions 4 and 5) to the right and promotes localized  
24 regions of increased  $\text{CO}_3^{2-}$  concentrations at the cell exterior. CA in the EPS [64] can  
25 further enhance local levels of  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  from incoming  $\text{CO}_2$  or  $\text{CO}_2$  that is  
26 leaked out from the cytosol [20]. In addition, both the EPS and S-layer contain  $\text{Ca}^{2+}$ -  
27 binding domains, e.g. glutamate and aspartate residues, which, together with the export of  
28  $\text{Ca}^{2+}$  through the  $\text{Ca}^{2+}/\text{H}^+$  translocator, raises the local  $\text{Ca}^{2+}$  concentration and serve as  
29 nucleation sites for  $\text{CaCO}_3$  precipitation. Formed  $\text{CaCO}_3$  can either precipitate as part of  
30 the EPS matrix or as calcified S-layers that shed from the cells, followed by subsequent  
31 synthesis of new S-layers. Cells that become completely embedded in  $\text{CaCO}_3$  and die due  
32 to their inability to take up nutrients have also been observed [57].

33 An inspection of reaction 1 above shows that production of  $\text{CaCO}_3$  results in the release  
34 of  $\text{CO}_2$ . Although less obvious, the same applies if carbonation proceeds from  $\text{CO}_3^{2-}$   
35 (reaction 2) [43,65]. As a consequence, the partial pressure of  $\text{CO}_2$  at the water surface  
36 rises. This calculation leads to the often puzzling and counterintuitive realization that  
37  $\text{CaCO}_3$  precipitation is associated with an increase in atmospheric  $\text{CO}_2$ . Simulations  
38 suggest that the *released  $\text{CO}_2$ :precipitated carbonate* ratio is close to 1 in freshwater but  
39 around 0.6 in marine waters, which are more buffered [43,65-67]. However, field and  
40 laboratory measurements revealed that biotic calcification exhibit *released*  
41  *$\text{CO}_2$ :precipitated carbonate* ratios between 0.1 and 0.006 [43]. This agrees with careful  
42 experimental analyses of carbon flux during cyanobacterial calcification that showed a  
43 significant net  $\text{CO}_2$  sequestration both in the field and laboratory [68]. The discrepancy  
44 between theoretic models and observed values most probably reflects the tight coupling  
45 between calcification and photosynthesis [43,66]. For example, the  $\text{CO}_2$  released during

1 calcification may be re-captured through photosynthesis [43]. Taking into account the  
2 combined effects of photosynthesis and calcification in seawater, Suzuki [66] presented a  
3 model showing that when the rate of photosynthetic biomass production (measured as  
4 *organic carbon production:calcification*) exceeds 0.6, the net effect is seawater  
5 absorption of atmospheric CO<sub>2</sub>. On the contrary, for long-term carbon sequestration it is  
6 important that as much CO<sub>2</sub> as possible be routed to calcification rather than to organic  
7 compounds [57,69].

## 8 **CCS using calcifying cyanobacteria**

9 Through photosynthesis and calcification, cyanobacteria have the potential to capture  
10 CO<sub>2</sub> from flue gas and store it as precipitated CaCO<sub>3</sub>. Calcium is abundant in many  
11 terrestrial, marine and lacustrine ecosystems. By using halophilic cyanobacteria, seawater  
12 or brines, e.g. agricultural drainage water, or saline water produced from petroleum  
13 production or geological CO<sub>2</sub> injections, can serve as potential calcium sources for the  
14 calcification process. Calcification can further be boosted by supplying calcium from  
15 gypsum [70] or silicate minerals, possibly in connection with biologically accelerated  
16 weathering [4].

17 However, successful implementation of calcifying cyanobacteria for point-source CCS  
18 are met with significant challenges that need to be addressed. For example, as seeing how  
19 alkalization of the EPS or S-layer depends on HCO<sub>3</sub><sup>-</sup> import (Fig. 1), the question arises  
20 as to whether calcification in cyanobacteria will occur also under high CO<sub>2</sub> conditions,  
21 e.g. when fed CO<sub>2</sub> from a flue gas stream. At high CO<sub>2</sub> levels, the CCM is not needed  
22 and cells will preferentially take up CO<sub>2</sub> rather than HCO<sub>3</sub><sup>-</sup>. The conversion of CO<sub>2</sub>  
23 during transport to the cytosol (Fig. 1) produces H<sup>+</sup> (reaction 6) that needs to be  
24 neutralized, possibly via export to the medium [18]. This counterbalances the subsequent  
25 and opposite alkalization reaction in the carboxysome. Also, rapid infusion of gaseous  
26 CO<sub>2</sub> into a cyanobacterial pond will probably lower the ambient pH, impeding  
27 alkalization at the extracellular surface. Cyanobacteria still calcify under elevated CO<sub>2</sub>  
28 levels but photosynthesis seems to exert little or no influence on the process [13,57].  
29 Furthermore, CaCO<sub>3</sub> precipitates were found to be more peripherally located on the  
30 extracellular surface and have a different morphology in cells predominantly taking up  
31 CO<sub>2</sub> instead of HCO<sub>3</sub><sup>-</sup> [57,71]. Whether reactions such as PET and Ca<sup>2+</sup> efflux suffice to  
32 generate extracellular alkaline microenvironments, to which extent CA activities in the  
33 EPS are involved, or if CaCO<sub>3</sub> precipitation during rapid CO<sub>2</sub> uptake becomes a passive  
34 process relying mainly of Ca<sup>2+</sup> binding and nucleation at the EPS or S-layer, remains to  
35 be clarified.

36 It will be important to unravel the mechanisms of calcification and how they are  
37 regulated in cyanobacteria growing under flue gas conditions, and in the presence of  
38 pulverized gypsum or calcium silicate minerals. Strategies to promote HCO<sub>3</sub><sup>-</sup> uptake  
39 would be to use strains where both the constitutive and inducible CO<sub>2</sub> uptake/conversion  
40 systems (Fig. 1) have been inactivated. Such mutants have been generated in  
41 *Synechococcus* PCC7942 and they exhibited HCO<sub>3</sub><sup>-</sup> but no CO<sub>2</sub> uptake capacity [72].  
42 Mutant cells grew at high CO<sub>2</sub> levels, but growth was not observed under CO<sub>2</sub>-limiting  
43 conditions. Another option might be to have the flue gas pass through a CA system so as  
44 to convert incoming CO<sub>2</sub> to HCO<sub>3</sub><sup>-</sup> prior to reaching the calcifying cyanobacteria. CA

1 could either be overproduced and secreted as extracellular enzymes directly into the  
2 solution by cyanobacteria or other bacteria, or immobilized on solid supports.

3 Another issue relates to scale. A 500 MW coal-fired power plant emits between 3 and 4  
4 Mt of CO<sub>2</sub> per year [73]. To be industrially relevant, ponds (or photobioreactors) with  
5 calcifying cyanobacteria have to produce large enough amounts of CaCO<sub>3</sub> to make an  
6 impact. Only a few attempts have been made at evaluating the rate of calcification in  
7 cyanobacteria. Extrapolating from whittings events in the Great Bahama Bank with an  
8 average of 70 km<sup>2</sup>, and microcosm experiments with the marine *Synechococcus* 8806 (*S.*  
9 8806), Lee et al. [7] estimated that calcification by *S.* 8806 could account for  
10 approximately 2.5 Mt CaCO<sub>3</sub> per year. This translates to sequestration of over half of the  
11 CO<sub>2</sub> produced from a 500 MW power plant [6,7]. Robust cyanobacterial strains or  
12 consortia need to be designed that exhibit maximized photosynthetic CO<sub>2</sub> uptake and that  
13 can fully utilize the plentiful calcium available in silicate minerals or gypsum.  
14 Calcification can be enhanced by increasing the number of carboxylate amino acids in the  
15 EPS that can be used as nucleation sites, and by increasing CA activities in the EPS. It is  
16 also crucial that strains be developed that have highly efficient light utilization and  
17 photoprotection properties. Cyanobacteria in general have low light requirements but  
18 when grown in ponds, cells below the surface will be light-limited while those at the top  
19 might experience excessive light intensities.

20 Furthermore, the information gained from studying calcification in cyanobacteria can be  
21 used for biomimetic approaches where artificial systems based on CA, CCM, EPS, or S-  
22 layers are designed for CO<sub>2</sub> capture and biomineralization. Crucial to these efforts is  
23 optimizing the long-term stability of the resulting carbonates [74]. For example, large  
24 calcite crystals containing an organic matrix similar to marine sediments are particularly  
25 stable and are highly desirable. Controlling the detailed morphology and composition of  
26 the organic (proteins, polysaccharide, etc.) and inorganic materials to result in highly  
27 stable carbonates is an important goal and may be achieved using biomimetic pathways  
28 to cyanobacterial mineralization. Ultimately such strategies could result in useful  
29 materials (i.e. bio-concrete).

## 30 **Conclusions**

31 Employment of cyanobacteria for point-source CCS of flue gas via calcification offers  
32 promising strategies for reducing anthropogenic CO<sub>2</sub> emissions. However, much research  
33 is urgently needed to further our understanding of the biochemical and physical processes  
34 in cyanobacteria that promote calcification, and that will allow us to select or design  
35 strains with optimized properties for specific applications and conditions using genetic  
36 engineering or directed evolution. For example, it is crucial that we determine the  
37 physiological functions of calcification in order to define conditions for maximal CaCO<sub>3</sub>  
38 production, and to be able to apply proper selection pressure for strain improvement. We  
39 also need to understand the different steps, that is nucleation, phase transition,  
40 crystallization, and aggregation in the biomineralization process, and the energy barriers  
41 for these stages so that we can identify bottlenecks in the overall process under different  
42 environmental conditions. We need to analyze the structural and functional  
43 characteristics of the EPSs and S-layers during calcification. We must investigate  
44 calcification at elevated CO<sub>2</sub> levels, such as in flue gas, and understand how



1 photosynthetic light harvesting and photoprotection can be improved in cyanobacteria  
2 growing in open pond cultures or in photobioreactors under such conditions. We need to  
3 identify the genes involved in calcification and utilize available batteries of 'omics  
4 technologies to obtain profiles for strains with different EPSs, S-layers, and capacities for  
5 calcification under various conditions.

6 Finally, it should not be expected that calcification by cyanobacteria and microalgae  
7 present an alternative to geological CCS. Rather biomineralization should most probably  
8 be viewed as a niche technology, preferably linked to small coal-fired power plants,  
9 natural gas systems, municipal solid waste combustion, and CO<sub>2</sub>-emitting industries such  
10 as cement manufacture, and iron and steel production. If nation-wide distributions of such  
11 units were to be deployed in countries such as the U.S.A., China and India, the impact in  
12 mitigation of global greenhouse gas emissions could be enormous.

### 13 Acknowledgements

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