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2 **Calcifying Cyanobacteria -**

3 The potential of biomineralization for Carbon Capture and Storage

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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**

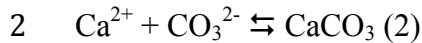
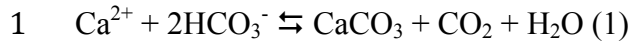
22 Strategies to reduce emissions of carbon dioxide (CO₂) from fossil fuels, and hence
23 mitigate climate change, include energy savings, development of renewable biofuels, and
24 carbon capture and storage (CCS). For CCS, several scenarios are being considered. One
25 approach is capture of point-source CO₂ from power plants or other industrial sources
26 and subsequent injection of the concentrated CO₂ underground or into the ocean [1]. An
27 alternative to this point-source CCS method is expansion of biological carbon
28 sequestration of atmospheric CO₂ 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 CO₂ 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 CO₂ by calcium carbonate (CaCO₃) 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 CaCO₃ can proceed by either or both the following reactions:

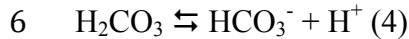
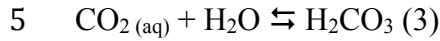
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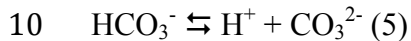
3 with reaction 2 being the principal path, at least in seawater [9,10].

4 Bicarbonate (HCO_3^-) is ubiquitous in water and is formed via dissolution of gaseous CO_2 :



7 The concentration of carbonic acid (H_2CO_3) is small so the dissolved CO_2 from reactions
8 3 and 4 occurs predominantly as HCO_3^- .

9 A fraction of HCO_3^- dissociates to form carbonate (CO_3^{2-}):

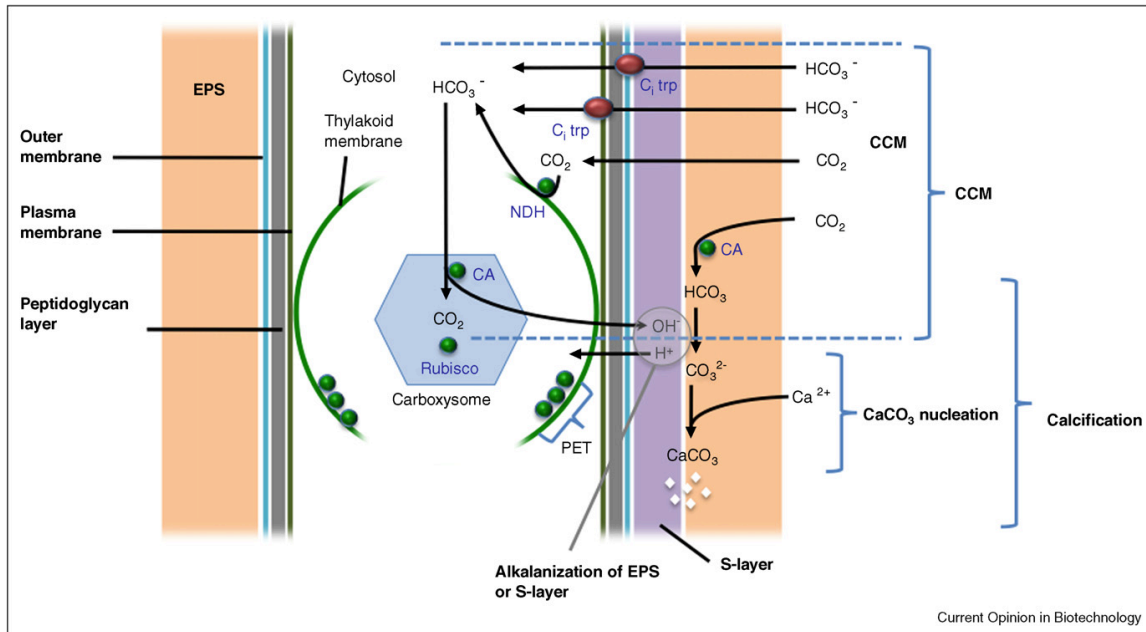


11 The lion's share of global calcification takes place through biotic processes in the oceans.
12 Although the oceans are supersaturated with Ca^{2+} and CO_3^{2-} , spontaneous precipitation of
13 CaCO_3 in the absence of calcifying (micro)organisms is rare owing to various kinetic
14 barriers [11]. The contribution of microorganisms, particularly cyanobacteria, in 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 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 CO_2 to recalcitrant calcium 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 (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



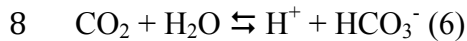
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2 **Fig. 1.** Model of the carbon-concentrating mechanism (CCM) and calcification in a
 3 cyanobacterial cell. CO₂ enters the cells mainly via active transport of HCO₃⁻ and also through
 4 diffusion of CO₂, which is converted to HCO₃⁻ during the uptake. Cytosolic HCO₃⁻ is
 5 subsequently imported to the carboxysome. CA, carbonic anhydrase; 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 CO₂ 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 CO₂ levels and are considered as attractive
 16 systems for CO₂ 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 CO₂ 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 CO₂ at the site of Rubisco (the first enzyme in the Calvin
 26 cycle that assimilates CO₂ 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 HCO₃⁻, the

1 major uptake form of C_i in cyanobacteria, across the outer membrane and the plasma
2 membrane, through HCO₃⁻/Na⁺ symports or ATP-driven uniports, as well as diffusion of
3 CO₂, into the cytosol. Conversion of cytosolic CO₂ is carried out by NADPH
4 dehydrogenase (NDH) complexes on the thylakoid and plasma membranes. HCO₃⁻ then
5 enters the carboxysome, the protein-enclosed compartment that houses most of the
6 Rubisco population, where it is converted to CO₂ in a reaction catalyzed by carbonic
7 anhydrase (CA)



9 The conversion of CO₂ to HCO₃⁻ via the NDH complexes relies on CA-like activities in
10 associated proteins [18,20]. The active transport of HCO₃⁻ 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_i transporters and the NDH complexes
13 together constitute the combination of constitutive and inducible HCO₃⁻ uptake systems
14 of the cyanobacterial CCM. When cells are exposed to CO₂/HCO₃⁻ limitation (<50 ppm
15 CO₂), 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 CO₂ concentrations, in some cases well above 50% CO₂
19 [21,24,25] might be found in the CCM. Inhibition of Rubisco through acidification under
20 high CO₂ 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-CO₂ tolerance of cyanobacteria and microalgae for
23 mitigation of CO₂ 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 CO₂ recycling is described and discussed [16,24,28-31]. Biomass
27 production and CO₂ uptake in cyanobacteria and microalgae exposed to elevated CO₂
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 CO₂
31 from sources such as flue gas; (2) many species are unaffected by the NO_x and SO_x
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 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 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 H^+ (or
19 produces 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 Ca^{2+} out (and H^+ in) in an effort to maintain an optimal 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 CO_3^{2-} concentrations at the cell exterior. CA in the EPS [64] can
25 further enhance local levels of HCO_3^- and CO_3^{2-} from incoming CO_2 or CO_2 that is
26 leaked out from the cytosol [20]. In addition, both the EPS and S-layer contain Ca^{2+} -
27 binding domains, e.g. glutamate and aspartate residues, which, together with the export of
28 Ca^{2+} through the $\text{Ca}^{2+}/\text{H}^+$ translocator, raises the local Ca^{2+} concentration and serve as
29 nucleation sites for CaCO_3 precipitation. Formed 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 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 CaCO_3 results in the release
34 of CO_2 . Although less obvious, the same applies if carbonation proceeds from CO_3^{2-}
35 (reaction 2) [43,65]. As a consequence, the partial pressure of CO_2 at the water surface
36 rises. This calculation leads to the often puzzling and counterintuitive realization that
37 CaCO_3 precipitation is associated with an increase in atmospheric CO_2 . Simulations
38 suggest that the *released 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 *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 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 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 CO₂. On the contrary, for long-term carbon sequestration it is
6 important that as much CO₂ 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 CO₂ from flue gas and store it as precipitated CaCO₃. 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 CO₂ 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 HCO₃⁻ import (Fig. 1), the question arises
20 as to whether calcification in cyanobacteria will occur also under high CO₂ conditions,
21 e.g. when fed CO₂ from a flue gas stream. At high CO₂ levels, the CCM is not needed
22 and cells will preferentially take up CO₂ rather than HCO₃⁻. The conversion of CO₂
23 during transport to the cytosol (Fig. 1) produces H⁺ (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 CO₂ into a cyanobacterial pond will probably lower the ambient pH, impeding
27 alkalization at the extracellular surface. Cyanobacteria still calcify under elevated CO₂
28 levels but photosynthesis seems to exert little or no influence on the process [13,57].
29 Furthermore, CaCO₃ precipitates were found to be more peripherally located on the
30 extracellular surface and have a different morphology in cells predominantly taking up
31 CO₂ instead of HCO₃⁻ [57,71]. Whether reactions such as PET and Ca²⁺ efflux suffice to
32 generate extracellular alkaline microenvironments, to which extent CA activities in the
33 EPS are involved, or if CaCO₃ precipitation during rapid CO₂ uptake becomes a passive
34 process relying mainly of Ca²⁺ 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 HCO₃⁻ uptake
39 would be to use strains where both the constitutive and inducible CO₂ uptake/conversion
40 systems (Fig. 1) have been inactivated. Such mutants have been generated in
41 *Synechococcus* PCC7942 and they exhibited HCO₃⁻ but no CO₂ uptake capacity [72].
42 Mutant cells grew at high CO₂ levels, but growth was not observed under CO₂-limiting
43 conditions. Another option might be to have the flue gas pass through a CA system so as
44 to convert incoming CO₂ to HCO₃⁻ 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 CO₂ per year [73]. To be industrially relevant, ponds (or photobioreactors) with
5 calcifying cyanobacteria have to produce large enough amounts of CaCO₃ 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 km², 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 CaCO₃ per year. This translates to sequestration of over half of the
11 CO₂ produced from a 500 MW power plant [6,7]. Robust cyanobacterial strains or
12 consortia need to be designed that exhibit maximized photosynthetic CO₂ 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 CO₂ 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 CO₂ 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 CaCO₃
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 CO₂ 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 CO₂-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.

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