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Role of bacterial carbonic anhydrase during CO_2 capture in the CO_2-H_2O-carbonate system

Taiming Shen [a], Wei Li [a,b,c,*], Weizhi Pan [a], Shengyan Lin [a], Min Zhu [a], Longjiang Yu [a,b,*]

[a] Institute of Resource Biology and Biotechnology, Department of Biotechnology, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China
[b] Key Laboratory of Molecular Biophysics, Ministry of Education, Wuhan 430074, China
[c] Karst Dynamics Laboratory, MLR and GZAR, Guilin 541004, China

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ABSTRACT

Atmospheric CO_2 are closely connected to climate change and global carbon cycle. Karst processes in CO_2-H_2O-carbonate system can absorb atmospheric CO_2. Carbonic anhydrase (CA) can efficiently catalyze the interconversion between CO_2 and HCO_3^-, which may help accelerate karst processes. To explore the influence of bacterial CA on CO_2 capture capacity in CO_2-H_2O-carbonate system, a flow dissolution experimental device was designed to simulate CO_2 absorption by rainwater infiltration or surface water flushing. Results showed that, when bacterial CA was added into the experimental system, it increased not only the rate but also the quantity of CO_2 absorption, and the CO_2 captured by limestone or dolomite dissolution increased by at least 18.9% and 22.1%, respectively. However, excessively high concentration of CO_2 was not conducive to carbonate rock dissolution by bacterial CA. In accordance with the fixed carbon caused by global carbonate dissolution, the contribution of soil CA to the CO_2 sinks may reach $0.567–1.938 \times 10^{14}$gC/a. In summary, the addition of microbial CA could increase the CO_2 capture in the form of DIC in CO_2-H_2O-carbonate system, thereby increasing the carbon sink potential of karst systems. The results help establish a promising approach for reducing CO_2 emission by microbial CA.

1. Introduction

As the most important anthropogenic greenhouse gas, CO_2 is closely connected to climate change and global carbon cycle [1]. Studies on the global carbon cycle, as well as atmospheric CO_2 dynamics, have indicated a missing sink of atmospheric CO_2 [2]. This missing sink may originate from terrestrial carbon sinks [3]. Dissolution of carbonate and silicate can absorb CO_2 in the atmosphere [4].The carbonate reservoir of the Earth's crust accounts for more than 90% of the global carbon storage [5], containing about 6.1×10^7 billion tons of carbon [6], is the largest carbon reservoir on Earth. Karstification has a significant effect on the capture and recovery of atmospheric CO_2. The dissolution of carbonate rocks absorbs large amounts of atmospheric CO_2 every year and forms a huge carbon sink of $1.1–6.08 \times 10^8$ ton C/a [7]. This event may be an important part of the global missing sink [8–12].

However, based on current studies of the carbon cycle, the increasing rate of atmospheric CO_2 caused by human activity is considered to be greater than the absorption rate of CO_2 in the natural weathering process, and karstification has little effect on hindering the elevation of atmospheric CO_2 concentration [13]. However, recent studies and observational data showed that karstification can sensitively reflect global environmental changes and is closely related to temperature, rainfall, and other factors [14,15]. Therefore, to clarify the actual contribution of carbonate dissolution to the capture of atmospheric CO_2, study on the CO_2 capture in CO_2-H_2O-carbonate systems is necessary.

Meanwhile, the accelerating effect of biological factors on karstification has received considerable attention among researchers. Some researchers isolated a variety of microorganisms from the surface of weathered carbonate rocks [16,17]. Moreover, some studies have shown that microbes and their carbonic anhydrase (CA) can accelerate limestone dissolution [18–21]; this finding indicates that microbes and their metabolites widely influence and participate in karst processes. CA is a zinc-containing metalloenzyme that can efficiently catalyze the interconversion reaction between CO_2 and HCO_3^- ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$) [22]. Thus, CA may accelerate carbonate rock dissolution. In previous studies,

* Corresponding authors at: Institute of Resource Biology and Biotechnology, Department of Biotechnology, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, 430074, China
E-mail addresses: hulwei_009@163.com (W. Li), yulongjiang@mail.hust.edu.cn (L. Yu).

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shaking flask test [23], rotating-disk test [24], and soil column test [18] were usually used in indoor simulation experiments to study the accelerating effect of various biological factors, such as CA, on carbonate rock dissolution. However, the rate of CO_2 capture in these experimental systems caused by carbonate rock dissolution was not calculated.

In our previous studies, the roles of microorganisms and their CA in carbonate rock dissolution were studied via shaking flask or soil column simulation experiments, respectively. Results showed that typical bacteria, fungi, and actinomycetes, which were isolated from karst soils, have a significant role in accelerating carbonate rock dissolution, and microbial extracellular CA is among the most important factors in this process [18–20].

To estimate the contribution of microbial CA to CO_2 capture in the experimental system, excluding the impact of other microbial metabolites on the experimental results, we designed a flow dissolution simulation test device. A gas mixture of air and CO_2 was bubbled through an aeration tube into the device, and the CO_2 absorption process of the CO_2-H_2O-carbonate system was simulated by rainwater infiltration or surface water flushing. The influence of CA, which was extracted from bacterial culture liquid, during CO_2 absorption in this simulation system, was investigated, and the contribution of bacterial CA to CO_2 capture was estimated. The results of this study provide a scientific basis for further studies on the contribution of microbial CA to karst carbon sinks.

2. Materials and methods

2.1. Preparation of bacterial CA

CA-producing bacterium *Bacillus cereus* GLRT202, which was screened and isolated from a karst soil in southwest China [25], was used in this study. The strain was inoculated into the sterilized liquid medium made by mixing beef extract (5 g), proteose peptone (10 g), NaCl (5 g) and zinc sulfate (10 μmol/L) in 1000 mL of distilled water. The final pH of the medium was 7.2. After incubation, the culture was centrifuged at 4 °C to remove the cells. Ammonium sulfate was added to the supernatant to precipitate the proteins. The precipitated proteins were collected and dissolved in Tris–H_2SO_4 buffer (pH 7.5) and then dialyzed at 4 °C for 48 h in Tris–H_2SO_4 buffer (pH 7.5). The dialysate was concentrated with PEG20000, placed into a pre-equilibrated anion exchanger DE-52 column for purification, and stored at 4 °C for future use. The purity of the enzyme protein was detected using SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis). The protein concentration and CA activity in the stock solution were analyzed.

2.2. Carbonate rock particles

The limestone used in the experiment belongs to the Rongxian group limestone, which was collected from the Guilin Yaji quarry. The dolomite used in the experiment was collected from the Guilin Xiongjia quarry. The rock samples were crushed and sieved between 250 and 180 μm meshes, washed with double-distilled water, and dried.

2.3. Experimental device and design

To study the effect of bacterial CA on CO_2 capture in the CO_2-H_2O-carbonate system, we designed a flow dissolution simulation test device (Fig. 2) based on the runoff modulus (average flow on unit drainage area, 11.67 $L\,s^{-1}\,km^{-2}$) of the karst area in South China [26]. The main component of the device is a glass column with a diameter of 3 cm and a length of 30 cm. The glass column was closed at both ends with a perforated rubber stopper and pre-loaded with carbonate rock particles. The glass column had an aeration tube and an inlet tube at the top, as well as an outlet tube at the bottom. Double-distilled water (Fig. 2b) was pumped into the glass column through an inlet tube with a constant flow pump (Fig. 2c) at 3 mL/min. The mixed gas of CO_2 and air was pumped into a glass column through an aeration tube (Fig. 2d) with an air pump (Fig. 2i) at 0.19 L/min. After mixing the mixed gas with double-distilled water, CO_2 reacted with H_2O and H_2CO_3 (carbonic acid) was produced in reaction region A (Fig. 2l). Then the mixture reacted with the carbonate rock particles in reaction region B (Fig. 2m). The produced Ca^{2+} in the reaction was pumped out with the liquid phase through a constant flow pump (Fig. 2c) and then entered into a collection tube (Fig. 2j).

The assembled test devices were divided into four groups named as A, B, C, and D. These devices were loaded with limestone particles or dolomite particles. The mixed gas of CO_2 and air was pumped into groups A, B, and C. The CO_2 concentrations of the mixed gas were 350, 2×10^5, and 1×10^6 ppm, respectively, representing different CO_2 partial pressure conditions. Simultaneously, double-distilled water was pumped into group A, which was considered a positive control. The purified bacterial CA enzyme solution with a final concentration of 3 U/mL was pumped into group B, which was taken as the bacterial CA test group. The commercial bovine CA(Sigma)enzyme solution with a final concentration of 3 U/mL was pumped into group C, which was considered the bovine CA test group. Only double-distilled water without the mixed gas was pumped into group D, which was regarded the negative control group. The double-distilled water used in the experiment was freshly prepared and sealed to isolate CO_2. During the experiment, the effluent samples were collected regularly from each group and then analyzed for HCO_3^- and Ca^{2+} concentrations. At the end of the experiment, the rock particles in the glass column were washed and dried. Microscopic morphology on the surface of the rock particles was then observed. The experiment was repeated three times.

2.4. Analytical methods

The CO_2 concentration in the mixed gas was measured with a ST-806 CO_2 analyzer. The pH was measured with a pH meter (Mettler Toledo EF 20A). The Ca^{2+} concentration was measured with an atomic absorption spectrophotometer (Analytik Jena AG novAA 400P) and by EDTA titration. The protein concentration of CA enzyme solution was determined by the Bradford method [27]. The CA activity was determined from a method modified from Li et al. [18]. The assay was carried out in a 4 °C coldroom. The CA activity was determined by the difference in the rates of decrease in pH in the assay medium, which containing 4.5 mL CO_2-saturated water at 4 °C, 5 mL barbital buffer (0.1 mol L^{-1} pH8.3) at 4 °C, and 0.5 mL boiling inactivated water sample or unboiled water sample, respectively. Units of activity were calculated according to the formula $U = 10\ (T_o/T_e - 1)$, where T_o and T_e represent time for pH change with boiling inactivated and unboiled water samples, respectively. The microscopic morphology of rock particles was observed using field emission scanning electron microscopy (FESEM; FEI, Nova NanoSEM 450).

2.5. Statistical analysis

The data in the results were standardized using averages of each variable. Single-factor analysis of variance (ANOVA) statistics were applied to determine if data in figures were statistically significant. Differences in Ca^{2+} concentration between different experimental groups were tested using T-test. The *P* values were calculated using the SPSS 18.0 statistical software package. Significant difference was defined at $P = 0.05$.