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Short Communication

Enhancement of Ca^{2+} release from limestone by microbial extracellular carbonic anhydrase

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Abstract

Three experimental systems were set up to investigate whether carbonic anhydrase (CA) from microorganisms actually plays the driving role in limestone dissolution. In one, redistilled water served as negative control. In a second, microbial origin CA enzyme solution supplemented with CA special inhibitor acetazolamide (AZ) served as positive control. A third contained a crude enzyme solution of microbial CA. The results showed that the amount of released Ca^{2+} from limestone in a CA non-inhibited system increased by 2.4 times compared to a CA inhibited system, and increased by 11.7 times compared to the redistilled water control. These experiments demonstrated that microbial origin CA significantly enhanced Ca^{2+} release from limestone ($P < 0.01$), and therefore, proved the significant driving effect of microbial CA on limestone dissolution. The results also suggested that microbially derived CA might exert an important influence on biokarst process.

Keywords: Carbonic anhydrase (CA); Microbial origin; Ca^{2+} release; Limestone dissolution; Biokarst

1. Introduction

Living organisms and their specific enzymes may play an important role in the operation of karst dynamic systems (Yuan and Jiang, 2000). It has been found that the dissolution rate of limestone could increase by about 10 times after adding bovine carbonic anhydrase (CA, EC4.2.1.1) to a karst system (Liu and Dreybrodt, 1997). Microorganisms exerted promoting effects on Ca^{2+} and Mg^{2+} migration in previous studies using simulated soil columns (Li et al., 2005). Correlation analysis showed that the mean activity of CA in leachates was significantly correlated with the total amount of Ca^{2+} in leachates. Moreover, scanning electron microscopy analysis showed that the surfaces of limestone cubes, which were put into soil columns containing microorganisms was corroded more than the surfaces of cubes in sterilized soil columns, and more than those not used in column experiments. These results suggested that microbial extracellular CA had an effect on limestone dissolution. However, since microorganisms existed in the soil columns, it would have been interesting to find out whether microbially derived CA actually had an effect on limestone dissolution. Therefore, the aim of this work was to remove microorganisms from the experimental systems and to separate the crude enzyme solution of CA from the microbial culture in order to investigate the dynamic process of limestone dissolution caused by microbial CA, and further to demonstrate the driving role of microbial CA in limestone dissolution.

2. Methods

2.1. Microbial strain

A bacterial strain, *Bacillus* sp GLRT102Ca was used in this study.

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2.2. Preparation of microbial extracellular carbonic anhydrase

The culture was inoculated with inoculum size of 10^6 cells ml^{-1} into the basic medium supplemented with 10 μmol L^{-1} zinc sulphate and incubated at 30 ± 1 °C on a rotary shaker at 120 rpm for 22 h. At the end of incubation, the bacteria were removed by centrifugation at $7000 \times g$ at 4 °C, and a crude enzyme solution containing extracellular CA was obtained. The CA activity of this crude enzyme solution was 0.385U mg^{-1}.

2.3. Limestone particles

The limestone was collected from a Qixing Rock at Guilin, China. It contained (g kg^{-1}): CaO, 545; MgO, 13.4; insoluble matter by acid, 3.8 (Yuan et al., 1996). The surface of the limestone was cleaned, air-dried, and then ground and sieved to obtain particles with diameter less than 0.25 mm. In consideration of exchangeable calcium presenting in the karst soil environments and limestone powders presenting at the interface between soil and rock, the limestone particles were not cleaned with redistilled water.

2.4. Simulated experimental systems

Three experimental systems were set up. Acid-washed 1 L flasks containing 0.08 g of limestone particles and 400 ml of solution were used in each experimental system. System A used redistilled water as dissolution medium, which served as negative control. System B used CA crude enzyme solution supplemented with CA specific inhibitor acetazolamide (AZ) as dissolution medium, which served as positive control. The final concentration of AZ in the solution was 1.0×10^{-4} mol L^{-1}. System C used crude enzyme solution of CA without the inhibitor as dissolution medium. All the flasks of three experimental systems were put on a shaker at room temperature at 120 rpm. Separate sets of samples were made up for analysis at sampling periods of every hour from 0 h. After each sampling period, the samples were centrifuged, decanted, and the supernatant was analysed for pH and Ca^{2+} concentration. The experiment continued for 9 h.

2.5. Analytic methods

Sample pH was determined with a Mettler model 320-S pH meter. Ca^{2+} concentration was measured using a Perkin Elmer model AA300 atomic absorption spectrophotometer.

3. Results

The pH of the two experimental systems taking the crude enzyme solution of CA as dissolution medium was approximately 1 unit higher than that of the control system taking redistilled water as dissolution medium (Fig. 1).

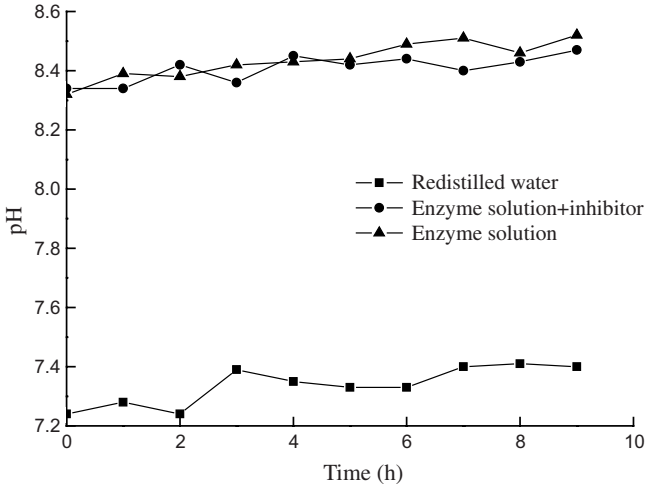

Fig. 1. Change of pH under different conditions.

Comparison of pH between the two enzyme solution systems indicated that pH of CA inhibited system was slightly lower than that of CA non-inhibited system. Moreover, all of the three experimental systems displayed an increasing trend in pH over the time.

The variation in Ca^{2+} concentration with time was quite small in the redistilled water system (Fig. 2). The concentration of Ca^{2+} at 9 h was slightly higher than the initial value, but all values were lower than 2 mg L^{-1}. The two enzyme solution systems had higher concentrations of Ca^{2+} than the redistilled water system, which was consistent with pH data. In the CA inhibited system, the variation in Ca^{2+} concentration was small. Although the concentration of Ca^{2+} fluctuated over time, the overall trend was toward increasing dissolved Ca^{2+} concentration. However, after 2 h during the experimental process, the Ca^{2+} concentration in the CA inhibited system was significantly lower than that in the CA non-inhibited system ($P < 0.01$). Moreover, the concentration of Ca^{2+} in the CA non-inhibited system increased gradually over time, and had the largest variation.

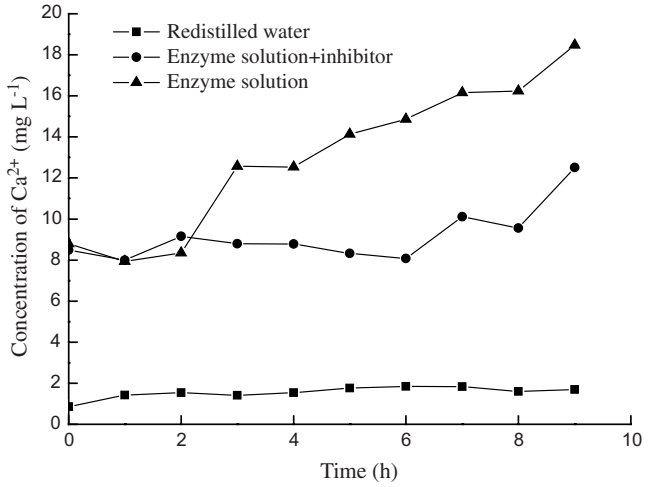

Fig. 2. Change of the concentration of Ca^{2+} under different conditions.