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Technical Note

Optimum conditions for microbial carbonate precipitation

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ABSTRACT

The type of bacteria, bacterial cell concentration, initial urea concentration, reaction temperature, the initial Ca^{2+} concentration, ionic strength, and the pH of the media are some factors that control the activity of the urease enzyme, and may have a significant impact on microbial carbonate precipitation (MCP). Factorial experiments were designed based on these factors to determine the optimum conditions that take into consideration economic advantage while at the same time giving quality results. *Sporosarcina pasteurii* strain ATCC 11859 was used at constant temperature (25 °C) and ionic strength with varying amounts of urea, Ca^{2+}, and bacterial cell concentration. The results indicate that the rate of ureolysis (k_{urea}) increases with bacterial cell concentration, and the bacterial cell concentration had a greater influence on k_{urea} than initial urea concentration. At 25 mM Ca^{2+} concentration, increasing bacterial cell concentration from 10^6 to 10^8 cells mL^{-1} increased the $CaCO_3$ precipitated and CO_2 sequestrated by over 30%. However, when the Ca^{2+} concentration was increased 10-fold to 250 mM Ca^{2+}, the amount of $CaCO_3$ precipitated and CO_2 sequestrated increased by over 100% irrespective of initial urea concentration. Consequently, the optimum conditions for MCP under our experimental conditions were 666 mM urea and 250 mM Ca^{2+} at 2.3×10^8 cells mL^{-1} bacterial cell concentration. However, a greater $CaCO_3$ deposition is achievable with higher concentrations of urea, Ca^{2+}, and bacterial cells so long as the respective quantities are within their economic advantage. X-ray Diffraction, Scanning Electron Microscopy and Energy Dispersive X-ray analyzes confirmed that the precipitate formed was $CaCO_3$ and composed of predominantly calcite crystals with little vaterite crystals.

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1. Introduction

Microbial carbonate precipitation (MCP) occurs as a byproduct of common microbial metabolic processes, such as photosynthesis (McConnaughey and Whelan, 1997), urea hydrolysis (Fujita et al., 2000; Hammes et al., 2003; Dick et al., 2006; De Muynck et al., 2007a,b; Ercole et al., 2007) and sulfate reduction (Castanier et al., 1999; Knorre and Krumbein, 2000; Hammes et al., 2003). Microorganisms whose net cell surface charge is negative have also been reported to act as scavengers for divalent cations including Ca^{2+} and Mg^{2+} in aquatic environment by binding them onto their cell surfaces, thereby making microorganisms ideal crystal nucleation sites (Ferris et al., 1986, 1987; Schultze-Lam et al., 1996; Stocks-Fischer et al., 1999; Ramachandran et al., 2001) and another source of MCP. Another basic advantage of MCP is its ability to sequestrate atmospheric CO_2 through calcium carbonate formation (Ferris et al., 1994; Rodriguez-Navarro et al., 2003; Manning, 2008). The uptake of CO_2 from the atmosphere by surface waters form carbonic acid which reacts with soluble products of weathered silicate minerals in the aquatic environment, and consequently raises the pH which creates a suitable condition for $CaCO_3$ precipitation.

MCP has been used for crack repair in concrete (Bang et al., 2001; Ramachandran et al., 2001; Bachmeier et al., 2002; DeJong et al., 2006), sand consolidation (Ferris and Stehmeier, 1992; Gollapudi et al., 1995; Stocks-Fischer et al., 1999; Nemati and Voordouw, 2003), repair of calcareous monuments (Le Metayer-Levrel et al., 1999; Tiano et al., 1999, 2006; Rodriguez-Navarro et al., 2003; De Belie et al., 2006; Dick et al., 2006; Jimenez-Lopez et al., 2008), concrete compressive strength improvement (Bang et al., 2001; Ramachandran et al., 2001; Ghosh et al., 2005; Jonkers et al., 2010), concrete durability improvement (De Muynck et al., 2007a,b), selective plugging for enhanced oil recovery (Gollapudi et al., 1995), wastewater treatment (Hammes et al., 2003), and soil improvement (Whiffin et al., 2007; Ivanov and Chu, 2008; DeJong et al., 2010).

MCP by urea hydrolysis has been used by many researchers especially its application in bioremediation because ureolytic bacteria are widespread in the environment (Fujita et al., 2000), and an in situ remediation scheme based on urea hydrolysis is not likely to require the introduction of foreign microorganisms. In addition, using ureolytic bacteria to increase pH is preferable to direct addition of a basic solution because the gradual hydrolysis of urea is

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likely to promote a wider spatial distribution of calcite precipitation in the subsurface than the direct addition of base (Ferris et al., 2003), and the rate and quantity of the carbonate precipitated can also be easily controlled (De Muynck et al., 2010).

Ureolytic bacteria especially *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*) and *Bacillus sphaericus* have generated a lot of interest in this area, and have been studied extensively (Fujita et al., 2000; Hammes et al., 2003; Dick et al., 2006; De Muynck et al., 2007a,b; Ercole et al., 2007). These facultative bacteria are able to precipitate calcite through the enzymatic hydrolysis of urea. The microbial urease enzyme hydrolyzes urea to produce dissolved ammonium, dissolved inorganic carbon and CO_2, and the ammonia released in the surroundings subsequently increases pH, leading to accumulation of insoluble $CaCO_3$ in a calcium rich environment. Quantitatively, 1 mol of urea is hydrolyzed intracellularly to 2 mol of ammonium (Eqs. (1) and (2)).

$$CO(NH_2)_2 + 2H_2O \xrightarrow{Urease} 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \xrightarrow{Urease} 2NH_4^+ + CaCO_3 \downarrow \quad (2)$$

These reactions occur under the influence of natural environmental factors that control the activity of the urease enzyme. Factors such as the type of bacteria, bacteria cell concentration, temperature, urea concentration, calcium concentration, ionic strength, and the pH of the media may have a significant impact on MCP. The bacteria should possess high ureolytic efficiency, alkalophilic (optimum growth rate occurs at pH around 9, and no growth at all around pH 6.5), non-pathogenic, and posses the ability to deposit calcite homogeneously on the substratum. The bacteria should also have a high negative zeta-potential (Dick et al., 2006; De Muynck et al., 2007a,b) to promote adhesion and surface colonization, and produce enormous amounts of urease enzyme in the presence of high concentrations of ammonium (Kaltwasser et al., 1972; Friedrich and Magasanik, 1977) to enhance both the rate of ureolysis and MCP (Nemati and Voordouw, 2003).

Urease-catalyzed ureolysis like any other enzymatic reaction is temperature dependent. However, the optimum temperature ranges from 20 to 37 °C depending on environmental conditions and concentrations of other reactants in the system. Ferris et al. (2003), Nemati and Voordouw (2003), and Mitchell and Ferris (2005) reported that increasing the temperature from 15 to 20 °C increased rate of ureolysis, k_{urea} 5 times (from 0.18 to 0.91 d^{-1}) and 10 times greater than k_{urea} at 10 °C (0.09 d^{-1}). It can therefore be emphasized that increasing temperature within the optimum range enhances rate of ureolysis.

Nemati and Voordouw (2003) established that increasing urea and Ca^{2+} concentration beyond 36 and 90 g L^{-1} respectively do not increase the amount of $CaCO_3$ obtained by MCP. In addition, since Ca^{2+} is not likely utilized by microbial metabolic processes, it would accumulate outside the cell where it would be readily available for MCP (Silver et al., 1975).

Ionic charge influences enzymatic reactions like temperature and concentration. In bacteria transport in porous media, the total interaction energy needed by microbial particles to adhere and attach themselves to solid surfaces as explained by the classical Derjaguin–Landau–Verwey–Overbeek theory, is composed of the repulsive electrostatic forces and the attractive Van Der Waals forces. High ionic strength increases electrical double layer (EDL) compression by decreasing EDL repulsive forces leaving attractive Van Der Waals forces to dominate, and in the process promotes bacterial adhesion and attachment to the substratum (Faibish et al., 1998; Foppen and Schijven, 2006). Increase in ionic strength from 0.1 to 1.0 may increase the equilibrium constant for ammonia speciation from 9.3 to 9.4 (Martell and Smith, 1974).

A pH increase is an indication of urea hydrolysis, and is an important property of alkalophiles (optimum growth at pH 9 and no growth below pH 6.5). At any media pH, NH_3 gas and dissolved NH_4^+ exist at different concentrations. Higher concentrations of NH_3 provide favorable conditions for MCP.

The main objective of this research is to determine the optimum conditions for urease catalyzed MCP. The urease enzyme will be supplied by the soil bacteria *S. pasteurii* strain American Type Culture Collection (ATCC) 11859, and the optimum conditions will be determined by the factorial experiments. The factorial experiments will be designed based on the important factors that affect MCP as previously been outlined.

2. Materials and methods

2.1. Stock culture

S. pasteurii strain ATCC 11859, (Manassas, VA) was grown at 30 °C for 72 h with agitation in brain heart infusion (BHI) broth. After growth, cells were platted in an agar plate to confirm their viability and storage.

2.2. Culture medium

The culture medium consisted of 3 g of BHI broth, 10 g of ammonium chloride, and 2.1 g of sodium bicarbonate (Fisher Scientific, Pittsburgh, PA) per liter of distilled water. A varied amount of urea was added to the mixture and the pH was adjusted to 6.5 using 1 N HCl (Fisher Scientific, Pittsburgh, PA) before addition of a varied amount of $CaCl_2$ (Fisher Scientific, Pittsburgh, PA) to avoid premature $CaCO_3$ precipitation. The mixture was then autoclaved at 121 °C for 20 min.

2.3. Factorial experimental design

Factorial experiments were designed based on the important factors that affect MCP (Table 1). Bacterial cell concentration was varied from 10^6 to 10^8 cells mL^{-1} by dilution using ultrapure water (Milli-Q Gradient, Molsheim, France) and quantified by measuring the absorbance (optical density) of the suspension using Spectronic Genesys five Spectrophotometer (Thermo Electron Corporation, Madison, WI) at 600 nm wavelength (OD_{600}). The concentration of cells suspended in the stock culture was estimated by the expression.

$$8.59 \times 10^7 \cdot Z^{1.3627} \quad (3)$$

(Ramachandran et al., 2001), where Z is reading at OD_{600}, and Y is the concentration of cells mL^{-1}.

For each test, 20 mL of the culture medium was mixed with 10 mL of the stock culture in a beaker, and the mixture was stirred slowly using a magnetic stirrer. A pH meter (accumet AB 15, Fisher Scientific, Pittsburgh, PA) and ammonia gas electrode (Cole Parmer, Vernon Hills, IL) were then dipped into the solution in succession to measure pH and ammonia concentration (in millivolts) of the mixture. Measurements were done after 15 min, 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, and every 24 h for 7 d. The ammonia gas concentration was converted to molarity using the ammonia electrode calibration curve provided by the manufacturer. Finally, NH_3 gas concentration was converted to [NH_4^+] by the equations derived from the chemistry of buffer solutions involving ammonium ion (pKa = 9.25). All experiments were done in triplicate.