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Ureolytic activities of a urease-producing bacterium and purified urease enzyme in the anoxic condition: Implication for subseafloor sand production control by microbially induced carbonate precipitation (MICP)

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ABSTRACT

Microbially induced carbonate precipitation (MICP) involves the hydrolysis of urea by indigenous or introduced urease-producing bacteria, which induces carbonate precipitation. By allowing this process to occur in the pores of unconsolidated sand, sand particles bond together, creating a sandstone like material. Although MICP has been explored recently for possible applications in civil and construction engineering, this study examines its application to sand production control during hydrate gas exploitation from subseafloor sediments. The major uncertainty is the ureolytic activities of bacteria and associated enzyme under the subseafloor condition. The main aim of this study was to quantify the ureolytic efficiency of a urease-producing bacterium and purified urease enzyme in the oxic and anoxic conditions. The purified urease enzyme and *Bacillus megaterium* were subject to bench shaking ureolyic activity tests in both conditions. Biochemical parameters including urea concentration, electric conductivity (EC), pH, and optical density at 600 nm (OD_{600}) of the solution at different time intervals were measured. As a quality control procedure, dissolved oxygen concentration (DO) of the final solutions was also measured. Results show that the effect of oxygen availability on ureolytic efficiency of purified urease enzyme is marginal. However, anoxic ureolytic performance of *B. megaterium* is better than its oxic counterpart. It is also found that higher concentration of urease and multi-amendment of bacteria help raise ureolytic efficiency. In order to sustain ureolytic efficiency and facilitate its up-scaled field application, several practice measures can be implemented including growing bacteria aerobically to exponential stage before implemented into the subseafloor sites, injecting larger bacteria cell number, and repeatedly supplying fresh bacteria cells.

1. Introduction

Sand production has been a major obstacle for the successful exploitation of weakly consolidated/unconsolidated oil and gas reservoirs worldwide. It is reported that 70% of the global hydrocarbon reservoirs are susceptible to sand production (Fattahpour et al., 2012). Typically, sand production is defined as sand particles in weakly consolidated subsea hydrocarbon-bearing sediments moving into the exploitation well along with the hydrocarbon and water flows, due to drilling and completion activities. The detachment of particles are usually induced by the combination of high pore fluid velocity and material degradation behavior (Rahmati et al., 2013). If it were to occur, sand production could result in troubles such as plugging of the perforations or production liner, wellbore instability, failure of sand control completions, and pipelines and surface facilities erosion (Rahmati et al., 2013). Several sand production control approaches have been developed by the petroleum industry and academia. These include the construction of sand screen, injection of chemical inhibitors, and setting up solid-fluid separation system. However, there is always a demand for more efficient, economic and durable solution for sand production control.

Recently microbially induced carbonate precipitation (MICP), a bacteria-generated bio-mineralization process, has been investigated extensively in geotechnical and environmental applications (Cuthbert et al., 2013; Jiang et al., 2014; Montoya et al., 2013; Al Qabany and Soga, 2013; Soon et al., 2014). The hydrolysis

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of urea by indigenous or introduced urease-producing bacteria (e.g., *Sporosarcina pasteurii* (*S. pasteurii*), *Sporosarcina aquimarina* (*S. aquimarina*) and *Bacillus megaterium* (*B. megaterium*)) is one of the most popular pathways used to induce carbonate precipitation (Hata et al., 2013; Soon et al., 2013). By allowing this process to occur in the pores of unconsolidated sand, sand particles bond together, creating a sandstone like material.

The carbonate precipitation via ureolysis involves several stages: synthesis of urease enzyme through bacteria metabolic activities (Krajewska, 2009); formation of ammonia (NH_3) and dissolved inorganic carbon (DIC) after urea catalyzed by urease enzyme (Eq. (1)); increase in alkalinity at the proximity of bacteria cells (Eqs. (2) and (3)); formation of carbonate precipitation on bacteria cell surfaces in the presence of available calcium source (Eq. (4)) (Ferris et al., 2004).

$$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2 \quad (1)$$

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \quad (2)$$

$$CO_2 + 2OH^- \leftrightarrow HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O \quad (3)$$

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3(s) \quad (4)$$

The distribution of produced carbonate precipitation has a preference around particle-particle contacts, which is primarily attributed to the microbe's preference to remain away from exposed particle surfaces and stay near smaller surface features (DeJong et al., 2010). Therefore, the particle-particle contacts contribute to stronger cementation within soils. Past studies show that MICP technique has the following highlighted features: (1) Enhancing soil strength and stiffness (Montoya et al., 2013; Al Qabany and Soga, 2013); (2) Retaining soil permeability (Martinez et al., 2013; Whiffin et al., 2007); (3) Creating expanded treatment zone (Martinez et al., 2013); (4) Fast bio-geochemical reaction rate (Martin et al., 2012).

The cementation tends to occur at particles and hence the pore spaces are kept open (DeJong et al., 2010). Therefore, MICP-treated sand provides resistance to erosion, but keeps the flow characteristics (i.e. permeability) similar to the original state for oil/gas production. This unique characteristic of MICP technique can benefit for the subseafloor sand production control, provided necessary technical issues are addressed.

The main issue for the application of MICP in the deep sea conditions is the degree of ureolysis activity of bacteria and pure enzyme at low temperature, high pressure and limited oxygen supply conditions. Hence, the primary objective of this study was to investigate the ureolytic activities of urease-producing bacteria and urease enzyme in oxic and anoxic conditions. The commercially purified urease enzyme and *B. megaterium* were subject to bench shaking ureolytic activity tests in both oxic and anoxic conditions. Biochemical parameters including urea concentration, electric conductivity (EC), pH, and optical density at 600 nm (OD_{600}) of the solution at different time intervals were measured. As a quality control procedure, dissolved oxygen concentration (DO) of the final solutions was also measured. By employing these variables, the ureolysis capacities of both purified urease enzyme and *B. megaterium* in the anoxic condition were assessed against in the oxic condition. It should be noted that, in this study, no cementation reagents were amended into the bacteria solution afterwards. This is to eliminate interference from precipitating calcium, as only ureolysis efficiency was under investigation. The process of calcite precipitation in deep sea conditions will be presented in future publication.

2. Materials and methods

2.1. Bacteria, enzyme and culture media

The investigation involves two series of tests. The first test series involve examination of activities of purified urease enzyme in oxic and anoxic conditions, whereas the second test results examination of ureolytic activities of urease-producing bacteria in oxic and anoxic conditions.

Urease enzyme is often found naturally in algae, fungi, bacteria, plants, and invertebrates (Krajewska, 2009). Commercially, urease has been commonly manufactured through beans purified from the jack bean meal. In this study, purified urease enzyme was supplied by Kishida Chemical, Osaka, Japan, which has an enzyme activity of 2950 U/g (Neupane et al., 2013). The investigation of purified urease enzyme in this work is based on the following two considerations: (1) the fundamental mechanism of MICP is ureolysis by urease enzyme regardless of originating from bacteria or industrial production; (2) the use of purified urease enzyme (instead of urease-producing bacteria) could be an alternative and straightway pathway to trigger carbonate precipitation.

In this study, *B. megaterium* (ATCC 14581) is used as the urease-producing microbe species. It is a Gram positive, rod-shaped soil bacterium with size ranging from 2 to 5 μm (Lian et al., 2006). Although past research has shown that *B. megaterium* has a relatively lower ureolysis rate than *S. pasteurii* (Bachmeier et al., 2002; Whiffin, 2004), the selection of *B. megaterium* is more relevant to this study, as it is used under the deep-sea conditions. This is due to the reason that *B. megaterium* can form endospores that are highly resistant to extreme environmental conditions. More specifically: (a) *B. megaterium* can grow at temperatures from 3 °C to 45 °C (Vos et al., 2009). It means that it can be potentially used at low temperature under deep sea condition while also adaptive to the heating environment during hydrate dissociation; (b) *B. megaterium* has the ability to grow on many carbon sources even including some waste (Vary, 1994); (c) *B. megaterium* is found to be able to survive toxic environments and may have potential as a detoxifying agent (Vary, 1994). (d) The large and elongated rod-shaped *B. megaterium* cell may provide the advantage of avoiding being flushed out during depressurization process during hydrate dissociation. Considering the adaptability of *B. megaterium* in the severe environment, it is a more reliable decision to use *B. megaterium* as a potential candidate for MICP application under deep sea conditions.

The culture media used in this study for the harvest of *B. megaterium* is ATCC-Medium 3. In the initial stage, freeze-dried culture was rehydrated in the nutrient broth solution, which consisted of 8.0 g nutrient broth in 1 L distilled water and had been autoclaved at 121 °C. Then, the rehydrated bacteria cells were grown on a plate which also contained nutrient agar (23 g in 1 L distilled water, sterilized at 121 °C) at 20 °C overnight. Afterwards, a single colony was transferred to the liquid media solution, which contained 8 g/L nutrient broth and 5 g/L NaCl. The bacteria solution was then harvested in a constant-temperature incubator until a final OD_{600} of 0.1 was achieved.

2.2. Shaking ureolytic activity test

The ureolytic activities of both purified urease enzyme and *B. megaterium* were investigated via the bench shaking test at constant ambient temperature of 20 °C. The schematic of the test procedures are shown in Fig. 1. For the oxic case, either purified urease enzyme in powder or bacteria solution were added into sterile Erlenmeyer flasks, which had been filled with 100 mL urea solution (for urease) or liquid media solution with urea (for bacteria solution). The flasks were then stoppered with foam plugs. For the anoxic case, either purified urease enzyme in powder or