#### Construction and Building Materials 82 (2015) 167-172

Contents lists available at ScienceDirect



**Construction and Building Materials** 

journal homepage: www.elsevier.com/locate/conbuildmat

# The influence of standing time and content of the slurry on bio-sandstone cemented by biological phosphates





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#### HIGHLIGHTS

• A new method was found to bind loose sand particles into bio-sandstone.

• Loose sand particles can be cemented by biological barium hydrogen phosphate.

• Bio-sandstones cemented by biological phosphates have good mechanical properties.

### G R A P H I C A L A B S T R A C T

Bio-calcite cement and portland cement can release ammonia and carbon dioxide respectively, and these gases have a negative impact on the environment. In response to these aspects, a new binder should be selected to cement loose particles, and bio-phosphate cement (microbial induced deposition phosphate) was taken into our consideration due to the presence of phosphate minerals in the nature. In this research, barium hydrogen phosphate by microbial deposition can well bind loose sand particles into sandstones.



#### ARTICLE INFO

Article history: Received 23 September 2014 Received in revised form 18 January 2015 Accepted 18 February 2015 Available online 9 March 2015

Keywords: Slurry Barium hydrogen phosphate Bio-sandstone Compressive strength Scanning electron microscope

#### ABSTRACT

Loose sand particles can be cemented by the microbial inducing the slurry of barium hydrogen phosphate precipitation into a bio-sandstone with certain mechanical strength, which is a new technique to bound loose sand particles with biological phosphates. This paper confirms the feasibility of biological phosphates binding loose sand particles and presents results from laboratory researches on the influence of standing time and content of the slurry on the mechanical properties of sandstone biological phosphates bound. The results show that standing time and content of the slurry have a strong influence on the mechanical properties of bio-sandstone. The best standing time, which is 24 h, of the slurry mixing with quartz sand and obtained higher compressive strength of sand column (bio-sandstone) than three other standing times. The maximum compressive strength of bio-sandstone is 2.1 MPa when the content of the slurry reaches 50% with 24 h standing time. In addition, the ingredients of the slurry and bio-sandstone are mainly hydrogen phosphate barium through XRD analysis. The corresponding variation of porous characteristics of sand column is observed by scanning electron microscope (SEM) and indicated that the cementitious materials between quartz sand are mainly irregular sphere clusters in shape. The technique can be used in other fields: including dust-fixing, foundation reinforcement, slope and so on.

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http://dx.doi.org/10.1016/j.conbuildmat.2015.02.038 0950-0618/© 2015 Elsevier Ltd. All rights reserved.

#### 1. Introduction

Loose particles are cemented by microbial induced calcium carbonate precipitation (MICP) into a bio-sandstone, which has been extensively applied in different fields [1–5]. It is rare for ureolytic bacteria to excrete urease. In most cases, urease is transported into the cell and hydrolyzed. *Helicobacter pylori* is an exception. Calcium carbonate is obtained when the carbonate reacts with calcium in the process of MICP [6,7]. The process will release ammonia and ammonium (NH<sub>3</sub>/NH<sub>4</sub>), and the gas has a negative impact on our human health and the environment when the concentration of ammonia and ammonium is higher than safety threshold. Based on cementation characteristic of MICP, this method can significantly improve the mechanical properties of bio-sandstone and has been used in soil improvement, anti-liquefaction of loose particles, bluff, slope, etc. [8–10].

MICP will release ammonia and ammonium (NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>) in reaction which is a serious disadvantage. Therefore, we proposed cementitious materials of biological phosphates to bind loose particles into a bio-sandstone which have certain mechanical properties. In this research, Bacillus subtilis was selected as the suitable microorganism, and which could produce alkaline phosphatase (EC 3.1.3.1) that constantly hydrolyzed phosphate monoester in bacterial solution, then  $PO_4^{3-}$  is obtained [11,12]. A part of phosphates has serious and extensive impact on human health and environment, for example, which can lead to human poisoning, eutrophication in lakes and so on. MHPO<sub>4</sub>, MPO<sub>4</sub> or M<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> are prepared when phosphate reacts with metal cations. Alkaline phosphates, such as barium hydrogen phosphate has been extensively studied in the past for their applications in different fields such as bioceramics, metal doped, corrosion resistance, ionic conductivity and ferroelectrics, so the barium ion is chosen [13–16]. Microbial-induced precipitation of barium phosphates is mainly barium hydrogen phosphate because of the acidic solution of BaCl<sub>2</sub>·2H<sub>2</sub>O. Different content of the slurry of barium hydrogen phosphate and quartz sand were evenly mixed under different standing time and then the mixture was added to 60 ml plastic syringe (inner diameter of 30 mm). After 15 d, loose sand particles were cemented into a sand column. The computer-controlled electronic universal testing machine and SEM were used to test and observe the compressive strength of all the specimens and the internal microstructure of sand columns respectively.

#### 2. Experiments and methods

#### 2.1. Materials

All raw materials were of analytically pure grade without further purification, and deionized water was self-made. *B. subtilis* was selected to apply for cementation experiment. The solution of phosphate monoester (0.125 ± 0.01 mol/l) was prepared before use. The aggregate used in this study was less than 300  $\mu$ m of quartz sand (grain size characteristics:  $d_{10} = 150 \,\mu$ m (10% of the grains was lower than a diameter of this size);  $d_{90} = 300 \,\mu$ m).

*B. subtilis* cultivation: 3 g of beef extract, 5 g of peptone, and 1.5 g of NaCl were completely dissolved in 1 L of deionized water. The mixed solution was then adjusted to a pH value of about 7.0 using diluted NaOH solution. Next, 1 L of mixed solution was added to two bottles (500 ml), and each bottle was wrapped by employing gauze and paper, and placed in an autoclave under 125 °C and 0.1 MPa conditions for 25 min. Until the above boiled culture was cooled down to ambient temperature, 3 ml of liquid strains was added to each bottle and cultivated in the oscillation incubator (170 r min<sup>-1</sup>) at  $30 \pm 2$  °C for 24 h. In general, the harvested microorganisms were kept in a refrigerator at  $4 \pm 0.2$  °C for stock prior to use. Microbe having OD<sub>600</sub> value of 0.8 and enzyme activity value of 0.4 mmol/(L min) was used in this study.

#### 2.2. Preparation of the slurry of barium hydrogen phosphate

The solution of substrate  $(0.125 \pm 0.1 \text{ mol/l}, 80 \pm 5 \text{ ml})$  was poured into a 1 L *B. subtilis* liquid, and the mixture solution was allowed to stand under static conditions for 2 h at ambient temperature of  $30 \pm 2$  °C. Two hours later, there was a large



Fig. 1. Loose sand particles to bio-sandstone cemented by cementitious material of biological phosphates.

number of sediment at once after  $BaCl_2 \cdot 2H_2O(20 \pm 0.1 \text{ mM})$  was added to the above solution. The precipitated solution was allowed to stand under static conditions at room temperature for 0, 12, 24, and 36 h. The precipitated slurry was obtained via removing away the supernatant of the precipitation. The weight at standing time of 0, 12, 24, and 36 h respectively, the average mass of dried slurry was corresponded to 8.13, 10.02, 10.07 and 10.05 g per 1 L bacteria.

#### 2.3. Specimen preparations

#### 2.3.1. Precipitated slurry of different standing time cemented quartz sand into biosandstone

Standing the precipitated slurry for 0, 12, 24, 36 h in the mixed solution of *B. subtilis* and phosphate monoester respectively and mixing with quartz sand. The quality of precipitation accounted for 20% and 40% of the total mass of quartz sand, and mechanical mixing until completely homogeneous, the mixed sand mortar was then poured into 60 ml plastic syringe. Each group of specimen including three cylinder samples of 30 mm in diameter × 60 mm in length was cast to determine the compressive strength. After casting, specimens were kept at  $30 \pm 2$  °C until the test ages was reached and the state before and after cementing loose sand particles is given in Fig. 1.

#### 2.3.2. Cement different dosage of precipitated slurry with quartz sand into biosandstone

Mixed different content of precipitated slurry with quartz sand evenly (the quality of precipitated slurry accounted for 10%, 20%, 30%, 40% and 50% of the total mass of quartz sand), and sand mortar was then poured into 60 ml plastic syringe. Each group of specimen including three cylinder samples of 30 mm in diameter  $\times$  60 mm in length was cast to determine the compressive strength. After casting, specimens were kept at 30 ± 2 °C until the test ages was reached, 15 specimens were obtained. All experiments designed in the paper were carried out in triplicate.



Fig. 2. XRD patterns of precipitated slurry for different standing time.

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