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Improving bio-desilication of a high silica bauxite by two highly effective silica-solubilizing bacteria

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ABSTRACT

Bacteria play an important role in bio-desilication of high silica bauxites. However, low bio-desilication efficiency and unknown mechanisms limit the use of bacteria in bio-desilication of bauxite. In this study, two highly effective Si-solubilizing bacteria (*Arthrobacter pascens* H19 and *Burkholderia anthina* G21) were obtained and characterized for their impact on Si and Al release from a high-silica (18.1%) bauxite and the mechanisms involved. Dissolved Si and Al concentrations in the culture medium were 3.3–13.9 mM and 0.04–0.41 mM, and total Si and Al released from the bauxite were 2.76–11.64 mg and 0.03–0.39 mg, respectively, in the presence of these strains. Strain G21 released more Si from the bauxite than strain H19 at the end of the experiment. These strains produced malic (791–1700 μM), citric (94–447 μM), and succinic (228–494 μM) acids, and exopolyssaccharides (EPS) (39–420 mg L^{-1}) during the bio-desilication processes. Furthermore, strain G21 produced more organic acids (19–65%) than strain H19, while strain H19 produced more EPS (1.7–4.9-fold) than strain G21. The metabolites (fermentation broth, organic acids, and EPS) of these strains also significantly increased dissolved Si concentration compared to the control. Notably, the ratios of Al_2O_3 to SiO_2 in the bauxite treated with strains H19 and G21 were 9.14 and 9.64, respectively, which met the requirement in the Bayer process for alumina production. The results showed distinct and high bio-desilication effectiveness of these strains and suggested that the highly effective Si-solubilizing bacterial strains increased bio-desilication efficiency through the production of organic acids and EPS.

1. Introduction

Bauxite resources are widely distributed in the world and are the main resources for aluminum production (Ma et al., 2009, 2011). Almost all of bauxite ores in China are diasporic-characterized and do not meet the demands in the Bayer process for aluminum production due to their high silica content and low Al_2O_3:SiO_2 ratio (Ma et al., 2009). Bayer process has the advantages of low energy consumption, simple procedure, and high-quality product and is the main method for the production of aluminum from bauxite in the world (Paz et al., 2017). The bauxite ores which have high alumina content and high Al_2O_3:SiO_2 ratio are needed in the Bayer process for aluminum production (Li et al., 2016). Bauxites are characterized by high Al and Si and low Al_2O_3:SiO_2 ratio (5–8) for most (80%) of bauxites in China (Zhao et al., 2010). Silicon is not only the main impurity in bauxite but also one of the most harmful impurities in the process of aluminum production using the Bayer process (Chen et al., 2016a). Furthermore, high silica bauxites which reactive silica content is more than about 8% need high caustic consumption and do not meet the requirement in the Bayer process for alumina or aluminum production (Smith, 2009). To solve this problem, many efforts have been made to improve the feed grade of the bauxites for the Bayer process (Ma et al., 2011; Chen et al., 2016a). In bauxite beneficiation, the physic-chemical processes are expensive and energy dissipation and not environmental-friendly (Ma et al., 2009; Smith, 2009). Bio-beneficiation has been considered to be environmentally benign and cost-effective for many types of low grade bauxites (Pradhan et al., 2006; Zhao et al., 2017).

Bio-beneficiation refers to the removal of undesirable mineral components from ores through the selective dissolution and removal of microorganisms, resulting in the enrichment of the desired mineral constituents in the solid ores (Anand et al., 1996). Bacteria and fungi have been found to play important role in the removal of Fe, Ca, and Si from clays, sands and bauxites (Anand et al., 1996; Groudev, 1999; Vasan et al., 2001). *Paenibacillus polymyxa* was reported to be efficient in removing Fe and Ca from bauxite (Anand et al., 1996; Vasan et al., 2001). Furthermore, Zhao et al. (2017) showed that *Paenibacillus*

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mucilaginosus BM-4 could release Si from aluminosilicate minerals. After aluminosilicate minerals were treated with *Paenibacillus mucilaginosus* BM-4, the Al/Si ratios of the minerals were improved from 3.05 to 8.60. Furthermore, the grade of bauxite could be enhanced by removing silica with *Bacillus polymyxa* (Anand et al., 1996). Bacteria promoted Si release from Si-bearing rocks and minerals through the production of microbial metabolites such as organic acids and expolyssaccharides (Gehrke et al., 1998; Pradhan et al., 2006; Sheng et al., 2008).

Although different microorganisms have been reported to be used in ore leaching, very few investigations have been reported on the use of microorganisms to remove silica from high silica bauxites. Bio-desilication by bacteria is a potential and promising technology for its characteristics of environmental protection, low energy consumption and sustainable development. However, low bio-desilication efficiency, unknown mechanisms of bio-desilication, and genetic instability of bacteria may limit the industrial use of this technology at present (Zhao et al., 2017). It is necessary to screen highly effective Si-solubilizing bacteria and evaluate their bio-desilication ability and the mechanisms involved for increasing the ratio of aluminum to silicon in bauxites in the Bayer process.

The objectives of the present study were to characterize the effectiveness of Si and Al release from a high silica bauxite in the presence of Si-solubilizing bacteria, to obtain highly effective Si-solubilizing bacteria, and to analyze the bio-desilication efficiency of the bauxite and the mechanisms involved. The results may give us to better understand the role of Si-solubilizing bacteria in Si removal from high silica bauxites and provide an environmentally friendly and cost-effective method for bio-beneficiation of high silica bauxites.

2. Materials and methods

2.1. Characterization of bauxite

The high silica bauxite was obtained from Shanxi Aluminum of Hejin, Shanxi province (China). The bauxite was crushed in a disc vibration grinder (RS 200; Retsch, Germany), sieved to isolate the 75- to 150-μm mineral powders, and cleaned based on the method of Sheng et al. (2008). The treated bauxite was then used for element analysis and bio-desilication experiments. The elemental composition of the bauxite was analyzed through total digestion based on the US EPA SW 846 Method 3050B (US EPA, 1996). A quadrupole inductively coupled plasma mass spectrometer (Q-ICP-MS, Perkin-Elmer, ELAN DRC-e) was used and further analysis was also made using an X-ray fluorescence spectrometer (Unique UX-230). The mineral compositions of the bauxite were analyzed by powder X-ray Diffraction (XRD) according to the method of Chen et al. (2016b).

2.2. Screening of highly effective Si-solubilizing bacteria

Twelve Si-solubilizing bacterial strains isolated from weathered rock (trachyte and mica schist) surfaces were tested for their Si and Al release from the bauxite. The Si and Al dissolution experiment of the bacterial strains was performed based on the method of Huang et al. (2014). Briefly, the strains were cultivated in liquid LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl, pH 7.0) and harvested by centrifugation respectively. Bacterial inoculum was washed in sterile distilled water two times to remove the nutrient and metabolic products from the medium. Cell pellets were then resuspended in sterile distilled water to a final concentration of 10^8 cells mL^{-1}. Triplicate 150-mL flasks containing 30 mL of sterilized SSM (1% sucrose, 0.1% $(NH_4)_2SO_4$, 0.001% NaCl, 0.05% $MgSO_4{\cdot}7H_2O$, 0.2% K_2HPO_4, 0.05% yeast extract, 0.001%$CaCO_3$, pH 7.2) (Zhao et al., 2013) (containing 0.1 g of 75- to 150-μm particles of bauxite) were each inoculated with 0.5 mL of bacterial suspension. The flasks were incubated at 28 °C on a rotary shaker at 150 rpm for 7 days for dissolved Si and Al determination. Controls containing bauxite and culture medium but no bacterial inoculum were treated in the same manner to monitor abiotic dissolution.

2.3. Identification of the highly effective Si-solubilizing bacteria

Based on Si and Al dissolution experiment of the bacteria, strains H19 and G21 were found to be the highly effective Si-solubilizing bacteria. Strains H19 and G21 were characterized and identified based on their morphological, physiological, and biochemical properties with reference to *Bergey's Manual of Determinative Bacteriology* (Holt et al., 1994) as well as by 16S rRNA gene sequences (Huang et al., 2014). The sequences obtained were subjected to similarity searches with the tool of the National Centre of Biotechnology Information (NCBI) BLAST program (http://www.ncbi.nlm.nih.gov) and EzTaxon (http://www.eztaxon.org) (Kim et al., 2012). Phylogenetic analysis was performed using the MEGA software (version 4.0) (Tamura et al., 2007) after multiple alignment of data using CLUSTAL X (Thompson et al., 1997). Distances were calculated according to the distance options with Kimura's two-parameter model and clustering with the neighbour-joining (Saitou and Nei, 1987) algorithms. The sequences of strains H19 and G21 have been deposited in the NCBI database under accession number of KC934811 and KM019723, respectively.

2.4. Bio-desilication experiment of the highly effective Si-solubilizing bacteria

The bio-desilication experiment was performed as described above with some modifications. The flasks were incubated at 28 °C on a rotary shaker at 150 rpm for 15 days. The dissolved Si and Al, pH, organic acid, and EPS in the culture medium were measured at different incubation times. In this study, we prepared individual flasks for each time point. To compensate for the evaporation of liquid from the culture medium during incubation, the volume in each flask was adjusted to 30 mL with distilled water at the time of sampling for the determination of Si and Al mobilization, pH, and organic acid and EPS production. At the end of the experiment, solid residue of the bauxite was separated by filtration through a 0.45-μm Millipore filter and washed gently in sterile distilled water, dried at 50 °C and analyzed for elemental composition of the bauxite.

2.5. Impact of bacterial metabolites on the bio-desilication of the bauxite

The role of bacterial metabolites in Si and Al removal from the bauxite was also assessed. The experimental design was as follows-treatment 1: 30 mL sterilized distilled water with 0.1 g bauxite (control); treatment 2: 30 mL sterilized bacterial fermentation broth of H19 (at 3 days of incubation in liquid SSM) with 0.1 g bauxite; treatment 3: 30 mL sterilized bacterial fermentation broth of G21 (at 3 days of incubation in liquid SSM) with 0.1 g bauxite; treatment 4: 30 mL mixed organic acids (the concentrations of malic, citric, and succinic acids were 900, 100, and 350 mg L^{-1}, respectively, the organic acid concentrations used were based on the organic acid production by strains H19 and G21 at 3 days of incubation) with 0.1 g bauxite; treatment 5: 30 mL 350 mg L^{-1} EPS produced by strain H19 (at 5 days of incubation, the EPS concentration in the cultural medium was 350 mg L^{-1} in the presence of strain H19) with 0.1 g bauxite; treatment 6: 30 mL mixed organic acids + EPS with 0.1 g bauxite. The bacterial fermentation broth was sterilized by autoclaving at 121 °C for 30 min. After 7 days of incubation, the dissolved Si and Al concentrations in the solution were analyzed.

2.6. Analytical methods

For Si and Al analysis, 20 mL of medium was collected from a given flask and filtered through a 0.45-μm Millipore filter. Of the filtrate, 15 mL was then centrifuged at 8943*g* for 10 min to remove the cells and