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Bioleaching phosphorus from fluorapatites with acidophilic bacteria

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ABSTRACT

Currently known high grade easily-acquirable phosphate rock reserves are depleting. The objective of this study was to evaluate the potential application of bioleaching technology to the recovery of phosphorus from different phosphate ore materials, and isolate indigenous phosphorus-solubilising bacterial strains from them. In the bacterial enrichment and isolation experiments no growth was detected in the medium designed for acidophiles. Heterotrophic phosphorus solubilising isolates were enriched from the fluorapatite concentrate, and all four isolates were most closely related to *Burkholderia fungorum*. Bioleaching of phosphorus from low grade fluorapatite ore containing 8.2% P_2O_5 and from fluorapatite concentrate containing 29.8% P_2O_5 was carried out in shake flasks. Supplemental elemental sulphur was added as an energy source for acid generation. Mixed and pure acidophilic bacterial cultures consisting of iron- and/or sulphur-oxidizing bacteria *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* were used in the experiments. These acidophiles are commonly used in bioleaching of sulphide minerals, but their application to phosphorus solubilisation has until now been limited. Phosphorus leaching was shown to be a pH-dependent phenomenon. Phosphorus bioleaching yields of up to 97% and 28% were obtained in 21 d for low grade fluorapatite ore and concentrate, respectively, in solutions with a 1% solid content. With adaptation, the bacterial mixture was acclimated to suspensions with a 10–30% solid content. These results indicate a potential for the application of bioleaching to phosphorus extraction of low grade materials.

1. Introduction

Currently known high grade easily-acquirable phosphate rock reserves required by the fertilizer industry may be depleted within a few decades (Cordell et al., 2009; EEA, 2013). Although there will undoubtedly be more phosphate discoveries after current identified reserves are depleted (Schröder et al., 2010), they will likely require progressively more effort and expense to extract and refine. The future supply of phosphate rock is expected to be of lower quality, contaminated foremost with cadmium and uranium, less accessible and more unequally distributed around the world (Cooper et al., 2011; EEA, 2013), thus creating a need to develop environmentally sustainable and cost-effective processes for exploitation of lower grade phosphorus containing materials.

Currently, the EU imports virtually all its phosphorus with the import dependency rate of approximately 92% (year 2011) (European Commission, 2013a) and, as reserves diminish, this will be the case for other countries that have large unsustainable phosphorus demands. 75% of the estimated phosphate reserves are located in Morocco and Western Sahara (Jasinski, 2013). The US is already heavily dependent on imports, and China is likely to increasingly depend on imported phosphorus in the future, increasing global pressures on the phosphate import market (European Commission, 2013b). Phosphate extraction from sub-economic reserves and other under-utilised sources may mitigate the pressure of global and regional phosphorus shortages. In addition to sub-economic rock phosphate reserves, waste rock from iron ore mining and steelmaking by-products enriched in phosphorus are largely unexploited potential sources of phosphorus. Targeted development and deployment of phosphorus bioleaching technologies that utilise these materials could reduce dependence on imported phosphorus as well as the pressure on conventional high-grade phosphorus resources.

In fertilizer production, the phosphorus concentrate is reacted with sulphuric acid in a wet chemical process to generate phosphoric acid. Sulphuric acid is generally either transported to the site or prepared in a sulphuric acid plant. However, chemical leaching is economic only when recoverable phosphorus is present at relatively high levels. The requirements for phosphate ore in the traditional wet process to contain >30% P_2O_5 and have a CaO/P_2O_5 ratio of <1.6, MgO content of <1%, and Fe_2O_3 and Al_2O_3 content of <2.5% are generally obtained through screening, scrubbing, heavy media separation, washing, roasting, calcinations, leaching and flotation (Gharabaghi et al., 2010).

Bioleaching with acidophilic, Fe- and S-oxidizing microorganisms has been proposed as an alternative treatment method for waste materials

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that contain relatively low levels of valuable metals, or are otherwise difficult to handle or treat. Due to the limited types of substrates available in mining environments, the predominant metal-sulphide dissolving microorganisms occurring in such environments are chemolithotrophic, extremely acidophilic bacteria using either inorganic sulphur compounds and/or ferrous iron (Fe^{2+}) ions as respiratory substrates. Mining biotopes, however, show greater microbial diversity than previously anticipated (Rohwerder et al., 2003). The commonly used leaching bacteria are *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*, which together with *Acidithiobacillus caldus* belong to γ-proteobacteria. *Acidiphilium*, *Leptospirillum*, *Acidimicrobium*, *Ferromicrobium* and *Sulfobacillus* species also belong to leaching bacteria, as well as archaeabacteria of the genus *Sulfolobales* (Rohwerder et al., 2003). Acidophilic iron and sulphur oxidizers have been widely used in bioleaching of sulphidic minerals (Rawlings, 2002); however, no industrial scale application of biological phosphorus leaching has been reported. In previous scientific studies, *A. ferrooxidans* and *A. thiooxidans* have been shown to leach phosphorus from fluorapatite ore (Bhatti and Yawar, 2010), calcium phosphate (Chi et al., 2006), sewage sludge ash (Zimmermann and Dott, 2009) and phosphorus containing iron ores (Wang et al., 2010). The rate of phosphorus dissolution is directly related to the rate at which the bacteria produce sulphuric acid (Bhatti and Yawar, 2010). Nevertheless, acid production is probably not the only mechanism involved.

Heterotrophic bacteria solubilise inorganic phosphorus via production of organic acids, like gluconic acid, acetic acid, oxalic acid, citric acid, lactic acid and itaconic acid (Khan et al., 2009). The chelating ability of organic acids is important, and e.g. Kim et al. (1997) showed that organic acids may be more effective in solubilising phosphorus than inorganic acids at the same pH. Strains from the genera of *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most powerful phosphate solubilisers (Illmer and Schinner, 1992; Rodríguez and Fraga, 1999), but also other heterotrophic bacterial genera and species have been shown to have phosphorus solubilising capability, like *Burkholderia* spp., *Clavibacter xyli*, *Enterobacter agglomerans*, *Serratia* and *Streptomyces* (Delvasto et al., 2008; Kim et al., 1997; Postma et al., 2010).

Most bacteria, including leaching bacteria, grow on surfaces of minerals and form multispecies biofilms. It has been shown that when substrate surface area is not limiting, >80% of the inoculum disappears from the solution within 24 h, forming a biofilm instead (Bagdigian and Myerson, 1986; DiSpirito et al., 1983; Gehrke et al., 1998). However, some cells also remain in the planktonic stage (Rohwerder et al., 2003). The non-contact bioleaching is exerted by planktonic bacteria, which oxidize ions in solution, whereas the contact mechanism of bioleaching takes place at the interface between the bacterial cell and the mineral surface (Rohwerder et al., 2003).

The objective of this study was to evaluate the bioleaching process for recovery of phosphorus from fluorapatite concentrate and low grade fluorapatite ore containing 29.8% and 8.2% P_2O_5, respectively. We also sought to isolate indigenous phosphorous leaching bacterial strains from the ore samples.

2. Materials and methods

2.1. Substrates for bioleaching

Initial bioleaching experiments were performed with hydroxyapatite (Sigma 21223) and $FePO_4$ (Sigma 436011). Subsequently low grade fluorapatite ore and fluorapatite concentrate from two different deposits were studied. The materials were stored in closed containers at room temperature and handled aseptically. The dominant mineral phases of the materials were identified by X-ray diffraction (XRD), and the P_2O_5 content of each was determined using X-ray fluorescence (XRF). Material pH was measured in deionized water at a 1:5 solid–solution ratio.

2.2. Bacterial strains

The bacterial strains and mixtures used in the study are described in Table 1. Pure cultures were obtained from the VTT Culture Collection (http://culturecollection.vtt.fi), and grown in their respective DSMZ (http://www.dsmz.de/) media for 7 d. A mixture of pure cultures (PCmix) was made by mixing 3 ml of each of the six pure culture suspensions. A natural mixture of bacteria (NATmix) had previously been enriched from acidic waters of the Talvivaara mine, Finland as described in Halinen et al. (2009). The enrichment culture contained bacteria related to *A. ferrooxidans*, *A. thiooxidans*, *A. caldus*, *L. ferrooxidans* and *Sulfobacillus thermotolerans* (Dopson et al., 2007; Halinen et al., 2009).

2.3. Isolation of bacteria from the ore samples

The isolation and enrichment of indigenous microbes from the ore samples were conducted within a month after the arrival of the samples. For isolation of heterotrophic phosphate solubilising microbes (PSM), the ores (5 g) were incubated in 100 ml NBRIP (National Botanical Research Institute's phosphate growth medium) broth (10 g glucose, 5 g $Ca_3(PO_4)_2$, 5 g $MgCl_2 \cdot 6H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.2 g KCl, 0.1 g $(NH_4)_2SO_4$ per litre; Nautiyal, 1999) for 21 d at 30 °C, agitated at 140 rpm. Following incubation the samples from the enrichment broth were cultured on NBRIP agar plates (NBRIP + 20 g agar l^{-1}) to isolate PSM. Ore samples were also directly cultured on NBRIP agar with either glucose or sucrose as a carbon source, and pH adjusted to 5 or 8. Ten grams of each ore were shaken (140 rpm) in 90 ml of Ringer's solution (Merck, Darmstadt, Germany) for 24 h, and cultured on NBRIP plates as a dilution series, and incubated at 30 °C for 12 d. PSM were identified based on the formation of visible halo/zone on agar plates.

For enrichment and isolation of autotrophic PSM, the ores (5 g) were incubated in modified 9 K medium (3 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g K_2HPO_4, 0.1 g KCl, 0.01 g $Ca(NO_3)_2$, 44.2 g $FeSO_4 \cdot 7H_2O$ and 10 g S^0 l^{-1}; (Silverman and Lundgren, 1959; Xiao et al., 2011) for 21 d at 30 °C, agitated at 140 rpm. The pH of the medium was adjusted to 1.8 with 0.5 M H_2SO_4. The enrichment cultures were followed by pH and redox potential measurements and by microscopy.

The obtained isolates with visible halo/zone on NBRIP agar plates were purified and identified by partial sequencing of their 16S rRNA gene. An approximately 500-bp fragment from the 5′ end of the 16S rRNA gene was amplified with universal primers BSF8/20 (5′-AGAGTT TGATCCTGGCTCAG-3′) and BSR534/18 (5′-ATTACCGCGGCTGCTGGC-3′). DNA extraction, PCR amplification and sequencing reactions have been described in detail in Katina et al. (2007). The obtained sequences were compared against GenBank sequences with BLASTN search (Camacho et al., 2009).

2.4. Bioleaching experiments

The experiments with the autotrophic strains were performed in modified 9 K medium without added phosphate (3 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g KCl, 0.01 g $Ca(NO_3)_2$, 44.2 g $FeSO_4 \cdot 7H_2O$ and 10 g S^0 l^{-1}; Silverman and Lundgren, 1959; Xiao et al., 2011). The pH of the medium was adjusted to 2.5 with 0.5 M H_2SO_4. Bioleaching experiments with the heterotrophic strain were carried out in NBRIP medium (Nautiyal, 1999) without added phosphate (10 g glucose, 5 g $MgCl_2 \cdot 6H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.2 g KCl, 0.1 g $(NH_4)_2SO_4$). The pH of the medium was adjusted to pH 8 with NaOH. The experiments were performed in Erlenmeyer flasks with 180 ml of medium, 2 g of sterilized ore sample (1% w/v), and 20 ml (10% v/v) of bacterial culture (approx. 10^7 bacteria/ml). The flasks were shaken for 21 d in 30 °C at 140 rpm. Samples of 10–12 ml were taken at days 0, 2, 5, 7, 9, 14, 16 and 21, and the pH and redox potential were measured. After centrifugation (2000 rpm, 5 min) and filtration (Whatman FP30/0.45 CA-S) the solubilised orthophosphate content of the samples was determined by absorbance at 882 nm using a Hach-Lange LCK349 kit (Hach