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Microbial mineralization of struvite: A promising process to overcome phosphate sequestering crisis

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ABSTRACT

Due to extensive exploitation of non-renewable phosphate minerals, their natural reserves will exhaust very soon. This necessitates looking for alternatives and an efficient methodology through which indispensable phosphorus can be harvested back. The current study was undertaken to explore the potential of a metallophilic bacterium *Enterobacter* sp. EMB19 for the recovery of phosphorus as phosphate rich mineral. A very low phosphate concentration strategy was adopted. The process led to the mineralization of phosphorus as homogeneous struvite crystals. For each gram of Epsom salt added, the cells effectively mineralized about 20% of the salt into struvite. The effect of different inorganic sources, culture profile and plausible mechanism involved in crystal formation was also explored. The synthesized struvite crystals typically possessed a prismatic crystal habit. The characterization and identification of the crystals were done using single crystal X-ray diffraction (SCXRD), powder X-ray diffraction (PXRD), energy dispersive X-ray analysis (EDAX) and fourier transform infrared (FTIR). The thermal characteristics were studied using thermo gravimetric analysis (TGA) and differential scanning calorimetric (DSC) processes. The synthesis of struvite by this bacterium seems to be a promising and viable strategy since it serves dual purpose (i) obtaining phosphorus and nitrogen rich fertilizer and (ii) conservation of natural phosphate reserves. This study is very significant in the sense that the process may be used for harvesting and synthesizing other valuable minerals. Also, it will provide new insights into phosphate biomineralization mechanisms.

1. Introduction

Phosphorus (P) is an indispensable macronutrient in all forms of living organisms. It plays a pivotal role in the biological system for the synthesis of various biomolecules, structural components, genetic processes, cycling and storage of energy (Elser, 2012). Till date it does not have any substitute in nature. Therefore, phosphorus is likely to act as a limiting factor in the flourishing of all organisms, whether microbes, plants or human. In many places due to short supply of phosphorus in soil, phosphate-based fertilizers have been used to increase agricultural yields (Dawson and Hilton, 2011). The process has led to extensive mining and exploitation of the finite phosphate reservoir. In 2009 itself, 23 million tonnes of phosphorus was mined around the world to furnish the global demand of

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phosphate fertilizers (Elser and Bennett, 2011). It has been anticipated that if the demand for the phosphate fertilizers continues at the current rate of 2.5–3% per annum for the next 5 years, the world's reserves will last only for around 125 years (Cordell and White, 2011). Also, about 40–60% of inevitable loss of phosphate occurs during the entire process of converting host phosphate rock into fertilizer, and even if these reserves could be processed economically, they are not renewable and hence will be exhausted in the near future (Gilbert, 2009). Not surprisingly there is an urgent need of looking at new and viable processes through which lost phosphorus can be harvested again into useful minerals.

Struvite (Magnesium ammonium phosphate hexahydrate; ($MgNH_4PO_4 \cdot 6H_2O$) is a valued and environmental friendly fertilizer (Parsons and Smith, 2008). It is rich in phosphorus and nitrogen content. The presence of magnesium makes it even more attractive than other commercial fertilizers, as it cannot be easily washed away by water. Hence, it can be effectively used in acidic, hilly or flooded lands (Lee et al., 2009; Pastor et al., 2008). Recently, the importance of struvite as fertilizer has been realized and industries like Unitika Limited in Japan have started selling struvite to American fertilizer companies (Lee et al., 2009). However, large scale industrial production or crystallization of struvite is still limited. This is mainly because of the problems related to its crystallization which need appropriate pH control and expensive reagents (Pastor et al., 2010).

Although nature has provided a vast resource of microbial phenomena, we have harvested very few of them for our betterment. Use of metallophilic microbes for struvite production and harvesting phosphorus from the environment seems to be a possible alternate strategy subjected to further refinements.

Metallophiles are endowed with various physiochemical and biochemical mechanisms, through which they can alter the physical and chemical nature of the metals or minerals. The process could lead to synthesis or transformation of different inorganic materials (Gadd, 2004; Haferburg and Kothe, 2007). Not surprisingly, many of the minerals in nature are biogenic in origin, and are of geological and industrial significance (Gadd, 2010). If we identify and harness these microbial systems for recovering phosphate from natural or contaminated source, it will be much more beneficial than excavating and exploiting the limited available natural phosphate sources. Previously, many microbial strains like *Trypanosoma cruzi* (Adroher et al., 1988), *Arthrobacter* sp. and *Pseudomonas* sp. (Pérez-Garcia et al., 1990), *Proteus mirabilis* (Sun et al., 2012), *Myxococcus* sp. (Gonzalez-Muñoz et al., 1993; Omar et al., 1998; Silva et al., 2000) have been employed for producing struvite either in-situ or ex-situ. However, most of them require higher external phosphate source for the formation of struvite.

Primary motivation towards current work was the recovery of phosphorus as struvite even from a medium containing low phosphate concentration. This has been achieved using a biotechnological approach by exploiting a metallophilic bacterium *Enterobacter* sp. EMB19. The strain and the process could also be exploited for the development of generic biotechniques for obtaining phosphate rich compounds.

2. Materials and methods

2.1. Materials

$MgSO_4 \cdot 7H_2O$ and NaCl was a procured from Qualigens Fine Chemicals (Glaxo India Ltd. Mumbai, India). The media components were purchased from HiMedia Laboratories (Mumbai, India). $NaH_2PO_4 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ were procured from Merck Limited, Mumbai, India. All other chemicals used were of analytical grade. All the glasswares used were soaked in 10% HNO_3 overnight and rinsed with double distilled water prior to the experiments.

2.2. Bacterial strain

A heavy metal resistant bacterium *Enterobacter* sp. EMB19, isolated from soil was used in the present study. The culture has been submitted to Microbial Type Culture Collection, IMTECH Chandigarh, India, with accession no. MTCC 10649. The lab culture was maintained at 4 °C on agar slants and subcultured at 15 day intervals.

2.3. Inoculum and culture conditions

A loopful from the slant was introduced into the culture medium containing (gL^{-1}): yeast extract, 3.0; peptone, 5.0; NaCl, 2.5; adjusted to pH 7.0 followed by incubation at 30 °C and 150 rpm. A 24 h grown culture having OD (optical density) ~1.0 was used as inoculum (mother culture). Culture medium (NB) consisted of (gL^{-1}): yeast extract, 3.0; peptone, 5.0; dextrose, 5.0; NaCl, 2.5; $MgSO_4 \cdot 7H_2O$, 0.5; and pH 7.0. In a 500 mL Erlenmeyer flask 100 mL of medium was taken and inoculated with 1% (v/v) mother culture. The inoculated medium was incubated at 30 °C with constant shaking at 150 rpm (Orbital Rotary Shaker, Orbitek, India).

To evaluate the heavy metal tolerance towards different metals, the isolated strain was inoculated into 250 mL Erlenmeyer flask containing 50 mL NB medium (pH 7.0) and supplemented with five different heavy metals at the following concentrations (mgL^{-1}): Zn^{2+} ($C_4H_6ZnO_4 \cdot 2H_2O$) 2.5, 5, 10, 15, 20; Hg^{2+} ($HgCl_2$) 10, 20, 30, 40, 50, 60; Pb^{2+} ($C_4H_6PbO_4 \cdot 3H_2O$) 10, 25, 50, 100, 125; Mn^{2+} ($MnCl_2 \cdot 4H_2O$) 10, 25, 50, 100, 150; Cd^{2+} ($C_4H_6CdO_4 \cdot 2H_2O$) 5, 10, 15, 30, 40. The cultures were incubated at 30 °C with constant shaking at 150 rpm for 96 h. The growth in each flask was determined by recording the OD at 660 nm.

For studying effect of medium composition on crystal formation, different inorganic sources were added into above medium as per Table 1. Control for each culture condition was run similarly without inoculating the medium with bacterial cells to rule out the abiotic crystallization of struvite if any.

2.4. Segregation and observation of crystals

The resultant crystals were harvested after 7 days. The culture flasks were kept still for an hour to settle the crystals. The supernatant was discarded and the crystals were recovered and washed thoroughly with Milli-Q water (Millipore Inc, Billerica, USA) by repeated resuspension and vortexing. Finally, the washed crystals were dried at room temperature.