



Evaluation of organic substrates to enhance the sulfate-reducing activity in phosphogypsum

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HIGHLIGHTS

- ▶ Evidences of sulfate-reducing bacteria (SRB) naturally occur in phosphogypsum.
- ▶ Experiments were conducted to enhance SRB activity using natural organic substrates.
- ▶ SRB were subjected to high Fe, Zn, Cu and Cd concentrations in a growth medium.
- ▶ Metal concentrations were depleted by precipitation of newly-formed sulfides.
- ▶ This paper confirms the potential role of SRB for bioremediation strategies.

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ABSTRACT

Several experiments were conducted to evaluate the activity and growth of sulfate-reducing bacteria (SRB) in a metal-rich culture medium (approx. 250 mg/L Fe, 75 mg/L Zn and Cu, 10 mg/L Cd) with phosphogypsum as bacterial inoculum. Phosphogypsum was collected from the stack covering the salt-marshes of the Tinto river (SW Spain). Three organic amendments were used as carbon sources, two low-cost wastes (horse manure and legume compost) and one sample of natural soil (vegetal cover). In the experiments, sulfate was reduced to sulfide during the growth of SRB populations, and concentrations were decreased in the solution. Metal concentrations also decreased to values below the detection limit. Metal removal took place by precipitation of newly-formed sulfides. Pyrite-S was the main sulfide component (approx. 200 μmol/g and 80% of pyritization) and occurred mainly as framboidal grains and rarely as isolated polyhedral crystals. Horse manure was the most successful organic substrate to promote SRB activity (sulfate removal of 61%), followed by vegetal cover (49%) and legume compost (31%). These findings propose the possibility of using naturally-occurring SRB in the phosphogypsum for bioremediation strategies based on natural soil covers with organic amendments.

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1. Introduction

Phosphate rock ore (mainly fluorapatite, $\text{Ca}_5(\text{PO}_4)_3\text{F}$) for the manufacture of phosphoric acid (H_3PO_4) in the fertilizer industry often contains high concentration of hazardous impurities such as As, Cd, Cu, Fe, Ni, Pb, and Zn and radionuclides of the U- and Th-decay series (Rutherford et al., 1994). The overall process involves the wet chemical attack of phosphate rock with sulfuric acid (H_2SO_4) and generates a by-product gypsum known as phosphogypsum ($\text{CaSO}_4 \cdot n\text{H}_2\text{O}$). Phosphogypsum inherits part of the potentially toxic elements from its parent phosphate ore, and hence, their potential effects on the environment (Oliveira et al., 2012a; Pérez-López et al., 2010; Silva et al., 2010). Efforts at assessing the phosphogypsum produced in large fertilizer plants around the world as a source of toxic elements focus on simulating its weathering by laboratory leaching experiments (Al-Hwaiti et al., 2010; Pérez-López et al., 2010;

Santos et al., 2006). The presence of impurities in the phosphogypsum leads to challenging its reutilization and storage.

The disposal options of phosphogypsum include direct pouring into the sea, backfill of open-pit mines or stacking in large areas (Frazier et al., 1977; Komnitsas et al., 1999; Oliveira et al., 2012b). The most widespread practice is to stack-pile the phosphogypsum near the industrial plant, which is often placed in coastal areas (Tayibi et al., 2009). Studies on the dynamic of contaminants in phosphogypsum should consider that the large disposal piles may exhibit different chemical gradients with depth. The unsaturated superficial zones likely show acidic and oxidizing weathering conditions; while water-saturation with neutral and anaerobic weathering conditions can be expected to develop in the deep zones of aged waste material (Lottermoser, 2007). In deep zones, stagnant and non-renewable waters near basement could lead to out-breaks of anaerobic bacteria communities that decompose the typically high concentration of organic matter contained in the soils or sediments supporting the stack (Pérez-López et al., 2011), along with the residual organic matter from phosphate rock ore (Rutherford et al., 1994).

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The contaminant mobility in groundwater must be strongly influenced by changes from aerobic to anaerobic conditions along the weathering profile of the phosphogypsum pile. Under waterlogged and anoxic conditions, aqueous sulfate from phosphogypsum could be reduced to aqueous sulfide by the activity of sulfate-reducing bacteria (SRB) and substantial retention of trace elements may be expected by precipitation of insoluble metal sulfides (Carbonell-Barrachina et al., 2002; Lottermoser, 2007; Pérez-López et al., 2011). These last authors recently revealed the existence of low bioavailability and toxic effects of contaminants under oxygen-restricted conditions in a phosphogypsum pile due to bacterial immobilization. Hence, designing a treatment strategy based on the possible activity of naturally-occurring SRB in the phosphogypsum could be a viable alternative to attenuate the release of contaminants into the environment. At the end of useful life of any phosphogypsum stack, restoration includes a cover of natural soil and vegetation to reduce landscape impact and erosion. The starting hypothesis would be to investigate the use of potential organic amendments that, added to natural soil covers, serve as a carbon source for growth of SRB in phosphogypsum piles.

Under anaerobic conditions, SRB oxidize simple organic compounds such as carboxylic acids or alcohols as carbon and energy sources to reduce sulfate to sulfide (White and Gadd, 1996; Widdel and Bak, 1992). Classically, research on SRB growth has recommended the use of lactate as pure carbon sources in *in vitro* lignin and experiments (Barnes, 1998; El Bayoumy et al., 1999; Postgate, 1984). However, it is not economically feasible to use lactate for a large-scale bioremediation treatment. For this reason, numerous investigations have focused on the search for low-cost organic wastes to replace lactate as carbon sources, e.g. molasses, bagasse, sewage sludge, wood chips, animal manure, vegetal compost (Annachatre and Suktrakoolvait, 2001; Cerqueira et al., 2011, 2012; Christensen et al., 1996; Coetser et al., 2006; Costa and Duarte, 2005; Dvorak et al., 1992; Hammack et al., 1994; Waybrant et al., 1998). Selecting an optimal carbon source for bioremediation by SRB activity depends on the reactivity and degradability of the organic substrate, as well as on its availability in the restoration area (Martins et al., 2009; Ribeiro et al., 2010).

In the current study, several laboratory experiments were conducted with the aims to (1) prove the occurrence of SRB in phosphogypsum wastes stack-piled on salt-marsh soils in a highly contaminated ecological environment, and (2) promote the SRB growth and activity using a cost-effective organic substrate to immobilize potentially toxic elements that are being currently released from these by-products to the environment.

2. Materials and methods

2.1. Phosphogypsum sampling

A set of samples were collected at different depths in a bore-hole drilled in the phosphogypsum stack-piled on the salt-marshes of the Tinto River (Huelva, Andalusia, SW Spain). This dump covers 1200 ha with approx. 120 Mt of phosphogypsum (Pérez-López et al., 2010, 2011). The deposition stopped in 1997 after four decades of operation in the fertilizer industry. Nearly 400 ha was restored in 1992 by direct application of a layer of fertile soil onto the substrate of gypsum and subsequent creation of a vegetal cover (Pérez-López et al., 2011). The core, with a length of 8 m from the surface to the marsh basement, was conducted in the non-restored zone. Samples were immediately transported to the laboratory and stored at room temperature in a N₂-purged glove box to prevent reaction with atmospheric oxygen. It is noteworthy that a previous study revealed evidence of sulfate-reducing activity at a depth of 7.4 m in this bore-hole (Pérez-López et al., 2011). Based on this hypothesis, the idea of the current study would be to prove the presence of SRB communities using a representative phosphogypsum sample as natural inoculum by stimulating their

growth, in highly metal-polluted solutions, with different natural organic substrates.

2.2. Organic substrates

Three different organic substrates were used as potential carbon sources in the laboratory experiments: horse manure, legume compost and vegetal cover. Horse manure was chosen due to its immediate availability and low-cost in the study area. Andalusia community has the highest horse population in Spain with around 228,000 animals registered in 2010, approx. 7000 kg of manure every year per each 500 kg of live weight (MARM, 2010). Legume compost consists of two *genera*, *Leucaena* and *Tagaste*, and both are a low-cost by-product from cleaning and composting agricultural wastes. Vegetal cover corresponds to a sample of the fertile soil with spontaneous growth plants, which was applied in the restored area of the phosphogypsum piles located on the Tinto marshes. This sample would serve to check whether it is currently acting as a carbon source that enhances SRB activity.

Representative subsamples of the three organic substrates were taken and subjected to analysis of lignin/cellulose content using the method of successive extractions described by Rahn et al. (1999), easily available substances (EAS) fraction according to the method proposed by Prasad et al. (1999), and volatile solids contents in accordance with the procedures outlined in Standard Methods (APHA, 1992). Biodegradable fraction of organic substrates was predicted from the lignin and volatile solids contents using the mathematical expression proposed by Chandler et al. (1980). The EAS fraction is considered as the organic fraction that can be readily used by microorganisms. In this sense, not all of the constituents in the EAS extract can readily be used by SRB, and hence, this fraction should be taken only as an approximate value.

2.3. Experimental design

Batch experiments were conducted to evaluate the potential of organic substrates as a carbon source for the growth of SRB using phosphogypsum as natural inoculum. These experiments were also carried out to check whether possible SRB populations are able to tolerate and remove high metal concentrations by precipitation of insoluble sulfides. Cultivations were performed in 250 mL glass-reactors with 5 g of phosphogypsum inoculum and 200 mL of growth medium (MTM; Sani et al., 2001). The growth medium, slightly modified for this study, contained 0.25 g/L yeast extract, 1 g/L NH₄Cl, 0.06 g/L CaCl₂·6H₂O, 1 g/L MgSO₄·7H₂O, 1 g/L Na₂SO₄, 5 g/L sodium lactate (with 0.05% sulfate) and 0.25 g/L sodium citrate. The pH of the culture medium was between 6.5 and 7. The final solution was deaerated with nitrogen for 3–5 min and then sealed with 10 mL of sterile liquid paraffin to prevent oxygen diffusion during the experimental run.

Five batch tests were undertaken simultaneously, each one with a different carbon source in the culture medium: (1) with 5 g/L of sodium lactate as above described for the composition of the growth MTM medium, (2) with 10 g of horse manure replacing lactate, (3) with 10 g of vegetal cover replacing lactate, (4) with 10 g of legume compost replacing lactate, and (5) without any carbon source. The experiment with lactate corresponds to a positive control where sulfate-reducing phenomenon is expected; while the experiment without carbon source corresponds to a negative control. All batch experiments were performed in duplicate to check for the reproducibility and these duplicates confirmed results obtained with standard deviations at the steady-state lower than 5%.

Metal concentrations in pore-water solutions in the phosphogypsum stack range between hundreds and thousands of mg/L for Fe, tens and hundreds of mg/L for Zn, and minor but toxic concentrations (tens to hundreds of µg/L) of other metals such as As, Cu, Cr, Cd, Pb, Mn and Ni (Pérez-López et al., 2011). In order to simulate extreme conditions, in addition to the metals contained in the phosphogypsum, additional amounts of some of these metals were added to the culture medium

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