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FEASIBILITY OF PbS NPs SYNTHESIS COUPLED TO A BIOREMEDIATION SYSTEM FOR ACID MINE DRAINAGE TREATMENT

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ABSTRACT

Nowadays, there is much attention to the development of protocols for the synthesis of nanocrystalline semiconductors, such as PbS and other metal sulphides, since they are emerging as a new class of materials with several recognized environmental applications, namely as photocatalysis and as additives to solar panels.

The use of Sulphate-Reducing Bacteria (SRB) for the bioremediation of metals and sulphate in highly contaminated effluents, such as Acid Mine Drainage (AMD), is well documented. The biological reduction of sulphate in the presence of metal ions leads to their precipitation as metal sulphides. However, depending on the metal and sulphate concentrations, these processes may generate excess sulphide, which may in turn pose an environmental issue that needs to be addressed.

In this context, the use of the surplus sulphide to synthesize useful materials, such as semiconductor metal sulphide nanoparticles (NPs) and nanocomposites, is of particular environmental relevance, as this will not only improve the remediation system from an economical point of view, but it also allows overcoming the problems generated by the excess of toxic H_2S produced by SRB.

This paper describes the successful synthesis of nanocrystalline PbS and PbS/TiO_2 nanocomposites, coupled to a continuous bioremediation system for AMD treatment, at room temperature and atmospheric pressure, yielding near complete metal precipitation.
According to Transmission Electron Microscopy (TEM) analysis, the particles obtained are in the range of 20 to 50 nm in size, for both the PbS nanoparticles and the PbS/TiO_2 composites.
This AMD bioremediation system with coupled synthesis of NPs presents obvious advantages in terms of economy and safety, due the production of functional materials from a toxic waste. Moreover, the simplicity of the process here described presents an attractive route for the synthesis of metal sulphide NPs, such as PbS NPs and the respective TiO_2 nanocomposites.

Keywords: PbS nanoparticles, PbS/TiO_2 nanocomposites, Bioremediation, Acid mine drainage, Sulphate-reducing bacteria

1. Introduction

These days, there is much attention to the development of protocols for the synthesis of nanocrystalline semiconductors (the so-called quantum dots) such as PbS, CdS and ZnS. These luminescent quantum dots are emerging as a new class of materials for biological detection and cell imaging (Chan *et al.* 2002), photocatalysis and solar cells.

Bioremediation processes for the treatment of Acid Mine Drainage (AMD) based on the use of Sulphate-Reducing Bacteria (SRB) have proven to be viable alternatives to traditional methods (Garcia *et al.*, 2001). SRB use sulphate as the terminal electron acceptor during the metabolism

of organic matter, resulting in the generation of sulphide ion, which in turn causes the precipitation of several metal ions as metal sulphides.
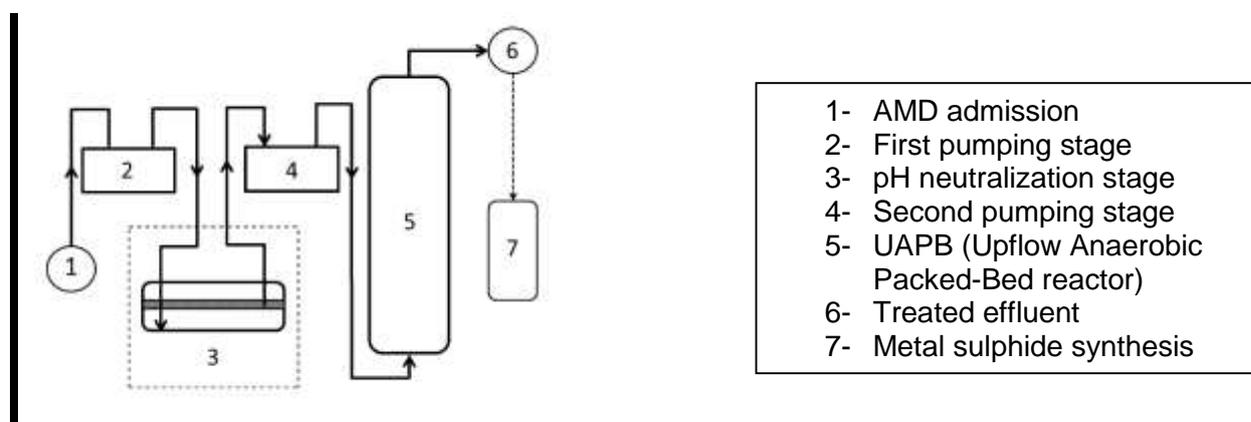
A chemical/biological combined process previously developed by members of this research team, has been optimized for the treatment of AMD from S. Domingos Mine, in Portugal (Vitor *et al.*, 2015), producing treated water complying with the Portuguese requirements for irrigation waters (Decreto-Lei nº 236/98, 1998). As the effluent produced in this bioremediation system presents an excess of sulphide ion (which in turn is object of environmental concern (Mandal *et al.*, 2006), studies have been underway in order to assess the feasibility of using this excess sulphide for the synthesis of specific metal sulphides, under controlled conditions, in order to produce functional nanoparticles and nanocomposites. This would serve a double purpose: on one hand, these metal sulphide NPs would have further applications, as semiconductors or as photocatalysts, among others (Chan *et al.*, 2002), thus contributing to the economic viability of the bioremediation process described, and on the other hand, the surplus sulphide would be consumed, removing or at the least minimizing the problem resulting from the excess of sulphide generated by the system.

Although several metal sulphide NPs have been successfully synthesised coupled to an AMD bioremediation system, this paper is focused on the synthesis of lead sulphide NPs and respective PbS/TiO_2 nanocomposites.

2. Experimental
2.1. Materials and synthesis procedure
The lead (II) solution used for synthesis was prepared by dissolving anidrous lead (II) nitrate ($Pb(NO_3)_2$, 99,8%, VWR Prolabo) in ultra-pure water (milli-Q), yielding lead concentration of ~1.53 mM.

NPs synthesis coupling to a bioremediation system for treatment of real acid mine drainage is described in the scheme of Figure 1.

1-	AMD admission
2-	First pumping stage
3-	pH neutralization stage
4-	Second pumping stage
5-	UAPB (Upflow Anaerobic Packed-Bed reactor)
6-	Treated effluent
7-	Metal sulphide synthesis

Figure 1: Schematic diagram of the AMD bioremediation system

Briefly, the AMD bioremediation system consists of a glass column packed with coarse sand and inoculated with a SRB community. Since the used SRB are intolerant to pH values lower than about 5 (Al-Zuhair *et al.*, 2008), this Upflow Anaerobic Packed-Bed reactor (UAPB) is preceded by a pH neutralisation stage, consisting of a shallow contact basin filled with limestone of appropriate grain size. Ethanol was added to the AMD as carbon source for the SRB (~5,6 g L^{-1}) and this mix was pumped to the neutralisation stage, in which pH rises to values near 7, optimal for SRB growth (Barton & Hamilton, 2007).

The composition of the AMD used to feed the bioremediation system during the synthesis is indicated in Table 1. It was collected in January 2015 in the stream of S. Domingos, located in the old mining village of S. Domingos, Mértola, Southeast Portugal, where an abandoned copper mine is located (N: 37º39'38"; W: 7º30'18").

The neutralised AMD is then pumped to the UAPB, inside which the sulphate ion is reduced to sulphide, due to SRB action, and this will in turn remove the remaining metals dissolved as

sulphides. The hydraulic residence time of the UAPB was adjusted to ~6.6 days, which assures that the concentrations of sulphate and dissolved metals in the treated AMD were below the Maximum Recommended Values (MRV) for irrigation waters, according to Portuguese legislation (Decreto-Lei nº 236/98, 1998). Effluent samples were collected regularly, to monitor the bioremediation system's performance. Table 1 also presents typical chemical parameters and concentration values for the bioremediation effluent used for the PbS synthesis.

Table 1: Main metals, sulphate and sulphide ions concentration and chemical parameters for the AMD used and typical values for the bioremediation effluent.

	Eh (mV)	pH	[Zn] (mg L^{-1})	[Fe] (mg L^{-1})	[Cu] (mg L^{-1})	[SO_4^{2-}] (mg L^{-1})	[S^{2-}] (mg L^{-1})
AMD	546	2,48	28	45	30	1848	-
Treated effluent	-286	6.25	0.3	0.1	0.1	10	385

For the NPs synthesis, a reaction vessel (Schott flask, 1L) containing the deaerated lead nitrate solution under agitation was added to the bioremediation system, and the effluent was collected on it drop-by-drop.

In order to try to prevent contamination of the synthesised particles (either with metal sulphide particles generated inside the bioreactor, or with bacteria), an inline filter system was also added between the bioreactor and the reaction vessel, consisting of a small (~14 cm long, 2 cm $\varnothing$) glass column with some glass wool pre-filter (for larger particles), followed by a 0,2 μm pore size syringe filter (Whatman Puradisc 25 AS).

The volume of sulphide-rich effluent added was calculated to yield a 1/1.2 ratio (lead/sulphide), based on the sulphide concentration measured at the end of the bioreactor and the metal solution concentration. The obtained suspensions were then centrifuged twice (Rotofix 32A, Hettich) for 15 minutes at 4000 rpm, washed twice with 70% ethanol and dried under vacuum at room temperature (APT. Line VD, Binder), saving a sample of each synthesis supernatant for further analysis.

Synthesis of nanocomposites was performed similarly, by adding 0.6 g of TiO_2 powder to the 500 mL of metal solution, prior to the deaeration step.

2.2. Analytical methods
Sulphide and sulphate ions concentrations were measured by UV-Visible spectroscopy (DR 2800, Hach-Lange) by the Methylene Blue Method at 665 nm and by the sulfaVer4 method at 450 nm, respectively, both using kits from Hach-Lange.

Metals concentration was determined by flame atomic absorption spectroscopy (Flame-AAS, Analytikjena NovAA 350).

The synthesized particles were analysed by X-Ray Powder Diffraction (XRD), using a PANalytical X'Pert Pro powder diffractometer, operating at 45 kV and 30 mA, with CuKα radiation filtered by Ni. XRD patterns were recorded using an X'Celerator detector, with a step size of 0.0334 º, and a time per step of 499,745 seconds. Peak analysis and crystalline phase identification were conducted using the HighScore Plus software, using the ICDD PDF-2 database.

Morphological and particle size characterization of precipitates was performed by electronic microscopy analysis. Transmission electron microscopy (TEM) coupled with EDX was performed with a Hitachi H8100 electron microscope at 160 kV of acceleration voltage. For the TEM measurements, samples were dispersed in distilled water using ultrasounds. A drop of this suspension was directly deposited in a copper grid and dried at room temperature. The elemental analysis was carried out by semi-quantitative EDS.

3. Results and discussion

In every synthesis run, in the presence or absence of the TiO_2 substrate, over 99 % of the initial lead(II) present in the solution was removed as insoluble PbS.

Figure 2 presents the obtained XRD patterns for the PbS nanoparticles and for the TiO_2/PbS nanocomposites. The difractograms confirm the presence of cubic PbS in both cases, as well as *anatase* and *rutile* TiO_2 crystalline phases in the composites.

The crystallite size of the PbS particles was estimated using the Scherrer equation (Scherrer, 1922), based on the peak at ~30⁰, indicating an average crystallite size of ~43 nm for the single particles and ~37 for the PbS particles in the composites.
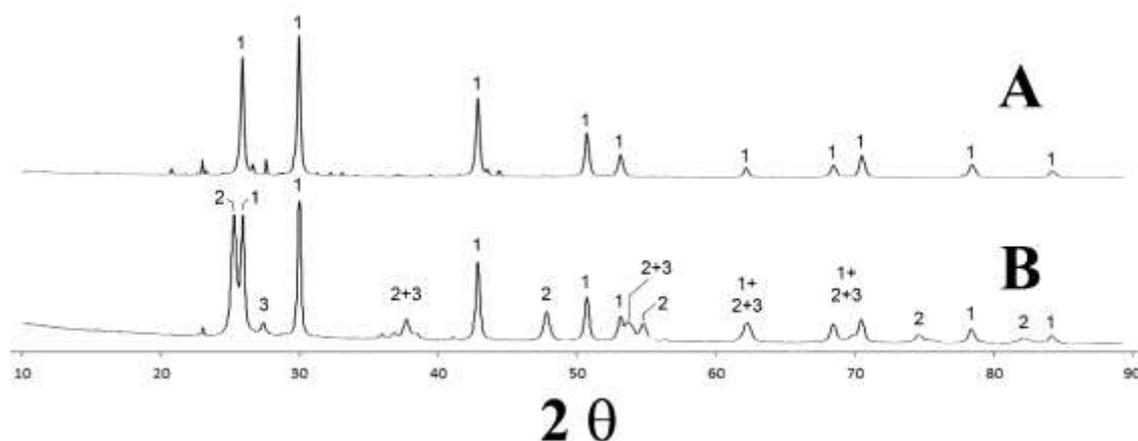
Figure 2 : X-ray powder diffraction patterns for the precipitate (A), and for the PbS/TiO_2 nanocomposite (B). '1' cubic PbS JCPDS# 03-065-0302; '2' *anatase* JCPDS# 01-072-7058 and '3' *rutile* JCPDS# 01-073-1782.

Figure 3 presents the images obtained by Transmission Electron Microscopy (TEM) for the PbS NPs (fig. 3A) and for the PbS/TiO_2 nanocomposites (fig. 3B).
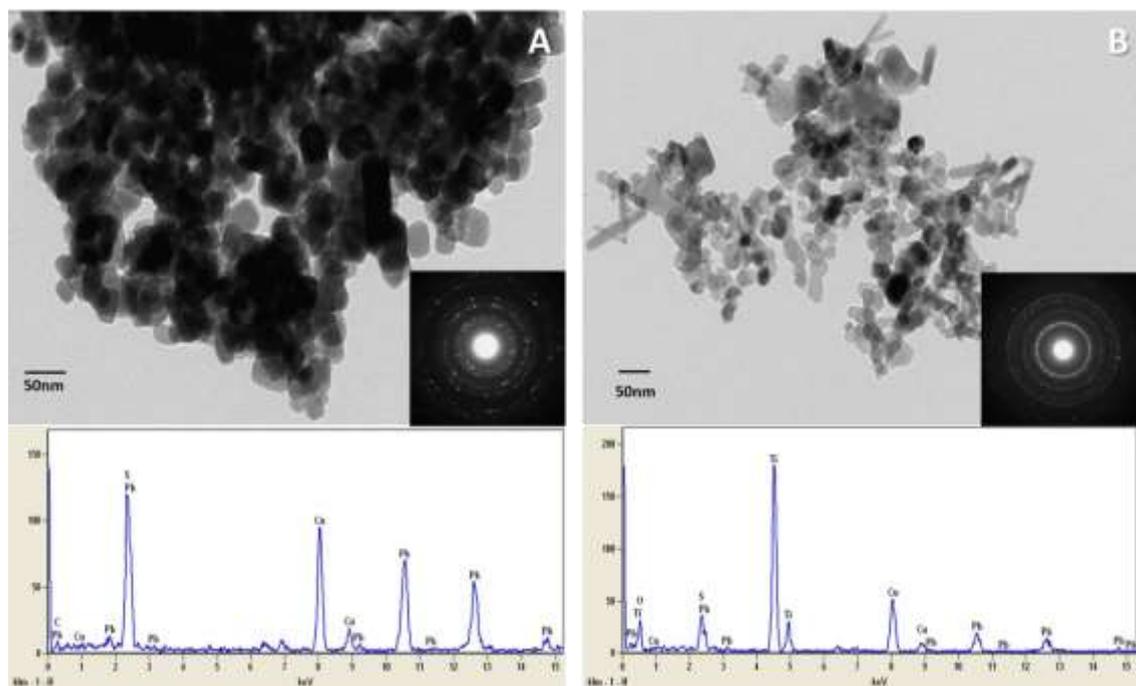
Figure 3: TEM images and EDS spectra of the PbS nanoparticles (A) and PbS/TiO_2 nancomposites (B). (Insets: SAED of the prepared powders).

As can be seen in fig. 3A, the sample is homogeneous and the PbS particles are mainly cubic shaped. The SAED and EDS analyses confirm the identity and crystallinity of the NPs. The morphology of the PbS/TiO$_2$ sample, however, is not homogenous, and together with the cubic PbS crystallites surrounding the TiO$_2$ pristine particles, small PbS needle-like particles can also be visualized. It can be seen in figure 3B that the PbS is segregated from the ~20 nm TiO$_2$ nanoparticles, as we can perfectly distinguish the particles with cubic morphology corresponding to PbS from the particles with spherical morphology corresponding to TiO$_2$. Particle size ranges between 20 and 50 nm for the cubic PbS, and 11 to 22 nm for the needle-like PbS particles. These values are in agreement with the values estimated by the Scherrer equation, confirming once again that the PbS was synthesized in the form of nanoparticles (Thakkar *et al.*, 2009).

Due to the interesting optical properties of the prepared powders (PbS and PbS/TiO$_2$), mainly in the visible range, these materials will be studied as photocatalysts for photocatalytic pollutants degradation processes.

4. Conclusions

Crystalline PbS NPs and the PbS/TiO$_2$ nanocomposites can be easily synthesized by coupling to a bioremediation system treating real acid mine drainage and adding the effluent containing excess sulphide, drop-by-drop, to a lead(II) solution. The synthesized PbS particles presented sizes ranging between 20 and 50 nm, with cubic shape morphology, both in the single PbS and in the nanocomposites. This method allows improving the economy and the environmental benefits of the bioremediation process.

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