Biologic Treatment Of Acid Mine Drainage: A Perspective Of Two Decades Of Research, Challenges And Opportunities

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Abstract

This article briefly reviews the work carried out over the last 20 years by the Environmental Technologies group at the Centre for Marine Sciences at the University of the Algarve on the biological treatment of acidic water from the São Domingos mine using sulfate-reducing bacteria. In a chronological sequence, its main challenges are presented, which included the search for and enrichment of metal-resistant sulfate-reducing bacteria and the search for efficient and available wastes as low-cost carbon sources. The opportunities that have resulted from these studies, namely the synthesis of functional nanoparticles of metal sulfides and the recovery of metals, among others, are also presented.

Keywords: São Domingos mine, acid mine drainage treatment, sulfate-reducing bacteria, carbon sources, metals and metal sulfide nanoparticles

Introduction

Mining activities are ancient in Portugal, dating back to the Pre-Roman and Roman periods. They played an important role in the Portuguese economy of the 19th and 20th centuries associated mainly with polymetallic sulfides in the south, and uranium minerals in the north. In the second half of the twentieth century, almost all the mining activities were suspended and consequently many mine sites were left untreated. The fact that much of Portugal mineralization is associated with sulfide minerals provided great potential for acid mine drainage (AMD) production and an estimate of about 14 % of the Portuguese mines were found to generate acidic waters (Pinto 2005). São Domingos mine, a deposit of polymetallic sulfides explored for Cu, Pb, Zn, S, Fe, Ag, and Au, located in Southeast Portugal in the Iberian Pyrite Belt, is an example of this situation. The inactive open-pit from the mining operations resulted in the creation of a large pit-lake with acid water with pH close to 2 containing high concentrations of sulfate and metals (mainly aluminium, iron, zinc and copper). The contamination is not only confined to the pit-lake since other water bodies located in the downstream area nearby (Fig. 1) are also affected (Tab. 1).

The problems associated to AMD in Portugal and in specially in São Domingos were the driving force that stimulated the studies of our research group for the development of alternative treatment processes based on the use of Sulfate-Reducing Bacteria (SRB), since traditional neutralization with alkaline agents do not address the reduction of sulfate, are expensive and result in the formation of high volumes of relatively soluble sludge. Moreover, the use of biologic techniques offers several opportunities, such as the possibility to recover the metals, namely as metal sulfides with functional applications.

Since our first studies several carbon sources have been tested, mainly wastes locally

Figure 1 *Aerial view of São Domingos mining area, AMD sampling and water bodies.*

available. In addition to the characterization of the consortia and their dynamics, other improvements were introduced to the bioremediation systems aiming to improve the efficiency of the process and the quality of the effluents, resulting in the installation *on-situ* of a pilot plant at São Domingos mine.

The present review gives a summarized perspective of the work evolution started twenty years ago by the Environmental Technologies research group of Centre of Marine Sciences at the University of Algarve, with the aim of developing simple and low-cost processes, based on the use of SRB, for the treatment of AMD from São Domingos mine site.

The biologic treatment of AMD using SRB: The main challenges

Selection of low-cost matrices

The research work of the Environmental Technologies group, started in 2004, has always been focused on the treatment of AMD from São Domingos mine and used bench scale fixed-bed column bioreactors, initially in a semi-continuous mode. Sewage, anaerobic sludge and soil from the mining area were tested as solid matrices, inoculum for SRB and as sources of substrates. Lactose was also added as a supplementary carbon and energy source because it could be easily available at zero or even at negative cost in

Table 1 *AMD typical composition collected in Site 1-6 (see Fig. 1) in different seasons.*

Chemical species	Concentration (mg/L)	
	Summer	Winter
Al	211	138
Fe	178	45
Zn	38	28
Cu	21	30
SO_4^{2-}	2323	1848
pH	2.58	2.48

effluents of local cheese industries. The results showed that in those conditions AMD was neutralized, and that the sulfide produced was enough for the precipitation of the main dissolved metals (Costa 2005). The use of soil as solid support suggested the possibility of using that system for the decontamination of both waters and soils, although the effluents have high organic load.

Later, the behavior of that bioreactor was compared with other three filled with "more inert" packaging materials: coarse sand, glass spheres and cereal straw in the absence or presence of three added carbon sources: lactate, ethanol and lactose (Costa 2008). The data confirmed that it was possible to grow SRB in the bioreactor containing sewage, anaerobic sludge, and soil in the absence and in the presence of the carbon sources, to remove the main metals present in AMD, although an incomplete sulfate removal was observed. When coarse sand or glass spheres were utilized, efficient copper and zinc removal was observed. Nevertheless, the incapacity of both systems to generate enough alkalinity did not allow to maintain their good performances in terms of iron removal and sulfate reduction. Thus, the incorporation of neutralizing and buffer capacity materials to the column matrices was recommended. Due to its low density, cereal straw was not suitable to obtain the necessary anaerobic environment inside the column for SRB activity and thus, coarse sand was selected as the preferable column matrix.

Selection of highly-metal resistant sulfate-reducing bacteria

Due to the high concentration of metals in AMD, the search for metals-resistant SRB was, at that time, considered important for the development of efficient bioremediation technologies. Therefore, samples from distinct anaerobic environments (sediments from the mining area, sludge from wastewater treatment plants and soil from a thermal place) were tested in batch aiming to obtain an SRB consortium resistant to Fe, Cu and Zn. The consortium enriched from an anaerobic lagoon of a local wastewater treatment plant was selected as the best metals-resistant SRB inoculum and its phylogenetic analysis of the dsr gene sequence revealed the presence of SRB species affiliated to *Desulfovibrio desulfuricans* and *Desulfobulbus rhabdoformis* (Martins 2009a). The results demonstrated that usually lethal concentrations of Fe (400 mg/L), Zn (150 mg/L) and Cu (80 mg/L) were not toxic and a very good efficiency in terms of sulfate reduction and metals removal was observed, while for other consortia enriched from different samples sulfate reduction was inhibited by the presence of copper and zinc. Both ethanol and lactate were efficiently used by the selected consortium. This highly metal-resistant SRB community was then used in the subsequent studies to inoculate the bioreactors to carry out AMD decontamination.

Neutralizing adjuvant for the biologic treatment of AMD

Marble stone powder (calcite tailing), a residual material resulting from the cutting and polishing of marble stone, an important Portuguese industry located not far from several national mining areas, was tested as a possible low-cost adjuvant for the biologic treatment of AMD. The performance of a combined chemical/biologic packed bed reactor containing the selected SRB inoculum and calcite tailing as neutralising agent mixed with coarse sand was assessed (Barros 2009). This work emphasized the key role played by the calcite waste in the inlet of the reactor by adjusting the pH to values adequate for SRB activity with low energy demand which would be impossible without its neutralisation action. Later the neutralizing and the biologic steps were separated to better control each stage of the treatment process, using two individual and sequential columns in an upflow continuous mode. Even later, during process optimization, the calcite column was replaced by a neutralization tank to avoid clogging (Fig. 2).

Selection of efficient low-cost carbon source

AMD is deficient in carbon sources/electron donors and thus requires the external addition for biologic sulfate reduction. However, the need of a carbon source is likely the most critical issue in AMD biologic treatment, determining the economic viability of the process which efficiency is highly dependent of the type of carbon and energy source utilized. To guarantee a viable economic process, wastes locally available at zero or even negative costs from the local food industry (wine wastes and cheese whey) were tested in batch as carbon sources (Martins 2009b). The results showed that these wastes could be efficiently used by these bacteria if calcite tailings were present as a neutralizing and buffer material: 95% and 50 % sulfate reduction were achieved within 20 days of experiment on media containing wine waste and cheese whey, respectively. Identification of the dissimilatory SRB community using the dsr gene revealed the presence of the species *Desulfovibrio fructosovorans*, *Desulfovibrio aminophilus* and *Desulfovibrio desulfuricans*. These findings demonstrated the potential of using wastes from the winery industry combined with calcite tailing for the development of cost-effective and environmentally friendly bioremediation processes.

The BIOMETAL DEMO project

Later, in the framework of BIOMETAL DEMO international project (Ballester 2017) five alternative industrial by-products and wastes from Portugal and Spain were tested: molasses from orange, molasses from sugar-beet, carbocal, domestic wastewater and olive mill wastewater (Carlier 2019). This work demonstrated the potential of new substrates largely available in the Iberian Peninsula and the respective required doses to feed SRB bioreactors in special for long-term passive bioremediation processes. Although relatively efficient, no one was able to compete with wine waste (Costa 2009), which remained the selected carbon source for the subsequent active systems tested in the laboratory (Fig. 2a) and for the pilot plant resulting from the process scale up further installed on situ in São Domingos. The pilot plant, set-up in collaboration of AGRI.PRO Ambiente, consisted of two serial neutralization tanks of 0.75 m^3 each filled with 450 kg of limestone followed by the biological treatment with SRB in one up-flow anaerobic packed bed reactor (UAPB) made of PEAD, with 4.5 m^3 capacity, filled with 8 metric tons of river stone, coarse sand, and limestone (Fig. 2b). In steady state conditions, water well below by the maximum values allowed by Portuguese legislation for irrigation (Decreto-Lei n.º 236/98, from 1 August, [SO_4^{2-}] = 575 mg/L; [Al] = 20 mg/L; [Fe] = 5 mg/L; [Zn] = 10 mg/L; [Cu] = 5 mg/L) was produced with one-week hydraulic retention time. When wine wastes were replaced by the domestic wastewater of the treatment plant where the pilot was installed, in a ratio of wastewater to AMD close to 1:1, about two weeks of hydraulic retention time was required to obtain water with similar quality. However, during operation, the system

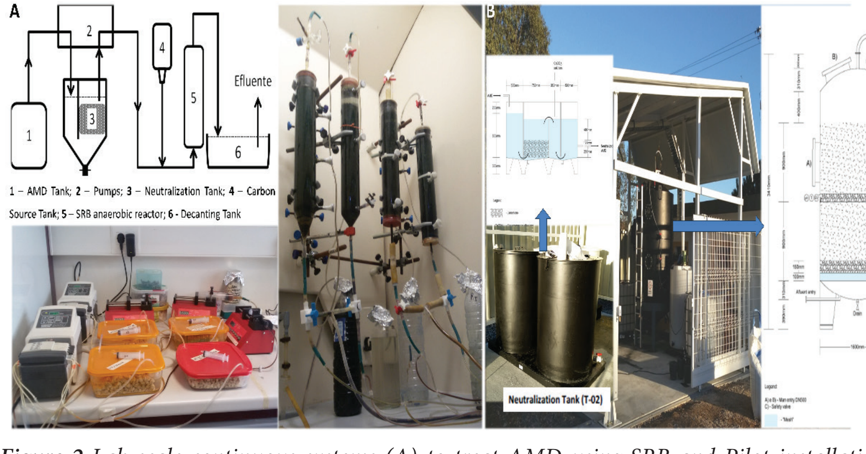

Figure 2 *Lab scale continuous systems (A) to treat AMD using SRB and Pilot installation (B) at São Domingos.*

faced problems mainly related to the low temperatures in winter and others associated to inefficient pH neutralization.

Dynamics of the bacterial populations

The dynamics of the bacterial populations in the UAPB during AMD treatment using wine wastes and ethanol as carbon source was investigated to better understand the bioremediation process (Martins 2011). The results demonstrated that the type of carbon source modulated the bacterial community. The bacterial diversity was higher in the bioreactor fed with wine waste where the dominant community was composed by bacteria affiliated with *Desulfovibrio* sp.. The presence of SRB and fermentative bacteria (*Clostridium* sp., Bacteroidales order, *Citrobacter* sp. and *Cronobacter* sp.) suggests a synergistic interaction between these bacterial groups and may be associated to the efficiency of such complex organic substrate.

The opportunities

Synthesis of metal sulfides NPs

During AMD treatment an excess of toxic hydrogen sulfide is produced which was the main reason to investigate its use to produce functional metal sulfides. Hence, batch tests where sulfide biologically generated by SRB was added to several unimetallic solutions demonstrated that ZnS (mainly sphalerite, 20–30 nm) (Costa 2012) and CuS (covellite, with a mean size of ~3.5 nm) (Costa 2013) nanocrystals could be synthesised in a simple way. Afterwards, the UAPB system used in AMD treatment was adapted (an "add-on" to the regular bioremediation system) to take advantage of the surplus H_2S contained in the treated effluent to produce zinc sulfide NPs (Vitor 2015) and other metal sulfides, or even NPs of reduced metals (Assunção 2016), under atmospheric temperature and pressure. With this, the environmental issue caused by the most problematic residue produced in AMD bioremediation, is not only minimized, but is also turned into an economic advantage, with the added benefit of dismissing the use of expensive and toxic chemicals and/or any sophisticated apparatus.

Recover of metals from extreme AMD

AMD waters have been regarded as a potential resource of metals due to the urgent need to recover or recycle metals from secondary resources benefiting the environment and stimulating circular economy and sustainability. Due to the presence of extreme AMD at São Domingos mine (impoundment next to the sulfur factory ruins at Achada do Gamo), with concentrations of copper higher than 5 g/L and zinc close to 2 g/L (Nobahar 2022a), a combination of chemical and biologic strategies based on the use of liquid-liquid extraction followed by SRB action was designed. In that process copper was first selectively extracted by ACORGA M5640 and then stripped using sulfuric acid to a purified aqueous phase, from where it was further precipitated and recovered as nanosized covellite with high purity, which may be used in a plethora of applications from medicine, catalysis and renewable energy devices.

Zinc recovery from the extreme copper free AMD was then attempted, but the high concentration of iron and their coextraction with several commercial extractants were the main drawbacks. The solution relied on the potential of AliCy, an ionic liquid, used to separate Fe(III) from the acidic multimetallic solution, followed by pH increase of the aqueous raffinate to values in the range of 3.25 to 3.50 (Nobahar 2022b). With this approach more than 90% of Fe(III) was separated and the remaining was removed by precipitation, with Zn losses lower than 18%. Thereafter, the highest Zn recovery from the medium resulting from such combination of processes was attained by precipitation using biogenic sulfide at pH = 3.5. The precipitates were identified as NPs of wurzite and sphalerite (ZnS) with sizes between 2 and 22 nm agglomerated into larger structures. These NPs may have applications as photocatalysts in H_2 production and in the degradation of toxic organic compounds, electrochemical sensors, among others.

Our group is currently investigating novel recovery options, focused on critical metals, and prioritizing green technologies, or others more environmentally friendly than those used until now, involving the use of new biomass and/or innovative materials not yet explored.

Cotreatments and acidophilic SRB

Since the BIOMETAL DEMO project where several wastes and domestic wastewater were used as carbon sources, the possibility of AMD cotreatment is being considered. We assessed the cotreatment of AMD and Olive Mill Wastewaters (OMWW), both coexisting in the same region, in SRB-enriched bioreactors (Carlier 2020). The process required a neutralizing agent to create optimal pH conditions for successful removal of the AMD's main contaminants. Sulfate, Al, Fe, Cu and Zn decreased below Portugal's maximum admissible values for irrigation waters, while phenol concentrations, the main pollutants in OMW, dropped to half their initial concentrations. Recently, an acid-tolerant SRB consortium, able to growth at pH 4.5 in the presence of methanol and mainly composed by *Desulfosporosinus* spp. was enriched from sediments from the confluence zone between its flow and treated sewage in São Domingos (Nobahar 2024). The use of acidophilic SRB may be promising for cotreatments by decreasing the required amounts of neutralizing agents and the ratios of wastewater:AMD. Presently, the potential use of acidophilic SRB consortia is under investigation and new projects aiming at their utilization in the cotreatment of AMD and other wastes are envisaged, expecting to bring new challenges and opportunities.

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