DEPHOSPHORIZATION OF AN IRON ORE BY A FILAMENTOUS FUNGUS.

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ABSTRACT

Phosphorus is a deleterious element for the manufacture of steel. The existing chemical processes to remove P from iron ores, the main raw material for steelmaking, are based on roasting and acid/alkaline leaching. In this paper, a biological dephosphorization route is proposed and investigated as an alternative to the traditional chemical routes. To accomplish these objectives, a fungal strain, identified as \emph{Aspergillus niger} HNA-1 was isolated from a Brazilian high phosphorus iron ore. The fungus was cultivated in a medium containing glucose (1 w\%) as carbon source with crushed iron ore (0.26 w\% of P) as sole source of P. The fungus was forced to obtain this limiting nutrient from the ore, so at the end of the process the ore would be dephosphorized to some extent. The time courses of the process of dephosphorization of the ore were registered for each condition tested. The amount of dissolved iron, the variation of pH and the accumulation of citric acid in the liquid medium were also monitored. The effects of the ore particle size (d) and the pulp density (PD) were also studied. The maximum dephosphorization efficiencies obtained ranged between 13.8\% and 33.2\% in 21 days of treatment. The interaction between the ore and the fungal biomass was studied by means of field emission scanning electron microscopy and X-ray diffraction. A high content (~18 w\%) of iron, precipitated as small oxide crystals, was found in the biomass. The evidence gathered was used to explain the overall process of dephosphorization.

KEYWORDS: Dephosphorization, high phosphorus iron ore, biobeneficiation, \emph{Aspergillus niger}. 
1. LITERATURE SURVEY

Iron ores are mainly composed of iron oxides and oxy-hydroxides, with other accessory gangue phases. Phosphorus may be incorporated either into the crystal lattice of iron oxides or into the gangue minerals (Dukino et al., 2000). During high-temperature reduction of iron ore, the thermodynamic conditions also allow the reduction and further concentration of P. This element has a deleterious effect on the workability of steel (Muhammed and Zhang, 1989). For that reason, in most places only premium low-P ores (less than 0.08 w% P) are extracted, leaving many iron mines around the world enriched in un-treatable, high-P iron ore (Cheng et al., 1999; Dukino et al., 2000).

P can be removed from iron ores through several processes generally referred to as beneficiation. Depending upon the degree of association of P with the minerals in the orebody, iron ore can be beneficiated either physically or chemically (Kokal, 1990; Fonseca et al., 1994). In the former case, comminution followed by wet magnetic separation or froth flotation is generally employed when the phosphatic gangue minerals appear as discrete inclusions in the iron oxide matrix (primary mineralization) (Kokal, 1990; Fonseca et al., 1994). However, when P is disseminated in the iron oxide structure, possibly forming cryptocrystalline phosphates or forming solid solutions with the Fe-oxide phases (secondary mineralization), the beneficiation can only proceed by chemical routes (Kokal, 1990; Fonseca et al., 1994; Dukino et al., 2000).

The chemical beneficiation consists in the hydrometallurgical processing of the ore, i.e. the selective leaching of P contained in the ore with a reagent, usually an acid or a base. Since early on in the XIX century, Julius Jacobi (Jacobi, 1872) suggested the use of H2SO4 to remove P compounds from lumps of iron ore. Nevertheless, a real scientific interest in hydrometallurgical processing of high-P iron ores can only be noticed after the last third of the XX century, when several papers and patents were published (Feld et al., 1968; Gooden et al., 1974; Muhammed and Zhang, 1989; Kokal, 1990; Fonseca et al., 1994; Cheng et al., 1999; Dukino et al., 2000). Ever since, traditionally low prices of iron ore products had impeded the large-scale industrial application of chemical dephosphorization. In the last few years, the situation of iron-ore markets has changed dramatically due to an increase on the world steel consumption, pushed up mainly by the economic growth of China and other Asian emerging markets. Currently, prices of iron ore are increasing in real terms and the production of this commodity is increasing as well (Poveromo, 2005). This new scenario for the iron mining industry has revived the interest in upgrading the quality of P-bearing iron ores by hydrometallurgical routes and, for example, at least one beneficiation plant to leach P from iron ore has been recently installed in Kazakhstan (Kokal et al., 2003).

Traditional chemical treatment for P removal comprises a heat-treating stage and a leaching stage. During the heat-treating stage, the ore is heated (roasted) up to a temperature between 300 °C and 1200 °C with the addition (or not) of an additive, usually a metal chloride or an alkaline metal hydroxide (Kokal, 1990). The intention of this procedure is to chemically modify the ore, in such a way that the P can migrate to crystal defects in the oxide lattice, grain boundaries, for example. P mobilized into these defects will react with the additives (or with other gangue materials) to form other mineral phases more amenable to leaching (Cheng et al., 1999). The second beneficiation stage, acid or alkaline leaching, can be performed in several manners ranging from static to dynamic leaching, room temperature to high-temperature leaching, and so on. Detailed explanations of chemical dephosphorization of iron ores can be found elsewhere (Kokal, 1990 and references therein).

On the search for more environmentally sound technologies for the mining industry, biological processes to extract metals from ores, pre-treating metallic ores or removing contaminants from metallic ores or industrial wastes have been developed for different metallic mineral resources, including: Cu, Zn, Au, Al, Ni, Co, Mn and so forth (Jain and Sharma, 2004). The removal of silica and alumina from iron ore through biological means has also been proposed (Natarajan et al., 2001; Pradhan et al., 2006).

The biological treatment of ores to remove contaminants, often referred to as biobeneficiation (Jain and Sharma, 2004), is another variant of the above mentioned chemical processing. In such a process the microorganisms produce, as a consequence of their metabolism, a chemical by-product (mineral acids, organic acids, polymers, enzymes, chelating agents, etc.). The chemical by-products, in turn, attack the gangue minerals contained in the ore, dissolving them and thus producing their selective removal (Jain and Sharma, 2004). The microorganism may, or may not, get some advantage from this solubilization process (such as a nutrient or energy source). In the iron mining industry, the use of microorganisms could offer an environmentally-friendly alternative to the traditional chemical dephosphorization processes (Delvasto et al., 2005).

In a P-limited environment, microorganisms will be obligated to extract P from mineral sources to supply their growth needs (Banfield et al., 1999) and this is the theoretical base for the biobeneficiation of high-P iron ores. Organic-acid-producing filamentous fungi have been used to remove P from ores in a series of reports (Parks et al, 1990; Buis, 1995; Delvasto et al., 2005). Bioactivation of the indigenous microflora of iron ores, by addition of proper nutrients, has also
been reported as a way to reduce their P content either in leaching columns (Benavides, 2003) or in shake flasks (Delvasto et al., 2005). Some other papers have reported the use of bacteria for the deposphorization of iron ore (He and Wei, 2000; Delvasto et al. 2005) and iron slag (Pradhan et al., 2004). Nevertheless, the mechanisms by which microorganisms can extract P from iron ore are not fully understood and more research is necessary to clarify the biogeochemical processes involved in the biodephosphorization if iron ores.

2. AIMS OF THIS WORK

In the present work, the biological route for deposphorization of a Brazilian high-P iron ore was explored using a filamentous fungus identified as Aspergillus niger strain HNA-1. This fungus was isolated from the indigenous microflora of the ore being beneficiated. The fungus was cultured in a medium where the only source of P was the iron ore. As a result, a P removal from the ore was expected because of the fungal development. The effect of process parameters such as the ore particle size and the pulp density was studied. Surface characterization techniques such as field emission scanning electron microscopy (FESEM) were used to visualize and explain the fungus-to-mineral interactions. The implications of these results for the bio-beneficiation processing of high-P iron ore are discussed.

3. EXPERIMENTAL

3.1 Iron Ore

The iron ore studied comes from the Jangada mine, Minas Gerais, Brazil. The samples used are the rejected fraction of a process of wet magnetic separation. X-ray diffraction (XRD) analyses of the ore revealed hematite (Fe₂O₃), quartz (SiO₂) and some goethite (FeOOH) as mineral phases. X-ray fluorescence spectroscopy (XRF) chemical analyses revealed the following elemental composition (w%): 55.3 Fe; 9.2 Si; 1.6 Al; 0.26 P; 0.26 Mn. Microprobe analyses indicated that P, Si, Al and Mn are mainly associated to goethite phases. For the bio-beneficiation experiments, the ore was sterilized by autoclaving at 121 °C for 30 min three consecutive times in 24 h intervals. Two particle sizes (d) were chosen for the experiments: A fraction of 100% -2.5 +2.0 mm (hereafter d= 2 mm) and another fraction of 100% -0.3 +0.2 mm (hereafter d= 0.2 mm)

3.2 Microorganism, Culture Medium and Inoculum

The acidophilic filamentous fungus Aspergillus niger HNA-1 was isolated from the iron ore associated microflora as described elsewhere (Delvasto et al., 2006). The strain was maintained on NBRJP agar plates (Nautiyal, 1999). The composition of the liquid culture medium used for bio-beneficiation experiments was (g/l): Glucose, 10.0; MgCl₂·6H₂O, 5.0; MgSO₄·7H₂O, 0.25; KCl, 0.20; (NH₄)₂SO₄, 0.10, deionized water to 1 l; without adjustment of the pH. The medium was autoclaved at 121 °C for 30 min. As inoculum, a fungal spore suspension (10⁶ spores/ml) was used.

3.3 Biobeneficiation Experiments

Experiments of bio-beneficiation were carried out in sterile, 250 ml Pyrex® glass flasks. Each flask received the sterile ore, 100 ml of sterile liquid medium and 0.250 ml of inoculum, under sterile conditions provided by a laminar-flow chamber. Un-inoculated flasks were left as blank controls. The flask caps were loosened to allow air exchange during the experiment. The flasks were incubated in a shaker at 150 rpm and at 30 °C. Three experimental conditions were tested: 1) d=0.2 mm ore solids were added at a pulp density (PD) of 5% (w/v); 2) d=0.2 mm ore solids were added at a PD= 10% (w/v) and 3) d= 2 mm ore solids were added at a PD= 10% (w/v). All experiments were triplicated and all flask contents were sampled every sampling time. Sampling was performed at fixed times of 0, 3, 7, 14 and 21 days for experiments with d= 0.2 mm iron ore, while the experiment with d= 2 mm iron ore was sampled at 0, 7, 14 and 21 days.

The variables monitored in the liquid culture were: pH (using a Crison Basic 20 pH meter with an Ag/AgCl electrode model 5202); total Fe in solution (by atomic absorption spectrophotometry, AAS); and citric acid in solution (by the enzymatic Boehringer Mannheim / R-Biopharm test-kit). The spent culture medium was not assayed for any other organic acid. The iron losses reported were calculated from a mass balance between the Fe originally contained in the ore and the leached Fe in solution. All values reported are mean of three replicates.

3.4 Phosphorus Determination
biomass was siphoned and the ore was recovered from the bottom of the container. The recovered ore was water-washed by centrifugation and dried at 100 °C for 24 h. The washed ore from three replicate flasks was pulverized and mixed. A representative composite sub-sample was then pressed at 392 kn per 20 s to form flat pellets of 37 mm in diameter and approximately 12 g in weight. The pellets were quantitatively analyzed for P by XRF using a PANalytical AXIOS XRF spectrometer under vacuum atmosphere. The values reported corresponded to a unique composite pellet. For all cases the standard deviation of the P values was not greater than 0.001 w%. The dephosphorization degree was calculated as:

$$\text{Dephosphorization degree (\%) = \frac{w\%P_{\text{initial}} - w\%P_{\text{final}}}{w\%P_{\text{initial}}} \times 100}$$

(1)

3.5 Characterization of the Fungus-to-Mineral Interactions

In the day 14th of incubation, a small portion of fungal biomass attached to ore particles was extracted before washing. To preserve the cellular morphology, the samples were dried using a Balzers CPD 030 critical point dryer with acetone as a transfer medium and CO2 as a transition liquid. For FESEM imaging, the dried samples were vapour-coated with a conductive composite layer of graphite and gold. FESEM examination was performed using a JEOL microscope model JSM-6335-F at an accelerating voltage of 10 kV. For chemical microanalyses, the samples were vapour-coated only with graphite and examined in a JEOL JSM-6400 scanning electron microscope (SEM) coupled with an energy dispersive X-ray (EDX) probe at an accelerating voltage of 20 kV. The mycelial biomass was also analysed by XRD to characterize the mineral inclusions observed in SEM. To determine the iron accumulated in the biomass by AAS, the biomass was digested in boiling HCl.

4. RESULTS AND DISCUSSION

4.1 Biobeneficiation Experiments

The time courses for the fungal dephosphorization process of the Jangada iron ore are shown in figure 1 (a). The strain *Aspergillus niger* HNA-1 was able to mobilize a maximum of 33.2 % of the P contained in the ore in 21 days of treatment, for the condition of d=0.2 mm ore size and 5% pulp density (PD). Two observations can be highlighted from the plots shown in figure 1 (a): i) At constant ore size (d), an increase of the pulp density (PD) adversely affects the biological P removal, and ii) At constant PD, a decrease in the ore size makes the P extraction from the ore more favourable. The first feature can be explained because of the fact that a higher solids-to-liquid ratio in the biological system may result harmful for the fungal development (Jain and Sharma, 2004). This feature might be due to higher probabilities of abrasive interactions between solids and fungal biomass. Also, the entrapped of ore particles by the fungal biomass should be considered, since at higher PD, biomass-encapsulated solids can form larger aggregates (Jain and Sharma, 2004) on which acidic fungal metabolites will difficulty access, lowering in such a way the biodephosphorization yield. For smaller ore sizes the dephosphorization yield improves because the ore phases containing P can be more readily accessed by the organic acids (citric acid) produced by the fungus during the incubation.

The aim of biobeneficiation is to remove unwanted elements keeping as low as possible the leaching (loss) of the metal value of the ore (Fe in this case). The monitoring of Fe losses during the dephosphorization process is shown in figure 1 (b). For all the conditions tested, the behaviour of Fe losses was almost the same during the first 14 days of treatment. Less than 1% of the Fe contained in the ore was lost during the biological process. In the experiments conducted with d=0.2 mm ore at 10% PD, a marked decrease of the iron extracted from the ore was measured in the 21st day of treatment, suggesting that a process of re-precipitation of the leached Fe might be taking place in the system during the biological treatment of the ore.

In environments where macronutrients such as P are poorly available (such as the one reproduced by the experimental conditions of this work), fungi can mobilize P from mineral sources through a mechanism of acidolysis and complexolysis that is driven by the production of organic acids (Banfield et al., 1999). The acidification and the accumulation of citric acid in the liquid medium are shown in figures 2 (a) and 2 (b) respectively. It is clearly visible that the isolate *A. niger* HNA-1 can acidify the surrounding medium reaching a minimum pH=2.10 at day 21st when d=2mm ore and a PD=10% were used. Figure 2 (a) also shows that PD had no major influence on the lowering of pH. On the other hand, an increase of the ore particle size promoted acidification. In addition, the fungus produced more citric acid with the d=2 mm ore, what explains why the iron losses, figure 1 (b), were higher for this experiment. The explanation for these features may lie in the fact that accessing P in larger ore particles requires more acid consumption,
as compared with small particle ore, on which P-bearing phases are more readily accessible to the mobilizing agent (citric acid). The comparison between the Fe mobilization, figure (1b), and the production of citric acid, figure 2(b), shows that both the variables are highly correlated. As previously mentioned, the ore contains mainly hematite and P-bearing goethite as accessory phase. During the process of biobeneficiation, Fe would be leached from the ore because citric acid would preferentially attack the more soluble phase, goethite, liberating not only some Fe in the liquid medium but also leaving available the P contained in this phase for the fungal uptake. In agreement with this explanation, Groudev (1999) confirms that, under biobeneficiation conditions, goethite is easier to dissolve than hematite.

![Figure 1](image1.png)

**Figure 1.** Time courses for (a) Dephosphorization and (b) Iron losses during the biobeneficiation of high-P iron ore with the indigenous acidophilic fungus *Aspergillus niger* HNA-1

![Figure 2](image2.png)

**Figure 2.** Evolution of liquid medium pH (a) and the release of citric acid into the liquid medium (b) during the biobeneficiation of high-P iron ore with the indigenous acidophilic fungus *Aspergillus niger* HNA-1

### 4.2 Fungus-to-Mineral Interactions During Biobeneficiation

The indigenous fungus *A. niger* HNA-1, developed into a mycelium made up of filaments (hyphae) when incubation with iron ore took place. This mycelial biomass colonized the iron ore particles, as shown in figure 3 (a). From a biogeochemical standpoint, these fungal filaments accomplish three main functions during the biobeneficiation process (Gadd, 2006): i) Sensing the environment, in order to find possible nutrient sources (i.e., P-bearing phases in the ore); ii) Excreting metabolites that may help to exploit the nutrient sources and release nutritive elements (for example organic acids that attack the P-bearing phases in the ore and liberate P in a soluble form) and iii) Taking in the solubilized nutrients for fungal growth. The attachment of the hyphae to the ore surface is essential to grant the fungal colonization and, as it can be seen in figure 3 (b), some defects in the iron ore particles, such as grooves, serve as anchoring location for the hyphae.
Figure 3. FESEM images of the fungus-to-mineral interactions during biobeneficiation of high-P iron ore after 14 days of incubation. (a) General view of an iron ore particle surrounded by fungal hyphae (filaments) of A. niger HNA-1. (b) Detail of a groove in an iron ore particle exhibiting some anchored hyphae. (c) and (d) Zoom view on the hyphae clearly showing the attachment of secondary iron oxide crystallites (arrow).

The biogeochemical processes involved during biobeneficiation of iron ore for P removal can be, however, somewhat complex. In figure 3 (c) and with more detail in figure 3 (d), arrow, a well known phenomenon of metal-mineral accumulation in fungal filaments is readily seen. Fungal filaments are usually covered of exopolymers of mucilage that may act either as particle binders or as dissolved-metal-complexation substrates (Banfield et al., 1999, Gadd, 2006). These complexation substrates may also facilitate the re-precipitation of metallic authigenic minerals when metals ions are found in solution (Banfield et al., 1999, Gadd, 2006). In this case, some iron is solubilized into the liquid medium because of the growth of the fungus in contact with the iron ore and its subsequent production of citric acid to facilitate a scavenging process of P. For example, it was determined that between 113 and 190 mg/l of Fe was concentrated in the liquid medium (~ 0.2 to 0.9 % of Fe losses) in 21 days of treatment. In this concentrated metal solution, conditions for the occurrence of metal biosorption and biomineralization phenomena are given. The typical EDS spectrum and chemical microanalysis of the mineral-bearing fungal filaments are shown in figure 4. Macroscopically, the dry mycelial biomass had a reddish colour. To determine quantitatively its Fe content, the debris-free biomass was digested in hot concentrated HCl and analysed by AAS. It was determined that the biomass contained around 18 w% (dry basis) of Fe, corroborating the semiquantitative chemical analysis shown in figure 4. The presence of Fe suggests the presence of Fe-compounds, other elements such as P are detected, indicating also that the P previously extracted from the parental iron ore is accumulated, either in the cellular material or in the authigenic iron crystals found attached to the biomass (see figure 3 (d)). Authigenic Fe compounds rather than the parental ore surface, are more likely to preferentially re-adsorb the P dissolved by the fungal action because of their higher surface area.

X-ray diffraction analyses of the fungal biomass (figure 4, embedded graph) indicated that Fe-crystallites accumulated in the surface of the hyphae are in fact hematite and goethite, likely to be precipitated by authigenic processes. Extensive examples of such authigenic processes of mineralization on fungal biomass can be found in the literature (Banfield et al., 1999, Gadd, 2006 and references therein). Summarizing, figure 5 shows the biogeochemical processes that might be taking place during the fungus-assisted dephosphorization of iron ores. The biogeochemical process depicted in figure 5 indicates that biomass plays a key role in the process by establishing a dynamic condition on which the elements extracted during the fungal action, Fe and P, are accumulated or mineralized (re-precipitated) into/onto the biomass. This dynamic process imposes the main drawback of this biological beneficiation process, i.e., biomass must be removed from the ore to grant the complete removal of P. In this work, the biomass removal was attained by the mechanical stirring of the biomass-ore mixture in a solution of an oxidative agent (NaClO). It was found that the NaClO post-treatment per se did not remove P from the ore and the determined dephosphorization was due to the fungal action.
Unlike other reports (Buis, 1995), in the present work P was determined by analysing the ore solids, instead of the leachate liquor. This provides a higher degree of confidence in the dephosphorization values reported herein.

![EDX spectrum](image)

**Figure 4.** Typical EDX spectrum of the *A. niger* HNA-1 hyphae after the biobeneficiation process. Embedded table shows the EDX semiquantitative chemical analysis of the hyphae. Typical X-ray diffractogram of the hyphal biomass extracted from biobeneficiation experiments is also embedded in the figure. Clear peaks corresponding to goethite and hematite can be readily identified.

**Figure 5.** Biogeochemical processes involved in the biobeneficiation of iron ore for P removal.

### 5. CONCLUDING REMARKS

The biological route to remove phosphorus from iron ore was found to be feasible using the fungus *A. niger* HNA-1. Interpretation of the information supplied by the examination of the fungus-to-mineral interactions indicated, however, that the beneficiation process could be affected if a post-treatment removal of the generated biomass is not performed. Biomass should be removed since fungal mycelium, and its associated re-precipitated minerals, may act as a sink for the P removed in the process. Dephosphorization yields were somewhat low and long term treatments (21 days) were needed to attain a dephosphorization degree of around 30%.
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