Mobilization of phosphorus from iron ore by the bacterium 
**Burkholderia caribensis** FeGL03


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**Abstract**

The bacterium *Burkholderia caribensis* FeGL03, isolated from a Brazilian high-phosphorus iron ore, was used to mobilize the phosphate contained in the same ore. The phosphate-mobilizing ability of the organism was tested in shake-flask cultures containing sterilized crushed iron ore in a chemically-defined liquid culture medium containing glucose (1 g/l) as carbon source and no phosphorus source except the ore itself. Phosphate removal from the ore was determined by measuring the residual phosphate contained in the ore after the bacterial treatment. The time course of the phosphate mobilization process was determined with two different particle sizes (2.0 mm and 0.2 mm mean size) of the crushed ore under conditions of unlimited and limited air exchange. Between 5% and 20% of the phosphorus originally contained in the ore was mobilized in 21 days of treatment. Other variables such as dissolved Fe, pH and cell counts were also monitored throughout the trials. It was also found that this bacterium accumulated gluconic acid in the spent broth. Scanning electron microscopy, revealed biofilms on the ore surface as a result of the production of exopolymorphic substances (EPS). Extraction of the EPS from the cultures and its analysis by Fourier transform infrared techniques revealed the presence of molecular functionalities capable of interacting with the ore surface and with the iron dissolved in the medium. It was found that dense biofilms, formed under limited air exchange, resulted in lower phosphate mobilization from the ore than under unlimited air exchange. This was found to be a consequence of a dynamic process of iron and phosphate re-precipitation within the formed biofilms.

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**Keywords:** High phosphorus iron ore; Bacteria; Bioleaching

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1. Introduction

Phosphate, which is frequently found as a constituent of iron ore, is deleterious in the manufacture of iron and steel (Kokal, 1990). Heat treatment and subsequent leaching is a way for upgrading high-P iron ores (Feld et al., 1968; Gooden et al., 1974; Muhammed and Zhang, 1989; Kokal, 1990; Araujo et al., 1994; Cheng et al., 1999; Kokal et al., 2003). Low prices in the recent past for this commodity discouraged industrial adoption of hydrometallurgical beneficiation of such ores. At the present time, an increase in world steel production has increased demand for iron ore with a consequent increase in the price for this commodity, making hydrometallurgical phosphate removal viable (Kokal et al., 2003). Biohydrometallurgy is an option for the removal of unwanted phosphate from iron ores because it is well established that many microorganisms, especially in nutrient-limited environments, are capable of mobilizing the phosphorus contained in minerals (Buijs, 1995) and iron-oxidizing bacteria (He and Wei, 2000). Heterotrophic phosphate bioleaching from an iron slag using the bacterium *Fratureia aurantia* has been reported by Pradhan et al. (2004). The organisms used in these previous studies were mostly isolated from sources other than the ore to be treated. From a practical standpoint, the use of microorganisms indigenous to an ore can have some advantage in biobeneficiation in terms of either adaptation time of the strains to a biobeneficiation process or in ecological disruptions in the surrounding area in an in situ biobeneficiation process (Delvasto et al., 2005; Delvasto et al., 2006a).

In the present study, the possibility of removing phosphate from a Brazilian iron ore (~0.2 wt% of P) using a heterotrophic bacterium indigenous to the ore, identified as *Burkholderia caribensis* FeGL03, is explored. This bacterium was incubated with crushed iron ore as sole P source and glucose as carbon source. The aim
was to force the bacterium to obtain P, a limiting nutrient, from the ore, resulting in a lowering in the P content of the ore. The influence of parameters such as particle size of the ore, and aeration condition was studied. Scanning electron microscopy (SEM) and chemical microanalyses were performed to study biofilm formation on the surface of the treated ore. Chemical analysis of the spent liquid medium for dissolved Fe, organic acids and pH and analysis of bacterially-produced exopolymeric substances (EPS) by means of Fourier transformed infrared spectroscopy (FTIR), was performed to follow the overall process of the phosphate mobilization from the iron ore by the bacteria. The implications of these findings for commercial biobeneficiation of this important raw material are discussed.

2. Materials and methods

2.1. Iron ore

The iron ore studied was the rejected fraction from a wet magnetic separation operation at the Jangada Mine, located in Minas Gerais State, Brazil. X-ray diffraction analysis (XRD) of the ore revealed hematite (Fe₂O₃) and quartz (SiO₂) as main mineral constituents. X-ray fluorescence chemical analysis of the ore (Table 1) indicated a mean phosphorus content of 0.23 wt%, almost three times higher than the maximum allowed in international iron ore commerce. Microprobe analysis of the ore (not shown) revealed that phosphorus was associated with low-grade iron oxide phases containing Al and Si.

2.2. Microorganism and culture medium

The isolation of the strain used in the phosphate-removal experiments from the ore was described elsewhere (Delvasto et al., 2006a). The exopolysaccharide-producing, heterotrophic bacterium with an ability to dissolve calcium phosphate in vitro was identified as B. caribensis FeGL03 by 16S rDNA gene sequencing (Delvasto et al., 2006b). A phosphorus-free, chemically-defined liquid culture medium, based on the NBRIP medium of Nautiyal (1999), was used in the phosphate-removal experiments. The composition of the medium was as follows (in g/l deionized water): Glucose, 10.0; MgCl₂·6H₂O, 5.0; MgSO₄·7H₂O, 0.25; KCl, 0.20; (NH₄)₂S₄O₆, 0.10. The medium was sterilized by autoclaving at 121°C for 30 min. The pH of the medium was around five after sterilization.

2.3. Phosphate removal in shake-flasks and phosphorus analysis

Phosphate removal was studied in cotton-stoppered 250-ml Erlenmeyer flasks. Each flask containing 7.5 g of crushed iron ore was autoclaved (121°C for 30 min) three times at 24-h intervals. After placing the autoclaved flasks in a laminar flow chamber, each of them received 150 ml of sterile liquid medium and 0.250 ml of bacterial inoculum. The inoculum was prepared by resuspending 10-day-old B. caribensis FeGL03 colonies, grown on NBRIP agar plates, in sterile deionized water. The cell concentration of the 10-day-old B. caribensis bacterial inoculum. The inoculum was prepared by resuspending of them received 150 ml of sterile liquid medium and 0.250 ml of the 0.2 mm-particle fraction was carried out in 250 ml Pyrex® flasks plugged with silicone stoppers. Uninoculated flasks served as controls in all cases. Incubation took place on an orbital shaker at 150 rpm and 30°C.

Sampling was performed during incubation on days 0, 1, 3, 7, 14 and 21. At every sampling time, three replicate inoculated flasks and two control flasks were removed from the orbital shaker and the entire content of each flask (spent broth and reacted ore) were collected separately by siphoning. The following parameters were monitored in the spent broth: cell concentration (using a Thoma-type hemacytometer), pH (using a Crison Basic 20 pH meter with an Ag/AgCl electrode model 5202) and total Fe in solution (determined by atomic absorption spectrophotometry). All measurements were reported as the mean of three replicate flasks ± standard deviation (SD).

At every sampling time, the ore collected from the bottom of each three replicate flasks, was washed repeatedly by centrifugation with deionized water, dried at 100°C for 24 h, pulverized and combined. A representative composite subsample was then pressed at 392 kN for 20 s to make flat pellets of 37 mm in diameter and approximately 12 g in weight. The pellets were quantitatively analysed for P by X-ray fluorescence (XRF) using a PANalytical ANOXS XRF spectrometer under a 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 wt%. From XRF data, the percentage of phosphorus mobilized from the ore was calculated as follows:

\[
\text{Phosphate mobilization (\%)} = \frac{\text{wt\%P}_{\text{initial}} - \text{wt\%P}_{\text{final}}}{\text{wt\%P}_{\text{initial}}} \times 100
\]

where, wt%P$_{\text{initial}}$, refers to the weight percentage of phosphorus originally contained in the ore and, wt%P$_{\text{final}}$, refers to the weight percentage of phosphorus contained in the ore after the bacterial treatment, at any given sampling time.

3. Electron microscopy

Before washing, some particles were removed from the flasks at day 14 and dried using a Balzers CPD 030 critical point dryer (CPD) with acetone as the transfer medium and CO$_2$ as the transition liquid. The dried particles were mounted on double-sided carbon tape and fixed on a brass holder. Mounted samples were then sputter-coated with a carbon–gold bilayer. SEM examination and energy dispersive X-ray (EDX) chemical microanalyses were performed using a JEOL JSM-6400 SEM coupled with EDX at an accelerating voltage of 20 kV.

To evaluate bacterial morphology, one drop from a 14-day-old culture was placed on a parafilm® strip. A copper micro-grid was then placed on the drop for 2–3 min to allow adsorption of the bacterial cells to the grid. The excess of liquid was drained away by placing the grid on cellulose filter paper. The grid was then placed on a drop of 2% uranyl-acetate dye for 2–3 min and then rinsed three to four times in distilled water, draining away the excess water with filter paper. The specimens were observed under a transmission electron microscope (TEM) JEOL-1010 at an accelerating voltage of 80 kV.

3.1. Analysis of organic acids and exopolymeric substances

The spent broth from the experiment carried out under limited air exchange was analysed using the Boehringer Mannheim/R-Biopharm Enzymatic BioAnalysis kits for detection of gluconic,

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<th>Element</th>
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<tr>
<td>Fe</td>
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<td>Mn</td>
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<tr>
<td>O</td>
<td>33.30</td>
<td>P</td>
<td>0.23</td>
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citric and acetic acids, following the manufacturer's directions. Exopolymeric substances accumulated in the spent broth of all experiments were precipitated by the addition of ethanol to the medium, according to the procedure of Kogan et al. (2002). A few minutes after the ethanol addition to the medium, an insoluble, cream-white colored, spaghetti-like mass was formed. This mass was washed by centrifugation with 1:1 ethanol–water solution, oven-dried at 45 °C for 24 h and analysed by Fourier transform infrared spectroscopy (FTIR) as described by Torres et al. (2005). The FTIR spectrum was recorded using a MIDAC Prospect-IR spectrophotometer.

4. Results and discussion

4.1. Phosphate removal from iron ore in shake flask with unlimited exchange of air

The time courses of growth of the bacterial population, accumulation of total dissolved iron, pH changes, and phosphate removal from the ore in percent are shown in Fig. 1a and b for the two different particle sizes tested, 2 mm and 0.2 mm, respectively. In both cases, the bacterial population increased very rapidly during the first day of treatment. Can be readily seen that after one day, both cultures reached the stationary phase of growth, followed by additional slight population growth up to day 7 of culturing and a slight decrease thereafter. The bacterial population in both cases stabilized around 2 × 10^9 cells/ml between days 1 and 21.

The pH under conditions of unlimited air exchange (Fig. 1a and b) showed a decrease of approximately 1.2 pH units between days 0 and 3 for both particle sizes, thereafter remaining nearly constant. This pH drop may have been due to the release of organic acids (O.A.) by the bacteria into the surrounding medium. Although O.A. were not measured in these two experiments, it is known that O.A. production constitutes an adaptation strategy by which bacteria and other microorganisms can extract limiting nutrients – such as P, K and Ca, from insoluble mineral matrices, by chemical attack of the crystal structure of the nutrient-containing host minerals (Banfield et al., 1999). In this case, such a strategy must have been used by the isolate B. caribensis FeGL03 in order to solubilize the P-containing phases within the iron ore to obtain enough phosphate to sustain its growth. In previously reported experiments, Delvasto et al. (2006b) showed that this isolate can mobilize phosphorus from an insoluble Ca-phosphate (hydroxyapatite). The mechanisms for such a mobilization were, however, not fully established.

The time required for phosphate mobilization (Fig. 1a and b) deserves special attention. For both particle sizes, a rapid decrease in the P content in the ore was observed on the first day of treatment. Specifically, 12.7% and 10.9% of the P were removed from the ore with particle sizes of 2 mm and 0.2 mm, respectively. Constructing a mass balance from the available data, it can be shown that in the first day of treatment the bacteria accumulated P in the culture medium at a maximum possible concentration of 14.6 mg/l and 12.5 mg/l, using the 2 mm and 0.2 mm particle sizes, respectively. This rapid phosphate mobilization stage coincided with a marked drop in pH in the medium during the rapid growth stage (1st day, Fig. 1a and b), suggesting a process of phosphate scavenging by which the bacteria generate a threshold phosphate concentration in the liquid medium that allows population growth (Delvasto et al., 2006b).

When grown on hydroxyapatite as P-source, B. caribensis FeGL03 showed a threshold phosphate concentration between 15 mg/l and 50 mg/l (Delvasto et al., 2006b), similar to the maximum possible concentrations of P estimated in the liquid for day 1, in the experiments shown in Fig. 1a and b. After day 1, the percentage of phosphate originally present in the ore that was mobilized by the bacteria decreased. This might result counterintuitive, since one would expect in an ideal biobeneficiation process for P removal, phosphate should be mobilized out from the ore in a con-

Fig. 1. Time courses of cell multiplication, and changes in pH, dissolved Fe and percentage of phosphate mobilization during treatment of high-P iron ore with Burkholderia caribensis FeGL03. Conditions tested: (a) Particle size 2 mm, unlimited air exchange; (b) particle size 0.2 mm, unlimited air exchange; and (c) particle size 0.2 mm, limited air exchange. Bars indicate ±SD of three replicates.
continuous fashion. The fact that phosphate removal from the ore exhibited risings and falls rather than being continuous, indicates that some of the phosphate bacterially extracted from the ore might be re-immobilized and released again. This suggests that a dynamic process is established between phosphate released and the parent ore surface or the microorganisms adhered on it. Re-adsorption of extracted phosphate by the ore surface is likely to occur because of the high phosphate-binding capacity of iron oxides (Taylor and Schwertmann, 1974). On the other hand, biofilms, i.e., bacterial assemblages bound to the ore surface, would also actively take up phosphate, because it is a key nutrient for cell processes. The accumulation of phosphates in biofilms will be discussed later, by examining evidences provided by scanning electron microscopy (SEM).

In the course of the process, phosphate mobilization from the ore continued, reaching a maximum value of 20% on the 21st day with the 2 mm particle size and 17% with the 0.2 mm particle size, showing that under these conditions the particle size had no major influence on the maximum amount of phosphate mobilization from the ore. Solubilized Fe in these two experiments was below 10 mg/l, indicating either that the phosphate removal by the bacteria was selective through preferential attack of phosphate-bearing phases, or that Fe was re-precipitated. In un-inoculated controls no Fe solubilization occurred and pH values remained around the initial value for the duration of the experiment. The phosphate content of the ore in un-inoculated flasks did not change during the course of the experiment.

4.2. Phosphate removal from iron ore in shake flasks with limited aeration

In an experiment conducted in closed vessels, i.e. under limited access to air, the bacterial population increased very rapidly during the first day of treatment, as with unlimited aeration. By contrast, the cell concentration after the first day increased very gradually with time for the remainder of the experiment, reaching a value of $7 \times 10^8$ on day 21 (Fig. 1c). The pH in this experiment rose on day 1 and decreased on day 3, with great variation in the readings on the different sampling days. The maximum drop in pH was only 0.4 units from the initial value of the medium at day 0 (pH ~ 5), much less than with unlimited aeration. This suggests a decrease in the amount of O.A. produced by the bacteria because of the lower concentration of oxygen in the liquid, when compared to the experiments conducted under unlimited access of air (Fig. 1a and b).

In this experiment conducted with limited aeration of the culture vessels, the concentration of gluconic acid that accumulated in the broth was determined on days 7, 14 and 21 and found to be 62 mg/l, 47 mg/l and 50 mg/l, respectively (SD = 4 mg/l). Analyses were also run for citric and acetic acids, but neither was detected in the broth. It can be seen that the concentration of gluconic acid remained approximately constant over the time interval from the 7th to 21st day, which is consistent with the almost constant pH of about 4.8 over that time interval (Fig. 1c).

It is known that mineral phosphate solubilizing Gram-negative bacteria oxidize glucose to gluconic acid through an aerobic process catalyzed by the enzyme glucose dehydrogenase located in the outer face of the cytoplasmic membrane (Goldstein et al., 1999; Goldstein, 2000). The restricted air supply in these cultures would then adversely affect gluconic acid production by *B. caribensis* FeGL03. Although it has been reported that phosphate removal by *Burkholderia* spp. from sediments containing mineral-phosphates was facilitated in the absence of aeration (Kim et al. (2005)), our results suggest the opposite in respect to phosphate mobilized from iron ore by our isolate. Kim et al. (2005) results would be explained because their *Burkholderia* isolates accumulated important amounts of acetic acid in the cultures when air was not supplied; and this acid, however, was not detected in our experiments.

As can be seen in Fig. 1c, the amount of phosphate removed from iron ore reached a maximum of 7.5% on day 3, and then dropped to a stable concentration between 4.8% and 4.3% for the remainder of the experiment. This indicates that extracted phosphate was sorbed by the ore as previously explained. In this experiment, the amount of Fe released into solution was higher than under unlimited aeration, reaching a maximum of 15.9 mg/l on day 7. The oscillating shape of the curve for Fe release was, however, similar to that of curves obtained under unlimited aeration (Fig. 1a and b), indicating that re-precipitation of Fe was occurring during phosphate removal. As with unlimited aeration, the control vessels did not show appreciable changes in pH or dissolved Fe, or in the final phosphate content of the ores.

4.3. Biofilm formation by SEM analysis

In nutrient-limited environments, such as the one in our experiments, bacteria must colonize mineral surfaces where phosphate is located in order to scavenge it (Banfield et al., 1999). They accomplish this through biofilm formation. Biofilms are complex aggregates of bacterial cells, bacterial exopolymers, mineral debris and other metabolites attached to a surface. As can be seen in Fig. 2, low-grade, phosphate-rich (~0.3 wt% as revealed by EDX analysis) iron ore particles were preferentially colonized by cells of *B. caribensis* FeGL03. Biofilm formation was clearly seen on the particles, in contrast to low-phosphate particles like quartz or hematite which remained almost uncolonized.

Biofilm is formed by secretion of exopolymERIC substance (EPS) by bacteria colonizing a surface. EPS facilitates the initial surface colonization by bacterial cells, since it acts as a surface conditioning agent creating primary or secondary bonds between the surface metal centres and the EPS functional groups (Omoike and Chorover, 2006). To analyse the effect of the bacterial colonization of an ore surface, a SEM analysis was performed. In Fig. 3a and b, SEM imaging reveals bacterial assemblages formed on the ore surface in the experiments with unlimited aeration. These biofilms consist of interconnected cells attached to the ore surface by a

![Image](Image308x106 to 546x297)

**Fig. 2.** SEM images of the preferential colonization of ore particles containing phosphate. Left: Low-grade iron ore particle containing 0.28 wt% phosphorus as revealed by EDX analysis (FeOx + Bio), heavily-colonized biofilm can be seen. Center: Phosphorus-free quartz particle (SiO2), un-colonized. Right: High iron containing particle, possibly specular hematite (Fe2O3), with 0.04 wt%P, poorly colonized. Experiment conditions: 14 days of incubation, air exchange permitted, ore size: 0.2 mm.
network of extracellular material. Special care should be taken, however, during Interpretation of SEM images from biological specimens. Sample preparation during CPD procedures may disrupt exopolymeric layers in biofilms (Bennett et al., 2006). Extracellular networks as those shown in Figs. 2–4 could result as a consequence of desiccation of hydrated structures such as bacterial slimes (Dohnalkova et al., 2005).

On the other hand, their regular and undistorted cable-like shape at higher magnification (Fig. 4b) suggest that extracellular networks observed could actually be due to the interconnection of proteinaceous cell appendages such as fibrils and anchors (Ishii et al., 2004) or pili (Chung et al., 2003; Tomish and Mohr, 2003). These structures are used by bacteria to strongly attach to surfaces during initial and intermediate stages of surface colonization (Davey and O'Toole, 2000; Dunne, 2002). As reported elsewhere (Miron et al., 2001; Levy et al., 2003; Ishii et al., 2004; Mora Bejarano and Schneider, 2004), cell appendages involved in surface colonization can give rise to clear extracellular networks similar to those observed in Fig. 4b.

According to the results shown by Ishii et al. (2004) and the detailed procedures given by Bennett et al. (2006), strongly adhesive bacterial interactions to surfaces, as those promoted by protein-rich appendages, are less prone to disruptions during CPD procedures. In any case, distribution density of the observed extracellular networks ought not to be strongly affected by sample preparation and, thus, the difference in relative density and cross-linking of the extracellular networks found in the biofilms formed by B. caribensis FeGL03 on iron ore particles may be regarded as representative of each condition tested.
phosphate-bearing phases (such as aluminous gangue minerals) may be driven either by H⁺ surface attack and/or by surface metal ion complexation by organic ligands (Banfield et al., 1999; White-law et al., 1999).

In the course of this investigation we found using microprobe analysis (data not shown) that phosphate in the Jangada iron ore was associated with iron phases in which Al was present. Proton attack of Al-bearing iron oxides would release into the liquid medium not only Fe³⁺ and/or Al³⁺, but also the phosphate occluded within these phases. Anions of gluconic acid accumulated by the bacteria may also assist mineral dissolution, either by interacting with the ore surface (complexing Al³⁺ and/or Fe³⁺) or complexing the cations released in solution by proton attack, lowering in such a way the saturation state and thus accelerating the mineral dissolution. Either of both processes requires the formation of stable metal complexes with gluconic acid. Whitelaw et al. (1999) studied the dissolution of Al, Ca and Fe phosphates with a gluconic-acid-producing fungus. These researchers found that solubilization of Fe-phosphate was poor, because of the lower Fe-chelating ability of gluconate, when compared to that of Al or Ca. They indicated that gluconate may form more stable complexes with Al³⁺ (equilibrium constant, \( k = 96.5 \)) than with Fe³⁺ (equilibrium constant, \( k = 3.2 \times 10^{-6} \)).

As a consequence of mineral dissolution, released metal ions (Fe³⁺ and/or Al³⁺) may be accumulated in the biofilm region. This feature was confirmed by EDX analyses performed in biofilm regions (colonized zones) in Fig. 3b. The chemical differences between the un-colonized (background) ore and the ore surface colonized by biofilms are shown in Table 2. Biofilm exhibited a higher concentration of C and O than the background ore as well as the presence of N, typical of biological assemblages. Cl was also detected in the biofilm, possibly incorporated by the bacteria from the culture medium. Other elements present in the biofilm, such as Fe, Al, Si and P would necessarily proceed from the ore dissolution.

Released metals can be accumulated in the biofilms by complexation with active moieties (such as carboxyl groups) present in EPS or other cellular material (Corzo et al., 1994; Comte et al., 2006). For example, extensive accumulation of Fe³⁺ in biofilm EPS through a complexation mechanism has been reported in the pyrite – Acidithiobacillus ferrooxidans system, and this feature modulated the bacterial colonization of the pyrite surface (Kinzler et al., 2003).

Biomineralization may constitute another way for the accumulation of metals in biofilms. According to several researchers (Banfield et al., 1999; Welch et al., 1999; Fortin, 2004; Fortin and Langley, 2005), EPS and cell walls provide nucleation sites for the precipitation of secondary authigenic minerals, through abiotic passive reactions. Corzo et al. (1994) studied the precipitation (gelling) of EPS from Bradyrhizobium spp. with several metal cations including Fe³⁺ and Al³⁺. These researchers indicated that some species originating in the hydrolysis of Fe³⁺ ion, such as Fe(OH)²⁺ could interact with COO⁻ groups of two different chains of acidic EPS, by creating divalent cationic bridges that facilitate gelling of EPS. They also found that at pH values between 4 and 5 (similar to those reached in the present study), the Fe³⁺/EPS ratio was maximum due to the possible presence of Fe-(oxyhydro)oxides in the gelled EPS. If saturation conditions are reached, acidic EPS may act as template in Fe³⁺biomineralization (Chan et al., 2004; Fortin and Langley, 2005). As previously explained, the preferential complexing of Al³⁺ by gluconate would leave enough Fe³⁺ or Fe(OH)²⁺ available to increase the saturation condition for Fe-(oxyhydro)oxides precipitation in the biofilm microenvironment. Re-precipitation of iron phases, would explain not only the low concentration of Fe detected in the liquid medium but also its fluctuation over time (Fig. 1a–c).

Polymer coated secondary iron oxides (SO) are readily observed in Fig. 4b, suggesting re-precipitation of Fe within the biofilms. The EDX spectrum of this SO is shown in Fig. 5, revealing an iron con-

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<th>Table 2</th>
<th>EDX chemical microanalyses (wt%) of the biofilm and background ore in Fig. 3b</th>
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![Fig. 5. Typical EDX spectrum and element quantification (embedded table) of the secondary iron oxide (SO) packet shown in Fig. 4b.](image-url)
tent of 38.5 wt% and a phosphorus content of 0.26 wt%. Other EDX chemical microanalyses performed on similar iron–polymer packets found within the biofilms (not shown) revealed iron contents between 30 wt% and 40 wt% and phosphate contents between 0.25 wt% and 0.51 wt%.

These evidences suggest that phosphate extracted from the original ore by bacterial action can be accumulated not only in cellular material (phospholipids, cell wall components, DNA, etc.) but also co-precipitated with biomineralized secondary Fe-oxides because of the higher surface area and reactivity of these SO when compared to the parental iron ore surface (Banfield et al., 2000). EPS itself can act as a reservoir of phosphate, as has been demonstrated by means of EDX techniques by Cloete and Oosthuizen (2001) in biological waste-water treatment processes. These dynamic processes of re-adsorption of solubilized phosphate onto ore biofilms (i.e. by the EPS and/or by the re-precipitated mineral debris) must be taken into consideration because they may also adversely affect the biobeneficiation process. Particles extensively covered by biofilm such as shown in Fig. 4a, could keep most of the dissolved phosphate from the parent ore after the mild after-treatment washing procedure used in this study. This last feature not only would explain why phosphate-removal efficiencies were poor, but also would explain why unexpectedly higher phosphate removal was observed with the larger iron-ore particle size tested (biofilms in this case were less dense than those found in the small particle size experiments). Possibly, a post-treatment washing with special reagents, capable of removing the biofilms formed on the ore surface, could increase the extent of phosphate removal that can be achieved.

4.4. FTIR analysis

B. caribensis is a polymer-producing bacterium, which has been identified as responsible for lump aggregation in soils (Vanhaverbeke et al., 2001). Fig. 6 shows a typical cell of B. caribensis FeGL03 that consists of the cell itself and a network of bound exopolymeric material surrounding it. In contrast to the problems associated to sample preparation for SEM, TEM images are more reliable since sample preparation is faster and less prone to desiccation-induced artifacts (Ishii et al., 2004). The main drawback of this technique is that TEM images provide only a two-dimensional projection of the cell/EPS assemblage (Ishii et al., 2004). Fig. 6 shows, however, that the shape of extracellular materials associated to a bacterial cell clearly resembles the network structures observed in biofilms shown in Figs. 3 and 4.

FTIR can be a powerful tool to reveal the chemical nature of the exopolymeric material surrounding a cell, and also other soluble exopolymers present in the liquid medium. Several EPS extraction methods have been proposed elsewhere (Comte et al., 2006). In our present study, the spent liquid medium was treated with ethanol. This enabled recovery of a bulk ethanol-insoluble fraction expected to be composed of cellular material, bound EPS and soluble EPS. The functional groups of this material that may interact with the ore at the moment of sampling can be determined. A typical FTIR spectrum of this ethanol precipitated bacterial substance is shown in Fig. 7. Similar spectra were obtained from all experiments. The apparent concentration of this material was around 280 mg/l on day 14 of culturing. The interpretation of the transmittance minima observed in the spectrum is shown in Table 3. IR band assignment was made by comparing with EPS and other cell material references, previously published in the literature (see Table 3 for details).

Fig. 6. Typical TEM image of a Burkholderia caribensis FeGL03 cell after 14 days of incubation with iron ore. The EPS sheath is clearly differentiable from the bacterial cell.

Fig. 7. Typical FTIR spectrum of the ethanol-precipitated bacterial substances accumulated in the Burkholderia caribensis FeGL03 culture medium after 14 days of incubation.
Several functionalities (Table 3) that play a key role in bacteria-to-mineral adhesion, mineral dissolution and/or metal complexation are readily seen in the spectrum in Fig. 7. Acidic functionalities associated to carboxylic acids and also their salts (probably due to the interaction of carboxyl moieties with the metals dissolved in the medium) are clearly seen. The EPS produced by B. caribensis comprises an acidic sugar and one acetyl group per repeating unit (Vanhaverbeke et al., 2001). It has been shown that acidic exopolysaccharides can increase the weathering rate of minerals when other metabolites, such as O.A., are present (Banfield et al., 1999; Parks et al., 1990; Buis, 1995; He and Wei, 2000; Pradhan et al., 2004; Delvasto et al., 2005). In our present study, the iron ore contained ~0.2 wt% of phosphate, requiring phosphate removal greater than 60% to make it suitable for international trade. Treatment of the ore with B. caribensis FeGLO3 resulted in phosphate removal of 20.3% in 3 weeks. These findings suggest that biological approaches could be applicable for long-term leaching treatments of marginal high-phosphorus iron ore in heaps or ponds.

5. Concluding remarks

Phosphorus mobilization from iron ores was found to be possible using bacteria although the extent of phosphate removal was low. Up to 20% of the phosphorus contained in the ore was extracted using a heterotrophic polymer-producing bacterium isolated from the ore, identified as B. caribensis FeGLO3, using glucose as carbon source. Production of gluconic acid, exopolysaccharides and the formation of biofilms were implicated in the mechanisms by which the bacteria extracted the phosphorus contained in the ore. Our results suggest that understanding the biogeochemical mechanisms involved in phosphate removal from iron ore is of paramount importance because the bacteria/mineral interactions are complex and the process of P extraction can be adversely affected by dynamic chemical processes, such as re-precipitation of Fe and phosphate, within a biofilm microenvironment.

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References


Table 3

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Main functional groups observed in IR spectrum of the ethanol-precipitated bacterial substances accumulated in the Burkholderia caribensis FeGLO3 culture medium after 14 days of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave number (cm⁻¹)</td>
<td>IR band assignment – Functional groups</td>
</tr>
<tr>
<td>3414</td>
<td>OH: Polymeric O–H stretching vibrationab</td>
</tr>
<tr>
<td>2977</td>
<td>Methyl (–CH₃): C–H asymmetric stretching vibration (fatty acids)ab</td>
</tr>
<tr>
<td>2934</td>
<td>Methylene (–CH₂): C–H asymmetric stretching vibration (fatty acids)ab</td>
</tr>
<tr>
<td>1724</td>
<td>Carbonyl(C=O): Stretching vibration of C=O (Carboxylic acids)ab,cd,ef</td>
</tr>
<tr>
<td>1638</td>
<td>Amide I spectral region: Mainly due to stretching vibration of C=O in peptide bond (proteins)g</td>
</tr>
<tr>
<td>1618</td>
<td>Carbonyl(C=O): Asymmetric stretching vibration of C=O (Carboxylic acid salt)h,j,k</td>
</tr>
<tr>
<td>1544</td>
<td>Amide II spectral region: Mainly due to stretching vibration of C–N and deformation vibration of N–H in peptide bond (proteins)i,j,k,loc</td>
</tr>
<tr>
<td>1538</td>
<td>Methyl (–CH₃): C–H scissoring (peptidoglycan, lipopolysaccharides or phospholipids)j,k,la–k,k</td>
</tr>
<tr>
<td>1381</td>
<td>Methyl (–CH₃): C–H symmetric bending vibrationa-e</td>
</tr>
<tr>
<td>1281</td>
<td>C–N stretching vibration (aromatic secondary amine)i</td>
</tr>
<tr>
<td>1229</td>
<td>C–O–C asymmetric stretching vibration of esters groups in cell wall asymmetric/stretching vibration of DNA phosphate groupsab</td>
</tr>
<tr>
<td>1184</td>
<td>Asymmetric stretching vibration of uncomplexed P–O bonds of phosphate groupa</td>
</tr>
<tr>
<td>1133–896</td>
<td>C–O–C (Glycosidic linkage), C–O, C–C vibrations (Polysaccharides)h,i,k,j,k</td>
</tr>
<tr>
<td>&lt;860</td>
<td>Phosphate or sulphur functional groupsab,k,k</td>
</tr>
</tbody>
</table>


4.5. Implications for biodynametallurgical processing of iron ores

Traditional hydrometallurgical processing of high-P iron ores, comprising a combination of heat treatment and leaching, results in phosphate removal amounting to between 60% and 97% (Feld et al., 1968; Gooden et al., 1974; Muhammed and Zhang, 1989; Kokal, 1990; Araujo et al., 1994; Cheng et al., 1999; Kokal et al., 2003). On the other hand, phosphate removal from iron ore using biodynametallurgical means may range from 1% up to 75% but require longer treatment times, ranging from weeks to a few months.

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