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Research paper

Refinement of industrial kaolin by microbial removal of iron-bearing impurities

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ABSTRACT

The commercial value of kaolin raw materials is greatly affected by the presence and content of iron-bearing impurities, which can have a detrimental effect on the whiteness and refractoriness of manufactured products. Because of the high cost and environmental impact of techniques currently used to remove these impurities, some effort is now targeted toward the development of alternative methods, such as biological processes. This paper reports a series of anaerobic microcosm experiments conducted to evaluate the suitability of iron-respiring bacteria (IRB) of the *Shewanella* species (*S. alga* BrY, *S. oneidensis* MR-1, *S. putrefaciens* CN32, and *S. putrefaciens* CIP 8040), in bioleaching iron-bearing impurities from raw kaolin. All tested bacterial strains were able to reduce and leach ferric iron present in the kaolin, thereby substantially improving its color properties. Among the tested bacteria, *S. putrefaciens* CIP8040 produced the greatest improvements, with increases in ISO brightness and whiteness from 74% to 79% and from 54% to 66%, respectively, in 5 days at 30 °C. Neither secondary mineral nor crystal-chemical alteration of the kaolinite was observed by X-ray diffraction and infrared spectroscopy. Observations of the biotreated kaolins by scanning electron microscopy showed that the original hexagonal shape of the clay particles became less regular. Further research and development should now focus on optimising the rate and extent of the bioleaching process before its application at a larger pilot or industrial scale. In particular, further studies should evaluate the environmental and economical benefits compared to currently used approaches, such as the chemical bleaching with sodium hydrosulfite.

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1. Introduction

Clay minerals are among the most important structural materials on the Earth's surface, being major components in soils and sediments, and the rocks formed from these (Moore and Reynolds, 1997). Clays play an important role in environmental, agricultural and industrial processes such as nutrient cycling, plant growth, petroleum production, and contaminant migration (Stucki, 2006; Stucki et al., 2002). Clay raw materials include kaolins, which are mined materials rich in kaolinite ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$), a clay mineral generally formed by the intense weathering or hydrothermal alteration of aluminosilicate minerals, such as feldspars and mica. Apart from kaolinite, kaolins as mined contain other mineral phases, such as quartz, micas and unaltered feldspars. They also contain minerals that act as pigments, including iron oxides or oxyhydroxides (lepidocrocite, goethite, and/or hematite), which give a brown colour, and the TiO_2 polymorphs anatase and/or rutile, which give a pink colour. Similarly to kaolinite,

iron oxides and oxyhydroxides are common products of intense weathering; their presence in kaolins is frequent but in low quantity (generally less than 5 m%).

Kaolins are exploited for a wide range of industrial applications, such as the production of paper (as a filler and/or a coating material), ceramics (to add strength, abrasion resistance, and rigidity), plastics (as a filler) and paints (as a filler and thickening agent). Before kaolin products can be used by manufacturing industry, the raw kaolins have to be refined to meet commercial specifications (Hosseini et al., 2007; Styriakova and Styriak, 2000). One of the key criteria for the industrial use of processed kaolins for paper manufacture and ceramics is a low content in iron-bearing minerals as such impurities substantially affect the whiteness and refractoriness of the product, affecting especially paper and ceramic applications. In particular, because of their particularly intense red or brownish color, it is essential that very low contents of the iron oxides and oxyhydroxides are achieved by processing following mining, either by blending or treatment during mineral production. Even a very low iron concentration in a kaolin may result in a significant reduction in the whiteness of a ceramic product as any coloration is intensified by the firing process (Lee et al., 2002).

Ferric iron (Fe^{III}) is only soluble in an acidic pH of 3 or below. Ferrous iron (Fe^{II}) is soluble over a wider range of pH but, at circum-neutral pH or above, it is only stable under reducing conditions. In the presence of oxygen, it is rapidly oxidised to the trivalent form and precipitated as an

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Fe(III)-bearing solid (Schwertmann and Taylor, 1989). The removal of Fe(III)-bearing impurities from industrial kaolins is generally achieved by the combination of physical techniques (magnetic separation, selective flocculation) with chemical treatments under acidic or reducing conditions. The reductive leaching of Fe from kaolins with sodium hydrosulfite ($\text{Na}_2\text{S}_2\text{O}_4$), alternatively known as sodium dithionite, is particularly efficient and is currently employed by the kaolin industry, in some cases giving very low iron concentrations (below 0.3% Fe_2O_3) and very high brightness values (above 94%) required for high specification kaolin products (Thurlow, 2001). However, these chemical treatments have economical, technological, and environmental disadvantages. In particular, sodium hydrosulfite is an expensive and dangerous chemical requiring specific and costly storage and transport arrangements. Iron leaching with this chemical is also fairly complex, requiring careful monitoring of the pH, the density of the kaolin slurry, the oxygen level, and the amount of added sodium hydrosulfite as the reaction of reduction of Fe(III) may be impaired by concurrent reactions (Conley and Lloyds, 1970). Its use also produces large amounts of effluents that have high concentrations of dissolved sulfates, requiring chemical treatment, often in large ponds, before disposal.

Over the last decade or so, some effort has been targeted on the development of alternative refining methods, such as biological processes, to remove iron impurities from raw kaolins (Arslan and Bayat, 2009; Camselle et al., 2003; Guo et al., 2010a,b; Hosseini et al., 2007; Lee et al., 2002; Musial et al., 2011; Stucki, 1988). The capital and energy costs of bacterial leaching are likely to be low and no or minimal environmental impact is expected. Such an approach is therefore considered to be of great potential for future mineral treatments (Bosecker, 1997; Hosseini et al., 2007). Most studies on the biological leaching of metal impurities from mineral materials have focused on acid-producing microorganisms, such as *Bacillus* sp., (Guo et al., 2010a; He et al., 2011) and *Aspergillus niger* (Arslan and Bayat, 2009; Guo et al., 2010b; Hosseini et al., 2007; Musial et al., 2011). The organic acids produced by their metabolism abiotically dissolve metals from solid materials, improving to some extent the quality of the raw material. In particular, the organic acids produced by the filamentous fungus *A. niger* (principally oxalic but also citric and gluconic acids) resulted in an increase in whiteness of another kaolin from 56.5% to 80% in 40 h (Camselle et al., 2003). Several technological problems were however identified, such as the use of two separate stages, one for the culture of the *A. niger* and the second for kaolin acid-leaching, and the need to maintain the pH around 3 through the addition of acid. Experiments using direct addition of oxalic acid (not biologically produced) required heating to 84 °C for more than 5 h to remove 44 wt.% of Fe from a raw kaolin (Terrazas Calderon et al., 2005). In the UK, unpublished work in the 1990s indicated costs of around £30/kg for treating kaolin with oxalic acid in heated reactors, compare to around £7/kg using sodium hydrosulfite. To evaluate the effectiveness of microbiological treatments in economically improving the quality and value of raw kaolin, new studies have to focus on different bacterial metabolisms. Biogeochemical evidence supports the potential importance of crystalline or amorphous Fe-bearing minerals as electron acceptors for Fe-reducing bacteria in soils and subsurface sediments (Lovley, 2000; Lovley and Phillips, 1988). A phylogenetically and physiologically diverse group of bacteria has been isolated that is capable of iron-respiring reduction. Iron-respiring bacteria (IRB) gain energy by coupling the oxidation of organic compounds or hydrogen to the reduction of ferric Fe oxides (Nealson and Myers, 1992; Nealson and Saffarini, 1994). These bacteria have been shown to reduce Fe(III) within the structure of clay minerals and associated phases (Jaisi et al., 2007; Kostka et al., 1996, 2002; Vorhies and Gaines, 2009). Moreover, some IRB were isolated from subsurface kaolin lenses (sedimentary kaolins, Georgia, USA) indicating their presence associated with raw kaolins in natural settings (Sheilbolina et al., 2007). Thus, the use of IRB may be of industrial interest for the removal of iron impurities from kaolins.

In the present study, the Fe(III) removal efficiency from a kaolin from SW England has been evaluated using different *Shewanella* IRB species (*S. alga* BrY, *S. oneidensis* MR-1, *S. putrefaciens* CN32, and *S. putrefaciens* CIP 8040). The microbial reduction of Fe(III) was monitored in batch cultures under non-growth conditions. The rate and extent of Fe(III) reduction were examined as a function of the *Shewanella* species and cell/kaolin ratio. The bio-treated materials were analysed by X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR) to investigate any mineralogical transformation. The whiteness and brightness indices of the kaolins were also assessed by spectrometry.

2. Materials and methods

2.1. Kaolin materials

The kaolin sample used in this study was provided by Imerys Minerals Ltd. (St. Austell, Cornwall, UK). It is a processed product ('Remblend') derived ultimately from a large open pit in kaolinised granite near St. Austell, Cornwall, UK (Psyrrillos et al., 1999). In this case, raw kaolin slurry was generated by washing the altered granite with a high-pressure water jet. The coarser grained and heavier sand, mica, feldspar, and other associated minerals were removed using a variety of processes combining gravity settling and subsequent mechanical separation to obtain a kaolinite-rich clay slurry. The kaolin slurry was then passed through hydrocyclones to collect the less than 53 μm fraction, consisting almost exclusively of kaolinite particles (Psyrrillos et al., 1999). The final kaolin slurries have a mineral content of around 250 g L⁻¹ and chemical analysis using X-ray fluorescence spectroscopy (XRF) revealed a low iron content (~ 1.05 m% Fe_2O_3) and the presence of few other impurities, as TiO_2 , K_2O , and MgO (Table 1).

2.2. Preparation of iron respiring bacteria inocula

Pure lyophilised cultures of *S. oneidensis* strain MR-1, *S. alga* strain BrY, *S. putrefaciens* strain CN 32 (*S. p.* CN 32), and *S. putrefaciens* strain CIP 8040 were obtained from NCIMB Ltd. (Aberdeen, Scotland) and DSMZ (Braunschweig, Germany). The cells were cultured as described in (Zegeye et al., 2007). Briefly, frozen cells from a stock (20% glycerol at -80 °C) were revived under aerobic conditions on tryptic soy agar (TSA). They were sub-cultured twice, and then the colonies were used to prepare a suspension with a target optical density of 0.55 ± 0.01 ($\lambda = 600 \text{ nm}$). Twenty millilitres of this suspension was inoculated in 200 mL of tryptic soy broth (TSB) in order to initiate the liquid culture. Cells were grown to a stationary growth phase (24 h) and harvested by centrifugation, washed twice with sterile NaCl 0.9% and concentrated in the same medium. Cells were purged for 30 minutes by bubbling with N_2 to reach anoxic conditions, sterilised by filtration through a membrane of pore size 0.2 μm (Millex FG50, Millipore) and used to inoculate batches with kaolin.

Table 1
XRF and specific surface area data of the kaolin used in this study.

Composition	Kaolin
SiO_2 (%)	48.7
TiO_2 (%)	0.08
Al_2O_3 (%)	35.8
Fe_2O_3 (%)	1.05
CaO (%)	0.06
MgO (%)	0.25
K_2O (%)	1.94
Na_2O (%)	0.07
Specific surface area (m^2/g)	8.19
> 53 μm	< 0.1%
> 10 μm	10%
< 2 μm	39%

2.3. Bioleaching experiments

The culture medium for the kaolin bio-reduction assays contained 10 mM of sodium methanoate as the electron donor, 10 or 20 g of kaolin as the final electron acceptor and 0.9% of NaCl. This medium was free of phosphate or carbonate-based buffer to limit as much as possible the formation of secondary minerals that would alter the colour properties of the kaolin. The medium was heat sterilised at 121 °C for 15 mn, purged with filter-sterilised N₂, and dispensed into sterile 100 mL flasks with butyl rubber stoppers. The flasks were then crimp sealed. AQDS (100 μM) sterilised by filtration (filter pore size 0.2 μm) and purged with filter-sterilised N₂ was introduced in the culture medium under aseptic conditions. The cell suspensions were added to obtain final concentrations ranging from $3.0 \pm 1 \times 10^6$ to $1.05 \pm 0.35 \times 10^7$ CFU mL⁻¹ depending on the assay, for a total volume of liquid of 80 mL. The pH, measured after all components had been mixed, was 7.0 ± 0.5 . The samples were incubated at 20 or 30 °C in the dark on an orbital shaker (320 rpm). The microbial reduction of Fe(III) associated with the kaolin was monitored for 5 days by measuring the concentration of Fe(II) in aliquots collected at selected time intervals. All assays were repeated in triplicate. A cell-free blank experiment was conducted under the same conditions as the biotic experiments.

2.4. Chemical analyses

One millilitre of the collected samples was injected directly into 1 mL of 2 M HCl and the concentration of HCl-extractable Fe(II) was measured by UV-Visible spectroscopy using an excess of 1,10-phenanthroline through the photochemical analysis of the [Fe(phen)₃]²⁺ complex (Fadrus and Maly, 1975).

2.5. Analyses of colour properties

Some of the kaolin samples were analysed for color properties (L*, a*, b*, ISO Brightness, and Whiteness) before and after bio-treatment and washing (three times, with ultrapure water), using a Technidyne ColorTouch PC Spectrophotometer. The ColorTouch PC system conforms to ISO standards 2469, 2470, 2471, and 3688. The system has three different apertures (27, 15, and 9.8 mm), a xenon flash lamp, holographic grating, scanning diode array, dual-beam ISO 2469 optics, and a built-in USB camera. The system was calibrated with certified paper standards (paper calibration kit) supplied by Carisbrooke Instrument Services Ltd. (Woking, Surrey, UK) on a quarterly basis.

Prior to measurement, the samples were dried in an oven at 110 °C overnight and pulverised for 30 sec using an IKA A11 Basic Analytical Mill (IKA-Werke, Stavfen, Germany). Around 10 g of the pulverised samples were then pressed into pellets 3.7 cm in diameter using a Technidyne Powder Press with a force of ~50 pounds/sq. inch (50 psi) for 5 sec following the recommended protocol issued by Carisbrooke Instrument Services Ltd. Each pellet was analysed 6 times to obtain average color analysis data with 2 standard deviation values. L*, a*, b* and whiteness values were acquired using a source D65 (outdoor daylight) and a D65/10° illumination and observer. ISO brightness was measured using a source C and a C/2° illumination and observer.

2.6. Mineralogical and textural analyses

2.6.1. X-ray diffraction (XRD)

Once collected on a millipore filter, the wet kaolin samples were spread out on glass plates and air-dried. The XRD patterns were collected over a range of 2° to 70° 2θ using Cu Kα radiation (λ = 0.15418 nm), with a 0.0334° step size and a nominal collecting time of 99.70 seconds per step. XRD scans were carried out using a

PANalytical X'pert PRO MPD system fitted with a PANalytical X'Celerator RTMS detector, which allowed the rapid acquisition of high quality.

2.6.2. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were obtained in transmission mode in the mid-infrared range (400 to 4000 cm⁻¹) using KBr pressed pellets (1–2 mg mineral material in ~200 mg KBr) with a resolution of 4 cm⁻¹ and 100 scans on an AVATAR 360 FTIR ESP spectrometer with a purge gas system to remove CO_{2(g)} and H₂O_(g) and an OMNIC software.

2.6.3. Scanning electron microscopy (SEM)

The micro-morphological features of the kaolin were examined before and after bioleaching using a CamScan series 4 SEM fitted with an Oxford Microanalysis X-ray dispersive system (EDS). The samples were coated with platinum with 5 nm of thickness prior to their analysis.

3. Results and discussion

3.1. Effect of *Shewanella* species on iron bioleaching rate

Bioleaching experiments were carried out using 20 g of kaolin and 4 different species of *Shewanella* sp (1.05×10^7 CFU mL⁻¹) at 20 °C. All tested bacteria were able to reduce iron in the kaolin and no extractable Fe(II) was detected in the blank sample indicating that the measured Fe(II) in all bio-treated samples resulted from bacterial activity (Fig. 1). However, the rate and extent of bioleaching were different depending on the bacterial species. The initial rate of reduction was computed from the first derivative of a nonlinear curve fit for Fe(II) versus time data to the following equation:

$$\text{Fe(II)}_t = \text{Fe(II)}_{\text{max}}[1 - \exp(-k_{\text{obs}}t)] \quad (1)$$

where Fe(II)_t is the concentration of total Fe(II) produced at time *t*, Fe(II)_{max} is the maximum Fe(II) concentration observed at the end of the reduction period, and *k*_{obs} is the pseudo first order rate constant.

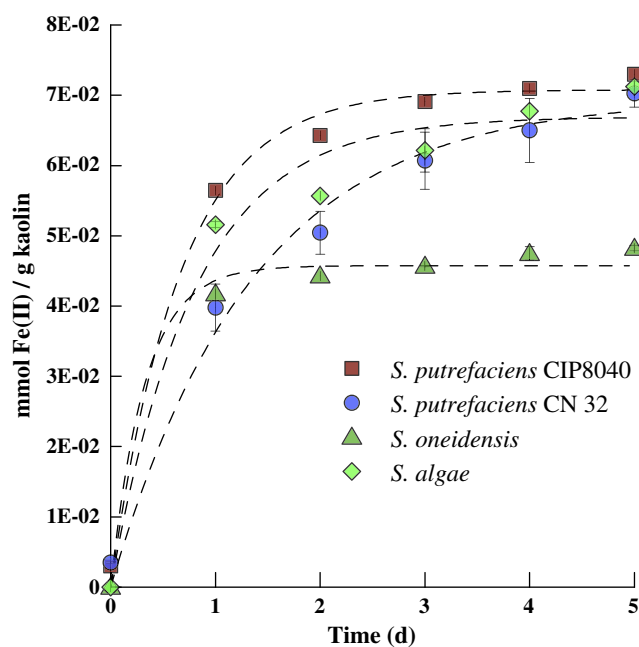


Fig. 1. Iron leaching from kaolin using various iron-reducing bacteria at 20 °C. All experiments were run with 20 g of kaolin and $(1.05 \pm 0.35) \times 10^7$ CFU mL⁻¹ of cell suspension. The initial rate of reduction was computed from the first derivation of a nonlinear curve fit represented by the dotted lines. Errors bars represent standard deviation of three triplicates incubations.

The assays run with *S. putrefaciens* CIP8040 and *S. oneidensis* displayed the highest rate of reduction ($0.106 \text{ mM Fe(II) g kaolin}^{-1} \text{ d}^{-1}$ and $0.100 \text{ mM Fe(II) g kaolin}^{-1} \text{ d}^{-1}$ respectively). Although the assays run with *S. putrefaciens* CN32 displayed a lower rate of reduction ($0.05 \text{ mM Fe(II) g kaolin}^{-1} \text{ d}^{-1}$), the extent of reduction after 5 days of incubation was one of the highest ($1.35 \text{ mM Fe(II) kaolin g}^{-1}$) alongside with those of *S. putrefaciens* CIP8040 and *S. algae*. On the other hand, *S. oneidensis* displayed the lowest extent of reduction, reaching the value of $0.93 \text{ mM Fe(II) per gram of kaolin}$ after 5 days of incubation. These results indicate that IRB are able to remove the iron impurities admixed with the kaolin by coupling the oxidation of the electron source, methanoate, to the reduction of Fe(III) during their respiration process. This is in agreement with other studies where the reduction of Fe(III), associated with clay minerals, by IRB is highlighted (Jaisi et al., 2007; Kostka et al., 1996, 1999) even if the rates of reduction calculated in the present study are low. The lower rates of reduction could be explained by the possible presence of other Fe(III) minerals, such as tourmaline or hematite. Alternatively, Fe(III) substituted in the kaolin structure could be less bio-available to IRB activity in comparison to the structural iron present in smectite (Kostka et al., 1999), illite, chlorite (Jaisi et al., 2007) or nontronite (O'Reilly et al., 2005). The admixed iron impurities in kaolins are often hematite, magnetite, pyrite (Muller and Callas, 1993), which are bio-reduced at a slower rate (Roden and Zachara, 1996). In addition, the presented bioleaching experiments were run in a medium containing exclusively 0.9% NaCl, which can be considered as a very poor medium, not suitable for optimal bacterial growth and activity. The use of vitamins and other supplements (oligoelements, trace metals) would likely optimise the rate and extent of the bioleaching process, and may allow reaching values similar to those reported in the literature for the bacterial reduction of Fe(III) in Fe-bearing clay minerals (Jaisi et al., 2007; Kostka et al., 1996). In addition, this first series of experiments was performed at 20°C , which is substantially lower than the optimum temperature reported for these *Shewanella* strains, which is 30°C (Venkateswaran et al., 1999). Increasing the temperature would therefore be expected to substantially improve the rate and extent of bioleaching. The use of higher temperature and additives would however increase the overall cost of the refinement procedure. Numerous supplemental bioleaching experiments would be required to evaluate the added benefits for each modification of the procedure (additives and temperature) and identify the optimum bioleaching conditions in terms of both benefits and costs.

Subsequent experiments focused on bioleaching with *S. putrefaciens* CIP 8040 because it was the most suitable bacterium in terms of rate and extent of iron reduction.

3.2. Effect of bacterial cells/kaolin ratio

Two series of experiments were conducted with 10 and 20 g of kaolin sample and different concentrations of *S. putrefaciens* CIP 8040 cells at the reported optimum temperature of 30°C . For an amount of 10 g of kaolin, the initial rate of reduction was similar for the 3 tested concentrations ($0.118 \pm 0.02 \text{ mM Fe(II) kaolin g}^{-1} \text{ d}^{-1}$), indicating that there was no direct relationship between the rate of reduction and the cell/kaolin ratio (Fig. 2). One possible explanation for this observation is that, at all tested cell concentrations, the Fe(III) sites on the surfaces of the iron-bearing mineral particles were probably saturated with cells such that any increase in cell number would not lead to any increase in the reduction rate. Regardless of the tested cell concentration, the extent of reduction reached after 5 days of incubation was about $75 \text{ mg of Fe}_2\text{O}_3 \text{ per } 100 \text{ g of kaolin}$, further demonstrating that the cell number was not the limiting factor during Fe(III) reduction but that the bio-accessibility of Fe(III) was likely governing the overall reduction kinetics. For the assays run with 20 g of kaolin, the rate of reduction increased from $0.016 \text{ mM Fe(II) kaolin g}^{-1} \text{ d}^{-1}$ to

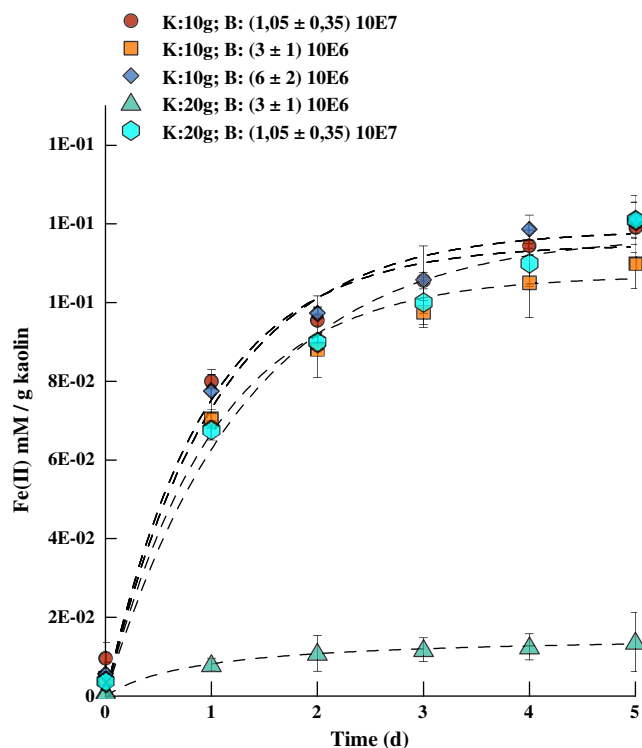


Fig. 2. Fe bioleaching of 10 and 20 g of kaolin at 30°C with different cell concentrations. The initial rate of reduction was computed from the first derivation of a nonlinear curve fit represented by the dotted lines. Errors bars represent standard deviation of triplicates batches incubations.

$0.124 \text{ mM Fe(II) kaolin g}^{-1} \text{ d}^{-1}$ when the cell concentration increased 3.5 fold (from 3×10^6 to 1.05×10^7). In this case, the reduction limiting factor was not the bio-accessibility of Fe(III) but the number of IRB. As expected, a substantially higher amount of Fe(III) was removed from the kaolin when the experiments were run at the optimum temperature of 30°C ($\sim 75 \text{ mg of Fe}_2\text{O}_3 \text{ per } 100 \text{ g of kaolin}$ after 5 days of incubation; Fig. 2) compared to the previous experiments at 20°C ($\sim 46 \text{ mg of Fe}_2\text{O}_3 \text{ per } 100 \text{ g of kaolin}$; Fig. 1, for the same cell/kaolin ratio). Additionally, an increase of 10% in the rate of reduction was calculated when the temperature of incubation was raised from 20°C to 30°C .

Colour analyses of the kaolin bioleached by the five cell concentrations after five days of incubation at 30°C showed a significant increase in whiteness and brightness compared to the untreated kaolin (Table 2). However, the improvement of the kaolin's colour properties did not follow a linear trend with the increase in bacterial cells/kaolin ratio (Fig. 3). Instead, 3 different behaviors were observed. First, there is an increase in the whiteness and brightness as a function of the bacterial cells/kaolin ratio (area 1). This indicates that the limiting factor was not the bio-accessibility of Fe(III) but the number of bacteria cells (Urrutia et al., 1998). The increase of the cell concentration led to a higher removal of Fe(III) which resulted in an increase in both the whiteness and brightness of the kaolin. Secondly, there is a plateau where only a slight variation of the whiteness and brightness is observed, which indicates that the limiting factor is the bio-accessibility of Fe(III) to bacterial cells. Because the same amount of Fe(III) is removed, the increase in the bacterial cells/kaolin ratio does not have an impact on the colour properties of the kaolin. Third, there is a slight decrease in both the whiteness and the brightness (area 3) even though a similar concentration of Fe(III), as in the area 2, was removed. This decrease in kaolin whiteness and brightness may not be explained by the impact of remaining iron impurities as those are likely comparable in both nature and content. Another explanation may be the presence of bacterial cells or organic

Table 2Colour analyses data of kaolin before and after bioleaching with different *Shewanella putrefaciens* CIP8040 concentration after 5 days of incubation at 30 °C.

	L*	A*	B*	%Whiteness	%Brightness (ISO)
Blank kaolin	91.81(0.012)	−0.08 (0.02)	5.56 (0.02)	54.31(0.08)	73.98 (0.02)
10 g kaolin/(3 ± 1) 10 ⁶ CFU mL ^{−1}	92.86 (0.02)	−0.41 (0.02)	3.53 (0.02)	66.30 (0.10)	78.63 (0.06)
10 g kaolin/(6 ± 2) 10 ⁶ CFU mL ^{−1}	92.78 (0.01)	−0.41 (0.02)	3.58 (0.02)	65.86 (0.08)	78.42 (0.04)
10 g kaolin/(1.05 ± 0.35) 10 ⁷ CFU mL ^{−1}	92.14(0.01)	−0.35 (0.02)	3.80 (0.02)	63.32 (0.10)	76.78 (0.02)
20 g kaolin/(3 ± 1) 10 ⁶ CFU mL ^{−1}	92.40 (0.01)	−0.23 (0.02)	5.27 (0.02)	57.10 (0.06)	75.60 (0.02)
20 g kaolin/(1.05 ± 0.35) 10 ⁷ CFU mL ^{−1}	93.00 (0.01)	−0.39 (0.02)	3.73 (0.01)	65.73 (0.06)	78.77(0.02)

by-products of bacterial activity, such as organic polymers, which could colour the material. Though the samples were washed extensively before the colour analyses, such organic materials may bind to the kaolin particles and remain in small amount in the washed bioleached kaolins. Being more abundant in this last sample (higher cell/kaolin ratio), their effect on colour properties would be more pronounced leading to a slight reduction in the colour measurements. The whiteness and brightness values reached their maximum (66.3% and 79.0%, respectively), indicating an increase of 22.07% for the whiteness and 6.5% for the brightness, for a cell/kaolin ratio of 24×10^6 CFU per g of kaolin. The whiteness improvement is comparable with results obtained by Guo et al. (2010b) where an increase of around 34% was observed using fermentative indigenous iron-reducing bacteria. However, although higher increases in whiteness, up to 70%, have been observed in other studies (Arslan and Bayat, 2009), a direct comparison of the whiteness improvement obtained in the present study with other published data is not straightforward. This is due to the fact that microorganisms can enhance kaolin quality, in terms of colour properties, by producing organic or inorganic acids or changing redox conditions to leach Fe(III) iron impurities. The bacterial metabolism used in the present study to refine the kaolin is anaerobic iron respiration (e.g. electron transfer) while the studies available in the literature emphasise fermentative metabolism (e.g. acid production) or mixed metabolism (e.g. soil consortium) where the relative importance of anaerobic and aerobic metabolism is not thoroughly identified. Additionally, the amount of Fe(III) impurities (admixed or structural) can vary from kaolin to kaolin, depending on the origin of the deposit and geological processes, hampering the comparison. Nevertheless, our study highlights that a metabolism

other than fermentative can be used to improve the quality of industrial kaolin even though supplementary research is needed to exploit this metabolism to its full potential.

3.3. Mineralogical analyses of the kaolin after Fe(III) bioleaching

The XRD patterns of the raw material and kaolin treated with *S. putrefaciens* CIP 8040 for 5 days at 30 °C are identical (Fig. 4), indicating that very little – if any – structural modification of the kaolinite occurred during the bioleaching procedure, and that no crystalline secondary material was formed, as elsewhere reported (Guo et al., 2010a).

FTIR analyses of the initial kaolin and of the blank samples after 5 days at 30 °C are very similar and characteristic of kaolinite with no measurable trace of organic material or bacterial cells (Fig. 5a). In addition, all spectra show a small shoulder at 3598 cm^{−1}, which is characteristic of AlFeOH vibrators (Fig. 5b), (Petit and Decarreau, 1990), therefore indicating that the small amount of structural Fe(III) present in the kaolinite crystal lattice was not removed during the bioleaching procedure. In fact, only a small part of this structural iron can be removed from the kaolin by industrial processing techniques (magnetic separation followed by sodium hydrosulfite leaching) as evidenced by Bertolino et al., (2010).

Observations of the initial kaolin by SEM revealed the presence of well-crystallised kaolinite consisting of hexagonal particles of different size and thickness (Fig. 6a). In comparison, the bioleached samples exhibited particles with similar size and thickness but with rounded and slightly damaged edges (Fig. 6b). Additionally,

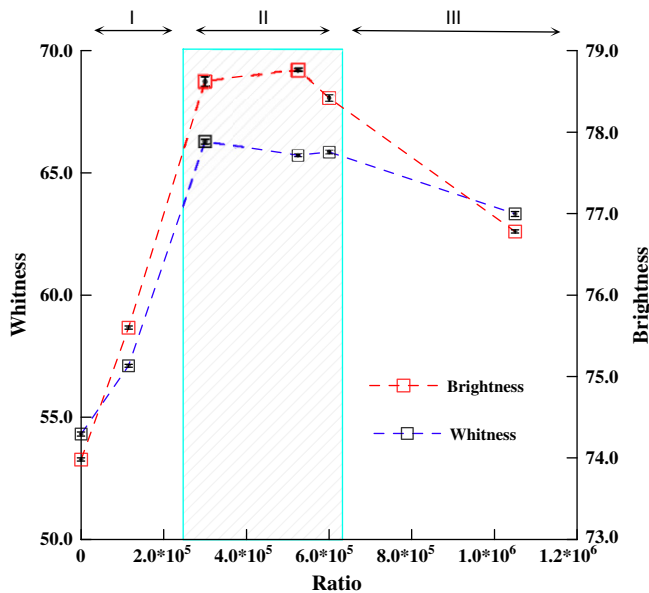


Fig. 3. Whiteness and brightness evolution after 5 days of incubation as the function of cell/kaolin ratio (CFU/g kaolin). The blue box represents the best cell/kaolin ratio identified in this study, to achieve a noticeable improvement of the kaolin colour properties. The error bars represent standard deviation of six separate analyses.

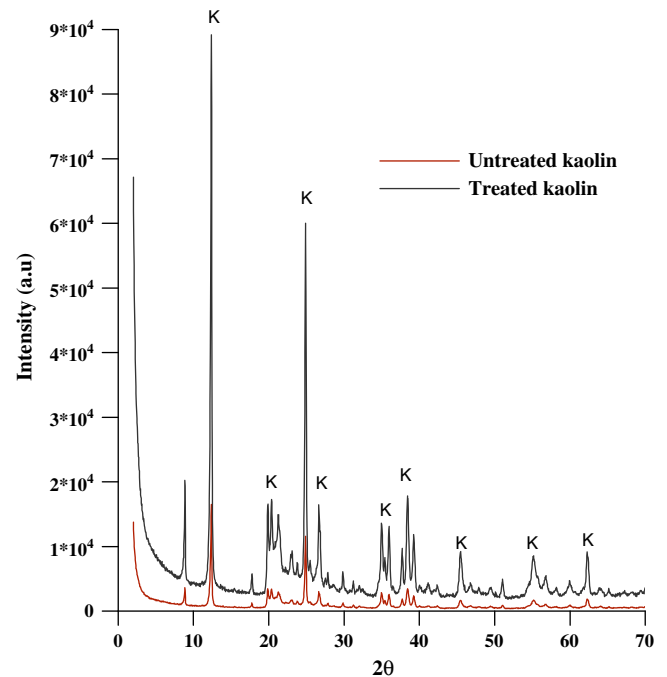


Fig. 4. XRD patterns of kaolin before and after its bioleaching for 5 days by *S. putrefaciens* CIP8040 at 30 °C using 20 g of clay, K = kaolinite peaks a.u = arbitrary units.

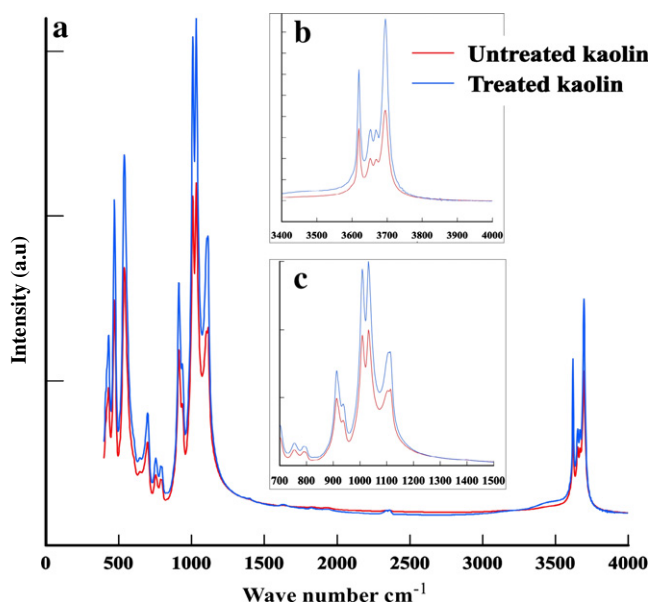


Fig. 5. FTIR spectra of the kaolin before and after its bioleaching for 5 days by *S. putrefaciens* CIP8040 at 30 °C using 20 g of clay. Inset (b) represents the region of OH stretching and (c) the region of structural vibration.

EDS analysis indicated that the bioleaching did not cause significant change in the chemical composition of the kaolin (Table 3) apart from a notable decrease of the amount of Fe impurities (Fe m%), from 1.12 m% to 0.60 m% after the bioleaching process.

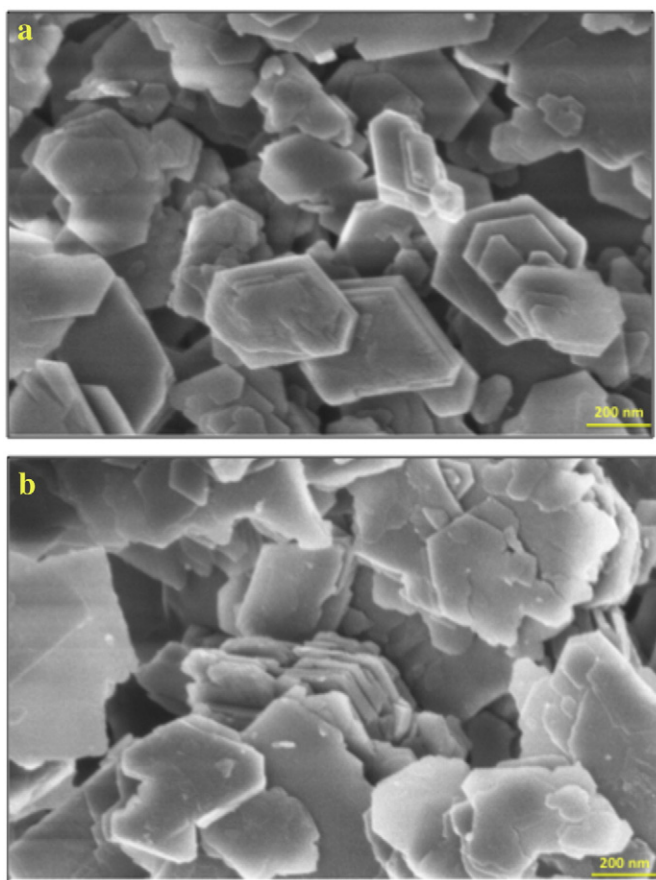


Fig. 6. SEM micrographs of kaolin before and after its bioleaching for 5 days by *S. putrefaciens* CIP8040 at 30 °C using 20 g of clay. (a) represents the untreated kaolin, and (b) the bioleached kaolin.

Table 3

EDS analysis of kaolin before and after its bioleaching for 5 days by *S. putrefaciens* CIP8040 at 30 °C using 20 g of clay.

Elements	Mass %		Atomic %	
	Untreated kaolin	Treated kaolin	Untreated kaolin	Treated kaolin
O	58.67	60.06	71.56	72.51
Al	17.66	17.04	12.77	12.20
Si	20.59	20.99	14.30	14.43
K	1.96	1.31	0.98	0.64
Fe	1.12	0.60	0.39	0.21

4. Conclusions

Two series of bacterial iron-reduction experiments have shown that all tested *Shewanella* sp. bacterial strains were able to leach iron impurities from industrial kaolins and that among the tested strains, *S. putrefaciens* CIP8040 is the most efficient in terms of both extent of Fe removal and improvement in the colour properties of the kaolin. Colour analyses after 5 days of incubation with *S. putrefaciens* CIP8040 at 30 °C suggest that the most positive results are obtained for a cell/kaolin ratio of 24×10^6 CFU/g of kaolin and a solid/liquid ratio of 250 g/L. These conditions result in an increase in ISO brightness from 74% to 79% and an increase in whiteness from 54% to 66%. Using additives may substantially increase the effectiveness of the bacterial refinement procedure but would also increase its overall cost. Supplemental bioleaching research and development studies are required to identify the optimum conditions in terms of rate and extent of iron removal but also in terms of product quality, and costs and benefits, before the procedure can be applied at the pilot or industrial scale. These studies will have to carefully evaluate the environmental and economical benefits compared to currently used approaches, such as the chemical bleaching with sodium hydrosulfite.

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