



Leaching of iron from kaolins by a spent fermentation liquor: influence of temperature, pH, agitation and citric acid concentration

Claudio Cameselle¹, M^a José Núñez¹, Juan M. Lema¹ and Jesús Pais²

¹Department of Chemical Engineering, University of Santiago de Compostela, Av. de las Ciencias s/n, E-15706 Santiago de Compostela, Spain and ²CAVISA, Vimianzo (A Coruña), Spain

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SUMMARY

The efficiency of a biological bleaching process on a highly contaminated iron oxides kaolin was analyzed. The bio-bleaching method consists of two steps: first, the fermentation of a chemically-defined medium by *Aspergillus niger* leads to a spent liquor which, in a second step, is employed as leaching agent for kaolin. In the leaching process 43% of iron oxides was removed when the kaolin was treated at 60 °C for 5 h and the whiteness index was increased to 67%. Extracellular enzymes were not responsible for leaching, which is basically due to organic acids produced during the fermentation.

INTRODUCTION

Kaolin (kaolinite) is a clay which comes from degradation of original parent-rock. Frequently, deposits of kaolin are contaminated by small amounts of iron-containing minerals. Kaolin is widely used as a raw material in the ceramic industry and as an additive in the pulp and paper industry. Both applications require a clear whiteness [17]. Iron oxides reduce the whiteness index of kaolin giving a brown-yellow coloration depending on the concentration of contaminant iron. Under these conditions the kaolin is useless for industrial applications and it becomes necessary to apply a bleaching process.

Currently, different methods are used to remove mineral iron, so that the kaolin reaches an adequate whiteness index for industrial applications. Physical-mechanical methods such as flotation or magnetic separation (High Gradient Magnetic Separation) are not suitable for all applications. Their efficiency depends on the properties of the kaolin to be treated and often is lower than the efficiency of chemical methods.

Chemical methods are based on the reduction of ferric iron to ferrous iron by reducing agents (sodium dithionite, sulfur dioxide, sodium acid sulfide) at low pH. A treatment with complexing agents prevents later reoxidation from ferrous to ferric iron, avoiding the undesirable color formation. If the kaolin is used in the ceramic industry, the iron should be extracted because it will be oxidized to ferric iron during the kilning phase, appearing as ferruginous spots.

Chemical methods are suitable to achieve high yields of iron removal and high whiteness indices, but they are expensive, and the technological conditions are harsh and dangerous

for the environment. The quantity of chemical reagents used in the bleaching process may be minimized in order to obtain economical benefits because they are expensive and the price of kaolin is low. When the iron content is high, the bleaching process by chemical methods may be uneconomical due to the large quantity of reagent necessary to reduce all contaminant mineral. Thus there is great interest in developing alternative processes to the classical methods or which can be used together with them to reduce cost and increase yield, and biotechnological techniques merit special attention [6,18].

A number of microorganisms are capable of reducing and/or dissolving heavy metals directly from minerals and the surface of rocks [1,3,5,12,15,16]. Precious metals, copper, nickel, iron and others are mobilized by the action of heterotrophic bacteria or fungi when they grow together with the mineral or if spent fermentation liquor is used as a leaching agent. Metal solubilization may be an indirect process, due to microbial production of organic acids, aminoacids, and other metabolites which dissolve heavy metals by direct displacement of metal ions from the ore matrix by hydrogen ions or by the formation of soluble metal complexes and chelates [5,6,12,15]. Alternatively, enzymes secreted in the medium can also act in reducing processes. Reduction of ferric to ferrous iron by enzymes from bacteria and fungi has been described by several authors [7,8,10,11]. Bacteria in the genera *Bacillus* and *Pseudomonas* and fungi in the genera *Aspergillus* and *Penicillium* were active in iron dissolution from limonite, hematite and goethite [18]. *A. niger* gave the best results in the bleaching of kaolins contaminated by iron oxides, since it secretes large quantities of citric and oxalic acids in a medium with low pH, thus avoiding re-oxidation of the solubilized iron.

The purpose of this investigation was to determine the ability of a strain of *A. niger* to bleach a kaolin with high content of iron oxides.

Correspondence to: C. Cameselle, Dept of Chemical Engineering, University of Santiago de Compostela, Av. de las Ciencias s/n, E-15706 Santiago de Compostela, Spain.

MATERIALS AND METHODS

Kaolin. High iron content kaolin (2.3% Fe₂O₃) was employed, supplied by CAVISA, a mining company in Vimianzo, La Coruña, Spain. Its whiteness index was 56.5%.

Microorganism. *Aspergillus niger* CBS 246-65 was maintained at 4 °C on a solid medium containing (w/v) 2% agar, 2% malt extract and 0.1% yeast extract.

Fermentation. The fermentation process was carried out in a Biostat E bioreactor (B. Braun, Melsungen, Germany) with a 2-L working volume, under the following conditions: 500 rev min⁻¹ stirring speed, 30 °C and 1 v.v.m. air flow, setting the value of dissolved oxygen at 75% saturation. The bioreactor was inoculated with 200 ml of a suspension of pellets grown in a proliferation medium (Table 1) in an orbital shaker. Methanol was added to proliferation medium because it stimulates citric acid production [9]. The fermentation medium is shown in Table 1.

Leaching agent. At the end of the fermentation process, the medium was filtered through a 0.45- μ m pore size membrane filter to remove biomass. A clear liquid was obtained, which was used as leaching agent. 1 N NaOH or concentrated HCl, and citric acid (as pure powder) were used to adjust the pH and citric acid concentration. To determine if enzyme activity was involved in the leaching process, kaolin was treated with the same clear liquid after denaturing the enzymes by autoclaving the spent medium at 121 °C for 20 min. Solutions of citric acid (10, 25 and 40 g L⁻¹) and oxalic acid (1–25 g L⁻¹) were used to clarify the role of each in the leaching process.

Leaching process. Experiments were carried out in 250-ml flasks incubated on a shaker with orbital speed and temperature control. Each flask contained 15 g of kaolin and 95 ml of leaching solution. The total operating volume was 100 ml. The operation time was 5 h, although in some experiments the process was longer in order to determine the maximum dissolving capacity of the leaching medium. The leaching processes to determine the activity of organic acids and enzymes were carried out at 45 °C and 200 r.p.m.

TABLE 1

Composition of the culture media used in inoculum preparation and fermentation process

	Proliferation	Fermentation	Units
Sucrose	40	150	g L ⁻¹
NH ₄ NO ₃	0.2	2	g L ⁻¹
FeSO ₄ ·7H ₂ O	0.1	0.1	mg L ⁻¹
ZnSO ₄ ·7H ₂ O	0.25	0.25	mg L ⁻¹
KH ₂ PO ₄	0.2	–	g L ⁻¹
MgSO ₄ ·7H ₂ O	0.3	–	g L ⁻¹
Methanol	3%	–	v/v

Analyses. Leached-kaolin suspensions were separated by filtration through a 0.45- μ m pore size membrane filter. Solubilized iron was determined in the liquid by atomic absorption spectrophotometry (Perkin-Elmer, ICP/5000, Überlingen, Germany). The solid was washed with deionized water to remove both organic material and the retained dissolved iron. The whiteness index was determined by light reflexion at 457 nm considering BaSO₄ as the reference to 100% whiteness [14].

Factorial design. The activity of the leaching medium produced by fermentation with *A. niger* was studied as a function of four variables, temperature (T), agitation (A), pH and citric acid concentration (C). Citric acid was selected because it is the major product of the fermentation and it is specially active in some mineral leaching processes. Agitation and temperature are related to operational conditions. They influence mass transfer and chemical reaction rate, respectively.

To analyze the behavior of the system quantitatively, an experimental plan at two levels was designed [2], therefore 2⁴ experiments may be carried out. The selected values were: temperature (T): 30 and 60 °C; agitation (A): 100 and 300 r.p.m.; citric acid concentration (C): 10 and 40 g L⁻¹; and pH: 0.5 and 2. Methodology of the factorial plan involves that coded variables (signed with *) should be employed. The coded values are –1 and +1, for lower and upper real values, respectively. Four additional experiments were carried out in the central point of the range selected for each variable (T = 45 °C, A = 200 r.p.m., C = 25 g L⁻¹ and pH = 1.2) to estimate the experimental error. They take 0 as coded value. Table 2 shows the experimental matrix corresponding to N experiments (N = 20).

The selected objective function to maximize is the concentration of dissolved iron after 5 h of treatment (**Fe**). The effect of the several factors can be determined within the selected experimental region by fitting experimental data to the function indicated in Eqn 1, where b_i are the main effects and b_{ij} are the interaction effects between two of the four variables.

TABLE 2

Experimental matrix

N	T*	A*	pH*	C*	N	T*	A*	pH*	C*
1	–1	–1	+1	–1	11	+1	–1	–1	–1
2	–1	–1	+1	+1	12	+1	–1	–1	+1
3	–1	–1	–1	–1	13	+1	+1	+1	–1
4	–1	–1	–1	+1	14	+1	+1	+1	+1
5	–1	+1	+1	–1	15	+1	+1	–1	–1
6	–1	+1	+1	+1	16	+1	+1	–1	+1
7	–1	+1	–1	–1	17	0	0	0	0
8	–1	+1	–1	+1	18	0	0	0	0
9	+1	–1	+1	–1	19	0	0	0	0
10	+1	–1	+1	+1	20	0	0	0	0

$$\begin{aligned}
 \text{Fe} = & b_0 + b_T T^* + b_A A^* + b_{pH} pH^* \\
 & + b_C C^* + b_{TA} T^* \cdot A^* + b_{TpH} T^* \cdot pH^* \\
 & + b_{TC} T^* \cdot C^* + b_{ApH} A^* \cdot pH^* \\
 & + b_{AC} A^* \cdot C^* + b_{pHC} pH^* \cdot C^* \quad (1)
 \end{aligned}$$

RESULTS AND DISCUSSION

Dissolved iron values obtained after 5 h of treatment are shown in Table 3, together with the values of dissolved iron at an extended time for certain experiments. Table 3 also shows the whiteness index obtained at the maximum treatment time.

Experiments were classified in three groups according to the operational temperature. Experiments 1–8 were carried out at 30 °C, 9–16 at 60 °C, and the experiments in the central point (17–20) were performed at 45 °C. Therefore, in each group the results depended on the orbital speed, pH and citric acid concentration effect.

The dissolved iron concentration for the first eight experiments ranged from 61.5 to 122.5 p.p.m. There was a large influence of pH which can be observed in the results of experiments 2 (pH = 2) and 4 (pH = 0.5), which differ only in this variable. The value of dissolved iron was 50% higher at pH = 0.5 than at pH = 2 and the same occurred in experiments 1 and 3.

When leaching was performed at 60 °C (experiments 9–

16), the iron concentration increased about five times, reaching up to 609 p.p.m. Thus, increased temperature exerts a noticeable improvement on the leaching process, more than any other variable. The effect of pH was less than that at 30 °C. In some pairs of experiments (e.g. 14 and 16) the increment of leached iron was very slight when the pH changed from 2 to 0.5. In both groups, agitation and citric acid concentration had little effect.

As expected, the experiments at 45 °C showed intermediate values for dissolved iron. Much more iron was leached when the temperature was changed from 45 °C to 60 °C than from 30 °C to 45 °C.

In some experiments, the treatment time was extended beyond 5 h in order to determine the maximum leach capacity of the medium. In all cases the dissolved iron increased without reaching a plateau. The highest value corresponded to experiment 12, the longest (11 h), where 780 p.p.m. of iron were dissolved. Thus, better results may be obtained with a longer treatment. It would be interesting, in a further study, to extend the treatment time to make use of the maximum leaching capacity of the spent fermentation medium.

As a consequence of iron dissolution, the whiteness index of the kaolin improved. Accordingly, the experiments carried out at 60 °C, which showed the best results for removing dissolved iron (37–47% of the initial iron) also showed the best whiteness index, 65.6% as mean value, and represent an increment of about 10 points with reference to raw kaolin (56.5%).

TABLE 3
Dissolved iron and whiteness indexes obtained from the experimental design

N	5 h treatment		Extended experiments		Whiteness index ^a
	Dissolved iron (p.p.m.)	% Extract iron	Time	Dissolved iron (p.p.m.)	
1	69.5	5.47			59.6
2	61.5	4.48			59.7
3	105.5	8.31			58.8
4	93.0	7.32	9	140	60.3
5	82.5	6.50	7	110	–
6	83.5	6.57	7	97	–
7	122.5	9.65	7	148	64.2
8	112.0	8.82	7	137.5	62.6
9	511.0	40.25			65.1
10	480.0	37.08			64.8
11	609.0	47.96			63.1
12	514.0	40.48	11	780	66.8
13	551.0	43.40			67.0
14	508.0	40.01			65.4
15	521.0	41.03			66.4
16	510.0	40.17			66.0
17	185.8	14.64			61.7
18	186.1	14.65			61.7
19	180.9	14.25			61.6
20	180.0	14.18			61.6

^a Whiteness indices are for the maximum treatment time in each experiment.

Figure 1 shows the effect of treatment time on leaching of iron in several representative experiments. The large influence of temperature and the improvement by using low pH at 30 °C (expts 6 and 8), are shown in this figure. The leaching rate was high during the first hour of treatment, reaching 200 mg L⁻¹ h⁻¹ in experiments carried out at 60 °C. At 45° and 30 °C the values were lower. The rate decreased with treatment time, being for the last hour 72 mg L⁻¹ h⁻¹ at 60 °C. This may be due to the fact that iron is present in different forms, some being easier to dissolve than others. Another possibility is that iron existing in the particle surface is easily dissolved, but the dissolution rate is slower when the iron is inside the kaolin particles.

By fitting the values for dissolved iron at 5 h of treatment (Table 3) to Eqn 1, numerical values of b_i and b_{ij} were determined:

$$\begin{aligned} \text{Fe} = & 308.37 + 217.12T^* + 2.94A^* - 15.00pH^* \\ & - 13.12C^* - 5.94T^* \cdot A^* \\ & + 2.00 T^* \cdot pH^* - 9.37T^* \cdot C^* - 9.94A^* \cdot pH^* \\ & - 5.19A^* \cdot C^* + 3.00pH^* \cdot C^* \end{aligned} \quad (2)$$

The coefficients indicate the relevance of each variable or pair of variables. Therefore, the most important factor is temperature because its coefficient b_T is higher than any other. The yield was improved at high temperatures.

Agitation had the least effect on iron leaching process. The agitation rate should simply be enough to maintain the kaolin in suspension to avoid mass transfer problems.

pH and citric acid concentration had a negative influence, so lower values offered better results. However, leaching was most effective at the lowest pH tested only for a temperature of 30 °C, but not in the other cases in contrast to a prior report saying that low pH improves the medium activity [6]. Probably, the HCl added leaches iron in a reaction parallel to that of the leaching medium. Therefore, when strong mineral acids are added, the leaching process becomes a chemical attack without relation to the metabolic activity of the fungus. Never-

theless, it is important to reach a low pH in the spent fermentation medium.

The effect of citric acid was surprising. Although citric acid shows an important activity in several leaching processes [3,5,15,16], and contributes to iron removal from kaolins [6], the negative value of b_C indicates that slightly better results were obtained at a lower level of citric acid (10 g L⁻¹). Although the lower concentration may be sufficient for the leaching process, another possibility is that citric acid is not the active component of the leaching medium, other substances present in the medium at lower concentrations being responsible.

To clarify these possibilities, kaolin was treated with three different concentrations of citric acid at temperature and agitation conditions corresponding with those of the central point (Table 4). The iron dissolved in 5 h represented only 7% of the amount leached with the fermentation medium under the same operational conditions (at the central point, 25 g L⁻¹ of citric acid, Table 3 shows 186.1 p.p.m. as the maximum value for dissolved iron). Therefore, the citric acid may contribute to the leaching process, but it is not a major factor in iron dissolution.

Enzymatic reduction of iron by soluble or membrane-bound enzymes from iron oxides occurs in cultures of fungi and bacteria [6-8,10,11]. In our cases, only extracellular enzymes were present in the leaching medium because the biomass was removed by filtration. We obtained the same results with native or autoclaved medium, so leaching is not an enzymatic process, and iron leaching from kaolins with a spent fermentation medium from *A. niger* is due to the action of certain metabolites, such as organic acids contained in the medium in lower concentrations than citric acid. Oxalic acid may be the leaching agent because it can remove several ions [12,13].

TABLE 4

Effect of citric acid concentration on iron leaching

Citric acid (g L ⁻¹)	pH	Dissolved iron ^a (p.p.m.)
10	2.0	8.1
25	1.8	13.3
40	1.7	18.3

^a After 5 h of treatment.

TABLE 5

Leaching of iron from kaolin with oxalic acid

Oxalic acid (g L ⁻¹)	pH	Dissolved iron ^a (p.p.m.)
1	2.0	129
10	1.2	356
25	1.2	472

^a After 5 h of treatment.

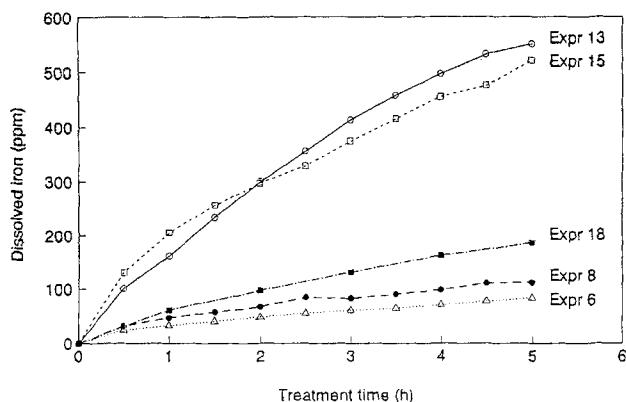


Fig. 1. Effect of treatment time on dissolved iron concentration in five experiments, whose conditions are shown in Table 3.

Oxalic acid is an active coal-solubilizing agent [4]. Several assays carried out with oxalic acid solutions ($1\text{--}25\text{ g L}^{-1}$) prove that it dissolves a significant quantity of iron. The pH of leaching solution does not dissolve the iron because the same pH does not cause leaching of iron in experiments with citric acid. Oxalic acid can be the main leaching agent for kaolin (Table 5).

CONCLUSIONS

A bioleaching method for iron oxides in kaolins presents interesting prospects. A significant reduction of iron oxides contained in kaolin was obtained and high temperature enhanced the process. Low pH (around 2) prevented precipitation of dissolved iron. Surprisingly, the influence of the citric acid is not as important as had been foreseen and oxalic acid could be the main leaching agent. The effect of this metabolite should be examined.

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