Effect of temperature on bioleaching of iron impurities from kaolin by Aspergillus niger fungal

Jalal Hajihoseini and Mahsa Fakharpour

Maybod Branch, Islamic Azad University, Maybod, Iran

ABSTRACT
The microorganisms used in this study are isolates and purified strains of Aspergillus niger pistachios and NCIM 548 A. niger. All experiments, including testing of the pH, iron, citric acid, oxalic acid and sucrose concentrations in kaolin samples, were performed at temperatures of 30°C and 25°C with three repetitions. After analyzing and disinfecting the samples with distilled water on the 28th day, X-ray fluorescence (XRF) was used to analyze kaolin, especially for iron content, as well as to conduct color measurements. The results of the kaolin powder XRF showed 52% iron removal from kaolin by A. niger fungal isolated from pistachio skin after 14 days and 47.7% iron removal after 28 days at a temperature of 25°C and 33.8% iron removal after 28 days at a temperature of 30°C. The results of white measurements of treated kaolin samples showed that kaolin powders treated with A. niger pistachio skin isolate at 25°C and 30°C and A. niger NCIM 548 with fungal cells at 25°C showed the highest L* of 75.41, 74.72 and 74.49, respectively, which were increased in comparison with the L* sample of untreated kaolin, which registered 71.94. Thus, the results showed that the A. niger fungal isolated from pistachio skin at 25°C and 30°C achieved better iron removal than the other samples and that a temperature of 25°C achieved better results than a temperature of 30°C.

1. Introduction
Kaolin is among the most important minerals, mainly in the mineral composition of aluminum silicate (Al₂Si₂O₅(OH)₄) [1–3]. The mineral properties, morphological and chemical properties of kaolin make it suitable for many industrial applications, such as production of ceramics, paper, paints, fillers, cosmetics and pharmaceuticals [3–6]. These applications are heavily influenced by the condition of the soil and its content of impurities, including iron. The presence of iron in kaolin decreases its brightness and refractoriness [1,4,7,8], and causes a significant reduction in its value [9]. Even 0.4% of oxide and iron hydroxides may cause red and yellow colors in the soil [10]. These iron oxide/hydroxides may be hematite (red), maghemite (reddish brown), goethite (brownish yellow), lepidocrocite (orange), ferrhydrate (brownish red), etc. Similarly, iron ores such as hematite may contain clays like kaolin as contamination that can cause problems in the operation of blast furnaces [10]. The first beneficial step in the production of commercially available raw materials is to effectively remove iron oxides from kaolin. The iron removal process can be conducted physically, chemically or in a combination of both [1,11]. Most industries have employed potent chemical reductants such as dithionite or hydrazine to remove iron impurities, but these chemicals are associated with iron reduction in nature [5]. Chemical techniques for the removal of iron have economical, technological and environmental disadvantages. Sodium hydrosulfite, in particular, 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, density of the kaolin slurry, oxygen level and amount of added sodium hydrosulfite, as the Fe (II) reduction reaction may be impaired by concurrent reactions [12]. Its use also produces large amounts of effluents containing high concentrations of dissolved sulfates that require chemical treatment, often in large ponds, before disposal.

In recent decades, biological methods have been used to remove iron impurities from kaolin [7,9,13,14]. Microbial leaching is more efficient compared to chemical routes because it is environmentally friendly: it neither uses nor releases dangerous chemicals, uses far less energy since it operates at room temperature, reduces initial and operational costs and maintains the crystal structure of clay to a large extent.

In a biological process, microorganisms reduce Fe (III), even in its structural form, for purposes other than assimilation of iron [3,15,16]. This occurs under anaerobic conditions in which soluble Fe (II) is produced by bacteria and removed from the kaolin [7,9,17,18]. These microorganisms can utilize hydrogen or organic reduction.
compounds such as sugars, amino acids and even monoo- 
aromatic compounds and long-chain fatty acids as elec-
tron donors and oxidize them to produce carbon dioxide 
with Fe\(^{III}\) serving as the sole electron acceptor 
[3,7,16,19]. The reactivity of Fe\(^{III}\) as an electron accep-
tor is related to its degree of crystallinity.

Most studies on microbiological leaching have been 
carried out using microorganisms that produce acids 
such as bacillus sp [9,20] and Aspergillus niger 
[9,13,21,22], which are resistant to heavy metals. 
Organic acids produced by these microorganisms dis-
solve metals. Organic acids produced by \(A.\) \(niger\) fungal, 
in particular, cause whitening of kaolin from 56.5\% to 
80\% within 40 h [23] because this fungus has the highest 
coefficient of oxalic acid production [3,21,22,24]. The 
process involves two steps: the first is culturing of the 
fungal and the second is kaolin acid leaching. The tem-
perature and pH of the environment must be controlled. 
Maximum oxalic acid production occurs at a pH of 6–7. 
But when the pH is lower, citric acid predominates 
[21,23]. The use of oxalic acid produced by the fungal 
to dissolve iron impurities from kaolin has been the 
subject of many studies. The important factors for iron 
removal from kaolin in microbiological tests are the pH, 
strain type, temperature, amount of kaolin and time 
allotted to add kaolin to the microbe culture. The exper-
imental results show that adding oxalic acid directly 
rather than biologically at a pH of 3 and heating 
the culture at up to 84°C for more than 5 h removes 
44 wt\% iron from raw kaolin [25].

Cameselle et al. [26] investigated the effects of the 
temperature, agitation rate, pH, citric acid and oxalic 
acid concentrations on iron dissolution. The dis-
solved iron concentration increased with increases 
in the temperature at a set pH. They also showed 
that the pH has a lower effect at low temperatures 
and that the oxalic acid concentration has greater 
importance. Iron dissolution increases with increases 
in the oxalic acid concentration.

Cameselle et al. [26] worked with only one fungi 
strain, but the present study used two different strains 
(NCIM 548 \(A.\) \(niger\) with and without fungal cells and \(A.\) \(niger\) fungal isolated from pistachio skin). The results 
were then compared with those for the initial sample.

The purpose of this study was to investigate the 
effects of temperature on the sucrose, citric acid, 
oxalic acid and pH concentrations in iron bioleaching 
by \(A.\) \(niger\) fungal. X-ray fluorescence (XRF) analyses 
were also performed at different temperatures at each 
stage to check the iron removal.

### Table 1. Results of XRF of the kaolin in this study.

| Composition | SiO\(_2\) | Al\(_2\)O\(_3\) | Fe\(_2\)O\(_3\) | CaO | Na\(_2\)O | K\(_2\)O | MgO | TiO\(_2\) | MnO | P\(_2\)O\(_5\) | S |
|-------------|---------|---------------|---------------|-----|---------|-------|-----|---------|-----|------------|---|
| %           | 54.01   | 17.38         | 5.26          | 4.7 | 3.11    | 2.44  | 3.91| 0.52    | 0.20| 0.07       | 0.89|

| Composition | Na | Mg | Al | Si | Mn |
|-------------|----|----|----|----|----|
| %           | 2.31| 2.35| 9.20|25.25|0.14|

| K | Ca | Ti | Fe | P | LOI | La & Lu |
|---|----|----|----|---|-----|----------|
| 2.03  | 3.36 | 0.31 | 3.68 | 0.03 | 7.51 | >1 |

### 2. Material and methods

#### 2.1. Kaolin materials

Samples of kaolin soil for testing were provided by 
the International Powder Technology Company. The 
mine from which the kaolin samples were taken was 
located in Iran’s Yazd province. After preparation of 
the kaolin samples, XRF spectroscopy was used to 
determine its mineral contents. The results are pre-
seated in Table 1. Based on these analyses, the kaolin 
soil samples contained concentrations of 5.26\% Fe\(_2\)O\(_3\) 
and 3.68\% Fe. The XRF analyses were carried out at 
the Razi Metallurgy Research Center in an environ-
ment with a temperature of 25°C and a humidity of 
30\%. The test reference standard is ASTM E1621-13. 
The microorganisms used in this study were isolates 
and purified strains of \(A.\) \(niger\) pistachios and 
NCIM548 \(A.\) \(niger\).

#### 2.2. Growth of microorganisms

Newly grown microorganism strains were used to 
 improve their leaching operation performance. For 
this purpose, microorganisms that had been stored 
in a refrigerator were first transferred to a fresh 
culture medium under a hood in the presence of a 
flame. The fungal strains were then placed in an 
incubator (IKA with KS 4000i) for 7 days at 30°C in 
preparation for transfer to a leaching culture med-
ium. Before transfer of the microorganisms to leach-
ing test flasks, the strains were first transferred to a 
flask containing a sterile solution of 0.1\% Tween 80 
and 0.9\% NaCl in order to disperse the cell spores 
and enable easier, more accurate counting under a 
microscope. The concentration of cells in the solution 
was adjusted to 10\(^7\) spores/cm\(^3\) by counting under a 
microscope. Appropriate volumes of this solution 
were entered into flasks containing the culture med-
ium (final volume: 100 ml) to obtain the desired 
concentration under hooded, sterile conditions.

#### 2.3. Culture medium for microbial leaching 
and performance of bioleaching

The fungal environment is presented in Table 2. 
Inoculation of the microorganisms into 500-ml flasks 
containing 100 ml of culture medium and 5 g of kaolin 
sample was conducted. All the flasks were then placed 
on a rotary shaker and shaken at a speed of 160 rpm at 
both temperatures, 30°C and 25°C.
2.4. Methods of analysis

In this study, an A. niger strain was used to remove iron from kaolin. It was also suggested that, in order to increase the removal rate of iron, a gross kaolin sample be added to the culture medium at the beginning of the experiment [22,27]. Sampling was carried out on days 2, 7, 14, 21 and 28. After removal of a small volume (10 ml) of liquid from the flasks and separation of the liquid phase from the solid phase by centrifugation, the pH, iron, citric acid, oxalic acid and sugar concentrations in the samples were measured to obtain the results presented below. For observation of changes in the mineralogy after bioleaching, X-ray diffraction (XRD) measurements were obtained (Philips X’pert PRO XRD system) from 5° to 80° 2θ CuKα, at 0.02° θ per step. Since the composition changes were very low after the bioleaching process, no effect was observed on the intensity of the XRD peaks. For this reason, XRF analyses were used to specify the percentages of compositions. After analysis and disinfection of the kaolin samples with distilled water on the 28th day of analysis, XRF (ARL 8410 instrument, tube node: Rh, 60 kv) was performed to measure the contents of the kaolin, especially the iron content, as well as color measurements. All experiments were performed at temperatures of 30°C and 25°C with three repetitions. The O-Phenantroline method was used to measure the iron concentration [28]. Finally, a PYE UNICAM spectrophotometer device (spectrophotometer with T80+ UV/ VIS spectrometer) was used at 510 nm to record the absorption. The results were compared with standard solutions to calculate the dissolved iron content, and the amount of dissolved iron was calculated. The Marrier and Boulet method [29] was used to measure the citric acid content in the medium. This method is based on the reaction reported by Ferret and Hermann, in which pyridine and pyridine acetic anhydride react with citric acid. Conducting this reaction, with the temperature controlled at 32°C ± 2°C for half an hour, produced a yellow solution. The absorption was measured at a wavelength of 425 nm using a spectrophotometer and compared to a control sample. Finally, the citric acid concentration was calculated and compared with that of the standard samples [29]. Oxalic acid measurement was performed using the method of Manganometry [28], and the Nelson and Somogyi method was used to measure the sugar content [30,31]. In this method, four solutions with the specifications given in reference [30,31] were combined. The absorbance was then recorded using a UNICAM PYE spectrophotometer at a wavelength of 500 nm and compared to that of the standard solutions.

2.5. Analyses of color properties

The kaolin powders were sent to the Color Science Technology Research Institute after 28 days of treatment with fungal strain. They were placed in a furnace set to 1000°C for 1 h if the microbial biomass mixed with the kaolin powder was burned at this temperature. The color coordinates were measured according to the ASTM E308, D2244 standard. The laboratory environment had a controlled moisture content of 29% and a temperature of 25°C. The samples were placed on a quartz glass powder and measured with an uncertainty of 95% with a spectrophotometer (XRite SP-64) using Color I Control software.

3. Results and discussion

3.1. Results for NCIM 548A. niger fungal without fungal cells

In order to evaluate the functions of the metabolites produced by fungal cells in the culture medium, NCIM 548 A. niger fungal cells were initially isolated from the culture medium in sterile condition after 7 days. Sterilized kaolin powder (5 g) was then added to the culture medium without fungal cells and samples were taken on specific days. The final results of kaolin bioleaching are given in Table 3. The table shows that, following a reduction in the temperature from 30°C to 25°C on day 7, the amounts of citric acid and oxalic acid increased and pH decreased as a result. After day 14, in addition to the increase in acidity, the iron concentration also increased. After day 7, the concentration of iron increased and the pH decreased, which corresponds to the results obtained by Hosseini et al. [22] and Cameselle et al. [26]. It can therefore be concluded that A. niger NCIM 548 fungal reacts better at a temperature of 25°C than at 30°C and is able to produce more organic acids, as a result of which its rate of iron removal is also higher. The XRF results for kaolin samples after 28 days at 30°C and 25°C are shown in Table 4. The results of XRF analysis of kaolin samples at 25°C (Table 4) show that microbial treatment reduced the Fe₂O₃ and Fe content in the samples by 25.6% compared to the original kaolin sample. Most of the treated sample constituents also show a significant reduction compared to the original kaolin sample. XRF analysis of kaolin samples at 30°C also shows a 36.5% reduction in Fe₂O₃ as well as in Fe, but other constituents show greater decreases at a temperature of 25°C.

It is reported in Ref. [26] that iron reduction increased with increases in temperature (from 30°C to 60°C). It can be concluded from the results of the
XRF samples in this study that the samples performed better at 30°C, which contradicts the results for iron, organic acids and pH measurements reported in Ref. [26]. The results for NCIM 548 A. niger fungal without fungal cells showed that iron dissolution increased with increases in the leaching time, which is consistent with the results of Ref. [26].

### 3.2. Results for NCIM 548A. niger fungal with fungal cells

The results of the previous series of the experiments shown for different times reveal that the amount of organic acids produced does not increase due to the absence of fungal cells. It is therefore likely that this will affect the amount of iron removal. It was consequently decided to add spores of the fungal as well as kaolin to the culture medium this time, and to conduct fungal growth analyses simultaneously and compare the results with those of previous tests. The results of bioleaching and the XRF results for the kaolin samples after 28 days at 30°C and 25°C temperatures are shown in Tables 5 and 6. Table 5 shows that the iron and sucrose concentrations increased and the pH decreased with decreases in the temperature after day 7 and that the amount of oxalic acid also increased in addition to the concentration of iron and sucrose after day 14. The amounts of iron and oxalic acid in the leaching culture medium were relatively high at 25°C compared to a temperature of 30°C, but the production of citric acid at a temperature of 30°C was slightly higher than at 25°C. pH reduction at 25°C was higher, however, than at 30°C. The XRF results for the treated kaolin sample show a 41.6% decrease in Fe and Fe content at 25°C, while other constituents also show a significant decrease compared to the standard. The XRF results for the kaolin sample show a 37.2% decrease in Fe and Fe content at 30°C. The reduction

### Table 3. Results of the kaolin bioleaching by NCIM 548 A. niger after 28 days without fungal cells.

| Time (days) | Oxalic acid (g/l) | Citric acid (g/l) | Dissolved iron concentration (ppm) | Sucrose (g/l) | Sucrose consumption (%) | pH |
|-------------|-------------------|------------------|-----------------------------------|---------------|-------------------------|-----|
| 2           | 2.70              | 0.735            | 32.25                             | 105           | 12.5                    | 3.28|
| 7           | 2.50              | 0.732            | 42.70                             | 95            | 20.8                    | 3.20|
| 14          | 2.83              | 0.790            | 58.90                             | 92.7          | 22.7                    | 3.30|
| 21          | 2.85              | 1.910            | 75.05                             | 91.5          | 23.7                    | 3.23|
| 28          | 3.08              | 2.150            | 85.08                             | 89.5          | 25.4                    | 3.18|

### Table 4. Results of the XRF analysis of the treated kaolin sample by NCIM 548 A. niger without fungal cells at 30°C and 25°C temperatures.

| Composition | SiO2 | Al2O3 | FeO | MgO | CaO | Na2O | K2O | MgO | TiO2 | S  |
|-------------|------|-------|-----|-----|-----|------|-----|-----|------|----|
| 25°C        | 57.34| 15.66 | 3.91| 2.93|     | 2.50 | 2.15| 2.87| 0.42 | 0.94|
|             | Na   | Mg    | Al  | Si  |     | K    | Ca  | Ti  | Fe   |    |
|             | 1.85 | 3.78  | 8.28| 26.81|     | 1.78 | 2.09| 0.25| 2.74 | 11.28|
| 30°C        | 56.06| 16.12 | 3.34| 3.25|     | 2.59 | 2.18| 3.91| 0.43 | 0.88|
|             | Na   | Mg    | Al  | Si  |     | K    | Ca  | Ti  | Fe   |    |
|             | 1.92 | 1.77  | 8.53| 26.21|     | 1.81 | 2.32| 0.26| 2.34 | 12.22|

### Table 5. Results of the kaolin bioleaching by NCIM 548 A. niger after 28 days with fungal cells.

| Time (days) | Oxalic acid (g/l) | Citric acid (g/l) | Dissolved iron concentration (ppm) | Sucrose (g/l) | Sucrose consumption (%) | pH |
|-------------|-------------------|------------------|-----------------------------------|---------------|-------------------------|-----|
| 2           | 1.81              | 0.881            | 40.80                             | 90.15         | 24.8                    | 3.07|
| 7           | 1.81              | 4.49             | 118.70                            | 41.92         | 65                      | 2.64|
| 14          | 6.98              | 3.65             | 162.40                            | 37            | 69.1                    | 2.96|
| 21          | 8.61              | 4.42             | 216.55                            | 19.8          | 83.5                    | 2.90|
| 28          | 10.5              | 4.26             | 159.55                            |               | 2.97                    |     |

### Table 6. Results of the XRF analysis of the treated kaolin sample by NCIM 548 A. niger with fungal cells at 30°C and 25°C temperatures.

| Composition | SiO2 | Al2O3 | FeO | MgO | CaO | Na2O | K2O | MgO | TiO2 | S  |
|-------------|------|-------|-----|-----|-----|------|-----|-----|------|----|
| 25°C        | 49.24| 10.64 | 3.07| 3.51|     | 2.3  | 1.52| 1.45| 0.42 | 0.94|
|             | Na   | Mg    | Al  | Si  |     | K    | Ca  | Ti  | Fe   |    |
|             | 1.37 | 0.87  | 5.63| 23.02|     | 1.26 | 2.51| 0.25| 2.15 | 26.88|
| 30°C        | 46.29| 11.22 | 3.3 | 1.63|     | 2.0  | 1.92| 1.42| 0.46 | 0.85|
|             | Na   | Mg    | Al  | Si  |     | K    | Ca  | Ti  | Fe   |    |
|             | 1.94 | 0.86  | 5.94| 21.64|     | 1.59 | 1.16| 0.28| 2.31 | 30.91|

JOURNAL OF ASIAN CERAMIC SOCIETIES
percentages of other components were also higher at 25°C. The results of measurements of the amount of iron in the culture medium indicate that iron reduction at 25°C was approximately twice as high as at 30°C, but these results show little change in the XRF analysis. The results for iron reduction at lower temperatures also contradict the results reported by Cameselle et al. [26].

Cameselle et al. [26] showed that iron dissolution increased with increases in leaching time, but own results for NCIM 548 A. niger fungal with fungal cells showed that iron dissolution decreased after 21 days at 25°C and 30°C.

### 3.3. Results for A. niger fungal isolated from pistachio skin

Similar experiments were carried out for A. niger fungal isolated from pistachio skin and sampling was conducted at different time intervals. The results after 1 month are presented in Table 7 according to fungal cell. XRF analysis of the treated kaolin samples was conducted after 14 days at 25°C and after 28 days at the two temperatures of 30°C and 25°C for purposes of comparison (Tables 8 and 9). Pellets were prepared from kaolin powder treated with A. niger isolated from pistachio skin at 25°C after 28 days and from a standard kaolin sample and heated in a furnace (NUVE MF207 model) at 1000°C for 1 h. After removal of the pellets from the furnace and cooling, the changes in color were observed. Images of the treated and standard kaolin samples are shown in Figure 1. The results of the iron XRF at 25°C after 14 days showed a reduction of 52% in Fe$_2$O$_3$ and Fe content and a reduction to 47.7% after 28 days. These results indicate that the time for the microbial culling with this fungus strain is 14 days, after which there is no significant reduction in the amount of iron in the environment. The results of iron measurements also confirm these results. The amount of sulfur in the treated kaolin sample after 14 days also shows a further decrease compared to other samples. The XRF results for a kaolin sample at 30°C showed a reduction of 33.8% in Fe$_2$O$_3$ and Fe content. The results of these experiments also indicate that a temperature of 25°C achieves better performance than 30°C. The results for A. niger fungal isolated from pistachio skin also showed that iron dissolution decreased after 14 days at 25°C and after 21 days at 30°C with no increase with increases in leaching time, which contradicts the results reported by Cameselle et al. [26]. In addition, the image presented in Figure 1 shows a significant change in the color of the treated sample compared to the original kaolin sample.

### 3.4. Results of XRD, reflection spectra and color coordinate analyses

To investigate any biogenic alteration of kaolin after bioreduction, mineralogical analyses were carried out using XRD (Figure 2). The mineral material exhibited the preferred orientation of the kaolin pattern with all peaks clearly resolved. Analysis of the XRD pattern revealed that the mineral was composed mainly of highly

### Table 7. Results of the kaolin bioleaching by A. niger isolated from pistachio skin after 28 days.

| Time (days) | Temperature (30°C) | Temperature (25°C) |
|-------------|-------------------|-------------------|
|             | Oxalic acid (g/l) | Citric acid (g/l) | Dissolved iron concentration (ppm) | Sucrose (g/l) | Sucrose consumption (%) | pH | Oxalic acid (g/l) | Citric acid (g/l) | Dissolved iron concentration (ppm) | Sucrose (g/l) | Sucrose consumption (%) | pH |
| 2           | 2.02             | 0.209             | 101.150             | 94           | 21.6                   | 3.20 | 2.025             | 0.166             | 70.3                   | 90           | 25                       | 3.60 |
| 7           | 3.83             | 0.616             | 252.15              | 25           | 79.1                   | 2.85 | 6.75              | 2.058             | 442.55                 | 28           | 76.6                     | 1.94 |
| 14          | 6.53             | 1.004             | 768.800             | 2            | 98.3                   | 2.29 | 7.43              | 2.220             | 1039.00                | 3.5          | 97.1                     | 1.80 |
| 21          | 8.78             | 1.725             | 806.500             | –            | –                      | 1.91 | 9.45              | 2.315             | 783.25                 | –            | –                        | 1.79 |
| 28          | 9.03             | 2.640             | 757.500             | –            | –                      | 1.85 | 10.5              | 2.430             | 752.750                | –            | –                        | 1.84 |

### Table 8. Results of the XRF analysis of the treated kaolin sample after 14 days by A. niger at 25°C temperature.

| Composition | SiO$_2$ | Al$_2$O$_3$ | FeO$_2$ | CaO | Na$_2$O | K$_2$O | MgO | TiO$_2$ | S  |
|-------------|---------|-------------|---------|-----|---------|-------|-----|---------|----|
| %           | 49.3    | 12.01       | 2.52    | 5.7 | 2.35    | 1.91  | 1.48| 0.46    | 0.71|
| Composition | Na      | Mg          | Al      | Si  | K       | Ca    | Ti  | Fe      | LOI|
| %           | 1.74    | 0.9         | 6.36    | 23.05| 1.38    | 4.0   | 0.28| 1.76    | 23.44|

### Table 9. Results of the XRF analysis of the treated kaolin sample after 28 days by A. niger at 30°C and 25°C temperatures.

| Composition | SiO$_2$ | Al$_2$O$_3$ | FeO$_2$ | CaO | Na$_2$O | K$_2$O | MgO | TiO$_2$ | S  |
|-------------|---------|-------------|---------|-----|---------|-------|-----|---------|----|
| %           | 51.71   | 11.72       | 2.75    | 4.67| 2.41    | 1.82  | 1.1 | 0.46    | 0.89|
| Composition | Na      | Mg          | Al      | Si  | K       | Ca    | Ti  | Fe      | LOI|
| %           | 1.79    | 0.67        | 6.2     | 24.17| 1.51    | 3.34  | 0.28| 1.93    | 22.47|
| 30°C Composition | SiO$_2$ | Al$_2$O$_3$ | FeO$_2$ | CaO | Na$_2$O | K$_2$O | MgO | TiO$_2$ | S  |
| %           | 47.71   | 10.26       | 3.48    | 3.99| 1.94    | 1.83  | 0.94| 0.48    | 1.01|
| Composition | Na      | Mg          | Al      | Si  | K       | Ca    | Ti  | Fe      | LOI|
| %           | 1.44    | 0.57        | 5.44    | 22.3| 1.52    | 2.85  | 0.29| 2.43    | 28.36|

| 25°C Composition | SiO$_2$ | Al$_2$O$_3$ | FeO$_2$ | CaO | Na$_2$O | K$_2$O | MgO | TiO$_2$ | S  |
| %           | 51.71   | 11.72       | 2.75    | 4.67| 2.41    | 1.82  | 1.1 | 0.46    | 0.89|
| Composition | Na      | Mg          | Al      | Si  | K       | Ca    | Ti  | Fe      | LOI|
| %           | 1.79    | 0.67        | 6.2     | 24.17| 1.51    | 3.34  | 0.28| 1.93    | 22.47|
| 30°C Composition | SiO$_2$ | Al$_2$O$_3$ | FeO$_2$ | CaO | Na$_2$O | K$_2$O | MgO | TiO$_2$ | S  |
| %           | 47.71   | 10.26       | 3.48    | 3.99| 1.94    | 1.83  | 0.94| 0.48    | 1.01|
| Composition | Na      | Mg          | Al      | Si  | K       | Ca    | Ti  | Fe      | LOI|
| %           | 1.44    | 0.57        | 5.44    | 22.3| 1.52    | 2.85  | 0.29| 2.43    | 28.36|

[26] Cameselle et al., 2016.
crystalline kaolinite, which is an accessory mineral found in both biotreated and blanc kaolin materials. Similarly, no crystalline by-product was produced during bioreduction. The XRD patterns obtained following treatment with *A. niger* appear to be unaltered with similar peaks before and after the treatment. This may be due to that the mineral is of high crystallinity.

Figure 3 of the reflection spectrum chart shows five samples produced at visible wavelengths from 400 to 700 nm in comparison with the control sample. As the wavelength rises from blue to red, the magnitude of the reflection increases. At wavelengths of 400–410 nm, the reflection has a steep slope at first and then a gradual slope. The powder treated with NCIM 548 *A. niger* fungal with fungal cell at a temperature of 30°C shows the highest reflection value and the powder treated with NCIM 548 *A. niger* fungal without cells at 25°C shows the lowest reflection value. Table 10 shows a comparison between color indices. The closer $L^*$ is to 100, the whiter the sample color. Also, $a^*$ and $b^*$ show the red and yellow indices of the sample, respectively. As shown in the table, sample 6 has the highest $L^*$, which is equivalent to 75.41, after which samples 5 and 4 show the highest values for $L^*$ of 74.72 and 74.49, respectively. $L^*$ of the original kaolin powder is 71.94. These three samples also show the smallest amount of $a^*$ and $b^*$ in comparison with other samples. Compared to the initial powder of kaolin (sample 7), $a^*$ shows decreases in samples 6 and 5 of 45% and 47% respectively, and a decrease of 35% in sample 4. Also, $b^*$ shows a 63% decrease in samples 6 and 5, moreover, and a 31.82% decrease in sample 4. It can be concluded, therefore, that kaolin powders treated with *A. niger* fungal isolated from pistachio skin at two temperatures of 25°C and 30°C as well as NCIM 548 *A. niger* fungal at 25°C show the highest degrees of whiteness compared to other samples. Pellets were prepared from the five treated samples and the original and placed in a furnace at a temperature of 1000°C for 1 h, as shown in Figure 4. As seen in the figure, samples 1, 2 and 5 show more color variations than the other samples and the control sample, which is consistent with the results for whitening and iron removal from the kaolin samples.

4. Conclusion
This study examines the effect of temperature on the removal of iron from kaolin by a biological method using *A. niger* strains isolated from pistachio skin and
The kaolin samples contained 5.26% iron oxide, and after kaolin treatment, the best iron concentration of 1039 ppm was obtained by \textit{A. niger} fungal isolated from pistachio skin at 25°C. The results for kaolin powder XRF showed 52% iron removal from kaolin after 14 days. But after treatment for 28 days with the same fungal, the iron concentration was 752.750 ppm, which XRF analysis showed to represent about 47.7% iron removal from the kaolin powder. The same fungal at 30°C showed iron content of 757.5 ppm after 28 days.

Table 10. Color indices of the samples after 28 days.

| N. | L*   | a*   | b*   | C*  | h*  | DL* | Da* | Db* | DC* | DH* | DE* |
|----|------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|
| 1  | 71.45| 4.77 | 13.57| 14.38| 70.63| D-0.49| G-0.70| B-2.52| D-2.61| R-0.16| 2.66 |
| 2  | 70.74| 5.32 | 14.37| 15.32| 69.69| D-1.20| G-0.15| B-1.73| D-1.68| R-0.44| 2.11 |
| 3  | 66.73| 6.71 | 12.95| 14.58| 62.6 | D-5.21| R1.25 | B-3.14| D-2.41| R-2.37| 6.21 |
| 4  | 74.49| 3.54 | 10.97| 11.53| 72.13| L2.55 | G-1.93| B-5.12| D-5.46| G0.22| 6.03 |
| 5  | 74.72| 2.88 | 5.94 | 6.6  | 64.19| L2.78 | G-2.95| B-10.15| D-10.39| R-1.30| 10.83 |
| 6  | 75.41| 3.01 | 5.81 | 6.55 | 62.62| L3.46 | G-2.45| B-10.28| D-10.45| R-1.59| 11.12 |
| 7  | 71.94| 5.47 | 16.09| 16.99| 71.24| –     | –    | –    | –    | –    | –    |

1 – Powder treated with \textit{A. niger} fungal isolated from pistachio skin at a temperature of 25°C; 2 – Powder treated with \textit{A. niger} fungal isolated from pistachio skin at a temperature of 30°C; 3 – Powder treated with NCIM548 \textit{A. niger} fungal without cells at 25°C; 4 – Powder treated with NCIM548 \textit{A. niger} fungal without cells at 30°C; 5 – Powder treated with NCIM548 \textit{A. niger} fungal with cells at 25°C; 6 – Powder treated with NCIM548 \textit{A. niger} fungal with cells at 30°C; 7 – Primary kaolin sample (Blanc).

Figure 3. Reflection spectrum of the samples after 28 days. 1 – Powder treated with \textit{A. niger} fungal isolated from pistachio skin at a temperature of 25°C; 2 – Powder treated with \textit{A. niger} fungal isolated from pistachio skin at a temperature of 30°C; 3 – Powder treated with NCIM548 \textit{A. niger} fungal without cells at 25°C; 4 – Powder treated with NCIM548 \textit{A. niger} fungal without cells at 30°C; 5 – Powder treated with NCIM548 \textit{A. niger} fungal with cells at 25°C; 6 – Powder treated with NCIM548 \textit{A. niger} fungal with cells at 30°C; 7 – Primary kaolin sample (Blanc).

Figure 4. Image of the pills prepared from kaolin powders after 28 days. 1 – Powder treated with \textit{A. niger} fungal isolated from pistachio skin at a temperature of 25°C; 2 – Powder treated with \textit{A. niger} fungal isolated from pistachio skin at a temperature of 30°C; 3 – Powder treated with NCIM548 \textit{A. niger} fungal without cells at 25°C; 4 – Powder treated with NCIM548 \textit{A. niger} fungal without cells at 30°C; 5 – Powder treated with NCIM548 \textit{A. niger} fungal with cells at 25°C; 6 – Powder treated with NCIM548 \textit{A. niger} fungal with cells at 30°C; 7 – Primary kaolin sample (Blanc).

\textit{A. niger} NCIM 548 fungal. The kaolin samples contained 5.26% iron oxide, and after kaolin treatment, the best iron concentration of 1039 ppm was obtained by \textit{A. niger} fungal isolated from pistachio skin at 25°C. The results for kaolin powder XRF showed 52% iron removal from kaolin after 14 days. But after treatment for 28 days with the same fungal, the iron concentration was 752.750 ppm, which XRF analysis showed to represent about 47.7% iron removal from the kaolin powder. The same fungal at 30°C showed iron content of 757.5 ppm after 28 days.

Kaolin powder treated with \textit{A. niger} NCIM 548 with fungal cells was also tested for the amount of iron in a 735 ppm culture medium after 21 days, and XRF results showed 41.6% iron removal from kaolin powder after 28 days at 25°C and 37.2% removal at 30°C. The results of white measurements of treated kaolin samples showed that kaolin powder treated with \textit{A. niger} with pistachio skin at 25°C and 30°C and \textit{A. niger} NCIM 548 with fungal cells at 25°C contained the highest \textit{L*}; the results showed 75.41, 74.72 and 74.49, respectively, which had increased
in comparison with the L* sample of the original kaolin, which was 71.94. Therefore, the results showed that the A. niger fungal isolated from pistachio skin at 25°C and 30°C achieved more efficient iron removal than the other fungal. Also, a temperature of 25°C showed better results than a temperature of 30°C. The results of the experiments therefore indicated that 14 days is suitable for removal of iron from kaolin powder by this fungal strain, after which period there is no increase in iron removal.

**Acknowledgments**

The authors express their appreciation to the Karaj Materials and Energy Institute and Dr Pazouki, who lent their support to this project.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**ORCID**

Mahsa Fakharpour [http://orcid.org/0000-0001-8226-5974](http://orcid.org/0000-0001-8226-5974)

**References**

[1] De Mesquita LMS, Rodrigues T, Gomes SS. Bleaching of Brazilian kaolins using organic acids and fermented medium. Miner Eng. 1996;9(9):965–971.

[2] Murray HH, editor. Developments in clay science. Elsevier; 2006.

[3] Zegeye A, Yahaya S, Fialips CI, et al. Refinement of industrial kaolin by microbial removal of iron-bearing impurities. Appl Clay Sci. 2013;86:47–53.

[4] Ryu HW, Cho KS, Chang YK, et al. Refinement of low-grade clay by microbial removal of sulfur and iron compounds using Thiobacillus ferrooxidans. J Ferment Bioeng. 1995;80(1):46–52.

[5] Kostka JE, Haefele E, Viehweger R, et al. Respiration and dissolution of iron (III)–containing clayminerals by bacteria. Environ Sci Technol. 1999(a);33(18):3127–3133.

[6] Štyriaková I, Štyriak I. Iron removal from Kaolins by bacterial leaching. Ceramics-Silikáty. 2000;44(4):135–141.

[7] Lee EY, Cho KS, Ryu HY. Microbial refinement of kaolin by iron-reducing bacteria. Appl Clay Sci. 2002;22(1–2):47–53.

[8] Mockovčiaková A, Iveta Š, Jiří Š, et al. Characterization of changes of low and high defect kaolinite after bioleaching. Appl Clay Sci. 2008;39(3–4):202–207.

[9] Guo MR, Lin YM, Xu XP, et al. Bioleaching of iron from kaolin using Fe (III)-reducing bacteria with various carbon nitrogen sources. Appl Clay Sci. 2010;48(3):379–383.

[10] Ambikadevi VR, Lalithambika M. Effect of organic acids on ferric iron removal from iron-stained kaolinite. Appl Clay Sci. 2000;16(3–4):133–145.

[11] Prasad MS, Reid KJ, Murray HH. Kaolin: processing, properties and applications. Appl Clay Sci. 1991;6(2):87–119.

[12] Conley RF, Lloyds MK. Improving in iron leaching in clays: optimizing processing parameters in sodium dithionite reduction. Eng Chem Process Des Dev. 1970;9(4):595–601.

[13] Arslan V, Bayat O. Removal of Fe from kaolin by chemical leaching and bioleaching. Clay Miner. 2009;57(6):787–794.

[14] Bergaya F, Lagaly G, editors. Handbook of clay science. Elsevier Science; 2013.

[15] Kostka JE, Stucki JW, Nealson KH, et al. Reduction of structural Fe (III) in smectite by a pure culture of Shewanella putrefaciens strain MR-1. Clays Clay Miner. 1996;44:522–529.

[16] Lovley DR, Holmes DE, Nevin KP. Dissimilatory Fe (III) and Mn (IV) reduction. Adv Microb Physiol. 2004;49:219–286.

[17] Lee EY, Cho KS, Ryu HW, et al. Microbial removal of Fe(III) impurities from clay by dissimilatory iron reducers. J Biosci Bioeng. 1999;87(3):397–399.

[18] Štyriaková I, Mockovčiaková A, Štyriak I, et al. Bioleaching of clays and iron oxidation coatings from quartz sands. Appl Clay Sci. 2012;61:1–7.

[19] Maurice PA, Vierkorn MA, Hersman LE, et al. Enhancement of kaolinite dissolution by an aerobic Pseudomonas mendocina bacterium. Geomicrobiol J. 2001;18(1):21–35.

[20] He QX, Huang XC, Chen ZL. Influence of organic acids, complexing agents and heavy metals on the bioleaching of iron from kaolin using Fe (III)-reducing bacteria. Appl Clay Sci. 2011;51(4):78–483.

[21] Musial I, Cibis E, Rymowicz W. Designing a process of kaolin bleaching in an oxalic acid enriched medium by Aspergillus niger cultivated on biodiesel-derived waste composed of glycerol and fatty acids. Appl Clay Sci. 2011;52(3):277–284.

[22] Hosseini MR, Pazouki M, Ranjbar M, et al. Bioleaching of iron from highly contaminated kaolin clay by Aspergillus niger. Appl Clay Sci. 2007;37(3–4):251–257.

[23] Cameselle C, Ricart MT, Nunez MJ, et al. Iron removal from kaolin. Comparison between “in situ” and “two-stage” bioleaching processes. Hydrometallurgy. 2003;68(1–3):97–105.

[24] Mulligan CN, Kamali M, Gibbs BF. Bioleaching of heavy metals from low-grade mining ore using Aspergillus niger. J Hazard Mater. 2004;110(1–3):77–84.

[25] Terrazas Calderon GD, Rodriguez JJ, Ortiz-Mendez U, et al. Iron leaching of a Mexican clay of industrial interest by oxalic acid. Adv Technol Mater Process. 2005;7(2):161–166.

[26] Cameselle C, Núñez MJ, Lema JM, et al. Leaching of iron from kaolins by a spent fermentation liquor: influence of temperature, pH, agitation and citric acid concentration. J Ind Microbiol. 1995;14(3–4):288–292.

[27] Pazouki M, Ganjkhanlou Y, Tofigh AA, et al. Optimizing of iron bioleaching from a contaminated kaolin clay by the use of artificial neural network. Int J Eng. 2012;25(2):81–88.

[28] Jeffery GH, Basset J, Mendham J, et al. Vogel’s textbook of quantitative chemical analysis. New York (NY): Longman Science and Technical; 1989.

[29] Marrier JR, Boulet M. Direct determination of citric acid in milk with an improved, pyridine acetic anhydride method. J Dairy Sci. 1958;41(12):1683–1686.

[30] Nelson N. A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem. 1944;132(2):375–380.

[31] Somogyi M. Notes on sugar determination. J Biol Chem. 1952;195:9–23.