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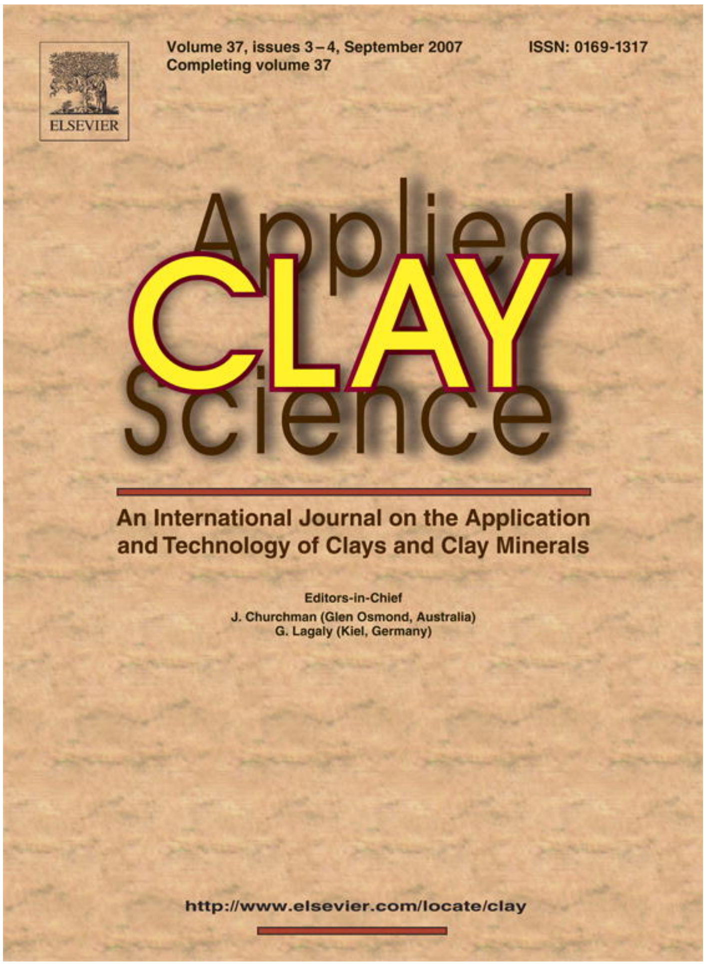
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Bioleaching of iron from highly contaminated Kaolin clay by *Aspergillus niger*

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Abstract

Kaolin is a clay mineral that has a wide application in the industry specially, in paper, ceramic, and porcelain manufacturing. One of the most important factors that affects the value of this raw material is its brightness. Unfortunately, with the iron oxides deposit on mineral particles during kaolin formation, much of this clay has become unusable for industries. So, several chemical methods have been applied in mineral processing plants to reduce these contaminants, but finding a more sustainable approach like biological methods have always attracted a great attention. In this work bioleaching of iron from a highly contaminated kaolin sample was carried out using two different strains of *Aspergillus niger*, and the effects of strain type, pulp density, and time of clay addition on the iron removal were investigated by employing a 2^3 full factorial design. Finally, it is concluded that strain type has the most significant effect on the response; also, the highest removal extent was 42.8% that was obtained by using the strain isolated from pistachio shell, and at the pulp density of 20 g/l when the clay was added at the beginning of the experiments.

Keywords: Kaolin; Bioleaching; *Aspergillus niger*; Iron removal

1. Introduction

Kaolin clay is a hydrous alumino silicate ($Al_2O_3\cdot 2SiO_2\cdot 2H_2O$) (Asmatulu, 2002), and an essential resource in porcelain and ceramic manufacturing (Ryu et al., 1995; Styriakova and Styriak, 2000), production of paper, pigments, and fillers. Clay is formed by the mechanical and chemical breakdown of rocks; also, depending on the atmospheric and geological condition of deposition, as well as the degree of alteration of the clay, iron (hydr)oxides (usually Fe^{3+} forms) are commonly precipitated or adsorbed to the clay surfaces or admixed as a separate phase (Murad, 1987; Stucki et al., 1988) that make much of the kaolin unusable for commercial application due to insufficient whiteness (Styriakova and Styriak, 2000), and the reduction of refractoriness of products (Lee et al., 2002). So, for the reasons mentioned above, the quality of kaolin is measured in terms of iron content (Lee et al., 2002).

Some researchers have developed different physical and chemical techniques (and recently microbiological) with the purpose of removing the ferric iron present as oxide or hydrated oxide in the clay. These techniques generally include magnetic separation, froth flotation, selective flocculation, size separation by hydrocyclones, and leaching (Styriakova and Styriak, 2000; Lee et al.,

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2002). However, the removal efficiency of iron is low because the iron in kaolin may be tightly adsorbed or it may be in a complexed form (Lee et al., 2002) that is not easily separated by the first four methods. Most of the industries have employed potent chemical reductants such as dithionite or hydrazine to remove iron impurities but these chemicals are not the cause of iron reduction in nature (Kostka et al., 1999); also, removal of only the poorly crystalline or more readily soluble iron oxide phases can be carried out using various methods such as ammonium oxalate or dilute HCl extractions (Stucki et al., 1988). Complete separation or removal of iron oxide phases by chemical means without in some way altering the remaining phase is unlikely (Manceau et al., 2000). Furthermore, the clay structure collapses during reduction in response to the reduction of structural Fe (Kostka et al., 1999); also, chemical methods have high operating costs and environmental impacts.

Field observations and laboratory experiments demonstrate that microbes can accelerate aluminum silicate mineral weathering reactions in direct contact with mineral surfaces, by producing organic and inorganic acids, creating metal-complex ligands, changing redox condition or mediating formation of secondary mineral phase (Styriakova and Styriak, 2000). Therefore, microorganisms which oxidize or reduce iron should be considered as a new and alternative method to remove iron impurities from kaolin (Lee et al., 2002). Microbial leaching is thought to be less energy-intensive than high temperature chemical processes and requires low capital and operating costs (Ryu et al., 1995), and causes less environmental problems (Mulligan et al., 2004).

In this work, the ability of two strains of *Aspergillus niger* to remove iron (hydr)oxides from a highly contaminated kaolin, and influence of some effective parameters on removal efficiency is studied.

2. Materials and methods

2.1. Kaolin sample

Kaolin sample with the particle size of 90% under 9.32 μm (d_{90}=9.32 μm) was supplied by Mehrkhak Company, Tehran, Iran from a deposit located in Damghan, Semnan Province, Iran that is unsuitable for using in ceramic body in sanitary ware manufacturing, because of its high content of iron oxides (11% Fe_2O_3).

2.2. Microorganisms and culture media

Two different strains of *A. niger* were used in the experiments. One *A. niger* NCIM 548 that was kindly provided by Dept. of Chem. Eng., IIT Madras, India, and the other was originally isolated from pistachio shell.

A solid media (malt extract, 30 g/l; meat peptone, 3 g/l and agar, 15 g/l, at pH 5.6) was employed for the growth and maintenance of the microorganism at 30 °C. A synthetic media (Cameselle et al., 2003) containing sucrose, 120 g/l; NH_4NO_3, 450 mg/l; KH_2PO_4, 100 mg/l; $MgSO_4 \cdot 7H_2O$, 300 mg/l; $FeSO_4 \cdot 7H_2O$, 0.1 mg/l; $ZnSO_4 \cdot 7H_2O$, 0.25 mg/l was employed as culture media.

2.3. Bioleaching of mineral samples

Fungal spores were suspended from a 7-day agar slant in a sterile solution (0.1% Tween80, and 0.9% NaCl) and enumerated by a microscope. Bioleaching experiments were carried out in 500-ml Erlenmeyer flasks containing 100 ml of culture media inoculated at a concentration of 10^6 spores/ml, and incubated at 30 °C, and 160 rpm on a rotary shaker. Amounts of 2 and 6 g of kaolin were added to the culture media in the beginning of the cultivation and on the third day. All experiments were performed in duplicate, and the average of the results reported, were with 2% deviation.

2.4. Methods of analysis

In order to determine the kaolin composition, and specially its iron contents, XRF analysis was done by ARL 8410 instrument, tube anode: Rh, and 60 kV (Table 1). Also, to determine kaolin particle size, particle size analysis made by Fritsch Particle Sizer "Analysette 22".

To analyze the amount of removable iron by a leaching process, iron was extracted from the kaolin by heating it for 15 to 20 min in 6 N HCl (Lee et al., 2002), and then total iron content was determined by the 1,10-phenanthroline method (Jeffery et al., 1989).

To register the changes in pH value, and dissolved iron concentration, sampling was done in determined intervals. Then the solid phase was separated, and the pH value of the liquor was measured by Metrohm pH meter model 744; also dissolved iron concentration was measured by the 1,10-phenanthroline method (Jeffery et al., 1989).

To calculate the removal percent of the iron, the measured dissolved iron concentration in the cultured media was divided

Table 1
Chemical composition of the clay sample

Component	P_2O_5	MnO	TiO_2	MgO	K_2O	Na_2O	CaO	Fe_2O_3	AL_2O_3	SiO_2
Percent	0.105	0.001	2.729	0.45	2.19	0.19	0.09	11.11	25.82	44.78

Table 2
Results of bioleaching by *A. niger* after 30 days

Run	Fungus	Pulp density* (g/l)	Time of clay addition (day)	Dissolved iron concentration** (ppm)	Iron removal (%)	Sugar consumption (%)	Citric acid (g/l)	Oxalic acid (g/l)
1	*A. niger* isolated from pistachio shell	20	First (0)	660.24	42.8***	43	5.95	4.67
2		60	First	672.47	14.6	35	5.22	4.41
3		20	Third (3)	462.85	30.1	45	4.92	2.65
4		60	Third	527.21	11.4	42	5.57	3.09
5	*A. niger* NCIM 548	20	First (0)	34.63	2.2	47	1.88	2.21
6		60	First	82.73	1.8	43	2.75	2.47
7		20	Third (3)	20.04	1.3	38	2.26	2.03
8		60	Third	50.37	1.1	38	2.00	2.29

* 2 g and 6 g of the clay were added to 100 ml of the media.
** Dissolved Fe concentration, $Fe=0.7Fe_2O_3$.
*** $Fe_2O_3=11\%$, $Fe_{total}=20*0.11*0.7=1.54$ g, percentage of iron removal=(660.24/1540)*100=42.8.

by 1.54, and 4.62 g/l (the total Fe contents of the clay) for the experiments with the clay density of 20, and 60 g/l, respectively.

To quantify the final concentration of organic acids exerted to the media, pyridine acetic anhydride method (Marrier and Boulet, 1958) was applied to determine citric acid concentration, and manganometry method (Jeffery et al., 1989) to measure oxalic acid concentration.

To determine total sugar contents, the spent media were hydrolyzed, and analyzed by colorimetry using the Nelson and Somogyi method (Nelson, 1944; Somogyi, 1952).

To measure the produced biomass in each flask, the flask solid phases were separated using a centrifuge at 3200 ×*g* for 10 min, and then the difference between their weights after drying at 100 °C, and after burning at 170 °C was calculated.

2.5. Design of experiments

To design the experiments, a full factorial design was employed, using three variables each at two levels (2^3) (Montgomery, 2005). The data of these experiments are presented in Table 2.

3. Result and discussion

To calculate the removal percentage of iron, the leachable iron (iron in oxyhydroxide form) was measured that was 11% of the mineral. This means that all the iron content (11%) that are specified by XRF can be removed.

3.1. Leaching by A. niger isolated from pistachio shell

Using *A. niger* isolated from pistachio shell in the first four experiments, the percentage of iron removal reached to 42.8% after 30 days in the best condition (run 1), where the density of pulp was 20 g/l, and clay was added in the beginning of the cultivation; also, iron concentration in the cultured media reached to 660.24 ppm, and the sugar concentration decreased from 120 g/l to 68.5 g/l. In the other experiment (run 3), kaolin was added to the culture at the third day that resulted in removal of 30.1% of iron content of the clay.

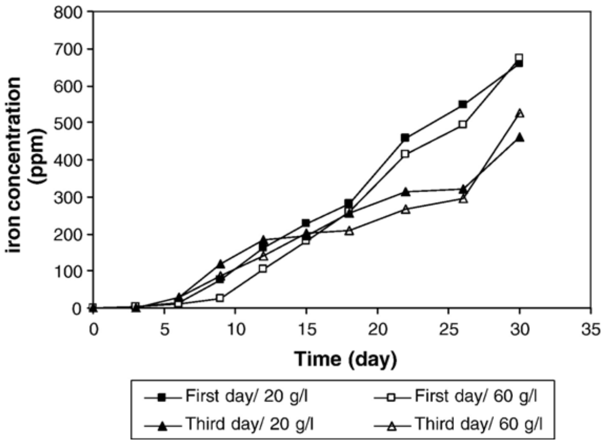

Fig. 1. Evolution of iron concentration in the cultured media of *A. niger* isolated from pistachio shell. Pulp densities were 20 g/l (full dots) and 60 g/l (empty dots), and the clay was added at the first (square dots) and third day (triangle dots).

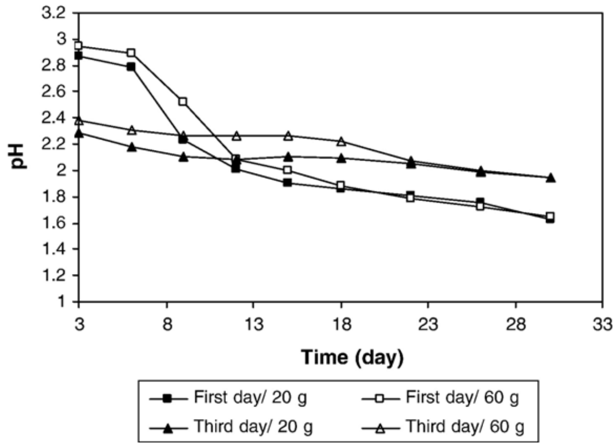

Fig. 2. Variation of pH with time for bioleaching by *A. niger* isolated from pistachio shell. Pulp densities were 20 g/l (full dots) and 60 g/l (empty dots), and the clay was added at the first (square dots) and third day (triangle dots).

Iron concentration in this run was 462.85 ppm, and the sugar concentration was reduced to 66.4 g/l. In the other two runs that the pulp density was 60 g/l, removal extent was even lower (Table 2). Fig. 1 shows the gradual increase of iron concentration in cultured media, and suggests that in the first 15 days the rate of iron dissolution of the samples in which kaolin was added at the third day was higher. This rate for run 1 and run 3 is 15.9, and 18.1 g/l/day, and for run 2 and run 4 is 11.6, and 17 g/l/day, respectively. However, after 15 days, the dissolution rate of the samples that the clay was added to them at the beginning (run 1 and run 2), caught up with the run 3 and run 4, and even exceeded them. The main reason is that the microorganisms are passing the adaptation process during the first 15 days. After that, the adapted microorganisms can perform better, but in the run 3 and run 4, the activity decreases after the 15th day, since the strains are not adapted to the new environment.

Fig. 2 illustrates the decrease in pH value over 30 days. Looking at this figure, it can be deduced that at the first half of the experiment time, the pH values of run 3 and run 4, are lower than run 1 and run 2. This observation can justify the higher iron dissolution rate of the former runs in the first 15 days. Finally, the pH value of culture media decreased from 5.51 (pH of the medium) to 1.64 for run 1 and run 2, and 1.95 for 3 and 4, inasmuch as the organic acid (oxalic acid, citric acid, gluconic acid) exerted into the culture media (Cameselle et al., 2003). For iron, oxalic acid was five times more effective than citric acid, and is capable of complexing and reducing iron (Mulligan et al., 2004). In order to dissolve a mole of iron, three equivalents of oxalic acid are necessary (Cameselle et al., 1997).

$$3C_2O_4^{2-} + Fe^{3+} \rightarrow [Fe(C_2O_4)_3]^{3-}$$

The final oxalic and citric acid concentrations of the experiments are presented in Table 2. These results show

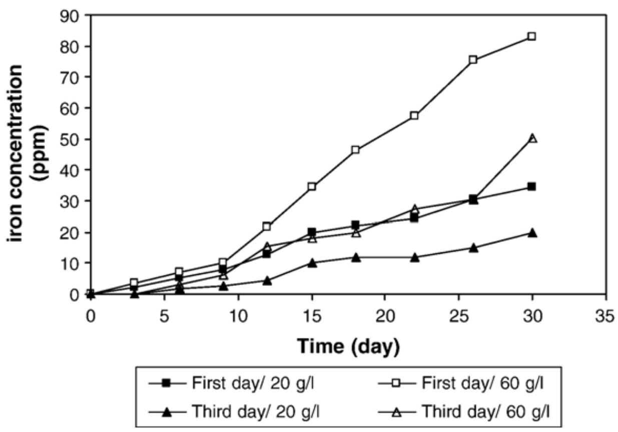

Fig. 3. Evolution of iron concentration in the cultured media of *A. niger* NCIM 548. Pulp densities were 20 g/l (full dots) and 60 g/l (empty dots), and the clay was added at the first (square dots) and third day (triangle dots).

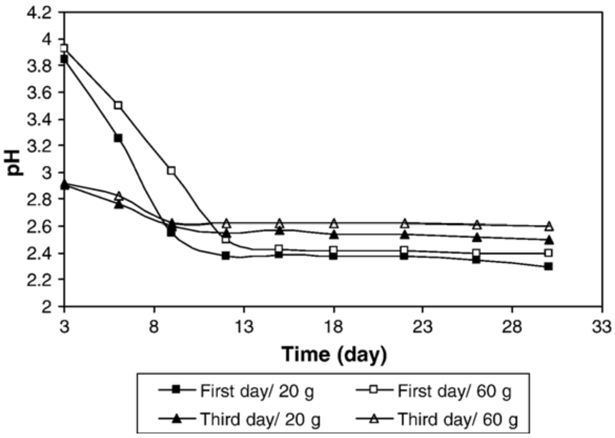

Fig. 4. Variation of pH with time for bioleaching by *A. niger* NCIM 548. Pulp densities were 20 g/l (full dots) and 60 g/l (empty dots), and the clay was added at the first (square dots) and third day (triangle dots).

that for those experiments in which the clay was added at the beginning of cultivation the average organic acid concentration (citric acid: 5.6 g/l, and oxalic acid: 4.54 g/l) is higher in comparison to those, where the clay was added at the third day (citric acid: 5.25 g/l, and oxalic acid: 2.87 g/l). These results correspond with the iron removal percentage that is higher for the run 1, and run 2. Furthermore, considering the results listed in Table 2, it can be found that the higher the particle loading, the lower the removal percentage of iron. The reason is that the culture media becomes highly viscous in response to increased loading of clay. It is suggested that increased viscosity represents a change in clay particle aggregation that decrease available surface area for organic compounds to be effective. It also, inhibits cells from making optimum contact with the clay mineral surface (Kostka et al., 1999).

At the end of cultivation, the biomass of all flasks was measured and each was 0.23, 0.20, 0.18, and 0.11 g for runs 1 to 4, respectively. It can be seen that for those experiments in which the clay was added in the beginning of cultivation, the produced biomass is also higher than the other.

Considering the iron removal percentage in Table 2, it can be said that this strain has a good ability to use for beneficiation of kaolin clay.

3.2. Leaching by *A. niger* NCIM 548

This strain did not show a good ability to remove iron efficiently from kaolin sample. Iron removed by this strain, was approximately 20 times lower than that removed by the strain isolated from pistachio shell. In the best condition, it only removed 2.2% of total iron of the clay. The best results attained when the pulp density was 20 and 60 g/l, and kaolin was added to the culture in the beginning of cultivation (Table 2).

The increase of iron concentration in this cultured media is presented in Fig. 3. The higher slope of the run 6 indicates the higher iron dissolution rate in this experiment. The reason is the higher pulp density, in consequence of addition of the higher amount of kaolin. Assuming that the particle size is constant, raising pulp density increases the contact surface between solid phase and microorganisms, and consequently accelerate the dissolution (Vaghar et al., 2000). Furthermore, the

Table 3
Analysis of variance table [partial sum of squares]

Source	Sum of squares	*df*	Mean of squares	*F* value	Prob > *F*
Model	3411.96	7	487.42	44.14	<0.0001
A	2134.44	1	2134.44	193.29	<0.0001
B	566.44	1	566.44	51.30	<0.0001
C	77.44	1	77.44	7.01	0.0293
AB	535.92	1	535.92	48.53	0.0001
AC	51.12	1	51.12	4.63	0.0636
BC	24.50	1	24.50	2.22	0.1747
ABC	22.09	1	22.09	2.00	0.1950
Pure error	88.34	8	11.04	–	–
Cor total	3500.30	15	–	–	–

Table 4
Coefficients of each variable and their confidence interval

Variable	Coefficient estimate	*df*	Standard error	α=0.05	
				High	Low
Intercept	13.16	1	0.83	15.08	11.25
A – fungus	−11.55	1	0.83	−9.63	−13.47
B – pulp density	−5.95	1	0.83	−4.03	−7.87
C – addition day	−2.20	1	0.83	−0.28	−4.12
AB	5.79	1	0.83	7.70	3.87
AC	1.79	1	0.83	3.70	−0.13
BC	1.24	1	0.83	3.15	−0.68
ABC	−1.18	1	0.83	0.74	−3.09

pH value of the flasks containing *A. niger* NCIM 548 was higher than *A. niger* isolated from pistachio shell. Also, referring to Table 2 it is clear that in the experiments with *A. niger* isolated from pistachio shell, average organic acid produced was higher (citric acid: 5.42 g/l, and oxalic acid: 3.71 g/l) than the others (citric acid: 2.22 g/l, and oxalic acid: 2.25 g/l). So, the lower acidity of *A. niger* NCIM 548 culture media, and consequently the lower iron dissolution by this strain is because of the lower production of organic acids which consequently caused lower dissolution of iron from clay. The pH value of the cultured media by this strain is illustrated in Fig. 4.

Finally, the biomass produced was 0.16 and 0.22 g for run 5 and run 6; also, 0.15 and 0.11 g of biomass was produced in run 6 and run 7, respectively.

3.3. Data analysis

As described before, experiments were done using a full factorial design (2^3), with three variables at two levels. Variables are fungal strain (*A*), pulp density (*B*), and the time of addition of kaolin (*C*). Also, lower levels are *A*=*A. niger* (pistachio shell), *B*=20 g/l, *C*=0 day, and upper levels are *A*=*A. niger* NCIM 548, *B*=60 g/l, *C*=3rd day. The removal percentage of iron content after 30 days was used as dependant variable called response (*R*).

Table 3 illustrates the results of analysis of variance, and indicates the significance of each variable. The Model *F*-value of 44.14 implies the model is significant. There is only a 0.01% chance that a “Model *F*-Value” of this large could occur due to noise. Values of “Prob>*F*” less than 0.0500 indicate model terms are significant. Referring to this table it can be concluded that all three main variables and the interaction between strain and pulp density is clearly significant, but the effect of time of clay addition has the least importance. Values greater than 0.10 indicate the model terms are not significant.

The effects of these three variables and their interactions can be determined by fitting experiment data to the function indicated in Eq. (1). Table 4 shows the coefficients of Eq. (1), and their statistical significance of these coefficients and equation itself.

Negative coefficients of *A*, *B*, and *C* imply that it is better to use *A. niger* isolated from pistachio shell; also,

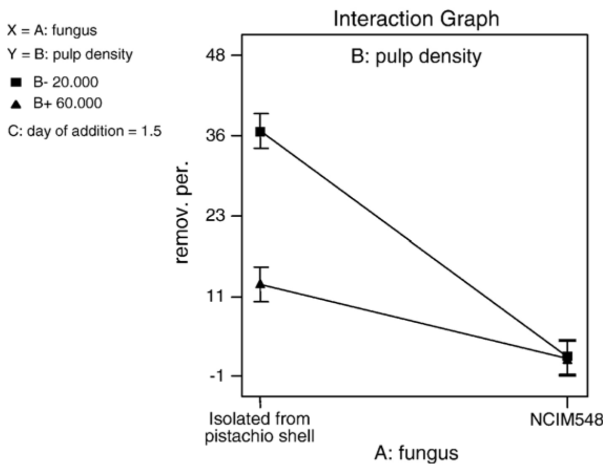

Fig. 5. Graph of interaction between fungus strain and pulp density.

set the pulp density at the lower amount (20 g/l), and add the clay in the beginning of cultivation, since the iron removal percentage is higher at the mentioned condition in all of the experiments. Also, considering the coefficients of *A*, and *C*, it is obvious that strain type has the most, and day of clay addition has the least importance among all the main variables.

Fig. 5 shows the interaction between fungus strain (*A*), and pulp density (*B*). Those points that have non-overlapping intervals are significantly different. In this case the spread of the points on the right side of the graph (where the fungus is *A. niger* NCIM 548) is smaller than the spread between the points at the left side of the graph (where fungus is *A. niger* isolated from pistachio shell). In other words, the effect of pulp density is less significant at the high level of fungus. Therefore, at the high level of fungus (NCIM 548) changing the pulp density does not change the removal percentage of iron.

The other variables are not significant, and do not have important influence on the response variable.

Final equation in terms of coded variables can be written as follows:

$$R = 13.16 - 11.55*A - 5.95*B - 2.20*C + 5.79*A*B + 1.79*A*C + 1.24*B*C - 1.18*A*B*C. \quad (1)$$

Also final equations in terms of actual variables are:
Strain: pistachio shell,

$$R = 57.00000 - 0.70750*(\text{pulp density}) - 5.87500 *(\text{day of addition}) + 0.08042*(\text{pulp density}) *(\text{day of addition}). \quad (2)$$

Strain: NCIM 548,

$$R = 2.47500 - 0.01125*(\text{pulp density}) - 0.35833 *(\text{day of addition}) + 2.08333E{-}003 *(\text{pulp density})*(\text{day of addition}). \quad (3)$$

4. Conclusion

The reduction of iron oxide contents of a highly contaminated kaolin clay was investigated using two different strains of *A. niger*. Also, statistical models for predicting the iron removal extent from the clay for both of the strains were presented. The bioleaching of iron oxides by *A. niger* isolated from pistachio shell resulted in removal of 42.8% of the total iron in the clay, and suggested that this strain has a good ability to remove iron impurities from kaolin. In comparison, the other strain, *A. niger* NCIM 548 did not show any promising result as in the best condition it only removed 2.2% of the iron contents of the clay.

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