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Bioleaching Performance of Titanium from Bauxite Residue Under a Continuous Mode Using *Penicillium Tricolor*

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Abstract
The present study performed a continuous mode of bioleaching to investigate the leaching efficiency of Titanium (Ti) from bauxite residue using *Penicillium Tricolor* at between 4% and 12% pulp densities during a 120-day running. Obtained results of the current study showed that increased pulp density led to a decrease in biomass, dissolved oxygen, and amount of leaching Ti as well as an increase in pH value. Further, it was found that efficiency of bioleaching can be enhanced by increasing the rate of aeration, retention time, and concentration of carbon source. However, it was also evident that, at high pulp density, excessive agitation did not give an expected leaching efficiency but a collapse of biomass. In addition, results of the present study showed that the maximum leaching amount of Ti was 3202 mg/L with a corresponding leaching ratio of 50.35% during the whole bioleaching process. Moreover, it was noted that the biomass showed a significant negative correlation with the pH value and dissolved oxygen. However, the biomass showed a significant positive correlation with leaching amount of Ti and thus indicate that microbial metabolic activities are the uppermost factor affecting the continuous leaching performance.

Keywords Bioleaching · Bauxite residue · Red mud · Titanium · Continuous leaching · Penicillium

Alumina refining of bauxite ores in Bayer or sintering processes leads to the generation of bauxite refinery residue which is a highly saline-alkaline solid or semi-solid waste, representing a main disposal problem in alumina industries. With the increasing global demand for alumina the storage volume of bauxite residue has reached over 4.0 billion tonnes and still rapidly increases with an annual rate of 120 million tonnes according to the latest reports (Xue et al. 2016, 2022). Therefore, there is an urgent need to develop safe and effective methods for recycling bauxite residue to decrease the huge storage amount.

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Bauxite residue is believed as ''a kind of artificial ore or polymetallic raw material with a complex content of oxides of aluminum, iron, titanium, silicon, and other valuable components, such as scandium, uranium, and thorium" in a metallurgy view (Klauber et al. 2011; Qu and Lian 2013). Therefore, it is highly desirable to extract and recover valuable metals from bauxite residue. Among these valuable metals, titanium (Ti), a kind of strong, light, bio-inert, and anticorrosion metal, is particularly worth recycling due to its high concentration in bauxite residue (Liu and Naidu 2014) and a high transaction value on the international markets (Borra et al. 2016; Klauber et al. 2011; Vakilchap et al. 2016). Although titanium commonly presents in minerals of Anatase, Perovskite, and Rutile with a stable form leading to expensive as well as complex recovery procedures, the advantages certainly outweigh the expense.

The extraction of titanium from bauxite residue has been widely reported using traditional hydrometallurgical and pyrometallurgical methods hence showing inevitable disadvantages such as high energy requirement, high investment, and operating cost, potential for environmental pollution as well as usage of hazardous chemical during the treatment (Borra et al. 2016; Klauber et al. 2011; Liu and Naidu 2014; Liu and Li 2015; Xue et al. 2016). By contrast, bioleaching approach is generally considered to be a ''green technology'' for the extraction of metals from solid materials in recent years. The approach offers many attractive features such as low energy requirement and cost, environmental benignity, operational flexibility, and simplicity even by unskilled workers (Borra et al. 2016; Pedram et al. 2020; Urík et al. 2017). Moreover, the approaches have been explored and are focused on the extraction of Al, Fe, and rare earth elements from bauxite residue (Pedram et al. 2020; Qu et al. 2019a, 2019b; Urik et al. 2015). However, it has been noted that only a few studies have addressed bioleaching of titanium (Pedram et al. 2020; Vakilchap et al. 2016).

Although bioleaching has been indicated to have the extraction potential for valuable metals from bauxite residue, most studies have only focused on examination of influencing parameters and mechanism through a batch bioleaching mode in lab-scale experiments (Qu et al. 2019a, 2019b, 2013; Qu and Lian 2013). There is limited research with respect to scale-up of bioleaching process under a continuous mode. Continuous bioleaching can be applied at an industrial scale as compared to batch bioleaching because it offer many advantages (Borja et al. 2019; Chen et al. 2021; Lotfalian et al. 2015; You et al. 2020) including: (1) high metabolic activities of bioleaching strains maintained in a logarithmic phase; (2) steady supply of energy sources and nutrients for the strains to achieve the fastest bioleaching rate; (3) high mixing and suspension system leading to a high mass transfer; (4) high efficiency of metal recovery under high solids loading or pulp densities. Therefore, there is urgent need to explore the bioleaching process operated in continuous mode for the treatment of huge quantity of bauxite residue and recovery of valuable metals.

To the best of our knowledge the present study is the first to evaluate the bioleaching performance of Titanium from bauxite residue in a continuous leaching mode. The leaching amount of Titanium, biomass, pH value, and dissolved oxygen were monitored under different operation conditions at between 4% and 12% pulp density in a continuous stirred-tank reactor (CSTR) using *Penicillium* as the leaching strain during a 120-day running.

Materials and Methods

The bauxite residue samples used in the present experiments were obtained from Zhongzhou Aluminum Corporation in Henan province, China (35°23'N, 113°23'E). The samples were fresh bauxite residue collected from the outlet of discharge pipe. They were transported to the laboratory, dried to constant weight in an oven at 80°C, and gently crushed as well as powdered to 200 mesh using a pestle. The RM powder was autoclaved at 121°C for 15 min prior to use.

The applied bioleaching strain (RM-10) was isolated from a bauxite residue depository of Bayer process and identified as *Penicillium Tricolor* (Qu and Lian 2013). It showed a favorable bioleaching performance of rare earth and radioactive elements from bauxite residue in the batch leaching test of our previous study (Qu and Lian 2013). The strain RM-10 was preserved on a 3.9% (w/v) potato dextrose agar (PDA) slant in a freezer. A 2 mL portion of the spore suspension was added to 100 mL of sterilized sucrose medium in a 250 mL Erlenmeyer flask before inoculation and incubated at 30℃ as well as at 120 rpm on a shaker for strain activization. The sucrose medium composition was 100, 0.5, 0.5, 2.0, and 2.0 g/L of sucrose, KNO_3, KH_2PO_4, yeast extract, and peptone, respectively. Sterilization was finally conducted in an autoclave at 121℃ for 15 min.

In the current study the acclimation and continuous bioleaching tests were carried out in a CSTR used in our previous study (Qu et al. 2015). The reactor was round-bottomed glass tank with height and diameter of 35 and 30 cm, respectively, equipped with an air distributor, a temperature controller, a pH and Dissolved Oxygen (DO) detector, as well as a mechanical stirring device mounted on a rotating shaft. Further, the feed was made up of bauxite residue slurry and sucrose medium solution. Moreover, it was stored in a 10-L reservoir connected to the reactor by a peristaltic pump and air was continuously injected into the liquid medium through the air distributor at the bottom of reactor.

Cell multiplication and acclimation were performed in a batch mode in the CSTR under conditions such as temperature of 30°C, agitation speed of 150 rpm, and aeration rate of 180 L/h. Further, a sucrose medium was used as the feed solution for microbial growth and acclimation, initially supplemented with bauxite residue (1% pulp density) as well as fungal culture solution (100 mL). In addition, the acclimation of fungal culture to higher pulp densities was performed through serial sub-culturing in the logarithmical phase of growth and by gradually increasing the pulp density from 1% to 8%. Lastly, each transfer during the acclimation process was made when the pH value was lower than 3.0.

Bioleaching tests were performed in a continuous mode in the CSTR. When the feed was continuously flowed into the reactor using a peristaltic pump, an equal volume of slurry was withdrawn at the exit. The initial operating conditions were set according to acclimation study and the available literature (Qu et al. 2015, 2019a, 2019b; Qu and Lian 2013; You et al. 2020). The parameters of residence time, agitation speed, aeration rate, and sucrose concentration were adjusted at the controlling pulp density of 4%, 8%, and 12%,

Table 1 Operating conditions during the continuous bioleaching tests

	Pulp density (w/w) (%)	Residence time	Air flow rate (L/h)	Agitation speed (rpm)	Sucrose concentration (g/L)
Phase 1					
Test 1	4	6	180	150	100
Test 2	4	3	180	150	100
Test 3	4	3	240	150	100
Phase 2					
Test 4	8	3	240	150	100
Test 5	8	3	240	300	100
Test 6	8	6	240	300	100
Phase 3					
Test 7	12	6	240	300	100
Test 8	12	6	300	300	100
Test 9	12	6	300	450	100
Test 10	12	9	300	300	100
Test 11	12	6	300	300	200

respectively. The detailed operating conditions are shown in Table 1.

Samples were periodically collected from the reactor for monitoring the pH, biomass, and concentration of metal ions. They were centrifuged at 5000 rps for 10 min and then filtered through a filter membrane. The pH value of bioleached filtrate was measured using a pH meter (PHS-3C). Concentration of Titanium ions was analyzed using an inductively coupled plasma mass spectroscopy (ICP-MS, Agilent 7800). The bioleaching efficiency of titanium was calculated from the mass balance considering the concentrations of input and output. The biomass was determined by the weight difference of residue collected from the filter paper between drying at 80℃ for 24 h and ashing at 500℃ for 4 h. The detailed information of analytical methods was as shown in our previous studies (Qu et al. 2015, 2019b).

Results and Discussion

The basic physicochemical characteristics and elemental composition of bauxite residue used in the present study were as shown in Table 2. The content of Ti in the samples of bauxite residue was 5.30%, which is higher than most the worldwide bauxite residue (0.98%–5.34%) (Liu and Naidu 2014). The high concentration of Ti in the samples of bauxite residue indicates that it is certainly worth a recovery. However, Ti is always present in a crystalline phase of anatase, rutile, and Perovskite coexisting with other minerals in a stable form, which is not easily bioleached by fungi (Gräfe et al. 2011; Liu and Naidu 2014; Pedram et al. 2020; Vakilchap et al. 2016). Furthermore, the high pH (11.35), EC (7.19 mS/cm), and ANC (3.12 mmol H^+/g) of the bauxite residue samples indicate a hostile habitat for the leaching strain and subsequently low microbial acidolysis and complexolysis under the bioleaching process. For the purpose of efficient leaching of Ti, the continuous bioleaching tests offering advantages of high microbial metabolism and solids loading were performed in the current study.

Results of the current study found that bauxite residue exhibit high levels of toxicity against microorganisms due to its high alkalinity, salinity, and metal toxicity (Dubey and Dubey 2011; Santini et al. 2015). Therefore, for the sake of pre-acclimation of *Penicillium Tricolor* in CSTR and prevention of washout at the beginning of continuous bioleaching, the serial sub-culturing of the leaching strain was performed by increasing the bauxite residue pulp density from 1% to 8%. It was found that at 1% pulp density, the pH value decreased to below 3.0 and the biomass increased to 26.48 mg/L after 72 h of cultivation. However, an increase in pulp density resulted in a prolongation of the acclimation phase. At 8% pulp density, it was noted that the pH value did not decrease to below 3.0 and the maximum biomass was only 8.13 mg/L even after 336-h cultivation. This indicates that 8% may be the threshold restricting the normal metabolic activity of *Penicillium Tricolor* under the batch bioleaching mode in CSTR. Therefore, to start smoothly, a

Table 2 Characteristics of bauxite residue used in the continuous bioleaching

pH	EC (mS/cm)	ANC (mmol H^+/g)	Ti (%)	Si (%)	Fe (%)	Al (%)	Ca (%)
11.35	7.19	3.12	5.30	17.20	15.73	20.88	14.15

lower pulp density of 4% was chosen as the initial value to perform the continuous bioleaching mode.

Pulp density is a crucial parameter which determines the overall performance of fungal bioleaching system (Hugues et al. 2002). The bauxite residue contains no energy source for fungal growth but possibly shows completely negative effects on bioleaching (Borra et al. 2016; Xue et al. 2016). Higher pulp densities of bauxite residue always give the lower leaching ratios of Al, Ti, Fe, V, and rare earth elements (Pedram et al. 2020; Qu and Lian 2013; Vakilchap et al. 2016). This is in comparison with sulphide ores that contains energy sources for the growth of autotrophic *Thiobacillus* and thus an appropriately higher pulp density is beneficial to efficiency of bioleaching (Bosecker 1997). However, higher pulp densities may provide sufficiently available react sites of minerals for the microbial-acidolysis reaction. This results in a high extraction amounts of metals in the bioleaching system and is conducive to subsequent recovery processes.

Therefore, the continuous bioleaching tests were performed under 3 phases with increasing pulp densities of 4%, 8%, and 12%, respectively. The operating parameters were adjusted to optimize the efficiency for the bioleaching of Ti at the controlling pulp density under each phase.

The biomass indicates the level of metabolic activity in CSTR during the continuous bioleaching process. To assess the impact of pulp density, residence time, agitation speed, aeration rate, and sucrose concentration on bioleaching performance and prevent the microbial washout, the biomass was closely monitored. Moreover, the variation of biomass during the continuous bioleaching experiments was as depicted in Fig. 1.

After a decrease in pulp density from 8% to 4% at the beginning of the continuous test, it was found that the biomass increased from 18.2 to 23.5 mg/L during the first 7 days and then remained relatively stable in test 1. This meant that the transition from batch acclimation phase to continuous bioleaching phase was successfully achieved and the biomass was accumulated. However, the biomass decreased from 25.9 to 14.6 mg/L and from 24.4 to 16.5 mg/L in the first 2 days of phase 2 (the pulp density increased from 4% to 8%) and phase 3 (the pulp density increased from 4% to 8%), respectively. Though the biomass was recovered after the shocks of higher pulp density, the maximum biomass was lower as compared with that at lower pulp densities. This is an indication that increasing pulp densities causes a negative effect on the growth of leaching strain.

Further, results of the present study found that the decreased residence time from 6 to 3 days resulted in a decrease in biomass from 22.8 to 17.4 mg/L within the first 4 days and then a slow increase to 20.7 mg/L during the next 6 days in test 2. This indicates that due to maintenance of logarithmic phase of leaching strain, an appropriate shortening of residence time is probably acceptable during the continuous leaching mode at low pulp density.

Moreover, it was noted that an increase in residence time from 3 to 6 days and 6 to 9 days resulted in an increasing biomass from 14.7 to 24.8 mg/L and 7.0 to 21.6 mg/L in test 4 and test 10 at 8% and 12% pulp densities, respectively. This suggests that when biomass is decreasing with a tendency of collapse at high pulp density (12% in test 10), the prolonged residence time can hence serve to recover the continuous leaching system.

It has been found that proper mixing is a prerequisite to achieve a sufficient solid–liquid mass transfer of metals in heterogeneous leaching system (Chen et al. 2021). However, the data obtained in the current study showed that the increased speed of agitation from 150 to 300 rpm led to a decreased biomass from 19.4 to 14.7 mg/L in test 5 at 8% pulp density. In addition, a higher agitation speed did not give the expected higher biomass, though a slight increase in leaching amounts of Ti was presented (Fig. 3). Furthermore, at 12% pulp density, an increase in speed of agitation from 300 to 450 rpm resulted in collapse of biomass from 18.6 to 7.1 mg/L in test 9. This indicates that an excessive hydrodynamic turbulence caused from agitation combined with high pulp densities is probably a stress factor for microbial

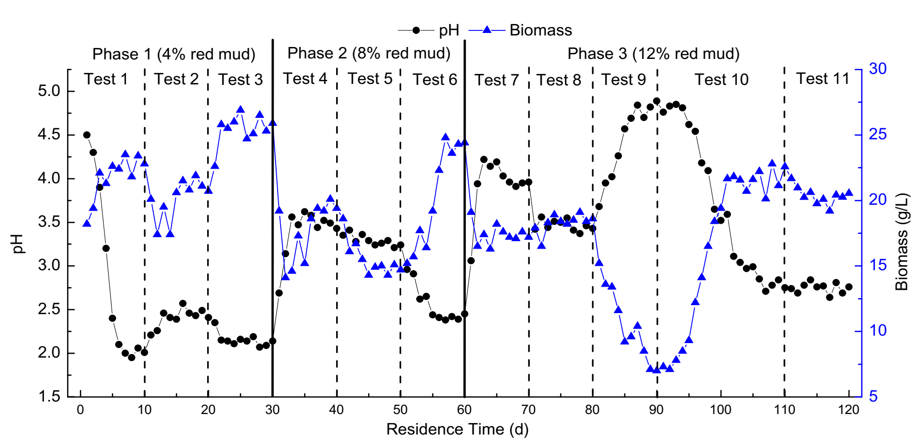


Fig. 1 Variation of biomass and pH value as a function of time during the continuous bioleaching experiments. The dashed vertical lines mark the 11 operating conditions and the bold vertical lines mark the transitions from Phase 1 to 2 and Phase 2–3, respectively

growth. Furthermore, this result is consistent with the previous report of a study that used bacteria *Sulfolobus* and *Acidianus infernus* to leach chalcopyrite (Hugues et al. 2002). It was also evident that a high mechanical force significantly limit the microbial productivity by damaging microbial cell wall and restraining growth pathways during continuous bioleaching (You et al. 2020).

However, after the collapse, biomass was recovered from 7.0 to 21.8 mg/L when the residence time and sucrose concentration were simultaneously increased from 6 to 9 days and 100–200 mg/L, respectively as well as decreased the agitation speed (450–300 rpm) during the first 12 days and then kept relatively constant in test 10. Although there was a slight decrease in biomass when the residence time was decreased from 9 to 6 days in test 11, it was found that the maximum biomass was still higher than that in test 7 and 8 under 12% pulp density. This indicated that high carbon resource is beneficial to the maintenance of biomass under high pulp density. It has been previously reported that high rates of organic carbon dose can compensate microbes for the energetic expenses of maintaining homeostasis and essential cellular functions at high pH as well as salinity and hence supporting a rapid neutralization of pH value of bauxite residue (Santini et al. 2016).

Theoretically, the pH value determines the rate and extent of acidolysis, which is the most important mechanism

Table 3 Correlation coefficients between running parameters

	Ti	pH	Biomass	DO
Ti		−0.084	0.203*	−0.019
pH	−0.084		−0.831**	0.096
Biomass	0.203*	−0.831**		−0.244**
DO	−0.019	0.096	−0.244**	

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

involved in bioleaching of metals using heterotrophic microbes. The surface of metal compounds covered by oxygen atoms are rapidly protoned and thus the metal and water combining with the protons and oxygen are separated from the surface of metal compound (Burgstaller and Schinner 1993; Qu et al. 2013).

The variation of pH value as a function of time was as shown in Fig. 1. Results of the present study showed that pH value had a significant negative correlation with biomass (Pearson's coefficient = −0.831, $p < 0.001$ in Table 3) throughout the continuous bioleaching process. This indicates that metabolic activity is the uppermost factor affecting the pH value. Further, according to our previous study, it has been reported that the main organic acids secreted by *Penicillium Tricolor* are oxalic, citric, and gluconic acids in a bauxite residue habitat (Qu and Lian 2013). However, it was found that the sole exception to this negative correlation was in test 5. Results of the present study found that an increase in agitation speed from 150 to 300 rpm led to a decrease in pH value from 3.43 to 3.21 along with a decrease in biomass from 19.4 to 14.3 mg/L. This indicates that a temperate agitation may stimulate production of organic acids though a decrease in the total biomass (Qu and Lian 2013; Urík et al. 2017). In addition to the organic acids, the pH value can also decrease through the carbonatation caused by microbial respiration and aeration (Santini and Banning 2016; Santini et al. 2015).

Dissolved oxygen is a key substrate for cultivation of the cells of leaching strain (You et al. 2020). The variation of DO during the continuous bioleaching experiments was as shown in Fig. 2. It was also found that increasing pulp density from 4% to 8% and 8%–12% resulted in a decrease in DO from 2.58 to 1.85 mg/L and 2.63–1.35 mg/L, respectively. This indicate that increased pulp densities increases the mass transfer resistance in the continuous bioleaching system. However, this challenge can be solved by an increased aeration rate. This is because rate of aeration is

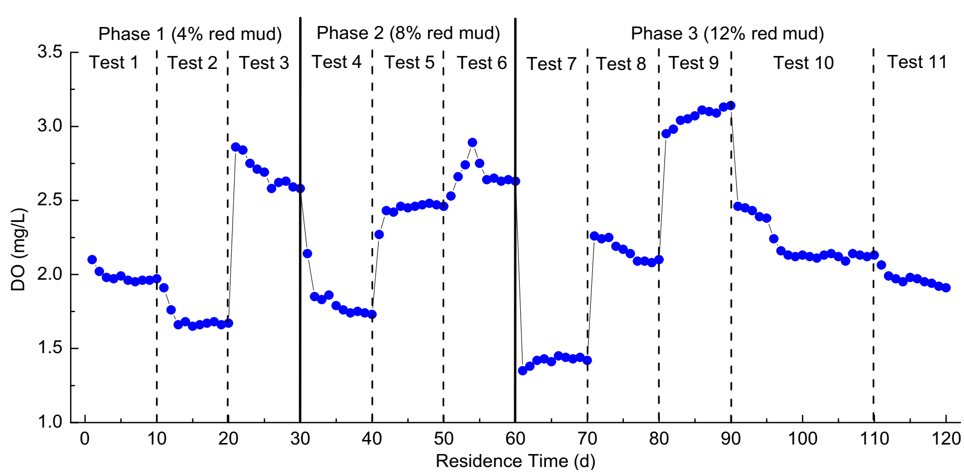


Fig. 2 Variation of DO value as a function of time during the continuous bioleaching experiments. The dashed vertical lines mark the 11 operating conditions and the bold vertical lines mark the transitions from Phase 1 to 2 and Phase 2–3, respectively

crucial to provision of the free form of dissolved oxygen (You et al. 2020). Results of the current study found that increasing the rate of aeration from 180 to 240 L/h and from 240 to 300 L/h led to an immediate increase in DO from 1.67 to 2.86 mg/L and 1.42–2.26 mg/L in test 3 and 8, respectively.

In the present study, results showed that biomass had a significant negative correlation with DO (Pearsons coefficient = −0.244, $p < 0.01$ in Table 3). It was evident that higher biomass consumed more oxygen to proceed catabolism resulting in a decrease in DO under the stable bioleaching condition. According to different previous studies the limiting concentration of dissolved oxygen with respect to optimal growth of most of biomining microorganisms is estimated at above 2.0 mg/L (Borja et al. 2019; Hugues et al. 2002). This indicates that there was still room for the further increase of biomass through elevating the rate of aeration under such tests which did not satisfy the minimum requirement of oxygen.

Figure 3 shows the leaching efficiency of Ti during the continuous bioleaching experiments. After the decrease in Ti concentration from 1858 to 1207 mg/L during the first 4 days acclimation, the continuous bioleaching system tended to be stable and the concentration of Ti gradually increased to 1311 mg/L in the remaining time in test 1. Further, the leaching amount of Ti immediately decreased from 1284 to 719 mg/L and from 1986 to 1524 mg/L with an increase in pulp density from 4% to 8% and then to 12% in test 4 and 7, respectively. This indicates that higher pulp density shows a completely negative effect on bioleaching system due to increased toxicity of bauxite residue imposed on leaching strains (Borra et al. 2016; Pedram et al. 2020; Vakilchap et al. 2016). However, this observation is on contrary to sulphide ores which show a positive effect on bioleaching with modestly increasing pulp density (Bosecker 1997; Krebs et al. 1997; Natarajan 2015).

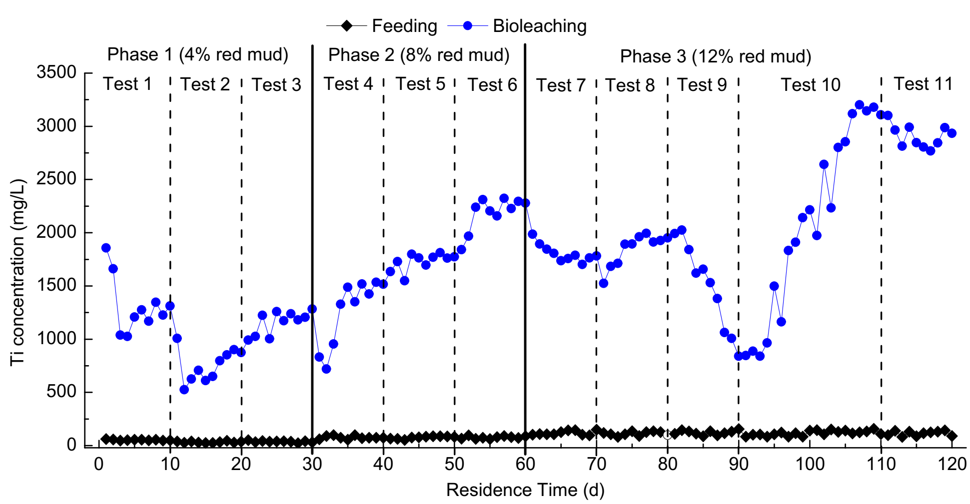
Nevertheless, the leaching efficiency in the present study was improved by optimizing the operating conditions. The increase in aeration rate (180–240 L/h) under phase 1, increase in retention time (3–6 days), and agitation speed (150–300 rpm) under phase 2, as well as increase in retention time (6–9 days), aeration rate (240–300 L/h), and carbon source concentration (100–200 mg/L) under phase 3, respectively, led to an increase in continuous bioleaching efficiency.

It has been previously shown that both liquid film diffusion and chemical reaction control the leaching of Ti from bauxite residue (Pedram et al. 2020). The result of the current study corroborates this fitting kinetic model. Therefore, from this view, the reaction rate of bioleaching can be enhanced through an increase in rate of aeration and speed of agitation to reduce the liquid film on the surface of bauxite residue particles, or/and through an increase in carbon source input to increase the number of reaction sites generated from increase in organic acids.

On the contrary to the expectations the leaching amount of Ti showed an insignificant correlation with pH value, but a significant correlation with biomass (Pearson coefficient = −0.203, $p < 0.01$ in Table 3). Therefore, results of the current study indicate that acidolysis may not be the only mechanism involved in bioleaching of metals from bauxite residue. However, bioaccumulation, bio-sorption, and biostimulation requiring the sufficient contact between microbial cells and bauxite residue particles may also play key roles during the continuous bioleaching process (Burgstaller and Schinner 1993; Pedram et al. 2020; Rezza et al. 2001).

In conclusion, it is evident that the highest leaching amount of Ti is 1347, 2294, and 3202 mg/L with a corresponding leaching ratio of 63.54, 54.10% and 50.35% at 4, 8, and 12% pulp densities, respectively. Previously, it has been reported that under a batch leaching mode, the optimum leaching ratio of Ti is 60% at 2% pulp density (Vakilchap

Fig. 3 Variation of Ti concentration as a function of time during the continuous bioleaching experiments. The dashed vertical lines mark the 11 operating conditions and the bold vertical lines mark the transitions from Phase 1 to 2 and Phase 2–3, respectively



et al. 2016). Therefore, the current study shows that continuous bioleaching mode has a more favorable efficiency for leaching of Ti especially at the high pulp density of bauxite residue as compare with batch mode.

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