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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Investigation of Bioleaching Kinetics of Valuable Metals in Boron Waste

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Abstract: This study utilized boron waste containing significant amounts of valuable metals as a secondary resource for metal recovery. Critical metals-lithium (Li), rubidium (Rb), and cesium (Cs)-identified in the boron waste were extracted via bioleaching using the fungus *Aspergillus niger* (*A. niger*). Biological methods such as bioleaching are considered low-cost and environmentally friendly and are particularly effective for metal recovery from secondary resources due to their efficiency in processing low-grade materials. Under the optimal conditions-31 days of incubation in a growth medium with *A. niger* using a shaking incubator at 30 °C and 125 rpm-bioleaching efficiencies of 78.67 % for Li, 37.44 % for Rb, and 30.47 % for Cs were achieved.
To identify the rate-controlling mechanisms during bioleaching, two kinetic models based on the Shrinking Core Model (SCM) were applied. The kinetic analysis revealed that the leaching of all three metals was primarily governed by surface chemical reactions rather than diffusion through a product layer, as indicated by higher correlation coefficients. The presence of two kinetic stages for Li suggests an initial low-reactivity period followed by an accelerated dissolution stage as the reaction progressed. The rate constants (k) for the chemical control model were 0.0019-0.0238 day^{-1} for Li, 0.0042 day^{-1} for Rb, and 0.0034 day^{-1} for Cs, indicating that Li reached higher rate constants and exhibited the highest apparent reactivity among the studied alkali metals.

Keywords: Bioleaching, boron waste, critical metal, shrinking core model.

Bor Atıklarındaki Değerli Metallerin Biyoliç Kinetiğinin Incelenmesi

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Öz: Bu çalışmada, değerli metaller içeren bor atığı, metal geri kazanımı için ikincil bir kaynak olarak kullanılmıştır. Bor atığında tespit edilen lityum (Li), rubidyum (Rb) ve sezyum (Cs) gibi kritik metaller, *Aspergillus niger* (*A. niger*) fungusu kullanılarak biyoliç yöntemiyle ekstrakte edilmiştir. Biyoliç gibi biyolojik yöntemler düşük maliyetli ve çevre dostu olarak Kabul edilir ve düşük tenörlü malzemelerin işlenmesindeki etkinlikleri sayesinde ikincil kaynaklardan metal geri kazanımında oldukça etkilidir. Optimal koşullar altında - 30°C sıcaklıkta ve 125 rpm'de çalkalamalı bir inkübatörde, büyüme besiyerinde *A. niger* ile 31 günlük inkübasyon sonucu – Li için %78,67, Rb için %37,44 ve Cs için %30,47 oranında biyoliç verimliliği elde edilmiştir.
Biyoliç sırasında hızı kontrol eden mekanizmaları belirlemek için, Küçülen Çekirdek Modeli'ne (KÇM) dayanan iki kinetik model uygulanmıştır. Kinetik analiz, incelenen üç metalin liçinin, daha yüksek korelasyon katsayılarıyla da gösterildiği üzere, bir ürün tabakasından difüzyondan ziyade öncelikli olarak yüzey kimyasal reaksiyonları tarafından kontrol edildiğini ortaya koymuştur. Li için iki kinetik aşamanın varlığı, başlangıçta düşük reaktiviteye sahip bir sürecin, reaksiyon ilerledikçe hızlanan bir çözünme aşaması olarak ilerlediğini göstermektedir. Kimyasal kontrol modeli için hız sabitleri (k) Li için 0,0019–0,0238 gün^{-1}, Rb için 0,0042 gün^{-1} ve Cs için 0,0034 gün^{-1} olarak belirlenmiş olup, bu değerler lityumun daha yüksek hız sabitine ulaştığını ve çalışılan alkali metaller arasında en yüksek görünür reaktiviteyi sergilediğini göstermektedir.

Anahtar kelimeler: Biyoliç, bor atığı, kritik metal, küçülen çekirdek modeli.

INTRODUCTION

Driven by the global energy transition and rapid technological advancements, lithium, rubidium, and cesium have emerged as critical elements in modern technology and industry (Tian et al., 2018). Lithium's central role in the modern energy industry—highlighted by the 2019 Nobel Prize in Chemistry—ensures its sustained global demand (Chaves et al., 2021). Meanwhile, rubidium and cesium are gaining importance in high-tech applications such as aerospace (Saliba et al., 2016), quantum computing (Xing et al., 2021), and biomedicine (Naidu et al., 2017) due to their unique physicochemical properties. Securing the supply chains of these strategic elements through improved extraction and recycling technologies has therefore become both an economic and strategic priority.

Commercially, lithium (Li) is primarily obtained from two sources: lithium-rich natural brines and pegmatitic minerals such as spodumene, petalite, lepidolite, and zinwaldite (Demirbaş, 1998). In contrast, rubidium and cesium are rarely found as major constituents of their own minerals and are typically recovered as by-products during the processing of lithium-bearing ores (Butterman & Reese, 2003). A major challenge in extracting Li, Rb, and Cs from brines lies in their low concentrations and the complexity of separating them from chemically similar elements. Conventional lithium extraction from continental brines is highly resource-intensive, requiring up to two years of evaporation and nearly 800 cubic meters of water per ton of lithium carbonate. Geothermal brines, though representing a small fraction of global reserves, have limited commercial utilization, and lithium extraction from seawater remains experimental due to its extremely low concentration (Yang et al., 2018). For example, although most of the world's rubidium resources occur in saline lake brines, their concentrations are typically below 100 mgL^{-1}, making recovery difficult. Moreover, rubidium and cesium often coexist with lithium and sodium in brines and geothermal waters, complicating their separation using conventional techniques such as fractional precipitation, ion exchange, and solvent extraction (Bin et al., 2008; Shan et al., 2013). Extraction of these elements from lepidolite (Yan et al., 2012) and boron waste (Ertan, 2020) has been achieved through chlorination roasting followed by water leaching. However, recycling spent lithium-ion batteries (LIBs)—a crucial secondary resource—using conventional pyrometallurgical and hydrometallurgical methods remains energy-intensive. These processes typically require high roasting temperatures (Su et al., 2020; Swain, 2017; Liu et al., 2022) or involve hazardous chemicals such as hydrofluoric acid (Guo et al., 2021). Pyrometallurgical methods incur high operational costs due to extreme energy consumption and gas emissions, while hydrometallurgical routes generate significant waste management challenges (Bharadwaj & Ting, 2013). Since both are poorly suited for low-grade materials, the development of innovative and environmentally friendly recovery technologies is essential (Ertan, 2023).

Bioleaching, a biohydrometallurgical process that employs microorganisms to dissolve and recover metals from ores or industrial wastes, presents a sustainable alternative. It operates at low temperatures with minimal energy input, produces fewer harmful emissions and reduces overall operational costs (Baniasadi et al, 2019).

Historically, bioleaching has been observed as a natural process for millennia, with evidence of its use in ancient copper extraction. Modern scientific understanding later revealed that microorganisms are the primary drivers of this phenomenon (Colmer & Hinkle, 1947). Bioleaching is an established industrial technology primarily employed for processing low-grade ores, typically with metal concentrations below 0.5%. Today, bioleaching is an established industrial technology, primarily applied to the recovery of metals such as copper, uranium, and gold from low-grade ores (Bosecker, 1997; MacGregor, 1966). In recent years, bioleaching has also been explored for the recovery of rare earth elements from various industrial wastes, including phosphogypsum, red mud, e-waste, coal fly ash, wastewater, fluorescent lamps, and cracking catalysts (Joshi et al., 2025). However, its potential for extracting lithium, rubidium, and cesium remains largely unexplored. Studies have demonstrated the feasibility of bioleaching lithium from silicate minerals using a variety of microorganisms. For example, fungi such as *Penicillium purpurogenum* and *Aspergillus niger* have been applied to spodumene (Rezza et al., 2001), while autotrophic bacteria have enhanced lithium recovery from zinnwaldite from 11% to 26% in a bioreactor (Reichel et al., 2017). A microbial consortium has also achieved limited success with lepidolite, extracting 60 µgL^{-1} of Li (Marcinčáková et al., 2015). Expanding on these findings, a recent study employed *Bacillus licheniformis* for the simultaneous recovery of Li, Rb, and Cs from boron waste, with process optimization achieved through response surface methodology and Box-Behnken design (Ertan, 2025). Despite these promising results, a notable limitation in the current bioleaching literature is the lack of detailed kinetic investigations (Faraji et al., 2018). While many studies empirically demonstrate metal extraction, the fundamental rate-controlling mechanisms remain poorly understood, hindering predictive modeling, process optimization, and industrial-scale application.

MATERIAL AND METHOD

Materials: The boron waste sample was collected from the -25 mm solid waste of the Eti Mine Boric Acid Operation's Espey concentrator in Kütahya Emet, following standard sampling procedures from different points. The samples were spread on a flat surface to dry, homogenized, and reduced using a sample splitter. The samples were dried at 110°C for 48 hours, followed by successive reduction steps through coning-quartering and splitting. The reduced samples were pulverized in a ring mill and passed through a 38-µm sieve to prepare them for analysis.

The *A. niger* strain used in this study was obtained from the Department of Molecular Biology and Genetics at Erzurum Technical University, Turkey. The culture was maintained on potato dextrose agar (PDA) in Petri dishes, with monthly subculturing performed by incubating at 30 °C for 10 days to preserve viability.

For the experiments, a preculture was prepared by incubating *A. niger* in a liquid medium containing (per liter): KH_2PO_4 (0.5 g), $MgSO_4{\cdot}7H_2O$ (0.025 g), KCl (0.025 g), $NaNO_3$ (1.5 g), yeast extract (1.6 g), and sucrose (100 g). Incubation was carried out in an incubator shaker (ZHWY-211D) at 30 °C and 125 rpm for 7 days. Prior to inoculation, all media, boron waste, and containers were sterilized by autoclaving at 121 °C and 1 atm for 15 minutes.

Methods: To fully characterize the boron waste, a multi-technique approach was employed: X-ray fluorescence (XRF) for bulk chemical composition, X-ray diffraction (XRD) for mineral phase identification, and inductively coupled plasma mass spectrometry (ICP-MS) with a Thermo Scientific iCAP RQ (serial number: ICAPRQ01205) with the following settings: nebulizer flow: 0.9950 $Lmin^{-1}$ (Argon), spray chamber temp.: 2.7 °C, peristaltic pump rate: 40 rpm, extraction lens voltage: −122 volt, plasma power: 1550 watt, and plasma gas flow: 14.8 $Lmin^{-1}$ (argon) for quantifying the critical metals lithium, rubidium, and cesium.

The bioleaching experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of medium inoculated with 2.5 mL of spore suspension and 1 g of boron waste (BW). The flasks were incubated at 30 °C with continuous shaking at 125 rpm. At predetermined time intervals (4, 10, 13, 18, 24 and 31 days), samples were withdrawn from the culture and subjected to centrifugation to separate the biomass. The resulting supernatant was then filtered through a 0.45-μm membrane filter, and the filtered leachate was stabilized by dilution in 0.65% nitric acid prior to measurement.

Metal recovery was calculated by the following equation, as shown in Equation 1

$$\%Metal\ recovery = \frac{Cs \times Vs}{Cf \times Mf} \times 100 \qquad (1)$$

where C_s (mgL^{-1}) is the concentration of metal in the leachate, V_s (L) is the volume of the leachate, C_f ($mgkg^{-1}$) is initial concentration of metal in SW, and M_f is the mass of the raw material (kg) (Bahaloo-Horeh & Mousavi, 2017).

Statistical Analysis: Statistical analyses were conducted using Minitab®22 Statistical Software. One-way ANOVA followed by Tukey's post-hoc test was applied to evaluate the significant of differences between time intervals at a 95% confidence level.

RESULTS AND DISCUSSION

Characterization of Boron Waste: XRD analysis identified the primary crystalline phases in the boron waste (BW) as colemanite ($2CaO{\cdot}3B_2O_3{\cdot}5H_2O$), muscovite ($KAl_3Si_3O_{10}(OH)_2$), quartz ($SiO_2$), and dolomite ($CaMg(CO_3)_2$). This mineralogical composition was consistent with the major elemental constituents (Si, Al, Ca, Mg) determined by chemical analysis. Based on the XRF esults, the boron waste primarily consisted of SiO_2 (28.7%), B_2O_3 (19.1%), CaO (8.80%), Al_2O_3 (8.43%), MgO (7.92%), and Fe_2O_3 (4.64%), with lower concentrations of K_2O (2.98%) and other oxides. ICP-MS analysis further revealed significant concentrations of critical metals. The lithium, rubidium, and cesium contents are given in Table 1.

Table 1. ICP-MS analysis of Li, Rb, and Cs.

Critical metal	Concentration ($mgkg^{-1}$)	95% C.L. (N=4)
Li	420.6	10.7
Rb	407.5	10.4
Cs	1280.8	5.4

C.L.: Confidence level; N: Number of replicates

Bioleaching Mechanism: Fungal metabolites such as amino acids, proteins, organic acids, and exopolysaccharides contribute to metal solubilization. Notably, organic acids serve a dual function: they directly promote metal dissolution by providing protons and ligands, while simultaneously alleviating metal toxicity for the microorganisms themselves through chelation and complexation processes (Welch et al., 1999; Gadd, 1999; Qu et al., 2013). The bioleaching activity of *A. niger* was primarily driven by organic acid secretion, with gluconic acid (21.90 $mgmL^{-1}$) being the dominant metabolite, followed by citric acid (0.90 $mgmL^{-1}$); oxalic acid was not detected. The absence of oxalic acid, despite its strong acidity (low pKa value), may be related to its potential to form stable and poorly soluble metal–oxalate complexes under certain conditions, as reported in copper leaching (Santos et al., 2005).

Metal dissolution occurs via two key mechanisms: acidolysis, where protons from the acids protonate the metal surface, and complexolysis, where acid anions form stable, soluble metal complexes (chelates). The presence of boron waste did not inhibit acid production, as evidenced by a corresponding pH decrease. The initial pH of the experiment was 5.78. The pH rose in the following days owing to the alkaline metal oxides present in the boron waste. However, after the 5th day, it began a steady decline, ultimately reaching a final value of 3.45 (Ertan, 2023).

Figure 1. Possible gluconate (left) and citrate (right) complexes formed in the presence of *A. niger*.

The relevant reactions of the organic acids with metal ions (M^{n+}) are as listed (Qu et al., 2013). (M^+ stands as a metallic ion with +1 valence such as Li, Rb, Cs, etc)

Gluconic acid dissociation (2) and complexation (3) reactions are,

$C_6H_{12}O_7 \rightarrow C_6H_{12}O_7^- + H^+$ Acidolysis (2)

$[C_6H_{12}O_7^-] + M^+ \rightarrow \mathrm{M}\,[C_6H_{12}O_7]$ Metal-gluconate complex (3)

Citric acid dissociation (4), (5), (6) and complexation (7), (8), (9) reactions are,

$C_6H_8O_7 \rightarrow C_6H_7O_7^- + H^+$ Acidolysis (4)

$C_6H_7O_7^- \rightarrow C_6H_6O_7^{2-} + H^+$ Acidolysis (5)

$C_6H_6O_7^{2-} \rightarrow C_6H_5O_7^{3-} + H^+$ Acidolysis (6)

$C_6H_7O_7^- + M^+ \rightarrow \mathrm{M}\,[C_6H_7O_7]$ Metal-citrate complex (7)

$C_6H_6O_7^{2-} + 2\,M^+ \rightarrow M_2[C_6H_6O_7]$ Metal-citrate complex (8)

$C_6H_5O_7^{3-} + 3\,M^+ \rightarrow M_3[C_6H_5O_7]$ Metal-citrate complex (9)

Hydrogen ions of the acids mobilize the metal ions and the hydrogen dissolution and complexolysis reactions for these acids are as follows. In acidolysis, the organic acids protonate the oxygen layer on the metal surface, facilitating dissolution. Simultaneously, the organic acid ligands form stable, water-soluble metal complexes through complexolysis, thereby enhancing metal release into the solution.

Bioleaching Kinetic: A kinetic study of the bioleaching process was conducted under the optimal conditions (30°C, 125 rpm, 0.01 solid/liquid rate) for 31 days, during which the aqueous concentrations of lithium, rubidium, and cesium were determined at predetermined time points (4, 10, 13, 18, 24 and 31 days), as shown in Figure 2. An increase in bioleaching yield over time was observed for all three elements. For lithium, the bioleaching efficiency was very low and nearly stagnant during the first 18 days, showing only a minimal increase. Subsequently, it rose sharply from 6.31% on day 18 to 43.71% on day 24, maintaining a steady increase thereafter. Consequently, it is appropriate to evaluate the bioleaching kinetics of lithium in two distinct phases. In contrast, rubidium bioleaching exhibited a slow but consistent increase, while cesium displayed a moderate and steady release rate throughout the process. At the end of the process, the highest amount of Li, Rb, and Cs bioleaching yields with *A. niger* were 78.67%, 37.4%, and 30.47%, respectively.

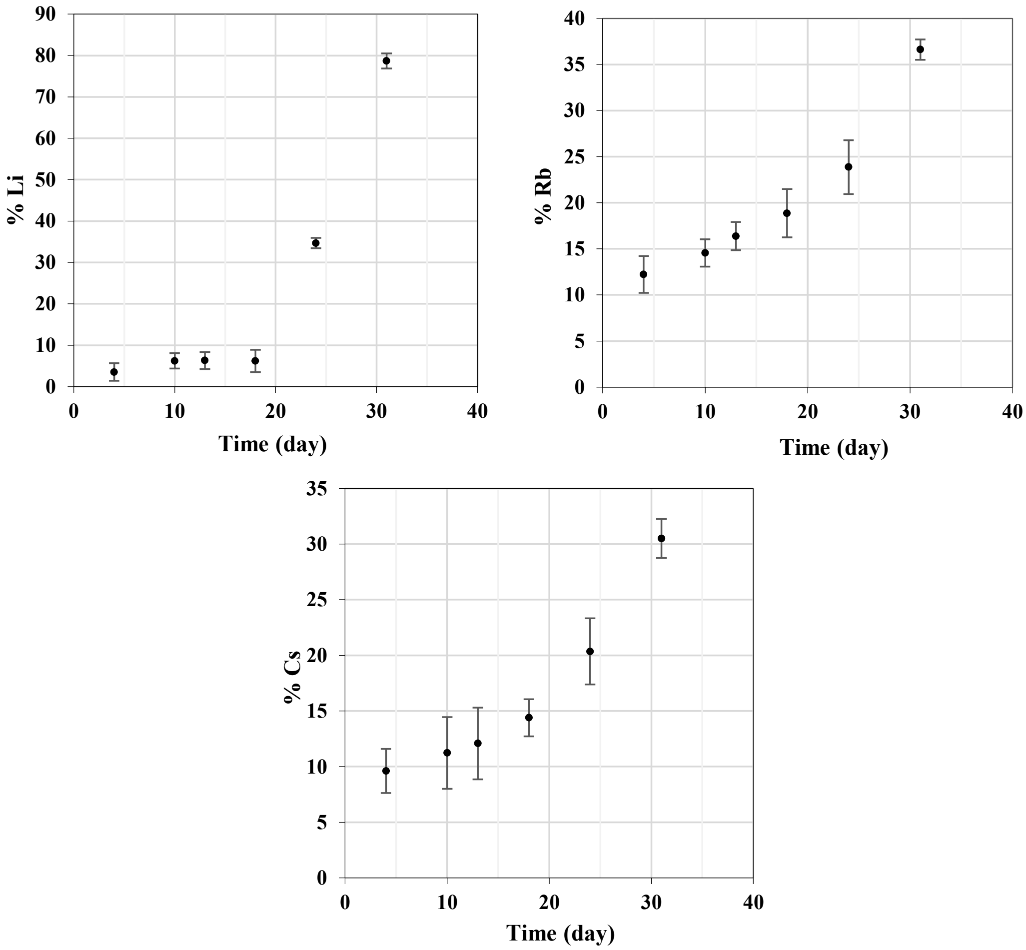

Figure 2. Bioleaching of Li, Rb, and Cs from boron waste by *A. niger.* Values represent the mean ± standard deviation (N=5).

The Shrinking Core Model (SCM) is a reaction kinetics model specifically used for solid-liquid or solid-gas reactions. This model is particularly applied in heterogeneous reactions – for instance, when a solid particle reacts with a liquid or a gas. The SCM assumes that the reaction begins at the outer surface of the solid particle and progresses inward. As the reaction proceeds, only an unreacted core remains from the reactant solid – this core gradually shrinks over time (Levenspiel, 1999). The kinetics of heterogeneous liquid-solid reactions involve two key stages: the transport of soluble reactants to the reaction site (via diffusion through surfaces or pores), followed by the surface chemical reaction itself. The slowest of these stages—be it diffusion through a product layer or the intrinsic chemical reaction rate—becomes the rate-controlling step for the entire leaching process (Levenspiel, 1999; Maccarthy et al., 2016). The two models of diffusion control and chemical control (presented with the Eqs. (10) and (11)) (Wadsworth et al., 2009) had the best fit on the data obtained for Li, Rb, and Cs bioleaching.

$$kt = 1 - (1 - X)^{1/3} \tag{10}$$

$$kt = 1 - 2/3X\text{-}(1\text{-}X)^{2/3} \tag{11}$$

where X is the fraction reacted, k is the kinetic constant of the reaction, and t is the reaction time. Experimental data obtained for all three metals were substituted into both equations of SCM model. The experimental data for Li, Rb, and Cs extraction using *A. niger* were evaluated against two fundamental kinetic models: diffusion control and chemical reaction control. Although both models were applied to the experimental data, the chemical reaction control model provided a significantly better fit than the diffusion control model. Changes of rate constant, k, (apparent from slopes of the plots), are clearly observable, as well. The constant of the reaction rate and the correlation coefficients of Li, Rb, and Cs dissolutions are given in Table 2.

In Figure 3a, a clear linear relationship is observed for both Part 1 and Part 2, with a notably steeper slope for Part 2. This suggests that the leaching process is likely controlled by a chemical reaction. In Figure 3b, however, the data remains almost flat, and no linear relationship is observed.This indicates that ash-layer (diffusion) control is not applicable in this case. Additionally, the much steeper slope of Part 2 compared to Part 1 implies that the reaction rate increases under the second period.

The results in Table 2 show that the chemical reaction control model fits the experimental data much better (R^2 = 0.9651-part 1 and 0.9883-part 2) than the diffusion control model (R^2 = 0.8981-part 1 and 0.9046-part 2). Therefore, the leaching process is predominantly controlled by the surface chemical reaction rather than by diffusion through the product layer.

The rate constant (k) for Part 2 is approximately twelve times higher than for Part 1, indicating a significant increase in reaction rate — most likely due to maybe more acidic media. This strong dependence on reaction conditions further supports a chemically controlled mechanism consistent with Arrhenius-type behavior.

For both Rb and Cs, the chemical reaction control model shows a significantly better fit (R^2=0.9716 for Rb and 0.9733 for Cs) than the diffusion control model (R^2=0.8530 for Rb and 0.8582 for Cs). This strong linearity in Figures 3c and e indicates that the leaching kinetics of both elements are primarily controlled by the surface chemical reaction.

In contrast, Figures 3d and f show poorer linearity and larger scatter from the fitted line, confirming that diffusion through the product (ash) layer is not the rate-controlling step. The rate constants (k) for the chemical reaction model (0.0042 day^{-1} for Rb and 0.0034 day^{-1} for Cs) are notably higher than those from the diffusion model, further supporting a chemically controlled mechanism.

Table 2. Reaction rate constant and correlation coefficients for Li, Rb, and Cs bioleaching.

	Chemical control model		**Diffusion control model**	
	R^2	**k (day^{-1})**	**R^2**	**k (day^{-1})**
Li (part 1)	0.9651	0.0019	0.8981	0.00003
(part 2)	0.9883	0.0238	0.9046	0.00690
Rb	0.9716	0.0042	0.8530	0.00005
Cs	0.9733	0.0034	0.8582	0.00030

To evaluate the statistical significance of the experimental findings, an analysis of variance (ANOVA) was performed. One-way ANOVA revealed that time had a statistically significant effect on the dissolution of all three metals. For lithium, a highly significant effect was observed (F=9593.99, p<0.001). Tukey's pos-hoc test indicated that days 10-18 formed a statistically homogeneous group, confirming the presence of an induction period prior to the active dissolution stage. In contrast, rubidium (F=2215.95, p<0.001) and cesium (F=1808.76, p<0.001) exhibited statistically significant differences between all-time intervals, suggesting a continuous and progressive dissolution behavior. The low standard deviations observed across replicates indicate high experimental reproducibility.

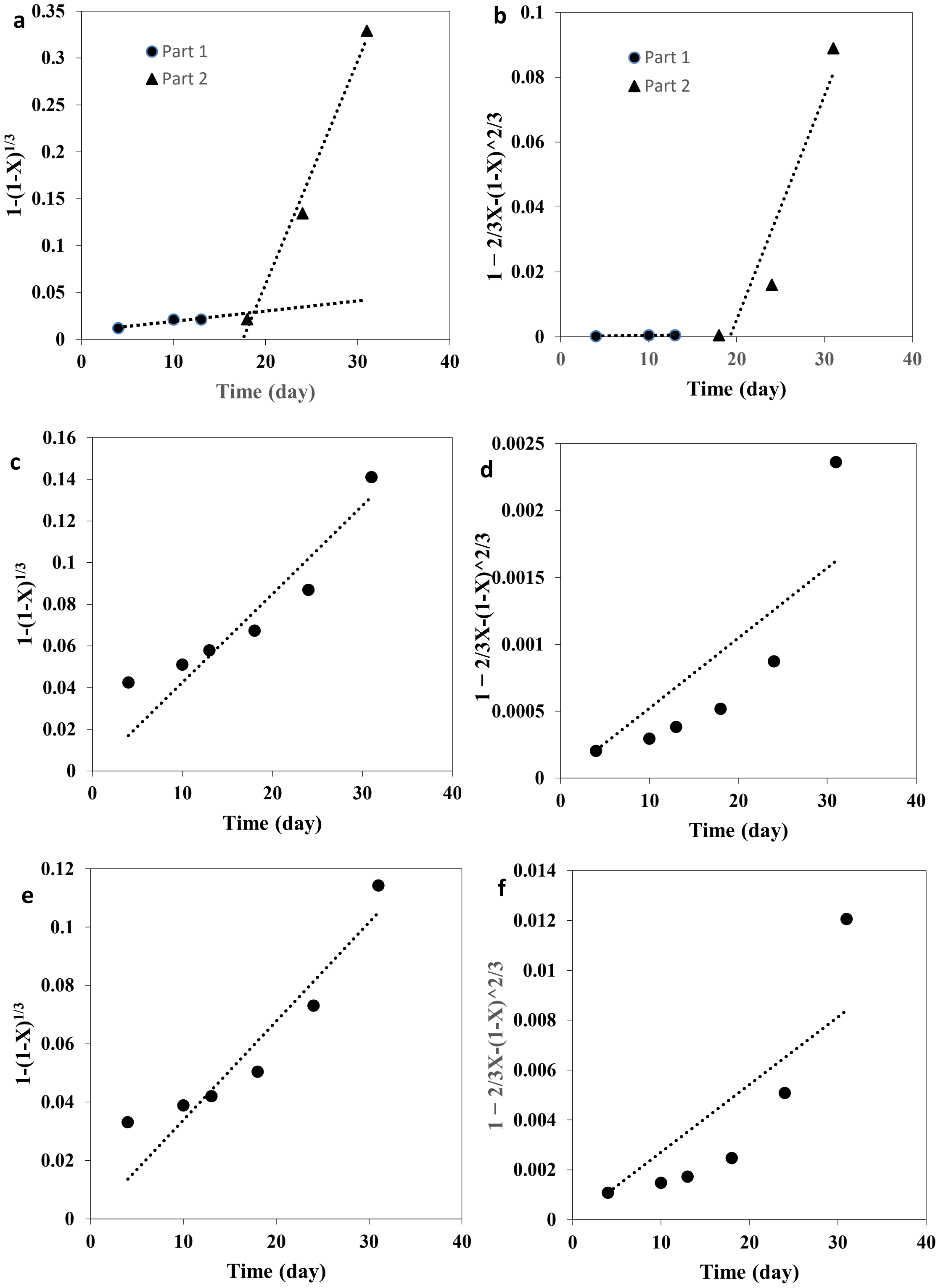

Figure 3. Kinetics of Li (a,b), Rb (c,d), and Cs (e,f) bioleaching from boron waste by *A.niger*. Chemical control model (a,c,e), diffusion control model (b,d,f).

CONCLUSION

Bioleaching of boron waste with *A. niger* successfully achieved high extraction efficiencies for lithium, rubidium, and cesium. The process proceeds via acidolysis and complexolysis mechanisms, driven by fungal-secreted gluconic and citric acids. The leaching kinetics follow the chemical reaction-controlled model of the Shrinking Core Model for all three metals, while diffusion control is not significant under the studied conditions. The higher rate constant in Part 2 confirms that the Li leaching process is sensitive to reaction parameters such as temperature or reagent concentration. The leaching behavior of both Rb and Cs follows the chemical reaction-controlled shrinking core model. The lower correlation coefficients and much smaller rate constants in the diffusion model confirm that diffusion is not the dominant kinetic step. The distinctly delayed bioleaching behavior of Li, compared with Rb and Cs, can be attributed to its intrinsic physicochemical characteristics rather than microbial or sample-related factors, since the same microorganism and solid matrix were used in all experiments. Lithium, having the smallest ionic radius and the highest charge density among the alkali metals, forms stronger bonds within the mineral lattice. This strong lattice incorporation makes Li more resistant to dissolution. Moreover, Li^+ possesses a very high hydration energy, which stabilizes it in the solid phase and hinders its transfer into the aqueous solution.

As a result, the leaching process is predominantly governed by surface chemical reactions rather than diffusion through the product layer for Li, Rb and Cs. The leaching of Li proceeds slowly during the initial stage, and significant dissolution occurs only after sufficient disruption of the mineral structure, reflecting the influence of its small ionic size, strong lattice bonding, and high hydration energy.

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AUTHORS' CONTRIBUTIONS

The entire process of the article was carried out by the corresponding author.

STATEMENT OF CONFLICTS OF INTEREST

The author declares that there is no conflict of interest.

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