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Aspergillus niger as an eco-friendly agent for potassium release from K- bearing minerals: Isolation, screening and culture medium optimization using Plackett-Burman design and response surface methodology

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ABSTRACT

The potential of Aspergillus niger, to enhance non-exchangeable potassium (K⁺) release from mineral structures were investigated as a cost-effective and environmentally friendly alternative to traditional chemical fertilizers. Optimizing the culture medium for maximum K⁺ release, alongside identifying potential mechanisms of action of the A. niger including the production of various organic acids and pH reduction in the minerals feldspar and phlogopite, were among the primary objectives of the present study. K⁺ dissolution from feldspar and phlogopite in the presence of Aspergillus niger were examined through a two-step experiment; impact of different carbon sources (glucose, sucrose, and fructose) on K⁺ release using the Plackett-Burman design (PBD) with 12 experimental runs and effect of other independent variables including pH (ranging from 5 to 10), carbon concentration (3–12.3 g l^{-1}), and incubation time (5–18 days) on K⁺ release using the central composite design (CCD). Our results indicated that the PBD demonstrated a strong predictive capacity (RMSE = 0.012–0.018 g l^{-1} and R^2 = 0.85–0.89) for K⁺ release. According to the CCD model, pH exerted a significant positive influence on increasing soluble K⁺ release (P < 0.001). The highest levels of K⁺ release (157.8 and 175.3 mg l⁻¹ in feldspar and phlogopite, respectively) were observed at the central levels (0) of time and carbon source, and at the $+\alpha$ level (+1.68) of pH. Furthermore, based on the CCD model, the optimal conditions for achieving high K⁺ release from feldspar and phlogopite in a medium were pHs of 10.36 and 10.31, sucrose concentrations of 11.23 and 11.32 g l^{-1} , and incubation times of 15 and 18 days, respectively. The determination coefficients of the CCD model indicated that 89.5% and 92.6% of the changes in soluble K^+ for feldspar and phlogopite, could be explained by this model, respectively. In the current study, the production of organic acids and the resulting pH reduction, along with the reduction in mineral particle size in feldspar and phlogopite, were identified as potential mechanisms influencing the enhancement of potassium solubility. The predominant acids in both feldspar and phlogopite were lactic acid (70.9 and 69.15 mg l^{-1}) and citric acid (40.48 and 22.93 mg l^{-1}), although the production levels of organic acids differed in the two minerals. Overall, our findings highlight the potential of A. niger to proficiently release non-

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exchangeable potassium from mineral matrices, indicating its promising potential in agricultural applications.

1. Introduction

Potassium (K^+) is a crucial macronutrient for plant growth and development, and its deficiency can lead to various detrimental effects such as chlorosis, weak roots, delayed maturity, reduced disease resistance, and decreased yields [1–3]. Despite being the seventh most abundant element in the earth's crust, a significant portion of potassium is locked within insoluble reserves in the form of K-bearing minerals in soils, including primary alumina silicates such as feldspar, mica, biotite, and muscovite. However, secondary alumina silicates consist of hydrous mica and a continuum of micaceous weathered or inherited products also act as K^+ sources in the soils [4].

The rapid expansion of agriculture, coupled with the imbalanced use of chemical fertilizers, has led to a decline in available potassium levels in soil, necessitating the exploration of alternative approaches to enhance potassium availability [1].

Soil-beneficial microorganisms, including bacteria, fungi, and algae, play a crucial role in the natural cycling of potassium. These microorganisms decompose silicates and release potassium, along with other essential elements such as iron, zinc, silica, and phosphorus [5–7]. Various species of microorganisms, such as *Arthrobacter*, *Thiobacillus*, *Rhizobium*, *Agrobacterium*, *Bacillus*, *Pseudomonas*, and *Aspergillus*, have been found to release substantial amounts of potassium from different minerals, thereby improving plant productivity [8–10]. They employ diverse mechanisms, including pH reduction, chelation enhancement, acidolysis, complexolysis, exchange reactions, organic and inorganic acid production, biofilm formation, and exopolysaccharide secretion to solubilize minerals [1, 4,11]. Additionally, certain microorganisms utilize carbonic acid, derived from carbon dioxide, to catalyze the chemical weathering of minerals [3].

K-solubilizing microorganisms have been found to produce various organic acids, such as oxalic acid, tartaric acid, malic acid, lactic acid, succinic acid, citric acid, gluconic acid, and fumaric acid, which are effective in releasing potassium. These organic acids play a vital role in the solubilization of potassium from minerals. This microbial-mediated approach holds promise for enhancing potassium availability in soils while reducing reliance on chemical fertilizers and mitigating their environmental impacts [12–15].

Research has demonstrated that various factors, including carbon and nitrogen sources, temperature, pH, aeration, and incubation time, influence the ability of microorganisms to solubilize potassium [1,16,17]. Parmar and Sindhu (2013) conducted a study to investigate the impact of different nutritional and environmental conditions on K^+ release by K-solubilizing bacteria isolated from the wheat rhizosphere. They utilized the Aleksandrov medium and observed that the highest solubilization of K^+ occurred in the presence of glucose as a carbon source at 25 °C temperature and a pH of 7.0. Additionally, in a liquid medium containing 0.5% potassium aluminum silicate, *Aspergillus niger* and *Aspergillus terreus* exhibited the highest available K^+ . Further solubilization achieved when grown in the presence of 1% feldspar [12]. This underscores the critical role of medium optimization in K^+ solubilization.

Conventional methods, involving the factorial combination of medium optimization by varying one variable at a time while keeping others constant, are laborious, time-consuming, and do not guarantee optimal conditions [18]. To address these limitations, statistical methods such as the Plackett-Burman design and response surface methodology (RSM) have proven to be valuable tools for screening and optimizing conditions to enhance the efficiency of microorganisms in biological activities [19]. RSM, in particular, has overcome the drawbacks of previous methods and has demonstrated its efficacy in optimizing the production of target metabolites [5].

Minerals serve as rich sources of elements such as potassium. Microorganisms can play a significant role in releasing these compounds. The primary objective of the present study was to explore the potential mechanisms involved in the release of potassium by fungi from two minerals, feldspar and phlogopite. The hypothesis put forth included the generation of organic acid compounds and a reduction in pH within the culture medium, leading to alterations in mineral size and an enhancement in specific surface area to facilitate effective potassium dissolution. Consequently, in order to eliminate extraneous factors impacting dissolution and to investigate the specific factors influencing the growth and development of fungi, the study was designed to be conducted under controlled conditions. To accomplish these objectives, several experiments were conducted as outlined below. I) the impact of different carbon sources on K^+ release by *A. niger* using the Plackett-Burman design (PBD), II) modeling the effects of incubation time, pH, and varying amounts of carbon sources on K^+ release using Central Composite Design (CCD), and III) determination of optimal conditions to enhance *A. niger*-mediated K^+ release through the production of organic acids and reduction of pH and reducing the particles size. Therefore, the main objectives of this study are to gain a deeper understanding of the factors influencing the solubilization of potassium ions (K^+) and to optimize the conditions to enhance the efficiency of *A. niger* in releasing K^+ by promoting organic acid production and reducing pH levels and particles size.

2. Material and methods

2.1. Preparation of K-bearing minerals

Feldspar and phlogopite, utilized in this investigation, were procured from the Zaminkav Company in Tehran-Iran. Subsequently, the minerals were ground and sieved through a 270 mesh sieve. To eliminate exchangeable K^+ , the samples were saturated in a calcium chloride solution (1 M, with a 2:1 ratio), followed by washing with 0.01 M HCL, and then dried at 50 °C for 48 h [20]. The elemental composition of the minerals was determined using X-ray fluorescence (XRF) and is presented in Table 1.

Total 99.28 99.47

2.2. Isolation and screening of K-solubilizing fungi

Fungal strains were isolated from the rhizosphere soil of potato in West Azerbaijan -Iran using the serial dilution plate method. A modified Aleksandrov medium, comprising 5.0 g glucose, 0.5 g MgSO₄.7H₂O, 0.1g CaCO₃, 0.006 g FeCl₃, 2.0 g Ca₃PO₄, 3.0 g insoluble mica powder as a source of K⁺, and 20.0 g agar per liter of deionized water, was employed for this purpose [21,22]. In the initial step, plates of the modified Aleksandrov medium containing mica powder were prepared to assess the qualitative release of K⁺. A loop of the 48-h-old fungal growth was inoculated onto the prepared plates, which were then incubated at 28 \pm 2 °C for ten days. Following the incubation period, the diameters of the colonies and halo zones were measured for strains that exhibited a clear zone. The solubilization index (SI) was calculated using Equation (1) to quantify the extent of solubilization [23].

$$SI = \frac{Colony \ diameter + Halo \ zone \ diameter}{Colony \ diameter} \tag{1}$$

In the subsequent step, for the quantitative estimation of K⁺ release, a loop of the 48-h-old fungal culture was inoculated into 100 ml of Aleksandrov broth medium containing K⁺ mineral (2 g l⁻¹ of K-feldspar) [21]. The inoculated flasks were incubated at 28 \pm 2 °C for ten days. Samples were withdrawn at 1, 2, 3, 5, 7, and 10 days, and the amount of released K⁺ was measured using a flame photometer (480, Corning, USA). Upon completion of the experiments, the strain demonstrating a larger halo zone, a higher SI, and a greater amount of released K⁺ was identified as the superior strain [24].

2.3. Inoculum preparation

Following isolation, the chosen fungus was re-cultured in a Potato Dextrose Agar (PDA) medium. A 48-h-old growth culture of the fungal strain was transferred to Erlenmeyer flasks containing 25 ml of Nutrient Broth (NB) medium. The flasks were shaken overnight at 120 rpm, and then 1 ml of inoculum (containing 4×10^7 spores per ml) was added to the Aleksandrov medium broth for the first and second steps of the experiment [5]. The Erlenmeyer flasks were incubated under shaking conditions at a temperature of 28 ± 2 °C and 120 rpm, using two different sources of K⁺ (feldspar and phlogopite). At the end of each incubation period, samples were centrifuged and filtered using Whatman filter paper (No. 41), and the released K⁺ was determined using a flame photometer [25]. The pH was measured with a Ruosull (RPB1000) pH meter.

2.4. Modeling and optimization of K^+ dissolution

Initially, 12 experiments were conducted using a combination of different carbon sources, including glucose, sucrose, and fructose at two levels of +1 (10 g l^{-1}) and -1 (5 g l^{-1}) as per the Plackett-Burman design. This allowed for the identification of the most effective carbon source in K⁺ release. All experiments were carried out according to the designed matrix (Table 2) using the first-order model (Equation (2)) [18].

$$Y = \beta_{o+} \sum \beta_i Xi \quad (i = 1, \dots, k)$$
⁽²⁾

where Y represents the predicted target, βo and βi are the model constants and regression coefficients, X is the independent factor, and k is the number of variables.

Subsequently, based on the results from the first step, response surface methodology with the CCD was employed to model and optimize the effects of the coded independent variables, including pH (3-10), incubation time (1–18 days), and carbon source (0.6–12 g l⁻¹) on K⁺ release from feldspar and phlogopite by *A. niger* [5,26,27]. The experiment was performed based on the coded values of the independent variables (Table 3). The variables are coded as in Equation (3).

$$X_i = \frac{x_i - x_0}{\Delta x_i} \tag{3}$$

where X_i represents the coded value of the independent variable, x_i is the actual value, x_0 is the value of X_i at the center point, and Δx_i is the step change value.

2.5. Detection of organic acids using HPLC and SEM imaging

The filtrated culture was analyzed for the presence of organic acids using the Agilent 1100 high-performance liquid chromatography (HPLC) [28]. For this purpose, $20 \mu l$ of the samples were injected into the C18 column, and the acetonitrile: water: 7: 3 was used

Table 1Elemental composition of minerals.

Minerals	SiO ₂	Al ₂ O ₃	K ₂ O	Na ₂ O	Fe ₂ O ₃	MgO	CaO	MnO	P_2O_5	TiO ₂	LOI ^a
Feldspar	64.50	17.44	13.67	2.76	0.35	0.01	0.11	0.01	_	0.01	0.42
Phlogopite	42.20	14.36	9.66	0.23	4.96	23.81	2.03	0.11	0.03	0.17	1.82

^a LOI Loss on ignition.

Table 2

The Plackett -Burman design variables with released K as a response.

Number of experiment	Source of Carbon	l		Released K (mg.1-	-1)
	Glucose	Sucrose	Fructose	Feldspar	Phlogopite
1	-1	-1	-1	0.255	0.765
2	$^{+1}$	$^{+1}$	$^{-1}$	0.255	0.510
3	$^{+1}$	-1	+1	0.255	0.510
4	-1	-1	+1	0.510	0.765
5	$^{-1}$	$^{+1}$	-1	0.765	1.27
6	$^{+1}$	$^{+1}$	+1	0.765	1.27
7	$^{-1}$	$^{+1}$	+1	0.255	0.510
8	$^{-1}$	+1	+1	0.510	0.765
9	$^{-1}$	$^{-1}$	-1	0.255	0.510
10	$^{+1}$	$^{+1}$	-1	0.510	0.765
11	$^{+1}$	$^{-1}$	-1	0.510	1.02
12	$^{+1}$	$^{-1}$	+1	0.255	0.765

+1 and -1 show 10 and 5 g l⁻¹ of carbon sources, respectively.

Table 3

Range of studied variables and their levels in RSM modeling.

Independent variable	Coded amount of variable	Range and amounts						
	Xi	$+\alpha$	+1	0	-1	-α		
Carbon source (g l^{-1})	X1	12.386	10	6.5	3	0.613		
pH	X ₂	10.36	9	7	5	3.63		
Incubation time (day)	X ₃	18.40	15	10	5	1.59		



Fig. 1. Schematic design of different stages of this study.

as the solvent of the mobile phase in isocratic condition with a flow rate of 1 ml min^{-1} . The column temperature was maintained at 27 °C, and the samples were detected at 210 nm using a UV detector. The authentic standards for oxalic acid, succinic acid, citric acid, lactic acid, maleic acid, malic acid, acetic acid, tartaric acid, and formic acid were run before the samples [18]. Additionally, to investigate the impact of microbial inoculation on the structural changes of the studied minerals, imaging was carried out using a Phenom Prox scanning electron microscope (SEM). The particle size in resulting images was determined using Image J software (ij154), and the histogram was plotted using PAST software (ver. 4.03).

2.6. Statistical analysis

The statistical ANOVA analysis was performed using MSTAT-C (version 4.1). The data gained from the experiments were analyzed using graphical and regression analysis, and the response surface and contour plots were examined using Design Expert 10 software.

3. Results and discussion

The study's various stages, including mineral preparation, fungal inoculation, optimization, and medium characterization, are depicted in the graphical diagram in Fig. 1. The results demonstrated successful potassium release from K-bearing minerals by *A. niger*.

3.1. Selection of superior strain

The qualitative and quantitative evaluation of K-releasing ability among different strains is presented in Figs. 2 and 3 and Table 4. Among the strains tested, potassium solubilizing fungi 3 (KSF3) exhibited higher SI and released a significant amount of K^+ , consequently, it was chosen for further study.

3.2. Plackett-Burman Design

The impact of various carbon sources on K^+ release from feldspar and phlogopite by *A. niger* was investigated using the Plackett-Burman Design (PBD) based on the first-order linear model. The model's predictive capability, as obtained from PBD for estimating K^+ dissolution, is depicted in Fig. 4A and B. The statistical analysis results of this design are also presented in Table 5. As shown in Fig. 4A and B, the PBD model accurately estimated the amount of K^+ dissolution (RMSE = 0.012 g l^{-1} and R^2 = 0.85 in feldspar, RMSE = 0.018 g l^{-1} and R^2 = 0.89 in phlogopite) in the 12 experiments of the first stage. According to the results in Table 5, no significant difference was observed between different carbon sources, indicating that each of them can be used interchangeably in the medium [5]. Consequently, sucrose was chosen to optimize the different levels of the medium's carbon source in the second stage of the experiment.

3.3. Central composite design (CCD) modeling

To evaluate the impact of time, pH, and carbon source on K⁺ release and dissolution by *A. niger*, 20 experiments were designed and conducted using the CCD (Table 6) following carbon source selection. The results revealed that the maximum K⁺ release was observed at the central levels (0) of time and carbon source and the $+\alpha$ level of pH (+1.68) (experiment 8, Table 6). The lowest K⁺ release rate from both minerals was observed at pH = 5 (-1 level) and pH = 3.63 (-1.68), indicating reduced fungal activity. In the RSM experimental design, the pH adjustment factor ranged from 3.63 to 10.36 across various experimental runs. A critical observation from the findings was the consistent decline in the final pH values across all treatments and experimental iterations. Specifically, this decline was noted within the ranges of 2.79–4.94 for Feldspar and 3.12 to 4.83 for phlogopite, suggesting the influence of organic acids generated by the fungus *A. niger* (Table 6). Furthermore, a noteworthy trend was the substantial reduction in potassium release associated with the final pH values. This phenomenon suggests that although potassium release tends to increase with environmental



Fig. 2. A view of the zone dissolved by potassium-solubilizing fungi in Alexandrov's medium.



Fig. 3. SI of K-solubilizing fungi.

Table 4			
Amount of rele	eased K (mg l⁻	⁻¹) by selec	ted strains.

Strains	Incubation time (day)									
	0	1	2	3	5	7	10			
Control (without inoculation)	0.36	0.37	0.34	0.39	0.39	0.43	0.44			
KSF1	0.61	1.38	1.71	2.31	2.75	3.33	3.60			
KSF2	0.61	1.40	1.63	2.29	2.52	3.20	3.45			
KSF3	0.61	1.81	1.98	2.31	2.48	3.24	3.72			
KSF4	0.61	1.11	1.85	2.06	2.52	2.95	3.35			

acidification, a disturbance in the continuity of fungal activity may occur at extremely acidic pH levels. Similarly it has been reported that organic acids produced in the periplasm could rapidly diffuse into the surrounding environment and facilitate the dissolution of insoluble forms of minerals such as feldspar and orthoclase [29–31], as well as feldspar and illite [1,32].

In the current study, even at high initial pH levels, a rapid decrease in pH during incubation was observed. The ability of the strain to reduce the medium's pH is considered an indicator of medium acidification [31]. KSMs can dissolve insoluble K^+ by producing significant amounts of organic acids. Organic acids resulting from the microorganism's metabolic activity lead to a decrease in the medium's pH, enhancing the total acidity and increasing the capacity to release cations such as K^+ [1,32–34]. This subject was confirmed by the findings of several researchers who reported that KSMs produce mono, di, and tri organic acids such as gluconic, acetic, oxalic, fumaric, tartaric, and citric, which lead to a decrease in the pH of the medium [29,35,36]. The *A. niger* strain studied in this research produces several types of organic acids, including oxalic, malic, lactic, citric, and succinic acids. Thus, it reduces the medium's pH, breaks the mineral structure, and imports Si⁴⁺ and K⁺ ions into the solution phase. The production of H⁺ during the hydrolysis of minerals in the medium is another reason for the decrease in pH [37].

The CCD's coefficients, significance level, and polynomial function's independent and response variables are presented in Table 7. The pH effect ($P \le 0.001$) on K⁺ release from minerals was significant in both the linear and quadratic parts. Although the interaction effects of independent variables on K⁺ release were insignificant, the interaction of time with carbon source in feldspar and the interaction of time with pH in phlogopite were greater than the interaction of other parameters due to the low *P* value.

Based on the significant coefficients outlined in Table 7, equations (4) and (5) predict the amount of K^+ release in the presence of feldspar and phlogopite, respectively. In these equations, X_1 , X_2 , and X_3 represent the coded values of time, pH, and carbon source. Notably, the equations demonstrate a positive correlation between pH (X_2) and the increase in K^+ release.

Released K from Feldspar (mg.l⁻¹) =
$$5.25 + 1.24X_1 + 30.56X_2 + 2.19X_3 + 0.60X_1X_2 + 24.81X_1X_3 - 2.52X_2X_3 - 2.48X_1^2 + 24.81X_2^2 - 2.52X_3^2$$

Released K from Phlogpite $(mg.l^{-1}) = 16.86 + 6.83X_1 + 36.28X_2 + 3.92X_3 - 4.72X_1X_2 + 4.56X_1X_3 - 0.98X_2X_3 - 1.78X_1^{2+25.26}X_2^{2-1.85}X_3^{2}$ (5)

Fig. 5A and B shows the comparison between the measured K⁺ in the CCD experiments and the predicted K⁺ amounts. The results



Measured Potassium (mg.l⁻¹)

Fig. 4. Measured and predicted concentration of released potassium using Plackett-Burman design A) in feldspar, B) in phlogopite.

Та	ble	5

Regression coefficients of Plackett-Burman model data.

Mineral	Parameter	Coefficient	<i>F</i> -Value	P-Value
Feldspar	Equation Constant	0.48	12.25	0.000
	Glucose	0.000	0.00	1.000
	Sucrose	0.095	2.45	0.070
	Fructose	-0.000	-0.00	1.000
Phlogopite	Equation Constant	0.85	17.67	0.000
	Glucose	0.016	0.33	0.756
	Sucrose	0.112	2.33	0.080
	Fructose	-0.048	-1.00	0.374

indicate that the CCD model effectively estimated the K⁺ content in feldspar ($R^2 = 0.89$) and phlogopite ($R^2 = 0.92$).

Furthermore, the effect of variable interactions on K⁺ release from feldspar and phlogopite was visually represented using threedimensional diagrams based on the CCD model (Fig. 6A–C and 6D-F). In these interaction diagrams, one variable was kept constant at its average value while the simultaneous effect of the other two variables on K⁺ release was observed. Specifically, Fig. 6A and D illustrate the combined effect of pH and incubation time on K⁺ release, demonstrating that an increase in pH enhances the K⁺ dissolution rate in both minerals. The production of organic acids by *A. niger* is also influenced by the medium's pH, with efficient production of oxalic acid and gluconic acid occurring at pH levels of 5–8. Conversely, oxalic acid is not produced at pH levels lower than 3 [38,39]. The minimal decline in pH observed in environments with lower adjusted initial pH levels in the present study can be ascribed to a diminished synthesis of organic acids. This reduction in production may potentially stem from the inhibitory influence of enzymes within the controlled in vitro environments, where a buffering mechanism akin to that found in soil is non-functional. Therefore, it appears that *A. niger* exhibits higher efficiency in secreting organic acids at higher pH levels. In the present study, the highest solubility activity of K was obtained at a pH of 10.36 and 10.31 in feldspar and phlogopite. Furthermore, the interaction effect plots showed that pH had the greatest impact among the selected factors on increasing solubility. The interaction of pH with the

Table 6

Matrix of coded variables amounts in central composite design modeling and released K as a response.

Experiment Number	Amounts of Coded Variables		Feldspar		Phlogopite		
	Time	pH	Carbon Source	Released K (mg.l ⁻¹)	Final pH	Released K (mg.l ⁻¹)	Final pH
1	1	1	-1	35.76	4.32	67.99	4.27
2	1	$^{-1}$	1	5.06	3.42	21.17	3.12
3	0	0	0	5.80	4.28	28.05	4.07
4	$^{-1}$	$^{-1}$	1	0.98	3.79	6.53	3.65
5	0	0	0	5.80	4.09	12.04	3.94
6	$^{-1}$	1	-1	37.58	4.64	52.71	4.68
7	0	0	0	2.55	4.07	15.03	3.88
8	0	1.68	0	157.85	4.54	175.33	4.27
9	0	0	0	1.30	4.14	7.04	3.93
10	0	0	1.68	6.54	3.7	23.52	3.75
11	0	-1.68	0	3.55	3.46	8.04	3.87
12	1.68	0	0	4.74	3.55	21.17	3.6
13	1	1	1	50.64	3.34	85.34	4.03
14	$^{-1}$	-1	-1	0.48	4.46	3.50	4.62
15	1	-1	-1	0.35	2.79	6.55	4.17
16	0	0	-1.68	1.30	5.1	7.29	5.96
17	$^{-1}$	1	1	40.10	3.99	45.14	3.94
18	-1.68	0	0	2.20	4.94	9.18	4.83
19	0	0	0	5.80	3.38	17.03	3.93
20	0	0	0	7.79	3.84	20.02	4.1

Fable 7	
Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of K solubilization.	

Model Parameters		Feldspar				Phlogopite				
	df	SS	MS	F-Value	P-Value	SS	MS	F-Value	P-Value	
Constant	9	22361.2	2484.5	9.46	0.0008>	28809.6	3201.1	14.01	0.0001	
A-Time	1	20.94	20.94	0.080	0.7834	637.71	637.71	2.79	0.1258	
B- pH	1	12759.1	12759.1	48.59	0.0001>	17977.6	17977.6	78.66	0.0001>	
C- Carbon Source	1	63.30	63.30	0.24	0.6340	205.46	205.46	0.90	0.3654	
AB	1	2.85	2.85	0.011	0.9191	178.55	178.55	0.78	0.3975	
AC	1	34.27	34.27	0.13	0.7254	166.68	166.68	0.73	0.4131	
BC	1	18.55	18.55	0.071	0.7958	7.73	7.73	0.034	0.8578	
A ²	1	88.65	88.65	0.34	0.5741	45.61	45.61	0.20	0.6646	
B ²	1	8907.1	8907.1	33.92	0.0002>	9231.87	9231.87	40.39	0.0001>	
C ²	1	82.98	82.98	0.32	0.5864	44.63	44.63	0.20	0.6679	

amount of carbon and also pH with incubation time indicated that pH is the determining factor in increasing potassium dissolution. Additionally, in the process of interaction effects, the non-significant effects of two factors, incubation time and the amount of carbon source, have led to the plots showing a relatively smooth trend without fluctuations, and an increase in the plots can be observed with an increase in pH. The results of the analysis of variance also showed a significant effect of X_2 -pH and X_2^2 . Similarly, the previous researches indicates that *A. niger* can thrive at pH levels below 2 and above 8 [40,41]. The role of organic acids, including oxalic acid, in the dissolution of rocks and minerals is linked to the presence of hydrogen ions and the formation of cationic complexes [31,42]. The attack of hydrogen ions leads to the release of structural cations, enabling the formation of cationic-organic complexes with oxalic acid, which possesses OH⁻ and COOH⁻ functional groups. The chemical adsorption of these complexes on mineral surfaces alters the electron density of the mineral structure, increasing the susceptibility of cation-oxygen bonds to hydrolysis [31]. Additionally, researchers have highlighted the involvement of increased H⁺ concentration (resulting in decreased pH) and the preferential complexation of aluminum in the presence of organic acids in the pH range of 4–9, as well as the high total dissolution of K⁺ in neutral pH, in the K⁺ dissolution process [43,44].

An increase in the carbon source amount has been observed to enhance the release of K^+ by *A. niger* (Fig. 6B and E). Given that carbon is an essential element for the growth and metabolic activity of the inoculated fungus, and its presence is crucial for the production of organic acids, it is imperative to identify the appropriate carbon source for the KSMs [45].

The release of K^+ varies at different incubation times. As the incubation time increases, the amount of K^+ released shows a gradual increase, eventually stabilizing after a certain period (Fig. 6C and F). The initial high release rate may be attributed to the discharge of K^+ from the edge and wedge-shaped regions of the minerals [46,47]. As the release intensifies, the adsorption energy of K^+ at interlayers increases, and the distance of K^+ from the edge of the minerals and diffusion distance also increases. These events ultimately lead to a decrease in the release rate [48,49]. The optimum pH range for *A. niger* activity has been observed to be < 2 to >8 [40,41]. Despite the increase in incubation time, the pH in the inoculated treatments gradually decreased due to the production of organic acids. However, it did not fall below the tolerance threshold of the fungus. According to this, the depletion of the carbon and energy





Fig. 5. Measured and predicted concentration of released potassium using Central composite design, A) in feldspar B) in phlogopite.

source, as well as the accumulation of toxic metabolites in the medium, are among the main reasons for the reduction in microbial activity and the stabilization of the K^+ release rate after a certain time [44].

At the central levels of independent variables, the amount of K^+ released from phlogopite was found to be 72% higher than that of feldspar (Fig. 6A- C and 6D- F). Phlogopite, being a trioctahedral mica, possesses a weak structure. On the other hand, since K^+ in micas is held by electrostatic forces, the anions produced by organic acids can dissolve aluminum in the octahedral sheet and release interlayer K^+ . This is in contrast to feldspars, where K^+ is held by a covalent bond, and its release depends on various factors including structural characteristics, chemical composition, particle size, and the release of weathering products. During the weathering of feldspar, the replacement of K^+ with hydronium ion weakens the feldspar structure, leading to feldspar expansion. The hydronium ions adsorbed on the surface can break the Al–O bonds, increasing the coordination number of aluminum from 4 to 6, and subsequently, pushing it out of the feldspar structure [50].

Studies have shown that microorganisms play a vital role in the weathering and dissolution process of rocks and minerals. Specifically, microorganisms can break down minerals containing potassium, phosphorus, and iron, such as mica and feldspar, leading to the release of these elements [51]. The mechanisms by which microorganisms participate in this process are diverse and include physical presence, attack using inorganic acids (e.g. sulfuric, nitric, and carbonic acids), attack using organic acids (e.g. acetic, citric, oxalic, and gluconic acids), attack by organic solvents (e.g. acetic and butyric acid, ethanol, propanol, and ketones), salinity stress caused by the reaction products of organic and inorganic acids, production of toxic compounds (e.g. hydrogen sulfide, nitrogen oxides), production of biofilling and biofilm, attack with exoenzymes, and production of chelating agents of suspended compounds [31, 44,52].

Through these mechanisms, microorganisms increase the release of K⁺ from minerals. Physical presence involves direct contact



Fig. 6. The three-dimensional presence of released K analyzed by central composite design in two types of mineral and variables interactions A) feldspar (time \times pH), B) feldspar (pH \times C source), C) feldspar (time \times C source), D) phlogopite (time \times pH), E) phlogopite (pH \times C source), F) phlogopite (time \times C source).

between microorganisms and minerals, while attacks using inorganic and organic acids result in the hydrolysis of materials. Organic solvents cause swelling and hydrolysis of materials, while salinity stress increases the probability of freezing and melting. Production of toxic compounds leads to the production of inorganic acids or deposition of metal sulfides and oxidants/reductants. Production of biofilling and biofilm results in localized surface corrosion, water retention in porous materials, hydrophobic effects on surfaces, reduction of heat transfer efficiency, reduction of flow rate, or an increase in pressure. Attack with exoenzymes leads to the distribution of insoluble organic compounds into small molecules soluble in water, while production of chelating agents of suspended compounds increases the solubility of insoluble or hydrophobic substances [31,44,52].

Optimum conditions to achieve the maximum K⁺ release by *A. niger* from feldspar and phlogopite in the medium predicted based on the RSM-CCD model (Fig. 7A and B). In the optimal conditions for potassium dissolution in feldspar, the dissolution performance was

130.98, achieved with an incubation time of 15.06, a pH of 10.36, and a carbon source of 11.23, with a desirability score of 0.829. Furthermore, in the optimal dissolution performance in phlogopite, the potassium dissolution occurred at an incubation time of 18.38, a pH of 10.31, and a carbon source of 11.32 g, resulting in an estimated performance of 176.5 in the optimal state. The softwarepredicted optimal conditions were subsequently employed to conduct experiments aimed at attaining maximum K⁺ release from different minerals present in the medium. The observed potassium release closely matched the predicted values of the models (130.98 vs 129.93 (mg. 1^{-1}) in feldspar; 176.05 vs 151.14 (mg. 1^{-1}) in phlogopite) and was utilized for further analysis using HPLC (Table 8).

The equation derived from the different concentrations of standard organic acids in Table 8 is provided. The corresponding R^2 for these equations (0.9797-0.9962) indicates the high correlation of the standard concentrations with their linear models. Organic acids produced by A. niger in both of feldspar and phlogopite were determined in optimize state by HPLC. The identified organic acids (mg l^{-1}) in feldspar and phlogopite respectively included oxalic acid (12.7 and 2.51), malic acid (0.5 and 1.9), lactic acid (70.9 and 69.15), citric acid (40.48 and 22.93), and succinic acid (4.44 and 11.53). Except for lactic acid, the other organic acids produced significant differences in the two evaluated minerals (p < 0.01). The most important organic acid secreted by A. *niger* was lactic acid, which could play a significant role in acidifying the culture medium towards the end of the incubation, highlighting its significance in the process. The reduction in pH at the conclusion of optimal incubation conditions supports the generation of organic acids, thereby inducing acidification of the culture media by A. niger in both studied minerals in optimize state (Table 8). Acidification of the culture media associated with high concentrations of lactic and citric acids observed in the medium (Table 8) (Fig. 8A-D). Various mechanisms have been proposed for K⁺ dissolution by fungi, with organic acid secretion by silicate-dissolving fungi being one of the suggested mechanisms [3,18,53]. Fungi generally increase mineral dissolution and element release by producing acid and reducing the pH of the environment [52,54], as well as via mineral decomposition by organic ligands [44,55] and mechanical crushing of particles [56]. The current study was related to the production of organic acids through pH reduction via potassium liberation and dissolution, in accordance with the aforementioned reports. Different fungal species can secrete various organic acids, such as oxalic, citric, and gluconic acids, which dissolve silicate, mica, and feldspar clays [22]. Previous studies have reported the production of oxalic, citric, malic, a-glutaric, and succinic organic acids by A. niger [54]. Additionally, high concentrations of lactic and oxalic acids, and low amounts of citric, formic, malic, and maleic acids, were reported in the medium containing A. niger [18]. Oxalic and lactic acids have also been proposed as the primary organic acids produced in the silicate minerals dissolution process [57]. Meanwhile, studies by Bakri have reported the secretion of oxalic, fumaric, malic, citric, and succinic acids under the conditions of A. niger inoculation [58]. In the

Fig. 7. Optimizing the values of model input parameters to obtain the maximum concentration of soluble potassium by fungus inoculation in the presence of A) feldspar, B) phlogopite.

Table 8

Organic acids identified by HPLC, related analysis by ANOVA, calibration equations, and R^2 for standards.

Culture media characteristics	Retention time (Min.)	Feldspar	Phlogopite	Mean square	Equation	R^2
HPLC identified organic acids (n	ıg. l ⁻¹)					
Oxalic acid	1.53	$12.70\pm2.77^{\mathbf{a}}$	2.51 ± 0.03	153.7***	y = 751.15x - 762.71	0.9962
Malic acid	2.23	0.505 ± 0.14	1.9 ± 0.13	2.76***	y = 1273.1x + 9340.8	0.9797
Lactic acid	2.79	$\textbf{70.9} \pm \textbf{2.04}$	69.15 ± 11.02	0.95 ^{ns}	y = 381.17x + 1421.5	0.9895
Citric acid	4.19	40.48 ± 0.65	$\textbf{22.93} \pm \textbf{2.22}$	449.4***	y = 2787.6x + 8826.2	0.9800
Maleic acid	4.57	nd	nd	-	y = 150.57x + 515.81	0.9836
Succinic acid	5.06	$\textbf{4.44} \pm \textbf{1.53}$	11.53 ± 1.17	73.6***	y = 1100.2x + 4758.4	0.9935
Potassium content (mg. l^{-1})						
Measured		129.93	151.14			
Predicted		130.98	176.05			
рН						
Initial		10.36	10.31			
Final		2.76	3.45			

nd means not detected.

*** shows significance at P < 0.001, ns means not significant.

^a Mean \pm Standard error.

current study, the predominant acids in both feldspar and phlogopite were lactic acid and citric acid. However, an interesting finding was that despite having the same strain, oxalic acid was the dominant third acid in feldspar, while succinic acid was predominant in the phlogopite environment. It was identified in this study that the fungus's activity for producing organic acids is dependent on the minerals present in the environment.

Structural changes in the studied minerals resulting from microbial inoculation were investigated through imaging using a scanning electron microscope (SEM) (Fig. 9A-D). The SEM images revealed that microbial inoculation led to flocculation and a reduction in the size of the mineral particles. The presence of fungal mycelium was evident in the images, with the mycelia entering the internal structure of the minerals to provide the necessary nutrients through the development of hyphae. This process resulted in the physical forces crushing the minerals, reducing the size of the particles, and creating new surfaces with high reactivity [56,59]. In the analysis of feldspar samples, it was observed that untreated treatment exhibited a prominent peak in particle size distribution around 100 μ m², whereas samples treated with fungus displayed a notable reduction in particle size, measuring approximately $12 \,\mu m^2$. A similar trend was noted in the examination of Phlogopite samples, with untreated variants showcasing a particle size of 925 μ m², which decreased significantly to 27.3 μ m² following fungal treatment (Fig. 10A–D). Furthermore, the introduction of fungus resulted in heightened surface roughness and an increased presence of pores within the mineral structure, consequently leading to a substantial augmentation in contact surface area. This enhanced surface area and porosity facilitated a more conducive environment for the organic acids excreted by the fungi and the decomposing enzymes they generated to exert a pronounced influence on the dissolution of potassium. It is evident that the morphological alterations induced by fungal treatment not only led to a reduction in particle size but also enhanced the accessibility of reactive sites on the mineral surface, thereby amplifying the efficacy of the biochemical processes involved in potassium dissolution. Numerous studies have been conducted to investigate the impact of microorganism inoculation on mineral structure. The results of these studies have consistently indicated a decrease in particle size distribution, flocculation, and transformation of minerals [60-62].

4. Conclusion

The use of K⁺ chemical fertilizers has been effective in improving plant productivity, but their excessive application is costly and contributes to environmental pollution. As a result, there is a growing interest in exploring potassium-solubilizing microorganisms (KSMs) as an alternative solution for addressing soil potassium deficiency. KSMs have the potential to enhance nutrient availability by facilitating the release of essential nutrients from mineral structures. Therefore, the isolation and identification of effective KSM species, along with quantitative modeling of various factors influencing the release of elements such as K⁺ from potassium-containing minerals, are of great importance. The present study revealed that KSF3 (A. niger) fungus exhibited superior quantitative and qualitative capabilities in the dissolution of potassium when compared to other fungal strains, prompting its selection for subsequent optimization experiments. Through the application of PBD and CCD techniques, the influential factors affecting potassium dissolution were effectively delineated. The findings underscored pH as the most fundamental factor in the potassium dissolution process, with higher pH levels showing more conducive to fungal activity. The mechanism by which Aspergillus niger fungus facilitates potassium dissolution can be ascribed to the generation of organic acids, pH modulation, particle size reduction, and augmentation of specific surface area. Notably, lactic acid (70.9 and 69.15 mg l^{-1}) and citric acid (40.48 and 22.93 mg l^{-1}) emerged as the predominant acids in both feldspar and phlogopite, albeit with variations in organic acid production levels between the two minerals. The study results indicated that the synthesis of organic acids by fungus is contingent upon the mineral type, with oxalic acid representing the third most abundant acid in feldspar and succinic acid in phlogopite. Microscopic analysis of particles under optimized conditions compared to controls revealed a substantial reduction in particle sizes by about 8 times for feldspar and by approximately 34 times for phlogopite. The diminished particle sizes enhance the capability of fungi and encourage their efficacy in dissolution processes. Additionally, higher concentrations of carbon sources were found to enhance K^+ release by A. niger. As for the effect of incubation time, the results

Fig. 8. Chromatograms of HPLC for minerals were exposed to A. niger, A, B) feldspar, C, D) phlogopite. Peaks corresponding to 1) oxalic acid, 2) malic acid, 3) lactic acid, 4) citric acid, 5) succinic acid.

demonstrated that K⁺ release by the fungus increased gradually with prolonged incubation time, eventually stabilizing after a certain period. In conclusion, creating optimal conditions for *A. niger* enhances its solubilizing capability. Given that the experiments of the present study were conducted under controlled laboratory conditions and demonstrated the effectiveness of *Aspergillus niger* fungus in potassium dissolution, further complementary studies are needed to extrapolate these findings to natural soil environments. Moreover, given the buffering capacity of soils rich in feldspar and phlogopite minerals with elevated pH levels, exploring the utilization of fungi in greenhouse and field settings on such soils holds promise for enhancing the accessibility of essential elements in soil. This results of the research could serve as a significant step towards harnessing the potential of fungi for improving soil fertility and nutrient availability.

Fig. 9. Scanning electron microscope image, A) feldspar (control), B) feldspar with fungus inoculation, C) phlogopite (control), D) phlogopite with fungus inoculation.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Sanaz Ashrafi-Saiedlou: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Data curation. MirHassan Rasouli-Sadaghiani: Supervision, Project administration, Conceptualization. Abass Samadi: Supervision, Project administration. Mohsen Barin: Supervision, Methodology. Ebrahim Sepehr: Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Fig. 10. The particle size histograms A) feldspar (control), B) feldspar with fungus inoculation, C) phlogopite (control), D) phlogopite with fungus inoculation.

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