

Received September 27, 2020; reviewed; accepted January 04, 2021

Screening of high-efficiency potassium-dissolving strains and optimization of the potassium-dissolving process

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Abstract: The biosolubilization of potassium feldspar (K-feldspar) by potassium-dissolving microorganisms has become a hot research topic. However, the screening of highly efficient potassium-dissolving strains from the soils of mining areas has not been reported. In this study, 82 strains with potassium-dissolving ability were screened from soils collected from a K-feldspar mining area in Suizhou, Hubei Province, China. One of them, JX-20, was a gram-positive is spherical bacteria with smooth edges, which was identified as a new strain by 16S rRNA gene sequencing. Simultaneously, the influences of temperature, initial pH value, inoculation volume, incubation time, shaking speed, K-feldspar concentration, K-feldspar granularity, and the ammonium sulfate dose on the potassium releasing ability of the JX-20 strain were investigated. The results showed that the JX-20 strain had an obvious dissolution effect on K-feldspar. The optimum conditions for the JX-20 strain to remove potassium from K-feldspar were as follows: cultured at 28-30°C for ten days, initial pH value of 7.4-8, 60 mL medium in a 250 mL conical flask, and 170 r/min shaking speed on a rotary shaker. The K-feldspar concentration, inoculation volume, K-feldspar granularity, and ammonium sulfate dose were 2 g/L, 20%, 0.02-0.03 mm, and 0.4 g/L, respectively. Under the above conditions, the highest corrosion efficiency of 39.75% was achieved.

Keywords: microorganism, potassium bacteria, potassium solubilization and release, optimization of potassium-dissolving process

1. Introduction

Potassium is one of the three macronutrients required for plant growth and development, and determines the growth rate of plants (Bakhshandeh et al., 2017). Potassium in plants mainly exists in the cell fluid as free K^+ , which promotes the growth metabolism of the plant. At the same time, it is an essential element in many chemical reactions in plants, not only activating various enzymes to accelerate the metabolic rate, but also promoting the absorption and balance of other elements (Xiao et al., 2018). It can enhance the drought resistance, cold resistance, and stress resistance of plants by regulating the water content of cells. Potassium therefore determines the growth rate of plants (Xiao et al., 2019).

In China, potassium in soils exists in different forms, i.e., ionic, chemical, and mineral, but only 2% of this potassium can be absorbed and used directly by plants. Most potassium is present in minerals in a slow-acting form, and is not easy to absorb and use by plants (Li et al., 2017). Although the soluble potassium resources in China's soil are seriously deficient, the silicate mineral resources, which are rich in insoluble potassium, are extremely rich and widely distributed, mainly in Yunnan, Guizhou, Hubei, and Shandong provinces. The silicate mineral that is most rich in potassium is potassium feldspar (K-

feldspar) (Zhang et al., 2014). The most effective and feasible way to solve the problem of soil potassium deficiency is to convert the slow-acting and inefficient potassium contained in minerals into a quick-acting form of potassium. The extraction of insoluble potassium from K-feldspar can be achieved using physical, chemical, and microbiological methods. In contrast to the other methods, the microbial methods have significant advantages, such as low cost, simplicity, feasibility, mild operating conditions, and little pollution (Zhang et al., 2014).

Some studies have documented the release of potassium during the degradation of silicate minerals by bacteria. Sun (2017) reported that nine strains of potassium-soluble bacteria could be isolated from the soil around crop roots, with the results showing that the optimum carbon and nitrogen sources were sucrose and peptone, respectively. It has been reported that JK-1 bacteria can decompose potassium from minerals to produce concentrations of 13.5 mg/L in solution (Sun et al., 2017). In the same year, a bacterial strain that dissolved potassium was isolated by Bagyalakshmi et al. (2017) from the soil of a tea plant crop in South India. Concentrations of 42 mg/L for potassium and 21 mg/L for phosphorus could be attained (Bagyalakshmi et al., 2017). In 2015, 38 strains of potassium-decomposing bacteria were isolated by Peng Tang from the soil of a mining area in Jishou City, Hunan Province. For one of the dominant *Bacillus* strains named L17, the effective potassium content reached 87.66 mg/L in the fermentation liquid (Tang et al., 2015).

Some researchers have reported the biological characteristics and the mechanisms by which potassium-dissolving bacteria act. However, there have been fewer studies of the silicate bacteria isolated from mining area soils. This is mainly due to the poor soils in mining areas, which makes it more difficult to screen the required microorganisms. In this study, highly efficient potassium-dissolving strains were obtained from soils collected from a K-feldspar mining area in Suizhou, Hubei Province, China. One of the strains, identified as a new strain by 16S rRNA gene sequencing, had a high potassium-dissolving ability, and could potentially be used to remove potassium from K-feldspar. The optimum conditions and the mechanism for removing potassium by the test bacteria was investigated. This study will not only contribute to resolving the major problem of the lack of soil soluble potassium resources in China, but also has great theoretical significance and can be applied to other studies.

2. Materials and methods

2.1. Materials, reagents and medium

Soil source: the root soils of rape, wormwood, and artemisia were collected from a K-feldspar mining area in Suizhou, Hubei Province, China.

Potassium feldspar was obtained from the Suizhou Mining Area, Hubei Province, China. After crushing and sieving, a powder with a grain size of 0.01-0.1mm was obtained. The grains were soaked in ultra-pure water and 3 mol/L hydrochloric acid for 24 and 72 h, respectively, to remove soluble ions from the mineral powder. The powder was then washed 3-5 times with ultra-pure water, until the natural pH was obtained, and the resulting material was dried and preserved (Sheng et al., 2008).

A potassium standard solution (1000 μ g/mL) was purchased from the National Nonferrous Metals and Electronic Materials Analysis and Testing Center.

Basic medium: glucose 10 g, K₂HPO₄ 0.2 g, NaCl 0.2 g, MgSO₄·7H₂O 0.2 g, FeSO₄·7H₂O 0.002 g, MnSO₄·7H₂O 0.2 g, CaCl₂ 0.2g, (NH₄)₂SO₄ 0.4 g, secondary distilled water 1000 mL, and pH 7.4-8.0. The medium was autoclaved at 121°C for 20 min (Lian et al., 2008).

Potassium-dissolving medium: glucose 10 g, K-feldspar powder 2 g, Na₂HPO₄ 0.2 g, NaCl 0.2 g, MgSO₄·7H₂O 0.2 g, FeSO₄·7H₂O 0.002 g, MnSO₄·7H₂O 0.2 g, CaCl₂ 0.2g, (NH₄)₂SO₄ 0.4 g, secondary ultra-pure water 1000 ml, and pH 7.4-8.0. The medium was autoclaved at 121°C for 20 min. Agar was added to a final concentration of 1.6% in the above media to form the solid medium.

All reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Characterization of K-feldspar before and after bacterial erosion

X-ray diffraction (XRD: D/Max-2200, Rigaku, Tokyo, Japan) was used to analyze the crystal structure of the K-feldspar. X-ray fluorescence (XRF: PW4400, Panalytical, Almelo, Netherlands) was used to analyze the type of elements and their concentration in the K-feldspar. Scanning electron microscopy (SEM: JSM-6700F, Jeol, Tokyo, Japan) was used to detect the morphologies and microstructures of the

samples. Fourier transform infrared spectroscopy (FTIR: FIRTracer-100, Shimadzu, Kyoto, Japan) was used for the analysis of the functional groups of metabolites, enabling the possible potassium-dissolving mechanism of the test bacteria to be investigated.

2.3. Characterization of the experimental methods

2.3.1. Screening and isolation of potassium-dissolving bacteria

Multiple enrichment culture: One-hundred-grams of mixed root soils was added to 1000 ml of sterile water. The solution was stirred evenly and filtered through a gauze. Five-milliliters of the soil suspension was added to a 250 ml conical flask containing 60 ml of sterilized basic medium. The suspension was then cultured at 30°C for two days in a rotary shaker (170 rpm), and was referred to as the first enrichment culture. Five-milliliters of the fermentation liquid was taken from the first enrichment culture solution, the amount of K_2HPO_4 in the basic medium was halved, and the suspension was then cultured under the same conditions. This suspension was referred to as the second enrichment culture. Multiple enrichments were cultured until the solution was clear. The amount of K_2HPO_4 was sequentially reduced to improve the adaptability of microorganisms on the potassium-dissolving medium.

Separation and purification of strains: after multiple enrichment with pipette tips, 200 ml of the clarified bacterial solution was evenly coated on a potassium-dissolving solid medium and cultured at 30°C for 3-5 days in a constant temperature incubator. Bacterial colonies were isolated and purified several times until single bacteria were visible under a microscope. The bacteria were then slant cultured at 30°C for 3-5 days and conserved at 4°C (Lian et al., 2008).

2.3.2. Morphological and gene sequencing identification of the dominant potassium-dissolving bacteria

Strains were identified by conventional methods according to their morphological, physiological, and biochemical characteristics. Genes were also sequenced by the Shanghai Meiji Biological Co., Ltd (Shanghai, China). The sequencing primers were 5'-AGAGTTTGATCCTGGCTCAG-3'(27F) and 5'-GGTACCTTGTTACGACTT-3'(1492R). The reaction conditions were as follows: 95°C for 5 min, 95°C for 30 sec, 56°C for 30 sec, 72°C for 90 sec, and 72°C for 10 min, over a total of 25 cycles. Finally, the species were identified by comparison with the National Center for Biotechnology Information -Nucleotide (NCBI-NT) database.

2.3.3. Determination of the potassium-dissolving ability of potassium-dissolving strains

The effective potassium concentration in solution was determined by atomic absorption spectroscopy (AAS). The potassium standard solution was diluted to 0.6 mg/L and a potassium calibration curve was constructed.

First, the preserved dominant potassium-dissolving strains were cultivated at 30°C for 3-5 days. Then a small amount of bacteria were removed with the inoculation loop to prepare a specific concentration of bacterial suspension for later use.

Second, the potassium-dissolving medium was prepared, and a certain amount of medium was placed in a 250 mL conical flask together with the different concentrations of potash feldspar and then autoclaved at 121°C for 20 min. The bacterium to be tested was inoculated into the sterilized liquid potassium-dissolving medium with an inoculation of the set volume, then cultured under different conditions. There were three parallel experiments per sample. The control experiment was not inoculated. After fermentation, the pH of the fermentation suspension was measured using a pH meter and then centrifuged at 9000 rpm for 20 min in a high-speed centrifuge. The supernatant was filtered through a 0.45 mm microfiltration membrane before the AAS analysis.

2.3.4. Optimization of the potassium dissolving process for the dominant potassium-dissolving strain

Using the single factor method, the effects of cultivation time, temperature, and shaking speed, as well as the inoculation volume (v:v %), K-feldspar concentration (g/L), granularity (mm), pH of the culture

medium, the ammonium sulfate dose and other factors on the potassium-dissolving ability of the dominant bacteria were investigated. Bacteria were cultured and measured by the methods described in Section 2.3.3. The control group was not inoculated. Each group was analyzed three times.

3. Results and discussion

3.1. Mineral phases

The mineral assembly used in this study was a potassium-rich shale (Table 1) collected from the Suizhou Mining Area, Hubei Province, China. The mineralogical and chemical compositions of the K-feldspar are shown in Tables 1 and 2.

Table 1. Mineralogical composition of the potassium feldspar

| Mineralogical composition (%) by XRD ^a | |
|---|------------------------------------|
| KAlSi ₃ O ₈ | NaAlSi ₃ O ₈ |
| 58.21% | 41.79% |

Table 2. Chemical composition of the potassium feldspar

| Chemical compositions (%) by XRF ^b | | | | | |
|---|--------------------------------|--------------------------------|-------|------------------|-------------------|
| SiO ₂ | Al ₂ O ₃ | Fe ₂ O ₃ | MgO | CaO | Na ₂ O |
| 64.81 | 18.07 | 1.63 | 0.17 | 0.82 | 3.67 |
| K ₂ O | TiO ₂ | P ₂ O ₅ | MnO | H ₂ O | ignition loss |
| 9.40 | 0.068 | 0.22 | 0.016 | 0.08 | 0.56 |

^a X-ray powder diffractometry, Rigaku D/Max-2200, CuKα at 40 kV and 30mA, and 30/min scan rate

^b X-ray fluorescence spectrometry, Panalytical Axios PW4400

Table 1 shows that the K-feldspar was mainly composed of albite and micro-plagioclase. The micro-plagioclase belonged to the triclinic system and had a stable structure. Table 2 shows that the mine was potassium-rich, with the composition of K₂O reaching 9.4%.

3.2. Screening and identification of potassium-dissolving strains

Eighty-two strains with potassium-dissolving ability were screened from the soil samples of the mining area, six of them had a higher potassium-dissolving ability under the same experimental conditions. At the same time, the six strains were respectively carried out to understand the potassium process optimization experiment, the results show that under the optimized conditions, the strain which named JX-20 with the highest potassium-dissolving ability and was selected for use in our experiments. A photograph of the potassium-dissolving solid medium and a gram stain of the JX-20 strain are shown in Fig. 1.

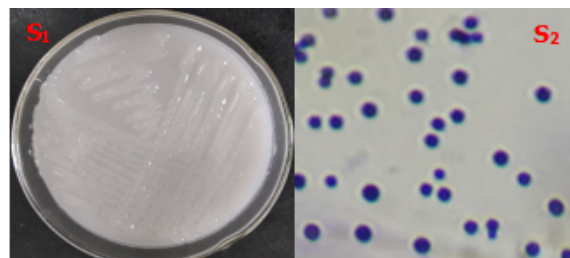


Fig. 1. Photograph of the potassium-dissolving solid medium and microscopic observation (x100) of the JX-20 strain. S1: Colony morphology; S2: Gram stain

Fig. 1 shows the colony morphology and microscopic observation (x100) of the JX-20 strain. The JX-20 strain was spherical bacteria, with a milky appearance. The surface of the colony was smooth,

transparent, and convex. A gram-positive bacterium was observed under a 100-fold magnification using oil immersion with a light microscope. The JX-20 strain was identified as a new strain by 16S rRNA gene sequencing.

3.3. Determination of the potassium-dissolving ability of the potassium-dissolving strain

The effectiveness of the potassium dissolving ability of the JX-20 strain was investigated for different culture times. The results were characterized by SEM, as shown in Fig. 2.

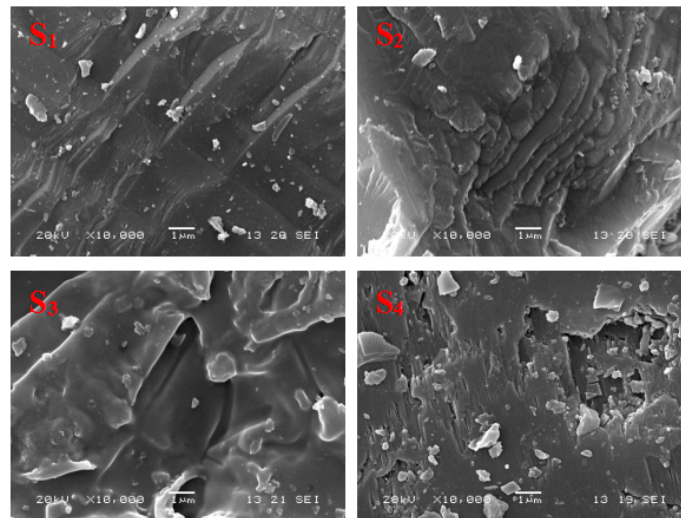


Fig. 2. Scanning electron micrograph of potassium feldspar decomposed by the JX-20 strain at different culture times. S1: 0 days, S2: 4 days, S3: 8 days, S4: 10 days

In Fig. 2, S1–S4 are SEM images of K-feldspar decomposed by the JX-20 strain under different culture times of 0, 4, 8, and 10 days, respectively. It was apparent that with an increase in the culture time, the surface of the K-feldspar displayed a more significant dissolution phenomenon. In the S1 SEM image the mineral surface had obvious edges and corners, without corrosion by the JX-20 strain. After 4 days, the surface was covered with bacteria. As the dissolution time increased, the mineral surface changed significantly. In the S3 SEM image, the mineral surface was covered with a film and pits following eight days of microbial erosion. It was speculated that the film may be metabolites of the microorganisms, with the mineral surface then destroyed by the metabolites, resulting in pits of different sizes on the surface (Xiao et al., 2018). After 10 days, this phenomenon was more obvious, and there were even potholes on the mineral surface. These images indicate that the mineral structure was destroyed due to erosion by the JX-20 strain.

To investigate the possible mechanism by which the bacteria dissolved potassium, the microbial metabolites were analyzed by FTIR. The results are shown in Fig. 3.

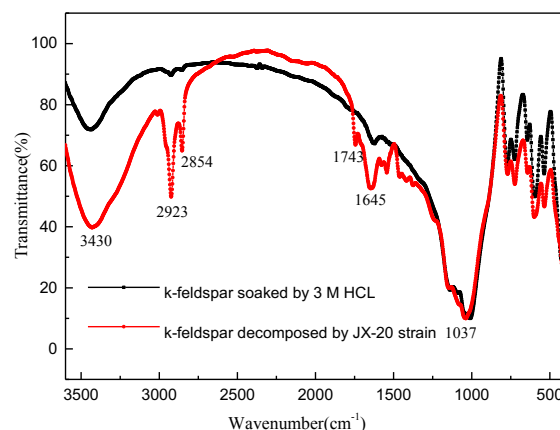


Fig. 3. FTIR curves of potassium feldspar soaked in hydrochloric acid and decomposed by the JX-20 strain

To produce the results shown in Fig. 3, K-feldspar was soaked in 3 M hydrochloric acid and decomposed by the JX-20 strain for 10 days. Several distinct absorption peaks were apparent at 2923, 2854, 1743, 1645, and 1037 cm^{-1} following corrosion by the JX-20 strain. It was surmised that the microbial metabolites may be carboxylates, with the pH of the fermentation liquid declining during microbial dissolution of K-feldspar. Therefore, the potential potassium dissolving mechanism of the bacteria may be as follows. Metabolites such as organic acids not only chemically degrade minerals, but also promote the active dissolution of minerals by microorganisms in the absence of potassium to promote their own growth. Bacterial-mineral complexes were formed with the proliferation of bacteria, which could promote mineral corrosion of k-feldspar, destroy the mineral lattice, and release potassium ions (Parmar et al., 2008; Xiao et al., 2017; Prajapati et al., 2012; Rajawat et al., 2016).

3.4. Optimization of the potassium dissolving process in the dominant potassium-dissolving strain

3.4.1. Construction of a potassium calibration curve

A potassium calibration curve was constructed using the standard material as described in Section 2.3.3. The regression equation was $y = 0.6922x + 0.0034$, $R^2 = 0.9999$.

3.4.2. Determination of total potassium in K-feldspar

To determine the total potassium in minerals, high temperature calcination and nitrification are commonly used methods (Xiao et al., 2011). The minerals were successively washed with water, hydrochloric acid, nitric acid, perchloric acid, and an acid mixture (nitric acid and perchloric acid). The total potassium in K-feldspar was found to be 69.73 mg/g k-feldspar, using the above method.

3.4.3. The growth curve of the JX-20 strain

According to the method described in Section 1.3.3, after inoculating the JX-20 strain under different incubation times, the optical density at 600 nm (OD_{600}) and pH of the fermentation liquid were determined every two days. The results are shown in Fig. 4.

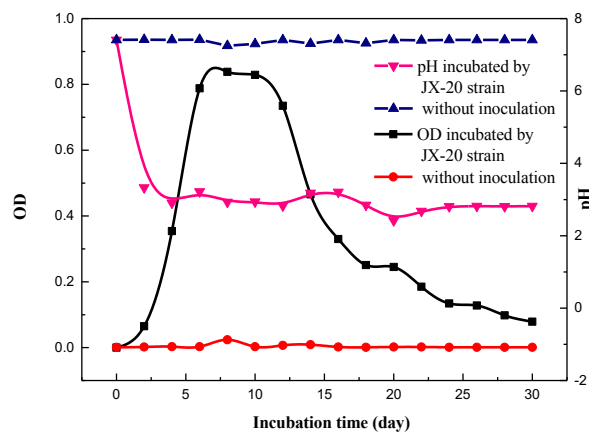


Fig. 4. The growth curve of the JX-20 strain

From Fig. 4, it can be seen that as the incubation time increased, the number of bacteria rapidly increased. The increase in the number of bacteria slowed after six days and gradually declined after 10 days. It was concluded that the first two days were a period of delayed bacterial growth, while the logarithmic growth phase of bacteria occurred over days 2-6, and days 6-10 represented a stable period, with the population beginning to decline after 10 days. After the inoculation of bacteria, the number of bacteria increased rapidly and the OD_{600} reached a maximum of 0.829 on the eighth day. This may be due to the lack of nutrients in the culture medium for bacterial growth, which restricted bacterial growth. As the incubation time increased to five days, the pH of the fermentation liquid rapidly declined. After five days, there was less change in the pH. The results proved that organic acid metabolites were produced during the microbial growth phase. Organic acids may effectively promote the microbial

dissolution of K-feldspar, which would be consistent with previous analyses of the possible mechanisms by which bacteria dissolve potassium (Xiao et al., 2017; Rajawat et al., 2016).

3.4.4. Effect of different culture times on the soluble potassium content of the culture medium

Following the method described in Section 1.3.4. Meanwhile, in the experiments, the cultivation temperature, shaking speed, pH of the culture medium, the K-feldspar concentration and granularity, the inoculation volume, the ammonium sulfate dose are set to 30 °C, 170 rpm, 7.4-8.0, 2 g/L, 0.02-0.03 mm, 20%, 0.4 g/L, respectively. After inoculating the JX-20 strain, the soluble potassium concentration the culture solution and pH of the fermentation liquid were measured every two days. The dissolution rate of the JX-20 strain was also obtained at the same time. The experimental results are shown in Fig. 5.

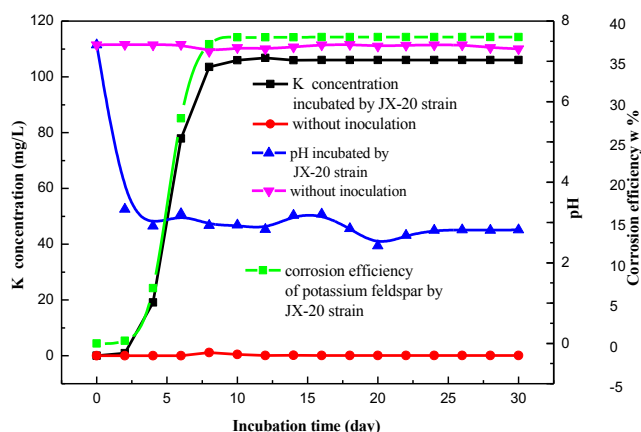


Fig. 5 Effect of different culture times on the soluble potassium content in the culture medium

From Fig. 5, it can be seen that as the incubation time increased, the soluble potassium content rapidly increased over the first eight days. Days 8-10 experienced a slower increase. After more than ten days, the potassium content in the solution changed only slightly, reaching a maximum of 106.06 mg/L. At the same time, the corrosion efficiency was 38.02%. According to our analysis, the consumption of the carbon sources reduced the amount of organic acids secreted by the microorganisms, and the potassium dissolving activity of the bacteria was also reduced (Liu et al., 2015; Zhou et al., 2006; Xiao al., 2015). As mentioned previously, the pH of the fermentation liquid rapidly declined in the first five days. After five days, there was less change in the pH, which was consistent with the increase in the organic acid content. This provides further evidence that the possible mechanism by which bacteria dissolve potassium is due to the secretion of organic acids (Xiao al., 2015).

3.4.5. Effect of the pH of the fermentation liquid on the soluble potassium content of the culture medium

To further clarify the effect of the amount of organic acid secreted by bacteria on the ability of bacteria to dissolve potassium, the relationship between the soluble potassium content of the culture medium and pH of the fermentation liquid are shown in Fig. 6.

From Fig. 6, it can be seen that as the pH of the fermentation liquid increased, the potassium content in the solution and the corrosion efficiency rapidly declined. At a pH above 3, there was almost no change. At pH 2.83, the potassium content in the solution and the corrosion efficiency reached maximum values of 106.06 mg/L and 38.02% respectively. This result was consistent with previous findings.

3.4.6. Effect of the cultivation temperature on the soluble potassium content of the culture medium

Using the method described in Section 1.3.4, the ability of microbes to dissolve potassium was studied under temperatures of 20, 24, 26, 28, 30, 32, 36, and 40°C. In the experiments, the culture times, shaking speed, pH of the culture medium, the K-feldspar concentration and granularity, the inoculation volume,

the ammonium sulfate dose are set to ten days, 170 rpm, 7.4-8.0, 2 g/L, 0.02-0.03 mm, 20%, 0.4 g/L, respectively. The soluble potassium content of the culture solution was measured after it was cultured on a rotary shaker at 170 rpm for 10 days. The experimental results are shown in Fig. 7.

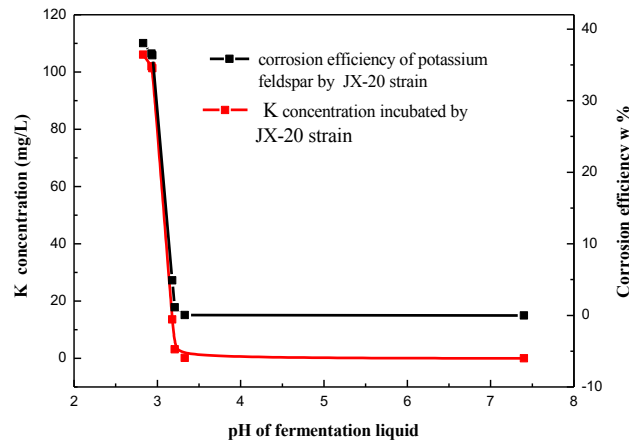


Fig. 6. Effect of the pH of the fermentation liquid on the soluble potassium content of the culture medium

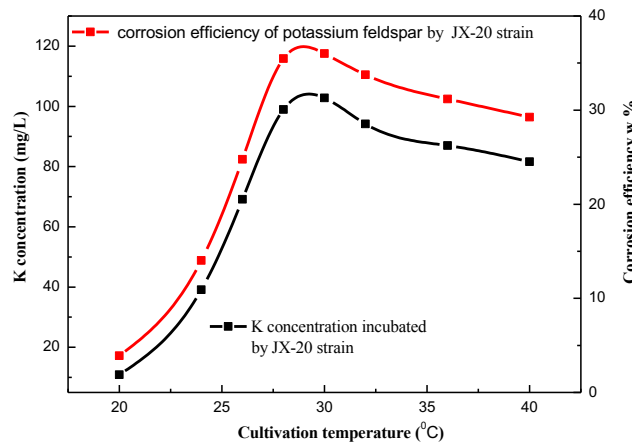


Fig. 7. Effect of the cultivation temperature on the soluble potassium content of the culture medium

It can be seen from Fig. 7 that as the cultivation temperature increased, the potassium content in the solution and the corrosion efficiency first increased and then decreased. The JX-20 strain had the strongest potassium-dissolving ability at a cultivation temperature of 28-30°C. The highest potassium content and corrosion efficiency were 98.95-102.80 mg/L and 35.48-36.01%, respectively. When the cultivation temperature was lower than 28-30°C the soluble potassium content decreased, while at temperatures higher than 28-30°C it still decreased, but not as substantially. It was considered that the cultivation temperature had a large effect on the growth of microorganisms. If the culture temperature was too high or too low, it was not conducive to the growth of microorganisms (Xiao et al., 2017). The ability of bacteria to dissolve potassium was also reduced under these conditions. An appropriate culture temperature is necessary for the growth of microorganisms and to facilitate their ability to dissolve potassium, further promoting the release of potassium from K-feldspar.

3.4.7. Effect of pH of the culture medium on the soluble potassium content of the culture medium

Using the method described in Section 1.3.4, before inoculating with the JX-20 strain the initial pH of the culture medium was adjusted to 3, 5, 6, 7, 7.4, 8, 9, or 10 by hydrochloric acid or sodium hydroxide. After inoculating at 30°C on a rotary shaker at 170 rpm for 10 days, and the K-feldspar concentration and granularity, the inoculation volume, the ammonium sulfate dose are 2 g/L, 0.02-0.03 mm, 20%, 0.4 g/L, respectively. The soluble potassium content of the culture solution was measured, the experimental results are shown in Fig. 8.

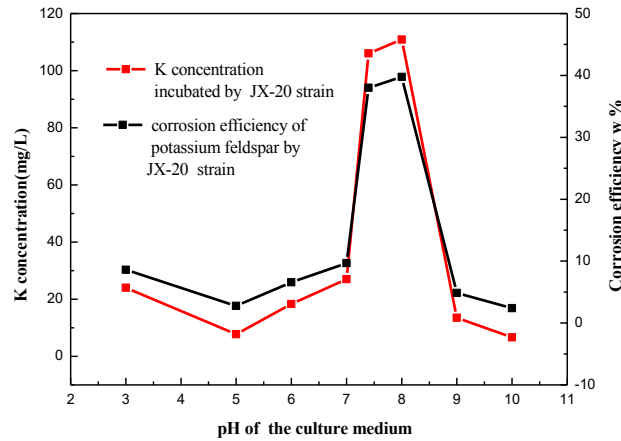


Fig. 8. Effect of pH of the culture medium, on the soluble potassium content of the culture medium

From Fig. 8 it can be seen that as the initial pH increased, the potassium concentration in the solution and the corrosion efficiency first increased and then decreased. The ability of bacteria to dissolve potassium was strongest when the initial pH was 7.4-8. Within this range, the soluble potassium content of the culture solution and the corrosion efficiency were highest, reaching maximum values of 106.06-110.85 mg/L and 38.02-39.75%. When the initial pH was higher or lower than 7.4-8, the soluble potassium content of the culture solution was significantly decreased. As with the cultivation temperature, pH has a strong impact on the growth and reproduction of microorganisms. If the initial pH was too high or too low, it was not suitable for microbial growth (Xiao et al., 2015). During the process of bacteria dissolving K-feldspar, a suitable initial pH was more conducive to the growth of microorganisms and the activity of potassium dissolution.

3.4.8. Effect of shaking speed during cultivation on the soluble potassium content of the culture medium

Using the method described in Section 2.3.4, the effect of shaking speed during cultivation on the soluble potassium content was investigated. In the experiments, the speed of the rotary shaker was set to 130, 150, 170, 190, or 210 rpm. A culture in a static device was used as the control. After inoculating for ten days at 30°C, and the the K-feldspar concentration and granularity, pH of the culture medium, the inoculation volume, the ammonium sulfate dose are set to 2 g/L, 0.02-0.03 mm, 7.4-8.0, 20%, 0.4 g/L, respectively. The soluble potassium content of the culture solution was measured. The results are shown in Fig. 9.

It can be seen from Fig. 9 that the JX-20 strain had the strongest potassium-dissolving ability at a rotation speed of 170 rpm. The soluble potassium content and the corrosion efficiency were highest at

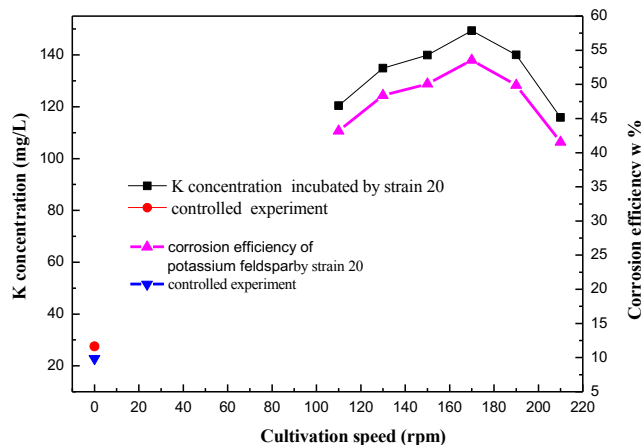


Fig. 9. Effect of shaking speed during cultivation on the soluble potassium content of the culture medium

170 rpm, with maximum values of 105.65 mg/L and 37.89%. When the rotation speed was lower than 170 rpm, an increase in the rotational speed increased the soluble potassium content. In contrast, when the rotation speed was higher than 170 rpm, an increase in the rotational speed decreased the soluble potassium content. This may be because the higher speed shear could reduce the number of bacteria, resulting in the ability to dissolve potassium to also be reduced (Xiao et al., 2019). However, compared with the static culture, the soluble potassium content and the corrosion efficiency were clearly higher than in a shaken culture. This was mainly because the JX-20 strain is aerobic bacteria, and shaking would stimulate the growth of bacteria, further improving the ability of the microorganisms to dissolve potassium.

3.4.9. Effect of the K-feldspar concentration on corrosion efficiency

Using the method described in Section 2.3.4, the soluble potassium content in the culture medium was studied under different K-feldspar concentrations. The K-feldspar concentration was 1, 2, 4, 6, 8, 10, or 12 g/L. After inoculating under 30°C on a rotary shaker at 170 rpm for ten days, and the K-feldspar granularity, the inoculation volume, pH of the culture medium, the ammonium sulfate dose are 0.02-0.03 mm, 20%, 7.4-8.0, 0.4 g/L, respectively. The corrosion efficiency was measured. The results are shown in Fig. 10.

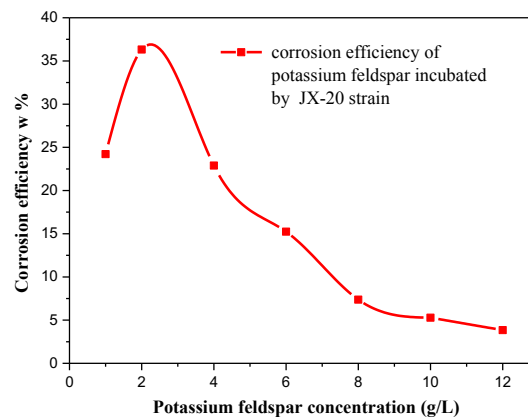


Fig. 10. Effect of the K-feldspar concentration on corrosion efficiency

From Fig. 10, it can be seen that the ability of bacteria to dissolve potassium was strongest when the K-feldspar concentration was 2 g/L, with the maximum corrosion efficiency reaching 36.32%. When the K-feldspar concentration was higher or lower than 2 g/L, the corrosion efficiency was significantly decreased. This may be due to microorganisms being continuously consumed in the process, and therefore even when the amount of K-feldspar increased there were no more microorganisms present to release potassium.

4.3.10. Effect of the ammonium sulfate dose on the soluble potassium content of the culture medium

According to the method described in Section 2.3.4 the effect of the ammonium sulfate dose on the soluble potassium content was investigated. The ammonium sulfate was applied at doses of 0.2, 0.4, 0.6, 0.8, 1.0 or 1.2 g/L. A medium without the addition of ammonium sulfate was used as the control. After being cultured at 30°C and at 170 rpm for ten days, after inoculating at 30°C on a rotary shaker at 170 rpm for 10 days, and the K-feldspar concentration and granularity, pH of the culture medium, the inoculation volume are set to 2 g/L, 0.02-0.03 mm, 7.4-8.0, 20%, respectively. the soluble potassium content in the culture solution was determined. The experimental results are shown in Fig. 11.

It can be seen from Fig. 11 that as the ammonium sulfate dose increased the potassium content in the solution and the corrosion efficiency first increased and then decreased. The soluble potassium content and the corrosion efficiency were highest when 0.4 g ammonium sulfate was added to 1 L of culture medium, with maximum values of 107.90 mg/L and 38.68%, respectively. The results for the control experiments were lower at only 82.64 mg/L and 29.63%, respectively. When the ammonium sulfate dose was higher or lower than 0.4 g/L, the potassium content in the solution and the corrosion

efficiency were significantly smaller (Bagyalakshmi et al., 2017). A suitable inorganic nitrogen dose could not only promote the growth of bacteria, but would also benefit the production of organic acids by bacteria.

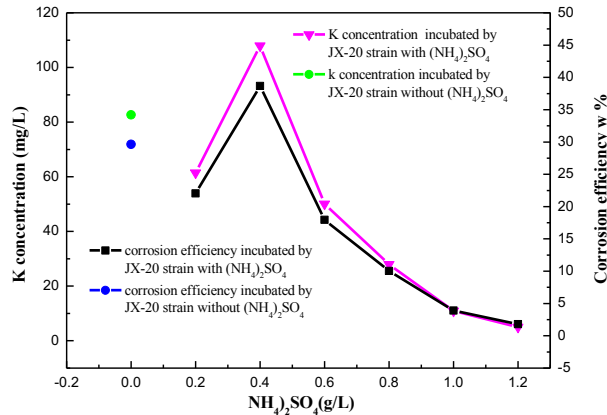


Fig. 11. Effect of the ammonium sulfate dose on the soluble potassium content of the culture medium

4.3.11. Effect of the inoculation volume on the soluble potassium content of the culture medium

Using the method described in Section 2.3.4, the effect of the inoculation volume on the soluble potassium content was studied under different inoculation volumes. The inoculation volume was 5, 10, 15, 20, 25, or 30 (v:v %). After inoculating the culture at 30°C on a rotary shaker at 170 rpm for ten days, and the K-feldspar concentration and granularity, pH of the culture medium, the ammonium sulfate dose are 2 g/L, 0.02-0.03 mm, 7.4-8.0, 0.4 g/L, respectively. The soluble potassium content of the culture solution was determined. The results are shown in Fig. 12.

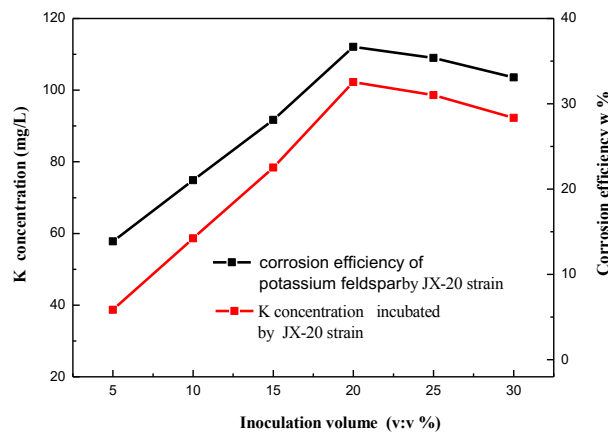


Fig. 12. Effect of the inoculation volume on the soluble potassium content of the culture medium

From Fig. 12, it can be seen that the soluble potassium content and the corrosion efficiency were highest at an inoculation volume of 20%, with maximum values of 102.26 mg/L and 36.67%, respectively. When the inoculation volume ranged from 5% to 20%, as the inoculation volume increased, the potassium content and the corrosion efficiency rapidly increased. When the inoculation volume exceeded 20%, the potassium content and the corrosion efficiency decreased as the inoculation volume increased, but the changes were small. This was largely because the culture medium could not maintain the growth and reproduction of bacteria under these conditions, and therefore the amount of organic acid secreted by bacteria was reduced, resulting in a decline in the potassium dissolution activity of the bacteria (Xiao et al., 2017).

4.3.12 Effect of K-feldspar granularity on the soluble potassium content of the culture medium

Using the method described in Section 2.3.4, the effect of K-feldspar granularity on the soluble potassium content was investigated. After inoculating the culture at 30°C on a rotary shaker at 170 rpm

for ten days, and the K-feldspar concentration, pH of the culture medium, the inoculation volume, the ammonium sulfate dose are 2 g/L, 7.4-8.0, 20%, 0.4 g/L, respectively. the soluble potassium content in the culture solution was measured. The results are shown in Fig. 13.

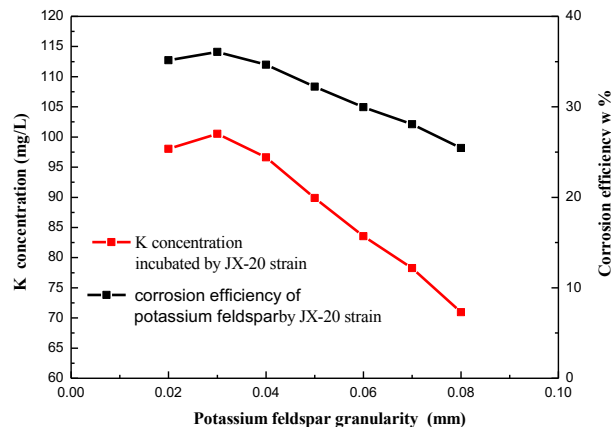


Fig. 13. Effect of K-feldspar granularity on the soluble potassium content of the culture medium

It can be seen from Fig. 13 that as the K-feldspar particle size increased, the potassium content and the corrosion efficiency first increased and then decreased. The ability of bacteria to dissolve potassium was highest at a K-feldspar granularity of 0.02-0.03 mm. Within this range, the soluble potassium content and the corrosion efficiency had maximum values of 98.05-100.54 mg/L and 35.16-36.06%, respectively. At a K-feldspar granularity above 0.04 mm, the ability of the microorganism to dissolve potassium declined. This was because the finer the ore powder, the larger the contact surface with the culture solution and the bacterial liquid, and the more soluble potassium was therefore released (Rajawat et al., 2016).

4. Conclusions

This study confirmed that the JX-20 strain isolated from a soil in a K-feldspar mining area in Suizhou, Hubei Province, China could efficiently solubilize potassium from a K-feldspar medium. The strain was a gram-positive spherical bacterium, with smooth edges. It was identified as a new strain according to its morphological, biochemical, and physiological characteristics, as well as the results of a 16S rDNA gene sequence analysis.

The optimum conditions for the JX-20 strain to remove potassium from K-feldspar were as follows: cultured at 28–30°C for ten days, initial pH value of 7.4–8, 60 mL medium in a 250 mL conical flask, and placed on a rotary shaker at 170 rpm. The k-feldspar concentration, inoculation volume, K-feldspar granularity, and ammonium sulfate dose were 2 g/L, 20%, 0.02-0.03 mm, and 0.4 g/L, respectively. The corrosion efficiency was highest under these conditions, with a maximum value of 39.75%. The thermodynamics and kinetics of the dominant potassium-dissolving strain will be determined in future studies.

Acknowledgments

This work was kindly supported by the National Natural Science Foundation of China (51674178) and National key R & D Program (2018YFC1801800). The authors also thank the center of testing and analysis, Wuhan Institute of Technology, and China University of Geosciences for their kind support.

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