



Bio-desilication of rutile concentrate and analysis of community structure in bio-desilication reactor

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Abstract: The original strain HY-7 was isolated from the bauxite mine drainage (BMD) taken from a reservoir in Sanmenxia Mine, Henan Province, China. The optimum temperature and pH for the growth of strain HY-7 were 30 °C and 7.0, respectively. The optimum UV radiating time was 20 s and the positive mutation rate was 23.0%. The growth curves show that strain HY-7 needs 144 h to reach the stationary phase after its mutagenesis, which is 24 h earlier than that of the original strain. Sequence homology analysis indicated that this community consisted of mainly two branches: one sharing high homology with *Paenibacillus stellifer* and the other sharing high homology with *Sporolactobacillus laevolacticus*. The experimental results showed that the TiO₂ grade of rutile concentrate increased from 78.21% to 91.80% and the recovery of TiO₂ reached 95.24% after 7 d of bioleaching. The bio-desilication process can not only effectively improve the TiO₂ grade of rutile concentrate but also meet the requirements of environmental protection.

Key words: bio-desilication; rutile concentrate; silicate bacteria; UV mutagenesis; community structure

1 Introduction

Bioleaching of sulfide ores with chemoautotrophic bacteria such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* is a well-known process in biohydrometallurgy [1,2]. It has been widely used to recover copper from low grade sulphide ores [3–5] and to treat gold concentrate by pre-biooxidation in order to increase the recovery of finely disseminated gold from refractory ores containing pyrite and arsenopyrite [6–8].

Compared with sulfide minerals, biohydrometallurgy of non-sulfide minerals has received little attention so far. Several studies on the bio-desilication of bauxite have been reported that the grade of bauxite could be enhanced by removing impurities such as silica, calcium, and iron with *Bacillus circulans* and *Bacillus mucilaginosus* [9–11].

However, to our best knowledge, the bio-desilication of rutile using silicate bacteria has not

yet been reported. The TiO₂ grades of rough concentrates recovered by the traditional enrichment processes are generally around 80% [12–14]. High quality rutile concentrate can only be obtained by the use of the acid leaching process, but this process can lead to serious environmental pollution. Therefore, it is of vital importance to develop a new and environmentally-friendly technology to purify the rutile concentrate.

In this research, a bio-desilication technology was employed for the purification of rough rutile concentrate. Silicate and other impurities were removed from the surface of rutile grains through the corrosive function of silicate bacteria. After 7 d of bioleaching, the TiO₂ grade of the concentrate increases from 78.21% to 91.80%, and the recovery reaches 95.24%. The optimum growth conditions of strain HY-7 were obtained through a series of experiments. Furthermore, analysis on the diversity of the microbial communities was conducted as well as the physiological and biochemical characterizations of strains.

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2 Experimental

2.1 Rough rutile concentrate

Rutile ore sample was obtained from the Nanzhao Rutile Mines in Henan Province, China. The raw rutile was crushed, ground and separated in our laboratory to obtain rutile concentrate used in this work. The chemical and mineralogical compositions of rutile concentrate are listed in Table 1.

Table 1 Chemical and mineralogical compositions of rough rutile concentrate

Chemical composition	Mass fraction/%	Mineralogical composition	Mass fraction/%
TiO ₂	78.21	Rutile	75–80
SiO ₂	14.84	Quartz	10–12
K ₂ O	1.66	Feldspar	5–7
MgO	0.21	Hornblende	1–2
CaO	0.67	Mica	1–2
Al ₂ O ₃	2.92	Limonite	1–2
Fe ₂ O ₃	1.91	Others	1

2.2 Culture medium

Solid medium used for isolation and purification of silicate bacteria is composed of the following ingredients: 2.0 g/L glucose, 1.6 g/L K₂HPO₄, 0.2 g/L MgSO₄, 1 g/L CaCO₃, 0.005 g/L FeCl₃, 1 g/L silica powder and 10–20 g/L agar. The pH of the medium was adjusted to 6.8–7.2 using 10% sulfuric acid solution. Solid medium was sterilized at 121 °C for 20 min before use.

Liquid medium for cultivation of silicate bacteria contains the following reagents: 5 g/L glucose, 0.2 g/L K₂HPO₄, 0.2 g/L MgSO₄, 0.1 g/L CaCO₃, 0.005 g/L FeCl₃, 1 g/L rutile and 1 g/L NH₄NO₃.

2.3 Silicate bacteria

Two reference strains (named strains I and II) belonged to *Bacillus edaphicus* and *B. mucilaginosus*, respectively. They were provided by the Soil and Fertilizer Institute, Chinese Academy of Agricultural Sciences. The bacterial strain (named HY-7) used in the experiments was isolated and conserved in our laboratory from the bauxite mine drainage (BMD) taken from a reservoir in Sanmenxia Mine, Henan Province, China.

Serial dilution was adopted to isolate the microorganism on the agar solid medium (1.5%, ratio of mass to volume). Strain HY-7 was cultured in 250 mL Erlenmeyer flasks containing culture media at pH 7 in a shaker at 200 r/min and 30 °C for 7 d. The appropriately diluted samples were monitored by counting with Hemacyte counter.

The total DNA of strain HY-7 and reference strains

were extracted and G+C content was analyzed by thermal denaturation temperature method [15]. The DNA–DNA homologies between HY-7 and the reference strains were analyzed by the re-association rate of the DNA [16] utilizing a UV300 ultraviolet spectrophotometer.

The strain HY-7 was grown in 250 mL Erlenmeyer flasks for 7 d in a shaker. The initial cell concentration was 1×10^3 cell/mL. Factors that influence microbial growth, such as temperature, environmental pH, rotation speed, and pulp density, were optimized for the strain HY-7.

2.4 UV mutagenesis of HY-7

The experiment was carried out under the following conditions. The UV wavelength was 2537 Å, the power was 15 W and the distance was 30 cm, and the radiation interval time was 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 s, respectively. The cultures were radiated by UV and stirred with a bent rod. After radiation, 1 mL culture was transferred to a solid medium plate for lethal rate calculation. Another 1 mL culture radiated by UV was transferred into 100 mL liquid medium in 250 mL Erlenmeyer flask. Additional 0.3% lithium chloride was added to assist the mutagenesis. The effectiveness of the positive mutation for strain HY-7 was evaluated by the desilication rate of rutile.

2.5 Bio-desilication of rutile concentrate

Bio-desilication experiments for purification of rutile concentrate were performed in 250 mL Erlenmeyer flasks using the mutation. The final cell density in culture medium was 1×10^9 cell/mL and pH was 7. The mineral concentration was adjusted to 10% (ratio of mass to volume) by adding rutile concentrate. Bio-desilication experiments were performed in a shaker at 200 r/min and 30 °C for 10 d. The abiotic controls were also designed in these experiments. The experiments were carried out in triplicate.

2.6 Analysis of community structure in leachate at end of bioleaching

2.6.1 DNA extraction and purification [17]

Total community DNA was extracted from filtered sediment using a protocol described by ZHOU et al [18]. The crude DNA was further purified using the Promega Wizard DNA clean-up system (Madison, WI, USA) according to the manufacturer's instruction. DNA quality was evaluated by the gel electrophoresis with 1% agarose gel stained with 0.5 µg/mL ethidium bromide. DNA quality and concentration were also evaluated by the absorbance ratios at $A_{260/280}$ and $A_{260/230}$ using a NanoDrop ND-1000 spectrophotometer (NanoDrop

Technologies Inc., Wilmington, DE). The purified DNA was stored at -80°C before use.

2.6.2 PCR amplification, purification, 16S rRNA gene amplification and cloning

PCR amplification was conducted in reaction mixtures containing 100 ng DNA template, 1×PCR buffer (10 mmol/L tris-HCl (pH 8.3), 50 mmol/L KCl and 2 mmol/L MgCl_2), 2 mmol/L dNTPs, 5 pmol/L forward primer and 5 pmol/L reverse primer, 2.5 U Taq polymerase (Invitrogen, Shanghai, China), and deionized water to a final volume of 50 μL . Universal primer for 16S rDNA was Bac 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and U1492R (5'-CGG TTA CCT TGT TAC GAC TT-3') [19]. The length of the amplification product was 1500 bp. The used thermal cycling protocol included an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 90 s. A final extension step of 72°C for 7 min was also used. Negative controls with DDH_2O as the template were performed for all PCR reactions [20].

Purified PCR products were later ligated into pCR[®]2.1 vector of TOPO TA cloning kit (Invitrogen Corporation, Carlsbad, CA) and then transferred to one Shot[®]TOP10 *Escherichia coli* competent cell (Invitrogen Corporation, Carlsbad, CA). Thereafter, the converted competent germ was spread on the LB solid medium containing ampicillin (100 mg/mL) and X-gal (15 mg/mL) for blue-white screening. Positive clones were picked to 0.5 mL LB medium by sterilized 200 μL spearhead. White colonies from each of these libraries were randomly selected and placed into the shaker and cultured at 250 r/min and 37°C for 24 h. Finally, they were added into the mixed liquor of 0.5 mL LB carrier and 40% glycerin and kept in -80°C refrigerator for sequencing together after all the clone libraries were finished.

2.6.3 Phylogenetic analysis

The nucleotide sequences were assembled and edited using Sequencer v.4.8 (GeneCodes, Ann Arbor, MI) and manually checked for chimeras using Ribosomal Database Project II. Identified chimeric sequences were discarded. Phylogenetic trees were built with MEGA-4 (1000 times of bootstrap) by using neighbor-joining analysis, which showed the phylogenetic relationships of bacterial 16S rRNA gene sequences recovered from the BMD samples [21,22].

2.7 Analytical methods

The physicochemical analyses of the samples were performed at Henan Provincial Rocks and Minerals Testing Center, China. Flame atomic absorption spectrometry was used for measuring metal ions. The pH value was measured with a pH meter (pHS-25). The lethal rate was determined by plating, and the

effectiveness of the positive mutation of strain HY-6 by the generation time.

3 Results and discussion

3.1 Microorganism strains

3.1.1 Morphological characteristics of strain HY-7

After culturing at 28°C for 48 h, the colonies of strain HY-7 were round and transparent with a regular edge and a moist surface (Fig. 1). The colonies were soft and stringy when they touched with an inoculating loop. This strain is extremely similar to reference strain I. The cells are rod-shaped with $(0.8\text{--}0.9)\mu\text{m} \times (2.2\text{--}2.4)\mu\text{m}$ in size (Fig. 2).

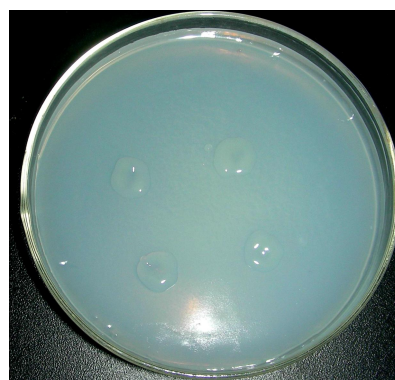


Fig. 1 Colony morphology of strain HY-7

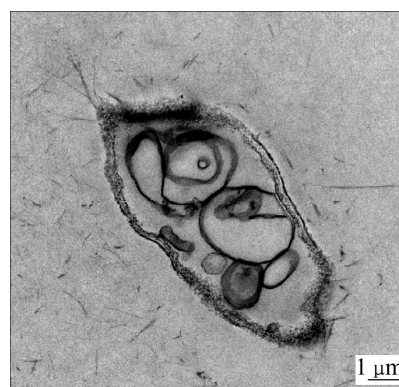


Fig. 2 TEM micrograph of strain HY-7

Strain HY-7 was inoculated into a liquid silicate medium (pH 7.0) and then cultured at 28°C and 150 r/min for 48 h, by which the cell concentration reached 1×10^8 cell/mL, indicating that the silicate bacteria grew well. These results indicated that strain HY-7 looked very similar to *B. mucilaginosus* on the morphological characteristics, and grew really well under right conditions.

3.1.2 Physiological and biochemical characterizations of strain HY-7

The physiological and biochemical characteristics of strain HY-7 showed that HY-7 could utilize glucose,

sucrose, fructose, maltose, sorbitol and amino alcohol, and decompose starch, gelatin, greases and some other large molecules (Table 2). It can also use carbon sources containing nitrogen and react with oxidase and catalase. However, there are some differences between HY-7 and reference strains I and II. These results indicated that the bacteria species of HY-7 could not be confirmed only according to the physiological and chemical characteristics for the differences from reference strains I and II.

3.1.3 G+C and DNA–DNA homology experiments

The results of homology experiments showed that the G+C contents of strain HY-7 and reference strains I and II were 51.7%, 53.2% and 54.9%, respectively. The extents of DNA hybridization of HY-7 with reference strains I and II were 79% and 25%, respectively. The standard of the National Committee for Classification of Bacteriology of China indicates that when the DNA–DNA homology between strains is above 70%, the strains are of the same species [23]. Consequently, we can assume that HY-7 is the same species as reference strain I (*Bacillus edaphicus*) and a different species from reference strain II.

3.2 Optimum growth conditions of strain HY-7

The initial bacteria density was 1×10^3 cell/mL, and culture time was 7 d. The effects of temperature, pH,

Table 2 Characteristics comparison of HY-7 and reference strains I and II

Strain	HY-7	I	II
Starch	+	+	+
Gelatin	+	+	+
Lipin	+	+	+
Urea	—	+	—
Milk	+	+	+
Glucose	+	+	+
Sucrose	+	+	+
Fructose	+	+	+
Maltose	+	—	+
Raffinose	—	—	—
Xylose	—	—	—
Arabinose	—	+	+
Sorbitol	+	+	+
Amino alcohol	+	—	—
Phenylalanine	—	—	+
Nitrate	+	+	+
Oxidase	+	+	+
Catalase	+	+	+

“+” indicates positive reaction and “—” indicates negative reaction

rotation speed and pulp density on the growth of strains HY-7 are illustrated in Fig. 3. These results indicated that the optimum growth conditions were 30 °C, pH 7.0, rotation speed of 300 r/min and pulp density of 10% for strain HY-7.

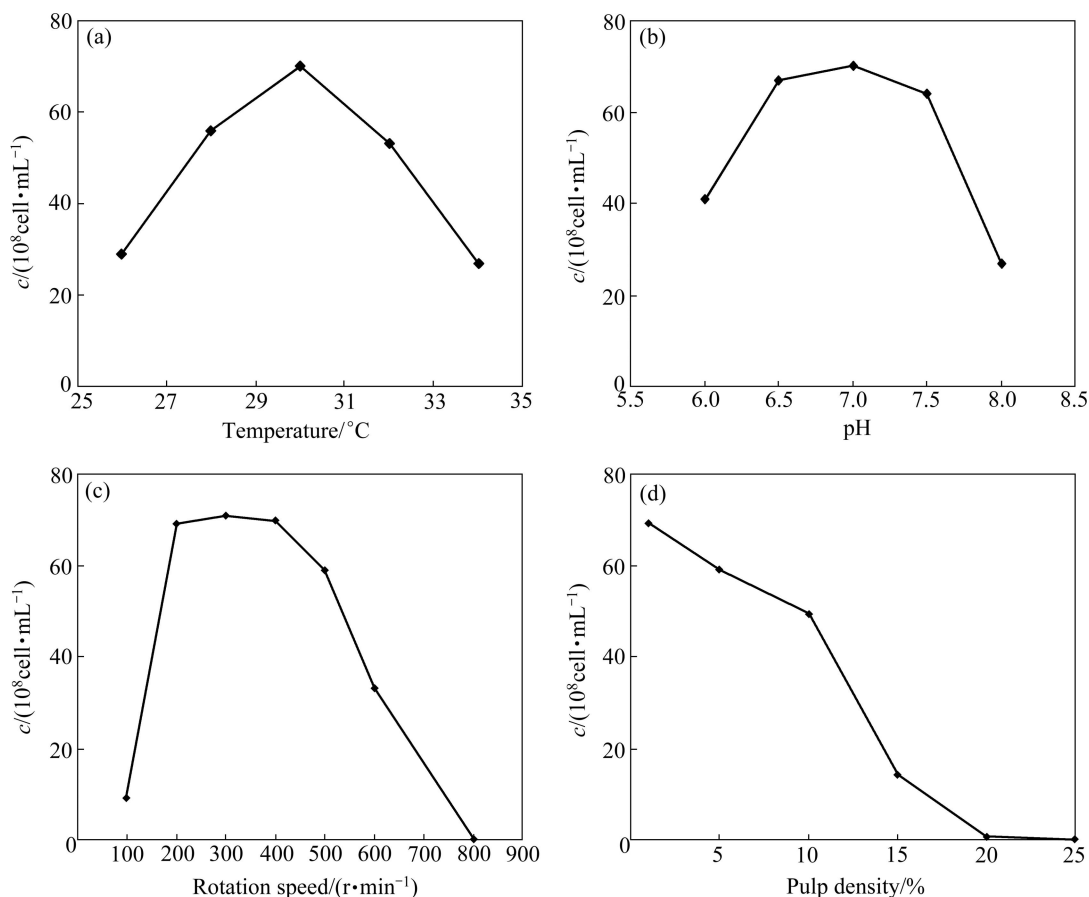


Fig. 3 Effects of temperature (a), pH (b), rotation speed (c) and pulp density (d) on growth of strains HY-7

3.3 UV mutagenesis of silicate bacteria

The UV lethal rate and the positive mutation rate for strain HY-7 are shown in Fig. 4. It is shown that the longer the radiation time of UV is, the lower the alive rate of the strain is. Generally, the UV lethal rate of 75%–95% is preferred [24,25]. So, the optimization radiation time was 20 s and the positive mutation rate was 23% for strain HY-7. In these experiments, the positive mutated colonies had higher cell density at stationary phase than the original strain.

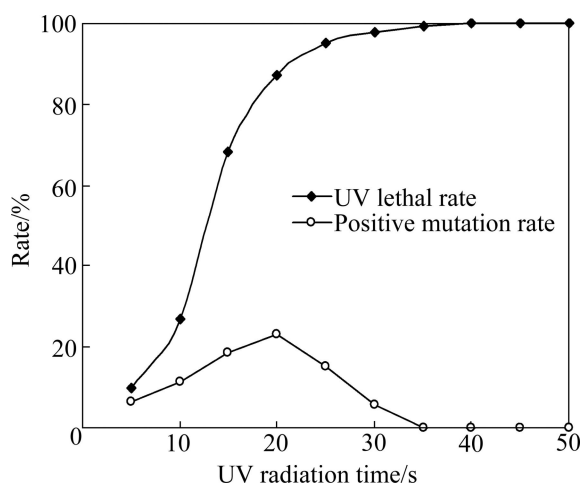


Fig. 4 Effect of UV-induced mutagenesis for strains HY-7

The strain which had the shortest generation time was chosen to leach the minerals from the positive mutation. The growth curves of strains HY-7 before and after UV-induced mutagenesis are shown in Fig. 5. It is shown that the strain after mutagenesis reaches stationary phase after 144 h, which is 24 h earlier than the original strain. It had higher cell density (10^{11} cell/mL) than the original strain (10^{10} cell/mL). These results indicated that

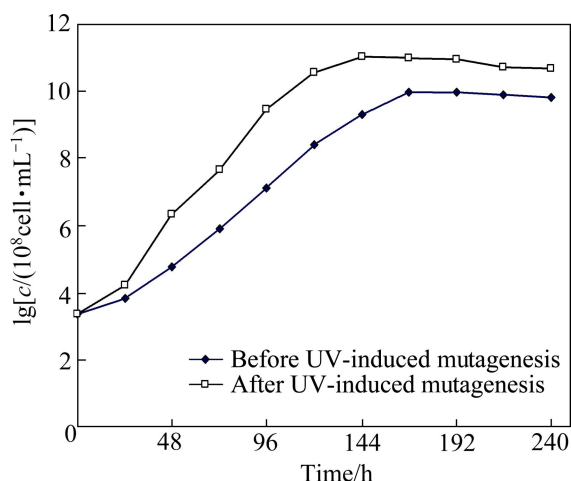


Fig. 5 Growth curves of strains HY-7 before and after UV-induced mutagenesis (c is bacteria concentration)

the strain after mutagenesis grew faster, and could have a better desiliconization capability.

3.4 Bioleaching experiment

The bioleaching results of rutile with the strains HY-7 after UV-induced mutagenesis are presented in Fig. 6. In the whole process, the TiO_2 grade of rutile continuously increases and the recovery decreases. From the first day to the 7th day, the TiO_2 grade of rutile concentrate increased quickly. After the 8th day, it became slow. After 7 d, the TiO_2 grade and recovery of rutile concentrate were 91.80% and 95.24%, respectively.

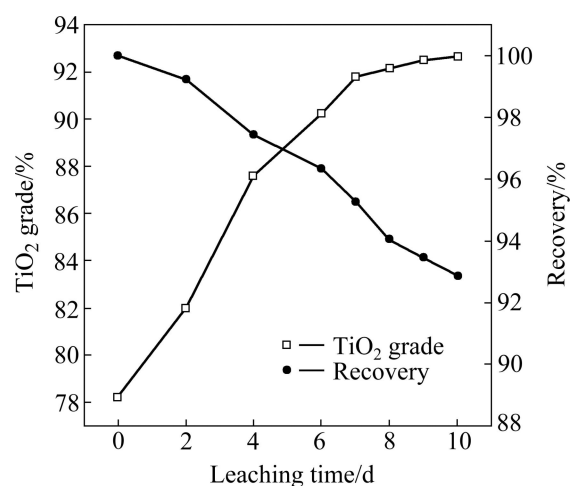


Fig. 6 Changes of TiO_2 grade and recovery of rutile concentrate during bioleaching experiments

To visualize the desiliconization effect of the bacteria on rutile, the surfaces of the rutile particles before and after bioleaching were observed using scanning electron microscopy (SEM), and the received SEM images are displayed in Figs. 7 and 8, respectively. The SEM images showed that the surfaces of the rutile particles had clearly changed after bioleaching. Before bioleaching, there were many zigzag cracks caused by the mechanical pressure, appearing on the surface of the rutile particles, while after bioleaching, the fine peaks on the surface of the rutile particle were pronouncedly decreased and considerable erosion was observed.

Energy dispersive spectroscopy (EDS) was used to identify the chemical compositions change of rutile particles during the bioleaching, and the changes of chemical compositions of rutile are shown in Table 3.

Table 3 reveals that after bioleaching, the average contents of Al and Si in the rutile concentrate decrease from 0.92% and 1.25% to 0.17%, respectively. While the content of Ti increases significantly from 38.73% to 54.77%. These results indicate that larger proportion of

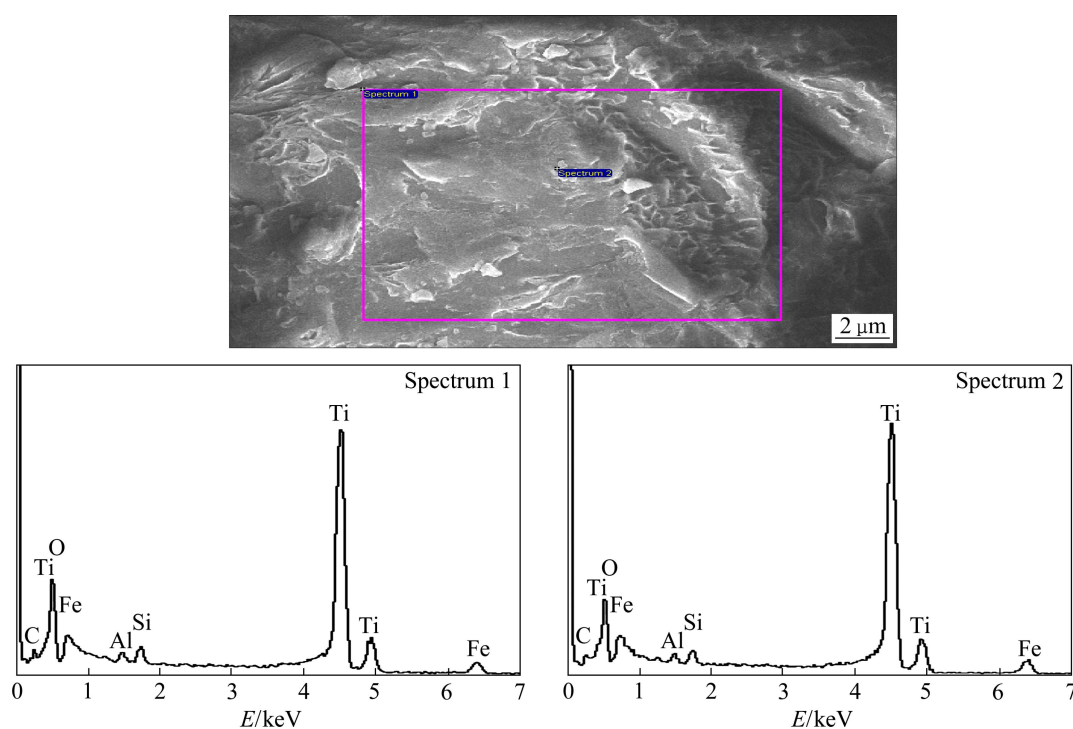


Fig. 7 SEM image and EDS analyses of rutile particle before bioleaching

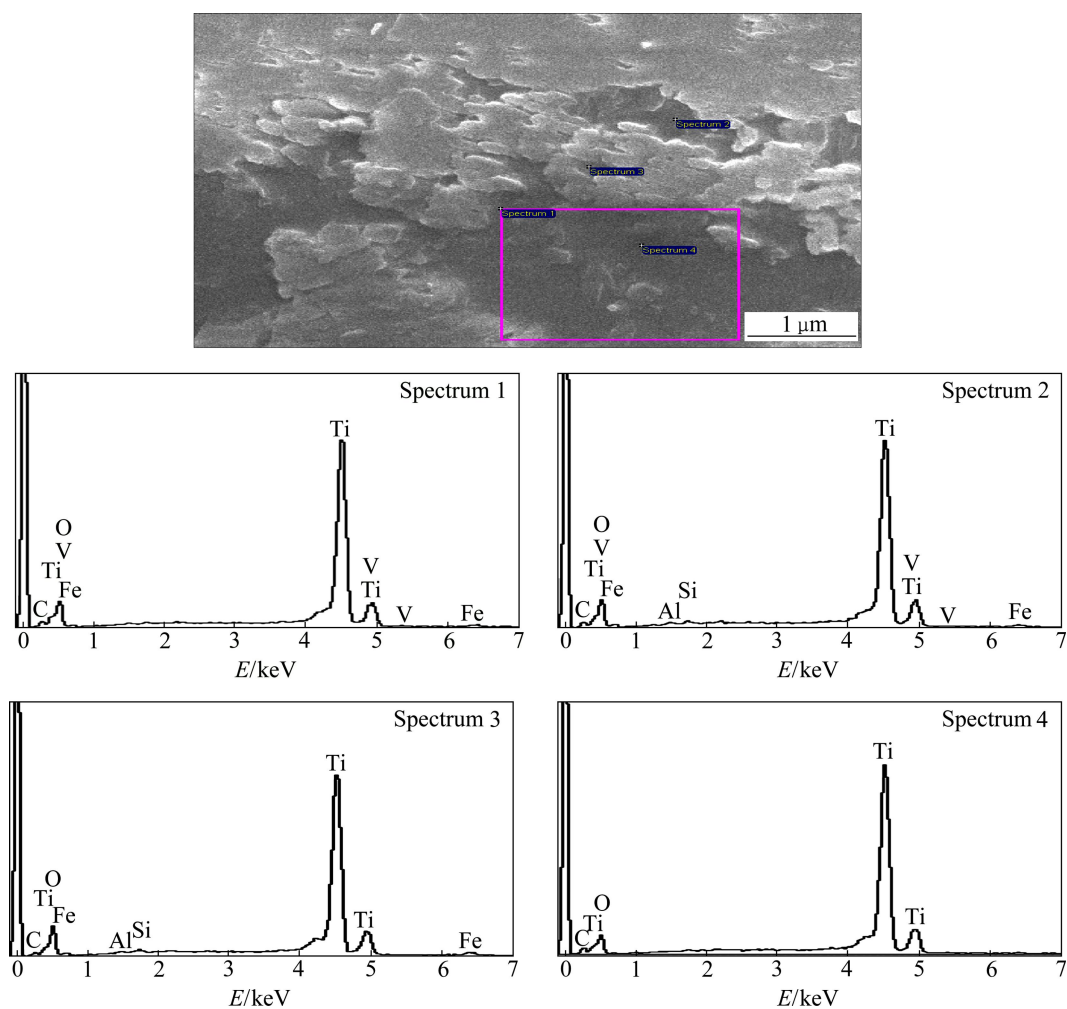


Fig. 8 SEM image and EDS analyses of rutile particle after bioleaching

siliceous materials in the rutile particles was removed by bacterial strain after bioleaching.

3.5 Phylogenetic analysis

The 16S rDNA nucleotide sequences of clones from 10 different measured macro-restriction maps were input into GenBank and Ribosomal Database Project II (<http://wdcm.nig.ac.jp/RDP/html/index.html>) database for

BLAST comparison. Phylogenetic tree was built by using the phylogenetic tree software MEGA 4 (1000 times of bootstrap). Sequence homology analysis showed that this community consisted of mainly two branches: one sharing high homology with *Paenibacillus stellifer* and the other sharing high homology with *Sporolactobacillus laevolacticus* (Fig. 9). From Fig. 9, it was found that after the NCBI database sequence

Table 3 EDS analysis results of rutile concentrate before and after bioleaching (Process options: All elements have been analyzed (normalized already))

Sample particular	Spectrum	Normal state	Mass fraction/%						
			C	O	Al	Si	Ti	Fe	Total
Before bioleaching	Spectrum 1	Yes	3.89	53.39	0.96	1.31	37.10	3.35	100.00
	Spectrum 2	Yes	4.39	48.97	0.89	1.20	40.35	4.21	100.00
	Average		4.14	51.18	0.92	1.25	38.73	3.78	100.00
	Maximum		4.39	53.39	0.96	1.31	40.35	4.21	
	Minimum		3.89	48.97	0.89	1.20	37.10	3.35	
After bioleaching	Spectrum 1	Yes	4.03	40.46	—	—	53.73	1.09	100.00
	Spectrum 2	Yes	3.95	40.48	0.36	0.31	53.25	0.94	100.00
	Spectrum 3	Yes	2.41	43.26	0.30	0.37	51.82	1.83	100.00
	Spectrum 4	Yes	5.02	34.72	—	—	60.26	—	100.00
	Average		3.85	39.73	0.17	0.17	54.77	0.97	
	Maximum		5.02	43.26	0.36	0.37	60.26	1.83	
	Minimum		2.41	34.72	0.00	0.00	51.82	0.00	

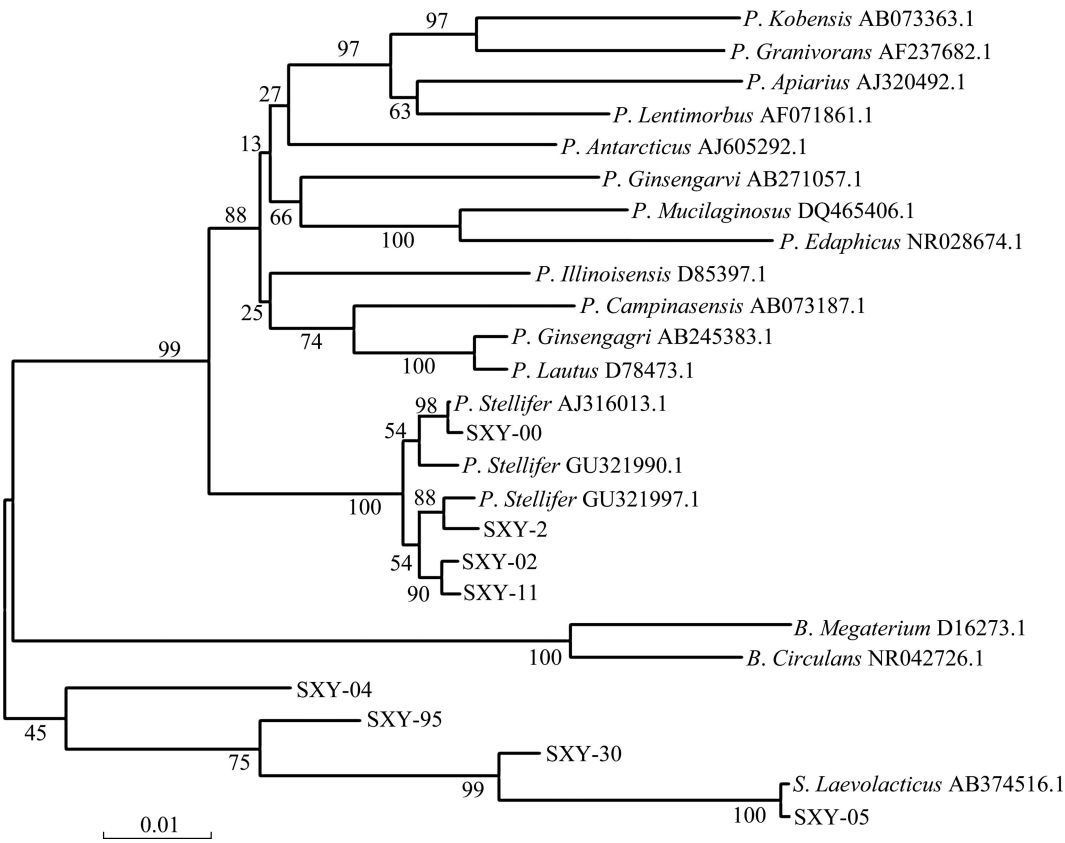


Fig. 9 Phylogenetic affiliation of prokaryotic 16S rDNA gene sequences obtained from bio-desilication reactor

Comparison, 79% unique clones shared high similarity with *Paenibacillus* while the others shared high similarity with *Sporolactobacillus*.

4 Conclusions

1) A silicate bacterium, strain HY-7, was isolated and purified from the mining waters, and identified as belonging to *Bacillus edaphicus* species. It had the optimal growth temperature of 30 °C and the optimal pH value of 7.0.

2) The optimum mutagenesis time was 20 s for strain HY-7. After the mutagenesis, the positive mutation rate was 23.0%. Strain HY-7 after mutagenesis reached the stationary phase after 144 h, which was 24 h earlier than the original strain. It also had higher cell density than the original strain.

3) The mutant of HY-7, which had significant desilicization capacity, was used in these bio-desilication experiments of rutile. Bioleaching experiments were carried out in a 10 L stirred stainless steel bioreactor by using the mutant strains. The pulp density of the rutile was adjusted to 10% (ratio of mass to volume), and the other test conditions included stirring rate of 80 r/min, aeration at 0.6 m³/h, original bacterial concentration of 7×10⁹ cell/mL, 30 °C and pH 7.0. After 7 d of bioleaching, the TiO₂ grade of rutile concentrate increased up to 91.80% from 78.21% with a recovery of 95.24%.

4) Phylogenetic analysis showed that this community consisted of mainly two branches: one sharing high homology with *Paenibacillus stellifer* and the other sharing high homology with *Sporolactobacillus laevolacticus*. After the NCBI database sequence comparison, 79% unique clones shared high similarity with *Paenibacillus* while the others shared high similarity with *Sporolactobacillus*.

5) Bio-desilication results showed that the biodesilicization process can not only effectively improve the grade of rutile concentrate but also meet the requirements of environmental protection. Further researches are needed to investigate the microorganisms involved in the bioleaching and to elucidate the physiological processes involved in desilicization.

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金红石精矿的生物脱硅提纯与脱硅微生物的群落结构分析

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摘 要: 初始菌株 HY-7 是从河南省三门峡矿区的铝土矿矿坑水中分离出来的硅酸盐杆菌。根据试验结果, 该菌的最佳生长温度为 30 ℃ 和 pH 值是 7.0, 紫外诱变改良时的最佳紫外照射时间为 20 s, 此时的正突变率为 23.0%。紫外诱变前后菌株的生长曲线对比表明, 诱变后菌株达到稳定状态的时间为 144 h, 比诱变前提早 24 h。细菌的序列同源性分析表明, 该群落组成主要可以分为两大支, 一支与类芽孢杆菌属有较高的同源性, 另一支与乳酸芽孢杆菌属有较高的同源性。生物脱硅试验结果表明, 经过 7 d 的生物浸出脱硅过程后, 金红石精矿中 TiO₂ 的品位从 78.21% 提高到 91.80%, 其回收率达到 95.24%。该生物脱硅工艺不仅有效地提高了金红石精矿的品位, 同时还很环保。

关键词: 生物脱硅; 金红石精矿; 硅酸盐杆菌; 紫外诱变; 菌群结构

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