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# Effect of Bioleaching on Alkalinene Copper Oxide Ores with Ammonia Producing Strain

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**There are mass of studies on ammonia leaching of copper, but few concerning bioleaching under alkalinene system. This paper presents an ammonia-producing strain isolated from soil, which is capable of decomposing urea, identified as Providencia.Sp and designated as strain AJT-1. Then bioleaching of high alkalinene copper oxide ores was carried out in the shake flask with Providencia JAT-1. The influencing parameters investigated include liquid-solid ratio, species and concentration of leaching aid and initial bacteria inoculation size. The results show that the 144h bioleaching yields a copper recovery of 42.35%, which is obtained at liquid-solid ratio of 7:1, ammonium sulfate of 0.024mol/L as the leaching aid, and initial bacteria inoculation of 20%. Based on the copper phase test of leaching residue, we can learn that the most easily leached copper dealing with ammonia strains is secondary copper sulfide, and its leaching rate is as high as 84.26%.**

**Key words:** Ammonia producing strain; Bioleaching; Alkalinene; Copper ore; Initial inoculation size.

With the exhausting of easy-separated sulfide copper ore, people are interested in the exploitation of low grade and refractory oxide copper ore (Zhan and Zhou, 2009; Cheng *et al*., 2006). As we all know, the acid leaching is a traditional way to deal with the oxide ores, but it is not suitable for those that abundant in alkaline gangue (Huang *et al*., 2010; Muir, 2011). The two main reasons are as follows: The ores must be pre-leached by acid to decrease pH value to make the best environment for leaching strains, yet the massive gangue minerals still consume lots of acid, that increases the costs of leaching (Sk³odowska *et al*., 2007; Ostrowski *et al*., 1993). On the other hand, quantities of slightly soluble compounds like $MgSO_4$ and $CaSO_4$ which formed during acid leaching will lead to blockage of leaching channels, and decrease of the leaching rate furthermore (Yan *et al*., 2012). Therefore, ammonia leaching method is[1] developed to treat the alkaline oxide copper ores (Fang *et al*., 2009; Zhang *et al*., 2010). High pressure ammonia leaching, though feasible in technology, turns out to be unreasonable in economy (Liu *et al*., 2003). And leaching under normal pressure, because of its strong volatility of ammonia, faces the problem of high cost, environmental pollution, energy-consuming and equipment investment (Zhao *et al*., 2010).

Bioleaching is a process based on the ability of microorganism to transform solid compounds in ores into soluble and extractable elements, which are then recovered. It represents a 'clean technology' in the mining industries with

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low cost and low investment. However, the studies and researches on copper bioleaching in alkalinene system are rare at present. S.Willscher *et al*. (Willscher *et al*.,2003) reported silicate material bioleaching with the strains isolated from alkaline waste. Bioleach of mo /ni by *P.simplicissimum*, and copper carbonate by urea decomposition bacteria were also studied by Amiri *et al*. (Amiri *et al*., 2011) and Groudeva *et al*. (Groudeva *et al*., 2007). On the other hand, the ammonia producing strains were mainly used on wastewater treatment, agricultural and medical studies (Saha, *et al*., 2012; Cong, *et al*., 2009; Shu, *et al*., 2003). The study of alkaline copper oxide ores bioleaching by the ammonia producing strain Providencia maybe is the first time.

In this paper, an ammonia producing bacteria was isolated and identified, and then used to leach the alkaline cooper ores. From the experiments, we can learn the influence factors and degree about alkaline copper bioleaching, understand the change of copper leaching rate under the optimum conditions.

## MATERIALS AND METHODS

### Micro-organisms

The bacteria used for experiment is isolated from the soil of Inner Mongolia, China. The identification results of 16Sr DNA were determined by Institute of Microbiology, Chinese Academy of Sciences (Beijing, China) as follows:

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1    ggaggagcgc tacacatgca agtcgagcgg taacagggga agcttgcttc tcgctgacga
61   gcggcggacg ggtgagtaat gtatggggat ctgcccgata gagggggata accactggaa
121  acggtggcta ataccgcata atctcttagg agcaaagcag gggaacttcg gtccttgcgc
181  tatcggatga acccatatgg gattagctag taggtggggt aatggctcac ctaggcgacg
241  atccctagct ggtctgagag gatgatcagc cacactggga ctgagacacg gcccagactc
301  ctacgggagg cagcagtggg gaatattgca caatgggcgc aagcctgatg cagccatgcc
361  gcgtgtatga agaaggcctt agggttgtaa agtactttca gtcgggagga aggcgttgat
421  gctaatatca tcaacgattg acgttaccga cagaagaagc accggctaac tccgtgccag
481  cagccgcggt aatacggagg gtgcaagcgt taatcggaat tactgggcgt aaagcgcacg
541  caggcggttg attaagttag atgtgaaatc cccgggctta acctgggaat ggcatctaag
601  actggtcagc tagagtcttg tagagggggg tagaattcca tgtgtagcgg tgaaatgcgt
661  agagatgtgg aggaataccg gtggcgaagg cggccccctg gacaaagact gacgctcagg
721  tgcgaaagcg tggggagcaa acaggattag ataccctggt agtccacgct gtaaacgatg
781  tcgatttgaa ggttgttccc ttgaggagtg gctttcggag ctaacgcgtt aaatcgaccg
841  cctggggagt acggccgcaa ggttaaaact caaatgaatt gacgggggcc cgcacaagcg
901  gtggagcatg tggtttaatt cgatgcaacg cgaagaacct tacctactct tgacatccag
961  agaatttagc agagatgctt tggtgccttc gggaactctg agacaggtgc tgcatggctg
1021 tcgtcagctc gtgttgtgaa atgttgggtt aagtcccgca acgagcgcaa cccttatcct
1081 ttgttgccag cgattcggtc gggaactcaa aggagactgc cggtgataaa ccggaggaag
1141 gtggggatga cgtcaagtca tcatggccct tacgagtagg gctacacacg tgctacaatg
1201 gcgtatacaa agagaagcga cctcgcgaga gcaagcggaa ctcataaagt acgtcgtagt
1261 ccggattgga gtctgcaact cgactccatg aagtcggaat cgctagtaat cgtagatcag
1321 aatgctacgg tgaatacgtt cccgggcctt gtacacaccg cccgtcacac catgggagtg
1381 ggttgcaaaa gaagtaggta gcttaacctt cgggagggcg ct
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The 16Sr DNA identification result and Phylogenetic tree(fig 1) of JAT-1 and its relative strains show the bacteria belongs to Providencia. Sp, and we named it as JAT-1. Urvashi (Urvashi *et al*., 2006) reported this kind of strain can be used for industrial wastewater treatment, but other roles about this strain are rare.

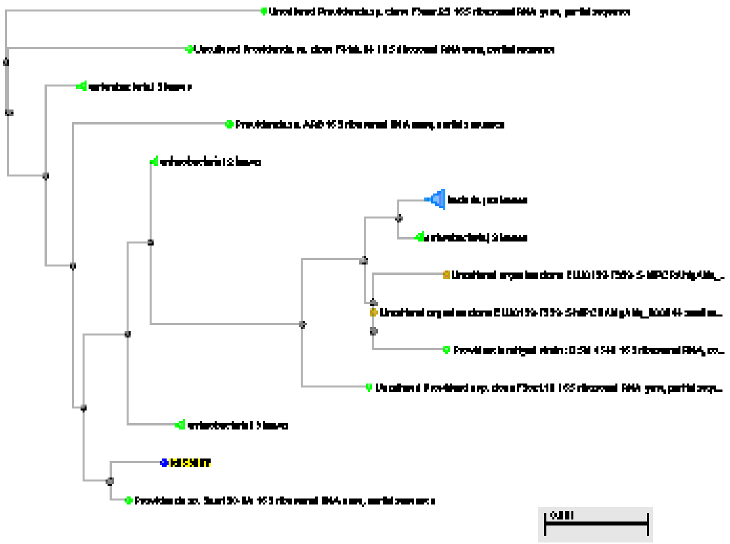

**Fig. 1.** Phylogenetic tree of JAT-1 and its relative strains

The bacteria is gram-negative(Fig.2), facultative anaerobic, metabolizing sodium citrate and urea as carbon and nitrogen source, and urea was decomposed as ammonia. After 24~48h Culture the strains were in logarithmic growth phase, and the concentration of ammonia in the solution reach the maximum between 48~60h.

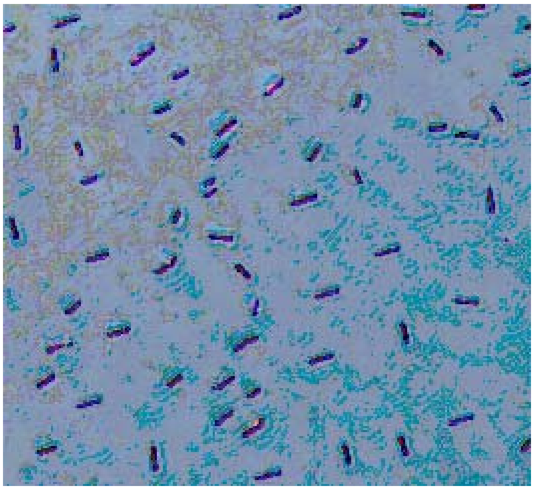

**Fig. 2.** Gram stain of ammonia producing strain JAT-1

### Ores

The oxide copper ores used for the experiment are collected from a mine in Yunnan province, crushed into 74¼m. Main mineral compositions of the sample are malachite, chrysocolla, chalcopyrite. The results of composition and copper phase analysis of the ore as Table 1 and Table 2.

The analysis results show that the ores are low grade of copper and typical high acid consumption and Oxidation. The MgO and CaO account for 12.03% of the ore, other compounds like $Fe_2O_3$ and $Al_2O_3$ are also exit in samples. Most of the ores are oxide copper, and contain primary sulfide copper and small secondary copper sulfide.

### Experiment methods

Bioleaching experiments were carried out in 250-ml shake flask containing ores and growth medium inoculated with microbial culture. Prior to leaching, media and ore samples were sterilized at 121°C for 20min. The flasks were incubated on a rotary shaker at 30°C at an operating speed of 150rpm. Liquid samples were taken at the end, filtered, and analyzed for $Cu^{2+}$ dissolved in each sample by atomic absorption. In the experiment, liquid-solid ratio, species and concentration of leaching aid and initial bacteria inoculation were investigated to understand influence factors and degree about alkaline copper bioleaching.

## RESULTS AND DISCUSSION

### Effect of liquid-solid ratio on copper leaching rate

The experiments were carried out in different liquid-solid ratio and other conditions

**Table 1.** Chemical composition analysis of copper ore (Mass fraction/% )

| Cu | FeO | MgO | CaO | $SiO_2$ | $Al_2O_3$ | Zn | S | As | $WO_3$ |
|---|---|---|---|---|---|---|---|---|---|
| 1.013 | 27.26 | 1.35 | 10.68 | 47.78 | 7.62 | 0.198 | 0.46 | 0.135 | 0.16 |

**Table 2.** Analyze of copper

| Phase | Free copper oxide | Combined copper oxide | Secondary copper sulfide | Primary copper sulfide | Total |
|---|---|---|---|---|---|
| Mass fraction /% | 0.352 | 0.289 | 0.076 | 0.296 | 1.013 |
| Account rate/% | 34.75 | 28.53 | 7.50 | 29.22 | |

keep consistent. Liquid samples were taken and analyzed after leaching, to calculate the leaching rate of copper and understand the relationship between liquid-solid ratio and copper leaching rate (Fig. 3).

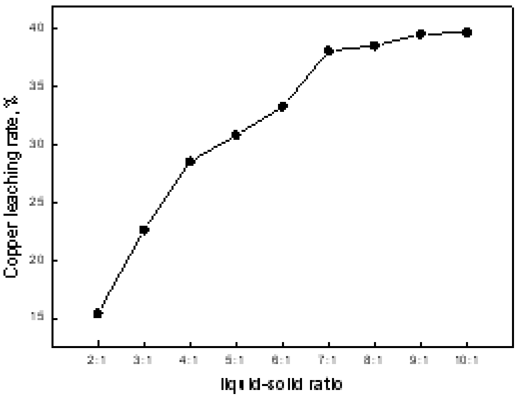


**Fig. 3.** Effect of liquid-solid ratio on copper leaching rate

The result show that copper leaching rate improves with the liquid-solid ratio increases, the change range becomes not obvious when the liquid-solid ratio is higher than 7:1. The reasons of this phenomenon are as follows: on the one hand, lower liquid-solid ratio means more solid substance in the solution that cased more serious friction for the cells during the flask, and dissolved oxygen content is also a problem for the microbial cultures that restrict the ammonia production. On the other hand, higher liquid-solid ratio means every unit mineral can be distributed more leaching solution in the system, in this case, more complete reaction of particles and solution can get better copper leaching rate. Therefore, by comprehensive consideration, the optimum liquid-solid ratio of copper bioleaching by ammonia producing bacteria is 7:1.

**Effect of leaching aid species on copper leaching rate**

Ammonium sulfate, ammonium carbonate, and ammonium bicarbonate were used as the candidates. In the experiments, $NH_4^+$ of each ammonium salt keep molar ratio of 1:5 with urea which contained 0.33mol/L in each flask. The liquid-solid ratio is 7:1 that determined in last part, and initial bacteria inoculation is 20%. The experiment results show that adding leaching aid can get better copper leaching effect than the control, and ammonium sulfate is the best leaching aid (Fig. 4).

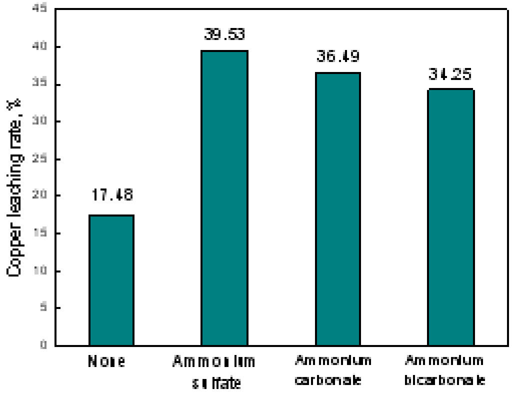


**Fig. 4.** Effect of leaching aid substance on copper leaching rate

The hydrolysis degree of each ammonium salt is different which contain identical molar concentration in each experiment, that case the concentration of $NH_4^+$ is different in each solution.

Ammonium sulfate is hydrolyzed most sufficiently because it is strong acid-weak base salt, that leads to more to the leaching system. However, ammonium carbonate and ammonium bicarbonate were double hydrolytic, so the released from the salts are limited. On the other hand, the solution contained ammonium sulfate shows lower pH compared with the other two. Therefore, ammonium sulfate is the most suitable leaching aid for the system of ammonia producing bacteria bioleaching.

**Effect of leaching aid concentration on copper leaching rate**

The bioleaching experiment was carried out in shake flasks with liquid-solid radio of 7:1, initial bacteria inoculation of 20%, urea of 0.33mol/L, and used various concentration of ammonium sulfate as the leaching aid. The process continued for 120h, and the leaching solution was collected to analyze the concentration of copper. The copper leaching rate influenced by leaching aid concentration as Fig. 5.

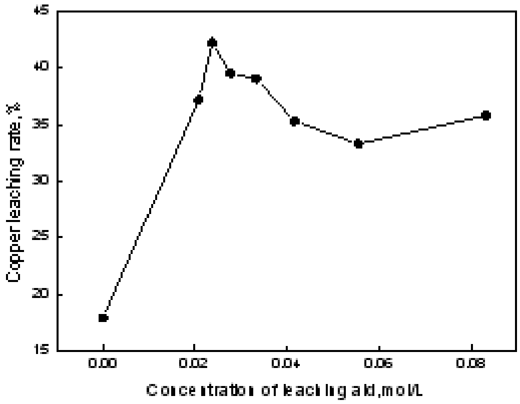


**Fig. 5.** Effect of leaching aid concentration on copper leaching rate

The result shows that ammonium sulfate which as the leaching aid has significant effect on copper leach compared with the control experiment. From the curve we can know that copper leaching rate fluctuating with the increase of ammonium sulfate. The optimal concentration of ammonium sulfate is 0.024mol/L. Overabundance of leaching aid will destroy the balance of cell pressure internal and external, hider compose of urease which plays important role in bioleaching process. Therefore, the leaching rate own to complexation of copper and ammonium salt in this condition. However, shortage of ammonium sulfate will cause leaching reaction end too early because of not enough providing of $NH_4^+$ for the leaching system.

**Effect of inoculation on copper leaching rate**

The inoculation of the strains infects the population quantity and ammonia production, thus lead to the strains play a very important role in the bioleaching system. The experiment carried out with various inoculation of the ammonia producing strain and the other conditions are consistent. The result shows as Fig. 6.

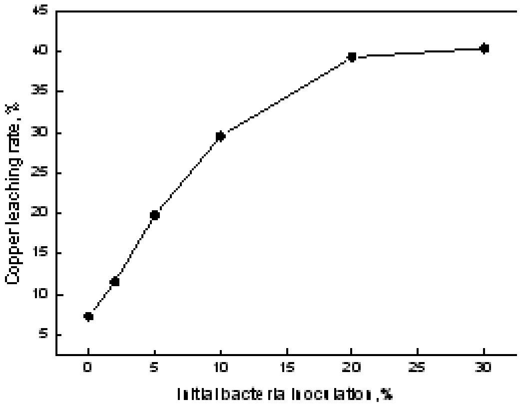


**Fig. 6.** Effect of bacteria inoculation on copper leaching rate

The copper leaching rate is significantly influenced by initial inoculation. From Fig. 6 we can know that copper leaching rate increases as the initial inoculation increases, while inoculation increases by 30%, copper leaching rate up slower. It is clear that excessive strains in the solution will lead to nutrient exhaustion faster, and the massive accumulation of metabolites will cause a limitation of strains' growth and ammonia producing. Comprehensive analysis of copper leaching rate and dealing cost, initial inoculation of 20% is best.

Change of copper leaching rate varied with time

Based on all above experiment results, we can understand the change of copper leaching rate with the dealing time (Fig. 7).

The result shows that copper leaching rate increases sharply during the starting period, and get the highest leaching rate while carried out by 144h, hereafter the leaching rate turns down as the time continue. Thus blame on the concentration of copper which leached in the solution absorbed by strains' metabolites, result in the error of copper concentration analysis.

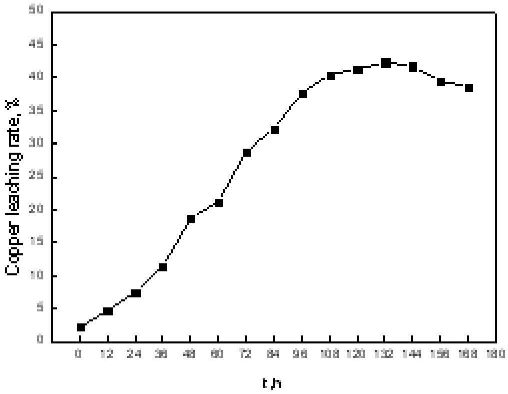


**Fig. 7.** Change of copper leaching rate with ammonia producing bacteria

Therefore, it is necessary to replace the culture solutions when the leaching deals about 144h, by fresh nutrient medium. Such procedure results in a better copper extraction and recovering.

**Change of copper phase of pre and post bioleaching**

Based on the copper phase test of leaching residue, we can make a comparative analysis of copper leaching rate on different copper phase (table 3). The result shows that the every phase of copper can be leached from the ores with ammonia producing strains indeed. The copper leached from high to low are: secondary copper sulfide, combined copper oxide, free copper oxide and primary copper sulfide, and the leaching rate is respectively 84.26%, 59.74%, 36.97% and 21.01%. As we all know, free copper oxide and combined copper oxide are easily extracted from the ores, and the copper sulfides are difficulty in leaching. However, it is interesting that our experiment results show the most easily leached copper dealing with ammonia strains is secondary copper sulfide, and the combined copper oxide takes second place Thus indicate the strains which exist in alkaline bioleaching system not only play the role of ammonia producing, but also take part in the catalysis processes of the copper leaching which difficulty carried out in traditional like copper sulfides. And what's more, the strains may absorb on ores and erosion them, or as the planktonic carrier transfer the protons in the solution, to promote the leaching procedure.

**Table 3.** Leaching rates of different copper phase

| Phase | Free copper oxide | Combined copper oxide | Secondary copper sulfide | Primary copper sulfide | Total |
|---|---|---|---|---|---|
| Mass fraction before leaching/% | 0.352 | 0.289 | 0.076 | 0.296 | 1.013 |
| Mass fraction after leaching /% | 0.222 | 0.116 | 0.012 | 0.234 | 0.584 |
| leaching rate/% | 36.97 | 59.74 | 84.26 | 21.01 | 42.35 |

## CONCLUSIONS

1. The bioleaching on alkaline copper oxide ores by Providencia JAT-1 is feasible. Copper leaching rate is affected by many factors, such as liquid-solid ratio, species and concentration of leaching aid, and initial inoculation.
2. The 144h bioleaching yields a copper recovery of 42.35%, which is obtained at liquid-solid ratio of 7:1, ammonium sulfate of 0.024mol/L as the leaching aid, and initial bacteria inoculation of 20%.
3. The bioleaching procedure should be controlled in 144h, replace the culture solutions by fresh nutrient medium after that period could get a better copper extraction and recovering.
4. The copper leached from high to low are: secondary copper sulfide, combined copper oxide, free copper oxide and primary copper sulfide, and the leaching rate is respectively 84.26%, 59.74%, 36.97% and 21.01%.

## ACKNOWLEDGEMENTS

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