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Valence variation of arsenic in bioleaching process of arsenic-bearing gold ore

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Abstract: The concentration and variational trend of As^{3+} and As^{5+}, the bacterial resistance for the As^{3+} and As^{5+} and converting conditions from As^{3+} to As^{5+} were analyzed. The additive was used to prompt the bacterial leaching efficiency by changing valence state of arsenic. The results show that the concentration of As^{3+} is larger than that of As^{5+} in the lag phase. The concentration of As^{3+} decreases in the log phase, and is lower than that of As^{5+}. HQ-0211 typed bacteria express better resistance for As^{3+} and As^{5+} and remain growing when the concentrations of As^{3+} and As^{5+} are above 6.0 g/L and 12.0 g/L, respectively. It is found that Fe^{3+} cannot oxidize As^{3+} singly as strong oxidant in the leaching system, but can cooperate with pyrite or chalcopyrite to do that. The oxidation of As^{3+} is prompted with addition of H_2O_2. The bacterial activity is improved in favor of bacterial leaching efficiency. NaClO restrains the bacterial growth to depress leaching efficiency because of the chloric compounds affecting bacterial activity.

Key words: As^{3+}; As^{5+}; bacterial leaching; arsenic resistance; oxidant; arsenic-bearing gold ore

1 Introduction

Arsenic-bearing gold ore is a common refractory gold ore. Arsenic of gold ore is very unfavorable to cyanide leaching[1−2]. Arsenic wraps gold grain in the form of arsenopyrite, which severely cuts off the cyanide from gold grain. Arsenic-bearing gold ore is easy to produce AsS_3^{3-}, CNS^-, $S_2O_3^{2-}$, AsO_3^{3-} and AsO_4^{3-} in cyanide solution. These products decrease the leaching efficiency because they exhaust cyanide and form compact film on the surface of gold grain to separate CN^- and O_2 from the grains in the leaching process. The oxidation pretreatment is used to depress or remove the arsenic in the gold ore in order that the gold is denuded before cyanide leaching[3−4]. Now pretreatment technologies for the ore are roasting oxidation, pressure oxidation and bacteria oxidation. Among them, the bacteria oxidation is famous for low cost, high efficiency and environmental protection[5−6]. Iron and sulfur in the ore are energy sources for bacteria to meet their metabolic demand. Then ore grade will be increased[7−8]. Refractory gold ore with high arsenic concentration has two impending problems to be solved, i.e. improving bacterial leaching efficiency and shortening oxidation period. Arsenic is the most influential compound on the bacterial activity in the leaching system and it is highly toxic substances. There are two types of As^{3+} and As^{5+} in the solution. The toxicity of As^{3+} is much higher than As^{5+}[9−11]. Accelerating the transition from As^{3+} to As^{5+} is very important for decreasing the concentration of As^{3+}, improving bacterial activity and increasing leaching efficiency. The present research of bacterial oxidation of arsenic-bearing gold ore is emphasized on the bacterial adsorption, bacterial extracellular polysaccharide and different single-mineral studies. In this work, a series of theoretical researches are emphasized on the valence state distribution of arsenic in the bacterial leaching process, bacterial resistance for As^{3+} and As^{5+}, transitional conditions from As^{3+} to As^{5+} and oxidant for converting As^{3+} and depressing bacterial toxicity. The research provides important theoretical consults for more mature, fast, steady, economic and high-performance bacterial oxidation pretreatment of the high arsenic

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refractory gold ore.

2 Materials and experimental

2.1 Materials

Bacteria HQ-0211 used by test, which are mixed with leaching bacteria dominated by *Thiobacillus ferrooxidans*. They are screened and have been domesticated for a long time in the laboratory.

The medium is 9K that was composed of 3.00 g/L $(NH_4)_2SO_4$, 0.10 g/L KCl, 0.50 g/L K_2HPO_4, 0.50 g/L $MgSO_4·7H_2O$, 0.01 g/L $Ca(NO_3)_2$ and 44.30 g/L $FeSO_4·7H_2O$[12]. pH is 1.8 regulated by sulfuric acid.

The ore sample comes from gold concentrate mine in Hunan Province, China, and the sample with size less than 0.038 mm is over 90%. The phase analysis shows that arsenic exists as arsenopyrite in the sample as shown in Fig.1. Table 1 lists the result of main elemental analysis.

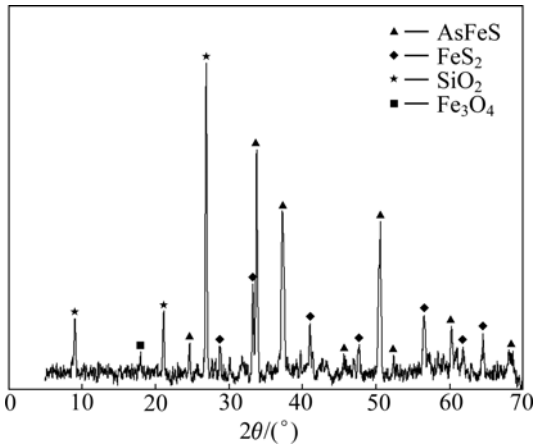

Fig.1 XRD pattern of gold concentrate

Table1 Result of main element analysis

Au	As	Si	Fe	S
129.10 g/t	16.05%	30.31%	21.60 %	17.71 %

The chemicals used in the experiments included As_2O_3, $Na_2HAsO_3·7H_2O$, H_2O_2, NaClO, NaOH, HCl, C_6H_5-CH_3 and I_2. All the chemicals were analytical reagents. All the aqueous solutions were prepared with the distilled water.

2.2 Experimental

2.2.1 Extractive separation of As^{3+} and As^{5+}

The toluene was used to extract the As^{3+} from the inorganic phase to the organic phase quantificationally in the condition of strong acid. The As^{5+} remained in the organic phase. The As^{3+} was back-extracted to aqueous phase from the inorganic phase and titrated by iodine titer in the condition of weak base[13]. The As^{5+} was measured by hypophosphite titrimetric method[14].

2.2.2 Arsenic resistance of bacteria

10% of HQ-0211 typed bacteria with high activity were injected into the 200 mL of 9K medium with different concentrations of As^{3+} and As^{5+} and were shake-flask cultured in constant temperature shaking incubator at 44 ℃. The concentration gradient of As^{3+} was 0, 1.5, 3.0, 4.5 and 6.0 g/L. The concentration gradient of As^{5+} was 0, 3.0, 6.0, 9.0 and 12.0 g/L.

2.2.3 Bacterial leaching of gold concentrate ore

10% of HQ-0211 typed bacteria were injected into 500 mL of shake flask with 200 mL of 9K medium in constant temperature shake incubator at 44 ℃. After activation of bacteria, 10 g of gold concentrate ore was added into the pulp.

2.3 Analytical method

The electric potential, pH and concentration of Fe^{2+}, TFe, As^{3+} and As^{5+} are determined in leach liquor in bioleaching and oxidant experiments per 24 h. Fe^{2+} and TFe are determined by dichromate method. The concentration of As^{3+} and As^{5+} are determined by toluene extraction-separation method.

The electric potential, pH and concentration of Fe^{2+} and TFe are determined in arsenic resistance experiment per 24 h. The bacterial reproduction is counted in blood counting chamber under the microscope. Growth curve is drawn.

3 Discussion

3.1 Concentration change of arsenic

Arsenic is the key negative factor in the biooxidation. The research of arsenic and its derivatives is the hotspot in the biohydrometallurgy. As^{3+} becomes the hotspots including its indigenous time, concentration change and transition to the arsenic in high valence because the toxicity of As^{3+} is stronger than that of As^{5+} [15−16]. The toluene extraction−separation method is used to pursue the concentration changes of As^{3+} and As^{5+} in the arsenic-bearing gold concentrate bioleaching process. Due to ferrous ion and sulfur element of ore as energy source for leaching bacteria, they obtained energy by oxidation them to grow, so the system redox potential and pH value changes can be indirectly reaction to the growth of bacteria and the disintegration of minerals. Bacteria adapted to the ore. Lag phase of bacteria only lasts three days. The fourth day is logarithm. At this time, arsenic-bearing ores are quickly oxidized and decomposed. Potential suddenly ascend,and pH value is declining. The fifth day and the sixth day are the stable phase. Due to ferrous ion and sulfur element are oxidized, no increase in potential. But bacteria are still in the production of acid phase, and pH value is declining as

shown in Fig.2. The concentration of As^{3+} is higher than that of As^{5+} in the former three days, namely the lag phase. The concentration of As^{3+} reaches the climax, 4.63 g/L, in the 3rd day. The concentration of As^{3+} steps down until below As^{5+} in the log phase of bacteria as shown in Fig.3. The concentration of As^{3+} increases then decreases compared with the escalation of As^{5+} in bioleaching process. The change of arsenic phase is $AsS^{2-} \rightarrow As^{3+} \rightarrow As^{5+}$ as shown in Fig.3. As^{3+} is produced and oxidized in bioleaching process. In the lag phase of bacteria, the indigenous rate is higher than the oxidized rate of the As^{3+} because of the weak activity of bacteria and oxidization. In the flushing log phase and stable phase of bacteria, escalating concentration of Fe^{3+} leads to the higher oxidizing rate than indigenous rate. The As^{5+} has higher concentration than As^{3+}. In other words, the activity of bacteria increases with the decrease of As^{3+} concentration and increase of As^{5+} concentration. Thus, transition from As^{3+} to As^{5+} has great influence on the biooxidation pretreatment. A large number of arsenopyrite, pyrite and other sulfide minerals are oxidized in bioleaching process. At the end of the experiment, the mass loss rate is 61.8% and the dearsenization rate is 98.2%.

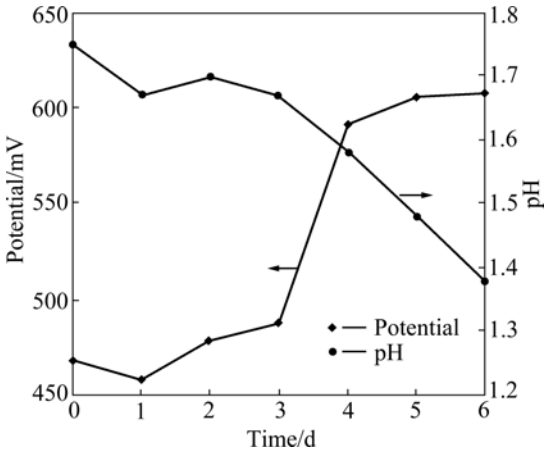

Fig.2 Potential and pH changes with bioleaching time

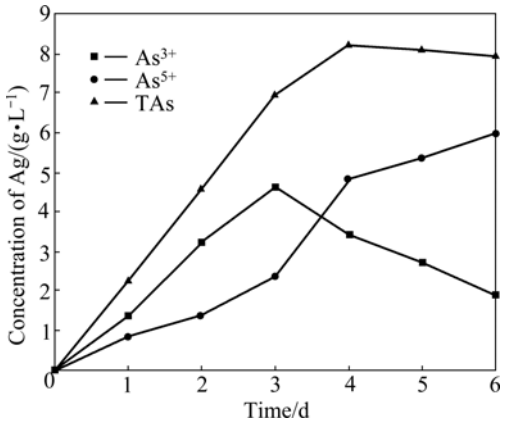

Fig.3 Concentration changes of As^{3+}, As^{5+} and TAs with bioleaching time

3.2 As^{3+} and As^{5+} resistances of bacteria

Because As^{3+} and As^{5+} coexist in the bioleaching system, As^{3+} and As^{5+} resistances of bacteria were studied further. This work will help with formulations of pulp density and ore blending in bioleaching process. The results of experiment show that the bacterial growth rate and the activity decrease with increasing concentrations of As^{3+} and As^{5+}. Bacterial activity is seriously affected with the gradual increase in the concentration of As^{3+}. Ferrous ion as energy cannot be oxidized, resulting in potential increased slowly. When the concentration of As^{3+} is 6.0 g/L, the bioleaching time of bacterial lag phase is 264 h. No restrain happens when the concentration of As^{3+} is below 1.5 g/L, as shown in Fig.4 and Fig.5. As^{5+} also has a great impact on the bacteria, but its toxicity is weaker than As^{3+}. When the concentration of As^{5+} is 12.0 g/L, the bioleaching time of bacterial lag phase is 264 h. No restrain happens when the concentration of As^{5+} is below 3.0 g/L, as shown in Fig.6 and Fig.7. The lag phase is extended with the increase of arsenic concentration because the bacteria

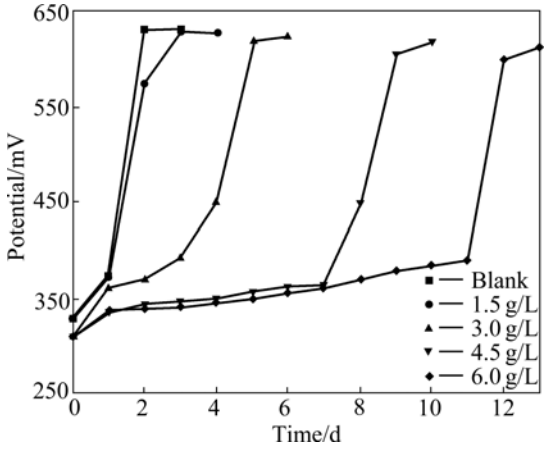

Fig.4 Potential change with bioleaching time at different As^{3+} concentrations

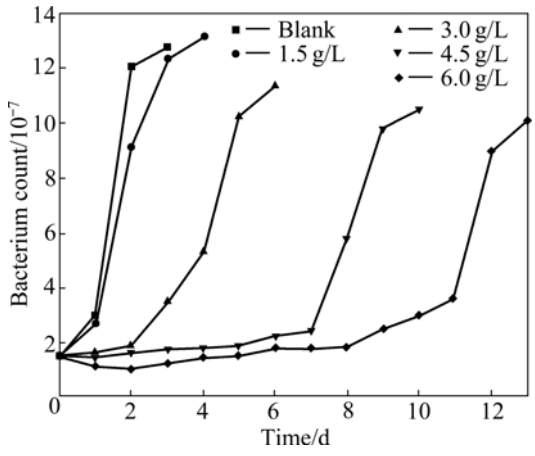

Fig.5 Bacteria count change with bioleaching time at different As^{3+} concentrations

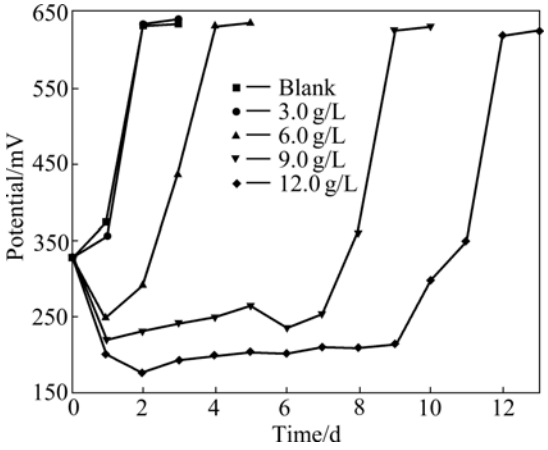

Fig.6 Potential change with bioleaching time at different As^{5+} concentrations

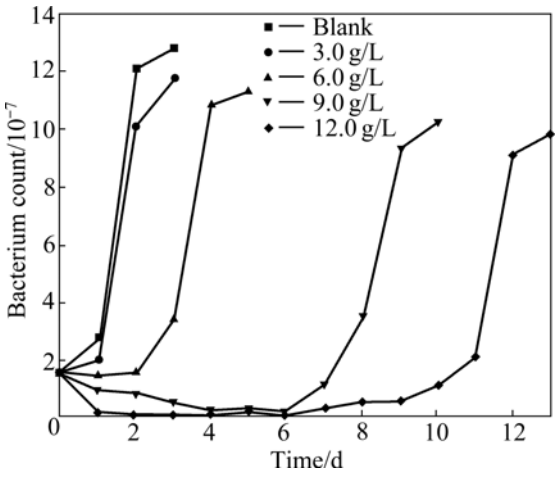

Fig.7 Bacteria count change with bioleaching time at different As^{5+} concentrations

need time to adjust metabolism and catalyze its enzyme, coenzyme and intermediate to adapt to new environment. In this phase, the bacterial growth is slow and the oxidizing efficiency is low for ferrous ion. The bacteria may meet the high arsenic gold concentrate ore with 16.05% arsenic in the section 3.1 because the resistances of As^{3+} and As^{5+} are above 6.0 g/L and 12.0 g/L as shown in Figs.3, 5 and 7. Toxicity of As^{3+} is 60 times higher than that of As^{5+} for the human body and animal body[17−19]. But the result of this work is 2 times for the bacterial leaching activity experiments as shown in Fig.5 and Fig.7. Thus, the further research is needed for the toxicity theory of arsenic ion for the leaching bacteria.

3.3 Analysis of converting conditions from As^{3+} to As^{5+}

The bacterial activity steps up from As^{3+} to As^{5+} in the process of biooxidation. Thus, it is important to study the converting conditions from As^{3+} to As^{5+} in the bacterial leaching process. The direct oxidation and indirect oxidation of the main body are bacteria and Fe^{3+} in bacterial oxidation system, therefore, to analyze As^{3+} to As^{5+} conversion conditions respectively, the bacteria, the medium of Fe^{3+} and pyrite often associated with gold ore were studied. The activated bacterial liquid is placed into four shake-flasks of 500 mL with 200 mL of 2 g/L As^{3+} standard liquid, as listed in Table 2.

Table 2 Different conditions of oxidation test for As^{3+}

No.	Concentration of $As^{3+}/(g\cdot L^{-1})$	Condition
$1^{\#}$	2	Primitive bacteria solution
$2^{\#}$	2	Sterile condition
$3^{\#}$	2	2 g pyrite
$4^{\#}$	2	4.64 g $FeSO_4\cdot 7H_2O$

The converting of As^{3+} in the bacteria system is compared with asepsis after the bacteria are filtrated in the $2^{\#}$ flask through microporous membrane. The conversion ratios are zero in the $1^{\#}$ and $2^{\#}$ flasks after three days germiculture. This shows that the leaching bacteria cannot convert As^{3+} to higher valence state in the simplex 9K culture medium and Fe^{3+} does not play its role as oxidant, as listed in Table 3. 2 g pyrite with Fe^{3+} as strong oxidant added into the $3^{\#}$ flask is compared with $4^{\#}$ flask with 4.64 g $FeSO_4\cdot 7H_2O$. This is the same amount of iron in 2 g pyrite. The conversion ratio of As^{3+} reaches 35.61% and progressively rises in the $3^{\#}$ flask after being cultured for three days, on the contrast, none in the $4^{\#}$ flask, as listed in Table 3. 2 g $FeSO_4\cdot 7H_2O$ is added into the $4^{\#}$ flask to ensure the mole ratio of iron to arsenic to convert As^{3+} to As^{5+} in the solution. After one day's culture, As^{3+} isn't oxidized in the ratio of 10:1 for iron to arsenic, and 480 mV in the $3^{\#}$ flask, which proves high concentration of Fe^{3+} and high electric potential are not preconditions to convert As^{3+} to As^{5+} in the leaching system. A part of As^{3+} is also oxidized after addition of chalcopyrite. O_2 in the air is an electron acceptor in addition to Fe^{3+} in leaching system. To analyze the electron acceptor in the process of $As^{3+}\rightarrow As^{5+}$, biooxidation of pyrite is analyzed. XPS shows that O^{2-} exists on the surface of pyrite when the flaky pyrite is oxidized by leaching bacteria as aerobic from low valence state to high valence state in the process of biooxidation. When the environment changes, EPS (extracellular polysaccharide) is secreted in the outer layer to promote oxidation of As^{3+}. 3% EDTA is used to extract the EPS in the $3^{\#}$ and $4^{\#}$ flasks. The results show that the EPS concentration in the $3^{\#}$ flask is two times higher than that in the $4^{\#}$ flask because the secretion is

stimulated by the addition of pyrite. Fig.8 shows that the bacteria are adhered to the surface of ore by the sticky EPS which controls the transference and exchange between the bacterial surface and environment. SAND et al[20] found that Fe^{3+} concentration in the EPS is much higher than that in the solution which prompts the oxidation of elements in low valence state in the system, and oxidoreductase secreted is congregated in the EPS to accelerate transference of electron in the bacterial enzyme system and to cause redox reaction to process easily. As exothermic reaction, the thermal energy relaxed by bacterial leaching catalyzes the reaction.

Table 3 Oxidation ratio of As^{3+} after being cultured for different time (%)

Time/d	1#	2#	3#	4#
0	0	0	0	0
1	0	0	22.92	0
2	0	0	31.71	0
3	0	0	35.61	1.46

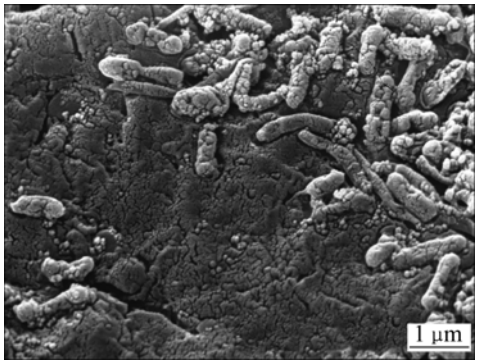

Fig.8 SEM image of bacteria adhering to surface of ore

3.4 Influence on bioleaching process by oxidant

The arsenic is notorious for toxicity and danger, and the oxidant is applied widely in the arsenic-bearing industrial wastewater. The section 3.1 shows that the trivalent arsenic exists in the former half part of the bioleaching process. The addition of oxidant oxidizes As^{3+} rapidly to depress the toxicity of arsenic and shorten the lag phase to enhance bacterial activity. It is very important to deal with long bioleaching period. It is easy to remove As^{5+} through coprecipitation, flocculation and absorption in the post processing. The oxidant has two sides because it promotes the oxidation of trivalent arsenic and restrains the bacterial growth. 30% of 0.3 mL H_2O_2 and 10% of 0.5 mL NaClO are added into at the 48th hour with high concentration of As^{3+}. H_2O_2 leads to the lower concentration of As^{3+} than the blank sample from the 3rd day to the end of bacterial leaching, as shown in Fig.9. The reduced As^{3+} leads to the enhanced bacterial activity in the shake-flask with H_2O_2 and large leaching oxidizing rate. The total arsenic concentration exceeds 0.39 g/L which is larger than that of the blank sample and trends to stabilize in the 3rd day after reaching maximum dearsenization rate, as shown in Fig.10. The concentration of As^{3+} in the shake-flask with NaClO is lower than that of the blank sample, but the total arsenic concentration in the shake-flask with NaClO is also lower than that of the blank sample, as shown in Fig.10. The results show that bacteria are unfit for the environment and the activity and the leaching efficiency decreases. The As^{3+} liberating from the ore leads to the lower concentration of As^{3+} than the blank sample. The observation through the microscope shows that the growth rate and activity are lower than those in the other two shake-flasks. H_2O_2 remains more stable in the acid environment than the alkaline, but lots of ferric ion, sulfur ion and arsenic ion catalyze the decomposition rapidly in the solution at 44 ℃. The purpose is to depress the toxicity of arsenic for the bacteria and to improve the bacterial activity because the oxidation of H_2O_2 disappears in a short time and does not restrain the bacteria. NaClO is decomposed to HClO, Cl_2 and Cl^-

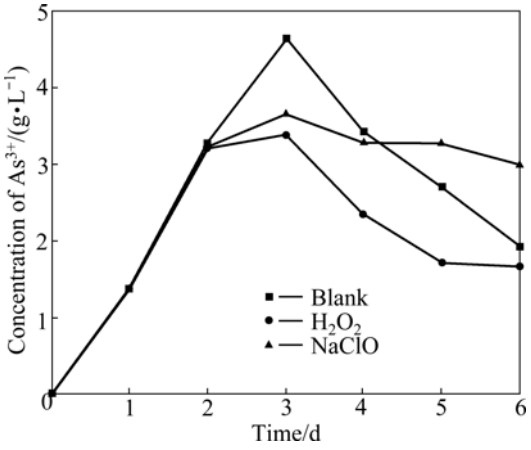

Fig.9 Influence of leaching time on concentration change for As^{3+} by H_2O_2 and NaClO

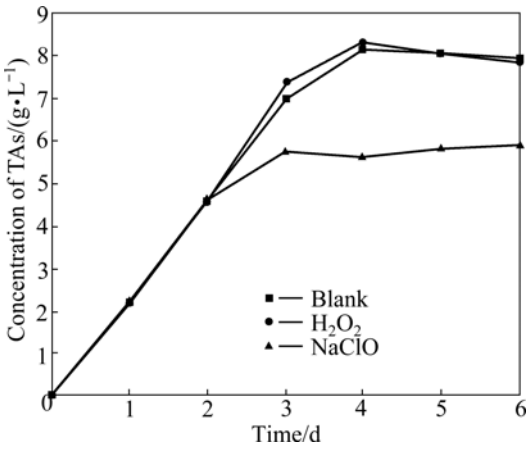

Fig.10 Influence of leaching time on total arsenic concentration change by H_2O_2 and NaClO

rapidly because of its instability and catalysis of other ions. The chloric compounds restrain bacterial activity strongly, which leads to low bacterial oxidizing rate and dearsenization rate.

4 Conclusions

1) The bacterial activity increases with the decrease of As^{3+} concentration and increase of As^{5+} concentration in the bioleaching process. In the lag phase, the concentration of As^{3+} is higher than that of As^{5+}, contrarily it is lower in the log phase and stable phase.

2) The bacteria HQ-0211 have perfect resistances for As^{3+} and As^{5+}. When the concentration of As^{3+} and As^{5+} are above 6.0 g/L and 12.0 g/L, the bacteria remain growth. Stable phase of the bacterial concentration may reach 1.0×10^8 cell/mL or more.

3) Fe^{3+} cannot convert As^{3+} to As^{5+} as strong oxidant in the simplex leaching system, but can cooperate with pyrite and chalcopyrite.

4) H_2O_2 has limited oxidation for As^{3+} and promotes the bacterial oxidizing leaching process. NaClO in the leaching system is unstable, and HClO, Cl_2, $Cl^-$and other chlorinated substances of decomposition products affect bacterial activity, leading to descend of leaching efficiency.

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