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Comparative study on effects of Tween-80 and sodium isobutyl-xanthate on growth and sulfur-oxidizing activities of *Acidithiobacillus albertensis* BY-05

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Abstract: Effects of two typical surfactants, Tween-80 and sodium isobutyl-xanthate (NaIBX), with different concentrations on the growth and sulfur-oxidizing activities of a new strain *Acidithiobacillus albertensis* BY-05, an acidophilic sulfur-oxidizing bacterium, were investigated. The results indicate that both surfactants can enhance the growth and sulfur-oxidizing activities of *A. albertensis* BY-05 only at some special concentrations, e.g., 10^{-4}−10^{-8} g/L for NaIBX and lower than 10^{-8} g/L for Tween-80, but were inhibited and even harmful at higher concentrations. Both surfactants can not be metabolized by *A. albertensis* BY-05. The contact between the bacteria and the sulfur particles may be dependent upon both the extracellular substance and the surfactants, both of which provide the amphiphilic environment improving the attachment for bacteria to the sulfur particles surface. These data could be significant for enlarging the applications of both *A. albertensis* BY-05 and some typical surfactants for industrial bioleaching of sulfides minerals.

Key words: *Acidithiobacillus albertensis* BY-05; surfactant; growth activity; sulfur-oxidizing activity

1 Introduction

It is known that during the acidic dissolution of metal sulfide ores, some elemental sulfur particles may form in the medium, which can inhibit the growth of the sulfur-oxidizing bacteria. The sulfur particles are prone to accumulate on the surface of ores and form sulfur-layer. The sulfur-layer can significantly inhibit the direct contact and reduce the efficiency of bioleaching of the metal sulfide ores by bacteria cells[1−4]. Elemental sulfur is hydrophobic and inert to abiotic oxidation in water without a strong oxidizing agent, and such sulfur oxidation can only be done by some acidophilic sulfur-oxidizing bacteria, typically for example, *Acidithiobacillus* spp[5], suggesting the significance of studying the bacterial oxidation of elemental sulfur.

The bacterial oxidation of the sulfur particles is believed to be initiated by the adhesion of the cells to the sulfur surface[6], then the sulfur transports into the periplasm where the sulfur atoms are oxidized with catalysis of a periplasmic sulfur dioxygenase to sulfite, further oxidized to sulfate by sulfite: acceptor oxidoreductase[7−9]. Such an initial adhesion of the cells to the hydrophobic surface of sulfur particles seems to be inhibited at beginning due to the hydrophilic extracellular layer of the bacterial cells. It was found that some amphiphilic extracellular polymeric substances (EPS) can be expressed with a high level in the sulfur-grown cells, which can assure an efficient contact between the cells and the inert sulfur particles[10]. The EPS of sulfur-grown cells contain considerably less sugar and uronic acids but much more fatty acids. These cells grown in sulfur are more hydrophobic than those in soluble substrates, e.g., ferrous sulfate[11]. This means that the bacteria need to adjust the composition and amount of their EPS to the different growth substrates.

The amphiphilic EPS of sulfur-grown cells assure the adaptation of the cells to the substrates, so it is reasonable to infer that such an adaptation would be modified or do not need any amphiphilic substances in the sulfur-containing medium, typically for example, non-ionic surfactant Tween-80 or ionic flotation agent xanthate. There is no doubt that such typical amphiphilic

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substances will change the surface properties of the ores and thus adjust the contact of the cells to the ores according to the industrial flotation practice. However, it is still unclear about how these amphiphilic substances affect the growth and sulfur-oxidizing activities of the cells. This paper just presents the investigation of the effect of Tween-80 and sodium isobutyl xanthate on the growth and sulfur-oxidizing activities of the typical *Acidithiobacillus* species, *A. albertensis* BY-05, a new strain recently isolated from an acid mine drainage at Baiying of Gansu Province, China, with high sulfur-oxidizing activity[12]. Our investigation may provide the pertinent basic data on the interaction between the minerals and the bacterial cells with typical surfactants and enlarge the application of the new sulfur-oxidizing strain *A. albertensis* BY-05.

2 Experimental

2.1 Bacterial strains and cultivation conditions

A. albertensis BY-05 was the isolate of the Key Laboratory of Biometallurgy of Ministry of Education of China, Central South University, Changsha, China. *A. albertensis* BY-05 was cultivated in 9K medium containing 5.0 g/L of elemental sulfur and the following components(g/L): $(NH_4)_2S0_4$, 3.0; $MgSO_4{\cdot}7H_20$, 0.5; KCl, 0.1; K_2HPO_4, 0.5 and Ca $(NO_3)_2$, 0.01. The initial pH of the media was adjusted to 3.8 with KOH. Incubation was conducted in 250 mL Erlenmeyer flasks containing 100 mL medium and performed on a reciprocating shaker at 180 r/min at 30 ℃. The concentrations of the bacterial cells were measured by light microscope (Olympus CX-31).

2.2 Pretreatment of elemental sulfur

In order to remove the contaminating inorganic sulfides and organic matter from the elemental sulfur surface, the elemental sulfur particles were previously treated three times by sequentially agitating them for 30 min in 1 mol/L of solutions of HCl and NaOH, respectively. After that, the sulfur particles were rinsed twice with di-distilled water and acetone, respectively, and dried at room temperature[13].

2.3 Determination of concentration of sulfate by barium sulfate turbidimetry

The concentration of sulfate was measured by barium sulfate turbidimetry[14]. 0.25 mL sample was added to a 50 mL flask, previously 0.1 g $BaCl_2$, and 1 mL stabilizing agent (75g NaCl dissolved in 300 mL di-distilled water, then 30mL concentrated HCl, 50 mL glycerol, and 100 mL 95% ethanol, mixing well), and then supplemented with di-distilled water to 50 mL. The solution was mixed well for 1 min and left at room temperature for another 15 min when it was transferred to a 2 mL quartz cuvette, and the absorbance was recorded precisely with UV-Vis spectrophotometer (UV−3000, Shimadzu, Japan).

2.4 FT-IR spectrometry

The sulfur particles, before or after being treated with *A. albertensis* BY-05, were measured at room temperature on FT-IR spectrometer (Nexus 670, Nicolet, USA) in the range of 400−4000 cm^{-1} by daubing the samples on potassium bromide slice. The elemental sulfur particles interacting with bacterial cells were thoroughly washed with di-distilled water and dried in vacuum before they were daubed into the KBr pellet slice, and the difference in spectra of the sulfur particles can reflect the absorption of the organic substance on the surface of the sulfur particles.

3. Results and discussion

3.1 Effects of NaIBX and Tween-80 on growth of *A. albertensis* BY-05

The growth curves of *A. albertensis* BY-05 cultivated in the sulfur-contained media with 0−10^{-2} g/L of surfactant NaIBX and Tween-80, and in the no-sulfur medium containing 10^{-4} g/L of NaIBX and Tween-80 are given in Fig.1 and Fig.2, respectively. Results in Fig.1 show that the growth rate of *A. albertensis* BY-05 increases along with the addition of NaIBX in the concentration range of 10^{-4}−10^{-8} g/L, but decreases somewhat at concentration of 10^{-8} g/L and is nearly repressed at the concentration of 10^{-2} g/L. Results in Fig.2 show that the growth rate of *A. albertensis* BY-05 increases with addition of 10^{-8} g/L Tween-80, but decreases with addition of Tween-80 at concentrations higher than 10^{-6} g/L and is nearly repressed at 10^{-2} g/L. This suggests that both NaIBX and Tween-80 can stimulate the growth of *A. albertensis* BY-05 in a limited range of concentrations. These results mean that surfactants can modify the surface properties and improve the contact between the bacterial cells and the energy substrate sulfur particles. Comparison in the effect on the growth by Tween-80 and NaIBX in terms of their concentrations shows that the concentration of Tween-80 that improves the growth of *A. albertensis* BY-05 is lower than that of NaIBX, suggesting that the influence on the growth rate of the non-ionic surfactant Tween-80 seems to be more obvious than the ionic surfactant NaIBX. Fig.1 and Fig.2 also show that *A. albertensis* BY-05 basically does not grow in the medium that has either NaIBX or Tween-80 without elemental sulfur, suggesting that both NaIBX and Tween-80 cannot be metabolized by *A. albertensis* BY-05.

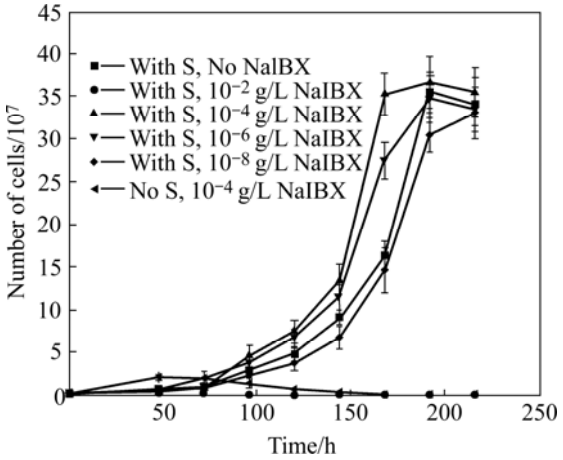

Fig.1 Growth curves of *A. albertensis* BY-05 in 9K medium with different concentrations of NaIBX

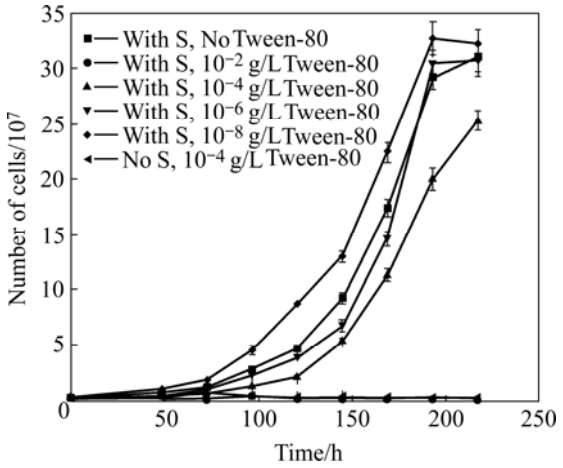

Fig.2 Growth curves of *A. albertensis* BY-05 in 9K medium with different concentrations of Tween-80

3.2 Effects of NaIBX and Tween-80 on sulfur-oxidizing activity of *A. albertensis* BY-05

As a bioleaching sulfur-oxidizing strain, *A. albertensis* BY-05 can oxidize sulfur to sulfuric acid, leading to the decrease in pH values of the solution. The effect of NaIBX or Tween-80 on the sulfur-oxidizing activity of *A. albertensis* BY-05 can be characterized by the changes in pH values and sulfate ion concentrations in the solution. Such changes under different concentrations of NaIBX and Tween-80 are shown in Fig.3 and Fig.4, respectively. Fig.3 and Fig.4 show that, compared with pH value without surfactant, 10^{-4} g/L of NaIBX and 10^{-8} g/L of Tween-80 lead to apparent decrease in pH values and increase in sulfate concentrations, while 10^{-4} g/L of Tween-80 leads to apparent increase in pH values and decrease in sulfate concentrations. It is suggested that the suitable concentrations of NaIBX improving the sulfur-oxidizing of *A. albertensis* BY-05 seem to be in the range of 10^{-4}–10^{-8} g/L. The suitable concentration for Tween-80 is much lower than that of NaIBX, even may be lower

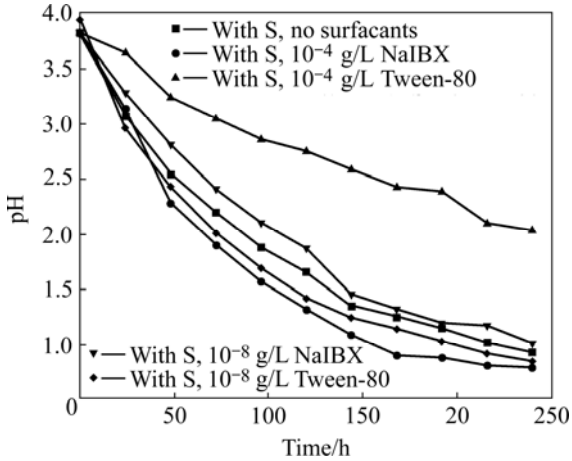

Fig.3 Change of pH values during cultivation of *A. albertensis* BY-05 in 9K medium with 10^{-4} g/L and 10^{-8} g/L of NaIBX or Tween-80

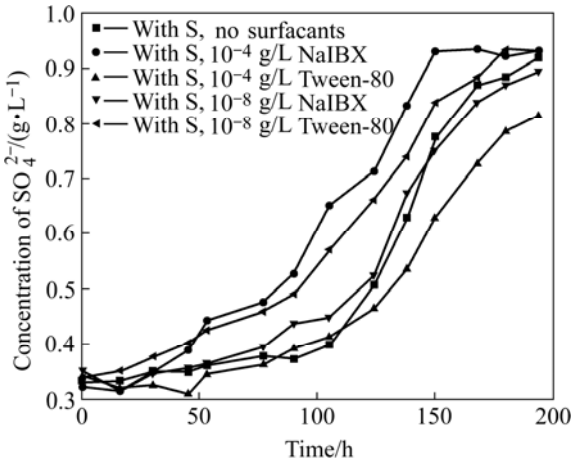

Fig.4 Change of sulfate concentration during cultivation of *A. albertensis* BY-05 in 9K medium with 10^{-4} g/L and 10^{-8} g/L of NaIBX or Tween-80

than 10^{-8} g/L, implying the higher efficient of non-ionic surfactant Tween-80 on the sulfur-oxidizing activity of *A. albertensis* BY-05 than the ionic surfactant NaIBX, and the higher toxicity of the non-ionic surfactant Tween-80 than the ionic surfactant NaIBX at relatively high concentrations.

The toxicity of fairly high concentration of flotation reagents to moderately thermophilic bioleaching microorganisms had been reported[15]. However, little is known about the improvement by these chemicals to the growth and sulfur-oxidizing activities of the acidophilic sulfur-oxidizing bacteria involved in bioleaching of minerals. In this work, just as described above, NaIBX ranging from 10^{-6} to 10^{-4} g/L seems to be optimal to the growth and sulfur-oxidizing activities of *A. albertensis* BY-05. The optimal concentration of Tween-80 for the growth and sulfur-oxidizing activities of *A. albertensis* BY-05 seems to be lower than 10^{-8} g/L, which is much lower than that of NaIBX, suggesting a possible

difference in modification of the surface properties of both sulfur particles and bacterial cells by these two typical surfactants. Table 1 lists the contact angles between several kinds of liquids and sulfur discs, which shows that the contact angles between the sulfur discs and the solution of Tween-80 are smaller and much more apparently decreased with the increase in the surfactant concentrations than those of NaIBX, suggesting that Tween-80 can modify the contact between the sulfur particles and the bacterial cells much more efficiently than NaIBX. By comparing the contact angles listed in Table 1 in more details, it can be found that though the potential of modification by the surfactants can be expected, i.e., the contact angles for both the surfactants decrease with increase in their concentrations, the optimal ranges of the surfactants can not be predicted. It is suggested that besides the effects of the surfactants, the contact between the sulfur particles and the bacterial cells may be also affected by the extracellular layer of the bacterial cells.

Table 1 Contact angles between several kinds of liquids and sulfur discs (°)

9K medium for sulfur oxidation	Concentration of Tween-80/(g·L^{-1})				Concentration of NaIBX/(g·L)		
	10^{-8}	10^{-6}	10^{-4}	10^{-2}	10^{-6}	10^{-4}	10^{-2}
78.8±1.9	85.9±2.0	73.9±1.5	42.0±1.6	11.3±0.9	81.4±1.8	76.0±1.3	76.2±1.6

3.3 FT-IR spectra of elemental sulfur before and after being treated by *A. albertensis* BY-05

The cells of *A. ferrooxidans* secreted more extracellular proteins when they were inoculated in the medium with pyrite or elemental sulfur after growing for a period of time in ferrous sulfate[16]. These proteins might take part in the interfacial action between cells and solid sulfides. In this case, just as shown by the FT-IR spectra in Fig.5, the sulfur particles after being treated by *A. albertensis* BY-05 cells have some apparent absorption zone form 1 536 to 500 cm^{-1}, which may be assigned for the formation of sulfur oxides. A strong absorption peak at 1 650 cm^{-1} is assigned to carbonyl. A wide absorption zone at 3 280 cm^{-1} might be related to the hydroxyl on the surface of elemental sulfur, indicating that the superficial properties of elemental sulfur are changed after it is treated by the bacterial cells. These new functional groups that modify the surface property of the sulfur particles are undoubtedly produced by the sulfur-oxidizing cells. These functional groups may actively adjust the secretion of their extracellular substance to attach the surface of elemental sulfur, so change their surface property from hydrophobicity to hydrophilicity.

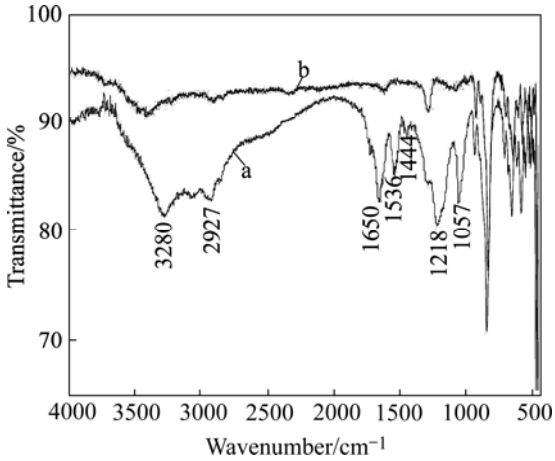

Fig.5 FT-IR spectra of elemental sulfur before (a) and after (b) being treated by *A. albertensis* BY-05 cells

It was reported that nonionic surfactants of Tween on the production and release of enzyme in industrial submerged culture or solid-state fermentation[17]. The study shows that ionic flotation agent xanthate at 10^{-4} mol/L can significantly improve the leaching efficiency of sphalerite[18]. Similar to the secreted extracellular substance, these surfactants added artificially to the medium may play as a bridge that provides the amphiphilic environment, leading to bacteria attachment to the sulfur particle surfaces. From the discussion above, it may be expected in the actual bioleaching trials that the addition of suitable concentrations of some surfactant to the sulfur-forming medium can enhance the growth and sulfur-oxidizing activities of *Acidithiobacillus* cells, and prevent elemental sulfur particles from aggregating, which may further deposit on the surface of sulfides minerals and inhibit the oxidation of the sulfides.

4 Conclusions

1) The effect of the surfactant NaIBX in different concentrations on the growth of *A. albertensis* BY-05 is much more obvious than that of Tween-80. The reason could be that Tween-80 can be much easier to modify the sulfur surface hydrophobicity and more convenient to contact the bacterial cells and sulfur particles. However, higher concentrations of Tween-80 are harmful to the bacterial cells.

2) Both surfactants can't be metabolized by *A. albertensis* BY-05. However, they can enhance the growth and sulfur-oxidizing activities of *A. albertensis* BY-05 in some limited concentrations, e.g., 10^{-4}–10^{-6} g/L for NaIBX and lower than 10^{-8} g/L for Tween-80.

3) These findings may be helpful to enhancing the applications of the new sulfur-oxidizing strain *A. albertensis* BY-05 and some typical surfactants to

industrial bioleaching of sulfides minerals.

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