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Research article

Interaction of *Acidithiobacillus ferrooxidans*, *Rhizobium phaseoli* and *Rhodotorula* sp. in bioleaching process based on Lotka–Volterra model

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A R T I C L E I N F O

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A B S T R A C T

Background: Nowadays, leaching-ore bacteria, especially *Acidithiobacillus ferrooxidans* is widely used to retrieve heavy metals, many researches reflected that extra adding microorganism could promote bioleaching efficiency by different mechanisms, but few of them discussed the interaction between microorganisms and based on growth model. This study aimed to provide theoretical support for the collaborative bioleaching of multiple microorganisms by using the Lotka–Volterra (L–V) model.
Results: This study investigated the interaction of *Acidithiobacillus ferrooxidans*, *Rhizobium phaseoli*, and *Rhodotorula* sp. Results showed that the individual growth of the three microorganisms fit the logistic curves. The environmental capacities of *A. ferrooxidans*, *R. phaseoli*, and *Rhodotorula* sp. were 1.88×10^9, 3.26×10^8, and 2.66×10^8 cells/mL, respectively. Co-bioleaching showed mutualism between *A. ferrooxidans* and *R. phaseoli* with mutualism coefficients of $\alpha = 1.19$ and $\beta = 0.31$, respectively. The relationship between *A. ferrooxidans* and *Rhodotorula* sp. could be considered as commensalism. The commensalism coefficient γ of the effect of *Rhodotorula* sp. on *A. ferrooxidans* was 2.45. The concentrations of *A. ferrooxidans* and *R. phaseoli* were 3.59×10^9 and 1.44×10^9 cells/mL in group E, respectively, as predicted by the model. The concentrations of *A. ferrooxidans* and *Rhodotorula* sp. were 2.38×10^9 and 2.66×10^8 cells/mL, respectively. The experimental peak values of the concentrations in microorganism groups E and F were detected on different days, but were quite close to the predicted values.
Conclusion: The relationship among microorganisms during leaching could be described appropriately by Lotka–Volterra model between the initial and peak values. The relationship of *A. ferrooxidans* and *R. phaseoli* could be considered as mutualism, whereas, the relationship of *A. ferrooxidans* and *R. phaseoli* could be considered as commensalism.

1. Introduction

Several originally existing organic compounds or metabolites produced in a solution might inhibit the activity and quantity of *Acidithiobacillus ferrooxidans* during bioleaching, thereby possibly affecting the efficiency of leaching [1,2]. In recent years, many studies on microbial collaboration between *A. ferrooxidans* and heterotrophic bacteria have been conducted. According to Okibe Naoko, the most effective bioleaching systems are consortia containing both autotrophic and heterotrophic moderate thermophiles [3]. Harrison studied the symbiotic mechanism between *Acidiphilium cryptum* and *A. ferrooxidans* and showed that *A. cryptum* can promote the growth of *A. ferrooxidans* [4]. Schrenk et al. [5] studied the bioleaching of pyrite and found that the leaching rate is higher with only *Thiobacillus ferrooxidans* than with both *T. ferrooxidans* and *Leptospirillum ferrooxidans*. Falco et al. [6] used *L. ferrooxidans* to leach copper with *A. ferrooxidans* and several other moderate thermophiles and found that the effect is more remarkable than any other single bacterium. Umanskii and Klyushnikov [7] researched the bioleaching process of uranium extraction from pyrite by a mixture of *A. ferrooxidans* and *A. thiooxidans* and found that the efficiency exceeded the results obtained by traditional acid leaching and single bacteria leaching. Our previous study demonstrated that *Rhizobium phaseoli*, as an acid-resistant chemoheterotrophic bacterium, can effectively metabolize the metabolites in the EPS of *A. ferrooxidans* into simple organic molecules to decrease its harmful effect to *A. ferrooxidans* in

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Table 1
The main element content of copper tailings.

Element	Content (mg/g)	Element	Content (mg/g)
Al	82.1786437	As	1.2221136
Ca	71.6768112	Cd	0.06403
Fe	141.931	Cu	3.49065
Mn	2.42879	Pb	0.33251
Si	250.35	Zn	1.3706
S	66.6592	Ni	0.06227
K	19.8302	Mg	4.57661

bioleaching solutions, and *R. phaseoli* could obtain energy by metabolizing the organic metabolites [8]. Previous study also showed that *Rhodotorula* sp. exhibits good ability to adsorb ions of Cd, Pb, and Cu in a solution, which are very harmful to *A. ferrooxidans*. The above-mentioned studies showed that composite microorganisms might increase leaching efficiency as the numbers of microorganisms were all changed during leaching using one single microorganism only. This leaching using a single microorganism is dependent on the different interactions among microorganisms, such as competition, predation, commensalism, and mutualism.

Thorough studies on the interacting growth models between single microorganism and populations during leaching are relatively few. Lotka [9] and Volterra [10] proposed a famous growth model that provides a new basis for the mathematical ecology of populations. Guerra [11] described the relationship between the absolute rates of *Lactococcuslactis* growth using the Lotka–Volterra (L–V) two predators-one prey model. Fujikawa et al. [12] described bacterial growth in a mixed culture of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* using the L–V model and found that the values of the competition coefficient in the model were stable. Mounier et al. [13] used the L–V model to evaluate microorganism interactions and proved the significant role of yeast-bacterium interactions in the establishment of this multispecies ecosystem on the cheese surface. Many researchers have studied the relationship between the two microorganisms using the L–V model. However, no research on the collaborative leaching of microorganisms has been conducted to date. Thus, based on these works, this study aimed to investigate the growth of *A. ferrooxidans*, *R. phaseoli*, and *Rhodotorula* sp. at a certain time during the bioleaching process and determine the relationships and interactions between the two microorganisms using the L–V model. This study also aimed to provide theoretical support for the collaborative bioleaching of multiple microorganisms.

2. Materials and methods

2.1. Materials

2.1.1. Sample

The tailing sample was collected from a copper mine reservoir in Yunnan province, China. Early sample analysis showed chalcopyrite as the main component, with 0.31% copper quality. However, the contents of other heavy metals especially toxic heavy metals (Cd 0.06403 mg/g, Pb 0.33251 mg/g, Ni 0.06227 mg/g and so on) were too little to affect the bacteria, the average particle size of this sample was 18.30 μm, and the content of sulfur was relatively high to provide the energy for *A. ferrooxidans*, so this tailing sample was suitable for bioleaching. The results of total content of heavy metals are listed in Table 1.

2.1.2. A. ferrooxidans

The strain was isolated from an acid mine drainage and stored in the biological lab of Chongqing University, China. At the beginning of the experiment, 9 K liquid medium was inoculated with the strain and then placed in constant temperature shaking with suitable environment. Only the bacteria in logarithmic phase were used for this experiment. The pictures under optical microscope are listed in Fig. 1.

2.1.3. R. phaseoli

The strain, which is a type of heterotrophic and aerobic bacteria, was obtained from Agricultural Culture Collection of China and initially isolated from nodules of kidney bean. The strain could use many types of carbon source and grow in acidic environment. After previous domestication, the strain could grow normally in a copper concentration of 0.5 g/L and pH value of 2.

2.1.4. Rhodotorula sp.

The strain is a type of aerobic fungus, which was obtained from Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences. The strain has good adsorption ability of heavy metal ions, and it could grow normally at the solution of pH = 2.

2.1.5. Medium

9 K liquid medium for *A. ferrooxidans* (composition: 3 g/L $(NH_4)_2SO_4$, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4 \times 7H_2O$, 0.01 g/L $Ca(NO_3)_2$, 44.3 g/L $FeSO_4 \times 7H_2O$, 0.1 g/L KCl and 1 L distilled water), Yeast morphology agar liquid medium for *R. phaseoli* (composition: 10 g/L mannitol, 1 g/L yeast powder, 0.5 g/L K_2HPO_4, 0.2 g/L $MgSO_4$ x $7H_2O$, 0.1 g/L $CaHPO_4$, 0.1 g/L NaCl, 4 mL 0.5% boric acid solution, 4 mL 0.5% sodium molybdate solution, 10 mL 0.4% Congo red and 1 L distilled water) and Maxwell culture medium for *Rhodotorula* sp. (composition: 1 g/L glucose, 1.8 g/L KCl, 0.5 g/L yeast powder, 8.2 g/L CH_3COONa, 0.01 g/L $Ca(NO_3)_2$ and 1 L distilled water).

2.1.6. Experimental equipment

Atomic fluorescence spectrometer (SK-2002B; Beijing, China), vertical pressure steam sterilizer (YXQ-LS-30S; Shanghai, China), constant temperature shaking (THZ-92A; Shanghai, China), pH-ORP tester (ORP-421; Shanghai, China), microscope (XSP-8C; Shanghai, China), thermostatic incubator (LRH-250-A; Shanghai, China), and hemocytometer (XB-R-25; Shanghai, China) were used in this experiment.

2.1.7. Analytical methods

The concentration of copper was tested with Atomic fluorescence spectrometer, leaching rate was defined as the copper concentration in leaching solution divided by the total copper content in the sample.

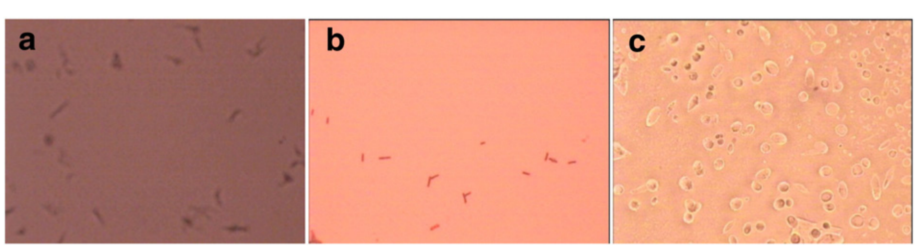

Fig. 1. Pictures of *A. ferrooxidans* (a), *R. phaseoli* (b) and *Rhodotorula* sp. (c) under optical microscope.