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

Xuecheng Zheng \textsuperscript{a,b}, Dongwei Li \textsuperscript{b,*}

\textsuperscript{a} College of Chemistry and Chemical Engineering, Southwest Petroleum University, Chengdu, China
\textsuperscript{b} College of Resource and Environment Science, Chongqing University, Chongqing, China

\begin{abstract}
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 \times 10^9, 3.26 \times 10^9, and 2.66 \times 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 \( \gamma = 1.19 \) and \( \gamma = 0.31 \) of the effect of Rhodotorula sp. on A. ferrooxidans was 2.45. The concentrations of A. ferrooxidans and R. phaseoli were 3.50 \times 10^9 and 1.44 \times 10^9 cells/mL in group E, respectively, as predicted by the model. The concentrations of A. ferrooxidans and Rhodotorula sp. were 2.38 \times 10^9 and 2.66 \times 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.

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\end{abstract}

\section{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...
biobleaching 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 Lactococcus lactis 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 biobleaching 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 biobleaching 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 biobleaching. 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 acid 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 (NH₄)₂SO₄, 0.5 g/L KH₂PO₄, 0.5 g/L MgSO₄ × 7H₂O, 0.01 g/L Ca(NO₃)₂, 44.3 g/L FeSO₄ × 7H₂O, 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 KH₂PO₄, 0.2 g/L MgSO₄ × 7H₂O, 0.1 g/L CaHPO₄, 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 CH₃COONa, 0.01 g/L Ca(NO₃)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.

### Table 1

The main element content of copper tailings.

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

**Fig. 1.** Pictures of *A. ferrooxidans* (a), *R. phaseoli* (b) and *Rhodotorula* sp. (c) under optical microscope.
We counted the number of bacteria/fungus by Hemocytometer measurement: at first, we centrifuged or diluted the bacteria/fungus liquid until the concentration was at the order of appropriate magnitude and dyed them with trypan blue, after that we counted them in the grids for 3 times and calculated the concentration with corresponding formula.

2.2. Experimental procedure

Tailing sample (10 g) was ground and divided into 18 flasks and six groups. Three flasks were included in each group for parallel tests. Group A was assigned as the sterile control group, and the five other groups were marked from B to F. We added 10 mL of yeast malt agar (YMA) medium, 10 mL of Maxwell culture medium, and bacterial liquid into each flask. The concentrations are listed in Table 2. We adjusted the volumes to 100 mL by adding non-iron 9 K liquid medium. We also adjusted the pH to 2.2 by adding concentrated sulfuric acid in all of the five groups. We then placed the 18 flasks in an air bath oscillator at 100 rpm and 25°C. The experiment lasted 25 d. The concentrations of copper and the number of bacterial/fungal cells were measured every 3 d. We calculated the mean values as the final test results after results with evident errors were omitted.

3. Results and discussion

3.1. Leaching results of copper

As shown in Fig. 2, the leaching rate of copper increased significantly in the first 3 d because the acid in solution preferentially reacted with minerals. Its reaction rate was much faster than the bio-catalytic reaction. The leaching rate in control group A barely increased after the 25th d was 16.8%. The growth of leaching rate in group D was almost the same as that in control group A. This result indicated that even using Rhodotorula sp., only could hardly influence the bioleaching process. The leaching rate in group D was generally a little higher than that in control group A. One possible reason could be the small molecular organic acids produced by R. phaseoli, which could damage mineral lattices to release copper into solution [14]. The leaching rate on the 25th d was 18%. The leaching rate of 22.5% in group B was significantly higher than that in groups A, C, and Don the 25th d. This result showed that A. ferrooxidans is an effective kind of bacteria during bioleaching. The leaching rates in groups E and F on the 25th d were respectively 34.1% and 24.3%, which is much higher than any other groups including group B. This result indicated that adding extra Rhodotorula sp. and R. phaseoli could further promote the leaching efficiency. Thus, to study the microorganisms themselves and their interactions in the leaching process is necessary.

3.2. Fitting results of single-microorganism growth curve and calculation of environmental capacity

The growth curves of A. ferrooxidans, R. phaseoli and Rhodotorula sp. in groups B, C and D are shown in Table 3 and Fig. 3.

Table 2
Initial cell numbers of bacteria/fungus in Group A–F (cells/mL).

<table>
<thead>
<tr>
<th>Group</th>
<th>A. ferrooxidans</th>
<th>R. phaseoli</th>
<th>Rhodotorula sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$1 \times 10^7$</td>
<td>$1 \times 10^7$</td>
<td>$1 \times 10^7$</td>
</tr>
<tr>
<td>B</td>
<td>$1 \times 10^7$</td>
<td>$1 \times 10^7$</td>
<td>$1 \times 10^7$</td>
</tr>
</tbody>
</table>

We used a logistic equation to fit the experimental results for calculating environmental capacity $K$. The logistic equation is as follows:

$N_t = \frac{K}{1 + ae^{rt}} \quad \text{[Equation 1]}$

where $r$ represents the population growth rate, $t$ represents the time, $N_t$ represents the instant population number, and $K$ represents the environmental capacity. When $t = 0, N = N_0 = K(1 + a)$; if $N_0 < K$, $N_{t(1)} < K$ is tenable regardless of the $r$ value; if $N_0 > K, N_{t(1)} > K$ is tenable regardless of the $t$ value. When $t \to \infty, \lim_{t \to \infty} N_t = K$, when $N_t = K$, microorganism population reaches the maximum, $dn/dt = 0$, and the population does not grow further at this moment. Microbial growth is not the unlimited $J$ type, but the smooth $S$ type, where in the curve is close to the $K$ value. The fitting results of A. ferrooxidans, R. phaseoli and Rhodotorula sp. are shown in Fig. 4:

The fitting equation of A. ferrooxidans:

$N_t = \frac{1.88013 \times 10^8}{1 + 45.33461 \times e^{-0.36154t}} \quad \text{R-square = 0.98} \quad \text{[Equation 2]}$

The fitting equation of R. phaseoli:

$N_t = \frac{3.25624 \times 10^8}{1 + 26.08856 \times e^{-0.67067t}} \quad \text{R-square = 0.93} \quad \text{[Equation 3]}$

The fitting equation of Rhodotorula sp.:

$N_t = \frac{2.65563 \times 10^8}{1 + 16.63705 \times e^{-0.3342t}} \quad \text{R-square = 0.98} \quad \text{[Equation 4]}$

$K$ of A. ferrooxidans, R. phaseoli and Rhodotorula sp. were respectively $1.88 \times 10^8, 3.26 \times 10^8$, and $2.66 \times 10^8$ cells/mL. The individual growth curves fit the logistic model and satisfied the precondition of the L-V model. We then performed a bioleaching experiment of A. ferrooxidans and R. phaseoli, A. ferrooxidans and Rhodotorula sp. to investigate their interaction. The information of these microorganisms is shown in Fig. 5.

Fig. 5 and Table 4 showed the increased maximum specific growth rate, maximum absolute growth rate, and the final cell numbers in the groups. Compared with Group B, the results indicated that the influences of both R. phaseoli and Rhodotorula sp. on A. ferrooxidans were positive. The maximum specific growth rate of R. phaseoli in Group C was even higher than that in Group E, whereas the growth almost stopped on the 15th d in Group C. The maximum absolute
growth rates and final cell numbers in Group C were both much lower than that in Group E. The reason might be because R. phaseoli needs longer adaptation period during adding extra A. ferrooxidans at the start of collaborative leaching. Then, the positive effect of A. ferrooxidans caused R. phaseoli to grow more rapidly, as shown in Fig. 5. The three indexes of Rhodotorula sp. cells were almost the same as in groups D and F, which indicated that A. ferrooxidans exhibit little effect on Rhodotorula sp.’s growth. Thus, the relationship between A. ferrooxidans and R. phaseoli could be considered as mutualism. The relationship between A. ferrooxidans and Rhodotorula sp. could be considered as commensalism before d 24 of bioleaching. Meanwhile, these three microorganisms can grow individually.

3.3. Calculation results of the L–V model

For simplicity, the promoting effects between microorganisms were all considered as providing food. Hence, the functions of quantitative changes were expressed in the following equations:

\[
\frac{dN_1}{dt} = r_1 N_1 \left( \frac{N_1}{K_1} + \alpha N_2 \right) \\
\frac{dN_2}{dt} = r_2 N_2 \left( \frac{N_2}{K_2} + \beta N_1 \right) \\
\frac{dN_3}{dt} = r_3 N_3 \left( \frac{N_3}{K_3} + \gamma N_4 \right) \\
\frac{dN_4}{dt} = r_4 N_4 \left( \frac{N_4}{K_4} \right)
\]

where 1 and 2 respectively represent A. ferrooxidans and R. phaseoli in group E; 3 and 4 respectively represent A. ferrooxidans and Rhodotorula sp. in group F; N is the instant population number; K is the environmental capacity of microorganisms; and r is the intrinsic rate of increase (the maximum instant growth rate). \( \alpha \) (mutualism coefficient) is the amount of food provided by unit R. phaseoli (N2) to fend A. ferrooxidans N1. We multiply \( \alpha \) by the amount of food consumed by unit A. ferrooxidans (N1); \( \beta \) (mutualism coefficient) is the amount of food provided by unit A. ferrooxidans (N1) to fend R. phaseoli N2. We multiply \( \beta \) by the amount of food consumed by unit (N2); \( \gamma \) (commensalism coefficient) is the amount of food provided by unit Rhodotorula sp. (N4) to fend A. ferrooxidans N3, we multiply by the amount of food consumed by unit (N3).

\( N_1 \) and \( N_2 \) remained unchanged when the number of the two species reached relative equilibrium. This phenomenon occurred because the nutrients provided by the tailing sample for the microorganisms were limited and the peak or equilibrium values of the number of cells could be obtained temporarily but could not be maintained permanently. \( f(N_1 N_2) \) and \( f(N_1 N_2) \) should be 0 at the same time. The intersection point of the two equations should also correspond to the equilibrium point of the two microorganisms.

\[
\frac{dN_1}{dt} = 0, \quad \frac{dN_2}{dt} = 0, \quad \frac{dN_3}{dt} = 0, \quad \frac{dN_4}{dt} = 0
\]  

[Equation 6]

Four equations are determined when growth rates were 0. The equations could not be solved explicitly although the previous experiment obtained real-time microbe numbers \( t_1 N_1, t_2 N_2 \) at different times for \( m + 1 \) (m = 7) times, \( i = 0,1,2,...,8 \), and \( N_1 = N_1(i+1) \), \( k = 1,2; \) \( i = 0,1,2,...,8 \). We sought the parameters using the method of inverse problem in differential equations, which meant using the data directly from the experiment to seek the approximate parameters in equations. The growth rates of \( N_1, N_2, N_3, N_4 \) are shown in Fig. 6:

Taking group E as an example, the equations of growth rates of A. ferrooxidans and R. phaseoli could be changed to time derivatives as follows:

\[
\begin{align*}
\frac{d\ln N_1}{dt} &= r_1 \left( 1 - \frac{N_1}{K_1} - \frac{\alpha N_2}{K_1} \right) \\
\frac{d\ln N_2}{dt} &= r_2 \left( 1 - \frac{N_2}{K_2} - \frac{\beta N_1}{K_2} \right)
\end{align*}
\]  

[Equation 7]

Then we estimated the parameters of equations and integrated them on interval \( (t_{i1}, t_i) \). Thus,

\[
\begin{align*}
\ln N_{1i} - \ln N_{1i-1} &= r_1 \left( t_i - t_{i-1} \right) \frac{A_{1i}}{K_1} + \frac{\alpha A_{2i}}{K_1} \\
\ln N_{2i} - \ln N_{2i-1} &= r_2 \left( t_i - t_{i-1} \right) \frac{A_{2i}}{K_2} + \frac{\beta A_{1i}}{K_2}
\end{align*}
\]  

[Equation 8]

where, \( A_i = \int_{t_{i-1}}^{t_i} N(t)dt; i = 1,2,...,8; k = 1,2 \). We obtained the equations of the parameters of [Equation 8]: \( AX = B1, \ Y = B2 \), then:

\[
A = \begin{pmatrix}
A_{11} & A_{12} \\
A_{21} & A_{22}
\end{pmatrix}, \quad Y = \begin{pmatrix}
r_1 \\
r_2
\end{pmatrix}, \quad X = \begin{pmatrix}
\frac{1}{K_1} \\
\frac{1}{K_2}
\end{pmatrix}, \quad B = \begin{pmatrix}
\ln N_{1i} - \ln N_{1i-1} \\
\ln N_{2i} - \ln N_{2i-1} \\
\vdots \\
\ln N_{8i} - \ln N_{8i-1}
\end{pmatrix}, \quad k = 1,2
\]

[Equation 9]

[Equation 9] has no solutions of general sense. Thus, we sought the least-squares solution:
\[ X = (A^T A)^{-1} A^T B_1, \ Y = (A^T A)^{-1} A^T B_2, \ A^T \] represents the transpose of \( A \).

\( A_{1i} \) and \( A_{2i} \) are solved using the trapezoid method formula in numerical integration:

\[ A_{ki} = \int_{t_{i-1}}^{t_i} N(t)\,dt \approx \frac{t_i - t_{i-1}}{2} (N_{ki} + N_{ki-1}) \]  

[Equation 10]

Among them, \( i = 1, 2, \ldots, 7 \) and \( k = 1, 2 \). Then we can obtain the approximate values of \( X, Y \):

\[
\tilde{X} = (\tilde{A}^T \tilde{A})^{-1} \tilde{A}^T B_1, \tilde{Y} = (\tilde{A}^T \tilde{A})^{-1} \tilde{A}^T B_2, \text{ and } \tilde{A} \text{ is a matrix by changing } A_{ki} \text{ to } \tilde{A}_{ki} \text{ in matrix } A.
\]

The two equation curves of \( N_1 = N_1(t) \) and \( N_2 = N_2(t) \) are continuous and smooth. When the time interval \( t_{i-1}, t_i \) was not too long, the error between \( A_{ki} \) and \( \tilde{A}_{ki} \) was small; Hence, we could obtain the approximate solutions of \( \alpha, \beta \) and \( \gamma \) using the least squares method in MATLAB, as follows: \( \alpha = 1.19, \beta = 0.31, \gamma = 2.45 \), which meant that unit \( A. \text{ferrooxidans} \) provided 0.31 times more food than that consumed by unit \( R. \text{phaseoli} \), whereas unit \( R. \text{phaseoli} \) provided 1.19 times more food than that consumed by unit \( A. \text{ferrooxidans} \).

As the growth rates were zero at the equilibrium state, \( dN/dt = 0 \), we could get obtain the following equation:

\[
\begin{cases}
1 - N_1 K_1 + \alpha N_2 K_1 = 0 \\
1 - N_2 K_2 + \beta N_1 K_2 = 0 \\
1 - N_3 K_3 + \gamma N_4 K_3 = 0 \\
1 - N_4 K_4 = 0
\end{cases}
\]  

[Equation 11]

The frontal fitting results showed the environmental capacities (cells/mL), as follows: \( K_1 = 1.88 \times 10^9; K_2 = 3.26 \times 10^8; \) and \( K_4 = 2.66 \times 10^8 \). After substituting these data in the previous equation sets, we could obtain the two lines determined by the following equation sets:

\[
\begin{cases}
N_2 = 0.84N_1 - 1.58 \times 10^3 \\
N_2 = 0.31N_1 + 3.26 \times 10^8 \\
N_4 = 0.408N_3 - 7.06 \times 10^8 \\
N_4 = 2.66 \times 10^8
\end{cases}
\]  

[Equation 12]
Fig. 7 reveals that the lines determined by the two isocline equations intersected with the coordinate axis at \((1.1 \times 10^9, 0)\) and \((0, 1.45 \times 10^9)\). The two lines also intersected with each other at equilibrium point \(P_1(3.59 \times 10^9, 1.44 \times 10^9)\). This finding indicated that the concentrations of \(A.\) ferrooxidans and \(R.\) phaseoli predicted by the model were \(3.59 \times 10^9\) cells/mL and \(1.44 \times 10^9\) cells/mL, respectively. At the end of the experiment on d 24, the concentrations of \(A.\) ferrooxidans and \(R.\) phaseoli were \(2.8 \times 10^9\) and \(1.3 \times 10^9\) cells/mL, respectively. As the leaching experiment in group E was extended until d 33, the concentration of \(A.\) ferrooxidans reached the peak value of \(3.31 \times 10^9\) cells/mL and became stable for 9 d. On d 42, the concentration of \(A.\) ferrooxidans decreased slowly because of insufficient nutrients. The number of \(R.\) phaseoli cells reached the peak value of \(1.3 \times 10^9\) cells/mL on d 36 and became stable for 3 d. On d 39, the number of \(R.\) phaseoli cells decreased slowly. The difference between the experimental values and the predicted values was small. This finding indicated the good fitting effect of the mutualism model. Furthermore, \(\alpha > \beta\) implied that \(A.\) ferrooxidans elicited a greater promoting effect on \(R.\) phaseoli than \(R.\) phaseoli did.

Fig. 7b shows a horizontal line parallel to the x-axis of the isocline of \(Rhodotorula\) sp. This condition showed the almost no effect of \(A.\) ferrooxidans to \(Rhodotorula\) sp. The two lines intersected with each other at the equilibrium point \(P_2(2.38 \times 10^9, 2.66 \times 10^8)\). This finding indicated that the concentrations of \(A.\) ferrooxidans and \(Rhodotorula\) sp. predicted by the model in equilibrium were \(2.38 \times 10^9\) and \(2.66 \times 10^8\) cells/mL, respectively. On d 24, the concentrations of \(A.\) ferrooxidans and \(Rhodotorula\) sp. were \(2.4 \times 10^9\) and \(2.7 \times 10^8\) cells/mL, respectively. The difference between the experimental values and the predicted values was very small, which indicated that the fitting effect of the mutualism model was also very

| Table 4 | Maximum specific growth rates \((d^{-1})\), maximum absolute growth rates \((\text{cells/mL}\cdot d^{-1})\) and final cell numbers \((\text{cells/mL})\) of the three microorganisms. |
|----------------|----------------|----------------|----------------|----------------|----------------|
| Group II | Group C | Group D | Group E | Group F |
| \(A.\) ferrooxidans | \(\mu = 0.34\) | \(\mu = 0.41\) | \(\mu = 0.35\) | \(\mu = 0.32\) |
| MAGR = \(1.7 \times 10^8\) | MAGR = \(2.3 \times 10^8\) | MAGR = \(2.4 \times 10^8\) | MAGR = \(2.2 \times 10^7\) |
| Cell = \(1.8 \times 10^9\) | Cell = \(2.8 \times 10^9\) | Cell = \(2.4 \times 10^9\) | Cell = \(2.6 \times 10^8\) |
| \(R.\) phaseoli | \(\mu = 0.67\) | \(\mu = 0.40\) | \(\mu = 0.31\) | \(\mu = 0.31\) |
| MAGR = \(5.5 \times 10^7\) | MAGR = \(1.2 \times 10^8\) | MAGR = \(2.2 \times 10^7\) | MAGR = \(2.2 \times 10^7\) |
| Cell = \(3.4 \times 10^9\) | Cell = \(1.3 \times 10^9\) | Cell = \(2.6 \times 10^8\) | Cell = \(2.7 \times 10^8\) |
| \(Rhodotorula\) sp. | \(\mu = 0.35\) | \(\mu = 0.30\) | \(\mu = 0.31\) | \(\mu = 0.31\) |
| MAGR = \(2.2 \times 10^7\) | MAGR = \(2.2 \times 10^7\) | MAGR = \(2.2 \times 10^7\) | MAGR = \(2.2 \times 10^7\) |
| Cell = \(2.6 \times 10^8\) | Cell = \(2.7 \times 10^8\) | Cell = \(2.7 \times 10^8\) | Cell = \(2.7 \times 10^8\) |
good. Moreover, the number of cells of the two microorganisms reached relative equilibrium on 24th d.

In Fig. 7, the two lines divided the first quadrant into four regions. In region $S_1$, $dN_1/dt > 0$ and $dN_2/dt < 0$ or $dN_2/dt > 0$ and $dN_3/dt < 0$. The number of $A. ferrooxidans$ in group E or A. ferrooxidans in group F increased, whereas $R. phaseoli$ in group E or Rhodotorula sp. in group F decreased at any position in this region. In region $S_2$, $dN_1/dt > 0$ and $dN_2/dt > 0$ or $dN_2/dt > 0$ and $dN_3/dt > 0$. The number of $A. ferrooxidans$ in group E or $A. ferrooxidans$ in group F and $R. phaseoli$ in group E or Rhodotorula sp. in group F increased at any position in this region. In region $S_3$, $dN_1/dt < 0$ and $dN_2/dt > 0$ or $dN_2/dt < 0$ and $dN_3/dt > 0$. The number of $A. ferrooxidans$ in group E or $A. ferrooxidans$ in group F and $R. phaseoli$ or Rhodotorula sp. in group F decreased at any position in this region. The arrows in Fig. 7 represent the increase or decrease in the number of cells. Regardless of the number of cells of the three microorganisms in any place in the quadrant, the numbers moved toward point $P$ and reached relative equilibrium.

4. Conclusions

This study fitted the growth of $A. ferrooxidans$, $R. phaseoli$, and Rhodotorula sp. in a leaching environment by using Lotka–Volterra model. Our results revealed that the individual growth curves of $A. ferrooxidans$, $R. phaseoli$, and Rhodotorula sp. fit the logistic pattern. In leaching process, the relationship of $A. ferrooxidans$ and $R. phaseoli$ could be considered as mutualism, and the mutualism coefficients of $A. ferrooxidans$ and $R. phaseoli$ were $\alpha = 1.19$ and $\beta = 0.31$, respectively. Whereas, the relationship of $A. ferrooxidans$ and $R. phaseoli$ could be considered as commensalism and the commensalism coefficient $\gamma$ of the effect of Rhodotorula sp. on $A. ferrooxidans$ was 2.45. This finding indicated that during the leaching process, $A. ferrooxidans$ elicited a greater promoting effect than $R. phaseoli$. A. ferrooxidans almost did not affect the growth of Rhodotorula sp., whereas Rhodotorula sp. could improve the growth of $A. ferrooxidans$. The predicted values of intersection points determined by isocline equations were quite close to the experimental values. Therefore, the L–V model could appropriately describe the relationships among the three microorganisms in bioleaching before they reached relatively stable peak values.

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Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ejbt.2016.06.004.

References


7] Umanskii AB, Klyushnikov AM. Bioleaching of low grade uranium ore containing
pyrite using A. ferrooxidans and A. thiooxidans. J Radioanal Nucl Chem 2013;295:

8] Zheng XC, Li DW. Synergy between Rhizobium phaseoli and Acidithiobacillus
http://dx.doi.org/10.1155/2016/9384767.

9659(95)00050-Z.

10] Lu ZY, Takeuchi Y. Permanence and global attractivity for competitive Lotka-Volterra
0362-546X(94)90053-1.

11] Perez GN. Modeling the batch bacteriocin production system by lactic acid bacteria
by using modified three-dimensional Lotka–Volterra equations. Biochem Eng J

12] Fujikawa H, Munakata K, Saka MZ. Development of a competition model for
org/10.4265/bio.19.61.

interactions within a cheese microbial community. Appl Environ Microbiol 2008;

14] Zhang L, Huang JC, Han YZ, Wu YK. Mobilization of potassium from soils by
stxb201109131338.