Electronic Journal of Biotechnology 22 (2016) 90–97

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Electronic Journal of Biotechnology

Research Article

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

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ARTICLE INFO

Article history:
Received 1 February 2016
Accepted 6 June 2016
Available online 02 July 2016

Keywords:
Cell concentration
Collaboration
Commensalism
Mutualism

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 1.88×10^9, 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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Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2016.06.004

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.

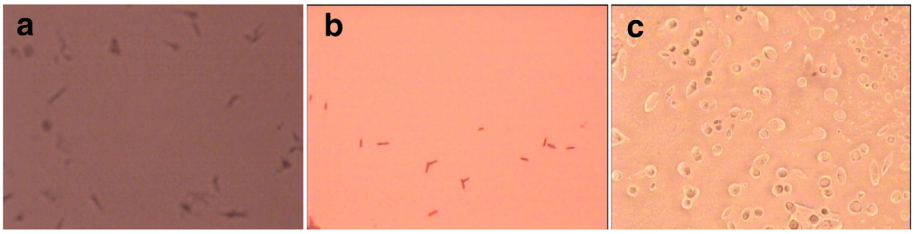

Fig. 1. Pictures of *A. ferrooxidans* (a), *R. phaseoli* (b) *and Rhodotorula* sp. (c) under optical microscope.

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 ninth day because the initial acid added was consumed and the acid reaction almost stopped. The leaching rate on 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 leachingrate 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).

	Group A	Group B	Group C	Group D	Group E	Group F
A. ferrooxidans		1×10^7			1×10^7	1×10^7
R. phaseoli			1×10^7		1×10^7	
Rhodotorula sp.				1×10^7		1×10^7

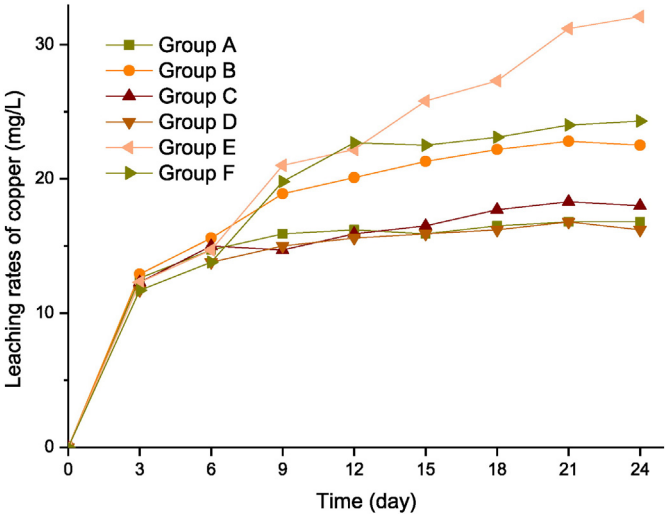

Fig. 2. Leaching rates of copper.

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 $N_0 < K$, $N_{(t)} < K$ is tenable regardless of the t value; if $N_0 > K$, $N_{(t)} > K$ 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:*

$$N = \frac{1.88013 * 10^9}{1 + 45.33461 * e^{-0.36154t}} \quad \text{R-square} = 0.98 \quad \text{[Equation 2]}$$

The fitting equation of *R. phaseoli*:

$$N = \frac{3.25624 * 10^8}{1 + 26.08856 * e^{-0.67769t}} \quad \text{R-square} = 0.93 \quad \text{[Equation 3]}$$

The fitting equation of *Rhodotorula* sp.:

$$N = \frac{2.65563 * 10^8}{1 + 16.63705 * e^{-0.33423t}} \quad \text{R-square} = 0.98 \quad \text{[Equation 4]}$$

K of *A. ferrooxidans*, *R. phaseoli* and *Rhodotorula* sp. were respectively 1.88×10^9, 3.26×10^8, and 2.66×10^8 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

Table 3
Numbers of microorganisms at individual growth (cells/mL).

	0 d	3rd d	6th d	9th d	12th d	15th d	18th d	21st d	24th d
A. ferrooxidans	1×10^7	4×10^7	3.9×10^8	6.7×10^8	1.1×10^9	1.7×10^9	1.6×10^9	1.9×10^9	1.8×10^9
R. phaseoli	1×10^7	6×10^7	7.0×10^8	1.4×10^8	2.1×10^8	2.4×10^8	2.7×10^8	2.5×10^8	2.6×10^8
Rhodotorula sp.	1×10^7	7×10^7	2.3×10^8	3.1×10^8	2.6×10^8	3.3×10^8	3.2×10^8	3.7×10^8	3.3×10^8

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:

$$\begin{cases} \frac{dN_1}{dt} = r_1 N_1 \left(1 - \frac{N_1}{K_1} + \frac{\alpha N_2}{K_1}\right) \\ \frac{dN_2}{dt} = r_2 N_2 \left(1 - \frac{N_2}{K_2} + \frac{\beta N_1}{K_2}\right) \end{cases} \quad \begin{cases} \frac{dN_3}{dt} = r_3 N_3 \left(1 - \frac{N_3}{K_3} + \frac{\gamma N_4}{K_3}\right) \\ \frac{dN_4}{dt} = r_4 N_4 \left(1 - \frac{N_4}{K_4}\right) \end{cases}$$

Group E (mutualism) Group F (commensalism)

[Equation 5]

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). α (mutualism coefficient) is the amount of food provided by unit *R. phaseoli* (N_2) to fend *A. ferrooxidans* N_1. We multiply α by the amount of food consumed by unit *A. ferrooxidans* (N_1); β (mutualism coefficient) is the amount of food provided by unit *A. ferrooxidans* (N_1) to fend *R. phaseoli* N_2. We multiply β by the amount of food consumed by unit (N_2); γ (commensalism coefficient) is the amount of food provided by unit *Rhodotorula* sp. (N_4) to fend *A. ferrooxidans* N_3, we multiply by the amount of food consumed by unit (N_3).

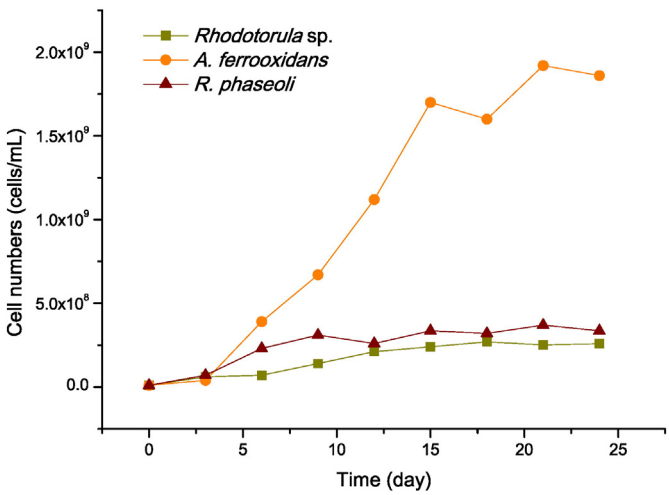

Fig. 3. Individual growth curves of *A. ferrooxidans*, *R. phaseoli* and *Rhodotorula* sp.

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.

$$\frac{dN_1}{dt} = 0, \frac{dN_2}{dt} = 0, \frac{dN_3}{dt} = 0, \frac{dN_4}{dt} = 0 \quad \text{[Equation 6]}$$

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_i,N_{1i},N_{2i}) at different times for $m + 1 (m = 7)$ times, $i = 0,1,2...,8$, and $N_{ki} = N_{k(ti)}$, $k = 1,2$; $i = 0,1,2...,8$. 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, and 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:

$$\begin{cases} dlnN_1 = \left[r_1 \left(1 - \frac{N_1}{K_1} + \frac{\alpha N_2}{K_1}\right)\right] dt \\ dlnN_2 = \left[r_2 \left(1 - \frac{N_2}{K_2} + \frac{\beta N_1}{K_2}\right)\right] dt \end{cases} \quad \text{[Equation 7]}$$

Then we estimated the parameters of equations and integrated them on interval (t_{t-1}, t_i). Thus,

$$\begin{cases} \ln N_{1i} - \ln N_{1i-1} = r_1 \left[(t_i - t_{i-1}) - \frac{A_{1i}}{K_1} + \frac{\alpha A_{2i}}{K_1}\right] \\ \ln N_{2i} - \ln N_{2i-1} = r_2 \left[(t_i - t_{i-1}) - \frac{A_{1i}}{K_2} + \frac{\beta A_{2i}}{K_2}\right] \end{cases} \quad \text{[Equation 8]}$$

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

$$A = \begin{pmatrix} t_1 - t_0 & -A_{11} & A_{21} \\ t_2 - t_1 & -A_{12} & A_{22} \\ \vdots & \vdots & \vdots \\ t_m - t_{m-1} & -A_{1m} & A_{2m} \end{pmatrix}, \quad X = \begin{pmatrix} r_1 \\ \frac{r_1}{K_1} \\ \frac{r_1 \alpha}{K_1} \end{pmatrix}, \quad Y = \begin{pmatrix} r_2 \\ \frac{r_2}{K_2} \\ \frac{r_2 \beta}{K_2} \end{pmatrix},$$

$$B = \left[\ln \frac{N_{k1}}{N_{k0}}, \ln \frac{N_{k1}}{N_{k0}}, ..., \ln \frac{N_{km}}{N_{km-1}}\right]^T, k = 1,2.$$

[Equation 9]

[Equation 9] has no solutions of general sense. Thus, we sought the least-squares solution:

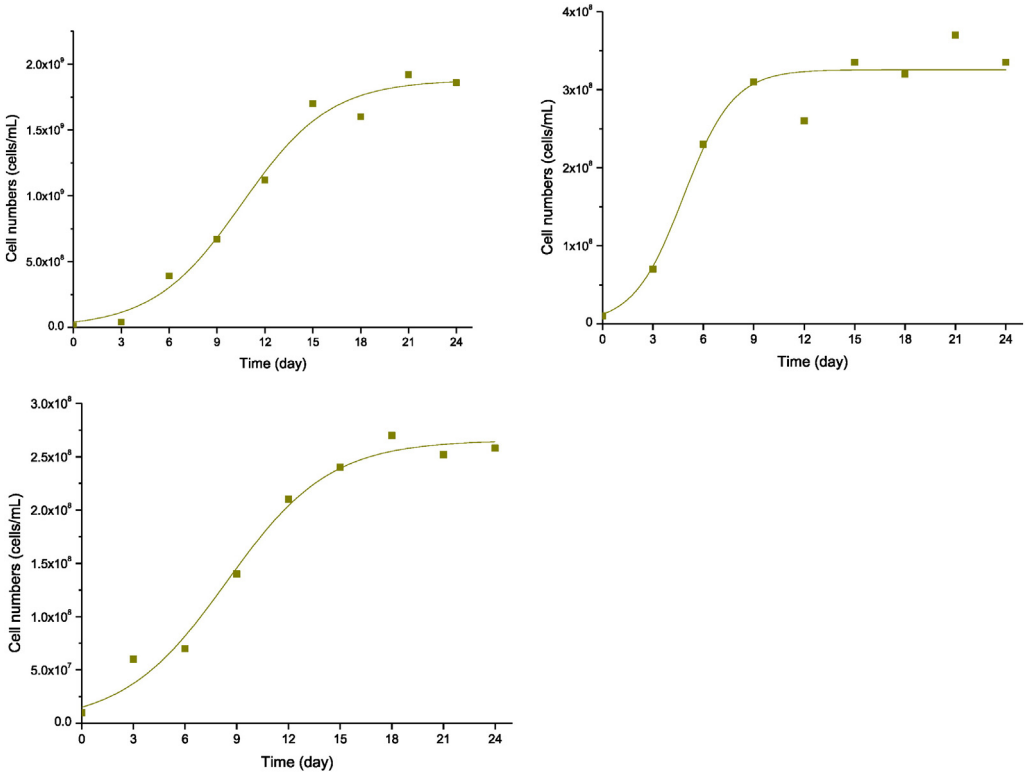

Fig. 4. Fitting result of individual growth curve of the three microorganisms.

$X = (A^TA)^{-1}A^TB_1$, $Y = (A^TA)^{-1}A^TB_2$, A^T represents the transpose of A. A_{1i} and A_{2i} are solved using the trapezoid method formula in numerical integration:

$$A_{ki}=\int_{t_{i-1}}^{t_i} N(t)dt \approx \tilde{A} = \frac{t_i - t_{i-1}}{2}(N_{ki} + N_{ki-1}) \qquad \text{[Equation 10]}$$

Among them, $i = 1,2\ldots,7$ and $k = 1,2$. Then we can obtain the approximate values of X,Y: $\tilde{X} = (\tilde{A}^T\tilde{A})^{-1}\tilde{A}^TB_1$, $\tilde{Y}=(\tilde{A}^T\tilde{A})^{-1}\tilde{A}^TB_2$, and $\tilde{A}$ is a matrix by changing A_{ki} to $\tilde{A}_{ki}$ 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-t_{i-1} was not too long, the error between A_{ki} and $\tilde{A}_{ki}$ was small; Hence, we could obtain the approximate solutions of α, β and γ using the least squares method in MATLAB, as follows: $\alpha = 1.19$, $\beta = 0.31$, $\gamma = 2.45$, which meant that unit *A. ferrooxidans* provided 0.31 times more food than that consumed by unit *R. phaseoli*, whereas unit *R. phaseoli* provided 1.19 times more food than that consumed by unit *A. ferrooxidans*. As the growth rates were zero at the equilibrium state, $dN/dt = 0$, we could get obtain the following equation:

$$\begin{cases} \left(1-\frac{N_1}{K_1}+\frac{\alpha N_2}{K_1}\right)=0 \\ \left(1-\frac{N_2}{K_2}+\frac{\beta N_1}{K_2}\right)=0 \end{cases} \text{and} \begin{cases} \left(1-\frac{N_3}{K_3}+\frac{\gamma N_4}{K_3}\right)=0 \\ \left(1-\frac{N_4}{K_4}\right)=0 \end{cases} \qquad \text{[Equation 11]}$$

The frontal fitting results showed the environmental capacities (cells/mL), as follows: $K_1 = K_3 = 1.88 \times 10^9$; $K_2 = 3.26 \times 10^8$; 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:

$$\begin{cases} N_2 = 0.84N_1 - 1.58 \times 10^9 \\ N_2 = 0.31N_1 + 3.26 \times 10^8 \end{cases}, \begin{cases} N_4 = 0.408N_3 - 7.06 \times 10^8 \\ N_4 = 2.66 \times 10^8 \end{cases}$$

[Equation 12]

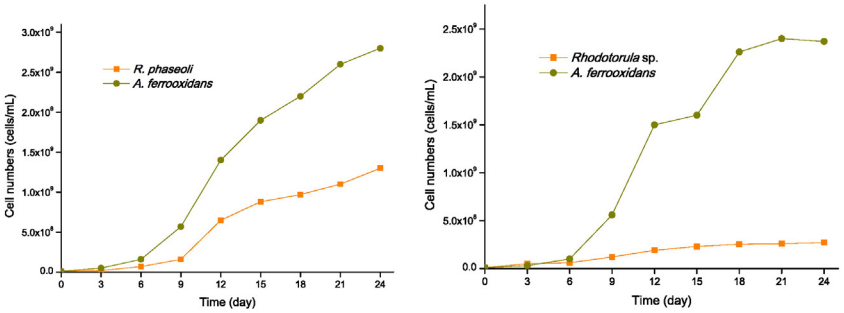

Fig. 5. Growth curves of microorganisms in groups E and F under joint leaching.

Table 4
Maximum specific growth rates (d^{-1}), maximum absolute growth rates ($cells \cdot mL^{-1}d^{-1}$) and final cell numbers (cells/mL) of the three microorganisms.

	Group B	Group C	Group D	Group E	Group F
A. ferrooxidans	$\mu = 0.34$ MAGR $= 1.7 \times 10^8$ Cell $= 1.8 \times 10^9$			$\mu = 0.41$ MAGR $= 2.3 \times 10^8$ Cell $= 2.8 \times 10^9$	$\mu = 0.35$ MAGR $= 2.4 \times 10^8$ Cell $= 2.4 \times 10^9$
R. phaseoli		$\mu = 0.67$ MAGR $= 5.5 \times 10^7$ Cell $= 3.4 \times 10^8$		$\mu = 0.40$ MAGR $= 1.2 \times 10^8$ Cell $= 1.3 \times 10^9$	
Rhodotorula sp.			$\mu = 0.32$ MAGR $= 2.2 \times 10^7$ Cell $= 2.6 \times 10^8$		$\mu = 0.31$ MAGR $= 2.2 \times 10^7$ Cell $= 2.7 \times 10^8$

Fig. 7a 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 3.59×10^9 cells/mL and 1.44×10^9 cells/mL, respectively. At the end of the experiment on d 24, the concentrations of *A. ferrooxidans* and *R. phaseoli* were 2.8×10^9 and 1.3×10^9 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 3.31×10^9 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 1.3×10^9 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. The two microorganisms reached relative equilibrium on d 36. 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 2.38×10^9 and 2.66×10^8 cells/mL, respectively. On d 24, the concentrations of *A. ferrooxidans* and *Rhodotorula* sp. were 2.4×10^9 and 2.7×10^8 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

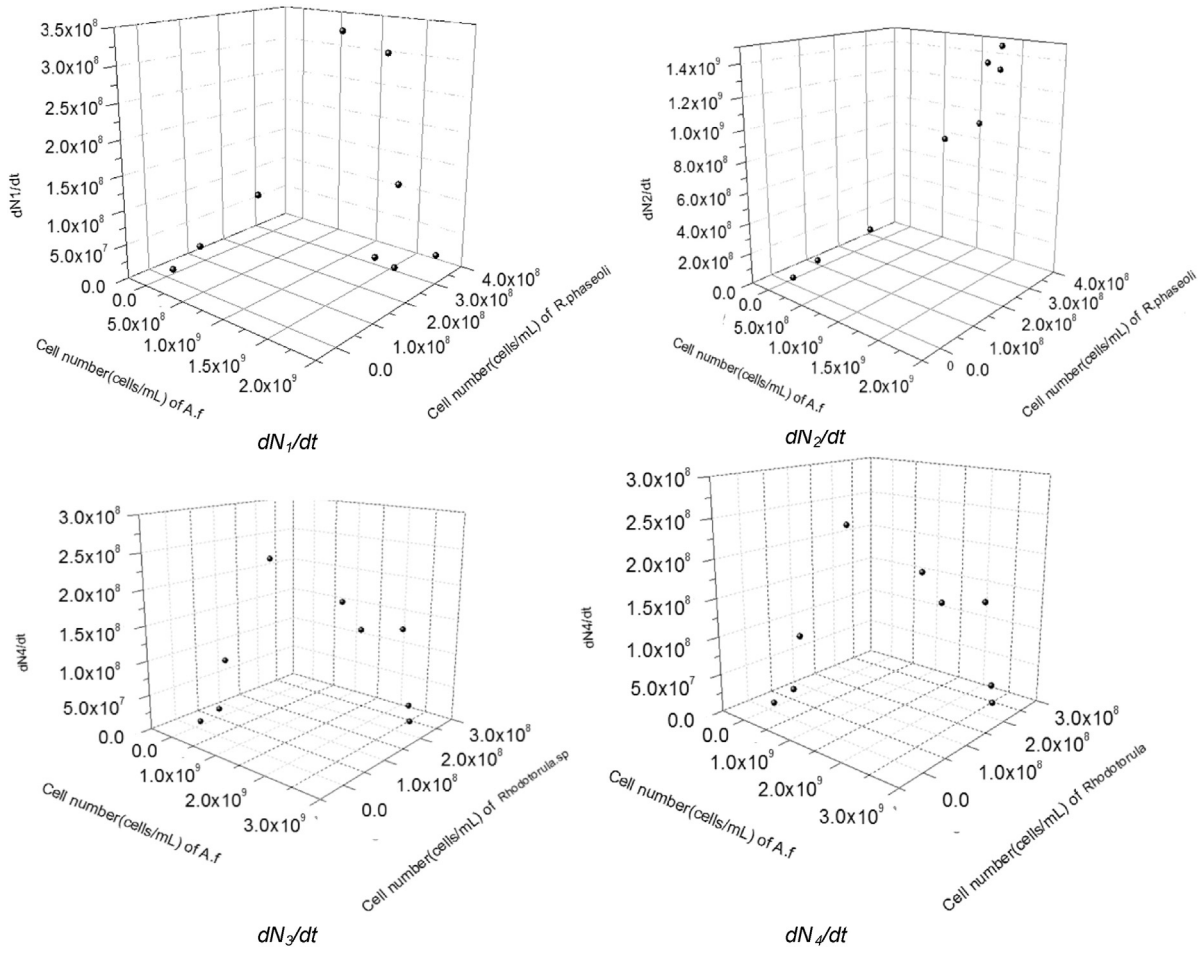

Fig. 6. Growth rates of microorganisms in Groups E and F.

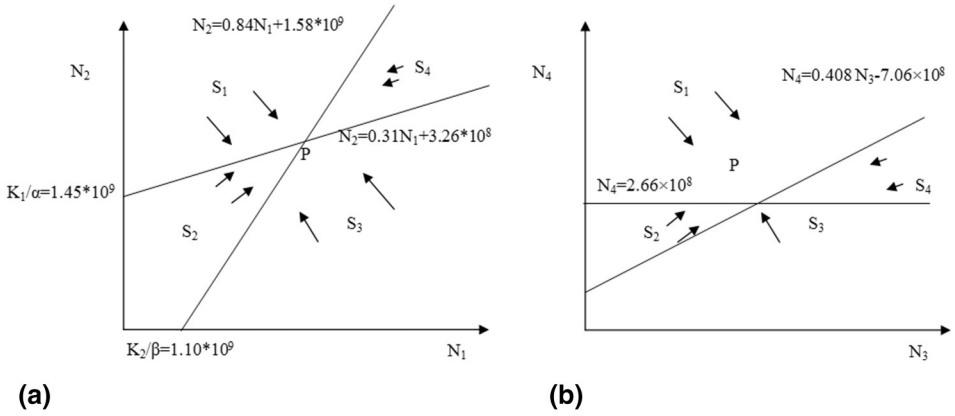

Fig. 7. Isoclines of *A. ferrooxidans* and *R. phaseoli* in Group E (a) and *A. ferrooxidans* and *Rhodotorula* sp. in Group F (b).

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 $dN_3/dt > 0$ and $dN_4/dt < 0$. 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 $dN_3/dt > 0$ and $dN_4/dt > 0$. 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 $dN_3/dt < 0$ and $dN_4/dt > 0$. The number of *A. ferrooxidans* in group E or *A. ferrooxidans* in group F decreased. By contrast, the number of *R. phaseoli* or *Rhodotorula* sp. in group F increased at any position in this region. In region S_4, $dN_1/dt < 0$ and $dN_2/dt < 0$ or $dN_3/dt < 0$ and $dN_4/dt < 0$. The number of *A. ferrooxidans* 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* respectively were $\alpha = 1.19$ and $\beta = 0.31$, while the relationship of *A. ferrooxidans* and *R. phaseoli* could be considered as commensalism, and the commensalism coefficient γ of the effect of *Rhodotorula* sp. on *A. ferrooxidans* was 2.45. This finding indicated that, in leaching process, *A. ferrooxidans* elicited a greater promoting effect than *R. phaseoli* did. *A. ferrooxidans* almost did not affect the growth of *Rhodotorula* sp. while *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, Lotka–Volterra model could appropriately describe the relationships between the three microorganisms in bioleaching before they reached relatively stable peak values.

This study fitted the growth of *A. ferrooxidans*, *R. phaseoli*, and *Rhodotorula* sp. in a leaching environment using the L–V model. Our results revealed that the individual growth curves of *A. ferrooxidans*, *R. phaseoli*, and *Rhodotorula* sp. fit the logistic pattern. In the 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 γ 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.

Financial support

The work was supported by the young scholars development fund of SWPU (No. 201499010071) and the Project of Education Department in Sichuan (No. 16ZB0086).

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.

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