A series piezoelectric quartz crystal response model for ferrous ion oxidation in the presence of T. ferrooxidans

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A novel and simple method, series piezoelectric quartz crystal (SPQC) has been used to monitor ferrous ion oxidation in the presence of T. ferrooxidans. By combination of a general SPQC response model with the kinetics of the bacterial growth, a SPQC response model for ferrous ion oxidation in the presence of T. ferrooxidans has been built. The response model can describe the bacterial growth process well. Some important kinetic parameters, such as $m$ (the maximum specific growth rate of bacterium), $K$ (saturation constant, i.e., the ferrous iron concentration at which the half-maximal growth rate occurs), $X_0$ / $Y$ (ratio of initial bacterial concentration to cell yield coefficient), have been obtained by fitting the model to the experimental curves.

Ferric ion is an effective oxidant in hydrometallurgy for dissolution of various minerals. As in the reaction ferric becomes ferrous ion, the rejuvenation of ferrous ion is necessary. This is often done with oxygen in acidic solution:

$$2 \text{Fe}^{3+} + \text{O}_2 + 2 \text{H}^+ = 2 \text{Fe}^{2+} + \text{H}_2\text{O} \quad \ldots (1)$$

The reaction, however, is very slow. So many catalytic methods have been presented to increase the oxidation rate of ferrous iron such as abiotic methods and biotic methods. The biotic methods are more effective and are based on the fact that some bacteria, e.g., T. ferrooxidans, oxidize ferrous iron to satisfy the energy requirements for the bacterial growth, as expressed in Eq. (2).

$$\text{Fe}^{2+} \xrightarrow{\text{enzyme}} \text{Fe}^{3+} + e^- \quad \ldots (2)$$

So a great deal of work has been done to study the kinetics of ferrous iron oxidation related to T. ferrooxidans growth. The concentration of ferrous iron, the rate of depletion of oxygen, acidification of the media or the accumulation of bacteria have been monitored. Ferrous iron, in general, is determined by using titrimetric, spectrophotometric and electrochemical methods. These methods, however, have some shortcomings involving sampling procedures and being time-consuming.

A SPQC method, therefore, for continuously monitoring the oxidation of ferrous iron in the presence of T. ferrooxidans was presented in our previous work. Piezoelectric quartz crystal (PQC) sensor is known as a microbalance as it can detect the mass change at mg level. SPQC sensor is one type of PQC sensor, which is constructed by connecting a quartz crystal and a conductivity electrode in series. Besides its sensitive response to mass, it can respond to many physical parameters of liquid medium including conductivity, permittivity, viscosity and density, and it has some advantages over classical conductometry. This method, which is based on the response of SPQC to conductivity changes in solution, however, is for the first time being applied in determination of T. ferrooxidans growth.

As the mobility of ferric iron is larger than that of ferrous iron, the total conductivity of the solution increases while the oxidation of ferrous iron is taking place. Consequently, the frequency of SPQC decreases. Compared with the existing methods such as titrimetric and spectrophotometric methods, this novel method is simpler.

In this work, a frequency response model of SPQC for ferrous iron oxidation in the presence of T. ferrooxidans has been proposed to study the kinetics of this process. By fitting the experimental results with the model, some kinetic parameters can
be obtained, such as the maximum specific growth rate of bacterium $\mu_m$, saturation constant $K$, ratio of initial concentration of bacterium to yield coefficient $X_0/Y$.

Materials and Methods

Apparatus

The SPQC was constructed by connecting an AT-cut quartz (Model JA-5, Beijing Factory No.707) with a conductivity electrode in series, to make up the feedback circuit of the oscillator. The resonant frequency was 9 MHz. The oscillator was of a homemade TTL-IC and the conductivity electrode was made of Pt with a cell constant of 1.119 cm. A universal frequency counter (Iwatsu Model SC-7201) was used to record the oscillating frequency of the oscillator.

Reagents

*F. ferrooxidans* strain (TD-03) used in the experiments was isolated from the arsenopyrite mine drainage in Guangdong Province, China and was maintained on 3-Leathen (L) nutrient media with the composition of (NH$_4$)$_2$SO$_4$ 0.45 g, K$_2$HPO$_4$ 0.15 g, MgSO$_4$·7H$_2$O 0.5 g, KCl 0.05 g, Ca(NO$_3$)$_2$ 0.01 g and FeSO$_4$·7H$_2$O as inorganic energy source 50 g in a liter solution. The pH value was adjusted to 2.0 using 1.0 mol/L H$_2$SO$_4$. The reagents used in the study were of analytical grade.

Experimental procedure

An experiment was carried out in a 250 ml Erlenmeyer flask containing 90 ml nutrient media and 10 ml inoculum of *F. ferrooxidans*. The bacteria were incubated at 25°C using an incubator agitated at 100 rpm to ensure enough O$_2$ for the bacterial growth. At predetermined time intervals, the water lost was compensated by addition of deionized water. SPQC was used to monitor the frequency shift of the solution continuously during *F. ferrooxidans* growth, with DJS-1 conductivity electrode as a probe in the solution. The equipment for the measurement is schematically shown in Fig. 1.

Results and Discussion

A general response model of SPQC

The oscillating frequency can be represented by Eq. (3) $^1$:

$$\begin{align*}
F &= F_0 \left[ 1 + \frac{\pi F_0 C_q (2\pi F_0 C_o) - y G}{G^2 + 4\pi^2 F_0^2 C_q (C_o + C_s) - 2\pi F_0 C_o G y} - \pi F_0 C_q R_q y \right] \quad (3)
\end{align*}$$

where $F$ is oscillating frequency, $F_0$ is resonant frequency, $C_q$, $C_o$ and $C_s$ are dynamic, state electric capacity in the air and the electric capacity in the solution, respectively, $G$ is the solution conductance, i.e., the reciprocal of $R_s$, $y$ is a parameter concerning the phase shift of the oscillator. In the low conductivity range there is a linear relation between frequency shift and specific conductivity, $F$ decreases with the increase in conductivity $\chi$. In the high conductivity range, however, the dependence of frequency shift on specific conductivity is not obvious. The linear relation between frequency shift ($\Delta F$) and the changes of conductivity ($\Delta \chi$) under certain experimental conditions can be obtained from Eq.(3), as expressed by Eq.(4).

$$\begin{align*}
\Delta F &= r \Delta \chi = r \sum l_i \Delta C_i \quad (4)
\end{align*}$$

where, $\Delta F$ is the frequency shift, $l_i$ is the equivalent conductivity of the $i$th ion, $C_i$ is the concentration of the $i$th ion and $r$ is the sensor sensitivity.

Ferrous ion decreases while ferric ion increases in concentration during the growth of *F. ferrooxidans*. As the conductivity of ferric ion is greater than that of ferrous ion, the total conductivity of the solution increases continuously. So this process can be monitored by the SPQC method.

Response model

As described above, the frequency shift of the SPQC is related to the concentration of ions in the solution. The changes of other ions in the nutrient solution are smaller than those of ferrous and ferric ion during the bacterial growth, so the variation in
frequency shift is caused mainly by the two ions. The linear relation, therefore, can be approximately expressed as:

\[ \Delta F_1 = -K_1[Fe^{2+}]_t + b_1 \]

\[ \Delta F_2 = -K_2[Fe^{3+}]_t + b_2 \]

where \( \Delta F_1 \) and \( \Delta F_2 \) are the frequency shifts, \([Fe^{2+}]_t\) and \([Fe^{3+}]_t\), are the concentrations of ferrous and ferric ion at time \( t \) and \( K_1, K_2, b_1, b_2 \) are constants.

A relative frequency shift at time \( t \) compared to initial time can be represented by:

\[ \Delta (\Delta F) = (AF)_t - (AF)_0 \]

\[ = -K_1[Fe^{2+}]_t - K_2[Fe^{3+}]_t + K_1[Fe^{2+}]_0 + K_2[Fe^{3+}]_0 \]

(7)

where \([Fe^{2+}]_0, [Fe^{3+}]_0\) are the initial concentrations. As \([Fe^{3+}]_0 = 0, [Fe^{2+}]_0 = [Fe^{2+}] - [Fe^{3+}]\), So,

\[ \Delta (\Delta F) = (K_1-K_2) ([Fe^{2+}]_0 - [Fe^{3+}]) \]

(8)

If \([Fe^{2+}]_0\) and \([Fe^{3+}]_0\) are expressed by \( S_0 \) and \( S \) respectively and \( K_1-K_2 \) is represented by \( K \), then

\[ S = S_0 - \Delta (\Delta F)_t / K \]

(9)

According to Monod model,

\[ \frac{dX}{dt} = \mu X \]

(10)

where \( X \) is the cell mass concentration and \( \mu \) is the bacterial specific growth rate. If it is assumed that nitrogen and phosphorus nutrients are adequate and the rate of aeration is sufficient to make the oxygen and carbon dioxide excessive, then the availability of ferrous ion limits the bacterial growth rate. The concentration of ferrous ion, \( S \), becomes the limiting substrate. According to Michaelis-Menten equation,

\[ \mu = \frac{\mu_m S}{K + S} \]

(11)

where \( \mu_m \) is the maximum specific growth rate and \( K \) is the saturation constant. So,

\[ \frac{dX}{dt} = \frac{\mu_m SX}{K + S} \]

(12)

A relationship between \( X \) and \( S \) is obtained by defining a yield coefficient \( Y \) which is assumed to be a constant:

\[ \frac{dX}{dt} = -Y \frac{dS}{dt} \]

(13)

Then Eq. (13) becomes,

\[ \frac{dS}{dt} = -\frac{\mu_m SX}{Y(K + S)} \]

(14)

If \( X_0 \) and \( S_0 \) are the concentrations of bacteria and substrate at zero time, then substituting Eq.(13) into Eq.(14), one gets

\[ \frac{dS}{dt} = -\frac{\mu_m S[X_0 + Y(S_0 - S)]}{Y(K + S)} \]

(15)

Equation (15) is integrated and Eq.(16) can be obtained:\

\[ \mu_m t = \frac{K}{(X_0/Y) + S_0} \ln \frac{S_0 - [(X_0/Y) + S_0]}{S - [(X_0/Y) + S_0]} + \ln \frac{S - [(X_0/Y) + S_0]}{S_0 - [(X_0/Y) + S_0]} \]

(16)

\[ \Delta F_{1,2} \text{ Hz} \]

Fig. 2—Simulated response curves with different \( \mu_m \) at \( K 0.6 \text{ g/L} \) and \( X_0/Y \text{ 2 g/L} \) [1. \( \mu_m = 0.03 \); 2. \( \mu_m = 0.035 \); 3. \( \mu_m = 0.04 \text{ hr}^{-1} \)]

\[ \Delta F_{1,2} \text{ Hz} \]

Fig. 3—Simulated response curves with different \( K \) at \( \mu_m \text{ 0.03 hr}^{-1} \) and \( X_0/Y \text{ 2 g/L} \) [1. \( K = 0.6 \); 2. \( K = 0.7 \); 3. \( K = 1.5 \text{ g/L} \)]
Substituting Eq. (9) into Eq. (16), one gets Eq. (17).

$$
\mu_m t = \frac{K + (X_0/Y) + S_0}{(X_0/Y) + S_0} \ln \left[ 1 + \frac{\Delta(F)}{K_3(X_0/Y)} \right] - \frac{K}{(X_0/Y) + S_0} \ln \left[ 1 - \frac{\Delta(F)}{K_3S_0} \right] \quad \ldots (17)
$$

It is the response model for SPQC to the oxidation of ferrous iron in the presence of *T. ferrooxidans*.

**Influence of parameters on response curve**

Effects of the parameters on the response curves are shown in Figs 2 to 4. It can be seen that the effects of $\mu_m$ and $X_0/Y$ are more obvious than that of $K$. The response curve increases and saturates more rapidly with the increase in $\mu_m$ and $X_0/Y$, but with the decrease in $K$.

**Estimation of parameters**

A typical response curve for SPQC to the ferrous oxidation in the presence of *T. ferrooxidans* at 25°C is shown in Fig. 5 (points). The frequency shift decreases slowly, then after acceleration reaches a constant value. It is in agreement with a sigmoid curve. As the conductivity of ferrous iron is lower than that of ferric iron, the total conductivity of the solution increases. As a result, the frequency shift increases. The sigmoid shape is related to the bacterial sigmoid growth.

The response model is used to fit the experimental results. The estimated values of $\mu_m$, $K$ and $X_0/Y$ are 0.028 h⁻¹, 0.578 g / l and 2.08 g / l respectively. The estimated theoretical response curve (line) obtained is also shown in Fig. 5. It can be seen that the fit of the response equation to the experimental data is good.

**Conclusions**

The response model presented in this paper can describe the ferrous iron oxidation in the presence of *T. ferrooxidans*, which was based on the general response model of SPQC and the kinetics of bacterial oxidation. By fitting the response model to the experimental data, parameters such as $\mu_m$, $K$ and $X_0/Y$ were obtained. It can be applied to monitor the growth of *T. ferrooxidans* continuously.

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**References**