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BIOTECHNOLOGY METHODS

Model-based evaluation of ferrous iron oxidation by acidophilic bacteria in chemostat and biofilm airlift reactors

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Abstract This article presents a model-based evaluation of ferrous iron oxidation in chemostat and biofilm airlift reactors inoculated with a mixed culture of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* bacteria. The competition between the two types of bacteria in the chemostat and in the biofilm airlift reactors together with the distribution of both bacteria along the biofilm thickness at different time sections has been studied. The bacterial distribution profiles along the biofilm in the airlift reactor at different time scales show that in the beginning *A. ferrooxidans* bacteria are dominant, but when the reactor operates for a long time the desirable *L. ferrooxidans* species outcompete *A. ferrooxidans* as a result of the low Fe^{2+} and high Fe^{3+} concentrations. The results obtained from the simulation were compared with the experimental data of continuously operated internal loop airlift biofilm reactor. The model results are in good agreement with the experimental results.

Keywords *Leptospirillum ferrooxidans* · *Acidithiobacillus ferrooxidans* · AQUASIM software · Ferrous iron · Biofilm airlift reactor · Chemostat reactor

Introduction

A well-known combined chemical–biological method to remove H_2S from gases is based on two steps. First step corresponds to absorption with chemical reaction of the gas in a solution of ferric sulfate, and subsequent step is the biological oxidation of ferrous ions in the solution to produce ferric ions again. [1, 5, 10, 14]. Biological oxidation of ferrous iron is an important sub-process in this integrated chemical–biological process for H_2S removal. The rates of biological ferrous ion oxidation obtained with suspended microorganisms are far too low to favor the biological regeneration concept in the integrated chemical/biological process suggested [14]. Therefore, adsorption of the bacteria onto porous and inert support materials could be the best solution to improve the biological oxidation rate. The application of biofilm-based bioreactors enables reactor operation at high biomass concentrations leading to high volumetric productivity and making it a viable option for the large-scale applications. Furthermore, in the integrated process for H_2S removal (Fig. 1), employing a biofilm type of reactor in the second stage prevents bacteria from entering the first stage (exposing to toxic H_2S) without the need to any external separator [6].

Several studies have proved the efficiency of immobilization and high oxidation rates via the setup of packed bed or fluidized bed reactors with various inert carrier matrix materials such as activated carbon, glass beads or ion-exchange resin [7]. By operating a fixed-bed reactor, the iron oxidation rate has been reported to be increased fivefold higher than suspended cells [8]. Nevertheless, the precipitation of ferric iron complexes even at very low pH values cannot be completely avoided and major clogging problems are likely to emerge in long-term operation of fixed-bed reactors. Alternatively, application of airlift reactors is a matter of major interest. In an airlift reactor clogging can be avoided, since there is a surface-attached immobilization, rather than entrapment. Interestingly, oxygen transfer capacity of an airlift reactor is far higher than that in a fixed-bed reactor. The maximum ferrous iron

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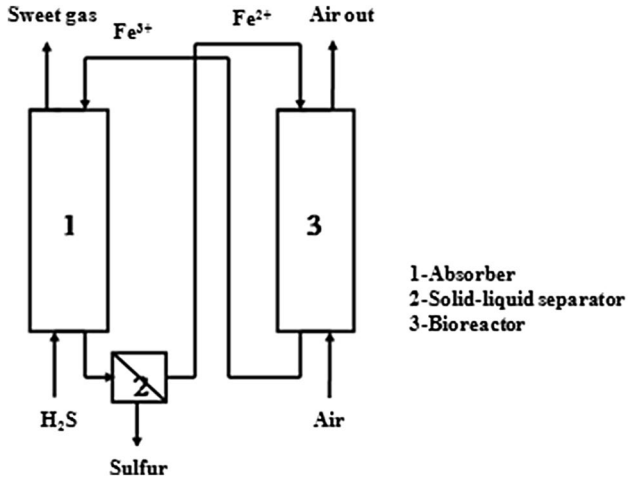

Fig. 1 Process scheme of chemo-biological process for H_2S removal

oxidation rate achieved in our previous study in biofilm airlift reactor was about 145 mol Fe^{2+}/m^3.h. This value is almost twice the one reported for fixed-bed reactors and roughly ten times higher than what has been obtained for suspended cells [6].

Two major types of acidophilic chemolithoautotroph bacteria for biological oxidation of ferrous iron are known, *A. ferrooxidans* and *L. ferrooxidans*. *A. ferrooxidans* has the ability to oxidize both ferrous iron and reduced sulfur compounds, while *L. ferrooxidans* can oxidize ferrous iron and pyrite, but not sulfur or its inorganic compounds [2, 6, 12]. In the integrated process for H_2S removal *A. ferrooxidans* is less desirable due to its ability to oxidize sulfur compounds and reducing the final product yield. Thus, *L. ferrooxidans* is the preferred iron-oxidizing bacteria due to non-oxidation of elemental sulfur. Nonetheless, the operation of a full-scale process with a pure culture is economically unviable. As such, monitoring the competition between both types of bacteria is of major interest [6].

In this study, the biological oxidation of ferrous iron was modeled and evaluated by AQUASIM software, a computer program for the identification and simulation of aquatic systems [16]. The competition between the two types of bacteria (mixed culture of *A. ferrooxidans* and *L. ferrooxidans)* both in the chemostat reactor and in the biofilm in the airlift reactor was studied. This included spatial distribution of the two bacteria along the biofilm thickness in the biofilm airlift reactor at different time sections. The results obtained from the simulation were compared with our previous experimental data of continuously operated internal loop airlift biofilm reactor [6].

Model development

Laboratory scale setup

The model results obtained from the simulation were compared with our previous experimental study. The experimental setup consists of a continuously operated internal loop airlift biofilm reactor, with a liquid volume of 3.0 l operated at a constant temperature of 30 °C. Air was introduced via a fine bubble aerator at the bottom of the reactor (5 and 10 l/min), further detail has been reported elsewhere [6].

Model description

The mathematical model describing the performance of the chemostat reactor and laboratory scale biofilm airlift reactor was implemented in the well-established AQUASIM simulation software for environmental applications [16]. At first, the model was used to demonstrate competition of *L. ferrooxidans* and *A. ferrooxidans* bacteria on the basis of substrate affinity in a chemostat reactor. Completely mixed reactor was used to simulate chemostat reactor.

Thereafter, the model was used to simulate the conversion processes which occur in a laboratory scale biofilm reactor and to compare the simulation result with those obtained experimentally under the same conditions. A biofilm compartment was defined containing the biofilm and some bulk liquid volume. The volume of this compartment was fixed at 3 l. The mixed compartment contained the reminder of the liquid volume. The biofilm compartment was linked to a gas compartment with diffusive link. Exchange of O_2 and CO_2 occurred via this link with a gas liquid mass transfer coefficient kLa 90 1/h corresponding to the maximum oxygen mass transfer capacity (~20 kg O_2/m^3.d) of the laboratory scale biofilm reactor. Special attention has been paid to simulate the dynamic distribution of two types of bacteria inside the biofilm and to compare with our previous Fluorescent in situ hybridization (FISH) studies [6]. This distribution gives insight into the overall reactor performance and its sensitivities.

Model reactions

Autotrophic ferrous-oxidizing bacteria feed on carbon dioxide as their sole carbon source to produce organic matter. The growth stoichiometric equation for ferrous iron oxidation by *A. ferrooxidans* and *L. ferrooxidans* can be derived from the elemental balances on, C, H, O, N, Fe, and the charge balance, assuming that the biomass composition can be represented by $CH_{1.8}O_{0.5}N_{0.2}$ [13]:

Table 1 Kinetic parameters for ferrous oxidation by *A. ferrooxidans* and *L. ferrooxidans* [9]

	A. ferrooxidans	*L. ferrooxidans*	Units
$q_{O_2}^{max}$	2.2	1.7	mol O_2/mol C/h
$q_{Fe^{2+}}^{max}$	8.8	6.8	mol Fe^{2+}/mol C/h
K_{O_2}	0.05	5e−4	–
$K_{Fe^{2+}}$	0.05	5e−4	–
Y_{ox}^{max}	0.051	4.5e−2	mol C/mol O_2
Y_{sx}^{max}	1.14e−2	1e−2	mol C/mol Fe^{2+}
m_o	0.1	2.6e−2	mol O_2/mol C/h
m_s	0.25	4.5e−1	mol Fe^{2+}/mol C/h

$$CO_2 + 0.2NH_4^+ + \frac{(1-4.2Y_{sx}^{max})}{4Y_{sx}^{max}}O_2 + \frac{1}{Y_{sx}^{max}}Fe^{2+} + \left(\frac{1}{Y_{sx}^{max}} - 0.2\right)H^+ \rightarrow CH_{1.8}O_{0.5}N_{0.2} + \frac{1}{Y_{sx}^{max}}Fe^{3+} + \left(\frac{1}{2Y_{sx}^{max}} - 0.6\right)H_2O \quad (1)$$

In this equation, Y_{sx}^{max} is the maximum biomass yield on iron (C-molX/mol Fe^{2+}).

In addition to growth, bacteria also use the energy created from ferrous iron oxidation for maintenance necessities. The simplified catabolic stoichiometric equation that applies to the bacterial maintenance reaction through the aerobic bacterial oxidation of ferrous iron can be written as:

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (2)$$

It has been shown that a Michaelis–Menten type rate of equation describes the specific rate of Fe^{2+} utilization of *A. ferrooxidans* and *L. ferrooxidans* bacteria in continuous cultures by incorporating ferric ion inhibition, and neglecting the relatively low threshold value of ferrous iron [3, 4, 15]:

$$q_{Fe^{2+}} = \frac{q_{Fe^{2+},max}}{1 + K_{Fe^{2+}} \cdot \frac{C_{Fe^{3+}}}{C_{Fe^{2+}}}} \quad (3)$$

$$q_{O_2} = \frac{q_{O_2,max}}{1 + K_{O_2} \cdot \frac{C_{Fe^{3+}}}{C_{Fe^{2+}}}} \quad (4)$$

where $q_{Fe^{2+},max}$ and $q_{O_2,max}$ are the maximum biomass-specific ferrous iron and the maximum biomass-specific oxygen consumption rates (mol O_2/C-mol/h), respectively. $K_{Fe^{2+}}$ is the ratio of the affinity constant for ferrous iron (K_s, mol Fe^{2+}/l) to the ferric iron inhibition constant (K_i; mol Fe^{3+}/l) and K_{O_2} is the ratio of the affinity constant for oxygen (K_s, mol O_2/l) to the water inhibition constant (K_i; mol H_2O/l).

By considering the biomass-specific substrate consumption rate, (q_S) and the biomass-specific maintenance coefficient (m_s; mol Fe^{2+}/C-mol.h) as independent rates and using the Pirt equation, the following relation for growth rate, $r_x = \mu C_x$, can be obtained [11]:

$$r_x = Y_{sx}^{max}(q_s - m_s)C_x = Y_{ox}^{max}(q_o - m_o)C_x \quad (5)$$

Substituting Eq. (4) into Eq. (5) gives:

$$r_x = \mu C_x = Y_{ox}^{max}(q_o - m_o)C_x = Y_{ox}^{max}\left(\frac{q_{O_2,max}}{1 + K \cdot \frac{C_{Fe^{3+}}}{C_{Fe^{2+}}}} - m_o\right)C_x = \left(\frac{\mu max + m_o.Y_{ox}^{max}}{1 + K \cdot \frac{C_{Fe^{3+}}}{C_{Fe^{2+}}}} - m_o.Y_{ox}^{max}\right)C_x \quad (6)$$

Consequently, the relations between conversion rates of the compounds can be obtained by coupling of the above growth and maintenance reactions, Eqs. (1) and (2):

$$-r_{Fe^{2+}} = r_{Fe^{3+}} = \frac{1}{Y_{sx}^{max}}r_x + m_s C_x \quad (7)$$

$$-r_{O_2} = \frac{(1-4.2Y_{sx}^{max})}{4Y_{sx}^{max}}r_x + \frac{m_s}{4}C_x \quad (8)$$

$$-r_{CO_2} = r_x \quad (9)$$

$$-r_{Fe^{2+}} = -4r_{O_2} - 4.2r_{CO_2} \quad (10)$$

The values of the kinetic and stoichiometric parameters describing ferrous iron oxidation by *L. ferrooxidans* and *A. ferrooxidans* are presented in Table 1. The main difference between the kinetics of both types of bacteria is that *L. ferrooxidans* has a higher affinity for ferrous iron and Fe^{3+} significantly inhibits the growth rate of *A. ferrooxidans*.

Results and discussion

Biological oxidation of ferrous iron in chemostat reactor

The competitive success of an organism in the competition for limiting resources is caused by the physiological response of the organisms to the available substrates. Therefore, if more organisms are present in the culture and the organisms must compete for the limited resources, the competitive success of an organism will depend on its (μ–s) curves or specific growth rate on the available growth-limiting mixture of substrates. For the two iron-oxidizing bacteria, *A. ferrooxidans* and *L. ferrooxidans*, μ–s curves are shown in Fig. 2.

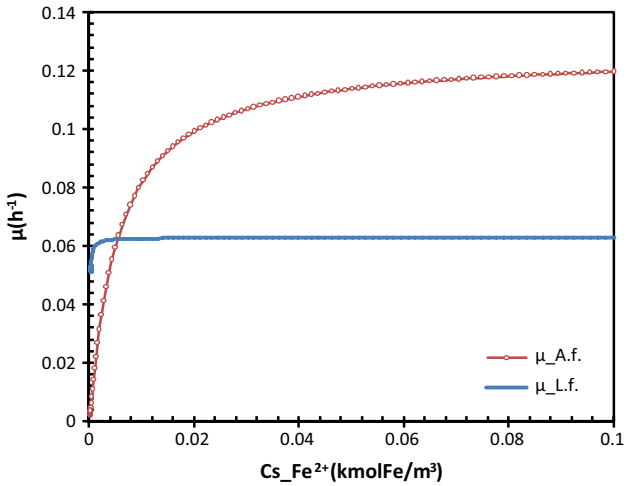

Fig. 2 The specific growth rate of *A. ferrooxidans* and *L. ferrooxidans* bacteria as a function of ferrous iron concentration

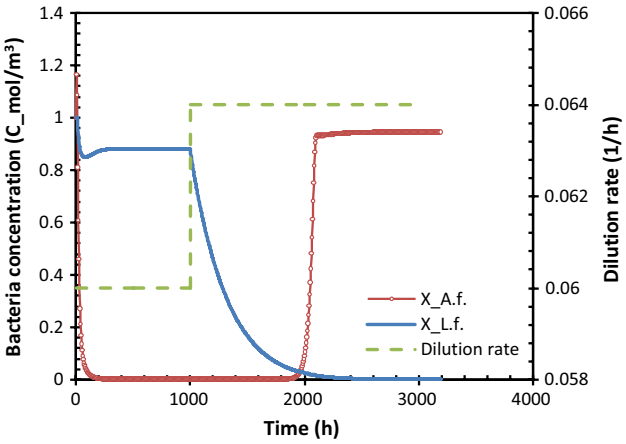

Fig. 3 Bacteria concentration in the chemostat

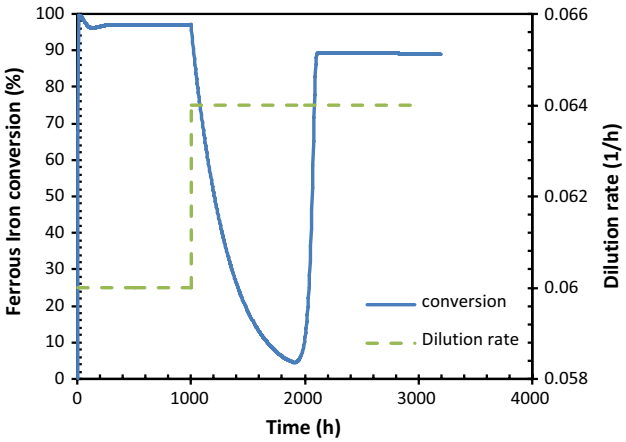

Fig. 4 Ferrous iron conversion in chemostat

If *A. ferrooxidans* and *L. ferrooxidans* are grown in batch culture (with a specific rate of μ_{max}), based on their specific (μ–s) curves, *A. ferrooxidans* will grow faster than *L. ferrooxidans* and, therefore, become dominant (assuming that both have started with equal numbers and neither has a lag phase). However, when *A. ferrooxidans* and *L. ferrooxidans* are grown in a continuous culture with growth limitation by substrate ferrous iron, then at low dilution rates, *L. ferrooxidans* will dominate. This can be attributed to higher affinity of L. *ferrooxidans* for the substrate than that of *A. ferrooxidans.* In other word, in spite of lower μ_{max} of *L. ferrooxidans* shown in Fig. 2, this organism will grow faster than organism *A. ferrooxidans* at low $C_{Fe^{2+}}$. This feature of *L. ferrooxidans* bacteria, which combines a low μ_{max} and a high affinity for the substrate, can be used in practical design of chemostat enrichment to select between *L. ferrooxidans* and *A. ferrooxidans*.

To demonstrate the competition of *A. ferrooxidans* and *L. ferrooxidans* in a chemostat reactor, the effect of operating dilution rate on bacteria concentration and iron concentration was modeled. A chemostat reactor with constant volume of 3 liter consisting of two microbial species, *A. ferrooxidans* and *L. ferrooxidans* was considered. The initial concentration of both microorganisms in the reactor was assumed to be 0.001 kmol C/m^3. The growth rates follow the Monod-type rate laws; with the kinetic parameters indicated in Table 1.

The dilution rate was increased in a stepwise from 0.06 1/h to 0.064 1/h while the inlet substrate concentration was assumed to be constant at 0.1 kmol Fe/m^3. Time course of bacteria concentration is shown in Fig. 3, as described by the model. The results imply that in the culture with low dilution rate (0.06 1/h), the *L. ferrooxidans* became dominant bacteria in the bioreactor. However, when the dilution rate is increased to 0.064 1/h *A. ferrooxidans* becomes dominant. When the two pure cultures were mixed, the culture appeared to behave like the original enrichment.

Modeling the variation of ferrous iron conversion in time due to changes in dilution rate is shown in Fig. 4. A sharp decrease in conversion of Fe^{2+} occurs as a result of the stepwise increase in dilution rate. Afterwards, the ferrous iron conversion increases to a new steady state level of 88 %. At this new steady state condition, the sole surviving culture in the reactor is *A. ferrooxidans* bacteria because of crossing μ–s curves as revealed in Fig. 3.

Biological oxidation of ferrous iron in biofilm airlift reactor

To demonstrate the biofilm formation and the competition of *A. ferrooxidans* and *L. ferrooxidans* in a biofilm airlift reactor, the airlift reactor with constant bulk volume of 3 l consisting of two microbial species, *A. ferrooxidans* and *L.*

ferrooxidans was simulated. It is assumed that the reactor is filled with carrier particles, so the biofilm area as a function of biofilm thickness on the particles becomes:

$$A = 4\pi n_{par}(r_{par}+z)^2 \quad (11)$$

In this equation, n_{par} is the carrier particle number (2×10^5), r_{par} is the particles radius (10^{-4} m) and z (m) is the depth coordinate in the biofilm.

The inlet substrate concentration and the density of both microorganisms were assumed to be 0.1 kmol Fe/m^3 and 2 kmol C/m^3, respectively. The initial biofilm thickness and the initial concentration of both bacteria were set at 10^{-4} m and 10^{-3} kmol C/m^3, respectively. The diffusion coefficients of the chemical components were considered to be 8.64×10^{-5} m^2/day.

By performing the simulation for 550 days, the time course of the ferrous iron conversion is shown in Fig. 5. Initially, the ferrous iron loading rate was kept constant at 100 mol Fe^{2+}/m^3.h. In the beginning, *A. ferrooxidans* bacteria were the dominant culture in the reactor. By developing biofilm inside the reactor, as the ferrous iron conversion increases, the ferrous iron concentration decreases. Therefore, the condition becomes favorable for *L. ferrooxidans* bacteria, which have high affinity for ferrous iron. Due to outcompeting of *L. ferrooxidans*, the conversion efficiency increases to 98 % after 50 days.

As far as loading rate is balanced with the amount of biomass to obtain high conversion rate, *L. ferrooxidans* will keep the dominancy in the system. For instance, at day 150, loading rate was increased to 110 mol Fe^{2+}/m^3.h, but no significant decrease in conversion was observed. However, if loading rate increases too sharply, (above maximum conversion capacity) which is limited by oxygen mass transfer rate, conversion will decrease in the system. Subsequently, the condition will be favorable for *A. ferrooxidans*. This was demonstrated by stepwise increase loading rate at day 300 as illustrated in Fig. 5. These model results are consist with our experimental results as described below.

The experimental results of the ferrous iron loading rate, conversion as a function of time during period of basalt attached biofilm are shown in Fig. 6, the measurements between days 9–26 and 65–85 were as off-line. The reactor was inoculated with a mixed culture of *A. ferrooxidans* and *L. ferrooxidans* in a 1:1 ratio. After 2 days of operation in batch mode, the reactor was then switched to continuous feeding with the ferrous iron loading rate of 5 mol Fe^{2+}/m^3.h. After 25 days of operation, the ferrous iron loading rate was increased to 25 mol/m^3.h. Then, the ferrous iron loading rate was increased to 45.5, 66.7, 100 and 200 mol Fe^{2+}/m^3.h on days 44, 52, 66 and 70, respectively.

During the first 40 days of operation, the treatment efficiency was limited to f 90 %. Fluorescent in situ hybridization (FISH) studies of the microbial community of the

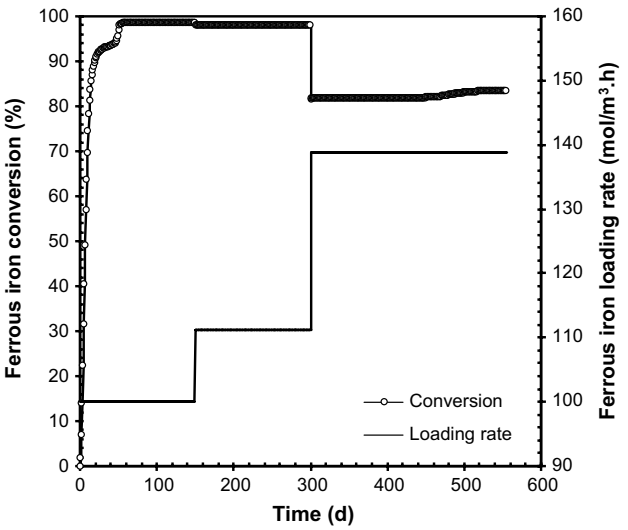

Fig. 5 The time course of the ferrous iron conversion by change in loading rate

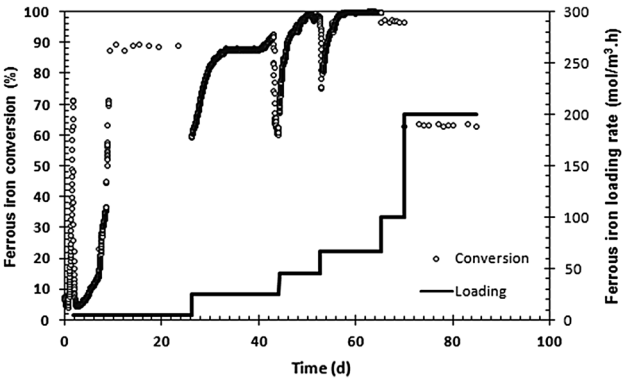

Fig. 6 Ferrous iron loading rate, conversion as a function of time during period of basalt attached biofilm; measurements between days 9–26 and 65–85 were as off-line

ferrous iron-oxidizing bacteria in the airlift biofilm reactor showed that *A. ferrooxidans* bacteria were the dominant culture in the reactor during this period.

However, when the reactor was operated for a long period of time at high ferrous iron conversion (>85 %), the treatment efficiency was started to increase (day 40) and reached to over 98 % (day 52).

FISH studies showed that this increase in the treatment efficiency was the result of outcompete of *L. ferrooxidans* species due to the low Fe^{2+} and high Fe^{3+} concentrations. When the volumetric loading rate was increased to above the corresponding oxygen mass transfer capacity of the reactor, on day 66 and 70 the treatment efficiencies were decreased to 96.5 and 63 %, respectively. The obtained experimental results and FISH studies of population

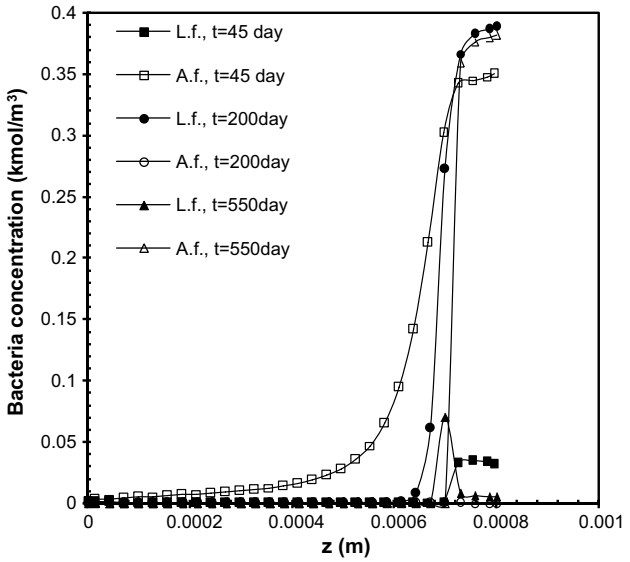

Fig. 7 The profile of bacteria concentration along the biofilm at different times

dynamics of bacteria in biofilm-based process are well described with model simulations of biofilm.

The model results of the profile of bacteria concentration along the biofilm at different time scales are shown in Fig. 7. The result demonstrates that before 50 days, *A. ferrooxidans* bacteria are dominant because of their high concentration in the biofilm. But when the reactor operates for long periods, the desirable *L. ferrooxidans* species can outcompete *A. ferrooxidans* due to low Fe^{2+} and high Fe^{3+} concentrations. After 400 days, *A. ferrooxidans* bacteria become dominant in the biofilm because of the rise in the loading rate beyond the optimal loading rate and oxygen mass transfer limitation. These results are again in good agreement with our previous FISH studies[6].

Conclusion

A model-based evaluation of ferrous iron oxidation in chemostat and biofilm airlift reactors, inoculated with a mixed culture of *A. ferrooxidans* and *L. ferrooxidans* bacteria was performed. The bacterial profiles along the biofilm in the airlift reactor show that in the beginning *A. ferrooxidans* bacteria are dominant, but when the reactor operates for longer time the desirable *L. ferrooxidans* species can outcompete due to low Fe^{2+} and high Fe^{3+} concentrations. At higher loading rates, the oxygen mass transfer from gas to liquid limits the ferrous iron oxidation rate and again *A. ferrooxidans* bacteria become dominant.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and Animal Rights This article does not contain any studies with human participants or animals performed by any of the authors.

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