Ferrous Iron Oxidation by Acidithiobacillus ferrooxidans Immobilized on Refractory Clay Tiles

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Abstract: Refractory clay tiles are composed of kaolin, which is the commercial name of clay, and this consists mainly of kaolinite mineral. Two such tiles and caowool packed in glass columns were used for the immobilization of Acidithiobacillus ferrooxidans cells in a ferrous iron medium, which was percolated through the supports. Colonization was carried out by several media replacements with no further inoculation until maximum ferric iron productivity was reached. One of the tiles was discarded due to the high iron precipitation during bacterial growth. The columns with the other supports were used for ferrous iron oxidation in batch and continuous flow modes of operation and these appeared to be promising supports for A. ferrooxidans. A ferrous iron oxidation rate of 14.5 mmol.l⁻¹.h⁻¹ was reached in one of the columns in the continuous culture. After being used for several cultures, pieces of tiles with immobilized cells were stored at 4 ºC. Samples at different times were incubated in ferrous medium and these showed high cell activity even after 6 months.

INTRODUCTION

Acidithiobacillus ferrooxidans is a chemolithoautotrophic acidophilic bacterium that is of great importance in bioremediation [1]. This microorganism is involved in the bioleaching of metal sulfide ores essentially due to its iron(II) oxidizing capacity [2]. Metal sulfides are dissolved by the oxidizing action of iron(III), which is continually regenerated by the cells. Iron(III) production by bacterial action can also be employed in bioremediation processes [3-4].

The natural tendency of A. ferrooxidans to grow on surfaces, not only on inert supports but also on minerals, makes it a suitable microorganism for cell immobilization and it is also useful to increase iron(III) productivity [5-7]. Different immobilization methods and several supports have been employed, including glass beads, activated carbon particles, sand, polystyrene, polyurethane, poly(vinyl alcohol), calcium alginate, ion-exchange resin, nickel alloy fibre, PVC and diatomaceous earth [8-17]. A. ferrooxidans cells immobilized on supports can grow at higher dilution rates and reach high iron(III) productivity.

Reactors with attached A. ferrooxidans that continually produce iron(III) have been employed to enhance metal recovery from ores as well as in other applications [18-24]. In most cases, the conditions under which the immobilization process was performed led to abundant levels of jarosite [basic iron(III) sulfate] precipitation. This precipitation is usually described as an unwanted phenomenon due to the decrease in the rate of diffusion of reactants and products through the layer of deposits and also to the blockage of pumps and valves. On the other hand, jarosite deposits participate in the biofilm formation process [25-27]. An interesting model proposed by Karamanev [27] describes the biofilm structure produced by A. ferrooxidans cells on the jarosite pores. Thus, support matrices with high porosity can be used to enhance jarosite precipitation and consequently the biofilm formation.

The main goal of the study described here was to assess the immobilization of A. ferrooxidans cells on highly porous refractory clay tiles (composed of kaolin). Iron(II) oxidation rates during the biofilm formation and the oxidative efficiency of this biofilm after storage at 4 ºC were also evaluated.

MATERIALS AND METHODS

Microorganism and Cultivation

A strain of A. ferrooxidans from Santa Rosa de Arequipa (DSM11477) was used for these experiments. The microorganism was grown and maintained on 9 K medium [2] at pH of 1.80.

Support Matrices

The supports used in this study were a ceramic fibre, the commercial name of which is caowool (S1), and two refractory clay tiles (S2 and S3). The compositions of the latter supports are as follows:

S2: SiO2: 27.7%, Al2O3: 50.8%, Fe2O3: 0.38%, TiO2: 0.25%, CaO: 17.1%, MgO: 0.14%, Na2O: 1.94% and K2O: 1.34%.

S3: SiO2: 54.7%, Al2O3: 42.8%, Fe2O3: 0.7%, TiO2: 0.7% and CaO: 0.6%.

Attachment to the Supports

Each support (20 mg) was added to the bacterial suspension (5.0 ml) previously filtered to remove jarosite. The test
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The percentages of attachment of A. ferrooxidans to the supports were 1.8%, 23.2% and 38.2% for S1, S2 and S3, respectively. The level of adhesion is dependent on the physico-chemical properties of the bacteria and the solid surfaces and it is responsible for the first and reversible phase of biofilm formation. After that phase, an irreversible phase should be formed in order to build a suitable biofilm. In the case of A. ferrooxidans, the latter phenomenon is strongly associated with the precipitation of jarosite [25], which is also related to the initial bacterial adherence. A high bacterial attachment to the support would produce rapid iron(II) oxidation very close to the surface, thus increasing pH and iron(III) concentration and the subsequent iron(III) precipitation. These deposits on the surface would allow the subsequent adsorption of cells on the pores and the formation of the biofilm. Although the initial attachment is important, there are other factors that must be considered. One of those factors that has a positive effect on the biofilm formation is the tendency of some supports (due to their specific physico-chemical characteristics) to increase jarosite precipitation on their surface regardless of the presence of attached bacteria. Once precipitation has occurred, cells can be adsorbed onto the surface of highly porous jarosite. However, the supports could inhibit the bacterial activity and consequently the biofilm formation. For these reasons, an experiment to study the bacterial growth in the presence of each support was carried out.

The results for the bacterial growth in the presence of each support are shown in Fig. (1). Although the supports did not affect the growth significantly, in the presence of support 2 the bacterial growth was slower than in the cultures with the other supports. Moreover, in this case the final pH value was the highest, which is consistent with the more basic characteristics of this support. The formation of iron(III) precipitates is highly dependent on pH as well as other factors such as ionic composition and concentration of the medium. It has been reported that pH values between 1.6 and 1.8 are appropriate to maintain high iron(II) oxidation rates and low levels of jarosite deposits [26]; at pH values above 2.0 the mass of jarosite increases with pH, whereas values below 1.6 cause a decrease in the biooxidation rate – probably due to inhibition by acidity [2] – and insufficient jarosite precipitation for the formation of a good biofilm [25]. In the case of support 2, the final pH was approximately 5 and, consequently, the amount of iron(III) precipitation was too high. Thus, a small amount of total iron remained soluble at the end of the bacterial growth. This
would be a serious disadvantage in biofilm bioreactors because the purpose of such systems is to produce higher iron(III) concentrations; as a result, support 2 was ruled out for the subsequent experiments.

From a thermodynamic point of view, jarosite is the most stable iron(III) precipitate at the final redox and pH conditions in this kind of culture. However, the precipitation of this material is slow and requires several hours even after the complete oxidation of iron(II) [28]. For this reason, the supports were kept in contact with the spent medium for a further 12 hours in order to analyze the iron(III) deposits produced during the growth. Values of 55%, 97% and 78% of the total iron were precipitated in the cultures with supports 1, 2 and 3, respectively. These solids were characterized as potassium and ammonium jarosite by X-ray diffraction. However, analysis by Mössbauer spectroscopy proved the existence of one or more phases of ferric oxohydroxides (the exact composition of which was not determined) joined to jarosite. These precipitates can be less stable than jarosite but their formation is usually faster [28]. According to the Mössbauer results, the percentages of jarosite in the solid residues were 41% (for S1) and 21% (for S2 and S3). The results for the consecutive batches run using the immobilized biomass on supports 1 and 3 with a rate between medium volume and mass support (V/m) of 5 ml/g are shown in Fig. (2). In the reactor with support 1, the productivity values show a tendency to decrease. This behavior indicates that there is only a small immobilized biomass, which is even further removed by successive washing after each cycle. For this reason, this support was discarded after six cycles. When support 3 was employed a steady increase in iron(III) productivity was observed up to a maximum value (after 6 to 7 cycles). The constant value was probably reached because the new iron(III) deposits preclude nutrient access. However, the iron(III) productivity and the final soluble iron are not sufficiently high in comparison with values reported (5-50 mmol·l⁻¹·h⁻¹) using other supports under similar, but not identical, conditions [16, 22, 24].

When the reactor operating in repeated batch mode reached its highest iron(III) productivity, the culture medium was replaced by fresh media and a continuous flow of medium through a peristaltic pump was started. The pH value and iron(II) concentration in the inlet medium were 1.80 and 9 g·l⁻¹, respectively.

Iron(III) productivity as a function of the dilution rate is represented in Fig. (3). It can be seen that the immobilized cells have an advantage in a continuous operation, reaching higher productivity values than those obtained in the batch mode. The washout occurred at a higher dilution rate than in free cell reactors (about 0.11 h⁻¹). It can also be seen that higher dilution rates lead to lower iron(III) precipitation levels.

In order to confirm this finding, new experiments in “batch mode” were carried out in which the rate V/m mass was modified by reducing the support. As shown in Fig. (4), iron(III) productivity was significantly enhanced by increasing V/m to 10 ml/g. Higher values of V/m did not give rise to improved performance and led to even worse results than the system with a V/m of 5 ml/g. The maximum iron(II) biooxidation rate of 14.5 mmol·l⁻¹·h⁻¹ achieved for a V/m of 10 ml/g is comparable with most values reported in literature and is even higher than many. The specific value taking into account the mass of the support was 145 mmoles.h⁻¹.kg⁻¹ support mass; this value is higher than most reported for similar conditions; for example, in a recent report by Mousavi et al. [22], only 37 mmoles of iron(II).h⁻¹.kg⁻¹ support mass were oxidized using low density polyethylene as a support. As expected, iron(III) precipitation was lower and this led to higher iron(III) concentrations at the end of each cycle. The iron(III) concentration in solution did not change even when the medium was kept in contact with the support for more than 24 hours after the iron(II) was exhausted. In a continuous mode of operation, similar behavior to that shown in Fig. (3) was obtained (data not shown).

One factor that must be taken in account in the industrial application of a reactor with immobilized biomass is its op-

![Fig. (2).](image-url) Fig. (2). Iron(III) productivity in bioreactors with immobilised biomass on supports 1 (white bars) and 3 (black bars) at 5 ml/g (volume/support mass). The values correspond to first (1st), third (3rd) and sixth (6th) cycle for support 1 and first (1st), fifth (5th) and eleventh (11th) for support 3. Each data value represents the mean of the duplicates. Standard deviations for iron productivities were between 1-7 % (5-12 % for cell populations).
eral stability after the operation is stopped for long periods. The reactor should be stopped temporarily and restarted whenever required without further manipulation. This characteristic should be demonstrated not only when the support is maintained wet with nutrient-lacking medium but also when the medium is totally drained from the column. In order to analyze the possibility of reactivation, the support with immobilized biomass was placed in a medium with iron after being stored for 2 and 12 weeks. The results of the iron(II) oxidation assay are shown in Fig. (5). It was observed that the support stored in open air only shows suitable activity after 2 weeks. In contrast, the cells immobilized in the wet support were reactivated very rapidly after storage 24 weeks, a long lag phase (7–15 days) was observed before cell reactivation.

**CONCLUSION**

In summary, certain refractory kaolin tiles can be employed as appropriate supports for the immobilization of *A. ferrooxidans* cells and allow the generation of an optimal biofilm. One of the supports was better than the others probably because it presents an intermediate basicity enough to allow an initial jarosite precipitation and consequently the biofilm formation but not so high to leave low concentration of soluble ferric iron. The biofilm produced on the best support achieved high iron(III) productivity in both batch and continuous modes of operation, with lower levels of iron(III) precipitation at higher dilution rates. The immobilized biomass on the support clearly retained its oxidative efficiency during storage at 4°C under dry or wet conditions for 2 or 24 weeks, respectively.

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**REFERENCES**


