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Increasing cell concentration does not affect specific ferrous iron oxidation rate in a continuously stirred tank bioreactor

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Abstract

Microbial oxidation of ferrous to ferric iron allows efficient oxidative processing of sulfide minerals under ambient conditions. This study determined the effect of cell concentration of a mixed mesophilic microbial culture on iron oxidation rate, and evaluated if there was a cell concentration threshold that dictates a maximal volumetric iron oxidation rate. A bioreactor with feedback-loading of ferrous media was operated at 30 °C to maintain a redox potential of +480 mV vs. Ag/AgCl at pH of 1.3. A positive and linear correlation (R = 0.955) between the cell concentration (6.8×10^7 - 7.1×10^9 cells mL^{-1}) and volumetric biological iron oxidation (up to 6.9 g L^{-1} h^{-1}) was observed. The specific iron oxidation was not affected by cell concentration, and no biocatalytic threshold was observed. This indicated that a high cell concentration can be used to achieve a high volumetric iron oxidation rate, enabling the use of a compact reactor size.

Keywords: bioleaching; bioreactor; iron oxidation; biocatalytic threshold; cell concentration

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1. Introduction

Ferric iron (Fe^{3+}) is an effective oxidant commonly used for the oxidation and dissolution of sulfide minerals (Dutrizac and MacDonald, 1974). The regeneration of the ferric iron is a critical component of many leach circuits (Kaksonen et al., 2014b). Iron oxidising microorganisms can increase the oxidation rate by approximately 10^5-10^6 times when compared to chemical iron oxidation with air especially at low pH of approximately 2 and below (Lacey and Lawson, 1970). As with enzyme-based biocatalytic processes (Lima-Ramos et al., 2014), the use of microbial cells as biocatalysts in iron oxidation may result in an increase in reaction rate up to a maximum threshold, beyond which further addition of cells does not further improve the reaction rate.

Biological iron oxidation kinetics has been investigated with reference to process conditions such as dissolved oxygen (Kinnunen and Puhakka, 2004), temperature, jarosite accumulation (Chowdhury and Ojumu, 2014), pH (Ojumu and Petersen, 2011), redox potential (Rawlings et al., 1999), and contaminants such as chloride (Gahan et al., 2010), sulfate (Ojumu et al., 2007), and cations (Ojumu et al., 2008). The effect of cell concentrations on ferrous iron oxidation rates at relatively low cell concentrations of 3×10^8 – 9×10^8 cells mL^{-1} has been previously evaluated (Okereke and Stevens, 1991). However, no studies have investigated the relationship between the cell concentration and iron oxidation rate at cell concentrations that are relevant to mineral bioprocessing (10^9-10^{10} cells mL^{-1}) (Batty and Rorke, 2006; Ly Arrascue and van

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Niekerk, 2006). It also remains unclear if a biocatalytic threshold exists for biological iron oxidation.

There are currently no industrial sulfide mineral bioprocesses where cell concentration is deliberately increased to increase biological iron oxidation rates. Most bioprocesses are managed simply by optimising the process conditions to enhance cell growth. In a conventional agitated tank leaching cascade, the cell numbers in each reactor increase by cell division and culture growth (Dew et al., 1997), while cells are simultaneously washed out from the reactor as a function of hydraulic retention time. As a result, the equilibrium between the growth rate and washout rate determines the prevailing cell concentration within a given process. Conceptual bioleaching process circuits have been described, where cells are recovered from bioreactor effluent and reintroduced into the mineral leaching reactor for increasing reaction rate and process performance (du Plessis, 2003). Such recovery and re-use of microbial cells result in a separation between the cell retention time and the reactor hydraulic retention time, and allow for the cell retention time to be controlled independently. Despite being successfully applied at industrial scale in activated sludge bioreactors for wastewater treatment to improve process efficiency and robustness (Metcalf and Eddy, 1991), this concept is yet to be demonstrated for the purposes of mineral bioprocessing. The objective of this study was to determine whether a positive linear correlation exists between cell concentration and ferrous iron oxidation rate up to 10^9 cells mL^{-1} range, and if a biocatalytic cell concentration threshold exists for ferrous iron oxidation at ambient temperature using a mixed mesophilic iron oxidising microbial consortium.

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2. Materials and Methods

2.1 Culture medium

The culture medium used in the iron oxidation experiments was 0K medium (modified 9K medium (Silverman and Lundgren, 1959)) supplemented with 8 g L^{-1} ferrous iron and pH 1.2 adjusted with H_2SO_4. The medium contained (g L^{-1}): 3.0 $(NH_4)_2SO_4$, 0.50 K_2HPO_4, 0.50 $MgSO_4 \cdot 7H_2O$, 0.10 KCl, 0.010 $Ca(NO_3)_2$, and 40 $FeSO_4 \cdot 7H_2O$.

2.2 Microbial cultures, reactor set up and operation

The mixed culture used for the experiments was obtained from ALS Metallurgy (Perth, Western Australia) and included *Ferroplasma* (*F.*) *acidarmanus/acidiphilum, Sulfobacillus* (*S.*) *thermosulfidooxidans, Leptospirillium* (*L.*) *ferriphilum* and *Acidithiobacillus* (*A.*) *caldus.* Microbial cells were harvested from 25 L of culture by centrifugation (Sorvall Lynx 6000 centrifuge, Germany) at 3,000 × g for 30 min. The cell pellets were resuspended in the 1 L of 0K medium containing 8 g L^{-1} ferrous iron (pH 1.2). The resulting 1 L culture was transferred to a 2 L glass reactor (working volume 1 L) which was kept in a water bath at 30 °C (Figure 1). The reactor was continuously mixed with an overhead stirrer (IKA RW20 digital) at 500 rpm. The reactor was aerated with humidified and pre-warmed compressed air to minimise evaporation. The aeration rate was adjusted to maintain dissolved oxygen (DO) concentration at a non-limiting level (DO >3 mg L^{-1}) for the bio-oxidation process (Witne and Phillips, 2001).

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Redox potential was controlled to +480 mV (vs. Ag/AgCl) by feedback dosing fresh 0K medium (supplemented with 8.0 g L^{-1} ferrous iron) to the reactor. The feedback control allowed the rate of iron oxidation in the reactor to control the rate of ferrous iron solution feed intake, resulting in the dilution of the culture over time. The working volume of 1 L was maintained by withdrawing effluent at a desired water level of the reaction liquor with a continuously operating peristaltic pump (Figure 1).

Redox potential was measured using a TPS meter, model MC-80 and IONODE ORP combination electrode, model PRFO (Ag/AgCl reference). Reactor pH was measured using a TPS miniCHEM controller and pH electrode (Ionode pH electrode, IJ44CT), calibrated using pH 1.68 and 4.01 standard buffers. The solution pH, redox potential and the consumption of 0K medium by the reactor were recorded using the LabVIEW software (National Instruments). Dissolved oxygen (DO) concentrations were measured using a HACH luminescent dissolved oxygen (LDO) HQ10 meter with HACH LDO probe.

2.3 Analytical methods

Solution samples (1 mL) were taken from the reactor (initially twice in an hour for the first 2.5 hours, then hourly until 6.5 hours and thereafter at decreasing frequency as the volumetric iron oxidation slowed down) and kept on ice prior to cell counting and ferrous iron and total iron analyses. Ferrous iron concentrations in filtered (0.2 µm) samples were measured spectrophotometrically using the phenanthroline method (APHA, 1992). Total soluble iron in filtered (0.2 µm) samples was measured using inductively coupled plasma – atomic emission spectrometry (ICP-AES) at the Analytical

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Services laboratories of CSIRO Process Science and Engineering at Waterford, Western Australia. Total cell counting was conducted in triplicate using a Thoma ruled counting chamber by phase contrast microscopy (DM4000 B, Leica, Germany) under 100 × objective.

2.4 Calculations

The Nernst equation was used to calculate ferric iron concentrations based on redox potentials, ferrous iron and total soluble Fe concentrations measured at selected time points during the experiment (Pesic et al., 1989; Watling et al., 2008; Equation 1).

$$E = E^0 + \frac{2.3RT}{nF} log \frac{Fe^{3+}}{Fe^{2+}} \qquad (1)$$

Here, E was solution redox potential determined experimentally, E^0 was the standard potential at experimental temperature (30°C) determined experimentally using redox potential, R was the universal gas constant (8.3144 J K^{-1} mol^{-1}), T was the temperature (303.15 K), n was the number of moles of electrons transferred in the reaction $Fe^{2+} \rightarrow Fe^{3+} + e^-$ (i.e. 1 mole), F was the Faraday constant (96485 K mol^{-1}), and Fe^{3+}/Fe^{2+} was the molar concentration ratio of Fe^{3+} vs. Fe^{2+}. The use of Nernst equation was appropriate in this study because 1) Fe^{3+}/Fe^{2+} is the major redox couple in the system, and 2) the total iron concentration remained stable as influent Fe^{2+} was the only iron source and pH remained low enough (average 1.31) to prevent iron precipitation.

Ferric iron concentrations calculated from Equation 1 were used to derive the volumetric Fe^{2+} oxidation rate R_v (mg L^{-1} h^{-1}) and the specific Fe^{2+} oxidation rate R_s (mg $cell^{-1}$ h^{-1}) according to Equations 2 and 3, respectively.

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$$R_v = \frac{C_{Ferric} \times Q}{V} \quad (2)$$

$$R_s = \frac{R_v}{D} \quad (3)$$

Here, C_{Ferric} was the Fe^{3+} concentration in reactor (mg L^{-1}), Q was the influent flow rate (L h^{-1}), V was the reactor working volume (L), and *D* was the cell density (cells L^{-1}).

3. Results and Discussion

3.1 Influent dosing by feedback control of redox potential

Initial cell concentration in the reactor was 5.8×10^9 cells mL^{-1}. Redox potential increased rapidly after reactor start up triggering the dosing of new influent when the redox potential reached 480 mV (vs. Ag/AgCl) 25 min after start up. The flow rate and cumulative consumption of influent solution fed into the reactor over time are shown in Figure 2A. As anticipated, the influent flow rate of the ferrous iron feed solution gradually decreased over time, which was a direct result of the dilution of microbial cells and the reduction of volumetric iron oxidation rate in the reactor. Redox potential set point was designed to be relatively low (i.e. corresponding to incomplete iron oxidation, i.e. ~50%) to facilitate also the growth of bioleaching microorganisms that may be sensitive to high redox potentials (Rawlings et al., 1999).

3.2 Solution pH control and acid demand

The solution pH decreased slightly during the experiment, with average, maximum and minimum values of 1.31 ± 0.05, 1.48 and 1.17, respectively (data not shown). The pH of the system was suitable for most of the microorganisms present in the mixed

culture (*F. acidarmanus* (pH <0-1.5, optimum 1.2), *F. acidiphilum* (pH 1.3-2.2, optimum 1.7), *S. thermosulfidooxidans* (pH 1.5-5.5, optimum 2), *L. ferriphilum* (pH optimum 1.3-1.8) and *A. caldus* (1.0-3.5, optimum 2.0-2.5)) (Schippers, 2007).

3.3 Dissolved oxygen demand

The initial aeration rate of 8 L min^{-1} could not maintain the desirable DO level at the beginning of the experiment due to the high microbial activity (Figure 2B). The aeration rate was therefore increased using multiple spargers to 18-20 L min^{-1} which increased the DO saturation. As the experiment proceeded, the DO saturation increased to 100%.

3.4 Ferrous iron oxidation rate as a function of cell density

The volumetric iron oxidation rate decreased from 6.9 g L^{-1} h^{-1} at the highest cell concentration to 0.06 g L^{-1} h^{-1} at the lowest cell concentration (Figure 3A). The cell density in the reactor initially increased from 5.8×10^{9} cells mL^{-1} to 7.1×10^{9} cells mL^{-1} within the first 27 minutes of operation. Thereafter, the cell density gradually decreased to 6.8×10^{7} cells mL^{-1} as the reactor contents were diluted with the influent (Figure 3A). All reported cell counts were recorded from the period where aeration rate was 18-20 L min^{-1} and DO was maintained above 3.5 mg L^{-1}, and hence the cell counts and iron oxidation were not impacted by possible changes in aeration rate or related turbulence.

The volumetric iron oxidation rate decreased as the influent was dosed to the reactor (Figure 3B), and was directly proportional to the decrease in cell number

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throughout the experiment. The maximum iron oxidation rate observed in this study compared favourably with other suspended cell bioreactors (Ebrahimi et al., 2005; Halfmeier et al., 1993; Kaksonen et al., 2014a; Kaksonen et al., 2014b), although higher iron oxidation rates have been observed with biofilm reactors and when using oxygen-enriched air (Kinnunen and Puhakka, 2004).

Interestingly, the specific iron oxidation rate, ranged from 6.2×10^{-10} to 1.5×10^{-9} mg Fe^{2+} $cell^{-1}$ h^{-1}, was relatively independent of the cell concentration present in the reactor. This indicated that the microbial consortium remained stable and active during the course of the experiment. For a comparison, the maximum specific iron oxidation rate reported for a mesophilic culture in a fluidized bed reactor operated at 37 °C was 1.0×10^{-8} mg Fe^{2+} $cell^{-1}$ h^{-1} (Kinnunen and Puhakka, 2004).

There was a strong positive linear correlation between cell density and volumetric iron oxidation rate (R = 0.955; Figure 4), which indicated that iron oxidation rate was directly related to cell density, affirming that by increasing (concentrating) the number of cells in a given reaction volume a higher iron oxidation rate can be achieved. This linear correlation also indicated that the system did not reach a biocatalytic threshold, i.e. there was no cell concentration detected (within the experimental range), at which the specific oxidation rate decreased. Further experimentation is required to delineate this threshold.

3.5 Industrial Process Implications

This study showed that higher cell concentrations allow higher iron oxidation rates. The final cell number at the end of the experiment was 6.8×10^{7} cells mL^{-1}, when

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approximately 46% of available ferrous iron was oxidised. The corresponding cell yield for 100% iron oxidation would have been 1.5 x 10^8 cells mL^{-1}. Higher cell concentrations (10^9 cells mL^{-1}) are achievable using mineral concentrates (Batty and Rorke, 2006; Ly Arrascue and van Niekerk, 2006) than when using soluble ferrous iron. This has important implications for mineral bioprocesses that rely on iron oxidation as the key driver for mineral dissolution and where high rates of mineral dissolution are required, particularly where continuous agitated tank reactors are used.

An increase of cell concentrations, beyond which is currently achievable in conventional agitated tank leaching, may be achievable using methods to recover and recycle suspended cells from the process discharge solution (du Plessis, 2004). Cell recovery may be achieved using a two-step process, involving initial removal of mineral solids from suspension by conventional mineral thickening, followed by cell recovery using continuous industrial centrifugation. Recovered cells can then be returned to the bioprocess to increase the cell concentration and thereby increasing the mineral processing oxidation rate. Although an increase in cell concentration will inevitably cause oxygen mass transfer challenges, the use of enriched oxygen in such mineral bioprocesses has already been successfully demonstrated to overcome this issue in tank mineral bioprocessing (Batty and Rorke, 2006).

4. Conclusions

This study showed that the biological iron oxidation rate showed a positive linear correlation with cell concentration in the studied range of 6.8×10^7 and 7.1×10^9 cells

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mL^{-1}. No threshold cell density was detected beyond which the specific biological iron oxidation rate would be detrimentally impacted. Thus, using high cell densities to achieve high volumetric iron oxidation rates can be a viable approach to achieve desired iron oxidation in smaller size bioreactors than what could be achieved with low cell densities. Operating at high cell densities would help to reduce the infrastructure footprint and hence capital costs of the process. Further research is recommended to define the impact of high cell densities on the operation and maintenance of reactors at the larger scale.

5. Acknowledgements

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Figure captions:

Figure 1. Schematic of the bioreactor system.

Figure 2. A) The flow rate and cumulative consumption of influent solution fed into the bioreactor, and B) dissolved oxygen (DO) saturation during biological iron oxidation experiment.

Figure 3. A) Volumetric iron oxidation rate and cell density over time during biological iron oxidation experiment and B) Volumetric iron oxidation rate and cell density for the initial 10 hours of the biocatalytic experiment.

Figure 4. Correlation between iron oxidation rate and cell counts during biological iron oxidation experiment.

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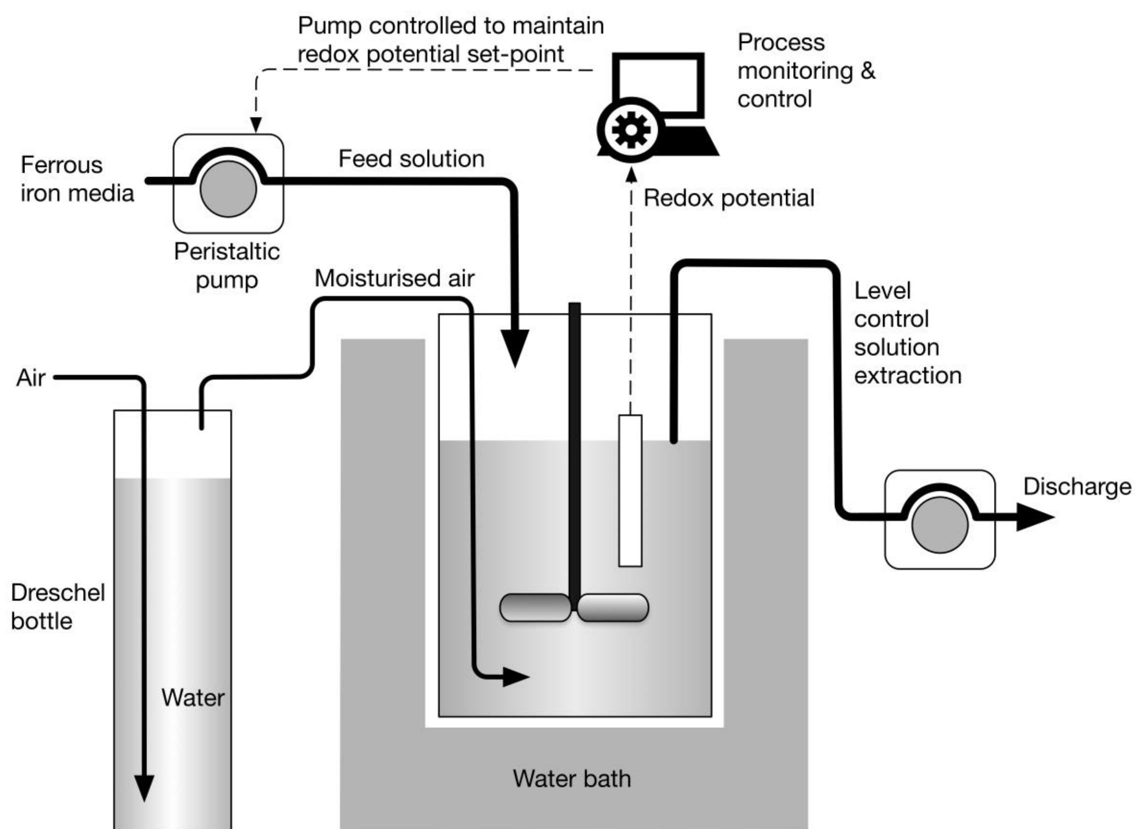

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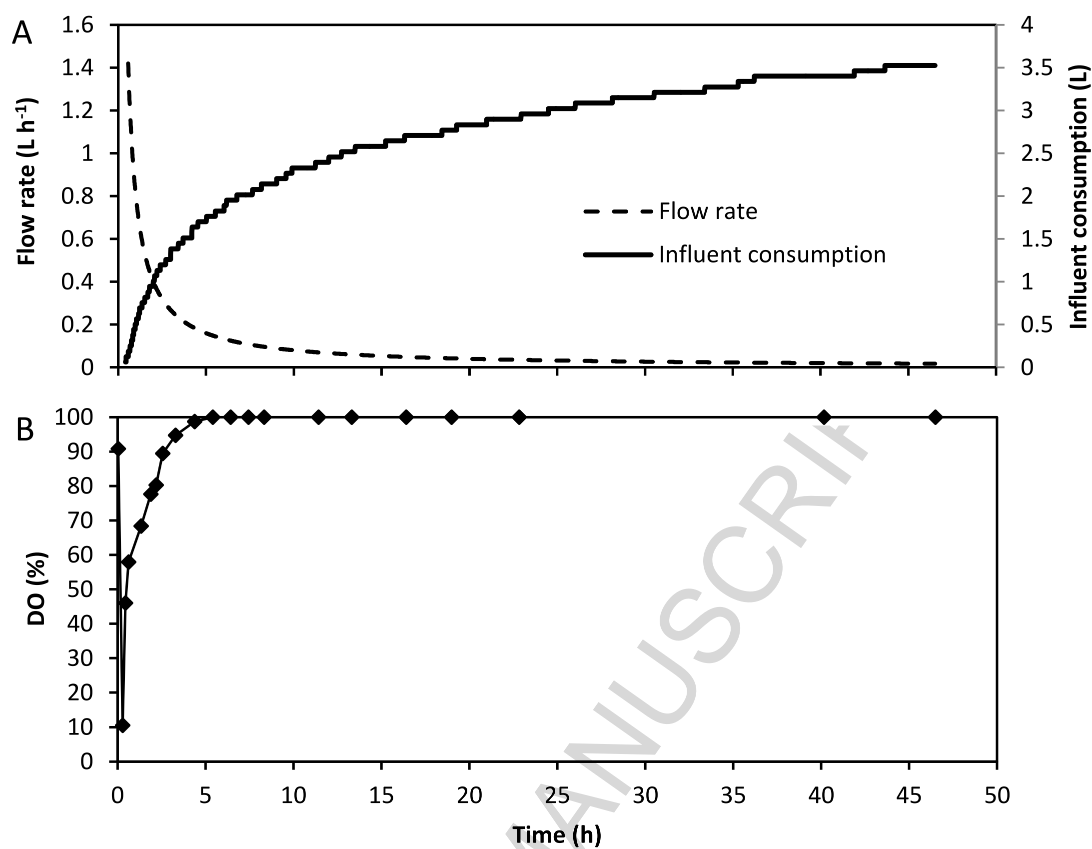

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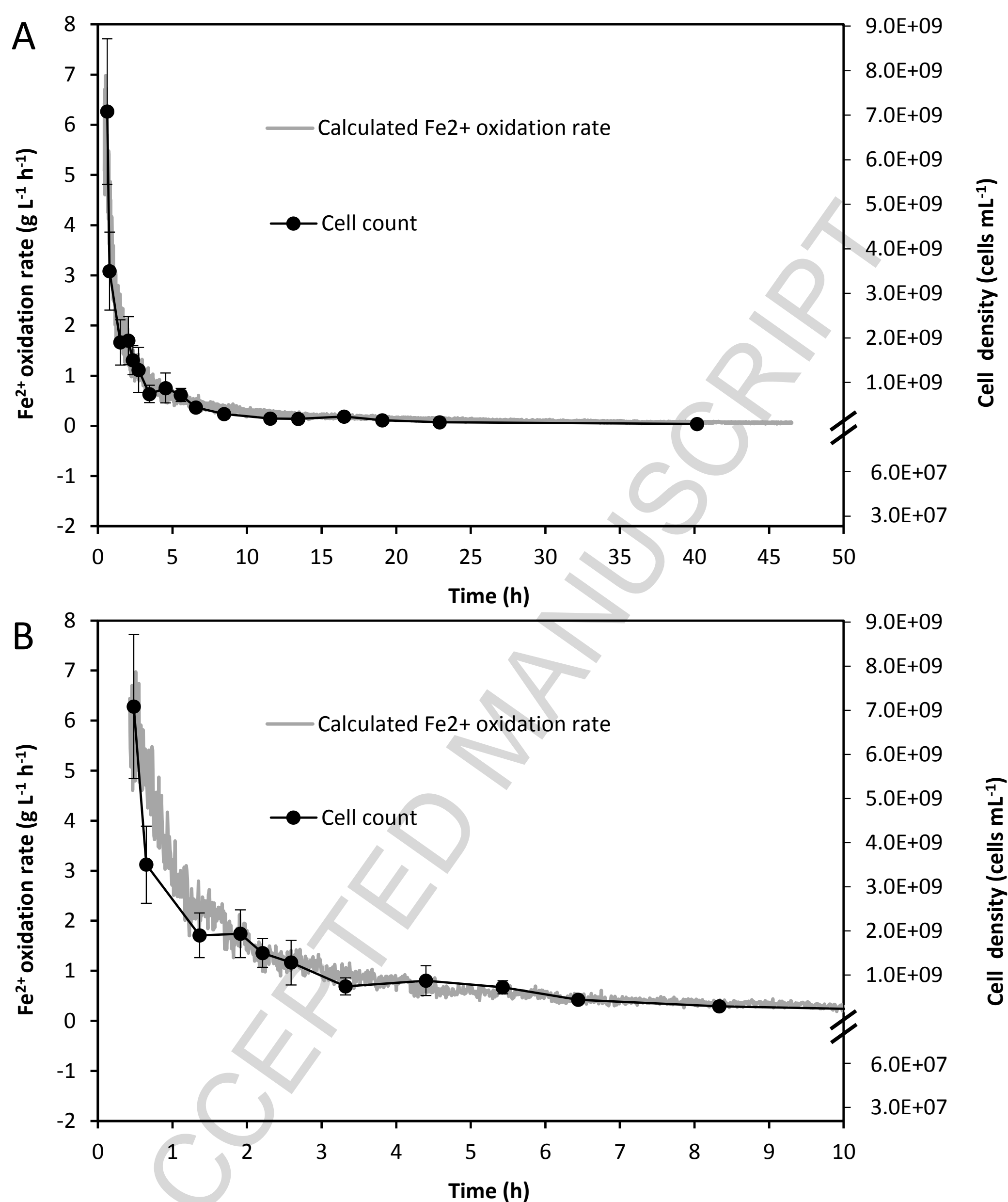

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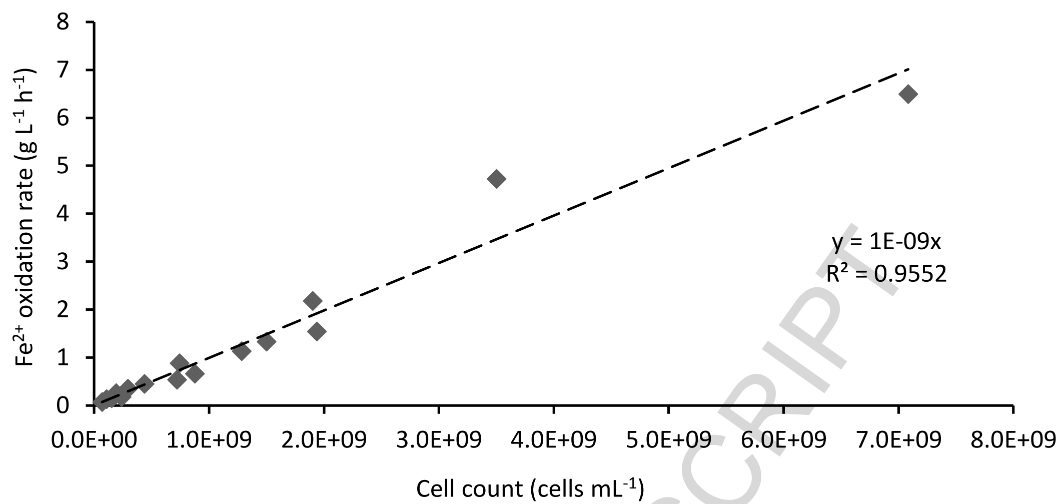

Figure 4. Correlation between iron oxidation rate and cell counts during biological iron oxidation experiment.

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Highlights

- The effect of cell concentration on biological iron oxidation rate was evaluated
- Cell concentration correlated positively with volumetric iron oxidation rate
- Specific iron oxidation was not notably affected by cell concentration
- No biocatalytic threshold was observed in the cell concentration range tested

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