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Oxidation of elemental sulfur, tetrathionate and ferrous iron by the psychrotolerant *Acidithiobacillus* strain SS3

Daniel Kupka [a], Maria Liljeqvist [b], Pauliina Nurmi [c,1], Jaakko A. Puhakka [c], Olli H. Tuovinen [c,d], Mark Dopson [b,*]

[a] *Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, SK-043 53 Košice, Slovakia*
[b] *Department of Molecular Biology, Umeå University, SE-901 87 Umeå, Sweden*
[c] *Department of Chemistry and Bioengineering, Tampere University of Technology, P.O. Box 541, FI-33101 Tampere, Finland*
[d] *Department of Microbiology, Ohio State University, 484 West 12th Avenue, Columbus, OH 43210, USA*

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Abstract

Mesophilic iron and sulfur-oxidizing acidophiles are readily found in acid mine drainage sites and bioleaching operations, but relatively little is known about their activities at suboptimal temperatures and in cold environments. The purpose of this work was to characterize the oxidation of elemental sulfur (S^0), tetrathionate ($S_4O_6^{2-}$) and ferrous iron (Fe^{2+}) by the psychrotolerant *Acidithiobacillus* strain SS3. The rates of elemental sulfur and tetrathionate oxidation had temperature optima of 20° and 25 °C, respectively, determined using a temperature gradient incubator that involved narrow (1.1 °C) incremental increases from 5° to 30 °C. Activation energies calculated from the Arrhenius plots were 61 and 89 kJ mol^{-1} for tetrathionate and 110 kJ mol^{-1} for S^0 oxidation. The oxidation of elemental sulfur produced sulfuric acid at 5 °C and decreased the pH to approximately 1. The low pH inhibited further oxidation of the substrate. In media with both S^0 and Fe^{2+}, oxidation of elemental sulfur did not commence until all available ferrous iron was oxidized. These data on sequential oxidation of the two substrates are in keeping with upregulation and downregulation of several proteins previously noted in the literature. Ferric iron was reduced to Fe^{2+} in parallel with elemental sulfur oxidation, indicating the presence of a sulfur:ferric iron reductase system in this bacterium.

Keywords: *Acidithiobacillus* strain SS3; Iron oxidation; Psychrotolerant; Redox coupling; Sulfur oxidation; Temperature dependency

1. Introduction

Bioleaching processes utilize acidophilic Fe^{2+} and elemental sulfur (S^0)-oxidizing microorganisms to aid in the solubilization of metals from sulfide minerals [27,31]. In bioleaching, the sulfur entity in sulfide minerals is oxidized chemically by Fe^{3+} or directly by bacteria with oxygen as the electron acceptor. Typical intermediates from these reactions are secondary sulfide minerals (e.g. CuS from $CuFeS_2$), thiosulfate ($S_2O_3^{2-}$), polythionates ($S_nO_6^{2-}$), polysulfides (S_n^{2-}) and elemental sulfur (S^0) [34]. These sulfur compounds are further oxidized to sulfate in acid-producing reactions. S^0 is biologically oxidized to sulfate in the presence of oxygen according to the net equation 1 (the major sulfate species in acidic solutions is bisulfate (pK_{a2} 1.92)).

$$2S^0 + 3O_2 + 2H_2O \rightarrow 2HSO_4^- + 2H^+ \quad (1)$$

The use of Fe^{3+} as an external electron acceptor for *Acidithiobacillus ferrooxidans* mediated oxidation of elemental sulfur (Eq. (2)) under oxic and anoxic conditions has been reported [6,28,35].

* Corresponding author.
E-mail addresses: dankup@saske.sk (D. Kupka), maria.liljeqvist@molbiol.umu.se (M. Liljeqvist), pauliina.nurmi@inbox.com (P. Nurmi), jaakko.puhakka@tut.fi (J.A. Puhakka), tuovinen.1@osu.edu (O.H. Tuovinen), mark.dopson@molbiol.umu.se (M. Dopson).
[1] Present address: MTT Agrifood Research Finland, Biotechnology and Food Research, FI-31600 Jokioinen, Finland.

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$$S^0 + 6Fe^{3+} + 4H_2O \rightarrow HSO_4^- + 6Fe^{2+} + 7H^+ \quad (2)$$

In bioleaching processes, S^0 can precipitate on sulfide mineral surfaces and slow down the diffusion of reactants and products to and from the mineral surface, thereby passivating the surface [22,33,42]. The passivating S^0 layer may be removed or partially alleviated by sulfur-oxidizing acidophilic bacteria and archaea [9,11], but oxidation of such sulfur rims is relatively slow. Bioleaching and associated iron oxidation involve acidic sulfate-containing solutions which promote the precipitation of schwertmannite (Eq. (3)) and jarosites such as K-jarosite (Eq. (4)).

$$8Fe^{3+} + SO_4^{2-} + 14H_2O \rightarrow Fe_8O_8(OH)_6SO_4 + 22H^+ \quad (3)$$

$$3Fe^{3+} + K^+ + 2SO_4^{2-} + 6H_2O \rightarrow KFe_3(SO_4)_2(OH)_6 + 6H^+ \quad (4)$$

The chemical and biological oxidation of the S-entity in sulfide minerals is central to metal dissolution, acid consumption or formation, and passivation in biological leaching processes. Understanding of the biological oxidation of inorganic sulfur compounds at low temperatures is, therefore, requisitely important for management of bioleaching processes and environmental problems with mine waste rocks in boreal environments. Bacterial oxidation of sulfide minerals at low temperatures has been reported [1,12,13,25] and a number of acidophilic iron-oxidizing strains have been isolated that are capable of growth at low temperatures [5,14]. In our previous work, we characterized growth and iron oxidation of an *A. ferrooxidans* strain SS3 at low temperatures [24]. Strain SS3 is psychrotolerant (optimum growth at 20–40 °C, but also grows at 4 °C). The 16S rRNA gene sequence of strain SS3 [24] aligned in a clade with other isolates from boreal environments, including clone T7 from a pilot scale bioheap [29] and strain NO-37 from an acid mine drainage site [19]. In this study, we have investigated the oxidation of S^0 and tetrathionate ($S_4O_6^{2-}$) by *Acidithiobacillus* strain SS3 and determined the corresponding activation energy values for the oxidation of these substrates. In addition, redox coupling between Fe^{3+} and S^0 was demonstrated in this work.

2. Materials and methods

2.1. Microorganisms and growth conditions

Acidithiobacillus strain SS3 [24] was used throughout this study. Based on 16S rRNA gene sequence similarity, strain SS3 aligns in a clade with the newly proposed '*Acidithiobacillus ferrivorans*' sp. nov. [16]. Cultures were incubated in mineral salts medium (pH 2.5) that contained (per liter) 3 g $(NH_4)_2SO_4$, 0.1 g KCl, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, and 0.01 g $Ca(NO_3)_2$. For routine subcultures, the medium was amended with 5 g S^0 L^{-1} (156 mM) and/or 80 mM Fe^{2+} as energy source. S^0 (precipitated) was from Lachema (Brno, Czech Republic; mean diameter of 30 μm and specific surface area of 0.175 m^2 g^{-1}).

2.2. Oxidation of tetrathionate and S^0 in temperature gradient incubator

Oxidation rates of tetrathionate and S^0 were measured over a temperature range of 5° to 30° ± 0.5 °C in a temperature gradient incubator (Test Tube Oscillator, Terratec®, Hobart, Australia). The temperature interval was approximately 1.1 °C and the oscillation at 35 min^{-1}. The mineral salts medium was adjusted to pH 2.5 with H_2SO_4 and supplemented with trace element solution [9] and filter-sterilized 5 mM $K_2S_4O_6$ or 5 g L^{-1} (156 mM) S^0 (autoclaved at 105 °C for 30 min). The media were inoculated with approximately 1×10^8 cells from a batch culture of *Acidithiobacillus* strain SS3 previously grown with the respective substrate. Cell numbers were determined by counting 4′-6-diamidino-2-phenylindole stained cells under epifluorescence microscopy. Changes in the tetrathionate concentration [21] and the pH were measured at intervals. The oxidation data were fitted to the Ratkowsky equation according to Eq. (5) [30].

$$\sqrt{\frac{1}{t}} = b \cdot (T - T_{\min}) \cdot \left(1 - e^{(c \cdot (T - T_{\max}))}\right) \quad (5)$$

where T = temperature (°C); $T_{\min}$ and $T_{\max}$ = the minimum and the maximum temperature, respectively; b and c = fitting parameters. The parameter time t is derived from the time it takes for half the tetrathionate concentration to be consumed or for the pH to be decreased by half. The greatest value for the square root of $1/t$ is derived from the optimum temperature (T_{opt}) shown by the inflection in the fitted curve. Chi-square (distribution when the null hypothesis is true) and R-square (index of correlation) values were calculated with a 95% confidence interval together with the errors for parameters b and c. Microcal Origin 6.0 software was used for construction of the Ratkowsky equation with a non-linear regression model.

Linearization of the Arrhenius equation (Eq. (6)) based on the rates of tetrathionate oxidation and pH decrease between 6° and 18 °C was used to calculate the activation energy (E_a).

$$\ln k = -\frac{E_a}{RT} + \ln A \quad (6)$$

where k = rate constant; A = frequency coefficient; E_a = activation energy (J mol^{-1}); R = gas constant; and T = absolute temperature (K). The rate constants for tetrathionate oxidation were calculated from half-life data, the time it takes for half the tetrathionate concentration to be oxidized ($T_{½} = 0.693/k$). The slope of the linear portion of the Arrhenius plot gives the E_a. Similarly, the rate constants for sulfur oxidation were calculated from the corresponding increases in sulfate concentration over time. Since tetrathionate and elemental sulfur oxidation produces sulfuric acid, rate constants were also estimated from time courses of decreases in pH.

2.3. Oxidation of elemental sulfur and iron

Elemental S^0 (5 g L^{-1}) was added to the mineral salts medium and autoclaved for 30 min at 105 °C. Filter-sterilized