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Catalytic Protein Film Electrochemistry Provides a Direct Measure of the Tetrathionate/Thiosulfate Reduction Potential

Julia M. Kurth,*[,†] Christiane Dahl,[†] and Julea N. Butt*[,‡]

[†]Institut für Mikrobiologie & Biotechnologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Meckenheimer Allee 168, D-53115 Bonn, Germany

[‡]Centre for Molecular and Structural Biochemistry, School of Chemistry, and School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, United Kingdom

Supporting Information

ABSTRACT: The tetrathionate/thiosulfate interconversion is a two-electron process: $S_4O_6^{2-} + 2\ e^- \leftrightarrow 2\ S_2O_3^{2-}$. Both transformations can support bacterial growth since $S_2O_3^{2-}$ provides an energy source, while $S_4O_6^{2-}$ serves as respiratory electron acceptor. Interest in the corresponding $S_2O_3^{2-}$ oxidation also arises from its widespread use in volumetric analysis of oxidizing agents and bleach neutralization during water treatment. Here we report protein film electrochemistry that defines the reduction potential of the $S_4O_6^{2-}/S_2O_3^{2-}$ couple. The relevant interconversion is not reversible at inert electrodes. However, facile reduction of $S_4O_6^{2-}$ to $S_2O_3^{2-}$ and the reverse reaction are catalyzed by enzymes of the thiosulfate dehydrogenase, TsdA, family adsorbed on graphite electrodes. Zero-current potentials measured with different enzymes, at three pH values, and multiple $S_4O_6^{2-}$ and $S_2O_3^{2-}$ concentrations together with the relevant Nernst equation resolved the tetrathionate/thiosulfate reduction potential as +198 ± 4 mV versus SHE. This potential lies in the ∼250 mV window encompassing previously reported values calculated from parameters including the free energy of formation. However, the value is considerably more positive than widely used in discussions of bacterial bioenergetics. As a consequence anaerobic respiration by tetrathionate reduction is likely to be more prevalent than presently thought in tetrathionate-containing environments such as marine sediments and the human gut.

There are numerous sulfur oxoacids, and many of those compounds have industrial significance.[1] Perhaps the most well-known is sulfuric acid. This chemical is a key constituent of lead-acid batteries and the production of phosphate fertilizers. However, other sulfur oxoanions are valuable reducing agents. A case in point is thiosulfate ($S_2O_3^{2-}$). This ion instantly neutralizes bleach in a reaction frequently exploited during water treatment and paper making. The final products of the reaction are tetrathionate ($S_4O_6^{2-}$), higher polythionates, and sulfate.[2] Tetrathionate is formed by oxidative conjugation of two molecules of thiosulfate with two electrons released in the corresponding half-reaction (eq 1):

$$S_4O_6^{2-} + 2e^- \leftrightarrow 2S_2O_3^{2-} \quad (1)$$

This half-reaction also underpins the widespread use of thiosulfate in analytical chemistry whereby stoichiometric reaction with I_2 produces 2 I^-. The corresponding color change is widely used for volumetric analysis of oxidizing agents in aqueous solutions of ecological and recreational interest. However, in other contexts, e.g., the extraction of gold and silver by ammoniacal thiosulfate leaching, the oxidation of thiosulfate to tetrathionate is detrimental and aims to be minimized.[3]

In addition to the industrial and analytical importance of the thiosulfate/tetrathionate interconversion, this reaction has considerable significance in the global biogeochemical cycling of sulfur.[4−6] Certain prokaryotes in aquatic and terrestrial habitats obtain energy by the oxidation of thiosulfate to tetrathionate. Other prokaryotes use the reverse reaction, namely tetrathionate reduction, to support anaerobic respiration. In this latter context two prominent examples are the human gut pathogens *Salmonella typhimurium*[7] and *Campylobacter jejuni*.[8] *S. typhimurium* reduces tetrathionate produced by vertebrate intestinal mucosa during inflammation, and this may confer a competitive growth advantage on the pathogen by supporting increased transmission through the faecal-oral route.[7]

The processes mentioned above have focused attention on the reduction potential ($E_{TT/TS}$) of the tetrathionate/thiosulfate couple. Pourbaix (reduction potential-pH) diagrams including this value have been presented.[3,9] $E_{TT/TS}$ is also included in redox towers. These compare the reduction potentials of different redox couples as a guide to the respiratory electron-transfer processes that may support bacterial colonization of a particular environment. However, there is ambiguity in the $E_{TT/TS}$ values that appear in such resources as they span a window exceeding 250 mV; from +24 to +300 mV versus SHE.[10−13] This variation stems largely from the irreversible nature of the thiosulfate/tetrathionate interconversion at an inert electrode.[9,14] The resulting behavior is inconsistent with the relevant Nernst equation, and this precludes direct measurement of $E_{TT/TS}$. As a consequence previously reported values relied completely on calculations from relevant thermodynamic data. However, free energies of formation for thiosulfate and tetrathionate are themselves constantly re-evaluated and published values cover ranges from approx-

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imately −510 to −600 kJ mol^{-1} and −1020 to −1055 kJ mol^{-1}, respectively.[12,15−18] Over the last four decades, an $E_{TT/TS}$ value of +24 mV has been most widely cited in the field of microbiology. This value was calculated based on free energies of formation published in the 1950s[15,17] and released in a highly influential seminal work on energy conservation in chemotrophic anaerobic bacteria.[10]

In order to address this situation by providing a direct measure of $E_{TT/TS}$ we have performed catalytic protein film electrochemistry of enzymes from the thiosulfate dehydrogenase, TsdA, family.[19] The TsdA proteins are *c*-type cytochromes carrying two heme groups.[19,20] An axial histidine/cysteine ligation of the central iron atom is characteristic for the active site heme. This type of ligation is rare among prokaryotes and appears to be of special importance in sulfur-based energy metabolism.[20] In many cases, TsdA is accompanied by another diheme cytochrome *c* (TsdB) that serves as the redox partner for TsdA.[19] In some instances, TsdA and TsdB form a fusion protein. All TsdA enzymes characterized to date catalyze both the oxidation of thiosulfate to tetrathionate and the reduction of tetrathionate to thiosulfate at measurable rates.[8,19] This reversibility is of crucial importance for the work presented here.

Samples of the purified TsdA enzymes from *Campylobacter jejuni* (*Cj*) and *Allochromatium vinosum* (*Av*) as well as the TsdBA fusion protein from *Marichromatium purpuratum* (*Mp*) were adsorbed as separate electrocatalytically active films on graphite electrodes. Cyclic voltammetry revealed clear catalytic currents when the enzyme-coated electrodes were placed in pH 5 solutions of equimolar tetrathionate and thiosulfate, Figure 1. These currents were absent when either the enzyme or the substrates were omitted from the experiment. Thus, the catalytic currents arise exclusively from enzyme-catalyzed tetrathionate reduction and/or thiosulfate oxidation and with that catalysis sustained by direct electron exchange between the enzyme and electrode.

For *Cj*TsdA the reductive (negative) catalytic currents have significantly larger magnitude than their oxidative (positive) counterparts, Figure 1A. As a consequence, it is immediately apparent that *Cj*TsdA is biased toward tetrathionate reduction relative to thiosulfate oxidation. By contrast *Mp*TsdBA displays higher catalytic rates for thiosulfate oxidation than tetrathionate reduction which reveals this enzyme's bias to oxidative catalysis, Figure 1B. However, *Av*TsdA displays the greatest bias toward thiosulfate oxidation of the enzymes studied here, Figure 1C. No evidence could be found for reductive catalysis by *Av*TsdA, and this was despite all three enzymes displaying comparable current magnitudes for thiosulfate oxidation. This agrees with results from colorimetric solution assays of *Av*TsdA activity that found a strong bias toward thiosulfate oxidation with very low specific activity for tetrathionate reduction.[8,20] Electrochemical resolution of catalytic reduction by *Av*TsdA is most likely to be precluded by the intrinsically low rate of tetrathionate reduction combined with low electrocatalytic coverage of the electrode by the enzyme. Indeed, none of the enzymes display detectable nonturnover waves in the absence of substrate, and this is consistent with low electrocatalytically active enzyme populations.

At high overpotentials the majority of the catalytic waves from all three enzymes fail to attain values that are independent of driving force for the relevant reaction. This is behavior that suggests heterogeneously oriented enzyme molecules displaying a range of interfacial electron-transfer kinetics.[21] Never the

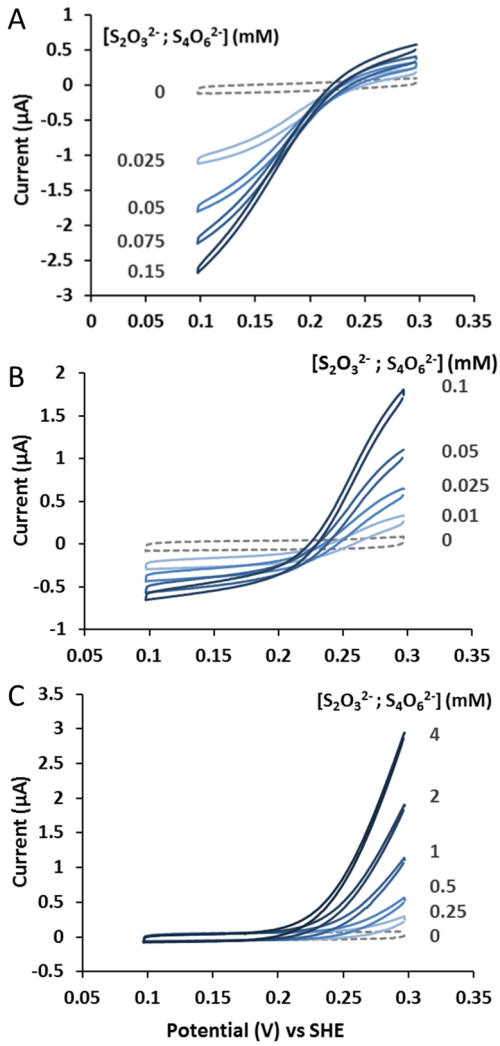

Figure 1. Representative protein film cyclic voltammetry of (A) *Cj*TsdA, (B) *Mp*TsdBA, and (C) *Av*TsdA in solutions containing equal concentrations of thiosulfate and tetrathionate as indicated (blue continuous lines) and prior to substrate addition (gray broken lines). Scan rate 10 mV s^{-1}, electrode rotation 500 rpm in 100 mM ammonium acetate, 50 mM NaCl, pH 5 at 25 °C for *Mp*TsdBA and *Av*TsdA and at 42 °C for *Cj*TsdA.

less, it is clear that films of *Cj*TsdA and *Mp*TsdBA catalyze rapid bidirectional interconversion of tetrathionate and thiosulfate. By visualizing such catalysis, the protein film electrochemistry defines a zero-current potential (E_{ZCP}) from which $E_{TT/TS}$ can be calculated using the relevant Nernst eq (eq 2):

$$E_{TT/TS} = E_{ZCP} - \frac{RT}{2F} \ln\left(\frac{[S_4O_6^{2-}]}{[S_2O_3^{2-}]^2}\right) \quad (2)$$

where *R*, *F*, and *T* have their usual meaning.[22−24] A number of factors contribute to defining catalytic bias,[25,26] but their resolution for the TsdA enzymes lies beyond the scope of the present work.

Values for E_{ZCP} were obtained by two methods as detailed in the Supporting Information. In one approach E_{ZCP} was defined as the points of intersection for cyclic voltammograms recorded in the presence and absence of substrates (averaged for each scan direction), e.g., Figure 1A,B. In the second approach the

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potential required to maintain zero current through the cell was measured directly. For all experiments the thiosulfate/tetrathionate mixtures were prepared immediately prior to use and with concentrations chosen to minimize the likelihood of any significant reaction between tetrathionate and thiosulfate.[27,28] Measurements by the second method were typically complete within 3 min, while those using the first method took ~15 min. No systematic differences were detected between E_{ZCP} values determined by the two approaches so the initial substrate concentrations were taken as those defining E_{ZCP}. The corresponding values of $E_{TT/TS}$ lie between +187 and +205 mV versus SHE, Figure 2 (black solid circles and triangles).

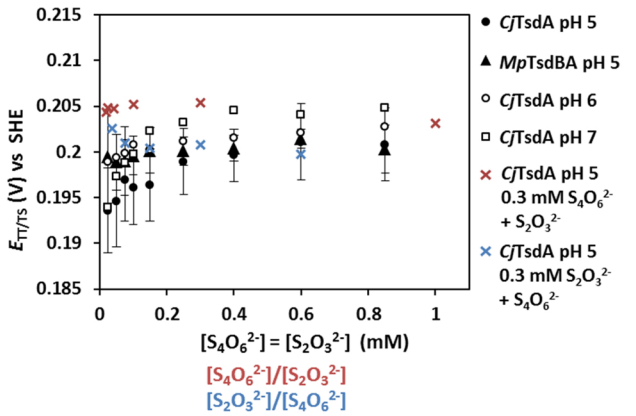

Figure 2. Values for the formal potential of the tetrathionate/thiosulfate couple, $E_{TT/TS}$, defined by protein film electrochemistry. Equal concentrations of $S_2O_3^{2-}$ and $S_4O_6^{2-}$ (black: symbols and *x*-axis title). Different concentrations of $S_2O_3^{2-}$ with $[S_4O_6^{2-}]$ = 0.3 mM (red: "×" and *x*-axis title). Different concentrations of $S_4O_6^{2-}$ with $[S_2O_3^{2-}]$ = 0.3 mM (blue: "×" and *x*-axis title). See text for details. Error bars were generated when at least two independent measurements were made.

Our analysis makes two assumptions. First, that the enzymes are true catalysts, changing the rate of attainment of equilibrium but not the position of equilibrium. Second, that the activities of thiosulfate and tetrathionate equate to their respective concentrations under our experimental conditions. Further experiments performed with *Cj*TsdA confirmed the validity of our approach. Thiosulfate was introduced to a solution that contained 0.3 mM tetrathionate but initially no thiosulfate, Figure 3A. Cyclic voltammetry quantified an increase in the ratio of oxidative relative to reductive catalysis on increasing the thiosulfate concentration from 0.3 to 12 mM. In addition E_{ZCP} was displaced by approximately −100 mV. In a separate experiment the tetrathionate concentration was increased from 0.05 to 0.5 mM in a solution containing initially 0.3 mM thiosulfate, Figure 3B. Here E_{ZCP} was displaced by approximately +30 mV. However, for both data sets the values of $E_{TT/TS}$ calculated from eq 2 were essentially independent of the thiosulfate:tetrathionate ratio, Figure 2 (red and blue "×"). The values were also in accord with those defined from the measurements with equal concentrations of both substrates.

The cyclic voltammograms presented above contain a wealth of information on the catalytic properties of TsdA enzymes. For example, thiosulfate is seen to inhibit tetrathionate reduction, Figure 3A, and tetrathionate to inhibit thiosulfate oxidation, Figure 3B. However, these features of the catalytic properties of *Cj*TsdA will be described more fully elsewhere (manuscript in

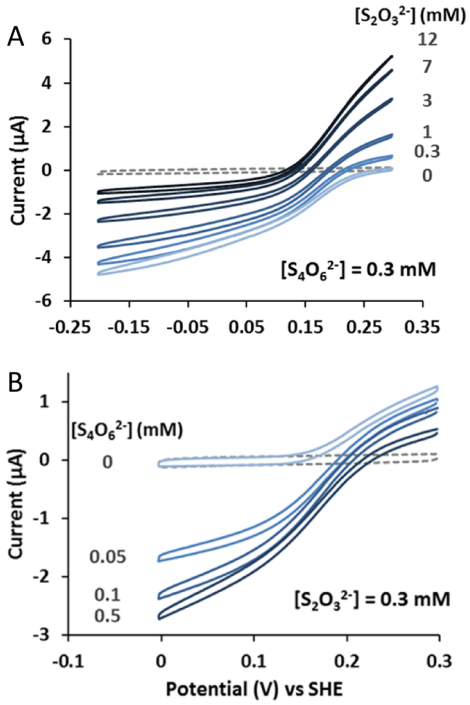

Figure 3. Protein film cyclic voltammetry for *Cj*TsdA in (A) 0.3 mM $S_4O_6^{2-}$ and (B) 0.3 mM $S_2O_3^{2-}$ with increasing concentration of the second substrate as indicated. Cyclic voltammetry recorded prior to substrate addition (gray broken lines). Scan rate 10 mV s^{-1}, electrode rotation, 500 rpm in 100 mM ammonium acetate, 50 mM NaCl, pH 5, 42 °C.

preparation). Here we retain a focus on the experimental resolution of $E_{TT/TS}$ and a final series of experiments that address the pH dependence of this parameter. Neither thiosulfate nor tetrathionate change their protonation state between pH 5 and 7.[9] As a consequence $E_{TT/TS}$ will be independent of pH in this range. Measurements in solutions of equal concentrations of thiosulfate and tetrathionate with *Cj*TsdA at pH 6 or at pH 7 generated $E_{TT/TS}$ values with the predicted behavior, Figure 2 (open circles and squares). Taking the average of the 111 data points represented in Figure 2, we determine a value for $E_{TT/TS}$ of +198 ± 4 mV versus SHE.

The range of oxidation states (−2 to +8) available to sulfur and the abundance of compounds containing multiple sulfurs with different oxidation states leads to a rich and complex chemistry of aqueous sulfur oxoanions.[1,2,29] By exploiting enzymes as selective catalysts for the tetrathionate/thiosulfate interconversion, we have been able to provide experimental resolution of a key thermodynamic parameter contributing to the description of such systems. The experimentally measured value of $E_{TT/TS}$ lies within the range of values calculated previously.[10−13] However, it is 174 mV more positive than the value of +24 mV[10] widely cited in the construction of redox towers. As a consequence more free energy is available to be harnessed during the respiratory reduction of tetrathionate than was previously recognized. At + 198 mV the formal reduction potential of the tetrathionate/thiosulfate couple is more positive than the corresponding values for several prevalent terminal respiratory electron-acceptor couples at neutral pH. These include fumarate/succinate (+33 mV),[10] trimethylamine oxide/trimethylamine (+130 mV),[30] and dimethyl sulfoxide/dimethylsulfide (+160 mV).[31] This can explain why *Salmonella enterica* grows by oxidation of propanediol or ethanolamine in

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the presence of tetrathionate, but not dimethyl sulfoxide, trimethylamine oxide, or fumarate, as terminal respiratory electron acceptor. This is despite *S. enterica* having capacity to respire on these alternate terminal electron acceptors when more reduced electron donor(s) such as glycerol are used.[32] Indeed tetrathionate may provide the respiratory electron acceptor in many more contexts than presently recognized. The lifestyles of pathogenic and commensal gut bacteria may benefit from respiratory reduction of the tetrathionate produced in the human intestine during inflammation.[6] In addition, for marine microbiota at neutral pH, the tetrathionate in sediments represents a more favorable electron acceptor than the high abundance compounds dimethyl sulfoxide and trimethylamine oxide.[33]

An exact, experimentally achieved $E_{TT/TS}$ value as provided here will also contribute to a better understanding of industrial applications involving thiosulfate. One prominent example is the use of thiosulfate instead of the hazardous cyanide as a lixiviant for gold. Despite extensive research work in this area, neither commercialization of the thiosulfate processes nor full knowledge of the underlying mechanism have been achieved.[3,34,35] This is due largely to the complexity of the chemical reactions accompanying the process and which include the formation of tetrathionate and other polythionates from the oxidation of thiosulfate in aqueous solutions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b08291.

Overproduction, purification and preparation of the thiosulfate dehydrogenases. Electrochemical procedures, resolution of E_{ZCP} and quantification of catalytic bias resolved from Figure 1 (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*j.butt@uea.ac.uk

*julia-kurth@gmx.de

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Harding, C.; Janes, R.; Johnson, D. *Elements of the P Block*; Royal Society of Chemistry: Cambridge, 2002.

(2) Varga, D.; Horváth, A. K.; Nagypál, I. *J. Phys. Chem. B* **2006**, *110*, 2467−2470.

(3) Aylmore, M. G.; Muir, D. M. *Miner. Eng.* **2001**, *14*, 135−174.

(4) Trudinger, P. A. *J. Bacteriol.* **1967**, *93*, 550−559.

(5) Barrett, E. L.; Clark, M. A. *Microbiol. Rev.* **1987**, *51*, 192−205.

(6) Podgorsek, L.; Imhoff, J. F. *Aquat. Microb. Ecol.* **1999**, *17*, 255−265.

(7) Winter, S. E.; Thiennimitr, P.; Winter, M. G.; Butler, B. P.; Huseby, D. L.; Crawford, R. W.; Russell, J. M.; Bevins, C. L.; Adams, L. G.; Tsolis, R. M.; Roth, J. R.; Bäumler, A. J. *Nature* **2010**, *467*, 426−429.

(8) Liu, Y. W.; Denkmann, K.; Kosciow, K.; Dahl, C.; Kelly, D. J. *Mol. Microbiol.* **2013**, *88*, 173−188.

(9) Pourbaix, M.; Pourbaix, A. *Geochim. Cosmochim. Acta* **1992**, *56*, 3157−3178.

(10) Thauer, R. K.; Jungermann, K.; Decker, K. *Bacteriol. Rev.* **1977**, *41*, 100−180.

(11) Kaprálek, F. *J. Gen. Microbiol.* **1972**, *71*, 133−139.

(12) Cobble, J. W.; Stephens, H. P.; McKinnon, I. R.; Westrum, E. F. *Inorg. Chem.* **1972**, *11*, 1669−1674.

(13) Williamson, M. A.; Rimstidt, J. D. *Geochim. Cosmochim. Acta* **1992**, *56*, 3867−3880.

(14) Bard, A. J.; Parsons, R.; Jordan, J. *Standard potentials in aqueous solution*; Marcel Dekker: New York, 1985.

(15) Mel, H. C.; Hugus, Z. Z.; Latimer, W. M. *J. Am. Chem. Soc.* **1956**, *78*, 1822−1826.

(16) Wagman, D. D.; Evans, W. H.; Parker, V. B.; Schumm, R. H.; Halow, I.; Bailey, S. M.; Churney, K. L.; Nuttall, R. L. *J. Phys. Chem. Ref. Data, Suppl.* **1982**, *11*, Supplement No. 2, pp 2-1−2-392.

(17) Latimer, W. M. *Oxidation potentials*, 2nd ed.; Prentice-Hall: New York, 1952.

(18) Oelkers, E. H.; Helgeson, H. C.; Shock, E. L.; Sverjensky, D. A.; Johnson, J. W.; Pokrovskii, V. A. *J. Phys. Chem. Ref. Data* **1995**, *24*, 1401−1560.

(19) Denkmann, K.; Grein, F.; Zigann, R.; Siemen, A.; Bergmann, J.; van Helmont, S.; Nicolai, A.; Pereira, I. A. C.; Dahl, C. *Environ. Microbiol.* **2012**, *14*, 2673−2688.

(20) Brito, J. A.; Denkmann, K.; Pereira, I. A. C.; Archer, M.; Dahl, C. *J. Biol. Chem.* **2015**, *290*, 9222−9238.

(21) Léger, C.; Jones, A. K.; Albracht, S. P. J.; Armstrong, F. A. *J. Phys. Chem. B* **2002**, *106*, 13058−13063.

(22) Hirst, J.; Sucheta, A.; Ackrell, B. A. C.; Armstrong, F. A. *J. Am. Chem. Soc.* **1996**, *118*, 5031−5038.

(23) Zu, Y.; Shannon, R. J.; Hirst, J. *J. Am. Chem. Soc.* **2003**, *125*, 6020−6021.

(24) Parkin, A.; Seravalli, J.; Vincent, K. A.; Ragsdale, S. W.; Armstrong, F. A. *J. Am. Chem. Soc.* **2007**, *129*, 10328−10329.

(25) Hexter, S. V.; Esterle, T. F.; Armstrong, F. A. *Phys. Chem. Chem. Phys.* **2014**, *16*, 11822−11833.

(26) Abou Hamdan, A.; Dementin, S.; Liebgott, P. P.; Gutierrez-Sanz, O.; Richaud, P.; De Lacey, A. L.; Roussett, M.; Bertrand, P.; Cournac, L.; Léger, C. *J. Am. Chem. Soc.* **2012**, *134*, 8368−8371.

(27) Zhang, H.; Jeffrey, M. I. *Inorg. Chem.* **2010**, *49*, 10273−10282.

(28) Fava, A.; Bresadola, S. *J. Am. Chem. Soc.* **1955**, *77*, 5792−5794.

(29) Xu, L.; Horváth, A. K. *J. Phys. Chem. A* **2014**, *118*, 6171−6180.

(30) Jones, C. W. *Bacterial respiration and photosynthesis*; Thomas Nelson and Sons, Ltd.: Walton-on-Thames, England, 1982.

(31) Wood, P. M. *FEBS Lett.* **1981**, *124*, 11−14.

(32) Price-Carter, M.; Tingey, J.; Bobik, T. A.; Roth, J. R. *J. Bacteriol.* **2001**, *183*, 2463−2475.

(33) Barrett, E. L.; Kwan, H. S. *Annu. Rev. Microbiol.* **1985**, *39*, 131−149.

(34) Grosse, A. C.; Dicinoski, G. W.; Shaw, M. J.; Haddad, P. R. *Hydrometallurgy* **2003**, *69*, 1−21.

(35) Feng, D.; Van Deventer, J. S. J. *Hydrometallurgy* **2010**, *105*, 120−126.

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