Analysis of the bacterial sulphur system

Rachel M. Candy[1,a]*; K. R. Blight[1,b]; David E. Ralph[1,c]

[1]School of Veterinary and Life Sciences, Chemistry Department, Murdoch University, 90 South Street, Murdoch, Western Australia, 6150, Australia

[a]R.Candy@live.com.au, [b]D.Ralph@murdoch.edu.au, [c]blightedhaggis@gmail.com

Keywords: Sulphur oxidation, Ionic strength, pH, CO_2 partial pressure, Chemolithotrophic bacteria, Kinetics

Abstract. Heterogeneous bacterial sulphur systems are inherently complicated. However, developing an understanding of the influence of environmental factors such as pH, *I* and P_{CO2} is important for a number of fields. Examples of these include minimising acid mine drainage and maximising metal recovery from low-grade sulphide minerals. Measuring the effect of these factors on sulphur (S) oxidation is complicated by the presence and nature of solid phase elemental S. The rate and extent of S oxidation can be determined indirectly via the reaction product, H_2SO_4, which was quantified using pH measurements in this study. The method was critically dependent on the quality of pH data but proved effective in providing rate constants for the catalysed S oxidation reaction and yield (biomass/substrate) estimates in the range pH > 1.5. Increasing *I* over the range 0.176 – 0.367 mol L^{-1} decreased bacterial cell yields but increased the rate of sulphur oxidation significantly. Partial pressures of CO_2 in the range of 0.039 – 1.18% v/v produced no significant effect on the rates of S oxidation or bacterial cell yields. Bacterial cell yields were not affected in the pH range 1.5 – 2.5, however the rate of S oxidation increased significantly from pH 2.0 – 2.5. In the range pH < 1.5 the batch cultures progressed and although no reliable pH data were recorded, cell yields decreased from 7.43×10^{12} to 2.05×10^{12} cells mol^{-1} at pH 1.5 to1.0 respectively.

Introduction

The extraction of metal values from sulphidic and refractory ores is intimately connected with the chemistry of sulphur species. Bioleaching is a common industrial process used to leach these types of ores and involves the use of chemolithotrophic bacteria to catalyse the oxidation of S. Elemental S is a common by-product generated during the oxidation of sulphide minerals, where the S atom changes oxidation state from -2 to +6 in the SO_4^{-2} form. Bacteria catalyse the conversion of elemental S to H_2SO_4, providing protons for the dissolution of the sulphide mineral matrix and solubilising metal values. The catalytic role of bacteria in these systems depends on the mineral undergoing treatment, but in all cases relies on the metabolic rate of the cells and the rate at which they auto-catalyse the rate determining step of the dissolution process.

The effect of solution conditions can be compared in replicate experiments where only the environmental factor of interest is varied. In bacterial S systems, measuring bacterial yields and the rate of elemental S oxidation is complicated by the presence and nature of the solid phase substrate. Bacteria adhere to the solid surface and require estimation before total cell numbers can be determined accurately. Measuring the amount of S oxidised over a period of time is also complicated because the system is heterogeneous.

A number of indirect methods have been developed to improve the estimation of yield and specific rates of S utilisation. Konishi *et al*. 1995 [1] used a Langmuir isotherm to predict the quantity of sessile cells present on the S surface from planktonic population and compared these results to the number of cells released into solution by the dissolution of S substrate using CS_2. An

adsorption isotherm was also reported by others [2, 3, 4], while Espejo *et* Romero [5] included labelled phosphorous in the medium and measured the partition of bacteria to the S surface. Suzuki *et al*. [6] measured the uptake of O_2 by bacteria and compared the relative effects of solution conditions on consumption rates. Estimates of yield and specific rate of S oxidation vary widely [7] and no general understanding of the affect of solution parameters on bacterial activity has emerged.

An alternative but less well studied method that can estimate the extent of S oxidation is the proton balance. Protons generated during S oxidation are consumed by protonation of SO_4^{-2} ions or during CO_2 reduction for the formation of biomass. Production of protons exceeds consumption and the system pH decreases. Excess proton production can be determined directly from the amount of base added to maintain the system at constant pH. Estimating the amount of S oxidised from the amount of base added relies on knowledge of the fraction of protons generated which are not neutralised as they are either associated with SO_4^{-2} or consumed in the formation of biomass. The fraction of protons associated with SO_4^{-2} can be determined empirically by replacing S oxidation with standard additions of H_2SO_4. Protons consumed in the formation of biomass can be determined using chemical oxygen demand (COD) analyses, provided that all S has been oxidised to SO_4^{-2}. The process of CO_2 reduction to create chemolithotrophic biomass (Eq. 1) is reversed under chromic acid oxidation and the stoichiometry allows estimation of the quantity of protons consumed during production of that biomass.

$$CO_2 + 0.25NH_3 + 4.86H^+ + 4.11e^- \rightarrow CH_{1.70}O_{0.42}N_{0.25} + 2.58H_2O. \quad (1)$$

Yields of chemolithotrophic bacteria are low and therefore the amount of H^+ consumed by CO_2 reduction is expected to be relatively small. The amount of S oxidised should then be a simple proportion of the amount of base added. The oxidation of solid S involves the dissolution of approximately spherical solid particles and the shrinking sphere model was applied to extract rate constants. The effect of environmental factors pH, *I* and P_{CO2} on yields and rate constants are reported in this study.

Materials and Methods

A heterogeneous culture of microbes was isolated from pyritic ore and maintained by repeated transfers in minimal media with elemental S as the sole energy source. In these experiments, bacteria were grown in minimal media, with pH and *I* adjusted using concentrated H_2SO_4 and Na_2SO_4 respectively. Recrystallised orthorhombic sulphur was crushed, sieved and a narrow size fraction used (38 to 63 μm). The small amount of S oxidised in each batch culture ensured that *I* remained constant while a static pH was maintained by NaOH additions. An auto-titrator (Dosimat; Metrohm model No. 776) was used to transfer base solution, with volume recorded over 10 minute intervals using a data logger (Datataker50) and computer interface (DeTerminal).

Known cell numbers were inoculated into the reactor and aeration and agitation commenced. A limiting amount of solid S was added (~ 0.5 g), the auto-titrator was connected and the oxidation continued for some days. After the exhaustion of solid S, manual cell counts (haemocytometer, Optic Labour) and tests for reduced S species were conducted (cyanolysis, Kamyshny *et al.* [8]). COD analyses were conducted on aliquots of the reactor fluid once all S was oxidised. The association constants of HSO_4^- were determined empirically using standard additions of H_2SO_4 to the reactor, with the auto-titrator activated.

Results and Discussion

Proton Balance.A proton balance was calculated for all experiments, with reasonable results obtained within the technical limitations of the commercial pH probes used. The system and methods were capable of achieving a balance within a few percent difference (Table 1) provided the media pH was 1.5 or higher. At pH < 1.5, variations in proton activity from S oxidation were no longer significant compared with the 'noise' and 'drift' associated with the pH probe.

Table 1: Proton mass balance for a range of pH, *I* and P_{CO2} conditions. Total number of protons generated from the complete oxidation of S is represented as H_T. The number of protons titrated and associated with SO_4^{-2} are represented as H_{Soln} and H_{Bio}, with H_{Obs} refering to the combined amount of protons neutralised and in biomass (H_{Soln} +H_{Bio}).

pH	*I* [mol L^{-1}]	P_{CO2} [% v/v]	H_T [mmol]	H_{Soln} [mmol]	H_{Bio} [mmol]	H_{Obs} [mmol]	$H_{Obs} - H_T$ [%]
2.0	0.11	0.04	13.5	10.9	2.76	13.7	1.5
2.0	0.19	0.04	14.1	11.1	2.54	13.6	-3.6
2.0	0.34	0.04	13.9	11.9	2.37	14.3	2.8
2.5	0.18	0.04	14.2	13.3	2.03	15.3	7.5
1.5	0.22	0.04	11.9	9.5	2.36	11.8	-0.8
1.0	0.37	0.04	5.81	0.00	3.13	3.13	-60
2.0	0.19	0.48	14.0	11.5	2.80	13.6	-2.9
2.0	0.19	1.18	14.0	11.5	2.33	13.8	-1.4

Influence of Environmental Conditions on Yields and Rates. The increased partial pressure of CO_2 from 0.04 to 1.18% v/v had no significant effect on the yield of cells or rate constant of S oxidation. The effects of pH and *I* on yield were similar in that neither variable showed an effect over the range investigated. At pH < 1.0, the yield was reduced significantly. The rate constant however, increased as pH increased to 2.5. Increasing *I* from 0.11 – 0.34 mol L^{-1}only marginally affected the yields observed but caused a doubling of the rate constant from $2.79 \pm 1.21 \times 10^{-6}$ to $6.67 \pm 1.45 \times 10^{-6}$ m s^{-1}.

Table 2: Effect of ionic strength, pH and CO_2 partial pressure on yield and rate constant (k)

pH	*I* [mol L^{-1}]	P_{CO2} [% v/v]	K [$\times 10^{-6}$ m s^{-1}]	Yield [$\times 10^{12}$ cells mol^{-1}]
2.0	0.11	0.04	2.98 ± 0.09	8.70 ± 0.09
2.0	0.19	0.04	2.79 ± 1.21	6.71 ± 2.09
2.0	0.34	0.04	6.67 ± 1.45	7.46 ± 0.09
2.5	0.18	0.04	11.2 ± 4.80	6.31 ± 1.09
1.5	0.22	0.04	3.46 ± 1.14	7.43 ± 0.01
1.0	0.37	0.04	NA	2.05
2.0	0.19	0.48	3.92 ± 3.38	6.56 ± 0.83
2.0	0.19	1.18	2.78 ± 2.02	7.33 ± 0.68

Yields obtained in this study compare closely to literature, with results falling well within the range of published values [1-5]. Rates of sulphur oxidation were significantly influenced by solution parameters pH and ionic strength, with CO_2 partial pressure having no discernible effect. The increase in the apparent rate constant with increasing pH and *I* provide some insight into the nature of the rate determining step in the S oxidation process. It can be inferred that protons are involved and additionally, that like-charged species are also involved.

References

[1] Y. Konishi, S. Asai, N. Yoshida, Growth kinetics of *Thiobacillusthiooxidans* on the surface of elemental sulfur, Applied and Environmental Microbiology. 61 (1995) 3617-3622.

[2] R. Gourdon, N. Funtowicz, Kinetic model of elemental sulfur oxidation by *Thiobacillus thiooxidans* in batch slurry reactors, Bioprocess Engineering. 18 (1998) 241-249

[3] P. Ceskova, M. Mandl, S. Helanova, J. Kasparovska, Kinetic studies on elemental sulfur oxidation by *Acidithiobacillusferrooxidans*: sulfur limitation and activity of free and adsorbed bacteria, Biotechnology and Bioengineering. 78 (2001) 24-30

[4] P. Ceskova, M. Mandl, J. Hubackova, Kinetic quantification of sulfur-oxidizing bacteria absorbed on sulfur, Biotechnology Letters. 22 (2000) 699-701

[5] R. T. Espejo, P. Romero, Growth of *Thiobacillusferrooxidans* on elemental sulphur, Applied and Environmental Micorbiology. 53 (1987) 1907-1912

[6] I. Suzuki, D. Lee, B. Mackay, L. Harahuc, J. Oh, Effect of various ions, pH, and osmotic pressure on oxidation of elemental sulphur by *Thiobacillusthiooxidans*, Applied and Environmental Microbiology. 65 (1999) 5163-5168

[7] B. Pokorna, M. Mandl, S. Borilova, P. Ceskova, R. Markova, O. Janiczek, Kinetic constant variability in bacterial oxidation of elemental sulphur, Applied and Environmental Microbiology. 73 (2007) 3752-3754

[8] A. Kamyshny, C. G. Borkenstein, T. G. Ferdelman, Protocol for quantitative detection of elemental sulphur and polysulphide zero-valent sulphur distribution in natural aquatic samples, Geostandards and Geoanalytical Research. 33 (2009) 415-435

[9] J. I. Partanen, A. K. Covington, Re-evaluation of stoichiometric dissociation constants from electrochemical cell data for propionic and *n*-butyric acids as (0 to 60) ^{o}C in aqueous sodium chloride solutions, Journal of Chemical Engineering Data. 49 (2004) 394-406