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APPENDICES

1. TOC ANALYSIS

Table 1 Instrument specifications and operating conditions for TOC analysis.

Function	Specification
Measurement principle	Wet chemical oxidation with NDIR
Measuring range	0-3500 mg/L
Detection limits	0.5 µg/L
Sample injection volume	2500 µL
Pre-treatment for IC	Automatic acid addition and sparging
Carrier gas	UHP nitrogen gas
Carrier gas flow rate	200 mL/min

2. SRB CHARACTERIZATION

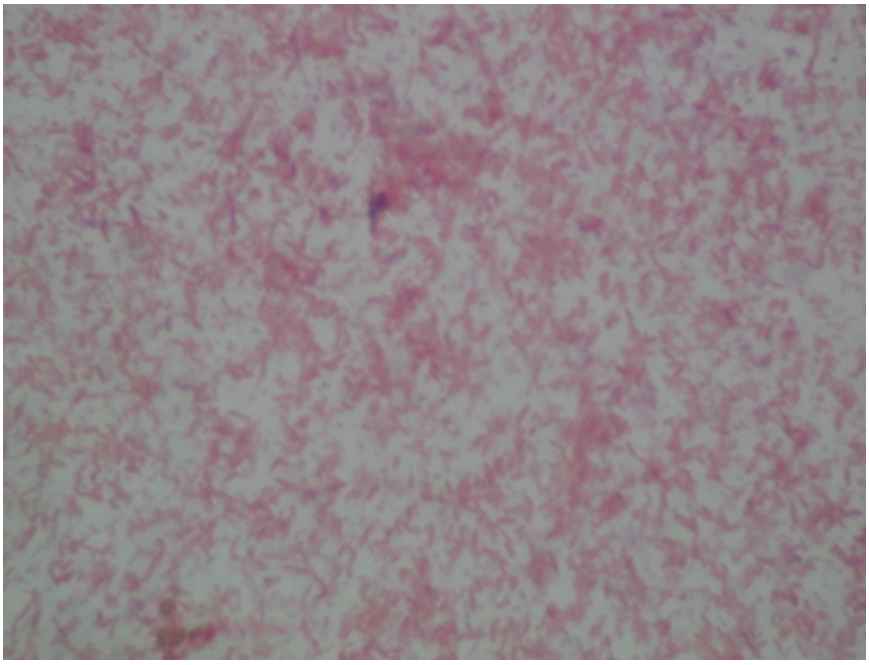

Figure 1: Gram staining image of a sulphate reducing bacteria consortium (magnification = X100).

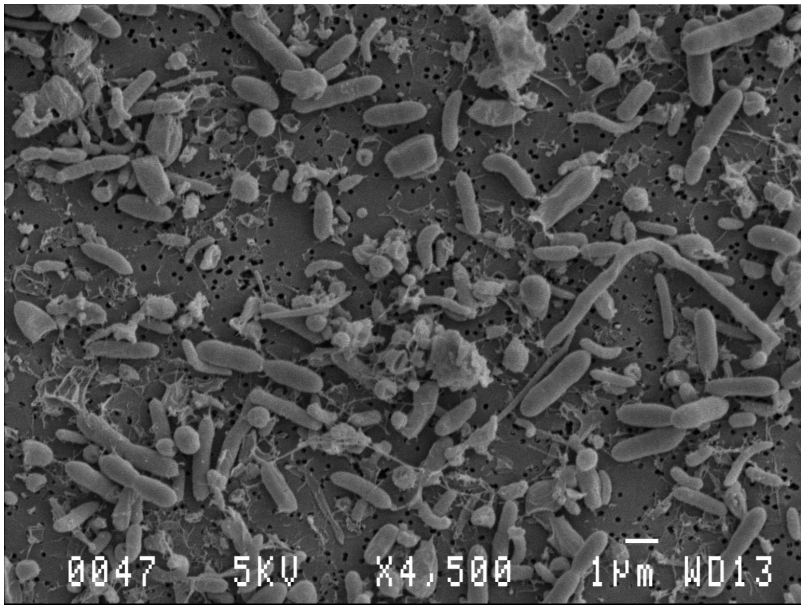

Figure 2: Scanning electron microscope image of sulphate reducing bacteria.

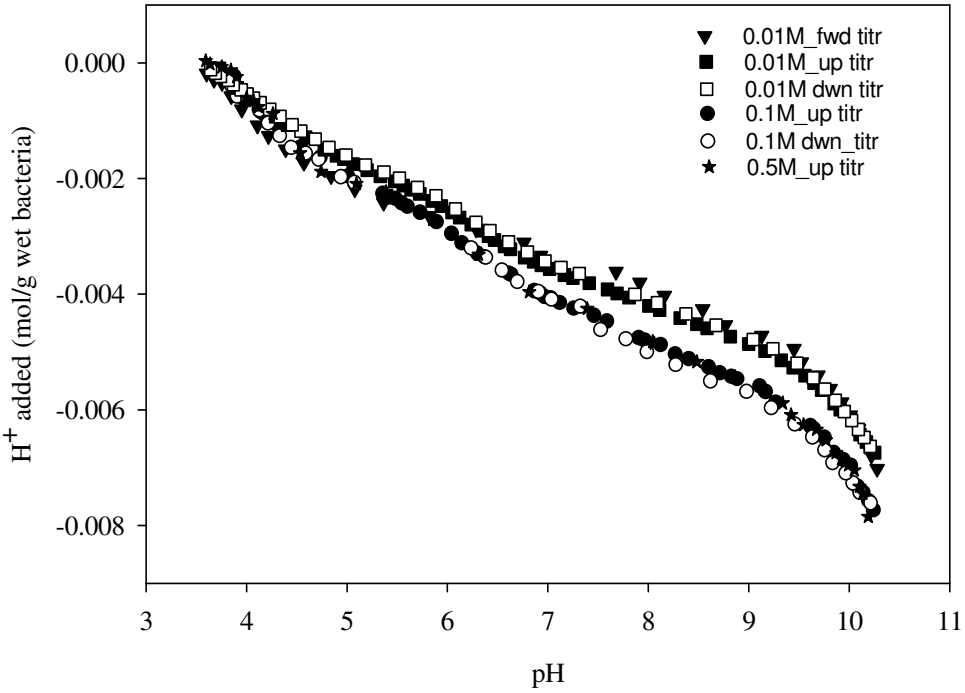

Figure 3: Experimental potentiometric titration data of variable masses of a viable SRB consortium in 0.01M, 0.1M and 0.5M $NaNO_3$ and 25°C.

Table 2 Absorption bands of SRB and functional groups assignment

Wavenumber (cm^{-1})	*Functional group/assignment*
~3414	Broad stretches of hydroxy group, H-bonded OH
~2925	Stretching vibrations of CH3 and CH2 due to fatty acid components of membranes
~1657	Primary amine, NH bend
~1545	Secondary amine, NH bend
~1453	Bending vibrations of CH, CH2 and CH3 due to a carboxylate (carboxylic acid salt)
~1400	Stretching vibrations of hetero-oxy compounds due to organic sulfates
~1240	Stretching vibrations of aromatic phosphates in polysaccharides and nucleic acids
~1081	stretching vibrations of phosphodiester, phosphorylated proteins, or polyphosphate products

3. AQUASIM SIMULATIONS

a. Description of parameters and input values

Table 2 Definition of variables used for SRB bioreactor processes model calibration.

Variable	*Variable Type*	*Description*	*Units*	*Initial value*	*Range*	*Std dev*	*Optimised value*
μ_{max}	Constant	Maximum specific growth rate coefficient	1/hr	0.4	0-20	1	0.985
$Y_{x/s}$	Constant	Bacterial yield coefficient	mg/mg	0.074	0-10	1	0.0735
K_s	Constant	Half velocity concentration	mg/L	150	0-1000	1	187
C	State	Metal concentration at time t	mg/L	N/A	N/A	1	N/A
C_{ini}	Constant	Initial concentration of metal	mg/L	75	0-100	1	0.976
C_{meas}	Real list	Experimental metal concentration	mg/L	N/A	N/A	1	N/A
S	State	Concentration of sulphate at time t	mg/L	N/A	N/A	1	N/A
S_0	Constant	Initial concentration of sulphate	mg/L	3000	2000-4000	1	3021
S_{meas}	Real list	Experimental sulphate concentration	mg/L	N/A	N/A	1	N/A
X	State	Concentration of bacteria at time t	mg/L	N/A	N/A	1	N/A
X_0	Constant	Initial concentration of biomass	mg/L	48	45-55	1	48.6
X_{meas}	Real list	Experimental bacterial concentration	mg/L	N/A	N/A	1	N/A
K_i	Constant	Inhibition coefficient	mg/L	0.1	0-10	1	0.616
k_C	Constant	Pseudo second-order rate coefficient	L/mg/h	0.0001	0-1	1	0.0004
t	Programme	Time	hr	N/A	N/A	1	N/A

b. Definition of Dynamic processes

Process	Description	Rate	Stoichiometry
Cell Growth	Bacterial growth rate	$(\mu_{max}*S*X/Ks+S)*I$	X: 1
Sulphate Reducti	Sulphate reduction rate	$(1/Y_{x/s})(\mu_{max}*S*X/Ks+S)*I$	S: -1
Metal Removal	Metal removal rate	k_C*C*X	C: -1

4. MODELLING METAL UPTAKE ONTO BACTERIAL SURFACES

a. Fitmod Input File (NEM) for Modelling the Acid Base Properties of SRB

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1
       0
       1
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       1
      90
       3       1       0       0       0
00001  0.0    1.44E-4   H1
00002  0.0    3.86E-4   H2
00003  0.0    2.70E-4   H3
00050  0.0    0.00E00    H

00050  0.0    050 01
00051  -13.90 050 -1
00001  0.0    001 01
00101  -4.2   001 01 050 -1
00002  0.0    002 01
00201  -6.5   002 01 050 -1
00003  0.0    003 01
00301  -8.2   003 01 050 -1


      03       3
     101
     201
     301
      01
      02
      03
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-0.000235023
-0.000374698
-0.000514372
-0.000654047
-0.000700605
-0.000747163
-0.000886837
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-0.001212744
-0.00130586
-0.001445535
-0.001538651
-0.001585209
-0.001631767
-0.001678326
-0.001818
-0.001911116
-0.002004233
-0.002097349
-0.002190465
-0.002237023
-0.002283581
-0.00233014
-0.002376698
-0.002423256
-0.002469814
-0.002516372
-0.00256293
-0.002609488
-0.002609488
-0.002656047
-0.002702605
-0.002749163
-0.002842279
-0.002888837
-0.002888837
-0.002981953
-0.003028512
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-0.003168186
-0.003261302
-0.003447535
-0.003587209
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-0.004099349
-0.004145907
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-0.004285581
-0.004378698
-0.004425256
-0.004518372
-0.00456493
-0.004658047
-0.004751163
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-0.004937395
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-0.005123628
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-0.005496093
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-0.006287581
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where:
Line 1-6: Program control input
Line 7-11: Matrix A components (Group of components, where Group I = components for which only T is known, Group II = components for which both X and T are known, and Group III = components for which only X is known).
Line 12: Blank card
Line 13-20 Matrix B components (Definition of species and reactions to form species)
Line 21: Blank card
Line 22: Blank card
Line 23: Definition of parameters for optimization (number of Log Ks and Ts)
Line 24-26: ID of 1st, 2nd and 3rd Log K values to be adjusted
Line 27-29: ID of 1st, 2nd and 3rd T values to be adjusted
Line 30: Serial data definition (number of data points, T serial data for 1 component, X serial data for 1 component, dilution factor serial data for 1 component).
Line 31: Total concentration of component (ID number)
Line 32-107: $[H^+]$ added concentration serial data points
Line 108: Log $[H^+]$
Line 109-184: Serial data points
Line 185-260: Dilution coefficient data
Line 261: Termination (00 00)

b. Extrapolation of Equilibrium Constants to Zero Ionic Strength

The deprotonation of a bacterial reactive surface site, e.g., a carboxyl site can be described by the following generic acid-base equilibrium:

$$\text{Carboxyl:} \qquad R-COOH^0 \Leftrightarrow R-COO^- + H^+ \qquad [1]$$

where: R = cell wall and –S-H = the protonated reactive surface site. The corresponding mass action equation for these deprotonation reactions can be expressed as:

$$K_1 = \frac{\left[R-COO^-\right]a_{H^+}}{\left[R-COOH^0\right]} \qquad [2]$$

where: K_1 = deprotonation constants for the reaction, a_i = activity of species *i* in the solution and the [ ] represents molal concentration of the surface species. The deprotonation constant were corrected to zero ionic strength using the Davies equation as follows:

$$\log \gamma_i = -Z_i^2 \left(\frac{A\sqrt{I}}{1+\sqrt{I}} - bI \right) \qquad [3]$$

where: γ = the activity (deprotonation) constant, A= Debye-Hückel constant, which incorporates temperature effects, and is equal to 0.5091 at 25°C (Fernandez et al., 1997), b = 0.1, and z_i = the ion charge. The ionic strength is defined as:

$$I = \frac{1}{2}\sum_k m_k Z_k^2 \qquad [4]$$

where: m_k = the molality (mol kg^{-1}) of ion k. At different temperatures, A can be expressed as:

$$A = 1.8252\times10^6\left(\frac{\rho_w}{\epsilon^3 T^3}\right) \qquad [5]$$

where: ρ_w = the density of water (g cm^{-3}), ϵ = the dielectric constant of water and T = the temperature in Kelvin. The latter is expressed as a function of temperature as follows:

$$\epsilon = \frac{5321}{T} + 233.76 - 0.929T + 1.417\times10^{-3}T^2 - 8.292\times10^{-7}T^3 \qquad [6]$$

c. Fitmod input file for modelling metal adsorption onto SRB cell surfaces

```
      1
      0
      1
      1
      1
     90
      6       1       1       0
00100  0.0    2.36E-3   H1
00200  0.0    2.12E-3   H2
00300  0.0    1.10E-3   H3
00400  0.0    3.58E-3   H4
00001  0.0    0.00E00   Sr2
00002  0.0    0.00E00   NO3-
00020  0.0    0.00E00   Srads
00050  0.0    0.00E00   H

00002  0.0    002 01
00050  0.0    050 01
00051  -13.91 050 -1
00100  0.0    100 01
00101  -4.32  100 01 050 -1
00200  0.0    200 01
00201  -6.38  200 01 050 -1
```

```
00300  0.0    300 01
00301  -8.05  300 01 050 -1
00400  0.0    400 01
00401  -10.18 400 01 050 -1
01000  0.0    001 01
01001  -13.29 001 01 050 -1
01002  -28.51 001 01 050 -2
01005  -4.47  001 02 050 -1
01022  -0.07  001 01 002 02
01111  -9.0   100 01 050 -1 001 01 020 01
01112  -9.0   200 01 050 -1 001 01 020 01
01113  -9.0   300 01 050 -1 001 01 020 01


      03        0
    1111
    1112
    1113
      12        3        1        1
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2.85E-04
2.85E-04
2.85E-04
2.85E-04
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2.85E-04
2.85E-04
2.85E-04
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2.85E-04
2.85E-04
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9.50E-05
1.24E-04
1.28E-04
1.34E-04
1.45E-04
1.57E-04
1.72E-04
1.83E-04
2.14E-04
2.30E-04
2.36E-04
2.39E-04
       2
```

```
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1.00E-01
1.00E-01
1.00E-01
1.00E-01
1.00E-01
1.00E-01
1.00E-01
1.00E-01
1.00E-01
1.00E-01
1.00E-01
      50
-4.75
-4.89
-5.02
-5.17
-5.42
-5.81
-6.02
-6.18
-6.86
-7.39
-8.21
-8.63
0.999
0.999
0.998
0.998
0.997
0.997
0.996
0.995
0.994
0.993
0.992
0.992
      00      00
```

where:

Line 1-6: Program control input

Line 7-15: Matrix A components (Group of components, where Group I = components for which only T is known, Group II = components for which both X and T are known, and Group III = components for which only X is known).

Line 16: Blank card

Line 17-35: Matrix B components (Definition of species and reactions to form species)

Line 36: Blank card
Line 37: Blank card
Line 38: Definition of parameters for optimization (number of Log Ks and Ts)
Line 39-40: ID of 1st and 2nd Log K values to be adjusted
Line 41: Serial data definition (number of data points, T serial data for 1 component, X serial data for 1 component, dilution factor serial data for 1 component).
Line 42: Total concentration of metal (ID = 1)
Line 43-54: [Metal ion] concentration serial data points
Line 55: Concentration of adsorbed metal
Line 56-67: Serial data points for adsorbed metal
Line 68: Nitrate concentration
Line 69-80: Nitrate concentration serial data points
Line 81: Log $[H^+]$
Line 82-93: Serial data for Log $[H^+]$
Line 94-105: Dilution coefficient data
Line 106: Termination (00 00)