

1 **Modelling microbial transport in simulated low-grade heap bioleaching** 2 **systems: the hydrodynamic dispersion model**

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14 **Abstract**

15 The hydrodynamic model was developed to describe microbial growth kinetics within heap
16 bioleaching systems. Microbial partitioning between the bulk flowing pregnant leach solution (PLS)
17 and ore-associated phases that exist within the low-grade chalcopyrite ore bed, as a function of
18 microbial transport between these identified phases, was investigated. Microbial transport between the
19 bulk flowing PLS and ore-associated phases was postulated to be driven by the microbial
20 concentration gradient between the phases, with advection and dispersion forces facilitating microbial
21 colonisation of, and transport through, the ore bed. The population balance model (PBM) was
22 incorporated into the hydrodynamic model to estimate mineral dissolution rates as a function of
23 available surface area appropriately. Temporal and spatial variations in microbial concentration in the
24 PLS and ore-associated phases are presented together with model predictions for overall ferrous and
25 ferric iron concentrations, which account for iron concentrations in the bulk flowing PLS and that in
26 the vicinity of the mineral surface. The model predictions for PLS and ore-associated microbial
27 concentrations are validated with experimental data, demonstrating the improvement of this model
28 over the previously presented ‘biomass model’. Based on Michaelis-Menten type kinetics, model-
29 predicted *true* maximum specific growth rates for *Acidithiobacillus ferrooxidans* in the PLS and ore-
30 associated phases were found to be 0.0004 and 0.019 h⁻¹, respectively. Estimated microbial
31 attachment and detachment rates suggest that microbial growth is more prolific in the ore-associated
32 phases with subsequent transport to the bulk flowing PLS. Sensitivity analysis of the hydrodynamic
33 transport model to changes in the advection mass transfer coefficient, dispersion coefficient and
34 inoculum size are discussed. For the current reactor configuration, increasing the irrigation rate from 2
35 to 2.5 L m⁻² h⁻¹, i.e. increasing the advection mass transfer rate, resulted in a significant decrease in
36 microbial retention within the ore bed.

37

38 **Keywords:** heap bioleaching; unsaturated packed beds; microbial colonisation; microbial transport
39 rates; microbial growth rates; microbial modelling

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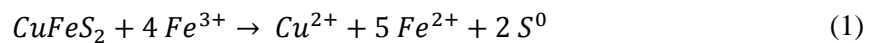
41 1. Introduction

42 Heap bioleaching is considered a feasible technology for the extraction of base metals from low-grade
43 mineral sulphide ores. Research is currently focussed on understanding the sub-processes governing
44 the dissolution of low-grade copper-bearing ores in heaps. Chalcopyrite is thought to be the most
45 abundant and refractory copper-bearing mineral resource (Wang, 2005, Watling, 2006). However,
46 lower mineral sulphide dissolution rates have been observed in commercial heaps (Chen and Wen,
47 2013, Panda et al., 2012, Watling, 2006) than previously obtained in tank bioleaching systems at
48 temperatures exceeding 50°C (Batty and Rorke, 2006) and pilot scale heaps (Dew et al., 2011), as a
49 result of poor temperature progression within the heap which limits efficacy.

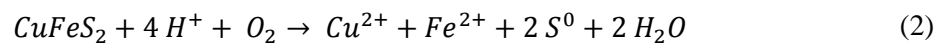
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51 In the dissolution of chalcopyrite, both ferric iron and hydronium ions react with the mineral sulphide,
52 as in Eqs. 1 and 2, respectively. Studies have shown that these reactions together with the microbial
53 oxidation of ferrous iron (Eq. 3), determine the ferric to ferrous iron ratio which, in turn, affects the
54 rate of chalcopyrite dissolution (Córdoba et al., 2008, Hiroyoshi et al., 2008). In addition, the
55 microbial oxidation of reduced sulphur species regenerates the hydronium ions (Eq. 4) responsible for
56 maintaining low pH conditions necessary for both optimum microbial oxidation and the prevention of
57 iron precipitation.

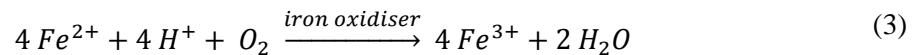
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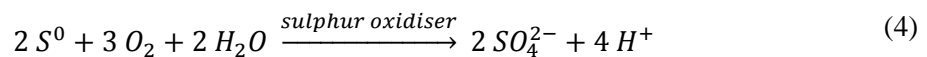
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61



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63 Although dump bioleaching has been applied successfully for the treatment of low-grade, copper-
64 bearing ores, previous studies have demonstrated the significance of variation in temperature, oxygen
65 concentration, concentration of chemical species, microbial activity and abundance across the length
66 and depth of test scale dumps (Bhappu et al., 1969, Murr, 1980, Murr and Brierley, 1978). Heap
67 bioleaching has begun to replace dump bioleaching as the more feasible technology (Chen and Wen,
68 2013, Norgate and Jahanshahi, 2010, Watling, 2006); however, the copper inventory within the heap

69 often requires months before metal recovery can occur. Decreasing the holdup of this copper
70 inventory has significant potential benefit to the industry.

71

72 Typically, commercial heap operations experience long heap start-up periods during which microbial
73 activity is low, leading to slow temperature progression within the heap and low mineral dissolution
74 rates. Energy loss from the heap during start-up may be minimised through effective management of
75 the solution irrigation rate and aeration of the heap (Dixon, 2000, Lizama, 2001, Muñoz et al., 1995).
76 The exothermic sulphide mineral dissolution reactions which generate energy within the heap are
77 controlled by the availability of chemical and microbial species, distributed within the heap by the
78 bulk flowing liquid stream as well as the degree of liberation and accessibility of the sulphide mineral.
79 Escobar et al. (1996) and van Loosdrecht et al. (1990) suggested that in a mineral leaching
80 environment, bioleaching micro-organisms may be concentrated in the phases associated with the
81 mineral.

82

83 Recent studies have demonstrated that bioleaching micro-organisms are present distributed non-
84 uniformly across the identified phases within the heap; namely, in the bulk flowing pregnant leach
85 solution (PLS), in the stagnant interstitial solution, and weakly and strongly attached to the mineral
86 surface (Chiume et al., 2012, Govender et al., 2013, Tupikina et al., 2014). These authors found that
87 the growth of *At. ferrooxidans* in the PLS did not represent that in the stagnant interstitial solution and
88 attached phases, with microbial abundance in the ore-associated phases (interstitial and attached) at
89 least two to three orders of magnitude greater than that in the PLS. In the study by Govender et al.
90 (2013), maximum specific growth rates in the PLS, interstitial and ore-associated phases were estimated
91 assuming no microbial transport between the identified phases. The authors concluded that the
92 specific growth rates in the PLS were exaggerated by the transport of micro-organisms from the ore-
93 associated phases to the bulk flowing solution and may therefore be regarded as *apparent* maximum
94 specific growth rates. The estimation of *true* maximum specific microbial growth rates in whole ore
95 leaching systems, therefore, requires accounting for microbial transport between the phases.

96

97 Previously, van Loosdrecht et al. (1990) proposed that a portion of new microbes grown in the
98 reversibly attached population may leave the vicinity of the mineral surface. The transport
99 mechanisms employed by micro-organisms to travel short distances, namely chemotaxis, Brownian
100 motion, attraction by electrostatic forces and hydrophobicity, are only effective at the micrometer
101 scale (Rossi, 1990). The conceptual understanding of microbial transport in mineral bioleaching
102 systems, as presented by van Loosdrecht et al. (1990) and Rossi (1990), was built on the work of
103 Marshall (1976), who interpreted numerous studies on porous soil systems aimed at pathogen removal
104 for the remediation of soils.

105

106 Expanding on the postulations by Marshall (1976), the advection-dispersion equation has been used to
107 model spatial and temporal variations in microbial concentration in both saturated and unsaturated,
108 porous soil systems (Tan et al., 1994, Taylor and Jaffé, 1990, Tufenkji, 2007). However, few studies
109 have modelled microbial transport in heap bioleaching systems (Leahy et al., 2004, Neuburg et al.,
110 1991, Petersen and Dixon, 2003). None of these cited studies have supported model predictions with
111 experimental validation of microbial population dynamics and growth in the ore-associated phases. In
112 addition, microbial transport from the bulk flowing solution to the solid medium was typically
113 modelled assuming either irreversible attachment, with no detachment term, or reversible attachment
114 described by equilibrium or kinetic terms. In each of the previous heap leaching models, microbial
115 transport was assumed to be governed by an advection-dispersion equation with microbial growth,
116 attachment, detachment and death rates described by a single empirical source/sink term. Although
117 these studies have acknowledged the microbial transport phenomena present in heaps, none have
118 decoupled the effect of transport on *true* microbial growth rates in the identified phases. Additionally,
119 the dynamic nature of the microbial community has not been described.

120

121 Recently, Soto-Rojo et al. (2013) presented a model to simulate microbial abundance and activity
122 within sequential lifts in a commercial heap as a function of physico-chemical conditions. The
123 proposed model was aimed at predicting the spatial variation in microbial population dynamics
124 between adjacent lifts, based on correlations obtained from pattern recognition in large data sets.
125 However, the authors obtained poor correlations (R^2 values between 0.53 and 0.71) in predicted
126 microbial abundance as model validation was based on the assumption that microbial abundance,
127 growth and activity in the PLS was representative of that within all phases in the heap.

128

129 This study builds on the findings of the biomass transport model presented previously in Govender et
130 al. (2014). The model was developed to validate the hypothesis that the driving force for microbial
131 transport between the PLS and ore-associated phases was largely due to the microbial concentration
132 gradients across the associated boundaries. Although the biomass model successfully predicted the
133 temporal variation in microbial abundance in the PLS and ore-associated phases, the microbial
134 transport rates between the phases were poorly estimated.

135

136 The current paper presents the development and validation of an improved mathematical model to
137 predict the microbial population dynamics within a simulated heap bioleaching environment. The
138 model is based on the advection-dispersion equation and describes the growth of *At. ferrooxidans* in
139 the PLS and ore-associated phases as a function of the physical parameters of the solution, ferric and
140 ferrous iron transport through the ore bed and microbial attachment and detachment. It builds on the
141 ‘biomass’ model reported previously (Govender et al. 2014) to include microbial transport between
142 the PLS and ore-associated phases as a function of the dispersive and advective properties of the bulk

143 solution. This microbial transport was assumed to be governed by the microbial concentration
144 gradient between these phases and the availability of ferrous iron at the ore surface, as described by
145 the population balance model.

146
147
148

149 **2. Methodology**

150 The experimental system used in this study consisted of multiple, agglomerate-scale mini-column
151 reactors (Govender et al., 2015b). Each mini-column reactor was loaded with *ca.* 150 g of
152 agglomerated ore sample comprised of 4 wt. % pyrite, 0.5 wt. % chalcopyrite, 0.3 wt. % covellite, 0.2
153 wt. % chalcocite and 0.1 wt. % each of bornite and enargite, together with quartz (44.8 wt.%) and
154 muscovite (28.6 wt.%) as the major gangue minerals . Each ore sample was re-constructed with
155 identical particle size distribution and mass from an acid pre-leached bulk ore sample with 93%
156 passing 16 mm and 12% passing 0.25 mm; the detailed particle size distribution is given by Govender
157 et al. (2015b). A single experiment consisted of multiple mini-column reactors, irrigated from a single
158 feed source and inoculated from the same sample of *At. ferrooxidans* (DSM 14882) culture. Each
159 mini-column reactor, therefore, represented a grab sample of a larger heap. The *At. ferrooxidans*
160 culture was grown on sterile autotrophic basal salts (ABS) medium containing trace elements
161 (Johnson et al., 2008), 0.5 g L⁻¹ Fe²⁺ (FeSO₄.7H₂O) and 1% (w/v) γ -irradiated pyrite concentrate (<
162 75 μ m).

163

164 Two sets of tests (A and B), each consisting of one abiotic control and seven biotic mini-column
165 reactors, were inoculated with approximately 10¹⁰ cells per ton dry ore and run under identical
166 conditions. Sterile feed solution containing 0.2 g L⁻¹ Fe³⁺ (Fe₂(SO₄)₃.xH₂O), ABS and trace elements
167 at pH 1.7 (96% H₂SO₄) was irrigated over the columns at a rate of 10 mL h⁻¹, equivalent to 2 L m⁻² h⁻¹.
168 Solution analysis included measurement of pH, redox potential, ferrous and total iron in solution, the
169 latter two using the modified ferric chloride assay described by Govender et al. (2012). Copper in
170 solution was measured by AAS. Details of sampling and analyses are given in Govender et al.
171 (2015b). At specified time intervals, a column from each test was sacrificed and the ore sample
172 analysed for microbial abundance. A column from test A was sacrificed every 72 h, whilst shorter
173 time intervals of 48 h between column sampling was chosen for test B.

174

175 For sampling of the ore-associated microbial population, a representative sample of the packed ore
176 bed from the sacrificed mini-column reactor was processed using a standardised detachment protocol
177 (Govender et al., 2013). This method is assumed to result in the extraction of a majority of the ore-
178 associated microbial community. The microbial concentrations in the effluent solution (PLS) and
179 associated with the ore were determined at regular intervals using the Miles-Misra serial dilution

180 plating protocol (Miles et al., 1938) for the enumeration of viable colony forming units (cfu) on iron
181 overlay plates containing approximately $1.5 \text{ g L}^{-1} \text{ Fe}^{2+}$ ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) (Johnson, 1995). Variance in
182 quantification of viable cell numbers using the Miles-Misra plating technique was determined by
183 analysing the PLS from multiple columns in an experiment at a single time point and by repeating
184 analysis of cells recovered from a single detached sample. The relative error associated with cell
185 enumeration in columns in the same test was found to be less than 13% (data not shown). A residence
186 time distribution (RTD) study was used to determine the mean residence time, τ , to be 1.45 h. This
187 study, together with compartment models, allowed for an estimation of the volumes of bulk flowing
188 and stagnant solution within the packed bed reactor to be *ca.* 11.7 and 2.3 mL, respectively (Govender
189 et al., 2015b).

190

191 The empirical data from tests A and B were presented and discussed previously in Govender et al.
192 (2013) and re-introduced for validation of the biomass transport model (Govender et al., 2014) and
193 the current model.

194

195 **3. Development of the hydrodynamic dispersion model**

196 At the particle scale, the interstitial phase includes the stagnant liquid boundary between the solid
197 mineral and the bulk flowing solution or PLS. Within this stagnant liquid boundary, the onset of
198 microbial attachment induces the production of extracellular polymeric substances (EPS) which
199 facilitate firm or strong attachment (van Loosdrecht et al., 1990). In a well-functioning heap, aside
200 from the presence of the chemical constituents of EPS, i.e. sugars, lipids and free fatty acids, the
201 concentration of ferric iron in this stagnant solution is proposed to be much greater than that in the
202 PLS (Gehrke et al., 1998, Kinzler et al., 2003, Sand and Gehrke, 2006, Tributsch, 2001). As an
203 experiment progresses, the higher microbial abundance and activity at the mineral surface is expected
204 to result in the rapid turnover of ferrous to ferric iron in this phase, depending on the extent of mineral
205 liberation.

206

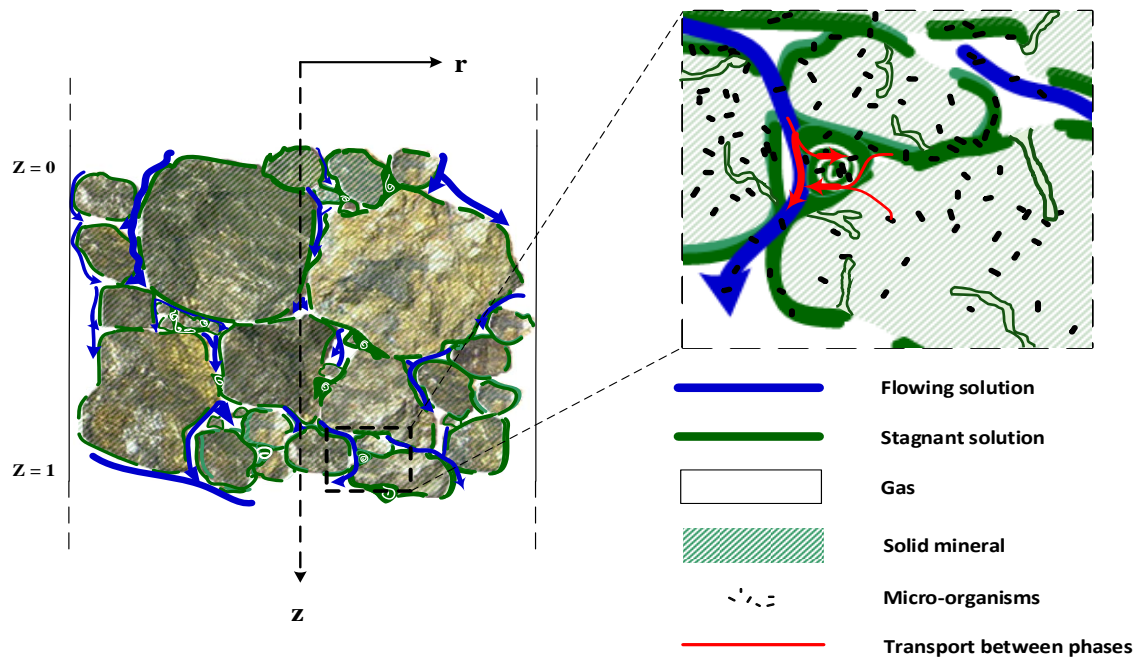
207 The hydrodynamic dispersion model presented in this study was developed to simulate the effect of
208 solution flow dynamics on the growth and transport of an *At. ferrooxidans* culture within a heap
209 environment. This model aims to describe the transport of chemical and microbial species from the
210 mineral surface across the largely stagnant interstitial phase surrounding the ore particles to the bulk
211 flowing PLS, as a function of dispersion and advection transport phenomena. Although dispersion
212 occurs in all spatial directions, conceptually the model is based on both radial (r-direction) and axial
213 (z-direction) diffusion of solution within the agglomerate-scale column reactor (Figure 1). This was
214 further simplified to account for dispersion in the axial direction only and dispersion in the radial
215 direction was ignored for two reasons: the uniform distribution of leach solution over the top of the

216 ore bed in the form of a fine mist, and the small height of the ore bed relative to the bed diameter, as
 217 described in Govender et al. (2015b).

218

219 In this study, the agglomeration of the ore sample and the presence of fine particles was expected to
 220 promote the formation of substantial solution hold-up within the ore bed (Bouffard, 2005, Bouffard
 221 and West-Sells, 2009, McFarlane et al., 2011). The solution hold-up or stagnant interstitial phase
 222 within the heap is constituted of a boundary layer of liquid surrounding individual particles as well as
 223 the stagnant solution held up in the void space between individual particles in a cluster forming the
 224 agglomerate, as illustrated in Figure 1.

225



226

227 **Figure 1: Illustration of the phases that exist within a cluster of particles at the agglomerate scale,**
 228 **describing the interaction between the bulk flowing solution, stagnant interstitial phase, solid mineral**
 229 **surface and gas phases, with radial dispersion in the r-direction and axial dispersion in the z-direction**
 230 **(Govender-Opitz et al., 2016).**

231

232 In developing the model for microbial transport within a heap, the measured ferric and ferrous iron
 233 concentrations in the PLS were proposed to be a poor representation of that in the vicinity of the
 234 mineral surface. This assumption was based on the theories postulated by Tributsch (2001) and Sand
 235 and Gehrke (2006) whereby the rapid turnover from ferrous to ferric iron near the mineral surface and
 236 in the EPS was not described adequately by that measured in the PLS. Thus far, rigorous experimental
 237 data supporting this theory has not been reported. Therefore, the overall ferric and ferrous iron
 238 balances include the iron concentrations measured in the PLS, $C_{i,PLS}$, and that estimated in the ore-
 239 associated phases, $C_{i,ore}$; each normalised with respect to the estimated volumes in each phase (Eq. 5).

240

$$C_{i,total} \cdot V^R = C_{i,PLS} \cdot V_{PLS} + C_{i,ore} \cdot V_{ore} \quad (5)$$

241

242 where C_i is the concentration of the soluble chemical species, either ferric iron, $C_{Fe^{3+}}$, or ferrous iron,
 243 $C_{Fe^{2+}}$, and V^R , V_{PLS} and V_{ore} are the volumes of total solution in the reactor, bulk flowing PLS and
 244 stagnant solutions, respectively. The volumes of PLS and stagnant solution within a mini-column
 245 reactor were estimated to be 11.7 and 2.3 mL, respectively (Govender et al., 2015b). These volumes
 246 were analysed using compartment model theory from residence time distribution (RTD) curves. The
 247 model predictions of overall ferric and ferrous iron concentrations may then be used to infer the ferric
 248 and ferrous iron concentrations at the mineral surface, which is expected to be significantly different
 249 from that in the bulk flowing solution.

250

251 Overall temporal and spatial changes in the ferrous and ferric iron concentrations were predicted
 252 using an advection-dispersion equation for solute flow through the heap (Eqs. 6 and 7). The model
 253 describes the rate of iron turnover within the ore bed as a function of the rate of mineral dissolution
 254 (r^R) and microbial substrate utilisation rate (r_{bio}). Solution-ore contacting results in solute transport out
 255 of the ore bed in the bulk flowing PLS *via* axial dispersion and advection mass transfer. The
 256 dispersion coefficient constant, D_z , was estimated from the RTD curves to be $4.24 \times 10^{-5} \text{ m}^2 \text{ h}^{-1}$, using
 257 the closed-closed boundary condition assumption. The advection mass transfer coefficient, v , was
 258 assumed to remain constant within the ore bed with time, and was determined from the solution
 259 irrigation rate of 10 mL h^{-1} , to be $2.46 \times 10^{-3} \text{ m h}^{-1}$. Dispersion in the radial direction was assumed to
 260 be negligible due to the small volume of the ore bed ($\phi = 80 \text{ mm}$, $h = 25 \text{ mm}$) in combination with the
 261 uniform distribution of feed solution over the top of the ore bed.

262

$$\frac{\partial C_{Fe^{3+}}}{\partial t} = D_z \cdot \frac{\partial^2 C_{Fe^{3+}}}{\partial z^2} - v \cdot \frac{\partial C_{Fe^{3+}}}{\partial z} - 4 \cdot r^R + 4 \cdot r_{bio} \quad (6)$$

263

$$\frac{\partial C_{Fe^{2+}}}{\partial t} = D_z \cdot \frac{\partial^2 C_{Fe^{2+}}}{\partial z^2} - v \cdot \frac{\partial C_{Fe^{2+}}}{\partial z} + 5 \cdot r^R - 4 \cdot r_{bio} \quad (7)$$

264

265 Stoichiometric coefficients presented in Eqs. 6 and 7 describe the mineral dissolution by ferric iron
 266 and microbial ferrous iron oxidation reactions as presented in Eqs. 1 and 3 respectively. Over the
 267 period of these experiments, whilst ferrous iron was available in solution for microbial ferrous iron
 268 oxidation, the concomitant reactions, i.e. mineral dissolution by acid attack (Eq. 2) and the oxidation
 269 of reduced sulphur species by *At. ferrooxidans* (Eq. 4), were assumed to be much slower than the
 270 aforementioned reactions (Moinier et al., 2013) and therefore, did not contribute to the corresponding
 271 rates of reaction. Initial ferric and ferrous iron concentrations in the feed solution, at $t = 0 \text{ h}$ and $z = 0$

272 m, were sourced from empirical measurements to be 3 mmol Fe³⁺ L⁻¹ and 0 mmol Fe²⁺ L⁻¹, while the
273 initial spatial boundary conditions at the bottom of the ore bed were assumed to be negligible.

274

275 The application of the advection-dispersion equation to describe the transport of chemical species in
276 unsaturated packed beds simulating heap leaching systems was most recently presented by Ilankoon
277 et al. (2013). In this study, positron emission particle tracking (PEPT) was used to quantify the
278 hydrodynamic dispersion coefficient in the axial (D_z) and radial direction (D_r). Although, the
279 radioactive tracer particles used in the aforementioned study were 400 μm in diameter, which did not
280 represent the flow of dissolved chemical species or micro-organisms in the packed bed, the authors
281 found that the estimation of the D_z using PEPT compared well with that achieved using a salt tracer.
282 No quantitative comparison of dispersion coefficients may be made since empirical estimations are
283 dependent on reactor configuration and irrigation rate, which differ between this study and that by
284 Ilankoon et al. (2013).

285

286 . For this study, the intrinsic rate of mineral dissolution was estimated from the ratio of total ferric to
287 total ferrous iron concentration (Hansford and Vargas, 2001) using the rate expression provided in Eq.
288 8, where k_m is the rate constant [$\text{mol m}^{-2} \text{h}^{-1}$] and n is the reaction order of 0.5, as proposed by
289 Williamson and Rimstidt (1994).

290

$$r''_{\text{mineral}} = k_m \cdot \left(\frac{C_{\text{Fe}^{3+}}}{C_{\text{Fe}^{2+}}} \right)^n \quad (8)$$

291

292 However, this approach assumes that all particles in the reactor are of the same size, with equal
293 reaction surface area in contact with the reaction environment over equal exposure times and
294 therefore, contribute equally to the overall mineral dissolution rate. In a system with non-uniform
295 particle size distribution, this assumption results in an over- or under-estimation of the overall mineral
296 leach rate (Kotsiopoulos et al., 2008, Kotsiopoulos et al., 2012). Therefore, the population balance
297 model (PBM) was applied to the current model to better approximate the overall mineral leach rate, r^R
298 [$\text{mol m}^{-3} \text{h}^{-1}$], using Eq. 9.

299

300 Solution flow dynamics within the mini-column reactors were characterised using RTD studies and
301 demonstrated relatively well-mixed, continuous reactor behaviour (Govender et al., 2015b).
302 Therefore, in the application of the PBM to describe the oxidation of low-grade mineral ores in a
303 heap, a segregation approach was adopted for the modification of the PBM to include both initial
304 particle size distribution, $f_0(l_0)$, and the change in particle reaction surface area with age, $\theta = t_0 - t$
305 (Kotsiopoulos et al., 2008). The rate contributions for each particle size fraction over a known age
306 range were summed up for the estimation of r^R .

307

$$r^R = \int_0^\infty \int_0^\infty r''_{\text{mineral}} A^p(\theta, l_0) \frac{M^p(\theta, l_0)}{V^R} \phi_{MS} N^T f_0(l_0) I(\theta) d\theta dl_0 \quad (9)$$

308

309 In Eq. 9, A^p [$\text{m}^2 \text{kg}^{-1}$] is the specific particle surface area, l_0 [m] the initial particle size, M^p [kg] the
 310 particle mass, V^R [m^3] the working volume of the reactor, ϕ_{MS} the fraction of chalcopyrite in the
 311 particles, $I(\theta)$ the internal RTD and N^T is an estimation of the total number of particles in the reactor.

312

313 In a well-functioning heap, microbial ferrous iron oxidation kinetics are assumed to contribute
 314 significantly to the changing ferrous and ferric iron concentrations in the heap. The overall microbial
 315 ferrous iron oxidation rate, r_{bio} [$\text{mol Fe}^{2+} \text{m}^{-3} \text{h}^{-1}$], was determined from the ratio of total ferric to total
 316 ferrous iron concentration estimated using Eqs. 6 and 7. This approach was used as it has been
 317 recognised that the ferric and ferrous iron concentrations in the PLS is not representative of that at the
 318 ore surface and experimental data are not available. The specific microbial ferrous iron oxidation rate,
 319 $q_{\text{Fe}^{2+}}$ [$\text{mol Fe}^{2+} \text{mol C}^{-1} \text{h}^{-1}$], was determined as a function of the total microbial population within all
 320 phases in the heap, $C_{x,\text{total}}$ [mol C m^{-3}]. The Michaelis-Menten type rate expression (Eq. 10) as
 321 proposed by Hansford (1997) was used to describe the specific microbial ferrous iron oxidation rate,
 322 $q_{\text{Fe}^{2+}}$, as a function of the ferric to ferrous iron ratio.

323

$$q_{\text{Fe}^{2+}} = \frac{-r_{\text{bio}}}{C_{x,\text{total}}} = \frac{q_{\text{Fe}^{2+}}^{\text{max}}}{1 + K_s \cdot \left(\frac{C_{\text{Fe}^{3+}}}{C_{\text{Fe}^{2+}}}\right)} \quad (10)$$

324

325 where $q_{\text{Fe}^{2+}}^{\text{max}}$ [$\text{mol Fe}^{2+} \text{mol C}^{-1} \text{h}^{-1}$] is the maximum specific microbial ferrous iron utilisation rate and
 326 K_s is the dimensionless form of the Michaelis-Menten rate constant. Although *At. ferrooxidans* is able
 327 to oxidise both ferrous iron and reduced sulphur species, ferrous iron was assumed to be the preferred
 328 substrate during the exponential phase of microbial growth, as recently demonstrated by Moinier et al.
 329 (2013). For the purposes of the current study, the assumption that ferrous iron was the preferred
 330 substrate was validated using copper extraction data from the base case experiments (Govender et al.,
 331 2014).

332

333 Specific microbial growth rates were assumed to be governed by ferrous iron availability, hence, the
 334 specific microbial ferrous iron oxidation rate, with the yield coefficient, Y_{sx} , serving as the
 335 proportionality constant. This was based on the assumption of negligible microbial culture
 336 maintenance requirements. Specific microbial growth rates were determined from calibrated
 337 parameters for yield coefficients and microbial ferrous iron oxidation rates, using Eq. 11.

338

$$\mu_{x,i} = Y_{sx,i} \cdot \frac{r_{bio}}{C_{x,i}} = Y_{sx,i} \cdot q_{Fe^{2+}} \quad (11)$$

339

340 where i represents the different locations for microbial growth, i.e. total, PLS and ore-associated
 341 phases. The yield coefficient for substrate utilisation by the total microbial population within the
 342 mini-column reactor, $Y_{sx,total}$, was determined empirically from base case experimental data to be 0.05
 343 mol $C_{x,total}$ (mol Fe^{2+})⁻¹. The yield coefficients for substrate utilisation by the microbial population in
 344 the PLS, $Y_{sx,PLS}$ [mol $C_{x,PLS}$ (mol Fe^{2+})⁻¹], and ore-associated phases, $Y_{sx,ore}$ [mol $C_{x,ore}$ (mol Fe^{2+})⁻¹],
 345 were estimated from the model fit and used to calculate maximum specific microbial growth rates,
 346 $\mu_{x,i}^{max}$ [h⁻¹], in each phase.

347

348 The model predictions for iron speciation in the ore-associated phases also inform the extent of
 349 microbial substrate utilisation and the magnitude of the yield coefficients in the different phases. In
 350 the absence of this supporting data, the model was developed using the assumption that the microbial
 351 substrate utilisation rate, r_{bio} , was representative of that within all phases in the heap, with separate
 352 yield coefficients used to distinguish the subsequent effect on microbial abundance in the bulk
 353 flowing PLS and ore-associated phases.

354

355 Advection-dispersion phenomena that incorporate microbial growth was applied to predict both
 356 temporal and spatial changes in microbial concentration within all boundaries within the agglomerate-
 357 scale heap, as in Eq. 12.

358

$$\frac{\partial C_{x,total}}{\partial t} = \mu_{x,total} \cdot C_{x,total} + D_z \cdot \frac{\partial^2 C_{x,total}}{\partial z^2} - v \cdot \frac{\partial C_{x,total}}{\partial z} \quad (12)$$

359

360 where $C_{x,total}$ [mol C L⁻¹] is the microbial concentration in the total reactor volume (V^R) and $\mu_{x,total}$ [h⁻¹]
 361 is the specific microbial growth rate of the total population within the mini-column reactor.

362

363 Building on the biomass transport model presented previously by Govender et al. (2014), the
 364 hydrodynamic model was developed assuming that the microbial concentration gradient between the
 365 phases is the driving force for microbial transport across the phase boundary. In the proposed
 366 mathematical model, changes to the microbial abundance in the bulk flowing PLS, $C_{x,PLS}$ [mol C L⁻¹],
 367 were described as a function of microbial growth in the PLS, microbial transport through the ore bed
 368 facilitated by bulk solution flow dynamics as well as microbial transport across the interface between
 369 the bulk flowing PLS and ore-associated phases (Eq. 13).

370

$$\frac{\partial C_{x,PLS}}{\partial t} = \mu_{x,PLS} \cdot C_{x,PLS} + D_Z \cdot \frac{\partial^2 C_{x,PLS}}{\partial Z^2} - v \cdot \frac{\partial C_{x,PLS}}{\partial Z} - k_{att} \cdot (C_{x,PLS} - C_{x,ore})$$

$$+ k_{det} \cdot (C_{x,ore} - C_{x,PLS})$$
(13)

371 Microbial transport from the bulk flowing PLS to the ore-associated phases is described using the
 372 attachment term, k_{att} [h^{-1}], whilst microbial transport from the ore-associated phases to the bulk
 373 flowing PLS is described using the detachment term, k_{det} [h^{-1}]. Eq. 13 may be simplified
 374 mathematically by replacing the k_{det} and k_{att} terms with a net rate of transport term, k_{net} , and a single
 375 concentration difference term. However, individual microbial transport terms may provide insight into
 376 the difference in magnitude of microbial transport rates between the phases.

377

378 Further changes in microbial abundance in the ore-associated phases, $C_{x,ore}$ [mol C L^{-1}], are similarly
 379 modelled as a function of microbial growth and microbial transport across the stagnant solution
 380 associated with the mineral and void space, as shown in Eq. 14. Once again, the source terms relating
 381 to microbial attachment and detachment can be reduced to a net rate of microbial transport, k_{net} , across
 382 the concentration gradient. For demonstration purposes, these terms are decoupled here in an effort to
 383 describe microbial transport across the phase boundaries.

384

$$\frac{\partial C_{x,ore}}{\partial t} = \mu_{x,ore} \cdot C_{x,ore} + k_{att} \cdot (C_{x,PLS} - C_{x,ore}) - k_{det} \cdot (C_{x,ore} - C_{x,PLS})$$
(14)

385

386 Although microbial transport along the height of the ore bed and out of the mini-column reactor was
 387 assumed to be induced by both the convective forces of the bulk flowing solution and the difference
 388 in microbial concentration between the phases, microbial transport to and from the ore-associated
 389 phases was assumed to be a result of a microbial concentration gradient only. It is proposed that the
 390 microbial concentration gradient was produced in response to the substrate concentration gradient;
 391 assuming a higher microbial production and simultaneous oxidation of ferrous iron in the ore-
 392 associated phases as compared to the bulk flowing PLS. *At. ferrooxidans* transport is facilitated *via*
 393 pili with “twitching motility” (Li et al., 2010) which may be triggered by cell to cell communication
 394 or quorum sensing in response towards a preferred environment with higher substrate availability,
 395 growth and maintenance requirements (Farah et al., 2005, Jerez, 2008, Soulère et al., 2008,
 396 Valenzuela et al., 2007).

397

398 Microbial transport in non-reactive porous soil systems has been modelled previously using the
 399 advection-dispersion equation (Tan et al., 1994, Taylor and Jaffé, 1990). The current model extends
 400 this approach by incorporating microbial growth kinetics as a function of the kinetics of microbial

401 substrate utilisation which, in turn, is limited by the rate of mineral dissolution, together with
 402 microbial attachment and detachment kinetics. The resulting estimation of *true* maximum specific
 403 growth rate (using Eq. 11) for the total microbial population within the agglomerate-scale heap was
 404 then applied to Eq. 15. As such, the theoretical ferric to ferrous iron ratio required to sustain the
 405 predicted microbial growth and abundance in the PLS and ore-associated phases can be estimated.
 406

$$\mu_{x,total} \cdot C_{x,total} \cdot V^R = \mu_{x,PLS} \cdot C_{x,PLS} \cdot V_{PLS} + \mu_{x,ore} \cdot C_{x,ore} \cdot V_{ore} \quad (15)$$

407
 408 The estimated volume of solution in the PLS, V_{PLS} , is 11.7 mL and that within the ore-associated
 409 phase, V_{ore} , is 2.3 mL, with a total reactor volume, V^R , of 14.0 mL (Govender et al., 2015b).
 410

411 **3.1. Application of the hydrodynamic transport model**

412 **3.1.1. Microbial growth and transport rates**

413 For the purposes of comparison, simplifying assumptions were used to convert the microbial
 414 concentrations in the PLS and ore-associated phases from cfu per millilitre [cfu mL⁻¹] and cells per
 415 gram dry ore [cfu g DO⁻¹] to mol carbon per litre [mol C L⁻¹] using the known reactor working
 416 volume and total mass of dry ore within the mini-column reactor. The cell to mole carbon conversion
 417 was assumed to be 4.8×10^{-15} mol C cell⁻¹, based on a dry mass of 1.18×10^{-13} g cell⁻¹ (Moon, 1995)
 418 which compares well with the value of 1.59×10^{-13} g cell⁻¹ reported by Blight and Ralph (2008).
 419

420 The mineral leaching rate constant (k_m), maximum specific ferrous iron utilisation rate ($q_{Fe^{2+}}^{max}$),
 421 Michaelis-Menten rate constant (K_m), overall yield coefficient ($Y_{sx,total}$), initial particle size distribution
 422 ($f_0(l_0)$), dispersion coefficient (D_z) and advection mass transfer rate (v) were estimated from data set
 423 Test A. Model fitting was performed using least squares regression analysis to estimate microbial
 424 yield coefficients in the PLS and ore-associated phases ($Y_{sx,PLS}$, $Y_{sx,ore}$) as well as microbial attachment
 425 (k_{att}) and detachment (k_{det}) rates. The data set from the repeated experiment Test B, and combined Test
 426 A and B data set, were then used to validate the model. The kinetic parameters used in the model are
 427 presented in Table 1.
 428

429
430

Table 1: Summary of kinetic parameter values required for input into the hydrodynamic dispersion model as determined from base case experimental data

Kinetic parameter	Units	Empirical value
Mineral leaching rate constant, k_m	$\text{mol m}^{-2} \text{ h}^{-1}$	1×10^{-6}
Maximum specific ferrous iron utilisation rate, $q_{\text{Fe}^{2+}}^{\text{max}}$	$\text{mol Fe}^{2+} \text{ mol C}^{-1} \text{ h}^{-1}$	1.61
Michaelis-Menten rate constant, K_m	Dimensionless	0.0025
Overall yield coefficient for substrate utilisation, $Y_{\text{sx}, \text{total}}$	$\text{mol C} (\text{mol Fe}^{2+})^{-1}$	0.05
Dispersion coefficient, D_z	$\text{m}^2 \text{ h}^{-1}$	4.24×10^{-5}
Advection mass transfer rate, v	m h^{-1}	2.46×10^{-3}

431

Note: Particle size distribution data was presented previously in Govender et al. (2013).

432

433 The maximum specific ferrous iron utilisation rate, $q_{\text{Fe}^{2+}}^{\text{max}}$, and the dimensionless Michaelis-Menten
434 rate constant, K_m , were determined from experimental data and have been presented previously as an
435 outcome of the biomass model (Govender et al., 2014). The $q_{\text{Fe}^{2+}}^{\text{max}}$ value of $1.61 \text{ mol Fe}^{2+} \text{ mol C}^{-1} \text{ h}^{-1}$
436 presented in this study, was lower than that of $8.8 \text{ mol Fe}^{2+} \text{ mol C}^{-1} \text{ h}^{-1}$, typically found for *At.*
437 *ferrooxidans* in bioleaching systems (Boon, 1996, Breed and Hansford, 1999, Hansford, 1997). This
438 result was expected since the cited studies were performed in continuous, well-mixed systems with
439 fully suspended, milled sulphide mineral particles, whilst this study was conducted on crushed low-
440 grade ore in the absence of supplementary ferrous iron in the feed, with pyrite occlusion in the low-
441 grade ore further limiting substrate availability.

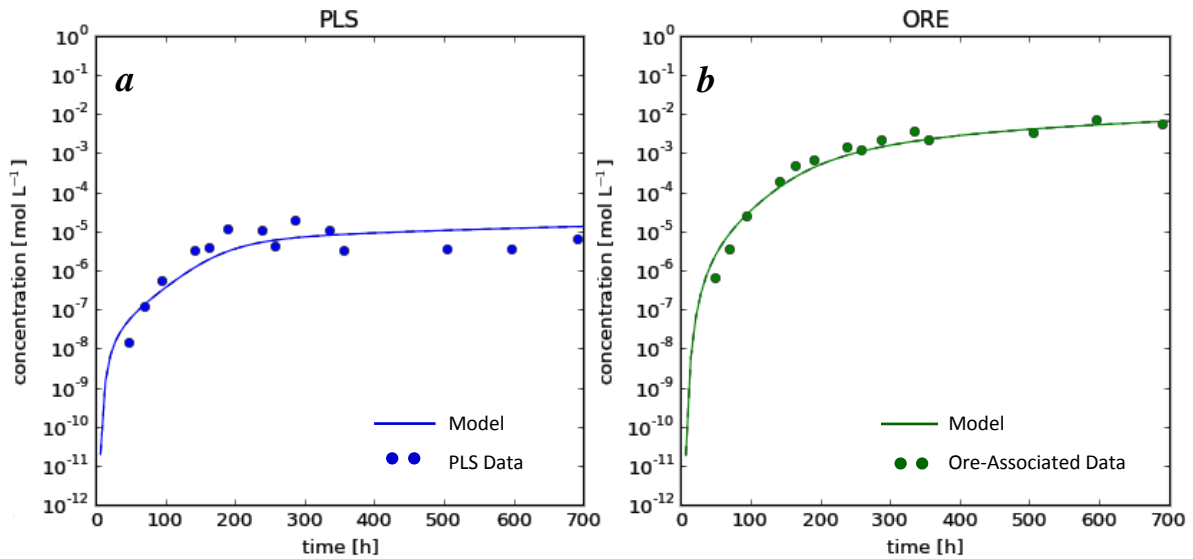
442

443 The total, PLS and ore-associated *apparent* maximum specific microbial growth rates were presented
444 previously in Govender et al. (2013). These growth rates are defined as *apparent* rates since the effect
445 of microbial transport between the phases was not taken into account in the calculation for specific
446 growth rates in each phase. This assumption resulted in exaggerated growth rates in the PLS and
447 under-estimated growth rates in the ore-associated phases. The current model aims to estimate these
448 microbial transport rates and present *true* maximum specific microbial growth rates on whole ore.

449

450 The model predictions for PLS and ore-associated microbial concentration profiles are compared to
451 those determined empirically, in Figure 2. From the onset, the model simulated the microbial
452 concentration profiles in both the PLS and ore-associated phases successfully; in particular, the
453 exponential growth region in both phases, the constant growth period observed after *ca.* 250 h in the
454 PLS (Figure 2a), and the continued but slow increase in microbial concentration in the ore-associated
455 phase (Figure 2b).

456



457
 458 **Figure 2: Model prediction of changes in microbial abundance in the (a) bulk flowing PLS and (b) ore-**
 459 **associated phases with time, using the combined base case experimental data set**
 460

461 A subset of the PLS microbial concentration data is presented in Figure 2a, with the complete data set
 462 presented previously in Govender et al. (2014) showing an initial dip in microbial abundance in the
 463 PLS, a result of initial and rapid microbial attachment. Although the model was able to predict low
 464 microbial concentrations over the first few hours of the experiment, it did not predict this period of
 465 initial and rapid attachment. This type of microbial behaviour, however, was predicted appropriately
 466 by the biomass transport model (Govender et al., 2014). The hydrodynamic dispersion model does
 467 predict the more rapid increase in the ore-associated population which may represent the initial period
 468 of attachment.

469
 470 The model outputs for yield coefficients, $Y_{sx,PLS}$ and $Y_{sx,ore}$, are 0.00027 and 0.012 mol C (mol Fe²⁺)⁻¹
 471 respectively. These yield coefficients correspond to *true* maximum specific growth rates in the PLS
 472 and ore-associated phases of 0.0004 and 0.019 h⁻¹ respectively. The apparent maximum specific
 473 growth rates as determined from empirical data without accounting for microbial transport between
 474 the phases may be compared to the *true* maximum specific growth rates as predicted by the biomass
 475 and hydrodynamic models (Table 2).

476
 477 The most notable difference in the hydrodynamic model output was the higher *true* maximum specific
 478 growth rates predicted for the ore-associated phase over the PLS (Table 2). This finding, together with
 479 the relatively low maximum specific growth rate in the PLS, suggests that microbial growth occurs
 480 primarily within the largely stagnant interstitial solution and mineral attached phases, with a
 481 proportion of the new cells being transported away from the mineral surface. The predicted *true*
 482 maximum specific growth rates in the ore-associated phase corresponded to a culture doubling time of
 483 36.5 h. The predicted ore-associated growth rate and doubling time is comparable to literature values

484 for microbial growth on pulverised low-grade Kennecott ore at similar substrate-free test conditions
 485 (Plumb et al., 2008), where the specific growth rate is estimated from further analysis of data to be
 486 0.028 h^{-1} , with a corresponding doubling time of 24.8 h.

487

488 Since microbial transport out of the ore bed through advection is incorporated into the equation
 489 describing the change in total microbial abundance within all phases in the heap (Eq. 12), the
 490 predicted total *true* maximum specific growth rate of 0.088 h^{-1} , with a doubling time of 7.9 h, was
 491 higher than that for the ore-associated phase of 0.019 h^{-1} , with a doubling time of 36.5 h. These
 492 doubling times (Table 2) are comparable to those reported previously for *At. ferrooxidans* growth on
 493 chalcopyrite mineral of 25 h (Plumb et al., 2008) and 13.9 h (McGoran et al., 1969), corresponding to
 494 specific growth rates of 0.028 and 0.050 h^{-1} respectively. The prediction for total *true* maximum
 495 specific growth rate from the current model compared more favourably to previously reported
 496 literature values, than that predicted by the biomass transport model (Govender et al. 2014).

497

498 **Table 2: Maximum specific growth rates as determined from experimental data and predicted by the**
 499 **biomass (Govender et al., 2014) and hydrodynamic microbial transport models, using Michaelis-Menten**
 500 **type rate kinetics**

		Test A & B combined	Biomass model	Hydrodynamic model
$\mu_{x,PLS}^{\max}$	h^{-1}	0.741 ± 0.011	0.736	0.0004
$\mu_{x,ore}^{\max}$	h^{-1}	0.054 ± 0.006	0.048	0.019
$\mu_{x,total}^{\max}$	h^{-1}	0.733 ± 0.013	0.739	0.088

501

502 The predicted rate of microbial transport from the PLS to the ore-associated phases *via* microbial
 503 attachment (k_{att}) was estimated to be $1.2 \times 10^{-7} \text{ h}^{-1}$, whilst the rate of microbial transport from the ore-
 504 associated phases to the bulk flowing PLS *via* detachment (k_{det}) was estimated to be $5 \times 10^{-5} \text{ h}^{-1}$. The
 505 higher rate of microbial transport from the ore-associated phases to the PLS, predicted by the
 506 hydrodynamic model, was in contrast to that previously predicted by the biomass model. The
 507 hydrodynamic model, therefore, supports the hypothesis that microbial growth is more prolific in the
 508 ore-associated phases with subsequent transport to the bulk flowing PLS.

509

510 The success of the current hydrodynamic model in predicting *true* maximum specific growth rates as
 511 a function of microbial transport may lie in the incorporation of attachment and detachment rates that
 512 are a function of the microbial concentration gradient, into the advection-dispersion model (Eqs. 13
 513 and 14). While it is recognised that attachment and detachment are also impacted by physicochemical
 514 surface properties of the microorganisms and mineral, surface topology of the mineral and fluid flow
 515 conditions, these are not varied in this study to allow the impact of concentration driving force to be
 516 determined. Current understanding suggests that in the first few hours after inoculation, the rate of

517 microbial attachment is significantly higher than the rate of microbial detachment, with $k_{att}(C_{x,PLS} -$
518 $C_{x,ore}) \gg k_{det}(C_{x,ore} - C_{x,PLS})$. Based on the attachment and detachment rates determined using the
519 model, it can be postulated that once steady state conditions are reached, the rate of microbial
520 detachment is much greater than microbial attachment, i.e. $k_{det}(C_{x,ore} - C_{x,PLS}) \gg k_{att}(C_{x,PLS} - C_{x,ore})$,
521 owing to growth at the mineral surface. The low microbial transport rates as compared to growth rates
522 may require further study for refinement; in this study, the attachment and detachment rate constants
523 (k_{att} and k_{det}) were postulated to remain unchanged despite the slopes of the concentration-time curves
524 change over the region of exponential microbial growth and constant growth (Figure 2a and b). The
525 use of a single value for each transport rate constant over the short duration of the experiments was
526 sufficient in the current study to decouple the effect of microbial transport from microbial growth.

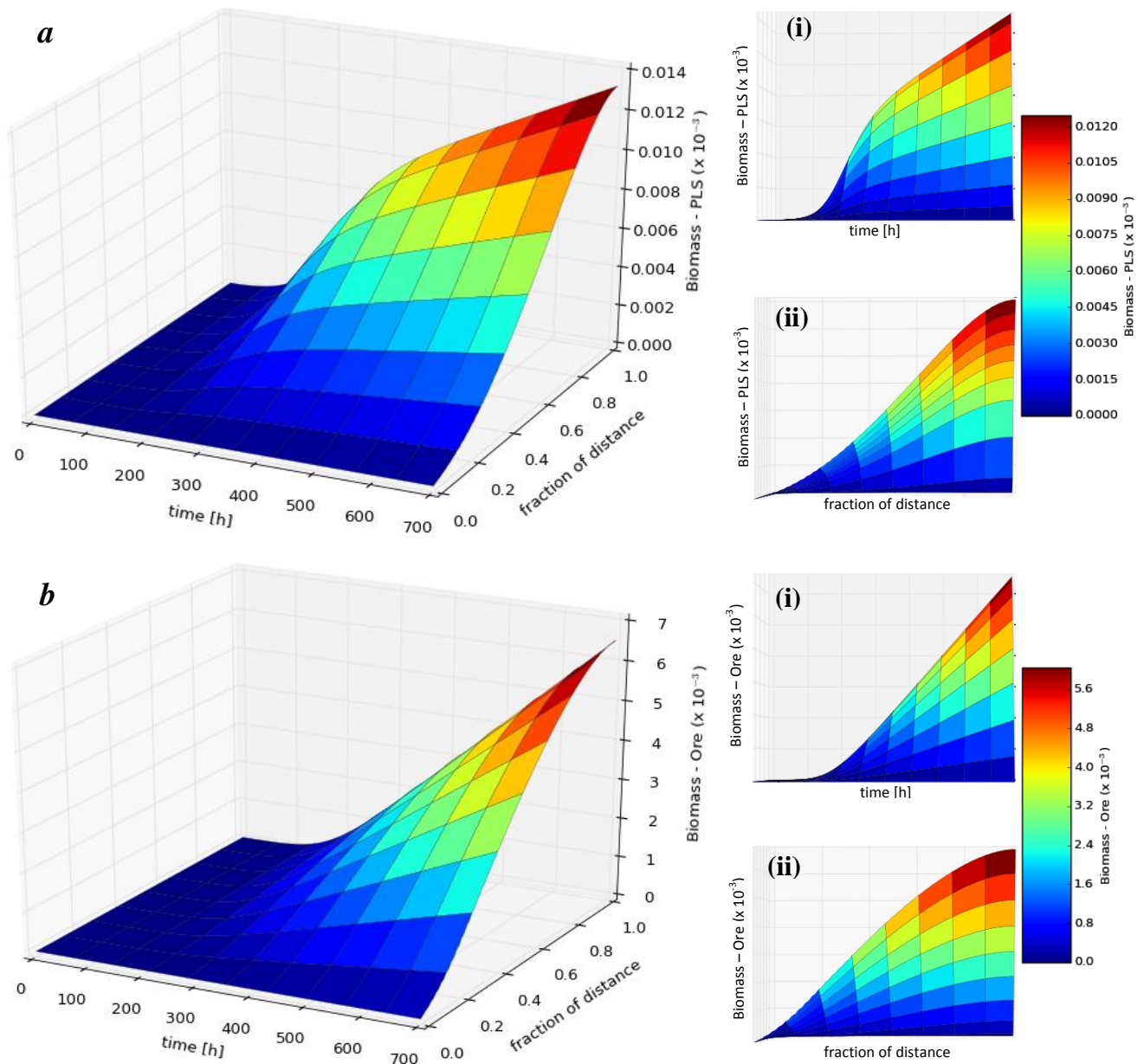
527

528 Using the hydrodynamic dispersion model, 3D plots were generated to demonstrate the change in
529 microbial concentration profiles in the PLS and ore-associated phases with time, as well as the change
530 in microbial distribution at $t = 700$ h with fractional distance from the top of the ore bed (top = 0.0).
531 The model predicted the rapid increase in PLS microbial concentration with time over the initial
532 period of *ca.* 250 h, followed by the period of slower increase (Figure 3b(i)). The microbial
533 distribution in the void PLS volume (Figure 3a (ii)) was observed to be *ca.* 10 times lower at the top
534 of the column than at the bottom, with the microbial abundance in the PLS increasing linearly with
535 the depth of the ore bed. This observation, however, may be subject to the hydrodynamic properties of
536 the bulk flowing solution which is largely influenced by both the height to diameter ratio of the ore
537 bed (Kappes, 2002) as well as flow rate of the irrigation solution (Pantelis and Ritchie, 1991, Pantelis
538 and Ritchie, 1992).

539

540 Microbial abundance in the ore-associated phases, shown in Figure 3b, was found to increase non-
541 linearly from the top to the bottom of the ore bed (Figure 3b(ii)), with a significantly higher microbial
542 abundance associated with the ore than that observed in the PLS. A high microbial concentration was
543 observed at the bottom of the ore bed by the end of the experiment. The observations suggest that for
544 this particular reactor configuration, the microbial population associated with the ore bed is not
545 uniformly distributed throughout the bed. This is consistent with the transfer of micro-organisms
546 through the bed, associated with fluid flow under gravity.

547



548 **Figure 3: 3D Plot illustrating changes to microbial concentration in the (a) bulk flowing PLS and (b) ore-**
 549 **associated phases with time and fraction of distance along the ore bed (top = 0.0) at the termination of the**
 550 **experiment. Inserts (i) highlights the change in biomass concentration with time and inserts (ii) highlights**
 551 **the change in biomass concentration with the depth of the bed.**

552

553 3.1.2. Ferric to ferrous iron concentrations within the ore bed

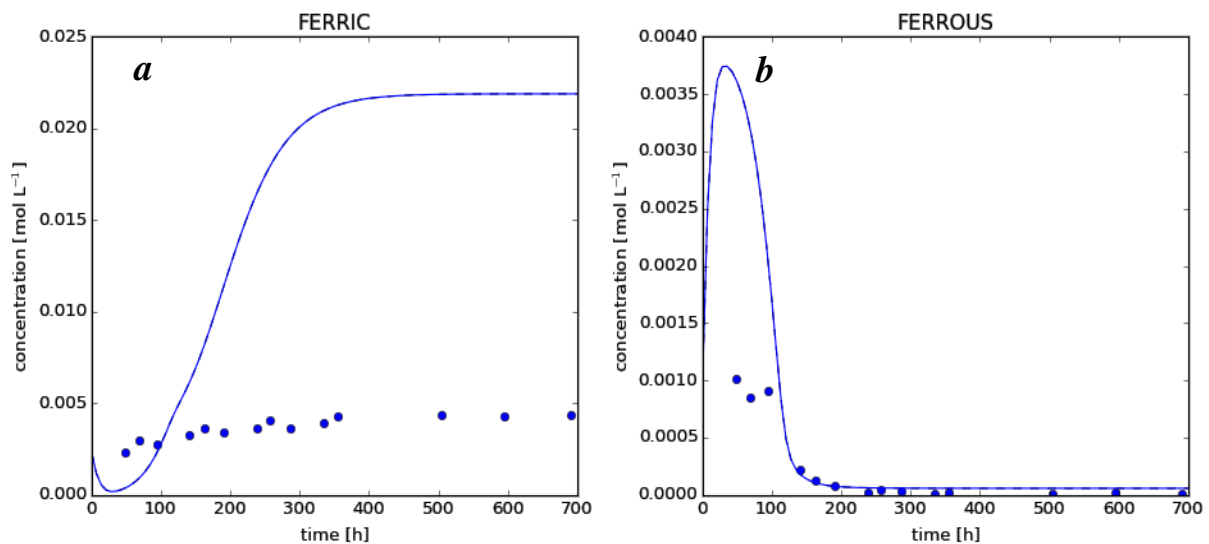
554 In an effort to decouple the concentrations of ferric and ferrous iron in the respective phases, model
 555 predictions of overall iron concentrations relative to those measured in the PLS, were compared
 556 (Figure 4). Using the combined data set, the ferric and ferrous iron concentrations measured in the
 557 bulk flowing PLS were used to estimate the mineral leach and microbial substrate utilisation kinetics
 558 for input into the model to describe the total microbial growth kinetics. The resulting model-predicted
 559 substrate utilisation kinetics in the PLS and ore-associated phases were then used to determine the
 560 ferrous and ferric iron concentrations required to sustain the combined PLS and ore-associated

561 microbial populations. The difference between the model prediction and the measured concentrations
562 was assumed to represent that in the ore-associated phases (Eq. 5).

563

564 The model prediction of higher overall ferric and ferrous iron concentrations than that measured in the
565 PLS supports the assumption of higher iron concentrations in the ore-associated phases. The predicted
566 overall iron concentrations follow similar trends to that shown by the measured data set. For example,
567 the model describes the expected initial production of ferrous iron (Figure 4b) as a result of
568 instantaneous oxidation of the mineral by ferric iron, depicted by the initial decrease in ferric iron
569 concentration (Figure 4a). The subsequent decrease in ferrous iron concentration in response to
570 increased microbial activity was also described by the model. In addition, the model predicts an
571 absence of residual ferrous iron concentration by *ca.* 200 h in the PLS and consequently, the ore-
572 associated phases.

573



574
575

576 **Figure 4: Model predictions for overall (a) ferric and (b) ferrous iron concentrations (—) in comparison**
577 **with ferric and ferrous iron concentrations measured in the bulk flowing PLS [•].**

578

579 3.2. Model sensitivity analysis

580 The trends presented in Figure 3 and Figure 4 are subject to the current test conditions, more
581 specifically, the dispersion and advection properties of the bulk flowing solution. The dispersion
582 coefficient, D_z , and advection mass transfer rate, v , were estimated from RTD experiments to be 4.24
583 $\times 10^{-5} \text{ m}^2 \text{ h}^{-1}$ and $2.46 \times 10^{-3} \text{ m h}^{-1}$, respectively (Table 1). A sensitivity analysis was performed to
584 determine the effect of varying these parameters on the model outcomes (Figure 5). For this analysis,
585 the dispersion coefficient was doubled to $8.48 \times 10^{-5} \text{ m}^2 \text{ h}^{-1}$ (Figure 5a) and halved to $2.12 \times 10^{-5} \text{ m}^2 \text{ h}^{-1}$
586 1 (Figure 5b). The advection mass transfer rate was varied through irrigation rates of $2 \text{ L m}^{-2} \text{ h}^{-1}$ to 2.5

587 $\text{L m}^{-2} \text{h}^{-1}$ and $1 \text{ L m}^{-2} \text{h}^{-1}$, respectively. As such, the equivalent advection mass transfer rates were 3.07
588 $\times 10^{-3} \text{ m h}^{-1}$ (Figure 5c) and $1.23 \times 10^{-3} \text{ m h}^{-1}$ (Figure 5d), respectively.

589

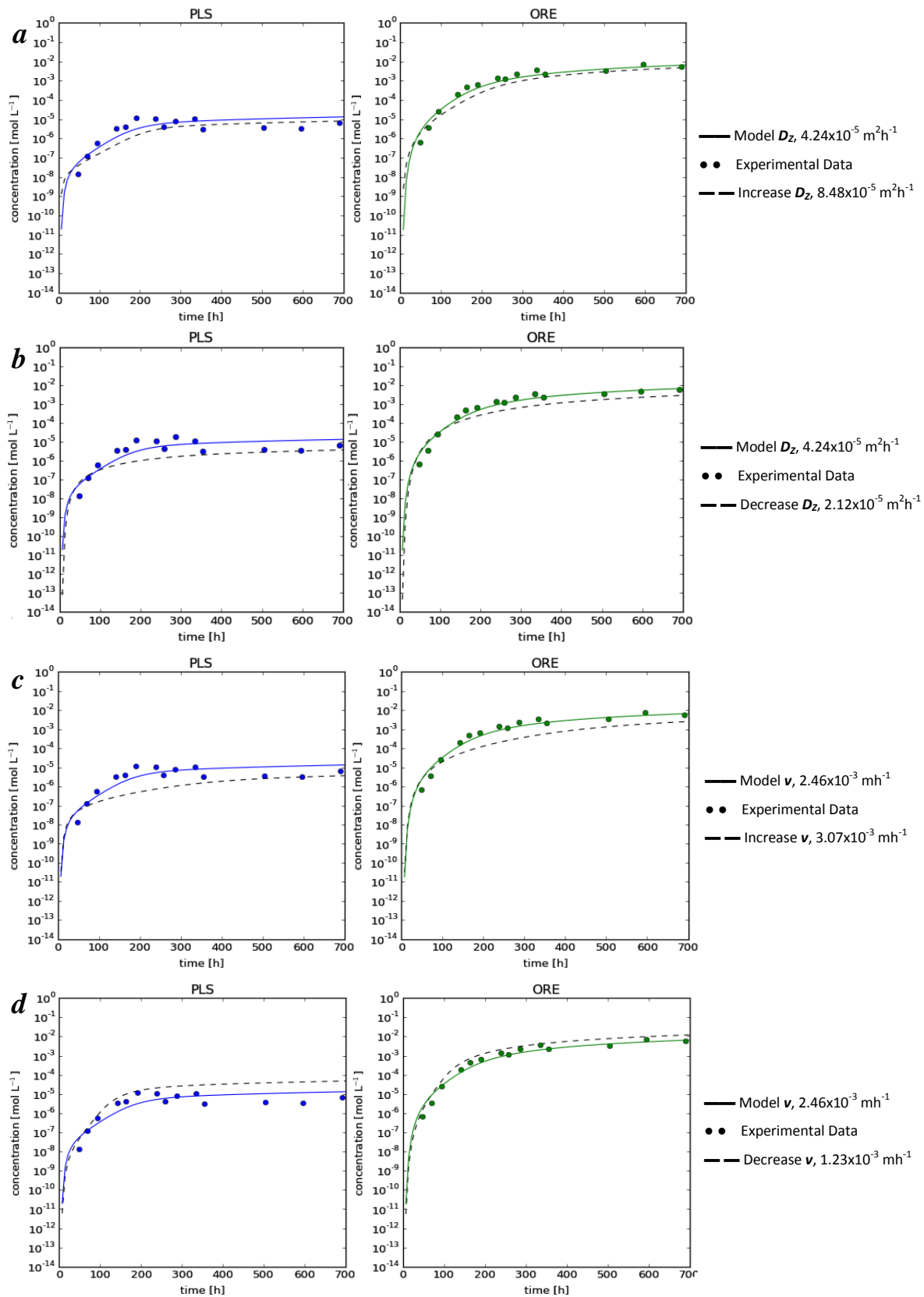
590 **3.2.1. Sensitivity of hydrodynamic model to variations in dispersion coefficient**

591 An increase in D_z resulted in an increase to the initial microbial concentrations found in the PLS and
592 ore-associated phases followed by a decrease in microbial abundance in both phases over the steady
593 state period, as illustrated in Figure 5a. Since increased dispersion increases the mixing or contacting
594 between the PLS and ore-associated phases, a larger portion of the inoculum is uniformly distributed
595 within the column initially, however, this will also facilitate increased transport of micro-organisms in
596 the bulk solution with depth of the ore bed. In addition, increased contacting would result in a higher
597 rate of attachment, k_{att} , with a larger population of micro-organisms in the PLS transporting to the ore-
598 associated phase. This would be observed by the decrease in the difference between the ore-associated
599 microbial abundance as predicted by the model and that of the model response to an increase in the
600 dispersion coefficient. Decreasing the dispersion coefficient (Figure 5b), decreased the retention of
601 inoculum in the mini-column reactor as a result of less contacting between the phases and possible
602 poor mixing or channelling of solution within the ore bed. Preferential flow within the ore bed would
603 result in greater detachment of the ore-associated population into the bulk PLS and out of the ore bed.

604

605 **3.2.2. Sensitivity of hydrodynamic model to variations in advection mass transfer coefficient**

606 The advection mass transfer coefficient (ν) was varied by increasing the feed irrigation rate from 2 L
607 $\text{m}^{-2} \text{h}^{-1}$ to $2.5 \text{ L m}^{-2} \text{h}^{-1}$ (Figure 5c) and decreasing the irrigation rate to $1 \text{ L m}^{-2} \text{h}^{-1}$ (Figure 5d). Beyond
608 these constraints, the model predictions for microbial concentration along the height of the ore bed
609 became unstable, yielding erratic concentration profiles. The variations resulted in significant changes
610 to the predicted microbial concentrations in the PLS and the ore-associated phases. A decrease in the
611 retention of inoculum within the mini-column with increasing ν was predicted, resulting in decreased
612 microbial abundance in the PLS and ore-associated phases (Figure 5c). By decreasing ν , a greater
613 population of micro-organisms was predicted to remain within the ore bed, resulting in an increase in
614 microbial abundance in the PLS and ore-associated phases (Figure 5d). The effect of varying
615 irrigation rates on inoculum retention and microbial colonisation was supported by the corresponding
616 change in the rate of ferrous iron oxidation at higher irrigations (results not shown).

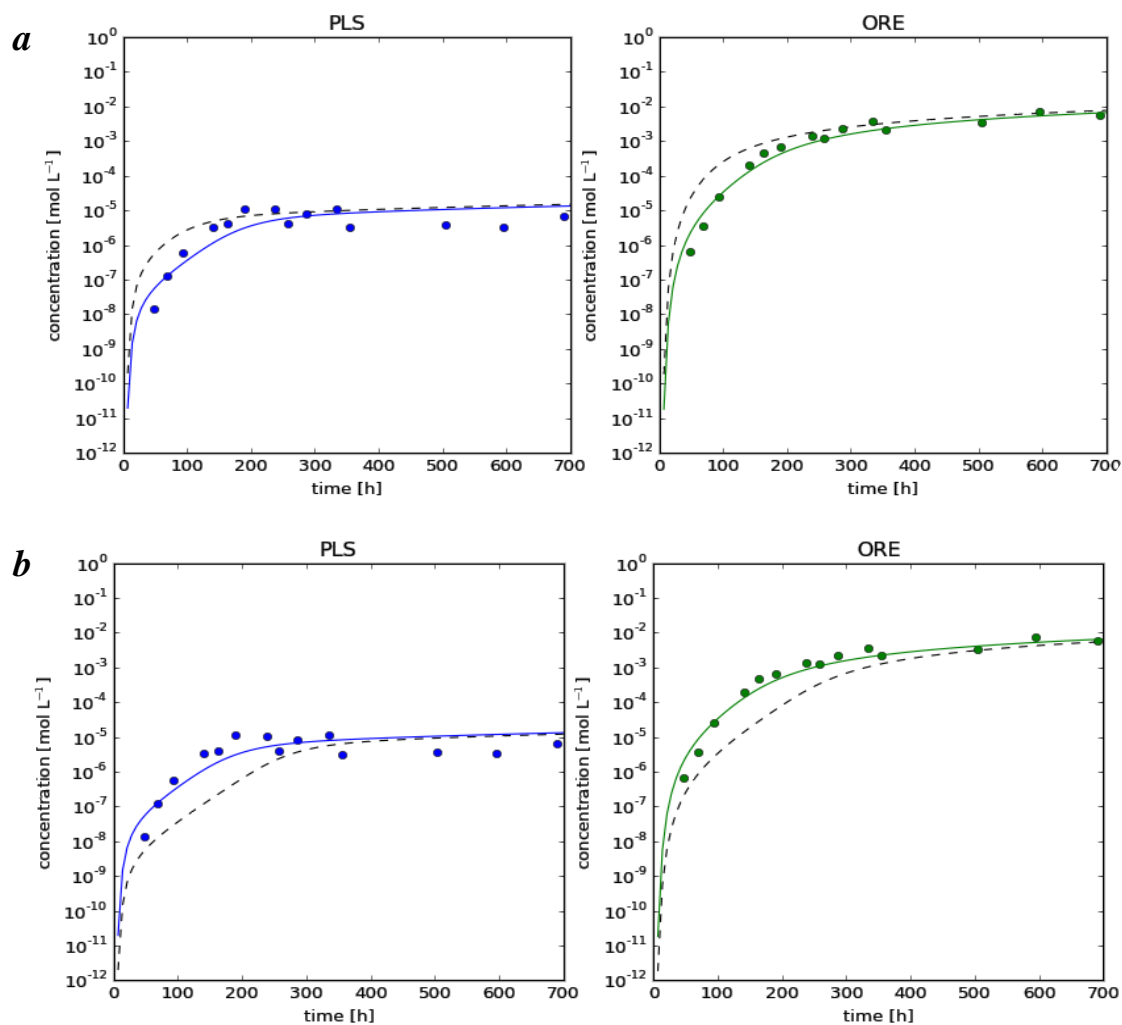


617 **Figure 5: Sensitivity of proposed model to changes in dispersion coefficient (D_Z) and advective transfer**
618 **rate (v), with (a) increasing D_Z to $8.48 \times 10^{-5} \text{ m}^2\text{h}^{-1}$, (b) decreasing D_Z to $2.12 \times 10^{-5} \text{ m}^2\text{h}^{-1}$, (c) increasing v**
619 **to $3.07 \times 10^{-3} \text{ mh}^{-1}$ and (d) decreasing v to $1.23 \times 10^{-3} \text{ mh}^{-1}$. The fitted model is represented by the solid line**
620 **and the model predicted outcome following a change in parameter by the dashed line.**

621

622 3.2.3. Sensitivity of hydrodynamic model to variations in inoculum size

623 The sensitivity analysis on the hydrodynamic model was extended to include the response of the
624 model to variations in an operating condition, in particular, to an increase and decrease in inoculum
625 concentration. The effect of varying inoculum size on microbial growth and activity using the current
626 experimental system has been discussed previously in Govender et al. (2015a); however, direct
627 comparison of the model predictions with experimental data cannot be made since the *At.*
628 *ferrooxidans* pre-culturing conditions and feed composition vary.
629



630 **Figure 6: Sensitivity of the proposed model predictions of the change in microbial concentrations in the**
631 **PLS and ore-associated phases, to (a) a ten-fold increase in inoculum concentration and (b) a ten-fold**
632 **decrease in inoculum concentration. The fitted model is represented by the solid line [—], experimental**
633 **data by shaded circles [•] and the model predicted outcome following a change in parameter by the**
634 **dashed line [- - -].**

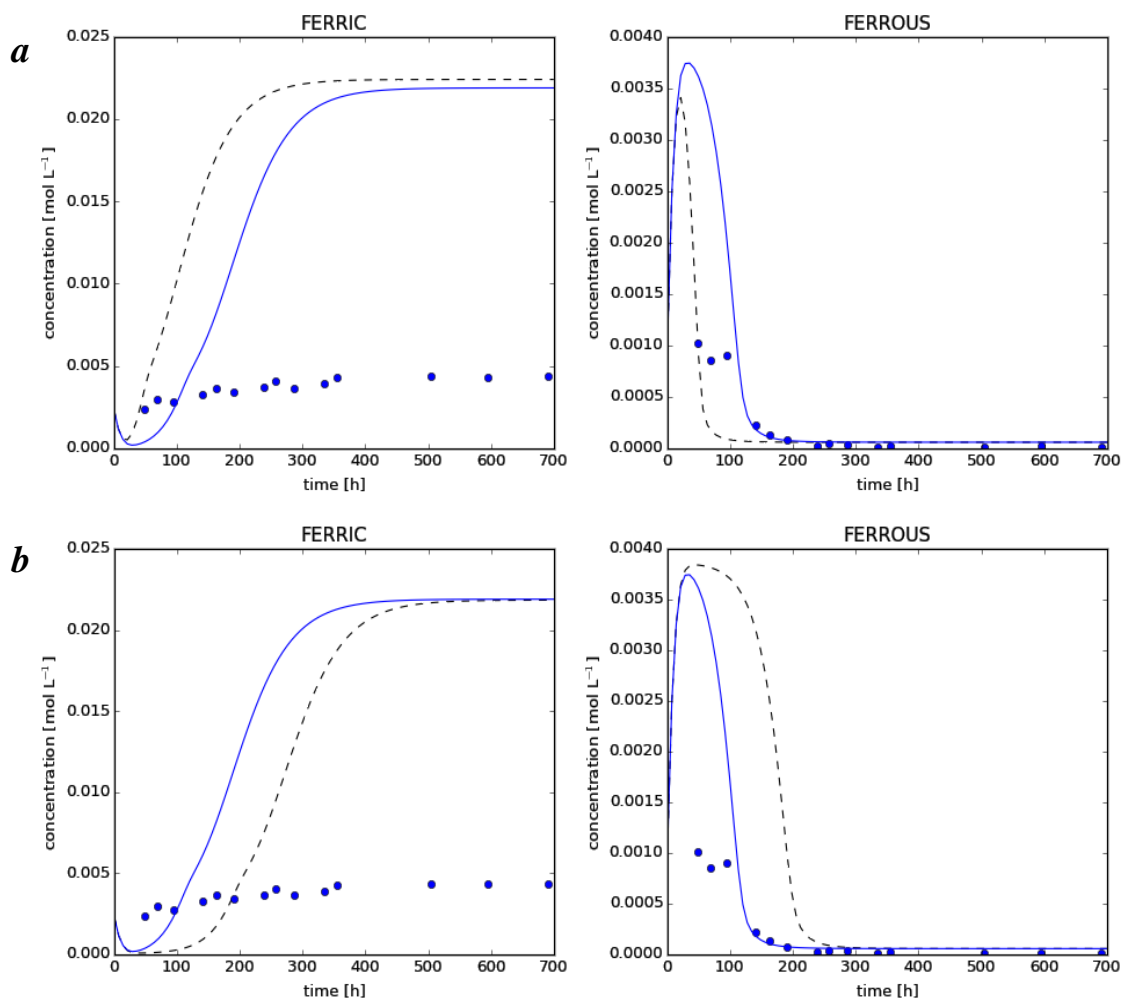
635
636 As observed from Figure 6a, an increase in inoculum size increased the initial microbial abundance in
637 each of the phases. An increase in inoculum size has been shown to produce a more rapid increase in
638 microbial number, which is in agreement with that observed in previous studies (Govender et al.,
639 2015a, Tupikina et al., 2014). This effect was not observed since the hydrodynamic model exhibits

640 poor resolution over this initial period. As previously mentioned, the model also did not simulate the
 641 initial decrease in PLS concentration as a result of rapid microbial attachment to the mineral (Figure
 642 2a). Initial microbial populations reporting to the PLS and ore-associated phases were observed to
 643 decrease with a decrease in inoculum size (Figure 6b) and the time required before microbial numbers
 644 increase substantially was expected to increase.

645

646 However, irrespective of inoculum size, the final maximum microbial concentrations in both the PLS
 647 and ore-associated phases remain unchanged. In addition, the model predicted that an increase in
 648 inoculum size results in the maximum microbial concentration being reached earlier than with a lower
 649 inoculum size. The abovementioned model responses to varying inoculum concentration were also
 650 observed in the empirical data presented previously (Govender et al., 2015a).

651



652 **Figure 7: Sensitivity of the proposed model predictions of overall ferric and ferrous iron concentrations,**
 653 **to (a) a ten-fold increase in inoculum concentration and (b) a ten-fold decrease in inoculum**
 654 **concentration. The fitted model is represented by the solid line [—], experimental data by shaded circles**
 655 **[●] and the model predicted outcome following a change in parameter by the dashed line [- - -].**

656

657 An increase in inoculum size resulted in a more rapid increase in microbial activity, as observed in the
658 predicted overall ferric and ferrous iron concentrations (Figure 7a). The ten-fold increase in inoculum
659 size produced an approximate decrease the time for onset of microbial activity of 50 h. Similarly, a
660 ten-fold decrease in inoculum size resulted in a predicted increase in time delay for onset of microbial
661 activity of *ca.* 100 h (Figure 7b).

662

663 **4. Conclusions**

664 Recent mineral bioleaching studies have demonstrated the significant difference between the
665 microbial populations in the PLS and ore-associated phases. This has impact on the interpretation of
666 the data obtained during monitoring of heap bioleaching systems. However, no studies have yet
667 developed and validated an appropriate mathematical model to decouple the effects of microbial
668 transport between these phases and microbial growth kinetics. The proposed hydrodynamic model
669 was developed assuming that the microbial concentration gradient between the PLS and ore-
670 associated phases was the driving force, together with substrate availability, for microbial attachment
671 to and detachment from the ore-associated phases, with transport being facilitated by advection-
672 dispersion properties of the bulk flowing solution. Mineral leach kinetics were assumed to be
673 dependent on the ferric to ferrous iron ratio and modified to include the population balance model
674 (PBM), thus providing a better estimation of the contribution of surface-based reactions to the overall
675 mineral leach rate. Microbial substrate utilisation kinetics were described assuming a Michaelis-
676 Menten type relationship with ferric to ferrous iron ratio and related to growth kinetics through the
677 yield coefficient.

678

679 This proposed model successfully predicted the changes in microbial concentration in the PLS and
680 ore-associated phases using an advection-dispersion equation, which incorporated terms for microbial
681 growth, attachment and detachment rates. The model prediction for the *true* maximum specific
682 growth rate in the ore-associated phases was found to be significantly greater than that predicted in
683 the PLS. This result is in accordance with the hypothesis that within a whole ore bioleaching system,
684 microbial growth and activity occurs primarily in the ore-associated phases, where ferrous iron
685 availability is enhanced. Hence, the microbial abundance in the PLS was a result of microbial
686 transport from the ore-associated phases to the PLS. This hydrodynamic dispersion model provides
687 insight into true estimates for microbial growth rates within heap bioleaching systems. With further
688 refinement and validation, the model, therefore, offers the potential for the estimation of the relative
689 microbial abundance in the ore-associated phases, given the microbial concentration and activity
690 measured in the PLS, and without the need to sample the heap for analysis the ore.

691

692 Since the model is based on the hydrodynamic properties of the bulk solution, the effect of variables
693 such as solution flow rate, bulk density and reactor configuration on the model outputs require further

694 study for refinement of these parameters for enhanced model predictions. These variables are
 695 particularly important for the estimation of dispersion coefficients and the advection transfer rates,
 696 which have a significant effect on the model outputs. In addition, the model may be improved through
 697 the inclusion of ferric and ferrous iron concentrations measured at the mineral interface, which will
 698 allow for further validation the model.

699

700 5. List of symbols

$\mu_{x,pls}$	Specific microbial growth rate in the PLS	h^{-1}
$\mu_{x,ore}$	Specific microbial growth rate in the ore-associated phase	h^{-1}
$\mu_{x,total}$	Specific microbial growth rate in the total population	h^{-1}
$\mu_{x,i}^{max}$	Maximum microbial specific growth rate for each phase	h^{-1}
ϕ_{MS}	Fraction of pure mineral sulphide in ore	dimensionless
τ	Mean residence time	h
θ	Age of particle	h
A^P	Particle specific surface area	$m^2 kg^{-1}$
$C_{Fe^{2+}}$	Ferrous iron concentration	$molFe^{2+} L^{-1}$
$C_{Fe^{3+}}$	Ferric iron concentration	$molFe^{3+} L^{-1}$
$C_{x,pls}$	Microbial concentration in the PLS	$molC L^{-1}$
$C_{x,ore}$	Microbial concentration in the ore-associated phase	$molC L^{-1}$
$C_{x,total}$	Microbial concentration in the total population	$molC L^{-1}$
D_r	Dispersion coefficient in radial direction	$m^2 h^{-1}$
D_z	Dispersion coefficient	$m^2 h^{-1}$
$f_0(l_0)$	The normal distribution representing the probability of particles in a specific size range	m^{-1}
$I(\theta)$	The internal age distribution of particles	h^{-1}
k_{att}	Microbial attachment rate constant	h^{-1}
k_{det}	Microbial detachment rate constant	$mol m^{-2} h^{-1}$
k_m	Mineral leach rate constant	$mol m^{-2} h^{-1}$
K_s	Michaelis-Menten constant for substrate utilisation	dimensionless
l_0	Initial particle size or diameter	m
M^P	Mass of a particle	kg
n	Order of mineral leach reaction	dimensionless
N^T	Total number of particles in the reactor	dimensionless
$q_{Fe^{2+}}$	Microbial specific ferrous iron oxidation rate	$molFe^{2+} molC h^{-1}$
$q_{Fe^{2+}}^{max}$	Maximum microbial specific ferrous iron oxidation rate	$molFe^{2+} molC h^{-1}$

r_{bio}	Rate of microbial ferrous iron oxidation	$\text{molFe}^{2+} \text{ L}^{-1} \text{ h}^{-1}$
r''_{min}	Intrinsic mineral surface reaction rate	$\text{mol m}^{-2} \text{ h}^{-1}$
r^R	Overall mineral leach rate	$\text{mol m}^{-3} \text{ h}^{-1}$
t	Time	h
v	Advection mass transfer coefficient	m h^{-1}
V^R	Total working liquid volume in reactor	m^3 or L
V_{PLS}	Volume of bulk flowing solution or PLS within ore bed	L
V_{ore}	Volume of stagnant interstitial solution within ore bed	L
$Y_{sx,pls}$	Microbial yield coefficient in PLS	$\text{molC}_{x,PLS} [\text{molFe}^{2+}]^{-1}$
$Y_{sx,ore}$	Microbial yield coefficient in ore-associated phase	$\text{molC}_{x,ore} [\text{molFe}^{2+}]^{-1}$
$Y_{sx,total}$	Microbial yield coefficient in all phase in ore bed	$\text{molC}_{x,total} [\text{molFe}^{2+}]^{-1}$
z	Depth of bed	m

701

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707

708 **7. References**

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