Kinetics of bacterial potentiometric titrations: the effect of equilibration time on buffering capacity of *Pantoea agglomerans* suspensions

Leon Kapetas[a,*], Bryne T. Ngwenya[a], Alan M. Macdonald[b] and Stephen C. Elphick[a].

[a] School of Geosciences, Earth Subsurface Research Group, Grant Institute, University of Edinburgh, West Mains Road, Edinburgh EH9 3JW, United Kingdom

[b] British Geological Survey, Murchison House, West Mains Road, Edinburgh EH9 3LA, United Kingdom

[*]Corresponding author. Tel: +44 (0) 1316508507, +30 6978651161, email: lekas11@gmail.com

Abstract

Several recent studies have made use of continuous acid-base titration data to describe the surface chemistry of bacterial cells as a basis for accurately modelling metal adsorption to bacteria and other biomaterials of potential industrial importance. These studies do not share a common protocol; rather they titrate in different pH ranges and they use different stability criteria to define equilibration time during titration. In the present study we investigate the kinetics of bacterial titrations and test the effect they have on the derivation of functional group concentrations and acidity constants. We titrated suspensions of *Pantoea agglomerans* by varying the equilibration time between successive titrant additions until stability of 0.1 or 0.001 mV s^{-1} was attained. We show that under longer equilibration times, titration results are less reproducible and suspensions exhibit marginally higher buffering. Fluorescence images suggest that cell lysis is not responsible for these effects. Rather, high DOC values and titration reversibility hysterisis after long equilibration times suggest that variability in

buffering is due to the presence of bacterial exudates, as demonstrated by titrating supernatants separated from suspensions of different equilibration times. It is recommended that an optimal equilibration time is always determined with variable stability control and preliminary reversibility titration experiments.

Keywords: Bacterial titrations; stability criteria; exudate production

1. Introduction

Potentiometric titrations have become a standard methodology for characterising proton exchange between bacterial surfaces and their surrounding solutions. Acidity constants and concentrations of functional groups are derived using Surface Complexation (SC) modelling of titration results. These parameters are then used in SC models to predict metal adsorption by the cell surfaces [1-9]. A survey of experimental practice shows that there is no standard protocol adopted in all titration studies. For instance, the temperature of titration experiments might vary between studies despite recent evidence of the temperature dependence of titration results [10, 11]. Similarly, there is no consensus regarding the pH range within which titrations should be carried out. In most studies suspension pH is dropped to a low value (between 2.4 and 4) by acidification or acid titration and then by base titration it is increased to approximately 10-11 [1, 2, 7, 12-22]. Fein et al. [23] carried out titrations to pH values as low as 2, since proton adsorption can occur at this low pH. However, in other studies it was preferred to titrate in both directions by separating the suspension into two equal volumes and starting titration from the immersion pH, for fear of denaturizing the cell membrane or promoting cell aggregation under extreme pH conditions [3, 6]. Borrok et al. [24] suggested that suspension pH values close to 3

can cause cell damage, indicated by elevated DOC values at this pH range. Similarly, for pH above 10 significant cell lysis can occur and may interfere with the buffering measurements [23].

Another significant issue is the stability criterion chosen to estimate equilibration time for continuous titrations. In most studies the equilibration time is controlled by the stability criterion of 0.1 mV s^{-1} [2-4, 8, 14, 16, 17, 20, 25]. In a few studies the stability criterion is set at 0.01 mV s^{-1} [7, 23], while in others it is more strict with values of 0.1 and 0.15 mV min^{-1} (0.0017 and 0.0025 mV s^{-1}) [18, 19]. Other studies use pH units when reporting stability criteria at 0.001 pH s^{-1}, approximately equivalent to 0.06 mV s^{-1} [1, 26]. Alternatively, a few studies have dealt with this problem by conducting discontinuous titrations in limited residence time reactors [27, 28]. This approach has been adapted from mineral surface charge studies [29-31]. Nevertheless, most studies use the traditional continuous titration approach, which is the focus of this study.

As yet, there has been no study that has rigorously investigated the amount of time required to reach equilibrium during bacterial titrations, i.e. investigate if the reaction of the surface with the acid/base proceeds after the stability criterion is met. Duc et al. [32] used oxide mineral surfaces to examine the effect of the acid-base titration speed on the pH measurements and on the surface charge calculation. They found that when the titration speed was increased, the measured pH value for the same added volume of acid increased. They differentiated between “fast” step reactions, attributed to the surface, and “slow” step reactions during titrations. By analogy, our study examines the effect that titration speed can have on bacterial surface proton exchange. Our

hypothesis tests whether longer equilibration times will lead to further acid/base consumption due to proton surface adsorption and/or intracellular diffusion/consumption. Alternatively, longer times could cause cell lysis and/or the production of exudates which might lead to higher buffering capacity than expected by the surface chemistry alone. We use a combination of titration results using different titration speeds, DOC measurements, titration reversibility and cell viability examination to investigate these hypotheses.

2. Materials and Methods

2.1. Bacterial potentiometric titrations

Fresh batches of *Pantoea agglomerans* (also known as *Enterobacter agglomerans* [33]) cells were cultured and titrated after the method of Tourney et al. [21]. Potentiometric titrations were carried out on fresh cell pellets suspended in 50 mL 0.01M $NaClO_4$ background electrolyte. Before titration, cell pellets were washed three additional times in $NaClO_4$ electrolyte of the same concentration used in the titration experiments. Also, the pH of the cell suspension was adjusted to approximately 4 by adding a known amount of standard 0.5 M HNO_3 solution. The pH was not reduced further in order to avoid cell lysis [24] and "acid shock" to the cells [34].

Titrations were carried out between pH values 4 and 10 in dynamic mode with a DL53 Mettler Toledo automatic titrator. Using this automated setting the volume of the successive aliquots of titrant standard 0.5 M NaOH added to the suspension was adjustable – it was increased when change of pH was small and it was reduced when pH shift was large. Titrations were carried out using two different stability criteria: 0.1 and 0.001 pH min^{-1} (equivalent to 0.1 and 0.001 mV s^{-1}), set as 0.1 mV in 1 s and 0.06 mV in 60 s respectively (a criterion of less than 0.06 mV should not be used due to meter precision limitations). The two stability criteria were chosen simply to represent the two extremes commonly used in the literature, as reviewed above. For the short equilibration time, a minimum and maximum equilibration time was set at 2 and 5 minutes respectively, while for the long equilibration time the minimum and maximum time was set at 2 and 12 minutes respectively. The upper time limit was set to allow the titration to proceed in case of continuous buffering of the base. It also guaranteed that the titrations would be completed within 4 hours, thus avoiding the

possibility of cell starvation leading to cell death [35]. Reversibility titrations were also conducted at the two equilibration times. Down-pH titrations were carried out using standard 0.5 M HNO_3 titrant.

2.2. Titration data modelling

Surface complexation modelling of titration data was carried out using the optimization program FITEQL 4.0 [36]. Titration data were plotted in terms of molar concentration of protons exchanged by the cell surface per gram of biomass according to Fein et al. [23]:

$$[H^+]_{exch} = (Ca - Cb + [OH^-] - [H^+])/m_b \quad (1)$$

where Ca and Cb are the concentrations of acid and base (mol L^{-1}) for each titration step, $[OH^-]$ and $[H^+]$ (mol L^{-1}) are the equilibrium concentrations of OH^- and H^+ at equilibrium and m_b is the dry biomass concentration (g L^{-1}). Hence, $[H^+]_{exch}$ has units of mol g^{-1}. Due to uncertainties with determining the surface potential [23], a non electrostatic model was chosen to derive the concentrations and acidity constants of the functional groups that exchange protons. We describe their deprotonation using a number of monoprotic discrete acids according to Borrok and Fein [37]:

$$R - AH \leftrightarrow R - A^- + H^+ \quad (2)$$

where *R* denotes the bacterium where the functional group type, *A*, is attached. *R-A*$^-$ and *R-AH* represent deprotonated and protonated sites of the functional group respectively. Acidity constants K_i (*i*= 1, 2, 3…) are calculated using the following expression:

$$K_i = \frac{[R - A^-][H^+]}{[R - AH]} \quad (3)$$

where brackets represent the concentrations of the sites at equilibrium and [H+] the activity of protons in solution. Cell surfaces are negatively charged even at low pH conditions [23]. Hence, titration data were corrected to account for initial protonation state [21, 23, 38, 39]. This correction vertically shifts the titration curves closer to the isoelectric point of most bacteria as determined by electrophoretic mobility measurements [22, 23, 40]. Models with varying number of functional group types were tested and the quality of fit criterion V(Y) of FITEQL 4.0 was used to select the most suitable model [8]. A value of V(Y) lower than 20 suggests a good model fit.

2.3. Dissolved organic carbon (DOC) measurements

DOC samples were taken from bacterial suspensions. The sample preparation and analysis technique is detailed in Tourney et al. [41]. Briefly, samples were filtered into ashed glass vials using a 0.22 μm cellulose acetate membrane syringe filters. Samples were then frozen until DOC analysis was carried out with a Shimadzu 6000 TOC/TDN analyser. The analyser incorporates a high temperature catalytic oxidation and non-dispersive infrared detector. External calibration standards prepared from a stock solution of 1 g L^{-1} potassium hydrogen phthalate were run. Blank samples were measured to correct sample DOC concentrations. The detection limit of the instrument is 24 μg L^{-1}, while the observed analytical precision was better than 2% in terms of relative standard deviation.

2.4. Fluorescence Microscopy

Live/dead bacteria viability tests were carried out on bacterial suspension samples taken at different pH values. Sample volumes of 1 mL were taken from the titration suspension and centrifuged for 5 minutes in ependorf microtubes. Cells were then

washed twice in 0.14 M NaCl solution and resuspended in 1 mL of 0.14 M NaCl. The resulting suspension was stained with 3 μL of a 50:50 mixture containing SYTO 9 green-fluorescent nucleic acid stain and Propidium Iodide red-fluorescent nucleic acid stain. The suspension was incubated according to the manufacturer's protocol [42] and cells were observed and photographed using a Zeiss Axioplan 2 microscope using FITC filter with excitation 485/20, emission 525/30 and the Texas Red filter with excitation 560/25, emission 607/36. A Photometrics Coolsnap HQ camera was used and all hardware was controlled by Metamorph 7.5. Images of live/dead bacteria for every slide were finally combined using ImageJ (National Institute of Health, USA). Bacterial counts were carried out using CellC software for quantification of labelled bacteria by automated image analysis [43]. This counting technique has a nominal 2% error. Comparisons between manual and CellC counts provided a 5% error in estimates, which is consistent with the results of Gorman-Lewis [44].

3. Results

3.1. Titrations at varying equilibration times

Titrations were carried out on cell suspensions of 6, 9 and 12 g L^{-1} dry mass under both stability criteria. As can be seen in figure 1a, titrations are well reproduced when fast equilibration is selected (low stability). On the other hand, figure 1b shows that high stability leads to larger variation in titration results. Moreover, the titration curve for the 12 g L^{-1} reveals notably higher buffering capacity than the titration data of figure 1a. Titration of the 12 g L^{-1} suspension at 0.001 pH min^{-1} exhibits higher buffering than the two titrations carried out with the same stability criterion, but with less biomass. Also, the 9 g L^{-1} at 0.001 pH min^{-1} data show a marginally higher buffering capacity than the 6 g L^{-1} at 0.001 pH min^{-1} data, though this difference is within experimental error.

Two cell suspensions of 12 g L^{-1} were titrated at 0.1 and 0.001 pH min^{-1} and their supernatants were titrated under the same criteria respectively. Figure 1c shows the negligible buffering capacity of a bacteria-free electrolyte solution and compares the buffering of the titrated supernatant suspensions. The supernatant titrated at 0.001 pH min^{-1} shows 2.5 times greater buffering than the one titrated at 0.1 pH min^{-1}. Hence, it is suggested that DOC is present in the supernatant and has a variable contribution to buffering depending on the stability criterion used. The concentration of exchanged protons shown in figure 1c is approximated by the concentration of base added to the supernatant solution. This approximation is required since the amount of base and acid added during the bacterial titration is not all transferred to the supernatant solution (cells had to be centrifuged and removed) and hence can not be calculated. Nonetheless, this practically does not affect the calculation of the buffering capacity

198 of the supernatant, as it is base concentration that dominates buffering, i.e. the
199 difference in H_{exch} between pH 4 and 10 is practically equal to the difference in C_b for
200 the same pH range. Note that correcting bacterial titrations for supernatant buffering
201 does not necessarily improve the quality of the titration results. This is because DOC
202 generated at high pH was not present for the entire duration of the bacterial titration,
203 i.e. no correction is required for the initial times.
204
205 Figure 1. Titration curves carried out using the (a) 0.1 pH min^{-1} and (b) 0.001 pH min^{-1}
206 stability criteria, shown in the top and middle figure respectively. Results have been
207 normalised by dry mass and corrected for initial protonation state. The titration curve
208 of 12 g L^{-1} carried out under low stability is plotted in figure 1b to allow comparisons
209 of the curve's shapes and of their buffering capacity. The normalised buffering
210 capacity of electrolyte and supernatant solutions are shown in the bottom figure (c).
211
212 The 0.1 pH min^{-1} stability criterion was met for all bacterial titration points within the
213 time limits set by the experimental protocol. The 0.001 pH min^{-1} stability criterion
214 was met in the beginning of the titration until approximately pH 5.5-6. Above this pH
215 value the time required for equilibration increased and the upper limit of 12 minutes
216 was met before proceeding to new titrant addition, i.e. "equilibrium" was many times
217 not attained under 0.001 pH min^{-1} criterion at pH values greater than 6. The fact that
218 equilibrium was not attained, also points to the production of DOC during the time
219 course of the titration, as will be shown in section 3.2. DOC in turn leads to the
220 displacement of the titration curves shown in figure 1b.
221
222 A quantitative understanding of the differences between the bacterial titration curves
223 is provided by comparing the acidity constants and concentrations of the functional

groups of the surface. Table 1a shows the results of the 4 site non electrostatic model which best described the titration curves. The average values of the acidity constants for both stability criteria are consistent with literature values of studies which made use of a four site non electrostatic model [16, 17, 21, 23, 38]. However, pK_1 is higher than those found in literature because titrations did not cover the low pH range. This is due to a limitation of the modelling approach, which does not effectively extrapolate results outside or near the edge of the pH range of the dataset [45]. There is an apparent shift to higher pK values associated with higher uncertainties when the stability criterion of 0.001 pH min^{-1} is used. The buffering capacity in the pH 4-10 range is comparable to that of gram negative *Pseudomonas putida* [46], however is an order of magnitude lower than that of *Pseudomonas aureofaciens* [40]. Moreover, there is an average increase in total buffering capacity by almost 10^{-4} mol g^{-1} when allowing longer equilibration. The difference in DOC buffering between the two supernatant titration curves (figure 1c) is large enough to account for this increase. The increase in buffering due to the presence of DOC is more notable at high biomass titrations because they last longer (more base consumed). Thus they allow more time for DOC production. Moreover, the calculated functional group concentrations carry high uncertainties in both cases, albeit with higher uncertainties in the 0.001 pH min^{-1} titrations.

Stability pH min^{-1}	V(Y)	pK_1	pK_2	pK_3	pK_4	T_1 mol g^{-1}	T_2 mol g^{-1}	T_3 mol g^{-1}	T_4 mol g^{-1}	$T_{(total)}$ mol g^{-1}
0.1										
Average	0.45	4.14	5.71	7.34	9.46	4.02E-04	1.76E-04	2.00E-04	1.73E-04	9.51E-04
Std. Dev.		0.03	0.03	0.05	0.01	1.71E-05	2.16E-05	1.62E-05	3.36E-05	1.13E-05
0.001										
Average	0.62	4.34	5.68	7.58	9.79	2.79E-04	2.88E-04	2.16E-04	2.53E-04	1.04E-03
Std. Dev.		0.24	0.24	0.19	0.09	4.08E-05	2.23E-05	5.09E-05	8.64E-05	1.24E-04

Parameter	pK_1	pK_2	pK_3	pK_4	$T_{(total)}$
p-value	0.30	0.88	0.17	0.03	0.36

Table 1. Values of acidity constants and site concentrations under the 0.1 pH min^{-1} and 0.001 pH min^{-1} stability criteria (top). Results are averages of the titration curves shown in Figure 1. The p-values in table 1 (bottom), derived from student's t-test, examine statistical differences between estimated parameters shown in table 1 (top).

Titration modelling results for 0.1 and 0.001 pH min^{-1} stability criteria were analysed with a t-test to examine whether they are statistically different. An F-test on the modelled values (table 1 top) suggested that they possibly have statistically different variances. Therefore, the Welch's t-test was applied, which is a specific solution of the Student's t-test for samples with different variances [47]. Table 1 (bottom) shows the results of the t-test in terms of the probability that the mean values under study are statistically similar. At 95% confidence interval, only the pK_4 values are statistically different between the two experimental protocols. It is apparent in figure 1b that deviation of titration curves carried out under different stability criteria takes place at the high pH range. Not surprisingly, this would be reflected in the values of pK_4 and/or T_4. Individual differences in site concentrations are not examined with the t-test since their values carry high uncertainties. Rather, it was preferred to compare the

total site concentrations between the two experimental protocols. As can be seen in table 1 (bottom), the difference in $T_{(total)}$ was not significant.

3.2. DOC results

Dissolved organic carbon (DOC) samples were taken at the initial pH~6.2 conditions of the suspension, at near pH 4 after the acidification step and at pH~10 conditions after the termination of the base titration. This was done for 9 g L^{-1} titration suspensions at both fast and slow titration rates. At the fast titration rate, DOC slightly increased after the acidification of the sample from 2.44 mg L^{-1} at immersion pH conditions (pH~6.2) to 4.62 mg L^{-1} at pH~4. After the titration of the sample with base, DOC was measured again and was found equal to 4.81 mg L^{-1} (pH~10). At the slow titration rate, DOC was initially measured at 1.82 mg L^{-1} (pH~ 6.2). After the sample was acidified to pH~4, DOC increased to 3.50 mg L^{-1}. DOC increased significantly to 22.80 mg L^{-1} after the suspension was slowly titrated with base to pH~10. It has been shown that DOC levels in *P. agglomerans* suspensions clearly increase with time, particularly at higher pH conditions [48]. Pokrovsky et al. also found that levels of DOC increased during the course of automatic titrations of cyanobacteria Gloeocapsa sp., which did not allow the quantification of surface proton adsorption with this method [28]. The increase in DOC can be attributed either to the lysis product of a portion of the titrated cells or to the release of exudates [48]. Claessens et al. reported an increase in base neutralisation at pH above 8 during static experiments and suggested that this was related to a metabolic response of Shewanella putrefaciens cells releasing organic compounds [13]. On the other hand, Wightman et al. showed that DOC does not contribute to proton buffering of B. subtilis surface titrations [49]. In this case, the production of DOC likely explains the variability in

the bacterial titration results between figure 1a and 1b, and is responsible for the proton buffering by the supernatants. The origin of the DOC measured in suspension at high pH after the long titrations (0.001 pH min^{-1}) is investigated below by examining viability of the cells in suspension.

3.3. Cell viability

Fluorescence images of cells used in titrations were taken in order to understand the effect that acidic and basic conditions or prolonged titrations can have on cell viability. Live and Dead cell counts were carried out for suspensions before acidification, after acidification and after completion of the base titration of a 9 g L^{-1} suspension at 0.001 pH min^{-1}. Glass slides were spotted with suspensions prepared from samples at initial pH~6.2, pH~4 and pH~10. Five images were taken per slide and more than 1000 cells were counted for every slide in order to obtain a representative idea of cell viability. Proportion of live/dead cells was essentially the same at immersion pH~6.2 (88% Live, 12% Dead), at pH~4 (86% Live, 14% Dead) after acidification, and at pH~10 (85% Live, 15% Dead) after titrating with base at 0.001 pH min^{-1}. Percentage differences are within error associated with the experimental technique and counting method. Results of this semi-quantitative analysis suggest that cell lysis does not take place during the prolonged duration of the titration carried out within the 4-10 pH range using the 0.001 pH min^{-1} criterion. Since cell lysis is not the source of DOC, we suggest exudate production as the source.

3.4. Reversibility of titrations

The presence of higher DOC concentration increases proton buffering, but might also affect the reversibility of titrations. Hence we decided to investigate this possibility.

Reversibility of titrations on 9 g L^{-1} bacterial suspensions was tested at both fast and low equilibration speed to investigate the potential effect of DOC. Figure 2 presents the acid and base titration curve results for both criteria. Results were corrected for initial protonation state assuming a 3 site non electrostatic model, as acid titrations did not converge under a 4 site model due to significant hysterisis. This difference does not affect buffering capacity of the titrations. While some reversibility hysterisis was exhibited during fast equilibration (low stability), the slow equilibration procedure (high stability) resulted in significant hysterisis, more pronounced at high pH where high DOC (exudates) production has been demonstrated. Bacterial titration irreversibility has been observed for other species during short equilibration times [13], although most studies have not shown such behaviour at either high [50] or low stability [2, 15, 25, 49, 51]. Further investigation is required to test whether such behaviour may be strain-dependent in terms of exudates production. Moreover, higher buffering was confirmed in the base titration with longer equilibration time (figure 2). Both observations suggest that a portion of the buffering during long equilibration times is not related to the exchange of protons from the bacterial surface. Instead, an interaction between protons and released DOC is believed to be taking place. The localisation of hysterisis to high pH suggests that the exudates contain primarily high pK_a functional groups which do not contribute to solution phase proton buffering at low pH. This suggestion is in agreement with the supernatant titration results, which show greater proton buffering at high pH and convergence of curves from different titration speeds at low pH.

Figure 2. Reversibility of acid-base titrations of 9 g L^{-1} dry weight cell suspensions equilibrated using 0.1 pH min^{-1} (closed symbols) and 0.001 pH min^{-1} (open symbols)

343 stability criteria. Circle and squares represent base and acid titration data respectively.
344 Results were normalised by dry weight to represent the proton exchange capacity per
345 gram biomass dry weight.
346
347

348 **4. Discussion and Conclusions**

349 When the stability criterion is set lower at 0.001 pH min^{-1} to allow more time for
350 equilibration, reproducibility of titrations is influenced adversely. The time limit for
351 equilibration (12 min) had to be invoked as equilibrium was not attained. High
352 stability is not suitable for this biomaterial due to inherent uncertainties related to its
353 metabolic activity. The total site concentration of the fast titrations is lower than the
354 total concentration of the slower titrations. However, as was shown this difference is
355 not statistically significant. This is because the exudates produced by these bacteria
356 have low buffering capacity (as demonstrated by titration of supernatants), such that
357 overall buffering remains dominated by the cell surface. Based on reversibility
358 experiments, exudates contribute to total buffering primarily at high pH with
359 maximum hysterisis around pK_3/pK_4 sites. This was also confirmed by titrating the
360 supernatant of cell suspensions separately. Furthermore, significant differences at a
361 95% confidence interval are observed only in the values of pK_4. This is anticipated
362 since the variability of deprotonation between titrations with different stability criteria
363 is more notable at the high pH range (~8-10).
364
365 The titration of the supernatants and the DOC measurements give an insight into the
366 possible origin of the observed variability between titrations at varying equilibration
367 times. When the duration of the titrations is increased, the amount of DOC in the
368 system increases significantly. The higher DOC values measured in our study suggest
369 that bacteria are metabolically releasing exudates. This inference is supported by the
370 fact that cell integrity between pH 4 and 10 is maintained in our experiments, since no
371 cell lysis takes place as shown by fluorescent imaging. The differences in cell
372 viability between initial, high and low pH are only marginal and within the precision

of the method. Moreover, the duration of the experiments is sufficiently short to exclude the possibility of cell starvation and death.

Exudates account for the slight increase in buffering capacity, but also for the lack of reproducibility between titrations carried out with the high stability criteria. As the exudates react with the titrating acid/base added, the buffering observed cannot be entirely attributed to the consumption of titrant by the cell surface. The larger reversibility hysterisis noticed at 0.001 pH min^{-1} accompanied by increase DOC values provides additional evidence to this.

We have tested whether increasing equilibration time during titration increases the exchange of protons with the surface. Our results suggest it is preferable to use fast equilibrium stability criteria, so that most of the apparent buffering capacity can be attributed to the surface reactions. However, this result is not necessarily transferable. Future studies should individually (i) check for the buffering contribution of the supernatant and (ii) examine the reversibility of titrations of each bacterial species under each culture and titration protocol, as titrations might be reversible over a short time scales but not over long periods [15]. It was shown that the selection of stability criterion depends on the demonstration of reversibility; nevertheless, reversibility was not attained during this study under neither stability criteria.

Strictly, the observed irreversibility under both criteria renders problematic the use of a thermodynamic approach on the titration curves, although the low contribution to buffering by exudates means that the differences are not statistically significant and thus only affect precision. This effect became apparent in the quality of surface

398 complexation modelling results which degraded due to the presence of non cell
399 surface related buffering. This has implications for determining surface sites for
400 bacteria that shed DOC to the electrolyte, e.g. EPS-producing ones [21]. Finally, we
401 suggest that small differences in site densities and pK_a values reported in bacterial
402 titration studies can, in some situations, be an artefact of different equilibration times.
403

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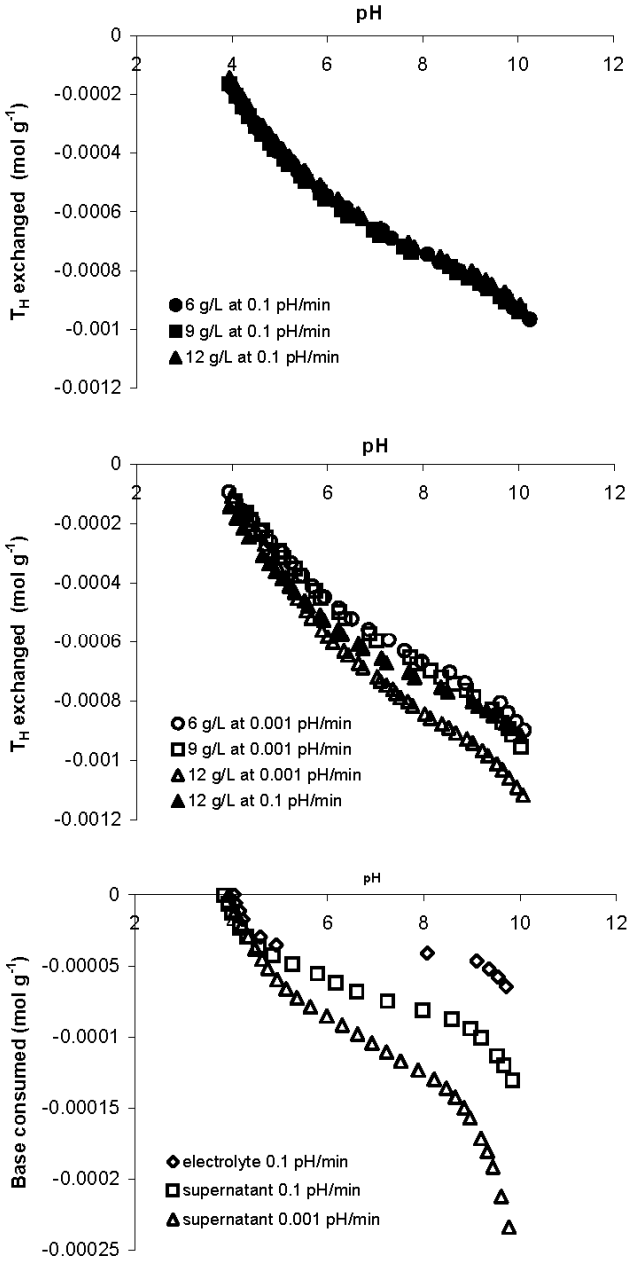

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496 Figure 1. Titration curves carried out using the (a) 0.1 pH min^{-1} and (b) 0.001 pH min^{-1}
497 stability criteria, shown in the top and middle figure respectively. Results have been
498 normalised by dry mass and corrected for initial protonation state. The titration curve
499 of 12 g L^{-1} carried out under low stability is plotted in figure 1b to allow comparisons
500 of the curve's shapes and of their buffering capacity. The normalised buffering
501 capacity of electrolyte and supernatant solutions are shown in the bottom figure (c).

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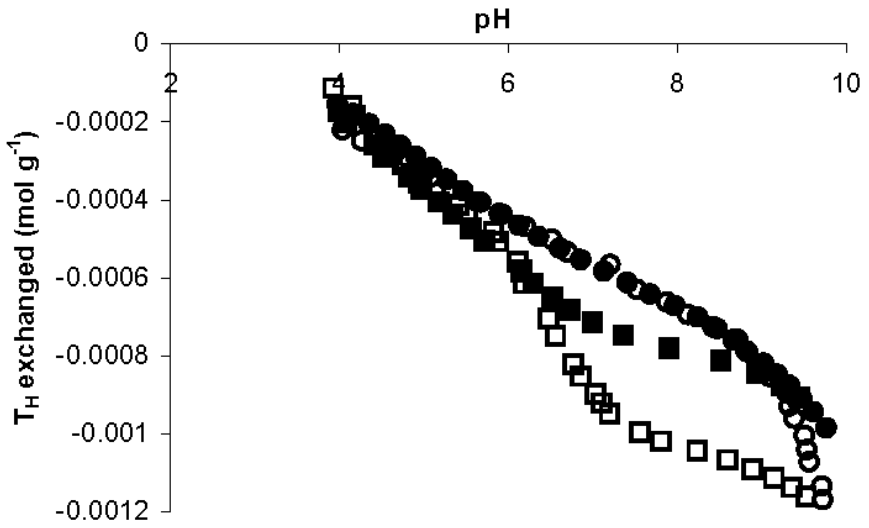

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504 Figure 2. Reversibility of acid-base titrations of 9 g L^{-1} dry weight cell suspensions
505 equilibrated using 0.1 pH min^{-1} (closed symbols) and 0.001 pH min^{-1} (open symbols)
506 stability criteria. Circle and squares represent base and acid titration data respectively.
507 Results were normalised by dry weight to represent the proton exchange capacity per
508 gram biomass dry weight.

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