CO₂ stripping abiotic tests

The titrimetric data obtained from the abiotic test performed to characterize the CO₂ stripping (I-2) was fitted using the following equations (Eqs. SI.1-SI.4) in order to estimate the corresponding overall mass transfer coefficient (Kₐa):

\[
\text{HP}_{\text{CO}_2} = V_{\text{Base}} \cdot C_{\text{Base}} - V_{\text{Acid}} \cdot C_{\text{Acid}} \quad (\text{SI.1})
\]

\[
\text{HPR}_{\text{CO}_2} = \frac{d(\text{HP})}{dt} \quad (\text{SI.2})
\]

\[
\text{HPR}_{\text{CO}_2} = \alpha \cdot 10^{-pH - pK_{c1}} \cdot \left(1 + 10^{pH - pK_{c2}}\right) \cdot \left(\left[C_{\text{CO}_2}\right]_0 - \left[C_{\text{CO}_2}\right]_{\text{eq}}\right) \cdot e^{-\alpha \cdot t} \quad (\text{SI.3})
\]

\[
\alpha = \frac{K_L a}{1 + 10^{pH - pK_{c1}} \left(1 + 10^{pH - pK_{c2}}\right)} \quad (\text{SI.4})
\]

where HP is the proton production (mmol), HPR_{CO₂} is the proton production rate associated to CO₂ stripping.
(mmol min\(^{-1}\)), \(V_{\text{base}}\) and \(V_{\text{acid}}\) are the accumulated base and acid dosage (mL), \(C_{\text{base}}\) and \(C_{\text{acid}}\) are the base and acid concentrations (M), \([\text{CO}_2]_{\text{eq}}\) is the CO\(_2\) concentration at equilibrium with the initial gas phase composition defined by Henry’s law (mM). A dimensionless Henry’s constant of 0.83 for CO\(_2\) was used (Ficara et al., 2003). \([\text{CO}_2]_0\) is the concentration of CO\(_2\) at time zero, \(pK_{c1}\) and \(pK_{c2}\) are the dissociation constants for the aqueous CO\(_2\) equilibriums and \(\alpha\) is a constant factor, described by Eq. (SI.4). This constant contains the parameter to be determined in order to characterize the stripping process, the \(K_{L,a}\).

**Respirometric tests**

The respirometer was submerged in a water bath to set a temperature of 30ºC (Polystat 24, Fisher Scientific). Software programmed in Visual Basic was used to control the pH at 7.5 through the addition of NaOH (0.5 M) and HCl (0.5 M) with an automatic dispensing burette (Multi-Burette 2S-D, Crison Instruments). The DO concentration and the pH in the respirometer were monitored with a CellOx® 325 (WTW) and a SenTix® 82 (WTW) sensors both connected to a bech-top multimeter (Inolab® Multi 740 – WTW). The volumes of acid and base added to control the pH were also monitored on-line. The schematic of the respirometer setup is presented in Fig. S1. The dissolved oxygen (DO) probe was used in order to ensure that oxygen was not entering into the respirometer and that respirometric tests were performed strictly under anoxic conditions.

![Fig. S1. Schematic of the respirometer](image-url)
The biomass used for the respirometric tests was obtained from the CSTR, centrifuged at 6500 rpm and resuspended in substrate-free buffered nutrient solution. The mineral medium composition was (g L\(^{-1}\)): NH\(_4\)Cl (0.40), KH\(_2\)PO\(_4\) (6.8), MgSO\(_4\) · 7H\(_2\)O (0.40), trace metal solution (2.0 mL L\(^{-1}\) of SL-4 for ATCC medium: 1255 *Thiomicrospira denitrificans*) with 10 mg N L\(^{-1}\) as either nitrite or nitrate and adjusted to pH 7.5 with NaOH (2M). After an endogenous phase of 2h and some “wake up” pulses (1 – 5 mg S\(^2-\) L\(^{-1}\)) to reactivate biomass enzymatic mechanisms, different initial concentrations of sulfide and nitrogen (as nitrate or nitrite) were tested to study the autotrophic sulfide oxidation under anoxic conditions.

**Mathematical methods**

Maximum specific growth rates, half-saturation constants and inhibition constants were estimated as well as the respirometric profiles were simulated by means of MATLAB 7.7 (Mathworks, Natik, MA). The differential equations were solved using a variable step Runge-Kutta method and the parameter estimation was carried out by using the Nelder-Mead Simplex search method algorithm (unconstrained non-linear optimization). The functions employed for the numerical solution were *ode45* and *fminsearch*, respectively. In this case, the fitting of the experimental data to model predictions considers not only the electron acceptors (nitrite and nitrate) but the substrates and the products of the reactions and it is based on seeking the minimum value of the objective function (F) (Eq. SI.5). This function is defined as the norm of the differences between the predicted values of the mathematical model and the experimental data:

\[
F = \sqrt{\sum_{i=1}^{N} (y_{\exp,i} - y_{\theta,i})^2}
\]  

(SI.5)

where N is the number of experimental data, \(y_{\theta,i}\) is the predicted value with the kinetic parameters to estimate (\(\theta\)) and \(y_{\exp,i}\) is the value experimentally measured.