

Quantification of the effect of CO₂ transfer on titrimetric techniques used for the study of biological wastewater treatment processes

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Abstract

Titrimetric methods are considered to be useful for the study of biological wastewater treatment processes, particularly those processes that have negligible influence on the dissolved inorganic carbon pool. However, the application of titrimetric methods for studying biological processes that produce/consume carbon dioxide is not straightforward as microbial activity affects the total amount of dissolved inorganic carbon with a proportioned change (determined by pH) in the concentration of every species of inorganic carbon. In this work, the impact of adjustments to the inorganic carbon pool on titrimetric data was assessed by considering a pH-stat titration of heterotrophic carbon oxidation. It was confirmed that at typical operating conditions (pH 7.5 and $K_L a_{CO_2} \approx 22.5 \text{ h}^{-1}$) carbon oxidation causes a marked increase in the rate of carbon dioxide transfer and consequently has impact on titrimetric data. Model simulation was used to quantify the impact for a wide range of operating conditions. It was found that only when a titration is operated at pH > 8 with a $K_L a_{CO_2} < 10 \text{ h}^{-1}$ can the interference that results from action of the bicarbonate system be neglected (< 5% error induced). Outside these operating conditions it is suggested that the interference be accounted for by either measurement or modelling of carbon dioxide transfer.

Keywords: bioprocess monitoring, sensors, titration, gas-liquid mass transfer, hydrogen ion production, aerobic processes

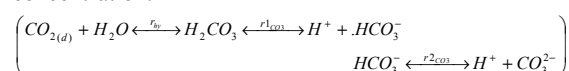
Nomenclature

APE	average percentage error
$K_L a$	mass transfer coefficient
m	fraction of dissociated acid A ⁻ in the liquid phase for a monoprotic acid HA
n	number of protons produced by the carbonate and bicarbonate systems per CO ₂ molecule dissolved
p	fraction of NH ₄ ⁺ among NH ₃ + NH ₄ ⁺ in liquid phase
pK	acid-base dissociation constant; general (pK_a), ammonium (pK_{NH_4}), carbonic acid ($pK_{H_2CO_3}$), bicarbonate (pK_{HCO_3}).
CTR	carbon dioxide transfer rate
CPR	carbon dioxide production rate
HPR	hydrogen ion production rate
Hp	cumulative net hydrogen ion production
H_{CO_2}	Henry's constant for carbon dioxide
OUR	oxygen uptake rate
pHsp	pH set point
r_{NH_4}	rate of dissociation of ammonium
r_{HA}	rate of dissociation of acid
r_{hy}	rate of hydration of carbon dioxide
$r_{CO_3}^1$	rate of dissociation of carbonic acid
$r_{CO_3}^2$	rate of dissociation of bicarbonate
r_{Ac}	rate of removal of acetic acid
r_{NH_3}	rate of removal of NH ₃
R	ideal gas constant

T	temperature
$Y_{H,c}$	biomass yield based on carbon
γ_s	degree of reduction of substrate $CH_f O_z$
γ_x	degree of reduction of biomass $CH_a O_b N_c$

Introduction

Microbial activity has a wide-ranging effect on pH. Invariably, the effect is a consequence of the action of acid-base buffering systems, whereby the biological consumption or production of components of these systems results in a change in hydrogen ion concentration (Pratt et al., 2003). For instance, when ammonia is consumed for biomass growth or oxidised during nitrification then dissociation of ammonium ions occurs, the result being an increase in hydrogen ion concentration; ($NH_4^+ \xrightarrow{r_{NH_4}} NH_3 + H^+$) when volatile fatty acids (VFA) are produced/consumed during anaerobic digestion then acid dissociation/formation results in a change in hydrogen ion concentration ($HA \xrightarrow{r_{HA}} A^- + H^+$); and even when carbon dioxide is produced/consumed during biological activity the carbonic acid concentration is altered, which again results in a change to the hydrogen ion concentration:



The examination of biological processes by the measurement of addition of titrant to counter the aforementioned effects on pH, formally referred to as pH-stat titration (Jacobsen et al., 1957), has been widely reported. A necessary aspect of pH-stat titration is eliminating the contribution to titrimetric data of background physico-chemical processes (Ficara et al., 2003), in particular the contribution, via action of the bicarbonate system,

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from the continuous interaction between carbon dioxide in the gas and liquid phases:

$$CTR = K_L a_{CO_2} (CO_{2(d)} - H_{CO_2} R.T. CO_{2(g)}) \quad (1)$$

Ficara et al. (2000), Massone et al. (1998) and Gernaey et al. (1997), in developing and applying a titrimetric method for examining ammonia oxidation, addressed the effect of background physico-chemical processes by proposing that the carbon dioxide transfer rate (*CTR*) is constant throughout an experiment, i.e. prior to ammonia addition, and during and after the ammonia oxidation. As such, its impact on the hydrogen ion production rate (*HPR*) is also assumed constant and is accounted for by measuring and eliminating the so-called background *HPR*: the *HPR* prior to substrate addition or immediately after substrate removal. The assumption was justified on the basis that the experiments were 'short', and so the driving force for CO_2 transfer would be maintained at a somewhat constant rate throughout the study. The method is considered to be particularly useful for the study of nitrification as autotrophic activity has just a small impact on the concentration of dissolved inorganic carbon.

Gernaey et al. (2002a; b) applied the method to aerobic carbon oxidation processes. However, the method is expected to be applicable under certain experimental conditions that minimise the variation in CO_2 transfer during the experiment. Such conditions may for example include the use of a high pH or a closed system (as applied by Bogaert et al. (1997) for the titrimetric study of denitrification). In more general circumstances, the relatively large amount of CO_2 that is produced during heterotrophic activity increases the total amount of inorganic carbon in the liquid phase with a proportioned increase (determined by pH) in the concentration of every species of inorganic carbon. The increase in dissolved CO_2 concentration results in an increase in *CTR* and consequently, a change to the background hydrogen ion production rate. Gernaey et al. (2002a; b) suggested a linearity check of the cumulative hydrogen ion production (*Hp*) data, and provided that the slopes of *Hp* obtained before and after an experiment are the same then the data obtained could be used for modelling purposes. However, direct experimental evidence to support the equivalence of linearity in the titration data and constant CO_2 stripping is still missing.

The objectives of this work are to:

- Assess the general validity of the assumption that *CTR* and consequently background proton production is constant for a short biological wastewater treatment experiment
- Determine the experimental operating conditions for which the assumption is best suited.

Particular attention is paid to the errors induced when applying the assumption in cases when biological activity has significant influence on the dissolved inorganic carbon pool. Assessment is made by quantifying the deviation in background *HPR* caused by changes in inorganic carbon concentration that result from biological carbon oxidation (in this case acetic acid oxidation).

Materials and methods

Fundamentals for interpreting titrimetric data

The hydrogen ion production rate observed for the aerobic degradation of acetic acid has been described by Gernaey et al. (2002a) as:

$$HPR = -mr_{Ac} + pr_{NH_3} + n(CPR - CTR) \quad (2)$$

where:

m mmol H^+ is consumed per mmol of acetic acid consumed:

$$m = \frac{10^{-pKa}}{10^{-pHsp} + 10^{-pKa}} \quad (3)$$

p mmol H^+ is released when ammonia is assimilated for biomass growth:

$$p = \frac{10^{-pKa}}{10^{-pHsp} + 10^{-pKa}} \quad (4)$$

and *n* represents the contribution of CO_2 production and transfer to the hydrogen ion production through the bicarbonate system. *n* is the number of protons per mol of CO_2 dissolved.

$$n = \frac{2 \times 10^{2 \times pHsp} + 10^{(pHsp + pK_2CO_3)}}{10^{2 \times pHsp} + 10^{(pHsp + pK_2CO_3)} + 10^{(pK_1CO_3 + pK_2CO_3)}} \quad (5)$$

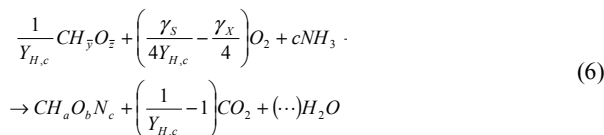
The CO_2 production rate (*CPR*) is associated with biomass production and/or intracellular polymer storage (Pratt et al., 2004).

Experimental work

An aerobic acetate oxidation experiment was carried out using a titrimetric and off-gas analysis (TOGA) sensor (see Pratt et al. (2003) for details), which allowed measurement of both *HPR* and the *CTR*. Mixed liquor (3 l) from a local domestic wastewater treatment plant was transferred to the TOGA reactor and aerated with a specialty gas, which was formed by mixing two gas streams: one stream with a flow rate of 140 ml·min⁻¹ containing 95% O_2 , 0.48% CO_2 and 4.52% Ar, and the other stream, containing He only, had a flow rate of 675 ml·min⁻¹. After 30 min, sodium acetate was added to the endogenously respiring sludge, resulting in an initial COD concentration of 210 mg·l⁻¹. The pH was controlled at 7.5 during the entire experiment. As well as *HPR* and *CTR*, the *OUR*, pH, DO and temperature were recorded frequently (at least every 8 s) during the experiment. The *OUR* resulting from exogenous activity was determined by subtracting the contribution of endogenous activity from the total *OUR*. Samples were regularly taken from the liquid phase for offline analysis of ammonium-nitrogen and volatile fatty acids (VFA). The nitrogen analysis was conducted using a Lachat QuickChem8000 Flow Injection Analyser. The VFA (in this case acetic acid) concentration was measured by gas chromatography with a DB-FFAP column at 140°C and an FID detector at 250°C.

Simulation

To assess the impact of mass transfer of CO_2 on titrimetric data obtained during aerobic carbon oxidation an acetic acid oxidation model was implemented using MATLAB/SIMULINK (Mathworks Inc.). The basis for the model is:



where:

$Y_{H,c}$ is the biomass yield based on carbon: 0.64 Cmol.Cmol⁻¹ (Van Aalst-Van Leeuwen et al., 1997);
 $Y_s = 4 + \bar{y} - 2\bar{z}$ is the degree of reduction of substrate CH_3O_2 : 4
 $Y_x = 4 + a - 2b - 3c$ is the degree of reduction of biomass
 $CH_aO_bN_c$: 4.2

The stoichiometric coefficient for O_2 was worked out using a redox balance. The model derivation has been reported in Pratt et al. (2004).

The CO₂ mass transfer model of Eq. (1) and a full model for the relevant weak acid/base systems including the acetate, ammonia and bicarbonate systems were also implemented.

Intracellular storage polymer formation was not considered. The production of polymer when acetate is available results in subsequent polymer oxidation and further CO₂ production (Van Aalst-Van Leeuwen et al., 1997), which complicates the interpretation of the *CTR* observed after the depletion of acetate.

To highlight the impact of CO₂ production and transfer on the hydrogen ion production, the following simplifying assumptions were made without losing general applicability:

- There is no endogenous respiration. Consequently, there is no background *CPR* ($CPR_{end} = 0$). It is further assumed that the gas/liquid transfer of CO₂ is in equilibrium prior to acetate addition, resulting in zero *HPR* prior to acetate addition. These assumptions will not affect the assessment as the impact of background processes on *HPR* is eliminated ultimately through subtracting the background *HPR* from the measured *HPR*.
- No NH₃ is assimilated during biomass growth. The *HPR* measured during acetate oxidation was thus solely caused by acetate consumption and CO₂ production and transfer.

While greatly simplifying the interpretation of the simulation data, the above assumptions have a minimal impact on the evaluation of the assumption that *CTR* is constant during the experiment.

In total, 176 simulations were performed using the model, each with a different $K_L a_{CO_2}$ value (ranging from 4 to 24 h⁻¹ with an increment of 2 h⁻¹) and/or a different pH set-point (ranging from 7.0 to 8.5 with an increment of 0.1). Air was used as the aeration gas. Each simulation started with a 2 h period without substrate addition, followed by the addition of an acetate pulse resulting in an initial acetate concentration in the reactor of 40 mgCOD·l⁻¹. Each simulation was then continued for three more hours. *CPR*, *CTR* and *HPR* were calculated and recorded. The maximum rate of biomass growth was 0.93 mgCOD·l⁻¹·min⁻¹, which is similar to that estimated in Gernaey et al. (2002a) (1.5

mgCOD·l⁻¹·min⁻¹ for acetate, 0.74 mgCOD·l⁻¹·min⁻¹ for dextrose). The duration of carbon oxidation was about 35 to 40 min.

Results and discussion

Experimental

Figure 1 shows the *CTR* and cumulative hydrogen ion production (*Hp*) measured prior to, during and after the addition of acetate in the experiment. Also shown in the figure are the oxygen uptake rate profile and the acetate and ammonia-nitrogen content profiles measured using lab analysis. From the measured DO (7.5 mg·l⁻¹ during endogenous respiration and 6.0 mg·l⁻¹ during exogenous respiration, data not shown) and *OUR* signals (Fig. 1), the oxygen transfer coefficient during the exogenous carbon oxidation was estimated to be 25 h⁻¹. This coefficient is not expected to be significantly different during periods prior to and after the carbon oxidation as the total gas flow rate into the reactor was unchanged (see the Material and Methods section). The CO₂ transfer coefficient was therefore estimated to be around 22.5 h⁻¹ (90% of the O₂ transfer coefficient, (Sperandio and Paul, 1997)) during the entire experiment.

It is important to observe that the *CTR* varied significantly during the relatively short experiment. Prior to substrate addition, the *CTR* decreased (3.3 to 3.0 mmol·h⁻¹ in 0.2 h) as the stripping of CO₂ from the liquid phase resulted in a reduced driving force for CO₂ transfer. With Eq. (1) it can be shown that this decrease in the *CTR* should be exponential. After acetate addition *CTR* increased exponentially (3.0 to 3.7 mmol·h⁻¹ in 1 h) as biological CO₂ production caused a gradual increase in the dissolved CO₂ concentration and thus a gradual increase in the driving force for CO₂ transfer. After the removal of acetate the *CTR* again decreased exponentially (3.7 to 1.8 mmol·h⁻¹ in 1.8 h) as a result of CO₂ stripping. It is expected that the *CTR* would continue to decrease until equilibrium between the gas and liquid phase CO₂ concentrations is reached. In the absence of endogenous respiration the *CTR* would reduce to zero in equilibrium. It is worthwhile to mention that an even more significant

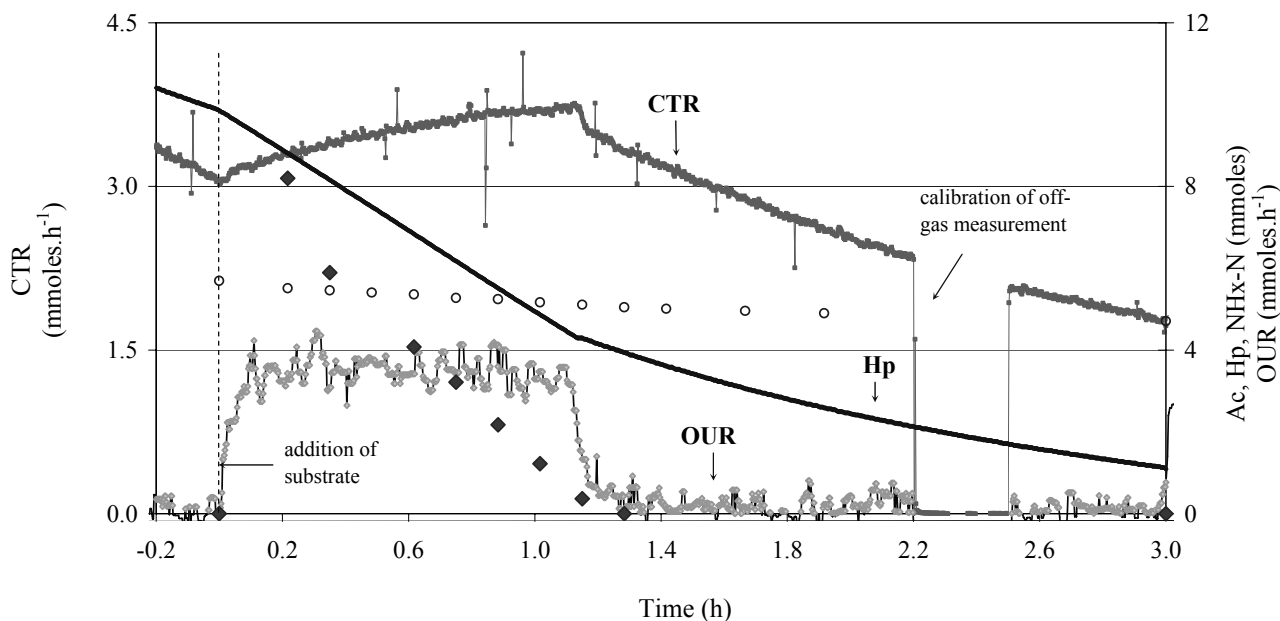


Figure 1
CTR and the accumulative hydrogen ion production (Hp) measured prior to, during and after acetate oxidation. The OUR resulting from exogenous activity and Ac (♦) and NHx-N (○) content are also shown.

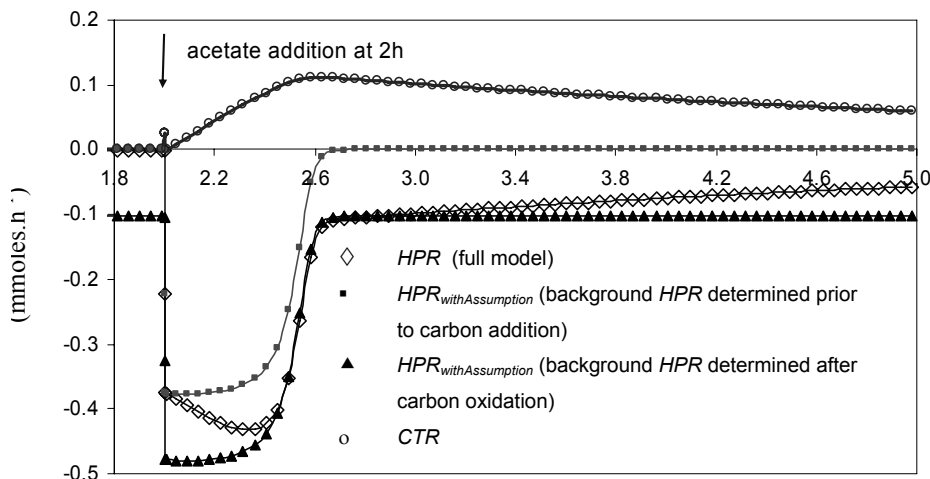


Figure 2
The simulated CTR and HPR for $pH_{sp} = 7.8$ and $K_L a_{CO_2} = 16 \text{ h}^{-1}$, in comparison with the HPR profiles predicted by assuming a constant background proton production. Note that the mismatches are solely caused by the assumption that CTR remains constant.

variation of CTR was observed in Beun et al. (2000), where $pH = 7$ was used.

This observed decrease of CTR prior to substrate addition indicates that CTR and consequently the background proton production are not constant. There are two reasons for the non-constant CTR in this particular experiment. First of all, the gas/liquid mass transfer of CO_2 had not reached equilibrium, causing a decreasing CTR before and also after the exogenous activities. Consequently, the HPR signal is not constant, causing non-linearity in the Hp signal. Based on the linearity check of the Hp signal suggested in Germaey et al. (2002a), this violation of the assumption of constant background HPR would have been detected, leading to the titration data being discarded.

The second reason for the variation in CTR is the CO_2 production by aerobic carbon oxidation, which caused a significant increase in CTR as shown in Fig. 1. It should be noted that the Hp data (which showed apparent linearity during exogenous activity) provided no indication of the variations in CTR. Therefore the linearity check suggested in Germaey et al. (2002a) would not detect this variation. As such, making direct linkages between Hp data and biological activity, as reported possible by Feitkenhauer and Meyer (2004), without properly accounting for variation in CO_2 transfer, would in this case result in substantial errors when interpreting titrimetric data, as will be further discussed through modelling and simulation studies.

Simulation

As an example, the simulated CTR and HPR for the case $pH_{sp} = 7.8$, $K_L a_{CO_2} = 16 \text{ h}^{-1}$ are shown in Fig. 2. Also shown in the figure is the HPR profiles predicted by assuming a constant background HPR:

$$HPR_{withAssumption} = -mr_{Ac} + nCPR + HPR_{background} \quad (7)$$

Any mismatches between the true HPR and $HPR_{withAssumption}$ can only be attributed to the assumption of constant CO_2 transfer. Two different $HPR_{background}$ values (determined from the HPR data prior to acetate addition and after acetate oxidation, respectively) were used in Eq. (7), resulting in two different predicted $HPR_{withAssumption}$ profiles (Fig. 2). Both have mismatches with the true HPR profile. The largest mismatch for each occurred during different periods of the experiment; when background HPR data was taken as the HPR before carbon addition then the largest mismatch was at the end of the experiment, whereas when background HPR data was taken as the HPR after the removal

of carbon then the largest mismatch was at the beginning of the experiment. For the quantification of the errors induced by assuming constant background proton production the HPR after acetate oxidation is used as $HPR_{background}$.

The CTR profile shown in Fig. 2 is clearly not constant. CTR started rising as soon as acetate was added, reaching its maximum when acetate was completely removed (data not shown) before gradually decreasing. The impact of this non-constant CTR on the HPR signal is clearly seen from the difference between the true HPR and $HPR_{withAssumption}$ profiles. To quantify the difference, the average percentage error (APE) defined below was used:

$$APE = \frac{\frac{1}{t_f - t_0} \int_{t_0}^{t_f} |HPR(t) - HPR_{withAssumption}(t)| dt}{\frac{1}{t_f - t_0} \int_{t_0}^{t_f} |HPR(t)| dt} \times 100\% \quad (8)$$

where:

The numerator is the average absolute error during the time interval $[t_0, t_f]$

The denominator is the average absolute HPR in the same period.

Given the fact that acetate was added at $t = 2 \text{ h}$, and completely removed at approximately $t = 2.7 \text{ h}$, $t_0 = 2 \text{ h}$, $t_f = 2.8 \text{ h}$ were chosen in the calculations. For the case presented in Fig. 2, for example, APE was calculated as 10.8%.

The calculation of the APE for all the simulated cases allowed the generation of the contour plot in Fig. 3, which shows the dependency of the APE on system pH and $K_L a_{CO_2}$. The APE increases with the increase in $K_L a_{CO_2}$ and/or the decrease in pH (because of the increased CTR), suggesting that the sensor should be operated at a high pH and a low $K_L a_{CO_2}$. In general, $pH > 8.0$ and $K_L a_{CO_2} < 10 \text{ h}^{-1}$ are required in order to keep APE within 5%.

When the assumption that CTR remains constant is not satisfied (for example when the biomass property at $pH = 7$ is studied), CTR needs to be quantified (Pratt et al., 2004) or properly modelled, or a closed system ($K_L a_{CO_2} = 0$) should be used (Ficara et al., 2003).

Conclusions

Titrimetric devices are popular tools for studying biological processes. However, to make use of titrimetric data, interferences from physico-chemical processes, like the bicarbonate system, need to be avoided or accounted for. Some authors have

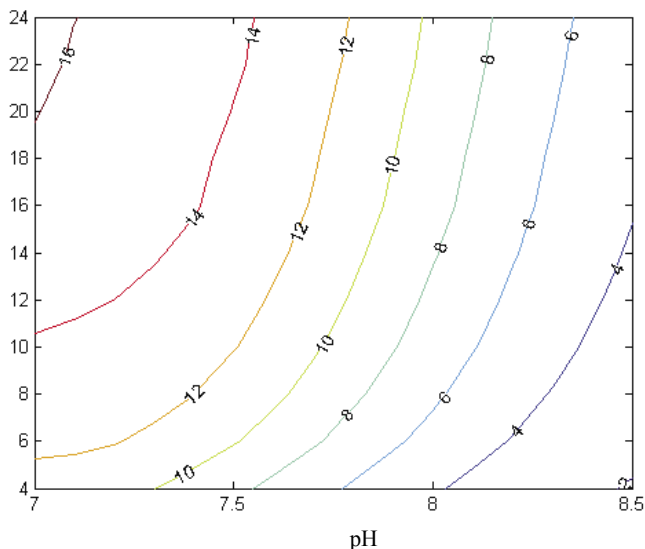


Figure 3

Average percentage error (as a function of pH and the CO_2 transfer coefficient) when predicting biologically instigated HPR; error caused by assuming a constant CTR.

suggested that the interference can be accounted for by assuming that it is constant throughout a titration experiment, which has been proposed to be verifiable through a linearity check of the titration data. This work indicates that, for the study of biological processes that have a significant impact on the dissolved inorganic carbon pool (e.g. heterotrophic COD oxidation processes), the interference that results from the action of the bicarbonate system is negligible (< 5% error induced) only when a titration is operated at high pH (pH > 8) and low gas transfer ($K_L a_{CO_2} < 10 \cdot h^{-1}$). Further, the linearity check proposed in the literature, while being able to verify if the CO_2 transfer prior to or immediately after aerobic COD oxidation is approximately constant, does not ensure that the CO_2 stripping rate during exogenous respiration is constant. Indeed, both experimental and simulation data presented in this work show that constant CO_2 stripping during aerobic carbon oxidation can only be achieved when the CO_2 transfer is minimised, which could be achieved by applying high pH (> 8) and low $K_L a$ ($K_L a_{CO_2} < 10 \cdot h^{-1}$) conditions. Outside these operating conditions it is suggested that the interference be accounted for by either measurement or modelling of CO_2 transfer.

References

- BEUN JJ, PALETTA F, VAN LOOSDRECHT MCM and HEIJNEN JJ (2000) Stoichiometry and kinetics of poly- β -hydroxybutyrate metabolism in aerobic, slow growing, activated sludge cultures. *Biotechnol. Bioeng.* **67** 379-389.
- BOGAERT H, VANDERHASSELT A, GERNAEY K, YUAN Z, THOYE C and VERSTRAETE W (1997) New sensor based on pH effects of denitrification processes. *J. Environ. Eng.* **123** 884-891.
- FEITKENHAUER H and MEYER U (2004) Software sensors based on titrimetric techniques for the monitoring and control of aerobic and anaerobic bioreactors. *Biochem. Eng. J.* **17** 147-151.
- FICARA E, ROZZI A and CORTELEZZI P (2003) Theory of pH-stat titration. *Biotechnol. Bioeng.* **82** (1) 28-37.
- FICARA E, MUSUMECI A and ROZZI A (2000) Comparison and combination of titrimetric and respirometric techniques to estimate nitrification kinetics parameters. *Water SA* **26** (2) 217-224.
- GERNAEY K, BOGAERT H, MASSONE A, VANROLLEGHEM PA and VERSTRAETE W (1997) Online nitrification monitoring in activated sludge with a titrimetric sensor. *Environ. Sci. Technol.* **31** (8) 2350-2355.
- GERNAEY K, PETERSEN B, NOPEN S I, COMEAU Y and VANROLLEGHEM PA (2002a) Modeling aerobic carbon source degradation processes using titrimetric and combined respirometric-titrimetric data: Experimental data and model structure. *Biotechnol. Bioeng.* **79** (7) 741-753.
- GERNAEY K, PETERSEN B, DOCHAIN D and VANROLLEGHEM PA (2002b) Modeling aerobic carbon source degradation processes using titrimetric data and combined respirometric-titrimetric data: Structural and practical identifiability. *Biotechnol. Bioeng.* **79** (7) 754-767.
- JACOBSEN C, LEONIS J, LINDERSTROM-LANG J and OTTESEN M (1957) The pH-STAT and its use in biochemistry. *Meth. Biochem. Anal.* **4** 171-210.
- MASSONE A, GERNAEY K, ROZZI A and VERSTRAETE W (1998) Measurement of ammonium concentration and nitrification rate by a new titrimetric biosensor. *Water Environ. Res.* **70** (3) 343-350.
- PRATT S, YUAN Z and KELLER J (2004) Modelling aerobic Carbon oxidation and storage by integrating respirometric, titrimetric, and off-gas CO_2 measurements. *Biotechnol. Bioeng.* **88** (2) 135-147.
- PRATT S, YUAN Z, GAPES D, DORIGO M, ZENG R and KELLER J (2003) Development of a novel titration and off-gas analysis (TOGA) sensor for study of biological processes in wastewater treatment systems. *Biotechnol. Bioeng.* **81** (4) 482-495.
- SPERANDIO M and PAUL E (1997) Determination of carbon dioxide evolution rate using on-line gas analysis during dynamic biodegradation experiments. *Biotechnol. Bioeng.* **53** (2) 243-252.
- VAN AALST-VAN LEEUWEN M, POT M, VAN LOOSDRECHT MCM and HEIJNEN JJ (1997) Kinetic modeling of poly(β -hydroxybutyrate) production and consumption by *Paracoccus pantotrophus* under dynamic substrate supply. *Biotechnol. Bioeng.* **55** (5) 773-782.

