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

Steven Pratt* and Zhiguo Yuan

1 Centre for Environmental Technology and Engineering, Massey University, New Zealand
2 Advanced Wastewater Management Centre, University of Queensland, Australia

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 Kₐ₁C₀₂ = 22.5 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 Kₐ₁C₀₂ < 10 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
Kₐ 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 CO₂ molecule dissolved
p fraction of NH₃ among NH₃ + NH₄⁺ in liquid phase
pK acid-base dissociation constant; general (pKa), ammonium (pKNH₄), carbonic acid (pK₁CO₃), bicarbonate (pK₂CO₃).
CTR carbon dioxide transfer rate
CPR carbon dioxide production rate
HPR hydrogen ion production rate
Hp cumulative net hydrogen ion production
H₄CO₂ Henry’s constant for carbon dioxide
OUR oxygen uptake rate
pHsp pH set point
r₄NH₄ rate of dissociation of ammonium
r₅ rate of dissociation of acid
r₆ rate of hydration of carbon dioxide
r₄CO₂ rate of dissociation of carbonic acid
r₅CO₂ rate of dissociation of bicarbonate
r₆ rate of removal of acetic acid
r₅NH₃ rate of removal of NH₃
R ideal gas constant
T temperature
Yₐ biomass yield based on carbon
Y_d degree of reduction of substrate CH₄ O₂
Y_g degree of reduction of biomass CH₄ O₂ N₃

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; \( [NH₄⁺] \rightleftharpoons [NH₃] + H⁺ \) when volatile fatty acids (VFA) are produced/consumed during anaerobic digestion then acid dissociation/formation results in a change in hydrogen ion concentration; \( [HA] \rightleftharpoons [A⁻] + H⁺ \); 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:

\[
\begin{align*}
CO₂ + H₂O \rightleftharpoons & H₂CO₃ \rightleftharpoons H⁺ + HCO₃⁻ \rightleftharpoons H⁺ + CO₂⁻ \\
& HCO₃⁻ \rightleftharpoons H⁺ + CO₂⁻ 
\end{align*}
\]

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,
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:

\[ HPR = -m \gamma_{H^+} + p \gamma_{NH_3} + n(CPR - CTR) \]  

where:

\[ CTR = K_{H^+}C(CO_\text{aq}) - H_{CO_\text{aq}}R.T.CO_{\text{H_2}} \]  

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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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).
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 $K_2 a_{CO_2}$ 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, 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
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 CO₂ 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 Gernaey 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 CO₂ 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 Gernaey 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 CO₂ 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_0 = 7.8, K_aCO₂ = 16 h⁻¹ are shown in Fig. 2. Also shown in the figure is the HPR profiles predicted by assuming a constant background HPR:

\[ \text{HPR}_{\text{with assumption}} = -mr_{dc} + n\text{CPR} + \text{HPR}_{\text{background}} \]  

(7)

Any mismatches between the true HPR and \( \text{HPR}_{\text{with assumption}} \) can only be attributed to the assumption of constant CO₂ transfer. Two different \( \text{HPR}_{\text{background}} \) values (determined from the Hp data prior to acetate addition and after acetate oxidation, respectively) were used in Eq. (7), resulting in two different predicted \( \text{HPR}_{\text{with assumption}} \) 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 \( \text{HPR}_{\text{background}} \).

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 \( \text{HPR}_{\text{with assumption}} \) profiles. To quantify the difference, the average percentage error (APE) defined below was used:

\[ \text{APE} = \frac{1}{t_f - t_0} \int_{t_0}^{t_f} \left( \text{HPR}(t) - \text{HPR}_{\text{with assumption}}(t) \right) \, dt \times 100\% \]  

(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 \( t = 2 \) h, and completely removed at approximately \( t = 2.7 \) h, \( t_0 = 2 \) h, \( t_f = 2.8 \) 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 \( K_aCO₂ \). The APE increases with the increase in \( K_aCO₂ \) 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 \( K_aCO₂ \).

In general, pH > 8.0 and \( K_aCO₂ < 10\cdot h^{-1} \) 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 (\( K_aCO₂ = 0 \)) should be used (Ficara et al., 2003).

**Conclusions**

Titrimetric devices are popular tools for studying biological processes. However, to make use of titrimetric data, interferes from physico-chemical processes, like the bicarbonate system, need to be avoided or accounted for. Some authors have...
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·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·h^-1) conditions. Outside these operating conditions it is suggested that the interference be accounted for by either measurement or modeling of CO_2 transfer.

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.

References


