Anoxic activated sludge monitoring with combined nitrate and titrimetric measurements

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Abstract An experimental procedure for anoxic activated sludge monitoring with combined nitrate and titrimetric measurements is proposed and evaluated successfully with two known carbon sources, acetate and dextrose. For nitrate measurements an ion-selective nitrate electrode is applied to allow for frequent measurements, and thereby the possibility for detailed determination of the denitrification biokinetics. An internal nitrate electrode calibration is implemented in the experiments to avoid the often-encountered electrode drift problem. It was observed that the best experimental design was with the carbon source in excess, since excess nitrate provoked nitrite build-up thereby complicating the data interpretation. A conceptual model could quantitatively describe the experimental observations and thus link the experimentally measured proton production with the consumption of electron acceptor and carbon source during denitrification.

Keywords Activated sludge; denitrification; ion-selective electrode; mathematical modelling; sensors; titrimetry

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Introduction

Respirometry, defined as the measurement and interpretation of the oxygen uptake rate (r_O), has turned out to be one of the most popular methods for the characterisation of wastewater composition and activated sludge biokinetics (Spanjers *et al*., 1998; Vanrolleghem *et al*., 1999). This is not surprising since the total respiration rate is affected by the concentration of all aerobically biodegradable components, to which the majority of wastewater components usually belong. However, for characterisation of denitrification under anoxic conditions respirometry fails. Thus, monitoring of nitrate utilisation rates (r_{NO3}) has been applied for denitrification monitoring. For example r_{NO3} has been used to determine the denitrification potential of a wastewater (Ekama *et al*., 1986; Kristensen *et al*., 1992; Naidoo *et al*., 1998; Spérandio, 1998; Urbain *et al*., 1998; Kujawa and Klapwijk, 1999). Alternatively, r_{NO3} measurements have been applied for biokinetic characterisation, and different studies have dealt with the comparison of r_O and r_{NO3} to assess anoxic rate reduction factors (e.g. McClintock *et al*., 1988; Kristensen *et al*., 1992; Orhon *et al*., 1996; Sözen *et al*., 1998). In these studies nitrate is typically measured via traditional analytical methods, and the sampling frequency is usually rather low (e.g. in the order of one sample every 5–10 min) compared to respirometric techniques (e.g. sampling every 10 sec). An exception may be cases where the traditional analytical method is incorporated in an on-line system, e.g. flow injection analysis systems allowing for more frequent data points (Pedersen *et al*., 1990). These systems, however, need sample preparation using filtration.

Another alternative to respirometry for characterisation of wastewater and activated sludge biokinetics is a titrimetric technique initially proposed by Ramadori *et al*. (1980). In this titrimetric method the amount of acid and/or base needed to keep the pH constant in an activated sludge sample where pH-affecting biological reactions occur, is monitored. This titrimetric method has been successfully applied for the monitoring of nitrification rates

and ammonium concentrations since nitrification has a well defined effect on the pH, resulting in titrimetric profiles that are rather straightforward to interpret (Massone *et al.*, 1995; Gernaey *et al.*, 1997). The titrimetric method has also been applied for monitoring of processes under anoxic conditions (Vanderhasselt, 1995; Massone *et al.*, 1996; Bogaert *et al.*, 1997; Devisscher *et al.*, 1998), and for the evaluation of the VFA concentration as readily biodegradable substrate in anaerobic digestion (Rozzi *et al.*, 1997). In yet another study the readily biodegradable COD available for denitrification was quantified, and control strategies for additional carbon source dosage were applied (Bogaert *et al.*, 1997). A complicating factor for the application of the titrimetric technique for denitrification monitoring is, however, that it is not a priori known whether the denitrification reaction will produce or consume protons as this depends on the carbon source and the actual pH of the activated sludge sample (Bogaert *et al.*, 1997).

In this study nitrate and titrimetric measurements were combined, thereby producing information-rich experiments for characterisation of denitrification kinetics. It is clear that this approach is equivalent to the combined titrimetric-respirometric measurements that have recently been applied successfully (Ficara *et al.*, 2000; Gernaey *et al.*, 2001a; Petersen *et al.*, 2001). This paper presents and evaluates the proposed methodology via validation experiments with additions of two known carbon sources, acetate and dextrose, to municipal activated sludge. A conceptual model linking proton production in the mixed liquor with nitrate and carbon source consumption is presented to explain the experimental observations quantitatively.

Materials and methods

A scheme of the experimental set-up is given in Figure 1. The set-up consists of a batch reactor with a volume of 2 litres. A cooling system is used to control the temperature. In order to obtain a high measurement frequency, it was chosen to measure nitrate directly via an ion-selective electrode submerged in the mixed liquor rather than to rely on a more complicated set-up (including filtration and an expensive chemical analysis or UV absorption method). The motivation to obtain frequent measurements is the necessity for a reliable determination of biokinetic parameters (Vanrolleghem and Spanjers, 1998). The nitrate

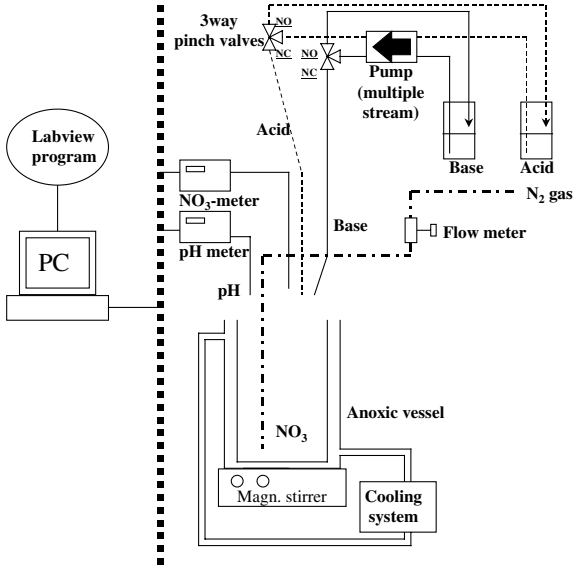

Figure 1 Illustration of the anoxic titrimetric set-up (NO = normally open; NC = normally closed)

and pH probe signals were logged by a PC equipped with the Labview software package (National Instruments).

A pH control system was also implemented in Labview. The pH was controlled within a narrow pH setpoint ± ΔpH region, as described by Gernaey *et al*. (1997). The pH setpoint was typically chosen between 7 and 8, and a ΔpH value of 0.03 pH units was used. When the pH was out of the pH setpoint ± ΔpH region, dosage of acid (0.05 N) or base (0.05 N) was done by opening an electromagnetic pinch valve for a short period (typically 1.5 s = 1 pulse). Acid and base solutions were continuously pumped around by a peristaltic pump to keep a constant liquid pressure in the tubes, and thus a constant dosage rate. When the valves are closed the acid and base flows are recycled to the storage vessels. Opening a valve diverts the acid or base flow to the bioreactor. Calibration of the dosage system was done by collecting the volume of acid or base dosed during 50 subsequent pulses. The cumulative amount of acid and base dosed during an experiment were logged.

For the experimental work activated sludge was sampled at the combined municipal–industrial wastewater treatment plant of Zele (operated by Aquafin NV, Aartselaar, Belgium). During the experiments small substrate pulses (e.g. 10 ml) of acetate or dextrose (10 g COD/l), and nitrate (10 g N/l) stock solutions were dosed to the activated sludge.

Conceptual proton production model

Models that link proton production during carbon source degradation to carbon source and electron acceptor consumption have for example been presented by Bogaert *et al*. (1997) for the denitrification process and by Gernaey *et al*. (2001b) for aerobic degradation of a carbon source. The conceptual model presented here is an extension to anoxic conditions of the proton production model of Gernaey *et al*. (2001b). The conceptual model (see Figure 2) is based on the assumption that all compounds will pass the cell wall as uncharged molecules. For denitrification of NO_3-N to N_2 gas, 4 processes will thus have an effect on the proton balance in the mixed liquor (which is assumed to have constant pH due to the action of the pH controller): (1) Uptake of the carbon source, (2) uptake of the electron acceptor, (3) uptake of NH_3 for growth and (4) production of CO_2. The proton consumption or pro-

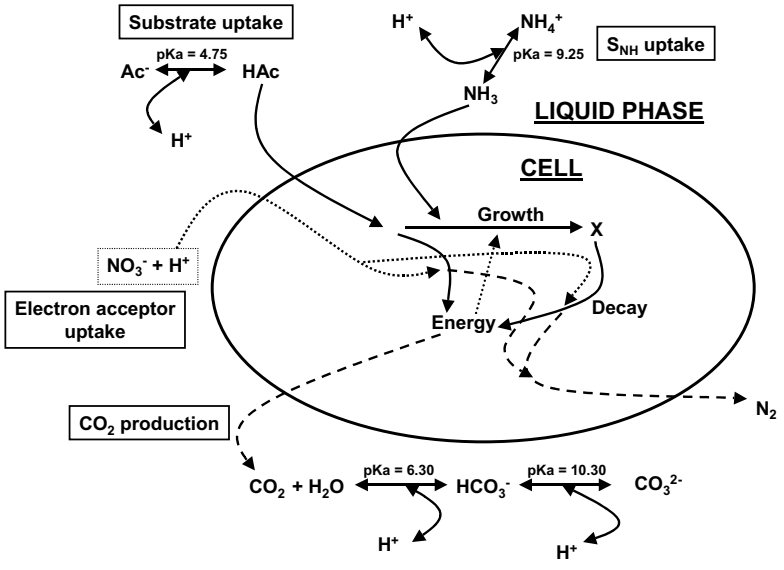

Figure 2 Illustration of the conceptual model (acetate as model component) that links proton production (or consumption) to electron acceptor uptake and substrate uptake during denitrification of NO_3-N to N_2 gas

duction due to each of these processes will depend on the actual pH of the mixed liquor. This will be illustrated for acetate as an example of a carbon source. The fraction of undissociated acetate in the liquid phase will increase as the pH of the mixed liquor decreases, especially when the latter varies around the pKa of acetic acid (4.75). During acetate uptake through the cell wall a proton will only be consumed for each dissociated acetate molecule that is taken up. Undissociated acetate will not result in proton consumption. The effect of acetate uptake on the proton balance in the mixed liquor will consequently depend on the actual pH of the mixed liquor: At high pH the effect will be more pronounced. Similar pH depending relationships will exist for NH_3 uptake and CO_2 production (see Gernaey *et al.* (2001b) for a detailed explanation). For denitrification with NO_3^- as electron acceptor it can be assumed that one proton is taken up per NO_3^- ion passing the cell wall since HNO_3 is a strong acid.

The reaction equation for the process illustrated in Figure 2 is given in Eq. (1). In that equation m, n and p are the result of the pH depending dissociation equilibria for carbon source, ammonia and CO_2 components.

$$\frac{1}{Y_H}S_S + \frac{1-Y_H}{2.86\cdot Y_H}S_{NO3} + i_{XB}S_{NH} \rightarrow X + \frac{1-Y_H}{2.86\cdot Y_H}S_{N2} + \left(-\frac{m}{C\cdot Y_H} - \frac{1-Y_H}{2.86\cdot 14\cdot Y_H} + \frac{p\cdot i_{XB}}{14} + \frac{n\cdot(1-Y_H)\cdot x}{C\cdot Y_H}\right)Hp \quad (1)$$

$$\text{with } m = \frac{[A^-]}{[HA]+[A^-]} = \frac{10^{-pKa}}{10^{-pH}+10^{-pKa}}$$

$$n = \frac{2\cdot 10^{2\cdot pH} + 10^{(pH+pK2CO2)}}{10^{2\cdot pH} + 10^{(pH+pK2CO2)} + 10^{(pK1CO2+pK2CO2)}};\ p = \frac{\left[NH_4^+\right]}{\left[NH_4^+\right]+\left[NH_3\right]} = \frac{10^{-pH}}{10^{-pH}+10^{-pKNH4}}$$

Based on the mass balance of Eq. (1) and specific stoichiometric parameters, the predicted proton production during denitrification of nitrate with acetate as carbon source can be plotted as a function of pH (Figure 3). Note that we applied the convention that proton production gets a positive sign whereas proton consumption (i.e. negative proton production) will get a negative sign for the conceptual model. The proton production (expressed as meq/meq NO_3-N) varies considerably when the pH of the liquid phase changes (Figure 3).

Results and discussion

Internal calibration of the nitrate electrode

It is known that nitrate electrodes may be difficult to operate due to electrode drift and the need for frequent calibration (Pedersen *et al.*, 1990). Furthermore, the electrode signal

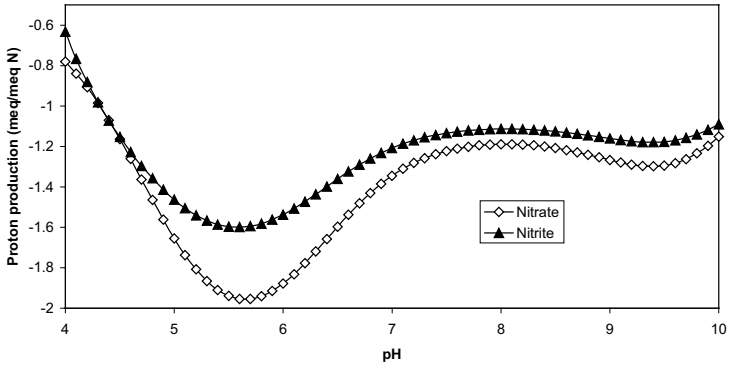

Figure 3 Variations of the proton production during denitrification with acetate as carbon source and nitrate or nitrite as electron acceptor according to the conceptual model of Figure 2 (Y_H = 0.67; i_{XB} = 0.086)

(more particularly the slope of the calibration line) depends on the sample it is submerged in due to, e.g., differences in ionic strength. This means that a calibration carried out with nitrate standards may not be transferable to an actual activated sludge sample. When determining nitrate in the laboratory via an ion-selective electrode the ionic strength of the sample is typically increased, via additions of a buffer solution (APHA, 1989). However, addition of buffer solution is not possible in tests for determination of the activated sludge biokinetics since the addition of buffer solution could change the biological activity of the sludge, which is obviously not desirable. These problems of unreliable electrode calibration and drifts were confirmed in this study. Thus, frequent recalibration is a necessity for this type of nitrate measurement, leading to undesirable interruption of the measurements. However, since the experiments to characterise the anoxic behaviour of the sludge are typically (fed-)batch experiments in which the nitrate is exhausted completely by the end of the experiment(s) (see below), the (re)calibration of the nitrate electrode becomes very simple. Before the experiment starts a zero reading is recorded (since nitrate is absent from the mixed liquor), and this is followed by addition of a known amount of nitrate (e.g. 10 mg NO_3-N/l). Thereby, the two data points that are necessary for electrode calibration are inherently present in the experimental data, and electrode calibration can be done in each experiment prior to the addition of carbon source. Note also that the titrimetric method maintains a constant pH in the sludge sample (see above) and thus favours good operation of the nitrate electrode since pH variations may influence the nitrate electrode readings (APHA, 1989).

Experiments with excess nitrate

Initially the purpose was to carry out the experiments with the electron acceptor (nitrate) in excess and to have the electron donor (carbon source) as the limiting factor, thereby having a system similar to respirometric tests where the electron acceptor (oxygen) is in excess during the degradation of the electron donor. Such an experimental approach could be used to obtain data that allow the identification of the maximum specific growth rate of the denitrifying bacteria together with the Monod half-saturation coefficient for the carbon source. However, in experiments with acetate as carbon source it appeared that the ratio of the amount of acid dosed (measured via the titrimetric method) to the amount of nitrate consumed (measured via the nitrate electrode) increased when e.g. three subsequent amounts of acetate were dosed under conditions where nitrate was in excess. Figure 4 illustrates this observation for one typical experiment. Furthermore, Figure 4 shows that the ratio of added COD to consumed nitrate nitrogen (corrected for endogenous respiration) is increasing for

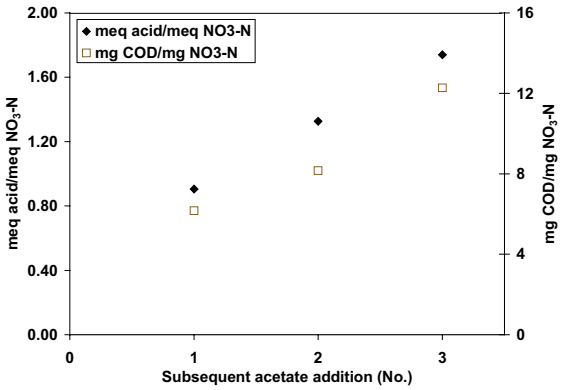

Figure 4 Ratio between amount of acid dosed during a titrimetric experiment and amount of NO_3-N consumed and between amount of COD added and amount of nitrate consumed for 3 subsequent experiments with nitrate in excess

three subsequent additions of acetate. This means that the stoichiometry of the reactions is not constant, which is not what one expects on the basis of microbial processes at equilibrium. In the work of Vanderhasselt (1995) similar phenomena were observed.

It was hypothesised that the change of this ratio was caused by nitrite build-up, and that the accumulated nitrite was only denitrified at the end of the experiment. Indeed, presence of nitrite was detected during the experiments via nitrite strips, but it was not analytically quantified. The assumption of nitrite build-up corroborates the data of Figure 4 because nitrite is not measured with the nitrate electrode used. Consequently, all COD that is used to denitrify nitrite to N_2 gas is not reflected in the nitrate measurements, and increased ratios of COD to nitrate nitrogen are observed as a result. The increased ratio between meq acid dosed and the measured meq NO_3-N consumed during the titrimetric experiment can be explained because denitrification of nitrite to N_2 gas will also result in considerable proton consumption.

In Figure 3 we have also illustrated the (slightly different) pH effect of denitrification of acetate with nitrite as electron acceptor. The NO_2^- curve in Figure 3 was obtained based on Eq. (2). Note that the proton production due to NO_2^- uptake will have a pH depending effect on the proton balance in the mixed liquor since (in contrast to HNO_3) HNO_2 is a weak acid. The fraction of NO_2^- in the mixed liquor is represented by q in Eq. (2).

$$\frac{1}{Y_H}S_S + \frac{1-Y_H}{1.72\cdot Y_H}S_{NO2} + i_{XB}S_{NH} \rightarrow X + \frac{1-Y_H}{1.72\cdot Y_H}S_{N2} + \left(-\frac{m}{C\cdot Y_H} - \frac{q\cdot(1-Y_H)}{1.72\cdot 14\cdot Y_H} + \frac{p\cdot i_{XB}}{14} + \frac{n\cdot(1-Y_H)\cdot x}{C\cdot Y_H}\right)Hp \qquad (2)$$

$$\text{with: } q = \frac{[NO_2^-]}{[HNO2]+[NO_2^-]} = \frac{10^{-pKHNO2}}{10^{-pH}+10^{-pKHNO2}}$$

In a study of Oh and Silverstein (1999) it was observed that nitrite accumulated in denitrifying activated sludge when the carbon source (acetate) was limiting. On the other hand, in the same study it was found that adding an excess of acetate led to a complete removal of nitrate at a constant rate without nitrite accumulation. Clearly, it is not desirable to have interference of nitrite accumulation in the experiments with combined nitrate and titrimetric measurements for characterisation of denitrification kinetics. In this respect it is noteworthy that experiments for anoxic activated sludge characterisation typically are based on monitoring of nitrate utilisation rates in the presence of excess nitrate (e.g. Ekama *et al*., 1986; Rozzi *et al*., 1997; Naidoo *et al*., 1998; Spérandio, 1998; Urbain *et al*., 1998; Kujawa and Klapwijk, 1999). However, from the above observations, it is important to reflect on the fact that the possible occurrence of nitrite build-up may limit the usefulness of the results derived from such tests, unless the nitrite concentrations are quantified as well. This was investigated in more detail in the work of Sözen and Orhon (1999) where different patterns of nitrite build-up were observed, and methods to correct for the nitrite accumulation on the evaluation of different parameters were discussed.

Experiments with excess carbon source

Our experimental approach was therefore changed, and the experiments were carried out with the carbon source in excess, and with nitrate as the limiting factor. Such experiments will still yield information on the maximum specific growth rate of the denitrifying bacteria (via the maximum nitrate utilisation rate) but will not yield information on the half-saturation coefficient for the carbon source. Instead, information on the half-saturation coefficient of nitrate will become available.

Figure 5 shows the combined nitrate and titrimetric measurements for experimental examples with additions of acetate and dextrose respectively. It is important to notice that the titrimetric data in Figure 5 are expressed as cumulative additions of acid and base respectively. During the experiments it indeed appeared that acid addition was needed for experiments with acetate, indicating proton consumption, while base addition was needed for experiments with dextrose, indicating proton production. These experimental observations were confirmed during calculations with the conceptual model for the experimental pH range. Moreover, Bogaert *et al.* (1997) calculated a similar effect with their model for these substrates. The main difference between dextrose and acetate as substrates for denitrification in the conceptual model of Figure 2 is that dextrose is present in the mixed liquor as an undissociated molecule. Consequently, dextrose uptake through the cell wall does not result in proton consumption, as is the case for acetate. In the case of dextrose, other processes such as CO_2 release and NH_3 uptake for biomass growth give rise to a proton production that is sufficiently large to compensate for the proton consumption due to nitrate uptake at pH values above 7. The conceptual model thus supports the data quantitatively at this point.

Figure 6 shows the observed linear relationship between the amount of nitrate dosed and the cumulative amount of acid dosed (proton consumption) for denitrification experiments at pH 7.5 with acetate as a carbon source. The linearity observed in Figure 6 proves that the proposed methodology works well. Moreover, the linear relationship between proton consumption for denitrification and nitrate consumption is a good indication that no nitrite build-up takes place during the experiments. Nitrite build-up, as discussed before, would result in a variable ratio between proton consumption for denitrification and nitrate consumption. The titrimetric data can therefore be used as a verification of the quality of the nitrate data and the experiment in general. Thus, variations in the ratio of proton consumption to nitrate consumption indicate nitrite build-up, and possible problems with the interpretation of the nitrate data, as was illustrated in Figure 4.

The slope in Figure 6, i.e. the ratio of acid dosage to nitrate consumption, was 1.01 meq H^+ consumed/meq NO_3-N. For the conceptual model presented in this paper a value of 1 meq H^+ consumed/meq NO_3-N is obtained for $Y_H = 0.59$. This value is lower than the reference value of 0.67 that is given for the parameter Y_H by Henze *et al.* (1987), but in accordance with Orhon *et al.* (1996) who reported that the biomass yield is lower under anoxic conditions. Thus, the conceptual model seems to be able to describe the stoichiometry of the denitrification reaction realistically. A similar linearity between proton production and nitrate consumption was found for the experiments with dextrose, where an average experimental value of 0.32 meq H^+ produced/meq NO_3-N was found. The prediction of the conceptual model gave a stoichiometric factor of 0.32 meq H^+ produced/meq NO_3-N for $Y_H = 0.64$.

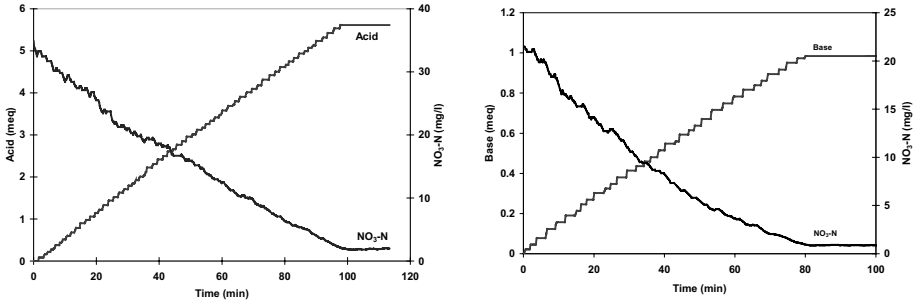

Figure 5 Example of combined titrimetric (cumulative addition of acid (for acetate) or base (for dextrose)) and nitrate measurements for experiments with addition of acetate (left) or dextrose (right) in excess

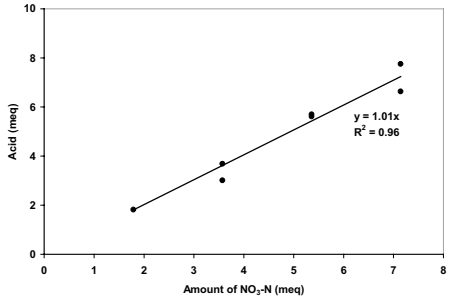

Figure 6 Cumulative amount of acid dosed during denitrification with acetate as a carbon source, plotted as function of the initial amount of nitrate nitrogen added

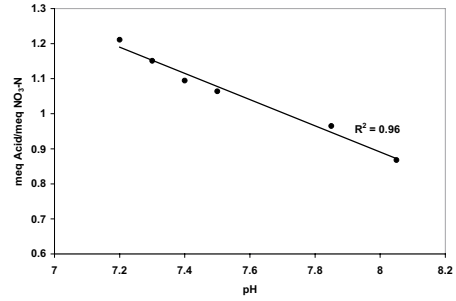

Figure 7 Ratio between meq acid added (which means the same as protons consumed) and meq NO_3-N consumed, plotted as function of pH, for acetate as denitrification carbon source

Finally, Figure 7 gives the experimentally observed dependency between the ratio of proton to nitrate consumption and the pH value, for acetate as carbon source. Such a pH dependency was also predicted by the conceptual model (see Figure 3). Figure 3 indeed shows that the proton consumption for denitrification of acetate with nitrate as electron acceptor decreases with increasing pH values for the pH range in Figure 7.

Conclusions and perspectives

Combined nitrate and titrimetric measurements were demonstrated to be a good tool to monitor the denitrification process in batch experiments with activated sludge. To avoid the often-encountered problems of nitrate electrode drift, a simple internal electrode calibration is implemented in the experimental strategy to ensure stable readings. It appeared that experiments with the carbon source rather than nitrate in excess is preferable to avoid the undesirable phenomenon of nitrite build-up. The proposed set-up provides data with a high frequency, which opens perspectives to further model-based data interpretation for the determination of biokinetic parameters. A conceptual model was proposed to link the titrimetric data to carbon source and electron acceptor consumption profiles. The model could quantitatively explain the experimental observations. Based on the combined nitrate and titrimetric data it should be possible to obtain the maximum specific growth rate of the denitrifying bacteria and the Monod half-saturation constant for nitrate.

Nomenclature

A^-	Monoprotic acid, dissociated
C	Conversion factor (g COD/mol)
COD	Chemical oxygen demand
HA	Monoprotic acid, undissociated
Hp	Proton concentration in mixed liquor (meq/l)
i_{XB}	Fraction of N in biomass (g N/g COD biomass)
m	Fraction of dissociated acid A^- in the liquid phase for a monoprotic acid HA
n	Number of protons produced per CO_2 molecule released
NH_3	Ammonia
NH_4^+	Ammonium
p	Fraction of NH_4^+ in liquid phase
pK1CO2	Negative logarithm of first acid dissociation constant for H_2CO_3
pK2CO2	Negative logarithm of second acid dissociation constant for H_2CO_3
pKa	Negative logarithm of acid dissociation constant
pKHNO2	Negative logarithm of acid dissociation constant for HNO_2

pKNH4	Negative logarithm of acid dissociation constant for NH_4^+
q	Fraction of dissociated HNO_2 in the liquid phase
S_{N2}	Gaseous nitrogen concentration (mg N/l)
S_{NH}	Ammonia + ammonium (concentration) (mg N/l)
S_{NO2}	Nitrite nitrogen concentration ($NO_2^- + HNO_2$) (mg N/l)
S_{NO3}	Nitrate nitrogen concentration (mg N/l)
S_S	Readily biodegradable substrate concentration (mg COD/l)
x	Number of carbon atoms per substrate molecule
X	Biomass concentration (mg COD/l)
Y_H	Yield coefficient for heterotrophic biomass (g COD/ g COD oxidised)

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