Modeling Aerobic Carbon Source Degradation Processes Using Titrimetric Data and Combined Respirometric–Titrimetric Data:

Experimental Data and Model Structure

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Abstract: Experimental data are presented that resulted from aerobic batch degradation experiments in activated sludge with simple carbon sources (acetate and dextrose) as substrates. Data collection was done using combined respirometric–titrimetric measurements. The respirometer consists of an open aerated vessel and a closed non-aerated respiration chamber for monitoring the oxygen uptake rate related to substrate degradation. The respirometer is combined with a titrimetric unit that keeps the pH of the activated sludge sample at a constant value by addition of acid and/or base. The experimental data clearly showed that the activated sludge bacteria react with consumption or production of protons during aerobic degradation of the two carbon sources under study. Thus, the cumulative amount of added acid and/or base could serve as a complementary information source on the degradation processes. For acetate, protons were consumed during aerobic degradation, whereas for dextrose protons were produced. For both carbon sources, a linear relationship was found between the amount of carbon source added and the amount of protons consumed (in case of acetate: 0.38 meq/mmol) or produced (in case of dextrose: 1.33 meq/mmol) during substrate degradation. A model taking into account substrate uptake, CO_2 production, and NH_3 uptake for biomass growth is proposed to describe the aerobic degradation of a $C_xH_yO_z$-type carbon source. Theoretical evaluation of this model for reference parameters showed that the proton effect due to aerobic substrate degradation is a function of the pH of the liquid phase. The proposed model could describe the experimental observations with both carbon sources. © 2002 Wiley Periodicals, Inc. *Biotechnol Bioeng* **79**: 741–753, 2002.
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INTRODUCTION

Respirometry is a generally applied tool to characterize aerobic degradation processes in activated sludge (Henze et al., 1987; Spanjers et al., 1998; Vanrolleghem et al., 1999). Respirometry is the measurement and interpretation of the respiration rate of activated sludge and is defined as the amount of oxygen per unit of volume and time that is consumed by the microorganisms in activated sludge. Respirometric applications have been developed for the characterization of aerobic carbon source degradation processes as well as for nitrification (oxidation of ammonium to nitrate with nitrite as intermediate product).

Besides respirometry, titrimetric experiments can also yield information about biological nitrogen removal processes in activated sludge (Bogaert et al., 1997; Gernaey et al., 1998; Massone et al., 1996; Ramadori et al., 1980). Indeed, the pH value of a biological system responds to microbial reactions, and evolution of the pH of a system often provides a good indication of some of the ongoing biological reactions. For aerobic degradation processes in activated sludge, the processes that mostly influence the pH of the liquid phase are as

follows: (1) nitrification, which causes a pH decrease due to proton production (Gernaey et al., 1998; Massone et al., 1996; Ramadori et al., 1980); (2) degradation of organic matter, which affects pH due to (a) the uptake of the carbon source through the cell wall of the bacteria, (b) the release of CO_2 resulting from respiration processes in the liquid phase, and (c) the uptake of ammonium for growth (Iversen et al., 1994; San and Stephanopoulos, 1984; Siano, 1995); and (3) stripping of CO_2 due to aeration.

The pH effects observed in a liquid medium can be related to the biological process rates and kinetics. However, one difficulty encountered with the observation of pH changes is the variable buffer capacity of the liquid medium due to the presence of several acid–base buffer systems with pH-dependent buffer capacity (Stumm and Morgan, 1981). The pH variation of the liquid medium during biological reactions is thus difficult to convert into a precise number of protons that is released or consumed. The problems caused by the pH depending buffer capacity of the liquid medium can be avoided by controlling the pH of the liquid medium at a constant pH setpoint through addition of acid and/or base. In that case, monitoring the acid and/or base consumption rate, which is needed to keep the pH constant, provides the rate of proton formation or consumption related to biological reactions.

Model-based analysis of titrimetric data can be used to estimate biokinetic parameters of the biological degradation processes. This approach has already been used in fermentation (Iversen et al., 1994). Specifically for wastewater treatment, a relatively simple model-based analysis of titrimetric data has been applied successfully to the nitrification process (Gernaey et al., 1998, 2001; Petersen, 2000; Petersen et al., 2000, 2001) based on the Activated Sludge Model No. 1 nitrification stoichiometry (Henze et al., 1987). When ammonium is added to an activated sludge sample, nitrification can be measured through both its oxygen consumption and its proton production. Thus combined respirometric–titrimetric data sets can be collected (Devisscher, 1997; Gernaey et al., 2001; Petersen et al., 2000). The most important advantage of a combination of respirometric and titrimetric measurements, however, is that two independent measurements are obtained simultaneously for the same process. This results in a higher information content of the experimental data and, therefore, more accurate determination of wastewater composition and biodegradation kinetics. It was illustrated for the nitrification process that the confidence intervals on the estimated biokinetic parameters improve significantly when combined respirometric and titrimetric data are applied, in comparison with a situation where only respirometric or only titrimetric data are available (Gernaey et al., 2001; Petersen et al., 2001).

For one available data set (e.g., oxygen uptake rate data resulting from a respirometric experiment, or titrimetric data), it has been shown that three combinations of parameters can be estimated from the data related respectively to the growth rate, the substrate affinity constant, and the initial substrate concentration (Dochain et al., 1995; Petersen et al., 2000). The biomass yield appears in all three parameter combinations and thus cannot be identified uniquely. However, for the nitrification process the autotrophic biomass yield becomes theoretically identifiable (i.e., can be identified uniquely assuming perfect noise-free data) from combined respirometric–titrimetric data sets without exact knowledge of the initial substrate concentration, by combining the information available from the separate data sets (Devisscher, 1997; Petersen et al., 2000). The practical identifiability of the autotrophic biomass yield from combined respirometric–titrimetric data was confirmed in Petersen et al. (2001), i.e., the autotrophic yield could be estimated uniquely from the available combined data sets. This is an important finding because the yield is an essential parameter in substrate degradation models. Indeed, the yield determines the distribution of consumed substrate between biomass growth and energy production.

With the developments that were done for the nitrification process in mind (Petersen et al., 2000, 2001), the first essential step for the estimation of biokinetic parameters for aerobic degradation of a carbon source from titrimetric data or combined respirometric–titrimetric data would be the availability of a model. If such a model were available, and if results obtained for the nitrification process could be confirmed, the heterotrophic biomass yield could be identified immediately on the basis of the data of one experiment in which a known carbon source is added to an activated sludge sample.

In this study a combined respirometric–titrimetric experimental set-up is described, and data collected with this set-up for two different carbon sources (acetate and dextrose) are shown. On the basis of an existing model for interpretation of respirometric data, a theoretical model is presented that links the degradation of a $C_xH_yO_z$-type carbon source to the proton consumption or production observed during the aerobic degradation process. In this paper the aims are thus to present the experimental methods used together with the resulting data and to qualitatively describe the proton production or consumption measured during aerobic degradation of both substrates. A more in-depth evaluation of the proposed model will be given in the following paper (this issue), which focuses on theoretical and practical parameter identifiability when using titrimetric and combined respirometric–titrimetric data to monitor aerobic carbon source degradation during batch degradation experiments (Gernaey et al., 2002).

MATERIALS AND METHODS

Set-up

Data were collected using a combined respirometric–titrimetric set-up (Gernaey et al., 2001). A schematic overview of the different components of the set-up is shown in Figure 1. The set-up consists of an aeration vessel (V = 2 L) and a respiration chamber (V = 0.5 L). The respiration chamber is completely closed and is not aerated. The contents of both vessels are mixed by magnetic stirrers with adjustable speed. A peristaltic pump with adjustable speed (pump 1 in Fig. 1) is used to continuously pump the activated sludge around in the set-up. A cooling system (Lauda WK1400) is used to control the temperature in the aeration vessel. The aeration vessel and the respiration chamber are both equipped with a dissolved oxygen electrode (Ingold/Mettler Toledo, Inpro 6400). The dissolved oxygen probes are connected to a transmitter (Knick 73 O_2 for the aeration vessel and Knick Stratos 2401 Oxy for the respiration chamber). The 4–20 mA transmitter signals are collected on a PC equipped with the Labview software package (National Instruments) and a combined A/D I/O card (National Instruments, AT-MIO-16XE-50).

The pH controller is installed in the aeration vessel. The pH in the aeration vessel is measured with a Mettler Toledo HA 405-DXK-S8/120 Xerolyte pH electrode connected to a Knick 73 pH transmitter. The 4–20 mA signal is logged with the same Labview software, and pH control was also implemented in Labview. The pH was controlled within a narrow pH setpoint ± ΔpH region, as described by Gernaey et al. (1997). Only the base dosage system is shown in Fig. 1 to prevent the scheme from being overloaded. The pH setpoint was typically chosen between 7.5 and 8.5, and a ΔpH value of 0.03 pH unit was used. When the measured pH value did not lie in the pH setpoint ± ΔpH region, acid (0.05 N) or base (0.05 N) was added by opening an electromagnetic pinch valve for a short period (typically 1.5 s = 1 pulse) to adjust the pH. Acid and base were continuously pumped around by a peristaltic pump (see Fig. 1) to keep a constant liquid pressure in the dosage system 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 aeration vessel. The dosage system was calibrated on the basis of the measurement of the volume of acid or base collected during 50 subsequent pulses (average dosage = 3.32 ± 0.013 mL/50 pulses for 19 calibrations). The cumulative amounts of acid and base dosed during an experiment were logged with the Labview software package.

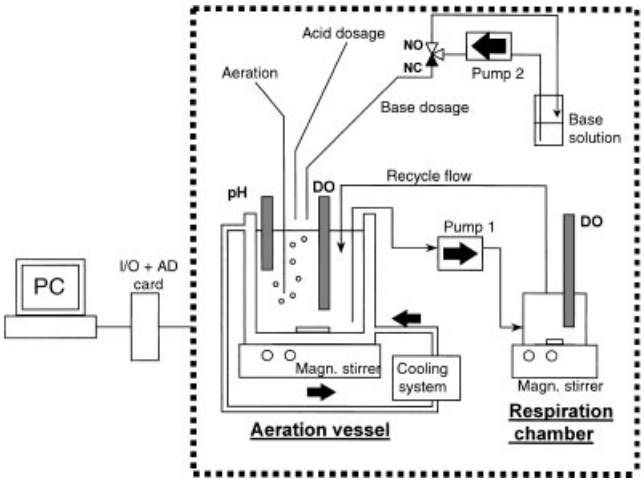

Figure 1. Schematic overview of the set-up for combined respirometric–titrimetric measurements.

Experimental Work

Activated sludge was sampled at the municipal wastewater treatment plant of Zele (operated by Aquafin NV, Aartselaar, Belgium) and transported to the laboratory. At the start of an experiment, the set-up was filled with 2.5 L of activated sludge. The activated sludge was aerated until the endogenous respiration phase was reached (typically 12 h or more). During the experiments small substrate pulses (e.g., 10 mL) of concentrated stock solutions (e.g., acetate 10 g COD/L, dextrose 10 g COD/L) were dosed to the activated sludge in the aeration vessel.

RESULTS

Data Set

The set-up was originally built to perform parallel respirometric and titrimetric experiments aiming to characterize nitrification in activated sludge. Results of these experiments can be found in Gernaey et al. (2001), Petersen (2000), and Petersen et al. (2000, 2001). The results described in this paper focus on the degradation of organic carbon sources by activated sludge.

Experiments were always done by addition of substrate pulses. As soon as the degradation of one substrate pulse was finished, another pulse was added. An example of a typical data set obtained with the set-up is given in Figure 2. In this experiment 0.781 mmol acetate (50 mg COD) was added at t = 0 to the aeration vessel of the set-up. The dissolved oxygen concentration measured in the aeration vessel ($S_{O,1}$) decreases immediately, and acid is added to compensate for a net proton consumption of the activated sludge (to maintain a constant pH). The dissolved oxygen concentration in the respiration chamber ($S_{O,2}$) decreases also, since sub-

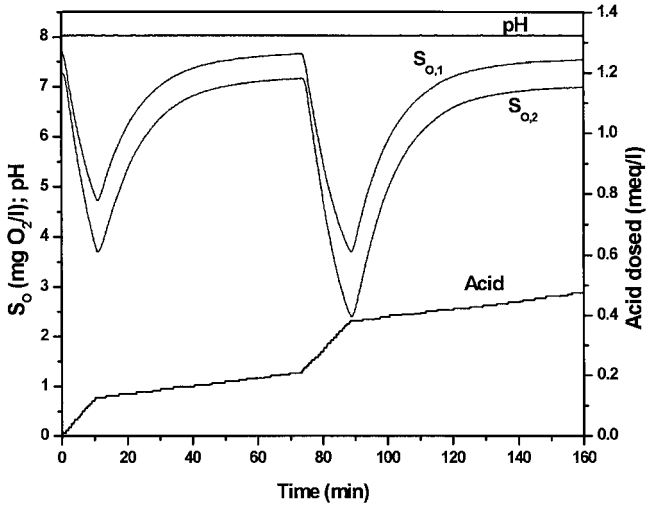

Figure 2. Typical data series collected during a batch substrate degradation experiment with the respirometric–titrimetric sensor following the addition of 0.781 mmol acetate (t = 0) and 1.172 mmol acetate (t = 74 min).

strate is transported to this vessel together with the activated sludge that is pumped around continuously between both vessels. In this case, all substrate is degraded at t = 10 min. From then on, the dissolved oxygen concentration increases again, due to input of oxygen via the continuous aeration in the aeration vessel. When the acetate degradation is finished, the acid dosage rate falls back to a background level. This background acid dosage level is related to CO_2 stripping and is influenced by the choice of the pH setpoint. Indeed, for this experiment CO_2 stripping caused the pH to increase, and acid dosage was needed to keep the pH of the mixed liquor constant. This background acid dosage was assumed to be constant for the short duration of each experiment. The fact that both the dissolved oxygen concentration and the acid addition rate react on the addition of the substrate clearly indicates that substrate degradation influences both measured variables.

A second substrate pulse of 1.172 mmol acetate (75 mg COD) was added to the aeration vessel at t = 74 min (Fig. 2), and the same phenomena as described for the first substrate addition can be observed again. The assumption that the background acid dosage (related to CO_2 stripping) is constant for the short duration of an experiment is supported by the experimental data, because the slope of the acid addition curve in Figure 2 is the same before addition of the second substrate pulse (slope of acid addition curve before t = 74 min) and after complete degradation of the second substrate pulse (slope of acid addition curve from t = 88 min onward).

Data Interpretation

A detailed description of the interpretation of the respirometric data can be found in Gernaey et al. (2001). The basic principles will be repeated here, for a better understanding of the results that will be described below. The presence of both an aeration vessel and a closed non-aerated respiration chamber in the respirometer, each equipped with a dissolved oxygen probe, allows a mass balance to be made for oxygen (S_O) over both vessels (Eqs. [1] and [2]). Subscripts 1 and 2 in these equations stand for the aeration vessel and the respiration chamber, respectively. Similar subscripts will be used throughout to indicate the concentration of biodegradable substrate in the aeration vessel ($S_{S,1}$) and the respiration chamber ($S_{S,2}$):

$$\frac{dS_{O,1}}{dt}=\frac{Q_{in}}{V_1}\cdot(S_{O,2}-S_{O,1})+K_La\cdot(S_O^0-S_{O,1})-r_{O,1} \quad (1)$$

$$\frac{dS_{O,2}}{dt}=\frac{Q_{in}}{V_2}\cdot(S_{O,1}-S_{O,2})-r_{O,2} \quad (2)$$

For substrate degradation processes, we are interested mainly in the oxygen uptake rate of the biomass (r_O). The mass balance for S_O over the aeration vessel (Eq. [1]) includes, in addition to the oxygen uptake rate and a transport term for oxygen, an aeration term that is generally difficult to measure because a K_La measurement is needed. The main motivation for operating such a complicated experimental set-up for these experiments, instead of, e.g., a continuously aerated flowing gas–static liquid respirometer (Vanrolleghem and Verstraete, 1993), is that the oxygen uptake rate of the biomass can also be obtained from the S_O mass balance over the respiration chamber without the need of a K_La value (Eq. [2]). The available S_O data of the aeration vessel ($S_{O,1}$) and the respiration chamber ($S_{O,2}$) are used to this purpose. The S_O measurements were corrected for the electrode response time, according to Spanjers and Olsson (1992), and $dS_{O,2}/dt$ was simply calculated with a moving window regression (over three data points). The short-term BOD, BOD_{st}, for each substrate addition was obtained as the area under the $r_{O,2}$ curve. The value of the endogenous oxygen uptake rate ($r_{O,end}$) was subtracted from $r_{O,2}$ in this last calculation step, such that the calculated area only reflected oxygen uptake related to substrate degradation ($r_{O,ex}$). The r_O data obtained via Eq. (2) will also be used for parameter estimation applications, as is discussed further in Gernaey et al. (2002). It should be noted here that parameter estimation on S_O data gives the most reliable parameter estimates compared to r_O data (Petersen et al., 2001). However, parameter estimation on S_O data is numerically difficult and initial parameter guesses need to be provided, e.g., from r_O or titrimetric data.

Figure 3A contains an S_O data set obtained by adding 1.563 mmol acetate (100 mg COD) to the aeration vessel of the set-up (V = 2.54 L) at t = 0. Figure 3B shows the $r_{O,2}$ data that were obtained from the S_O data by applying the mass balance given in Eq. (2). The calculated

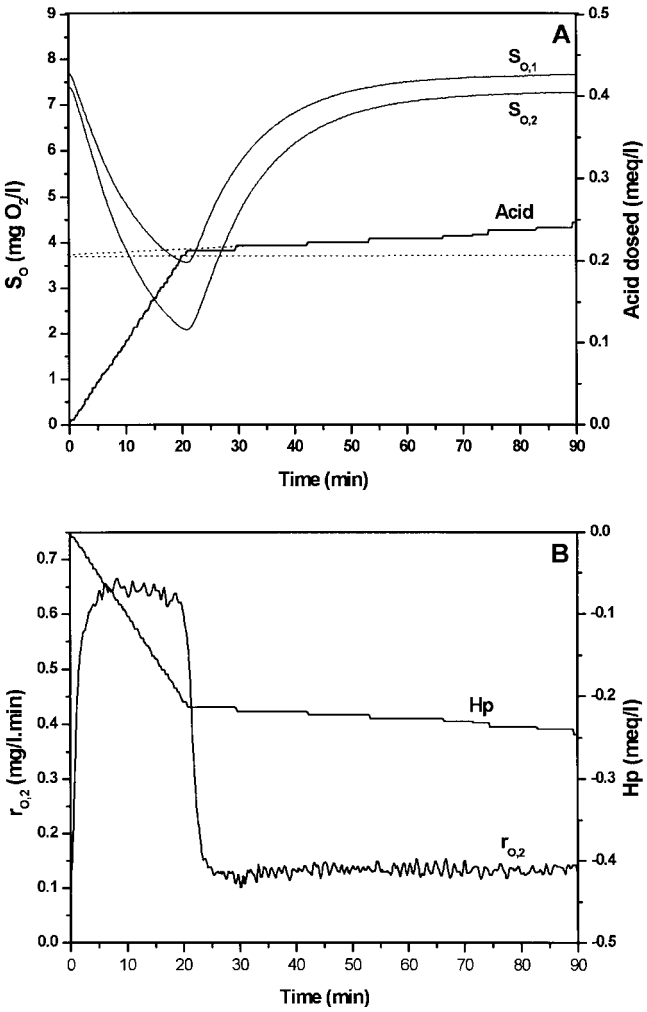

Figure 3. (A) Data series collected with the respirometric–titrimetric sensor following the addition of 1.563 mmol acetate (100 mg COD) to 2.54 L of activated sludge. (B) $r_{O,2}$ and proton production data resulting from the respirometric–titrimetric data set.

$r_{O,2}$ value is about 0.64 mg O_2/L · min during substrate degradation and decreases to an $r_{O,end}$ level of about 0.12 mg O_2/L · min when the endogenous respiration phase is reached (t = 22 min in Fig. 3B).

The titrimetric data set obtained from the same substrate addition experiment is shown in Figure 3A. Interpretation of such titrimetric data is relatively easy because the different slopes of the titration curves can be extrapolated to t = 0 (the dotted line in Fig. 3A), and thus the amount of acid or base dosed during the degradation of the substrate can be obtained, as was also shown by Gernaey et al. (1997). For the example of Figure 3A, the substrate degradation caused an extra acid addition of about 0.21 meq/L. Acid and base addition data were subsequently converted into proton production data, where acid addition during substrate degradation indicates a proton consumption (a "negative proton production") and base addition corresponds to proton production. Figure 3B contains an example of proton production data for the experimental acetate data of Figure 3A.

Experiments with Carbon Sources (Acetate and Dextrose)

Several experiments consisting of addition of different amounts of acetate to activated sludge in endogenous state were performed at different pH setpoints. The pH setpoints during the experiments ranged from 7.5 to 8.5. The results are given in Figure 4, and they were obtained using the slope extrapolation procedure illustrated in Figure 3A. As mentioned above, acid was added to the mixed liquor sample during degradation of acetate to keep the pH at the pH setpoint, which means that acetate degradation resulted in proton consumption by the biomass. The amount of acid added during acetate degradation increases linearly with the amount of acetate that was added to the activated sludge sample at the beginning of an experiment. For the pH range studied in the experiments, it was not possible to detect significant pH setpoint-depending differences in the results of the titration experiments, and therefore experimental results obtained at different pH setpoints are not given different symbols in Figure 4. The slope of the linear regression curve in Figure 4 equals 0.0060 meq acid dosed during acetate degradation per mg COD acetate added (r^2 = 0.95), which corresponds to 0.38 meq acid/mmol acetate (or 0.0064 meq acid/mg HAc).

For the experiments with acetate, a linear increase of BOD_{st} was observed when increasing amounts of acetate were added to the respirometer (data not shown; see Gernaey et al., 2001). The slope of this curve represents

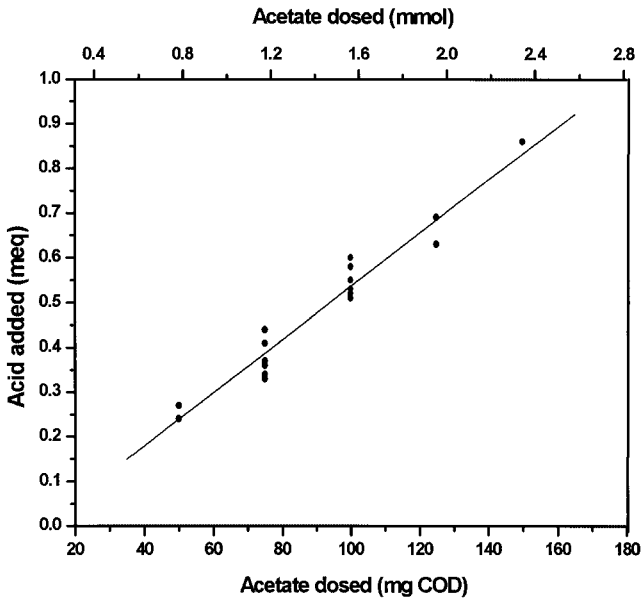

Figure 4. Amount of acid dosed during acetate degradation as a function of the initial amount of acetate added at the beginning of an aerobic batch substrate degradation experiment.

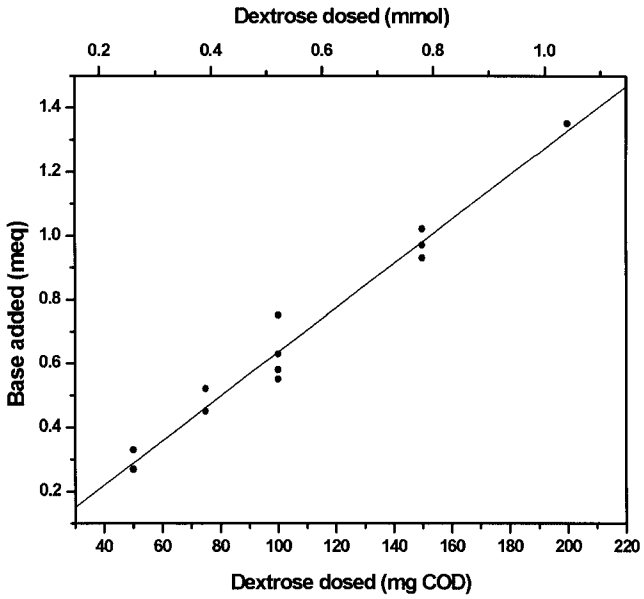

Figure 5. Amount of base dosed during dextrose degradation as a function of the initial amount of dextrose added at the beginning of an aerobic batch substrate degradation experiment.

the oxygen demand per unit of COD and allows the calculation of the biomass yield (via $BOD_{st} = [1 - Y_H] \cdot S_S[0]$). The average biomass yield that was obtained for this data series based on the respirometric data is 0.74.

Figure 5 summarizes the results of titration experiments performed with different amounts of dextrose. The pH setpoint used during the experiments varied from 8.05 to 8.35. As for acetate, it was not possible to detect a significant difference in the response of a titrimetric experiment depending on the pH setpoint. However, in contrast to the acetate titrimetric data, base was added during the degradation of dextrose, indicating a net proton production by the biomass cells during dextrose degradation. On average, 0.0069 meq base was dosed per mg COD dextrose added during dextrose degradation, which corresponds to 1.33 meq base/mmol dextrose (or 0.0074 meq base/mg dextrose). The BOD_{st} calculations resulted in a biomass yield of 0.88.

Model Development

The degradation of a readily biodegradable substrate, S_S, can be described by the stoichiometric matrix given in Table I. Table I is based on Activated Sludge Model No. 1 (ASM1; Henze et al., 1987) with some modifications: (1) X_B is used to indicate biomass instead of X_{BH}; (2) protons (H_p) are used instead of alkalinity (S_{ALK}), which means that stoichiometric factors used for S_{ALK} in ASM1 will change sign when working with H_p since consumption of S_{ALK} corresponds to production of H_p; (3) Monod functions for S_O were omitted from the process rates. The latter simplification was made because all experiments were done for non-limiting S_O concentrations ($S_O > 2$ mg/L). Based on the model presented in Table I, the oxygen consumption observed during substrate degradation (exogenous oxygen consumption, $r_{O,ex}$) can be related to substrate degradation kinetics (Eq. [3]), and substrate degradation kinetics can thus be extracted from respirometric data (e.g., Spanjers and Vanrolleghem, 1995; Sperandio and Paul, 2000; Vanrolleghem and Verstraete, 1993):

$$r_{O,ex} = \frac{1 - Y_H}{Y_H} \cdot \frac{S_s}{S_s + K_s} \cdot \mu_{max\,H} \cdot X_B \qquad (3)$$

However, in case proton consumption or production rates are measured, different effects should be taken into account in a model that relates proton consumption or production to substrate degradation. It might be clear from the experimental data presented in Figures 4 and 5 that the ASM1-based model presented in Table I cannot describe titrimetric data accurately. Indeed, the model in Table I would only describe a pH effect related to the uptake of NH_3 for biomass growth during aerobic substrate degradation. According to the ASM1 model all carbon sources will cause a production of protons during their aerobic degradation. However, the experimental data presented in Figures 4 and 5 show that some carbon sources will cause a proton production during degradation (e.g., dextrose, Fig. 5), while others will induce proton consumption (e.g., acetate, Fig. 4). A model that can describe these data should obviously contain extra components in addition to NH_3 uptake for biomass growth to accurately describe the experimental observations resulting from the titrimetric method. It should be explicitly added here that it was never the intention of the ASM1 model to describe such phenomena accurately. The ASM1 model is just used as a reference model in this paper and serves as a basis for further model developments. As a consequence, the ASM1 matrix notation will be used for the titrimetric model described in this paper, with substrate and biomass expressed in COD units and biomass (X_B) as the reference component in the model equations (see Table I).

Table I. ASM1-based process matrix for the aerobic degradation of a readily biodegradable carbon source.

	Component					
Process	1. X_B	2. S_S	3. S_O	4. S_{NH}	6. H_P	Process rate
1. Heterotropic growth with S_S as substrate	1	$-\frac{1}{Y_H}$	$-\frac{1 - Y_H}{Y_H}$	$-i_{XB}$	$\frac{i_{XB}}{14}$	$\mu_{max\,H} \cdot \frac{S_S}{K_S + S_S} \cdot X_B$

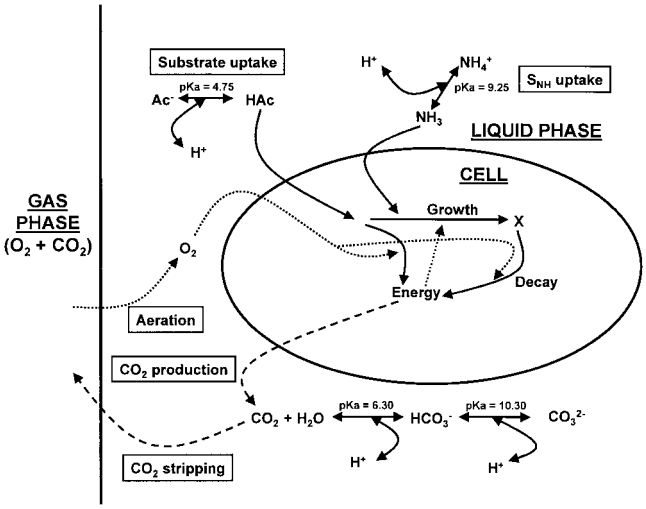

Figure 6. Schematic representation of the processes that will influence the proton balance during acetate degradation.

Generally, it will be assumed below that substrate uptake, CO_2 production and NH_3 uptake for biomass growth are the main processes that will influence the proton equilibrium (or the pH) in the liquid phase during degradation of a $C_xH_yO_z$ substrate compound. These three processes are illustrated in Figure 6 for the example of acetate as a biodegradable carbon source. Each of these processes and its influence on the proton balance in the liquid phase will be discussed separately below. It should be noted that other processes are included in Figure 6. These processes (aeration, CO_2 stripping, biomass decay) are discussed separately in Gernaey et al. (2002), and all are related to endogenous respiration ($r_{O,end}$, observable in the oxygen uptake rate data when substrate degradation is completed, see, e.g., Fig. 3A) and background acid or base dosage (observable in the titrimetric data when substrate degradation is completed; see Fig. 3). This paper focuses explicitly on substrate degradation related phenomena.

Substrate Uptake

It is assumed that the substrate is taken up through the cell wall in its undissociated form (Cramer and Knaff, 1991). Uptake of a substrate that is present in the liquid phase in undissociated form will thus cause no effect on the proton balance in the liquid phase, and consequently, it will not be visible in the titrimetric data. However, a weak acid, HA, present in the liquid phase in its dissociated form, A^-, will pass the cell wall together with a proton. In other words, the cell will extract a proton from the liquid phase during the substrate uptake process. This proton will be measured through addition of acid (to maintain the pH setpoint) when the titrimetric measurement technique is applied. The fraction m of a substrate (monoprotic acid) present in the dissociated form A^- can be expressed as a function of the pH of the liquid phase and the pK_a of the acid (Eq. [4]).

$$m = \frac{[A^-]}{[HA] + [A^-]} = \frac{10^{-pK_a}}{10^{-pH} + 10^{-pK_a}} \quad (4)$$

Consequently, for a known pH value the proton effect related to uptake of a substrate will be equal to m meq protons consumed per mmol substrate and can be included in a model that links proton production to substrate degradation (process 1.1 in Table II).

CO_2 Production

All carbon that is respired by the cells is converted into CO_2. For the model substrate $C_xH_yO_z$, x mmol CO_2 will be produced per mmol substrate that is completely respired. The number n of protons released per molecule of CO_2 produced will depend on the pH of the liquid phase and can be obtained using Eq. (5) (Iversen et al., 1994).

$$n = \frac{2 \times 10^{2pH} + 10^{(pH+pK_{2CO_2})}}{10^{2pH} + 10^{(pH+pK_{2CO_2})} + 10^{(pK_{1CO_2}+pK_{2CO_2})}} \quad (5)$$

The proton production effect related to CO_2 release can thus be included in a model that links proton production to substrate degradation (process 1.2 in Table II). The structure of the stoichiometric coefficient in the H_p column for process 1.2 already shows that the heterotrophic biomass yield Y_H will be of major influence on the amount of CO_2 produced through the factor $(1 - Y_H)$. Indeed, the higher the Y_H, the lower the amount of

Table II. Process matrix for the model developed to interpret combined respirometric–titrimetric data obtained during degradation of a $C_xH_yO_z$ carbon source.*

Process	1. X_B	2. S_S	3. S_O	4. S_{NH}	5. H_P	Process rate
1.1 Substrate uptake		$-\frac{1}{Y_H}$			$-\frac{m}{C \cdot Y_H}$	$\mu_{max} H \cdot \frac{S_S}{K_S + S_S} \cdot X_B$
1.2 CO_2 production			$-\frac{1 - Y_H}{Y_H}$		$\frac{n \cdot (1 - Y_H) \cdot}{C \cdot Y_H}$	$\mu_{max}\ H \cdot \frac{S_S}{K_S + S_S} \cdot X_B$
1.3 NH_3 uptake for growth	1			$-i_{XB}$	$\frac{p \cdot i_{XB}}{14}$	$\mu_{max}\ H \cdot \frac{S_S}{K_S + S_S} \cdot X_B$
1.1 Hetrotrophic growth with S_S as substrate	1	$-\frac{1}{Y_H}$	$-\frac{1 - Y_H}{Y_H}$	$-i_{XB}$	$-\frac{m}{C \cdot Y_H} + \frac{n \cdot (1 - Y_H) \cdot x}{C \cdot Y_H} + \frac{p \cdot i_{XB}}{14}$	$\mu_{max}\ H \cdot \frac{S_S}{K_S + S_S} \cdot X_B$

*Factors m, n, and p in the H_p column are pH-dependent functions; see Eqs. (4)–(6).

substrate that is respired to provide energy because a fraction Y_H of the substrate is converted into new biomass, and thus the lower the amount of CO_2 that will be released.

It can be noted that CO_2 will also be removed from the mixed liquor via stripping because the mixed liquor sample is continuously aerated. However, the pH effect related to CO_2 stripping is assumed to be constant for the short duration of each experiment. The validity of this assumption is illustrated in Figure 2.

NH_3 Uptake

According to standard activated sludge models (ASM1; Henze et al., 1987), biomass will grow during substrate degradation and incorporate a nitrogen fraction i_{XB} in the new biomass that is produced. Similar to ASM1, it is assumed that nitrogen incorporated into new biomass is taken up from the mixed liquor as NH_3. Because NH_3 is in equilibrium with NH_4^+ in the mixed liquor, a pH-dependent fraction p of protons will be released in the mixed liquor per mole of NH_3 taken up to form new biomass (Eq. [6]).

$$p = \frac{[NH_4^+]}{[NH_4^+] + [NH_3]} = \frac{10^{-pH}}{10^{-pH} + 10^{-pK_{NH4}}} \qquad (6)$$

The protons released due to NH_3 uptake for biomass formation can thus also be included in a model that links proton production to substrate degradation (process 1.3 in Table II). Note that there is no pH function p included in the original ASM1 model (Table I), which means that only NH_4^+ is assumed to be present in the liquid phase. The latter will be valid for pH values around 7 but not for pH values around 8 or higher.

In summary, the three above-mentioned model components can be added together, resulting in the overall process 1 in Table II. Compared to the model in Table I, the H_p stoichiometric factor has been extended in the new model to better describe titrimetric data.

Evaluation of the Influence of pH on Proton Production Related to Substrate Uptake, CO_2 Production, and NH_3 Uptake

Acetate: Theoretical Model Predictions versus Experimental Results

Each of the above-mentioned model components (substrate uptake, CO_2 production, and NH_3 uptake) was studied in more detail to theoretically evaluate how the contribution of each model component varies as a function of pH. Acetate ($C_2H_4O_2$) was considered as a first model compound. The constants used for the theoretical study with acetate are given in Table III.

Table III. List of constants used for theoretical model study with acetate.

Constant	Value
pK_a	4.75
C (g COD/mol)	64
x	2
pK_{1CO_2}	6.30
pK_{2CO_2}	10.30
pK_{NH_4}	9.25

The calculated variation of the net proton production as a function of the pH of the liquid phase for substrate uptake, CO_2 production, and NH_3 uptake is given in Figure 7 for acetate. The proton production is expressed as meq protons produced per mmol substrate, and a negative sign is attributed to proton consumption. It should be noted also that the values in Figure 7 were obtained using default ASM1 parameter values for Y_H (0.67) and i_{XB} (0.086 g N/g COD biomass).

Proton consumption due to acetate uptake increases drastically between pH 4 and 6 (Fig. 7). At pH 4, most acetate is present in its undissociated form. However, the higher the pH, the higher the fraction of dissociated acetate and thus the higher the amount of protons consumed per mmol acetate. At pH 6, almost all acetate is present in dissociated form. Consequently, the proton consumption related to acetate uptake is rather constant for pH values above 6.

According to the model, the amount of protons produced due to CO_2 formation during degradation of acetate depends strongly on the pH (Fig. 7). This can be explained by the formation and dissociation of H_2CO_3. The proton production due to CO_2 formation increases considerably between pH 5.5 and 7.5, because the first

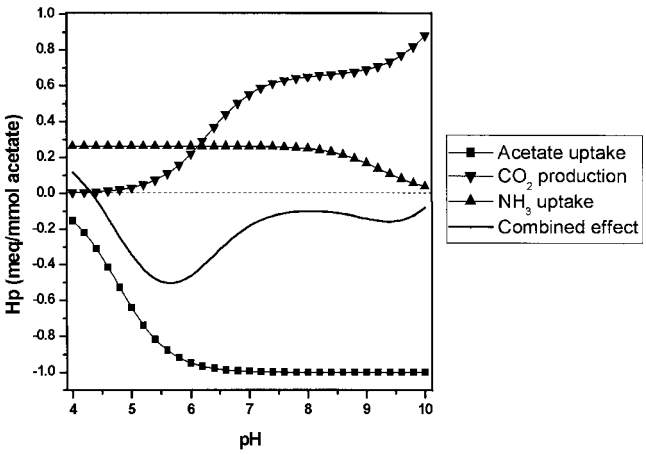

Figure 7. Calculated variation as a function of the pH of the liquid medium of the amount of protons produced (H_p) due to substrate uptake, CO_2 production, and NH_3 uptake for biomass growth during acetate degradation. Calculations were done using ASM1 reference parameters (Y_H = 0.67, i_{XB} = 0.086 mg N/mg COD). The combined effect of the three processes (acetate uptake + CO_2 release + NH_3 uptake) is also shown.

acid dissociation constant of H_2CO_3 will be influential in this pH region (pK_{1CO2} = 6.30, see Table III). However, according to the model the CO_2 production effect on the proton balance is more or less constant between pH 7.5 and 9. In this pH region, about one proton is produced for each substrate degradation related CO_2 molecule that is released by the biomass. Above pH 9, the amount of protons produced per CO_2 molecule that is released increases again, because the pH values are approaching the second acid dissociation constant of H_2CO_3 (pK_{2CO2} = 10.3, see Table III). Above pH 9, the average number of protons produced per released CO_2 molecule therefore becomes higher than 1.

The proton production due to NH_3 uptake is rather constant for pH values below 8 (Fig. 7). For pH values below 8 one proton is produced per NH_3 molecule that is incorporated into new biomass, because mainly NH_4^+ is present in the solution. Above pH 8, proton production due to NH_3 uptake decreases. Indeed, above pH 8 the NH_4^+ fraction that will be present in the liquid phase in dissociated form will increase with pH (pK_{NH4} = 9.25, see Table III), and the average number of protons released per NH_3 that is incorporated into biomass will thus decrease.

The total proton consumption due to acetate degradation calculated according to the proposed model, consisting of the sum of the three separate model components, is also shown in Figure 7. For the pH range that was studied in the experiments (pH 7.5–8.5), the theoretical model predicts a proton consumption, which agrees with the laboratory observations for acetate. Moreover, according to the model, proton consumption does not vary much between pH 7.5 and 8.5. This is also in agreement with the laboratory results (Fig. 4), where it was observed that the total amount of acid added during acetate degradation did not change significantly when the pH setpoint in the combined respirometric–titrimetric set-up was varied between 7.5 and 8.5. For example at pH 8, the model predicts a proton consumption of 0.1 meq/mmol acetate, which is considerably lower than the proton consumption of 0.38 meq/mmol acetate that was observed experimentally. The difference between the experimental observations and the model predictions can be explained by differences in the model parameters Y_H and i_{XB}. The calculations were done with a biomass yield of 0.67 and an i_{XB} value of 0.086, without calibrating the model to the available data. For example, from the respirometric data a biomass yield of about 0.74 was obtained, which is significantly higher than the biomass yield applied in the calculations, and this will have an important influence on the proton production measured during substrate degradation, as will be illustrated below (Fig. 8). Note also that a detailed calibration of the model is described in Gernaey et al. (2002).

The influence of a variation of the parameters Y_H and i_{XB} on the acetate degradation induced proton consumption predicted by the model presented in Table II was evaluated (see Figs. 8 and 9, respectively).

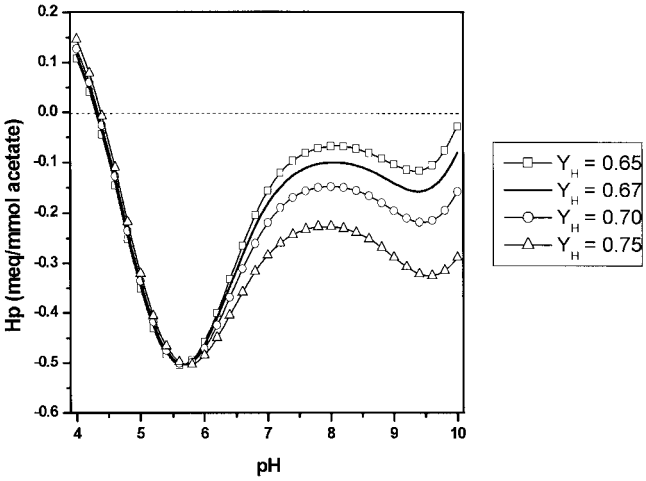

Figure 8. Calculation of the influence of the biomass yield Y_H on the proton production (H_p) during acetate degradation (i_{XB} = 0.086 mg N/mg COD).

An increase of Y_H resulted in an increase of the proton consumption related to acetate degradation (Fig. 8). Indeed, when looking at the process scheme of Figure 6, an increase of the parameter Y_H means that a higher fraction of the substrate consumed by the biomass is converted into new biomass. Thus, a lower fraction of the substrate is oxidized, or less CO_2 is produced per unit of substrate that is consumed by the biomass. As a consequence, the proton production due to CO_2 release decreases because less CO_2 is produced per substrate unit consumed. The latter could explain the trend in Figure 8. However, one should notice that an increase of Y_H also results in an increased uptake of NH_3 because more biomass is formed per unit of substrate consumed, which means that more protons are released from NH_3 uptake. The extra release of these protons can by far not

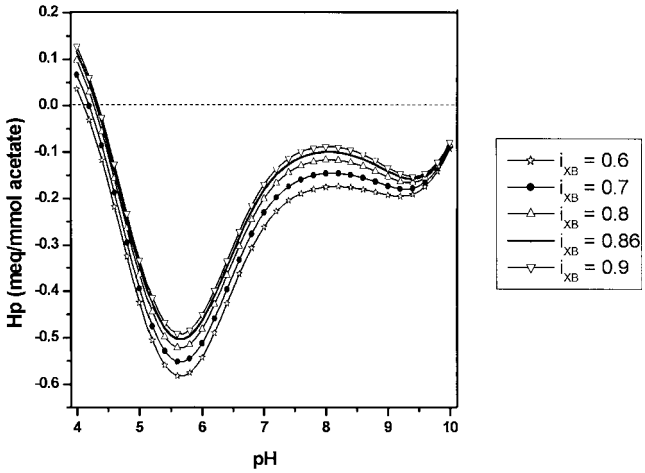

Figure 9. Calculation of the influence of the nitrogen fraction in the biomass (i_{XB}) on the proton production (H_p) during acetate degradation (Y_H = 0.67).

compensate for the effect on the proton balance related to the decrease in CO_2 production. According to the proposed model, an increase of Y_H from 0.67 to 0.74 (the Y_H value resulting from the respirometric tests) at pH 8 reduces the proton production due to CO_2 formation from 0.650 to 0.512 meq/mmol acetate consumed, while the proton production due to NH_3 uptake for biomass growth increases simultaneously from 0.249 to 0.275 meq/mmol acetate consumed. The net effect of an increase of Y_H from 0.67 to 0.74 is an increase of the predicted proton consumption from 0.10 to 0.21 meq/mmol acetate consumed, which is already closer to the experimental observations (0.38 meq/mmol acetate consumed). Variation of Y_H (and also of i_{XB}) does not influence the number of protons consumed during the substrate uptake process (process 1.1 in Table II). Indeed, for the substrate uptake process, Y_H appears in the denominator of the stoichiometric factor for both S_S and H_p (Table II), which means that Y_H will disappear when dH_p/dt is expressed as a function of dS_S/dt for this process.

Finally, the data of Figure 8 show that the influence of Y_H on the amount of protons consumed per unit of substrate consumed during acetate degradation increases with increasing pH values. According to Eq. (5) and Figure 7, more protons are released per molecule of CO_2 when pH increases, and this explains why an increase of Y_H and the corresponding decrease of the CO_2 production has a more pronounced effect on substrate degradation related proton production when pH values increase.

A decrease of the parameter i_{XB} will lead to a decrease of the amount of protons released due to uptake of NH_3 for biomass growth, because less nitrogen is incorporated in the biomass according to the model. As a consequence, acetate degradation results in higher proton consumption per unit of acetate consumed when i_{XB} values decrease (Fig. 9). The effect of a lower i_{XB} value on the total proton consumption during acetate degradation is less pronounced with increasing pH values. The ratio between $[NH_4^+]$ and $([NH_4^+] + [NH_3])$ decreases with increasing pH, and in the proposed model it is assumed that only the NH_4^+ fraction will leave a proton behind in the liquid phase when taken up by the biomass (see Fig. 6). At pH 8, and for a biomass yield Y_H of 0.67, a decrease of i_{XB} from 0.086 to 0.07 mg N/mg COD biomass resulted in a calculated decrease of the proton production related to NH_3 uptake for biomass growth from 0.249 to 0.203 meq/mmol acetate consumed. When all three processes are considered (substrate uptake, CO_2 production, and NH_3 uptake for biomass growth), a decrease of i_{XB} from 0.086 to 0.07 mg N/mg COD biomass results in an increase of the proton consumption from 0.100 to 0.146 meq/mmol acetate consumed, or, in other words, a decrease of the parameter i_{XB} results in model predictions that are closer to the experimental observations.

Conclusively, the proposed model could describe the experimental observations for acetate batch degradation experiments (0.38 meq protons consumed/mmol acetate) on condition that values for the parameters Y_H and i_{XB} are higher or lower, respectively, than the reference values ($Y_H = 0.67$ and $i_{XB} = 0.086$) of Henze et al. (1987). Calculations with the proposed model showed that for pH 8 a parameter combination of $Y_H = 0.74$ and $i_{XB} = 0.034$ mg N/mg COD biomass result in the experimentally observed proton production of 0.38 meq/mmol acetate consumed. The respirometric data collected in parallel with the titrimetric data confirm the Y_H value of 0.74. For a more in-depth analysis of the parameter values, the reader is referred to Gernaey et al. (2002).

Dextrose: Theoretical Model Predictions versus Experimental Results

Figure 10 shows the influence of pH on the proton production during aerobic dextrose ($C_6H_{12}O_6$) degradation. It was assumed that dextrose is present in the mixed liquor as a neutral component, which means that substrate uptake neither consumes nor produces any protons in the mixed liquor. The remaining two processes that are considered in the model, CO_2 production and NH_3 uptake, will both result in a production of protons. This is corroborated by the experimental observations, because base was added to the mixed liquor to keep the pH at the constant pH setpoint value during dextrose degradation experiments, indicating a production of protons during the dextrose degradation process. Similar to acetate, the calculated proton production due to aerobic dextrose degradation is also rather constant between pH 7.5 and 8.5 (Fig. 10).

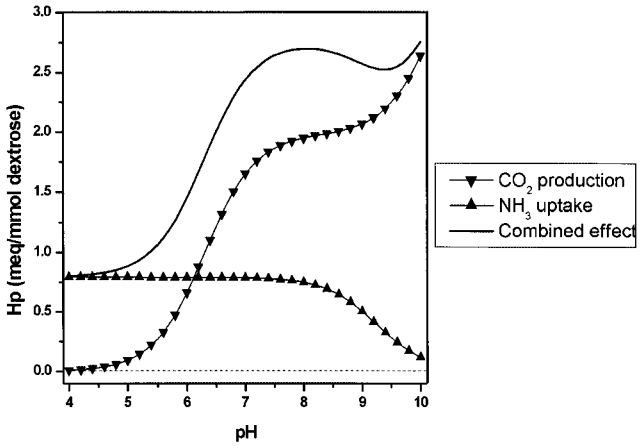

Figure 10. Calculated variation of the amount of protons produced (H_p) due to CO_2 release and NH_3 uptake during dextrose degradation as a function of the pH of the liquid medium for ASM1 reference parameters ($Y_H = 0.67$, $i_{XB} = 0.086$ mg N/mg COD). The net amount of protons produced as a result of the two processes (CO_2 release + NH_3 uptake) is also shown.

Finally, the model predicts a proton production of about 2.70 meq/mmol dextrose at pH 8 (Y_H = 0.67 and i_{XB} = 0.086), which is considerably higher than the proton production of 1.33 meq/mmol dextrose that was observed experimentally. However, the respirometric data that were collected in parallel with the respirometric data resulted in a value of 0.88 for Y_H, which is considerably higher than the reference value of 0.67. A similar observation resulted from the acetate batch degradation experiments. For Y_H = 0.88 a proton production of 1.69 meq/mmol dextrose was calculated, which is already much closer to the experimental observations. Also in this case, a decrease of the parameter i_{XB} will bring the model predictions closer to the experimental observations. A detailed calibration of the model for dextrose is described in Gernaey et al. (2002).

DISCUSSION

An important detail related to the respirometric experiments is that the respirograms collected in this study during batch substrate degradation experiments do not show a "tail" as was observed by Dircks et al. (1999). In the study of Dircks et al. (1999) the oxygen uptake rate from activated sludge to the addition of acetate could be divided into two phases. The first phase was assumed to reflect the degradation of added acetate, while the second phase (the tail) was assumed to originate from degradation of internal polymers, like polyhydroxyalkanoates, stored in the cells during the first phase when acetate was still present. The shift from phase one to two was assumed to be due to the depletion of the exogenous substrate (acetate). In this study, however, only one phase was observed in the oxygen uptake rate profiles. This would indicate that substrate storage does not take place with this activated sludge and the substrates used in these experiments, and it makes it easier to apply the straightforward data interpretation to the respirometric data, i.e., the short-term BOD was equal to $(1 - Y_H) \cdot S_S(0)$. In addition, if substrate storage had taken place in this study, a second bending point would have been expected in the titrimetric profile, which is also not the case. In case substrate storage would take place, the proposed model would have to be extended with this process.

On the basis of the experimental titrimetric data, it became clear that both acetate and dextrose affect the proton balance in the liquid phase when they are degraded. The respirometric data that were recorded in parallel with the titrimetric data provided the first confirmation that the observed proton consumption (for the example of acetate) or proton production (for the example of dextrose) is related to substrate degradation, because both experimental responses begin and end at exactly the same moment (e.g., Fig. 2). The good correlations that were found between the amount of protons consumed or produced during substrate degradation and the amount of COD that was initially added to the activated sludge sample provided the second confirmation that the observed titrimetric phenomena are clearly related to substrate degradation (Figs. 4 and 5).

A model was proposed to describe the experimental observations. The model contains three different processes that will consume or produce protons: substrate uptake, CO_2 production due to substrate degradation, and NH_3 uptake for biomass growth. These three processes are influenced by the actual pH of the liquid phase, and this was discussed in detail on the basis of a theoretical study of the proposed model (Fig. 7). Compared to the proton model described by Drtil et al. (1995) for the denitrification process, the proposed model is different because it adds the pH dependency of the proton production or consumption of the different processes that are involved. Bogaert et al. (1997) also studied the pH effect of the denitrification process. However, in their approach only pH effects related to CO_2 production and nitrate or nitrite reduction were considered, while pH effects related to substrate uptake or NH_3 uptake were not included.

The experimental observations for acetate (0.38 meq protons consumed/mmol acetate) and dextrose (1.33 meq protons produced/mmol dextrose) could be explained with the proposed model by using a biomass yield Y_H of 0.74 and 0.88, respectively, combined with a decrease of i_{XB}. In both cases the use of relatively high Y_H values in the model was confirmed by the respirometric data that were collected in parallel with the titrimetric data.

The proposed model structure implies that titrimetric data can provide information about aerobic degradation of a carbon source. The titrimetric method could thus be useful to quantify substrate biodegradation kinetics in activated sludge with specific substrates. This will be discussed in detail in Gernaey et al. (2002). For biotechnological processes in general, the method could furthermore be useful to quantify substrate consumption kinetics of pure cultures, e.g., in the frame of fermentation processes. The model could also be extended for anoxic conditions, with nitrate or nitrite as electron acceptor, by including a pH effect related to uptake of nitrate or nitrite in the model. This could be important for simpler kinetic characterization of the denitrification process, as respirometry cannot be used to characterize anoxic degradation processes, and operation of nitrate electrodes to monitor the denitrification process is rather cumbersome.

CONCLUSIONS

A combined respirometric–titrimetric method was used to monitor the aerobic degradation of known carbon

sources (acetate and dextrose) by activated sludge during batch substrate degradation experiments. For both carbon sources, a good correlation was found between the response of the titrimetric method and the initial amount of substrate added at the beginning of an experiment, indicating that titrimetric data can yield information about aerobic carbon source degradation processes.

A model that includes substrate uptake, CO_2 production, and NH_3 uptake for biomass growth as factors that produce or consume protons is proposed to describe titrimetric data obtained from aerobic degradation experiments with a known $C_xH_yO_z$-type carbon source. Besides wastewater treatment processes, similar models could also be useful for application in all kinds of biotechnological processes provided that the proton stoichiometry of the reactions is known.

With modification of the parameters Y_H and i_{XB}, the proposed model can quantitatively describe the experimental data for acetate (proton consumption of 0.38 meq/mmol acetate) and dextrose (proton production of 1.33 meq/mmol dextrose). The relatively high values for Y_H of 0.74 and 0.88 for acetate and dextrose, respectively, that were used in the model were confirmed by respirometric data.

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NOMENCLATURE

ASM1	Activated Sludge Model No. 1
A^-	monoprotic acid, dissociated
BOD	biochemical oxygen demand
BOD_{st}	short-term biochemical oxygen demand
C	conversion factor (g COD/mol)
COD	chemical oxygen demand
H_p	proton concentration in mixed liquor (meq/L)
HA	monoprotic acid, undissociated
i_{XB}	fraction of N in biomass (g N/g COD biomass)
K_La	oxygen transfer coefficient (1/min)
K_S	heterotrophic half-saturation substrate concentration (mg COD/L)
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
pK_a	negative logarithm of acid dissociation constant
pK_{NH4}	negative logarithm of acid dissociation constant for NH_4^+
pK_{1CO2}	negative logarithm of first acid dissociation constant for H_2CO_3
pK_{2CO2}	negative logarithm of second acid dissociation constant for H_2CO_3
Q_{in}	flow rate of liquid entering the system (L/min)
$r_{O,end}$	endogenous oxygen uptake rate (mg/L · min)
$r_{O,ex}$	exogenous oxygen uptake rate (mg/L · min)
r_O	oxygen uptake rate (mg/L · min)
$r_{O,1}$	oxygen uptake rate in the aeration vessel (mg/L · min)
$r_{O,2}$	oxygen uptake rate in the respiration chamber (mg/L · min)
S_{ALK}	alkalinity concentration (meq/L)
S_{NH}	ammonium (concentration) (mg N/L)
S_O	dissolved oxygen concentration in the liquid phase (mg/L)
$S_{O,1}$	dissolved oxygen concentration in the aeration vessel (mg/L)
$S_{O,2}$	dissolved oxygen concentration in the respiration chamber (mg/L)
S_S	readily biodegradable substrate concentration (mg COD/L)
$S_{S,1}$	readily biodegradable substrate concentration in the aeration vessel (mg COD/L)
$S_{S,2}$	readily biodegradable substrate concentration in the respiration chamber (mg COD/L)
$S_S(0)$	readily biodegradable substrate concentration at $t = 0$ (mg COD/L)
S_O^0	saturation dissolved oxygen concentration (mg/L)
V	volume
V_1	volume of the aeration vessel
V_2	volume of the respiration chamber
x	number of carbon atoms per substrate molecule for a $C_xH_yO_z$ carbon source
X_B	biomass concentration (mg COD/L)
y	number of hydrogen atoms per substrate molecule for a $C_xH_yO_z$ carbon source
Y_H	yield coefficient for heterotrophic biomass (g COD/gCOD)
z	number of oxygen atoms per substrate molecule for a $C_xH_yO_z$ carbon source
$\mu_{max\ H}$	maximum specific growth rate for heterotrophic biomass (1/min)

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