Anal Bioanal Chem (2005) 383: 605–618
DOI 10.1007/s00216-005-0009-6

ORIGINAL PAPER

Usama E. Zaher · Peter A. Vanrolleghem

Automatic initialisation of buffer composition estimation for on-line analysis of unknown buffer solutions

Received: 2 March 2005 / Revised: 1 July 2005 / Accepted: 7 July 2005 / Published online: 24 September 2005

Abstract An automatic initialisation procedure for extracting useful information about buffer composition from a titration experiment is presented in this paper. The initialisation procedure identifies which buffering components are present in the sample from a relatively long list of buffers expected in the system monitored. The procedure determines approximate pK_a values of the buffers and evaluates their maximum and minimum concentrations. This information is then used to start an optimisation procedure to fit the model of the buffer components to the titration data and to accurately determine buffer concentrations and pK_a values. The procedure has been integrated as a software layer around the buffer capacity optimum model builder (BOMB) that fits a buffer-capacity model to a measured buffer-capacity curve to estimate model properties (pK_a values and concentrations). The reliability and robustness of the resulting buffer capacity software (BCS) were tested using a titrimetric analyser simulator (TAS). The BCS was then validated off-line and on-line.

Keywords Anaerobic digestion · Buffer capacity · Software sensor · Titration · Wastewater treatment

Introduction

Buffer components are weak acids and bases that do not dissociate completely to their ions in aqueous solutions. Water itself is a buffer component that partially dissociates into H^+ and OH^-. Dissociated and undissociated species of buffer components will be held in an equilibrium that tends to shift to release any stress exerted by introduction of other buffer components, strong acids, strong bases, or other external factors, e.g. temperature. Buffer components therefore have a substantial effect on the chemical properties of a solution. Buffer components play an essential role in many, if not all, biological systems either because of their availability as substrates or because of their toxic effect on a living species. Therefore, quantification of buffer components is deemed to be very important for chemical, physical, and biological process engineering.

Among other analytical techniques, titrimetric methods are the most direct for quantifying buffer components in aqueous solutions, because they are directly related to their chemical equilibrium properties. Other techniques, for example ion chromatography, that can be used to measure most buffers, and gas chromatography, that can be used to measure volatile buffers, e.g. volatile fatty acids (VFA), provide accurate results compared with titrimetric techniques. On the other hand, titrimetric techniques are far more economical, especially when considered for on-line applications.

Detailed titration data and advanced interpretation techniques are therefore desirable to improve the accuracy of the titrimetric techniques and extend them to the determination of a wide range of buffer components. Detailed titration data can now be obtained on-line because of current advances in instrumentation technology.

In the fields of environmental engineering, water quality and wastewater treatment, determination of different combinations of buffer components is of significant importance. In such systems buffer components are subject to dynamics and transitions from one combination of buffers to another. Developed measurement methods should, therefore, be reliable when applied to this variety of applications and sufficiently robust to cope with the rapid dynamics and transitions. In anaerobic digestion applications, for example, many dynamics and transitions are observed. Bicarbonate and VFA are important buffers used to monitor process dynamics. Accumulation of VFA is important indicator of digester overload. During overload, lactate will also start to build up in the reactor [1, 2]. Phosphorus may be released into the digester as a result

U. E. Zaher (✉) · P. A. Vanrolleghem
Department of Applied Mathematics,
Biometrics and Process Control
(BIOMATH), Ghent University,
Coupure Links 653,
9000 Gent, Belgium
e-mail: usama.zaher@biomath.ugent.be
e-mail: peter.vanrolleghem@ugent.be

of polyphosphate hydrolysis and degradation of organic solids. Consequently, its quantification is important to assess recycling of digester overflows to a biological phosphorus removal plant [3] or to study mineral precipitation problems in the pipe network and the sludge-drying equipment of a treatment plant [4]. In some applications of anaerobic digestion, toxicant buffers are present at high concentrations in the influent wastewater and, therefore, their quantification is important. Ammonia is toxic to aceticlastic methanogenesis [5]. Different types of waste, for example piggery waste, poultry and cattle manure, and abattoir wastes, contain high concentrations of ammonia [6–9]. They are, however, suitable for anaerobic digestion. Cyanide and phenol are also toxic buffers that occur in many industrial applications. In many crop-processing activities, for example the production of starch from cassava, cyanide is produced and is therefore present in the wastewaters produced. They contain anaerobically degradable substrates but cyanide is inhibitory to methanogenesis [10]. Therefore, a titrimetric sensor capable of quantifying different combinations of these buffers would be useful for monitoring anaerobic digestion processes.

Classification of the interpretation techniques for titrimetric monitoring of anaerobic digestion [2] has shown that nonlinear fitting of buffer models is an advanced technique for improving the accuracy of the titrimetric measurement. A buffer-capacity-based multipurpose hardware and software sensor for environmental applications was developed [11]. The software sensor fits a general buffer-capacity model to a buffer-capacity curve that is evaluated from a detailed titration experiment and estimates the characteristics of buffer components, e.g. concentrations and pK_a values. The interpretation method depends on prior information about the buffer components to define and initialise the buffer-capacity model. To deal with the expected limitation of prior information about the buffer systems in any application, the method was extended by model-selection techniques [12]. Model selection starts with optimisation of an initial model that is defined and initialised on the basis of the information available about buffer components in a certain system. The model-selection process detects, stepwise, possible extensions to the initial model as a result of other buffers introduced to the system and gives new information about the most possible extension, e.g. pK_a values of the new buffers. Both optimisation and model selection are built into the software sensor BOMB (buffer capacity optimum model builder).

This paper presents an initialisation procedure that directly determines the buffer model and estimates minimum and maximum limits for the concentrations of buffer components in a sample. This information is then used to run an optimisation algorithm to quickly and accurately estimate the concentrations of the buffers present in the titrated sample. In this study the procedure was integrated with BOMB. The result is buffer capacity software (BCS) that can work off/on-line with many titrimetric analysers. BCS is suitable for on-line titrimetric monitoring of bioprocesses in which buffer systems will frequently shift from one buffer combination to another. In such transition cases, BCS will not need any user interaction, e.g. for definition of the model or its parameter limits. In the work discussed in this paper BCS was tested using a titrimetric analyser simulator (TAS) [14, 15]. The test with the TAS was designed to assess the linearity and robustness of BCS in the measurement of a wide range of buffer components under rapid transition conditions that may occur in anaerobic digestion applications. The test will illustrate the advantages of automatic initialisation to eliminate problems caused by buffer interferences Finally, results from off-line and on-line validation of BCS (initialisation plus BOMB) are presented.

Methods

General model

A general buffer-capacity model was derived elsewhere [11]. The model is obtained in three steps. First, a charge balance is formulated after addition of strong acid or base considering ions of monoprotic, diprotic, and triprotic buffers. Second, ion concentrations are substituted in the charge-balance equation as functions of their total buffer concentrations. Third, the charge-balance equation is derived with respect to the pH. The result is the general model that comprises three terms for monoprotic, diprotic, and triprotic buffers, as presented by the model Eq. (1):

$$\beta = 2.303[H^+]\cdot\left(\begin{array}{l} 1+\sum_{i=1}^{l} C_i\left(K_a\frac{1}{\left([H^+]+K_a\right)^2}\right)_i+\sum_{j=1}^{m} C_j\left(K_{a1}\frac{[H^+]^2+4K_{a2}[H^+]+K_{a1}K_{a2}}{\left([H^+]^2+K_{a1}[H^+]+K_{a1}K_{a2}\right)^2}\right)_j \\ +\sum_{k=1}^{n} C_k\left(K_{a1}\frac{[H^+]^4+4K_{a2}[H^+]^3+(K_{a1}+9K_{a3})K_{a2}[H^+]^2+(4[H^+]+K_{a2})K_{a1}K_{a2}K_{a3}}{\left([H^+]^3+K_{a1}[H^+]^2+K_{a1}K_{a2}[H^+]+K_{a1}K_{a2}K_{a3}\right)^2}\right)_k \end{array}\right) \quad (1)$$

where β is the buffer capacity (eq $L^{-1}pH^{-1}$), $[H^+]$ the hydrogen ion concentration (mol L^{-1}), $C_{i,\,j,\,k}$ the concentration of monoprotic, diprotic, and triprotic weak acids, respectively (mol L^{-1}), and K_a is the acidity constant.

The measurement principle is to successively measure pH as a function of stepwise addition of acid or base. In this way the titration curve is built. From this measured titration curve (typically approximately 30 to 50 points), the buffer

capacity at each pH point is calculated as the derivative of the amount of base or acid needed to change the pH (meq L^{-1} pH^{-1}), Eq. (2), and a buffer-capacity curve is produced. On the basis of the initialisation procedure developed below in this paper, a buffer-capacity model is defined using the general form of Eq. (1) and model optimisation is initialised. The concentrations and pK_a values are estimated by fitting the model to the buffer-capacity curve.

The method was extended with an automatic model-building procedure based on model-selection techniques [12]. Model selection will act as a second barrier to warn the user on the rare occasion that the initialisation procedure fails to initialise other existing buffers. If the model selection detects such buffers, it will provide an estimate of their pK_a values and concentrations and these will be used to adapt the parameters of the initialisation procedure.

Monoprotic model-based initialisation

This procedure can be defined in three main steps. The first step is generation of a smooth and uniform buffer-capacity curve (BC) from the raw titration data. The titration experiment is stepwise addition of acid or base to the sample. At each step pH is measured so that data points are recorded as pairs of pH and volume of acid or base added. Knowing the experimental conditions, acid normality and sample volume, the buffer capacity is evaluated at each point using Eq. (2). With a reasonable number of calculated buffer-capacity points, a smooth BC is obtained by parabolic interpolation in steps of 0.1 pH units. To this end, the BC is constructed and distributed at regular pH intervals between the experiment's minimum pH (TCmin) and maximum pH (TCmax):

$$\beta = -\frac{\Delta C_a}{\Delta \mathrm{pH}} \tag{2}$$

where C_a is the acid concentration.

The BC is a $n\times 2$ array in Eq. (3):

$$\begin{matrix} BC_{j,1} = pH_j = (\mathrm{TC_{min}}, \mathrm{TC_{min}} + 0.1, \ldots, \mathrm{TC_{max}}) \\ BC_{j,2} = \beta_j \end{matrix}, \quad j = (0, 1, 2, \ldots, n-1) \tag{3}$$

The second step is successive detection of the maxima of the BC. On detection of a maximum, an assumed monoprotic model is subtracted at this point. Accordingly, a next maximum point can be easily detected. Another monoprotic model is then subtracted. The subtracted monoprotic model has the maximum buffer capacity after the previous subtraction. This step starts with the subtraction of the water buffer that usually has the maximum buffering capacity at the extreme ends of the pH axis. In general the water buffer is the most dominant buffer at pH<2.5 and at pH>10.5.

For simplicity, in this initialisation step only, a buffer-capacity model is assumed in which all the buffers are regarded as monoprotic:

$$\begin{aligned} \beta_j = 2.303[H^+]\Bigg(1 + C_w K_w^{\dagger}\frac{1}{\left([H^+]+K_w^{\dagger}\right)^2} \\ + \sum_i^m C_i K_i \frac{1}{([H^+]+K_i)^2}\Bigg) \\ \text{where}: \begin{matrix} [H^+] = 10^{-pH_j} = h_j(\mathrm{pH}), \\ i = (1, 2, \ldots, m) \end{matrix} \end{aligned} \tag{4}$$

It is assumed that m buffers exist in the sample. Each buffer has a concentration C_i and acidity constant K_i. In addition, the water buffer, B_w, exists with concentration C_w=55.5 mol L^{-1} and $K_w^{\dagger}$ is a function of the water acidity constant, Eq. (5):

$$K_w^{\dagger} = \frac{K_w}{C_w} \tag{5}$$

Subtracting the water buffer and hydrogen ion effect from the raw BC results in another curve ($BC_{j,step,0}$), as in Eq. (6):

$$\forall j : BC_{j,step,0} = BC_{j,2} - B_{w,j} \tag{6}$$

If plotted against $\forall j{:}pH_j$, the maxima of the $BC_{j,step,0}$ curve are clear and the corresponding pH points are the p$K_{a,i}$ of the buffering systems, assuming all buffer systems are distant enough (minimum overlap of adjacent buffers). Finding the maximum value of $BC_{j,step,0}$ at point j, the maximum concentration limit of the maximum buffering component can be determined and its pK_a will be pH_j as defined in Eq. (7) and its maximum concentration value is calculated from Eq. (8).

$$\exists i, j : BC_{j,step,0} = \max\left(BC_{j,step,0}\right) \rightarrow \{pK_{a,i}, C_{i,\max}\} \tag{7}$$

for ($H_j^+ = K_i$):

$$C_{i,\max} = \frac{1}{2.303} \cdot BC_{j,step,0} \cdot \frac{\left(H_j^+ + K_i\right)^2}{H_j^+ \cdot K_i}$$

$$= \frac{4}{2.303} \cdot BC_{j,step,0}, \quad (8)$$

Equation (6) is applied again to subtract the buffer determined by use of Eqs. (7) and (8), enabling determination of $BC_{step,l}$ and a new buffer. The steps involving Eqs (6) to (8) will be repeated until the maximum concentrations and pK_a values are determined for all the buffers. From Eq. (4) each buffer $B_i[j]$=b(H_i^+,K_i,C_i). Therefore, repetition of the steps can be generalised in Eq. (9) for $L=(0)\cup(i)$:

$$\forall l, j : BC_{j,step,l+1} = BC_{j,step,l} - B_{j,l+1}$$
$$\forall l \exists i, j : BC_{j,step,l} = \max\left(BC_{step,l}\right) \rightarrow \{pK_{a,i}, C_{i,\max}\} \quad (9)$$
$$C_{i,\max} = \frac{4}{2.303} \cdot BC_{j,step,0}$$

Searching for buffers is stopped when the last buffering capacity value is less than a predetermined value. In the implementation this value is fixed at 10% of the highest buffer capacity value detected. Knowing the pK_a values enables identification of the buffers present in the system. Therefore, at the end of this second step the model to be optimised is defined. For optimisation of buffer concentrations, however, range and initial value must be defined. At this point, only the maximum limit for the concentration is known.

The third step defines the minimum possible BC and concentration of each detected buffer component, B_i, by looping over the detected buffers (r=1:m, $r \neq i$) and subtracting the maximum BC that can be introduced by other existing buffers at the pK_a point under consideration. This procedure is formulated in Eq. (10) assuming m detected monoprotic buffers:

$$B_{i,\min} = \frac{2.303}{4} \cdot C_{i,\max} - \sum_{r=l, r\neq i}^{m} 2.303\left[H_i^+\right] \times \left(1 + \frac{C_{r,\max} K_{a,r}}{\left(\left[H_i^+\right] + K_{a,r}\right)^2}\right) \quad (10)$$

From this, a set of buffer objects is defined, Eq. (11), so that a model can be defined. In addition, the concentration range of each buffer has been defined so that concentrations can be estimated by a minimisation algorithm that aims at fitting the BC model to the BC data:

$$\text{Buffers} : B_i = \{B_1, B_2, \ldots, B_m\}$$
$$\text{Buffer characteristics} : \forall i : B_i = \{pK_{a,i}, C_{i,\max}, C_{i,\min}\} \quad (11)$$

As will be shown later in the results, it is found that $C_{i,\max}$,$C_{i,\min}$ are usually close to the actual concentration. This is useful, because most of the minimisation algorithms quickly find a global minimum and, therefore, the correct concentration. This narrow range can trouble some minimisation algorithms, however. For example, setting a narrow range can trouble the Praxis minimisation algorithm [13] used by the BOMB software. It is therefore decided to use the detected minimum concentration $C_{i,\min}$ as the initial value for the optimisation and to extend the minimum limit to a significantly smaller value, i.e. allowing more freedom to the optimisation from the lower-concentration end. This approach works well for definition of the most probable model if the buffers are so distant that each is present as a clear peak in the BC (Fig. 2).

If, however, the buffers are not sufificiently distant, adjacent buffers will overlap and form one peak, for instance as shown in Fig. 3. One pK_a value will be evaluated between their true pK_a values. Because the detected peak does not correspond to one buffer, subtraction of the monoprotic buffer model at the evaluated pK_a will result in large residuals on both sides. The residual peaks will be erroneously detected by the above procedure as more buffers. Therefore, if overlap (interference) occurs, the initialisation procedure should be extended with logic-based rules for accurate detection of interfering buffers.

Logic-based rules

The logic in this extension of the initialisation is threefold. First, for the system that is intended for application (e.g. anaerobic digestion), a set of all possible buffers should be defined.

Second, for each pK_a of the possible buffers, two acceptable ranges are defined. For initialisation purposes, a wide range for each pK_a is assumed and overlap is allowed for any two adjacent buffers. It should be stressed that areas of overlap are not allowed for more than two buffers. Otherwise, a high correlation may make the optimisation fail. Another range is determined for parameter estimation and is used to deal with the expected variation of external factors, e.g. temperature and ionic strength. These optimisation ranges should not overlap, because this is an essential requirement to guarantee the convergence of the optimisation algorithm. For example, for lactate (pK_a= 3.86) and VFA (pK_a=4.75), their wide initialisation ranges could be 3.4–4.4 and 4.2–5.3 respectively. For estimation

of the pK_a of lactate and VFA, reasonable ranges for optimisation are 3.6–4.2 and 4.4–5, respectively. It should be noted that some buffer components are diprotic or triprotic. Therefore, the corresponding pK_a and pK_a ranges should be defined twice or three times, respectively. But at the same time, their concentration is only defined once. For a number q of pK_a definitions for possible buffers, the suggested characteristics are defined in Eq. (12).

$$\begin{aligned} &B_p = \{B_{p,1}, B_{p,2}, \ldots, B_{p,s}\} \\ &\text{where} \\ &\forall_s : B_{p,s} = \{pK_{a,p,s}, pK_{a,s,\max}, pK_{a,s,\min}, pK_{a,s,in\_\max}, \\ &\qquad pK_{a,s,in\_\min}, C_{p,s,\min}, C_{p,s,\max}\} \\ &\text{with } s = 1:q \end{aligned} \tag{12}$$

The characteristics of possible buffers are defined on the basis of optimisation and interference:

$pK_{a,p,s}$: the initial value for optimisation pK_a evaluated under standard conditions

$pK_{a,s,\max}$, $pK_{a,s,\min}$: the maximum and minimum pK_a values allowing a possible shift from standard conditions; overlap of adjacent ranges is not allowed

$pK_{a,s,in\_\max}$, $pK_{a,s,in\_\min}$: logical initialisation range for the detection of interference; overlap of adjacent ranges is allowed

$C_{p,s,ini}$, $C_{p,s,\min}$, $C_{p,s,\max}$: are respectively the initial, minimum, and maximum concentrations of a buffer to initialise the optimisation of its concentration.

Last, the detected buffers, B_i, determined in the monoprotic model-based initialisation, will be used in view of the initialisation of the pK_a values of possible buffers. The result is a definition of the initial model for optimisation and an initialisation of their concentration. If B_i is generated by two interfering buffers, it will initialise two buffers of B_p using the switch $pK_{a\_hits,s}$. This switch has a default value of 0 and it changes to 1 the first time the detected buffer corresponds to one of these particular two buffers B_p. This switch helps to test the hypothesis that only one of the two buffers is actually present. Also, it helps to adjust the maximum concentration limit of the different B_p's to the highest detected value. Initialised buffers will be defined for optimisation, $opt(B_p,s)$. Therefore, if the buffer's $pK_{a,i}$ is within the initialisation range of a B_p buffer ($pK_{a,s,in\_\min} < pK_{a,i} < pK_{a,s,in\_\max}$), the logic procedure can be simplified for two situations, Eq. (13). In the first situation ($pK_{a\_hits,s}=0$), the buffers that will be optimised and their concentration ranges are defined. In the second situation ($pK_{a\_hits,s}=1$), the concentration limits are adjusted.

$$pK_{a\_hits,s}\begin{cases} = 0 \Rightarrow \begin{cases} opt(B_{p,s}) \\ C_{p,s,ini} = C_{i,\min} \\ C_{p,s,\min} = C_{i,\min} \\ C_{p,s,\max} = C_{i,\max} \end{cases} \\ = 1 \Rightarrow \begin{cases} (C_{p,s,\max} < C_{i,\max}) \overset{Change}{\longrightarrow} C_{p,s,\max} = C_{i,\max} \\ C_{p,s,ini} = 0 \\ C_{p,s,\min} = 0 \end{cases} \end{cases} \quad \begin{aligned} &\text{with :} \\ &i = 1:m \\ &s = 1:q \end{aligned} \tag{13}$$

Further rules must be defined, depending on the implementation and the software into which the initialisation procedure is to be integrated. For example, some rules are needed to define the settings of the optimisation algorithm, estimate the ionic strength of the titrated samples when using BOMB, ... etc. For the current implementation, interference detection has been integrated with the monoprotic model-based initialisation procedure, programmed in C++, supplemented with other interface modules and combined as a software layer around the BOMB software.

On-line implementation

Figure 1 shows the flow chart of the BCS. After a titration experiment has been performed by a titrimetric analyser the titration curve is logged into a computer. The user starts the BCS and chooses the run mode: off-line or on-line, according to the analyser to which BCS is connected. For each instance of the internal loop, BCS manages different procedures for calculation, result output, and logging the information, necessary to assess the quality of the titration experiment. All calculation procedures and modules are managed by the integration module. The calculation modules and procedures comprise the initialisation module, the BCS parameters procedure, the interference detection module, and the buffer capacity optimum model builder (BOMB) procedure. The BCS parameters procedure reads the parameters needed for other modules and procedures from a standard initialisation file. The initialisation and interference detection modules work interactively with the BCS parameters procedure to update its objects according the information abstracted from the titration data and the defined logic rules. The appropriate initial model, parameters, and data are then passed to BOMB.

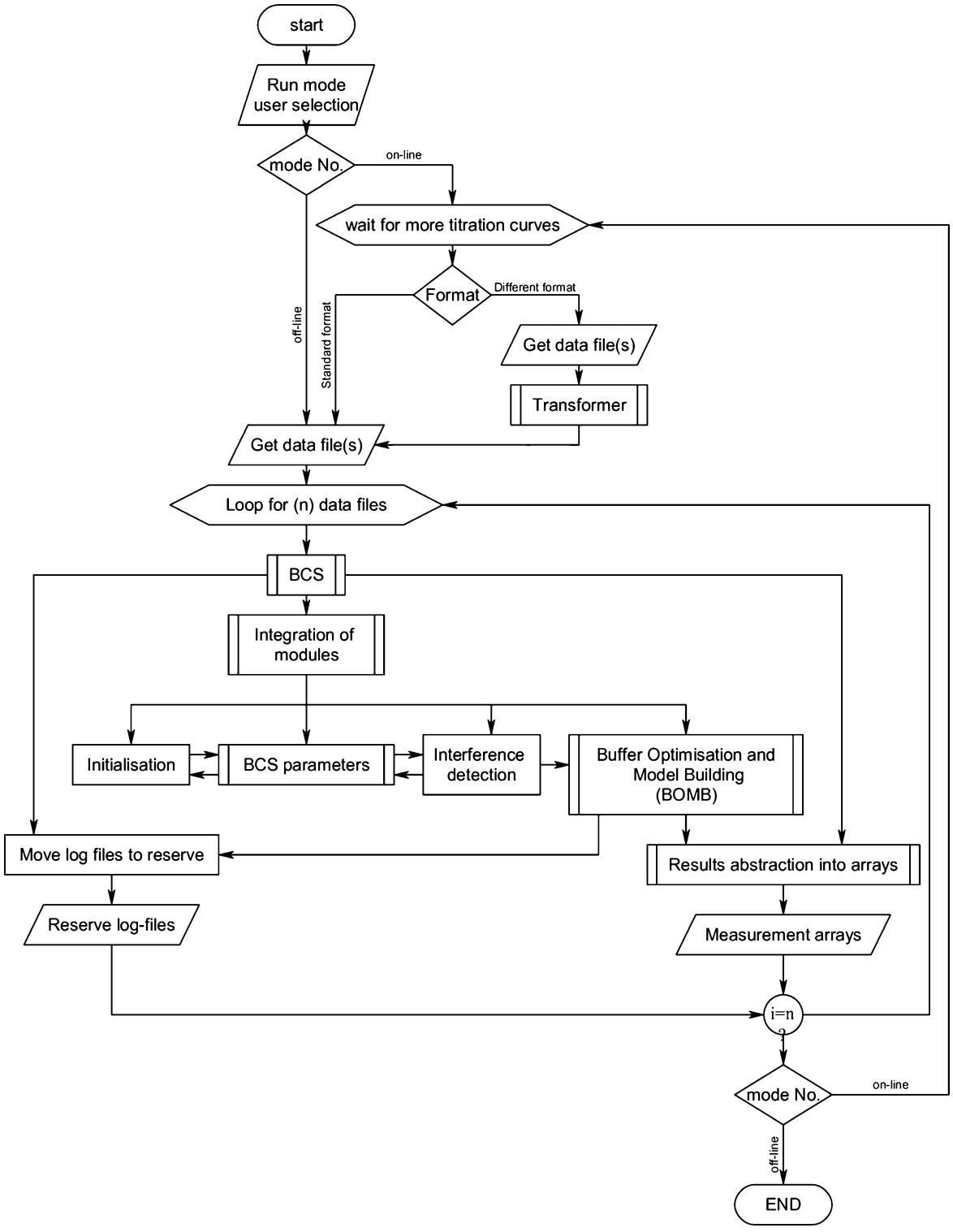

Fig. 1 Flow chart of the buffer capacity software (BCS)

BOMB optimises the initialised model to fit the buffer capacity data that are calculated from the titration experiment. As a check point BOMB applies advanced model-selection techniques to evaluate the optimisation results of the initial model and to propose a model extension if deemed useful to get a better fit. The optimisation results and quality data of the titration curve are stored in log files. The results are stored in separate arrays for each buffer concentration and pK_a value so that each measurement can be dealt with as an on-line mono-sensor output that is useful for data validation, e.g. detection of outliers, shifts, drift, ... etc.

Validation methods

Validation has been performed in three ways. First, a titrimetric analyser simulator (TAS) was built using WEST modelling software (Hemmis, Kortrijk, Belgium). The detailed implementation of TAS can be found elswhere [14, 15]. The simulator generates ideal titration curves for any buffer combination defined in the simulator parameters and using the sampled input concentration. In this work external factors such as temperature and ionic strength were not considered. The BCS, with the initialisation software layer, is then used to analyse the virtual titration curves and its results are compared with simulator parameters and inputs.

Second, titration experiments were performed with various combinations of buffer standard solutions. A Metrohm laboratory titrimetric analyser was used for the titration experiments. These laboratory titration experiments were designed to test the detection of the most interfering buffers.

Third, BCS was tested with titration data collected from the on-line titrimetric analyser AnaSense (AppliTek, Nazareth, Belgium). The analyser was installed on-line with a laboratory-scale UASB reactor which was fed with synthetic wastewater made from wine and starch (COD of the influent varies between 5000 and 10000 mg L^{-1}). The temperature of the reactor was 37°C and pH was stabilized at approximately 7.2. The BCS results are then compared with the bicarbonate and VFA measurement provided by the on-line analyser using two other methods to interpret the raw titration curves [16]. The analyser measurement method 1 was developed by INRA (Institut National de la Recherche Agronomique, Narbonne, France) and is based on the Kapp method for VFA measurement, extended for bicarbonate measurement [17]. The analyser method 2 was developed by AppliTek (Nazareth, Belgium), based on the method of McGhee [18]. Bicarbonate is stripped in the form of CO_2 from the anaerobic samples at pH<5 by use of compressed air. Because of the stripping of CO_2 the pH will tend to rise. The quantity of acid added till pH 5 can be related to the bicarbonate alkalinity. The VFA can then be directly measured by a down-titration, because it is directly related to the volume of acid added between pH 5 and pH 4 [18].

Results and discussion

Simulated titration curves

Ideal titration curves were produced by the titrimetric analyser simulator (TAS) and then used to test the initialisation and optimisation procedure. It can be seen in Fig. 2 that, as expected, a perfect fit could be achieved.

Figure 2a shows the BC of 0.1 mol L^{-1} VFA and 0.1 mol L^{-1} carbon system mixture. In this combination, three peaks are very clear for VFA, bicarbonate, and carbonate at pH points that correspond to their approximate pK_a values. The carbon system is a diprotic buffer and therefore produces two peaks. Table 1 shows the detected buffer characteristics using the monoprotic model-based approach, the initialised model, and the optimisation results. The detected pK_a values were used to define the components of the buffer model. For optimisation of the pK_a values, they were initialised to their standard value. Their optimisation ranges (minimum and maximum values) were set to predefined values to accommodate possible shifts because of external factors such as temperature and ionic strength of the solution. The detected buffer characteristics are from the monoprotic model-based initialisation approach. The detected minimum and maximum concentrations are very close to the real concentrations.

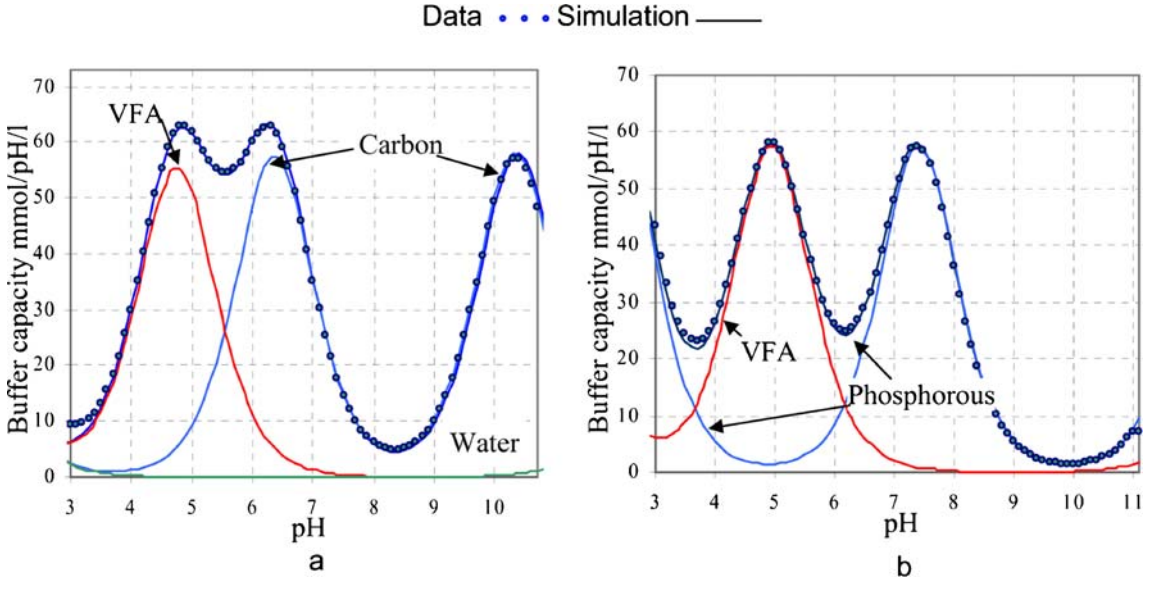

Fig. 2 Examples of sufficiently distant buffers that can be accurately initialised by the monoprotic model approach. (**a**) 0.1 mol L^{-1} VFA and 0.1 mol L^{-1} carbon. (**b**) 0.1 mol L^{-1} VFA and 0.1 mol L^{-1} phosphorus

Table 1 Initialisation and optimisation results for 0.1 mol L^{-1} VFA and the 0.1 mol L^{-1} carbon mixture

Buffer: no./component	Detected buffer characteristics			Buffer model initialisation		
	0	1	2	VFA	Carbonate	Bicarbonate
$pK_{a\ detected/initial}$	4.9	10.3	6.4	4.75	10.33	6.361
$pK_{a\ min}$	–	–	–	4.1	10.1	5.6
$pK_{a\ max}$	–	–	–	5.5	10.9	6.6
Maximum BC (mmol L^{-1}/pH)	62.78	56.69	61.62	–	–	–
Maximum concentration (mol L^{-1})	0.109	0.098	0.107	0.109	0.107	
Minimum BC (mmol L^{-1}/pH)	55.45	56.66	54.13	–	–	–
Minimum concentration (mol L^{-1})	0.096	0.098	0.094	0.00096	0.00094	
Initial concentration	–	–	–	0.096	0.094	
Optimisation results:			pK_a	4.745	10.36	6.344
			pK_a STD	0.001	0.001	0.000
			Concentration	0.099	0.100	
			Conc. STD	0.00086	0.00060	

The detected minimum concentrations were used as initial values for the optimisation problem. The minimum limits were set to 1% of the initial value and the maximum limits were maintained as detected. The carbon system was detected by both its species, i.e. bicarbonate and carbonate.

Automatically, the initialisation program recognised that the carbon system is diprotic and thus the concentration data were assigned only once, for both of them. The maximum limit was chosen as the larger of the two and the minimum and initial concentrations were chosen as the lowest from the detected values of bicarbonate and carbonate. With this accurate and concise initialisation, the minimisation algorithm reached a global minimum quickly and gave accurate results.

Figure 2b shows the BC of a VFA and phosphorus mixture. For this mixture, two peaks will be very clear for VFA and phosphorus at pH points that correspond to their approximate pK_a values. Although phosphorus is triprotic and hence has 3 pK_a values, only the middle one will be clear because the other two, at 2.15 and 12.35, cannot be seen in the BC because they will be in the ranges of high interference by the water buffer. The initialisation and optimisation results for this example are listed in Table 2. These results lead to a similar interpretation as described for Table 1 except that the phosphorus concentration was directly initialised according to the only detected middle peak (pK_{a2}).

Figure 3 shows two examples of buffer interferences. Accurate initialisation leads to accurate optimisation results as shown in Table 3. It can also be seen from Fig. 3 that a perfect fit could be reached. Figure 3a shows the BC of a mixture of 0.05 mol L^{-1} VFA, 0.1 mol L^{-1} sulfide and 0.05 mol L^{-1} carbon system. For this combination three peaks are clear. The peaks of VFA and carbonate are clear at their approximate pK_a values. Sulfide interferes with bicarbonate and both are shown as one peak between their pK_a values. Table 3 shows the detected buffer characteristics using the monoprotic model-based approach, the initialised model, and the optimisation results. In addition to the steps illustrated for the buffer combinations that are presented in Tables 1 and 2, the logic-based rules are implemented to deal with the interference between bicarbonate and sulfide. The pK_a of the detected buffer 0 hits the initialisation ranges defined for both sulfide and bicarbonate and therefore both buffers were activated for optimisation. The pK_a values are initialised with standard condition values. The pK_a optimisation ranges are set to the predefined values. The detected maximum concentration of buffer 0 is used as

Table 2 Initialisation and optimisation results for 0.1 mol L^{-1} VFA and 0.1 mol L^{-1} phosphorus mixture

Buffer: no./component	Detected buffer characteristics		Buffer model initialization	
	0	1	VFA	Phosphorus
$pK_{adetected/initial}$	4.8	7.2	4.75	7.206
$pK_{a\ min}$	–	–	4.1	7.1
$pK_{a\ max}$	–	–	5.5	8.0
Maximum BC (mmol L^{-1}/pH)	58.02	57.56	–	–
Maximum concentration (mol L^{-1})	0.101	0.010	0.101	0.010
Minimum BC (mmol L^{-1}/pH)	55.54	56.63	–	–
Minimum concentration (mol L^{-1})	0.096	0.098	0.00096	0.00098
Initial concentration	–	–	0.096	0.098
Optimisation results:		pK_a	4.75	7.20
		pK_a STD	0.000	0.000
		Concentration	0.100	0.100
		Conc. STD	0.00048	0.00044

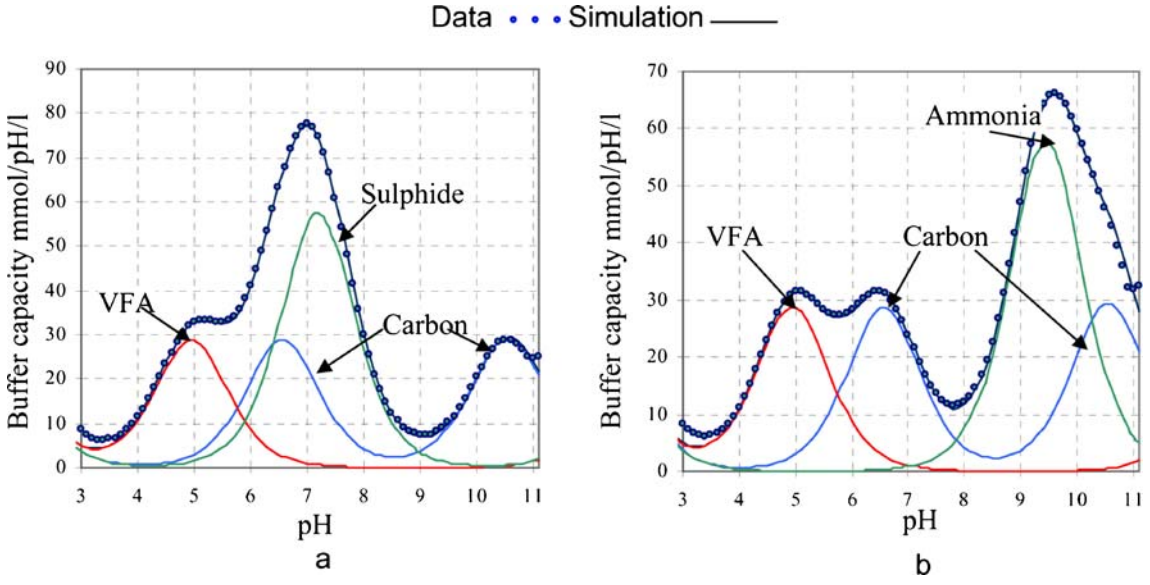

Fig. 3 Examples of mixtures with interfering buffers that can accurately be initialised by integrating the monoprotic model approach and the logic-based approach. (**a**) 0.05 mol L^{-1} VFA, 0.05 mol L^{-1} carbon and 0.1 mol L^{-1} sulfide. (**b**) 0.05 mol L^{-1} VFA, 0.05 mol L^{-1} carbon, and 0.1 mol L^{-1} ammonia

the upper limit of both sulfide and carbon system concentration. Initial and minimum values are set to very small values (to numerically approximate zero). This initialisation of concentrations corresponds to asking the optimiser two questions:

- Is the detected area of interference because of both interfering buffers or only one of them?
- What are the concentrations, provided that it should not exceed the specified maximum concentration?

Then, the optimiser gives its answer in the light of the best fit of this peak of interference and within the constraints of the specified pK_a optimisation ranges. It can be seen from the optimisation results in Table 3 that accurate concentration results of all buffers can be reached.

Figure 3b shows the BC of a mixture of 0.05 mol L^{-1} VFA, 0.05 mol L^{-1} carbon, and 0.1 mol L^{-1} ammonia; Table 4 shows the detected buffer characteristics. In this combination, two peaks for VFA and bicarbonate are very clear at pH points that correspond to their approximate pK_a values. Ammonia and carbonate appear as one peak. Despite this interference, they were detected by the monoprotic model-based approach and therefore concise concentration ranges could be defined for optimisation. The reasons for this good result are that the approach applies successive subtraction of buffers and the approximate 1 pH unit difference between the ammonia pK_a and carbonate pK_a enables their detection in the monoprotic model-based approach.

Table 3 Initialisation and optimisation results for 0.05 mol L^{-1} VFA, 0.1 mol L^{-1} sulfide, and 0.05 mol L^{-1} carbon mixture (logic-based rules are applied)

Buffer: no./component	Detected buffer characteristics			Buffer model initialization			
	0	1	2	VFA	Sulfide	Carbonate	Bicarbonate
p$K_{a\ detected/initial}$	6.8	4.8	10.3	4.75	6.9	10.33	6.36
p$K_{a\ min}$	–	–	–	4.3	6.7	10.1	5.6
p$K_{a\ max}$	–	–	–	5	7	10.9	6.6
Maximum BC (mmol L^{-1}/pH)	77.62	32.86	28.47	–	–	–	–
Maximum concentration (mol L^{-1})	0.135	0.057	0.045	0.057	0.135	0.135	
Minimum BC mmol L^{-1}/pH)	76.30	29.82	28.37	–	–	–	–
Minimum concentration (mol L^{-1})	0.132	0.052	0.0493	0.00052	1e-05	1e-05	
Initial concentration	–	–	–	0.052	1.1e-05	1.1e-05	
Optimisation results:			pK_a	4.747	6.987	10.35	6.36
			pK_a STD	0.013	0.006	0.012	0.000
			Concentration	0.050	0.100	0.050	
			Conc. STD	0.00066	0.00099	0.00054	

Table 4 Initialisation and optimisation results for 0.05 mol L^{-1} VFA, 0.1 mol L^{-1} ammonia, and 0.05 mol L^{-1} carbon mixture

Buffer: No./component	Detected buffer characteristics				Buffer model initialisation			
	0	1	2	3	VFA	Ammonia	Carbonate	Bicarbonate
pK_a detected/initial	9.4	6.3	4.7	10.5	4.75	9.252	10.33	6.361
p$K_a$min	–	–	–	–	4.3	8.5	10.1	5.6
p$K_a$max	–	–	–	–	5	9.7	10.9	6.6
Maximum BC (mmol L^{-1}/pH)	66.23	31.65	30.75	38.72	–	–	–	–
Maximum concentration (mol L^{-1})	0.115	0.055	0.053	0.067	0.053	0.115	0.067	
Minimum BC (mmol L^{-1}/pH)	55.57	28.49	27.72	20.65	–	–	–	–
Minimum concentration (mol L^{-1})	0.096	0.050	0.048	0.036	0.00050	0.00096	0.00036	
Initial concentration	–		–	–	0.0481	0.096	0.0359	
Optimisation results:				pK_a	4.748	9.267	10.4	6.357
				pK_a STD	0.014	0.007	0.024	0.000
				Concentration	0.050	0.101	0.050	
				Conc. STD	0.00066	0.00104	0.00062	

Robustness during rapid transitions

On the basis of simulations, the usefulness of BCS and the developed initialisation procedure for monitoring anaerobic digestion is illustrated. Also, the BCS with the initialisation modules is tested for the automatic detection of rapidly changing buffer combinations. The test is performed on the hypothetical evaluation of buffer concentrations that is shown by the solid lines in Fig. 4. The evaluation was designed to imitate different scenarios that would be relevant in practice when monitoring the anaerobic digestion process under rapid transitions of operating conditions. Indeed, these dynamics would be very severe to an anaerobic digester but the idea is to test BCS and the initialisation procedure on such hypothetical extreme case. The dynamics were designed as a combination of triangular waves of different buffer concentrations. Two peaks of amplitude 0.105 mol L^{-1} VFA with a minimum of 0.005 mol L^{-1} VFA are accompanied by similar but inverse waves of carbon. This imitates the dynamics in a digester during overloads—bicarbonate alkalinity is consumed during VFA accumulation. Then the alkalinity recovers when the VFA concentration drops. The first peak of VFA is accompanied by a peak of lactate, which is also expected to accumulate during overload. The second VFA peak is accompanied by a peak of ammonia that is toxic to metha-

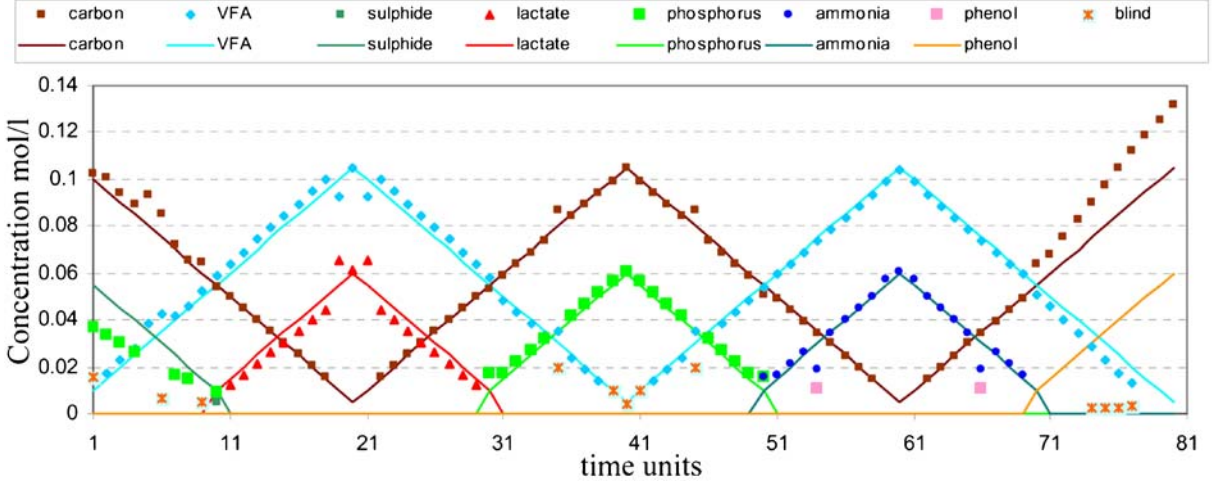

Fig. 4 Robustness test of BCS under conditions of rapid transitions of buffer combinations. *Lines* are simulated buffer concentrations and *symbols* are BCS estimated concentrations

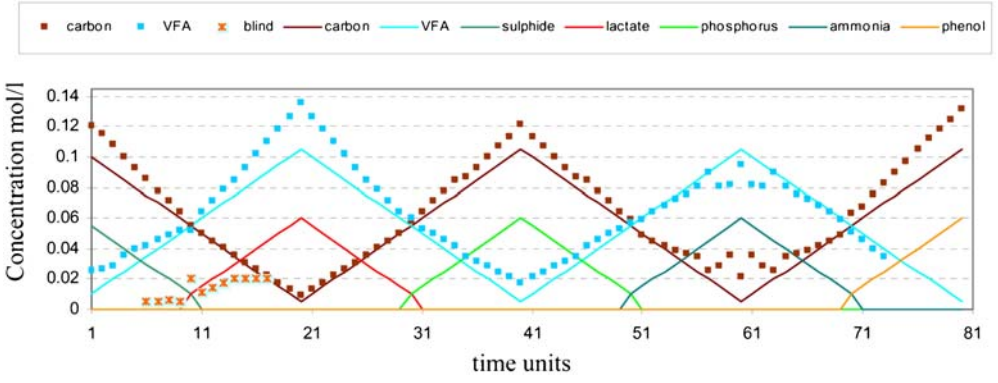

Fig. 5 Possible results from BOMB under conditions of rapid transitions of buffer combinations. *Lines* are simulated buffer concentrations and *symbols* are BOMB estimated concentrations

nogenesis and would therefore cause such VFA accumulation. Between the two peaks of VFA, a peak of phosphorus is introduced to imitate a case of phosphorus release, VFA uptake, and alkalinity increase that would happen in a digester fed by sludge from a biological phosphorus-removal plant. During the first 10 time units hydrogen sulfide is added as a source of toxicity to the anaerobic digestion process during the feed with a high sulfate concentration. Also, during the last 10 time units phenol is added as a source of toxicity that may be expected in some types of wastewater.

The evolution was simulated by WEST with the TAS running simulations of titration experiments at the normal sampling rate. At each sampling point an ideal titration curve is simulated for the corresponding buffer combination. All titration curves are subsequently evaluated by BCS with the same pK_a optimisation and initialisation ranges $pK_{a,p,s}$, $pK_{a,s,\max}$, $pK_{a,s,\min}$, $pK_{a,s,in\_\max}$, $pK_{a,s,in\_\min}$. The proper model is activated for optimisation at each sampling point. Other initialisation data $C_{p,s.ini}$, $C_{p,s,\min}$, $C_{p,s,\max}$ were determined automatically by the monoprotic model-based approach and the logic-based rules extension. It can be seen from Fig. 4 that the BCS measurements correlate with the simulated dynamics of the different buffer combinations.

Figure 5 shows the results obtained under the designed dynamics when using BOMB without BCS initialisation or human interaction to improve its initial model. It was found that it was not possible to successfully run BOMB with all buffers defined for optimisation. The initial model was then defined for BOMB with the two main buffers (the carbon system and the VFA). Except for a few sampling points that correspond to lactate interference, the model selection performed by BOMB was not powerful enough to extend the initial model with the necessary (blind) buffers for this particular set of complicated interferences. Despite this, the concentrations for bicarbonate (carbon system) and VFA determined by BOMB show a reasonable correlation with the simulated dynamics of the two components. Visually, it is clear, however, that the initialisation by BCS gives better results. This will be evaluated statistically below.

To reflect the extent of the linear relationship between the expected concentrations and the BCS and BOMB measurements, the Pearson product moment correlation coefficient, r, was calculated for each buffer component:

$$r = \frac{n \cdot (\sum XY) - (\sum X) \cdot (\sum Y)}{\sqrt{\left[n \cdot \sum X^2 - (\sum X)^2\right] \cdot \left[n \cdot \sum Y^2 - (\sum Y)^2\right]}} \tag{14}$$

where X is the independent value (the expected measurement) and Y is the dependent value (the observed measurement).

Table 5 shows the r values for each buffer except phenol, because phenol could not be detected when present in combination with carbonate (see below). The high linearity of the BCS data can be deduced from the high r values. Sulfide has the lowest value, because it was confused with phosphorus and calculated as such. From investigating the optimised pK_a values, however, it can be observed that they are usually equal to the lower limit of the optimisation range of the pK_a of phosphorus. Indeed, three buffers at the same peak cannot be accurately measured, even if the three can be automatically initialised. The high correlation will trouble the minimisation algorithm (i.e. Praxis will be trapped in a local minimum) and the three interfering components will not be estimated accurately. The pK_a of sulfide at 7 is close to that of phosphorus at 7.2 and does not enable initialisation of both besides bicarbonate at pK_a 6.35. It is, therefore, up to the user to decide which component exists. In another situation, if both sulfide and phosphorus must be

Table 5 Pearson correlation coefficient of BCS linearity with the real concentrations in cases of ideal titration curves

Buffer	Carbon	VFA	Sulfide*	lactate	Phosphorus	Ammonia
r (BCS)	0.982828	0.99038	0.901139	0.984847	0.943402	0.991353
r (BOMB)	0.981025	0.945581	–	–	–	–

*Detected as phosphorus because of severe interference of three buffers (bicarbonate, sulfide, and phosphorus)

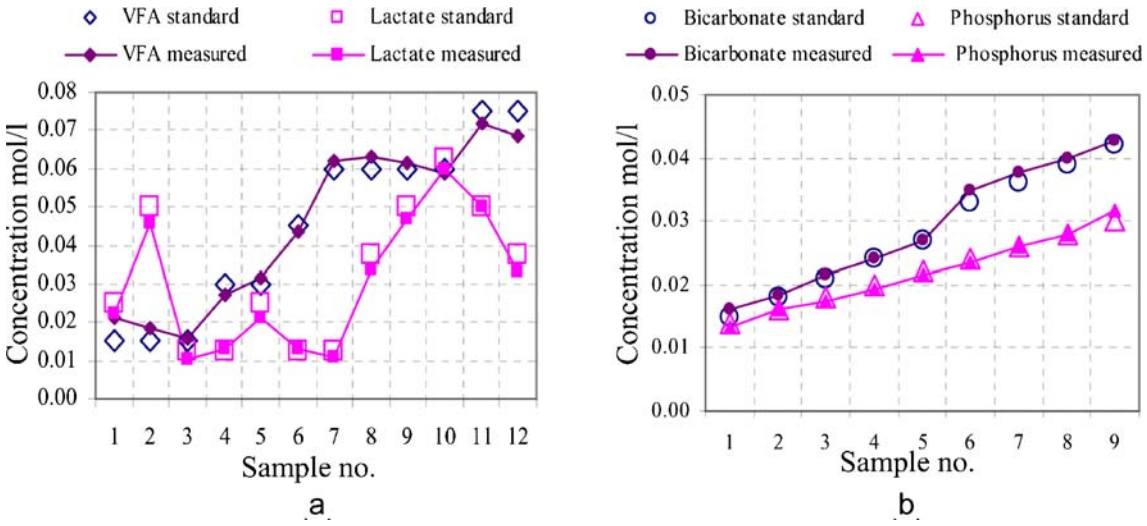

Fig. 6 Validation of BCS with standard solutions. (**a**) VFA and lactate, (**b**) bicarbonate and phosphorus

determined, an additional titration experiment must be performed for the same sample. The additional experiment should be performed by first stripping of CO_2 and H_2S at low pH. In this way phosphorus could be determined accurately. Then a second experiment is performed without stripping and phosphorus is fixed in the model building at the value detected from the first experiment. Bicarbonate and sulfide can then be estimated. Handling of nested titration experiments is, however, beyond the scope of this approach to automatic initialisation. Similarly, phenol could not be detected because of interference of its pK_a of 9.97 with carbonate pK_a of 10.35 and the high interference of the water buffer at pH>10.5. Also, in this situation, an additional titration experiment with CO_2 stripping would be required.

It can be seen from Fig. 4 that some outliers appear. Also, drift and shift can be seen on introduction of some buffers that cannot be initialised because of their interferences with other buffers. Examples, shown in Fig. 4, are H_2S and phenol as discussed above. Therefore, on-line mono-sensor validation procedures to detect outliers, drift, and shift can be applied to ensure that improper data are not passed on to an automatic control system [19]. It will be shown later in the on-line validation section that such procedures are also useful to filter out other bias arising from anomalies in the titration experiments.

Validation with standards

The initialisation procedure and BCS was further validated by titrating standard solutions with known buffer concentrations. The experiments with standards aim to test com-

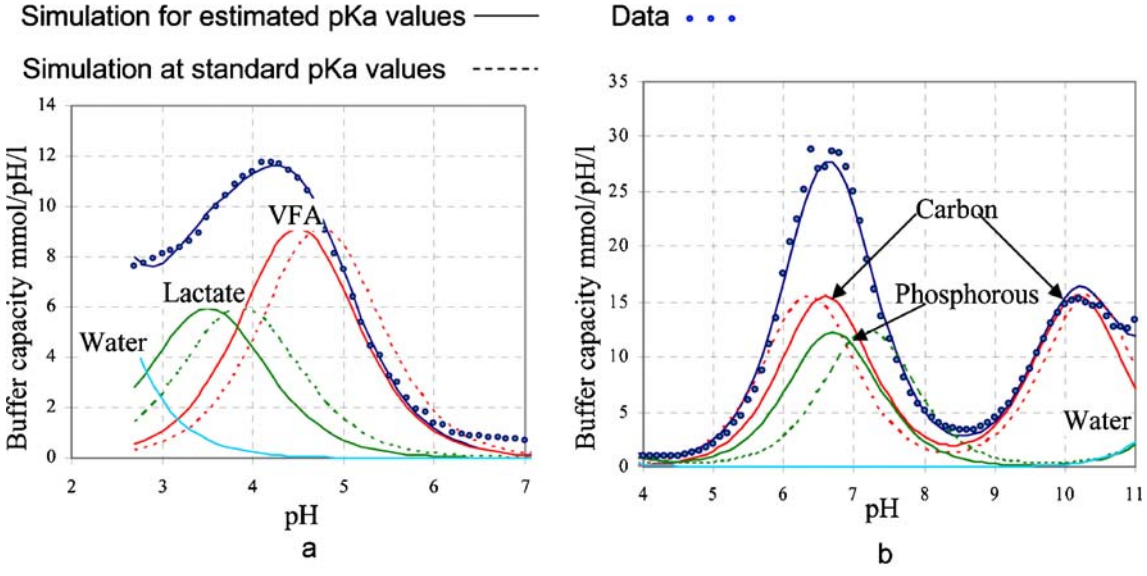

Fig. 7 BC results for measuring standard solutions while having pK_a shifts. (**a**) VFA and lactate, (**b**) carbon and phosphorus

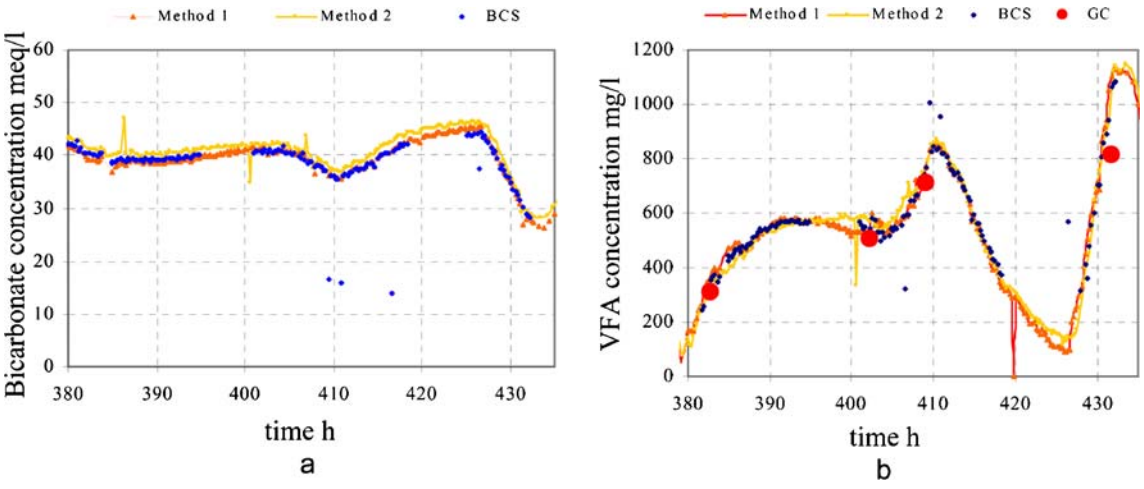

Fig. 8 On-line validation analysis results using AnaSense built-in methods 1and 2, BCS and gas chromatography (GC)

binations of important interfering buffers that are expected in anaerobic digestion.

The main buffer components in anaerobic digestion are bicarbonate and VFA. The buffer that interferes most with VFA is lactate, which can be regarded as an indicator of digester overload [2]. Figure 6a gives an overview of BCS measurements obtained for VFA and Lactate mixtures. The results cover a wide range of VFA/lactate ratios. The linearity of the results was tested with Eq. (14). For VFA, r is 0.99 and for lactate r is 0.995.

Phosphorus can be regarded as the buffer that interferes most with bicarbonate. Figure 6b shows the linearity of the BCS measurements for bicarbonate and phosphorus mixtures. Again, linearity was tested. For bicarbonate r is 0.998 and for phosphorus r is 0.996.

When performing a titration, the actual pK_a values may differ from their standard values because the titration is not performed under standard conditions. Also, a shift in pH measurement (because of drift in the electrode) will change the detected pK_a value. Such shifts will not affect the detected results as long as the shifts are within the pK_a initialisation ranges. Indeed, the pK_a value will be estimated so as to fit the measured BC data optimally. Figure 7a shows the simulated and measured BC curves of sample 3 in Fig. 6a. VFA and lactate buffers could be initialised and accurately measured although there was a shift from the standard pK_a values. Similarly, in Fig. 7b, the bicarbonate and phosphorus values could be initialised and accurately estimated despite the shift in pK_a values. Figure 7b shows the measured and the simulated BC curves of sample 5 in Fig. 6b.

On-line validation

The AnaSense (AppliTek, Nazareth, Belgium) titrimetric analyser was installed on-line with an UASB reactor [16] to monitor bicarbonate and VFA concentrations. The AnaSense performed the titration experiments on-line, starting from the pH of the reactor (maintained at approximately 7) and titrating down to pH 3.5 with stepwise addition of acid every 8 s. Titration data were collected from the titrimetric analyser during three periods (382–395 h, 401–418 h, and 427–433 h). The BCS analysed the collected titration data and results are shown in Fig. 8a for bicarbonate and in Fig. 8b for VFA. The results are compared with the results from interpretation methods 1 and 2 that are built-in in the analyser [16]. Similar results of the BCS and the analyser results are obtained for bicarbonate, Fig. 8a. Figure 8b shows the VFA results of the three methods compared with the GC results. The three methods follow the same dynamics and results correspond to those obtained by GC.

The relationship between the on-line measurements given in Fig. 8 was evaluated. The correlation matrix is given in Eq. (15) for the on-line bicarbonate measurements (R_{HCO_3}) and in Eq. (16) for the on-line VFA measurements (R_{VFA}). The correlation matrices are arranged in the sequence of AnaSense methods 1 and 2 and BCS, respectively. Correlations are larger than 0.97 for bicarbonate and larger than 0.98 for the VFA measurements. In this experiment, however, no other buffers were interfering with the main buffers in the digester (bicarbonate and VFA) and, therefore, the AnaSense built-in methods and the new BCS method give the same results.

$$R_{HCO_3} = \begin{bmatrix} 1 & 0.9768 & 0.9809 \\ 0.9768 & 1 & 0.9748 \\ 0.9809 & 0.9748 & 1 \end{bmatrix} \quad (15)$$

$$R_{VFA} = \begin{bmatrix} 1 & 0.9819 & 0.9860 \\ 0.9819 & 1 & 0.9820 \\ 0.9860 & 0.9820 & 1 \end{bmatrix} \quad (16)$$

Conclusion

The initialisation procedure presented in this paper is able to extract useful information from simple titration experiments. The required information concerns the likely buffers

to be present in the titrated sample and their expected concentration range. This information is shown to be sufficient to initialise an optimisation procedure that enables accurate determination of the buffer concentrations.

The procedure does not require frequent interaction with the user to study prior analytical results or information about the system to update the optimisation model. The model is defined and initialised automatically which therefore increases its robustness for on-line application.

The initialization procedure has been implemented in a software layer and integrated to a buffer capacity software sensor, buffer capacity optimum model builder (BOMB), which uses modelling and optimisation techniques to analyse buffer systems observed in titration data. The resulting buffer capacity software (BCS) was tested in three ways—with a titrimetric simulator, a laboratory analyser, and an on-line analyser. BCS showed its potential to accurately measure a wide range of buffer components and combinations. It also has a high measuring quality, even under fast transitions between different buffer combinations. For biological processes such as anaerobic digestion that require continuous and on-line monitoring of buffering substrates or toxic compounds, BCS is a good solution.

Acknowledgements The authors would like to thank EU TELEMAC project IST-2000-28156 for financial support.

References

1. Bjornsson L, Murto M, Jantsch TG, Mattiasson B (2001) Evaluation of new methods for the monitoring of alkalinity, dissolved hydrogen and the microbial community in anaerobic digestion. Water Res 35:2833–2840
2. Zaher U, Bouvier JC, Steyer J-P, Vanrolleghem PA (2004) Titrimetric monitoring of anaerobic digestion: VFA, alkalinities and more. In: Proceedings of 10th World Congress on Anaerobic Digestion (AD10), August 29–September 2 2004, Montreal, Canada, Vol. 1 330–336
3. Wild D, Kisliakova A, Siegrist H (1997) Prediction of recycle phosphorus loads from anaerobic digestion. Water Res 31 (9):2300–2308
4. van Rensburg P, Musvoto EV, Wentzel MC, Ekama GA (2003) Modelling multiple mineral precipitation in anaerobic digester liquor, Water Res 37:3087–3097
5. Batstone DJ, Keller J, Angelidaki RI, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, Sanders WTM, Siegrist H, Vavilin VA (2002) Anaerobic Digestion Model no1, IWA publishing, London, UK
6. Ahn YH, Bae JY, Park SM, Min KS (2004) Anaerobic digestion elutriated phased treatment of piggery waste, Water Sci Technol 49(5–6):181–189
7. Atuanya EI, Aigbirior M (2002) Mesophilic biomethanation and treatment of poultry waste-water using pilot scale UASB reactor, Environ Monit Assess 77(2):139–147
8. Borja R, Sanchez E, Weiland P (1996) Influence of ammonia concentration on thermophilic anaerobic digestion of cattle manure in upflow anaerobic sludge blanket (UASB) reactors. Process Biochem 31(5):477–483
9. Wang Z, Banks CJ (2003) Evaluation of a two stage anaerobic digester for the treatment of mixed abattoir wastes. Process Biochem 38(9):1267–1273
10. Gijzen HJ, Bernal E, Ferrer H (2000) Cyanide toxicity and cyanide degradation in anaerobic wastewater treatment. Water Res 34(9):2447–2454
11. Van Vooren L, Van De Steene M, Ottoy JP, Vanrolleghem PA (2001) Automatic buffer-capacity model building for the purpose of water quality monitoring, Water Sci Technol 43(7): 105–114
12. Van De Steene M, Van Vooren L, Ottoy J-P, Vanrolleghem PA (2002) Automatic buffer-capacity model building for advanced interpretation of titration curves, Environ Sci Technol 36:715–723
13. Brent RP (1973) Algorithms for minimization without derivatives, Prentice-Hall, Englewood Cliffs, New Jersey
14. Zaher U (2005) 'Modelling and monitoring the anaerobic digestion process in view of optimisation and smooth operation of WWTP's', PhD thesis, Ghent University, Ghent, Belgium p 346
15. Zaher U. and Vanrolleghem P.A.: 2005, General ion recruiting procedure for pH-based calculation, Environmental Modelling & Software (in press)
16. De Neve K, Lievens K, Steyer J-P, Vanrolleghem PA (2004) Development of an on-line titrimetric analyser for the determination of volatile fatty acids, bicarbonate, and alkalinity. In: Proceedings 10th World Congress on Anaerobic Digestion (AD10). August 29-September 2, 2004, Montreal, Canada, Vol. 3, 1316–1318
17. Bouvier JC, Steyer JP, Delgenes JP, (2002) On-line titrimetric sensor for the control of VFA and/or alkalinity in anaerobic digestion processes treating industrial vinasses, VII Latin American Workshop and Symposium on Anaerobic digestion, October 23–25, 2001,Merida, Mexico, pp 65–68
18. McGhee TJ (1968) A method for approximation of the volatile acid concentrations in anaerobic digesters. Water Sew Works 115:162–166
19. Olsson G, Newell B (1999) Wastewater treatment systems-modelling diagnosis and control, IWA Publishing