Modelling of an EGSB treating sugarcane vinasse using first-order variable kinetics
Iván López and Liliana Borzacconi

ABSTRACT
An expanded granular sludge bed (EGSB) anaerobic reactor treating sugar cane vinasse was modelled using a simple model with two steps (acidogenesis and methanogenesis), two populations, two substrates and completely mixed conditions. A first-order kinetic equation for both steps with time-variant kinetic coefficients was used. An observer system was used to estimate the evolution of kinetic constants over time. The model was validated by comparing methane flow predictions with experimental values. An estimation of evolution of populations of microorganisms was also performed. This approach allows calculation of specific kinetic constants that reflect biological activity of microorganisms. Variation of specific kinetic constants reflects the influence of the fraction of raw vinasse in the feed. High salt concentrations in the reactor may have inhibited the process.

Key words | EGSB, kinetic, modelling, observers

INTRODUCTION
Bioethanol production is very important for industrial applications and in the substitution of energy-renewable sources for fossil fuels. The main objective of the distillation process is production of alcohol, but a concentrated wastewater called vinasse or stillage is simultaneously generated. About 15 L of vinasse are produced per litre of distillate alcohol (Van Haandel 2004; Pant & Adholeya 2007) over a concentration range that can vary widely according to the origin of the raw material. The organic matter concentration in vinasse apparently depends strongly on the design and operating conditions of each distillery and the origin of the vinasse. The concentration of organic matter shows a wide range of values: 20 to 33 g Chemical Oxygen Demand (COD)/L for cane juice vinasse and 48 to 120 g COD/L for vinasse from molasses, with a widely varying biodegradable fraction (Wilkie et al. 2000). Sulphate and potassium concentrations also show very high values. Because of the high organic load, anaerobic treatment with biogas recovery is recommended (Van Haandel & Van Lier 2006; Satyawali & Balakrishnan 2008). Anaerobic systems are particularly suitable for handling high concentrations of organic matter and are highly favourable from the standpoint of sustainability (Fernández-Polanco et al. 2005).

Most industrial applications of anaerobic technology for vinasse treatment are based on the Upflow Anaerobic Sludge Bed (UASB) reactor concept, widely used for wastewater treatment (Laubscher et al. 2001; Wolmarans & de Villiers 2002). The Expanded Granular Sludge Bed (EGSB) reactor is an extension of the upflow sludge bed concept that increases upflow liquid velocity. The higher upflow liquid velocity allows fluidisation of the sludge bed with a more intensive contact between liquid substrate and solid microorganisms. Industrial applications are rapidly increasing, and organic loads for the EGSB reactor are higher than for traditional UASB reactors. Nevertheless, stable operation of the reactor is challenging. Modelling efforts to understand the dynamic behaviour of the EGSB reactor are critical.

High levels of potassium are reported to inhibit the operation of the reactor, probably because of the neutralisation effect on the membrane potential (Chen et al. 2008). Release of micronutrients because of the exchange capacity of potassium with other ions has been reported (Ilango van & Noyola 1993). As reported by Chen et al. (2008), the potassium IC50 (i.e. the concentration of inhibitor that decreases the biological activity to half) for acetate-utilising microorganisms was found to be between 0.15 and 0.74 mol/L but the degradation rate of glucose was virtually unaffected.

Despite the great increase in anaerobic applications, the comprehensive and practical modelling of these systems is still developing. An important milestone in this field was
the IWA ADM1 (Batstone et al. 2002, 2006; Batstone 2006). This structured model includes multiple steps describing biochemical and physicochemical processes involving at least 26 dynamic state variables and many parameters. Although the complexity of anaerobic processes is reflected in the ADM1 model, direct application for modelling and control purposes is difficult. The identification of model parameters under actual operating conditions is quite difficult. On the other hand, the ADM1 model fails to depict all of the complex phenomena that occur in an anaerobic system.

Simpler models with a reduced set of state variables and parameters have been proposed (Dalla Torre & Stephanopoulos 1986; Angelidakis et al. 1993; Bernard et al. 2001; Noykova et al. 2002; Haag et al. 2005). Morel et al. (2006) have used a first-order kinetic model with time-variant parameters to model the UASB reactor. Although simple models do not represent the complexity of real processes, parameter identification and model validation are more straightforward in the simple models.

Usually, from the hydrodynamic point of view, these models are based on completely stirred tank reactor (CSTR) conditions (Ojha & Singh 2002; Chowdhury & Mehrotra 2004; Batstone 2006). A few authors have considered other hydrodynamic behaviours (Wu & Hickey 1997; Keshtkar et al. 2003; Pontes & Pinto 2006). As the kinetic models used are very simple, there is no need to consider deviations from complete mixing behaviour (López & Borzacconi 2010).

Beyond the selection of an appropriate model to represent the system in a simple form, identification of the values of parameters is a complex task. Simplifications introduced in the model are probably reflected in parameter variations when environmental conditions change. Simpler models imply that less generalisation of results is necessary when model parameters are fixed. In particular, if rate expressions are simplified with respect to the dependence of state variables, parameters should ‘absorb’ this simplification to maintain the same confidence level. The change in the history of microorganisms will then be transferred to the parameters, which will evolve over time. A simplified kinetic approach can be used but kinetic parameters will probably vary over time. How can the path of this evolution be determined? Independent batch tests are tedious and are not practical for continuous monitoring. However, observers or software sensors can provide estimates of unmeasured variables or unknown parameters based on knowledge of the process dynamics. Asymptotic observers are based on a linear transformation of state variables that allows the material balance to be written in an independent form from the reaction kinetics (Dochain 2005). When a decoupled parameter estimation (Perrier et al. 2000; Morel et al. 2006) is used, the evolution of specific growth coefficients can be determined simultaneously.

### MATERIALS AND METHODS

#### Experimental reactor

A bench scale EGSB reactor with a working volume of 6 L and a total volume of 12 L (including phase separator) was used for the treatment of sugarcane vinasse. The reactor was placed in a temperature-controlled cabinet at 30 ± 1 °C. Characterisation of the vinasse used to feed the reactor is shown in Table 1. The reactor was fed with diluted vinasse, increasing the percentage of raw vinasse over time and maintaining the volumetric flow at an average value of 4.4 L/d (standard deviation of 2.4 L/d). For monitoring purposes, Chemical Oxygen Demand (COD), Volatile Fatty Acids (VFA), alkalinity and pH were measured at the inlet and the outlet of the reactor. COD was determined by the reflux method (APHA, AWWA, WEF 1995), and VFA and alkalinity were determined by a simplified titration method (DiLallo & Albertson 1961). Gas flow was recorded by a wet-type gas meter (Schlumberger, Germany), and gas composition was determined by gas chromatography (Shimadzu GC 14B, Japan). Samples from the sludge bed were periodically collected, and solids content in the reactor was determined. Additionally, volatile suspended solids (APHA, AWWA, WEF 1995) in the reactor effluent were determined routinely.

#### The model

A simple two-step model (acidogenesis – methanisation) was used. In the first step, acidogenic bacteria ($X_1$) consume the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (g/L)</td>
<td>56.4–65.8</td>
</tr>
<tr>
<td>BOD$_5$ (g/L)</td>
<td>17.5–27.0</td>
</tr>
<tr>
<td>N-NH$_4^+$ (g N/L)</td>
<td>0.04–0.19</td>
</tr>
<tr>
<td>NTK (g N/L)</td>
<td>1.4</td>
</tr>
<tr>
<td>VFA (g HAc/L)</td>
<td>7.75–12.2</td>
</tr>
<tr>
<td>SO$_4^{2–}$ (g/L)</td>
<td>2.09–2.24</td>
</tr>
<tr>
<td>K$^+$ (g/L)</td>
<td>3.9–4.5</td>
</tr>
<tr>
<td>pH</td>
<td>4.0–4.3</td>
</tr>
</tbody>
</table>
complex organic substrate ($S_1$) and produce volatile fatty acids (VFA, $S_2$) and more bacteria. Next, a methanogenic population ($X_2$) consumes the VFA and produces methane (and more microorganisms). The model reduces the complexity of the network of anaerobic reactions to only two biological reactions in series.

Considering the simplicity and the mathematical benefits derived from a linear system, we propose to use a first-order kinetic equation to represent the reaction rates instead of the most common Monod- or Haldane-based kinetic equation. A first-order kinetic equation is the simpler option, to reflect the dependence on the reactant concentration. Remember also that the most common kinetic expressions used in a biological process, like Monod or Haldane kinetics, approximate to a first-order kinetic equation when the substrate concentration is low relative to the semi-saturation constant. Volumetric rates are

\[ r_1 = k_1S_1 \]  
\[ r_2 = k_2S_2 \]

where $S_1$ represents the concentration of the complex organic substrate (expressed as g COD/L), $S_2$ is the concentration of the VFA (also expressed as g COD/L) and $k_1$ and $k_2$ (in d$^{-1}$) are the first-order kinetic constants for both reactions. For the calculation of $S_1$, only 90% of the non-VFA COD was considered anaerobically biodegradable.

Loss of complexity due to linear behaviour must be compensated by relaxing the fixed values of constant parameters to maintain the same confidence level in the model. At the same time, the number of microorganisms and their biological activity must be reflected in the values of the constants. We propose dynamic behaviour of kinetic constants to encompass biomass quantity and variation in environmental conditions.

Many phenomena cannot be represented by the model due to its simplicity. Variation in pH is not considered. Inhibition of the reaction caused by pH variation, by the presence of chemicals or product inhibition are not represented explicitly by the model.

Continuously stirred tank reactor (CSTR) behaviour is assumed for the liquid phase. The high recirculation ratio in an EGSB reactor is the dominant factor in producing a significant amount of mixing. The production of biogas, uniformly distributed inside the reactor due to fluidisation, also contributes to mixing. Equations for the dynamic model are:

\[ \dot{S}_1 = D(S_{1in} - S_1) - k_1S_1 \]  
\[ \dot{S}_2 = D(S_{2in} - S_2) + k_1S_1 - k_2S_2 \]

where $D$ (in d$^{-1}$) is the dilution rate, i.e., the inverse of the hydraulic retention time. The $in$ subscript shows inlet conditions, and the dot variables are the respective time derivatives.

The solid phase also achieved a high degree of mixing due to fluidisation. If the reactor is well designed, the biomass is retained in the reactor. However, a small fraction of the solids content can be washed out with the liquid effluent. To incorporate the effect of solids retention in the reactor, the $\alpha$ parameter (which represents the solid fraction that leaves the reactor) was introduced, as in the work of Bernard et al. (2001). Two additional equations can be written to reflect the biomass dynamic:

\[ \dot{X}_1 = Y_1k_1S_1 - \alpha DX_1 \]  
\[ \dot{X}_2 = Y_2k_2S_2 - \alpha DX_2 \]

where $X_1$ is the concentration of acidogenic bacteria (in g Volatile Suspended Solids (VSS)/L), $X_2$ is the concentration of methanogenic bacteria (in g VSS/L), $\alpha$ is the solid fraction that leaves the reactor and $Y_1$ and $Y_2$ are the substrates to biomass yield of each type of microorganism.

Assuming that the kinetic constants are proportional to the respective biomass quantities, we can write equations for specific kinetic constants

\[ k'_1 = k_1/(X_1V) \]  
\[ k'_2 = k_2/(X_2V) \]

both constants in (g VSS d)$^{-1}$.

**Kinetic estimation using observers**

Model Equations (3) and (4) can be rewritten in a more compact matrix form as

\[
\begin{bmatrix}
  \dot{S}_1 \\
  \dot{S}_2
\end{bmatrix} =
\begin{bmatrix}
  -S_1 & 0 \\
  S_1 & -S_2
\end{bmatrix}
\begin{bmatrix}
  k_1 \\
  k_2
\end{bmatrix}
+ D
\begin{bmatrix}
  S_{1in} - S_1 \\
  S_{2in} - S_2
\end{bmatrix}
\]

where
\[
D = \begin{bmatrix}
  D & 0 \\
  0 & D
\end{bmatrix}
\]
Observers provide software estimations of unmeasured variables or unknown parameters based on knowledge of process dynamics. Asymptotic observers are based on a linear transformation of state variables that allows the material balance to be written in a form independent of the reaction kinetics. We write the following observer system to estimate the kinetic constants (Morel et al. 2006):

\[
\begin{bmatrix}
\dot{S}_1 \\
\dot{S}_2 \\
\end{bmatrix} =
\begin{bmatrix}
-S_1 & 0 \\
0 & -S_2 \\
\end{bmatrix}
\begin{bmatrix}
\dot{k}_1 \\
\dot{k}_2 \\
\end{bmatrix} +
D
\begin{bmatrix}
S_{\text{lin}} - S_1 \\
S_{\text{lin}} - S_2 \\
\end{bmatrix}
+
\begin{bmatrix}
\omega_1 & 0 \\
0 & \omega_2 \\
\end{bmatrix}
\begin{bmatrix}
S_1 - \dot{S}_1 \\
S_2 - \dot{S}_2 \\
\end{bmatrix}
\]

\[
\begin{bmatrix}
\dot{k}_1 \\
\dot{k}_2 \\
\end{bmatrix} =
\begin{bmatrix}
-1/S_1 & 0 \\
1/S_2 - 1/S_2 & 0 \\
\end{bmatrix}
\begin{bmatrix}
\gamma_1 & 0 \\
0 & \gamma_2 \\
\end{bmatrix}
\begin{bmatrix}
S_1 - \dot{S}_1 \\
S_2 - \dot{S}_2 \\
\end{bmatrix}
\]

where the ‘hat’ variables are estimated and \(\omega_i\) and \(\gamma_i\) are the gains of the observers. Following the suggestion of Perrier et al. (2000), \(\gamma_i\) can be considered equal to \(\omega_i^2/4\). Then only two parameters must be adjusted. Knowing the inlet and the reactor concentrations over time, the equations can be integrated. The evolution of the kinetic constants is thus obtained.

The methane flux \(q_M\) (in L/d) can be related to the \(S_2\) degradation rate:

\[
q_M = 0.38(1 - 1.42 \times Y_2)k_2S_2V_R
\]

where 0.38 is the conversion factor to convert g COD to litres of methane at 30°C, 1.42 is the conversion factor to convert VSS to COD for biomass, and \(V_R\) is the active reactor volume. If we have experimental data for methane flow, we can validate the model results.

The calculation algorithms were implemented and the optimisation runs were performed using MATLAB®. Because the concentration determinations are not continuous, a spline interpolation was used to apply the model.

### RESULTS AND DISCUSSION

Figure 1 shows the experimental values of inlet and outlet COD. A relatively low organic load (an average of 3.7 g COD/L·d referred to the active reactor volume) was applied during the first 50 days to acclimate the biomass to vinasse. Organic load was then progressively increased for 40 days until an organic load of 18 g COD/L·d was reached, and this approximate value was maintained for 30 days. Between days 120 and 160, some operational problems were encountered that forced us to diminish the organic load by half. Thereafter, the organic load was maintained at approximately 20 g COD/L·d. After day 200, the reactor was fed with a COD concentration of approximately 40 g/L, diminishing the volumetric flow. During this period, the VFA concentration in the reactor increased and bicarbonate addition was necessary to maintain alkalinity. Finally, VFA concentrations reached values up to 3 g COD/L, and destabilisation occurred although the load was lowered. The pH was maintained in the range between 7 to 8 in the reactor without alkali addition, even though the feed was at a pH of approximately 4.5.

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**Figure 1** | Reactor performance. –Inlet COD (g COD/L), --- outlet COD (g COD/L), --- volumetric load (g COD/L·d).
Adjustment of the observer parameters $\omega_i$ was made empirically in an attempt to achieve an acceptable fit with the experimental variables and smooth behaviour of the estimated parameter. Figure 2 shows that a very good adjustment was achieved with $\omega_1 = 2$ and $\omega_2 = 3$, providing an estimate of the evolution of the kinetic constants over time. The results do not vary significantly when $\omega_i$ parameters show minor variations. Optimisation of these results is not warranted because there is too little change in the general behaviour of variables and parameters.

Low values of kinetic constants in the first 50 days correspond to an acclimation period with a low organic load and consequent low biological activity. After the first 50 days of acclimation, kinetic constant $k_1$ shows values between 1.5 and 3 d$^{-1}$. In the last period, when reactor failure occurs, the $k_1$ constant appears to fall. After the acclimation period, the methanogenic constant $k_2$ fluctuates mainly between 5 and 10 d$^{-1}$, with some peaks that exceed 10 d$^{-1}$. Variations in reactor working conditions are reflected in the variations of the kinetic parameters. After day 210, when raw vinasse dilution is diminished, the methanogenic constant definitely tends to decrease. With this fall in the value of $k_2$, the model reflects the destabilisation of the reactor: the second step of the model (methanogenesis) works with a low rate, and accumulation of acids occurs. The simple model not explains the reasons for this destabilisation but reflects the facts appropriately.

Model validation was performed using Equation (11). An empirical factor $m = \text{mean}(q_{M,\text{exp}}/k_2S_2) = 4.29$ L$_{\text{CH}_4}$/g COD/L was used to fit experimental values of methane flow with $S_2$ removal via the second reaction. Figure 3 shows an acceptable fit between experimental and predicted values despite the simplicity of the model. Beyond this general good fit, in the last 100-day period some peaks in the simulation seem not to correspond to experimental values. However, the average trend of the simulated line follows the line of experimental values. The experimental values tend to average variations instantaneously. The model probably tends to amplify variations in working conditions when a high load is applied to the reactor. From the empirical $m$ factor, a value of $Y_2 = 0.042$ g VSS/g COD$_{\text{rem}}$ was obtained, in agreement with the value found in literature data for yield of methanogenic microorganisms (Pavlostathis & Giraldo-Gomez 1991).

Experimental data for exit of reactor solids and content of reactor solids was used to estimate biomass evolution. Assuming that all biomass consists of $X_1$ and $X_2$, summation of Equations (5) and (6) yields

$$\dot{X} = Y_1k_1S_1 + Y_2k_2S_2 - aDX$$  \hspace{1cm} (12)
Since there was a systematic evaluation of the solids exit, the $aDX$ term was known. Periodic measurement of total solids content in the reactor allowed us to perform a least squares fit to estimate the $Y_1$ value. The result was $Y_1 = 0.057$ g VSS/g COD$_{rem}$, a reasonable value for carbohydrate fermentation according to literature data (Pavlostathis & Giraldo-Gomez 1994).

If the initial population distribution is known, Equations (5) and (6) can be integrated separately. Unfortunately, these data are not available. Actually, the initial value seems to have little impact after the first adjustment period: virtually identical values were reached even with very different initial values as shown in Figure 4. In Figure 4(a) the initial conditions are equal masses of $X_1$ and $X_2$; in Figure 4(b) the initial conditions are 10% of $X_1$ and 90% of $X_2$. We can observe that after the first hundred days, the curves of individual populations are virtually identical for both sets of initial conditions.

On this basis, calculation of a specific kinetic constant according to Equations (7) and (8) was performed from day 100 onward, i.e., the kinetic constants were expressed per biomass unit:

$$k_1^* = k_1/(X_1V_R)$$
$$k_2^* = k_2/(X_2V_R).$$

These specific kinetic constants reflect more precisely the biomass activity and are presented in Figure 5.

Until day 210, the reactor was fed with a maximum of 40% raw vinasse, but from day 210, more concentrated vinasse was used (Figure 5). Highly concentrated vinasse seems to inhibit the activity of microorganisms, as expressed by the decrease in the $k_i$ values. Methanogenic organisms are more sensitive, and this sensitivity is reflected in $k_2$ and is not observed as a rise after raw vinasse concentrations are lowered. This lack of a rise could be explained by irreversible damage to the cellular machinery of the microorganisms or by a significant disease of the microorganisms, probably due to the increase in osmotic pressure (Martin et al. 1999).

Moreover, in the period after day 260, there seems to be an increase in the activity, corresponding to an increase in acid-forming bacteria, as seen in the increase of the $k_1^*$ values. This increase in acid production (reaching values up to 3 g COD/L) is not balanced by the corresponding acid consumption by methanogenic microorganisms because $k_2^*$ does not rise.
It is highly probable that the main inhibiting factor associated with raw sugarcane vinasse is the high concentration of a wide spectrum of substances, probably due to the increase in the osmotic pressure. Potassium concentration could be a strong inhibitor, and raw vinasse usually shows values between 3 to 5 g K\(^+\)/L. Potassium is not consumed in the reactor, and potassium concentration in the reactor follows the percentage of raw vinasse in the inlet.

In Figure 6, specific kinetic constants were plotted jointly with the potassium concentration.

Are values of specific kinetic constants reasonably? Is coherent that \( k_1^* \) is about four times smallest than \( k_2^* \) when normally we assume that acidogenesis is faster than methanogenesis? In a first-order kinetic model, kinetic constants are not directly associated with the microorganism growth rate as in the Monod model or similar models. Then, the fact that acidogenic bacteria grow faster than methanogenic microorganisms not implies that \( k_1 \) is greater than \( k_2 \) in our model. In a series of reactions, the rates of production and consumption of the intermediate product must be compared. The key to a successful process is that there is no accumulation of the intermediate product. For balanced reactor operation, acid formation must be compensated by acid consumption. In our experiment the concentration of acidogenic population \( X_1 \) is approximately the same as the concentration of the methanogenic population \( X_2 \). On the other hand, substrate concentration \( S_1 \) is approximately three times higher than the concentration of \( S_2 \). Then,
reaction rates \( r_1 = k_1^c X_1 S_1 \) and \( r_2 = k_2^c X_2 S_2 \) are similar in magnitude, and no accumulation occurs except in the last destabilisation period. In this last period, \( k_1^c \) rises but \( k_2^c \) remains the same. The rate of acid production is not balanced by the rate of acid consumption, and acidification occurs.

**CONCLUSIONS**

The EGSB reactor for treatment of sugarcane vinasse can be modelled using the hypothesis of complete mixing with a simplified kinetic model consisting of two reactions in series with first-order kinetics and variable coefficients. Estimation of these first-order kinetic coefficients over time can be performed using a system of observers with calibration done empirically. The model is validated by comparing predicted and experimental methane production.

By knowing the solids output in the exit stream over time, the cell yield coefficients of the populations associated with both reactions can be estimated. We can also estimate the evolution of individual populations; it is not necessary to know initial conditions because the system approaches the same values independent of the starting point.

Specific reaction constants for each reaction can be estimated. These specific constants are indicators of the activity of the microorganism population. The constants are strongly affected by the percentage of raw vinasse used in the reactor feed. Inhibition of the reaction could be attributed to high concentrations of a wide spectrum of substances (such as potassium) that increase osmotic pressure.

The model with first-order kinetics and variable coefficients transfers the complexity of biological kinetic expressions to the variation over time of the coefficients. Despite the simplicity of the model, the model can predict the reactor behaviour and reflect the periods of malfunction.

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