Determining anaerobic degradation kinetics from batch tests

Iván López Moreda

ABSTRACT

Data obtained from a biomethane potential (BMP) test were used in order to obtain the parameters of a kinetic model of solid wastes anaerobic degradation. The proposed model considers a hydrolysis step with a first order kinetic, a Monod kinetic for the soluble organic substrate degradation and a first order decay of microorganisms. The instantaneous release of methane was assumed. The parameters of the model are determined following a direct search optimization procedure. A 'multiple-shooting' technique was used as a first step of the optimization process. The confidence interval of the parameters was determined by using Monte Carlo simulations. Also, the distribution functions of the parameters were determined. Only the hydrolysis first order constant shows a normal distribution.

Key words | anaerobic digestion, modelling, Monte Carlo methods, multiple shooting

INTRODUCTION

At present, global changes in the world (e.g. greenhouse gases, the energy crisis) require a more efficient use of biotechnologies (Holm-Nielsen et al. 2009). Concepts such as 'biorefinery', based on the biotechnological transformation of biomass have replaced the traditional refinery scheme based on the transformation of oil and its derivatives. Additionally, anaerobic technology has proven to be more efficient than the aerobic treatment of wastes from the point of view of greenhouse gas generation (Cakir & Stenstrom 2008) and can even compete with other biofuels (Power & Murphy 2003). In this scenario, anaerobic digestion plays a key role because the generated products (e.g. hydrogen, methane) can be used as energy sources in boilers, internal combustion engines or fuel cells (Wheeldon et al. 2007) or as raw material for other processing options (e.g. the production of biopolymers or other organic substances). For these reasons, anaerobic technology constitutes the core of organic waste treatment systems (Verstraete et al. 2005).

The modelling of biological systems is a more complex task than modelling just chemical systems, because of the multiplicity of biochemical reactions, the number of microbial populations involved and the incidence of multiple environmental factors (Angelidaki et al. 1993; Mata-Alvarez et al. 2010). As a rule, the model is a set of differential equations with a large number of parameters and little experimental data. In anaerobic processes, quantitative data of microbial populations are difficult to obtain; on the other hand, isolation and enrichment of pure cultures is a very complicated task and, furthermore, reaction networks and syntrophism are destroyed in this case.

Despite increasing anaerobic applications, the comprehensive and practical modelling of these systems is still developing. An important milestone in this field was the development of the International Water Association Anaerobic Digestion Model 1 (ADM1) (Batstone et al. 2002). This structured model included multiple steps describing the biochemical and physicochemical processes, which involved at least 26 dynamic state variables and many parameters. Although the complexity of anaerobic processes was reflected in the ADM1, direct applications for modelling and control purposes are difficult to use. Additionally, the identification of model parameters under actual operational conditions is difficult. Simpler models with reduced sets of state variables and parameters have been proposed (García-Dieguez et al. 2013; Momoh et al. 2013). Although simple models do not represent the complexity of real processes, the identification of parameters and model validations are more straightforward (Noykova et al. 2002; Donoso-Bravo et al. 2013).

The methods of parameter determination are generally based on the minimisation of an objective function, usually least squares minimisation between experimental and model data (Donoso-Bravo et al. 2011). However, if a large number
of parameters must be determined, there is a great risk of determining just a local minimum and not the global minimum; the parameter set will probably lack a physical mean (Noykova et al. 2002). The multiple-shooting methods attempt to extract more information from the experimental data than could be obtained in a usual optimization. Typically, from a vector of initial values of the state variables, equations are integrated obtaining model points at different times which are compared with the N experimental values; the sum of squared errors is then minimized. Conversely, in the multiple-shooting method N integrations are performed, starting from each experimental point (Müller et al. 2002). Thus, the initial point to start the search is less significant since the problem is divided into N small coordinated problems.

Due to experimental errors and disturbances that introduce noise in the model, the optimum value found from experimental data could not be the true value. Then, another task in the determination of a model parameter is the setting of a confidence interval that gives, with a defined level of probability, the range of values including the true value. Usually, the confidence interval is obtained by performing several experimental runs and computing the standard deviation. However, this requires a lot of time and the expenditure of resources. Monte Carlo methods constitute an alternative way that only consumes computational time and allows ‘pseudo-experimental’ runs to be performed, generating a lot of results that could be statistically analysed (Dimov 2008; Preacher & Selig 2012).

Biomethane potential (BMP) tests (Angelidaki et al. 2009) are widely used for the characterisation of wastes in order to act as substrates for methane production. The substrate to be degraded and the anaerobic inoculum are introduced into a flask in anaerobic conditions, and then methane production is followed through time. The cumulative methane production at the end of the experiment constitutes the methane potential of the substrate. This assay provides information on how much and how fast the material can be degraded under optimal batch conditions, which are valuable parameters in the design and operation of a biogas plant (Strömberg et al. 2014). The primary task is to determine the potential of methane production from a substrate; however, the test may be considered as an experiment which can result in kinetic data.

There are two main purposes to this paper: first, to test a methodology of parameter determination and, second, to extract the information of a commonly performed analysis (BMP test) in order to validate a simple kinetic model of anaerobic degradation.

**MODEL**

Biodegradability tests are typically performed in batch conditions (Angelidaki et al. 2009; Labatut et al. 2011). The solid substrate and the anaerobic sludge (i.e. the inoculum) are added to batch reactors in anaerobic conditions. The cumulative methane production is monitored over time and the ultimate value is employed to calculate the methane potential. The transformation of solid substrates into methane involves a complex series of sub-transformations, whereas hydrolysis is generally the step that determines the overall kinetics. Typically, a single first-order-kinetic model represents the hydrolysis of the particulate (Eastman & Ferguson 1981):

\[ r_h = k_h X_b \]  

where \( r_h \) is the hydrolysis rate, \( k_h \) (d\(^{-1}\)) is the first order kinetic constant and \( X_b \) (gVSS/L; VSS: volatile suspended solids) is the biodegradable solid substrate concentration.

The simplest manner to include the microorganisms is to consider a lumped biomass concentration \( X \) (gVSS/L) growing following Monod kinetic:

\[ r_X = \mu_m X \frac{S}{K_s + S} \]  

where \( \mu_m \) (d\(^{-1}\)) is the maximum specific growth rate, \( S \) (gCOD/L; COD: chemical oxygen demand) is the liquid substrate concentration and \( K_s \) (gCOD/L) is the affinity constant or half-saturation constant. Microorganism decay rate \( (r_d) \) can be represented as a first order kinetic:

\[ r_d = m X \]  

where \( m \) (d\(^{-1}\)) is the decay rate constant.

It can be assumed that substrate consumption is proportional to microorganism growth, being \( Y \) (gVSS/gCOD), the yield coefficient. Also, taking into account the low solubility of methane it can be assumed that the release of methane to the gaseous phase is instantaneous; then the rate of liquid substrate consumption is the rate of methane production. Performing a COD mass balance in a batch system the following equations constitute the model of the system:

\[
\frac{dX_b}{dt} = -k_h X_b + mX
\]

\[
\frac{dS}{dt} = 1.42k_h X_b - \frac{\mu_m X S}{Y K_s + S}
\]
\[ \frac{dX}{dt} = \mu_{m}X \frac{S}{K_s + S} - mX \]  \hspace{1cm} (6)

\[ \frac{dM}{dt} = \mu_{m}X \frac{S}{YK_{s} + S} \]  \hspace{1cm} (7)

where \( M \) is the methane produced expressed in COD and the factor 1.42 in the Equation (5) corresponds to COD to VSS ratio.

The proposed model is relatively simple but is capable of retaining the main processes of the anaerobic digestion. However, one key of the modelling is to have a reliable set of parameters. A methodology that can allow determining the parameters without the need of extra experimental work other than the traditional BMP test could constitute a substantial contribution. Having to perform a BMP test for the determination of the biodegradability of a certain substrate, much more information can be derived from the experimental data in the form of the proposed model, and could be used for design purposes.

**METHODS**

**Batch assay**

Sludge purge of an activated sludge wastewater plant (Minas City, Uruguay) was used as substrate. Inoculum was selected from a modified up flow anaerobic sludge bed (UASB) reactor receiving dairy industry wastewater (Passeggi et al. 2009), with a maximum methanogenic activity of 0.3 gCOD-CH₄/gVSS.d. Batch biodegradability tests were performed in 150 mL flasks, which were filled with substrate and fresh inoculum. An inoculum to substrate ratio of 3 on gVSS basis was selected to ensure no limitations on the amount of microorganisms. The pH was adjusted to 7.0 and the volume adjusted to 100 mL with a bicarbonate buffer solution. The flasks were gassed with nitrogen to achieve anaerobic conditions and were sealed and placed in a shaker at 30 °C. Monitoring of the methane production was performed using a pressure transducer to measure the increase of pressure generated by the biogas production, and the biogas composition was determined by gas chromatography. A Porapack Q column with Argon gas as a carrier was used in a GC14 Shimadzu gas chromatograph with an thermal conductivity detector (TCD).

**Parameter identification**

The regular procedure followed to determine the parameters of a differential equation system is the following: the ordinary differential equations or partial differential equations are solved from known values of the initial conditions, followed by numerical integration of the equations over time. Results of this integration are compared with experimental values, and the optimization method is applied to determine parameter values. The multiple shooting method implies the disaggregation of experimental data in order to consider different initial values. This fact also allows integration over different periods of the experiment. In this way, more experimental information is retained and a global optimum approach facilitated.

The multiple shooting method was adapted as follows: first, considering previous knowledge or a previous direct search with all the experimental data, a set of initial parameters \( p_0 \) were taken; with this parameter set, the integration was performed using each experimental data point as an initial value and by considering the time differences between experimental data points as the time span in each integration. Assuming a least squares criterion for optimization, a new parameter set \( p_1 \) was obtained. Starting from this new parameter set, a new integration was performed involving all experimental points, and a new set of parameters \( p_2 \) was constructed.

To determine parameters from the batch tests, Scilab software was used to perform an optimization routine based on the Nelder and Mead Simplex method (Baudin 2010). The least squares function between experimental and simulated values was selected as the objective function to be minimized. Constraints were added in order to comply with physical restrictions: all parameters must be positive and yield must be less than one. Penalisation functions are included to take into account these restrictions in the direct search process.

Initial values of variables are determined experimentally: biomass \( X \) was determined as VSS; soluble substrate was determined as soluble COD; volumetric methane was measured and also expressed as COD in order to state all substances on the same unit basis (calculations were made considering that 1 g COD is equivalent to 0.350 L of methane in standard conditions); finally, biodegradable...
solids were estimated considering that at the last experimen-
tal time 90% of biodegradable solids were transformed in
methane. Considering the VSS initially disposed in the
vial, a 42% solid degradation was obtained.

Taking into consideration the high non-linearity of the
model, error estimation of the parameters cannot be
performed through the usual calculation of the covariance
matrix (Müller et al. 2002). In experiments with small sample
sizes, it is better to apply Monte Carlo simulations (Noykova
et al. 2002). The idea of Monte Carlo simulations is that the prob-
ability distribution of the estimated parameters is approximately
the same as the true values. Hence, a Monte Carlo method was
used in order to obtain the distribution function for each par-
parameter. From this distribution function, a confidence interval
was obtained. This method has been shown to be a useful way
for determining the probability distribution in biotechnological
models (López & Borzacconi 2010; López et al. 2015). It was
assumed that the error distribution around each point of the
cumulated methane curve (from the batch experiment), calcu-
lated with the parameters obtained from the optimization, is
similar to the error of the real value. Also, the distribution of
errors around that point is assumed to be normal, taking 5%
standard deviation. Then, considering the value calculated by
the model as a centre of this error distribution, new sets of
pseudo-experimental data were randomly generated, adding a
random noise to the point defined by the model in order to simu-
late many data sets closely related to the original data set. With
each pseudo-experimental data set, a new optimization was per-
formed, and a new set of optimal parameters was found.

Repeating the procedure a sufficiently large number of times,
the distribution function for each parameter was found. A
normal distribution was verified using the chi-square criterion
(Himmelblau 1970). Considering a 90% probability, the confi-
dence intervals of the parameters were determined.

RESULTS

The BMP test used as experimental set-up is a well established
tool to evaluate the potential of methanisation for an organic
substrate. Although there are some variation in its application
in laboratories, the BMP test is widely used in the world. The
experimental procedure used in this paper follows the sugges-
tions formulated by Angelidaki et al. (2009), in an effort to
standardize the procedure.

Figure 1 shows the first and last step of multiple shoot
integrations. Common results found in literature are taken
into account for the choice of $p_0$ values. It can be observed
that the final points of integrations are widely spaced from
the experimental values in the initial step. However, accord-
ing to the search progress, the final points of each
integration tend to come near the experimental ones.

The values obtained in the last step of multiple-shooting
integration are assumed as the initial point for the traditional
optimization involving all experimental data. Figure 2 shows
the final result of this phase of the optimization process. The
model is always capable of reproducing the experimental
results with an error less than the experimental error
(0.025 gCOD/L reactor).

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**Figure 1** | Left: initial multiple-shoot integrations with $p_0 - (k_0, \mu_m, K_s, Y, m) = (0.05, 0.005, 0.050, 0.02, 0.001)$. Right: last multiple-shoot integrations, $p_1 - (k_0, \mu_m, K_s, Y, m) = (0.0198, 0.606, 0.064, 0.098, 3.9 \times 10^{-3})$. Circles: experimental data; continuous line: model simulation.
A Monte Carlo method was applied in order to evaluate the confidence interval of the optimal values of the model parameters. Six hundred simulations were performed and the distribution of the results are presented in Figure 3. In the same figure, Gaussian curves are represented in order to compare the results obtained with the normal distribution. Clearly, the $k_h$ results are close to normal distribution but the other parameters show a weak visual correlation. To confirm this visual impression, a statistical test was applied using the $X^2$ distribution. Values of the histograms are compared with normal distribution using statistic
\[
\frac{\sum_{i=1}^{k} (n_i - n_{i*})^2 / n_{i*}}{n_i}
\]
where $n_i$ is the number of observations in the $i$ interval of the histogram and $n_{i*}$ is the corresponding value of the normal distribution with the same mean and standard deviation, and comparing it with the $X^2$ value with $k-1$ degrees of freedom. Statistics values for $k_h$, $\mu_m$, $K_s$, $Y$, and $m$ are 3.26, 38.5, 117.4, 60.7 and 49.0 and the $X^2$ value is 33.2; then, only $k_h$ presents a normal distribution. However, distributions obtained from

![Figure 2](https://iwaponline.com/wst/article-pdf/73/10/2468/461758/wst073102468.pdf)

**Figure 2** | Optimal results with $p_2 = (k_h, \mu_m, K_s, Y, m) = (0.0138, 0.0068, 0.0256, 0.0206, 7.8 \times 10^{-5})$. Circles: experimental data; continuous line: model simulation.

![Figure 3](https://iwaponline.com/wst/article-pdf/73/10/2468/461758/wst073102468.pdf)

**Figure 3** | Distribution of parameters obtained with Monte Carlo simulations. Continuous curves are Gaussian curves with the same mean and standard deviation values.
Monte Carlo simulations allow achievement of a confidence interval beyond whether distribution is normal or not. Final results are presented in Table 1 and they allow evaluation of the precision of parameter values.

The model structure is able to be applied to other substrates. But the parameter values are specific to the substrate and inoculum and must be determined in each case. However, as BMP tests are commonly performed routinely in anaerobic digestion, the model is easily implemented for any substrate and inoculum.

Obviously, different substrates and inocula will correspond to different parameter values. But the order of magnitude of these values must be comparable, according to the accumulated experience. According to this point of view, despite the differences in the substrate composition (and probably in some environmental conditions), the results obtained in this work could be compared with other values found in literature. As an example, Tomei et al. (2008) working at a 0.1 inoculum/substrate ratio and 35 °C achieved a first order constant of 0.029 d⁻¹ for activated sludge waste; Sambusiti et al. (2014) found values between 0.049 d⁻¹ and 0.146 d⁻¹ for sorghum degradation using different inoculum sources; Ferreira et al. (2013) found a value of 0.069 d⁻¹ at 35 °C for anaerobic degradation of wheat straw with no pre-treatment. Jokela et al. (2005) found values between 0.024 and 0.107 d⁻¹ for different substrates at 35 °C. Borja et al. (2005) reports a value of 0.054 ± 0.005 d⁻¹ for the hydrolysis of olive pomace. It can be seen that a wide range of values is found in literature corresponding to different substrate composition. However, the order of magnitude of the literature values is coherent with the results found in this work.

The hydrolysis constant could be affected by the nature of the substrate in different ways. For example, lipids require specific enzymes to be hydrolysed, enzymes that must be developed in the inoculum; so, a low value of the hydrolysis constant is expected to be found in a non-acclimated inoculum. On the other hand, high lignin content in cellulosic materials is associated with a low value of the hydrolysis constant as a rule.

A typical value of 0.03 gVSS/gCOD is reported by Pavlostathis & Giraldo-Gómez (1991) for the methanogenic step in their classical anaerobic kinetic review. The same authors also report a typical value of 0.050 gCOD/L as a $K_s$. However, the typical value reported for $\mu_m$ is 0.4 d⁻¹, much larger than that obtained in this work, although the range of values is large. Probably, other limitations such as mass transfer resistances or the presence of complex materials to be degraded are influencing the low values obtained in this work. Regarding $\mu_m$, it depends on the microbial composition of the inoculum, reflecting the methanogenic activity of the sludge. However, this activity is not an absolute notion but it refers to methanogenesis of a specific substrate. Similar comments can be made about the $K_s$ constant, which reflects the affinity between the substrate and the microorganisms.

Finally, the values of the decay coefficient found in this work are too low, suggesting that it is not a significant parameter of the model. In consequence, a simplified model excluding the decay rate of microorganisms could be considered without loss of significance.

### CONCLUSIONS

Anaerobic digestion of solid waste is a complex process but simple models can be used to describe the methane production. A model that involves the hydrolysis rate of solid biodegradable material to produce soluble substrate, which is then consumed by the microorganisms to produce methane, was proposed. The variables of the model are the biomass, the solid biodegradable substrate, the soluble organic concentration and the methane production.

Experimental data obtained from a classic BMP can be used to adjust this simple models and to determine the kinetic parameters.

Due to the high non-linearity of the model, an alternative method based on the multiple-shooting approach was used in order to obtain more information from the limited experimental data and to find the optimal values of the model parameters.

A Monte Carlo simulation was useful to obtain the confidence intervals of the parameters. Only the first-order hydrolysis constant showed a normal distribution.

### REFERENCES


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