

Kinetic Study on Methane Fermentation of Pre-Hydrolyzed Lignocellulosics

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ABSTRACT

The anaerobic fermentations of hemicellulose and starch hydrolyzates mixtures, simulating the composition of agricultural wastes, have been studied supposing first order kinetics.

Results of previous works demonstrate that the equation of chemical oxygen demand (COD) consumption rate should take into account, for a satisfactory kinetic description, the COD value at the end of batch digestion. The First Order model has accordingly been adapted and checked in this study.

The modified model shows a better fit than the simple First Order model to the experimental data of COD consumption for the substrate under consideration. It is very simple and easy to use but requires further experimentation to identify the phenomenon actually responsible for the observed kinetics.

INTRODUCTION

Anaerobic digestion of organic wastes has been receiving increasing interest during the last years.¹ It was initially employed to simultaneously treat and recover the energy contained in the sludges from municipal wastewater treatment plants^{2,3} and subsequently extended with success to the organic fraction of municipal solid wastes^{4,5} and lignocellulosic residues.⁶

The correct design of fermenters to carry out any of these processes cannot leave information on substrate

degradation and biogas production kinetics out of consideration. Unfortunately, however, the biological events responsible for anaerobic digestion constitute on the whole such a complex phenomenon that they cannot satisfactorily be described by the most common kinetic models. An effective model should be simple enough to make data interpretation as easy as possible. For practical purposes it must contain as many parameters as possible to be actually and usefully applicable.

As far as the degradation of the organic fraction of municipal solid waste (MSWOF) is concerned, Cecchi et al.⁷ have demonstrated that a step diffusional model, taking into account the different steps of anaerobic digestion—acidogenesis, acetogenesis, and methanogenesis—is able to provide the best macroscopic description of the process when compared with the well-known models of Chen and Hashimoto,⁸ Monod,⁹ Inhibition,¹⁰ First Order,¹¹ Diffusion,¹² and Singh.¹³

In the case of lignocellulosic and woody wastes, which need preliminary hydrolysis to ensure satisfactory digestion rates,¹⁴ some important peculiarities should be considered. First, the complex organic matter, mainly constituted by hemicellulose and lignin besides cellulose, is present in the form of simple organic chemicals. Second, the recalcitrant fraction of organic matter may account for a relevant part of the feed, ranging from 25 to 50% according to the conditions.¹⁵

As the hydrolysis of the organic polymers is often, under various conditions, the rate-limiting step of digestion,¹⁶ common biological kinetic models are unsuitable for describing the degradation of these substrates.

One of the problems often arising during the kinetic study of anaerobic fermentation of difficult materials is the inability of the common models to describe chemical oxygen demand (COD) consumption as well as biogas development, due to the existence in the feed material of a relevant fraction of organic substances resistant to digestion. On the other hand, the approach using the integral methane production instead of COD consumption requires the knowledge of the ultimate methane yield.⁸

IMPLICATIONS

The kinetics of the COD consumption during the anaerobic digestion of pre-hydrolyzed agricultural wastes are studied. Due to the presence of a relevant fraction of recalcitrant substances in this material, the models available in the literature for this end need to be suitably modified to take this fraction into consideration. A comparative analysis of the kinetic parameters calculated in this study demonstrates that a modified version of the First Order model can be a useful theoretical tool to follow the kinetics of the methane fermentation of recalcitrant materials.

Unfortunately, this parameter can only be measured at the end of biodegradable substances fermentation with much difficulty, mainly due to the subsequent and additional very slow degradation of recalcitrant materials. Because of these difficulties, a model derived from the First Order model has been proposed in this study to describe COD degradation versus time. It implies that the system reaches a final COD value ascribable to the recalcitrant fraction in the feed.

MATERIALS AND METHODS

Experimental Setup

Fermentations were carried out simultaneously using two Pyrex glass digesters with working volumes of 3 L, both filled with active anaerobic sludges rich in methanogenic microorganisms from the municipal anaerobic secondary digester of Volpara (Genoa, Italy). The reactors were fed with a mixture of hemicellulose and starch hydrolyzates suitably diluted with tap water in order to simulate the composition of agricultural wastes.

Batch tests were carried out at the end of fed-batch operation in order to follow the COD consumption versus time. Digestion temperature was kept at 37 °C by means of a water bath where the reactor was almost completely submerged.

Hydrolysis of Starchy and Woody Residues

The procedure used for enzymatic hydrolysis of starchy residue, coming from both corn overproduction and cultivation wastes, was previously described.¹⁷ The hydrolysis of woody wastes was performed according to the dilute acid Tennessee Valley Authority procedure.^{18,19} The average chemical compositions of the liquid fractions of both hemicellulose and starch hydrolyzates are listed in Table 1.

Table 1. Characteristics of the hydrolyzates used to simulate the composition of hemicellulosic and cellulosic fractions of agricultural waste.

	Wood Hydrolyzate	Starch Hydrolyzate
Density (g/l)	1,050	1,560
Soluble COD (g/L)	193.7-200.2	680.0-740.0
*Dry solids (g/L)	135.0-140.0	550.0-600.0
*Volatile solids (g/L)	131.6-136.5	547.5-597.3
Glucose (g/L)	5.0	488.8
Pentoses (g/L)	109.3	-
Acetic acid (g/L)	31.2	-
Maltose (g/L)	-	14.9
Oligosaccharides (g/L)	-	43.1
Proteins (g/L)	4.10	6.32

*Values refer to total solids.

The nutrient for batch runs was prepared by mixing 0.678 L of the liquid fraction of the hemicellulose hydrolyzate and 0.299 L of the starch hydrolyzate and subsequently diluting the mixture with tap water up to a final volume of 3 L. The fraction of substances of this mixture that are difficult to digest can reasonably be estimated at 30–40%. A few milliliters of this suspension were introduced into the digester to achieve starting CODs of 6.3, 13, 18, and 30 g/L.

To simulate a feed with a percentage of recalcitrant substances of 50–60% and with a much higher concentration of pentoses, successive batches of residue from wood acid hydrolysis were washed through a countercurrent washing scheme.¹⁹⁻²¹ This substrate was used only for the batch test with a starting COD of 7.8 g/L. As suggested by the almost negligible final fraction of insoluble residue, this treatment was able to ensure a nearly complete solubilization and a greater availability of organic substances in the form of monomers.

Measuring Methods

COD, total dry solids, and volatile solids concentrations were determined as described in American Public Health Association (APHA) Standard Methods.²² Biogas production was followed by measuring its developed volume by liquid level displacement. The fractions of methane, carbon dioxide, and nitrogen were determined by gas chromatography, using a Fractovap model M (Carlo Erba, Milan) equipped with a column packed with Cromosorb 1102, using helium as carrier gas and known mixtures of CH₄, CO₂, and N₂ as internal standards. Monitoring of sludge pH revealed that this parameter did not change significantly during each run.

THEORETICAL MODELS

During a batch run, substrate concentration, C_s, decreases according to the fermentation kinetics, which may be described by several models proposed in the past for anaerobic digestion. A further model is proposed in this study due to the particular composition of the substrate fed into the digesters (containing a significant fraction of organic substances that are hard to ferment). The suitability of both the First Order model and its modified version has been checked by estimation of the square of the correlation coefficient (r²), referred to as the difference between experimental and theoretical values taken on the whole for each run.

First Order Model

The First Order model, first utilized by Pfeffer¹¹ to describe the anaerobic fermentation of domestic refuses, consists of an overall mass transfer kinetic approach

which considers the biomass as a catalyst. It is based on the equation:

$$-\frac{dC_s}{dt} = k_1 C_s \quad (1)$$

where k_1 is the first order kinetic constant, C_s the concentration of soluble organic substances, and t the fermentation time.

The first order constant has been calculated from the slope of the straight line obtained by plotting $\ln C_s$ versus time.

Modified First Order Model

The composition of Table 1 clearly shows the existence of a relevant fraction of hardly degradable organic substances in the nutrient. Because in the presence of a mixture of substrates, the one most easily biodegradable is primarily consumed,^{14,23} a first order kinetic model is proposed:

$$-\frac{dC_s}{dt} = k_2 (C_s - f C_s^0) \quad (2)$$

where k_2 is the corresponding first order kinetic constant. This model implies a final COD value at the end of fermentation due to the presence of a fraction of recalcitrant substances in the feed ($f C_s^0$).

RESULTS AND DISCUSSION

A comparison between the above kinetic models and the experimental data was carried out to find the model which best describes the behavior of batch anaerobic fermentations of pre-hydrolyzed lignocellulosic wastes.

The substrate used for the tests at $COD_0 = 6.3, 13, 18,$ and 30 g/L was obtained by mixing and diluting hemicellulose and starch hydrolyzates as described in Materials and

Table 2. Values of the kinetic parameters calculated for the anaerobic fermentation of pre-hydrolyzed lignocellulosics.

COD_0 (g_{COD}/l)	6.3	7.8	13.0	18.0	30.0
Substrate	A	B	A	A	A
First Order (eq 1)					
k_1 (h^{-1})	0.0314	0.0163	0.0195	0.0186	0.0147
r^2	0.923	0.895	0.874	0.957	0.977
Modified First Order (eq 2)					
k_2 (h^{-1})	0.1177	0.1140	0.0787	0.0459	0.0383
f (-)	0.405	0.615	0.331	0.314	0.383
r^2	0.998	0.997	0.995	0.996	0.997

A = Mixture of hydrolyzates with 30–40% recalcitrant substances.

B = Mixture of hydrolyzates with 50–60% recalcitrant substances.

The determination coefficients (r^2) refer to linear interpolation by the integrated forms of eqs 1 and 2.

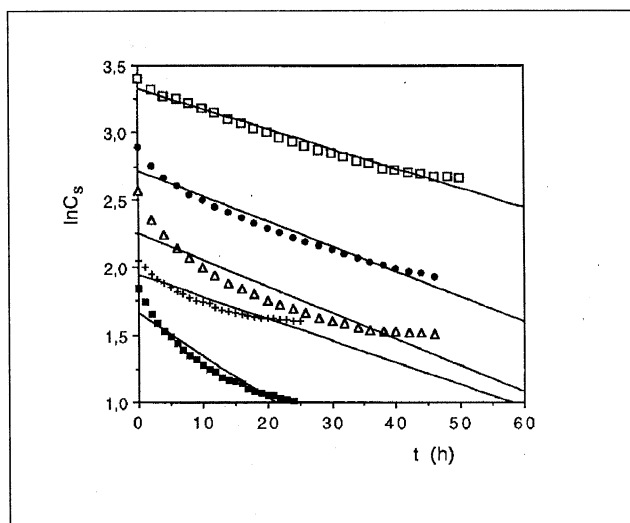


Figure 1. Application of the First Order model to the COD consumption during the anaerobic fermentation of pre-hydrolyzed lignocellulosics. Starting COD (g/L): (■) 6.3; (+) 7.8; (Δ) 13.0; (●) 18.0; (□) 30.0.

Methods. It contained an estimated fraction of recalcitrant organic substances of about 30–40%, mainly coming from lignin and hemicellulose hydrolysis (e.g., slowly metabolizable pentoses, phenols, furfural, hydroxymethylfurfural, incompletely hydrolyzed polymers).

To study the possible influence of the fraction of recalcitrant substances on the kinetic constants, an additional test at $COD_0 = 7.8 \text{ g/L}$ was carried out using a mixture of hydrolyzates containing about 50–60% of recalcitrant materials. This substrate was prepared submitting the wood hydrolyzate to the above countercurrent washing scheme.

These data have then been used to calculate the kinetic parameters of both models (Table 2). Besides the comparative analysis of the values calculated for each of the kinetic parameters at the different COD_0 values, the validity of the above models has been checked by the estimation of the determination coefficient (r^2), which can range from 0 to 1. The values of r^2 , calculated by linear interpolation of experimental data, are also listed in Table 2.

Figure 1 shows the application of the First Order model to the COD consumption at different starting COD levels. As shown by the values of the first-order kinetic constant listed in Table 2, k_1 decreases considerably with increasing starting COD. This behavior of the kinetic constant raises doubts on the ability of this model to shed light on the actual phenomenon limiting the anaerobic digestion process. In fact, this model, which proved to apply very well to sugar digestion in a biofilm reactor²⁴ and to brewery by-products,²⁵ should show values of k_1 not depending on COD_0 .

Two different diffusion-limited phenomena are consistent with pseudo-first order kinetics in such a

reactor, where the digesting mass can behave, due to poor mixing, as a biofilm with constant thickness: a deep biofilm with a very low C_s value, or a fully penetrated biofilm with a very low C_s value. In both cases, the decrease of k_1 with increasing COD_0 could be the result of a growing importance of the recalcitrant fraction in reducing the average diffusivity of the biodegradable substances. This phenomenon would be confirmed by the significantly lower value of k_1 obtained using the hydrolyzate with the higher fraction of recalcitrant substances ($COD_0 = 7.8$ g/L).

The same first order kinetics could be consistent with two other phenomena: the bioreaction is the limiting step and the biocatalyst concentration in the digester is so high that C_s quickly reaches negligible levels with respect to K_s in Monod's equation, or the diffusion through the cell wall is limiting and the substrate concentration in the inside of methanogenic bacteria cells is higher than zero. Both situations can reasonably be considered unlikely because the concentration of biodegradable substances in the cell can be considered negligible, unless metabolic disorders take place.

Examination of the error structure for the First Order model shows that it suffers the presence of systematic errors. In fact, Figure 1 shows a concave shape to the data, thus indicating that the response has a higher reaction order than first.

Although a theory able to attribute physical meaning to a reaction order higher than first is not available in the literature for comparable biological systems, one can learn about the source of the error examining the structure of these errors. To this purpose, the reaction order (n) able to ensure the lowest deviations has

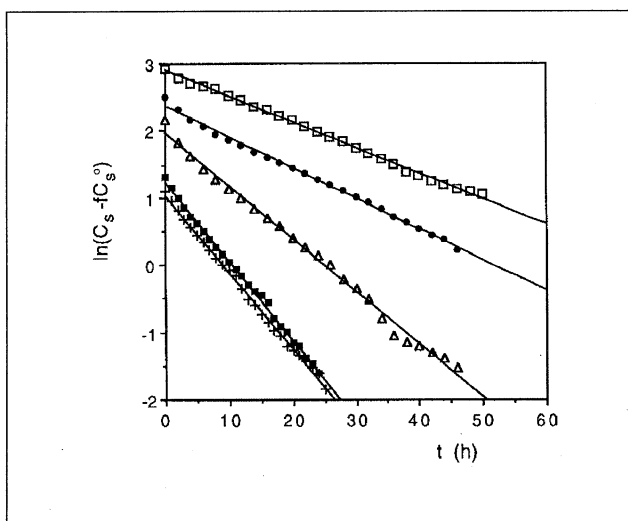


Figure 2. Application of the unfermentable COD fraction to the First Order model. For symbols see Figure 1.

been estimated for each starting COD by the equation:

$$-\frac{dC_s}{dt} = k_3 (C_s)^n \quad (3)$$

where k_3 is the kinetic constant of the n -order reaction. While the values of k_3 listed in Table 3 cannot be directly compared because they have different units, the reaction order seems to be scarcely influenced by the starting COD, ranging only between 2.9 and 3.7. On the other hand, when using a more recalcitrant feed (substrate B), the reaction order nearly doubles, which provides additional support for the inhibiting role of the recalcitrant fraction. This result, however, does not add any information about the phenomena involved in the process because of its mere empirical nature. For this reason, the successive effort to better describe the digestion kinetics of the substrate under consideration has been addressed to a simple modification of the First Order model.

According to the results presented in Table 2 and Figure 2, the Modified First Order model, taking into account the fraction of recalcitrant substances in the feed (fC_s^0), better describes the progress of the digestion at all starting COD levels. To ascertain which of the phenomena described above is most likely responsible for the observed first order kinetics referred to the biodegradable fraction alone, further studies are programmed at different biomass concentrations in the reactor.

The values of f calculated by this model that ensure the best linear interpolation for both lignocellulose hydrolyzates ($0.31 < f < 0.40$ for substrate A and $f = 0.61$ for substrate B) are in satisfactory agreement with the fractions of recalcitrant compounds that can be estimated for these wastes on the basis of their compositions (30–40% and 50–60%, respectively).

CONCLUSIONS

This study has demonstrated that the simple First Order

Table 3. Reaction orders and related kinetic constants calculated at different starting COD values by eq 3.

Substrate	COD_0 (g/L)	n (-)	k_3 ($g_{COD}^{1-n} L^{n-1} h^{-1}$)	r^2
A	6.30	3.72	$9.53 \cdot 10^{-4}$	0.884
A	13.0	3.62	$1.93 \cdot 10^{-4}$	0.950
A	18.0	2.92	$2.44 \cdot 10^{-4}$	0.927
A	30.0	3.01	$3.43 \cdot 10^{-5}$	0.669
B	7.80	6.12	$2.21 \cdot 10^{-6}$	0.875

A = Mixture of hydrolyzates with 30–40% recalcitrant substances.

B = Mixture of hydrolyzates with 50–60% recalcitrant substances.

model cannot satisfactorily describe the kinetics of batch digestion of pre-hydrolyzed lignocellulosic wastes. Nevertheless, thanks to the preliminary hydrolysis, the microorganisms inside the reactor are able to directly metabolize monomeric organic compounds. So, the hydrolysis is no longer the limiting step and the anaerobic digestion may take place at a higher rate.

The introduction of a correction factor accounting for the presence of substances that are difficult to biodegrade in the nutrient has been proposed in this study to enhance the suitability of first order kinetics.

Further studies are in progress to confirm the suspicion that the phenomenon responsible for the observed kinetics is the reduction of the average diffusivity of the biodegradable substances, provoked by the recalcitrant compounds present in this material.

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