

EFFECT OF pH AND VFA ON HYDROLYSIS OF ORGANIC SOLID WASTE

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ABSTRACT: The anaerobic hydrolysis rate of organic solid waste was studied at fixed volatile fatty acid (VFA) concentrations ranging from 3 to 30 g COD/L and fixed pH values between 5 and 7. For separate control of both VFA and pH, a special completely mixed reactor was designed. In this way, it was possible to distinguish between the inhibitory effects of pH, total VFA, and undissociated VFA on anaerobic hydrolysis. It was shown that hydrolysis of the organic solid waste followed first-order kinetics. Using a statistical analysis, it was found that the hydrolysis rate constant was pH dependent but was not related to the total VFA and undissociated VFA concentrations.

INTRODUCTION

Anaerobic solid state fermentation is a promising method for treatment of municipal solid wastes (MSW) (White et al. 1995). In The Netherlands, an organic fraction of biological origin makes up 50% of the total amount of MSW. This part is source separated (so-called biowaste) because this strategy provides the best quality compost with respect to heavy metals and plastics (Roosmalen and Langerijt 1989). Four metabolic stages can be distinguished in the overall anaerobic digestion of biowaste:

- Hydrolysis—complex insoluble organic material is solubilized by enzymes excreted by hydrolytic microorganisms.
- Acidogenesis—soluble organic components including the products of hydrolysis are converted into organic acids, alcohols, hydrogen, and carbon dioxide.
- Acetogenesis—the products of the acidogenesis are converted into acetic acid, hydrogen, and carbon dioxide.
- Methanogenesis—methane is produced from acetic acid, hydrogen, and carbon dioxide as well as directly from other substrates of which formic acid and methanol are the most important.

In a well-balanced anaerobic digestion process, all products of a previous metabolic stage are converted into the next one without significant build up of intermediary products. The overall result is a nearly complete conversion of the anaerobically biodegradable organic material into end products like methane, carbon dioxide, hydrogen sulfide, and ammonia.

In general, hydrolysis is the rate-limiting step if the substrate is in particulate form (Ghosh and Klass 1978; Eastman and Ferguson 1981; Arntz et al. 1985; Noike et al. 1985). The rate of hydrolysis is a function of factors such as pH, temperature, composition and particle size of the substrate, and high concentrations of intermediate products (Veeken and Hamelers 1999). Various models have been proposed to model hydrolysis. First-order kinetics with respect to the remaining biodegradable particulate substrate is the simplest and most

widely applied approach in describing the hydrolysis rate. According to Eastman and Ferguson (1981), the first-order hydrolysis function is a pure empirical expression that reflects the cumulative effect of many processes. The Monod equation also is sometimes used for degradation of particulate matter (Ghosh and Klass 1978; Lin 1991). Hobson (1983) proposed a model with two Monod equations that contains distinctions among nondegradable, rapidly degradable, and slowly degradable fractions. Chen and Hashimoto (1980) developed their own equation derived from the Contois model. Four types of hydrolysis kinetics (first-order, Monod, Contois, and two-phase, taking into account colonization of the particles by bacteria) were compared by Vavilin et al. (1996), and they concluded that all types of hydrolysis kinetics could fit a variety of experimental data comparatively well. This fact seems to justify a broad application of first-order kinetics, as this approach is the simplest way to describe the hydrolysis rate.

Several modifications of the simple first-order kinetics were proposed by Mata-Alvarez (1987) and Llabrés-Luengo and Mata-Alvarez (1988), as high concentrations of volatile fatty acids (VFA) accompanied by low pH may inhibit hydrolysis. They tested the Monod, first-order, and Hashimoto's models. Because all showed a significant lack of fit to their experimental data, Llabrés-Luengo and Mata-Alvarez (1988) proposed a kinetic model where the hydrolysis rate was proportional to the substrate volatile solids and biomass concentrations and inversely proportional to the VFA concentration. The proposed model together with fitted parameters adequately represented the hydrolysis process at all tested conditions. It was concluded that hydrolysis kinetics followed a VFA inhibition model; however, in this model, it is not possible to separate the effect of pH and VFA concentration on inhibition of hydrolysis.

A problem with research on inhibition of microbial processes by VFA is that a VFA concentration increase results in a pH decrease. Therefore, it cannot be distinguished if VFA and/or pH are inhibiting hydrolysis; i.e., inhibition of VFA also could be caused by a lower pH. Very little data can be found in the literature on inhibition of anaerobic hydrolysis of

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Note. Associate Editor: Robert G. Arnold. Discussion open until May 1, 2001. To extend the closing date one month, a written request must be filed with the ASCE Manager of Journals. The manuscript for this paper was submitted for review and possible publication on April 9, 1999. This paper is part of the *Journal of Environmental Engineering*, Vol. 126, No. 12, December, 2000. ©ASCE, ISSN 0733-9372/00/0012-1076-1081/\$8.00 + \$.50 per page. Paper No. 20682.

TABLE 1. Undissociated VFA Concentration (mM) in Selected Series of Experiments

Total VFA (g COD/L) (1)	Undissociated VFA (mM)		
	pH 5 (2)	pH 6 (3)	pH 7 (4)
30	Not tested	18.5	2.0
10	40.0	6.2	0.7
3	12.1	1.8	0.2

Note: Calculations were made using average composition of VFA formed during fermentation [acetate:propionate:butyrate:valerate = 0.51:0.21:0.22:0.06 (g COD/L)] as determined by ten Brummeler et al. (1991) and corresponding dissociation constants of VFA components.

particulate matter by VFA, whereas hydrolysis is the rate-limiting step in solids digesters and thus determines the solids retention time of the reactor. The objective of this work was to design an experimental setup to study the separate effects of pH and VFA on the inhibition of biowaste hydrolysis. Separate controls of VFA and pH were organized in the experimental setup. Moreover, special attention was paid to ensure complete mixing of the liquid in the reactor to avoid the possible influence of nonhomogeneous distribution of pH and VFA concentrations inside the biowaste bed. To fulfill this requirement, the application of a reactor system with high recirculation flows was chosen to make sure that no aggregates larger than the individual particle size of the biowaste constituents are present. A series of experiments were conducted for conditions where inhibition of hydrolysis was expected, namely, the VFA concentrations of approximately 3, 10, and 30 g/L COD were evaluated at pHs of 5, 6, and 7 (Table 1). If hydrolysis follows first-order kinetics, it should be possible to find out whether pH, total VFA, or undissociated VFA inhibits hydrolysis of biowaste in the pH and VFA ranges studied.

MATERIALS AND METHODS

Experimental Setup

Reactor design. A schematic representation of the anaerobic solid waste reactor (ASWR) is shown in Fig. 1. The ASWR, made from acrylonitril-butadiene styrene, had a volume of 25 L and operated in an upflow mode. At the top and bottom of the reactor, gravel-filled sections were installed that were separated from the biowaste by a perforated stainless steel plate and cheesecloth. The bottom section dispersed the incoming leachate. The cheesecloth was added to keep all the particulate matter in the reactor and prevent clogging at the reactor outlet. The top section served as a gas-liquid separator. The gravel helped to keep the cheesecloth and perforated plate in place. The reactor was placed in a temperature-controlled room of $28^\circ \pm 2^\circ\text{C}$.

The ASWR was completely filled with biowaste, approximating the biowaste as a static bed, where hydrolysis products are leached from the solid bed to the liquid phase. Because biowaste was used as the substrate, pH control in the reactor itself is not possible. A separate control tank (4-L homemade Perspex reactor) equipped with a stirrer was inserted in the leachate recirculation line, and a pH gel electrode was immersed in the control tank. To separate the gas from the liquid, the control tank also was provided with a gas-liquid separator and water lock in the effluent outlet. A recirculation rate of approximately $120 \text{ m}^3/\text{m}^3$ biowaste/day was imposed on the system to ensure complete mixing of pH-control chemicals, produced VFA, and added replacement liquid. In such a way, uniform conditions for hydrolysis of particulate polymers were imposed in every part of the hydrolysis reactor. The total system should be considered as an ideally mixed batch reactor with constant volume, where part of the liquid is replaced by acid, base, and replacement liquid to keep pH and VFA concentration in the reactor constant. Particle-size measurements revealed that no significant agglomeration of particles took place in the ASWR under applied recirculation flows (data not shown), thus ensuring that the determined hydrolysis rate constant could not be influenced by this factor.

Retention time distribution test. To determine if the reactor is ideally mixed, retention time distribution of Li^+ was determined by injecting pulses of LiCl as tracer. The Li^+ response curves were normalized and analyzed using the "tanks-in-series" model of Levenspiel (1972) to calculate the mean hydraulic residence time HRT_m , dead space fraction of reactor volume τ , and theoretical number of mixers N_{th} , according to equations described by Grobicki and Stuckey (1992).

Inoculum. Because acid formation typically starts within 2 h after anaerobic conditions are established in the reactor (ten Brummeler 1993), no additional inoculation of the substrate was necessary.

Substrate. Biowaste, originating from Assen, The Netherlands, was collected at VAM, Wijster, The Netherlands. The composition of biowaste for all experiments is shown in

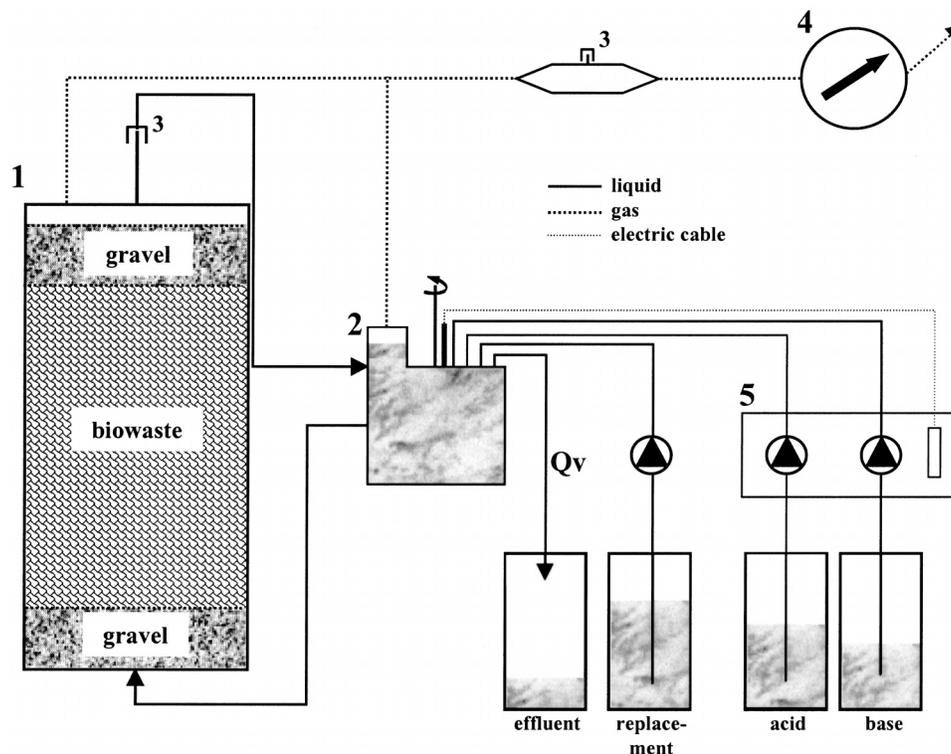


FIG. 1. Schematic Representation of Anaerobic Solid Waste Reactor: 1—Hydrolysis Reactor, 2—Control Tank, 3—Gas Sampling Points, 4—Gas Meter, 5—pH Controller

TABLE 2. Characteristics of Biowaste Used in Hydrolysis Experiments

Parameter (1)	Experiment			
	Runs 1 and 2 (2)	Runs 3 and 4 (3)	Runs 5 and 6 (4)	Runs 7 and 8 (5)
TS (%)	39.6	33.1	36.1	30.7
VSS (%)	16.7	18.2	18.8	19.9
VSS/ash (kg/kg)	0.73	1.22	1.09	1.84

Note: Experimental conditions for each run are given in Table 4.

Table 2. Before filling the reactor, the branches, leaves, roots, and lumps of grass were cut by hand to a maximum size of about 5 cm.

pH control. Leachate pH was controlled by an automatic pH controller, which corrected for positive and negative deviations from the set point by means of acid (HCl) or base (mixture of NaOH and KOH) addition to the leachate, respectively.

VFA control. As VFA is continuously produced under anaerobic conditions and has to be controlled at a fixed concentration, it is necessary to use a replacement liquid to keep the reactor at a fixed VFA level. A fixed VFA level in the reactor was maintained by measuring the VFA concentration two to three times a day and adjusting the effluent rate accordingly. To keep the effects of changing the mineral composition of the liquid on the kinetics of hydrolysis to a minimum, a replacement liquid was used which had a similar composition to the solution of salts found in biowaste (Veecken 1998). The replacement liquid was composed of 5.5-mM $(\text{NH}_4)_2\text{SO}_4$, 5.9-mM CaHPO_4 , 6.0-mM CaCl_2 , 14.4-mM MgCl_2 , and HCl to adjust the pH to 7. Additionally, a solution of trace elements, regularly used in anaerobic wastewater treatment research, was added to the replacement liquid (ten Brummeler 1993). The base for the pH control contained sodium and potassium hydroxides in a ratio of 1:3.6 (weight to weight) according to the Na/K ratio in biowaste.

Sampling and measurements. Preliminary experiments showed that it took 6–10 days before a VFA concentration of 30 g COD/L was reached. Because it was intended to limit the duration of each experiment to 2 weeks, prior to starting the experiments at 10 and 30 g VFA-COD/L, the corresponding VFA concentrations were reached by an external slug addition of the VFA mixture. To obtain a constant VFA concentration during the experiment, VFAs were analyzed three times a day in the initial stage of the experiment and twice daily when their concentrations were fairly constant. Leachate samples were taken daily for analysis of COD, formate, lactate, and alcohols. Three times a week gas samples were taken and monitored for O_2 , N_2 , CO_2 , CH_4 , and H_2 . Furthermore, volumes of gas, replacement liquid, base, acid, and leachate effluent were measured. Based on these data, the production rates of monomer and fermentation products could be determined, from which hydrolysis rates were calculated.

Analyses

Total solids, volatile solids (VS), and COD were determined according to *Standard methods* (APHA et al. 1985). The VFA, alcohols, and biogas composition were measured by gas chromatography, and lactic and formic acids were measured by high pressure liquid chromatography, as described by ten Brummeler et al. (1991). Lithium was determined by atomic

absorption spectrometry (Weltz 1985). The biogas volume produced was measured by a wet gas meter (Meterfabriek Schlumberger) and recalculated to standard pressure and temperature (STP: 0°C and 1 atm). The amounts of base, acid, replacement liquid, and leachate effluent were measured by weighing the containers on a balance. Weights were recalculated to volumes through the specific gravity of each liquid.

Determination of First-Order Hydrolysis Rate Constant

A simplified scheme for the first two steps of anaerobic degradation (Gujer and Zehnder 1983) is given in Fig. 2. To compare production and degradation rates, all components are expressed in grams of COD. As the contribution of hydrogen and methane was small compared to the other fermentation products, COD production of both components was neglected for the calculation. For the ASWR under study, the general equation for the mass balance is adapted as leachate effluent is leaving the reactor system

$$V_r \frac{dC_i}{dt} = -Qv \cdot C_i + r_i V_r \quad (1)$$

where V_r = reactor volume (m^3); Qv = leachate effluent flow rate ($\text{m}^3 \cdot \text{day}^{-1}$); C_i = concentration of component i ($\text{g COD} \cdot \text{m}^{-3}$); and r_i = production rate of component i ($\text{g COD} \cdot \text{m}^{-3} \cdot \text{day}^{-1}$). Applying (1) to the mass balances for the components of Fig. 2 and taking into account that the particulate matter was retained in the ASWR (Qv for P is equal to zero) gives

$$\frac{dP}{dt} = -r_p \quad (2)$$

$$V_r \frac{dM}{dt} = -Qv \cdot M + r_p V_r - r_M V_r \quad (3)$$

$$V_r \frac{dF}{dt} = -Qv \cdot F + r_M V_r \quad (4)$$

where P = particulate organic matter concentration ($\text{g COD} \cdot \text{m}^{-3}$); M = monomer products concentration ($\text{g COD} \cdot \text{m}^{-3}$); F = fermentation products concentration ($\text{g COD} \cdot \text{m}^{-3}$); r_p = hydrolysis rate of P ($\text{g COD} \cdot \text{m}^{-3} \cdot \text{day}^{-1}$); and r_M = fermentation rate of monomers ($\text{g COD} \cdot \text{m}^{-3} \cdot \text{day}^{-1}$). The concentration of fermentation products equals the sum of measured VFA, lactate, formate, and alcohols. The concentration of monomers equals the total soluble COD minus the concentration of fermentation products. Combination of (3) and (4) results in

$$r_p V_r = V_r \frac{dM}{dt} + Qv \cdot M + V_r \frac{dF}{dt} + Qv \cdot F \quad (5)$$

As the concentration of M equals the total soluble COD minus the concentration of F , (5) can be replaced by

$$r_p V_r = V_r \frac{d(\text{COD} - F)}{dt} + Qv \cdot (\text{COD} - F) + V_r \frac{dF}{dt} + Qv \cdot F \quad (6)$$

and the hydrolysis rate of particulate organic matter thus can be expressed

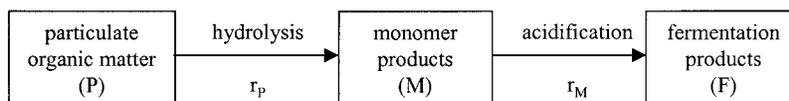


FIG. 2. Simplified Scheme of First Two Steps of Anaerobic Degradation

$$r_p = \frac{d(\text{COD})}{dt} + \frac{Qv}{V_r} \cdot \text{COD} \quad (7)$$

where COD is the total soluble COD concentration (g COD · m⁻³). Integration of (7) results in

$$\int r_p dt = \int d(\text{COD}) + \int \left(\frac{Qv}{V_r} \right) \text{COD} \cdot dt \quad (8)$$

The result of this integration can be approximated by

$$\int r_p dt \approx (\text{COD}_j - \text{COD}_0) + \frac{1}{V_r} \sum_{j=1}^n (Qv_j \text{COD}_j \Delta t_j) \quad (9)$$

or

$$\int r_p dt \approx (\text{COD}_j - \text{COD}_0) + \frac{1}{V_r} \sum_{j=1}^n (Ve_j \text{COD}_j) \quad (10)$$

where COD_j = total soluble COD at time *j*; COD₀ = COD at time zero; and Ve_j = volume of effluent removed during the *j*th period *i*. Assuming the first-order degradation of particulate organic matter, the concentration of *P* can be expressed by

$$P = P_0 \cdot \exp(-k_H \cdot t) \quad (11)$$

where P₀ = concentration of degradable particulate organic matter at the start of the experiment; *t* = time (day); and k_H = first-order hydrolysis rate constant (day⁻¹). The rate of degradation of *P* based on (11) after its integration is then given by

$$\int r_p dt = P_0(1 - \exp(-k_H \cdot t)) \quad (12)$$

Parameter values for P₀ and k_H of (12) were estimated using nonlinear least-squares curve fitting of the experimental data of (10) by the spreadsheet program Excel. Statistical analysis was based on multiple linear regression with the computer program Statgraphics.

RESULTS AND DISCUSSION

Retention Time Distribution

Table 3 shows the results of the retention time distribution with lithium. The number of mixers of 1.22 indicates that the reactor strongly resembles complete mixing. The lithium recovery of 0.89 indicates that the reactor has a dead volume of 20%, probably due to the incorporation of Li⁺ into the intracellular water of biowaste. Thus, the developed experimental setup rules out the possibility of nonhomogeneous distribution of VFA and pH concentrations inside the biowaste bed, which could have an effect on the measured hydrolysis constants.

Hydrolysis of Particulate Polymers

Table 4 shows the accuracy in maintaining the conditions, which were set during the experiments. It is seen that pH con-

TABLE 3. Analysis of Retention Time Distribution of Li⁺ for ASWR

Parameter (1)	Value (2)
Reactor liquid volume (m ³)	0.027
C ₀ (g/m ³)	3.66
Flow rate (m ³ /h)	0.0012
HRT _{th} (h)	22.57
HRT _m (h)	18.01
Variance RTD	0.82
N _{th}	1.22
Dead volume (%)	20.18
Recovery of Li tracer	0.89

TABLE 4. Experimental Conditions and Determined First-Order Hydrolysis Rate Constants and Starting Concentration of Biodegradable Particulate Polymers

Run (1)	pH (2)	VFA (g COD/L) (3)	Undissociated VFA (mM) (4)	k _H (day ⁻¹) (5)	P ₀ (g/L) (6)
2	7.0 ± 0.0	2.8 ± 0.9	0.18	0.108	20.2
4	6.9 ± 0.2	10 ± 2	0.81	0.154	39.5
1	7.0 ± 0.1	28 ± 1	1.7	0.245	20.2
8	6.0 ± 0.1	3.2 ± 0.8	1.9	0.113	30.6
3	6.0 ± 0.1	11 ± 2	6.4	0.086	39.5
6	6.0 ± 0.1	25 ± 2	14.8	0.096	37.5
5	5.0 ± 0.1	3.1 ± 0.8	11.8	0.098	37.5
7	5.1 ± 0.1	10.4 ± 0.6	40.3	0.060	30.6

Note: Experimental conditions are expressed as mean ± standard deviation.

control was accurate because pH was on-line measured and controlled. Control of the VFA concentration was only possible within a certain range and could not be narrowed down to a specific value. Even with three VFA samples per day, followed by adjustment of the replacement liquid flow rate, fluctuations of the VFA level still occurred.

Fig. 3 shows the total production of soluble COD and fermentation products calculated by using (10) in time for all runs at fixed pH and VFA concentrations. The curves of production of total soluble COD and fermentation products show a typical first-order rate pattern, namely, a high rate at the start, which then levels down in time. For runs at pHs 6 and 7 and VFA levels of 3 and 10 g COD/L, the production of total soluble COD and fermentation are almost equal. This means that no accumulation of monomeric products (Fig. 2) occurs or, in other words, it indicates that fermentation of monomers proceeds faster and the hydrolysis of particulate polymers is the rate-limiting step. However, for runs at pH 5 and VFA levels of 30 g COD/L, the produced fermentation products rise slower than total soluble COD, showing some accumulation of monomers. As the accumulation increases for lower pH and higher VFA levels, this suggests that the fermentation is in some extent inhibited under these conditions. This is in accordance with the observation of several authors who found slightly acid conditions optimal for hydrolysis (Zoetemeyer 1982; Arntz et al. 1985). The reason might be a decreasing fermentative ability of acidogenic bacteria while the exoenzyme activity for hydrolysis still remains high. The results of the determination of the first-order hydrolysis rate constants k_H and concentration of degradable particulate organic matter P₀ on the basis of the total soluble COD data of Fig. 3 are presented in Table 4. It is assumed that the value of P₀ was the same for the runs where the same biowaste was used (Table 2). The first-order hydrolysis rate constant ranges from 0.06 to 0.24 day⁻¹. These rate constants are two to five times higher than the rate constants of 0.038 and 0.048 day⁻¹ found at 30°C by ten Brummeler et al. (1991) for biowaste in 5-m³ reactors without recirculation. This is not totally unexpected, because increased leachate recirculation rates enhance the anaerobic degradation (Veeken and Hamelers 2000). However, the values obtained in this study are in good agreement with the data of Veeken and Hamelers (1999) who determined the first-order hydrolysis rate constants for several typical constituents of biowaste (bread, orange peels, leaves, and bark) in optimal batch conditions. Their values at 30°C varied in the range of 0.076–0.264 day⁻¹.

The first-order rate constants were statistically analyzed to determine the influence of pH, total VFA concentration, undissociated VFA concentration, and starting material. Among the factors studied, only pH had a significant influence on the hydrolysis rate constant. On the contrary, neither VFA (total

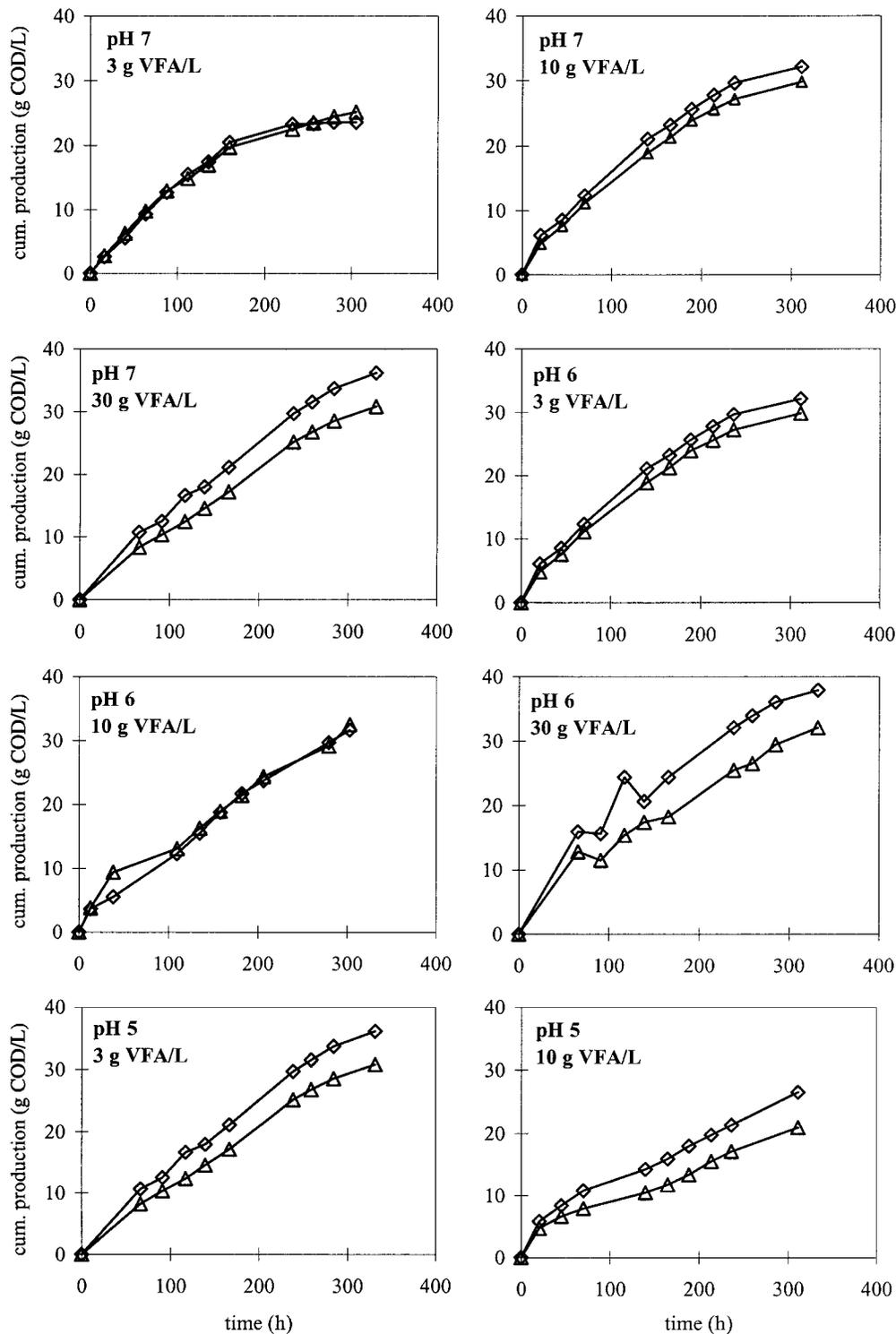


FIG. 3. Cumulative Production of Total Soluble COD and Fermentation Products for Fixed pH and VFA Concentrations (Numbers in Left Corner of Each Panel)

or undissociated) nor starting material had a statistically reliable effect on the hydrolysis rate of biowaste within pHs of 5–7 and VFA concentrations of 3–30 g COD/L. Summarizing the results of statistical analysis, the following formula for k_H was deduced (Fig. 4) for the pH and VFA ranges tested in this research (day^{-1}):

$$k = 0.048 \text{ pH} - 0.172 \quad (13)$$

This formula was significant for a P value of 0.06.

Although the absence of VFA influence on the hydrolysis rate constant was unexpected, the subsequent desk analysis

showed that this finding did not contradict the data available in the literature. According to de Baere et al. (1985), the maximum concentration of organic acids that can be attained in anaerobic digestion is around 30 g/L. Similarly, ten Brummeler et al. (1991) found that hydrolysis of biowaste was severely inhibited under VFA concentrations up to 33 g/L. Veeken and Hamelers (2000) also observed that a complete inhibition of anaerobic hydrolysis of biowaste was met at VFA levels of 40–50 g VFA-COD/L. However, such severe inhibition regimes were always accompanied by not only high VFA concentrations, but also low pHs (5.0–5.5). Thus, in the

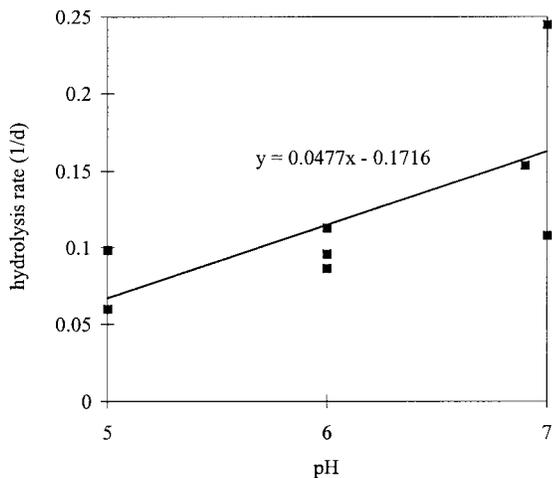


FIG. 4. First-Order Hydrolysis Rate Constant k_h as Function of pH

light of the findings here, it is likely that pH was a dominant factor of hydrolysis inhibition observed in the abovementioned works.

CONCLUSIONS

The designed experimental setup was appropriate to study the separate inhibitory effect of pH and VFA on the hydrolysis rates of biowaste. All mass flows could be accounted for in the batchwise reactor with accurate pH control and control of the VFA concentration within a certain range. A retention time distribution test confirmed that the reactor was completely mixed.

For the pH and VFA ranges studied, the hydrolysis rate of biowaste depended on the pH value. However, no dependence of the hydrolysis rate on VFA could be revealed between pHs of 5–7 and VFA concentrations up to 30 g COD/L. These findings imply that the pH is the primary process variable in controlling the hydrolysis rate of the anaerobic solid state fermentation process, not the VFA concentration. Of course, the VFA concentration via chemical equilibrium influences the pH in the waste and, for a specific waste composition, the VFA concentration and pH can be related to each other. However, this relationship depends on the composition of the waste, which may differ from waste to waste and may even change during the process. For instance, degradation of protein will lead to an increase of buffer capacity through the release of ammonia. Lowering of the pH below neutral gives no clear enhancement of the hydrolysis rate. From this perspective, it would seem unnecessary to look for two-phase systems.

ACKNOWLEDGMENT

This work was partially supported by The Netherlands Organization for Scientific Research, Den Haag, The Netherlands, project N299971.

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