Available online at www.sciencedirect.com
ScienceDirect
Journal of the Chinese Institute of Chemical Engineers 38 (2007) 21–27
JOURNAL OF THE CHINESE INSTITUTE OF CHEMICAL ENGINEERS
www.elsevier.com/locate/jcice

Kinetics of enzymatic hydrolysis of polysaccharide-rich particulates

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Received 5 December 2005; accepted 3 July 2006

Abstract

Both pH and volatile fatty acid (VFA) inhibit the hydrolysis of particulate organic waste. It is not clear yet whether VFA or pH is the dominant factor in hydrolysis inhibition. The effects of pH and acetate on the enzymatic hydrolysis of potato samples containing mainly carbohydrate were studied at fixed pH values (5–9) and with/without 20 g L^{-1} acetate. The leaching liquors were refreshed at prescribed intervals. Experimental results showed that both pH and acetate influenced the hydrolysis of carbohydrate, but that pH was more influential than acetate.

Numerous kinetic models could fit the hydrolysis data during the first 40 h of hydrolysis when inhibitive effects were not significant. The simplified Chen–Hashimoto model, $-dS/dt = K_H S/(K_S(S_0 - S) + S)$, closely fit all of the experimental data. Also, the fitting achieved by applying the non-competitive inhibition model ($K_H = K_H^0/(1 + I/K_I)$) on three inhibitors (H^+, OH^-, total/undissociated/dissociated acetate) was excellent for carbohydrate hydrolysis.

Keywords: pH value; Acetate; Enzymatic hydrolysis; Inhibition; Particulate organic waste; Kinetic models

1. Introduction

Substrate hydrolysis is achieved with extracellular hydrolytic enzymes excreted by fermentative microorganisms, whose essential metabolism, called acidogenesis, yields various metabolites, including volatile fatty acids (VFA), lactate, alcohols, ammonia, and other monomeric substances, which change the suspension's pH (Horiuchi *et al.*, 2002; Lata *et al.*, 2002; Traverso *et al.*, 2000; Zoetemeyer *et al.*, 1982). Both the suspension pH and acidogenic products influence microbial activity in an anaerobic environment (Aguilar *et al.*, 1995; Barredo and Evison, 1991; Batstone *et al.*, 2002; Mladenovska and Ahring, 2000; Mösche and Jördening, 1998, 1999; Pind *et al.*, 2003a). The very complex nature of the interactions between organic substances and pH makes predicting the inhibitory levels of acidogenic products difficult, if not impossible (Pind *et al.*, 2003b).

Some studies have addressed the effects of pH and acidogenic products on the hydrolysis of substrates. Palenzuela-Rollón (1999) stated that pH did not significantly affect the rate of hydrolysis of protein-rich wastewater from fish processing when the pH < 8. Boon found that the optimal pH in the hydrolysis of primary sludge was 6.5 (as reviewed by Sanders *et al.*, 2002). Elefsiniotis *et al.* (1996) found that reducing the pH from 5.1 to 4.5 did not influence the hydrolysis or rate of acidogenesis of primary sludge, while an increase in pH from 5.1 to 6.1 increased the rate of hydrolysis but reduced the rate of acidogenesis. Veeken *et al.* (2000) were the first to evaluate separately the roles of pH, total VFA, and undissociated VFA in the hydrolysis of biowaste. Their statistical analysis of data obtained at pH 5–7 showed that the rate of hydrolysis depended on the pH value, but was not related to the total VFA (3–30 g-COD L^{-1}) or the undissociated VFA. Babel *et al.* (2004) found that adding anaerobic inoculums reduced the amount of volatile solids in pineapple samples by 42% at 2500 mg L^{-1} VFA and by 48% at 5000 mg L^{-1} VFA at pH 6.5–7.5. Restated, VFA did not affect the rate of hydrolysis. Zou *et al.* (2003) and Veeken and Hamelers (2000) observed a decline in the hydrolysis rate caused by the accumulation of VFA. González *et al.* (2005) found that the hydrolysis of soluble protein was completely inhibited when the concentration of acetate exceeded

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doi:10.1016/j.jcice.2006.07.001

Nomenclature

A	exponent in the A-order biomass kinetic equation
B	concentration of biomass or enzyme (mol L^{-1})
f_h	biodegradable fraction of the substrate
$[H^+]$	concentration of hydronium ion (mol L^{-1})
H_S	hydrolytic flux, g-dry solid (cm^{-2} h^{-1})
K_B	saturation constant for biomass or enzyme (mol L^{-1})
K_H	hydrolysis rate constant (h^{-1})
K_H^0	hydrolysis rate constant without inhibition (h^{-1})
$K_{I,i}$	inhibition constant for the i inhibitory agent (mol L^{-1})
K_S	saturation constant for the substrate (mol L^{-1})
k_H	specific hydrolysis rate constant (h^{-1})
I_i	concentration of the i inhibitory agent (mol L^{-1})
$[OH^-]$	concentration of hydroxyl ion (mol L^{-1})
r	radius of a particle at time t (cm^{-1})
r_0	initial radius of a particle (cm^{-1})
S	substrate concentration (mol L^{-1})
S_0	initial substrate concentration (mol L^{-1})
S_{surf}	surface area of the organic solid (cm^2)
t	time (h)
v	hydrolysis rate (mol L^{-1} h^{-1})
v_{max}	maximal hydrolysis rate (mol L^{-1} h^{-1})
Y	growth yield coefficient (g-dry cell g^{-1}-substrate)

Greek symbols

γ	exponent
μ_{max}	maximum specific growth rate (h^{-1})
ρ	density of the organic solid (g cm^{-3})
ϕ	dimensionless particle radius, equal to the ratio of the radius of the particle at time t to the initial radius of the particle

1000 mg L^{-1}. Anaerobic Digestion Model No. 1 (ADM1) revealed that pH could inhibit hydrolysis, if required (Batstone *et al.*, 2002). However, ADM1 did not incorporate the pH-dependent inhibition of hydrolysis by acidogenic products.

Numerous kinetic models have been used to describe the hydrolytic kinetics same of them are listed in Table 1. In fact, these hydrolytic models were effective for the data by the corresponding authors and were sometimes equivalent to each other in effectiveness or essence (Cecchi *et al.*, 1990; Valentini *et al.*, 1997; Vavilin *et al.*, 1996). For example, if the μ_{max} and Y in the Monod equation is taken as being constant, then it has the same form as the Monod and Michaelis–Menten equations. The Haldane equation can be expressed as $-dS/dt = k_H B/(1 + K_S/S + S/K_I)$. Also, the shrinking core model, flux model, and surface based kinetics model can all be expressed in the same form $S_0^{1/3} - S^{1/3} = \int B\,dt$, where S_0, S, and B were the initial substrate concentration, substrate concentration, and biomass concentration, respectively.

Many inhibitive mechanisms have been proposed for biological processes, but discussion of the inhibition of the hydrolysis of biowaste has been limited. The effect of pH on hydrolysis was usually qualitatively described or empirically linearly regressed with data (Veeken *et al.*, 2000). The non-competitive inhibition model, $K_H = K_H^0/(1 + I/K_I)$, was applied by Angelidaki *et al.* (1999) and González *et al.* (2005) to interpret their hydrolysis data. González *et al.* (2005) found that Levenspiel model, $K_H = K_H^0/(1 - I/K_I)$, and Luong model, $K_H = K_H^0/(1 - (I/K_I)^\gamma)$, could fit their data. Competitive or incompetitive inhibitions, however, have seldom been applied in hydrolysis studies.

The above-cited works indicate that the effects of suspension pH and VFA on substrate hydrolysis and acidogenesis have not been conclusively determined. More works on substrate hydrolysis and bioreactor design and operation were recently published (Chen, 2006; Chen *et al.*, 2006; Huang, 2005; Hwang *et al.*, 2005; Kao *et al.*, 2006; Lan *et al.*, 2005; Lin *et al.*, 2005; Liu *et al.*, 2004; Ng and Tsai, 2005; Ruaan and Yu, 2004; Su *et al.*, 2006). In particular, hydrolysis often occurs together with acidogenesis, and the products of the latter further inhibit the hydrolysis of the substrate, making data interpretation more difficult. It is not clear yet whether VFA or pH is the dominant factor in hydrolysis inhibition. In this study, we sought to find out how the pH and the amount of acetate, one major acidogenic product, influence the enzymatic hydrolysis of particulate organic waste. The kinetic equations frequently accepted in the literature were tested to determine their fit with the hydrolysis data, in order to demonstrate the inhibition kinetics of this system

2. Materials and methods

2.1. Materials

Fresh potato, the testing substrate, was cut into cubes 2–3 mm in size and stored at 4 °C for 12–24 h. Each potato sample contained 0.87 g carbohydrate g^{-1} volatile solids, 0.12 g protein g^{-1} volatile solids, and 0.005 g lipid g^{-1} volatile solids.

Hydrolysis enzymes were extracted from the returned activated sludge in the secondary treatment stage of a municipal wastewater treatment plant. The sludge was gravitationally precipitated for 30 min, and the supernatant was discarded. The residual sludge was centrifuged at 1000 × g for 30 min, and the supernatant was again discarded. The residual sludge was washed with an equal volume of 0.9% NaCl solution and centrifuged at 1000 × g for another 30 min, with the sludge cake collected. Five hundred grams of the collected sludge cake was mixed with 2000 mL potassium phosphate buffer (pH 7.2, 0.1 mol L^{-1}) and then allowed to settle for 1 h at room temperature with frequent agitation. The aqueous phase, i.e., here referred to as the enzymatic extracts, was recovered through centrifugation at 1200 × g for 30 min. The activities of α-amylase and protease were measured. The former was found to be 72.6 U L^{-1}, while the latter was not detected in this extract. It was found that the activities of hydrolytic enzymes could hydrolyze the solid substrates (Goel *et al.*, 1998).

Table 1
Kinetic models in the literature for describing hydrolysis

Name	Expression	References
Chemical first-order	$-dS/dt = K_H S$	Eastman and Ferguson (1981)
Biological first-order	$-dS/dt = k_H SB$	Valentini *et al.* (1997)
Half-order biomass kinetic	$-dS/dt = k_H SB^{0.5}$	Rozzi and Verstraete (1981)
A-order biomass kinetic	$-dS/dt = k_H SX^A$	Valentini *et al.* (1997)
Michaelis–Menten equation	$-dS/dt = k_H SB/(K_S + S)$	Valentini *et al.* (1997)
Monod equation	$-dS/dt = \mu_{max} SB/(Y(K_S + S))$	Hobson (1983)
Haldane equation	$-dS/dt = \mu_{max} B/(Y(1 + K_S/S + S/K_i))$	Andrews and Graef (1971)
Contois model	$-dS/dt = k_H SB/(K_S B + S)$	Henze (1995)
Chen–Hashimoto model	$-dS/dt = k_H SB/(K_S(S_0 - S) + S)$	Chen and Hashimoto (1980)
Two phase model	$-dS/dt = k_H SB/((K_S + S)(K_B + B))$	Vavilin *et al.* (1996)
Step diffusion equation	$-dS/dt = [\nu_{max}^2 + K_H(S_0 - S)]^{1/2}$	Cecchi *et al.* (1990)
Shrinking core model	$-dS/dt = 3k_H S_0 \phi^2 B$ $-d\phi/dt = k_H B$	Negri *et al.* (1993)
Flux model	$-dS/dt = k_H S_{surf} B\rho$	Terashima and Lin (2000)
Surface based kinetics model	$-dS/dt = k_H S_{surf}$	Sanders *et al.* (2000)

2.2. Hydrolysis tests

Two batches of hydrolysis tests with different concentrations of added acetate were conducted. The batch I tests were conducted using leaching liquor at fixed pH values and recorded as I-pH 5 to I-pH 9, while the batch II tests were conducted at fixed pH values with liquors containing externally dosed acetate of 20 g L^{-1} and recorded as II-pH 5 to II-pH 9. For batch I, every 50 mL of leaching liquor consisted of 5 mL of enzymatic extracts and 45 mL of 0.1 mol L^{-1} phosphate buffers. For batch II, every 50 mL of leaching liquor consisted of 5 mL of enzymatic extracts and 45 mL of acetate buffers.

In each test, samples containing 20 g of wet potato were wrapped in gauze bags (80-mesh) and hung in bottles with 50-mL of leaching liquor. The bottles were shaken at 100 rpm, and the temperature was kept at 37 ± 0.2 °C. The leaching liquor was replaced with fresh liquor (containing enzyme extracts, dosed acetates, etc.) after 4, 8, 12, 16, 24, 32, 40, 48, 60, 72, 96, and 144 h of hydrolysis, respectively. In each liquor replacement, the changes in pH and the acetate level of the suspension were regarded as minimal.

2.3. Analytic methods

The collected leaching liquors were filtered using 0.45 μm polyester film and measured to determine the pH, the amount of dissolved carbon, dissolved nitrogen, reducing sugar, and amino acid. The suspension pH was measured with a pH meter (OAKTON, USA). Dissolved carbon and dissolved nitrogen were measured with a TN_b/TC multi N/C 3000 Analyzer (Analytik Jena AG, Germany). Reducing sugar was spectrophotometrically determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1956). The Lowry method was used to determine the amount of amino acid (Lowry *et al.*, 1951). After 144 h of testing, the potato particulates were removed from the gauze bags and tested.

The total amount of carbohydrate in the substrate was determined with the method described by Mösche and Jördening (1999) with glucose used as a standard. The total amount of proteins was determined with the Lowry method (Lowry *et al.*, 1951), and that of lipid was gravimetrically determined by the Soxlett extraction (APHA, 1998). The α-amylase activity of the enzymatic extracts was assayed according to Bernfeld (1955). One unit (U) of α-amylase activity was defined as the amount of enzyme that released 1 mmol L^{-1} of reducing sugar, equivalent to maltose, per min. The protease activity was determined using casein as a substrate according to the method of McDonald and Chen (1965). One unit (U) of protease activity was defined as the amount of enzyme that released 1 mg L^{-1} of tyrosine per min.

3. Results and discussion

3.1. Particulate hydrolysis

The 0.45 μm filtered, dissolved carbon of leaching liquors was cumulated to access the total organic carbon hydrolyzed into aqueous phase (Fig. 1).

With regular replacement of leaching liquors, one may assume that hydrolysis will proceed until only residues are left. However, this was not the case in the test. The cumulative dissolved carbon approached a saturated value. This phenomenon suggested structural transformation might have happened to the solid substrate, a factor that was not under control during the reaction (Gallant *et al.*, 1997). The total hydrolyzed dissolved carbon of batch I was higher than that of batch II with 20 g L^{-1} of added acetate at a corresponding pH, indicating the inhibitive role of acetate. The cumulative dissolved carbon of batch I and batch II followed the descending sequence pH 7 > pH 8 > pH 9 > pH 6 > pH 5; i.e., pH 7 was preferable for enzymatic hydrolysis, and the inhibition caused by pH was weak in the alkali solution.

The generation rate of reducing sugar in batch reactors represented the amylase activity in the suspension. The total amount of produced reducing sugar (RS) of batch I without acetate fell within 1500–3200 mg-C L^{-1}. The production rate dRS/dt followed the sequence pH7 > pH 8 > pH 9 > pH 5 ≈ pH 6 as shown in Fig. 2, which is consistent

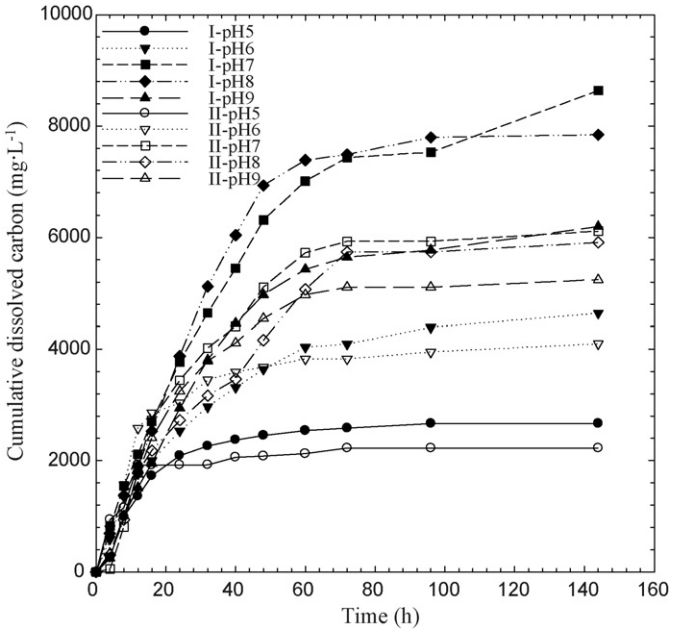

Fig. 1. The cumulative dissolved carbon with or without acetate addition at different pH values.

with the cumulative dissolved carbon result (Fig. 1). The amount of produced reducing sugar of batch II with 20 g L^{-1} acetate within 1000–2000 mg-C L^{-1}. dRS/dt followed the sequence pH 5 > pH 7 > pH 6 > pH 8 > pH 9, but the difference among the dRS/dt data sets was not obvious.

In all of the batches, dRS/dt gradually decreased with time to zero, indicating that enzymes were not efficient during the latter period of hydrolysis.

3.2. Effects of pH and acetate on the hydrolysis of carbohydrate

The data for I-pH 7 (i.e., pH 7 and without acetate) were used to compare the hydrolysis efficiency. The hydrolysis of carbohydrate was indicated by the change in the dissolved carbon content: the quantity of hydrolyzed carbon was 30% less at pH 7 with 20 g L^{-1} of extrinsic acetate, 10–12% or 36% less at pH > 7 without acetate or a high acetate concentration, respectively, and 50 or 60% less at pH 5 without or with high concentrations of acetate, respectively. Dissociated acetate dominated at pH > 6. Therefore, 10^{-5} mol L^{-1} [H^+] inhibited carbohydrate hydrolysis by approximately 50%, while 20 g L^{-1} of dissociated acetate inhibited carbohydrate hydrolysis by 30%. Additionally, undissociated acetate did no noticeably inhibit carbohydrate hydrolysis.

3.3. Kinetic model

The carbon in the particulate sample was the substrate to be hydrolyzed. The amount of solid carbon was determined as the initial quantity of solid carbon minus the amount of dissolved carbon in the aqueous phase. Since the same quantity of enzymatic extracts was added during the refreshment of leaching liquors, the activity of enzyme (B) could be regarded

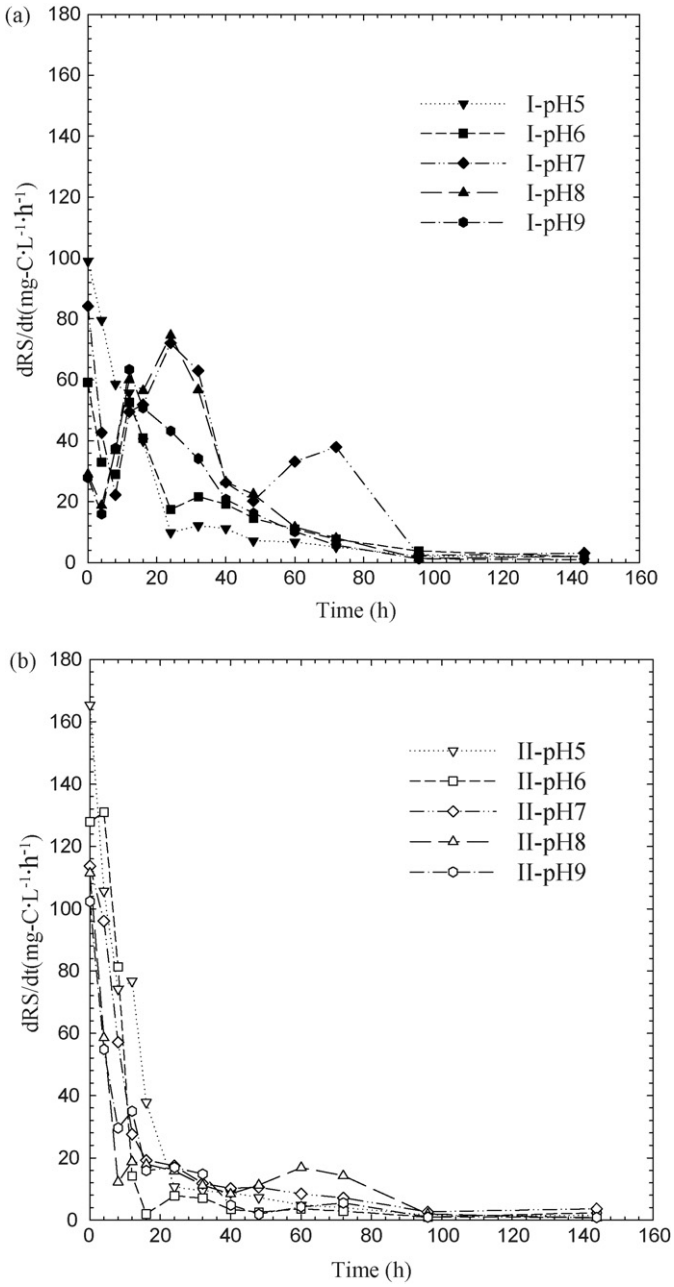

Fig. 2. The rate of production of reducing sugar (dRS/dt) of (a) batch I and (b) batch II.

as being constant. However, as revealed in Fig. 2, the generation rate of reducing sugar, which represented the in situ enzymatic activity according to the definition of α-amylase (Bernfeld, 1955), was not stable. Thus, the concentration of B was determined under either constraint: B = constant or B = the generation rate of reducing sugar.

Under constant B, the biological first-order and A-order biomass kinetics correspond to a first-order chemical reaction with the kinetics $-dS/dt = K_H S$. The Michaelis–Menten, Monod, Contois and two-phase models use $-dS/dt = K_H S/(K_S + S)$. Of all the kinetic models tested here, the Chen–Hashimoto model most closely fitted the original data ($R^2 = 0.999$), followed by the step diffusion model ($R^2 = 0.994$). Other models fitted in the first

40 h but not thereafter because the inhibitive effects increased with time. Nonlinear regressions of the tested models were conducted to obtain a least-squares fit to all of the kinetic data. The chemical first-order and flux model yielded unsatisfactory R^2 values of 0.860 and 0.849, respectively. Although the Michaelis–Menten and Haldane equations exhibited a high R^2 value of over 0.99, negative kinetic parameters, which lack physical meaning, were obtained. Roques *et al.* (1982) also asserted that anomalies may arise when the saturation constant K_S in the Monod model is determined by fitting because it is not a true constant but depends on the age of the sludge and the total amount of biomass in the reactor.

Sanders *et al.* (2002) considered the effect of the biodegradability of the substrate in the model of hydrolysis by introducing a constant parameter—the biodegradable fraction f_h. Fitting the chemical reaction by means of a first-order equation improved R^2 from 0.860 to 0.980 at $f_h = 0.32$. However, as indicated by the saturation value of dissolved carbon in Fig. 1, for an initial substrate concentrate S_0 of 27 g L^{-1}, the regressed f_h value ranged from 0.08 to 0.32, so the value was not constant as proposed but depended on the pH and the amount of acetate.

When the non-linear regressions were conducted, using the rate of generation of the reducing sugar measured in Fig. 2 as the concentration of the enzyme, the regression coefficient R^2 of the biological first-order and half-order kinetics and in the flux model were improved to 0.979, 0.946, and 0.980, respectively. The improvement in the fitting was attributable to the slow drop in the rate of generation of reducing sugar, so the simulated substrate concentration approached an asymptote. The Michaelis–Menten, two-phase and Haldane models produced negative or overly large fitted parameters. The Chen–Hashimoto model yielded a high R^2 value of 0.999. The Contois model yielded a R^2 value of 0.862 and reasonable kinetic parameters, so fitting was much better than that at a constant B, which yielded negative parameters.

Table 2 lists the best-fit parameters of the Chen–Hashimoto model and the step-diffusion model in all of the tests at constant B. The Chen–Hashimoto model fitted all of the tests with high R^2 value (>0.998). Also, the fitted saturation constant K_S was 1.04 ± 0.00192, so one parameter, the hydrolysis constant K_H, sufficed to characterize the process. However, the step diffusion model could not fit tests with acetate at pH 5 or 6, where hydrolysis was severely inhibited. Also, the obtained fitted parameters, K_H and v_{max}, followed no clear trend under various pH values. Therefore, the simplified Chen–Hashimoto model, $-dS/dt = K_H S/(K_S (S_0 - S) + S)$, was suitable for modeling the hydrolysis data.

The best-fit of the Chen–Hashimoto model indicated a possible substrate limit in the test, with emphasis of the influence of the initial concentration of the substrate (S_0). In the present tests, the cumulative dissolved carbon of all of the batches progressed to an asymptote (Fig. 1), and the enzymatic activity (indicated by the generation rate of reducing sugar, Fig. 2) to a void, indicating impaired biodegradability or limited availability of the substrate, although the concentration of the substrate remained high enough.

The competitive or incompetitive models indicated that the inhibition resulted in a fluctuating saturation constant, K_S. However, Table 3 shows that all of the best-fitted K_S values were close to each other. Therefore, these two inhibition mechanisms can be excluded from our discussion.

Fitting using the combined non-competitive inhibition model of three inhibitors (1st: H^+, 2nd: OH^-, 3rd: total/undissociated/dissociated acetate) yielded an R^2 value of greater than 0.9. The model showed a combination of inhibition by organic acid and independent, non-competitive inhibition by H^+ and OH^- ions. Restated, hydrolysis was inhibited by three inhibitors: H^+, OH^-, and organic acid, each followed a non-competitive inhibition mechanism. The inhibitory effects of total acetate were also significant. Some model fitting schemes were noted by Mösche and Jördening (1999).

Table 3 lists the corresponding kinetic parameters. The concentrations of undissociated, dissociated, and total acetate needed to inhibit hydrolysis were 61, 9490, and 9615 mg L^{-1}, respectively. Other inhibitive models, including those of non-competitive inhibition using one or two inhibitors, such as the Levenspiel model or the Luong model, failed to model the effects of pH and acetate simultaneously.

Table 2
Kinetic parameters of the Chen–Hashimoto model and step diffusion model of the hydrolysis of carbohydrate[a]

	pH	5	6	7	8	9
Chen–Hashimoto model of carbohydrate hydrolysis						
Without acetate	R^2	0.999	0.999	0.999	0.998	0.999
	K_H (h^{-1})	1.06E − 05	7.93E − 05	3.86E − 04	2.04E − 04	1.44E − 04
	K_S	1.04E + 00	1.04E + 00	1.04E + 00	1.04E + 00	1.04E + 00
With 20 g L^{-1} acetate	R^2	0.999	0.999	0.999	0.999	0.999
	K_H (h^{-1})	1.17E − 05	3.02E − 05	1.23E − 04	1.35E − 04	7.69E − 05
	K_S	1.04E + 00	1.04E + 00	1.04E + 00	1.04E + 00	1.04E + 00
Step diffusion model of carbohydrate hydrolysis						
Without acetate	R^2	0.757	0.915	0.975	0.977	0.975
	K_H (h^{-1})	1.49E − 03	1.90E − 03	2.82E − 03	3.35E − 03	2.36E − 03
	v_{max}	6.96E − 02	9.79E − 02	1.58E − 01	1.72E − 01	1.25E − 01
With 20 g L^{-1} acetate	R^2	0.081	0.617	0.958	0.981	0.940
	K_H (h^{-1})	1.41E − 03	2.27E − 03	2.64E − 03	2.07E − 03	2.46E − 03
	v_{max}	6.33E − 02	1.08E − 01	1.34E − 01	1.14E − 01	1.22E − 01

[a] 95% confidence interval.

Table 3
Kinetic parameters of combined non-competitive inhibition of pH and acetate in the hydrolysis of carbohydrate[a]

Inhibitor	Carbohydrate			Protein
	H^+, OH^-, HAc	H^+, OH^-, Ac^-	H^+, OH^-, total acetate	H^+, OH^-, HAc
R^2	0.899	0.930	0.930	0.797
K_H^0 (h^{-1})	$1.50E-03$	$1.51E-03$	$1.51E-03$	$2.30E-05$
$K_{I,H}^+$ (mol L^{-1})	$1.50E-07$	$1.23E-07$	$1.23E-07$	$2.51E-05$
$K_{I,OH}^-$ (mol L^{-1})	$6.68E-08$	$8.14E-08$	$8.11E-08$	$3.57E-08$
$K_{I\text{-acetate}}$ (mol L^{-1})	$1.18E-03$	$1.93E-01$	$1.95E-01$	$3.56E-05$

[a] 95% confidence interval.

Based on this inhibition model, using undissociated acetic acid or dissociated acetate as an inhibitor, an acetate level below 12,600 or 17,400 mg L^{-1} would not markedly affect carbohydrate hydrolysis at pH values between 5.9 and 7.7, respectively.

4. Conclusion

This work examined the effects of pH and the acetate concentration on the enzymatic hydrolysis of potato samples which contained 87% carbohydrate and 12% protein, at 37 °C. Replacing the leaching liquors at fixed pH (5–9) and acetate levels (0 or 20 g L^{-1}), yielded separate rates for the hydrolysis of carbohydrate in potato and revealed the possible inhibitive effects of pH and acetate.

The drop in the amount of volatile solids was maximal at neutral pH, without or with 20 g L^{-1} acetate. The amounts of cumulative dissolved carbon in both tests of the effect on pH followed the order pH 7 > pH 8 > pH 9 > pH 6 > pH 5.

The Chen–Hashimoto model and the step diffusion model correlated with the kinetic hydrolysis data. Introducing a correction factor, such as substrate biodegradability, or considering the change in enzyme activity during the test, improved the model fitting. The non-competitive inhibitive model with three inhibitors (H^+, OH^-, total/undissociated/dissociated acetate) best fit the inhibition of carbohydrate hydrolysis.

Acknowledgement

This work was financially supported by the National Hi-Tech Research and Development Program of China (program no. 2001AA644010, no. 2003 AA644020 and no. 50538080, PR China) and Project of National Natural Science Foundation of China (no. 50578115).

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多醣顆粒物之酵素催化水解動力

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摘　要

溶液酸鹼度及揮發性有機酸皆可能為有機顆粒物水解之抑制因素，但其水解抑制之相對重要性則尚未釐清。本研究探討含大量多醣之馬鈴薯在固定 pH 值(5-9)與外加 20 g L^{-1} 醋酸條件下之水解動力，發現 pH 值為較醋酸更重要之抑制因素。簡化 Chen-Hashimoto model: $-dS/dt=K_H S/(K_S(S_0-S)+S)$可最適當描述抑制效應不明顯之馬鈴薯初期水解反應數據，而包含三個抑制因子(H^+, OH^-, 總/未解離/解離醋酸)之非競爭性抑制模型 ($K_H=K_H^0/(1+I/K_I)$)則可描述後期抑制效應明顯時之水解數據。