

# Lactate inhibits hydrolysis of polysaccharide-rich particulate organic waste

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Received 4 February 2007; received in revised form 29 April 2007; accepted 29 April 2007

Available online 19 June 2007

## Abstract

This work reveals that, at pH 5–9, a lactate level of up to 30 g l<sup>-1</sup> retarded hydrolysis rates in polysaccharide-rich potato samples. Lactate substantially limited carbohydrate hydrolysis and enhanced the hydrolysis of proteins. Statistical analysis identified the significance of numerous process factors in substrate hydrolysis. At fixed pH, dissociated lactate affected hydrolysis rates more strongly than its molecular counterpart. At a fixed lactate level, an alkaline environment favors carbohydrate hydrolysis; the effect of pH is secondary. Significant effects of lactate on substrate hydrolysis may be evident in fermenting organic substrates with high carbohydrate content. © 2007 Elsevier Ltd. All rights reserved.

**Keywords:** Hydrolysis; Partial least squares; Acid toxicity; Fermentation; Anaerobic digestion

## 1. Introduction

Substrate hydrolysis reduces the efficiency of anaerobic digestion of particulate organic waste (Mata-Alvarez, 2003; Miron et al., 2000; Sanders et al., 2003; Vavilin et al., 1996). The anaerobic digestion model (ADM) No. 1 model does not consider the inhibitory effects of substrate or the products of substrate hydrolysis (Batstone et al., 2002). However, recent investigations have shown that substrate or products affect substrate hydrolysis (González et al., 2005; Lokshina et al., 2003; Rao, 2003; Sanders et al., 2003; Vavilin et al., 2001, 2003, 2006; Veeken and Hamelers, 2000). For instance, the pH of a suspension has been identified as an important inhibitory parameter (Elefsiniotis et al., 1996; Veeken and Hamelers, 2000; Veeken et al., 2000). Additionally, volatile fatty acids (VFA) reduce substrate hydrolysis rates (González et al., 2005; He et al., 2006; Rao, 2003; Vavilin et al., 2006).

Lactate, an intermediate in the anaerobic digestion of organic substrate, is consumed to generate acetate and pro-

pionate, and is formed under excessively high organic or hydraulic loading, or under loading shocking (Batstone et al., 2002). Lü et al. (2006) demonstrated in fermentation tests with vegetable waste (total solids, TS = 10%) that the lactate content could reach 21 g l<sup>-1</sup> at pH 5–9, accounting for 40–50% of total organic carbon in acidogenic products. Zhang et al. (2005) noted that lactate from digested kitchen waste could reach a concentration of 8.7 g l<sup>-1</sup> at pH 7, or to 20.5 g l<sup>-1</sup> at pH 11. Lactate can reach even higher concentrations in anaerobically digested kitchen wastes herein than were measured by Zhang et al. (2005): concentrations of 30 g l<sup>-1</sup> in anaerobic incubation (Wang et al., 2002) 27–45 g l<sup>-1</sup> at pH 7 (Sakai et al., 2004) and 63 g l<sup>-1</sup> at pH 7 (Zhang et al., 2007b) have been obtained. Ohkouchi and Inoue (2007) claimed that high levels of lactic acid could be produced using carbohydrate-rich stocks. Zhang et al. (2007a) demonstrated the negative effects of accumulated lactic acid (13 g l<sup>-1</sup>) on the performance of the two-phase anaerobic digestion of kitchen wastes.

The inhibition by lactate of the formation of product by fermentative microorganisms has been described (Moon, 1983; Narendranath et al., 2001; van Maris et al., 2004; Warnecke and Gill, 2005). A few works have examined

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the inhibition by lactate of cellulose activity (Iyer and Lee, 1999; Moldes et al., 2001; Takagi, 1984; Yeh et al., 1991). The inhibition by lactate of  $\alpha$ -amylase and glucoamylase activities has also been observed (Anuradha et al., 1999).

This study elucidates how lactate levels and pH reduce the rates of the enzymatic hydrolysis of particulate organic substrate. Partial least square (PLS) modeling is employed to investigate the correlation between response variables (such as hydrolysis efficiency) and input factors (such as pH and lactate levels) (Ecke et al., 2003; Jolliffe, 2002; Quinn and Keough, 2002).

## 2. Methods

### 2.1. Materials

Fresh potato, as the test substrate, was cut into cubes of size 2–3 mm and stored at 4 °C for 12–24 h before testing. The potato contained substantial amounts of carbohydrate and some proteins (Table 1).

Enzymes for enzymatic hydrolysis were prepared from sludge that was obtained after secondary treatment at a municipal wastewater treatment plant in Shanghai, China, since no significant difference existed between hydrolytic enzyme activity under anaerobic and aerobic incubations (Goel et al., 1997, 1998). The details of the enzyme extraction procedure have been published elsewhere (He et al., 2006). Table 1 lists the physiochemical characteristics of the enzyme extracts.

### 2.2. Hydrolysis test

This investigation involved three hydrolysis tests, each with a different initial lactic acid concentration. In each test, 20 g of wet potato was wrapped in gauze bags (80-mesh), and hung in bottles with 50 ml of extracted liquors. The extracted liquors employed in the batch I tests were in suspensions at pH 5–9 (recorded as I-pH 5–I-pH 9), or without pH control (I-pH unadjusted). Batches II and III tests were performed in a manner similar to batch I tests, but with initial lactate concentrations of 15 and 30 g l<sup>-1</sup>, respectively. Each test was conducted with 50 ml of extracted liquors that were made of 5 ml enzyme extracted

from sludge and 45 ml of 0.1 M phosphate buffer with added lactate.

In the hydrolysis tests, sample bottles were shaken at 100 rpm at 37 ± 0.2 °C. Extracted liquors were refreshed using fresh liquors at 4, 8, 12, 16, 24, 32, 40, 48, 60, 72, 96 and 144 h of testing. With liquor replacement, the pH and lactate level of suspensions in the tests were periodically recovered, minimizing the effects of the surrounding liquor on hydrolysis efficiencies.

### 2.3. Analytical methods

The collected extracted liquors were filtered using a 0.45 µm polyester film, and measured for pH, the concentrations of dissolved carbon, dissolved nitrogen, reducing sugar (RS), and amino acid (AA), and the activities of  $\alpha$ -amylase and protease. The suspension pH and oxidation–reduction-potential (ORP) were measured using a pH/ORP meter (OAKTON Instruments, IL, USA). Dissolved carbon and dissolved nitrogen concentrations were measured by first filtering a sample suspension through 0.45 µm filter paper followed by making the measurements using a TN<sub>b</sub>/TC Multi N/C 3000 Analyzer (Analytic Jena AG, Jena, Germany). Reducing sugar concentration was spectrophotometrically determined using the 3,5-dinitrosalicylic acid (DNS) approach (Miller, 1959). The Lowry method was utilized to determine the amino acid content (Lowry et al., 1951). The activities of  $\alpha$ -amylase were assayed using the Bernfeld method (Bernfeld, 1955). Protease activity was determined using casein as a substrate, according to the method developed by Lowry (McDonald and Chen, 1965).

After 144 h of testing, residual potato particulates were tested for total solid (TS) content, volatile solid (VS) content, and elemental composition (C/H/N/S/O). Both TS and VS contents were determined by drying at 70 °C for 48 h and at 550 °C for 6 h, respectively. The elemental composition was measured using a LECO CHNS-932 (LECO Corporation, MI, USA).

### 2.4. Data analysis

Partial least squares modeling (PLS) statistically reveals whether process parameters, such as pH or lactate, significantly affect the hydrolysis rates of potato samples. The experimental data matrix comprised 234 observations made at different initial pHs, total lactate concentrations, and test times. The initial pH (pH<sub>initial</sub>), final pH (pH), concentrations of hydronium ions (H<sup>+</sup>), lactic acid (HLA) and lactate ions (LA<sup>-</sup>), total lactate, ORP and test time were the input factors. Cumulative dissolved carbon, cumulative dissolved nitrogen, RS and AA and their production rates (dC/dt, dN/dt, dRS/dt, and dAA/dt),  $\alpha$ -amylase activity, protease activity, and fall in amount of VS were the dependent variables in the analysis. Simca-P v.11.0 (Umetrics, Umeå, Sweden) was used for PLS analysis.

Table 1  
Physiochemical characteristics of the experimental materials

Potato	Enzymatic extracts	
TS (g g <sup>-1</sup> – wet sample)	0.18	Total organic carbon (mg l <sup>-1</sup> ) 162
VS (g g <sup>-1</sup> – TS)	0.96	Total nitrogen (mg l <sup>-1</sup> ) 50
Carbohydrate (g g <sup>-1</sup> – VS)	0.87	$\alpha$ -Amylase activity (U l <sup>-1</sup> ) 72.6
Protein (g g <sup>-1</sup> – VS)	0.12	Proteinase activity (U l <sup>-1</sup> ) 0.0
Lipid (g g <sup>-1</sup> – VS)	0.005	
Element C (g g <sup>-1</sup> – VS)	0.41	
Element N (g g <sup>-1</sup> – VS)	0.14	

### 3. Results and discussion

#### 3.1. Particulate hydrolysis

The hydrolysis efficiencies of potato samples after 144 h of testing depended on both pH and lactate level. In batch I tests without acid addition, the fall in VS was maximal at pH 7–8, reaching a level of 61%. The fall in amount of VS declined to 50.8% at pH 9. In an acidic environment, the fall in VS amount was reduced to 49.6%, 40.8% and 35.5% at pH 6, 5 and unadjusted pH, respectively. In batch II tests with  $15 \text{ g l}^{-1}$  lactate, the VS reduction was maximal at pH 8 (42.9%) and pH 9 (37.1%). Under neutral conditions, the hydrolyzed VS was 29.1% at pH 7. In an acidic environment, the fall in VS amount was 27.7%, 18.6% and 17% at pH 6, 5 and unadjusted pH, respectively. In batch III tests with  $30 \text{ g l}^{-1}$  lactate, the VS reduction was significantly reduced to  $16.7\% \pm 2.7\%$  and was independent of pH. Externally dose acetate (He et al., 2006) reduced the fall in VS amount to 23.2% (pH unadjusted), 26.6% (pH 5), 34.1% (pH 6), 47.3% (pH 7), 43.1% (pH 8) and 42.7% (pH 9) at  $20 \text{ g l}^{-1}$ . Accordingly, lactate was more suppressive than acetate.

The amounts of hydrolyzed dissolved carbon in batch I tests (Fig. 1a) were higher than those in batch II (Fig. 1b) and batch III (Fig. 1c) tests over the pH range. The cumulative amount of dissolved carbon in batch I tests followed the order pH 7–8 > pH 9 > pH 6 > pH 5 > unadjusted pH, which is consistent with order of the effects of pH on the drop in the amount of VS. The cumulative amount of dissolved carbon in batch II tests followed an order similar to that in batch I, except that the efficiency at pH 7 was close to that at pH 8–9. The cumulative amount of dissolved carbon in batch I reached a plateau at 40–80 h, whereas that in batch II reached a lower plateau after 120 h. The cumulative amounts of dissolved carbon were lower for batch III than for batches I and II.

The cumulative amounts of dissolved nitrogen in batches I–III tests were also analyzed (Fig. 2). The presence of high levels of lactate enhanced the hydrolysis of substrate that contained nitrogen. The plateau values for batches I and II were  $229 \pm 72 \text{ mg N l}^{-1}$ , and that for batch III was  $1010 \pm 365 \text{ mg N l}^{-1}$ . Large fluctuations were present in the data from the samples, suggesting intrinsic differences among the tested samples rather than pH-controlling protein extraction efficiencies.

The activities of  $\alpha$ -amylase during 144 h of tests were highest in batch I at pH 7 ( $189 \pm 49 \text{ U l}^{-1}$ ) and pH 8 ( $171 \pm 49 \text{ U l}^{-1}$ ), followed by pH 9 ( $115 \pm 39 \text{ U l}^{-1}$ ), pH 6 ( $66 \pm 32 \text{ U l}^{-1}$ ) and pH 5 ( $33 \pm 12 \text{ U l}^{-1}$ ). In batch II tests with  $15 \text{ g l}^{-1}$  lactate, the activities of  $\alpha$ -amylase were highest at pH 8 ( $102 \pm 49 \text{ U l}^{-1}$ ), followed by pH 9 ( $69 \pm 25 \text{ U l}^{-1}$ ), pH 7 ( $69 \pm 30 \text{ U l}^{-1}$ ), pH 5 ( $62 \pm 17 \text{ U l}^{-1}$ ) and pH 6 ( $46 \pm 10 \text{ U l}^{-1}$ ). In batch III tests with  $30 \text{ g l}^{-1}$  lactate, the amylase activities were overall lower than  $50 \text{ U l}^{-1}$  (pH 5,  $39 \pm 10 \text{ U l}^{-1}$ ; pH 6,  $42 \pm 10 \text{ U l}^{-1}$ ; pH 7,  $50 \pm 10 \text{ U l}^{-1}$ ; pH 8,  $50 \pm 10 \text{ U l}^{-1}$ ; pH 9,  $45 \pm 10 \text{ U l}^{-1}$ ).

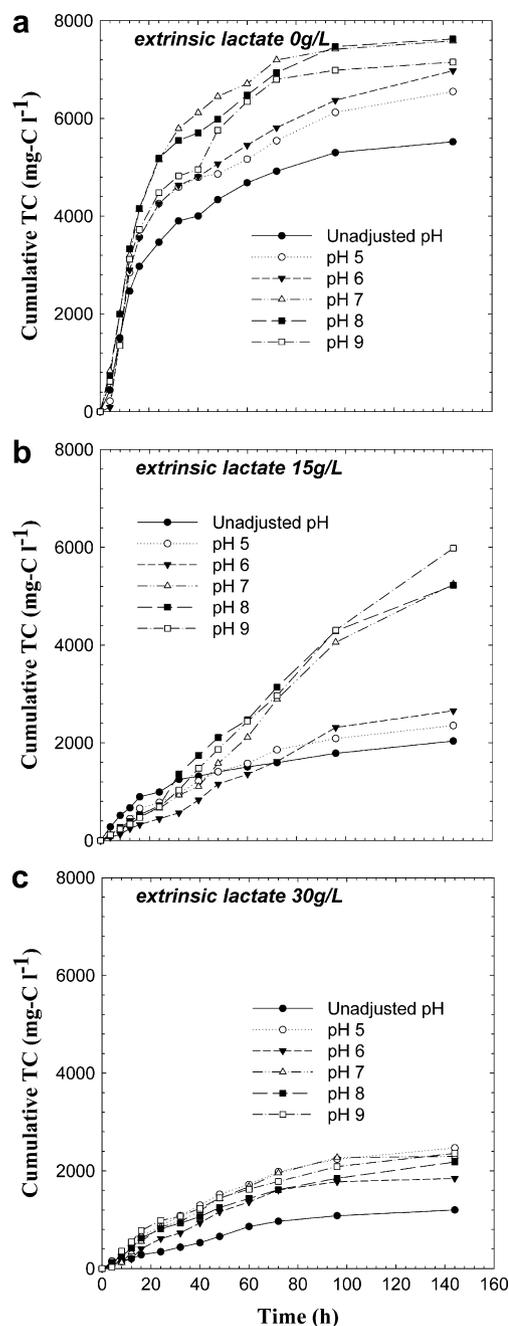


Fig. 1. Effect of pH and lactate levels in extracted liquors on cumulative amounts of dissolved carbon.

The activities of protease during 144 h of tests were highest in batch III with  $30 \text{ g l}^{-1}$  lactate (pH 5,  $8726 \pm 5527 \text{ U l}^{-1}$ ; pH 6,  $7421 \pm 3238 \text{ U l}^{-1}$ ; pH 7,  $6568 \pm 3056 \text{ U l}^{-1}$ ; pH 8,  $7421 \pm 3646 \text{ U l}^{-1}$ ; pH 9,  $5930 \pm 3559 \text{ U l}^{-1}$ ), decreasing as the lactate dose fell in batch II and batch I: the results were (pH 5,  $5516 \pm 3836 \text{ U l}^{-1}$ ; pH 6,  $5254 \pm 3465 \text{ U l}^{-1}$ ; pH 7,  $4649 \pm 2558 \text{ U l}^{-1}$ ; pH 8,  $6016 \pm 3663 \text{ U l}^{-1}$ ; pH 9,  $4943 \pm 3159 \text{ U l}^{-1}$ ) and (pH 5,  $1743 \pm 989 \text{ U l}^{-1}$ ; pH 6,  $1981 \pm 1019 \text{ U l}^{-1}$ ; pH 7,  $2117 \pm 1604 \text{ U l}^{-1}$ ; pH 8,  $1903 \pm 1514 \text{ U l}^{-1}$ ; pH 9,  $7347 \pm 3559 \text{ U l}^{-1}$ ), respectively.

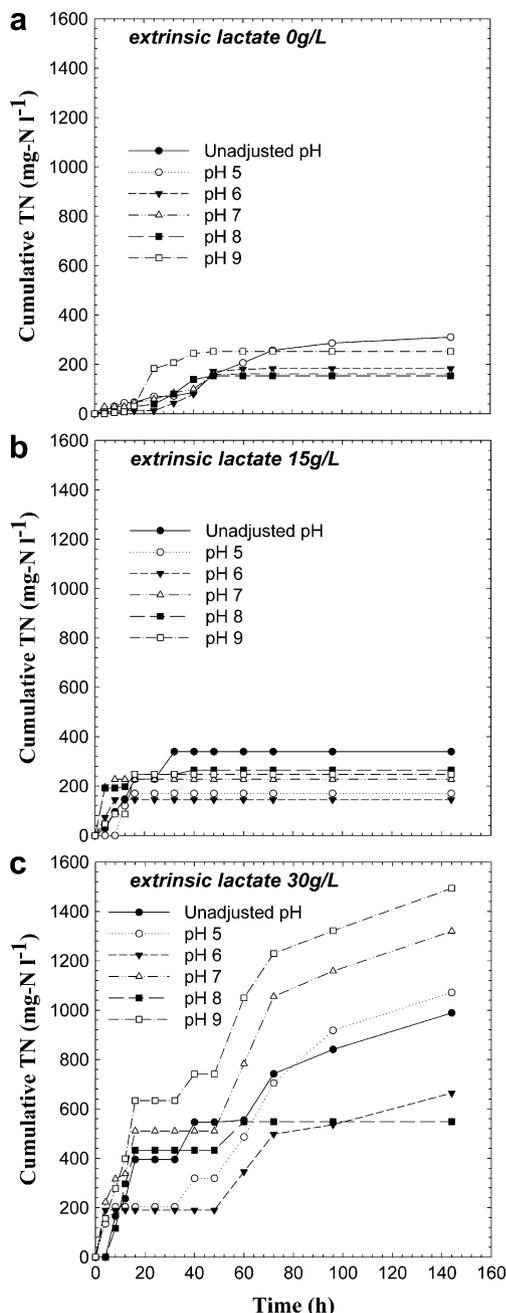


Fig. 2. Effect of pH and lactate levels in extracted liquors on cumulative amounts of dissolved nitrogen.

According to the data fluctuation, the activities of these enzymes generally correlated with hydrolysis efficiencies (Figs. 1 and 2). Lactate suppressed carbon hydrolysis particularly in an acidic environment. Conversely, the presence of lactate promoted nitrogen hydrolysis, and was weakly correlated with suspension pH.

3.2. Statistical analysis

The PLS modeling of carbohydrate hydrolysis demonstrated that three principal components, PC1, PC2 and PC3, accounted for 21.3%, 12.7% and 7.0% of the variation

in the data, respectively. The presented model revealed unresolved variability associated with noise, the impact of factors that were not considered, or the setting of certain parameters to constants. The loading plot (Fig. 3a) indicates that the hydrolysis of carbohydrate depended substantially on pH, lactic acid concentration, ORP and test duration. The loading of responses was high in PC1 (21.3% of the data variation), and the total lactate and dissociated lactate ( $LA^-$ ) loadings were higher in PC1 than any of the factors considered, suggesting that lactic acid most strongly affected the carbohydrate hydrolysis rate. The score contribution plot (Fig. 3b) also demonstrates that total lactate and dissociated lactate had higher scores than pH and HLA. Hence, the suppressive effects of carbon hydrolysis examined in this work should be the result of the chemical nature of lactate, rather than the reduced pH.

The PLS model of protein hydrolysis generated three principal components, which explained 19.4%, 8.6% and 1.7% of the data variation, respectively, or 29.9% together. The loading plot (Fig. 4a) revealed that the effect of pH was

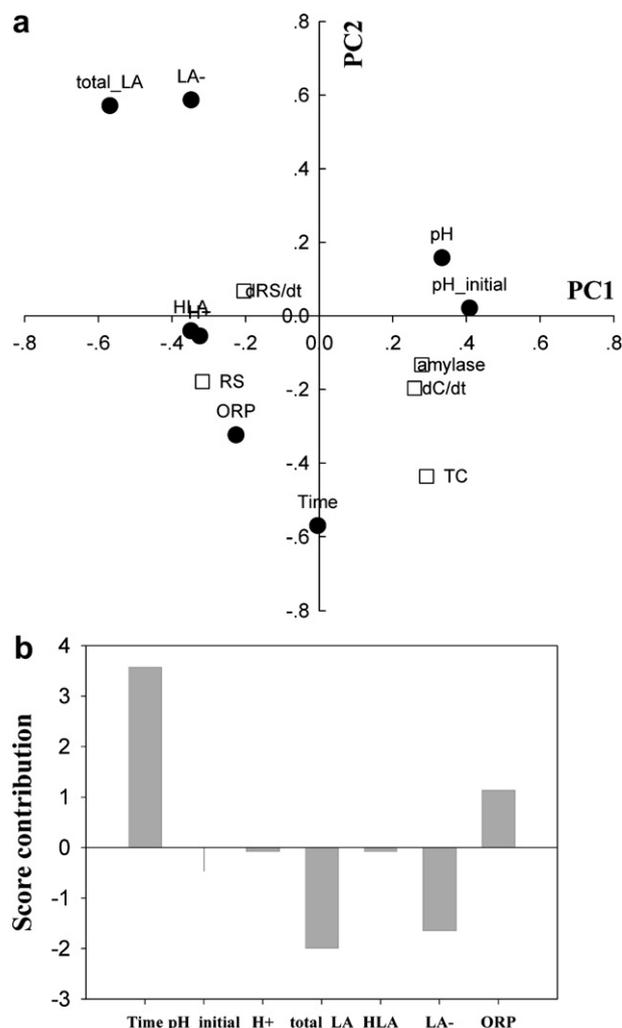


Fig. 3. Partial least squares analysis for carbohydrate hydrolysis. (a) Loadings plot of first and second principal components: ● factors; □ responses. (b) Score contribution of variables.

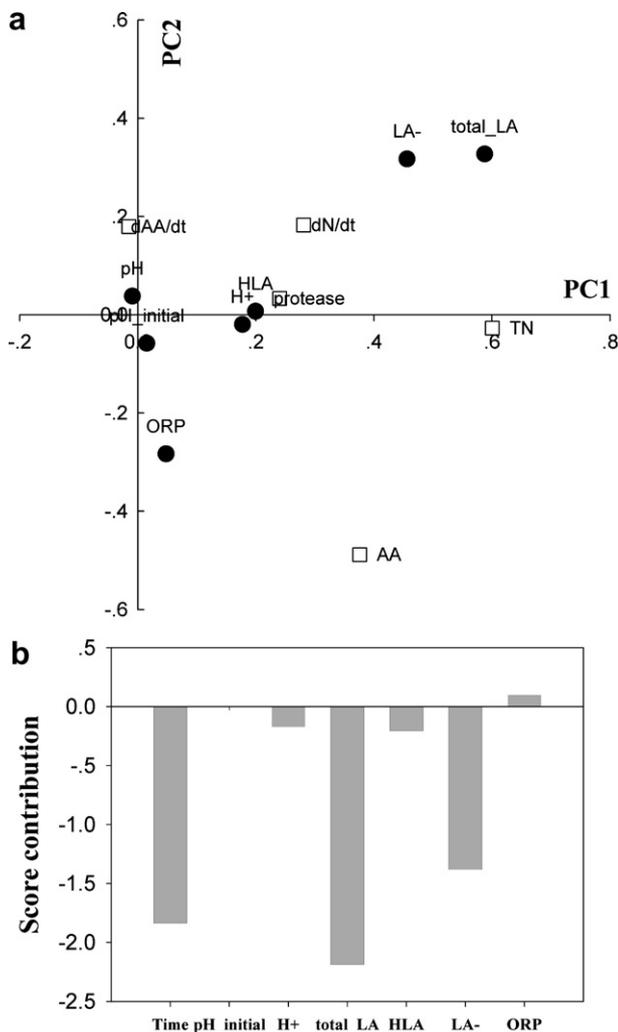


Fig. 4. Partial least squares analysis for protein hydrolysis. (a) Loadings plot of first and second principal components: ● factors; □ responses. (b) Score contribution of variables.

minor in PC1 and PC2, whereas total lactate and dissociated lactate were more strongly weighted than  $H^+$  and HLA, revealing that lactate rather than pH determined the hydrolysis of protein. Furthermore, lactate- and nitrogen-related parameters (TN, AA, and protease) located on the same side of PC1. Accordingly, the protein hydrolysis efficiency was positively correlated with the lactate concentrations. The score plot also verified this correlation (Fig. 4b).

The PLS analysis results demonstrated that pH and lactate differently influenced the hydrolysis rates of carbohydrates and proteins. Both pH and lactate affected carbohydrate hydrolysis, and the lactate was dominant and more inhibitory. Conversely, lactate and not pH enhanced the hydrolysis of protein.

When only hydrolyzed VS was considered as a response factor, PLS modeling revealed two principal components, which were responsible for 75.5% and 7.4% of the data variation, respectively, or a total 82.9% of the data variation (Fig. 5). Total lactate and dissociated lactate more strongly

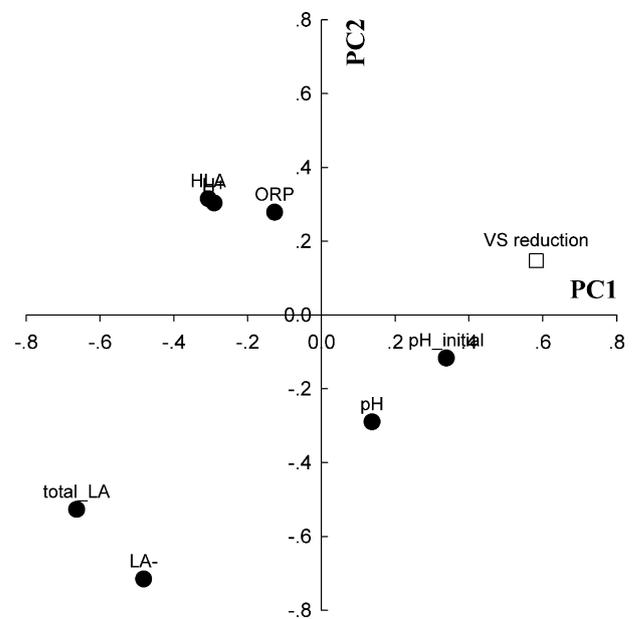


Fig. 5. Partial least squares analysis for volatile solid hydrolysis: ● factors; □ responses.

reduced the amount of VS than did pH and the undissociated lactate. Lactate was negatively correlated and pH was positively correlated with VS reduction.

### 3.3. Inhibitory hydrolysis with lactate or acetate

He et al. (2006) demonstrated the inhibitive effects of externally dosed acetate on the hydrolysis of organic particulates. With  $20 \text{ g l}^{-1}$  acetate, the VS reduction of potato samples was higher under alkaline than under acidic conditions; the reduction ratio was 42–47% at pH 7–9 and 26–34% at pH 5–6. Accordingly, the presence of lactate or acetate suppresses the rate of hydrolysis of polysaccharide-rich, potato samples. In contrast with the conclusions drawn for lactate in Sections 3.1 and 3.2, He et al. (2006) concluded that induced pH changes more strongly inhibited carbohydrate hydrolysis than dose acetate. Lactate ( $pK_a = 3.86$  at  $25^\circ\text{C}$ ) inhibited the hydrolysis efficiency of tested potato samples more than did acetate ( $pK_a = 4.74$  at  $25^\circ\text{C}$ ) on an equal weight or equal molar basis. Additionally, acetate inhibits and lactate enhances protein hydrolysis. Hence, the effects of lactate and acetate as intermediates during the anaerobic digestion of organic substrates, in inhibiting substrate hydrolysis differ.

## 4. Conclusions

The study suggested that the suppression of carbohydrate hydrolysis by 15 or  $30 \text{ g l}^{-1}$  lactate was stronger than that by acetate or by a change in pH. Lactate stimulated protein hydrolysis and the effect of pH on protein hydrolysis was insignificant. Accordingly, the influence of lactate on carbohydrate or protein hydrolysis was associated primarily with the presence of lactate species, rather than

the corresponding fall in pH. Since lactate can be produced in significant amounts during the fermentation of organic waste with a high solid content, the bi-directional role of lactate in hydrolysis should be regarded as non-trivial in comprehensive modeling, such as that in ADM No. 1.

### Acknowledgements

This work was financially supported by the National Sci. & Tech. Supporting Program of China (2006BAJ04A06), Key Project of Chinese Ministry of Education (107122), Shanghai-Rhone Alpes Region International Scientific Research Cooperation Fund (06SR07105) and Program for Young Excellent Talents in Tongji University (2006KJ032).

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