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Partial least square modeling of hydrolysis: analyzing the impacts of pH and acetate

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Abstract: pH and volatile fatty acids both might affect the further hydrolysis of particulate solid waste, which is the limiting-step of anaerobic digestion. To clarify the individual effects of pH and volatile fatty acids, batch experiments were conducted at fixed pH value (pH 5—9) with or without acetate (20 g/L). The hydrolysis efficiencies of carbohydrate and protein were evaluated by carbon and nitrogen content of solids, amylase activity and proteinase activity. The trend of carbohydrate hydrolysis with pH was not affected by the addition of acetate, following the sequence of pH 7>pH 8>pH 9>pH 6>pH 5; but the inhibition of acetate (20 g/L) was obvious by 10%—60 %. The evolution of residual nitrogen showed that the effect of pH on protein hydrolysis was minor, while the acetate was seriously inhibitory especially at alkali condition by 45%—100 %. The relationship between the factors (pH and acetate) and the response variables was evaluated by partial least square modeling (PLS). The PLS analysis demonstrated that the hydrolysis of carbohydrate was both affected by pH and acetate, with pH the more important factor. Therefore, the inhibition by acetate on carbohydrate hydrolysis was mainly due to the corresponding decline of pH, but the presence of acetate species, while the acetate species was the absolutely important factor for the hydrolysis of protein.
Keywords: inhibition; hydrolysis; pH; volatile fatty acids; partial least square

Introduction

Environmental and economic advantages highlight anaerobic digestion as a sustainable technology for solid waste treatment (Edelmann *et al.*, 2000). Anaerobic digestion could be described as four sequential steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis, among which the hydrolysis is recognized as the rate-limiting step for particulate organic waste (Jain *et al.*, 1992; Mata-Alvarez, 2000). Hydrolysis occurs commonly together with the acidogenesis, while the products of the latter would result in the change in pH. Both pH and the acidogenic products may affect the microbial activity as well as the hydrolysis in an anaerobic environment (Veeken and Hamelers, 2000). The very complex nature of the interactions between organic substances and pH makes the prediction of inhibitory levels of acidogenic products difficult (Pind *et al.*, 2003). Some references discussed the effects of pH or VFA on the substrate hydrolysis (Palenzuela-Rollón, 1999; Boon, 1994; Elefsiniotis *et al.*, 1996; Veeken and Hamelers, 2000; Babel *et al.*, 2004; González *et al.*, 2005). Their works suggest that the roles of pH and VFA on substrate hydrolysis and acidogenesis are not conclusive. To clarify the roles of hydrolysis inhibition by pH and/or acidogenic products is not only of academic interest, but has significantly practical implementation. For instance, it could suggest the processes of bioreactor landfill, which recycles leachate, untreated or pretreated, back to the landfill layers (Zou *et al.*, 2003; Veeken and Hamelers, 2000). If the major inhibitory factor could be determined to be pH, then buffering the recycled leachate is a key to enhance the hydrolysis efficiency. If it were VFA (volatile fatty acid)-type inhibition, the removal of organic matters from leachate before recycling is essential to the success of bioreactor landfill.

This work investigated how pH and the level of acetate, one major acidogenic product, affected the enzymatic hydrolysis of particulate organic waste. Partial least square modeling (PLS) was used to identify the significance of the correlation between the response variables and the input factors (Jolliffe, 2002; Quinn and Keough, 2002; Ecke *et al.*, 2003), accordingly, to compare the importance of each factor.

1 Materials and methods

1.1 Materials

Fresh potato was the testing substrate, cut into cubes of size 2—3 mm and stored at 4℃ for 12—24 h. Enzyme extracts for enzymatic hydrolysis was prepared from the returned sludge following secondary treatment of a municipal wastewater treatment plant. The physiochemical characteristics of the substrate and enzymatic extracts are listed in Table 1.

1.2 Hydrolysis tests

Two batches of hydrolysis tests with different concentrations of added acetate were conducted. Notably, the batch I tests were conducted using leaching liquor at fixed pH recorded as I-pH 5 to I-pH 9, while the batch II was tested with liquors at fixed pH and containing externally dosed acetate (20 g/L) recorded as II-pH 5 to II-pH 9. For batch I, every 50 ml leaching liquor consisted of 5 ml enzymatic

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Table 1 Physiochemical characteristics of experimental materials

Potato		Enzymatic extracts	
TS, g/g wet sample	0.18	TOC, mg/L	162
VS, g/g TS	0.96	TN, mg/L	49.9
Carbohydrate, g/g VS	0.874	α-Amylase activity, U/L	72.6
Protein, g/g VS	0.121	Proteinase activity, U/L	0.0
Lipid, g/g VS	0.005		
Element C, g/g VS	0.41		
Element N, g/g VS	0.14		

Notes: TS. total solid; VS. volatile solid

extracts and 45 ml of 0.1 mol/L phosphate buffers. For batch II, every 50 ml leaching liquor consisted of 5 ml enzymatic extracts and 45 ml acetate buffers.

In each test, 20 g of wet potato was wrapped in gauze bags (80-mesh), and hung in bottles with 50 ml leaching liquors. The bottles were shaken at 100 r/min at (37±0.2)℃. The leaching liquor was replaced by fresh one (containing enzyme extract, dosed acetates, etc.) after 4, 8, 12, 16, 24, 32, 40, 48, 60, 72, 96 and 144 h of hydrolysis, respectively. With the liquor replacement, the changes in pH and the acetate level in the test could be regarded minimal.

1.3 Analytical methods

The collected leaching liquors were filtered using 0.45 μm polyester film to measure pH, oxidation and reduction potential (ORP), dissolved carbon, dissolved nitrogen, reducing sugar, amino acid, α-amylase activity and proteinase activity. pH and ORP was measured by a pH/ORP meter (OAKION, USA). Dissolved carbon and dissolved nitrogen were measured with TN_b/TC multi N/C 3000 Analyzer (Analytik Jena AG, German). The α-amylase activity was assayed according to Bernfeld (1955). Proteinase activity was determined using casein as a substrate according to the method by McDonald and Chen (1965).

After 144 h testing, the potato particulates were unwrapped from the gauze bags and tested for total solid (TS), volatile solid (VS) and C/H/N/S/O elemental composition. The TS and VS were determined by drying at 70℃ for 48 h and 550℃ for 6 h, respectively. The elemental composition was measured with LECO CHNS-932 determinator (LECO Ltd., USA).

2 Results and discussion

2.1 Hydrolysis of carbohydrate

The hydrolysis of carbohydrate was indicated by the evolution of residual carbon in substrate potato, i. e., the initial carbon quantity of the substrate minus dissolved carbon in aqueous phase. As shown in Fig.1, the total hydrolyzed carbon of batch I was higher than that of batch II with added acetate (20 g/L) at corresponding pH, indicating the inhibitory role of acetate. The hydrolyzed carbon of batch I followed the descending sequence of pH 7 > pH 8 > pH 9 > pH 6 > pH 5 (Fig.1a), indicating neutral condition (pH 7) was preferable for enzymatic hydrolysis and the inhibition by pH was weaker in alkali solution than in acidic solution. The hydrolyzed carbon of batch II followed the descending sequence of pH 7 > pH 8 > pH 9 > pH 6 > pH 5 (Fig.1b), similar to that of batch I, suggesting that the effect of pH on hydrolysis was not affected by the addition of acetate.

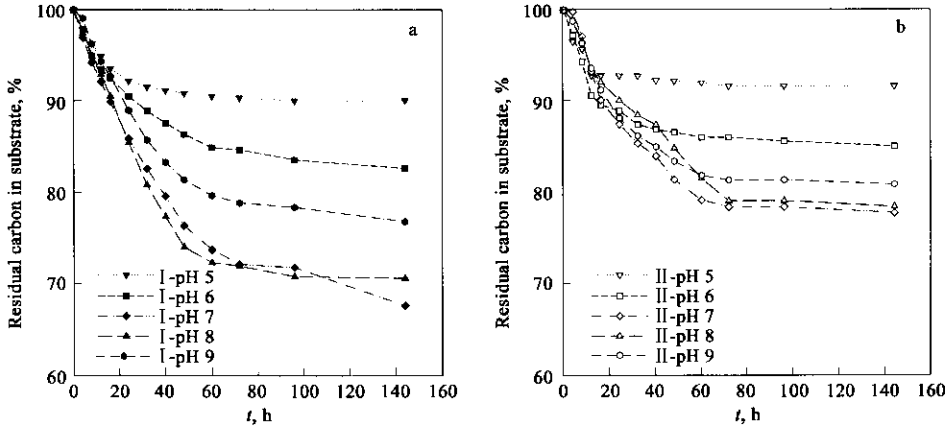

Fig.1 Temporal evolution of residual carbon in the substrate

Taking pH 7 and no acetate addition as the comparison basis, we could find that the quantity of hydrolyzed carbon was 30% less when pH=7 with extrinsic acetate, 10%—12% or 36% less when pH > 7 and without or with high concentration of acetate, respectively; and 50% or 60% less at pH 5 and without or with high concentrations of acetate. Dissociated acetate is the dominant form at pH > 6. Hence, 10^{-5} mol/L [H^+] could inhibit about 50% of carbohydrate hydrolysis, while dissociated acetate would inhibit

30% of carbohydrate hydrolysis. Additionally, the inhibition effect of undissociated acetate on carbohydrate hydrolysis was not noticeable.

2.2 Hydrolysis of protein

Fig.2 illustrates the effects of pH and acetate on the hydrolysis of protein, indicated by the evolution of residual nitrogen in substrate potato, i.e., the initial nitrogen quantity of substrate minus dissolved nitrogen in aqueous phase. In batch I (without addition of acetate), pH has no significant role on the residual nitrogen, suggested by a nearly constant, saturated value of 90.4% ± 0.2% in all tests. As Fig.2a reveals, acidic environment is a slightly not favorable for protein hydrolysis. Addition of acetate (batch II) reduced the total hydrolyzed carbon compared with batch I without acetate (Fig.2b). Least residual nitrogen occurred at pH 5 to 95.9%, followed by at pH 6 to 98.7%. Enzymatic hydrolysis of nitrogen was not found at alkaline condition.

Dependence of pH and acetate on inhibition to

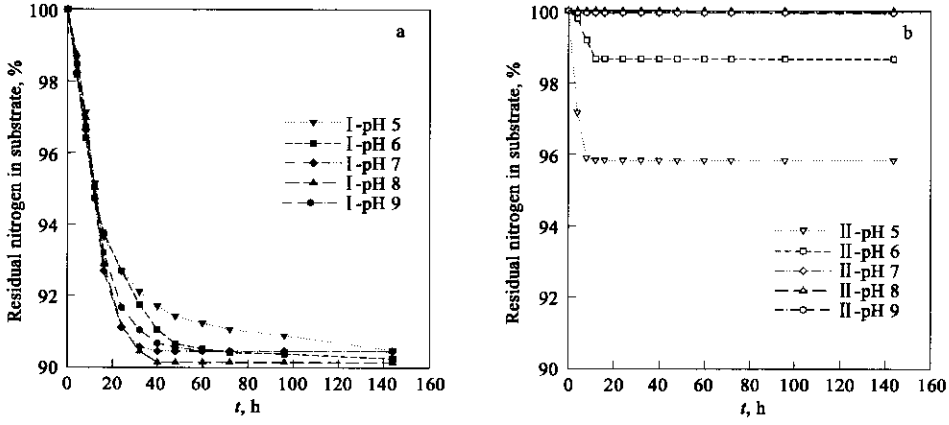

Fig.2 Temporal evolution of residual nitrogen in the substrate

protein hydrolysis is different from those on carbohydrate. The hydrolysis of protein would not be inhibited by pH at low acetate concentration. With acetate (20 g/L) hydrolysis reaction of protein was completely inhibited at pH>7, or above 50% inhibition to occur at pH<5. Therefore, [H$^+$] did not inhibit the hydrolysis of protein, while dissociated acetate could significantly inhibit the hydrolysis.

2.3 Enzymatic activity assay of α-amylase and proteinase

The activities of hydrolytic enzymes including α-amlyase and proteinase were assayed to demonstrate the straight effects of pH and acetate on the key catalysts of hydrolytic reactions. As shown in Fig.3a, for batch I without acetate, the α-amlyase activity at pH 7 was the highest, followed by pH 8, 9, 6 and 5, indicating the weak inhibition at alkali conditions and acute inhibition at acidic conditions. For batch II (Fig. 3a), the α-amlyase activities were seriously impaired compared to batch I at similar pH 7—9, while not affected at pH 5—6, suggesting the inhibition from dissociated acetate. The effects of pH and acetate on the α-amlyase activity were consistent with that on the evolution of residual carbon (Fig.1). The proteinase activities are shown in Fig.3b. The differences at each pH and with or without acetate addition were not obvious, except that the proteinase activity extruded a lot at pH 9. Although the hydrolysis of protein was completely depressed at alkali conditions (Fig.2b), the proteinase activity was only slightly declined at pH 7—9, compared to pH 5—6 (Fig.3b), indicating the parameter of proteinase activity was insensitive to the stress of pH and acetate.

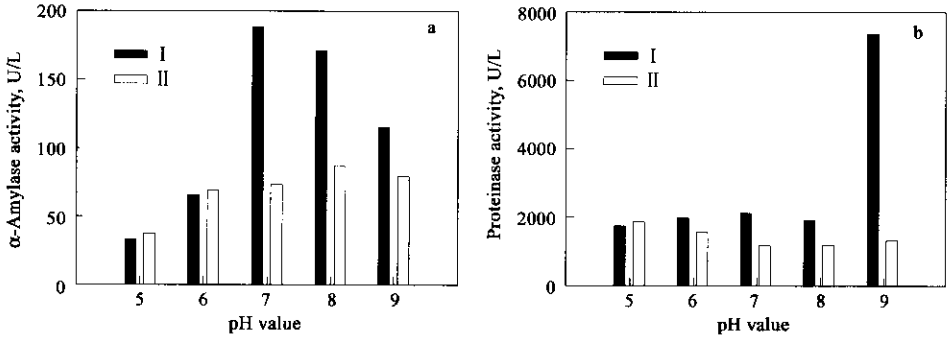

Fig.3 α-Amylase and proteinase activity

2.4 Partial least square analysis

In this section, we utilized the PLS modeling to statistically reveal how significantly pH or acetate would inhibit the hydrolysis of potato.

The experimental data matrix consisted of 130 observations at individual initial pH value, total acetate concentrations, and testing times. The initial pH value, the actual pH value, the concentration of hydronium ion (H^+), undissociated acetate (HAc), dissociated acetate (Ac^-) and total acetate, oxidation and reduction potential (ORP) and time were input factors. The residual carbon, residual nitrogen, reducing sugar (RS) or amino acid (AA) as well as their generation rate dC/dt, dN/dt, dRS/dt and dAA/dt were set as response variables in separated analysis considering hydrolysis of carbohydrate or protein. Data were standardized by the automatic transformation of scaling and centering. SimcaP v. 11.0 (Umetrics, USA) was used to conduct PLS analysis.

The PLS modeling on carbohydrate hydrolysis resulted in two principal components (PC1 and PC2) comprising 29.1% and 14.1% of the data variation, respectively. The received model totally explained 43.2% of the data variation, i.e., there still remained unresolved variability, due to either noise or the impact of factors that were not considered or had been set at constant conditions. The loading plot (Fig.4a) illustrated the impact of the factors on the response variables, indicating that the carbohydrate hydrolysis depended greatly on pH, acetate, and testing time. The loadings of responses were the high in PC1 (explaining 29.1% of the data variation), while the loadings of pH were highest in PC1 among the factors, suggesting that pH was the most important variable on carbohydrate hydrolysis rate. The score contribution plot (Fig.4b) also showed that pH (including initial pH, actual pH and H^+) had higher scores than acetate (including total acetate, HAc and Ac^-). Hence, the corresponding pH decline owing to acetate production in acidogenesis mainly corresponded to the hydrolysis inhibition. However, the superiority of pH upon acetate was not marked, suggesting that the inhibition of acetate could not be neglected in the case of carbohydrate hydrolysis. Comparing the first 24 h of testing and the latter 120 h of testing, the importance of time was greatly reduced at latter stage of test. The score contribution of pH and acetate were both slightly strengthened at the latter stage of hydrolysis than the former stage.

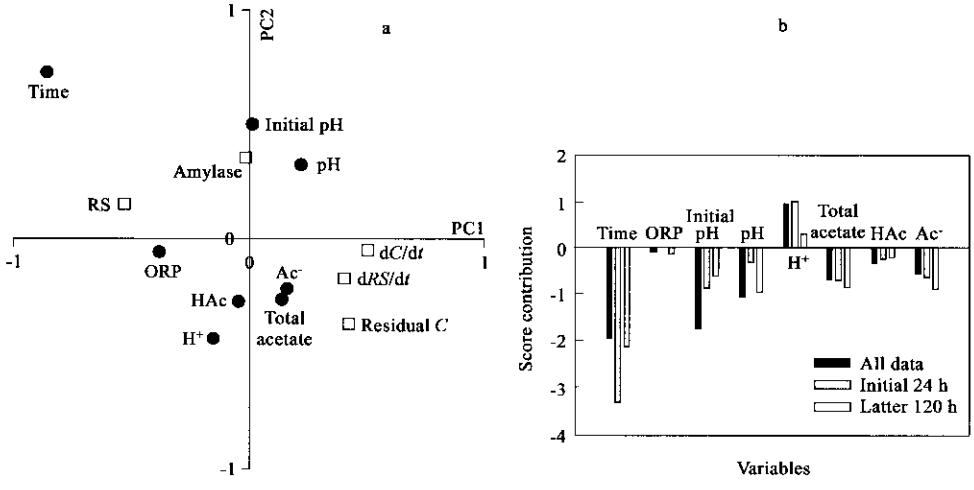

Fig.4 Partial least square analysis for carbohydrate hydrolysis

The PLS modeling on protein hydrolysis resulted in two principal components comprising 27.7% and 13.5% of the data variation, respectively, totally covering 41.2% of the data variation. The loading plot (Fig.5a) showed that the effect of pH was much weaker than acetate in PC1, indicating that the protein hydrolysis was dominantly inhibited by acetate but not by pH. The score contribution confirmed this conclusion (Fig.5b). In the latter period of hydrolysis (Fig.5b), the score contributions of pH and acetate variables were both enhanced, whereas the improvement of pH was becoming more significant, compared with the initial 24 h of testing (Fig.5b).

The PLS analysis demonstrated that the effects of pH and acetate on hydrolysis of carbohydrate or protein were different. The hydrolysis of carbohydrate was both affected by pH and acetate, with pH the more important variable. Therefore, the inhibition by acetate on carbohydrate hydrolysis was mainly due to the corresponding decline of pH, but the presence of acetate species. The hydrolysis of protein, on the other hand, was strongly affected by acetate. Its inhibition on protein hydrolysis was pH-independent.

3 Conclusions

It was investigated in this work how pH and the

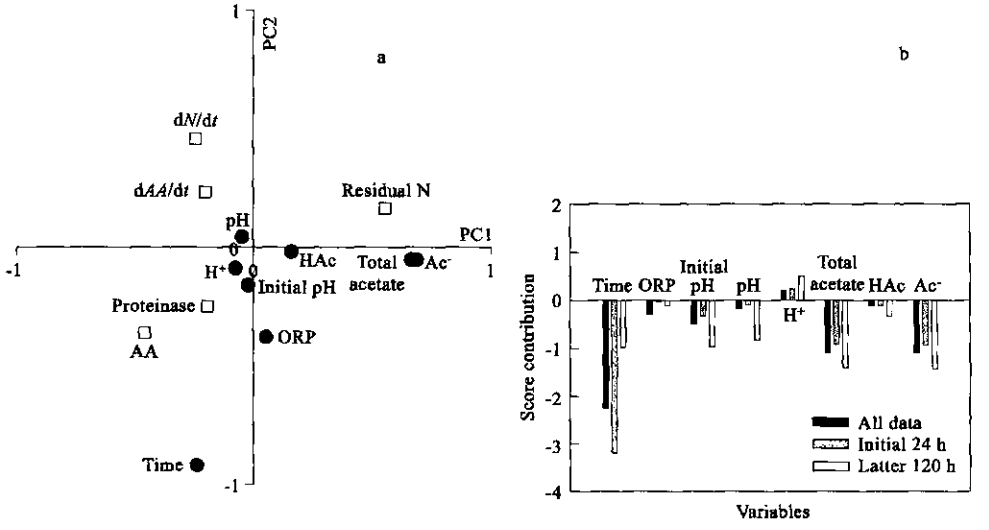

Fig.5 Partial least square analysis for protein hydrolysis

level of acetate affected the enzymatic hydrolysis of potato sample. By replacing the leaching liquors at fixed pH (5—9) and acetate levels (0 or 20 g/L), the hydrolysis rates of carbohydrate and protein fractions of potato were compared separately and illustrated the possible inhibitory effects of pH and acetate. The experimental data and the PLS modeling demonstrated that the hydrolysis of carbohydrate was both affected by pH and acetate, with pH the more important factor, while acetate species was far more important than pH for the hydrolysis of protein. Therefore, the inhibition by acetate on carbohydrate hydrolysis was mainly due to the corresponding decline of pH, but the presence of acetate species, while the acetate species was the absolutely important factor for the hydrolysis of protein.

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