PERGAMON

Water Research 37 (2003) 55–66

WATER RESEARCH

www.elsevier.com/locate/watres

# Acidogenesis of gelatin-rich wastewater in an upflow anaerobic reactor: influence of pH and temperature

Han Qing Yu[a,*], Herbert H.P. Fang[b]

[a] *Lab of Environmental Engineering, Department of Chemistry, The University of Science & Technology of China, Hefei, Anhui, 230026 China*

[b] *Department of Civil Engineering, Centre for Environmental Engineering Research, The University of Hong Kong, Hong Kong*

Received 24 January 2002; received in revised form 11 June 2002; accepted 19 June 2002

**Abstract**

The influence of temperature and pH on the acidification of a synthetic gelatin based wastewater was investigated using an upflow anaerobic reactor. Gelatin degradation efficiency and rate, degree of acidification, and formation rate of volatile fatty acids and alcohols all slightly increased with temperature. Temperature affected the acidogenesis of gelatin according to the Arrhenius equation with an activation energy of 1.83 kcal/mol. Compared with temperature, pH had a more significant effect on the acidogenesis. Gelatin degradation efficiency substantially increased with pH, from 60.0% at pH 4.0 to 97.5% at pH 7.0. The degree of acidification increased from 32.0% at pH 4.0 to 71.6% at pH 6.5, but dropped to 66.8% when pH increased to 7.0. The optimum pH for the overall acidogenic activity was found to be 6.0, close to 5.9, the optimum pH calculated using a semi-empirical model. Operation at pH of 4.0–5.0 favored the production of propionate, hydrogen, whereas the operation at pH 6.0–7.0 encouraged the production of acetate, butyrate, and i-butyrate. The region between pH 5.0 and 6.0 was the transition zone.


*Keywords:* Acidogenesis; Gelatin; pH; Temperature; Wastewater

## 1. Introduction

The efficient anaerobic degradation of organic matters is dependent upon the coordinated metabolisms of acid-forming and methane-forming bacteria. Imbalances in the metabolic rates of these two bacterial groups have largely been responsible for the instabilities associated with anaerobic digestion. These imbalances can lead to the accumulation of intermediary acid products which will eventually cause the inhibition of methanogenic bacteria. For this reason the separation of the acidogenic and methanogenic phases into separate reactors, was proposed [1]. Process stability and overall degradation rates can thus be increased by separately optimizing conditions for each bacterial group [2]. The formation of certain intermediary products have been shown to be energetically more favorable, higher biogas production and better effluent quality could be obtained [3]. However, product formation by a mixed acidogenic population is a very complex process and is greatly influenced by many factors. These factors include wastewater specificity, reactor configuration, hydraulic retention time (HRT), influent organic concentration, organic loading rate, pH, temperature, oxidation–reduction potential, and nutritional requirements [4–7].

Temperature effect on the maximum substrate utilization rates of methanogens has been observed [8,9]. Lowering operational temperature generally leads to a decrease in the maximum specific growth and substrate utilization rate. In addition, methanogenic sludge yield has been shown to decrease with decreasing temperature [8]. Temperature also affects the maintenance

*Corresponding author. Tel.: +86-551-3607592; fax: +86-551-3631760.

*E-mail address:* hqyu@ustc.edu.cn (H.Q. Yu).

0043-1354/02/$ - see front matter 
PII: S0043-1354(02)00256-7

requirements of methanogens. Specific maintenance rate has been shown to give linear Arrhenius plots over a limited temperature range [10]. However, the temperature effect studies have been focused on overall anaerobic degradation process or methanogenesis, rather than acidogenesis.

Since pH affects growth rate, pH changes may cause drastic shifts in the relative numbers of different species in a heterogeneous population such as is present in the acidogenic reactor [11]. Many aspects of microbial metabolism are greatly influenced by pH variations over the range within which the microorganisms can grow. These aspects include utilization of carbon and energy sources, efficiency of substrate degradation, synthesis of proteins and various types of storage material, and release of metabolic products from cells [12]. Moreover, pH variation can affect cell morphology and structure and, therefore, flocculation and adhesion phenomena [13]. A substantial number of studies have been carried out on the effect of pH on acidogenesis of carbohydrate-rich wastes [14–18], but little attention has been paid to the influence of pH on acidogenesis of protein-laden wastes [19]. However, many industrial and agricultural wastewater also contain appreciable quantities of protein. Treating protein-rich wastewater often results in formation of scum accumulated inside the reactor, and causes sludge washout [19]. This problem has significantly hindered the application of the anaerobic process to the treatment of wastewaters from dairy and slaughter industries. In addition, proteins are degraded slower than carbohydrates under acidogenic conditions [20].

Since both pH and temperature are important factors affecting microorganisms, it is essential to study the effects of pH and temperature on the acidogenesis before an improved acidification process for protein-rich wastewaters can be developed. However, information about the influence of pH and temperature on the acidogenesis of protein-rich wastewaters. The purpose of this study was thus to investigate such an influence on the acidogenesis of gelatin, a model protein. Gelatin is a protein originating from animal connective tissue, and is rich in slaughter and meat-processing wastewaters.

## 2. Materials and methods

### 2.1. Reactor and wastewater

The continuous experiment was conducted in a 2.8-l upflow reactor, which had an internal diameter of 84 mm and a height of 500 mm [5]. The reactor was water-jacketed. A synthetic proteinaceous wastewater was prepared by using gelatin as the sole carbon source. The wastewater also contained balanced nutrient and trace metals following the formulation used in the previous study [21]. Since gelatin contained enough nitrogen, no nitrogen was dosed to the wastewater.

During the start-up, the acidogenic condition was controlled by keeping the pH at $5.5 \pm 0.1$ so that methanogenesis was suppressed and acidogenic bacteria were enriched. Throughout the experiment, the influent chemical COD and hydraulic retention time were kept at 4 g/l and 12 h. The reactor was seeded with the sludge taken from a conventional methanogenic reactor treating a synthetic dairy wastewater for another study [6]. The seed sludge contained 30.2 g volatile suspended solids (VSS), resulting in an initial VSS concentration of 10.8 g/l. The amount of biogas produced was recorded daily using water replacement method. The VSS concentrations of the effluent and in various heights of the reactor were measured weekly. To ensure representative mixed liquor samples were taken, each sampling line was flushed with 5 ml of mixed liquor, before a 30 ml sample was taken for analysis of the VSS concentration in the reactor.

This study was divided into two phases. In phase I, the operational pH level was kept at 5.5, seven runs were conducted to examine the influence of temperature at 20°C, 25°C, 30°C, 37°C, 45°C, 50°C and 55°C; In phase II, the temperature was kept at 37°C, seven runs at pH ranging from 4.0 to 7.0 were undertaken. The pH of the mixed-liquor was controlled by titration using a solution of 4 N NaOH or 4 N HCl. The reactor was operated at each temperature or pH level for 36–43 days to ensure reaching steady state before changing the temperature or pH level to the next level.

### 2.2. Analyses

The contents of $H_2$, $CH_4$, $CO_2$ and $N_2$ in the biogas were analyzed by a gas chromatograph (Hewlett Packard, Model 5890 Series II) equipped with a thermal conductivity detector and a $2\,m \times 2\,mm$ (inside diameter) stainless-steel column packed with Porapak N (80–100 mesh). Injector and detector temperatures were respectively kept at 130°C and 200°C, while column temperature was increased from 90°C to 110°C.

The concentrations of volatile fatty acids (VFA), including acetate, propionate, butyrate, i-butyrate, valerate, i-valerate, caproate and lactate, and alcohols, including methanol, ethanol, propanol and butanol, were determined by a second gas chromatograph of same model equipped with a flame ionization detector and a $10\,m \times 0.53\,mm$ HP-FFAP fused-silica capillary. Samples were filtered through a 0.2 μm membrane, acidified by formic acid, and measured for free acids. The temperatures of the column was initially 70°C for 4 min, followed by 140°C for 3 min, and lastly 170°C for 4 min. The temperature of injector and detector were

both 200°C. Helium was used as the carrier gas at a flow rate of 25 ml/min. The detectable levels were 1 mg/l for individual VFA (from C2 to C7) and 3 mg/l for individual alcohols. The formate concentration was measured by the colorimetric method [22]. Protein was measured by the Lowry–Folin method [23]. Concentration of amino acids was measured using an HPLC (Shimadzu LC-6A) equipped with an UV detector (Shimadzu SPD-6A) and a column (Asahipack GS-220H). However, the repeatability of amino acid analytical results was not as good as expected. The amino acid data were thus used for qualitative analysis to confirm the production of amino acids during the acidogenesis of gelatin, not appropriate for quantitative analysis. Measurements of COD, pH, $NH_3$–N, and VSS were performed according to the Standard Methods [24].

## 3. Results

### 3.1. Temperature effect

Fig. 1 illustrates the effect of temperature on (a) gelatin degradation efficiency, (b) specific gelatin degradation rate, and (c) degree of acidification, whereas Fig. 2 illustrates: (a) partial pressures of $H_2$, $CH_4$ and $CO_2$, (b) fraction of influent COD converted to biogas, and (c) biomass yields at various temperatures.

#### 3.1.1. Gelatin degradation and VFA/alcohol production

As shown in Fig. 1a, gelatin degradation efficiency slightly increased with temperature. In all runs, the gelatin degradation efficiencies exceeded 90%, indicating that gelatin was readily degraded under the tested

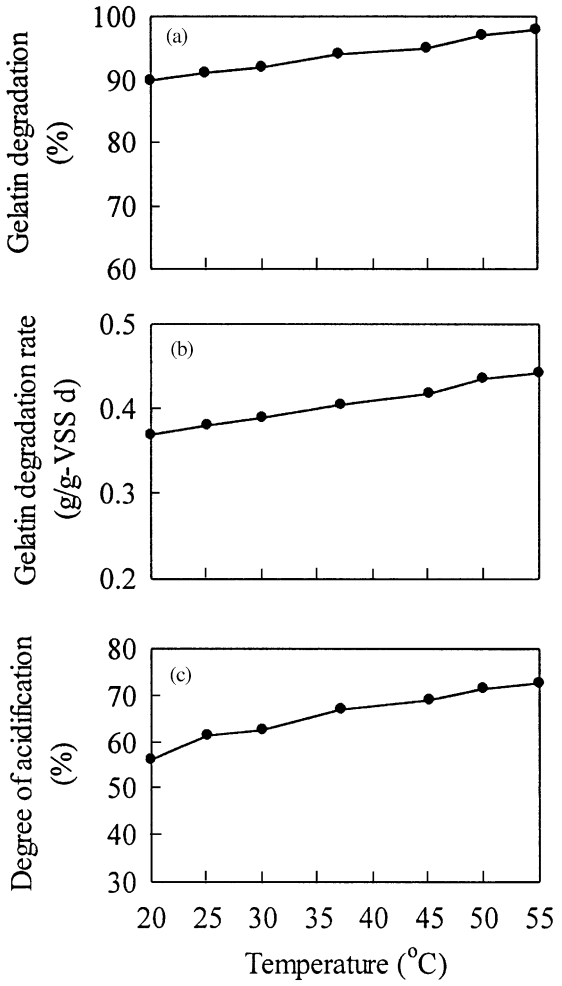


Fig. 1. Performance of the acidogenic reactor at various temperatures: (a) gelatin degradation efficiency; (b) specific gelatin degradation rate; (c) degree of acidification.

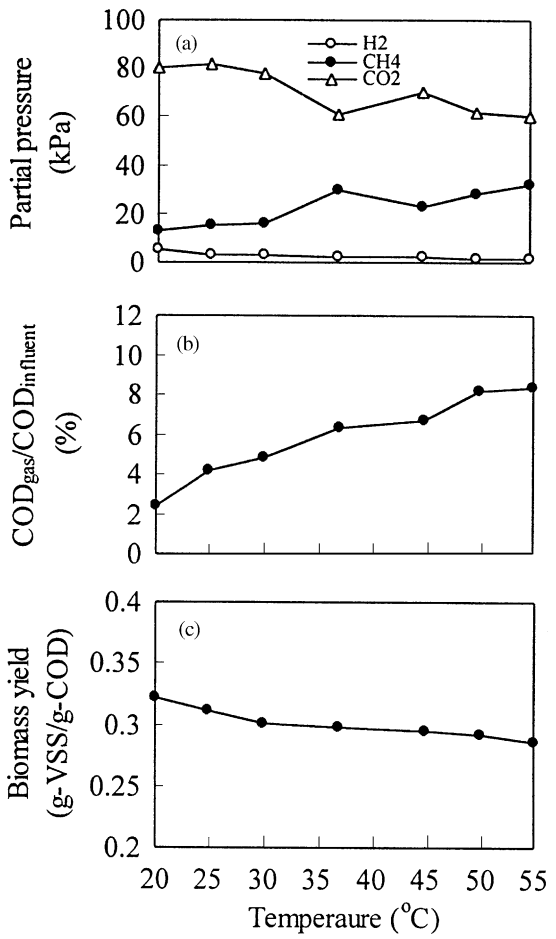


Fig. 2. Performance of the acidogenic reactor at various temperatures: (a) partial pressures of $H_2$, $CH_4$ and $CO_2$; (b) fraction of influent COD converted to biogas; (c) biomass yields.

Table 1
Distribution of VFA and alcohols at various temperatures

| Temp. (°C) | VFA + alcohols (mg/l) | HFr (%) | HAc (%) | HPr (%) | HBu (%) | i-HBu (%) | HVa (%) | i-HVa (%) | HCa (%) | Mol (%) | Eol (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 1294 | 3.2 | 19.8 | 18.2 | 10.3 | 13.4 | 10.7 | 12.3 | 8.3 | 0 | 4.3 |
| 25 | 1376 | 1.3 | 22.9 | 17.3 | 11.6 | 15.5 | 9.7 | 8.7 | 8.2 | 0 | 5.4 |
| 30 | 1392 | 1.4 | 26.1 | 14.6 | 11.6 | 12.7 | 10.9 | 11.0 | 9.1 | 0 | 2.3 |
| 37 | 1470 | 2.0 | 25.4 | 12.3 | 12.5 | 13.4 | 11.8 | 12.5 | 7.3 | 1.3 | 3.2 |
| 45 | 1485 | 0 | 27.3 | 13.9 | 10.7 | 11.5 | 11.9 | 9.6 | 9.3 | 2.2 | 3.3 |
| 50 | 1498 | 0 | 23.8 | 13.3 | 12.8 | 10.4 | 10.6 | 14.5 | 9.1 | 0 | 2.0 |
| 55 | 1531 | 0 | 21.6 | 15.0 | 14.8 | 13.3 | 13.8 | 9.4 | 8.5 | 2.2 | 3.3 |

Note: HFr = formate, HAc = acetate, HPr = propionate, HBu = butyrate, i-HBu = i-butyrate, HVa = valerate, i-HVa = i-valerate, HCa = Caproate, Mol = methanol, Eol = ethanol.

conditions, and that temperature had little influence on the gelatin degradation efficiency. Fig. 1b illustrates that the specific gelatin degradation rate also increased with temperature, from 0.370 g/g-VSS d at 20°C to 0.443 g/g-VSS d at 55°C. As illustrated in Fig. 1c, the degree of acidification slightly increased with temperature, from 56.4% at 20°C to 72.6% at 55°C.

### 3.1.2. Distribution of VFA and alcohols

The percentages (by weight) of individual VFA and alcohols in the effluent are summarized in Table 1. For the main products, acetate was in a range of 20–27% with a mean value of 24%, propionate of 12–18% with a mean value of 15%, butyrate of 10–15% with a mean value of 13% and i-butyrate of 10–15% with a mean value of 12%, respectively. Valerate, i-valerate, caproate and ethanol were present at lower levels, averaging 11%, 11%, 8% and 3%, respectively. Formate and methanol were found in certain runs, whereas propanol and butanol were not detected. These results demonstrate that temperature did not have a significant effect on the product distribution.

### 3.1.3. Biogas and biomass production

The hydrogen partial pressure was measured and illustrated with methane and carbon dioxide partial pressures in Fig. 2a. The hydrogen partial pressure decreased when temperature increased, from 5 kPa at 20°C to 1.5 kPa at 55°C. The methane partial pressure generally has an opposite trend to the hydrogen partial pressure with an exception at 37°C, while carbon dioxide partial pressure ranged from 60 to 82 kPa.

The proportion of influent COD converted to methane/hydrogen, ranging 2.4–8.4%, increased with temperature, as illustrated in Fig. 2b. Compared with a methanogenic reactor, a much smaller proportion of protein was converted to gaseous products in this acidogenic reactor. In the methanogenic reactor, 86–90% of protein was converted to methane [5].

The sludge yield in this reactor as a function of temperature was illustrated in Fig. 2c. The yield increased when temperature dropped, from 0.286 g/VSS/g-COD at 55°C to 0.298 g-VSS/g-COD at 37°C, and to 0.322 g-VSS/g-COD at 20°C. This result concurs with some in literature [8,25]. High temperature results in low biomass yield, which is attributed to increased lysis of cells and greater maintenance requirements at elevated temperatures [13].

### 3.1.4. Mass balance

Tables 2 and 3, respectively summarize the overall COD and effluent COD balances at various temperatures. For the overall COD balance (Table 2), since the effluent COD, biomass-COD and biogas-COD were all measured, it is easy to calculate the overall recovery [(effluent COD + biomass-COD + biogas-COD)/influent COD × 100%]. Over 93.0% of substrate in COD was recovered, except at 55°C, where 91.9% COD recovery was obtained.

The amount of effluent COD should be equal to the sum of COD in (1) VFA measured; (2) alcohols measured; (3) residual gelatin; and (4) unknown metabolites. Among them, the first two could be calculated by summing the COD values of individual acids and alcohols respectively, the third could also be calculated according to the COD equivalent of gelatin (1.00 g gelatin equals to 1.36 g COD). The quantity of the fourth group equals to the effluent COD minus the summary of the COD equivalents of VFA, alcohols and gelatin in the effluent. The difference between the effluent COD and the summary of the COD equivalents of VFA, alcohols and gelatin in the effluent, was the COD equivalent of unknown metabolites. Table 3 lists the COD constituents in 1-l of effluent at various temperatures. The effluent COD recovery generally increased with temperature: 76.6% at 20°C to 91.8% at 55°C. A certain amount of unknown metabolites was present in the effluent. For instance, at 37°C,

Table 2
Overall COD balances for 1-l influent at various temperatures

| Temp. (°C) | Influent ($A$) (g) | Effluent ($B$) (g) | Gas ($C$) (g) | Biomass ($D$) (g) | ($B+C+D$) (g) | Recovery $(B+C+D)/A$ (%) |
|---|---|---|---|---|---|---|
| 20 | 4 | 3.340 | 0.097 | 0.303 | 3.740 | 93.5 |
| 25 | 4 | 3.320 | 0.166 | 0.304 | 3.790 | 94.8 |
| 30 | 4 | 3.204 | 0.190 | 0.328 | 3.722 | 93.0 |
| 37 | 4 | 3.183 | 0.252 | 0.345 | 3.780 | 94.5 |
| 45 | 4 | 3.136 | 0.268 | 0.362 | 3.766 | 94.2 |
| 50 | 4 | 3.104 | 0.328 | 0.372 | 3.803 | 95.1 |
| 55 | 4 | 2.888 | 0.336 | 0.453 | 3.677 | 91.9 |

Table 3
Effluent COD balances for 1-l influent at various temperatures

| Temp. (°C) | Effluent ($A$) (g) | Protein ($B$) (g) | VFA ($C$) (g) | Alcohol ($D$) (g) | $B+C+D$ (g) | Recovery $(B+C+D)/A$ (%) |
|---|---|---|---|---|---|---|
| 20 | 3.340 | 0.397 | 2.074 | 0.086 | 2.557 | 76.6 |
| 25 | 3.320 | 0.360 | 2.175 | 0.115 | 2.650 | 79.8 |
| 30 | 3.204 | 0.319 | 2.276 | 0.046 | 2.641 | 81.5 |
| 37 | 3.183 | 0.231 | 2.307 | 0.120 | 2.658 | 83.5 |
| 45 | 3.136 | 0.200 | 2.365 | 0.125 | 2.690 | 85.8 |
| 50 | 3.104 | 0.120 | 2.489 | 0.051 | 2.660 | 85.8 |
| 55 | 2.888 | 0.080 | 2.443 | 0.127 | 2.650 | 91.8 |

Table 4
Nitrogen balances for 1-l influent at various temperatures

| Temp. (°C) | Influent protein ($A$) (g) | Effluent protein ($B$) (g) | Effluent $NH_3$ ($C$) (g) | Biomass ($D$) (g) | $B+C+D$ (g) | Recovery $(B+C+D)/A$ (%) |
|---|---|---|---|---|---|---|
| 20 | 0.556 | 0.055 | 0.354 | 0.030 | 0.439 | 79.0 |
| 25 | 0.556 | 0.050 | 0.368 | 0.030 | 0.448 | 80.1 |
| 30 | 0.556 | 0.044 | 0.393 | 0.033 | 0.470 | 84.5 |
| 37 | 0.556 | 0.032 | 0.424 | 0.035 | 0.491 | 88.3 |
| 45 | 0.556 | 0.028 | 0.413 | 0.036 | 0.478 | 85.9 |
| 50 | 0.556 | 0.017 | 0.436 | 0.037 | 0.490 | 88.1 |
| 55 | 0.556 | 0.011 | 0.456 | 0.045 | 0.512 | 92.1 |

approximately 0.525 g-COD/l of unknown metabolites were formed.

Ammonium was produced during the acidification of protein. As shown in Table 4, the effluent ammonium concentration also increased with temperature. This is consistent with the variation of protein degradation efficiency as shown in Fig. 1a. The nitrogen recovery increased with temperature, from 79.0% at 20°C to 92.0% at 55°C. This tendency is also consistent with the variation of protein degradation efficiency as shown in Fig. 1a, because nitrogen in amino aids, possible intermediates from gelatin hydrolysis, was not recovered in this nitrogen balance.

*3.1.5. Modeling*

In order to quantify the effect of temperature, the Arrhenius equation was used.

$$r = A \exp\left(\frac{-E_a}{RT}\right), \quad (1)$$

where $r$ is the reaction rate; $A$ is the frequency factor (same unit as $r$); $E_a$ is the apparent activation energy (kcal/mol); $R$ is the gas constant ($=0.001987$ kcal/mol K); and $T$ is the absolute temperature (K).

Since acidogenesis produces not only acids and alcohols in the effluent but also hydrogen and methane in the biogas, the overall acidogenic activity should take

Table 5
Calculation procedures of acidogenic activity for Arrhenius equation

| Temp. (°C) | COD equivalent (g/l) | | | | MLVSS (g/l) | $r$ | Ln $r$ | $1/T(\times 10^{-3})$ |
|---|---|---|---|---|---|---|---|---|
| | VFA ($A$) | Alcohols ($B$) | Biogas ($C$) | $A+B+C$ ($D$) | | (D × 1000)/(HRT × MLVSS) (mg-COD/g-VSS d) | | |
| 20 | 2.074 | 0.086 | 0.097 | 2.257 | 11.103 | 406.6 | 6.01 | 3.41 |
| 25 | 2.175 | 0.115 | 0.166 | 2.456 | 11.104 | 442.4 | 6.09 | 3.36 |
| 30 | 2.276 | 0.046 | 0.190 | 2.512 | 11.128 | 451.5 | 6.11 | 3.30 |
| 37 | 2.307 | 0.120 | 0.252 | 2.679 | 11.145 | 480.8 | 6.18 | 3.23 |
| 45 | 2.365 | 0.125 | 0.268 | 2.758 | 11.162 | 494.2 | 6.20 | 3.14 |
| 50 | 2.489 | 0.051 | 0.328 | 2.868 | 11.172 | 513.4 | 6.24 | 3.10 |
| 55 | 2.443 | 0.127 | 0.336 | 2.906 | 11.253 | 516.5 | 6.25 | 3.05 |

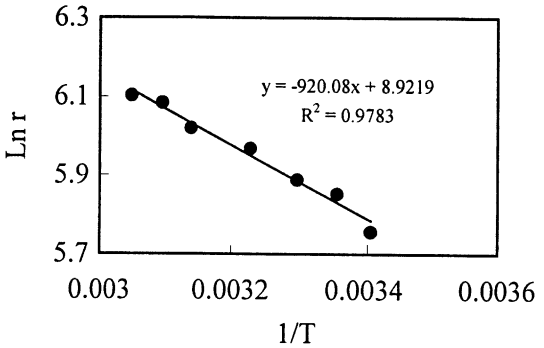


Fig. 3. Arrhenius plot for the overall acidogenic activity

products in both effluent and biogas into account. When $r$ is equal to the overall acidogenic activity, expressed by the total COD equivalent of VFA, alcohols, hydrogen and methane (mg-COD/g-VSS d), the values of $r$ were calculated according the procedures shown in Table 5. The following equation is obtained using regression (Fig. 3):

$$r = 7480 \times \exp\left(\frac{-1.83}{RT}\right). \quad (2)$$

The activation energy of the reaction was found to be 1.83 kcal/mol. The high correlation coefficient value, 0.978, suggests that the temperature affected the acidogenesis of gelatin according to the Arrhenius equation.

### 3.2. pH effect

Fig. 4 illustrates the effect of pH on (a) gelatin degradation efficiency, (b) specific gelatin degradation rate, (c) degree of acidification, whereas Fig. 5 illustrates: (a) partial pressures of $H_2$, $CH_4$ and $CO_2$, (b) fraction of influent COD converted to biogas, and (c) biomass yields at various pH levels.

#### 3.2.1. Gelatin degradation and VFA and alcohol production

Fig. 4a illustrates the gelatin degradation efficiency as a function of pH value. Gelatin was readily converted with an efficiency higher than 94% when pH was higher than 5.5. After pH was lowered to 5.0, the gelatin degradation decreased to 85%; a further decrease of pH resulted in a significant reduction of gelatin degradation efficiency. This shows that the degradation of gelatin was sensitive to pH at low levels. As illustrated in Fig. 4b, the specific gelatin degradation rate also increased with from 0.287 g/g-VSS d at pH 4.0 to 0.406 g/g-VSS d at pH 6.0; further increase in pH resulted in a lower specific gelatin degradation rate: 0.361 g/g-VSS d at pH 6.5 and 0.350 g/g-VSS d at pH 7.0.

Fig. 4c illustrates that the degree of acidification also considerably increased with pH, from 32.0% at pH to 71.6% at pH 6.5; a further increase of pH 4.0–7.0 resulted in a slight reduction of degree of acidification to 66.8%. This suggests sensitive response of the acidogenesis of gelatin at the lower pH range.

#### 3.2.2. Distribution of VFA and alcohols

The concentrations and fractions of individual VFA and alcohols at various pH levels are listed in Table 6. Acetate, propionate, butyrate and i-butyrate were the main products, whereas valerate, i-valerate and caproate were the next important products. Formate, ethanol and methanol were found in low-pH runs, while propanol and butanol were not detected. At pH 4.0, the effluent products were composed of 67% of acetate, propionate, butyrate and i-butyrate by weight in total; at pH 5.0, they comprised 68% of the total VFA and alcohols; after pH increased to 7.0, the fraction of the four main VFA increased to 78%.

Differing from the above temperature-effect experimental results, pH had a substantial influence on the distribution of VFA and alcohols (Table 6). Acetate and butyrate both increased with pH, with percentages of 15% and 10%, respectively, at pH 4.0 to 35% and 22%,

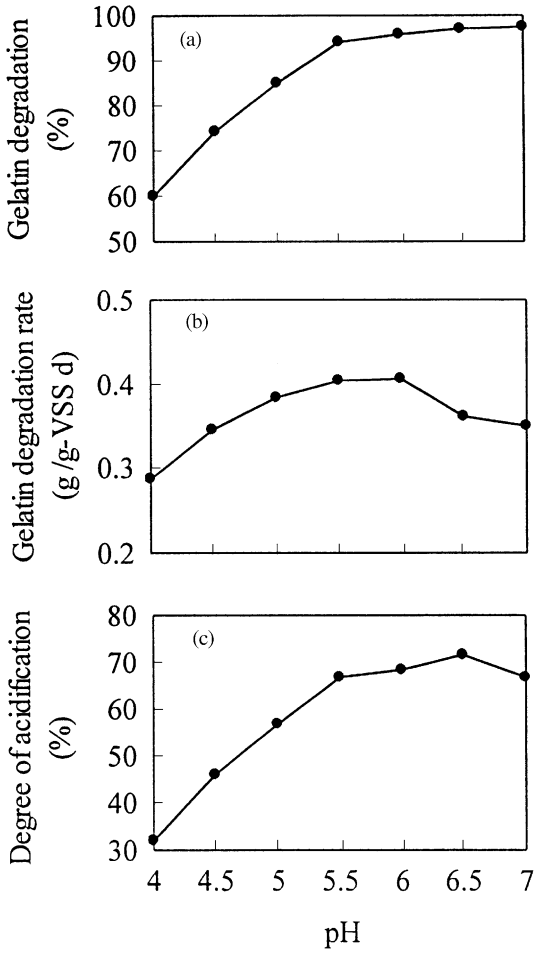


Fig. 4. Performance of the acidogenic reactor at various pH levels: (a) gelatin degradation efficiency; (b) specific gelatin degradation rate; (c) degree of acidification.

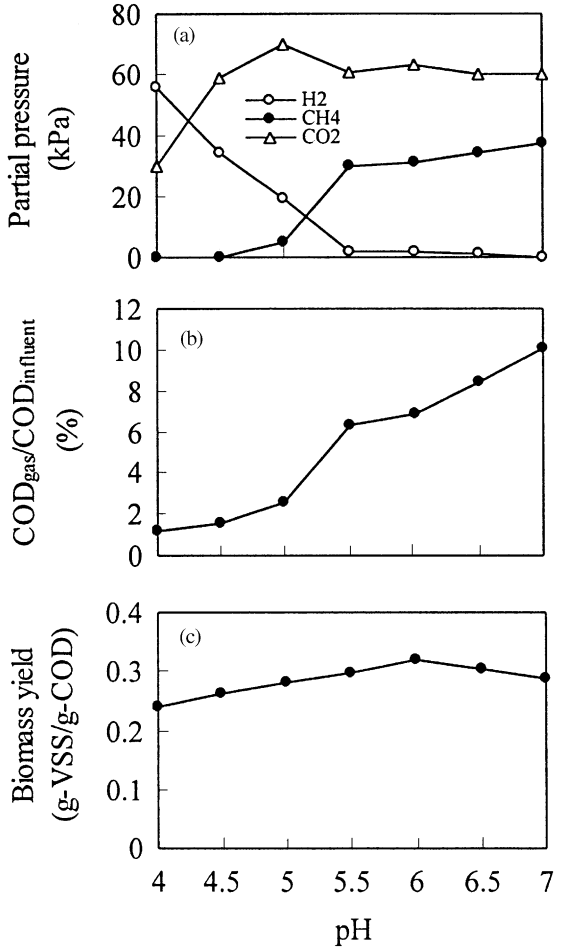


Fig. 5. Performance of the acidogenic reactor at various pH levels: (a) partial pressures of $H_2$, $CH_4$ and $CO_2$; (b) fraction of influent COD converted to biogas; (c) biomass yields.

respectively, at pH 7.0. On the other hand, propionate production was depressed by low pH levels, dropping from 32% at pH 4.0 to 9% at pH 7.0. The variation of pH level had little effect on the production of i-butyrate, valerate, i-valerate and caproate.

### 3.2.3. *Biogas and biomass production*

The gas composition was markedly influenced by pH as shown in Fig. 5a. At pH 4.0, the gas phase was composed of 30% carbon dioxide and 56% hydrogen, but no methane. With pH increase, hydrogen fraction decreased, whereas methane fraction increased. At pH 7.0, methane fraction was 37%, but no hydrogen was detected. The operation beyond pH 5.5 significantly encouraged the activity of the methane-producing bacteria.

As illustrated in Fig. 5b, the production of biogas ($H_2$ and $CH_4$) only accounted for a reduction in the influent COD of 1.2% to 10.1% at pH ranging from 4.0 to 7.0. This fraction increased with pH.

The sludge yield as a function of pH was illustrated in Fig. 5c. The yield increased from 0.239 g-VSS/g-COD at pH 4.0 to 0.321 g-VSS/g-COD at 6.0, the highest sludge yield in this reactor; afterwards it slightly declined to 0.305 g-VSS/g-COD at pH 6.5, and 0.289 g-VSS/g-COD at pH 7.0.

### 3.2.4. *Modeling*

As illustrated in Fig. 6, the overall acidogenic activity, expressed by the total COD equivalents of VFA, alcohols, hydrogen and methane, increased from 216 mg-COD/g-VSS d at pH 4.0 to 395 mg-COD/g-VSS d at pH 6.0, but a further increase in pH resulted in a decrease to 362 mg-COD/g-VSS d at pH 6.5, and 351 mg-COD/g-VSS d at pH 7.0.

Table 6
Distribution of VFA and alcohols at various pH values

| pH | VFA + alcohols (mg/l) | HFr (%) | HAc (%) | HPr (%) | HBu (%) | i-HBu (%) | HVa (%) | i-HVa (%) | HCa (%) | Mol (%) | Eol (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4.0 | 774 | 2.2 | 14.8 | 32.0 | 10.2 | 10.1 | 10.4 | 10.3 | 7.0 | 0.9 | 3.2 |
| 4.5 | 1102 | 1.9 | 17.8 | 27.3 | 10.6 | 10.7 | 8.7 | 9.2 | 6.2 | 1.8 | 4.4 |
| 5.0 | 1311 | 1.3 | 23.1 | 19.6 | 11.6 | 12.7 | 9.9 | 8.7 | 9.4 | 1.4 | 2.3 |
| 5.5 | 1470 | 2.0 | 25.4 | 12.3 | 12.5 | 13.4 | 11.8 | 12.5 | 7.3 | 1.3 | 3.2 |
| 6.0 | 1508 | 0 | 28.3 | 12.9 | 15.7 | 13.0 | 9.9 | 9.6 | 9.1 | 0 | 1.2 |
| 6.5 | 1573 | 0 | 31.9 | 11.3 | 20.8 | 13.4 | 9.4 | 8.5 | 6.1 | 0 | 0 |
| 7.0 | 1560 | 0 | 35.0 | 9.3 | 22.1 | 11.3 | 9.8 | 7.3 | 5.9 | 0 | 0 |

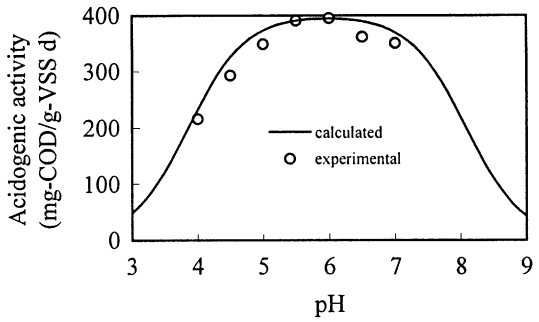


Fig. 6. The overall acidogenic activity as a function of pH.

The bacterial activities may be controlled by the overall enzymatic activity. Since enzymes are made of amino acids, their activities are thus pH dependent, as shown in the following:

$$E^+ \leftrightarrow E + H^+, \tag{3}$$

$$E \leftrightarrow E^- + H^+, \tag{4}$$

where $E$ represents the active enzyme, and $E^+$ and $E^-$ are the less active forms of charge-carrying enzyme [12]. Assuming $K_H$ and $K_{OH}$ are the respective equilibrium constants of reactions (1) and (2), the enzymatic activity can be expressed as:

$$r = \frac{r_{max}}{1 + \left(K_{OH}/[H^+]\right) + \left([H^+]/K_H\right)}, \tag{5}$$

where $r$ is the overall acidogenic activity (mg/g-VSS d), $r_{max}$ is the maximum overall acidogenic activity (mg/g-VSS d). Using the data in Fig. 6, parameters $K_H$, $K_{OH}$, and $r_{max}$ can be determined using nonlinear regression methods as $1.395 \times 10^{-4}$ M, $8.327 \times 10^{-9}$ M, and 399 mg/g-VSS d, respectively. This regression had a correlation coefficient of 0.936. These parameters were used to draw the solid curve shown in Fig. 6. The calculated maximum overall acidogenic activity of 399 mg/g-VSS d was close to the experimental maximum value of 395 mg/g-VSS d, whereas the predicted optimum pH of 5.9 $[(pK_{OH} + pK_H)/2]$ was close to the experimental optimum pH of 6.0.

### 3.2.5. Mass balance

Table 7 lists the effluent COD, COD equivalents of biomass and biogas, and overall COD balances at various pH levels. From 93.0% to 95.0% of substrate in COD was recovered in all the runs. As shown in Table 8, from 80.9% to 89.5% of effluent COD was recovered from the summaries of the COD equivalents of effluent protein, VFA and alcohols. Again, the effluent COD recovery at each pH level was lower than the corresponding overall COD recovery.

Table 9 shows that the effluent ammonium concentration increased with pH. This is consistent with the variation of protein converted at each pH level as illustrated in Fig. 4a. The nitrogen balance, listed in Table 9, also increased with pH from 60.0% at pH 4.0 to 92.1% at pH 7.0.

## 4. Discussion

The comparison between in Tables 2 and 3, as well as Tables 6 and 7, indicates that the overall COD recovery was significantly higher than the effluent COD recovery in both temperature- and pH-effect tests. The low effluent COD recovery suggests that a certain amount of unknown metabolites should be present in the effluent. For instance, at pH 4.0, approximately 680 mg-COD/l of unknown metabolites were formed. A variety of VFA and alcohols were measured in this study, but some possible soluble acidogenic products, such as ketones, aldehydes and glycerol, were not detected. However, amino acids, formed by hydrolysis of gelatin, were found to be the main uncovered COD by analyzing the HPLC data. In the effluent COD balance calculations, amino acids were not included and thus caused the low effluent COD recoveries.

In the acidogenesis of carbohydrate-rich wastewaters, the production of i-butyrate, valerate, i-valerate and caproate was not as significant as those of acetate,

Table 7
Overall COD balances for one liter influent at various pH levels

| pH | Influent ($A$) (g) | Effluent ($B$) (g) | Gas ($C$) (g) | Biomass ($D$) (g) | $B+C+D$ (g) | Recovery $(B+C+D)/A$ (%) |
|---|---|---|---|---|---|---|
| 4.0 | 4 | 3.561 | 0.048 | 0.150 | 3.758 | 94.0 |
| 4.5 | 4 | 3.480 | 0.064 | 0.193 | 3.737 | 93.4 |
| 5.0 | 4 | 3.360 | 0.104 | 0.256 | 3.720 | 93.0 |
| 5.5 | 4 | 3.183 | 0.252 | 0.345 | 3.780 | 94.5 |
| 6.0 | 4 | 3.122 | 0.276 | 0.401 | 3.799 | 95.0 |
| 6.5 | 4 | 3.039 | 0.336 | 0.417 | 3.792 | 94.8 |
| 7.0 | 4 | 2.881 | 0.404 | 0.460 | 3.745 | 93.6 |

Table 8
Effluent COD balances for 1-l influent at various pH levels

| pH | Effluent ($A$) (g) | Protein ($B$) (g) | VFA ($C$) (g) | Alcohol ($D$) (g) | $B+C+D$ (g) | Recovery $(B+C+D)/A$ (%) |
|---|---|---|---|---|---|---|
| 4.0 | 3.561 | 1.601 | 1.229 | 0.053 | 2.882 | 80.9 |
| 4.5 | 3.480 | 1.040 | 1.677 | 0.106 | 2.823 | 81.1 |
| 5.0 | 3.360 | 0.600 | 2.097 | 0.065 | 2.762 | 82.2 |
| 5.5 | 3.183 | 0.231 | 2.307 | 0.120 | 2.658 | 83.5 |
| 6.0 | 3.122 | 0.162 | 2.437 | 0.025 | 2.624 | 84.0 |
| 6.5 | 3.039 | 0.118 | 2.645 | 0 | 2.645 | 87.0 |
| 7.0 | 2.881 | 0.099 | 2.579 | 0 | 2.579 | 89.5 |

Table 9
Nitrogen balances for 1-l influent at various pH levels

| pH | Influent protein ($A$) (g) | Effluent protein ($B$) (g) | Effluent $NH_3$ ($C$) (g) | Biomass ($D$) (g) | $B+C+D$ (g) | Recovery $(B+C+D)/A$ (%) |
|---|---|---|---|---|---|---|
| 4.0 | 0.556 | 0.222 | 0.091 | 0.015 | 0.328 | 60.0 |
| 4.5 | 0.556 | 0.144 | 0.248 | 0.019 | 0.411 | 74.1 |
| 5.0 | 0.556 | 0.083 | 0.358 | 0.026 | 0.467 | 84.0 |
| 5.5 | 0.556 | 0.032 | 0.424 | 0.035 | 0.491 | 88.3 |
| 6.0 | 0.556 | 0.022 | 0.432 | 0.040 | 0.495 | 89.0 |
| 6.5 | 0.556 | 0.016 | 0.448 | 0.042 | 0.506 | 91.0 |
| 7.0 | 0.556 | 0.014 | 0.452 | 0.046 | 0.512 | 92.1 |

propionate and butyrate [16,18,26]. However, in the present study, as shown in Tables 1 and 6, i-butyrate, valerate, i-valerate and caproate were significant composites in the effluent, totally ranging from 34% to 45% of total VFA/alcohols. Furthermore, i-butyrate was of almost same level of butyrate in most runs; valerate and i-valerate were also of same levels, but with less concentrations compared with butyrate or i-butyrate. The production of i-butyrate, i-valerate and caproate were largely associated with the acidification of gelatin. They could be produced either via reductive deamination of individual amino acids or by an oxidation–reduction reaction between amino acid pairs, known as the Stickland reaction [20]. For instance, i-valerate can be formed through Stickland reaction from leucine as a donor, while caproate can formed from leucine through Stickland reaction from leucine as an acceptor [27]. Leucine makes up 5–6% (in mol) of total amino acids in gelatin [20]. The VFA isomers are also possible from the acidification of the aromatic amino acids, such as tyrosine and tryptophan, which are also the constituents in gelatin [27].

Tables 1 and 6 also show that production of alcohols was much lower than that of VFA. Ethanol was the main alcohol produced, but never exceeded 5%; methanol production was observed in certain runs with a less concentration; propanol and butanol were not detected at any runs. In the acidogenic reactors treating

carbohydrate-rich wastewaters, alcohols are often produced, even with a higher percentage than VFA, especially when hydrogen partial pressure is higher than 30 kPa [28]. The present study indicates that VFA, rather than alcohols, are the main products of acidogenesis of protein. This result is consistent with findings of Breure and Andel [19].

It has been reported that there are two optimum temperature regions for anaerobic degradation process: a mesophilic range with an optimum temperature around 35–37°C and a thermophilic range with an optimum temperature around 55–60°C. Beyond these two temperature ranges, e.g. 45–50°C, the degradation efficiency and rate decrease sharply [7]. In most cases, methanogenesis is the rate-limiting step for the overall degradation process, anaerobic reactor should be operated around 37°C or 55°C to ensure methanogens to grow at their optimum temperatures [29]. However, as demonstrated in this study, acidogens are not sensitive to temperature changes as methanogens. Operation at 45°C or 50°C did not result in a lower degree of acidification or VFA/alcohol formation rate compared with at 37°C (Fig. 1c). At the overall ranges tested, temperature had little influence on gelatin degradation and degree of acidification. This might be partially attributed to a temperature compensation effect. This effect means that at decreased temperature, the specific activity of sludge still remains high, despite the significantly lower maximum specific activity [29]. Such a temperature compensation effect has been found for methanogenic reactors with both pure methanogens, such as *Methanosarcina barkeri* [30], and mixed cultures [31,8]. The engineering implication of this observation is that temperature control may not be essential for an acidogenic reactor treating protein-rich wastewaters.

The calculated activation energy, 1.83 kcal/mol, is at the lower end of the range of activation energy range, 1–80 kcal/mol, reported in literature for anaerobic microorganisms [2,29,32]. The low value of the activation energy implies that the acidogenic biomass copes more easily with temperature variations than the methanogenic biomass. Both mesophilic and thermophilic acidification is feasible for gelatin acidification. In general, mesophilic operation should be chosen, because the slightly higher thermophilic rates cannot outweigh the mesophilic advantages of greater stability and especially a much lower energy requirement. Thermophilic acidification should be chosen for hot industrial wastes.

Gelatin degradation was considerably affected by pH, compared with temperature. This result is in agreement with those of Breure and Andel [19], Eastman and Ferguson [4]. When gelatin was acidified in a continuously-stirred tank reactor, the protein degradation increased with pH and the maximum protein degradation occurred at pH 7.0 [19]. A similar trend was observed for the degradation of protein present in primary sludge at pH values between 4.5 and 7.0 [4]. The acidogenesis of gelatin was more sensitive at the lower pH range, which might be due to the limited enzymatic activities for substrate hydrolysis and fermentation under these conditions. The optimum pH range for the acidogenesis can be affected by the characteristics of the wastewater and operating conditions. This study shows that high VFA and alcohols were produced at pH between 6.0 and 6.5, which can be considered as an "optimum pH range". For carbohydrate-rich wastes, the optimum pH range of 5.0–5.5 has been found for glucose [7], lactose [16], and sucrose [33].

The distribution of effluent products was also substantially influenced by pH, and the relative amount of the four main VFA was strongly dependent on pH. Acetate, butyrate, and i-butyrate predominated above pH 6.0, whereas propionate predominated below pH 5.0, the region between pH 5.0 and 6.0 was the transition zone. Significant change in product distribution was also found for glucose acidogenesis [18]. The change in dominant products might be due to either in the metabolism of the same population or a change in the population itself or a combination of these both changes. Furthermore, since the significant changes in product distribution occurred in the narrow pH 4.0–7.0, pH control should be important for the production of a stable effluent composition from an acidogenic reactor.

Products of acidification have to be consumed in the subsequent methanogenic reactor. Thus, operational conditions for an acidogenic reactor should be maintained for more production of products suitable for methanogens. The productions of acetate and butyrate were favored over propionate at high pH values, and vice versa. Since the methanogenesis of propionate is slower compared with acetate and butyrate, propionate was regarded not to be a desirable end-product of the acidogenesis for the subsequent methanogenic reactor [34]. The engineering implication of this result is that high pH level should be selected for low production of propionate and high production of acetate and butyrate.

## 5. Conclusions

Experimental results showed that temperature and pH had a different influence on the acidification of the gelatin-rich wastewater. Gelatin degradation efficiency and rate, degree of acidification, and formation rate of volatile fatty acids and alcohols all slightly increased with temperature. Temperature affected the acidogenesis of gelatin according to the Arrhenius equation with a low activation energy of 1.83 kcal/mol. This might be partially attributed to a temperature compensation effect. The engineering implication of this observation is that temperature control may not be essential for an

acidogenic reactor treating protein-rich wastewaters. Compared with temperature, pH had a more significant effect on the acidogenesis. Gelatin degradation efficiency substantially increased with pH, from 60.0% at pH 4.0 to 97.5% at pH 7.0. The degree of acidification increased from 32.0% at pH 4.0 to 71.6% at pH 6.5, but dropped to 66.8% when pH increased to 7.0. The optimum pH for the overall acidogenic activity was found to be 6.0, close to 5.9, the optimum pH calculated using a semi-empirical model. Operation at pH of 4.0–5.0 favored the production of propionate, hydrogen, whereas the operation at pH 6.0–7.0 encouraged the production of acetate, butyrate, and i-butyrate. Since the significant changes in product distribution occurred in the narrow pH range of 4.0–7.0, pH control should be important for the production of a stable effluent composition from an acidogenic reactor treating protein-rich wastewaters.

## Acknowledgements

The authors wish to thank the Natural Science Foundation of China (Grant No. 20122203) and the China Academy of Sciences for the partial financial support of this study.

## References

[1] Pohland FG, Ghosh S. Developments in anaerobic stabilization of organic wastes, the two-phase concept. Environ Technol 1971;1:255–66.
[2] Dinopoulou G, Rudd T, Lester JN. Anaerobic acidogenesis of a complex wastewater: 1. The influence of operational parameters on reactor performance. Biotechnol Bioeng 1988;31:958–68.
[3] Pipyn P, Verstraete W. Lactate and ethanol as intermediates in two-phase anaerobic digestion. Biotechnol Bioeng 1981;23:1145–54.
[4] Eastman JA, Ferguson JF. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. J Water Pollut Control Fed 1981;53:352–66.
[5] Yu HQ, Fang HHP. Thermophilic acidification of dairy wastewater. Appl Micorbiol Biotechnol 2000;54:439–44.
[6] Yu HQ, Fang HHP. Acidification of mid- and high-strength dairy wastewaters. Water Res 2001;35:3697–705.
[7] Zoetemeyer RJ, Arnoldy P, Cohen A. Influence of temperature on the anaerobic acidification of glucose in a mixed culture forming part of a two-stage digestion process. Water Res 1982;16:313–21.
[8] Lin CY, Noike T, Sato K, Matsumoto J. Temperature characteristics of the methanogenesis process in anaerobic digestion. Water Sci Technol 1987;19:299–310.
[9] Rebac S, van Lier JB, Janssen MGJ, Dekkers F, Swinkels KTM, Lettinga G. High-rate anaerobic treatment of malting wastewater in a pilot-scale EGSB system under psychrophilic conditions. J Chem Tech Biotechnol 1997; 68:135–46.
[10] Conrad R, Wetter B. Influence of temperature on energetics of hydrogen metabolism in homoacetogenic, methanogenic, and other anaerobic bacteria. Arch Microbiol 1990;155:94–8.
[11] Horiuchi J, Shimizu T, Kanno T, Kobayashi M. Dynamic behavior in response to pH shift during anaerobic acidogenesis with a chemstat culture. Biotech Tech 1999;13:155–7.
[12] Baily JE, Ollis DF. Biochemical engineering fundamentals, 2nd ed.. New York: McGraw-Hill, 1986.
[13] Gottschalk G. Bacterial metabolism, 2nd ed.. New York: Springer-Verlag, 1986.
[14] Hsu ST, Yang ST. Propionic acid fermentation of lactose by *Propionibacterium acidipropionici*: effects of pH. Biotechnol Bioeng 1991;38:571–8.
[15] Joubert WA, Britz TJ. The effect of pH and temperature manipulation on metabolite composition during acidogenesis in a hybrid anaerobic digester. Appl Microbiol Biotechnol 1986;24:253–8.
[16] Kissalita WS, Lo KV, Pinder KL. Acidogenic fermentation of lactose. Biotechnol Bioeng 1987;30:88–95.
[17] Tang IC, Okos MR, Yang ST. Effects of pH and acetic acid on homoacetic fermentation of lactose by *Clostridium formicoaceticum*. Biotechnol Bioeng 1989;34:1063–74.
[18] Zoetemeyer RJ, van den Heuvel JC, Cohen A. pH influence on acidogenic dissimilation of glucose in an anaerobic digestor. Water Res 1982;16:303–11.
[19] Breure AM, van Andel JG. Hydrolysis and acidogenic fermentation of a protein, gelatin, in an anaerobic continuous culture. Appl Microbiol Biotechnol 1984;20: 45–9.
[20] McInerney MJ. Anaerobic hydrolysis and fermentation of fats and proteins. In: Zehnder AJB, editor. Biology of anaerobic microorganisms. New York: Wiley, 1988. p. 373–416.
[21] Fang HHP, Yu HQ. The effect of hydraulic retention time on acidogenesis of simulated dairy wastewater. J Environ Eng 2000;126:1245–9.
[22] Lang E, Lang H. Spezifische farbreaktion zum directen nachweis der ameisensure. Z Anal Chem 1972; 260:8–10.
[23] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[24] APHA, AWWA and WEF. Standard methods for the examination of water and wastewater, 18th ed. Washington, DC: American Public Health Association, 1992.
[25] van Lier JB, Rebac S, Lens P, van Bijnen F, Elferink OSJW, Stams AJM, Lettinga G. Anaerobic treatment of partly acidified wastewater in a two-stage expanded granular sludge bed (EGSB) system at 8°C. Water Sci Technol 1997;36:317–24.
[26] Cohen A, Breure AM, van Andel JG, van Deursen A. Anaerobic digestion of glucose with separated acid production and methane formation. Water Res 1979;13: 571–80.
[27] Ramsay IR. Modelling and control of high rate anaerobic wastewater treatment systems. Ph.D. Thesis, University of Queensland, Brisbane, Australia, 1997. p. 11–28.
[28] Jones DT, Woods DR. Acetone-butanol fermentation revisited. Microbiol Rev 1986;50:484–524.

[29] Pavlostathis SG, Giraldo-Gomez E. Kinetics of anaerobic treatment: a critical review. Crit Rev Environ Sci Technol 1991;21:411–90.

[30] Westermann P, Ahring BK, Mah RA. Temperature compensation in *Methanosarcina barkeri* by modulation of hydrogen and acetate affinity. Appl Environ Microbiol 1989;55:1262–6.

[31] Kettunen RH, Rintala JA. The effect of low temperature (5–29°C) and adaptation on the methanogenic activity of biomass. Appl Microbiol Biotechnol 1997;48: 570–6.

[32] Veeken A, Hamelers B. Effect of temperature on hydrolysis rates of selected biowaste components. Biores Technol 1999;69:249–54.

[33] Zoetemeyer RJ, Borgerding PH, van den Heuvel JC, Cohen A, Boelhouwer C. Pilot scale anaerobic acidification of waste water containing sucrose and lactate. Biomass 1982;2:201–11.

[34] Cohen A, van Gemert JM, Zoetemeyer RJ, Breure AM. Main characteristics and stoichiometric aspects of acidogenesis of soluble carbohydrate containing wastewater. Proc Biochem 1984;19:228–32.