Mesophilic acidification of gelatinaceous wastewater

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Abstract

The influence of hydraulic retention time (HRT) and gelatin concentration on the acidification of gelatinaceous wastewater in an upflow anaerobic reactor was investigated at pH 5.5 and 37 °C. The degree of gelatin degradation increased with the HRT, from 84.1% at 4 h to 89.6% at 24 h, but decreased with the increase of the gelatin concentration in the influent from 65.2% at 2 g-COD l^{-1} to 51.9% at 30 g-COD l^{-1}. The degradation of gelatin followed the Monod kinetics with a maximum rate of 1.10 g (g-VSS·d) $^{-1}$ and a half-rate constant of 0.23 g l^{-1}. The overall production rate of VFA and alcohols decreased with HRT, from 0.33 g (g-VSS·d) $^{-1}$ at 4 h to 0.15 g (g-VSS·d) $^{-1}$ at 24 h, but increased with gelatin concentration in the influent, from 0.10 g (g-VSS·d) $^{-1}$ at 4 g-COD l^{-1} to 0.58 g (g-VSS·d) $^{-1}$ at 30 g-COD l^{-1}. The key acidification products were acetate, propionate and butyrate, plus *i*-butyrate, valerate, *i*-valerate, caproate and ethanol in smaller quantities. Formate, methanol, propanol and butanol were found only in certain runs. Only 4.5–7.8% of COD in wastewater was converted to hydrogen and methane. The sludge yield was estimated as 0.320 ± 0.014 g-VSS (g-COD) $^{-1}$. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Acidification; Gelatin; Hydraulic retention time; Protein; Volatile fatty acid

1. Introduction

Since the introduction of the anaerobic filter (Young and McCarty, 1969), anaerobic processes have become viable for the treatment of high-strength industrial wastewater. A number of high-rate processes have been successfully commercialized in the past decade (Lettinga, 1995; Fang and Liu, 2000). Most of the full-scale anaerobic reactors were designed for treating wastewaters from the sugar, starch, and brewery industries, the main pollutants of which are carbohydrates. However, many industrial and agricultural wastewaters also contain appreciable quantities of protein. Treating protein-rich

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wastewater often results in the formation of scum, which accumulates inside the reactor, and causes sludge washout (Lettinga and Hulshoff Pol, 1991). 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 more slowly than carbohydrates. Anaerobic degradation of proteins is a complex process involving various groups of microorganisms. Proteins are first hydrolyzed and degraded by proteolytic enzymes into peptides and individual amino acids (McInerney, 1988). The peptides and amino acids are then acidified into volatile fatty acids (VFA), hydrogen, ammonium, and reduced sulfur. The VFA are further converted by acetogens into acetate and H_2/CO_2, both of which are lastly converted to methane by methanogens. The initial hydrolysis is the rate-limiting step in protein degradation (Gujer and Zehnder, 1983), and the overall degradation rate is slow (Harper and Pohland, 1986; McInerney, 1988).

To improve the process efficiency, a two-stage anaerobic process (Pohland and Ghosh, 1971) has been developed for the treatment of protein-rich wastewaters. In such a process, hydrolysis and acidification are carried out in the first reactor, the effluent of which is subsequently further treated in the second reactor for acetogenesis and methane production. Gelatin, a protein rich in animal connective tissue, is the main constituent in slaughterhouse and meat-processing wastewaters. Acidogenesis of gelatin in a continuously stirred tank reactor (CSTR) has been studied (Breure and van Andel, 1984). Results show that pH is crucial to the acidogenesis efficiency and product distribution. Furthermore, gelatin hydrolysis was found to be suppressed by the presence of glucose, because the latter is the preferred substrate for the hydrolytic bacteria (Breure et al., 1986). However, little information is available on the acidogenesis of gelatin in wastewaters in continuously fed upflow reactors. This work was thus conducted to investigate the effects of hydraulic retention time (HRT) and gelatin concentration in wastewater on the acidification of gelatin in a mesophilic upflow anaerobic reactor.

2. Materials and methods

2.1. Reactor and wastewater

The continuous experiment was conducted for 412 days in a 2.8 l upflow reactor (84 mm inside diameter and 500 mm height) used previously for methanogenic anaerobic degradation (Fang et al., 1994). The reactor was water-jacketed and operated at 37 °C. Synthetic wastewater was prepared by using gelatin as the sole carbon source, plus balanced nutrient and trace metals, following the formulation used in the previous study (Fang et al., 1994). Throughout the experiment, the pH of the mixed liquor was kept at 5.5 ± 0.1 in order to suppress methanogenesis. The reactor was seeded with the sludge taken from a conventional methanogenic reactor treating dairy wastewater for a previous study (Fang and Chung, 1999). The initial sludge concentration was 10.8 $g l^{-1}$ of volatile suspended solids (VSS). The sludge retention was controlled at 15 days, by wasting one-fifteenth of the sludge in the reactor daily.

This study was conducted in two phases: in phase I, the influent COD (chemical oxygen demand) was kept at 4 $g l^{-1}$, while the HRT was decreased stepwise from the initial 24 h to 16, 12, 8, 6, and lastly 4 h; in phase II, the HRT was kept at 12 h, while the wastewater COD was increased stepwise from 2 $g l^{-1}$ to 9, 15, 20, and lastly 30 $g l^{-1}$. The reactor was operated at each HRT or COD level for 33–44 days to ensure reaching steady state before changing the HRT or COD to the next level.

2.2. Analysis

The production of biogas was measured daily by the water displacement method. 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 × 2 mm (inside diameter) stainless-steel column packed with Porapak N (80–100 mesh). Injector and detector temperatures were kept at 130 and 200 °C, respectively, while column temperature was increased from 90 to 110 °C.

The concentrations of individual acidogenic products in the effluent were determined by a second gas chromatograph of same model equipped with a flame ionization detector and a 10 m × 0.53 mm HP-FFAP fused-silica capillary. The products were mostly VFA, including acetate, propionate, butyrate, *i*-butyrate, valerate, *i*-valerate, caproate and lactate, and alcohols, including methanol, ethanol, propanol and butanol. Effluent samples were filtered through a 0.2 μm membrane, acidified by formic acid, and measured for free acids. The temperature 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 temperatures of injector and detector were both 200 °C. Helium was used as the carrier gas at a flow rate of 25 ml min^{-1}. The detectable levels were 1 mg l^{-1} for individual VFA (from C2 to C7) and 3 mg l^{-1} for individual alcohols. The formate concentration was measured by the colorimetric method (Lang and Lang, 1972). Protein was measured by the Lowry–Folin method (Lowry et al., 1951).

Measurements of COD, pH, NH$_3$-N, and VSS were performed according to the Standard Methods (APHA, AWWA and WEF, 1992).

3. Results and discussion

3.1. Overall performance

During anaerobic degradation, gelatin was converted into VFA and alcohols in the effluent, plus H$_2$/CO$_2$ in the biogas and biomass. Fig. 1 illustrates: (a) HRT; (b) gelatin concentration in influent; (c) gelatin concentration in effluent; (d) total VFA and alcohol concentration in effluent; and (e) biogas production rate throughout this study. Results in Fig. 1c illustrate that over 84.1% of 4 g-COD l^{-1} of gelatin in the wastewater was degraded for HRT as low as 4 h, and over 89.6% of gelatin up to 30 g-COD l^{-1} in wastewater was degraded at 12 h of HRT. Fig. 1d illustrates that the effluent VFA/alcohol concentration increased with both HRT and influent gelatin concentration. On treating wastewater containing 4 g-COD l^{-1} of gelatin, the effluent VFA/alcohol

concentration decreased from 1.67 g l^{-1} at 24 h of HRT to 0.96 g l^{-1} at 4 h of HRT; at 12 h of HRT, the effluent VFA/alcohol concentration increased from 0.76 g l^{-1} at 2 g l^{-1} of gelatin in wastewater to 7.76 g l^{-1} at 30 g-COD l^{-1}.

Fig. 1e illustrates that the total biogas production rate increased with gelatin concentration in wastewater, but decreased when HRT increased. Compared to the conventional methanogenic process, the acidogenic process produces a much lower amount of gas due to the suppression of methane production. For example, in a previous
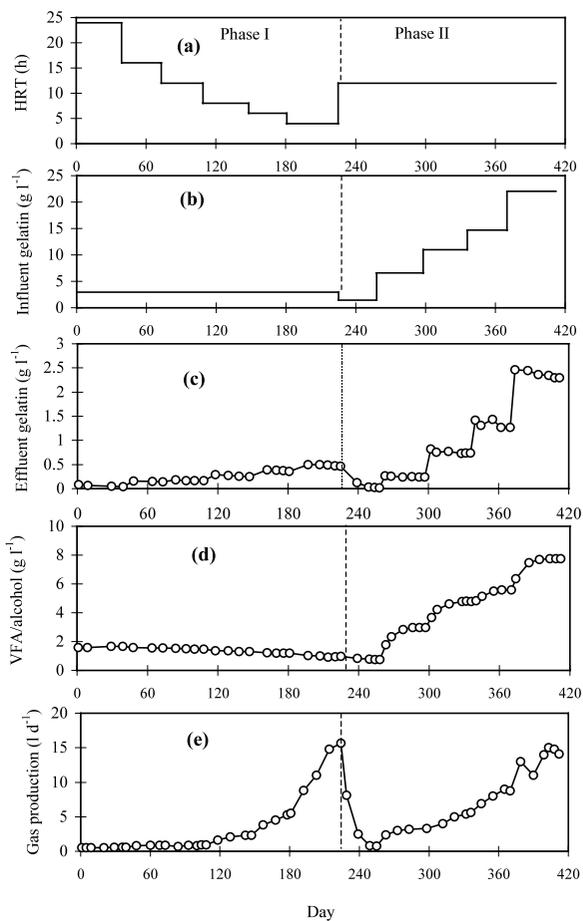

Fig. 1. Operational conditions and performance of the acidification reactor: (a) HRT; (b) gelatin concentration in influent; (c) gelatin concentration in effluent; (d) total VFA/alcohol concentration in effluent; and (e) biogas production rate.
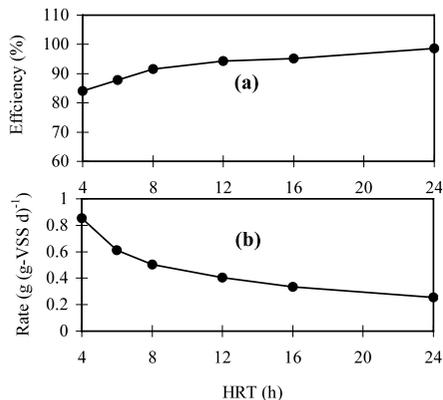
Fig. 2. Effect of HRT on: (a) degradation efficiency; and (b) specific gelatin degradation rate.

study on mesophilic methanogenesis of proteinaceous wastewater using the same reactor (Fang et al., 1994), the gas production reached 16.70 l (l·d)$^{-1}$ at 10 h of HRT and 10 g l^{-1} of protein in wastewater, whereas the gas production of this acidogenic reactor was only 5.60 l (l·d)$^{-1}$ at 12 h of HRT and 15 g l^{-1} of protein in wastewater.

3.2. Gelatin degradation

In anaerobic degradation, gelatin is first hydrolyzed, and the products of this reaction are further fermented into acids and alcohols. It was found in this study that, gelatin degradation efficiency increased with HRT, from 84.1% at 4 h to 94.3% at 24 h (Fig. 2a), but decreased with the increase of gelatin concentration in wastewater, from 98.9% at 2 g-COD l^{-1} to 89.6% at 30 g-COD l^{-1} (Fig. 3a). The degradation efficiency ranging from 84.1 to 98.6% indicates that gelatin was easily hydrolyzed under acidogenic conditions.

Fig. 2b illustrates that the specific gelatin degradation rate treating 4 g-COD l^{-1} of gelatin decreased with the increase of HRT, from 0.85 g (g-VSS·d)$^{-1}$ at 4 h to 0.25 g (g-VSS·d)$^{-1}$ at 24 h. Fig. 3b, on the other hand, illustrates that the specific gelatin degradation rate at 12 h of HRT increased with gelatin concentration in wastewater, from 0.19 g (g-VSS·d)$^{-1}$ at 2 g-COD l^{-1} to 1.49 g (g-VSS·d)$^{-1}$ at 30 g-COD l^{-1}. The rates in

this study were considerably lower than the value 3.653 g (g-VSS·d)$^{-1}$ found in another study treating gelatin at pH 5.3, 4 h of HRT and 3.04 g l^{-1} of gelatin in a complete-mix reactor (Breure and van Andel, 1984). The lower rates of this study are likely due to the lack of mixing in the upflow reactor. However, the rates found in this study were still considerably higher than the protein degradation rate of 0.172 g (g-VSS·d)$^{-1}$ in the acidogenesis of a dairy wastewater using a similar upflow reactor (Yu and Fang, 2000). This is due to the absence of carbohydrate in the wastewater of this study. Carbohydrates tend to suppress the synthesis of exopeptidases, a group of enzymes facilitating protein hydrolysis (McInerney, 1988); their presence in dairy wastewater lowers the protein degradation rate.

The results of Figs. 2a and 3a show that gelatin degradation efficiency had a near-linear relationship with both HRT and gelatin concentration in wastewater. Based on linear regression analysis, it can be expressed as follows:

$$\text{efficiency} = 2.37 \times \text{HRT} - 0.63 \times S_i + 47.05$$

$$r = 0.947 \tag{1}$$

where S_i represents the gelatin concentration in wastewater. Eq. (1) shows that gelatin degradation is more dependent on HRT (h) than S_i (g l^{-1}). Therefore, longer HRT is required to increase the gelatin degradation efficiency.
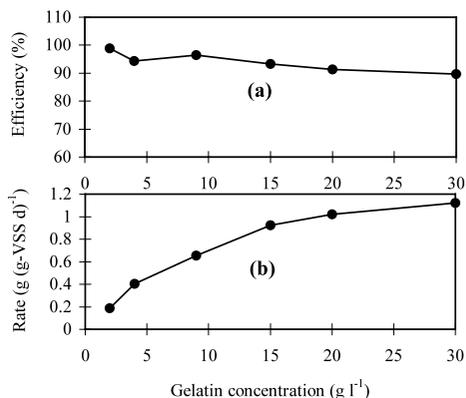

Fig. 3. Effect of gelatin concentration in wastewater on: (a) degradation efficiency; and (b) specific gelatin degradation rate.
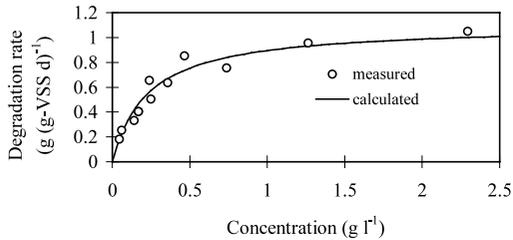
Fig. 4. Gelatin degradation kinetics.

3.3. Gelatin degradation kinetics

At steady state, the mass balance of gelatin in the acidogenic reactor may be expressed, assuming that the effluent and waste gelatin concentrations are equal, as follows:

$$RM = QS_i - (Q - Q_w)S_e - Q_w S_e \qquad (2)$$

or

$$R = Q(S_i - S_e)/M \qquad (3)$$

where Q and Q_w are the flow rates $(l\,d^{-1})$ of effluent and waste sludge, respectively; S_i and S_e are the influent and effluent gelatin concentrations $(g\,l^{-1})$, respectively; R is the specific gelatin degradation rate $(g\,(g\text{-VSS}\cdot d)^{-1})$; M is the total biomass (g-VSS). The gelatin degradation rate may follow the Monod model as:

$$R = R_{max}S_e/(K_s + S_e) \qquad (4)$$

where R_{max} is the maximum gelatin degradation rate $(g\,(g\text{-VSS}\cdot d)^{-1})$; S_e is the effluent gelatin concentration $(g\,l^{-1})$; and K_s is the half-rate concentration $(g\,l^{-1})$. To best-fit the degradation data, R_{max} and K_s were determined as:

$$R_{max} = 1.10\ g\,(g\text{-VSS}\cdot d)^{-1}$$

$$K_s = 0.23\ g\,l^{-1}$$

Fig. 4 illustrates that the degradation rates calculated from Eq. (4) using the aforementioned parameters fit the measured data satisfactorily. In a recent study on acidification of lactose using a similar upflow reactor and operational conditions (Fang and Yu, 2001), the R_{max} and K values were found to be 4.39 g $(g\text{-VSS}\cdot d)^{-1}$ and 1.97 g l^{-1}, respectively. This suggests that acidification rate of lactose was significantly higher than that of

gelatin. In another study on acidification of gelatin using a complete-mix reactor (Breure and van Andel, 1984), the R_{max} and K values were estimated as 0.29 g $(g\text{-VSS}\cdot d)^{-1}$ and 3.43 g l^{-1}, respectively. The R_{max} value is substantially lower than that in the present study. This could be due to the differences in reactor configuration and operational conditions. In the upflow reactor of the present study, the gelatin concentration decreased as the wastewater flowed upward, and the gelatin concentration in the effluent was substantially lower than the average concentration in the reactor; but in a complete-mix reactor, the gelatin concentration in the effluent equals that in the mixed liquor.

3.4. Production and distribution of VFA and alcohols

Acidification of gelatin produced not just VFA but also alcohols. Fig. 5a illustrates that the overall production rate of VFA and alcohols decreased with HRT, from 0.33 g $(g\text{-VSS}\cdot d)^{-1}$ at 4 h to 0.15 g $(g\text{-VSS}\cdot d)^{-1}$ at 24 h. Fig. 5b, on the other hand, illustrates that the production rate increased with gelatin concentration in wastewater, from 0.10 g $(g\text{-VSS}\cdot d)^{-1}$ at 4 g-COD l^{-1} to 0.58 g $(g\text{-VSS}\cdot d)^{-1}$ at 30 g-COD l^{-1}.

Tables 1 and 2 summarize, respectively, the data of total VFA/alcohols concentration in the
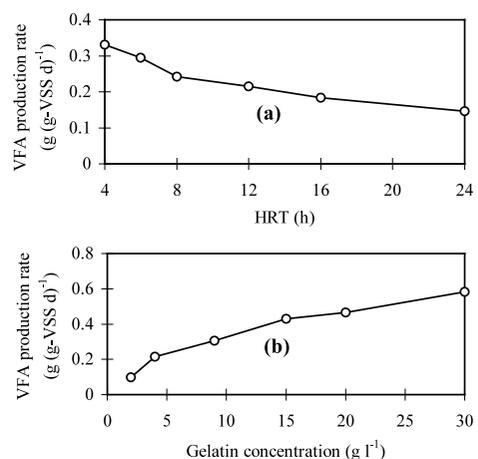

Fig. 5. Specific VFA/alcohol production rate at various: (a) HRTs; and (b) gelatin concentrations.

Table 1
Distribution of VFA and alcohols in the effluent at various HRTs

HRT (h)	VFA/alcohols (mg l^{-1})	HFr (%)	HAc (%)	HPr (%)	HBu (%)	i-HBu (%)	HVa (%)	i-HVa (%)	HCa (%)	Mol (%)	Eol (%)	Pol (%)	Bol (%)
4	960 ± 29	1.9 ± 0.1	17.8 ± 1.1	20.3 ± 1.0	9.2 ± 0.1	10.6 ± 0.1	13.2 ± 0.2	14.7 ± 0.2	7.0 ± 0.1	1.3 ± 0.0	3.8 ± 0.1	0	0
6	1193 ± 51	2.7 ± 0.1	22.7 ± 1.2	16.3 ± 0.5	11.2 ± 0.1	10.7 ± 0.2	9.1 ± 0.1	11.9 ± 0.1	10.3 ± 0.2	0	4.6 ± 0.1	0	0
8	1292 ± 73	0	23.3 ± 0.9	13.5 ± 0.2	12.5 ± 0.2	14.8 ± 0.3	11.3 ± 0.1	12.6 ± 0.2	7.9 ± 0.1	1.9 ± 0.1	3.3 ± 0.1	0	0
12	1470 ± 82	2.1 ± 0.1	25.2 ± 1.0	12.4 ± 0.3	12.1 ± 0.1	13.8 ± 0.1	11.2 ± 0.2	12.7 ± 0.2	7.2 ± 0.1	1.1 ± 0.0	2.5 ± 0.1	0	0
16	1547 ± 80	0	29.9 ± 1.6	11.9 ± 0.4	12.0 ± 0.1	14.5 ± 0.1	10.6 ± 0.1	10.1 ± 0.1	8.8 ± 0.1	0	2.9 ± 0.1	0	0
24	1670 ± 105	0	35.1 ± 1.4	11.2 ± 0.5	11.4 ± 0.1	14.7 ± 0.2	7.7 ± 0.1	10.8 ± 0.1	6.4 ± 0.1	0	4.7 ± 0.1	0	0

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; Pol = propanol; Bol = butanol.

Table 2
Distribution of VFA and alcohols in the effluent at various gelatin concentrations in wastewater

Gelatin in wastewater (g-COD l^{-1})	VFA/alcohols (mg l^{-1})	HFr (%)	HAc (%)	HPr (%)	HBu (%)	i-HBu (%)	HVa (%)	i-HVa	HCa (%)	Mol (%)	Eol (%)	Pol (%)	Bol (%)
2	761 ± 33	3.6 ± 0.1	33.7 ± 0.9	10.6 ± 0.1	15.0 ± 0.2	11.8 ± 0.1	8.5 ± 0.1	7.5 ± 0.1	7.7 ± 0.1	0	2.1 ± 0.0	0	0
4	1470 ± 82	2.1 ± 0.1	25.2 ± 1.0	12.4 ± 0.3	12.1 ± 0.1	13.8 ± 0.1	11.2 ± 0.2	12.7 ± 0.2	7.2 ± 0.1	1.1 ± 0.0	2.5 ± 0.1	0	0
9	2984 ± 113	0	26.3 ± 0.5	13.7 ± 0.2	11.8 ± 0.1	12.7 ± 0.2	12.0 ± 0.1	13.2 ± 0.2	7.2 ± 0.1	0	4.2 ± 0.1	0	0
15	4782 ± 201	3.3 ± 0.1	22.4 ± 0.6	12.1 ± 0.1	10.2 ± 0.1	11.5 ± 0.1	11.3 ± 0.2	12.1 ± 0.1	11.0 ± 0.2	2.3 ± 0.1	3.9 ± 0.1	0.9 ± 0.0	0
20	5586 ± 327	1.2 ± 0.1	21.9 ± 0.7	12.9 ± 0.2	9.4 ± 0.1	10.4 ± 0.1	10.8 ± 0.1	10.4 ± 0.1	8.2 ± 0.1	2.1 ± 0.0	5.8 ± 0.1	4.7 ± 0.1	2.3 ± 0.1
30	7758 ± 388	0	20.1 ± 0.6	12.3 ± 0.1	9.0 ± 0.1	10.3 ± 0.1	12.9 ± 0.2	13.3 ± 0.2	7.9 ± 0.1	0	6.1 ± 0.2	4.9 ± 0.1	4.1 ± 0.1

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; Pol = propanol; Bol = butanol.
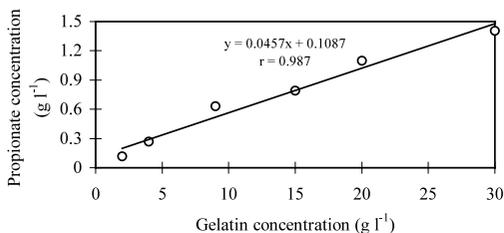
Fig. 6. Effluent propionate concentration at various gelatin concentrations.

effluent plus percentages of individual VFA and alcohols at various HRT and gelatin concentrations in wastewater. It shows that acetate was the main acidification product, accounting for 17.8–35.1% of total VFA/alcohols, with an average of 27.4%. The next important VFAs were propionate, butyrate and *i*-butyrate ranging from 10.6 to 20.3%, 9.0 to 15.0% and 10.6 to 14.7%, respectively. Valerate, *i*-valerate and caproate and ethanol were found at lower percentages. Formate, methanol, propanol and butanol were found only in certain runs, at less than 5%, whereas lactate was not detected in all runs.

The product distribution was dependent upon the operational parameters. For instance, the acetate concentration increased with both HRT and gelatin concentration in wastewater; but by contrast, the concentration of propionate in the effluent was independent of HRT, but increased with the gelatin concentration, as illustrated in Fig. 6. Since the methanogenesis of propionate is slower compared with acetate and butyrate, propionate was an undesirable intermediate product in the two-stage anaerobic process (Cohen et al., 1984; Harper and Pohland, 1986). The engineering implication of this observation is that low

production of propionate might only be achieved when less dilute substrate concentration is used.

Tables 1 and 2 also show that production of alcohols was much lower than that of VFA. Ethanol was the main alcohol produced, accounting for only 2–6% of total VFA/alcohols. Methanol was produced only in certain runs, and never exceeded 2.5%. Propanol and butanol were not detected when gelatin concentration was at 4–8 g-COD l^{-1}. However, at 15 g-COD l^{-1} and higher concentrations, propanol was produced. The proportions of propanol and butanol increased with gelatin concentration, but were independent of HRT. In the acidogenic reactors treating carbohydrate-rich wastewaters, alcohol concentrations could sometimes exceed those of VFA, especially when hydrogen partial pressure is higher than 30 kPa (Dabrock et al., 1992; Jones and Woods, 1986). However, results of this study show that acidification of gelatin produced mostly VFA and substantially lower amount of alcohols, as observed previously by others (Breure and van Andel, 1984; Jain and Zeikus, 1989).

3.5. Ammonium production

Ammonium was produced during the acidification of protein. As shown in Table 3, the effluent ammonium concentration increased with gelatin concentration, but decreased with the increase of HRT, as expected from the gelatin degradation patterns. The ammonium concentration in the effluent ranged from 0.23 to 2.50 g l^{-1}, which is substantially below the threshold level of 5 g l^{-1} above which it becomes toxic to acidogens (Breure and van Andel, 1984; Koster and Lettinga, 1988). Thus, the production of ammonium

Table 3
Effluent ammonia concentrations at various HRTs and gelatin concentrations in wastewater

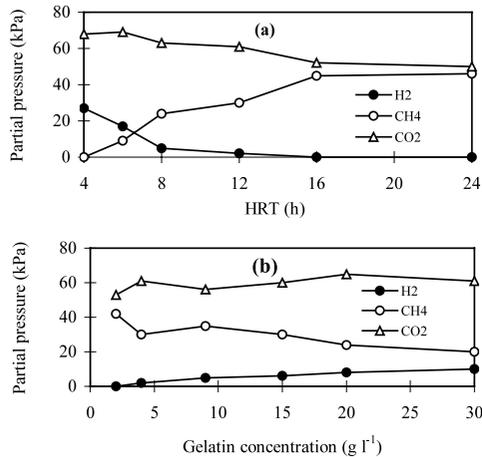| HRT (h)	NH$_3$-N (g l^{-1})	Gelatin in wastewater (g-COD l^{-1})	NH$_3$-N (g l^{-1})
4	0.295 ± 0.015	2	0.233 ± 0.017
6	0.349 ± 0.026	4	0.424 ± 0.031
8	0.386 ± 0.028	9	0.964 ± 0.057
12	0.424 ± 0.031	15	1.443 ± 0.078
16	0.409 ± 0.030	20	1.791 ± 0.095
24	0.448 ± 0.037	30	2.501 ± 0.106

Fig. 7. Partial pressures of hydrogen, methane and carbon dioxide at various: (a) HRTs; and (b) gelatin concentrations.

appears to have no influence on the acidification of gelatin in this study. This is also due to the acidic condition in the acidogenic reactor, under which little ammonium is converted to the more toxic form of ammonia.

However, methanogens are more vulnerable to the ammonia toxicity and the increase of pH in the methanogenic reactor would convert ammonium into toxic ammonia. Thus, controlling the ammonium concentration could be critical in feeding the acidified effluent to the methanogenic reactor in the two-stage process.

3.6. Gas production

Fig. 7a illustrates that on treating gelatin at 4 g-COD1^{-1}, the hydrogen partial pressure decreased with the increase of HRT, becoming un-

detectable at 16 h. Fig. 7b illustrates that on treating gelatinaceous wastewater at 12 h of HRT, the hydrogen partial pressure increased with gelatin concentration, from undetectable at 2 g-COD1^{-1} to 10 kPa at 30 g-COD1^{-1}. The methane partial pressure followed an opposite trend to that of hydrogen, while carbon dioxide partial pressure was steady, ranging from 50 to 69 kPa.

The total COD of hydrogen and methane accounted for only 4.5–7.8% of COD in wastewater. It increased with HRT, but decreased with the increase of gelatin concentration. The COD conversion to hydrogen and methane in acidification was considerably lower than the 86–90% found in a methanogenic reactor treating proteinaceous wastewater (Fang and Chung, 1999).

3.7. Sludge yield

Sludge yield in an anaerobic treatment system can be estimated based on COD balance (Fang et al., 1994). COD is a wastewater parameter indirectly measuring the amount of electrons that are available for oxidation. In a strict anaerobic process, no electron acceptor is added to the system. In such a case, although the COD in the influent is transformed into VFA, alcohols, hydrogen, methane and biomass, the overall COD should remain unchanged. As a result, the COD difference between influent and effluent should be equal to the COD in biogas, i.e. hydrogen and methane, and COD in the biomass. The biomass COD can, thus, be estimated from the other three terms of COD, all of which can be accurately measured. Assuming the chemical formula of sludge to be $C_5H_7NO_2$, each gram of biomass is equivalent to 1.42 g of COD, and the sludge yield can be estimated accordingly.

Table 4
Sludge yields at various HRTs and gelatin concentrations in wastewater

HRT (h)	Yield (g-VSS (g-COD)$^{-1}$)	Gelatin in wastewater (g-COD1^{-1})	Yield (g-VSS (g-COD)$^{-1}$)
4	0.326 ± 0.015	2	0.311 ± 0.018
6	0.323 ± 0.020	4	0.316 ± 0.020
8	0.323 ± 0.008	9	0.320 ± 0.007
12	0.316 ± 0.012	15	0.322 ± 0.018
16	0.310 ± 0.007	20	0.330 ± 0.021
24	0.301 ± 0.011	30	0.338 ± 0.013

Table 4 summarizes the estimated yield of gelatin-acidifying sludge at various HRTs and gelatin concentrations. The average yield was 0.320 ± 0.014 g-VSS (g-COD)$^{-1}$, which is comparable to that of acidogenic sludge reported in literature ranging from 0.230 to 0.324 g-VSS (g-COD)$^{-1}$ (Cohen et al., 1980; Zoetemeyer et al., 1982; Kissalita et al., 1989; Fang and Yu, 2001; Yu and Fang, 2000).

4. Conclusions

At pH 5.5 and 37 °C, the degree of gelatin degradation increased with the HRT, from 84.1% at 4 h to 89.6% at 24 h, but decreased with the increase of the gelatin concentration in the influent from 65.2% at 2 g-COD l^{-1} to 51.9% at 30 g-COD l^{-1}. The degradation of gelatin followed the Monod kinetics with a maximum rate of 1.10 g (g-VSS·d)$^{-1}$ and a half-rate constant of 0.23 g l^{-1}. The overall production rate of VFA and alcohols decreased with HRT, from 0.33 g (g-VSS·d)$^{-1}$ at 4 h to 0.15 g (g-VSS·d)$^{-1}$ at 24 h, but increased with gelatin concentration in influent, from 0.10 g (g-VSS·d)$^{-1}$ at 4 g-COD l^{-1} to 0.58 g (g-VSS·d)$^{-1}$ at 30 g-COD l^{-1}. The key acidification products were acetate, propionate and butyrate. Only 4.5–7.8% of COD in wastewater was converted to hydrogen and methane. The sludge yield was estimated as 0.320 ± 0.014 g-VSS (g-COD)$^{-1}$.

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