

Comparative performance of mesophilic and thermophilic acidogenic upflow reactors

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Abstract

Two anaerobic acidogenic reactors, one mesophilic (37 °C) and one thermophilic (55 °C), were operated with a synthetic wastewater at a series of organic loading rates (OLRs). There was almost no difference between the two reactors for chemical oxygen demand (COD) reduction, and degree of acidification at any given OLRs. However, the thermophilic reactor had a higher substrate degradation rate, biogas production rate, and specific formation rate of aqueous products than the mesophilic reactor. The predominant acidogenic products in the two reactors were acetate, propionate, butyrate, and ethanol. The distribution of acidogenic aqueous products was significantly influenced by OLR, but not by temperature. Only 1.7–7.9% of the COD in wastewater was converted to hydrogen/methane for the mesophilic reactor, and 2.5–8.8% of the COD for the thermophilic reactor. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In anaerobic degradation, complex organics, such as polysaccharides, proteins, and lipids, are first hydrolyzed by enzymes, forming sugars, amino acids, and fatty acids. These intermediate products are then degraded by acidogens, forming volatile fatty acids (VFA), which are further degraded by acetogens, forming acetate, carbon dioxide, and hydrogen. Finally, both acetate and H₂/CO₂, are converted by methanogens to methane [1]. Acidogens grow relatively faster and are less sensitive to pH variation than acetogens/methanogens [2]. Hence, it is difficult to maintain the proper balance between acidogens and acetogens/methanogens at high substrate loading rates in an anaerobic digester [1]. Instability or failure of single-phase anaerobic digesters due to the unbalance between the rates of production and consumption of VFA has been widely reported for a variety of wastewaters [2–4]. Therefore, it

has been proposed that the two phases are physically separated by using two reactors in series: one for VFA production and another for methane production [3]. A two-phase anaerobic process has been successfully applied to the treatment of municipal sludge [3] and various wastewaters, including trout processing wastewater [4], cafeteria wastewater [5], olive oil mill effluent [6], and vegetable-processing wastewater [7]. However, nearly all of these studies have been focused on mesophilic acidogenesis, while information for the thermophilic acidogenesis is very limited [8].

Food processing wastewaters, such as wastes from coffee processing, palm oil mill, cannery, distillery, and ice-cream, are often discharged at or above ambient temperatures. Treating these effluents under conventional mesophilic conditions requires costly pre-cooling, and has the risk of losing biomass activity should the cooling system break down. Therefore, thermophilic treatment is an attractive alternative for these wastewaters [9]. Furthermore, in the thermophilic range, reaction rates proceed faster than under mesophilic conditions, so that the organic loading potentials of the anaerobic reactors are substantially higher [10]. On

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the other hand, the thermophilic process is reported to be less stable to environmental changes than the mesophilic process. In addition, the thermophilic process is more favourable for the production of propionate, which is more slowly converted to methane in the subsequent methanogenic reactor than other VFAs [11]. Thermophilic acidogenesis of municipal sludge was found to be effective [3,12]. Compared with a single-phase digester, a thermophilic acidogenic reactor followed by a mesophilic methanogenic reactor, produced a significant improvement in the degradation of organic matter [5,13]. However, these studies were limited to digestion of municipal sludge. The purpose of this research was to evaluate the significance of organic loading rate (OLR) in the sizing of upflow acidogenic reactors, and to compare the performance and operating characteristics of reactors operated at mesophilic and thermophilic temperatures. Laboratory experiments were conducted over a 10-month period using dry milk as a substrate.

2. Materials and methods

2.1. Reactor

The experiment was conducted in parallel in two upflow anaerobic reactors as shown in Fig. 1. Each Plexiglas-made reactor had a working volume of 2.8 l with an internal diameter of 84 mm and a height of 500

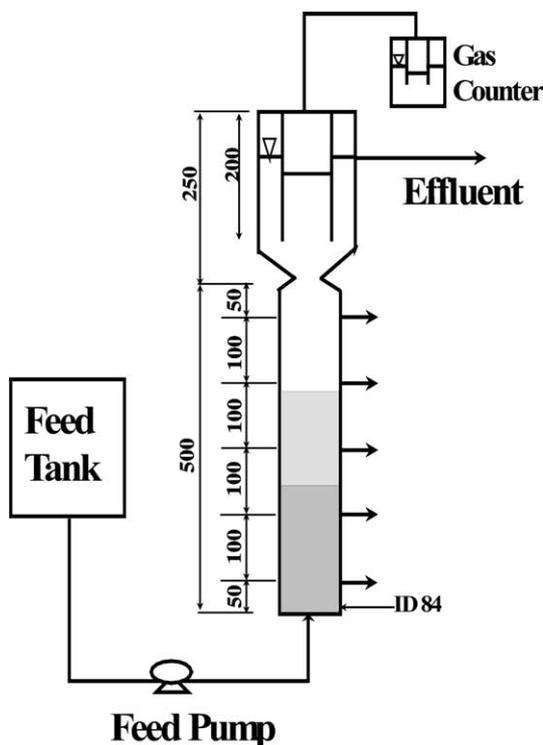


Fig. 1. Experimental setup (unit, mm).

mm. Five evenly distributed sampling ports were installed over the height of the column. Total biomass in the reactors was estimated based on the profile of the volatile suspended solids (VSS) of the samples taken from the sampling ports. Under the cap of the reactors was a gas–liquid–solid separator with an internal diameter of 114 mm and a height of 250 mm making a filled volume of 2.55 l. The reactors were water-jacketed, and one operated at a constant temperature of 37 °C and another operated at 55 °C. The other operating conditions for the two reactors were identical except the temperature.

2.2. Wastewater and seed sludge

A synthetic wastewater was prepared using full cream powdered milk supplied by Nestle Corp. Since the milk contained sufficient amounts of nitrogen, minerals and vitamins, only phosphorus as KH_2PO_4 was added to ensure the ratio of chemical oxygen demand (COD) to P to be 200:1. Acidogenic bacteria were enriched in the reactor by controlling the pH of the mixed liquor at $\text{pH } 5.5 \pm 0.1$ using 2N HCl and NaOH solutions.

Each reactor was seeded with 1-l sludge taken from an upflow anaerobic reactor treating the same wastewater for another study [14]. The seed sludge contained 26.2 g VSS, resulting in an initial VSS concentration of 9.5 g/l. After seeding, the OLR was increased stepwise from the initial 4 gCOD/l d to 6, 8, 12, 16, and lastly 24 gCOD/l d. The reactors were operated at each OLR level for 31–40 days to reach pseudo-steady state before increasing OLR to the next level. The data are based on arithmetic means of six or more measurements at each substrate level. The solids retention time of the reactors was maintained at 15 days by wasting one fifteenth of the sludge blanket volume everyday.

2.3. Characterization of wastewater

Throughout the experiment the influent COD was kept at 4000 mg/l, equivalent to 2860 mg/l milk. The lactose and total carbohydrate concentrations were measured as 1041 mg/l and 1107 mg/l, respectively; lactose represented 94.0% of the total carbohydrate. One milligram of lactose equals to 1.12 mg of COD. Hence, the COD due to carbohydrate in the wastewater was estimated as 1239 mgCOD/l, which was 30.9% of total COD.

The protein concentration was estimated by multiplying the total nitrogen (TN) concentration by 6.25 [11]. As the TN concentration in the wastewater was determined as 112 mg/l, the total protein concentration was calculated as 701 mg/l. Since 1 mg of protein was measured as 1.35 mg of COD, the COD due to the presence of protein in the wastewater was 947 mg/l, which was 23.6% of the total COD.

Lipid in the wastewater was extracted from the acidified wastewater using trichlorotrifluoroethane, and was then measured for COD after the solvent was evaporated. Since 1 mg of lipid was measured as 2.25 mg of COD, 745 mg/l of lipid in the wastewater was equivalent to 1676 mgCOD/l, i. e. 41.9% of the total COD.

The above analyses of components indicate that carbohydrate, protein and lipid were the three major components in the wastewater. A total of 94.6% of the COD in the wastewater was accounted for carbohydrate, protein, and lipid.

2.4. Analysis

The amount of biogas produced in the reactors was recorded daily using the water displacement method. The contents of H₂, CH₄, and CO₂ in the biogas were analyzed with a gas chromatograph (Hewlett Packard, Model 5890) equipped with a thermal conductivity detector and a 25 m × 0.53 mm CarboPlot P7 column. The concentration of 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 equipped with a flame ionization detector and a capillary column. The formate concentration was measured by the colorimetric method of Lang and Lang [15].

Carbohydrate and protein were measured by a phenol–sulphuric method [16] and the Lowry–Folin method [17], respectively. Lipid was extracted by the Bligh–Dyer method from the acidified sample [18], and was then measured gravimetrically after the solvent was evaporated at 80 °C. The lipid measured also accounted for the long-chain fatty acids. Measurements of COD, pH, TN, and VSS were performed according to the Standard Methods [18].

3. Results and discussion

3.1. Overall performance

During anaerobic degradation, substrate was converted into VFA and alcohols in the effluent, plus H₂/CO₂ in the biogas and biomass. Fig. 2 illustrates (a) OLR, (b) total VFA and alcohol concentration in effluent, and (c) biogas production rate throughout this study. Fig. 2b illustrates that the VFA/alcohol concentrations of the two reactors had similar changing patterns. Both of them initially increased with OLR and reached maximum at 6 gCOD/l d; thereafter, they declined as OLR increased.

Fig. 2c illustrates that the total biogas production rates of the two reactors increased with OLR until

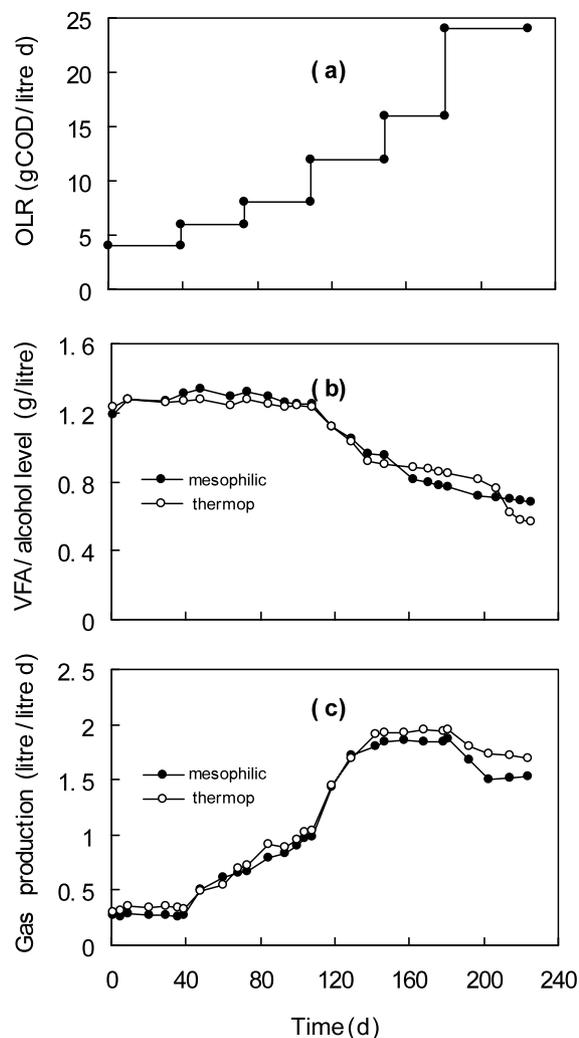


Fig. 2. Operational conditions and performance of the two reactors: (a) OLR; (b) total VFA/alcohol concentration in effluent, and (c) biogas production rate.

reaching a maximum at 12 gCOD/l d. 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 study on mesophilic methanogenesis of identical wastewater using the same reactor [19], the gas production reached 12.40 l/l d at 8.9 gCOD/l d, whereas gas production was only 0.98 l/l d for the mesophilic acidogenic reactor and 1.22 l/l d for the thermophilic reactor at 8 gCOD/l d in the present study. The biogas production rate in the thermophilic reactor was greater than that in the mesophilic reactor.

3.2. COD reduction

In an acidogenic reactor, although substrate is converted to intermediates such as VFA and alcohols, the COD of the liquid phase is conservative; the only loss of COD from the influent comes from the reduced gases

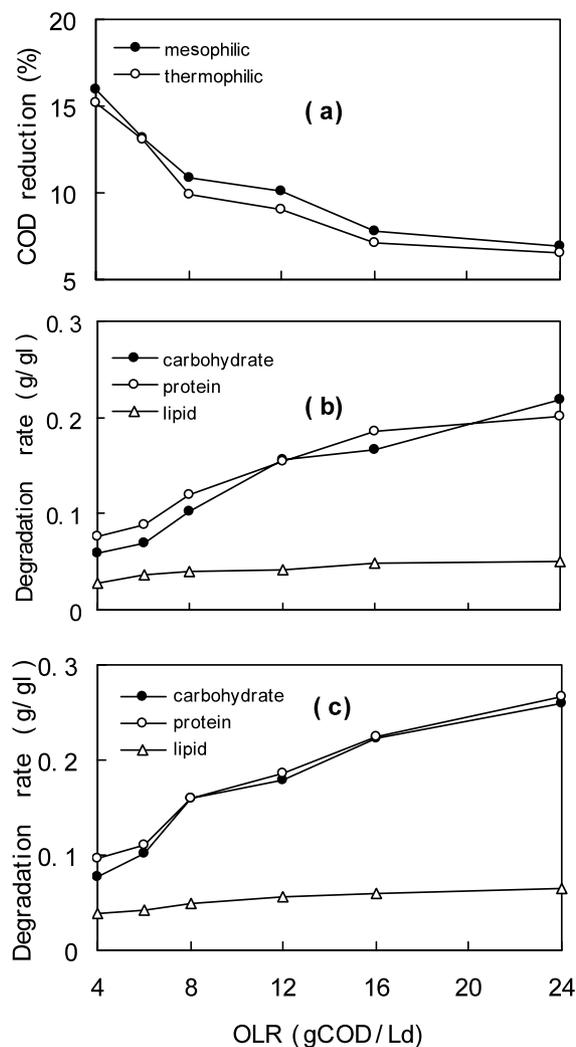


Fig. 3. (a) COD reductions of the two reactors; (b) specific degradation rates of substrate in mesophilic reactor; and (c) specific degradation rates of substrate in thermophilic reactor.

(hydrogen or/and methane) and the production of biomass. As shown in Fig. 3a, the maximum COD removal efficiencies were 16% for the mesophilic reactor and 15% for the thermophilic reactor, indicating that only small amount of substrate was converted to biomass and biogas. With the increase of OLR, the COD reductions of the both reactors slightly declined. There was almost no difference between the two reactors for the COD reductions at any given OLRs.

3.3. Substrate degradation

Table 1 lists the degradation efficiencies of carbohydrate, protein, and lipid as a function of OLR in the two reactors. The degradations of carbohydrate, protein and lipid increased individually with the decrease of OLR, following the order of carbohydrate > protein > lipid. For the mesophilic reactor, carbohydrate was readily degraded at all loading rates, from 93.0% at 24 gCOD/

l d to 98.9% at 4 gCOD/l d. However, protein degradation was significantly influenced by the OLR, decreasing from 88.0% at 4 gCOD/l d to 59.1% at 24 gCOD/l d. Similarly, lipid degradation also decreased with the increase of OLR, from 46.9% at 4 gCOD/l d to only 19.8% at 24 gCOD/l d. For the thermophilic reactor, the degradation efficiencies of carbohydrate, protein and lipid had a similar trend to those of the mesophilic reactor, as shown in Table 1.

Fig. 3b and c respectively illustrates the specific degradation rates of carbohydrate, protein, and lipid by the acidogenic biomass in the two reactors. Their specific degradation rates of increased with increasing OLR. These tendencies are the opposite to those of the degradation efficiencies. For the mesophilic reactor, the specific degradation rates were 0.059–0.218 g/gVSS d for carbohydrate, 0.076–0.202 g/gVSS d for protein, and 0.028–0.051 g/gVSS d for lipid. All increased with OLR. For the thermophilic reactor, however, the specific degradation rates were 0.077–0.259 g/gVSS d for carbohydrate, 0.096–0.266 g/gVSS d for protein, and 0.038–0.065 g/gVSS d for lipid. The specific degradation rates of the three components in the thermophilic reactor were slightly greater than the corresponding values in the mesophilic reactor, suggesting that the increase of operating temperature accelerated the acidogenic degradation of the substrate.

3.4. Aqueous products

VFA and alcohols are the main aqueous products of acidogenesis. In this study the VFA/alcohols identified included: formate, acetate, propionate, butyrate, i-butyrate, valerate, i-valerate, caproate, lactate, methanol, ethanol, propanol, and butanol. The overall performance of an acidogenic reactor can be evaluated using the term 'degree of acidification', which is quantified by comparing the COD equivalent of the acidogenic products, i.e. VFA/alcohols plus hydrogen and methane in the biogas, to the influent COD [7]. Table 2 lists that the degree of acidification decreased with an increase of OLR, from 59.1% at 4 gCOD/l d to 28.2% at 24 gCOD/l d for the mesophilic reactor, and from 60.8% at 4 gCOD/l d to 27.1% at 24 gCOD/l d for the thermophilic reactor. There was almost no difference between the two reactors for the degree of acidification at any given OLRs.

Specific VFA/alcohol production rate, defined as the rate of VFA/alcohol production per day per unit amount of VSS in the reactor (g/gVSS d), can also be used to evaluate the VFA/alcohol generating capability of an acidogenic reactor [2,7]. The specific VFA/alcohol production rate, as shown in Table 2, increased with OLR, from 0.109 g/gVSS d at 4 gCOD/l d to 0.203 g/gVSS d at 24 gCOD/l d for the mesophilic reactor, and from 0.117 g/gVSS d at 4 gCOD/l d to 0.214 g/gVSS d at

Table 1
Degradation efficiencies of carbohydrate, protein, and lipid in the two reactors

OLR (gCOD/l d)	Degradation efficiency (%)					
	Mesophilic reactor			Thermophilic reactor		
	Carbohydrate	Protein	Lipid	Carbohydrate	Protein	Lipid
4	98.9	88.0	46.9	98.7	89.2	46.1
6	97.7	86.2	45.7	98.5	87.0	43.6
8	95.6	81.7	42.2	98.0	84.0	39.1
12	95.4	71.3	27.7	97.2	75.4	28.7
16	95.0	64.8	21.6	96.0	72.3	21.2
24	93.1	59.1	19.8	92.1	66.9	18.9

24 gCOD/l d for the thermophilic reactor. The specific VFA/alcohol production rates in the thermophilic reactor were slightly greater than the corresponding values in the mesophilic reactor. This confirms that the increase of operating temperature accelerated the acidogenic reaction rate.

Table 3, summarizing the fractions of the VFA and alcohols in the effluents from the two reactors at each OLR, shows that the three main acidogenic products were acetate, propionate and butyrate, accounting for 61–65% of total VFA and alcohols in the two reactors. The percentages of acetate and propionate in the effluent products were highly influenced by the variation of OLR. For the mesophilic reactor, acetate accounted for 17% of the total VFA/alcohols in the effluent at 24 gCOD/l d, and 40% at 4 gCOD/l d. On the other hand, the percentage for propionate decreased from 32% at 24 gCOD/l d to 10% at 4 gCOD/l d, suggesting that higher OLR favoured the production of propionate. These results show that the OLR had a significant effect on the distribution of effluent products, as reported by Elsfinitis and Oldham [20]. The percentage of butyrate in the effluent products was rather steady, ranging from 8–12%.

It has been reported that the propionate concentration in the effluent from a conventional single-phase thermophilic reactor is much higher than that from a mesophilic reactor [11]. However, as shown in Table 3, the two acidogenic reactors had similar propionate

levels at any given OLRs, indicating that elevated temperature did not enhance the production of propionate in an acidogenic reactor.

Table 3 shows that ethanol was also an important product of acidogenesis, each respectively accounting for about 11 and 12% of the total effluent VFA/alcohols in the mesophilic and thermophilic reactors. Results of this study show that variation of OLR had little effect on the effluent ethanol concentration.

3.5. Gaseous products

In the acidogenic reactors, biogas is mostly composed of the acidogenic by-products, carbon dioxide and hydrogen. As shown in Table 4, at 24 gCOD/l d, the hydrogen partial pressure was 33 kPa for the mesophilic reactor and 40 kPa for the thermophilic reactor, and there was no detectable methane in the biogas of the two reactors. Table 4 also shows that methanogenic activity increased with further decrease of OLR. Hydrogen was consumed by the methanogens as electron donors for the formation of methane. The hydrogen partial pressure decreased, along with the increase of methane, as OLR increased. At 4 gCOD/l d, the hydrogen partial pressure was only 0.4 kPa for the mesophilic reactor and 0.5 kPa for the thermophilic reactor, whereas methane was increased to 31 kPa for the mesophilic reactor and 34 kPa for the thermophilic reactor at 24 gCOD/l d.

Table 2
Degree of acidification and specific VFA/alcohol production rates in the two reactors

OLR (gCOD/l d)	Degree of acidification (%)		VFA/alcohol production rate (g/gVSS d)	
	Mesophilic reactor	Thermophilic reactor	Mesophilic reactor	Thermophilic reactor
	4	59.1	60.8	0.109
6	56.9	56.0	0.127	0.172
8	54.3	55.1	0.174	0.196
12	43.4	41.9	0.184	0.205
16	34.8	37.3	0.195	0.209
24	28.2	27.1	0.203	0.214

Table 3
Percentages of the VFA and alcohols in the effluents from the two reactors

OLR (gCOD/l d)	Reactor	HFr	Hac	HPr	Hbu	i-Hbu	HVa	i-HVa	Hca	HLa	Mol	Eol	Pol	Bol
4	Mesophilic	1	40	10	12	3	5	2	3	8	2	11	2	0
6		1	31	16	11	6	5	2	2	7	2	10	0	0
8		2	30	18	9	5	4	5	3	8	1	12	1	2
12		3	21	26	10	6	4	5	3	7	0	12	2	0
16		2	18	30	8	4	5	4	1	8	0	12	2	1
24		1	17	32	9	4	4	7	4	9	1	13	0	0
4	Thermophilic	2	34	16	10	3	5	3	4	6	5	12	0	0
6		2	32	16	12	4	4	5	6	3	4	10	2	0
8		3	28	18	9	4	3	4	6	7	5	12	2	0
12		2	23	20	9	4	4	2	7	6	8	10	3	1
16		3	24	26	9	3	2	2	5	5	8	12	0	2
24		2	17	32	9	4	2	1	8	7	1	11	3	4

Note: HFr, formate; Hac, acetate; HPr, propionate; Hbu, butyrate; i-Hbu, i-butyrate; HVa, valerate; i-HVa, i-valerate; Hca, caproate; HLa, lactate; Mol, methanol; Eol, ethanol; Pol, propanol; Bol, butanol.

Table 4
Partial pressure (kPa) of biogas in the two reactors

OLR (gCOD/l d)	Mesophilic reactor			Thermophilic reactor		
	H ₂	CH ₄	CO ₂	H ₂	CH ₄	CO ₂
4	0.4	31	67	5	34	58
6	2	12	85	19	14	65
8	6	10	82	20	7	73
12	20	3	74	24	2	73
16	24	0	73	34	0	64
24	33	0	66	40	0	59

However, the overall conversion of hydrogen and methane from the substrates was insignificant. Fig. 4 illustrates that only 1.7–7.9% of the COD in wastewater was converted to either hydrogen or methane in the mesophilic reactor, and 2.5–8.8% of the COD in the thermophilic reactor.

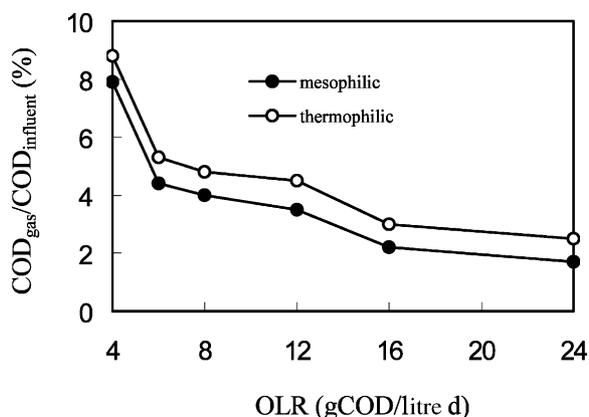


Fig. 4. Ratio of biogas COD equivalent to influent COD as a function of OLR.

3.6. Effluent COD balance

By mass balance, the amount of aqueous COD in the effluent should equal to the sum of COD in (1) VFA; (2) alcohols; (3) residual carbohydrate, protein and lipid; and (4) unknown metabolites. Amongst them, the first two may 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 carbohydrate, protein and lipid. The quantity of the unknown metabolites equaled to the remaining COD. Fig. 5 illustrates the four groups of COD in the effluent at various OLRs. At an OLR of 12 gCOD/l d or less, VFA and alcohols were the majority of the effluent organic matters. For example, in the mesophilic reactor, the COD in the form of VFA/alcohols was 55.2% of effluent COD at 8 gCOD/l d, but was reduced to 36.6% at 16 gCOD/l d and 26.6% at 24 gCOD/l d. By comparison, the fraction of the unknown acidification products in the effluent increased with OLR, from 9.9% at 4 gCOD/l d to 24.0% at 24 gCOD/l d. Glycerol, ketones, aldehydes and amino acids were likely among these unidentified metabolites. Increased hydrogen partial pressure in reactors would suppress the further degradation of many amino acids [21,22]. Since the partial pressure of hydrogen consistently exceeded 0.4 kPa in this study, accumulation of amino acids in the effluent is expected.

3.7. Comparison of the two reactors

For the two reactors, the degradation efficiencies of carbohydrate, protein and lipid were similar, indicating that temperature had little influence on substrate conversion. Similarly, the two reactors had identical degrees of acidification. This might be partially attributed to a temperature compensation effect. This effect means that at decreased temperature, the specific activity of sludge

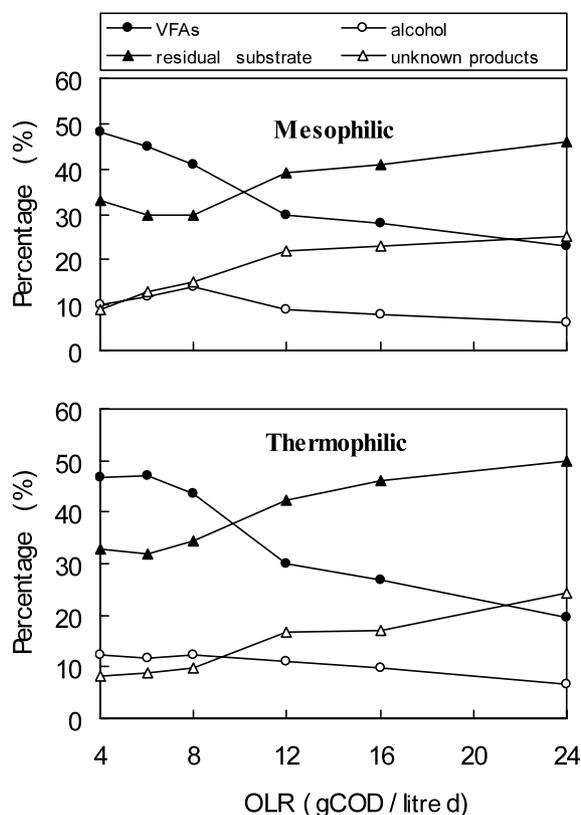


Fig. 5. Effluent COD balances at various OLRs.

still remains high, despite the significantly lower maximum specific activity [23]. Such a temperature compensation effect has been found for methanogenic reactors with both pure methanogens, such as *Methanosarcina barkeri* and mixed cultures [23,24]. The engineering implication of this observation is that temperature control may not be essential for an acidogenic reactor treating dairy wastewater.

In the two reactors the predominant acidogenic products were acetate, propionate, butyrate, and ethanol. Lactate was not detectable in the present study. The distribution of acidogenic aqueous products was not significantly influenced by temperature. However, in Zoetemeyer et al.'s study with a glucose-fed acidogenic reactor [25], proportions of lactate and ethanol both increased, from 4 and 10% at 20 °C to 13 and 19% at 60 °C. The difference in predominant acidogenic products might be partially attributed to the different substrates used in the two studies. These results imply that both mesophilic and thermophilic acidogenesis is feasible for the acidification of dairy wastewater. 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. However, thermophilic acidogenesis should be chosen for hot industrial wastes.

4. Conclusions

The degree of acidogenesis of synthetic wastewater decreased as OLR increased. The biodegradability of the major constituents in wastewater also decreased when OLR increased, following the order of carbohydrates > protein > lipid. Only 1.7–7.9% of the COD in wastewater was converted to hydrogen/methane for the mesophilic reactor, and 2.5–8.8% of the COD for the thermophilic reactor.

There was almost no difference between the two reactors for COD reduction, and degree of acidification at any given OLR. However, the thermophilic reactor had a higher substrate degradation rate, biogas production rate, and specific VFA/alcohol production rate than the mesophilic reactor. The predominant acidogenic products were acetate, propionate, butyrate, and ethanol. The distribution of acidogenic aqueous products was significantly influenced by OLR, but not by temperature. These results suggest that the food processing wastewaters discharged above ambient temperatures could be acidified under either mesophilic or thermophilic conditions.

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