



# ANAEROBIC TREATMENT OF PROTEINACEOUS WASTEWATER UNDER MESOPHILIC AND THERMOPHILIC CONDITIONS

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## ABSTRACT

Experiments were conducted in two 2.8 liter UASB (upflow anaerobic sludge blanket) reactors treating proteinaceous wastewaters at 37° and 55°C with 9 hours of hydraulic retention. Results showed that the mesophilic reactor consistently removed 83.5-85.1% of COD (chemical oxygen demand) at loading rates ranging 8-22 g COD l<sup>-1</sup> d<sup>-1</sup> (corresponding to 3000-8250 mg l<sup>-1</sup> of proteinaceous COD in wastewater), whereas the thermophilic reactor removed only 68.5-82.7%. At 32 g COD l<sup>-1</sup> d<sup>-1</sup> (i.e. 12000 mg COD l<sup>-1</sup>), the removal efficiencies were lowered to 75.7% in the mesophilic reactor and 65.1% in the thermophilic reactor. At 42 g COD l<sup>-1</sup> d<sup>-1</sup>, severe sludge washout occurred in the mesophilic reactor; at the same loading rate, the thermophilic reactor removed only 53.8% of COD even though sludge washout was under control. The degradation rate in the both reactors was limited by the initial hydrolysis of proteins. However, batch tests showed that thermophilic sludge had slightly higher methanogenic activities than mesophilic sludge in treating proteins and intermediate acids, except propionate. The sludge yields in mesophilic and thermophilic reactors were 0.066 and 0.099 g VSS g COD<sup>-1</sup>, respectively. Observations by scanning electron microscopy indicated that both types of sludge granules were of irregular shape. There was little noticeable difference between the two granules; both had neither a layered microstructure nor a predominant bacterial species. © 1999 Published by Elsevier Science Ltd on behalf of the IAWQ. All rights reserved

## KEYWORDS

Anaerobic treatment; mesophilic; peptone; proteinaceous wastewaters; thermophilic; UASB.

## INTRODUCTION

Due to the development of high-rate reactors and a better understanding of microbiology, anaerobic technology has advanced considerably in the past decade for industrial wastewater treatment (Speece, 1983). Most of these treatability studies were conducted under conventional mesophilic conditions (35°C to 40°C). However, effluents from food industries are often discharged at elevated temperatures. Treating these effluents under conventional mesophilic conditions would require pre-cooling. Furthermore, a sudden breakdown of the cooling system could have a long-lasting damaging effect on the activity of the biomass. It thus seems to be more natural to treat these effluents under thermophilic conditions, which is also presumably more effective for the degradation of organics and the killing of pathogens (Wiegant *et al.*, 1985; Lettinga *et al.*, 1991).

Among the high-rate reactors, the UASB (upflow anaerobic sludge blanket) process (Lettinga *et al.*, 1980; Fang and Chui, 1993) is most commercially successful. Hundreds of full-scale treatment plants have been installed in the past decade for the treatment of various wastewaters (Lettinga and Hulshoff Pol, 1991). The process has been investigated for the thermophilic treatment of wastewater in bench-scale (Wiegant, 1985; Van Lier *et al.*, 1992; Shi and Forster, 1993; Fang and Lau, 1996) as well as in pilot-scale reactors (Souza *et al.*, 1992; Ohtsuki *et al.*, 1994). However, its application to the thermophilic treatment of wastewater from food industries is still very limited.

This study was thus conducted for the treatment of concentrated proteinaceous wastewater in two parallel UASB reactors under mesophilic and thermophilic conditions, respectively, in order to compare the treatment efficiency and sludge characteristics in both reactors.

## MATERIALS AND METHODS

### Experimental conditions

Experiments were conducted in two 2.8 liter UASB (upflow anaerobic sludge blanket) reactors at 37° and 55°C, respectively. Figure 1 illustrates the reactor setup. Mesophilic sludge from a pilot UASB reactor for a separate study treating carbohydrate-containing wastewater was used to seed both reactors. Throughout the experiment, peptone was used as the proteinaceous substrate for both reactors, and the hydraulic retention time was kept at 9 hours. The wastewater also contained balanced nutrient, trace metals and buffering chemicals following the formulation used in previous studies (Fang and Chui, 1993). The mixed liquor pH in both reactors were kept with the range of pH 7.2-7.5 throughout the experiment.

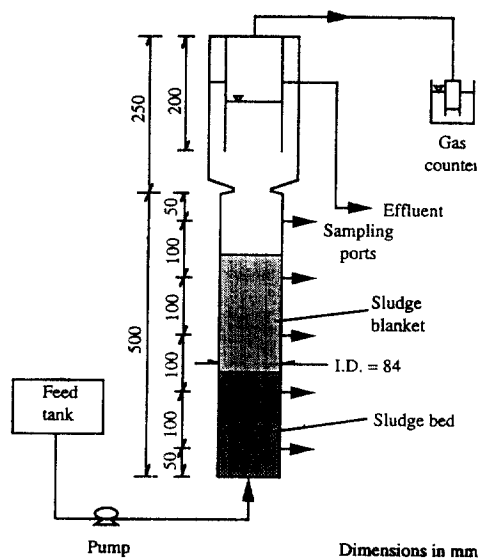


Figure 1. Experimental setup.

The startup phase lasted six months, allowing the sludge to acclimate to the proteinaceous substrate and temperature. During this period, the mesophilic reactor was kept at the constant temperature of 37°C, while the temperature of the thermophilic reactor was increased gradually from 37°C to 55°C. After the startup, the peptone concentration was step-increased in six increments over a five-month period from 3000 mg l<sup>-1</sup> of chemical oxygen demand (COD), corresponding to 8 g l<sup>-1</sup> d<sup>-1</sup>, up to 15800 mg l<sup>-1</sup> of COD, corresponding to 42 g l<sup>-1</sup> d<sup>-1</sup>. However, the mesophilic reactor experienced severe sludge washout at the last loading rate, and had to be terminated. For loading rates up to 32 g l<sup>-1</sup> d<sup>-1</sup>, The average volatile suspended solids (VSS) contents were 35.90 g in the mesophilic reactor and 39.89 g in the thermophilic reactor.

### Analytical methods

The amount of biogas produced in each reactor was recorded daily using a water replacement method. The contents of methane, CO<sub>2</sub> and N<sub>2</sub> in the biogas were analyzed by a gas chromatograph (GC, Hewlett-Packard, Model 5890 Series II) equipped with a thermal conductivity detector and a 10 m stainless steel column packed with HayeSepQ (80/100 mesh). Helium was used as the carrier gas at a flow rate of 22 ml min<sup>-1</sup>. The column was operated at a temperature program of 90°C for 1.2 minutes followed by 2 minutes at 110°C. The respective temperatures of injector and detector were 130°C and 200°C.

The concentration of volatile fatty acids (VFA), ranging from acetic to heptanoic acids, were determined by a second gas chromatograph (Hewlett Packard, Model 5890 Series II) equipped with a flame ionization detector and a 10m × 0.53mm HP-FFAP fused-silica capillary column. Samples were filtered through a 0.2 µm filter, acidified by phosphoric acid, and measured for free acids. The initial temperature of the column was 70°C for 4 minutes and then 140°C for 3 minutes, and finally 170°C for 4 minutes. 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<sup>-1</sup>. The column used in this study was unable to detect formic acid.

The COD of the effluent and the VSS of the mixed liquor were measured following the standard methods (APHA, 1985). Biogranules were sampled from both reactors for examinations by scanning electron microscopy, following the procedures reported previously (Fang *et al.*, 1994). The specific methanogenic activities (SMA) of biogranules sampled from the UASB reactors were measured in 120 serum vials in duplicate using the method of Dolfing and Bloemen (1985) adapted from Owen *et al.* (1979).

## RESULTS AND DISCUSSIONS

### COD removal efficiency

Figure 2 illustrates, respectively, the COD removal efficiencies at 37°C and 55°C at loading rates varied from 8 to 42 g COD l<sup>-1</sup> d<sup>-1</sup>. During this period, the biomass contents averaged 35.90 g in the mesophilic reactor and 39.89 g in the thermophilic reactor. The pH in both reactors was within the range of pH 7.2-7.5. Figure 2 clearly shows that the mesophilic reactor was superior to the thermophilic reactor in COD removal. The former reactor consistently removed 83.5-85.1% of COD at loading rates ranging 8-22 g COD l<sup>-1</sup> d<sup>-1</sup>, whereas the latter reactor removed only 68.5-82.7%. At 32 g COD l<sup>-1</sup> d<sup>-1</sup>, the removal efficiency by the mesophilic reactor was lowered to 75.7%, which, however, was still better than the 65.1% by the thermophilic reactor. When the loading rate was further increased to 42 g COD l<sup>-1</sup> d<sup>-1</sup>, the mesophilic reactor had severe sludge washout and the experiment had to be terminated. Although there was no noticeable sludge washout in the thermophilic reactor at this loading rate, its COD removal efficiency was further lowered to only 53.8%. The experiment was terminated on day 152.

### Residual acids in effluent

Degradation of protein is a very complex process. Proteins are first hydrolyzed by acidogens forming intermediate fatty acids, which are further converted by acetogens producing acetate and H<sub>2</sub>/CO<sub>2</sub>. Both acetate and H<sub>2</sub>/CO<sub>2</sub> are finally converted to methane by respective methanogens. The residual COD in both reactors was mainly attributed to the intermediate fatty acids. Table 1 summarizes the concentrations of total, as well as individual, residual acids (from C<sub>2</sub> to C<sub>7</sub>) in the effluent of mesophilic reactor. It shows that at loading rates up to 16 g COD l<sup>-1</sup> d<sup>-1</sup> the effluent contained very low concentrations of VFA (no more than 40 mg l<sup>-1</sup>, or 80 mg COD l<sup>-1</sup>). The large majority of COD in the effluent was thus the unhydrolyzed peptone, because of only 9 hours of hydraulic retention. This indicates that hydrolysis/acidogenesis was the rate-limiting step in the degradation of protein at these loading rates. Once protein was acidified, the intermediate VFAs were readily converted to acetate, and then methane. The residual VFAs increased as loading rate further increased to 22 and later 32 g COD l<sup>-1</sup> d<sup>-1</sup>, as indicated by the lowering of COD removal. At 32 g COD l<sup>-1</sup> d<sup>-1</sup>, the main constituents of VFA in the effluent were acetate (267 mg l<sup>-1</sup>) and propionate (115 mg l<sup>-1</sup>).

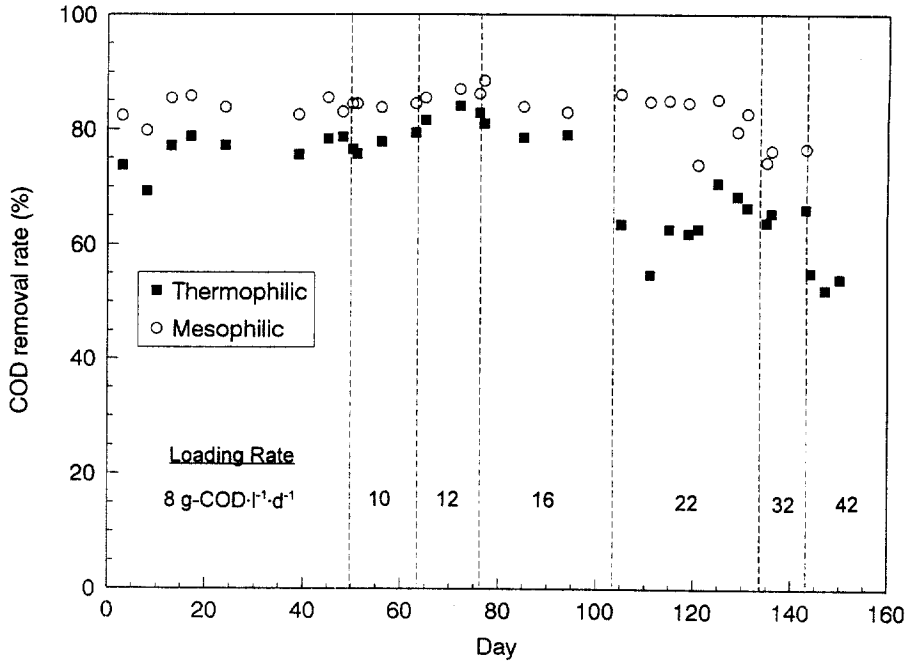


Figure 2. COD removal efficiency.

Table 1. VFA contents in mesophilic reactor effluents

| org. loading<br>(g COD l <sup>-1</sup> d <sup>-1</sup> ) | total VFA<br>(mg l <sup>-1</sup> ) | acetate<br>(mg l <sup>-1</sup> ) | propionate<br>(mg l <sup>-1</sup> ) | butyrate<br>(mg l <sup>-1</sup> ) | valerate<br>(mg l <sup>-1</sup> ) | caproate<br>(mg l <sup>-1</sup> ) | heptanoate<br>(mg l <sup>-1</sup> ) |
|--|------------------------------------|----------------------------------|-------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------|
| 31   | 8                                  | 3                                | 5                                   | 4                                 | 4                                 | 7                                 | 8                                   |
| 10   | 35                                 | n/a                              | 5                                   | 5                                 | 8                                 | 6                                 | 11                                  |
| 12   | 40                                 | 8                                | 11                                  | 5                                 | 5                                 | 7                                 | 4                                   |
| 16   | 28                                 | n/a                              | 4                                   | 3                                 | 9                                 | 3                                 | 9                                   |
| 22   | 160                                | 68                               | 37                                  | 12                                | 4                                 | n/a                               | 39                                  |
| 32   | 481                                | 267                              | 115                                 | 44                                | 47                                | n/a                               | 8                                   |

Table 2 summarizes the corresponding VFA data in the effluent of the thermophilic reactor. The residual VFA concentration in the effluent was also very low (less than 120 mg l<sup>-1</sup>) at loading rates up to 16 g COD l<sup>-1</sup> d<sup>-1</sup>. At each loading rate, the effluent VFA concentration in the thermophilic reactor was higher than that in the mesophilic reactor, which is consistent with the COD data. However, as the loading rate further increased, the residual VFA concentration increased drastically. At 32 g COD l<sup>-1</sup> d<sup>-1</sup>, the effluent contained 1488 mg l<sup>-1</sup> of residual VFA, i.e. over 200% more than that in the mesophilic reactor effluent. Most of these residual VFA was propionate, which had a concentration, i.e. 1012 mg l<sup>-1</sup>, considerably higher than that in the mesophilic reactor effluent (115 mg l<sup>-1</sup>). This seems to suggest that acetogenesis in thermophilic reactor was more sensitive to the increase of COD loading rate. At 42 g-COD l<sup>-1</sup> d<sup>-1</sup>, the residual VFA in the effluent further increased to 2524 mg l<sup>-1</sup>.

Table 2. VFA contents in thermophilic reactor effluents

| org. loading<br>(g COD l <sup>-1</sup> d <sup>-1</sup> ) | total VFA<br>(mg l <sup>-1</sup> ) | acetate<br>(mg l <sup>-1</sup> ) | propionate<br>(mg l <sup>-1</sup> ) | butyrate<br>(mg l <sup>-1</sup> ) | valerate<br>(mg l <sup>-1</sup> ) | caproate<br>(mg l <sup>-1</sup> ) | heptanoate<br>(mg l <sup>-1</sup> ) |
|--|------------------------------------|----------------------------------|-------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------|
| 8  | 62                                 | 33                               | 13                                  | 6                                 | 3                                 | 8                                 | n/a                                 |
| 10   | 94                                 | 50                               | 23                                  | n/a                               | 12                                | 9                                 | 4                                   |
| 12   | 81                                 | 31                               | 17                                  | 8                                 | 22                                | 3                                 | n/a                                 |
| 16   | 114                                | 66                               | 36                                  | 4                                 | n/a                               | n/a                               | 7                                   |
| 22   | 1102                               | 462                              | 547                                 | 30                                | 29                                | 14                                | 20                                  |
| 32   | 1488                               | 381                              | 1012                                | 47                                | 32                                | 3                                 | 13                                  |
| 42   | 2524                               | 633                              | 1680                                | 74                                | 54                                | 11                                | 40                                  |

### Methane production and sludge yields

The COD is a quantitative measurement of electrons in wastewater available for oxidation. In a strict anaerobic degradation process, there are no electron acceptors available to remove the COD from the system. Although the overall electrons available in the anaerobic reactor would remain unchanged throughout the process, they are transferred from substrate to intermediate VFA, methane and biomass.

Figure 3 illustrates the correlations between specific methane production rates and substrate utilization rates in both reactors.

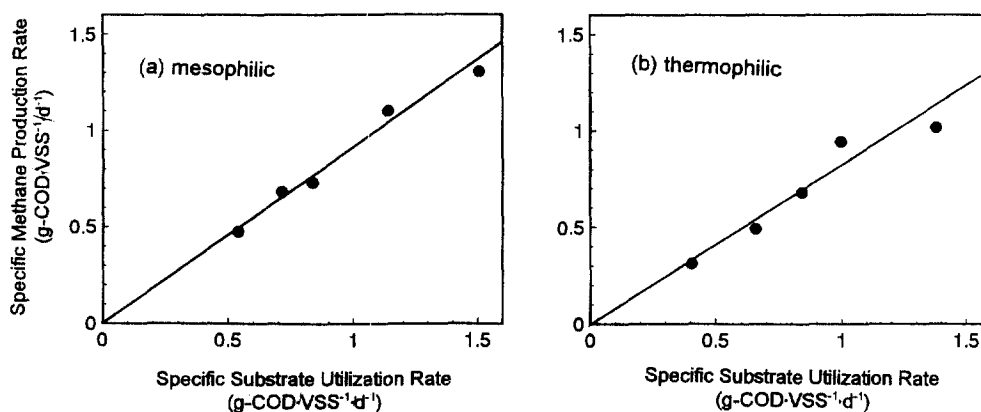


Figure 3. Methane production rates at various substrate utilization rates.

The slope in Figure 3a indicates that, under mesophilic conditions, 90.6% of COD removed was converted to methane. The remaining 9.4% was presumably converted to biomass. Assuming a chemical formula of  $C_5H_7O_2N$ , the biomass has a COD-equivalent of 1.42 mg COD/mg VSS. Thus, the yield for the mesophilic sludge was estimated as 0.066 mg VSS/mg COD-removed ( $0.094/1.42=0.066$ ). Similarly, Figure 3b illustrates 86.0% of COD conversion to methane under thermophilic conditions. The corresponding yield for the thermophilic sludge was thus estimated as 0.099 mg VSS/mg COD-removed.

### Specific methanogenic activities

Sludge was granulated in UASB reactors. The methanogenic activities of the granular sludge in both reactors were analyzed using the SMA methods (Owen, *et al.* 1979). Four substrates were selected for these tests, including peptone, acetate, propionate, and butyrate.

Table 3. Specific methanogenic activity of protein-degrading granular sludge

| Substrate  | This study |      |       |
|------------|------------|------|-------|
|            | 37°C       | 55°C | 37°C* |
| Peptone    | 0.51       | 0.67 | 0.59  |
| Acetate    | 0.92       | 1.31 | 0.89  |
| Propionate | 0.37       | 0.21 | 0.39  |
| Butyrate   | 0.55       | 1.40 | 0.46  |

All measurements in g methane COD g VSS<sup>-1</sup> d<sup>-1</sup>

\*Fang *et al.* (1994).

Results in Table 3 show that the mesophilic sludge of this study had comparable methanogenic activities to another mesophilic protein-degrading sludge in a previous study (Fang *et al.*, 1994). It is interesting to note that, however, the thermophilic sludge exhibited higher SMA, using peptone, acetate and butyrate as substrates, than the two mesophilic sludges. All sludges had the lowest SMA values using propionate as substrate; this was most noticeable for the thermophilic sludge. This explains the reason for the accumulation of residual propionate in the effluents of high loadings, especially in the effluent of the thermophilic reactor.

#### Scanning electron microscopic observations

Mesophilic protein-degrading biogranules obtained in this study were 1-2 mm in size, similar to those of a previous study (Fang, *et al.*, 1994). The thermophilic biogranules were in the range of 0.5-1 mm in size. Both types of biogranules did not exhibit any layered microstructure, nor any predominant species inside. Instead, densely packed clusters of *Methanothrix*- and *Methanosarcina*-like bacteria, and colonies of bacteria with various morphologies were found scattered throughout the cross-section of biogranules, as illustrated in Figures 4a (mesophilic granule) and 4b (thermophilic granule). There was no noticeable difference between the two granules.

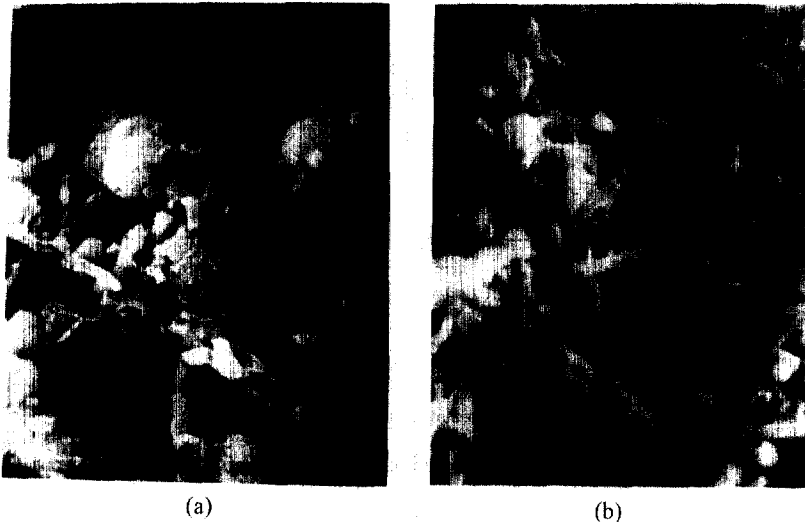


Figure 4. SEM Photos of (a) mesophilic granule and (b) thermophilic granule.

#### CONCLUSIONS

Results of experiments conducted in UASB reactors showed that treating proteinaceous wastewaters at 37°C was in general more effective than treating at 55°C. With 9 hours of hydraulic retention, the mesophilic reactor consistently removed 83.5-85.1% of COD at loadings of 8-22 g-COD l<sup>-1</sup> d<sup>-1</sup>, whereas the

thermophilic reactor removed only 68.5-82.7%. Performance of both reactors deteriorated as the loading rate further increased. At 42 g COD l<sup>-1</sup> d<sup>-1</sup>, severe sludge washout occurred in the mesophilic reactor and the removal efficiency in the thermophilic reactor was further deteriorated to 53.8%. The degradation rate in both reactors was limited by the initial hydrolysis of proteins. On the other hand, batch tests showed that thermophilic sludge had slightly higher methanogenic activities than mesophilic sludge in treating proteins and intermediate acids, except propionate. Observations by scanning electron microscopy indicated that there was little difference between the two types of granular sludge; both sludges had neither a layered microstructure nor a predominant bacterial species.

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