Thermophilic high-rate anaerobic treatment of wastewater containing long-chain fatty acids: impact of reactor hydrodynamics

C.-S. Hwu*, G. Molenaar, J. Garthoff, J.B. van Lier and G. Lettinga

Department of Environmental Technology, Wageningen Agricultural University, Bomenweg 2, 6703 HD Wageningen, The Netherlands

The liquid superficial upflow velocity (V_{up}) and hydraulic retention time (HRT) on the thermophilic treatment of oleate in expanded granular sludge bed (EGSB) reactors were investigated. The highest methane conversion rate of oleate, 93 mg CH_4-COD/g VSS.d, was attained in a reactor operating at a V_{up} of 1 m/h and an HRT of 24 h. The typical EGSB reactor hydrodynamics ($V_{up} > 4$ m/h and HRT < 10 h) inhibited the treatment performance, mainly due to biomass washout in particulate form.

Introduction

Since its introduction two decades ago, the upflow anaerobic sludge bed (UASB) reactor has become the most widely applied, high-rate, anaerobic wastewater treatment process. The success of the UASB reactor can be attributed to its excellent biomass retention in the reactor systems, which is attained by mutual attachment of bacteria, resulting in the formation of granular sludge. More than nine-hundred industrial scale UASB reactors are currently under operation (L.H.A. Habets, personal communication), successfully treating a wide variety of (easily biodegradable) wastewaters. However, treatment failure of UASB reactors has been reported both at laboratory and industrial scale, following shock loads of long-chain fatty acids (Rinzema *et al.*, 1989) or milk fats (Samson *et al.*, 1985).

Long-chain fatty acids are products of lipid hydrolysis. They can inhibit anaerobic micro-organisms at millimolar concentrations (Koster and Cramer, 1987; Hwu *et al.*, 1996a). Apart from the inhibitory effect, long-chain fatty acids can cause severe flotation/washout of granular sludge due to their adsorption onto the granules (Rinzema *et al.*, 1989; Hwu *et al.*, 1996b). When comparing the anaerobic digestion of sodium laurate in UASB and expanded granular sludge bed (EGSB) reactors, Rinzema *et al.* (1993) recommended the use of EGSB reactors under thermophilic conditions for the treatment of long-chain fatty acids. The EGSB process is a modified-UASB process, conceptually operated at relatively higher liquid superficial upflow velocities ($V_{up} > 4$ m/h; Lettinga, 1996) and shorter hydraulic retention times (HRT < 10 h; Van Haandel and Lettinga, 1994). The better biomass-substrate contact in EGSB reactors, compared to conventional UASB reactors, allows anaerobic treatment of wastewaters containing lipids and/or higher fatty acids (Lettinga, 1995) and even recalcitrant or toxic substrates (Lettinga, 1996).

When considering the predominant long-chain fatty acid in wastewaters (Viswanathan *et al.*, 1962; Quéméneur and Marty, 1994), oleate was used as a model compound in a previous study (Hwu *et al.*, 1997a). In contrast to Rinzema *et al.* (1993), EGSB hydrodynamic parameters (i.e., $V_{up} = 4$–7 m/h or HRT = 0.6–6 h) gave a less favorable reactor performance compared to the typical UASB operational parameters ($V_{up} = 1$ m/h or HRT = 24 h). The reasons for the controversies between the two reactor concepts were not well understood. Therefore the effects of the EGSB reactor hydrodynamics on the treatment were investigated systematically. The present study considered the impact of the hydrodynamic parameters (V_{up} and HRT) on removal efficiencies, methane conversion and biomass characteristics. The results of four thermophilic (55°C) EGSB-type reactors with six test runs (three in each two) operating at different V_{up} and HRT treating oleate (82%, w/w) are presented.

Materials and Methods
Biomass

Anaerobic granular sludge was taken from a thermophilic (55°C) EGSB reactor (Hwu *et al.*, 1997a). Before inoculation, the sludge had been exposed to sodium oleate (95% purity, Sigma), with a COD of 2.0 g/l, for 16 days. The inoculum had an initial specific methanogenic activity (SMA) with acetate of 0.23 g COD per g volatile suspended solids per day.

Media

The composition of the basal medium was described previously (Hwu *et al.*, 1996a). Initially, sodium butyrate (2.8 g/l) was continuously supplemented. To avoid cumulative inhibition of sodium ions in the reactors during the test runs in V_{up} study, butyric acid with the same concentration was used. During all test runs, technical grade sodium oleate (Riedel de Haën, Germany), analyzed with a mixture of c. 82% oleate (w/w) and 18% palmitate, was supplemented by pulse injection (V_{up} study) or continuously (HRT study) by a step-wise increase of the COD from 0.5 to 1.0 and finally to 2.0 g/l. Influent pH was adjusted to 7.0 (± 0.1), except for the runs using butyric acid, where the pH eventually ranged 6.2 (± 0.1) in the mixing flasks (see below).

Reactor runs to study the impact of V_{up}
Start-up

Due to the rather low methanogenic activity of the inoculum, probably because of inhibition by oleate (2.0 g COD/l), a start-up procedure was required to revive the activity and to estimate the pseudo-steady state ratio of COD recovered as methane to COD added as butyrate. Two identical EGSB reactors (diameter, 40 mm; height, 965 mm; liquid volume, 1.4 l), each inoculated with 19.2 g volatile suspended solids, were operated with an HRT of 24 h and a V_{up} of 1.3 m/h. No long-chain fatty acids was fed to the reactors, operating at a volumetric loading rate (B_v) of 4 g COD/l.d. The reactor temperature (55°C) was controlled by a thermostat with water recirculation through the reactor jacket. Figure 1a depicts the reactor set-up. The mixing flask was not installed during the start-up period. Influent was pumped from a cooled (5°C), stirred feed tank and effluent was discharged after the siphon. From day 44 onwards, the V_{up} of a reactor (R2) was increased to 7.9 m/h. When c. 60% of COD recovery as methane was constantly maintained over 10 days in both reactors, the start-up was terminated (day 92). An equal amount of sludge was withdrawn from each reactor, resulting in an initial biomass content of 16.1 g volatile suspended solids per reactor for the remaining experiments.

Test runs

After start-up, the reactor set-up was modified to a virtually closed system (Fig. 1a) in order to monitor the impact of the V_{up} while minimizing the influence of the HRT. Effluent was collected in a 1.3 l flask, mixed with the cosubstrate and then recirculated. Highly concentrated butyric acid (250 g COD/l) was pumped constantly (22 ml/d), so that the total liquid volume of the system was nearly unaltered. Occasionally, anaerobic tap water was added to maintain the level. An N_2/CO_2 (70/30, v/v) gas bag was connected to each flask to balance the gas pressure and liquid bufferity. The concentrated (but well soluble) oleate solution was fed by pulse injection into the butyric feed line. Two injections with each of the three designated final concentrations were performed: day 0 & 6 with 0.5 g COD/l, day 12 & 19 with 1.0 g COD/l and day 40 & 47 with 2.0 g COD/l. The time interval between two injections was large enough to reach a pseudo-steady state. The total time period of the three test runs (I, II and III) was 53 d. In each test run the impact of the V_{up} was evaluated, based on the difference (R1–R2) between the change of COD recovery ratio as methane (due to oleate injection) in reactor R1 (V_{up} = 1.3 m/h) and the change in reactor R2 (V_{up} = 7.9 m/h). The change in each reactor was calculated by subtracting the ratio with butyrate (before oleate injection, mean value) from the ratio with butyrate plus oleate (maximum value).

Reactor runs to study the impact of HRT
Start-up

Two identical EGSB reactors (diameter, 56 mm; liquid volume, 4.4 l) were used to study the impact of the HRT (Fig. 1b), one inoculated with 30.3 g volatile suspended solids (R3) and the other (R4) with 35.6 g volatile suspended solids. In the beginning of start-up period, both reactors were operated with an HRT of 24 h and a V_{up} of 1 m/h. From day 52 onwards, the HRT of R4 was shortened to 6 h while the B_v (4 g COD/l.d) remained constant by equivalent dilution. When over 99% of the butyrate was removed and c. 80% of COD recovery as methane was maintained constantly over 7 d in both reactors, the start-up was terminated (day 91).

Test runs

Three oleate B_v (0.5, 1 and 2 g COD/l.d) were tested, respectively denoted by test run IV, V and VI. Thus the total B_v in each run was 4.5, 5.0 and 6.0 g COD/l.d for both reactors. Each run was operated for 21 days.
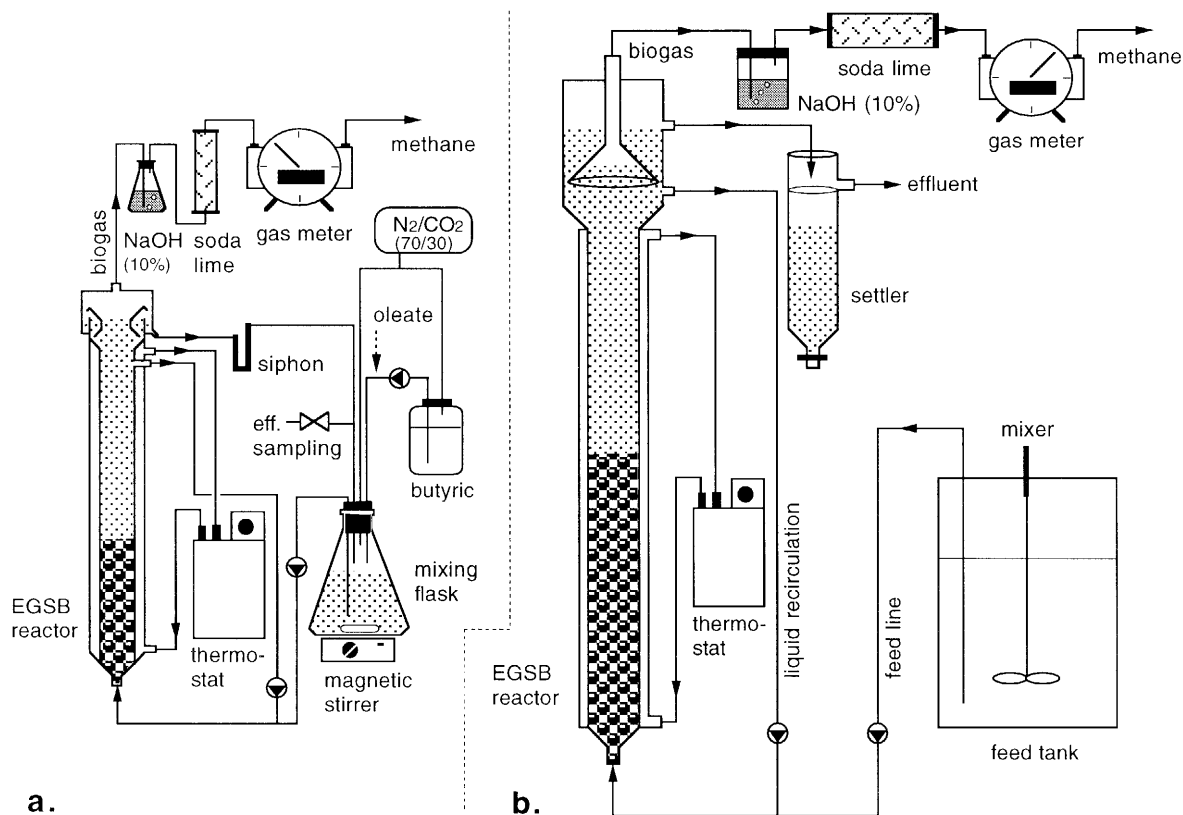
Figure 1 Schematic diagram (not to scale) representing reactor set-up, (a) in V_{up} and (b) in HRT study.

Analyses

Determinations for COD and volatile suspended solids followed the procedures described in the standard method (APHA, 1992). For COD determinations, well mixed influent samples were used. Effluent samples were membrane-filtered (pore size 0.45 μm, Schleicher & Schuell, Germany) in V_{up} and paper-filtered (pore size 4.4 μm, same supplier) in HRT runs. In the V_{up} test runs, the reactor biomass was not quantified. In the HRT runs, volatile suspended solids were determined upon starting and finishing a run, and averaged to calculate the conversion rate per unit biomass.

Results and discussion
Impact of the V_{up}

In all test runs, the oleate injection induced a significant increase in methane production in the following 1–3 days (data not shown). Subsequently, it returned to its background level from butyrate, suggesting that the added oleate were biodegraded without detectable inhibition. Table 1 compares the reactor performance at the two distinct V_{up}. It is not surprising that excellent COD removal efficiencies (94–98%) were achieved in the

thermophilic digestion of butyrate. The methane conversion rates can be regarded as the pseudo-steady state, background levels during each test run in each reactor. Though fluctuating, the conversion rates in reactor R2 increased with time course. This trend was not found in reactor R1, suggesting that the higher V_{up} (7.9 m/h) led to the increase in butyrate conversion.

Interestingly, no significant difference was found between the change of methane recovery following an oleate injection in R1 and that in R2. The difference was never larger than 6% (Table 1), indicating that the two reactors made a similar response in the degradation of oleate, regardless of the applied V_{up}. These results differ with a previous study on oleate conversion (Hwu *et al.*, 1997a), where methane recovery ratio in an EGSB reactor operating at a V_{up} of 1 m/h was 1.8 times as high as that at 7.2 m/h. It should be noted that in the virtually closed system, complete biomass retention prevailed in both reactor systems. This probably explains for the similarity in the reactor performance. Also this deduces the importance of retention of oleate-degraders which are susceptible to higher upflow velocities.

Table 1 Comparison of the reactor performance operating at different V_{up} (R1, 1.3 m/h; R2, 7.9 m/h)

Test run	Butyrate conc. (g COD/l)	Oleate conc.[a] (g COD/l)	COD removal[b] (%)		Methane conversion rate[b,c] (mg CH₄-COD/g VSS.d)		Difference R1–R2 (%)[d]
			R1	R2	R1	R2	
I	4.0	0.5	95 ± 6	94 ± 4	195.2 ± 20.7	142.6 ± 20.1	4.7 ± 0.8
II	4.0	1.0	96 ± 9	95 ± 5	209.9 ± 26.2	184.4 ± 18.3	1.9 ± 0.2
III	4.o	2.0	98 ± 9	98 ± 7	188.1 ± 23.0	225.9 ± 38.3	5.8 ± 0.6

[a]Pulse feed.
[b]Based on butyrate only.
[c]Mean value of methane production from butyrate divided by the initial amount of biomass.
[d]Absolute value of the difference between the change of methane recovery in R1 and that in R2.

Table 2 Comparison of the reactor performance operating at different HRTs (R3, 24h; R4, 6h)

Test run	B_v (g COD/l.d)		COD removal* (%)		Methane conversion rate* (mg CH₄-COD/g VSS.d)		Methane recovery* (%)	
	Butyrate	Oleate	R3	R4	R3	R4	R3	R4
IV	4.0	0.5	94 ± 3	95 ± 2	370.1 ± 10.1	289.0 ± 7.7	94 ± 4	88 ± 4
V	4.0	1.0	95 ± 2	96 ± 0	415.9 ± 9.5	326.7 ± 6.7	87 ± 4	83 ± 5
VI	4.0	2.0	96 ± 2	97 ± 0	415.4 ± 51.0	361.4 ± 29.6	71 ± 10	76 ± 7

*Calculation based on total influent COD.

Flotation of granular sludge did not occur under the considerably high V_{up} (7.9 m/h). Hwu *et al.* (1996b) speculated that a longer HRT would minimize LCFA adsorption and consequently result in less granular sludge flotation. This study confirms their speculation, considering the very long HRT (123 d, cosubstrate flow basis) applied in the present system. However, more small particulates were washed-out at the higher V_{up}, as indicated by the greater turbidity in the mixing flask (Fig. 1) of reactor R2. Moreover, compared to mesophilic granules, thermophilic ones have a lower structural strength (Quarmby and Forster, 1995) and perhaps a more loosely open structure (Macario *et al.*, 1991). An increased V_{up} enhances the liquid and particle shear forces, leading to abrasion or breaking of granules; thus resulting in washout of particulates or formation of smaller granules. Indeed, microscopic examination upon finishing test run III showed that the average granular size in the reactor operating at 7.9 m/h (R2, average diameter = 1 mm) was half of the granules present in R1, operating at 1.3 m/h (average diameter = 2 mm). The sludge characteristics in reactor R2 due to higher V_{up} probably illuminate the increase in methane conversion rate (Table 1), as reported by van Lier *et al.* (1996) that biomass with smaller size has higher butyrate conversion rate due to better mass transfer.

Impact of the HRT

The results of the HRT test runs are summarized in Table 2. An increase in the methane production was generally found on the next day when the oleate concentration was increased (data not shown). In runs IV and V, the peak disappeared and returned to its base-line. In contrast, the methane production in run VI decreased throughout the test run, as indicated by the lower ratios of COD recovery as methane with the bigger standard deviation (Table 2). The drop of methane recovery was deeper in R3 (HRT = 24 h) than in R4 (HRT = 6 h), because one-fourth of the oleate concentration was applied to R4. This agrees with Rinzema *et al.* (1994) and Hwu *et al.* (1996b), who reported that LCFA toxicity is merely concentration dependent.

With respect to the HRT, R3 gave better results both in methane recoveries and conversion rates during runs IV and V. Assuming the pseudo-steady state methane recovery obtained during the start-up was not altered during these test runs, the methane conversion rates originating solely from oleate in runs IV and V amounted to 73.7 (±9.7) and 92.8 (±7.5) in R3, and 50.4 (±7.5) and 83.2 (±5.2) mg CH₄-COD/g VSS.d in R4, respectively. These data show that methane conversion from the added oleate did take place in

both reactors, but R3 had over 40% higher LCFA degradation rates. This agrees with Elefsiniotis and Oldham (1994), who reported that a longer HRT results in a better methanogenesis of the lipids present in primary sludge.

Also in R3 or R4, flotation of granular sludge did not prevail. Washout of particulates (diameters ranging between 50–100 μm) occurred, however, already at a V_{up} as low as 1 m/h. These particulates were found to have a higher oleate degradability than the granules present in the reactor (Hwu *et al.*, 1997b). The mean washout rate during test runs was 0.03 and 0.08 g volatile suspended solids per day, respectively, in R3 and R4. These different rates may mirror the effect of the HRT on the hydrodynamics, as the dilution factor in R4 was four-fold that of R3. The washout rate in R4 was rather high compared to the washout rate (0.11 g volatile suspended solids per day) in an anaerobic fluidized bed reactor fed with acetate, operating at a V_{up} of 14.5 m/h (Shieh and Hsu, 1996). Angelidaki and Ahring (1995) postulated that the growth rate of thermophilic LCFA-degraders is as slow as 0.3 d^{-1}. Therefore it is very likely that these specific substrate-utilizing biomass, oleate-oxidizers, suffered greatly from washout, more significantly at a shorter HRT.

Table 2 shows that the conceptual EGSB reactor operational parameter, a short HRT, was found unfavorable for a good treatment 0f oleate. It should also be noted that the highest oleate conversion rate (93 mg CH_4-COD/g VSS.d) in R3 is far below that (135 mg CH_4-COD/g VSS.d) obtained in batch tests (Hwu *et al.*, in preparation). The optimization of high-rate reactor treatment of LCFA towards these high conversion rates is presented in the following paper (Hwu *et al.*, 1997b).

References

APHA (1992). *Standard Methods for the Examination of Water and Wastewater*, 18th edn Am Publ Health Assoc, Washington, DC.

Angelidaki I and Ahring BK (1995). *Appl Environ Microbiol* **61**: 2442–2445.

Elefsiniotis P and Oldham WK (1994). *J Environ Engng ASCE* **120**: 645–660.

Habets LHA (1997). Personal communication. Paques, POB 52, 8560 AB Balk, The Netherlands.

Hwu C-S, Donlon B and Lettinga G (1996a). *Wat Sci Tech* **34**: 351–358.

Hwu C-S, Tseng S-K, Yuan C-Y, Kulik Z and Lettinga G (1996b). In: *Proc 1st IAWQ Conf Adsorption in Water Environment and Treatment Processes*, pp 91–98, Wakayama, Japan.

Hwu C-S, van Lier JB, Kulik Z, Mishra PK and Lettinga G (1997a). In: *Proc 8th Intl Conf Anaerobic Digestion*, Sendai, Japan.

Hwu C-S, van Beek B, van Lier JB and Lettinga G (1997b). The following paper.

Koster IW and Cramer A (1987). *Appl Environ Microbiol* **53**: 403–409.

Lettinga G (1995). Antonie van Leeuwenhoek **67**: 3–28.

Lettinga G (1996). *Wat Sci Tech* **33**: 85–98.

Macario AJL, Visser FA, van Lier JB and Conway de Macario E (1991). *J Gen Microbiol* **137**: 2179–2189.

Quarmby J and Forster CF (1995). *Enzyme Microb Technol* **17**: 493–498.

Quéméneur M and Marty Y (1994). *Wat Res* **28**: 1217–1226.

Rinzema A, Alphenaar A and Lettinga G (1989). *J Chem Tech Biotechnol* **46**: 257–266.

Rinzema A, Alphenaar A and Lettinga G (1993). *Process Biochem* **28**: 527–537.

Rinzema A, Boone M, van Knippenberg K and Lettinga G (1994). *Water Environ Res* **66**: 40–49.

Samson R, van den Berg B, Peters R and Hade C (1985). In: *Proc 39th Ind Waste Conf*, JM Bell, ed pp 235–241, Boston: Butterworth.

Shieh WK and Hsu Y (1996). *Wat Res* **30**: 1253–1257.

Van Haandel AC and Lettinga G (1994). *Anaerobic Sewage Treatment*, Chichester: Wiley.

Van Lier JB, Sanz Martin JL and Lettinga G (1996). *Wat Res* **30**: 199–207.

Viswanathan CV, Meera Bai B and Pillai SC (1962). *J Water Pollut Control Fed* **34**: 189–194.