0273-2289/99/76/0015/$14.50

# Anaerobic Treatment of Low-Strength Brewery Wastewater in Expanded Granular Sludge Bed Reactor

**Mario T. Kato,*[,1] Salih Rebac,[2] and Gatze Lettinga[2]**

*[1]Federal University of Pernambuco, Department of Civil Engineering, Av. Acadêmico Hélio Ramos s/n, 50670-530 Recife PE, Brazil, E-mail: kato@npd.ufpe.br; [2]Wageningen Agricultural University, Department of Environmental Technology, Wageningen, The Netherlands*

**Received March 6, 1998; Accepted August 19, 1998**

## Abstract

Anaerobic treatment of low-strength brewery wastewater, with influent total chemical oxygen demand (COD) ($COD_{in}$) concentrations ranging from 550 to 825 mg/L, was investigated in a pilot-scale 225.5-L expanded granular sludge bed (EGSB) reactor. In an experiment in which the temperature was lowered stepwise from 30 to 12°C, the COD removal efficiency decreased from 73 to 35%, at organic loading rates (OLR) of 11–16.5 g COD/L/d. The applied hydraulic retention time (HRT) and liquid upflow velocity ($V_{up}$) were 1.2 h and 5.8 m/h, respectively. Under these conditions, the acidified fraction of the $COD_{in}$ varied from 45 to 90%. In addition to the expected drop in reactor performance, problems with sludge retention were also observed. In a subsequent experiment set at 20°C, COD removal efficiencies exceeding 80% were obtained at an OLR up to 12.6 g COD/L/d, with $COD_{in}$ between 630 and 715 mg/L. The values of HRT and $V_{up}$ applied were 2.1–1.2 h, and 4.4–7.2 m/h, respectively. The acidified fraction of the $COD_{in}$ was above 90%, but sludge washout was not significant. These results indicate that the EGSB potentials can be further explored for the anaerobic treatment of low-strength brewery wastewater, even at lower temperatures.

**Index Entries:** Anaerobic treatment; expanded granular sludge bed reactor; brewery wastewater; acidified low-strength wastewater; low temperature.

## Introduction

Low-strength effluents can be considered as those containing chemical oxygen demand (COD) concentrations below 2000 mg/L, though many

*Author to whom all correspondence and reprint requests should be addressed.

contain concentrations of even less than 800 mg/L *(1)*. Dilute wastewater streams may be discharged at a number of industrial processes *(2–5)*. Important examples are effluents from some alcoholic and soft drink bottling industries, paper recycling and papermaking mills, fruit and vegetable canneries, and malting and brewing processes *(4,6–8)*. Some wastewaters may have a broad concentration range, because the COD of industrial effluents chiefly depends on the technological process, especially because of the water used and recycled, as well as the internal sources of wastewaters. Typical examples are brewery industry wastewaters, which can have COD concentrations as low as 0.6–0.9 g/L or as high as 160 g/L, because these effluents may consist of a mixture of process streams from the malting and brewing processes, and spent grain, and hops-pressing liquor wastes. Moreover, many breweries also include a soft drink bottling section, which also discharges dilute wastewaters *(6,9–14)*. Regardless of the concentration, brewery-like wastewaters are attractive for anaerobic treatment processes, because they contain mostly soluble, readily degradable simple substrates *(15,16)*. The anaerobic treatment processes are well-established methods for the elimination of easily biodegradable organic matter from wastewater. Since its earlier development, the upflow anaerobic sludge bed (UASB)-like systems have been more widely applied in practice than other anaerobic systems *(16,17)*. However, in order to improve the applicability of the UASB reactor, some modifications have been proposed. High hydraulic mixing intensity is important in the treatment of dilute wastewater, because the gas production is lower compared with that generated from higher-strength wastewaters.

A modification of the UASB reactor is the expanded granular sludge bed (EGSB) reactor, in which the granular sludge bed is expanded and the hydraulic mixing is intensified in order to improve the wastewater–biomass contact *(18,19)*. A higher superficial liquid velocity is achieved by applying effluent recirculation or by using taller reactors. In the UASB reactor, the liquid upflow velocity ($V_{up}$) is usually in the range of 0.5–1.5 m/h; the EGSB utilizes $V_{up}$ exceeding 5–6 m/h *(19)*.

Earlier experiments with EGSB reactors showed the importance of high $V_{up}$, and that such reactors can potentially be applied for the treatment of several wastewaters. Experiments with dilute vinasse at 8°C showed the importance of a high $V_{up}$ of 5 m/h compared to when 0.5 m/h was applied for influents with a low COD *(19)*. Other investigations were carried out with complex wastewaters such as lipid-containing effluents and presettled domestic sewage *(20)*. A higher treatment performance was obtained in the EGSB compared with the UASB reactor for the degradation of sodium caprate and sodium laurate solutions at 30°C *(21)*. The experiments with presettled domestic sewage were conducted at temperatures ranging from 8 to 20°C *(20)*. The influent total COD ($COD_{in}$) concentration ranged from 100 to 650 mg/L, but with a soluble COD fraction (membrane filtered) of

around 50%. The results showed that the removal efficiency of the soluble COD fraction was between 62 and 95% at hydraulic retention time (HRT) of 1.0–3.5 h, when the temperature was above 13°C and the COD was above 350 mg/L. The feasibility of EGSB reactors treating ethanol-containing wastewaters was demonstrated at 30°C. COD removal efficiency above 80%, at organic loading rate (OLR) up to 12 g COD/L/d, was achieved with COD concentrations as low as 100–200 mg/L *(22)*. A common aspect of these studies is that the high performance of EGSB reactor treating dilute wastewaters can be attributed to the very high mixing intensity and efficient contact between the biomass and the substrate.

An additional important aspect is that low temperatures in anaerobic treatment have always been associated with low methanogenic sludge activity. However, this does not necessarily mean that psychrophilic wastewater treatment is unfeasible *(18,19)*. Further investigation of the treatment of dilute acidified wastewater in an EGSB reactor at 10–13°C, fed with COD ranging from 600 to 900 mg/L, showed COD removal efficiency of approx 100% after 100 d of operation *(20)*. The HRT applied was less than 2 h and the OLR was in the range of 10–13 g COD/L/d.

Because intense mixing is very important for the treatment of low-strength and cold wastewaters, the EGSB does fulfill that requirement. The objective of this study was to evaluate the application of the EGSB reactor for the treatment of low-strength brewery-type wastewater. The experiments were conducted in a 225.5-L pilot-scale reactor at a temperature range of 30–12°C.

## Materials and Methods

### *Experiments in EGSB Reactors*

EGSB experiments were conducted with brewery wastewater in a pilot-scale 225.5-L polyacrylate reactor, with a height of 7.5 m and 20-cm inner diameter. The experiments were conducted with the temperature varying from 30 to 12°C, and later set at 20°C. Figure 1 shows the schematic diagram of the EGSB reactor system. The total amount of biomass inoculated, including 6 kg of fines (granules with diameter below 0.8 mm), was 74 kg wet granular sludge, which corresponded to 24.6 g volatile suspended solids (VSS)/L reactor. Wastewater, originating from Bavaria Brewery, Lieshout, The Netherlands, was stored in two 3-$m^3$ tanks at ambient temperature. Its total COD was 60 g/L, of which ethanol and volatile fatty acids (VFA) concentrations were 71 and 12%, respectively. During the course of the experiments, concentrations and composition of the original wastewater fed to the EGSB reactor changed, because of sedimentation of suspended solids and acidification in the storage tanks. When the system was started up, ethanol and VFA concentrations of the original wastewater had already changed to 30 and 45% of the total COD, respectively.

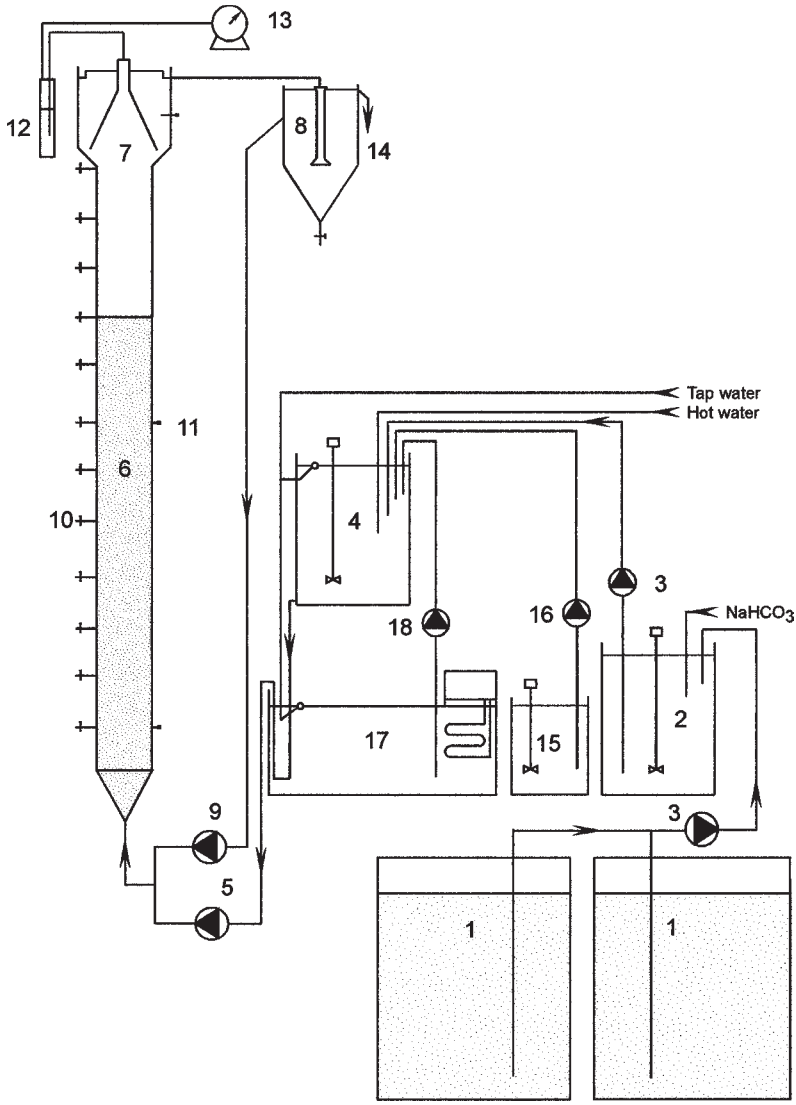


Fig. 1. Schematic diagram of the 225.5 $dm^3$ pilot-scale EGSB system used in this study. 1, Wastewater tanks; 2, pH neutralization tank; 3, concentrated wastewater pumps; 4, mixing and dilution tank; 5, main flow wastewater pump; 6, EGSB reactor; 7, gas–liquid separator; 8, external settler; 9, recirculation pump; 10, reactor sampling points; 11, thermocouple; 12, water seal; 13, wet gas meter; 14, effluent; 15, nutrient tank; 16, nutrients pump; 17, cooling bath; 18, cold water pump.

## *Inoculum*

All the experiments were conducted using anaerobic granular sludge obtained from a full-scale UASB reactor treating alcohol distillery wastewater at 35°C (Nedalco, Bergen op Zoom, The Netherlands). The composition of the distillery wastewater on a COD basis was 50% butane-diol;

20% higher alcohols; 4% acetone; 20% acetic acid; and 2% propionic acid. The sludge was stored at 4°C for 4 mo before starting the experiments. The VSS content was 7.5% of the weight of wet sludge. Wet sludge refers to the solids before drying overnight in an oven at 100–103°C. The mean density, settling velocity, and granule diameter were 1019 g/L sludge, 35 m/h, and 1.2 mm, respectively. The granule size distribution was 28% 0.7–1.0 mm; 42% 1.0–1.5 mm; 30% 1.5–1.9 mm.

### *Experimental Design*

In the first experiment, the reactor temperature was decreased step-by-step from 30 to 12°C. In the second experiment, the temperature was maintained at 20°C. Monitoring consisted of daily reactor influent and effluent sampling. COD removal efficiency is based on the effluent COD of the internal settler supernatant relative to $COD_{in}$.

### *Batch Methanogenic Activity Assay*

The specific methanogenic activity of the sludges was determined using 0.6-L glass serum flasks supplier-sealed with a rubber septum and a screw cap. Sludge was added to flasks, and the liquid volume was brought to 0.5 L with the basal mineral medium solution. Final substrate and sludge concentrations were 4 g COD/L and 1.5 g VSS/L, respectively. Acetate and VFA solutions were neutralized. The composition of the VFA-mixture solution was 24 acetate ($C_2$):34 propionate ($C_3$):41 butyrate ($C_4$) on a COD basis. After flushing the medium with nitrogen gas, the flasks were sealed and incubated in a temperature-controlled room at 20 and 30 ± 2°C. The flasks were provided with a second feeding when more than 80% of the substrate COD supplied in the first feeding was converted to methane. Monitoring consisted of periodic measurements of methane production by modified Mariotte flasks. Flasks contained a 3% (w/v) NaOH solution to remove the carbon dioxide from the biogas. Maximum specific methanogenic activity was calculated from the slope of the methane production vs time curve. Assays were conducted in duplicate and were not mixed.

### *Granule Size Distribution*

A sedimentation assay was performed in duplicate or triplicate to determine the particle size distribution and the mean granule diameter of the sludge, as described by Hulshoff Pol et al. *(23)*. The method is based on relating sedimentation velocities to the size and density of the granules.

### *Basal Media*

For batch methanogenic activity assays, a concentrated stock solution of essential inorganic macro- and micronutrients was prepared. After a fivefold dilution, the basal medium solution contained (in mg/L): 280 $NH_4Cl$, 327.4 $K_2HPO_4 \cdot 3H_2O$, 100 $MgSO_4 \cdot 7H_2O$, 10 $CaCl_2 \cdot 2H_2O$, 100 yeast extract, 0.05 $H_3BO_3$, 2 $FeCl_2 \cdot 4H_2O$, 0.05 $ZnCl_2$, 0.05 $MnCl_2 \cdot 4H_2O$, 0.05 $(NH_4)6Mo_7O_{24}$

· $4H_2O$, 0.09 $AlCl_3$ · $6H_2O$, 2 $CoCl_2$ · $6H_2O$, 0.05 $NiCl_2$ · $6H_2O$, 0.03 $CuCl_2$ · $2H_2O$, 0.1 $NaSeO_3$ · $5H_2O$, 1 EDTA, 0.2 resazurin, and 0.001 mL/L 36% HCl. Alkalinity was provided as sodium bicarbonate in an amount depending on the type and concentration of the substrate utilized. The bicarbonate concentration in mg $NaHCO_3$/L was 2100, using ethanol, and 400 for acetate and VFA-mixture solutions.

For the reactor experiments, the following nutrients were added to the substrate (brewery wastewater) (in mg/L influent): 45 $NH_4Cl$, 16 $K_2HPO_4$, 10 $(NH_4)SO_4$, 4 $CaCl_2 \cdot 2H_2O$, 9 $MgCl_2 \cdot 6H_2O$. Alkalinity was also provided as sodium bicarbonate, in an amount (336–924 mg $NaHCO_3$/L influent) depending on the influent COD concentration.

### *Analyses and Chemicals*

Ethanol and VFA were determined with Hewlett Packard 5890 gas chromatograph (Palo Alto, CA) using columns and operating conditions, as described elsewhere *(1,22)*. Measurement of biogas composition was based on a 200-mL glass sampler used for collection prior to the analyses. The $CH_4$, $CO_2$, and $H_2S$ were analyzed with the same sample of 100 µL injected into a Packard Becker 433 chromatograph (Delft, The Netherlands). Concentrations of ethanol and VFA, as well as the methane production, are referred to in COD units. Conversion factors utilized were 2.087 g COD/g ethanol, 1.067 g COD/g $C_2$, 1.515 g COD/g $C_3$, and 1.820 g COD/g $C_4$. For methane, a factor of 2.577 g COD/L $CH_4$ at 30°C was utilized. This factor was corrected for other temperatures. The values of soluble COD, ethanol COD, and VFA-COD of reactors are referred to samples centrifuged at 13,000*g* for 3 min. Measurements of pH were conducted immediately after sampling with a Knick 510 pH/mV-meter (Berlin, Germany) and a Schott Nederland N61 double electrode (Tiel, The Netherlands). The COD, solids, and other analyses were determined according to standard methods *(24)*. All chemicals were of analytical grade and purchased from Merck (Darmstad, Germany); yeast extract from Gist-Brocades (Delft, The Netherlands); resazurin from Fluka (Buchs, Switzerland); gases from Hoekloos (Schiedam, The Netherlands); and sodium bicarbonate (99.5%) added to the influent of the reactor, from Boom (Meppel, The Netherlands).

## Results

### *EGSB Reactor Treatment Efficiency*

Operational conditions and performance of the pilot-scale EGSB reactor treating brewery wastewater are shown in Figs. 2–4. Average values of applied $COD_{in}$, HRT, OLR, and SLR, and treatment efficiencies during the two experiments are also summarized in Table 1.

During the first experiment, each period corresponded to one range of temperature. The reactor was fed with a $COD_{in}$ concentration ranging from 550 to 825 mg/L, and the applied HRT was maintained at 1.2 h, resulting

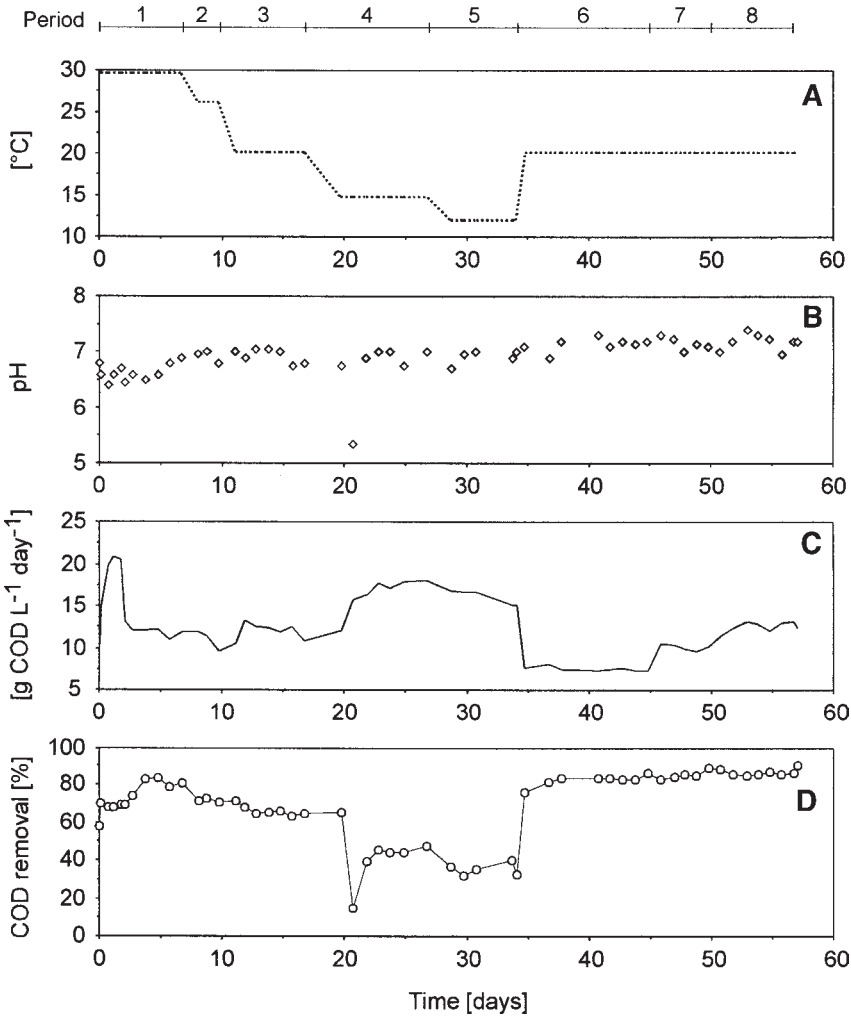


Fig. 2. Operational conditions and performance of the EGSB system. **(A)** Temperature. **(B)** pH in the reactor. **(C)** Organic loading rate. **(D)** Removal efficiency, based on $COD_{tot}$.

in a $V_{up}$ value of 5.8 m/h in all five periods (Table 1). No recirculation was applied. Decreasing the temperature from 30 to 20°C corresponded to a gradual decrease in the COD removal efficiency from 73 to 66% (periods 1–3, Table 1), when operating at an OLR and SLR of 11.0–14.4 g COD/L/d and 0.53–0.62 g COD/g VSS/d, respectively. It should be noted that in addition to the influence of low temperatures in those periods, the OLR values also significantly increased to 16.1–16.5 g COD/L/d as a result of the increase in $COD_{in}$ concentrations, which resulted in overloading of the reactor in periods 4 and 5. Significant sludge washout occurred during these periods. Because the sludge was not returned to the reactor, the applied SLR (0.94–1.07 g COD/g VSS/d) was 40–60% higher, compared to the initial three

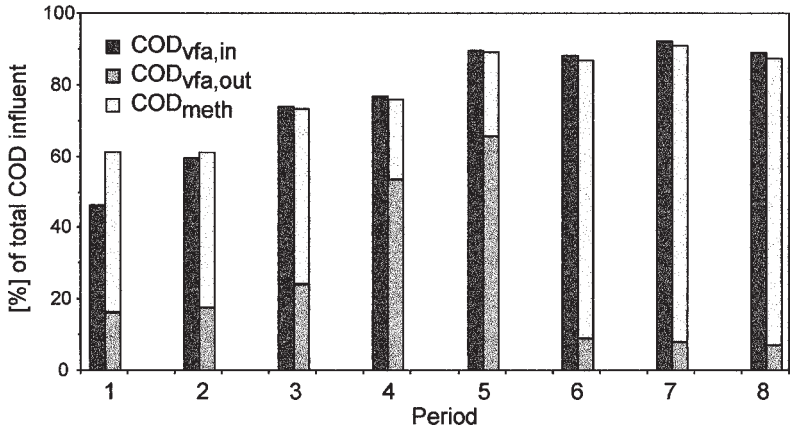


Fig. 3. Fate of $COD_{sol}$ in the EGSB system. First bar, $COD_{vfa}$ (▓) of the influent; second bar, effluent $COD_{vfa}$ (▓) + $COD_{meth}$ (▒) expressed as percentage of average influent total COD in the experimental periods.

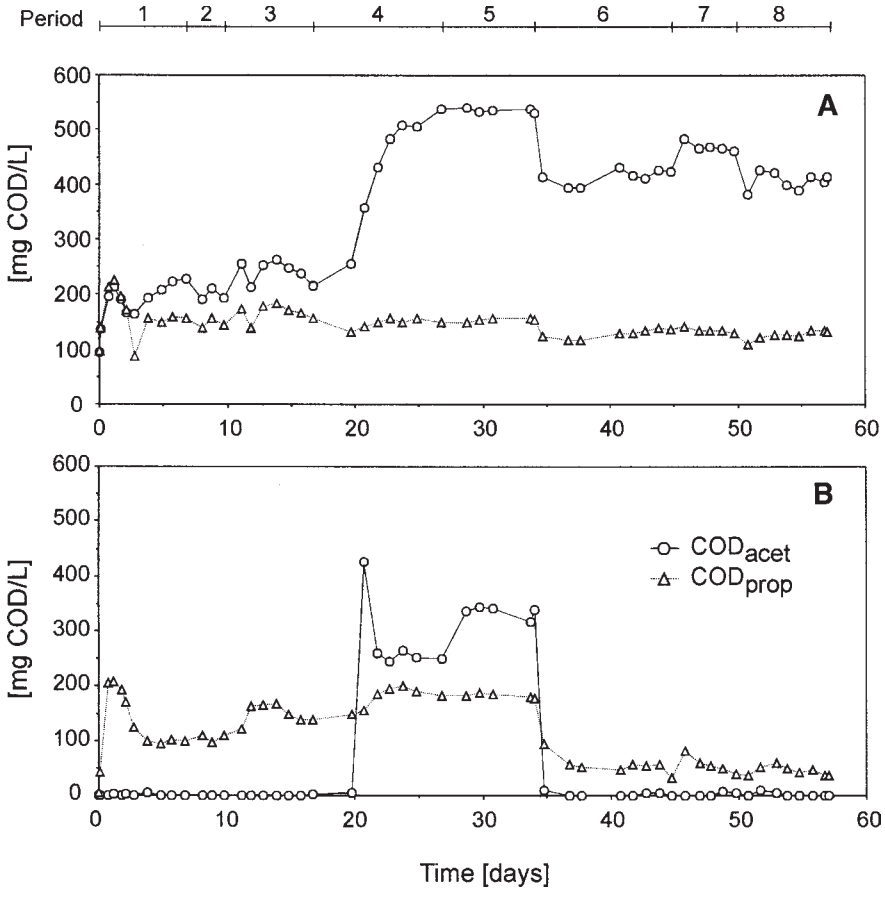


Fig. 4. Evolution of the VFA concentration in the influent **(A)** and the effluent **(B)** as a function of time: (○) $COD_{acet}$, (△) $COD_{prop}$.

Table 1
Average Values of Operational Conditions and Reactor Performance of Pilot-Scale EGSB Reactor Treating Brewery Wastewater[a]

| | Experiment I, period | | | | | Experiment II, period | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Days | 0–7 | 8–10 | 11–17 | 20–27 | 29–34 | 35–45 | 46–50 | 51–57 |
| Temperature (°C) | 30 | 26 | 20 | 15 | 12 | 20 | 20 | 20 |
| HRT (h) | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 2.1 | 1.7 | 1.2 |
| $V_{up}$ (m/h) | 5.8 | 5.8 | 5.8 | 5.8 | 5.8 | 4.4 | 6.2 | 7.2 |
| $COD_{in}$ (mg/L) | 720 | 550 | 600 | 825 | 805 | 656 | 715 | 630 |
| OLR (g COD/L/d) | 14.4 | 11.0 | 12.0 | 16.5 | 16.1 | 7.5 | 10.1 | 12.6 |
| SLR (g COD/g VSS/d) | 0.62 | 0.53 | 0.60 | 0.94 | 1.07 | 0.56 | 0.77 | 1.01 |
| COD removal (%) | 73 | 71 | 66 | 43 | 35 | 81 | 85 | 86 |

[a]SLR was corrected for the sludge washed out, and values refer to the end of each experimental period.

periods. At d 21 (period 4), an unintended pH drop (Fig. 2B) caused by a failure of the bicarbonate pump resulted in a very sharp decrease in COD removal efficiency (Fig. 2D). This might have contributed to the poor performance in that period, despite the apparent recovery of the reactor system after 24 h to the level of 43% of COD removal, which remained until the end.

The degradation of acetate on a COD basis was almost complete in periods 1–3, but decreased to about 50% in periods 4 to 5 (Fig. 4), when operating at 15°C (Fig. 2A). In the case of propionate, a slight degradation occurred in periods 1 and 2; however, in periods 3–5, the degradation was completely retarded (Fig. 4A and B). It was also observed that the VFA–COD influent increased from 45% of the total COD at the beginning of period 1 to 90% of the total COD at the end of period 5 (Fig. 3). In this experiment, decreasing the temperature from 30 to 12°C resulted in a decrease in the $COD_{in}$ converted into methane, from 45 to 16% (Fig. 3). This corresponded to a reduction in biogas production from 833 L/d to 42 L/d.

During the second experiment, the reactor temperature was set at 20°C, and different HRT values, ranging from 2.1 h to 1.2 h, were applied in periods 6–8 (Table 1). Differences in the applied liquid $V_{up}$ were obtained by using effluent recirculation in a ratio up to 0.8. Compared to experiment I, the reactor was fed with $COD_{in}$ levels of 630–715 mg/L, corresponding to OLRs up to 12.6 g COD/L/d. A good treatment performance, with a COD removal efficiency exceeding 80%, was obtained at an OLR of 7.5 g COD/L/d, when the reactor was operated at a $V_{up}$ of 4.4 m/h (period 6). Even when the reactor was operated up to 12.6 g COD/L/d, the efficiency exceeded 85%. In this case, the reactor was operated at a $V_{up}$ of 7.2 m/h (period 8). Much less sludge washout occurred in this experiment, compared to previous periods. Nevertheless, because of the accumulated sludge washed out prior

to the beginning of period 8, which was not returned to the reactor, the applied SLR achieved about 1.01 g COD/g VSS/d. In this experiment, the wastewater fed into the reactor can be considered as completely acidified, because more than 90% of $COD_{in}$ was composed of VFA (Fig. 3). The degradation of acetate was almost complete in these last three periods; in the case of propionate, the degradation increased from 0% at the beginning of period 6 up to 60–70% at the end of period 8 (Fig. 4A and B).

### *Sludge Washout*

Throughout the experiments, an operational difficulty observed was related to sludge washout, especially during specific days of experiment I. The total amount of sludge washed out collected in an external settler in periods 1–5 was 546, 314, 378, 935, and 359 g VSS, respectively. This signifies that, at the end of experiment I, about 45% of the seed sludge was washed out. Significant sludge washout mostly occurred during the days when reactor temperature changed from one experimental period to another. The occurrence of high washout at the liquid $V_{up}$ of 5.8 m/h applied in the whole of experiment I, was caused by the excessive expansion of the sludge bed. Moreover, during the days of maximal washout, sludge flotation caused by gas bubbles attached to granules was also observed. The flotation resulted in buoyancy forces driving part of the sludge bed upward from the bottom of the reactor. When this occurred, sludge washout occurred in a very short period of time. In the second experiment, despite even higher $V_{up}$ values being applied, less expansion of sludge bed occurred, since the initial amount of sludge was already reduced by almost one-half. The sludge washout in experiment II was decreased to only 10% of the values measured in periods 3–5, corresponding to an average effluent concentration of only 0.006 g COD/L.

### *Changes in Sludge Characteristics*

During the course of the experiments, changes in reactor sludge characteristics were observed (Tables 2 and 3). Most changes were related to methanogenic activity and the granule size of reactor sludge.

The maximum specific methanogenic activity of reactor sludge samples, with ethanol as the substrate at 20°C, are shown in Table 2. The assays were conducted with the seed sludge, the bottom and the top sludges from the EGSB reactor at d 34 and d 57. The sludge activity with ethanol decreased from d 0 to d 34 by approx 15%, either for the bottom or top reactor sludge. At d 57, the decrease was 30% for the bottom sludge, but, in contrast, activity of the top sludge increased by 40%, compared with that of d 0. The decreases of sludge activity on ethanol as substrate may be caused primarily by changes of the characteristic of the brewery wastewater fed into the reactor during experimental periods. The increased activity of the top sludge sample at the end of experiment II may be caused by the higher granule segregation that occurred in the sludge bed in the last days of the experiments, compared with that of d 0 and d 34. Apparently, the smaller granules in the top of the reactor

Table 2
Maximum Specific Methanogenic Activity
of Seed and EGSB Sludge with Ethanol as Substrate

| | | Maximum specific methanogenic activity ($g CH_4$-COD/g VSS/d) Ethanol | |
|---|---|---|---|
| Time | Temperature (°C) | 1st feeding[d] | 2nd feeding |
| Day 0[a] | 30 | 1.780 (0.086) | 1.760 (0.367) |
| | 20 | 0.629 (0.021) | 0.666 (0.022) |
| Bottom sludge | | | |
| Day 34[b] | 20 | 0.533 (0.047) | 0.585 (0.062) |
| Day 57[c] | 20 | 0.434 (0.003) | 0.351 (0.038) |
| Top sludge | | | |
| Day 34 | 20 | 0.532 (0.113) | 0.549 (0.002) |
| Day 57 | 20 | 0.863 (0.006) | 0.950 (0.038) |

Standard deviation is given between parentheses.
[a]Nedalco seed sludge after 4 mo of storage at 4°C.
[b]End of experiment I.
[c]End of experiment II.
[d]Product spectra of ethanol biodegradation by these corresponding sludges are given in Fig. 5.

Table 3
Maximum Specific Methanogenic Activity
of Seed and EGSB Sludge with VFA Mixture as Substrate

| | | Maximum specific methanogenic activity ($g CH_4$-COD/g VSS/d) VFA mixture | |
|---|---|---|---|
| Time | Temperature (°C) | 1st feeding | 2nd feeding |
| Day 0[a] | 30 | 0.334 (0.013) | 0.875 (0.020) |
| | 20 | 0.142 (0.018) | 0.160 (0.005) |
| Day 57[b] | 20 | 0.346 (0.005) | 0.403 (0.019) |

Standard deviation is given between parentheses.
[a]Nedalco seed sludge after 4 mo of storage at 4°C.
[b]End of experiment II with mixed sludge from the EGSB reactor.

were characterized by a higher sludge activity, compared to the larger granules at the bottom of the reactor. The batch activity tests also showed that the ethanol conversion pathway changed during experimental periods (Fig. 5). Acetate was the primary accumulated intermediate during ethanol conversion by the seed sludge. However, a significant shift occurred in the intermediate formation in the tests with sludges sampled at d 34 and d 57. Propionate accounted for 25 and 38% of the initial ethanol COD, respectively, for the bottom reactor sludge. Propionate accumulated up to 21 and 19%, respectively, for the top reactor sludge.

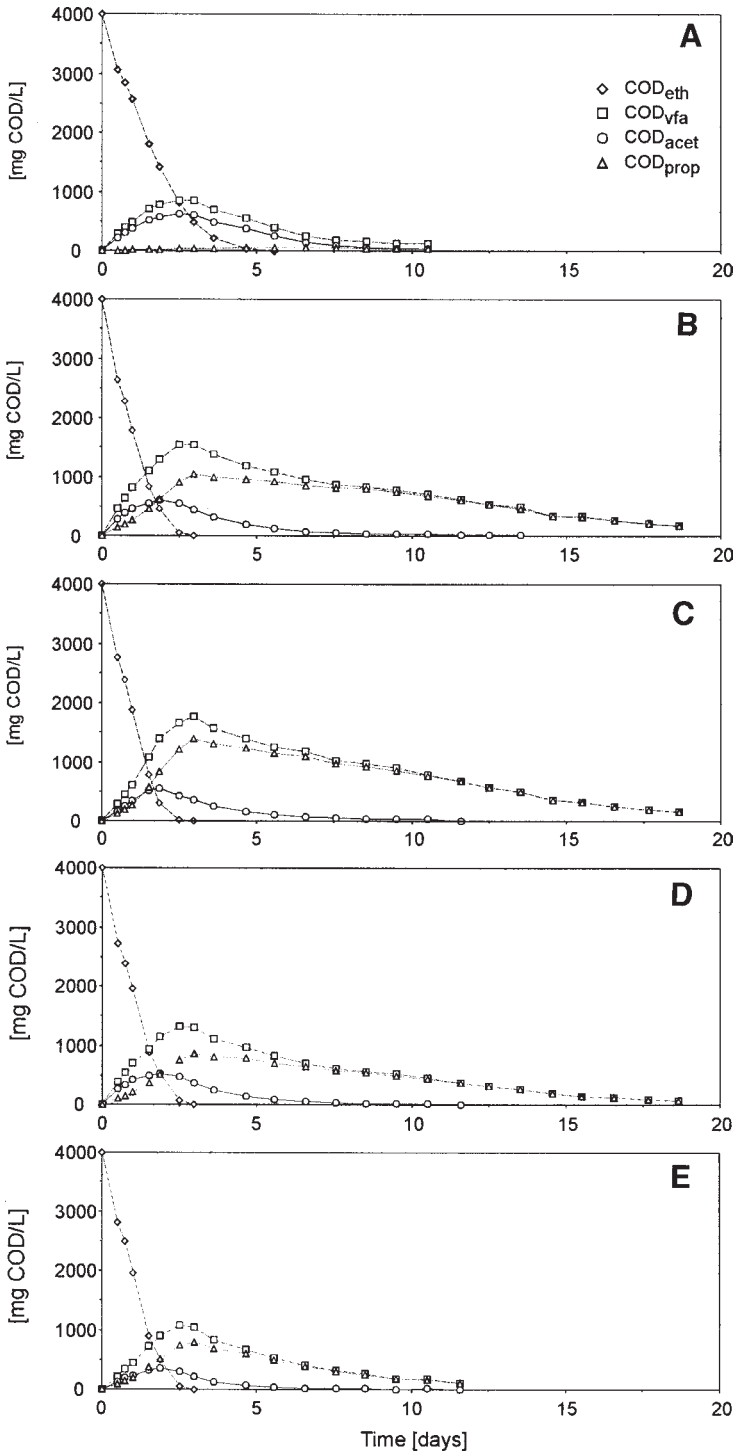


Fig. 5. Degradation of ethanol in batch experiments. **(A)** seed sludge. **(B)** Bottom sludge at d 34. **(C)** Bottom sludge at d 57. **(D)** Top sludge at d 34. **(E)** Top sludge at d 57. (◇) $COD_{eth}$, (□) $COD_{vfa}$, (○) $COD_{acet}$, (△) $COD_{prop}$.

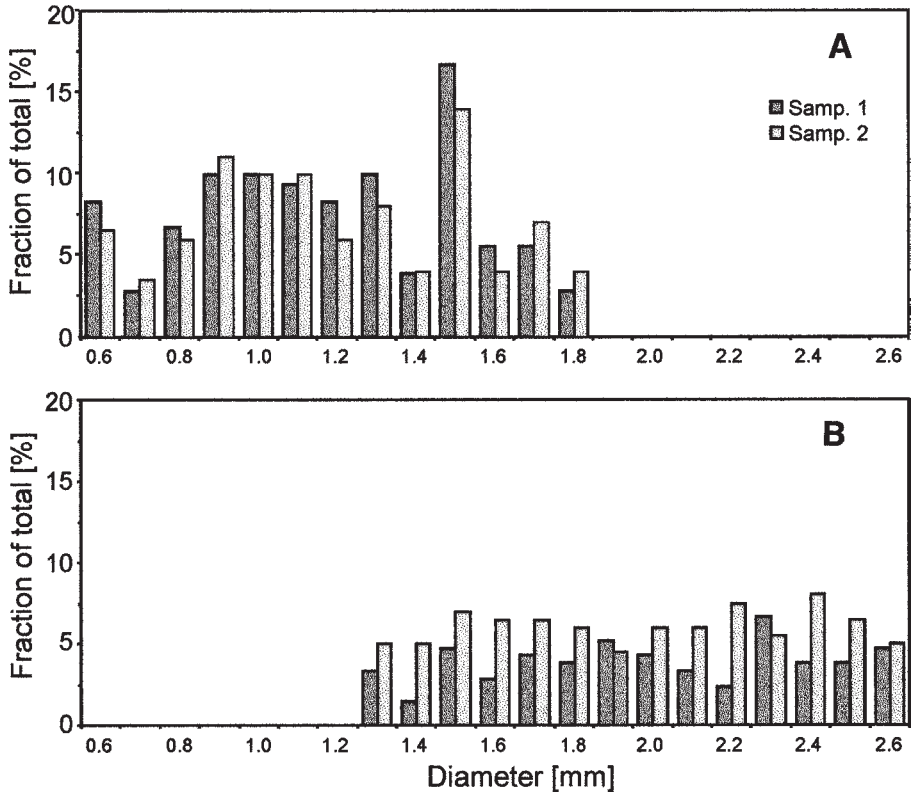


Fig. 6. Size distribution of mixed granular sludge at the start of the experiment **(A)** and at d 57 **(B)**, expressed in percentage of the biomass weight represented by the granules. Duplicate samples are presented by bars ▮▮.

The maximum specific methanogenic activity of the seed sludge and reactor sludge sampled on d 57, assessed with the VFA mixture, are presented in Table 3. The results reveal that methanogenic activity of seed sludge at 20°C was very low, even in the second feeding. However, activity was increased by a factor of 2.5 in a short period of time (34 d), taking into consideration that the sludge was overloaded (periods 4 and 5) with a new type of wastewater at the low temperatures applied (12–15°C).

The granular size distribution at the end of experiment II (d 57) was compared with that at the start of the experiment. Figure 6 shows that the seed sludge was formed by granules of 0.7–1.9 mm, with mean diameter of 1.2 mm; the final sludge was formed by granules of up to 2.6 mm, with mean diameter of 1.6 mm. Additionally, the size distribution was more uniform for the final sludge, compared with that of the seed sludge, because the fractions of granules between 1.3 and 2.6 mm were approximately the same. Moreover, although in the seed sludge, the granules with diameter below 1.2 mm were 40% of the total weight, in the final sludge the granules in that diameter range were only 20%. Aside from the increase in sludge diameter, the fines were washed out with the effluent during the course of the experi-

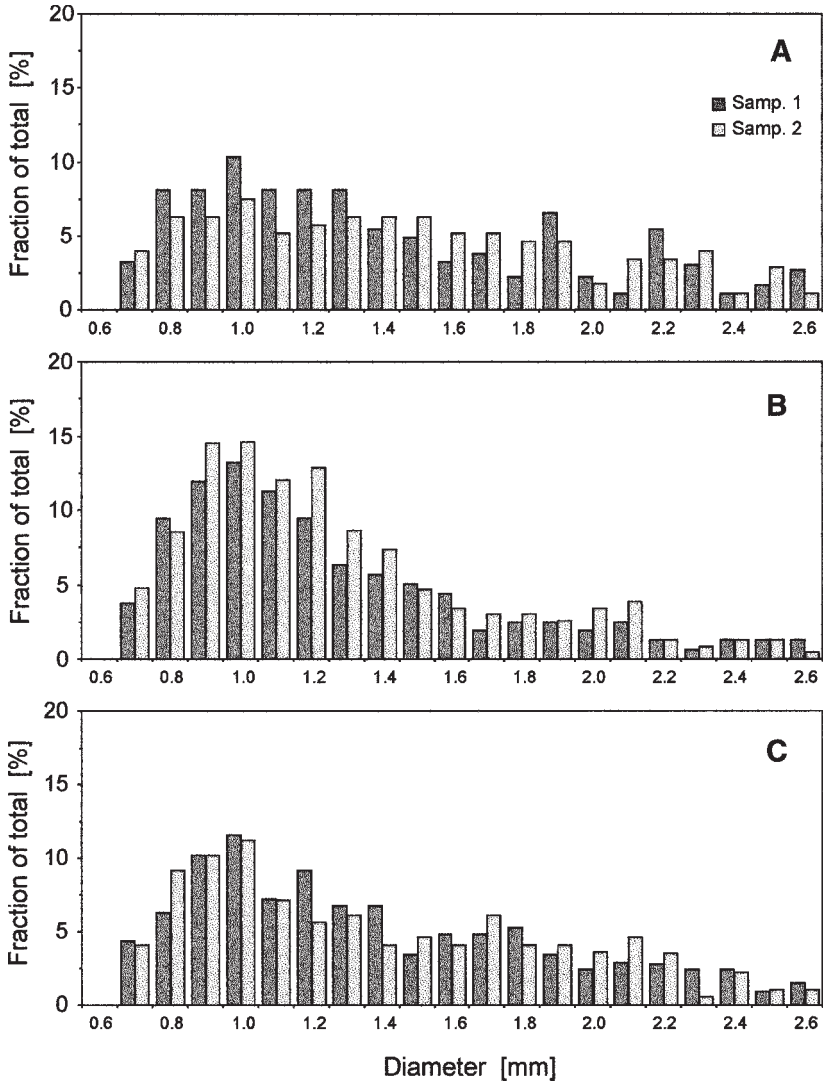


Fig. 7. Size distribution of washout of granular sludge during experiments **(A)** period 1, d 0–7; **(B)** period 3, d 11–17; **(C)** period 5, d 29–34, expressed in percentage of the biomass weight represented by the granules. Duplicate samples are presented by bars ▮▮.

mental periods, especially in the first experiment. Figure 7 shows the result of the size distribution assay from the sludge washed out during periods 1, 3, and 5. The fraction of smaller granules with diameter below 1.2 mm was 31, 75, and 55% of the total sludge weight, respectively.

## Discussion and Conclusions

This study showed that the EGSB reactor system is feasible for direct treatment of low-strength brewery wastewater, with some or most COD

consisting of VFA (<1000 mg COD/L influent) at lower temperatures (12–20°C). Decreasing the temperature from 30°C to 20°C resulted in a drop of the COD removal efficiency of about 10% (Table 1). A more serious drop in COD removal efficiency (43%) occurred when the operating system temperature was lowered to the range of 15–12°C. The decreasing efficiencies when lowering the temperature can be attributed first to overloading. The very high OLR applied (16.5 g COD/L/d) was above the maximum biological capacity of the reactor at almost any time, resulting in decreasing values of specific methanogenic activity of the sludge (Tables 2 and 3). Second, the reactor capacity was also influenced by severe sludge washout. Third, the lower activities may be caused by the gradual change in the wastewater composition, because of the increasing degree of acidification (Fig. 3). Partially acidified wastewater (300–400 mg VFA-COD/L) is important for obtaining high treatment efficiency at low temperatures, as shown in further experiments conducted in the same EGSB system with malting wastewater *(25)*. In the present experiments, the influent contained VFA concentrations above the 300–400 mg COD/L range. However, the reactor did not remove propionate (Fig. 4B), which is consistent with low activity tests (Table 3, 20°C). Thus, the shift in ethanol fermentation product from acetate to propionate (Fig. 5) also contributed to the lower COD removal capacity of the EGSB reactor (Fig. 2D). These results indicate that lower organic loads should be applied to achieve a higher treatment performance of low-strength acidified brewery-type wastewater at temperatures lower than 20°C in EGSB reactor system. Application of higher values of OLR (>8 g COD/L/d) requires long-term operation, which allows the development of a specific bacterial population in the granular sludge, as demonstrated with an EGSB system treating low-strength malting wastewater at low temperatures (20–13°C), when very good and stable reactor performance was obtained after 60 d of operation *(25)*.

In this study, when treating low-strength acidified brewery wastewater at 20°C, the EGSB reactor system showed good COD removal efficiency, exceeding 80% at organic loading rates up to 12.6 g COD/L/d. Such high organic loads could be accommodated at an HRT as low as 1.2 h at liquid $V_{up}$ applied up to 7.2 m/h. These results reveal that the potential of anaerobic treatment for various types of low-strength wastewaters at lower temperatures may be further explored using the EGSB reactor system, as was already shown in experiments with malting and acidified wastewaters *(25,26)*. One common observation in such experiments was that adequate hydraulic mixing is essential for achieving high COD removal efficiencies. Previous research with EGSB reactors also showed the importance of meeting that condition *(22)*. Because gas production is low at low temperatures, high hydraulic loads are to be applied to improve mixing intensity. The present results indicate that a higher COD removal efficiency was achieved when lower HRTs combined with higher $V_{up}$ using effluent recirculation, were applied, despite the increased organic loads (Fig. 2D). The primary conditions of adequate expansion of the sludge bed and good sludge holdup

were also met, because substantially less washout occurred, compared to the previous experiment of this study. Good reactor performance can also be explained by the increased reactor sludge activity. Specific methanogenic activity of the sludge at the end of this experiment, with acetate or the VFA mixture as substrates in batch tests at 20°C (Table 3), increased about three-fold, compared with that of the seed sludge. This may be an indication that some growth and enrichment of methanogens and acetogens occurred, probably mostly after the reactor was set at 20°C in experiment II. This agrees with the increase in granule diameter (Fig. 6). Additionally, almost no acetate was detected in the effluent, and more than 60% of the influent propionate was removed at the end of the experiments (Fig. 4).

The changes occurring in the specific methanogenic activity of the reactor sludge during the experiments can be attributed to the changes in temperature and wastewater characteristics, which influenced the formation of intermediate products and, consequently, the microbial populations. The considerable change in the ethanol degrading pathway in the batch activity tests, especially concerning the formation of propionate as intermediate and its further degradation (Fig. 5), might be caused by the presence of sulfate at very low concentration in the reactor influent. The latter might support the growth of *Desulfobulbus propionicus* in the granular sludge, a bacterium known to form propionate during the anaerobic degradation of ethanol. Similar low influent sulfate concentrations and shifts in ethanol-degrading pathway have also been observed during the treatment of malting wastewater *(25)*.

The increased mean diameter of reactor sludge at the end of the experiments confirms the effect of high liquid $V_{up}$ applied in the EGSB, as shown in other studies *(22,25,27)*. High $V_{up}$ enhances the shear forces on granules that may result in erosion and some granule segregation in the sludge bed. The fact that a significant amount of the washed-out sludge was of small particles may be attributed to erosion of the seed granules, resulting in fines having diminished settleability (Fig. 7). A large amount of fines was observed visually in the collected washed-out sludge. Very fine suspended anaerobic solids with powder-like appearance floated on the surface of the external settler. The apparently more polished and uniform size distribution of final reactor sludge also indicates the effect of higher shear forces (Fig. 6).

The granular segregation in the expanded sludge bed, caused by the liquid $V_{up}$ applied, was also observed in EGSB reactor experiments *(22,25)*. First, despite the high values of $V_{up}$ in the experiments, resulting in an expansion of the bed over the whole reactor height, the lower part remained less expanded, compared to the upper part. This was especially the case when the reactor was started up, and until the end of experiment I, because the inlet system was probably not optimal. The poor expansion of the lower bed may have caused dead zones, channeling, and gas pockets. Much of the sludge washout was hydraulically assisted, because the expansion was already excessive. However, the strong washout observed was probably caused by the gas accumulated in the dense unexpended lower sludge bed,

because it occurred only on certain days. Gas pockets exploded and sludge bed moved upward as a result of the buoyancy forces dragging up the particles. Examples of such occurrence were observed in periods 1 and 2, when higher gas production up to 833 L/d resulted in pocket volumes up to 35 L every hour. This volume represented about 33% of the volume occupied by the sludge in the reactor. Later, in experiment II, because the sludge washed out was not returned to the reactor, resulting in a smaller sludge bed, and because recirculation started to be applied, increasing the $V_{up}$, the expansion bed was more homogeneous, causing significantly fewer problems associated with sludge holdup. Second, the size and settleability of granules may indicate segregation by gravitational forces. Based on the sludge size, a clear segregation between the top and down part of reactor bed was observed previously *(22,25)*, and in this work. Higher granule diameters were found for the sludge at the bottom, compared with those of the top. Based on the results of this study, higher treatment efficiencies can be expected using the EGSB reactor system, in the case of low-strength acidified brewery-type wastewater at low temperatures. The results of this and other EGSB experiments confirm the requirement of high hydraulic loads to ultimately enhance the wastewater–biomass contact. In addition to setting the proper operational conditions needed to meet that requirement, the EGSB reactor also needs an improved device for solid–liquid–gas separation. The development of a good system for sludge holdup will minimize the limitation for the EGSB application in practice.

## Acknowledgments

Financial aid of the Ministry of Education/CAPES, Brazil to M. T. K. and the Wageningen Agricultural University to S. R. is gratefully acknowledged. The authors thank J. van der Laan and Ilse Bennehey for technical assistance with the chromatographs, and Nedalco and Bavaria B. V., The Netherlands, for their cooperation.

## References

1. Kato, M. T. (1994. ), PhD Thesis, Landbouwuniversiteit Wageningen, The Netherlands.
2. Bhaskaran, T. R. (1973), *Guidelines for the Control of Industrial Wastes. 8. Brewery Wastes*. World Health Organization (WHO/WD/73. 15), Geneva, Switzerland.
3. García, P. A., Rico, J. L., and Fernandez-Polanco, F. (1991), *Environ. Technol.* **12,** 355–362.
4. Isaac, P. C. G. and McFiggans, A. (1981), in *Food Industry Wastes: Disposal and Recovery*, Herzka, A. and Booth, R. G., eds., Applied Science, London, pp. 144–160.
5. Vereyken, T. L. F. M., Swinkels, K. Th. M., and Hack, P. J. F. M. (1986), in *Proceedings of the Water Treatment Conference, Anaerobic Treatment a Grown-up Technology, AQUATECH '86*. Amsterdam, The Netherlands, pp. 283–296.
6. Craveiro, A. M., Soares, H. M., and Schmidell, W. (1986), *Water Sci. Technol.* **18,** 123–134.
7. Lettinga, G. and Hulshoff Pol, L. (1986), *Water Sci. Technol.* **18,** 99–108.
8. Maaskant, W. and Zeevalkink, J. A. (1983), in *Proceedings of the European Symposium on Anaerobic Waste Water Treatment*, van den Brink, W. J., ed., Noordwijkerhout, The Netherlands, pp. 430–439.

9. Cheng, S. S., Lay, J. J., Wei, Y. T., Wu, M. H., Roam, G. D., and Chang, T. C. (1989), in *Proceedings of the International Symposium on Waste Management Problems in Agro-Industries*, Istanbul, Turkey, pp. 213–220.
10. Fang, H. H. P., Guohua, L., Jinfu, Z., Bute, C., and Guowei, G. (1990), *J. Environ. Eng.* **116,** 454–460.
11. Hanaoka, T., Nakahata, S., Shouji, T., Sasaki, M., Maaskant, W., and Wildschut, L. (1994), in *Proceedings of the Seventh International Symposium on Anaerobic Digestion*, Cape Town, South Africa, pp. 518–527.
12. Hulshoff Pol, L. W., Dekkers, F., and Tersmette, T. (1991), in *Proceedings of the Sixth International Symposium on Anaerobic Digestion*, São Paulo, Brazil (poster).
13. Keenan, J. D. and Kormi, I. (1981), *J. Water Pollut. Control. Fed.* **53,** 66–77.
14. Lovan, C. R. and Foree, E. G. (1971), in *Proceedings of the 26th Industrial Wastes Conference* (Bell, J. M., ed.), Purdue University, West Lafayette, IN, pp. 1074–1086.
15. Lettinga, G. (1982), in *Anaerobic Reactor Technology*, Agricultural University, Wageningen, The Netherlands, pp. 88–95.
16. Lettinga, G. and Hulshoff Pol, L. W. (1991), *Water Sci. Technol.* **24,** 87–107.
17. Lettinga, G., Rinzema, A., and Hulshoff Pol, L. W. (1992), in *Proceedings of the Third Workshop Working Group on Biogas Production Technologies*. *CNREE Network on Biogas Production and Conversion for Energy*, Baader, W., ed., Braunschweig, Germany, pp. 73–90.
18. Last, A. R. M. van der and Lettinga, G. (1991), in *Proceedings of the Sixth International Symposium on Anaerobic Digestion*, São Paulo, Brazil, pp. 153–154.
19. Man, A. W. A. de, Last, A. R. M. van der, and Lettinga, G. (1988), in *Proceedings of the Fifth International Symposium on Anaerobic Digestion*, Bologna, Italy, Hall, E. R. and Hobson, P. N., eds., Pergamon, Oxford, England, pp. 197–209.
20. Lettinga, G., Man, A. de, Last, A. van der, Kato, M., Wang, K., and Rebac, S. (1993), in *The Second Japan/Dutch Workshop on Water Quality Management*, Ube, Japan.
21. Rinzema, A. (1988), PhD Thesis, Landbouwuniversiteit, Wageningen, The Netherlands.
22. Kato, M. T., Field, J. A., Versteeg, P., and Lettinga, G. (1994), *Biotechnol. Bioeng.* **44,** 469–479.
23. Hulshoff Pol, L. W., Worp, J. J. M. van de, Lettinga, G., and Beverloo, W. A. (1986), in *Proceedings of the Water Treatment Conference AQUATECH '86*, Amsterdam, The Netherlands, pp. 89–101.
24. American Public Health Association (1985), *Standard Methods for the Examination of Water and Wastewater*, 16th ed., Washington, D. C.
25. Rebac, S., Lier, J. B. van, Janssen, M. G. J., Dekkers, F., Swinkels, K. Th. M., and Lettinga, G. (1997), *J. Chem. Tech. Biotechnol.* **68,** 135–146.
26. Rebac, S., Ruskova, J., Gerbens, S., Lier, J. B. van, Stams, A. J. M., and Lettinga, G. (1995), *J. Ferm. Bioeng.* **80,** 499–506.
27. Guiot, S. R., Arcand, Y., and Chavarie, C. (1992), *Water Sci. Technol.* **25,** 897–906.