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ANAEROBIC TREATMENT OF SYNTHETIC SULFATE-CONTAINING WASTEWATER UNDER THERMOPHILIC CONDITIONS

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ABSTRACT

The anaerobic treatment of a sulfate-containing waste water using a UASB reactor was studied at 55 °C. As substrate, acetate and a mixture of acetate, propionate and butyrate were used. With acetate as substrate it was shown that sulfate reducers are capable of using acetate as substrate at 55 °C, and that, under the conditions applied, they even outcompete acetoclastic methanogens. Batch-activity measurements with the sludge revealed temperature optima for acetate, propionate and butyrate degradation of ± 56-59, < 40 and 52-54 °C respectively.
After switching the substrate to a mixture of acetate, propionate and butyrate, the reactor pH dropped from 8.3-8.6 to 7.6-7.9 and the methane production recovered. After the establishment of a pseudo-steady state situation the part of COD removed by methane production and sulfate reduction was ± 60 and 40 %. Results of batch activity experiments showed that the methanogenic activity dropped sharply at pH ≥ 8 and ≤ 6 causing a predominance of sulfate reducers at pH ≥ 8.

KEYWORDS

Anaerobic thermophilic treatment ; UASB reactor ; sulfate reduction optimum temperature ; methanogenesis ; pH ; competition.

INTRODUCTION

A better understanding of the sulfate reduction process in anaerobic reactor systems is becoming increasingly important. This applies both for mesophilic as well as thermophilic systems.

In the anaerobic treatment of sulfate-containing waste water sulfate reducers have to compete with methanogens for hydrogen and acetate. Research in this field has been focussed so far mainly on competition under mesophilic conditions. Experimental data on marine and freshwater sediments (Winfrey and Zeikus, 1977; Banat, 1981; Lovley et al., 1982) and in completely mixed reactors (CSTR's) without biomass retention (Middleton and Lawrence, 1977; Olthof et al., 1985) show that in presence of excess sulfate, sulfate reducers will outcompete methanogens ; sulfide is then the end product of the anaerobic mineralization process. Experimental data obtained with anaerobic reactors with sludge retention (Upflow Anaerobic Sludge Blanket Reactor (UASB), Anaerobic filter) reveal that the hydrogen produced as an intermediate in anaerobic digestion is generally completely used by sulfate reducers, while the utilization of acetate is rather unpredictable. It most cases it was found that acetate is completely converted into methane (Mulder, 1982; Hoeks et al., 1984; Rinzema and Lettinga, 1988), while in a few cases it was observed that acetate was used by sulfate reducers (Rinzema and Schultz, 1987).

Compared with mesophilic anaerobic treatment, only few experimental data are available about thermophilic anaerobic treatment of sulfate-containing waste water. Although a number of thermophilic methanogens have been isolated and described, only a few pure cultures of thermophilic sulfate reducers are known, Desulfotomaculum nigrificans, Desulfovibrio thermophilus, Thermodesulfobacterium commune (Widdel, 1988) and Desulfotomaculum thermoacetoxidans (Min and Zinder, 1990), the only thermophilic sulfate reducer known capable of using acetate.

The aim of this study was to describe the anaerobic breakdown of sulfate-containing waste water at 55 °C and to assess the competition between thermophilic sulfate reducers and methanogens.

MATERIAL AND METHODS

Apparatus

A 5.75 litre upflow anaerobic sludge blanket (UASB) reactor was used, equipped with a stirring blade which was intermittently used (5 seconds every 30 minutes at 100 rpm). The temperature was controlled by a thermostat bath - circulator connected to the double wall of the reactor. Methane production was measured with a wet test gasmeter (Meterfabriek Dordrecht, The Netherlands) at ± 25 °C after contacting the evolved biogas with a 1 M NaOH solution and a column of soda lime pellets. (Fig. 1)

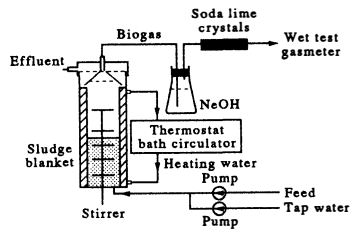

Fig. 1. Scheme of the 5.75 l UASB reactor

Biomass

The reactor was seeded with 2.5 l mesophilic granular sludge from the UASB reactor of the Aviko potato processing factory at Steenderen, The Netherlands.

Medium

A concentrated substrate stock solution of pH 6-6.2 containing volatile fatty acids (acetate or a mixture of acetate, propionate and butyrate) and sodium sulfate was used.

A basal nutrient solution (60 ml/l) and a trace element solution (10 ml/l) were added to the stock solution. The basal nutrient solution consisted of (g/l) : NH_4Cl (174), KH_2PO_4 (28), $(NH_4)_2SO_4$ (28), $MgCl_2.6H_2O$ (25), KCl (45) and yeast extract (3). The trace element solution consisted of (mg/l) : $FeCl_2$ (2000), $MnCl_2$ (500), resazurin (500), EDTA (500), Na_2SeO_3 (100), H_3BO_3 (50), $ZnCl_2$ (50), $(NH_4)_6Mo_7O_{24}.4H_2O$ (50), $AlCl_3$ (50), $CoCl_2$ (50), $CuCl_2$ (50) and HCl 36 % (1 ml/l). The concentrated stock solution was diluted with tap water in situ. The tap water contained about 35 mg Ca^{++}/l.

All the chemicals were of analytical grade and were supplied by Merck, Darmstadt, FRG, except yeast extract which was supplied by Gist Brocades, Delft, The Netherlands.

Batch assays experiments

Two sets of batch activity assays were used. In the first set the maximum specific sludge activity was assessed in relation to the temperature. The test was performed in a 0.1 L serum bottle at a sludge content of ≈ 3 g VSS/l, using acetate, propionate or butyrate (2 g COD/l) and sodium sulfate (4 g SO_4/l) as substrate. A nutrient solution was added to the serum bottle (10 ml/100 ml). This nutrient solution consisted of (g/l) : $NaHCO_3$ (100), K_2HPO_4 (3), KH_2PO_4 (4), basal nutrient solution (60 ml/l) and trace element solution (10 ml/l). During the test volatile fatty acid and sulfate concentrations were followed in time.

In the second set of assays the maximum specific methanogenic sludge activity with acetate as substrate, was assessed as a function of the pH. The methanogenic activity was measured with the "headspace method" as has been described by Koster et al (1986).

Analysis

Volatile fatty acid concentrations were measured by gas-chromatography (glass column 2m x 4mm, packed with Supelcoport (100-200 mesh) coated with 10 % Fluorad FC 431, temperatures (°C) : column 130, injection port 220, flame ionization detector 240, carrier gas (50 ml/min) nitrogen saturated with formic acid). Sulfate was measured by ion-chromatography (packing Chrompack Ionosphere, differential refractometer, eluent potassium biphthalate 0.04 M, pH 4.2). Sulfide was measured photometrically (Trüper and

Schlegel, 1964). pH of the samples was determined with a pH-electrode immediately after taking the sample.

RESULTS

UASB reactor

Table 1 summarizes the average influent composition, effluent pH and H_2S concentrations, and the treatment results achieved in the UASB reactor.

The reactor performance throughout the experiment is shown in Figure 2 (loading rate), Figure 3 (total sulfide and H_2S concentrations in the effluent), Figure 4 (effluent pH) and Figure 5 (treatment results). The hydraulic retention time of the reactor during the experiments was 8-9 hours. The sludge in the reactor, seeded with granular mesophilic sludge, remained granular throughout the experiment.

TABLE 1 Influent Values, Effluent Values And Treatment Results In The UASB Reactor

	INFLUENT				EFFLUENT		COD-REMOVAL EFF.			
day	C2	C3	C4	SO_4	H_2S	pH	COD	CH_4COD	SO_4COD	T
	g COD/l			g/l	mg S/l		%	%	%	°C
0-21	2	0	0	1	2	7.5-8	95	75-85	0-3	30
22-115	2	0	0	4	5-10	8.3-8.6	90	0-2	65-75	55
116-235	0.7	0.7	0.7	4	15-25	7.6-7.9	95	50-60	30-40	55

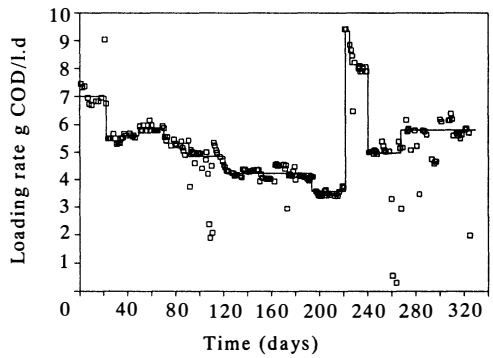

Fig. 2. The applied organic loading rate

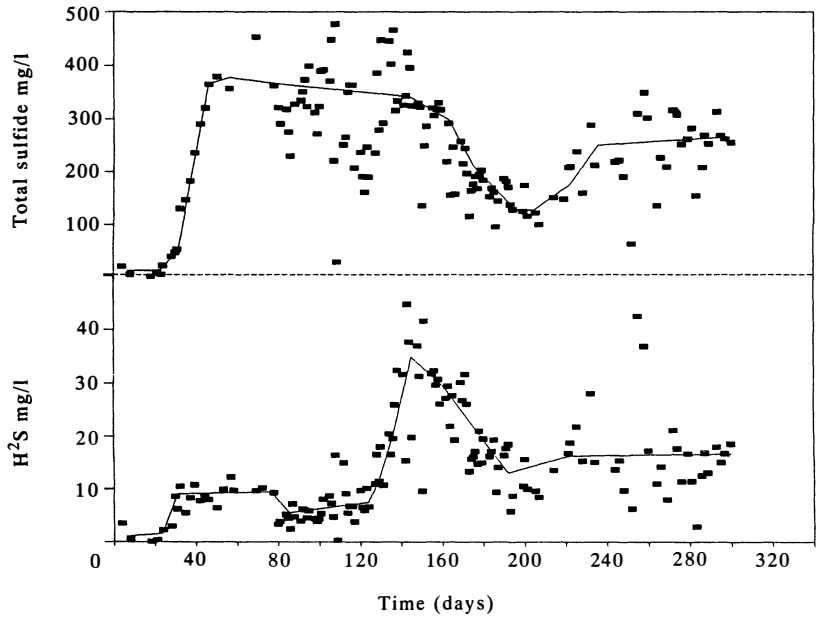

Fig. 3. Sulfide concentrations in the effluent

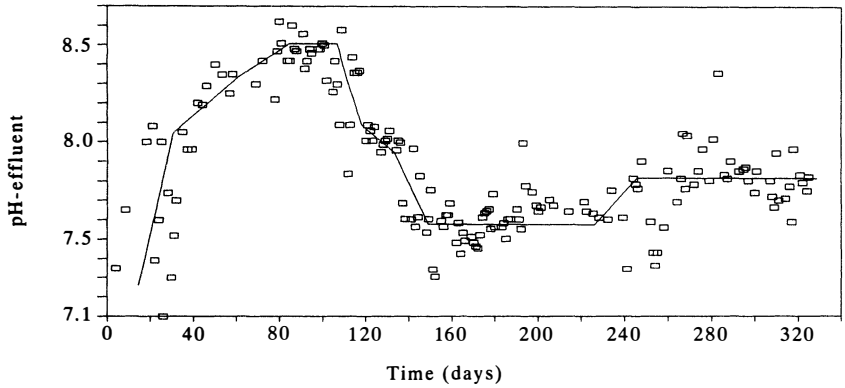

Fig. 4. Effluent pH

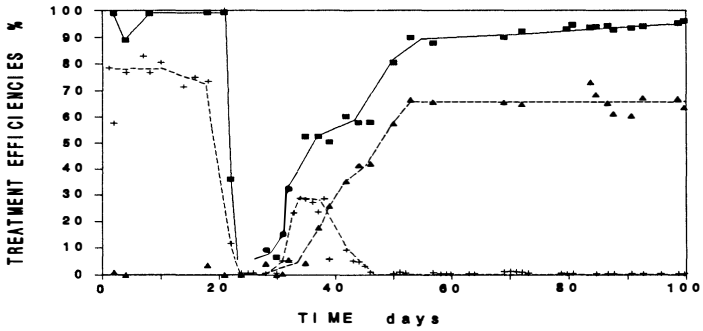

Fig. 5a.

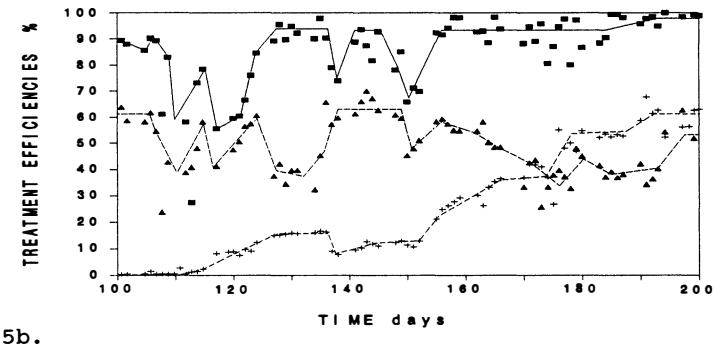

Fig. 5b.

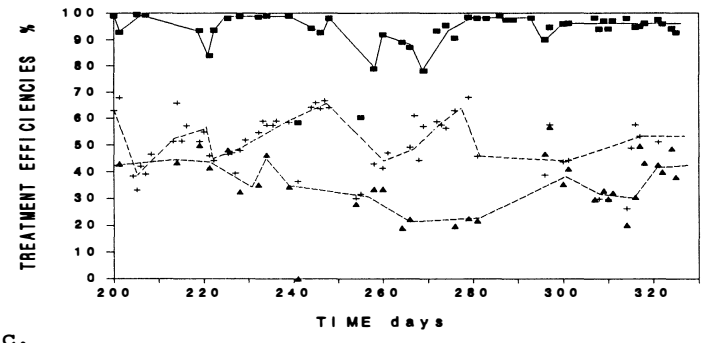

Fig. 5c.

Fig. 5 a,b,c. Removal efficiencies : Total COD removal efficiency (■), COD removal efficiency by methane production (+) and by sulfate reduction (▲) during day 0-100 (Fig. 5a.), day 100-200 (Fig. 5b.) and day 200-325 (Fig. 5c.).

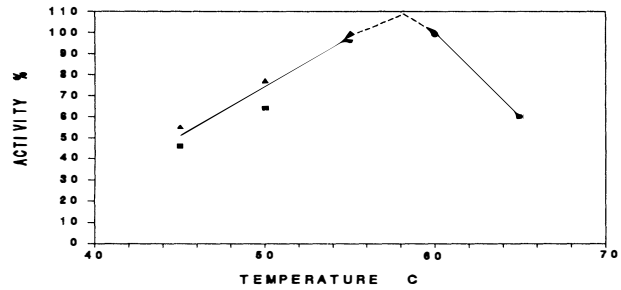

Fig. 6a. 100 % = 0.13 g COD/g VSS.d
0.10 g SO_4COD/g VSS.d

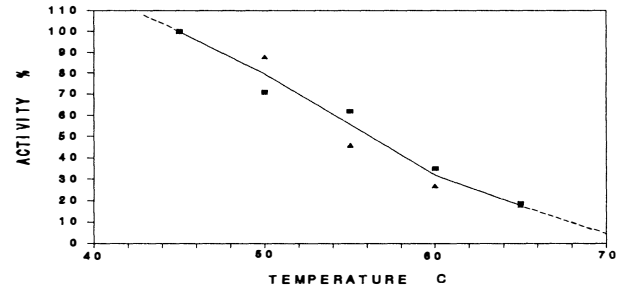

Fig. 6b. 100 % = 0.04 g COD/g VSS.d
0.04 g SO_4COD/g VSS.d

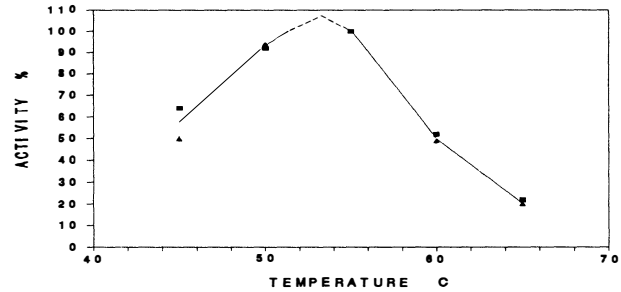

Fig. 6c. 100 % = 0.10 g COD/g VSS.d
0.09 g SO_4COD/g VSS.d

Fig. 6 a,b,c. Sludge activity based on VFA measurements (■) and sulfate measurements (▲) as a function of the temperature for acetate (Fig. 6a.), propionate (Fig. 6b.) and butyrate (Fig. 6c.) as substrate.

Batch experiments

Figure 6 shows the sludge activities in relation to the temperature for acetate (Fig 6a), propionate (Fig 6b) and butyrate (Fig 6c) as substrate. Figure 7 shows the acetoclastic methanogenic activity at 55 °C as a function of the pH.

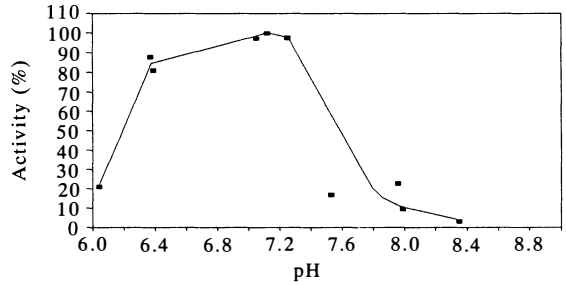

Fig. 7. Methanogenic activity as a function of the pH with acetate as substrate

DISCUSSION

The reactor was started up under mesophilic conditions (30 °C) using as feed a solution of acetate and sulfate. Under these conditions acetate removal was accomplished almost completely by methanogenic bacteria. Only about 0-3 % of the acetate removal was accomplished by sulfate reducing bacteria (Fig. 5a.).

After three weeks of operation at 30 °C the temperature was increased to 55 °C, resulting in an almost immediate and complete cessation of the (mesophilic) methane production and acetate removal. However, after about 1 week the acetate breakdown and methane production recovered, while at the same time sulfate reduction was observed. Both the acetate breakdown and sulfate reduction gradually improved further, but the contrary occurred with the methane production. The methanogenesis even stopped completely at day 45. From then onwards acetate degradation was accomplished completely by sulfate-reducing bacteria (Fig. 5a.), indicating that at 55 °C and under the conditions prevailing in the system, sulfate reducers can outcompete acetoclastic methanogenic bacteria. Specific activity assays with acetate as substrate confirmed the predominance of sulfate-reducing bacteria in the system. If no sulfate was added during the activity test, acetate breakdown did not occur.

The sulfate-reducing sludge was found to exert a maximum specific activity for acetate, propionate and butyrate breakdown at ± 56-59 °C (Fig. 6a.), ≤ 40 (Fig. 6b.) and 52-54 °C (Fig. 6 c.) respectively. These results suggest that breakdown of propionate can mainly be attributed to mesophilic rather than thermophilic organisms. The results are in accordance with findings of lab-scale experiments in which sludge cultivated on a mixture of

propionate, acetate and sulfate at 55 °C, a temperature drop to 30 °C did not show any effect as far as the propionate removal efficiency is concerned. The acetate removal efficiency of the sludge however decreased sharply (A. Visser unpublished results).

After 115 days of operation the feed was changed from acetate to a mixture of volatile fatty acids. This substrate switch resulted in a decrease in effluent pH from 8.3-8.6 to 7.6-7.9 (Fig. 4.). This decrease in pH was accompanied by a resuming of the methane production (Fig. 5b.), and it gradually increased to 50-60 % COD-conversion into methane, resulting in a drop in sulfate reduction to 35-45 % of the COD-conversion (Fig. 5 b,c.).

These results suggest a strong effect of the pH on the competition between thermophilic sulfate reducers and methanogenic bacteria. Apparently sulfate reducers predominate at high pH (8.3-8.6) while a kind of equilibrium between sulfate reducers and methanogens is established at more neutral pH (7.6-7.9). After a steady state situation was reached, the methanogenic activity of the sludge was measured at 55 °C as a function of the pH . The results (Fig. 7.) obtained clearly reveal the important role of the pH in the competition between sulfate-reducing and methanogenic bacteria.

The methanogenic activity of the sludge was found to be relatively low at pH $\geq$ 8 and pH $\leq$ 6. Obviously, pH values $\geq$ 8 inhibit methanogenesis, giving sulfate-reducing bacteria the opportunity to outcompete methanogens in the UASB reactor. pH values $\leq$ 6 also inhibit methanogenesis, but so far no experimental data are available for the effect of low pH values on sulfate reducers, so that it is uncertain yet whether or not at pH $\leq$ 6 sulfate reduction would predominate.

The observed sharp decrease in methanogenic activity at pH $\geq$ 8 was not observed for a thermophilic sludge cultivated on volatile fatty acids in the absence of sulfate ; in that case a more gradual decrease in methanogenic activity was found (J. van Lier, unpublished results). It looks like the occurrence of sulfate reduction leads to the development of a more pH-sensitive population of methanogens, than in the case of absence of sulfate reduction.

Another factor that can influence competition between sulfate reducers and methanogens might be found in the presence of sulfide, an inhibitory compound for methanogens. The toxic effect of sulfide is believed to be caused by the undissociated H_2S. H_2S levels measured in the reactor ranged from 5-10 mg/l at pH 8.3-8.6 to 15-25 mg/l at pH 7.6-7.9 (fig 3). It can be seen that in the absence of methane production (pH 8.3-8.6) the H_2S concentration is lower than when 50-60 % of the COD is removed by methanogenesis (pH 7.6-7.9). This finding suggests a much lower effect of sulfide than of the pH. So far no experimental data about sulfide toxicity for thermophilic methanogens are available, so that the effect of sulfide on competition between sulfate reducers and methanogens remains uncertain.

CONCLUSIONS

During anaerobic treatment of sulfate-containing waste water at 55 °C sulfate reducers have to compete with methanogens. This competition is influenced by the pH. pH values ≥ 8 strongly inhibit methanogenesis, giving sulfate reducers sufficient advantage to outcompete methanogens. At more neutral pH values an equilibrium between sulfate reduction and methanogenesis is established.

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