Sulfidogenic volatile fatty acid degradation in a baffled reactor

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Abstract The effect of staging the sludge bed on volatile fatty acid degradation by sulfidogenic reactors was evaluated in a baffled reactor. In a 5.4 l baffled reactor, containing three equal compartments, a volatile fatty acid (VFA) mixture (acetate:propionate:butyrate ratio 1:2:2 on COD basis; pH 8) was treated under mesophilic (30°C) and sulfidogenic (COD:SO₄²⁻ ratio: 0.5) conditions for 38 days. At a specific sludge loading rate of 0.50 g COD.gVSS⁻¹.d⁻¹, a COD and sulfate removal of 85% and 30%, respectively, was obtained. In the baffled reactor, staging of the sulfidogenic VFA degradation occurred. Propionate and butyrate were mainly degraded in the first compartment. Their degradation was incomplete, resulting in elevated acetate concentrations in compartment I. In the second and third compartment of the baffled reactor, a net degradation of acetate took place. Acetate was the sole substrate present in compartment III and residual acetate concentrations of about 200 mg/l were present in the effluent at a specific sludge loading of 0.50 g COD.gVSS⁻¹.d⁻¹. Sludges with different maximum specific VFA and acetate degrading activities developed in the first and second compartment. These maximal specific activities were almost equal for sludge present in compartment II and III.

Keywords Anaerobic digestion; baffled reactor; sulfate reduction; VFA

Introduction
In sulfidogenic reactors, organic matter is predominantly removed by sulfate reducing bacteria (SRB). These anaerobic reactor systems are applied when a maximum sulfate reduction is wanted, i.e. for the removal of sulfur and/or heavy metals from wastewaters (Vallero et al., 2003; Hulshoff Pol et al., 1998). In high-rate sulfidogenic reactors supplied with organic matter as electron donor, acetate degradation is the rate limiting factor (Visser et al., 1993; Dries et al., 1998). The maximum specific acetate (C₂) removal rate at which a complete acetate removal is achieved amounts to 0.4 gC₂-Chemical Oxygen Demand (COD) per g volatile suspended solids (VSS) per day (Omil et al., 1996). Higher acetate loading rates result in residual effluent acetate levels of the sulfidogenic reactor, which imposes the need for further treatment of the effluent.

Lens et al. (1998) investigated the option to stage the VFA degradation in an upflow staged sludge bed (USSB) reactor to optimise the treatment performance of sulfidogenic reactors. Since acetate is the most recalcitrant VFA, it can be expected to be the main substrate in the last compartments of a USSB reactor, thus allowing the proliferation of a specific population of acetotrophic SRB (ASRB). The USSB reactor was shown to be effective with respect to staging of the VFA conversions (Lens et al., 1998), but the specific acetotrophic SRB population developed rather slowly. In the present study, the VFA removal efficiency as well as staging of their degradation was investigated in a horizontally staged reactor configuration, i.e. a baffled reactor.

Materials and methods
Experimental set-up
A baffled PVC reactor with three compartments of 1.8 l each was used for the experiments.
The reactor, described in detail in Lens et al. (2000), had one large lid on top, containing gas exits and openings for pH electrodes and sampling ports. The reactor design did not separate the gas phases of the different compartments, so that all three compartments contained the same gas phase. The baffled reactor was fed using Watson Marlow pumps (Watson Marlow, Falmouth, Cornwall, UK) and operated at 30°C using a thermostat bath (type 202 U). No effluent recirculation was installed. The pH was measured in compartment I using a sulfide resistant Flushtrode pH electrode (Hamilton, Hilkomij bv, Rijswijk, The Netherlands).

Medium
The baffled reactor was fed with a synthetic influent (pH 8.0) consisting of a mixture of acetate, propionate and butyrate (COD ratio of 1:2:2), sodium sulfate (COD/sulfate ratio 0.5) and macro and micronutrients (Visser et al., 1993). The influent COD and SO$_4^{2-}$ concentrations varied, as a function of the organic loading rate (OLR), between 0.6 to 1.5 g COD.l$^{-1}$ and between 1.2 to 3.0 g SO$_4^{2-}$.l$^{-1}$.

Inoculum
The baffled reactor was inoculated with 3.0 l granular sludge from a sulfidogenic UASB reactor (Lens et al., 1998), cultivated on the same synthetic wastewater as used in this study. The inoculum sludge had a VSS content of 14.6 mgVSS/g wet sludge and the total reactor was inoculated with 43.8 g VSS. The inoculum sludge was equally distributed over the three compartments.

Experimental design
The influent flow rate to the baffled reactor was adjusted to obtain a mean flow rate of 0.5 m/h at the cross section just below the middle baffle of a compartment. The value of 0.5 m/h was chosen as it is commonly used as the upflow velocity in conventional upflow anaerobic sludge bed (UASB) reactors (Lettinga, 1995). The applied flow rate corresponded to a mean upflow velocity of 0.14 m/h between the baffles. The baffled reactor was started up at a specific OLR (OLR$_u$, according to the amount of inoculum) of 0.33 gCOD.gVSS$^{-1}$.d$^{-1}$, which was doubled at day 14 (Figure 1). The sulfate loading rate (SLR) of the baffled reactor was the double of the OLR throughout the experiment, as the reactor was fed a synthetic wastewater with a COD/SO$_4^{2-}$ ratio of 0.5.

Maximal specific activity tests
Tests to determine the maximal specific activity of the sludges were performed in duplicate at 30°C as described by Omil et al. (1997a). Serum bottles (315 ml) with 250 ml basal medium (Omil et al., 1997b) (final pH 8) were supplemented with sludge (1.0–1.5
VSS and substrate (2 gCOD.l\(^{-1}\)). Activity tests were performed with a VFA mixture (acetate, propionate and butyrate in a COD ratio of 1:2:2) or acetate as the sole substrate in the presence and absence of sulfate. When sulfate was added, its initial concentration was adjusted to a COD/SO\(_4^{2-}\) ratio of 0.5. The overall activity (OA) and sulfidogenic activity (SA) were calculated from substrate depletion and sulfide production curves, respectively, as described by Visser et al. (1993). The maximal specific methanogenic activity (MA) was determined by the pressure bottle technique (Colleran and Pistilli, 1994). The partitioning of the fraction of COD used by SRB or methanogenic bacteria (MB) was calculated according to the method of Visser et al. (1993).

**Analysis**

VSS were analysed according to standard methods (APHA, 1985). Sulfide was measured photometrically as described by Trüper and Schlegel (1964). VFA and sulfate were measured by gas chromatography and high pressure liquid chromatography, respectively, as described by Omil et al. (1996).

**Results**

**Performance of the baffled reactor**

The COD removal efficiency of the baffled reactor gradually increased to about 85% during the 38 days of operation (Figure 2) at an average OLR up to 0.5 gCOD.gVSS\(^{-1}.d^{-1}\). The sulfate removal efficiency was about 30% throughout the experiment (Figure 2B). Both propionate and butyrate were hardly present in the effluent of the baffled reactor (Figure 3). In contrast, acetate was the main contributor to the effluent COD (Figure 3C). Figure 3 shows that almost all propionate and butyrate were degraded in the first compartment. In contrast, there was a net accumulation of acetate due to incomplete propionate and butyrate degradation in compartment I (Figure 3A). Subsequently, there was a net degradation of acetate in compartment II and III. The total reactor system removed 40% and 80% of the influent and total (influent + produced) acetate, respectively (Figure 2A). The total specific acetate loading rate (C\(_{2totLRs}\)) consists of acetate present in the influent and acetate formed during propionate and butyrate break-down. The C\(_{2tot}\) removal efficiency amounted to 80 (± 5)% at a total specific acetate loading rate (C\(_{2totLRs}\)) of 0.50 gC\(_2^-\)COD.gVSS\(^{-1}.d^{-1}\).

**Specific sulfidogenic and methanogenic activities**

Figure 4 shows the evolution of the substrate and product concentration during the batch degradation of a VFA mixture by sludge from compartment III after 38 days of operation. Propionate and butyrate were degraded instantaneously (Figure 4A), accompanied by a decrease of the sulfate concentration and accumulation of sulfide (Figure 4B). The acetate concentration decreased only after a lag phase of two days (Figure 4A), whereas methane production started from day one onwards (Figure 4C).

Table 1 shows that the specific sulfidogenic activity of sludge from the different compartments of the baffled reactor with VFA and acetate as the sole substrate only slightly increased during the course of the experiment. The total activity of the sludge present in compartment III had increased by 17% and 8% for, respectively, VFA and acetate. The contribution of the SRB to the total activity (% SA) is quite similar for the inoculum and the final sludges present in the baffled reactor.

Table 1 further illustrates that after 38 days of reactor operation, compartmentalisation of the maximal specific activities on VFA and acetate had developed only between compartment I and II. Both the sulfidogenic and methanogenic activities increased to the same extent between compartment I and compartment II. Sludge present in compartment II
and compartment III had a similar maximal specific activity both on VFA and on acetate
(\(\Delta\)). \(C_2\): acetate, \(C_{2\text{tot}}\): total acetate (present in the influent and what can theoretically be formed out of propionate and butyrate), \(C_3\): propionate and \(C_4\): butyrate

**Table 1** Effect of staging and sulfate on the maximum specific activities (gCOD\(gVSS^{-1}.d^{-1}\)) of the inoculum and sludges from the baffled reactor (after 38 days of operation) with VFA (OA) and acetate (AOA) in the presence and absence of sulfate. The contribution of the methanogenic (MA) and sulfidogenic (SA) activity to the activities are given in % of the total activity

<table>
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<tr>
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<th>VFA and sulfate</th>
<th>Acetate and sulfate</th>
<th>Acetate</th>
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<tr>
<td></td>
<td>OA (gCOD(gVSS^{-1}.d^{-1}))</td>
<td>MA (%)</td>
<td>SA (%)</td>
<td>AOA (gCOD(gVSS^{-1}.d^{-1}))</td>
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<tr>
<td>Inoculum</td>
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<td>Baffled reactor</td>
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<td>Compartment 1</td>
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<tr>
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<td>16</td>
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<tr>
<td>Compartment 3</td>
<td>0.511</td>
<td>8</td>
<td>92</td>
<td>0.320</td>
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N.D. Not determined

Figure 2 Removal efficiency of the baffled reactor for (A) individual VFA and (B) total COD (o) and sulfate (\(\Delta\)). \(C_2\): acetate, \(C_{2\text{tot}}\): total acetate (present in the influent and what can theoretically be formed out of propionate and butyrate), \(C_3\): propionate and \(C_4\): butyrate

Discussion

The overall COD removal capacity of the baffled reactor (Figures 2 and 3) is comparable to that of other sulfidogenic systems, i.e. UASB (Omil et al., 1996) or USSB (Lens et al., 1998) reactors, operating at the same temperature (30°C) and treating the same synthetic wastewater as in this study. The staged degradation of the VFA (Figure 2) resulted in the development of different biomass types in compartments I and II of the baffled reactor (Table 1). The specific sulfidogenic and methanogenic activities of the sludges present in...
the three compartments of the baffled reactor (Table 1) are in the same range as those found for other sulfidogenic sludges present in UASB (Omil et al., 1997) or USSB (Lens et al., 1998) reactors. The increase in activity as well as the compartmentalization was comparable to that in a sulfidogenic USSB reactor, where the acetate degrading activity increased by only 13% after 5 months of operation (Lens et al., 1998). It was, however, much less pronounced compared to that of another baffled reactor, operating for 90 days at a OLR of about 1.1 gCOD.qVSS –1.d –1 (Lens et al., 2000).

In comparison to other baffled reactor systems, comparable COD removal efficiencies were obtained in this study at the OLR (maximum OLR of 15 gCOD.l–1.d–1) applied. Nachaiyasit and Stuckey (1995) reported a 98% COD removal efficiency for a baffled reactor treating sucrose-peptone synthetic wastewater at 35°C at an OLR of 4.8 gCOD.l–1.d–1. The COD removal efficiency dropped to 93% when the operation temperature was decreased to 25°C when operating at the same organic loading rate (Nachaiyasit and Stuckey, 1997).

The sulfidogenic acetate degrading capacity of sludge developed rather poorly, even in compartment III where acetate was the sole substrate present (Figure 3C). As ASRB have a low growth rate (Widdel, 1988), the experimental run-time (38 days) might have been insufficient to allow the development of a large ASRB population, as postulated by Omil et al. (1998) based on theoretical calculations with the growth kinetics of ASRB. This work

Figure 3 VFA concentration (A) at the outlet of compartment I, (B) at the outlet of compartment II and (C) in the effluent of the baffled reactor. C2: acetate, C3: propionate and C4: butyrate
confirms that the development of a specific ASRB population in anaerobic granular sludge is a time consuming process.

Acetate degradation is the rate limiting step of sulfidogenic reactors (Hulshoff Pol et al., 1998; Dries et al., 1998). Indeed, at a C_{2totLRs} of about 0.50 gC_2-COD.gVSS^{-1}.d^{-1}, the COD removal efficiency of the baffled reactor amounted to only 80%. Omil et al. (1996) were able to reach acetate removal efficiencies of over 90% at C_{2totLRs} values up to 0.78 gC_2-COD.gVSS^{-1}.d^{-1}. In that case, however, MB also contributed to the organic matter removal. In the present study, the acetate effluent concentration still exceeded 200 mg/l (Figure 3C), despite the intrinsic methanogenic capacity of the sludge present in compartment III (Table 1).

Thus, other strategies have to be applied to increase the acetate removal capacity of sulfidogenic reactors. Omil et al. (1997) tried, unsuccessfully, to colonize granular sludge with a pure culture of the acetate degrading SRB Desulforhabdus amnigenus. The addition of the strain did not lead to an increase of the sulfidogenic acetate-removal capacity of the sludge. The authors postulated that the wash out of dispersed biomass or individual bacteria by selection pressure present in UASB reactors (Hulshoff Pol et al., 1988) diminished the possibilities of incorporation of the newly introduced SRB into the sludge. Further research towards inoculation techniques is required to apply bioaugmentation strategies in sludge.

Figure 4 Substrate and product profiles during batch degradation of the VFA mixture in the presence of sulfate by sludge of compartment III. (A) Total VFA, acetate (C_2), propionate (C_3) and butyrate (C_4). (B) Sulfate removal (o) and total sulfide (A) production. (C) Methane production.
bed based reactors. Alternatively, the acetotrophic sulfidogenic activity can be increased by triggering of the activity of aerobic (Takahashi and Kyosai, 1991) or denitrifying (Lens et al., 2000) populations by adding minor amounts of, respectively, oxygen and nitrate, to the last compartment of a baffled reactor.

Conclusions
The staged degradation of VFA was observed in the baffled reactor, leading to sludges with different metabolic properties in each compartment. Acetate degradation was the rate limiting process. The low acetate degradation in the last compartment of the baffled reactor warrants further research on alternative ways to induce acetotrophic activity, e.g. bioaugmentation with ASRB or addition of alternative electron acceptors.

References