Biodegradation **11:** 429–439, 2000.

Performance of a thermophilic sulfate and sulfite reducing high rate anaerobic reactor fed with methanol

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Accepted 19 October 2000

Key words: bioreactor, desulfurisation, methanol, sulfate reduction, sulfite, thermophilic

Abstract

Thermophilic sulfate and sulfite reduction was studied in lab-scale Expanded Granular Sludge Bed (EGSB) reactors operated at 65 °C and pH 7.5 with methanol as the sole carbon and energy source for the sulfate- and sulfite-reducing bacteria. At a hydraulic retention time (HRT) of 10 h, maximum sulfite and sulfate elimination rates of 5.5 gSO_3^{2-} L^{-1} day^{-1} (100% elimination) and 5.7 gSO_4^{2-} L^{-1} day^{-1} (55% elimination) were achieved, resulting in an effluent sulfide concentration of approximately 1800 mgS L^{-1}. Sulfate elimination was limited by the sulfide concentration, as stripping of H_2S from the reactor with nitrogen gas was found to increase the sulfate elimination rate to 9.9 gSO_4^{2-} L^{-1} day^{-1} (100% elimination). At a HRT of 3 h, maximum achievable sulfite and sulfate elimination rates were even 18 gSO_3^{2-} L^{-1} day^{-1} (100% elimination) and 11 gSO_4^{2-} L^{-1} day^{-1} (50% elimination). At a HRT of 3 h, the elimination rate was limited by the biomass retention of the system. 5.5 ± 1.8% of the consumed methanol was converted to acetate, which was not further degraded by sulfate reducing bacteria present in the sludge. The acetotrophic activity of the sludge could not be stimulated by cultivating the sludge for 30 days under methanol-limiting conditions. Omitting cobalt as trace element from the influent resulted in a lower acetate production rate, but it also led to a lower sulfate reduction rate. Sulfate degradation in the reactor could be described by zeroth order kinetics down to a threshold concentration of 0.05 g L^{-1}, while methanol degradation followed Michaelis-Menten kinetics with a K_m of 0.037 gCOD L^{-1}.

Abbreviations: EGSB – expanded granular sludge bed, HRT – hydraulic retention time, COD – chemical oxygen demand, SRB – sulfate reducing bacteria, MA – methanogenic archaea, OLR – organic loading rate, VSS – volatile suspended solids

Introduction

The use of sulfur-containing fossil fuels as energy source, for example in electrical power production, leads to the production of large amounts of sulfur dioxide. As sulfur dioxide is an environmentally hazardous compound, i.e., as one of the main causes of acid rain, the emission of sulfur dioxide is restricted which results in the need for flue-gas desulfurisation. At present, the limestone/gypsum process covers 85% of the installed flue gas desulfurisation capacity in the power-generating sector. In this process, the flue-gas is brought into contact with an $CaCO_3$-suspension, which results in the formation of gypsum. The major disadvantage of the limestone/gypsum process is that it requires the input of huge amounts of non-renewable chemicals. In addition, impurities such as fly-ash and dust originating from the flue gas may become restrictive for the application of the produced gypsum. Disposal of the waste then becomes the only alternative, resulting in additional costs and new environmental problems. In view of this, the last decade efforts are made to develop a biological alternative for

the limestone/gypsum process for flue-gas desulfurisation (Dries et al. 1998; Lens et al. 1998; de Smul et al. 1997). This biological desulfurisation process makes use of the following sulfur conversions:

$$SO_2 + HCO_3^- \Rightarrow HSO_3^- + CO_2 \quad (1)$$

$$HSO_3^- + \tfrac{1}{2}O_2 \Rightarrow SO_4^{2-} + H^+ \quad (2)$$

$$HSO_3^- + CH_3OH \Rightarrow HS^- + HCO_3^- + H_2O + H^+ \quad (3a)$$

$$3\,SO_4^{2-} + 4\,CH_3OH \Rightarrow 3\,HS^- + 4\,HCO_3^- + 4\,H_2O + H^+ \quad (3b)$$

$$HS^- + \tfrac{1}{2}O_2 \Rightarrow S + OH^- \quad (4)$$

In the first step of biological flue-gas desulfurisation, sulfur dioxide is scrubbed from the flue gas with a bicarbonate solution (reaction 1). Presence of oxygen in the flue-gas results in oxidation of part of the sulfite into sulfate (2). In the subsequent step, sulfite and sulfate are reduced under anaerobic conditions with an added electron donor (here: methanol) to sulfide by sulfate reducing bacteria (3a and b). Thereafter, the produced sulfide is partially oxidized to elemental sulfur by autotrophic sulfur bacteria like *Thiobacillus* spp. in a micro-aerobic reactor with concomitant production of hydroxide (4). Separation of the solid sulfur particles from the medium enables the recovery of elemental sulfur as a valuable product. The remaining bicarbonate solution, with a pH of about 9, can be reused for scrubbing of sulfur dioxide. Because besides SO_2, also heat is transferred from the flue-gas to the scrubbing solution, it is attractive to operate the desulfurisation process at thermophilic conditions (50–65 °C), so that cooling is not required.

A critical factor determining the economic feasibility of biological desulfurisation represent the costs of the added electron donor used for sulfate reduction in the anaerobic step, and the market price of the produced biological sulfur. Methanol is a relatively cheap bulk chemical and therefore represents an attractive substrate for use in biotechnological processes (Dijkhuizen et al. 1985). It is for instance successfully used in denitrification processes (Germonpre et al. 1991; Klapwijk et al. 1981), and has been proposed as electron donor in biological sulfate reducing processes (Hard et al. 1997). Compared to other alternatives such as ethanol (de Smul et al. 1997), H_2/CO_2 (van Houten et al. 1997), acetate (Dries et al. 1998) or higher volatile fatty acids (Omil et al. 1996; Visser et al. 1993), the use of methanol has the advantage that it is the most cost-effective and it allows the use of a relatively simple reactor design such as the EGSB-reactor. Hence, we selected methanol as electron donor for reduction of sulfate and sulfite in our investigations.

Previously, we found high sulfate and sulfite elimination rates of 14 $gSO_4^{2-}\ L^{-1}\ day^{-1}$ and 18 $gSO_3^{2-}\ L^{-1}\ day^{-1}$ with methanol in thermophilic anaerobic high-rate reactors at a hydraulic retention time (HRT) of 4 h (Weijma et al. 2000b). Methane production was low (<2% of methanol consumed), while 10–13% of the consumed methanol was converted to acetate. In this paper, we will describe investigations dealing with the maximum attainable sulfate and sulfite elimination rate at HRTs of 10 and 3–4 h and with the minimisation of acetate formation as well.

Materials and methods

Reactors

Three EGSB-reactors (IA, IB, and II) were used for continuous experiments. All reactors had a working volume of 4 L. A detailed description of the experimental set-up, including the reactor, can be found elsewhere (Weijma et al. 2000a). The reactor was equipped with a screen (circle openings 1 mm) placed below the gas-solids separator device. In all reactors, effluent recycling was applied to increase the liquid upflow velocity to 3 m h^{-1}. Temperature and pH in the reactor were kept at 65 °C and 7.5.

Reactors were fed with a basal medium consisting of (g L^{-1}): NaCl (7), $MgCl_2.6H_20$ (1.2), KCl (0.5), NH_4Cl (0.3), $CaCl_2$ (0.15), Na_2SO_4 (2.8 or 5.6), KH_2PO_4 (0.2), and a trace element solution (1 mL L^{-1}) containing (mg L^{-1}): $FeCl_2.4H_2O$ (1500), $CoCl_2.2H_2O$ (190), $MnCl_2.4H_2O$ (100), $ZnCl_2$ (70), H_3BO_3 (62), $Na_2MoO_4.2H_2O$ (36), $NiCl_2.6H_2O$ (24), $CuCl_2.2H_2O$ (17), EDTA (500) and HCl 37% (7 mL L^{-1}). Cobalt was omitted from the medium of EGSB-IA during day 0 to 12. Demineralised water was used to prepare media and stock solutions. Stock solutions containing methanol (5 M) and Na_2SO_3 (1 M) were pumped into the influent using a Gilson Minipuls3 Peristaltic Pump (Gilson, Villiers-le-Bel, France) at a flow rate needed to obtain the desired influent concentration. Yeast extract was dissolved in the methanol stock solution to give a concentration of 20 mg L^{-1} in the influent. All chemicals were analytical grade from Merck (Darmstadt, Germany) except for yeast extract that was obtained from Life Technologies (Paisly, UK), and methanol that was obtained from Labscan Ltd. (Dublin, Ireland).

The reactors EGSB-IA, IB and II were inoculated with 300, 300 and 430 mL, respectively, of a floc-

culent sludge that was precultivated for 3 months on a methanol/sulfate mixture at 65 °C. The operational parameters for the three reactors are summarized in Tables 1 and 2 of the results section. From day 30 to 34, 15–18 mL min^{-1} nitrogen gas (nitrogen 3.0, Hoek Loos, the Netherlands) was bubbled into EGSB-IA, using a gas inlet positioned at 1–2 cm above the dynamic sludge bed. From day 70 onwards, the lower 10 cm of the sludge bed of EGSB-IA was intermittently (5 seconds per 50 seconds) stirred at 45 rpm with 3 two bladed turbines (dimension stirrer blade 1.5 $*$ 2 cm) to prevent coagulation of the sludge bed that manifested around day 70.

EGSB-II was operated batchwise on day 13 and 14. On day 13, the recycling flow was terminated for 1.5 h and a medium containing 2.4 gCOD L^{-1} methanol and 1.92 gSO_4^{2-} L^{-1} (ratio COD : sulfate = 1.25) was pumped into the reactor at an HRT of 10 min. During the following 12 h, operation of EGSB-II was continued in batch mode. At time intervals, samples were taken for the determination of the total sulfide, methanol, acetate and sulfate concentration. The decrease in medium volume in the reactor due to sampling was less than 1% during batch operation. Subsequently, continuous operation was resumed, applying the same organic and sulfate loading rate as before the batch operation. At day 14 a second batch experiment was performed in EGSB-II, but now at initial methanol and sulfate concentrations of 1.2 gCOD L^{-1} and 3.84 gSO_4^{2-} L^{-1}, respectively (ratio COD : sulfate = 0.30).

Volumetric methanogenic and acetogenic conversion rates in the reactors were calculated as described previously (Weijma et al. 2000a). The sulfidogenic COD-conversion rate was defined as the actual use of reducing equivalents for sulfite and sulfate reduction per unit of time per volume of reactor. Thus, the sulfidogenic COD-conversion rate was calculated by subtracting the amount of sulfite-COD converted in the reactor per unit of time per volume of reactor from the sulfide-COD leaving the reactor via the effluent and the biogas per unit of time per volume of reactor.

Activity assays

The specific sulfidogenic and methanogenic activity of sludge were assayed in 120-mL vials as described previously (Weijma et al. 2000a).

Analyses

A detailed description of the analytical procedures for determination of methanol, acetate, sulfide, biogas composition and VSS and TSS have been described elsewhere (Weijma et al. 2000a). Sulfate and thiosulfate were analysed using a HPLC (Spectra Physics) equipped with a VYDAC Ion Chromatography column (nr. 302 IC, 250 $*$ 4.6 mm). The temperature of the column and detector (Waters 431 conductivity detector) were 20 °C and 35 °C, respectively. As eluent 18 mM potassium biphthalate, at a flow rate of 1.2 mL min^{-1}, was used. Samples were fixed by 2- to 4-fold dilution with a 0.1 M zinc acetate solution, centrifuged (3 min, 10000 g) and diluted with demineralised water to a concentration below 500 mg L^{-1}. Sulfite was determined semi-quantitatively using test strips (Merckoquant cat. nr. 1.100–13).

The SVI of sludge was determined according to Clesceri et al. (1998). The polysaccharide content of sludge was determined with the phenol-sulfuric acid method. 2 mL of sludge was vigorously mixed with 2 mL phenol solution (5 vol% in demineralised water), followed by addition of 10 mL of 18 M sulfuric acid. Then, the mixture was heated to 105 °C for 10 min. After cooling, the extinction of the sample was measured at 480 nm. Calibration curves were made with glucose.

Methanol degradation kinetics

Methanol depletion data from the batch experiment in EGSB-II were fitted to an integrated solution of the Michaelis-Menten equation $V^*_{max}t = S_0 - S + K^*_m \ln(S_0/S)$ using nonlinear regression analysis, as described by (Robinson & Characklis 1984), where V_{max} = maximum consumption rate; t = time; S_0 = initial substrate concentration; S = substrate concentration; K_m = half-saturation constant.

Results

Maximum sulfate and sulfite elimination rate at HRTs of 10 and 3–4 h

Reactor EGSB-IA was started to determine the maximum attainable sulfate and sulfite elimination rates at HRTs of 10 and 3–4 h. Results are presented in Figures 1A–D and in Table 1. In the start-up period, from day 0 to day 12, the organic, sulfate and sulfite loading

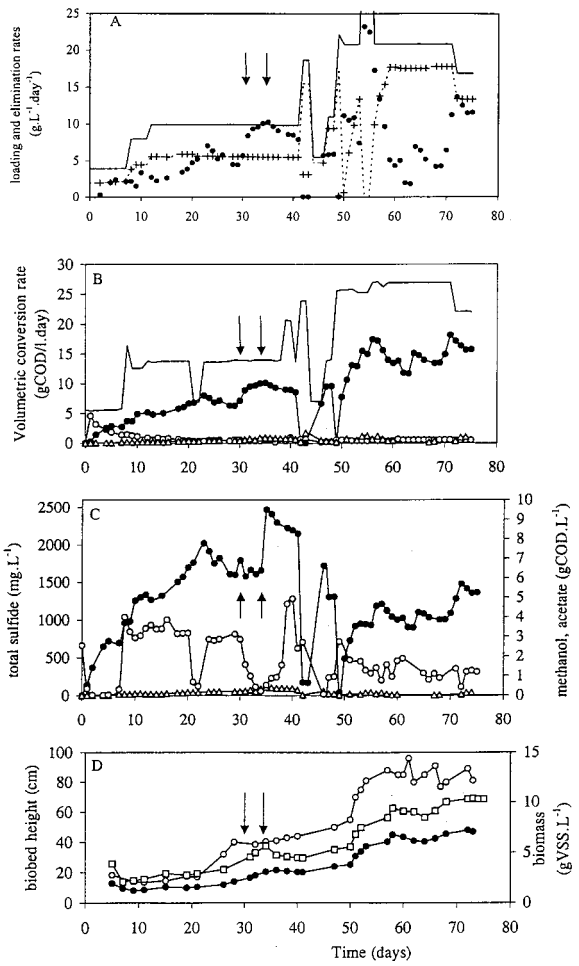

Figure 1. Performance EGSB-IA. Arrows indicate start and termination of H_2S-stripping with N_2. On day 54 and 55, the sulfate loading rate was increased to 42 gSO_4^{2-} L^{-1} day^{-1} (not shown in Figure 1) A. Sulfate (—) and sulfite (- - - -) loading rate, sulfate (●) and sulfite (+) elimination rate. B. Organic Loading Pate (—), Volumetric COD-conversion rates for Sulfidogens (●), Methanogens (○) and Acetogens (△). C. Sulfide (●), methanol (○) and acetate (△) effluent concentration. D. Biomass concentration (□), static (●) and dynamic (○) biobed height.

rates were stepwise increased to 14 gCOD L^{-1} day^{-1}, 10 gSO_4^{2-} L^{-1} day^{-1} and 5.5 gSO_3^{2-} L^{-1} day^{-1}, respectively (Table 1). Methane production increased rapidly from the start, but following day 2 it decreased (Figure 1B). From day 15 onwards, only 0–3% of the consumed methanol was used for methanogenesis. Acetate formation accounted for 5.5 ± 1.8% of methanol conversion during the entire experiment. Activity assays conducted at day 75 revealed that the sludge cultivated in EGSB-IA did not develop any acetotrophic sulfidogenic activity with sulfate (data not shown). Throughout the entire experiment, sulfide was the main product in the reactor (Figure 1B), accounting for about 90% of the degraded methanol.

During the period from day 20 to day 30, the sulfidogenic COD-conversion rate remained stable at 7.0 ± 0.6 gCOD L^{-1} day^{-1} (Figure 1B), at a total sulfide concentration of 1770 ± 140 mgS L^{-1} (Figure 1C). The imposed temporary decrease of the OLR to 6.9 gCOD L^{-1} day^{-1} during day 21 and 22 resulted in a drop in the methanol concentration in the reactor from 3.2 to 0.6 gCOD L^{-1} (Figure 1C), but this did not strongly affect the calculated methanogenic, sulfidogenic and acetogenic COD-conversion rates. In Figure 1A it is shown that from day 20 to day 30 the calculated sulfite degradation rate amounted to 5.5–5.9 gSO_3^{2-} L^{-1} day^{-1} (100% elimination), whereas sulfate was reduced at a rate of 4.4–7.0 gSO_4^{2-} L^{-1} day^{-1} (45–70% elimination). H_2S was stripped from the bioreactor with nitrogen gas during day 30 to day 34. H_2S-stripping was effective, as the sulfide production steadily increased from 7.1 gCOD L^{-1} day^{-1} on day 30 to 10 gCOD $L^{-1}day^{-1}$ on day 34 (Figure 1B), corresponding to 100% elimination of both sulfate and sulfite (Figure 1A). Termination of H_2S-stripping on day 34 did not immediately affect the sulfidogenic COD-conversion rate, which led to an increase of the sulfide concentration in the reactor from 1660 mgS L^{-1} on day 34 to 2470 mgS L^{-1} on day 35. However, the sulfide concentration gradually declined to 2150 mgS L^{-1} on day 41 and, accordingly, the sulfidogenic COD-conversion rate gradually decreased to 8.5 gCOD L^{-1} day^{-1}. In order to assess methanol toxicity, the OLR was temporarily increased to 21 gCOD L^{-1} day^{-1} during day 39 and day 40, resulting in elevated methanol levels of 4.8 gCOD L^{-1} (100 mM) in the reactor (Table 1). This did not affect the slightly downward trend of the volumetric sulfidogenic COD-conversion rate that already had started on day 35, as shown in Figure 1B. On day 41 the OLR was again set at 21 gCOD L^{-1} day^{-1}, as it had been previous to day 39. The slightly downward trend of the volumetric sulfidogenic COD-conversion rate also continued on day 41.

The HRT was decreased to 3 h on day 42, corresponding to an increase of the OLR to 24 gCOD L^{-1} day^{-1} and of the sulfate and sulfite loading rates to 18.7 gSO_4^{2-} L^{-1} day^{-1} and 15.5 gSO_3^{2-} L^{-1} day^{-1}. From the results shown in Figure 1B it appears that the sulfidogenic activity of the biomass became severely inhibited, presumably

Table 1. Operational parameters and effluent concentrations for reactor EGSB-IA. Values are averages in the respective periods. The index 'eff' denotes effluent

Operational parameter	Day 1–7	Day 8–11	Day 12–20,	Day 21–22	Day 30–34	Day 35–38	Day 39–40	Day 42–43	Day 44–46	Day 47–48	Day 49	Day 50–53	Day 54–55	Day 56–58	Day 59–71	Day 72–75
HRT[a] (h)	10	10	10	10	10	10	10	3	11	5.5	3	3	3	3	3	4
OLR[b] (gCOD L^{-1} day^{-1})	5.5	14	14	6.9	14	14	21	24	7	14	26	26	26	27	27	22
SO_4^{2-} loading (gSO_4^{2-} L^{-1} day^{-1})	4.0	7.9	9.9	9.9	9.9	9.9	9.9	19	5.5	11	22	21	42	21	21	17
SO_3^{2-} loading (gSO_3^{2-} l^{-1} day^{-1}	2.0	4.3	5.5	5.5	5.5	5.5	5.5	16	4.7	9.4	17	0→13	0	10→15	18	13
$[methanol]_{eff}$ (gCOD L^{-1})	0.2	3.3	2.3	0.6	2.3	2.3	4.8	2.7	0.1	0.9	2.7	1.8	1.2	3.8	1.2	1.0
$[SO_4^{2-}]_{eff}$ (gSO_4^{2-} L^{-1})	1.3	2.6	3.0	4.0	3.0	3.0	3.7	1.6	0	1.2	ND[e]	1.2	2.4	1.1	2.0	0.8
$[SO_3^{2-}]_{eff}$ (gSO_3^{2-} L^{-1})	0	0	0	0	0	0	0	>0.4	0	0	>0.4	0	0	0	0	0
Ac_{eff}^{c} (mgCOD L^{-1})	0.03	0.09	0.15	0.16	0.25	0.39	0.36	0.03	0.22	0.09	0.02	0.07	0.15	0.11	0.04	0.11
TS_{eff}^{d} (mg L^{-1})	510	1121	1658	1763	1660	2391	2211	171	1721	1306	44	772	936	1173	1038	1404

[a] HRT = hydraulic retention time.
[b] OLR = Organic loading rate.
[c] Ac = Acetate.
[d] TS = total sulfide.
[e] ND = not determined.

due to toxicity of sulfite, which was detected in the effluent at a concentration above 400 mg L^{-1}. By contrast, the sulfite concentration remained below the detection limit at the applied HRT of 10 hr before day 41. The prevailing methanol concentration of about 2.5 gCOD L^{-1} (50 mM) was not the cause of the drop ofthe sulfide production, because in the period from day 10 to day 30 it was already shown that the biomass was not affected by such a methanol concentration. As the sulfidogenic activity did not recover within two days, we decided to increase the HRT to 11 h, corresponding to a drop in the organic, sulfate and sulfite loading rates to 7 gCOD L^{-1} day^{-1}, 5.5 gSO_4^{2-} L^{-1} day^{-1} and 4.7 gSO_3^{2-} L^{-1} day^{-1} at day 44. The sulfide production recovered within 2 days, but subsequent stepwise decreases of the HRT to 3 h with concomitant increases of the loading rates to final values of 26 gCOD L^{-1} day^{-1}, 22 gSO_4^{2-} L^{-1} day^{-1} and 17 gSO_3^{2-} L^{-1} day^{-1} on day 49 once again resulted in a nearly complete inhibition of the sulfide production. However, after switching to an influent containing merely methanol and sulfate on day 50, the sulfide production recovered completely within one day, clearly showing that the inhibition was due to the presence of sulfite. During day 51–53, the sulfite loading rate was increased in small steps from 0 to 13.4 gSO_3^{2-} L^{-1} day^{-1} without occurrence of inhibition. However, within hours following a moderate increase in the sulfite loading rate at day 54, the sulfite concentration in the reactor increased to 40–80 mg L^{-1}, and concomitantly, the sulfidogenic COD-conversion rate dropped dramatically (not shown). After ceasing the sulfite addition, the activity recovered almost immediately. The sulfidogenic COD-conversion rate with sulfate alone, as determined on day 54 and 55 at a sulfate loading rate of 42 gSO_4^{2-} L^{-1} day^{-1}, amounted to 15 gCOD L^{-1} day^{-1}. Based on this value, a maximum sulfite elimination rate of 25 gSO_3^{2-} L^{-1} day^{-1} should be possible to achieve theoretically. In order to establish the sulfite reducing potential of the system, the sulfate loading rate was decreased to 21 gSO_4^{2-} L^{-1} day^{-1} on day 56, while the sulfite loading rate was stepwise increased from 0 to 18 gSO_3^{2-} L^{-1} day^{-1} during days 56–59, well below the calculated maximum sulfite elimination rate. By following this procedure, sulfite inhibition could be prevented, viz. the sulfite was completely eliminated from day 56 onwards. Moreover, the results in Figure 1A reveal that the increase in the sulfite elimination rate during days 56–59 completely balanced the drop

in the sulfate elimination rate (Figure 1A), indicating that sulfite is the preferred electron acceptor for the SRB. The sulfidogenic COD-conversion rate from day 59 to 71 remained relatively constant at a value of 17.5 ± 1.7 gCOD L^{-1} day^{-1}. Maximum sulfite and sulfate elimination rates of 18 gSO_3^{2-} L^{-1} day^{-1} (100% elimination) and 11 gSO_4^{2-} L^{-1} day^{-1} (50% elimination) were attained on day 71. The increase of the HRT to 4 h on day 72, corresponding to a lower organic, sulfate and sulfite loading rates of 22 gCOD L^{-1} day^{-1}, 17 gSO_4^{2-} L^{-1} day^{-1} and 13 gSO_3^{2-} L^{-1} day^{-1}, improved the sulfate elimination efficiency from 50% to 70–80% from day 72 to 75, while sulfite elimination remained at 100%.

The VSS concentration increased from 4 gVSS L^{-1} on day 41 to about 10 gVSS L^{-1} on day 61 (Figure 1D). In order to prevent expansion of the sludge bed into the gas-solid separator, excess sludge was frequently removed from the reactor during the period from day 61 to day 75. As a result, the VSS concentration in the reactor remained in the range 9 to 10 gVSS L^{-1} from day 61 onwards. The calculated (from the data presented in Figure 1B and 1D) specific sulfidogenic activity of the sludge during this period amounted to 1.52 ± 0.19 gCOD $gVSS^{-1}$ day^{-1}. The maximum specific sulfidogenic activity of the sludge, as determined in activity assays on day 75, was very similar, with a value of 1.47 ± 0.15 gCOD $gVSS^{-1}$ day^{-1}.

Attempts to minimise acetate formation

As the formation of acetate is highly unwanted, attempts were made to minimise this. To this purpose we studied the effect of cobalt on the anaerobic conversion of methanol in EGSB-IA, by omitting the supply of cobalt in the feed of this reactor, while the feed of reactor EGSB-IB contained 0.07 mg L^{-1} cobalt. Cobalt may stimulate unwanted methane and acetate formation from methanol, analogous to the effect of cobalt on methanol degradation under mesophilic conditions (Florencio 1994). Operating conditions and effluent concentrations of EGSB-IB are shown in Table 2. A high methane production was found in EGSB-IA and IB merely during the first days (Figure 2A), but in both reactors these rapidly declined to a value of less than 1 gCOD L^{-1} day^{-1} on day 12. Apparently, cobalt does not strongly affect methane production. However, in contrast, cobalt strongly affects the formation of sulfide and acetate because omission of the cobalt supply led to a substantial lower production of these compounds after day 8 (Figure 2B and 2C). From day 12 onwards, cobalt was also added to the feed of EGSB-IA, and as can be seen from the results shown in Figure 1B, the sulfidogenic conversion rate in EGSB-IA indeed increased within the next 11 days to about the level found in EGSB-IB on day 12.

Table 2. Operational parameters and effluent concentrations of reactor EGSB-IB and EGSB-II. Values are averages in the respective periods. The index 'eff' denotes effluent

	EGSB-IB		EGSB-II
Operational parameter	Day 1–7	Day 8–12	Day 1–35
HRT[a] (h)	10	10	12
OLR[b] (gCOD L^{-1} day^{-1})	5.5	14	5.2
SO_4^{2-} loading (gSO_4^{2-} L^{-1} day^{-1})	4.0	7.9	7.6
SO_3^{2-} loading (gSO_3^{2-} L^{-1} day^{-1})	2.0	4.3	–
$[methanol]_{eff}$ (gCOD L^{-1})	0.17	2.4	0.05
$[SO_4^{2-}]_{eff}$ (gSO_4^{2-} L^{-1})	1.6	1.3	0.2
$[SO_3^{2-}]_{eff}$ (gSO_3^{2-} L^{-1})	0	0	–
Ac_{eff}[c] (gCOD L^{-1})	0.02	0.19	0.21
TS_{eff}[d] (mg L^{-1})	439	1830	1150

[a] HRT = hydraulic retention time.
[b] OLR = organic loading rate.
[c] Ac = acetate. [d] TS = total sulfide.

As 3 to 8% of the consumed methanol was used for acetate formation in reactor EGSB-IA, and a higher efficiency of electron donor utilisation for sulfate reduction is needed, we attempted to stimulate acetotrophic sulfidogenic activity of the sludge by imposing methanol-limiting conditions to the sludge, keeping sulfate in excess. For this purpose, reactor EGSB-IA was operated for 35 days under methanol-limiting conditions. Operating conditions and effluent concentrations of EGSB-IB are shown in Table 2. The methanol concentration in the effluent of EGSB-II did not exceed 0.1 gCOD L^{-1} (Figure 3), whereas the sulfate concentration was always > 0.5 g L^{-1}. The relative acetate production (the percentage acetogenic methanol conversion of total methanol conversion) increased from 5% on day 3 to 8.5% on day 17, but from then onwards until day 35 it remained in the range of 8 to 9% (Figure 3). The sludge did not develop any acetotrophic activity under the methanol limiting conditions applied in EGSB-II, as assessed in batch assays (data not shown).

Methanol and sulfate depletion kinetics

On day 13, methanol, sulfate, sulfide and acetate concentrations of 2.4 gCOD L^{-1}, 1.92 g L^{-1}, 10 mgS L^{-1}

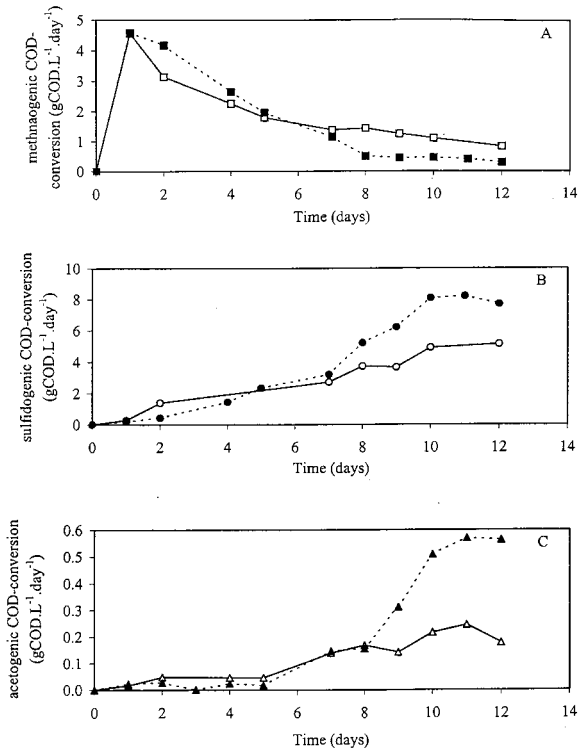

Figure 2. Performance EGSB-IA and IB during the first 12 days of operation. The influent of EGSB-IA did not contain cobalt as trace element. (A) Volumetric metiatnogenic COD-conversion rate in EGSB-IA (□) and EGSB-IB (■). (B) Volumetric sulfidogenic COD-conversion rate in EGSB-IA (○) and EGSB-IB (●). (C) Volumetric acetogenic COD-conversion rate in EOSB-IA (△) and EGSB-IB (▲).

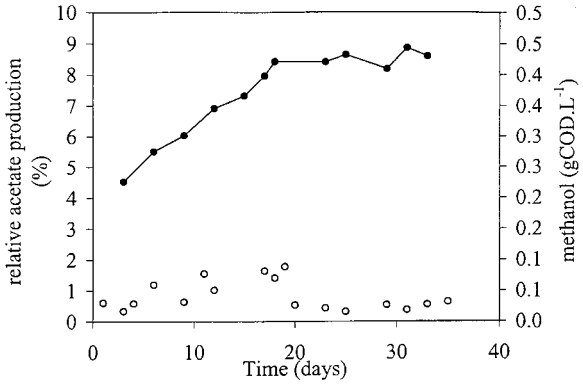

Figure 3. Acetate formation under methanol limiting conditions in EGSB-II. Relative acetate prodriction (●) and methanol concentration effluent (○).

and <0.02 gCOD L^{-1}, respectively, were measured in EGSB-II. The course of the sulfate, methanol and acetate concentration during the subsequent 13 h batch-mode operation of EGSB-II are shown in Figure 4A-I. The sulfate depletion corresponds to zero-order kinetics down to a concentration of at least 0.1 g L^{-1} (Figure 4A-II). Sulfate depletion was not found at concentration <0.05 g L^{-1}. During the period of sulfate reduction, about 14% of the consumed methanol was used for acetate formation. However, once the residual sulfate concentration had dropped to 0.05 g L^{-1}, the methanol degradation rate decreased by a factor 4, while the acetate formation remained almost the same. In the batch experiment carried out in EGSB-II at day 14, the initial methanol, sulfate, sulfide and acetate concentrations were 1.2 gCOD L^{-1}, 3.84 g L^{-1}, 30 mgS L^{-1} and <0.02 gCOD L^{-1}, respectively. The results are depicted in Figure 4. From the data in Figure 4B-II a K_m of 0.037 ± 0.012 gCOD L^{-1} (0.78 ± 0.25 mmol L^{-1}) for methanol degradation was calculated. Here, about 12% of the consumed methanol was used for acetate formation, but when methanol was depleted completely, acetate accumulation did not occur.

Biomass characteristics

The sludge developed in the EGSB-reactors consisted of small (<1 mm in diameter) floes with a loose structure. Dense aggregates or granules did not develop. As can be expected for a flocculent type of sludge, the expansion of the sludge bed at the imposed superficial liquid velocities was high; at the applied upflow liquid velocity of 3 m h^{-1}, the height of the dynamic sludge bed was twice as high as that of the static sludge bed (Figure 1D). The VSS content of the sludge from EGSB-IA gradually increased from 75% of TSS at the start, to 84% at the end of the experiment. At day 75, samples were taken from the sludge bed at a height of 10, 40 and 80 cm from the bottom of the sludge bed. The specific sulfidogenic and methanogenic activity, VSS-content, polysaccharide content and sludge volume index of these samples were determined, and the results are summarised in Table 3.

Discussion

Our results show that methanol represents an attractive electron donor for thermophilic sulfate and

Table 3. Characteristics of sludge taken from EGSB-IA at day 75

Sample point	SSA[a] gCOD $gVSS^{-1}$ day^{-1}	SMA[b] gCOD $gVSS^{-1}$ day^{-1}	VSS-content $gVSS^{-1}$ $GTSS^{-1}$	PS-content[c] gPS $gVSS^{-1}$	SVI[d] mL $gTSS^{-1}$[e]
Upper	1.54 ± 0.24	0.00 ± 0.00	0.90 ± 0.01	0.040 ± 0.003	27 ± 4
Middle	1.48 ± 0.04	0.03 ± 0.02	0.86 ± 0.00	0.072 ± 0.001	21 ± 0
Lower	1.40 ± 0.10	0.05 ± 0.03	0.77 ± 0.01	0.076 ± 0.003	17 ± 0

[a] SSA = specific sulfidogenic activity.
[b] SMA = specific methanogenic activity.
[c] PS = polysaccharide.
[d] SVI = sludge volume index.
[e] TSS = total suspended solids.

sulfite reduction. High maximum sulfite and sulfate space elimination rates of 18 gSO_3^{2-} L^{-1} day^{-1} (100% elimination) and 11 gSO_4^{2-} L^{-1} day^{-1} (50% elimination) were found at an HRT of 3 h. In this situation, sulfate elimination was limited by the amount of biomass retained in reactor EGSB-IA. This can be concluded from the good agreement between the specific sulfidogenic activity of the reactor sludge (1.52 ± 0.19 gCOD $gVSS^{-1}$ day^{-1}), calculated from the sulfidogenic COD-conversion rate and the average biomass concentration in the reactor, and the maximum specific sulfidogenic activity of the sludge as determined in batch assays (1.47 ± 0.15 gCOD $gVSS^{-1}$ day^{-1}). Although a high methane formation rate was found in the first days of reactor operation, it rapidly dropped to a very low level, confirming our previous results that methanogens are outcompeted by sulfate reducing bacteria for the substrate methanol at 65 °C (Weijma et al. 2000a). The rates of sulfate and sulfite reduction with methanol are about twice as high as the rates found with H_2/CO_2 at 55 °C (van Houten et al. 1997). Moreover, these authors found that about half of the added hydrogen is used for methanogenesis.

At an HRT of 10 h, it was furthermore found that the sulfate and sulfite reducing biomass can tolerate high sulfide levels of up to 1600–1900 mgS L^{-1} at pH 7.5, resulting from reduction of approximately 6 gSO_3^{2-} L^{-1} day^{-1} (100% elimination) and 6 gSO_4^{2-} L^{-1} day^{-1} (50% elimination). The inhibiting effect of sulfide was revealed by the increase of the sulfate elimination to 100% once H_2S was stripped from the reactor. However, after ceasing the stripping, the sulfate elimination did not immediately revert to the level before stripping was applied, resulting in a high sulfide concentration of 2440 mg S^{-1}. Apparently, the activity of the sulfate reducing biomass is not instantaneously affected by such extremely high sulfide concentrations, but it was clearly detrimentally affected in the subsequent week. The sulfidogenic COD-conversion rate then dropped with 10%. These results point to sulfide-induced uncoupling of growth and activity of the sulfate reducing biomass. This can also be interpreted as a higher biomass decay rate at increasing sulfide concentrations. The net (i.e., growth minus decay) growth rate of the sulfate reducing biomass presumably becomes too low at the high sulfide concentration of 2440 mgS L^{-1} to compensate for biomass washout, and as a result, a decrease of the biomass concentration will occur and accordingly, a decrease of the sulfidogenic COD-conversion rate. Uncoupling of growth and activity of sulfate reducing biomass by sulfide was previously demonstrated by Okabe et al. (1995) for the mesophilic sulfate reducer *Desulfovibrio desulfuricans*. Also Visser (1995) found a similar uncoupling for mesophilic sulfate reducing sludge cultivated on acetate, propionate, and butyrate.

In addition to the high rates of sulfite and sulfate reduction that can be achieved in the process, also a high sulfate removal efficiency is possible with the reactor design used in this study. This follows from the sulfate depletion kinetics, that could be described with zeroth order kinetics down to a threshold concentration of 0.05 g L^{-1}. This threshold value is so low that for practical applications the rate of sulfate reduction can be regarded as independent on the sulfate concentration in the reactor. On the other hand, the rate of methanol degradation coupled to sulfate reduction is dependent on the methanol concentration following classic Michaelis-Menten kinetics, with an *apparent* K_M-value of 0.037 gCOD L^{-1}. However, still more than 80% of the maximum specific sulfidogenic activity of the sludge can be employed at a methanol concentration in the reactor exceeding 0.16 gCOD L^{-1}.

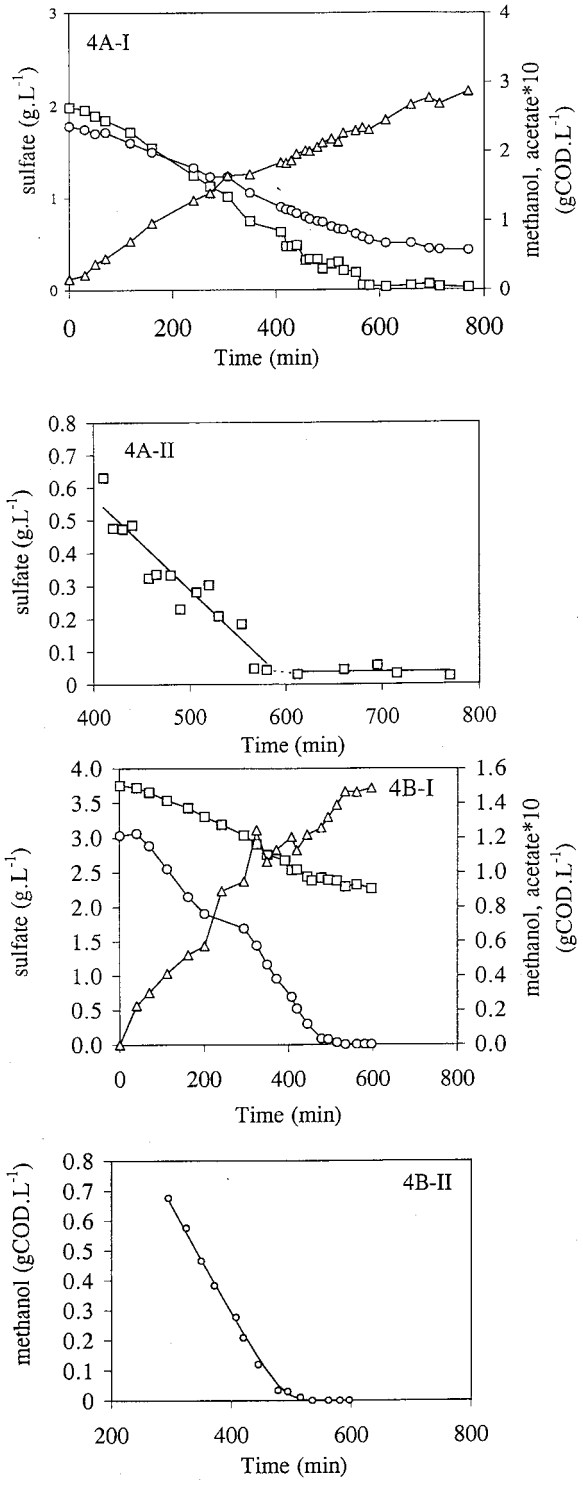

Figure 4. Batch-mode operation EGSB-II. A-I and A-II: initial ratio COD/sulfate = 1.25 (excess methanol). B-I and B-II: initial ratio COD/sulfate = O.30 (excess sulfate). Symbols: (□): sulfate; (○): methanol; (△): acetate.

A clear disadvantage of the use of methanol is the formation of acetate of up to 0.45 gCOD L^{-1} as a by-product, which is highly undesirable as it negatively affects the efficiency of methanol utilisation for sulfate and sulfite reduction. A more serious problem is that the presence of acetate in the effluent of the anaerobic reactor of the biological desulfurisation process will deteriorate the performance of the sulfide oxidising reactor, due to growth of heterotrophic sulfur and sulfate reducing bacteria (Janssen et al. 1997). The lack of any acetotrophic sulfate reducing activity in the sludge may indicate that growth of acetotrophic SRB or MA is impeded under the prevailing conditions. This cannot be the applied temperature of 65 °C, because such a high temperature does not represent a barrier for anaerobic acetotrophic growth. For instance, Nozhevnikova & Chudina (1984) and Min & Zinder (1990) demonstrated growth on acetate at 65 °C of MA and SRB, respectively. The lack of methanogenic acetate degrading activity in the thermophilic sludge can be attributed to the low tolerance of thermophilic acetotrophic methanogens for hydrogen sulfide, as was found by Colleran et al. (1998). Likewise, the lack of acetotrophic sulfidogenic activity may be explained by a high susceptibility of acetotrophic SRB for (hydrogen) sulfide (Maillacheruvu & Parkin 1996), although the evidence for this was obtained with experiments conducted under mesophilic conditions. By contrast, Visser et al. (1992) did find substantial acetotrophic sulfate reduction at 55 °C and pH 7.5. It therefore seems that the lack of acetotrophic sulfate reduction of the thermophilic sludge cultivated on methanol cannot solely be attributed to a high susceptibilty towards sulfide. Possibly, sulfate reducing species capable of utilizing acetate as growth substrate were absent in the seed sludge in sufficiently high numbers.

Only the omission of the supply of cobalt from the influent lowered the acetogenic COD-conversion rate. However, the rates of sulfide formation decreased to the same extent, indicating that sulfide and acetate formation were coupled. This suggests that formation of acetate is an intrinsic feature of thermophilic methanol degradation under sulfate reducing conditions. Thus, it appears that the formation of acetate from methanol cannot be reduced without diminishing the formation of sulfide as well. Therefore, for application of methanol in biological desulfurisation of flue-gases, a compromise must be found between the formation of sulfide and acetate. Alternatively, measures can be taken to remove acetate at some stage in the process,

e.g., by implementing an additional anaerobic reactor prior to the methanol-fed reactor, in which acetate is used as electron donor for sulfite and sulfate reduction.

The results clearly reveal that sulfite overloading conditions cause a severe inhibition of the sulfidogenic biomass, which already will manifest at sulfite concentrations in the reactor as low as 40–80 mg L^{-1}. This observation is in agreement with results of Widdel & Bak (1992), who reported complete inhibition of growth of SRB at 40 mg L^{-1} sulfite. Inhibition by sulfite seems to be reversible, because the results show a rapid recovery of the sulfidogenic activity once the sulfite addition was stopped, even when the sludge had been exposed to high (>400 mg L^{-1}) sulfite concentrations for more than 24 h. To our knowledge, such a reversibility of sulfite inhibition of SRB has so far not been reported. The reversible character of the inhibition shows that sulfite does not cause any serious damage to the sulfate and sulfite reducing biomass. In order to prevent inhibition by sulfite, it is necessary to avoid any accumulation of sulfite in the process, which can easily be achieved by applying sulfite underloading to the reactor.

In view of the flocculent nature of the sludge, the retention of biomass in EGSB-IA was still relatively good, as indicated by the maximum value of the biomass concentration of 9–10 gVSS L^{-1}. This can be attributed to the very low biogas production. Also the lower viscosity of aqueous media at increasing temperatures contributes to a better sludge retention in thermophilic high-rate reactors compared to mesophilic ones. The flocculent nature of the sludge resulted in a large expansion of the sludge bed of 100% at an upflow liquid velocity of 3 m h^{-1}. Evidently, higher biomass concentrations than the maximum found value of 9 to 10 gVSS L^{-1} can be attained when a granular rather than a flocculent sludge had developed. Although we applied high liquid upflow velocities in the reactors to provide a selection pressure for development of granules (Lettinga 1995), we did not observe such a granulation. Apparently, the thermophilic biomass cultivated on methanol does not have the intrinsic ability to form granules under the applied conditions in the reactor. The polysaccharide content of the sludge (4 to 8% of VSS) lies within the range of values (0.6 to 20%) that is normally found for the extracellular polymers content of granular sludge (Schmidt & Ahring 1995). Therefore, a lack of extracellular polymers appears not to be the reason for the inability to form granules. The lack of growth of *Methanosaeta* species in the sludge, that presumably initiate granulation (Hulshoff Pol 1989) or the lack of growth of acidifiers, which are believed to be involved in granulation as well (Vanderhaegen et al. 1992), may explain why granules did not develop.

Conclusions

Methanol represents, from the technological point of view, an attractive electron donor for thermophilic sulfate and sulfite reduction, because high rates of sulfite and sulfate reduction can be achieved at a high sulfite and sulfate removal efficiency. Furthermore, the sulfate and sulfite reducing biomass can tolerate high sulfide levels of up to 1600–1900 mgS L^{-1} at pH 7.5. Therefore, there is no need to integrate a sulfide removal technique into the process.

A disadvantage of the use of methanol is the formation of acetate as undesired byproduct. The results indicate that formation of acetate is an intrinsic feature of thermophilic methanol degradation under sulfate and sulfite reducing conditions. Therefore, for application of methanol in biological desulfurisation of flue-gases, a compromise must be found between the formation of sulfide and acetate.

Sulfite concentrations in the reactor as low as 40–80 mg L^{-1} were already inhibitory for the sulfate and sulfite reducing biomass. However, inhibition by sulfite is completely reversible, which means that lowering the sulfite load to the process will result in rapid recovery of the process performance.

Acknowledgments

This research was supported by the Technology Foundation STW, applied science division of NWO and the technology program of the Ministry of Economic affairs. The authors thank C.J.N. Buisman and H. Dijkman from Paques Bio Systems, Balk, the Netherlands for valuable discussions.

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