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# Use of sulfate reducing cell suspension bioreactors for the treatment of $SO_2$ rich flue gases

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**Abstract**

This paper describes a novel bioscrubber concept for biological flue gas desulfurization, based on the recycling of a cell suspension of sulfite/sulfate reducing bacteria between a scrubber and a sulfite/sulfate reducing hydrogen fed bioreactor. Hydrogen metabolism in sulfite/sulfate reducing cell suspensions was investigated using batch activity tests and by operating a completely stirred tank reactor (CSTR). The maximum specific hydrogenotrophic sulfite/sulfate reduction rate increased with 10% and 300%, respectively, by crushing granular inoculum sludge and by cultivation of this sludge as cell suspension in a CSTR. Operation of a sulfite fed CSTR (hydraulic retention time 4 days; pH 7.0; sulfite loading rate 0.5–1.5 g $SO_3^{2-}$ $l^{-1}$ $d^{-1}$) with hydrogen as electron donor showed that high (up to 1.6 g $l^{-1}$) $H_2S$ concentrations can be obtained within 10 days of operation. $H_2S$ inhibition, however, limited the sulfite reducing capacity of the CSTR. Methane production by the cell suspension disappeared within 20 days reactor operation. The outcompetition of methanogens in excess of $H_2$ can be attributed to $CO_2$ limitation and/or to sulfite or sulfide toxicity. The use of cell suspensions opens perspectives for monolith or packed bed reactor configurations, which have a much lower pressure drop compared to air lift reactors, to supply $H_2$ to sulfite/sulfate reducing bioreactors.

## Introduction

To minimize the negative impact of fossil fuel combustion, a variety of off-gas desulfurization technologies have been developed (Pandey & Malhotra 1999). The recurring costs of chemicals and catalysts needed make physico-chemical desulfurization processes rather expensive. Biological desulfurization techniques are a cheap alternative (Pandey & Malhotra 1999; Janssen et al. 2000). They rely on the microbiological treatment of the scrubber liquor in a two step process, a biological sulfate reduction step, converting $HSO_3^-$/$HSO_4^-$ to $H_2S$, followed by a $H_2S$ removal step. In case of partial sulfide oxidation, elemental sulfur can be recovered (Janssen et al. 2000). The scrubber liquor is an inorganic wastewater (as it contains hardly organic matter) and contains sulfite formed out of the dissolved $SO_2$. Between 10 and 20% of the sulfite is further oxidized to sulfate, as also some of the oxygen present in the off gas (3–15% $O_2$) dissolves during the scrubbing.

The biological sulfate reduction process has developed over the past 15 years to a point that it successfully competes with other sulfate removal technologies for the full-scale treatment of inorganic sulfate rich wastewaters (Hulshoff Pol et al. 1998; Lens et al. 2000). Based on design considerations as mass transfer and biomass immobilization, gas-lift reactors were selected as the most appropriate reactor type for $H_2/CO_2$ based sulfate reduction (van Houten et al. 1994, 1996). In these $H_2$ utilizing sulfate reducing bioreactors, the sulfate reduction efficiency is mainly determined by two factors: mass transfer resistance of the supplied substrate $H_2/CO_2$ and the amount of

$H_2$ lost by its consumption by non sulfate reducing bacteria. The latter depends on the activity of other $H_2$ utilizing bacterial populations present in anaerobic sludges: Methanogenic Archeae (MA) and Homo Acetogenic Bacteria (HAB). Although many methods to steer the competition between sulfate reducing bacteria (SRB), MA and HAB have been investigated (Lens et al. 2000), only few methods are available that can be used in practise.

The overall sulfate conversion rate in $H_2$ utilizing bioreactors is often determined by the mass transfer capacity for $H_2$ (van Houten et al. 1996). This is especially the case in bioreactors that rely on biomass retention by cell immobilization, as e.g. gas lift or upflow anaerobic sludge bed (UASB) reactors. In these reactors, three types of mass transfer can be distinguished (Nicolella et al. 2000): (i) gas ($H_2$) to liquid (reactor liquid) phase mass transfer; (ii) mass transfer in the liquid phase because of transport of dissolved $H_2$ to the biofilm/granule; and (iii) diffusional mass transfer resistance within the biofilm/granule. For high rate sulfite/sulfate reduction, $H_2/CO_2$ supply can become the rate limiting or cost determining step (van Houten et al. 1996; Janssen 2000), so that methods that improve mass transfer also result in more efficient sulfate reducing bioreactors.

Bioreactors using cell suspensions, consisting of individual cells or small flocs (Nicolella et al. 2000), have lower mass transfer resistance compared to biofilm reactors, as the only mass transfer barrier in cell suspension bioreactors is transfer of compounds from the gas to the liquid phase. Cell suspension bioreactors have thus far only limited applications in environmental biotechnology, as biomass retention is a prerequisit to obtain high conversion rates when treating diluted wastewaters. In waste gas treatment, however, the polluted gas stream is uncoupled from the liquid (scrubber liquor and bioreactor liquid) loop. Therefore, there is no strict need for biomass retention and bioreactors using cell suspensions can be applied. In these cell suspension bioreactors, a closed liquid loop between the scubber and the bioreactor is installed (Figure 1), where the cell suspension acts both as scrubbing liquor (uptake of pollutants) and bioconversion medium (biodegradation of pollutants).

This paper describes this novel wastegas treatment bioreactor concept for the treatment of $SO_2$ rich flue gases. The metabolic properties of hydrogen fed sulfite/sulfate reducing cell suspensions are determined in batch and continuous experiments. The operation window of this novel bioreactor concept is then further elaborated.

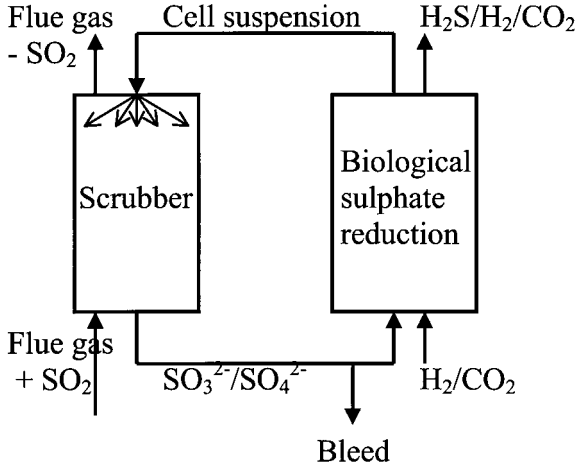


*Figure 1.* Schematic representation of the cell suspension bioreactor concept for flue gas desulfurization.

## Materials and methods

### *Inoculum*

Experiments were performed with intact or crushed anaerobic granular sludge grown in a UASB reactor treating paper mill wastewater at Industrial Water NV (Eerbeek, The Netherlands). The cell suspension was obtained by blending (Braun, 160 Watt) the granular sludge for 3 min under anaerobic conditions, thus yielding a suspension of individual cells and small flocs (<20 mm).

### *Continuous stirred tank reactor*

The cell suspension was grown in a hard PVC plastic CSTR with a liquid volume of 3.6 l (liquid height 0.225 m, inner diameter 0.16 m). The reactor was continuously stirred (using a Heidolph RZR 1 motor) at 235 rpm to keep the biomass in suspension. In order to improve the mixing of the reactor mixed liquor, it was also continuously recycled at a flow rate of 115 ml $min^{-1}$ using a Watson-Marlow peristaltic pump (type 503 S, Watson Marlow, Falmouth, Cornwall, UK). The reactor was operated at 30 °C by recirculation of water from a thermostatic bath (Haake DC 3) at that temperature through the reactor mantle. The pH of the reactor liquid was controlled at 7.0 (±0.2) with a sulfide resistant Flushthrode® pH-electrode (Hamilton Flushtrode, Hilkomij bv, Rijswijk, The Netherlands)

and a controller with two changeable set points to correct the pH by adding concentrated NaOH or HCl solutions.

The CSTR treated a synthetic wastewater containing (in g $l^{-1}$): $Na_2SO_3$ (4.97), $Na_2HPO_4 \cdot 2H_2O$ (0.53), $KH_2PO_4$ (0.41), $NH_4Cl$ (0.30), KCl (0.37), $MgCl_2 \cdot 6H_2O$ (0.10), $CaCl_2 \cdot 2H_2O$ (0.11) and $NaHCO_3$ (2.00). Micronutrients (2 ml $l^{-1}$) were added according to Fedorovich et al. (2000). The synthetic wastewater was pumped into the reactor with a minipulse 2 Gilson thin tubing pump. Pure $H_2$ gas was used as the influent gas and its gas flow was set with a mass flow controller (Brooks thermal mass flow meter, type 5850TR, Brooks Instruments, Veenendaal, The Netherlands), connected to a read out/control unit (Brooks type 5876, Brooks Instruments, Veenendaal, The Netherlands). $H_2$ was added in excess, as three times the stoichiometric amount of $H_2$ needed to reduce the supplied amount of sulfite was added to the reactor. $H_2$ was introduced at the bottom of the reactor by sparging through a diffusor. The effluent gas flow was monitored with a wet-type precision gas meter (type 1, Meterfabriek Schlumberger Industries, Germany) and its composition was analyzed by gas chromatography.

The CSTR operated at a hydraulic retention time (equal to the solid retention time) of 4 days. In period I (Days 1–34), the CSTR was inoculated with 108 g wet weigth inoculum sludge, corresponding to 6 g volatile suspended solids (VSS) per $l_{reactor}$, and was initially operated at a volumetric sulfite loading rate (VSLR) of 1.1 g $SO_3^{2-}$ $l^{-1}$ $d^{-1}$. The VSLR was increased to 1.5 g $SO_3^{2-}$ $l^{-1}$ $d^{-1}$ after 10 days of operation. In period II (Days 35–65), the reactor was reinoculated with 1008 g wet weigth (16 g VSS $l^{-1}$) and a VSLR of 0.5 g $SO_3^{2-}$ $l^{-1}$ $d^{-1}$ was applied.

### *Determination of the maximal specific hydrogenotrophic activity*

Batch experiments to determine the maximal specific hydrogenotrophic activity of the sludge were conducted at 30 °C, using the same medium (pH 7.0) as fed to the reactor, except for the electron acceptor. Activity tests were done in the absence of electron acceptor as well as in the presence of sulfite (1 g $SO_3^{2-}$ $l^{-1}$) or sulfate (1 g $SO_4^{2-}$ $l^{-1}$).

Pure $H_2$ was introduced as the substrate in the headspace at an overpressure of 1.5 bar. At the beginning of a batch test, the headspace of each serum bottle was flushed for 5 min by passing $H_2$ gas through each bottle ($\sim$500 cm3.$min^{-1}$) using two syringe needles as injector and vent, respectively. The gas vent was then removed and the injector was left in place until the headspace was filled with $H_2$ gas at a 1.5 bar overpressure.

The decrease of the gas pressure of the headspace was recorded as a function of time using a Wal-BMP pressure meter (0–4 bar; Wal, Germany). Depending on the activity of the sludge, the headspace pressure was measured twice or more daily during 12–14 days. Tests were terminated when the gas pressure in the headspace had stabilized. At the end of the experiment, the gas phase was analyzed and liquid samples were taken and analyzed immediately for sulfide and pH. For other analysis, samples were centrifuged at 10,000 rpm for 10 min and analyzed for the sulfate, volatile fatty acid (VFA) and VSS concentration.

After converting the pressure decrease to the amount of hydrogen gas ($gH_2$ COD) consumed, the maximal specific activity of the sludge ($gH_2$ COD $gVSS^{-1}$ $d^{-1}$) was calculated by relating the amount of $H_2$ consumed to the time needed for its utilization and the amount of biomass present. The partitioning of the electron flow was calculated from the theoretical amount of $H_2$ needed to produce the amount of product (sulfide, methane or acetate) formed upon termination of the tests using stoichiometric relations (Thauer et al. 1977).

### *Analytical techniques*

Sulfide was determined photometrically using a modified method of Trüper & Schlegel (1964). Sulfate and sulfite were measured by high-pressure liquid chromatography (HPLC) as described in Omil et al. (1996). VSS were analyzed according to standard methods (APHA 1985). VFA and gas phase composition ($H_2$, $CO_2$, $H_2S$, $CH_4$ and $N_2$) were determined using gas chromatography as described by Omil et al. (1996). All gas measurements are expressed at 0 °C and standard pressure (760 mm Hg). Granular sludge anatomy was investigated with a Leica MZ8 (Rijswijk, The Netherlands) stereomicroscope as described by Alphenaar et al. (1993).

*Table 1.* Effect of sulfite and sulfate on the maximal specific hydrogenotrophic activity (MSHA, mg $H_2$ COD $gVSS^{-1}$ $d^{-1}$) of intact and crushed inoculum sludge as well as of a CSTR cell suspension after 30 days of operation at a VSLR of 1 g $SO_3^{2-}$ $l^{-1}$ $d^{-1}$

| Electron acceptor | Granular inoculum sludge | Crushed inoculum sludge | CSTR cell suspension |
|---|---|---|---|
| *Basal medium* | | | |
| Control | 23.4 ± 2.7 | 25.1 ± 3.2 | 194.3 ± 40.1 |
| Sulfite | 95.6 ± 17.6 | 106.3 ± 31.6 | 379.1 ± 37.8 |
| Sulfate | 70.2 ± 4.6 | 78.6 ± 4.2 | 225.8 ± 5.5 |
| *Basal medium – $NaHCO_3$* | | | |
| Control | 13.1 ± 0.5 | 19.2 ± 1.3 | 68.0 ± 2.4 |
| Sulfite | 24.3 ± 1.5 | 116.9 ± 8.9 | 343.7 ± 21.2 |
| Sulfate | 32.5 ± 1.9 | 43.5 ± 7.9 | 202.5 ± 19.3 |
| *Basal medium + acetate* | | | |
| Control | 36.8 ± 0.6 | 48.8 ± 4.0 | 313.1 ± 28.9 |
| Sulfite | 70.4 ± 1.1 | 64.0 ± 8.8 | 415.9 ± 23.5 |
| Sulfate | 67.7 ± 11.0 | 85.1 ± 8.1 | 278.4 ± 21.0 |

## Results

### *Characterization of $H_2$ utilizing processes in the inoculum sludge*

#### *Effect of crushing and sulfite/sulfate on the maximum hydrogenotrophic activity*

Crushing of the inoculum sludge caused an increase in maximal hydrogenotrophic activity of 7, 14 and 10% for respectively the control, sulfite and sulfate incubated sludge samples (Table 1). Interestingly, $H_2$ utilization for sulfide production was almost doubled with suspended sludge compared to granular sludge, both with sulfite and sulfate as the electron acceptor (Table 2).

Table 1 further shows that the maximal hydrogenotrophic activity of intact granular inoculum sludge is about 4 and 3 times higher with, respectively, sulfite and sulfate as the electron acceptor than in the absence of electron acceptor. Table 2 shows that, for intact inoculum sludge, the largest part (up to 79%) of the $H_2$ is consumed for the production of methane by the MA. In the presence of sulfate or sulfite, less methane is formed, but significantly larger amounts of acetate are produced compared to incubations in the absence of sulfite/sulfate (Table 2).

#### *Effect of acetate and $NaHCO_3$ on hydrogenotrophic activity*

Addition of a small amount (100 mg $l^{-1}$) of acetate in the basal medium had no stimulating effect on the maximum specific hydrogenotrophic activity (Table 1). However, it considerably increased (doubled) the fraction of $H_2$ used for sulfite/sulfate reduction by intact sludge (Table 2). Omitting $NaHCO_3$ from the medium resulted in a decrease of the maximun specific hydrogenotrophic activity with about 75%, except for the cell suspension with sulfite (Table 1). Table 2 shows that the methanogenic activity was almost completely repressed in the $NaHCO_3$ deprived incubations (Table 2).

#### *Effect of pH on hydrogenotrophic activity*

Figure 2 gives the maximal hydrogenotrophic activity of the inoculum as a function of the pH with sulfite and sulfate as the electron acceptor. The optimum pH of the maximum hydrogenotrophic activity using sulfite as the electron acceptor was around 6.5–7.5 for both the granular and crushed sludge (Figure 2A). Outside that narrow pH range, the $H_2$ utilizing activities were very low (around 15 mg $H_2$ COD $gVSS^{-1}$ $d^{-1}$). For both crushed and granular sludge, sulfite reduction clearly dominated at more neutral pH's, but was also important at lower and higher pH (Figure 3A and 3B).

Through the curves with sulfate as electron acceptor, a parabolic curve could be fitted (Figure 2B). The pH optimum of the granular sludge was broad, ranging between pH 5 and pH 7. The optimum of the suspended sludge is more narrow with a peak at pH 6.5–7.0. Again, both intact and crushed sludge still had hydrogenotrophic activity at pH 3, 4 and 9, but this activity was rather small (Figure 2B) and mainly methanogenic (Figure 3C and 3D). In contrast to sulfite, $H_2$ was mainly used for methanogenesis and the differences in the fraction of $H_2$ used for methane production at various pH's is fairly small. For both crushed and granular sludge, $H_2$ utilization by sulfate reduction had a rather sharp optimum, with a maximum of 50% around pH 6.5 (Figure 3C and 3D). Acetate production by crushed sludge slightly increased with increasing pH. In contrast, it had an optimum at pH 7.5 with granular sludge (Figure 3C).

#### *Continuous stirred tank reactor*

Figure 4 shows the performance of the CSTR reactor as a function of time. During period I, the $H_2S$ concentration raised to 880 mg $l^{-1}$ within 10 days

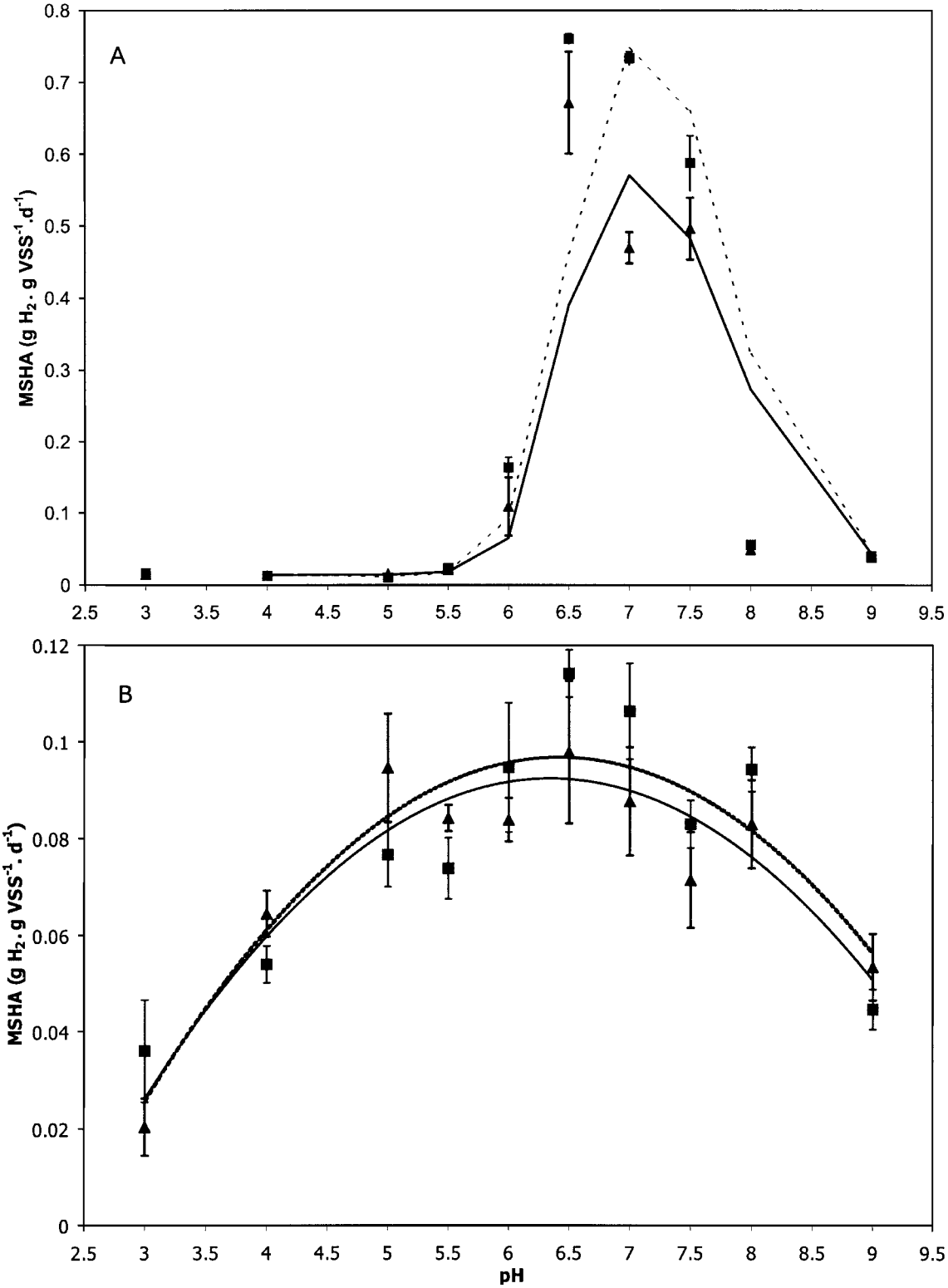


*Figure 2.* Effect of pH on the maximal specific hydrogenotrophic activity of intact (—) and crushed (- - -) anaerobic granular sludge with sulfite (A) and sulfate (B) as the electron acceptor.

*Table 2.* Effect of sulfite and sulfate on the partitioning of $H_2$ utilization by intact and crushed inoculum sludge as well as of the CSTR cell suspension after 30 days of operation at a VSLR of 1 g $SO_3^{2-}$ $l^{-1}$ $d^{-1}$

| Electron acceptor | Granular inoculum sludge | | | Crushed inoculum sludge | | | CSTR cell suspension | | |
|---|---|---|---|---|---|---|---|---|---|
| | MA (%) | SRB (%) | HAB (%) | MA (%) | SRB (%) | HAB (%) | MA (%) | SRB (%) | HAB (%) |
| *Basal medium* | | | | | | | | | |
| Control | 92.5 | 5.7 | 1.8 | 80.5 | 18.4 | 1.1 | N/D | N/D | N/D |
| Sulfite | 67.3 | 13.7 | 19.0 | 61.0 | 27.1 | 11.9 | 0 | 99.9 | 0.1 |
| Sulfate | 79.6 | 7.0 | 13.4 | 77.3 | 14.6 | 8.1 | 5.9 | 85.4 | 8.7 |
| *Basal medium – $NaHCO_3$* | | | | | | | | | |
| Control | 69.1 | 10.5 | 20.4 | 67.2 | 22.4 | 10.4 | N/D | N/D | N/D |
| Sulfite | 0.0 | 81.9 | 18.1 | 0.0 | 96.8 | 3.2 | 0.0 | 98.5 | 1.5 |
| Sulfate | 16.2 | 75.9 | 7.9 | 0.0 | 94.5 | 5.5 | 0.0 | 99.0 | 1.0 |
| *Basal medium + acetate* | | | | | | | | | |
| Control | 83.3 | 2.5 | 14.2 | 69.8 | 9.4 | 20.8 | 17.8 | 7.0 | 75.2 |
| Sulfite | 58.2 | 26.1 | 15.7 | 53.3 | 34.5 | 12.2 | 0.0 | 99.7 | 0.3 |
| Sulfate | 53.6 | 38.8 | 7.6 | 76.5 | 20.4 | 3.1 | 5.2 | 85.4 | 9.4 |

MA = methanogenic archeae; SRB = sulfate reducing bacteria; HAB = homoacetogenic bacteria; N/D = not determined.

of operation (Figure 4C). After this high $H_2S$ concentration was achieved, and increasing the VSLR furthermore, the sulfide concentration dropped considerably. Because no biomass retention was provided, a gradual sludge wash-out was observed (Figure 4B), and after 15 days of operation, only 3.6 g VSS $l^{-1}$ remained present in the reactor. Fresh inoculum sludge was added again on day 15 in order to increase the biomass concentration to 4.8 gVSS $l^{-1}$ (Figure 4B). This resulted in an immediate sulfide production up to 950 mg $l^{-1}$, but the sulfide concentration dropped again after this peak concentration to values of 125 mg $H_2S$ $l^{-1}$ (Figure 4C). The VSS concentration of the effluent was also quite high and at the end of period I, the CSTR mixed liquor contained only about 0.8 g VSS $l^{-1}$ (Figure 4B).

The wash-out of the sludge resulted in a steady increase of the specific SLR (SSLR) up to 2.3 g $SO_3^{2-}$·gVSS$^{-1}$ $d^{-1}$ at the end of period I (Figure 4A). As this is a very high SSLR, the performance of the CSTR might have been affected by an overloading of the toxic $SO_3^{2-}$. Therefore, the VSLR was lowered to 0.5 g $SO_3^{2-}$ $l^{-1}$ $d^{-1}$ in period II (Figure 4A) and the reactor was reinoculated with 16 g VSS $l^{-1}$ fresh inoculum sludge. Again, high sulfide concentrations (500 mg $l^{-1}$) were obtained in 5 days, which further increased to 1600 mg $l^{-1}$ within 10 days of operation (Figure 4A). After reaching this maximum, the sulfide concentration dropped drastically to values of about 150 mg $l^{-1}$.

Figure 4C shows that the effluent always had a low acetate concentration of maximal 200 mg $l^{-1}$. Furthermore, no $CH_4$ could be detected in the gas phase of the reactor throughout the reactor run (Figure 4C).

### *Characterization of $H_2$ utilizing processes in the CSTR suspension*

#### *Evolution of hydrogenotrophic activities as a function of time*

Table 3 shows that the maximal specific hydrogenotrophic activity of the CSTR biomass considerably increased during reactor operation. Interestingly, the CSTR sludge did not have any methanogenic activity anymore after 20 days of operation at a high SSLR (Table 4). Even if the sludge was incubated for a prolonged period of time, no hydrogenotrophic methane production could be detected (Table 4). When operating at a low SSLR (period II), some methanogenic activity was observed after prolonged incubation of the sludge, especially with sulfate as the electron acceptor. A small biofilm developed on the wall of the CSTR, which had a similar metabolic spectrum as the CSTR mixed liquor biomass (Table 4).

#### *Effect of sulfite/sulfate on the hydrogenotrophic activity*

The CSTR sludge, present in the reactor for 30 days at a VSLR of 1.5 $SO_3^{2-}$ $l^{-1}$ $d^{-1}$, had the highest activity with sulfite as the electron acceptor (Tables 1 and 3). In

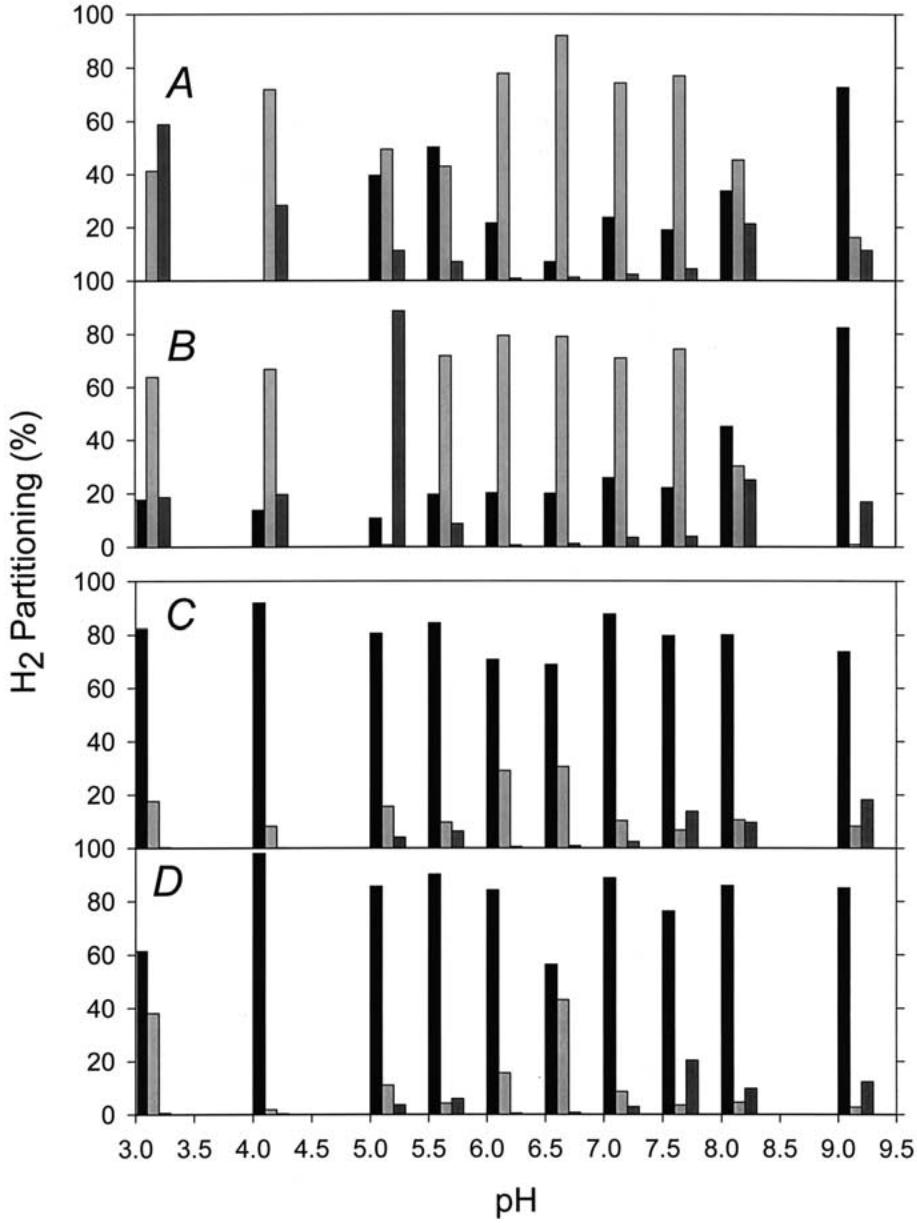


*Figure 3.* Partitioning of the electron flow between sulfate reduction (dark), methanogensis (light grey) and homoacetogenesis (dark grey) as a function of pH during hydrogenotrophic sulfite reduction by (A) intact and (B) crushed sludge as well as hydrogenotrophic sulfate reduction by (C) intact and (D) crushed sludge.

case of sulfite, all $H_2$ was consumed for sulfite reduction, whereas a small fraction of the $H_2$ was consumed by MA and HAB in case of sulfate as the electron acceptor (Table 2).

*Effect of acetate and $NaHCO_3$ on hydrogenotrophic activity*

In the presence of 100 mg $l^{-1}$ acetate, the maximum specific hydrogenotrophic activity of the CSTR suspension increased with 20% (Table 1). However, the partitioning of the electron flow was similar to that of the inoculum sludge: only sulfite reduction with sulfite as the electron acceptor, and also some methanogenic

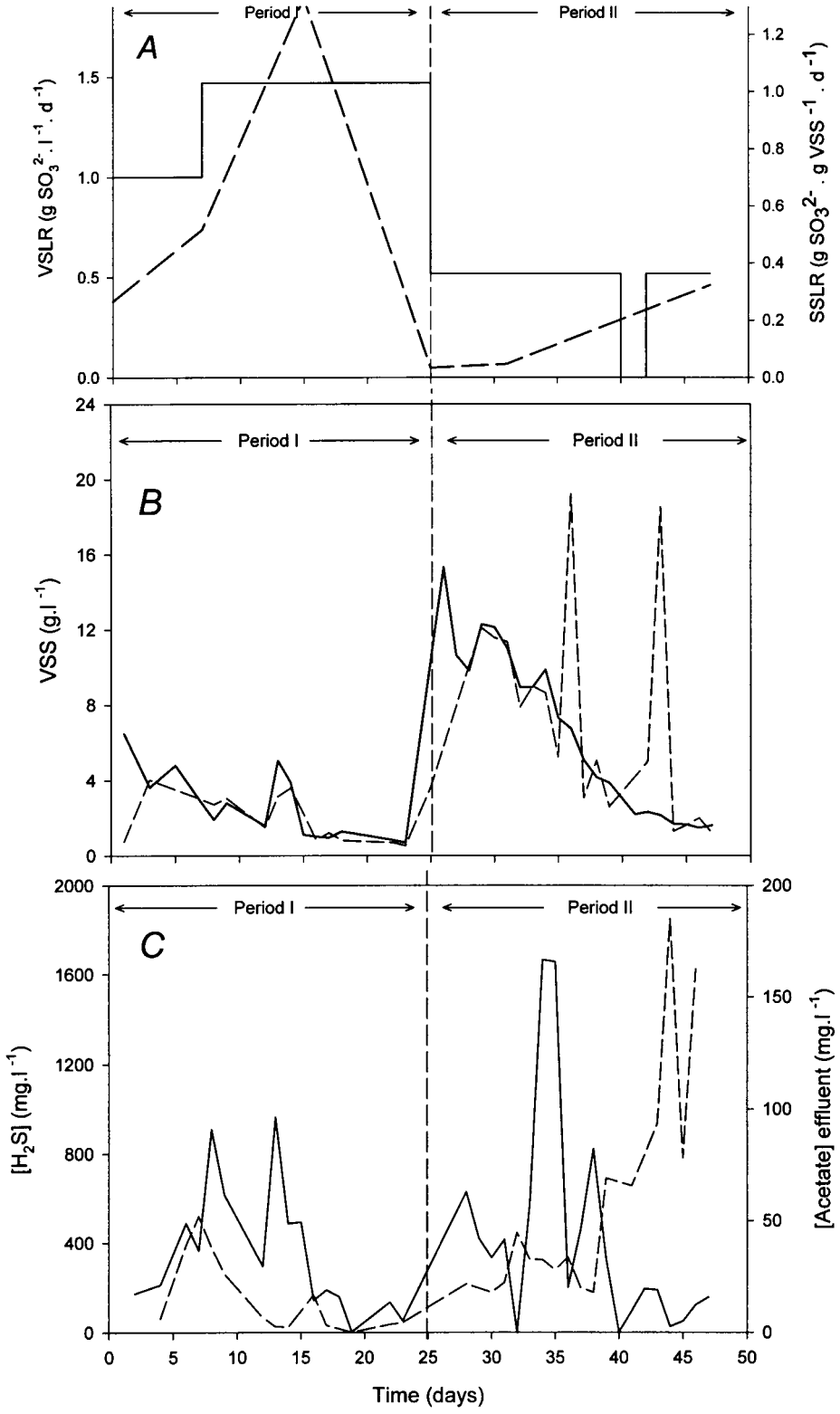


*Figure 4.* Performance of the CSTR reactor. (A) Sulfite loading rate, (B) VSS content in the CSTR reactor influent (—) and effluent (- - -), (C) Effluent $H_2S$ (—) and acetate (- - -) concentration.

*Table 4.* Evolution of the partitioning of the $H_2$ utilization by CSTR sludge

| Time (days of operation) | Incubation time (days) | Sulfite MA (%) | Sulfite SRB (%) | Sulfite HAB (%) | Sulfate MA (%) | Sulfate SRB (%) | Sulfate HAB (%) | Control[1] MA (%) | Control[1] SRB (%) | Control[1] HAB (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| CSTR reactor sludge | | | | | | | | | | |
| 14 | 2 | 0 | 94.7 | 5.3 | 0 | 96.2 | 3.8 | N/D | N/D | N/D |
| | 7 | 0 | 95.6 | 4.4 | 0 | 97.2 | 2.8 | N/D | N/D | N/D |
| 24 | 3 | 0.02 | 96.8 | 3.2 | 0 | 94.3 | 5.7 | N/D | N/D | N/D |
| 55 | 2 | 0 | 99.3 | 0.7 | 0 | 99.7 | 0.3 | 0 | 36.5 | 63.5 |
| | 9 | 0.8 | 95.9 | 3.3 | 2.8 | 89.7 | 7.5 | 13.0 | 34.2 | 52.8 |
| 65 | 2 | 0 | 99.8 | 0.2 | 0.1 | 98.3 | 1.6 | 11.1 | 37.2 | 51.7 |
| | 32 | 0.6 | 98.2 | 1.2 | 1.2 | 96.0 | 2.8 | 12.5 | 52.2 | 35.3 |
| Biofilm on CSTR reactor wall | | | | | | | | | | |
| 24 | 2 | 0 | 94.6 | 5.4 | 0 | 96.2 | 3.8 | N/D | N/D | N/D |
| | 7 | 0 | 95.6 | 4.4 | 0 | 97.2 | 2.8 | N/D | N/D | N/D |
| 65 | 3 | 0 | 96.8 | 3.2 | 0 | 94.3 | 5.7 | N/D | N/D | N/D |

[1]Control = incubation in the absence of sulfite or sulfate.
HMA = methanogenic archeae; HSRB = sulfate reducing bacteria; HAB = homoacetogenic bacteria; N/D = not determined.

*Table 3.* Evolution of the maximal hydrogenotrophic activity (MSHA, mg $H_2$ COD $gVSS^{-1}$ $d^{-1}$) with sulfite and sulfate of CSTR sludge

| Time (days of opereation) | Sulfite | Sulfate | Control[1] |
|---|---|---|---|
| *Reactor sludge* | | | |
| 14 | $145.8 \pm 26.7$ | $96.0 \pm 12.0$ | N/D |
| 24 | $472.9 \pm 94.8$ | $318.0 \pm 29.5$ | N/D |
| 55 | $238.5 \pm 14.4$ | $332.7 \pm 29.2$ | $221.8 \pm 3.7$ |
| 65 | $142.8 \pm 37.8$ | $206.1 \pm 42.0$ | $170.5 \pm 40.1$ |
| *Biofilm on reactor wall* | | | |
| 24 | $284.6 \pm 31.7$ | $159.0 \pm 28.2$ | $145.1 \pm 20.7$ |
| 65 | $66.6 \pm 9.8$ | $92.8 \pm 11.0$ | $127.3 \pm 18.6$ |

[1]Control = incubation in the absence of sulfite or sulfate.
N/D = Not determined.

and homoacetogenic activity with sulfate as the electron acceptor (Table 2). As for the inoculum sludge, the maximum specific hydrogenotrophic activity was lower in the absence of $NaHCO_3$ (Table 1), and no methanogenesis prevailed (Table 2).

## Discussion

### *Sulfite/sulfate reduction in cell suspensions*

This study showed that, despite the toxic properties of sulfite, sulfite/sulfate containing wastewaters can be treated successfully in cell suspension reactors at a VSLR of 0.5 $SO_3^{2-}$ $l^{-1}$ $d^{-1}$ (Figure 4). It confirms the results obtained with immobilized cell bioreactors, which have been used to treat both organic (Athanassopoulos et al. 1989; Eis et al. 1983) and inorganic (van Houten et al. 1996; Weijma et al. 2000) sulfite rich wastewaters. The fast adaptation and high sulfite conversion efficiency can presumably be attributed to the proliferation of the SRB population in the sludge, which also uses sulfite as electron acceptor (Widdel 1988). Sulfide concentrations in the reactor liquid rose rapidly to very high values (up to 1.6 g $l^{-1}$ in 10 days in period II; Figure 4). This confirms the fast sulfite/sulfate reduction rates that can be obtained with hydrogen as the electron donor, as reported by van Houten et al. (1996). These high sulfide concentrations are, however, inhibitory and caused a collapse of the sulfite reduction efficiency (Figure 4C). The potential $H_2S$ toxicity makes an efficient removal of this reaction product a prerequisite when using cell suspensions for sulfate reduction. This can be achieved by operating the bioreactor in counter current mode using the $H_2/CO_2$ gas as the stripping gas. Alternatively, an extra gas stream (e.g. $N_2$) can be introduced

into the cell suspension, from which $H_2S$ has to be scrubbed (e.g. by bubbling through a $FeCl_3$ solution) during its recirculation. The introduction of extractive $H_2S$ membranes (De Smul & Verstraete 1999; Chuichulcherm et al. 2001) into the bioreactor mixed liquor might be an elegant and effective $H_2S$ removal method, that allows a direct recovery of elemental sulfur if a $Fe^{3+}$ solution is used as the extraction solution.

The low effluent acetate concentration (Figure 4D), together with the absence of acetoclastic methanogenic and acetotrophic sulfate reduction of the CSTR sludge (data not shown), suggests that homoacetogens did not play an important role in the sludge. However, some hydrogenotrophic SRB are heterotrophic and utilize acetate as the carbon source in stead of $HCO_3^-$ utilizing autotrophic SRB (Van Houten et al. 1994; Fedorovich et al. 2000). Thus, the growth of heterotrophic $H_2$ utilizing SRB depends on the activity of HAB. Tables 1 and 2 show that the presence of a small amount of acetate (100 mg $l^{-1}$) in the medium increased the fraction of $H_2$ used for sulfite/sulfate reduction in the inoculum sludge. Moreover, HAB activity accompanied sulfite/sulfate reduction in the CSTR cell suspension and the biomass that developed on the reactor wall (Table 4). This suggests that acetate has a stimulatory effect on sulfite/sulfate reduction, but further research is needed to unravel the exact mechanism of this stimulation.

The disappearance of methanogenic activity in the CSTR sludge (Tables 2 and 4) is somewhat surprising, as MA activity is known to be very difficult to suppress in high-rate sulfate reducing granular sludge (Omil et al. 1998; Lens et al. 2000) and gas lift (van Houten et al. 1994, 1996) reactors. It is, however, in agreement with previous work using a CSTR, where $H_2$ was supplied to a cell suspension of the same inoculum sludge via hydrophobic membranes (Fedorovich et al. 2000). Apparently, suspended cell based bioreactor concepts are an effective way to develop fully sulfate reducing sludges. Although selective wash-out of MA from the reactor can not be ruled out as the selection mechanism, it is rather unlikely to be the sole factor determining the outcompetition of MA, as the applied HRT (4 days) is longer than the doubling time of hydrogenotrophic MA (about 1 day) and MA activity could also not be detected in the CSTR biofilm (Table 4), which has a much longer SRT. Other reasons for the disappearance of the MA from the CSTR sludge might be toxicity of sulfide or sulfite (complete inhibition of methanogenesis in nonadapted sludge occurs at 75 mg $SO_3^- \cdot l^{-1}$; Yang et al. 1979) or $CO_2$ limitation. Indeed, the supplied amount of $HCO_3^-$ (2 g $l^{-1}$) was insufficient to support the growth of both SRB and MA. Moreover, the activity tests confirmed that no $CH_4$ is produced in the absence of $HCO_3^-$ (Table 2). However, as $HCO_3^-$ is formed during other microbial processes, i.e. sulfate reduction, $HCO_3^-$ limitation might be partly overcome and still support the methanogenic population.

*Use of cell suspensions in bioscrubbing*

Because no sludge retention was applied, large amounts of sludge washed-out from the CSTR reactor (Figure 4C). This agrees with CSTR theory, as the amount of biomass that can be obtained in a CSTR equals the yield of the bacteria (Colleran et al. 1995). At the applied VSLR of 1 g $SO_3^{2-}$ $l^{-1}$ $d^{-1}$, and assuming a yield of about 100 mg VSS. $g^{-1}$ $SO_3^{2-}$ for SRB (Widdel 1988), this will give a biomass concentration of only 100 mg $l^{-1}$. It should be noted that such low biomass concentrations are intrinsic for CSTR reactors, and higher sulfite loading rates will lead to higher biomass concentrations.

In the closed loop bioscrubber concept (Figure 1), the SRT will be set by the amount of bleed required to avoid toxicity from accumulated salts, dust and potential toxicants ($F^-$, heavy metals) in the scrubber liquor (cell suspension). Consequently, the solid retention time (SRT) will not be infinite, but a function of the bleed required to avoid toxicity. Alternatively, the bleeding can be used to set the SRT at a desired value. This is of particular interest if two populations with different growth rates are present in the sludge. If the applied SRT is lower than the growth rate of the slowest growing species, but longer than that of the fastest growing, the former will wash out and the suspension will solely contain the fast growing species. As the mean doubling time of hydrogenotrophic MA is longer than that of hydrogenotrophic SRB (Widdel 1988), a CSTR might be a way to get a fully sulfate reducing sludge. Thus, the SRT, which is equivalent to the hydraulic retention time (HRT) in CSTR systems, is a tool to steer the competition between the different bacterial populations. This steering method has already been applied for nitrification of ammonia rich wastewaters in the Single reactor High activity Ammonia Removal Over Nitrite (SHARON) process (Hellinga et al. 1998).

When a bioreactor has to be designed for the cell suspension supported hydrogenotrophic sulfite/sulfate

reduction process, it has to be a gas-liquid (slurry of bacteria) reactor with a low pressure drop, high mass transfer rates between gas and liquid phase (high $k_1a$) and a sufficient residence time for microbiological conversion. Several reactor designs comply to these criteria, including monolith or packed bed reactors (Moulijn 1993; Lebens et al. 1998). Monolith packings consist out of many parallel channels of uniform dimensions and are used in chemical gas phase catalyses (Moulijn 1993). The shape and the geometry of the individual channels of monoliths are adjusted to the needs of the individual application. For a bioreactor, these are mainly governed by the potential clogging of the channels by fouling. In addition, plastic packings or ceramic structures are prefered for sulfate reducing bioreactors, as metals will corrode by the reaction product $H_2S$. Theoretical calculations on hydrogen mass transfer from the gas to the liquid phase show that the mass transfer of $H_2$ in these reactor configurations exceeds the hydrogenotrophic sulfite/sulfate reduction efficiency with a factor 10 (Van Zundert 2001). Thus, if hydrogenotrophic sulfite/sulfate reduction are applied in cell suspension monolith bioreactor types, methods that accelerate the hydrogenotrophic sulfite/sulfate reduction efficiency are required.

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