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Isolation of a carbon disulfide utilizing *Thiomonas* sp. and its application in a biotrickling filter

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Abstract The carbon disulfide (CS_2)-oxidizing bacterium *Thiomonas* sp. WZW was enriched and isolated using activated sewage sludge as inoculum. Growth of *Thiomonas* sp. WZW was observed on CS_2, thiosulfate, dimethylsulfide (DMS), dimethyldisulfide (DMDS), and H_2S. No growth occurred on dimethylsulfoxide, methanol, acetate, and on complex media with glucose, yeast extract, or tryptone. DMDS-grown cells respired CS_2, DMS, and DMDS, while thiosulfate-grown cells did not respire CS_2. Chemostat cultures growing on thiosulfate could be rapidly adapted to growth on CS_2. Growth was observed between pH 6 and 8. The K_s values for CS_2, thiosulfate, and sulfide of CS_2-grown cells were between 5 and 10 μM. CS_2 was inhibitory above 0.3 mM. A lab-scale biotrickling filter with lava stone as carrier material for treatment of CS_2-polluted air was inoculated with *Thiomonas* sp. WZW. A rapid start up (95% removal in 1 week) was obtained at an inlet CS_2 concentration of 2 cmol l^{-1} and an initial space velocity (SV) of 54 h^{-1}. Subsequent thiosulfate addition for a week during start up increased the removal to 99%. The step-wise increase of SV to 130 h^{-1} and a CS_2 concentration to 3 μmol l^{-1} resulted in a stable performance with a removal efficiency of 95%. Feeding mixtures of volatile sulfur compounds showed simultaneous conversion of H_2S, CS_2, dimethyldisulfide (DMDS), and DMS, with a preference in this order.

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Introduction

Carbon disulfide (CS_2), a volatile sulfur compound, is emitted to the atmosphere from both natural and anthropogenic sources. The total CS_2 emission to the atmosphere amounts to about 5 Tg annually (Kelly et al. 1994). The atmospheric lifetime of CS_2 is 7–12 days, during which it is oxidized to carbonyl sulfide (COS). COS is the most abundant atmospheric sulfur compound, with a lifetime of about 1 year (Kelly and Smith 1990; Mihalopoulos et al. 1992). Natural sources of CS_2 include soils, marine sediments, salt marshes, and plants (Kelly and Smith 1990). Worldwide industrial production of CS_2 is estimated at 400,000 tonnes per year, of which about 80–90% is used in the production of viscose and cellophane (Grothaus et al. 1982). Industrial process schemes show that from every kilogram of CS_2 consumed, 0.56 kg is emitted to the atmosphere. From this, it can be calculated that the worldwide annual emission of the viscose industry is at least 200,000 tonnes CS_2. Due to the toxicity of CS_2 (maximum acceptable concentration value 10 ppm; Beauchamp et al. 1993) and increasing stringent rules on the emission of harmful gases to the environment, it is necessary to treat CS_2-containing waste gases. An attractive alternative is the use of CS_2-utilizing microorganisms in a biological filter. Until now, the number of microorganisms reported to use CS_2 as a growth substrate and energy source is very small: only strains of *Thiothrix ramosa*, *Paracoccus denitrificans*, *Thioalkalivibrio*, and various *Thiobacillus* species were able to grow chemolitho-autotrophically on CS_2 (Hartikainen et al. 2000; Jordan et al. 1995, 1997; Odintsova et al. 1993; Plas et al. 1993; Smith and Kelly 1988; Sorokin et al. 2002). The isolation and partial characterization of a *Thiomonas* sp. able to utilize CS_2 as

a carbon and energy source, and its application in a biotrickling filter for the removal of CS_2 from waste gases, is described in this paper.

Materials and methods

Media and culture conditions

A mineral medium was used for enrichment and cultivation. The medium contained (in g l^{-1}) K_2HPO_4, 0.4; KH_2HPO_4, 1.0; NH_4Cl, 0.4; $MgSO_4.7H_2O$, 0.05; $CaCl_2.2H_2O$, 0.005; and Na_2CO_3, 0.5, and 2 ml l^{-1} trace element solution. The trace element solution was prepared according to Suylen and Kuenen (1986). The final pH was adjusted to 7.1. Enrichments and cultures were maintained in 120-ml serum bottles filled with 30 ml mineral medium and closed with gray butyl rubber stoppers (Cat. No. V9034.0612.00, International Glass Trading, Zeewolde, The Netherlands). The cultures were incubated on a rotary shaker (150 rpm) at 30°C. CS_2 was always added in portions and the liquid concentration did not exceed 0.3 mM. Other growth substrates tested were thiosulfate (10 mM), thiocyanate (20 mM), tetrathionate (5 mM), methanol (50 mM), acetate (20 mM), dimethylsulfide (DMS) (0.5 mM), DMDS (0.8 mM), dimethylsulfoxide (1.3 mM), and H_2S (0.3 mM). The culture was transferred every 3–4 weeks to fresh medium. All chemicals were analytical grade and obtained from Merck AG (Darmstadt, Germany) or Sigma (St. Louis, MO, USA) unless stated otherwise.

Enrichment and isolation

CS_2-utilizing bacteria were enriched at room temperature in mineral medium containing CS_2 at low concentration using activated sludge as inoculum. For the first three transfers, the liquid CS_2 concentration was 0.01 mM, while for the later transfers, up to 0.3 mM was used. From the culture obtained after seven transfers, a dilution series was made in the same medium and growth was observed up to 10^6 dilution. The 10^5 dilution was used to inoculate agar plates composed of the same mineral medium, to which 1.5% (w/v) agar (MP Biomedicals, Solon, OH, USA) was added. The agar plates were incubated at room temperature (20–23°C) in a closed jar containing 10 μmol l^{-1} CS_2 in the gas phase. Alternatively, agar plates were used with 10 mM of thiosulfate, yeast extract (ICN Biomedicals, Aurora, OH, USA), and tryptone (Gibco BRL, Paisley, UK) (each 2 g l^{-1}). Single colonies were picked and streaked again on agar plates and checked for utilization of CS_2. The purity of single colonies was checked microscopically after growth in mineral medium supplemented with CS_2. Additional purity tests were performed with mineral medium enriched with glucose, yeast extract, and tryptone, each at 2 g l^{-1}. The latter medium does not support growth of autotrophic CS_2-utilizing bacteria.

16S rRNA gene sequence analysis

DNA was isolated from strain WZW and used as template for PCR amplification of 16S rRNA genes. PCR was performed with a T gradient thermal cycler (Biometra, Göttingen, Germany) using the primers and protocols published by Juretschko et al. (1998). The 16S rRNA gene sequence was aligned with homologous 16S rRNA gene sequences of closely related organisms using the ARB program package (Ludwig and Strunk 2004). The 16S rRNA gene sequence has been deposited in the GenBank/European Molecular Biology Laboratory database under accession number DQ902578.

Continuous cultivation

Continuous cultivation was performed at 30°C in a 1-l glass fermentor with a liquid volume of 350 ml. The fermentor was operated either as a chemostat or as a pH-auxostat. For chemostat experiments, thiosulfate was added to sterile mineral medium to a final concentration of 36 mM. When CS_2 was used as a growth substrate, an air stream containing CS_2 was passed through the medium via a sparger positioned close to the stirrer bar. The CS_2 concentration in the air stream amounted up to 180 nmol ml^{-1}, resulting in an outlet concentration of 20 nmol ml^{-1}. The gasflow was set at 25 ml min^{-1}. The pH was maintained at 7 by automatic titration with 1 M NaOH. For pH-auxostat experiments, the pH was maintained by titration with a combination of medium and NaOH (40–50 mM final concentration) giving OD_{600} values of 0.3–0.4. For kinetic experiments the inlet concentration of CS_2 was varied between 20 and 100 nmol ml^{-1} at a gasflow of about 190 ml min^{-1}.

Respiration measurements

Respiration rates of whole cells were measured polarographically in a respiration chamber (1.2 ml) thermostatted at 30°C with a Clark-type oxygen electrode. Rates were expressed as nanomoles O_2 per minute per milligram of protein and were corrected for endogenous respiration.

Preparation of cell-free extracts

A cell suspension in Tris–HCl buffer (pH 8.0) was passed through a French pressure cell at 110 MPa. After centrifugation at 40.000×*g* at 4°C for 20 min, the supernatant (crude extract) was either directly used or stored at −20°C.

Enzyme assay

CS_2 oxidative activity of crude extracts was measured in Tris–HCl buffer (pH 8.0) at 30°C in stoppered bottles, to which CS_2 was added. The decrease in CS_2 was measured gaschromatographically. In some experiments, the production of COS and H_2S was also measured.

Analytical procedures

CS_2, DMDS, DMS, and H_2S were determined gas chromatographically as described by Derikx et al. (1990). Protein was determined with the Bio-Rad (Bio-Rad, Richmond, CA, USA) protein reagent using bovine serum albumin as a standard. Thiosulfate was determined according to Kelly et al. (1969).

Biotrickling filter operation

A lab-scale biotrickling filter was constructed of a glass-cylinder (90 mm ø×450 mm) packed with lava stones (8–16 mm ø). The biofilter was maintained at 25°C by a water jacket. CS_2-containing air, saturated with water, was supplied at the bottom of the cylinder. Airflow is expressed as space velocity (SV), i.e., the flow rate divided by the reactor volume. The sulfuric acid produced in the filter was removed by intermittent recirculation of the medium. Every 20 min, 0.5 l of medium was sprayed on top of the biofilter via a perforated plate. This took about 30 s. The pH of the medium was regulated between 6.8 and 7.1 by simultaneous addition of fresh medium and NaOH (88 mM). Other volatile sulfur compounds were supplied to the filter in a similar way as CS_2. The filter was inoculated by replacing 900 ml of the recirculation fluid with a CS_2-grown bacterial culture. Two different experiments (including start-up) were performed. Operational details are given in Table 1.

Table 1 Biotrickling filter operation

	Day	Inlet CS_2 ($\mu mol\ l^{-1}$)	Space velocity (SV, h^{-1})
Experiment 1	1	2.0	4.8
	8–14	2.0	4.8→27
	16	1.1	54
	105	4–8	54
	136	10–16	54
	166–379[a]	1.5	54
Experiment 2	1	2.0	54
	7–14[b]	2.0	54
	14–136	2.0	54→130
	250	3.0	80

[a] In this period, experiments with simultaneous additions of other volatile sulfur compounds were performed
[b] In this period, thiosulfate was added to stimulate growth

Results

Isolation and characterization of a CS_2-utilizing bacterium

A bacterium able to use CS_2 was successfully enriched using activated sludge as inoculum in a mineral medium with CS_2 as the sole carbon and energy source. A pure culture was obtained by streaking the final enrichment on agar plates. The application of CS_2 in the headspace of closed jars did not result in bacterial colonies on agar plates. On agar plates with thiosulfate, single colonies were observed. Bacteria from these colonies were capable of growth on CS_2 in liquid media. A pure culture of a CS_2-utilizing bacterium, isolate WZW, was obtained from these colonies. Upon light microscopic examination, colonies of this isolate consist of bacteria of the same morphology. Cells were motile rods with polar flagella and stained negative in the Gram-reaction. Under certain conditions, especially sudden excess of both thiosulfate and CS_2, cells produce elemental sulfur and tended to center around sulfur globules (microscopic observations). Colonies on thiosulfate agar plates were small, hard, and white. It was found that growth on plates with purified agar (washed twice with bidistilled water) gave much better results. Isolate WZW was unable to grow on complex media containing glucose, yeast extract, and tryptone. Growth was observed on CS_2, thiosulfate, DMS, DMDS, and H_2S. Poor growth with S^0 production was obtained on tetrathionate and thiocyanate. No growth occurred on dimethylsulfoxide, methanol, and acetate. Acetate (20 mM) had no effect on the growth rate with either CS_2 or thiosulfate as substrate. Thiosulfate (50 mM) and sulfate (above 85 mM) inhibited growth, while NaCl (100 mM) had no effect. Growth was observed between pH 6 and 8, with an optimum at 7.0.

To establish the identity of our isolate, we sequenced the 16S rRNA gene. An alignment was made with homologous sequences of selected species, and this alignment was used to construct a phylogenetic tree (Fig. 1). The alignment showed that the strain clusters within the genus *Thiomonas* and is most closely related to *Thiomonas* sp. B3 (99.5% identity, Bruneel et al. 2003). On the basis of this analysis and the morphological and growth characteristics, our strain was assumed to be a *Thiomonas* species, and is further referred to as *Thiomonas* sp. WZW.

Respiration and kinetic parameters

Thiomonas sp. WZW oxidized a number of sulfur compounds. Respiration rates are given in Table 2. CS_2-grown cells hardly respired DMDS, DMS, or methanethiol, but

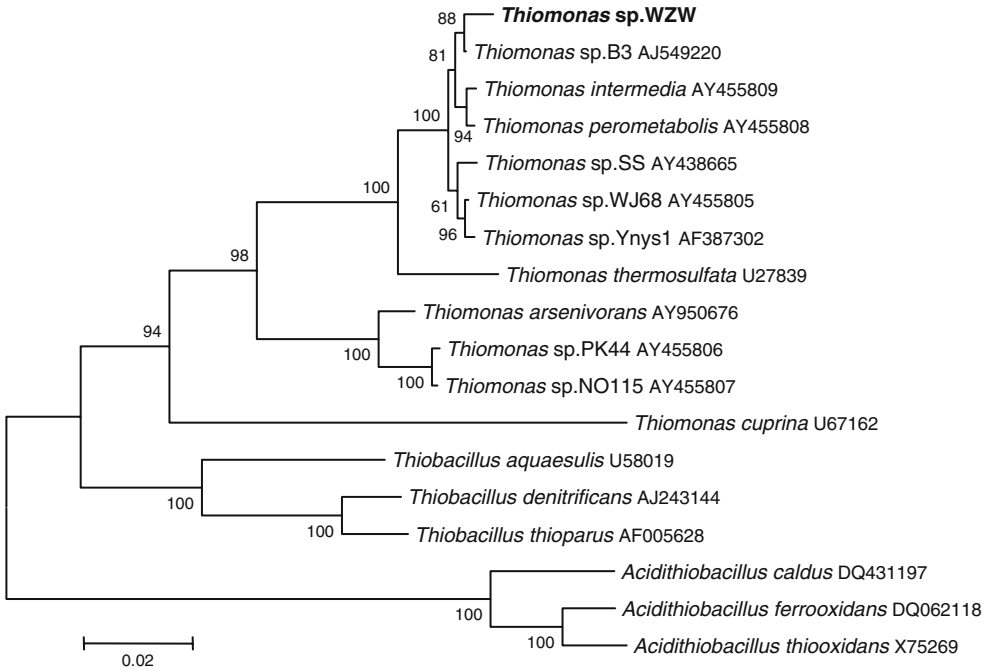

Fig. 1 Neighbor-joining phylogenetic tree based on an alignment of 16S rRNA gene sequences of *Thiomonas* sp. WZW and closely related bacteria (accession numbers are included in the figure). *Scale bar* represents 2 base substitutions per 100 bases. Bootstrap values (500 replicates) are indicated at the *branches*

thiosulfate and H_2S were oxidized. DMDS-grown cells respired CS_2, DMS, and DMDS. Thiosulfate-grown cells did not respire CS_2. However, chemostat cultures growing on thiosulfate could be adapted rapidly to growth on CS_2. Maximal respiration on CS_2 was obtained within 75 h. The substrate affinity constants (K_s) for thiosulfate and sulfide of CS_2-grown cells were estimated from the respiration rates at different substrate concentrations and amounted to 11 and 5–10 μM, respectively.

CS_2 appeared to be a toxic substrate. The transfer of cells that had been inactive for some days to fresh media containing more than 0.1–0.3 mM CS_2 did not result in growing cultures. At 30°C and pH 7, the maximum specific growth rate (μ_{max}) in a pH-auxostat was 0.07 h^{-1} at outlet CS_2 concentrations of 30–40 μmol l^{-1}, which would reflect 30–40 μM liquid concentration, assuming there is a gas–liquid equilibrium for CS_2. Half μ_{max} was reached at CS_2 concentrations of about 6 nmol ml^{-1} (6 μM in the liquid), i.e., the K_s value. This μ_{max} value may be influenced by inhibition because, at 60 nmol ml^{-1} in the outlet, the growth rate dropped to only half the μ_{max}. An apparent K_s of 7 μM was estimated from CS_2 conversion rates in batch incubations at different initial concentrations (0.5–15 μmol/l). When judged from respiration rates at different CS_2 concentrations (5–200 μM), a value of 20 μM was estimated. Oxygen consumption ranged from 3–3.5 mol per mol CS_2.

In a chemostat on thiosulfate, the μ_{max} was 0.09 h^{-1}. The growth yield (Y_{max}) on thiosulfate amounted to 7.4±0.6 g dry weight mol^{-1} (n=6). The yield on CS_2 was higher and amounted to 11.7 g dry weight mol^{-1} (mean of two experiments) in pH-auxostat at μ=0.04 h^{-1}.

Table 2 Respiration of *Thiomonas* sp. WZW grown under various conditions

Substrate	Respiration rate (nmol O_2 min^{-1} mg dry $weight^{-1}$)		
	CS_2-grown	DMDS-grown	Thiosulfate-grown
CS_2	390	488	<5
DMDS	7	570	<5
Thiosulfate	147	n.d.	283
Thiocyanate	<5	n.d.	<5
DMS	<5	68	<5
H_2S	310–700	n.d.	235

n.d. not determined

Enzymatic conversion of CS_2

A suspension of cells grown on CS_2 was able to convert CS_2 under both aerobic and anaerobic conditions (Fig. 2). Under aerobic conditions, a transient accumulation of small amounts of H_2S and COS was found (Fig. 2a), whereas, under anaerobic conditions, H_2S and COS accumulated to much higher concentrations (Fig. 2b), indicating that these products were not further degraded. The accumulation of these intermediates finally resulted in an inhibition of CS_2 degradation. Taking into account the dissolved sulfide in the liquid phase, more than 90% of the CS_2 converted was recovered as H_2S in anaerobic incubations. These results suggest that oxygen is not involved in the conversion of CS_2 to COS and H_2S. To substantiate the evidence for a hydrolytic cleavage of CS_2, enzymatic studies were performed with crude extract. Crude extracts of CS_2-grown cells

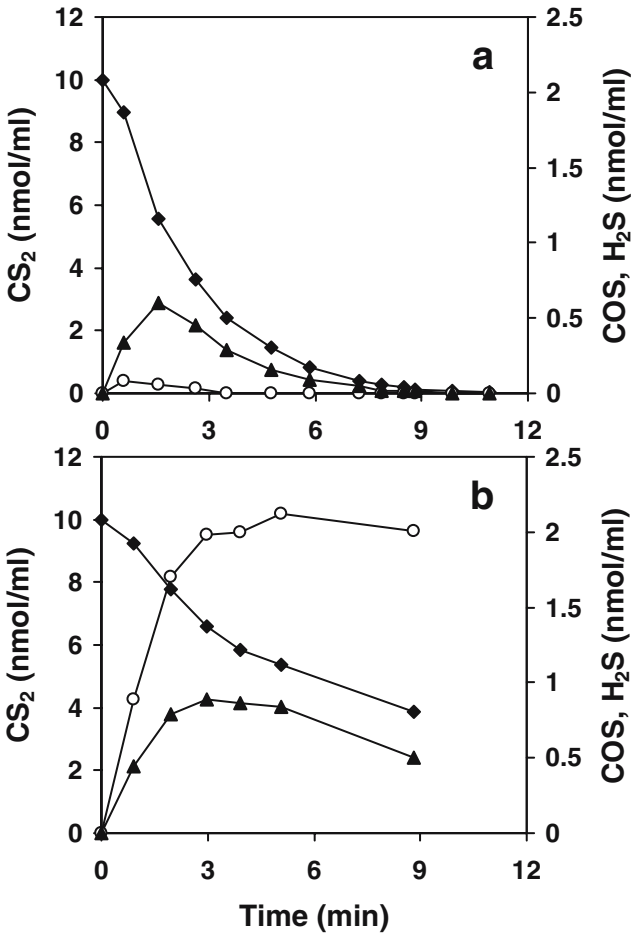

Fig. 2 Conversion of CS_2 by a *Thiomonas* sp. WZW cell suspension under aerobic (**a**) and anaerobic (**b**) conditions. Cells were grown on CS_2. Changes in the headspace concentration of CS_2 (*diamonds*), COS (*triangles*), and H_2S (*circles*) are plotted against time

were able to degrade CS_2 under both aerobic and anaerobic conditions. Initial rate measurements demonstrated that the amount of COS plus H_2S formed was twice the amount of CS_2 degraded. The K_m and V_{max} values for CS_2 in crude extracts were estimated from Lineweaver–Burk plots and yielded values of 10–15 μM and 80 nmol min^{-1} mg $protein^{-1}$, respectively. The crude extract was also able to degrade COS. K_m and V_{max} values for COS were found to be 20 μM and 195 nmol min^{-1} mg $protein^{-1}$, respectively.

Application of *Thiomonas* sp. WZW in a biotrickling filter

A biotrickling filter (2.6 l volume) with lava stones as carrier material was started up by inoculation with a CS_2-adapted culture of *Thiomonas* sp. WZW (34 mg of protein). The CS_2 concentration at the inlet of the filter was kept at 2 μmol l^{-1} and the initial airflow was set at a SV of 4.8 h^{-1}. Within 7 days, a removal of 99% was obtained. At that time, the SV was step-wise increased in the course of a week to 27 h^{-1}. This resulted in a transient decrease in the CS_2 removal to about 95%. At day 16, the SV was increased to 54 h^{-1}, and from then on the inlet concentration was maintained at 1.05± 0.08 μmol l^{-1}. Again, a transient increase of CS_2 in the outlet was observed (77% removal), but the biotrickling filter recovered in about 2 weeks and showed stable operation for another 3 weeks (removal 92–93%). Two months later the removal still fluctuated around these values. To stimulate biomass growth, the inlet concentrations were increased to around 4–8 μmol l^{-1} for a month (outlet concentrations between 2 and 4 μmol l^{-1}) and then for another month to around 10–16 μmol l^{-1} (outlet concentrations 8–12 μmol l^{-1}). At inlet concentration of 8–16 μmol l^{-1}, the removal capacity was more or less constant at 200–250 μmol l $reactor^{-1}$ h^{-1}, indicating maximum capacity. After this period, the inlet concentration was lowered to around 1.5 μmol l^{-1}. The biotrickling filter now showed an increased removal efficiency of 93–96%.

The simultaneous addition of H_2S (0.8 μmol l^{-1}) at 1.5 μmol l^{-1} CS_2 had a negative effect on the efficiency of CS_2 removal only during the first week. Thereafter, the removal percentage increased again to 95%. Furthermore, from the start of the experiment, H_2S removal was 99% or higher. Subsequently, a mixture of volatile sulfur compounds was applied to the filter: CS_2, 1–4 μmol l^{-1}; H_2S, 1–2 μmol l^{-1}; DMS, 1–3 μmol l^{-1}; and DMDS, 0.9–1.3 μmol l^{-1}. The capacity for DMS appeared to be low (13% removal, which may even be caused by losses), but increased rapidly in a few days. DMDS oxidation was observed from the start of the experiment (about 60% removal). After 7 weeks, it appeared that, for CS_2, the capacity decreased from 90 to 82%, for H_2S the capacity remained at >99%, for DMS the capacity increased from 13 to 47%, and for DMDS the capacity increased from 60 to 75%.

By varying the inlet concentrations of CS_2 from 1–20 μmol l^{-1}, the maximum conversion rate of the filter was estimated to be 220 μmol l $reactor^{-1}$ h^{-1}. When the reactor was adapted for a week to high inlet concentration (18 μmol l^{-1}), the maximum was estimated at 310 μmol l $reactor^{-1}$ h^{-1}. Similar maximum rates were obtained in bottle incubations with samples taken from the reactor; 230 μmol l $reactor^{-1}$ h^{-1} for the lower part and 275 μmol l $reactor^{-1}$ h^{-1} for the upper part of the reactor. Starting with 40 nmol CS_2 ml^{-1} in these incubations, the degradation rates decreased below 10 nmol/ml and the apparent affinity constant was estimated to be about 4 μM.

In an attempt to increase the biotrickling filter capacity, the lower part (volume 0.6 l) was filled with lava stones with a diameter of 8–16 mm and smaller lava stones (2–5 mm ø) were used to fill the upper part (volume of 2 l). Operation of the filter was started by inoculation with 25 ml of a CS_2-grown culture of *Thiomonas* sp. WZW (about 1–2 mg of protein) at an inlet CS_2 concentration of 2 μmol l^{-1} and a SV of 54 h^{-1} (Fig. 3). After 3 days, the removal efficiency was

95%. After a week, thiosulfate (20 mM) was added via the recirculation medium for a period of 7 days to increase the biomass content. During this treatment, the efficiency dropped to about 75%, but increased in a few days after thiosulfate addition was stopped to a removal of 99%. In the course of the subsequent 4 months, the SV was stepwise increased to 130 h^{-1}, which resulted in increased capacity, a removal efficiency of about 95%. Stopping the spraying cycle for 7 h had a positive effect on the performance, which increased from 95 to 98.5%, despite the fact that after the first subsequent spray cycle the collected drain water had a pH of 3.3. However, when the biotrickling filter was run overnight with the normal spraying cycle but at pH 3.3, the performance dropped to about 85%. After restoring the pH to 7, it took about a day to reach the initial performance of about 95%.

After 8 months of operation, the biotrickling filter started to clog by a combination of sulfur deposition and biomass growth, as was evident from the hold up and channelling of circulation water. Because the pressure in the column forced the gas through the water outlet (12 cm water column), the high gas flow could not be maintained and the filter had to be operated at an SV of 80 h^{-1}. At a CS_2 inlet concentration of 3 μmol l^{-1}, performance was still more than 98%. The kinetics of the biotrickling filter were studied by several series of increasing and decreasing CS_2 inlet concentrations (0.3–40 μmol l^{-1}). From these experiments the maximum conversion rate was estimated to be 1,300–2,000 μmol l reactor^{-1} h^{-1} and the half-saturation constant 5–15 μmol l^{-1} (i.e., 7–20 μM in the liquid phase). COS and H_2S were detected in the outlet after such sudden increases (up to 1 μmol l^{-1} at 15 μmol l^{-1} CS_2). Prolonged exposure to such high concentration resulted in the formation of elemental sulfur, which partly deposited on the lava stones and reactor wall. Eventually, after the CS_2 load was reduced, the sulfur was slowly further oxidized to sulfate. The inhibiting effect of salt was investigated by stepwise increasing the NaOH concentration of the titration liquid. For a period of 17 days, a maximum concentration of 0.2 M Na_2SO_4 in the recirculation liquid was tested. The removal efficiency was not significantly affected.

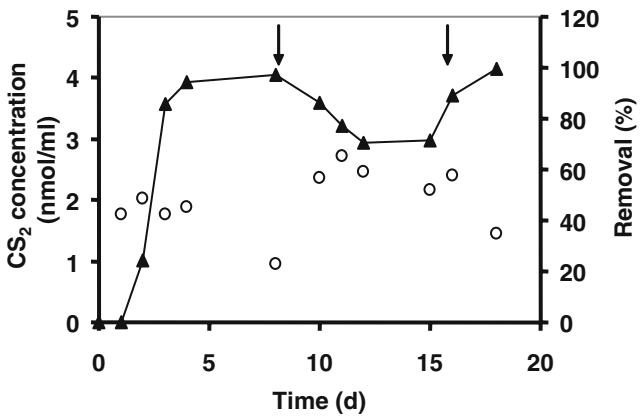

Fig. 3 Start-up of a biotrickling filter with *Thiomonas* sp. WZW as an inoculum and different sizes of lava stones as carrier material. The biofilter inlet CS_2 concentration and SV were maintained at 2 μmol l^{-1} and 54 h^{-1}, respectively. Every 20 min, 0.5 l of medium was sprayed on top. After 3 days, the removal efficiency was 95%. On day 7, thiosulfate addition was started for a period of 7 days (*arrows*). Inlet CS_2 concentration (*circles*); removal (*triangles*)

Discussion

An autotrophic bacterium able to use CS_2 as sole carbon and energy source was isolated from activated sludge. Phylogenetic analysis demonstrated that the isolate is a *Thiomonas* strain. *Thiomonas* is a new genus that was recently described and comprises several mixotrophic thiobacilli that were reassigned to this genus (Moreira and Amils 1997). In the absence of organic compounds, these bacteria grow autotrophically using S^0, reduced inorganic sulfur compounds, or arsenite (Battaglia-Brunet et al. 2006; Chen et al. 2004). CS_2-oxidation is a new feature within this genus and it would be interesting to test the other representatives in this respect. *Thiomonas* sp. WZW fits the morphological and growth characteristics of *Thiomonas*, although it is the first nonmixotrophic species in this genus. The CS_2-utilizing microorganisms described so far comprise *T. ramosa*, *P. denitrificans*, *Thioalkalivibrio* species, and *Thiobacillus* species. Several of these strains are able to grow heterotrophically, but a few are strict chemolithoautotrophs (Jordan et al. 1995, 1997; Odintsova et al. 1993; Plas et al. 1993; Smith and Kelly 1988; Sorokin et al. 2002). Besides growth on CS_2, our isolate was able to grow on thiosulfate, DMS, DMDS, and H_2S. No growth was observed on DMSO, methanol, and acetate. The yield (Y_{max}) value of about 7.5 g mol^{-1} observed after growth on thiosulfate is within the range reported for many *Thiobacillus* species. The yield on CS_2 (11.7 g dry weight mol^{-1}) is comparable to the yield reported for *P. denitrificans* KL1 (11.1 g dry weight mol^{-1}, Jordan et al. 1995, 1997), *Thiobacillus thioparus* (9.5 g cell protein mol^{-1}, Smith and Kelly 1988) and *T. ramosa* (6 g cell protein mol^{-1}, Odintsova et al. 1993).

The K_s estimated from growth rates in chemostat culture was 6 μM. This value may be an underestimation because it was based on gas outlet concentrations of the chemostat. The liquid concentration is most likely not in full equilibrium with the gas phase. Hartikainen et al. (2000) reported a K_s as low as 1 μM, determined from growth rates at various CS_2 concentrations. Although K_s values and maximum respiration rates for CS_2 are in the range reported for other CS_2-utilizing strains, the maximum growth rate of *Thiomonas* sp. WZW on CS_2 (0.07 h^{-1}) is significantly higher. Substrate toxicity for CS_2 seems to be a general

phenomenon (Jordan et al. 1995). Concentrations at which inhibition was observed, 50 μM in the pH-auxostat and 100 μM in batch culture, are in the range reported for other strains. As we did not observe immediate inhibition effects of high CS_2 concentrations (up to 250 μM) in the respiration experiments, the toxicity may be related to long-term effects or toxic intermediates like H_2S.

Cells grown on CS_2 were able to respire thiosulfate, sulfite, and H_2S, but hardly showed respiratory activity with MT and DMDS. Cells grown on DMDS respired DMDS, CS_2, and DMS. Thiosulfate-grown cells respired thiosulfate, sulfite, and H_2S, but were unable to oxidize CS_2, DMDS, and DMS. These results indicate that the enzymes responsible for the oxidation of the various sulfur compounds have to be induced. *Thiomonas* sp. WZW cells were able to convert CS_2 under aerobic and anaerobic conditions. CS_2 could be enzymatically converted under anaerobic conditions. Initial rate experiments in crude cell-free extracts under anaerobic conditions showed that equimolar amounts of H_2S and COS were formed. These data point to a hydrolytic cleavage of CS_2 to COS and H_2S as a first step in CS_2 degradation. Most likely, COS is further hydrolyzed to CO_2 and H_2S. Oxygen is required to oxidize the H_2S formed to sulfate, and in this way, energy is generated, which enables the organism to grow on CS_2. This reaction mechanism is similar to that proposed for *T. thioparus* TK-m (Smith and Kelly 1988). For *P. denitrificans*, evidence was obtained for CS_2 oxidation driven by an NADH-dependent oxygenase (Jordan et al. 1997).

Our *Thiomonas* isolate appears to be a promising organism for application in biofiltration of industrial air streams polluted with CS_2 and H_2S at neutrophilic conditions. The inocula for biotrickling filters can be easily grown with CS_2 or thiosulfate as a substrate. The addition of thiosulfate to the recirculation fluid during start-up increased biomass growth and reactor performance. The reactor performance was strongly improved using smaller lava stones as carrier material, resulting in maximal conversion rates between 1,300 and 2,000 μmol l reactor^{-1} h^{-1}. These rates are higher than the 350–900 μmol l reactor^{-1} h^{-1} reported for comparable systems (Lobo et al. 1999; Windsperger 1990). Hartikainen et al. (2001) obtained higher conversion rates (up to 2,900 μmol l reactor^{-1} h^{-1}) in a peat biofilter under acidophilic conditions. However, as a result of sulfuric acid formation, the lifetime of the peat biofilter was limited to several weeks. The advantage of the biotrickling filter over peat biofilters in this respect is the simple solution to the problem: recirculation of medium together with a neutralizing agent through the trickling filter continuously removes the acid (Cho et al. 1992; Pol et al. 1994). Furthermore, recirculation allows control of optimal physiological conditions (i.e., pH, medium addition), which ensures optimal microbial activity.

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