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Isobutyraldehyde as a competitor of the dimethyl sulfide degrading activity in biofilters

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Abstract

Upon inoculation with *Hyphomicrobium* MS3, the elimination capacity of a lab-scale biofilter for the odorant dimethyl sulfide (Me_2S) can be strongly increased from less than 10 to more than 35 and 1000 g $m^{-3}d^{-1}$ using wood bark and compost as a carrier material, respectively. However, upon supplementation of isobutyraldehyde (IBA) as a second gaseous substrate, sequential degradation profiles of IBA and Me_2S in physically separated sections were observed in the *Hyphomicrobium* MS3-inoculated wood bark and compost biofilters. Contrary to this, the biofiltration efficiency for Me_2S remained unaffected upon the supplementation of toluene as a second gaseous substrate. Batch experiments with the liquid *Hyphomicrobium* MS3 culture confirmed the competitive effect of IBA on the Me_2S degrading activity: in the presence of both compounds, *Hyphomicrobium* MS3 preferred degradation of the carbonyl compound. In technical terms, this means that the complete purification of a waste gas stream containing both IBA and Me_2S should be performed using sufficiently high or bistage *Hyphomicrobium* MS3-inoculated biofilters. Design criteria have to be conceived in this respect.

Abbreviations: CDW – cell dry weight; EC – elimination capacity; IBA – isobutyraldehyde; Me_2S – dimethyl sulfide; OL – organic loading rate; RT – retention time; VOSC – volatile organic sulfur compound

Introduction

Biofiltration is a biotechnological waste gas treatment technology, mainly used to treat large volumes of low concentrated waste gases as they can be found in the odorous emissions from rendering plants. In these waste gases, volatile sulfur compounds and carbonyl compounds represent the main groups of odorous volatiles present, while also aromatic, aliphatic and halogenated hydrocarbons can be detected (Van Langenhove et al. 1991). In full-scale biofilters treating these complex rendering waste gases, the removal efficiency for carbonyl compounds and hydrogen sulfide is high (> 98%) while the efficiency is low and unpredictable for volatile organic sulfur compounds (VOSCs) as e.g. dimethyl sulfide (Me_2S) (Van Langenhove et al. 1991). As a result of the very low odour threshold values of these VOSCs (0.6–40 ppbv for Me_2S) (De Zwart & Kuenen 1992) and their unpleasant hedonic character, the concomitant overall odour removal efficiency of these full-scale biofilters will be insufficient.

In agreement with the reported full-scale biofiltration results, high elimination capacities (EC) for carbonyl compounds (e.g. 2160 g butanal m^{-3} d^{-1}) and very low EC for VOSCs (e.g. 10 g Me_2S m^{-3} d^{-1}) were reported in lab-scale single-compound biofiltration experiments (Weckhuysen et al. 1993; Smet et al. 1996a, b). Upon inoculation of the biofilter material with *Hyphomicrobium* MS3, however, performance of the lab-scale biofilters towards VOSC-degradation was strongly enhanced, yielding EC of 35 and 1000 g Me_2S m^{-3} d^{-1} when using compost and wood bark as a carrier material, respectively. The applicability of the culture *Hyphomicrobium* MS3 to inoculate full-scale biofilters requires however more insight into the sta-

bility of its Me_2S degrading capacity in complex waste gas streams.

Since different classes of compounds often are degraded by different groups of micro-organisms, interspecies inhibition (as e.g. by the production of antibiotica or toxic/acidifying metabolites) and interspecies competition (for available space, substrates, oxygen, nutrients, . . .) can occur in biofilters loaded with complex waste gases (Robertson et al. 1988; Van Groenestijn & Hesselink 1993). These microbial interactions result in the colonisation by the different active micro-organisms of physically separated zones in the biofilter and the subsequent sequential degradation of the compounds involved (Bendinger 1992). According to Van Groenestijn & Hesselink (1993), micro-organisms with a broad substrate specificity will convert the easily degradable compounds at the inlet of the filter, while specialized organisms will hardly be able to develop as they are excluded by competition or inhibition.

Next to this, the biofiltration of a specific pollutant can be influenced by the presence of more attractive alternative substrates for the active micro-organism. At cellular level, the organism can prefer one substrate above another one for reasons of transport, energy supply, . . . (Goldstein et al. 1985; Bally et al. 1994).

A sequential degradation is also expected in a biofilter when the presence of a specific waste gas component inhibits the degradation of another one as e.g. VOSCs that inhibit the nitrification of ammonia (Bremner & Bundy 1974; Juliette et al. 1993). Van Langenhove et al. (1989) reported a 60% decrease in hexanal biofiltration efficiency in the presence of 40 ppmv SO_2, while a concentration of 100 ppmv SO_2, resulted in a complete and permanent inhibition. In this case, complete elimination of the toxic compound has to be achieved before the others can be degraded (Bendinger 1992).

In this work, the influence of the presence of the carbonyl compound isobutyraldehyde (IBA) and the aromatic hydrocarbon toluene on the EC for Me_2S in *Hyphomicrobium* MS3-inoculated lab-scale biofilters was studied in order to investigate the applicability of this inoculum to be used in full-scale biofilters treating rendering and other complex VOSC-containing waste gases.

Materials and methods

Enrichment culture

The enrichment procedure for *Hyphomicrobium* MS3 was described in a previous paper (Smet et al. 1996a). The culture consisted of *Hyphomicrobium* species and converted Me_2S stoichiometrically into H_2SO_4. *Hyphomicrobium* MS3 was maintained for batch- and biofilter experiments by passing Me_2S-containing air ($\pm$ 120 ppmv) at a flow rate of 1 l min^{-1} through an erlenmeyer flask containing 5 l of inoculated mineral medium. The culture was magnetically stirred. Every day, the pH was readjusted to 7.0, the volume was readjusted to 5 l and 100 ml of mineral medium was renewed by a fill and draw procedure. The mineral medium used for cultivation contained (in g l^{-1}): K_2HPO_4: 3.00; KH_2PO_4: 3.00; NH_4Cl: 3.00; $MgSO_4 7H_2O$: 0.50; $FeSO_4 7H_2O$: 0.01; final pH 7.0.

Biofilter experiments

Two lab-scale biofilters, consisting of plexi-glass columns with an internal diameter of 0.19 m, were filled up to a height of 0.7 and 0.6 m with wood bark and compost as a carrier material, respectively. The compost biofilter was supplemented with 10% (w/w) limestone powder. Characteristics of both carrier materials are given in Table 1. The buffer capacity was defined as the amount of HCl (expressed as meqkg^{-1} material) needed to reduce the pH of the fresh materials to 4.0. Gas sampling points were installed at the influent and effluent side of both biofilters and at a height of 10, 30, and 50 cm in the filled part of the wood bark biofilter. For the compost biofilter, gas sampling points were installed every 10 cm in the filled part of the column. Gas samples were taken at a horizontal depth of 7 cm ($\pm$ at the center of the cross-sectional area), using a 500 μl Hamilton 1700 series gas syringe (Alltech Ass.) with a needle of 9 cm length. Volatiles (Me_2S, IBA, toluene) were dosed by bubbling a calibrated N_2-flow through a thermostated gas bubbling bottle containing the pure liquid compound. The air passing through both biofilters was humidified in a scrubber and was blown from bottom to top in the bioreactors. The superficial gas flow rate was 24 and 80 m^3 m^{-2} h^{-1} in the wood bark and compost biofilter, respectively (corresponding to an apparent gas residence time of 105 and 27 s, respectively). Both biofilters were inoculated by mixing the carrier materials with *Hyphomicrobium* MS3 ($\pm$ 120 g CDW m^{-3} biofilter).

Table 1. Characteristics of the compost and the wood bark used as carrier materials

Parameter	Compost	Wood bark
pH	7.8	6.7
Buffer capacity (meqkg^{-1} wet)	250	50
Moisture content (%)	53	69
Bulk density (kgm^{-3} wet)	579	310
C/N-ratio	15.2	119.1

Batch experiments

For the batch experiments, 20 ml of the *Hyphomicrobium* MS3 culture, pregrown on Me_2S, were brought in penicillin bottles (120 ml). The penicillin bottles were sealed with Teflon-lined Mininert valves (Alltech Ass.) and the culture was magnetically stirred. Volatiles were dosed using stock mixtures in water. After vortexing these stock mixtures, appropriate amounts were transferred into the penicillin bottles with a liquid syringe and allowed to equilibrate between gas and liquid phase.

Gas chromatography

Analysis of volatile compounds was carried out with a Varian 3700 gas chromatograph, equipped with a flame ionisation detector (flow rates: H_3 30 mlmin^{-1}; air 295 mlmin^{-1}). A 30 m DB-1 bounded phase column (100% dimethylpolysiloxane, internal diameter 0.53 mm, film thickness 1.5 μm) with He as a carrier gas (flow rate 4.9 mlmin^{-1}) was used. The oven temperature was 40 °C for mixed Me_2S (retention time RT 4′ 52″) and IBA (RT 5′ 16″) analysis and 80 °C for mixed Me_2S (RT 3′ 22″) and toluene (RT 6′ 23″) analysis.

Results

Simultaneous biofiltration of Me_2S and IBA in the Hyphomicrobium *MS3-inoculated wood bark biofilter*

At an organic Me_2S loading rate of 24 g m^{-3} d^{-1}, the elimination efficiency of the *Hyphomicrobium* MS3-inoculated wood bark biofilter was 67% (EC = 16 g Me_2S m^{-3} d^{-1}). When IBA was dosed as a second substrate to this biofilter at an organic loading rate of 1100 g m^{-3} d^{-1} (± 450 ppmv) during a 4-hour period, the Me_2S elimination efficiency strongly decreased from 67% to 8%. The IBA removal efficiency was high (> 90%), although an increasing amount was breaking through at the end of this 4-hour period (Figure 1). As a result of desorption processes taking place in the biofilter, IBA was detected in the outlet gas stream for as long as 1 hour after interruption of the IBA dosing. In Figure 2, it can be seen that the EC for Me_2S decreased from 16 to 2 g m^{-3} d^{-1} during the 4-hour IBA loading, while it took up to 5 days after interruption of the IBA dosing before the biofilter regained its original EC for Me_2S.

After restabilisation of the Me_2S-degradation in the *Hyphomicrobium* MS3-inoculated wood bark biofilter,

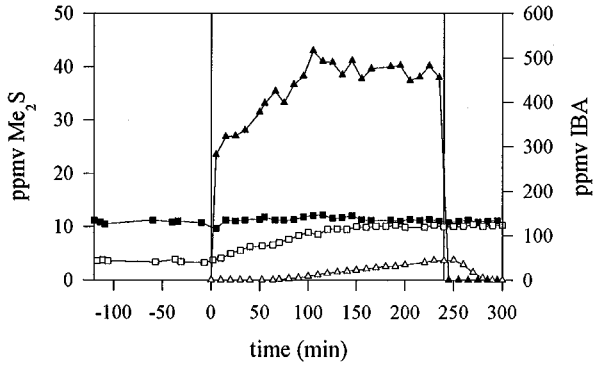

Figure 1. Removal of Me_2S in the *Hyphomicrobium* MS3-inoculated wood bark biofilter before, during (t = 0 → 240 min) and after the 4-hour additional loading with IBA. Symbols: Me_2S influent (■) and effluent (□) concentration (ppmv); IBA influent (▲) and effluent (△) concentration (ppmv).

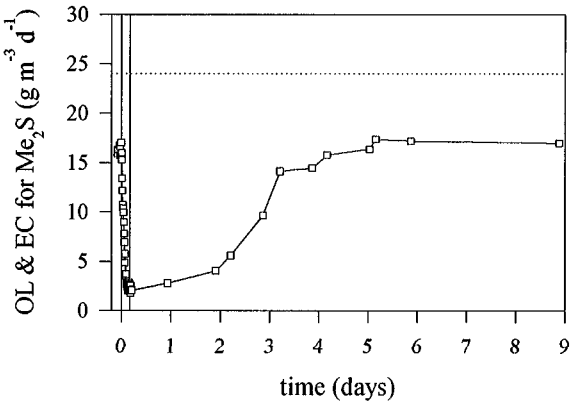

Figure 2. Me_2S elimination capacity EC (□) (g m^{-3} d^{-1}) in the *Hyphomicrobium* MS3-inoculated wood bark biofilter before, during (vertical lines on day 0 and 0.17) and up to 9 days after the 4-hour additional IBA loading. The dotted line represents the organic Me_2S loading rate OL (g m^{-3} d^{-1}).

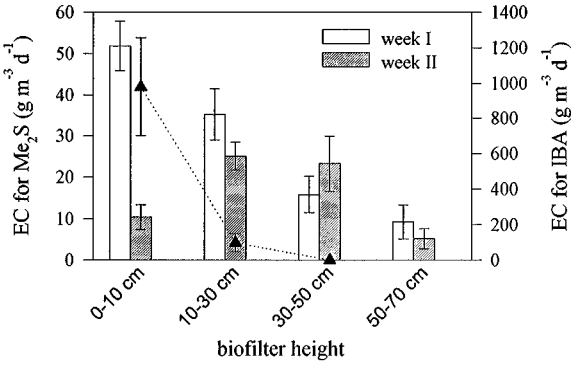

Figure 3. Elimination capacity EC for Me_2S (g m^{-3} d^{-1}) ± standard deviation (n = 4) in the different sections of the biofilter before (week I) and during (week II) the supplementary loading with IBA. (▲) represents the EC for IBA (g m^{-3} d^{-1}) ± standard deviation (n = 4) in the different sections during week II.

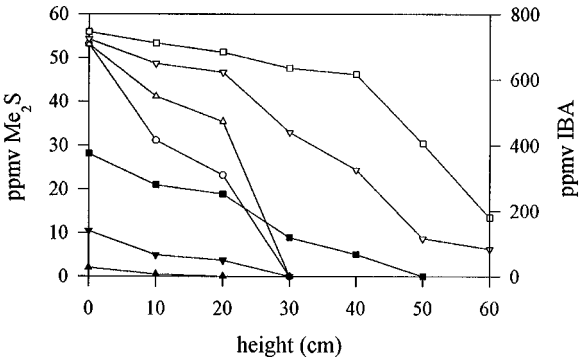

Figure 4. Vertical Me_2S (○, △, ▽, □) and IBA (▲, ▼, ■) concentration profiles (ppmv) in the *Hyphomicrobium* MS3-inoculated compost biofilter. Initially, the reactor received only Me_2S (○). The vertical concentration profiles in the biofilter when supplemented with IBA for 1.5 h are indicated with (△, ▲) at an IBA load of 262 g m^{-3} d^{-1}, with (▽, ▼) at an IBA load of 1384 g m^{-3} d^{-1} and with (□, ■) at an IBA load of 3710 g m^{-3} d^{-1}.

the organic Me_2S loading rate was adjusted to 49 g m^{-3} d^{-1}. After one week dosing of Me_2S as a single substrate, IBA was supplementary dosed at an organic loading rate of 153 g m^{-3} d^{-1} during the subsequent 7-day period. Over this 14-day period, the vertical Me_2S- and IBA-concentration profiles within the biofilter bed were measured regularly and allowed comparison of the degradation of both compounds in the different sections (height 0–10; 10–30; 30–50 and 50–70 cm) of the biofilter during both periods. During week I, the EC for Me_2S was 52 g m^{-3} d^{-1} in the lower section (0–10 cm) of the biofilter and decreased to 9 g m^{-3} d^{-1} in the highest part (50–70 cm) of the biofilter, giving an overall EC of 25 g m^{-3} d^{-1} for the complete biofilter (Figure 3). During week II, 85% of the IBA was degraded in the lower section of the biofilter while the remaining 15% was degraded in the section 10–30 cm. Comparison of the EC for Me_2S in the different sections of the biofilter before and during the supplementary loading with IBA revealed a 81% decrease of the Me_2S-degrading activity (EC dropped from 52 to 10 g Me_2S m^{-3} d^{-1}) in the lower 10 cm. A 29% decrease was observed in the section 10–30 cm, while no statistically significant change in EC for Me_2S was measured in the upper 40 cm of the biofilter. The overall EC of the biofilter for Me_2S decreased from 25 g m^{-3} d^{-1} in week I to 17 g m^{-3} d^{-1} in week II.

Simultaneous biofiltration of Me_2S and IBA in the Hyphomicrobium *MS3-inoculated compost biofilter*

After inoculation of a lab-scale compost biofilter, colonisation of the culture over the complete biofilter height was obtained by imposing a high organic Me_2S loading rate of 1500 g m^{-3} d^{-1}. After several weeks, the organic Me_2S loading rate was readjusted to 411 g m^{-3} d^{-1} (± 56 ppmv), resulting in a 100% elimination efficiency at a biofilter height of 30 cm (Time period 1) (Table 2, Figure 4). Upon the supplementation of IBA to the biofilter during a 1.5-hour period at an organic loading rate of 262 (Time period 2), 1384 (Time period 3) and 3710 g m^{-3} d^{-1} (Time period 4), respectively, the overall Me_2S elimination efficiency of the biofilter was 100, 89 and 76% (Table 2). Even at the highest IBA dose, 100% elimination efficiencies were obtained for this compound. The vertical concentration profiles within the biofilter bed during these experiments revealed the migration of the Me_2S-degrading activity towards higher parts in the biofilter as the IBA loading rate and the concomitant IBA penetration height in the biofilter increased (Figure 4).

Simultaneous biofiltration of Me_2S and toluene in the Hyphomicrobium *MS3-inoculated compost biofilter*

At an organic loading rate of 1168 g m^{-3} d^{-1} (± 160 ppmv), the elimination efficiency for Me_2S as a single compound in the inoculated compost biofilter was

Table 2. Organic loading rate (OL) and elimination capacity (EC) (g m^{-3} d^{-1}) of the *Hyphomicrobium* MS3-inoculated compost filter for Me_2S and IBA upon simultaneous biofiltration of both compounds

Time period	OL Me_2S (g m^{-3} d^{-1})	OL IBA (g m^{-3} d^{-1})	EC Me_2S (g m^{-3} d^{-1})	EC IBA (g m^{-3} d^{-1})
1 (0.0 h–1.5 h)	411	0	411	0
2 (1.5 h–3.0 h)	453	262	453	262
3 (3.0 h–4.5 h)	463	1384	410	1384
4 (4.5 h–6.0 h)	477	3710	362	3710

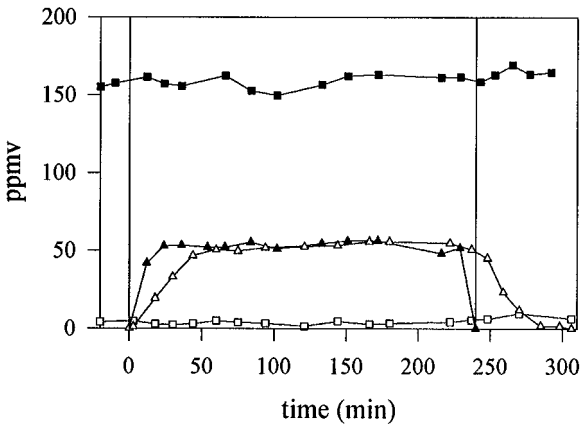

Figure 5. Removal of Me_2S in the *Hyphomicrobium* MS3-inoculated compost biofilter before, during (t = 0 → 240 min) and after the 4-hour additional loading with toluene. Symbols: Me_2S influent (■) and effluent (□) concentration (ppmv); toluene influent (▲) and effluent (△) concentration (ppmv).

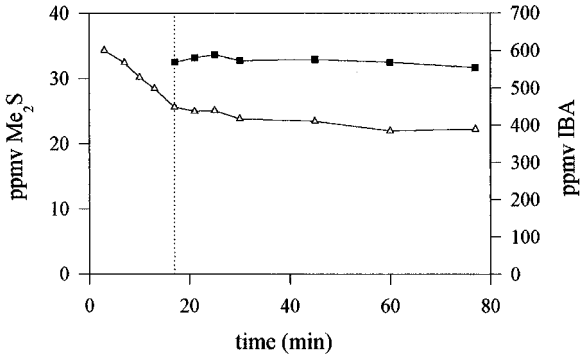

Figure 6. Effect of the supplementation of 570 ppmv IBA (■) in the headspace (dashed line at t = 17 min) on the Me_2S-degradation of *Hyphomicrobium* MS3 in liquid culture (△). Shown data are headspace gas concentrations.

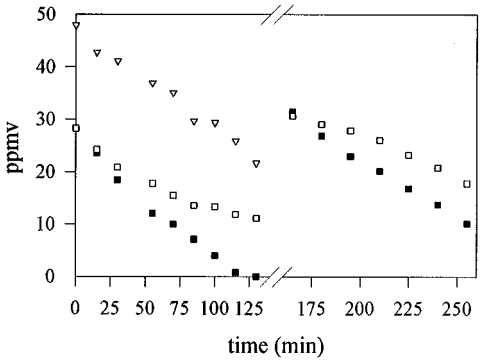

Figure 7. Me_2S degradation by *Hyphomicrobium* MS3 in liquid culture with (□) and without (■) the presence of IBA (▽) as a second substrate. At t = 130 min, both bottles were opened and volatiles were stripped out, while at t = 150 min, bottles were re-incubated with Me_2S as a single substrate. Shown data are headspace gas concentrations.

97%. The supplementation of toluene as a second substrate at an organic loading rate of 564 g m^{-3} d^{-1} (± 50 ppmv) during a 4-hour period was not affecting the EC for Me_2S, while toluene itself was not degraded (Figure 5). The influence of sorption phenomena in the biofiltration process are illustrated as the apparent positive and negative elimination efficiency for toluene at the start and immediately after the 4-hour toluene dosing period, respectively.

Degradative properties of Hyphomicrobium *MS3*

An overview of the degradative properties of *Hyphomicrobium* MS3 towards a selection of volatile compounds is given in Table 3. As can be seen, the culture was able to degrade several other sulfur compounds next to Me_2S, as hydrogen sulfide, methanethiol, dimethyl disulfide and dimethyl trisulfide. Besides these sulfur compounds, *Hyphomicrobium* MS3 degraded methanol and IBA. In Figure 6, it can be seen that the degradation of Me_2S by *Hyphomicrobium* MS3 in liquid culture was strongly (90%)

Table 3. Degradative properties of *Hyphomicrobium* MS3 towards volatile compounds from different chemical groups

Group	Compound	Degradation
Sulfur compounds	hydrogen sulfide	+
	methanethiol	+
	dimethyl sulfide	+
	dimethyl disulfide	+
	dimethyl trisulfide	+
	carbon disulfide	-
	thiofene	-
Carbonyl compounds	isobutyraldehyde	+
	methyl isobutyl ketone	-
Alcohol compounds	methanol	+
Aromatic compounds	benzene	-
	toluene	-

inhibited upon the addition of 570 ppmv IBA as a second substrate. In the presence of 48 ppmv IBA, the Me_2S-degradation by *Hyphomicrobium* MS3 slowed down, while IBA was efficiently degraded (Figure 7). This observation suggests that *Hyphomicrobium* MS3 is shifting its metabolism towards the degradation of IBA in the presence of the latter. Upon reincubation with Me_2S as a single substrate, Me_2S-degradation restarted, illustrating the reversability of the metabolic shift. *Hyphomicrobium* MS3 was not able to degrade toluene or benzene as a single compound (Table 3), while the co-presence of 55 ppmv benzene or toluene did not affect the Me_2S-degradation (data not shown).

Discussion

An almost complete (88%) inhibition in Me_2S removal was observed in the *Hyphomicrobium* MS3-inoculated wood bark biofilter upon the supplementation of IBA as a second compound at an organic loading rate of 1100 g m^{-3} d^{-1} (Figures 1, 2). When IBA was supplementary dosed at an organic loading rate of 153 g m^{-3} d^{-1}, a 81% inhibition in Me_2S removal was observed in the lower biofilter section (0–10 cm) which was responsible for 85% of the IBA removal (Figure 3). No inhibition in Me_2S removal was observed in the higher sections of the biofilter where no IBA was present. A similar illustration of the sequential degradation of both compounds was obtained in the *Hyphomicrobium* MS3-inoculated compost biofilter (Figure 4). For toluene as a second substrate at an organic loading rate of 564 g m^{-3} d^{-1}, however, no inhibition of the Me_2S-degradation was observed, while toluene itself was not degraded during this 4 hours period. An inhibition of the Me_2S removal can, however, be expected at very high toluene concentrations due to toxicity. In relation to this, Weber (1995) reported a 50% inhibition in growth rate of *Rhodococcus* S5 and *Pseudomonas* S12 at toluene gas concentrations of 68 and 82 gm^{-3}, respectively.

These biofiltration experiments with mixed substrates were in accordance with the metabolic properties of the inoculum *Hyphomicrobium* MS3 as determined in batch experiments (Table 3). Indeed, the Me_2S-degradation by the culture was not affected by the co-presence of moderate concentrations (55 ppmv) of toluene or benzene, while the co-presence of IBA resulted in a metabolic shift of *Hyphomicrobium* MS3 towards degradation of the latter compound. After complete degradation of the aldehyde, however, *Hyphomicrobium* MS3 restarted to degrade Me_2S without any lag-period (Figure 7), suggesting the IBA to perform a competitive inhibition on the Me_2S-degrading enzyme system in *Hyphomicrobium* MS3. Apparently, the aldehyde is a more attractive substrate for *Hyphomicrobium* MS3 than Me_2S. Similar competitive inhibition phenomena were observed by Zhang et al. (1991), who reported a decrease in Me_2S removal efficiency in a *Hyphomicrobium* I55-inoculated peat biofilter from 80% to 25% by the co-presence of hydrogen sulfide and methanethiol due to a preferential degradation of the latter compounds. In a peat biofilter inoculated with *Thiobacillus thioparus* DW44, however, the elimination capacity for Me_2S was inhibited by the presence of methanethiol, but accelerated by the presence of hydrogen sulfide (Cho et al. 1991). More recently, Deshusses et al. (1995) studied the biofiltration of mixtures of carbonyl compounds and reported marked influences of the compounds on each other's removal rates due to competition by the active biomass for both substrates.

The most probable cause for the 5-day inhibition period of the Me_2S-degradation in the *Hyphomicrobium* MS3-inoculated wood bark biofilter after the 4-hour aldehyde dosing (Figure 1) is the preferential degradation by *Hyphomicrobium* MS3 of slowly desorbing IBA molecules from the wood bark material, prior to Me_2S-degradation. According to Chiou & Kile (1994), sorption and desorption of volatiles into high-organic-content materials as e.g. wood bark are indeed very slow (up to several weeks) processes.

It can be concluded that, in *Hyphomicrobium* MS3-inoculated biofilters treating mixed waste gases, the

Me_2S-degrading activity will be negatively influenced by the presence of more attractive substrates as IBA and probably several other volatiles. This is a potential explanation for the low and unpredictable removal efficiency observed for VOSCs in biofilters treating complex rendering waste gases. The observed sequential degradation of IBA and Me_2S in separated parts of the biofilter implicates that sufficiently high or bistage biofilters have to be designed to achieve a complete purification of this mixed waste gas stream. Microbial understanding of the degradation processes taking place in biofilters loaded with complex waste gas streams should ultimately determine process engineering of biofiltration by predicting the height of the packed bed or the gas residence time required for complete elimination of the different volatiles present.

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