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Enhancement of ethene removal from waste gas by stimulating nitrification

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Abstract

The treatment of poorly water soluble waste gas compounds, such as ethene, is associated with low substrate concentration levels in the liquid phase. This low concentration level might hamper the optimal development of a microbial population. In this respect, the possible benefit of introducing nitrifying activity in the heterotrophic removal of ethene at moderate concentrations (< 1000 ppm) from a waste gas was investigated. Nitrifying activity is known to be associated with (i) the production of soluble microbial products, which can act as (co-)substrates for heterotrophic micro-organisms and (ii) the co-oxidation of ethene. The used reactor configuration was a packed granular activated carbon biobed inoculated with the heterotrophic strain *Mycobacterium* E3. The nitrifying activity was introduced by regular submersion in a nitrifying medium prepared from (i) compost or (ii) activated sludge. In both cases a clear enhancement of the volumetric removal rate of ethene could be observed. When combined with a NH_3 dosage on a daily basis, a gradual increase of the volumetric removal rate of ethene could be observed. For a volumetric loading rate of 3 kg ethene-COD$\cdot m^{-3}\cdot d^{-1}$, the volumetric removal rate could thus be increased with a factor 1.8, i.e. from 0.72 to a level of 1.26 kg ethene-COD$\cdot m^{-3}\cdot d^{-1}$.

Abbreviations: GAC – granular activated carbon; SMP – soluble microbial products

Introduction

In recent years, several studies concerning the biological treatment of waste gases containing *V*olatile *O*rganic *C*ompounds (VOC) have been reported, e.g. Kanagawa & Mikami (1989), Diks & Ottengraf (1991), Hartmans & Tramper (1991), Kirchner et al. (1991), Shareefdeen et al. (1993), Weckhuysen et al. (1993) and Zilli et al. (1993). However, few reports are concerned with the treatment of VOC for which the dimensionless air/water partition coefficient (m) is greater than 1, like e.g. ethene (Van Ginkel et al. 1987) and methane (Sly et al. 1993). The high m value of these VOC is a reflection of their poor water solubility and/or high volatility. This poor water solubility hampers in the first place the transfer of the VOC from the gas phase to the bioactive phase in the waste gas treatment system where biodegradation takes place (e.g. a biofilm supported by an inert packing material). In the second place, the low concentration in the bioactive phase might hamper the development of a microbial population showing a high biodegradation activity. In the case of ethene, for which m at 20 °C is about 7.6 (l'Air Liquide 1976), the equilibrium liquid concentration of a waste gas containing 100 ppm ethene is equal to 13 $\mu g\cdot l^{-1}$, corresponding to 45 μg COD$\cdot l^{-1}$. This concentration is very low in comparison with e.g. a biological waste water treatment system where substrate concentrations are generally in the order 10–50 mg COD$\cdot l^{-1}$ (Verstraete & Van Vaerenbergh 1986). Of the few bacteria whose ability to grow at very low substrate concentrations have been studied, threshold concentrations have been found to be ca. 2 $\mu g\cdot l^{-1}$ or much higher (Schmidt & Alexander 1985). An explanation for the existence of a threshold concentration is that energy is obtained too slowly from oxidation of the low substrate concentration to meet the cellu-

lar energy demands for growth (Schmidt & Alexander 1985; Boethling & Alexander 1979).

A strategy to overcome starvation due to low substrate concentration levels is the supply of co-substrates. However, the selection of an appropriate co-substrate is not straightforward since excessive growth of common micro-organisms, not showing the degradative capacity for the targetted pollutant, should be avoided. The co-substrate should therefore be characterised by a poor biodegradability and a limited availability. In principle, co-substrates can be supplied indirectly by micro-organisms which in the treatment system are co-existing with the primary degrader of the contaminant of interest. Micro-organisms are known to leak metabolic products through their semi-permeable cell-membrane (Atlas & Bartha 1992), which subsequently become available as co-substrates in the community. In this view, the introduction of autotrophic nitrifying micro-organisms in the treatment system shows an interesting potential. First, they are not heterotrophic competitors for the limited available organic carbon. Second, their presence and activity in the treatment system can be well controlled by the supply of ammonia. In addition, the ammonia monooxygenase of *Nitrosomonas europaea* is known to co-oxidize ethene.

This work investigates the possibility that the heterotrophic removal of poorly water soluble waste gas compounds, using ethene (C_2H_4) as a model, can be stimulated by the introduction of autotrophic nitrifying activity. The reactor used for experimental investigation was a packed *G*ranulated *A*ctivated *C*arbon (GAC) biobed (De heyder et al. 1994) inoculated with the heterotrophic ethene-degrading strain *Mycobacterium* E3.

Materials and methods

Analyses

Ethene was analysed chromatographically as described by De heyder et al. (1994). The water content of the GAC biobed samples was determined using an infra-red drying balance (Sartorius). The nitrite, nitrate and ammonia concentrations were determined semi-quantitatively using Merckoquant test-strips (Merck).

Mineral media

Physiological solution (PS) was composed of 8.5 g $NaCl \cdot l^{-1}$. All mineral salts media contained the following salts in 1 l of deionized water: 0.2 g $MgSO_4 \cdot 7H_2O$, 1.5 mg $CaCl_2$, 1 mg $FeSO_4 \cdot 7H_2O$, 5 μg $CuSO_4 \cdot 5H_2O$, 10 μg H_3BO_3, 70 μg $ZnSO_4 \cdot 7H_2O$, 10 μg $MnSO_4 \cdot 5H_2O$ and 100 μg $Na_2MoO_4 \cdot 2H_2O$ (De Bont 1976). For mineral medium MM1 the following salts were also added: 2.0 g $NaNO_3$, 0.5 g K_2HPO_4 and 0.5 g KH_2PO_4. For mineral medium MM2 the following salts were also added: 2.57 g $(NH_4)_2SO_4$, 22.28 g K_2HPO_4, and 13.61 g KH_2PO_4. Mineral medium MM3 was composed as MM1 with 2.57 g $(NH_4)_2SO_4$ added.

Microbial cultures and nitrifying media

The ethene-degrading strain *Mycobacterium* E3 is described by Habets-Crützen et al. (1984) and was kindly provided by Dr. Hartmans (Wageningen Agricultural University, Wageningen, The Netherlands). Batch cultures of this strain were grown at 25 °C in MM1 in shaken serum flasks sealed with rubber septa. The flasks contained 10% ethene in the gas phase.

Nitrifying mixed cultures originated from two sources. A first source was DP-compost (Gellens & Verstraete 1995). When required, 160 g compost was suspended during 3 h in 1 l MM2 in a shaken batch recipient. Subsequently, the nitrite, nitrate and ammonia concentrations were checked to evaluate the presence of nitrification activity. The suspension was sieved several times to reduce the suspended solid fraction (2 mm sieve). The sieved suspension was diluted five-fold with MM2. The obtained nitrifying suspension based on compost is further referred to as the compost medium (CMM2). A second source was activated sludge. The activated sludge was grown in a 1 l aerated fed-batch reactor. Each day, the aeration was stopped to settle the sludge and half of the supernatant was replaced by tap water. The nitrite, nitrate and ammonia concentrations were checked to evaluate the presence of nitrification activity. Subsequently, 1.35 g NH_4Cl, 0.45 g $CaCO_3$, 0.15 g KH_2PO_4 and 0.1 g Nutrifloc 50S (Vansever et al. 1995) were added and the aeration was restarted. Overnight, a pH drop was prevented by dosing NaOH. When required, 300 ml of the nitrifying activated sludge was diluted eleven-fold in MM3. The obtained nitrifying suspension based on activated sludge is further referred to as the activated sludge medium (ASMM3). The use of activated sludge medium (ASMM3) allowed a more reproducible preparation of a nitrifying medium in comparison with compost medium (CMM2).

Reactor set-up

The reactor consisted basically of a plastic column (diameter 9.4 cm, length 50 cm) packed over a height of 24.5 cm with Granular Activated Carbon (GAC) (Chemviron Carbon F30/470) as a carrier for the micro-organisms and is further referred to as the GAC biobed. The waste gas stream was created by dosing ethene (C_2H_4) into a stream of laboratory air. The laboratory air was characterised by a relative humidity of 50–60%. A humidifier allowed humidification of the laboratory air before dosing ethene. The auxilliary equipment necessary to dose ethene, to humidify the laboratory air, to measure the waste gas flow rate, etc. is described by De heyder et al. (1994). Extra equipment was provided to:

- measure continuously the temperature and relative humidity of the waste gas influent and effluent using a thermometer and hygrometer, respectively and
- dose NH_3 in the waste gas influent (see further).

The influent and effluent waste gas streams were sampled just before and after the GAC biobed, respectively, as described by De heyder et al. (1994). Three samples were taken for each stream to determine the mean influent ($C_{g,i}$) and effluent ($C_{g,e}$) concentrations. The corresponding volumetric loading (L_v) and removal (R_v) rate was calculated as kg COD·m^{-3}·d^{-1} based on the equations (with the necessary unit correction factors, e.g. 1 g of ethene corresponds to 3.42 g COD):

$$L_V = \frac{C_{g,i}}{\theta_p} \quad (1)$$

$$R_v = \frac{C_{g,i} - C_{g,e}}{\theta_p} \quad (2)$$

where θ_p equals the pseudo residence time of the waste gas in the GAC biobed, i.e. the ratio of the GAC biobed volume (1.7 l) and the waste gas flow rate. The comparison of R_v for a certain value of L_v at two different moments, assumes that the respective values of $C_{g,i}$ and θ_p are not differing to a wide extent.

NH_3 dosing in the waste gas influent

The dosing of NH_3 was based on stripping of NH_3 with a separate ambient air stream from a saturated $(NH_4)_2SO_4$ solution buffered at pH 10 and was started on day 209. The stripped NH_3 was subsequently mixed with the waste gas influent. The NH_3 stripping rate was quantified in a separate experiment where during a certain time the stripped NH_3 was absorbed in a boric-acid solution. Back titration of the boric acid solution with HCl, as in the determination of Kjehldahl nitrogen (Bremner & Keeney 1965), allowed to determine the absorbed amount of NH_3. The calculated NH_3 stripping rate corresponded to 697 μg NH_3-N·min^{-1}. The NH_3 dosing rate in the waste gas influent was set according to the ethene removal assuming that 50% of the removed carbon is incorporated into biomass with a composition of $C_5H_7O_2N$. The dosing of NH_3 was limited to this load not to create adverse effects such as nitrification associated acidification of the GAC biobed. The NH_3 dosing was controlled by a time clock which activated the stripping during 5 times 15 minutes, corresponding to 31 g NH_3-N·m^{-3}·d^{-1}. The dosage was done overnight, in order to prevent disturbance of the measurements during the day.

Start-up and operational state of the reactor set-up

Before inoculation with *Mycobacterium* E3, the GAC packing was pH neutralised by continuous recirculation of liquid over the packing. The pH of the recirculating liquid was checked at regular intervals and corrected with HCl. In total, approximately 26.2 μeq HCl·g^{-1} GAC were added over a period of 5 days to obtain a stabilised pH value around 6.9–7.1. The original inoculation of the GAC packing was done by recirculation of a batch-grown *Mycobacterium* E3 suspension over the packing. The sorption of micro-organisms on the packing was indicated by the overnight disappearance of the turbidity of the recirculating liquid. During a period of about 5 months, the GAC biobed was subjected to different operating conditions for preliminary experiments which implied extra inoculations with *Mycobacterium* E3 and addition of an extra nutrient source. The experiments described in this report were performed after this initial period and are based on short submersions of the GAC biobed with liquid medium. These submersions implied that approximately 2.25 l liquid medium was pumped into the GAC biobed from the bottom. During the submersion the waste gas influent was disconnected from the GAC biobed. After a certain time, the medium was pumped out of the column and the waste gas influent reconnected. The submersion of the GAC biobed with the compost medium (CMM2) or the activated sludge medium (ASMM3) allowed to inoculate the GAC biobed with nitrifying activity.

Table 1. Details of the different submersions noted in Figure 1

N^o	Time (d)	Duration (min)	%[1]	Medium
(1)	2.7	75	100	MM1
(2)	7.7	60	100	CMM2
(3)	14.5	75	100	PS
(4)	20.7	67	100	CMM2
(5)	33.7	10	100	PS
(6)	41.7	30	100	MM2
(7)	51.6	35	100	MM2
(8)	64.7	40	100	PS[2]
	64.7	40	100	CMM2
(9)	76.6	10	29	PS
(10)	83.6	10	41	PS
(11)	90.6	20	100	MM1

[1] The percentage corresponds to the height of the GAC biobed that was submerged.
[2] During submersion (8), the submersion in PS was done separately just before the submersion in CMM2.

First experimental phase

During the first experimental phase, the performance of the GAC biobed was followed by regular determination of L_v and R_v. The values of L_v and θ_p were held constant around 1 kg COD·m^{-3}·d^{-1} and in the range 70–80 s, respectively. The waste gas influent was not pre-humidified. The low relative humidity of the waste gas influent resulted in a visible dehydration of the GAC biobed. Excessive dehydration was prevented by the above-mentioned submersions in the different media (PS, MM1, MM2, compost medium (CMM2)). The GAC biobed was submerged completely or partially (i.e. only a lower part of the GAC biobed), during a period of maximal 2 h.

Second experimental phase

The second experimental phase differed from the first in three ways. First, the performance of the GAC biobed was evaluated by recording the removal function (RF). The RF gives R_v as a function of the L_v at a constant θ_p, and allows thus to evaluate the performance over a range of L_v. For this, the GAC biobed is loaded with a L_v step-function by increasing the waste gas influent concentration every 55–60 minutes. After breakthrough of each new effluent concentration, R_v is determined. The relative short time period of this experimental procedure, maximum approximately 9 h, allowed to assume constant operating conditions, especially with respect to the biomass concentration. In between the recordings of the different removal functions, the values of L_v and θ_p were held around 1 kg COD·m^{-3}·d^{-1} and in the range 60–80 s, respectively. The exponential equation:

$$R_v = A \cdot [1 - e^{-B \cdot L_v}]$$

was fitted to the recorded removal functions for presentation purposes. The fitting and estimation of parameters A and B was performed using the Marquardt-Levenberg algorithm incorporated in the software Sigmaplot 1.01 (Jandel Scientifique). No physical interpretation is given to the estimated values of A and B.

Second, the GAC biobed was submerged with MM1 or activated sludge medium (ASMM3). The submersion procedure was also standardised to allow a better comparison of the obtained results: the submersion lasted 10 minutes after which the liquid was pumped out slowly and the GAC biobed was dried during 30 minutes by pumping a laboratory air stream through the GAC biobed from the top to the bottom.

Third, the waste gas influent was pre-humidified to (i) 100% using water or (ii) 92% using a saturated Na_2CO_3 solution. The measured relative humidity when using the saturated Na_2CO_3 solution was, however, higher than the theoretically expected value. In this respect it should be noted that the used hygrometer had only an accuracy of a few percent of relative humidity. After the recording of some removal functions the water content of GAC biobed samples from the bottom, middle and top of the bed were determined. The same time scale is used for presentation of the results of both the first and second experimental phase. On this time scale, the start of the first experimental phase corresponds to day zero.

Results

First experimental phase

Figure 1 shows θ_p, L_v and R_v as a function of time together with the different times of submersion. The conditions of the different submersions are described in Table 1.

At the beginning of the experiment, the operational state of the GAC biobed was such that a regular complete submersion of the GAC biobed with MM1 result-

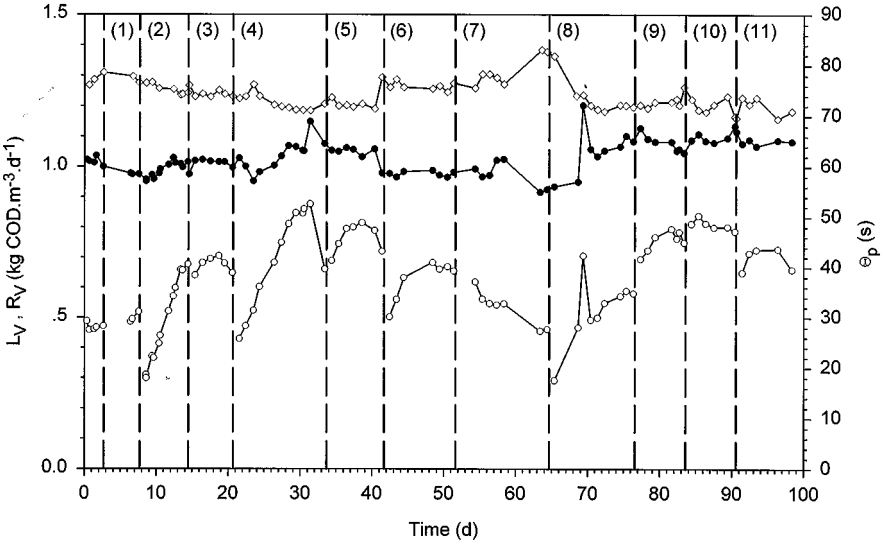

Figure 1. Evolution of L_v (●), θ_p (◇) and R_v (○) during the first experimental phase. The time of the different submersions is indicated by a vertical dashed line with the corresponding number of the submersion (see Table 1).

ed, for the given θ_p and L_v, in a R_v of approximately 0.5 kg COD·m^{-3}·d^{-1}. The latter is illustrated by the first submersion (Figure 1).

The second submersion, with the nitrifying compost medium (CMM2), resulted first in a sharp decrease of R_v. The decrease, however, was compensated by a subsequent stronger increase, resulting in an overall positive effect of submersion in compost medium (CMM2).

The third submersion, with PS, allowed to check the mineral nitrogen in the GAC biobed. The latter is based on the extraction of mineral nitrogen from the GAC biobed during submersion in PS. The PS is collected after submersion and the extracted mineral nitrogen semi-quantitatively determined with test strips. Since the mineral N compounds are probably not extracted completely, the method does not allow to determine directly their conversion over a certain time period. Their concentration in the collected PS, however, reflects their availability in the GAC biobed. The collected PS showed the following composition: 10 mg NO_2^-·l^{-1}, 250 mg (NO_2^- + NO_3^-)·1^{-1} and 5 mg NH_4^+·l^{-1}.

The fourth (CMM2) and the fifth (PS) submersions were repetitions of the second and the third submersions, illustrating the same effects. The collected PS showed the following composition: 0 mg NO_2^-·l^{-1}, 50 mg (NO_2^- + NO_3^-)·l^{-1} and 0 mg NH_4^+·l^{-1}.

The sixth and seventh submersions were performed with MM2. These subsequent submersions, however, seemed on a longer time scale to have a decreasing effect on the removal.

The eighth submersion was a repetition of the second and fourth submersion, performed with compost medium (CMM2). The eighth submersion, however, was preceeded by a separate submersion in PS. The collected PS showed the following composition: 0 mg NO_2^-·l^{-1}, 75 mg (NO_2^- + NO_3^-)·l^{-1} and 5 mg NH_4^+·l^{-1}. As a result of the submersion in compost medium (CMM2), R_v again showed a decrease followed by an increase, as could be observed for the second and fourth submersion.

The ninth and tenth submersions, corresponding with only a partial submersion in PS, resulted in no decrease of R_v, and allowed for R_v to further increase and reach a constant level. The corresponding composition of the collected PS corresponded in both cases to 0 mg NO_2^-·l^{-1}, 25 mg (NO_2^- + NO_3^-)·l^{-1} and 0 mg NH_4^+·l^{-1}.

Finally, the eleventh submersion, with MM1 as in the beginning of the first experimental phase, illustrated that submersion in this medium did not result in a further enhancement of the removal.

Table 2. Operating conditions and R_v values corresponding with the different removal functions

RF or submersion	Day	Temperature waste gas influent (°C)	Pre-humidification medium	Relative humidity waste gas influent	θ_p (s)	Water content of GAC biobed samples (%)			Estimated R_v value as a function of L_v (kg COD·m^{-3}·d^{-1})		
						Bottom	Middle	Top	L_v = 1	L_v = 2	L_v = 3
MM1	124			(%)							
1	131	20.8	water	100	86.2	48	47.1	47.3	0.45	0.64	0.72
ASMM3	169										
2	176	20.4	water	98	76.8	50	49.3	n.d.	0.55	0.83	0.97
3	183	19.5	water	100	80.9	n.d.	n.d.	n.d.	0.47	0.64	0.69
MM1	195										
4	203	19.5	sat. Na_2CO_3 sol.	95.2	94	48.7	48.9	46.4	0.51	0.76	0.87
ASMM3	209										
5	217	17.9	sat. Na_2CO_3 sol.	94	79.3	48.5	47.3	47.2	0.63	1.01	1.25
ASMM3	294										
6	301	22.9	sat. Na_2CO_3 sol.	98.5	65.8	46.2	45.6	43	0.72	1.08	1.26
7	308	22.2	sat. Na_2CO_3 sol.	97	66	44.1	40.8	45.2	0.83	1.20	1.35

n.d.: Not Determined.

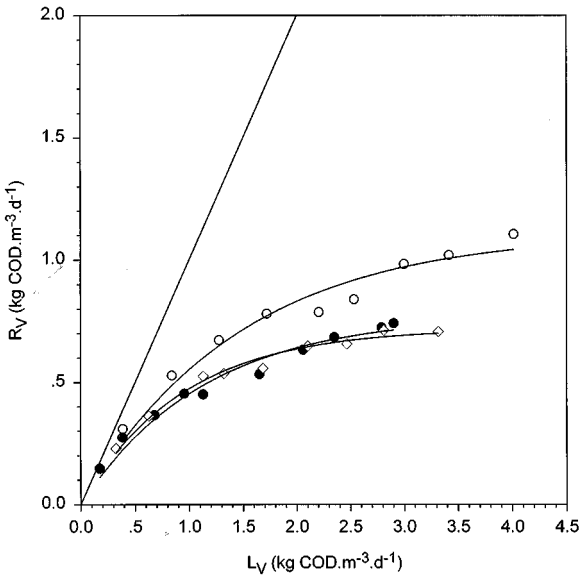

Figure 2. Effect of submersion in MM1 and ASMM3 on the removal function during the first period of the second experimental phase: (●) RF1 recorded after submersion in MM1, (○) RF2 and (◊) RF3 recorded after submersion in ASMM3 (see Table 2). (—) Fitted curves.

Second experimental phase

In the period between the first and the second experimental phase, the GAC biobed was three times partially submerged with PS (days 99, 105 and 111). The second experimental phase begins on day 117 when the GAC biobed was completely submerged with MM1 and the pre-humidification of the waste influent restarted. Table 2 shows the operating conditions corresponding to the recording of the different removal functions. Although the obtained relative humidity level, when using the saturated Na_2CO_3 solution, was higher than the theoretically expected values, the expected decreasing trend of relative humidity can be observed (Table 2). This trend can also be observed in the water content of the GAC biobed samples (Table 2).

During a first period (days 117–194), the waste gas influent was pre-humidified using water (Table 2). The high pre-humidification level slowed down the dehydration of the GAC biobed. The effect of the first submersion in activated sludge medium (ASMM3) on the removal function during this period is shown in Figure 2. The removal function RF1, recorded before submersion in activated sludge medium (ASMM3), corresponds to the reference mode of operation, i.e. regular submersion in MM1. Considering that the last submersion in an ammonium containing medium was done during the first experimental phase on day 64, it was assumed that no actively stimulated nitrifying activity was present when RF1 was recorded. Removal function RF2, recorded after submersion in activated sludge medium (ASMM3), shows clearly an enhanced removal, although only for L_v larger than 1 kg COD·m^{-3}·d^{-1}. Removal function RF3, recorded 1 week after RF2, showed that the removal function had decreased again to a level comparable with RF1.

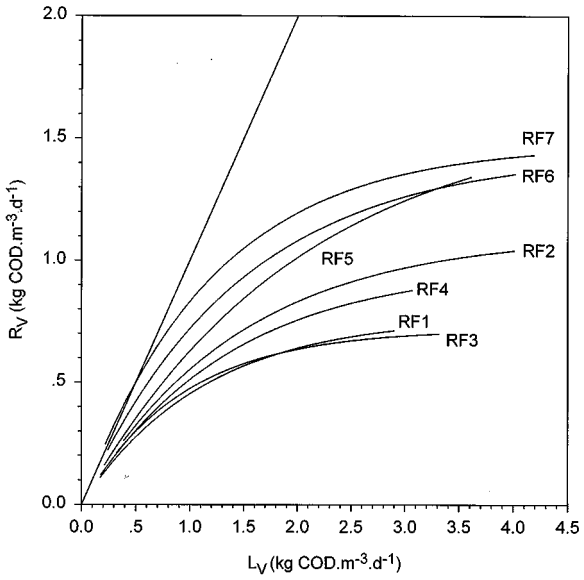

Figure 3. Overview and comparison of the different fitted removal functions (see Table 2).

The results in Figure 2 illustrate typically the fit of the experimental equation to the experimental data. For the further experiments, only the fitted curves are compared in Figure 3.

During a second period (days 195–217), it was investigated if the higher removal capacity of the GAC biobed obtained after submersion in activated sludge medium (ASMM3) could be maintained by daily dosage of NH_3 in the waste gas influent. This would allow a more continuous support of the nitrifying activity. Since during this second period the waste gas influent was pre-humidified using a Na_2CO_3 saturated solution (Table 2), the removal corresponding with the reference mode of operation had to be characterised again by submersion in MM1. It was assumed that no actively stimulated nitrifying activity was present since no NH_4^+ had been dosed to the GAC biobed for 34 days. The effect of submersion in activated sludge medium (ASMM3) combined with the daily dosage of NH_3 is shown in Figure 3 (RF5). The results show clearly a higher removal capacity in comparison with the reference mode of operation for L_v larger than 1 kg COD·m^{-3}·d^{-1} (Figure 3) (RF4). The removal capacity characterised by RF5 is also higher than the removal capacity characterised by RF2 (Figure 3).

During a third period, starting the day after recording RF5 (day 218), the ethene removal could during approximately 2 months be held at a higher level as characterised by removal function RF5. In these two months, the waste gas influent was also pre-humidified using a Na_2CO_3 saturated solution, and the GAC biobed was only submerged one time with MM1 (day 257). After these two months, however, the ethene removal started to decrease (Table 3). This decrease was probably caused by a malfunctioning of the NH_3-dosage system as was detected during this period. As a result of this malfunctioning, an unknown smaller amount of NH_3 was being dosed. After repairing the NH_3-dosage system, the GAC biobed was submerged with activated sludge medium (ASMM3). The resulting ethene removal capacity is illustrated in Figure 3 by removal functions RF6 and RF7. Both removal functions show a slightly higher removal capacity in comparison with RF5 (Figure 3).

Table 3. Evolution of R_v after recording removal function RF5

Day	L_v (kg COD·m^{-3}·d^{-1})	θ_p (s)	R_v (kg COD·m^{-3}·d^{-1})
225	0.920	84.0	0.748
240	1.037	77.6	0.832
267	1.137	67.1	0.884
280	1.266	67.1	0.693
285	1.211	63.7	0.469
292	1.238	64.6	0.368

Discussion

The second, fourth and eighth submersion during the first experimental phase (Figure 1) clearly showed that submersion of the GAC biobed in the compost medium (CMM2) resulted in a decrease and subsequent increase of R_v. The observed decrease, however, was smaller than the increase, so that the overall effect was positive. Comparison with the first (MM1) and eleventh (MM1) submersions suggests that submersion in compost medium (CMM2) enhances the ethene removal capacity of the GAC biobed. Submersion in the activated sludge medium (ASMM3) during the second experimental phase, also resulted in higher ethene removal capacities (RF1 & RF5 – Figure 3) in comparison with the corresponding submersion in MM1 (RF2 & RF4 – Figure 3). An initial decreasing effect was not observed during the second experimental phase where each removal curve was recorded several days after submersion.

The initial decrease of R_v observed after submersion with the compost medium CMM2 during the first experimental phase can in principle be caused by the wetting of the GAC biobed. The latter results in less optimal conditions with respect to mass transfer of ethene from the waste gas to the biofilm (De heyder et al. 1994). The relative small decrease of R_v after the third submersion (PS) (Figure 1), however, illustrated that the effect of wetting is quite limited under the given circumstances. The dry waste gas influent results in a rapid elimation of the excess amount of water after submersion. A possible explanation for the initial decrease of R_v is stress due to a shock loading of mineral salts.

The enhancing effect of submersion in a nitrifying medium can be caused by at least 4 phenomena. In the first place, since the mineral nitrogen concentration is higher in the nitrifying media (compost medium CMM2 0.55 g $N{\cdot}l^{-1}$; activated sludge medium ASMM3 0.88 g $N{\cdot}l^{-1}$) than in the medium MM1 (0.33 g $N{\cdot}l^{-1}$), it is probable that submersion in the nitrifying media allows a better supply of nitrogen. However, the following figures illustrate that the nitrogen supply during a submersion in MM1 or a nitrifying medium, i.e. the compost medium (CMM2), is probably sufficient. During the third submersion (PS) of the first experimental phase (Figure 1) approximately 0.138 mg N was extracted from the GAC biobed. This extracted amount corresponds to the order of magnitude of nitrogen demand in between the different submersions assuming that 50% of the removed carbon is incorporated in new biomass with a composition of $C_5H_7O_2N$. The extraction of this amount of nitrogen does not hamper the subsequent level of R_v which was situated between that of the third and the fourth submersion (Figure 1). Also the decreasing R_v after the sixth (MM2) and seventh (MM2) submersions suggests that the positive effect of submersion in the compost medium (CMM2) is probably not strictly an effect of higher mineral nitrogen supply.

In the second place, the increase of R_v could be caused by the introduction of extra heterotrophic strains showing a biodegradative capacity towards ethene. Based on the results of the first and second experimental phase, however, it seems that these strains would require the presence of an active nitrifying culture.

In the third place, the increase of R_v could be caused by the co-oxidation of ethene by the nitrifying mixed culture. According to Keener & Arp (1993) the co-oxidation of ethene by *Nitrosomonas* is characterised by an affinity constant of 658 μM in the liquid phase, corresponding to approximately 150,000 ppm in the gas phase, and a maximum specific oxidation rate of 1,560 nmole $(min{\cdot}mg\ protein)^{-1}$. Since the ethene gas concentration corresponded to the order of magnitude of 200 ppm, co-oxidation of ethene by the nitrifying mixed culture would occur at a rate of maximum 2 nmole $(min{\cdot}mg\ protein)^{-1}$. For *Mycobacterium* E3 the kinetic parameters for ethene degradation estimated by Van Ginkel et al. (1986), namely affinity 100 ppm and maximum specific degradation rate 50 nmole $(min{\cdot}mg\ protein)^{-1}$, would result in an ethene degradation rate of 33 nmole $(min{\cdot}mg\ protein)^{-1}$. Although the exact contribution of the nitrifiers to the ethene removal depends on the concentration of the nitrifying biomass, these figures indicate the potential interference of the nitrifiers in the ethene removal process.

In the fourth place, the increase of R_v could be caused by the supply of an extra nutrient or carbon component present in the nitrifying media. Since the two nitrifying media were prepared from a different source, it is less probable that the extra nutrient or carbon component would be exclusively related to one of these two sources. The nutrient or carbon component could also be produced as a result of the metabolic activity of the nitrifying mixed culture. Fumurai & Rittmann (1992) addressed these compounds as *S*oluble *M*icrobial *P*roducts (SMP), representing a group of compounds which can be consumed by heterotrophs. Based on model simulations, they stated that in activated sludge systems heterotrophs can grow with very low influent organic substrate concentration if SMP are available from nitrifiers. Also Schmidt & Alexander (1985) conducted experiments that indicated that secondary substrates and uncharacterised dissolved organic carbon may play an important role in controlling the rate and the extent of biodegradation of organic compounds at low concentration. Fumurai & Rittmann (1994) applied the concept of SMP production by nitrifiers in a biofilm model. The model theoretically confirmed trends observed in experimental data concerning trace level removal. Using chemostat experiments, Rittmann et al. (1994) demonstrated experimentally the hypothesis that nitrifiers produce SMP that can support heterotrophic bacteria. Rittmann et al. (1994) distinguish two kinds of SMP being produced. Utilization-associated products (UAP) are formed directly as a result of substrate utilization, while biomass-associated products (BAP) are generated as a part of biomass maintenance and decay. The UAP for-

mation rate coefficient for nitrifiers is estimated to be 0.14 mg $COD_p \cdot (mg\ N)^{-1}$, where the subscript p refers to SMP. This coefficient refers to the complete nitrification of ammonia to nitrate. The BAP-formation rate coefficient for nitrifiers is estimated to be 0.1 mg COD_p (mg $COD_x \cdot d)^{-1}$, where the subscript x refers to biomass. Considering that during e.g. the second experimental phase 53 mg NH_3-$N \cdot d^{-1}$ was dosed to the GAC biobed with the NH_3-stripping system, only 7.4 mg $COD_p \cdot d^{-1}$ of UAP would be produced. This amount is neglible compared to the removed amount of COD supplied as ethene, namely 850 mg $COD \cdot d^{-1}$ corresponding to a volumetric removal rate of 0.5 kg COD $(m^3\ d)^{-1}$. It is therefore more probable that BAP formation would be responsible for a supply of extra or stimulating co-substrates.

The gradual increasing removal capacity obtained during the second experimental phase after each submersion in the activated sludge medium (ASMM3), illustrated by RF2, RF5 and RF6 (Figure 3), shows that the enhancing effect is accumulating. This gradual increase allowed to obtain a volumetric removal rate which, for a volumetric leading rate of 3 kg $COD \cdot m^{-3} \cdot d^{-1}$, is a factor 1.8 higher (RF6) in comparison with initial removal capacity (RF1) (Table 2). This gradually increasing removal capacity is probably an indication for a gradually increasing biomass concentration in the GAC biobed. An increased biomass concentration might explain the large difference in removal capacity between RF2 and RF5 (Figure 3) and between RF4 and RF1 which characterise the removal capacity for the reference mode of operation. The daily dosage of NH_3 started after recording RF4, allowed in principle a more continuous nitrifying activity and SMP production. The importance of daily NH_3 dosage is illustrated by the decrease of the removal capacity as a result of a malfunctioning of the NH_3 dosage system (Table 3).

Finally, it can be remarked that the drier operating conditions throughout the second experimental phase did probably not interfere to a large extent with the observed gradual increase of removal capacity. This is because drier operating conditions do not necessarily increase the biomass concentration. The biomass concentration determines in principle the maximum R_v-level that can be obtained at the higher levels of L_v. Drier operating conditions enhance, in principle, the diffusion rate of ethene from the gas phase to the biofilm and thus the rate by which the maximal R_v level is obtained (De Heyder et al. 1994).

Conclusions

The volumetric ethene removal rate by the GAC biobed was enhanced by regular submersion in a nitrifying medium. Combined with daily dosage of NH_3, the enhanced removal rate could be maintained for a long term. The enhanced removal rate is probably the result of co-oxidation of ethene by the nitrifiers or an enhanced growth of the heterotrophic ethene degrading *Mycobacterium* E3. This might possibly be caused by the *in-situ* autotrophically generated co-substrates under form of SMP. However, further research is required to make a strict discrimination between the latter effects and other possible interfering effects such as the extra supply of nitrogen or the introduction of extra heterotrophic ethene degrading strains with the nitrifying medium. The concept of *in-situ* production of co-substrates remains in principle attractive for treatment of poorly water soluble compounds, especially in the case of packed bed reactor systems that use a non-biodegradable packing material as support for the micro-organisms. In these systems, the level of substrate concentration in the liquid phase, as supplied by the influent waste gas, might be limiting for an optimal development of the microbial population.

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