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The influence of NaCl on the degradation rate of dichloromethane by *Hyphomicrobium* sp.

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Abstract

The degradation of dichloromethane by the pure strain *Hyphomicrobium* GJ21 and by an enrichment culture, isolated from a continuously operating biological trickling filter system, as well as the corresponding growth rates of these organisms were investigated in several batch experiments. By fitting the experimental data to generally accepted theoretical expressions for microbial growth, the maximum growth rates were determined. The effect of NaCl was investigated at salt concentrations varying from 0 to 1000 mM. Furthermore the dichloromethane degradation was investigated separately in experiments in which a high initial biomass concentration was applied. The results show that microbial growth is strongly inhibited by increased NaCl concentrations (50% reduction of μ_{max} at 200–250 mM NaCl), while a certain degree of adaptation has taken place within an operational system eliminating dichloromethane. A critical NaCl concentration for growth of 600 mM was found for the microbial culture isolated from an operational trickling filter, while a value of 375 mM was found for the pure culture *Hyphomicrobium* GJ21. The substrate degradation appears to be much less susceptible to inhibition by NaCl. Even at 800 mM NaCl relatively high substrate degradation rates are still observed, although this process is again dependent on the NaCl concentration. Here the substrate elimination is due to the maintenance requirements of the microorganisms. The inhibition of the dichloromethane elimination was also investigated in a laboratory scale trickling filter.The results of these experiments confirmed those obtained in the batch experiments. At NaCl concentrations exceeding 600 mM a considerable elimination of dichloromethane was still observed for during several months of operation. These observations indicate that the inhibition of microbial growth offers a significant control parameter against excessive biomass growth in biological trickling filters for waste gas treatment.

Introduction

A biological trickling filter for the elimination of biodegradable compounds from waste gases consists of a packed column of inert materials (e.g. plastics), onto which a suitable microbial flora is immobilized. The waste gas is forced to rise through this packed bed in cocurrent, countercurrent or cross flow with a water phase, which is continuously recirculated through the system. The pollutants, which are introduced via the gas phase, are absorbed in the liquid and transferred to the biofilm on the carrier material, where degradation takes place (Fig. 1).

This type of reactor is especially suitable in those cases where the microbial degradation of the polluting compounds, e.g. H_2S, NH_3 or halogenated hydrocarbons, results in the formation of acids. In case of chlorinated hydrocarbons such as dichloromethane the chlorine is excreted by the microorganisms in the form of hydrochloric acid. This acid is continuously washed from the filterbed by the water phase, after which it is neutralized. The neutralization process is most easily carried out with a sodiumhydroxide solution, which results in the accumulation of NaCl in the reactor system.

For many organisms adjusted to fresh water environments such accumulation and the presence of high

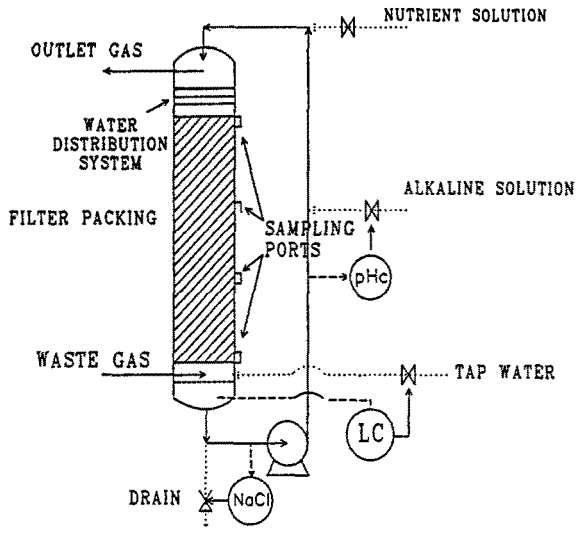

Fig. 1. Experimental set-up of a biological trickling filter system. The packing material consists of structured PVC elements.

salt concentrations may have considerable effects on their activity. For example the growth rate and yield coefficient of *Klebsiella pneumoniae* (aerogenes) was strongly reduced upon the addition of NaCl to the growth medium (Esener et al. 1981). This inhibition phenomenon is, at least for some bacterial species, caused by the dehydration of the cells (Abbe 1989). Microbial strains of the genus *Hyphomicrobium*, to which also some dichloromethane degrading microorganisms belong, are generally found in soil, fresh water, aquarium water, acid mine water and sewage, but also in brackish water and sea water (Moore 1981). These last two sources indicate that a certain resistance towards increased ionic strength exists in certain strains. It is reported that several *Hyphomicrobia* can grow in the presence of 2.5% w/w NaCl (Hirsch 1989). Nevertheless for *Hyphomicrobium* DM2 the degradation of dichloromethane in a fluidized bed reactor was inhibited by 100 mM NaCl, which led to a washout of biomass (Gälli et al. 1985). The inhibition of the activity by a large number of organic and inorganic compounds was tested for a strain B552 isolated from soil, one from fresh water, AE 617, and one from brackish water ZV850 (Moore 1989). It is reported that e.g. K_2SO_4 and KNO_3 concentrations of 80 mM resulted in 50% inhibition (found by plate counting), whereas $CaCl_2$ was found not to inhibit growth even at saturation (± 6.8 M). In this paper the degradation of dichloromethane by *Hyphomicrobium* GJ21 is discussed. Dichloromethane is converted by these bacteria into formaldehyde and hydrochloric acid. The former is readily converted into biomass and CO_2, while the latter is released into the medium (Stücki et al. 1981; Gälli et al. 1985). It has been reported for this microorganism that a concentration of only 50 mM of $CaCl_2$ and 300 mM of NaCl were found to be completely growth inhibiting (Ottengraf et al. 1986; Janssen et al. 1987). The inhibition phenomenon described above may lead to the conclusion that the NaCl concentration within a trickling filter system should be kept as low as possible in order to maintain a maximum microbial activity. However, experimental investigations also reveal that increased NaCl concentrations strongly enhance the immobilization of the microorganisms and the formation of a dichloromethane degrading biofilm. The latter is very important as it significantly adds to the stability of the reactor system.

Therefore the influence of NaCl on the rate of dichloromethane degradation by several microbial cultures, in batch as well as in a continuously operated, laboratory scaled trickling filter, was more closely investigated. Experiments recording both microbial growth and dichloromethane degradation were carried out in a NaCl concentration range of 0–1000 mM.

Intrinsic kinetics

The following set of equations gives a general description of microbial substrate degradation (Cooney 1981):

$$-r_s = \frac{\mu(S) \cdot X}{Y_{s/x}} + m_s \cdot X \qquad (1)$$

$$r_x = [\mu(S) - k_2] \cdot X \qquad (2)$$

$$\mu(S) = \mu_{max} \cdot \frac{S}{K_s + S} \qquad (3)$$

in which $\mu(S)$ is the microbial growth rate, m_s the maintenance substrate consumption rate, k_2 the rate of endogenous respiration or decay and $Y_{s/x}$ the yield coefficient. The Monod expression given in equation (3) (Monod 1949) relates the microbial growth to the concentration of the growth-limiting substrate, in which μ_{max} reflects the maximum growth rate, while K_s is referred to as the Monod constant.

Apart from growth, equations (1) and (2) also account for maintenance, which reflects the continuous flow of energy in the cells required for the basic cellular metabolism (Moser 1981). Two different ways

of describing this maintenance process are incorporated in the above-given set of equations. Herbert (Herbert 1958) assumes that energy for maintenance is produced by the respiration of cell reserves and hence a decrease of biomass growth, i.e. endogenous respiration or decay (k_2, equation (2)) results. This implies that the substrate degradation aims at biomass production only ($m_s = 0$, equation (1)). However, if external substrates are available, it is more likely that the energy required for basic metabolism is, at least partly if not all, covered by substrate degradation rather than by endogenous respiration. For this reason Pirt (Pirt 1965) proposed that the microbial growth rate $r_x(S)$ remains unchanged ($k_2 = 0$, equation (2)), whereas the maintenance requirement m_s completely results from substrate degradation. Recently an intermediate formulation was presented by Beeftink (Beeftink et al. 1990), who assumes that basic metabolism is supplied with energy from both biomass and substrate degradation, the ratio of which is dependent on the environmental conditions. Hence k_2 and m_s are both a function of the substrate concentration using a Monod type of expression.

Concerning the experiments described in this paper, the above-given set of equations can be simplified. For the microbial degradation of dichloromethane by several strains of *Hyphomicrobium* and *Pseudomonas* the Monod constant appears to amount to 10^{-3}–10^{-2} mM (Diks 1992; Hartmans 1991). As the following experiments were carried out at 1 mM dichloromethane, $S >> K_S$, and hence $\mu = \mu_{max}$ in equation (3). Moreover, as external substrate is available, the models according to Pirt and Beeftink indicate that substrate is the main source for the supply of energy for growth as well as for maintenance, i.e. $k_2 \approx 0$. This assumption is supported by k_2 values determined under substrate-limited conditions, which shows that k_2 is in the range of 0.02–0.07 day^{-1} (compare μ_{max} = 1.9 day^{-1}; T = 21° C) (Diks 1992).

When studying the microkinetics of DCM degradation in shaking flasks, the gas-water distribution of this volatile compound should be accounted for. If S_i is the initial concentration in the liquid phase before the partition of the volatile compound over both phases, the actual liquid phase concentration is then given by:

$$S_o = S_i \cdot \frac{V_l}{m \cdot V_g + V_l} = S_i \cdot \alpha \qquad (4)$$

in which α reflects the air-water distribution of the volatile substrate in the batch system concerned, while m is the air-water distribution coefficient.

If the following dimensionless parameters are applied:

$$\gamma = \frac{S}{S_0} \quad \chi = \frac{X}{Y \cdot S_0} \quad M = \frac{m_s \cdot X}{\mu_{max} \cdot S_0} \quad \tau = \mu_{max} \cdot t$$

the substrate and biomass concentrations in a batch system are, according to rate equations (1) and (2), given in a dimensionless form as:

$$-\frac{d\gamma}{d\tau} = \alpha \cdot [\chi + M] \qquad (5)$$

$$\frac{d\chi}{d\tau} = \chi \qquad (6)$$

The boundary conditions are:

$$\tau = 0 : \gamma = 1 \text{ and } \chi = \chi_0 : M = M_0 \qquad (7)$$

Integration of equation (6) yields:

$$\chi = \chi_0 \cdot e^{\tau} \qquad (8)$$

Combination of equations (5) and (8) yields after integration, using equation (7):

$$\gamma = 1 - \alpha \cdot (\chi_0 + M_0) \cdot (e^{\tau} - 1) \qquad (9)$$

Equation (9) holds as long as $\gamma \geq 0$. If $\gamma = 0$ at $\tau = \tau_e$, which is the end point of the zeroth order degradation curve, equation (9) can be rewritten as:

$$\gamma = 1 - \frac{1 - e^{\tau}}{1 - e^{\tau e}} \qquad (10)$$

In case a high initial biomass concentration is applied in batch experiments ($\alpha \cdot \chi_0 >> 1$), biomass growth becomes negligible, and it can be shown that under these circumstances the path of the substrate concentration for $\tau \leq \tau_e$ is given by:

$$\gamma = 1 - \alpha \cdot (\chi_0 + M_0) \cdot \tau \qquad (11)$$

Materials and methods

Set-up batch experiments

The experiments were carried out in 0.5 dm^3 serum flasks containing 0.2 dm^3 of culture medium. After the addition of pure dichloromethane into the medium, using a 25 μl liquid syringe (Hamilton & Co; UK), the bottles were sealed by a screw cap containing two septa. The one in contact with the content of

the serum flask was made of Viton rubber, being inert towards the chlorinated compound concerned, while a second septum, of ordinary rubber, was put on top in order to guarantee sealing. Hereafter the flasks were vigorously shaken by hand until the compound had dissolved. Unless stated otherwise the concentration of dichloromethane amounted to 1 mM (based upon the addition to the liquid phase, without gas-liquid distribution). The serum flasks were shaken horizontally at 120 rpm, thus resulting in a continuous equilibrium of volatile compounds between both gas and liquid phases (data not shown).

Set-up trickling filter experiments

The 70 dm^3 laboratory-scale trickling filters (Fig. 1) were constructed and operated as described elsewhere (Diks et al. 1991a–b; Diks 1992). At normal operating conditions, the inlet DCM concentration was set at 2.8 g/m^3, while the superficial gas- and liquid flow rates amounted to 200 m/h and 7 m/h respectively. The NaCl concentration in the liquid phase was controlled by the residence time of the liquid phase. For an increase of the NaCl concentration a concentrated salt solution was added step-wise to the liquid phase in order to prevent the biomass from an osmotic shock.

The performance curve of the system was determined in a short term experiment (< 1 day) at pseudo-steady-state conditions, as described by Diks (Diks et al. 1991a–b).

Bacteria

The degradation of dichloromethane under septic conditions was investigated; the strain used was *Hyphomicrobium* GJ21 (Ottengraf et al. 1986; Janssen et al. 1987), which was kindly supplied by Dr. D.B. Janssen and Drs. A.J. van den Wijngaard, Rijksuniversiteit Groningen. The culture was kept on sealed mineral agar plates or tubes containing dichloromethane.

Furthermore enriched cultures were cultivated from the microbial flora which had developed in a laboratory trickling filter degrading dichloromethane. Although this system was initially inoculated with *Hyphomicrobium* GJ21, the enriched culture is most likely not a pure one and will further be referred to as 'TF-enrichment culture'. Finally experiments were also performed at a high initial concentration of biomass. This biomass was either cultivated on high dichloromethane concentrations, using the above-described cultures, or directly harvested from the laboratory trickling filter. This biomass, which is referred to as 'TF-sludge', does not only contain the primary dichloromethane degrading organism, but also dead biomass, debris, other bacteria and higher organisms. *Inoculation* with bacteria took place by injecting 2 ml of a precultured microbial suspension through the septa. Previous to the actual experiment this preculture had been cultivated at room temperature at the same initial dichloromethane concentration, after the addition of a negligibly small amount of inoculum. After a complete degradation of the dichloromethane, the actual experiment was inoculated. Following this procedure, each experiment is provided with an active inoculate and hence the occurrence of a lag phase is minimized. The serum flasks for the investigation of pure cultures were sterilized for 15 minutes at 120° C before the addition of the dichloromethane, while the inoculation occurred with aseptic syringes.

Medium

The inorganic medium applied in the batch growth experiments was composed according to Janssen (Janssen et al. 1984). All inorganic compounds were of analytical grade (Merck, Germany), while the dichloromethane was of technical grade (Merck, Germany).

Carbon source analysis

As for the volatile compounds an equilibrium exists between the gas and the liquid phases during the experiments, the dichloromethane depletion can be followed by determining the gas phase concentration of this compound as a function of time. Therefore headspace gas samples of 100–200 μl were drawn with a gastight syringe (Hamilton, UK) and injected in a gaschromatograph (Carlo Erba 2000, Italy), that was provided with an FID and connected to a Milton-Roy CI-10 integrator. The injector and detector temperature amounted to 170° C, while the oven temperature was 130° C. A 1 m stainless steel column packed with Cromosorb 101 was used, while the carrier gas was nitrogen. The retention times were typically less than 1 min.

Biomass concentrations

After centrifugation (5000 rpm for 10 min) the liquid phase in each experiment was filtered over a predried glass fibre micropore filter (Schleicher & Schull Nr. 6, Germany; dried at 60° C and weighed). After washing

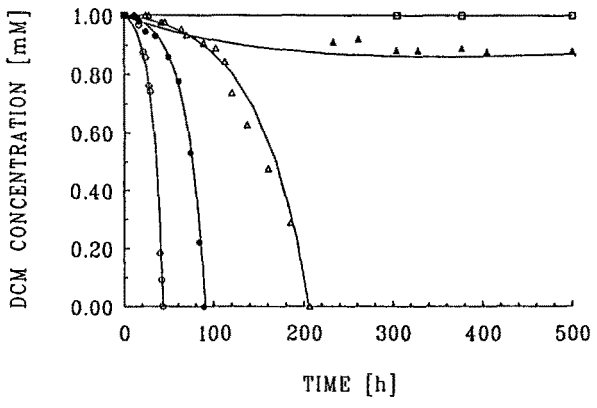

Fig. 2. The batch degradation of 1 mM dichloromethane by the TF-enrichment culture at different NaCl concentrations: 0 (○); 200 (•); 400 (△); 600 (▲) and 800 (□) mM. Solid lines calculated according to eq. (10).

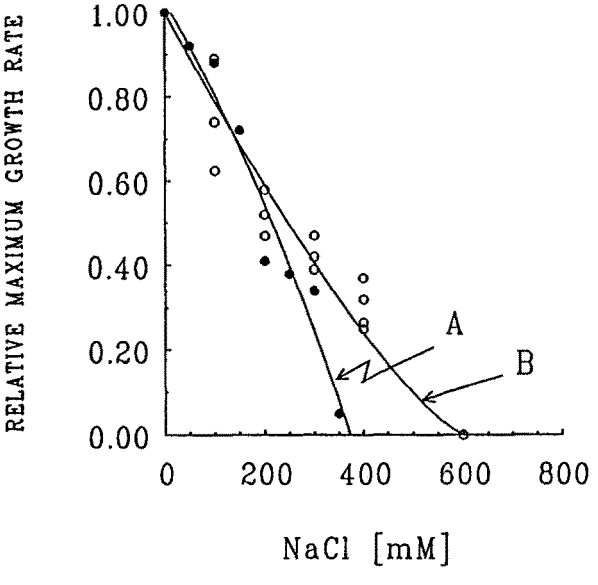

Fig. 3. The influence of the NaCl concentration on μ_{max} of *Hyphomicrobium* GJ21 (•) and the TF-enrichment culture (○), as calculated according to equation (11); (A)– (B): inhibition models (see Table 1).

the sample by filtering 0.1 dm^3 of distilled water, the biomass content followed after drying the filter at 60° C for at least 24 hours till constant weight and again weighing.

Results

In a first series of experiments the inhibition of the dichloromethane degradation by NaCl was investigated at a small inoculation concentration of biomass, taken from a preculture. In these experiments, which involved *Hyphomicrobium* GJ21 as well as the TF-enrichment culture, the NaCl concentration was varied between 0 and 800 mM. After inoculation the degradation of 1 mM dichloromethane was followed as a function of time. For the TF-enrichment culture Fig. 2 shows the results of one of the three experiments which was carried out using this culture.

The path of the dichloromethane concentration shows an increasing slope which is caused by microbial growth. The substrate degradation can therefore be described by zeroth order degradation kinetics and growth as explained above and the experimental data were therefore evaluated by means of equation (10). Applying the SAS package (SAS Inst. Inc. Cary, USA) the maximum growth rate was found by non-linear curve fitting. The results of this procedure are given in Table 1, which lists the maximum growth rates at different NaCl concentrations. For the TF-enrichment culture the values of μ_{max} given are the mean of three independent experiments, the range of which is indicated below Table 1.

Taking μ_{max} at NaCl = 0 as a reference value, the relative maximum growth rate can be calculated from these experimental data. In Fig. 3 this magnitude is plotted versus the concentration of NaCl for both *Hyphomicrobium* GJ21 and the TF-enrichment culture. This graph shows that for *Hyphomicrobium* GJ21 growth only occurs below a NaCl concentration of 350 mM, while the activity of the TF-enrichment culture appears to be less inhibited. At 400 mM the maximum growth rate still amounts to 30% of its value at [NaCl] = 0.

As the absolute value of μ_{max} at NaCl = 0 mM for the TF-enrichment culture has also increased as compared to the μ_{max} of *Hyphomicrobium* GJ21, a certain degree of adaptation towards the conditions in the trickling filter may have taken place. This suggestion is supported by the fact that the trickling filter, from which the enriched culture was obtained, had already been operational for about two years at a NaCl concentration of 100–200 mM.

The results so far suggest that NaCl concentrations in a biological trickling filter should be kept as low as possible in order to achieve an optimal microbial growth and hence substrate elimination. On one hand

Table 1. The inhibiting effect of NaCl on the maximum growth rate and specific activity of the dichloromethane degrading cultures; a) T = 21° C; $K_s = 10^{-2}$ mM: $S_0 >> K_s$; percent coefficient of variation < 5%; b) absolute deviation ± 0.1–0.25 day^{-1}, T = 22° C; c) Specific activity of TF-enrichment culture, cultivated on 5 mM DCM: average value of three independent experiments; range of k_{obs} at NaCl = 0: 0.45–0.6 gDCM/(gTSSh), Fig. 6; d) representative example for TF-sludge; range of k_{obs} at NaCl = 0: 0.05–0.1 gDCM/(gTSSh); e) curve A in Fig. 3; f) curve B in Fig. 3; g) curve B in Fig. 6; h) curve A in Fig. 6.

NaCl [mM]	*Hyphomicrobium* GJ21 μ^a_{max} [day^{-1}]	TF-enrichment culture μ^b_{max} [day^{-1}]	TF-enrichment culture k^c_{obs} [g/(g·h)]	TF-sludge k^d_{obs} [g/(g·h)]
0	1.40	1.90	0.57	0.04
50	1.28			
100	1.23	1.43	–	–
150	1.00			
200	0.57	0.99	0.34	0.028
250	0.53			
300	0.48	0.81	–	–
350	0.07			
400	–	0.57	0.24	0.017
600	–	0.0	0.12	0.009
800	–	–	0.05	0.004
1000	–	–	0.03	–
1200	–	–	0.02	–

Inhibition models	Parameters				
Levenspiel (1980)	C_R [mM]	358[e]	601[f]	1900[g]	–
	n [–]	0.7[e]	1.2[f]	4.0[g]	–
Yano & Koga (1969)	K_i [mM]	–	–	282[h]	–
	n [–]	–	–	2.2[h]	–

this would require a high liquid dilution rate, while also the biofilm formation is less stable (Heijnen 1984). Therefore the laboratory trickling filters were generally operated at 100–300 mM NaCl.

In Fig. 4 a representative example is given of a DCM performance curve, i.e. the elimination capacity as a function of the inlet gas concentration, at 100 mM NaCl in the liquid phase, which shows a maximum elimination capacity of 115 g/(m^3h). After raising the NaCl concentration to a level of 600 mM, by adding a concentrated salt solution, it appeared that the performance curve of the system was only slightly influenced by the high NaCl concentration, even after 12 days. Although the slope of the curve in Fig. 4 is somewhat lower, a high maximum elimination capacity could still be obtained. Data analyses, based on the Uniform Concentration Model for the trickling filter system (Diks et al 1991a–b), indicates that these results are due to an inhibition of the microbial dichloromethane degradation rate, which is partly compensated by an increase of the amount of immobilized biomass (Diks 1992). The latter was supported by the strong decrease of the suspended biomass concentration in the trickling filter liquid, which was observed within a few days after increasing the NaCl concentration. Nevertheless, the inhibition of the activity of the microbial population in the trickling filter thus amounted to only 50%, which is significantly less than can be expected from the results presented in Fig. 3.

Several biological phenomena reported in literature may be the cause of these results. In the trickling filter the microorganisms are immobilized as a biofilm, instead of being suspended in the liquid phase. The immobilization as such may result in an increased resistance against increased ionic strength. Also the production of specific compounds that increase the osmotic resistance (so-called 'osmoprotectants') of the mixed culture may be involved. Microbial cells can respond

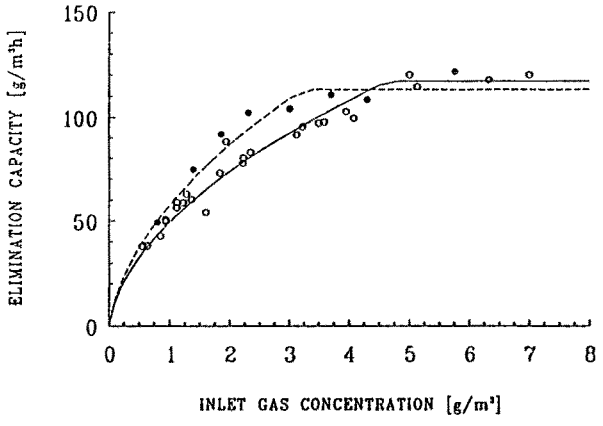

Fig. 4. The short term effect (5–12 days) of an increased [NaCl] on the DCM performance curve in a 1m BTF; NaCl = 100 mM (•); 600 mM (○); lines calculated using the UCM model (Diks et al. 1991a–b).

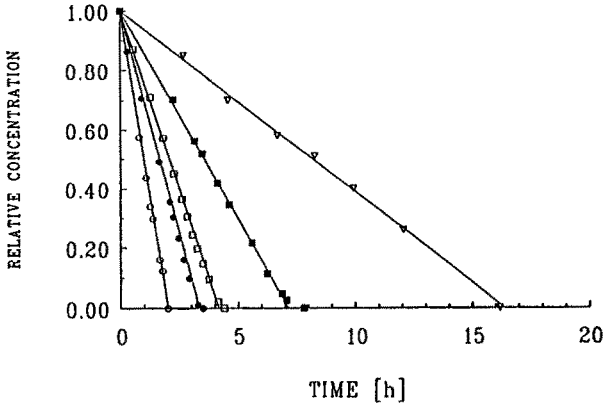

Fig. 5. The degradation of 1 mM dichloromethane by the TF-enrichment culture at different NaCl concentrations: 0 (○); 200 (•); 400 (□); 600 (■) and 800 (▽) mM; solid lines calculated according to eq. (11).

to a high osmotic pressure by the accumulation of such osmotically active solutes and thus retain growth (Abbe 1989). In a second series of experiments the inhibition of the dichloromethane degradation was therefore investigated applying high concentrations of biomass. Trickling filter sludge was obtained from grown packing elements, which were removed from the filter bed after shutting down the filter system. Thereafter the biomass adhered to the packing elements was resuspended in mineral medium, which resulted in a biomass concentration of about 1 gTSS/dm^3. A similar experiment was carried out with the TF-enrichment culture, which was cultivated on 5 mM dichloromethane. After the addition of NaCl to samples of the two suspensions (0.2 dm^3) in concentrations that varied between 0 and 1200 mM, the depletion of 1 mM of dichloromethane was recorded versus time. From the substrate depletion rate and the biomass concentration, the specific activity was calculated as gDCM degraded per gTSS and hour. These experiments, which were carried out several times during the total period of operation of the laboratory trickling filters, were evaluated by means of equation (11).

Figure 5 shows the results of a representative example, in which the NaCl concentration was varied between 0 and 800 mM. The results again show a strong decrease of the specific dichloromethane degradation activity at higher NaCl concentrations. This result was obtained for both the TF-enrichment culture and the TF-sludge. It must be noted here, that the absolute values of the specific activity differed about one order of magnitude in both experiments (e.g. 0.04 gDCM/(gTSSh) for the TF-sludge, versus 0.57 gDCM/(gTSSh) for the TF-enrichment culture at [NaCl] = 0 mM). Obviously the major part of the trickling filter sludge consists of inactive material and secondary organisms (LaMotta 1976; Bishop et al. 1981; Diks 1992).

Figure 6 summarizes the relative specific dichloromethane degrading activity as a function of the NaCl concentration, for all the experiments carried out at a high initial biomass concentration. Mathematical expressions describing the substrate (equation 12) and product inhibition (equation 13) were taken from literature. The model of Levenspiel (equation 13) (Levenspiel 1980), which starts from product inhibition, appeared fairly well able to describe the inhibition of μ_{max}, while the model of Yano & Koga (equation 12) could quite well describe the effect of NaCl on the specific dichloromethane degrading activity (Yano & Koga 1969).

$$\frac{\mu}{\mu_{max}} = \frac{1}{1 + (\frac{C}{K_i})^n} \qquad (12)$$

$$\frac{\mu}{\mu_{max}} = \left(1 - \frac{C}{C_R}\right)^n \qquad (13)$$

Table 1 lists the values of the parameters according to both models, as obtained from fitting the experimental data. Recently Niemann reported a K_i value of 800 mM, according to equation 12 with n = 1, for the inhibition of the volumetric dichloromethane removal in a fluidized bed reactor (Niemann 1993). Although

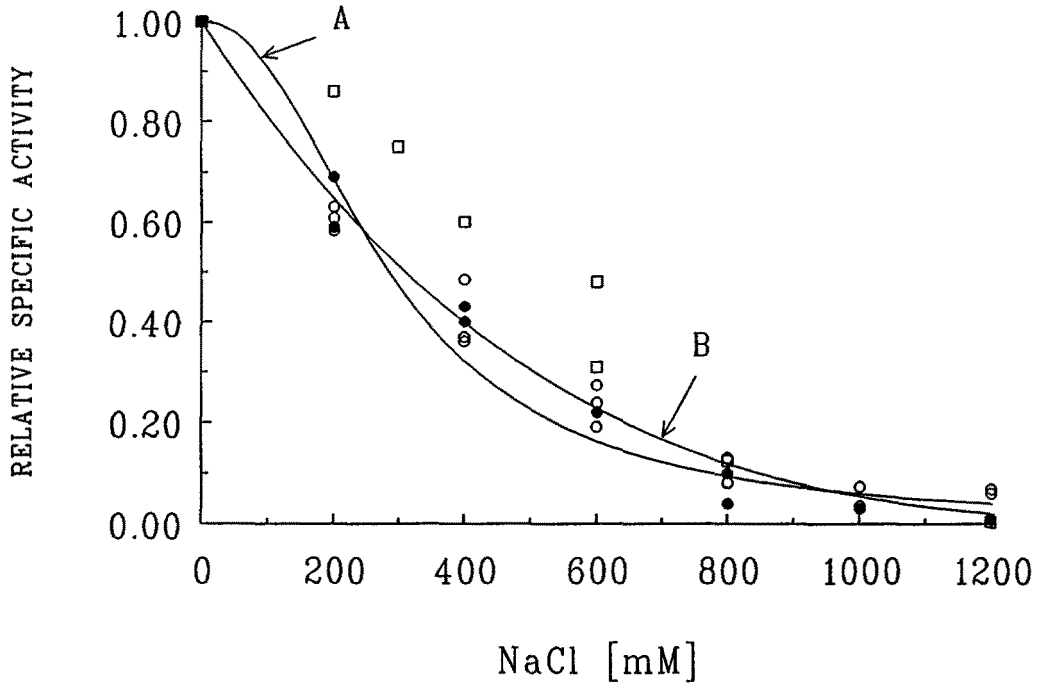

Fig. 6. The NaCl inhibition of the specific activity of the TF-biomass at 26° C (□, 22° C (•) and the TF-enrichment culture 22° C (○). Inhibition models according to Yano & Koga (A, eq. 12); Levenspiel (B, eq, 13).

this value is of the same order of magnitude, further interpretation is complicated, as the liquid residence time and hence biomass concentration does influence the removal rate as well.

Although the set of experimental data in Fig. 6 shows some scattering, it can be seen that the inhibition phenomenon is less severe than shown in Fig. 3 and hence a dichloromethane degrading capacity is still observed at NaCl concentrations exceeding 600 mM (see also Table 1). This result is comparable to the influence of NaCl on the trickling filter performance mentioned earlier (Fig. 4).

It is therefore concluded that the immobilization of the biomass as such is not responsible for the increased NaCl resistance. Moreover, as the influence on the relative activity of the TF-enrichment culture equals the effect on the activity of the mixed population taken from the trickling filter (Fig. 6), the presence of this mixed population and cross-interactions apparently do not affect the NaCl resistance either. Similar results as presented in the Figs 3 and 5 have been found for the inhibiting effect of $CaCl_2$ and $MgCl_2$ upon the growth of and dichloromethane degradation rate by *Hyphomicrobium* GJ21 and the TF-enrichment culture (Oever et al. 1989). Already at 150 mM growth was completely inhibited, whereas a 30% specific substrate degrading activity was still observed at 250 mM $CaCl_2$ in batch experiments performed with a high initial concentration of biomass.

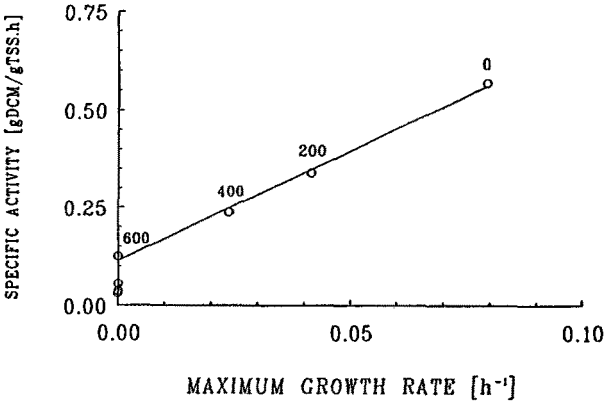

Fig. 7. The average, specific dichloromethane degrading activity of the TF-enrichment culture (Fig. 6) versus the average maximum growth rate (Fig. 3); parameter NaCl concentration [mM].

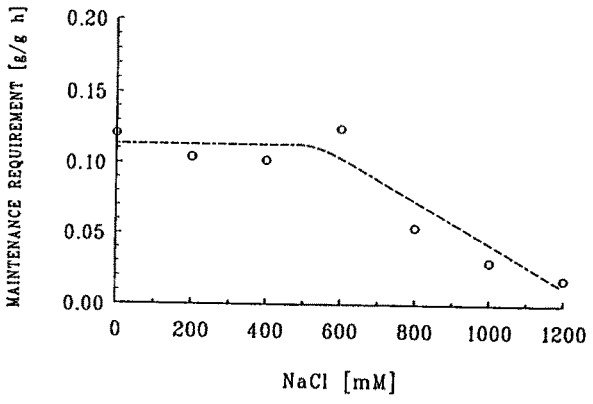

Fig. 8. The influence of the NaCl concentration on the maintenance requirement of the TF-enrichment culture consuming dichloromethane, as calculated from Fig. 7 and Table 1, according to equation (1).

Discussion

Inhibition

As it appears that above 600 mM NaCl the microbial growth on dichloromethane by the TF-enrichment culture is completely inhibited by NaCl, the question remains as to what process is responsible for the dichloromethane degradation observed at higher salt concentrations. Apparently the dichloromethane consumption is due to non-growth-related processes or maintenance, i.e. m_s in equation (1). Once the NaCl concentration exceeds 600 mM, the μ_{max} of the TF-enrichment culture decreases to zero and according to equation (1) the substrate degradation rate should then result from the maintenance requirement only. Moreover, the continuous decrease of the relative activity in Fig. 6 indicates that not only the growth rate, but also m_s is a function of the NaCl concentration.

One should be able to distinguish between substrate degradation for growth and for maintenance purposes at lower NaCl concentrations. As the true yield coefficient $Y_{s/x}$ is nearly a constant (Fiechter 1981) and $S >> K_s$ in equation (3), this parameter as well as m_s should follow from a plot of $-r_s(S)/X$ versus μ_{max} according to equation (1). The slope of the straight line reflects $1/Y_{s/x}$, while the vertical intercept equals the maintenance requirement at $\mu_{max} = 0$.

In Fig. 7 the average specific activity (- 1/XdS/dt) of the TF-enrichment culture from Fig. 6 is plotted versus the average μ_{max} of this culture, as can be found from Fig. 3. From the experimental data presented in Fig. 7, $Y_{s/x}$ and m_s are calculated as 0.18 $gTSS/g_{substrate}$ and 0.11 g/(gh) respectively, for $\mu_{max} > 0$ and [NaCl] < 600 mM. Defining the apparent yield coefficient Y as $Y = -r_x/r_s$, it can be derived from equation (1):

$$\frac{1}{Y} = \frac{1}{Y_{s/x}} + \frac{m_s}{\mu(S)} \qquad (14)$$

According to the results from Fig. 7 and $\mu_{max} = 1.9$ day^{-1}, this apparent yield coefficient at [NaCl] = 0 mM is calculated as 0.16 $gTSS/g_{DCM}$. This is in agreement with the experimentally determined overall value of 0.17 $gTSS/g_{DCM}$, which appeared to be nearly constant at [NaCl] $\leq$ 400 mM (Diks 1992).

The overall effect of NaCl on the rate of dichloromethane consumption due to maintenance is plotted in Fig. 8. For [NaCl] > 600 mM, i.e. when growth is negligible, m_s equals the specific activity given in Table 1. At low NaCl concentrations m_s can be calculated for each experiment according to equation (1), as both $Y_{s/x}$ and μ_{max} are known at each value of $-r_s/X_0$ (K_{obs} in Table 1). These results are plotted in Fig. 8 as well. The trend which emerges from this graph is that m_s seems to be quite constant when microbial growth takes place, while its value decreases at NaCl concentrations exceeding 600 mM.

The specific activities which are calculated from Fig. 6 for [NaCl] > 600 mM are assumed to reflect the dichloromethane consumption due to maintenance. However, at high NaCl concentrations cell deactivation may also be involved, and hence the dehalogenation of the dichloromethane may proceed only (Wijngaard 1991), while the complete metabolic conversion is suppressed. Moreover, on a longer time scale the negative effect of growth inhibition is also likely to overrule the initial positive effect of improved biomass immobilization, which was observed after raising the NaCl concentration in the biological trickling filters (BTF) described earlier.

A long-term trickling filter experiment was therefore carried out. During 300 days the influence of different NaCl concentrations on the elimination of DCM was investigated in a 1 m BTF, filled with 1″ polypropylene-Super Torus saddles (Raschig Gmbh, Germany). During the experiment the inlet gas concentration of dichloromethane was maintained at 0.36 g/m^3, while the NaCl concentration was stepwise increased from 250 to 1000 mM. At superficial gas and liquid flow rates of 200 m/h and 7 m/h respectively, the elimination capacity was daily determined by measuring

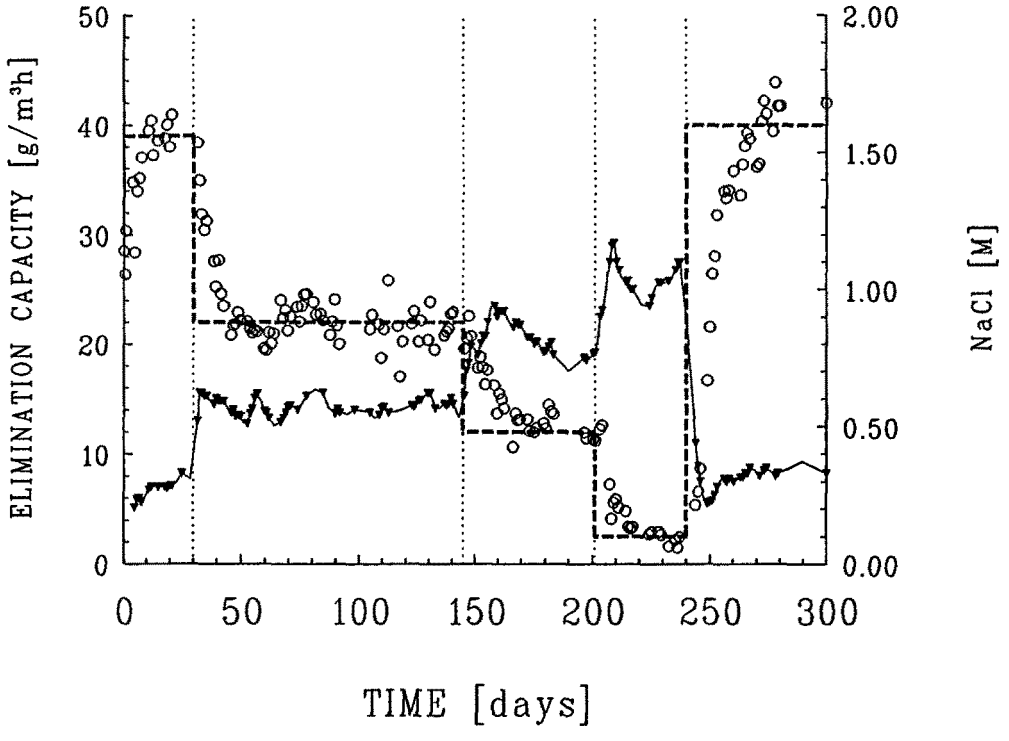

Fig. 9. The long term effect of the NaCl concentration (▼) on the EC (○) in a 1 m BTF, containing 1″ PP-Super Torus saddles; C_{go} = 0.36 g/m^3; v_g = 200 m/h; v_l = 7 m/h.

Table 2. The inlet dichloromethane concentration, NaCl concentration, average temperature and the dichloromethane elimination capacity finally reached in each of five consecutive experimental runs carried out in a BTF; v_g = 200 m/h; v_l = 7 m/h; BTF filled with 1″ PP-Super Torus saddles (see Fig. 9).

run	Period [days]	C_{go} [g/m^3]	EC [g/(m^3·j)]	NaCl [mM]	T [° C]
1	0– 30	0.37	39	262	17.7
2	30–145	0.37	22	569	16.3
3	145–201	0.36	12	811	16.6
4	201–240	0.35	2.5	1039	17.6
5	240–280	0.34	40	335	18.9

the consumption rate of NaOH and the degree of conversion in the gas phase.

For the total period of operation the elimination capacity is plotted versus time in Fig. 9. It can be seen that each time the NaCl concentration was increased, the BTF performance gradually decreased. This decrease can only partly be attributed to direct inhibition of the microbial activity, as the elimination capacity further decreased once the NaCl concentration was established. Although this is probably due to decay, a constant elimination capacity was each time achieved. At 600 mM and 800 mM NaCl a stable DCM degradation rate of 22 g/m^3·h) and 12 g/(m^3·h) respectively was recorded, while at the lowest NaCl concentration (250 mM) a value of 39 g/(m^3·h) was observed (Table 2). As no growth was observed at NaCl concentrations exceeding 600 mM, the substrate degrading capacity should gradually decrease in time to zero, due

to biomass decay. As this was not observed experimentally, a retention of the activity has apparently taken place and hence the DCM degrading capacity at high NaCl concentrations seems indeed the result of an active maintenance process instead of a remaining dehalogenating activity.

Kinetics

The results of the NaCl inhibition experiments reveal that for *Hyphomicrobium* growing on dichloromethane the maintenance coefficient according to the Pirt model amounts to aproximately 0.11 g/gTSS·h. This implies that at optimal growth conditions ($S > K_s$, 0 mM NaCl), 20% of the dichloromethane degradation rate results from the microbial maintenance requirement. As these figures have been derived from average data on both the specific activities and the maximum growth rates, the absolute results should be used with care. Nevertheless it should be verified to what extent the μ_{max} determined, using equation (10), deviates from its real value if maintenance is not neglected.

From an evaluation of the kinetic equations it appears that the determination of μ_{max} at high substrate concentrations, as applied during the experiments described in this paper, is not effected by the assumption of $m_s = 0$ at all. It can be shown that the introduction of the boundary condition $\gamma\, 0$ at $\tau = \tau_e$ in both cases results in equation (10) (Diks 1992). Moreover, using Monod kinetics, μ_{max} is mainly determined by the path of the substrate concentration at the point where the substrate gradient starts to change rapidly, thus at relatively high concentrations, and hence it can be expected that in this case μ_{max} is hardly effected either. Finally, from numerical procedures solving the complete set of differential equations (1) – (3) and fitting all parameters using SAS, it appeared that the maintenance parameter is statistically indeterminable, with reference to the experimental procedure applied. Apparently the Monod model, with or without the assumption of $K_s = 0$, can quite adequately describe the substrate degradation in batch experiments. The rate of maintenance cannot directly be determined from the results of batch growth experiments with a small inoculum, but the decrease of the microbial growth rate by the addition of NaCl allows for an estimation of m_s as shown in Fig. 7.

Comparing the results of μ_{max} at [NaCl] = 0 in Table 1 with literature data, it can be seen the value thus found for *Hyphomicrobium* GJ21 is somewhat lower than the μ_{max} reported previously (Ottengraf et al. 1986; Janssen et al. 1987). The growth rate of the TF-enrichment culture is comparable to that of other *Hyphomicrobia* (Hartmans et al. 1991). As compared to the results of experiments using the TF-enrichment culture at dichloromethane concentrations in the range of 0.02–0.1 mM, i.e. when Monod kinetics become important, identical values for μ_{max} have been obtained (Diks 1992), thus confirming the theoretical approach discussed above. It can hence be concluded that the kinetic modelling of the kinetics according to equation (10) is applicable at the dichloromethane concentrations applied here ($S_o/K_s = 100–1000$).

Conclusions

Based on the results reported in this paper it can be concluded that the growth rate of *Hyphomicrobium* GJ21 can adequately be determined applying zeroth order kinetics, using the end point method. The occurrence of maintenance does not influence the results of μ_{max} using this procedure.

The elimination of dichloromethane by *Hyphomicrobium* GJ21 is strongly inhibited at NaCl concentrations exceeding 350 mM. For the TF-enrichment culture this inhibition is less severe as growth is observed up to 600 mM. At salt concentrations exceeding these critical values dichloromethane degradation is still observed. Long-term trickling filter experiments show that cell decay takes place at such conditions, but the results of both the batch growth and trickling filter experiments clearly indicate that active maintenance, which still takes place, is responsible for the continued dichloromethane degradation. Designing a biological trickling filter, the effect of inhibition should be accounted for. A high NaCl concentration will lead to a decrease of the volumetric liquid drain and an improved biomass immobilization. More important is the fact that inhibition by NaCl offers a significant control parameter against excessive biomass growth in biological trickling filters for wastegas treatment. Nevertheless, a reduced elimination capacity should be accounted with, which will require an increase of the reactor volume. Consequently the final design strongly depends upon the system economics in specific cases.

Finally, when comparing the results for pure *Hyphomicrobium* GJ21 and the 'TF-enrichment' culture, it can be concluded that some adaptation towards increased salt concentrations seems to have taken place within continuously operating trickling filters.

List of symbols

A	area for mass transfer in the batch experiment	m^2
C	inhibitor concentration (equations 12–13)	mol/m^3
C_{go}	inlet gas phase concentration in BTF	g/m^3
C_R	critical inhibitor concentration (eq. 14)	mol/m^3
EC	elimination capacity	$g/(m^3 \cdot h)$
EC_{max}	maximum elimination capacity	$g/(m^3 \cdot h)$
k_2	rate of decay at substrate limited conditions	h^{-1}
K_s	Monod constant for substrate	g/m^3
K_{obs}	specific activity of suspended biomass	gDCM/(gTSS· h)
K_i	inhibition constant (eq. 13)	mol/m^3
k_{ol}	overall mass transfer coefficient in the batch experiment	m/s
m	gas-liquid distribution coefficient	m_l^3/m_g^3
m_s	rate of substrate degradation due to maintenance	g/(gh)
M	dimensionless rate of maintenance	
n	power number for inhibition kinetics	
[NaCl]	concentration of NaCl	mol/m^3
r_s	substrate production rate	$g(m^3 \cdot s)$
r_x	biomass production rate	$g(m^3 \cdot s)$
S	substrate concentration in batch experiments	mol/m^3
t	time	h or s
t_e	extrapolated end point of zeroth order substrate degradation in batch	h
T	temperature	K or ° C
TSS	Total Suspended Solids	g/m^3
v_l	superficial liquid flow rate	m/h
v_g	superficial gas flow rate	m/h
V_l	liquid volume	m^3
V_g	gas volume	m^3
X	concentration of suspended biomass	g/m^3
Y	overall yield coefficient on substrate	gTSS/gDCM
$Y_{s/x}$	true yield coefficient on substrate	gTSS/gDCM
Greek symbols		
α	correction factor water-air substrate distribution	
γ	dimensionless substrate concentration	
γ_s	dimensionless Monod constant	
μ	microbial growth rate	h^{-1}
μ_{max}	maximum growth rate	h^{-1}
τ	dimensionless batch reaction time	
τ_e	dimensionless, extrapolated end point of substrate degradation in batch	
χ	dimensionless biomass concentration	

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