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Modelling of cometabolic transformation of *ortho*-xylene in a denitrifying biofilm system

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Abstract

A model describing the cometabolic biotransformation of *o*-xylene with toluene as primary carbon source in a continuously fed fixed biofilm reactor is presented. The model is based on the concept of competitive inhibition between *o*-xylene and toluene. The proposed model simulated successfully the transformation of *o*-xylene and the associated by-products formation, as well as the toluene degradation. However, it appears that an accurate measurement of active biomass density and distribution in the biofilm is needed, since these factors dramatically affects the modelling. The modelling of various kinetic experiments indicates that the active biomass (or toluene degraders) is accumulated on the top of the biofilm, leading to the conclusion that only a minor part of the biofilm thickness was active. The calibrated model is able to predict the removal of toluene and *o*-xylene for concentrations ranging from 0 to 30 mg/L. For higher concentrations toxicity phenomena may decrease the accuracy of the model.

Introduction

An increasing number of organic chemicals are introduced in the environment as pesticides, chlorinated derivatives or gasolines. Many of these compounds are recalcitrant or need long adaptation times before biodegradation will occur. However, the degradation of some pollutants may be enhanced if a suitable carbon source is amended in order to support bacterial growth. For example, a chlorinated ethene like trichloroethylene (TCE), can be degraded in groundwater by introducing methane in order to stimulate the growth of methanotrophic bacteria (Wilson & Wilson 1985). The cometabolic transformation of *o*-xylene has been reported in aerobic groundwater (Jørgensen & Aamand 1991), and, under nitrate reducing condition with toluene as the primary carbon source (Evans et al. 1991), (Jørgensen 1992). In order to quantify a cometabolic degradation, and to design engineered systems, mathematical models able to capture the feature of cometabolism are required. Many models have been proposed in order to model the kinetics of cometabolic transformation by resting cells, based on a Michaelis-Menten expression (Alvarez-Cohen & McCarty 1991), or a zero-order expression (Flyvbjerg et al. 1993). For growing cells, a competition for the same enzyme often occurs because of the simultaneous presence of the primary substrate and the co-substrate (Broholm et al. 1990; Arcangeli & Arvin 1993b). Field experiments corroborated competitive inhibition between methane and TCE (Semprini et al. 1991) and *o*-xylene and toluene (Barbaro et al. 1992). Broholm et al. (1992) proposed a mathematical model for the degradation of TCE and methane by methanotrophic bacteria, taking competitive inhibition into account. Criddle (1992) developed a model for both resting and growing cells incorporating competitive inhibition, lag time and deactivation of resting cells. This model was successfully applied by Chang et al. (1993) for investigating the kinetics of biodegradation of benzene, toluene and *p*-xylene by a pure culture. In a recent paper, the cometabolic biotransformation of *o*-xylene in a biofilm system has been reported (Arcangeli & Arvin 1993b).

The aim of this work was to develop a steady-state mathematical expression, able to simulate the simultaneous degradation of a growth- and a non-growth substrate in a continuously fed biofilm reactor, and to quantify substrate interactions between the primary substrate, toluene, and *o*-xylene. The modelling is based on two sets of kinetic experiments where the following was studied: (i) influence of toluene concentration on the *o*-xylene removal rate, tests X2 and X4. (ii) influence of *o*-xylene concentration on the toluene removal rate, test X3. The experimental results have been discussed elsewhere (Arcangeli & Arvin 1993b).

Mathematical model

The reaction rate of the primary substrate, toluene, r_{tol}, which support the biomass growth can be described by the following equation, assuming competitive inhibition from the secondary substrate, *o*-xylene (Bailey & Ollis 1977). The inhibition coefficient of the competitive inhibitor is approximated by its single-substrate half-saturation coefficient, as proposed by Broholm et al. (1992) and suggested by Chang et al. (1993).

$$r_{tol} = k_{X(tol)} X_f \frac{S_{tol}}{S_{tol} + K_{S(tol)} \left(1 + \frac{S_{o-xyl}}{K_{S(o-xyl)}}\right)} \quad (1)$$

Where:

$k_{X(tol)}$	:	Maximum toluene utilisation rate, (mg toluene/mg biomass/day)
X_f	:	Cell concentration in biofilm (g/m^3)
S_{tol}	:	Toluene concentration (mg/L)
S_{o-xyl}	:	*o*-Xylene concentration (mg/L)
$K_{S(tol)}$	:	Toluene saturation constant (mg/L)
$K_{S(o-xyl)}$	:	*o*-Xylene saturation constant (mg/L)

The *o*-xylene cannot serve as a sole carbon source for the mixed nitrate-reducing culture investigated in this work. However, transformation of *o*-xylene could be enhanced when toluene was supplied as a primary substrate. It is hypothesized that the enzyme that initiate the transformation of *o*-xylene is synthesized in the presence of toluene only (Jørgensen 1992). Enzyme kinetics is described by a Michaelis-Menten type of equation (Lehninger 1972). The *o*-xylene transformation rate is described by the following expression which takes the competitive inhibition and the stimulating effect from toluene into account.

$$r_{o-xyl} = k_{X(o-xyl)} X_f \frac{S_{o-xyl}}{S_{o-xyl} + K_{S(o-xyl} \left(1 + \frac{S_{tol}}{K_{S(tol)}}\right)} \frac{S_{tol}}{S_{tol} + K_{S(tol)}} \quad (2)$$

Hence, the optimum toluene concentration at which the *o*-xylene degradation is maximized can be found by differentiation of Equation (2).

$$S_{opt} = \sqrt{K_{S(tol)}^2 \frac{(S_{o-xyl} + K_{S(o-xyl)})}{K_{S(o-syl)}}} \quad (3)$$

Where:

$k_{X(o-xyl)}$	:	Maximum *o*-xylene utilisation rate, (mg *o*-xylene/mg biomass/day)
S_{opt}	:	Optimum toluene concentration (mg/L)

For a biofilm system the diffusion transport of substrate into the biofilm has to be taken into account. Furthermore, the following assumptions are made: (i) The biofilm is homogeneous and liquid film diffusion is negligible. (ii) The transport of substrate into the biofilm only takes place by diffusion. (iii) Only the substrate and the co-substrate are assumed to be rate limiting.

The profiles of toluene and *o*-xylene in a biofilm are shown under circumstances where the primary substrate, toluene, does not fully penetrate the biofilm (Fig. 1). The *o*-xylene biotransformation only occurs in area '2'. In area '1' the toluene is lacking and in area '3' the toluene inhibits *o*-xylene degradation nearly completely.

The concentration profile in the biofilm for toluene and *o*-xylene can be calculated by integration of Equations (4) and (5), respectively.

$$\frac{d^2 S_{tol}}{dx^2} = \frac{k_{o,f(tol)}}{D_{f(tol)}} r_{tol} \quad (4)$$

$$\frac{d^2 S_{o-xyl}}{dx^2} = \frac{k_{o,f(o-xyl)}}{D_{f(o-xyl)}} r_{o-xyl} \quad (5)$$

Where:

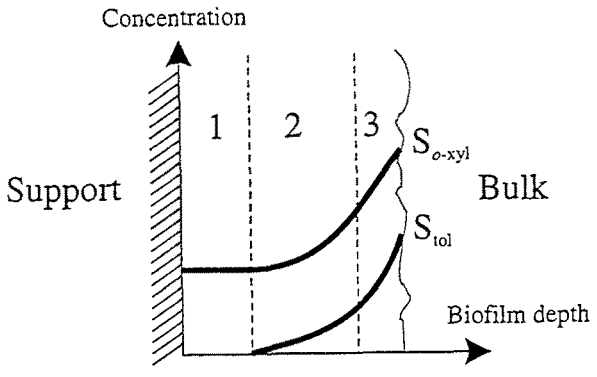

Fig. 1. Concentration profile of toluene and *o*-xylene for a cometabolic reaction. The *o*-xylene profile in area 3 is linear. This is due to its molecular diffusion into the biofilm.

$D_{f(tol)}$	:	Diffusion coefficient of toluene in biofilm (m^2/d)
$D_{f(o-xyl)}$	:	Diffusion coefficient of toluene in biofilm (m^2/d)
$k_{o,f(tol)}$	:	Toluene zero-order reaction rate ($= X_f \times k_{X(tol)}$)
$k_{o,f(o-xyl)}$	:	*o*-xylene zero-order reaction rate ($= X_f \times k_{X(o-xyl)}$)

An analytical solution for Equations (4) and (5) is not possible, since they are non-linear. However, they can be solved numerically. For that purpose, the computer programme BIOSIM from EAWAG, Switzerland is used (Reichert et al. 1989). It is an interactive programme for the simulation of the dynamics of mixed culture biofilm systems. The programme was implemented on an IBM PS/2, model 386 or 486 with a coprocessor. The operator has to specify characteristics of the reactor system, the characteristics of particulate and dissolved components, the biochemical model, influent concentrations and boundary conditions.

Model parameters

The characteristics of the reactor system are summarized elsewhere (Arcangeli & Arvin 1993b). The biological model consists of Equations (1) and (2) which express the degradation and the interaction between toluene and *o*-xylene. The substrate diffusion coefficients in the water were estimated from the Wilke & Chang method (Perry & Green 1987). A value of 8.73×10^{-5} m^2/d and 7.92×10^{-5} m^2/d was found for toluene and xylene, respectively. The diffusion coefficient was calculated to 5×10^{-5} m^2/d for the *o*-xylene by-products (*o*-methyl-benzaldehyde and *o*-methyl-benzoic acid). The relative substrate diffusivity was estimated to 0.47 (Fan et al. 1990). Furthermore, this biological model developed admits two biomass: inert (exopolymers – EPS – and dead cells) and active (toluene degraders). The solid phase density required in the model is characteristic for the particulate components, namely the mass of dry solids per unit solid phase volume. This value was estimated to 1.11×10^5 g/m^3 for the biomass (heterotrophic and inert). This calculation assumes a liquid volume fraction of 71% estimated according to an average dry weight content of 31851 g/m^3, an average dry weight of 2.5×10^{-13} g/cell and a cell volume of 2.25 μm^3 (*Escherichia-coli*) (Characklis & Marshall 1990). The liquid film diffusion did not influence the process because of the fast speed of the rotor (200 RPM).

Results

Modelling of the toluene effect

Two tests, X2 and X4, were modelled. Kinetic constants are summarized in Table 1. The toluene maximum growth rate constants, $\mu_{max(tol)}$, and the *o*-xylene maximum utilization rate, $k_{x(o-xyl)}$ were higher in test X4 than X2, indicating a higher metabolic activity in the biofilm. The difference between test X2 and X4 was the biofilm thickness (311 and 379 μm for X2 and X4, respectively) and its age: test X2 was performed 3 weeks after the reactor was inoculated in contrast to 8 weeks for test X4. The modelling of the toluene and *o*-xylene removal rate and the associated products formation is illustrated in Fig. 2A, B. Generally speaking, the comparison between modelled and experimental data indicates a good agreement.

The transformation of *o*-methyl-benzaldehyde to *o*-methyl-benzoic acid has been found to be independent of the toluene concentration (Jørgensen 1992). In the model, a first-order reaction is proposed, and the *o*-methyl-benzoic acid is assumed to be an end product of the *o*-xylene transformation. The *o*-methyl-benzaldehyde could be well modelled. However, the scattered data on the *o*-methyl-benzoic acid formation did not allow us to conclude whether the *o*-xylene was further transformed or not.

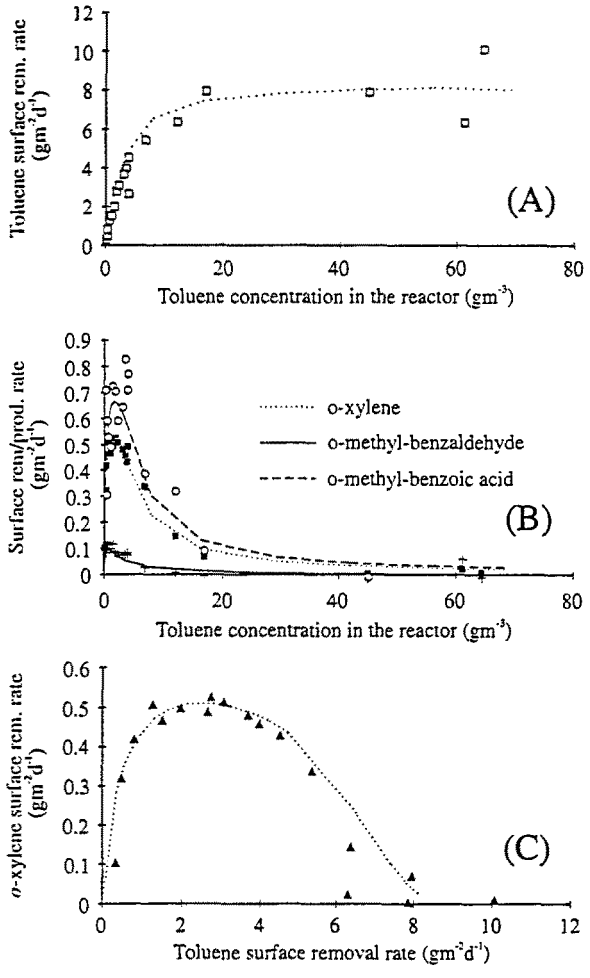

Fig. 2. Kinetic experiment X2. Modelling of toluene removal rate (A). Modelling of *o*-xylene removal and associated by-product formation rate (B). Modelling of the *o*-xylene removal rate vs. the toluene removal rate (C). Toluene (□); *o*-xylene (■); *o*-methyl-benzaldehyde (+); *o*-methyl-benzoic acid (o). Solid or dashed lines: model.

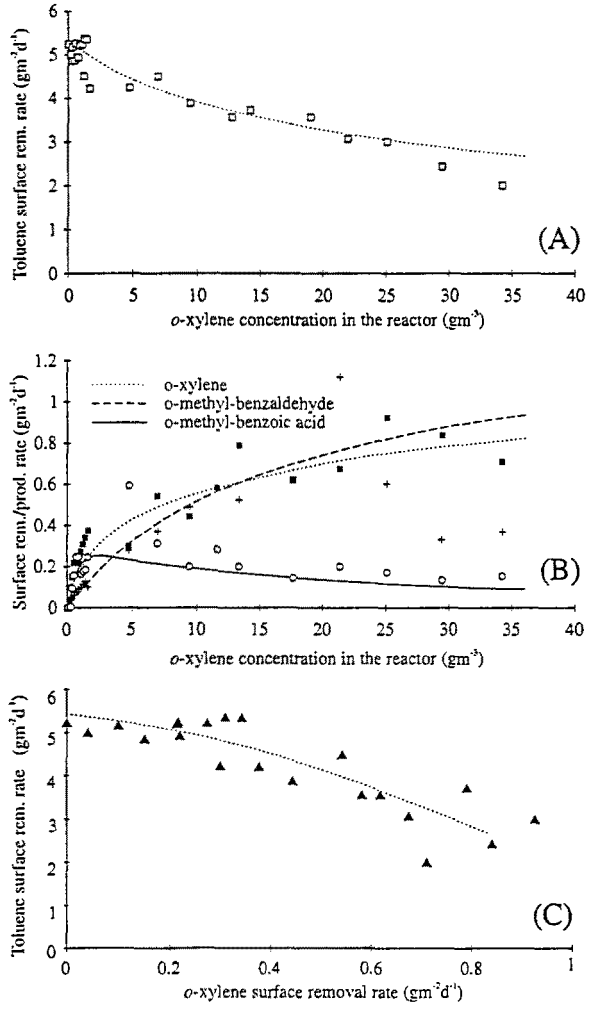

Fig. 3. Kinetic experiment X3. Modelling of toluene removal rate (A). Modelling of *o*-xylene removal and associated by product formation rate (B). Modelling of the toluene removal rate vs. the *o*-xylene removal rate (C). Toluene (□); *o*-xylene (■); *o*-methyl-benzaldehyde (+); *o*-methyl-benzoic acid (o). Solid or dashed lines: model.

Effect of O-xylene concentration

In test X3, the *o*-xylene concentration was increased in the reactor whereas the toluene concentration was maintained constant in the reactor inlet. Experimental and modelled data are shown in Fig. 3A,B. Kinetic parameters are summarized in Table 1. The *o*-xylene and the toluene removal rate were modelled with the same half-saturation constants used for tests X2 and X4. However, the decrease of the maximum growth constants $\mu_{max(tol)}$ and the *o*-xylene maximum utilisation rate $k_{x(o-xyl)}$ for test X3 indicates a toxicity phenomenon, most likely from *o*-xylene. In addition, the *o*-xylene at concentrations above 5 mg/L seems to inhibit the oxidation of *o*-methyl-benzaldehyde into *o*-methyl-benzoic acid. In order to model this latter phenomenon, an inhibition expression (similar to Equation 1) was used, where *o*-xylene inhibits competitively the *o*-methyl-benzaldehyde biotransformation.

Table 1 summarizes the kinetic parameters for experiments X1, X2, and X3. The modelling of three independent kinetic experiments could be performed without changing the toluene and *o*-xylene half-saturation constants. Based on this modelling, the average toluene maximum growth constant, $\mu_{max(tol)}$, was 4.8 ± 0.9 d^{-1}. For *o*-xylene, however, the max-

Table 1. Estimated kinetics parameters for experiment X1, X2, and X3.

Run	$K_{S(tol)}$ (mg/L)	$K_{S(o-xyl)}$ (mg/L)	$K_{S(omba)}$ (mg/L)	$\mu_{max(tol)}$ (d^{-1})	$k_{x(o-xyl)}$ [1] ($mg_x/mg_s\ d^{-1}$)	$\mu_{max(omba)}$ (d^{-1})
X2	0.11	0.32	nc	4.75	2.15	1.25
X3	0.11	0.32	0.5	3.7	1.4	2
X4	0.11	0.32	nc	6	7.5	nc

[1] Concentration in COD units
NB: The temperature in all these tests was 25 °C ± 1 °C.
nc: not calculated

imum substrate utilisation rate, $k_{x(o-xyl)}$, indicates a stronger variability.

The toluene removal rate is plotted versus the *o*-xylene transformation rate for tests X2 and X3 (Figs. 2C and 3C). This plotting allows a better adjustment of the modelled curves to the experimental data and thus a better estimation of the kinetic parameters. More practically, it also permits to assess the optimal toluene degradation rate in order to maximize the *o*-xylene transformation rate (see Fig. 2C).

Sensitivity test

A sensitivity test has been performed for this model. The test consisted of altering independently various parameters used in the model, and to estimate how much these variations affected the modelled curves. The test was accomplished in relation to kinetic experiment X2; i.e., under circumstances where the toluene concentration was gradually increased in the reactor and *o*-xylene concentration held constant.

Factors investigated were kinetic parameters, substrate diffusivity, dry weight density and active biomass distribution in the biofilm. These factors were independently changed to three levels. For each change the toluene and *o*-xylene removal rate were simulated. The variation range and results are summarized in Table 2.

The density of the active biomass and its concentration profile in the biofilm have the strongest effect on the removal of both substrates (Fig. 4A-D). The kinetic parameters related to toluene ($\mu_{max(tol)}$ and $K_{S(tol)}$) influence the *o*-xylene removal. However, the toluene removal is not significantly affected by the kinetic parameters of *o*-xylene (Figs. 6 and 7). The substrate diffusivity does not have any significant influence (curves not shown).

Discussion

Modelling of kinetic experiments

The advantage of this model is its simplicity. The cometabolic degradation of *o*-xylene and substrate interactions could be modelled by only calibration of the toluene and *o*-xylene half-saturation constants. The modelling of three kinetic experiments could be performed without changing the value of the half-saturation constants for both substrates. Only the adjustment of the toluene maximum growth rates, μ_{max}, and the *o*-xylene maximum utilization rate were necessary.

The variation of the toluene half-saturation constant, $K_{S(tol)}$, has a significant effect on the toluene removal rate (Fig. 6A-D). This suggests that the value reported in Table 2 is accurate. The kinetics of toluene degradation under denitrifying condition has been studied in a batch system by Flyvbjerg (1992). He determined the toluene half-saturation constant, $K_{S(tol)}$ to 0.2 mg/L. This is in accordance with this present modelling.

For toluene, an average $\mu_{max(tol)}$ of 4.8 ± 0.9 d^{-1} was determined. The decrease of $\mu_{max(tol)}$ between tests X2 and X3 may be explained by a toxic inhibition due to the increasing *o*-xylene bulk concentration. This has been discussed elsewhere that *o*-xylene, even at very low concentration, inhibits the toluene removal. (Arcangeli & Arvin 1993a,b).

Concerning *o*-xylene, the estimation of the maximum utilisation rate, $k_{x(o-xyl)}$, indicates a significant variability. This constant was lower in test X3 than X2. A likely explanation may be substrate inhibition, where *o*-xylene at high concentration inhibits its own removal. In opposition, the high $k_{x(o-xyl)}$ found in test X4 suggests a high cometabolic activity; i.e., the amount of *o*-xylene transformed per unit of toluene

Table 2. Effect of kinetic factors on the toluene and *o*-xylene removal rate.

Factors	Estimated -text X2-	Variation range	Toluene deg. rate	*o*-Xylene deg. rate
$k_{x(o-xyl)}$ ($mg_X/mg_S\ d^{-1}$)[a]	2.15	1-3	-	++
$K_{S(o-xyl)}$ (mg/L)	0.32	0.11-0.95	+	++
$\mu_{max(tol)}$ (d^{-1})	4.75	3-6.5	++	+
$K_{S(tol)}$ (mg/L)	0.11	0.48-0.96	++	++
$X_{(deg)}$[b]	Fig. 5	Fig. 5	++	++
$X_{(deg)}$ density (gm^{-3})[c]	1.11×10^5	$0.71\text{-}1.43 \times 10^5$	++	++
Toluene diffusivity (m^2/d)	8.73×10^{-5}	$5.73\text{-}12 \times 10^{-5}$	+	+
o-xylene diffusivity (m^2/d)	7.92×10^{-5}	$4\text{-}11.5 \times 10^{-5}$	-	+

[a] Concentration in COD units.
[b] Distribution of toluene degraders in the biofilm.
[c] Active biomass solid phase density.
-: Insignificant effect.
+: Moderate effect.
++: Significant effect.

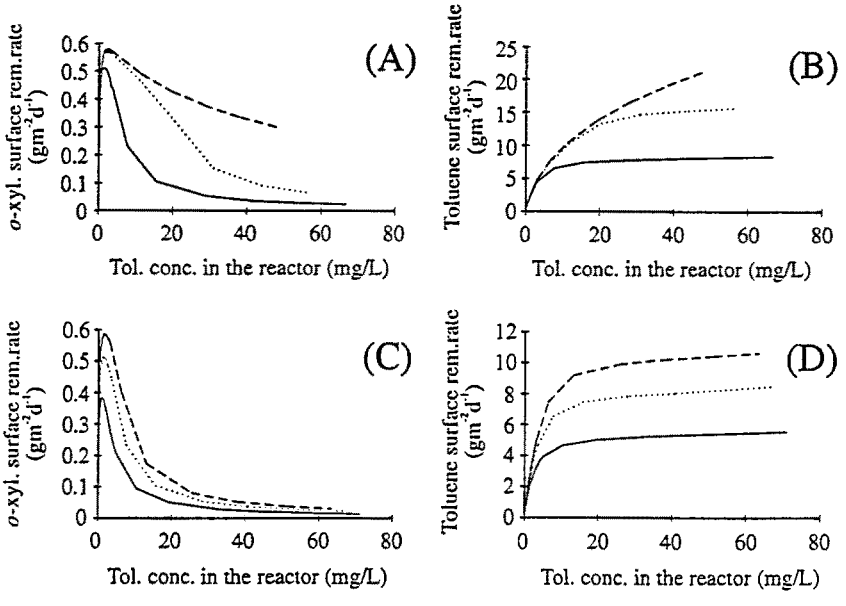

Fig. 4. Kinetic experiment X2. Effect of the active biomass depth in the biofilm on *o*-xylene (A) and toluene (B) removal rate. 77 μm (—); 155 μm (...); 311 μm (- - -). Effect of the active biomass solid phase density on *o*-xylene (C) and toluene (D) removal rate. 71 kg/m^3 (—); 111 kg/m^3 (...); 143 kg/m^3 (- - -).

degraded was higher in test X4 than X2. This higher activity is corroborated by a molar balance (Arcangeli & Arvin 1993b) which showed a larger quantity of *o*-xylene removed compared to test X2, but also a further transformation and/or mineralisation of *o*-xylene. The test X4 was performed as the biofilm was old (8 weeks), contrary to tests X2 and X3. Moreover, between tests X3 and X4, kinetic experiments with a mixture of BTEX were performed. Therefore, the main reason of the higher removal of *o*-xylene in test X4 may be due to an adaptation of the biomass, and/or a natural selection of bacteria which is able to mineralize *o*-xylene. Evidence of mineralization of *o*-xylene under denitrifying conditions with toluene as primary source has been reported by Hutchins (1993).

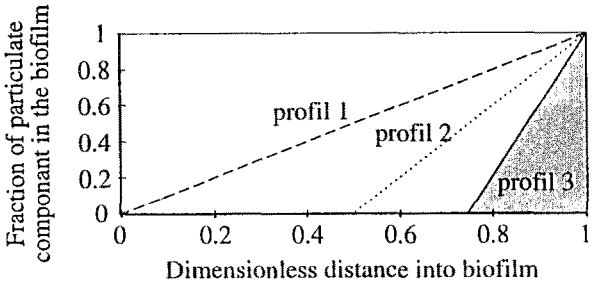

Fig. 5. Active biomass profile in the biofilm. All three profiles were used for simulating results presented in Fig. 4A,B. Example for profile 3: the dark area symbolize the fraction of active biomass (toluene degraders) in the biofilm, whereas the light area symbolize the fraction of inactive biomass.

Biomass density and active biomass

Equations (1) and (2) reveal that an independent estimation of the maximum growth constant and active biomass concentration (toluene degraders) is not possible. The biomass characterization is thus the bottleneck of this modelling. The main parameter which describes the biomass is the density. This density can vary widely depending on the nature of the bacterial growth, biofilm depth, biofilm age, and the presence of other particles in the biofilm matrix. The literature reports values from 5,000 to 130,000 g/m^3 (Characklis et al. 1989). In this study, the analytical methods used did not allow an exact determination of the active biomass concentration in the biofilm, but rather the total particulate concentration in the biofilm. This includes active and inactive biomass (polymers, dead cells...). However, Fig. 4A-D reveals how the independent variation of the active biomass density can alter the toluene and *o*-xylene removal rate significantly. Consequently, as a result of the inaccurate determination of the active biomass concentration in the biofilm, the toluene maximum growth rate constants, μ_{max}, and the *o*-xylene maximum utilisation rate, $k_{x(o-xyl)}$, deduced from this modelling are also uncertain, but they reflect well the relative activity between the experiments.

Shallow active thickness

The modelling of tests X2 and X4 indicates that the active biomass (or toluene degraders) accumulated in the biofilm top in a thin layer of approximately 80–100 μm where the bioreaction takes place. Fig. 4A,B shows that the depth of this active biomass dramatically affects the modelling of the toluene and the *o*-xylene removal rate. Comparing Fig. 2A,B and Fig. 4A,B, it can be seen that the thinner this active layer is, the better the model fits the experimental data. The depth of this active layer could be determined according to model calibration and assuming that the active biomass was distributed linearly in the biofilm (Fig. 5). The modelling of tests X2 and X4 could be performed assuming the same profile and depth of toluene degraders in the biofilm.

Experimental data corroborate a thin active layer: although the biofilm thickness was different in experiments X2 and X4 (311 and 379 μm) results show that the maximum *o*-xylene transformation rate was obtained for an optimum toluene concentration, S_{opt}, of 1.5–2 mg/L, independently of the biofilm thickness. The modelling of these experiments with an active biomass distributed uniformly in the biofilm lead to higher S_{opt} because of the diffusion resistance in the biofilm matrix (results not showed). Computer simulations demonstrate clearly the dependency of S_{opt} with the active biomass depth (Fig. 4A). In addition, if an equally distributed active biomass is assumed in the biofilm, the modelling of the toluene degradation would require a diffusion coefficient of 1.8×10^{-4} and 4.7×10^{-4} m^2/d for runs X2 and X4, respectively. These values represent 2 to 5 times the toluene diffusion coefficient in pure water which suggest that the degradation occurred in the biofilm top layer.

A likely explanation for this shallow active layer may be that the biofilm was grown with a low toluene concentration. This leads to a partly substrate penetrated biofilm when the biofilm thickness is above a certain value. A typical active layer of 100 μm can be calculated for a toluene bulk concentration of 5 mg/L. This is calculated based on kinetic results related to tests X2 and X4 reported elsewhere (Arcangeli & Arvin 1993b), and assuming a half-order reaction in the biofilm (Harremoës 1978). Hence, the toluene degraders accumulate in the biofilm top where the substrate is not limiting. The rear part of the biofilm mainly consists of polymers and dead cells unable to degrade toluene.

Simulation

Based on this modelling different series of simulations have been made. The goal of these simulations was to predict the concentration of toluene in the reactor and thus, the amount which has to be amended in order to enhance the degradation of a certain concentration of *o*-

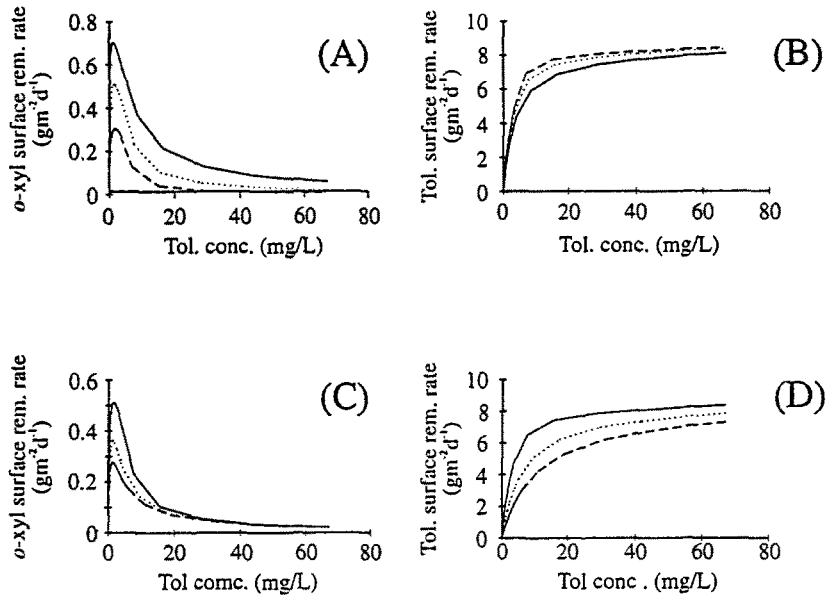

Fig. 6. Kinetic experiment X2. Effect of the *o*-xylene half-saturation constant on *o*-xylene (A) and toluene (B) removal rate. 0.11 mg/L (—); 0.32 mg/L (...); 0.95 mg/L (- - -). Effect of the toluene half-saturation constant on *o*-xylene (C) and toluene (D) removal rate. 0.11 mg/L (—); 0.48 mg/L (...); 0.96 mg/L (- - -).

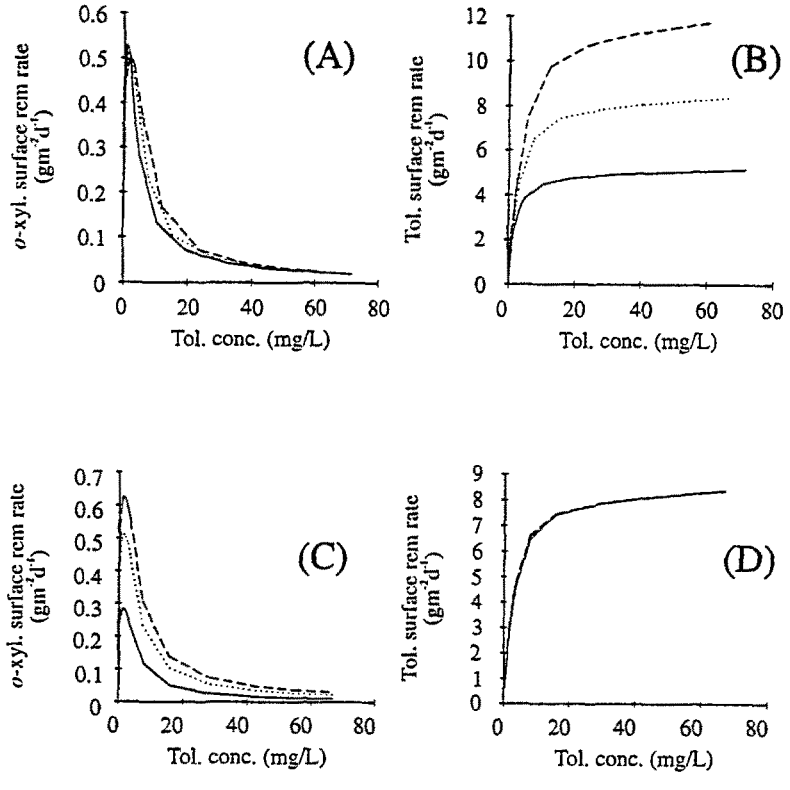

Fig. 7. Kinetic experiment X2. Effect of the toluene maximum growth constant on *o*-xylene (A) and toluene (B) removal rate. 3 d^{-1} (—); 4.75 d^{-1} (...); 6.5 d^{-1} (- - -). Effect of the *o*-xylene maximum growth constant on *o*-xylene (C) and toluene (D) removal rate 1 d^{-1} (—); 2.15 d^{-1} (...); 3 d^{-1} (- - -).

xylene. Kinetic parameters from test X2 were used for that simulation. Results are displayed in Fig. 8A,B. The amount of *o*-xylene degraded may be overestimated, since this model did not take into account the substrate inhibition effect of *o*-xylene. However, this approximation leads to minor errors. For example, test X2 reveals an *o*-xylene removal rate of 0.9 $gm^{-2}d^{-1}$ for an *o*-xylene and a toluene concentration in the range of 30 mg/L and 8 mg/L, respectively. The model predicts an *o*-xylene removal rate of 1 $gm^{-2}d^{-1}$ for the same input data (Fig. 8A). Additionally, it appears that the optimum toluene concentration, S_{opt}, which is associated to the maximum *o*-xylene removal rate, increased in the range from 1.1 to 2 mg/L for an *o*-xylene concentration of 0.1 and 65 mg/L, respectively. Equation (3) predicts a value of 0.13 and 1.58 mg/L. Consequently, a rough approximation might be to neglect the substrate diffusion in the biofilm when high concentrations of pollutants are encountered. However, such an approach may rapidly lead to significant errors for a thick active thickness or low substrate concentrations.

It can be concluded, that the use of this model is a useful engineering tool for design of treatment processes for groundwater remediation or for industrial wastewater. However, it should be noted that the estimated constants for this mixed denitrifying culture may be valid only for this specific culture, under these specific conditions. Besides, this model is limited to substrate concentrations reported in this study. For higher concentrations, toxicity effects have to be taken into account such as substrate inhibition or non-competitive inhibition.

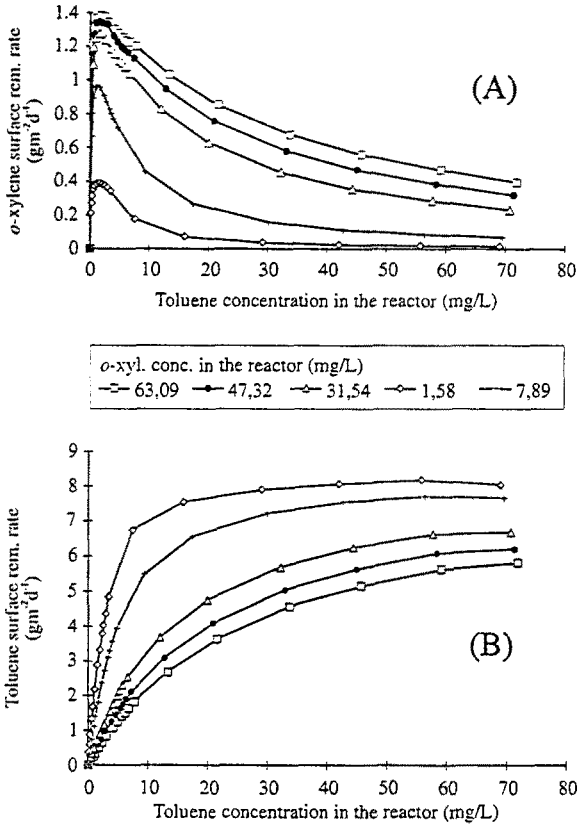

Fig. 8. Simulation of *o*-xylene removal rate (A) and toluene removal rate (B) versus various *o*-xylene concentrations in the reactor input.

Conclusions

The cometabolic biotransformation of *o*-xylene in a denitrifying biofilm system with toluene as primary carbon source has been modelled using a mathematical expression which takes the competitive inhibition and the stimulating effect from toluene into account. The proposed model was able to predict not only the degradation rate of *o*-xylene and toluene but also interaction phenomena between toluene and *o*-xylene as well as the associated by-products formation. Based on three independent simulations, a good agreement between modelled and experimental data was found.

This model requires two biomasses: active (toluene degraders), and inactive (dead cells and polymers). The modelling of various kinetic experiments suggested that the active biomass is accumulated on the biofilm top layer, indicating that only the upper part of the biofilm was active. Furthermore, it appears from the sensitivity test that a precise determination of the active biomass density and distribution in the biofilm is needed since these factors dramatically affect the modelling.

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