

The relation between pore-scale heterogeneity, bioavailability and bacterial mobility: a numerical modelling approach

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Abstract Desirable reactive subsurface processes, e.g. nutrient cycling or degradation of organic contaminants, are driven by microorganisms populating the soil matrix. These environments are characterized by heterogeneities at various scales which influence the transport of chemical species and the spatial distribution of microorganisms. As a result, the biodegradation rate of contaminants at large scales does not only depend on the degradation capacity of the indigenous microbial population, but also on their distribution patterns and the heterogeneities of the hosting media. Many of these organisms are motile and exhibit a chemotactic behaviour driven by gradients of substrate and fellow organisms. In this study, we developed a reactive transport pore-network model to study the bioavailability effects resulting from structural heterogeneity of the pore space. Then, we included an individual-based modelling approach to simulate the bacterial pattern conformation in heterogeneous porous media. By varying the degree of structural heterogeneities or the chemotactic sensitivities of the bacteria, we explored how the degradation performance is affected and what population distributions emerge.

Key words individual-based modelling; pore network model; bioavailability; reactive transport model; spatial distributions; pore-scale heterogeneity; microbial patterns

INTRODUCTION

One of the common strategies to clean polluted groundwater aquifers is *in situ* bioremediation. The basic principle of this technique is to use indigenous microorganisms to transform water-dissolved organic contaminants to harmless end-products. Hence, physical access of the microbial cells to the contaminant is particularly essential for the efficiency of *in situ* biodegradation. Natural porous media exhibit high spatial heterogeneities of the pore space down to the micro scale. This might affect the distribution of contaminants within the pore scale and consequently the access of microorganisms to the contaminants. Such limitation of contaminant bioavailability can reduce effective degradation rates and thus the success of *in situ* biodegradation.

Overall degradation can be improved if bacteria are able to detect contaminant concentration gradients and migrate towards regions of higher concentrations. This process, called chemotaxis, has been shown to be crucial in the context of bioremediation (e.g. Wang *et al.*, 2008). Bacteria in soil often create microcolonies that can be dynamically formed by active aggregation. Such aggregation can be mediated by a second chemotactic process in which bacteria are attracted to signal molecules that are excreted by the cells themselves (e.g. Mittal *et al.*, 2003). At the pore scale, the distribution of bacteria in porous media might therefore not only be influenced by the distribution of the contaminant substrate, but also by the migration dynamics of the bacterial population itself. Structural heterogeneities and their influence on microbial distribution patterns within the porous medium might, therefore, also be important controlling factors for the bioavailability of contaminants.

In this study we developed and used a pore network reactive transport model to simulate and analyse the fate of chemical compounds and chemotactic bacteria in porous media. We coupled a newly developed pore network model simulating the transport of dissolved species in heterogeneous pore assemblies with the Biogeochemical Reaction Network Simulator (BRNS), an established flexible numerical tool for the simulation of kinetic and equilibrium reactions of arbitrary size and complexity (Regnier *et al.*, 2002; Centler *et al.*, 2010). The resulting reactive transport pore network model (PNBRNS) is suitable for simulating a wide range of biogeochemical reactions in heterogeneous porous media including microbial contaminant degradation, and allows the use of any user-defined reaction rate law or thermodynamic equilibrium constraint. In this study, we employed PBRNS to simulate and determine (i) the

bioavailability effects resulting from structural heterogeneity, (ii) the influences that medium heterogeneity causes on the formation of microbial patterns. For the latter, the PNBRNS was expanded to allow for the individual-based modelling of bacterial chemotaxis. Here we consider besides a random diffusion-type movement of the cells, two antagonistic chemotactic processes: chemotaxis towards the substrate which leads to bacterial dispersal, and chemotaxis towards fellow microorganisms which allows for bacterial aggregation.

Model description

The model describes the soil texture as a 2D network of inter-connected cylindrical micro-tubes in which every micro-tube represents a pore. Three pores are connected at each node symmetrically and form a hexagonal pore network with coordination number 3 (Fig. 1). The length of pores is identical, while their radius can vary allowing the creation of heterogeneous networks. The medium is assumed to be isothermal and fully water saturated. Water flow in the cylindrical pores is considered to be laminar following Hagen-Poiseuille equation. For the transport simulation of dissolved solutes we implemented a finite volume method. The resulting equations were implemented in MATLAB using its numerical routines.

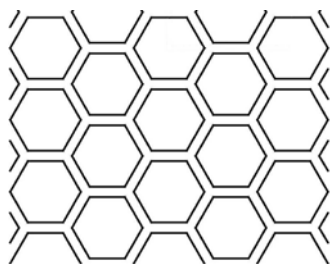


Fig. 1 Hexagonal pore network assembly.

Microbial degradation (or any other reactive process) which takes place in each pore is described by coupling the flow and transport model to the biogeochemical reaction solver BRNS (Regnier *et al.*, 2002; Centler *et al.*, 2010). For the coupling, an operator splitting technique was used following a sequential non-iterative procedure. In each time step, the transport calculations for the pore network were performed in MATLAB. Then the problem specific BRNS dynamic library is called for each pore to accomplish the reactive step. The concentration changes are computed according to the defined chemical kinetics and reactions, the updated concentrations are then passed back to the transport module in MATLAB (for further details see Gharasoo *et al.*, 2012).

For the individual-based modelling of bacterial mobility a similar concept from Centler *et al.* (2011) and Schofield *et al.* (2002) was used and adapted for the hexagonal pore network geometry (Gharasoo *et al.*, 2013). The chemotactic flux towards the substrate is described as $J_c = \chi_c \cdot b \cdot \nabla c$ with the constant rate parameter χ_c and substrate concentration c ; analogously the flux due to chemotaxis towards chemoattractants excreted by the cells is described as $J_b = \chi_b \cdot b \cdot \nabla b$ with χ_b as rate parameter and b as concentration of bacterial cells. The random dispersion of bacteria is described in analogy to Fick's diffusion law along with a flux $J_D = -D_b \cdot \nabla b$ where D_b is bacterial "diffusion" coefficient.

Combining all the above equations together, the spatial movement of bacteria is described by:

$$\frac{\partial b}{\partial t} = D_b \cdot \nabla^2 b - \chi_c \cdot \nabla \cdot (b \nabla c) - \chi_b \cdot \nabla \cdot (b \nabla b) \quad (1)$$

Based on this equation, the probabilities p_0 of a single bacterial cell to stay in the current pore or p_j to move to the neighbouring pore j within the time step Δt are derived as:

$$\begin{aligned}
p_0 &= 1 - \frac{2D_b\Delta t}{l^2} \pm \chi_c\Delta t \left\{ \left(\frac{V_0}{V_R+V_0} - \frac{V_0}{V_L+V_0} \right) \frac{\nabla c}{l} + \nabla^2 c \right\} \pm \chi_b\Delta t \left\{ \left(\frac{V_0}{V_R+V_0} - \frac{V_0}{V_L+V_0} \right) \frac{\nabla b}{l} + \nabla^2 b \right\} \\
p_j &= \frac{D_b\Delta t}{l^2} \frac{V_j}{V_j} \pm \frac{\chi_c\Delta t\nabla c}{l} \frac{V_j}{V_j+V_0} \pm \frac{\chi_b\Delta t\nabla b}{l} \frac{V_j}{V_j+V_0}
\end{aligned} \quad (2)$$

with $V_{j,0}$ as volume of the individual pores and l as (constant) pore length. The $V_{L,R}$ denotes the total pore volume of all neighbouring pores at the left or at the right hand side of a given pore, respectively, and V_j is the total volume of all pores located at the same side of neighbouring pore j . Subsequently all probabilities are normalized by Σp_k and used to simulate the random movement of the individual cells. In case a pore contains more cells than its maximum carrying capacity after the completion of migration processes, excess cells are randomly moved from the overcrowded pore to its neighbouring pores. Expanding the operator splitting approach to the individual-based model, cell migration is simulated for each time step following the transport and reactive processes. Microbial growth is simulated as part of the biogeochemical reaction step and a constant cell density per volume is used for conversion between bacterial biomass concentrations and bacterial cell numbers.

SIMULATED SCENARIOS

Biodegradation in heterogeneous pore networks

The reactive transport pore network model PNBRS, was used to simulate the microbial degradation of an organic carbon substrate and to investigate the influence of pore scale heterogeneities on the bioavailability of the substrate. For this purpose, the FFT-based concept of generating random fields (Dietrich & Newsam, 1993) is used to produce heterogeneous pore networks with variable pore radii distributions. Considered heterogeneities include two different standard deviations of the pore size distribution (28% and 45% of the mean value of 160 μm), and three different spatial correlation lengths (1 (no correlation), 2.5 and 5 times the pore length of 1 mm). For each case of heterogeneity, simulations were performed for five different realizations. Furthermore, a homogeneous network was simulated as a reference.

In this scenario, bacterial biomass in each network was considered to cover homogeneously the walls of the pores without any growth and/or migration of the bacteria. All pore networks were constantly flushed by a degradable substrate assuming constant-flux boundary condition at the inflow and zero-flux boundary condition at network sides. All parameters describing flow, transport and the degradation of the substrate were derived from a column experiment in the literature (Harms & Zehnder, 1994). For each network, the flow boundary and total biomass were adjusted to ensure the same residence times and degradation capacities in all networks.

The biodegradation rate at the micro-scale is assumed to depend on the substrate concentration following Michaelis-Menten kinetics. Intra-pore diffusive mass transfer processes can limit the bioavailability of substrates inside a pore. The resulting effect on microbial degradation can be expressed by combining a linear exchange model with Michaelis-Menten kinetics (Bosma *et al.*, 1997; Hesse *et al.*, 2010). The final degradation rate is then given by the Best equation (Best, 1955):

$$R(c) = \frac{k_{tr}}{2} (c + K_t + k_{max}/k_{tr}) \cdot \left\{ 1 - \sqrt{1 - \frac{4c k_{max}/k_{tr}}{(c + K_t + k_{max}/k_{tr})^2}} \right\} \quad (3)$$

where c is substrate concentration in a pore, k_{max} is maximum degradation rate, and K_t is Michaelis-Menten constant. Simulations were performed assuming $k_{tr} = \pi^2 D a_v / 4r$, as proposed by Hesse *et al.* (2010) (r is the pore radius, a_v the specific surface of the pore wall, and D the molecular diffusion coefficient of the substrate), and compared to the results obtained for the standard Michaelis-Menten kinetics (equal to $k_{tr} \rightarrow \infty$, assuming no intra-pore mass transfer limitations).

The steady state outflow concentration of the substrate was used as an indicator of the total microbial degradation activity within the pore network.

Chemotaxis and microbial distribution patterns

The reactive transport pore network model is also implemented to simulate the microbial pattern formation in both homogeneous and heterogeneous pore networks (generated as above using a standard deviation of 40 μm and a correlation length of 5 mm). For these scenarios, substrate was supplied to each pore using a capacitive source term with a constant rate parameter, which would in the absence of heterogeneous biomass distribution lead to a uniform substrate concentration along the network. No advective flow was considered between the pores due to the zero-flux condition applied to the network boundaries. Substrate transport was thus limited only to diffusion. Bacterial cells were assumed to be associated with the water phase and were able to grow and migrate between the pores following the mechanisms described in section “Model description”. Parameters describing the activity and mobility of the bacteria are adapted from Keymer *et al.* (2006) and Berg & Turner (1990). Simulations were performed for different combinations of χ_c and χ_b , i.e. for different affinities of the cells toward concentration gradients of the substrate and of the bacterial cells. Microbial patterns were analysed once a steady state (or a steady temporal pattern) was achieved.

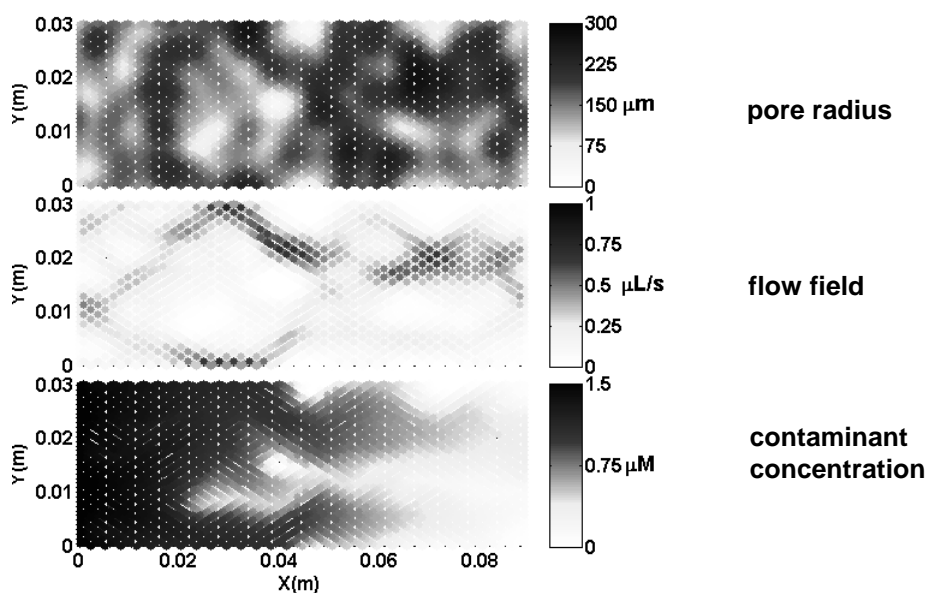


Fig. 2 Top: generated heterogeneous pore network (28% standard deviation, 5 mm correlation length). Middle: resulting flow pattern. Bottom: steady state distribution of a biodegradable contaminant.

RESULTS AND DISCUSSION

Model results for heterogeneous pore networks show that a preferential flow pattern progressively emerges when the standard deviation and correlation length increase (see Fig. 2 for a single realization example). Regardless of whether intra-pore mass transfer limitations are considered or not, an increase in standard deviation or correlation length always leads to a decrease of substrate bioavailability (Fig. 3). In all cases (i.e. regardless of the heterogeneity of the porous medium) intra-pore processes contribute significantly to the overall bioavailability restrictions. This indicates that even in the presence of larger scale limitations, pore-scale effects might still have a significant impact on the bioavailability of the substrate. For the tested pore size distributions the bioavailability restrictions attributed to the combined effect of intra- and inter-pore mass transfer

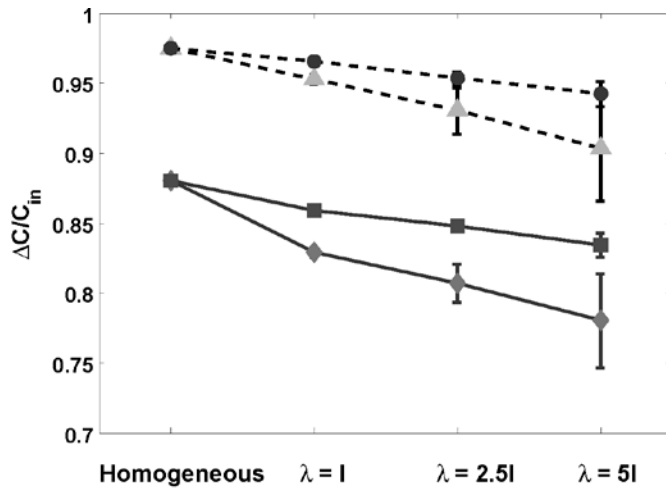


Fig. 3 Total normalized biodegradation (concentration change) along the network for homogeneous and heterogeneous pore networks using different spatial correlation lengths λ (1 denotes the pore length). Solid lines and dotted lines correspond to results with and without intra-pore bioavailability limitations, respectively. Symbols \blacklozenge and \blacktriangle indicate higher (45%) while \blacksquare and \bullet indicate lower (28%) standard deviations of the pore radius distribution. Error bars indicate variations between individual realizations (standard deviation from the average).

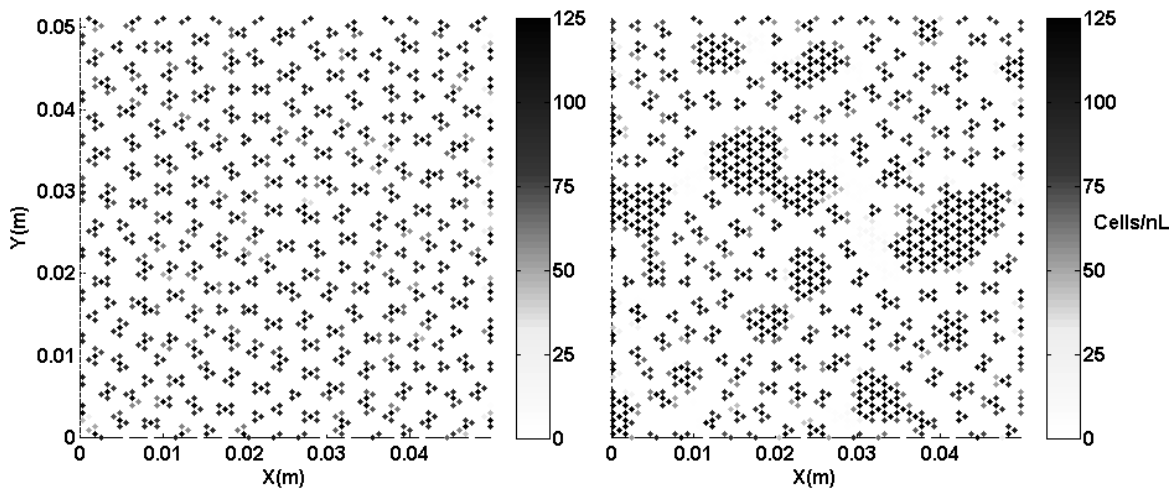


Fig. 4 Example of bacterial pattern formation due to chemotaxis in a homogenous (left) and a heterogeneous (right) pore network. An identical set of parameters is applied in both cases. Every single dot represents a single pore with colours indicating the volumetric concentration of bacteria in the pore.

lead to a reduction of effective biodegradation rates of almost 20%. This suggests that major changes between expected and observed *in situ* biodegradation rates must be attributed to extra processes.

Model simulations on the chemotactical formation of microbial distribution patterns showed that depending on the values of χ_c and χ_b , highly heterogeneous bacterial distribution patterns can also be found in the absence of any structural heterogeneity of the pore networks (results not shown). The results for homogenous pore networks are in good agreement with results obtained for continuum models (Centler *et al.*, 2011; Gharasoo *et al.*, 2013) confirming that the relation between bacterial motility, bacterial physiology and environmental factors controls the pattern formation. For a particular range of χ_c and χ_b values, results for heterogeneous pore networks differed from those of homogeneous networks (see an example in Fig. 4), while for all other χ_c and χ_b values results showed little variation between homogeneous and heterogeneous networks. This

suggests that the structural heterogeneity of a porous medium can have an impact on distribution patterns of bacteria. The magnitude of impact is significant when chemotactic parameters are in a specific range of values. Otherwise, the impact is limited and microbial patterns are mainly controlled by the chemotactic behaviour of the bacteria. This observation might change, if such structural heterogeneities exhibit an influence on microbial living conditions in the pore space (e.g. via the transport of a substrate).

In summary, these results show that the heterogeneity of porous media at the pore scale can have a significant impact on biodegradation observed at the larger scale. Pore network models provide the opportunity to study pore scale processes in heterogeneous media and the resulting impact on, e.g. effective biodegradation rates.

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