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Simulating the influence of dormancy on microbial community dynamics

Invloed van niet-actieve micro-organismen op microbiële gemeenschap dynamiek: Een model simulatie studie

(met samenvatting in het Nederlands)

Proefschrift

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door

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geboren op 18 oktober 1981 te Protvino, Rusland

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Co-promotor: Dr. M. Thullner

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Talk about what is clear for you,
otherwise keep silence.
Leo Tolstoy

Иногда я лежу в кровати и думаю, что ничто не заставит меня встать.
А потом чувствую, как подо мной становится мокро,
и понимаю, что ошибался.
Гомер Симпсон

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Introduction and thesis outline

General background

Nearly all natural subsurface environments are characterized by a large variety of reactive processes which are directly or indirectly driven by microbial activity. In particular, the degradation of organic matter (including organic contaminants) in nearly all subsurface water bodies in terrestrial (groundwater, soil moisture, water within very low permeability bedrock, and deep-geothermal or oil formation-derived water) and marine environments is controlled by the abundance and functional capability of resident microorganisms. Microbial processes have also a central function in the global fluxes of key biogenic greenhouse gases (carbon dioxide, methane and nitrous oxide), which requires the understanding of these processes to predict and eventually to influence them in order to mitigate anthropogenic climate changes (Singh et al., 2010).

Furthermore, understanding the dynamics of microbial processes in groundwater systems is of critical importance, as it can have a profound effect on the water quality, and thus on the drinking water supply to many of the world's communities. Besides groundwater, soil is a very important resource. Microorganisms are critical for the maintenance and function of natural soils because of their involvement in crucial processes such as structure formation, decomposition of organic matter, toxin removal, and the turnover of carbon, nitrogen, phosphorus, and sulphur (Garbeva et al., 2004). For example, nitrogen fixation provided by microorganisms that reside in the rhizosphere is a crucial function for plant growth and productivity (Barea et al., 2005).

Soils and aquifers represent a highly heterogeneous environment for the inhabiting microbiota. The various components of the solid fractions in soil provide a countless number of different microhabitats. Beside that, all natural systems are subject to periodical fluctuations of living conditions such as e.g., tides, and daily or seasonal cycles. In combination, spatial heterogeneities and fluctuating living conditions can have a profound influence on the abundance and activity of microbial communities and thus on their reactive performance.

In addition to the aforementioned spatial and temporal variations, natural bacterial communities are highly diverse. Nevertheless, our knowledge on microbial diversity is limited, e.g., only approximately 1% of the soil bacterial population can be cultured using standard laboratory practices (Kirk et al., 2004). Torsvik et al. (2002) estimated that one gram of soil may harbor up to 10 billion microorganisms of thousands of different species, although further development of analytical techniques in molecular microbiology may suggest an even higher diversity. As there is a limited number of biochemical pathways, many bacterial groups are involved in the same process which implies a direct competition between different microbial species. Nevertheless, these species can exhibit a large variability in their activity at given environmental conditions and thus, representing them as one single functional group may not be adequate.

Due to both the high complexity of these subsurface environments and the limited knowledge on the bacterial community, natural bacterial systems are far too complex to be fully described by a theoretical model, and, even the development of simplified models is a big challenge. However, the activity of the catalyzing microorganisms has a crucial impact on the overall rate of underground processes, thus bacterial activity requires comprehensive research. In this respect, there is a need to find suitable theoretical approaches which are able to describe the most relevant features of bacteria systems while maintaining an adequate level of simplicity and flexibility. Finding such an effective approach without oversimplifying the system complexity is of paramount importance when studying microbial activity at *in situ* conditions.

Bacterial growth and activity

Replication represents one of the main bacterial functions, which implies the division of one bacterium into two daughter cells. Bacteria grow to a fixed size and then reproduce through binary fission, a form of asexual reproduction (Koch, 2002). Under optimal conditions and after a potential lag phase with cells having to adapt to new environmental conditions (Prats et al., 2006), bacteria can grow and divide rapidly, exhibiting exponential growth with some populations doubling as fast as every 10 minutes (Eagon, 1962). This rapid exponential growth is associated with high activity rates and thus a high degradation capacity of the bacterial community. Furthermore, nutrients are metabolized until one of the nutrients is depleted and starts limiting growth. The subsequent stationary phase is characterized by nutrient depletion, where the cells decrease their metabolic activity and use the remaining degradation capacity mainly to maintain the existing microbial biomass. Further depletion of one or more nutrients will eventually lead to starvation and cell death unless the bacterial metabolism allows for survival during periods of starvation stress. A transition of bacterial cells from an active state to an inactive stress-response state is known as dormancy and is one of the most effective bacterial survival strategies.

The microbial degradation capacity of an environmental system depends on the bacterial abundance and activity. As a consequence, quantitative descriptions of microbially-driven (degradation) processes should not only include depictions for chemical species and bacterial abundance but also of the fraction of bacteria present in an active state. An appropriate description of the ability of bacterial cells to switch between an active and a dormant state is therefore of essential importance for understanding degradation processes in highly-transient natural systems.

Dormancy as a survival strategy

In nature, most organisms live under regularly and irregularly fluctuating living conditions and typically experience circumstances that are suboptimal for growth and reproduction (Cowan et al., 2004). Organisms commonly respond to environmental stress by entering a reversible state of reduced metabolic activity known as dormancy (Lennon and Jones, 2011). By doing so, these organisms can

drastically lower their energetic expenditure and endure unfavorable conditions such as high/low temperature, low osmotic pressure (e.g. substrate availability), extreme light and pH that would otherwise reduce the health of the population.

Dormancy is a widespread phenomenon and allows microorganisms in natural settings to contend with harsh environmental conditions (Stevenson, 1977). The importance of this phenomenon is highlighted by the fact that in many environments a large fraction of the resident microorganisms is metabolically inactive under natural conditions (Fig. 1.1, Lennon and Jones 2011).

To study the impact of dormancy on the ecology and evolution of complex microbial communities it is necessary to have a basic understanding of the cellular processes that control dormancy. Active bacterial cells may detect changes in external environmental conditions such as temperature, substrate concentration, and pH via highly dedicated sensors. Signals from these sensors elicit an intracellular response that leads to changes in gene expression and protein synthesis (Mascher, 2006), which can ultimately trigger dormancy (Boon et al., 2001).

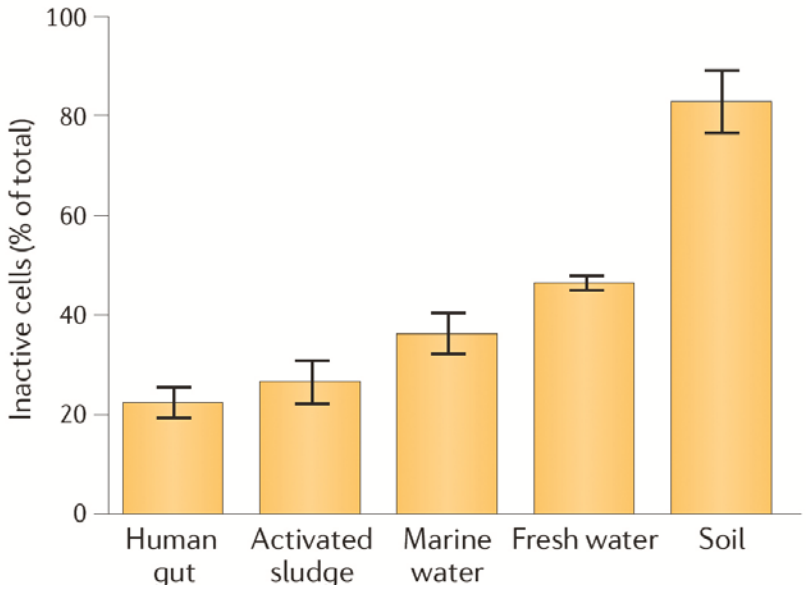

Figure 1.1. Percentages of dormant cells in various environments (after Lennon and Jones 2011).

Dormancy describes the transition of metabolically active cells into a dormant state. Dormant cells can remain in an inactive state for prolonged periods. To use dormancy as a successful strategy, dormant cells must be able to resuscitate and return to the actively growing pool of cells if environmental conditions become again sufficiently favorable. Nevertheless, dormancy is not a cost-free strategy. Reactivation requires a sufficient amount of available substrate which is typically higher then concentrations needed for maintenance of active cells. Furthermore, the inactive fraction usually contains a percentage of cells incapable of reactivating (Bakken et al.,1987). Organisms must invest resources in resting structures and in the metabolic machinery that is needed for transitioning into and out of a dormant state. In addition, dormant organisms must be able to interpret and respond to signals associated with favorable conditions, otherwise they will miss opportunities for growth and reproduction. Beside that, the rate of reactivation of dormant bacteria also depends on the duration of dormancy (Kaprelyants et al., 1993b). This resuscitation time can be a part of the so-called lag phase of bacterial growth. Despite these general tradeoffs, microbial species from all domains of life have evolved the ability to use dormancy during periods of environmental stress.

Addressed research questions

As knowledge on microbial processes and their interactions expands, it becomes possible to develop increasingly-detailed model representations of biogeochemical reaction systems. Although natural systems are inherently complex, simplifications of these systems can be used in order to better understand and quantify particular phenomena within the system. Chemical and microbial species (variables) and their properties (parameters), which are inherently interconnected in biogeochemical environments, are typical components of microbial models. The development of detailed biogeochemical models helps close the gap between fundamental research in biogeochemistry, microbial ecology and molecular biology, and application-oriented reactive transport modeling.

The aim of this thesis is to develop more general mathematical representations of microbial activity in biogeochemical models, which account for the diversity, physiological state and ecological interactions of microorganisms. These concepts

are used to study the dynamic of microbially-driven processes in systems with spatially or temporarily changing environmental conditions. A particular focus is given on the effects of dormancy and reactivation of individual bacterial species on the abundance and composition of bacterial communities and their biodegradation activity. These phenomena have hardly been addressed conceptually so far, especially not in the context of biogeochemical models of environmental systems.

Methodological approach

Modeling approaches which predict the fate of organics and related changes in redox conditions have to account for the effects of microbial activity on the degradation kinetics, as well as for the spatial and temporal distributions of the chemical species that control microbial activity (Thullner et al., 2007). Models are used to describe the behavior of microorganisms under different physical or chemical conditions (e.g., substrate availability, temperature, or pH), as well as the resulting changes in the chemical composition of the modeled system. Computational modeling is not only used for predicting or reproducing the dynamics of an environmental system, as some degree of experimental validation will always be needed to confirm any model results. Rather, models provide the ability to test hypotheses on and to obtain new insights into the underlying mechanistic basis of observed biogeochemical phenomena. Microbial models for natural environments face many challenges, including the development of realistic representations of microbial reaction kinetics, microbially-driven reaction networks, scale-dependent mixing processes, as well as chemical, biological and physical heterogeneities.

There are two major classes of models used to simulate bacterial growth and activity. The first considers the live cycle of each cell, as well as its biochemical activity and transport individually. These type of models are broadly referred to as Individual-based Models (IbM). IbMs make no assumptions about population properties and constraints but instead consider macroscopic behavior/patterns that emerge from microscopic rules. IbMs can be used to make predictions for complex systems based on usually simpler description of the lower levels of organization. These models can be very useful for understanding the bioavailability of nutrients, the dynamic of biofilms and interrelations within the group of bacteria. Nevertheless,

these models lose efficiency due to computational demands when considered at macroscopic scales of naturally occurring systems.

Another class of models, the one used in this thesis, uses a differential equation-based approach to describe bacterial populations, depicted as macroscopic (functional) groups, as well as the dynamics and activity of these bacterial groups. In particular it considers active cells growing and utilizing dissolved organic carbon (DOC) or the production/consumption of any other biologically-active species according to a generalized reaction rate expression. The growth and turnover rates of the involved chemical species are typically assumed to follow standard Michaelis-Menten- or Monod-type kinetics (Regnier et al., 2005; Thullner et al., 2007 and references therein). Within this concept, active and dormant biomass fractions are included as independent reactive species, and kinetic expressions are derived to account for the deactivation and reactivation of the microorganisms. These kinetic expressions are linked to the energy yields of the corresponding respiration pathways, as well as to the maintenance requirements of the organisms. In the model applications presented in this thesis, the catabolic energy supply of the cells is assumed to represent the main factor controlling the transition of cells between the active and inactive states. The developed concepts and kinetic expressions are first tested using data from laboratory experiments and are subsequently explored to study *in silico* the implications of dormancy as survival strategy on the dynamics of microbial communities for different environmental settings.

In the present thesis the biogeochemical reaction network simulator (BRNS) is used as a general tool to model biogeochemical processes. The BRNS has been developed to numerically simulate biogeochemical processes coupled to one-dimensional flow and species transport (Aguilera et al., 2005, and references therein). The equations describing the transformations of the chemical and biotic species can be of arbitrary form allowing the use of complex kinetic expressions as derived within this thesis. Simulations were performed either for perfectly mixed conditions (batch experiments) or for porous media considering one-dimensional transport using a finite difference approach. In order to determine the influence of pore scale heterogeneities on the studied process interactions, the reactive transport pore network model PNBRNS (Gharasoo et al., 2012) is used. PNBRNS couples the

BRNS with advective-diffusive transport of chemical species in a two-dimensional heterogeneous network of cylindrical pores (Fig. 1.2).

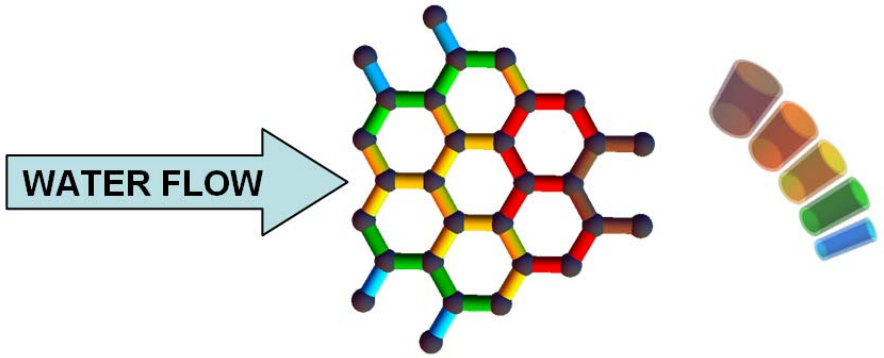

Figure 1.2. Schematic representation of the two-dimensional hexagonal lattice used to construct the heterogeneous pore networks. Colors represent pore sizes assigned individually to each pore.

Thesis outline

This thesis addresses the implications of microbial dormancy on abundance, dynamics and diversity of bacterial systems.

Chapter 2 introduces a modeling concept that explicitly accounts for the bacterial ability to switch between active and dormant states. This concept is then implemented into a numerical model to simulate the growth and activity of bacteria using dormancy as a survival strategy. The model was successfully used to reproduce published data from laboratory experiments and to simulate the dynamic behavior of hypothetical microbial communities. These simulations showed that the ability to switch to and from the dormant state substantially widens the spectrum of possible community structures and population dynamics. Beside that, an extended sensitivity analysis of the involved parameters was performed, which allows determining the largest source of uncertainties for the model results.

Chapter 3 focuses on the impact of pore size heterogeneities in natural porous media such as soils and aquifers on the distribution of abundance and activity of a bacterial community consisting of competing species. These species respond to external environmental stress periods by switching from an active into an inactive or

dormant state and back. Simulation results particularly show that such pore scale heterogeneities have a strong impact on bacterial abundance and activity creating a complex structure of microbial niches. This leads to an increased coexistence of microbial species at both the micro (pore) and the macro (porous medium) scale. Furthermore, this chapter shows that not a single but various sets of external conditions may be optimal for the competitive behavior of species.

Chapter 4 of this thesis describes the response of bacterial model systems containing two and three competing species with different growth and de-/reactivation efficiencies to different regimes of intermittent changes of environmental conditions. Besides identifying conditions which allow for a coexistence of competing species, the chapter focuses on the explicit influence of so-called suppressed species on dominating species. Particularly it shows that even though the biomass of some suppressed species is below a typical detection threshold, the species may still play a considerable role for the dynamics of the community. Moreover, suppressed species may affect the behavior of dominating species more than would be anticipated from their low abundance.

Finally Chapter 5 deals with the optimal levels of model complexity. Though many studies considered this problem from a theoretical standpoint and have produced some useful guidelines, this chapter investigates the problem under a different angle which focuses on the determination of end/key species involved in substrate degradation. The study considers a highly diverse artificial bacterial community promoting one substrate degradation pathway within a one-dimensional transport system, and compares its dynamics with results using a simpler representation of the microbial community. The results of the chapter reveal the minimum requirements for simplified models needed for a sufficient reproduction of the dynamics of a reference system. This allows identifying the limits of the use of these simplified model systems.

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2

Incorporating dormancy in microbial community dynamic models

with Paula Martinez-Lavanchy, Hermann J. Heipieper,
Philippe Van Cappellen and Martin Thullner

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Abstract

Biogeochemical activity in natural and engineered systems depends on the abundances, functional capabilities and physiological states of the indigenous microorganisms. Typically, only a fraction of the microbial population is active at any given time. As environmental conditions change, previously active microorganisms may switch to an inactive or dormant state, while dormant ones may become active. Here, we present an extended modeling concept for the growth and decay of microorganisms that explicitly accounts for their ability to switch between active and dormant states. The equations describing the switching between physiological states are implemented into a biogeochemical reaction simulator. The model was used to reproduce the published data from laboratory experiments in which microorganisms were subjected to intermittent substrate supply or reactivated after a prolonged period of starvation. Fitting procedure let us estimate unknown parameters of these systems and use them in further hypothetical modeling experiments. Results for hypothetical microbial communities consisting of two competing species exposed to periodic feeding imply that, under certain conditions, an effective dormancy-reactivation strategy may have a competitive advantage over a fast growth strategy.

That is, organisms that can switch rapidly in response to fluctuations in external conditions may outcompete fast-growing organisms. Furthermore, certain combinations of growth and dormancy strategies may lead to the long-term coexistence of the two competing species. Overall, the simulated population dynamics show that dormancy is an important feature of microbial communities, which can lead to complex responses to environmental fluctuations.

2.1. Introduction

Microbial activity plays a crucial and often dominant role in the cycling of carbon (Griffith et al., 2010), nutrient elements (Ganzert et al., 2011) and contaminants in the environment (Borch et al., 2010). The activity of microorganisms is controlled by many external factors, such as the availability of energy substrates, electron acceptors and nutrients, as well as physical and geochemical variables (e.g., temperature, water saturation, salinity, pH, and redox state) and the presence of inhibiting or toxic substances (Paul and Clark, 1996). In most near-surface environments, these external factors vary significantly over a range of temporal scales. In addition, oligotrophic conditions are the rule rather than the exception in natural systems, in contrast to the nutrient replete conditions under which microorganisms are typically studied in the laboratory (Harder and Dijkhuizen, 1983). Microorganisms in the environment must therefore cope with a variety of stresses and must be able to continuously adapt their metabolism to the conditions in their immediate surroundings.

In order to survive unfavorable environmental conditions, microorganisms can switch to an inactive or dormant state. Dormancy represents a reversible state of low to zero metabolic activity, in which cells can persist for extended periods of time without dividing. Dormancy often corresponds to a state in which cells are neither operationally alive, in the sense of being able to form a colony when plated on a suitable medium, nor dead as they can revert to a metabolically active state when conditions become more favorable (Kaprelyants et al., 1993a). Dormancy is most obviously connected to morphologically specialized microbial structures such as spores and cysts. Vegetative, non-sporulating bacteria, however, can also exhibit dormant states (Kaprelyants et al., 1993a). In fact, the ability to switch between active and dormant states may be an essential adaptation for microorganisms exposed to periodically changing environmental conditions (Balaban et al., 2004, Metris et al., 2001) as encountered, for example, in upland and riparian soils, shallow water sediments or estuaries.

Numerical simulation models that represent microbially-mediated transformations are available for a wide variety of environmental settings (see e.g., Thullner et al.,

2007 and literature cited therein). Some models directly link the rates of microbial reactions to the abundances of the corresponding functional groups of microorganisms. With few exceptions (e.g., Wirtz, 2003), however, these models do not consider possible changes in the physiological state of the microorganisms (Borch et al., 2010, Dale et al., 2006, Regnier et al., 2005, Thullner et al., 2005 and Thullner et al., 2007). That is, cells are either alive and active or dead. For environmental systems with frequent or long lasting periods of nutritional shortage or otherwise unfavorable conditions, models that neglect the ability of the microorganisms to become dormant may not be able to capture the dynamic responses of biogeochemical activity and microbial community structure to changing external conditions.

Bär et al. (2002) previously presented a simple mathematical model to account for dormancy of bacterial populations intermittently exposed to external stresses. These authors showed that for cyanobacterial crusts in drylands exposed to severe water shortage, dormancy may offer a competitive advantage compared to generic populations that are incapable of dormancy. Here, we build on this earlier work by developing a more general model based on bioenergetic concepts. The potential implications of dormancy are then explored by incorporating the ability to switch between active and inactive states into a biogeochemical reaction model. The latter is used to simulate the population dynamics and biogeochemical activity of heterotrophic communities that experience periodic inputs of degradable organic matter.

2.2. Model description

2.2.1. Concept

In this study, we consider active and inactive cells (Kaprelyants et al., 1993a). Active cells are capable of division and can form colonies on an agar plate without a preceding resuscitation phase. Inactive or dormant cells are viable cells that need a resuscitation phase before they can resume cell division or colony formation. Non-viable (dead) cells act as a sink for biomass, but are not explicitly included in the mathematical treatment. The conceptual model describing the dynamics of the two-

state microbial system is shown in Figure 2.1. Note that only active cells contribute to biomass growth. While both active and inactive cells can decay, the intrinsic decay rate of inactive cells is assumed to be significantly slower than that of active cells. The reactivation of dormant cells depends on the potential supply of catabolic energy to the cells (Section 2.2.2.1).

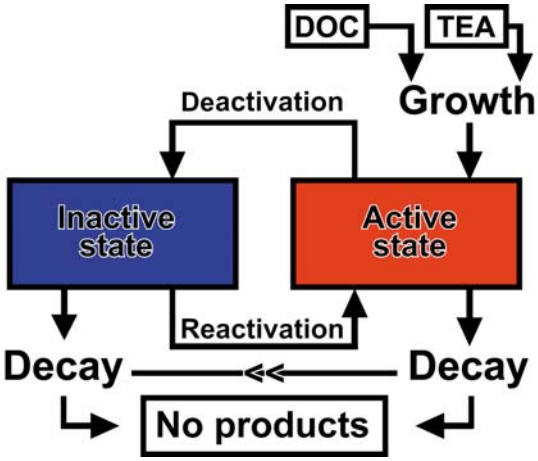

Figure 2.1. Schematic representation of the simulated microbial processes.

The two-state microbial model will be illustrated for the special case of heterotrophic bacteria that rely on organic compounds for both their carbon and energy requirements. Henceforth, the terms 'starvation' and 'starving cells' will refer to the environmental conditions where cells experience a shortage of organic substrates, rather than to a physiological state. Thus, starving cells (or cells that have suffered other stresses) do not necessarily have to be dormant. Note further that the two-state model is generic and therefore not restricted to the heterotrophic and chemoorganotrophic bacteria considered here, but can be applied to other types of microorganisms as well.

2.2.2. Mathematical description

2.2.2.1. Growth and decay

We consider active cells growing by utilizing a (dissolved) organic compound (DOC) according to the generalized reaction

$$\text{DOC} + \text{TEA} \rightarrow Y_{eff} \bullet B + (1 - Y_{eff}) \bullet \text{DIC} \tag{2.1}$$

where TEA is the terminal electron acceptor (e.g., O_2), DIC stands for (dissolved) inorganic carbon, B is the biomass of active bacteria and Y_{eff} is the effective growth yield factor. DOC, DIC and B are all expressed in units of mass carbon per unit volume. Note that it is the oxidation of the (1 - Y_{eff}) fraction of DOC to DIC that generates energy for the cells.

The rate of DOC degradation is assumed to follow standard Michaelis-Menten- or Monod-type kinetics (Regnier et al., 2005; Thullner et al., 2007 and references therein):

$$\frac{dC_{DOC}}{dt} = -\theta \cdot k_C \cdot B \cdot \frac{C_{TEA}}{K_{TEA} + C_{TEA}} \cdot \frac{C_{DOC}}{K_{DOC} + C_{DOC}} \tag{2.2}$$

$$\left(\frac{dB}{dt}\right)_{\text{growth}} = -Y_{eff} \cdot \frac{dC_{DOC}}{dt} = \theta \cdot Y_{eff} \cdot k_C \cdot B \cdot \frac{C_{TEA}}{K_{TEA} + C_{TEA}} \cdot \frac{C_{DOC}}{K_{DOC} + C_{DOC}} \tag{2.3}$$

where C stands for concentration, B is the concentration of active cells (biomass), k_C is the maximum specific rate of degradation of DOC by the active cells using terminal electron acceptor *TEA*, and K_{DOC} and K_{TEA} are half-saturation constants for DOC and TEA utilization, respectively. The possible transition of active cells into a dormant state is explicitly accounted for in equation 2.2 via the switch function θ, which determines the fraction of active cells that contributes to DOC degradation (see Section 2.2.2.3 for the mathematical formulation of θ). Under conditions favorable for the given microbial group, θ approaches 1, under non-favorable conditions, θ approaches zero. In the latter case, the majority of active bacteria are shutting down to the dormant state.

The biomass growth of active cells is linked to the substrate consumption rate through the effective growth yield factor Y_{eff} as it given in the equation 2.3. As shown by Kleerebezem and Van Loosdrecht (2010) the amount of new biomass that can be

synthesized depends on the energy yield of the catabolic pathway. We therefore assume that the effective yield factor is positively related to the energy released by the oxidation of the organic electron donor. In addition, growth is only possible when the minimum maintenance energy requirements of the cells are met. The equation 2.4 exhibits the required behavior, where Y_{max} represents the highest possible cell yield for the given microbial group, G is the maximum rate of Gibbs energy release per unit biomass, and G_0 is a corresponding minimum threshold value. Note that G and G_0 are positive. Further note that equation 2.4 only applies when $G \geq G_0$; when $G < G_0$, $Y_{eff} = 0$.

The maximum rate of energy release is computed as

$$Y_{eff} = Y_{\max}\left(1 - \frac{G_0}{G}\right) \tag{2.4}$$

$$G = \Delta G \cdot \left.\frac{dC_{DOC}}{dt}\right|_{\theta_i=1;Y_{eff}=0} \cdot \frac{1}{B} = -\Delta G \cdot k_C \cdot \frac{C_{TEA}}{K_{TEA}+C_{TEA}} \cdot \frac{C_{DOC}}{K_{DOC}+C_{DOC}} \tag{2.5}$$

where ΔG is the Gibbs energy change of the oxidation of DOC into DIC by the given TEA. The value of ΔG depends on the actual concentrations of DOC, TEA, DIC and additional products of the catabolic reaction.

Intrinsic decay of active and inactive cells is described by first order kinetics:

$$\begin{aligned}\left(\frac{dB}{dt}\right)_{\text{decay}} &= -\mu_{dec} \cdot B \\ \left(\frac{dB^{in}}{dt}\right)_{\text{decay}} &= -\mu_{dec}^{in} \cdot B^{in}\end{aligned} \tag{2.6}$$

where μ_{dec} stands for the specific cell decay rate and the superscript *in* indicates inactive (dormant) bacteria.

The above equations apply to the growth and decay of a single (functional) microbial group. However, additional microbial groups can be introduced, each with

its own set of parameter values. Similarly, the model can be expanded to include additional energy substrates, carbon sources and TEAs.

2.2.2.2. Deactivation and reactivation

The deactivation of cells, that is, the transformation of active into inactive cells, is treated as a first order rate process:

$$\left(\frac{dB}{dt}\right)_{deac} = -\left(\frac{dB^{in}}{dt}\right)_{deac} = -(1-\theta)\cdot \mu_{deac}\cdot B \qquad (2.7)$$

where μ_{deac} is the specific rate of deactivation and θ is the switch function introduced in equation 2.2. As conditions become unfavorable, the (1 - θ) term increases implying that an increasing fraction of the active cells begin to transform into inactive ones.

The reactivation of dormant cells is calculated using the following rate expression:

$$\left(\frac{dB^{in}}{dt}\right)_{reac} = -Y_{reac}\cdot\left(\frac{dB}{dt}\right)_{reac} = -Y_{reac}\cdot\theta\cdot\mu_{reac}\cdot S\cdot B^{in} \qquad (2.8)$$

where Y_{reac} is the reactivation cell yield, μ_{reac} is the specific rate of reactivation, and S is a variable representing the “depth” of dormancy (see below). Note that Y_{reac}, μ_{reac} and S all have values between 0 and 1. The Y_{reac} factor is introduced to account for the incomplete reactivation of dormant cells. Bakken and Olsen (1987), for instance, observed that only a fraction of dormant cells isolated from a soil was capable of forming colonies on agar, that is, some cells failed to reactivate even under favorable conditions.

The rate of reactivation of dormant bacteria also depends on the duration of dormancy (Kaprelyants et al, 1993b). For example, *Micrococcus luteus* cultures fully reactivates within 50 hours after 2 months of starvation, but requires 80 hours after a starvation period of 4.5 months (Kaprelyants et al, 1993a, Votyakova et al, 1994). In order to describe this behavior referred to as ‘deep dormancy’ mathematically we introduce the variable S in equation 2.8. For very long periods of unfavorable conditions, S approaches 0, while for short periods it approaches 1. The value of S thus depends on the environmental conditions experienced by dormant cells prior to their reactivation and, hence, is related to the time-dependent changes in the switch

function θ. Although a variety of formulations are possible, we use the following expression to calculate the changes in S with time:

$$\frac{dS}{dt} = S \cdot \left(k_{incr} \cdot \theta \cdot \left(1 - \theta_S\right) - k_{decr} \cdot \left(1 - \theta\right)\right) \tag{2.9}$$

$$\mu_{dec}^{in} = S \cdot \mu_{dec,0}^{in} + \mu_{dec,\min}^{in} \tag{2.10}$$

where k_{incr} and k_{decr} are first order rate parameters describing the increase and decrease of S under favorable ($\theta \approx 1$) or unfavorable ($\theta \approx 0$) conditions, respectively. The additional switch function θ_S is introduced to avoid values of S >1.

As shown by Schippers et al. (2005), significant numbers of sub-seafloor prokaryotes remain viable, even in very old (16 million years) and deep (> 400 m below the seafloor) sediments. This phenomenon is explained by a large drop in the decay rate of dormant cells exposed to very long periods of starvation. In the model, the decay rate of inactive cells is therefore dependent on the depth of dormancy as it given in equation 2.10, where $\mu_{dec,0}^{in}$ and $\mu_{dec,\min}^{in}$ are the initial and minimum specific decay rates of inactive cells.

2.2.2.3 Switch function

In the model applications considered here, the catabolic energy supply of the cells is assumed to represent the main factor controlling the switching of cells between the active and inactive states. When the energy requirements of the organisms are no longer met by the energy supply from the oxidation of the organic substrate by the TEA, cell activity slows down and active cells increasingly convert to the inactive state. In turn, when the energy supply increases, dormant cells can reactivate and start contributing to biomass growth. As shown by equation 2.5, the energy supply rate to the cells depends on the concentrations of energy substrate (DOC) and TEA, the efficiency with which the cells can utilize the substrate (k_C) and the Gibbs energy of the catabolic reaction (ΔG).

The required behavior of the switch function is captured by the following smoothed step function adapted from Fermi-Dirac statistics (see, e.g., Carter et al., 2001):

$$\theta = \frac{1}{\exp\left(\frac{-G + G_0}{st \cdot G_0}\right) + 1} \tag{2.11}$$

where G and G_0 are defined as before (Section 2.2.2.1), and st is a non-dimensional parameter controlling the steepness of the step function as shown in Figure 2.2. equation 2.11 yields θ values approaching 1 under conditions favoring biomass growth, i.e. when the energy supply is high, and 0 under unfavorable conditions (i.e. starvation). In the model applications, we arbitrarily use a value of st = 0.1, which results in a narrow but finite "switching zone" (Figure 2.2). Variations in st may be due to differences in microbial species or differences in environmental conditions such as temperature, salinity, pH, water content or the exposure to toxic chemicals or radioactive radiation.

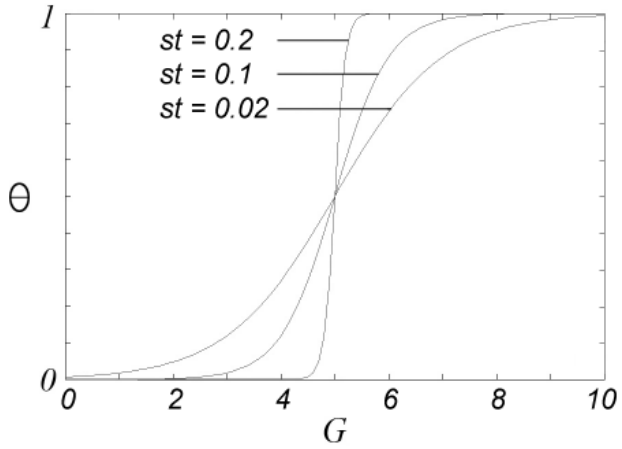

Figure 2.2. Switch function θ used to control the change of cells between active and inactive states, as a function of the Gibbs energy release per unit biomass and unit time, G, and different values of st, the parameter describing the steepness of the switch function. Note that G is given in arbitrary units and G_0=5 in all cases.

The model assumes that a single threshold energy supply G_0 controls both the deactivation and reactivation of the cells. This is likely an oversimplification, as the energy requirements of active and dormant cells may differ. However, little information exists on maintenance energy requirements of natural microbial communities in energy- or nutrient-limited environments.

2.2.3 Numerical simulations

The mathematical expressions described above are implemented into the Biogeochemical Reaction Network Simulator (BRNS) (Regnier et al., 2002; Aguilera et al., 2005), an adaptive simulation environment that can handle complex, mixed kinetic-equilibrium reaction networks (Thullner et al., 2005; Jourabchi et al., 2005; Dale et al., 2006). However since the present paper deals with biochemical transformations only the simulations are performed for perfectly mixed batch conditions and transport processes are not explicitly included.

2.2.4 Experimental References

The model is applied to two published experimental data sets (Martínez-Lavanchy, 2009 and Kaprelyants et al. 1993b), to assess the overall performance of the model and to derive first estimates for the unknown parameters controlling the de- and reactivation dynamics of microorganisms.

One data set describes the response of a culture of *Pseudomonas putida* to transient amendments of toluene and oxygen. Growth and activity of *P. putida* were monitored in a batch experiment supplied by pulses of toluene and oxygen, in order to reproduce the daily variability in oxygenation and contaminant concentration that might be encountered in the rhizosphere (Figure 2.3A; Martínez-Lavanchy, 2009).

The other data set relates results of the reactivation of *Micrococcus luteus* cells from deep dormancy. Kaprelyants et al. (1993b) report results from a batch experiment in which cells of *M. luteus* were resuscitated after a relatively long period of starvation. Cells were grown to stationary phase in a minimal medium with lactate and then starved (without washing) for 2 months, before reactivation was initiated by the amendment of lactate. Prior to reactivation, the *M. luteus* culture contained a

high abundance of small cells, characteristic of a state of deep dormancy (Kaprelyants et al., 1993a).

2.2.5 Sensitivity analysis

Sensitivity analysis is broadly used in microbiological researches (Aljundi et al., 2010, Kalinin et al., 2009 and Min et al., 2009). For a multi-parameter model as the one presented here, testing the sensitivity of model outcomes one parameter at the time is not only time-consuming, but may also overlook interactions among parameters that may affect the results. Factorial design provides an alternative approach to perform a global sensitivity analysis on the complete set of model parameters (Box et al., 1978). In factorial design, model sensitivity is determined by monitoring the response of a pre-defined model variable (for example, a reaction rate, a chemical species concentration or a biomass) to perturbations of n model parameters each defined by an upper and lower value. For each of parameter the model response to perturbations is further assumed to be approximately linear within the range defined by the corresponding upper and lower values (Dale et al., 2006). Thus, any effects that deviate significantly from a linear trend can be attributed to parameters or combinations of parameters that significantly impact the model results. These parameters are therefore the most critical model parameters to be constrained. A detailed account of the theory of factorial design can be found in Box et al. (1978).

The factorial design analysis performed here aims to identify the kinetic and thermodynamic parameters that most sensitively affect the dynamics of cell switching between active and inactive states. The sensitivity analysis is applied to a model system consisting of a single microbial species that experiences an intermittent supply of the limiting substrate. All model features introduced above are considered. The six parameters tested include the rate parameters for de- and reactivation of cells (μ_{deac} and μ_{reac} respectively), the reactivation yield factor (Y_{reac}), the energy rate threshold value G_0, and the rate parameters controlling the evolution of the dormancy variable S (k_{incr} and k_{decr}, respectively).

With six parameters, a total of 2^6=64 simulation runs are necessary to perform the analysis for a given scenario. Analyses are performed for intermittent substrate supply scenarios with injections frequencies of 10^{-3}, 10^{-2}, 10^{-1}, and 1 h^{-1}. Each single feeding event corresponds to an instantaneous substrate addition of 0.1mM. The simulations are performed until an average steady state is reached, that is, when the variations in the microbial biomasses exhibit a constant, repetitive temporal pattern. The resulting long-term average concentration of the active biomass of the microbial population is then used as indicator of model sensitivity.

2.2.6 Hypothetical scenarios

Intuitively, one would expect dormancy to offer an advantage to microorganisms to overcome periods of starvation (or other stressful conditions). To determine whether or not this advantage can compensate for competitive disadvantages in growth rates, a hypothetical microbial system consisting of two competing species is considered. Both species are capable of performing the same set of processes (growth, decay, deactivation and reactivation), following the kinetic descriptions presented above. The first species referred to as “growers” has a fast and efficient metabolism, characterized by relatively high values for the substrate utilization rate, growth yield and decay rate. The second species referred to as “switchers” is able to rapidly switch between active and dormant states.

The parameter values assigned to the two species can be found in Table 2.4. The selected parameter values fall within the ranges used in the sensitivity analysis (Table 2.2). Both species are assumed to compete for the same carbon substrate, the concentration of which is considered to be the only growth-limiting factor. The cells are intermittently fed with carbon substrate pulses of 0.1mM each, at three different feeding frequencies (1/15 h^{-1}, 1/3 h^{-1} and 1 h^{-1}). Transient simulations are performed until stable repetitive concentration patterns with constant averages are obtained. The results shown correspond to these “steady state” conditions.

2.3. Results

2.3.1. *Pseudomonas putida*

The measured data include the concentrations of toluene and oxygen in the medium, the total biomass of *P. putida* as well as their level of activity (Figures 2.3B and 2.3C). As a measure of activity, the relative expression of the gene for toluene-monooxygenase, xylM, measured by real time quantitative PCR, RT-qPCR, is used (see Martínez-Lavanchy et al., 2010, for details on the experimental procedures).

Toluene degradation mainly occurs during the initial and final phases of the experiment (0-12 hours and >24 hours, Figure 2.3C). The degradation of toluene is accompanied by net biomass growth (Figure 2.3B). From 12 to 24 hours, when anaerobic conditions prevail, much more limited bacterial activity and biomass growth are observed. The features of the data can be reproduced by the model as shown by the curves in Figures 2.3B, 2.3C and 2.3D. The parameter values corresponding to the model curves are listed in Table 2.1. Because of the relatively short duration of the experiment, cell decay is neglected. In addition, the effective growth yield factor (Y_{eff}) is kept constant and assigned the experimentally estimated value.

Fitting of the experimental results implies that deactivation and reactivation of the bacteria occurs on time scales of a few hours. The model results predict a near-complete conversion of active to inactive cells during the anaerobic phase (Figure 2.3B). When oxygen is reintroduced after 24 hours in the toluene-rich medium, the rapid recovery of active cells is mainly due to the instantaneous rise of the switch function θ from near 0 to a value of 1 (Figure 2.3D). This contrasts with the much slower drop in θ, when toluene is introduced in the oxygen-poor medium at 12 hours.

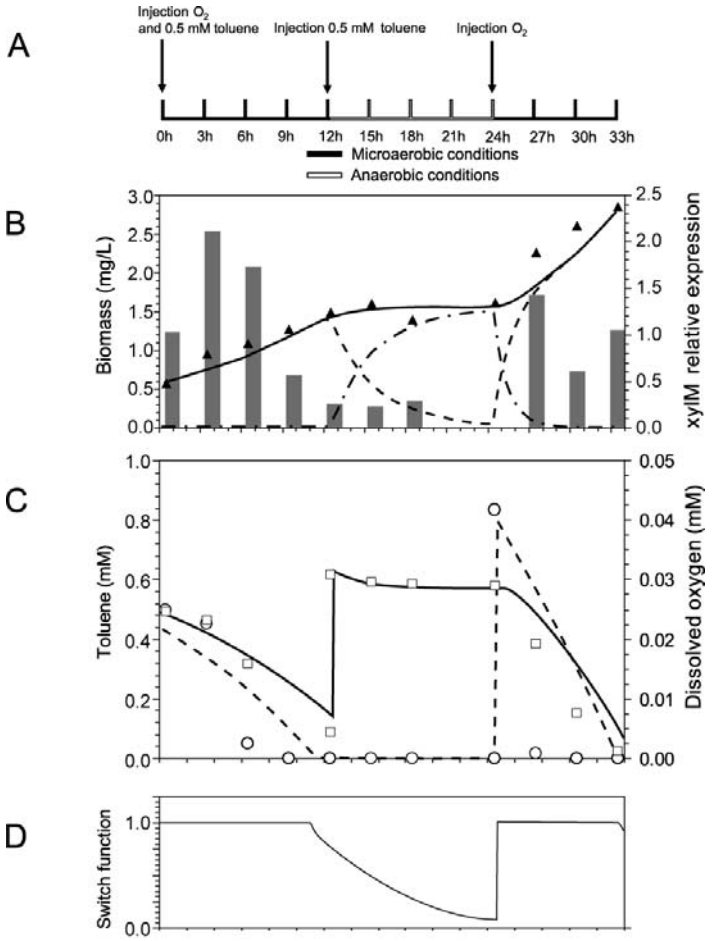

Figure 2.3. Growth and activity of Pseudomonas putida under conditions of fluctuating toluene and oxygen concentrations. The experimental results are from Martinez-Lavanchy (2009).A: Experimental procedure. B: measured (closed triangles) and simulated (solid line) total biomass, dashed line – simulated active fraction, dash-dot line – simulated dormant fraction, relative expression of xylM gene (bars), corresponding to active bacteria. C: measured (open boxes) and simulated (solid line) concentratrions of toluene; measured (open circles) and simulated (dashed line) concentrations of oxygen. D: simulated value of the switch function θ (used to control the switch of cells between active and inactive states).

Parameters	Value	Source
Maximum reaction rate of degradation of toluene by Pseudomonas putida, k_C	*0.146 h^{-1}*	*measured*
Half-saturation constant for toluene, K_{DOC}	*3 µM*	*fitted*
Half-saturation constant for oxygen, K_{TEA}	*1 µM*	*fitted*
Growth yield factor (constant), Y_{eff}	*0.5*	*measured*
Thermodynamic threshold, G_0	*0.34 $kJ \cdot mol_{biomass}^{-1} \cdot h^{-1}$*	*fitted*
Reactivation rate parameter, μ_{reac}	*1 h^{-1}*	*fitted*
Deactivation rate parameter, μ_{deac}	*3 h^{-1}*	*fitted*

Table 2.1. Parameter values used for simulation of the batch experiment with Pseudomonas putida. Values for the maximum growth rate and the growth yield factor were obtained from additional growth experiments with P. putida (Martinez-Lavanchy, 2009, data not shown).

2.3.1.2. *Micrococcus luteus*

After the two-month starvation period of *M. luteus*, it took more than 30 hours for all the cells to reactivate (Figure 2.4). The recovery was much slower than observed for shorter starvation periods when bacteria had not yet reached a deep dormant state and the percentage of small cells was negligible (Kaprelyants et al., 1993a, data not shown).

The slow recovery after starvation is well reproduced by the model (Figure 2.4). Because no significant changes in total cell counts were observed, the simulation assumes that during the recovery period growth is negligible. We further assume that (1) after the addition of lactate the available energy is much higher than the threshold value and, therefore, $\theta = 1$, and (2) the variable representing the "depth" of dormancy, S, reaches its maximum value ($S = 1$) 43 hours after the onset of reactivation when the percentage of small cells drops below 50% (Kaprelyants et al.,

1993b, data not shown). The initial value of S is set to an arbitrarily low initial value of 10^{-9}.

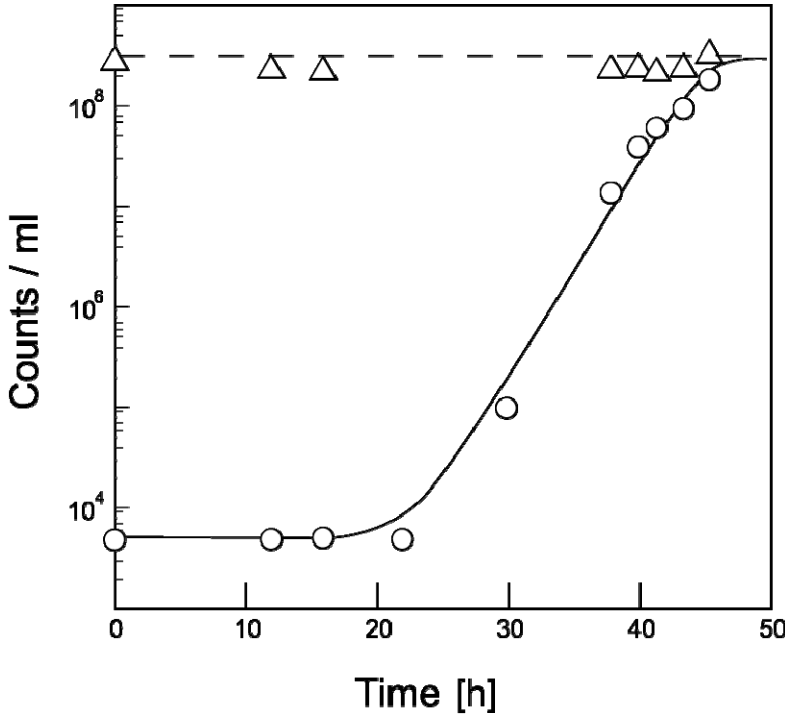

Figure 2.4. Resuscitation of Micrococcus luteus after 2 months of starvation. Measured total (triangles) and dormant (circles) cell densities; simulated total (broken line) and dormant (solid line) cell densities.

With these assumptions, fitting of the time evolution of the concentration of active cells yields a value for the reactivation rate parameter μ_{reac} of 0.4 h^{-1}. This value is of the same order of magnitude as the μ_{reac} value obtained from fitting the data for toluene degradation by *P. putida*, despite the vast differences in experimental design and conditions. The model fit of the *M. luteus* recovery further provides a rough estimate for the rate parameter, k_{incr}, which controls the increase in the dormancy variable S ($k_{incr} \approx 0.5$ h^{-1}). As the model fit in Figure 2.4 is insensitive to k_{decr}, we assume in what follows that this parameter is of the same order of magnitude as k_{incr}.

2.3.2. Parameter sensitivity

Upper [+] and lower [-] bounds for the six tested parameters are selected based on the parameter values obtained from fitting the experimental data of the *P. putida* and *M. luteus* cultures, plus the following constraints. All scenarios should yield some de- and reactivation, as well as long-term survival and non-zero activity of the microbial population. In addition, the long-term abundance of microorganisms should be controlled by the model parameters and not by the imposed initial concentration of microorganisms or any other external constraint.

Parameters	[-] Value	[+] Value
Biomass loss during reactivation, Y_{reac}	*0.1*	*1*
Thermodynamic threshold, G_0	*0.1 $kJ \cdot mol_{biomass}^{-1} \cdot h^{-1}$*	*25 $kJ \cdot mol_{biomass}^{-1} \cdot h^{-1}$*
Reactivation rate parameter, μ_{reac}	*0.1 h^{-1}*	*2 h^{-1}*
Deactivation rate parameter, μ_{deac}	*0.1 h^{-1}*	*2 h^{-1}*
Dormancy rate parameter, k_{incr}	*0.05 h^{-1}*	*0.5 h^{-1}*
Dormancy rate parameter, k_{decr}	*0.05 h^{-1}*	*0.5 h^{-1}*

Table 2.2. Upper [+] and lower [-] limits of the parameters tested in the sensitivity analysis.

The resulting set of upper and lower values of the parameters is given in Table 2.2. All other parameters used are those listed in Table 2.1. Table 2.3 presents the 64 combinations of [+] and [-] values considered in the sensitivity analyses. The results of the sensitivity analyses are illustrated in Figure 2.5 for the four frequencies at which the limiting substrate is supplied.

Sim. #	Y_{reac}	G_0	μ_{react}	μ_{deac}	k_{decr}	k_{incr}	Sim. #	Y_{reac}	G_0	μ_{reac}	μ_{deac}	k_{decr}	k_{incr}
1	--	--	--	--	--	--	33	--	--	--	--	--	+
2	+	--	--	--	--	--	34	+	--	--	--	--	+
3	--	+	--	--	--	--	35	--	+	--	--	--	+
4	+	+	--	--	--	--	36	+	+	--	--	--	+
5	--	--	+	--	--	--	37	--	--	+	--	--	+
6	+	--	+	--	--	--	38	+	--	+	--	--	+
7	--	+	+	--	--	--	39	--	+	+	--	--	+
8	+	+	+	--	--	--	40	+	+	+	--	--	+
9	--	--	--	+	--	--	41	--	--	--	+	--	+
10	+	--	--	+	--	--	42	+	--	--	+	--	+
11	--	+	--	+	--	--	43	--	+	--	+	--	+
12	+	+	--	+	--	--	44	+	+	--	+	--	+
13	--	--	+	+	--	--	45	--	--	+	+	--	+
14	+	--	+	+	--	--	46	+	--	+	+	--	+
15	--	+	+	+	--	--	47	--	+	+	+	--	+
16	+	+	+	+	--	--	48	+	+	+	+	--	+
17	--	--	--	--	+	--	49	--	--	--	--	+	+
18	+	--	--	--	+	--	50	+	--	--	--	+	+
19	--	+	--	--	+	--	51	--	+	--	--	+	+
20	+	+	--	--	+	--	52	+	+	--	--	+	+
21	--	--	+	--	+	--	53	--	--	+	--	+	+
22	+	--	+	--	+	--	54	+	--	+	--	+	+
23	--	+	+	--	+	--	55	--	+	+	--	+	+
24	+	+	+	--	+	--	56	+	+	+	--	+	+
25	--	--	--	+	+	--	57	--	--	--	+	+	+
26	+	--	--	+	+	--	58	+	--	--	+	+	+
27	--	+	--	+	+	--	59	--	+	--	+	+	+
28	+	+	--	+	+	--	60	+	+	--	+	+	+
29	--	--	+	+	+	--	61	--	--	+	+	+	+
30	+	--	+	+	+	--	62	+	--	+	+	+	+
31	--	+	+	+	+	--	63	--	+	+	+	+	+
32	+	+	+	+	+	--	64	+	+	+	+	+	+

Table 2.3. Combinations of upper [+] and lower [-] values of parameters used in the different simulations of the sensitivity analysis.

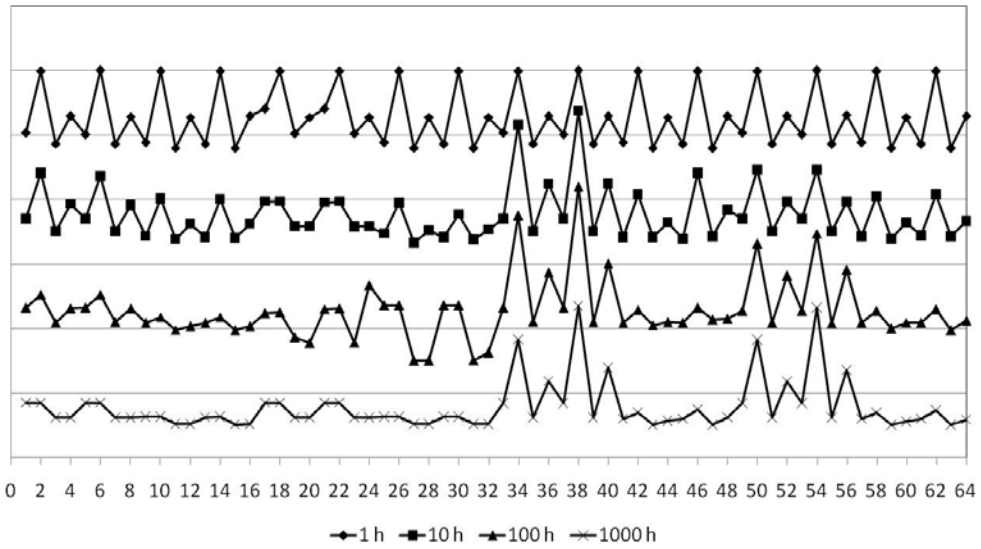

Figure 2.5. Normalized model response (amount of active bacteria at "steady state", averaged over a period of time much longer than a single carbon substrate supply cycle) to the four substrate supply scenarios, for the different combinations of parameter values used in the global sensitivity analysis. The numbers on the horizontal axis correspond to the parameter combinations listed in Table 2.3.

For all four frequencies, the selected model outcome (the biomass of active bacteria) is most sensitive to Y_{reac} and G_0, with a combination of high Y_{reac} and low G_0 resulting in the highest amounts of active biomass. For the highest frequency of supply (1 h^{-1}) the values of Y_{reac} and G_0 dominate the model outcome and a relatively simple response pattern of the sensitivity analysis is obtained (Figure 2.5, topmost curve). For lower frequencies of supply, the patterns become more irregular suggesting an increased sensitivity towards additional parameters. Nonetheless, for the three lower frequencies, the highest active biomasses are found for the same combinations of parameters (e.g., runs # 2, 6, 34, 38, 46, 50, 54), implying

no profound change in parameter sensitivity. The parameter combinations resulting in low biomass concentrations (e.g., runs # 11, 15, 31, 43, 45, 57, 59), however, are all characterized by a high value for μ_{deac}, indicating the relevance of this parameter at lower frequencies of substrate supply. Non-linear interactions among the parameters tested are not observed.

2.3.3. Growers versus switchers

The parameter values assigned to the two species can be found in Table 2.4.

Parameters	"growers"	"switchers"
Maximum reaction rate of degradation of DOC by microbial group, k_C	*0.2 h^{-1}*	*0.05 h^{-1}*
Half-saturation constant for carbon substrate, K_{DOC}	*1 µM*	*3 µM*
Growth yield factor (max), Y_{max}	*0.8*	*0.5*
Decay rate of active bacteria, μ_{dec}	*0.01 h^{-1}*	*0.01 h^{-1}*
Initial decay rate of inactive bacteria, $\mu_{dec,0}^{in}$	*1e-4 h^{-1}*	*1e-4 h^{-1}*
Thermodynamic threshold, G_0	*25 $kJ \cdot mol_{biomass}^{-1} \cdot h^{-1}$*	*12 $kJ \cdot mol_{biomass}^{-1} \cdot h^{-1}$*
Reactivation rate parameter, μ_{reac}	*2 h^{-1}*	*2 h^{-1}*
Deactivation rate parameter, μ_{deac}	*0.1 h^{-1}*	*2.0 h^{-1}*
Dormancy rate parameter, k_{incr}	*0.05 h^{-1}*	*0.5 h^{-1}*
Dormancy rate parameter, k_{decr}	*0.5 h^{-1}*	*0.05 h^{-1}*

Table 2.4. Parameter values used to represent the two competing microbial species in the simulations of batch systems with periodic substrate supply.

The highest substrate supply frequency ($1h^{-1}$) results in a stable total biomass of the growers (Figure 2.6). The total biomass is approximately equally divided between active and inactive cells. While the abundance of active cells exhibits regular fluctuations, they are of limited amplitude (Figure 2.6B). In effect, the rapid succession of substrate additions prevents large readjustments in population size. The biomass of switchers is negligible compared to that of the growers (not shown). The results thus imply that the cells exhibiting the fastest growth are those dominating the community.

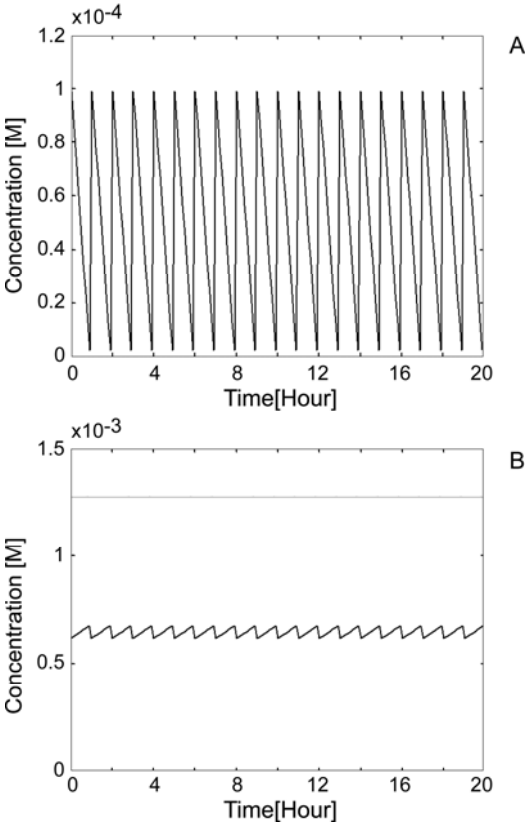

Figure 2.6. Simulation results for a bacterial community with two competing species and a substrate supply frequency of 1 h-1. A: concentration of the dissolved organic carbon substrate, B: biomass concentrations of active (black) and total (grey) growers.

For a feeding frequency of 1/3 h^{-1} very different temporal patterns of the concentrations of the carbon substrate and the two microbial species are obtained (Figure 2.7). For the substrate concentration, each single feeding cycle can be recognized, but the overall pattern is quite irregular (Figure 2.7A). Irregular patterns are also observed for the microbial species.

While the total biomass of the growers remains relatively constant, the active fraction of growers exhibits large and irregular variations (Figure 2.7B). Similarly to the 1 h^{-1} scenario, active cells represent approximately half of the biomass of the growers. The biomass of switchers is similar to values found for the growers, which indicates that both species coexist at this supply frequency. As for the growers, large and irregular variations are seen in the active fraction of switchers (Figure 2.7C). The active fraction contributes anywhere from nearly 100% down to 20% of the biomass of switchers.

Results of a Fourier analysis (Körner 1988) of the temporal pattern of the carbon substrate concentration indicates that the variations are dominated by the frequency of the supply events, with some minor lower frequency contributions in the range 0.05 – 0.1 h^{-1} (results not shown). Fourier analysis of the active biomass fraction also shows that the observed temporal pattern is dominated by the feeding frequency, although the frequency spectrum shows significant contributions from frequencies below 0.25 h^{-1} (Figure 2.8). The latter contributions are more pronounced for the growers, hence explaining the more irregular pattern of active growers, compared to active switchers.

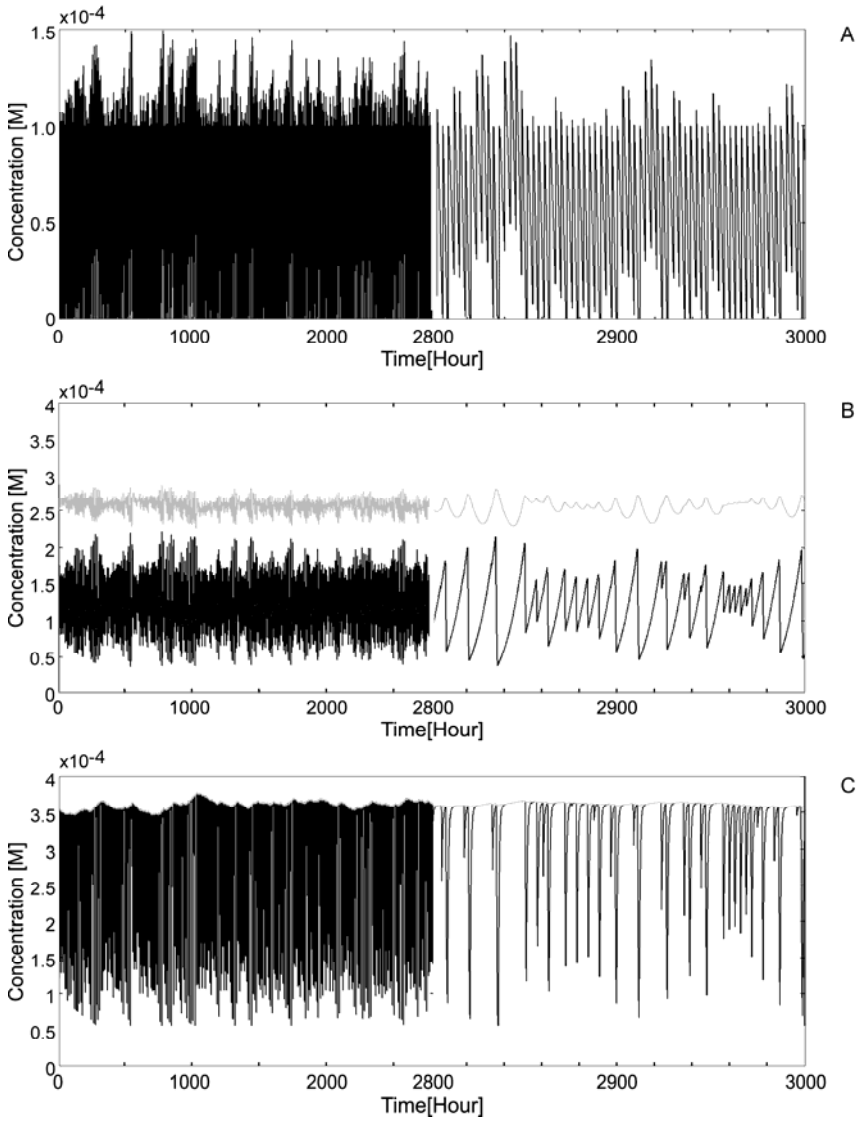

Figure 2.7. Simulation results for a bacterial community with two competing species and a substrate supply frequency of 1/3 h-1. A: concentration of the dissolved organic carbon substrate, B: biomass concentrations of active (black) and total (grey) growers, C: biomass concentrations of active (black) and total (grey) switchers. Note that the horizontal axis is not linear.

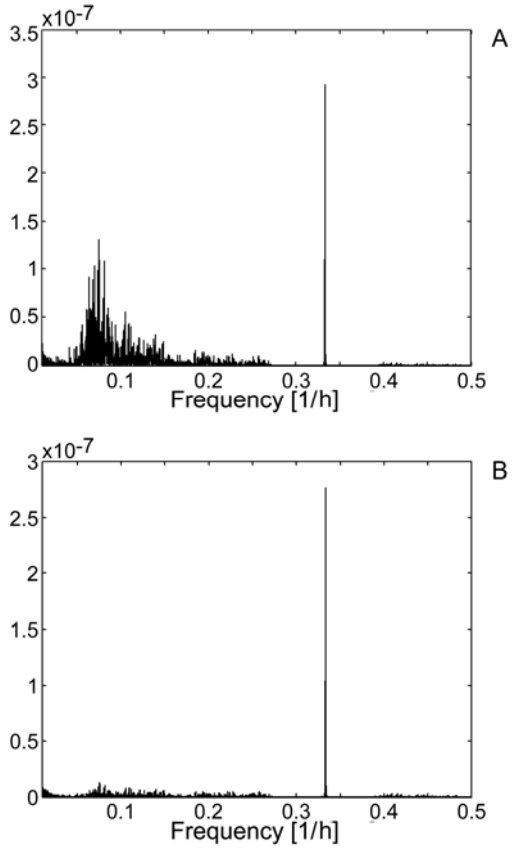

Figure 2.8. Frequency spectra for the concentrations of active biomasses for a substrate supply frequency of 1/3 h-1. A: growers, B: switchers.

For the lowest frequency considered (1/15 h^{-1}) substrate and biomass concentrations exhibit quite regular patterns (Figure 2.9). After each supply event, the substrate concentration drops to zero, yielding a regular saw-tooth pattern that follows the substrate supply frequency (Figure 2.9A).

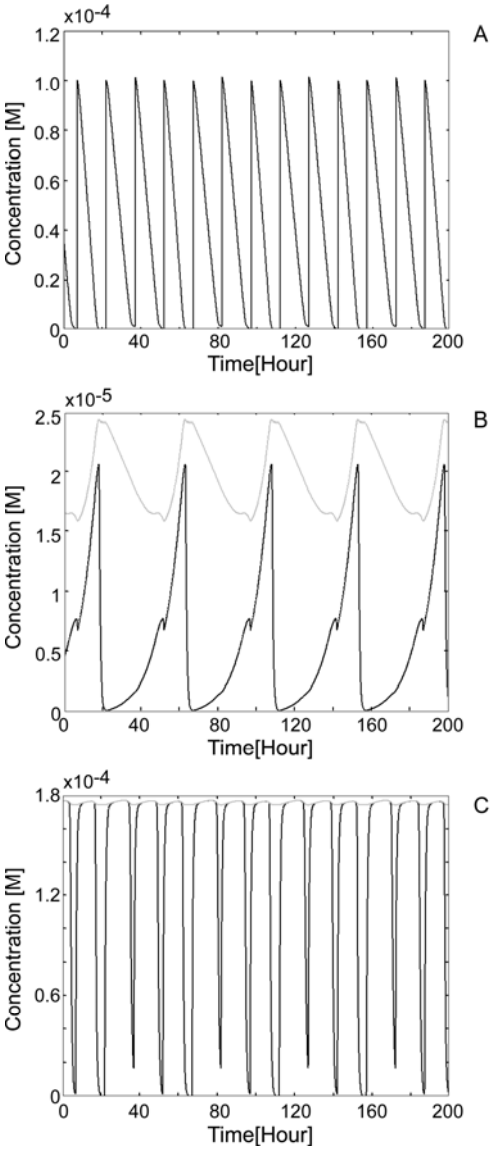

Figure 2.9. Simulation results for a bacterial community with two competing species and a substrate supply frequency of 1/15 h^{-1}. A: concentration of the dissolved organic carbon substrate, B: biomass concentrations of active (black) and total (grey) growers, C: biomass concentrations of active (black) and total (grey) switchers.

In contrast, the variations in total and active biomass of the growers exhibit patterns that are repeated every three feeding cycles. The dominant frequency for the active biomass of switchers appears again to be the feeding frequency. A Fourier analysis confirms that the temporal changes in the active biomass of the growers is dominated by a frequency three times smaller than the feeding frequency (0.022 rather than 0.066 h^{-1}) (Figure 2.10A). (Note that for any real frequency *v* the Fourier analysis returns multiples n·*v* (Körner 1988), and thus such multiples are not further considered here.) In contrast, for the active switcher biomass the dominant frequency is the substrate supply frequency (1/15 h^{-1}), the lower frequency (0.022 h^{-1}) being of lesser importance. For the carbon substrate concentration the Fourier analysis only returns the supply frequency (not shown).

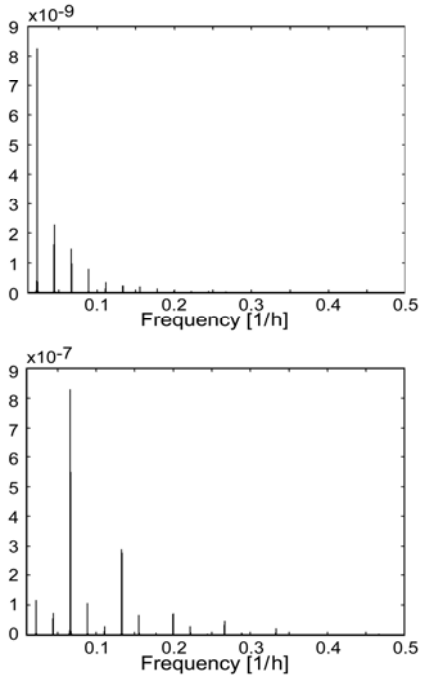

Figure 2.10. Frequency spectra for the concentrations of active biomasses for a substrate supply frequency of 1/15 h^{-1}. A: growers, B: switchers.

As for the other feeding frequencies, variations of the total biomasses of growers and switchers at the lowest frequency are smaller than for the corresponding active biomasses. The total biomass of switchers is approximately one order of magnitude larger than for growers.

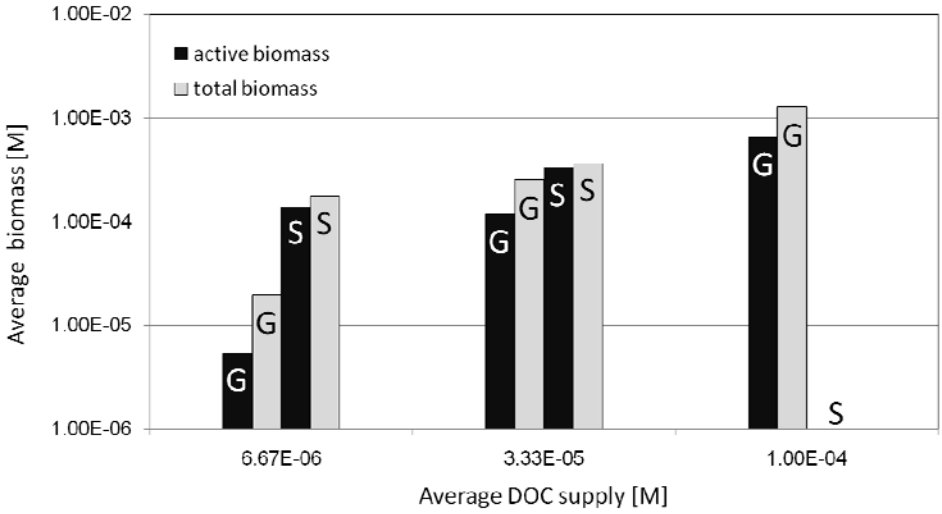

Figure 2.11. Average steady state active and total biomasses of growers and switchers versus the average organic carbon substrate supply. Note the logarithmic scale for the biomasses.

Thus, although both species still coexist, conditions are more favorable for switchers than for growers. The average total and active biomasses of growers and switchers for the three feeding scenarios are compared in Figure 2.11. The figure clearly shows the emergence of very different community structures under the variable substrate supply regimes.

2.4. Discussion

The ability of microorganisms to survive unfavorable environmental conditions is broadly recognized (Bakken and Olsen, 1987; Harder and Dijkhuizen, 1983; Kaprelyants et al., 1993a; Chmielewsky and Frank, 1995). Viable microorganisms recovered from deep, energy-starved subsurface settings (the deep biosphere) demonstrate that microbial cells can stay dormant for geological periods of time (Schippers et al., 2005). Also in near-surface environments microorganisms are often found under conditions that preclude their metabolic activity. For example, aerobic bacteria are known to survive in highly anoxic sediments (Koretsky et al., 2005). The ability of functionally diverse microbial communities to respond to changing conditions by switching metabolic pathways on and off is implicit in many biogeochemical models of surface and subsurface environments (Van Cappellen and Gaillard, 1996; Thullner et al., 2005). However, few microbial reaction models explicitly account for the switching of microorganisms between active and inactive states.

The proposed model treats active and inactive (dormant) cells as physiologically distinct states. It assumes that only active cells contribute to biomass growth, and it accounts for the progressive slowing down of the reactivation of dormant cells with increasing duration of inactivity (Kaprelyants et al., 1993b). The model reproduces the features of two experimental data sets on bacterial de- and reactivation reasonably well. Estimates of the newly introduced parameter values can be used as initial guesses in future applications of the model. Based on the results of the global sensitivity analysis, a reduction in parameter uncertainty would benefit most from experimental determinations of the energy threshold controlling the switch between active and inactive states, the rate of deactivation, and the efficiency with which inactive cells can be reactivated.

The proposed model relies on simple, empirical representations of the switch between physiological states. Deactivation and reactivation are complex processes, however, including many biochemical and physiological changes as reflected, for instance, in significantly different sizes of active and dormant cells, the formation of cysts, or the production of specific growth factors that induce the resuscitation of dormant cells (Mukamolova et al., 1999; Shleeva et al. 2004). There is thus ample room for further theoretical developments as our understanding of the mechanisms and drivers of dormancy advances. In particular, there is a need to provide a more fundamental basis to predict the various adjustable parameters currently included in the model.

The simulation results show that the ability to switch to and from the dormant state substantially widens the spectrum of possible community structures and population dynamics (Figures 2.5-2.11). Even in simple microbial systems, the interactions between growth, decay and the inter-conversion between active and inactive states may cause the emergence of complex behavior that is no longer related in a simple way to the changes in external forcing (e.g., Figure 2.7). This obviously complicates the relationship between geochemical conditions and microbial community composition, as observed, for example, in salt marsh sediments (Koretsky et al., 2005).

Dormancy contributes to the resilience of microbial communities (Bär et al., 2002). In all the simulations, the microbial community comprises a significant fraction of dormant cells. The transformation of active cells into inactive ones limits the loss of biomass and metabolic functions when environmental conditions become unfavorable (Jones et al., 2010). The reservoir of dormant cells in turn allows the microbial system to respond to an improvement in environmental conditions without the need of *ab initio* biomass production. A related outcome is that microorganisms that are efficient at switching to and from the dormant state may outcompete

microorganisms with better growth performance in environments characterized by recurrent and prolonged periods of starvation or stress (Lennon et al., 2011). As many laboratory enrichment experiments preferentially select for organisms with the best growth performance, the extrapolation of such laboratory results to environmental systems is questionable (Kaprelyants et al., 1993a).

Many near-surface microbial communities experience environmental changes at a variety of different frequencies (e.g., from tidal, via seasonal, to interannual changes). According to the results presented here, these conditions may promote the coexistence of species with different growth and dormancy strategies. The coexistence of competing species is commonly explained by a spatial differentiation of the species (Crane and Grover 2010), but the results presented here suggest this does not have to be the case in environmental systems affected by intermittent substrate limitation or stress conditions.

In summary, the proposed model shows that it is possible to quantitatively account for changes in the physiological state of microorganisms under changing environmental conditions. The simulations illustrate the role of dormancy in the functioning of microbial systems ranging from laboratory monocultures to multi-species communities. Because of the current paucity of observational data, we limit ourselves to simple hypothetical microbial systems. Nevertheless, even for these systems, the results emphasize the potentially key role of cell deactivation and reactivation in linking diversity, resilience and function of natural microbial populations. The mathematical implementation of dormancy presented here can easily be combined with existing reactive transport models in order to simulate microbially mediated biogeochemical processes in the environment.

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3

The impact of pore size heterogeneities on the spatio-temporal variation of microbial metabolic activity in porous media

with Mehdi Gharasoo and Martin Thullner

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Abstract

Natural porous media such as soils or aquifers are characterized by various pore scale heterogeneities. This includes the temporal variability of substrate supply, and the spatial heterogeneity of pore sizes. As a consequence, microbial growth conditions in natural porous media environments may differ from typical laboratory setups used to study microbial behavior. Pore size heterogeneities and the resulting transport regime can lead to highly complex distribution patterns of substrates and corresponding microbial growth conditions including the frequent occurrence of stress periods for the microbial population. Microorganisms can respond to such stress periods by switching from an active into an inactive or dormant state, and the corresponding microbial abundance and dynamics may exhibit rather complex temporal and spatial patterns. This study applies an extended modeling concept for the growth and degradation activity of microbial species able to switch between two different physiological states. The concept is implemented into a pore network model which allows simulating the changes in microbial growth conditions in heterogeneous porous media. The model is used to study the impact of pore size heterogeneities on the distribution and activity of a bacterial community consisting of two competing

species. Simulation results show that such pore scale heterogeneity has a strong impact on bacterial abundance and activity creating a complex structure of microbial niches. This leads to an increased coexistence of microbial species at both the micro and the macro scale while at the macro scale the total biomass concentration is less affected by these heterogeneities.

3.1. Introduction

Microbial activity plays a crucial and often dominant role in the cycling of carbon, nutrient elements and contaminants in the environment (Borch et al., 2010). Many of these reactive processes are taking place in porous environments like soils or aquifers. These environments are characterized by spatial heterogeneities at various scales down to the pore scale (King et al., 2010, Caruso and Rillig, 2011). Such heterogeneities can have an important influence on overall microbial activity. For instance, the success of *in-situ* bioremediation (i.e. the achievement of sufficiently high biodegradation rates of the contaminants) requires among several other factors (i.e. metabolic capacity of the microorganisms, presence of suitable reaction partners etc.) a sufficient access of the microbial cells to the contaminant (e.g., Semple et al., 2003). A limitation of this access or more generally the bioavailability of a chemical compound in a porous medium can be caused by the physico-chemical properties of the compound (Bonneville et al., 2004; Haws et al., 2006) or by mass transfer processes taking places at various scales within the porous medium (Bosma et al., 1997; Hesse et al., 2009, 2010). In turn, the interaction between heterogeneities and mass transfer processes can lead to the formation of microhabitats providing within close vicinity distinct living conditions for microorganisms and controlling the competitiveness of microbial species. The heterogeneity of porous media was experimentally demonstrated e.g., by gas adsorption (Groen et al., 2003), MRI and X-ray tomography (Wildenschild et al., 2002) or water retention curves (Mualem, 1986; Van Genuchten, 1980). Experimental analyses of microbial distributions at the pore scale are more limited but the available evidence points toward a rather heterogeneous distribution of microorganisms at the pore scale (Dechesne et al., 2008, Caruso and Rillig, 2011). However, a link between microbial abundance and the living conditions in such microhabitats has not been established, yet.

Despite of its high capacity for microbial ecosystem services, conditions in soils or aquifers can not generally be recognized as plentiful for the resident microorganisms, and subsurface bacteria must cope with a variety of stresses, such

as periodic lacks of substrate, nutrients and/or water (Martínez-Lavanchy et al., 2010). Therefore, they must be able to continuously adapt their metabolism to the conditions in their immediate surroundings, and in order to survive unfavorable environmental conditions microorganisms can switch to an inactive or dormant state (Lennon and Jones, 2011). Dormancy represents a reversible state of low to zero metabolic activity, in which cells can persist for extended periods of time without dividing. It has been shown that for a given set of environmental conditions microbial dormancy can lead to a rather complex response of microbial abundance and activity to changes of the environment (Jones and Lennon, 2010, Stolpovsky et al., 2011).

The aim of the present study is to explore the interaction between pore scale heterogeneities leading to different microhabitats within a porous medium and the dynamics of microbial communities exhibiting dormancy behavior. For this purpose concepts describing the ability of microorganisms to switch into an inactive state (Stolpovsky et al., 2011) are implemented into an advanced reactive transport model simulating microbial processes within heterogeneous pore networks (Gharasoo et al., 2012). The resulting modeling approach is used to study how the ability of the microorganisms to fall into a dormant state and the heterogeneity of the pore scale affect the spatio-temporal distribution of microbial metabolic activity in porous media.

3.2. Model description

3.2.1 Reactive transport pore network model

The applied reactive transport pore network model PNBRNS (Pore network biogeochemical reaction network simulator; Gharasoo et al., 2012) describes a porous medium as a two-dimensional network of interconnected pores. All pores are assumed as cylindrical micro-tubes of identical length, but the radius of each pore is assigned individually thus allowing for heterogeneous pore networks. Each pore is treated as a finite volume, while the nodes connecting the pores are considered to be volumeless. The pores are arranged to a regular hexagonal honeycomb structure with coordination number 3 (Fig. 3.1).

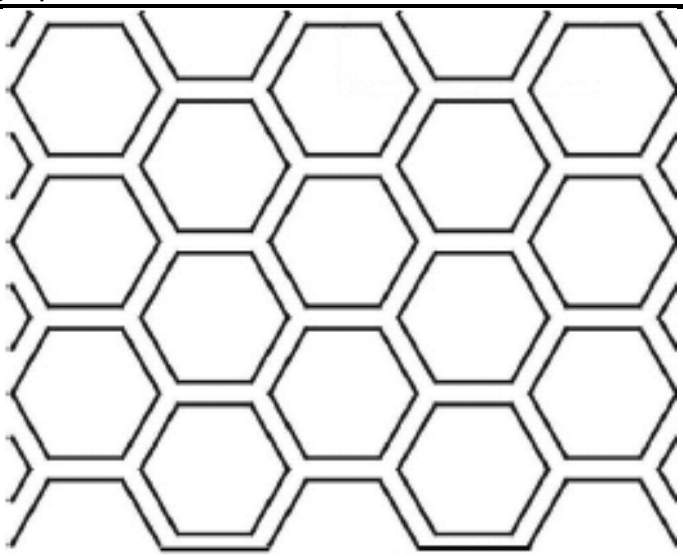

Figure 3.1. Two dimensional hexagonal lattice used to construct the pore network.

Water flow is assumed at steady state considering an isothermal system where an incompressible and laminar water flow passes through the pores. Laminar flow through each individual pore is described using the Hagen-Poiseuille equation in which the hydraulic properties of each pore are derived from its size and geometry. The determination of hydraulic pressures at each node allows calculating the water flow velocity in every pore. The solution of the flow problem is obtained assuming fixed pressure conditions on inlet and outlet boundaries providing a steady flow of water through the medium, while the other boundaries on the sides are treated as zero-flux.

The reaction-advection-diffusion equation describes the transient reactive transport of solutes in a system. A finite volume method (FVM) is implemented to solve the conservative transport of dissolved species along the pore network. Once the magnitude and direction of the flow velocities in every pore is determined, the advective transport of dissolved species within the medium can be simulated. A Courant-Friedrichs-Lewy criterion is applied to ensure the numerical convergence for a given time step. Fick's second law is implemented to simulate the diffusive transport of the salute through the pores. To compute the solute concentration

gradient along a pore, the nodal concentrations are calculated as a weighted average of all neighboring pores. This leads to two independent diffusive fluxes toward or away from the center of a pore (depending on the direction of flux vectors). The scalar summation of these two diffusive vectors determines the total change of concentration in a pore due to the diffusion.

Reactive processes taking place in each individual pore are described by coupling the flow and transport model to the Biogeochemical Reaction Network Simulator (BRNS) (Regnier et al., 2002; Aguilera et al., 2005; Centler et al., 2010), an adaptive simulation environment that can handle complex, mixed kinetic-equilibrium reaction networks (Thullner et al., 2005; Jourabchi et al., 2005; Dale et al., 2006). To solve the reactive part, an operator splitting technique is used to couple the transport module to biogeochemical reaction solver BRNS following a sequential non- iterative procedure. In each time step, the transport calculations for the pore network are performed in MATLAB. Then the premade 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. More details on the structure of the network and its transport conditions are provided in Gharasoo et al. (2012).

3.2.2 Simulated processes

In this study, we consider microbial cells to be represented by two different physiological states: active and inactive (Fig. 3.2). Active cells are able to grow/divide, to consume available substrate and are subject to decay/cell death. Inactive or dormant cells do not exhibit any growth or degradation activity while their decay/death rate is highly reduced compared to their active state.

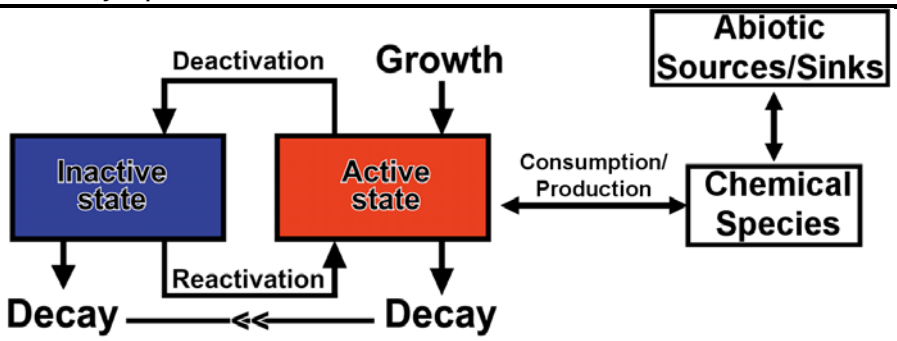

Figure 3.2. Schematic representation of the simulated microbial processes.

Depending on the environmental conditions cells are assumed to switch between these two states using the potential supply of catabolic energy to the cells as control variable.

In each pore the rate of degradation of dissolved organic carbon (DOC) is assumed to follow standard Michaelis-Menten- or Monod-type kinetics (Regnier et al., 2005; Thullner et al., 2007 and references therein):

$$\frac{dC_{DOC}}{dt} = -\theta \cdot k_C \cdot B \cdot \frac{C_{DOC}}{K_{DOC} + C_{DOC}} \cdot \left(1 - \frac{B_{tot}}{B_{\max}}\right) \qquad (3.1)$$

where C_{DOC} (M) is the concentration of DOC, B (M) is the concentration of active cells (biomass organic carbon), k_C, (h^{-1}) is the maximum specific rate of degradation of DOC by the active cells, K_{DOC}, (M) is the half-saturation constant for DOC utilization, B_{tot}, (M) is the total biomass (all species) in the pore, and B_{max}, (M) is the highest possible concentration of biomass in the pore. The later allow for considering a maximum carrying capacity limiting the biomass activity in each single pore. Assuming the microorganism to be bound to the surface of the solid matrix (pore walls) the carrying capacity is assumed to be related to the specific surface area of a pore. The pore specific maximum carrying concentration B_{max} is thus calculated as $B_{max}=B_{max}^{av} \cdot R_{av}/R$, where B_{max}^{av} is the carrying capacity in a pore having the average radius R_{av}. As a consequence B_{max} is decreasing for increasing radii R. θ is a unitless switch function, see below for further explanation. All concentrations refer to the (total) volume of an individual pore.

A dependency of the growth rate on the concentration of terminal electron acceptors or any other chemical species is not considered assuming these species to be present at sufficient amounts along the simulated domain, i.e. the entire domain is assumed to belong to a single redox zone. However, the concept could easily be expanded to consider such additional dependencies.

The biomass growth of active cells is linked to the substrate consumption rate through the effective growth yield factor Y_{eff}:

$$\left(\frac{dB}{dt}\right)_{\text{growth}} = -Y_{eff} \cdot \frac{dC_{DOC}}{dt} = \theta \cdot Y_{eff} \cdot k_C \cdot B \cdot \frac{C_{DOC}}{K_{DOC} + C_{DOC}} \cdot \left(1 - \frac{B_{tot}}{B_{\max}}\right) \tag{3.2}$$

$$Y_{eff} = Y_{\max}\left(1 - \frac{G_0}{G}\right) \tag{3.3}$$

where Y_{max} represents the highest possible cell yield for the given microbial group, G is the maximum rate of Gibbs energy release per unit biomass, and G_0 is a corresponding minimum threshold value. G is calculated as follows:

$$G = \Delta G \cdot \left.\frac{dC_{DOC}}{dt}\right|_{\theta_i = 1; Y_{eff} = 0} \cdot \frac{1}{B} = -\Delta G \cdot k_C \cdot \frac{C_{DOC}}{K_{DOC} + C_{DOC}} \tag{3.4}$$

Where ΔG as the Gibbs energy of the oxidation of DOC For the present simulations a fixed value of ΔG = 522 *kJ/mol* is used using the aerobic oxidation of toluene as reference. Note that G and G_0 are positive. Further note that equation 3.3 only applies when $G \geq G_0$; when $G < G_0$, $Y_{eff} = 0$. The deactivation of cells, that is, the transformation of active cells into inactive cells, is treated as a first order rate process and regulated by a switch function θ which is directing the process forward or backward depending again on the ratio of G and G_0, more details are given in Stolpovsky et al. (2011). This energy supply is assumed to represent the main factor controlling the switching of cells between the active and inactive states: when the energy requirements of the organisms are no longer met by the energy supply from the degradation of the organic substrate ($G/G_0 < 1$), cell activity slows down and active cells increasingly convert to the inactive state. In turn, when the energy supply increases ($G/G_0 > 1$), dormant cells can reactivate and start contributing to substrate degradation and biomass growth.

Furthermore, the length of the starvation period slows down the reactivation and (residual) decay rate of the dormant cells gradually entering a so-called deep dormancy. Active and dormant biomasses are included in the model as state variables. The mathematical expressions describing growth, decay of active and (at significantly lower rate) inactive cells, deactivation of active and reactivation of dormant biomass (except of growth all following by first-order kinetics with respect to the specific biomass concentration), and the consumption of the substrate are implemented into the model as described by Stolpovsky et al. (2011). There, also more details are provided on the mathematical representations functionally linking the modeled microbial processes to the energy yields of the corresponding respiration pathways and to the maintenance requirements of the organisms.

3.3. Simulated scenarios

3.3.1 Flow and transport processes

Simulations were performed using a hexagonal pore network of 8.9 cm length and 3.1 cm width. The length of pores was set to 1 mm. At the inflow of the network constant hydraulic pressure boundaries were assumed while no flow conditions were considered for the other boundaries.

Independent to the employed experimental method, pore size distribution functions (PSD) are often following either the log-normal (e.g., Vogel et al., 2010, Ezeuko et al., 2011) or normal distributions (e.g., Vogel et al., 2010, Ezeuko et al., 2011, Yanuka et al., 1986, Øren P. and Bakke S. 2002). The mean values and variances of PSD depend on type of soil and the degree of compaction and thus exhibit high variabilities between different samples. In this study, we consider either a homogeneous medium with all pores having a constant pore radius of 160 μm, or heterogeneous media with a normal distribution of the pore sizes defined by a mean value of 160 μm, correlation lengths of 1 mm (i.e. no spatial correlation) or 5 mm (i.e. spatially correlated distribution) and a standard deviation of 45 μm and 70 μm. The homogeneous medium is a fully hypothetical case and considered here as a reference for comparison while heterogeneous media may represent conditions found in natural soil or aquifer systems (Vogel, 2000). For each combination of

standard deviation and correlation length two random realization of pore size distribution were simulated.

Parameters	Value
Number of pores	*3162*
Length of medium	*0.089 m*
Width of medium	*0.031 m*
Average pore radius	*160 µm*
Standard deviation of pore radius distribution	70 µm
Maximum carrying capacity for a pore with average radius	*10.0 µM*
Fluid velocity	*$1.85 \cdot 10^{-3}$ (= 0.089/48) m/h*
Duration of starvation	*22 h*
Duration of feeding	*2 h*
Concentration of DOC during feeding	*80.0 µM*

Table 3.1. Parameters describing flow and transport in the pore networks.

Values for the hydraulic pressure were adjusted for each individual network to ensure an average water residence time of 48 hours in each network. The concentration of the incoming dissolved organic carbon substrate is periodically switching between its maximum value (set to *80* µM for all scenarios) and zero representing a succession of feeding and starvation periods. The length of the feeding period is 2 hours; the period of starvation is set to 22 hours resulting in a total cycle length of 24 hours. Parameters describing the considered flow and transport conditions are summarized in Table 3.1.

3.3.2 Microbial species and processes

Two different bacterial strains each using different growth/survival strategies are considered in this study: One strain referred to as ‘Growers’ exhibiting a relative

effective metabolism i.e. higher growth rate and more efficient substrate turnover, and one strain referred to as 'Switchers' exhibiting a more effective and opportune switching between active and inactive states. Parameters describing the behavior of these two bacterial strains are summarized in Table 3.2, parameter values are within the range used in a previous study (Stolpovsky et al., 2011) and are adapted from a batch experiment (Martínez-Lavanchy et al., 2010).

Parameters	"Growers"	"Switchers"
Maximum DOC degradation rate , k_C	*0.2 h^{-1}*	*0.05 h^{-1}*
Half-saturation constant for DOC, K_{DOC}	*1.0 µM*	*3.0 µM*
Growth yield factor (max), Y_{max}	*0.8*	*0.5*
Decay rate of active bacteria, μ_{dec}	*0.02 h^{-1}*	*0.002 h^{-1}*
Initial decay rate of inactive bacteria, $\mu_{dec,0}^{in}$	*$1 \cdot 10^{-3}$ h^{-1}*	*$2 \cdot 10^{-4}$ h^{-1}*
Thermodynamic threshold, G_0	*25.0 $kJ \cdot M_{biomass}^{-1} \cdot h^{-1}$*	*12.0 $kJ \cdot M_{biomass}^{-1} \cdot h^{-1}$*
Reactivation rate parameter, μ_{reac}	*0.1 h^{-1}*	*2.0 h^{-1}*
Deactivation rate parameter, μ_{deac}	*2.0 h^{-1}*	*2.0 h^{-1}*
Dormancy rate parameter, k_{incr}	*0.05 h^{-1}*	*0.5 h^{-1}*
Dormancy rate parameter, k_{decr}	*0.5 h^{-1}*	*0.05 h^{-1}*

Table 3.2. Parameter values describing the two competing microbial species.

Nevertheless, it is important to note that although the microbial groups used in this study are characterized by realistic parameters, they do not directly refer to any naturally observed bacterial species. In the model, each pore is represented as a perfectly mixed batch system and the incoming carbon substrate is consumed by the two competing (immobile) bacterial strains. Note that all resulting biomass concentrations are too small to have any impact on the volume and hydraulic properties of single pores (Thullner, 2010). All simulations were performed for a period of at least 6000 hours (= 250 days) a constant/repetitive spatial-temporal pattern of all state variables (concentration of DOC and bacterial biomass) was observed and shown results refer to these steady patterns.

Correlation matrices were calculated for three selected microbial variables (total biomass; overall biomass activity, i.e. ratio between sum of active biomass and total biomass; (log of) ratio between total biomass of Growers and total biomass of Switchers), and five potential driving factors/indicators of microbial dynamics (pore radius; pore water velocity; distance from inlet boundary; average DOC concentration (within a 24 hour cycle); standard deviation of single pulse DOC concentration). For each scenario single node values were analyzed using the program Maple.

When analyzing the results either for the coexistence of both species or for the dominance of a single species a threshold ratio of 1/100 (or 100/1) is used. This threshold ratio is derived from typical limits of molecular profiling methods for the detection of single species in microbial communities from the field (Blackwood et al, 2007). When a total biomass concentration ratio of 100/1 of one species with respect to the other is reached, this is assumed to represent the case when one species is dominant, otherwise both species are considered to coexist.

3.4. Results

3.4.1 Simulated spatio-temporal concentration distributions

For the homogeneous medium, simulated biomass distributions show for the total biomass only negligible variations within a single feeding cycle. The amount of biomass is decreasing moderately between inflow and outflow (Fig. 3.3) showing (as expected) no variations along the width of the medium.

Also the contribution of the two bacteria species to the total biomass is constant in time with Switchers dominating the total biomass for most of the length of the medium, while Growers are dominant in the vicinity of the outlet, only. In contrast, the concentration of the DOC substrate as well as the concentration of active bacteria exhibit strong variations during each feeding cycle (Fig. 3.4).

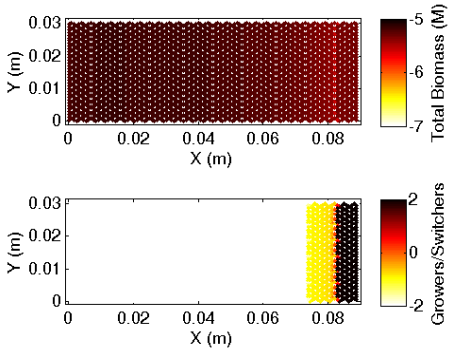

Figure 3.3. Homogeneous medium: simulated biomass distribution (steady state). Top – Log_{10} of distribution of total biomass, bottom – Log_{10} of ratio between total Growers and total Switchers. The main flow direction is from left to right.

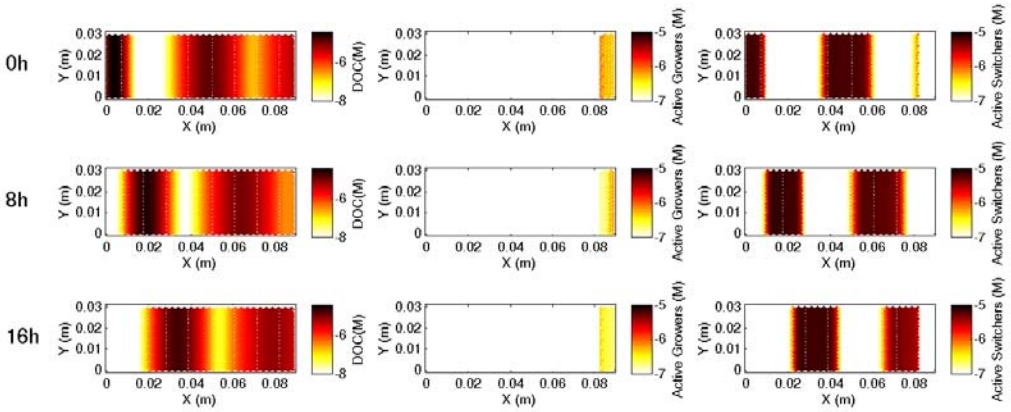

Figure 3.4. Homogeneous medium: simulated concentration changes during a single 24 hour feeding cycle. Left - Log_{10} of substrate (DOC) distribution, middle - Log_{10} of distribution of active Growers, right – Log_{10} of distribution of active Switchers. Top – 0 hours, middle - 8 hours, bottom – 16 hours after onset of the cycle. The main flow direction is from left to right.

The concentration of active Switchers is increased at locations where the DOC pulses migrating along the medium are reaching high local concentrations, too.

Concentrations of active Growers only exhibit changes close to the outlet of the medium with variations less pronounced than those found for the Switchers in the other parts of the medium.

For the heterogeneous medium with 1 mm correlation length (uncorrelated heterogeneity), pore water fluxes show a relatively dense pattern of flow paths along the medium (Fig. 3.5). The resulting total biomass distribution is again not depending on time showing a relatively homogeneous distribution with only little spatial variations at the small scale.

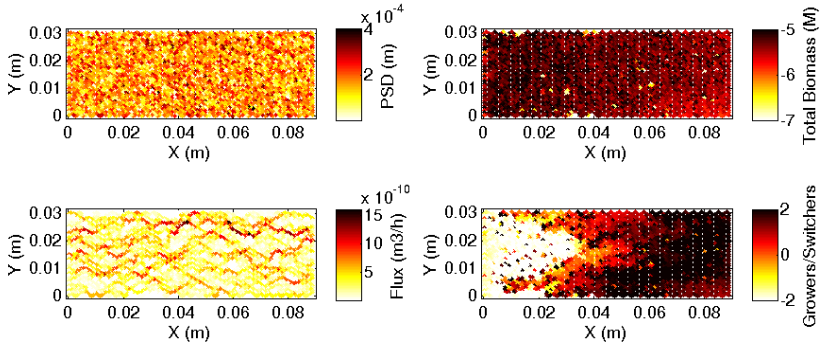

Figure 3.5. Heterogeneous medium (1 mm correlation length, 70 µm standard deviation of PSD): physical characteristics and simulated biomass distribution (steady state). Top-left – pore size distribution (PSD), bottom-left – pore water flux, top-right – Log_{10} of distribution of total biomass, bottom-right – Log_{10} of ratio between total Growers and total Switchers; values beyond ± 2 are shown as maximum/minimum of the displayed range. The main flow direction is from left to right.

Along the length of the medium, concentration shows a slightly decreasing trend from the inlet to the outlet. In contrast, the contribution of Growers and Switchers to total biomass shows no temporal but high spatial variations along the length and width of the medium. Next to various small scale variations a general trend from Switcher dominated regions at the vicinity of the inlet to Grower dominated regions at the vicinity of the outlet can be observed. Concentrations of DOC and active bacteria again vary during a feeding cycle (Fig. 3.6) with DOC pulses now becoming increasingly diffuse when migrating along the medium. High concentrations of active

Switchers again coincide with time and location of highest DOC concentrations while active Growers appear less variable in space and time with no obvious link to DOC concentrations.

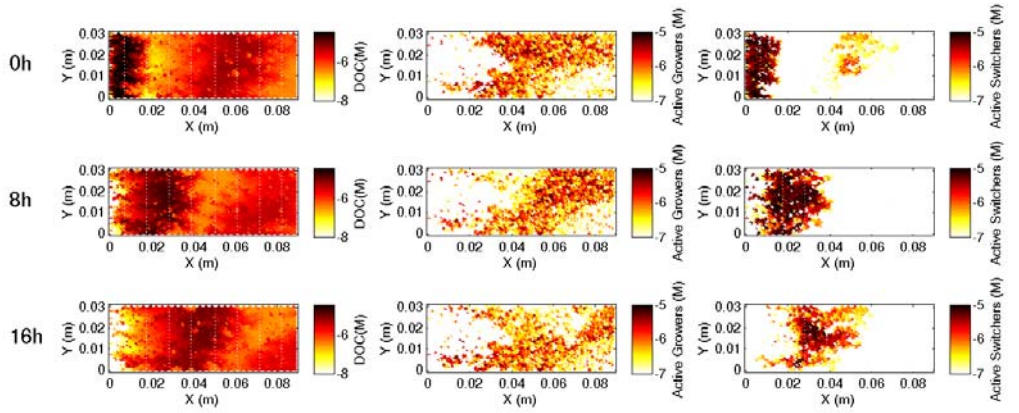

Figure 3.6. Heterogeneous medium (1 mm correlation length, 70 µm standard deviation of PSD): simulated concentration changes during a single 24 hour feeding cycle. Left - Log_{10} of substrate (DOC) distribution in, middle - Log_{10} of distribution of active Growers, right – Log_{10} of distribution of active Switchers. Top – 0 hours, middle - 8 hours, bottom – 16 hours after onset of the cycle. The main flow direction is from left to right.

For the heterogeneous medium with 5 mm correlation length (correlated heterogeneity), pore water fluxes are mainly restricted to few preferential flow paths connecting clusters of large pore sizes (Fig. 3.7). The resulting total biomass distribution is reflecting the pore size distribution but showing only mild variations except for a few regions with very low biomass concentration. Variations of the ratio between Growers and Switchers are again more pronounced than those of the total biomass indicating that along the preferential flow paths and closer to the inlet Switchers are dominating while Growers dominated areas apart from the preferential flow paths and/or closer to the outlet. Exceptions are the low biomass areas where Switchers are dominating. The spatio-temporal distribution of DOC now follows the preferential flow pattern (Fig. 3.8). As for the 1 mm correlation length, high

concentration of active Switchers coincided with highest DOC concentrations while active Growers exhibit more complex variations.

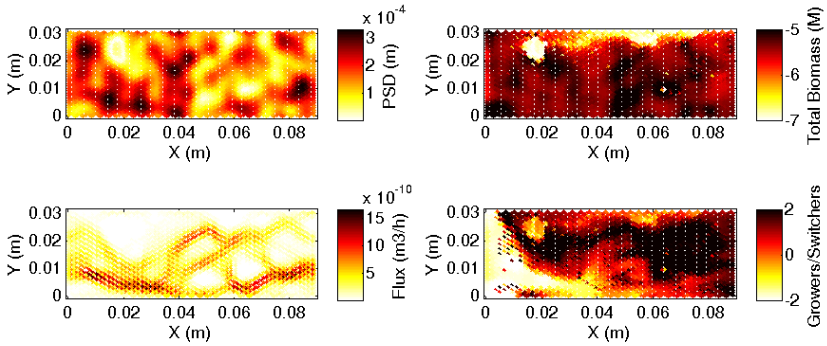

Figure 3.7. Heterogeneous medium (5 mm correlation length, 70 μm standard deviation of PSD): physical characteristics and simulated biomass distribution (steady state). Top-left – pore size distribution (PSD) (m), bottom-left – pore water flux (m^3/h), top-right – Log_{10} of distribution of total biomass, bottom-right – Log_{10} of ratio between total Growers and total Switchers; values beyond ± 2 are shown as maximum/minimum of the displayed range. The main flow direction is from left to right.

Figures 3.5 and 3.6, and 3.7 and 3.8 represent the results for a single random realization for each correlation length and a standard deviation of 70 μm. The results obtained for the second random realization simulated for each correlation length and results obtained for a standard deviation of 45 μm are qualitatively the same for as the presented examples (data not shown).

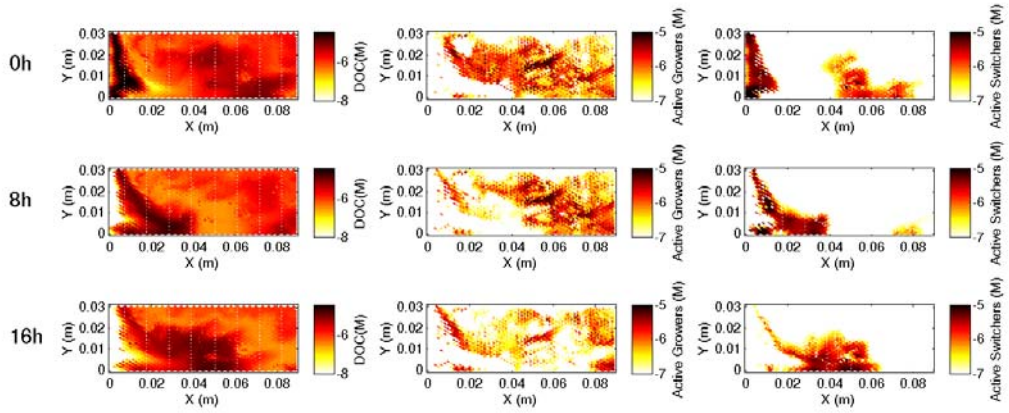

Figure 3.8. Heterogeneous medium (5 mm correlation length, 70 µm standard deviation of PSD): simulated concentration changes during a single 24 hour feeding cycle. Left - Log_{10} of substrate (DOC) distribution in, middle - Log_{10} of distribution of active Growers, right – Log_{10} of distribution of active Switchers. Top – 0 hours, middle - 8 hours, bottom – 16 hours after onset of the cycle. The main flow direction is from left to right.

3.4.2 Spatially averaged analyses

Average total biomass concentrations show for (nearly) all simulated realizations a slight decrease of biomass between inlet and outlet of the media (Fig. 3.9). These gradients seem to become less obvious for increasing correlation lengths but variations between different realizations of the same heterogeneity are too pronounced to fully establish this trend. For all realizations these average concentrations indicate Switchers to dominate regions at the inlet of the media while Growers dominate at the outlet (Fig. 3.9). In contrast to the homogeneous medium, where most regions are Switcher dominated and the transition to the Grower dominated region occurs within 1-2 cm, the heterogeneous media exhibit a gradual transition between the two extremes with most regions of the medium showing at this average scale rather a coexistence of both species (log of ratio around 0) than the dominance of a single one. For the larger correlation length changes are slightly

smoother with most regions of the media indicating a coexistence of species at this averaged level.

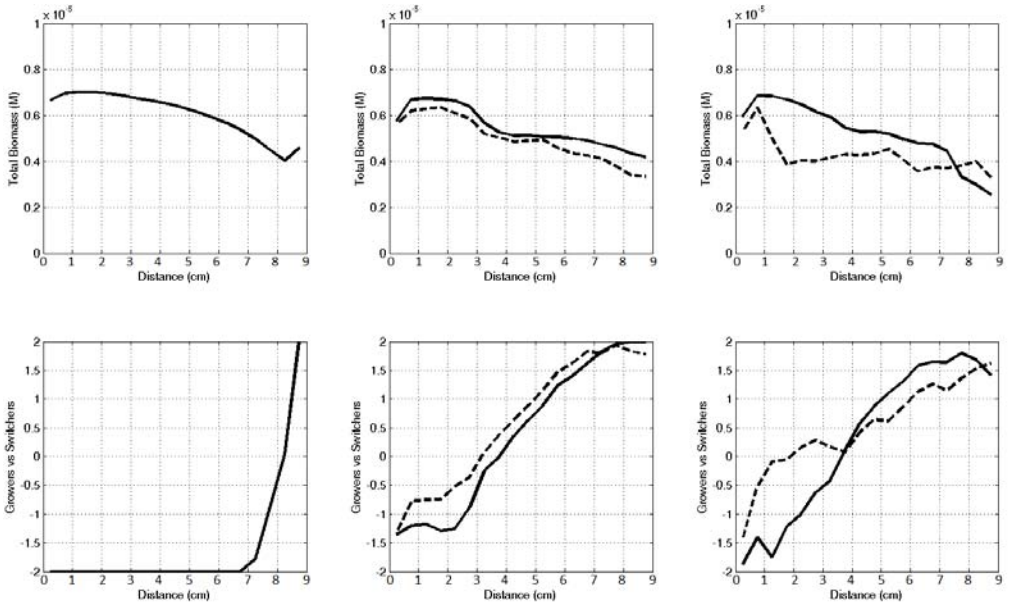

Figure 3.9. Average concentration of total biomass over 5 mm slices versus distance from the inlet (top) and Log_{10} of ratio between Growers and Switchers (buttom); values beyond ± 2 are shown as maximum/minimum of the displayed range: Left – homogeneous media, middle - heterogeneous media with correlation length 1 mm, right – heterogeneous media with correlation length 5 mm. Solid line - standard deviation of 45 μm, dashed line - standard deviation of 70 μm.

The analysis of species coexistence in single pores (Fig. 3.10) revealed that for the homogeneous medium the biomass is in most pores dominated by a single species with only less than 20 % of the pores showing a coexistence of the two species. In contrast, for the heterogeneous media the biomass in most of the pores (60-75 %) is composed by both species and only a minor fraction of all pores is dominated by a single species.

The correlation analysis indicated that in the heterogeneous media total biomasses are most correlated with the pore radius for all tested heterogeneities (correlation

coefficients of approx. -0.5 to -0.7) except for PSD with 45 µm standard deviation and 5 mm correlation length (Tab. 3.3a and 3.3b). Note that for all pores biomass concentrations remain below 70% of the carrying capacity exhibiting no dependency of this percentage on the individual pore sizes (data not shown). The correlation between average bacterial activity and pore radius is lower (correlation coefficients of up to approx. 0.4) with correlation coefficients between bacterial activity and other tested parameters reaching similar values (depending on realization) not supporting the assumption of a single driving factor here. The ratio between Growers and Switchers is strongest correlated with the average DOC concentration (correlation coefficients of approx. -0.8), its standard deviation (most correlation coefficients of approx. 0.7 - 0.8) and the distance to the inlet (most correlation coefficients of approx. 0.7 – 0.8) with always slightly stronger correlations observed for the lower correlation length and the larger standard deviation.

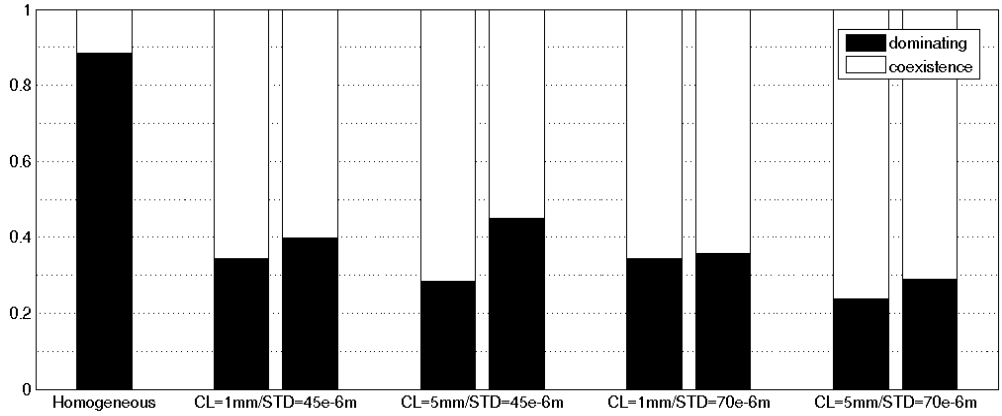

Figure 3.10. Fractions of pores containing dominating species (either Growers or Switchers) in homogeneous and different realizations of heterogeneous pore networks. A species is assumed to be dominating if its biomass is more then 100 times higher then the concentration of the suppressed species, in other case both species are considered as coexisting.

For the smaller standard deviation also a cross correlation between bacterial activity and the ratio between Grower and Switcher was observed (correlation coefficient of up to -0.6 depending on realization). The combined dependency of the

ratio between Growers and Switchers is also shown in Figure 3.11, indicating that for the heterogeneous networks Switcher dominate not only for high DOC concentrations/low standard deviations of DOC concentrations, but also for the opposite part of the DOC concentration/standard deviation range high relative abundances of Switcher are observed. In turn, intermediate DOC values support the dominance of Growers and the coexistence of both species is found between these "dominance" regions.

Correlation length 1 mm

	Tot. Biomass	Ratio	Activity	Radius	Av. DOC	Std. DOC	Velocity	Distance
Tot. Biomass	1.000	-0.335	0.085	**-0.738**	0.335	-0.270	-0.178	-0.345
Ratio	-0.335	1.000	**-0.536**	0.015	**-0.949**	**0.828**	-0.098	**0.899**
Activity	0.085	**-0.536**	1.000	0.221	**0.520**	-0.259	-0.004	**-0.528**
Radius	**-0.738**	0.015	0.221	1.000	-0.005	0.006	0.159	0.024
Av. DOC	0.335	**-0.949**	**0.520**	-0.005	1.000	**-0.847**	0.111	**-0.939**
Std. DOC	-0.270	**0.828**	-0.259	0.006	**-0.847**	1.000	-0.157	**0.802**
Velocity	-0.178	-0.098	-0.004	0.159	0.111	-0.157	1.000	0.004
Distance	-0.345	**0.899**	**-0.528**	0.024	**-0.939**	**0.802**	0.004	1.000

Realization 1

	Tot. Biomass	Ratio	Activity	Radius	Av. DOC	Std. DOC	Velocity	Distance
Tot. Biomass	1.000	-0.339	0.076	**-0.758**	0.345	-0.287	-0.186	-0.368
Ratio	-0.339	1.000	**-0.512**	-0.035	**-0.952**	**0.843**	-0.120	**0.899**
Activity	0.076	**-0.512**	1.000	0.238	0.483	-0.253	0.049	**-0.516**
Radius	**-0.758**	-0.035	0.238	1.000	0.043	-0.038	0.195	-0.018
Av. DOC	0.345	**-0.952**	0.483	0.043	1.000	**-0.865**	0.118	**-0.929**
Std. DOC	-0.287	**0.843**	-0.253	-0.038	**-0.865**	1.000	-0.140	**0.832**
Velocity	-0.186	-0.120	0.049	0.195	0.118	-0.140	1.000	-0.013
Distance	-0.368	**0.899**	**-0.516**	-0.018	**-0.929**	**0.832**	-0.013	1.000

Realization 2

Correlation length 5 mm

	Tot. Biomass	Ratio	Activity	Radius	Av. DOC	Std. DOC	Velocity	Distance
Tot. Biomass	1.000	-0.323	0.112	-0.019	0.447	-0.421	-0.205	-0.452
Ratio	-0.323	1.000	-0.342	0.009	**-0.876**	**0.749**	-0.030	**0.869**
Activity	0.112	-0.342	1.000	-0.007	0.292	-0.197	0.284	-0.309
Radius	-0.019	0.009	-0.007	1.000	-0.026	0.023	-0.306	0.024
Av. DOC	0.447	**-0.876**	0.292	-0.026	1.000	**-0.900**	0.105	**-0.964**
Std. DOC	-0.421	**0.749**	-0.197	0.023	**-0.900**	1.000	-0.142	**0.875**
Velocity	-0.205	-0.030	0.284	-0.306	0.105	-0.142	1.000	0.002
Distance	-0.452	**0.869**	-0.309	0.024	**-0.964**	**0.875**	0.002	1.000

Realization 1

	Tot. Biomass	Ratio	Activity	Radius	Av. DOC	Std. DOC	Velocity	Distance
Tot. Biomass	1.000	-0.418	0.271	0.000	0.401	-0.241	-0.198	-0.482
Ratio	-0.418	1.000	**-0.620**	0.028	**-0.889**	**0.819**	-0.122	**0.757**
Activity	0.271	**-0.620**	1.000	-0.020	0.493	-0.336	0.064	-0.409
Radius	0.000	0.028	-0.020	1.000	-0.030	0.020	-0.316	0.024
Av. DOC	0.401	**-0.889**	0.493	-0.030	1.000	**-0.860**	0.129	**-0.870**
Std. DOC	-0.241	**0.819**	-0.336	0.020	**-0.860**	1.000	-0.164	**0.792**
Velocity	-0.198	-0.122	0.064	-0.316	0.129	-0.164	1.000	0.007
Distance	-0.482	**0.757**	-0.409	0.024	**-0.870**	**0.792**	0.007	1.000

Realization 2

Table 3.3.a: Correlation matrices obtained for individual random realizations with PSD of 45 µm standard deviation. Av. DOC and Std. DOC refers to average DOC concentrations and their standard deviations. Highlighted values represent correlation coefficients > 0.5 (or < -0.5).

Correlation length 1 mm

	Tot. Biomass	Ratio	Activity	Radius	Av. DOC	Std. DOC	Velocity	Distance
Tot. Biomass	1.000	-0.034	-0.116	**-0.568**	0.156	-0.072	-0.195	-0.225
Ratio	-0.034	1.000	-0.355	-0.057	**-0.843**	**0.761**	-0.151	**0.771**
Activity	-0.116	-0.355	1.000	0.353	0.372	-0.188	0.209	-0.323
Radius	**-0.568**	-0.057	0.353	1.000	0.056	-0.077	0.218	0.021
Av. DOC	0.156	**-0.843**	0.372	0.056	1.000	**-0.837**	0.202	**-0.865**
Std. DOC	-0.072	**0.761**	-0.188	-0.077	**-0.837**	1.000	-0.244	**0.718**
Velocity	-0.195	-0.151	0.209	0.218	0.202	-0.244	1.000	-0.007
Distance	-0.225	**0.771**	-0.323	0.021	**-0.865**	**0.718**	-0.007	1.000

Realization 1

	Tot. Biomass	Ratio	Activity	Radius	Av. DOC	Std. DOC	Velocity	Distance
Tot. Biomass	1.000	-0.036	-0.168	**-0.584**	0.142	-0.075	-0.196	-0.208
Ratio	-0.036	1.000	-0.232	-0.074	**-0.866**	**0.762**	-0.116	**0.792**
Activity	-0.168	-0.232	1.000	0.387	0.230	-0.091	0.193	-0.252
Radius	**-0.584**	-0.074	0.387	1.000	0.068	-0.078	0.154	-0.006
Av. DOC	0.142	**-0.866**	0.230	0.068	1.000	**-0.844**	0.167	**-0.872**
Std. DOC	-0.075	**0.762**	-0.091	-0.078	**-0.844**	1.000	-0.209	**0.721**
Velocity	-0.196	-0.116	0.193	0.154	0.167	-0.209	1.000	-0.002
Distance	-0.208	**0.792**	-0.252	-0.006	**-0.872**	**0.721**	-0.002	1.000

Realization 2

Correlation length 5 mm

	Tot. Biomass	Ratio	Activity	Radius	Av. DOC	Std. DOC	Velocity	Distance
Tot. Biomass	1.000	-0.082	0.125	-0.433	0.295	-0.169	-0.177	-0.141
Ratio	-0.082	1.000	-0.345	-0.164	**-0.774**	**0.675**	-0.257	**0.602**
Activity	0.125	-0.345	1.000	0.353	0.359	-0.260	0.215	-0.110
Radius	-0.433	-0.164	0.353	1.000	0.336	-0.419	0.392	0.085
Av. DOC	0.295	**-0.774**	0.359	0.336	1.000	**-0.847**	0.343	**-0.555**
Std. DOC	-0.169	**0.675**	-0.260	-0.419	**-0.847**	1.000	-0.466	0.371
Velocity	-0.177	-0.257	0.215	0.392	0.343	-0.466	1.000	-0.003
Distance	-0.141	**0.602**	-0.110	0.085	**-0.555**	0.371	-0.003	1.000

Realization 1

	Tot. Biomass	Ratio	Activity	Radius	Av. DOC	Std. DOC	Velocity	Distance
Tot. Biomass	1.000	0.090	-0.055	-0.348	0.250	-0.302	0.065	0.011
Ratio	0.090	1.000	-0.079	-0.270	**-0.727**	**0.595**	-0.123	**0.574**
Activity	-0.055	-0.079	1.000	0.445	0.248	-0.158	0.268	-0.113
Radius	-0.348	-0.270	0.445	1.000	0.448	-0.382	0.287	-0.174
Av. DOC	0.250	**-0.727**	0.248	0.448	1.000	**-0.903**	0.352	**-0.514**
Std. DOC	-0.302	**0.595**	-0.158	-0.382	**-0.903**	1.000	-0.361	0.437
Velocity	0.065	-0.123	0.268	0.287	0.352	-0.361	1.000	0.093
Distance	0.011	**0.574**	-0.113	-0.174	**-0.514**	0.437	0.093	1.000

Realization 2

Table 3.3.b: Correlation matrices obtained for individual random realizations with PSD of 70 µm standard deviation. Av. DOC and Std. DOC refers to average DOC concentrations and their standard deviations. Highlighted values represent correlation coefficients > 0.5 (or < -0.5).

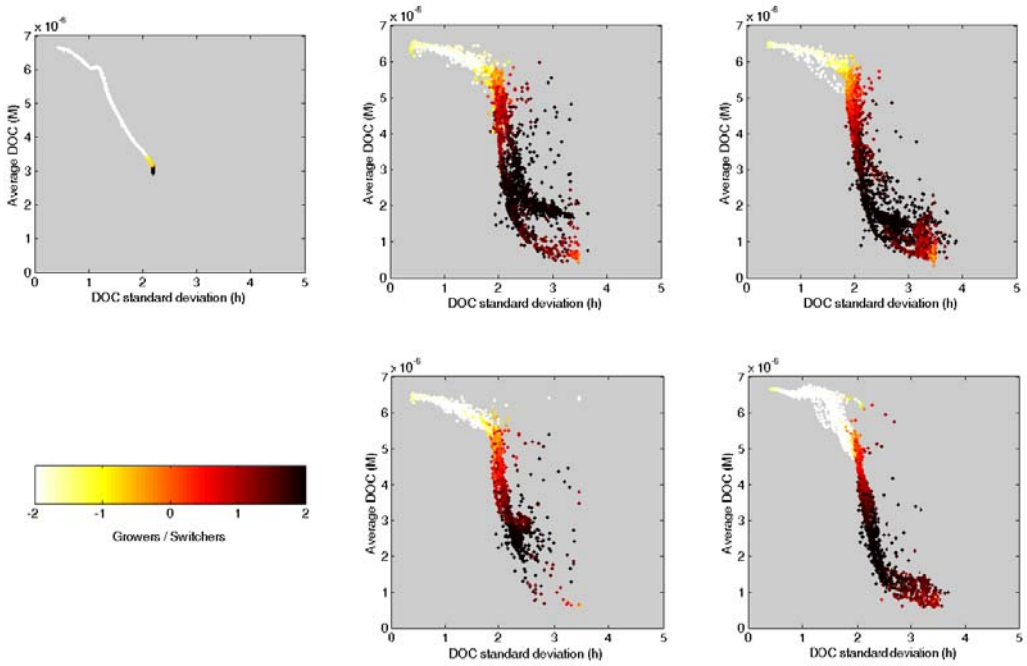

Figure 3.11. Dependency of the ratio between Growers and Switchers (color of dots) on the map of standard deviation of dissolved substrate (horizontal axis) and average concentration of dissolved substrate (DOC) (vertical axis). Each panel shows result of one single realization. Left - homogeneous media, middle - heterogeneous media with correlation length 1 mm, right – heterogeneous media with correlation length 5 mm. Top row - standard deviation of 45 μm, bottom row - standard deviation of 70 μm.

3.5. Discussion

Microbial dormancy is a typical phenomenon in soils and other environments, where concentrations of substrates are frequently lower than concentrations required for the maintenance the cellular function of active bacteria. This specifically includes environments exposed to intermittent stresses due to e.g., daily or seasonal cycles. The ability of bacteria to survive in permanently or intermittently stressed environments was broadly discussed in numerous works (Bakken and Olsen 1987,

Harder and Dijkhuizen 1983, Kaprelyants et al. 1993), but so far research on incorporating this type of behavior into microbial reaction models is limited. In this study, we apply a recently developed modeling concept to simulate the response of a bacterial population to periodic starvation conditions in heterogeneous porous media aiming at the reflection of a natural soil system. Structural heterogeneities and the resulting preferential flow patterns at various scales are a common observation in the terrestrial subsurface (e.g, Webb and Anderson, 1996, Lee et al., 2001), and such structural heterogeneities are well described by the geostatistical approach as used in this study (Knudby and Carrera, 2005). However, the presented simulations certainly represent a strong simplification of microbial habitats in soils and the shown examples can not cover the variability of such habitats in terms of pore scale heterogeneities and substrate conditions. Nevertheless, the shown examples represent reasonable conditions to be found in soils (e.g., daily fluctuations, heterogeneous pore size distribution) and the presented results indicate the potential dynamics of microbial communities at such conditions.

3.5.1 Heterogeneity, dormancy and bacterial abundance

The spatial distribution patterns observed in this study generally confirm results from earlier studies (e.g., Gharasoo et al. 2012, Bundt et al., 2001), which show that preferential flow patterns caused by the heterogeneity of the pore space have a high impact on microbial activity. When considering dormancy our results do however also show that even when substrate transport occurs mainly via preferential flow paths microbial abundance in general may still be rather homogeneous and does not reflect the observed flow and transport pattern. This would suggest that microbial abundance as prerequisite of any microbial ecosystem services (e.g., biogeochemical cycling of elements, degradation of contaminants) is not controlled by the type of pore scale heterogeneities investigated in this study.

In turn heterogeneity does have an impact on the distribution and activity of individual bacterial species. Even if these distributions do not directly reflect the preferential flow patterns the heterogeneous media lead to much more complex distribution patterns for each of the two considered species and a clear spatial

sequence between a Switcher dominated and a Grower dominated region is hardly observed for the heterogeneous media. The spatial and temporal activity patterns for both species are certainly triggered by the migration of the substrate pulses along the media and more complex transport patterns thus lead to more complex activity patterns. The activity of the Switchers is more obviously linked to the distribution of the substrate than the activity of the Growers, which reflects the Switchers having the better ability to respond to intermittent stress periods. However, both species respond to these stress periods by switching from an active into an inactive or dormant state which helps them to endure periods of unfavorable environmental conditions (Kaprelyants et al, 1993). This shows that the competitiveness of microbial species is not only controlled by their growth performance under plentiful conditions but also by their ability and readiness to respond to periods of unfavorable environmental conditions.

3.5.2 Bacterial competition

DOC is considered as the only growth limiting substrate and the ability to compete for this substrate is controlling the abundance of bacteria in the porous medium. An individual pore to be dominated by a single species, which is more competitive at the given conditions, is therefore an expected result and explains for the homogeneous network the observation of two distinct regions each dominated by one of the species. The increased coexistence observed for heterogeneous networks (Fig. 3.9) can in part be explained by the heterogeneity leading to a closer vicinity of single pores or pore assemblies dominated by different species. Spatial averaging then leads to a pseudo-coexistence on the average scale, and effect commonly used for explaining the coexistence of competing species (Tiedje et al., 1999). However, heterogeneity also leads to a larger number of individual pores exhibiting the coexistence of both species (Fig. 3.10). In the homogeneous medium the few pores with coexistence of both species are located at the transition between the region dominated by the Switchers and the region dominated by the Growers. In the heterogeneous media, the larger number and closer vicinity of small regions or pore assemblies dominated by a single species lead to a much larger number of pores

located at the interface between such regions, which explains the higher total number of pore exhibiting species coexistence. Interestingly, within the tested range of heterogeneities the extent of the heterogeneity (standard deviation and/or correlation length) had only little influence on species coexistence with all heterogeneous examples showing rather similar results (Figs. 3.9 and 3.10)

That the competitive performance of microbial species can vary with changing substrate conditions has been observed previously (e.g., Dechesne et al., 2008). The results of the present study indicate that dormancy or the ability to endure unfavorable growth conditions has also a strong impact on microbial competition in case of intermittent substrate supply. These results thus confirm that the observation of this phenomenon made for no-transport systems (Stolpovsky et al., 2011) also holds for soils or other porous media affected by advective transport of substrates.

3.5.3 Driving factors for microbial behavior

The correlation analysis presented in this study implies a simplified linear dependency between the tested variables and thus might not fully reflect the complexity of the relation between these variables. Nevertheless, this analysis indicates that for heterogeneous media not all variables describing the microbial community as a whole can be related to abiotic features of the porous medium. When using a correlation coefficient of ± 0.5 to determine if two variables are correlated (an arbitrary but typical threshold for biological systems (Gianoulisa et al., 2009)) microbial activity and total biomass are not linked to any other of the tested variables, for all 5 mm correlation length scenarios. For the 1mm correlation length scenarios, some correlations could be observed for microbial activity but correlation coefficients are quite close to the used threshold value and for establishing (or denying) any trends more realizations would be needed. In contrast, the ratio between Growers and Switchers seems to be controlled by DOC. This is also supported by a direct comparison of the single pore ratios between the species and the DOC conditions in a pore (Fig. 3.11). The later also shows that the dependency between ratio and DOC is not monotonous (and thus not linear) but indicates for the Switchers at least two favorable conditions: high concentrations applied in short

pulses and (less pronounced) low concentrations applied in long pulses. With intermediate concentration pulses favoring the Growers there are thus also two sets of DOC conditions supporting the coexistence of species which also explains the high number of individual pores showing a coexistence of both species in heterogeneous media. Ecological theories commonly consider only a single set of conditions to be optimal for the competitive behavior of species (Horner-Devine et al., 2004 and Lynch et al., 2004) and the multiple optima observed in the present study might contribute to the explanation of the high diversity of species observed in the terrestrial subsurface. The results do not allow determining if the average amount or the duration of the single-pore substrate pulses is more important for the competitive behavior the two bacterial species, as the two DOC variables are correlated in the simulated media. The fact that both DOC variables are correlated with the distance from inlet supports the assumption that the observed correlation between this distance and the ratio between species is not due to any direct interaction but reflects the DOC dependency of the ratio. It is also worth noticing that the pore radius – although being the ultimate feature defining the heterogeneity of the media – has no direct impact on the ratio between the species or their activity. Only for the total biomass the radius appears to have some direct influence. This is attributed to lower water and thus solute fluxes entering (regions of) small pores. The pore size specific carrying capacity appears to have little influence on the results here but for higher substrate and thus biomass concentrations (or lower carrying capacities) this observation might have been different.

In this study we controlled microbial competition by the available carbon substrate, only. The size of individual redox zones can vary between more than 100 m (e.g., in contaminant plumes; Christensen et al., 2000) and less than 1 cm (Thullner et al., 2002; Bauer et al., 2008). This suggests that assuming no redox gradients along the transport path of 9 cm considered in this study is reasonable for many natural systems. Results would also be similar for microbial communities competing for a single electron acceptor in parts or porous media where an excess of carbon substrate is available. In porous media where both, carbon substrate and other species such as electron acceptors, exhibit concentration changes relevant

degradation rates microbial competition the resulting abundances and activities are expected to be different than presented here.

3.6. Summary and conclusion

An advanced modeling concept for growth and activity of microorganisms exhibiting dormancy behavior (Stolpovsky et al., 2011) was implemented into the flexible reactive transport model for pore networks PNBNRS (Gharasoo et al., 2012). The model was used to simulate the response of a hypothetical microbial community of two species competing for a single substrate intermittently supplied to the porous medium. The obtained results show that the ability of the microorganisms to switch into a dormant/inactive state during periods of starvation has a strong impact on their competitiveness. Depending on the heterogeneity of the porous medium microbial abundance exhibits a complex spatial distribution of the individual species. In particular, heterogeneity leads not only to the formation of a larger number of niches dominated by a single species but also to a higher number of pores simultaneously inhabited by both species, both effects contributing to the coexistence observed a the larger scale. The main driving factor for the competitive behavior of the microorganisms is the substrate supply in each individual pore, while other parameters defining heterogeneity (pore radius, flow field) have only an indirect impact via their influence on substrate transport. Furthermore, we found that not a single set of substrate conditions may be optimal for the competitive behavior of species with the found multiple optima contributing to the coexistence of species observed at the large scale. While abundances of species being constant in time their activity can rapidly respond to the intermittent sequence of feeding and starvation. This complex spatio-temporal distribution of microbial activities and thus their ecosystem service potential indicates that an extrapolation of observation from simple laboratory systems to real soils or aquifers is highly challenging.

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4

Microbial competition under intermittent substrate supply:
Insights from model simulations

with Ingo Fetzer , Philippe Van Cappellen and Martin Thullner

submitted to Ecological Complexity

Abstract

Microorganisms are responsible for a wide range of ecosystem services. However the ability of microbial communities to provide any of such functions depends on the composition of the specific community. Many environmental systems are characterized by frequent changes of their abiotic environment. Microorganisms can respond to such changes by switching their physiological state between activity and dormancy allowing them to endure periods of unfavorable abiotic conditions. As a consequence, the competitiveness of microbial species is not only controlled by their growth performance under favorable conditions but also by their ability and readiness to respond to periods of unfavorable environmental conditions.

The present study investigates the relevance of factors controlling the abundance and activity of individual bacterial species competing for an intermittently supplied substrate. For this purpose, numerical simulations were performed addressing the response of microbial systems – containing up to three competing species with different growth characteristics and de-reactivation efficiencies – to regularly applied feeding pulses. Simulation results show that community dynamics may exhibit a non-trivial link to the frequency of the external constraints and that for a certain

combination of these environmental conditions coexistence of species is possible. Furthermore, the results show that introducing an additional competitor into a community affects the realized niche of the individual species even when the abundance of the added competitor is negligible. A potential ecological implication of our results is that even non-dominant and therefore often undetected species can have a strong influence on realized species composition of dominant key species and by this affecting provided function of the system.

4.1. Introduction

Many ecological relevant processes are catalyzed by the activity of microorganisms, which includes remineralisation processes and the degradation of organic compounds in nearly all terrestrial and aquatic environments (e.g., Paul and Clark 1996). Although the dynamics of such degradation processes differ with the involved chemical compounds and environmental conditions, the general activity of the catalyzing microorganisms has a crucial impact on the overall rate of the process (Paul and Clark 1996). Therefore, a sound theoretical description and/or the prediction of such microbial rates require knowledge on community composition, species abundances, individual traits of the involved microorganisms, and on the factors controlling these variables.

Experimental studies in the laboratory are commonly performed at constant, nutrient enriched conditions, which rarely exhibit a coexistence or coactivity (i.e., simultaneous activity) of competing species (Codeço et al., 2001). In contrast, natural microbial systems are rather oligotrophic, and are usually characterized by frequent changes of abiotic conditions for the microbial community, facilitating the coexistence of competing microbial species (Kaprelyants et al., 1993). Environmental changes can differ in their regularity and vary in both, their frequency and amplitude. Changes may include natural variations ranging from daily cycles (e.g. precipitation, daylight triggered plant activity and tides) to seasonal changes - (e.g. submergences and droughts) as well as anthropogenic activities across different time scales (e.g., De Biase et al., 2011). Since microorganisms often cannot physically escape from local changes by emigration, they can respond to such changes by switching their physiological state from active to dormant (Lennon et al., 2011). This allows them to endure periods of unfavorable environmental conditions (Stevenson, 1997; Kaprelyants et al., 1993) and in many natural environments the largest fraction of the microbial population is found to be metabolically inactive (Lennon and Jones 2011). As a consequence, the competitiveness of microbial species and consequently also their abundance and activity is not only controlled by their growth performance under favorable conditions but also by their ability and

readiness to respond to periods of unfavorable environmental conditions (Stolpovsky et al., 2011).

Although microbial communities represent the most diverse type of communities on the Earth (Curtis et al., 2002; Dykhuizen, 1998.), the estimation of the biodiversity of natural communities the factors controlling it in the field is still a challenge. There are several methods determining functional and structural composition of a microbial community (Forney et al., 2004). Despite their common use, methods typically used in microbiological investigations usually are able to detect only the most dominating microbial groups (e.g. > 1% of total microbial biomass) (Blackwood et al., 2007). Although low abundant groups usually remain undetected these groups may still play a significant role in microbial communities (Forney et al., 2004).

Coexistence and dominance patterns of the co-occurring species in a microbial community are generally explained by mechanisms such as competition for equal resources, predation and abiotic stress. Generalizations of these mechanisms form the basis of various fundamental ecological theories as e.g. intermediate disturbance hypothesis, insurance hypothesis, niche partitioning, etc. are formed on the basis of changes in species coexistence (Bruno et al., 2006, Chapin et al., 2000, Ives et al., 2007, Loreau 2000, 2001, 2009). In particular, the ecological niche concept plays a central theme in ecology and evolutionary biology (Sillero, 2011), describing a niche as the range that is physiologically available for (potential niche) and used by (realized niche) a species or population in an environment (Grinnell, 1917). The distribution and overlaps of physiological niches and competition strength of species finally explains theoretical long-term coexistence under given environmental conditions (Gage 1996). Furthermore it was recently shown that even in the absence of any spatial ecological niches intermittent substrate feeding can also lead to the coexistence of competing species in time by deactivation mechanisms during periods of starvation (Stolpovsky et al. 2011). Simulation of microbial community dynamics in porous media suggested that the intermittent substrate supply is also at the pore scale the main driver for microbial competition but results did not allow attributing the observed effects to either the magnitude or the variation of substrate concentrations (Stolpovsky et al., in press).

Computational modeling is a promising method to be applied in the field of microbiology and ecology and models are increasingly used to theoretically describe the behavior of microorganisms under different physical or chemical conditions (Jamieson et al., 2004, Nielsen and Villadsen 1992). These modeling approaches enable not only predictions of microbial systems behavior but provide also the ability to give new insights into the underlying mechanisms for the observed biological phenomena. The aim of the present studies is to investigate how the characteristics of periodically changing environmental conditions might affect the coexistence of competing microbial species, and if species with negligible abundance and activity might still have an influence on the ecological behavior of their competitors. For this purpose, we use a computational model based on simplified ecological concepts allowing us understanding the principal behavior of ecological systems. The applied numerical simulation approach is used to identify factors controlling the dynamics, and the coexistence (i.e. total abundance) and coactivity (i.e. abundance of active cells) of competing species within a range of different intermittent feeding conditions. In particular, we investigate the dynamics of species dominance patterns in a system containing up to three competing bacterial species using different strategies in terms of resource use efficiency, growth rate and ability to switch between active and inactive (dormant) states.

4.2. Model description

4.2.1 General concept

Simulations were performed for microbial systems containing different bacterial species competing for a common, intermittently supplied carbon substrate. Each of these bacterial species is able to switch into a dormant state. The conceptual model describing the dynamics of these two-state (active/dormant) microbial species considers i) growth of active cells coupled to the consumption an energy yielding carbon substrate, ii) intrinsic mortality of active cells, iii) intrinsic mortality of inactive cells (considered to be significantly slower than for active cells), iv) deactivation of active cells controlled by the energy supply of the cells, and v) reactivation of inactive cells controlled by the potential energy supply of the cells (Fig. 4.1). This

conceptual approach has been introduced by Stolpovsky et al. (2011) where a detailed description is provided. Thus, we here give only a short overview of the underlying assumptions and used equations.

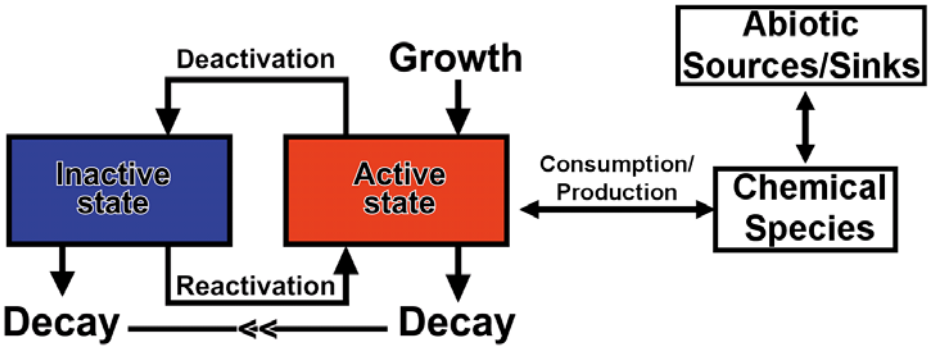

Figure 4.1. Schematic representation of the microbial processes simulated for each bacterial species.

Active cells are able to build up biomass by growth and/or cell division, to consume available substrate and are subject to mortality by cell death. Inactive (dormant cells) do not exhibit any growth and degradation activity while their death rate is highly reduced compared to their active state. Depending on the environmental conditions cells are assumed to switch between these two states using the potential supply of catabolic energy to the cells as control variable. Active and dormant biomasses are included as state variables, and species growth is described by established approaches (Thullner et al., 2007; Stolpovsky et al., 2011). These approaches are combined with kinetic expressions describing the deactivation and reactivation of the simulated species. The kinetic expressions are functionally linked to the energy yields of the corresponding respiration pathways, as well as to the maintenance requirements of the organisms. We also take into consideration variations in the yield factor, depending on the energy budget and consider loss of biomass during reactivation.

The rate of degradation of dissolved organic carbon (DOC) is assumed to follow standard Michaelis-Menten- or Monod-type kinetics (e.g., Thullner et al., 2007 and references therein):

$$\frac{dC_{DOC}}{dt} = -\theta \cdot k_C \cdot B \cdot \frac{C_{DOC}}{K_{DOC} + C_{DOC}} \tag{4.1}$$

where *C* stands for concentration, *B* is the concentration of active cells (biomass), k_C is the maximum specific rate of degradation of DOC by the active cells, K_{DOC} is the half-saturation constants for DOC utilization. Maximum bacterial concentration is set to be 10 *μM*. The biomass growth of active cells is linked to the substrate consumption rate through the effective growth yield factor Y_{eff}:

$$\left(\frac{dB}{dt}\right)_{\text{growth}} = -Y_{eff} \cdot \frac{dC_{DOC}}{dt} = \theta \cdot Y_{eff} \cdot k_C \cdot B \cdot \frac{C_{DOC}}{K_{DOC} + C_{DOC}} \tag{4.2}$$

$$Y_{eff} = Y_{max}\left(1 - \frac{G_0}{G}\right) \tag{4.3}$$

where Y_{max} represents the highest possible cell yield for the given microbial group, *G* is the maximum rate of Gibbs energy release per unit biomass, and G_0 is a corresponding minimum threshold value. Note that *G* and G_0 are positive. Further note that Equation (4.3) only applies when $G \geq G_0$; when $G < G_0$, $Y_{eff} = 0$. The deactivation of cells, that is, the transformation of active cells into inactive cells, is treated as a first order rate process and regulated by a switch function *θ* which is directing the process forward or backward depending on the level of the catabolic energy supply of the cells which is assumed to represent the main factor controlling the de-/reactivation of cells: when the energy requirements of the organisms are no longer met by the energy supply from the oxidation of the organic substrate by the electron acceptor, cell activity slows down and active cells increasingly convert to the inactive state. In turn, when the energy supply increases, dormant cells can reactivate and can subsequently contribute to biomass growth and substrate degradation. Furthermore, the length of the starvation period slows down the reactivation and (residual) mortality rate of the dormant cells gradually entering a so-called deep dormant state. Active and dormant biomasses are included in the model as state variables. The mathematical expressions describing mortality of active and (at significantly lower rate) inactive cells, deactivation of active and reactivation of dormant biomass are expressed as described by Stolpovsky et al. (2011). There,

also more details are provided on the mathematical representations functionally linking the kinetics of microbial processes to the energy yields of the corresponding respiration pathways and to the maintenance requirements of the organisms. This concept of microbial degradation and population dynamics is implemented into the numerical simulation software BRNS (Biogeochemical Reaction Network Simulator; Regnier et al., 2002; Aguilera et al., 2005) and the model is used to simulate the dynamics of the microbial models systems for different periods between substrate supply events.

4.2.2 Simulated scenarios

The simulated generic microbial systems is considered to contain one to three bacterial species: the first species (termed 'Growers') has a fast and effective metabolism, i.e. relatively high growth rate, yield factor and mortality rate; in contrast, the second species (termed 'Switchers') is characterized by an effective dormancy, i.e. profitable parameter values for de-/reactivation and deep dormancy; the third species (termed 'Intermediate') has no specific advantages, but represents an average between characteristics and performance of Growers and Switchers. All bacterial species are considered to compete for the same carbon substrate, the concentration of which is assumed as the only growth limiting factor. Parameters values describing the behavior of these three bacterial strains (Table 4.1) are within the range of values used before in Stolpovsky et al. (2011) and are adapted from a batch experiment (Martínez-Lavanchy et al., 2010). Nevertheless, it is important to note that although the microbial species used in this study are characterized by reasonable parameters, they do not directly refer to any naturally observed bacterial species.

Simulations were performed for virtual batch type systems inhabited by bacteria and intermittently supplied with carbon substrate pulses, while no other chemical species are considered to limit bacterial growth and activity. The amplitude and frequency of the intermittent carbon supply scheme were constant in each scenario but differed between the different scenarios.

Parameters	"Growers"	"Intermediate"	"Switchers"
Maximum reaction rate of degradation of DOC by microbial group, k_C	*0.2 h^{-1}*	*0.12 h^{-1}*	*0.05 h^{-1}*
Half-saturation constant for carbon substrate, K_{DOC}	*1.0 µM*	*2.0 µM*	*3.0 µM*
Growth yield factor (max), Y_{max}	*0.8*	*0.65*	*0.5*
Mortality rate of active bacteria, μ_{dec}	*0.02 h^{-1}*	*0.015 h^{-1}*	*0.01 h^{-1}*
Initial mortality rate of inactive bacteria, $m^{in}_{dec,0}$	*1.0e-4 h^{-1}*	*1.0e-4 h^{-1}*	*1.0e-4 h^{-1}*
Thermodynamic threshold, G_0	*25.0 $kJ \cdot M_{biomass}^{-1} \cdot h^{-1}$*	*18.0 $kJ \cdot M_{biomass}^{-1} \cdot h^{-1}$*	*12.0 $kJ \cdot M_{biomass}^{-1} \cdot h^{-1}$*
Reactivation yield	*0.1*	*0.5*	*1.0*
Reactivation rate parameter, μ_{reac}	*0.1 h^{-1}*	*1.0 h^{-1}*	*2.0 h^{-1}*
Deactivation rate parameter, μ_{deac}	*2.0 h^{-1}*	*2.0 h^{-1}*	*2.0 h^{-1}*
Dormancy rate parameter, k_{incr}	*0.05 h^{-1}*	*0.25 h^{-1}*	*0.5 h^{-1}*
Dormancy rate parameter, k_{decr}	*0.5 h^{-1}*	*0.25 h^{-1}*	*0.05 h^{-1}*

Table 4.1. Parameter values used to simulate the different competing microbial species.

Considered amplitudes and frequencies of feeding events lay within a wide range and may represent different natural condition. For each scenario the transient behavior of the microbial system was simulated. Simulations were run until simulated concentrations reached stable average concentrations of all considered species (referred to as "quasi-steady-state").

The obtained "quasi-steady-state" results were analyzed for the contribution of the single species to the total/active biomass. For this purpose a detection threshold of 1% was used (in analogy to typical experimental detection limits reported for field studies (Blackwood et al., 2007)) to characterize the abundance/activity of a species: species contributing to the total/active biomass less than this threshold value are considered to be of negligible abundance/activity. Species with biomass contributions higher than this threshold are either considered to dominate the bacterial community as the only abundant species (exceeding the threshold) or to

coexist in a community of multiple species (each of them exceeding the threshold). The temporal pattern of active biomass concentration changes was analyzed for each species with respect to the full period length (or frequency) of any repetitive patterns. A pattern was considered as repetitive with a period length t_p if the concentrations c(t) and c(t+t_p) differed for all times t by less than 1%. If no repetitive pattern was found for $t_p \leq 5$ (times the period length of the carbon feeding) the temporal biomass concentration changes were considered as irregular/chaotic. The maximum difference of 1% was selected arbitrarily as a small number representing a graphically non-detectable difference. Tests revealed that using a tenfold increased threshold value did not lead to considerable changes in the presented results, on the other hand further decreasing of this threshold quickly turned most of responses to be chaotic.

4.3. Results

4.3.1 Single-species system

As a reference case we use single-species systems populated with either Growers or Switchers. These systems exhibited amounts of total biomass generally following the average amount of carbon substrate supplied to the system, with the activity of the species showing strong fluctuations due to the intermittent substrate supply (data not shown).

The most remarkable behavior of the system populated with Growers was the discrepancy between frequencies of the external constraint and the internal response i.e. between the substrate supply and changing of active and total biomass concentrations (Tab. 4.2). A match between these frequencies was only found for the highest substrate supply frequency or for a combination of high frequencies with low amplitudes of the substrate pulses. For all other pulse characteristics, the changes of the active biomass followed a more complex temporal pattern. For intermediate pulse frequencies (approximately between 0.1 and 0.5 h^{-1}) biomass changes appeared rather irregular or chaotic (except for very low pulse amplitudes), while at lower frequencies most scenarios exhibited regular patterns of the biomass changes but their period length was 2 to 4 times increased compared to the

substrate supply. Examples representing the different types of frequency responses are given in Figure 4.2. In contrast, systems inhabited by Switchers showed an exact match between frequencies of external constraint and internal response for the entire range of simulated conditions (data not shown).

Freq. ($hours^{-1}$) → Amlitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
1.00E-06	1	1	1	1	4	4	4
2.00E-06	1	1	1	>5	>5	>5	2
5.00E-06	1	1	>5	5	3	3	2
1.00E-05	1	>5	>5	>5	>5	3	2
2.00E-05	1	>5	>5	>5	>5	3	2
5.00E-05	1	>5	>5	>5	4	3	2
1.00E-04	1	>5	>5	>5	2	3	2
2.00E-04	1	>5	>5	>5	2	3	2
5.00E-04	1	>5	>5	>5	2	4	2
1.00E-03	1	>5	>5	>5	2	4	2
2.00E-03	1	>5	>5	>5	2	4	2
5.00E-03	1	>5	>5	>5	2	4	2
1.00E-02	1	>5	>5	>5	2	4	2

Table 4.2. Factors linking period lengths of concentration changes of active biomass of Growers to period lengths of substrate supply for the single-species system. '>5' is considered as irregular concentration changes.

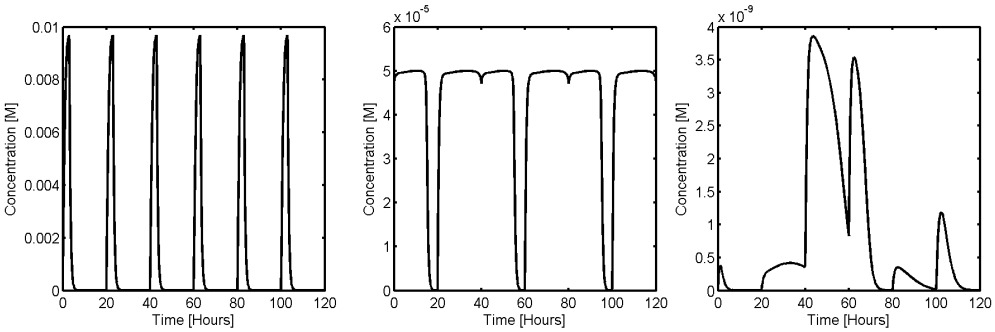

Figure 4.2. Examples for different responses of active biomass concentration changes to the frequency of the feeding pulses as periodical external stimulation. Feeding frequency is 1/20 h^{-1} for all shown examples. Left – regular response with same frequency as external stimulation, middle – regular response with period length increases by factor 2 compared to external stimulation, and right – irregular response.

4.3.2 Two-species system

Simulation results revealed that a long-term coactivity (coexistence of active fractions) of Growers and Switchers is found at substrate concentration pulse amplitudes around 10^{-4} M (Tab. 4.3a). For lower amplitudes Growers were dominating the activity of the community while higher amplitudes led to a dominance of the Switchers. Little dependency of activity results was found on the pulse frequency showing only a slight decrease of the amplitudes allowing for species coactivity when decreasing the pulse frequency from 1 h^{-1} to 1/100 h^{-1}. For frequencies of 0.05 h^{-1} and higher the active biomass fraction (active Growers plus active Switchers) represented 0.1 or more of the total biomass, while for lower frequencies active fractions were below 0.01 (data not shown).

Analysis of results obtained for total biomass revealed a more complex response (Tab. 4.3b). Here the pulse frequency and amplitude range of Growers presence and dominance expanded to some of the values ranges where Switchers were more active than Growers. On the other hand pulse characteristics leading to Growers dominated activities occasionally showed a coexistence of Growers and Switchers (e.g., at lowest amplitudes).

The frequency analysis of the active biomass concentration showed for the Switchers a discrepancy between the frequencies of the external carbon pulses and of the active biomass concentration changes (Tab. 4.4). For a frequency of 1 h^{-1} the frequency of the active biomass matched the frequency of the carbon supply.

For decreasing pulse frequencies active biomass concentrations became less regular exhibiting for most pulse amplitudes first a rather irregular or chaotic temporal pattern while further decrease led again to concentration patterns which were stable/repetitive but at period lengths 2 to 4 times larger than the period of each carbon supply cycle. These changes in pattern stability with decreasing carbon pulse frequency occurred first at pulse amplitudes in the order of 10^{-5} M. For amplitudes of smaller or larger order of magnitude pattern changes occurred at increasingly lower pulse frequencies. For the Growers a similar frequency behavior was observed (data not shown).

a)

Freq. (hours^{-1}) → Amlitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
1.00E-06	G	G	G	G	G	G	G
2.00E-06	G	G	G	G	G	G	G
5.00E-06	G	G	G	G	G	G	G
1.00E-05	G	G	G	G	GS	G	G
2.00E-05	G	G	GS	GS	G	GS	GS
5.00E-05	G	G	GS	GS	GS	GS	GS
1.00E-04	GS	GS	GS	GS	S	S	S
2.00E-04	GS	GS	S	S	S	S	S
5.00E-04	S	S	S	S	S	S	S
1.00E-03	S	S	S	S	S	S	S
2.00E-03	S	S	S	S	S	S	S
5.00E-03	S	S	S	S	S	S	S
1.00E-02	S	S	S	S	S	S	S

b)

Freq. (hours^{-1}) → Amlitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
1.00E-06	GS	GS	GS	GS	GS	G	GS
2.00E-06	GS	GS	GS	G	G	G	G
5.00E-06	G	G	G	G	G	G	G
1.00E-05	G	G	G	G	G	G	G
2.00E-05	G	G	GS	G	G	G	G
5.00E-05	G	G	GS	GS	GS	G	G
1.00E-04	GS	GS	GS	GS	S	G	G
2.00E-04	GS	GS	GS	S	S	GS	GS
5.00E-04	S	S	GS	S	S	GS	GS
1.00E-03	S	S	GS	GS	S	GS	GS
2.00E-03	S	GS	GS	GS	S	GS	GS
5.00E-03	GS	GS	S	S	GS	GS	GS
1.00E-02	S	S	S	S	S	GS	GS

Table 4.3. Abundance of a) active biomass fractions and b) total biomass of Growers and Switchers for the tested feeding pulse periods and amplitudes. Threshold of existence – 1% of active or total biomass. 'G' – dominance of Growers, 'S' – dominance of Switchers. 'GS' – coactivity or coexistence of Growers and Switchers.

Freq. (hours^{-1}) → Amlitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
1.00E-06	1	1	1	1	2	4	4
2.00E-06	1	1	1	>5	>5	>5	2
5.00E-06	1	1	1	5	3	3	2
1.00E-05	1	>5	>5	>5	>5	3	2
2.00E-05	1	>5	>5	>5	>5	>5	2
5.00E-05	1	>5	>5	4	2	3	2
1.00E-04	1	2	4	>5	3	3	2
2.00E-04	1	1	1	>5	>5	>5	>5
5.00E-04	1	1	1	1	2	>5	3
1.00E-03	1	1	1	1	1	>5	>5
2.00E-03	1	1	1	1	1	2	>5
5.00E-03	1	1	1	1	1	1	1
1.00E-02	1	1	1	1	1	1	1

Table 4.4. Factors linking period lengths of concentration changes of active biomass of Switchers to period lengths of substrate supply for the two-species system. '>5' is considered as irregular concentration changes.

All obtained results were independent of the initial conditions. In particular, for all considered scenarios adding a seed concentration of Growers to a population of Switchers having reached a "steady-state" led to the same results than obtained for adding a seed concentration of Switchers to a "steady-state" Grower population. The presented activity and abundance patterns (Tab. 4.3a, 4.3b, 4.5a and 4.5b) did in general not depend on the used detection threshold (1% for the shown results), with lower threshold values showing only an increase in the range of pulse characteristics (amplitude, frequency) leading to coexistence and higher threshold values having the opposite effect (data not shown).

4.3.3 Three-species system

Simulating the response of an established two-species system to the introduction of the third Intermediate species showed that the resulting community allowed for five out of seven possible combinations of species activity and abundances at "steady state" (Tab. 4.5a and 4.5b). Only coexistence or coactivity of Switchers and Intermediates, or a dominance of Intermediates was not observed.

As for the two-species system higher (medium to high) feeding pulse amplitudes led to activities dominated by Switchers, while lower amplitudes allowed the Growers to contribute to the activity either as single, dominant species (pulses with low amplitudes and high frequencies), or together with one of the other species (Intermediates at low amplitudes and low frequencies, or Switchers at medium amplitudes and high frequencies) (Tab. 4.5a). When combined with lower frequencies medium amplitude pulses also allowed for a few scenarios with all three species contributing to total activity. Compared to the activities in of the two-species scenarios, most three-species scenarios show either the same dominance/coactivity of Growers and Switchers or exhibit an additional activity contribution of the Intermediates. Only for few scenarios (medium amplitudes and lower frequencies) a coactivity of Growers and Switchers is (partially) replaced by a coactivity of Growers and Intermediates.

Similarly to the two-species scenarios, contributions of species to total abundance (biomass) showed a more complex dependency on feeding pulse frequencies and amplitudes (Tab. 4.5b). For most pulse characteristic the same results were found for the three-species and for the two-species scenarios (i.e., the Intermediates were not abundant and did not affect the abundance of Growers and/or Switchers). In contrast, some scenarios allowed for an abundance of the Intermediates in addition to the Growers (low to medium amplitudes and lower frequencies) or in addition to Growers and Switchers (low amplitudes and higher frequencies, high amplitudes and low frequencies, or medium amplitudes and medium frequencies). Less often a community of coexisting Growers and Switchers was replaced by a community of Growers and Intermediates (medium amplitudes and medium frequencies). On the other hand, for some scenarios a dominance of Growers in a two-species system was replaced by a coexistence of Growers and Switchers (but no abundance of the Intermediates) in the corresponding three-species system (medium amplitudes and low frequencies). The frequency analysis of the active biomass concentration exhibited a behavior which was very similar to one observed for two-species systems (data not shown).

a)

Freq. (hours^{-1}) → Amlitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
1.00E-06	G	G	G	G	G	GI	G
2.00E-06	G	G	G	G	G	GI	GI
5.00E-06	G	G	G	GI	GI	GI	GI
1.00E-05	G	G	G	GI	GI	GI	GI
2.00E-05	G	G	GI	GI	GI	GI	GI
5.00E-05	G	G	GS	GSI	GI	GSI	GSI
1.00E-04	GS	GS	GS	GS	S	S	S
2.00E-04	GS	GS	S	S	S	S	S
5.00E-04	S	S	S	S	S	S	S
1.00E-03	S	S	S	S	S	S	S
2.00E-03	S	S	S	S	S	S	S
5.00E-03	S	S	S	S	S	S	S
1.00E-02	S	S	S	S	S	S	S

b)

Freq. (hours^{-1}) → Amlitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
1.00E-06	GSI	GSI	GSI	GSI	GS	G	G
2.00E-06	GS	GS	GS	G	G	G	G
5.00E-06	G	G	G	GI	GI	G	G
1.00E-05	G	G	G	GI	GI	GI	GI
2.00E-05	G	G	GI	GI	GI	GI	GI
5.00E-05	G	G	GS	GSI	GI	GS	GS
1.00E-04	GS	GS	GS	GS	S	GS	GS
2.00E-04	GS	GS	GS	S	S	GS	GS
5.00E-04	S	S	GS	S	S	GS	GS
1.00E-03	S	S	GS	S	S	GS	GS
2.00E-03	S	GS	GS	S	GS	GS	GSI
5.00E-03	GS	GS	S	GS	GS	GS	GSI
1.00E-02	S	S	S	GS	GS	GS	GSI

Table 4.5. Abundance of a) active biomass fractions and b) total biomass of Growers and Switchers for the tested feeding pulse periods and amplitudes. Threshold of existence – 1% of active or total biomass 'G'– dominance of Grower; 'S' – dominance of Switchers; 'GS' – coexistence of Growers and Switchers; 'GI' – coexistence of Intermediate species and Growers. 'GSI' – coexistence of all tree microbial species.

The frequency analysis of the active biomass concentration exhibited a behavior which was very similar to one observed for two-species systems (data not shown).

4.4. Discussion

4.4.1 Driving effects

Simulation results show that the characteristics of the intermittent substrate feeding control the competition between the considered microbial species. Both, the amplitude and the frequency of feeding pulses have a considerable impact on the composition of abundance and activity of the microbial community, and thus on the dominance and coexistence/coactivity of species. However, within the tested range of these two parameters, variations of pulse amplitudes has a much higher influence on the competitive activity of species in the system (Tab. 4.3a and 4.5a). On the other hand, although the frequency of feeding was of lesser importance for the competitive behavior, an intermittent supply of substrate is the prerequisite for Switchers to coexist with or to dominate over Growers, while at any constant substrate supply Growers would dominate over Switchers due to their better growth performance (Tab. 4.1). The parameter set describing the growth, activity and dormancy behavior is not only controlling the competitiveness of the single species but also their ability to follow with their activity the frequency of the external feeding, which leads to the complex frequency response observed for the Grower's activity already in the single-species system. Initial conditions (i.e. starting concentration of species) had no impact on the shown results but considering species which turn out to be non-abundant or non-active may influence the long term results of the other species (frequency behavior and/or in some cases also the abundance of species).

4.4.2 Ecological implications

The coexistence of competing species is a know phenomena in systems allowing for a spatial differentiation and leading to the implementation of realized niches (Stolpovsky et al, in press, Tiedje et al., 1999, Adler et al., 2007, Chesson, 1992). The presented simulations confirm previous modeling results (Stolpovsky et al,

2011) showing that intermittent feeding conditions can lead to a long term coexistence of competing microbial species even in the absence of spatial differentiation. Simulations also confirm common observations that active biomass represents only a sub-fraction of the total microbial biomass (Lennon and Jones, 2011), which also explains the difference observed for some scenarios in the composition of active and total biomass. Although there are more scenarios or feeding pulse characteristics allowing for coexistence than for a coactivity of competing species there are quite a number of tested conditions allowing for more than one competing species to contribute to the total activity of the community. For the two-species system (and to lesser extent for the three-species system, too) the dependence of the activity patterns on pulse amplitudes confirm predictions from intermittence disturbance theory with moderate pulse amplitudes best suited to allow for a coactivity of both species. Surprisingly, the frequency of the pulses has – for the tested range of values - less influence of the composition of the resulting communities. It appears that in case of small pulses the ability of the Growers to grow faster and more efficiently allows them to consume most of the substrate and to compensate the delayed reactivation. For a larger pulse the faster and more effective reactivation and lower energy requirements allow Switchers to be active in sufficient amounts to suppress Growers. If these processes take place the same way during each single feeding cycle it would to some degree explain the limited influence of the pulse frequency. However, for many scenarios a rather irregular response of the microbial dynamics to the external feeding frequency has been observed which challenges such straightforward explanations of the observed activity patterns. This irregular frequency response also shows that individual species especially when within interacting communities can exhibit a temporal behavior which is controlled by external forcing dynamics but does not allow establishing a direct causality link. A phenomenon which might also be relevant in the analysis of temporal fluctuations observed in many existing natural ecosystems. Such irregular frequency responses of the Switcher have been found also in case where the abundance and activity of the Growers are considered as negligible although in single species systems the Switchers have shown regular frequency

responses at all tested conditions. This emphasizes the need to assess as many as possible of the species and processes of an ecological system to understand its dynamics and functioning. The potential relevance of species with negligible abundance also becomes apparent in the comparison of the community composition of the two- and the three-species systems. While for most of the tested scenarios introduction of the Intermediate species does not change the composition patterns of active (71 out of 91 scenarios) or total biomass (60 out of 91 scenarios) those scenarios exhibiting a change do not only show the expectable results of Intermediate species coexisting with one or both of the other species: for some scenarios (frequency ≤ 0.02 h^{-1}; amplitude = $5 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ M) a community formerly dominated solely by Growers in the two-species system turns by introducing a subdominant and, thus, indictable species suddenly into exhibiting a coexistence of Growers and Switchers under the same environmental conditions. Similarly, for one scenario (frequency = 0.01 h^{-1}; amplitude = $1 \cdot 10^{-6}$ M) the coexistence of Growers and Switchers for the two-species system turns into a Grower dominated community for the three-species system. For these scenarios the introduction of the Intermediates as an additional competitor has affected the relative advantage or disadvantage between Growers and Switchers in sense that the 'enemy of my enemy is my friend'. When regarding these results the other way round: the ecological consequences for our findings would give evidence to that the coexistence of important key species, and thus, the ecosystem services they provide, can completely fail in an ecosystem if some unimportant subdominant, species become extinct by e.g. human activities.

An opposite situation is observed in simulations with a low DOC (favorable conditions for Growers in two-species system) where we can observe either quantitative coexistence of Growers and Switchers or common quantitative coexistence of all tree species (Table 4.5b).

4.4.3 Comparison of ecological niches

In accordance to the ecological niche theory, species may occupy only subunits of the habitat containing the environmental conditions that enable them to survive

(potential vs. realized niche). Depending on competition strength under the given environmental conditions they dominate over or coexist with other species (Sillero, 2011). As already shown above, the two species system allows distinguishing between conditions leading to a dominance of either Growers or Switchers with coexistence/coactivity found mainly at the transition between the conditions allowing for single species dominance. This is in agreement with considering separated and only partially overlapping niches for each single species (Loreau, 2000). When the third, Intermediate species is introduced it does not affect the dominance patterns of Growers and Switcher, i.e. their abundance is not affected by the presence of the Intermediates. The only exceptions - besides the few examples discussed above – are some scenarios where a coexistence/coactivity of Growers and Switchers is replaced by Growers and Intermediates, indicating a slight reduction in the realized niche size of the Switchers. In all other cases, the Intermediates only represent an additional contribution to the community not being able to outcompete the other species and to occupy their own niche – as suggested by niche theory (Gage, 1996). As a coexistence of Intermediate and Switchers only is not observed, the Intermediates are obviously limited to intruding areas where Growers dominate (Fig. 4.3).

Dormancy is known to be one of the relevant bacterial strategies (Lennon et al., 2011) and under different conditions it could be replaced by or combined with any other advantage, for instance high efficiency of biomass building or bacterial ‘educability’. Considered in this context, the Intermediate species to represent a generalist species while Growers and Switchers represent end members of specialist species, the obtained simulation results suggest that when in competition with both end members a generalist strategy does not lead to any advantages in case of regularly occurring intermittent environmental conditions. Similar observation was suggested in Legan and Owens, 1988. Further investigations will have to show if irregular conditions (irregular changing amplitudes and/or frequencies of feeding pulses; e.g., Wardle, 1995) might lead to different observations.

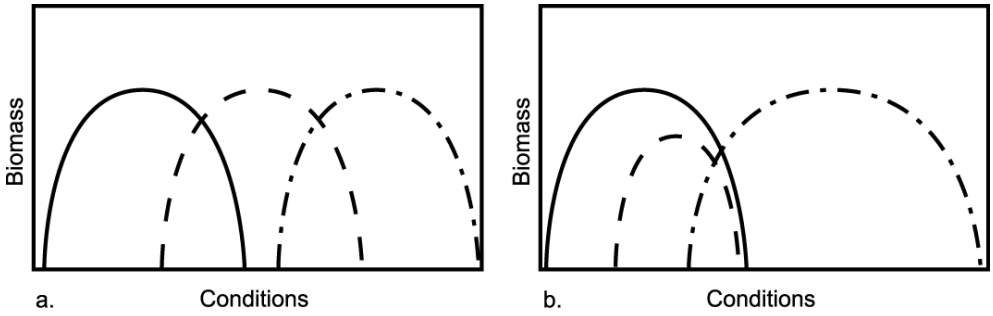

Figure 4.3. Distribution of niches. a) Theoretical prediction (REF), b) niches as found in the simulated scenarios. Solid lines represent Growers, dash-doted lines – Switchers, and dashed - Intermediate species.

4.5. Summary and conclusion

In the present study we showed that intermittent environmental conditions, represented here by the feeding regime, have a high impact on the composition of microbial communities. Depending on the frequency and magnitude of the regularly applied feeding pulses, communities of only up to three different competing microbial species may exhibit rather complex dominance and coexistence patterns and may also exhibit dynamics different from the external forcing. The study also revealed that species abundances below a broadly used threshold value of 1% of the total community (molecular profiling method, Blackwood et al., 2007) may still have an important impact on the competitive performance of the dominating species.

The presented results are theoretical predictions and the discussed hypotheses eventually need to be tested experimentally – a task which is beyond the scope of this study. However, the shown observations do suggest that the characterization of as many species from a community as possible might be necessary to understand the behavior of the community as a whole, and the behavior of its key players responsible for any ecosystem services. This raises the question for the best theoretical approaches to describe the functional dynamics of a complex microbial system: What is the required number of microbial species we need to take into account to obtain sufficiently precise theoretical results? Future research must show

which might be the best compromise between the accuracy of the results, the computational demands and the information needed for simulating the behavior of multi-species microbial communities.

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5

Influence of bacterial diversity on degradation performance and bacterial abundance in biogeochemical models

with Philippe Van Cappellen and Martin Thullner

in preparation

Abstract

Natural environments such as soils and aquifers are highly complex systems characterized by a dynamic interplay between abiotic and biotic processes. In particular, the level of detail required to efficiently capture the dynamics of bacterial abundance and degradation activity in biogeochemical models has not been well defined. Presently, it is not clear how to find a balance between capturing the high complexity of soils and aquifers on the one hand, and pure knowledge of the involved processes on the other. This issue is more acute due to frequent changes of most environmental systems, as fluctuating living conditions causes temporal and spatial coexistence of different bacterial strains involved in the same degradation pathway. Competing species may have very different behaviour so they can not be modelled by a single generalized bacterial species.

The present study investigates model approaches to describe a diverse community of bacterial species. All species are able to switch between active and dormant states, and they are all involved in the degradation of the same substrate supplied under intermittent conditions. For this purpose numerical simulations of a one-dimensional transport system subject to regularly applied substrate feeding pulses were performed. For this transport system the response of microbial communities

containing up to one hundred competing species with different growth characteristics and de-/reactivation efficiencies was simulated. Simulation results showed that a diverse system can be well-represented by so-called end-member species, while considering a single species community leads to less accurate representations. Generally, the simplified models are more efficient in reproducing concentrations of the degraded substrate than in reproducing biomass abundance. This suggests that the dynamics of a microbial community can be adequately described by a simplified community representation but that an acceptable simplification of the microbial systems depends on the purpose of the particular modeling study.

5.1. Introduction

The use of numerical models as quantitative assessment tools in the management of contaminated soils and groundwater aquifers is well established. However, understanding the required complexity and reliability of biogeochemical models is still a common research problem due to the direct involvement of resident microorganisms in the degradation processes. Though complex models containing sufficient levels of detail have become available due to increased computing power, they may be subject to many uncertainties as a large number of parameters is not or only poorly constrained. On the other hand, simple models may oversimplify real world systems and their results are of limited use for assessing real environments (Raicka et al., 2006).

Soils and aquifers are highly complex environments, characterized by a broad variety of chemical and microbial species and functional interconnections between them, temporal variation of concentrations of chemical compounds, and spatial heterogeneity of both bacterial communities and chemical species. It is important to consider microbial abundance and activity, as bacteria play a crucial role in the degradation of organic matter (including contaminants) in nearly all subsurface water bodies of terrestrial environments such as groundwater, soil moisture, water in very low permeability bedrock, and deep geothermal or oil formation water. As less than 1% of the microorganisms observed under the microscope is cultivated and characterized, soil ecosystems are, to a large extent, uncharted. It was estimated that one gram of soil may harbor up to 10 billion microorganisms of possibly thousands of different species (Torsvik et al. 2002). However, many of these species perform under certain conditions the same geochemical reaction (e.g., degradation of a specific organic carbon substrate). On the one hand, involvement of different microbial species in the same degradation pathway simplifies the designing of representations of the system, but still, modeling the transformations of reactants in subsurface environments faces many challenges, including the development of realistic representations of microbial communities and chemical and biological heterogeneities. However, numerous principal modeling approaches that are used to

simulate the degradation of organic matter in porous media such as soils and aquifers were suggested within last decades (Thullner et al., 2007).

Finding the optimal levels of model complexity remains a major question in describing biochemical and ecological systems and has received some attention in theoretical systems over the last 40 years (Fulton et. al., 2003). Many studies considered this problem from a theoretical standpoint and have produced some useful guidelines (O'Neill and Rust 1979, Cale and Odell 1980, Gardner et al. 1982, Cale et al. 1983, Iwasa et al. 1987, Bartell et al. 1988, Rastetter et al. 1992, Fulton 2001), but to our knowledge most of these focus on finding an efficient way to maximize the number of microbially driven reactions within available computational resources. In contrast to theses studies, in the present paper we try to quantify how the results obtained for a simplified model consisting of the major degraders deviate from the results obtained for a highly diverse artificial bacterial community promoting a single substrate degradation pathway.

Despite the high variety of geochemical reactions observed in real systems, conditions in soils or aquifers cannot generally be recognized as plentiful for the resident bacteria, and thus subsurface microorganisms must cope with a variety of stresses, such as periodic lacks of substrate, nutrients and/or water (Martínez-Lavanchy et al., 2010). In other words, they must be able to continuously adapt their metabolism to the conditions in their immediate surroundings, and in order to survive unfavorable environmental conditions microorganisms can switch to an inactive or dormant state (Lennon and Jones, 2011). Dormancy represents a reversible state of low to zero metabolic activity, in which cells can persist for extended periods of time without dividing.

Stolpovsky et al. (2011) indicates that the ability to switch to and from the dormant state substantially widens the spectrum of possible community structures and population dynamics. Even in simple microbial systems, the interactions between growth, decay and the inter-conversion between active and inactive states may cause the emergence of complex behavior that is no longer related in a simple way to the changes in external forcing. In other words, dormancy leads to formation of new ecological niches and allows for the competition between bacteria with high

growth performance and microbial strains with low metabolism and more effective use of the dormancy strategy. This obviously complicates the relationship between geochemical conditions and microbial community composition, as observed, for example, in salt marsh sediments (Koretsky et al., 2005).

Numerical modeling is an approach of growing importance in the field of biogeochemistry. These models are increasingly used to theoretically describe the behavior of bacterial communities under different physical or chemical conditions (Jamieson et al., 2004, Nielsen and Villadsen 1992). Modeling in biogeosciences gives a new insight into the underlying mechanisms for the observed biological phenomena. However, many modeling studies do not consider the contribution of individual bacterial species. In other words, the degradation rate of all bacterial species involved in a degradation pathway is lumped into an cumulative rate of all species involved in the degradation process (e.g. Yeh et al., 2011). In a reaction-based formulation, all biogeochemical processes are conceptualized and transformed into a reaction network (Fang et al., 2003). The aim of the present study is i) to evaluate limitations of such simplified systems and ii) to find the minimum number of species or functional groups needed in a simplified system to allow for an adequate simulation of the abundance and activity of a complex and diverse bacterial community (reference system) controlling a single degradation pathway.

For this purpose the concepts describing the ability of microorganisms to switch into an inactive state (Stolpovsky et al., 2011) are implemented into a one-dimensional transport reactive transport model (Aguilera et al., 2005) simulating microbial processes within a homogeneous column.

5.2. Model description

5.2.1 Simulated processes.

In this study, we consider microbial cells to be represented by two different physiological states: active and inactive. Each bacterial species is able to switch between active and dormant states. Active cells are able to grow/divide, to consume available substrate and are subject to decay/cell death. The rate of growth and

substrate consumption is assumed to follow standard Michaelis-Menten- or Monod-type kinetics (Regnier et al., 2005, Thullner et al., 2007 and references therein);

$$\frac{dC_{DOC}}{dt} = -\theta \cdot k_C \cdot B \cdot \frac{C_{DOC}}{K_{DOC} + C_{DOC}} \cdot \left(1 - \frac{B_{tot}}{B_{\max}}\right) \tag{5.1}$$

where C_{DOC} (M) is the concentration of dissolved organic carbon (DOC) considered as substrate, B (M) is the concentration of active cells, k_C (h^{-1}) is the maximum specific rate of degradation of DOC by the active cells, K_{DOC} (M) is the half-saturation constant for DOC utilization, B_{tot} (M) is the total biomass (all species) at a given depth, and B_{max} (M) is the highest possible concentration of biomass. The biomass growth of active cells is linked to the substrate consumption rate through the growth yield factor Y:

$$\left(\frac{dB}{dt}\right)_{\text{growth}} = -Y \cdot \frac{dC_{DOC}}{dt} = \theta \cdot Y \cdot k_C \cdot B \cdot \frac{C_{DOC}}{K_{DOC} + C_{DOC}} \cdot \left(1 - \frac{B_{tot}}{B_{\max}}\right) \tag{5.2}$$

where θ is a unitless switch function possessing values between 0 (conditions unfavourable for growth) and 1 (conditions favourable for growth), governing growth of active bacteria and the switching of bacteria between active and dormant state and back. Inactive or dormant cells do not exhibit any growth or degradation activity while their decay/death rate is highly reduced compared to their active state. Depending on the environmental conditions cells are assumed to switch between these two states using the potential supply of catabolic energy to the cells as the control variable.

The rate laws of the individual processes are taken from Stolpovsky et al. (2011). There, also more information on the simulated processes and all governing equations can be found. In contrast to Stolpovsky et al. (2011) the present study does not consider a deep dormant state and variable growth yield factors.

The microbial processes described above are implemented into the Biogeochemical Reaction Network Simulator (BRNS) (Regnier et al., 2002; Aguilera et al., 2005), an adaptive simulation environment that can handle complex, mixed kinetic-equilibrium reaction networks. Briefly, this simulation environment consists of a generalized finite difference algorithm for one-dimensional advective-dispersive

transport coupled to a multi-component reaction solver capable of handling the reaction network. The transport and reaction components are solved in sequence within a single time step of numerical integration, following the Sequential Non-Iterative Algorithm (SNIA, Steefel and MacQuarrie, 1996).

5.2.2 Simulated system.

Simulations were performed for microbial systems containing different bacterial species competing for a common, intermittently supplied carbon substrate. A reference system is represented by an artificial bacterial community promoting a single substrate degradation pathway within a one-dimensional transport system. The reference system contains 100 species each degrading the same dissolved organic carbon (DOC) as substrate, which is assumed to be the only specific growth limiting factor (growth is further limited by a maximum carrying capacity). We chose this number of species as it is much higher than the typical number of species usually taken into consideration in modeling while still allowing for sufficiently short computer simulation times.

The simulated generic microbial system is defined by two end-member species: one species (termed 'Growers') has a fast and effective metabolism, i.e. relatively high growth rate, yield factor and mortality rate; in contrast, the second species (termed 'Switchers') is characterized by an effective dormancy, i.e. profitable parameter values for de-/reactivation (Tab. 5.1). The parameter values describing these end members are within the range of the values presented in Stolpovsky et al. (2012). Growers and Switchers have different functional advantages (Tab. 5.1), and also all other considered species are not supposed to have both advantages (i.e. high growth performance and effective switching between activity and dormancy) at the same time. Thus, we consider all further species to have kinetic parameter values placed on the linear gradient between the corresponding parameter values of Growers and Switchers. For example, if some parameter value ranges are given by X_G=1 and X_S=5 (for Growers and Switchers, respectively), then the values for a 5 species system would be given by X = 1, 2, 3, 4 and 5 correspondingly. Analogously the parameter values of the 100 species systems were determined.

Parameters	"Growers"	"Switchers"
Maximum reaction rate of degradation of DOC by microbial group, k_C	0.2 h^{-1}	0.06 h^{-1}
Half-saturation constant for carbon substrate, K_{DOC}	1.0 µM	3.0 µM
Growth yield factor (max), Y_{max}	0.8	0.5
Decay rate of active bacteria, μ_{dec}	1.0e-2 h^{-1}	1.0e-3 h^{-1}
Decay rate of inactive bacteria, μ_{dec}^{in}	1.0e-3 h^{-1}	1.0e-5 h^{-1}
Reactivation yield, Y_{reac}	0.2	1.0
Reactivation rate parameter, μ_{reac}	0 h^{-1}	5.0 h^{-1}
Deactivation rate parameter, μ_{deac}	0 h^{-1}	5.0 h^{-1}

Table 5.1. Parameter values used to represent the two extreme microbial species.

The dynamics of the bacterial communities is simulated using the one-dimensional reactive transport model BRNS (see Section 5.2.1) considering a 0.5 meter long transect along a water filled porous medium. This transport system is exposed to an intermittent substrate supply. Previous studies showed that the composition of a community does not have a strong correlation with the period length of the transient feeding pattern if this period length varies within the range of 1 to 100 h (Stolpovsky et al., submitted). For this study we chose a feeding period length of 1 h as a shorter period of disturbance shortens the required simulation time (the system reaches stable average concentrations of all considered species faster). The magnitude and duration of the individual feeding events was set to 10^{-3} M and to 0.1 h, respectively, to achieve nearly full degradation of the substrate along the simulated transport path. An overview of all parameters describing the properties of the simulated transport system is given in Table 5.2. Note that the flow direction is arbitrarily considered from top to bottom using the term "depth" to characterize the distance from the upstream boundary. Results do equally apply to any other (horizontal) one-dimensional transport system described by the used parameter values.

5.2.3 Simulated scenarios

Simulations were performed for the reference system containing a 100-species community as well as for simpler systems containing either 1 or 2 species. This

study considers two major types of simulations. First, simulations were run until simulated concentrations reached stable average concentrations of all considered species (referred to as “quasi-steady state”). These simulations were performed in order to determine the species dominating the system in the long run, and to analyze the deviation between results obtained for the reference system and quasi-steady state results obtained for systems of 1 or 2 species. Subsequently transient simulations were performed for a relatively short period of 3 months (2160 hours) using different initial conditions. Again focus was given to the deviation between results of the reference system at different initial conditions and the results obtained for the simplified systems with 1 or 2 species, only.

Parameter	Value
Depth	0.5 m
Water flow	0.01 m/h
Longitudinal dispersivity	1.0e-7 m
Water filled porosity	0.4
Amplitude of feeding	1e-3 M
Period of feeding	1 h
Duration of feeding	0.1 h
Maximum biomass carrying capacity	1e-2 M

Table 5.2. Physical characteristics of the transport system.

All simulations were performed considering the same transport conditions (Tab. 5.2). Deviations *D* between concentration profiles obtained for different community sizes are calculated as:

$$D = \frac{\sqrt{(c_r(d,t) - c_s(d,t))^2}}{c_{max}} \tag{5.3}$$

where $c_r(d,t)$ and $c_s(d,t)$ are the concentration of either DOC or biomass at depth *d* and time *t* in the reference system and the analyzed simplified system, respectively, and c_{max} is a maximum possible concentration of either DOC or biomass used for normalization of the result. For the analysis of DOC concentrations c_{max} is the feeding concentration used as intermittent boundary condition, for the analysis of biomass concentration c_{max} is the maximum biomass carrying capacity (Tab. 5.2).

5.3. Results and discussion

Figure 5.1 shows the general behavior of the reference system at quasi-steady state. The top layer up to 15 cm depth exhibits higher concentrations of DOC and total biomass with DOC and active biomass concentrations periodically changing at the frequency of the feeding pulses. In turn, at depths below 15 cm total biomass concentrations drop by approximately a factor of 2 and active biomass concentrations are constant. This strict zonation in the distribution of activity and abundance of the microbial community accompanied by a strong difference in the community composition: the top zone is occupied solely by Switchers and below 15cm depth the system is mostly occupied by Growers with trace contribution of species closely related to Growers (results not shown).

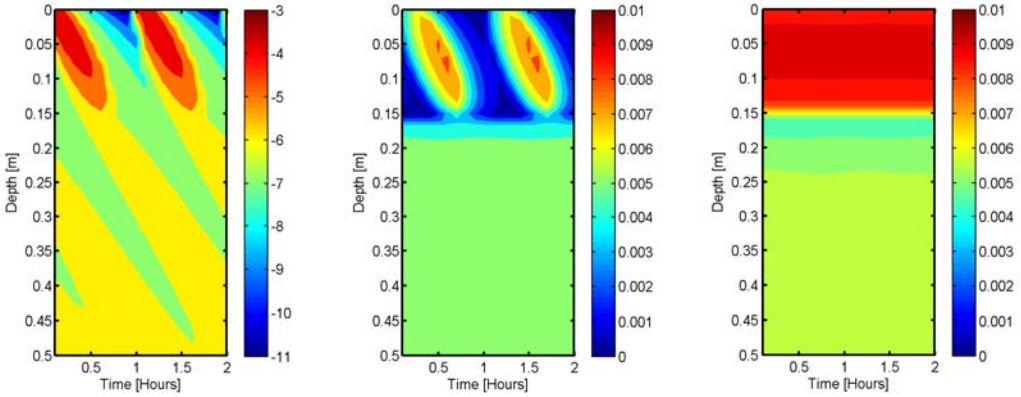

Figure 5.1. General behavior of reference system in quasi-steady state: left - DOC (Log scale, M), middle - active biomass (M), right - total biomass (M).

Analyzing the total biomass in the entire model domain shows that - independently of the initial conditions - the key players in the reference system are the two end member species - Growers (#1) and Switchers (#100) (Fig. 5.2). This bi-modal community composition was obtained regardless of any variations of parameters (data not shown).

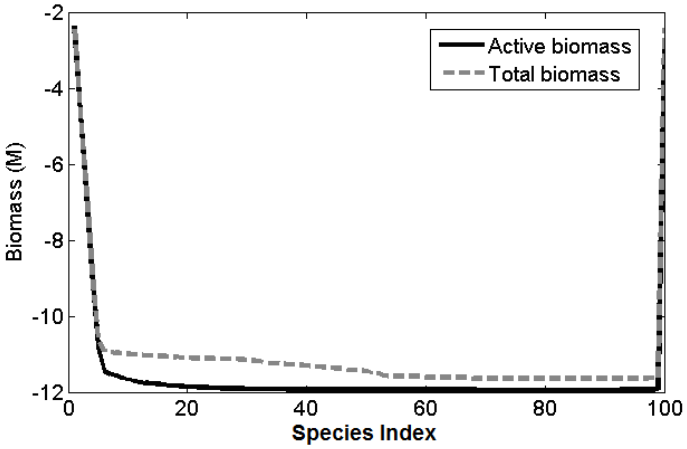

Figure 5.2. Active and total biomass (log scale) of different species in the reference system at quasi-steady state (spatially integrated results). Species 1 represents Growers and Species 100 represents Switchers.

Quasi-steady state results obtained for simulation of the two-species system (results not shown) with Growers and Switchers only are very close to the results obtained for the reference case (Fig. 5.1) and thus provide a very good match for both DOC and biomass (Fig. 5.3). This is in agreement with these two species being the dominant species and thus controlling the concentrations in the reference system. Residual differences are highest for the active biomass (up to 3%). This deviation may be explained by trace activities of the species closely related to the Growers (Fig. 5.2). Though suppressed species introduce visible changes, they do not affect the competitive behavior of dominating species, as was observed in Stolpovsky et al. (submitted).

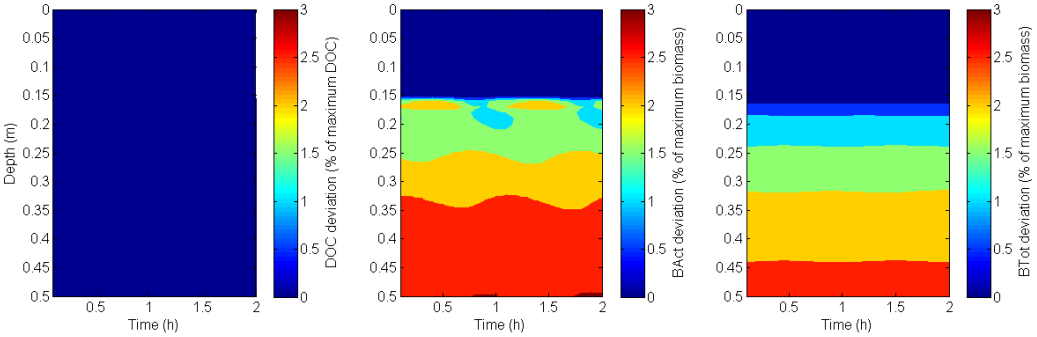

Figure 5.3. Deviation of results of the 2-species system (Growers and Switchers) from results of the reference system at quasi-steady state: DOC concentration (left), active (middle) and total (right) biomass concentration.

Results of the reference system were further compared with results from single-species systems considering as single species either Growers, Switchers or an Intermediate species characterized by the averaged Growers and Switchers parameters.

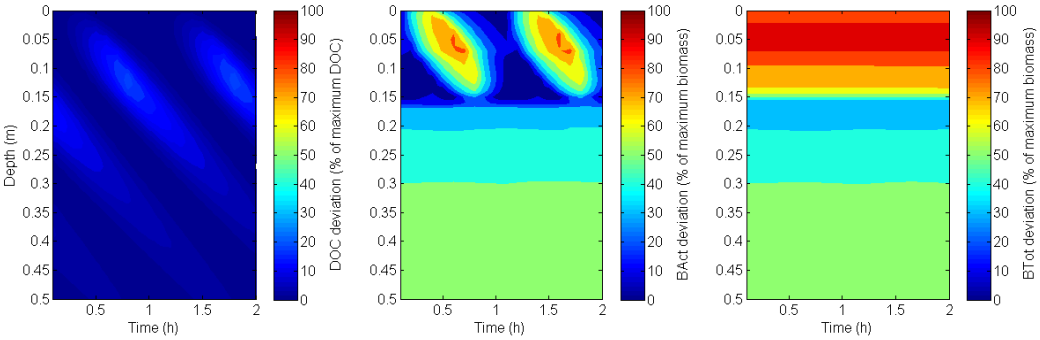

Figure 5.4. Deviation of results of the single-species system (Growers) from results of the reference system at quasi-steady state: DOC concentration (left), active (middle) and total (right) biomass concentration.

The deviations between quasi-steady state results obtained for the reference case and three single-species systems (Growers, Switchers and Intermediate species; Figs 5.4, 5.5 and 5.6) show that none of the single-species systems is able to

represent degradation performance and bacterial abundance and activity of the reference system as well as the two-species system (Fig. 5.3) though the Switchers alone represented degradation performance of the reference system reasonably well (Fig. 5.6).

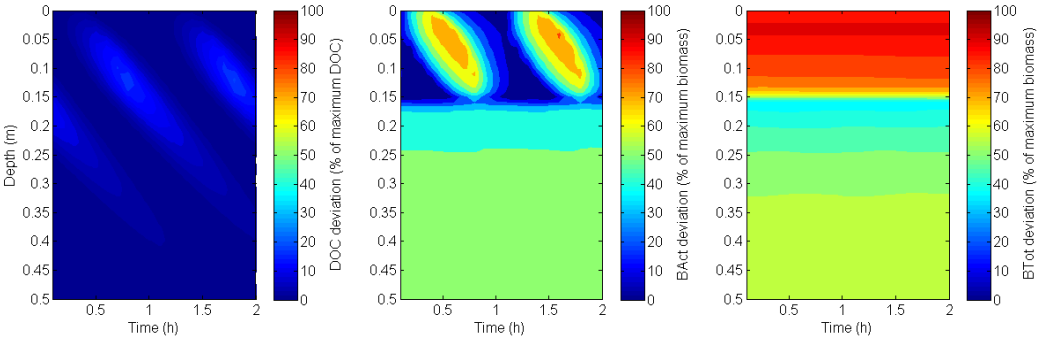

Figure 5.5. Deviation of results of the single-species system (Intermediate species) from results of the reference system at quasi-steady state: DOC concentration (left), active (middle) and total (right) biomass concentration.

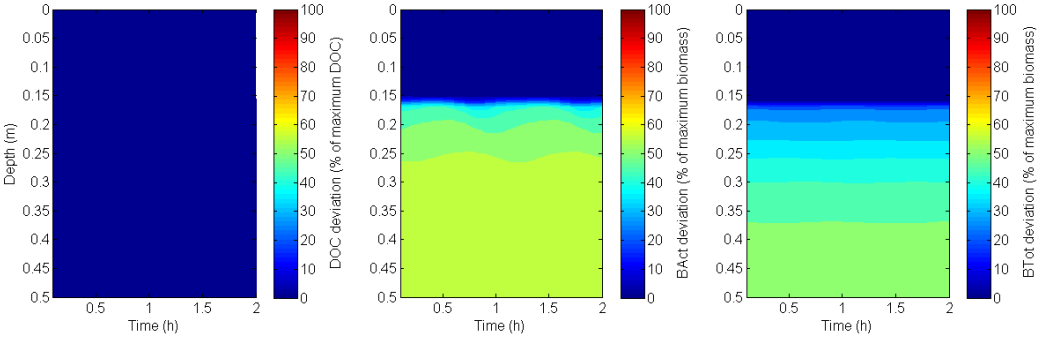

Figure 5.6. Deviation of results of the single-species system (Switchers) from results of the reference system at quasi-steady state: DOC concentration (left), active (middle) and total (right) biomass concentration.

However, quasi-steady state conditions are not likely to be found in natural systems due to long term variations (e.g. seasonal fluctuations) of the environmental conditions, imposing further selective stress for the microbial community (beyond the

variation of substrate supply). As a result, the composition of the microbial community might be more complex than inferred from the quasi-steady state results as different species might respond differently to such additional stress factors (e.g., temperature, nutrient supply, pH) and their variations. For these reasons transient simulations of the reference systems were performed for a period of 3 months (2160 hours) considering three different initial community compositions (and imposed zonation of species, Fig. 5.7; a random distribution, Fig. 5.9; and an even distribution with all species having the same initial concentration). In each simulation, the initial total biomass concentration at each depth was the same and obtained from the quasi-steady state simulations.

In case that initially all 100 species are distributed following a distinct zonation (Fig. 5.7), the maximum deviation with each feeding cycle between transient simulations of the reference system and the two-species system (with initial conditions equal to the results of the quasi-steady state) was used to compare the results for different communities. Similarly, maximum deviations were obtained for random initial conditions (Fig. 5.10) and for an even initial distribution of species (Fig. 5.11). Though the deviation of DOC concentrations gradually approaches zero (left pannel in Figs 5.8, 5.10 and 5.11) which indicates the reference system approaching its quasi-steady state, the period of 3 month (2160 hours) is definitely insufficient for the reference system to reach its quasi-steady state as bacterial abundances (right pannels in Figs 5.8, 5.10 and 5.11) and even more pronounced bacterial activities (middle pannels in Figs 5.8, 5.10 and 5.11) still show strong deviations. On the other side, results obtained for different initial conditions are rather similar, this indicates that the end-member species, i.e. Growers and Switchers, quickly suppress other species and then compete with each other at a longer time scale trying to settle their niches. The period until all intermediate species are suppressed by the end-member species explains is characterized by initial (0 - 500 h) peaks of deviation found for both, active and total biomass, at the top layers (0 - 15 cm) in the transient simulations (Figs 5.8, 5.10 and 5.11). Note that in all cases initial total biomass is identical to the results obtained for the quasi-steady state simulations. In general, these results indicate that in case the composition of the community is not well

known, its replacement by a simplified community consisting of the end members only leads to mispredictions of DOC concentrations in the range of 20% or less. This errors, although not negligible are in the range of other uncertainties associated with *in situ* microbial degradation rates cause e.g. by the limited bioavailability of the substrate (Gharasoo et al., 2012).

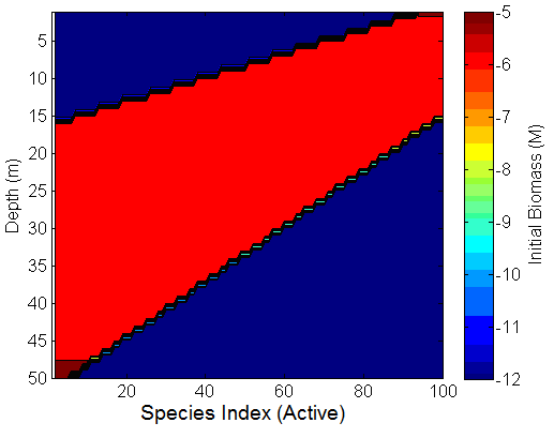

Figure 5.7. Initial concentration of active biomass (concentrations of inactive biomass is set to zero initially) of the reference system considering an imposed zonation.

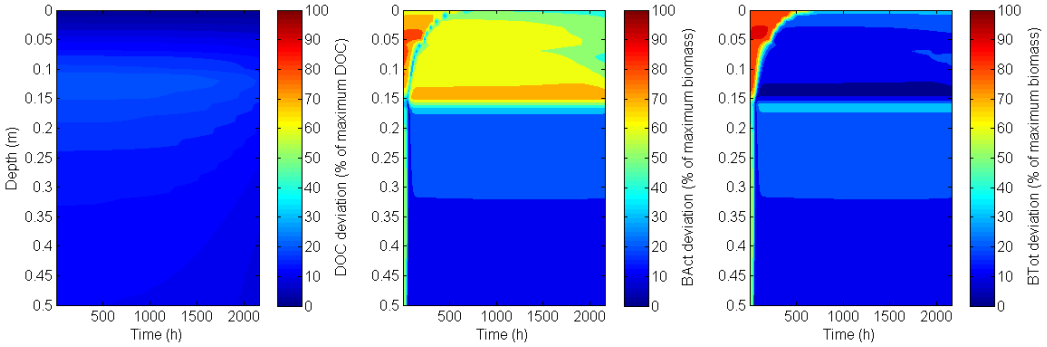

Figure 5.8. Maximum deviation from the reference system of DOC (left), active (middle) and total (right) biomass concentration, obtained for transient simulations of a two-species system and a reference systems with imposed initial zonation of species.

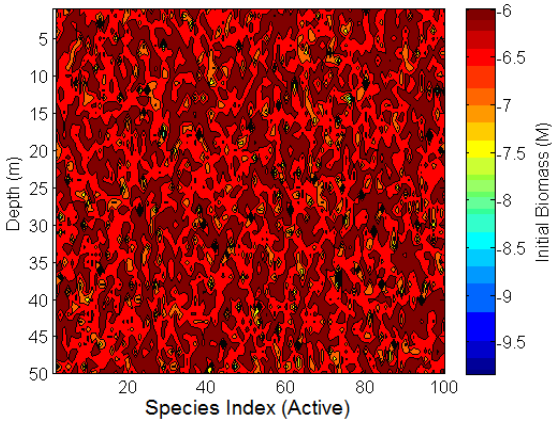

Figure 5.9. Initial concentration of active biomass (concentration of inactive biomass is set to zero initially) of the reference system considering a random distribution.

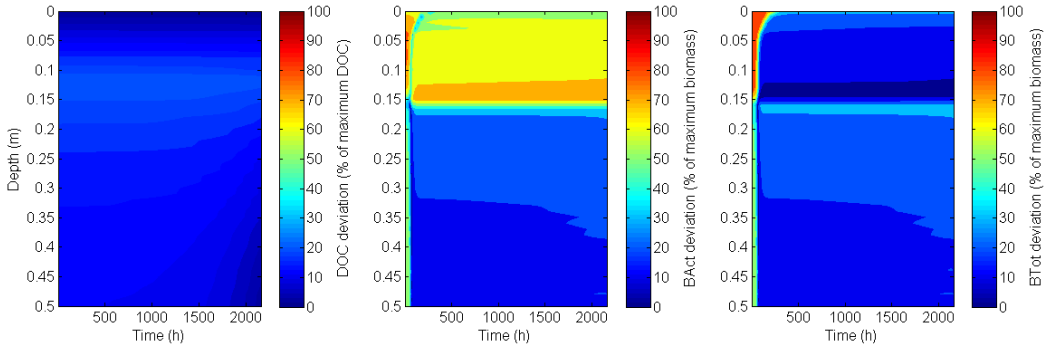

Figure 5.10. Maximum deviation from the reference system of DOC (left), active (middle) and total (right) biomass concentration obtained for transient simulations of a two -species system and a reference systems with random initial distribution of species.

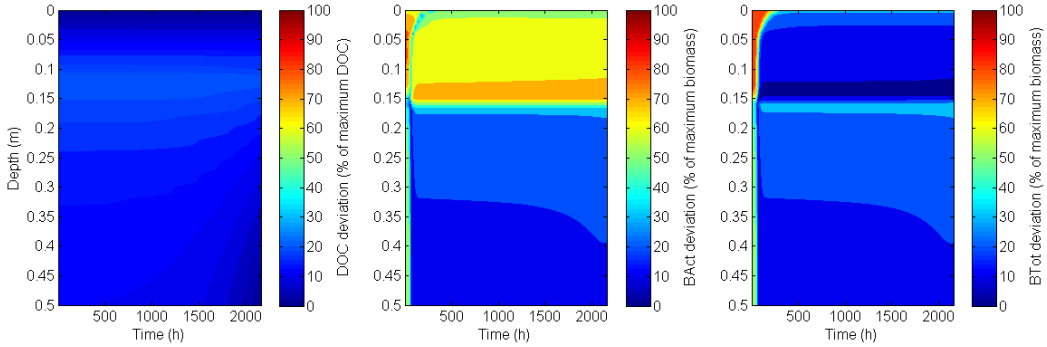

Figure 5.11. Maximum deviation from the reference system of DOC (left), active (middle) and total (right) biomass concentration obtained for transient simulations of a two-species system and a reference systems with an even initial distribution of species.

5.4. Summary and conclusions

In the present study we showed the influence of bacterial diversity in biogeochemical models. It needs to be pointed out that the reference system considered in this study is still too simple to introduce the diversity inherent to natural systems, which is further emphasized by reference system characterized at steady state by only two end-member species (i.e. Growers and Switchers, Fig. 5.2). This is explained by the relative simplicity of the model, since it does not take into consideration factors such as the spatial heterogeneity of the inhabited environment, the variety of chemical pathways, the ability of bacteria to be respond differently to fluctuations of physical factors (temperature, pH), the various interconnections between different species (symbiosis, predation, educability) and further more.

Though the reference system ended up with a relatively simple composition of the bacterial community, it was sufficient to conclude that the end-member or key species of a community can sufficiently well describe the degradation performance of the system. Thus, knowledge of the range of variability of the metabolic potential of species within a community and the recognition of key species has a crucial impact on the accuracy of the model. Negligence of any key species may lead to significant deviation of the results specifically when focus is given to distribution of

microbial abundance and activity. However, determination of any common representative species (e.g., Switchers in the case of this study) or even excluding bacteria from the system and replacing it with degradation capacity of the media (Yu et al., 2010) can be a good alternative if the aim of observation is to estimate dynamics of chemical species only. Though the question of prediction of number and quality of key species lies beyond the scope of this study, we may assume that it is determined by the number of different survival strategies used by members of the bacterial community (Chesson, 1992). The present study has shown that in a general case, the key species are a necessary and sufficient set of bacterial species needed to successfully simulate both degradation performance and bacterial abundance and activity and that end members of the spectrum of species within microbial communities might be good candidates for such key species.

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6

Summary

Even though any sample taken from the terrestrial subsurface is usually a composite of many microhabitats that change with many temporal and spatial characteristics of the sampled system (e.g., water content, flow and transport paths, plant effects, distance to surface), modeling microbial activity in soils and aquifers is an essential tool to unravel the relationships between different microbial species and degradation pathway, to quantitatively assess microbially driven reactions and to determine the factors influencing them.

Nowadays, different modeling techniques are rapidly expanding in numbers, reliability and sensitivity. Moreover, the rapidly increasing pool of genetic information concerning many microorganisms provides the opportunity to understand ecological processes at all scales of biological organization. However, microbial model systems still contain numerous difficulties such as lack of physiological details, insufficient spatial resolution, inability to consider individual microbial strains in natural media, etc. The aim of this thesis is to build a more general mathematical representation of bacterial behavior, addressing in particular microbial dormancy and its inclusion into microbial modeling concepts. This gives a chance to determine which characteristics of a microbial community or of individual species determine the overall carbon degradation activity of the community, and to test hypotheses on and to obtain new insights into the factors controlling the dynamics of biogeochemical processes. Moreover, simulations were performed to determine the level of model complexity needed for an efficient approximation of natural biogeochemical systems.

Chapter 2 focuses on an extended batch modeling concept which is used to show the basic effects of microbial growth and decay, and which explicitly accounts for their ability of microorganisms to switch between active and dormant states. In summary, the proposed model shows that it is possible to quantitatively account for

changes in the physiological state of microorganisms under changing environmental conditions. Specifically, this chapter shows that (1) a conceptual approach describing the de- and reactivation of bacteria is able to explain dormancy behaviors observed in experiments; (2) simulation results indicate that the ability to switch to and from the dormant state increases the overall resilience of the microbial system; (3) the long term activity of microbial performance can be dominated by bacteria having the most efficient dormancy adaptation; and (4) bacteria with the best growth performance may be suppressed.

Chapter 3 considers the impact of pore size heterogeneities of natural porous media such as soils or aquifers on the distribution of abundance and activity of a bacterial community. This community consists of competing species able to respond to external environmental stress periods by switching from an active into an inactive or dormant state and back. This chapter shows that (1) observed spatial distribution patterns confirm results from earlier studies, showing the impact of heterogeneity and preferential flow patterns on microbial abundance and activity; (2) intermittently changing environmental conditions lead to strong fluctuations of bacterial activity but to stable distributions of bacterial abundance; (3) heterogeneity leads to a larger number of small niches dominated by a single species and to an increased number of individual pores with species coexistence; (4) competitive advantage of single species is not controlled by a single environmental factor but by a combination of different factors (characteristics of DOC dynamics); and finally (5) not only a single set of environmental conditions might be optimal for the competitive behavior of species.

Chapter 4 deals with the response of microbial model systems containing two and three competing species with different growth and de-/reactivation efficiencies on different regimes of intermittent changing environmental conditions. The chapter mainly focuses on conditions controlling the coexistence/dominance of species and on the explicit influence of so-called suppressed species on dominating species. This chapter showed that (1) intermittent environmental conditions, represented here by the feeding regime, have a high impact on the composition of microbial communities: depending on the frequency and magnitude of the regularly applied

feeding pulses, communities of up to three different competing microbial species may exhibit rather complex dominance and coexistence patterns and may also exhibit dynamics that differ from the external forcing; (2) species abundances below a broadly used threshold value of 1% of the total community may still have an important impact on the competitive performance of the dominating species; (4) the characterization of as many species from a community as possible may be necessary to understand the behavior of both, the community as a whole and the key players responsible for any ecosystems.

Finally, Chapter 5 investigates the influence of bacterial diversity in biogeochemical models and considers the interactions of different microbial species involved in a common degradation process. This chapter showed that (1) a highly diverse artificial bacterial community (the reference system) promoting one reactive pathway of substrate degradation can be sufficiently well described with the system of end-member or key species; (2) some simpler single-species systems can describe the degradation performance of the reference system well enough, but prediction of bacterial abundance is still of poor quality.

Samenvatting

Een monster van de terrestrische ondergrond bevat meestal vele microhabitats. Deze microhabitats kunnen veranderen afhankelijk van de tijd en plaats van de bemonstering van het systeem (bijvoorbeeld door veranderingen in watergehalte, stroming en transport wegen, effecten van planten, afstand naar het oppervlak). Het modelleren van microbiële activiteit in bodems en aquiferen is daarbij een belangrijk middel om de relaties tussen de verschillende microbiële soorten en degradatiewegen te ontrafelen, om zo microbieel gedreven reacties te kwantificeren en de factoren te bepalen die deze reacties beïnvloeden.

Er is tegenwoordig een enorme groei in de hoeveelheid, betrouwbaarheid en gevoeligheid van modelleertechnieken. De toename in genetische informatie van micro-organismen biedt bovendien de mogelijkheid om ecologische processen van alle biologische niveaus te doorgronden. Er zijn echter ook nog tal van problemen met het modelleren van microbiële systemen, zoals het gebrek aan fysiologisch detail, onvoldoende ruimtelijke resolutie, gebrek aan mogelijkheden om individuele microbiële stammen in natuurlijke media mee te nemen, etcetera. Het doel van dit proefschrift is om een meer algemeen wiskundig model te maken van bacterieel gedrag, met in het bijzonder een focus op microbiële ruststadia en het integreren van deze ruststadia in een conceptmodel. Dit geeft de mogelijkheid om te doorgronden welke karakteristieken van een microbiële gemeenschap en/of een individuele soort precies de algehele koolstof afbraak van een gemeenschap beïnvloeden. Tevens geeft het de mogelijkheid om hypothesen te testen en nieuwe inzichten te vergaren over welke factoren de dynamiek van biogeochemische processen bepalen. Bovendien zijn er simulaties uitgevoerd om te bepalen welk niveau van model complexiteit nodig is om een inschatting te maken van natuurlijke biogeochemische systemen.

Hoofdstuk 2 is gericht op een uitgebreid groepsmodelconcept dat wordt gebruikt om de basis effecten van microbiële groei en afbraak te presenteren, maar ook expliciet rekening houdt met het vermogen van micro-organismen om te schakelen tussen een actieve en inactieve toestand. Het voorgestelde model laat zien dat het mogelijk is om kwantitatief rekening te houden met veranderingen in de

fysiologische toestand van microben onder variërende milieucondities. In het bijzonder laat dit hoofdstuk zien dat (1) een conceptuele benadering van de de- en reactivatie van micro-organismen het inactieve gedrag zoals geobserveerd in experimenten kan verklaren; (2) dat het vermogen om te schakelen van en naar de rusttoestand de gehele veerkracht van het microbiële systeem toe laat nemen; (3) op lange termijn de activiteit van microbiële prestatie gedomineerd kan worden door bacteriën die de meest efficiënte 'slaap' adaptatie hebben; en (4) bacteriën met de beste groeiprestaties mogelijkerwijs onderdrukt kunnen worden.

Hoofdstuk 3 neemt de impact van de heterogeniteit van poriegrootte van natuurlijk poreus materiaal, zoals bodems of aquifers, op de distributie van voorkomen en activiteit van de bacteriële gemeenschap in beschouwing. Deze gemeenschap bestaat uit concurrerende soorten die kunnen reageren op stress periode in hun buitenmilieu door te schakelen van een actieve toestand naar een rusttoestand en weer terug. Dit hoofdstuk toont aan dat (1) de geobserveerde ruimtelijke distributie patronen bevestigen eerdere studies, wat de impact aantoont van heterogeniteit en preferente stroom patronen op het voorkomen en de activiteit van micro-organismen; (2) sporadisch veranderende milieucondities leiden tot sterke variaties in bacteriële activiteit, maar ook tot stabiele patronen waar bacteriën voorkomen; (3) heterogeniteit leid tot grotere aantallen van kleine niches die gedomineerd worden door één soort en een toenemend aantal individuele poriën met soort coëxistentie; (4) het competitie voordeel van een enkele soort wordt niet gecontroleerd door één enkele milieufactor, maar door a combinatie van factoren (karakteristieken van DOC dynamieken); en tenslotte (5) niet alleen één enkele set van milieucondities hoeft optimaal te zijn voor het competitieve gedrag van soorten.

Hoofdstuk 4 beschrijft de reactie van microbiële modelsystemen, bestaande uit twee of drie concurrerende soorten met verschillende rendementen wat betreft groei en de-/reactivatie, op verschillende patronen van periodiek veranderende milieucondities. Het hoofdstuk focust vooral op die condities die de co-existentie of dominantie van soorten bepalen en op de expliciete invloed van zogeheten onderdrukte soorten op dominante soorten. Dit hoofdstuk toont aan dat (1) periodiek veranderende milieucondities, die hier worden geïmplementeerd als veranderingen

in het voedingspatroon, een grote invloed hebben op de samenstelling van microbiële gemeenschappen: afhankelijk van de frequentie en omvang van de regelmatig toegepaste voedingspulsen kunnen gemeenschappen bestaande uit maximaal drie concurrerende soorten vrij ingewikkelde patronen van dominantie en co-existentie laten zien, die in sommige gevallen anders zijn dan het patroon van de externe condities; (2) een soort met een lagere abundantie dan de algemeen gebruikte drempelwaarde van 1% van de totale gemeenschap nog steeds een belangrijke invloed kan hebben op de competitieprestaties van de dominante soort; (3) de karakterisering van zoveel mogelijk verschillende soorten uit een gemeenschap nodig kan zijn om het gedrag van zowel de totale gemeenschap als van de essentiële soorten van elk ecosysteem te begrijpen.

In hoofdstuk 5 wordt ten slotte het effect van bacteriële diversiteit in biogeochemische modellen bestudeerd. Ook wordt er gekeken naar de interacties tussen verschillende microbiële soorten die bij hetzelfde degradatieproces betrokken zijn. Dit hoofdstuk toont aan dat (1) een zeer diverse, artificiële, bacteriële gemeenschap (het referentiesysteem), die een bepaald reactiepad bij de degradatie van het substraat bevordert, toereikend kan worden beschreven met alleen de essentiële of 'end-member' soorten; (2) sommige eenvoudigere systemen met één soort wel de mate van degradatie in het referentiesysteem goed kunnen beschrijven; de voorspellingen van de hoeveelheden bacteriën zijn daarentegen van slechte kwaliteit.

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There is only my name on the cover of this thesis, but it doesn't mean that I did it alone, so I can imagine that this is the part that will be red by most.

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Curriculum Vitae

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