Doctoral Thesis

Experimental and numerical investigations of bioclogging in porous media using two-dimensional flow fields

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EXPERIMENTAL AND NUMERICAL INVESTIGATIONS OF BIOCLOGGING IN POROUS MEDIA USING TWO-DIMENSIONAL FLOW FIELDS

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH
for the degree of
DOCTOR OF NATURAL SCIENCES

presented by
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2001
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Zusammenfassung


Zur Interpretation der experimentellen Beobachtungen wurde eine Vielzahl verschiedener Modelle entwickelt, die jedoch nicht in der Lage waren, die in den Experimenten beobachteten Reduzierungen der hydraulischen Leitfähigkeit zufriedenstellend zu erklären. Es wurde angenommen, dass dieser Missstand teilweise dadurch zu begründen ist, dass diese Modelle keine Verbindung zwischen den Poren in mehr als einer Dimension annehmen. Als weiterer Grund wurde genannt, dass diese Modelle lediglich das Wachstum der Biomasse in Form eines Biofilms berücksichtigen, es aber denkbar wäre, dass unter Annahme einer alternativen räumlichen Verteilung der Biomasse (z.B. Mikrokolonien) eine bessere Übereinstimmung zwischen Simulation und Experiment erzielt werden kann.


Die Ergebnisse in Kapitel 4 zeigten ausserdem die Notwendigkeit einer hohen räumlichen Messauflösung, um Bioclogging in mehrdimensionalen Strömungsfeldern zu untersuchen. Um Bioclogging im Feld zu untersuchen, ist es daher notwendig eine möglichst hohe Auflösung unter Feldbedingungen zu gewährleisten. Aus diesem
Gegrundet wurde in Kapitel 6 die "Dual Pumping Technique" hinsichtlich ihrer Anwendbarkeit zur Messung vertikaler Konzentrationsprofile im Grundwasser untersucht.

Als ein Beispiel für Bioclogging auf einer größeren Skala als im Labor wurde das mikrobiell bedingte Verstopfen von Langsamfiltern in der Trinkwasseraufbereitung untersucht. In den obersten Zentimeter der Filter war das Clogging am stärksten, wodurch der wiederholte und aufwendige Abtrag dieser Schicht notwendig war.
Summary

The change of hydraulic conductivity and porosity of a saturated porous medium due to growth of microbial biomass is known as bioclogging. Microbial biomass is composed of bacterial cells and extracellular polymeric substances (EPS), the latter being produced by the bacteria. Until now bioclogging has been investigated mainly in laboratory systems that resembled a one-dimensional flow field. Results of these studies showed that biomass was able to reduce hydraulic conductivity up to three orders of magnitude.

To interpret these experimental observations several different models have been developed in the past. These models were not able to explain the observed reductions of hydraulic conductivity sufficiently. It was assumed that this failure is caused partly by the fact that these models did not take into account pore interconnectivities in more than one dimension. In addition, it was suggested that assuming the biomass to grow not only as a biofilm, but also in form of micro-colonies, may yield a better agreement between experimental data and simulations.

The focus of this thesis was on the investigation of bioclogging in two-dimensional flow fields. Bioclogging effects were investigated theoretically using numerical simulations, and experimentally in the laboratory. In Chapters 2 and 3 bioclogging was theoretically investigated using two-dimensional pore-network simulations, assuming the biomass to grow in colonies and as a biofilm, respectively. Results from these simulations were different from results of previous clogging models, showing better agreement with experimental data from the literature, especially for the case of biomass growth in colonies.

In Chapter 4 bioclogging was investigated experimentally in a flow cell with a two-dimensional flow field. The flow cell (56 cm × 44 cm × 1 cm) was operated under a continuous flow of a mineral medium containing nitrate as electron acceptor. To initiate microbial growth, a glucose solution was injected through an injection port, simulating a point source of carbon substrate. During the experiment biomass distribution and the migration of a tracer dye were observed using visible light transmission. In contrast to studies in one-dimensional flow fields, flow bypass of
the clogged areas was possible, resulting in different observations than those reported before. At the end of the experiment (after 30 days), porous media samples were analyzed for abundance of bacterial cells, bacterial cell volume and concentration of polysaccharides and proteins. The analysis of the biomass composition showed that the majority of the biomass was composed of EPS. Thus, the clogging effects were assumed to be caused by these substances and not by the bacteria.

In Chapter 5 the clogging models derived in Chapter 2 as well as a previously published model were used to simulate the experiment described in Chapter 4. A comparison between simulation and experimental results showed that the colony model introduced in Chapter 2 yielded the best agreement with regard to the biomass distribution and its influence on the flow field. The biofilm model introduced in Chapter 2 was also able to reproduce the measured data but with different parameter values than determined by the pore network simulations. The third model, which was taken from the literature, was strongly underestimating the clogging potential of the biomass. These results indicate that it is necessary to include multi-dimensional effects already on the pore scale in order to get an appropriate description of bioclogging in porous media.

Results from Chapter 4 showed also that investigating bioclogging in multi-dimensional flow fields requires sampling with high spatial resolution. To be able to study bioclogging in the field, it is therefore necessary to achieve high spatial resolution under field conditions, too. Therefore, in Chapter 6 the dual pumping technique was validated to measure vertical concentration profiles in groundwater.

Finally, an example was given for bioclogging on a scale larger than in the lab (Chapter 7). In this Chapter bioclogging of slow sand filters used for drinking water treatment was investigated. Clogging occurs mainly in the top centimeters of the filters, which makes it necessary to remove this top layer periodically.
Chapter 1

Introduction

In a lot of countries groundwater is an important natural resource. In Switzerland for example approximately 40\% of the drinking water are obtained directly from groundwater (Swiss Federal Statistical Office 1998). The protection of this resource is of great importance and thus the remediation of groundwater pollution is often required (e.g. Swiss Council of Ministers 1998; U.S. Environmental Protection Agency 1996). Among several other techniques, in situ bioremediation is used for the remediation of aquifers, contaminated by organic pollutants. The application of this technique may be associated with an increased production of biomass in the subsurface (e.g. Zarda et al. 1998) and this has the potential to plug the aquifer’s pores thus reducing the hydraulic conductivity of the aquifer. This effect is called bioclogging. The aim of this thesis is to provide an improved understanding of this effect.

1.1 Remediation of contaminated aquifers

1.1.1 Sources of groundwater contamination

Groundwater contamination is a major problem in many countries, especially if the groundwater is used for drinking water production or if groundwater is flowing into surface waters (e.g. rivers or lakes). In analogy to Wiedemeier et al. (1999) most sources of groundwater contamination are given by one or more of the following
scenarios:

• Aqueous phase release to the subsurface. Under this scenario aqueous phase contaminants enter the subsurface and reach groundwater via areal groundwater recharge or via recharge through an injection well (Fig.1.1a).

• NAPL release to vadose zone only. Here contaminants enter the vadose zone as NAPLs (non-aqueous phase liquids), which are then dissolved by water from recharge and act as a source of contamination of this water. Via this recharge the contaminants finally reach the groundwater (Fig.1.1b).

• LNAPL release to the water table. An LNAPL (lighter-than-water NAPL) penetrates through the vadose zone and forms a pool on the water table. If the water table is fluctuating this NAPL pool is partly distributed within a smear zone. As groundwater gets into contact with the NAPL it dissolves the contaminants and the contamination is spread by the water flow (e.g. Schluep 2000). This is the typical scenario for petroleum hydrocarbon contaminations (Fig.1.1c).

• DNAPL release to the saturated zone. If DNAPLs (denser-than-water NAPLs) enter the subsurface in sufficient amount, they may penetrate the water table and move further down into the saturated zone until they reached the aquitard or the entire DNAPL body is immobilized by capillary forces or dissolved (Schwille 1988). Water getting in contact with the DNAPL dissolves components of the DNAPL resulting in a contamination of the groundwater. Typical DNAPL contaminants are chlorinated hydrocarbons (Fig.1.1d).

Additionally groundwater may become contaminated by changes in groundwater pH due to acid mine drainage or rain, which result in a mobilization of toxic minerals or metal ions. At some sites the degradation of the primary pollutant may cause the production of a secondary pollutant, which is more toxic than the primary one.
1.1.2 Available remediation technologies

The remediation of a contaminated groundwater site is often required (e.g. Swiss Council of Ministers 1998; U.S. Environmental Protection Agency 1996). As each contaminated site may have its own specific characteristics, a variety of different remediation techniques have been developed. These remediation techniques may in-
clude physical, chemical or biological means to remove the contamination. Physical means include e.g. the excavation and incineration of contaminated aquifer material or the extraction of contaminated water via pumping wells. An example of chemical means are reactive walls containing ferric iron, which catalyze a reductive dechlorination of chlorinated hydrocarbons. Biological remediation techniques use the ability of microorganisms to degrade many organic contaminants. An additional way to classify remediation techniques is to distinguish between in situ and ex situ technologies. Tab.1.1 gives an overview on common remediation techniques. A detailed review of available remediation techniques is e.g. given in DVWK Schriften (1991), and in situ bioremediation is also discussed in Section 1.1.3. Which technique or which combination of techniques is applicable at a given site depends on the kind of contamination, the risk associated with the contamination, the hydrogeology of the site, the infrastructure, the time available for remediation and the legal requirements.

1.1.3 In situ bioremediation

At sites contaminated with organic products (e.g. petroleum hydrocarbons), in situ bioremediation may be an applicable option. This technique uses the ability of microorganisms to degrade organic contaminants inside the aquifer (Fig.1.2). Whether bioremediation is successful at a specific site depends on a combination of the properties of the contaminants, the indigenous microorganisms and the site (Hinchee et al. 1994). For a sustained in situ degradation to take place the following conditions have to be fulfilled:

- The indigenous microorganisms must be able to degrade the contaminant.

- Sufficient amounts of oxidants (e.g. oxygen, nitrate, iron, sulfate) and nutrients (e.g. ammonium, phosphate) must be present in the aquifer.

- The contaminant must be available to the microorganisms.

- Contaminant concentration should not reach levels, which are toxic to the microorganisms.
The groundwater flow field should provide sufficient nutrient delivery to the microorganisms to support high biodegradation rates.

If a contaminated site fulfills all these requirements, intrinsic in situ bioremediation may be considered as remediation technique. In this case no active remediation is necessary, but an extensive monitoring program is needed, which has to be maintained for a long period of time. If a lack of oxidants or nutrients prevents the biodegradation of the contaminants or if the intrinsic rates of degradation are too

<table>
<thead>
<tr>
<th>Technique</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excavation</td>
<td>contaminated soil or aquifer material is permanently removed from the site and treated elsewhere</td>
<td>contamination is permanently removed from the site</td>
<td>high costs, not applicable if buildings etc. are on the site, soil is destroyed</td>
</tr>
<tr>
<td>Pump and treat</td>
<td>contaminated groundwater is pumped out of the aquifer, cleaned and then injected again</td>
<td>no advanced subsurface engineering necessary if contaminants are present as NAPLS or adsorbed on the matrix no sustainable cleanup possible</td>
<td></td>
</tr>
<tr>
<td>Encapsulation</td>
<td>contaminated parts of the aquifer are surrounded by subsurface walls</td>
<td>no long term engineering necessary</td>
<td>not applicable to widespread contaminations or in deep aquifers</td>
</tr>
<tr>
<td>Funnel and gate</td>
<td>contaminant groundwater flow is forced to pass a reactive wall, which initiates the degradation of the contaminants</td>
<td>no continuous activities necessary</td>
<td>remediation efficiency of the walls may decrease over time (also due to clogging)</td>
</tr>
<tr>
<td>Air sparging</td>
<td>air is injected into the aquifer to allow volatilization of contaminants and to stimulate biodegradation of contaminants</td>
<td>low costs, no water or matrix material treatment necessary</td>
<td>very local effect</td>
</tr>
<tr>
<td>Engineered in situ biodegradation</td>
<td>microbial degradation of contaminants is stimulated by injection of oxidants and/or nutrients</td>
<td>no above ground treatment of water or material necessary, contaminants are degraded without waste production</td>
<td>long term engineering necessary, success limited by aquifer heterogeneity</td>
</tr>
<tr>
<td>Intrinsic in situ bioremediation</td>
<td>indigenous microorganisms degrade the contaminants with oxidants present in groundwater or on the matrix</td>
<td>no engineering necessary</td>
<td>no fast remediation success, long term monitoring necessary</td>
</tr>
</tbody>
</table>

Table 1.1: Selected remediation techniques
slow, engineered in situ bioremediation might be an alternative. In this case oxidants or nutrients are supplied to the aquifer (usually via injection wells) in order to stimulate microbial activity and hence the biodegradation of the contaminants. To reach a uniform distribution of these oxidants or nutrients, the heterogeneity of the aquifer must be small (U.S. National Research Concil 1993). Otherwise the injected substances may bypass contaminated parts of the aquifer. Another method to increase in situ biodegradation rates is the injection of microorganisms, known as bioaugmentation, a method which is considered mainly for chlorinated hydrocarbon contaminations (Ellis et al. 2000; Salanitro et al. 2000).

As in situ bioremediation often leads to an increased biomass production in the aquifer, an interaction between the biomass and the water flow may take place, influencing the success of the bioremediation.
1.2 Definition of bioclogging

The interaction between water flow and solute transport in porous media is a well described phenomenon (Bear 1972; Scheidegger 1974). Reactive processes (e.g. chemical reactions or microbial growth) in porous media are controlled by the presence of dissolved reactants or nutrients (e.g. Chapelle 1993). Therefore these reactive processes depend on solute transport and thus also on the water flow in the porous medium. Descriptions of these interactions usually assume that the hydraulic properties of the porous medium are constant and not affected by transport or reactive processes. Nevertheless the hydraulic properties of porous media may change over time. The causes of this change can be divided into three classes: physical, chemical and microbial (Vandevivere and Baveye 1992c). Physical causes may be particle deposition or media compaction, chemical causes may be dissolution of the porous media matrix or precipitation of solutes, microbial causes may be production of gas bubbles by microorganisms or the production of large amounts of biomass plugging the pores of the porous medium. The focus of this thesis is on the latter cause, the production of biomass. The ability of microorganisms to reduce the saturated hydraulic conductivity of porous media is known and defined as biological clogging or bioclogging (Baveye et al. 1998). Although gas production and biomass accumulation often occur simultaneously, the term bioclogging will be used in this thesis exclusively for the reduction of hydraulic conductivity due to biomass accumulation. Understanding of bioclogging in porous media therefore requires the study of interactions between water flow and solute transport, and solute transport and microbial growth but also the feedback between microbial growth and water flow (Fig1.3).

1.3 Occurrence and significance of bioclogging

Bioclogging was first discovered in laboratory tank experiments by Slichter (1905) and it may also be an important process on the field scale. Reductions of hydraulic conductivities in the field, which were at least partly attributed to bioclogging, were observed at the bottom of artificial water reservoirs (Mirtskhulava et al. 1972) or
Bioclogging was mostly investigated in connection with the water flooding of petroleum reservoirs and with in situ bioremediation of contaminated aquifers. In secondary oil recovery projects petroleum reservoirs are flushed with water (Willhite 1986) in order to replace and recover the oil. The efficiency of this method may be reduced due to regions of high hydraulic conductivity establishing preferential flow paths. Zones of low hydraulic conductivity are bypassed by the water flow and oil from these zones cannot be recovered. To avoid this, bioclogging is stimulated to plug preferential flow paths in order to get a more homogeneous flow field and thus a higher oil recovery (Jack et al. 1983; Torbati et al. 1986; Updegraf 1983), a
method also investigated and referred to as bacterial profile modification (Lappan and Fogler 1994; Lappan and Fogler 1996).

The success of projects dealing with in situ bioremediation of contaminated aquifers may also be influenced by bioclogging. The microbial degradation of contaminants such as hydrocarbons is limited by the availability of oxidants and nutrients. In engineered systems these substances are often injected via wells and bioclogging of the aquifer around the injection wells may lead to increased injection pressures or decreased flow rates, thus limiting the efficiency of this remediation method (Bedient et al. 1994; McCarty et al. 1998; U.S. National Research Council 1993). To obtain high degradation rates, contaminants as well as oxidants and nutrients must be available for the microorganisms simultaneously. Thus the success of engineered as well as intrinsic bioremediation projects may be reduced by preferential flow paths in an aquifer, which can bypass zones of high contamination. Bioclogging is suspected to increase this flow bypass effect as biologically active zones may be encapsulated due to bioclogging (Baveye et al. 1998). These negative bioclogging effects can not be prevented by biocides as the latter can not be used in bioremediation. In contrast to these negative implications, there is evidence that bioclogging may reduce aquifer heterogeneity by plugging preferential flow paths (Lappan and Fogler 1996; MacLeod et al. 1988), which increases the efficiency of bioremediation. Controlled bioclogging may even be used to build up biobarriers to control the migration of contaminants in an aquifer (Brough et al. 1998; Johnston et al. 1997).

For these reasons it is important to investigate the interactions between biomass, hydraulic conductivity and porosity. Understanding these interactions would allow bioclogging to be avoided, where it has negative consequences, or for controlled bioclogging to be stimulated, where it has positive consequences.

1.4 Experimental studies on bioclogging

Most of the studies investigating bioclogging of saturated porous media were performed in laboratory systems. Recent studies on bioclogging in laboratory columns
include those of Cunningham et al. (1991), Holm (1999), Taylor and Jaffé (1990a) and Vandevivere and Baveye (1992c). All these authors observed significant reduction of hydraulic conductivity, which was related to the accumulation of biomass in the pores. Taylor and Jaffé (1990a) operated their columns with constant flow rates. They observed hydraulic conductivity reductions of up to three orders of magnitude, which were correlated with an increase in bacterial organic carbon. In contrast Cunningham et al. (1991) performed experiments for a constant hydraulic head difference between the inlet and outlet of their columns. They observed the homogeneous growth of a biofilm which reduced the porosity of the media by 50 to 96%. The hydraulic conductivity decreased between 92 and 98%. Vandevivere and Baveye (1992c) performed column experiments for constant flow and constant hydraulic head conditions. In their study hydraulic conductivity reductions up to three orders of magnitude could be observed, whereas porosity reduction, caused by heterogeneously distributed aggregates of bacteria, was less than 10%. More recently Holm (1999) investigated bioclogging in sand columns operated at constant flux. A decrease in hydraulic conductivity of again up to three orders of magnitude was observed. This decrease correlated with an increase in bacterial cells and total organic carbon (TOC).

<table>
<thead>
<tr>
<th>Technique</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>biofilm thickness</td>
<td>Cunningham et al. (1991)</td>
</tr>
<tr>
<td>bacterial carbon content</td>
<td>Taylor and Jaffé (1990a)</td>
</tr>
<tr>
<td>phospholipid content</td>
<td>Vandevivere and Baveye (1992c)</td>
</tr>
<tr>
<td>plate counting</td>
<td>Brough et al. (1997)</td>
</tr>
<tr>
<td>confocal laser scanning microscopy</td>
<td>DeLeo and Baveye (1997)</td>
</tr>
<tr>
<td>epifluorescent microscopy</td>
<td>Wu et al. (1997)</td>
</tr>
</tbody>
</table>

Table 1.2: Biomass detection methods used in bioclogging studies

For the different studies cited above, quantification of biomass and monitoring of its distribution in the porous medium was done in several different ways (Tab.1.2). Usually only one single parameter was used to quantify total biomass (Cunningham
et al. 1991; Taylor and Jaffé 1990a; Vandevivere and Baveye 1992c). Since biomass is composed of bacterial cells and extracellular polymeric substances (EPS), one single parameter may not give sufficient information on biomass distribution in a porous medium. This limits the possibility to estimate the volume of biomass based on the measured data, and thus a comparison of results from different studies is difficult. Fig. 1.4 shows the relation between saturated hydraulic conductivity (relative to initial values) and volume of biomass (relative to total pore volume) as taken from Vandevivere et al. (1995) for studies of Cunningham et al. (1991) and Vandevivere and Baveye (1992b).

![Relation between saturated hydraulic conductivity and biovolume](image)

Figure 1.4: Relation between reduction of saturated hydraulic conductivity $k_f$ (relative to initial hydraulic conductivity $k_{f_i}$) and biovolume $v_{bio}$ (relative to total pore volume $v_{pore}$) as taken from Vandevivere et al. 1995. Data are based on measurements of Cunningham et al. (1991) (for 1mm beads and 0.12 - 0.7 mm sand) and Vandevivere and Baveye (1992a) (for 0.09 mm sand). In comparison the theoretical prediction of the model by Clement et al. (1996) is shown.

Recently bioclogging was also investigated in a two-dimensional flow tank experiment (Kildsgaard and Engesgaard 2002). The authors stimulated microbial growth
and could show a change in the flow field throughout the experiment, an effect which was attributed to the accumulation of biomass in the system. The amount and distribution of biomass were not measured in this study.

Artificial micromodels of pore networks are another kind of laboratory system used to investigate bioclogging. Such systems provide the opportunity to observe the distribution of biomass inside the pores. In studies of Dupin and McCarty (2000) and Kim and Fogler (2000) a significant reduction in hydraulic conductivity of these pore networks was observed, correlating to a production of biomass in the pores. Kim and Fogler (2000) observed growth of biomass in the form of colonies whereas Dupin and McCarty (2000) observed different growth morphologies (biofilm, aggregates or filaments) depending on the pH, but in both studies the amount of biomass was not quantified.

The investigation of bioclogging in the field is much more difficult than in the laboratory. In the field, distinguishing between bioclogging and other clogging effects is often not possible. In addition, it is not always possible to measure hydraulic conductivity and biomass concentration with a sufficiently high spatial resolution in the field. A review of studies dealing with clogging in the field is given in Baveye et al. (1998).

1.5 Modeling of bioclogging

To model the reduction of hydraulic conductivity of porous media due to bioclogging, it is necessary to describe the accumulation of biomass in the porous medium as well as the influence of the biomass on the saturated hydraulic conductivity. A variety of models exist, which describe the growth of bacteria in porous media. To include the dependence of microbial growth on the presence of one or even more growth limiting substances, recent models describe bacterial growth with the help of Monod-type kinetics (e.g. Brun et al. 1994; Chiang et al. 1991; Kindred and Celia 1989; Kinzelbach et al. 1991; Lensing et al. 1994; MacQuarrie et al. 1990; Schäfer et al. 1998a; Widdowson et al. 1988). This of course means that these models also have to calculate the transport of these substances as well as their
consumption due to microbial growth. In addition it may be necessary to include concentration changes due to other non-biological reactive processes into the model. Other factors, which may influence the distribution of biomass in porous media, are biomass detachment by shear forces (Rittmann 1982) and the transport and attachment of mobile biomass. A more detailed description of reactive transport in porous media is given in Section 1.5.1, followed by a review of published concepts to describe the reduction of hydraulic conductivity due to biomass accumulation (Section 1.5.2).

1.5.1 Reactive transport in saturated porous media

Flow and transport

Water flow in porous media is considered as laminar flow and with the theory of laminar flow it is possible to describe the flow in single pores. Description of a large amount of interconnected pores is theoretically possible but practically not feasible for the description of macroscopic porous media. To describe flow in porous media on the macroscopic scale the concept of the representative elementary volume (REV) is used. The size of an REV is determined by the length scale at which processes occurring in single pores can be neglected. An REV is assumed to be a continuum containing two phases, the pore space and the solid matrix. The porosity \( n \) is the fraction of pore volume on the total volume of the REV.

On the REV scale the flow of a fluid with constant density in a saturated porous medium with constant porosity is described by the equation:

\[
\nabla \cdot \left( \mathbf{K}_f(\bar{x}) \nabla h(\bar{x}, t) \right) = S_0(\bar{x}) \frac{\partial h(\bar{x}, t)}{\partial t} - w(\bar{x}, t)
\]

(1.1)

\( \mathbf{K}_f \) is the tensor of hydraulic conductivity, \( S_0 \) the specific storage coefficient and \( w \) a volume source and sink term. The hydraulic head \( h \) at location \( \bar{x} \) and time \( t \) is the variable to be calculated. If \( h \) is known the flow velocity \( \bar{v}_D \) can be calculated using Darcy’s law

\[
\bar{v}_D(\bar{x}, t) = -\mathbf{K}_f(\bar{x}) \cdot \nabla h(\bar{x}, t)
\]

(1.2)
If the solution of the flow problem is known, the concentration $c$ of a solute can be calculated using the transport equation

$$\frac{\partial c(\vec{x}, t)}{\partial t} = -\vec{\nabla} \cdot (\vec{u}(\vec{x}, t)c(\vec{x}, t)) + \vec{\nabla} \cdot \left( (D(\vec{x}) + D_m(\vec{x})) \vec{\nabla} c(\vec{x}, t) \right) + r(\vec{x}, t) \quad (1.3)$$

$D$ is the dispersion tensor and $D_m$ the molecular diffusion coefficient. The transport velocity $\vec{u}$ is related to the flow velocity via the equation $\vec{u} = \frac{\vec{v} \rho}{\rho}$. The term $r$ includes all sources and sinks caused by reactive processes.

**Reactive processes**

Reactive processes in porous media include sorption of solutes, radioactive decay, dissolution of NAPLs, precipitation or dissolution of minerals, and biological processes. These biological processes include growth and decay of biomass as well as biologically mediated chemical reactions, i.e. the consumption of nutrients and oxidants (Fig.1.5).

![Conceptual model of aquifer phases, major processes, exchange relations and species residing in the phases](image)

Figure 1.5: Conceptual model of aquifer phases, major processes, exchange relations and species residing in the phases (taken from Schäfer et al. (1998a)).

For the description of each of these processes a variety of approaches exist in the literature. The sorption of solutes on the matrix surface is often defined by isotherms, which describe the equilibrium between dissolved concentration $c_s$ and sorbed concentration $c_a$. Commonly the Freundlich isotherm

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\[ c_s = F \cdot c_a^m \]  

is used with \( F \) as the Freundlich constant and \( m \) as a measure of the nonlinearity involved (Schwarzenbach et al. 1993). The dissolution of NAPLs into the pore water is determined by its maximum solubility in water. If the NAPL is a mixture of several different substances, the effective solubility of each substance is given in analogy to Raoult’s law (e.g. Mott 1995; Schluep 2000).

The equilibrium of chemical reactions is described by the law of mass action as well as the conservation of mass. If several chemical reactions are taking place simultaneously, Morel (1983) introduced a concept, which uses chemical components to describe a system of chemical reactions. If the time scale of the chemical reactions, adsorption or NAPL dissolution is small compared to the time scale of solute transport, it is justified to assume an instantaneous equilibrium between the different substances involved. In case this assumption is not justified, an expression for the kinetics of the process must be added to describe the entire process explicitly.

For the description of microbial growth and the change of solute concentration and microbial activity, the kinetic behaviour of the process is of importance. The time dependence of growth of the microorganism \( X \) on the concentration \( c_i \) of different growth limiting substrates is given by Monod kinetics (Monod 1942)

\[
\frac{\partial X}{\partial t} = \mu_{\text{max}} \prod_i \frac{c_i}{K_i + c_i}
\]

with \( \mu_{\text{max}} \) being the maximal growth rate and \( K_i \) the so-called Monod constants. The incorporation of organic carbon from a substrate into the biomass is then linked by a yield or turnover coefficient to the microbial growth. In case that biochemical reactions are catalyzed by the microorganisms, the rate of these reactions is coupled to the kinetics of microbial growth.

### 1.5.2 Hydraulic conductivity reduction

The reduction of hydraulic conductivity due to biomass accumulation in the pores has been studied theoretically by several authors. In Taylor et al. (1990c) two
different models were presented. For the first model Taylor et al. (1990c) assumed that the porous medium is built by uniform spheres with diameter $d$ and with a geometrically regular packing. Changes of porosity $n$ and specific surface $S$ due to biomass growth were calculated by assuming the spheres to be coated by a biofilm with uniform thickness $L_f$. Changes in $n$ and $S$ due to growth of the biofilm were expressed by the equations

$$n = 1 - \frac{\pi}{\alpha_m} \left[ \frac{(2 - m)}{12} \left( \frac{2L_f}{d} \right)^3 + \frac{(4 - m)}{8} \left( \frac{2L_f}{d} \right)^2 + \frac{1}{2} \left( \frac{2L_f}{d} \right) + \frac{1}{6} \right]$$  \hspace{1cm} (1.6)

and

$$S = \frac{\pi}{\alpha_m d} \left[ \frac{(2 - m)}{2} \left( \frac{2L_f}{d} \right)^2 + \frac{(4 - m)}{2} \left( \frac{2L_f}{d} \right) + 1 \right]$$  \hspace{1cm} (1.7)

$m$ is the number of contact points a sphere has with its neighbors and $\alpha_m$ is a packing arrangement factor, both depending on the geometry of the packing arrangement.

By using the Kozeny-Carman equation (Carman 1937)

$$k_f = \frac{\rho g \, n^3}{\eta \, 5.5^2}$$  \hspace{1cm} (1.8)

which relates hydraulic conductivity $k_f$ to porosity and specific surface of the medium ($\rho$ is the mass density and $\eta$ the viscosity of the fluid, $g$ is the acceleration due to gravity), it was possible to calculate the reduction of hydraulic conductivity due to bioclogging.

For the second model Taylor et al. (1990c) assumed the biomass to grow as a homogeneous biofilm inside cylindrical pores (Fig.1.6). The pores are arranged in bun-
Figure 1.7: Schematic realization of a pore bundle with different pore radii
dles of parallel pores with radii given by a pore-size distribution function (Fig.1.7).
According to the "cut-and-random-rejoin" model (Childs and Collis-George 1950)
these pore bundles were cut into planes and these planes were then put together
in a random fashion. Due to this procedure some random number of pores from
the two planes became interconnected (Fig.1.8), while other pores became dead end
pores and were removed. Based on results of Mualem (1976), Taylor et al. (1990c)
presented a relation linking the reduction of hydraulic conductivity to porosity and
the thickness of the biofilm by the equation

\[ k_f = \frac{\kappa \pi^2}{8} \left[ \int_{r_{0_0}}^{R-L_f} \frac{r^3}{(r + L_f)^2} f(r + L_f) dr \right]^2 \]  

(1.9)

\( \kappa \) is a dimensionless constant, \( r_0 \) the smallest and \( R \) the largest pore radius. \( f(r) \)
is the pore size distribution function and \( r_{0_0} = \max[r_0 - L_f, 0] \).

The models of Taylor et al. (1990c) were evaluated in Vandevivere et al. (1995),
who compared the experimental results shown in Fig.1.4 with the model predictions.
It was shown that except for the 1 mm glass beads system both models "... vastly
underestimate the clogging in all other cases." It was suggested that two reasons
may be responsible for this failure, the idealized geometry and the assumption that
the biomass is growing in an uniform biofilm. As a conclusion Vandevivere et al.
Figure 1.8: Example of two pores with different radii $r_1$ and $r_2$ after "cut-and-random-rejoin" (1995) suggested to assume the biomass to grow as plugs, which may form flow barriers inside the pores (Fig.1.9). They presented a simple model with bacterial plugs clogging a bundle of uniform pores, and could show that this assumption lead to a stronger clogging efficiency of the biomass. It was also suggested that concepts from percolation theory (Berkowitz and Balberg 1993; Sahimi 1995) should be used to get a better description of the porous media structure. This theory describes generally the properties of a system of interconnected elements (e.g. networks of pores or electrical resistors). In particular, percolation theory relates the overall properties of such a system to the interConnectivity between various regions of the system. Fig.1.10 shows an example of a (pore) network as it is initially and after reducing the interconnectivity by removing a number of individual bonds (pores). Pore network simulations were used by Lappan and Fogler (1996) as well as Suchomel et al. (1998a) and Suchomel et al. (1998b) to investigate bioclogging effects caused by biofilm growth. These authors showed that their simulations were able to reproduce experimental data, but an upscaled functional relation between changes in hydraulic conductivity and porosity was not presented.

Clement et al. (1996) presented a macroscopic interpretation of the "cut-and-random-rejoin" model, which did not assume a specific morphology for microbial growth. The model is based on the assumption that clogging occurs preferentially in the largest pores as observed by Torbati et al. (1986), although in Torbati et al. (1986) microorganisms were grown under no flow conditions. Furthermore the soil-water retention functions from van Genuchten (van Genuchten 1980) and Brooks-Corey (Brooks and Corey 1964) respectively, which describe the relation between...
Relative water saturation and capillary pressure head in a porous medium, were used to derive a pore size distribution function, which in turn was used to express the hydraulic conductivity according to Mualem (1976). Regardless of the soil-water retention function used the change of relative hydraulic conductivity $k_{f_{rel}}$ due to increasing volume of biomass $v_{bio}$ is expressed by the function

$$k_{f_{rel}} = \left(1 - \frac{v_{bio}}{v_{pore}}\right)^{10}$$

(1.10)

with $v_{pore}$ being the total pore volume. Within a wide range of pore heterogeneities this expression was nearly identical to the model of Taylor et al. (1990c), which is based on Mualem (1976). This means that also this macroscopic model was not able to explain the clogging effects observed in the experiments of Cunningham et al. (1991) and Vandevivere and Baveye (1992b) (Fig.1.4). Kildsgaard and Engesgaard 2001 were able to reproduce the experimental data of Kildsgaard and
Engesgaard (2002) with the model of Clement et al. (1996), but as no biomass measurements were presented in Kildsgaard and Engesgaard (2002), the simulated amount of biomass had to be fitted to reproduce the clogging effects.

1.6 Scope and outline of the thesis

The majority of existing experimental and theoretical studies was focusing on bioclogging in one-dimensional flow fields. As natural flow systems usually do not behave like one-dimensional systems the investigation of bioclogging in systems with flow fields with a greater number of dimensions is of importance. Such investigations should be done experimentally and theoretically. In addition, taking different growth morphologies into account may lead to a better description of bioclogging processes.

The thesis investigates bioclogging effects in two-dimensional flow fields. This is done on two different scales. The pore- or micro-scale, where pore network simulations were used to investigate bioclogging theoretically, and the lab- or macro-scale, where bioclogging was investigated in a flow cell experiment with a two-dimensional flow field. Results from the flow cell experiment were compared to simulation results using a continuous medium model, which was developed based on the results of the pore network simulations. For future investigations of bioclogging in the field, it
will be necessary to be able to measure distributions of biomass as well as solute concentration in a three-dimensional spatial resolution. Therefore the dual-pumping technique was validated concerning its applicability for measuring vertical concentration profiles in the field. In addition, bioclogging was investigated in slow sand filters used for drinking water treatment, as such filters are nearly a field scale but allow detailed measurements with the required high spatial resolution.

In particular in the next chapters of the thesis the following research questions are addressed:

Chapter 2: Pore network simulations were used to investigate theoretically the influence of microbial growth on the hydraulic conductivity of the pore networks. The question was addressed, whether assuming multi-dimensional flow fields on the pore scale yields hydraulic conductivity vs. porosity relations fundamentally different to those found in the literature. In addition the influence of the morphology of microbial growth on these relations was investigated.

Chapter 3: As results from pore network simulations shown in Chapter 2 focused only on the changes in hydraulic conductivity due to bioclogging, in this chapter the impact of bioclogging on the dispersivity and surfaces of pore networks was shown.

Chapter 4: The interaction between water flow and microbial growth was observed in an experiment using a flow cell with a two-dimensional flow field. Water flow and biomass distribution were monitored in situ using visible light transmission. In addition the spatial distribution and composition of biomass was investigated destructively at the end of the experiment.

Chapter 5: The experimental results from Chapter 4 were used to validate a newly developed reactive transport groundwater model, which includes bioclogging. Simulation results using different hydraulic conductivity vs. porosity relations taken from Chapter 2 and from literature were compared to experimental results.

Chapter 6: For the investigation of bioclogging on a field scale, measurements with
a high spatial resolution are required. Therefore the dual pumping technique was investigated concerning its applicability to the measurement of vertical concentration profiles in groundwater. This technique was applied in a contaminated aquifer in Menziken, Switzerland. Results from an established ram technique were used as a reference to validate the applicability of the dual pumping technique.

**Chapter 7:** As an example for bioflocculation on a scale larger than in the lab, the influence of microbial growth on the hydraulic conductivity of slow sand filters used for drinking water treatment was investigated.

**Chapter 8:** Results from the previous Chapters are compared and discussed in general. Possible consequences concerning the effect of bioflocculation on bioremediation are discussed. Finally open questions are addressed and an outlook on possible further research is given.
Chapter 2

Influence of microbial growth on hydraulic properties of pore networks


2.1 Abstract

From laboratory experiments it is known that bacterial biomass is able to influence the hydraulic properties of saturated porous media, an effect called bioclogging. To interpret the observations of these experiments and to predict possible bioclogging effects on the field scale it is necessary to use transport models, which are able to include bioclogging. For these models it is necessary to know the relation between the amount of biomass and the hydraulic conductivity of the porous medium. Usually these relations were determined using bundles of parallel pore channels and do not account for interconnections between the pores in more than one dimension.

The present study uses two-dimensional pore network models to study the effects of bioclogging on the pore scale. Numerical simulations were done for two different scenarios of the growth of biomass in the pores. Scenario 1 assumes microbial growth in discrete colonies clogging particular pores completely. Scenario 2 assumes microbial growth as a biofilm growing on the wall of each pore. In both scenarios
the hydraulic conductivity was reduced by at least two orders of magnitude, but for the colony scenario much less biomass was needed to get a maximal clogging effect and a better agreement with previously published experimental data could be found. For both scenarios it was shown that heterogeneous pore networks could be clogged with less biomass than more homogeneous ones.

2.2 Introduction

The ability of bacterial biomass to change hydraulic properties of a saturated porous medium has been observed in laboratory studies (Baveye et al. 1998). The possible influence of this phenomenon - called bioclogging - on the applicability of bioremediation techniques can be envisioned in two contrary ways. Bioclogging may reduce the success of bioremediation because contaminated parts of an aquifer can clog due to microbial activity and rates of degradation can decrease (Baveye et al. 1998). On the other hand, controlled clogging of an aquifer may be used to clog preferential paths and get a more homogeneous sweep or to build up biobarriers which could be used to increase the success of remediation (Johnston et al. 1997).

The decrease in hydraulic conductivity due to microbial growth has been investigated in column experiments. Several authors reported a significant reduction of hydraulic conductivity due to bioclogging (Cunningham et al. 1991, Taylor and Jaffé 1990a, Vandevivere and Baveye 1992c, Brough et al. 1997, Johnston et al. 1997, Wu et al. 1997). While circumstances necessary to clog the test columns varied, Cunningham et al. (1991) used a column inoculated with bacteria and applied a constant head difference between inflow and outflow. A decrease of more than 90% in hydraulic conductivity and 50-90% in porosity was observed. Taylor and Jaffé (1990a) used columns with a constant influx. Reduction in hydraulic conductivity by three orders of magnitude was observed along with an increase of biomass. In addition a change in dispersivity was observed. Vandevivere and Baveye (1992c) performed similar column tests with varying boundary conditions. They observed a reduction of hydraulic conductivity by three orders of magnitude but only a maximum porosity reduction of less than 10%. In a series of 35 column experiments,
Brough et al. (1997) observed a decrease of hydraulic conductivity of between 28% and 79%. It was found that a procedure in which activated sludge microorganisms were added during the filling of the column with sand was the best method to reach the desired high reductions of hydraulic conductivity. Johnston et al. (1997) investigated the production of polysaccharides in aquifer material under different nutrient supply. In a column experiment performed under anaerobic conditions they observed a reduction of hydraulic conductivity by a factor of about 14. Wu et al. (1997) performed column experiments with constant head boundaries. A reduction in hydraulic conductivity of about one order of magnitude was observed but due to gas production this reduction could not be attributed to the biomass alone (Bav- eye and Dumestre 1998). In addition to these column studies Dupin and McCarty (2000) and Kim and Fogler (2000) investigated microbial growth and its influence on the hydraulic properties of microscopic pore networks. Both authors observed high reductions in hydraulic conductivities. Dupin and McCarty (2000) could show that the morphology of microbial growth depends on the pH-value, whereas Kim and Fogler (2000) were focusing on the effect of shear forces and nutrient feeding, showing that biofilms may resist in the pores even when nutrient supply has stopped.

Besides bioclogging there are also non biological mechanisms reducing the hydraulic conductivity of a porous medium. Such processes may be e.g. particle deposition as investigated in connection with deep bed filtration (e.g. Tien and Payatakes 1979), particle deposition in combination with chemically induced processes which is causing formation damage of petroleum reservoirs (e.g. Chang and Civan 1997; Wojtanowicz et al. 1988) or the capillary instability of wetting films causing pore blocking in gas-condensate systems (e.g. Coskuner 1997). The mechanisms causing these non biological clogging processes are well investigated but differ from the mechanisms causing the clogging of pores due to biomass production.

Several models have been introduced to simulate the observed interactions between biomass and the hydraulic properties of the porous media. Usually these models are based on the assumption of a homogeneous biofilm covering the surface of the grains (e.g. Taylor et al. 1990c). A reduction of hydraulic conductivity caused by biofilm growth was theoretically derived assuming bundles of parallel pores. Es-
pecially for fine-textured materials these models could not predict observed hydraulic conductivity reductions satisfactorily (Vandevivere et al. 1995; Vandevivere 1995). In Clement et al. (1996) a clogging model was presented, which made no explicit assumptions on the distribution of the biomass in the pores. The prediction of hydraulic conductivity reductions of the model of Clement et al. (1996) was nearly identical to predictions of Taylor et al. (1990c), and thus the criticism of Vandevivere et al. (1995) also applies to the model of Clement et al. (1996). In Vandevivere et al. (1995) it was assumed that the discrepancy between model predictions and experimental results is caused by the fact that microorganisms can also form colonies, which influence the hydraulic conductivity differently than a biofilm. In addition, it was suggested to use pore networks instead of pore bundles in order to account for interpore connections (Vandevivere et al. 1995, Loehle and Johnson 1994). In Suchomel et al. (1998a) a pore network model was introduced and applied to previously published data (Suchomel et al. 1998b). The model produced realistic results assuming the growth of a biofilm on the walls of cylindrical pores. Kim and Fogler (2000) could also reproduce their experimental data with pore network simulations assuming biofilm growth. In contrast to this Dupin and McCarty (2000) showed that their experimental observations could be explained with network model simulations assuming the growth of biomass in aggregates, whereas assuming a biofilm could not explain their observations.

Until now no theoretically derived hydraulic conductivity vs. porosity relations are published, which account for interpore connections in more than one dimension and which account for microbial growth morphologies different to a biofilm. The available relations could not explain experimentally observed clogging effects. Thus the question arises whether approaches, which include a multi-dimensional pore structure and alternative growth morphologies, yield a better description of hydraulic conductivity reduction due to the biomass.

For the present project we used pore network models as a tool to simulate the change of the hydraulic properties due to microbial growth. The investigation of general properties of such networks is a subject of percolation theory (Stauffer and Aharony 1992). Results from percolation theory were already used for the descrip-
tion of hydraulic properties of porous media (Berkowitz and Balberg 1993, Sahimi 1995). Pore network models were already used successfully for the simulation of non biological clogging processes (e.g. Burganos et al. 1995; Ewing and Gupta 1994; Imdakm and Sahimi 1991; Kaiser 1997; Lee and Koplik 2001; Rege and Fogler 1987; Rege and Fogler 1991; Wang and Mohanty 1999).

As the existing hydraulic conductivity vs. porosity relations were not able to reproduce experimental observations available in literature, we were focusing on the question whether relations derived from pore network simulations were generally able to explain the observed small reductions of porosity already causing a large reduction in hydraulic conductivity. In addition, we wanted to investigate the influence of the morphology of microbial growth on the results of the pore network simulations. For this reason we decided to use two-dimensional pore networks consisting of cylindrical pores as a simplified representation of a porous medium. To investigate the influence of the morphology of microbial growth, we chose two different scenarios describing biomass growth. Following the suggestions made in Vandevivere et al. (1995) we assumed for the first scenario the biomass to grow as micro-colonies, which plug pores entirely. Generally it would be possible to assume different ways to correlate the size of a pore with its probability of becoming clogged (e.g. preferential clogging of the largest pores, preferential clogging of the smallest pores or no correlation at all). As experimental observations showed that relatively small amounts of biomass could cause high reductions in hydraulic conductivity, we decided to let the micro-colonies plug the smallest pores preferentially, giving the biomass a high clogging efficiency. For natural porous media this scenario corresponds to a preferential plugging of the pore throats, which are the bottle necks for the water flow.

In comparison to this colony scenario, we assumed for the second scenario the biomass to grow as a biofilm on the walls of each pore. For both scenarios we calculated the changes of hydraulic conductivity and pore volume of the entire pore networks to derive hydraulic conductivity vs. porosity relations, which could be compared to results published in literature.
2.3 Model description

2.3.1 Model geometry

The model used in this study consists of cylindrical pores. The pores are connected in a 2-dimensional rectangular grid. All pores are assumed to have the same length $l$, the radius $r_i$ of the pore $i$ is taken from a lognormal distribution. The sites connecting the pores (in the following referred to as sites) are assumed to have no volume.

2.3.2 Flow model

The volume $v_i$ of a single pore $i$ is given by $r_i$ and $l$ following the equation

$$v_i = \pi r_i^2 l$$  \hspace{1cm} (2.1)

The flow rate $q_i$ in a pore $i$ is given by Hagen-Poiseuilles law

$$q_i = -c_i \frac{\Delta h_i}{l}$$  \hspace{1cm} (2.2)

where $\Delta h_i$ is the hydraulic head difference between the sites at each end of the pore and $c_i$ the hydraulic conductivity given by

$$c_i = \frac{\pi \rho g r_i^4}{8\eta}$$  \hspace{1cm} (2.3)

where $\rho$ is the density, $\eta$ the viscosity of the fluid and $g$ the gravitational acceleration.

As shown by Suchomel et al. (1998a) this formulation together with mass conservation leads to a system of linear equations which can be solved for the hydraulic heads $h^j$ at each site $j$ efficiently by a successive overrelaxation (SOR) method. In this study the system of flow equations was solved assuming constant head boundaries at two opposite sides of the grid, while the other two sides were no flow boundaries. With the calculated heads $h^j$ it is possible to determine the flow rates $q_i$ and the average velocities $s_i = \frac{q_i}{\pi r_i^2}$ in each pore.

In addition the hydraulic conductivity $C$ of the entire grid can be calculated in analogy to Darcy’s law by the expression

$$C = \frac{QL}{A\Delta H}$$  \hspace{1cm} (2.4)
where $Q$ is the total flow rate through the grid, $\Delta H$ the total hydraulic head difference between the constant head boundaries, $L$ the total distance between these boundaries and $A$ the total cross sectional area of the grid. It can be seen that $C$ is proportional to $Q$ and so relative changes in $C$ can be inferred from relative changes of $Q$.

### 2.3.3 Transport model

Using the results of the flow model the system of equations describing the transport of a solute within the pore grid can be derived by calculating the solute mass balances for each pore $i$

$$\frac{\partial m_i}{\partial t} = v_i \frac{\partial c_i}{\partial t} = q_i c^j - q_i c_i$$  \hfill (2.5)

with $m_i$ being the solute mass and $c_i$ being the solute concentration in the pore $i$. $c^j$ is the concentration of solute entering the pore from site $j$. It can be calculated as the flux weighted average of the concentration in the pores with flux towards site $j$ assuming perfect mixing at the site.

In Eqn. 2.5 no diffusive processes are included. This simplification was made because numerical tests showed that the addition of a diffusion term to Eqn. 2.5 did not influence the results of the simulation. In particular single realization results simulating clogging due to a biofilm (Section 2.4.2) were identical concerning the reduction of hydraulic conductivity and pore volume of the entire network.

This leads to the time discretized equation

$$\frac{\Delta c_i}{\Delta t} = q_i \frac{\sum_k q_k c_k}{\sum_k q_k} - q_i c_i$$  \hfill (2.6)

with $k$ as the index of the pores with flux towards the upgradient site $j$ of pore $i$.

The size of the time step is determined by the equation

$$\Delta t = \frac{l}{v_{\text{max}}}$$  \hfill (2.7)

where $v_{\text{max}}$ is the maximum of the average pore velocities $v_i$.

This system of linear equations was solved with an explicit solver.


2.3.4 Incorporation of biological growth

For this study two different types of microbial growth were incorporated into the model. Like other authors (Taylor and Jaffé 1990b, Clement et al. 1996, Suchomel et al. 1998a) we did not divide the biomass into bacteria and extracellular polymers (EPS) but regarded them as a unit for both scenarios.

**Microbial growth in colonies**

For this scenario the growth of the microorganisms is not coupled to the concentration of a nutrient in the pores but to the radius of the pores. It is assumed that growth occurs in the smallest pores first. Pores in which growth occurs are assumed to be completely filled with biomass whereas all other pores are assumed to be void of biomass. Thus such a network represents a bond percolation model (Stauffer and Aharony 1992). In practice the presence of a certain amount of biomass in the network is simulated by selecting a number of pores to be filled with biomass and consecutively removing the smallest pores from the grid until a pre-selected volume of biomass is reached. Here it is implicitly assumed that nutrient conditions allow the production of this amount of biomass. The case that the biomass production would be limited by the presence of a nutrient would result in an upper limit for total biomass in the pore network.

**Microbial growth in a biofilm**

In this scenario it was assumed that the microorganisms were growing in a biofilm covering the wall of each pore and therefore reducing the initial radius. The microbial growth was coupled to the presence of a growth limiting solute nutrient. Assuming that only the amount of nutrient within a limited distance $\delta = exch\Delta t$ to the surface of the biofilm is available for the microorganisms the mass $m_{bio}$ of nutrient available for the microorganisms in pore $i$ within the time step $\Delta t$ is

$$m_{bio} = c_i l \pi \left( r_i^2 - (r_i - exch\Delta t)^2 \right)$$

(2.8)

where $exch$ is the exchange parameter.
The net change of biomass is described by the following equation:

\[
\frac{\Delta b_t}{\Delta t} = \frac{\Delta b^+_t}{\Delta t} - \frac{\Delta b^-_t}{\Delta t} - \frac{\Delta b_{\text{shear}}}{\Delta t} = b_i \mu^+ - b_i \mu^- - s_i z 2\pi r_t l \tag{2.9}
\]

with \(b_t\) as the biomass and \(\mu^+\) and \(\mu^-\) as the first order rate constants for microbial growth and decay. The nutrient consumption \(\Delta m_t\) caused by the microbial growth \(\Delta b^+_t\) is linked to the change in biomass by \(\Delta m_t = \frac{\Delta b^+_t}{Y}\) with \(Y\) as a yield factor. \(\Delta b^+_t\) is limited by the assumption that \(\Delta m_t\) should not exceed \(m_{i\text{bio}}\). Thus in case \(\Delta b^+_t = \Delta m_t Y\) would become larger than \(m_{i\text{bio}} Y\), bacterial growth is expressed by a zeroth order rate of \(\frac{m_{i\text{bio}} Y}{\Delta t}\) instead of the first order rate \(b_i \mu^+\). This description of microbial growth is a slight simplification of the Monod kinetic (Monod 1942), which is widely used for the simulation of microbial growth (e.g. Brun et al. 1994; Chiang et al. 1991; Kindred and Celia 1989; Kinzelbach et al. 1991; Lensing et al. 1994; MacQuarrie et al. 1990; Schäfer et al. 1998a; Suchomel et al. 1998a; Widdowson et al. 1988). As also done by most of these authors, bacterial decay is described by a first order expression. The term \(\Delta b_{\text{shear}}\) depending on the average velocity \(s_i\) and the surface of the biofilm in the pore describes the detachment of biomass due to shear forces with \(z\) as a rate constant.

By giving the biomass a constant density \(d\) (in analogy to Characklis and Marshall (1990) expressed as dry mass per wet volume) it was possible to calculate its volume \(v_{i\text{bio}}\) and the resulting change of the radius \(r_t\) of each pore. Although Characklis and Marshall (1990) reported on an increase of biomass density by a factor of three along the thickness of a biofilm, this effect is not considered in the model, because the typical biofilm thickness is in the range of the pore radii, which are about one order of magnitude smaller than the biofilm thickness presented in Characklis and Marshall (1990). If the average biomass density would increase with an increasing biofilm thickness, the pore radii reduction due to biofilm growth would be overestimated when assuming a constant biomass density.
2.3.5 Coupling of flow, transport and microbial growth

Microbial growth in colonies

As microbial growth was not coupled to specific nutrient concentration in each pore, it was not necessary to include transport calculations for this scenario. The influence of a certain amount of biomass on the hydraulic conductivity of a given pore network was calculated using a three step procedure.

1. The initial hydraulic conductivity \( C_{init} \) was calculated for the entire network assuming no biomass was present. The initial pore volume of the entire grid \( V_{init} \) was determined by integration of the single pore volumes \( v_i \).

2. A certain number of pores were removed from the grid. Smallest pores were removed first.

3. The hydraulic conductivity \( C_{reduced} \) of the remaining percolation cluster was determined. If no percolation cluster was found, which means that no flow path between the inflow and outflow boundary existed, the hydraulic conductivity was set to \( C_{reduced} = 0 \). The volume of the pores clogged by the biomass \( V_{bio} \) and the volume of the remaining unclogged pores \( V_{reduced} \) was calculated (note that \( V_{reduced} + V_{bio} = V_{init} \)).

Each of these procedures returned a single pair of values: the relative hydraulic conductivity \( C_{rel} = \frac{C_{reduced}}{C_{init}} \) and the relative pore space \( V_{rel} = \frac{V_{reduced}}{V_{init}} \).

Microbial growth in a biofilm

For this scenario the simulation of flow, transport and microbial growth had to be coupled. A complete simulation step consisted of the following substeps:

1. The flow problem was solved according to the actual pore radii of each pore.

2. Based on the results of the flow simulation the transport problem was solved.

3. Using the results of substeps 1 and 2 biofilm growth in each pore and thus the changes of the pore radii were calculated.
This results from substep 3 were then used for the next simulation step to calculated the solution of substep 1 and 2. To increase calculation efficiency an option was implemented into the model, which allowed to skip substep 1 of a simulation step. For such simulation steps, substeps 2 and 3 were using the flow solution from the last simulation step, which included substep 1. The changes in the hydraulic conductivity and the pore space of the entire grid were calculated after each simulation of substep 1.

2.4 Simulation of microbial clogging scenarios

2.4.1 Scenario 1: Clogging due to microbial colonies

Numerical simulations of hydraulic conductivity reduction

To investigate the influence of microbial colonies on the hydraulic conductivity of the pore networks, simulations were done for 50x50 pore networks. The log of the radius of each pore was determined randomly, with random numbers following a normal probability density function. The resulting lognormal pore radii distribution had an average or mean dimensionless radius of 1. For standard deviations we used $\sigma = \frac{1}{3}, \frac{1}{2}$ and $\frac{2}{3}$. These $\sigma$-values are in the range of values typical for sand packs with different heterogeneities (Brutsaert 1966; Imdakm and Sahimi 1991; Sugita et al. 1995).

For each $\sigma$ a set of 5000 realizations was created. For each of these realizations a volume of biomass was selected randomly (with random numbers equally distributed between 0 and $V_{init}$) and - as described above - a number of pores was removed from the grid and the change in hydraulic conductivity and pore volume was calculated.

Afterwards the range of $V_{rel}$ for which nonzero $C_{rel}$-values could be observed was divided into 100 equal intervals. For each of these intervals the $C_{rel}$-values were averaged to reduce statistical effects.

Fig.2.1 shows the reduction of hydraulic conductivity due to the pore clogging for $\sigma = \frac{1}{3}, \frac{1}{2}$ and $\frac{2}{3}$. For all investigated values of $\sigma$ a reduction of hydraulic conductivity by more than two orders of magnitude could be observed. The pore volume, for which the maximum reduction of hydraulic conductivity could be achieved, strongly
depended on the value of $\sigma$. The higher the heterogeneity the less biomass was
needed to reduce the hydraulic conductivity to a given degree.

Figure 2.1: Reduction of hydraulic conductivity due to clogging of pores by microbial
colonies

To express the numerical data with an analytical function we could not use a
power law, as done typically to express changes of hydraulic conductivity of pore
networks (Sahimi 1995). The numerical data exhibited a behaviour similar to a
power law for small values of hydraulic conductivity, but with increasing $V_{rel}$ a
power law would not have been able to reproduce the change in curvature observed
for the numerical data. Therefore a function was needed which fulfilled the following
constraints:

1. $C_{rel}(V_{rel} = 1) = 1$ and $C_{rel}(V_{rel} = V_0) = 0$

2. $\frac{dC_{rel}}{dV_{rel}} = 0$ for $V_{rel} = V_0$

3. $\exists V_{rel} \epsilon [V_0, 1]$ with $\frac{d^2C_{rel}}{dV_{rel}^2} = 0$

The function

$$C_{rel}(V_{rel}) = a \left( \frac{V_{rel} - V_0}{1 - V_0} \right)^3 + (1 - a) \left( \frac{V_{rel} - V_0}{1 - V_0} \right)^2$$

(2.10)
satisfies all of these constraints for $a < -0.5$. As the existence of local minima
for $V_{rel} \epsilon [V_0, 1]$ would have been physically unrealistic, $a > -2$ was an additional
requirement. For \(a = 1\) and \(a = 0\) Eqn.2.10 would be an ordinary power law, but not fulfilling constraint 3, as mentioned above. The parameter \(a\) therefore describes the difference between Eqn.2.10 and a power law. \(V_0\) contains the information, for which pore volume, and thus volume of biomass, the hydraulic conductivity reaches 0.

Eqn.2.10 was chosen to fit the numerical results with \(a\) and \(V_0\) as the fitting parameters. Best fits for \(\sigma = \frac{1}{3}, \frac{1}{2}\) and \(\frac{2}{3}\) were also shown in Fig.2.1. To monitor the sensitivity of the fitting parameters to \(\sigma\) we also fitted numerical results using \(\sigma = 0, \frac{1}{10}\) and 1 (graphs not shown). The optimal values for \(a\) and \(V_0\) are given in Tab.2.1. For all values of \(\sigma\) the numerical data could be fitted with a correlation coefficient of \(R^2=0.998\). Both parameters show a significant correlation to \(\sigma\). \(V_0\), which is the relative pore volume at which \(C_{rel}\) becomes 0, increases with \(\sigma\) and therefore the heterogeneity of the network, whereas \(a\) decreases with \(\sigma\).

<table>
<thead>
<tr>
<th>(\sigma)</th>
<th>(a)</th>
<th>(V_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>-1.07</td>
<td>0.422</td>
</tr>
<tr>
<td>0.10</td>
<td>-1.28</td>
<td>0.513</td>
</tr>
<tr>
<td>0.33</td>
<td>-1.66</td>
<td>0.706</td>
</tr>
<tr>
<td>0.50</td>
<td>-1.83</td>
<td>0.815</td>
</tr>
<tr>
<td>0.67</td>
<td>-1.90</td>
<td>0.891</td>
</tr>
<tr>
<td>1.00</td>
<td>-1.94</td>
<td>0.970</td>
</tr>
</tbody>
</table>

Table 2.1: Scenario 1:Parameters used for fitting of numerical results

Calculation of biomass needed for maximum reduction of hydraulic conductivity

From percolation theory it is known that if the density of open pores \(p\) in an infinite network is below a critical value, called percolation threshold \(p_c\), no percolation cluster exists. In such a case there is no continuous flow path and hydraulic conductivity is 0. For \(p > p_c\) a percolation cluster exists and hydraulic conductivity has a value greater than 0. The value of \(p_c\) depends on the dimensionality and the geometry
of the pore network. For the network type used in this study $p_c$ has a value of 0.5 (Stauffer and Aharony 1992).

For the present scenario it was assumed that the smallest pores are clogged first. Using this assumption it is possible to calculate the volume of biomass (and therefore the reduction of pore volume) needed to reduce the density of open respectively unclogged pores to a certain value $p$. Generally the volume $V$ of a given number of pores would be $V = \sum_i v(r_i)$. For infinite networks this can be replaced by $V = \int v(r)\xi(r)dr$, which is the average volume per pore and where $\xi(r)$ is the probability distribution of the pore radius $r$. If $\xi$ is not expressed as a function of $r$ but instead as a function of $z = f(r)$ the pore volume can be expressed by

$$V_{tot} = \int_{z_{min}}^{z_{max}} v(z)\xi(z)dz$$ (2.11)

In the case of a lognormal distribution of the pore radii the probability density function is

$$\xi(z) = \frac{1}{\sqrt{2\pi}\sigma} \exp \left(-\frac{z^2}{2\sigma^2}\right) dz$$ (2.12)

with $z = \ln \left(\frac{r}{r_{av}}\right)$ and $r_{av}$ as the average pore radius. With this and the assumption that clogging occurs in the smallest pore first Eqn.2.11 can be transformed for $V_{clogg}$ as the volume of the clogged pores

$$V_{clogg} = \sqrt{\pi} \frac{r_{av}^2}{\sigma} \int_{-\infty}^{z_n} \exp \left(-\frac{z^2}{2\sigma^2} + 2z\right) dz$$ (2.13)

where $z_n$ is the $z-$value corresponding to the fraction $n$ of pores clogged by the biomass.

The volume $V_{bio}$ of the clogged pores as a fraction of total pore volume can therefore be written as:

$$V_{bio} = \frac{V_{clogg}}{V_{tot}} = \frac{1}{2} \left(1 + \text{erf} \left(\frac{z_n}{\sqrt{2}\sigma} - \sqrt{2}\sigma\right)\right)$$ (2.14)

with erf$(x)$ as the error function.

To determine, which $z_n$ corresponds to a certain fraction $n$ of clogged pores the integral

$$n = \int_{-\infty}^{z_n} \xi(x)dx$$ (2.15)
must be calculated. This equation can be solved for $z_n$ resulting in

$$z_n = \sqrt{2\sigma \text{erf}^{-1}(2n - 1)}$$  \hspace{1cm} (2.16)

where $\text{erf}^{-1}$ is the inverse error function. With this Eqn.2.14 can be transformed to

$$V_{bio} = \frac{1}{2} \left( 1 + \text{erf} \left( \text{erf}^{-1} (2n - 1) - \sqrt{2\sigma} \right) \right)$$  \hspace{1cm} (2.17)

for the volume of biomass needed to clog a given fraction of pores of a pore network. To avoid percolation between the in- and outflow boundary of the network $n$ has to be equal to or larger than the percolation threshold $p_c$ (note that $n = 1 - p$).

All relations discussed are valid for infinite size pore networks. Whether the finite size networks used for the numerical simulation approximate them will be discussed later.

### Comparison of theoretical and numerical results

Fig.2.2 shows the volume of pores clogged by biomass as a function of the density of clogged pores $n$ (ratio of clogged pores to total number of pores) for the numerical simulations compared to the theoretical values (Eqn.2.17). It can be seen that these values are in good agreement. It can also be seen that even for $n > p_c$ data points from the numerical simulations exist. As only data for $C_{rel} > 0$ are plotted, this contradicts the findings of percolation theory, which says that no percolation should be possible for $n > p_c$. This effect is caused by the finite size of the pore networks (e.g. Reynolds et al. 1980; Stauffer and Aharony 1992). Fig.2.3 shows that increasing the size of the pore networks used for simulation yields a better approximation of the percolation threshold for infinite networks, $p_c = 0.5$. For the 50x50 pore networks used in this study the threshold values for $n$ are about 0.54 (Fig.2.3). Volumes calculated with Eqn.2.17 for the pore radii distribution used in this study are shown in Tab.2.2. It can be seen that the $V_{rel}$-values for $n = p_c$ are larger than $V_0$ determined from the numerical simulations (Tab.2.1), but for $n = 0.54$ the calculated values for $V_{rel}$ and $V_0$ correspond very well.
Figure 2.2: Comparison of numerically and theoretically calculated volumes of biomass as a function of density of clogged pores

<table>
<thead>
<tr>
<th>( n = p_c = 0.5 )</th>
<th>( n = 0.54 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \sigma )</td>
<td>( V_{bio} )</td>
</tr>
<tr>
<td>0.33</td>
<td>0.252</td>
</tr>
<tr>
<td>0.50</td>
<td>0.159</td>
</tr>
<tr>
<td>0.67</td>
<td>0.091</td>
</tr>
</tbody>
</table>

Table 2.2: Scenario 1: Theoretically calculated volumes of biomass needed for maximal reduction of hydraulic conductivity

2.4.2 Scenario 2: Clogging due to a biofilm

To investigate the influence of biofilm growth on hydraulic conductivity, simulations were done on 50x50 pore networks with lognormally distributed pore radii, in analogy to Scenario 1. As in Scenario 1, the standard deviations of the lognormal distributions were \( \sigma = \frac{1}{3}, \frac{1}{2} \) and \( \frac{2}{3} \). The average pore radius, as defined in Sec.2.4.1, was \( r_{av} = 30 \mu m \) and the pore length was \( l = 1.5 mm \) for all realizations. In each pore an initial biofilm thickness of \( 1 \mu m \) was assumed. All other parameters used for the simulation (Tab.2.3) were adjusted to achieve the following effects:

- The reduction of hydraulic conductivity of the entire pore network should be
Figure 2.3: Hydraulic conductivity as a function of density of clogged pores, for different sizes of $N \times N$ pore networks

- Biomass should not grow preferentially in the vicinity of the inflow of the pore networks.

The parameters $\Delta H$ and $z$ were controlling the extent of biofilm detachment by shear forces and high values of these parameters would have prevented the development of thick biofilm. $C_{in}$ and $exch$ controlled the amounts of nutrient available to the microorganisms in the biofilm. A lower amount of nutrient supply would also have avoided the buildup of thick biofilms, whereas a higher value of $exch$ would have caused high biomass production close to the inflow of the pore network, consuming nearly all of the nutrient already there. Parts of the pore network closer to its outflow would not have been supplied with the nutrient and no biomass growth would have taken place there. From the parameters $\mu^+$, $\mu^-$ and $d$, $Y$, describing properties of the biomass, $d$ was the most important as it determined the biofilm thickness associated to the amount of biomass in a pore. Higher densities would have avoided bioclogging as the produced biomass would not have been able to change pore radii significantly. After the adjustment these values were kept constant for all simulations. For each $\sigma$, simulations were performed for 11 realizations. Numerical
tests showed that single realization results did not change when the solution of the flow problem was only recalculated every 40th time step. For the results presented in this paper the flow field was recalculated after 20 simulation steps of transport and biofilm growth.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta H$</td>
<td>$3 \cdot 10^{-2}m$</td>
<td>piezometric head difference between inflow and outflow</td>
</tr>
<tr>
<td>$C_{in}$</td>
<td>$1 Mm^{-3}$</td>
<td>inflow concentration of nutrient</td>
</tr>
<tr>
<td>$exch$</td>
<td>$7 \cdot 10^{-10}ms^{-1}$</td>
<td>rate constant for exchange between pore water and biofilm</td>
</tr>
<tr>
<td>$\mu^+$</td>
<td>$10^{-4}s^{-1}$</td>
<td>rate constant for microbial growth</td>
</tr>
<tr>
<td>$\mu^-$</td>
<td>$0.1\mu^+$</td>
<td>rate constant for microbial decay</td>
</tr>
<tr>
<td>$Y$</td>
<td>0.1</td>
<td>yield factor</td>
</tr>
<tr>
<td>$z$</td>
<td>$10^{-5}Mm^{-3}$</td>
<td>rate constant for shear forces</td>
</tr>
<tr>
<td>$d$</td>
<td>$10^2Mm^{-3}$</td>
<td>density of biomass (dry mass per wet volume)</td>
</tr>
</tbody>
</table>

Table 2.3: Scenario 2: Parameters used for simulation of biofilm growth; masses are given in units of a reference mass M

Results of these simulations are shown in Fig.2.4 for all values of $\sigma$. It can be seen that for all realizations a reduction in hydraulic conductivity of about two orders of magnitude could be achieved. The amount of biomass needed to get a maximal clogging effect again depended on the heterogeneity of the pore radii distribution. The smaller $\sigma$ the more biomass (or pore volume reduction) was needed to reduce the hydraulic conductivity. Nevertheless the simulation results indicated that the hydraulic conductivity was not dropping below a certain threshold and thus the pore network did not get completely clogged. Another observation was that with increasing $\sigma$ the differences between the $C_{rel} - V_{rel}$ curves for different single realizations, having the same $\sigma$, were increasing.
Figure 2.4: Reduction of hydraulic conductivity due to clogging of pores by a biofilm. Single realization results are compared to fits using Eqn.2.18 with parameters given in Tab.2.4.

To express the numerical data analytically a power law could be used, which was only modified to take into account the existence of a lower (non zero) threshold $C_{rel}^{min}$ for the relative hydraulic conductivity. Therefore the function

$$C_{rel}(V_{rel}) = \left( \frac{V_{rel} - V_0}{1 - V_0} \right)^b + C_{rel}^{min} \frac{1}{1 + C_{rel}^{min}}$$  \hspace{1cm} (2.18)

was used for fitting, with $V_0$, $C_{rel}^{min}$ and $b$ as fitting parameters. In analogy to Scenario 1, $V_0$ is the relative pore volume, for which the relative hydraulic conductivity reaches its minimum, and thus $1-V_0$ is the relative volume of biomass needed to reach the full clogging effect. To give the data points near the minimal $C_{rel}$ a higher weight, the log-values of $C_{rel}$ were fitted. Fitting was done for each single realization and the resulting parameters were then averaged for every $\sigma$ (Tab.2.4). It can be seen that $V_0$ and less strongly $C_{rel}^{min}$ increase with increasing $\sigma$, whereas $b$ is nearly not affected by the degree of heterogeneity of the pore network. Fig.2.4 shows plots of Eqn.2.18 using these averaged parameters in comparison to the single realization results.

To monitor the influence of the biofilm growth on the heterogeneity of the pore radii, one single realization was selected for each initial value of $\sigma$. The change of
\[ \sigma \quad b \quad C_{rel}^{\min} \quad V_0 \]

<table>
<thead>
<tr>
<th>( \sigma )</th>
<th>( b )</th>
<th>( C_{rel}^{\min} )</th>
<th>( V_0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.33</td>
<td>1.77 ± 0.01</td>
<td>(0.68 ± 0.04) ( \times 10^{-2} )</td>
<td>0.178 ± 0.004</td>
</tr>
<tr>
<td>0.50</td>
<td>1.78 ± 0.02</td>
<td>(0.93 ± 0.06) ( \times 10^{-2} )</td>
<td>0.301 ± 0.009</td>
</tr>
<tr>
<td>0.67</td>
<td>1.79 ± 0.02</td>
<td>(1.01 ± 0.07) ( \times 10^{-2} )</td>
<td>0.436 ± 0.014</td>
</tr>
</tbody>
</table>

Table 2.4: Scenario 2: Parameters used for fitting of numerical results

\( \sigma \) of the pore radii distribution was monitored for different reductions of hydraulic conductivity (Fig. 2.5). With increasing reduction of the hydraulic conductivity the pore radii became more heterogeneous until the highest values for \( \sigma \) were reached at \( C_{rel} \approx 0.1 \). Then the heterogeneity decreased again slightly, but remained clearly higher than for the unclogged networks.

![Figure 2.5: Change of heterogeneity of pore radii distribution for single pore networks clogged by a biofilm](image)

**2.5 Discussion**

Using pore network models to investigate the effects of bioclogging on a pore scale for different scenarios of microbial growth shows that the change of hydraulic properties of the entire pore network strongly depends on the morphology of microbial growth
in the pores.

### 2.5.1 Clogging due to microbial colonies

In Scenario 1 where the biomass was assumed to be present in form of microbial colonies, which occupy the smallest pores of the network first, it was possible to simulate reductions of hydraulic conductivity of more than two orders of magnitude. The amount of biomass needed to achieve a certain reduction of hydraulic conductivity strongly depended on the heterogeneity of the pore network. The higher the heterogeneity (within this study expressed by the standard deviation of the lognormal pore radii distribution) the less biomass was needed to clog the entire network. A function was presented, which could be successfully fitted to the numerical data by adjusting two independent parameters $V_0$ and $a$ only. $V_0$ is interpreted as the relative pore volume for which the hydraulic conductivity reaches 0, and thus $1-V_0$ is the relative volume of biomass needed to clog the entire network. The parameter $a$ quantifies the difference between Eqn.2.10 and a power law. In addition, $a$ contains the information how the hydraulic conductivity decreases initially for small changes of pore volume. The slope of this initial decrease $(\frac{dC_{rel}}{dV_{rel}})$ is $\frac{a+2}{1-V_0}$ for $V_{rel} = 1$. For homogeneous networks $a$ was close to -1 and thus this slope was approximately $\frac{1}{1-V_0}$, which would be the slope when expressing a linear decrease between $C_{rel} = 1$ at $V_{rel} = 1$ and $C_{rel} = 0$ at $V_{rel} = V_0$. With increasing heterogeneity, $a$ approaching -2, which corresponded to a slope of 0. This means that for homogeneous networks the removal of a small number of pores contributes equally to the decrease in $C_{rel}$ and $V_{rel}$, whereas for heterogeneous networks removing a small number of pores almost does not change $C_{rel}$. This behaviour is obviously determined by the way the pores are selected for removal. In this study the smallest pores were plugged first, and thus a removal of only a small number of pores effected $C_{rel}$ less than $V_{rel}$ (note that the hydraulic conductivity of a single pore $i$ scales with $r_i^4$, whereas its volume scales with $r_i^3$), especially for high degrees of heterogeneity. As a consequence $a$ is not only affected by the heterogeneity of the pore radii but also by the way pore plugging and pore radius are correlated. A preferential clogging of the smallest pores results
in values of $a$ closer to $-2$, and a weaker correlation between pore radii and clogging probability would result in values of $a$ closer to $-1$.

For practical reasons one can summarize that the key parameter $V_0$ is given by the minimum of the $C_{rel} - V_{rel}$ curves. To determine $1-V_0$, which is the volume of biomass needed to get the maximal clogging effect, it is possible to use results from percolation theory. For a large variety of network geometries percolation thresholds are known (Sahimi 1995) and if in addition the pore size distribution is known, Eqn.2.17 provides the possibility to predict the volume of biomass necessary to clog these networks. If $V_0$ is known, the parameter $a$ can be estimated from the slope of the $C_{rel} - V_{rel}$ curve for $V_{rel}$ close to 1.

\[ 2.5.2 \quad \text{Clogging due to a biofilm} \]

In Scenario 2 it was assumed that biomass grows as a biofilm at the walls of each pore. Results of the simulations could show that this biofilm was able to reduce the hydraulic conductivity of the entire network by up to two orders of magnitude. For this scenario it was possible to find an analytical expression linking hydraulic conductivity and pore volume using a power law, which could be fitted to the numerical results successfully. Parameters determined by this procedure again show the importance of determination of $V_0$ as the parameter varying most sensitively with $\sigma$. The exponent $b$ of the used power law seems to be independent of the heterogeneity of the pore network. Whether this is a general behavior for networks with biofilm growth can only be answered by further studies. For the upscaling of pore network results to the scale of a representative elementary volume this question is of great importance. Simulation results of this study indicate that biofilm growth is increasing the heterogeneity of a network's pore radii distribution. If these results can be confirmed by further investigations (numerical simulations or preferably experimental studies), they would contradict assumptions made by Clement et al. (1996), where preferential clogging of the larger pores was assumed, which led to higher volumes of biomass needed to reduce the hydraulic conductivity.
2.5.3 Comparison between numerical and experimental results

Comparing the results of both scenarios to experimental results reviewed in Vandevivere et al. (1995) and to the clogging model of Clement et al. (1996) (Fig. 2.6), the most significant difference is the different amount of biomass volume needed to reduce the hydraulic conductivity of the entire network. Microbial colonies are much more efficient in clogging a pore network than a continuous biofilm. The reduction of hydraulic conductivity derived for the colony scenario is similar to hydraulic conductivity reductions measured for porous media with grain sizes of less than 1 mm. The fact that for the presented experiments hydraulic conductivity was not reduced to 0 but maintained a small residual conductivity can not be explained with results from the colony scenario. Assuming that pores completely filled with biomass retain a very small but finite hydraulic conductivity, a residual conductivity can be reproduced within the colony scenario, too.

The existence of a residual hydraulic conductivity can be explained using the biofilm scenario, but the general decrease in hydraulic conductivity is strongly underpredicted by the biofilm scenario for grain sizes below 1 mm. In contrast, the reduction of hydraulic conductivity for a grain size of 1 mm is only slightly overpredicted by the biofilm scenario. This is at least an indication that for porous media with grain sizes below 1 mm, the assumption is more likely that the biomass is growing in colonies and not in a biofilm. For a porous medium made of 1 mm beads the measured decrease in hydraulic conductivity was less severe than for smaller grain sizes and predictions assuming a biofilm growth in the pores are better suited to describe the clogging for this bigger grain size. This would indicate that the growth morphology changes from colony growth to biofilm growth with increasing grain sizes and increasing average pore radii. As the formation of large plugs, able to clog pores with large radii, is less likely than the formation of small plugs, the assumption is reasonable that porous media made of coarse grains can not be clogged by microbial colonies.

Results for the biofilm scenario were similar to predictions of the model of
Figure 2.6: Comparison of measured (A) and predicted (B) reductions of hydraulic conductivity. Measured data were taken from Vandevivere et al. (1995), the Clement model is taken from Clement et al. (1996).

Although in Clement et al. (1996) no assumptions were made according to the distribution of biomass in the pores, it was shown in their study that their model predictions were very similar to results of the biofilm model of Taylor et al. (1990c), which was derived for bundles of non-interconnected pores. This indicates that in case of porous media clogged by a biofilm the pore interconnectivity has only small influence on the clogging effects.
The initial heterogeneity of the pore radii distribution had the same effect in both scenarios. The higher the initial heterogeneity the less biomass was needed to clog the entire network. The influence of biomass itself on the pore radii distribution differed between the two scenarios. For the colony scenario small pores were clogged preferentially and thus the heterogeneity of the remaining unclogged pores was reduced due to the bioclogging. In contrast it was shown in this study that for the biofilm scenario the heterogeneity of the pore radii distribution was increased due to bioclogging.

2.5.4 Limitation of pore network results

Results of this study were based on simplified assumptions on the growth of biomass in the pore space and the geometry of the used pore networks was also a simplification of a real porous medium. It must be assumed that this may affect the $C_{rel} - V_{rel}$ relations derived from the pore network simulations. The hydraulic properties of pore networks mainly depend on the coordination number $Z$ (number of pores per node) (Jerauld et al. 1984; Jerauld et al. 1984) and thus increasing the dimensionality of the rectangular network used in this study from two to three would increase its coordination number from $Z = 4$ to $Z = 6$. This increases the degrees of freedom for the water to flow through the network and it is reasonable to assume that a network can be clogged less easily then. The fact that the percolation threshold is getting smaller with increasing coordination number supports this assumption. Nevertheless this does not necessarily mean that natural porous media have a higher coordination number than the pore networks used in this thesis. Sahimi (1995) reported that the average coordination number for sandstones was found to be between 4 and 8, whereas Vogel and Roth (1998) found an average coordination number of approximately 2.5 for the A- and B-horizon of a forest soil. For the colony scenario we present a function (Eqn.2.17), which links the change in $V_0$ to the geometry and coordination number of the network. As Eqn.2.17 is only limited by assumptions regarding the biomass distribution, it can be used to estimate the volume of biomass needed to clog a given network. Only the knowledge of
the percolation threshold and the pore size distribution of the network is required. To determine, which type of network is the best representation of a natural porous medium, several techniques are available. Techniques for investigating porous media structures are e.g. mercury injection, electron microscopy or NMR; a method transferring the topology of a natural soil, analyzed by image analysis of serial sections, into a pore network model is e.g. described in Vogel (1997) and Vogel and Roth (1998). In this study we did not assume a spatial correlation of the pore radii. In case of porous media with spatial correlation lengths larger than the length of a pore, preferential flow may occur within the pore network. Especially for the colony scenario this would reduce the ability of the biomass to clog the entire network, as preferential flow paths contain mainly large pores. For the biofilm scenario the occurrence of preferential flow paths may have less influence on the clogging effects.

Another aspect may influence the results derived from pore network simulations in general. In this study the sites connecting the pores of the pore network were assumed to have no volume. If they contributed significantly to the total pore volume of the network, the $C_{rel} - V_{rel}$ relations would be different. Assuming that the pressure loss along these sites would be negligible, the biomass would have a higher clogging efficiency and thus the maximum reduction of hydraulic conductivity would be reached for smaller reductions of porosity. In addition it must be pointed out that simulations for the biofilm scenario were done for constant head boundary conditions. Therefore the flow velocity in the pores was decreasing with decreasing $C_{rel}$, and thus biofilm detachment by shear forces was not a dominant process. Assuming constant flux boundaries would cause the flow velocity in the pores to increase with increasing biofilm thickness. In this case biofilm detachment by shear forces as well as the transport and reattachment of the detached biomass would become an important process for the evolution of the clogging effects.

The absolute values of the parameters obtained in this study may not be representative for microbial growth in a natural porous medium (e.g. sand). Nevertheless, the general functional relationships provide an explanation for measured data such as those shown in Fig.2.6. For a further confirmation of the validity of the concept more experiments are necessary. Such experiments may be performed on a lab scale.
or on artificial pore networks (e.g. Wilson 1996; Dupin and McCarty 1999; Dupin and McCarty 2000; Kim and Fogler 2000).

2.6 Conclusion

Pore network simulations performed in this study could show that the high reductions in hydraulic conductivity of porous media, which for grain sizes below 1 mm were observed already for small reductions in porosity (Vandevivere et al. 1995), can be explained theoretically by assuming a colony scenario for microbial growth. In contrast, assuming microbial growth in a biofilm was underpredicting the experimentally observed clogging effects, except of those of a porous medium with a grain size of 1 mm. This indicates that the assumption of microbial colonies causing the bioclogging is more likely for porous media with grain sizes below 1 mm.

$V_0$ is the key parameter for both scenarios. It may depend on the geometry of the pore networks. At least for the colony model a function was presented in this study, which described the dependence of $V_0$ on the geometry and heterogeneity of the pore network.

Acknowledgements

The authors like to thank Geri Wagner, Israel Institute of Technology, Haifa, Israel for his support. This study was funded by the Swiss Federal Institute of Technology (ETH) Zurich, Switzerland.
Chapter 3

Further results of pore network simulations

Results shown in Chapter 2 were focusing on the changes of hydraulic conductivity of pore networks due to bioclogging. For the description of reactive transport in porous media, parameters such as the dispersivity or the specific surface of the pores may be important, too. This chapter focuses on the influence bioclogging may have on these parameters. Results are given for selected single realizations. The goal of this chapter is that pore network simulations can be used to investigate changes of these parameters, too.

3.1 Changes of flow pattern and transport properties due to bioclogging

3.1.1 Flow and transport properties of unclogged networks

To get reference data for the unclogged pore networks but also to verify the consistency of the flow and transport model, the transport of a nonreactive solute was simulated for pore networks without any microbial growth taking place. Using a 50×50 pores network with lognormally distributed pore radii, simulations were performed for a single realization with standard deviations of the natural logarithm of
\( \sigma = \frac{1}{3}, \frac{1}{2} \) and \( \frac{2}{3} \); the average pore radius was \( r_{\text{av}} = 30 \mu m \) and the pore length was \( l = 1.5 mm \). The distribution of the pore fluxes for these examples is given in Fig.3.1. A Dirac pulse of solute was injected at the inflow boundary and its breakthrough at the outflow boundary was observed. Results are shown in Fig.3.2 for piezometric head gradients \( G = 4 \cdot 10^{-2} \) and \( G = 4 \cdot 10^{-3} \). In analogy to Bear (1972) the function

\[
c(x, t) = \frac{c_0 u T}{\sqrt{4\pi Dt}} \exp \left( -\frac{(x - ut)^2}{4Dt} \right)
\]  

(3.1)

should express the cross-sectionally averaged concentration \( c(x, t) \) and was used here to fit the simulated concentration at the outflow. For the calculated examples \( c_0 = 1 \) was the injected concentration, \( T = 1 \) the injection interval for the bigger head gradient, \( T = 10 \) the injection interval for the smaller gradient and \( x = 0.0735 m \) the distance between inflow and outflow. The dispersion coefficient \( D \) and the average pore velocity \( u \) were fitted (Tab.3.1). It can be seen that the simulated data agree well with the theoretically proposed breakthrough. For a given pore size distribution, \( D \) and \( u \) show a linear relation with \( \alpha_L = \frac{L}{u} \) being the longitudinal dispersivity (Tab.3.1). \( \alpha_L \) increases with increasing \( \sigma \), which corresponds to the increasing heterogeneity of the pore size distribution.

![Figure 3.1: Distribution of pore fluxes for different degrees of heterogeneity; Greyscales indicate the absolute value for each pore compared to the maximum value of the specific network](image)

Figure 3.1: Distribution of pore fluxes for different degrees of heterogeneity; Greyscales indicate the absolute value for each pore compared to the maximum value of the specific network
3.1.2 Microbial growth in colonies

For the example shown in Fig.3.1 the changes in the flow field caused by the clogging of the pores are illustrated in Fig.3.3 (\(C_{rel}\) is between 0.11 and 0.13 for each \(\sigma\)). For these grids the breakthrough of a tracer was simulated as described in Section 3.1.1. Gradients were increased to achieve the same flux as for a gradient of \(G = 4 \cdot 10^{-2}\) in the unclogged networks. The simulated concentration at the outflow is shown in Fig.3.4, the parameters obtained by fitting Eqn.3.1 to the numerical results are given in Tab.3.2 together with values for gradients and pore volume. It was still possible to reproduce the numerical data by the analytical fitting, but with less accuracy than for the unclogged networks. Comparing these results with the parameters describing the unclogged grids (Tab.3.1) the examples for \(\sigma = \frac{1}{3}\) and \(\frac{1}{2}\) show an increase of \(\alpha_L\) due to the pore clogging whereas for \(\sigma = \frac{2}{3}\), \(\alpha_L\) is smaller for the clogged grid. This corresponds to the flow pattern where for \(\sigma = \frac{2}{3}\) only one major flow path remained,
Table 3.1: Transport parameters of the pore networks

<table>
<thead>
<tr>
<th>$\sigma$</th>
<th>$G$</th>
<th>$u$ [m/s]</th>
<th>$D$ [m$^2$/s]</th>
<th>$\alpha_L$ [m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.33</td>
<td>$4 \cdot 10^{-2}$</td>
<td>$1.02 \cdot 10^{-3}$</td>
<td>$2.42 \cdot 10^{-6}$</td>
<td>$2.37 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>0.33</td>
<td>$4 \cdot 10^{-3}$</td>
<td>$1.02 \cdot 10^{-4}$</td>
<td>$2.36 \cdot 10^{-7}$</td>
<td>$2.32 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>0.50</td>
<td>$4 \cdot 10^{-2}$</td>
<td>$7.97 \cdot 10^{-4}$</td>
<td>$2.39 \cdot 10^{-6}$</td>
<td>$3.00 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>0.50</td>
<td>$4 \cdot 10^{-3}$</td>
<td>$7.97 \cdot 10^{-5}$</td>
<td>$2.39 \cdot 10^{-7}$</td>
<td>$3.00 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>0.67</td>
<td>$4 \cdot 10^{-2}$</td>
<td>$5.82 \cdot 10^{-4}$</td>
<td>$2.86 \cdot 10^{-6}$</td>
<td>$4.92 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>0.67</td>
<td>$4 \cdot 10^{-3}$</td>
<td>$5.82 \cdot 10^{-5}$</td>
<td>$2.86 \cdot 10^{-7}$</td>
<td>$4.92 \cdot 10^{-3}$</td>
</tr>
</tbody>
</table>

Thus reducing dispersion effects in the network.

Figure 3.3: Distribution of pore fluxes in networks clogged by microbial colonies for different degrees of heterogeneity; Greyscales indicate the absolute value for each pore compared to the maximum value for the specific network.

3.1.3 Microbial growth in a biofilm

Analogously to Section 3.1.2, tracer breakthrough was simulated for the sample grids now clogged by a biofilm ($C_{rel}$ between 0.12 and 0.15). Flow patterns are given in Fig.3.5 and outflow concentrations in Fig.3.6. Again Eqn.3.1 was used to fit the simulated concentration with results of this procedure given in Tab.3.3. The flow patterns as well as the transport data indicate that the biofilm growth is increasing the heterogeneity of the pore network causing an increased dispersion length.
3.2 Changes of pore surfaces of clogged pore networks

The changes in the pore surfaces of networks clogged by microbial colonies can be calculated theoretically.

In a way analogous to the procedure described in Chapter 2 the surface of the unclogged pores $S_{rel}$ in relation to the initial surface of all pores can be expressed as

$$S_{rel} = \frac{1}{2} \left(1 - \text{erf} \left( \frac{\sigma}{\sqrt{2}} \right) \right)$$  \hspace{1cm} (3.2)

Fig.3.7 shows $S_{rel}$ as a function of $V_{rel}$. It can be seen that for the more heterogeneous pore networks a given pore volume reduction causes a higher decrease in pore surface area than for more homogeneous pore networks.

Simulated reduction of pore surfaces $S_{rel}$ of networks clogged by a biofilm is
Table 3.2: Transport parameters of the pore networks clogged by microbial colonies

<table>
<thead>
<tr>
<th>$\sigma$</th>
<th>$G$</th>
<th>$V_{rel}$</th>
<th>$u_{m,s}$</th>
<th>$D_{m,s}$</th>
<th>$\alpha_L [m]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.33</td>
<td>3.3 · $10^{-1}$</td>
<td>0.78</td>
<td>$3.50 \cdot 10^{-3}$</td>
<td>$1.39 \cdot 10^{-5}$</td>
<td>$3.99 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>0.50</td>
<td>3.7 · $10^{-1}$</td>
<td>0.85</td>
<td>$3.22 \cdot 10^{-3}$</td>
<td>$1.55 \cdot 10^{-5}$</td>
<td>$4.82 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>0.67</td>
<td>3.6 · $10^{-1}$</td>
<td>0.91</td>
<td>$2.88 \cdot 10^{-3}$</td>
<td>$1.13 \cdot 10^{-5}$</td>
<td>$3.93 \cdot 10^{-3}$</td>
</tr>
</tbody>
</table>

Figure 3.5: Distribution of pore fluxes in networks clogged by a biofilm for different degrees of heterogeneity; Greyscales indicate the absolute value for each pore compared to the maximum value for the specific network given for single realization results. $S_{rel}$ decreases with decreasing pore volume $V_{rel}$ showing only small differences for different $\sigma$, with a larger decrease of pore surfaces for larger $\sigma$ (Fig.3.8).

3.3 Summary of results

These results indicate that for both scenarios the presence of biomass is initially increasing the dispersivity of a pore network. For networks clogged by microbial colonies the dispersion length is decreasing again when reaching the largest decrease in hydraulic conductivity. This can be explained with the reduction of available flow paths by reaching the percolation threshold. For networks clogged by a biofilm where pores are not clogged completely this effect does not take place. Nevertheless the flow patterns were also changed due to clogging by a biofilm. The flow patterns became more heterogeneous due to the biofilm growth, which corresponds to the
observed increase in pore size heterogeneity (Chapter 2). These results are different from results given in Taylor and Jaffé (1990b). With their biofilm model these authors predict a much higher dispersivity increase of several orders of magnitude due to the bioclogging, which agreed with experimental observations also shown in (Taylor and Jaffé 1990b).

Reductions in pore surfaces were larger for networks clogged by a biofilm. In both scenarios, these reductions were increasing with an increasing heterogeneity of the networks.

The examples presented show that regardless of the morphology of microbial growth the biomass is influencing not only the hydraulic conductivity of porous media but also other parameters important for reactive transport. Pore network models can be used for the investigation of these questions, too.
Table 3.3: Transport parameters of the pore networks clogged by a biofilm

<table>
<thead>
<tr>
<th>$\sigma$</th>
<th>$G$</th>
<th>$V_{rel}$</th>
<th>$u[\frac{m}{s}]$</th>
<th>$D[\frac{m^2}{s}]$</th>
<th>$\alpha_L[m]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.33</td>
<td>$2.9 \times 10^{-1}$</td>
<td>0.45</td>
<td>$2.32 \times 10^{-3}$</td>
<td>$7.52 \times 10^{-6}$</td>
<td>$3.24 \times 10^{-3}$</td>
</tr>
<tr>
<td>0.50</td>
<td>$3.2 \times 10^{-1}$</td>
<td>0.51</td>
<td>$1.78 \times 10^{-3}$</td>
<td>$1.22 \times 10^{-5}$</td>
<td>$6.87 \times 10^{-3}$</td>
</tr>
<tr>
<td>0.67</td>
<td>$3.3 \times 10^{-1}$</td>
<td>0.61</td>
<td>$1.31 \times 10^{-3}$</td>
<td>$1.53 \times 10^{-5}$</td>
<td>$1.17 \times 10^{-2}$</td>
</tr>
</tbody>
</table>

Figure 3.7: Theoretical proposed reduction of pore surface for pore networks clogged by microbial colonies
Figure 3.8: Relation of pore surface and pore volume for single pore networks clogged by a biofilm
Chapter 4

Interaction between water flow and spatial distribution of microbial growth in a two-dimensional flow field in saturated porous media


4.1 Abstract

Bacterial growth and its interaction with water flow was investigated in a two-dimensional flow field in a saturated porous medium. A flow cell (56 cm × 44 cm × 1cm) was filled with glass beads and operated under a continuous flow of a mineral medium containing nitrate as electron acceptor. A glucose solution was injected through an injection port, simulating a point source contamination. Visible light transmission was used to observe the distribution of the growing biomass and water flow during the experiment. At the end of the experiment (on day 31) porous
medium samples were destructively collected and analyzed for abundance of total and active bacterial cells, bacterial cell volume and concentration of polysaccharides and proteins. Microbial growth was observed in two stripes along the length of the flow cell, starting at the glucose injection port, where highest biomass concentrations were obtained. The spatial distribution of biomass indicated that microbial activity was limited by transverse mixing between glucose and nitrate media, as only in the mixing zone between the media high biological activities were achieved. The ability of the biomass to change the flow pattern in the flow cell was observed, indicating that the biomass was locally reducing the hydraulic conductivity of the porous medium. This bioclogging effect became evident when the injection of the glucose solution was turned off and water flow still bypassed the area around the glucose injection port, preserving the flow pattern as it was during the injection of the glucose solution. As flow bypass was possible in this system, the average hydraulic properties of the flow cell were not affected by the produced biomass. Even in the vicinity of the injection port, the total volume of the bacterial cells remained below 0.01% of the pore space and was unlikely to be responsible for the bioclogging. However, the bacteria produced large amounts of extracellular polymeric substances (EPS), which likely caused the observed bioclogging effects.

4.2 Introduction

Biodegradation is often a desired process for the remediation of aquifers contaminated by organic compounds (Anderson and Lovley 1997). These contaminants may serve as a carbon source for microorganisms, and increased biomass production rates are possible given that suitable electron acceptors are present (Zarda et al. 1998; Bolliger et al. 2000). Transverse mixing between the contaminant and the electron acceptor was found to be an important processes controlling degradation rates on the pore scale (e.g. Raje and Kapoor 2000) as well as on the field scale (e.g. Thorton et al. 2001). Theoretical studies could identify dispersion as the dominating process for transverse mixing (Cirpka et al. 1999; Oya and Valocchi 1998).

Reduction of hydraulic conductivity and porosity of a saturated porous medium
due to microbial growth, hereafter referred to as bioclogging, has been observed under various conditions (Baveye et al. 1998). Bioclogging may influence the feasibility of bioremediation techniques in several ways. For example, microbial activity in contaminated parts of an aquifer can clog porous media and thus limit the availability of oxidants to microorganisms. Conversely, controlled clogging of an aquifer may be used to block preferential flow paths to produce a more homogeneous sweep (Lappan and Fogler 1996), or to build up biobarriers that could be used to intensify contact between bacteria and contaminant, which may increase the success of remediation. For these reasons, it is necessary to investigate and understand interactions between biomass (bacteria and extracellular polymeric substances (EPS) produced by bacteria) and flow in porous media.

Several authors have reported on significant reduction of hydraulic conductivity due to bioclogging (Taylor and Jaffé 1990a; Cunningham et al. 1991; Vandevivere and Baveye 1992c; Brough et al. 1997; Johnston et al. 1997; Wu et al. 1997). All of these studies were performed in laboratory systems using one-dimensional flow fields (i.e. columns). Models for interpretation of these data exist (e.g. Taylor and Jaffé 1990b), but they do not appear to reproduce reductions of hydraulic conductivity properly (Vandevivere et al. 1995). Model shortcomings were attributed to microorganisms growing in colonies, which influence the hydraulic conductivity differently than a biofilm, and to the lack of interpore connections in the models (Vandevivere et al. 1995). Microbial growth and its influence on hydraulic properties of microscopic pore networks was investigated by Dupin and McCarty (2000) and Kim and Fogler (2000). Dupin and McCarty (2000) observed a correlation between hydraulic conductivity and biological growth and morphology, Kim and Fogler (2000) also observed a decrease in hydraulic conductivity, which was only partially reversed under bacterial starving conditions. They attributed irreversible clogging effects to the gelation of the EPS, which makes the EPS more resistant to shear forces. In studies by Taylor and Jaffé (1990b) and Sharp et al. (1999) it was shown that biomass is not only able to reduce hydraulic conductivity and porosity, but may also lead to an increased dispersivity of the porous medium.

As natural flow systems usually do not behave like one-dimensional systems,
investigation of bioclogging in two- or three-dimensional systems is of considerable interest. In such systems clogged parts of the porous medium can be bypassed by water flow. This may produce effects that cannot be observed in quasi-one-dimensional systems, in which potential flow bypassing of clogged parts of the porous medium is difficult to resolve.

To demonstrate that observed clogging effects are caused by biomass, it is necessary to determine the presence of biomass in the porous medium. In previous studies different parameters were used to quantify biomass: bacterial carbon content (Taylor and Jaffé 1990a), phospholipid content (Vandevivere and Baveye 1992c) and biofilm thickness (Cunningham et al. 1991). To monitor biomass distribution, confocal laser scanning microscopy (De Leo and Baveye 1997), epifluorescent microscopy (Wu et al. 1997) and plate counting (Brough et al. 1997) have been employed. Except for direct visual measurements in Cunningham et al. (1991), all of these methods are destructive and thus can be performed only once at the end of the experiments. Furthermore, only one parameter was measured in each study to describe the biomass distribution, thus assuming that this parameter is characteristic for total biomass. However, since biomass composition depends on the growth conditions (Christensen and Characklis 1990), one single parameter may not give sufficient information on biomass distribution in a porous medium. As not only the bacterial cells but also EPS are assumed to be responsible for bioclogging (Baveye et al. 1998), their presence must be quantified, too. In order to relate observed clogging effects to the presence of biomass it may therefore be desirable to measure a set of different parameters to quantify the biomass distribution in porous media. In addition, to avoid the limitations of destructive methods, application of a non-invasive method during an experiment may also increase our understanding of microbial growth in porous media.

The goal of this study was to investigate the interaction between water flow and microbial growth in a two-dimensional flow field. In particular, we wanted to visualize water flow and biomass distribution for a simulated point source contamination. We performed an experiment in a flow cell filled with glass beads as a porous medium. To assess biomass distribution present in the porous medium, we measured changes
in visible light transmission throughout the experiment. In addition, we used a color
dye to visually observe flow patterns. Finally, upon termination of the experiment,
we destructively sampled the porous medium and measured the number of total
and active bacteria, bacteria cell volume and concentrations of polysaccharides and
proteins.

4.3 Materials and Methods

4.3.1 Growth medium and inoculum

The mineral medium consisted of KNO₃, 51 mg/l; KH₂PO₄, 5 mg/l; NH₄Cl, 63
mg/l; NaCl, 25 mg/l; MgCl₂·6H₂O, 10 mg/l; CaCl₂·2H₂O, 38 mg/l; KCl, 20 mg/l;
and NaSO₄, 5 mg/l dissolved in de-ionized water. After autoclaving the medium
at 120 °C, a sterile mixture of trace elements, vitamins, selenite-tungstate solution
and NaHCO₃ solution was added and pH was adjusted to 7.5. The mineral medium
was sparged with argon gas and then kept under argon atmosphere to reduce its
nitrogen and oxygen gas content.

The inoculum was prepared 24 h before inoculation of the porous medium. For
this purpose Pseudomonas strain PS⁺ (Deutsche Sammlung von Mikroorganismen
und Zellkulturen No. 12877) was grown in 2 l of mineral medium with 1 g/l glucose
as substrate and nitrate as electron acceptor on an orbital shaker at 30 °C.

4.3.2 Setup and operation of the flow cell

The experiment was conducted in a flow cell (Fig.4.1; height: 56 cm; width: 44
cm; thickness: 1 cm) that consisted of two parallel polycarbonate plates mounted
on an aluminum frame. In addition, two pairs of aluminum braces were used to
stabilize the plates from the outside (not shown in Fig.4.1). The bottom of the flow
cell was first filled with 303 g coarse glass beads followed by 3865 g fine beads (all
packed under saturated conditions) to achieve a total filling height of 55 cm. The
coarse beads had a diameter of 1 mm and the fine beads a diameter of 0.4-0.6 mm.
Both types of glass beads had a density of 2.5 kg/l. Due to pressure forces and

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the elasticity of the polycarbonate plates the total volume of the filled flow cell was increased from the calculated 2464 ml to 2720 ml (determined gravimetrically by filling the flow cell with water). Using this adjusted volume, the mass and density of the glass beads, the total porosity was calculated to be 0.38.

Figure 4.1: Diagram of the experimental setup (only flow cell and objects inside flow cell are drawn to scale). Aluminum frame and braces are not shown.

The flow cell was operated at a constant temperature of 22 °C in an upward-flow mode under water saturated conditions throughout the experiment. A peristaltic pump (IPN-4, Ismatec, Glattbrugg, Switzerland) was used to inject a mineral medium containing nitrate at four inflow ports at the bottom of the flow cell (Fig. 4.1). The pumping rate at each port was kept constant at 500 ml/day.

An additional injection port was placed 10 cm above the bottom and equidistant to the sides of the cell. At this port glucose solution (1 g/l) was injected at a constant pumping rate of 40 ml/day using a peristaltic pump (IPC-16, Ismatec). The outflow port at the center on top of the flow cell was kept at constant hydraulic head. Manometers were placed close to the top and bottom of the flow cell within
the fine beads pack (Fig. 4.1).

After packing of the flow cell and initiation of mineral medium flow, de-ionised water containing a color dye (Brilliant Blue FCF, Eriogluacine A, Fluka, Buchs, Switzerland) was injected at the glucose injection port and its distribution was monitored as a reference for glucose distribution during the experiment (see also section 4.3.3). Furthermore, the breakthrough of chloride, injected at the four inflow ports with a total pumping rate of 2000 ml/day, was measured at the outflow to ensure the hydraulic functioning of the system and to determine the residence time of water in the flow cell.

Prior to starting the experiment, 2 l of the inoculum were injected via the inflow ports at the bottom of the flow cell using the same pumping rate as for the mineral medium. The experiment began when injection of glucose solution was initiated (day 1). On days 4, 9, 17 and 22 color tracer tests were performed by adding the color dye to the glucose to trace its distribution in the flow cell. An additional tracer tests was performed, for which the glucose injection was temporarily stopped (days 28-29). For this additional test, the glucose injection port was partially withdrawn and dyed de-ionized water was injected approximately 4 cm below the former glucose injection port at a flow rate of 40 ml/day. From day 29 on the experiment was performed as before this tracer test and ended on day 31.

4.3.3 Water flow and microbial growth visualization

Visible light transmission was used for water flow and biomass visualization. A light table was placed behind the flow cell (in an upright position). Light transmitted through the flow cell was recorded by a digital camera (JVC TK-C 1380E with Leica 1.2/12.5-75 mm lens) connected to a PC via a framegrabber (Leutron Picport, all from Leica Microsystems, Glattbrugg, Switzerland). Color pictures (red, green and blue channel (RGB), resolution 752 x 582 pixels) of the flow cell were taken and processed in two steps using Photoshop 5.5 (Adobe). The first step of picture processing was background subtraction with a background picture. In the second step, the RGB-color pictures were separated by color channel, the red channel (maximum
of contrast) was converted into a gray scale picture.

For visualization of the flow field, the injected fluids was dyed using Brilliant Blue FCF. Background pictures were taken just before dye injection. After background subtraction, the contrast of each single grey scale picture was maximised by linear amplification such that the darkest pixel(s) would attain a grey value of 255.

Biomass was visualized by taking a picture of the flow cell and subtracting a background picture, which was taken at the beginning of the experiment. All grey scale pictures showing biomass had grey values ranging between 0 and 60. To increase the contrast, the grey value range was linearly amplified from 0 to 60 to the full range of 0 to 255 for each picture in identical fashion. To avoid interference between signals from the biomass and the color dye, pictures for biomass visualization were taken when no dye was present in the flow cell.

4.3.4 Water and porous medium sample collection

Water samples were collected once per day from inflow (2-3 ml per sample) and outflow (10 ml per sample) sampling ports. During the chloride tracer test, the sampling interval at the outflow sampling port was between 0.5 and 7 h.

On day 31 day (end of the experiment), the flow cell was drained (flow rate approx. 1 l/h), placed into horizontal position and opened by removing the top plate. Forty-nine porous medium samples were collected (7 transects x 7 samples per transect, see Fig.4.1). Each porous medium sample of 3 cm³ in volume (3 cm x 1 cm x 1 cm, approx. 5 g wet weight) was homogenized and divided into four sub-samples (2.0, 2.0, 0.5 and 0.5 g). The two 2 g sub-samples were immediately used for polysaccharide and protein quantification, while the two 0.5 g sub-samples were fixed for subsequent bacterial counts according to the method of Zarda et al. (1997).

4.3.5 Chemical and biological analyses

Water samples were analyzed for nitrate and chloride using a Dionex DX-100 ion chromatograph (Dionex, Sunnyvale, CA, USA), equipped with an electrical conduc-
tivity detector. All biological analysis were performed using nanopure-water and acid washed glass material. Results are averages of triplicates ± standard error.

Total concentration of polysaccharides (EPS and bacteria) in the sampled porous medium were measured using the Dubois method (Dubois et al. 1956). Absorbance of light (wavelength 495 nm) was measured with a spectrometer (UVicon, Kontron Instruments, Switzerland) against the reagent blank. Measured values were compared against fresh glucose solution of 0.0, 3.5, 7.0, 17.0, 28.0, and 35.0 µg per ml water as standards.

Total concentration of proteins (EPS and bacteria) were measured using the Lowry method (Lowry et al. 1951). Absorbance of light (wavelength 750 nm) against the reagent blank was measured using the same spectrometer as previously mentioned. Measured absorbance values were compared against standards of 1, 2, 5, 10, 15, 20 and 25 mg/l Bovine Serum Albumine.

Number and volume of total bacteria were investigated using DAPI staining (4',6-diamidino-2-phenylindole, Porter and Feig 1980) according to the method of Zarda et al. (1997) combined with image analysis. Slides mounted with citifluor were examined at 400× magnification (Zeiss Plan-Neofluar oil) with a Zeiss Axiophot microscope fitted for epifluorescence with a 50 W high-pressure mercury bulb and filter set 02 (Zeiss, G 365, FT 395, LP 420). At least 400 bacteria per slide triplicate were manually counted. Bacterial volumes were measured by an image analysis system in 5 images per triplicate with up to 100 cells per image (Schönholzer et al. 1999). Cell volume was determined based on measurements of area and perimeter for each organism or bacterial aggregate. After subsequent determination of fiberlength and fiberwidth, bacterial volumes were calculated (Analytical vision 1992; Russ 1995).

To determine whether bacteria were metabolically active in the system, the number of ETS-active bacteria (i.e. bacteria with an active electron transport system) in porous medium samples was measured using CTC staining (5-cyano-2,3-ditolyl tetrazolium choride, Rodriguez et al. 1992). Fresh samples were incubated for 3 h in the dark at room temperature with CTC solution (1.4 mg/ml). Samples were diluted with 0.1% pyrophosphate. Bacteria were detached from the glass beads us-
ing ultrasonification (Sonifer B-12, Branson sonic power Company, Danbury, CT, USA) (Mermillod-Blondin et al. 2001). Aliquots were spotted onto gelatin-coated slides. Slides mounted with citiflour were examined as previously described with the microscope fitted with filter set Hq Cy3 (AHF, Germany; G 546, FT 560, BP 575-640). At least 400 bacteria (10 × 40 fields) were counted per slide triplicate.

4.4 Results

4.4.1 Hydraulic and chemical data

Chloride breakthrough was measured at the beginning of the experiment to check the hydraulic functioning of the system and to determine the residence time of water in the flow cell and the longitudinal dispersivity of the porous medium. Measured chloride concentration at the outflow sampling port increased smoothly between 10 and 15 h from background concentration to inflow concentration (Fig.4.2). To compare the measured and theoretical breakthrough curves, flow was assumed to be one-dimensional within the cell. An analytical solution to solute transport of a conservative tracer in a one-dimensional flow field taken from Bear (1972) was fitted to the measured data to determine values for pore water velocity \( u \) and the longitudinal dispersion coefficient \( D \). For \( u=1.04 \text{ m/day} \) and \( D=0.002 \text{ m}^2/\text{day} \) the best fit was achieved (Fig.4.2). The total residence time \( T \) of water in the entire flow cell (length: \( x=0.56 \text{ cm} \), including the top zone filled with water only) is therefore \( T = \frac{x}{u} = 0.54 \text{ days} \). Given the total pumping rate of 2000 ml/day, this results in a computed average porosity of 0.39 for the porous medium, which is slightly higher than the porosity of 0.38 calculated from the weight of the glass beads. Assuming the relation \( D = \alpha_L u \), we computed a longitudinal dispersivity \( \alpha_L \approx 0.2 \text{ cm} \).

Manometer measurements showed no changes in hydraulic head during the entire experiment (not shown). The head difference between the manometers at the top and the bottom of the flow cell was approximately 1 mm with no visible horizontal differences. Based on this value the hydraulic conductivity of the porous medium was estimated at 2.3 \( \times 10^{-3} \text{ m/s} \).
Analysis of nitrate concentration at the inflow and outflow sampling ports indicated that nitrate consumption occurred in the flow cell. The inflow concentration was between 450 and 500 $\mu$M throughout the experiment, whereas the outflow concentration was generally smaller than the inflow concentration (not shown). Between days 17 and 27 the average nitrate concentration was approximately 450 $\mu$M at the inflow and 225 $\mu$M at the outflow. Using the total pumping rate of 2000 ml/day this corresponds to a nitrate consumption of approximately 0.45 mmol/day (50% of the injected amount). Assuming that the oxidation of glucose with nitrate is described by the equation

$$5C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 30CO_2 + 12N_2 + 42H_2O \quad (4.1)$$

nitrate consumption of 0.45 mmol/day corresponds to a glucose oxidation of approximately 0.09 mmol/day or 42% of the amount of glucose injected per day. Between days 28 to 29 the glucose injection was stopped. During this period the outflow concentration of nitrate exhibited an increase, approaching the inflow concentration (not shown).

Figure 4.2: Breakthrough of chloride at the outflow sampling port of the flow cell. Injection of concentration $c_0$ began at $t=0$. 

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4.4.2 Visual observations

About one week after the start of the flow cell experiment, an orange-brown colored substance was observed in the flow cell. This substance, assumed to be biomass, was initially located close to the glucose injection port, but later biomass was observed in a stripe pattern along the entire length of the flow cell starting near the glucose injection port (Fig.4.3). Biomass was also found in the free water phase on top of the porous medium in the direct vicinity of the outflow port. About three weeks after the start of the experiment, gas bubbles could be observed in the upper regions of the flow cell, which interfered with the optical signals from the biomass. Subsequent measurement of the N₂ gas content in water collected from the outflow sampling port showed that the water was oversaturated with N₂ gas. Thus, the observed gas bubbles were attributed to N₂ gas production, showing that the argon sparging of the mineral medium was not sufficiently effective. However, the argon sparging seemed to reduce the N₂ gas content of the mineral medium at least partly as we observed no gas bubbles (but high amounts of biomass) in a zone close to the glucose injection port. Therefore, the results described below will focus on this observation zone, indicated by the rectangles in Fig.4.3.

Within the observation zone the optical signals were attributed to biomass only. Pictures of visible light transmission indicate that during the experiment the biomass increased significantly, developing in a U- to ring-shaped pattern with its origin directly below the glucose injection port (Fig.4.4). From the picture taken at day 30 we extracted the pixel values along the transects A and B. In Fig.4.5 these values are shown as averages for the height (3 cm) of the transects. For transect A, a double peak pattern is clearly visible, whereas data for transect B show only a slight tendency to exhibit this double peak pattern. When comparing these data to the average pixel values for the area of the individual porous medium sampling points, the double peak pattern for transect A is much less pronounced, and for transect B only a single peak in the middle of the transect remains.

The color tracer tests with dyed glucose solution showed no significant changes in the distribution of the glucose solution throughout the experiment (Fig.4.6). The
Figure 4.3: Biomass (and partially gas) distribution in the flow cell. Pictures show a 46cm × 20cm area in the center of the flow cell; rectangles mark the observation zone used for detailed analyses.

Figure 4.4: Biomass distribution in a 14.5cm × 8.5cm observation zone around the glucose injection port. Rectangles show the position of the porous media sampling point of transects A and B in this zone.
Figure 4.5: Pixel values for transects A and B obtained by picture analysis. x-axes show distance to left border of flow cell.

Pixel values along the center line were slightly more scattered towards the end of the experiment, but these effects were not pronounced enough to be considered significant. Data shown in Fig.4.6 represent the quasi steady-state distribution of the glucose at the end of each tracer test, but also observations during the early stages of these tracer tests showed no changes in the flow of the glucose solution (data not shown). Conversely, during the color tracer test with dyed de-ionized water on days 28 to 29 (injected via the partially withdrawn glucose injection port), an influence of the biomass on the flow field could be observed (Fig.4.7). The flow of the dyed water tended to surround the area containing the biomass (note that Figs.4.4, 4.6 and 4.7 show the same area of the flow cell), but this clogging effect was not strong enough to prevent dyed water from penetrating also the clogged area. Nevertheless, the flow pattern of the dyed water indicated that the biomass increased the flow resistance of the porous medium in that region.

4.4.3 Biological analyses

At the end of the experiment, porous medium samples were collected and analyzed for total number of bacteria, number of active bacteria, concentration of polysaccharides, and concentration of proteins. Results of these analyses show a strong
Figure 4.6: Distribution of dyed solution injected at the glucose injection port before the experiment (day 0) and on selected days. Pictures were taken one day after injection of color dye was started. Grey values show pixel values along the black line in the center of the observation zone.

variability of measured parameters along the transects (Fig.4.8). All measured parameters show a two-stripe shaped growth zone in the center of the flow cell with highest values found in direct vicinity of the glucose injection port. Highest measured bacterial numbers were $190 \cdot 10^6$ cells/g beads for total bacteria and $17 \cdot 10^6$ cells/g beads for active bacteria, maximum concentrations were $29 \mu g/g$ beads for polysaccharides and $53 \mu g/g$ beads for proteins.

In addition, Fig.4.9 shows the results for the two transects, A and B, located close to the glucose injection port. Results for transect A show a double peaked pattern for all measured parameters. For transect B concentration of polysaccharides and to some extent concentration of proteins generate only a single peak in the middle of the transect. Total and active bacteria numbers exhibit a double peak pattern.

The average volume per bacterial cell was determined to be $0.082 \pm 0.006 \mu m^3$. Using average volume per bacterial cell, porosity, glass bead density and total number of bacteria, we calculated the volume fraction of the pore space occupied by bacteria to be less than 0.01%.
Figure 4.7: Water flow surrounding the zone clogged by the biomass. Glucose injection was turned off and dyed water was injected via the partially withdrawn glucose injection port, starting at day 28. Legends show time after the start of color dye injection.

4.5 Discussion

Results from the chloride tracer test showed that the porous medium pack was highly homogeneous. Only a small discrepancy between measured and fitted chloride concentration towards the end of the breakthrough curve was observed, which we were unable to attribute to a specific cause. The computed dispersivity $\alpha_L \approx 0.2$ cm was slightly smaller than the value of 0.43 cm found by Sharp et al. (1999) for a similar system, supporting the assumption that our packing was highly homogeneous. Therefore, dispersive mixing between mineral medium and glucose solution affected the media’s distribution only slightly. The fact that the nitrate concentration at the
outflow approached the inflow concentration during the break in glucose injection indicates that nitrate consumption was coupled to glucose oxidation, suggesting a microbially mediated processes.

The distribution of the biomass was strongly influenced by the boundary conditions determining the flow field in the flow cell (Fig.4.3). This was caused by the separate injection of electron donor (glucose) and acceptor (nitrate). Only where water from both origins mixed, microbial growth was possible. Thus, microbial growth was generally limited to a zone in the center of the flow cell starting from the glucose injection port upward. Biomass production occurred initially (days 8-14) in all parts of this zone, indicating that at least to a certain extent mixing took place. During the experiment, biomass production shifted to the boundary of this growth zone (Fig.4.4). This may be explained by a consumption of the nitrate by the bacteria located at the boundary of the growth zone, which resulted in nitrate depletion in internal regions of the growth zone. Another plausible explanation is that clogging of the pores reduced mixing of water containing nitrate and water containing glucose.

The lack of changes in the spatial distribution of the glucose solution (Fig.4.6) indicated that the produced biomass did not significantly affect the initial flow field
Figure 4.9: Measured biological parameters for transects A and B. x-axes show distance to left border of flow cell.

during the experiment, as long as the flow boundary conditions (represented by the position of the injection and inflow ports and their flow rates) were not modified. Throughout the entire experiment the glucose was migrating between the two stripes of microbial growth. However, it was possible to show an influence of the biomass on the flow field when the boundary conditions for the flow were modified by changing the position of the glucose injection port (Fig.4.7). This clearly indicated that biomass was generally able to influence the hydraulic properties of a porous medium and bioclogging took place as previously reported by several authors (e.g. Taylor and
Jaffé 1990a; Vandevivere and Baveye 1992c). In case of a two-dimensional flow-field, as in the present experiment, bioclogging had a stabilizing effect on the flow field. This means that the initial flow field was not changed due to bioclogging but the flow pattern defined by the initial flow boundary conditions was preserved even when these flow boundary conditions were changed.

In a similar study Kildsgaard (1999) also investigated bioclogging in a two-dimensional flow field, but they injected the electron donor and acceptor together at the same injection port and clogging effects in their study were different from the results of our study. In Kildsgaard (1999), the buildup of a clogged zone in the flow path of the nutrients was observed, forcing the water to bypass this zone while the flow boundary conditions were not changed. The differences between the results of the current study and the study of Kildsgaard (1999) show that the occurrence of bioclogging is highly dependent on the method of nutrient addition. In contaminated aquifers electron donor and acceptor are often from different sources (e.g. for the case of a point source petroleum hydrocarbon contamination or for the case of an engineered remediation system where the electron acceptor is injected as a point source). A laboratory system that uses separate injection systems for electron donor and acceptor, as was the case in this study, is therefore better suited to simulate a point source contamination or an engineered system, as mentioned above.

Although the hydraulic parameters were not measured locally, data shown in Fig.4.7 suggest that the clogged part of the system was less permeable to flow. As indicated by the tracer test on days 28 and 29 (when no glucose but dyed water was injected), the clogging must be attributed to the accumulation of biomass in the pore space. Nevertheless, dyed water eventually entered this clogged part, which indicates that the reduction in hydraulic conductivity did not reach the extent reported elsewhere for column experiments. Reduction of hydraulic conductivity of more than three orders of magnitude, as observed e.g. in experiments of Taylor and Jaffé (1990a) and Vandevivere and Baveye (1992c), should have nearly completely prevented dyed water from entering the clogged part of the flow cell. Conversely, flow bypass of a clogged zone, which can only take place in multi-dimensional flow fields, appears to play an important role for flow in considerably clogged porous me-
Due to the possibility of flow bypass, the hydraulic conductivity of the entire flow cell remained essentially constant, showing that clogging can take place in a part of a multi-dimensional flow system without influencing the properties of the system on a larger scale.

The analysis of biological parameters at the end of the experiment showed that the biomass was mainly located in a growth zone, divided into two stripes, in the center of the flow cell (Fig. 4.8). This agrees with results from picture analysis (Fig. 4.3). Focusing on transects A and B, a comparison between the original pixel values and the average pixel value, which corresponds to a sample (Fig. 4.5), shows that the pattern observed by the picture analysis could only partly be resolved by the porous medium sampling scheme. Therefore, a higher spatial resolution of the measured biological parameters would have been desirable. However, the sample size and number used in this study had to be a compromise between a high spatial resolution and the minimum amount of sample material needed for the different analyses. Pixel values, averaged for the area of the sampling points (Fig. 4.5), agree well with the concentration of polysaccharides and proteins whereas the measured bacterial numbers show a slightly different pattern (Fig. 4.9). This suggests that EPS and not the bacteria were observed by picture analysis. The fact that the total volume of bacteria did not exceed a fraction of 0.01% of the porosity also supports this assumption.

To further determine, which fraction of the biomass was dominant, bacteria or EPS, we estimated the total amount of organic carbon and the amount of bacterial carbon for transects A and B. We assumed that with the exception of polysaccharides and proteins other types of organic carbon can be neglected. Thus, the concentration of total organic carbon would be the sum of carbon in the polysaccharides and the proteins. From the molecular structure of polysaccharides and proteins (Stryer 1990), the average carbon contents were assumed to be $0.40 \pm 0.05$ g C/g polysaccharides and $0.50 \pm 0.20$ g C/g proteins. Fig. 4.10 shows that for transect A organic carbon concentration reached 30-40 $\mu$g/g beads, and was much higher compared to transect B with maximum concentration of approximately 10 $\mu$g/g beads. For the calculation of bacterial carbon, the average carbon content per volume of the bacteria
was an important variable. Values for this conversion factor published in literature (reviewed in Fry 1990) vary between 0.05 and 0.65 mg C/cm\(^3\) bacterial cells with most of the values being between 0.05 and 0.35 g C/cm\(^3\). Therefore, we used an estimate of 0.20 ± 0.15 g C/cm\(^3\) bacterial cells, which resulted in a high uncertainty associated with the bacterial carbon concentration. Results of these calculations showed that bacterial carbon concentration did not exceed 4 \(\mu\)g/g beads (Fig.4.10) and the fraction of bacterial carbon in the total organic carbon is < 10% for most of the sampling points and with an average of approximately 5%, again indicating that the bulk of the biomass is formed by EPS and not by bacteria, which agrees with findings in Bakke et al. (1984). In addition, these results support observations made by Vandevivere and Baveye (1992a), who compared clogging efficiency of different bacterial strains in sand columns, showing that all strains produced similar amounts of bacterial mass, but only the EPS producing strain was able to clog the porous medium. Differences in the distribution of the bacteria and the EPS within the growth zone indicated that the bacteria and especially the active fraction of the bacteria were much more sensitive to the concentration of electron donor and acceptor as they could be found mainly in the mixing zone between the mineral medium and the glucose solution. Especially for transect B the EPS concentration exhibited a different distribution than the bacteria. A possible reason for this behaviour may be different growth or decay rates for bacteria and EPS (Kim and Fogler 1999) or consumption of EPS by the bacteria (Walker and Pulkownik 1973). Furthermore, the comparison between the total carbon concentration for transect A and B indicated that the total organic carbon had already decreased drastically between the two transects. This provides evidence that the area in direct vicinity of the glucose injection port was the zone of highest biological activity as suggested by data shown in Fig.4.8 and by visual observations (Fig.4.3).

To estimate the total amount of organic carbon in the porous medium at the end of the experiment we assumed that the biological activity was limited to a 45 cm × 5 cm × 1 cm zone in the flow cell. Further assuming an organic carbon concentration of 40 \(\mu\)g/g beads (approximate maximum from transect A) as a conservative estimate for all parts of this zone, the total organic carbon in the flow cell would only have
been 13.7 mg or 3.5% of the total carbon injected as glucose. As only 42% of the injected glucose were oxidized with nitrate, not only nitrate but also glucose must have reached the outflow.

In addition, the buildup of biomass in the water reservoir close to the outflow was observed during the experiment. This indicates that mixing between the glucose and nitrate was the limiting process for microbial activity within the porous medium. Transverse mixing was therefore not only determining the spatial distribution of the biomass, but also the consumption of the glucose must be assumed to have taken place in the mixing zone only. This confirms findings of studies showing that the degradation of a contaminant plume is highly limited by transverse mixing (e.g. Cirpka et al. 1999; Thorton et al. 2001). This suggests that the size of the stripes, where biological activity took place, was correlated to the transversal dispersivity.
of the porous medium. As the chloride breakthrough curve could be used only to estimate the longitudinal dispersivity, the transversal dispersivity of the porous medium pack was not known. In case of a longer flow cell, it must be assumed that biomass would have continued to grow in the stripe pattern as observed in this study, until the glucose was completely consumed. From our observation there is no indication that clogging processes could become more relevant in that case.

Finally, it must be pointed out that the comparison between bacterial carbon and total organic carbon was done in terms of masses and not volumes. The biomass occupying the pore space was clearly visible towards the end of the experiment and this suggests that the total volume of biomass must have been orders of magnitude higher than the bacterial volume alone. This indicates that the density (expressed in mass of carbon per volume) must have been much smaller for the EPS than for the bacteria. Moreover, the small bacterial volume measured at the end of the experiment can not explain the observed clogging effects, which confirms that the EPS and not the bacteria were responsible for the bioclogging in this experiment.

4.6 Conclusion

Results of this study show that for a two-dimensional flow field water flow and thus solute transport are dominating the spatial distribution of biomass in porous media. In addition, bioclogging effects in two-dimensional flow fields may drastically differ from observations made in one-dimensional flow fields. In a two-dimensional flow field bioclogging must not necessarily change a given flow pattern, but can also stabilize this flow pattern in case of changes in flow boundary conditions. Another difference between one- and two-dimensional flow fields is the effect of flow bypass of a clogged zone, which was observed in the present study. Due to flow bypass, clogging effects occurring locally did not change the hydraulic parameters of the entire system.

Data shown in this study demonstrate that it is necessary to determine both the distribution of bacteria and EPS to interpret bioclogging effects observed in an experiment. In particular, quantification of the bacterial carbon alone may drastically
underestimate the amount of biomass present in the pore space. For a qualitative determination of growth and distribution of the total biomass, picture analysis has shown to be a suitable non-destructive method.

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Chapter 5

Modeling of a microbial growth experiment with bioclogging in a two-dimensional saturated porous media flow field


5.1 Abstract

Results from a bioclogging experiment in a flow cell with a two-dimensional flow field were used as a data base to verify the simulation results of a reactive transport model, which includes bioclogging. Simulations were performed using several different hydraulic conductivity vs. porosity relations published in literature; a relation taken from Clement et al. (1996), which did not include pore connectivity in more than one dimension, and relations derived from pore network simulations assuming the biomass to grow in discrete colonies and as a biofilm respectively. The results showed that it was generally possible to simulate the experimental results with a numerical model. The best simulation of the experimental data could be achieved
with the hydraulic conductivity vs. porosity relation derived from pore network simulation assuming the biomass to grow in colonies. The relation derived from pore network simulations assuming the biomass to grow as a biofilm was also able to reproduce the experimental data but with different parameter values than in the pore network simulations. With the relation from Clement et al. (1996) the clogging ability of the biomass was strongly underestimated. These findings indicate the need to include the effect of multi-dimensionality already on the pore scale to get an appropriate description of bioclogging.

5.2 Introduction

The change in hydraulic conductivity and porosity of a saturated porous medium due to microbial growth is commonly referred to as bioclogging (Baveye et al. 1998). Previous investigations have demonstrated that bioclogging may influence the success of bioremediation in aquifers (Anderson and Lovley 1997), the performance of sand filters (Urfer et al. 1997) or the effectiveness of water flooding of oil reservoirs (Lappan and Fogler 1994).

Thus, it is important to understand the mechanisms involved in bioclogging. Most of the studies on bioclogging were performed in laboratory systems with one-dimensional flow fields (e.g. Cunningham et al. 1991; Taylor and Jaffé 1990a; Vandevivere and Baveye 1992c). These authors observed high reduction in hydraulic conductivity due to biomass production. Microbial growth and its influence on hydraulic properties of microscopic pore networks was investigated by Dupin and McCarty (2000) and Kim and Fogler (2000). Both observed a decrease in hydraulic conductivity and Dupin and McCarty (2000) also reported on a correlation between growing conditions and the morphology of bacterial growth. Recently Kildsgaard and Engesgaard (2002) and Thullner et al. (2002a) investigated bioclogging in systems with two-dimensional flow fields. In these systems bioclogging was also observed, but in contrast to one-dimensional systems, effects like flow bypass played an important role in the latter. Whereas no measurements of the biomass were performed by Kildsgaard and Engesgaard (2002) , Thullner et al. (2002a) observed the
distribution and quantified the amount of biomass in the porous medium, showing that it was mainly formed by extracellular polymeric substances (EPS).

Several models have been introduced to simulate the experimentally observed interaction between biomass and the hydraulic properties of porous media. Usually these models are based on the assumption of a homogeneous biofilm covering the surface of the grains (Taylor et al. 1990c), and a reduction of hydraulic conductivity caused by biofilm growth is theoretically derived assuming bundles of parallel pores. Especially for fine-textured materials these models were unable to predict observed hydraulic conductivity reductions satisfactorily (Vandevivere 1995; Vandevivere et al. 1995). It was assumed that this is caused by microorganisms growing in colonies, which influence the hydraulic conductivity differently than a biofilm (Vandevivere 1995; Vandevivere et al. 1995). In addition it was suggested to use pore networks instead of pore bundles in order to account for interpore connections (Loehle and Johnson 1994; Vandevivere et al. 1995). Clement et al. (1996) presented a macroscopic generalization of the approach of Taylor et al. (1990c), but their prediction of hydraulic conductivity was nearly identical to the biofilm model of Taylor et al. (1990c). In Suchomel et al. (1998a) a pore network model was introduced and applied to previously published data (Suchomel et al. 1998b). The model produced realistic results assuming the growth of a biofilm on the walls of cylindrical pores. In contrast to this Dupin and McCarty (2000) stated that their experimental observations could be explained with network model simulations assuming the growth of biomass in aggregates, whereas assuming a biofilm could not explain their observations. However, they did not present a functional relation between reductions in hydraulic conductivity and porosity.

In a recent study Thullner et al. (2002b) used pore network models to investigate the influence of biomass on hydraulic conductivity. Two different hydraulic conductivity vs. porosity relations were presented (in the following referred to as \( k_f - n \) relations), which predict the decrease of hydraulic conductivity caused by porosity reductions, depending on the morphology of microbial growth. Both relations predict higher reductions of hydraulic conductivity for a given reduction of porosity than the relations published previously (e.g. Clement et al. 1996).
In the present study the reactive transport groundwater model TBC (transport, biochemistry, and chemistry) by Schäfer et al. (1998a) was used as the basis for developing the model TBCC (transport, biochemistry, chemistry and clogging), which now includes bioclogging. TBCC was used to simulate the two-dimensional experimental results from Thullner et al. (2002a). By using different hydraulic $k_f - n$ relations (Clement et al. 1996; Thullner et al. 2002b) we wanted to investigate whether the results of the experiment (i.e. measured biomass concentration and observed changes of the flow field due to bioclogging) could be reproduced by the model in general and whether $k_f - n$ relationships derived from pore network models were able to predict experimental results better than $k_f - n$ relations derived from models not accounting for interpore connections.

5.3 Review of the two-dimensional bioclogging experiment

A bioclogging experiment was performed in a flow cell (56 cm × 44 cm × 1 cm, Fig.5.1) filled with water a water saturated glass bead packing and operated under a continuous flow of a mineral medium containing nitrate (injected via four inflow ports at the bottom of the flow cell, creating a two-dimensional flow field) (Thullner et al. 2002a). Simultaneously, glucose solution was injected through an injection port, simulating a point source of carbon substrate. Visible light transmission was used to observe qualitatively the distribution of the growing biomass and water flow during the experiment. At the end of the experiment, porous medium samples were destructively collected along several transects and analyzed for abundance of bacterial cells, bacterial cell volume and concentration of polysaccharides and proteins. Microbial growth was observed in a stripe along the length of the flow cell, starting at the glucose injection port, where the highest values were obtained. The ability of the biomass to change the flow pattern in the flow cell was observed. This bioclogging effect became evident when the injection of the glucose solution was turned off and water flow still bypassed the area around the glucose injection port, preserving
the flow pattern as it was during the injection of the glucose solution. Analyses of the porous media samples showed that only 5% of the total organic carbon was present as bacterial biomass, whereas the remaining 95% were attributed to EPS. The total volume of the bacterial cells remained below 0.01% of the pore space even in the vicinity of the injection port. Therefore, the observed clogging effects were assumed to be caused mainly by EPS.

Figure 5.1: Diagram of the experimental design as given in Thullner et al. (2002a) (only flow cell and objects inside flow cell are drawn to scale).

5.4 Model description

5.4.1 General concept

The model TBCC (transport, biochemistry, chemistry and clogging) used in this study is based on the reactive transport model TBC (transport, biochemistry, and chemistry) by Schäfer et al. (1998a). TBC was successfully used for simulations of a laboratory column study on microbial carbon degradation (Schäfer et al. 1998b)
as well as a field experiment on bioremediation of a contaminated aquifer (Thullner and Schäfer 1999). This numerical model uses a finite element approximation to simulate three-dimensional saturated groundwater flow and transport following the equations

$$\nabla \cdot (k_f \nabla h) = S_0 \frac{\partial h}{\partial t} - w$$  \hspace{1cm} (5.1)$$
for water flow and

$$\frac{\partial c_{mob}}{\partial t} = -\nabla \cdot (\bar{u} c_{mob}) + \nabla \cdot (D \nabla c_{mob}) + r$$  \hspace{1cm} (5.2)$$
for the transport of a solute in the water, where $S_0$ is the storage coefficient and $w$ is a source and sink term; $D$ is the dispersion tensor including molecular diffusion. The transport velocity $\bar{u}$ can be calculated using $\bar{u} = \bar{v}_D$, where $\bar{v}_D$ is the Darcy velocity. The term $r$ includes all sources and sinks caused by reactive processes (e.g. microbial consumption or chemical reactions). The piezometric head $h$ and the solute concentration in water $c_{mob}$ were the variables to be calculated.

In TBC the volume of each element is divided into three phases, the water phase (where transport of solvents takes place), the solid matrix phase and a biophase. The biophase is assumed to include all microorganisms and EPS. All biological processes, especially the degradation of organic carbon are assumed to take place in this biophase. Within a phase, species are assumed to be distributed homogeneously. The exchange of solvents between the water phase and the biophase is simulated using the equations

$$\frac{\partial c_{bio}}{\partial t} = -\frac{\lambda}{n_{bio}} (c_{bio} - c_{mob})$$  \hspace{1cm} (5.3)$$
and

$$\frac{\partial c_{mob}}{\partial t} = \frac{\lambda}{n} (c_{bio} - c_{mob})$$  \hspace{1cm} (5.4)$$
where $\lambda$ is a rate parameter. $c_{mob}$ and $c_{bio}$ is the concentration of a solute in the water phase and in the biophase respectively. $n_{bio}$ is the volume fraction of the biophase.
Microbial growth is assumed to follow Monod-type kinetics and thus

$$\frac{\partial X}{\partial t} = \mu_{\text{max}} X \prod_i \frac{c_{\text{bioi}}}{K_i + c_{\text{bioi}}} - \mu_{\text{dec}} X$$  \hspace{1cm} (5.5)$$
is used to express changes in bacterial mass $X$. $K_i$ are the Monod constants for the growth limiting solutes and $c_{\text{bioi}}$ their concentrations in the biophase. $\mu_{\text{max}}$ and $\mu_{\text{dec}}$ are the rate constants for maximum growth and first-order decay, respectively. Substrate consumption and release of metabolic products are coupled to microbial growth via yield coefficients and stoichiometric relations.

In order to simulate bioclogging effects the model TBC was modified getting the new model TBCC. Changes in porosity are calculated by converting biomass into biovolume, which directly reduces the porosity. The hydraulic conductivity $k_f$ is assumed to be a function of porosity $n$. In this paper three different relations between hydraulic conductivity and porosity are used. In Clement et al. (1996) a macroscopic model was introduced relating the change of relative hydraulic conductivity $k_{frel} = \frac{k_f}{k_{fini}}$ to the change of relative porosity $n_{rel} = \frac{n}{n_{ini}}$ ($k_{fini}$ and $n_{ini}$ are the values when no biomass is present). According to Clement et al. (1996) $k_{frel}$ is given by the function

$$k_{frel}(n_{rel}) = n_{rel}^{10}$$  \hspace{1cm} (5.6)$$
In case of biomass growing in colonies, which occupy pores entirely, changes in $k_{frel}$ are given by (Thullner et al. 2002b)

$$k_{frel}(n_{rel}) = a \left( \frac{n_{rel} - n_0}{1 - n_0} \right)^3 + (1 - a) \left( \frac{n_{rel} - n_0}{1 - n_0} \right)^2$$  \hspace{1cm} (5.7)$$
where $n_0$ and $a$ are adjustable parameters. In case of biomass growing as a homogeneous biofilm the changes in $k_{frel}$ are given by the function

$$k_{frel}(n_{rel}) = \left( \frac{n_{rel} - n_0}{1 - n_0} \right)^b + k_{f_{\text{min}}} \frac{1}{1 + k_{f_{\text{min}}}}$$  \hspace{1cm} (5.8)$$
with $n_0$, $k_{f_{\text{min}}}$ and $b$ as adjustable parameters. For the latter two relations, $1-n_0$ is interpreted as the volume of biomass (relative to initial pore volume), needed to get the maximum reduction of hydraulic conductivity. In case of $n_{rel} \leq n_0$ it was
assumed that $k_{f_{rel}}$ is constantly equal to 0 in Eqn.5.7 or $k_{f_{rel}} = k_{f_{min}}$ in Eqn.5.8, respectively.

In case of Eqn.5.7 it is assumed that for $n_{rel}$. When using Eqn.5.8 $k_{f_{rel}} = k_{f_{min}}$ is assumed for $n_{rel} \leq n_{0}$.

A comparison between hydraulic conductivity reductions predicted by Eqns.5.7 and 5.8 with parameter values used by Thullner et al. (2002b), and hydraulic conductivity reduction predicted by Eqn.5.6 is given in Fig.5.2.

Figure 5.2: Comparison of predicted reductions of hydraulic conductivity. The Clement model refers to Eqn.5.6 (Clement et al. 1996); the colony and the biofilm model refer to Eqn.5.7 and Eqn.5.8 respectively (Thullner et al. 2002b). $\sigma$ is the standard deviation of the lognormal pore radii distribution used for the pore network simulations.

Using an expression from Rittmann (1982), we found that the biomass detachment rate was in our experiment several orders of magnitude smaller than the assumed biomass decay rate. For this reason we did not include biomass detachment into the model.
5.4.2 Implementation of model modifications

TBCC solves the flow and reactive transport problem independently for each time step. During each time step all flow and transport parameters are kept constant. Changes of these parameters (and all other parameters which depend on the phase volumes) due to microbial growth are calculated at the end of each simulated time step and the updated values are used for the next time step.

By giving each component of the biophase a specific density, the volume of the biophase is calculated for each simulated time step. The volume of the matrix phase is assumed to be constant for each element and thus the volume of the water phase and the porosity respectively can be calculated. As the biomass mainly consists of water (Characklis and Marshall 1990) it is assumed that the increasing biophase is incorporating the volume of water being removed from the water phase; a decreasing biophase is releasing water to the water phase respectively. This procedure allows to neglect changes in hydraulic pressure due to biomass growth or decay. In addition, this exchange of water causes also an exchange of solute mass between the phases. Similar to Schäfer (1992) this mass exchange between the bio phase and the water phase is calculated by assuming that solute concentrations in the shrinking phase as well as total solute masses in an element remain constant. The porosity is assumed to be equal to the volume fraction of the water phase. Based on these porosity changes the changes in hydraulic conductivity are calculated using either Eqn.5.6, 5.7 or 5.8.

5.5 Numerical simulations

5.5.1 General procedure

Analogously to the experiment each simulation started on day 1 and ended on day 31. Between day 28 and 29 the glucose injection was stopped and instead a tracer solution was injected at the tracer injection port. Simulations were performed for the different $k_f - n$ relations. When using Eqn.5.6 the model will be referred to as Clement model, when using Eqn.5.7 the model will be referred to as colony model.
and when using Eqn.5.8 the model will be referred to as biofilm model. In addition, we also performed simulations assuming no changes of hydraulic conductivity during the experiment. All parameters describing flow, transport and reactive processes were kept constant for all simulations.

5.5.2 Model discretization and parameters for flow and transport

Preliminary simulations, in which the entire flow cell was modeled with a spatial discretization of 0.5 to 1.0 cm, indicated that simulation results using this discretization showed for all used models a biomass distribution which was similar to the experimental observations growing in a stripe like pattern. Simulated nitrate concentration at the outflow of the flow cell were similar to values reported in Thullner et al. (2002a), showing only negligible variations between the different models (data not shown). Nevertheless, a detailed simulation of the experimental observations and especially the clogging processes was not possible with this discretization.

To get a more detailed simulation of the clogging processes, which took place mainly in the vicinity of the glucose injection port we selected a 14 cm × 56 cm × 1 cm area in the center of the flow cell, which was modeled with a refined grid of 45 × 84 × 2 nodes (Fig.5.3). The spacing in x- and y-direction was 0.2 cm in an observation zone close to the glucose injection port and increased to up to 10 cm. The size of the observation zone was 8.4 cm × 14.4 cm and corresponded to the observation zone described in Thullner et al. (2002a). The spacing in z-direction was again 1.1 cm. At the bottom of the flow cell we now assumed a constant influx boundary. The flow rate of the influx boundary was set to 410 lm−2 day−1 in order to reproduce the initial head distribution in the observation zone; results from the simulation of the entire flow cell were used as a reference. Flow rate for the glucose injection port 0.041/day. The time step size was kept constant at 0.001 days. Unless stated otherwise, all simulation results presented in this study were calculated using this refined grid.

In the experiment the longitudinal dispersivity was determined to be 2·10−3 m,
for the transverse dispersivity we assumed a value of $2 \cdot 10^{-4}$ m.

### 5.5.3 Reactive processes

To simulate the consumption of nitrate and glucose as well as the production of biomass we assumed that the oxidation of glucose with nitrate, given by the equation

$$5C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 30CO_2 + 12N_2 + 42H_2O$$

is catalyzed by bacteria. The total biomass was assumed to consist of two components, bacteria and EPS. As in the experiment bacterial carbon only contributed
5% of the total organic carbon, we assumed that the growing bacteria also produced EPS at a production rate of 20 times the rate of bacterial growth. The parameters used for the simulation of the reactive processes are given in Tab.5.1. For the bacterial carbon density we took the same value as in Thullner et al. (2002b) and for the density of the EPS we took the smallest value for dry mass per wet volume presented in Characklis and Marshall (1990) and assumed a dry mass carbon content of 50%. All other parameters given in Tab.5.1 were fitted to get an optimal reproduction of the measurements (i.e. observed biomass buildup in the flow cell, biomass concentration at the end of the experiment, and nitrate concentration at the outflow).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{biom}$</td>
<td>$2.5 \times 10^{-7}$</td>
<td>initial volume of biomass (relative to total pore space)</td>
</tr>
<tr>
<td>$\rho_{bac}$</td>
<td>200 g/l</td>
<td>carbon density of bacteria</td>
</tr>
<tr>
<td>$\rho_{EPS}$</td>
<td>2.5 g/l</td>
<td>carbon density of EPS</td>
</tr>
<tr>
<td>$\mu_{max}$</td>
<td>1 day$^{-1}$</td>
<td>maximum growth rate of bacteria</td>
</tr>
<tr>
<td>$\mu_{dec}$</td>
<td>0.1 day$^{-1}$</td>
<td>decay rate for bacteria and EPS</td>
</tr>
<tr>
<td>$K_{nit}$</td>
<td>0.01 mM</td>
<td>Monod constant for nitrate</td>
</tr>
<tr>
<td>$K_{glu}$</td>
<td>0.06 mM</td>
<td>Monod constant for glucose</td>
</tr>
<tr>
<td>$Y$</td>
<td>0.2</td>
<td>carbon conversion rate between glucose and biomass</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>100 day$^{-1}$</td>
<td>exchange rate for solutes between water and biophase</td>
</tr>
</tbody>
</table>

Table 5.1: Parameters used for the simulation of reactive processes

5.5.4 Bacterial clogging

Parameter values of $a=-1.7$, $k_{f_{min}}=0.01$, and $b=1.8$ in Eqns.5.7 and 5.8 were taken from the most homogeneous case (standard deviation of $\sigma=0.33$ for the lognormal pore radii distribution) presented in Thullner et al. (2002b), assuming that the
nearly constant diameter glass bead packing used in Thullner et al. (2002a) is best represented by this case. In contrast the parameter \( n_0 \), which is the relative porosity for which the hydraulic conductivity reaches its minimum, was fitted to get an optimal reproduction of the measurements.

5.6 Results

5.6.1 Biomass distribution

For all models the general pattern of biomass distribution was similar, showing a V- to U-shaped structure starting close to the glucose injection port (Fig.5.4). The biomass showed a gradual increase until approximately day 23 and only small changes between days 23 and 30. Highest peak values for biomass volumes (36% to 37% of initial porosity filled with biomass) were reached with simulations assuming no clogging or using the Clement model. Using the colony or biofilm model (with a value of \( n_0 = 0.75 \) in Eqn.5.7 and 5.8, respectively) resulted in smaller peak values for biomass volumes of approximately 24%. In addition, Fig.5.5 shows the biomass distributions at day 30 simulated with the colony and the biofilm model for different values of \( n_0 \). With one exception the general pattern was similar in both models for all values of \( n_0 \). Only for the colony model with \( n_0=0.9 \) the result was slightly different. In that simulation the distance between the two branches of the biomass distribution pattern had a minimum at a few cm above the glucose injection port. For all other simulations this distance was constantly increasing between the glucose injection port and the top of the observation zone. Peak values for biomass volumes were decreasing constantly for increasing \( n_0 \). For the colony model, the highest biomass volumes were 34% of initial porosity for \( n_0=0.6 \) and 12% of initial porosity of \( n_0=0.9 \). For the biofilm model the highest biomass volumes were 33% of initial porosity for \( n_0=0.6 \) and 22% of initial porosity of \( n_0=0.9 \).
Figure 5.4: Distribution of biomass volume (for the simulation results given in % of initial porosity) in the observation zone for the different hydraulic $k_f - n$ relations.
Figure 5.5: Distribution of biomass volume (given in % of initial porosity) the observation zone at day 30 for the colony and biofilm model using different values of $n_0$.

5.6.2 Organic carbon concentration

To compare simulation results with data from Thullner et al. (2002a) we calculated the average organic carbon concentration at the end of the experiment for the areas given in Fig.5.3, which corresponded to porous media samples analyzed by Thullner et al. (2002a). Results of this procedure are given in Fig.5.6 for the different models (colony and biofilm model with $n_0=0.75$) together with the experimental data. For
transect A the organic carbon concentration showed a dual peak pattern for all of the models similar to that of the measured data. Highest simulated concentrations for transect A ranged between 28 $\mu$g/g beads for the biofilm model and 39 $\mu$g/g beads for the model without clogging. For transect B all the simulations showed a dual peak pattern, too, whereas the measured data showed a single peak in the middle of the transect only. Highest simulated organic carbon concentrations for transect B ranged between 14 $\mu$g/g beads for the model without clogging and 21 $\mu$g/g beads for the colony model. When comparing simulation results of the colony and biofilm model using different values for $n_0$ (Fig. 5.7), the dual peak pattern for transects A and B could be observed for all simulations, with the highest concentrations depending on $n_0$. Using the colony model the concentration maximum for transect A decreased from 35 $\mu$g/g beads ($n_0=0.6$) to 24 $\mu$g/g beads ($n_0=0.9$), whereas peak concentrations for transect B were highest (22 $\mu$g/g beads) for $n_0=0.8$ and decreased to 20 $\mu$g/g beads for $n_0=0.6$ and 18 $\mu$g/g beads for $n_0=0.9$. Using the biofilm model the highest organic carbon concentration was 31 $\mu$g/g beads for $n_0=0.6$ and decreased to 21 $\mu$g/g beads for $n_0=0.9$. For transect B peak concentrations were around 20 $\mu$g/g beads for $n_0$ between 0.6 and 0.8, but for $n_0=0.9$ only 17 $\mu$g/g beads were reached.

5.6.3 Tracer migration

Simulation results for the tracer test on days 28 and 29 are shown in Fig. 5.8 for the different models together with the experimental observations. Depending on the model used for simulation the influence of biomass on the tracer migration was different. Assuming no clogging in the model, tracer flow was straight upward with only dispersive mixing increasing the width of the tracer plume. In comparison the tracer distribution was only slightly different for the Clement model, but a small increase in the width of the tracer plume, starting close to the glucose injection port, was visible. Results from the colony and biofilm model (both with $n_0=0.75$) showed that the tracer plume initially (1 h after the start of injection) tended to split into two branches when reaching the vicinity of the glucose injection port where
the biomass was located. This initial trend disappeared with further migration of the tracer plume, but the width of the tracer plume was clearly increased. When comparing the simulation results for the tracer migration using the colony and the biofilm model with different values for $n_0$, we observed that the influence of the biomass on the tracer migration was increasing with increasing $n_0$ for both models (Fig. 5.9). For values of $n_0$ between 0.6 and 0.8 the general pattern of a branched tracer plume (1 h after the start of the tracer injection) and the increased width of the tracer plume (3 h and 5 h after start of the injection) could be observed for all simulations, but these effects became more pronounced for higher $n_0$ (with

Figure 5.6: Organic carbon concentration at the end of the experiment for the different $k_f - n$ relations compared to measured data. x-axes show distance to left border of flow cell.
only small differences between the colony and the biofilm model). For $n_0=0.9$ the colony and the biofilm model showed different results. For the colony model the tracer plume was split into two branches even 5 h after start of tracer injection. In contrast, for the biofilm model the tracer did not change the general pattern observed for smaller values of $n_0$. 

Figure 5.7: Organic carbon concentration at the end of the experiment for the colony and biofilm model using different values of $n_0$. x-axes show distance to left border of flow cell.
Figure 5.8: Tracer migration in the observation zone for the different $k_f - n$ relations. For the simulation results, grey scales show relative tracer concentration ($c/c_0$). Data show tracer distribution 1 h, 3 h, and 5 h after start of tracer injection.
Figure 5.9: Tracer migration in the observation zone for the colony and biofilm model using different values of $n_0$. Grey scales show relative tracer concentration ($c/c_0$). Data show tracer distribution 1 h, 3 h, and 5 h after start of tracer injection.

5.7 Discussion

To compare the simulation results the experimental data from Thullner et al. (2002a) were divided into three groups; pattern of biomass distribution in the flow cell, measured organic carbon concentration at the porous media sampling points and the results from the tracer test conducted on days 28 and 29. In contrast to the nitrate concentration at the outflow, which could be reproduced with all of the models, the model, which served the best fit of the measured data, varied from group to group. The measured pattern of biomass distribution as well as the kinet-
ics of biomass growth could generally be reproduced with all models, but only the colony model with \( n_0 = 0.9 \) was able to reproduce at least slightly the tendency of the two branches of the pattern to nearly re-unify, as observed in the experiment. Comparing experimental and simulated data for the organic carbon concentration along transects A and B the best agreement could be achieved assuming no clogging in the flow cell. When clogging was assumed the simulation results were still close to the measured values for the Clement model as well as for the colony and the biofilm model for \( n_0 \leq 0.8 \). For larger values of \( n_0 \) the decrease in organic carbon concentration between the transects A and B, which was observed in the experiment, could not be reproduced satisfactorily by the simulations. Nevertheless, the measured single peak pattern of organic carbon concentration could not be found in any of the simulations. For the tracer migration best simulation results compared to the experiment could be achieved with the colony and the biofilm model for high values of \( n_0 \). The simulated tracer migration for the colony model (\( n_0 = 0.8 \)) and the biofilm model (\( n_0 = 0.8 \) or \( n_0 = 0.9 \)) reproduced the measured tracer migration very well. The colony model with \( n_0 = 0.9 \) already overestimates the change of tracer migration due to bioclogging, whereas the colony and the biofilm model for \( n_0 < 0.7 \) but also the Clement model were underestimating the change of tracer migration due to biomass. The biomass had of course no influence on the tracer migration if no clogging was assumed in the model.

To decide, which model allowed the best reproduction of all data groups measured by Thullner et al. (2002a), one had to find a compromise, which reproduces all data groups satisfactorily. Due to the fact that in the experiment the tracer migration was definitely changed by the biomass, the models, which do not reproduce this effect at least partly, were assumed to be inappropriate for simulating the flow cell experiment. In contrast to this the colony and the biofilm models allowed a better reproduction of the tracer data, especially for \( n_0 > 0.7 \), but for higher values of \( n_0 \) the simulated organic carbon concentration for transects A and B did not fit to the measured data in an acceptable way. The simulation results indicated that the colony and the biofilm model with \( n_0 = 0.75 \) were best suited to simulate the flow cell experiment, because these models were able to reproduce all groups of measured
data in an acceptable approximation.

The simulation results from the colony and the biofilm model were very similar for a given $n_0 < 0.9$. Therefore there was no direct indication to decide, whether Eqn.5.7 or Eqn.5.8 was the more appropriate function for describing the reduction of hydraulic conductivity due to biomass growth. Values for $n_0$, for which an appropriate reproduction of the experimental data could be achieved are closer to values used in Thullner et al. (2002b) for describing the influence of microbial colonies in pore networks. For the most homogeneous pore networks investigated in Thullner et al. (2002b) $n_0$ was 0.7 for the colony model and 0.2 for the biofilm model. This would suggest that the assumption of biomass growing in colonies as described in Thullner et al. (2002b) was better suited to describe the flow cell experiment. This finding suggests that the assumption of biomass growth in form of colonies and not as a biofilm is more reasonable, to describe the flow cell experiment. Therefore results of this study agree with the observations of Thullner et al. (2002a), who reported on a better agreement between the colony model and experimental data in case of porous media having a grain diameter of less than 1 mm. We did not explicitly investigate the sensitivity of the simulation results towards variations of parameters other than $n_0$ in Eqn.5.7 and Eqn.5.8. Nevertheless the small differences between the colony and biofilm model for a given $n_0$ indicated that simulation results were not sensitive towards the way expressing the decrease of $k_f$ for porosity reductions between $n = 1$ and $n = n_0$. This indicates that the sensitivity of the simulation results towards the parameters mentioned above must have been small, too, as most of these parameters only modify the $k_f$ decrease between $n = 1$ and $n = n_0$, but they do not alter the position of the minimal $k_f$.

Our results confirm the assumption of Vandevivere et al. (1995) that models, which account for pore connectivity in more than one dimension and which assume biomass growth not only in a biofilm, may allow better predictions than models as those of Clement et al. (1996) and Taylor et al. (1990c). We showed that the biofilm and especially the colony model, which were derived from pore network simulations, were able to reproduce measured clogging effects, whereas the Clement model was underestimating the bioclogging.
Results from this study showed that an experimental data set, which included measurements of biomass distribution and the flow field but did not include measurement of hydraulic conductivity changes, could be used to verify a model including bioclogging. As the measurement of local hydraulic conductivities may be impossible in multi-dimensional systems this finding is of importance. In contrast, this study also showed the importance of measuring a variety of different data in order to make an accurate verification of a clogging model, because each of the data groups was best reproduced with a different model and the combination of all data groups was needed to decide, which model was the most adequate. This may also explain the fact that Kildsgaard and Engesgaard (2001) were able to simulate the clogging experiment of Kildsgaard and Engesgaard (2002) using the Clement model. As no biomass measurements were available for their experiment, the biomass could be used as a fit parameter in their simulations.

For transforming the mass of EPS into volume of EPS we had to make an assumption on the density of EPS. As reviewed by Characklis and Marshall (1990), values for this density (expressed in dry weight per wet volume) vary between 5 kg/m$^3$ and 130 kg/m$^3$. For the density of EPS in this study we decided to use the smallest value of 5 kg/m$^3$, giving the EPS and therefore the total biomass a maximum of volume. In case that the density of the EPS was higher in the flow cell experiment than assumed for the simulations, the total volume of biomass would have been smaller than simulated in this study. To explain the observed clogging effects in such a case would be possible by adjusting $n_0$ towards higher values in the colony or biofilm model. The Clement model, which already underestimated the bioclogging effects for small EPS densities would by far not be able to explain the bioclogging effects for high EPS densities, again indicating that the colony and biofilm models are more appropriate than the Clement model to describe bioclogging in porous media. As the contribution of bacterial volume to total biomass volume was negligible, simulation results were not sensitive to the density of bacteria.

Finally, the simulations results showed that two parameters were important for the description of bioclogging. The first one is the density of EPS or more general the density of the biomass. As the volume of a given amount of biomass directly
depended on its density, simulated porosity reductions were highly sensitive to the biomass density. The second parameter was \( n_0 \) in Eqns 5.7 and 5.8. This parameter contains the information of how much biovolume was needed to clog the porous medium. These results suggest that experimental determination of biomass densities and how much biovolume is present in a clogged porous medium would strongly reduce the uncertainty of the numerical simulations describing the reduction of hydraulic conductivity, especially because the sensitivity of the simulations results was low to other parameters.

5.8 Conclusion

Results from this study showed that it was generally possible to simulate the experimental results from Thullner et al. (2002a) with a numerical model. A detailed comparison between the experimental data from Thullner et al. (2002a) and the simulation results using different models to describe the change of hydraulic conductivity due to biomass growth showed that best reproduction could be achieved with a model introduced by Thullner et al. (2002b) assuming microbial growth in colonies. A biofilm model (also introduced by Thullner et al. (2002b)) was also able to simulate the experimental data, but the value for the most sensitive parameter was quite different to that of the study of Thullner et al. (2002b). The model published by Clement et al. (1996) was strongly underestimating the clogging effects observed in the experiment. This finally suggests that it is necessary to include multi-dimensionality effects already on the pore scale, as was done in the pore network simulations by Thullner et al. (2002b), in order to get an appropriate description of bioclogging in porous media.

Acknowledgements

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Chapter 6

Validation of the dual pumping technique for level-determined groundwater sampling in a contaminated aquifer


6.1 Abstract

The dual pumping technique (DPT) was introduced recently as a new and inexpensive technique to measure the level-determined solute concentrations in groundwater. Using two pumps, one placed near the groundwater table and one placed near the bottom of a fully screened well, this technique allows to determine vertical concentration profiles for solutes in groundwater given that additional information about the influx distribution into the well is known. Until now, however, the DPT was applied only in an aquifer with a thickness of about 40 m and the validation was weakened by the lack of a reliable reference system. The present study aims to investigate the applicability of the DPT for shallow (thickness < 10 m) and unconfined aquifers and to validate the results of the DPT with a better reference system. For
this purpose the DPT was applied in Menziken, Switzerland to a gravel aquifer contaminated with petroleum hydrocarbons. Solute concentrations measured at that site with an established drive point sampling technique (ram technique) were taken as a reference to demonstrate the applicability of the DPT. Results for 8 different solutes showed a reasonable agreement between both techniques. An algorithm was developed that allowed the computation of a single solute concentration profile incorporating measured data from both pumps. It was possible to demonstrate that this alternative algorithm can improve the quality of solute concentration profiles obtained by the DPT. This indicates that the DPT is a useful tool for determining vertical concentration profiles in groundwater for conditions similar to those in Menziken (unconfined gravel aquifer, screen length less than 10 m).

6.2 Introduction

Accurate measurement of the vertical distribution of solute concentrations in an aquifer is a major problem at sites equipped with fully-screened observation wells. Nearly all types of pollutants show a vertical concentration gradient. Especially in the case of groundwater contamination by petroleum hydrocarbons, the assumption of a homogeneous vertical distribution of solutes is not justified. Most petroleum hydrocarbons are lighter-than-water nonaqueous-phase liquids (LNAPLs) and float on the water table where their dissolution into groundwater takes place. Dissolved petroleum hydrocarbons therefore often exhibit distinct vertical concentration profiles with maximum solute concentrations near the water table. Typically, chemical and microbiological processes associated with the degradation of dissolved petroleum hydrocarbons also exhibit vertical gradients within the aquifer (Cho et al. 1997; Eganhouse et al. 1996). Usually, measurements taken in fully screened observation wells represent the flux weighted average solute concentration for the screened section of the aquifer independently from the position of the pump (Barczewski and Marshall 1989). These data contain no information about the vertical gradients of the measured parameters. If the assessment of a contaminated site is based only on such measurements, there is a significant risk of misinterpreting the extent and
the attenuation of the contaminant plume (Barczewski and Marshall 1989; Martin-Hayden and Robbins 1997).

Several methods have been developed in recent years for measuring vertical solute concentration profiles in aquifers more accurately (Lerner and Teutsch 1995). A well established and easy to use tool is the so-called piezometer nest or multilevel sampling well (e.g. Pickens et al. 1978). Here, a set of several different piezometer tubes, each screened at a different depth, is used to obtain highly accurate vertical solute concentration profiles. Disadvantages of this tool include its inflexibility due to the fixed sampling levels and its high cost compared to a single fully-screened observation well. A number of different methods have been presented for getting depth resolving measurements when only fully-screened observation wells are available. Published methods include the multiport sock samplers (e.g. Schirmer et al. 1995; Teutsch and Ptak 1989), packer systems (e.g. Andersen 1982; Price and Williams 1993), a modular passive multilevel sampler (Ronen et al. 1987) and the separation pumping technique (SPT), in which two or three pumps are used simultaneously (Bishop et al. 1992; Nilsson and Jakobsen 1990; Nilsson et al. 1995). Methods like the drive-point sampling technique (ram technique) (Kretzer 1992) do not require the construction of a well and can be used to extract groundwater samples from a defined depth. Kretzer (1992) and Kretzer and Niederleithinger (1995) demonstrated the suitability of the ram technique for measuring vertical solute concentration gradients in groundwater. A similar technique was introduced and applied for groundwater sampling by Pitkin et al. (1994). In addition to these established sampling techniques, the dual pumping technique (DPT) offers a new approach to measure vertical concentration profiles in groundwater (Rapp et al. 1998). This new method is similar to the SPT, however, the DPT is based on the idea to use only two pumps simultaneously for sampling a fully screened well (Kinzelbach et al. 1988). One pump is located near the groundwater table, the other one near the bottom of the well. Both pumps are operated simultaneously with variable pumping rates while the combined pumping rate is held constant. A water divide develops between the two pumps; its location depends on the ratio of the pumping rates between the two pumps. The position of the water divide maybe measured with a flowmeter.
Rapp et al., 1998 introduced an algorithm, which allows to compute the vertical solute concentration profile based on the DPT measurements taken for several different pumping rate ratios. On a theoretical basis it was shown that this algorithm generates correct results for a hypothetical concentration distribution. In addition Rapp et al. (1998) conducted a field experiment to validate the applicability of the DPT in a well with a screen length of about 40 m. The well was located in a sandy aquifer contaminated mainly with chlorinated hydrocarbons. Measurements from a multilevel sampling well were used as a reference for the profiles determined with the DPT. Unfortunately, this multilevel well was about 50 m away from the well where the DPT measurements were performed and therefore it was not possible to compare the results unequivocally. Nevertheless, the data presented by Rapp et al. (1998) suggest that the DPT may be an adequate tool to determine vertical solute concentration profiles in groundwater.

The main objective of this study was to further validate the DPT as a reliable tool for obtaining accurate vertical solute concentration profiles in an aquifer. In particular, we wanted to apply the DPT method to a set of different field conditions, compared to those presented in the study of Rapp et al. (1998), to answer the following questions:

- Is it possible to apply the DPT in a shallow aquifer at a well with a screened section of about 6m?

- Can the DPT results be validated by the results of an independent method applied in close proximity? - Does the reduction of the water table due to the pumping limit the reliability of the DPT results in the vicinity of the water table?

6.3 Site description

The site used to validate the DPT method is located in Menziken, Switzerland (Fig.6.1). The subsurface at the site generally consists of glaciofluvial outwash deposits, mainly poorly sorted gravel and sand which overly an aquitard of tightly
packed till. The uppermost 2-3 m of the subsurface are composed by loamy sediments. The unconfined aquifer has an average thickness of about 5 m. The water table is between 3 and 4 m below surface with high temporal fluctuations. The regional ground water flow direction is from south to north with an average gradient of 2-3%. Hydraulic conductivity is $4.5 \pm 2.5 \times 10^{-3}$ m/s and porosity was assumed to be 0.19 (Hunkeler et al. 1999). This specific aquifer is representative for the hydrogeological situation of the perialpine belt of Switzerland.

![Site map of the aquifer in Menziken, Switzerland.](image)

Figure 6.1: Site map of the aquifer in Menziken, Switzerland. Locations of the contaminated area and the wells are taken from Hunkeler et al. (1999). Water table elevations (in m above sea level) were measured before the pumping experiment at all of the shown wells. Contour lines calculated with Surfer (Golden Software, Golden, CO, USA)
In 1988, a contamination of the aquifer by a diesel fuel spill of about 10-12 m³ was detected. After excavation of the most severely contaminated area and the removal of parts of the spill by pumping, it was estimated that about 5 m³ diesel fuel remained in the subsurface acting as a long-term source of groundwater contamination. The vertical location of the bulk LNAPL was near the groundwater table with a variable thickness of about 1 m on average as determined from drilling cores (Hunkeler et al. 1999).

A set of 18 observation wells was installed for the evaluation of the extent of the contamination as well as for later monitoring and remediation activities. Between 1989 and 1995, active in situ remediation of the contaminant plume was performed (Bregnard et al. 1996; Hunkeler et al. 1995; Hunkeler et al. 1997; Hunkeler et al. 1999; Höhener et al. 1998). Since 1995, no further activities were performed on this site except for monitoring of the contamination.

The well KB3 with an inner diameter of 11.5 cm was chosen to apply the DPT. It is located near the centre of the plume. The well has a total length of about 10 m and is fully screened (Fig.6.2). Just prior to pumping, the groundwater table in this well was at 3.8 m below ground surface. The ram profile was measured about 5 m away from well KB3 (Fig.6.1).

### 6.4 Experimental procedures

#### 6.4.1 The DPT method

Two pumps (Grundfos MP1 (Bjerringbro, Denmark)) were placed in well KB3 (Fig.6.2). The bottom pump was lowered to an intake depth of about 8.3 m below ground surface. In principle, this pump should be placed as deep as possible (Rapp et al. 1998). However, to prevent any extraction of mud out of the mud pit at the bottom of the well a distance of about 2 m to the bottom of the well was chosen. The top pump was located just below the water table during the pumping with the intake at about 4.7 m below the ground surface. Because only the part between the two pumps can be investigated, concentrations were measured over a
vertical distance of 3-4 m. The total pumping rate of both pumps together was kept constant at 30 l/min throughout the experiment. This caused a drawdown of the groundwater table from 3.80 m to 4.45 m below surface. The pumping rate of each pump was controlled by its operating unit and measured independently using an inductive flowmeter or volumetric measurements.

Samples were collected at the following pumping rate ratios $\gamma_t = 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.50$ (sampled twice), 0.60 and 1.0, with $\gamma_t$ defined as

$$\gamma_t = \frac{Q_t}{Q_t + Q_b}$$
where $Q_t$ and $Q_b$ being the pumping rates of the top and bottom pumps, respectively. Measurements were taken from both pumps except at $\gamma_t = 0$ where only the bottom pump was extracting water and $\gamma_t = 1$ where only the top pump was extracting water. The pumps were connected to flowcells which were equipped with an electrode to measure dissolved oxygen (Clark type electrode, WTW Oxi 96, Weilheim, Germany). After purging of at least 2 volumes contained within the well casing and surrounding sand pack, water samples were taken from these flowcells for further analysis.

The water divide between the two pumps was measured using an impeller flowmeter (Intergeo-Haferland AG, Holzhusern, Switzerland). For each $\gamma_t$ measurements were performed with the impeller flowmeter moving along the well with two different velocities (2 m/min and 3.5 m/min). For each velocity two measurements of the impeller signals were made, one for the flowmeter moving upward and one for the flowmeter moving downward. Data were collected online and saved in a PC-notebook.

The DPT experiment lasted for about 6h. During this time the total water amount of about 10m$^3$ were extracted via the pumps.

6.4.2 The Drive Point Sampling Technique (Ram Technique)

Using a conventional ram device, stems with a diameter of 32 mm were driven into the ground. A probe head with the same diameter was attached at the bottom of the stems. The probe head had a screened section of 40 mm length. Tubing placed inside the stem connected the probe head with an above-ground vacuum pump. Due to the used vacuum technique this system allowed water extraction from depths up to about 7 m below ground surface only. The depth resolution of this technique was about 20 cm (Kretzer 1992).

To collect groundwater samples the probe was rammed down in consecutive steps to the desired depths until the maximum depth of 7 m was reached. At each sampling depth, approximately 250 ml of water were extracted and discarded to
flush the tubing. Thereafter the same amount was extracted and collected in a glass bottle from which subsamples were taken.

Due to the high gravel fraction in the subsurface, only one of the four attempts made to lower the ram probe down to the depth of interest was successful. During this attempt a single ram profile was obtained with samples taken from depths of 4.5 m, 4.8 m, 5.0 m, 5.4 m, 6.0 m, 6.5 m and 7.0 m below ground surface within a period of 1.5 h. These measurements were performed 16 h before the beginning of the DPT experiment.

6.4.3 Preparation and analysis of water samples

Samples for the analysis of dissolved species were filtered immediately after sampling using 0.22 mm polyvinylidenefluoride filters (Millipore, Bedford, MA, USA). Samples for the analysis of cations were acidified on site. Concentrations of the anions nitrate, sulfate and chloride and the cations sodium, potassium, magnesium and calcium were determined using a Dionex DX-100 ion chromatograph (Dionex, Sunnyvale, CA, USA) (Bolliger et al. 1999; Hunkeler et al. 1999). Alkalinity was measured by potentiometric titration using Gran plots for graphical determination of the end point (Stumm and Morgan 1996). Standard deviations were 2% for anions and alkalinity and 5% for cations.

6.5 DPT data analysis

The transformation of single DPT measurements into a vertical solute concentration profile is described in detail in Rapp et al. (1998). To illustrate this method and the improvements made here we present results for nitrate as an example. All other chemical parameters were evaluated analogously. In a first step the measured solute concentration for the top pump \( c_t(\gamma_t) \), for the bottom pump \( c_b(\gamma_t) \) and the flux weighted average solute concentrations of both pumps

\[
C_{ave}(\gamma_t) = \frac{c_t(\gamma_t) \cdot Q_t + c_b(\gamma_t) \cdot Q_b}{Q_t + Q_b}
\]

were plotted against the relative pumping rate of the upper pump \( \gamma_t \) (Fig.6.3A).
To eliminate the negative influence of the scattering of the data on the calculated concentration profile, measured data were normalized to a constant value of the flux weighted average concentration. This constant value was calculated as the mean value of the flux weighted average concentration. These normalized concentrations were plotted for each pump against the relative pumping rate of the top pump. Afterwards data for each pump were interpolated with two different functions, a second-order polynomial and a third-order polynomial (Fig. 6.3B). These functions were chosen as the simplest functions which can approximate a change in concentration gradient without the danger of overfitting. Results are given for both functions to demonstrate the sensitivity of the method and therefore the uncertainty of the results.

Following the algorithms presented in Rapp et al. (1998) the concentration profiles can be calculated according:

$$c_t(z(\gamma_t)) = C(\gamma_t) + \frac{dC(\gamma_t)}{d\gamma_t} \gamma_t$$

(6.1)
and

\[ c_b(z(\gamma_b)) = C(\gamma_b) + \frac{dC(\gamma_b)}{d\gamma_b} \gamma_b \]  

(6.2)

where \( c_\ldots(z(\ldots)) \) are the calculated concentrations at depth \( z(\ldots) \) which is the depth of the water divide at the specific pumping rate ratio, \( C(\ldots) \) denotes the measured concentration at the specific pump and \( dC(\ldots)/d\ldots \) the derivatives taken from the interpolated measurements. \( \gamma_\ldots \) and \( \gamma_b \) are the pumping rate ratios (note that \( \gamma_b = 1 - \gamma_\ldots \)). Theoretically the profiles calculated with the two different equations should be identical, practically they will differ at least slightly. In addition we here introduce an interpolation between the two profiles given in Eqn.6.1 and 6.2. This new profile is calculated as:

\[ c^{\text{int}}_\ldots(z(\gamma_\ldots)) = c_\ldots(z(\gamma_\ldots))(1 - \gamma_\ldots) + c_b(z(\gamma_b)) \gamma_b \]  

(6.3)

\( c_\ldots(z(\ldots)) \) is the concentration at depth \( z \) calculated with Eqn.6.1 or 6.2 resp. and \( c^{\text{int}}_\ldots(z(\ldots)) \) the average of the single profiles weighted with the pumping rate ratio of the other pump.

After determining the depths of the water divides for the different pumping rate ratios we obtained three concentration profiles for each interpolation function, one based on the data of the upper pump only, one based on the data of the lower pump only and a profile based on the data of both pumps simultaneously (Figs.6.5A and B).

### 6.6 Results

Analysis of the flowmeter data revealed that a precise estimate of the water divides in the well was not possible due to the bad signal to noise ratio of the impeller flowmeter. Nevertheless the absolute error of this measurement is about 1 m and therefore it is in the same range as it was in the study of Rapp et al. (1998). In Tab.6.1 the depth of the water divides estimated from the flowmeter measurements of both velocities were averaged and compared to calculated depths of the water divide using a given influx distribution. These calculations were done for three model concepts of the observed aquifer: A homogenous aquifer and two types of
heterogeneous aquifers. Both heterogeneous aquifers are assumed to be divided into two layers of equal thickness. For one of these aquifers (type I) the upper layer has a hydraulic conductivity reduced by a factor of 2 compared to the lower layer of the aquifer, for the other aquifer (type II) the upper layer has a hydraulic conductivity increased by a factor of 2 compared to the lower layer of the aquifer. One can see for each $\gamma$ that the estimated and calculated values for the homogeneous aquifer correspond to each other within the error range of 1 m. For the two heterogeneous aquifer types (especially for type II), the agreement between the measured and the calculated depths of the water divide is worse than for the homogeneous aquifer. It is therefore justified to assume homogenous inflow into the well. To demonstrate the sensitivity of the vertical concentration profiles against the uncertainty in the determination of the depth of the water divide, concentration profiles for nitrate were calculated using the different data for the depth of the water divide as given in Tab.6.1. (Fig.6.4). For small depths the DPT profiles differ only slightly from each other whereas the differences between the profiles tend to increase with depth. The profile calculated using the assumption of a homogeneous inflow into the well corresponds best to the profile based on the flowmeter data. All other presented DPT concentration profiles are based on the assumption of a homogenous water inflow into the well.

<table>
<thead>
<tr>
<th>Aquifer type</th>
<th>$\gamma$</th>
<th>0.05</th>
<th>0.10</th>
<th>0.15</th>
<th>0.20</th>
<th>0.25</th>
<th>0.30</th>
<th>0.35</th>
<th>0.40</th>
<th>0.50</th>
<th>0.60</th>
</tr>
</thead>
<tbody>
<tr>
<td>unknown</td>
<td>$z(\gamma)_{\text{measured}}$ [m]</td>
<td>4.75</td>
<td>5.00</td>
<td>5.25</td>
<td>5.50</td>
<td>5.50</td>
<td>6.00</td>
<td>6.25</td>
<td>7.25</td>
<td>7.50</td>
<td>8.00</td>
</tr>
<tr>
<td>homogeneous</td>
<td>$z(\gamma)_{\text{calculated}}$ [m]</td>
<td>4.79</td>
<td>5.07</td>
<td>5.36</td>
<td>5.64</td>
<td>5.93</td>
<td>6.21</td>
<td>6.50</td>
<td>6.78</td>
<td>7.35</td>
<td>7.92</td>
</tr>
<tr>
<td>heterogeneous type I</td>
<td>$z(\gamma)_{\text{calculated}}$ [m]</td>
<td>4.93</td>
<td>5.36</td>
<td>5.79</td>
<td>6.21</td>
<td>6.64</td>
<td>7.07</td>
<td>7.42</td>
<td>7.64</td>
<td>8.06</td>
<td>8.49</td>
</tr>
<tr>
<td>heterogeneous type II</td>
<td>$z(\gamma)_{\text{calculated}}$ [m]</td>
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<td>4.93</td>
<td>5.14</td>
<td>5.36</td>
<td>5.57</td>
<td>5.79</td>
<td>6.00</td>
<td>6.21</td>
<td>6.64</td>
<td>7.07</td>
</tr>
</tbody>
</table>

Table 6.1: Depth of water divides as taken from impeller flowmeter measurements compared to depth of water divides calculated assuming a given influx distribution into the well.

The flux weighted average concentration in the pumps is constant within the scattering of concentrations caused by small scale heterogeneities in the distribu-
Figure 6.4: Vertical DPT-concentration profiles for nitrate using different data sets for the position of the water divide. The DPT-profiles were calculated using Eqn.6.3 and a third-order polynomial fitting function.

...tion of the solutes in the subsurface and by the accuracy of the sampling analysis (Fig.6.3A for nitrate). These values represent the concentration one would measure when using a single pump. A fairly constant concentration indicates that no temporal changes in concentrations occurred during the time period of the experiment. Another important aspect is the fact that the flux weighted average concentration does not depend on the pumping rate ratio $\gamma$. This expected behaviour was observed for all measured concentrations and served as a control that no systematic error was made during the experiment.

Vertical solute concentration gradients were observed only for some of the measured species (Figs.6.5A-6.7D). They include some of the species involved in the mineralization of petroleum hydrocarbons (nitrate, sulfate) and some coupled geochemical reactions (alkalinity, calcium, magnesium) (Hunkeler et al. 1999). The results of the different sampling techniques show generally acceptable agreement between the DPT and ram technique profiles. The general trend of the concentration profile is the same for both methods. Only for potassium a systematic offset
between the results of the DPT and the ram technique was observed. For nitrate and chloride, the small concentration peak measured with the ram technique could not be reproduced properly with the DPT. In addition the decrease in alkalinity and calcium concentration measured at a depth of 7 m below surface with the ram technique could not be reproduced with the DPT.

![Diagram showing vertical concentration profiles for nitrate, sulfate, and chloride determined with the DPT and the ram technique.](image)

Figure 6.5: Vertical concentration profiles for nitrate, sulfate, and chloride as determined with the DPT and the ram technique. DPT profiles in figures A, C, and E are calculated using a second-order polynomial fitting function; DPT profiles in figures B, D, and F are calculated using a third-order polynomial fitting function. DPT results are shown for the different algorithms given in Eqns.6.1-6.3.

The general agreement between the results of both techniques does not depend on
Figure 6.6: Vertical concentration profiles for alkalinity, sodium and potassium as determined with the DPT and the ram technique. DPT profiles in figures A, C and E are calculated using a second-order polynomial fitting function; DPT profiles in figures B, D and F are calculated using a third-order polynomial fitting function. DPT results are shown for the different algorithms given in Eqns.6.1-6.3.

the used interpolation method. Considering all measured profiles it is not possible to decide which interpolation function produces better results. In some of the profiles, especially the profiles for nitrate and sulfate, it can be seen that for small depths the calculated DPT profile based on the measurements of the top pump matches the ram profile better than the profile calculated with the bottom pump. For greater depths this trend is reversed. This indicates that measurements taken from the pump which is located closer to the depth of interest are more suitable to evaluate
Figure 6.7: Vertical concentration profiles for magnesium, calcium and oxygen as determined with the DPT and the ram technique. DPT profiles in figures A, C and E are calculated using a second-order polynomial fitting function; DPT profiles in figures B, D and F are calculated using a third-order polynomial fitting function. DPT results are shown for the different algorithms given in Eqns.6.1-6.3.

the concentration gradient at that depth. Considering the total investigated length the DPT profiles calculated based on the measured concentration of both pumps following Eqn.6.3 show a better agreement with the ram profiles than the DPT profiles based on the measurements of one pump only (Figs.6.5A - 6.7D).
6.7 Discussion

This study presents the vertical concentration profiles of eight different groundwater solutes. The concentration profiles were measured with two independent techniques, the DPT and a ram technique. A comparison of both techniques for results obtained from a field experiment shows that generally acceptable agreement between the DPT and the ram technique could be achieved. Especially if the concentration of a species changes with depth significantly, it was possible to reproduce the trend, measured by the reference technique, with the DPT. Only for potassium a remarkable offset could be observed. We were not able to fully explain these differences. Generally, small-scale horizontal gradients can be a reason for such measurements, but at the test site in Menziken no evidence for this could be found (Hunkeler et al. 1999). In general the measured concentration profiles, especially those of the electron acceptors nitrate and sulfate, correspond to the history of the site, i.e. a diesel fuel spill close to the water table. The microbial degradation of these contaminants and therefore the consumption of the electron acceptors can be expected to have the highest rates in the upper part of the aquifer (Bennet et al. 1993).

No indications were found that the presence of residual (immobile) hydrocarbons limits the applicability of the DPT.

Using the ram technique as a reference the presented data demonstrated that the DPT is an adequate method to measure vertical concentration profiles in fully screened observation wells even for screen lengths of less than 10m. The drawdown of the groundwater around the well seemed to have no general influence on the quality of the DPT data but the observed discrepancy between the results of the two techniques for nitrate and chloride at small depths might be caused by the drawdown of the water table, but the concentrations measured at Menziken do not answer this question definitely.

The use of two different interpolation functions (a second and a third-order polynomial) and the fact that the calculated concentration profiles do not depend on the function used for interpolation indicates the stability of the algorithm introduced by Rapp et al. (1998). We presented here an improvement of this algorithm. The new
algorithm, which calculates one single profile as a weighted average of the profiles based on the data of only one pump, solves a limitation of the algorithm used by Rapp et al. (1998), i.e. that 50% of the measured data remain unused or two different profiles needed to be calculated. In theory these profiles should be equal but in reality they will differ at least slightly. It is not possible to decide a priori which one of these two profiles is more representative for the real concentration profile. Results of the present study indicate that the calculated profiles based on the measurements of the top pump yields better results for smaller depths whereas profiles calculated with data taken from the bottom pump agree better with reference measurements for greater depths. The profile calculated with the improved algorithm respects all the measured data and takes into account the appropriately weighted advantages of the other two profiles.

A problem that became obvious was the determination of the water divide between the two pumps. The measured impeller flowmeter data could only be used for a rough estimation of the water divide because of a bad signal to noise ratio. This problem could not be solved by increasing the total pumping rate in order to get a better flowmeter signal because this would have lowered the water table to an unacceptable level. Nevertheless the assumption of a homogenous influx distribution along the screen into the well agreed reasonably well with the flowmeter measurements. These flowmeter measurements were the most time consuming part of the DPT measurements and the free water table which is not constant for long-term monitoring makes it necessary to repeat these measurements at every sampling. It must also be pointed out that the costs for sampling are increased by the use of a flowmeter. These aspects suggest the conclusion that a flowmeter-based evaluation of the water divide is only meaningful if the screen length of a well in the groundwater exceeds a certain value or if strong vertical heterogeneities of the aquifer must be assumed. For the aquifer investigated in this study with a screen length of about 6 m, use of a flowmeter did not appear warranted. For small screen lengths and for homogeneous aquifers the assumption of a homogeneous inflow of water into the well seems to be appropriate. Another advantage of not measuring but calculating the water divide is the fact that without using a flowmeter the total pumping rates can
be decreased which allows to use smaller and cheaper pumps requiring less technical supply. Whether it is efficient to use a flowmeter for measuring the position of the water therefore largely depends on the aquifer to be investigated.

For aquifers, in which flow is dominated by preferential flow paths, the position of the water divide is highly sensitive to a variation of the pumping rate ratio. To state, whether DPT data is sufficient to measure vertical concentration profiles even in such aquifers, requires additional studies.

The total amount of extracted water during the 6 h lasting DPT experiment was about 10 m³. The time required for the measurements and the amount of extracted water can be reduced significantly if no flowmeter measurements are performed. In such a case the amount of extracted water for a concentration profile with n data points would be about n times the amount of water extracted by a single pump sampling at this well using the same total pumping rate for all samplings.

Compared to the study of Rapp et al. (1998), this study provides an independent reference for the results of the DPT. It is therefore possible to validate the accuracy of the DPT results. Due to this reference it was possible to improve the algorithm used to calculate the DPT based vertical concentration profiles. Another new feature of this study compared to Rapp et al. (1998) is the successful application of the DPT on a site contaminated with petroleum hydrocarbons, equipped with wells with screen lengths of less than 10 m and with a vertical concentration gradient close to the free water table.

Finally these results lead to the conclusion that the DPT is a useful and effective technique to investigate depth profiles of concentrations in groundwater. For accurately measuring vertical concentration profiles in wells with small screen lengths the DPT only requires two pumps, each with adjustable flowrate. No further equipment must be installed inside the well. An advantage of the DPT compared to the ram method can be demonstrated with the profile of the oxygen concentration as an example (Fig.6.7E and F). This parameter could only be measured with the DPT because the ram technique uses a vacuum pump which extracts only a small amount of water and the oxygen concentration could therefore not be investigated with this method. The ram technique is also limited to aquifers without a high fraction of
coarse gravel.

6.8 Conclusion

From our results we conclude that the DPT is a useful tool for measuring vertical concentration profiles in groundwater. We demonstrated that the DPT is applicable to unconfined gravel aquifers for wells with screen lengths of less than 10 m.

In our experiments, the use of an impeller flowmeter for measuring the water divide in a well did not increase the accuracy of the DPT results because of the high signal to noise ratio of the flowmeter data.

Improvement of the algorithm used to calculate the DPT-based vertical concentration profile increased the agreement between the results of the DPT and the reference profile measured with the ram technique.

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Chapter 7

Clogging of slow sand filters used for drinking water treatment


7.1 Abstract

Slow sand filtration is an established technique for the treatment of drinking water. However, clogging of these filters requires extensive maintenance. The clogging and hydraulic characteristics of slow sand filters were investigated in a drinking water plant that processes lake water. Reasons for the clogging were evaluated by measuring physical, chemical and biological parameters of the interstitial water and the filter matrix. The biomass in the filters was characterised by quantifying bacterial abundance and activity as well as the concentration of extracellular polymeric substances (EPS). Results of this study showed that the clogging effects were mainly attributed to the presence of EPS. This microbial biomass reduced the pore space in the highly clogged parts of the filters by at least 7%. Although the most severe clogging occurred in the top 5-10 cm of the filters where bacterial abundance and activity were highest, deeper layers of the filters were clogged, too.
7.2 Introduction

Slow sand filtration is one of the oldest technologies for treating water in drinking water plants (Weber-Shirk and Dick 1997b). The simplicity of slow sand filtration makes the process attractive and therefore it is widely used throughout the world. Prior to the progress of disinfection techniques, filtration was the principal defence against the spread of disease by water, and the majority of research focused on bacterial removal mechanisms (Datta and Chaudhuri 1991; Palmateer et al. 1999; Urfer et al. 1997; Weber-Shirk and Dick 1997a). However, the infiltration capacity of slow sand filtration is seriously limited by clogging processes. Despite the high maintenance costs, regular removal of the upper heavily clogged part of the filter is a useful method to re-establish a good hydraulic conductivity. The economic efficiency of slow sand filtration may be increased when clogging mechanisms could be prevented.

Clogging is defined as the decrease of hydraulic conductivity in a porous medium, and occurs commonly in a wide range of systems (e.g. review in Baveye and Dumestre 1998). The progressive augmentation of a medium’s physical resistance to water flow results from the reduction in the size of pore space. The mechanisms responsible for these changes are usually classified into physical, chemical and biological factors or a combination of thereof (Baveye and Dumestre 1998; Weber-Shirk and Dick 1997a).

Biological processes can be the major reason of clogging and they were extensively studied in laboratory experiments (e.g. Kildsgaard and Engesgaard 2002; Taylor and Jaffé 1990a; Thullner et al. 2002a; Vandevivere and Baveye 1992c). However, the majority of field studies on clogging did not investigate hydraulic conductivity because soil heterogeneities make this measurement difficult. Field determinations of hydraulic conductivity were only made for confined systems such as ponds (Chang et al. 1974; Siegrist 1987; Wood and Bassett 1975). Although, it was possible to monitor the overall clogging of such systems by measuring the water infiltration rate, researchers did not locate precisely the clogging processes in the sediment profile.

In the present study, we investigated the change of hydraulic conductivity in slow sand filters of a drinking water plant in Zurich, Switzerland. Physical, chemical, and
biological parameters potentially related to the decrease of hydraulic conductivity were investigated in the slow sand filters at different stages in the evolution of clogging. Microbial abundance, microbial activity and extracellular polymeric substances (EPS) were quantified to elucidate the contribution of biological parameters to clogging.

7.3 Methods

7.3.1 Major steps of water treatment

Raw water is collected in the Lake Zrich at a depth of 30 m (more than 20 m below thermocline) and pumped to the water treatment plant Lengg. The different steps of water treatment are given in Fig.7.1, and the water quality within the system is summarised in Tab.7.1. A first ozonation is used for disinfection prior to the treatment by rapid filters. These filters, made of pumice and quartz sand, are designed for a coarse cleaning. Before activated carbon filtration a second ozonation (0.6 mg O₃ l⁻¹) is conducted. For the mechanical and biological superfine cleaning, slow sand filters are used (Fig.7.1). The filters are constructed on top of a floor of special bricks that serve as a drainage area. The filters consist of four layers with increasing grain size from the top to the bottom: 50 to 85 cm of fine sand (diameter 0.2-2 mm), 5 cm of coarse sand (diameter 4-8 mm), 5 cm of fine gravel (diameter 8-15 mm), and 5 cm gravel (diameter 15-30 mm). Thickness of fine sand layer varied according to number of cleaning cycles (removal of the top 5 cm). Thus, the total thickness of the slow sand filter varies between 65 and 100 cm. After 10 to 15 years the remaining sand in a filter must be removed and cleaned. The plant Lengg has 14 slow sand filters (operating in parallel) designated as SSF1 to SSF14. Each filter has an area of 1120 m² and a maximum daily load capacity of around 16 m³ m⁻². They are kept at constant water level. The whole system is capable of treating 250,000 m³ d⁻¹.
7.3.2 Hydraulic behaviour

To study clogging processes, two filter types defined as highly and less clogged were sampled. The highly clogged filters (C+) are old filters (age > 9 years) with fast cleaning cycles of 2-3 years (Fig.7.2). These filters had an average hydraulic conductivity around 0.5 m h$^{-1}$ at the sampling dates (Tab.7.2). The less clogged filters (C-) are newly built (age < 8 years), never cleaned, and were only partially clogged at the sampling dates with respect to the C+ filters. The dynamics of the system can be visualised by the hydraulic behaviour of the slow sand filters (Fig.7.2).

Newly constructed filters (clean sand) exhibit an initial hydraulic conductivity of approximately 4 to 7 m h$^{-1}$. During the following years, the hydraulic conductivity decreases gradually to 0.5 m h$^{-1}$, indicating the increasing clogging of the system. At this point, filters are drained and cleaned by removing the first 5 cm of the sand. Cleaning rises the hydraulic conductivity by 1-2 m h$^{-1}$ for up to three years. However, this method does not restore the original infiltration capacity.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lake water (1)</td>
</tr>
<tr>
<td>Ammonium-N (μM)</td>
<td>0.3 ± 0.19 a</td>
</tr>
<tr>
<td>Nitrate-N (μM)</td>
<td>54.6 ± 3.39 a</td>
</tr>
<tr>
<td>Nitrite-N (μM)</td>
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<td>Phosphate-P (μM)</td>
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<tr>
<td>Sulfate (μM)</td>
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<td>Dissolved oxygen (mg l⁻¹)</td>
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</tr>
<tr>
<td>Dissolved organic carbon (mg l⁻¹)</td>
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<tr>
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</tr>
<tr>
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<tr>
<td>Temperature (C)</td>
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</tbody>
</table>

<sup>a</sup> n=12, <sup>b</sup> n=23, <sup>c</sup> n=22

Table 7.1: Physical and chemical characteristics of water along the treatment processes (numbers refer to the location on Fig.7.1). Averages and standard deviations for the year 2000.

### 7.3.3 Sampling procedure

Samples were collected on three dates: 2 November 2000, 20 March 2001 and 17 May 2001. The last two sampling dates were chosen before and after lake stratification. For each filter type (C+ and C-), three different filters were selected. Samples of sand and interstitial water were taken for each filter at 3 points spaced approximately 1 m apart, forming an equilateral triangle. The triplicate samples of each filter were pooled for subsequent analyses. Interstitial water was collected using porous candles (length 50 mm, diameter 15 mm) placed at 0 to 5 cm and 15 to 20 cm below the surface of the sand. Sand was collected by coring with a plexiglass tube (diameter 50 mm). The cores were separated, and two parts (0-5 cm and 15-20 cm) were preserved in an icebox for later analysis. The top layer (0-5 cm) corresponded to the sand removed when slow sand filters are cleaned. Analysis of fine sand profiles (0-1 cm,
Figure 7.2: Evolution of hydraulic conductivity for highly and less clogged filters based on measured head differences from inlet to outlet with a constant water flow of 500 m³ h⁻¹ (Zurich waterworks, personal communication). Arrows indicate cleaning by removing the top 5 cm of sand.

1-2 cm, 2-3 cm, 3-4 cm, 4-5 cm) revealed that microbial products (polysaccharides, proteins, and cells) and activities were maximal between 1-2 cm (data not shown). Differences within the first 5 cm were relatively small and never exceeded 30% of the maximum value. Preliminary measurements of profiles of polysaccharides content
and bacterial numbers showed no significant change below 10 cm (data not shown).

### 7.3.4 Measurements of interstitial water characteristics

All chemical and biological analyses were performed using nanopure water (MilliQ), and acid washed glass material. Nitrate, sulphate and phosphate in free water were quantified using an ion chromatograph (Dionex DX-100). Dissolved organic carbon (DOC) was measured with a "Total Carbon Analyser" (Himadzu, Shimadzu) after removing the inorganic carbon with hydrochloric acid (2M, 1 ml ml⁻¹) and stripping with oxygen for 10 min. Refractory dissolved organic carbon (RDOC) was measured as the remaining fraction after 20 days of incubation with the natural bacterial consortia at 20°C (Servais et al. 1987). The biodegradable organic carbon (BDOC) fraction was calculated as DOC minus RDOC. The amount of fine particles in the inlet water was measured by filtering 25 l of water with a 0.2 mm filter (Supor) and calculating the increase in weight.

<table>
<thead>
<tr>
<th></th>
<th>Begin of operation</th>
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<th>Hydraulic conductivity (m h⁻¹)</th>
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<td></td>
<td></td>
<td>November</td>
<td>March</td>
</tr>
<tr>
<td>Highly clogged filters (C+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>June 1985</td>
<td>October 1997</td>
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</tr>
<tr>
<td>SSF6</td>
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<tr>
<td>SSF8</td>
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<td>April 1999</td>
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</tr>
<tr>
<td>Less clogged filters (C-)</td>
<td></td>
<td></td>
<td></td>
</tr>
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<tr>
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<td>1.00</td>
</tr>
<tr>
<td>SSF13</td>
<td>October 1994</td>
<td></td>
<td>1.54</td>
</tr>
</tbody>
</table>

* by removing the first 5 cm of sand

Table 7.2: Maintenance and average hydraulic conductivity of the slow sand filters
7.3.5 Physical and chemical parameters

Hydraulic conductivity (K) of the entire filter was estimated by measuring the difference in water level between the inlet and outlet of each filter at a constant flux rate of 500 m$^3$ h$^{-1}$. To investigate the change of K with increasing depth, piezometers were placed at approximately 1, 2, 3, 4, 5, 10, 15, 20, 30, 50 and 80 cm below the sand surface. The hydraulic conductivity was determined by measuring the piezometric head profile by using Darcy’s law:

$$\frac{Q}{A} = K \cdot \frac{\Delta H}{\Delta z}$$

(7.1)

where $Q$ is the flow rate, $A$ is the surface of slow sand filter, $H$ is the head loss, and $z$ is the depth below the sand surface. Measurements were conducted at different flow rates (500 and 150 m$^3$ h$^{-1}$) to verify that computed $K$ values were constant.

Grain size distribution of the sand was determined for dried sand from the top 5 cm that was separated with a sieve series (0.063, 0.125, 0.25, 0.5, and 1 mm). Sieving was performed until each size class comprised at least 10 g of dry sand. Water content was determined by loss of weight after drying (24 h at 60°C). In the sand carbon is present as total organic matter (TOM) or calcite. TOM of the sand was measured by loss of weight after ignition (4 h at 550°C; Bretschko and Leichtfried 1987 of 10 g of dry sand. Calcite content of the sand was estimated as the carbon content of burned samples (4h at 550°C) measured with a carbon analyser (CHNS-932, Leco).

7.3.6 Polysaccharide and protein quantification

Polysaccharides were quantified within four hours of sampling using the Dubois method (Dubois et al. 1956). Sonication (2 min, power 7, Sonifer B-12 Branson) was applied to detach the polysaccharides from 10 g of wet sand placed in 50 ml of water (Mermillod-Blondin et al. 2001). 0.5 ml of the supernatant was mixed with phenol solution and 95% sulphuric acid, and incubated at room temperature in the dark for 1 h. Absorbance was measured at $\lambda=495$ nm with a photometer.
Polysaccharide content was expressed as mg of glucose equivalent per gram of dry sand.

Proteins, extracted by the same sonication procedure, were quantified using the Lowry method (Lowry et al., 1951). Protein content was expressed as mg of bovine serum albumin (BSA) equivalent per gram of dry sand.

### 7.3.7 Bacterial abundance and volume

Number of total bacteria were counted using DAPI (4',6-diamidino-2-phenylindol) staining (Porter and Feig 1980). Wet sand (1 g) fixed with formaldehyde (final concentration: 4%) was diluted with 100 ml 0.1% pyrophosphate. Bacteria were detached by sonication as described previously, and aliquots were spotted onto slides and stained with DAPI solution (Schönholzer et al. 1999). The slides were examined at 400x magnification with a microscope (Zeiss Axioshot Plan Neofluar) fitted for epifluorescence measurements with a 50 W high pressure mercury bulb and a filter set 02 (Zeiss, FT 395, LP 420). At least 40 randomly selected fields per triplicate measurement were counted. The number of bacteria was expressed per gram of dry sand.

Bacterial volumes were analysed in samples from May by image analysis of 5 images per triplicate with up to 100 cells per image (Schönholzer et al. 2002). Cell volumes were determined based on measurements of area and perimeter for each organism or bacterial agglomerate (Bloem et al. 1995; Russ 1995). Volume of bacterial cells was expressed as percentage of pore space.

### 7.3.8 Bacterial activity

Numbers of ETS-active bacteria (i.e. bacteria with an active electron transport system) were measured using CTC (5-cyano-2,3-ditolyl tetrazolium chloride) staining (Rodriguez et al. 1992). Wet sand (1 g) was incubated for 3 h in the dark at room temperature with 1 ml CTC solution (1.4 mg ml$^{-1}$). The reaction was stopped by adding 0.25 ml of 7% formaldehyde. Samples were stored at -20°C before analysis. Samples were diluted with 50 ml 0.1% pyrophosphate, and bacteria were
detached from the sand particles by sonication as previously described. Ten micro-
liter aliquots were spotted onto slides. The preparations were allowed to air dry, and slides were mounted with citifluor. The preparations were examined at 400x magnification as described above with the filter set Hq Cy3 (FT 560, BP 575-640). At least 40 randomly selected fields were counted per triplicate measurement. The number of ETS-active bacteria was expressed per gram of dry sand. Percentage of ETS-active bacteria was calculated by dividing abundance of ETS-active bacteria by total number of bacteria determined by DAPI staining.

### 7.3.9 Hydrolytic activity

Hydrolytic activity of microorganisms was measured using fluorescein diacetate (FDA) as substrate for hydrolases (Fontvieille et al. 1992). Wet sand (1 g) was incubated in 3 ml of phosphate buffer (pH = 7.6) with 0.1 ml of FDA solution (2 mg ml$^{-1}$) and kept at 20°C and darkness until the green color of the fluorescein was visible (0.5-2 hours). The reaction was stopped by addition of 3 ml of mercuric chloride solution (200 mg l$^{-1}$). The supernatant was filtered (0.45 mm, HAWP Millipore), and absorbance of the solution was measured at $\lambda=490$ nm. Results were expressed as mmoles of FDA hydrolysed per hour and gram of dry sand.

### 7.3.10 Data analysis and statistical methods

Results are averages of measurements made in 3 filters of each type (C+ or C-) ± standard errors. Spatial and seasonal variations of parameters were examined using the Tukey test (multiple comparison procedure) and analysis of variance (ANOVA) after checking normality and homoscedasticity (Statview 4, Abacus). Spatial variations for each sampling season were tested using ANOVA2 (filter type x sampling depth) by grouping data from the three filters of each type. Temporal variation was investigated by ANOVA1 (date) for each combination of filter type and sampling depth.

For the determination of biovolumes we assumed that with the exception of polysaccharides and proteins other types of microbial substances can be neglected.
Volumes of biofilms extracted by sonication from the sand were calculated by using the amount of polysaccharides and proteins, and reported biofilm densities which range from 5 to 130 kg dry mass m\(^{-3}\) wet volume (Christensen and Characklis 1990). Volume of biofilm was expressed as percentages of pore space.

7.4 Results

7.4.1 Physical and chemical properties of water

Interstitial water had similar chemical characteristics in all filters (Fig.7.3). The average of all interstitial water samples was 57.6 ± 4.6 mM for nitrate, 163 ± 10 mM for sulphate, and 3.4 ± 0.4 mg l\(^{-1}\) for BDOC. Water chemistry depicted only small changes during the infiltration and showed few temporal changes. The inlet water contained 0.4 mg l\(^{-1}\) of fine particles in average (Tab.7.3). Detailed statistical analyses of data given in Fig.7.3 (and of data given in Figs.7.5 and 7.6) are presented in Tab.7.4. In November nitrate and DOC showed significant changes during infiltration, which were significantly higher in the deep layer compared to the top layer (Fig.7.3). Nitrate and sulphate concentrations were significantly higher in the top layer of C+ filters in March compared with other sampling dates, whereas DOC was significantly lower in the deep layer of C- filters in May compared with November. Amounts of imported particles were not statistically different according to the filter type (Tab.7.3).

7.4.2 Physical and chemical properties of the filter matrix

The majority of the observed clogging occurred in the first centimeters of infiltration demonstrated by a drastic head loss in those layers (Fig.7.4). Average hydraulic conductivity was calculated using Darcy’s law where \(\frac{\Delta H}{\Delta z}\) was given by the slope of linear regression of head loss vs. depth for defined layers. K was calculated from points in three layers from approximately 0-10, 10-50, and 50-80 cm below sand surface, with values of 0.24, 1.42, and 8.49 m h\(^{-1}\) in the C- filter, and 0.09, 0.87 and 6.66 m h\(^{-1}\) in the C+ filter, respectively (Fig.7.4). K increased by a factor of
Figure 7.3: Chemical characteristics of the top (0-5 cm) and deep (15-20 cm) layers of the two filter types: highly clogged (C+, dark grey) and less clogged (C-, pale gray). Bars identified by different letters are significantly different (Turkey test, 5%).

6-10 from one layer to the next. The C+ filter showed smaller conductivities in all respective layers. The sand of the upper part of all filters exhibited a similar grain
<table>
<thead>
<tr>
<th>Water</th>
<th>Fine particles Content of fine particles (&gt;0.2 mm) in the inlet water (mg l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly clogged filters (C+)</td>
<td>1.08 ± 0.91 0.44 ± 0.11 0.25 ± 0.17 0.87 ± 0.91 0.44 ± 0.13 0.14 ± 0.19</td>
</tr>
<tr>
<td>Less clogged filters (C-)</td>
<td>0.44 ± 0.08 0.09 ± 0.11 0.14 ± 0.11 0.22 ± 0.19</td>
</tr>
<tr>
<td>Sand</td>
<td>Grain size distribution expressed as cumulative percentage of the dry sand</td>
</tr>
<tr>
<td>Grain size diameter ((\mu)m)</td>
<td>&lt;63  63-125  125-250  250-500  500-1000  &gt;1000</td>
</tr>
<tr>
<td></td>
<td>0.15 ± 0.11  0.44 ± 0.37  5.52 ± 1.37  46.51 ± 4.36  93.12 ± 4.14  100.00</td>
</tr>
<tr>
<td></td>
<td>0.24 ± 0.04  0.47 ± 0.20  3.12 ± 0.54  52.50 ± 4.53  84.14 ± 4.03  100.00</td>
</tr>
<tr>
<td></td>
<td>0.04 ± 0.11  0.29 ± 0.17  4.82 ± 1.68  45.30 ± 4.36  90.44 ± 5.21  100.00</td>
</tr>
<tr>
<td></td>
<td>0.11 ± 0.08  0.31 ± 0.37  6.18 ± 2.38  48.10 ± 4.36  89.23 ± 5.21  100.00</td>
</tr>
<tr>
<td></td>
<td>0.16 ± 0.10  0.48 ± 0.35  3.38 ± 0.17  5.72 ± 2.33  74.12 ± 4.14  100.00</td>
</tr>
<tr>
<td></td>
<td>0.03 ± 0.19  0.35 ± 0.38  4.39 ± 1.03  52.57 ± 4.46  83.01 ± 8.81  100.00</td>
</tr>
<tr>
<td></td>
<td>0.08 ± 0.09  0.38 ± 0.10  4.74 ± 2.33  52.57 ± 4.46  85.56 ± 8.81  100.00</td>
</tr>
<tr>
<td></td>
<td>0.11 ± 0.10  0.48 ± 0.35  3.38 ± 0.17  5.72 ± 2.33  74.12 ± 4.14  100.00</td>
</tr>
</tbody>
</table>

Table 7.3: Particle content of inlet water, and grain size distribution and calcite content of sand

size distribution, and a similar amount of calcite (Tab.7.3). Calcite content was around 6% of the weight of sand and depicted no trend in C+ or C- filters. Pore volume per weight of dry sand was calculated based on measurements of porosity (0.21) and bulk density of sand (2.87 kg l\(^{-1}\)). The total volume occupied by 1 g of dry sand was 0.44 ml, with 0.35 and 0.09 ml for sand matrix and pore space, respectively.

### 7.4.3 Biological properties of the filter matrix

Temporal pattern: Biological characteristics are shown in Figs.7.5 and 7.6. Polysaccharide content depicted small temporal variations with highest values in November (Fig.7.5). This trend was present for both filter types and sampling depths, but significant only for the top layer of the C+ filters and the deep layer of C- filters. In the top layer of C- filters polysaccharide content shows a tendency to decrease. Most of the measured biological characteristics changed significantly according to the sampling date (Tab.7.4). Protein content displayed a similar trend throughout the sampling period for both filter types (Fig.7.5). In the top layer of C+ filters
protein content was significantly lower in November than in March and May, while protein content in the top layer of the C- filters increased continuously from November to May. In the deep layer of C+ and C- filters maximal values were recorded in March. Bacterial abundance increased from November to May for both filter types and sampling depths (Fig.7.6). Bacterial abundance rose in the top layer from 2 \(10^8\) cells [g dry sand]\(^{-1}\) in November to 1.9 \(10^9\) cells [g dry sand]\(^{-1}\) in May, in the deep layer from 2 \(10^8\) cells [g dry sand]\(^{-1}\) in November to 1.5 \(10^9\) cells [g dry sand]\(^{-1}\) in May. Growth was especially evident in the 2 months between the March and the May sampling compared to the 4.5 months between the November and March sampling. Number of ETS-active bacteria showed a different temporal pattern (Fig.7.6). In the top layer bacterial activity was maximal in March, whereas in the deep layer bacterial activity increased from November to March and remained at that level in May. Because bacterial abundance and activity had different temporal patterns the fraction of ETS-active bacteria on total number of bacteria varied between sampling dates: 9.4 ± 1.4% in November, 12.7 ± 1.9% in March, and 2.4 ± 0.3% in May.

The pore space occupied by EPS (proteins and polysaccharides) was calculated
Figure 7.5: EPS produced by microorganisms of the top (0-5 cm) and deep (15-20 cm) layers of the two filter types: highly clogged (C+, dark grey) and less clogged (C-, pale grey). Bars identified by different letters are significantly different (Turkey test, 5%).

As the minimum volume by using the highest density reported in literature (130 kg m$^3$, Christensen and Characklis 1990. Pore space occupied by bacterial compounds (Tab.7.5) did not change much according to the sampling date in the top layer of the C+ filters. In the top layer of C- filters bacterial compounds occupied an increasing percentage of the pore space over time. The percentage of occupied pore space almost doubled in the 6.5 months from November till May. The almost four-fold increase of protein content accounted for this change. In the deep layer bacterial compounds occupied the same pore space in November and in May, but surprisingly significantly more pore space was occupied in March. Between March and May the pore space occupied by bacterial compounds in the deep layer decreased by about 50% for both filter types. The volume of bacteria cells was minuscule compared with the volumes of EPS.
Figure 7.6: Microbial characteristics of the top (0-5 cm) and deep (15-20 cm) layers of the two filter types: highly clogged (C+, dark grey) and less clogged (C-, pale grey). Bars identified by different letters are significantly different (Turkey test, 5%).

Spatial pattern: All biological characteristics, EPS (Fig.7.5), bacterial abundance and activity, number of ETS-active bacteria and hydrolytic activity (Fig.7.6), significantly changed with the sampling depth. For both filter types all biological characteristics were significantly higher in the top layer (Tab.7.4). Microbial compounds (bacterial cells, polysaccharides and proteins) occupied an average of 6 to 14% of the pore space of the top layer, and an average 3 to 9% of the pore space of the deep layer (Tab.7.5). Differences between filter types were less pronounced, but some were significant especially in the top layer. C+ filters contained significantly more EPS than C- filters, except the protein content in the top layer in May. C+
Table 7.4: Statistical differences of chemical and microbial characteristics between sampling depths, dates, filter types (highly clogged C+, and less clogged C-) and depths tested by ANOVA1 and 2 filters also depicted higher activity (number of ETS-active bacteria and hydrolytic essay) in March and higher bacterial abundance in May than the C- filters (Tab.7.4).

<table>
<thead>
<tr>
<th>Chemical characteristics</th>
<th>All dates</th>
<th>November</th>
<th>March</th>
<th>May</th>
<th>ANOVA2 (filter type x depth)</th>
<th>ANOVA1 (dates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>0.4427</td>
<td>0.7873</td>
<td>0.6853</td>
<td>0.3581</td>
<td>0.7116</td>
<td>0.7956</td>
</tr>
<tr>
<td>Sulphate</td>
<td>0.6597</td>
<td>0.7256</td>
<td>0.8671</td>
<td>0.1482</td>
<td>0.2386</td>
<td>0.0571</td>
</tr>
<tr>
<td>Dissolved organic carbon (DOC)</td>
<td>0.6319</td>
<td>1.3530</td>
<td>0.6617</td>
<td>0.0049***</td>
<td>0.3627</td>
<td>0.6144</td>
</tr>
<tr>
<td>Refractory DOC</td>
<td>0.7087</td>
<td>0.8947</td>
<td>0.9202</td>
<td>0.8990</td>
<td>0.8672</td>
<td>0.7865</td>
</tr>
<tr>
<td>Biodegradable DOC</td>
<td>0.6698</td>
<td>0.4147</td>
<td>0.2726</td>
<td>0.3112</td>
<td>0.2608</td>
<td>0.9659</td>
</tr>
<tr>
<td>% Biodegradable DOC</td>
<td>0.6018</td>
<td>0.8445</td>
<td>0.2215</td>
<td>0.7119</td>
<td>0.7635</td>
<td>0.8785</td>
</tr>
<tr>
<td>Total organic matter</td>
<td>0.9277</td>
<td>0.1457</td>
<td>0.8794</td>
<td>0.1830</td>
<td>0.9442</td>
<td>0.1628</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biological characteristics</th>
<th>Polyaspartic acids</th>
<th>Proteins</th>
<th>Total bacteria</th>
<th>% of ETS-active bacteria</th>
<th>Hydrolytic activity</th>
<th>Pore space occupied</th>
</tr>
</thead>
<tbody>
<tr>
<td>All dates</td>
<td>&lt;0.0001***</td>
<td>0.0007*</td>
<td>0.5343</td>
<td>0.0003**</td>
<td>0.1058**</td>
<td>0.0005*</td>
</tr>
<tr>
<td>November</td>
<td>0.0234*</td>
<td>0.0008**</td>
<td>0.3141</td>
<td>0.0162*</td>
<td>0.0332*</td>
<td>0.0001***</td>
</tr>
<tr>
<td>March</td>
<td>0.0948</td>
<td>0.0012**</td>
<td>0.6865</td>
<td>0.0018**</td>
<td>0.2060</td>
<td>0.0013**</td>
</tr>
<tr>
<td>May</td>
<td>0.4250</td>
<td>0.0014**</td>
<td>0.9686</td>
<td>0.0044***</td>
<td>0.1492</td>
<td>0.0001**</td>
</tr>
<tr>
<td>ANOVA2 (filter type x depth)</td>
<td>0.9432**</td>
<td>0.0013**</td>
<td>0.8270</td>
<td>0.0094***</td>
<td>0.5845</td>
<td>0.0003***</td>
</tr>
<tr>
<td>ANOVA1 (dates)</td>
<td>0.0090*</td>
<td>0.0013**</td>
<td>0.8960</td>
<td>0.0002***</td>
<td>0.0235*</td>
<td>0.0029*</td>
</tr>
</tbody>
</table>

*0.05 cm, **0.01 cm

Values: **p<0.01, *p<0.05, ^p<0.10
### Microbial substances

<table>
<thead>
<tr>
<th></th>
<th>Bacterial cells(^a)</th>
<th>Proteins</th>
<th>Polysaccharides</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Top layer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Highly clogged C+</strong></td>
<td>November 0.01 ± 0.001</td>
<td>4.2 ± 0.70</td>
<td>5.8 ± 0.57</td>
<td>10.0 ± 1.27</td>
</tr>
<tr>
<td></td>
<td>March 0.05 ± 0.002</td>
<td>8.4 ± 0.19</td>
<td>4.1 ± 0.62</td>
<td>12.5 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>May 0.16 ± 0.034</td>
<td>8.7 ± 0.12</td>
<td>4.8 ± 0.28</td>
<td>13.6 ± 0.40</td>
</tr>
<tr>
<td><strong>Less clogged C-</strong></td>
<td>November 0.01 ± 0.001</td>
<td>2.4 ± 0.88</td>
<td>4.1 ± 0.86</td>
<td>6.5 ± 1.74</td>
</tr>
<tr>
<td></td>
<td>March 0.05 ± 0.001</td>
<td>6.8 ± 0.99</td>
<td>2.8 ± 0.35</td>
<td>9.6 ± 1.34</td>
</tr>
<tr>
<td></td>
<td>May 0.17 ± 0.037</td>
<td>8.9 ± 0.18</td>
<td>3.1 ± 0.84</td>
<td>12.0 ± 1.02</td>
</tr>
</tbody>
</table>

| **Deep layer**       |                        |           |                 |          |
| **Highly clogged C+**| November 0.01 ± 0.0002 | 2.5 ± 0.49| 3.1 ± 0.95      | 5.6 ± 1.43 |
|                      | March 0.03 ± 0.004     | 5.6 ± 0.51| 3.6 ± 0.32      | 9.2 ± 0.83 |
|                      | May 0.12 ± 0.018       | 3.0 ± 0.16| 1.9 ± 0.99      | 4.9 ± 1.17 |
| **Less clogged C-**  | November 0.01 ± 0.004  | 1.1 ± 0.11| 2.6 ± 0.71      | 3.8 ± 0.88 |
|                      | March 0.03 ± 0.007     | 4.5 ± 0.42| 3.0 ± 0.68      | 7.5 ± 1.11 |
|                      | May 0.12 ± 0.033       | 1.6 ± 0.36| 1.5 ± 0.51      | 3.2 ± 0.87 |

\(^a\) Bacterial volume was based on the average biovolume of May samples (0.06 mm\(^3\) cell\(^-1\)).

\(^b\) To calculate the minimum volume occupied by proteins and polysaccharides, the highest density reported in literature was used (130 kg m\(^3\), Christensen and Characklis 1990).

Table 7.5: Calculation of the percentage of pore space occupied by bacteria, proteins and polysaccharides

### 7.5 Discussion

#### 7.5.1 Hydraulics

The average hydraulic conductivity of all sand filters exhibited a strong decrease over time (Fig.7.2). The initial values of the hydraulic conductivity were between 4 and 7 m h\(^{-1}\), which was only slightly higher than the theoretically predicted value.
of 3 m h\(^{-1}\) using the Kozeny-Carman equation in a version given in Bear (1972)

\[
K = \frac{\rho g d^2 n}{\mu 180(1 - n)^2}
\]  

(7.2)

with \(d=1\) mm as the typical grain diameter and \(n=0.21\) as porosity. \(\rho\) is the density of water, \(g\) the gravitational acceleration and \(\mu\) the dynamic viscosity of water. This indicates that the initial hydraulic conductivity of the sand filter did not vary with depth. Measurement of the piezometric head profile and thus the hydraulic conductivity along the depth of the sand filters revealed that the fine sand layer of a sand filter could be divided into three sub-layers (Fig.7.4), each with a different development of hydraulic conductivity over time. The uppermost layer, with a thickness of approximately 5-10 cm, showed the highest reduction of hydraulic conductivity, but also for the second layer, which ends at a depth of approximately 50 cm, a reduction of hydraulic conductivity could be observed. Below a depth of 50 cm the measured hydraulic conductivity was even slightly higher than the average hydraulic conductivity of a newly built filter, suggesting that the hydraulic conductivity in this layer did not change over time. The varying extent of clogging depending on the layer explains the effect that the removal of the top 5 cm of a sand filter has on the average hydraulic conductivity. The top 5 cm constitutes the highest clogged part of a filter. After the removal, the average hydraulic conductivity of the sand filters increased to 1-2 m h\(^{-1}\), which was similar to the values measured for the second layer from 10-50 cm depth. As we observed no changes of hydraulic conductivity with depth within each of these layers, it is likely that clogging processes proceed homogeneously within each layer. Thus, samples taken from a certain depth were assumed to be representative for the entire respective layer. In particular samples taken from the top 5 cm represented the top layer (0-10 cm depth) and samples taken at a depth of 15-20 cm represented the deeper layer from 10-50 cm depth.

### 7.5.2 Clogging due to physical and chemical causes

To verify whether the particles suspended in the infiltrating water were contributing to the clogging of the sand filters, the measured particle mass had to be transferred into a volume. Assuming 1 mg l\(^{-1}\) of particles (which is more than the average for
the C+ and C- filters) with the same density as sand and an average flow rate of 150 m$^3$ h$^{-1}$, a volume of 38 l of particles entered each filter every month. Supposing that all these particles were deposited within the top layer of 10 cm thickness and that the deposition had a porosity of 0.3, the total pore space in the top layer would have been reduced by 7% during the typical length of 2.5 years between two cleaning cycles. In this case particle deposition would have contributed to the clogging of the top layer of the sand filters but not of the deeper layers. Thus, the clogging of the deep layers was due to other processes than particle deposition. In contrast, assuming that the particle deposition took place homogeneously in the top and the deep layers, a pore volume reduction of only 1-2% would result in 2.5 years. In this case particle deposition would not have caused major clogging effects, mainly because large pores may still retain their full water carrying capacity (Goldberg et al. 1992).

For hard waters, such as water from the Lake Zurich, calcite precipitation must be assumed to contribute to the clogging of sand filters (Weber-Shirk and Dick 1997b). In the present study we observed that calcite contributed to the weight of the sand grains by approximately 6%. Assuming that the density of the sand was not changed due to the calcite, 6% of the sand volume were composed by calcite. In case that the calcite was built during the operation of the filters, calcite precipitation may have caused a reduction of the hydraulic conductivity of the filters. Potential clogging due to calcite precipitation was considered to occur uniformly along the depth of the filters because the measured calcite content and the grain size distribution were similar in all samples. Therefore, we infer that calcite precipitation was not the cause for the different degree of clogging in the different layers of the sand filters.

### 7.5.3 Clogging due to biological causes

Biological activities in the sand filters were in the same range as reported values for comparable natural environments like aquifers with river water infiltration (Mauclaire et al. 2000; Mauclaire and Gibert 2001). We observed a decrease of biomass in the deep layer between the sampling in March and in May 2001. As this decrease
was observed simultaneously for all filters, it could not be regarded as a random fluctuation. A possible reason for this effect may be a change in the composition of the lake water (e.g. nutrient, quorum sensing), caused by the seasonal stratification of the Lake Zurich, which took place at the beginning of May 2001 (F. Schanz, Limnology, University of Zurich, personal communication, 2001).

As a consequence of biological activity produced biomass reduced the pore space of the sand filters. The volume of bacterial cells did not exceed 0.2% of the pore volume, but the EPS produced by the bacteria occupied at least 7% of the top layer and 3% of the deep layer of the sand filters. For column experiments (e.g. Vandevivere and Baveye 1992c and results reviewed in Vandevivere et al. 1995 it has been reported that the hydraulic conductivity of sand can be reduced by up to three orders of magnitude due to biomass, which occupies only a small fraction of the pore space (the volume occupied by biomass needs to be precise). This was theoretically explained by assuming that biomass preferentially plugged the bottlenecks of the water flow (Thullner et al. 2002b).

In addition, it must be emphasised that the presented EPS volumes in this study were determined using a density of 130 g l\(^{-1}\). This calculation of biovolume is strongly driven by the conversion factor used for EPS density. Values reported in literature range from 5 to 130 kg m\(^{-3}\) (Christensen and Characklis 1990). In order to be stringent in our conclusions we calculated a minimum biomass volume by using the maximum biofilm density reported. So biovolumes tended to be underestimated on the whole and consequently, the occupied pore space was potentially much larger. The finding that the majority of the biomass was composed of EPS agrees with other studies that investigated bioclogging of porous media (Thullner et al. 2002a; Vandevivere and Baveye 1992a). These studies attributed the observed clogging effects to the EPS produced by the bacteria, whereas the bacterial cells by themselves were found to have only negligible influence on occupied pore space and hydraulic conductivity.

Another biological activity potentially contributing to clogging of the sand filters is the production of gas by bacteria in excess of the solubility in water. In such cases the clogging due to gas bubbles may be more important than the clogging due to
the biomass (Soares et al. 1989). However, we found no indication that gas bubble production occurred in the sand filters. In particular, the high concentration of dissolved oxygen measured in the outflow of the filters suggests that anaerobic processes were negligible. Additionally, nitrate concentration did not change between inflow and outflow of the filters and we could never detect any foul smell during sampling. Thus, neither nitrogen gas, sulphide or methane production nor fermentation was likely to occur.

7.5.4 Comparison of causes of clogging

Comparing the different causes of the clogging of the sand filters showed that particle deposition, calcite precipitation and biomass production may all have been responsible for the clogging (Fig. 7.7). The extent to which these different causes potentially contributed to the clogging of the filters varied. As calcite precipitation could not explain the different clogging in the top and deep layer, it is not likely that it had a major impact on the hydraulic conductivity of the sand filters in general. Nevertheless, it may have supported the formation of floes composed of EPS, bacterial cells and minerals, which potentially affect the hydraulic conductivity of the sand matrix (Rinck-Pfeiffer et al. 2000). Hard waters with little organic content, such as that of the Lake Zurich, have been reported as favorable for particle-particle attachment (Ali et al. 1985). The deposition of particles was shown to potentially contribute to the clogging of the top layer, whereas for the deep layer particle deposition could not explain the clogging. In contrast, we observed considerable amounts of biomass, which could account for the clogging of the top and the deep layer, even without other clogging processes taking place.

These results indicated that the bioclogging was the major reason for the observed decrease in the hydraulic conductivity of the sand filters (Fig. 7.7). This conclusion agrees with Rice (1974), who inferred that the clogging was mainly due to biological rather than physical and chemical causes for water containing a low amount of suspended particles, with a slow development of clogging taking place not only in the top centimeters of the filters. Another indication that the observed
clogging was mainly attributed to the presence of biomass was the correlation between the amounts of biomass and the different degrees of clogging in the top and deep layers as well as the different degrees of clogging in the two filter types C+ and C- (Fig.7.7).

7.6 Conclusion

Clogging was mainly due to microbial activity, and in average about 10% of the pore space in the top layer was occupied by microbial substances. The development of clogging in the highly clogged filters (C+) was different from that of the less clogged filters (C-). We were able to demonstrate that this difference correlated with varying amounts of biomass found in the two filter types. It appears that the development of biofilms can not be explained solely by environmental variables, suggesting that this process is also controlled at the organism level by the diversity of microbial population and fauna. Build-up of biofilms in well-defined systems like slow sand filters may be a powerful model for exploring how bacterial and faunal diversity and community structure relate to bioclogging. Further research must verify this.
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Chapter 8

General Discussion and Outlook

8.1 Differences between bioclogging in one-dimensional and in two-dimensional flow fields

8.1.1 Micro-scale

Comparing the bioclogging effects observed in this thesis for two-dimensional flow fields with published results obtained for one-dimensional flow fields showed significant differences on the micro-scale, where processes within each single pore were resolved, as well as on the macro-scale, where processes occurring in single pores were neglected.

In this thesis bioclogging effects on the micro- or pore-scale were investigated theoretically. By using pore network simulations it was possible to obtain hydraulic conductivity vs. porosity relations, which were different from results available in literature (e.g. Clement et al. 1996; Taylor et al. 1990c). Regardless of the microbial growth morphology (biofilm or discrete colonies), the minimum of hydraulic conductivity was reached before the entire pore space was filled with biomass. In contrast to this, the models of Clement et al. (1996) and Taylor et al. (1990c) predict that the biomass has to fill the entire pore space in order to reduce the hydraulic conductivity to zero. Nevertheless the biofilm model introduced in Chapter 2 was
still underpredicting most of the experimentally observed reductions of hydraulic conductivity (Fig. 2.6), as did the biofilm model of Taylor et al. (1990c) and the model of Clement et al. (1996). The relatively small differences between the one-dimensional biofilm model of Taylor et al. (1990c) and the two-dimensional biofilm model introduced in Chapter 2 indicates that predictions of a biofilm clogging model were only slightly affected by the dimensionality of the flow system.

For the models introduced in this thesis the amount of biomass needed to reduce the hydraulic conductivity to its minimum value depended mainly on the morphology of the microbial growth (discussed in Section 8.3), but the heterogeneity of the pore size distribution was also an important parameter. The higher the heterogeneity, the less biomass was needed (compared to the initial pore volume) to get the maximum clogging effect. Extrapolating this finding to a natural porous medium (e.g. sand) means that the medium with the higher pore-scale heterogeneity (e.g. wider grain size distributions) can be clogged more easily compared to a more homogeneous medium having the same initial porosity. In contrast, Taylor et al. (1990c) predicted the opposite correlation between porous media heterogeneity and reduction of hydraulic conductivity. Their model predicts that for a given porosity reduction the hydraulic conductivity reduction is higher for a homogeneous medium compared to a more heterogeneous one, but it must be pointed out that the models of Taylor et al. (1990c) are less sensitive to the heterogeneity of the porous medium. This differences between the models may be due to the different dimensionality of the models. In the biofilm model of Taylor and Jaffé (1990a) a few large pores may determine the hydraulic conductivity of the whole bundle. As the authors assumed the biofilm was of the same thickness in all pores these large pores are less affected by the biofilm than the smaller pores. The higher the heterogeneity of the pore size distribution, the more large pores were in the bundle and thus the less influence the biofilm had on the hydraulic conductivity of the entire bundle. In case of pores arranged in a network of interconnected pores, as used in Chapter 2, small pores may act as bottle necks for the flow. A growing biofilm may occupy nearly the entire volume of the small pores reducing the hydraulic conductivity of the pore network as a whole. The higher the heterogeneity the more of these bottle necks exist and
thus the more influence the biofilm has on the hydraulic conductivity of the entire pore network. As pore bundles are a stronger simplification of a porous medium than pore networks are, it is likely that results from pore network simulations are more realistic concerning the influence of the pore scale heterogeneity on bioclogging. In contrast, in Clement et al. (1996) changes of the pore size distribution due to bioclogging were ignored and thus in their model the heterogeneity of the porous medium has no influence on the bioclogging. Finally, experiments focusing on the effect of pore scale heterogeneity on bioclogging would provide means to evaluate, which one of the theoretical approaches is better justified.

8.1.2 Macro-scale

Not only on the micro-scale but also on the macro-scale bioclogging effects observed in a two-dimensional flow field differ from those observed in literature for one-dimensional flow fields. In this thesis clogging on the macro-scale was investigated in the laboratory during a flow cell experiment (Chapter 4). Results from this experiment showed that significant bioclogging also occurred in a two-dimensional flow field, but flow-bypass of clogged zones was an important effect. Due to this flow-bypass the average hydraulic conductivity of the flow cell was not affected by localized bioclogging that took place in only some parts of the flow cell. This flow-bypass is an observation, which could not be made using columns (e.g. Cunningham et al. 1991; Taylor and Jaffé 1990a; Vandevivere and Baveye 1992c). Due to the one-dimensionality of the flow field in these studies, the average hydraulic conductivity of the column was reduced, even if only a part of the column was affected by bioclogging.

In the flow cell experiment (Chapter 2) the oxidant and the carbon source were injected separately. Therefore these substances had to mix to enable microbial activity and in particular to allow bioclogging to take place. Consequently, the distribution of biomass was strongly influenced by the initial flow field and bioclogging did not significantly impact the initial flow field. Nevertheless, bioclogging took place and could be visualized by changing the flow injection and thus the hydraulic
boundary conditions. Conversely, in the above mentioned published column studies the one-dimensional flow field experiments were performed with the oxidant and carbon source being mixed before the injection. Thus, the biomass distribution had a gradient only along the length of the column and was therefore less complex than that observed for the flow cell experiment.

These observations demonstrate that investigations using multi-dimensional flow fields show a much more complex picture of bioclogging in porous media than the investigations using only one-dimensional flow fields. Comparing the results of this thesis with the results of Kildsgaard and Engesgaard (2002) shows that bioclogging in two-dimensional flow fields is strongly influenced by the way the oxidants and carbon source are injected. Kildsgaard and Engesgaard (2002) used two-dimensional flow fields, too, but in contrast to this thesis they mixed the substances before the injection, causing the bioclogging to change the flow field in a different way than observed in this thesis. Generalizing, this means that bioclogging in multi-dimensional flow fields is highly sensitive to the boundary conditions of flow and transport, whereas for one-dimensional systems the flexibility in changing the boundary conditions is small and thus the general observations were similar for the different columns studies published in the literature.

It could be shown in this thesis that hydraulic conductivity vs. porosity relations derived from pore network models were suitable to simulate bioclogging in a two-dimensional flow field. This finally indicates that investigating bioclogging in multi-dimensional flow fields has to be done on both scales, the micro- and the macro-scale, because the micro-scale effects influence the results on the macro-scale. The fact that it was possible to reproduce all the results of the flow cell experiment with a numerical model indicates that the important aspects influencing bioclogging in porous media are covered by the model.
8.2 Expected changes for three-dimensional flow fields

Flow fields in natural porous media are usually three-dimensional and therefore bioclogging effects observed in these flow fields may differ from results of the thesis. Nearly no studies investigating bioclogging in three-dimensional flow fields are available. Therefore, it is here only possible to speculate about possible differences between bioclogging in two- and in tree-dimensional flow fields. For the pore-scale the influence of the geometry of the pore network has been discussed (Chapter 2). The hydraulic properties of pore networks mainly depend on the coordination number $Z$ (number of pores per node) (Jerauld et al. 1984; Jerauld et al. 1984), which depends on the geometry of the network. Examples for two-dimensional networks having different geometries and coordination numbers are shown in Fig.8.1. Therefore, the most important impact of increasing the dimensionality of a network is the modification of the coordination number. E.g. for the rectangular network used in this study an increase in dimensionality from two to three would increase the coordination number from 4 to 6. This increases the degrees of freedom for the water to flow through the network and it is reasonable to assume that such a network is clogged less easily. As already discussed in Chapter 2 average percolation numbers obtained for natural porous media vary at least between 2.5 and 8, and thus results presented in this thesis may already be similar to results one would get for three-dimensional flow fields in porous media with an average coordination number close to 4.

On the macro-scale the degrees of freedom for the water to flow through the network is greater in three- than in two-dimensional flow fields. Thus it can be expected that the effect of flow-bypassing may become even more important than already observed in this thesis. This would mean that if bioclogging takes place only locally, it would have even less of an effect on the hydraulic conductivity of the porous medium on a larger scale. Nevertheless, there are no indications that in three-dimensional flow fields bioclogging would produce effects, which could not be observed already in two-dimensional flow fields.
8.3 Biomass distribution and composition

From the pore network simulations it became obvious that the morphology of microbial growth and thus the distribution of biomass in the pores is important concerning the ability of the biomass to reduce the hydraulic conductivity of a porous medium. Within this thesis two different microbial growth scenarios were investigated - microbial growth in micro-colonies, having a high clogging efficiency by plugging the smallest pores preferentially, and microbial growth as a biofilm, covering the walls of each pore independent of its size. It could be shown that for the colony scenario the biomass needed to reach the highest reduction in hydraulic conductivity was two to three times smaller than for the biofilm scenario. This suggests that not knowing the correct description of biomass growth inside the pores causes a high degree of uncertainty in the hydraulic conductivity vs. porosity relation. This uncertainty seems to be much higher than the uncertainties caused by using a simplified pore network geometry. In Watnick and Kolter (2000) a biofilm is described as a complex object, which has a three-dimensional structure within itself. In addition, Dupin and McCarty (2000) showed that the morphology of microbial growth depends on the pH and thus on the environmental conditions. This suggests that growth morphologies other than those investigated in this thesis might occur in porous media. Thus, the potential impact of the growth morphology on bioclogging might be even higher than described in this thesis. Nevertheless using the results obtained from
the pore network simulations for the colony scenario could successfully explain the experimental results of previously published column experiments (Fig. 2.6) as well as results of the flow cell experiment described in this thesis. This at least suggests that during these experiments the biomass was growing preferentially in form of micro-colonies, plugging pores entirely.

Investigating biomass distribution on the macro-scale showed that the zones of microbial activity were strongly related to the presence of the growth limiting solutes, and thus on the water flow in the porous medium. As the glass bead packing of the flow cell (Chapter 4) was very homogeneous, dispersive mixing was not a dominant process. Therefore the bacterial activity was limited to a small mixing zone, where the carbon source and the oxidant were present simultaneously. This caused sharp gradients for the biomass distribution. As an interpretation of the bioclogging effects requires the knowledge of the biomass distribution, it is necessary to measure the biomass distribution with a high spatial resolution. Otherwise averaged values for biomass, or biomass concentration measured at the wrong place may lead to a misinterpretation of the observed clogging phenomena. In addition the distribution of solutes also showed a high spatial variability and required a high spatial sampling resolution. In this study the biomass distribution was observed in situ via light transmission and sampled at the end of the experiment. The sampling scheme was adapted to these visual observations and could reproduce the gradient in biomass across the flow-cell. Only due to these detailed measurements was it possible to distinguish between the validation of the different clogging models.

In addition, not only the micro-scale distribution of the biomass but also its composition affects its ability to reduce the hydraulic conductivity of a porous medium. Results from Chapter 4 demonstrated that not the bacteria but the EPS, produced by the bacteria, were reducing the hydraulic conductivity of the porous medium. It was therefore possible to confirm the findings of Vandevivere and Baveye (1992a), who observed that EPS producing strains had a higher ability to clog sand columns than non EPS producing strains. In the flow cell experiment more than 90% of the biomass was contributed by EPS, when expressed in terms of organic carbon. As EPS have a lower density than bacteria (5-130 g/l for EPS (Characklis and Marshall...
the total volume of biomass was clearly dominated by the EPS. Therefore the EPS density was a sensitive parameter for the simulation of the flow cell experiment (Chapter 5). Measurement of the in situ density of EPS requires the measurement of the in situ volume of EPS. As the sampling procedure used in this study did not preserve the volume of EPS the density of the EPS had to be estimated. The density of the bacteria had to be estimated too, but due to the small volume fraction of bacteria on total biomass, this uncertainty did not influence the simulation results. Nevertheless the bacteria were responsible for the production of EPS and the consumption of solutes. These results demonstrated that a quantification of both fractions, the bacteria and the EPS, is necessary for bioclogging studies, especially as the composition of biomass may vary from study to study. In the case of EPS production in the porous medium, the quantification of EPS, and especially its volume, would be more important for the description of bioclogging processes than the quantification of bacteria.

8.4 Possible implications of bioclogging on bioremediation

As biodegradation of contaminants in an aquifer is associated to an increased production of biomass, the question arises, whether bioclogging has any impact on bioremediation in the field. In Chapter 4, a flow cell experiment analogously to a point source contamination (Fig.1.1a) was performed. Results from this experiment and the subsequent simulations shown in Chapter 5 indicated that the bioclogging did not influence the consumption of nitrate and therefore also of glucose. This finding suggested that bioclogging may not affect degradation rates in the case of such contaminations. If one were to take the same hydraulic design, but inject the oxidant and nutrients via a point source and the organic contaminant via the basic groundwater flow, one would arrive at the usual design for the engineered bioremediation of a plume of dissolved contaminants. In this case the distribution pattern of
the carbon source and the oxidant would be the inverse of the distribution observed for the flow cell experiment. It must be assumed that in this case the influence of bioclogging on the degradation rates of the contaminants would be low, too. In particular, it was observed in the flow cell experiment that bioclogging does not lead to a better mixing rate between the contaminants and oxidants. This emphasized the importance of achieving the desired mixing via hydraulic means (e.g. injection galleries (Hunkeler et al. 1999)).

In cases where the pollutants do not reach the aquifer via a point source, an influence of bioclogging on the rates of biodegradation may be assumed. In field studies the biomass is usually measured in terms of bacterial numbers. Highest bacterial numbers measured in the flow cell experiment were approximately $1.9 \times 10^8$ bacteria/g beads. These numbers were also found in the field for contaminated aquifers (e.g. Zarda et al. (1998) measured up to $2.4 \times 10^8$ bacteria/g core material for a core taken from the saturated part of a contaminated aquifer). This indicates that bioclogging may take place in such aquifers. As the release of contaminants by dissolution from the NAPL body to the aqueous phase depends on the flow rate of water passing the NAPL body, a possible interaction between bioclogging and the NAPL dissolution may here in fact affect the rates of biodegradation and thus the success of bioremediation.

Generally the sharp gradients for the distribution of biomass but also for solute concentration, as observed in the flow cell experiment, suggest that investigating the implications of bioclogging on bioremediation also requires field measurements with a high spatial resolution. As the installation of a multilevel sampling well is more expensive than the installation of a fully screened well, contaminated sites are often equipped with fully screened wells only. It could be shown in this thesis (Chapter 6) that for such sites the dual pumping technique may serve as a method for the measurement of vertical concentration profiles of solutes, which might eventually allow bioclogging effects to be investigated.
8.5 Suggestions for further research

In this thesis bioclogging was investigated on different scales, showing that an accurate description of pore-scale processes is necessary to describe bioclogging on a larger scale. Thus, further investigations should also be done on different scales in order to further improve the understanding of the phenomenon. On the pore scale the use of pore network simulations has been shown to be a suitable framework to study bioclogging effects theoretically. As already pointed out, this study was using a simplified pore network geometry and therefore further research should investigate the influence of geometry on the derived hydraulic conductivity vs. porosity relations. Nevertheless, results from this thesis indicated that the morphology of microbial growth had the strongest impact on the results of the pore network simulations and thus future research should focus on determining the correct description for the distribution of biomass within the pores. This also requires experimental studies, to backup theoretical predictions based on pore network simulations. Artificial micromodels of pore networks as those used by Dupin and McCarty (2000) and Kim and Fogler (2000) have the advantage that their geometry is known and thus they can easily be simulated. Nevertheless future research finally has to answer how the biomass distribution is in natural porous media.

Another important problem which has to be solved is the quantification of the in situ volume of biomass. Future research should focus on this question by finding a way to measure biomass (bacteria and especially EPS) volumes or porosity reductions directly or by finding a method to determine the in situ density of biomass. For the quantification of porosity changes due to biomass growth NMR or soil-water retention curves may be used and should be investigated with regard to this.

These investigations should finally answer the question, how much biomass is needed to reduce the hydraulic conductivity down to a minimum value. Knowing this value would allow bioclogging effects to be predicted quite well. Results from this thesis indicated that the sensitivity of the simulation results to this parameter was high, whereas the type of function used to express the reduction of hydraulic conductivity had only little impact on the simulation results.
For the study of bioclogging on a macro-scale a flow cell was used in this thesis. This flow cell had the advantage that it resembled a two-dimensional flow field, which introduced already a certain complexity, but the flow field and even the biomass could be observed easily. It would therefore be interesting to use this experimental setup for further investigations. These investigations may focus on the interaction between porous media heterogeneity and bioclogging, but also on factors supporting or inhibiting bioclogging. Such factors may be the influence of chemicals or the hydraulic conditions or the porous medium itself. Focusing on the influence of bioclogging on bioremediation, it would be interesting to investigate bioclogging for different scenarios of contamination sources. As many contaminants enter the aquifer as NAPLs, the interaction between bioclogging and NAPL dissolution would be of interest. It should be pointed out that results of such investigations may strongly depend on the organisms, substrates and hydraulic conditions used. Future research must show, which influence these items have on bioclogging.

The investigation of bioclogging on the field scale requires the understanding of the important processes under laboratory conditions, first. Nevertheless, it would be interesting to study sites, where bioclogging may occur. Such studies should answer the question whether bioclogging actually takes place at a given site and for which environmental conditions bioclogging can be found in nature. Besides these basic questions, the significance of bioclogging for slow sand filters in water works, for river bank filtration and bioremediation are questions of applied research, where results of this thesis may be applied to (e.g. Chapter 7). As the evaluation of bioclogging in the field also requires porous media sampling with a high three-dimensional spatial resolution, progress in the development of field sampling methods is essential for the investigation of bioclogging, too.
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Curriculum vitae

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