

Bacteria transport under unsaturated conditions

Von der Fakultät für Mathematik, Informatik und Naturwissenschaften der
Rheinisch-Westfälischen Technischen Hochschule Aachen zur Erlangung des
akademischen Grades eines Doktors der Naturwissenschaften genehmigte
Dissertation

vorgelegt von

Dottoressa in Chimica Industriale Grazia Gargiulo
aus Castellammare di Stabia (Napoli) Italia

Berichter: Universitätsprofessor Dr. Andreas Schäffer
Universitätsprofessor Dr. Harry Vereecken
Tag der mündlichen Prüfung: 19. Oktober 2006

Diese Dissertation ist auf den Internetseiten der Hochschulbibliothek online verfügbar.

Abstract

The aim of this work was to study the bacteria transport behaviour in different conditions using an unsaturated porous media. A column based system able to keep the unsaturated conditions was designed and developed to perform the experiments. Two bacteria strains *Deinococcus radiodurans* and *Rhodococcus rhodochrous* strongly different in hydrophobicity were employed. During the experiments the bacteria concentration in the outflow was continuously on-line measured and after the experiment the column has been dismantled to determine the retention profile. The observed data were fitted using two different models (*Tufenkji et al. 2003, 2004, 2005* and *Bradford et al. 2003*) and the resulting coefficients were used to elucidate the transport mechanisms.

Following aspects concerning the bacteria transport behaviour were investigated: (i) the influence of matrix saturation; (ii) the role of the bacteria surface characteristic; (iii) the effect of matrix grain size; (iv) the transport behaviour of metabolically active bacteria; for the first time, fresh bacteria cells supplied with nutrients during the experiments were used to be more close to the real situation experienced in the soil. (v) the role of bacteria surface the protein; bacteria were treated with the enzyme α -Chymotrypsin to remove the surface protein.

It was found that bacteria transport through variably saturated porous media was directly related to water content. The trend observed for both strains was that decreasing water content inside the porous media led to a decreasing cells effluent concentration and an increasing amount of retained bacteria. This effect was more pronounced for hydrophobic bacteria. Concerning the retention profile it was established that the bacteria location inside the packing did not follow a disposition predictable with the classical filtration theory. The most of the bacteria amount was found in the first sand centimetres below the inlet and a monotonical decrease of the bacteria amount with the depth was observed. This effect was directly related to the packing water content and the bacteria hydrophobicity: decreasing the water content a higher bacteria amount was found close to the column inlet and this effect was more pronounced in the case of more hydrophobic bacteria. According to the “straining” model, at fully saturation the more hydrophobic strain showed a higher adhesion rate compared with the hydrophilic one. The results highlight that for the hydrophilic strain the adhesion to the air-water-solid interface was the main removal mechanism. In contrast, the main removal mechanism for the hydrophobic strain was the straining: due to their aggregation behaviour the cells were filtered out from the solution bulk. The coefficients resulting from the fitting using the dual deposition mode model

showed that the fraction of bacteria with a fast adhesion increased decreasing the saturation. The hydrophobic strain showed always the higher adhesion rate and for both strains the adhesion rate increased with decreasing saturation. The packing grain size was an important factor for the bacteria transport: different pore size led to a different interaction between the bacteria and the grain surface. In the case of fine sand (330 μm) a strong filter-out effect in the first centimetres after the inlet was observed causing a strongly reduced bacteria transport. For the coarse sand (607 μm) the interaction bacteria/sand surface was reduced and nearly all the cells were able to pass the packing. The fitted parameter calculated with the “straining” model showed that both the adhesion rate and the straining rate increased when the grain size decreased. Applying the dual deposition mode model, two different approaches were used to fit the experimental data concerning the fine sand. In the first case two *discrete* rate coefficients were used and both fitting parameters increased with decreasing sand grain size. In the second case only one attachment rate was supposed to be the important for the bacteria adhesion through the porous system and this approach could better describe the experimental data. Metabolically active bacteria during the transport showed a different behaviour compared with “resting cell” bacteria. Cells in the *log* phase were retained in the column more than in the *stationary* phase and a continuous release after the breakthrough curve was observed in the outflow. During the transport bacteria in active phase did not show blocking evidences. The growing cells showed an increasing hydrophobicity during the *log* phase. This effect was attributed in particular to the changes in amount and type of the proteins present on the bacteria surface. Chemically treated bacteria without protein showed less adhesion to the sand surface and the transport was enhanced by this treatment. According to the “straining” model the bacteria enzymatically treated showed a decrease in the adhesion rate while the straining rate was not effected. Using the dual deposition mode model both the adhesion rates decreased in the case of the treated bacteria. Further experiment should be performed under these conditions with other strains to better understand the effect of proteins and eventually protein associated macromolecules on the bacteria adhesion to the surface.

Table of contents

Introduction	1
1 Processes governing bacteria transport	5
1.1 Bacteria transport under unsaturated conditions	5
1.2 Bacteria adhesion	6
1.2.1 Bacteria growth phase and hydrophobicity	7
1.2.2 Role of the surface protein in the bacteria transport	7
1.2.3 Effect of different grain size on the bacteria transport	8
2 Theory of bacteria transport and deposition	11
2.1 Colloid-filtration theory.....	11
2.2 Modelling transport of colloids with straining	13
2.3 Fitting experimental data using straining	15
2.4 DLVO theory for colloids.....	17
2.5 Modelling bacteria and colloids deposition using Dual Deposition Mode Model	19
2.6 Fitting experimental data using Dual Deposition Mode Model	22
3 Materials and methods.....	25
3.1 Solid grain materials.....	25
3.2 Set-up description of column technique	26
3.3 Measured quantities in column experiments	31
3.4 Experimental conditions	32
3.5 Bacteria determination in the porous media	33
3.6 Bacteria used.....	34
3.6.1 <i>Deinococcus radiodurans</i> (DSMZ 20539)	34
3.6.2 <i>Rhodococcus rhodochrous</i> (DSMZ 11097).....	34
3.6.3 Column experiments.....	35
3.7 Enzymatic treatment of <i>Rhodococcus rhodochrous</i>	35
3.8 Bacteria characterization	36
3.8.1 Hydrophobicity measurements	36
3.8.2 Bacteria counting and size determination.....	37
3.8.3 Electrophoresis measurement	37
3.8.4 Fourier Transform infrared (FT-IR) spectroscopy ATR technique	38
3.8.5 Fluorescence microscopy technique	38

4 Results and discussion	41
4.1 Bacteria characterization	42
4.2 The effect of moisture content on the transport behaviour of <i>Deinococcus radiodurans</i> and <i>Rhodococcus rhodochrous</i>	43
4.2.1 A conceptual model for colloid transport considering reversible attachment, straining and Langmuirian blocking	47
4.2.2 Dual deposition mode model	53
4.3 The effect of grain size on the transport of <i>Rhodococcus rhodochrous</i> under unsaturated condition	56
4.3.1 Modelling of breakthrough curve and retention profile for <i>Rhodococcus rhodochrous</i> at different mean grain size	59
4.4 Transport behaviour of metabolically active <i>Deinococcus radiodurans</i> under unsaturated condition: role of the growth phase	63
4.4.1 <i>Deinococcus radiodurans</i> growth curve measurements	64
4.4.2 Hydrophobicity measurements during bacteria growth using M.A.T.H test	65
4.4.3 <i>Deinococcus radiodurans</i> transport under unsaturated conditions: the influence of growth phase	66
4.4.4 Fitting experimental data using straining	69
4.5 Bacteria transport under unsaturated conditions: the role of surface protein	72
4.5.1 Characterization of <i>Rhodococcus rhodochrous</i> treated with α -Chymotrypsin	73
4.5.2 Transport experiment of <i>Rhodococcus rhodochrous</i> enzymatic treated cells	75
4.5.3. Modelling of breakthrough curve and retention profile for <i>Rhodococcus rhodochrous</i> treated with α -Chymotrypsin	77
4.5.4 Dual deposition mode model	78
5 Summary and conclusion	81
List of references	85
Appendix I	91
Appendix II	99
Acknowledgement	101
Curriculum Vitae	103

Introduction

The vadose zone comprises the subsurface environment, situated between the land surface and the saturated subsurface zone (groundwater). The vadose zone is the first subsurface environment encountered by contaminants released through human activities. Groundwater will be vulnerable to even more widespread contamination in the future as population and industrial impacts continue to increase. One of the major objectives of vadose zone research is to understand and to predict the fate of contaminants and to identify creative and cost-effective options for remediation before the groundwater becomes contaminated. Groundwater provides half of the world's population with drinking water. Its sustainable use and protection is therefore of great concern, but the vadose is one of the least understood hydrogeological zones due to its inherent complexity of processes and the multi-scale character of its properties.

The microbial contamination of groundwater is a serious problem that can result in significant outbreaks of waterborne disease [24,25, 29 and 37]. Sludge and water sewage contain many pathogenic organisms with bacteria and viruses being the most prevalent [39,37]. The present trend of disposing of wastewater through infiltration in the soil is opening up a new dimensions in the range of traditional environmental problems. The recharge of aquifers by infiltration of sewage effluents has been practiced in numerous locations [7, 25]. Residential septic tanks and cesspools are another source of potential contamination. Many are not lined to prevent wastewater from leaking into the soil and, because all earth materials are permeable to some degree, wastewater leaks into the subsurface and may reach the groundwater. Many bacteria can beneficially serve to degrade or immobilize environmental contaminants [7, 29, and 20]. One of the methods to remediate subsurface soils polluted with recalcitrant chemicals is to inoculate the site with specific bacteria, which are able to degrade those compounds. One of the main problems is the distribution of the inoculated bacteria through the soil profile and ensuring adequate contact between specialized bacteria and the target compound. Although bioremediation holds great promise for dealing with intractable environmental problems, it is important to recognize that much of this promise has yet to be realized. Specifically, much needs to be learned about how microorganisms interact with different hydrologic environments. As this understanding increases, the efficiency and applicability of bioremediation will rapidly grow.

The assessment of radionuclide transport in deep geological systems has become an important subject in the risk evaluation of radioactive waste repositories. It has recently been discovered that the temporal and the spatial distribution of radionuclides were significantly affected by microbial activity and transport. In particular, microbes can adsorb contaminants and maintain them in the mobile phase during their movement through the subsurface transport.

The transport and mobility of bacteria in the vadose zone is presently poorly understood due to the complex interplay of chemical, biological and physical factors. Especially the role of unsaturated flow conditions on mobility and transport in porous media is presently not well understood [2,5]. To better understand the bacteria movement in the vadose zone, experiments using a model unsaturated system have been performed to study the transport behaviour of two bacteria strains (*Rhodococcus rhodochrous* and *Deinococcus radiodurans*). The aims of this work regarding the transportation and deposition of micro-organisms in unsaturated porous media include:

- How different air/water ratios in the porous media affect the transport of bacteria and which are the mechanisms for bacteria adhesion and retention in the porous unsaturated media;
- How the different matrix mean grain sizes influence bacteria transport and which physical-chemical mechanisms occur during the transport process with different pore sizes. To perform these experiments different grain size distributions were utilized for the porous media;
- The importance of the single strain characteristics for transportation, in particular surface hydrophobicity. To investigate this aspect, two bacteria strains, similar in shape and size but extremely different concerning hydrophobicity were employed;
- The role of the growth phase and in particular the difference in the transportation behaviour between metabolically active bacteria compared to *resting cells*. Since bacteria in the real environmental come in contact with nutrients and become metabolically active these experiments are closer to the natural environmental conditions where bacteria cells are able to reproduce, to divide and to change their surface characteristics during the transportation process;
- Protein present on the bacteria surface can influence the adhesion and, on the macroscopic scale, both the transportation and the retention profile.

For all the experiments considered, the spatial distribution of the bacteria in the porous media after the breakthrough was measured. A further aim of this work was to give a theoretical description to the observed data using mathematical models. The experimental data was fitted using two recently developed modelling approaches [41-48] and the resulting coefficients were discussed taking microbiological aspects into consideration.

The challenge for the scientific community is to increase their understanding of these processes in order to predict bacterial distribution in the vadose zone using mathematical models [1-4]. This knowledge will be important in formulating adequate responses to protect water supplies from anthropogenic pollution stemming from civil and industrial activities and also to improve the risk management processes when promoting the use of bioremediation processes.

Chapter 1 Processes governing bacteria transport

1.1 Bacteria transport under unsaturated conditions

Bacteria are typically considered to be colloids. Colloids by definition are particles that range from 1 nm to 10 μ m. Included in this range are: mineral fines, organic macromolecules, bacteria and viruses [2]. Colloid transport experiments in partly saturated porous media showed that effluent concentration diminishes with decreasing moisture content [1, 2, and 4]. The role of the air-water interface on colloidal sorption and transport was studied showing an increase in the retention of both hydrophilic and hydrophobic colloids with air content increasing in the porous medium [5, 6, and 11]. Hydrophobic colloids compared with hydrophilic ones are retained more in the porous media under otherwise similar conditions [12, 26]. The transport of silica colloids and decreasing outflow concentration has been investigated [38] with decreasing water saturation has been attributed to the irreversibility of colloid trapping at the air-water interface. The authors suggested film straining is a dominant mechanism. Concerning virus transport under unsaturated conditions it has been stated [39] that virus and grain surface characteristics were more important than matrix water content. Previous bacteria transport studies conducted under unsaturated conditions have indicated the importance of soil water content. *Powelson et al. (1996)* reported that the recovery of microbes in the outflow was lower under unsaturated conditions and that sorption appeared to increase with bio-colloid hydrophobicity [5]. *Schäfer et al. (1998)*, *Jewett et al. (1998)* assumed that the reason for the increasing retention of bacteria in porous media under unsaturated conditions is the accumulation at air-water interfaces due to long range hydrophobic interactions [6,10]. In particular, *Schäfer et al. (1998)* proposed as possible explanations for a reduced bacteria transport the immobile water portions or *thin water films* formed that cannot be accessed by the bacteria [6]. Additionally, previous research under unsaturated conditions has provided very little data on the correlation of bacteria retention with depth [10].

1.2 Bacteria adhesion to solid surfaces

Many literature studies deal with the identification of mechanisms determining the adhesion of bacteria to solid surfaces [13-16]. *Van Loosdrecht et al. (1987a)* found no clear correlation between the electrophoretic mobility of bacteria and adhesion to solid surfaces [12]. In successive works [13, 14, 15] it was recognized that hydrophobic cells adhere to solid surface to a greater extent than hydrophilic cells. *Van Loosdrecht et al. (1989b)* assumed that the initial stage of bacteria adhesion could be described using DLVO theory [14,55]. The adhesion process can be considered as a combination of hydrophobicity and electrostatic interactions [55]. Surface hydrophobicity was recognized as the dominant characteristic, however it was reported that for more hydrophilic cell surfaces the electrokinetic potential becomes more influential [36]. *Rijnaarts et al. (1999)* evaluated different types of interactions in the adhesion including *Van der Waals* attractions, electrostatic repulsion and steric interactions between the outer cell surface macromolecules and the substrata [14].

The air-water interface plays an important role in both colloid and bio colloid adhesion. Experimental studies [1, 2 and 5] reported that bacteria were preferentially adhering to the gas-water interface rather than the solid-water interface. In a more recent study [26] the effect of cell hydrophobicity on adhesion has been described as being less a function of the cell surface interaction, but rather a function of the bacteria cell expulsion from the bulk of water due to the strong tension at the cell-water interface.

Crist and his collaborators [8, 9] questioned previous interpretations based on their pore scale visualization in three-dimensional porous media. They suggested that colloids were not retained at air-water interface but rather near the air-water-solid interface located at the menisci pendular ring. Visualization using a light transmission technique showed that for negatively charged hydrophilic carboxylated latex microspheres, trapping occurred in the thin film of water where the air-water interface and the solid interface meet. The greater retention of the hydrophobic colloids was attributed to a *filtering out* effect rather than adhesion to the air-water interface. The strong attractive hydrophobic interaction forces between colloids leads to the formation of aggregates that can be easily trapped in the narrow passages between grains.

Chen et al. (2005) suggested that colloids were mostly likely to be retained by attachment to the sediment caused by repulsive interaction exerted by the liquid-gas interface [58]. It was stated

that when the water film is comparable in thickness to colloid diameter, repulsive interactions between colloids and the liquid-gas interface aided colloids in overcoming the repulsive electrostatic interaction barrier with the sediments and allowed the colloids to come closer to the sediment surface.

1.2.1 Bacterial growth phase and hydrophobicity

The change in hydrophobicity during growth has been observed in other bacteriological studies: It was reported [17,50] that hydrophobicity for four different bacteria strains increases during growth. The cell's hydrophobicity is directly related to adhesion to solid surfaces [29, 30]. Experimental studies [23, 17] showed that bacteria cells tend to adhere more to solid surfaces at the maximum growth rate than *stationary phase* cultures. *Smets et al. (1999)* presented experimental results indicating that the adhesion of a *Pseudomonas* to glass was significantly more favourable in the exponential growth phase than in the stationary or decay phase [21]. However an adhesion decrease has been reported [59] during the starving phase. In this recent work the influence of the growth phase on the initial stage of bacteria adhesion was investigated and it was found that the *stationary phase* cells for a mutant *Escherichia coli k12* were more adhesive than *mid-exponential phase* cells. Less hydrophobicity was measured in the *mid-exponential* than in the *stationary-* phase cells.

1.2.2 The role of the surface protein on bacteria transport

The change in the surface hydrophobicity and the resulting change in the bacteria adhesion behaviour to solid surfaces can be attributed to the variation in surface molecular composition during the different bacteria life phases [16, 28, and 32]. The nature of cell surface coating determines the complexity of the *steric interactions* between bacteria and surfaces, which may reduce the rate of deposition, but at the same time, can cause irreversible attachment [33, 34 and 35]. *Norde et al. (1989)* found that the interactions between the outer bacteria protein compound and the solid surface play a dominant role in the microbial adsorption process [16].

Surface layers (S-layers) are the outer component of the cell wall of bacteria and consist primarily of proteins. A close relationship between bacteria surface hydrophobicity and protein

content has been established in several studies [23, 50, and 53]. How bacteria cell surface hydrophobicity is caused by nitrogen-rich groups, most notably protein has been reported [17, 22 and 23]. *Dignac et al. (1998)* reported that protein and amino acids are hydrophobic components of the EPS and *Sanin et al. (2003)* showed that the increase of protein and amino acids in EPS causes an increase in surface hydrophobicity [35,17]. Consequently, the presence of protein on the surface is necessary for bacteria adhesion [32,33]. The decreased adhesion during the bacteria starvation was explained by *Castellanos et al. (2000)* who concluded that starvation changed the concentration of several cell surface proteins, but there was no induction leading to the synthesis of new ones [23]. *Caccavo et al. (1999)* reported adhesion experiments where the bacteria were enzymatic and chemically treated to damage both the protein and the lipo-polysaccharides on the bacteria surface [34]. In most cases the treatment to damage the protein led to a decrease in adhesion in bacteria. However, *Walker et al. (2005)*, found a decrease in the adhesion and hydrophobicity of *E.coli* during the *mid-exponential* compared with the *stationary*-phase [59]. The effect was justified with the assumption that on the outer membrane of *E.coli* hydrophilic (acidic) proteins are present that decrease with the culture age leading to a decrease in hydrophobicity and consequently in adhesion. Since contrasting results are reported, further investigations are necessary to define the role of the surface protein for both bacteria hydrophobicity and adhesion. No studies have been reported about the influence of the surface proteins on bacteria transportation behaviour.

1.2.3 Effect of varying grain size distribution on bacteria transportation

The mean grain size of the porous medium is an important factor in determining the transportation of bacteria [18, 19 and 20]. Grain size distribution and pore size can affect the mechanisms of the bacteria translocation such as straining [47,48 and 62]. Straining is the trapping of colloid particles in down gradient pore throats that are too small to allow particle passage.

Fontes et al. (1991) studied the role of grain size (0.013-0.2 mm) on bacteria transportation under saturated conditions [18]. In their experiments they claimed that grain size was the most important factor controlling the transportation of bacteria in comparison to ionic strength, bacteria cell size and the presence of heterogeneities within the porous media. *Sharma et al.*

(1994) observed that the small pore throats restrict microbial activity and penetration using 5 different mean sizes of acid-washed glass beads (116, 192, 281, 398 and 767 μm)[19]. However in a successive work [40] it was concluded that the effect of grain size on maximum bacteria retention capacity under saturated conditions was not significant, two grain sand distributions were used respectively 0.42-0.50 mm and 0.707-0.850 mm. *Bradford et al. (2003)*, reported that for colloid transportation under saturated conditions, the effluent colloidal concentration curves and the final retention profile is highly dependent on the mean soil grain size (respectively $d_p = 0.45, 1.00, 2.00$ and $3.20 \mu\text{m}$) [47]. These observations were attributed to the increased straining of the colloids. Under unsaturated conditions colloid transportation is more efficient in a porous medium with larger size grains (425-500 μm) than that with smaller grains (150-212 μm) due to differences in water film thickness [4,10]. However data about bacteria transport dependency on grain size distribution under unsaturated conditions is not present in the literature.

Chapter 2 Theory of bacteria transport and deposition

Physicochemical filtration has been recognized as the dominant mechanism responsible for the removal of microbial particles from the pore fluid. Because bacteria are colloidal in size, colloid-filtration theory [60,61] is the most commonly used model for describing bacteria filtration.

2.1 Colloid-filtration theory

According to the colloid filtration theory the concentration of suspended and deposited particles can be modelled using a one-dimension *advection–dispersion* equation with first-order deposition [60,61]:

$$\frac{\partial C}{\partial t} + \frac{\rho_b}{\theta} \frac{\partial S}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} \quad (1)$$

$$\frac{\rho_b}{\varepsilon} \frac{\partial S}{\partial t} = kC \quad (2)$$

Where θ is the volumetric water content, ρ_b soil bulk density [g/ ml], t the time [min], v the flow rate [ml/ cm], x is the spatial coordinate [cm], D is the dispersion coefficient [cm^2/min], C is the bacteria concentration in the aqueous phase [$\text{N}_c \text{L}^{-3}$], S is the solid phase concentration [$\text{N}_c \text{M}^{-1}$], ε the bed porosity.

The particle deposition rate coefficient, k , is related to the commonly used single collector efficiency η :

$$k = \frac{3(1 - \theta)v}{2d_c} \eta \quad (3)$$

Where θ is the volumetric water content, v pore water velocity, d_c is the mean diameter of the collector grain. Note that the single collector efficiency (determined experimentally) is

commonly expressed as $\eta = \alpha \eta_0$, with α being the collision efficiency and η_0 the single collector efficiency.

In the original derivation of colloid-filtration theory [60,61] a filtration equation using a mass balance has been developed based on particle removal by an isolated sphere, assuming that a packed bed is an assemblage of isolated spheres. In the classic *clean bed filtration theory* (CFT) it is typically assumed that the rate of particle deposition is both spatially and temporally invariant, the removal of suspended particles is represented by first-order kinetics resulting in a concentration of suspended and retained particles that declines exponentially with distance [60,6].

It has been shown that deposition rates often change with time, indicating that the presence of previously deposited particles can indeed affect deposition rates. Although previously deposited particles have been observed in a few cases to increase deposition rates, a process referred to as filter ripening, more common is the observation that attached particles reduce deposition rates. To consider this observed behaviour, the colloid-filtration model has been modified to account for decreasing deposition rates due to previously attached particles. A *Langmuir-type* model is used in which deposition rates are modelled as decreasing linearly with the concentration of deposited particles.

$$B(\theta) = \frac{\theta_{\max} - \theta}{\theta_{\max}} \quad (4)$$

Where $B(\theta)$ is the blocking function, $\theta_{\max}$ is the maximum attainable fractional surface coverage.

Despite the massive use of this model, several studies [41-48 and 62] have recently revealed that the observed colloid retention profiles are not always in agreement with this theory. Experimental data on the transportation of abiotic colloids and bacteria have shown *monotonical non-exponential* retention profiles. To overcome this incongruence *Bradford et al. (2003)* and *Tufenkji et al. (2003, 2004, and 2005)* have been developing two independent models, the subject of a sparkling discussion in the latest literature publications [41-48].

In accordance with *Bradford et al. (2003)* some of the discrepancies between colloid transport data and CFT may be due to the fact that the CFT does not account for physical *straining*.

Tufenkji et al. (2003, 2004, and 2005) postulated in their theoretical work that the deviation from the classical colloid theory could be caused by the existence of a dual, instead of a unique, value in the particle deposition rate [41-48]. The existence of both *favourable* and *unfavourable* colloid –chemical interactions was considered [41-46, 37 and 38].

2.2 Modelling the transportation of colloids with straining

Hydrus-1d code [63] is a finite element model for simulating the one-dimensional movement of water, heat, and multiple solutes in variably saturated media. The code numerically solves the *Richards'* equation for saturated-unsaturated water flow and *Fickian*-based advection-dispersion equations for the solute transport.

The solute transport equations consider advective-dispersive transport in the liquid phase. The transport equations also include provisions for non-linear and/or nonequilibrium reactions between the solid and liquid phases. The code may be used to analyze water and solute movement in unsaturated, partially saturated, or fully saturated porous media. The governing flow and transport equations are numerically solved using the *Galerkin* type of linear finite element schemes. Integration in time is achieved using an implicit (backwards) finite difference scheme. The code allows us to fit the breakthrough curve and the retention profile simultaneously using a non –linear least square optimization routine based upon the *Levenberg-Marquardt* algorithm.

Bradford et al. (2003) modified Hydrus-1d to account for colloid attachment, detachment, blocking, straining and exclusion. In this conceptual model the colloid attachment and detachment is modelled using first-order rate expressions, whereas straining is described using an irreversible first-order term that is depth dependent [47].

In this dissertation these modifications will be extended to examine microbial transport in unsaturated porous media. For simplicity the equations presented below are for a single type of microorganism. Separate simulations with different model parameters are needed for each microorganism.

In this study bacteria transport was modelled using a modified form of the convection-dispersion equation, in which two adsorption sites s_1 and s_2 , respectively were considered. The mass balance equation is defined as:

$$\frac{\partial \theta c}{\partial t} + \rho_b \frac{\partial s_1}{\partial t} + \rho_b \frac{\partial s_2}{\partial t} = \frac{\partial}{\partial x} \left(\theta D \frac{\partial c}{\partial x} \right) - \frac{\partial qc}{\partial x} \quad (5)$$

Where θ is the volumetric water content, ρ_b soil bulk density [m^3/L^3], t the time [T], q the flow rate [L/T], x is the spatial coordinate [L], D is the dispersion coefficient [cm^2/min], c is the bacteria concentration in the aqueous phase [$\text{N}_c \text{L}^{-3}$], $s = (s_1 + s_2)$ is the solid phase concentration [$\text{N}_c \text{M}^{-1}$], N_c is the number of bacteria. Mass transfer between the aqueous and both solid phases can be described as:

$$\rho \frac{\partial s}{\partial t} = \theta k_a \psi c - k_d \rho_b s \quad (6)$$

Where k_a is the first-order deposition (attachment) coefficient [T^{-1}], k_d is the first-order detachment coefficient [T^{-1}] and ψ is a dimensionless colloid retention function. To simulate reductions in the attachment coefficient due to the filling of favourable sites, ψ is sometimes assumed to decrease with increasing colloid mass retention. A Langmuirian dynamics (Adamczyk *et al*, 1994) equation has been proposed for ψ to describe this blocking phenomenon [64]:

$$\psi_1 = \frac{s_{\max} - s}{s_{\max}} \quad (7)$$

In which $s_{\max}$ is the maximum solid phase concentration [$\text{N}_c \text{M}^{-1}$].

Bradford *et al*. (2003) hypothesized that the influence of straining and attachment processes on colloid retention can be separated into two distinct components [47,48]. They suggested the following depth-dependent blocking coefficient for straining process:

$$\psi_2 = \left(\frac{d_c + x - x_0}{d_c} \right)^{-\beta} \quad (8)$$

Where d_c is the mean diameter of the sand grains [L], x_0 is the coordinate of the location where the straining process starts in this case the surface of the soil profile, and β is an empirical factor controlling the shape spatial distribution. The modified code allowed us to make a linear combination between the two equations to describe at the same time both the effect due to the straining (ψ_2) and to the Langmuirian dynamic (ψ_1). The model described above using equation (1) can be used in many different ways. In our case we used the two adsorption sites s_1 and s_2 to describe respectively attachment/detachment and irreversible straining.

2.3 Fitting experimental data using straining

In this work experiments have been performed on the effect of: (i) the porous media saturation, (ii) matrix grain size, (iii) the bacteria growth phase and (iv) surface protein, on the transport behaviour of two different bacteria strains.

(i) Transport experiments were performed with three different saturations and with two different bacteria strains that differ in hydrophobicity. To fit the experimental data the advection-dispersion equation was as formulated in equation 5. Two different adsorption sites s_1 and s_2 , respectively, were used (equation 6) one reversible site for attachment / detachment and one irreversible site for straining. The dimensionless colloid retention function (ψ) was used as linear combination between the equation 7 and 8.

(ii) To inversely determine the process parameters for the experiments with four different sand grain sizes, the advection-dispersion equation was used as formulated in equation 5. The dimensionless colloid retention function (ψ) was used without the Langmuirian blocking coefficient in equation 7, but only the coefficient for straining equation 8 was considered. Two different adsorption sites s_1 and s_2 , respectively, were used: one reversible site for attachment / detachment and one irreversible site for straining.

(iii) To fit the experimental data about bacteria in growing condition in equation 5, two degradation terms as growing coefficients were employed: μ_w , μ_s .

$$\frac{\partial \theta c}{\partial t} + \rho_b \frac{\partial s_1}{\partial t} + \rho_b \frac{\partial s_2}{\partial t} = \frac{\partial}{\partial x} \left(\theta D \frac{\partial c}{\partial x} \right) - \frac{\partial qc}{\partial x} \mu_w \theta c - \mu_s \rho_b (s_1 + s_2) \quad (9)$$

Normally μ_w , μ_s represent the degradation term for the substance transported in the solution and adsorbed to the matrix, respectively. This degradation term μ has been used as negative parameter in our case ($\mu < 0$) in order to emulate the growth instead of the degradation. The growth of bacteria cells were considered both in the solution and attached to the sand grains. No Langmuirian blocking coefficient was considered in the dimensionless colloid retention function (ψ). This assumption in the model was based on microbiological considerations. It is well-known that the bacteria are capable of forming a biofilm. According with the '*quorum sensing*' theory, bacteria secrete and sense small signalling molecules for communication and to promote biofilm formation when they are in a favourable growing condition. The capability to form a biofilm is important for a bacteria strain to secure species survival. In this optic the cells should not tend to reject each other and the deposition of one layer of cells should not inhibit the next layer deposition. The cells try to get as close together as possible in order to form a resistant structure.

In order to emulate this effect in the dimensionless colloid retention function (ψ) the Langmuirian dynamics equation 7, introduced to emulate the blocking phenomenon, was omitted and only a depth dependent blocking coefficient for straining equation 8 was used. A different approach was tested and a *ripening* effect was introduced in the dimensionless colloid retention function (ψ) to test if this theoretical assumption could correctly describe both the breakthrough curve and the retention profile. *Ripening* was described using a functional form of ψ that increases with an increasing mass of retained colloids:

$$\psi = \max(1, S^{\max}) \quad (10)$$

$S_{\max}$ is the maximum solid phase concentration [$N_c M^{-1}$]. However, this approach failed to describe the observed data.

In the successfully used approach two adsorption sites were used, one for attachment/detachment and the other for straining. Both were considered to be reversible. The concept of *reversible straining* was introduced and the $K_{str det}$ rate was considered in the mass transfer equation 9. In fact we supposed that bacteria should be able to release themselves from

the death end pore due to multiplication and the more dynamic situation. While the mother cell had no possibility to be released after having been trapped in the death end pores, the same situation was not experienced by the daughter cells that can be remobilized again. This assumption could produce a satisfactory data match.

(iv) To understand how protein absence from the bacteria surface could influence the fitted coefficients and what in microbiological terms that means, an approach similar to (i) was used to fit the experimental data. The advection-dispersion equation was used as formulated in equation 5. Two different adsorption sites s_1 and s_2 , respectively, were used in equation 6 with one reversible site for attachment / detachment and one irreversible site for straining. The dimensionless colloid retention function (ψ) was used as linear combination between the equations 7 and 8.

2.4 DLVO theory for colloids and bacteria

Colloidal interactions formulated on the basis of the DLVO (*Derjaguin-Landau-Verwey-Overbeek*) theory are frequently invoked to investigate a vast array of natural and engineered phenomena, such as particle aggregation, heterocoagulation, colloid deposition, and a host of processes involving colloidal stability and transport [55]. The thermodynamics leading to colloid-surface interactions are traditionally described using the classical DLVO theory [14], which characterizes total interaction energy as the sum of the electrostatic double layer (EL) and Lifshitz-van der Waals (LW) interaction energies and their decay length with separation distance (figure 2.1). The Van der Waals interaction energy per unit area between two infinite flat plates separated by a distance h is given by:

$$E(h) = -\frac{A_h}{12\pi h^2} \quad (11)$$

Where the Hamaker constant A_h , is the most important parameter determining the LW interaction which is a material property. Calculation of electrostatic interactions (EL) requires knowledge of the electrostatic surface potential of the interacting surfaces.

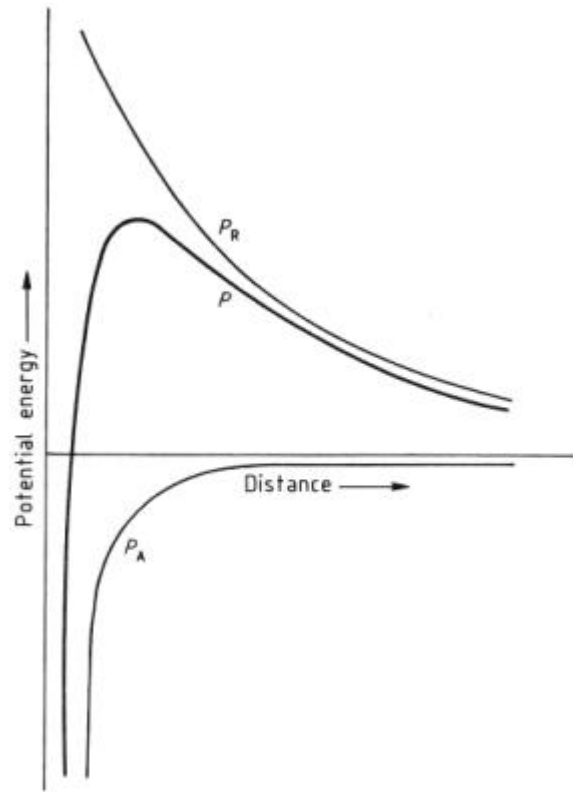


Figure 2.1. DLVO interaction energy between two negatively charged particles. P_R is the repulsive electrostatic interaction and P_A is the attractive interaction. P is the resulting net interaction.

Microbial surface thermodynamics is theoretically based on colloid surface thermodynamics using the classical theory of colloidal stability [58]. An extended DLVO theory, the so-called XDLVO also included Lewis-acid base or hydration interaction not considered in the classical theory. The value of the Hamaker constant for microbial cell surfaces is currently being estimated from contact angle measurements. The electrostatic surface potential of microbial cell surfaces cannot be measured directly but is commonly estimated from measured electrophoretic or electro-osmotic velocities observed during dynamic measurements in electric fields. Despite the above facts, it appeared that the DLVO framework could not be used to provide an accurate description of cell adhesive behaviour. Application of DLVO theory to explain bacterial adhesion to solid substrata has only been successful for a limited number of strains and species [58]. The Van der Waals interactions energy is always attractive and therefore, when they exceed repulsive electric double layer interactions energy between bacteria and charged surfaces, bacteria can adhere better than theoretically expected. Furthermore, it was observed that experimental particle deposition rates were many orders of magnitude greater than

theoretical predictions based on the DLVO theory as reported by *Tufenkji et al. (2004)* (figure 2.2) [42].

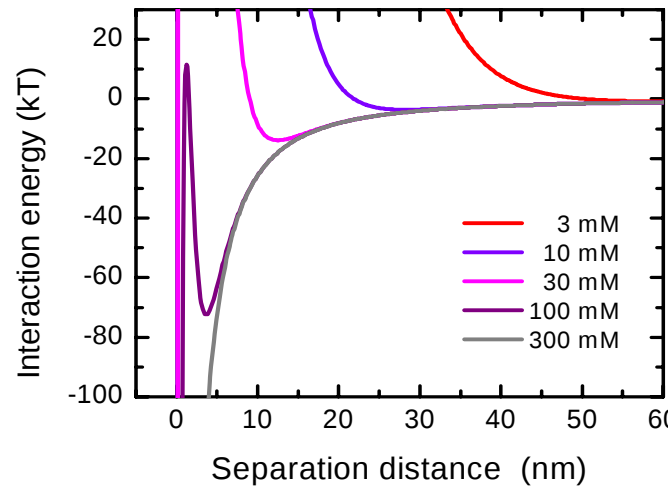


Figure 2.2. DLVO interaction energy calculated for glass beads at different ionic strengths.(from ref *Tufenkji et al 2004*)[45].

In general, the applicability of the DLVO theory to explain bacterial adhesion frequently decreases as the complexity of the cell surface of the organisms under consideration increases. A major problem is that cells cannot be considered as smooth structures when they are separated by a distance less than 10 or 20 nm, corresponding to the primary and even secondary minimum. Indeed, intrinsic membrane proteins may in principle raise several tens of nanometres above the lipid bi-layer [16, 28].

2.5 Modelling bacteria and colloid deposition using the Dual Deposition Mode Model

A theoretical model has been proposed by *Tufenkji and Elimelech (2004)* to highlight the motivations of the deviations from the CFT which have been widely observed in colloid deposition studies [42]. Theoretical predictions have been compared with experimental data on the transport of polystyrene latex colloids in different physicochemical conditions and protozoa (*Cryptosporidium oocysts*) under saturated conditions.

The transport of colloidal particles, including bacteria has been described by accounting for particle advection, hydrodynamic dispersion and filtration. Due to the relatively low particle

concentration and moderate ionic strength, *blocking* and *ripening* are not considered to be important and particle release is negligible. Under these conditions the concentration of suspended and deposited particles at depth x and time t can be modelled by a one dimension advection-dispersion equation with a term for first-order particle deposition kinetics (chapter 2.1 equation 1, 2 and 3). According to this model the discrepancy between the CFT and the observed data may result from the fact that not only a *single* but a *dual* deposition rate is present. These dual rates are both *normally distributed* around two discrete values (figure 2.4). This dual distribution may be caused by variability in the microbial particle-collector grain interaction. To explain this finding, heterogeneity in particle population has been considered. In particular it has been proposed that a fraction of the particle population exhibits a *slow* and another fraction a *fast* deposition rate.

This *fast* and *slow* adhesion rate concept can be explained in term of DLVO interaction. In the presence of repulsive DLVO interaction (figure 2.2), particles with sufficient energy can overcome the repulsive energy barrier and reach the primary energy minimum (figure 2.3b). This bacteria population will show a *slow* deposition rate. The other fraction shows a *fast* deposition rate due to the presence of a deep secondary energy minimum (figure 2.3a). Furthermore, *Tufenkji et al. (2004)* highlighted how the surface charge heterogeneity plays an important role in the kinetics of particles depositions and suggested that deposition occurs preferentially on energetically *favourable* sites [46].

This can explain the difference in magnitude observed between the DLVO prediction and the experimental particle deposition rate. The presence of *favourable* sites due to charge heterogeneity results in a deposition rate, which is higher than predicted, based on the average collector surface potential. The concurrent existence of *favourable* and *unfavourable* colloid-chemical interactions may cause a significant deviation from CFT.

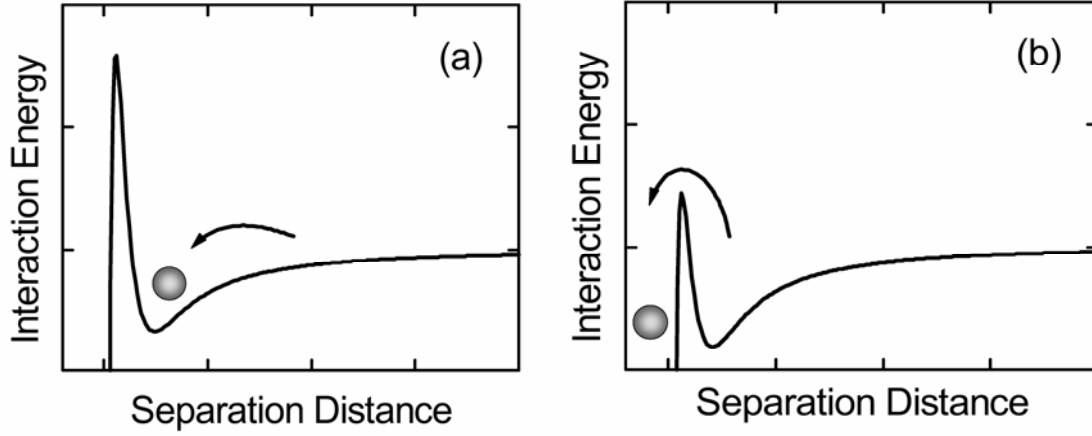


Figure 2.3. Schematic representations of DLVO interaction energy plotted as a function of separation distance between collector and particle. (a) Particle deposition in the secondary minimum. (b) Particle with enough energy to “jump” the primary energy minimum. (From Tufenkji *et al.*, 2004)[46].

These heterogeneity effects can be incorporated into *classical colloid filtration* theory by inclusion of a distributed deposition rate coefficient k around the two discrete values of k_{slow} and k_{fast} (figure 2.4).

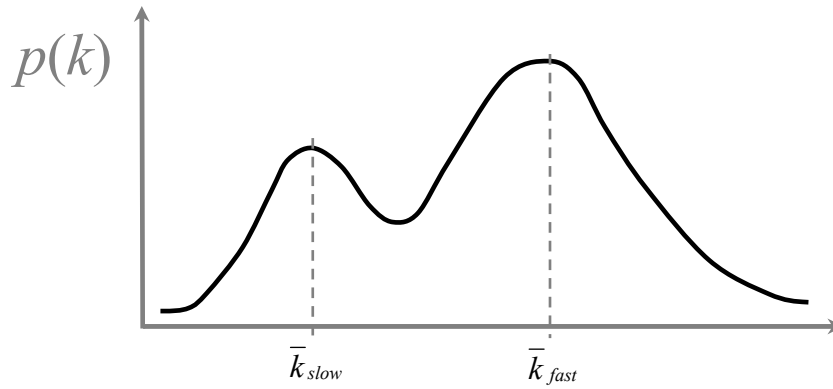


Figure 2.4. Representation of the bimodal distribution in k . The $p(k)$ function is expressed as the sum of two Gaussian distributions around the value respectively of k_{slow} and k_{fast} . (from Tufenkji *et al.*, 2004)[46].

The occurrence of both favourable and unfavourable conditions can be considered by including a bimodal distribution of k in the classical colloid filtration theory eq 1 and 2:

$$C(x) = C_0 \int_0^\infty \exp\left[-\frac{k}{v}x\right] p(k) dk \quad (12)$$

$$S(x) = \frac{\varepsilon t_0 C_0}{\rho_b} \int_0^\infty k \exp\left[-\frac{k}{v} x\right] p(k) dk \quad (13)$$

Here v is the interstitial particle velocity [L/T], D is the hydrodynamic dispersion coefficient [L²/T], ε is the bed porosity, ρ_b is the porous medium bulk density, and k is the particle deposition rate coefficient [T], t_0 is the injection time [T], C_0 is the initial concentration [M L⁻³]. Function $p(k)$ is the linear combination of two normal (Gaussian) distributions.

$$p(k) = f_{low} \frac{1}{\sigma_{low} \sqrt{2\pi}} \exp\left[-\frac{1}{2} \left(\frac{k - k_{low}^-}{\sigma_{low}}\right)^2\right] + f_{high} \frac{1}{\sigma_{high} \sqrt{2\pi}} \exp\left[-\frac{1}{2} \left(\frac{k - k_{high}^-}{\sigma_{high}}\right)^2\right] \quad (14)$$

Where ε is the volumetric water content [-], t_0 is the injection time [T], C_0 is the initial concentration [M L⁻³], k_{low}^- and k_{high}^- is the mean deposition rate coefficients [T], σ_{low} and σ_{high} are the corresponding standard deviations and f_{low} and f_{high} are the fractions of the total population associated with each mode.

2.6 Fitting experimental data using the Dual Deposition Mode Model

To fit the experimental data, the following equation was used:

$$F(x) = f_{fast} \frac{\varepsilon t_0 C_0}{\rho} k_{slow} \exp\left[\left(\frac{-k_{slow} x_i}{v}\right)\right] + (1 - \alpha_{slow}) k_{fast} \frac{\varepsilon t_0 C_0}{\rho} \exp\left(\frac{-k_{fast} x_i}{v}\right) \quad (15)$$

Where ε is the volumetric water content [-], t_0 is the injection time [T], C_0 is the initial concentration [M L⁻³], f_{slow} and f_{fast} are the fractions of the total population associated with each mode, α is the sticking efficiency and k_{fast} and k_{slow} is the adhesion rate for favourable and unfavourable sites.

Ddm model uses only 2 fitting parameters: the adhesion rate for the slow bacteria (k_{slow}) and the fraction of bacteria expressing the fast rate (f_{fast}). In this model the BTC is not fitted *a priori*, but the measured value of the relative effluent concentration C/C_0 at pore volume=1.8 is compared with the one predicted in the model. The C/C_0 relative concentration was calculated using the steady-state filtration with no dispersion equation:

$$v \frac{dC}{dx} + kC = 0 \quad (16)$$

Here v is the interstitial particle velocity, k is the adhesion rate, and C is the concentration. The inlet boundary condition $C=C_0$ and $x=0$ were applied. For a continuous particle injection at concentration C_0 and a time period t_0 the solution for a column initially free of deposited particles is:

$$C(x) = C_0 \exp\left[\frac{-k}{v} x\right] \quad (17)$$

Here v is the interstitial particle velocity, k is the adhesion rate, C_0 is the initial concentration. For the first time instead of a distribution, discrete values for k_{slow} and k_{fast} were used and the results obtained with $\sigma = 15\%$ (mean value) did not effect the fitting. Sensitivity analysis has shown that *Ddmm* calculations are not effected by the width of the distribution of k when the coefficient of variation (σ) is less than 30% [41-46].

The figure 2.5 illustrates the differences between the two models [41-48] used to fit the experimental data. According to *Bradford et al. (2003)*, in the first sand centimetres the straining is the most important removal mechanism, and in figure 2.5B the red line visualizes this area [47]. After the first sand centimetre along the column the bacteria are not filtered out from the death end pore and are retained in the sand mostly due to adhesion [47,48]. The blue line represents the area were the main removal mechanism is attributed to the bacteria adhesion to the sand surface rather than straining. In the model developed by *Tufenkji et al (2003, 2004, 2005)* bacteria expressing a k_{fast} adhesion rate are mostly retained in the first centimetre [41-46]. In figure 2.5A the red line represents the area in the column where this phenomenon is present. According to [41-48] the bacteria expressing k_{slow} is retained mostly along the column length and this effect is illustrated by the blue line.

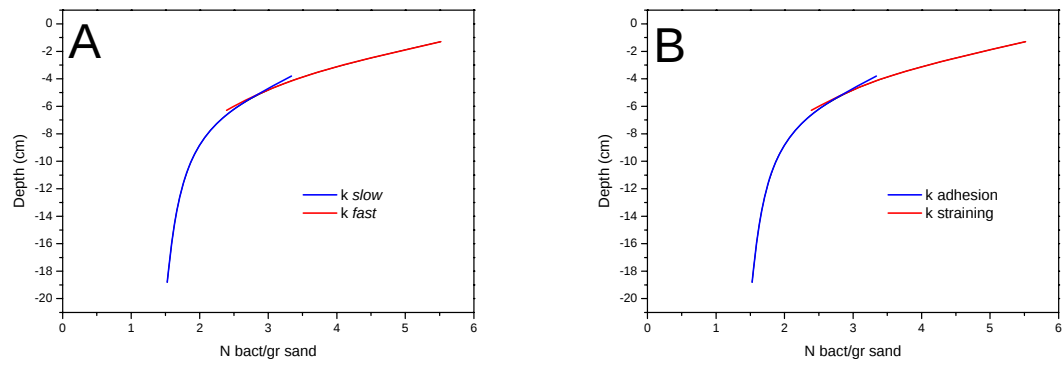


Figure 2.5. Difference between (A) the dual deposition mode model and (B) the *Bradford et al.* (2003) model [47].

Chapter 3 Materials and methods

Bacteria transport experiments using an unsaturated system were performed. This chapter provides an extensive description of the experimental set-up, the employed materials, the experimental techniques and the preparation of the experiments.

3.1 Solid grain materials

Sterile fused silica sand (*Teco-Sil*, *C-E Minerals Greenville, USA*) was used as a granular matrix (figure 3.1). Two different grain sizes named -50+100 (fine) and -30+50 (coarse) respectively (figure 3.1) were chosen. The grain size distribution was determined by sieve analysis. The grains resemble broken glass and have smooth surfaces (figure 3.2). Except for the experiment where the effect of different grain size distribution was studied (chapter 5.2), all the other experiments used a 1:1 (W: W) mixture between the coarse and fine sand (table 3.1).

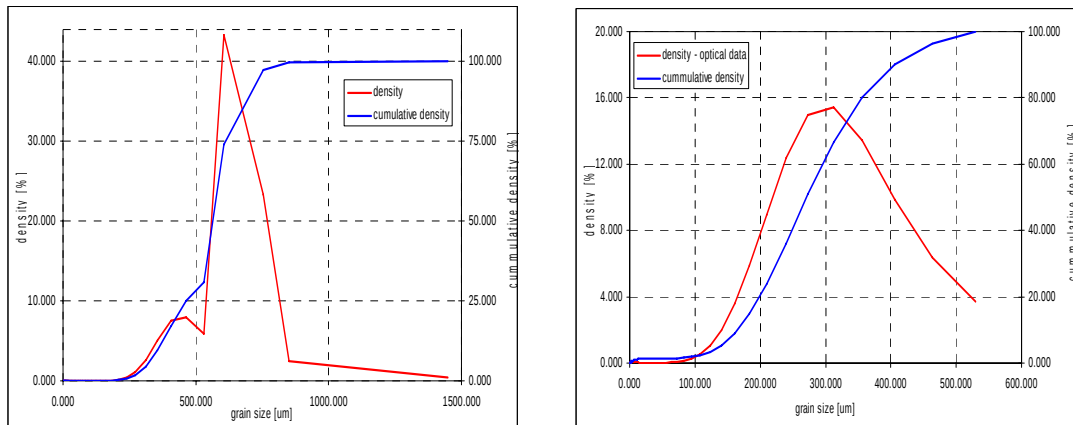


Figure 3.1. Mean grain size distribution of the silica sand *Teco-Sil* -30+50 (left), *Teco-Sil*-50+100 (right).

Table 3.1. Different mean grain size of the sand used for the different experiments

Composition	Mean grain size μm
100% of (-30+50)	607
50 % of (-50+100) and 50 % of (-30+50)	567
66.5 % of (-50+100) and 33.5 % of (-30+50)	524
100 % (-50+100)	330

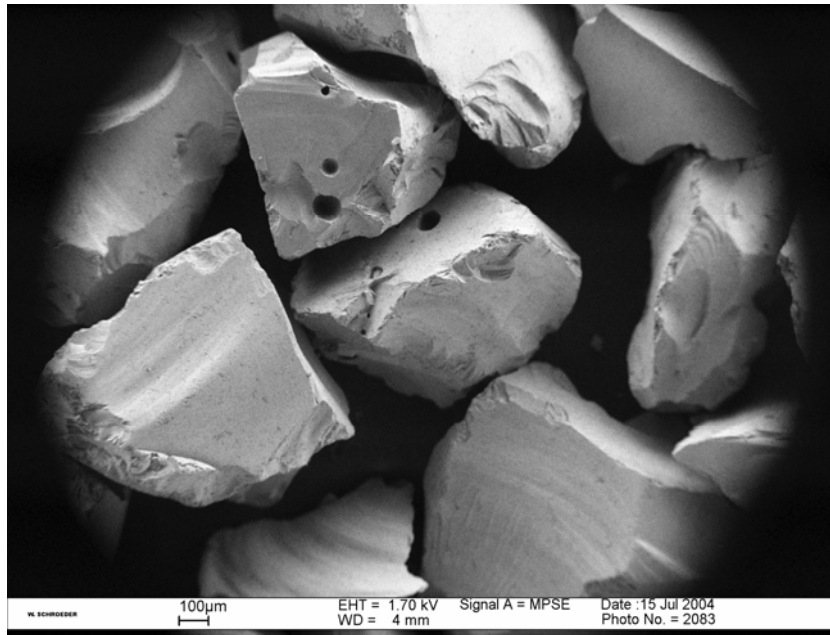


Figure 3.2. E-M image of silica sand grain surface.

3.2 Set-up description of the column technique

The experimental set-up consisted of a Plexiglas column (figure 3.4 left) able to ensure unsaturated conditions, filled with fused silica sand (figure 3.2). The column bottom was connected with PVC tubes (2 mm inner diameter) to a conductivity probe (WTW D823662 *Heilheim LDM/S*), spectrophotometer (*Tidas Spectralitic*) and UV detector (*Hitachi –Merck f-1050*), respectively (figure 3.3). Both the bacteria and the tracer outflow concentration were monitored online.

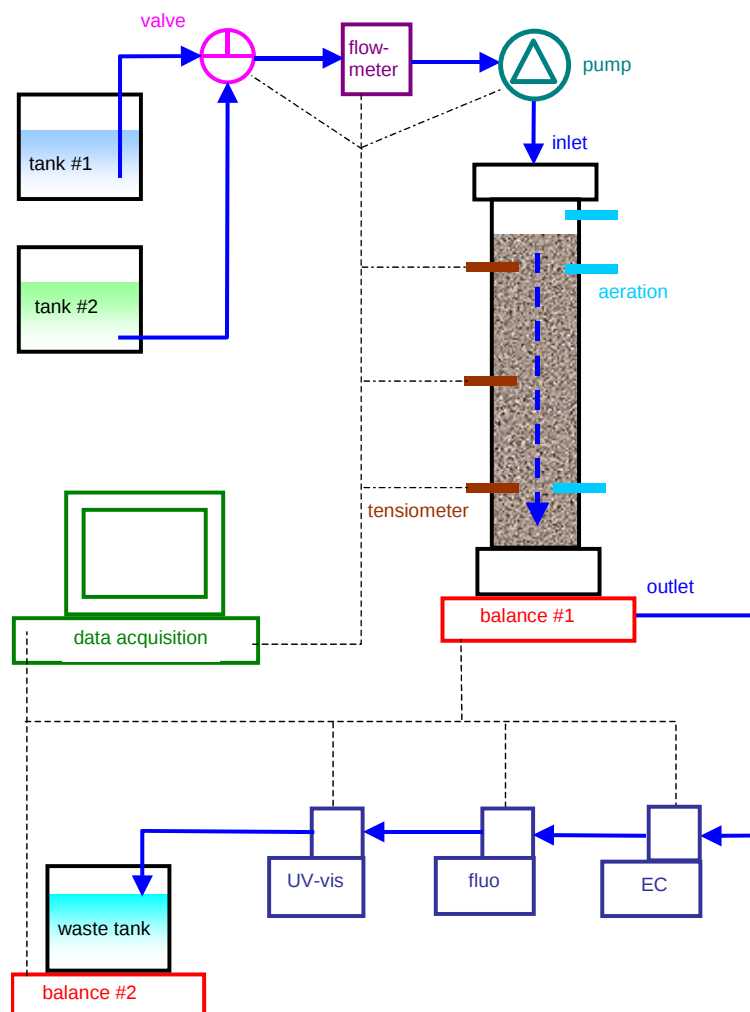


Figure 3.3. Experimental set-up schematic representation.

The experimental column was manufactured from Plexiglas (diameter 8.0 cm and length 22.5 cm) and consisted of 4 filled sections 5 cm in length and one empty section 2.5 cm in length (figure 3.4). The bottom was formed of a stainless-steel plate covered with a suction membrane (polyester fabric, mesh size 15 μm) (figure 3.5). The hydrophilic membrane was used to trap the air inside the column to ensure unsaturated conditions during the experiments. Prior to the experiments the polyester fabric was tested for the retention of bacteria and air entry pressure (-3 kPa).

At the top of the column a sprinkling plate equipped with 80 stainless-steel needles evenly distributes the influent (figure 3.4 right). The column was provided with pressure probes (*tensiometers with Druck, PMP 4070 pressure transducers*) near the top and near the bottom

(figure 3.4). The air phase was maintained at static atmospheric pressure by means of two aeration openings (figure 3.4).

Since the suction pressure at the outflow was achieved gravimetrically, the detection chain (electrical conductivity probe, UV-vis and fluorescence photometer) was installed directly behind the column and remained accessible (figure 3.3).



Figure 3.4. (Left) Plexiglas column. (Right) Sprinkler supplied with stainless steel needles.

Column filling procedure. As showed in figure 3.6 the dry sand was filled into the column by continuous free falling through a filling tube that was equipped with two homogenizing screens in the lower part. The tube was continuously elevated during the filling procedure to keep a constant distance of 10 cm to the rising sand surface. The tensiometers were inserted into the free gap between the sand surface and the filling tube and embedded in the falling sand. Since the tube had the same diameter as the column, the sand was evenly distributed over the entire cross-section of the column resulting in compact and homogeneous packing.



Figure 3.5. Stainless-steel plate covered with a suction membrane 15 μ m mesh size.



Figure 3.6. Column filling procedure.

Saturation procedure. Different saturation procedures were used to generate either fully or partially saturated packing. For full water saturation (100%), the column was completely evacuated using a vacuum pump (figure 3.7 left) and subsequently flushed from the top with degassed buffer solution. For partial water saturation, the column containing dry sand was slowly filled at a constant flow rate supplied through the bottom. The latter procedure resulted in a water saturation (approx. 80%) containing residual entrapped air bubbles (figure 3.7 right).

Water saturation of 40% was obtained by gradually reducing the inflow rate at the top while at the same time increasing the suction pressure at the bottom. The unit gradient conditions were achieved when the suction pressures at both tensiometer ports were identical. The packing was then equilibrated by flushing it with several pore volumes of $M=10^{-4}$ phosphate buffer pH =7.



Figure 3.7. Full saturation procedure (Left): The column is emptied using a vacuum pump from the bottom and degassed water is injected from the top. Procedure to achieve 80% saturation (Right): The column filled with dry sand is successively filled with water from the bottom to the top.

3.3 Measured quantities in column experiments

The measured quantities included water saturation, porosity, water pressure, water flow rate, and concentration of dissolved tracer, concentration of suspended bacteria and the concentration of retained bacteria. All quantities were monitored non-destructively and online. The data was logged into a PC via data multiplexers and served as a feedback to automatically control the experiments via a PC. Long-term continuous experimentation could be conducted under this configuration without interruption at night.

The **water saturation** was determined by online weighing of the column and controlling the water pressure via the tensiometers positioned on the top and on the bottom.

The overall **porosity** was determined gravimetrically from the weight difference of the dry and saturated packing. The tensiometer transmitted both negative and positive **water pressure** inside the packing to a pressure transducer. The transducer converted the difference between an internal and an external pressure into a voltage signal. The signal was logged via a data multiplexer in a PC for further processing. The pressure transducer sends a linear signal:

$$\Delta h = h_i - h_e = aU + b \quad (19)$$

Where Δh denotes the pressure difference head [m] h_i and h_e is the internal and external pressure head acting on transducer's membrane. U is the signal voltage [V] and a [m V^{-1}], b [m] are calculated calibration constants.

The water flow rate was monitored using a flow meter (*Flow meter Earl Laaser & co. GmbH*) and online by weighting the cumulative outflow difference within a given time period. For this, water outflow at the bottom of the column was collected in a tank positioned on a high-resolution digital balance. The mean outflow rates were obtained from two consecutive balance and time readings.

$$Q = \frac{\Delta V}{\Delta t} = \frac{m_2 - m_1}{\rho_w(t_2 - t_1)} \quad (20)$$

Where Q is the outflow rate (total flux) [$L^3 T^{-1}$], ΔV is the increment of the outflow volume [L^3], Δt is the corresponding time period [T]. m_2 , m_1 denote the weight [kg] at the end and at the beginning of the time period. t_2 and t_1 refer to the corresponding time readings. The specific flux through the cell is given as:

$$q = \frac{Q}{A} = \frac{m_2 - m_1}{\rho_w A (t_2 - t_1)} \quad (21)$$

Where q is the specific flux [$L^3 T^{-1}$] and A denotes the total cross-sectional area of the packing [L^2].

3.4 Experimental conditions

During each experiment the flow rate at the top and the suction pressure at the bottom were kept constant after achieving unit gradient conditions (i.e. identical suction pressures at both the lower and the upper port). Since the sand was homogeneously packed, the packing's hydraulic functions were assumed to be spatially invariant and the capillary pressure could be used as an indicator of local water content. The porosity of the packing was determined gravimetrically at the beginning (*Sartorius® CP12001S digital balance*); the same procedure was used to monitor online the total water amount inside the packing. The water and the solution were supplied by a piston pump (*Ismatec Reglo CPF* see fig) and the flow rate measured independently using a flow meter (*softflow.de GmbH, SF-591*) and a digital balance (*Sartorius® CP4202S*), the value was fixed for each experiment to between 165-170 ml/h.

The transport of both a conservative tracer and the bacteria cells solution was investigated by applying a continuous injection onto the column. The injected volume ranged between 3.0 and 3.5 filled pore volumes. Unlike the conservative tracer, the cell concentration could not be adjusted to be exactly identical for all experiments, but was of $4\text{-}5 \cdot 10^7$ cells/ml. The bacteria concentration in the outflow was monitoring online during the experiment using a UV-Vis spectrophotometer (*Tidas Spectralitic*) and a fluorescent detector (ex. 360nm em. 520nm) (*Hitachi –Merck f-1050*).

Tracer breakthrough curve. A solution of demineralized water was used as a negative conservative tracer against the PBS solution. The tracer concentration in the effluent was monitored online by measuring its electrical conductivity. For this purpose, a conductivity probe, *WTW D823662 Heilheim LDM/S*, connected with a thermally compensated conductometer (*CDM 83 Radiometer Copenhagen*) was installed at the outflow.

Bacteria breakthrough curve. The bacteria concentration in the outflow liquid was photometrically monitored online during its passage through the vial without taking samples. A flow-through vial was inserted in the outflow tube directly after the column. The light absorption was measured every 20 seconds in the range between 350 and 750 nm. The photometer was calibrated before and after the experiment between the buffer and the maximum concentration applied in the experiment.

3.5 Bacteria determination in the porous media

To determine the bacteria retention profile, the column was cut in 8 different slices. The content of each slice was accurately mixed in order to obtain a homogeneous concentration. Each sample was about 160 grams. The TOC content inside the sand was determined and about 0.5 grams of wet sand from each slice was measured using solid-state TOC analysis (*RC-412 Multiphase Determinator*). After the analysis the amount of water in the sand was subtracted from the total mass and the carbon concentration for grams of sand was determined and related to the bacteria number for grams of sand. The bacteria percentages in the effluent and in the porous media were calculated independently. The bacteria percentage in the outflow was calculated by integrating the outflow relative concentration area and comparison with the integration of the inlet pulse area. The bacteria number for grams of sand was calculated, therefore determining the carbon content.

A calibration curve was achieved using step dilution of a bacteria suspension of well-known concentration. The percentage of retained bacteria was calculated from the sum of bacteria number found in each sand slice compared with the total number of bacteria injected, determined with the coulter counter.

3.6 Bacteria used

3.6.1 *Deinococcus radiodurans* (DSMZ 20539)

Gram-positive, non-motile, non-spore-forming, spherical bacterium (diameter 1.5 - 3.5 μm) which form pairs and tetrads when grown in rich liquid medium (figure 3.8 left). They belong to the family of *Deinococcaceae* that include widespread soil organisms with an extraordinary ability to be able to survive in dry, nutrient-poor environments. This bacteria strain is well known to be able to tolerate lethal effects caused by DNA-damaging agents like ionizing radiation. *D. radiodurans* is resistant to ionizing radiation because it has the capacity to repair the DNA damage introduced during dehydration.

Medium preparation: Casein peptone, tryptic digest (10.0 g), yeast extract (5.0 g), glucose (5.0 g), NaCl (5.0 g), Agar (15.0 g), Distilled water (1000.0 ml).

3.6.2 *Rhodococcus rhodochrous* (DSMZ 11097)

Gram positive, soil, spherical bacteria able to form large three-dimensional aggregates of 10 μm (figure 3.8 right).

This strain was isolated from soil contaminated with alkenes (in Germany) by *Kästner* [57]. Rhodococci are aerobic, gram-positive actinomycetes of high G+C content, capable of morphological differentiation in response to their environment. The genus *Rhodococcus* is a group of bacteria that exhibit a diverse range of metabolic activities. Rhodococci have the ability to degrade a variety of organic compounds, including man-made xenobiotic compounds.

Medium preparation: mineral medium (Brunner) Na_2HPO_4 (2.44 g), KH_2PO_4 (1.52 g), $(\text{NH}_4)_2\text{SO}_4$ (0.50 g), $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ (0.20 g), $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$ (0.05 g), Trace element sol. (10 ml), Glucose (5.0 g), Distilled water (1000 ml).

For both bacteria strains the **agar dishes** preparation was identical: Medium 220: CASO AGAR (Merck 105458) Peptone from casein (15.0 g), Peptone from soy meal (5.0 g), NaCl (5.0 g), Agar (15 g), distilled water (1000.0 ml). Both strains were cultivated at 30°C and agitated at 130 rpm using a thermo stated shaker (*Certomax HK BRAUN®*).

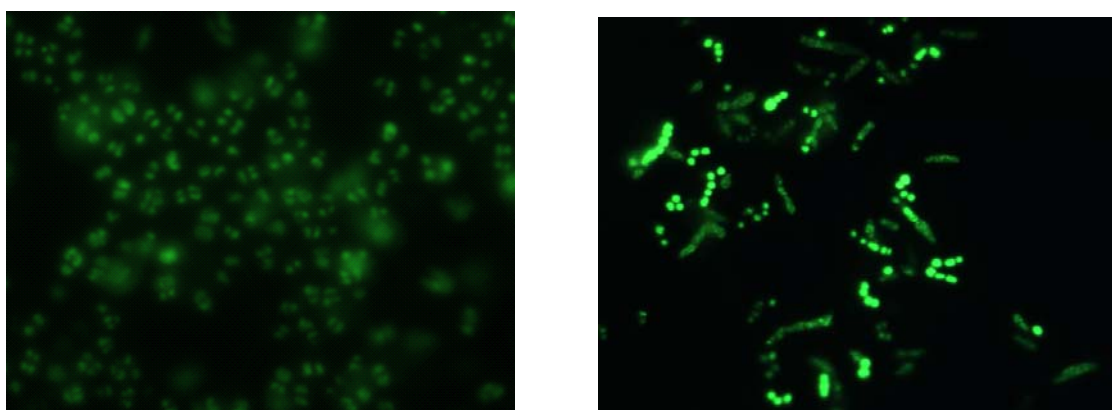


Figure 3.8. Images using Nikon ECLIPSE E 1000 epifluorescent microscopy (Left) *Deinococcus radiodurans*. (Right) *Rhodococcus rhodochrous*.

3.6.3 Column experiments

Column experiments without substrata: The bacteria were harvested in the late stationary phase by centrifugation (10 min, 8000 rpm, 20 °C) and suspended in the 10^{-4} M phosphate buffer saline, pH=7. In this phase, the substrate has been consumed and suspended, the cell culture has stopped growing, reaching a resting cell mode. Since no substrate was available inside the sterile sand packing, the suspension was considered to possess constant properties with respect to the cell concentration, size and surface properties.

Column experiments with substrata: the bacteria were harvested in the early Log phase by centrifugation (10 min, 8000 rpm, 20 °C) and re-suspended in the growing media.

3.7 Enzymatic treatment of *Rhodococcus rhodochrous*

The bacteria cells of *Rhodococcus rhodochrous* were harvested in the late stationary phase by centrifugation (10 min, 8000 rpm, 20 °C), the pellet of cells was re-suspended in a Chymotrypsin solution (100 U/ml). The solution was put in darkness at 37°C for 2 hours and agitated at 130 rpm using a thermostated shaker (*Certomax HK BRAUN®*). Then the bacteria cells were washed twice with a 10^{-4} M phosphate buffer saline solution, pH=7.

Chymotrypsin solution preparation: Chymotrypsin powder 350 U/mg (Sigma) a solution of 100 U/ml in 0.1 M Tris HCl buffer pH =7 was prepared.

0.1 M Tris HCl buffer pH =7 preparation: 12.1 g of Trizma base was diluted in 1 L of deionized and sterile water, 4.1 ml of HCl 30% was added.

3.8 Bacteria characterization

3.8.1 Hydrophobicity measurements

The hydrophobicity of *Deinococcus radiodurans* and *Rhodococcus rhodochrous* has been measured using the M.A.T.H. approach (microbial adhesion to hydrocarbon) [27, 49]. The test is based on the determination of the relative equilibrium partitioning coefficient between an aqueous (polar) and a hydrocarbon (non-polar) phase (similar to the K_{ow} value for dissolved chemicals) (figure 3.9). The test was performed under the following conditions: The microbial cultures were successively harvested at different growth stages by centrifugation (8000 rpm, 10 min and 20°C) and resuspended in a 10^{-1} M NaCl solution. A glass test tube (diameter 10 mm) was filled with 3 ml of the bacteria suspension and the optical density of the bacteria solution was measured at 600 nm in a detector (*Spectrophotometer DU800, Beckman Coulter*). Next, 300 μ L of n-hexadecane (Merck) was added to the suspension and the glass tube was stirred for 2 minutes (vortex GENIE 2). When the phases in the glass tube were clearly separated, a sample of the aqueous phase was carefully taken out of the tube with a sterile glass capillary and the sample's optical density at 600 nm was determined again. The relative hydrophobicity H_r was then calculated from:

$$H_r = \left(1 - \frac{od_f}{od_i} \right) \times 100\% \quad (22)$$

Where $OD_{initial}$ denotes the optical density of the original suspension and OD_{final} is the optical density of equilibrated aqueous phase after partitioning.

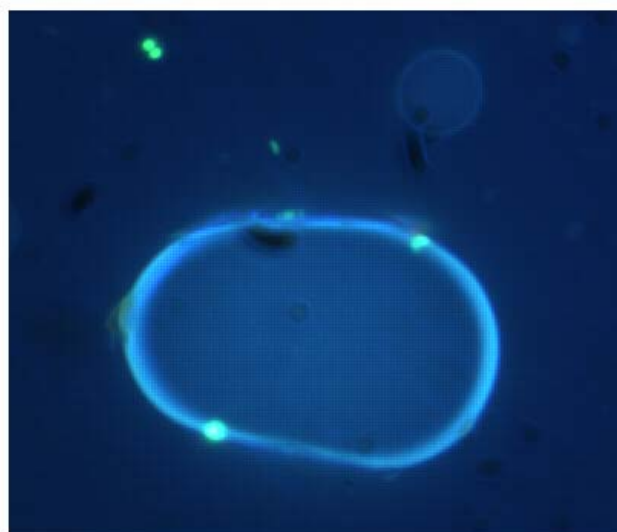


Figure 3.9. N-hexadecane drops in aqueous solution. The green spots are the stained bacteria. The picture shows some bacteria positioned at the hydrocarbon-water interface and others in the solution bulk.

3.8.2 Bacteria counting and size determination

Before each transport experiment, the bacteria concentration was determined using a Coulter counter (*BECKMAN COULTER Multisizer 3*). Using the Coulter principle it was possible to gain information not only about the number of particles in solution, but also about the particle size. A small volume of bacteria solution (100 μ L) was injected in the measurement vase using the Coulter counter (*BECKMAN COULTER Multisizer 3*).

3.8.3 Electrophoresis measurement

The bacteria Z-potential measurements were made using Lazer Zee Meter (Pen Kem, Model 501) equipped with a video system. The bacteria were harvested in the late stationary phase by centrifugation and the cell pellets were resuspended in 10^{-4} M phosphate buffer and pH=7 and injected in the electrophoresis chamber.

3.8.4 Fourier Transform infrared (FT-IR) spectroscopy ATR technique

The FT-IR (ATR) technique was used to gain information about the chemical composition of the outer surface of the bacteria. Internal reflection spectroscopy or attenuated total reflectance (ATR) is a versatile non-destructive technique for obtaining the infrared spectrum of the surface of a material.

The bacteria were selected at the late stationary phase by centrifugation and the cell pellets were obtained. The bacteria were washed twice with 10^{-4} M phosphate buffer pH=7. The cells were harvested with a sterile loop and scraped on for the FT-IR scan on s ATR (ZnSe) –crystal for reflexion. The infrared spectra of the samples were recorded using a (Bruker Equinoxs) Fourier transforms spectrometer. Typical measurement time while recording the spectra was about 4 min (60 scan) at a resolution of 4 cm^{-1} . The data was taken and transformed using OPUS software version 5.5.

3.8.5 Fluorescence microscopy technique

Fluorescent dyes and epifluorescent microscopy have been used to visualize the bacteria cells before and after the experiment. Using this technique the morphology of the bacteria strains used for the transport experiment was determined.

The microscope used was a PC-supported Nikon ECLIPSE E 1000 with a motorised and PC – controlled three-axis cross table from Merzenhäuser (Germany). The images were captured with a CCD camera, Sony DXC-9100 P, and a Matrox Corona Framegrabber. The crossable, microscope and image acquisition were controlled by the LUCIA 32 4.11 software (Nikon, Germany).

According to *Klauth et al. 2004* an aliquot of the sample was sonicated on ice for 1 min before incubation with the dye [54]. The sample was pipetted into a FinStar staining reactor containing 4 ml of phosphate buffered saline (PBS; pH 7). For filtration, an Anodisc filter (*Whatman*, 0.2 μm , 25-mm diameter) was used. The liquid sample was sucked off with 130 mbar under pressure and the retained particles were washed twice with 4 ml PBS buffer (pH 7). After washing, the filter was coated with 1.5 ml of 5 AM Sybr green solution in PBS buffer (pH 7)

and incubated for 15 min. Two washing steps were performed subsequently as described above. The filter was mounted into a drop of immersion oil on a microscope slide after drying and a drop of immersion oil was put on the surface. A cover slide was used and an additional drop of immersion oil was put on the cover slide. The sample was viewed under blue excitation light (Nikon B-2A, excitation 450–490 nm, dichroic mirror 505 nm, longpassN520 nm) at 600-fold (in some cases 450- or 1000-fold) magnification.

Chapter 4 Results and discussion

Column experiments were used to investigate the effect of different degrees of saturation on bacteria transport in porous media. Two bacteria strains of different hydrophobicity-namely *Deinococcus radiodurans* and *Rhodococcus rhodochrous* were used to study the role of the solid-liquid and the gas-liquid interfaces on bacteria movement in soil. Both strains are coccoid-shaped, having a diameter of 1 μm .

The hydrophobicity of both strains was measured using the M.A.T.H. test. The transport behaviour of bacterial cells was studied in partially saturated columns packed with synthetic silica sand. Steady state flow conditions were imposed on the column and bacteria were applied in suspension on the top of the column as a pulse.

After the breakthrough the resident bacteria concentration inside the packing was determined layer wise, resulting in a vertical bacteria retention profile. The experimental data was fitted using an inverse calculation to determine a set of parameters that made the measured and calculated data match. Two different modelling approaches were used (chapters 2.2, 2.5). The first model (chapter 2.2) was developed by *Bradford et al. (2003)*[47]. It implements the influence of physical straining, blocking and reversible attachment on colloid retention and introduces a depth-dependent coefficient for the straining process. The second model (chapter 2.5) was developed by *Tufenjki and Elimelech (2005)* and is based on the assumption of a bimodal distribution in the bacteria adhesion coefficient to the collector [43].

4.1 Bacteria characterization

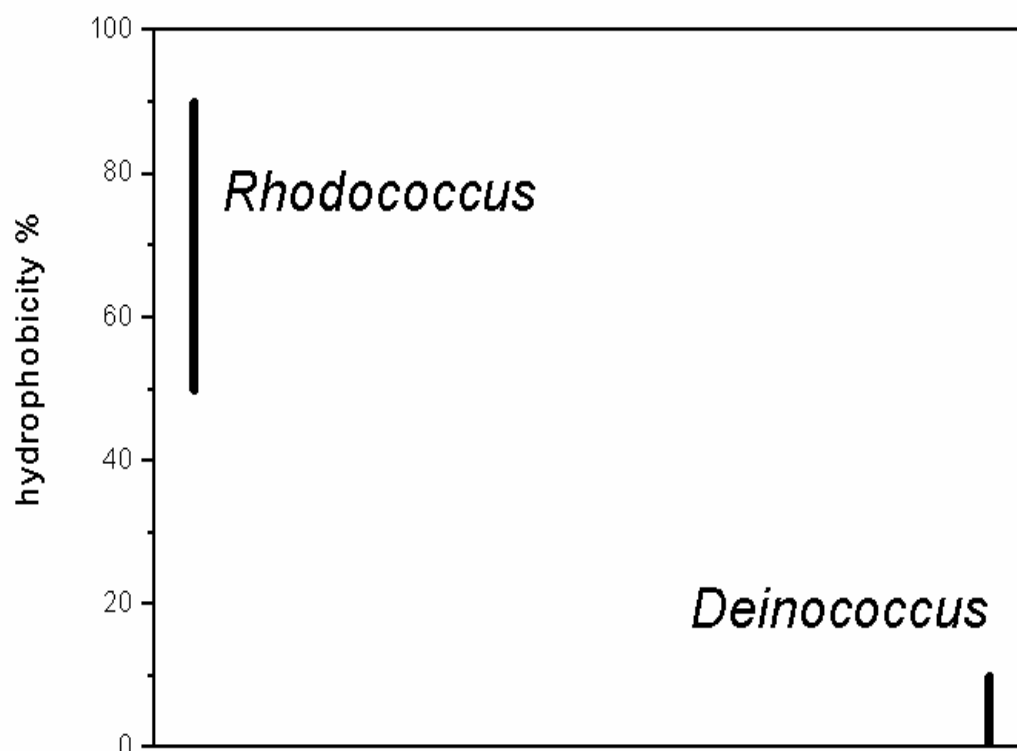


Figure 4.1. Hydrophobicity measurements using the MATH test for *Deinococcus radiodurans* and *Rhodococcus rhodochrous*.

Figure 4.1 shows that the two bacteria strains have a very different value in the hydrophobicity percentage. The hydrophobicity percentage was measured using the M.A.T.H. test (chapter 3.8.1). Bacteria hydrophobicity was monitored during the complete bacteria lifespan. For *Rhodococcus rhodochrous* it was possible to observe that the minimum measured was 55% and the maximum value was around 90%. A lower value was measured for *Deinococcus*, the hydrophobicity percentage value over the bacteria's life oscillated between 0% and 8%.

4.2 The effect of moisture content on the transport behaviour of *Deinococcus radiodurans* and *Rhodococcus rhodochrous*

The effect of different water saturation on the transport behaviour of a hydrophilic strain *Deinococcus radiodurans*, and the more hydrophobic *Rhodococcus rhodochrous* was investigated.

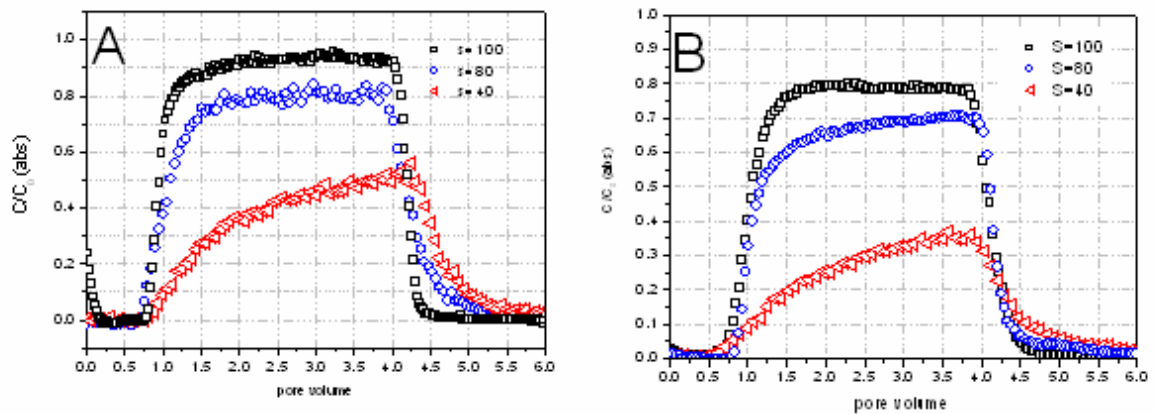


Figure 4.2. Breakthrough curves at three different saturations ($\square$) 100% ($\circ$) 80% (Δ) 40 %. (A) *Deinococcus radiodurans* (B) *Rhodococcus rhodochrous*.

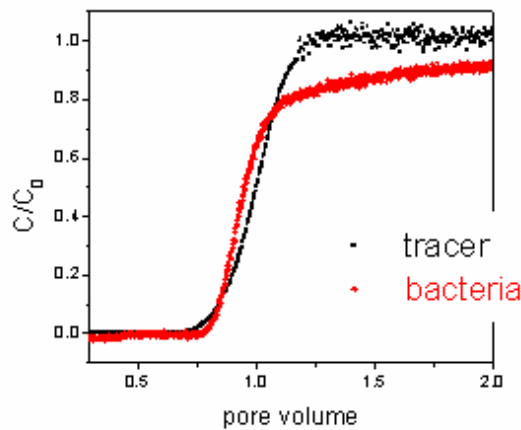


Figure 4.3. Breakthrough curves for conservative trace (NaCl) and *Rhodococcus rhodochrous* at 80% saturation.

The experimental breakthrough curves obtained for the three different saturations are shown in figure 4.2. Essentially, the mean travel time of suspended bacteria was equal to the mean travel time of the conservative tracer (figure 4.3). Obviously, the interaction between bacteria and the porous packing was not subject to an equilibrium sorption that would have led to an apparent retardation. Still the bacteria transport was clearly non-conservative because different amounts of bacteria were filtered through the packing at different water contents.

Figure 4.2 shows that a reduction in moisture content leads to a breakthrough curve having a smaller maximum in effluent concentration (reduced plateau). This effect is independent of the bacteria strain. However, the hydrophobic strain (*Rhodococcus rhodochrous*) is more retained in the soil column compared with the hydrophilic strain (*Deinococcus radiodurans*). For *Deinococcus radiodurans* in the saturated case, the breakthrough profile of the cell relative concentration increased until a plateau value of about 0.9 was reached. *Rhodococcus rhodochrous* in the saturated case reached a plateau value with a relative concentration of 0.8. At 80% water saturation, the breakthrough curve of *Deinococcus* reached a plateau at 0.8 (C/C_0) while *Rhodococcus rhodochrous* attained a plateau of $C/C_0 = 0.7$. However both the rising and the falling front is slowed down and the plateau is reached clearly later compared to the saturated case, namely after 2 pore volumes for *Deinococcus radiodurans* and after 3 pore volumes for *Rhodococcus rhodochrous*. For the lowest saturation considered (40%) a clear plateau was not reached for neither strains since the front dynamic was too slow. The maximum relative concentration reached was 0.5 for *Deinococcus radiodurans* and 0.35 for *Rhodococcus rhodochrous* (figure 4.2).

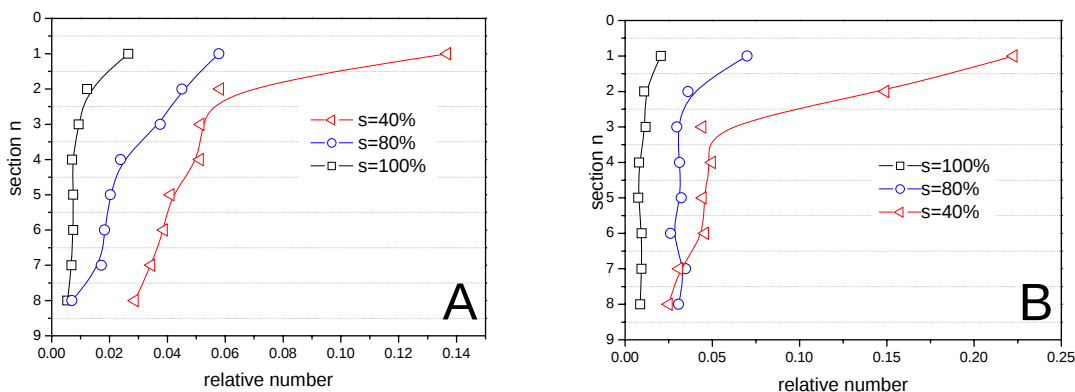


Figure 4.4. Bacteria retention profile for three different saturations. ($\square$) 100% ($\circ$) 80% (Δ) 40%. (A) *Deinococcus radiodurans*. (B) *Rhodococcus rhodochrous*.

The final spatial distribution of retained bacteria was measured (chapter 3.5) following each transport experiment and is given in figure 4.4. Unlike the temporal bacteria retardation, the bacteria retention inside the column was strongly influenced by the packing's moisture content (figure 4.4). A reduction of moisture content leads to an increase in the retained bacteria independent of strain which is coherent with the findings of *Lenhart et al. (2002)* for silica particles transport and of *Jewett et al. (1999)*, *Schäfer et al. (1998)* for bacteria transport under unsaturated conditions [38,11 and 6]. In these studies the first effect observed was the increasing number of bacteria found inside the packing with decreasing saturation. This effect has been explained with the increasing of the air-water interface that has been widely demonstrated [1,2,5,6 and 11] as an attractive site for colloids and bacteria. The highest relative number of bacteria was found in the top layer. This effect is independent from the strain (figure 4.4). This observation is in accordance with other experimental works [11,46] that have reported enhanced colloid retention at the surface. It was observed [42] that the strongest retention of *Oocystis* occurred in the sand adjacent to the column inlet. However for *Rhodococcus rhodochrous*, the more hydrophobic strain, this effect is more pronounced (figure 4.4 B), where a higher relative number of bacteria compared with the hydrophilic strain is found on the top layer.

The classical Colloid Filtration Theory (CFT) describes the particle attachment rate by a first order kinetics and predicts an exponentially decrease of retained concentration along the flow path. However, the experimental results did not show this trend. In figure 4.4 it is clearly visible that the fractional bacterial retention decreased non-exponentially with depth. It has been reported [41-48] that the spatial distribution of retained colloids under saturated conditions did not follow a simple exponential decrease with depth in accordance with the prediction of the classical clean-bed filtration model. Other sources also suggested that the deposition behaviour of microbial particles is inconsistent with the CFT [62].

Table 4.1. Percentage of bacteria cells found in the outflow and retained in the column

Saturation	<i>Rhodococcus rhodochrous</i>		<i>Deinococcus radiodurans</i>	
	outflow	retained	outflow	retained
100	90%	8%	95%	6%
80	63%	33%	88%	14%
40	36%	65%	47%	53%

The shape of cell retention profile for the unsaturated column was found to be similar to the saturated case (figure 4.4), except that the cell retention in the first slice was higher the drier the conditions were (table 4.1). To calculate the relative portion of bacteria mass present in the eluate the outflow concentration was integrated and compared with the integrated inlet injection. The percentage of retained cells was calculated as the sum of the number of cells found in each slice compared with the total cell number injected (chapter 3.3).

For both investigated strains a higher bacteria mobility was observed in the saturated case, but this was more pronounced for the hydrophilic strain. The highest mobility was observed for *Deinococcus* under fully saturated conditions figure 4.1): 95% of the total bacteria injected were found in the outflow and only 6% were retained in the slices (table 4.1). Decreasing the packing saturation from 100% to 80% resulted in a decreasing percentage of cells found in the column out flow: 88% instead of 95% of the cells could breakthrough. This reduction in the cell effluent concentration with decreasing saturation was valid for the lowest saturation (40%) as well, where 53% of the injected cells were retained in the column and only the 47% were detected in the outflow. These finding are in agreement with observations on the transport of latex micro spheres and bacteria and viruses through unsaturated sand [3, 4, 6, and 11].

Comparing the two different strains, for *Rhodococcus rhodochrous* at saturation 100%, only 86% instead of 95% of injected cells were found in the outflow (table 4.1). The lower amount of cells in the outflow found for *Rhodococcus* compared with *Deinococcus* was observed for all the saturations (table 4.1) and with the saturation decrease the difference between the strains became more marked (table 4.1).

4.2.1 Conceptual model for colloid transport considering reversible attachment, straining and Langmuirian blocking.

To fit the experimental data the code Hydrus 1-d was used [63]. The code allowed us to fit the parameters k_a , k_d and k_{str} simultaneously from the breakthrough curve and the retention profile. Mean pore water velocity and dispersivity were obtained by fitting the CDE for chloride tracer btc data (figure 4.2). Both the breakthrough curves and the retention profiles were weighted and incorporated into the optimization procedure.

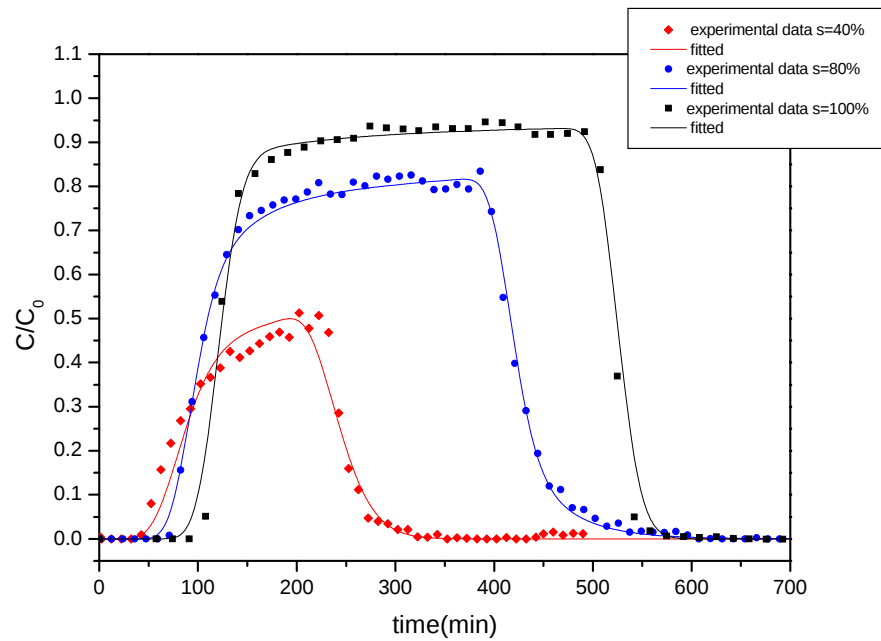


Figure 4.5. Observed and fitted effluent concentration curve for *Deinococcus radiodurans* at different saturations. Outflow relative concentration plotted as a function of time.

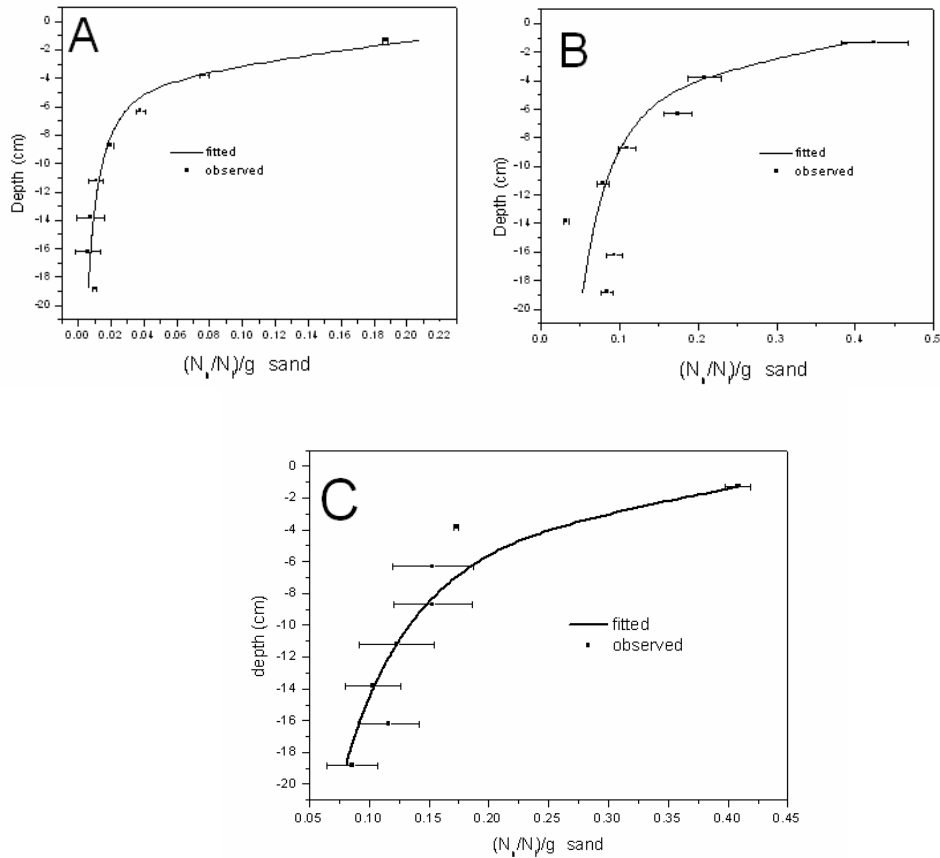


Figure 4.6. *Deinococcus radiodurans* retention profiles. (■) Observed data (—) model fitted. The different graphs represent different saturations (A) 100% (B) 80% (C) 40%.

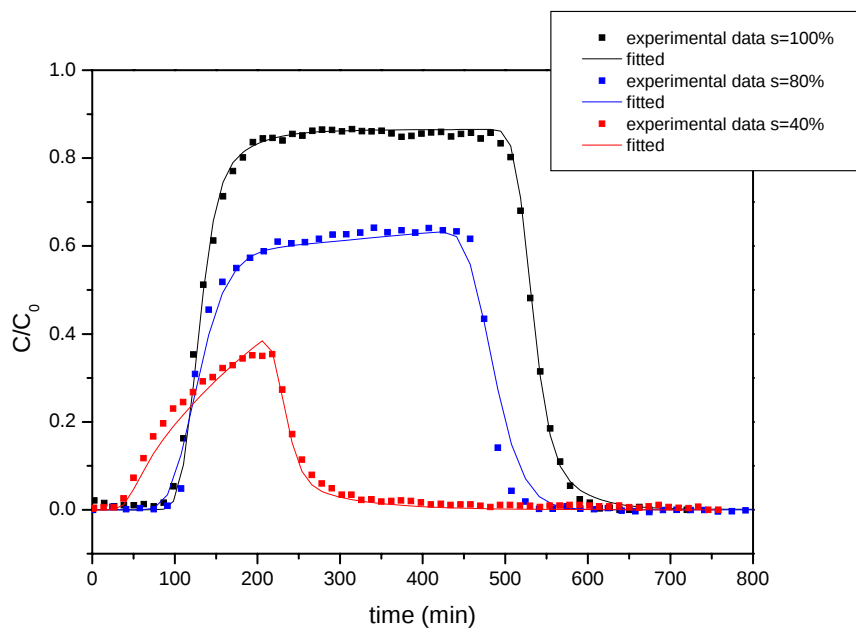


Figure 4.7. Observed and model fitted effluent concentration curve for *Rhodococcus rhodochrous* at different saturations. Outflow relative concentration is plotted as function of time.

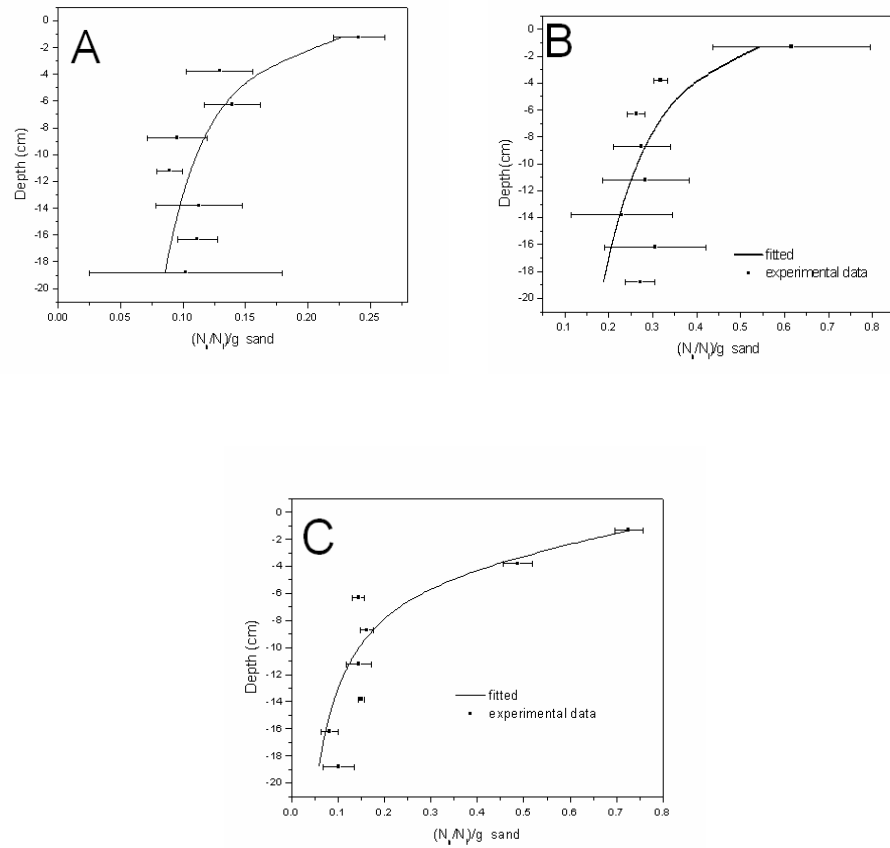


Figure 4.8. *Rhodococcus rhodochrous* retention profile. (■) Observed data. (—) fitted data by the Bradford model (chapter 2.2, equation 8). The different graphs represent different saturations (A) 100% (B) 80% (C) 40%.

The experimental data was fitted by accounting for the effect of straining, reversible attachment and blocking contemporary. This conceptual model was developed by *Bradford et al. (2002 and 2003)* for colloid transport under saturated conditions [47,48]. The straining was described as mass removal rate using first order depth dependent, irreversible rate term (chapter 2.2 equation 8). A Langmuirian approach has been proposed to describe the blocking effect as reduction in the attachment coefficient due to the filling of favourable sorption sites (chapter 2.2 equation 7). The values of the estimated parameters are given in table 4.2.

Table 4.2. Fitted parameters using the irreversible depth dependent straining for *Rhodococcus rhodochrous* and *Deinococcus radiodurans* using the experimental breakthrough curve and retention profile (*Bradford et al. 2003*).

Saturation %	K_a (min^{-1})		K_d (min^{-1})		K_{str} (min^{-1})	
	<i>Deinococcus radiodurans</i>	<i>Rhodococcus rhodochrous</i>	<i>Deinococcus radiodurans</i>	<i>Rhodococcus rhodochrous</i>	<i>Deinococcus radiodurans</i>	<i>Rhodococcus rhodochrous</i>
100	1.73E-05	1.38E-03	1.56E-03	0.024	0.07	3.9E-3
80	1.84E-03	2.81E-03	1.35E-02	0.07	0.09	2.32
40	0.109	2.82E-03	0.152	0.011	0.101	2.8

The resulting parameters are consistent with the expected trends discussed below. It was not possible to distinguish between the adhesion to soil-water and to the air-water interface. Therefore, the fitted coefficient k_a is a lumped parameter and both effects are expressed as a sum in the adhesion rate. For both strains the attachment and detachment coefficients (k_a and k_d) were found to be strongly dependent upon the water content: the attachment significantly increases with decreasing water content (table 4.2). This effect was due to the fact that the water film size became thinner with the decrease of the saturation and the water film size reduction allowed the bacteria to get closer to the sand grain surface and this increased the bacteria-collector interaction. Comparing the two strains, k_a for *Deinococcus radiodurans* had a lower value in the saturated case ($1.73 \times 10^{-05} \text{ min}^{-1}$) than for *Rhodococcus rhodochrous* ($1.38 \times 10^{-03} \text{ min}^{-1}$). This result is consistent with the higher hydrophobicity of *Rhodococcus rhodochrous* compared with *Deinococcus radiodurans* (figure 4.1). These values predict that *Rhodococcus* has a higher sticking coefficient α because for both systems the single collector efficiency η is the same.

This result is consistent with the higher hydrophobicity percentage resulting for this strain (figure 4.2). As reported in the literature, hydrophobicity directly influences the bacteria adhesion behaviour [14, 15, 18, and 26]. A similar conclusion about bacteria adhesion and hydrophobicity was reported by *Unc et al. (2003)* where slower attachment and detachment rates were found for hydrophilic compared to hydrophobic strains [7].

It should be considered that (according to *Bradford et al. 2003*) both processes, namely straining and reversible attachment act simultaneously and with respect to particle removal the

mechanism is also similar [47]. However is very important to notice (table 4.2) the different behaviour of k_a and k_{str} for the two different strains when lowering the matrix saturation. For *Deinococcus* k_a increases of several orders of magnitude were noted with the decreasing saturation ($k_a = 1.84 \cdot 10^{-03}$ at 80% and $k_a = 0.109$ at 40% saturation). This trend is coherent with the increasing of the air-water-solid interface and with the reduction of water pore volume as explain before. However the same increase in k_a was not observed for *Rhodococcus rhodochrous*; in this case k_a remained in the same order of magnitude in the water saturation range considered (table 4.2). The missing dynamics in the k_a of *Rhodococcus* was compensated by the dynamics of k_{str} . Indeed k_{str} for the case of *Rhodococcus* (table 4.2) dramatically increased, passing from $3.9 \cdot 10^{-3} \text{ min}^{-1}$ under saturated conditions to 2.32 min^{-1} at 80% saturation, but no remarkable increase was observed between the unsaturated cases 80 % and 40%. In contrast, for *Deinococcus* only a small increase was observed in the k_{str} with decreasing saturation (table 4.2). Unlike for *Deinococcus* the strong dependence of k_a on water saturation was not observed in the case of *Rhodococcus*, probably due to the fact that different transport mechanisms were involved. In the case of *Deinococcus* the increasing k_a value may reflect the attraction between the bacteria surface and the air-water-solid (AWS) interface, which can reasonably be considered to be the most important removal mechanism for hydrophilic colloids. This hypothesis is in accordance with pore-scale visualization in three-dimensional porous media [9] where it was reported that hydrophilic colloids were retained at the AWS interface near the menisci and the pendular rings rather than at the AW interface. The hydrophilic bacteria during the transport process tend to remain in the bulk solution and are transported mainly in the filled pore channels. Thus, the reduction in the water pore size due to decreasing moisture content did not significantly affect k_{str} (table 4.2). A different situation was observed for *Rhodococcus rhodochrous*, the k_{str} in this case increased passing from the saturated to the unsaturated conditions of several orders of magnitude (table 4.2). Within the unsaturated condition only a slight increase was observed when passing from 80% to 40% saturation. This bacteria strain revealed a high value in hydrophobicity (figure 4.1) and a marked tendency to aggregation (figure 3.8 right). Most of the cells in solution are in the form of three-dimensional aggregates that can reach from 10 to 50 times the size of a single cell (figure 3.8 right). This behaviour explains the strong increase in the straining when lowering the water saturation: the cells due to their aggregation tend to be *filtered out* from the solution bulk. The retention of aggregates by straining at pore throats has been postulated by *Bradford et al. (2002 and 2003)*[47,48]. This assumption is in accordance with other observations: *Crist et al. 2005* reported for hydrophobic colloids that the strong attachment force resulted in the formation of

larger colloid aggregates which can be more easily filtered by the relative narrow pore spacing representing the major deposition mechanism [9]. The adhesion rate of *Rhodococcus* did not show a remarkable increase under unsaturated conditions. Thus, it is reasonable to suppose that straining plays the main role on bacteria retention inside the packing.

The use of the Langmuir-type blocking model provided relatively good fits of the observed data (figure 4.7). The linear blocking has also been successfully utilized by other authors [45, 78]. *Schäfer et al. (1998)* used this approach to fit the experimental data [6]. The comparison of the breakthrough curves (figure 4.2) with those presented by *Schäfer et al. (1998)* show the same differences in the shape as a function of saturation [6]. In that work [6] the curve measured under full saturation has a linear positive slope clearly indicating that the solid surface was rendered less attractive by attached bacteria. The blocking mechanism was claimed to cause the positive slope in the breakthrough curve [6]. In our case this positive slope was not observed (figure 4.2). This difference could be attributed to the fact that in our case the adhesion sites were not completely occupied since only 3.2 pore volumes of bacteria solution were injected instead of 8 as in the previous work [6]. The concentration was in both cases close to 4×10^7 bacteria cell/ml. *Schäfer et al. (1998)* used a different grain size (between 250 and 500 μm) while in our case the mean grain size was 567 μm [6]. The breakthrough curve maximum relative concentration was different. In our experiments more bacteria were transported through the column (figure 4.2). These differences may be due to the bigger pore size present in our column.

Concerning the retention profile (figure 4.8) the results are consistent with the assumption of *Bradford et al. (2003)*: the majority of the bacteria are trapped close to the column inlet because they are strained by impassable pores at the sand surface [47]. The sand close to the column inlet is the first surface to come in contact with the bacteria and the first deposited cells decrease the pore volume. The new bacteria injected encounter a different pore volume compared with the bacteria injected in a clean column. According to this assumption straining plays a more important role in the first centimetres close to the inlet. This effect is explained in the straining equation with the introduction of a depth dependency factor (chapter 2.3 equation 8). After the first centimetres the bacteria able to pass are transported through the main flow path and adhesion becomes the most important removal mechanism.

4.2.2 Dual deposition mode model

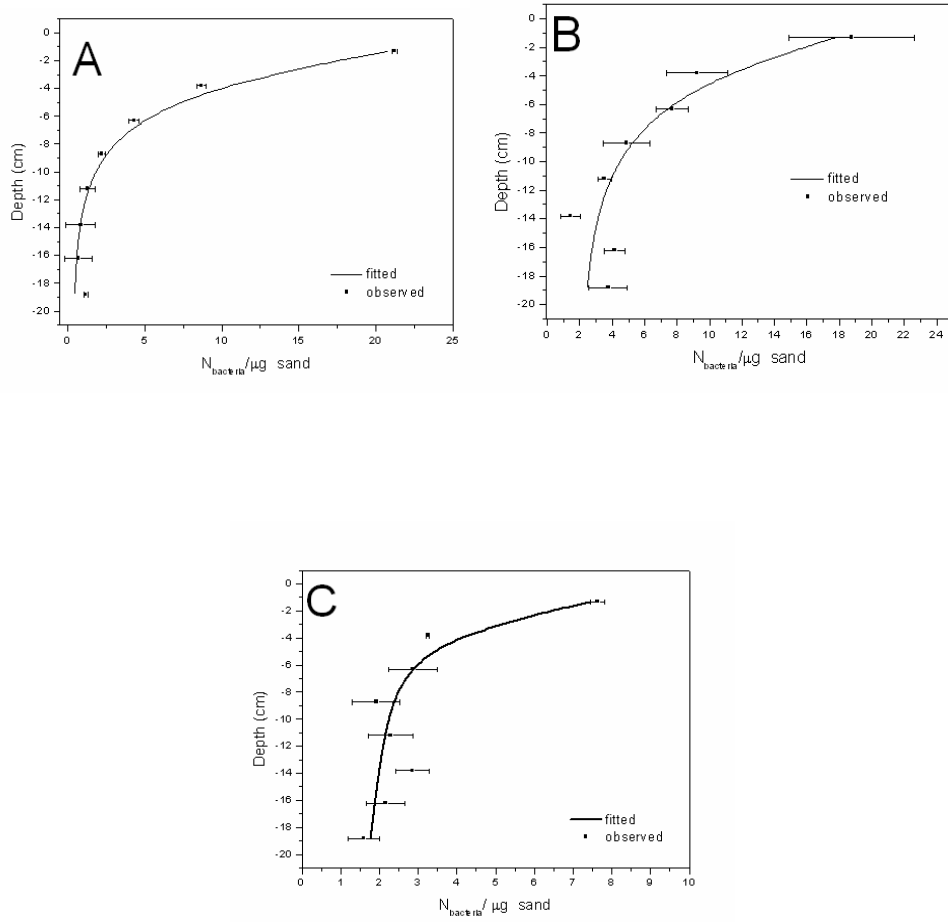


Figure 4.9. *Deinococcus radiodurans* retention profile. (■) Observed (—) fitted data by Ddm model. The distribution of bacteria along the packing was plotted as the number of bacteria found in each slice per gram of sand. The different graphic represents different saturation (A) 100% (B) 80% (C) 40%.

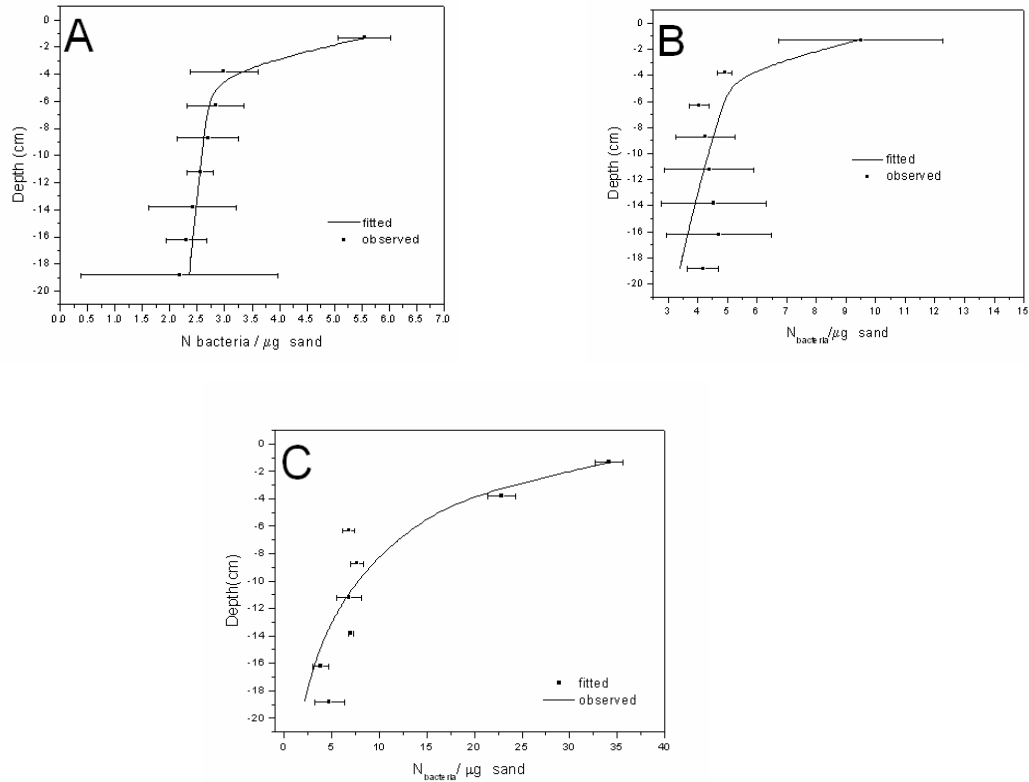


Figure 4.10. *Rhodococcus rhodochrous* retention profile. (■) Observed (—) fitted data by Ddm model. The distribution of bacteria along the packing was plotted as the number of bacteria found in each slice per gram of sand. The different graphic represents different saturations (A) 100% (B) 80% (C) 40%.

Table 4.3 Dual deposition mode model fitted parameters for *Deinococcus radiodurans* and *Rhodococcus rhodochrous* retention profile observed data.

Sat. %	C/C_0 calculated		C/C_0 experimental		f_{slow}		K_{fast} (Min ⁻¹)		K_{slow} (Min ⁻¹)	
	<i>Deinococcus radiodurans</i>	<i>Rhodococcus rhodochrous</i>	<i>Deinococcus radiodurans</i>	<i>Rhodococcus rhodochrous</i>	<i>Deinococcus radiodurans</i>	<i>Rhodococcus rhodochrous</i>	<i>Deinococcus radiodurans</i>	<i>Rhodococcus rhodochrous</i>	<i>Deinococcus radiodurans</i>	<i>Rhodococcus rhodochrous</i>
100	0.94	0.78	0.91	0.85	0.95	0.96	1.37E-05	4.67E-05	9.60E-09	4.55E-07
80	0.8	0.54	0.76	0.61	0.89	0.93	2.00E-05	6.23E-05	3.78E-07	1.29E-06
40	0.55	0.04	0.33	0.24	0.85	0.70	7.78E-05	3.33E-04	2.34E-06	1.60E-05

The Ddmm was used to fit the experimental data for *Rhodococcus rhodochrous* and *Deinococcus radiodurans* transport under unsaturated conditions. The results are presented as vertical retention profiles in figure 4.8 and figure 4.9. The values of the fitted parameters are showed in table 4.3. Both coefficients k_{fast} and k_{slow} decrease with moisture content. This effect was observed for both strains and is, purely qualitatively, in accordance with the trend of the

fitted parameters k_{att} and k_{str} in the model developed by *Bradford et al. (2003)* (chapter 4.2.1)[47]. According to the calculations made using the model developed by *Bradford et al. (2003)* decreasing water saturation leads to increasing adhesion rates (table 4.2)[47].

For all the saturations most of bacteria population from both strains exhibited a *slow* adhesion rate and the fraction of *slow* bacteria always decreased with moisture content decrease (table 4.3). For *Deinococcus* a relative small difference was observed passing from the saturated condition ($f_{slow} = 0.95$) to 80% ($f_{slow} = 0.89$) and to 40% ($f_{slow} = 0.85$) saturation. For *Rhodococcus* the situation is different: the f_{slow} changed from 0.96 at 100% to $f_{slow} = 0.93$ at 80% and to $f_{slow} = 0.7$ at 40% saturation (table 4.3). Indeed, at the lowest saturation there were a significant number of *Rhodococcus* cells adhering at the secondary energy minimum. The decrease of saturation led to an increase in both adhesion rates (k_{fast} and k_{slow}) and in the fraction of bacteria having a *slow* rate for both strains (table 4.3). *Rhodococcus* showed the higher values for both k_{fast} and k_{slow} for all the saturation considered. This trend is consistent as discussed before (chapter 4.2.1) with the higher hydrophobicity of this strain. According to the fitted parameters only a small fraction of bacteria exhibited a *fast* adhesion rate (table 4.3). This amount of bacteria did not overcome the primary energy maximum and thus they are the first to be attached to the collector at the column inlet. The rest of the bacteria with a *slow* attachment rate adhered to the column. The value of α (sticking efficiency) fitted for *Deinococcus* had a value of 1 for all saturation levels. For *Rhodococcus* this value increased with decreasing water content as was the case for the three saturations (100%, 80% and 40%) respectively 2, 3.4 and 4. These values were found to be unexpectedly high because other physical mechanisms such as straining had played the main role during the transport process. K_{fast} is responsible for increasing adhesion close to the column inlet (chapter 2.4). For both strains the increasing of this value decreases with the saturation, reflecting the experimental data where the mass concentration in the first slice increases with the decrease in moisture content (figure 4.3). For *Rhodococcus* k_{fast} , for all the saturations considered, the results were higher than with *Deinococcus* (table 4.3). The result is consistent with the observed data where a higher mass concentration was found at the column inlet for *Rhodococcus* than for *Deinococcus* (figure 4.3). The increasing in the value for k_{fast} and k_{slow} with decreasing saturation has been attributed to the bacteria's affinity for the air-water interface which implies for the cells a general increase of their adhesion rate. The Ddmm did not allow us to fit the experimental breakthrough curve, but only to calculate the expected C/C_0 . However not a complete correlation was found between the experimental and the calculated C/C_0 in particular in the case of the more hydrophobic strain.

The calculated data tends to underestimate the bacteria concentration in the outflow (table 4.3). The value of C/C_0 calculated using this model represents the value of the plateau value if this had been reached. This discrepancy has been attributed to neglecting the blocking mechanism in the Ddm model.

4.3 The effect of grain size on the transport of *Rhodococcus rhodochrous* under unsaturated conditions.

In the literature the role of grain size has never been taken into account concerning bacteria transport under unsaturated conditions. The experiments reported in the literature about bacteria or colloid transport as a function of the grain size have been performed under saturated conditions [57,45,21] .

The bacteria used for these experiments were *Rhodococcus rhodochrous* cells in resting cell-mode. This strain has the peculiar habit of forming tri-dimensional aggregates in solution which can achieve a length of 10 μ m (figure 3.8 right). Four different sand mean grain sizes (607 μ m, 567 μ m, 524 μ m and 330 μ m) were used. All the experiments were performed at 80% saturation. Both the effluent concentration and the distribution inside the packing were measured and evaluated as a function of the grain size. Both the breakthrough curve and the retention profile were modeled using two different modelling approaches (chapter 2.2, 2.6). The resulting fitting coefficients, obtained with an inverse calculation based on the experimental data, have been discussed.

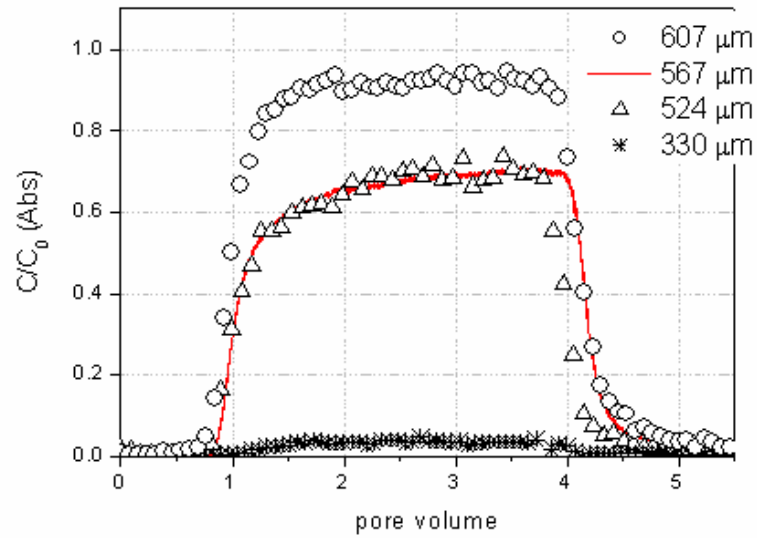


Figure 4.11. Breakthrough curve of *Rhodococcus rhodochrous* on quartz sand with different grain sizes under unsaturated conditions ($S=80\%$) Mean grain size: ($\circ$) $607\ \mu\text{m}$ ($-$) $567\ \mu\text{m}$ (Δ) $524\ \mu\text{m}$. ($*$) $330\ \mu\text{m}$.

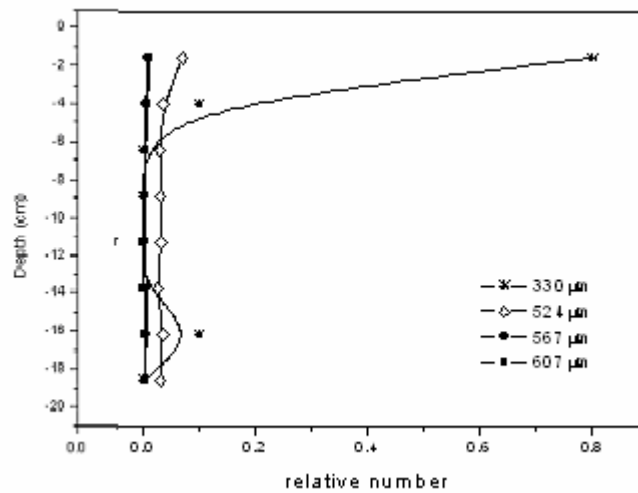


Figure 4.12. Retention profile of *Rhodococcus rhodochrous* on quartz sand with different grain sizes under unsaturated conditions ($S=80\%$) Mean grain size: ($\circ$) $607\ \mu\text{m}$ ($-$) $567\ \mu\text{m}$ (Δ) $524\ \mu\text{m}$. ($*$) $330\ \mu\text{m}$.

The mean front of suspended bacteria arrived at the same time for all the grain size distributions considered (figure 4.11). The bacteria were neither retarded nor accelerated due to the different pore sizes. However the comparison between the relative effluent concentrations (C/C_0) with different mean grain sizes showed that this parameter does play a fundamental role in bacteria transport under unsaturated conditions (figure 4.11).

The column filled with coarse sand (607 μm) had a higher cell effluent recovery compared with the column filled with fine sand (330 μm); most of bacteria were found in the outflow (95%). The bacteria outflow relative concentration reached a maximum of 0.92. The C/C_0 maximum value markedly decreased passing from 607 μm to 567 μm (figure 4.11), whereas the plateau reached only 0.7 (C/C_0). No difference was observed between the breakthrough curves of the column filled with 567 μm and 524 μm . Both the shape and the relative amount of bacteria in the outflow were similar (figure 4.11 and 4.12) and this case will therefore be no longer discussed separately. Thus no difference in the transport mechanism was considered. This observation was consistent with other experimental data obtained under saturated conditions [18]. In figure 4.11 the bacteria retention profiles at different grain size are shown. A decreasing relative concentration (C/C_0) and an increasing cell retention passing from the coarse to the fine sand was measured. In particular the retention profile showed a higher colloidal mass removal near the column inlet passing from the coarse to the fine grain (figure 4.12). For the case with the coarser grain size an almost uniform distribution with depth was observed. For the finest grain size (330 μm) a clearly different distribution was observed: nearly all bacteria were found inside the packing and only 1% could be detected in the outflow (figure 4.12 and 4.13). For the fine sand most of bacteria cells (98%) were found in the first slice, close to the column inlet. The biomass was simply *filtered-out* in the first centimeters due to the small pore size (figure 4.13). The cells were easily trapped in the death-end pore throat. This observation is proof in favor of colloidal straining as important mechanism occurring in fine sand [47,56] and the depth dependency of this physical phenomena [62]. Along the column depth only a small amount of bacteria was found (figure 4.12). The case of 567 μm was already extensively discussed (chapter 4.2.1) and the 524 μm did not show any difference in the transport mechanism compared with the 567 μm . This result was probably due to the similar value in the pore size that was not enough to cause any difference in both the breakthrough curves and the retention profiles (figure 4.11 and 4.12).



Figure 4.13. Column experiment under unsaturated conditions ($S=80\%$) with *Rhodococcus rhodochrous*, the sand grain size was $330\ \mu\text{m}$. the sand surface close to the injection point is shown. (A). Surface of the first sand layer. (B) Transversal cut of the first slice.

4.3.1 Modelling of a breakthrough curve and retention profile for *Rhodococcus rhodochrous* at different mean grain sizes

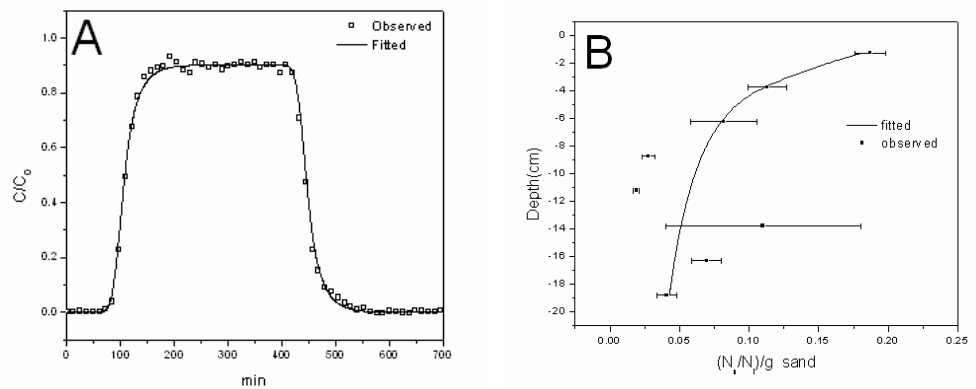


Figure 4.14. *Rhodococcus rhodochrous*, unsaturated condition (80%) mean grain size $607\ \mu\text{m}$. Observed and model fitted effluent breakthrough curve (A) and retention profile (B) using Bradford *et al.* (2003) model.

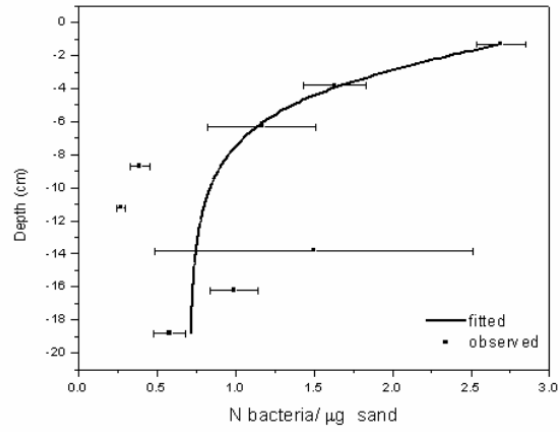


Figure 4.15. Retention profiles of *Rhodococcus rhodochrous*, 80% saturation, sand grain size 607 μm . Observed data and fitted using Ddmm model.

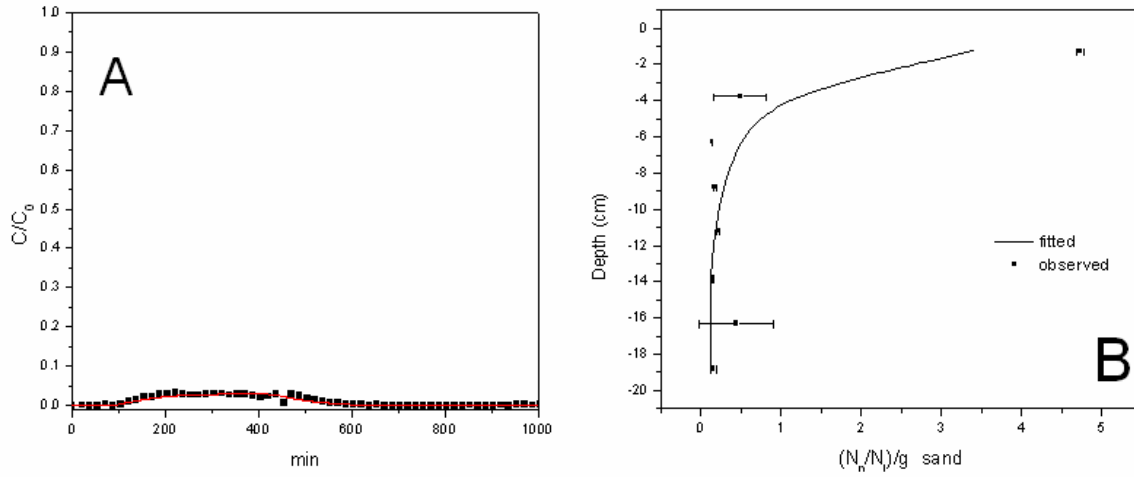


Figure 4.16. *Rhodococcus rhodochrous*, grain size 330 μm , saturation 80%. Observed and model breakthrough curve (A) and retention profile (B) using the model developed by Bradford *et al.* (2003).

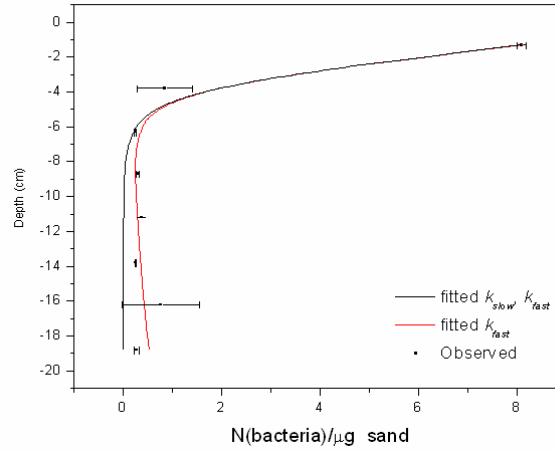


Figure 4.17. *Rhodococcus rhodochrous*, grain size 330 μ m, saturation 80%. Observed and model and retention profile using a Ddm model. (-). Data fitted using dual discrete values for the adhesion rate, k_{fast} and k_{slow} . (-) Data fitted using only a discrete value for the k_{fast} .

Table 4.4. Coefficient calculated with straining model developed by Bradford et al. (2003) for different sand grain experiment.

Sand diameter (mm)	K att (min^{-1})	K det (min^{-1})	K str (min^{-1})
0.607	4.7E-3	4.5E-2	8.28E-03
0.567 (0.524)	2.81E-3	0.07	0.118
0.330	0.109	0.152	1.83

Table 4.5. Coefficient calculated with Dual deposition mode model for different sand grain experiments.

Sand diameter(mm)	C calc	C sper	f slow	k slow (min^{-1})	k fast (min^{-1})
0.607	0.96	0.91	0.99	2.22e-7	2.02E-05
0.567(0.524)	0.54	0.61	0.93	1.29E-06	6.23E-05
0.330 dual rate	3.90E-08	0.037	0.99	3.69E-05	2.68e-4
0.330 single rate	1.8e-8	0.037	0	0	3.57e-5

To fit the experimental data concerning the transport of *Rhodococcus rhodochrous* at 80% saturation as a function of grain size, both models previously described (chapter 2.2, 2.6) were used. The breakthrough curve for coarse sand (607 μ m) indicated a lack of blocking (figure 4.11), thus this case was modeled without considering the Langmuirian dynamics (chapter 2.2 equation 7). In the dimensionless colloid retention function ψ (chapter 2.2) only a depth-dependent coefficient for the straining was considered (chapter 2.2 equation 8). The same conditions were used to fit the experimental data for the fine sand (330 μ m). Contrary to the 567

μm , the Langmuirian dynamics for blocking were taken into account (chapter 2.2 equation 7). These differences in the fitting approach can be explained with the diversity in the transport mechanisms due to the different pore sizes. As presented in table 4.4 passing from the coarse to the fine sand a significant increasing was noticed in the k_a (from $4.7\text{e-}3$ to 1.83 min^{-1}). This effect was attributed to the different pore geometry and sizes. In the fine sand, due to the smaller pore volume, the bacteria and the grain surface were in close contact and a strong interaction was present. In the $607\mu\text{m}$ case the collector-bacteria interaction was not so intense; due to the larger average pore diameter the bacteria were allowed to be transported in the pore vein, this caused a weaker interaction and consequently less attachment (as resulted in the k_a value). The adhesion to the grains surface was limited and the available sites did not have the possibility to be completely occupied. Considering this transport mechanism in the case of coarse sand, it is reasonable to omit the blocking. The situation was different in the case of the fine grain sand: the blocking could be neglected because the straining was considered the principle removal mechanism. In the fine sand the small pore volume increased dramatically the possibility for the bacteria to be filtered-out. In this situation it is not really reasonable to describe the filling of any available site for adhesion because the most of bacteria cells were already trapped in the first centimeters after the column inlet (figure 4.13). Nearly all the biomass was found in the first centimeters of sand and a low cell concentration was detected both along the column length (figure 4.12) and in the outflow (figure 4.11). Moreover the resulting coefficients showed how passing from the coarse to the fine sand the k_{str} an increasing of three orders of magnitude was observed and the value passed from $8.28\text{e-}03$ to 0.118 min^{-1} (table 4.4). These results highlight that straining played the main role in bacteria transport through a porous media filled with fine sand ($330\mu\text{m}$) and this factor is depth dependent as postulated in *Bradford et al. (2003)*[47].

In the case of the sand with mean grain size of $524 \mu\text{m}$ the blocking was considered to fit the experimental data. In this case it was considered that both straining and adhesion played an important role. In this situation it is more reasonable to describe the filling of available sites by using Langmuirian dynamics because the bacteria not strained were interacting with the collector and competed for the available surface sites.

The other model used to fit the experimental data was the *Dual deposition mode model* (chapter 4.2). Two different approaches were used to fit the experimental data concerning the fine sand. In the first case two *discrete* (*Tufenkji et al. 2005*) rate coefficients: k_{fast} and k_{slow} were used and in the second case only *one attachment rate* was considered to be important [43]. This

novel use of the model was possible due to the extraordinary shape and the notable filtration observed in the case of the fine sand (figure 4.12 and 4.13). These experimental results clearly highlight that adhesion was not the only mechanism occurring but it was reasonable to suppose that straining had played a significant role as well. A unique value for the adhesion to fit the experimental data was considered to mimic the strong “clogging” occurred in the first slice (figure 4.13). The approach with *one attachment rate* could better fit the experimental data (figure 4.17). Using the two *discrete* rate coefficients, k_{fast} and k_{slow} no biomass was supposed to be located along the column depth (figure 4.17).

The value for both k_{fast} and k_{slow} increased as sand grain size decreased (table 4.5). With the coarse sand the bacteria were transported in the pore volume vein and the contact with the sand grain was not favored, while in the case of fine sand the pore volume decrease led to a more intimate contact between the bacteria and the grain surfaces. This implied an increasing in the k_{slow} and k_{fast} , expression of an irreversible attachment rate. These results are consistent with the fitting parameter result using the model developed by *Bradford et al. (2003)*, where k_a and k_{str} respectively increased with sand grain decrease (table 4.4)[47]. The *Ddm* model gave a better result in terms of fitting of the retention profile even if the C/C_0 value was underestimated in the calculation (table 4.5).

4.4 Transport behaviour of metabolically active *Deinococcus radiodurans* under unsaturated conditions: The role of the growth phase

To better understand how metabolically active bacteria can be transported in a porous media, transport experiments were performed under unsaturated conditions with *Deinococcus radiodurans* cells harvested during the *log* phase and continuously supplied with nutrients during the duration of the experiment. Two different saturations were used (80% and 40%). In all the experiments the breakthrough curve and the retention profile were determined. The observed data was fitted using a model developed by *Bradford et al. (2003)*[47]. New concepts and hypothesis were introduced to include the biological aspects connected with bacteria growth inside the porous media.

4.4.1 *Deinococcus radiodurans* growth curve measurements

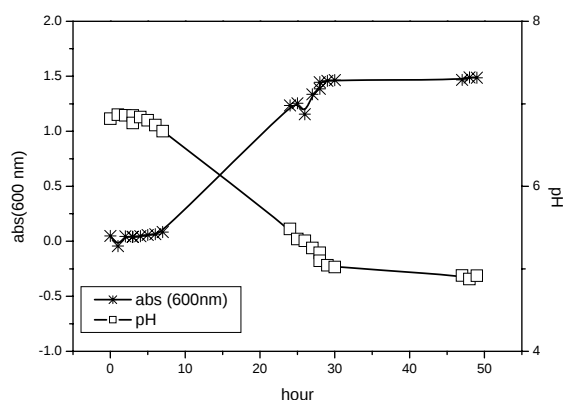


Figure 4.18. Growth curve of *Deinococcus radiodurans* measured by optical density and related to the pH changes. The optical density (left line) and the pH (right line) were monitored as a function of time.

Figure 4.18 shows the growth curve of *Deinococcus radiodurans* measured when monitoring both optical density (O.D.) and pH changes. During the bacteria growth an increasing O.D. (measured at 600nm) and a decreasing pH was found. The media acidification was an indication of bacteria metabolic activity due to the enrichment in the growing media of an acidic metabolite. An O.D. increase was detected between 6 and 7 hours, the value passed from 0.06 to 0.08 denoting the starting point for bacteria binary division (*log phase*). After 28-29 hours the O.D. stopped increasing and also the pH decrease. The metabolically activity had ended, showing clear evidence that the *log phase* was over. Since this point the bacteria culture has entered the *stationary phase*. After 32 hours only a weak metabolically activity was noted and the bacteria number was not seen to be increasing.

4.4.2 Hydrophobicity measurements during bacteria growth using M.A.T.H test

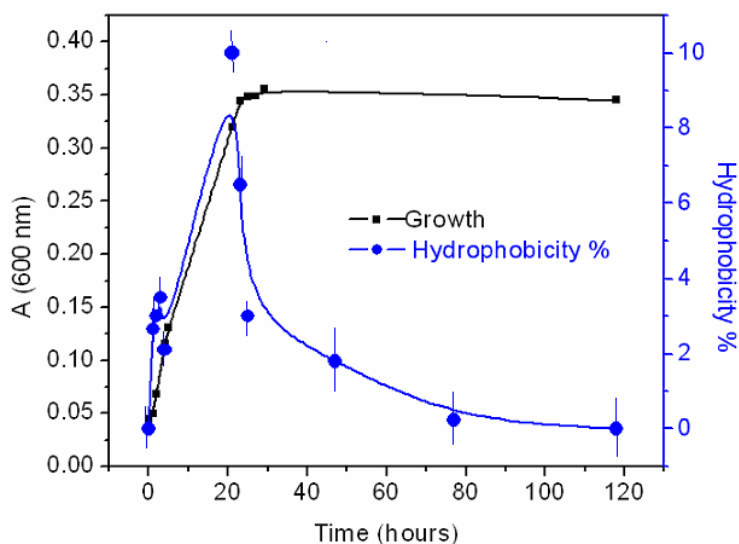


Figure 4.19. M.A.T.H. test conducted with n-hexadecane and 10^{-4} M NaCl suspension for *Deinococcus radiodurans* (■) bacteria growth curve measured as O.D. as function of time (hours), (□) hydrophobicity %.

Figure 4.19 shows how the hydrophobicity percentage of *Deinococcus radiodurans* changes during the cells lifetime. The hydrophobicity percentage was measured using the M.A.T.H. test [27,49]. *Deinococcus radiodurans* showed a change in hydrophobicity percentage depending on the growth phase. The hydrophobicity percentage increased during *logarithmic growth* until a maximum at the beginning of the *stationary phase*. During the *stationary phase* and later during bacteria starvation a decrease in the hydrophobicity percentage value was observed (figure 4.19).

The initial hydrophobicity percentage value was zero and after about 20 hours the maximum was reached with the value of 9%. During the *stationary phase*, the hydrophobicity percentage rapidly decreased and the cells showed a value close to zero after about 60 hours. In this phase, the nutrients in the growth media were depleted and the starving cells reached the resting mode. The hydrophobicity dependence on growth agrees with others bacteriological studies [17, 23 and 36]. The change in the hydrophobicity and consequently in the bacteria adhesion behaviour could be attributed to the different surface molecular composition that changes during the different life phases of the bacteria (chapter 1.2.1). Protein and polysaccharides are described as molecules involved in the interaction of cells with interfaces [28,30]. The protein and the amino acids are the hydrophobic component of the EPS; therefore an increase of these

macromolecules causes a slight increase in surface hydrophobicity [34]. Moreover it has been observed that bacteria starvation causes a diminishing in the concentration of surface protein [23]. The bacteria hydrophobicity observed during growth (figure 4.19) was attributed to the initial increase in the protein amounts on the bacteria surface during the *log* phase. The successive decrease in hydrophobicity was due to the successive diminishing in the surface protein during cell starvation.

4.4.3 *Deinococcus radiodurans* transport under unsaturated conditions: The influence of the growth phase

In this section the effect of the growth phase in the breakthrough behaviour of *Deinococcus radiodurans* is presented. Figures 4.20 and 4.21 show the active bacteria breakthrough curve and the retention profile plotted together with the transport data of *stationary phase* cells for both 80% and 40% saturation respectively.

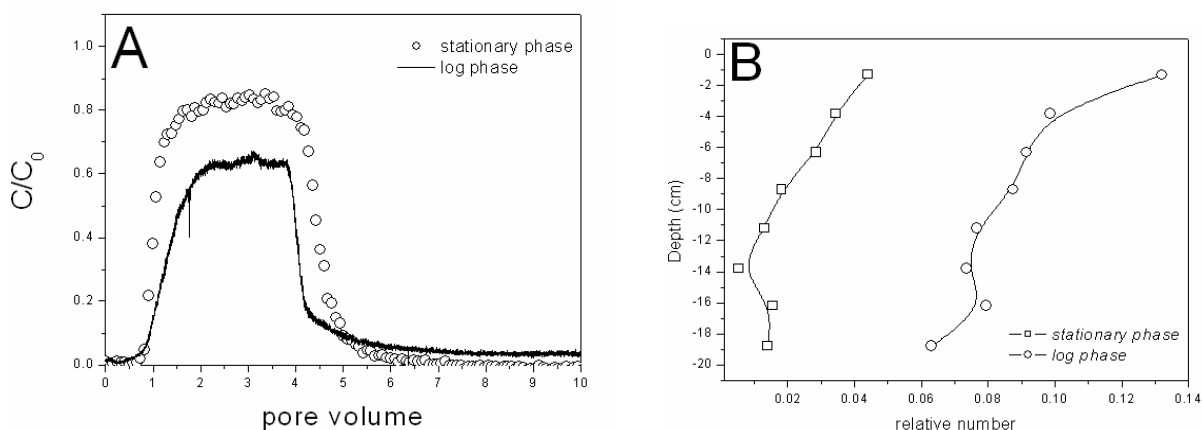


Figure 4.20. Comparison between *Deinococcus radiodurans* cells in the *stationary* and *Log phase* (saturation 80%). (A) Breakthrough curve. (B) Retention profile.

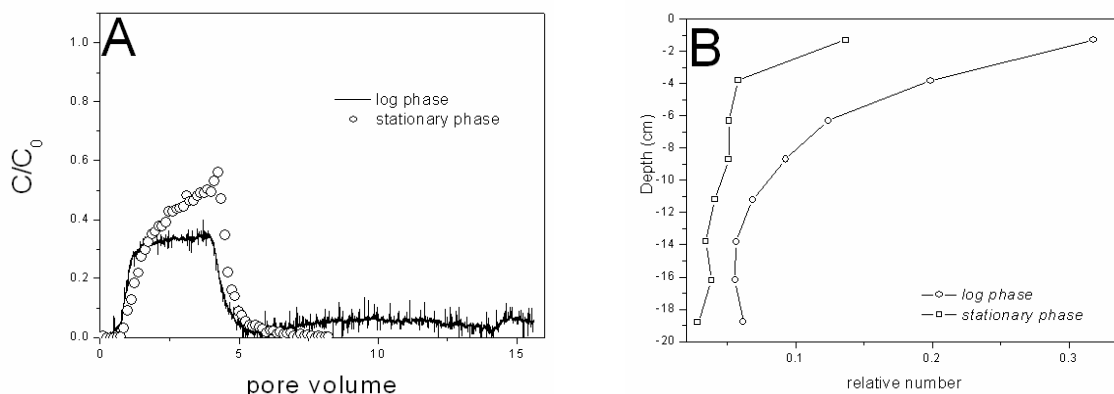


Figure 4.21. Comparison between *Deinococcus radiodurans* cells in the *stationary* and *Log phase*. (Saturation 40%). (A) Breakthrough curve (B) Retention profile.

The breakthrough curve shapes demonstrated a strong difference between the *stationary phase* and the metabolically active bacteria for both saturations (figure 4.20 and 4.21). For the 80% saturation the breakthrough curve as in the case of *stationary phase* bacteria reached a plateau (figure 4.20), however the relative concentration (C/C_0) was different: $C/C_0 = 0.63$ for the *log* and 0.83 for the *stationary phase*. For the 40% saturation the outflow maximum relative concentration (C/C_0) demonstrated a notable difference between the *stationary* ($C/C_0 = 0.5$) and *log phase* ($C/C_0 = 0.3$) bacteria. The slope shape before reaching the plateau was also different, in particular a stronger removal was observed in the case of metabolically active bacteria (figure 4.20 and 4.21). This effect was attributed to different factors: For the active bacteria the absence of a blocking mechanism due to biofilm formation and enhanced hydrophobic interaction due to increased hydrophobicity (figure 4.19). The “*bridging effect*” due to the presence of biopolymers on the bacteria surface also should be taken into consideration. In the case of active bacteria these macromolecules show a higher extension compared with bacteria in the *resting cell mode* [23,59].

The observation of the breakthrough curves with bacteria in both phases can highlight a blocking mechanism for the cells in the *stationary phase* and its absence for the bacteria in *log phase* (figure 4.21). From the mechanical point of view, when the bacteria are metabolically active, the adsorbed cells on the sand surface did not hinder the adhesion of the successive adhesion layer. This conclusion is consistent with the bacteria’s tendency to form a bio film when nutrients are present and in converse to repulse each other when starving as a survival strategy [27, 28]. Johnson *et al.* (1996) noticed as well how starving bacteria are more easily transported and Van Loosdrecht *et al.* (1987a) reported how the detachment of bacteria in soil

and sediment during starvation allows the organisms to be transported with the pore water in order to reach an environment richer in nutrients [30,12].

As in the case of 80% saturation for bacteria in the *log* phase, after the main breakthrough peak occurs, a continuous concentration of bacteria was detected in the outflow for the 40% saturation. Contrary to the *stationary state*, the *log phase* bacteria showed a pronounced breakthrough tailing and the outflow concentration did not reach the zero after 15-pore volume (figure 4.20a and 4.21a). This phenomenon was observed for the first time and was attributed to cell growth due to the continuous bacteria multiplication in the outflow solution and to the cells release from the matrix. It was hypothesized that the daughter cells could be remobilised from the enhanced water pore velocity due to the pore size restriction caused by bacteria growth itself. Besides this, the increasing number of cells inside the dead end pores could be easily “washed out” by the pore flow. In addition, recent microbiological literature [29] a mechanism is recognized called *Cell-division mediated transport* where the mother cell already attached to the mineral surface grows, divides and the daughter cells are released into the aqueous phase.

It is important to note that the complete cell budget in the case of cells in the *log phase*, where the binary division occurred during the experiment, exceeded 30% in the total cell number. Concerning the retention profile for the 80% saturation, a remarkable difference in the bacteria number and in the shape was observed.

The retention profile in the case of *log phase* bacteria showed a more pronounced presence of bacteria at the column inlet (figure 4.20b). This effect can be explained by the “*bridging effect*” due to the more extensive protein molecules present on the bacteria surface during the *log phase* compared with the *stationary phase*. It has been reported [26] that for *A. lipoferum* a change in the protein in the first 24 hours of starvation and after 72 hours starvation the cell surface hydrophobic protein could not be detected. A difference between the two phases was also observed in the retention profile for the 40% saturation (figure 4.20b). The relative number of bacteria present inside the packing in the case of *log phase* cells is higher compared to the *stationary phase* cells. The bacteria presence at the column inlet was higher in the case of 80% saturation. Compared to the *stationary phase* the active bacteria showed an increase in attachment along the column length. This observation can be attributed to the different surface

characteristics of the metabolically active bacteria compared with the *resting mode cells* and in particular to the role played by the protein on the surface. The protein present enhanced adhesion along the packing. The role of the protein on the bacteria surface will be better highlighted in the next chapter (4.5).

4.4.4 Fitting experimental data using straining

The model developed by *Bradford et al. (2003)* was used to fit the experimental data [47]. A first-order reversible attachment rate was considered. No blocking was considered as opposed to the previous case, where the bacteria cells were in resting cell mode,. Bacteria in favourable growing conditions should show the capability to form a biofilm and tend not to repel each other; the deposition of one cell layer does not inhibit the next layer deposition. In order to emulate this effect in the model, specifically in the dimensionless colloid retention function (ψ), the Langmuirian dynamics, introduced to emulate the blocking phenomenon was omitted (chapter 2.2 equation 8). Only a depth dependent blocking coefficient for straining *Bradford et al. (2003)* was considered in the dimensionless colloid retention function (ψ) (chapter 2.2 equation 7) [47]. The concept of *reversible* straining was introduced and the $k_{str\ det}$ coefficient was introduced in the mass transfer equation (chapter 2.2 equation 6) between the aqueous and the solid phase. This assumption was justified by the active phase during the experiments. Due to the bacteria multiplication during the transport process a more dynamic situation was present. It was supposed that the bacteria should be able to release themselves from the death-end pores (figure 4.22). While the resting mode cell has no possibility of being released after have been trapped in the death-end pores the same is not experienced by the daughter cells that can be remobilised again (figure 4.22). To fit the experimental data the convective-dispersive (equation 9) was used. μ_w , μ_s represent respectively the degradation term for the substance transported in the solution and adsorbed. This degradation term μ has been used in this case as a negative parameter ($\mu < 0$) in order to emulate growth. Beside this we considered the bacteria in solution able to growth as well the bacteria already attached to the grain surface.

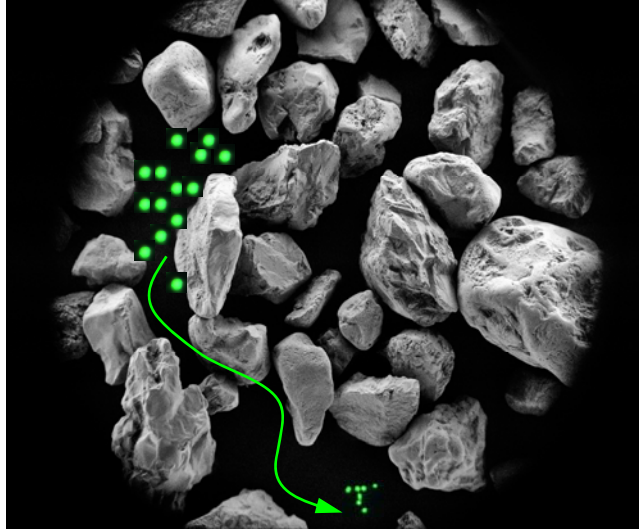


Figure 4.22. In the figure the concept of *reversible* straining applied to the daughter cells is represented. While the mother cells are trapped in the death end pores the new generation due to their smaller size and the dynamic condition resulting from growth are transported away in the vein of the pore volume.

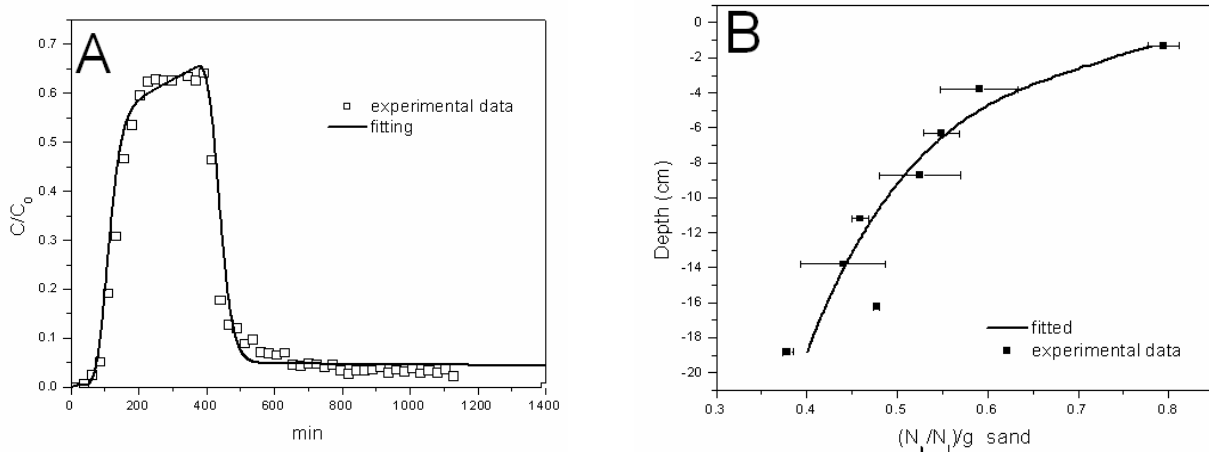


Figure 4.23. Transport experiment conducted with metabolically active cells of *Deinococcus radiodurans* in log phase at 80% saturation (A) Breakthrough curve. (B) Retention profile.

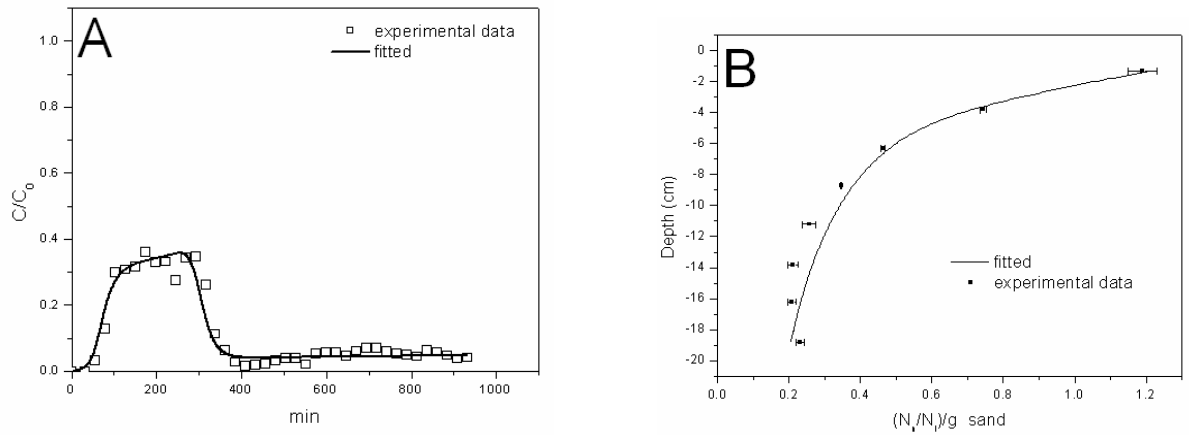


Figure 4.24. Transport experiment conducted with metabolically active cells of *Deinococcus radiodurans* in log phase at 40% saturation (A) Breakthrough curve. (B) Retention profile.

Table 4.6 . *Deinococcus radiodurans* experimental breakthrough curve and retention profile. Fitted parameters using Hydrus -1d.

Saturation %	K_{att}	K_{det}	$K_{str att}$	$K_{str det}$
80%	5.54×10^{-3}	0.043	0.021	2.64×10^{-4}
40%	0.012	0.096	0.128	3.47×10^{-4}

The data fitted using the straining model [47] properly described the experimental data (figure 4.23 and 4.24). This model approach was used for the first time for metabolically active bacteria under unsaturated conditions. The breakthrough curve and the retention profile were contemporarily fitted. A good agreement between the calculated and experimental data indicated that the hypothesis formulated was correct. k_a and k_{str} as observed before (chapter 4.2.1), increase with saturation decrease. For both saturations (80% and 40%) the bacteria tailing in the outflow concentration after the breakthrough curve could be fitted (figures 4.23a and 4.24a). It was hypothesized that the tailing was due to the bacteria reproduction and releasing in the liquid phase and the bacteria attached to the soil was considered able to reproduce and to be remobilized. This mechanism has been recognized in recent microbiological literature as *Cell-division mediated transport*, where the mother cell already attached to the mineral surface grows, divides and the daughter cell is released into the aqueous phase.

The existence of the *blocking* mechanism was omitted in order to simulate bacteria cell-cell attraction due to biofilm formation. However no evidence of *ripening* was found. Ripening implies that colloid retention increases with increasing attached phase concentration, producing a mass accumulation at the column inlet. This effect was found in our experimental data. However a decrease in the colloid effluent concentration with increasing time of colloid addition should have been noticed, but this was not the case for the breakthrough curves observed in the experiments (figure 4.23a and 4.24a).

A new concept was introduced in the model to fit the data: *reversible straining*. This effect implies that the bacteria already trapped in the death-end pores, could be re-immobilized due to the growth and reinserted in the pore vein flow. The bacteria growing effect can alter hydrodynamic conditions inside the column, leading to a more dynamic situation: Initially, pore clogging can change the flow path and the pore flow velocity, which can be a first cause of re-immobilization. Furthermore, the strained bacteria trapped in a death-end pore are confined in volume. During growth the number of cells in this volume increases, which might cause the microorganism *wash out*. However, further investigations should be performed to better clarify this mechanism. The data shown in this section were the results of the first ever experiments, to our knowledge about the transport and modelling of metabolically active bacteria, able to reproduce during an experiment under unsaturated conditions.

4.5 Bacteria transport under unsaturated conditions: The role of surface protein

The rate and the extent of bacteria adhesion to surfaces, which affects the transport behaviour on the macro scale, is governed by molecular-scale interactions between the bacteria's outer layer and the surface structure. These interactions are poorly understood especially the role of cell surface polymers [39]. These macromolecules can inhibit or promote adhesion in accord with their affinities for the substratum and depending upon the aqueous solution conditions such as ionic strength [36]. We focused our attention on the protein component of the outer surface because these macromolecules play an important role in bacteria adhesion and hydrophobicity [20].

The result regarding bacteria transport in the different growth phases (chapter 4.4) demonstrated the necessity to better clarify the role of surface proteins, as this molecules changes during the bacteria's lifecycle. In order to fulfil this gap, investigations with bacteria without the surface protein component were performed. The bacteria surface was therefore treated with α -*Chymotrypsin*, a protolytic enzyme able to digest the protein in peptide fragments (chapter 3.7). The hydrophobicity percentage of the treated bacteria was measured, and a transport experiment under unsaturated condition (80% saturation) was performed. The experimental data was fitted using the two different approaches mentioned above (chapter 2.2 and 2.6) and the calculated coefficients were compared with those of the untreated bacteria (chapter 4.2.1).

4.5.1 Characterization of *Rhodococcus rhodochrous* treated with α -*Chymotrypsin*

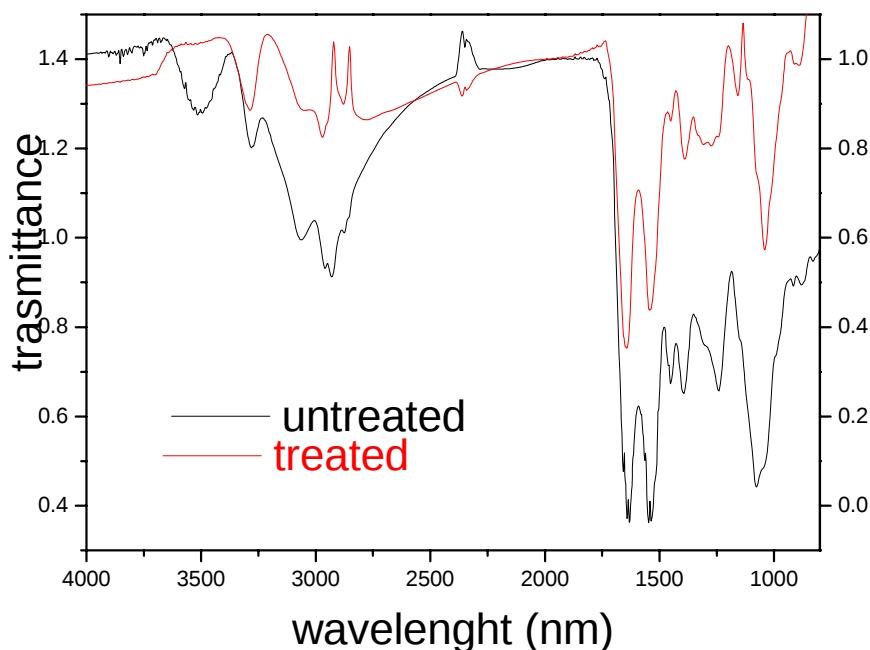


Figure 4.25. IR analysis of *Rhodococcus rhodochrous* cells. The difference between the *treated* cells surface with α -*Chemotrypsin* and *untreated*.

Several papers have been published in the last decade on the use of *Fourier-transform* infrared (FT-IR) spectroscopy as a means of rapidly identifying micro-organisms [51]. Using this technique it is possible to characterize the surface and to obtain important information about the

bacteria's surface chemical structure [52]. In the transmittance spectrum (figure 4.25) were individuate different spectral regions. The bands between 3000 and 2800 cm^{-1} are related to the alkyl hydrocarbons groups, the so called 'fatty acid region'. In this part the bands at 2956, 2934 and 2875 cm^{-1} characterize asymmetric CH_3 stretching, asymmetric CH_2 stretching and symmetric CH_3 stretching. Between 1700 and 1500 cm^{-1} a 'proteins region' was assigned. A very intense band between 1700 and 1600 cm^{-1} indicates the presence of -C=O group.

In the transmittance spectrum regarding treated bacteria the diminishing of several peaks was detected (figure1). At 1450 and 1245 cm^{-1} the bands in the treated bacteria have completely disappeared, while the band at 1390 cm^{-1} is still present. The band at 3071 cm^{-1} is assigned to the symmetric stretching of the -NH_2 group and for this a strong decreasing was found for the treated cells. The decreasing of this band is reasonable due to the diminishing of proteins on the surface as expected from protease treatment. A shoulder at 1035 and 986 cm^{-1} disappeared after treatment, but a band at 1450 appeared, probably this band was overlapped by the transmission of other groups. The treatment of the bacteria surface with α -Chymotrypsin strongly affected the spectral area assigned to the alkyl hydrocarbons groups and proteins. It was reasonable to suppose the presence of hydrocarbons chains associated with the protein structure removed as well. In particular a stronger decreasing in the band intensity at 2934 and 2875 cm^{-1} compared with the decreasing at 2956 cm^{-1} was an indication that long alkyl chains have been removed. This effect founded from the FT-IR could explain the decreasing in the hydrophobicity percentage observed with the MATH test (table 4.7) due to the removing of hydrocarbons chains. Nevertheless the proteins are hydrophobic molecules and their removal from the surface may played a role in the decreased hydrophobicity percentage measured.

In the *treated bacteria*, the surface proteins were digested using α -Chymotrypsin, an enzyme able to degrade the peptidic bond (chapter 3.5). MATH test results showed how the treatment had affected the surface hydrophobicity. The hydrophobicity percentage passed from 33% to 21% (table 4.7). This tendency was consistent with other observations: *Flint et al. (2002)* reported treatment with *Trypsin* or sodium dodecyl sulphate to remove cell surface proteins resulting in a 100-fold reduction in the number of bacteria cells attaching [52].

Table 4.7. Hydrophobicity measurement with MATH test for *Rhodococcus rhodochrous* cells treated with α -Chymotrypsin and untreated, measured during the *stationary phase*.

Treated cells	21%
Untreated cells	33%

4.5.2 Transport experiment of *Rhodococcus rhodochrous* enzymatic treated cells

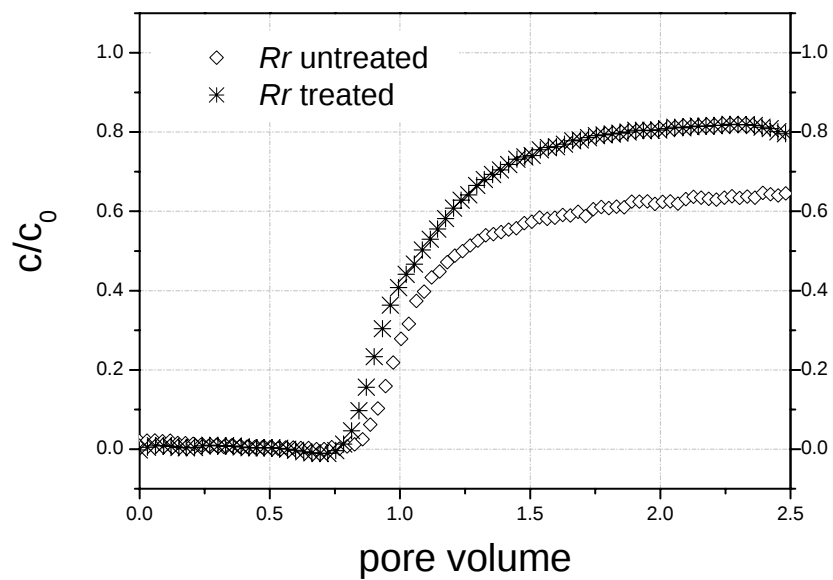


Figure 4.26. *Rhodococcus rhodochrous* breakthrough curves for cells treated with α -Chemotrypsin and untreated ones at 80% saturation.

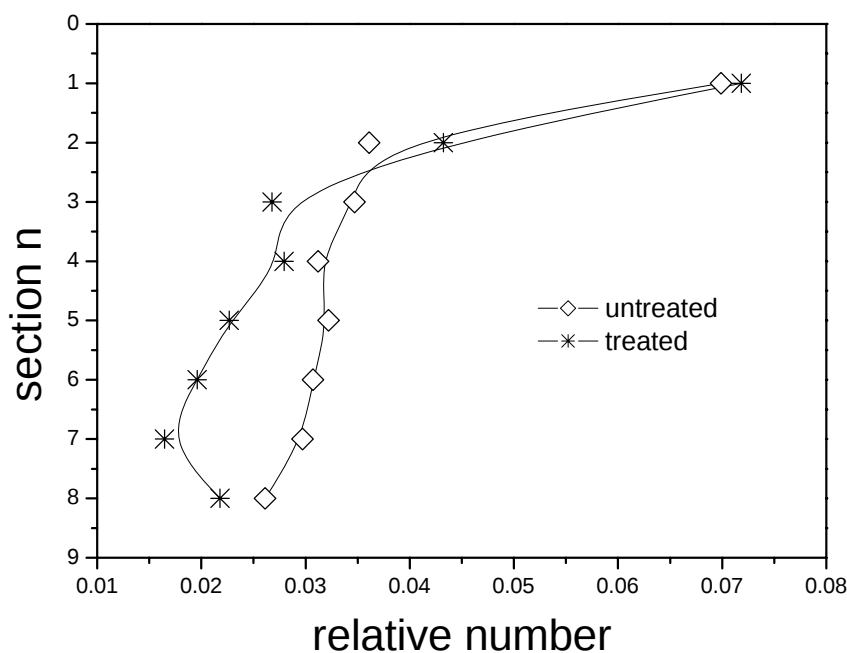


Figure 4.27. *Rhodococcus rhodochrous* retention profiles for cells treated with α -Chemotrypsin and untreated at 80% saturation.

The mean front of treated bacteria arrived at the same time as the untreated (figure 4.26). However the maximum relative concentration (C/C_0) was different for treated and untreated bacteria. For the treated cells the C/C_0 value was 0.8 and for the untreated bacteria it was less (0.65) (chapter 4.2.1). The breakthrough curve showed the same shape (figure 4.26): a plateau value was reached in both cases. Despite the protein absence on the surface, the bacteria transport had the same mechanisms, but different a intensity of adhesion. The *Rhodococcus rhodochrous* treated cells showed a higher tendency to be transported compared with the untreated cells. As had been observed in the M.A.T.H. test the bacteria showed a lower hydrophobicity percentage (table 4.6), the well-known relationship between hydrophobicity and adhesion gives the basis to understand this phenomenon [17,21]. Protein represents the hydrophobic part in the bacteria surface [18,21and 22], the 10% decrease in hydrophobicity percentage (table 4.7) reduced bacteria adhesion to the sand surface observed along the column depth (figure 4.26). Additionally the absence of appendices on the surface could cause less marked “steric interactions” between bacteria and collector [31]. The enhancement of transport for cells without protein was coherent with the observation for bacteria in different growing conditions (chapter 4.4). The increasing concentration in the outflow observed for cells in *stationary phase* has been attributed to the decreased concentration of protein on the bacteria surface due to starvation [17]. The relative concentration measured in the outflow and the retention profiles were indicative of less attachment (figure 4.26). In the first slice close to the

column inlet the relative bacteria concentration for the treated and untreated bacteria showed a very similar value (figure 4.27). The main difference was measured along the packing where the treated bacteria amount was less. This experimental data suggests that, in the first centimetres a physical mechanism such straining occurred. For the cells able to pass through the initial death-end pores, adhesion played a decisive role as a mass removing mechanism.

4.5.3 Modelling of breakthrough curves and retention profiles for *Rhodococcus rhodochrous* treated with α -Chymotrypsin

Two different modelling approaches were used (chapters 2.2, 2.5) to fit the experimental data.

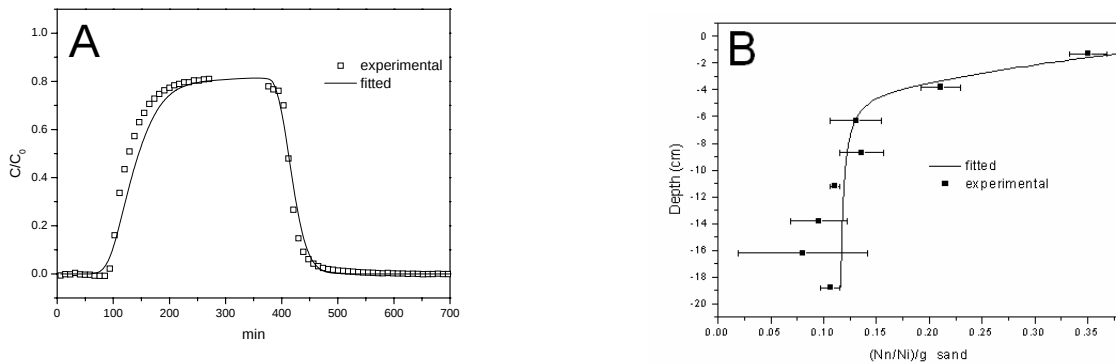


Figure 4.28. *Rhodococcus rhodochrous* breakthrough curves and retention profiles for cells treated with α -Chymotrypsin at 80% saturation. Experimental data was fitted using the *Bradford et al. (2003)* model [47].

The model developed by *Bradford et al. 2003* properly fitted the experimental data (figure 4.28)[47]. The resulting coefficients for the *treated* bacteria cells were compared with those of *untreated* cells (chapter 4.2.1). In the first centimetres near the column inlet the most important removal mechanism was attributed to the straining of bacteria in the death end pores. The k_{str} value was not very different for the *treated* and *untreated* bacteria (table 4.8), and the corresponding retention profiles showed a comparable relative amount present in the first slice for both cells (figure 4.27). The main difference was observed in the bacteria concentration along the column depth (figure 4.27). Along the column depth the adhesion to grain surfaces was considered to be the most important removal mechanism. In particular the *treated* bacteria showed less adhesion compared with *untreated*. This finding was confirmed by the calculated

adhesion coefficients (table 4.8). The k_a for the *treated* bacteria was three orders of magnitude less compared with the *untreated*. These results showed the importance of protein on the bacteria surface for adhesion. It was reasonable to consider the straining occurring at the column inlet independent from the surface protein removal. The presence of nearly the same amount of bacteria on the top for both the *treated* and *untreated* cells (figure 4.27) highlights how effectively straining could have been the physical mechanism occurring. Straining is a physical effect mainly due to the difference between the particle size and the pore volume. The proteins cut did not affect the bacteria size, only a macromolecular layer had been removed from the surface. The decreasing adhesion resulted was consistent with other studies [34].

By performing these experiments, it has been demonstrated for the first time the role played respectively from the adhesion to the sand grain surface and straining in bacteria transport under unsaturated conditions.

4.5.4 Dual deposition mode model

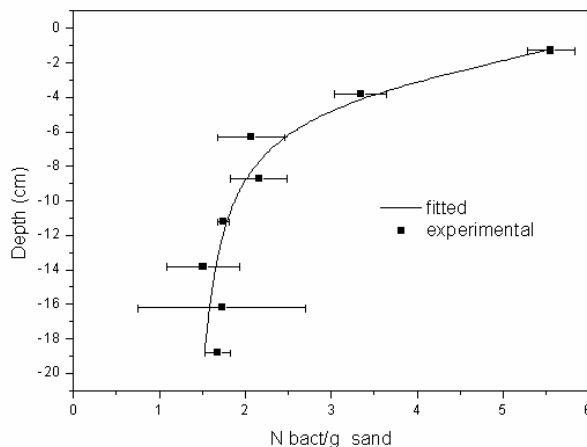


Figure 4.29. *Rhodococcus rhodochrous* breakthrough curves and retention profile for cells treated with α -Chymotrypsin at 80% saturation. Experimental data was fitted using the *dual deposition mode* model.

Table 4.8 .Dual deposition mode model fitted parameters for treated and untreated *Rhodococcus rhodochrous* cells.

	C/C ₀ calculated	C/C ₀ experimental	f_{slow}	k fast (Min ⁻¹)	k slow (Min ⁻¹)
Untreated	0.54	0.61	0.93	6.23E-05	1.29E-06
treated	0.74	0.80	0.91	1.61E-05	5.14E-07

The *dual deposition mode* model properly described the experimental data for treated and untreated bacteria (figure 4.29). A good agreement resulted between the calculated and the experimental relative concentrations (C/C₀) (table 4.8). These results were also consistent with the fitted parameters resulting from the model developed by *Bradford et al. (2003)* (table 4.7)[47]. Both the k_{fast} and k_{slow} values decreased in the case of the treated bacteria (table 4.8). The fraction of bacteria with a *slow* adhesion rate was nearly the same for both cases considered (table 4.8). An order of magnitude difference resulted between the k_{slow} for treated and untreated bacteria. For the k_{fast} the untreated bacteria showed a 6 times higher adhesion rate and an order of magnitude difference in the k_{slow} . According to the *dual deposition mode* model in the column top, close to the inlet, the accumulation has been hypothesized given by the bacteria having a *fast* adhesion rate (figure 2.5). In the k_{fast} values calculated for treated and untreated cells no notable difference was observed (table 4.8). The cell adhesion along the depth, instead, is attributed (chapter 2.6) more to the cells having a *slow* adhesion rate (figure 2.5). Consistent with this hypothesis between treated and untreated cells a notable difference was observed in the k_{slow} value (table 4.8).

In conclusion, the coefficients calculated with both models (table 4.6 and 4.7) showed how treatment with the α -*Chemotrypsin* had affected bacteria adhesion capability, confirming the role of the surface protein in this process. As is shown in table 4.7 the k_a value decreases passing from the untreated to the treated bacteria and the same decreasing trend is observed in the k_{slow} value when the *dual deposition mode* model is considered. Concerning the retention profile it was noticed that the lack or damage of these macromolecular structures led to a decreasing bacteria quantity along the packing but did not affect the bacteria accumulation in the first slice (figure 4.27). In the breakthrough curve the maximum relative concentration changed, but the shape was the same. Treatment with α - *Chemotrypsin* did not affect the cell

size and thus the straining effect should have been the same for treated and untreated bacteria, which is consistent with the assumption made by *Bradford et al. (2003)* [47]. This assumption was confirmed by the k_{str} value resulting from the fitting of the experimental data (table 4.6). As is possible to observe in table 4.7 the k_{str} showed nearly the same value for the treated and untreated bacteria. While on the contrary, the k_a value revealed three orders of magnitude less for treated bacteria, confirming the decreased adhesion capability for the treated bacteria (table 4.6). According to the *dual deposition mode* model the protein's removal did not affect the amount of bacteria being capable of interacting with the collector due to the secondary minimum of energy. Considering both models a parallel was observed between the k_{slow} and the k_a , and between the k_{fast} and the k_{str} .

Chapter 5 Summary and conclusion

The aim of this work was to study bacteria transport behaviour in different conditions using an unsaturated porous media. A column-based system able to ensure unsaturated conditions was designed and developed in order to perform the experiments. Two bacteria strains *Deinococcus radiodurans* and *Rhodococcus rhodochrous*, which were strongly different in hydrophobicity were employed. During the experiments the bacteria concentration in the outflow was continuously measured online and after the experiment the column was dismantled to determine the retention profile. The observed data was fitted using two different models (*Tufenkji et al. 2003, 2004, 2005* and *Bradford et al. 2003*) and the resulting coefficients were used to elucidate the transport mechanisms [41-47].

The following aspects concerning bacteria transport behaviour were investigated: (i) the influence of matrix saturation; (ii) the role of the bacteria surface characteristics; (iii) the effect of matrix grain size; (iv) the transport behaviour of metabolically active bacteria; for the first time, fresh bacteria cells supplied with nutrients during the experiments were used to ensure conditions close to real situation experienced in the soil, and (v) the role of bacterial surface protein;

The bacteria transport through variably saturated porous media was directly related to water content. The trend observed for both strains was that water content decrease inside the porous media led to a decreasing of the cells effluent concentration and an increasing amount of retained bacteria. This effect was more pronounced for hydrophobic bacteria. Concerning the retention profile it was established that the bacteria location inside the packing did not follow a disposition predictable with classical filtration theory. Most of the bacteria were found in the first centimetres below the inlet and monotonically decreased along the column depth. This effect was directly related to the packing water content and the bacteria hydrophobicity: the decrease of the water content lead to a higher concentration of bacteria close to the column inlet and this effect was more pronounced in the case of more hydrophobic bacteria. According to the “straining” model developed by *Bradford et al. 2003*, at fully saturation the more hydrophobic strain (*Rhodococcus rhodochrous*) showed a higher adhesion rate (k_a) compared with the hydrophilic one (*Deinococcus radiodurans*) [47]. When diminishing the saturation, a continuous increasing in k_a for *Deinococcus* only a small increasing for *Rhodococcus* was observed.

Contrary to *Rhodococcus* the straining rate (k_{str}) strongly increased with the decrease of moisture content. The results highlight the fact that for the hydrophilic strain, adhesion was the main removal mechanism; in particular the air-water-solid interface appeared to be an attractive site for the cells, while the main removal mechanism for the hydrophobic strain rather was straining: Due to their aggregation behaviour the cells were filtered out from the solution bulk. The coefficients resulting from the fitting using the dual deposition mode model showed that the fraction of bacteria with a k_{fast} increased with a decreasing of saturation. Consistent with the hydrophobicity values for all the saturations observed, *Rhodococcus* always showed the higher adhesion rates (both k_{fast} and k_{slow}) compared with *Deinococcus*. For both strains k_{fast} and k_{slow} increased with decreasing saturation.

The packing mean grain size is an important factor, which strongly determines physical processes during bacteria transport. Different pore sizes led to a different interaction between the bacteria and the grain surfaces. In the case of fine sand (330 μ m) a strong filtering-out effect in the first centimetres after the inlet was observed causing a strongly reduced bacteria transport. For the coarse sand (607 μ m) the bacteria/sand surface interaction was reduced and nearly all the cells were able to pass the packing. The fitted parameter calculated with the “straining” model showed that both the adhesion rate (k_a) and the straining rate (k_{str}) increased with grain size decrease. In the fine sand, due to the smaller pore volume, the bacteria and the grain surfaces were in close contact and a strong interaction was present, so both adhesion and straining were enhanced. In the 607 μ m case, due to the larger average pore diameter the bacteria were allowed to be transported in the pore vein causing a weaker collector-bacteria interaction and consequently less attachment. By applying the “dual deposition mode model”, two different approaches were used to fit the experimental data concerning the fine sand. In the first case two *discrete* rate coefficients, k_{fast} and k_{slow} were used and both fitting parameters increased with decreasing sand grain size. These results were consistent with the fitting parameter resulted in the straining model where k_a and k_{str} respectively increased with sand grain decrease. In the second case only one attachment rate was supposed to be the important for bacteria adhesion through this porous system. These results clearly highlighted the fact that for 330 μ m grain size, straining played a significant role concerning transport.

Bacteria which were metabolically active and able to divide during transport showed different behaviour compared with *stationary phase* bacteria. Bacteria in the *log* phase were retained more than in the *stationary* phase and a continuous cell release in the outflow was observed

after the breakthrough curve. This phenomenon was attributed to cell division. During the transport, bacteria in the active phase did not show evidence of blocking. This result is consistent with the mechanism of biofilm formation: The adhesion of one cell layer does not prevent successive cell adhesion and layering. The straining model successfully fitted the experimental data. The strained cells were considered able to be remobilized due to the growth and no blocking was supposed. In the resulting coefficients both values the k_a and k_{str} increased with the saturation decrease and both the calculated breakthrough curve and retention profile described the experimental data.

The growing cells showed increasing hydrophobicity during the *log* phase. This effect was attributed to changes in the amount and type of the protein present on the bacteria surface. Chemically treated bacteria without protein showed less adhesion to the sand surface and transport was enhanced by this treatment. According to the “straining” model the enzymatically treated bacteria showed a decrease in the adhesion rate k_a while the k_{str} value was not effected. Using the ddmm both the k_{fast} and k_{slow} values decreased in the case of the treated bacteria. These results were consistent with the resulting coefficients calculated with the straining model. Further experimentation should be performed under these conditions and with other strains in order to better understand the effects of protein and eventually protein associated macromolecules on the bacteria adhesion to the surfaces.

In this work purified quartz sand was used as the model matrix. The experimental system used allowed us to keep the experimental conditions under control, but the results achieved have a limited application to real systems. In soil and aquifers heterogeneity factors such as the presence of organic material and preferential flow paths influence bacteria movement. Thus, for a better understanding of the key mechanisms of cell transport in the vadose zone, experiments with a natural undisturbed soil column should be performed. Besides this an experimental scale-up would be necessary to better estimate the cells transversal movement. An interesting extension of these experiments could be achieved using metabolically active cells in undisturbed unsaturated soil using a lysimeter. This data could be the basis for further model development.

List of References

- 1) Wan, J., Wilson J. L, Visualization of the role of the gas-water interface on the fate and transport of colloids in porous media, *Water Resour. Res.* (1994). 30, 11-23.
- 2) Wan, J., Wilson J. L, and Kieft T. L. Influence of the gas water interface on transport of micro-organisms through unsaturated porous media. *Appl. Envir. Microbiol.* (1994). 60, 509–516.
- 3) Wan, J., Wilson J. L, Colloid transport in unsaturated porous media. *Water Resour. Res.* (1994). 30, 857-864,
- 4) Wan, J., and Tokunaga T. K. Film straining of colloids in unsaturated porous media: Conceptual model and experimental testing. *Environ. Sci. and Technol.* (1997). 31, 2413–2420.
- 5) Powelson, D. K., and A. L. Mills. Bacterial enrichment at the gas-water interface of a laboratory apparatus. *Appl. Environ. Microbiol.* (1996). 62, 2593-2597.
- 6) Schaefer, A., Ustohal P., Harms H., Stauffer F., Dracos T., and Zehnder A. J. B. Transport of bacteria in unsaturated porous media. *J. Contam. Hydrol.* (1998). 33, 149-169.
- 7) Unc, A., and M. J. Goss. Movement of faecal bacteria through the vadose zone. *Water, Air, and Soil Pollution* (2003). 149, 327-337.
- 8) Crist, J.T., J.F. McCarthy, Y. Zevi, P. Baveye, J.A. Throop, and T.S. Steenhuis. Pore-scale visualization of colloid transport and retention in partly saturated porous media. *Vadose Zone J.* (2004). 3, 444–450.
- 9) Crist, J. T., Y. Zevi, J. F. McCarthy, J. A. Throop, and T. S. Steenhuis. Transport and Retention Mechanisms of Colloids in Partially Saturated Porous Media. *Vadose Zone Journal* (2005). 4, 184-195.
- 10) Jewett D. G., Logan B. E., Arnold R. G. Comment on Application of Clean-Bed Filtration Theory to Bacterial Deposition in Porous Media. *Environmental Science Technology* (1995). 27(5), 984-985,199

- 11) Jewett D. G., Logan B. E., Arnold R. G., and Bales R. C. Transport of *Pseudomonas fluorescens* strain P17 through quartz sand columns as a function of water content. *J. Contam. Hydrol.* (1998). 36, 73–89.
- 12) Van Loosdrecht, M. C. M., Lyklema J., Norde W., Schraa G., and Zehnder A. J. B. The role of bacterial cell wall hydrophobicity in cell attachment. *Appl. Envir. Microbiol.* (1987). 53, 1893-1897.
- 13) Van Loosdrecht, M. C. M., Lyklema J., Norde W., Schraa G., and Zehnder A. J. B. Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. *Appl. Environ. Microbiol.* (1987). 53, 1898-1901.
- 14) Rijnaarts, H.H.M., W. Norde, J. Lyklema, and A.J.B. Zehnder DLVO and steric contributions to bacterial deposition in media of different ionic strengths. *Colloids and Surfaces B: Biointerfaces* (1999). 14, 179-195.
- 15) Rijnaarts, H. H. M., W. Norde, E. J. Bouwer, J. Lyklema, and A. J. B. Zehnder. Reversibility and mechanism of bacterial adhesion. *Colloids and Surfaces B: Biointerfaces* (1995). 4(1), 1-63.
- 16) Norde W. and J. Lyklema. Protein adsorption and bacterial adhesion to solid surfaces: a colloid-chemical approach. *Colloids and Surfaces* (1989). 38, 1-13.
- 17) Sanin, S.L., Sanin F.D., Bryers J.D. Effect of starvation on the Adhesive Properties of xenobiotic degrading Bacteria. *Process Biochemistry* (2003). 38, 909-914.
- 18) Fontes, D. E.; Mills, A. L.; Hornberger, G. M.; Herman, J.S. Physical and chemical factors influencing transport of microorganisms through porous media. *Appl. Environ. Microbiol.* (1991). 57, 2473-2481.
- 19) Sharma P. K, McInerney M. J. Effect of grain size on bacterial penetration, reproduction and metabolic activity in porous media glass bead chambers. *Appl. Environ. Microbiol.* (1994). 60, 1481-1486.
- 20) Sharma P. K, McInerney M. J, Knapp R. M. In situ growth and activity and modes of penetration of *Escherichia coli* in unconsolidated porous materials. *Appl Environ Microbiol.* (1993). 59, 3686–94.
- 21) Smets, B.F., Grasso, D., Engwall, M.A., and Machinist, B.J. Surface physicochemical properties of *Pseudomonas fluorescens* and impact on adhesion and transport through porous media. *Colloids Surf. B: Biointerfaces* (1999). 14, 121–139.

- 22) Thomas D. and Y Surdin-Kerjan. Metabolism of sulfur amino acids in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* (1997). 61, 503-532.
- 23) Castellanos T., Ascencio F., Bashan Y. Starvation-induced changes in the cell surface of *Azospirillum lipoferum*. *FEMS Microbiology Ecology* (2000). 33, 1-9.
- 24) Jenneman Gary E, Michael J. McNerney, and Roy M. Knapp Microbial Penetration through Nutrient-Saturated Berea sandstone. *Appl Environ Microbiol.* (1985). 50(2), 383–391.
- 25) Reynolds P.J., P. Sharma, G. E. Jenneman, and M J McNerney. Mechanisms of microbial movement in subsurface materials. *Appl Environ Microbiol.* (1989). 55(9), 2280–2286.
- 26) Fattom, A. and Shilo M. Hydrophobicity as an adhesion mechanism of benthic cyanobacteria. *Appl. Environ. Microbiol.* (1984). 47, 135-143.
- 27) Malmqvist T. Bacterial hydrophobicity measured as partition of palmitic acid between the two immiscible phases of cell surface and buffer. *Acta Pathol. Microbiol. Immunol. Scand. [B]* (1983). 91(1), 69–73.
- 28) Neu TR. Significance of bacteria surface active compounds in interactions of bacteria with interfaces. *Microbiological reviews* (1996) 60, 151-166.
- 29) Ginn, T.R., Wood, B.D., Nelson, K.E., Scheibe, T.D., Murphy, E.M. and Clement, T.P. Processes in microbial transport in the natural subsurface. *Advances in Water Resources* (2002). 25, 1017-1042.
- 30) Johnson W. P., M J. Martin, M. J. Gross and Bruce E. Logan. Facilitation of bacterial transport through porous media by changes in solution and surface properties. *Colloids and surfaces A: Physiochem. Eng. Aspects* (1996). 107, 263-271.
- 31) DeFlaun M.F, A.S. Tanzer, A.L. McAteer, B. Marshall and S.B. Levy .Development of an adhesion assay and characterization of an adhesion-deficient mutant of *Pseudomonas fluorescens*. *Applied and Environmental Microbiology* (1990). 56, 112-119.
- 32) Salerno M.B., Logan, B.E., Velegol, D. Importance of Molecular Details in Predicting Bacterial Adhesion to Hydrophobic Surfaces. *Langmuir* (2004). 20, 10625-10629.

- 33) Samuelson P., E. Gunneriusson, P Nygren and S. Ståhl. Display of proteins on bacteria. *Journal of Biotechnology* (2002). 96, 129-154.
- 34) Caccavo, F. Jr. Protein-Mediated Adhesion of the Dissimilatory Fe (III)-Reducing Bacterium *Shewanella alga* BrY to Hydrous Ferric Oxide. *Appl. Environ. Microbiol.* (1999). 65, 5017-5022.
- 35) Dignac M. F., V. Urbain, D. Rybacki, A. Bruchet, D. Snidaro and P. Scribe. Chemical description of extracellular polymers: implication on activated sludge floc. *Water Science and Technology* (1998). 38, 45-53.
- 36) Huysman F. and Verstraete W. Water-facilitated transport of bacteria in unsaturated soil columns: Influence of cell surface hydrophobicity and soil. *Soil Biol. Biochem.* (1993). 25, 83-90.
- 37) Logan M.B. Transport and fate of *Cryptosporidium parvum* oocysts in intermittent sand filters. *Wat. Res.* (2001). 35, 18, 4359–4369.
- 38) Lenhart, J.J., and J.E. Saiers. Transport of silica colloids through unsaturated porous media: Experimental results and model comparisons. *Environ. Sci. Technol.* (2002). 36, 769–777.
- 39) Chu, Y., Y. Jin, M. Flury, and M.V. Yates. Mechanisms of virus removal during transport in unsaturated porous media. *Water Resour. Res.* (2001). 37(2), 253–264.
- 40) Bolster Carl H., A. L. Mills, G. M. Hornberger, J. S. Herman. Effect of surface coatings, grain size, and ionic strength on the maximum attainable coverage of bacteria on sand surfaces. *Journal of Contaminant Hydrology* (2001). 50, 287–305
- 41) Tufenkji, N. and Elimelech, M. Breakdown of Colloid Filtration Theory: Role of Secondary Energy Minimum and Surface Charge Heterogeneities. *Langmuir* (2005). 21, 841–852.
- 42) Tufenkji, N., G. F. Miller, J. N. Ryan, R. W. Harvey, and M. Elimelech Transport of *Cryptosporidium* Oocysts in Porous Media: Role of Straining and Physicochemical Filtration. *Environ. Sci. Technol.* (2004). 38, 5932-5938.

- 43) Tufenkji, N. and Elimelech, M. Spatial Distributions of *Cryptosporidium* Oocysts in Porous Media: Evidence for Dual Mode Deposition. *Environ. Sci. Technol.* (2005). 39,3620–3629.
- 44) Tufenkji, N. and Elimelech, M. Correlation Equation for Predicting Single-Collector Efficiency in Physicochemical Filtration in Saturated Porous Media. *Environ.Sci.Technol.* (2004). 38, 529-536.
- 45) Tufenkji, N. and Elimelech, M. Deviation from the Classical Colloid Filtration Theory in the Presence of Repulsive DLVO Interactions. *Langmuir* (2004). 20, 10818-10828.
- 46) Tufenkji, N., Redman J. A and Elimelech M. Interpreting Deposition Patterns of Microbial Particles in Laboratory-Scale Column Experiments. *Environ. Sci. Technol.* (2001). 37, 616-623.
- 47) Bradford, S. A., Šimůnek J., Bettahar M., Van Genuchten M. Th., and Yates S. R. Modeling colloid attachment, straining and exclusion in saturated porous media. *Environ. Sci. Technol.* (2003). 37, 2242–2250.
- 48) Bradford, S. A., Yates S. R., Bettahar M., and Šimůnek J. Physical factors affecting the transport and fate of colloids in saturated porous media. *Water Resources Research* (2002). 38(12), 1327. doi: 10.1029/2002WR001340.
- 49) Rosenberg, M., Rosenberg E., and Gutnick D. Bacterial adherence to hydrocarbons. In R.C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent (ed.), *Microbial adhesion to surfaces*. Society of Chemical Industry, London. (1980).
- 50) Selim L. Sanin, F. Dilek Sanin and James D. Bryers. Effect of starvation on the adhesive properties of xenobiotic degrading bacteria. *Process biochemistry* (2003). 38, 909-914.
- 51) Ngo-Thi N. A, C. Kirschner and D. Naumann. Characterization and identification of microorganisms by FT-IR microspectrometry. *Journal of Molecular Structure* (2003). 661-662, 371-380.
- 52) Sharma P. K., A. Das, K. Hanumantha Rao and K. S. E. Forssbe. Surface characterization of *Acidithiobacillus ferrooxidans* cells grown under different conditions *Hydrometallurgy* (2003). 71, 285-292.
- 53) Flint, S., J. Palmer, K. Bloemen, J. Brooks, and R. Crawford. The growth of *Bacillus stearothermophilus* on stainless steel. *J. Appl. Microbiol.* (2001). 90, 151-157.

- 54) Klauth, P., R. Wilhelm, E. Klumpp, L. Poschen, and J. Groeneweg. Enumeration of soil bacteria with the green fluorescent nucleic acid dye Sytox green in the presence of soil particles. *Journal of Microbiological Methods* (2004). 59(2), 189–198.
- 55) Hermansson, M. The DLVO theory in microbial adhesion. *Colloids Surf. B* (1999). 14, 105-119.
- 56) Foppen J.W.A, A. Mporokoso, J.F. Schijven. Determining straining of *Escherichia coli* from breakthrough curves. *Journal of contaminant hydrology* (2005). 76, 191-210.
- 57) Kästner, M.. Anreicherung und Isolierung von Chlorkohlenwasserstoffe abbauenden Mikroorganismen unter verschiedenen physiologischen Bedingungen - Abbaukinetiken und Test auf technische Nutzbarkeit zur Sanierung kontaminierter Grundwässer. *Dissertation an der Technischen Universität Braunschweig*. (1989).
- 58) Chen G. and M. Flury. Retention of mineral colloids in unsaturated porous media as related to their surface properties. *Colloids and surfaces A: physicochem. Eng. Aspects* (2005). 256, 207-216.
- 59) Walker, S.L., Hill, J., Redman, J.A. and Elimelech, M., The Influence of Growth Phase on Adhesion Kinetics of *Escherichia coli* D21g. *Applied and Environmental Microbiology* (2005). 71, 3093-3099.
- 60) Yao, K., M. T. Habibian and C. R. O'melia. Water and wastewater filtration: Concepts and applications. *Environ. Sci. Technol.* (1971). 1105-I 112.
- 61) Rajagopalan, R., and C. Tien. Trajectory analysis of deep-bed filtration with the sphere-in-a-cell porous media model. *AIChE J.* (1976). 22, 523-533.
- 62) Johnson, W. P., M. Tong, and X. Li. Colloid Deposition in Environmental Porous Media: Deviation from Existing Theory is the Norm, Not the Exception. *EOS* (2005). 86(18), 179-180.
- 63) Šimůnek, J., Van Genuchten M. Th., and Šejna M. The HYDRUS-1D software package for simulating the one-dimensional movement of water, heat, and multiple solutes in variably saturated media. Version 3.0, HYDRUS Software Series 1. (2005). Department of Environmental Sciences, University of California Riverside, Riverside, CA, 270 pp.
- 64) Adamczyk, Z., Siwek B., Zembala M., and Belouschek P. Kinetics of localized adsorption of colloid particles. *Adv. Colloid Int. Sci.* (1994). 48, 151–280.

Appendix I

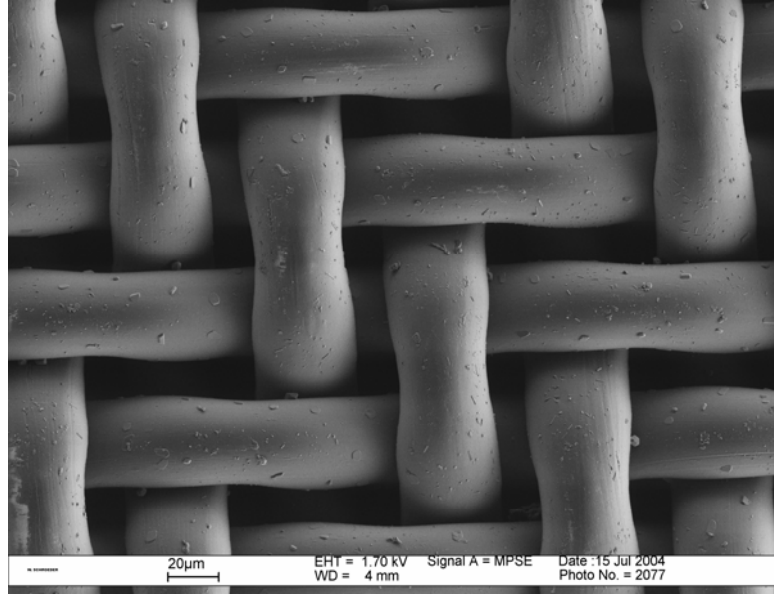


Figure A1. Suction membrane (polyester fabric, mesh size 15 μm).



Figure A2. Tensiometer with 1 μM membrane.

Salt tracer concentration

The intensity of the electrical current I [A] in a probe formed by two electrodes plunged into an aqueous solution of dissociating salts depends on:

$$I = I(A_e, I_e, U, T, c_m) \quad (23)$$

Where A_e denotes the surface area of the electrodes [m^2], I_e the distance between them [m], U is the potential difference (voltage) [V], T is the temperature [K] and c_m the molar concentration

of the solute[mol m^{-3}]. For a probe of fixed geometry kept at a constant voltage and temperature, the relationship reduces to $I=I(c_m)$. In diluted solutions, the dissociated ions are completely mobile and the current intensity is linearly dependent on the total amount of charges available in the solute. Applied to a dissociating tracer dissolved in solvent containing dissociated ions in background, one obtains:

$$\kappa = \frac{IC_p}{U} = GC_p = \lambda_t c_t + \lambda_b c_b \rightarrow c_t = \frac{\kappa - \lambda_b c_b}{\lambda_t} \quad (24)$$

In (24) κ refers to the specific electrical conductivity [S m^{-1}], G is the electrical conductivity [S], λ_b, λ_t denote the molar conductivities of the tracer and the background [$\text{S m}^2 \text{mol}^{-1}$], c_t, c_b are the molar concentrations of the tracer and the background, C_p is a probe constant [m^{-1}] depending on the geometry and arrangement of the electrodes. Since the relationship between the electrical conductivity G and the salt concentration is linear, for the injected solution, G_0 was measured as reference and the tracer breakthrough curve was expressed as G/G_0 .



Figure A3. Conductivity probe, *WTW D823662 Heilheim L*.

The Lambert-Beer-Bouguer law describes the reduction of the light intensity due to its passage through a transparent medium containing dispersed particles.

$$I = I_0 \times \exp(-\alpha d) = I_0 \times \exp(-\varepsilon cd) \quad (25)$$

Where I_0 denotes the unreduced light intensity (in pure liquid), I the light intensity reduced due to the suspended bacteria, α is the absorption coefficient [m^{-1}], and d the thickness of the absorbing medium. The extinction coefficient is denoted by ε [$\text{m}^2 \text{kg}^{-1}$] and c corresponds to the concentration of the suspended bacteria [kg m^{-3}]. The output quantity of the photometer was the extinction E [-], defined as follows:

$$E = \varepsilon cd = \ln\left(\frac{I_0}{I}\right) = \ln\left(\frac{I_0}{I_1}\right) - \left(\frac{I}{I_1}\right) \quad (26)$$

Where I_0 , I_1 are the calibration light intensities in pure water and at the maximum bacteria concentration, respectively.

TOC Analysis

The bacteria concentration inside the sand was detected using the TOC technique. The bacteria amount was measured as organic carbon content. All the molecules, with the exception of dipolar species such as O_2 , N_2 , and H_2 absorb in the infrared region. Because no two compounds absorb the same way, infrared spectrometry is an extremely useful method for qualitative and quantitative analysis. As radiant energy is projected through the sample material, an infrared absorption spectrum is produced. Since all molecules have a characteristic spectrum, the identity and quantity of a compound can be determined. This principle is normally applied to determine in a sample the organic carbon content. By analyzing the sample in an oxidizing atmosphere, all forms of carbon are converted to CO_2 organic forms produce H_2O as well as CO_2 when oxidized. Therefore, the presence of organic carbon may be verified by finding coincident peaks in H_2O and CO_2 . After the bacteria solution passage through the column the microbial concentration inside the sand was related with the amount of organic carbon. The RC-412 Multiphase Carbon, Hydrogen and Moisture Determinator were used for this aim. The RC-412 employs a furnace control system that permits the temperature of the furnace to be stopped and ramped. The temperature profile has been programmed as shown in the figure A4.

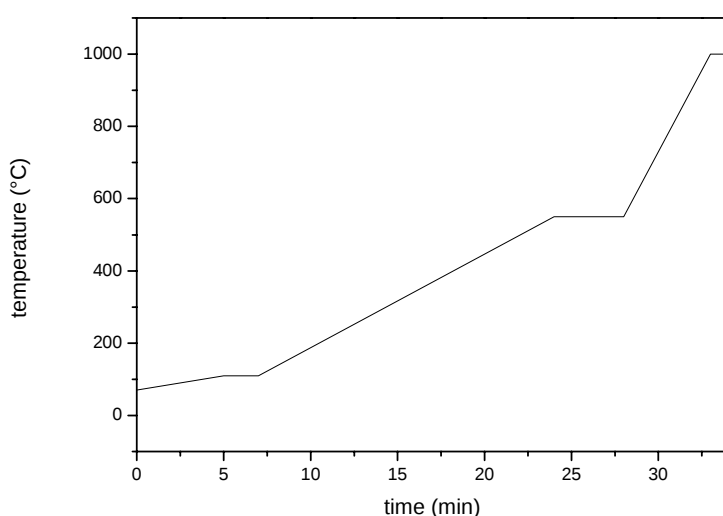


Figure A4. Temperature profile inside the furnace for total organic carbon content detection.

After combustion tube the measured gas passes through an infrared detector cell, where the sources of infrared energy are emitted using a nichrome wire heated to 850°C.

Gasses absorb the infrared radiations they pass through the cell chamber, producing a spectrum. The detector responds to energy changes between the carrier gas and the measured gas to ultimately determine the concentration of CO₂. The cell output decreases proportionally with the amount of carbon as CO₂ present in the IR cell. After measurement the results are displayed as percent carbon.

The Coulter Principle

The Coulter Principle also known as the Electrical Sensing Zone (or ESZ) method has become the accepted "Reference Method" for particle size analysis. The Coulter Principle often serves as a reference in the evaluation of both other particle size analyzers and sizing techniques. The Coulter method of sizing and counting particles is based on measurable changes in electrical resistance produced by nonconductive particles suspended in an electrolyte. In the ESZ method a suspension is made to flow through a small cylindrical opening (the aperture) separating two electrodes between which an electric current flow. Although the magnitude of this current may be small, (typically about 1 mA), the resistance, or “pinch”, created by the restriction separating

the electrodes produces a considerable current density within the aperture. As each particle passes through the aperture (or “sensing zone”) it displaces its own volume of conducting liquid, momentarily increasing the impedance of the aperture. This change in impedance produces a tiny but proportional current flow into an amplifier that converts the current fluctuation into a voltage pulse large enough to measure accurately. The Coulter Principle states that the amplitude of this pulse is directly proportional to the volume of the particle that produced it. Scaling these pulse heights in volume units enables a size spectrum to be acquired and displayed. In addition, if a metering apparatus is used to draw a known volume of the suspension through the aperture, a count of the number of pulses will yield the concentration of particles per unit volume in the suspension. Data is acquired and processed using proprietary DSP circuitry and may be archived, displayed and reanalyzed on a PC.

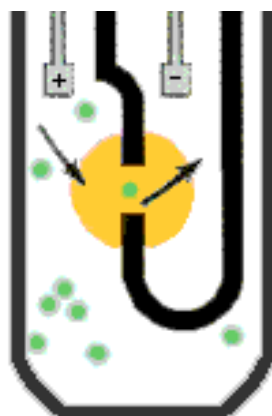


Figure A5. Schematic illustration of the Coulter Principle applied in the Multisizer 3.

The Z-potential of colloidal particles is determined using the rate at which these particles move in a known electric field. The colloid to be measured is placed in an electrophoresis chamber consisting of two electrode compartments and a connecting chamber. A voltage is applied between two electrodes, one located in each compartment. The applied voltage produces a uniform electric field in the connecting chamber and the charged particles respond by moving towards one or the other electrode. The speed of the particles is directly proportional to the magnitude of the particle charge or zeta potential.

Zeta potential principle

Zeta potential is the electrical potential that exists at the *shear plane* of a particle, which is some small distance from the surface. Colloidal particles dispersed in a solution are electrically charged due to their ionic characteristics and dipolar attributes. The development of a net charge at the particle surface affects the distribution of ions in the neighbouring interfacial region, resulting in an increased concentration of counter ions (ion of charge opposite to that of the particles) close to the surface. Each particle dispersed in a solution is surrounded by oppositely charged ions called fixed layer. Outside the fixed layer, there are varying compositions of ions of opposite polarities, forming a cloud-like area. Thus an electrical double layer is formed in the region of the particle-liquid interface. When a voltage is applied to the solution in which particles are dispersed, particles are attracted to the electrode of the opposite polarity, accompanied by the fixed layer and part of the diffuse double layer. The potential at the boundary between this unit, that is to say at the above-mentioned *shear plane* between the particle with its ion atmosphere and the surrounding medium, is known as the Zeta Potential. Zeta potential is a function of the surface charge of a particle, any adsorbed layer at the interface and the nature and composition of the surrounding medium in which the particle is suspended. Zeta potential can be calculated with the following *Smoluchowski's* formula:

$$\zeta = \frac{4\pi\eta}{\varepsilon} \times U \times 300 \times 300 \times 1000 \quad (27)$$

Where ζ = zeta potential, η = viscosity of solution, ε = Dielectric constant, U = electrophoretic mobility

$$U = \frac{v}{V/L} \quad (28)$$

v = speed particle (cm/sec), V = Voltage (V), L = the distance of electrode

The principle of determining zeta potential is very simple. A controlled electric field is applied via electrodes immersed in a sample suspension and this causing the charged particles to move towards the electrode of opposite polarity. Viscous forces acting upon the moving particle tend to oppose this motion and equilibrium is rapidly established between the effects of the

electrostatic attraction and the viscosity drag. The particle therefore reaches a constant terminal velocity.

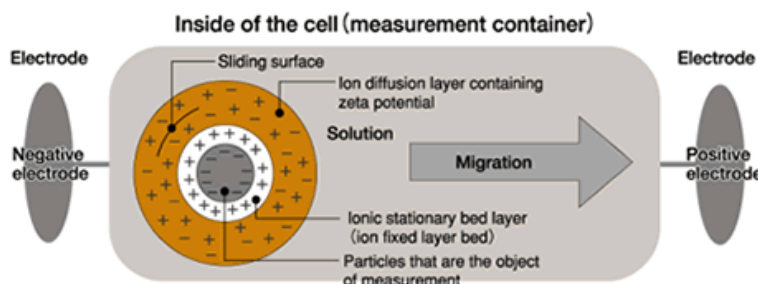


Figure A6. Scheme of the zeta potential cell.

Fourier Transform infrared (FT-IR) principles.

In a molecule, the atoms are not held rigidly apart. Instead they can move, as if they are attached by a spring of equilibrium separation R_e . This bond can either bend or stretch. If the bond is subjected to infrared radiation of a specific frequency (between $300 - 4000 \text{ cm}^{-1}$), it will absorb the energy, and the bond will move from the lowest vibrational state, to the next highest. In a simple diatomic molecule, there is only one direction of vibrating, stretching. This means there is only one band of infrared absorption. Weaker bonds require less energy, as if the bonds are springs of different strengths. If there are more atoms, there will be more bonds, and therefore more modes of vibrations. This will produce a more complicated spectrum. There is one important restriction; the molecule will only absorb radiation if the vibration is accompanied by a change in the dipole moment of the molecule. A dipole occurs when there is a difference of charge across a bond. If the two oppositely charged molecules get closer or further apart as the bond bends or stretches, the moment will change. To calculate the frequency of light absorbed requires *Hooke's law*:

$$\nu = \frac{1}{2\pi} \sqrt{k \frac{m_1 + m_2}{m_1 m_2}} \quad (29)$$

Appendix II

Internal reflection spectroscopy

During the studies of total reflecting light at the interface between two media of different refractive indices, it was discovered that an evanescent wave extends in the less dense medium beyond the reflecting interface. The internal reflection spectroscopy is based on the optical absorption spectra which could conveniently be obtained by measuring the interaction of the evanescent wave with the external less dense medium. In this technique, the sample is placed in contact with the internal reflected, generally several times, and the sample interacts with the evanescent wave resulting in the absorption of radiation by the sample at each point of reflection. The internal reflection element is made from a material with a high refractive index; zinc and germanium are the most commonly used. To obtain the total internal reflection the angle of the incident radiation θ must exceed the critical angle θ_c defined as

$$\theta_c = \sin^{-1} \frac{n_2}{n_1} \quad (30)$$

Where n_1 is the refractive index of the internal reflection element and n_2 is the refractive index of the sample.



Figure A7. The epifluorescence microscope (Nikon ECLIPSE E 1000 with a motorised and PC controlled. The images were captured with a CCD camera, Sony DXC-9100 P, and a Matrox Corona Framegrabber.

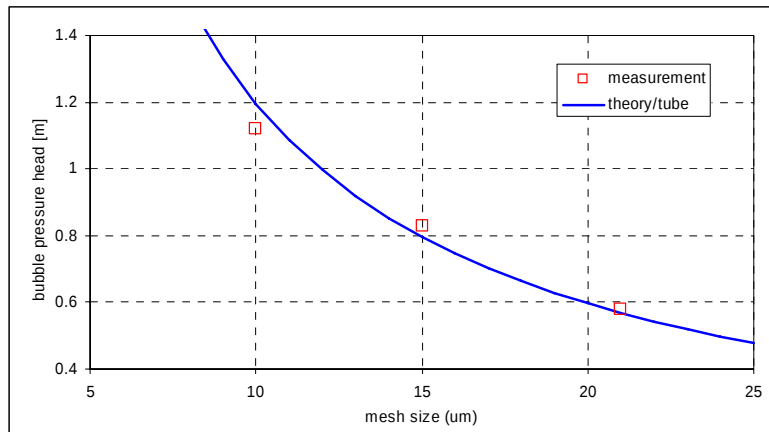


Figure A8. Polyester fabric test for air entry pressure.

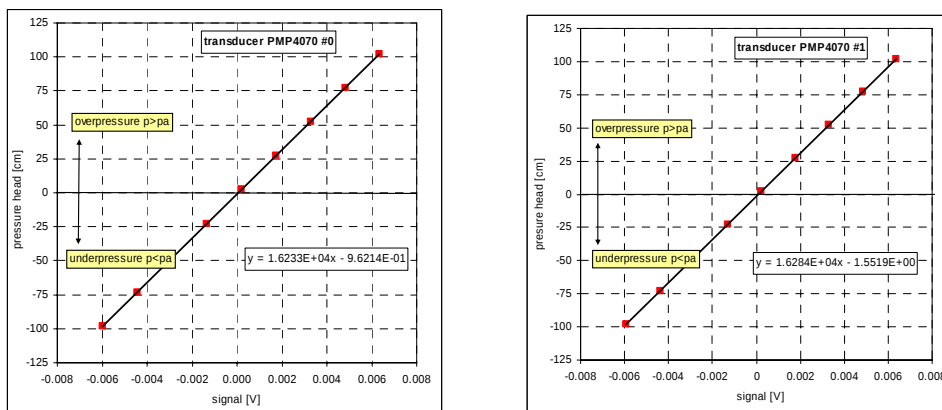


Figure A9. Pressure head acting on transducer's membrane.

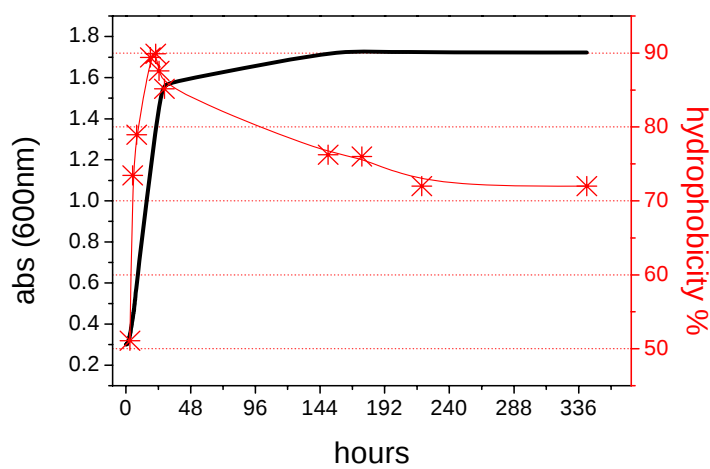


Figure A10. Hydrophobicity percentage (M.A.T.H. test) and growth curve (measured as O.D.) of *Deinococcus radiodurans* as a function of time. (*) hydrophobicity percentage.

Acknowledgement

This work has been done during my PhD research at the Institute of Chemistry and Dynamics of the Geosphere - Agrosphere of the Forschungszentrum Juelich GmbH, Juelich, Germany.

My sincerest gratitude goes to Dr. Petr Ustohal, my supervisor for the first part of this work. Thanks to him it was possible to realize and use the unsaturated system. He has been a silent shadow for all the duration of my PhD ready to support me in the hardest moments.

I would like to thank:

Dr. Scott Bradford, Prof. Jirka Simunek and Prof. Nathalie Tufenkji for the happy and rewarding time I had working in collaboration with them.

Dr. Anne Berns for her help and support and in particular because she made possible the final realization of this manuscript.

Mr. Manfred Michulitz for the I.R. measurements he performed for this work.

Mr. Gary Evans for the english editing of this work.

My family, my friends and Kevin for the support during this period.

Finally many thanks to my referees Prof. Harry Vereecken and Prof. Andreas Schaeffer for the chance to conduct this research at their institutes.

Curriculum vitae

Personal information

Name	Grazia Gargiulo
Nationality	Italian
Date of birth	01\07\1977
Place of birth	Castellammare di Stabia (Napoli)- Italy
Email	grazgar@yahoo.it

Academic background

25 th October 2002	Dottoressa in Chimica Industriale (Masters degree in Industrial Chemistry) at Bologna University (Universita' degli studi Bologna), Italy
19 th October 2006	Ph-D (Dr rer. nat.) at the RWTH Aachen, Germany

Work experience

Since July 2006	Senior Environmental Officer Environmental Protection Agency (EPA) Contaminated Land Unit Brisbane QLD, Australia
-----------------	---