Transport d'*E. coli* en milieux poreux complexes et artificiels : Expérimentations et modélisations.

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***Escherichia coli* transport in artificial and complex porous media: experiments and modelling**

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Avant propos

Le chapitre 3 a révélé l'impact des caractéristiques d'un milieu poreux sur le transport bactérien. Ainsi la forme irrégulière des grains de sables permet de coincer d'avantage de cellules que des billes de verre parfaitement sphériques. Ce résultat soulève la problématique de la simplification des conditions expérimentales par rapport à la réalité du terrain. Qu'en est-il pour des environnements encore plus complexes comme un véritable sol ? En effet dans la littérature comme pour les travaux des chapitres 2 et 3 le transport bactérien est généralement étudié dans des systèmes artificiels : billes de verre ou grains de sables de taille et de caractéristiques bien définies. En revanche l'étude du transport de cellules bactériennes dans des conditions plus réalistes est beaucoup plus rare. Des expériences ont été menées pour comparer le transport bactérien en milieu artificiel (billes de verre et sable calibré) avec le transport en milieu complexe (colonnes de sol avec des agrégats de diverses tailles et sol non remanié). Les objectifs sont d'évaluer la compétition entre les phénomènes hydrodynamiques et physicochimiques. L'ensemble des données obtenues a également permis de tester un modèle de transport bactérien en milieu poreux.

Abstract

The transport of a single negatively charged bacterial strain (*E. Coli*) was examined for columns made up of glass beads, sand, soil aggregates of various sizes and undisturbed soil. Experiments were performed at two ionic strengths relevant to environmental conditions and the water flow was adjusted so as to have similar velocities whatever the porous media porosities. Bacterial enumeration in the effluents was obtained by flow cytometry. Tracer breakthrough curves were obtained to characterize the porosity and flow patterns of the various columns. Modeling of experiments accounted for kinetic deposition and release of cells. The model offers the possibility to account for two types of sites differing by their rates (fast and slow). Tracer and cells breakthrough curves indicate that the cells are transported at a rate similar to the tracer for the glass beads and the sand columns. For the columns packed with aggregates, the bacterial cells are transported much faster than the tracer. Also, cells retention in the glass beads and the sand columns depends largely on ionic strength, while this dependence is weak in the columns packed with soil aggregates. In all the porous media, breakthrough curves display long tails revealing a slow release of bacterial cells. Modeling of the breakthrough curves indicates that retention occurs in two types of sites. One is exchanging rapidly with the flow and could correspond to stagnant flow regions or sites with weak interaction. These sites are responsible for the curvature of the breakthrough curve immediately before the tail begins. The second type is associated with classical deposition and characterized by slower rates and in particular release from these sites explains the long tails.

1. Introduction

Understanding and predicting bacterial transport in porous media has become an important subject of research for its applications in domains such as water source protection and bioremediation (Fontes *et al.* 1991). Since soils are supposed to act as a filter groundwater has been assumed to be free of pathogenic bacteria, but several contaminations of water sources with micro-organisms were possibly linked to bacterial transport through the unsaturated zone (Krapac *et al.* 2002). (Unc et Goss 2004). Controlling bacterial fate in soils has also interesting prospects for agronomic issues such as bio control with plant growth promoting strains (Amarger 2002). Concerns are also growing about the use of waste water for irrigation in Mediterranean regions suffering water shortages. Indeed waste water carrying pathogenic strains could contaminate drinking wells when used for irrigation. Thus understanding the mechanisms involved in bacterial displacement and modeling are important for practical applications. During the last two decades several models have been developed to describe micro organism transport in porous media (Chen et Strevett 2003; Johnson *et al.* 1995; Sen *et al.* 2005; Tian *et al.* 2002; Tufenkji *et al.* 2003). Among all the phenomena involved in bacteria displacement, one important mechanism is the deposition on the solid phase. This has been studied for a long time and the deposition rate has been shown to depend on several parameters among which: flow velocity, solid grain diameter, bacterial dimension, collector efficiency, etc… Correlations have been proposed to relate collector efficiency to elementary mechanisms controlling colloid approach to the solid (Rajagopalan 1976; Tufenkji et Elimelech 2004). In simple porous media made up of glass beads or sand grains, and under favourable conditions (i.e. absence of repulsive electrostatic barrier) the deposition rate has been shown to adequately predict bacterial retention. However, it remains the problem of estimating the collision efficiency under unfavourable conditions (Dong *et al.* 2002). Many experiments have shown that retention could happen in situation presenting unfavourable conditions (repulsive electrostatic energy barrier) (Tong et Johnson 2006). Experiments have also shown that bacteria were not retained although the conditions seemed favourable (Tong *et al.* 2005). Hence, the deposition rate is still a subject of study. The release rate of previously retained bacteria is another problem that is being analyzed (Tong *et al.* 2005). As well, heterogeneity of bacterial

population properties and in particular of surface charges are also being considered to explain deposition or transport deviation from theory (Brown et Abramson 2006; Li *et al.* 2004; Simoni *et al.* 1998; Tong *et al.* 2005). Sand or glass beads columns are porous medium with very simple and homogeneous porosity patterns in which chemical effects are important determinants of bacterial displacement. Transport experiments of colloids or bacteria in porous media presenting more complicated porosity patterns are scarce (Artz *et al.* 2005; Dong *et al.* 2002; Fuller *et al.* 2000).

This paper presents transport experiments at two ionic strengths, in order to obtain favourable and unfavourable conditions for deposition, with a single bacterial strain. Porous media ranged from glass beads and sand columns to columns repacked with soil aggregates of increasing size and ultimately to undisturbed soil columns. Results are analyzed with a model previously proposed (Li *et al.* 2005) and with a model accounting for two types of deposition sites. Results show that physical mechanisms can be dominant over chemical mechanisms when the complexity of the porosity patterns increases.

2. Material and methods

2.1. Strain, plasmid and fluorescent labeling

The bacterial strain used in this study was *Escherichia coli* K12 MG1655 *ompR234* (Vidal *et al.* 1998) (PHL1314) and was kindly given by P. Lejeune (French Institute of Applied Science, Lyon, FRANCE). This strain was selected for its ability to be transported in porous media as described previously (Jacobs *et al.* 2007). PHL1314 surface tension components were described in (Jacobs *et al.* 2007). The *E. coli* strain used in this study was genetically fluorescently labeled using the pDsred-express plasmid (Clonetech, USA). Cells were transformed using the chemical TSS method (Transformation Storage Solution: PEG3350 10%, $MgCl_2$ 10mM, $MgSO_4$ 10mM, DMSO 5%). The pDsred-express plasmid inserted is carrying the *dsred* gene witch encodes for the red fluorescent protein from *Discosoma* sp. reef coral. Dsred maximum excitation wavelength is 556nm and maximum emission is 586nm. The bacterial cells were fluorescently labeled in order to perform flow cytometry enumeration. The pDsred-express plasmid also provides resistance to ampicillin antibiotic. Prior to transport

Characteristics	Glass beads	Sand	Soil (0.3-1) mm	Soil (1-3) mm	Soil (3-5) mm
Grain Size (µm)	250	230-310			
Column volume (cm^3)	294,5	294,5	294,5	294,5	294,5
Dry solid mass(g)	467	480	340	340	340
Solid density ($g.cm^3$)	2,52	2,65	2,65	2,65	2,65
Column bulk density ($g.cm^3$)	1,59	1,63	1,15	1,15	1,15
Total porosity	0,371	0,385	0,566	0,566	0,566
Inter aggregates porosity	-	-	0,342	0,342	0,342
Total pore volume (cm^3)	109,2	113,4	166	166	166
Water flux ($cm^3.mn^{-1}$)	3,3	3,6	2	2	2

Table 1: Porous media and column characteristics

experiments, bacteria were cultivated overnight in 100ml of half diluted Luria Bertani media at 30°C, 100rpm. Ampicillin (100mg.L^{-1}) was added to ensure the selection of cells carrying the pDsred-express plasmid.

2.2. Electrophoretic surface properties of cells and porous medium measurements

ζ potentials (mV) of bacteria were determined by micro-electrophoresis using a Zetaphoremeter model (II) (CAD Instrumentation, Limours, France). The electrophoretic mobility was measured with the bacteria collected after overnight culture, suspended in two NaCl ionic strength (10^{-1} and 10^{-2} mol.l^{-1}) adjusted at pH7. Bacterial cells were placed in a measurement cell under a microscope equipped with a CCD camera. Their displacements in response to an applied electric field of 8V/cm were recorded and the velocity of individual cells was calculated. The ζ potentials were calculated with the conventional Smoluchowski theory for each cell. About one hundred cells were followed at each measurement. The experiment was repeated three times for each of six separate cultures.

The electro kinetic properties of the sand and the glass beads used in transport experiments were measured by a streaming potential analyzer Zetacad (CAD Instrumentation, Limours, France) in two NaCl solutions (10^{-1} and 10^{-2} mol.l^{-1}). The zeta potential was calculated from the streaming potential experiments as described by Elimelech *et al.* (Elimelech *et al.* 2000). Due to the soil heterogeneity it was impossible to measure its zeta potential.

2.3. Saturated column experiments

The experimental setup consisted in Plexiglas columns (Ø = 50mm, length = 150mm) filled either with glass beads, sand, or soil aggregates. Porosity and porous media characteristics are listed in table 1. In the case of the soil three types of columns were realized. We used three different sizes of aggregates (0.3 to 1mm, 1 to 3mm and 3 to 5mm) to obtain columns having the same total porosity, the same total inter-aggregates porosity but different patterns of this porosity. The columns packed with the smallest aggregates displayed a fine porosity approaching that obtained with the sand. Inversely,

the columns packed with the largest aggregates displayed large inter-aggregates voids as can be met in undisturbed soils. Before packing into the columns, soil aggregates were rewetted on a suction table at a water potential of -30 hPa. Next, the aggregates were compacted by layers of 3cm in the column. The bulk densities are given in table 1. Last, the columns were vacuum saturated with sterile solutions. For glass beads and sand, the columns were filled under water. Experiments were carried out at 10^{-1}M and 10^{-2}M NaCl concentrations for the background solution. Water fluxes used are given in table1.

Bacterial cells were collected during the stationary growth stage, centrifuged for 5min at 5000tr/min and suspended in a sterile 10^{-1}M or 10^{-2}M NaCl solution. Before use, concentration of the bacterial solution was adjusted approximately to 5.10^{7} cells.ml^{-1}. Injection of the bacteria in the column lasted 10 to 12 minutes depending on the columns which corresponded to 0.3, 0.33, 0,12 pore volume for the glass bead, the sand and the soil columns, respectively. Application of bacteria was followed by the injection of 4 to 5 pore volumes of the background solution. Upward circulation of the solution through the column was obtained by mean of a peristaltic pump Ismatec SA reglo digital MS-4 (Switzerland). Each column experiment was repeated at least three times with bacteria cells from separate cultures. Bacterial concentration was measured by taking 0.5ml samples at the outlet of the column for flow cytometer immediate enumeration. Standard microspheres (Bacterial Counting Kit, Molecular Probes, Oregon, USA) were used for cytometer calibration. The flow cytometer (Beckman Coulter, EPIC XL, Fullington, USA) settings were as follows: excitation source 488nm, fluorescence detection on FL2 channel (emission 560-590nm), analysis time 1minute, no compensation was used.

Breakthrough curves with a tracer (Potassium Bromide) were also obtained for the various porous media. The breakthrough curves were measured for two purposes. First, tracer breakthrough curves reveal the porosity patterns and thus the differences between the porous medium. Second, a comparison of these curves with those obtained for bacteria is useful for revealing phenomena involved in bacteria displacement and in particular the competition between physical and chemical mechanisms. A convection dispersion model was used to analyze the breakthrough curves obtained on sand and glass beads columns. Curves obtained on columns packed with aggregates were analyzed with a two-regions (mobile-immobile water) model.

2.4. Undisturbed columns with rain simulation

In order to get closer to conditions occurring in the field, bacterial transport experiments through intact soil columns in response to a rain fall were carried out. Two undisturbed soil columns (15 cm in diameter and length of 30 cm) were extracted from the surface layer of a nearby field (Avignon, FRANCE). The soil (calcisol) extracted contained randomly distributed roots, worm holes or some stones. Soil composition is 11% clay, 37% loam and 52% sand. A rain simulator consisting of 57 hypodermic needles was used to apply water. De-ionized water was used. The application rate was 20mm/h for one column and 30mm/h for the other and lasted 1 hour for both columns. In the seconds preceding the rain, a solution containing the bacteria (10 ml and concentration of about 5. 10^8 cells.ml^{-1}) was carefully and as homogeneously as possible distributed at the soil surface. As for saturated experiments, solution samples were collected at the bottom of the column and cells enumerated with the flow cytometer. Samples were taken at regular time increments except during the decrease of the flows.

2.5. Model development

The transport models used for simulating bacterial cells displacement in porous media combine the convection dispersion equation with various representations of the attachment and detachment processes. Deposition of cells on the solid is usually modeled with a first-order equation, the rate constant of the process being either calculated with the classical correlations or fitted to breakthrough curves. Detachment kinetic of the deposited cells is included in some models (Becker *et al.* 2004; Bradford et Bettahar 2006; Chen et Strevett 2003; Mallen *et al.* 2005; Tong et Johnson 2006). Among these models, (Becker *et al.* 2004) proposed a detachment rate decreasing when the residence time of the cell on the solid increases. Instantaneous reversible adsorption is sometimes used (Chen *et al.* 2003; Chen et Strevett 2002; Mallen *et al.* 2005).

The model we developed considers that two types of sites can be involved in bacterial cells retention. As usual, we assume that there are sites where bacteria can be kinetically deposited and eventually detached. Deposition and release rates can be different. In addition, we assume there are sites or regions where bacteria can be temporarily retained but that, at these sites, they do not experience strong interaction with the solid phase, thus

Porous medium	Ionic Strength : 0.01M	Ionic Strength : 0.1M
Glass beads	94.9 (3.8)	0.85 (0.01)
Sand	90.4 (2.6)	0.0 (0.0)
Aggregates (0.3 – 1)mm	10.5 (1.5)	0.27 (0.07)
Aggregates (1 – 3)mm	40.2 (2.8)	22.25 (1.2)
Aggregates (3 – 5)mm	50.7 (6.4)	31.8 (4.9)

Table 2: Mean percentage of bacteria recovered at the outlet of the columns for the various porous media and ionic strength used.

The standard deviation is given between the parentheses.

Experiments	k(cm^3/g)	ω (h^{-1})	k_d (h^{-1})	k_r (h^{-1})	θ	θ_{tracer}	λ (cm)
GB 0.01M	0,0155 (0,009)	16,7 (11,02)	0,125 (0,01)	0,068 (0,018)	0,352 (0,015)	0,373 (0,007)	0,042
GB 0.1M	0,07 (0,0144)	5,42 (1,33)	9,545 (0,015)	0,01 (0)	0,35		
SAND 0.01M	0,0334 (0,0052)	5,93 (0,9)	0,21 (0,045)	0,098 (0,03)	0,38 (0,003)	0,37 (0,014)	0,046
SAND 0.1M	ND	ND	40	ND	0,38		
(0.3 – 1) 0.01M	0,0692 (0,03)	6,2 (3,7)	7,48 (0,7)	0,016 (0,016)	0,15	0,36	0,13
(0.3 – 1) 0.1M	0,46 (0,2)	0,115 (0,06)	12,87 (0,63)	0,03	0,244 (0,017)		
(1 – 3) 0.01M	0,0345 (0,0097)	17,2 (7)	2,67 (0,5)	0,0187 (0,003)	0,158	0,325	0,415
(1 – 3) 0.1M	0,086 (0,01)	48,2 (21,7)	7,16 (0,77)	0,03	0,15		
(3 – 5) 0.01M	0,063 (0,003)	16,5 (5,7)	2,48 (0,62)	0,063 (0,016)	0,15	0,339	0,559
(3 – 5) 0.1M	0,072 (0,01)	62,7 (35,6)	4,38 (0,44)	0,03	0,14		
Undisturbed (20 mm/h)	0	0	13,8	3,67	0,03		
Undisturbed (30 mm/h)	0	0	13,7	3,94	0,03		

Table 3: Fitted model parameters for the various experiences: θ_{tracer} is the water content fitted from the tracer experiments.

For the glass beads (GB) and sand columns the C-D model was used to analyse tracer experiments. For the columns made up of aggregates, θ_t corresponds to the mobile water fitted by the MIM model. Numbers between parentheses are SD.

making their release in the flow an easy and relatively rapid process. Exchange between the bulk flow and these retention sites is also assumed to obey a first order process. We do not consider instantaneous reversible adsorption.

Assuming that the transport is modeled by the convection dispersion equation, the mass balance equation writes:

$$\theta \frac{\partial C}{\partial t} = \theta D_h \frac{\partial^2 C}{\partial z^2} + q \frac{\partial C}{\partial z} - \rho \left(\frac{\partial S_1}{\partial t} + \frac{\partial S_2}{\partial t} \right)$$

with t time (T), z depth (L), θ water content ($L^3.L^{-3}$), ρ bulk density (M. L^{-3}), q water flow ($L^3.L^{-2}.T^{-1}$), D_h hydrodynamic dispersion ($L^{-2}.T^{-1}$), C (cells.L^{-3}) the amount of cells in the solution and S_1, S_2 (cells.M^{-1}) the quantity of bacteria on the weak interaction sites and deposited on the solid, respectively.

The equation for type 1 sites is:

$$\frac{\partial S_1}{\partial t} = \omega_1 (kC - S_1)$$

with k the partition constant ($L^3.M^{-1}$) and ω_l (T^{-1}) the rate constant for reversible exchange. For sites of the second type we have the following equation:

$$\rho \frac{\partial S_2}{\partial t} = k_d \theta C - k_r \rho f S_2$$

with k_d (T^{-1}) the rate constant for deposition, k_r (T^{-1}) the rate constant for release and f the fraction of deposited bacteria that can be released. Note that we assume that for sites of the first type (weak interaction between the cells and the solid) the rate constant is the same for attachment and detachment, while for the sites of the second type (deposition) the attachment and detachment rate constants may be different and in particular, setting k_r (T^{-1}) to 0 allows irreversible deposition. Also, setting parameter ω_l to zero, the model reduces the model proposed by (Tong et Johnson 2006).

These equations are solved with an implicit finite difference schema. The solution was coupled with a least-squares minimization algorithm for estimation of parameters.

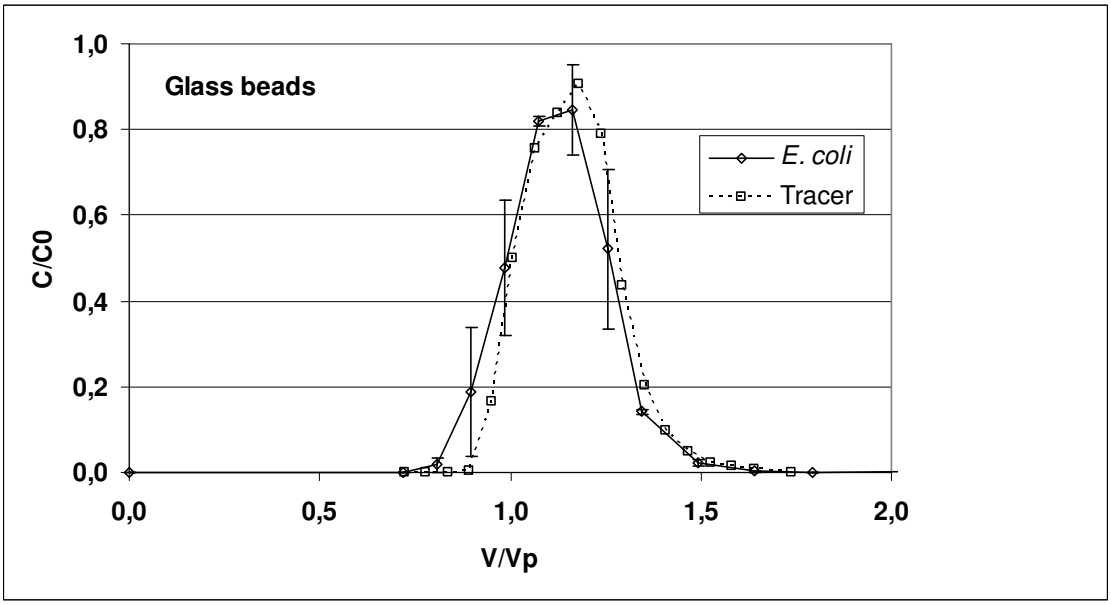

Figure 1 : Tracer and *E. coli* breakthrough curves for the glass beads columns.

Error bars indicate the range of observed cells concentrations.

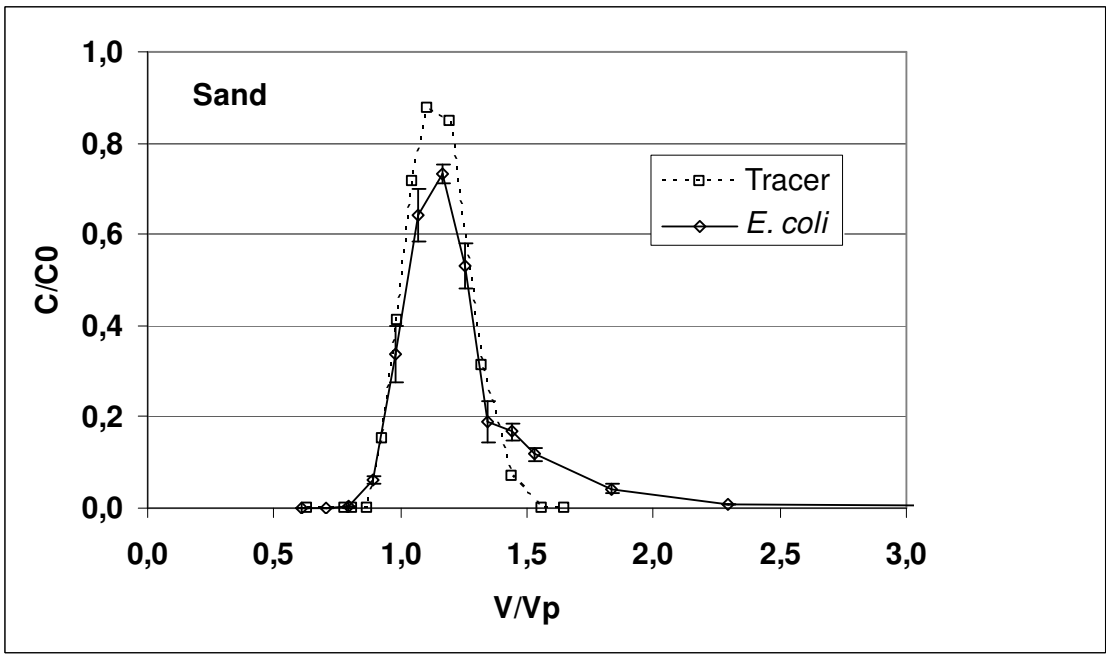

Figure 2: Tracer and *E. coli* breakthrough curves for the sand columns.

Error bars indicate the range of observed cells concentrations.

3. Results and discussion

3.1. Saturated column experiments

Whatever the porous medium and the ionic strength, bacterial cells transport experiments were very repeatable (Table 2, Fig. 1, 2, 3, 4, and 5).

The breakthrough curves recorded for the tracer in the columns made up of glass beads and sand were similar (Fig. 1, 2). This was expected as the sizes of sand grains and glass beads were almost the same. So, any differences in observed bacterial cells transport between these two porous media can only originate from differences in retention processes (intensity and/or mechanisms). For the glass beads, the bacterial cells breakthrough curves recorded at an ionic strength of 0.01M are very similar to the tracer breakthrough curves (Fig.1). Only a slight shift to the left is observed indicating a possible exclusion from a part of the porosity. Longer columns would be required to make sure that this is really the case. This differential advection has been reported for transport of an adhesion deficient bacteria through undisturbed cores (30 cm in length) (Dong *et al.* 2002) although their observations also display cases where differential advection is not noticeable. Observations reported by (Mallen *et al.* 2005) for in situ transport of *E. coli* and *P. putida* do not display any accelerated cells transport as compared to the tracer. The amount of recovered bacteria (table 2) is large. Only a small part remains in the porous medium. Filtration is not responsible for this retention (Jacobs et al. 2007). Given their negatives charges, the bacteria and the solid particles experienced high repulsive interactions. In the condition of the experiment (ionic strength and surface charge of solid and bacteria), the secondary minimum calculated with the DLVO theory is shallow (1kT) and can be easily overcome by drag forces as calculated according to the work of Bergendahl and Grasso (Bergendahl et Grasso 2000). Hence, we think that the bacteria retained in the porous media are not retained in secondary minima, but blocked at beads junctions (Choi *et al.* 2007; Johnson *et al.* 2007).

For the sand columns, the behavior is slightly different (Fig. 2). The bacterial cells breakthrough curve is similar to the tracer breakthrough curve but display a tail indicating that bacteria are temporarily retained in the porous media and are being reversibly

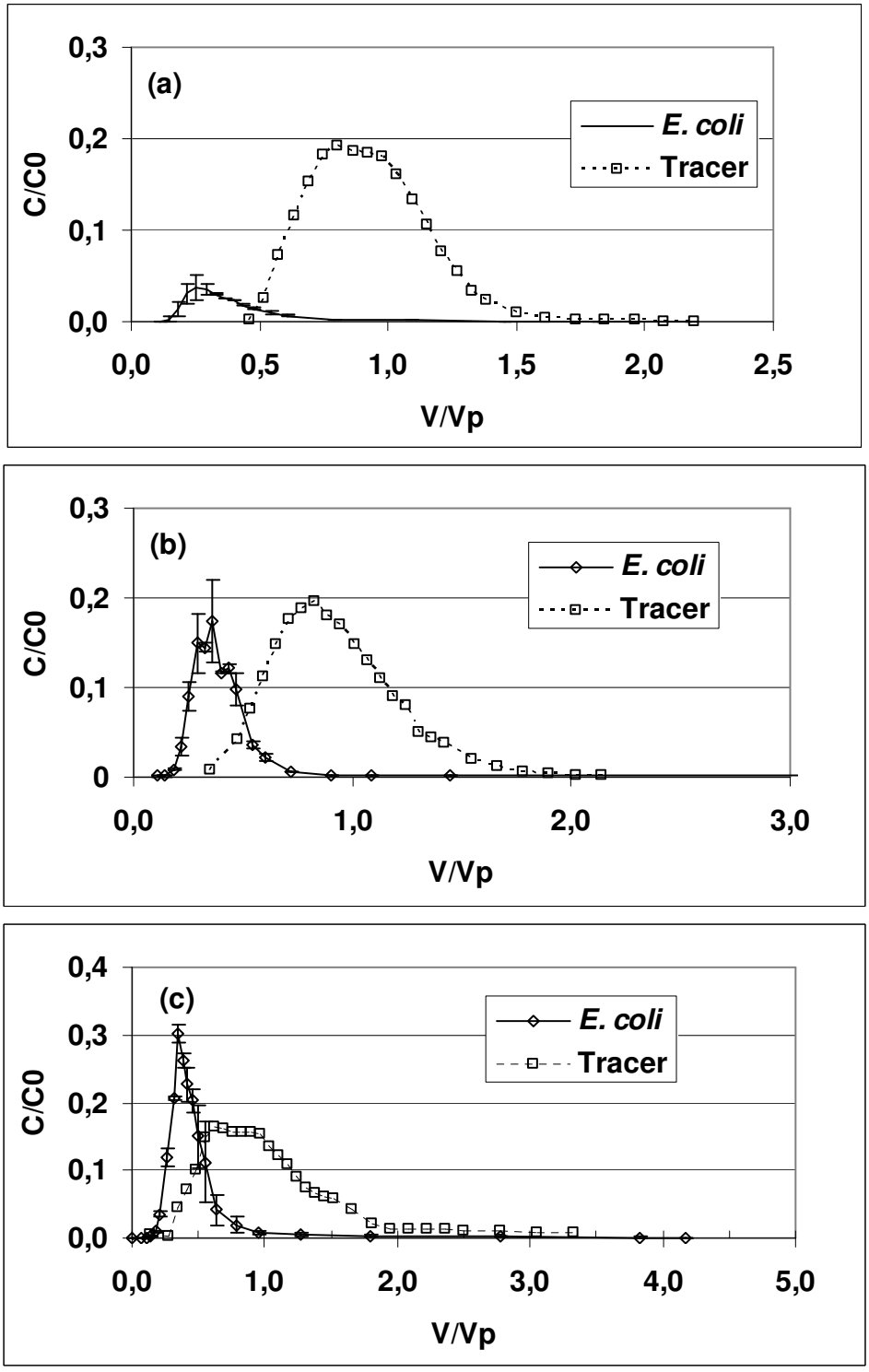

Figure 3: Tracer and *E. coli* breakthrough curves for the columns packed with aggregates.

(a) 0.3 – 1mm, (b) 1 – 3 mm, (c) 3 – 5 mm. Error bars indicate the range of observed cells concentrations.

exchanged between the bulk flow and retention regions. In addition, the amount of bacteria irreversibly retained is slightly larger than for the glass beads columns (table 2). These differences originate from the roughness properties of the sand grains that enhance retention as compared to the smooth glass beads. This behavior has already been observed at a microscopic scale (Choi *et al.* 2007) and predicted by a small scale modeling of colloids interactions with the solid phase (Johnson *et al.* 2007). The breakthrough curves indicate also that there are two retention mechanisms acting in parallel: irreversible or slowly reversible trapping revealed by the recovery percentage and relatively fast reversible exchange revealed by the shape of the curve (curvature just before the tail).

The breakthrough curves measured for the tracer in the columns made up of aggregates correspond to weakly structured porous media with early breakthrough and tailing being more and more apparent as the size of the aggregates increases (Fig. 3a, b, c). For all the columns with soil aggregates, the breakthrough curves of the cells are markedly shifted to the left of the tracer curves. This indicates that transport of cells occurs in a portion only of the soil water. Differential advection is important in these cases. The columns were packed to obtain the same intra and inter aggregate porosities. As a consequence, the first bacteria were detected at the outlet of the columns at similar times (0.1 pore volume) (Fig. 3a, b, c). Also, the curves are asymmetrical with a sharp increase followed by a tail. This indicates that a fraction of the bacteria are being exchanged between reversible retention sites and the bulk flow as for the sand columns. At the ionic strength of 0.01M, the amount of bacteria retained in the porous media depends largely on the size of the aggregates (Fig. 4, table 2). The quite large retention measured for the columns made up of small aggregates indicates the existence of favorable sites within the soil. As compared with the sand and glass beads columns, the electrostatic repulsions are probably not so important in this type of porous media. At least, one may think that there are sites with positive charges for which the interaction energy with the bacteria is negative (attraction). However, despite the presence of favorable sites, a part of the bacteria are not retained. When the size of the aggregates increases, the amount of bacteria retained decreases markedly. This indicates that retention of bacteria in a porous medium depends not only on the chemical properties of the solid and the cells, but also on the flow pattern.

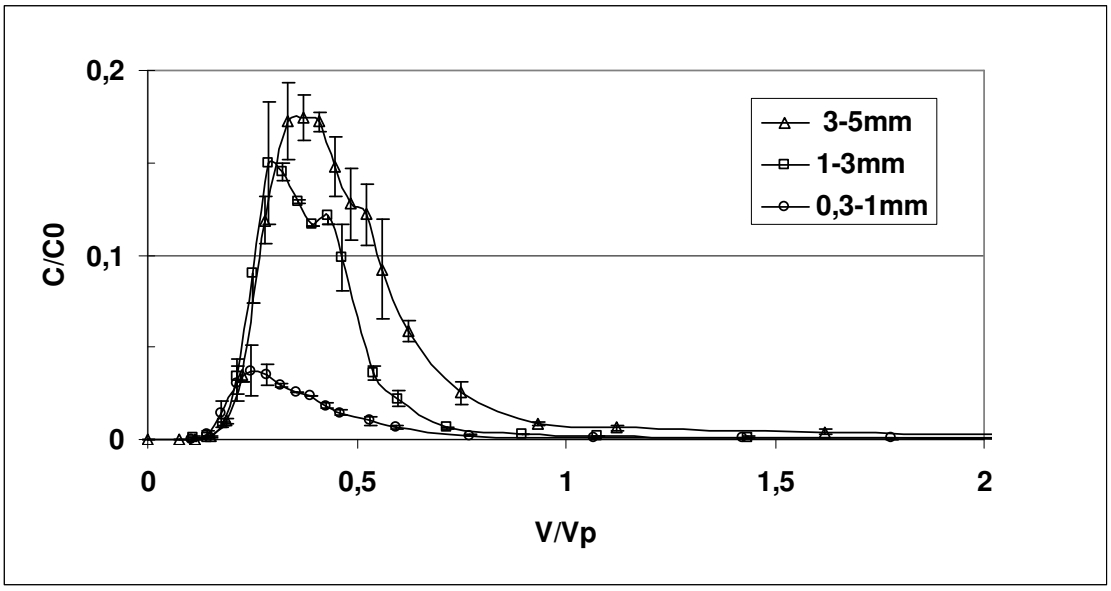

Figure 4: Comparison of E. coli breakthrough curves for the various aggregates sizes at an ionic strength of 0.01M.

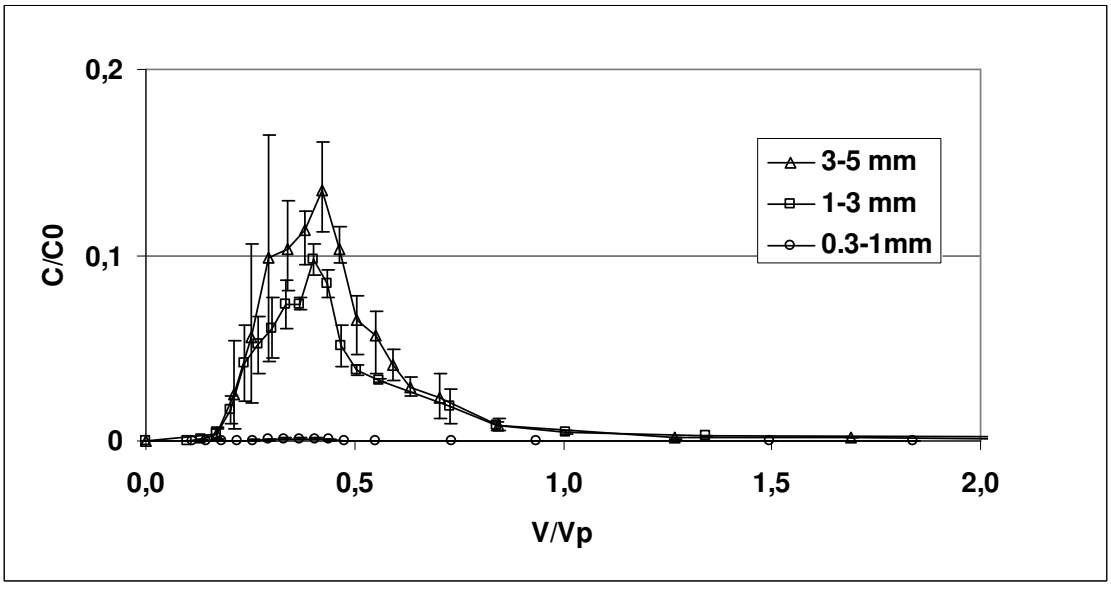

Figure 5: Comparison of *E. coli* breakthrough curves for the various aggregates sizes at an ionic strength of 0.1M.

At an ionic strength of 0.1M, the behavior is completely different. For the sand and glass beads columns, retention of bacteria is almost complete (Table 2). Despite the unfavorable conditions, (positive free energy of interaction), the bacteria are trapped in the porous media. The free energy of interaction between the cells and the solid were calculated using the DLVO theory (van Oss 1994) and the previously determined cell and porous media surface properties (Jacobs *et al.* 2007). The depth of the secondary minimum is of about -3kT in these conditions, which is not very large, but the Debye-Huckel length is severely reduced as compared with the 0.01M conditions. So despite the high electrostatic repulsive forces, the bacteria can approach the solid phase close enough to have the opportunity to establish contact and to have physical interactions. It should be noted that filtration is not the mechanism responsible for retention. One argument for this is that the bacteria are not retained at 0.01M. In addition, the size of the cells and of the solid grains were such that filtration should not occur (Bradford *et al.* 2002). For the columns made up of aggregates, retention is only slightly improved when the ionic strength is increased (Table 2, Fig. 5). For the smallest aggregates, bacteria are almost completely retained, but still about 1% of the injected cells are recovered at the outlet. For the larger aggregates, the percentage of recovery is still larger than 20%. So, for the soil, the role of the ionic strength is not as important as observed for retention in the sand and glass beads columns. These observations show that in porous medium displaying a porosity network not as simple as in sand or glass beads columns; the flow pattern plays a major role among the mechanisms determining cells retention.

All the breakthrough curves obtained, whatever the solid phase and the ionic strength, display a long tail with very low concentration (10^{-3} to 10^{-4} times the input concentration). Figure 6 a,b,c,d,e illustrate this phenomena. These tails indicate that a fraction of the bacteria, which were retained in the porous medium, are slowly released in the bulk flow.

3.2. Undisturbed columns experiments

Figure 7a, b show the bacterial concentration and the water flux measured at the outlet of the two columns as a function of time. At the end of drainage, the cumulative quantity of

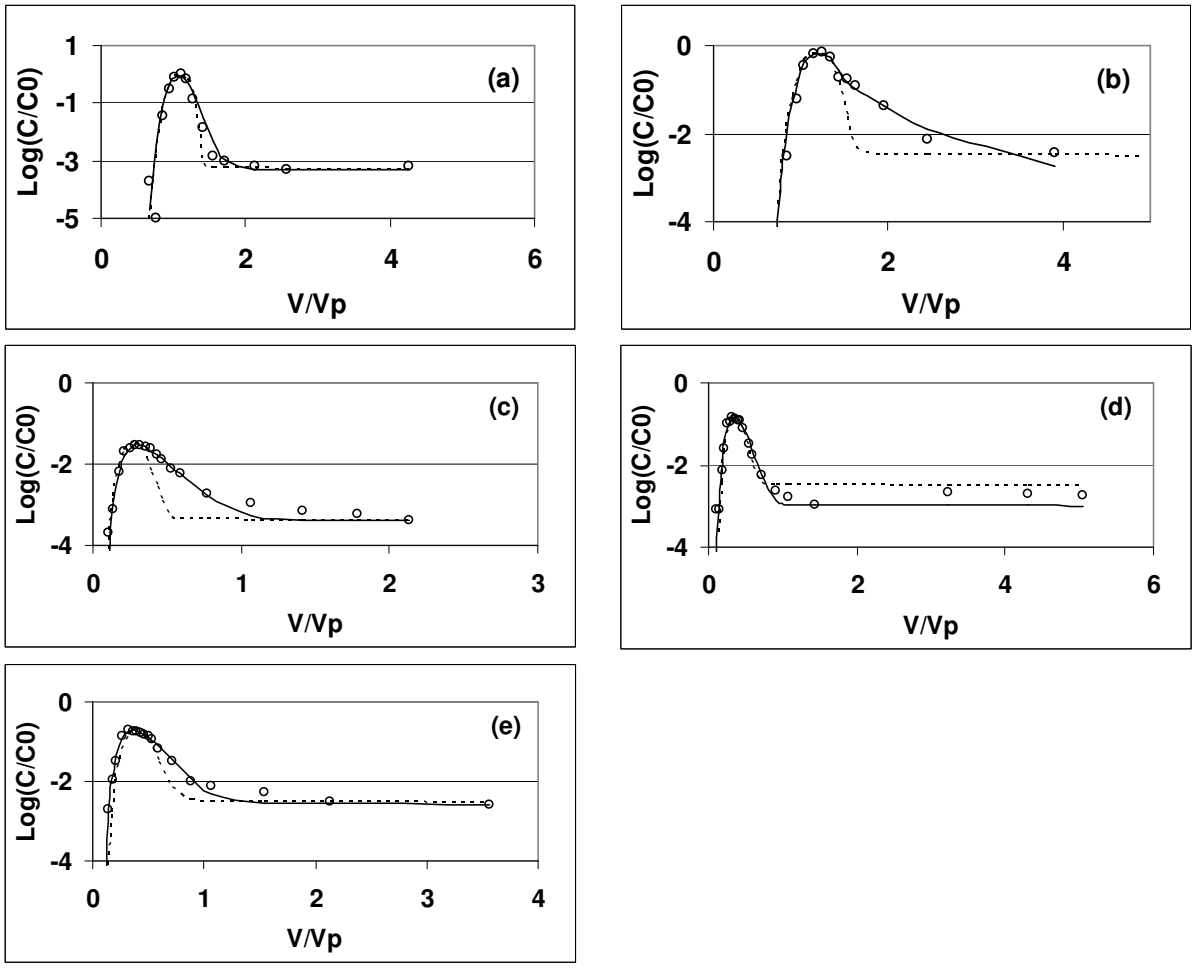

Figure 6: Comparison of observed and simulated breakthrough curves for the various porous medium (a) glass beads, (b) sand, (c) 0.3-1mm aggregates, (d) 1-3 mm aggregates, (e) 3-5 mm aggregates.

The circles are the observations, the solid line is the two-site model and the dashed line the model assuming that the fast sites are not present.

bacteria recovered was 1% and 5.5% of the injected amount for the 20mm.h^{-1} and the 30mm.h^{-1} columns, respectively. For both columns, the peak of concentration appeared very early. For the 30mm.h^{-1} column it corresponded to the first water sample collected and to the second water sample for the 20mm.h^{-1} column. In both cases, the peak was followed by a slow decrease of the concentration. For the highest flow rate the peak was one order of magnitude higher than for the other column. Hence it seems that bacteria retention diminishes when the flow rate increases. In both case the water content participating to transport is very low. Using the measured outflow curves and knowing the flux applied, one can assess this water content as the difference between the cumulated input and cumulated output when the plateau is reached. These values are 0.03 and 0.04 for the 20mm.h^{-1} and 30mm.h^{-1} columns. These values are close to those obtained when fitting a kinematics waves dispersion model (Di Pietro *et al.* 2003) which gives a mobile water content of about 0.02. So with these experimental conditions (high velocity and low ionic strength), a large amount of bacteria could be expected at the outlet. The small quantity recovered and the curves indicate that bacteria are rapidly trapped and then released at a decreasing rate.

3.3. Model comparisons

The fitted parameters were the water content θ, the partition constant k ($L^3.M^{-1}$) and the rate constant ω (T^{-1}) for fast reversible sites, the rate constant for deposition k_d (T^{-1}) and the rate constant for detachment k_r (T^{-1}). The parameter f was taken equal to unity. The model with the two sites successfully represented the various breakthrough curves observed. Figures (6abcde) present some examples of the model restitution for the saturated columns. Simulation results are also presented in figure 7 for the undisturbed soil columns. The fitted parameters are given in table 3. For the sand column at a 0.1M ionic strength experiments, the deposition coefficient k_d was set to the lower value for which the model predicted a zero concentration at the outlet.

The water content that were fitted for the experiments carried out with the glass beads and the sand are very close to those obtained from the tracer experiments. They are also almost identical to the porosity calculated from the mass of solid (0.371 and 0.385 for glass beads and sand columns respectively). This confirms that in sand and glass

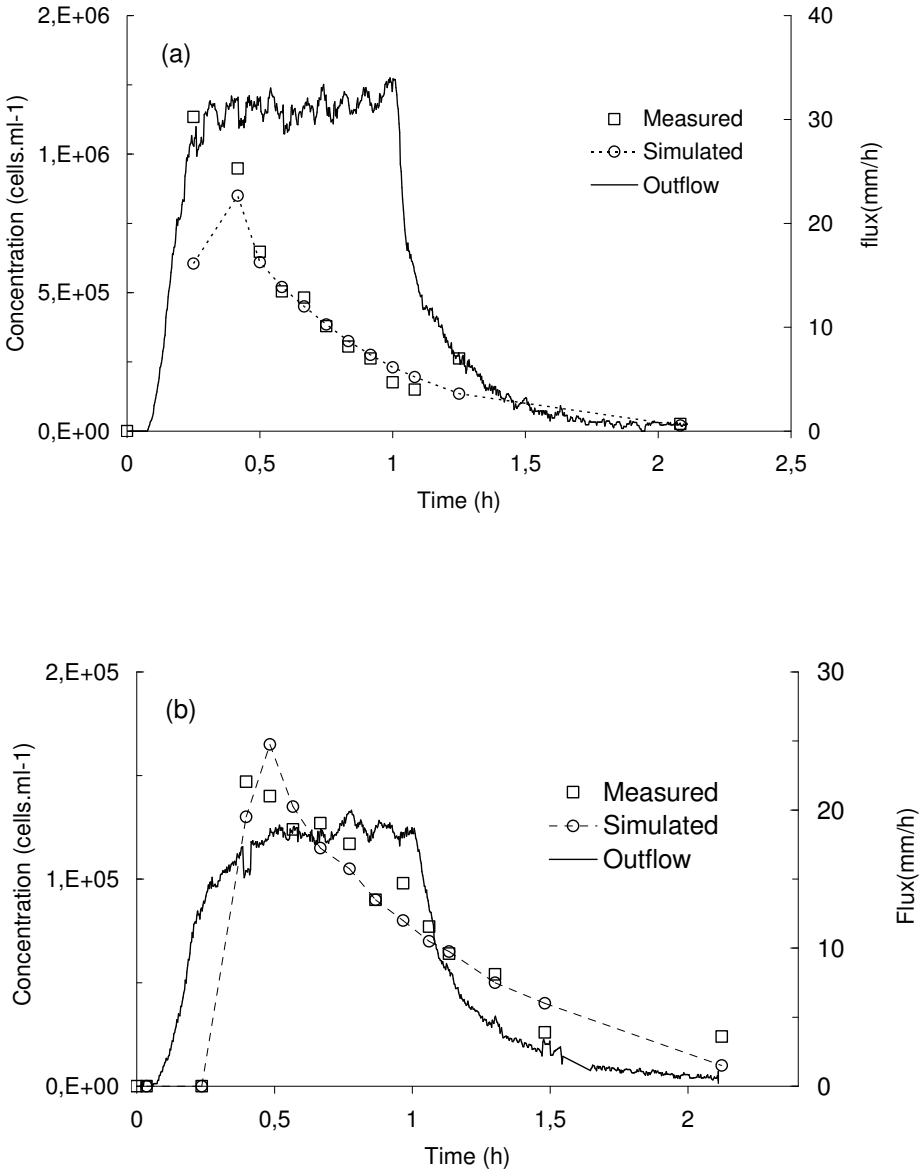

Figure 7: Water flow and observed and measured cell concentrations at the outlet of the two undisturbed soil columns: (a) Input flow is 30mm/h (b) Input flow is 20mm/h

beads columns, the volume of water transporting the bacteria is close to that for the tracer. For the columns made up of soil aggregates, the mobile water content fitted for the tracer agrees with the inter-aggregates porosity. For the cells, the fitted water content is significantly lower than the inter-aggregates porosity, indicating that the transport occurs mainly in a reduced fraction of the mobile water. The fitted water content is almost the same whatever the size of the aggregates at 0.01M and amounts to about half the mobile water calculated with the tracer. A slightly larger value was obtained at 0.1M for the small aggregates.

Although no clear trend seems to emerge from the parameters k and ω obtained for the various columns the following points may be underlined. These parameters allow accounting for a rapid and reversible exchange of bacteria between the bulk flow and retention sites with weak solid-bacteria interaction. If they are set to zero, the other parameters being fitted, the model cannot reproduce the part of the curves just before the tail. Figure 6 illustrates this point. The effect is most visible for the sand and for the small aggregates. The values fitted for ω indicate that it is a relatively rapid process as compared to deposition. Also, the k values seem to be larger for the 0.1M ionic strength experiments. This can be explained by temporary retention in a secondary minimum energy that is deeper du to compression of the electrical double layer when the ionic strength is high. Bacteria retained in secondary minimum can be easily released in the flow as they have not established strong links with the solid phase. This is in agreement with previous observations (Jacobs *et al.* 2007; Redman *et al.* 2004).

The fitted deposition coefficients show a strong dependence on ionic strength, especially for the glass beads and the sand. The deposition rate coefficient in a porous medium can be estimated as:

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

where θ is porosity, d_c is collector grain diameter, v is velocity and α is collision efficiency. Correlations (Rajagopalan 1976; Tufenkji et Elimelech 2004) are available for estimating η. For the sand, the deposition coefficient in favorable condition (α=1) calculated with the above relationship was k_d =31.3 h^{-1} (R-T) or k_d =19.2 h^{-1} (T-E). These estimations agree with the fitted value for the 0.1M ionic strength experiments (k_d >20 h^{-}

^{1}). For the glass beads at an ionic strength of 0.1M, the fitted deposition coefficient (k_d =9.5 h^{-1}) is lower than the values calculated from the correlations indicating a collision efficiency lower than unity. At this ionic strength the electrostatic repulsions are still present but the width of the electrical double layer is of about 1nm, hence allowing the cells to approach closely the solid phase. Despite the presence of the energy barrier, the retention observed in these columns can be explained by cells being trapped at grain junctions (Tong et Johnson 2006), this mechanism being more efficient with irregular and rough sand grains than with spherical and smooth glass beads. For the soil columns, the deposition depends largely on the size of aggregates; the smaller the aggregates the better the deposition. In addition, the effect of the ionic strength is not as important as for the sand and the glass beads. In particular the deposition that is already important at an ionic strength of 0.01M with the small aggregates is only slightly improved when the ionic strength is 0.1M. This reveals a relative dominance of physical versus chemical effects on the transport of cells in porous media with complex flow structure as opposed to what is observed in porous media made up of identical particles. The release coefficient does not show any clear trend as function of solid or ionic strength.

4. Conclusions

The measured breakthrough curves indicate that the volume of water involved in the transport of bacterial cells can vary considerably from one media to another. While this volume is very similar to that of a tracer in sand and glass beads columns it is much lower than mobile water in aggregated porous media probably because the diffusion of the cells is orders of magnitude lower than that of the ions. Hence, one must be careful when modeling the transport of these colloids in heterogeneous porous media.

For all the porous media, the breakthrough curves and the comparison with the model indicate that two types of retention sites are present. One type is characterized by a rapid and reversible exchange of bacteria and explains the curvature of the breakthrough curves just before the tail begins. This temporary retention is probably related to stagnant flow regions and low energy secondary minima. The second type corresponds to usual deposition with slow release of the cells. Also, for all the porous media, a tail was observed corresponding to the release of deposited cells.

The effect of the ionic strength is very apparent for the glass beads and the sand. In these media with fine grains and a simple hydrodynamics, retention is almost complete (collision efficiency factor close to 1) for an ionic strength of 0.1M while it is very weak at an ionic strength of 0.01M. Conversely, the effect is much less for the columns packed with aggregates. In these porous media characterized by a more complicated hydrodynamics and larger particles, transport seems to be the dominant factor. In addition, the experiments on sand and glass beads columns confirm that retention is possible in presence of a high energy barrier; wedging being the anticipated phenomena.
The model with the two types of site correctly reproduced the various experiments while the model considering deposition only was unable to provide a reasonable restitution of the observed curves. The fitted deposition parameters in favourable conditions (high ionic strength) agree with those calculated from classical correlations.

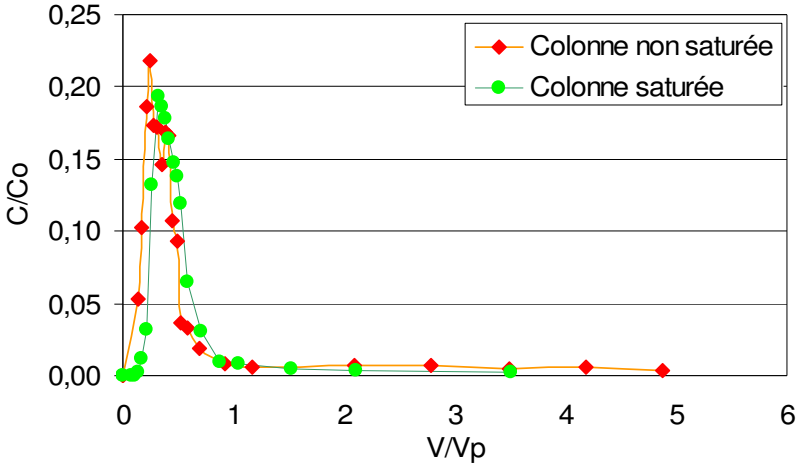

Figure 8 : Transport de la souche *E. coli* PHL1314 dans un sol Poirson (3-5mm) saturée et non saturée.

Le graphique présente la concentration cellulaire normalisée en fonction des volumes de pores.

5. Résultats complémentaires :

Toutes les expériences de transport en colonnes n'apparaissent pas dans l'article. Cette partie présente une série d'expériences qui n'ont pas fait l'objet d'une publication. La première série est dédiée à des expériences en colonnes de sol non saturée. La seconde concerne le transport en milieu poreux de la souche *Rhizobium* YAS3401 dans différentes conditions.

5.1. Le transport microbien dans une colonne de sol non saturée.

Les résultats présentés dans l'article montraient une série d'expériences de transport de bactérien dans des colonnes de sol Poirson saturée. Dans ce cas de figure il n'y a que 2 phases : le sol et la solution. Pour obtenir de telles condition expérimentales les colonnes ont été saturée sous vide afin d'éviter le piégeage d'air dans les pores du sol. Cependant lors de premières expériences, la mise sous vide de la colonne a échoué par deux fois donnant un sol non saturé. Il existe alors 3 phases : l'eau, le sol et l'air. La littérature a montré que les bactéries pouvaient être piégées aux interfaces eau air (voir § 2.4 du chapitre état de l'art). Malgré la présence d'air dans les colonnes le transport de la souche *E. coli* PHL1314 a été réalisé et les résultats sont présentés ici. Ces résultats n'ont toutefois pas fait l'objet d'une publication car il était impossible de localiser et de mesurer la taille des bulles d'air piégées.

Les expériences ont été réalisées dans des colonnes de sol Poirson avec des agrégats de 3 à 5mm. Les dispositions expérimentales sont identiques à celles décrites dans la section matériel et méthode de l'article. La figure 8 montre la comparaison entre le transport d'*E. coli* PHL1314 dans un milieu saturé et un milieu non saturé.

En colonne non saturée 77% des cellules d'*E. coli* ont été récupérées contre seulement 50% dans la colonne saturée. De plus le transport de la bactérie est plus rapide dans la colonne non saturée. La présence d'air dans les pores diminue le volume poral ce qui a eu deux effets : (i) réduction des chances de rencontre entre les cellules et la surface des agrégats du sol, (ii) augmentation de la de la vitesse de circulation de la solution. Ces

deux effets contribuent à la sortie plus rapide des bactéries dans le milieu non saturé par rapport au milieu saturé et à une diminution de la quantité retenue. En outre l'augmentation de la vitesse peut aussi induire une augmentation des forces de cisaillement et ainsi diminuer l'adhésion des bactéries sur les agrégats du sol.

Les résultats obtenus ne vont pas dans le sens de ce qui est rapporté dans la littérature (plus de rétention en non saturé). Cette différence est peut-être la conséquence de l'augmentation de la vitesse qui entre en compétition avec la cinétique d'attachement.

5.2. Le transport de Rhizobium YAS3401 dans différentes conditions

Tout comme pour la souche *E. coli* PHL1314 nous avions entrepris d'étudier le transport de la souche *Rhizobium* YAS3401 dans du verre, du sable et le sol Poirson du fait que cette souche présentait un comportement d'adhésion très différent de *E. coli* PHL1314 (cf Chapitre1). La souche *Rhizobium* YAS3401 a été fournie par le Laboratoire d'Ecologie Microbienne de la Rhizosphère et d'environnements extrêmes (LEMIRe) du CEA de Cadarache. Cette souche avait été transformée au LEMIRe avec un plasmide portant le gène *gfp* codant pour la protéine fluorescente GFP permettant le comptage des cellules par cytométrie de flux. Cette souche a été isolée de la rhizosphère du tournesol et a la particularité de pouvoir produire de grandes quantités d'EPS (Alami *et al.* 2000).

5.2.1. Matériel et méthode

Le mode opératoire des expériences de transport en milieu poreux est identique à la méthode expliquée dans l'article de ce chapitre. Pour rappel des colonnes de sables, de verre et de sol Poirson ont été préparées et saturée avec une solution NaCl 10^{-1}M ou 10^{-2}M stérile. Puis une solution de *Rhizobium* YAS3401 (environ 7.10^7 cellules.ml^{-1}) a été injectée pendant 10 minutes dans chaque milieu poreux avec un débit de 3ml.min^{-1} environ. La culture de *Rhizobium* YAS3401 a été réalisée en milieu LB adapté (dilué 5 fois, sans NaCl et contenant 15mg.l^{-1} de Kanamycine pour sélectionner les cellules contenant le plasmide) à 30°C pendant une nuit. La culture a ensuite été centrifugée à 5000rpm pendant 5 minutes. Le culot de cellules obtenu est remis en suspension avec une

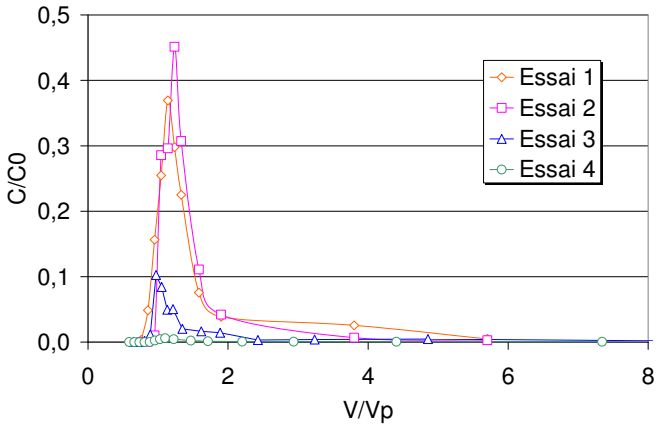

Figure 9 : Transport de la souche *Rhizobium* YAS3401 dans des colonnes de sable à 10-2M de NaCl.

Le graphique présente la concentration cellulaire normalisée en fonction des volumes de pores. Les 4 essais correspondent à des cultures indépendantes

Milieu poreux	Pourcentage de cellules transportées
Sable E1	79.89
Sable E2	24.51
Sable E3	2.90
Sable E4	19.05
Verre E1	39.15
Verre E2	40.53
Sol E1	77.39
Sol E2	65,12

Table 4: Pourcentage de cellules de *Rhizobium* YAS récupérées en sortie de colonne dans différents milieux poreux (sable, verre et sol Poirson).

Transport bactérien réalisé à 10^{-2}M NaCl avec pour chaque essai des cellules issues de cultures indépendantes.

solution de NaCl 10^{-1}M ou 10^{-2}M stérile. Le comptage des cellules en sortie de colonne a été effectué au cytomètre de flux comme pour *E. coli* PHL1314.

5.2.2. Résultats

La figure 9 présente 4 essais de transport de *Rhizobium* YAS3401 dans des colonnes de sables à 10^{-2}M de NaCl. Chaque essai correspond à une culture différente. L'ensemble des résultats de transport de *Rhizobium* YAS3401 pour le sable, le verre et le sol Poirson sont résumés dans le tableau 4. On constate que les résultats sont peu reproductibles et différents de la souche *E. coli* PHL1314. En effet contrairement à *E. coli* PHL1314, *Rhizobium* YAS3401 circule mieux dans le sol Poirson et moins bien dans le verre. Mais tout comme pour la souche *E. coli* PHL1314, le transport en milieu poreux de *Rhizobium* YAS est très sensible aux variations de la force ionique de la solution (figure 10). Dans le verre comme dans le sable une diminution de la force ionique provoque le décrochage des cellules vraisemblablement retenue dans le minimum secondaire comme expliqué dans l'article du chapitre 2. Il est intéressant de noter que *Rhizobium* YAS3401 est mieux transporté dans le sable qu'à travers une colonne remplit de billes de verre. Un résultat qui est également très différent de celui obtenue avec la souche *E. coli* PHL1314 et peu vraisemblable. L'ensemble de ces observations peu cohérentes entre elles, nous a amené à remettre en question la reproductibilité des propriétés de surface de cellules issues de cultures différentes et en conséquence à nous intéresser au rôle des EPS sur le transport.

5.2.3. Discussion

Les résultats de transport obtenus avec *Rhizobium* YAS3401 sont peu reproductibles et souvent inconsistant avec le comportement de la souche *E. coli* PHL1314 dans les mêmes conditions expérimentales. On suppose que le niveau de production d'EPS par la souche *Rhizobium* YAS3401 est à l'origine des résultats obtenus. En effet on a remarqué que lors de certaines cultures le culot bactérien était tantôt « d'aspect gluant », tantôt très « sec ». Lorsque le culot était « gluant », attestant à priori d'une production abondante d'EPS par les cellules, la bactérie était mieux transportée sur le sable. Dans le cas contraire avec un culot « sec » les cellules sont d'avantage retenues par les milieux poreux testés. Nous avons alors émis l'hypothèse que de petites variations dans les conditions de cultures ou

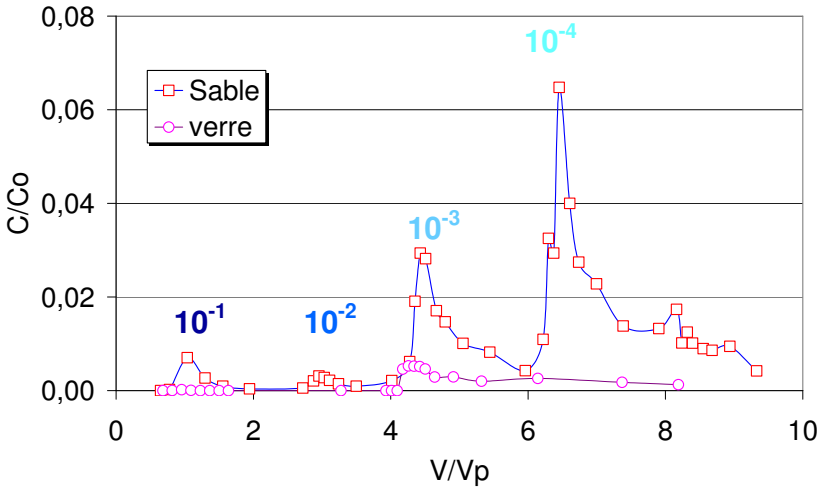

Figure 10 : Impact de la diminution de la force ionique sur le transport de la souche *Rhizobium* YAS3401 dans du verre et du sable.

A chaque diminution de la force ionique du milieu une portion de cellules se décroche et se manifeste par un pic à la sortie de colonne.

de centrifugation étaient peut être à l'origine des quantités variables d'EPS sur les cellules et donc d'un comportement différent lors du transport en colonne. Deux protocoles expérimentaux ont été mis au point pour vérifier ces hypothèses. Le premier pour mesurer l'effet de la centrifugation (possibilité de perte d'EPS par les cellules sous l'effet de la centrifugation), le second sur les conditions de cultures.

5.3. 1ère Expérience : vérification de l'effet centrifugation sur le transport de *Rhizobium* YAS3401 sur du sable

- Culture de *Rhizobium* YAS3401 en milieu LB adapté (dilué 5 fois, sans NaCl, 15mg.l^{-1} Kanamycine) pendant une nuit à 30°C et sous agitation (100rpm).
- Le lendemain la culture est divisée en 2 parties. Uniquement une des deux parties est centrifugée (5000rpm pendant 5 minutes).
- Le culot issu de la centrifugation est repris dans 50ml d'une solution de NaCl 10^{-2}M stérile. Puis une suspension cellulaire ajustée à environ 1.10^7 cellules.ml^{-1} est préparée à partir de la solution mère.
- Environ 1ml est prélevé de la partie non centrifugée et dilué dans 100ml d'une solution de NaCl 10^{-2}M de NaCl. La solution est ensuite ajustée à environ 1.10^7 cellules.ml^{-1}.
- Circulation des deux préparations microbiennes à travers des minis colonnes de sable en circuit fermé pendant 30 minutes avec un débit d'environ 3.5ml.min^{-1}. Ce mode opératoire est identique aux expériences en mini colonne utilisées dans l'article du chapitre 1.
- Comptage des cellules dans la solution à la fin de l'expérience.

Résultats et interprétations

Le culot obtenu pour la partie centrifugée avait un aspect « gluant » suggérant la production d'EPS par les cellules. Après le transport en mini colonne 42% des cellules ont été retenu dans le sable pour la solution bactérienne ayant subit la centrifugation. Pour la solution bactérienne n'ayant pas subit de centrifugation seulement 31% des cellules ont été retenues par le sable. Bien que peu significative cette différence pourrait

Culture	Glucose	Durée	Aspect culot	cellules retenues
culture 1	Avec	15h	gluant	37%
culture 2	Sans	15h	gluant	45%
culture 3	Sans	18h	sec	74%
culture 4	Sans	21h	sec	71%

Tableau 5 : Résultats du transport en mini colonne de sable de la souche *Rhizobium* YAS3401 en fonction de différentes conditions de culture.

indiquer un léger effet de la centrifugation sur la physiologique des cellules et induire un changement de comportement du transport en milieu poreux. Toutefois par manque de temps aucune répétition de cette expérience n'a été faite. De plus la quantification des EPS produits par les cellules et de la quantité d'EPS éventuellement perdus sous l'effet de la centrifugation n'a pu être réalisée.

5.4. 2ère Expérience : vérification de l'effet des conditions de culture sur le transport de Rhizobium YAS3401 sur du sable

- 4 Cultures de *Rhizobium* YAS3401 en milieu LB adapté (dilué 5 fois, sans NaCl, 15mg.l^{-1} Kanamycine) sont préparées au même moment à 30°C sous agitation (100rpm). La durée des cultures varient (15h, 18h ou 21h) et une des cultures est enrichie avec 0.2% de glucose (pour favoriser la production des EPS). Chaque culture est ensuite centrifugée (5000rpm pendant 5minutes) et l'aspect du culot est inspecté pour vérifier la présence d'EPS.
- Des suspensions cellulaires ajustées à environ 1.10^{7} cellules.ml^{-1} sont préparées à partir des culots obtenus à l'étape précédente
- Circulation de chaque suspension cellulaire à travers les minis colonnes de sable en circuit fermé pendant 30 minutes avec un débit d'environ 3.5ml.min^{-1}. Ce mode opératoire est identique aux transports en mini colonne utilisé dans l'article du chapitre 1.
- Comptage des cellules dans les solutions à la fin de l'expérience

Résultats et interprétations

Le tableau 5 montre les résultats obtenus. Premier constat : les cultures les plus courtes (15 heures) ont donné des culots d'aspect gluant suggérant une production abondante d'EPS par la bactérie dans ces conditions. Les cultures plus vieilles (18 heures et 21 heures) ont donné des culots secs indiquant à priori des quantités d'EPS bien moindres. Il est possible que dans les cultures 3 et 4 les sources carbones aient été épuisées incitant les bactéries à consommer les EPS.

Une différence de comportement de transport significative apparaît entre les cellules d'un culot sec et les cellules d'un culot gluant. En effet les cellules issues de la culture 1 ou 2 sont presque 2 fois moins retenues dans le sable que celles issues des cultures 3 et 4. De plus la culture 1, enrichie en glucose pour favoriser la production d'EPS, est la moins retenue. Ces résultats suggèrent que la présence d'EPS diminue la capacité d'adhésion des cellules de *Rhizobium* YAS3401 et favorise leur transport. Dans ce cas les EPS pourraient être à l'origine d'interactions stériques répulsives entre la cellule bactérienne et la surface des grains de sables comme expliqué par la figure 6 page 32.

5.5. Conclusion

Ces premiers résultats constituent des pistes intéressantes sur l'implication des EPS dans les phénomènes d'adhésion et de transport bactérien en milieu poreux. Pour continuer ces travaux une quantification des EPS à la surface des cellules est indispensable en utilisant par exemple le marquage au calcofluor. Il faudra également être capable d'enlever les EPS des cellules et disposer d'un mutant ne produisant pas d'EPS pour avoir un témoin négatif. Enfin il faudra déterminer la composition chimique des EPS qui peut avoir une importance comme l'atteste la littérature. Par manque de temps et de moyens techniques ces travaux n'ont pu être réalisés.