Transport et devenir en milieux poreux d'une communauté bactérienne issue de boues de stations d'épuration

Le chapitre 5 correspond à un article à soumettre pour publication dans la revue

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Transport and fate of a sludge bacterial community in porous media.

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Avant propos

Le chapitre 5 correspond à la partie appliquée de la thèse. Les chapitres 2, 3 et 4 ont permis d'aborder certains mécanismes qui régissent le transport bactérien en milieux poreux. Comment ces mécanismes influent-ils sur la communauté bactérienne issue de boues de stations d'épuration lors de son transport en milieux poreux? Y-a-t-il une migration différentielle des bactéries comme le laisse penser les résultats précédents ? Peut-on isoler certaines souches transportées et identifier leurs propriétés de surface ? Est-ce cohérent avec nos résultats sur l'adhésion et le transport ?

Pour cela des expériences de transport on été réalisées avec des boues prélevées à la station d'épuration de Caromb (Vaucluse, France). Des méthodes de biologie moléculaire ont été utilisées pour analyser la modification de diversité microbienne après transport et tenter d'identifier des souches transportées.

Abstract

Sludge from waste water plant contains bacteria which may contaminate drinking water sources when transported through soils. In this study the fate of microbial communities from three different sludge samples in saturated porous media was investigated. Microbial communities were analysed using SSCP techniques before and after their transport through either sand or soil media. Bacterial counting was performed with flow cytometer while thermo tolerant coliforms were counted on m-FC medium and identified by sequencing. Results showed that the abundance of species in the bacterial communities was severely reduced by transport through both porous media used. Simpson diversity indexes of the sludge decreased from over 6 to 4. Less than 40% and 10% of the bacteria were able to travel through the sand and the soil respectively. Despite the porous media acting as a efficient barrier against bacterial transport for a major part of the microbial communities studied still some species travelled through both sand and soil media. One strain, identified as *Rhodoccocus sp.* Represented almost 7 to 9% of the SSCP patterns. Moreover three different *E. coli* strains and a *Enterococcus sp.* Were also identified from blue colonies on the m-FC agar plates. The consequences of these results on waste water effluents spreading are discussed.

1. Introduction

Spreading of sludge from waste water treatment units on open fields is widely practiced in France and other countries. Such process may diminish the volume of pollutants throw back directly to natural water resources of rivers or lakes. However waste water plant effluents are rich in pathogenic microorganisms and other bacteria uncommon to soil environments (Dumontet 2001). As a result spreading of sludge can cause drinking water contamination or modification of the soil ecology by bacterial transport through aquifers. Coliforms are commonly found in waste water plants and some are often responsible for gastrointestinal infection outbreaks (Crockett 2007). Coliform bacteria are microorganisms that primarily originate from the intestines of warm-blooded animals but can survive in other environments. By testing for coliforms, especially the well known thermotolerant coliform *Escherichia coli*, one can determine if the water has probably been exposed to fecal contamination (Tian *et al.* 2002; Unc et Goss 2004). The WHO (World Health Organisation) recommends *E. coli* detection in water to track fecal contamination. Bacteria from sludge able to travel through soils can thus negatively impact human health and the environment if not under control. Bacterial transport studies usually focus on one type of bacterial strain instead of an entire bacterial community like in sludge (Abu-Lail et Camesano 2003; Bradford *et al.* 2006; Stevik *et al.* 1999). Most of such studies describe two main mechanisms that are responsible for preventing bacterial transport: filtration and adsorption (Stevik *et al.* 2004). Filtration typically involves the physical blocking of bacterial movement through small pores. Filtration depends on the cell to porous media grain size ratio (Bradford *et al.* 2006), water degree saturation (Smith 1985) or clogging (Rijnaarts *et al.* 1996). Adsorption involves the bacterial cells to be attracted towards the porous media grain surface and to stick on it. Many approaches have been used to explain bacterial adhesion on solid surfaces. Among them the physicochemical approach which can be interpreted using the DLVO theory (van Loosdrecht 1989). Hydrophobic attraction and electrostatic repulsions have been reported to be major factors influencing bacterial adhesion (Li et Logan 2004; van Loosdrecht 1990). It has been widely admitted that most bacterial cells and sediments are typically negatively charged at groundwater low ionic strength and mild pH, resulting in electrostatic repulsions occurring between bacterial cells and soil particles

Characteristics	Sand	Soil
Grain Size (µm)	230-310	300-1000
Column volume (cm^3)	294,5	294,5
Dry solid mass(g)	480	340
Solid density ($g.cm^3$)	2,65	2,65
Column bulk density ($g.cm^3$)	1,63	1,15
Total porosity	0,385	0,566
Inter aggregates porosity	-	0,342
Total pore volume (cm^3)	113,4	166
Water flux ($cm^3.mn^{-1}$)	3,6	2

Table 1: Porous media characteristics and hydrodynamics

(Choi *et al.* 2007). In such conditions bacterial transport is enhanced. Jacobs *et al.* showed that differences in transport behaviours through sand among various bacterial strains were partly due to the physicochemical cell surface properties (Jacobs *et al.* 2007). They concluded that small bacterial cells with hydrophilic and negatively charged surface characteristic are most likely to be transported on long distances. Despite the increasing number of bacterial transport related articles, the literature lacks any reference about the fate of bacterial sludge community in soil environments. Waste water plant sludge is known for housing riche bacterial communities suggesting all sorts of transport behaviours depending on each bacterial strain. In this paper we are interested in studying the transport and fate of bacterial sludge community through two porous media (sand and soil). SSCP was used to characterize the community before and after transport through the porous media. We also looked for the presence of thermo tolerant coliforms as it is a valuable tool used for tracking recent fecal contamination.

2. Material and methods

2.1. Saturated column experiments

The experimental setup consisted in Plexiglas columns (Ø = 50mm, length = 150mm) filled either with sand or soil aggregates. Porosity and porous media characteristics are listed in table 1. Before use, the sand was thoroughly rinsed with milliQ water on a 40µm filter (VWR international, 11cm, type 417) then heat treated and oven dried for at least 2 hours at 120°C. For sand, the columns were filled under water. 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. Last, these columns were vacuum saturated with sterile solutions. The bulk densities are given in table 1. Experiments were carried out with a sterile 10^{-4} $mol.l^{-1}$ $CaCl_2$ 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 two times with each sample of sludge. Three samples of sludge were obtained from a rural waste water plant (Caromb, south of

France) in November 2006, January and February 2007 respectively. Each sample of sludge was used for the experiments the day it was produced at the waste water plant. For each experiment 1g of sludge was mixed with 20ml of the 10^{-4} mol.l^{-1} $CaCl_2$ solution (referred as sludge solution) and injected in the column at about 2ml.min^{-1} (corresponding to about 60mm.h^{-1}) followed by the bacterial free solution (10^{-4} mol.l^{-1} $CaCl_2$). Each experiment lasted 90 minutes which corresponds to about 3 pore volumes of the porous media used. Controls consisted of the same column experiments as described above but without the sludge. For each experiment, enumeration and other molecular biological techniques were carried out on aliquots of the whole water recovered at the outlet of the columns.

2.2. Bacterial counting and detection methods

Two methods to count bacterial cells were used: mFC Agar (Fluka, Sigma-Aldrich, St Louis, USA) was used for the detection and enumeration of thermo tolerant coliforms on agar plates. Flow cytometer techniques were used to count stainable cells with the green fluorescent SYTO^{TM} BC dye (Bacterial counting kit B-7277, Molecular Probes^{TM}, Oregon, USA). Bacterial counting with both methods was performed on the sludge solutions prior to the experiments and on the solutions recovered at the outlet of the columns at the end of the experiments. For the enumeration of thermo tolerant coliforms samples were diluted 10^1 to 10^5 times and each dilution was incubated at 44.5°C for 24h. Each dilution was repeated three times. For the enumeration with the flow cytometer (EPICS XL, Beckman Coulter, Fullerton, USA) the samples were diluted 10^2 times prior to the analysis and filtered (40µm filter, 11cm, type 417, VWR international) to avoid obstruction of the device. Samples of 1ml were stained with the 1µl of the SYTO BC dye and counted using latex Standard Beads following instructions from the manufacturer (Bacterial Bacterial Counting Kit, Molecular Probes, Oregon, USA). The flow cytometer settings were as follows: excitation source 488nm, fluorescence detection on FL1 channel (emission 510-530nm), analysis time 1minute, no compensation was used.

Primers	Sequences	Amplification conditions
w49	5' ACGGTCCAGACTCCTACGGG 3'	1 cycle : 94°C 2minutes 25 cycles : 94°C 30 seconds 61°C 30 seconds 72°C 30 seconds final cycle : 72°C 10 minutes
w104	5' *FAM*-TTACCGCGGCTGCTGGCAC 3'	
w31	5’ TTACCGCGGCTGCTGGCAC 3’	
T7	5’ TAATACGACTCACTATAGGG 3’	1 cycle : 94°C 2minutes 25 cycles : 94°C 30 seconds 61°C 30 seconds 72°C 30 seconds final cycle : 72°C 10 minutes
T3	5’ ATTAACCCTCACTAAAGGGA 3’	
S1	5’ AGAGTTTGATC (A, C) TGGCTCAG 3’	1 cycle : 94°C 3minutes 25 cycles : 94°C 40 seconds 55°C 50 seconds 72°C 1 minute final cycle : 72°C 10 minutes
S2	5’ GG (A,C)TACCTTGTTACGA(T,C)TTC 3’	

Table 2: PCR primers and amplification conditions used in this study.

2.3. DNA extraction

The recovered solutions from the column experiments were vacuum filtered with Nalgene® filter units (Nalgene®, Rochester, USA) on 0.22µm filters (type GS, Millipore®, Bedford, USA) to collect bacterial cells. Next the filters were cut to fit into 1.5ml eppendorf tubes under sterile condition to start DNA extraction. DNA extraction was performed using Kit amp™ (Qiagen®, Germany) and according to instructions of the manufacturer. Extracted DNA was frozen until analysis.

2.4. DNA analysis

Bacterial community from the sludge solutions and the recovered solutions from the column experiments were analysed using SSCP techniques (Single Strand Conformation Polymorphism). The total DNA extracted as explained previously was amplified with one set of primers: W49 and W104 (table 2). Reverse primer W104 was labelled with 5'fluorescin phosphoramidite 6*FEM*. All primers were synthetized by Eurogentec® (Liège, Belgium). Pfu turbo Dna polymerase (Stratagene, Europe, Amsterdam Zuidoost, Netherlands) was used for these PCR amplifications. PCR was performed in a total volume of 50µl containing 36.9µl of pure water, 5µl of 10X Pfu Turbo buffer, 4µl of dNTP mix (2.5mM), 1.3µl of each primer ($100ng.\mu l^{-1}$), 1µl of the DNA extract and 0.5µl of the pfu turbo ($2.5U.\mu l^{-1}$). All the reaction mixtures were subjected to 30 amplification cycles in a thermocycler (thermocycler 2720, AB applied Bioscience, Foster City, USA). Further PCR amplification conditions are reported in table 2. PCR products (expected band strip of 200pb) were controlled by electrophoresis on 0.8% agarose gel and visualised with UV light.

2.5. SSCP capillary electrophoresis

PCR products were purified with a Strata Prep™ PCR purification Kit (Stratagene Europe, Amsterdam Zuidoost, Netherlands) following the manufacturer's instructions. A mixture containing 18.75 µl deionised formamide (Applied Biosystems, Foster City, USA), 0.25 µl internal DNA molecular weight standard Genescan 400 HD Rox (Applied Biosystems, Foster City, USA) and 1 µl purified PCR product was denatured for 5 min at

95 °C and immediately cooled on ice. The mixture was then electrophoresed by SSCP capillary electrophoresis on an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, USA) as described elsewhere (Duthoit 2003). Assignation of dominant peaks was performed as described hereafter.

Dominant peaks in the SSCP patterns were assigned by comparing their migration with the migration of cloned 16S RNA. A clone library was prepared using genomic DNA extracted from the recovered sand column solution A which showed the highest probability to identify the major peaks of the SSCP profiles. The 16S rRNA gene was amplified with universal primers w31 and w49. In a total volume of 50 µl, the PCR mixture contained containing 36.9µl of pure water, 5µl of 10X Expand High Fidelity enzyme buffer, 4µl of dNTP mix (2.5mM), 1.3µl of each primer (100ng.μl^{-1}), 1µl of the DNA extract from the sand column and 1µl Expand High Fidelity DNA polymerase (2.5U.μl^{-1}) from Roche Applied Science. All the reaction mixtures were subjected to 25 amplification cycles in a thermocycler (thermocycler 2720 AB applied Bioscience). Further PCR amplification conditions are reported in table 2. The amplified product was cloned into pCR 4-TOPO vector (TOPO TA cloning Kit for sequencing) and transformed in *Escherichia coli* cells (one shot TOPO 10 chemical competent *E. coli*) following the manufacturer's instructions (Invitrogen SARL, Cergy Pontoise Cedex, France). Transformed cells were cultivated for 24H at 37°C on Luria Bertani media containing Kanamycine. Plasmid inserts of 30 obtained clones were amplified by PCR with T7 forward and T3 reverse (see table 2) to identify the strains. Next, the obtained PCR product was amplified with w49 and w104 primers to allow SSCP analysis as explained above. Finally the 16S rRNA gene clones corresponding to dominant peaks of the SSCP patterns were sequenced. Each sequence was compared with sequences in the Genbank data base using the NCBI blast program.

2.6. M-FC agar grown colonies identification

E. coli grow into bleu colonies on m-FC agar media. First Bleu colonies were frozen (-18°C) in ultra pure water. Then each colony was identified by amplifying and sequencing the RNA 16S using primers S1 and S2. Further PCR instructions are given in table 2.

		Sand			Soil		
		Injected	Outlet	Recovery (%)	Injected	Outlet	Recovery (%)
Sludge A	FCM	2.16e+09	7.02+08	32.50	NA	NA	NA
	m-FC 44°	1.48e+05	3.30e+03	2.23	1.48e+05	1.20e+03	0.81
Sludge B	FCM	2.89e+09	1.15e+09	39.79	2.89e+09	1.86e+08	6.44
	m-FC 44°	3.60e+04	7.66e+02	2.13	3.60e+04	4.60e+02	1.28
Sludge C	FCM	7.35e+09	6.34e+08	8.63	7.35e+09	1.75e+08	2.38
	m-FC 44°	5.11e+05	5.15e+03	1.01	5.11e+05	3.21e+02	0.06
control (*)	FCM	0	0	-	0	5.63e+06	-
	m-FC 44°	0	0	-	0	0	-

(*) The injected solution for the control consisted of the sterile 10^{-4} CaCl2 solution without sludge
FCM = Flow CytoMeter

Table 3: Results of bacterial counting with flow cytometer method and results of thermo tolerant coliform counting on m-FC agar plates at 44°C.

Results indicated correspond to an average obtained from duplicated column experiments.

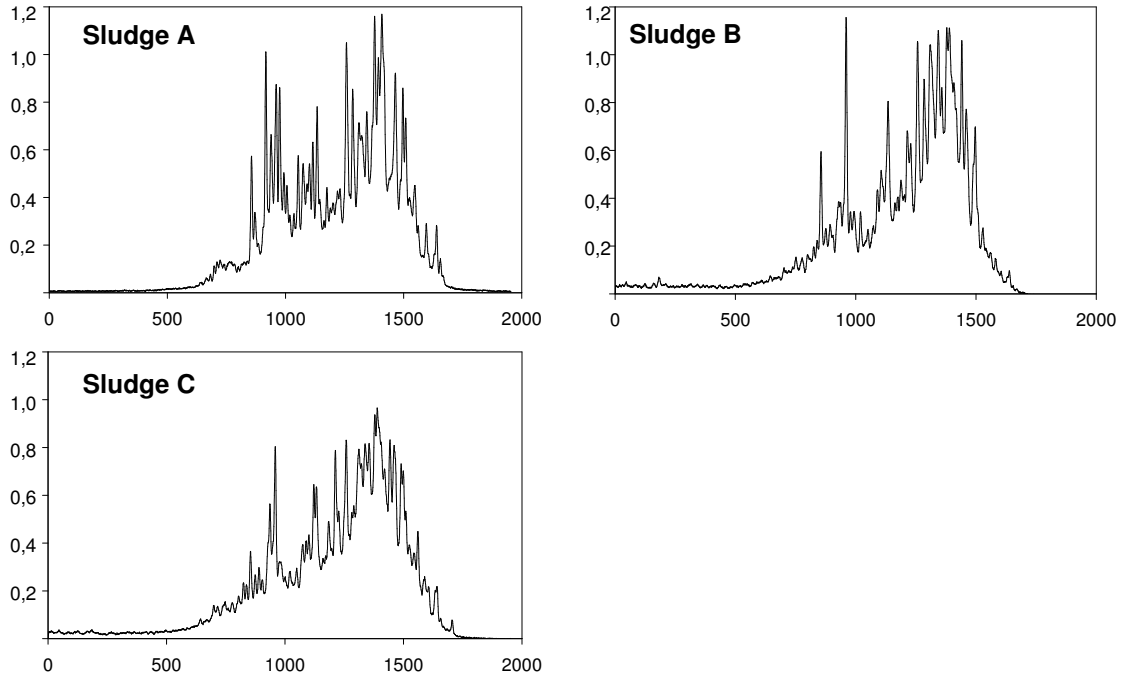

Figure 1: SSCP patterns of PCR amplified 16S rRNA gene fragments from bacterial communities of sludge A, B and C.

3. Results

3.1. Bacterial counting

Both counting methods showed that the amount of bacterial cells in the sludge solutions were severely reduced after it was transported through both porous media used. Counting results are listed in table 3. Cell counting with the cytometer indicated that over 60% of the injected bacteria were retained by the sand and over 90% by the soil. About the same amounts of cells were transported for sludge A and sludge B meanwhile bacteria from sludge C were slightly less transported. Also more cells were counted with the cytometer in sludge C than A or B (table 3). Column experiments were repeated two times and counting results were repeatable (Results not shown). Flow cytometer counting did not detect any cells in the recovered control solution from the sand columns while the amount of cells from the soil was low (table 3).

The sludge solutions contained between 3.6 10^4 and 5.11 10^5 CFU.ml^{-1} of bacteria able to grow on m-FC agar at 44.5°C. Only a few percent of these bacteria were recovered from either the sand or the soil columns (table 3). It's also interesting to notice that bacteria able to growth on m-FC agar at 44.5°C represented only a negligible fraction of the total amount of bacteria counted with the flow cytometer. The controls (transport experiment where the sludge solution was replaced with a sterile 10^{-4}M $CaCl_2$ solution) indicated that the out coming solution from both porous media did not contain bacteria able to cultivate on m-FC agar (44°C).

3.2. SSCP pattern analysis

SSCP patterns were obtained with the w49 and the w104 primers amplifying the V3 region of the 16S rRNA gene. Patterns were analyzed using GeneScan 3.1 Software (Applied Biosystems) and the results are compiled in figure 1 and 2. Simpson's Diversity Index was used as a measure of diversity and takes into account the number of species

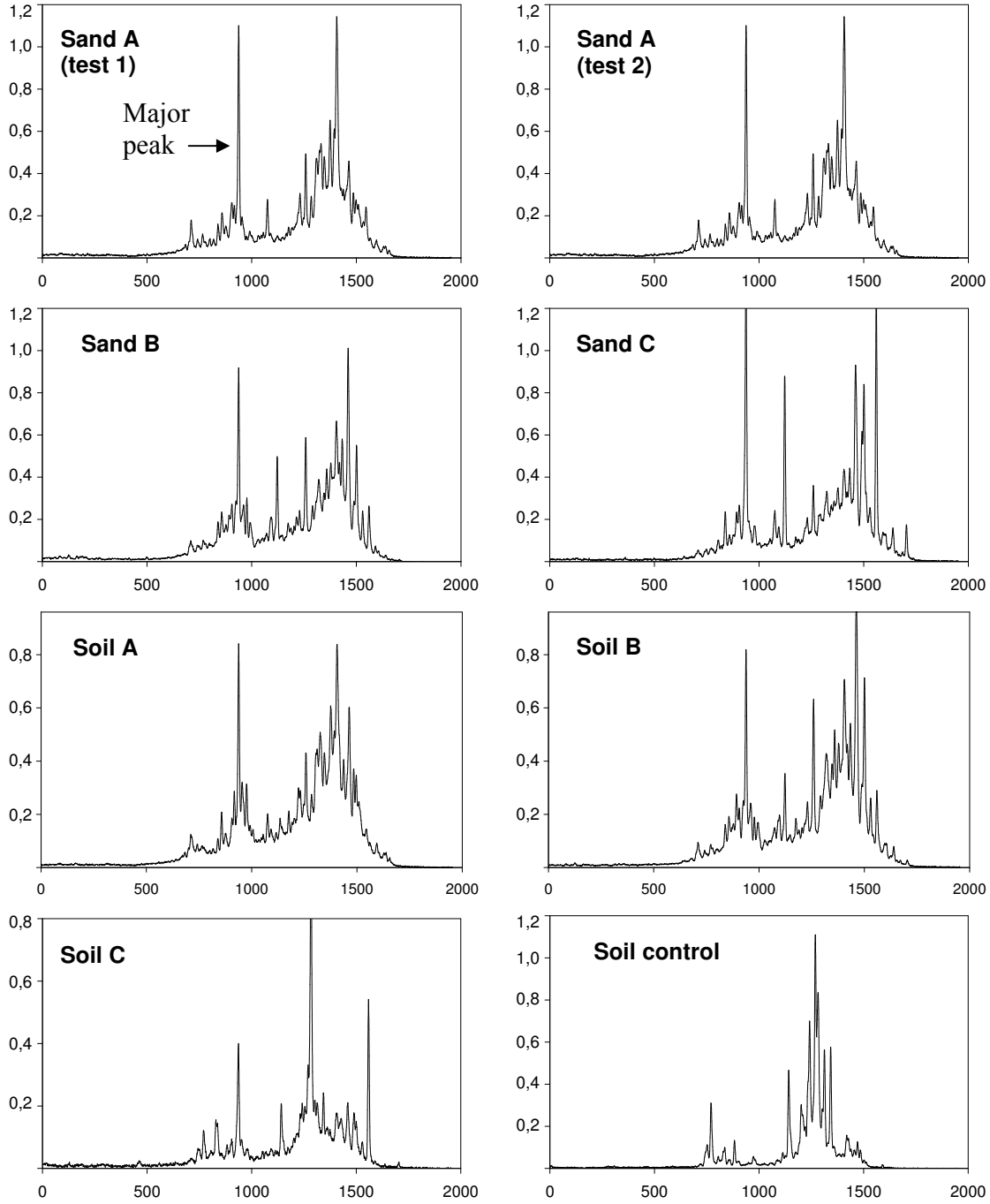

Figure 2: SSCP patterns of PCR amplified 16S rRNA gene fragments from bacterial communities of sludge A, B and C after their transport through sand or soil media.

The Soil control corresponds to the same experiments but without sludge.

present, as well as the abundance of each species. Simpson index measurements results are listed in table 4. Bacterial communities of sludge A, B and C showed great diversity (figure 1) with Simpson indexes above 6 and over 32 major peaks. Sludge A and B were very alike with a Simpson index of 6.27 and 6.35 respectively. Sludge C presented a higher diversity with a Simpson index of 7.45 and 38 major peaks.

SSCP analysis showed that the bacterial sludge community was modified after transport on either sand or soil porous media. Figure 2 shows the SSCP patterns of out coming solutions. The Simpson indexes for the recovered solutions from the column experiments are summarized in table 3. During transport through sand porous media the microbial diversity of either A, B or C sludge was reduced (table 3 and 4). The greatest reduction of the microbial diversity occurred for sludge C whose Simpson index was decreased from 7.45 to 4.72. These results are in line with those obtained with the bacterial counting experiments which showed bacteria from sludge C were less transported than bacteria from sludge A and B. Microbial diversity reduction for sludge A and B on the sand column experiments were about the same (table 4). Also SSCP patterns entitled “sand A test 1” and “sand A test 2” in figure 2 were obtained from two distinct column experiments and illustrates that the results were repeatable (other duplicated column experiments are not shown but displayed similar repeatable results as for sand A).

Transport through the soil led to a microbial diversity reduction different from observed with the sand columns. It should be kept in mind that the soil was not sterile as opposed to the sand which was heat treated before use. While the control from the sand showed no SSCP signal (result not shown), the control of the soil had a Simpson index of 4.47 with 15 major peaks (figure 2). This result indicates that the out coming solution was containing autochthonous soil bacteria. However it was still possible to observe that transport through the soil media strongly modified the sludge bacterial communities (Soil A and Soil B and Soil C in figure 2).

All the SSCP patterns (excepted from the control) presented one identical dominant peak around abscissa 950 (Fig 2). This peak represented an estimated 5 to 8% of the total microbial population for all the recovered solutions. It showed that the bacterial strain

Sludge solutions		Sand	Soil
Sludge A (6.27)	column 1	5.60	6.52
	column 2	5.45	6.12
Sludge B (6.35)	column 1	5.62	5.35
	column 2	5.33	5.12
Sludge C (7.45)	column 1	4.72	4.69
	column 2	NA	4.69
control		0,00	4.47

Table 4: Simpson indexes obtained from SSCP pattern analysis for each out coming solution from the sand and the soil columns. Each experiments was repeated two times (column 1 + column 2), value in parenthesis indicate Simpson index of the respective sludge solutions. The control was performed without sludge solution.

corresponding to the peak is particularly well transported in porous media and was identified as *Rhodococcus sp*. However we failed to identify bacterial strains corresponding to other peaks.

3.3. Identification of M-FC Agar blue colonies

X bleu colonies isolated on mFC agar plates were assigned to a species, based on a >97% similarity between their partial 16S rRNA gene sequences and those in the databases (NCBI databases using the BLAST program). 3 different types of *E. coli* strains (*E. coli* HS, *E. coli* B ctg116 and *E. coli* UTI89) and 1 *Enterobacter sp.* were identified in all the out coming solutions from both porous media studied. No other thermo tolerant coliforms corresponded to the blue colonies grown on mFC agar. The same strains were identified in the all the three sludge samples.

4. Discussion

Both bacterial counting results and SSCP analysis showed great quantitative and qualitative reduction of bacterial sludge community when transported through soil or sand. Previous studies have shown that cell surface characteristics (Becker *et al.* 2004; Chen et Strevett 2001) (i.e. hydrophobicity, electrophoretic mobility, polymers…) and size (Gannon 1991) are important parameters influencing bacterial transport in porous media. Sludge from waste water plant contains thousands of different bacterial strains which display a wide range of cell characteristics. As a result transport behaviours through porous media of bacteria from sludge might be very variable depending on cell properties of each strain. More than 60% and 90% of the bacterial cells were not transported in the sand and soil media respectively. Thus we assume the majority of the bacteria displayed cell characteristics unfavourable for their transport through porous media. According to several authors, hydrophobicity, low electrophoretic mobility and increasing cell size contribute to bacterial deposition in porous media. Other authors described the influence of cell wall polymeric substances (EPS, LPS…) for preventing bacterial transport in a porous matrix (DeFlaun *et al.* 1999; Tsuneda *et al.* 2003). It should be kept in mind that with a 10^{-4}M $CaCl_2$ back ground solution conditions were

unfavourable for bacterial adhesion as low ionic strength result in strong electrostatic repulsions between the cells and the porous media grains. Despite these conditions bacterial retention in both porous media occurred nevertheless and other parameters than cell characteristics or physicochemical interactions are presumably involved.

Flocculation of bacterial cells might also help for preventing bacterial transport. Bacterial cells sticking together will give shape to colloids with a great diameter and so filtration in porous media might occur. Flocculation in waste water treatment process is very common. Standing alone cells are not filtrated as their diameter is far inferior to the average diameter pore size of the porous media used. According to Bradfrord *et al.* filtration occurs when particle diameter is higher than 5×10^{-3} times the mean diameter of porous media grains (Bradford *et al.* 2002). Using this criteria with our porous material, bacterial cells with a diameter smaller than 2 µm should not be strained.

Despite the majority of the cells being retained, the amount of bacterial strains able to travel through both the soil and the sand media should not be neglected. Several authors consider decreasing cell size and increasing negative electrophoretic mobility as the most important cell properties favourable for bacterial transport. One strain was identified as very capable of being transported through both porous media used as it represented 7 to 9% of the total bacterial communities in the out coming solutions: *Rhodococcus sp.*. *Rhodococcus sp.* transport in porous media has already been studied previously (Priestley 2006; Rijnaarts *et al.* 1996; Schafer *et al.* 1998). Under certain nutrient limited growth conditions, Priestley *et al*. observed almost 100% of the *Rhodococcus sp.* DN22 injected cells recovered at the outlet of the sand columns (Priestley 2006). Their results support our observations of *Rhodococcus sp.* transport behaviour through sand and soil media. Shafer *et al.* showed that their *Rhodococcus sp.* Strain (C125) displayed very negative zeta potential (-44mV in low ionic conditions) (Schafer *et al.* 1998). As most sediment have also negative surface strong electrostatic repulsion occur between such bacteria and the porous media grains which is a well known factor for enhancing bacterial transport. Moreover strong negative charged cell surface may avoid flocculation of *Rhodococcus sp.* cells with other bacteria as electrostatic repulsions also operate between cells (van Oss 1989). In a previous study we found a good correlation between the zeta potentials from 21 bacterial strains and their respective rate of adhesion on sand grains (Jacobs *et al.* 2007). We also found that two different *E. coli* strains showed the best transport capacity

in sand among the 21 bacterial strains studied, presumably because of their highly negatively charged cells surface. In this study three different *E. coli* strains were identified in the out coming solutions from both porous media. The zeta potentials of these three *E. coli* strains were measured according to the method described previously by Jacobs *et al.* **(Expériences en cours)**. It seems that *E. coli* cells are easily transported through porous media which makes it a good indicator for fecal pollution of soils.

5. Conclusions

The majority of the bacterial strains from the sludge communities studied were retained by both the sand and the soil media. But despite the fact that porous media seems to act as an efficient barrier against bacterial transport still some strains were able to travel through the porous media used. Among them we identified *Rhodococcus sp.* and *E. coli* strains. Regular disposal of sludge from waste water plants may have a negative impact on the environment and on autochthonous soil bacteria if accumulation of bacteria from sludge in soils occurs. Moreover pathogenic organisms with cell characteristics close to the *E. coli* strains identified in this study are likely to be transported through soils and contaminate drinking water sources. Research should focus on these particular strains displaying great transport capabilities. In light of this study spreading of waste water effluents on fields is not without consequences and such practices should be better monitored.

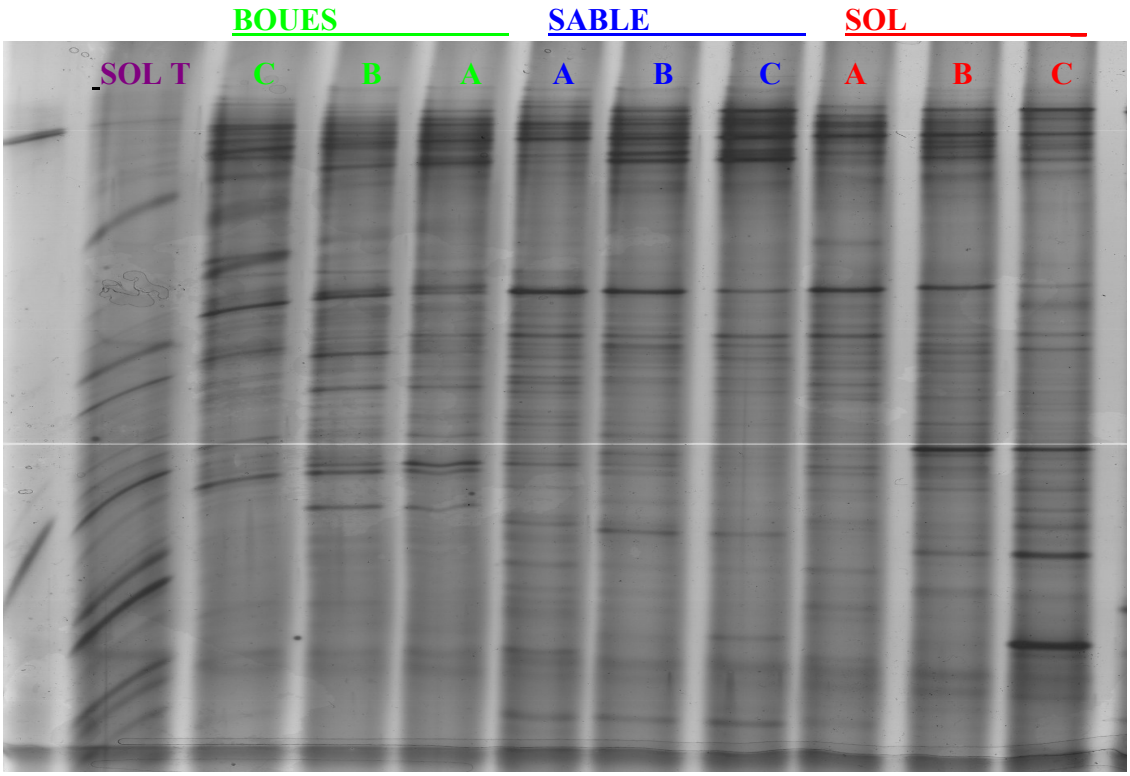

Figure 3: Profils DGGE des Boues A, B et C de la station d'épuration de Caromb (Vaucluse) ainsi que des solutions A, B et C issues des colonnes de sable et du sol. Profils obtenue à partir d'ADN extrait. SOL T correspond au témoin sol.

6. Résultats complémentaires

Parallèlement à la technique SSCP une approche identique avec la méthode DGGE (Denaturing gradient gel electrophoresis) a été tentée. La DGGE est une technique d'électrophorèse permettant la séparation de molécules d'acides nucléiques (ADN ou ARN) de même taille mais de séquence nucléotidique différent. Le principe de séparation est basé sur l'ouverture des doubles brins par un agent dénaturant (par exemple l'urée) dont la concentration dans le gel augmente avec la distance de migration. L'ouverture du brin (qui dépend de la proportion de liaisons GC dans la séquence du brin) provoque l'arrêt de la migration.

La figure 3 illustre les profils DGGE obtenus pour l'extrait d'ADN des boues A, B et C ainsi que l'extrait d'ADN des solutions de boues A, B et C après leurs transport soit sur du sable soit sur le sol. Le sol témoin (Sol T) correspond à la solution transportée sur le sol sans boue.

On constate que les boues A et B ont des profils DGGE semblables mais différents de la boue C. Ce résultat est en concordance avec les résultats obtenus en SSCP. Le nombre de bandes détecté dans les profils des boues est plus importantes (une trentaine) que dans les solutions issues du sable ou du sol (une vingtaine). Ces résultats sont en analogies avec les profils SSCP qui ont montrés que la diversité microbienne d'une communauté bactérienne d'une boue diminue sensiblement après son transport sur du sable ou un sol.

Certaines bandes détectées dans les boues se retrouvent sur les profils DGGE des solutions issues des milieux poreux signifiant que les souches correspondant à ces bandes n'ont pas été retenu ni par le sable ni par le sol. Nous avons tenté de séquencer ces souches mais sans véritable succès. En effet il faut tenir compte que deux espèces peuvent correspondre à une seule bande si elles ont leurs brins d'ADN ont une proportions de liaisons GC identiques. Aussi nous avons décidé d'abandonner l'approche DGGE au profit de la méthode SSCP dont les résultats obtenus furent meilleurs.