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Effect of Surface Properties and Flow Regime on the Transport of Bacteria in Groundwater: An Experimental Approach

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Abstract

Bacteria are transported with groundwater and this phenomenon has potential engineering applications such as bioremediation. The success of bioremediation can be related to transport distance or conditions that determine bacterial adhesion to the soil surfaces. In this study it is experimentally shown that different bacterial surface properties as well as different adsorption and transport characteristics can be obtained from the same species by using different growth conditions. Xenobiotic degrading bacteria, *Pseudomanas* strain D, capable of biodegrading hazardous waste (s-triazine) had more hydrophobic surface properties in the absence of carbon and less hydrophobic surface properties in the absence of nitrogen. Their transport through porous media was also affected as a result.

Key words: Bacterial transport, Groundwater, Bioremediation, Flow regime, Bacterial surface properties, MATH test.

Introduction

Native as well as non-native bacterial species capable of degrading hazardous chemicals are used in bioremediation applications (Bolster *et al.*, 2001). In the application, the success of bioremediation is determined by the transport of bacteria in groundwater and their adsorption to the surfaces. Early adsorption can be detrimental to the remediation process.

Transport of bacteria in groundwater is a combination of flow rate, water chemistry and physical and chemical properties of porous media, as well as bacterial size, shape and surface properties (Travos *et al.*, 1990). Bacteria behave different from dissolved chemicals in groundwater because they are (i) colloidal particles and (ii) they interact with the surface of the porous structure during transport. The difference in behavior is a result of several processes such as straining, dispersion, chemotaxis, and viability, as well as sorption processes. Depending on the existing conditions one or more of the processes mentioned above can be dominant. Differences in transport are also a result of bacterial surface properties.

The importance of bacterial surface properties, in addition to the water and soil chemistry in studies in related fields with different focuses such as the environment, biology, ecology and agriculture are emphasized in the literature (Mehmannavaz *et al.*, 2001).

For the success of engineering applications such as bioremediaiton the parameters that effect the transport of microorganisms in groundwater must be known and different processes must be preferred and enhanced accordingly (Wang *et al*, 2002). Experimental and field studies clearly indicate the complex interaction between the contributing parameters in bioremediation studies, (Schijven *et al.*, 2002).

When the parameters that affect the bacterial transport examined, the difficulty in changing the soil structure can easily be understood; this can only be possible for limited local applications (Li *et al.*, 1999). Therefore, variety in species and form, biodegradation ability and different surface properties make bacteria a preferred control parameter for

engineering applications (Burk *et al.*, 200; Sanin *et al.*, 2003).

Bacterial surface properties become more important when pumping is not used or when the transport distance is long (Dong, 2002). In addition to the surface properties the effect of bacterial physiology on the transport is also emphasized in the literature. According to the findings the transport of bacteria is effected by the growth phases and transport is observed as a combination of bacterial physiology and physicochemical interactions of the system (Sandrin *et al.*, 2001).

In this study, the effect of bacterial surface properties on the interaction with the sand was investigated experimentally during bacterial transport in porous media. Flow rate and surface properties were varied while the other control parameters mentioned above were kept constant. The differences observed in the transport profile of the selected bacterial culture were examined and reported.

Experimental Methods

Reactors

The reactors used in the study were produced from clear plexiglas and the properties of the reactors and experimental set-up are given in Table 1 and Figure 1, respectively. The columns were prepared with 20-30 mesh (650 μm-825 μm) precleaned Ottowa sand. The column to particle ratio used in the reactors was calculated at 128. Flow was gravitational in the columns. Laminar flow conditions were satisfied in the reactors. Reynolds numbers used in the experiments are given in Table 1. The reactors were filled using the wet filling method in order to ensure the homogeneous packing in columns. A peristaltic pump was used for water circulation.

Microorganisms and growth conditions

In this study *Pseudomonas* strain D (NRRLB-12228), an s-triazine degrader isolated from soils exposed s-trazine during agricultural activities for 3 to 7 years,was used. This species, which is known as a polymer producer, is defined as a strain with an average growth rate and capable of biodegrading several s-triazine compounds.

The composition of the growth media used in the experiments was (g/l) $MgSO_4$, 0.05; $Na_2HPO_4.2H_2O$, 0.04; $KH_2P\ O_4$, 0.037; $CaCl_2$, 0.01; $MnSO_4.H_2O$, 0.005; $FeSO_4.7H_2O$, 0.001; $CuSO_4.5H_2O$, 0.0005; and $ZnSO_4.7H_2O$, 0.0005. The pH of the system was 6.8.

Bacteria used in the transport studies were prepared under 2 different conditions. Once the bacteria reached the lag-phase stage they were harvested, washed twice and transferred to either a carbon (glucose) or a nitrogen (s-triazine) source deficit batch reactor and incubated at 20 °C for 1 week under constant stirring.

Analytical methods

The flow regimes in the column reactors were determined by tracer tests. Methylene blue was used as the tracer during the experiments. Methylene blue injected into the reactors was determined spectroscopically at 625 nm.

Bacteria in the water samples were determined by measuring optical density at 600 nm. Bacterial surface properties were determined by the Microbial Attachment to Hydrocarbon test (MATH). Details of the test was given by Sanin *et al.,* (2003). Briefly, three milliliters of bacterial suspension was transferred to a 10 mm round bottom test tube. After the initial turbidity (optical density—OD) of the system was determined, 0.3 ml of *n*-Hexadecane was added and the mixture was vortexed for 2 min. After 15 min of settlement at room temperature the ODs of the solutions were determined at 600 nm. The results are given in percentages calculated using Eq. (1);

Table 1. Reactor design parameters and experimental conditions used in the study.

Column size (cm)	5		
Column height (cm)	27		
Particle size (μm)	700		
Flow regimes used in the experiments		Flow rate (ml/s)	Reynolds Number
	Slow	0.014	5.3 x 10-3
	Medium	0.084	0.03
	Fast	0.32	0.121

$$\text{Hydrophobicity } (\%) = 100(1 - OD_{\text{final}}/OD_{\text{initial}}) \quad (1)$$

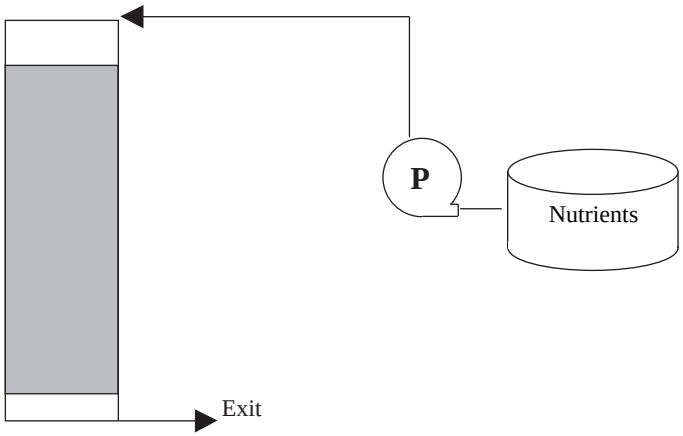

Figure 1. Schematic representation of Experimental set-up.

Results and Discussion

Experiments to characterize the flow regime

Before injection of bacteria into the column, tracer tests were conducted in the reactors. Obtained data from these tests were also used to verify porosity. In these experiments 0.32, 0.084 and 0.014 ml/s discharges were used for fast, medium and slow flow rates, respectively. The tracer test results are given in Figure 2. As the flow rate increased the residence time in the reactors decreased and minimum dispersivity was observed in the fastest flow regime. Since the tracer was not inert (chemical reaction, diffusion, adsorption) observable dispersion was reported with the decreasing flow rate. Residence times observed in the reactors for slow, medium and fast flow rates were 231, 40 and 10 min, respectively. These residence time values obtained from tracer tests were used as references in observing bacterial residence time changes in the column due to growth conditions.

Darcy's law, given in Eq. (2), an empirical approach that defines laminar flow conditions for groundwater, was satisfied in the system. Reynolds number, given in Table (1), defined as the ratio of inertial forces to viscous forces, must be less then 10 for the validity of the Darcy's law, were also satisfied in the column reactor set-up. Reynolds numbers used in the experiments were calculated to be 1.2 x 10^{-1}, 3.2 x 10^{-2} and 5.3 x 10^{-3} for fast, medium and slow flow regimes, respectively. Cross-sectional area, viscosity and particle size used in the experiments were 20.26 cm^2, 0.89 x 10^{-2} cm^2/s and 0.069 cm, respectively.

$$Q/A = -K(dh/dl) \quad (2)$$

The porosity of the columns was calculated by weighing the reactors before and after filling with water and was also verified by the tracer tests. The calculated porosity of the experimental setups was 38% . The range reported in the literature for the porosity of sand is between 20 and 50% .

Bacterial transport experiments

Pseudomonas strain D was grown under carbon and nitrogen deficit conditions in 2 separate batch reactors and at the end of incubation period bacteria were injected into the prepared columns under 3 different flow regimes, results were given in Figures 3-5. Water passing through the columns was sampled and optical densities were measured at specific time intervals.

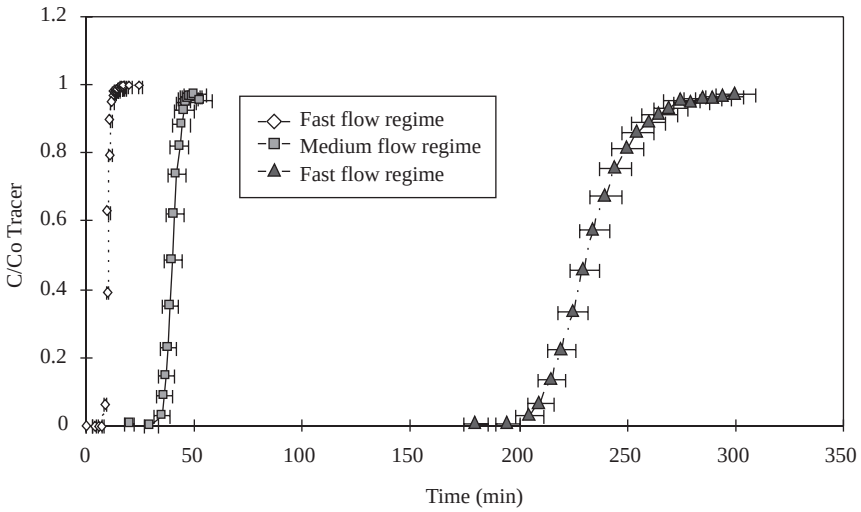

Figure 2. Tracer test results under fast, medium and slow flow regimes.

Pseudomonas strain D grown until the early stages of the lag phase in the presence of glucose and nutrients was injected into the columns as reference. During the experiments, patterns obtained for the bacterial transport were similar to the tracer test results, which indicate the effect of hydrodynamic forces on bacterial transport in porous media. The flow rates used during the experiments were selected to mimic the flow rates of bioremediation applications and natural groundwater movement. During the high flow regime experiments 5% of injected bacteria was retained in the column. The retained bacteria increased to around 10% in medium flow regimes. When the flow regime was reduced to typical groundwater flow rates (slow flow regime) around 20% of the injected bacteria were retained in the column. These observations clearly indicate the dominance of groundwater velocity on bacterial transport. With the reduction in the flow rate early breakthrough and tailing in the bacterial transport curves were observed. Deviation in breakthrough curves were significant under the slow flow regime in all bacteria cultures.

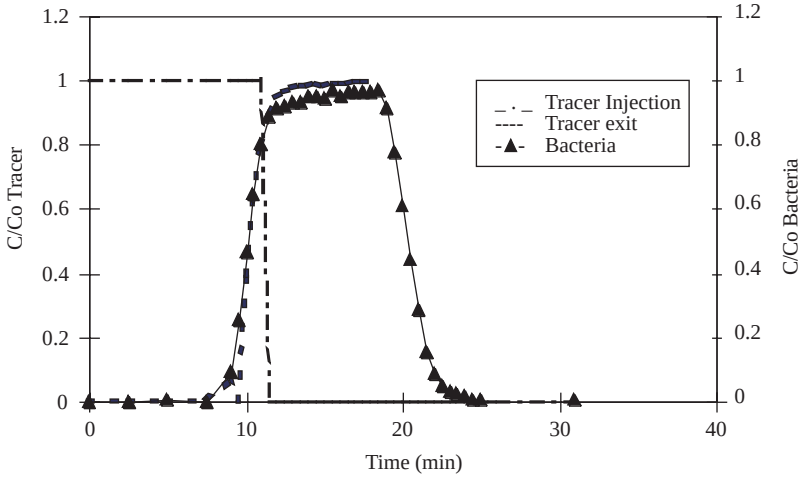

Figure 3. High flow regime tracer and bacterial transport profile.

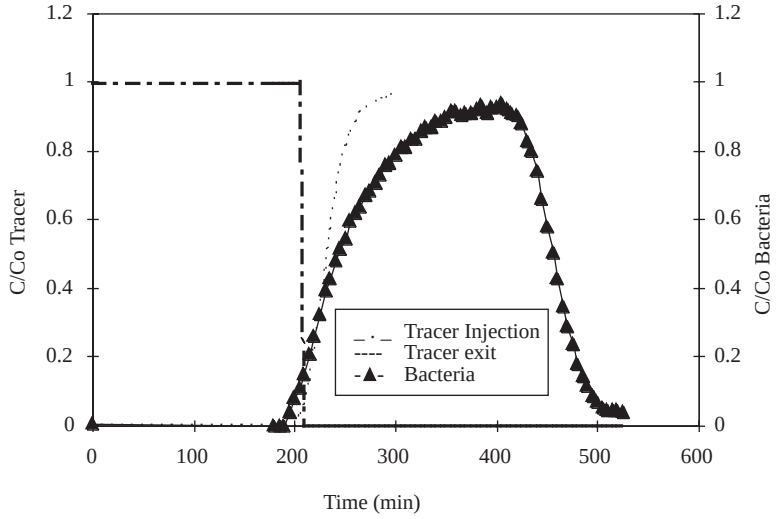

Figure 4. Medium flow regime tracer and bacterial transport profile.

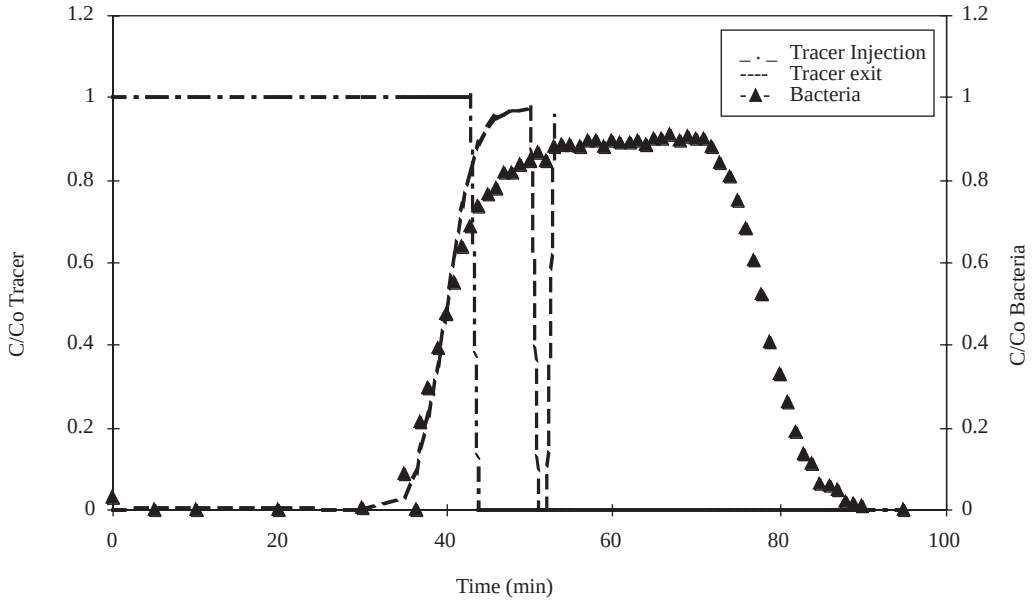

Figure 5. Slow flow regime tracer and bacterial transport profile.

In the second phase of the study interaction between bacterial surface properties and flow rate was investigated. These comparative studies were carried out by overlapping the breakthrough profiles of bacteria subjected to different environmental conditions before injection into the column. The results of these experiments are given in Figures 6-8 for high, medium and slow flow conditions, respectively. The first observation of these experiments was the change (shape deviation) in the bacterial and tracer breakthrough profiles with the decrease in the flow rate. Early breakthrough was significant for the cultures grown in both carbon and nitrogen deficit conditions compared to the lag phase population. This phenomenon, known as chromatographic exclusion, has been reported in all experiments. Bacterial injections delivered as step injections, had early and late breakthrough due to dispersion.

It can be conclude from the figures that bacteria with low surface hydrophobicity values have a similar flow profile to that of the tracer, where as more hydrophobic bacteria, although they have an early

breakthrough, stay longer in the column and create a significant tail on the profile. This may be due to the interaction of the bacteria with the sand surface in the column. It is known that surface hydrophobicity increases the interaction of bacteria with the natural surfaces and the findings of this study support those from the literature. Flocculation is frequently observed in hydrophobic bacteria, as a result floc size increase, and microorganisms may be strained and slow down while they pass through the column. The residence time of the bacterial population in the reactors during these studies is given in Table 2. The residence times of bacteria and tracer show no difference under high flow rates. The residence times of bacteria and tracer started to deviate with the decrease in water velocity. According to these results bacteria prepared at the beginning of the lag phase had similar residence times to those of the tracer in the columns under all flow regimes. Bacteria prepared in nitrogen deficit batch reactors had the shortest residence time (180 min) in the column reactors under slow flow regimes. No significant difference was observed under fast and medium flow rates. The bacterial population exposed to carbon deficiency stayed longer (256 min) in the column reactors under both medium and slow flow rates compared to dissolved tracer residence time.

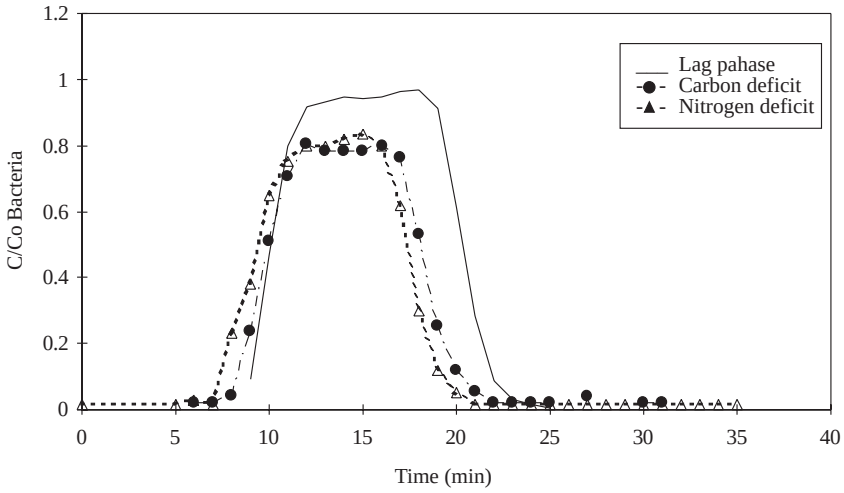

Figure 6. Transport profile of *Pseudomonas* strain D with different surface properties in the high flow regime.

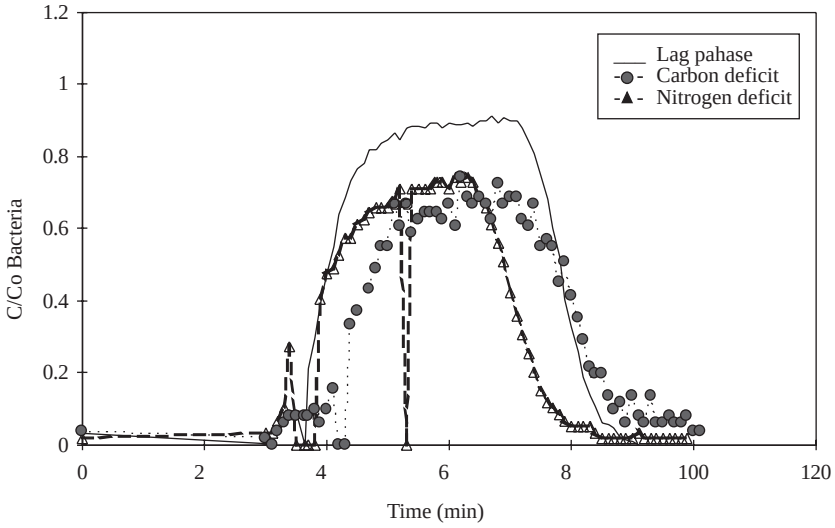

Figure 7. Transport profile of *Pseudomonas* strain D with different surface properties in the medium flow regime.

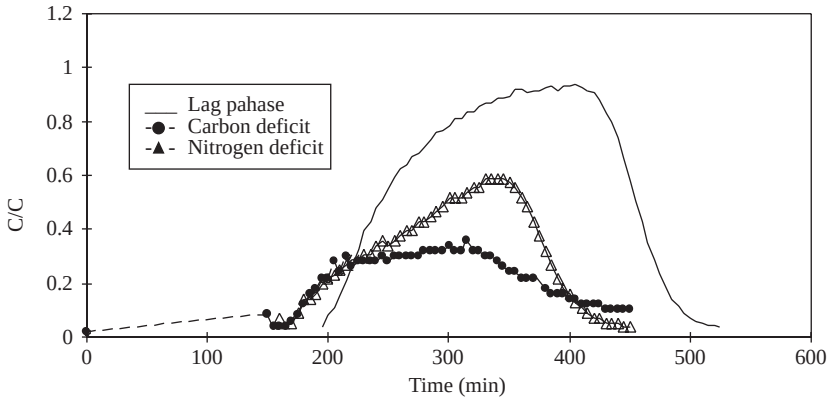

Figure 8. Transport profile of *Pseudomonas* strain D with different surface properties in the slow flow regime.

Table 2. Average residence times of tracer and bacteria in the column.

	Time (min)		
	Fast Flow (0.32 ml/s)	Medium Flow (0.084 ml/s)	Slow Flow 0.014 ml/s)
Tracer	10	40	231
Bacteria grown to lag phase	10	40	238
Bacteria grown without nitrogen	9	38	180
Bacteria grown without carbon	10	46	256

Conclusions

The results of this study indicate that (i) different surface characteristics can be obtained from the same bacterial species and (ii) such differences cause different transport behavior in groundwater. This can have significant uses in environmental engineering applications. Bacteria can be sent to predetermined subsurface locations. Travel time and distance of injected bacteria can be controlled by the surface properties of bacteria and the flow rate. Although bacterial transport in porous media is a complex phenomenon certain processes can be selected depending on the purpose and can be used to control application.

Nomenclature

Re	reynolds number (vD_p/ν)
v	flow velocity (Length/Time)
D_p	particle size (Length)
ν	kinematic viscosity (Length^2/ Time)
Q	flow rate (Length^3/ Time)
A	cross-sectional area (Length^2)
h	pressure head (Length)
l	transport distance (Length)
K	hydraulic conductivity coefficient (Length / Time)

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