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FEMS Microbiology Reviews 20 (1997) 461–472

FEMS MICROBIOLOGY REVIEWS

Microorganisms as tracers in groundwater injection and recovery experiments: a review

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Abstract

Modern day injection and recovery techniques designed to examine the transport behavior of microorganisms in groundwater have evolved from experiments conducted in the late 1800s, in which bacteria that form red or yellow pigments were used to trace flow paths through karst and fractured-rock aquifers. A number of subsequent groundwater hydrology studies employed bacteriophage that can be injected into aquifers at very high concentrations (e.g., 10^{13} phage ml^{-1}) and monitored through many log units of dilution to follow groundwater flow paths for great distances, particularly in karst terrain. Starting in the 1930s, microbial indicators of fecal contamination (particularly coliform bacteria and their coliphages) were employed as tracers to determine potential migration of pathogens in groundwater. Several injection and recovery experiments performed in the 1990s employed indigenous groundwater microorganisms (both cultured and uncultured) that are better able to survive under in situ conditions. Better methods for labeling native bacteria (e.g. by stable isotope labeling or inserting genetic markers, such as the ability to cause ice nucleation) are being developed that will not compromise the organisms' viability during the experimental time course.

Keywords: Microorganism; Groundwater; Tracer; Aquifer; Transport; Subsurface

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1. Introduction

Microorganisms were first introduced into groundwater as tracers in order to gain information about the hydrology of complex groundwater environments. However, recent interest in microbial migration through aquifers has clearly shifted to the transport behavior of the microorganisms themselves. The

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0168-6445/97/$32.00 © 1997 Federation of European Microbiological Societies. Published by Elsevier Science B.V.
PII S0168-6445(97)00026-0

shift is due largely to the now recognized importance of subsurface microbial transport in aquifer restoration, pathogen contamination of water supply wells, and oil recovery. Although considerable information on subsurface microbial transport has derived from column experiments, there is a growing awareness that factors controlling subsurface microbial transport behavior can be operative on scales of space, time, and complexity that are not conducive to laboratory study. This is leading to more in situ experiments involving the injection into aquifers of microbes that are either labeled or are clearly discernible from indigenous populations. Most controlled groundwater additions and recoveries of microbes are conducted over limited distances relative to those of interest for pathogen transport to water supply wells, microbially enhanced oil recovery from petroleum reservoirs, or using introduced bacteria for aquifer restoration. However, continued advances in our understanding of microbial transport behavior as a result of ongoing small-scale injection and recovery studies are facilitating larger-scale field experiments. For the purposes of this review, controlled injections of specific microorganisms into groundwater environments in order to enhance our understanding of subsurface transport behavior of other microbes of interest are considered to be uses of microorganisms as tracers.

The purpose of this review is to provide information about the history, specific uses, experimental methodologies, and geohydrologic and physiologic considerations pertaining to the use of microorganisms as tracers in groundwater environments. A number of studies in which 'tracer' microorganisms have been used to delineate geohydrologic aspects of transport behavior in saturated subsurface material have involved columns and are summarized elsewhere [1]. The focus of this review involves the field use of 'tracer' microorganism in groundwater injection and recovery experiments. Particular emphasis is placed upon the uses of viruses, bacteria, protozoa, and yeast to delineate flow paths in aquifers or to enhance our understanding of some of the processes controlling subsurface microbial transport. The use of spores as groundwater tracers is beyond the scope of this review and is not discussed. The interested reader is referred to a paper by Käss and Reichert [2] for a delineation of the history and methodologies involving the use of spores in hydrologic studies of aquifers.

2. History of microbial additions to groundwater

Although aquifer (well water) microorganisms were first observed by van Leeuwenhoek in the late 1600s [3], interest regarding the manner in which microorganisms move through aquifers did not develop until considerably later. Two paths of research led to the development of methodology for injection and subsequent recovery of microorganisms from aquifers, i.e., the role of groundwater in the spread of waterborne disease and the manner in which groundwater moves through aquifers dominated by preferential flow paths. The use of microorganisms as tracers to delineate subsurface microbial transport behavior in support of aquifer biorestoration did not occur until the last two decades. A history of the addition of microorganisms to groundwater for hydrologic, public health, industrial, and environmental purposes is depicted in Fig. 1. Reviews by Keswick et al. [4] and Gerba [5] discuss a number of the earlier (pre-1981) groundwater tracer studies involving the use of microorganisms. Experimental results of more recent experiments involving use of microorganisms as tracers in injection and recovery experiments in fractured rock and in granular aquifers are summarized in Table 1. For geohydrologic reasons discussed in the subsequent section, recent injections of microorganisms into aquifers characterized by preferred flow paths were made primarily for the purposes of providing convenient tracers to delineate groundwater flow. In contrast, the rationale for microbial addition to and recovery from aquifers characterized by more-homogeneous, granular media has often been to gain information about the subsurface transport potential of microbial pathogens or, more recently, of microorganisms that may facilitate biorestoration. This is because, in the latter type of aquifer, conservative (non-reactive) solute tracers that can be easily measured and do not decay or sorb to surfaces have advantages over microbial tracers as hydrologic tools. However, the division between hydrologic, public health, and environmental research involving additions of microorganisms to aquifers is not always clear. Indeed many recent mi-

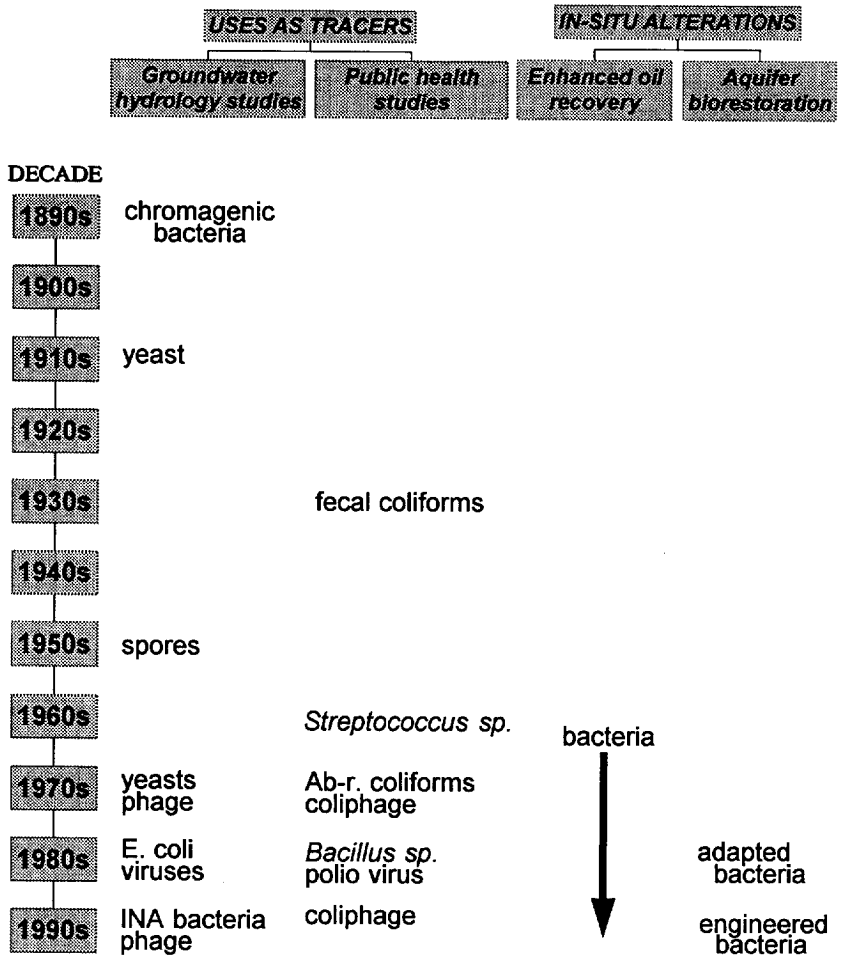


Fig. 1. History of published additions of microorganisms to aquifers. Microbial enhanced oil recovery refers to processes whereby surfactant-producing or pore-clogging bacteria are injected into oil-bearing formations in order to increase oil recovery from aging wells. Presently, the introduction of waste-adapted or genetically engineered bacteria into organically contaminated aquifers to enhance the rate of waste degradation is still experimental. Abbreviations: INA, ice-nucleation active; Ab-r, antibody-resistant.

crobial transport experiments have important implications for aquifer hydrology, subsurface transport potential of pathogens, aquifer biorestoration, origins of indigenous groundwater microorganisms, and aquifer microbial ecology. Therefore, the separation of subsurface microbial transport experiments into the two categories listed below is for organizational purposes only.

2.1. *Microbial tracers of groundwater flow*

Microorganisms were first introduced into groundwater in the latter part of the nineteenth century for the purpose of delineating flow paths in aquifers characterized by preferential flow, e.g., in karst or fractured rock. The first such studies employed bacteria that form red or yellow pigments; the results of the studies were published in the 1890s [6,7]. Bacteria are still the most commonly used biological tracers in hydrologic studies of aquifers. Until recently, non-indigenous bacteria were preferred as groundwater tracers because of the simplicity of culturing them in large quantities, the ease of detection (using light microscopy techniques or plating on selective media), and their general absence in groundwater systems. For example, antibiotic-resistant strains of *Escheri-*

chia coli, which may be enumerated as colony forming units on antibiotic-containing solid agar, were used in conjunction with solute tracers to provide hydrologic information concerning the movement of groundwater in fractured crystalline rock at the Chalk River Nuclear Laboratory site in Ontario, Canada [8] and in saturated hillslope soil [9]. Although easily differentiated from the indigenous bacteria, future use of *E. coli* and other potentially pathogenic enteric bacteria as groundwater tracers may be limited because of concerns about groundwater quality [5]. A number of more recent experiments have used labeled indigenous groundwater bacteria that do not pose a water quality threat and are more suitable for survival in the aquifer. Uncultured, fluorescently labeled communities of free-living groundwater bacteria that may be enumerated by direct count procedures and are already adapted to transport in the aquifer have been used extensively as tracers in the sandy aquifer at the USGS ground-water hydrology study site on Cape Cod, MA, USA [10–12]. A benzoate-degrading, sulfate-reducing bacterium fortuitously present in the injection water system and absent in a fractured oil-bearing formation beneath the North Sea was used as a groundwater tracer to follow penetration through the subsurface and into the oil reservoir [13]. Labeling of groundwater bacteria with stable isotopes or genetic 'markers' may allow for microbial tracers that are able to survive in the aquifer and be detected in very low numbers. At the time of this writing a series of injection and recovery experiment performed in a sandy aquifer in Oyster, VA, USA is being conducted using a ^{13}C-labeled groundwater bacterium [14]. Also, the tracing of groundwater flow in fractured karst (eastern Tennessee, USA) is being facilitated by use of the ice-nucleating active (INA) bacterium *Pseudomonas syringae* [15].

The use of yeast and protozoa as groundwater tracers in hydrologic studies has been limited, largely because of size and survival considerations. The first use of yeast to delineate groundwater flow is not clear. However, Reichert [16] makes reference to the use of 'yeast bacteria' in groundwater tracer studies conducted in the early 1900s. In 1975, 2–3 μm *Saccharomyces cerevisiae* (baker's yeast) was used in a hydrologic study of secondary permeability features in a heterogeneous sand and gravel aquifer partly cemented with calcium carbonate and interspersed with clay [17]. The advantage of using baker's yeast was that a large mass (16 kg) could be easily obtained and added to the aquifer. Survival in the short-duration (7 h) forced-gradient experiment was not a factor. More recently, hydroethidine- and DAPI-stained flagellates were injected into the Cape Cod aquifer under natural-gradient conditions for the purpose of learning more about the behavior of indigenous protists [18,19].

The use of viruses in hydrologic studies of aquifers started in the early 1970s. Although animal viruses

Table 1
Use of microbial tracers in selected recent (1986–1996) injection and recovery experiments involving fractured and granular aquifers

Formation	Microorganism	Type of test	Distance	RF[a]	Ref.
Fractured media					
clay-rich till	MS-2 and PRD1 (bacteriophage)	Natural-gradient	4 m	0.01	[22]
crystalline rock (granite)	*Escherichia coli*	Forced-gradient	13 m	0.1	[8]
North Sea oil-bearing formations	str. 4502 (sulfate reducing bacterium)	Forced-gradient	1–4 km	n/a[b]	[13]
Granular media					
alluvial sand	f2 (bacteriophage)	Forced-gradient	5 m	n/a	[24]
alluvial gravel	*E. coli* PB922 (rifampicin-resistant)	Natural-gradient	42 m	n/a	[46]
well-sorted sand and gravel	indigenous bacterial community	Natural-gradient	6 m	1.0	[10]
	PRD1 (bacteriophage)	Natural-gradient	12 m	1.0	[25]
	P32-labeled PRD1	Natural-gradient	4 m	1.0	[42]
	indigenous flagellates (protozoa)	Natural-gradient	1 m	4.7	[18]
Bassendean sand (high Si, low pH)	vaccine-type polio virus	Artificial recharge	2 m	n/a	[39]

[a]Retardation factor, $RF = V_{bromide}/V_{microbe}$, where $V_{bromide}$ and $V_{microbe}$ are the observed average velocities of the peak concentrations of bromide and microbe, respectively, that appear at a downgradient well.
[b]n/a, the data were not reported.

have been used for this purpose, some can be pathogenic to humans. Consequently, bacteriophage are generally preferred for use as groundwater tracers. The first uses of bacteriophage in hydrologic studies in the early 1970s clearly demonstrated their potential as groundwater tracers for delineating flow paths over considerable distances. For example, using only a ten-liter suspension of a type 2 phage of *Aerobacter aerogenes* injected into sandstone, groundwater flow was traced through 0.6 km of porous media at a site in South Wales [20]. Similarly, the coliphage T_4 was used to successfully trace groundwater flow for 1.6 km in the karst terrain of southern Missouri, USA [21]. The advantages of using bacteriophage as groundwater tracers include their small size (20–100 nm), negligible impact upon water quality, well-defined surface characteristics, the ability to add them at high concentration, and the ability to detect them in low numbers (by a priori concentration using positive-charge filters). Indeed, the ability to add viruses at much higher concentrations (e.g., at 10^{13} phage ml^{-1}) [22] to an aquifer than would be possible using bacteria or yeast, coupled with the ability to collect them from large volumes of groundwater (e.g., from 2000 l) [23], and concentrate them into a few ml means that they can be tracked after many log units of reduction. The advective movement of bacteriophage in a sandy aquifer recently has been determined at concentrations of less than one plaque-forming unit (pfu) per ml of groundwater [24]. Current uses of phages as tracers in hydrologic studies have involved both granular [24,25] and fracture-flow [22] aquifers.

2.2. Microbial tracers of fecal contamination

The first evidence for groundwater transport of bacterial pathogens was collected in 1854, when it was found that a cholera epidemic in Central London was the unfortunate result of a contaminated public well on Broad Street [26]. However, the use of bacterial tracers to investigate the potential for subsurface pathogen transport did not occur until considerably later. Coliform bacteria were first used as surrogate tracers for examining pathogen transport potential in contaminated groundwater in the 1930s (e.g., [27]). Since then, numerous studies have examined the migration in groundwater of bacterial indicators of fecal contamination (e.g., *E. coli*, *Streptococcus faecalis*, *Salmonella typhi*, and the coliform and fecal coliform groups). Yates and Yates [28] summarize pre-1987 observations of the extent of subsurface migrations of these pathogenic and indicator bacteria away from known contaminant sources. As expected, the lateral extent of observed migrations of these bacteria increases greatly with hydraulic conductivity. Observed advective transport of 3 m or less appear to be common for silt or very fine sand deposits [29–32], whereas transport between 0.1 and 1 km has been reported for coarse gravel, pebbles, and weathered limestone [32,33].

Over 100 types of pathogenic viruses are known to occur in water that has been contaminated with fecal material [23]. Therefore, the use of nonpathogenic viral indicators of fecal contamination has served as an important tool in public health studies involving groundwater resources. Snowdon and Cliver [34] review the merits of and techniques for using coliphage (viruses that infect specific coliform bacteria) as indicators of human enteric viruses in groundwater. The uses of 'viral indicators' to trace the lateral extent of fecal contamination in the subsurface began in the 1970s (e.g., [35,36]). As is the case for bacteria, viral indicators of fecal contamination can travel considerable distances in coarse-textured and karstic terrain [28].

Many of the earlier observations of the extent of virus transport in aquifers made use of human or animal enteric viruses and coliphages that were already present in the contamination sources. Unfortunately, the loading histories in these situations were often poorly understood, which limited what could be concluded about the transport behavior of the virus being monitored. However, there were several notable exceptions in which the loading history involved a controlled injection of a nonpathogenic indicator virus. For example, Noonan and McNabb [37] injected the T_4 coliphage into an alluvial aquifer at an on-land sewage disposal site in New Zealand and traced the virus to an observation well over 900 m downgradient. Schaub and Sorber [38] did a continuous 7-day addition of the f2 coliphage into a rapid infiltration sewage disposal site and monitored its concentration in an adjacent observation well. More recently, a recharge basin located in high silica, very low pH sand in Western Australia was seeded

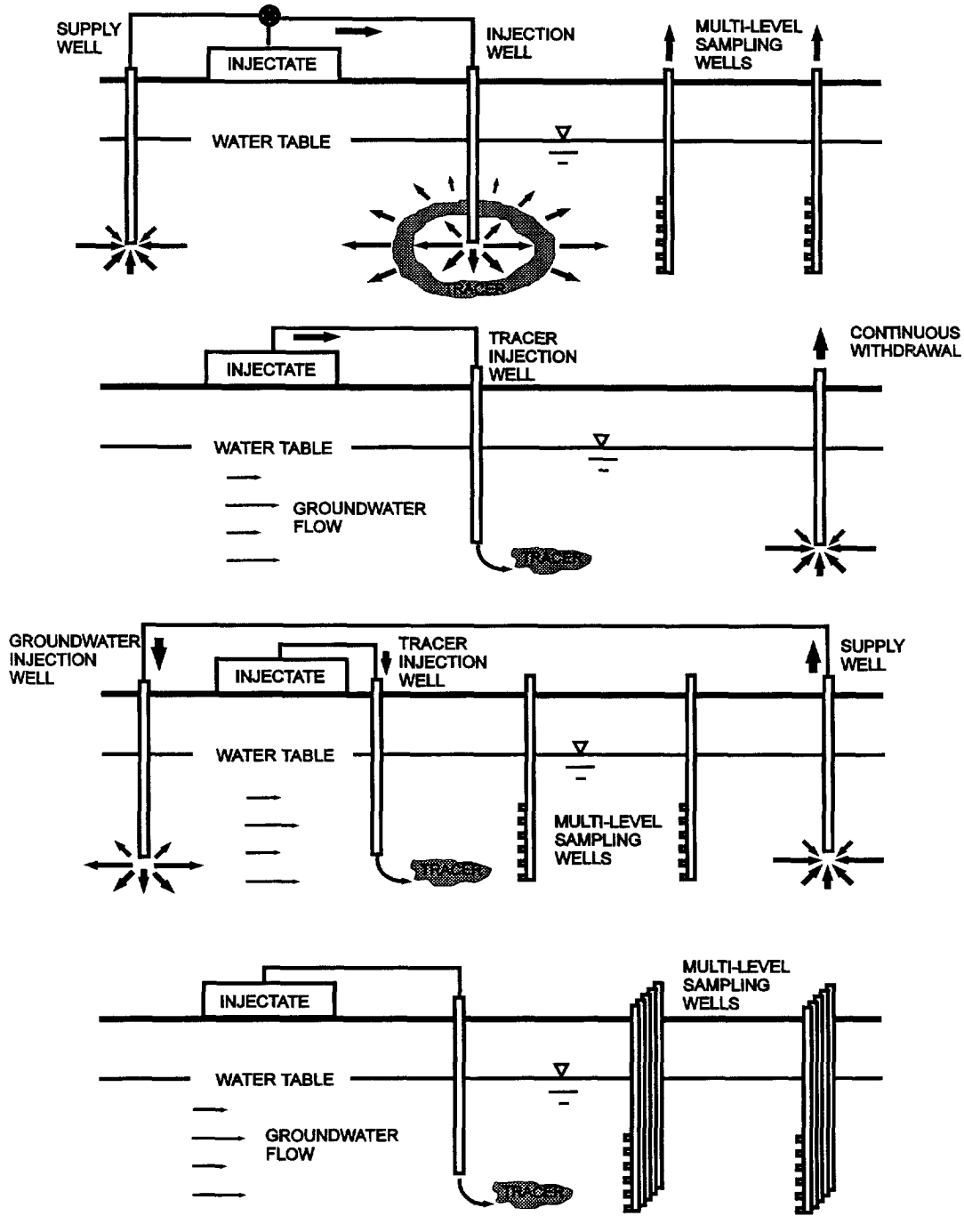


Fig. 2. Hydrologic designs for small- and intermediate-scale injection and recovery experiments for examining microbial transport behavior in groundwater (reproduced from Ref. [1] with permission from the American Society for Microbiology). A: Divergent, forced gradient; B: convergent, forced-gradient; C: doublet cell, forced-gradient; D: natural-gradient tracer test designs.

with vaccine-type poliovirus and the extent of travel in the underlying aquifer was monitored by sampling observation wells downgradient from the basin [39].

3. Methods of injecting and recovering microbial tracers

Early uses of microorganisms as tracers often involved a simple addition of the organism to the aquifer and a search for its appearance downgradient. In contrast, modern methodology often involves addition of the microorganism of interest under well-understood or controlled hydrologic conditions together with a conservative tracer (e.g., bromide, chloride, or iodine) and sometimes with similar-sized microspheres, which serve as a particulate analog. Complete concentration histories of the injectate constituents are often collected from a number of points downgradient from the point of injection. In granular aquifers, the concomitant use of conservative tracers and a three-dimensional sampling grid greatly facilitates determination of the microbe's dispersion, retardation, and immobilization rates. This allows for more information to be gathered about the transport behavior of the microorganism being used as the tracer [1].

There are several types of injection and recovery tests that may be employed to study the transport behavior of microbial tracers in groundwater (Fig. 2). In forced-gradient tests, high-volume pumping is used to control the flow field. Continuous pumping may be done at the point of injection, point of withdrawal, or both. The advantage of forced-gradient tests is a substantially shortened time frame required for observing complete breakthrough of the tracer microorganism and conservative tracer. The forced-gradient test is often preferred for use with aquifers characterized by fracture-flow, because a complete understanding of the flow paths under natural-gradient conditions is not necessary in order to decide where to place the sampling well(s). Indeed, with the exception of the experiment involving virus transport through fractured clay, the recent microbial tracer experiments listed in Table 1 that involve fracture-flow were all performed under forced-gradient conditions. The major disadvantages of forced-gradient transport experiments are that the resulting flow-fields are non-uniform and the tracer microorganisms are subjected to a considerable degree of forced dispersion. This can limit the distance over which the microbial tracer can be followed and can make modeling of transport more difficult.

Forced-gradient conditions can be engineered by using a variety of pumping schemes and include divergent (Fig. 2A), convergent (Fig. 2B), and doublet

Table 2
Comparison of transport parameters for labeled-indigenous groundwater bacteria in injection and recovery experiments involving a sandy aquifer and one characterized by preferential flow

Location	Aquifer type	Test type	Relative breakthrough[a] (RB)	Collision efficiency[b] (α, $\times 10^{-3}$)	Retardation factor[c] (RF)
Cape Cod (MA)	Sand and gravel	Natural-gradient			
8.5 m bls[d]			0.15	8.5	0.99
9.1 m bls			0.21	5.4	0.97
Oahu (Hawaii)[e]	Fractured, layered basalt	Convergent	~ 1.0	< 0.1	0.62

[a]Relative breakthrough (RB) is the ratio of the integral of the dimensionless bacterial concentration to that of bromide.

[b]Collision efficiency, $\alpha = (d[[1-2(A_L/x_1)\ln(RB)]^2-1])/(6(1-\theta\eta A_L)$, where d is the median grain size (cm), x_1 is the travel distance from point of injection to the point at which the concentration history was monitored (6.7 and 27 m for the Cape Cod and Hawaiian experiments, respectively), θ is the porosity and η is the single collector efficiency. The latter parameter is the rate at which the labeled-bacteria strike a single sand grain divided by the rate at which they move toward the grain and represents the physical factors determining collision. Apparent longitudinal (direction of flow) dispersion (A_L) for the bacteria was estimated using $A_L = (x_1(\Delta t/t_{peak})^2)/16 \ln 2$, Δt is the duration of breakthrough when $C(t) > 1/2$ peak concentration and t_{peak} is the time to peak concentration.

[c]Retardation factor, $RF = V_{bromide}/V_{bacteria}$, where $V_{bromide}$ and $V_{bacteria}$ are the observed average velocities of the peak concentrations of bromide and labeled-bacteria, respectively, that appear at a downgradient well.

[d]Depth below land surface.

[e]Data from Harvey, Voss and Souza, unpublished.

cell (Fig. 2C) tests. In a divergent test, known quantities of microbial tracers are added quickly to a continuous stream of groundwater that is being injected back into the aquifer to create the gradient. This produces radially divergent flow and forces the gradient in all directions. An example of this type of test was conducted with stained-groundwater bacteria and bromide at an uncontaminated site in the Cape Cod aquifer [12]. The convergent test involves the continuous withdrawal at the sampling well. An advantage of this type of test is that all of the conservative tracer can be theoretically recovered at the sampling well allowing a true mass balance to be calculated for the experiment. A convergent tracer test was run to observe the below-described transport behavior relative to bromide of stained, cultured groundwater bacteria in a fractured, layered basalt aquifer in Oahu (Table 2). Finally, an example of a doublet cell was an experiment set up to look at the transport behavior of *E. coli* relative to bromide in a fractured granite aquifer in Ontario [8].

For relatively homogeneous, granular aquifers, natural-gradient tests (Fig. 2D) can be run in which the injectate is added slowly to the aquifer and the natural flow of groundwater advects the injectate cloud past rows of multi-level samplers (MLS) placed in rows perpendicular to the flow path. The latter type of experiment is best suited for situations in which the gradient can be predicted within reasonable limits and where the water table is shallow enough to permit the use of peristaltic pumps to sample water from each level of the MLS. Construction and use of MLS for groundwater studies involving microorganisms are discussed by Pickens et al. [40] and Smith et al. [41]. Natural-gradient tests have been used to examine the transport behaviors of protozoa, bacteriophage, and bacteria through organically contaminated aquifer sediments at the Cape Cod site [10,18,25,42]. More complete description of methodologies for using microorganisms in injection and recovery experiments, including labeling, physiological, and analytical considerations are given elsewhere [1].

4. Geohydrologic considerations

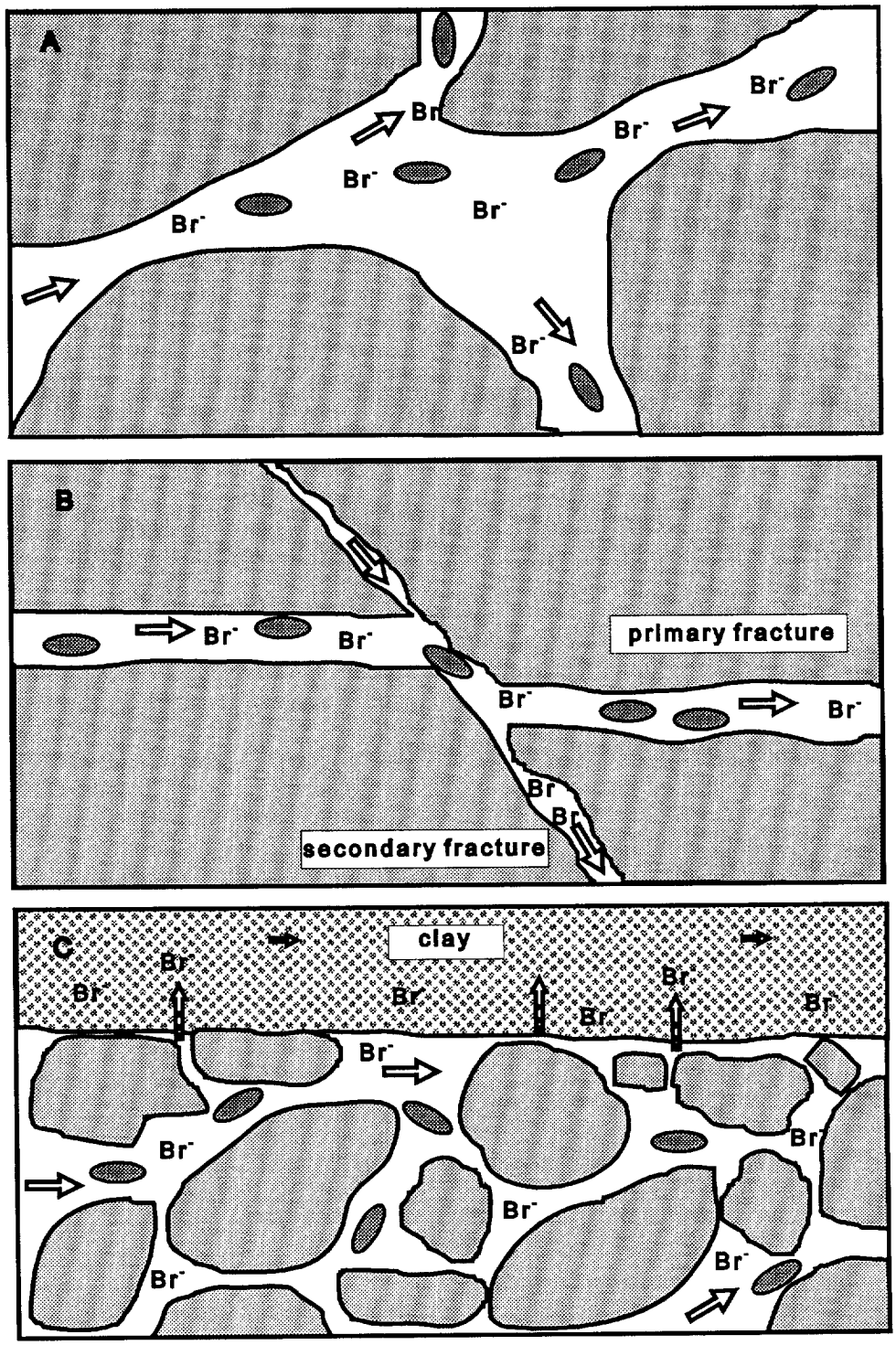
Whereas the suitability of a microorganism to help predict the subsurface transport potential of a pathogen depends largely upon its physical, physiological, and surface chemical characteristics, the utility of microbial tracers in hydrologic studies of aquifers can often depend upon the geohydrologic character of the aquifer. Because microorganisms in the aquifer can be retarded [10], immobilized [25], confined to preferential flow paths on the basis of size [17], grow in situ [43], and fall prey to protozoa [44], it is useful to compare the transport behavior of the microbe with the conservative tracers. It has been found in both the laboratory columns [45] and in small-scale injection and recovery tests performed in the field [10] that physical heterogeneity increases the dissimilarity between the transport behavior of microorganisms and the conservative tracers.

The pore-scale movement of bacteria and bromide in injection and recovery tests involving aquifers consisting of relatively homogeneous granular material (e.g., uniform sand), fractured media, and stratified granular material consisting of adjacent layers of fines and coarser sand are schematically depicted in Fig. 3A,B,C, respectively. In relatively homogeneous sandy media, the microbial tracers that do not become immobilized within the matrix can exhibit concentration histories at downgradient samplers that resemble that of the conservative tracer (Fig. 3A). An example of this type of matrix is the one in Cape Cod, Massachusetts, where the bacteriophage PRD1 and labeled groundwater bacteria injected into the aquifer in small-scale, natural-gradient experiments closely tracked the movement of bromide [25]. In this type of system, microorganisms and dissolved tracers take the same flow paths and consequently the conservative tracers yield important information about where and when to sample for the microbial tracers.

In contrast, the transport behaviors of microbial and solute tracers in fractured media are often dissimilar, sometimes to the point where conservative

→

Fig. 3. Schematic depiction of bacteria and bromide being advected through aquifer material. A: Relatively homogeneous granular material; B: fractured media; C: layered fine (clay or fine silt) and coarse granular media. Broken arrows in the bottom figure (vertical) indicate bromide diffusion into the matrix. Large arrows indicate direction of groundwater flow.


A
Br⁻
B
primary fracture
secondary fracture
C
clay

tracers are of limited value in deciding when to sample for the microorganisms that were coinjected with them. This is because the pore volume along the flow path that is kinetically or spatially available for the microorganism can be substantially smaller than that available for the conservative tracer. Conservative tracers like bromide are small enough to advect or diffuse into some the fine fractures that are connected to preferential flow paths, but are inaccessible to the microorganisms (see Fig. 3B). The greater transport velocities observed for microorganisms versus conservative tracers results in apparent retardation factors for microbial transport in fractured media that are substantially less than one (see Table 1). For example, in a forced-gradient experiment, *E. coli* was observed to travel almost an order of magnitude faster than bromide [7]. The smallest retardation factors are typically observed where the preferred flow paths capable of allowing microbial transport represent a very small fraction of the total porosity. For example, it was recently shown in fractured clay that bacteriophage could travel almost two orders of magnitude faster than bromide [22]. This has important implications for potential transport of pathogens; clearly, the time frame required for microbial pathogens from a known source to reach drinking water wells located in areas having fractured media can be much less than would be predicted based upon first detection of conservative tracers.

The movement of bromide into fine-porosity that contributes little to bulk movement of groundwater (matrix diffusion) has been used to mathematically describe the differences between bromide and virus transport in flow-through columns of fractured tuff [24]. Table 2 illustrates the fundamental differences in transport behavior of fluorescently labeled groundwater bacteria (relative to bromide) between the sandy aquifer on Cape Cod and a fractured, layered basalt aquifer on the island of Oahu (Hawaii) using data collected from injection and recovery experiments. The injection at the latter site was made in a highly conductive layer of rubble that was sandwiched between layers of fractured basalt. In the Hawaiian experiment, almost all of the labeled bacteria injected into the aquifer were recovered at the pumping well located 27 m downgradient. Judging from this negligible loss of mass, the shape of bacterial breakthrough curve, and the average velocity relative to bromide, it appears that the bacteria were confined to the highly transmissive layer into which they were injected. The high groundwater velocity and low specific surface area of the highly conductive layer afforded relatively few opportunities for bacterial attachment. This was not the case for the sandy aquifer in Cape Cod, where 79–85% of the bacteria were immobilized over the first 7 m of travel. In the Hawaiian experiment, the peak bacterial concentration traveled substantially faster than that of bromide, which continued to breakthrough long after all the labeled bacteria had been collected at the pumping well. This suggests that a substantive fraction of the bromide moved into fractures in the adjacent intact basalt layers and (or) into the interior matrices of the rubble within the transmissive layer. The opposite was observed at the Cape Cod site, i.e., the bacteria continued to appear at the downgradient sampler after the bromide cloud had passed by.

Physical heterogeneity in granular media can also result in dissimilarities between the transport behavior of microbial and conservative tracers. For example, Wood and Ehrlich [17] found that yeast cells traveled up to five times faster (at peak concentration) through sand and gravel cemented with calcium carbonate than did the iodide tracer. They concluded that most of the yeast cells traveled through preferred flow paths caused by solution of the calcium carbonate. Even in the relatively homogenous sandy glacial outwash aquifer on Cape Cod, differences in degrees of physical heterogeneity among different sand and gravel strata can result in depth-specific differences in the relative transport behavior of microbial tracers. For example, in a natural-gradient experiment involving the coinjection of labeled groundwater bacteria and bromide into overlying strata, the peak in bacteria at 6 m downgradient lagged behind the bromide peak at the 8.8 m depth, preceded the bromide peak at the 9.0 m depth, and coeluted with the bromide peak at the 9.5 m depth [11]. Differences in transport behavior between microbial and dissolved tracers in the presence of both fine and coarse granular material are schematically depicted in Fig. 3C. In the figure, bromide is shown as moving into very fine-grained material (clay or fine silt), whereas the bacteria are confined largely the preferential flow path composed of coarser sand or gravel.

5. Conclusions

Microbial tracers tend to be excluded on the basis of size from much of the fine porosity outside preferred flow paths and, consequently, can have an advantage over dissolved tracers for helping delineate major flow paths through fracture-rock and limestone. However, there is an increasing interest in the subsurface transport behavior of the microorganisms themselves, because of their recognized or potential role in biorestoration of contaminated aquifers, spread of waterborne disease, origin of subsurface microbial populations, enhanced oil recovery, and aquifer ecology. This had led to increasing numbers of controlled experiments in which microorganisms are injected into aquifers and recovered downgradient. Although there have been numerous static and flow-through column experiments delineating specific controls of subsurface microbial transport, there is a growing realization that many of these processes can be interrelated and operative on spatial and temporal scales not conducive to small-scale studies in the laboratory. The need for better information from field studies has necessitated the use of different types of tracers (e.g., conservative solutes such as bromide and particulate analogs such as microspheres) that are typically coinjected with the microorganism into the aquifer, improved field instrumentation, and a better understanding of the system hydrology. Better methods are also being developed to label microorganisms in a manner that will not compromise their viability in the aquifer.

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