Environmental Science & Technology

ARTICLE

pubs.acs.org/est

Differential Effects of Dissolved Organic Carbon upon Re-Entrainment and Surface Properties of Groundwater Bacteria and Bacteria-Sized Microspheres during Transport through a Contaminated, Sandy Aquifer†

Ronald W. Harvey,*,‡ David W. Metge,‡ Arvind Mohanram,§ Xiaodong Gao,⊥ and Jon Chorover⊥

‡National Research Program, U.S. Geological Survey, 3215 Marine Street, Boulder, Colorado 80303, United States

§Department of Molecular Bioscience and Bioengineering, 1955 East-West Road, Agricultural Science 218, University of Hawaii at Manoa, Honolulu, Hawaii 96822, United States

⊥Department of Soil, Water and Environmental Science, University of Arizona, Tucson, Arizona 85721, United States

ABSTRACT: Injection-and-recovery studies involving a contaminated, sandy aquifer (Cape Cod, Massachusetts) were conducted to assess the relative susceptibility for in situ re-entrainment of attached groundwater bacteria (*Pseudomonas stuzeri* ML2, and uncultured, native bacteria) and carboxylate-modified microspheres (0.2 and 1.0 μm diameters). Different patterns of re-entrainment were evident for the two colloids in response to subsequent injections of groundwater (hydrodynamic perturbation), deionized water (ionic strength alteration), 77 μM linear alkylbenzene sulfonates (LAS, anionic surfactant), and 76 μM Tween 80 (polyoxyethylene sorbitan monooleate, a very hydrophobic nonionic surfactant). An injection of deionized water was more effective in causing detachment of micrsopheres than were either of the surfactants, consistent with the more electrostatic nature of microsphere's attachment, their extreme hydrophilicity (hydrophilicity index, HI, of 0.99), and negative charge (zeta potentials, ζ, of -44 to -49 mv). In contrast, Tween 80 was considerably more effective in re-entraining the more-hydrophobic native bacteria. Both the hydrophilicities and zeta potentials of the native bacteria were highly sensitive to and linearly correlated with levels of groundwater dissolved organic carbon (DOC), which varied modestly from 0.6 to 1.3 mg L^{-1}. The most hydrophilic (0.52 HI) and negatively charged (ζ -38.1 mv) indigenous bacteria were associated with the lowest DOC. FTIR spectra indicated the latter community had the highest average density of surface carboxyl groups. In contrast, differences in groundwater (DOC) had no measurable effect on hydrophilicity of the bacteria-sized microspheres and only a minor effect on their ζ. These findings suggest that microspheres may not be very good surrogates for bacteria in field-scale transport studies and that adaptive (biological) changes in bacterial surface characteristics may need to be considered where there is longer-term exposure to contaminant DOC.

INTRODUCTION

The role of dissolved organic carbon (DOC), both natural organic matter (NOM) and organic contaminants, upon subsurface bacterial transport has important environmental and public health implications. This is because migration of bacterial pathogens to water supply wells and bacterial transport within contaminated aquifers undergoing natural or engineered restoration often involve groundwater with elevated levels of DOC. The ability of humic material to enhance advective bacterial transport through saturated granular media has been well documented (e.g., ref 1). However, to assess the role of DOC in bacterial transport at scales that are environmentally relevant, it is useful to consider also the contributions of contaminant organic compounds such as surfactants, some of which affect bacterial attachment behavior in aquifer sediments at "trace" concentrations.[2] Although the majority of studies examining the roles of DOC on subsurface microbial transport indicate decreased attachment to grain surfaces (e.g., ref 3), implying enhanced transport, some organics have the opposite effect.[4] Clearly, the extent to which DOC promotes or inhibits bacterial transport depends on a complex suite of poorly understood interactions among the aqueous chemistry, experimental conditions, and natures of the DOC, bacteria, and grain surfaces.

More information is also needed on the roles of DOC in the subsurface transport of microspheres, which are often employed as colloidal surrogates for bacteria in field-scale transport tests involving a variety of aquifer types[5] because they are easy to detect, chemically inert, available in a wide variety of sizes, and nonhazardous in tracer applications.[6] Studies of the effect of DOC upon the transport of microspheres through saturated granular material are less numerous than those involving bacteria. However, it appears that both NOM and surfactants can enhance

† Part of the Nanoscale Metal-Organic Matter Interaction Special Issue

Special Issue: Nanoscale Metal-Organic Matter Interaction

Received: August 31, 2010
Accepted: January 4, 2011
Revised: December 10, 2010

dx.doi.org/10.1021/es102989x | *Environ. Sci. Technol.* XXXX, XXX, 000–000

microsphere transport in aquifer sediments.[7] To date, most studies of DOC effects upon bacterial transport in groundwater have involved column experiments. Because of the chemical and physical complexities of field sites, more in situ studies are needed to better understand the roles of DOC upon the attachment and re-entrainment of bacteria and bacteria-sized microspheres.

This report describes a field study involving a surfactant-contaminated, sandy aquifer in which re-entrainment of attached carboxylated microspheres and 4′,6-diamidino-2-phenylindole (DAPI)-stained groundwater bacteria in response to injections of groundwater (hydrodynamic perturbation), deionized water (ionic strength, I_c, alteration), Tween 80, and LAS were compared. Charge densities (ζ), relative hydrophilicities, and surface functional group composition for bacterial communities collected from organically contaminated and uncontaminated zones of the aquifer were also assessed to ascertain the longer-term (biological) effects of aquifer DOC upon bacterial surface properties. Our first hypothesis was that the presence of surfactants would affect the detachment behavior of bacteria and microspheres differently because of fundamental differences in the microsphere and bacterial surfaces. Our second hypothesis was that the longer-term presence of modestly elevated, labile DOC within a contaminant plume could substantively affect the hydrophilicity and surface charge of adapted groundwater bacterial communities thereby altering their transport potential.

EXPERIMENTAL SECTION

Field Site. The field experiments were performed at the U.S. Geological Survey (USGS) Cape Cod groundwater hydrology study site (Falmouth, MA) within a large (50 m × 200 m) array of 15-port multilevel samplers (MLS) constructed of 6.5-mm (diameter) polyethylene tubes that allow discrete sampling at 25 cm depth intervals. Mean grain size, average porosity, hydraulic conductivity, and groundwater velocities are ∼0.5 mm, 0.38, ∼0.1 cm/s, and 0.3−1.0 m/d, respectively. Prior to its demolition in 1995, a treatment plant discharged secondary effluent onto sand loading beds that resulted in a 6-km-long plume of organic contaminants characterized by elevated levels of DOC (up to 4 mg/L), specific conductance (up to 400 μS cm^{-1}), and temperature (up to 18 °C) relative to adjacent uncontaminated groundwater (<1 mg/L, <80 μS/cm, and 10 °C, respectively).[8] Although the aquifer sediments were made up largely of quartz and feldspar, the grains were coated to varying degrees with Al- and Fe-bearing minerals, both polycrystalline and pure mineral crystals, that vary in thickness from <10 nm to 30 μm.[9] Fractional surface coverage of metal oxide coatings (mixture of Fe-, Al-, Si-, and Mn- containing phases) on the grains in the contaminated zone was reported as 3.5 ± 11.1% in ref 10. The ζ for the sediment grains (measured by streaming potential at pH 6−6.2) were ∼−11 mv for the uncontaminated zone and −23 mv for the contaminated zone.[11]

In-Situ Detachment Studies. Two tests were conducted that involved sequential, 100-L injections into MLS located in the upgradient portion of the aforementioned large array. Injections were made into ports GN and R, screened within the suboxic (<0.3 mg/L dissolved oxygen, DO) contaminant plume at 8.7 and 9.0 m, respectively, below land surface, bls). The injection procedures involved collecting 100 L of groundwater from the aforementioned ports in a collapsible, N_2-sparged, gas-impermeable bladder, mixing with injectate constituents, and slowly adding the injectate back into the aquifer (1 L/min for 1.7 h) at the collection depths. Bromide (added as 1.9 mM NaBr) and chloride (added as 4.2 mM NaCl) were used as nonreactive tracers to establish the direction of groundwater flow and degrees of dispersion. After the first 1.7 h, natural-gradient conditions were allowed to prevail. Based upon conservative tracers, average groundwater flow at 8.7−9.0 m bls ranged from 0.4 to 0.7 m/d during the tests.[10] Samples were taken daily 1.0 m downgradient at the same depths as those used for the injections. For each sequential injection, relative breakthroughs (RB) of the fluorescently labeled bacteria and microspheres were calculated as[12]

$$\mathrm{RB} = \int_{t_0}^{t_f} \frac{C(t)}{C_0}\,\mathrm{dt} \Big/ \int_{t_0}^{t_f} \frac{[Tr]_t}{[Tr]_0}\mathrm{dt}$$

where C_0 and $[\mathrm{Tr}]_0$ are labeled-colloid (bacteria or microspheres) and tracer (Cl^- or Br^-) concentrations in the injectate, C(t) and $[\mathrm{Tr}]_t$, are concentrations at time t, and t_0 and t_f are elapsed times from injection to the beginning and end of breakthrough.

Detachment in the Presence of LAS. The first field study examined re-entrainment behavior of bacteria and microspheres in the presence of an anionic surfactant (LAS) and was performed concomitantly with a virus transport study.[11] *Pseudomonas stuzeri* ML2 (4.8×10^6 mL^{-1}), isolated from a fractured-rock aquifer[13] and labeled with DAPI (a fluorochrome that is highly specific for DNA and does not affect bacterial surface properties or attachment in granular media[14]), and 0.2-μm fluorescent microspheres (1.9×10^9 mL^{-1}) were allowed to attach to grain surfaces between the injection well M4-15 and the sampling well M4A-15 (Figure 1A). The initial injection was followed 14 days later by injections of bromide-amended (2.5 μM) groundwater to ascertain the effect of a hydrodynamic perturbation. The third injection occurred at day 23 and consisted of a 77 μM mixture of LAS isomers (C_{10} to C_{14}, Vista Chemical Co., Alston, TX) and NaCl (5.2 μM).

Detachment in the Presence of Tween 80. The second field study assessed re-entrainment of microspheres and attached native bacteria in response to sequential injections of deionized water and a nonionic surfactant (Tween80). M3-11 and M3A-11 served as the injection well and sampling wells, respectively (Figure 1B). The initial injection involved adding to the aquifer DAPI (29 μM) in order to fluorescently label the attached native bacteria; carboxylated microspheres (1.0 μm type YG, 2.3×10^6 mL^{-1}); and NaBr (1.9 μM) in filter-sterilized groundwater. At 12 days after the initial injection, a second injection consisting of deionized water was made to assess the effects of a reduction in I_c. This was followed at day 22 with an injection of Tween 80 (76 μM) and NaBr (1.9 μM).

Analysis of Field Study Constituents. Analyses were made in the nearby USGS field laboratory (Otis Air Base). DAPI-labeled *P. stuzeri* and native bacteria (340−380 nm excitation) and fluorescent microspheres (390−490 nm excitation) were enumerated on black Nuclepore filters (25-mm diameter, 0.2-μm pore size) at a precision of ±10% using a Nikon model Optiphot-2 epifluorescence microscope at 500−1250× magnification.[15] Filtered sample volumes varied from 5 to 200 mL. Lower limits of detection using 200-mL sample volumes were ∼10/mL for bacteria and ∼1/mL for microspheres. Specific conductance was determined using an electrical conductivity meter. Bromide and chloride concentrations were assessed at ≤5% using specific ion eletrodes (Orion models 9435 and 961713, respectively) LAS concentrations were determined at ±15% using methylene blue

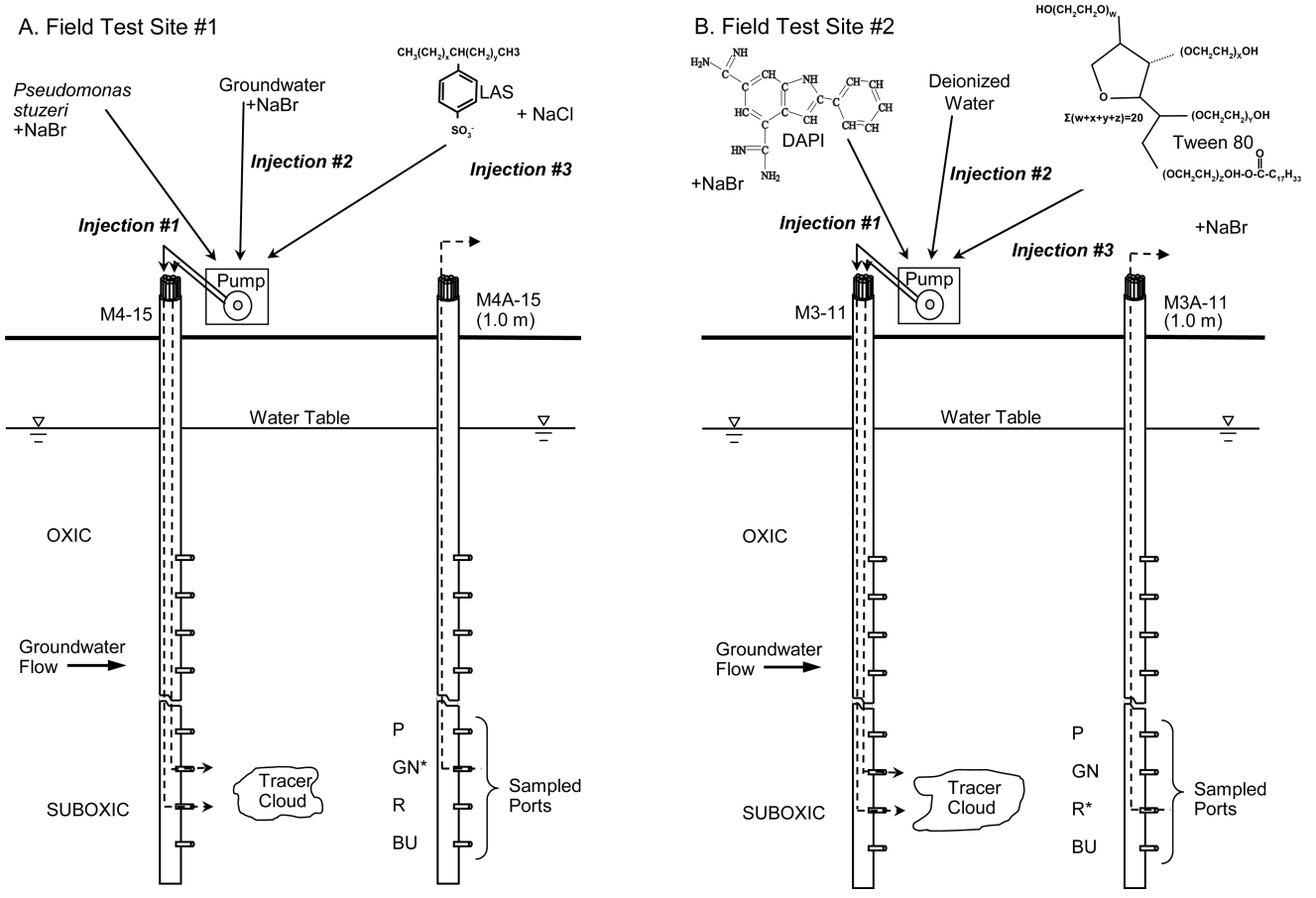

Figure 1. Design of injection-and-recovery studies (USGS Cape Cod, MA Groundwater Study Site) for assessing re-entrainment of attached bacteria and carboxylated microspheres. (A) Re-entrainment of fluorescently labeled *Pseudomonas stuzeri* and microspheres in response to a hydrodynamic perturbation and to injection of LAS (anionic surfactants). (B) Re-entrainment of microspheres and DAPI-labeled native bacteria in response to injections of deionized water and Tween80 (nonionic surfactant). "*" indicates the depths corresponding to the highest breakthroughs of the conservative tracers.

active substances (MBAS) kits (CHEMetrics, Inc.). DOC was measured by an OI carbon analyzer.[16]

Bacteria and Microsphere Surface Properties. To ascertain the effect of groundwater DOC upon the surface properties of native bacteria, groundwater was collected from three locations in July 2010. Pristine groundwater with low (0.6 mg/L) DOC was collected from F605-MO1-R. Two groundwater samples with slightly elevated DOC (0.9 and 1.3 mg/L) were collected from S469-51 and F347-46, respectively, that sample the former plume. Although cessation of the contaminant source occurred in 1995, levels of DOC are still elevated because of a 60-year buildup of particulate organic carbon.[17] Native bacteria were removed by filtration (Osmonics AcetatePlus filter, 0.45-μm pore size), stained with DAPI, resuspending in a filter-sterilized 1 mM NaCl solution, and stored at 4 °C. Bacterial abundances used for MATH and ζ assays were between 2 and 8 $\times$ 10^6 mL^{-1}.

Zeta Potential. Electrophoretic mobilities for bacteria collected from F605, S469, and F347 and of 1.0-μm carboxylated microspheres suspended in filter-sterilized groundwater from the same three wells were measured at 22 °C by laser Doppler micro electrophoresis (Zeta Pals-Zeta Potential Analyzer, Brookhaven Instruments) using 5 runs at 50 cycles each. Conversions were made to ζ using the Smoluchowski equation.[18]

Microbial Adhesion to Hydrocarbon (MATH) Test. Hydrophilicities of bacteria and microspheres were assessed at 22 °C using the microbial adhesion to hydrocarbon (MATH) assay.[19] Briefly, 500 μL of a suspension of bacteria in 5 mM NaHCO3 (pH 6.6−7.0) or microspheres (in filter-sterilized well water) were added to 1.5-mL-capacity microcentrifuge tubes along with 500 μL of hexadecane and mixed rapidly for 30 s. After allowing 5 min for phase separation, hexadecane and aqueous phases were gently and sequentially withdrawn by pipet. Bacterial or microsphere abundances in the aqueous phases were determined and compared to the initial loading to calculate fractional partitioning to the aqueous phase. Three to five replicates were made for each determination.

FTIR Spectroscopy Measurements. The surface functional group compositions for the bacteria and microspheres in aqueous solution were obtained using in situ attenuated total reflectance (ATR)-FTIR spectroscopy. All ATR-FTIR experiments were conducted using a Magna-IR 560 Nicolet spectrometer (Madison, WI) equipped CsI beam splitter and a deuterated triglycine sulfate (DTGS) detector. A 500-μL aliquot of the bacteria or microsphere suspension (1 to 4 $\times$ 10^7 mL^{-1}) in 10 mM NaCl background electrolyte was applied directly by pipet to the ZnSe internal reflection element (IRE) (56 mm $\times$10 mm $\times$ 3 mm) (Pike Technologies, Inc.). All spectra were acquired at 4.0 cm^{-1} resolution with 400 scans over the spectral range of 4000−800 cm^{-1} without ATR correction for wavenumber dependence. A final sample spectrum was obtained by subtracting the

dx.doi.org/10.1021/es102989x |*Environ. Sci. Technol.* XXXX, XXX, 000–000

Table 1. Physical and Chemical Parameters for DAPI-Labeled Bacteria and Microspheres Used in the Sequential Injection-and-Recovery Studies

parameter	*P. stuzeri* (field test 1)	native bacterial community (field test 2)	type YG carboxylated microspheres
length or diameter (μm)	0.92 ± 0.02	0.46 ± 0.02	0.2 (test 1), 1.0 (test 2)
aspect ratio	1.6	1.1	1.0
buoyant density (g cm^{-3})	1.05	1.02−1.08[a]	1.05
zeta potential at pH 7 (mv)	−19.5 to 30.3[b]	−38	−44 to −49
hydrophilicity index (%)	83.9[c]	17.4	99.9

[a] Measured by Harvey et al.[37] [b] Measured by Matsuda et al.[38] [c] Measured by Tattawasart et al.[39], average for six strains.

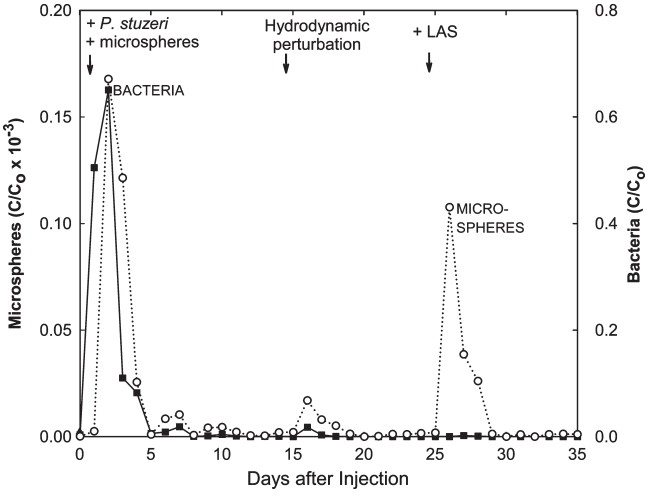

Figure 2. Recovery (level GN) of 0.2-μm carboxylated microspheres and DAPI-labeled *Pseudomonas stuzeri* 1.0 m downgradient from the injection well, M4-15, in field study 1. Arrows represent times of injections. Surfactant was C_{10}−C_{14} linear alkyl benzene sulfonates (LAS) at 77 μM. The secondary microsphere peaks at day 18 and day 27 represent re-entrainment in response to the groundwater and LAS injections, respectively. Note the absence of a bacterial peak at day 27.

appropriate background spectrum from the spectrum of the microsphere or bacteria suspension.

RESULTS

Comparison of Bacterial and Microsphere Characteristics. Salient characteristics of the two types of bacteria and microspheres employed in the field studies are listed in Table 1. Although the buoyant densities of the colloids were comparable, the cultured *P. stuzeri* had much larger aspect ratios (1.6) than either the uncultured native bacteria or the microspheres (1.1 and 1.0, respectively). Both cultured *P. stuzeri* and uncultured, indigenous populations had less negative ζ than the microspheres and exhibited more hydrophobicity.

Detachment in Response to Perturbation and Anionic Surfactant (Field Study 1). Dimensionless concentration histories of *P. stuzeri* and microspheres re-entrained in response to sequential injections of groundwater (hydrodynamic perturbation) and LAS are depicted in Figure 2 for M4A-15GN. During the initial loading phase, microsphere attachment was more extensive than that of *P. stuzeri*, judging from 2 to 3 log unit differences in fractional recoveries (Table 2). Relative breakthroughs (RB) of re-entrained *P. stuzeri* in response to groundwater injections were ∼2- and 10-fold greater (level GN and R, respectively) than those corresponding to the LAS injection. In contrast, RB of

Table 2. Relative Breakthrough (RB)[a] for DAPI-Stained *Pseudomonas stuzeri* and Microspheres for Field Sequential-Injection-and-Recovery Study 1 at USGS Wells Site M4A-15

sampled port (depth)	colloid	initial (loading) injection	groundwater injection	LAS injection
GN (8.7 m bls)	bacteria	∼1.0	0.0195	0.0018
	microspheres	0.0025	0.0003	0.0008
R (9.0 m bls)	bacteria	0.1617	0.0022	0.0013
	microspheres	0.0016	0.0004	0.0008

[a] A mathematical definition for RB is given in the Experimental section text.

re-entrained microspheres in response to the groundwater injections was, respectively, ∼2 and ∼3-fold lower than that corresponding to the LAS injections.

Detachment in Response to Ionic Strength Change and Nonionic Surfactant (Field Study 2). Concentration histories of previously attached native bacteria and microspheres re-entrained in response to sequential injections of deionized water and Tween 80 are depicted in Figure 3 for M3A-11R. Relative breakthroughs for the previously attached, indigenous bacteria could not be determined because the source term (C_0) consisted of an unknown number of bacteria that were labeled in situ. However, there were substantially (1.65 fold) more recovered bacteria that were re-entrained as a result of the Tween 80 injection relative to the deionized water injection. Conversely, there was 11.5 fold greater recovery of microspheres re-entrained as a result of the DI water injection as compared with the Tween 80 injection. The RB of microspheres at M3A-11GN and M3A-11R were 0.0003 and 0.0012, respectively, following the first (loading) injection and were 0.0002 and 0.0003, respectively, following the third (Tween80) injection. Relative breakthroughs for the second (deionized water) injection could not be determined because decreasing the ionic strength within the aquifer precluded use of conservative (halide) tracers.

Bacterial and Microsphere Surface Characteristics as a Function of DOC. The ζ and aqueous/hexadecane partitioning of bacteria harvested from the three wells are depicted in Figure 4. Partitioning to the aqueous phase (hydrophilicity) decreased and ζ increased with increasing groundwater DOC. The most hydrophilic and negatively charged bacteria were those obtained from the uncontaminated zone having the lowest DOC. The FTIR spectra for the three bacterial communities sorbed onto a flat ZnSe IRE surface (Figure 4, inset) show differences in surface functional group chemistries. The spectrum for bacteria from the low-DOC control well (F605) exhibits stretching bands at 1739 and 1243 cm^{-1} corresponding to the carboxylate and phosphate stretchings, respectively, that were absent in the spectra for the two contaminant plume communities.

dx.doi.org/10.1021/es102989x |*Environ. Sci. Technol.* XXXX, XXX, 000–000

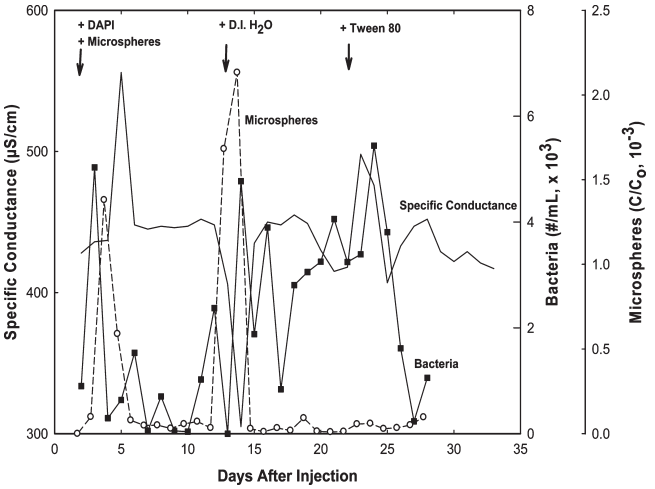

Figure 3. Recovery (level R) of 1.0-μm carboxylated microspheres and DAPI-labeled native bacteria at 1.0 m downgradient from the injection well, M3-11, in field study 2. Arrows represent times of injections. Initial injection of DAPI was to fluorescently label the attached, indigenous bacteria within the aquifer sediments. The secondary microsphere and bacteria peaks at day 13 represent re-entrainment in response to the deionized water. Secondary bacteria peaks at day 24 represent re-entrainment in response to the subsequent Tween 80 injection. Note the sparsity of microspheres detaching in response to the presence of Tween 80.

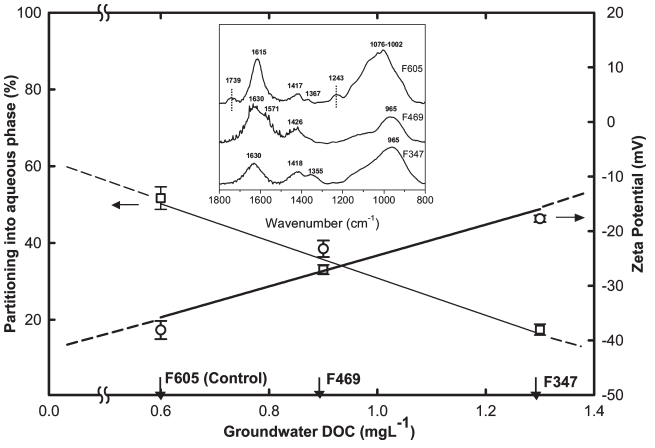

Figure 4. Surface properties (relative hydrophilicity, as determined by the MATH assay, and ζ) of the unattached bacterial communities from the control well F605 (pristine groundwater) and wells S469 and F347 (former plume) as a function of the groundwater DOC. Inset: FTIR spectra for bacteria harvested from F605, S469, and F347 that were dried onto ATR crystal (ZnSe). The spectra exhibit bands corresponding to carboxylate (stretching of C=O of ester or carboxylic acids at 1739 cm^{-1}; symmetric stretching of COO^- at 1426−1417 cm^{-1}), amide I (1630−1615 cm^{-1}), amide II (1571 cm^{-1}, phosphate (1243 cm^{-1}), and polysaccharides (1100−900 cm^{-1}) functional groups, respectively.

Figure 5 shows the effect of groundwater DOC upon the hydrophilicity and ζ of micropheres suspended in filter-sterilized groundwater collected from the aforementioned three wells. Unlike the bacteria, the highly hydrophilic microspheres all partitioned into the aqueous phase irrespective of DOC concentration. However, there was a slight decrease (from −49 to −44 mV) in ζ with increasing DOC. The FTIR spectra for microspheres in aqueous solution and dried on ZnSe IRE are

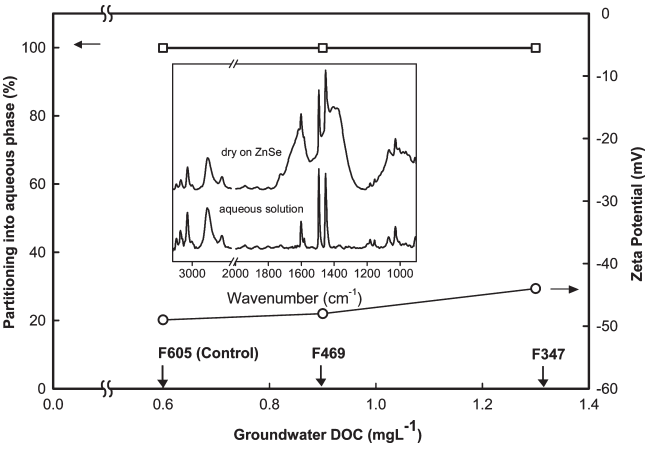

Figure 5. Surface properties (relative hydrophilicity, as determined by the MATH assay, and ζ) of microspheres from the control well F605 (pristine groundwater) and wells S469 and F347 (former plume) as a function of the groundwater DOC. Inset: ATR-FTIR spectra for microsphere suspensions in 10 mM NaCl solution and dried on ZnSe IRE surface.

shown in Figure 5 inset. The spectrum of microspheres in aqueous solution is almost identical to that of pure polystyrene [20] and surface carboxylate groups are not detectable. However, when the microspheres were dried on the ZnSe IRE, two broad bands emerge from the polystyrene spectrum at ~1613 and 1400 cm^{-1} corresponding to the asymmetric and symmetric stretching, respectively, of deprotonated carboxyl groups (COO^-).

DISCUSSION

Microspheres as Bacterial Surrogates in Organics-Contaminated Aquifers. Microspheres that are carboxylate-modified are most often employed in field tests, in part, because they are negatively charged over the pH range of most natural waters, as are most aquatic bacteria. Also, microspheres are available in diameters that bracket the sizes of bacteria of environmental and (or) clinical interest. Although in situ use of microspheres has been useful in helping to explain the abiotic (colloidal) aspects of subsurface bacterial transport behavior, dissimilarities in transport behaviors between microspheres and bacteria have been reported for both column [21] and field [12] scales. In an earlier transport study at the Cape Cod study site, breakthrough of 0.2−1.35-μm carboxylated microspheres was several orders of magnitude more attenuated than that of DAPI-labeled, uncultured groundwater bacteria, but characterized by more substantive and protracted tailing,[12] suggesting that microspheres may be subject to more frequent attachments to grain surfaces. However, the protracted tailing in breakthrough in the earlier study suggested that a fraction of the attachments are clearly characterized by weak interactions with the surface, such as would be the case for localization in the secondary minima associated with uncoated quartz-grain surfaces. In the present study, results from the two sequential injection-and-recovery studies suggest that the re-entrainment behaviors of microspheres and groundwater bacteria in response to physical and (or) chemical perturbations can also be quite different.

In addition to the disparities in surface properties between the very hydrophilic carboxylate-modified microspheres and the more hydrophobic groundwater bacteria (Table 1), the spherical morphology of the rigid microspheres would limit its contact

area on a grain surface. In contrast, the rod-shaped groundwater bacteria can have many points of surface-to-surface contact, which can result in repulsive steric interactions caused by surface macromolecules[22] that are not present on the microsphere. Li et al.[23] observed that deposition of carboxylated microspheres under unfavorable conditions (also the case for microspheres contacting quartz surfaces in the present study) results in increased vulnerability to hydrodynamic drag and increased re-entrainment with increased flow rate. Consequently, the low (0.0003−0.0004) RB of microspheres in response to a hydrodynamic perturbation that caused a much greater recovery (RB 0.0022−0.0195) of previously attached bacteria (Table 2) was somewhat unexpected, although a much more modest release in response to increased flow rate would be expected for the 0.2-μm microspheres (field study 1) relative to the larger 0.9-μm *P. stuzeri* or the 1.0-μm microspheres (field study 2) simply on the basis of size.

Given the much greater propensity of microspheres to attach during transport through Cape Cod aquifer, a correspondingly larger fraction of remobilized microspheres would be expected to reattach during transport to the sampling well. Therefore, it is necessary to examine the differences between bacteria and microspheres in terms of their respective patterns of RB for the different types of physicochemical-perturbation injections within the same field study. For example, in the first study (Figure 2), injection of LAS was up to 3 times more effective in remobilizing attached microspheres than was the preceding injection of groundwater (Table 2), whereas the LAS injection was up to 10-fold less effective in remobilizing attached bacteria than was the preceding groundwater injection. Although the lack of re-entrainment of *P. stuzeri* in response to the LAS injection in the current field study is unclear, it is possible that their cell envelopes may be less amenable to modification by LAS than those of the more surface-active and hydrophobic native bacteria.

In the second study, more of the microspheres were re-entrained and subsequently recovered in the response of the DI water injection than in response to the subsequent Tween 80 injection, whereas the opposite was observed for the hydrophobic, native bacteria. The microspheres, which are much less hydrophobic and more negatively charged at the pH of the Cape Cod aquifer than the indigenous bacteria (Table 1, Figures 4 and 5), may attach electrostatically to a greater degree. Consequently, they may be more vulnerable to re-entrainment in response to abrupt decreases in I_c and the accompanying increases in the thickness of the electrical double layer associated with the grain surfaces. Some studies suggest that large (orders of magnitude) changes in I_c are necessary to influence transport of bacteria-sized colloids in subsurface granular media.[24] In our study, the injected plume of deionized water only decreased the I_c of groundwater appearing at sampling ports 1 m downgradient by 2−5-fold, i.e., from 0.9[25] to 0.2−0.4 mM, because of mixing with ambient groundwater and transfer of cations and anions from the grain surfaces. Nevertheless, a large peak in re-entrained microspheres was observed coincident with the arrival of the dip in I_c. (Figure 3). Collectively, the different patterns of re-entrainment between groundwater bacteria and microspheres suggests different vulnerabilities to re-entrainment in the presence of chemical purturbations, such as changes in ionic strength and the presence of contaminant surfactants.

The higher efficacy of the Tween 80 injections to re-entrain bacteria versus microspheres (Figure 3) is consistent with its use to detach bacteria from surfaces.[26] Gross and Logan[27] observed that constituents such as Tween surfactants that made *Alcaligenes paradoxus* (bacterium) more hydrophobic, decreased its propensity for attachment to borosilicate beads. Tween 80 is quite hydrophobic and has been shown to substantively reduce the surface hydrophobicity of soil bacteria,[28] although its effect on bacterial attachment can also involve modifications of the solid surface in a manner that alters the surface free energy of bacterial adhesion.[29] Tween 80 has been used in high concentrations for in situ aquifer restoration.[30] In the present study, the concentration of Tween 80 needed to cause substantive re-entrainment of bacteria was only 76 μM at point of injection.

One additional consideration when using microspheres as bacterial surrogates in field-scale transport/re-entrainment studies is that, unlike microspheres, abundance of injected bacteria can be substantively affected by ecological and biological effects. For example, microspheres and bacteria have different susceptibilities to grazing by bacterivorous protozoa. In a transport study involving *Comamonas* DA001 labeled with a vital stain and injected into an oxic zone of a sandy aquifer (Oyster, VA), it was observed that nanoflagellate "blooms" appeared at downgradient sampling wells following the peaks in labeled bacteria.[31] This was not observed in our study, even though nanoflagellates are known to inhabit the aquifer.[32] However, the bacteria employed in field study 1 were labeled with DAPI, which is lethal to the nanoflagellates,[33] and the injections occurred in the suboxic zone of the aquifer. Bacteria can also grow in situ, even under the low DOC conditions present in the aquifer. We did not monitor growth in our field studies. However, DAPI severely hampers cell metabolism[33] and DAPI-stained groundwater bacteria incubated in groundwater collected from the plume showed no appreciable change in abundance during a month-long earlier test.[34]

Bacterial Surfaces Changes Resulting from Long-Term Exposure to Elevated DOC. Although the surfaces of both bacteria and microspheres may undergo modest chemical changes because of sorption of labile groundwater DOC, bacteria can also respond biologically. The link between bacterial hydrophobicity and nutritional status has long been recognized (e.g., ref 22). However, much of what is known about the dependence of bacterial hydrophocity on nutrient conditions derives from studies involving pure cultures grown with high levels of carbon substrate. It was reported that the cell surface hydrophobicity of stationary-phase *Zymomonas mobilis* 113S increased from 15.6 to 39.8% when the growth medium was switched from 0.16 M to 0.56 M glucose.[35] In our study, the almost 3-fold increase in the apparent hydrophicity (as estimated by the MATH assay) and concomitant 2-fold decrease in ζ in response to increases in groundwater DOC from 0.6 to 1.3 mg L^{-1} suggests that substantive changes in the surface properties of aquifer bacteria can result from modest changes in very low levels of DOC (Figure 4).

The predominance of surface carboxyl groups (Figure 5 insert) helps explain the high hydrophilicity of the microspheres relative to the more-hydrophobic groundwater bacteria. Polar and ionizable functional groups, such as carbonyl, hydroxyl, carboxyl, and phosphoryl are the major surface functional groups of microbial cells that control surface charge and hydrophilicity.[36] Thus, the increased presence of acidic functional groups in the spectrum for bacteria from well 605 (Figure 4 inset) is consistent with observation that these populations are the most hydrophilic and negatively charged among the three communities. That the hydrophilicity of the microspheres was unresponsive and the surface charge only slightly responsive to the same increase in DOC suggests that longer-term biological changes are likely to have contributed to the differences in surface properties for the

unattached bacteria from different locations within the aquifer. This underscores the need to consider the longer-term, biological effects of organic contaminants on bacterial transport, which is often overlooked.

AUTHOR INFORMATION

Corresponding Author

*Fax: 303-541-3084; Phone: 303-541-3034; E-mail: rwharvey@usgs.gov.

ACKNOWLEDGMENT

The advice and assistance of Denis LeBlanc of the USGS Massachusetts/Rhode Island District and Kathy Hess (USEPA), David Kinner (Western Carolina University), Annie Pieper, and Joseph Ryan (University of Colorado) for their help on the field studies are gratefully acknowledged. Funding for the field work was provided, in part, by the USGS Toxics Substances Hydrology Program. Use of brand names is for informational purposes only and does not constitute product endorsement by the authors, USGS, or the publisher.

REFERENCES

(1) Foppen, J. W.; Liem, Y.; Schijven, J. Effect of humic acid on the attachment of *Escherichia coli* in columns of goethite-coated sand. *Water Res.* **2008**, *42* (1−2), 211–219.

(2) Harvey, R. W.; Metge, D. W.; Barber, L. B.; Aiken, G. R. Effects of altered groundwater chemistry upon the pH-dependency and magnitude of bacterial attachment during transport within an organically contaminated sandy aquifer. *Water Res.* **2010**, *44* (4), 1062–1071.

(3) Johnson, W. P.; Martin, M. J.; Gross, M. J.; Logan, B. E. Facilitation of bacterial transport through porous media by changes in solution and surface properties. *Colloids Surf., A* **1996**, *107*, 263–271.

(4) Marshall, S. J.; House, W. A.; White, G. F. Role of natural organic matter in accelerating bacterial biodegradation of sodium dodecyl sulfate in rivers. *Environ. Sci. Technol.* **2000**, *34* (11), 2237–2242.

(5) Harvey, R. W.; Metge, D. W.; Shapiro, A. M.; Renken, R. A.; Osborn, C. L.; Ryan, J. N.; Cunningham, K. J.; Landkamer, L. Pathogen and chemical transport in the karst limestone of the Biscayne aquifer: 3. Use of microspheres to estimate the transport potential of *Cryptosporidium parvum* oocysts. *Water Resour. Res.* **2008**, *44* (8), 12.

(6) Behrens, H.; Beims, U.; Dieter, H.; Dietze, G.; Eikmann, T.; Grummt, T.; Hanisch, H.; Henseling, H.; Käss, W.; Kerndorff, H.; Leibundgut, C.; Müller-Wegener, U.; Rönnefahrt, I.; Scharenberg, B.; Schleyer, R.; Schloz, W.; Tilkes, F. Toxicological and ecotoxicological assessment of water tracers. *Hydrogeol. J.* **2001**, *9*, 321–325.

(7) Metge, D. W.; Harvey, R. W.; Aiken, G. R.; Anders, R.; Lincoln, G.; Jasperse, J. Influence of organic carbon loading, sediment associated metal oxide content and sediment grain size distributions upon *Cryptosporidium parvum* removal during riverbank filtration operations, Sonoma County, CA. *Water Res.* **2010**, *44* (4), 1126–1137.

(8) LeBlanc, D. R., Digital modeling of solute transport in a plume of sewage-contaminated ground water. In *Movement and Fate of Solutes in a Plume of Seqage-Contaminated Ground Water*; USGS Open File Report 84-475; LeBlanc, D. R., Ed.; U.S. Geological Survey: Reston, VA, 1984; pp 11−46.

(9) Coston, J. A.; Fuller, C. C.; Davis, J. A. Pb^{2+} and Zn^{2+} adsorption by a natural aluminum-bearing and iron-bearing surface coating on an aquifer sand. *Geochim. Cosmochim. Acta* **1995**, *59* (17), 3535–3547.

(10) Ryan, J. N.; Elimelech, M.; Ard, R. A.; Harvey, R. W.; Johnson, P. R. Bacteriophage PRD1 and silica colloid transport and recovery in an iron oxide-coated sand aquifer. *Environ. Sci. Technol.* **1999**, *33* (1), 63–73.

(11) Pieper, A. P.; Ryan, J. N.; Harvey, R. W.; Amy, G. L.; Illangasekare, T. H.; Metge, D. W. Transport and recovery of bacteriophage PRD1 in a sand and gravel aquifer: Effect of sewage-derived organic matter. *Environ. Sci. Technol.* **1997**, *31* (4), 1163–1170.

(12) Harvey, R. W.; George, L. H.; Smith, R. L.; Leblanc, D. R. Transport of microspheres and indigenous bacteria through a sandy aquifer - Results of natural-gradient and forced-gradient tracer experiments. *Environ. Sci. Technol.* **1989**, *23* (1), 51–56.

(13) Becker, M. W.; Metge, D. W.; Collins, S. A.; Shapiro, A. M.; Harvey, R. W. Bacterial transport experiments in fractured crystalline bedrock. *Ground Water* **2003**, *41* (5), 682–689.

(14) Chen, J.; Koopman, B. Effect of fluorochromes on bacterial surface properties and interaction with granular media. *Appl. Environ. Microbiol.* **1997**, *63* (10), 3941–3945.

(15) Harvey, R. W.; Kinner, N. E.; Macdonald, D.; Metge, D. W.; Bunn, A. Role of physical heterogeneity in the interpretation of small-scale laboratory and field observations of bacteria, microbial-sized microsphere, and bromide transport through aquifer sediments. *Water Resour. Res.* **1993**, *29* (8), 2713–2721.

(16) Aiken, G. R. Chloride interference in the analysis of dissolved organic carbon by the wet oxidation method. *Environ. Sci. Technol.* **1992**, *26*, 2435–2439.

(17) Repert, D. A.; Barber, L. B.; Hess, K. M.; Keefe, S. H.; Kent, D. B.; Leblanc, D. R.; Smith, R. L. Long-term natural attenuation of carbon and nitrogen within a groundwater plume after removal of the treated wastewater source. *Environ. Sci. Technol.* **2006**, *40* (4), 1154–1162.

(18) Masliyah, J. H.; Bhattacharjee, S. *Electrokinetic and Colloid Transport Phenomena*; Wiley-Interscience: New York, 2006; p 673.

(19) Vandermei, H. C.; Vandebeltgritter, B.; Busscher, H. J. Implications of microbial adhesion to hydrocarbons for evaluating cell-surface hydrophobicity 0.2. Adhesion mechanisms. *Colloids Surf., B* **1995**, *5* (3−4), 117–126.

(20) Jordan, R.; Ulman, A.; Kang, J. F.; Rafailovich, M. H.; Sokolov, J. Surface-initiated anionic polymerization of styrene by means of self-assembled monolayers. *J. Am. Chem. Soc.* **1999**, *121* (5), 1016–1022.

(21) Tong, M. P.; Li, X. Q.; Brow, C. N.; Johnson, W. P. Detachment-influenced transport of an adhesion-deficient bacterial strain within water-reactive porous media. *Environ. Sci. Technol.* **2005**, *39* (8), 2500–2508.

(22) Rijnaarts, H. H. M.; Norde, W.; Lyklema, J.; Zehnder, A. J. B. DLVO and steric contributions to bacterial deposition in media of different ionic strengths. *Colloids Surf., B* **1999**, *14* (1−4), 179–195.

(23) Li, X. Q.; Zhang, P. F.; Lin, C. L.; Johnson, W. P. Role of hydrodynamic drag on microsphere deposition and re-entrainment in porous media under unfavorable conditions. *Environ. Sci. Technol.* **2005**, *39* (11), 4012–4020.

(24) Jewett, D. G.; Hilbert, T. A.; Logan, B. E.; Arnold, R. G.; Bales, R. C. Bacterial transport in laboratory columns and filters - Influence of ionic strength and pH on collision efficiency. *Water Res.* **1995**, *29* (7), 1673–1680.

(25) Scholl, M. A.; Harvey, R. W. Laboratory investigations on the role of sediment surface and groundwater chemistry in transport of bacteria through a contaminated sandy aquifer. *Environ. Sci. Technol.* **1992**, *26* (7), 1410–1417.

(26) Jayasekara, N. Y.; Heard, G. M.; Cox, J. M.; Fleet, G. H. Association of micro-organisms with the inner surfaces of bottles of non-carbonated mineral waters. *Food Microbiol.* **1999**, *16* (2), 115–128.

(27) Gross, M. J.; Logan, B. E. Influence Of Different Chemical Treatments On Transport Of Alcaligenes Paradoxus In Porous-Media. *Appl. Environ. Microbiol.* **1995**, *61* (5), 1750–1756.

(28) Jana, T. K.; Srivastava, A. K.; Csery, K.; Arora, D. K. Influence of growth and environmental conditions on cell surface hydrophobicity of *Pseudomonas fluorescens* in non-specific adhesion. *Can. J. Microbiol.* **2000**, *46* (1), 28–37.

(29) McEldowney, S.; Fletcher, M. Variability of the influence of physicochemical factors affecting bacterial adhesion to polystyrene substrata. *Appl. Environ. Microbiol.* **1986**, *52* (3), 460–465.

(30) Pinto, L. J.; Moore, M. M. Release of polycyclic aromatic hydrocarbons from contaminated soils by surfactant and remediation of this effluent by Penicillium spp. *Environ. Toxicol. Chem.* **2000**, *19* (7), 1741–1748.

(31) Zhang, P. F.; Johnson, W. P.; Scheibe, T. D.; Choi, K. H.; Dobbs, F. C.; Mailloux, B. J. Extended tailing of bacteria following breakthrough at the Narrow Channel focus area, Oyster, Virginia. *Water Resour. Res.* **2001**, *37* (11), 2687–2698.

(32) Kinner, N. E.; Harvey, R. W.; Shay, D. M.; Metge, D. W.; Warren, A. Field evidence for a protistan role in an organically-contaminated aquifer. *Environ. Sci. Technol.* **2002**, *36* (20), 4312–4318.

(33) Harvey, R. W.; Mayberry, N.; Kinner, N. E.; Metge, D. W.; Novarino, F. Effect of growth conditions and staining procedure upon the subsurface transport and attachment behaviors of a groundwater protist. *Appl. Environ. Microbiol.* **2002**, *68* (4), 1872–1881.

(34) Harvey, R. W.; Garabedian, S. P. Use of colloid filtration theory in modeling movement of bacteria through a contaminated sandy aquifer. *Environ. Sci. Technol.* **1991**, *25* (1), 178–185.

(35) Zikmanis, P.; Shakirova, L.; Auzina, L.; Andersone, I. Hydrophobicity of bacteria *Zymomonas mobilis* under varied environmental conditions. *Process Biochem.* **2007**, *42* (4), 745–750.

(36) Karaman, M. E.; Pashley, R. M.; Bustamante, H.; Shanker, S. R. Microelectrophoresis of *Cryptosporidium parvum* oocysts in aqueous solutions of inorganic and surfactant cations. *Colloids Surf., A* **1999**, *146* (1−3), 217–225.

(37) Harvey, R. W.; Metge, D. W.; Kinner, N.; Mayberry, N. Physiological considerations in applying laboratory-determined buoyant densities to predictions of bacterial and protozoan transport in groundwater: Results of in-situ and laboratory tests. *Environ. Sci. Technol.* **1997**, *31* (1), 289–295.

(38) Matsuda, N.; Agui, W.; Ogino, K.; Kawashima, N.; Watanabe, T.; Sakai, H.; Abe, M. Disinfection of viable *Pseudomonas stutzeri* in ultrapure water with ion exchange resins. *Colloids Surf., B* **1996**, *7* (1−2), 91–100.

(39) Tattawasart, U.; Maillard, J. Y.; Furr, J. R.; Russell, A. D. Development of resistance to chlorhexidine diacetate and cetylpyridinium chloride in *Pseudomonas stutzeri* and changes in antibiotic susceptibility. *J. Hosp. Infect.* **1999**, *42* (3), 219–229.