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Microbial and Geochemical Investigations of Dissolved Organic Carbon and Microbial Ecology of Native Waters from the Biscayne and Upper Floridan Aquifers

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Contents

Figures

Tables

Conversion Factors

Multiply	By	To obtain
Length		
centimeter (cm)	0.3937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
meter (m)	1.094	yard (yd)
Volume		
liter (L)	0.2642	gallon (gal)
liter (L)	61.02	cubic inch (in^3)
Flow rate		
liters per day (L/d)	0.264	gallon per day (gal/d)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows: °F=(1.8×°C)+32

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows: °C=(°F-32)/1.8

Abbreviations and Symbols

ASR	aquifer storage and recovery
BA	Biscayne Aquifer
CFU	Colony-forming units
CO_2	carbon dioxide
DAPI	4',6-diamidino-2-phenylindole
DIC	dissolved inorganic carbon
DO	dissolved oxygen
DOC	dissolved organic carbon
DPD	deployment-protection device
DPM	disintegrations per minute
FDC	frequency of dividing cells
FI	fluorescent index
GPM	gallons per minute
HIBA	hydroxyisobutyric acid
HPOA	hydrophobic organic acid
L	liters
LPD	liters per day
LPM	liters per minute
mbs	meters below surface
MCL	maximum contaminant level
MDWSD	Miami-Dade Water and Sewer Department
min	minutes
NMR	nuclear magnetic resonance
ORP	oxidation-reduction potential
POC	particulate organic carbon
SUVA	specific UV absorbance
TOC	total organic carbon
TPIA	transphilic organic acid
TTHM	total trihalomethanes
TTHMFP	total trihalomethane-formation potential
UFA	Upper Floridan Aquifer
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
UV	ultra-violet
VFA	volatile fatty acids
VLP	virus-like particles
Ω	solubility ratio

Microbial and Geochemical Investigations of Dissolved Organic Carbon and Microbial Ecology of Native Waters from the Biscayne and Upper Floridan Aquifers

John T. Lisle, Ron W. Harvey, George R. Aiken, and David W. Metge

Abstract

Groundwater resources in the United States are under ever-increasing demands for potable, irrigation, and recreational uses. Additionally, aquifer systems are being used or targeted for use as storage areas for treated surface waters and (or) groundwaters via injection (for example, aquifer storage and recovery). To date, the influence that the nutrients, including carbon, in the injected water have on native microbial communities and the biogeochemistry in the subsurface zones used for storage of the injectate has not been determined. In this report, we describe a series of experiments that establishes a baseline dataset for the quantity and quality of organic and inorganic carbon and nutrients in the Biscayne Aquifer (BA) and Upper Floridan Aquifer (UFA) in south Florida.

The most significant differences between the BA (26 meters below surface) and UFA (366 meters below surface) are the average specific conductance (0.552 and 6.12 microsiemens per centimeter, respectively), dissolved oxygen (1.6 and 0 milligrams per liter, respectively), and oxidation-reduction potential (40.3 and -358 millivolts, respectively). The dissolved organic carbon from the BA is characterized by carbon originating from terrestrial sources and microbial activities, while the UFA has a distinctive microbial signature. Acetate and lactate are the dominant carbon constituents in both aquifers. Additionally, components of the dissolved organic carbon from the UFA have a total trihalomethane-formation potential that is approximately threefold greater than the maximum contaminat level of 80 µg per liter established by the U.S. Environmental Protection Agency.

The average native bacterial abundances in the aquifers are similar with 4.69×10^4 cells per milliliter in the BA and 1.33×10^4 cells per milliliter in the UFA. The average bacteriophage abundances are also similar with 1.15×10^5 virus-like particles in the BA and 1.92×10^5 virus-like particles in the UFA. Interestingly, ciliated protozoa are present in both aquifers. The average abundance of ciliates in the BA (2.97×10^3 ciliates per milliliter) is approximately twentyfold greater than abundances in the UFA (1.39×10^2 ciliates per milliliter). Collectively, these data indicate that microbial processes are the dominant contributor to the cycling of carbon and

inorganic carbon in the BA and may be the only carbon cycling process in the UFA, as this aquifer has not had a terrestrial influx of carbon for more than 15,000 years.

The rates of carbon, in the form of acetate, utilization by the native microbial communities are significantly different between the two aquifers. Based on data from ^{14}C-acetate-utilization experiments, the microbial communities in the BA turn over the native acetate in 2.5 years, whereas communities in the UFA turn over native acetate in 6.8 years. These data support the hypothesis derived from the microbial-abundance data, in that the carbon for bacterial maintainence and growth is recycled from bacterial biomass released during cell lysis, especially in the UFA.

An in situ diffusion chamber was designed to retain bacterial cells within the chamber while allowing native water constituents to move through the chamber. A series of 1-week deployments of chambers filled with fluorescent beads, inactivated native bacteria and laboratory grown and viable bacteria into the UFA, permitted by the State of Florida Environmental Protection Agency, was successfully completed. This was the first time this type of deployment into an aquifer system that is used for potable water supply has been permitted within the United States. This technology will allow, for the first time, in situ studies on the survival of microbial indicators of fecal pollution and true pathogens in groundwater systems.

Introduction

Groundwater resources are the major sources for potable and irrigation waters in Florida. As the population of Florida continues to increase, especially in the Miami-Dade County region, the demand for adequate and high-quality groundwater resources increases as well. These increases in demand have forced municipalities to extract groundwater from more than one aquifer.

Groundwater quality in the BA and UFA can be degraded by several processes that include, but are not limited to, non-point-source introduction of untreated surface waters, mechanical penetration of confining layers, or injection processes such as aquifer storage and recovery (ASR). Regardless of the mechanism through which non-native waters are introduced into the aquifers, or through which two previously isolated aquifers become mixed, the influence mixing has on the geochemical equilibria and microbial communities can be significant.

Changes in the microbial communities will influence alterations in the geochemical equilibria. The most significant nutrient component that microbial communities respond to is dissolved organic carbon (DOC). Interestingly, the current carbon-related criterion for injected waters in the State of Florida is only total organic carbon (TOC), which includes DOC. However, the majority of the carbon that makes up TOC is not immediately assimilable by bacteria (Tranvik, 1990; Aiken, Kaplan, and Weishaar, 2002). Therefore, this aspect of TOC makes it a relatively insensitive surrogate for determining the impact that introduced carbon will have on the native bacterial communities and, ultimately, on the quality of the recovered water. All TOC is not composed of the same carbon substrates, and the same is true for DOC. Studies on the composition of DOC have provided insight into differences the influence that water source has on the composition of DOC and how bacterial communities preferentially metabolize different classes of carbon compounds within the DOC (McKnight, Aiken, and Smith, 1991; Aiken, Kaplan, and Weishaar, 2002; Weishaar and others, 2003; Anesio and others, 2005). Because the oxidation of carbon is a significant part of bacterial metabolism, understanding which carbon substrates are available in injected, native, and blended groundwaters should assist in modeling

changes in geochemistry and water quality (for example, changes in oxidation/reduction potential, development of anoxic conditions, and changes in the rates of carbonate dissolution and precipitation).

With changes in the concentrations and availability of carbon, other organic and inorganic nutrients, and oxygen, following the mixing of water from the two aquifers or from injected waters, bacterial-biomass production and physiological capacities will also change. These changes will eventually promote changes in the structure of the bacterial communities. Responses of the bacterial communities to water constituents have also been shown to alter transport and survival in aquifer systems (Harvey and Harms, 2002a; Becker and others, 2004; Ford and Harvey, 2007). Field tracer and laboratory studies using fluorescent microspheres, bacteria, bacteriophage, and encysted protozoan parasites have shown the following to have significant influences on the fates and transport rates of microorganisms in aquifers: (1) nutrient concentration, (2) geochemical and physical characteristics of the groundwaters, (3) composition and physical structure of the aquifer matrix, and (4) physicochemical characteristics of the groundwaters (Harvey and George, 1989; Scholl and Harvey, 1992; Ryan and others, 2002; Harvey and Harms, 2002a; Harvey and Harms, 2002b; Becker and others, 2004; Harvey and Ryan, 2004; Renken and others, 2005; Abudalo and others, 2005; Ford and Harvey, 2007; Maxwell, Welty, and Harvey, 2007). A better understanding of native geochemical characteristics and microbial ecology within groundwater systems will provide a dataset on which treatment and regulatory decisions can be made. Examples include (1) specific public health issues such as bacterial indicator or pathogen survival and transport and (2) mobility of toxic elements (for example, arsenic) that have been shown to be enhanced by specific bacterial activities (Akai and others, 2004; Islam and others, 2004; Oremland and Stolz, 2005; Keimowitz and others, 2007).

Purpose

The Miami-Dade Water and Sewer Department (MDWSD) withdraws groundwater from the karstic BA and UFA for treatment for a potable product. One of their groundwater-extraction facilities, the Southwest Well Field, also has an ASR facility that has been shown to be vulnerable to contamination, based upon field hydraulic-transport studies (Renken and others, 2005). Current monitoring requirements do not provide pertinent data on the geochemical and nutrient variables that are known to significantly influence microbial fate and transport and carbon assimilation. Establishing a baseline dataset for geochemical, nutrient, and microbiological variables that can be used to model geochemical and microbiological processes is necessary, as the stress on and need for these resources increases.

Objectives

The objectives of this project are to characterize the carbon, nutrient, and geochemical compositions of the native groundwaters and establish baseline data on the respiration rates and fate and transport of native microbial populations in the sampled groundwater systems. In addition, a novel design of a diffusion chamber, which contains laboratory-grown bacteria, is deployed in the UFA for three 1-week periods. This proof-of-concept task, completed successfully, provides a technology for studies on bacterial survival under in situ conditions in groundwater systems. The following sections outline the methods used to achieve these objectives and explain the resulting data.

Methods

Site Description

Two wells in the Southwest Well Field of the MDWSD were selected for access to the BA and UFA (table 1). Experiments were conducted on site starting January 2008 through June 2009. Water was collected from the BA well through the pump housing located within a pump house. The pump ran continuously, so the well casing was completely flushed at all sample collection times. A stainless steel valve was installed in the pump housing, from which all samples were collected after purging the sample line for 15 minutes (min). The UFA well is artesian with a fully exposed well head. This well is also a monitoring well, which is permitted to flow continuously to the potable water-treatment facility as a drinking-water source. The collection-zone depth for this study was approximately 366 meters below surface (mbs). The flow rate from this well is approximately 151.4 liters per minute (LPM). The estimated flow or purge rate for this well is approximately 218,000 liters per day (LPD), which is equivalent to approximately 12 casing volumes per day.

Field Data Collection

During each site visit, data were collected for temperature, specific conductance, dissolved oxygen, pH, and oxidation/reduction potential (ORP) from each well using an YSI 556 MPS system attached to a flow cell. The multiprobe system was calibrated prior to

Table 1. Well locations and characteristics, Biscayne and Upper Floridan Aquifers. [mbs, meters below surface; cm, centimeters]

Aquifer	Well Identification	Latitude	Longitude	Bore Hole Depth (mbs)	Casing Type (mbs)	Casing Diameter (cm)	Collection Interval (mbs)
Biscayne	Well #25	25° 69' 01.374 N	80° 39' 29.193 W	26	Single zone	61	24-26
Upper Floridan	MW-1	25° 69' 01.374 N	80° 39' 29.193 W	366	Dual zone	30.5 15.3	259-274 338-366

attaching to the flow cell for each parameter as required by the manufacturer. The flow cell was attached to the respective sample valves and allowed to equilibrate to in situ conditions for at least 2 hours prior to initiating an automatic data-collection interval of 5 min for at least an hour.

Geochemical and Nutrient Data Collection

Samples were collected and preserved as required by the two contracted laboratories, Florida Spectrum Environmental Laboratory (Ft. Lauderdale, FL) or TestAmerica Laboratory (Tampa, FL). All samples were transferred to representatives of the respective laboratories within 4 hours of sample collection. The following analyses were conducted on each sample: chloride, nitrate, nitrite, sulfate, bicarbonate, ammonia, ammonium, Kjeldahl nitrogen, total phosphorus, sulfide, chemical oxygen demand, ferric iron, ferrous iron, total iron, silica, calcium, magnesium, manganese, potassium, and zinc. Separate water samples from each well were collected for determination of dissolved inorganic carbon (DIC) and total alkalinity (Yates and Halley, 2003) at the U.S. Geological Survey (USGS) laboratory in St. Petersburg, FL.

Calculation of the Calcite and Aragonite Saturation Index

The geologic matrix through which the BA and UFA flow is predominantly karstic (carbonate). Carbonate-based substrates are susceptible to dissolution due to reductions in pH or increases in carbon dioxide (CO_2). On the contrary, these systems may also precipitate carbonate if the geochemical balance favors the precipitation of calcite and aragonite from the water phase. Because changes in pH and CO_2 concentrations can be directly influenced by injected waters and (or) by bacterial respiration, an understanding of carbonate-saturation state in these native waters is of interest. Data that were collected on salinity, temperature, pressure, total phosphorus, total silicates, total alkalinity, DIC, and pH were entered into the software program CO2SYS, which calculates the saturation state of calcite and aragonite, the major forms of carbonate in karstic-aquifer matrices (Pierrot, Lewis, and Wallace, 2006). The general criterion for determining whether the carbonate chemistry promotes dissolution or precipitation is that if the solubility ratio (Ω) for either calcite or aragonite is < 1, the system trends toward undersaturation or

dissolution of the aquifer matrix. Values > 1 indicate the system favors oversaturation and precipitation of the respective carbonate species.

Quantification and Characterization of Dissolved Organic Carbon (DOC)

Routine monitoring of groundwater systems includes quantification of TOC. Though of general interest from an operational perspective, this parameter provides little insight into how carbon is utilized by the microbial populations in groundwater systems. The percentage of TOC that is assimilable for microbial populations is most often very small, making this parameter relatively insensitive when trying to assess microbial processes. The DOC component of TOC has been shown in all ecosystems studies to be the preferred source of carbon for microbial populations. However, not all DOC is available to microbes for assimilation, so an understanding of which constituents of the DOC are reactive is of interest.

Due to the relatively low concentrations of DOC in groundwaters, large volumes of native water must be collected to allow the concentration of the DOC to reach preparative quantities that can be easily analyzed. Accordingly, 200 liters (L) of native water were obtained from the BA and 500 L from the UFA. Samples were collected in sterile containers, packed in coolers, and shipped overnight to the USGS laboratory in Boulder, CO. The samples were processed upon receipt by passing the water through a series of XAD™ resin columns for the separate isolation and elution of hydrophobic organic acid (HPOA) and transphilic organic acid (TPIA) fractions of the total DOC (Aiken, 1992; Aiken and others, 1992). The different eluted samples were characterized by elemental, molecular weight, titration, and ^{13}C nuclear magnetic resonance (NMR) analyses.

The specific ultra-violet (UV) absorbance (SUVA) was also determined for each eluted fraction at a wavelength of 254 nanometers (nm) (Weishaar and others, 2003). This analysis was used to estimate the quantity of dissolved aromatic-carbon constituents in the water. Also, the fluorescence index (FI) was determined for the total and eluted fractions of the DOC (McKnight and others, 2001). The FI provides information as to the source (terrestrial versus microbial) of fluorescing organic matter in the sample. FI values normally are in the range of 1.0 to 2.0. FI

values in the range of 1.0 to 1.3 indicate DOC from a terrestrial source, while values in the range of 1.7 to 2.0 indicate a microbial source. FI values in the range of 1.4 to 1.6 can be considered an indicator of a mixture of DOC that originates from terrestrial and microbial sources.

Determination of the Total Trihalomethane (TTHM) Formation Potentials

Potential health effects from ingestion of total trihalomethanes (TTHMs) in water include liver, kidney, and central nervous system problems, as well as an increased risk of cancer. The maximum contaminant level (MCL) of 80.0 micrograms per liter (µg/L) was set based on the potential for an increased risk of these health effects. The U.S. Environmental Protection Agency (USEPA) has not conducted a cancer assessment for the TTHMs. However, the individual TTHM constituents have been evaluated. Qualitative descriptors of their carcinogenicity are as follows: (1) bromodichloromethane ($CHCl_2Br$) and bromoform ($CHBr_3$) are likely to be carcinogenic to humans by all routes of exposure; (2) there is suggestive, but insufficient, evidence to assess carcinogenicity of dibromochloromethane ($CHClBr_2$); and (3) chloroform ($CHCl_3$) is likely to be carcinogenic to humans by all routes of exposure under high-dose conditions, which lead to cytotoxicity and regenerative hyperplasia in susceptible tissues. Chloroform is not likely to be carcinogenic to humans by all routes of exposures at a dose level that does not cause cytotoxicity and cell regeneration.

For the TTHM formation potential (TTHMFP) experiments, DOC from the native water sample from each aquifer was used in one set of experiments, and the HPOA and TPIA fractions of the respective aquifer DOCs were also used in separate sets of experiments. A standard protocol for determining the TTHMFP was used where known concentrations of the different organic-carbon constituents were added to a set volume of reagent-grade water. To each of these samples, hypochlorus acid (chlorine) was added to a final concentration of 5.0 milligrams per liter (mg/L). All samples were incubated in the dark at room temperature. At the end of the incubation period, a subsample was taken to analyze for the residual-chlorine concentration. The chlorine in each sample was then inactivated by the addition of sodium thiosulfate. Each

sample was processed for analyses on a gas chromatograph that was set up and optimized for the detection and quantification of each of the four compounds that make up TTHMs.

Volatile Fatty-Acid (VFA) Data Collection

Volatile fatty acids (VFAs) are short carbon-chain compounds that are byproducts of and nutrient sources for bacterial communities in groundwater systems. Samples were collected (100 milliliters, mL) from each well, appropriately preserved, packaged, and shipped overnight to Microseeps, Inc. (Pittsburg, PA). This laboratory offers a specialized analytical technique that quantifies concentrations of VFAs significantly below the detection limits of other environmental analysis laboratories. This additional analysis was necessary because one of the VFAs, acetic acid or acetate, was used as the substrate to determine bacterial-community respiration rates. For the derived respiration-rate data to be meaningful, the concentration of acetate added to the assay must be significantly lower than the native concentration. Also, the presence and relative concentrations of VFAs provide insight into the microbial physiologies (for example, fermentation, acetogenesis, and sulfate reduction) that dominate in aquifer systems.

Bacterial and Bacteriophage Abundance Data Collection

A 50-mL sample was collected from each well and immediately frozen and stored, without a preservative, in liquid nitrogen. Upon return to the USGS laboratory in St. Petersburg, FL, samples were allowed to thaw at room temperature. Once completely thawed, each was filtered and stained using SYBR® Gold (Molecular Probes, Inc.) for the enumeration of bacteria and bacteriophage (Lisle and Priscu, 2004). All prepared slides were enumerated using an epifluorescent microscope equipped with a filter-cube set specifically designed to optimize the visualization of the SYBR® Gold stain.

Ciliated-Protozoa Data Collection

A 50-mL sample was collected from each well during two sampling periods and preserved with glutaraldehyde (final concentration: 1% volume/volume), stained with 4’,6-diamidino-2-

phenylindole (DAPI) (final concentration: 0.15 millimoles, mM), and transported to the USGS laboratory in St. Petersburg, FL. Each stained sample was gently filtered [< 13 millimeters (mm) Hg] through a 25-mm-diameter, 0.8-micrometer (μm)-pore-size filter. The ciliates on each filter were counted under an epifluorescent microscope equipped with a filter-cube set specifically designed to optimize the visualization of DAPI-stained organisms.

Diffusion-Chamber Deployment and Bacterial Survival in the Upper Floridan Aquifer

The survival and inactivation of microorganisms in groundwater systems under in situ conditions are currently some of the least studied aspects of ASR and deep well injection programs. Understanding if and how microorganisms of public health concern survive once introduced into groundwater systems has significant implications from a regulatory perspective. To date, survival and inactivation studies in groundwater systems in Florida and the United States have been limited to laboratory studies where the physical (that is, pressure) and geochemical (that is, anaerobic and highly reduced waters) aspects are most often compromised. The development of an in situ device that can withstand harsh aquifer conditions while retaining the microorganism of interest for later study is needed if data on microbial indicator and (or) pathogen survival and inactivation rates are to be reliable. The USGS diffusion-chamber system was designed to address these issues.

The USGS diffusion-chamber system was used in a three-phase study to demonstrate that the chamber design could tolerate the conditions in the UFA and retain bacteria within the chamber during extended in situ incubation periods. This approach was mandated by the State of Florida Department of Environmental Protection prior to its acceptance of this technology for routine use in aquifer systems within the State.

The chamber design used for this study had final constructed dimensions (width x length x depth) of 4.0 cm x 12.0 cm x 2.3 cm (fig. 1). The internal chamber that contains the sample (for example, fluorescent beads, inactivated bacteria, living bacteria, and such) measures 2.0 cm x 7.4 cm x 1.0 cm and has a maximum volume of approximately 15.0 mL. The central chamber and outer plates of the diffusion chamber are clear acrylic. The polycarbonate membranes

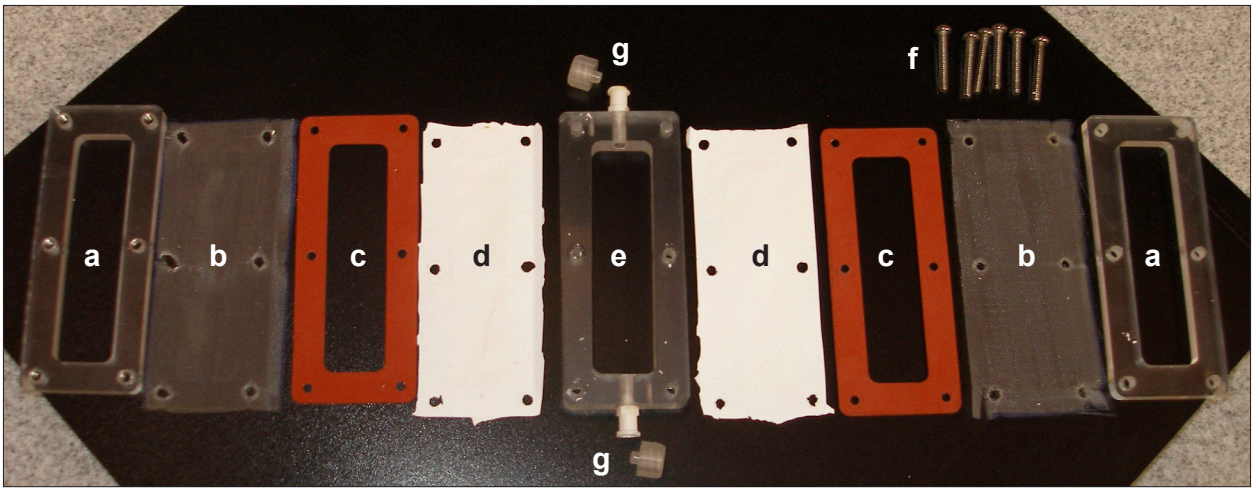

Figure 1. Diffusion-chamber construction. Each chamber was constructed by placing a membrane filter (d) on the central chamber (e), on which the silicon gasket (c) is placed. A polycarbonate screen (b) was placed on the gasket and the top chamber plate (a) placed on the screen. The same sequence of construction is repeated for the other side of the central chamber and the completed chamber fastened and sealed with six stainless steel bolts (f). The nylon syringe fittings on either end of the central chamber are sealed with polycarbonate caps (g).

used to retain the sample within the chamber while excluding the microorganisms outside the chamber allow the equilibration of the dissolved constituents (for example, nutrients, gases) within the chamber with those in the aquifer. The gaskets used to form a water-tight seal between the contents of the inner chamber and the native water are silicon. The screens used to provide physical protection for the membranes are polycarbonate (fig. 1). The USGS diffusion-chamber system is composed of a series of diffusion chambers, a deployment protection device (DPD) in which the chambers are secured, allowing them to be easily deployed to any depth while preventing direct contact of the chambers with the well casing, and a deployment/retrieval winch (fig. 2).

The first phase of this study used fluorescently labeled polycarbonate beads (0.5-µm diameter) to load into each of three diffusion chambers. A known concentration of beads (1×10^4 beads) was loaded into each chamber. All chambers were secured in the DPD and lowered into the UFA, at a rate of approximately 4.9 meters per second (m/sec), to a depth of approximately 366 mbs. The chambers were left in place for 7 days, after which they were retrieved at the same rate as they were deployed. Once back on the surface, each chamber was removed from the

DPD, placed in a 1-L bottle filled with source water, and returned to the USGS laboratory in St. Petersburg, FL. The content of each chamber was retrieved using a sterile syringe and transferred to a sterile 50-mL centrifuge tube.

Each chamber was then tested for membrane integrity by filling it with a 1.0% weight/volume (w/v) solution of methylene blue and immediately submerging the chamber in a

Figure 2. Diffusion-chamber deployment (clockwise from the upper left corner). The constructed and filled chambers were secured in the deployment protection device, connected to the deployment winch cable, inserted into the well head of the Upper Floridan Aquifer well, and deployed to approximately 366 meters below surface using the winch system.

1-L beaker filled with 800 mL of reagent-grade water. Samples (1.0 mL) were collected and transferred to cuvettes that were immediately analyzed for absorbance using a spectrophotometer set at a wavelength of 668 nm. The absorbance values were compared to a standard curve for diffusion rate that had been generated from absorbance data collected from diffusion chambers that were known to be intact and filled with the same methylene-blue solution. Following the integrity test, the solution within each chamber was removed with a syringe and the volume transferred to the respective 50-mL tubes. Each chamber was dismantled, and the two membranes were transferred to the 50-mL tube. Finally, the inner surface of the chamber was rinsed and gently brushed in 10-15 mL of sterile water that was subsequently transferred to the 50-mL tube. The tube was then vigorously mixed repeatedly to remove the beads from the membrane surfaces, and the membranes were removed. The total volume from each chamber was filtered through separate 25-mm-diameter, 0.2-µm pore-size filters, rinsed several times with reagent-grade water, and the beads counted under an epifluorescent microscope.

The second phase of the project followed the same procedures as described for the first phase, except the diffusion chambers were filled with inactivated native bacteria. A 1-L water sample from the UFA had been collected prior to this deployment event. This sample was immediately preserved with formalin (final concentration: 2% v/v), a 5-mL subsample was stained with DAPI, and the number of bacteria was counted. The preserved and stained sample was then used to fill each diffusion chamber, with an approximate bacterial abundance of 1×10^4 cells mL^{-1}, prior to deployment. The diffusion chambers were deployed to the same depth and retrieved at the same rate. After retrieval, each diffusion chamber was processed for recovery of the bacterial cells and testing of integrity.

The third and last phase of the project followed the same procedures as for the previous two phases, except the diffusion chambers were filled with a viable, laboratory-grown culture of *Pseudomonas putida* KT244. This strain was selected because it has been routinely used in the bioremediation of contaminated groundwaters and is a commonly isolated bacterium from groundwater systems. Just prior to filling each chamber with *P. putida* KT244, a series of

dilutions of this culture was plated onto R2A agar (Reasoner and Geldreich, 1985) to determine the number of culturable bacteria at the beginning of the experiment. A second subsample (2.0 mL) was collected and immediately preserved with formalin (final concentration 2.0% v/v) for determination of total cell counts following staining with DAPI. After the 1-week incubation period in the UFA, the chambers were retrieved. A sample from each chamber was removed and immediately plated onto R2A agar plates along with a second sample for determination of total cell counts. All R2A plates were incubated at room temperature in the dark and counted each day until colony development had ceased. Each chamber was processed for integrity testing.

Bacterial Respiration, Carbon Turnover, and Carbon-Dioxide Production Rates

Respiratory rates of the native bacterial populations were determined using radio-labeled substrates, appropriate for the geochemical conditions in both aquifers. ^{14}C-labeled acetate was the choice for these groundwater systems because the volatile fatty acid is naturally present and is assumed to be a carbon source that native bacterial populations could easily utilize. The samples were processed and analyzed (Wright and Hobbie, 1966; Hobbie, 1973; Wright, 1978), with the following modifications. Each sample was split. One split was filtered through a 1.0-μm pore-size syringe filter to remove the ciliates. This sample was a control for estimating the effects that grazing has on the bacterial populations, thereby reducing the bacterial abundances and possibly the resulting respiration rates. Both samples were processed to prepare a set of duplicate samples for seven time points, including a time-zero set of duplicates. A 70-mL sample for both types of samples from each aquifer was dosed with ^{14}C-acetate to a final concentration of 20 nM, gently mixed, and 5.0 mL transferred to each 25-mL serum bottle. Each bottle was immediately sealed with a butyl rubber stopper through which a plastic cup containing a piece of fluted filter paper had been placed. The stopper was then sealed with an aluminum cap that was crimped tight. The time-zero replicate set of bottles was immediately processed by first adding 200 μL of phenyleneamine, which absorbs CO_2, to the fluted filter paper using a syringe to pierce the rubber stopper. The reaction was then terminated by adding 500 μL of 2N H_2SO_4 to the water sample, which drives the CO_2 into the head space of the bottle. After adding the

acid, each bottle was gently shaken on a rotary shaker overnight. The following day, the filter paper was transferred to a scintillation vial and 10 mL of scintillation fluid added. The water, which contained the bacterial cells, was filtered through a 25-mm-diameter, 0.20-µm pore-size filter, rinsed three times with sterile water, briefly dried, then placed in a scintillation vial, and 10 mL of scintillation fluid were added. All scintillation vials were allowed to set overnight at room temperature to quench, then were read the next day in a scintillation counter to record disintegrations per minute (DPM).

Determination of the Frequency of Dividing Cells (FDC) in Native Groundwaters

Determining the frequency of dividing cells (FDC) is another approach for estimating growth rates in bacterial populations. A 1-L sample was collected from the UFA into sterile polypropylene bottles and immediately placed in a cooler with a non-ice coolant. A second 50-mL sample was also collected, preserved with formalin (final concentration 2.0% v/v), and placed in the cooler with the other samples. All samples were shipped by overnight carrier to the USGS laboratory in Boulder, CO. Upon receipt at the laboratory, the preserved sample was stained with DAPI and counted under an epifluorescent microscope. At set time points, subsamples were removed from the 1-L sample, preserved with formalin, stained with DAPI, and counted. The criterion for counting a bacterial cell as dividing was the occurrence of an obvious invagination within the cell but with no separation at the point of invagination.

Results and Discussion

Water Quality

There are distinct differences between the general water qualities of the aquifers (table 2). The most obvious are the dissolved oxygen (DO) and oxidation-reduction potential (ORP) data. The BA contains DO, whose concentration is variable. The variable concentration is due to the hydraulic connections of the aquifer to surface waters and rapid infiltration rates of surface waters into the aquifer. The ORP values for the Biscayne Aquifer support the presence of DO

at measurable concentrations because all samples collected indicate this aquifer is relatively oxidized. In contrast, the UFA is anaerobic because no DO was detected. The ORP values indicate the UFA, where sampled, is highly reduced by the respiratory activity of microbial populations within the aquifer.

The geochemical and nutrient data from the two aquifers are also significantly different (table 3). The most obvious differences are in the presence and concentrations of nutrients (nitrate, phosphorus) and trace minerals (for example, iron) required for microbial productivity. The BA contains the constituents, in the presence of dissolved oxygen, that support aerobic metabolism and biomass production. In contrast, the UFA is anaerobic, highly reduced and dominated by sulfate as an electron donor for microbial energy and biomass production and contains a relatively high concentration of sulfides. Collectively, these conditions indicate that sulfate reduction is the dominant biogeochemical process in this zone of the aquifer. Also, the absence of detectable phosphorus classifies the UFA as an oligotrophic ecosystem.

When considering that the general composition of the geologic matrix through which both aquifers flow is karstic, understanding the extent to which the groundwaters interact with solid surfaces along flow paths is of interest from not only a geochemical but also a process-control perspective (well-production efficiency). One interaction of concern is the potential for the native

Table 2. Water-quality data collected by the YSI 556 MPS system, Biscayne and Upper Floridan Aquifers. [°C, degrees Celcius; mS/cm, millisiemens per centimeter; mg/L, milligrams per liter; mV, millivolts]

Aquifer	Date	Temp (ºC)	Specific Conductance (mS/cm)	Dissolved Oxygen (mg/L)	pH	Oxidation Reduction Potential (mV)
Biscayne	3/28/08	25.2 (0.1)[1]	0.545 (<0.001)	1.16 (0.10)	7.19 (0.01)	+49 (10)
	5/21/08	25.5 (0.1)	0.555 (<0.001)	2.60 (0.10)	7.28 (0.01)	+38 (3)
	3/10/09	24.9 (0.1)	0.555 (<0.001)	1.14 (0.08)	7.02 (0.01)	+34 (4)
Upper Floridan	3/28/08	22.3 (0.3)	6.070 (<0.001)	0	7.58 (<0.01)	-313 (11)
	5/21/08	22.3 (0.1)	6.056 (<0.001)	0	7.66 (<0.01)	-369 (9)
	3/10/09	22.2 (0.1)	6.226 (<0.001)	0	7.49 (<0.01)	-391 (8)

[1] Data in parentheses are ± standard deviation values.

Table 3. Geochemical and nutrient data, Biscayne and Upper Floridan Aquifers.

Parameter [1]	Biscayne Aquifer	Upper Floridan Aquifer
Chloride	37	1500
Nitrate (as N)	0.28	< 0.10
Nitrite (as N)	< 0.006 [2]	< 0.10
Sulfate	15	528
Bicarbonate	184	154
Total Phosphorus	0.04	< 0.004
Sulfide	< 0.005	3.2
Silica	4.7	12.5
Calcium	85	120
Total Iron	0.04	0.35
Magnesium	3.7	130
Manganese	< 0.00006	0.004
Potassium	4.4	54.5
Zinc	< 0.00099	< 0.00099

[1] All parameters are expressed as milligrams per liter.

[2] < = less-than values represent the detection limits for this assay.

waters that are in contact with karstic matrices either to dissolve or precipitate carbonates. We used a software program, CO2SYS, to calculate the saturation ratios (Ω) for the two constituents of the bicarbonate/carbonate system (calcite and aragonite) from input of data collected in the field (table 4). This approach is routinely used by those interested in the carbonate chemistry in pelagic and coastal-marine systems because these interactions are major components of carbon-cycling and ocean-acidification processes in these ecosystems. In general, when the Ω values are < 1.0, the respective constituents are undersaturated in the water phase and dissolution of the matrix is favored. Values > 1.0 are oversaturated and precipitation is favored. Dissolution of carbonates is preferred in the BA, with aragonite being the more soluble of the two components. In the UFA, both saturation states are significantly greater than those in the BA, with precipitation of calcite being favored in the UFA.

Dissolved Organic-Carbon (DOC) Quantification and Characterization

DOC is a component of the total organic carbon (TOC), which is of regulatory significance and is a required variable to monitor in potable and source waters. However, not all TOC or DOC is equal. The methods currently required for analyzing for these organic-carbon constituents do not characterize the carbon compounds that make up TOC and DOC. One approach to assessing the composition of DOC is specific UV absorbance (SUVA) at a wavelength of 254 nm (Weishaar and others, 2003). The SUVA analysis data provide insight into the aromatic structure of the organic matter that makes up the DOC but do not correlate with reactivity with oxidants, like chlorine, and the formation of disinfection byproducts.

The dissolved organic-carbon (DOC) analyses show that both aquifers contain DOC at concentrations that can support microbial communities, even in the oligotrophic UFA (table 5). The BA has approximately threefold more DOC than the UFA, which may be due to the BA having more direct and indirect hydraulic connections with surface waters that contain carbon from plant biomass. These carbon concentrations are sufficient to support the aerobic and anaerobic microbial communities, though at relatively reduced rates. The UV spectrometric analyses of the DOC in both aquifers indicate there is a difference in aromatic constituents, as

Table 4. Carbonate-chemistry data, Biscayne and Upper Floridan Aquifers.
[‰, parts per thousand; dbars, decibars; µmol/kgSW, micromoles per kilogram of seawater]

Parameter	Units	Biscayne Aquifer	Upper Floridan Aquifer
Salinity [1]	‰	0.27	3.38
Temperature [1]	ºC	25.2	22.4
Pressure[1]	dbars	10.1	10.1
Total Alkalinity [1]	µmol/kgSW	4059.9	3342.6
Total CO_2 [1]	µmol/kgSW	4400.9	3414.2
pH [1]		7.16	7.58
Ω Calcite [2]		0.23	1.22
Ω Aragonite [2]		0.14	0.69

[1] Data taken from field measurements.

[2] Saturation solubility (Ω) values were calculated by the CO2SYS program.

Table 5. Dissolved organic-carbon (DOC) data, Biscayne and Upper Floridan Aquifers. [nm, nanometer; ppm, parts per million; %, percent]

Aquifer	Ultra-violet Absorbance (at 254 nm)	Dissolved Organic Carbon (ppm)	Whole Water Specific Ultra-violet Absorbance (at 254 nm)	Hydrophobic Organic Acid Specific Ultra-violet Absorbance (at 254 nm)	Hydrophobic Organic Acid (%)	Transphilic Organic Acid (%)	Fluorescent Index
Biscayne	0.053	3.1	1.7	2.6	36	1.8	1.5
Upper Floridan	0.024	1.1	2.2	2.2	59	1	1.8

observed in the data for the whole-water and the HPOA and TPIA fractions. The fluorescent index (FI) value may provide the most insight into the characteristics of the DOC in the respective aquifers. The UFA FI of 1.8 indicates the DOC in this aquifer is of microbial origin. These data support the contention that the UFA is hydraulically isolated from surface-water intrusion. The isotopically determined age of the water in the UFA is ~15,000-20,000 years. The FI value for the BA is in the transition zone for DOC-source determination and indicates a mixture of DOC from terrestrial and microbial sources.

Total Trihalomethane-Formation Potential (TTHMFP)

A volume of the respective native waters and a fraction of each DOC component that was extracted during the DOC-characterization process were used to determine which component of the DOC had the greatest potential to form the disinfection byproducts, total trihalomethanes (TTHM), during exposure to hypochlorus acid (chlorine) (table 6). The DOC components of the native whole-water fraction and the HPOA and TPIA fractions of the BA sample produced limited concentrations of TTHM. All samples produced similar concentrations of TTHM, and no value exceeded the maximum contaminate level (MCL) for TTHM (80.0 µg/L). These data indicate that, though there is a detectable concentration of DOC in the BA, the constituents of the DOC are not readily reactive with oxidants commonly used in drinking-water disinfection

Table 6. Total trihalomethane-formation-potential (TTHMFP) data, Biscayne and Upper Floridan Aquifers. [mg/L, milligrams per liter; µg/L, micrograms per liter; HPOA, hydrophobic organic acid; TPIA, transphilic organic carbon]

Aquifer	Sample Date	Fraction	Dissolved Organic Carbon (mg/L)	Residual Chlorine (mg/L)	$CHCl_3$ (µg/L)	$CHCl_2Br$ (µg/L)	$CHBr_2Cl$ (µg/L)	$CHBr_3$ (µg/L)	TTHMFP (µg/L)
Biscayne	5/29/08	Whole water	3.1	2.8	18.3	17.5	12.8	2.6	51.2
		HPOA	11.2	3.5	54.8	3.9	< 0.3	0.3	59.2
		TPIA	6.1	3.1	58.9	5.5	0.3	0.3	64.9
Upper Floridan	7/30/08	Whole water	1.1	1.9	0.3	0.8	4.1	57.1	62.3
		HPOA	7.2	2.3	50	75.8	90.9	49	265.6
		TPIA	2.3	1	4.7	20.7	68.3	116	209.6

processes. On the contrary, though the native whole waters from the UFA did not produce TTHM at concentrations greater than those of the BA, the HPOA and TPIA fractions of the UFA DOC did produce these regulated disinfection byproducts at concentrations that were 2.6- to 3.3-fold greater than the regulatory MCL. These constituents, most likely associated with, or produced by, microbial processes, are highly reactive with chlorine and would need to be accounted for and removed by the treatment processes used to produce potable water if the water from this zone of the UFA was used as a drinking-water source.

Volatile Fatty Acids (VFA)

The DOC and SUVA analyses quantify and, to a limited extent, characterize the DOC constituents. We also employed a direct analysis of both native waters for the presence and concentration of a range of volatile fatty acids (VFA) that have been shown to be associated with the microbial processing of carbon substrates through oxidative, fermentative, and anaerobic metabolic pathways. Table 7 lists the suite of VFAs detected and quantified. In both aquifers, acetic acid (acetate), hexanoic acid, and lactic acid + hydroxyisobutyric acid (HIBA) were detectable.

Table 7. Volatile fatty-acid (VFA) data, Biscayne and Upper Floridan Aquifers.

Parameter [1]	Biscayne Aquifer	Upper Floridan Aquifer
Acetic acid	0.12	0.38
Butryic acid	< 0.07 [2]	< 0.07
Hexanoic acid	0.13	0.16
i-Hexanoic acid	< 0.10	< 0.10
i-Pentanoic acid	< 0.07	< 0.07
Lactic acid + HIBA	0.44	0.24
Pentanoic acid	< 0.07	< 0.07
Proprionic acid	< 0.07	< 0.07
Pyruvic acid	< 0.07	< 0.07

[1] All parameters are expressed as milligrams per liter.

[2] Less-than value is the detection limit for the assay.

The total VFA concentration in each aquifer represents approximately 22.3 percent of the DOC in the BA and 70.9 percent of the DOC in the UFA. Considering the ability of bacteria to directly utilize VFA, the percentage of the DOC theoretically assimilable in the UFA is relatively high. The geochemical, nutrient, and DOC conditions described for both aquifers would permit fermentative and anaerobic processes to proceed by specific bacterial genera (for example, *Clostridium* sp.) and specialized metabolic capabilities (for example, acetogens).

Bacterial, Bacteriophage, and Ciliate Abundances

Samples analyzed from both aquifers contained bacteria, viruses that infect only bacteria (i.e., bacteriophage or phage), and ciliated zooplankton (table 8). The number of bacteria living in both aquifers is similar, with an average abundance of 4.69×10^4 cells mL^{-1} in the BA and 1.33×10^4 cells mL^{-1} in the UFA. These bacterial abundances are also similar to those recovered from a series of aquifer samples taken from ASR wells in central and south Florida (Lisle, 2005). The proportion of the bacterial population in the respective aquifers that was culturable on R2A agar was significantly different with 4.7 percent of the population being culturable in the BA, while only 0.2 percent were capable of growth on this medium in the UFA. This difference

Table 8. Microbiological data, Biscayne and Upper Floridan Aquifers. [ND, no data; CFU/mL, colony-forming units per milliliter; VLP/mL, virus-like particles per milliliter; mL^{-1}, per milliliter]

Aquifer	Date	Bacteria (CFU/mL)	Bacteria (cells/mL)	Bacteriophage (VLP/mL)	Virus to Bacteria Ratio	Ciliates (mL^{-1})	Bacteria to Ciliates Ratio
Biscayne	3/28/08	ND	1.31×10^{4}	ND		ND	
	6/30/08	3.80×10^{3}	8.07×10^{4}	1.15×10^{5}	1.4	2.97×10^{3}	27.7
Upper Floridan	2/18/08	ND	1.55×10^{4}	1.57×10^{5}	10.1	ND	
	3/28/08	ND	5.78×10^{3}	ND		1.03×10^{2}	
	6/30/08	40	1.86×10^{4}	2.27×10^{5}	12.2	1.74×10^{2}	106.9

may be attributable to the differences between the carbon and nutrient constituents and the geochemical conditions of the two aquifers. The physiologically associated stresses of survival in the UFA, relative to those in the BA, would require a starvation phenotype that most often results in low culturability rates, reduced cell volumes, and significantly reduced rates of metabolism (Nystrom, 2004; Finkel, 2006).

The phage abundances in the BA, based upon the enumeration of virus-like particles (VLP) under epifluorescent microscopy, were similar to those found in lake-water systems (Mathias, Kirschner, and Velimirov, 1995; Tapper and Hicks, 1998) (table 8). The VLP data for the UFA were not significantly different from those in the BA and were similar to VLP abundances recovered from deep granitic groundwater (450 mbs) (Kyle and others, 2008). The virus-to-bacteria (V/B) ratios, however, were significantly different. The low V/B ratio in the BA is indicative of a host bacterial population that may have a higher specific growth rate (therefore greater abundance) than the population in the oligotrophic and relatively harsh conditions in the UFA. Interestingly, the V/B ratios in the UFA are very similar to those calculated for deep granitic groundwaters (Kyle and others, 2008). V/B ratios this high have been shown to be indicative of an ecosystem within which the phage are actively infecting host bacteria that are metabolically active, though their surrounding conditions are relatively harsh.

Ciliated zooplankton, or ciliates, were also recovered from both aquifers. Though microorganisms in this trophic level do not have a significant impact on the consumption of DOC, they do represent a significant source for recycling particulate organic carbon (POC) into their biomass while releasing DOC as a byproduct of their feeding style. Bacteria and phage can be associated with the POC fraction of any aquatic system. Regarding the dominant biological components of the trophic level in these aquifer systems (i.e., bacteria and phage), ciliates exert a significant influence on the cycling of carbon because they feed upon bacteria and phage through relatively nondiscriminant grazing. Based upon previously determined grazing rates for ciliates in a more shallow groundwater system, approximately 0.8 bacteria/ciliate per hour were removed from the system (Kinner and others, 1998). Judging from that grazing rate, approximately 2.38×10^3 and 111 bacteria would be removed from the BA and UFA, respectively, each hour. The bacterial-removal rate may be possible within the BA due to the hydraulic connections to surface waters that bring not only pulses of carbon and nutrients into the aquifer but also bacteria, phage, and ciliates. However, when considering the age of the water in the collection zone of the UFA in which this study was conducted, this grazing rate cannot be possible without an actively growing bacterial population because bacterial abundance in the bulk phase of this aquifer would be removed in less than a year. Another explanation could be that the grazing rate in the UFA is significantly less than that found in the shallower aquifer and, in fact, grazing in these ciliates may be nondetectable, as has been shown in other oligotrophic and extreme aquatic environments (Laybourn-Parry and others, 1997). This study did not quantify the grazing rates of the ciliates, though their presence in experiments that would be influenced by this activity was accounted for by pre-filtering the water prior to starting the respective experiments.

Carbon-Utilization Rates of Native Aquifer Bacterial Communities

The rates of carbon mineralization (production of CO_2) and assimilation (incorporation into biomass) were quantified in both aquifers using radio-labeled acetate. Acetate was the choice of carbon substrate since it is readily utilized by all bacterial species and is naturally present in both aquifers. Based upon the assimilation rates, the concentrations of acetate in the BA and

UFA would turn over in 2.5 and 6.8 years, respectively (table 9). These rates may seem to be slow, relative to the age of the aquifer waters and the fact that the UFA has been isolated from surface-water influx over geologic time scales, but the rates are relatively rapid. This apparent contradiction is indicative of microbially dominated ecosystems, where microorganisms are rapidly recycling native carbon and terrestrial carbon pulses in the BA and strictly recycling native carbon in the UFA. The FI for the DOC from the respective aquifers supports this hypothesis: the FI value for the UFA DOC indicated a microbial source, while the BA FI value indicated a more terrestrial source for the DOC.

These turnover rates are also indicative of metabolically active bacterial populations in both aquifers, contradicting a common misconception in groundwater systems like the BA and UFA. The mineralization rates in the BA indicate this bacterial population is increasing in biomass (increasing in abundance). Only 15.1 percent of the acetate is converted to CO_2, while the majority of the remaining acetate is used to increase biomass by increase in cell size and (or) cell division. The growth-rate estimate for the BA also supports this hypothesis. The bacterial growth yield is 2.5-fold greater than that in the UFA. The mineralization and growth-yield values for the UFA samples indicate these native bacterial populations utilize a larger percentage of the available carbon source for maintaining energy for cellular processes versus diverting the carbon into biomass. This results in a relatively lower growth yield in the UFA. This physiological

Table 9. Bacterial carbon utilization-rate data, Biscayne and Upper Floridan Aquifers. [yr, year; µM/yr, micromoles per year; % µg C/L; percent micrograms of carbon per liter]

Carbon Utilization Parameter	Units	Biscayne Aquifer	Upper Floridan Aquifer
Acetate turnover rate	yr	2.5	6.8
CO_2 production rate	µM/yr	0.45	0.72
Mineralization to CO_2	% µg C/L	15.1	38.6
Growth yield		6.6	2.6

response is common in bacterial populations that reside in oligotrophic ecosystems like this aquifer.

Frequency of Dividing Cells (FDC)

The frequency of dividing cells (FDC) is an indirect measure of the mean growth rate of bacterial communities in water samples. This technique detects bacterial cells capable of replication without the requirement of their being culturable. The FDC data in table 10 indicate that the percentage of dividing cells is very similar in both aquifers. The FDC percentages are similar to those found in other groundwater systems and support the conclusions derived from the data in table 8 that the bacterial populations in both aquifers are metabolically active and capable of replication under native conditions.

When compared to the culturability data (table 8), the FDC data for the BA are similar, indicating there is a high percentage of the dividing cells in this aquifer that are also culturable. However, this same relation does not hold for the UFA because only 0.2 percent of the cells were culturable. This non-culturability phenomenon is common in bacterial populations that inhabit stressful environments such as that of the UFA.

Deployment of Diffusion Chambers into the Upper Floridan Aquifer

The most novel task of the project was the proof-of-concept demonstration of the structural integrity of a diffusion-chamber design that had been deployed into the UFA for 1 week. The

Table 10. Frequency of dividing-cell (FDC) data, Biscayne and Upper Floridan Aquifers. [cells/mL, cells per milliliter; %, percent]

Aquifer	Total Bacteria (cells/mL)	Dividing Bacteria (cells/mL)	FDC (%)
Biscayne	1.86×10^{5}	4.27×10^{3}	2.3
Upper Floridan	1.88×10^{4}	571	3.04

objectives were to systematically, via three separate deployments, expose a set of diffusion chambers into the UFA containing inert beads, followed by inactivated native bacteria, and finally, a laboratory-grown strain of bacteria known to inhabit groundwater systems. The metrics for a successful deployment were to demonstrate that the diffusion chambers retained their structural integrity (did not release their contents into the UFA) and retained a high recovery rate for the contents that were loaded into the diffusion chambers for the respective deployments.

There were no detectable failures in the structural components or membrane filters of any diffusion chamber tested. All of the methylene-blue integrity test-sample values were close to the standard curve values for the respective sample-time intervals. In fact, the diffusion rates were lower than those predicted by the standard curve for all diffusion chambers. This may be attributable to the growth of biofilms on the surfaces of the membrane filters used in the diffusion chambers, thereby obstructing flow through pores of the membrane.

The recovery efficiencies for the fluorescent-bead and inactivated native-bacteria deployments were between 80 and 97 percent of the abundances loaded into the diffusion chambers (table 11). Admittedly, while recoveries were not 100 percent, the development of an optimized recovery method was not part of this study. The objectives were to use the recovery efficiencies to complement the integrity tests following each deployment. The final deployment provided data on two aspects of investigations of microbial processes using an in situ device like the diffusion chambers. The first was to confirm that living bacteria could be deployed in the UFA and successfully retained within the diffusion chamber for extended periods of time. The

Table 11. Diffusion-chamber data, Upper Floridan Aquifer. [mL^{-1}, per milliliter; %, percent]

Chamber Content	Abundance Loaded (mL^{-1})	Abundance Recovered (mL^{-1})	Recovery Rate (%)
Fluorescent beads	1.0×10^{4}	9.1×10^{3} (± 551)	85.5-96.5
Inactivated native bacteria	1.0×10^{4}	8.5×10^{3} (± 423)	80.8-89.2
Viable bacteria (cells)	9.0×10^{9}	8.7×10^{8} (± 321)	~ 90.0
Culturable bacteria	8.4×10^{9}	3.1×10^{6} (± 280)	0.03-0.04

second aspect was to investigate the applicability of the diffusion-chamber design to study the decay or die-off kinetics of native or introduced microoganisms (for example, total coliforms, fecal coliforms, *Cryptosporidium* sp., *Giardia* sp.). The *Pseudomonas putida* KT244 strain not only was successfully retained within the diffusion chamber but also retained some degree of culturability after a week under the conditions of the UFA. This culturability rate is similar to that of the native bacteria in the UFA (table 8).

The application of this type of diffusion chamber has significantly advanced the study of microbial ecology in deep groundwater systems. To date, all studies on the microbial interactions in groundwater systems like the BA and UFA have been conducted on the bench top, where native conditions cannot be replicated, much less maintained. The diffusion-chamber deployments during this study have proven this technology is a reliable method for conducting studies in the subsurface that have until now been impractical, if not impossible.

Future Research Directions

1. Understanding the processes that drive the carbon cycle in the aquifer systems underlying Florida and especially in the UFA is very important. This study provides the baseline data on which to build a more comprehensive model for how native and introduced (for example, ASR) microbial populations may alter the geochemistry and, thereby, water quality in these aquifers. More detailed studies to determine the dominant physiologies that drive these ecosystems (for example, sulfate reduction, methanogenesis, syntrophic relationships) and bacterial speciation (i.e., nucleic-acid sequencing) will help determine which species are driving these processes.

2. Further and more detailed characterization of the DOC may lead to the design and (or) optimization of treatment processes that can remove the specific components of the DOC that can significantly influence the production of TTHMs, permitting the production of a safer potable product.

3. The interactions between the native and introduced microbial populations and the geology of the aquifer systems have a significant influence on the water quality of these groundwater systems. Though most often the perception of the association of microbes with water quality is negative, the ability of native microbes to improve the quality of native or injected waters while in the subsurface could benefit from continued studies.

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