

## Microbiological Response to Well Pumping

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### Abstract

To better constrain sampling strategies for observing biologically sensitive parameters in ground water, we vigorously pumped for 120 h a lightly pumped well completed in a confined glacial aquifer while observing how various physical and chemical parameters evolve in the water produced. The parameters commonly monitored when sampling a well stabilized within about an hour, after 5 wellbore volumes were produced; these parameters include temperature, pH, dissolved oxygen, oxidation-reduction potential (Eh), and electrical conductivity. The concentrations of ferrous iron, sulfide, and sulfate and various biological or biologically sensitive parameters, including the concentrations of dissolved hydrogen and methane, direct cell counts, and the microbial community profile, in contrast, required more than 8 h or 36 well volumes to stabilize. We interpret this result to mean that the zone of influence of the wellbore on biologic processes in the aquifer extends beyond the commonly recognized zone where physical properties are affected. A second period of adjustment of these biologically sensitive parameters began after about 50 h of pumping, following displacement of 230 wellbore volumes, and continued to the end of the experiment. During this period, the cell density and the composition of the microbial community suspended in the water samples changed. This finding indicates that the microbial community in and near the wellbore changed in response to pumping and the changes affected aspects of the composition of water produced from the well. The study demonstrates the importance of allowing adequate pumping time when sampling ground water for the analysis of biologically sensitive parameters.

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### Introduction

Ground water hydrologists have long appreciated that obtaining representative samples for chemical analysis from an unpumped or lightly pumped well requires that the wellbore first be purged several times. This requirement arises because the chemical environment in the well and proximal aquifer can differ considerably from that in the aquifer as a whole. Water in the well

reacts over time with the well's casing and screen and the concrete or other sealing materials used in construction (Barcelona et al. 1985). Oxygen from the atmosphere can diffuse into the bore of static wells, surface water may enter the well, and surface and shallow ground water may seep down the well along the outside of the casing.

These effects can change the temperature, pH, dissolved oxygen (D.O.), oxidation-reduction potential (ORP, or Eh), electrical conductivity (EC), and total dissolved solids content of water in the well. Standard protocol calls for pumping three to five times the wellbore volume before taking a sample for chemical analysis (Puls and Barcelona 1996) to ensure that the sample is representative of ground water in the aquifer. It is common, furthermore, to wait until one or more easily observed parameters such as water temperature, pH, D.O., ORP, EC, or the density or size of colloidal particles have stabilized (Robin and Gillham 1987; Powell and Puls 1993; Kearn et al. 1992).

In recent years, there has been considerable interest in the microbial communities that inhabit the subsurface and, as such, in chemical parameters that reflect microbial activity in ground water. These parameters include dissolved hydrogen (H<sub>2</sub>), methane (CH<sub>4</sub>), organic

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molecules, iron, sulfide, and so on (Chapelle 2001; Lovley and Chapelle 1995). Such analytes are influenced not only by the factors already mentioned but also by the action of the microbes resident in and near the well. The microbial community there almost certainly differs significantly from that in the pristine aquifer (Alfreider et al. 1997).

It is important, therefore, to consider how the microbial community reacts to purging a well and the effect it has on the measurement of the biologically sensitive analytes. If the community grows or changes in composition during purging, the H<sub>2</sub> concentrations observed, for example, may vary in response over time, even after the parameters not influenced by biologic activity have stabilized.

To date, there has been little study of the influence of microbes during well pumping on ground water chemistry. How long should a well be purged when sampling ground water for biologically sensitive parameters? Can an analysis obtained from a continuously pumped well be compared to one from a well in the same aquifer that is pumped intermittently? In this study, we approach such questions directly by monitoring ground water chemistry and sampling suspended microbes as we vigorously pump a well that is normally pumped only lightly.

## Methods

### Study Site and Sampling

We collected ground water samples from a research well in Urbana, Illinois, located next to the Newmark Civil Engineering Laboratory at the University of Illinois. The well is 46 m (152 feet) deep and completed in unconsolidated sand and gravel of the Glasford formation. According to drilling records, mainly clay was encountered to a depth of 37.5 m (123 feet), below which deposits of medium to coarse sand were found (Figure 1). The area is overlain by about 1.5 m of glacial loess (Kempton et al. 1982).

The well had been pumped continuously at 0.5 L/min (0.03 m<sup>3</sup>/h) for several years prior to the experiment. Higher flow rates were used occasionally for short periods of time to obtain ground water samples for other studies. In this experiment, the flow rate was increased to 6 L/min (0.36 m<sup>3</sup>/h) for 120 h and subsequently to 12 L/min (0.72 m<sup>3</sup>/h) for 3 h, prior to returning to baseline conditions. Ground water residence time in the wellbore at the 6 L/min flow rate was about 13 min, as calculated from the dimensions of the screened bore (2 × 2 inch × 3.14 × 32 feet = 5.08 × 5.08 × 3.14 × 975 cm = 79 L). Thirteen water samples were collected at irregular intervals over the course of the experiment, starting 30 min after flow was increased, after stagnant water in the pipes had been cleared.

### Chemical Analysis

At each sampling time, we measured temperature, D.O., ORP, and EC in situ using a HYDROLAB Data-sonde 4A probe. The probe cluster was allowed to equilibrate with the flowing ground water for 30 min before the measurements were made. We determined the total inorganic carbon content of samples by titrating them with 0.1 N HCl.

We analyzed samples preserved with 0.5 N HCl for ferrous iron concentration by Ferrozine assay (Stookey 1970). We determined sulfide concentration in samples shortly after collection by the methylene blue method, and sulfate by the turbidimetric method with barium chloride as described previously (Eaton et al. 1995). We analyzed ferrous iron, sulfide, and sulfate immediately after collection using a portable spectrophotometer (LaMotte Company, Chestertown, Maryland).

We used a gas stripping method (Chapelle et al. 1997) to analyze for dissolved H<sub>2</sub> and CH<sub>4</sub>. We injected 30 mL of ultrapure nitrogen gas into a gas collection bottle containing ground water flowing at a rate greater than

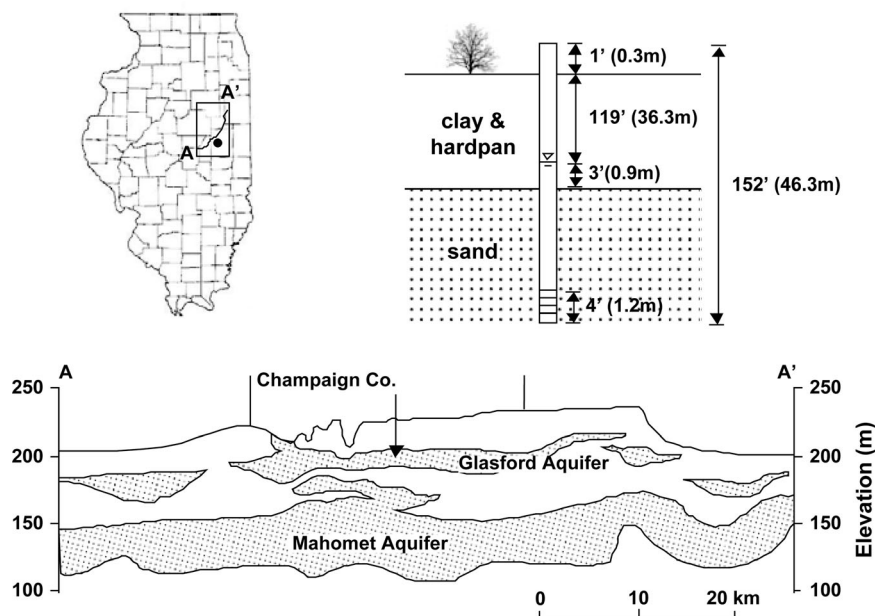


Figure 1. The location and stratigraphy of the study area; arrow indicates the well location.

300 mL/min (0.018 m<sup>3</sup>/h). After the gas bubble had equilibrated for 30 min, we withdrew 10-mL aliquots of the gas and injected the gas into two vacuumed glass tubes sealed with butyl rubber stoppers. We determined H<sub>2</sub> and CH<sub>4</sub> concentrations in the gas sample by gas chromatography (Perkin Elmer, Wellesley, Massachusetts) with a reduction gas detector and a flame ionization detector (Trace Analytical, Menlo Park, California), respectively, within 1 week of collection.

## Microbial Analysis

### Total Number of Microbes

To count the total number of microbes suspended in a ground water sample, we preserved 65 mL of each sample by adding 60 mL of ethanol (100%) in 125-mL high density polyethylene bottles. We transferred the fixed samples to the laboratory and stored them at -20°C in a freezer. We stained the microbes by adding 40 µL of buffered SYBR Green I solution (SYBR Green I:pH 8 tris-acetate-EDTA buffer = 1:200) to 30 mL fixed ground water samples and filtering the mixture through 0.2-µm black membrane filters (47-mm Isopore<sup>TM</sup> Millipore filters) with vacuum filtration units. A drop of mounting solution (ethanol:glycerol = 1:9) was placed on the center of a membrane filter and the filter covered by a cover glass. We counted the microbes using epifluorescence microscopy (Nikon eclipse E800) at a magnification of ×900 using a UV fluorescence excitation (filter combination 365/395/397 nm). More than 20 fields were selected randomly over the filter, photographed, and the counts per field averaged.

### Genetic Analysis of Microbial Communities

Terminal-restriction fragment length polymorphism (T-RFLP) is a polymerase chain reaction (PCR)-based technique used to characterize genetically the microbial community from an environmental sample such as ground water or sediment (Lueders and Friedrich 2000; Rees et al. 2006; Takai et al. 2001) that has yielded community profiles of high reproducibility and quality (Marsh 1999; Osborn et al. 2000). In this method, the bulk DNA from a sample is extracted, and the DNA that codes for small subunit ribosomal RNA from different microbial populations is amplified using PCR. Once amplified, the 16S rDNA is labeled at one end with a fluorochrome that fluoresces under UV light of a specific wavelength.

The DNA is then cut into several fragments with a series of restriction enzymes that cleave the strands only in certain locations where nucleotides occur in specific sequences. The terminal fragments, those from the end of the DNA that has been labeled with the fluorochrome, can fluoresce. The fragments are sorted by size in a gel across which an electrical potential is maintained, and the size distribution of the terminal fragments is determined quantitatively from the intensity at which the size fractions fluoresce under UV light.

Each microbial species yields a terminal fragment of a specific size, so the T-RFLP results reflect the various species that ensemble make up the microbial community in a sample. T-RFLP, then, provides a unique genetic

fingerprint representing the community's composition: each fragment size is derived from a species and the intensity with which those fragments fluoresce reflects the species' relative abundance in the sample. This community fingerprint can be compared rather easily to those from samples collected at different times or from different places.

For T-RFLP analysis, we collected biomass by filtering the retentate from the 20-inch ultrafiltration unit with a 0.2-µm filter (Pall Gelman Laboratory, Ann Arbor, Michigan) for 30 min. The average flow rate through the ultrafiltration unit was initially 0.6 L/min (0.036 m<sup>3</sup>/h) and was increased to 0.7 L/min (0.042 m<sup>3</sup>/h) after 120 h. We preserved the filters in a 50-mL sample tube on dry ice until the filters could be stored at -30 °C in the lab. We extracted the total community DNA from the filters using an UltraClean<sup>TM</sup> water DNA kit (Mo Bio Laboratories Inc., Carlsbad, California).

We amplified the 16S rDNA by PCR using a Master Taq<sup>®</sup> kit (Eppendorf AG, Hamburg, Germany). To amplify eubacterial DNA, we used eubacterial primer 8F (5'-AGAGTTTGATYMTGGCTCAG-3') and universal primer 1492R (5'-GYTACCTTGTTACGACTT-3') from Integrated DNA Technologies Inc., Coralville, Iowa. For amplification of archeal DNA, we used primers 109F (5'-ACKGCTCAGTAACACGT-3') and 912R (5'-CTCC-CCCCCAATTCCTTTA-3') from the same source. We used 5 µL of DNA template in 50 µL reactions and amplified the DNA for 20 cycles with unlabeled primers. Next, we used 2 µL of the PCR product from the 20 cycle reactions as template for 30 cycle 100 µL reactions with 6-carboxyfluorescein (FAM)-labeled forward primers (i.e., nested PCR). For all reactions, we used a 55°C annealing temperature. We purified the PCR products of the 30 cycle reactions using QIAquick purification kit (Promega, Madison, Wisconsin), and then we performed T-RFLP as described by Liu et al. (1997) using restriction enzymes *HhaI* and *RsaI* (Invitrogen, Carlsbad, California) and *MspI* (Promega). The University of Illinois Biotechnology Center analyzed the digests using a Prism 377 automated sequencer and GeneScan analysis software (Applied Biosystems Instruments, Foster City, California) to produce electropherograms showing the abundance and length of the fluorescently labeled terminal fragments.

To compare the similarity of T-RFLP peak profiles of each sample over time, we generated a distance matrix using the Bray-Curtis model with a square root transformation of the relative peak heights using primer 5. We then graphically displayed this relationship between sample terminal restriction fragment (TRF) community profiles using nonmetric multidimensional scaling (MDS) to show how the composition shifted with time. Each MDS ordination was run with 30 random starting configurations and proceeded through multiple iterations until the fit of a nonparametric regression of *d* (distances between samples on the MDS) against  $\delta$  (similarities in the Bray-Curtis matrix) could not be improved (Klaus et al. 2007).

## Results

Table 1 shows the physical and chemical properties of the fluid measured as the well was sampled, at hours

	0.5 h	8 h	50 h	120 h	123 h
Pumping rate (L/min)	6	6	6	6	12
Water quality parameters					
pH	6.80	6.78	6.81	6.82	6.75
Temp (°C)	18.5	18.0	18.0	18.0	16.5
ORP (mV)	48	24	21	20	17
EC (µS/cm)	500	545	546	545	544
D.O. (mg/L)	0.3	0.03	0.02	<0.01	<0.01
Alkalinity (mg/L, as CaCO <sub>3</sub> )	325	325	325	325	325
Ion concentration					
Fe(II) (µM)	35	25	25	25	24
Sulfate (µM)	200	174	170	154	186
Sulfide (µM)	0.9	2.1	2.0	2.1	2.8
Dissolved gas concentration					
H <sub>2</sub> (nM)	48	95	88	35	95
CH <sub>4</sub> (µM)	62	72	72	80	83
Microbial population (cells/L)	5.3 × 10 <sup>6</sup>	5.3 × 10 <sup>6</sup>	5.3 × 10 <sup>6</sup>	9.4 × 10 <sup>6</sup>	1.10 × 10 <sup>7</sup>
Organics					
Acetate (µM)	~125 <sup>1</sup>	ND <sup>2</sup>	ND	ND	ND

<sup>1</sup>Concentration estimated based on DOC measurements of 3.0 mg/L, assuming all DOC is acetate.  
<sup>2</sup>Not determined.

0.5, 8, 50, 120, and 123. Ground water temperature decreased slightly from 18.5 °C to 18.0 °C in the first half hour, then remained around 18 °C. When the pumping rate was increased to 12 L/min at 120 h, temperature decreased to 16.5 °C. pH remained constant at 6.8 ± 0.2 throughout the experiment. D.O. was 0.3 mg/L initially and then decreased to below the detection limit of 0.01 mg/L within 1 h. ORP trended downward slightly, from 48 mV at the onset to 17 mV at 121 h, but was stable at around 30 mV for most of the experiment. Electrical conductivity was 500 µS/cm at the onset and stabilized at 545 µS/cm after half an hour of pumping. Alkalinity was stable at 325 mg/L (as CaCO<sub>3</sub>).

#### Biologically Sensitive Chemical Parameters

The concentrations of ferrous iron and sulfide changed sharply at the beginning of the experiment, then remained nearly constant; sulfate showed a similar but less dramatic trend (Figure 2). The concentration of ferrous iron, 35 µM at the onset, decreased to 25 µM within 1 h. Sulfide concentration increased from 0.9 µM to around 2.1 to 2.2 µM within 8 h. Sulfate showed a trend similar to ferrous iron, falling from 200 to 175 µM within 8 h. The concentrations of each analyte remained stable after the initial adjustment period.

H<sub>2</sub> and CH<sub>4</sub> concentrations stabilized after the initial adjustment period, then, after an interval of stability, began to change once again. H<sub>2</sub> concentration increased from 48 to 100 nM within 8 h, remained approximately constant until 50 h, and then began a steady decrease (Figure 3A); at 120 h into the experiment, the H<sub>2</sub> concentration was 35 nM. CH<sub>4</sub> concentration increased from 62 to 72 µM in the first 8 h (Figure 3B). It stabilized at about

69 µM between 8 and 50 h of continuous pumping, after which, in contrast to H<sub>2</sub>, it began to steadily increase, reaching 80 µM by 100 h.

#### Microbial Community Changes

The total number of microbes suspended in the water held stable at 5 × 10<sup>6</sup> to 6 × 10<sup>6</sup> cells/L for 50 h, then continuously increased to 9.4 × 10<sup>6</sup> cells/L at 120 h (Figure 3C). The results of T-RFLP microbial community analysis indicate that the bacterial community in the ground water changed in composition over time (Figure 4). Using the Bray-Curtis model, the graphic MDS output shows this change with time (Figure 5). During the first 4 h, the community profile varied little. After 4 h, the community began to change significantly, stabilizing again between 30 and 51 h. Later, from 96 to 120 h, microbial community composition began to shift again and continued to change until the experiment ended.

Using archaeal-specific PCR primer sets, two major populations of archaea, likely methanogens, were detected with TRF fragment lengths (*RsaI* digest) of 180 and 500 (Figure 6). The archaeal population corresponding to the 180 bp TRF was observed throughout the experiment, suggesting that this organism maintained its place in the community regardless of pumping rate.

#### Effect of Doubling Ground Water Pumping Rate

When the pumping rate was increased from 6 to 12 L/min for the last 3 h of the experiment (Table 1), the concentration of H<sub>2</sub> increased by a factor of 2.7. This increase is similar to that observed at the onset of the experiment (Figure 3). The concentrations of methane, sulfide, and sulfate increased slightly, whereas the

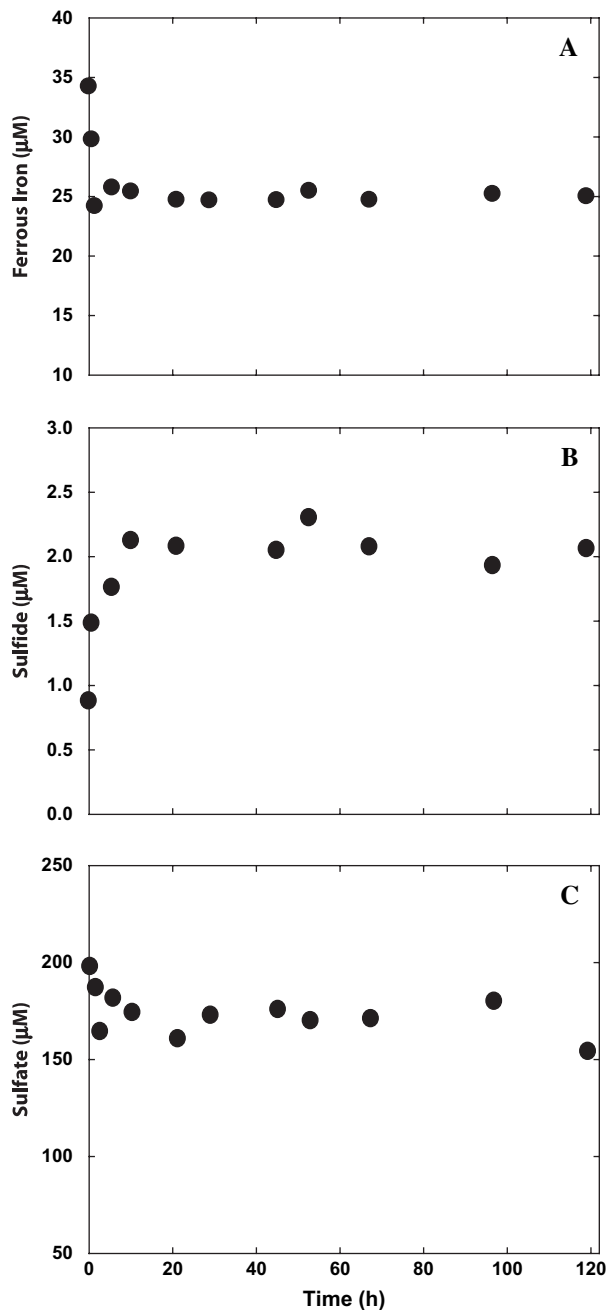


Figure 2. Variation in (A) ferrous iron, (B) sulfide, and (C) sulfate concentration in water sampled from the well, over the course of the experiment.

concentration of ferrous iron decreased when the pumping rate rose. The microbial cell count also increased slightly, perhaps due to microbes being displaced from sediment surfaces as shear forces associated with fluid movement increased.

## Discussion

### Microbiological Response to Well Pumping

The data indicate that in response to increased well pumping, biologically sensitive parameters, and by inference, the subsurface microbiological community, evolved for at least 120 h. Prior to onset of the experiment, chemical and biological processes near the well may have been

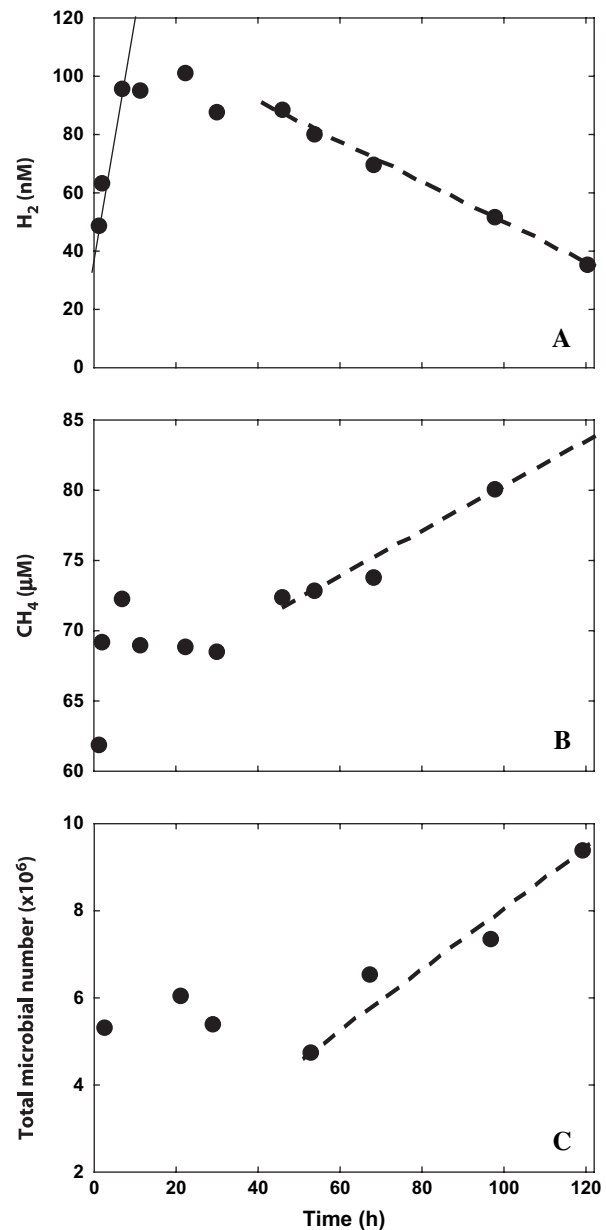
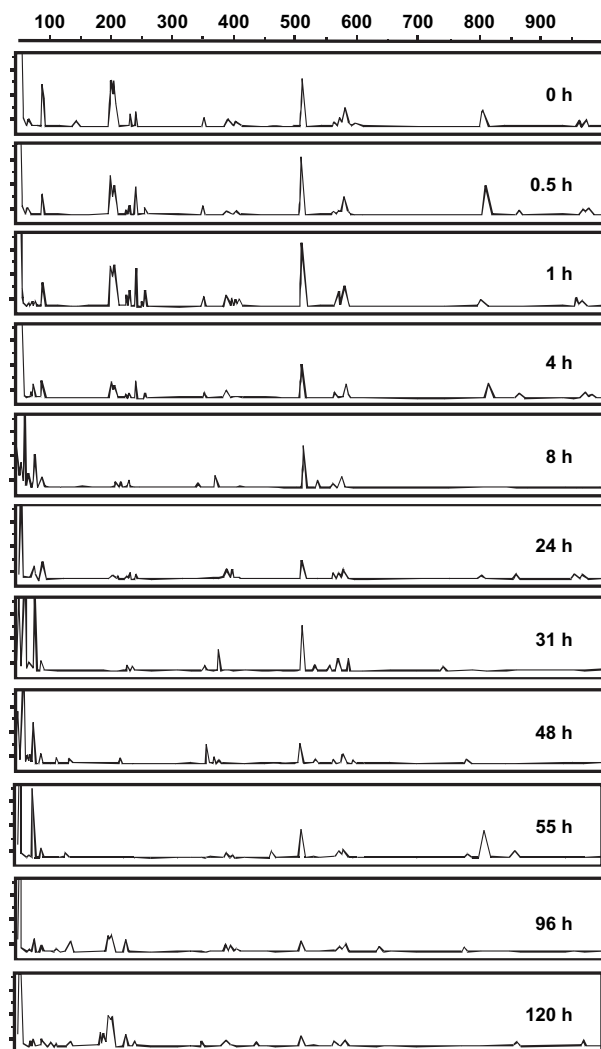


Figure 3. Variation in (A) dissolved dihydrogen, (B) dissolved methane, and (C) total microbial cell counts over the experiment. Solid line (A) shows the initial increase in H<sub>2</sub> concentration (slope = 13.5 nM/h); dashed line in (A) shows later decrease in H<sub>2</sub> levels, apparently in response to growth of the microbial community (0.7 nM/h). In (B), dashed line shows increase in CH<sub>4</sub> concentration in apparent response to the microbial growth (0.15 µM/h). The dashed line in (C) indicates the growth in microbial cell counts after 50 h of pumping.

in a near steady-state condition, since the pump rate had been maintained in previous years at 0.5 L/min. Increasing the pumping rate disrupted conditions near the well, and, in response, chemical and biological processes there adjusted, leading to the observed change in the biologically sensitive parameters.

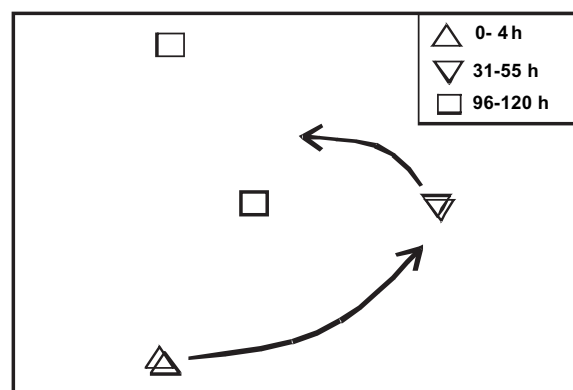
An alternative explanation is that these parameters varied as a result of macroscopic heterogeneity in the aquifer. If microbial communities and activities are distributed unevenly in the subsurface (Goldscheider et al. 2006), beginning to pump the well rapidly might have



**Figure 4.** Electrophoretograms showing the TRF variation of bacterial (nonarchaeal) communities (*HhaI* digest) over time. Each peak indicates a bacterial member among bacterial communities in the ground water, and the peak height indicates relative abundance of each member at each time. Values on the x-axis are sizes in base pairs (terminal restriction fragment length); the y-axis shows signal strength in arbitrary units.

drawn in water from disparate portions of the aquifer, causing water composition to change with time. The aquifer system studied, however, is known to be quite homogeneous (Figure 1) (Kempton et al. 1982). More notably, such an interpretation does not offer a ready explanation of why the physical and chemical parameters stabilized relatively quickly whereas the biologically sensitive parameters changed more slowly.

Parameters commonly observed during well sampling—temperature, pH, D.O., ORP, and EC—stabilized within an hour of initiating pumping, consistent with previous studies. The concentrations of ferrous iron, sulfide, and sulfate took about 8 h to stabilize, in contrast, and a steady state was maintained for the duration of the experiment. Concentrations of  $H_2$  and  $CH_4$  also stabilized within 8 h but later began to drift again. These parameters are particularly sensitive to biological activity. Since the second adjustment period appears to result from changes



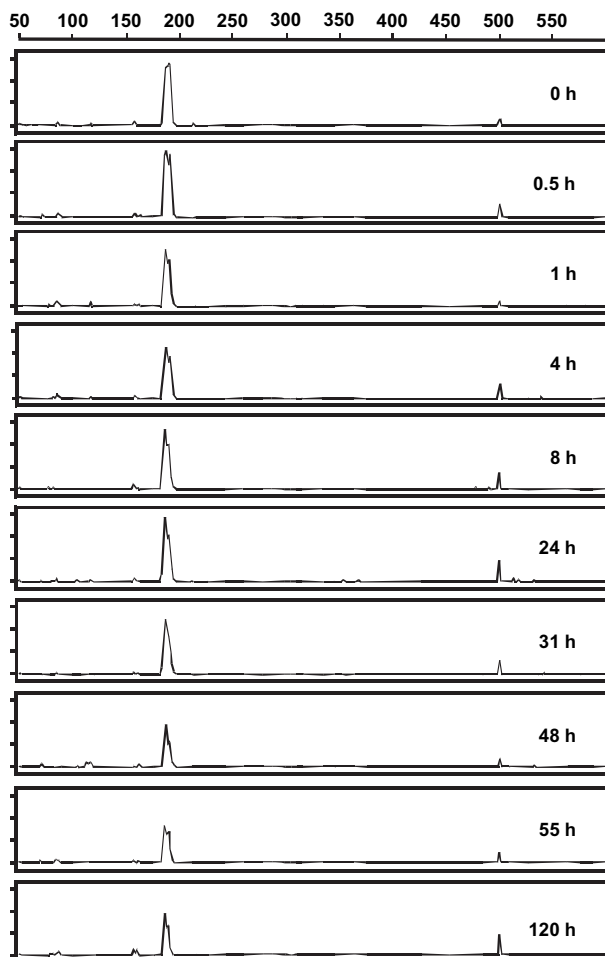
**Figure 5.** Nonmetric MDS plot of bacterial community change, as indicated by variation over time in the experiment of T-RFLP analyses. Distance between symbols scales with the dissimilarity among communities. Arrows indicate changes observed between initial conditions (0 to 4 h) and later time points in the experiment. Metastable community profiles are apparent from 0 to 4 h, and again from 31 to 55 h.

to the microbial community composition near the well, it might be argued that the values observed after the first adjustment period, when the parameters stabilized at about 8 h, are the observations most representative of conditions in the bulk aquifer.

Results of this study suggest that common sampling practice, which calls for flushing three to five well volumes before samples are taken, may be sufficient for temperature, pH, D.O., ORP, and EC measurements. Considerably more time, however, is needed to obtain representative concentrations of ferrous iron, sulfide, sulfate,  $H_2$ , and  $CH_4$ . Considering that microbial metabolisms influence each of these analytes, the change of the concentrations over 8 h indicates that microbial activity near the wellbore is not the same as in the bulk aquifer. Measurements obtained after a short period of pumping, then, do not reflect the true nature of ground water in the aquifer as a whole.

In the case of  $H_2$ , the concentration nearly doubled after 8 h, which corresponds to purging the wellbore about 36 times, compared to measurements made at less than 4 h of pumping. If normal sampling protocols that require purging the wellbore only five times were to be used, the mass of electron donor available in the ground water would be greatly underestimated.

Under anaerobic conditions,  $H_2$  is produced by fermentative microorganisms, which metabolize organic matter in the subsurface, and consumed by anaerobic respiration (Lovley and Goodwin 1988). In our experiment, as the pumping rate increased and the well was flushed with ground water, the  $H_2$  concentration increased rapidly, stabilizing after about 8 h (Figure 3A). This concentration may come close to reflecting the actual ambient conditions in the aquifer. By providing substrates like  $H_2$  to the vicinity of the wellbore, however, the pumping promotes microbial growth there, as reflected by the increase in microbial cell count between 50 and 120 h of pumping (Figure 3C). The range of microbial cell counts for the ground water is of the magnitude seen in other ground water studies (Stevens et al. 1993; Pedersen and Ekendahl

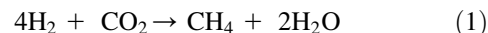


**Figure 6.** Electrophoretograms showing the TRF variation of archaeal communities (*RsaI* digest) in the experiment over time. Each peak indicates a bacterial member among archaeal communities in the ground water, and the peak height indicates relative abundance of each member at each time. Values on the *x*-axis are sizes in base pairs (terminal restriction fragment length); the *y*-axis shows signal strength in arbitrary units.

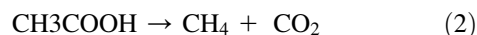
1990; Olson et al. 1981). Over the initial 55 h of pumping, the number of microorganisms ranged from  $5 \times 10^6$  to  $6 \times 10^6$  cells/L. Between 55 and 120 h, the total microbial numbers in the ground water samples increased to  $9.4 \times 10^6$  cells/L (Figure 3C).

If we assume that methanogens are a dominant group of microorganism, we can estimate the group's growth from the increase in  $\text{CH}_4$  concentration, which rose from 70 to 85  $\mu\text{M}$  from 55 to 120 h. Based on known stoichiometric relationships associated with the coupling of methanogenesis to growth (Rittmann and McCarty 2001), given this increase, we would expect the population of methanogens to rise about 20%, a value significantly less than observed. The discrepancy may reflect the fact that planktonic microbial populations in ground water samples do not necessarily reflect microbial densities in subsurface systems (Alfreider et al. 1997) because most microorganisms in an aquifer live attached to solid surfaces. The result may also indicate that actively growing microorganisms are relatively easily detached from aquifer surfaces, contributing to their being overrepresented in the planktonic fraction.

After 30 h, the  $\text{H}_2$  concentration decreased and  $\text{CH}_4$  concentration increased (Figures 3A and 3B). The decrease of  $\text{H}_2$  could be explained in part by increased rates of hydrogenotrophic methanogenesis:



This is the case even though the measured  $\text{H}_2$  concentration (35 to 100 nM) is in the range of the  $\text{H}_2$  threshold concentration for methanogenesis (5 to 95 nM) (Conrad 1996; Ralf et al. 1988; Lovley 1985; Lovley and Goodwin 1988). This reaction, however, can account for only a small fraction of the increase in  $\text{CH}_4$  concentration because more  $\text{CH}_4$  is gained than  $\text{H}_2$  lost (+15  $\mu\text{M}$  vs. -60 nM). The bulk of the methane produced, therefore, may arise from acetoclastic methanogenesis:



Acetate can be produced by acetogenic bacteria in natural anaerobic environments as a byproduct of biomass decay (Conrad 1999), where it becomes available for use by acetoclastic methanogens. The dissolved organic carbon (DOC) concentrations in this ground water are 3.0 mg/L (Najm et al. 1993), which if present purely as acetate would yield 125  $\mu\text{M}$  of  $\text{CH}_4$  by Reaction 2. The hydrogenotrophic reduction of  $\text{CO}_2$  to acetate, another potential source of acetate, is not likely in this case since the  $\text{H}_2$  concentrations in this aquifer are too low for the reaction to occur (Dolfing 1988) and the amount of acetate possibly produced would account only for about 15 nM  $\text{CH}_4$  generation, 1/1000 of that observed.

The results of T-RFLP analysis in our study show that the bacterial community composition remained stable for the first 4 h (Figures 4 and 5). Between 30 and 50 h, the composition of the bacterial community was also stable; however, it differed considerably from the initial community. After 50 h, the community changed again as growth due to increased substrate flux became significant. Some members of the initial bacterial community reestablished themselves in the final stage of the experiment; however, the overall community profile differed considerably from the initial profile.

In contrast to the results for bacteria, the archaeal community of presumed methanogens was largely invariant throughout the experiment (Figure 6). This result is somewhat surprising because  $\text{H}_2$  consumption and  $\text{CH}_4$  production in the ground water (Figures 3A and 3B) indicate that methanogenesis increased. This could be interpreted in two ways: (1) acetoclastic methanogens are dominant and a small increase in the hydrogenotrophic populations may not significantly change the community structure or (2) all the methanogens in the community grew at approximately equal rates in response to the increased availability of  $\text{H}_2$  and potentially acetate. Since T-RFLP provides no indication of absolute biomass concentration, we were not able to tell which of the possibilities is more likely. This situation demonstrates the usefulness of using  $\text{H}_2$  and  $\text{CH}_4$  concentrations as an indicator of microbial activity. No clear change in archaeal T-RFLP patterns is apparent, but the change in  $\text{H}_2$  and  $\text{CH}_4$  concentrations seems to require an increased rate of methanogenesis.

Parameter	<1 h	1–8 h	8–50 h	50–120 h
Water quality parameters				
pH, ORP, alkalinity	—	—	—	—
D.O., temperature	↘	—	—	—
EC	↗	—	—	—
Ion concentration				
Fe(II)	↘	↘	—	—
Sulfate	↘	↘	—	—
Sulfide	↗	↗	—	—
Gas concentration				
H <sub>2</sub>	↗	↗	—	↘
CH <sub>4</sub>	↗	↗	—	↗
Microbial population (cells/L)	—	—	—	↗

Note: ↗, increase; ↘, decrease; and —, stable.

In summary, variation in H<sub>2</sub> and CH<sub>4</sub> concentrations, T-RFLP profiles, and microbial cell counts demonstrate that there were two intervals of adjustment in the well over the course of the experiment. During the initial adjustment period, H<sub>2</sub> and CH<sub>4</sub> concentrations initially reflected conditions near the well. Concentrations immediately started to increase toward conditions in the bulk aquifer away from the well. Following a period of stability, there is a second period of acclimation marked by the increase in cell density and a change in the composition of the microbial community near the well.

#### Implications For Sampling Ground Water

Chemical and biological analytes in the experiment evolved over four distinct time periods (Table 2). If we are concerned with only dissolved ions (e.g., ferrous iron, sulfide, and sulfate), the sampling can be done after displacement of about 36 well volumes (8 h of pumping this well). For dissolved ions, gas concentrations (e.g., H<sub>2</sub> and CH<sub>4</sub>), and the microbial community, the optimum sampling interval continued until the wellbore had been displaced about 230 times (50 h for this well).

The optimum interval for obtaining a sample representative of these parameters can be expected to vary depending on well setting (e.g., the hydrogeologic environment and subsurface microbial community) and pumping rate. Our results, however, suggest that it can be identified by tracking when metabolic indicators such as H<sub>2</sub>, CH<sub>4</sub>, and Fe(II) stabilize, perhaps at around 36 wellbore displacements. These parameters may begin to change again in response to new microbial growth near the well, and the growth may be accompanied by a shift in the microbial community composition.

#### Conclusions

This study shows that rapid pumping of ground water from a well affects various physical and chemical

parameters of the ground water at different rates. Parameters commonly monitored when sampling a well (temperature, pH, D.O., ORP, and EC) stabilized within about an hour, after five wellbore volumes were produced. Biological and biologically sensitive parameters (ferrous iron, sulfide, sulfate, H<sub>2</sub>, CH<sub>4</sub>, direct cell counts, and the microbial community profile), in contrast, required more than 8 h, or 36 wellbore volumes to stabilize.

This result suggests that the zone over which the well influences biologic processes in the aquifer extends beyond the commonly recognized zone where physical properties are affected. This zone may be particularly sensitive to extended periods of rapid pumping, since the biologically sensitive parameters started to change again after about 50 h, following displacement of 230 wellbore volumes. The shift appears to be directly associated with increases in the cell density and variations in the composition of the microbial community. This study, of course, considers the dynamics of only a single well, and further work is needed to confirm how wells in general respond to the onset of pumping.

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