

현장 Single Well Push-Pull 실험을 통한 탈질산화반응 각 단계의 반응속도 측정

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<Abstract>

Quantifying rates of microbial processes under subsurface conditions is difficult, and is most commonly approximated by laboratory studies using aquifer materials. In this study a single-well, "push-pull" test method is adapted for the in situ determination of denitrification rates in groundwater aquifers. The rates of stepwise reduction of nitrate to nitrite, nitrous oxide, and molecular nitrogen were determined by performing a series of push-pull tests at an experimental well field of Korea University. A single Transport Test, one Biostimulation Test, and four Activity Tests were conducted for this study. Transport tests are conducted to evaluate the mobility of solutes used in subsequent tests. These included bromide (a conservative tracer), fumarate (a carbon and/or source), and nitrate (an electron acceptor). At this site, extraction phase breakthrough curves for all solutes were similar, indicating apparent conservative transport of the solutes prior to biostimulation. Biostimulation tests were conducted to stimulate the activity of indigenous heterotrophic denitrifying microorganisms. Biostimulation was detected by the simultaneous production of carbon dioxide and nitrite after each injection. Activity tests were conducted to quantify rates of nitrate, nitrite, and nitrous oxide reduction. Estimated zero-order degradation rates decreased in the order nitrate > nitrite > nitrous oxide. The series of push-pull tests developed and field tested in this study should prove useful for conducting rapid, low-cost feasibility assessments for in situ denitrification in nitrate-contaminated aquifers.

Key word : in situ determination of denitrification rate, single-well push-pull test, fumarate, a nitrate-contaminated aquifer

1. INTRODUCTION

Quantifying rates of microbial processes under subsurface conditions is difficult, and is most

commonly approximated by laboratory studies using aquifer materials. The laboratory method has several disadvantages that limit its routine use for feasibility assessment and remedial design. For example, core samples are often difficult to obtain, and may be too small to provide representative information on subsurface conditions. This fact makes it difficult to assess whether rate measurements of microbial process that are determined in the laboratory are relevant to actual in situ rates.

In this study a single-well, "push-pull" test method is adapted for the in situ determination of denitrification rates (Kim et al., 2001, 2003). The rates of stepwise reduction of nitrate to nitrite, nitrous oxide, and molecular nitrogen were determined by performing a series of push-pull tests. A push-pull test consists of the controlled injection of a prepared test solution into an aquifer followed by the extraction of the test solution/ground water mixture from the same location. The injected test solution consists of ground water containing a nonreactive tracer and one or more biologically reactive solutes selected to investigate specific processes of interest. The test solution is injected ("pushed") into the aquifer where it flows radially outward from the well and penetrates a volume of aquifer material adjacent to the well. During the extraction phase, flow is reversed; the test solution/ground water mixture is extracted ("pulled") from the same location, and concentrations of tracer, reactants, and reaction products are measured as a function of time. Reaction rate coefficients are computed from the mass of reactant consumed and/or product formed (Haggerty et al. 1998; Istok et al. 1997; Kim et al., 2003). A rest phase (with no pumping) may be included between the injection and extraction phases, to allow time for a particular reaction to proceed.

The objective of this study was to evaluate in situ rates of stepwise reduction of nitrate to nitrite, nitrous oxide, and molecular nitrogen. The test series consists of (1) a short-duration (~ 5 hrs) Transport test to evaluate the mobility of fumarate (CS/ED) and nitrate (an electron acceptor, EA) in the absence of biological activity; (2) longer-term (~ weeks) Biostimulation tests to evaluate the ability of fumarate and nitrate additions to stimulate heterotrophic denitrifying microorganisms; and (3) intermediate-term (~ 8 hrs) Activity tests, to quantify in situ rates of nitrate, nitrite, and nitrous oxide degradations.

2. METHODS

Field tests. A single Transport Test, one Biostimulation Test, and four Activity Tests were conducted. Transport Test was first conducted, and was followed by a Biostimulation period and a series of Activity Tests. Field equipment consisted of a helium gas, a carboy (800 L), a collapsible metalized-film gas-sampling bag (Chromatography Research Supplies, Addison, IL), a peristaltic pump to inject the test solution into the well and extract the groundwater/test solution mixtures from the same well.

Transport test. A short-duration transport test was conducted in a well to compare the relative mobility of bromide, nitrate and fumarate in the aquifer prior to subsequent tests. Site groundwater was used to prepare a 400-L test solution containing known concentrations of bromide (KBr, Spectrum Chemical Mfg. Corp. Gardena, CA) to serve as a nonreactive tracer, nitrate (NaNO_3 , Dong Yang Chemical Inc., Seoul, Korea) as an EA, fumarate as an CS/ED. Four-hundred liters of test

solution (prepared as described above) were injected at 4 L/min. Ten samples of the injected test solution were collected using a 40-mL VOA vial with a Teflon -lined cap during the injection phase, and analyzed to determine test solution composition. The extraction phase began 1 hour after the end of the injection phase to minimize the time available for microbial degradation of injected nitrate and fumarate. During the extraction phase the test solution/groundwater mixture was extracted from the well using a peristaltic pump at a rate of 4 L/min. Samples collected during the extraction phase were analyzed and used to prepare breakthrough curves for each injected solute.

Biostimulation period. During the Biostimulation Period one addition of fumarate and nitrate was performed in the well to stimulate the activity of indigenous denitrifying bacteria. Test solutions were prepared and injected as described above and contained known concentrations of bromide, fumarate and nitrate. Periodic sampling of the test solution/groundwater mixture was used to ensure the stimulation of denitrifying microorganisms.

Activity tests. Following the Biostimulation period, a series of four Activity tests (1st Nitrate Activity test, Nitrite Activity test, 2nd Nitrate Activity test, and Nitrous oxide Activity test) were conducted to quantify in situ rates of nitrate, nitrite, and nitrous oxide degradation. After a 7 hr rest phase with no pumping, the test solution/ground water mixture was extracted from the well at a rate of 4 L/min. Samples collected during the extraction phase were analyzed and used to prepare breakthrough curves for each injected solute and products formed in situ.

Data Analysis. Mass balance calculations were performed by integrating measured solute concentrations and injection and extraction volumes. For plotting purposes, normalized concentrations, C^* , were computed using

$$C^* = [(C - C_{BG}) / (C_o - C_{BG})] \quad (1)$$

where C is a measured concentration in an extraction sample, C_o is average injected concentration, and C_{BG} is the background (pre-injection) concentration of the same solute. Overall zero-order reaction rates (r) for injected solutes were calculated using the method of Istok et al. (1997):

$$r = \frac{M_{inj} - \{M_{ext} / R_{tracer}\}}{(V_{inj})(t^*)} \quad (2)$$

where M_{inj} is total mass of solute injected, M_{ext} is the total mass of the solute extracted, V_{inj} is volume of injected test solution (L), R_{tracer} is the mass recovery fraction of the conservative tracer (extracted tracer mass divided by injected mass) and t^* is the mean residence time defined as the elapsed time from the midpoint of the injection phase to the centroid of the conservative tracer breakthrough curve during the extraction phase. Additional details of this calculation are provided by Istok et al. (1997) and Haggerty et al. (1998).

3. RESULTS AND DISCUSSIONS

Transport tests. Extraction phase breakthrough curves for all injected solutes were similar during transport tests indicating conservative transport of all injected solutes prior to biostimulation (Figure 1). These results are important because they mean that measured concentrations of the substrates and metabolites can be adjusted for dilution using measured bromide concentrations (Haggerty et al.,

1998). The tests demonstrated approximately 90% recovery of the injected solutes upon extraction after they resided in the aquifer for a mean residence time (t^*) of 5.4 hours.

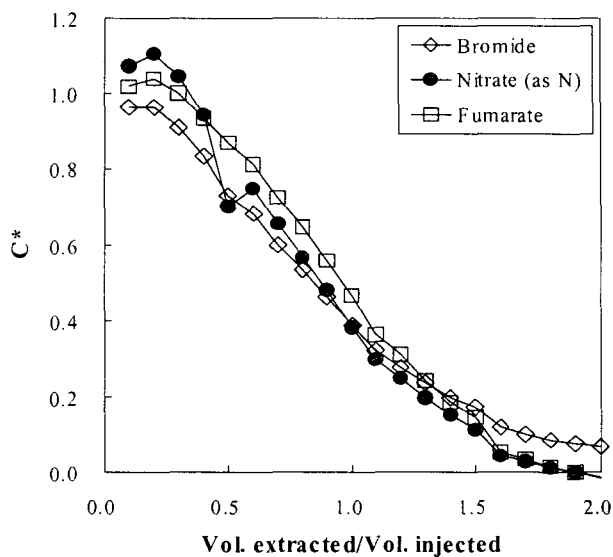


Figure 1. Pull phase normalized concentrations during the Transport test showing conservative transport of fumarate and nitrate.

Activity tests. Four Activity tests were performed to confirm the stimulation of indigenous denitrifying activity through Biostimulation period and to quantify the rates of nitrate, nitrite, and nitrous oxide degradation. The tests were performed in sequence: 1st Nitrate Activity test, Nitrite Activity test, 2nd Nitrate Activity test, and Nitrous oxide Activity test. The mean residence time for the activity tests were similar to the transport test.

During the 1st Nitrate Activity test, substantial fumarate and nitrate degradations were observed, and significant amounts of carbon dioxide and nitrite were produced, indicating biostimulation of indigenous heterotrophic denitrifying microorganisms (Figure 2). Zero-order rates of fumarate and nitrate degradation during the test were computed as described in the Data Analysis section. Computed rates of nitrate and fumarate degradation were 0.37 and 0.36 mmol/L/hr, respectively. Computed value of the ratio (nitrate utilization rate)/(fumarate degradation rate) was very close to the theoretical value of 1.0 computed using the energetic model of Rittmann and McCarty (2001), which assumes no toxicity of substrate (e.g. nitrite and nitrous oxide) on cell activity.

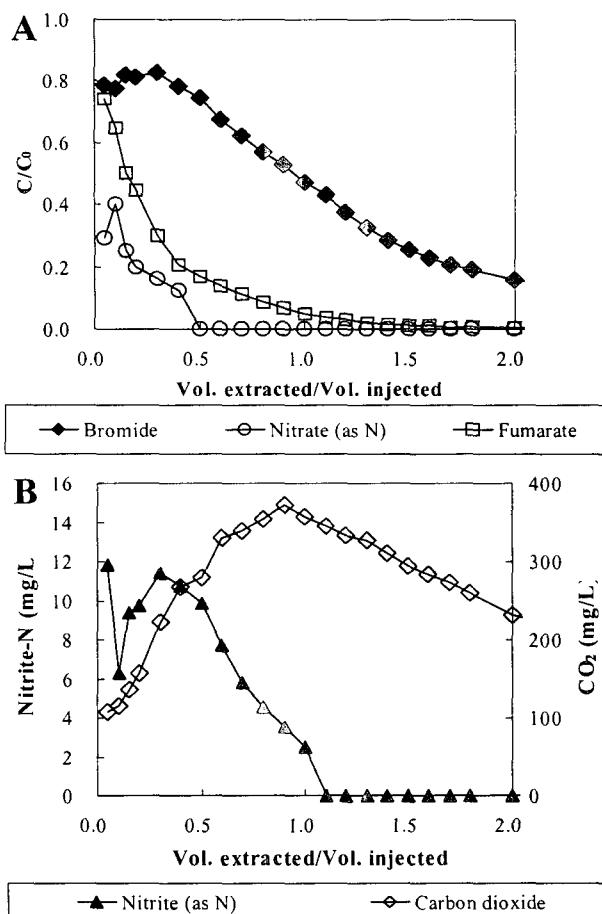


Figure 2. Extraction phase normalized concentrations of bromide, nitrate, and fumarate (A) and concentrations of nitrite and carbon dioxide (B) during the 1st nitrate Activity Test.

The Nitrite Activity test was conducted in the absence of added nitrate or nitrous oxide. The results of the test indicated that significant amount of injected nitrite was degraded (data not shown), however, no nitrous oxide and nitrate was detected during the extraction phase, indicating all nitrite may be degraded in situ to molecular nitrogen, and nitrate in the site groundwater may also degraded during the extraction phase. Computed rate of nitrite degradation was a factor of 2.7 smaller than the nitrate degradation rate, and fumarate degradation rate was slightly greater than that computed from the results of the 1st Nitrate Activity test. Computed value of the ratio (nitrite utilization rate)/(fumarate degradation rate) was 0.29 that is a factor of 4.7 smaller than the theoretical value computed using the energetic model, which does not assume substrate toxicity. These suggest that more amount of fumarate may be required to degrade nitrite than nitrate due to nitrite toxicity on cell activity.

The Nitrous oxide Activity test was conducted in the absence of added nitrate or nitrite (data not shown). Although 400-L test solution containing 7.2 mg-N/L of nitrous oxide was injected into the aquifer, no nitrous oxide was detected during the extraction phase. Nitrate was not detected, either. These results suggest that all nitrous oxide injected and nitrate in the site groundwater may be rapidly degraded. The rate of fumarate degradation was a factor of 1.7 greater than that of nitrate degradation, while much less production of CO₂ was observed. Computed value of the ratio (nitrous

oxide degradation rate)/(fumarate degradation rate) was 0.12 that is a factor of 12 smaller than the theoretical value of 1.4. These suggest that more amount of fumarate may be required to degrade nitrous oxide than nitrate and nitrite.

4. CONCLUSIONS

The series of push-pull tests developed and field tested in this study should prove useful for conducting rapid, low-cost feasibility assessments and obtaining important design parameters for in situ denitrification in a nitrate-contaminated aquifer. Estimated zero-order degradation rates decreased in the order nitrate > nitrite > nitrous oxide. Degradation of nitrous oxide might be more toxic than nitrite degradation, thus, these toxicities need to be considered in design in situ bioremediation of a nitrate-contaminated aquifer.

REFERENCES

- Haggerty, R., M. H. Schroth, and J. D. Istok. 1998. Simplified method of "push-pull" test data analysis for determining in situ reaction rate coefficients. *Ground Water*. 36: 314 - 324.
- Istok, J.D., M.D. Humphrey, M.H. Schroth, M.R. Hyman, and K.T. O'Reilly. 1997. Single-well, "push-pull" test method for in situ determination of microbial metabolic activities. *Ground Water*. 35: 619 - 631.
- Kim, Y., J. D. Istok, L. Semprini. 2001. Assessing the feasibility of in-situ aerobic cometabolism of chlorinated solvents by single well push-pull and natural gradient drift tests in McClellan AFB, CA. Abstract B42B-0140. American Geophysical Union 2001 Fall Meeting, San Francisco, CA.
- Kim, Y., J. D. Istok, and L. Semprini. 2003. Push-Pull Tests for Assessing In Situ Aerobic Cometabolism. In Press. *Ground Water*.
- Rittmann, B. E. and P. L. McCarty. 2001. *Environmental Biotechnology: Principles and Applications*. McGraw-Hill, New York, NY.