A single well push–pull test method for in situ determination of denitrification rates in a nitrate-contaminated groundwater aquifer

Y. Kim*, J.H. Kim*, B.H. Son** and S.W. Oa**

*Department of Environmental Engineering, Korea University, 208 Seochang-Dong, Jochiwon, Choong-Nam 339-800, Korea
**Department of Civil and Environmental Engineering, Woosong University, Dong-Gu, Daejeon 300-718, Korea

Abstract: In this study a single-well, “push–pull” test method is adapted for determination of in situ 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. The method consists of the controlled injection of a prepared test solution (“push”) into an aquifer followed by the extraction of the test solution/ground water mixture (“pull”) from the same location. The injected test solution consists of ground water containing a nonreactive tracer and one or more biologically reactive solutes. Reaction rate coefficients are computed from the mass of reactant consumed and/or product formed. 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). 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 and consisted of injection of site ground water containing fumarate and nitrate. 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.

Keywords: In situ denitrification rate; push–pull test; fumarate; a nitrate-contaminated aquifer

Introduction

Nitrate is the most prevalent groundwater contaminant in freshwater aquifers. In widespread areas of Korea, 17% of all wells sampled contain nitrate in excess of 10 mg-N/L, while long-term trends indicate increasing nitrate concentrations in groundwater in all parts of the country.

To remediate nitrate-contaminated groundwater, biological denitrification has been known to be more cost-effective than other processes such as ion exchange (Fonseca et al., 2000; Smith et al., 2001). Denitrification, the microbial respiratory pathway in which NO$_3^-$ is successively degraded to NO$_2^-$, N$_2$O, and N$_2$, is the predominant mechanism for removal of fixed nitrogen from groundwater. 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 determination of in situ 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 (Istok et al. 1997; Haggerty et al. 1998; 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.

In our previous microcosm study, several potential growth substrates such as formate, acetate, propionate, lactate, ethanol, methanol, and hydrogen were screened to determine the effective substrate for stimulating denitrification activity. Fumarate was turned out to be the most effective substrate. In fumarate-fed microcosms, higher nitrate degradation rate and fewer amounts of nitrite and nitrous oxide accumulated were observed than the other microcosms tested. Thus, in this field test, fumarate was used as a carbon source and/or electron donor (CS/ED).

The objective of this study was to evaluate 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 rates of nitrate, nitrite, and nitrous oxide degradations.

**Methods**

**Site description**

Push–pull tests were performed in an experimental well field at Korea University in Korea. The aquifer at this site is contaminated with nitrate (~10 mg-N/L), and the groundwater is aerobic (~5 mg/L dissolved oxygen). The aquifer consists primarily of alluvial deposits, and is unconfined with a water table depth ranging from 180 cm to 350 cm below ground surface. The aquifer porosity and bulk density are 0.28 and 1.65 g/cm³, respectively. The regional hydraulic gradient is approximately 0.004 m/m, and the average groundwater (Darcy flux) velocity is approximately 0.65 m/d. Tests were conducted in a single monitoring well constructed of 6.5 cm polyvinyl chloride casing with a 4 m long screen.

**Field tests**

A single Transport Test, one Biostimulation Test, and four Activity Tests were conducted (Table 1). Transport Test was first conducted, and was followed by a Biostimulation period and a series of Activity Tests (Table 1). 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.
Table 1 Test solutions compositions used for field push–pull tests

<table>
<thead>
<tr>
<th>Test type</th>
<th>Injection volume (L)</th>
<th>Fumarate (mg/L)</th>
<th>$^3$Nitrate (mg/L)</th>
<th>Nitrite (mg/L)</th>
<th>Nitrous oxide (mg/L)</th>
<th>$^1$Oxygen (mg/L)</th>
<th>Br$^{-}$ (mg/L)</th>
<th>$t$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport test</td>
<td>400</td>
<td>50 ± 0.1</td>
<td>30 ± 0.4</td>
<td>$^3$NI</td>
<td>NI</td>
<td>$^4$NA</td>
<td>99 ± 0.7</td>
<td>5.4</td>
</tr>
<tr>
<td>Biostimulation period</td>
<td>1200</td>
<td>488 ± 4.7</td>
<td>30 ± 0.8</td>
<td>NI</td>
<td>NI</td>
<td>6.3 ± 0.7</td>
<td>88 ± 2.4</td>
<td>–</td>
</tr>
<tr>
<td>First nitrate activity test</td>
<td>400</td>
<td>451 ± 4.1</td>
<td>45 ± 1.9</td>
<td>NI</td>
<td>NI</td>
<td>6.5 ± 0.9</td>
<td>175 ± 1.6</td>
<td>7.9</td>
</tr>
<tr>
<td>Nitrite activity test</td>
<td>400</td>
<td>409 ± 5.3</td>
<td>Ni</td>
<td>15 ± 3.8</td>
<td>Ni</td>
<td>2.9 ± 0.3</td>
<td>129 ± 2.5</td>
<td>7.9</td>
</tr>
<tr>
<td>Second nitrate activity test</td>
<td>400</td>
<td>399 ± 1.7</td>
<td>30 ± 2.0</td>
<td>Ni</td>
<td>Ni</td>
<td>3.8 ± 1.5</td>
<td>112 ± 1.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Nitrous oxide activity test</td>
<td>400</td>
<td>464 ± 3.9</td>
<td>Ni</td>
<td>Ni</td>
<td>5.9 ± 0.6</td>
<td>1.9 ± 0.1</td>
<td>62 ± 0.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

1 The background average dissolved oxygen concentration was 6.0 mg/L.
2 The background average NO$_2$ (as N) concentration was 8 mg-N/L.
3 NI indicates not included.
4 Not analyzed.
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 (Table 1). 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 (NaNO₃, 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 (Table 1). 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 (first Nitrate Activity test, Nitrite Activity test, second Nitrate Activity test, and Nitrous oxide Activity test) were conducted to quantify in situ rates of nitrate, nitrite, and nitrous oxide degradation (Table 1). Care needs to be taken in comparing relative degradation rates of nitrite and nitrous oxide, since high nitrite level may result in toxic effect on cell activity. To limit the adverse effect of nitrite activity test on the estimation of nitrous degradation rate, the second nitrate activity test was performed after the nitrite activity test. Test solutions were prepared and injected as described above. After a ~7 h rest phase with no pumping, the test solution/groundwater 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.

Analysis

Nitrate and nitrite concentrations were quantified on a shimadzu 10A (Chiyoda-Ku, ToKyo, Japan) ion chromatograph (IC) equipped with a conductivity detector. A Shodex IC SI-90 4E column (Shodex, Showa Denko K.K., Tokyo, Japan) that utilized a regenerant containing H₂SO₄ and an eluent consisting of a mixture of Na₂CO₃ and NaHCO₃ was used. The aqueous samples were dispensed into 1.5-mL polypropylene flattop microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA), and filtered with a 0.2 μm filter paper. The supernatant (250 μL) was then manually injected into the IC.

For nitrous oxide and carbon dioxide analysis, a Hayesep Q 80/100 (Alltech Associates Inc., Deerfield, IL) attached to the thermoconductivity detector (TCD) was used. The following GC operating conditions were used: injector temperature, 120°C; detector temperature, 120°C; carrier gas, helium; carrier gas flow rate, 20 mL/min; initial oven temperature, 40°C for 1 min; 10°C/min up to 220°C. The methods were
calibrated using external standards. A 3-mL of aqueous sample was taken from a VOA vial to create a headspace, and the VOA vial was then placed in a 20°C incubator in order to equilibrate temperature. A 500-μL headspace gas sample was taken with a 1001-gastight syringe (Hamilton, Reno, NV), and manually introduced into the GC. The total mass of nitrous oxide and carbon dioxide in the VOA vial was determined by mass balances assuming Henry’s law equilibrium partitioning. Henry’s law constant values for nitrous oxide and carbon dioxide were reported by Lide and Frederikse (1995).

Fumarate in aqueous samples was determined using high performance liquid chromatography (HPLC). A Shimadzu 10A HPLC (Shimadzu, Chiyoda-Ku, Tokyo, Japan) equipped with an UV absorbance detector operated at 210 nm. Separation of acids was made using an IC Sep 87H column (300 × 7.8 mm) and 0.008 NH₂SO₄ (pH 3.0) as an eluent at the flow rate of 1 mL/min.

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

\[ C^+ = \frac{1}{2} \left( \frac{C - C_{BG}}{C_o - C_{BG}} \right) \]  

where C is a measured concentration in an extraction sample, C₀ 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):

\[ r = \frac{M_{inj}}{V_{inj}} - \frac{M_{ext}/R_{tracer}}{t^*} \]  

where M_{inj} is total mass of solute injected, M_{ext} is the total mass of the solute extracted or produced in the aquifer and 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).

Results and discussion

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 (Tables 1 and 2).

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: first nitrate activity test,
nitrite activity test, second nitrate activity test, and nitrous oxide activity test (Table 1). The mean residence time for the activity tests were similar to the transport test.

During the first 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

![Figure 1](https://iwaponline.com/wst/article-pdf/52/8/77/434459/77.pdf)

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

Table 2 Summary of mass balance and rate calculations

<table>
<thead>
<tr>
<th>Test type</th>
<th>Quantities</th>
<th>Fumarate (mmol)</th>
<th>Nitrate-N (mmol)</th>
<th>Nitrite-N (mmol)</th>
<th>Nitrous Oxide-N (mmol)</th>
<th>Bromide (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport test</td>
<td>mass injected (mmol) 175</td>
<td>867</td>
<td>0</td>
<td>0</td>
<td>494</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mass extracted (mmol) 158</td>
<td>1125</td>
<td>0</td>
<td>0</td>
<td>445</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% recovery</td>
<td>90</td>
<td>130</td>
<td>–</td>
<td>–</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>rate (mmol/L/hr)</td>
<td>0.36</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>First nitrate</td>
<td>mass injected (mmol) 1582</td>
<td>1281</td>
<td>0</td>
<td>0</td>
<td>878</td>
<td></td>
</tr>
<tr>
<td>activity test</td>
<td>mass extracted (mmol) 436</td>
<td>125</td>
<td>229</td>
<td>0</td>
<td>916</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% recovery</td>
<td>28</td>
<td>9.8</td>
<td>–</td>
<td>–</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>rate (mmol/L/hr)</td>
<td>0.36</td>
<td>0.37</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrite activity</td>
<td>mass injected (mmol) 1535</td>
<td>0</td>
<td>433</td>
<td>0</td>
<td>648</td>
<td></td>
</tr>
<tr>
<td>test</td>
<td>mass extracted (mmol) 225</td>
<td>0</td>
<td>6.3</td>
<td>0</td>
<td>626</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% recovery</td>
<td>15</td>
<td>–</td>
<td>1.4</td>
<td>–</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>rate (mmol/L/hr)</td>
<td>0.48</td>
<td>–</td>
<td>0.14</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Second nitrate</td>
<td>mass injected (mmol) 1400</td>
<td>869</td>
<td>0</td>
<td>0</td>
<td>560</td>
<td></td>
</tr>
<tr>
<td>activity test</td>
<td>mass extracted (mmol) 310</td>
<td>153</td>
<td>209</td>
<td>0</td>
<td>604</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% recovery</td>
<td>22</td>
<td>18</td>
<td>–</td>
<td>–</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>rate (mmol/L/hr)</td>
<td>0.34</td>
<td>0.22</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>mass injected (mmol) 1626</td>
<td>–</td>
<td>–</td>
<td>34</td>
<td>311</td>
<td></td>
</tr>
<tr>
<td>activity test</td>
<td>mass extracted (mmol) 1087</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>307</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% recovery</td>
<td>67</td>
<td>–</td>
<td>15</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rate (mmol/L/hr)</td>
<td>0.73</td>
<td>–</td>
<td>0.04</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

1When bromide recovery is greater than 100%, a value of Rtracer in an equation 2 is assumed as 1.00.
2Nitrate recovery was greater than 100% due to nitrate in the site groundwater.
3Rates for nitrate, nitrite, and nitrous oxide degradation have a unit of mmol N/L/hr.
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 (Table 2). 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.

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 (Fig. 3A), however, no nitrous oxide and nitrate was detected during the extraction phase (Fig. 3B), 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 first 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 second nitrate activity test was performed after the nitrite activity test. The rate of nitrate degradation was approximately 60% of that computed in the first nitrate activity test, while fumarate degradation rate was very similar. Much less amount of CO₂ was

**Figure 2** Extraction phase normalized concentrations of bromide, nitrate, and fumarate (A) and concentrations of nitrite and carbon dioxide (B) during the first nitrate Activity Test.
produced compared to the previous nitrate activity test. These results confirm that nitrite may be adversely effect on denitrification activity of the stimulated microorganisms.

The nitrous oxide activity test was conducted in the absence of added nitrate or nitrite. Although 400-L test solution containing 3.7 mg-N/L of nitrous oxide was injected into the aquifer, some nitrous oxide was detected during the extraction phase. These results suggest that all nitrous oxide injected rapidly be degraded. The rate of fumarate degradation was a factor of 2 greater than that of nitrate degradation, while much less production of CO₂ was observed (Figures 2 and 4). Computed value of the ratio (nitrous oxide degradation rate)/(fumarate degradation rate) was 0.05 that is a factor of 26 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.

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 nitrate-contaminated aquifers. 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.
Acknowledgements
The research was supported by a research grant from Korea Ministry of Environment (2003-2004). This article has not been reviewed by the agency, and no official endorsement should be inferred.

References


Figure 4 Extraction phase normalized concentrations of bromide, nitrous oxide, and fumarate (A) and concentrations of nitrate, nitrite and carbon dioxide (B) during nitrous oxide activity test.
