Modelling bioremediation of nitrate-contaminated waste and groundwater

Matthew L. Duthy

Bioremediation of an aquifer, through the injection of labile carbon and subsequent induced denitrification, is one method of effecting a cleanup of nitrate-polluted groundwater. A one-dimensional computer model that incorporates a detailed description of nitrate biodegradation kinetics has been developed. The program is a first step towards a final model that will enable the transport and fate of a groundwater nitrate plume and the effect of aquifer bioremediation strategies to be usefully predicted. The model considers three microbially mediated reactions involving nitrate (nitrification, deoxygenation and denitrification); the transport and reactions of ammonium, nitrate, oxygen and ethanol; and the growth of nitrifying and denitrifying bacteria. Model simulations of two separate laboratory soil column denitrification experiments were able to reproduce the observed transient concentration distributions of nitrate and ethanol. Some considerations for applying a bioremediation model to the field are briefly mentioned.

Introduction

The contamination of aquifers by nitrate derived from point sources of pollution is a significant problem in Europe and Northern America, and has also been observed in parts of Australia, such as near the town of Mount Gambier in the southeast of South Australia (Lawrence, 1983; Dillon & others, 1991). Prolonged consumption of drinking water containing elevated nitrate levels is associated with a number of human health problems. Although the prevention of nitrate pollution of groundwater used for drinking is the best way of avoiding potential problems, there will still be instances where pollution has already occurred, sometimes for a prolonged period of time, or else is not easily prevented. In these instances, some form of treatment of the groundwater is necessary.

The so-called ‘in situ bioremediation’ method for effecting a cleanup of nitrate polluted groundwater involves the introduction of a labile carbon source (and possibly certain nutrients) into the aquifer through one or more injection wells. The carbon, once mixed with the nitrate contaminated groundwater, stimulates the growth of bacteria in situ, which consume both the carbon and the nitrate by the denitrification reaction, with inorganic carbon (i.e. CO$_2$) and gaseous nitrogen products (N$_2$, N$_2$O) resulting. The nitrate-free water may then be abstracted and subjected to further above-ground treatment, such as aeration and chlorination before use. The advantage of in situ bioremediation over other conventional nitrate treatment methods is that no costly or technically advanced treatment works are required, and no undesirable waste is produced if care is taken, since the entire remediation process occurs within the zone of pollution.

In order to design bioremediation strategies for a nitrate pollutant plume, a computer model is needed to simulate the transport and fate of the plume and the effects of bioremediation. The model should account for the solute transport of nitrate and related species, the relevant reaction processes for all modelled species and the growth of the mediating bacteria. Such a model would act as a valuable predictive tool and would provide a rational basis for managerial decision making concerned with a particular contaminated site. The initial development of such a model in one dimension, and its testing on selected laboratory column experiments, is the subject of this paper.

\[ \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} + S \]  

\( (1) \)

where

- \( C = C(x,t) \) = solute concentration (ML$^{-3}$)
- \( D = D(t) \) = dispersion coefficient (LT$^{-1}$)
- \( v = v(t) \) = average porewater velocity (LT$^{-1}$)
- \( S = S(x,t) \) = general reaction source/sink term (ML$^{-3}$T$^{-1}$)
- \( x = \) Cartesian coordinate (L)
- \( t = \) time (T).

The initial and boundary conditions used when applying...
the model to laboratory soil column experiments are:

\[ C = C_0 \text{ for } t = 0, \quad 0 \leq x \leq L \]  
\[ -D \frac{\partial C}{\partial x} + v C = v C_{in} \text{ for } t > 0, \quad x = 0 \]  
\[ \frac{\partial C}{\partial t} = 0 \text{ for } t > 0, \quad x = L \]  

where

\[ C_0 = C_0(x) = \text{initial concentration of solute (ML}^{-2}\text{)} \]  
\[ C_{in} = C_{in}(t) = \text{inlet concentration of solute (ML}^{-2}\text{)} \]  
\[ L = \text{length of column (L)} \]  

The finite difference scheme of Stone & Brian (1963) is used to implement Equation (1), together with Equations (2) to (4).

Reactions

Three key microbially mediated reactions were identified as being important to a description of a nitrate plume in groundwater. Each of these reactions involves the oxidation of one species by the bacteria in order to provide energy for maintenance and growth of the bacterial population. Another solute species is consequently reduced. In addition, the bacteria also bring about an assimilation reaction in which nutrients (such as carbon, oxygen and nitrate) are taken up by the bacteria in order to produce new cellular material.

Aquifers may be polluted either by nitrate directly, or by nitrate forming from other forms of nitrogen within the aquifer. Usually, the aquifer becomes contaminated when a reduced form of nitrogen, such as organic nitrogen and/or ammonium, is applied to the soil profile. The nitrogen source is then oxidized to nitrate, as it leaches to the water table. The nitrate formation reaction from reduced forms of nitrogen is termed nitrification. The nitrification reaction was incorporated into the model, because a description of nitrate plume formation was thought to be as pertinent to a bioremediation model as a description of nitrate plume removal. Nitrification is a two-step process in which ammonium is oxidised to nitrite and then to nitrate, at the expense of molecular oxygen. Each step of the reaction is mediated by a different genus of autotrophic bacteria, e.g. *Nitrosomonas* and *Nitrobacter*, though normally the two bacterial types are found to coexist. Since the second step is faster than the first, nitrite does not normally accumulate and is commonly ignored in modelling. A balanced stoichiometric equation that combines the nitrification energy reaction with the nutrient assimilation reaction is given below (derived from Equations 5 and 6 of McCarty & Haug, 1971):

\[ \text{NH}_4^+ + 1.856\text{O}_2 + 0.103\text{CO}_2 = 0.021\text{C}_3\text{H}_7\text{O}_2\text{N} + 0.979\text{N}_3 + 0.938\text{H}_2\text{O} + 1.979\text{H}^+ \]  

where \( \text{C}_3\text{H}_7\text{O}_2\text{N} \) is an empirical representation for bacterial cells.

The microbially mediated nitrate reduction reaction is termed denitrification. The reduction proceeds via a number of intermediate nitrogen compounds, most notably nitrite, and is most commonly brought about by heterotrophic bacteria. The carbon source, which acts as the electron donor for the reaction, is oxidised to carbon dioxide. Ethanol was the carbon source used for both the model development and the experimental program. This substrate was selected because of its proven lability, ready availability, high solubility, and moderate cost. The ability of different nitrogen reducing bacteria to denitrify varies, with not all species able to complete all steps of the nitrate reduction. However, a naturally occurring heterogeneous population would normally be able to fully convert nitrate to nitrogen gas. The buildup of nitrite concentrations has not been considered in the model development, even though nitrite is actually more harmful than nitrate. McCarty & others (1969) conducted batch denitrification experiments using a range of carbon sources, including ethanol. They found that for an initial \( \text{NO}_3^-\text{N} \) concentration of 25 mg/L, a transient peak of 10 mg/L \( \text{NO}_2^-\text{N} \) resulted after 11 days. However, after 40 days both the \( \text{NO}_3^-\text{N} \) and \( \text{NO}_2^-\text{N} \) concentrations were zero. This indicates that as long as enough ethanol is supplied to stoichiometrically convert all of the nitrate to nitrogen gas, the presence of non-zero nitrite concentrations will only be a transient phenomenon.

The denitrifying bacteria are also facultative anaerobes, meaning that any oxygen present will be used by the bacteria in preference to nitrate as the electron acceptor in the bacteria’s respiratory process. A certain amount of carbon source will also be consumed as the electron donor in this reaction before any denitrification can begin. Therefore, it is also necessary to consider this oxygen consumption reaction as well as the denitrification reaction in a model of nitrate bioremediation. The combined (energy plus synthesis) equations for the oxygen consumption and denitrification processes are, respectively,

\[ 0.5\text{C}_3\text{H}_7\text{OH} + \text{O}_2 + 0.071\text{NO}_3^- + 0.071\text{H}^+ \Rightarrow 0.071\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.643\text{CO}_2 + 1.286\text{H}_2\text{O} \]  
\[ 0.583\text{C}_3\text{H}_7\text{OH} + \text{NO}_3^- + \text{H}^+ \Rightarrow 0.087\text{C}_2\text{H}_5\text{O}_2\text{N} + 0.732\text{CO}_2 + 0.457\text{N}_2 + 1.946\text{H}_2\text{O} \]  

where \( \text{C}_3\text{H}_7\text{OH} \) (ethanol) has been selected to represent the labile carbon source. Equations (6) and (7) were developed by using the concept of the “consumptive ratio” as set out in McCarty & others (1969), together with the appropriate half equations for oxidation, reduction and cell synthesis. The model considers the three reactions given by (5), (6) and (7), and four solute species: ammonium \( \text{NH}_4^+ \), nitrate \( \text{NO}_3^- \), oxygen \( \text{O}_2 \), and ethanol \( \text{C}_3\text{H}_7\text{OH} \). The nitrifying bacteria, \( \text{Xaut} \), and the deoxygenating/denitrifying bacteria, \( \text{Xhet} \), are also modelled, making the total number of modelled species equal to six.

Reaction kinetics

A model of reaction kinetics is necessary to simulate the time dependent changes in reactant and product concentration levels. A literature review of models of nitrogen biodegradation kinetics shows that the most common approach has been to adopt a simple power rate model, usually zero-order or first-order kinetics. The values of the rate constants are then determined by attempting to match the kinetic model simulations to the observed concentration distributions. However, the rate constants then merely become fitting parameters, and such a model would have little predictive capacity for experiments conducted under different conditions. Starr & others (1974) explained: "Many factors such as microbial growth kinetics, temperature, \( \text{pH} \), and the supply of oxygen and carbon as well as a
MODELLING BIOREMEDIATION OF NITRATE

The solution algorithm adopted follows that used by Kinzelbach and coworkers (Kinzelbach & others, 1989; Kinzelbach & Schäfer, 1989; Kinzelbach & others, 1991). An overall model flow diagram is given by Figure 1. The

Figure 1. Flow diagram for one dimensional. Reactive solute transport model.
basis of the solution algorithm is the splitting of the
reactive transport equations into a transport component and
a reaction component. The separate components are solved
for sequentially and the final concentrations so obtained are
equivalent to those that would result from solving the
single reactive transport equation. This is illustrated by the
following equations, in which Equation (14) is simply the
sum of Equations (12) and (13).

\[
\begin{align*}
0.5 \frac{\partial C}{\partial t} &= L(C) \\
0.5 \frac{\partial C}{\partial t} &= S \\
\frac{\partial C}{\partial t} &= L(C) + S
\end{align*}
\]

where

\[L(\cdot) = \text{linear transport operator} = D \frac{\partial^2}{\partial x^2} - v \frac{\partial}{\partial x}.
\]

Iteration between the two steps was found to improve the
solution convergence. This is done by incorporating
explicit reaction terms in the transport step that are
calculated from the final concentrations obtained from the
previous global iteration. These explicit terms are then
subtracted from the reaction step before an updated global
solution is calculated. The process continues until the
values of the final global concentrations converge.

Application of the transport step results in a set of linear
simultaneous equations that are solved by the very efficient
Thomas (1949) algorithm. The system of nonlinear equa•
tions resulting from the application of the reaction step is
solved iteratively by a multidimensional Newton-Raphson
method. An adaptive timestep is used to control numerical
oscillations and smearing, as well as to avoid zero
concentrations from being reached within a timestep.

One-dimensional experimentation

All aspects of the model, including each of the three
microbially mediated reactions given by Equations (5), (6)
and (7), were tested by a series of experiments in
zero-dimension (batch) and/or one-dimension (column).
For the present purposes, the results from two separate
denitrification column experiments only are presented.

Denitrification laboratory soil column experiments were
conducted to confirm that nitrate removal from a saturated
porous medium through the introduction of labile carbon
was feasible. The experiments also provided a means of
testing whether the denitrification component of the
one-dimensional reactive nitrate transport model that was
developed was able to adequately simulate actual observed
behaviour. The soil column was intended to act as a
simplified one-dimensional microcosm of an aquifer
system, since the work presented here was undertaken in
the context of development of a model that could be applied
to bioremediation of nitrate contaminated groundwater.

The complete set of model equations is simplified when the
denitrification reaction only is considered and ammonium,
oxogen and nitrifying bacteria are not modelled. The
remaining equations are:

\[
\begin{align*}
\frac{\partial NO_3}{\partial t} &= L(NO_3) - \left[ \frac{1}{Y_{NO_3 \text{hetan}}} \frac{\partial X_{\text{hetan}}}{\partial t} \right] \\
\frac{\partial C_{\text{org}}}{\partial t} &= L(C_{\text{org}}) + \frac{1}{Y_{C_{\text{org}} \text{hetan}}} \frac{\partial X_{\text{hetan}}}{\partial t} + X_{\text{use}} \frac{\partial X_{\text{dec}}}{\partial t} \\
\frac{\partial X_{\text{hetan}}}{\partial t} &= \frac{\partial X_{\text{hetan}}}{\partial t} - \frac{\partial X_{\text{hetdec}}}{\partial t} \\
\frac{\partial X_{\text{hetdec}}}{\partial t} &= \mu_{\text{max hetan}} \frac{K_{NO_3 \text{hetan}} + NO_3 \text{het}}{X_{\text{hetan}}} - \frac{X_{\text{hetan}} - c_{\text{org}}}{K_{C_{\text{org}} \text{hetan}} + C_{\text{org}} \text{het}} \\
\frac{\partial X_{\text{hetdec}}}{\partial t} &= \mu_{\text{dec}} X_{\text{het}}
\end{align*}
\]

where

\[NO_3 = \text{concentration of nitrate (ML}^{-3}\)]

\[C_{\text{org}} = \text{concentration of organic carbon (ML}^{-3}\)]

\[X_{\text{hetan}} = \text{concentration of heterotrophic bacteria (ML}^{-3}\)]

\[K_{\text{bug}} = \text{bacterial inhibition constant (ML}^{-3}\)]

\[X_{\text{use}} = \text{fraction of heterotrophic biomass reusable as}
\text{organic carbon (-)}
\]

\[\text{The experimental setup is illustrated diagrammatically in}
\text{Figure 2. The soil column was formed from a 50 cm long}
\text{segment of PVC pipe of internal diameter 8 cm. The}
\text{column was packed with a uniformly fine-grained (0.1 mm)
clean mineral sand to a bulk density of approximately}
\text{1.6 g/cm}^3 \text{and porosity 0.4. The pipe was sealed by plate}
\text{chambers at either end.}
\]
The soil column was orientated vertically and saturated with a solution representing a mineral salts medium minus any organic carbon. The composition of the medium was adapted from that of Bowman & Focht (1974) and contained 499 mg/L NO$_3$-N. Such a high level of nitrate-nitrogen is unlikely to be present in nitrate polluted groundwater, but was used in the column experiments so that the effect of prolonged microbial growth and uptake upon reactant concentrations would be clearly defined over a longer period of time. The absolute effects of analytical and experimental errors upon concentration levels was also minimised by using elevated reactant concentrations. The mineral salts medium was contained in a mariotte bottle arrangement and was maintained in an anaerobic state by bubbling nitrogen gas through it. The solution was applied to the base of the column by a peristaltic pump that maintained a relatively constant flow rate. Pumping continued until a uniform distribution of nitrate was present along the column. At this point, the pump was turned off and the column lines clamped. A fixed volume of denitrifying bacteria solution of known concentration was injected by needle into each of 13 rubber sealed sampling ports spaced at 4 cm intervals along the column, in order to produce a uniform initial bacterial condition. The bacteria were allowed to attach to the sand particles overnight before pumping once again resumed. However, this time the solution applied also contained 1600 mg/L ethanol.

The denitrifying bacteria used in the experiments were isolated from soil sampled from the grounds of the CSIRO Water Resources Division at Urrbrae, Adelaide. The bacteria were not identified and probably consisted of several types of nitrate reducers, since more than one species might be expected to grow on a combination of ethanol and nitrate. However, consideration should be given to denitrifier species identification for any field bioremediation unless the denitrified water is then chlorinated, in order to avoid the possibility of the growth of pathogenic organisms.

Average porewater velocities for the experiments were generally 0.8–1.1 cm/hr, corresponding to a residence time within the column of approximately 1.9–2.6 days. Liquid sample sets were taken at regular intervals, which involved extracting 2 ml of liquid from the column by syringe and needle at each of the 13 sample ports at any one time. Solid samples were taken every second sampling period by removing the port Subaseals, sampling 1–2 cm$^3$ by push tube, replacing the void with clean sand and then rescaling the ports. All samples were frozen until assayed. Nitrate-nitrogen concentrations were obtained spectrophotometrically through the hydrazine reduction method of Kamphake & others (1967). This method in fact determines the sum of nitrate-nitrogen and nitrite-nitrogen concentrations. Ethanol concentrations were determined by gas chromatography.

The determination of bacterial concentration levels was attempted by using the method of ATP (adenosine triphosphate) measurement. The results of the assay were highly variable and this was probably due to the sampling regime adopted. Solid samples were taken for bacterial analysis, since it was expected that most cells would be attached to the solid grains (Dillon & others, 1991). However, only very small cored samples could be taken at the edges of the column; samples were therefore likely to have been non-representative and minus any microbial flocs in suspension. Therefore, no bacterial results are presented here.

**Denitrification Experiment A**

In this experiment, the denitrifier medium solution containing both nitrate and ethanol was applied to the soil column for the duration of the experiment after the period of bacterial incubation.

Figure 3 shows the distribution of simulated and observed nitrate-nitrogen and ethanol concentrations at an elapsed time of 48 hours. Note that the calculated initial conditions were $C(NO_3^-)$ = 499 mg/L, $C(CH_3OH)$ = 0 mg/L and $C(X_{het})$ = 0.4 mg/L. After 48 hours, the ethanol front had almost reached the end of the column. Denitrification had meanwhile caused the depletion further upstream of both the nitrate-nitrogen and the ethanol concentration levels.

![Figure 3. Denitrification experiment A. Simulated versus observed concentrations at an elapsed time of 48 hours.](image)

Allowing for differences in vertical scale, the correspondence between the shapes of the nitrate-nitrogen and ethanol curves suggests that their depletions were indeed linked. The reason for the depletions was the significant growth of the denitrifier population at and immediately downstream from the column inlet. Simulated bacterial concentrations only are shown since practical difficulties meant that accurate measured concentrations were not obtainable.

Figure 4 shows that at an elapsed time of 164 hours the bacterial concentration had increased tremendously at the two sample ports nearest the column inlet. This was confirmed visually when the column was subsequently dismantled and a heavy bacterial sludge was found in the base plate chamber. Any nitrate and almost all of the ethanol entering the column was immediately degraded by the bacteria. The depleted profile then continued to propagate downstream. The measured nitrate-nitrogen concentrations were zero downstream, whilst the measured downstream ethanol concentrations were relatively constant and averaged 120 mg/L. From Equation (7), the amount of residual ethanol that was expected can be calculated as 956 mg/L. The large amount of extra carbon was probably assimilated by the bacteria to form the numerous large microbial flocs that were observed by 164 hours to be passing out of the column suspended in the mineral salts medium.
The model simulations were able to closely reproduce the observed concentrations at both the intermediate time of 48 hours and at the final time of 164 hours. The largest discrepancy is that the observed ethanol front at 48 hours is somewhat ahead of the simulated front. This may be attributed to slightly non-uniform packing and/or saturation of the column, resulting in preferential flow channels developing and an apparent greater average pore water velocity.

Table 1 gives the values of all biochemical model parameters used in the simulations of Denitrification Experiment A. All parameters were taken from the literature, except the values of Xuse and Kbug which were assumed, and the values of \( \mu_{\text{max het an}} \) and \( X_0\text{het} \) which were fitted. The value of \( Y_{\text{corg het an}} \) was lower than the value of 0.37 calculated from Equation (7). This was done to reflect the higher than expected ethanol consumption observed during the experiment.

### Table 1. Values of biochemical model parameters used in denitrification experiments A and B.

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Experiment A</th>
<th>Experiment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_{\text{max het an}} )</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>( \mu_{\text{dec}} )</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>( X_0\text{het} )</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>( K\text{bug} )</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>( X\text{use} )</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>( K_{\text{het an}}^{\text{cor}} )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( K_{\text{het an}}^{\text{corg}} )</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>( Y_{\text{corg het an}} )</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>( Y_{\text{het an}}^{\text{corg}} )</td>
<td>0.24</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Table 2 gives the values of the transport parameters for Denitrification Experiment A. The values of \( v \) were back-calculated from the measured discharge rates of the column effluent. The values of \( D \) were then able to be calculated by using a value of longitudinal dispersivity determined from a separate column tracer test.

### Table 2. Values of transport model parameters. Used in denitrification experiments A and B.

<table>
<thead>
<tr>
<th>Transport parameter</th>
<th>Range for experiment A</th>
<th>Range for experiment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>( v )</td>
<td>0.83-0.93</td>
<td>0.91-1.17</td>
</tr>
<tr>
<td>( D )</td>
<td>0.17-0.19</td>
<td>0.19-0.25</td>
</tr>
</tbody>
</table>

**Denitrification Experiment B**

This experiment was the same as Denitrification Experiment A, but with one important difference. For Denitrification Experiment B, the application of ethanol after the short period of bacterial incubation was pulsed for the duration of the experiment, rather than continuous. For approximately one fifth of the time, the solution applied to the column contained 1600 mg/L ethanol; for the remaining time the solution contained no ethanol. The nitrate-nitrogen level was 499 mg/L at all times as for Denitrification Experiment A.
This indicates that denitrification activity was smaller than for Denitrification Experiment A, but was also decrease at those locations where the carbon peaks were present. The nitrate–nitrogen concentrations had also begun to decrease at those locations where the carbon peaks were present, but not to the extent of Denitrification Experiment A. This was because, as shown by the simulated distribution curve, the bacterial population increase was smaller than for Denitrification Experiment A, but was also much more evenly distributed along the column compared to the distribution shown in Figure 3.

Figure 6 shows the distributions at a time of 124 hours. The carbon at this stage had become almost completely degraded downstream from the column inlet (at x=38 cm). Nitrate–nitrogen levels had also significantly decreased at the locations of the carbon pulses. These locations are also the positions at which the bacterial numbers are highest (e.g. x=10 cm). This indicates that denitrification activity only occurs where there is carbon, nitrate and denitrifiers all present. It is apparent that a pulsed application of ethanol will avoid excessive bacterial numbers building up at any one location, with the subsequent risk of clogging of the porous medium. However, significant nitrate concentrations were still being propagated downstream between the carbon pulses. This was because the pulses used for Denitrification Experiment B were too far apart, and also were not concentrated enough in relation to the nitrate levels present. An average concentration of 956 mg/L ethanol is required to ensure complete denitrification of 499 mg/L nitrate-nitrogen according to Equation (7); the mean concentration of ethanol present for the pulsed experiment was only 320 mg/L.

As for Denitrification Experiment A, the observed ethanol and nitrate-nitrogen profiles are somewhat shifted forwards from the simulated profiles. It would therefore appear that the cross-sectional area of the column subject to flow was significantly less than expected, causing a lower effective porosity and higher pore velocity. By comparing the positions of the simulated and measured carbon peaks, the true porosity may be estimated as 0.30 compared to a calculated value of 0.39. Adjustment of the value of porosity for comparisons between model predictions and experimental observations may therefore be justified. Apart from this, there was again a very good agreement between simulated and observed concentrations for both nitrate–nitrogen and ethanol. As shown in Table 1, the values of the biochemical model parameters used for simulating Denitrification Experiment B were the same as for Denitrification Experiment A, except for minor differences in $\mu_{\text{max}}$, $X_0$ and $Y_{\text{org}}$. This indicates that the biochemical model parameters used for one experiment are applicable to those of other experiments that use the same bacterial type but operate under different biochemical experimental conditions. Table 2 shows that the flow in Denitrification Experiment B was only slightly greater than that in Denitrification Experiment A, so that transport processes did not significantly differ between the experiments.

**Conclusions**

Health risks are present when humans are exposed to nitrate-contaminated groundwater. Treatment of groundwater used for drinking purposes is required where pollution has already occurred or where the prevention of further pollution is not possible. One such method of treatment is *in situ* bioremediation, in which bacteria present within the aquifer are stimulated to consume the nitrate by denitrification once a labile carbon source is injected into the groundwater. To be able to both predict the transport and fate of a nitrate plume, and design bioremediation strategies for its removal, a reactive solute transport computer model of the nitrate system is required. The development and laboratory testing of such a model formed the subject of this paper.

**Table 3. Sensitivity analysis of selected biochemical parameters for denitrification experiment A.**

<table>
<thead>
<tr>
<th>$\mu_{\text{max}}$ (hr$^{-1}$)</th>
<th>$X_0$ (mg/L)</th>
<th>RMSSR</th>
<th>% Change from optimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.130</td>
<td>0.40</td>
<td>84.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.130</td>
<td>0.36</td>
<td>85.3</td>
<td>1.5</td>
</tr>
<tr>
<td>0.130</td>
<td>0.44</td>
<td>84.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.117</td>
<td>0.40</td>
<td>104.1</td>
<td>23.9</td>
</tr>
<tr>
<td>0.143</td>
<td>0.40</td>
<td>96.9</td>
<td>15.4</td>
</tr>
</tbody>
</table>

**Figure 5. Simulated versus observed concentrations at an elapsed time of 76 hours.**

**Figure 6. Denitrification experiment B. Simulated versus observed concentrations at an elapsed time of 124 hours.**
A one-dimensional model of reactive nitrate transport in saturated porous media has been developed. The model is capable of modelling three microbiologically mediated reactions associated with the presence of nitrate in groundwater (nitrification, deoxygenation and denitrification) as well as the transport and reactions of four solute species (ammonium $\text{NH}_4^+$, nitrate $\text{NO}_3^-$, oxygen $\text{O}_2$ and ethanol $\text{C}_2\text{H}_5\text{OH}$) and the growth and decay of two bacterial species (nitrifiers $\text{Xaut}$ and denitrifiers $\text{Xhet}$).

Although a two-dimensional model is required for most general field applications, it would be relatively straightforward to increase the dimensionality of the model. Instead, the focus of the model development has been on a detailed description of reaction kinetics. The reaction module that has been developed is superior to a simple zero-order or first-order rate constant module (as is commonly used for nitrate system soil column experiments), because it is able to mechanistically describe the processes of reactant consumption and product formation as a consequence of the growth of the mediating bacteria. The parameters of the model therefore have a real physical meaning in contrast to a zero-order or first-order rate constant which is effectively a fitting parameter only. The one-dimensional model as it stands, although developed in the context of groundwater bioremediation, may be applied to a description of a nitrate system in other liquid or porous media in either batch mode or subject to one dimensional flow. It therefore would have use in the modelling of nitrogenous wastewater treatment.

The model has been applied to a series of nitrification, deoxygenation and denitrification experiments in both zero and one-dimension. The application of the model to the simulation of denitrification in two separate one dimensional laboratory soil column experiments has been presented here. In each of the experiments, nitrate was denitrified by heterotrophic bacteria through the addition of ethanol. This indicates that bioremediation is a feasible process for removing nitrate from a saturated porous medium subject to flow. The simulated distributions of nitrate-nitrogen, ethanol and heterotrophic bacteria were compared to the measured values of nitrate-nitrogen and ethanol. No accurate measured values of bacterial concentrations were able to be obtained for the experiments. In all cases, the model was able to accurately reproduce the spatial and temporal changes in solute concentrations resulting from the transport and reaction processes.

In the first experiment, the ethanol was applied to the column continuously, whilst in the second experiment it was applied in the form of discrete pulses. A continuous application of ethanol resulted in the total removal of nitrate from the column, but gave rise to very high bacterial numbers near the column inlet. A pulsed application of ethanol resulted in a more even distribution of simulated bacterial numbers along the length of the column. This meant that column clogging was avoided, though non-zero nitrate levels continued to reach the column outlet between the pulses. The model was able to reproduce the observed concentration distributions of the modelled solute species for both experiments using very similar sets of biochemical model parameters.

The model therefore has predictive capacity. If incorporated into a two-dimensional coupled flow and solute transport model, it has the potential for simulating the bioremediation of nitrate-contaminated groundwater through injection of labile carbon. Such a package would form a useful consulting tool that has potential application to sites both in Australia and overseas.

However, there are a number of limitations in scaling up model performance from simulations of laboratory column experiments to a field application. The spatial domain of laboratory experiments is much smaller, and reactions typically occur under ideal conditions and at a much greater rate than they would in the field. Average porewater velocities are often greater, and dispersion coefficients smaller, in the laboratory than in the field. Thus, the spatial and temporal discretisations, and the coefficients in the solute transport equation, may all differ between the laboratory and the field. This has important implications for the numerical accuracy of a computer model.

In addition, a field situation is physically more complex than a laboratory experiment, since both large and small-scale heterogeneities are present. The reaction components of the model is also sensitive to the magnitude of the initial bacterial concentrations, the distribution of bacteria and particularly to the maximum specific bacterial growth rate. Therefore, the accuracy of field measurements of porosity, dispersivity and bacterial numbers, and laboratory measurements of other biochemical parameters, will also determine the usefulness of model predictions.

**Acknowledgements**

The author wishes to thank Dr Geoffrey D. Smith, Division of Biochemistry and Molecular Biology, Australian National University, and Mr S.G. Ferris, Agricultural Research Centre, Tamworth, for their review of this paper.

**References**


Monod, J., 1949 — The growth of bacterial cultures.
Nitrogen transformations during continuous leaching.
Proceedings of the Soil Science Society of America, 38,
283–289
of convective transport problems. Journal of the
American Institute of Chemical Engineers, 9, 5, 681–
688.
Thomas, L. H., 1949 — Elliptic problems in linear
difference equations over a network. Watson Scientific
Computing Laboratory, Columbia University, N.Y.