Integrated modeling of biogeochemical reactions and associated isotope fractionations at batch scale: A tool to monitor enhanced biodenitrification applications

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ABSTRACT

Enhanced in-situ biodenitrification (EIB) is a potential technology for remediating nitrate-polluted groundwater. EIB aims to create optimal biodenitrification conditions through the addition of carbon sources, enabling the autochthonous microbial community to degrade nitrate via different redox pathways. Biogeochemical numerical models are useful tools for predicting and designing such biodenitrification applications. Compound-specific stable isotope analysis (CSIA) is another valuable method for determining the degree of nitrate transformation. Therefore, incorporating isotope fractionation in biogeochemical models combines the two tools and is a key step in the development of reactive transport models of EIB under field conditions. In this work, we developed such an integrated model using the Phreeqc code and calibrated the model with batch scale experimental data using either ethanol or glucose as external carbon sources. The model included the following: microbiological processes —exogenous and endogenous nitrate respiration coupled to microbial growth and decay; geochemical processes —precipitation or dissolution of calcite; and isotopic fractionation —$\delta^{15}N-NO_3^-$, $\delta^{18}O-NO_3^-$, and $\delta^{13}C$-DIC, incorporating the full $\delta^{13}C$ isotope geochemistry involved in EIB. The modeled results fit well with the hydrochemical and isotopic experimental data. The model also incorporated nitrite accumulation observed during the glucose experiment. The biogeochemical model indicates that, depending on the added carbon source, calcite precipitates (using ethanol) or dissolves (using glucose). In both cases, changes in hydraulic conductivity can be induced for actual and longterm EIB applications. The incorporation of isotope fractionation in the model better enables to account for other natural attenuation processes, such as dilution and dispersion, in EIB applications at field scale. Both calibrated enrichment factors ($+8‰$ for ethanol and $+17‰$ for glucose) suggest that an inverse fractionation effect occurred (in which the heavy isotope reacts faster than the light isotope) during their oxidation.

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1. Introduction

The presence of nitrates in groundwater is a worldwide environmental problem (European Environment Agency, 2007; Organisation for Economic Co-operation and Development, 2008; Otero et al., 2009). Intensive farming and agricultural activities with high fertilizer application and animal waste disposal are the major causes of such pollution in rural areas (Foster, 2000; Otero et al., 2009). Wastewater disposal and poor maintenance of septic tanks and sewage systems (MacQuarrie and Sudicky, 2001) also cause nitrate pollution of groundwater after reduced nitrogen compounds are oxidized (Wakida and

Lerner, 2005; Otero et al., 2009). Excessive nitrates and nitrites consumed with drinking water (nitrate is converted to nitrite in the human body) increase the risk of methemoglobinemia and stomach cancer (Fan and Steinberg, 1996; Höring and Chapman, 2004). Consequently, maximum drinking water nitrate and nitrite concentrations have been established: 50 mg/l for nitrate and 0.1 mg/l for nitrite within the European Union; and 10 mg/l for nitrate (measured as nitrogen) and 1 mg/l for nitrite (measured as nitrogen) within the USA.

Biodenitrification is the reduction of nitrate to gaseous dinitrogen by anaerobic facultative bacteria that use nitrate as an electron acceptor and that are ubiquitous in surface water, soil and groundwater (Beauchamp et al., 1989). Denitrifying bacteria are generally heterotrophic and utilize organic matter as an electron donor (Reaction 1). A limited number of bacteria are also capable of carrying out autotrophic denitrification using electron donors such as reduced sulfur, dihydrogen

gas, ferrous iron and uranium (IV) (Straub et al., 1996; Zumft, 1997; Beller, 2005).

$$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O \qquad (Reaction 1)$$
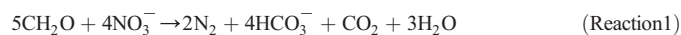
Aquifers vary widely in their denitrifying capacities, but biodenitrification appears to be possible anywhere bacteria thrive, electron donors are present and oxygen levels are low (Korom, 1992). Nevertheless, under natural aquifer conditions, a major limiting factor for biodenitrification to occur is the lack of electron donors to provide energy to heterotrophic microorganisms. To solve this problem, enhanced *in-situ* biodenitrification (EIB) creates optimized conditions through the addition of organic carbon sources and by controlling/monitoring other environmental parameters (*e.g.*, oxidant concentrations, pH, micro-nutrients). EIB is thus a potential technology for cleaning nitrate-polluted groundwater and achieving drinking water standards (Matějů et al., 1992; Khan and Spalding, 2004; Vidal-Gavilan et al., 2013). This *in situ* technology is particularly desirable because it offers a potentially safe and cost-effective remedy for nitrate-contaminated groundwater. Moreover, EIB disturbs the sites around single-household water supply wells minimally (Killingstad et al., 2002), making EIB more useful/desirable than pump and treat approaches (Smith et al., 2001). EIB is thus a suitable technology for increasing drinking water availability at the local scale.

For successful biodenitrification, the aquifer environmental parameters must be controlled. Denitrification is thermodynamically less favorable than the reduction of dissolved oxygen, and oxygen, therefore, will be preferred over nitrate as an electron acceptor. Denitrification will then occur when dissolved oxygen concentrations are less than 1–2 mg/l (Korom, 1992; Cey et al., 1999). Denitrifiers are not particularly pH sensitive, exhibiting an optimal pH range between 5.5 and 8.0 (Rust et al., 2000). Heterotrophic denitrifying bacteria obtain the energy needed for metabolism and growth from the oxidation of organic matter. Their metabolic requirements for nitrogen can be met through the direct assimilation of nitrate (Rittmann and McCarty, 2001; Rivett et al., 2008). They also require phosphorous, sulfur, and other micronutrients (such as B, Cu, Fe, Mn, Mo, Zn, and Co) for effective metabolism. Most groundwater contains these necessary elements at concentrations adequate for supporting microbial growth (Champ et al., 1979). However, phosphorus availability can be a limiting factor (Rivett et al., 2008); therefore, it is sometimes added as an external nutrient in EIB (Vidal-Gavilan et al., 2013).

Bioreduction of nitrate proceeds through different intermediate nitrogen compounds (Reaction 2). The nitrogen of nitrate is increasingly reduced, eventually arriving at dinitrogen gas. Notably, if optimal conditions do not exist (*e.g.*, inadequate supply of organic carbon or the presence of oxygen), intermediate nitrogen compounds such as nitrite can temporally accumulate. Nitrite is significantly more toxic than nitrate, and this toxicity is reflected in its considerably lower standard of 0.1 mg/l (Fan and Steinberg, 1996).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO_{(g)} \rightarrow N_2O_{(g)} \rightarrow N_{2(g)} \qquad (Reaction 2)$$
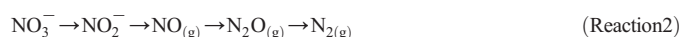
In this context, the development of biogeochemical models to reliably predict the rates of biodenitrification would be useful for designing EIBs and monitoring their performance. Several studies have evaluated biodenitrification at different scales using numerical models (Smith et al., 2001; Killingstad et al., 2002; Lehmann et al., 2003; Chen and MacQuarrie, 2004; Lee et al., 2006; Calderer et al., 2010; André et al., 2011; Mastrocicco et al., 2011). However, most of these studies (except Smith et al., 2001; Calderer et al., 2010; Mastrocicco et al., 2011) focused exclusively on naturally attenuating biodenitrification systems and not on enhanced ones. Furthermore, only a few studies examined the effects of geochemical interactions on biodenitrification within the aquifer matrices (Chen and MacQuarrie, 2004), and only a few explored the potential use of isotope fractionation for monitoring biodenitrification (Lehmann et al., 2003; Chen and MacQuarrie, 2004).

Geochemical interactions can occur between the biodenitrification reactants and the porous geologic medium in response to biodegradation reactions. These interactions may play a critical role in EIB implementation. Because of inorganic carbon production and pH alteration, carbonate mineral dissolution/precipitation is induced by heterotrophic biodenitrification.

Another important factor when monitoring EIB in the field is dilution resulting from nutrient injection, hydrodynamic dispersion, mixing, or other processes. Because of dilution, a decrease in nitrate concentration cannot always be attributed to degradation. Monitoring the changes in the nitrogen and oxygen isotope ratios of nitrate ($\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$) allows nitrate transformation and dilution to be distinguished (*e.g.*, van Breukelen, 2007). As NO_3^- is consumed, the residual NO_3^- becomes enriched in the heavy isotopes ^{15}N and ^{18}O, and the denitrification reaction follows a Rayleigh distillation process (Eq. (1)):

$$R_s = R_{s,0}\, f^{(\alpha-1)} \qquad (1)$$

where R_s is the stable isotope ratio of the fraction of molecules, f, remaining at time t; $R_{s,0}$ is the initial isotopic composition of the molecule; and α is the kinetic isotopic fractionation factor of the transformation process, which is often represented as the kinetic isotopic enrichment factor ε (in permil, ‰), where $\varepsilon = (\alpha - 1)$. Furthermore, monitoring nitrogen and oxygen isotopes may also allow for assessing the origin of nitrate pollution as different sources have different isotopic compositions (Kendall et al., 2007).

The inclusion of dissolved inorganic carbon isotopes ($\delta^{13}C\text{-}DIC$), which are involved in both direct (oxidation of organic carbon) and indirect (carbonate mineral interaction) processes of enhanced biodenitrification, in the biogeochemical model is expected to better allow the evaluation of the overall model consistency due to the central role of $\delta^{13}C\text{-}DIC$ in the reaction network.

To control the direct and indirect processes that are associated with enhanced biodenitrification, complete models that consider all interactions (*i.e.*, microbiological, geochemical, and isotopic) need to be developed. In this paper, we developed an integrated model for enhanced biodenitrification. We calibrated and validated the model with batch scale experimental data obtained from Vidal-Gavilan et al. (2013), who used either ethanol or glucose as external carbon sources. This biogeochemical model represents a key step towards simulating EIB at the field scale as a core of reactive transport models (RTMs). Our model considered: 1) microbiological processes —exogenous and endogenous respiration; 2) geochemical processes —calcite precipitation and dissolution; and 3) isotopic fractionation —$\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$, and $\delta^{13}C\text{-}DIC$, while considering full $\delta^{13}C$ isotope geochemistry. To our knowledge, our model is the first that simulates all isotopes of relevance in monitoring EIB.

2. Model construction

2.1. Model code

The Phreeqc-2 model code (Parkhurst and Appelo, 1999) was used to simulate the evolution of groundwater chemistry and isotopic composition during enhanced biodenitrification. The model was calibrated using the parameter estimation software PEST (Doherty, 2005). Previously, this approach has been successful in calibrating reactive transport models (van Breukelen et al., 2004; Karlsen et al., 2012). PEST adjusts model parameters until a fit between model outputs and data observations (in this study: nitrate, ethanol, biomass, calcium, DIC, $\delta^{13}C\text{-}DIC$) is obtained through the method of weighted least squares. Weights were applied as in Karlsen et al. (2012) and Matott and Rabideau (2008). These researchers used the inverse of the standard deviation, considering the concentration measurement errors as 5%. For $\delta^{13}C\text{-}DIC$, we considered the measurement errors to be 0.2‰. PEST uses a nonlinear