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Induced nitrate attenuation by glucose in groundwater: Flow-through experiment

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ABSTRACT

Endorheic basins are frequently exposed environments to nitrate (NO_3^-) pollution where groundwater may be the primary fresh water resource. The Pétrola basin (Central Spain) is an outstanding example of this type of basin that is affected by NO_3^- pollution where natural attenuation capacity observed in the field is limited. A three-stage flow-through experiment was developed to evaluate the viability of induced heterotrophic denitrification to remove NO_3^- using chemical, microbial and isotopic tools. The proposed biostimulation involves periodically injecting glucose to act as an electron donor to promote complete NO_3^- removal. The C/N ratio tested is nearly stoichiometric to avoid the generation of undesirable compounds such as NO_2^- or H_2S. Nitrate reduction was achieved after 13 days, along with transient NO_2^- accumulation that was observed until day 27. In addition to attenuating NO_3^-, the glucose injection also represses the dissimilatory nitrate reduction to ammonium (DNRA), reducing the NH_4^+ concentration in the outflow. Changes in the C/N ratio during the experiment reduced the amount of glucose discharged from the system. However, despite these changes, NO_3^- attenuation continued because secondary carbon sources (dissolved organic carbon in the input water or biomass) were present during the experiment and accounted for approximately 30% of the total attenuated NO_3^-. Isotopic characterisation of the sulphate (SO_4^{2-}) proved that the SO_4^{2-} reduction did not occur, even though carbon excess and low redox conditions were present. This is attributed to the lack of time for SO_4^{2-} reduction to occur inside the column. The N and O isotopic fractionation obtained during the induced attenuation were −8.8‰ and −8.0‰, respectively; these values were lower (in absolute values) than the fractionation from natural denitrification processes observed in the Pétrola basin. This variation was caused by differences in the experimental conditions that affected the denitrification rate. Overall, periodically injecting glucose might be a feasible method to remove NO_3^- from groundwater; a pilot-scale test should be performed to verify its applicability during long-term treatments in the field.

1. Introduction

Nitrate (NO_3^-) is one of the most common groundwater pollutants. Anthropogenic activities increase the NO_3^- concentration, reducing water quality. Frequently, the sources for the NO_3^- pollution in groundwater are linked to the extensive use of synthetic and organic fertilisers, inappropriate placement of animal waste, and spills from septic system effluents. High NO_3^- ingestion causes adverse health effects, such as methemoglobinemia, in infants and young children (Comly, 1945; Magee and Barnes, 1956) and may also promote cancer (Ward et al., 2005). Moreover, NO_3^- impacts the environment, contributing to the eutrophication of surface water bodies (Vitousek et al., 1997). The NO_3^- concentration threshold established by Directive 98/83/CE for human water supplies is 0.81 mM. This limit is exceeded by many aquifers worldwide because NO_3^- is highly mobile in groundwater and often persists in aquifers where the concentration of dissolved oxygen is over 0.06 mM and/or there are few electron donors available, such as labile organic carbon, sulphides and Fe(II)-bearing minerals (Korom, 1992). Consequently, Europe has proposed actions to reduce NO_3^- pollution (Directive 91/976/ECC). Of the different strategies, one of the most efficient treatments for removing NO_3^- involves enhanced biological denitrification within the aquifer using biodenitrification technologies (Tartakovsky et al., 2002; Khan and Spalding, 2004; Vidal-Gavilan et al., 2013; among others).

Denitrification is a redox reaction driven by autotrophic or heterotrophic bacteria that reduce NO_3^- to nitrogen gas (N_2) under suboxic conditions. Autotrophic bacteria promote denitrification using reduced sulphur compounds. Heterotrophic denitrification occurs through a number of sequential reactions where bacteria use organic matter as the electron donors for NO_3^- reduction. In both processes, NO_3^- is initially converted to nitrite (NO_2^-), which is more toxic than NO_3^- (De Beer et al., 1997). The maximum allowed NO_2^- concentration in drinking

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water is 0.01 mM (Directive 98/83/CE). The next reaction transforms NO_2^- into nitric oxide gas (NO), and NO is subsequently converted into nitrous oxide gas (N_2O); both species are greenhouse gases. Finally, N_2O is transformed into N_2. Usually, this reaction sequence is presented as a single reaction (Eqs. (1) and (2)).

$$5FeS_2 + 15NO_3^- + 5H_2O \rightarrow 10SO_4^{2-} + 7.5N_2 + 5FeOOH + 5H^+ \quad (1)$$

$$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O \quad (2)$$

Chemical data, when coupled with multi-isotopic studies of the solutes involved in the reactions, are an effective tool to identify and describe heterotrophic and autotrophic denitrification, as well as secondary processes, such as SO_4^{2-} reduction (Mariotti et al., 1988; Aravena and Robertson, 1998; Pauwels et al., 2000; Vitòria et al., 2008). Stable isotopes are commonly measured as the ratio between the heavier and the most-abundant isotope (lighter isotope), e.g., ^{15}N against ^{14}N. These ratios are established in accordance with international standards using delta notation (δ) (Eq. (3)).

$$\delta^{15}N = [(R_{sa} - R_{std})/R_{std}] \times 1000 \quad (3)$$

where $R = {}^{15}N/{}^{14}N$ in the sample (sa) and the standard (std).

In addition, the isotopic fractionation (ε) of the N and O in dissolved NO_3^- is essential for determining the rate of denitrification. During denitrification, while NO_3^- is consumed, any residual NO_3^- becomes enriched in the heavier isotopes (^{15}N and ^{18}O). This process can be expressed as a Rayleigh distillation process (Eqs. (4) and (5)) (Mariotti et al., 1988).

$$\delta^{15}N_{residual} = \delta^{15}N_{initial} + \varepsilon \ln f \quad (4)$$

$$\delta^{18}O_{residual} = \delta^{18}O_{initial} + \varepsilon \ln f \quad (5)$$

where f is the residual NO_3^- divided by the initial NO_3^- concentration and ε is the fractionation factor that depends on the aquifer's materials and media characteristics.

In natural systems, denitrification is predominantly restricted by the availability of electron donors (Knowles, 1982). To overcome this natural limitation, different field-scale treatments were tested to remove NO_3^- from both ground- and wastewaters by adding an external electron donor to promote denitrification with significant success (Tartakovsky et al., 2002; Istok et al., 2004; Leverenz et al., 2010; Borden et al., 2011; Vidal-Gavilan et al., 2013). From the different remedial strategies tested, biostimulation of heterotrophic denitrification has been commonly used because it is the most economical and easily performed technique. However, some issues must be taken into account during induced treatments to avoid increasing the toxicity of the treated water by generating undesirable compounds, such as NO_2^-, $N_2O(g)$ or hydrogen sulphide (H_2S). Furthermore, the processes that reduce NO_3^- beyond denitrification, such as the dissimilatory nitrate reduction to ammonium (DNRA or ammonification), should be avoided. DNRA is enacted by fermentative bacteria, reducing NO_3^- to NO_2^- before the final reduction to NH_4^+. Therefore, before any field treatment, exhaustive laboratory experiments must be performed to avoid these adverse effects. Consequently, in recent decades, several studies have introduced different carbon sources as electron donors and/or a specific bacterial strain to promote heterotrophic denitrification. Frequently tested electron donors included alcohols, sugars, or other organic compounds (Akunna et al., 1993; Lee and Welander, 1996; Aesoy et al., 1998; Gómez et al., 2000; Peng et al., 2007; Osaka et al., 2008; Martin et al., 2009; Fernández-Nava et al., 2010; Ge et al., 2012; Vidal-Gavilan et al., 2013; among others). Complex organic compounds, such as pine bark, compost or sawdust have also been studied (Schipper and Vojvodic, 2000; Trois et al., 2010). Few of the induced studies have utilised multi-isotopic characterisation to identify and describe the denitrification reactions (Delwiche and Steyn, 1970; Barford et al., 1999; Torrentó et al., 2011; Vidal-Gavilan et al., 2013).

Enhanced denitrification may be applied in areas affected by NO_3^- pollution where attenuation is absent or limited but environmental conditions in the natural system are met. Examples of areas exposed to NO_3^- pollution include the endorheic basins located in arid and semiarid regions. These basins are common in central Spain. The Pétrola basin is an excellent example of an endorheic system affected by NO_3^- pollution. Previous work performed in this basin has indicated that, although heterotrophic denitrification occurs naturally, it is limited; the NO_3^- attenuation ranges from 15% to 60% with an average value of approximately 20% (Carrey et al., 2013). To enhance the heterotrophic denitrification in the Pétrola basin, a biostimulation treatment is proposed. Before any field application, a detailed laboratory characterisation is required. Consequently, the present work sought to design an efficient strategy for inducing biostimulation. To simulate field conditions, the experiment was performed on a flow-through system. The selected carbon source was glucose which has been previously used as an electron donor in denitrification batch experiments (Akunna et al., 1993; Ge et al., 2012; Vidal-Gavilan et al., 2013). However, to the best of our knowledge, long-term denitrification experiments using glucose have not been assessed. The major goal of this experiment was to evaluate the viability of periodically injecting glucose to promote denitrification in ground water. Different C/N ratios were tested to achieve complete NO_3^- elimination while preventing the generation of undesirable compounds, such as NO_2^- or H_2S. The second goal of this study was to obtain the isotopic fractionation factor (ε) for N and O during the induced denitrification reaction to evaluate the efficiency of future field tests.

2. Methodology/methods

2.1. Experimental set-up

The experiment consisted of a glass cylindrical column (40 cm high, 9 cm inner diameter) filled with a homogeneous mix of sediment and clean silica (siliceous) sand (Panreac®) (Fig. 1). Before the biostimulation experiment, the system's natural denitrification potential was evaluated. The sediment had a limited capacity for inducing denitrification. This preliminary experiment lasted for one year, ending when the electron donors were exhausted (Carrey et al., 2013). After that interval but before the biostimulation experiment was set-up, the column was operated for 3 months at 0.1 mL/min and with constant NO_3^- input (0.88 mM) to verify that the sediment was no longer able to naturally undergo denitrification.

The experiment was developed in an anaerobic glove box filled predominantly with argon. The temperature ranged from 18 to 27 °C. Oxygen was removed once a day to maintain a partial pressure between 0.0% and 0.4%. The inflow and outflow rates were controlled with a peristaltic pump (Micropump Reglo Digital 4 channels ISMATEC). The flow

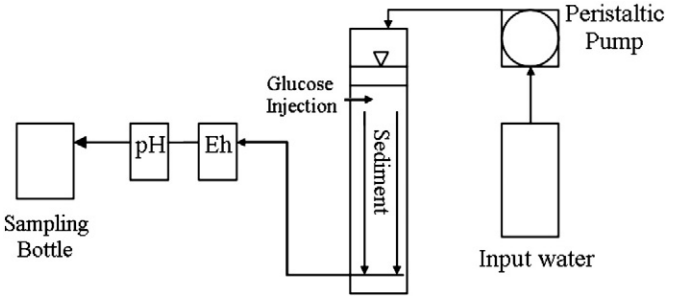

Fig. 1. Set-up of the column experiment. Glucose injection was performed in the top of the sediment. Water was sampled in agricultural well; NO_3^- concentration was 0.88 mM. Flow rate in the experiment was controlled by a peristaltic pump.