Induced nitrate attenuation using a wine industry by-product as electron donor

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Abstract

The feasibility of inducing nitrate attenuation using a by-product of the wine industry was tested at laboratory scale by means of a flow through experiment. Periodical injections of the wine industry by-product (mainly consisting on ethanol) were performed. The experiment lasted for 80 days and different C/N ratios were used. Results showed complete nitrate attenuation with transient nitrite accumulation, though nitrite was only detected in the first 12 days of sampling. The isotopic fractionation of nitrogen and oxygen of dissolved nitrate was determined. The ^{13}C isotopic compositions of the C source (ethanol), dissolved inorganic carbon, dissolved organic carbon and biomass were also determined. The evolution of the isotopic composition of organic C was modeled to study the role of biomass itself as an electron donor for denitrification.

Introduction

Nitrate (NO_3^-) is one of the most common groundwater pollutants. NO_3^- can cause health problems in humans and contributes to the eutrophication of surface water bodies. Frequent sources of NO_3^- pollution are linked to extensive application of synthetic and organic fertilizers, inappropriate placement of animal waste and spills from septic system effluents. Denitrification is an effective process that reduces NO_3^- to nitrogen gas (N_2) under anaerobic conditions. However, in natural systems denitrification is mainly restricted by the availability of electron donor (Knowles et al., 1982). To overcome this natural limitation biostimulation of heterotrophic denitrification by means of adding an external electron donor has been commonly used. Frequently tested electron donors included pure compounds such as alcohols (ethanol, methanol) or sugars (glucose, sucrose) among others (Akunna et al., 1993, Fernandez-Nava et al., 2010). During the last years, there is an increasing interest in alternative sources of organic carbon such as compost or sawdust (Trois et al., 2010). In this context, the use of industry by-products as electron donors provides economic and environmental benefits compared with pure compounds. The main goal of this work was to evaluate the feasibility of using a wine industry by-product (WIB) as organic C source to promote denitrification, preventing the generation of adverse compounds such as nitrite (NO_2^-) or hydrogen sulfide (H_2S). The secondary goal was to calculate the isotopic fractionation factor (ε) of N and O of dissolved nitrate and to study the C isotopic evolution of the organic carbon source during the induced denitrification reaction. The study was performed at laboratory scale by means of a flow-through experiment.

Materials and methods

The experiment design consisted on a glass cylindrical column (70 cm high, 9 cm inner diameter) filled with clean silica balls. The WIB, that had a dissolved organic carbon content (DOC) of 29 g/L, was injected every 4 days. Four different C/N ratios were tested. Initially (stage I, day 0 to 20), the C/N molar ratio used was about 7, over the stoichiometric value in order to stimulate

biomass growth. From day 20 to day 40 (stage II) and from day 40 to day 60 (stage III), the by-product addition was diminished to reach a C/N ratio of 2 and 0.6 respectively. Finally, from day 60 to day 80 (stage IV) no further injections were performed. Chemical (pH, NO_3^-, NO_2^-, NH_4^+, SO_4^{2-}, Cl^-, DOC, DIC, Ethanol) and multi-isotopic ($\delta^{15}N$, $\delta^{18}O$, $\delta^{13}C_{Eth}$, $\delta^{13}C_{DIC}$, $\delta^{13}C_{DOC}$, $\delta^{13}C_{Biom}$) analyses of the outflow water were performed periodically.

Results and discussion

Nitrate attenuation

After the first injection, NO_3^- concentration decreased rapidly. Complete NO_3^- attenuation was achieved after 5 days (2nd injection) showing that organic C from the WIB was easily degraded. An important NO_2^- accumulation was observed at the beginning of the experiment (up to 1.7 mM). The NO_2^- generation persisted in the outflow even after NO_3^- was completely removed. This lag in the NO_2^- reduction was produced because nitrite reductase is repressed when NO_3^- and NO_2^- are available (Strohm et al., 2007). After day 12 (3rd injection) NO_2^- was not observed in the outflow. Complete NO_3^- attenuation with no NO_2^- accumulation remained for stage II (C/N ratio was below the stoichiometric) and III (C/N ratio was below the stoichiometric). This implied that the biomass growing inside the column was used as a secondary organic carbon source. The use of biomass as organic C source was also relevant during stage IV. During this period, the consumption of the biomass as carbon source could not promote complete NO_3^- reduction, increasing nitrate concentration in the outflow up to 1.3 mM. However, after 24 days from the last injection, nitrate removal was still up to 0.5 mM of NO_3^- (almost 40% of the input NO_3^-).
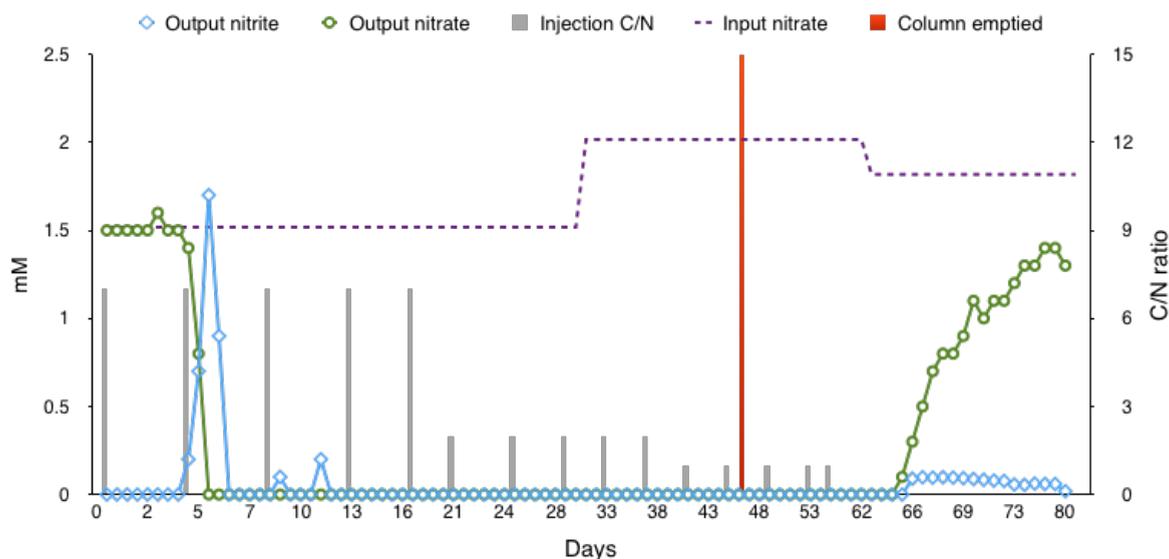

Fig. 1. Evolution of nitrate concentration (mM) in the input water (dashed line) and the outflow water, together with nitrite concentration in the outflow. The vertical grey lines represent the injections of the WIB using the different C/N rations (right y-axis). The red line represents the moment when the water of column was emptied.

The dissolved organic carbon (DOC) showed a cyclical behaviour with several increase-decrease trends due to the injection strategy. The amount of DOC in the outflow decreased as the C/N molar ratio decreased. However, even when C/N ratio was below stoichiometric values, DOC was still

detected in the outflow water. This excess of DOC may develop other redox reactions such as sulphate reduction. Nevertheless, the sulphate concentration in the outflow was similar to the input and, therefore, no sulphate reduction was produced during the experiment. However, at field-scale, the excess of DOC in the outflow is undesirable therefore an appropriate C/N ratio should be used in biostimulation treatments.

Isotopic results

During NO_3^- reduction the isotopic composition increased from +16.9‰ to +38.4‰ for $\delta^{15}N_{NO3}$, and from +27.5‰ to +49.9‰ for $\delta^{18}O_{NO3}$. The $\delta^{15}N_{NO3}$ showed a linear relationship with $\delta^{18}O_{NO3}$, with a slope of 1.04 (R^2=0.70), a common slope for denitrification reaction (Böttcher et al., 1990). The isotopic fractionation (ε) of nitrogen and oxygen, calculated using the Rayleigh equation (Mariotti et al., 1981) was -7.2‰ and -8.1‰ respectively. Results of the $\delta^{13}C_{Eth}$ ranged form -28.2‰ to -23.1‰. The $\delta^{13}C_{DIC}$ in the outflow varied form   -29.9‰ to -8.9‰, and the $\delta^{13}C_{DOC}$ in the outflow ranged between -26.8‰ to -20.4‰. In addition, the biofilm generated was isotopically analysed (8 samples) and the $\delta^{13}C_{Biom}$ varied from -25.8‰ to -28.8‰. The isotopic fractionation (ε) of C was determined in the ethanol of the outflow for the different C/N ratios. The shift in the isotopic fractionation of the outflow ethanol indicated the shift of the C source between the WIB and the biomass itself.

Conclusions

Periodical injections of a wine industry by-product may be suitable to remove NO_3^- from groundwater. This strategy was tested at laboratory-scale and achieved complete NO_3^- removal after 5 days. The complete NO_3^- attenuation remained even with a C/N ratio below the stoichiometric value. No sulphate reduction was detected during the experiment; however, the DOC available once NO_3^- was completely consumed may be high and should be controlled. The ε_N and ε_O of dissolved nitrate during denitrification was determined to. The study of the isotopic fractionation of the different C-species (ethanol, DOC, DIC, and biomass) confirmed the shift in the source of organic C used to reduce nitrate between the wine industry by-product and the biomass itself.

Acknowledgements

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