

## INSPIRATION bulletin

CL:AIRE's INSPIRATION bulletins describe practical aspects of research which have direct application to the management of contaminated soil or groundwater in an agricultural context. This bulletin describes a laboratory method to determine denitrification rates and identify different processes during nitrate reduction in aquifer sediments.

Copyright © CL:AIRE.

# Experimental quantification and kinetics of nitrate reduction potential by reduced species in soil samples obtained from sandy aquifers

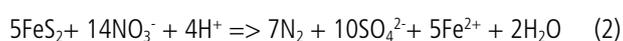
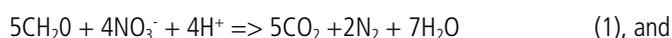
## 1. Introduction

Many areas of Germany are intensively used by agriculture and in recent decades nitrate contamination of groundwater has become a relevant environmental issue. Intense agricultural activity has led to increasing fertiliser utilisation and, as a consequence, nearly 30% of the shallow groundwater bodies in Germany have a nitrate concentration above the 50 mg/l threshold value set by the Drinking Water Directive 98/83/EC (BMU, 2012). High levels of nitrate in drinking water can be hazardous to human health and increase the risk of methaemoglobinemia, which prevents haemoglobin to bind and transport oxygen in infants. In principle denitrification processes in the subsurface can mitigate the impact of high nitrate inputs. In this context, the main aim of this work was to investigate nitrate reduction capacities (sulfide-/disulfide sulfur and organic carbon) and reactivities of different aquifer sediments using different batch experiments in the laboratory.

## 2. Denitrification

Denitrification is a microbiologically-driven process that contributes to nitrate attenuation (Rivett et. al., 2008), and can occur when the dissolved oxygen concentration falls below 1-2 mg/l (Korom, 1992). Most denitrifying bacteria are heterotrophic and consume organic matter as their energy source, whereas those which utilise inorganic compounds (e.g. reduced sulfur and iron) as their energy source are classified as autotrophic. Although there are still areas where nitrate reduction potential is available, with continuing input of nutrients, energy sources for bacteria can be depleted and denitrification potential can be significantly decreased.

Denitrification by organic matter and by reduced sulfur species (e.g. pyrite) can be written as:



respectively.

Although under anaerobic aquifer conditions, iron sulfide (pyrite) is expected to be the electron donor (Korom, 1992), the susceptibility of pyrite to oxidation depends on its microscopic structure; therefore not all pyrite in the aquifer sediment may be available for reaction (Kölle *et al.*, 1985). For instance, framboidal pyrite has a higher degradation capacity (for denitrification) than idiomorphic minerals due to its larger surface area (Houben *et al.*, 2017). Moreover, the pH (Torrentó *et al.*, 2010) and spatial distribution of pyrite (Jang *et al.*, 2017) can be also the controlling factors for autotrophic denitrification in an aquifer.

Similarly to autotrophic denitrification, nitrate removal by heterotrophic denitrification using organic carbon is also affected by many factors (eg. pH, chemical composition). It has been shown that nitrate reduction rates are most often affected by the dissolved or soluble organic carbon content of the subsurface rather than the total solid organic carbon content (Rivett et. al., 2008). The degradation rate is assumed to be higher including low-molecular weight compounds (eg. acetate) in the reactions since these were found to be biologically more reactive than the high-molecular weight compounds (Kaiser et. al., 2002).

Studies have shown that reactive solid phases that can be significant for denitrification are present especially in the soil fraction of <2 mm. In addition to the redox potential and the concentration of complexing agents, pH is an important factor for the release of heavy metals. If pH decreases, more heavy metals can be released (Fe, Mn, As, Ni, Cu, Cr) and increased concentrations can lead to further environmental problems.

## 3. Laboratory method

Soil samples were collected in a shallow aquifer in northern Germany down to a depth of 12 m. Denitrification potential was measured in batch experiments containing sediments with different concentrations of the above-mentioned electron donors, pyrite and organic carbon. A method was developed to achieve anaerobic conditions and completely exclude oxygen from the processes. With this step, oxidation of pyrite and organic carbon by oxygen was prevented, ensuring nitrate was the main electron acceptor.

# INSPIRATION bulletin

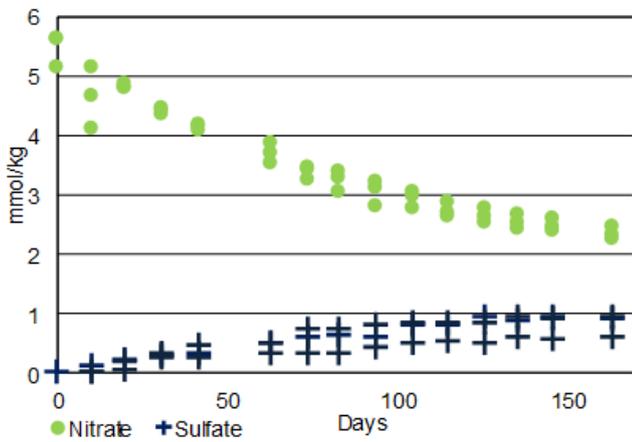


Figure 1: Nitrate reduction due to pyrite oxidation, with a corresponding release of sulfate (experiments were performed in triplicates).

Furthermore, an oxygen sensing system was used to monitor oxygen concentrations during the experiments. Water samples were taken periodically and the concentrations of major anions, cations and heavy metals were measured.

Degradation of nitrate could be observed in all experiments. Figure 1 is a typical example for substantial contribution of autotrophic denitrification to the overall denitrification, where pyrite oxidation coupled to nitrate reduction results in the release of both sulfate and heavy metals (data not shown). Nitrate degradation rates were estimated by linear regression approach (Fig. 2). For most samples there was a good correlation between the observed nitrate degradation rate and initial electron donor (pyrite, TOC) concentration.

A difference was found in the efficiency of different reactive phases. Based on the same mass of electron donors, degradation of nitrate by organic carbon is slower than by sulfides. However, due to the substantially higher organic carbon content typically found in the samples, a larger amount of nitrate can potentially be degraded using the organic carbon.

#### 4. SEM analysis

Sediment samples were examined using a scanning electron microscope (SEM) and in some cases a raspberry-shaped mineral, called framboidal pyrite was found (Fig. 3). Framboids are densely packed, spheroidal aggregates of microcrystals (Ohfuji and Rickard, 2005) and can be the dominant form of pyrite in anoxic environments.

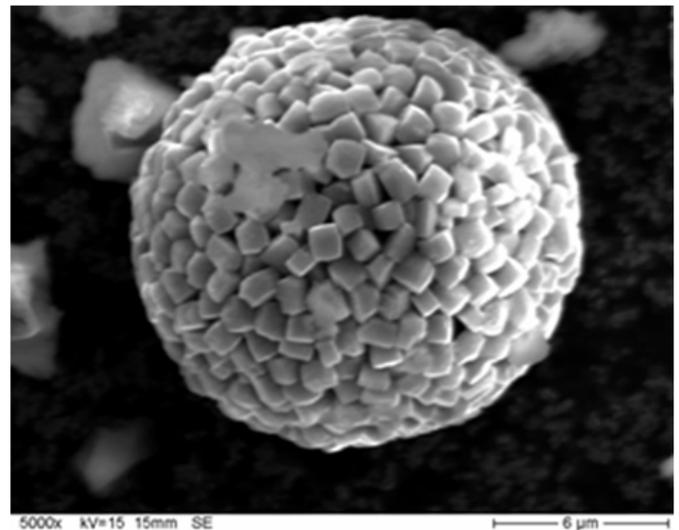


Figure 3: Framboidal pyrite observed under SEM.

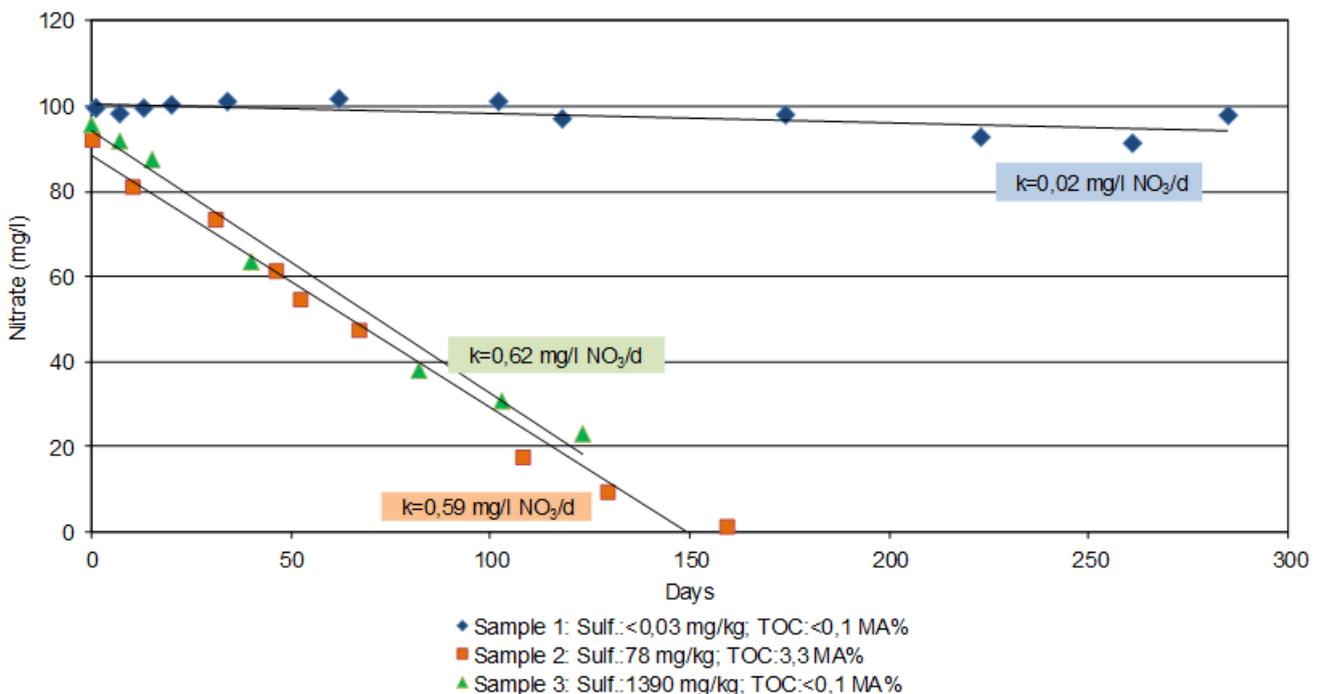


Figure 2: Nitrate concentration changes in samples in relation to electron donor concentration (k value: estimated degradation rate based on linear regression approach).

# INSPIRATION bulletin

## 5. Conclusion

The laboratory method developed in this study is suitable for the determination of denitrification rates and the identification of different processes during denitrification. It can be applied to deduce the role of denitrification by autotrophic and heterotrophic degradation of nitrate from different sources in groundwater. The results showed that there are reactive phases in the sediments (pyrite, organic carbon), but there is difference in their efficiency with respect to denitrification (Fig. 2).

Currently, isotopic analyses ( $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}-\text{NO}_3$ ) of groundwater samples are being performed to determine the isotopic composition during denitrification and quantify N and O isotopic enrichment factors. Besides this, synthesised framboidal pyrite is being used in ongoing experiments to check its reactivity.

## Acknowledgments

This research has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 675120 for the project entitled "Managing soil and groundwater impacts from agriculture for sustainable intensification – INSPIRATION".

## References

- BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) & BMELV (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz) 2012. Nitratbericht 2012. Gemeinsamer Bericht der Bundesministerien für Umwelt, Naturschutz und Reaktorsicherheit sowie für Ernährung, Landwirtschaft und Verbraucherschutz.
- Houben, G. J., Sitnikova, M. A., Post, V. E. 2017. Terrestrial sedimentary pyrites as a potential source of trace metal release to groundwater – A case study from the Emsland, Germany. *Applied Geochemistry*, 76, 99-111.
- Jang, E., He, W., Savoy, H., Dietrich P., Kolditz O., Rubin Y., Schüth C., Kalbacher T. 2017. Identifying the influential aquifer heterogeneity factor on nitrate reduction processes by numerical simulation, *Advances in Water Resources* 99, 38-52.
- Jørgensen, P.R., Urup, J., Helstrup, T., Jensen, M.B., Eiland, F., Vinther, F.P. 2004. Transport and reduction of nitrate in clayey till underneath forest and arable land. *J. Contam. Hydrol.* 73, 1-4. 207-226.
- Kaiser, K., Guggenberger, G., Haumaier, L., Zech, W. 2002. The composition of dissolved organic matter in forest soil solutions: changes induced by seasons and passage through the mineral soil. *Org. Geochem.* 33 (3), 307-318.
- Korom, S.F. 1992. Natural denitrification in the saturated zone: a review. *Water Resour. Res.* 28 (6), 1657-1668.
- Kölle, W. O., Strelbel, O., Böttcher, J. 1985. Formation of sulfate by microbial denitrification in a reducing aquifer, *Water Supply*. 3 (1). 35-40.
- Ohfuji, H., Rickard, D. 2005. Experimental syntheses of framboids - a review. *Earth-Science Reviews* 71. 147-170.
- Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.W.N., Bemment, C.D. 2008. Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. *Water Res.* 42. 4215-4232.

For more information on the INSPIRATION Project, please visit:  
[www.inspirationitn.co.uk](http://www.inspirationitn.co.uk)

If you would like further information about other CL:AIRE publications please contact us at the Help Desk at [www.claire.co.uk](http://www.claire.co.uk)