Nitrate consumption by the oxidation of sulfides during an enhanced natural attenuation project at a contaminated site in Berlin, Germany

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Abstract
Background: Organic pollutants at contaminated sites are often eliminated naturally by biological degradation. The redox processes responsible can be enhanced by infiltrating electron acceptors such as nitrate or sulfate into the aquifer. However, the addition of oxidative agents can lead to undesired side-effects in the saturated soil zone such as the consumption of nitrate by the oxidation of sulfides contained in the aquifer. Laboratory-scale 1D column experiments in up flow mode were performed to evaluate the potential consumption of nitrate and the related kinetics by the oxidation of sulfides during an enhanced natural attenuation project at a site contaminated with monoaromatic compounds and trimethylbenzene. Water containing nitrate was infiltrated into aquifer soil material containing sulfides. To study side reactions, experiments were conducted with low levels of organic hazardous compounds.

Results: The results indicate that sulfide was oxidized with the simultaneous formation of sulfate by nitrate-consuming processes. The degradation rate of sulfide was calculated to be 1.26 mg kg\(^{-1}\) per exchanged pore volume, corresponding to nitrate consumption of 8.5 mg kg\(^{-1}\) in the case of incomplete denitrification and 3.4 mg kg\(^{-1}\) in the case of complete denitrification.

Conclusion: The presence of sulfides contained in the soil leads to a nitrate-consuming redox reaction following a linear function in case of sufficient availability of nitrate. This information is helpful for planning ENA projects at contaminated sites to reduce the risk of under- or overdosing the electron acceptor nitrate, which may lead to a lack of nitrate needed to enhance the biodegradation of contaminants in the aquifer or to the deterioration of groundwater quality.

Keywords: Column experiments, Sulfide oxidation, Nitrate, Enhanced in situ bioremediation, Contaminated aquifer

Background
The remediation of sites contaminated with benzene, toluene, ethylbenzene, and xylene (BTEX) as well as trimethylbenzene (TMB)—such as former wood preservation facilities, gasworks, gasoline spill sites, etc.—is sometimes carried out using alternative methods based on natural processes [1]. In these cases, a process known as enhanced natural attenuation (ENA) is applied with or without the simultaneous addition of microorganisms [2–4]. One of the factors that may limit bioremediation is the availability of nitrogen and phosphorous [5]. Improving the metabolism of autochthonous microorganisms as well as the removal of contaminants can be significantly increased by the addition of electron acceptors [6, 7]. Due to its high water solubility and redox potential, nitrate is...
often used, which seems to be more effective than sulfate or ferric salt [8].

However, the natural nitrate-consuming process of chemolithotrophic denitrification may limit the availability of electron acceptors for enhanced bioremediation. This applies in particular to contaminated aquifers containing sulfides in the form of iron disulfide (FeS₂) or pyrite, the most abundant mineral on the Earth's surface [9].

The phenomenon of nitrate-consuming processes driven by sulfide oxidation has been known for decades and extensively described in the literature [10–17]. However, research has mostly focused on the desired reduction of nitrate input into the aquifer by these processes. The issue of the lack of nitrate, respectively, electron acceptors required for enhanced bioremediation due to consumption by redox reactions taking place simultaneously has not been addressed.

In anoxic groundwater environments, denitrifying microorganisms use the energy released by the oxidation of sulfides for their metabolic process [18]. The reduced sulfur from iron monosulfide and disulfide (S²⁻, S¹⁻) is used as an inorganic electron donor. Due to their properties, reduced sulfur compounds can act as denitrification hotspots in anoxic groundwater environments [19].

Finally, chemolithotrophic denitrification leads to the formation of either sulfur and sulfate or solely sulfate, depending on the sulfide–nitrate ratio [20, 21]. Released sulfate can serve as an electron acceptor in contaminated aquifers, supporting biodegradation in the contamination plume [22]. This effect has been observed for example at a gasworks site [16].

In addition, reduced Fe²⁺ is released in the process of denitrification and oxidized to Fe³⁺ by available NO₃⁻. This reaction is catalyzed by microorganisms [18, 23] or occurs abiotically [24]. Nitrite also appears to play an important role in the conversion of nitrate as an intermediate within the biodegradation pathway [25].

The best-known chemolithotrophic denitrifiers in groundwater are *Thiobacillus denitrificans* [10, 26] and *Thiomicrospira denitrificans* [20, 27]. In addition, there are microorganisms that catalyze elemental sulfur (S₀), thiosulfates (S₂O₃²⁻), and sulfites (SO₃²⁻) during denitrification [20, 28, 29].

The described process of chemolithotrophic denitrification may result in insufficient nitrate being available for bioremediation or chemoorganotrophic degradation, including the degradation of BTEX and TMB. Therefore, the correct dosage of nitrate is important during enhanced bioremediation measures, but difficult to calculate due to uncertainties about the degree and kinetics of nitrate consumption by the oxidation of sulfides. This is particularly important given the toxicity of nitrate, for its excessive infiltration could lead to permissible limits being exceeded. Under the European Nitrates Directive (91/676/EEC), drinking water in the European Union may not contain more than 50 mg L⁻¹ nitrates [30].

To correctly calculate nitrate consumption when preparing an ENA project involving the infiltration of nitrate as an electron acceptor at a site contaminated with BTEX and TMB, and with an aquifer containing sulfides, a better understanding of the mechanisms of this undesired reaction is necessary. For this reason, laboratory-scale 1D column experiments were performed while taking into account the particular hydrogeological and geochemical conditions at this site. Water containing nitrate was infiltrated into aquifer soil material containing sulfides. Experiments were conducted with various consortia of denitrifying microorganisms (chemotrophic and heterotrophic).

The main objective of the investigations was to establish how much nitrate is consumed in the presence of sulfides—and how fast. This study is intended to show the extent to which sulfate oxidation can contribute to nitrate consumption at contaminated sites where ENA schemes are carried out. To calculate a mass balance, we wanted to determine whether nitrate consumption was related to sulfate formation. Furthermore, the oxidation sulfides were to be analyzed with respect to nitrite as the intermediate. The findings obtained from this study ought to provide an indication of the potential consumption of nitrate in ENA schemes involving the oxidation of sulfides in the aquifer. However, since this was a site-specific case study, the aim was not to generalize the results.

**Materials and methods**

**Site description**

The contaminated site (75% of the total area of 70,000 m²), located north-west of Berlin, Germany, was the site of a tank farm for the storage of petroleum products (especially fuel derivatives) from 1920 to 1976. Additionally, a tar distillation facility had been operated there for the first few decades.

Investigations at the site revealed that the main contamination was localized in the upper aquifer, which was separated from the deeper, second aquifer by glacial till. A contamination plume (250 m long and 80 m wide) currently exists in the upper aquifer, which is characterized by quaternary sediments mainly composed of fine-to-medium sand (average hydraulic conductivity K 2 × 10⁻⁴ m s⁻¹). The depth to the water table is about 2–3 m and the thickness of the aquifer averages about 7–8 m. The groundwater has a temperature in the range of 10–12 °C, and flows with a low gradient (about 0.05%) and a velocity of 0.15 m d⁻¹ in a south-westerly direction. Soil and water in the vicinity of the
pollutant plume is contaminated with gasoline hydrocarbons originating from leaking underground storage tanks and surface spills. The main components are the BTEX compounds (benzene, toluene, ethyl benzene, and xylene isomers) and the three TMB isomers (1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene and 1,2,3-trimethylbenzene) (Table 1). Furthermore, polycyclic aromatic hydrocarbons (PAH) and alkyl phenols are detectable at significantly lower concentrations in the upper aquifer.

Anoxic conditions predominate in the vicinity of the contaminant plume. The levels of potential electron acceptors are in the range of 0.5–10 mg L$^{-1}$ for nitrate and 10–100 mg L$^{-1}$ for sulfate. Due to the reducing conditions, iron is only present in bivalent form, with concentrations ranging from 13 to 17.5 mg L$^{-1}$.

The electron acceptors nitrate and sulfate were infiltrated into the aquifer as part of an ENA project to boost the degradation of the pollutants. The successful anaerobic degradation of BTEX and TMB under nitrate and sulfate reducing conditions was confirmed in [31].

### Table 1

|          | Benzene | Toluene | Ethyl-benzene | m,p-Xylene | o-Xylene | 1,3,5-TMB | 1,2,4-TMB | 1,2,3-TMB | PAH    | Alkyl-phenols |
|----------|---------|---------|---------------|------------|----------|-----------|-----------|-----------|--------|--------------|
| GW       | 0.6/2.5 | 2.2/6.9 | 1.2/2.5       | 1.4/6.4    | 0.7/2.8  | 0.08/0.36 | 0.2/0.5   | 0.15/0.4  | 0.05/0.15 |              |
| Soil     | 13/28   | 21/64   | 7/25          | 70/210     | 30/90    | 50/120    | 50/125    | 45/130    | 3/63   | 2/74         |

GW groundwater

### Column experiment—setup

Two stainless steel columns (one biologically active, the other abiotic as a blank) with a length of 47 cm and an internal diameter of 7 cm were used for the experiment. The inflow and outflow pipes were also made of stainless steel (Fig. 1).

The columns were filled with sandy soil material with low pollutant loading (Table 2) taken by tube core drilling at the edge of the contaminant plume from a depth of 5–6 m. The soil material was homogenized and packed into the columns by excluding oxygen. Before the beginning of the actual experiment, the hydraulic properties of the soil material were determined by means of sieving analysis [32] and tracer tests [33] (Table 2).

Anaerobic groundwater with low contamination, and distilled anaerobic water concentrated with nitrate were infiltrated into the test column by means of piston pumps in up flow mode to prevent channeling and differential gravity flow. The residence time of the infiltrated water in the column was 3 days, corresponding to the average velocity of the groundwater at the site of 15.6 cm d$^{-1}$. 

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**Fig. 1** Experimental setup
Pressure equalization in the flask containing the nitrate solution was carried out by means of a bag filled with nitrogen. To remove residual oxygen from the gas bag and the pipes, the nitrogen was passed through a solution of 10 mg L\(^{-1}\) sodium dithionite via a silicone tube. The test columns were operated at a temperature of 10–12 °C, simulating the conditions in the aquifer.

Sampling to analyze aromatic hydrocarbon as well as nitrate, nitrite, and sulfate took place at the inflow (P0) and outflow of the columns. In order to prevent the BTEX and TMB outgassing in the outflow, the samples were collected in gas-tight bags.

### Column experiment—operating system

#### Conditioning phase (CP)

In the conditioning phase, anaerobic groundwater with low contamination taken from the edge of the contaminant plume was infiltrated without the addition of nitrate (Table 3). Specific stainless steel containers were used to transport large quantities of groundwater under strict anaerobic conditions from the site to the laboratory. The tanks were equipped with gas sampling bags to prevent the evaporation of volatile compounds. The tanks and gas bags were flushed with nitrogen to remove the oxygen before sampling. The water in the abiotic control column also contained sodium azide with a concentration of 1 g L\(^{-1}\), a common method to prevent biological activity [34].

Soil pore water associated with leaching BTEX and TMB from the soil material was exchanged until only low concentrations of these compounds were detectable in the outflow. This was expected to minimize the consumption of infiltrated nitrate by the biodegradation of BTEX and TMB in the following main experimental phase. Approximately 15 pore volumes were exchanged.

#### Main experimental phase (MEP)

Initially, oxidation of the bivalent iron contained in the anaerobic groundwater due to the addition of nitrate was observed. Therefore, distilled anaerobic water concentrated with nitrate (in the form of KNO\(_3\)) in a concentration of 300 mg L\(^{-1}\) was used instead of anaerobic groundwater in the columns during the following main experimental phase to prevent blockage due to the precipitation of iron. In addition, the macro-nutrient phosphate (in the form of Na\(_2\)HPO\(_4\)) and a trace element solution was dosed into the column at a concentration of 1 mg L\(^{-1}\) (Table 4) to provide ideal conditions for the microorganisms [35].

The components of the trace element solution were extended to the elements magnesium, tungsten, selenium, and aluminum.

The consumption of nitrate for the biodegradation of BTEX was avoided by infiltrating pollutant-free water. Furthermore, the oxidation of the bivalent iron contained in the anaerobic groundwater and the resulting precipitation were prevented.

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**Table 2** Hydraulic properties and of the soil material used

| Hydraulic properties | Biologically active column | Abiotic column | Pollutant loading | Biologically active column | Abiotic column |
|----------------------|----------------------------|----------------|-------------------|---------------------------|----------------|
| Bulk density (g cm\(^{-3}\)) | 1.65 | 1.62 | BTEX (mg kg\(^{-1}\)) | 1.7 | 1.9 |
| Hydraulic conductivity K (m s\(^{-1}\)) | 3 \times 10^{-4} | 5 \times 10^{-4} | TMB (mg kg\(^{-1}\)) | n.d | n.d |
| Longitudinal dispersivity \(a_L\) (m) | 0.009 | 0.0075 | PAH (mg kg\(^{-1}\)) | n.d | n.d |
| Hydraulic effective porosity \(n_e\) [-] | 0.34 | 0.345 | Alkyl-phenols (mg kg\(^{-1}\)) | 0.3 | 0.3 |
| Pore volume PV (cm\(^3\)) | 614.0 | 624.0 | TOC (mg kg\(^{-1}\)) | 4.1 | 4.2 |

n.d.: not detectable

**Table 3** Concentrations of the compounds in the infiltrated groundwater

| Benzene | Toluene | Ethyl-benzene | m,p-Xylene | o-Xylene | 1,3,5-TMB | 1,2,4-TMB | 1,2,3-TMB | PAH | Alkyl-phenols | NO\(_3^–\) | SO\(_4^{2−}\) | Fe\(^{2+}\) |
|---------|---------|---------------|------------|----------|-----------|-----------|-----------|-----|--------------|---------|---------|---------|
| \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) | \(\mu g\) L\(^{-1}\) |
| 45 | 9.8 | < 5.0 | 11 | 9.6 | < 5.0 | 26 | < 5.0 | 34.5 | 10 | 4.4 | 6.8 | 16 |

**Table 4** Composition of the trace element solution (TES) in mg L\(^{-1}\)

| Component | Concentration (mg L\(^{-1}\)) |
|-----------|-------------------------------|
| ZnSO\(_4\)*7H\(_2\)O | 100 |
| CaCl\(_2\)*H\(_2\)O | 197 |
| FeSO\(_4\)*7H\(_2\)O | 100 |
| NiCl\(_2\)*6H\(_2\)O | 10 |
| H\(_3\)BO\(_3\) | 20 |
| CuSO\(_4\)*5H\(_2\)O | 10 |
| Na\(_2\)MoO\(_4\)*2H\(_2\)O | 100 |
| Na\(_2\)WOC\(_2\)*2H\(_2\)O | 10 |
| MnSO\(_4\)*H\(_2\)O | 500 |
| Co(NO\(_3\))\(_2\)*6H\(_2\)O | 10 |
| AlK(SO\(_4\))\(_2\)*12H\(_2\)O | 10 |
| Na\(_2\)SeO\(_3\) | 1 |
The soil pore water was exchanged until a concentration difference between the infiltrated sulfate and the sulfate contained in the pore water was no longer detectable. Approximately 45 pore volumes were exchanged.

Analysis
Aromatic hydrocarbon analysis was performed according to [36]. A Hewlett Packard 6890 gas chromatograph (GC) equipped with a splitless injection port, a 0.53 mm × 29.8 m DB624 capillary column with a film thickness of 3 μm, and a flame ionization detector was used. The chromatographic conditions were injection port temperature 250 °C, initial column temperature 90 °C, initial time 10.5 min, heating rate 5 °C min⁻¹, final temperature 250 °C, final time 1.5 min, and column flow rate 4 mL min⁻¹ helium. The detection limits for all the identified compounds varied from 0.005 to 1 mg L⁻¹.

The ions nitrate, nitrite, and sulfate were analyzed according to [37]. A Metrohm 733 Separation Center equipped with a Metrosep separation column Chrompack 7414 (4.6 × 75 mm) and a conductivity detector were used for analysis. The detection limits were 0.5–25 mg L⁻¹ for nitrate, sulfate, and phosphate, and 0.1–25 mg L⁻¹ for nitrite.

Sulfate-S and sulfide-S concentrations in the soil were analyzed on an EA 2000 elemental analyzer multi (AnalytikJena GmbH, Jena, Germany) using the NDIR (non-dispersive infrared spectrometry) method. In the induction furnace, the sample was melted in a stream of pure oxygen at temperatures of 600 and 1400 °C, the sulfide-S and sulfate-S contained reacting to form sulfur dioxide (SO₂). The sulfur dioxide was detected in infrared measuring cells, the detection limit being 2 mg kg⁻¹.

Calculation of mass balance
The plausibility of nitrate consumption and sulfate formation for the oxidation of sulfides was analyzed by a mass balance between the inflow and outflow of the columns. This was done using Eqs. 1 and 2 based on the redox reaction of nitrate with iron disulfide (FeS₂). It was assumed that nitrate was completely converted into nitrite (Eq. 1) or nitrogen (Eq. 2) by chemolithotrophic denitrification [10–12]:

\[
7\text{NO}_3^- + \text{FeS}_2 + \text{H}_2\text{O} \rightarrow 7\text{NO}_2^- + \text{Fe}^{2+} + 2\text{SO}_4^{2-} + 2\text{H}^+ \tag{1}
\]

\[
14\text{NO}_3^- + 5\text{FeS}_2 + 4\text{H}^+ \rightarrow 10\text{SO}_4^{2-} + 5\text{Fe}^{2+} + 7\text{N}_2 + 2\text{H}_2\text{O} \tag{2}
\]

The additional consumption of nitrate by the oxidation of the bivalent iron formed according to Eqs. 1 and 2 was calculated according to Eq. 3 [18, 23, 24]:

\[
2\text{NO}_3^- + 10\text{Fe}^{2+} + 24\text{H}_2\text{O} \rightarrow \text{N}_2 + 10\text{Fe(OH)}_3 + 18\text{H}^+ \tag{3}
\]

Furthermore, nitrate consumption by chemooorganotrophic denitrification must be taken into account when calculating mass balance. Nitrate consumption occurs through the oxidation of organic electron donors such as dissolved organic carbon (DOC) or bound organic material in the soil (BOM). Since the composition and structure of the carbon is often unknown, the stoichiometric calculation of nitrate consumption is done according to Eq. 4 [9, 26, 38]:

\[
4\text{NO}_3^- + 5\text{CH}_2\text{O} \rightarrow 2\text{N}_2 + 4\text{HCO}_3^- + \text{CO}_2 + 3\text{H}_2\text{O} \tag{4}
\]

Results and discussion
Sulfide-S and sulfate-S levels in the soil
Elemental analysis was performed on four samples each of the soil used for the experiments to determine the sulfate and sulfide levels. The concentrations were in the range of 29.7–42.0 mg kg⁻¹ for sulfide-S and 132.6–148.1 mg kg⁻¹ for sulfate-S (Table 5). The concentrations of sulfide are relatively low compared to levels of up to 120 mg kg⁻¹ found in samples taken from the contaminated site. Due to low sulfate concentrations in the pore water (Table 3), it can be assumed that the high sulfite-S content in the soil consists of poorly soluble sulfates.

Conditioning phase (CP)
The infiltration of less contaminated anaerobic groundwater with low nitrate and sulfate concentrations was carried out for 15 exchanged pore volumes (EPV) (Fig. 2A–C). The almost uncontaminated soil was leached in order to minimize the consumption of nitrate for the biodegradation of the BTEX and TMB contained in the soil in the following main experimental phase.

The observed data for BTEX and TMB (Fig. 2A) indicate that the inflow and outflow concentrations were the same for the first five EPV in both columns. Based on the results of the abiotic column, no leaching of BTEX or TMB took place, and the concentrations in the outflow

| Table 5 Sulfide-S and sulfate-S concentrations in 4 samples from the soil used in mg kg⁻¹ |
|----------------|----------------|----------------|
|                | Biologically active column | Abiotic column |
| Sample         | Sulfide-S | Sulfate-S | Sulfide-S | Sulfate-S |
| S1-1           | 38.7      | 135.2     | 40.8      | 148.0     |
| S1-2           | 41.5      | 142.6     | 29.7      | 142.4     |
| S1-3           | 36.5      | 144.9     | 41.0      | 140.4     |
| S1-4           | 42.0      | 147.7     | 37.7      | 132.6     |
corresponded to those in the inflow. Later on, the concentration of the BTEX and TMB decreased in the outflow of the biologically active column compared to the abiotic column, reflecting the degradation of these substances.

Moreover, the inflow and outflow concentrations of sulfate were almost identical in the biologically active and abiotic column, indicating that no sulfide oxidation occurred in this phase.

**Main experimental phase (MEP)**

When artificial groundwater containing nitrate was infiltrated, the situation changed fundamentally. Neither BTEX nor TMB were infiltrated in either column, corresponding to the nearly undetectable substances in the effluent.

In the biologically active column, 50–100 mg L\(^{-1}\) of the infiltrated 300 mg L\(^{-1}\) of nitrate was consumed by the oxidation of sulfide contained in the soil (Fig. 2B), whereas only very low consumption was observed in the abiotic column. Consumption fluctuated over the 45 exchanged pore volumes.

Sulfate concentrations in the outflow of the biologically active column were significantly higher than in the inflow (Fig. 2C), indicating the oxidation of sulfide by nitrate. No formation of sulfate was registered in the outflow of the abiotic column, indicating that the oxidation of sulfide in the soil is driven by microorganisms.

The oxidation of sulfide was coupled with the production of nitrite as an intermediate due to the incomplete denitrification process (Fig. 2D), with no formation of nitrite being observed in the abiotic column.

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**Fig. 2** Total concentrations of aromatic compounds (BTEX and TMB) (A), nitrate concentration (B), sulfate concentration (C), and nitrite concentration (D) in the biologically active and abiotic column during the conditioning phase and the main experimental phase (phases separated by dotted lines).
Mass balance of nitrate, sulfate, and sulfide

A mass balance for the main experimental phase based on the obtained data and Eqs. 1–3 was performed (Table 6). The values determined in the biologically active column were corrected by those of the abiotic column. Since different pore volumes were exchanged and therefore different amounts of nitrate were infiltrated into both columns, correction took place by the percentage loss of nitrate and the formation of sulfate in the abiotic control column.

A total of 391 mg sulfate was formed during the exchange of 45 pore volumes in the biologically active column. According to Eqs. 1 and 2, this corresponds to 244.4 mg oxidized iron disulfide (FeS$_2$) and 130.5 mg oxidized sulfide (S$_2$). Assuming a soil mass of 3 kg in the column, this results in a concentration in the soil of approximately 82 mg kg$^{-1}$ for iron disulfide and 43.7 mg kg$^{-1}$ for sulfide. This tallies well with the sulfide levels measured in the soil (Table 5, average 39.7 mg kg$^{-1}$), considering the uneven distribution in the soil used.

According to Eqs. 1 and 2, 883.7 mg (incomplete chemolithotrophic denitrification) or 353.5 mg (complete chemolithotrophic denitrification) nitrate was necessary to oxidize the total amount of iron disulfide contained in the soil. Furthermore, 25.2 mg nitrate was consumed by the oxidation of the bivalent iron formed according to Eq. 3. The consumption of nitrate for the oxidation of additional dissolved organic carbon (DOC) in the infiltrated water (chemoorganotrophic denitrification) amounted to 401.8 mg.

Therefore, the mass of nitrate consumed (1341.2 mg) by chemolithotrophic denitrification, the oxidation of the bivalent iron formed, and chemoorganotrophic denitrification was only slightly higher than theoretically needed (1309.9 mg).

Kinetics of sulfide oxidation

The kinetics of sulfate formation and sulfide oxidation can be described by a linear regression in the form of the function $y = a + b \times x$, where $a$ is the intercept and $b$ the slope of the regression line. The criterion for the fitting quality was the coefficient of determination. Both the elimination and the formation curve followed a weak sigmoid shape possibly caused by temporary adsorption effects.

Based on the data obtained, a sulfate formation rate of 11.3 mg per EPV was determined (Fig. 3A). This resulted in an oxidation rate of 1.26 mg kg$^{-1}$ per EPV and 0.027% for the sulfide contained in the sediment (Fig. 3B).

Based on Eqs. 1 and 2, this corresponds to nitrate consumption of 8.5 mg kg$^{-1}$ per EPV (incomplete denitrification) or 3.4 mg kg$^{-1}$ per EPV (complete denitrification). A comparable value of 7.56 mg kg$^{-1}$ d$^{-1}$ for autotrophic denitrification was demonstrated by [39]. However, a much lower rate of 0.12–0.19 mg kg$^{-1}$ d$^{-1}$ was determined in [10].

Table 6 Mass balance of nitrate, sulfate, and sulfide for the main experimental phase (mg)

|                      | Biologically active column | Abiotic column |
|----------------------|----------------------------|----------------|
| Sulfate formation    |                            |                |
| $\text{SO}_4^{2-}$ inflow | 249.9                     | 226.0          |
| $\text{SO}_4^{2-}$ outflow | 666.6                     | 251.6          |
| $\text{SO}_4^{2-}$ formation | 416.6                     | 25.6           |
| Sulfide oxidation    |                            |                |
| Total amount of oxidized iron disulfide | 260.5 | 16.1 |
| Total mass of oxidized iron disulfide in the soil | 87.4 (mg kg$^{-1}$) | 5.4 (mg kg$^{-1}$) |
| Total amount of oxidized sulfide | 139.1 | 8.6 |
| Total mass of oxidized sulfide in the soil | 46.6 (mg kg$^{-1}$) | 2.9 (mg kg$^{-1}$) |
| Nitrate consumption  |                            |                |
| Incomplete chemolithotrophic denitrification (Eq. 1) | 941.8 | 58.1 |
| Complete chemolithotrophic denitrification (Eq. 2) | 376.7 | 23.2 |
| Oxidation of bivalent iron formed (Eq. 3) | 26.9 | 1.7 |
| Chemoorganotrophic denitrification (Eq. 4) | 404.8 | 3.8 |
| $\text{NO}_3^-$ consumption calculated according to Eqs. 1, 3, 4 | 1373.5 | 63.6 |
| $\text{NO}_3^-$ consumption calculated according to Eqs. 2, 3, 4 | 808.4 | 28.7 |
| $\text{NO}_3^-$ inflow | 7262.6 | 7630.8 |
| $\text{NO}_3^-$ outflow | 5797.2 | 7506.2 |
| $\text{NO}_3^-$ consumption measured | 1465.4 | 124.2 |
Conclusion
The results of the investigations indicate that the presence of soils containing sulfides leads to a nitrate-consum ing redox reaction, as confirmed by the formation of sulfate resulting from the oxidation of iron disulfide. Given the sufficient availability of nitrate, the kinetics of the processes follow a linear function corresponding to zero-order kinetics. This information is helpful for planning ENA projects requiring the correctly calculated amount of the electron acceptor nitrate to be infiltrated at contaminated sites.

In the case of sulfide in the soil, both the number of pore volumes to be exchanged for the complete oxidation of the sulfides and the amount of nitrate additionally required for the oxidation of the sulfides can be determined. This reduces the risk of under- or overdosing nitrate, which may lead to a lack of nitrate needed to enhance the biodegradation of contaminants in the aquifer or to the deterioration of groundwater quality. However, to generalize the results and to establish the limiting factors of the reactions, further investigations with different boundary conditions such as temperature, flow rate, and soil type are required.

Abbreviations
BTEX: Benzene, toluene, ethylbenzene, xylene; TMB: Trimethylbenzene; ENA: Enhanced natural attenuation; PAH: Polycyclic aromatic hydrocarbons; GW: Groundwater; CP: Conditioning phase; MEP: Main experimental phase; GC: Gas chromatograph; NDIR: Non-dispersive infrared spectrometry; DOC: Dissolved organic carbon; BOM: Bound organic material in the soil; EPV: Exchanged pore volumes.

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Authors’ contributions
The concept for the study was developed by TF and ARF. TF performed the experiment including analysis, data curation, interpretation and validation. TF and ARF were the major contributors in writing the original draft of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Competing interests
The authors declare that they have no competing interests.

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