

# Bio-electro-remediation: electrokinetic transport of nitrate in a flow-through system for enhanced toluene biodegradation

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**Abstract** The availability of electron acceptors and nutrients is often limiting the microbiological clean-up of polluted groundwater at contaminated sites. In this study, the feasibility of electrokinetic processes to improve mass transfer was demonstrated in a model system simulating laminar groundwater flow conditions. Electrokinetic nitrate transport and enhanced biodegradation of toluene under denitrifying conditions was studied as function of voltage gradient in a new flow-through system. The study was done in a three-dimensional anaerobic model aquifer system filled with coarse sand. The influent area was divided into seven chambers thus enabling a separate addition of toluene and nitrate. Mesh electrodes were inserted laterally in order to form an electric field perpendicular to the flow direction with voltage gradients of 0, 0.125 and 0.25 V cm<sup>-1</sup>, respectively. Biodegradation was studied after inoculation with a denitrifying microbial mixed culture. Application of the electric field resulted in nitrate migration into areas containing toluene. In the presence of denitrifying bacteria, the availability of nitrate in toluene polluted areas resulted in toluene biodegradation, demonstrated by nitrite formation and decreased toluene concentration.

**Keywords** Electrokinetic transport · Bioremediation · Nitrate · Nitrite · Laminar flow conditions

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## 1 Introduction

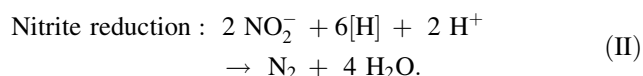
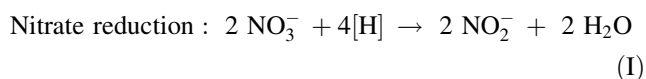
Improper waste disposal, leakages, and accidents resulted in the formation of thousands of contaminated sites worldwide in the past decades. Today, in particular in countries using groundwater resources for their drinking water supply, contaminated sites cause problems as the pollutants often form long plumes of polluted groundwater.

Due to the high costs of ex situ treatment or conventional pump and treat techniques in situ strategies become more important and attractive. Bioremediation is a widely used and effective soil and groundwater remediation strategy for organic pollutants. Biodegradation is the key process for pollutant elimination in engineered and natural systems under aerobic and anaerobic conditions [1–5]. The availability of electron acceptors such as oxygen, nitrate, or sulfate, and the availability of nutrients such as phosphorus and ammonium are the key factors for a successful microbiological clean-up of polluted sites. In polluted aquifers, often a non-uniform distribution of microorganisms, contaminants, and nutrients is observed. Transport of substances occurs in flow direction and only a very limited transversal mixing is observed by dispersion and diffusion [6–9]. Therefore, mass transfer limitations are most pronounced under in situ conditions where a heterogeneous spatial distribution of microorganisms, contaminants, nutrients, and electron acceptors is observed already at a micrometer to millimeter scale [10, 11].

The application of electrodes represents a new approach to overcome the mass transfer limitations. Electrokinetic soil remediation, i.e. the controlled application of lower DC electric fields to polluted soils, is an emerging in situ technology that previously has attracted interest for example for the remediation of heavy metal contaminated sites [12, 13]. The electric field causes electrokinetic transport processes such as movement of charged species by electromigration and

transport of non charged compounds by electroosmosis [14, 15]. In several studies, also the electrokinetic transport of nitrate [16–18], sulfate and ammonium [17–20] has already been shown. However, most of the electrokinetic studies were done in batch systems [e.g. 17, 18, 21] that do not reflect the hydrodynamic conditions at most contaminated sites. The pH gradients that develop in static batch systems are a major drawback with respect to microbial activity. It is well known that values of pH 3 and pH 11 rapidly develop at the anode and the cathode, respectively [16–19].

For this study, a new flow-through model system was developed in order to simulate in situ groundwater flow conditions. Nitrate was chosen as a model compound since it is known to be utilized as a terminal electron acceptor by many pollutant degrading microorganisms [2, 22–25]. During microbial nitrate utilization, in the first step nitrate is reduced to nitrite that is further reduced to gaseous nitrogen. The reactions are shown in Eqs. I and II.



The objective of this study was to study the electrokinetic transport of nitrate, the pH effect, and

microbial nitrate utilization during toluene degradation in the flow-through model system. The applied voltage gradients varied between 0 and  $0.25 \text{ V cm}^{-1}$ , and the system was operated at a flow rate of  $30 \text{ cm day}^{-1}$  representing often observed field conditions in sandy soils.

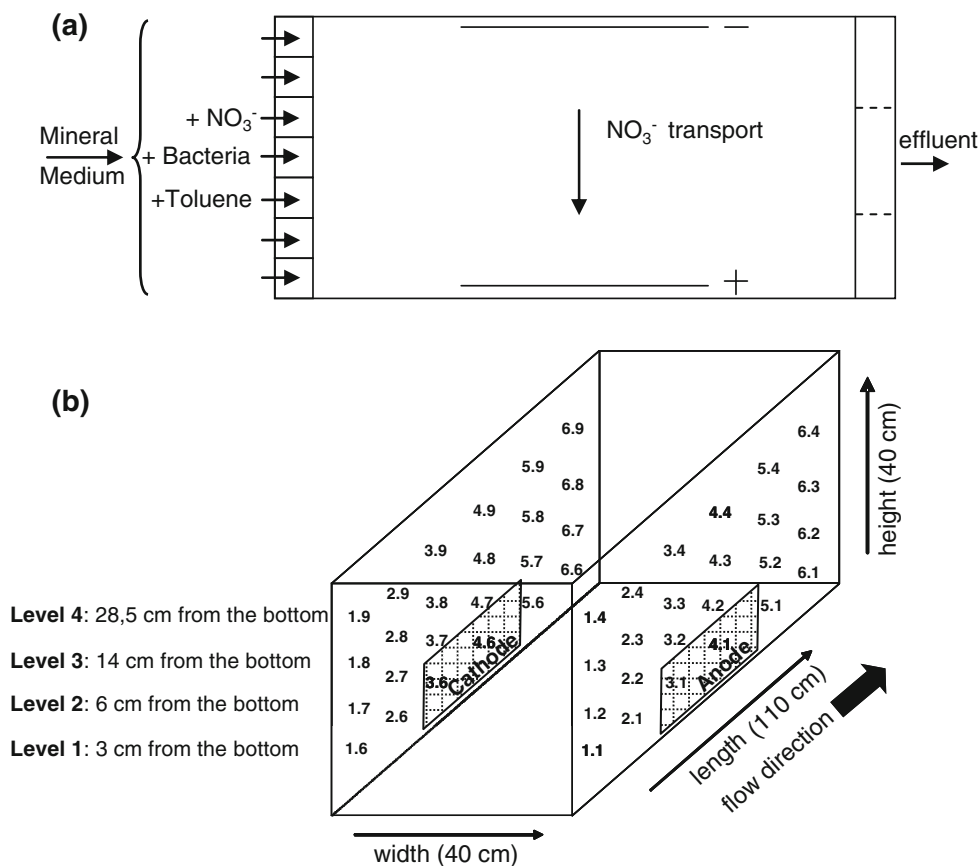
## 2 Materials and methods

### 2.1 Experimental setup

The three-dimensional anaerobic model aquifer system (length: 110 cm; width: 40 cm; height: 40 cm) was filled with coarse sand (DORSILIT® 9S silica sand free of silt, particle density  $2.65 \text{ g cm}^{-3}$ , particle sizes  $> 0.1$  and  $< 1 \text{ mm}$ ) and equipped with 48 sampling ports. Figure 1 shows a schematic illustration of the flow-through system.

The mineral medium was pumped with a flow rate of  $13 \text{ L day}^{-1}$  out of a reservoir by a peristaltic pump, resulting in a laminar flow of  $30 \text{ cm day}^{-1}$  in the model aquifer. Oxygen in the mineral medium was removed by passing through a 30 m silicon tube in dissolved  $\text{Na}_2\text{SO}_3$  [26]. In addition, the head space of the model aquifer system was purged with nitrogen every 48 h for 1 h to maintain anaerobic conditions. The influent area of the flow-through system was divided into seven chambers thus enabling a

**Fig. 1** Schematic illustration of the flow-through system; **a** top view with the influent chambers; **b** three-dimensional illustration with the location of the sampling ports



**Table 1** Overview of the performed experiments

Experimental purpose	Addition of			Applied voltage (V cm <sup>-1</sup> )	Results shown in
	Nitrate	Toluene	Bacteria		
Electrokinetic nitrate transport and pH shift	+	–	–	0	Figures 2 and 3
	+	–	–	0.125	
	+	–	–	0.25	
Electrokinetic stimulation of microbial degradation	+	+	+	0	Figure 4
	+	+	+	0.125	Figure 5

separate addition of toluene (chamber 3) and nitrate (chamber 5). For adding toluene and nitrate to the influent chambers, stock solutions of approximately 300 mg L<sup>-1</sup> toluene and 1 g L<sup>-1</sup> NaNO<sub>3</sub>, respectively, were pumped with a low flow rate into the chambers. Due to varying flow rates and influent concentrations, the maximum concentration observed at the ports ranged between 227 and 395 mg L<sup>-1</sup> (nitrate) and between 44 and 104 mg L<sup>-1</sup> (toluene).

Stainless steel mesh electrodes (length: 40 cm, height: 10 cm) were inserted laterally in order to form an electric field perpendicular to the flow direction. The electrodes were connected to a DC power supply (model 1145, Heiden power GmbH) by a stainless steel wire. Abiotic experiments were performed with voltage gradients of 0, 0.125 and 0.25 V cm<sup>-1</sup>, respectively, in order to examine electrokinetic transport rates and pH shifts. Abiotic experiments were conducted 3 times without a voltage gradient (0 V cm<sup>-1</sup>), twice with 0.125 V cm<sup>-1</sup> and once with 0.25 V cm<sup>-1</sup>. Biodegradation was studied after inoculation with a denitrifying microbial mixed culture (chamber 4) with and without application of an electric field. Both conditions were conducted twice. An overview of the experimental conditions is shown in Table 1.

## 2.2 Sampling methods and chemical analysis

Samples were taken with a syringe via Teflon tubes which were distributed in the flow-through system (Fig. 1b). Nitrate and nitrite samples were filtrated by 0.2 µm cellulose acetate filter and analyzed with an ion chromatograph (Metrohm 761 Compact IC, Metrohm) equipped with a MetrohmA-Supp-5 column (length: 150 mm, 4 mm inside diameter) and a suppression system and conductivity detector (quantification limit for nitrate: 1 mg L<sup>-1</sup>, for nitrite: 0.5 mg L<sup>-1</sup>). The mobile phase of the ion chromatography was a carbonate eluent (3.2 mmol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub>, 1 mmol L<sup>-1</sup> NaHCO<sub>3</sub>, 2 vol.% acetone). Toluene was determined by a gas chromatograph (Hewlett Packard, 5890 Series II, Headspace Sampler HP 19395A) equipped with a flame ionization detector. Samples were preheated at 70 °C for 3 h before headspace analysis. The GC column was a HP Pona (length: 50 m, 0.2 mm inside diameter, film thickness:

0.5 µm), operated with the temperature program: 15 min at 35 °C, 1.5 °C min<sup>-1</sup> up to 60 °C (hold for 12 s), 15 °C min<sup>-1</sup> up to 130 °C (hold for 7 min) and 30 °C min<sup>-1</sup> up to 200 °C (hold for 5 min). Measurement of pH was done with a multimeter (WTW).

## 2.3 Denitrifying culture and mineral medium

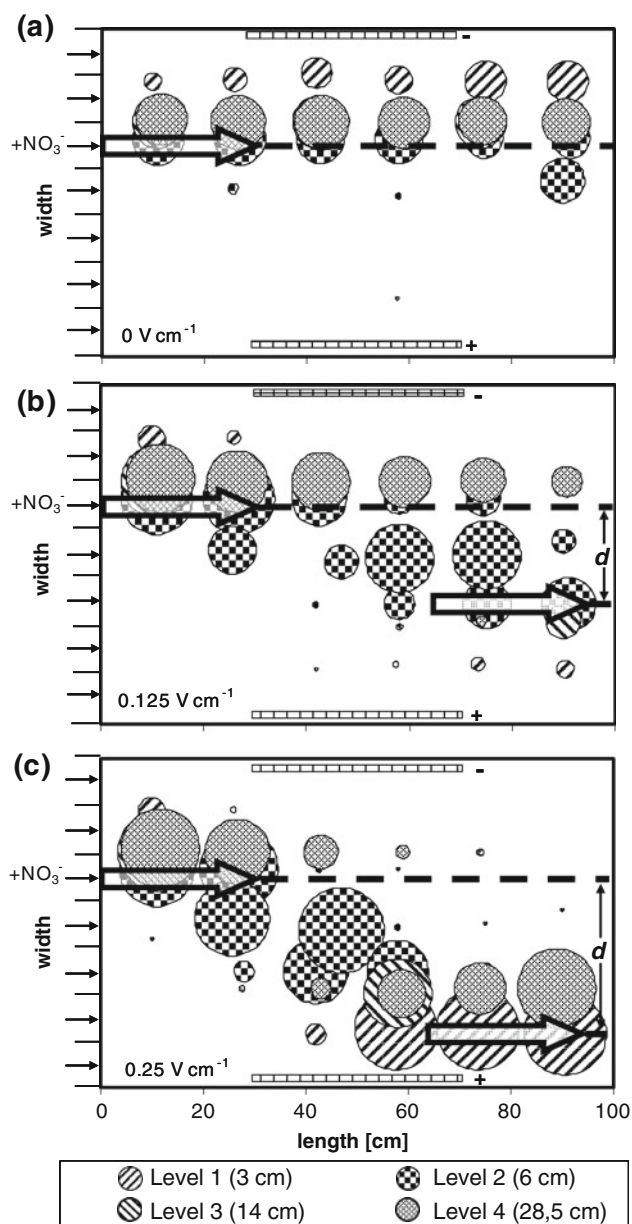
The denitrifying culture was originally isolated from a contaminated site where microbial degradation of toluene took place. The culture was incubated in a mineral medium according to Lochhead and Chase [27] containing the following constituents per liter of demineralized water: 1 g K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 0.2 g KH<sub>2</sub>PO<sub>4</sub>, 0.04 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.02 g CaCl<sub>2</sub>·2H<sub>2</sub>O, and 1 mL trace elements solution. A transfer of the denitrifying culture into fresh mineral medium was done periodically, and toluene as organic substrate and nitrate as electron acceptor were added.

## 3 Results and discussion

### 3.1 Nitrate transport

Figure 2 shows the nitrate distribution at applied voltage gradients of 0, 0.125 and 0.25 V cm<sup>-1</sup>. Without applied voltage, nitrate was mainly found downgradient chamber 5 in which nitrate was added (Fig. 2a). In contrast, electrokinetic nitrate transport perpendicular to the flow direction was observed in experiments when a voltage gradient was applied. Due to their negative charge nitrate ions were transported from the cathode to the anode in the electric field. In Fig. 2b and c electrokinetic nitrate transport perpendicular to the flow direction is illustrated. With 0.125 V cm<sup>-1</sup> nitrate was transported in the electric field towards the anode up to the centerline of the system (Fig. 2b). Applying 0.25 V cm<sup>-1</sup> led to high nitrate concentrations near the anode and downgradient the anode (Fig. 2c).

Previously electrokinetic transport of nitrate was studied in a static system, i.e. without any hydraulic gradient [17, 18]. Experiments in the static system were conducted at average voltage gradients of 2 and 1 V cm<sup>-1</sup> in



**Fig. 2** Distribution of nitrate (*top view*) during abiotic experiments with **a**  $0 \text{ V cm}^{-1}$ , **b**  $0.125 \text{ V cm}^{-1}$  and **c**  $0.25 \text{ V cm}^{-1}$  (areas of the *circles* correspond to the concentration of the analytes). Electrokinetic nitrate transport distance  $d$  is illustrated

demineralized water and groundwater [17]. In the static system, the same model sandy soil was used as in the flow-through system. Calculating transport parameters of nitrate in the static system revealed that electrokinetic transport rate was a linear function of the applied voltage. At  $2 \text{ V cm}^{-1}$  average electrokinetic nitrate transport was  $1.3 \text{ cm h}^{-1}$ , and applying  $1 \text{ V cm}^{-1}$  led to  $0.7 \text{ cm h}^{-1}$ . In the flow-through system, operated with mineral medium, also a linear relationship between voltage gradient and transport rate was observed. The flow velocity of  $30 \text{ cm day}^{-1}$  led to a hydraulic residence time between the

electrodes of 32 h (calculated with a hydraulic pore volume of 35%). At  $0.25 \text{ V cm}^{-1}$  the electrokinetic transport distance  $d$  was approximately 20 cm (Fig. 2c). Thus the electrokinetic transport rate was  $0.6 \text{ cm h}^{-1}$ . Applying  $0.125 \text{ V cm}^{-1}$  resulted in a transport distance  $d$  of approximately 10 cm (Fig. 2b). The calculated electrokinetic transport rate at  $0.125 \text{ V cm}^{-1}$  was  $0.3 \text{ cm h}^{-1}$ . The observed differences in transport rates between electrokinetic experiments in the static system and the flow-through system might be due to the different pH gradients since a neutral pH results in higher transport rates [18]. In the static system, pH near the cathode increased up to pH 12 and in the anode region pH 2 was observed [17, 18]. Due to the continuous water flow in the model aquifer system used in this study, less pronounced pH gradients developed.

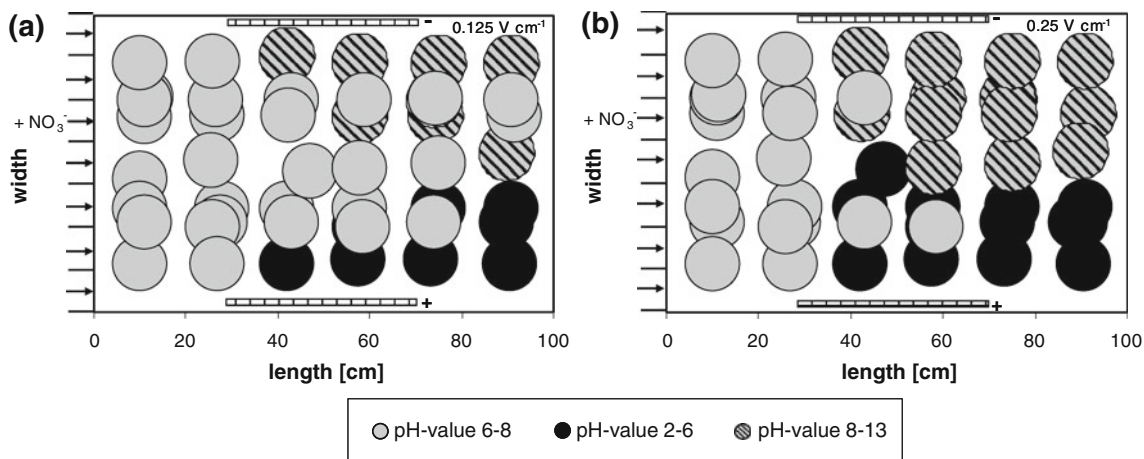
### 3.2 pH shifts in the system

The pH of the mineral medium pumped through the system was about 7.4. Significant pH changes were observed in experiments with applied voltage. Changes in pH result from water electrolysis at the electrodes where  $\text{H}^+$  and  $\text{OH}^-$  are generated at the anode and cathode, respectively. The distribution of the pH during the experiments with applied voltage is shown in Fig. 3. Applying  $0.25 \text{ V cm}^{-1}$  caused a strong pH shift downgradient the electrodes. High pH values about pH 11 were measured downgradient the cathode. Downgradient the anode the pH values were about pH 2. In the center of the system high pH values were observed (Fig. 3b). Applying  $0.125 \text{ V cm}^{-1}$  also resulted in a shift in pH. However, the extreme pH values were only found near and downgradient the electrodes, whereas in the center pH 6 to 8 remained (Fig. 3a).

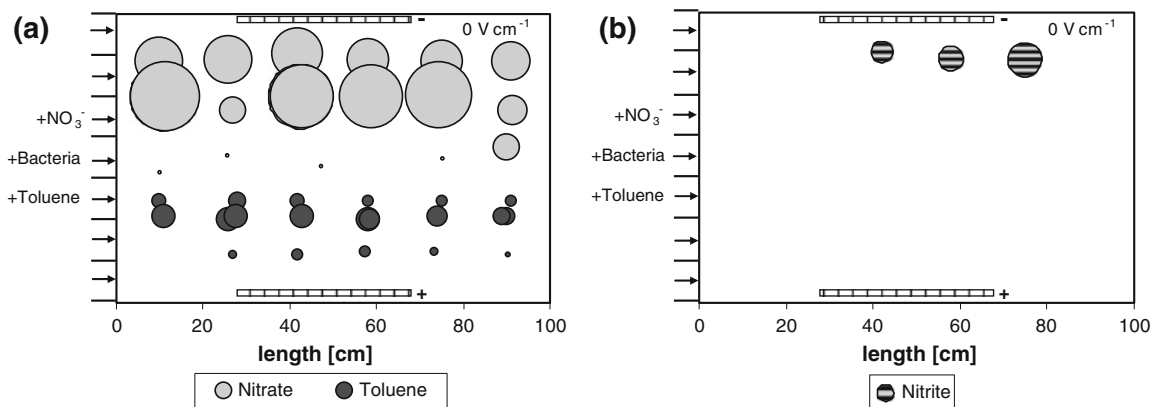
The abiotic experiment revealed that applying  $0.125 \text{ V cm}^{-1}$  was sufficient to transport nitrate into toluene polluted areas, while the pH in this area remained neutral. Therefore, subsequent experiments with the addition of bacteria were conducted with a voltage gradient of  $0.125 \text{ V cm}^{-1}$ .

### 3.3 Simultaneous application of nitrate, toluene and bacteria

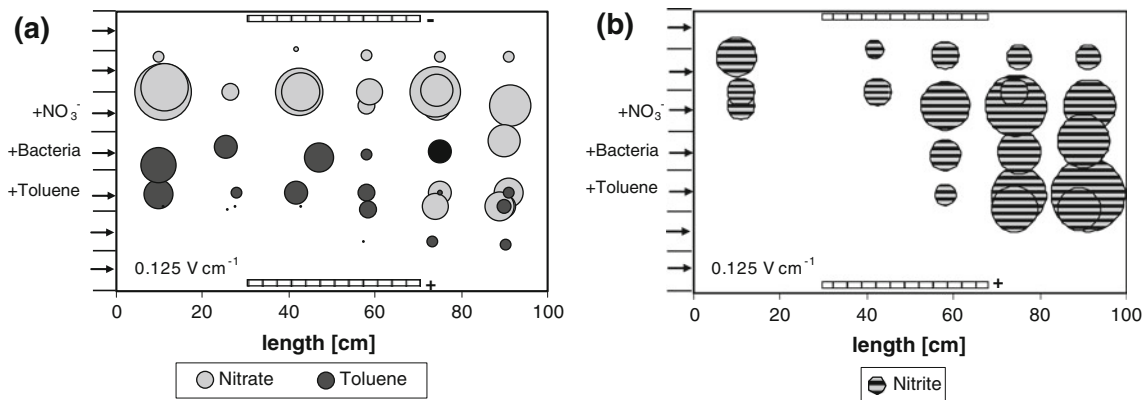
Without voltage gradient, a laminar water flow with spatially separated nitrate and toluene was observed (Fig. 4a). No microbial degradation of toluene occurred as the areas of the model pollutant (toluene) and the electron acceptor (nitrate) did not overlap. Nitrite, an intermediate compound indicating microbial denitrification (Eq. I), was only observed in low concentration (maximum:  $1.6 \text{ mg L}^{-1}$ ) in a small area (Fig. 4b) in sampling ports near the interphase of sand/water and the gas phase (i.e. level 4; 28.5 cm from



**Fig. 3** Distribution of pH (*top view*) during abiotic experiments with **a**  $0.125 \text{ V cm}^{-1}$  and **b**  $0.25 \text{ V cm}^{-1}$



**Fig. 4** Simultaneous application of nitrate, toluene and bacteria (*top view*); without electric field; distribution of nitrate and toluene (**a**) and nitrite (**b**) (areas of the *circles* correspond to the concentration of the analytes)



**Fig. 5** Simultaneous application of nitrate, toluene and bacteria (*top view*); applying  $0.125 \text{ V cm}^{-1}$ ; distribution of nitrate and toluene (**a**) and nitrite (**b**) (areas of the *circles* correspond to the concentration of the analytes)

the bottom). Most probably, toluene was available in this area due to volatilization into the gas phase followed by dissolution into the water at the top of the model aquifer.

Application of the electric field resulted in nitrate migration into areas with toluene as shown in Fig. 5a. In the presence of denitrifying bacteria, the availability of

nitrate in toluene polluted areas resulted in toluene biodegradation, demonstrated by significant nitrite formation (Fig. 5b). Nitrite was found at the ports downgradient the electrodes. The maximum nitrite concentration was  $8.1 \text{ mg L}^{-1}$  at level 2 (6 cm from the bottom) of the sampling ports. In this area, nitrite has been below the detection limit in the abiotic control experiments with  $0.125 \text{ V cm}^{-1}$ .

A sufficient mass balance of toluene degradation and nitrate consumption could not be obtained. One reason was the high volatility of toluene, resulting in losses in particular in the effluent area of the flow-through system. On the other hand, calculating microbial nitrate utilization was not possible as the influent concentrations of nitrate varied. However, the microbiological nitrite formation clearly demonstrated that applying  $0.125 \text{ V cm}^{-1}$  resulted in a stimulation of denitrifying toluene biodegradation. In previous studies, enhanced microbiological chloroethene degradation in electric fields was shown due to the electrochemical formation and microbiological utilization of the electrolysis products hydrogen and oxygen [5, 28, 29]. This study demonstrates that electrokinetic processes can also be used to stimulate microbiological degradation of aromatic hydrocarbons such as toluene. In the flow-through model system, electrokinetic transport of nitrate was demonstrated over distances in the centimeter to decimeter scale. Since biodegradation limitation in the field occurs in the micro- to millimeter scale [7–11] due to the spatial separation of pollutants, electron acceptors, and microorganisms even lower voltage gradients might be suitable to improve bioremediation at contaminated sites.

#### 4 Conclusions

A new flow-through model system equipped with electrodes proved to be suitable to study electrokinetic transport under laminar flow conditions. Electrokinetic transport of the microbial electron acceptor nitrate perpendicular to the water flow direction was demonstrated. Nitrate transport was observed already at voltage gradients not resulting in pH shifts inhibiting microbial activity. In conclusion, the study demonstrates the feasibility of electrokinetic approaches to stimulate microbial pollutant degradation.

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