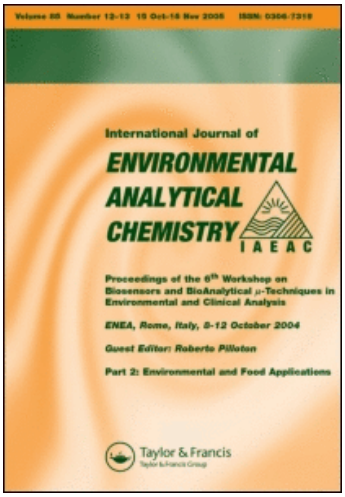

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Monitoring Of Nitrifying Processes In Ground Water By Ion Chromatography

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MONITORING OF NITRIFYING PROCESSES IN GROUND WATER BY ION CHROMATOGRAPHY

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The removal of ammonia from mineral medium containing known concentrations of ammonia (up to 300 mg/L) and from ground water by biological oxidation was studied. Nitrifying bacteria were isolated from ground water containing ammonia.

Ammonium ion was determined by a standard titration technique while nitrite and nitrate ions were determined by ion chromatography (IC Supersep anion column) using 1.5 mM phtalic acid solution containing 5 % acetonitril as eluent.

Depending on its concentration in water biooxidation of ammonia lasted from 48 hours till three weeks.

KEY WORDS: Anion-exchange ion chromatography, nitrite and nitrate determination, nitrifying bacteria.

INTRODUCTION

The European Guideline on Drinking Water (EEC 80/778) fixed the standards for nitrogen compounds in potable water in 25 mg/L of nitrate (NO_3^--N) and 0.05 mg/L of ammonia (NH_4^+-N), but levels twice as high for nitrates and ten times as high for ammonia are tolerated[1]. Because of the widespread occurrence of ammonia in ground water[2-4], these quality requirements initiated intensive research on nitrogen removal methods.

The recycling of nitrogen compounds within the nitrogen cycle after ammonification has taken place, i.e. the oxidation of ammonia into nitrites and nitrates, is an important step. The nitrates are further converted to organic nitrogen compounds in the microbial cells[5].

Nitrification has been well recognized as a beneficial treatment for the removal of ammonia in municipal sewage. In addition, research at several European and American water treatment facilities has shown that controlled nitrification provides an effective means of removing ammonia from the raw water[2,3,6-8]. This is a two-step process that is most commonly carried out by two distinct groups of chemolithotrophic bacteria. In the first step, ammonia is oxidized to nitrite:

$$NH_4^+ + 3/2\ 0_2 \longrightarrow NO_2^- + H_2O + 2H^+ \text{ (- 65 Kcal/mole)}$$

Nitrosomonas is the most frequently identified organism associated with this step, but other genera, such as *Nitrosolobus*, *Nitrosococcus*, *Nitrosovibrio* and *Nitrosospira*, can also oxidize ammonia to nitrite autotrophically. In the second step of the process, nitrite is oxidized to nitrate:

$$NO_2^- + H_2O \longrightarrow NO_3^- + 2H^+ \text{ (- 20 Kcal/mole)}$$

In soil and freshwater, this step is carried out exclusively by members of the genus *Nitrobacter*[5,9].

Incomplete or partial nitrification in distribution systems can adversely affect water quality because nitrite rapidly reduces free chlorine, interfere with the measurement of free chlorine, causes corrosion of pipelines and increases heterotrophic plate counts and anaerobic conditions[10].

The demand for the determination of NO_2^--N, NO_3^--N or NH_4^+-N in a variety of aqueous environments is increasing rapidly and, as a result, there is an expanding need for automated or semiautomated analysis of chemical plant streams, environmentally important waters such as waste streams, rivers, lakes or fluids of biological interest such as blood, urine, etc. There are many examples where is a continual need for routine analysis of common species such as NO_2^--N and NO_3^--N.

Ion exchange resins have a well known ability to provide excellent separations of ionic species and there are a number of instances where ion exchange chromatography has been successfully applied[11–15]. The chromatographic approach is simple, versatile and has the added advantage of being applicable over a wide range of concentrations while the other methods or techniques have limited useful concentration ranges[16].

This paper deals with the investigation of the biological oxidation of ammonia in mineral medium and ground water with determination of NO_2^- + NO_3--N by anion-exchange ion chromatography. Ion chromatographic methods for determination of nitrites and nitrates have been modified because these anions are determinated in mineral media for bacterial growth.

EXPERIMENTAL

Apparatus

Anion exchange ion chromatography was performed by using a 690 Ion Chromatograph (Metrohm, Switzerland) with IC Anion Column Super Sep and 697 IC pump. The column packing was polymethacrylate with quarternary ammonium groups and the dimension was 100 × 4,5 mm. An IC Anion precolumn Super-Sep was used and coupled to the analytical anion column. Ion exchange materials of low capacity with particle sizes of 5–10 μm were employed. The conductivity detector followed the two columns. The chromatograms were treated on a C–R 5A Chromatopac system (Shimadzu, Kyoto, Japan).

Reagents

All inorganic salts used were of analytical grade. Standard solutions of inorganic anions were prepared from stock solutions (1–50 g/L) of the sodium salts and for dilutions and washings demineralized water was used. All eluents were filtered through a 0.45 μm filter FP 030/2 (Schleicher and Schuell, Germany) to ensure that the solvent was degassed and that particulate matter was not present. All glassware were soaked in 10 % hydrochloric acid and rinsed copiously with high purity water prior to use.

Basal medium: $MgSO_4 \times 7H_2O$ 0,2 g; KH_2PO_4 1,0 g; $MnCl_2 \times 4H_2O$ 0,02 g; $CaCl_2$ 0,02 g; $NaMnO_4 \times 2H_2O$ 0,001 g; phenol red 0.001 g; in 1000 mL deionized water.

Additions: for ammonia oxidizing bacteria, NH_4Cl 1,5 g/L and chalk in traces; for nitrite oxidizing bacteria, $NaNO_2$ 1, 0 g/L.

These mineral media will be subsequently mentioned as mineral medium I and II.

Operating conditions

The NO_2^--N and NO_3^--N ions in ground water and complex matrices were determined by means of ion chromatographic analysis. The column and the precolumn were eluted with 1.5 mM phthalic acid solution containing 5 % (v/v) acetonitrile as eluent and pH=7.5. Calibrations by standard additions and external standard were used.

The optimization of the chromatographic system resulted in a flow-rate of 1.5 mL/min, a pressure of 20 bar (valid for the column alone) and a total retention time of 9.5 min, which enabled the sulphate peak, the last to elute. The volume injected was 100 μL.

Samples with NO_2^--N and NO_3^--N concentrations higher than 10 mg/L were diluted prior to determination of these ions, in order to avoid column saturation effects[17], because of high concentrations of other salts present in the medium. Ammonium ion was determined by the usual acidimetric method[18].

Isolation of nitrifying bacteria

Nitrifying bacteria were isolated from ground water containing ammonia. Raw water samples before conditioning were collected in sterile bottles from Kutina drinking water distribution plant (Croatia), filtered through a 0.2 μm pore-size cellulose acetate membrane (Sartorius, Germany) and transferred into previously sterilized mineral medium I, for enrichment of bacteria which oxidize ammonia, and mineral medium II for bacteria which oxidize nitrites. Cells were grown in the dark on a rotary shaker "Gallenkamp" IH–460 (200 rpm) at 25 °C for 14 days. The medium was adjusted to pH 8.0 with K_2CO_3 (1M).

Beginning with the fourth day after inoculation, chemical reactions for the presence of nitrite and nitrate were regularly conducted. Griess reagent served as a reagent for nitrite while nitrate was detected by reaction with pyrogallol[19]. When positive reactions for nitrite and nitrate were recorded, a survey of the developed microflora was carried aut under a microscope. After that, cultures were spread on silicic acid plates and treated with mineral

medium I and mineral medium II, respectively. When pure cultures of ammonia and nitrite oxidizing bacteria were obtained, they were used for preparing the inoculum for testing the efficiency of nitrification in submerged culture and in laboratory scale sand filter.

Nitrification process

Pure cultures of ammonia and nitrite oxidizing bacteria, obtained through enrichment of cultures from ground water, were transferred separately into 100 mL mineral medium with final NH_4^+-N and NO_2^--N concentrations of 3 mg/L, 30 mg/L and 300 mg/L, respectively. 10 mL of bacterial suspension were filtered through a 0.2 μm membrane to concentrate the cells and rinsed three times with 50 mL of sterile buffer to remove NO_2^--N and NO_3^--N ions developed through enrichment of cultures. The membranes were placed into appropriate medium in 500 mL Erlenmeyer flasks. The cultures were incubated from 48 to 384 hours (depending on initial ammonia and nitrite concentrations) at pH 8.0, 25 °C and 200 rpm, in a rotary shaker incubator. To maintain the environmental conditions constant for nitrifiers, $NaHCO_3$ and NaOH (stock solution 70 g/L $NaHCO_3$ in 0.32 % NaOH) were added until a pH-value of 8.0 was reached. This provided simultaneously the necessary CO_2 supply for these autotrophic bacteria.

The presence of $NO_2^- + NO_3^-$-N in the medium was determined at certain intervals by ion chromatography. Prior to determination, samples were filtered through a 0.2 μm membrane. Concentrations of nitrifying bacteria were determined by optical density measurement at a wavelength of 610 nm using an "Iskra" MA 9502 colorimeter. The determinations were made in an optical density range of less than 0.25, where the Beer-Lambert law applies, and the cell concentrations are reported in terms of units of optical density (UOD/mL).

Biological sand filter

Biooxidation of ammonia in the sample of a raw ground water (Kutina, Croatia) was carried out in a laboratory scale sand filter. A glass column (20 cm height, 7 cm diameter and working capacity 0.5 L) was filled with 3–5 mm grain-size quartz sand (Puconci, Slovenia) and seeded with 100 mL suspension of ammonia and nitrite oxidizing bacteria. The attachment of nitrifying bacteria on the sand particles under the aerated conditions lasted 48 hours. After that, the column was backwashed with sterilized deionized water to remove unattached biomass and medium in which cells were previously grown. The glass column was wrapped with aluminium foil to shield the cultures from direct ambient light. The prepared column was filled with 0.5 L of raw ground water containing 2.9 NH_4^+-N mg/L and pH 7.5. The system was aerated with a membrane pump type AP-2 and dissolved oxygen (DO) was measured with an oxygen electrode (YSI Model 54 A). The experiment was carried out in batch system at 20 °C for the first 48 hours and after that in continuous system.

RESULTS AND DISCUSSION

Identification of nitrifying bacteria

Nitrifying bacteria isolated from ground water were morphologically different, but both isolates were gram-negative and rod-shaped bacteria. The first one was 0.8 by 1.5 μm in size. Cells usually occurred singly or in small clumps, grow readily in a fluid medium without organic matter, on silicic acid gel and form small sharply defined colonies brownish in colour. Gel taken from a zone of clearing yields a positive reaction for nitrite. Microscopic analyses indicated that these bacteria had morphologic features characteristic of the chemolitotrophic ammonia oxidizing bacteria[8,9].

The second isolate was 0.6 by 1.2 μm in size. Cells usually occurred singly, some of them pear-shaped. On silic acid gel colonies were smaller greyish and round. The gel around these bacteria gives a positive reaction for nitrate, which is indicative of members of the chemolithotrophic nitrite oxidizing bacteria[9,19].

Nitrification process

Experiments with cells of ammonia oxidizing bacteria and nitrite oxidizing bacteria were separately conducted because nitrification is a two step process. Ammonia oxidation (Figures 1. a, b, c), nitrite oxidation (Figures 2. a, b, c) and bacterial growth (Figures 3. a, b) in the medium were investigated during 384 hours. Experiments were carried out at different NH_4^+-N and NO_2^--N concentrations, (3 mg/L, 30 mg/L and 300 mg/L, respectively).

At lower NH_4^+-N and NO_2^--N concentrations 100 percent ammonia and nitrite removal were achieved. At the lowest concentration biooxidation lasted 48 hours (Figures 1.a and 2.a), while at higher concentration 96 hours (Figures 1.b and 2.b). An exponential bacterial growth was also observed (Figures 3. a and b). The results obtained showed that ammonia oxidation with ammonia oxidizing bacteria and nitrite oxidation with nitrite oxidizing bacteria, which were separately carried out, finished at the same time what is very important for complete nitrification. In uncomplete nitrification, the remaining nitrite rapidly reduces free chlorine in a distribution water system[10] and this compound can react with secondary amines to produce nitrosoamines, a potent class of carcinogens[20].

At the highest concentration 70% of ammonia-N was removed approximately (Figure 1.c) while only 42 % of nitrite-N (Figure 2.c). Though the experiment lasted for 384 hours complete nitrification was not achieved. For the first 120 hours nitrifying bacteria grew well but after that no changes in their number were observed (Figures 3.a and 3.b). Nevertheless, the pH of media were maintained between 7.5–8. Nitrifying bacteria in the process of oxidation secrete organic compounds, in addition to producing nitrogen byproducts, which may inhibit further biooxidation in batch culture[9,21].

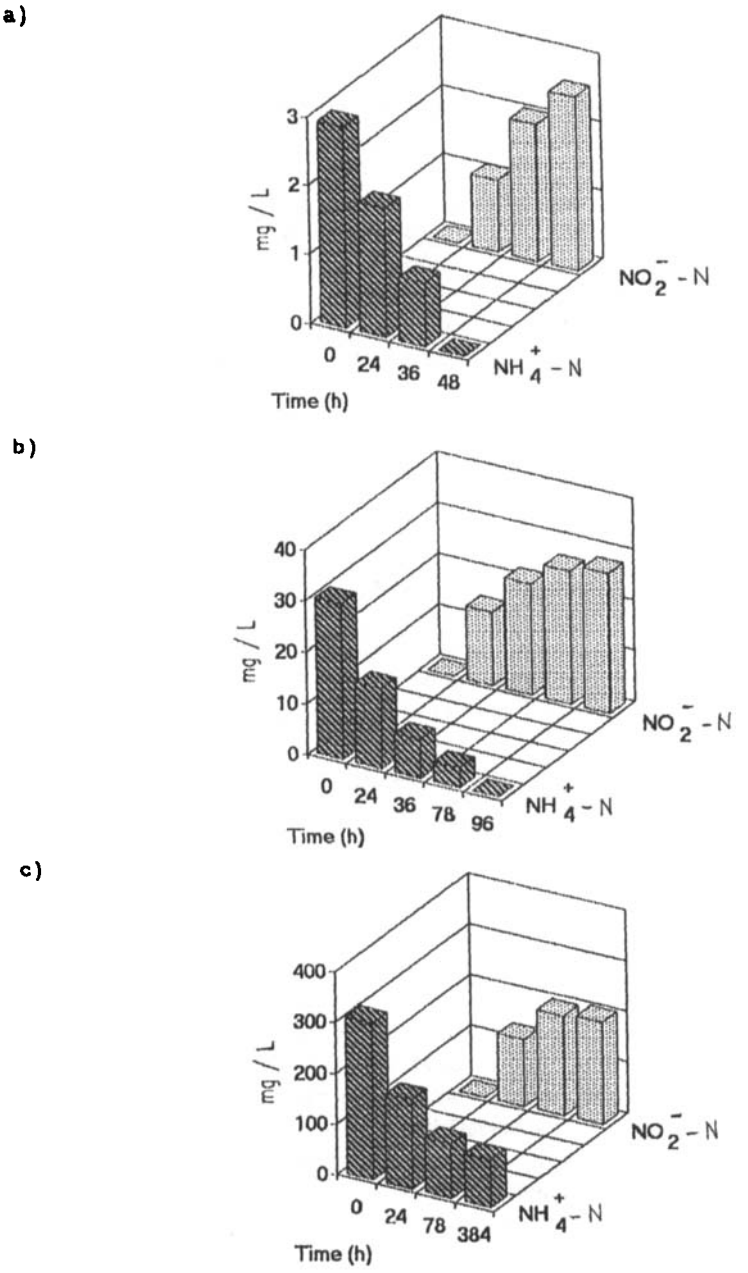

Figure 1 Ammonia oxidation at different initial concentrations 3 mg/L (a), 30 mg/L (b) and 300 gm/L (c) in medium by cells of ammonia oxidizing bacteria during 384 hours at 25 °C and pH 8.

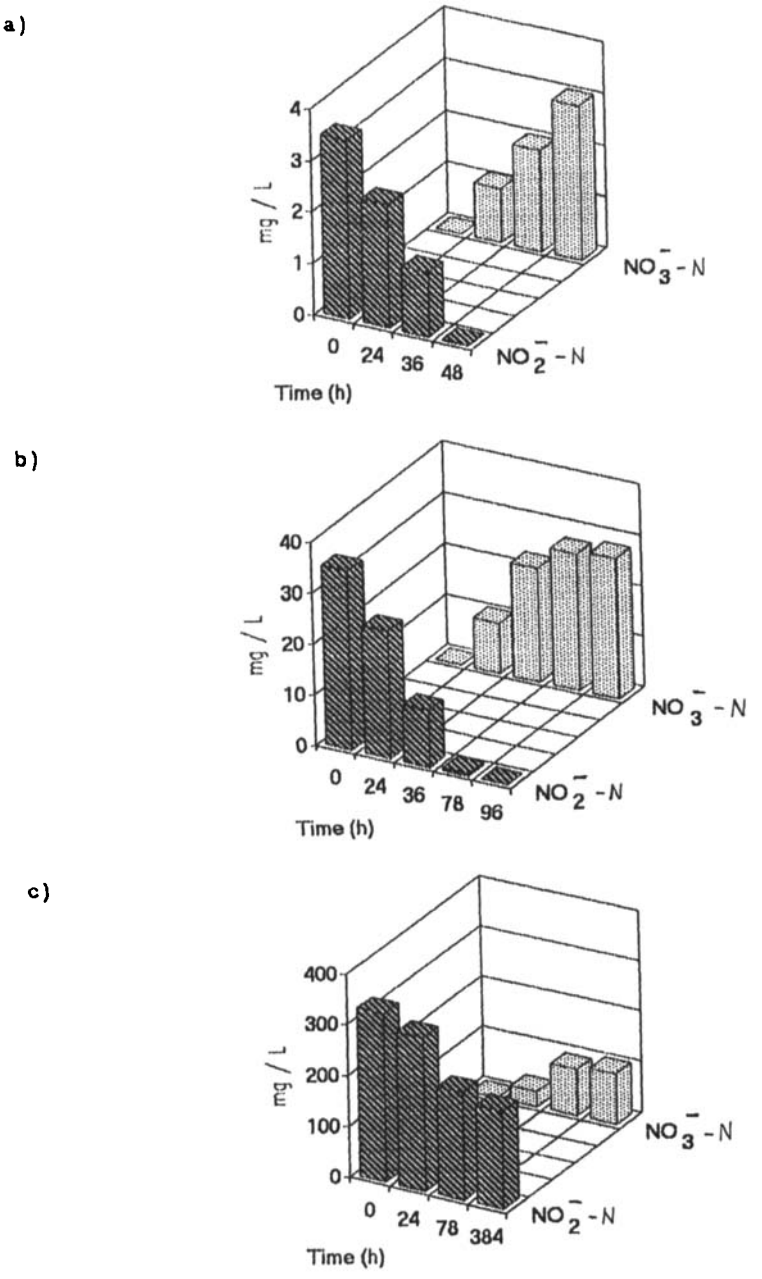

Figure 2 Nitrite oxidation at different initial concentrations 3 mg/L (a), 30 mg/L (b) and 300 gm/L (c) in medium by cells of nitrite oxidizing bacteria during 384 hours at 25 °C and pH 8.

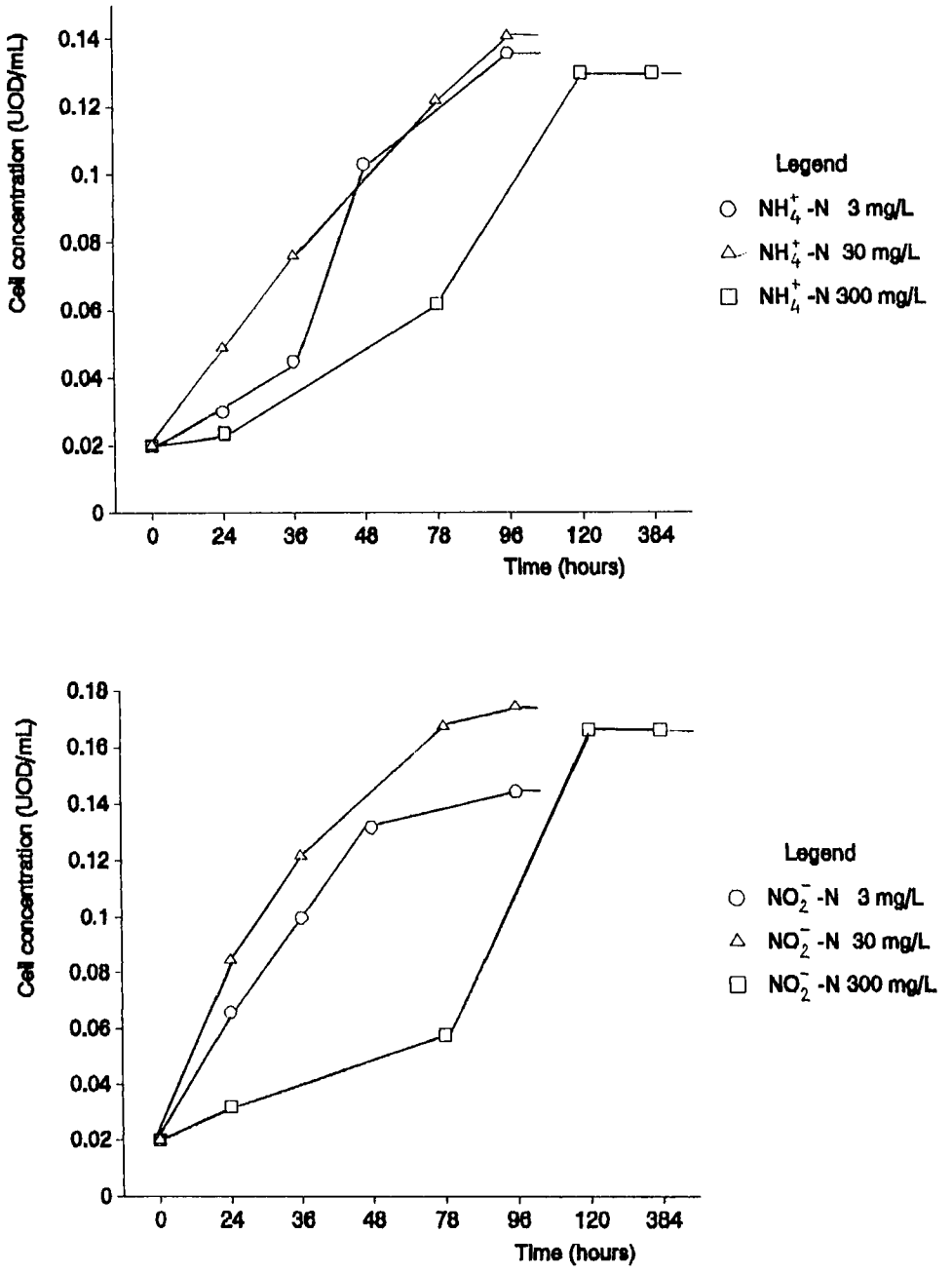

Figure 3 Cell concentrations of ammonia oxidizing (a) and nitrite-oxidizing (b) bacteria during the nitrification processes.

Biological sand filter

The nitrification process in the fixed-bed biological sand filter has been carried out for the first 48 h in a batch reactor, to determine the nitrification velocities in the sample of raw ground water containing 2.9 mg/L of ammonia-N at 20 °C, DO 2–4 mg O_2/L and pH 7.5. After that, the experiment was run in the continuous system.

The concentration pattern of ammonium-N, nitrite-N and nitrate-N with time of a batch experiment is given in Figure 4.

In the first 24 h 68 % of the initial NH_4^+-N concentration was oxidized and at the same time 36 % of NO_2^--N and 27 % of NO_3^--N were formed by biooxidation. Further results showed that the first step nitrification decreased while at the same time the second one increased. After 48 hours only nitrate ion (2.7 mg NO_3^--N/L) was determined. Bacterial attachment on the sand particles, which resulted in biomass accumulation, as well as the aerated environment, enabled a good nitrification which is in accordance with the literature[3,7]. Though the optimal temperature for growth of nitrifying bacteria is between 25–28 °C, ammonia removal (50–100 %) in the biological sand filter was achieved at temperatures from 5 to 21 °C[7].

Sand particles having grain sizes of 3–5 mm were selected favouring bacterial adherence and limiting head loss in sand filter. Prior to the determination of NO_2^--N and NO_3^--N ions by ion chromatography, samples were filtered through a 0.2 μm membrane to avoid bacterial contamination of the column.

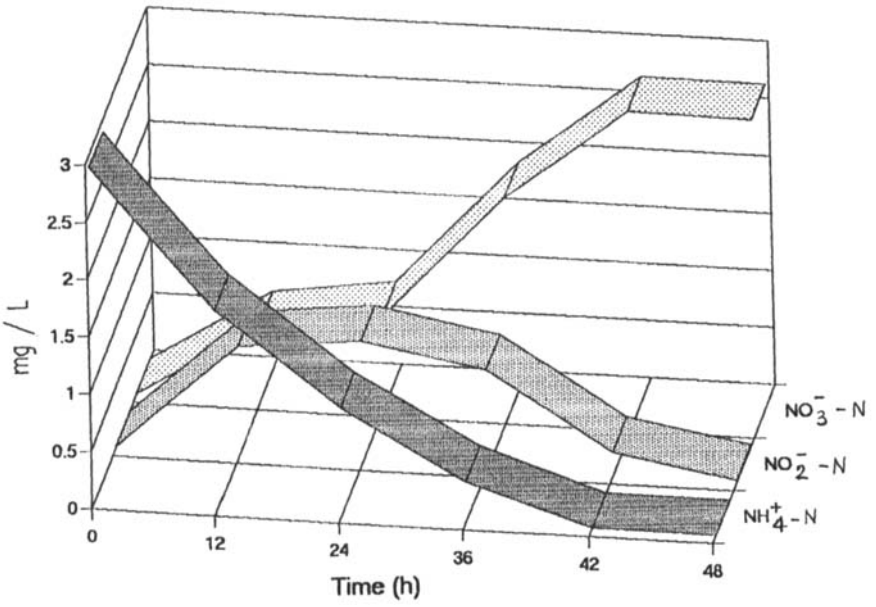

Figure 4 Removal of NH^+_4-N ion from ground water in biological sand filter at 20 °C and pH 7.5.

Determination of nitrite and nitrate

Complex matrices continue to be one of the most challenging areas in analytical chemistry[22]. The evaluation of anion-exchange ion chromatography necessitates the investigation of several aspects of the technique. The resin could be used not only to preconcentrate but also to separate nitrites and nitrates of complex matrices prior to instrumental analysis by conductometry. Relative background conductance and detector responses are predicted with different concentrations of phthalic acid solution and pH ranges in the mobile phase.

Great concentrations of salts in basal medium and different relations of anions and cations in the samples, have influenced the retention time of nitrites (t_1) and nitrates (t_2).

A measure of the quality of the separation actually found is the resolution R. The width of the Gaussian-shaped peaks is determined from the chromatogram as the standard deviation σ, width at half height $w_{0.5}$ ($w_{0.5} = 2.354\ \sigma$) or base width w ($w=4\sigma$):

$$R = \frac{2\ (t_2 - t_1)}{(w_2 + w_1)}$$

At a resolution of 0.5, two maxima can still be perceived separately. For quantitative analysis, a resolution of up to 1.5 is desirable, greater values leading only to unnecessarily long analysis times.

The retention time and width (w) of individual components are dependent essentially on the elution strength of the mobile phase, i.e. changing the concentration of phthalic acid and pH of eluent rise the resolution.

The experimental conditions used in the present study are shown in Table 1. As it can be seen the optimum conditions are obtained at pH = 7.5 of mobile phase.

Injecting 100 µL of the ground water sample, using 1.5 mM of phthalic acid solution containing 5 % (v/v) acetonitril at a flow rate of 1.5 mL/min, nitrite was eluted after 3.17 min and nitrate after 4.42 min (Figure 5).

Pfaff and Brackhoff[15] reported that nitrite-N was eluted after 3.3 min and nitrate-N after 5.8 min at a flow rate of 1.5 mL/min but using a different eluent (1.4 mM sodium carbonate and 0.2 mM sodium bicarbonate).

The determination of these ions by ion chromatography gives reproducible results over a wide concentration range. The technique involves minimal handling and sample prepara-

Table 1 Relation of pH-value of mobile phase and retention times of examined anions.

pH mobile phase	t_1 *(NO_2^--N) min*	t_2 *(NO_3^--N) min*	(w_2-w_1) *cm*	*R*
4.5	2.51	3.50	1.94	1.02
5.5	2.88	3.96	1.44	1.50
7.5	3.17	4.42	0.71	3.50
8.5	3.26	4.15	1.69	1.05
9.5	3.32	4.10	1.48	0.78

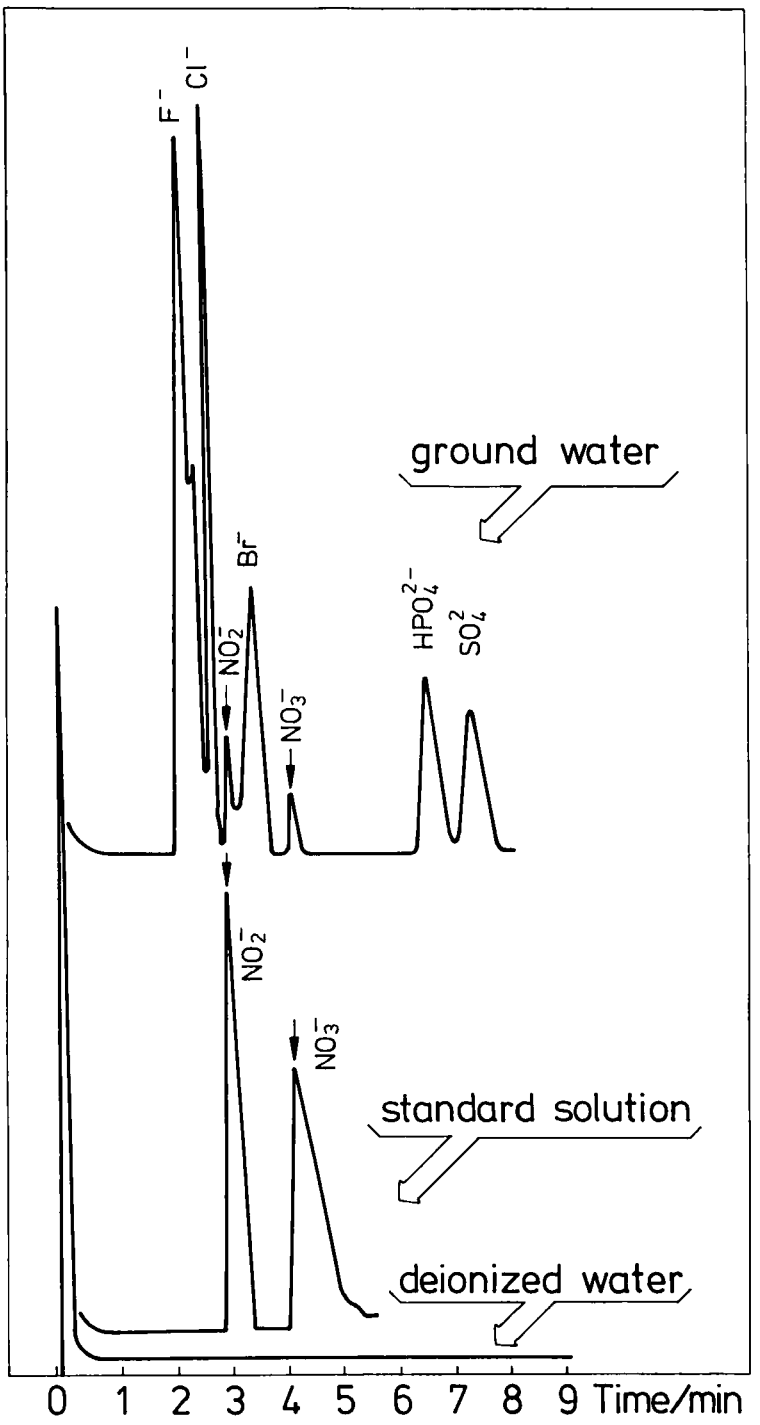

Figure 5 Chromatograms of standard solution (NO^-_2-N and NO^-_3-N concentration 5 mg/L) and of ground water from sand filter (0.8 mg/L NO^-_2-N and 1.9 mg/L NO^-_3-N).
Arrows indicate nitrite and nitrate retention time (Injection vol. 100 µL; flow rate 1.5 mL/min; Full scale 5 µS/cm).

tion[16]. The additional benefit is the possibility for simultaneous determination of fluoride, chloride, bromide, phosphate and sulphate during the same analytical run.

The results obtained in this contribution on ammonia biooxidation in mineral medium and in ground water showed a good reproducibility. They have given us the optimal conditions of the process and represent a basis for the study of ammonia removal in a pilot water treatment plant.

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