PN ABL-109
93229

ANNUAL REPORT

Covering Period: August 1993 - August 1994

Submitted to the Office of The Scientific Advisor
US. Agency for International Development

CELLULOSE-DEPENDENT BIOLOGICAL RESTORATION OF NITRATE POLLUTED AQUIFERS

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Project Number: C13-157
Grant Number: TA-MOU-C13-157

A.I.D. Grant Project Officer:

Project Duration: 11 August 1993 - 10 August 1997

Executive Summary

The objective of this project is to develop a cost effective and simple microbial process for the removal of nitrate from contaminated drinking water by using cellulose-rich substrates as the sole carbon and energy source. The project comprises three years of laboratory work and will culminate with one year of field work when a pilot reactor will be operated in Hungary.

The main activities to be carried out during the laboratory stage of the project include the selection of: (a) inoculum, (b) substrate, (c) substrate pretreatment, (c) reactor configuration, (d) field site in Hungary, and (e) guidelines for the field reactor.

During this first year of the project, various inocula were cultured for the selection of the most efficient cellulose-dependent denitrification in the following substrates: paper, wheat straw, unprocessed cotton and maize leaves (the later in Hungary only). A preliminary comparison of columns packed with one of the three first substrates showed that all supported good rates of denitrification and appeared to offer the potential for future application. An in depth study was therefore initiated for each substrate, starting with printed newspaper. Thus most of the first year was devoted to the investigation of denitrification in columns packed with newspaper.

The results obtained show that printed newspaper can effectively and solely support denitrification. The cellulose degradation/denitrification process is markedly affected by temperature, and in a field facility the decreased capacity at lower temperatures should be compensated by increasing the contact time (longer reactor or lower flow rate). None of the pretreatments studied (diluted alkali, diluted acid and autoclave) improved the performance of the newspaper-packed columns.

The reactor configuration selected was the following: columns kept in the vertical position with flow directed upwards by means of peristaltic pumps. Shredded newspapers were the sole packing material. Gases were allowed to escape through an opening adjacent to the outlet and clogging of the matrix was not observed. The Hungarian group has selected the site for the field trial.

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Section I

A) Research Objectives

The overall objective of this project is to develop a system for the microbial removal of nitrate from contaminated drinking water by using cellulose wastes as the energy source. The specific research objectives are:

(1) Achieve efficient degradation of the cellulose substrate.

(2) Design and operate simple reactors where cellulose degradation and denitrification will be coupled.

(3) Obtain nitrate-free water that will require minimal post-treatment.

B) Research Accomplishments

According to the technical work plan approved for this first year of research, the following activities were carried out:

(1) Selection of efficient cellulose degrading/denitriying inocula.

(2) Selection of substrate and substrate pretreatment.

(3) Selection of a nitrate contaminated well in Hungary.

(4) Study of bacteria isolated from the denitrification reactors.

A manuscript covering the results obtained so far is in preparation and will be submitted before the end of 1994 to the journal Water Research. An abridged version of the manuscript is attached (see Appendix).

C) Scientific Impact of Collaboration

The scientists involved in this project have carried out their activities as two independent but collaborating groups. While similar methodologies were adopted by the two laboratories, the natural conditions prevailing in each country were respected. Thus the selection of inoculum was carried out in each country. In addition, some of the substrates used in the preliminary comparative studies differed.

D) Description of Project Impact

The results obtained are part of the data to be compiled and compared in order to define the guidelines for the field application of the method we propose to develop.

E) Strengthening of Developing Country Institutions

The project investment has allowed the Hungarian institution to undertake the basic research required to back up the practical solution of a very pressing local problem. The exchange of ideas and the meeting in Israel between the two groups has strengthened their collaboration capacity in general and their managerial skills in particular. It is expected that during the coming years Hungarian students will be able to take advantage of the funds budgeted by the Israeli laboratory for the purpose of training.

F) Future work

Based on the experience and results obtained so far, the work plan has been slightly altered:

Activity	Initial work plan	Revised
Selection of cellulose source	1st	1st, 2nd
Selection of reactor configuration	2nd	1st

The performance of packed reactors operated in a continuous mode has been satisfactory. These reactors are easy to build, easy to operate and yield effluents of

high quality. For these reasons, it is felt that there is no need to study also a fluidized reactor, as originally proposed. The operation of a fluidized reactor is more complicated, and its up-scaling more difficult. In order to maintain the fluidized conditions, continuous energy impute in the form of stirring would be required, and a clarification stage would have to be added.

A short comparison of various cellulose substrates was initially planned for the first year. When this was carried out, the results showed that all the substrates tested (wheat straw, newspaper, unprocessed cotton and corn straw) were promising. Bearing in mind that not only the efficiency of a substrate but also its cost and availability are important in the application of the technology we propose to develop, it was decided to carry out an in-depth study of each cellulose substrate. Operational parameters such as flow rates, hydraulic retention times and temperature are being manipulated. It is felt that only in this way can a trustworthy evaluation of each substrate can be achieved.

Apart from the two points raised above, the work plan during the first year ran as scheduled.

During the coming year we will study denitrification in columns packed with wheat straw or unprocessed cotton. The advantages of each substrate will be assessed and compared with those offered by newspaper. Continuing the study of bacteria isolated from the reactors, we will address the following basic question: is the nitrate removal the result of a concerted activity of cellulose degraders and denitrifiers, the first breaking down cellulose to products used by the latter? Or is the removal of nitrate carried out primarily by cellulose degraders capable of denitrifying?

Section II

A) Managerial Issues

Slight changes in the work plan have been introduced and have been discussed under the section entitled "Future work". Changes in the budget are described below.

B) Budget

The following changes in the budget occurred:

1) Hungarian group

Equipment -- The funds approved for the purchase of a microscope were not used. The Hungarian Principal Investigator requests permission to use this sum ($19,500) as partial payment for an Ion Chromatograph.

2) Israeli group

Training -- No Hungarian students were sent to the Israeli laboratory. The funds approved fro training ($ 7,000) will be used for the same purpose at a later stage.

C) Special concerns

No change in the protocols was or is required.

D) Collaboration, Travel, Training and Publications

Throughout the year the two groups have exchanged results and methodology. In August, the Hungarian group comprising Dr. Bitskey, Dr. Novak and Ms. Szocs visited the Israeli group. The Israeli Principal Investigator has postponed her visit to Hungary until after the meeting in Israel. She will travel to Hungary in October.

Contrary to the initial plans, no Hungarian students were sent to the Israeli laboratory during the first year. The Hungarian Principal Investigator will select two suitable candidates for the coming year.

6) Request for A.I.D. or BOSTID Actions

At this stage of the project we have not yet determined how to benefit from the assistance of the A.I.D. or BOSTID staff in promoting project productivity.

Appendix

MATERIALS AND METHODS

Selection of inoculum

Various inocula of cellulose degrading bacteria (e.g., forest and garden soil, wood degraded by termites) were studied for the selection of the most efficient cellulose-dependent denitrification. Cultures were prepared in 150 ml capacity Erlenmeyer flasks containing 75 ml of tap water supplemented with 100 mg/l nitrate (as KNO_3), 3 mg/l phosphate (as KH_2PO_4), 2.5 mg/l cyclohexemide (to prevent fungal growth), and one of the following cellulose substrates, after sterilization in the autoclave: cotton wool (0.4 g), straw (0.5 g) and shredded newspapers (1 g). Cultures were kept on a Labquake shaker (Labindustries, Inc., Marietta, Ohio) inside an anaerobic chamber at 27°C. Media aliquots were removed periodically (2 - 3 times a week) and assayed for nitrate and nitrite. Upon depletion of nitrate, an appropriate volume of a solution containing 1000 mg/l nitrate and 30 mg/l phosphate was added.

The performance of the various inocula was monitored for 4 weeks. From the beginning, it became clear that the inoculum obtained from wood degraded by termites brought about the highest rates of denitrification with all three cellulose substrates, while the inoculum derived from the pine forest soil was equally good with straw. These two inocula were then studied in denitrification columns packed with straw. The columns were operated for 40 days, during which the column containing the inoculum derived from the wood degraded by termites showed the best performance; this inoculum was chosen for further studies.

Experimental apparatus

The laboratory reactors routinely used were 55 cm height, 10 cm dia PVC columns; smaller glass columns (44.5 cm height, 4.5 cm dia) were used in the study on the effect of various pretreatments applied to newspaper. The columns were packed with newspapers shredded to ribbons (0.4 cm width) by means of an office-type paper

shredder. The weight of newspaper packed into the columns varied and is indicated for each experiment. Glass wool plugs were placed at both ends of the packing material. Columns were fed with tap water amended with 100 mg/l nitrate and 3 mg/l phosphate. Flow was regulated by peristaltic pumps and was directed to create an up-flow through the columns; values are presented as Darcy's flux (q, m/day) calculated as $q = (Q/A)$, where Q is the measured flow rate (m^3/day) and A the section area of the column (m^2). The columns were dosed with medium prior to the moment of inoculation, when the void volume was replaced by a suspension prepared by blending in water a small amount of matrix from an active denitrification column (original inoculum obtained as described above). The inoculated columns were allowed to stand for 2-3 days before flow through was initiated. Samples were usually collected twice a week from the inlet and the outlet and routinely assayed for nitrate, nitrite, ammonia, pH and TOC. Bacterial counts were carried out less frequently; room temperature was continuousiy recorded.

Initially, experiments were carried out at room temperature and the temperatures shown are daily averages; later, the columns were kept under controlled temperature (26±1°C, unless indicated otherwise).

Pretreatment of newspaper

The following pretreatments were tried: ball milling, diluted acid, diluted base, and steaming in an autoclave. Ball milling was carried out with a Pascal Eng., UK., mill; the other treatments are summarized in Table 1.

Analytical determinations

Nitrate was determined with an Orion (Orion Research Inc., Boston, MA, USA) nitrate selective electrode or by the colorimetric method described by Cataldo *et al.* (1975). Nitrite and ammonia were assayed according to Standard Methods (APHA, 1989). Carbon was determined by means of a Dohrmann DC-190 (Rosemount Analytical Inc., Santa Clara, CA, USA) high-temperature TOC analyzer.

The chemical composition of newspaper was determined in samples ground to pass a 40-mesh screen in a Wiley Intermediate mill (Thomas Scientific Apparatus, Swedesboro, NJ, USA). Extractive components, acid-detergent fiber, lignin and ash were determined by the Goering-Van Soest method as described by Kirk and Obst (1988). Potential sugars were determined by the method of Scott (1976).

Bacterial counts

Total bacteria in the effluent and in the influent were determined by fluorescent microscopy counts using acridine orange stained samples (Hobbie *et al.*, 1977).

RESULTS

Nitrate removal and water quality

The results presented on Figures 1 and 2 illustrate the performance of a column packed with 400 g of shredded newspapers kept at room temperature and at varying q. During the first days of operation, total removal of nitrate was observed (q= 0.55 m/day) without accumulation of nitrite (Fig. 1) while relatively high concentrations of TOC were measured (Fig. 2). As the initial excess of soluble TOC washed out, breakthrough of nitrate started and was further aggravated by the decrease in room temperature observed around day 26. The subsequent increase in temperature was accompanied by an improvement in the performance of the system (Fig. 1), while successive increments in q (up to 1 m/day) brought about breakthrough of nitrate and nitrite. Complete removal of nitrate with no accumulation of nitrite was again observed in the five-month old column although then at a lower q(0.4 m/day) and at a higher temperature, the reason being that the column had past its period of peak performance and was deteriorating. This decay was observed in all columns operated for longer than 3-4 months. The reasons for the relatively rapid and apparently irreversible deterioration are under investigation. In the studies described below, the columns were

operated for a period of up to three months only, and the results compared were obtained during the "stable" period, usually in the second and third months.

Effect of temperature

Temperature affects the rate of the cellulose degradation/denitrification process. Fig. 3 illustrates losses of nitrogen per day in a column operated at maximum capacity at varying temperatures. The column was packed with 300 g newspaper and operated at a q value of approximately m/day and kept under controlled temperature. The concentration of nitrate in the influent (18 - 38 mg/l) was adjusted to allow breakthrough of nitrate.

Within the range tested, a marked increase in removal of nitrate was observed between 23 and 26 °C. In Israel, ground water temperature is in the order of 20-22°C but higher temperatures will often prevail in an above ground field reactor. The lower activity during the colder winter months in Hungary will be compensated by increasing the contact time in the reactor (by decreasing q or increasing the reactor length).

Effect of flow rate

The effect of q on the removal of nitrate was studied in a column packed with 200 g newspaper by varying the flow rate while supplying a constant concentration of nitrate (100 mg/l approximately) (Figs 4 and 5). Complete removal of nitrate was achieved at q values of up to 0.8 m/day. Breakthrough of nitrate occurred at higher q and, within the range tested, a linear relationship was observed between the percentage of nitrate removal and q. At the highest flux tested, 1.5 m/day, the concentration of nitrate decreased by 30% only.

In terms of amounts of nitrate removed per day, values increased with q up to 1.2 m/day; thereafter, a sharp decline was observed (Fig 5).

Effect of newspaper pretreatment

The effect of pretreating newspaper was investigated. The methods assessed were: ball milling, swelling with 1% NaOH, swelling with 1 N HCl and heat treatment in an autoclave.

Ball milling of newspaper, in our opinion, is not a viable option: the process is extremely slow, therefore, too expensive in terms of energy required. Milling gradually pulverized the paper fragments from the edges (the rest remaining practically intact) and the fine matrix of the final product would require a complicated reactor design and operation. For these reasons, this pretreatment was not pursued.

Table 1: Treatments applied to newspaper prior to packing into the experimental columns. During treatment, the ratio of newspaper to liquid was 100 g/4 l. Treatments were followed by rinsing with tap water. In experiment 1, the acid- and base-treated paper was washed until no change was observed in the pH of the rinsing water, the volume of water used was measured and an equal volume was used for rinsing the control and the autoclave-treated paper.

Column	Experiment	Treatment
Control	1	Water for 24 h in a shaker at room temperature
Autoclave	1	Autoclaved in water for 2h
Acid	1	1N HCl for 24 h in a shaker at room temperature
Base	1	1% NaOH for 24 h in a shaker at room temperature
Control	2	Water for 2 h at room temperature
Autoclave	2	Autoclaved in water for 2h

The effects of the other methods were studied in small columns packed with newspaper subjected to the treatments described on Table 1. In experiment 1, four columns were packed with one of the following types of newspaper: control, autoclave-treated, diluted acid-treated and diluted base-treated. The columns were operated at identical flow rates.

Neither diluted acid nor the diluted base pretreatment improved the denitrification capacity of the columns (Figs 6 and 7). In fact, a negative effect was observed after the first week of operation, with lower removal of nitrate and higher accumulation of nitrite. As was shown in Fig. 2, an excess of soluble TOC is available and eluted from the columns during the first week. During this period, total removal of nitrate was achieved by all the columns (Fig. 7).

The results were inconclusive as to the effect of pretreating newspaper in the autoclave: as compared to the control, a transient slight improvement of the denitrification rates with lower nitrite accumulation may have occurred; however, from the time at which no nitrate breakthrough was observed the true capacity of each column could not determined (from day 35 onwards, both columns reached overcapacity at the flow rates employed).

To clarify this point two new columns (Table 1, experiment 2; Fig. 8) one control and one packed with autoclave-treated paper, were operated at flow rates allowing breakthrough of nitrate (thus comparison of their denitrification capacities was possible). The results obtained showed that the autoclave pretreatment negatively affected the overall removal of nitrate.

Except for a small decrease in the extractable fraction of the paper treated by diluted alkali or base, the pretreatments appeared to cause no change in the chemical composition of newspaper (results not shown here). The appearance of the printed newspaper suggested that diluted alkali and diluted base removed ink; this would explain the lower proportion of extractables.

Conclusions:

Cellulose wastes in the form of printed newspaper serve as efficient substrates for denitrification.

The cellulose degradation/denitrification process is markedly affected by temperature. The decreased rates of denitrification at lower temperatures can be compensated by increasing contact time in the reactor (lower q or longer columns)

Fixed-bed reactors packed with untreated newspaper achieve high rates of denitrification and yield water of high quality.

References

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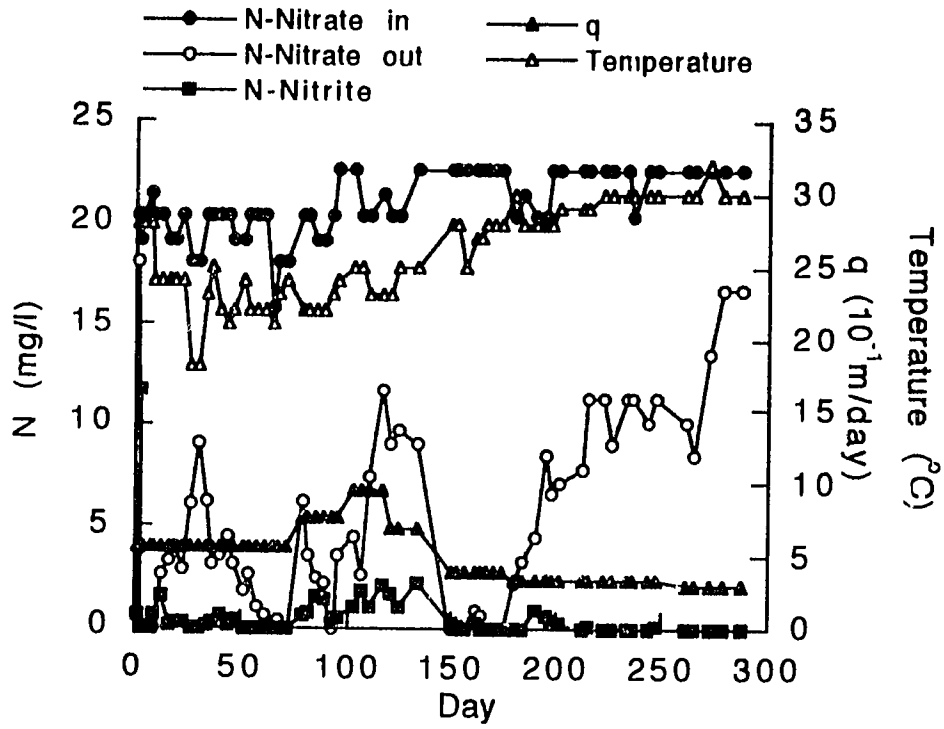

Figure 1: Inlet concentration of nitrate and outlet concentrations of nitrate and nitrite in a column packed with 400 g of shredded printed newspaper and operated at room temperature.

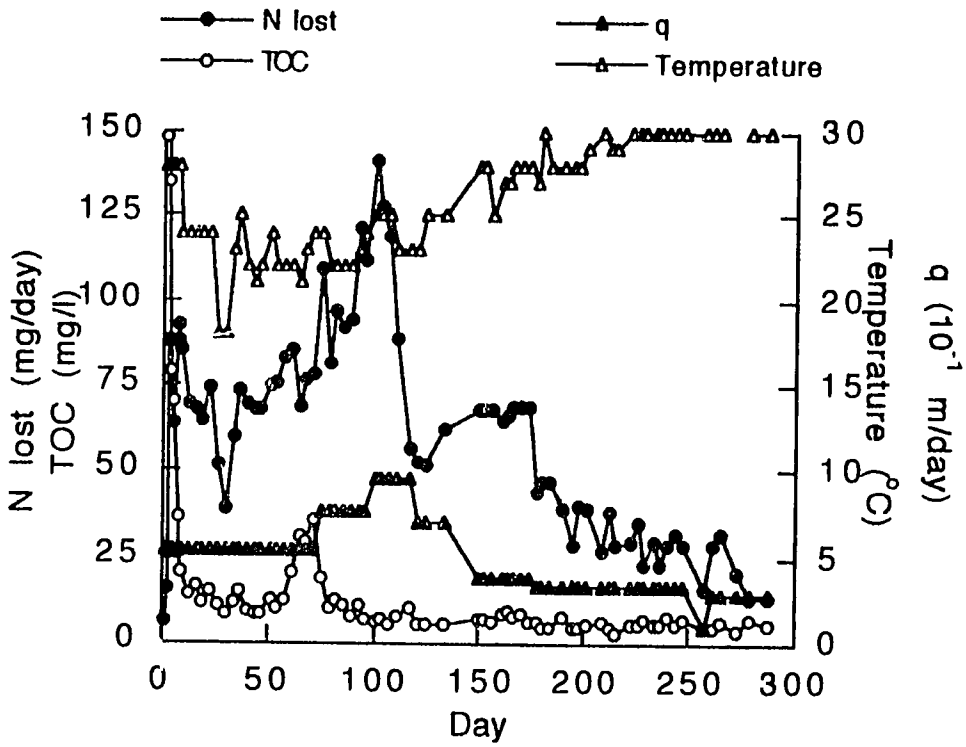

Figure 2: Nitrogen removal and effluent TOC concentrations in the column described in Fig. 1.

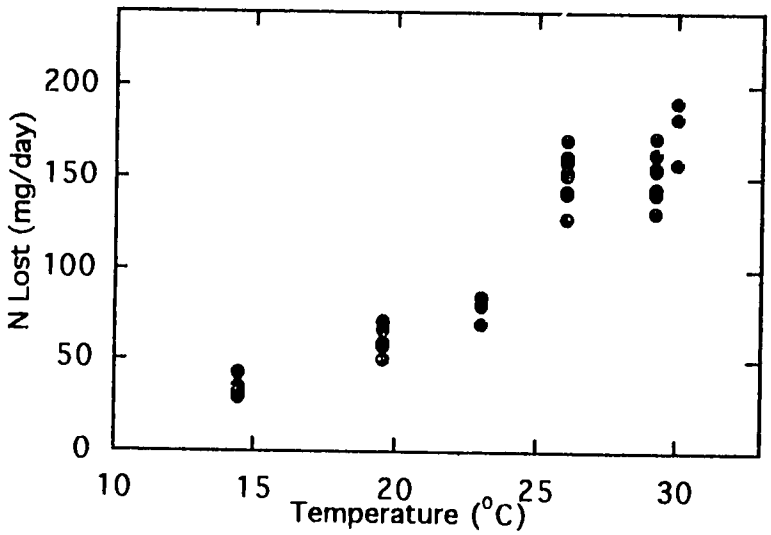

Figure 3: Effect of temperature on the removal of nitrogen in a column packed with 300 g shredded newspaper.

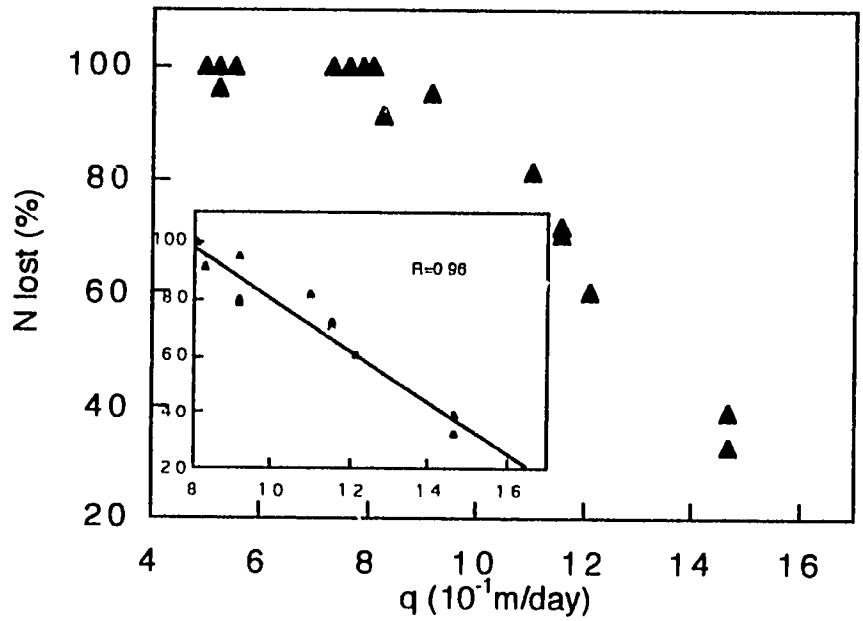

Figure 4: Effect of flow rate on the percentage of nitrogen removal in a column packed with 200 g shredded newspaper and kept at 26±1°C.

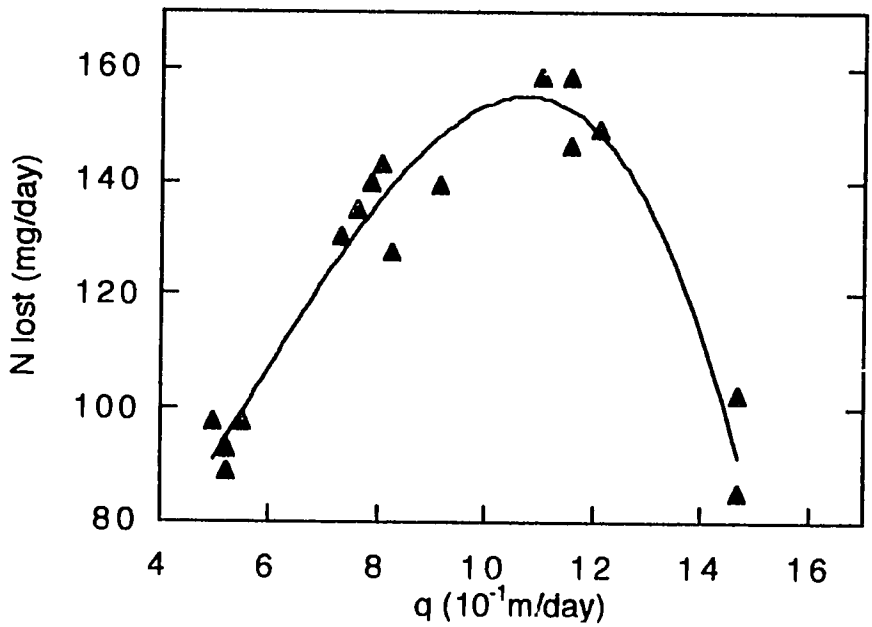

Figure 5: Effect of flow rate on the amount of nitrogen removed per day in the column described in Fig. 4.

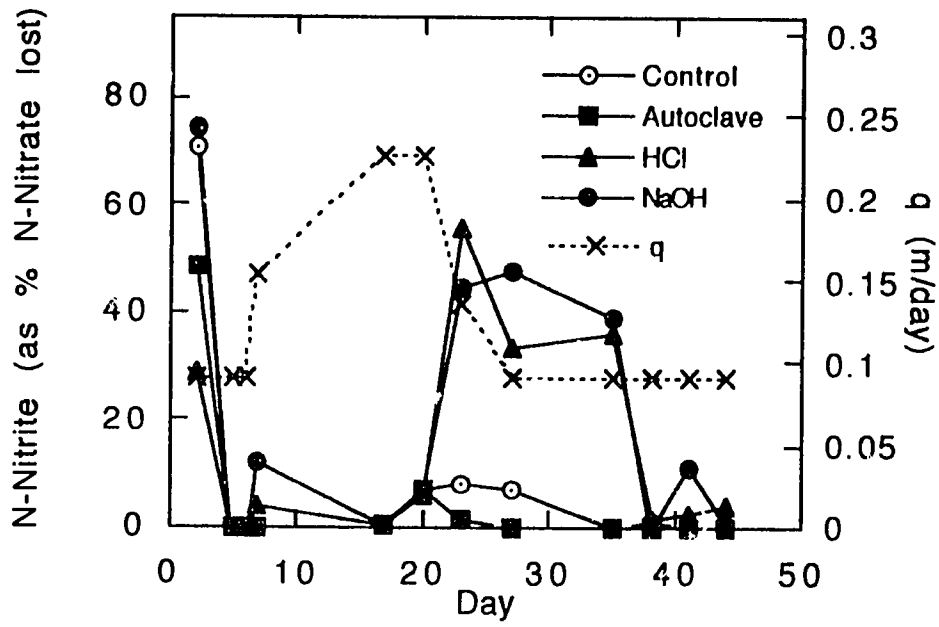

Figure 6: Accumulation of nitrite in small columns packed with 154 g of newspaper pretreated as described in Table 1 (experiment 1) and kept at 26±1°C.

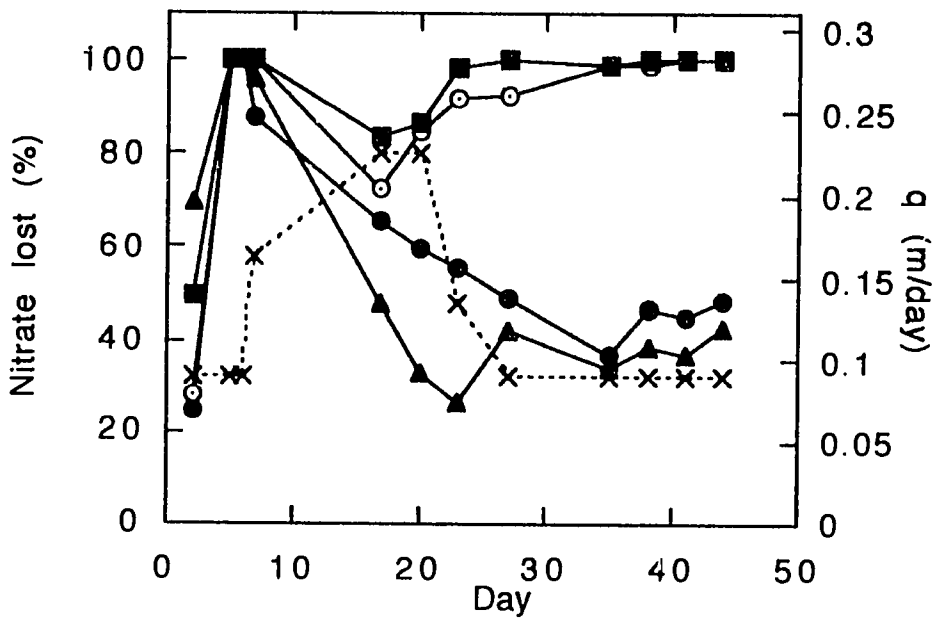

Figure 7: Removal of nitrate in the columns described in Fig. 6 (legends as in Fig.6).

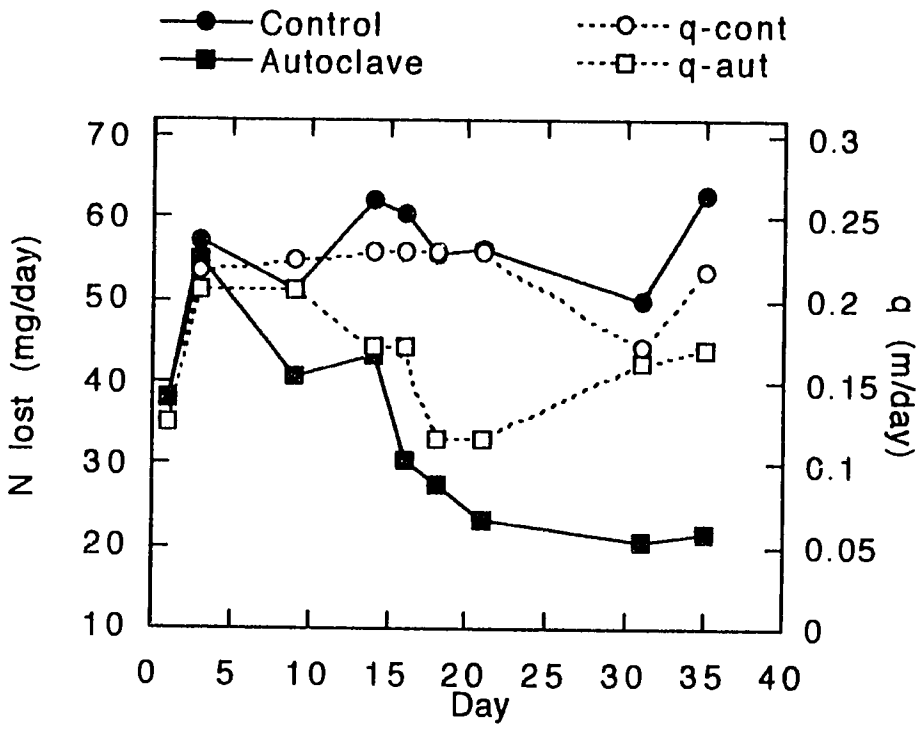

Figure 8: Nitrogen removal in small columns packed with 100 g of newspaper treated as described in Table 1 (experiment 2) and kept at 26±1°C.