PN ACS-998
101391

ANNUAL REPORT

Covering Period January 1998 - December 1998

Submitted to the Center for Economic Growth
U S Agency for International Development

Hydrogen-dependent denitrification of drinking water

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Grant Number TA-MOU-C15-045

Project Duration 1 January 1996 - 31 December 1999

Executive Summary

The objective of this study is to develop an efficient, cost effective microbial process for removal of nitrate from polluted ground water A small-scale biological system is being developed in which denitrification is achieved by autotrophic bacteria using hydrogen as their energy source

Generation of the required hydrogen is an integral part of the denitrification setup and is achieved by electrolysis of the water to be treated in an electrolysis cell (EC) The EC is divided in two compartments by a cation-exchange membrane which prevents oxygen formed in the anode from reaching the cathode and the water to be treated (oxygen inhibits denitrification), water is pumped through the cathodic compartment where it is enriched with hydrogen

Two system configurations were studied (1) a single-reactor with the biomass in the cathodic compartment of the electrochemical cell (ECBR) and (2) a system consisting of two distinct units, an electrochemical cell and a bioreactor (EC+BR) In both systems granulated activated carbon (GAC) served as physical support for the biomass

The establishment of various inocula on the surface of GAC was studied as well as the overall performance of the denitrification systems with different inocula

The main practical conclusions that can be drawn from the studies carried out during the past year are

- The two-reactor configuration is the most suitable for upscaling and long-term operation
- The establishment of biomass on GAC is rapid, and the start up phase of the reactors lasts a few days only
- Among the inocula tested, a mixture of strains (isolated from a previous reactor which had been inoculated with an enrichment culture from an oxidation pond) was the most effective, yielding the highest rates of denitrification and the lowest breakthrough of nitrite

A setup for the measurement of dissolved hydrogen was developed in collaboration with T Bejerano, the electrochemist adviser to the Israeli group

Istvan Kiss, a Ph D student form the Hungarian group has been in the Israeli laboratory since April

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Section I

A) Research Objectives

The overall objective of this project is to develop and standardize a simple, small-scale biological system for the treatment of nitrate contaminated drinking water This will be achieved by denitrifying bacteria using hydrogen as the energy source The specific research objectives are

(a) Design and operate small-scale reactors where hydrogen production and denitrification will be coupled

(b) Obtain nitrate- and nitrite-free water that will require minimal post-treatment

B) Research Accomplishments

Selection of the most suitable configuration for the denitrification system and selection of the most effective bacterial inoculum were the main research accomplishments of the past year Special attention was also devoted to the establishment of biomass in the reactors Preliminary characterization of bacteria isolated from the reactors was carried out

Introduction

Increasing nitrate contamination of current and potential drinking water sources is rapidly reducing the water resources in many parts of the world The process is particularly accelerated in developing countries where rapid demographic and economic changes are taking place and the concentration of human activities in fast growing urban settlements is seriously threatening the

the quality of the water supplies The establishment of intensive agriculture as well as the use of effluents in irrigation pose an increasing problem of nitrate contamination of the aquifers Excessive levels of nitrate in some countries have required the capping of drinking water wells which in turn forces people to resort to contaminated surface water

In Hungary, elevated nitrate level in natural water sources is a widespread phenomenon Several dozen of the country's small villages are already so heavily contaminated that bottled drinking water has to be shipped in Denitrification of drinking water is not a common practice even at the water treatment plants of large settlements, and the adverse public health effect of the steadily increasing nitrate accumulation reaches a growing number of consumers Finding alternative non-polluted aquifers and building long water conduits have been considered to be very costly and often impossible the present mode of transferring potable water in plastic bags is neither satisfactory nor sustainable Thus, nitrate removal from the contaminated water sources is urgently needed, and simple, cost-effective methods are required

The biological method generally applied in nitrate removal relies on the capacity of certain bacteria to use nitrate under anaerobic conditions as a terminal electron acceptor in their respiratory processes This is achieved through a series of enzymatic steps (denitrification) and results in the reduction of nitrate to N_2

Denitrifiers are abundant in nature (Gamble *et al*, 1982) and are primarily heterotrophs - an organic carbon substrate is required for both energy and cell synthesis Known exceptions are sulfur oxidizers which can utilize elemental sulfur and thiosulfate (or even sulfide in limited quantity) as the electron donor for denitrification, and hydrogen oxidizers which can use hydrogen as the electron donor for denitrification

Hydrogen gas is an ideal energy substrate for denitrification in the sense that it is completely harmless to potable water and no further steps are required to remove either excess substrate or its derivatives which is the case with other organic (e g , methanol, acetate, ethanol) and inorganic (e g , sulfur) substrates In addition, since only a few species can carry out H_2-dependent denitrification, the production of biomass is very low

In spite of the obvious advantages the use of H_2 is limited by two of its inherent properties formation of flammable and explosive mixtures with oxygen, and very low solubility in water (1 6 mg l^{-1} at 20°C)

In recent years, two new approaches have been described which avoid the disadvantages associated with the use of H_2 The first, proposed by the Hungarian partner and co-workers, uses an immobilized mixed bacterial population containing specially selected H_2 producing bacteria and denitrifiers, H_2 is safely and effectively delivered to the denitrifying bacteria by interspecies H_2 transfer but organic carbon is required for the production of H_2 (Kovacs and Polyak, 1991)

In the second approach, described by Sakakibara and Kuroda (1993), H_2 was produced by the electrolysis of water A film of denitrifying bacteria was allowed to develop on the surface of a cathode while the anode was kept clean, electric current was then applied, and the hydrogen produced at the cathode was immediately taken up by the microorganisms on its surface The current study is based on the approach of Sakakibara and Kuroda (1993)

Stoichiometry of the process

The net chemical reaction describing the hydrogen-dependent denitrification reaction is

$$2\ NO_3 + 5\ H_2 + 2\ H^+ \rightarrow N_2 + 6\ H_2O \qquad (1)$$

The hydrogen is provided by the cathodic reaction

$$10\ e + 10\ H_2O \rightarrow 5\ H_2 + 10\ OH \qquad (2)$$

Combining equations (1) and (2) and rearranging gives

$$10\ e + 2\ NO_3 + 12\ H^+ \rightarrow N_2 + 6\ H_2O \qquad (3)$$

Assuming that the sole anodic reaction is

$$5\ H_2O \rightarrow 21/2\ O_2 + 10\ H^+ + 10\ e \qquad (4)$$

and that there is no separation between the anode and cathode (undivided electrochemical cell) then the net reaction is given by

$$2\ NO_3 + 2\ H^+ \rightarrow N_2 + 21/2\ O_2 + H_2O \qquad (5)$$

In this case, one equivalent of acid is needed for each mole of nitrate

If the EC is separated by a cation-exchange membrane, and dilute sulfuric acid is used as an anolyte, then most of the current — about 95% — will be carried through the membrane by protons Thus, similar demand for acid is expected in both divided and undivided electroreactors Only minor pH changes are expected at the concentrations of nitrate used in these studies

Materials and Methods

Experimental set up

Two system configurations were studied (1) a single reactor (Fig 1) with the biomass in the cathode compartment of the electrochemical cell (ECBR), and (2) a system consisting of two distinct units (Fig 2), an electrochemical cell and a bioreactor (EC+BR)

The basic design of the electrochemical cell (EC) is schematically represented in Fig 3 and was identical in both systems, except for differences in dimensions described below A Nafion 350 cation-exchange membrane (Du Pont Wilmington, DE) separated the two electrodes and Hypalon rubber spacers intercalated between the membrane and the electrodes created cathodic and anodic chambers Plates of rubber (3 mm thick) PVC (1 cm thick) and stainless steel (1 cm thick), in this order, followed each electrode, and the different layers were held together by four stainless steel screws and bolts The rubber plates and spacers were 3 mm thick except for the spacer in the cathodic side of the ECBR system which was 6 mm thick The anode was made from a plate of 2 mm thick titanium coated with platinum-iridium oxide and the cathode was made from 5 mm thick graphite plates The dimensions of the electrodes and of the cathodic chambers in the two denitrification systems are summarized in Table 1 The electrodes were connected to a Sunrise RPS 3025 power supply

The EC was kept in the vertical position and peristaltic pumps regulated the flow of the solutions 0 1 N H_2SO_4 was recirculated in the anodic chamber and the feed solution flowed through the cathodic chamber

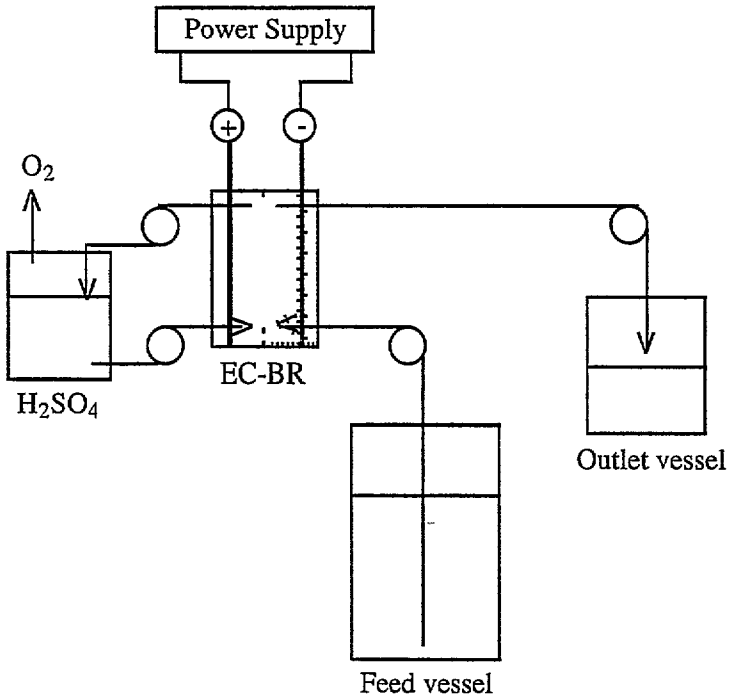

Fig 1 Schematic representation of the single reactor system (ECBR)

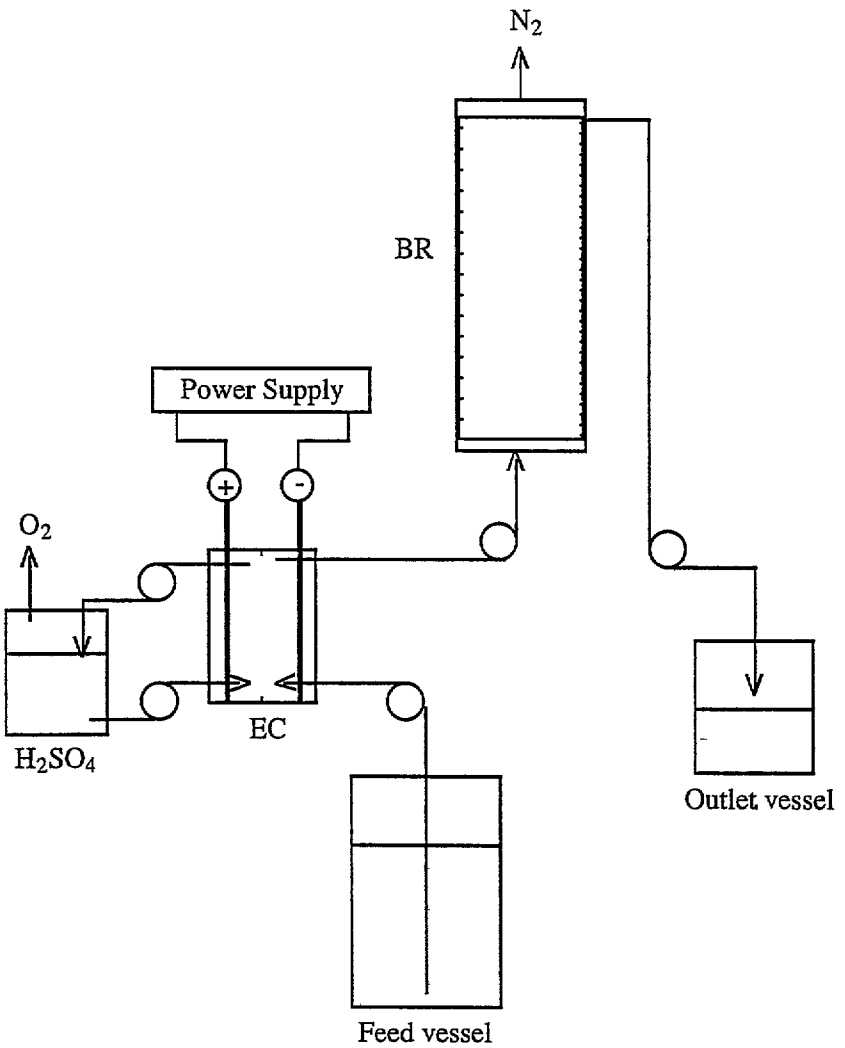

Fig 2 Schematic representation of the two-reactor system (EC+BR)

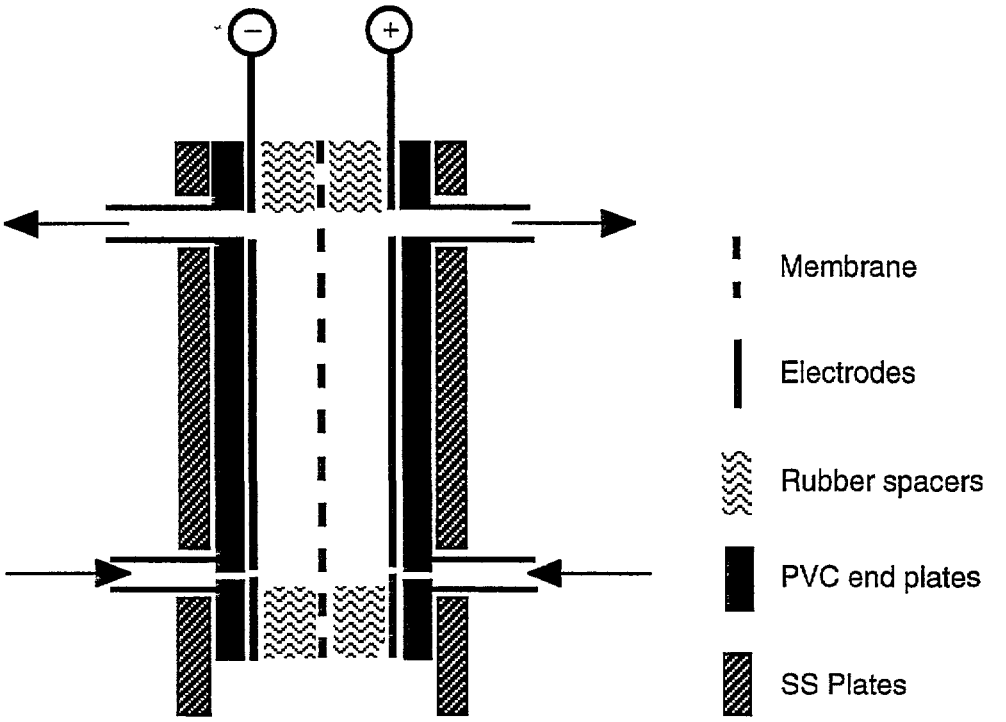

Fig 3 Schematic representation of the electrochemical cell

The feed solution consisted of tap water amended with phosphate (1g KH_2PO_4 l^{-1}), bicarbonate (0 07 g $NaHCO_3$ l^{-1}) and nitrate (usually around 0 16 g KNO_3 l^{-1}), the feed reservoir was gently sparged with N_2 (to remove dissolved oxygen)

Pre-washed 0 85 - 1 70 mm matrix granulated activated carbon (GAC) served as biomass substratum In the single-reactor system (Fig 2) the cathodic compartment was filled with 35 g GAC In the two-reactor system the feed solution was enriched with hydrogen in the EC prior to entering a column packed with 4 5 cm diameter and 45 cm high bed of GAC (Fig 3) The reactors were kept at 25±1° C

Table 1 EC characteristics in the single-reactor system (ECBR) and in the two-reactor system (EC+BR)

		ECBR	EC+BR
Electrodes	Dimensions (cm)	17 x 9	12 x 7
	Area (cm^2)	1 5 3	8 4
Cathodic chamber	Dimensions (cm)	14 x 7 x 0 6	9 x 5 x 0 3
	Volume (cm^3)	6 0	1 3 5
	GAC (g)	3 5	-
	Void volume (cm^3)	1 7	-

The following water parameters were routinely monitored concentrations of nitrate, nitrite, ammonia dissolved organic (DOC) and inorganic carbon (DIC), and pH Bacterial counts were carried out less frequently

Inoculum

Various inocula were studied (1) *Paraccocus denitrificans* 22, (2) strains A4, M2, M3 and P11 isolated from previous reactors which had been inoculated with an enrichment culture from an oxidation pond) and (3) various combinations of the strains under (2)

The effect of the inoculum pregrowth conditions, autotrophic and heterotrophic, was studied by cultivating cells in mineral medium (Schlegel *et al*, 1961) or in nutrient broth Routinely, cells were grown in nutrient broth, harvested by centrifugation followed by washing and resuspension in mineral medium The suspension was then recirculated through the reactors for 3-5 days unless when indicated otherwise

Batch cultures in GAC

Batch cultures were prepared in 250 ml Erlenmeyer flasks containing 15 g GAC and 100 ml of liquid medium The media tested were the mineral medium of Schlegel *et al* (1961) in which ammonia was replaced by 45 mg nitrate-N l^{1} and enriched with 0 2 % (w/v) of either acetic acid or sucrose, or nutrient broth containing 45 mg nitrate-N l^{1} The cultures were incubated for 24 days at 25°C under static conditions with media replacements on days 5, 12 and 17

Adsorption of bacteria to GAC

Mixed and single strain cultures were prepared in nutrient broth containing 45 2 mg nitrate-N l^{1} After 48 h the cells were harvest by centrifugation (15 min at 4 500 rpm), washed with 0 85% NaCl and resuspended in mineral medium Each suspension was then recirculated at a rate of 1 ml min^{1} through a (50 or 75 cm high, 0 8 cm diameter) column packed with 16 g GAC Changes in OD_{600} of the suspensions were monitored during 24 h and the initial readings were normalized In one experiment with strain A4, the pH of the final suspension was 3 5, 7 25 (control pH of mineral medium), 8 4 or 9 9

The columns were then supplied with feed solution (tap water amended with nitrate, bicarbonate and phosphate) at a rate of 3 ml min^{1} After 24 h, GAC samples were removed for determinations of protein, polysaccharides and denitrification activity

In the experiment with chitosane-treated GAC, a stock solution of 0 5% (w/v) chitosane in 1% (v/v) acetic acid was diluted 1 10 and 1 100 with mineral medium, 25 g GAC were added to 100 ml of each dilution and stirred for 6 h Controls without chitosane were also prepared The various batches of GAC were air dried prior to packing into 50 cm high 0 8 cm diameter columns An inoculum was prepared and divided into four batches, all the following steps of the experiment were as described above

Characterization of bacterial isolates

Isolates were characterized using standard methods (Gerhardt *et al*, 1994) The following parameters were studied appearance of colonies on different media, shape of bacteria, Gram reaction,

oxidase activity (by the addition of 1% aqueous tetramethyl-p-phenylenediamine on filter paper) catalase activity (formation of bubbles with a 3% hydrogen peroxide) and accumulation of poly-β-hydroxybutyrate granules (staining with Nile Blue) The ability to denitrify was determined as described by Tiedje (1982) using Durham tubes and nutrient broth supplemented with nitrate or nitrite, tests showing gas accumulation were confirmed by assaying for the detection of nitrate and nitrite (Morgan, 1930) Additional physiological characteristics were determined by means of API 20 NE (Biomerieux France) and Biolog (Biolog, USA) diagnostic kits

Assay of denitrification activity

Measurements of rates of denitrification were by the method of C_2H_2 inhibition of N_2O reduction Triplicate assays were carried out in 9 ml glass bottles (with butyl rubber septa in screw caps) containing 3 ml of mineral medium of Schlegel *et al* (1961) (as described by Aragno and Schlegel 1992) supplemented with 113 mg nitrate-N l^{1} and 0 2% (v/v) acetic acid (pH readjusted), 1 2 mg l^{1} chloramphenicol and the GAC sample (0 3 0 6 g dry weight) to be tested The bottles were flushed with N_2 to remove all traces of O_2 Approximately 0 6 ml of acetylene was injected into the gaseous phase (10% final concentration) and the flasks were incubated at 25°C After 24 h the flasks were shaken (to ensure equilibration of N_2O between gas and aqueous phase) and the concentration of N_2O was determined in 0 1 ml samples removed from the gaseous phase The total amount of N_2O formed (M) was calculated using the Bunsen absorption coefficient according to the following equation

$$M = C_g\ (V_g + V_l\ \alpha)$$

where C_g is the concentration of N_2O in gas phase, V_g is the volume of gas phase, V_l is the volume of liquid phase, and α is the Bunsen absorption coefficient The Bunsen absorption coefficient for N_2O in water at 25°C was 0 544 (Wilhelm *et al*, 1977)

Analytical determinations

Nitrate was determined as described by Cataldo *et al* (1975), nitrite and ammonia were assayed according to Snell and Snell (1949) and direct Nesslerization (APHA, 1992) respectively and the concentrations of DOC and DIC were determined with a high-temperature TOC analyzer (Dohrmann DC-190, Rosemount Analytical Inc , Santa Clara, California)

Protein was determined by the method of Lowry *et al* (1951) using crystalline bovine serum albumin as standard GAC samples were transferred to small test tubes and treated with 1 ml of 1 N NaOH, for 30 min in a shaking water bath at 60 °C, after centrifugation protein concentrations were determined in 0 2 ml aliquots of the supernatant

Polysaccharides were determined in GAC samples treated with 1 ml of 0 01 M EDTA for 30 min in a shaking water bath at 60 °C The concentration of total sugars was determined in 0 5 ml aliquots by the phenol-sulfuric method (Dubois *et al*, 1956)

Nitrous oxide determinations were carried out with a gas chromatograph (GOW-MAC Instrument Co , Terrance, CA, USA) equipped with a thermal conductivity detector and a Porapak Q (Supelco, Inc , Bellefonte PA, USA) column The detector temperature was 50° C and the flow rate of

the carrier gas, helium, was 25 ml min^{-1} The gas chromatograph was connected to a Hewlett Packard (Avondale, PA, USA) 3390A integrator

The weight of GAC samples used in the assay of denitrification activity and in the determination of protein concentrations was determined by drying to constant weight at 105 °C

Amperometric analyzer for dissolved hydrogen in aqueous solutions

As commercial analyzers for dissolved hydrogen in aqueous solutions are very expensive we decided to try to construct ourselves an amperometric, analyzer similar in principle to those for dissolved oxygen The electrochemical cell, Fig 4, comprised a platinum working electrode (WE) in the shape of a ring, and a Ag/AgCl reference electrode (RE), both concentrically enclosed in a glass tube filled with about one ml of a 3M KCl solution A porous polypropylene membrane sealed the cell with the aid of a Viton O-ring

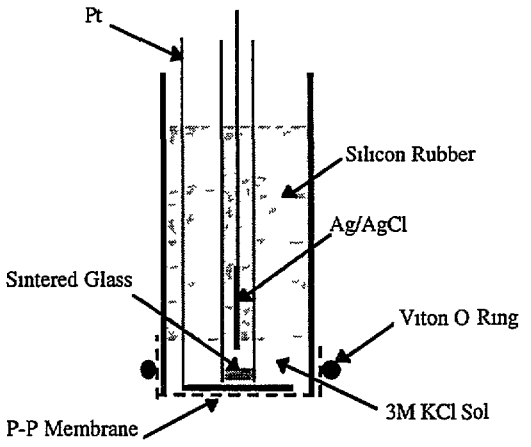

Fig 4 Assembly of the electrochemical cell of the hydrogen analyzer

The cell was controlled by a potentiostat (microstat Model 1503) A constant potential of + 0 6V was applied on the WE versus the Ag/AgCl RE To enable measurement of the expected low currents, in the range of 10^{-5} Amps, a precise resistance of 10^6 Ohms was connected between the counter electrode-CE and the RE outputs of the potentiostat (Fig 5) Measurement of the potential drop on the resistance, by a high impedance voltmeter such as pH meter, served as the current monitor, (1mV corresponds to 1nA) The current through the RE output is negligible - less than 0 01 pA

The current in this system is controlled mainly by the diffusion through the membrane, with only minor influence of the stirring of the analyzed solution, therefore it is expected that a linear dependence will exist between the current and the hydrogen concentration In Fig 6 a calibration curve done with various dilutions of a H_2 saturated solution is shown As expected the experimental dependence was close to linear Our objective was not to determine exact concentrations of H_2 for mass

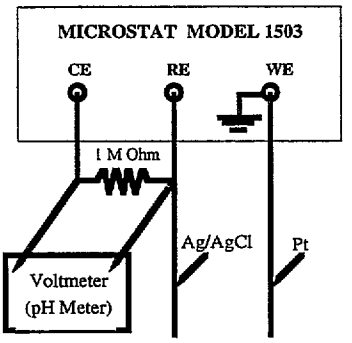

Fig 5 Connection of the microstat to the cell in Fig 4 and to the current measuring set-up

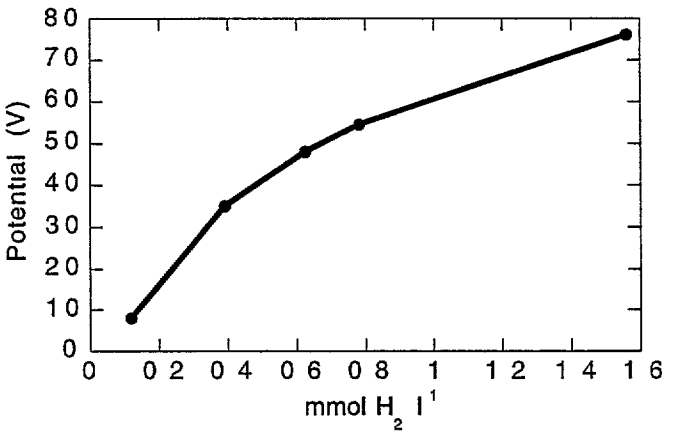

Fig 6 Hydrogen calibration curve

balance calculations but rather to detect H_2 in the reactors effluent as a means to verify that the supply of electron donor was not limiting

Bacterial counts

Colony forming units were counted in water samples by standard plating techniques on R2A agar (Difco Laboratories) The numbers of denitrifiers were estimated by the most probable numbers counts in the medium of Focht and Josef (1973) as described by Tiedje (1982), tubes were incubated in a Forma Scientific (Mallincrodt Inc , Marietta, Ohio) anaerobic chamber

Results and Discussion

The main points studied during the past year were

- Inoculum type single strains, mixtures of known strains, enrichment culture
- Biofilm establishment
- Long-term stability and performance of reactors

- Process design – single reactor and two-reactor systems
- Characterization of bacterial isolates obtained from the reactors

Throughout these studies the ultimate aim of the project – to develop a simple and user friendly denitrification process – was kept in mind Thus, instead of carrying out a systematic and sequential study of all the parameters listed above, various experiments were run in parallel and usually the simplest approaches turned out to be satisfactory and were selected An example was the type of inoculum Preliminary single-strain studies carried out in liquid cultures showed that strain A4 was the most efficient denitrifier and the strain was used in a number of studies on biofilm establishment The use of single strains in "real life" would be less practical but the effort might be justifiable by the superiority of the performance However, as it is shown below, the reactors inoculated with A4 were not more efficient than those inoculated with an enrichment (mixed) culture and the research on A4 was discontinued

Summarized below are some of the results obtained

Biofilm Establishment

Batch Experiment

The objective of this experiment was to determine if any of the denitrifying isolates tested was a faster colonizer than the others Thus, the establishment on GAC of strains A4, M2, M3 and P11 was studied in batch cultures in mineral medium with acetate or glucose as sole carbon source The concentrations of protein and polysaccharides and denitrification activity in samples of GAC removed from the cultures were used as indicators of biofilm establishment (Fig 7)

The concentration of protein increased with time in all cultures with the highest growth in nutrient broth and the lowest in acetate (Fig 7 row 1) The concentration of polysaccharides oscillated, and in the acetate and nutrient broth cultures it tended to be higher during the first two weeks In glucose the pattern was not clear and some of the oscillations may have been due to the interference of glucose in the polysaccharide detection method (Fig 7, row 2) The denitrification capacity in GAC samples removed from the batch cultures was expressed in two ways as mg N_2O-N g^{-1} GAC (Fig 7, row 3) or as mg N_2O-N mg^{-1} protein (Fig 7, row 4) With the former method of expression, an increase in activity was observed during the first two weeks followed by approximately constant rates When expressed on a protein basis, the highest denitrification rates were observed around days 5 to 10 (Fig 7, row 4) and this discrepancy might be explained by mass transfer limitations following the increase in biofilm thickness as indicated by the increase in protein (Fig 7, row 1) From the denitrification activity results one can conclude that although strain A4 appeared to be the most active, no major differences existed among the various strains and treatments and that the thin biofilms were the most efficient

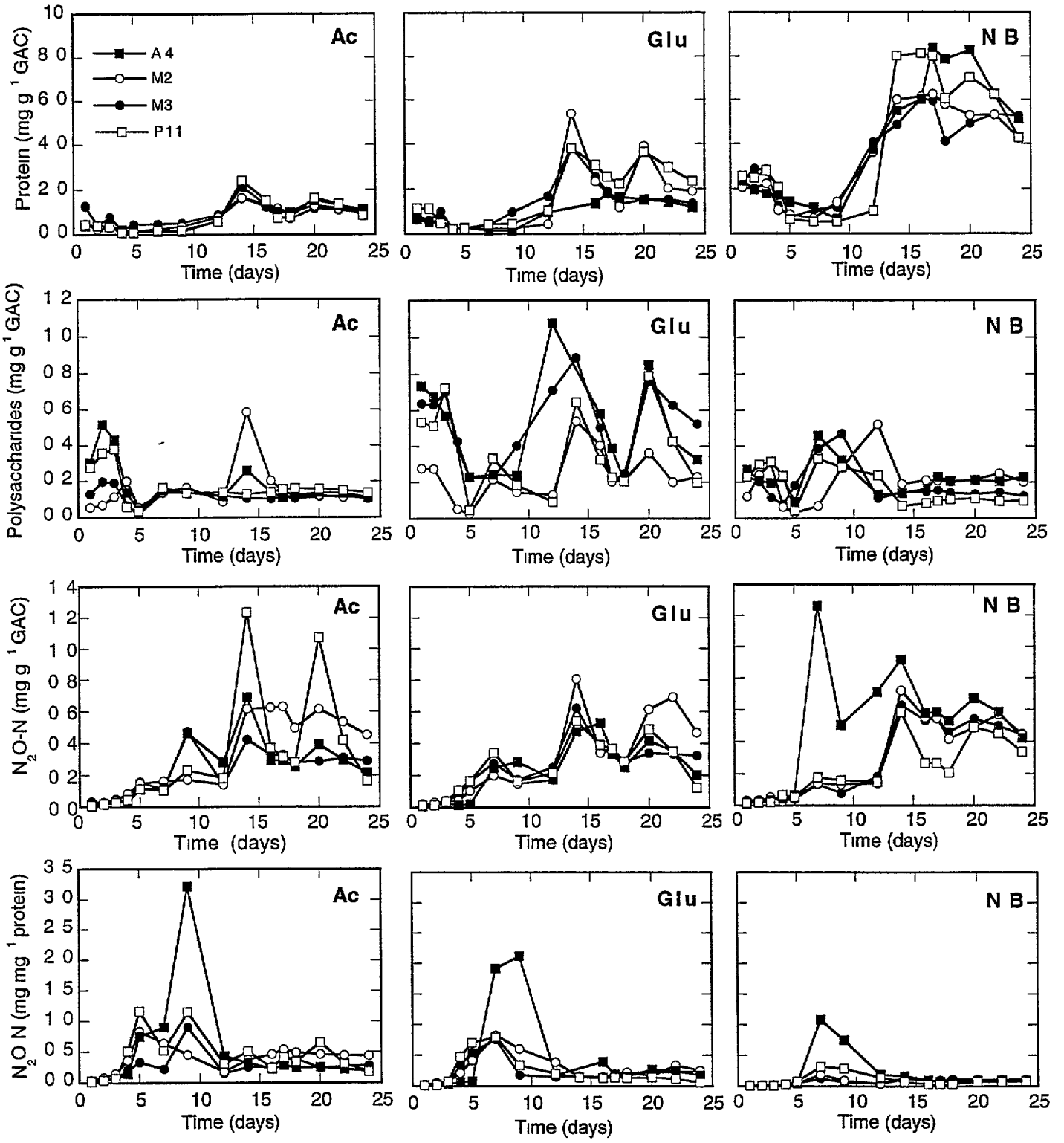

Fig 7 Protein (row 1) and polysaccharides (row 2) in GAC samples from batch cultures of strains A4, M2, M3 and P11 incubated either in medium containing acetate or glucose as sole carbon source or in nutrient broth, and their denitrification activity (rows 3 and 4)

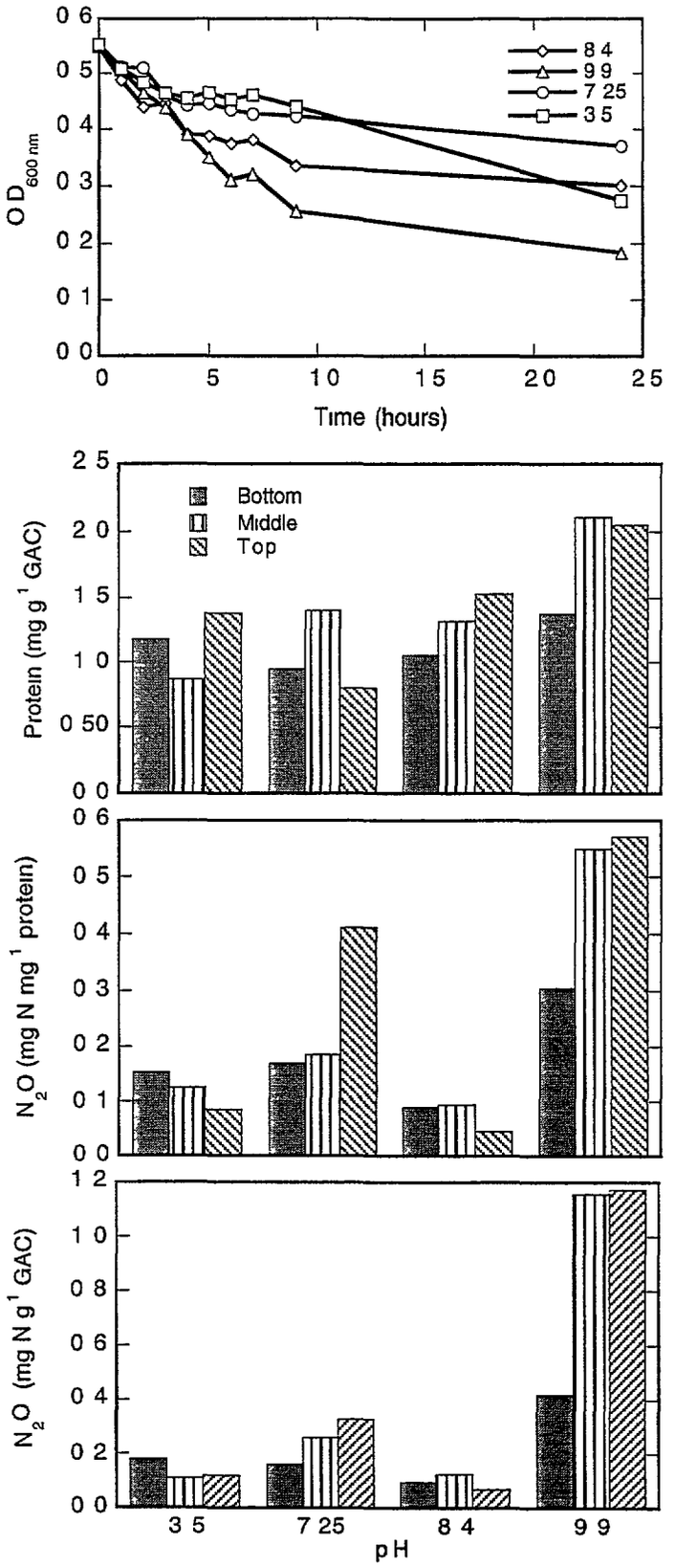

Fig 8 Effect of pH on the attachment of bacterial strain A4 to a GAC column Changes on OD_{600} of an inoculum which was recirculated for 24 h (line graph), the columns were then operated for 24 h prior to determinations of protein concentration and denitrification and activity in GAC samples (bar graphs)

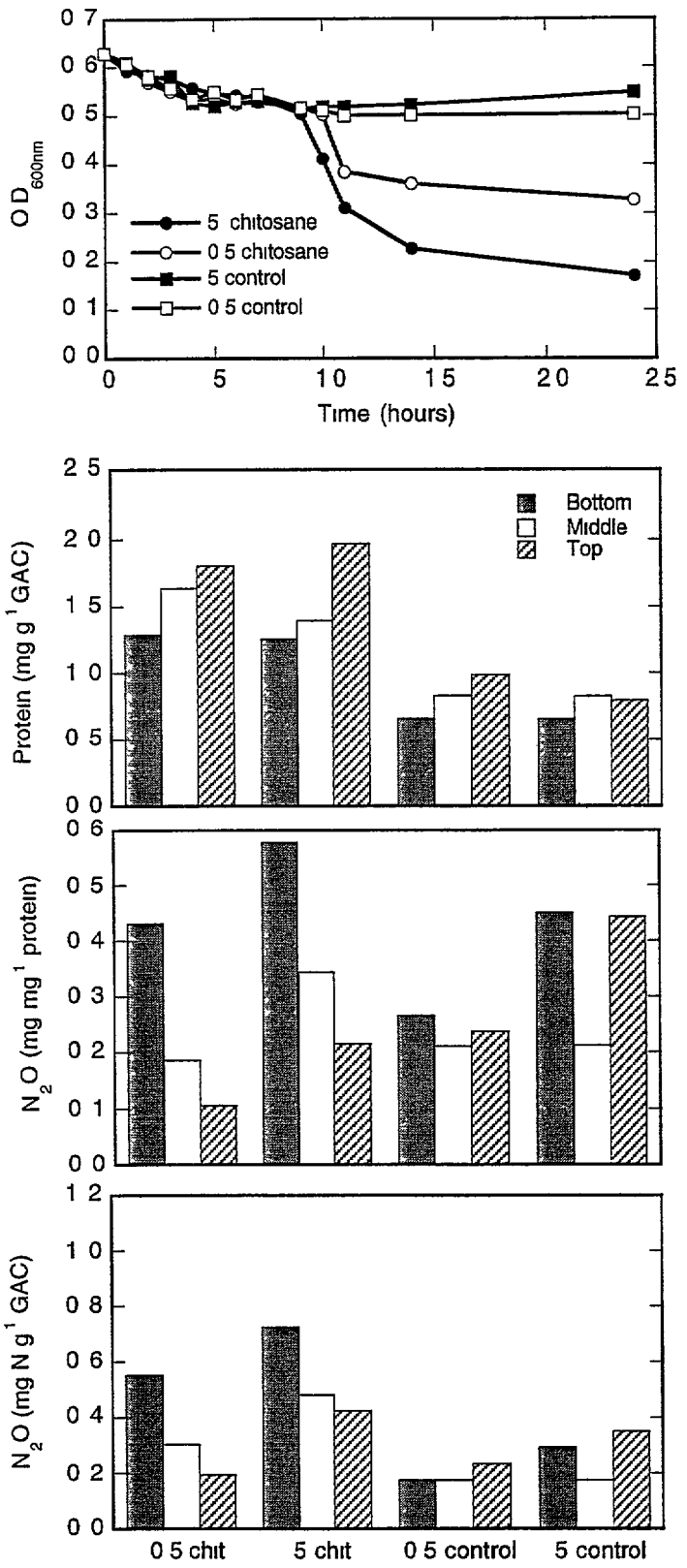

Fig 9 Effect of chitosane on the establishment of biofilm on a GAC column Attachment of strain A4 as shown by changes on OD_{600} of the inoculum which was recirculared during 25 h (line graph), the columns were then operated for 24 h prior to determinations of protein and denitrification activity in GAC samples (bar graphs) Treatments 0 5 or 5 mg chitosane l^{-1} and controls without chitosane

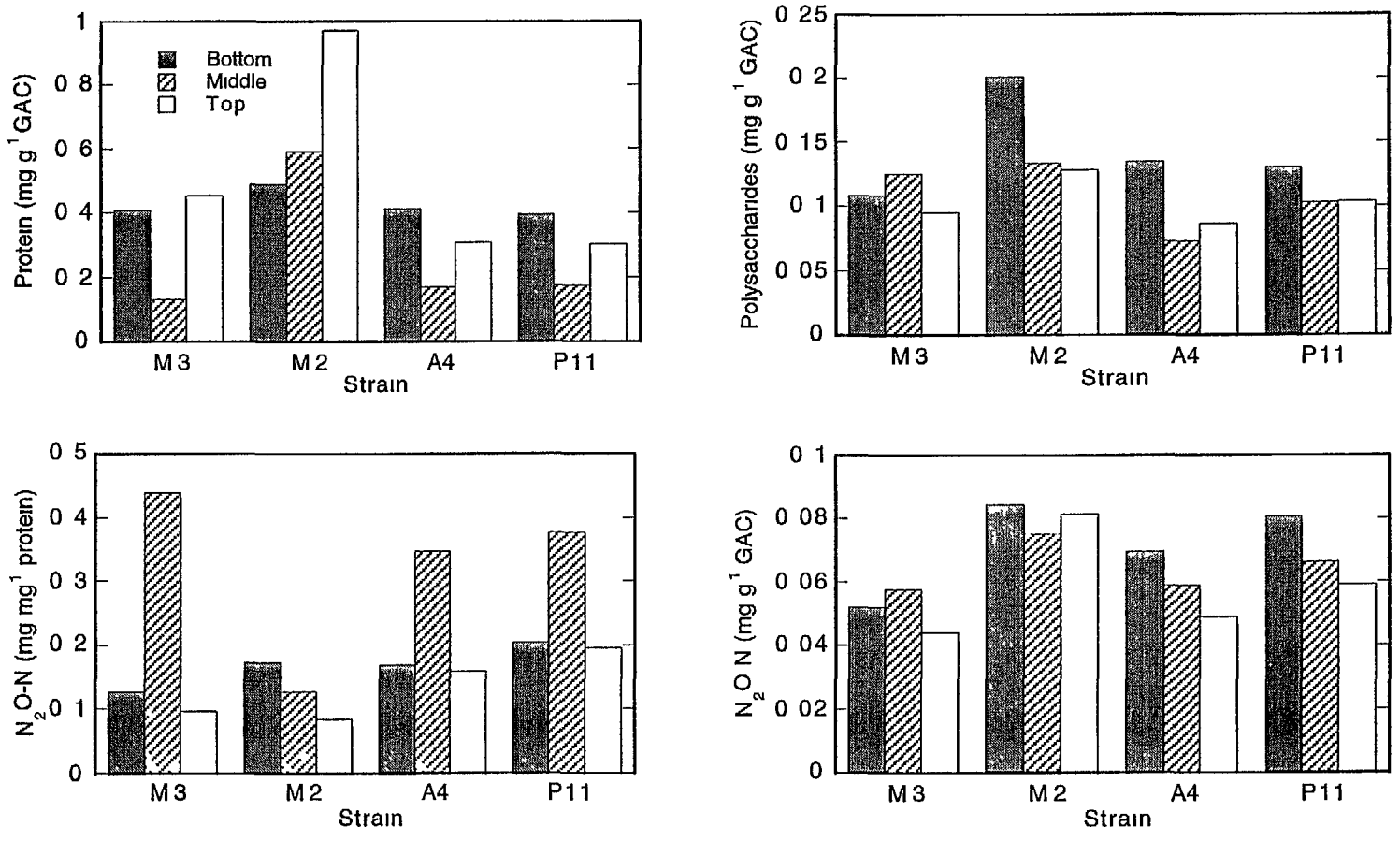

Fig 10 Concentrations of protein and polysaccharides and denitrification activity in GAC samples from different levels of 75 cm high columns inoculated with strain A4, M2, M or P11

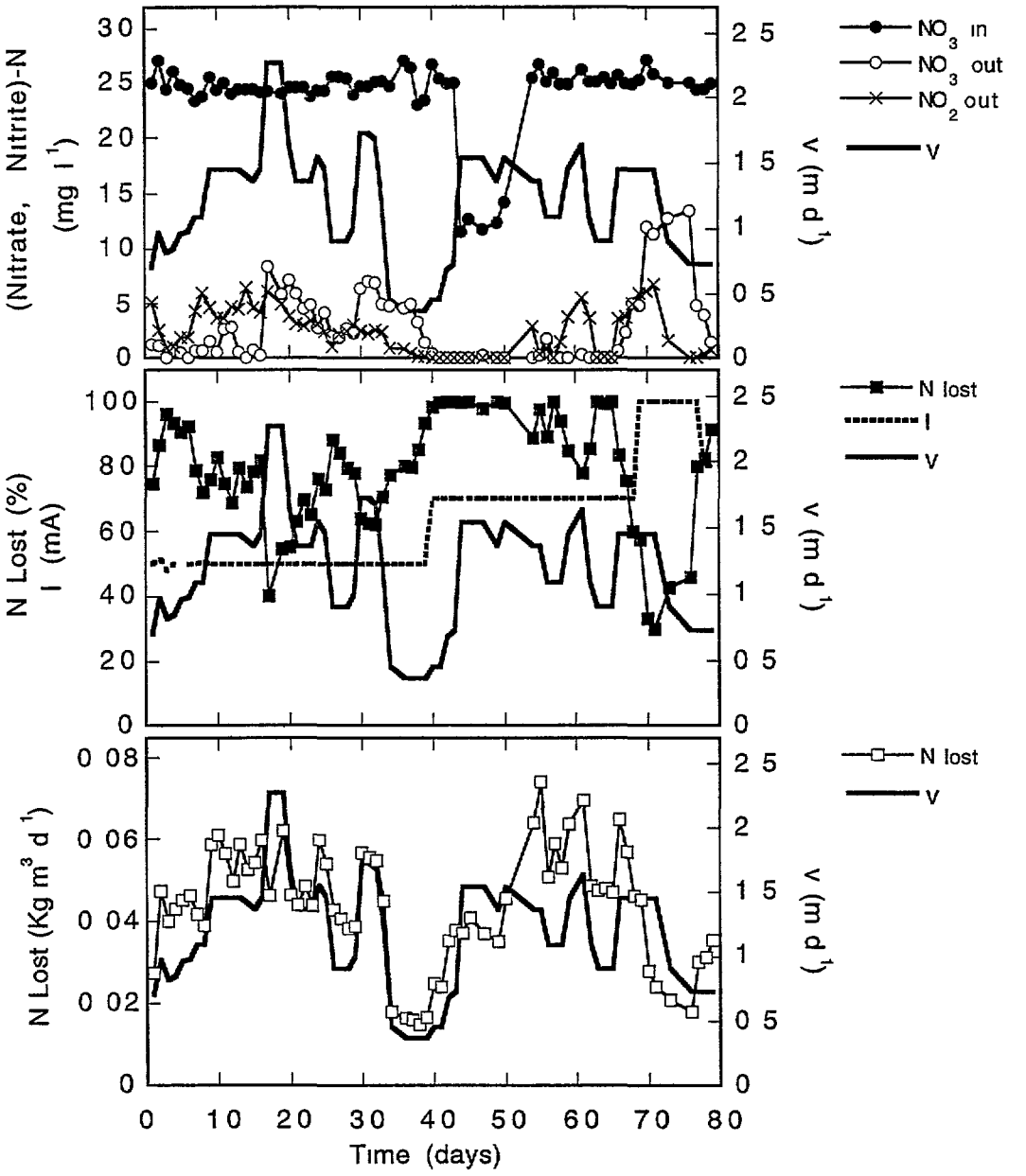

Fig 11 Denitrification in a EC+BR system inoculated with strain A4

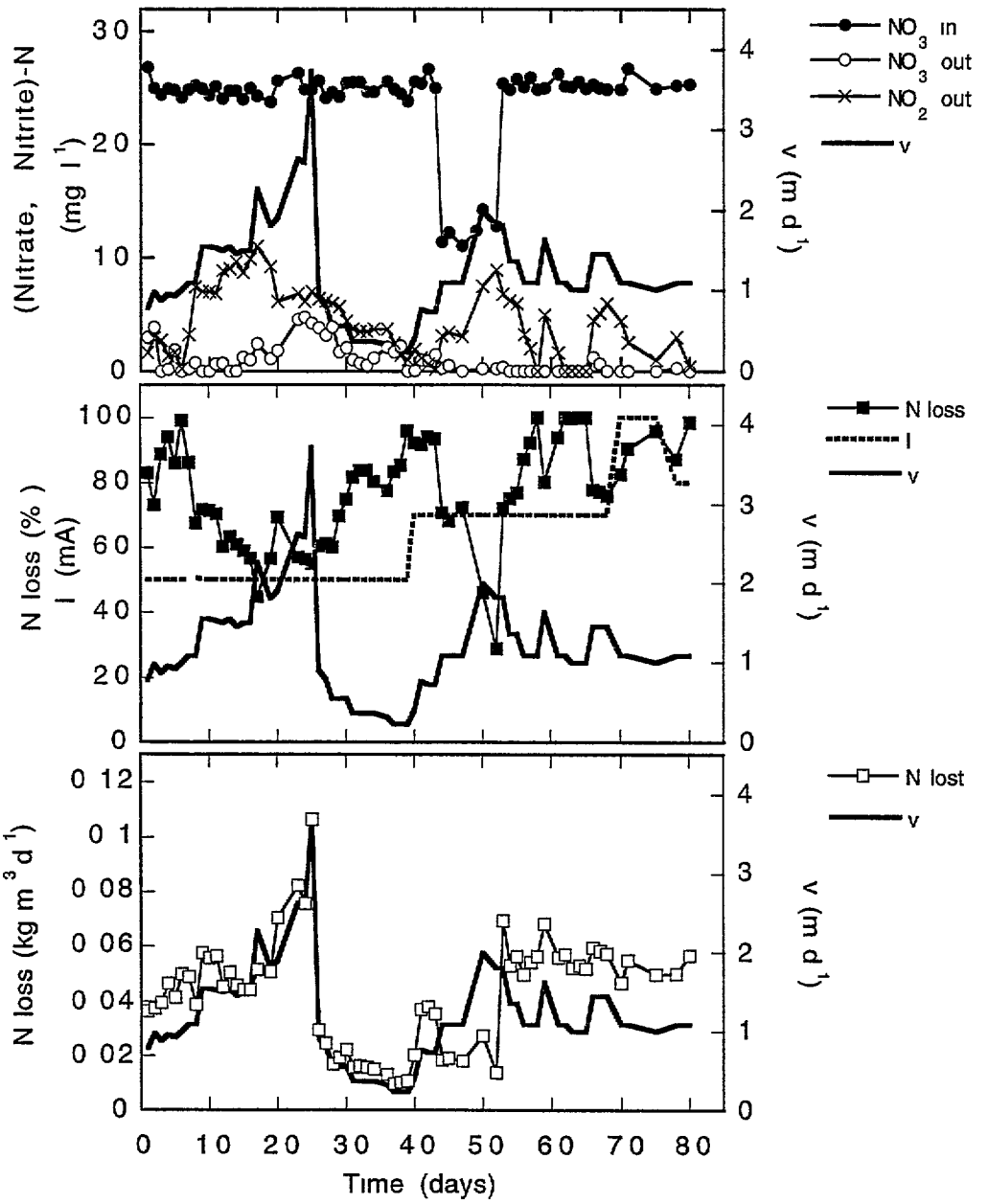

Fig 12 Denitrification in a EC+BR system inoculated with strain P11

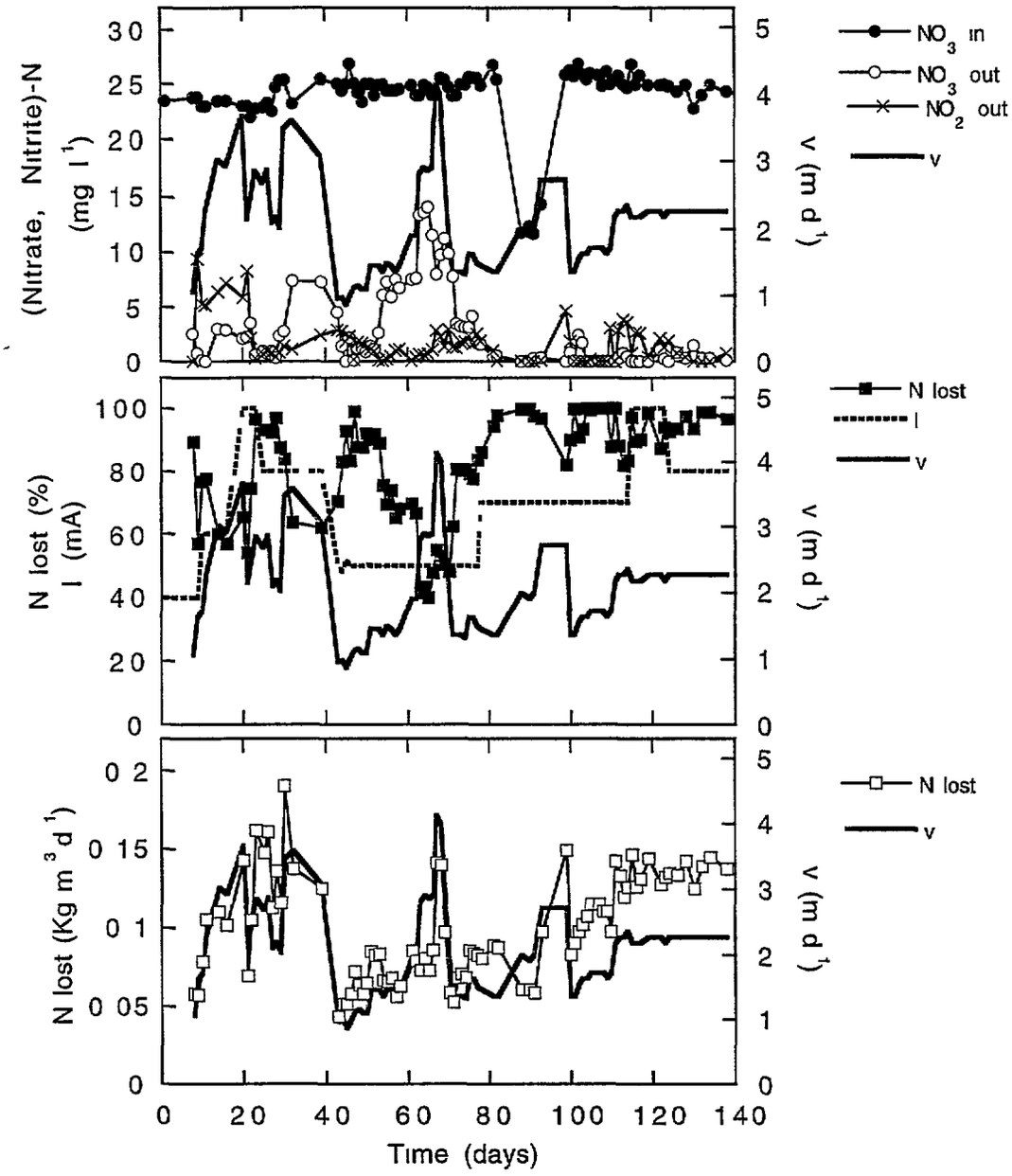

Fig 13 Denitrification in a EC+BR system inoculated with a mixed inoculum

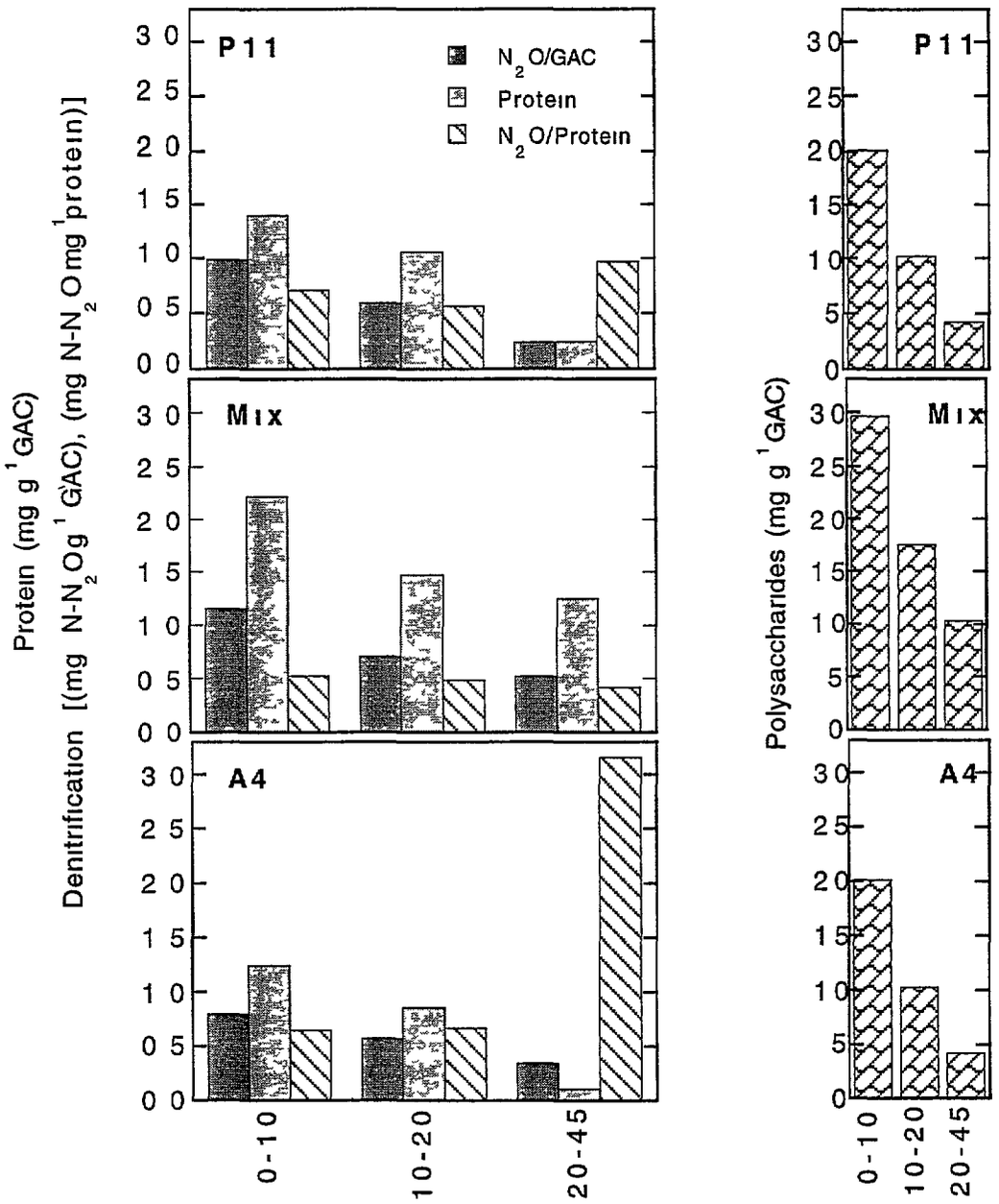

Fig 14 Concentrations of protein and polisaccharides and denitrification activity in GAC samples from various levels of the columns in Figs 11-13

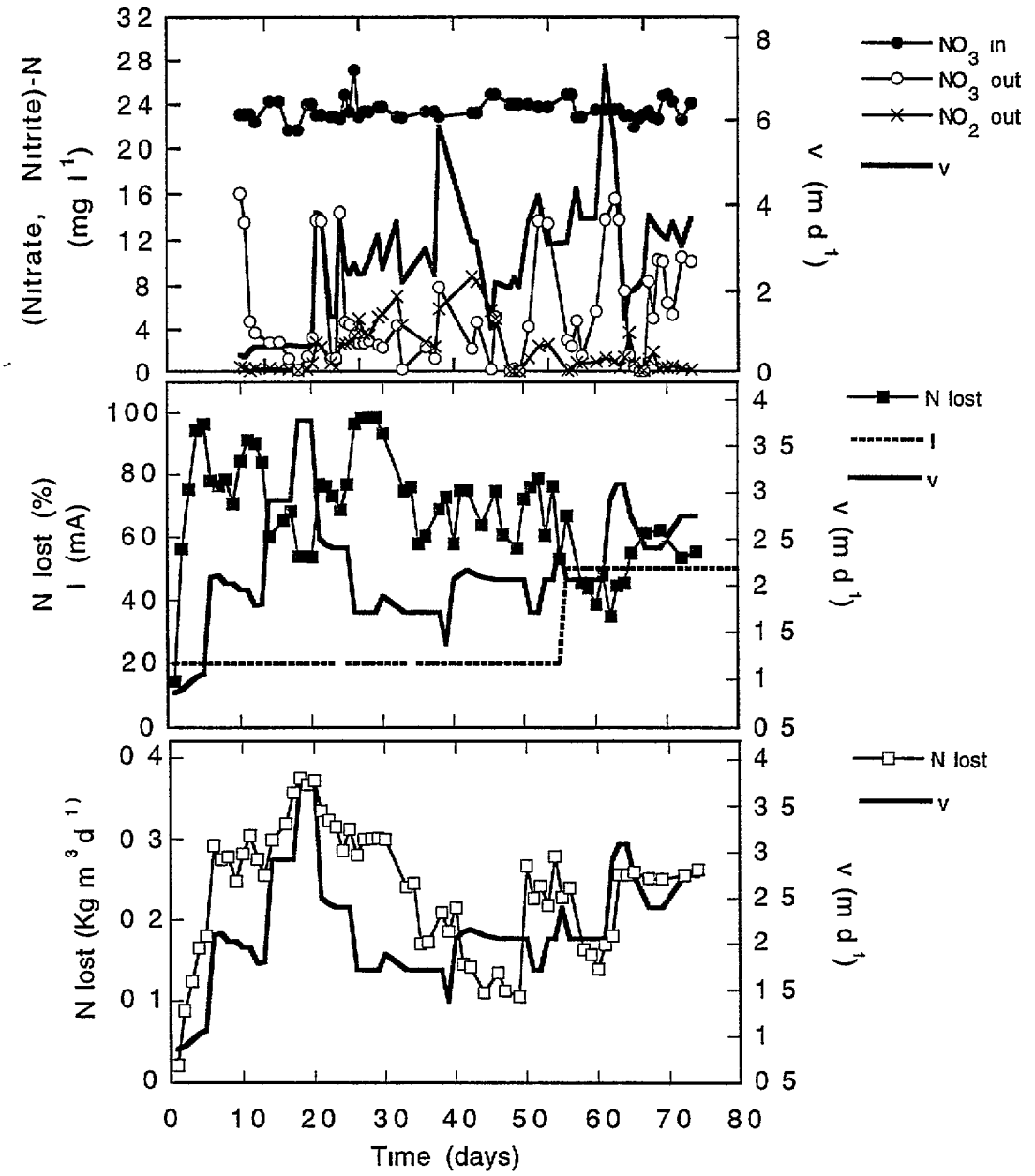

Fig 15 Denitrification in a ECBR system inoculated with A4

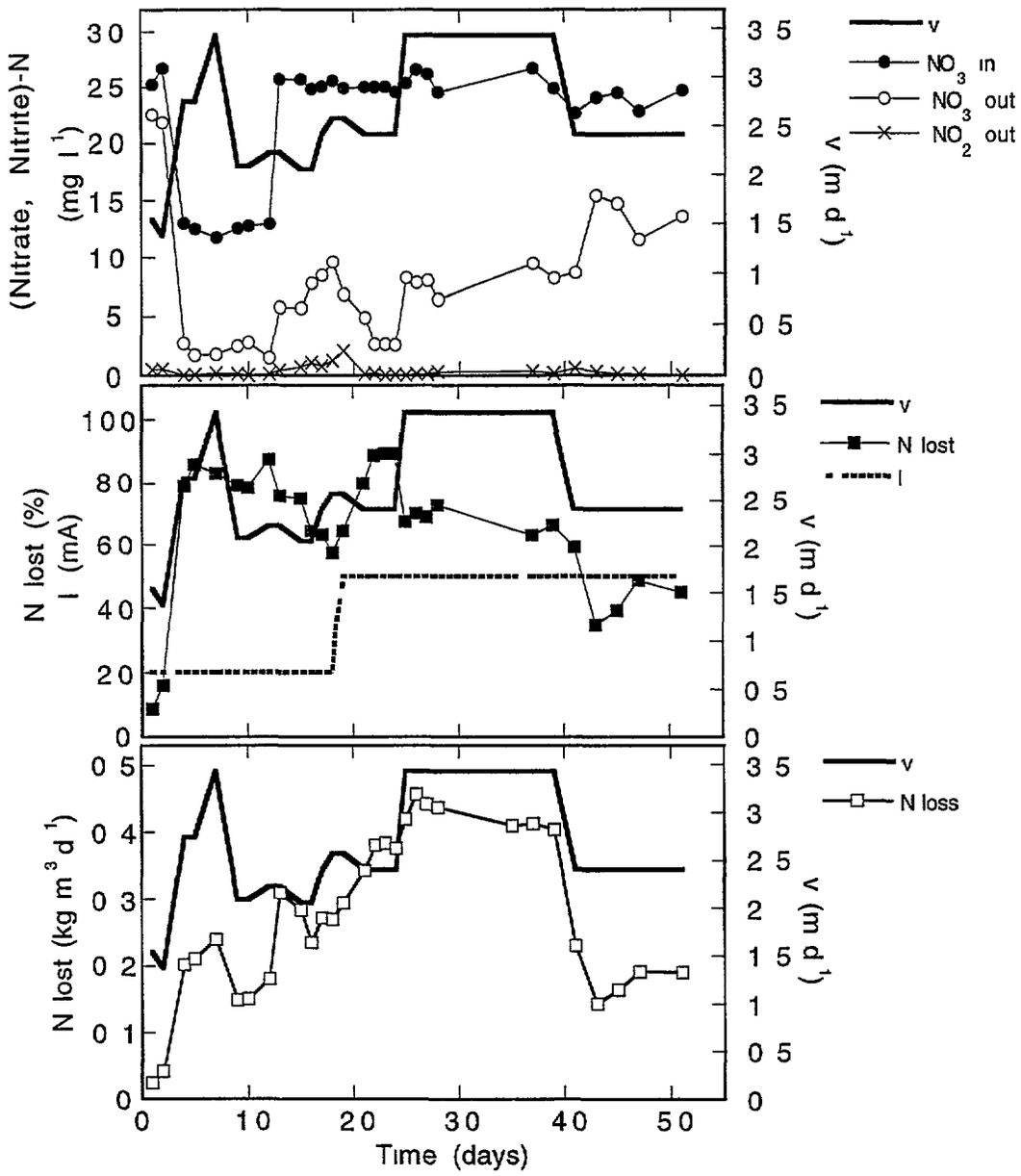

Fig 16 Denitrification in a ECBR system inoculated with a mixed inoculum

Column experiments

Effect of pH

A short-term experiment was carried out to determine the effect of pH on the bacterial colonization of GAC After pregrowth in nutrient broth, A4 bacteria were harvested, washed and resuspended in standard mineral medium (pH 7 25) or mineral medium adjusted to pH 3,5, 8 4 or 9 9 The optical density at 600 nm of each suspension was measured prior to pumping into GAC-packed columns for 24 h in a recirculation mode, changes in optical density were monitored (Fig 8, line graph) The columns were then operated under identical standard conditions for 24 h after which concentrations of protein and denitrification activity were measured in GAC samples removed from the bottom, middle and upper sections of the columns (Fig 8, bar graphs) The highest adsorption of the bacterium occurred at the highest pH tested (9 9) has shown by the decrease in OD during the first 10 h (Fig 5, line graph) By the end of the experiment, a similar trend existed in the accumulation of protein and the rates of denitrification the column which had been inoculated at pH 9 9 showed significantly higher values (Fig 8, bar graphs)

Effect of Chitosane

Chitosane, a soluble deacylated form of the natural polymer chitin, contains amino groups which have been used as reactive groups in microcarriers for cell cultivation (Gemeiner *et al*, 1994)) Tests were carried out to determine if chitosane would improve the adsorption of inoculum to the reactor bed Chitosane was solubilized in acetic acid and applied to GAC as described under materials and methods A stock solution was prepared containing chitosane and acetic acid and diluted to give final concentrations of 0 5 or 0 05 mg chitosane l^{1}, controls contained acetic acid and no chitosane were also prepared and each of the four solutions was used to treat a batch of GAC Four columns were then packed and inoculated as follows cells grown in nutrient broth were harvested, washed, resuspended in mineral medium and divided into four batches which were recirculated during 24 h through each column Only after 9 h the optical densities of the suspensions passing through chitosane-treated GAC became lower than those of the controls suggesting that the chitosane effect was not due to simple adsorption (Fig 9, line graph) The columns were operated for further 24 h under routine conditions (i e , fed with tap water amended with nitrate, bicarbonate and phosphate) prior to removal of GAC samples for analysis The levels of protein were higher in chitosane-treated GAC the effect on denitrification activity was not clear (Fig 9 bar graphs)

Effect of different bacterial inocula

Columns inoculated with a suspension of one of the isolates A4, M2, M3 and P11 (pregrown in nutrient broth and washed as described under materials and methods) were operated for one week, after which samples of GAC were analyzed for protein, polysaccharides and denitrification activity (Fig 10) No major differences were found on the levels of polysaccharides and of denitrification activity per weight of GAC although those of M2 might be somewhat higher

Denitrification systems

The reactors presented here were inoculated with strains P11 or A4 or with the mixed inoculum and they are designated accordingly However it should be stressed the experiments were conducted under non-sterile room conditions

Two-reactor system (EC+BR)

The reactors were operated for 80 or 140 days at various feed rates and current intensities (Figs 11-13) The overall performance of the system inoculated with the mixed inoculum was superior with rates of N removal m^3 reactor d^1 some five-fold higher than these in the reactors inoculated with A4 or P11 Furthermore, the mixed inoculum resulted on the lowest accumulation of nitrite, even at high breakthrough of nitrate (Fig 13, days 54-70) Low accumulation of nitrite is an important consideration in drinking water treatment although it is sufficient to lower the concentration of nitrate-N to 11 29 mg l^1 (WHO 1984) in practice breakthrough of nitrate is often accompanied by the undesirable presence of nitrite

By the time the last measurements were taken (after more than 3 months of operation) the system was stable and was operating under capacity Experiments with strictly controlled feed rates are in progress in order to establish the relationship between v and N removal and between I and N removal

The concentrations of polysaccharides and the denitrification activity in samples of GAC were also higher in the system inoculated with the mixed inoculum than in those inoculated with A4 or P11 In general, denitrification activities and biomass were higher at the inlet end of the columns and decreased towards the outlet (Fig 14)

No signs of clogging of the bed were observed Entrapment of gas bubbles occurs in fine matrixes (Soares *et al*, 1989, 1991) but the GAC particles were large enough to preclude this problem

Single-reactor system (ECBR)

Summarized in Figs 15 and 16 are the results obtained in reactors inoculated with strain A4 and with the mixed inoculum In the latter, nitrite was seldom detected, even in the presence of high concentrations of nitrate In general, the rates of N removed m^3 d^1 were in the same order of magnitude in both reactors The deterioration observed after day 40 in the "mix" reactor appeared to be due to the formation of scale which covered part of the cathode and solidified in a hard block the GAC granules in the lower half of the bed This reduced the effective surface area of the electrode and the biofilm and limited mass transfer

Conclusions

In short, the main practical conclusions that can be drawn from the above results are

(1) Although the calculated rates of denitrification per m^3 of reactor per day were higher in the single-reactor system, the two-reactor system is superior The EC is the most complex and expensive component, and increasing the volume of the bed will be more costly than using a small EC with a larger BR Furthermore, a comparison between Figs 13 and 16 shows that the calculated rates of denitrification in EC+BR system were an underestimation since most of the time nitrate was limiting, this was not the case in the ECBR system which was operated at over-capacity The bed volume in the EC+BR system was ten times larger than that in ECBR and a higher volume of water was treated per energy input

The formation of scale in the ECBR system brought about an irreversible deterioration of its performance In the EC+BR system the accumulated salts were not in direct contact with the biomass and could be easily removed by disconnecting the BR from the EC while passing a diluted acid solution through the cathodic chamber

(2) Nitrite was absent or in very low concentration in the effluents from reactors inoculated with the mixed inoculum, even when the concentration of nitrate was relatively high

(3) The startup phase of the reactors was short This was due to the rapid establishment of biomass

(4) The mixed inoculum was more effective than each one of its components alone

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C) Scientific Impact of Collaboration

Istvan Kiss, a Hungarian Ph D student who has been in the Israeli laboratory, has been a good bridge between the two groups On his return to Hungary at the end of March 1999, Istvan Kiss will set up reactors similar to the ones built in the Israeli laboratory Thus the Hungarian group will take full advantage of the advisory services of Dr T Bejerano, the electrochemist adviser to the Israeli group

D) Description of Project Impact

As described under E) the project has upgraded the research capacity of the Hungarian institution It has introduced a new line of applied research and has allowed training of young scientists It is expected that by the end of project a blue print will be drawn for the immediate application of the denitrification technology which is being developed

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 It has provided the funds to support two Ph D students in the Hungarian institution These students have also been invited to join the Israeli team where they have the opportunity to work and interact with a larger and diverse group of scientists with long experience on water treatment In our view, this has been one the most important and lasting contributions to the developing country institution

F) Future work

The project is progressing on schedule During the coming year long-term performance of the reactors will be studied The effects of water velocity and current intensity on the removal of nitrogen will be analyzed Guidelines for the system upscaling will be drawn The dynamics of the bacterial population will be studied and isolates will characterized

Section II

A) Managerial Issues

No special managerial issues have arisen during this year The project schedule has not been changed

B) Budget

No budgetary changes took place or were requested by us during the last year

C) Special concerns

No change in the protocols was or is required

D) Collaboration, Travel, Training and Publications

Istvan Kiss, a Ph D student from the Hungarian group has been in the Israeli laboratory since April, he will leave at the end of March when another Hungarian Ph D student, Szilvia Szekeres will join the Israeli group

The Israeli PI visited the Hungarian group in May and another visit is planned to take place in May 1999

A paper will be prepared for presentation at the 7th International Conference of The Israel Society for Ecology and Environmental Quality Sciences held in conjunction with the Regional Conference of the International Water Resources Association on Water and Environmental Cooperation, in Jerusalem, 13-18 June 1999, and for subsequent publication in Water Science and Technology

6) Request for American Embassy Tel Aviv, A I D or BOSTID Actions

For the time being we do not request assistance of the A I D or BOSTID staff