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CELLULOSE-DEPENDENT BIOLOGICAL RESTORATION OF NITRATE POLLUTED AQUIFERS

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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 culminates with one year of field work with a pilot reactor operated in Hungary.

The results obtained until now have shown that unprocessed short-fiber (low quality) cotton is superior to the other cellulose-rich sources studied (newspaper, wheat straw and maize leaves) as substrates in the treatment of nitrate-contaminated drinking water. However, cotton is not grown in Hungary and the Hungarian partners have decided to use wheat straw in the field study to be carried out next year. For this reason, efforts during the past year concentrated mainly on the use of wheat straw.

Microbial denitrification of drinking water was studied in laboratory columns packed with wheat straw. The highest rates of denitrification were observed in fresh reactors and the efficiency of the process declined with time; replenishment of the reactor with periodic additions of fresh substrate brought about a more constant performance. The rate of denitrification was also affected by the flow rate and decreased at flow rates above a certain threshold. Increased wash out of bacteria appears to be one of the reasons for this phenomenon. The Israeli group continued the studies on bacterial isolates from cotton reactors. The two most abundant cultivable isolates were identified as *Cellulomonas uda* and what appears to be a new species of *Comamonas*; the first degrades cellulose but can only reduce nitrate to nitrite while the second has no cellulose degrading capability but can reduce nitrate and nitrite to N_2. Mixed cultures of both isolates are capable of growing and sustaining high rates of denitrification when cotton is the sole carbon and energy source.

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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 a simple reactor where cellulose degradation and denitrification are be coupled.

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

B) Research Accomplishments

During this third year of the project, the Israeli group has carried out a detailed study on wheat straw-dependent denitrification of drinking water.

A manuscript summarizing the results obtained is in preparation and a preliminary draft is attached. Considerable effort has been invested in the isolation, characterization and physiological studies of bacterial isolates from cotton-packed reactors. The two most abundant cultivable isolates were identified as *Cellulomonas uda* and what appears to be a new species of *Comamonas*; the first degrades cellulose but can only reduce nitrate to nitrite while the second has no cellulose degrading capability but can reduce nitrate and nitrite to N_2. Mixed cultures of both isolates are capable of growth and sustaining high rates of denitrification when cotton is the sole carbon and energy source. This work is still in progress and the overall results will be presented in next year's report.

The Hungarian group has also studied the use of wheat straw and their their results are also attached.

C) Scientific Impact of Collaboration

Both groups are carrying out all stages of the experimental plan. The methodologies used have been discussed as well has the results obtained. The personal contacts during the visits between the two teams have been invaluable in establishing a work relationship.

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. Some concrete guidelines have been defined and will be tested during the coming year.

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 purchase of the Ion Chromatography apparatus has made possible the fast handling of numerous chemical analysis and has markedly improved the capabilities of the Hungarian laboratory.

2. Volokita M., Abeliovich, A. and M. I. M. Soares. 1996. Denitrification of groundwater using cotton as energy source. J. Water Science and Technology 34 (1-2). In Press.

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

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

STRAW AS SUBSTRATE FOR THE MICROBIAL REMOVAL OF NITRATE FROM WATER

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Running head: Straw as substrate for water denitrification.

ABSTRACT

Microbial denitrification of drinking water was studied in laboratory columns packed with wheat straw. Straw served as the sole chemical and physical support for the microbial population. The highest rates of denitrification were observed in fresh reactors and the efficiency of the process declined with time. Replenishment of the reactor with periodic additions of fresh substrate brought about a more constant performance. The rate of denitrification was also affected by the flow rate and decreased at flow rates above a certain threshold. Increased wash out of bacteria appears to be one of the reasons for this phenomenon.

Key words -- Nitrate contamination, denitrification, microbial water treatment, wheat straw, cellulose.

INTRODUCTION

Removal of nitrate is often required in polluted drinking water supplies in order to meet the standard of 11.29 mg nitrate-N per liter in water for human consumption (WHO, 1984). Among the various methods available (physical, chemical, chemical-physical and biological) for the removal of nitrate microbial removal (denitrification) is considered to be the most economical and environmentally sound process, and to be feasible in a large scale. Denitrification is the reduction of nitrate to N_2 carried out by facultative aerobic bacteria which in the absence of oxygen utilize nitrate as a terminal electron acceptor in their respiratory processes (Payne, 1981).

The majority of microbial denitrification treatments rely on heterotrophic bacteria which require an organic carbon source. Usually, a simple carbon compound (e.g., ethanol, methanol, acetate) is employed but complex carbon sources such as cellulose-rich materials can also be used. In recent laboratory studies we have investigated the feasibility of using newspaper (Volokita *et al.*, 1996 a) and cotton (Volokita *et al.*, 1996 b) in the restoration of nitrate-polluted groundwater.

Cellulose is a basic component of all plant materials and constitutes the most abundant renewable resource in the world with an estimated syntheses rate of $4*10^{10}$ tons per year (Couglan, 1985).

Cellulose is a linear glucose polymer with hydrogen bonding between hydroxyl groups of neighboring parallel chains. Native cellulose is organized in fibers and occurs in close association with lignin and

hemicellulose (cotton is an exception being the purest form of naturally occurring cellulose). Wheat straw is a complex mixture of cellulose, hemicelluloses (including xylan), pectines, and lignins, of which xylans and other xylose polymers constitute ca. 25% (Buchala and Wilkie, 1973).

The potential for the utilization of wheat straw in denitrification of contaminated water has been shown by other authors. Boussaid *et al.* (1988) described field and laboratory reactors packed with wheat straw in the treatment of nitrate-contaminated ground water; in their process straw was mixed with sand or with sand maërl. Avnimelech *et al.* (1993) observed removal of nitrate in wheat straw filters designed to remove particulate matter from effluents prior to their application on drip irrigation. In the current study wheat straw was the sole carbon and energy substrate as well as the only physical support for bacteria in laboratory denitrification reactors supplied with nitrate-amended tap water.

MATERIALS AND METHODS

Experimental apparatus

Laboratory reactors were cylindrical perspex columns, 50 cm high and 8 cm dia, except for one experiment in which a PVC column, 55 cm high and 10 cm dia was used. The reactors were packed with wheat straw (a glass wool layer was placed at each end) and inoculated with a small amount of material from an active denitrification column (original inoculum obtained as described by Volokita *et al.*, 1996 a). The freshly packed columns were filed with nitrate- and phosphate-amended tap water and allowed to stand for 2-3 days in a recirculation mode before start up. The columns were fed with tap water amended with nitrate (22.58 mg N l^{-1}) and phosphate (3 mg

l^{-1}). Flow rate was regulated by peristaltic pumps and was directed to create an up-flow through the columns. Flow rates are presented as Darcy's flux ($q = Q/A$, in m day^{-1} where Q is the measured flow rate and A is the cross section of the column. In the perspex columns, a q value of 1 m day^{-1} corresponds to a flow rate of 3.5 ml min^{-1}, in the PVC column 1 m day^{-1} corresponds to 5.5 ml min^{-1}.

Unless otherwise indicated, ambient temperature was maintained at 24±1°C. Influent and effluent samples were usually collected twice a week and routinely assayed for nitrate, nitrite, ammonia, pH, dissolved organic carbon (DOC) and bacterial counts.

The amount of N lost per day was as calculated as follows:

N lost (mg day^{-1}) =

[nitrate-N in - (nitrate-N out + nitrite-N out)] (mg l^{-1}) x Volume day^{-1} (l)

Analytical determinations

Nitrate was determined by the method of Cataldo *et al.* (1975) and nitrite and ammonia were assayed according to Standard Methods (APHA, 1989). Dissolved organic carbon was determined by means of a high-temperature TOC analyzer (Dohrmann DC-190, Rosemount Analytical Inc., Santa Clara, CA, USA).

The chemical composition of straw was determined in samples ground to pass a 40-mesh screen with 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).

Bacterial counts

Colony forming units were counted by standard plating techniques on R2A agar (Difco Laboratories). Bacteria producing extracellular xylanase were detected in 1.5% (w/v) agarose plates containing mineral medium supplemented with 0.2% (w/v) remazol brilliant blue-xylan prepared as described by Biely *et al.* (1988). Release of cells from straw was achieved by blending with 0.75 % NaCl (w/v) containing 0.01% (w/v) Tween 80.

RESULTS

Nitrate removal and water quality

A column packed with 103 g of wheat straw was inoculated and maintained in recirculating mode for 2 days prior to start up (day 0) at q 1.4 m d^{-1}. The column was fed with approximately 22.6 mg nitrate-N l^{-1} and operated for nine weeks (Fig. 1). The flow rate was varied to allow breakthrough of nitrate and the determination of the capacity of the column to remove nitrogen.

Effluent samples were first collected after 24 h and by then most of the soluble and readily available fraction of DOC had been eluted; thereafter, the accumulation (concentration in the influent subtracted from the concentration in the effluent) of DOC was consistently bellow 10 mg l^{-1} (Fig. 1b). The highest rates of nitrogen removal were observed during the first days when q was increased to 2.06 m d^{-1} and little nitrate and nitrite washed out (Fig. 1a). By day 5, however, breakthrough of nitrate-N reached 6 mg l^{-1} and q was lowered to 1.65 m d^{-1}. From then on, decreasing rates of nitrogen removal were observed.

Among possible reasons for the deterioration in the capacity of the column to remove nitrogen are quantitative and qualitative changes in the substrate. Indeed, by the end of the experiment, the dry weight of straw in the column was down to 61 g, representing a loss of 40% of the original dry weight.

Breakthrough of nitrite was lower than that of nitrate and was never observed in the absence of nitrate breakthrough (Fig. 1b). Ammonia seldom reached detection limits and its concentration never exceeded 0.3 mg l^{-1}. During the first week of operation the effluent showed a light yellow colour. The number of colony forming bacteria was in the order of 10^{-6} bacteria ml^{-1}.

Effect of flow rate

The effect *q* on the removal of nitrogen was studied in a column packed with 103 g straw and fed with approximately 22.6 mg nitrate-N l^{-1} (Fig. 2). The fresh column was allowed to stabilize for 13 days during which the flow rate was adjusted (1.12 -1.72 m d^{-1}) to assure non-limiting supply (e.g., a small breakthrough) of nitrate (Fig. 2). The flow rate was then lowered until complete removal of nitrogen was achieved prior to its gradual increase from 0.72 to 2.81 m d^{-1} (days 14 to 34).

During this experiment, breakthrough of nitrate and nitrite started when *q* reached 1.29 m d^{-1} with N concentrations close to 0.9 mg l^{-1} and 0.5 mg l^{-1} respectively. Further increases in *q* resulted on increasing concentrations of nitrate while the concentration of nitrite-N leveled off at below 5 mg l^{-1} (Figs 2 and 3a). At the highest *q* tested, 2.81 m d^{-1}, the effluent nitrate-N concentration was 12 mg l^{-1} approximately (Fig. 3a).

The data presented on Fig. 2 were used to calculate the relation between flow rate and denitrification rate. Maximum rate of nitrogen removal was achieved at q 1.29 m d^{-1} when approximately 134 mg of nitrogen were removed per column per day (Fig. 3b). Decreasing amounts of nitrogen were removed as q was increased up to 2.21 m d^{-1} at which point the rate of nitrogen removal was just 78 mg N d^{-1}; further increase of q up to 2.81 m d^{-1} had no marked effect on the total amount of nitrogen removed per column per day. Thus at the higher q tested, 2.81 m d^{-1}, removal of nitrogen was approximately 60% of the maximum rate which was observed at q 1.29 m d^{-1}. However this decrease in efficiency was somewhat exaggerated by changes taking place in the substrate during the experiment. This became evident when on day 34 (Fig. 2) q was lowered to bellow 0.97 m d^{-1} and breakthrough of nitrate and nitrite were observed; earlier on, on day 14, complete removal of nitrogen had been achieved at that flux. By day 40 the dry weight of straw packed into the column had been reduced by 42%.

Possible reasons for the decline in nitrogen removal observed at high flow rates (Fig. 3b) are: wash out of bacteria, wash out of extracellular enzymes, wash out of solubilized substrate, and discrepancy between reaction rates and flow rates. To investigate whether the marked decrease on nitrogen removal efficiency observed on Fig. 3b at q around 2.0 m d^{-1} was due to wash out of bacteria, a shift up experiment was carried out in a column similar to that on Fig. 3. In this experiment q was increased from 0.89 to 2.89 m d^{-1} for 90 min, and then brought back to 0.89 m d^{-1}. During the first 90 min, the number of bacteria (colony forming units) was counted, and the concentrations of nitrate and nitrite were determined in the effluent (collected in batches every 5 min), and then again 2 h after the shift down of q (Figs 4 and 5). At time 0, the number of colony forming bacteria in the effluent was around $2.3 * 10^6$ ml^{-1} and a threefold increase was

observed during the 5 min following *q* increase (Fig. 4). Less bacteria were eluted during subsequent 5 min periods although counts similar to the first 5 min were observed again between 70 and 90 min; 2 h after the shift down of *q* the number of bacteria in the effluent was close to the value at 0 time (Fig. 4).

During the 90 min at high flux a tenfold increase was observed in the effluent nitrate concentration, from approximately 0.5 to 5 mg nitrate-N l^{-1}, and a slight decrease was apparent two hours after returning the flux to 0.89 m d^{-1} (Fig. 5). Nitrite was not detected in the effluent prior to the shift up of the flow rate, from then on, its concentration gradually increased and leveled after 70 min at approximately 1 mg nitrite-N l^{-1} (Fig. 5).

Long term operation

It was shown above that the rates of nitrogen removal in a freshly packed straw column declined steadily with time. A marked decrease in dry weight of the carbon substrate was also observed. Thus, a strategy to maintain the highest rates of denitrification might be to periodically add fresh substrate to the columns. These additions should be carried out at the bottom of the reactor in order to minimize the wasteful and undesirable wash out of the readily soluble fraction of carbon present in fresh straw. Two columns (PVC, 2 cm broader than those in all the other experiments) were packed with 177 g of straw, inoculated and started in the usual mode. One of the columns, the control was operated in the usual mode, to the other, 20 g amounts of fresh straw were added on days 18, 25, 32, 77, 92 and 101 (Fig. 6). The influent nitrate concentration was identical in both columns and the flow rates were regulated to allow breakthrough of nitrate. Flow rates during the first two months of operation were approximately the same

in both columns; later, lower fluxes were maintained in the control column so that breakthrough of nitrate-N would not exceed 6 mg l^{-1}. During the first days, both columns removed approximately the same amount of nitrogen and the additions of fresh substrate to the test column on days 18, 25 and 32 had no visible effect. After day 49, a marked decline was observed in the performance of the control column while the replenished column maintained approximately constant denitrification rates for two weeks longer before showing clear signs of decline. At this stage, additions of fresh straw were resumed and were carried out on days 77, 92 and 101. Partial restoration of the denitrification rates was then observed in the test column while the control column continued to decline. By day 105, removal of nitrogen in the control column had reached 30 mg N d^{-1} and approximately 190 mg N d^{-1} in the test column (the experiment is still in progress).

Even though the fresh straw additions were carried out at the inlet end of the column, a considerable amount of soluble carbon washed out. Figure 7 shows effluent concentrations of nitrate-N, nitrite-N and DOC (corrected for DOC in the inlet) before and following the addition of 20 g of fresh straw to a column similar to that in Fig. 6. The addition was carried out on day 56 and previous replenishments had been carried out on days 29 and 43. The flow rate of 3 m d^{-1} allowed the breakthrough of nitrate at a high concentration (11.5 mg N l^{-1}) at time 0. Following the addition of substrate, the effluent was collected in hourly batches for 5 h, and sampled again on the following day. During the first 2 h, a marked decrease was observed in the concentration of nitrate and a small increase in the concentration of DOC; from then on, the concentration of DOC increased dramatically reaching approximately 80 mg l^{-1} during the following 2 h, and declined

thereafter. During this time, nitrate and nitrite were depleted. On the following day, the concentrations of nitrite and DOC were similar to time 0 and that of nitrate was somewhat lower (Fig. 7).

Discussion

Wheat straw can serve as the only physical and chemical substrate in the microbial removal of nitrate from contaminated water. The treated water contains low concentrations of DOC and bacterial numbers in the order of magnitude of these found in conventional drinking water denitrification (e.g., with simple organic substrates) treatments. Clogging of the straw matrix by gas (Soares *et al.*, 1989; 1991) was never observed under the conditions tested.

The rate of flow had a marked effect on the rate of denitrification and increasing amounts of nitrate, and to a lesser extent of nitrite, were eluted from the column at flow rates above 1 m d^{-1} (Fig. 3a). In terms of total nitrogen removal per day per column, the maximum rate obtained was 134 mg d^{-1} at the flow rate of 1.28 m d^{-1} ; relatively constant denitrification rates, around 80 mg N d^{-1}, were observed at q 2.0 to 2.8 m d^{-1}, the highest value tested in this experiment (Fig. 3b).

Among the possible reasons for the decrease in denitrification rates observed at high flow rates - wash out of bacteria, wash out of extracellular enzymes, wash out of solubilized substrate, and discrepancy between reaction rates and flow rates- wash out of bacteria was investigated. High flow rates appeared to increase wash out of bacteria. The column described on Fig. 4 had a void volume (at the time of packing) of 2.7 l and a flux of 2.89 m d^{-1} corresponded to 10 ml min^{-1}. The increase on bacteria counts observed during the first 5

min (50 ml of effluent) was due to the displacement of bacteria on the upper surface of the column, probably from the fiberglass layer covering the straw, from where non-cellulolytic (e.g., loosely attached) bacteria could be easily washed out at high flow rate. The gradual increase in the number of bacteria observed later reflected wash out from the column proper. The high flow rate was maintained for 90 min only, and the higher bacterial counts during the last 15 min appears to suggest that more bacteria were detached from the lower sections of the column. However, the heterogeneous nature of the matrix and the existence of preferential canals would have made it difficult to arrive to definitive conclusions even if the high flux had lasted for a period long enough to completely elute the void volume. It is important to note that once the flow rate was lowered back to 0.89 m d^{-1} the wash out of bacteria also decreased (Fig. 4).

Thus, wash out of bacteria appears to be one of the reasons for the decline on denitrification efficiency at high flow rates. The almost immediate and steady increase on nitrate breakthrough (Fig. 5) may suggest a discrepancy between reaction rates and flow rates although, as mentioned above, channeling in the heterogeneous matrix complicate the interpretation of these observations.

A time-dependent decline in the efficiency of the straw reactors was observed with the highest rates of denitrification being measured during the first days of operation. This was accompanied by a decrease on the dry weight and, possibly, by qualitative changes of the substrate

High rates of denitrification could be maintained by periodic replenishment of the substrate (Fig. 6). The fresh undegraded straw had a lasting effect (Fig. 6) while its readily soluble fraction was partially

"wasted" and brought only a small and short lived increase on the rate of denitrification (Fig. 7). Depletion of nitrate appeared to be the reason for this elution of soluble organic carbon. Studies are in progress to determine an adequate strategy to deal with the bursts of DOC following the replenishments of substrate. The biodegradability of this DOC and its removal with activated carbon are being investigated.

Xylan, an important component of wheat straw (Buchala and Wilkie, 1973) appears to be used in the reactors; screening of bacteria removed from the substrate of active columns showed that the capacity to degrade xylan is widely distributed among their bacterial populations (results not shown).

The denitrifying process described here is based on reactors filled with cellulosic material serving both as matrix and carbon source and such a reactor is expected to have a complex community structure. Understanding the metabolic and spatial interrelations among the major participants in the process is important for the evaluation of its potential and operation limitations. Studies in progress attempt to clarify these microbial processes.

Acknowledgments

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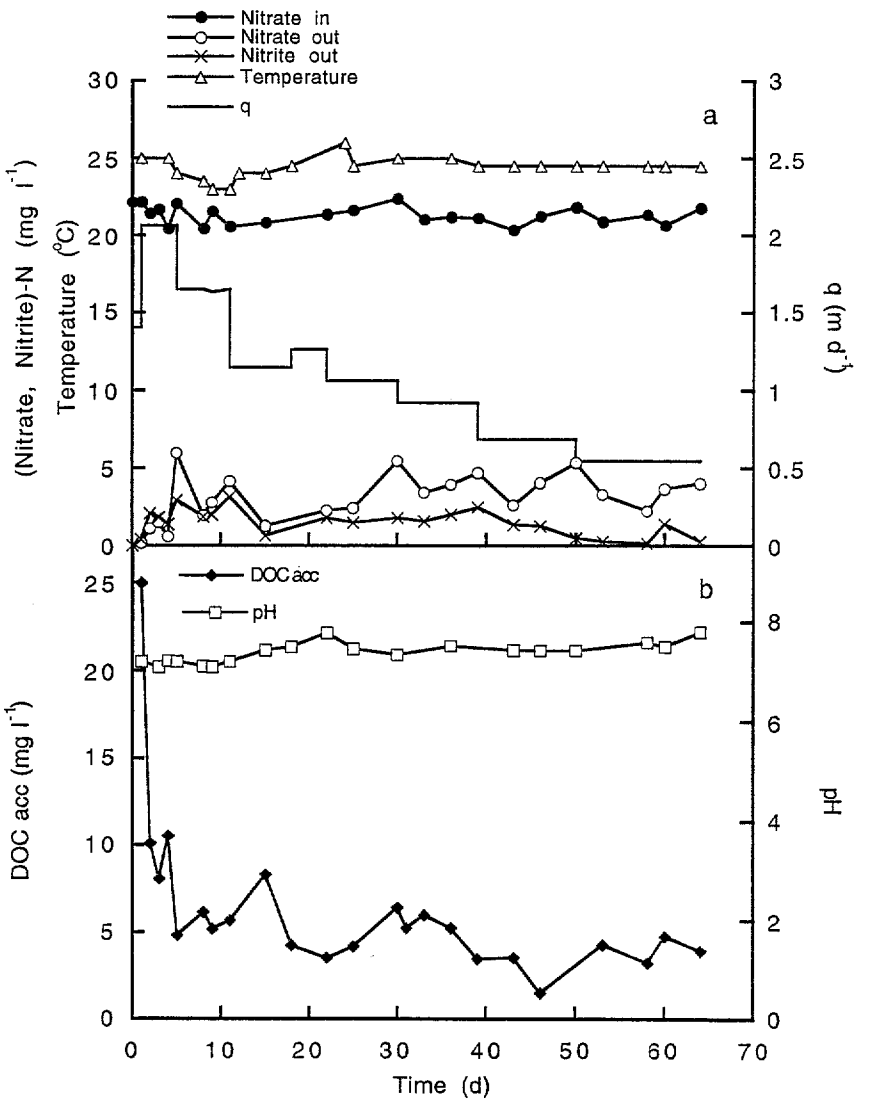

Figure 1: Concentration of nitrate-N in the influent, and pH and concentrations of nitrate-N, nitrite-N and DOC in the effluent of a column packed with 103 g of wheat straw and operated at the temperatures and flow rates indicated.

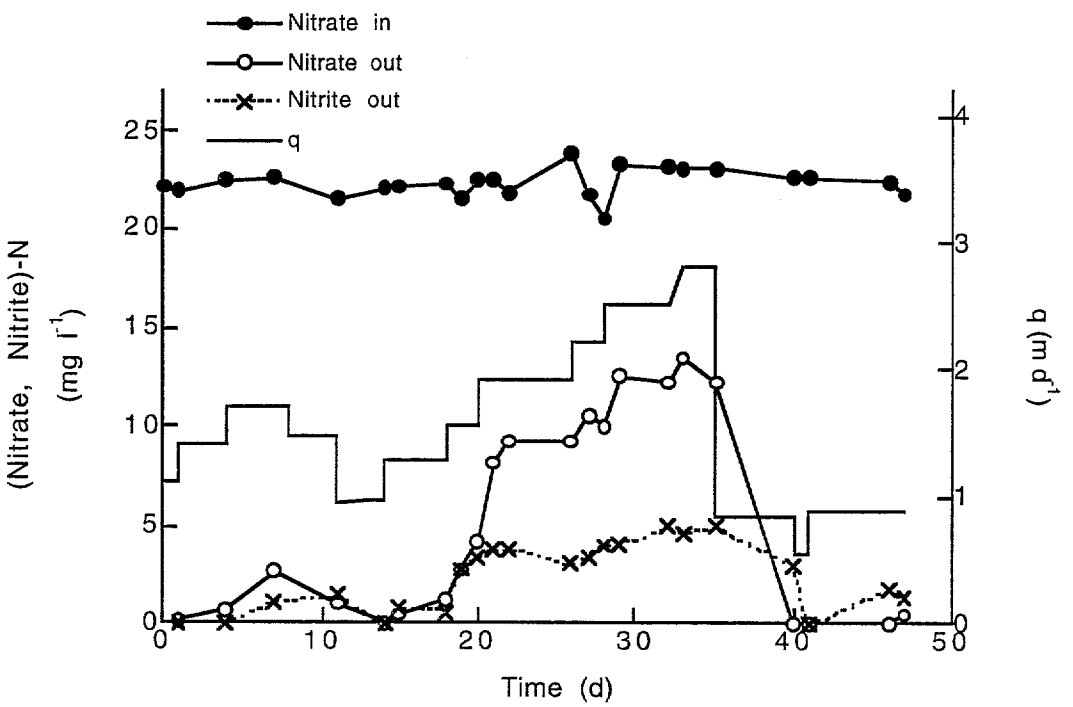

Figure 2: Concentration of nitrate-N in the influent, and concentrations of nitrate-N and nitrite-N in the effluent of a column packed with 103 g of wheat straw and operated at various flow rates and at the temperature of 24±1 °C.

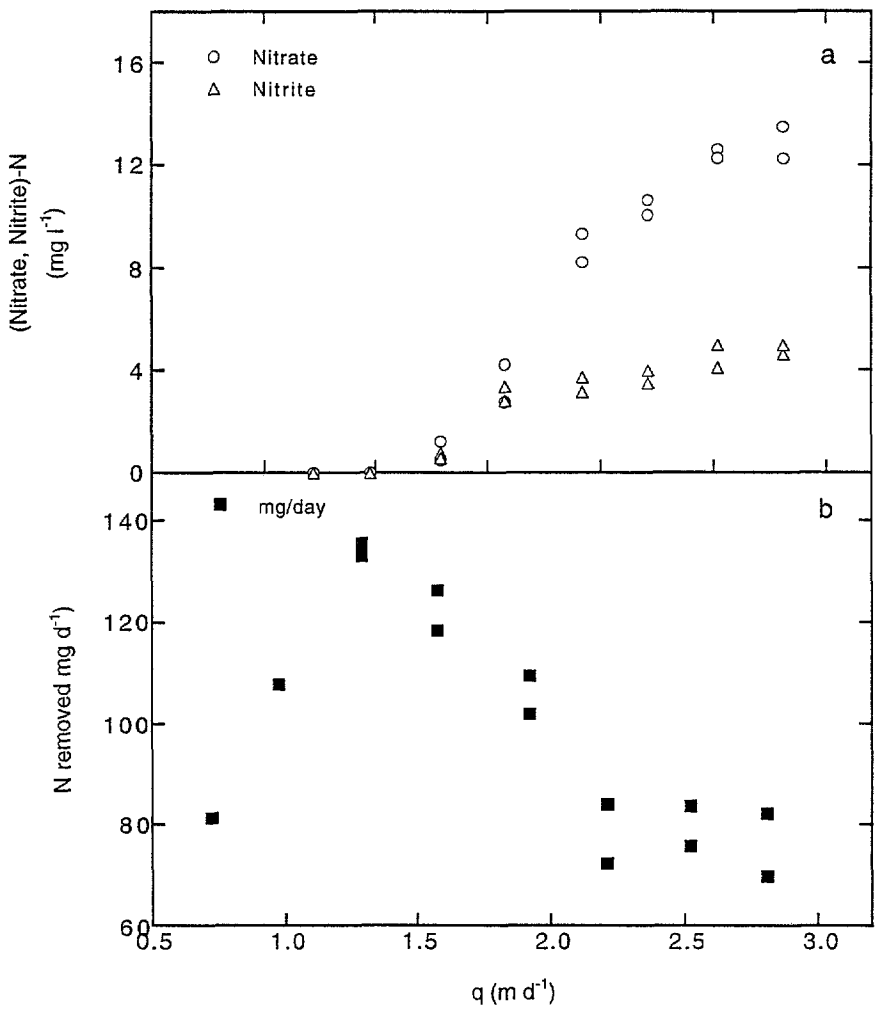

Figure 3: Effect of flow rate on the removal of nitrogen in the column described on Fig. 2. a, concentrations of nitrate-N and nitrite-N in the effluent; b, nitrogen removed per day.

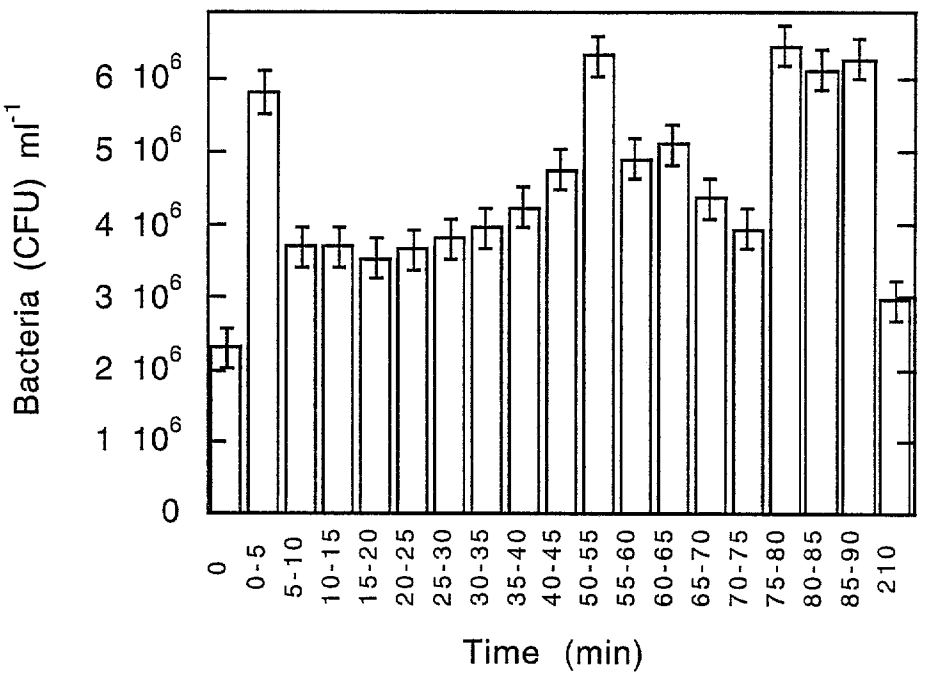

Figure 4: Breakthrough of bacteria (colony forming units) upon increase of q from 0.89 to 2.89 m d^{-1} in day 37 of operation in a column similar to that discribed in Fig. 2.

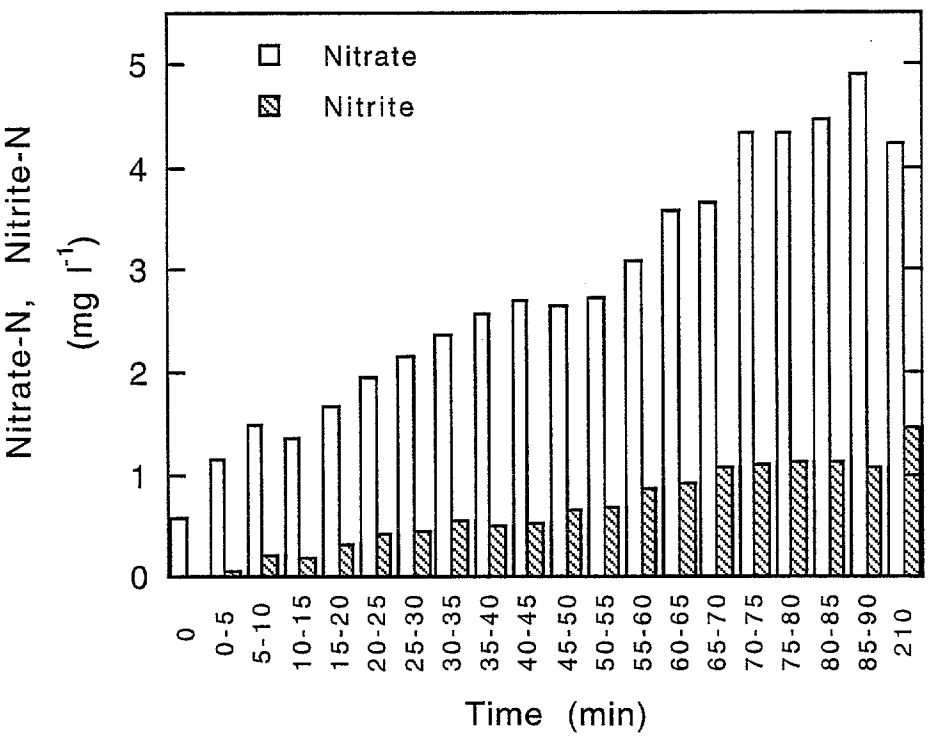

Figure 5: Breakthrough of nitrate-N and nitrite-N under the conditions described in Fig. 4.

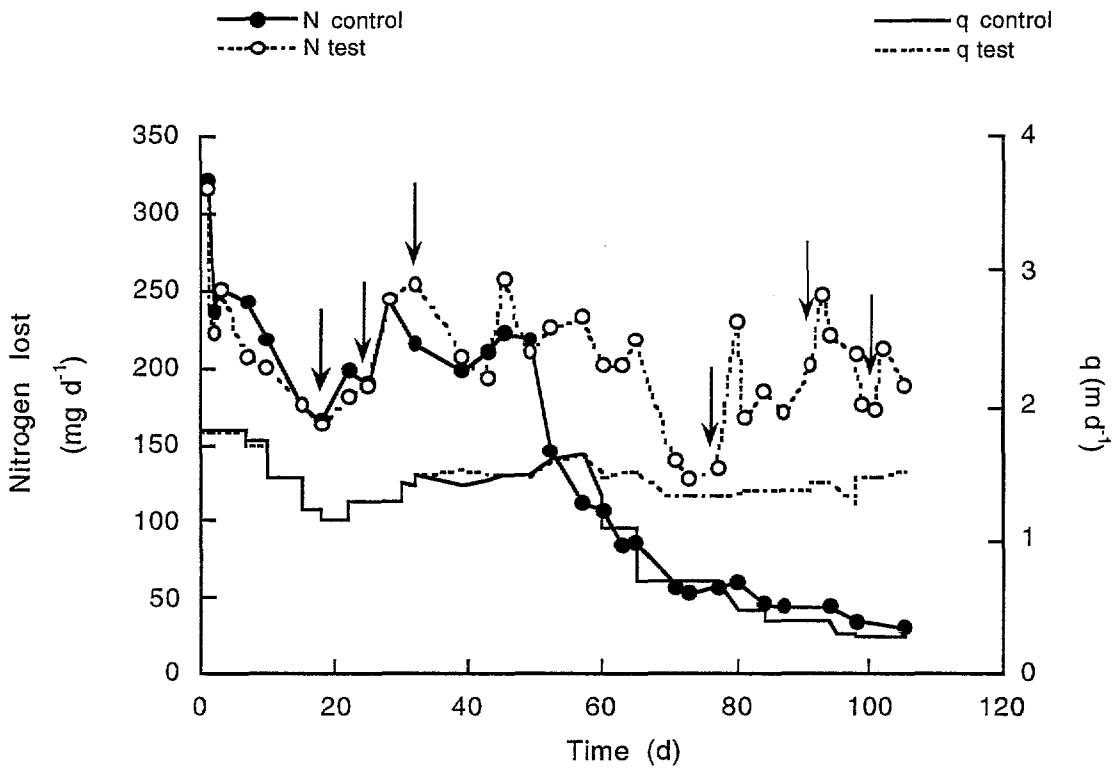

Figure 6: Removal of nitrogen in two columns, control and test, packed with 133 g of wheat straw and operated at the flow rates indicated. Arrows indicate the addition of 20 g of fresh straw to the test column.

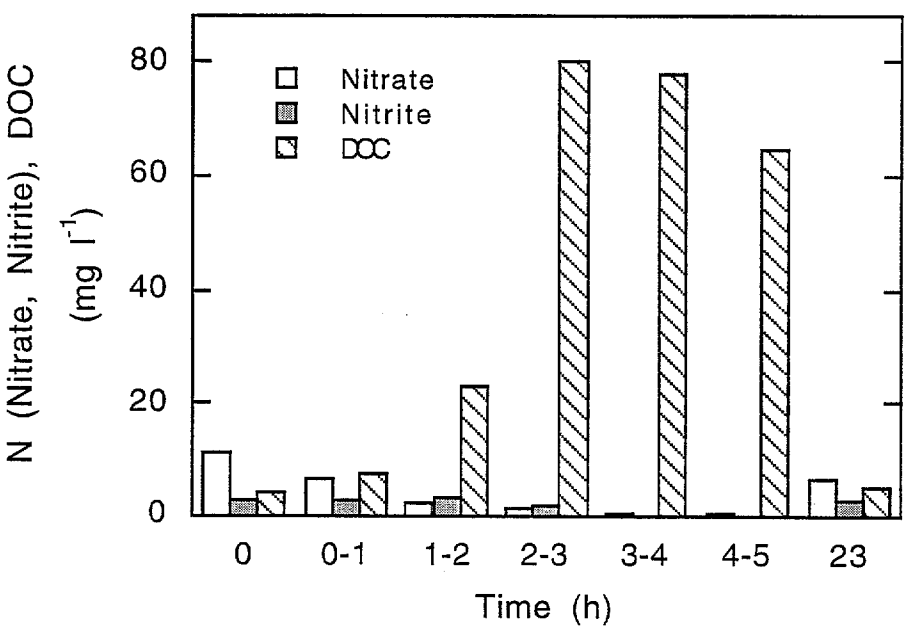

Figure 7: Elution of nitrate-N, nitrite-N and DOC before and following the addition of 20 g of fresh straw on day to a column similar to that in Fig. 6. Flow rate, 3 m d^{-1}.

REAQUA

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Report on the period November '95-end of July '96

SUBPERIODS: 29 (because of changes of filling straw, or alterations of residence time=flow-rate, or in Fe component of the medium); period length with flow rates: 1, 2, 3, 6, 10, or 13; with filling changes:
4, 5, 6 (3x), 7, 10, 15, or 20 days

RESIDENCE TIME (flow rate): 2 h (1440 mm/h), 4 h (770 mm/h), 10 h (285 mm/h) with the three column system, while 0.5 h (860 mm/h), 1 h (430 mm/h), 1.5 h (287 mm/h), 2 h (215 mm/h) or 3.5 h (126 mm/h).

CIRCUMSTANCES ADJUSTED: 3 subsequent narrow column (4,5 X 72 cm), or 1 broad column (12X44 cm); N2 was daily eliminated (by let the fluid to flow out from the column into a reservoir and thereafter pumped back) excluding some week-ends; bubbles were continuously trapped between the columns of the three column system. In the broad single column the filling space was divided into three parts by two perforated iron plates (to make possible of 1/3, 2/3 or 3/3 straw changes, however it caused a spontaneous Fe supply because of corrosion). Medium as earlier (Fe salt incorporated), or Fe chelate extra added in the indicated amounts.,

SAMPLING : daily before and after the individual columns (some week-ends omitted).,

PARAMETERS MEASURED (7): NO3- , NO2, PO4, SO4 (ion-chromatography), NH3 (nesslerization), pH (potenciometry), chemical oxygen demand COD (permanganometry). (SO4 although was measured was not evaluated].

SAMPLES and **DATA:** 311 samples, 2548 primary (measured) data, 2541 secondary (computed and published) data (SO4 omitted).,

EVALUATION of EFFICIENCY (considering about 100 ppm NO3- content gener-ally in Hungarian sources and the hygienical limits):
NO3- elimination capacity (C): 201 ppm or more = e< (more than excellent),

151-200 ppm = e (excellent),
101-150 ppm = g (good),
51-100 ppm = m (medium),
50 ppm or less = w (weak),

Hungarian standards of NO3-, NO2, NH3 and COD in "drinking" water:

Pollutant	"Ground Water" (GW)	Surface Source (SS)	Defended Layer (DL)
NO3- ppm	120/40	120/40	120/20
NO2- ppm	0.10/0.30	0.10/0.30	0.50/1.0
NH3 ppm	0,10/0,20	0,20/0,50	1,0/2,0
COD ppm	2.50/3.50	3.50/4.50	10.0/15.0

1fraction nominator = acceptable, denominator = tolerable (5a1/5t1),

NO3- vel in the effluent (L): 10 ppm or less = bb (super for babies),

11-20 ppm = b (for babies; limit 20),
21-40 ppm = a (for adults; limit 40),
41-60 ppm = w (wrong),
61 or more = vw (very wrong),

NO2- level in the effluent (L): 0.10 ppm or less = 5a 1for GW & SS,

0,10-0,30 ppm = 5t for GW & SS,
0,30-0,50 ppm = 5a for DL only,
0,50-1,00 ppm = 5t for DL only,
1,0 ppm or more = w (wrong) ,

NH3 level in the effluent (L): 0.1 ppm or less = 5a 1for GW,

0.10-0.20 ppm = 5a for GW & 5t 1for SS,
0.20-0.50 ppm = 5t 1for SS,
0.50-1.00 ppm = 5t 1for DL only,
1.00 ppm or more = w (wrong),

COD level in the effluent (L): 2.50 ppm or less = 5a 1for GW,

2.50-3.50 ppm = 5t 1for GW,
3.50-4.50 ppm = 5t 1for SS,
4,50-10.0 ppm = 5a 1for DL,
10.0-15.0 ppm = 5t 1for DL only,

These reported levels are not for direct qualifying, but for orientation as two additional facts should also be considered:

i). for a resource water probably the more rigorous standard will be applied;

ii). in our case a strong post-reactor water-cleaning process,

i.e. a plant will be inserted, thus drinking quality is guaranteed.,

RESULTS

I. and II. period (08.11.95-11.11.95 and 12.11.95-14.11.95): Tree columns, residence time 2 or 4 h., N2 elimination, bubble trappings. The system were in the stage of lag: NO3- C: m or w (less than 30%), NO3- L: vw, however no NO2 was produced, while considerable NH3 evolved, NH3-L: DL-t or DL-t<. COD-L: GW-t-DL-t<.

III. period (15.11.95): Residence time 4 h., no bubble trapping. Results similar to the previous: NO3- C: m, NO3- L: vw (although better than before), No NO2, while NH3: DL-t<, COD-L: DL-a,

IV. Period (16.11.95-17.11.95): Residence time 10 h., no N2 elimination and bubble trapping. Short start; NO3- C: g and m, NO3- L: vw (although on 16 better than on 17), with no NO2, but more NH3 production as NH3-L: DL-t and DL-t<, while the increase of COD refers to starting bacterial reproduction, as COD-L: from GW-t to DL-a.

V. Period (18.11.95): Residence time 10 h., N2 eliminated, no bubble trapping. Slight improvement. NO3- C: g, NO3-L: vw (although better than before), no NO2, NH3-L: DL-t or DL-t<, COD-L: DL-a.

VI. Period (19.11.95-25.11.95): New straw in c-3, others c-3 → c-2, c-2 → c-1, c-1 → discharged, on 19, 20 and 22 no N2 elimination good NO3- C: e< and NO3-L: bb, but with very high NO2-L: DL-t<< (although in c-3 only, while in c-2 GW-t = SS-t), and NH3. COD increased (NH3-L: DL-t< COD-L: DL-a, again in c-3 only). At 20: NO3- C: g, NO3- L: vw, NO2-L: DL-t< (again the c-3 is the worst), NH3-L: DL-t<, COD-L: DL-t< (to which growing microbes contribute too). At 21: Medium good results, NO3- C: g, NO3- L: w, NO2-L: GW-t= SS-t, NH3-L: DL-t< (slightly), COD-L: DL-t< (in the last only). At 23: Better, NO3- C: e, NO3- L: b, NO2-L: GW-t = SS-t, NH3-L: DL-a, COD-L: DL-t. At 24 and 25: System tended to get exhausted.

VII. Period (26.11.95-03.12.95): New straw in c-1, others c-2 → c-3, c-1→ discharged. At 26 and 27: strong NO3- elimination (NO3- C:e and e, NO3- L: bb both (no residue !), with good NO2-L: GW-t = SS-t and GW-<a = SS-<a, while NH3-L: DS-t<< and DS-t<, COD-L: DL-t< both. At 29 and 30: NO3- C: g both, but NO3- L: b and a, with good NO2-L: GW-t = SS-t, while NH3- L: DL- t< and DL-t, COD-L: DS-t< and DS-t. At 02.12 and 03.12: decreasing activity, NO3- C: g both, NO3- L: vw both, NO2-L: GW-t = SS-t both, NH3-L: DL-t both, COD-L: dl-a both.

VIII. Period (04.12.95-10.12.95): New straw in c-1, others c-1→ c-2, c-2→ c-3, c-3 → discharged.. At 04, 05, 06, 07, 08 and 10: activity increases and slowly decreases, NO3- C: e<, e<, e, e, e and e; NO2-L: GW-t = SS-t, GW-t = SS-t, DL-a, DL-t<, DL-a and GW-t = SS-t; NH3-L: DL-t<, DL-t<, DL-t<, DL-t<, DL-t and DL-t; COD-L: DL-t<<, DL-t<<, DL-t<, DL-t, DL-t< and DL-t.

IX. Period (29.01.96-13.02.96): One (broad column, autoclaved new straw (inoculum taken from old), residence time 2 h, flow rate 170 mm/h. At 30.01 and 31.01: system starts, NO3- C; a and g, NO3- L: vw<< and vw<, NO2-L: DL- t<< both, NH3-L: DL-t< both, COD-L: DL-t<< both. At 01.02, 02.02, 05.02, 06.02, 07.02, 12.02 and 13.02: Gradual decrease of activity, NO3- C: e, m, w, m, m, w and w; NO3- L: b, vw<< all; NO2-L: DL-t<<, GW-t = SS-t all; NH3-L: DL-t<, DL-t<, DL-t, DL-t, DL-t, SS-t and DL-a (but high influx level in all); COD- L: DL-t<, DL-t, DL-a, DL-a, DL-a, SS-t and SS-t.

X. Period (19.03.96-29.03.96): 3 columns, again, new straw, residence time 2 h, flow rate 1440 mm/h, new straw. At 20: Weak activity as start. NO3- C: m, NO3- L: vw<<, NO2-L: DL-t<, NH3-L: DL-t<, COD-L: DL-t<<. At 25, 26, 27, 28 and 29: Slow exhaustion, NO3- C: e<, e all; NO3- L: b, bb, bb, b and a; NO2-L: DL-t, GW-a = SS-a, GW-t = SS-t, DL-t and DL-a; NH3-L: DL-a, DL-a, DL-a, DL-t and DL-a (although influx high); COD-L: DL-t<, DL-t<, DL-t<, DL- t<, dL-t.

XI. Period (30.03.96-04.04.96): New straw. At 01, 02, 03 and 04: medium good results, NO3- C: g, g, e and e; NO3- L: w, a, a and w; NO2-L: GW-t = SS-t, DL-t, GW-t = SS-t, GW-a = SS-a; NH3-L: DL-a all; COD-L: DL-t, DL-t<, DL-t, DL-t. ,

XII. Period (10.04.96-16.04.96): New straw. Activity decreased. At 11, 15 and 16: NO3- C: g, m and m; NO3- L: a, vw< and vw<<; NO2-L: GW-a = SS-a, DL-a and DL-a; NH3-L: DL-t, DL-a and SS-t; COD-L: DL-t<, DL-a and DL-a. ,

XIII. Period (09.05.96-14.05.96): Single, broad column, new straw. At 10, 12, 13 and 14: NO3- C: e, e, e and e; NO3- L: a, w, a and a; NO2-L: GW-t = SS-t, DL-a, DL-t and DL-t; NH3-L: DL-t<, SS-t, DL-a and DL-a; COD-L: DL-t<<, "DL-t", DL-t and DL-t. ,

XIV. Period (15.05.96-16.05.96): New straw, flow rate ca. doubled. At 15 and 16: NO3- C: g and missing, NO3- L: vw and missing; NO2-L: DL-t<< and DL-t; NH3-L: DL-t and DL-t; COD-L: DL-t< and DL-t<.

XV. Period (17.05.96-18.05.96): Flow rate slightly decreased. At 17 and 18: NO3- C: e< and e<<; NO3- L: a and a; NO2-L: DL-a and DL-t; NH3-L: DL-a and DL-a; COD-L: DL-t< and DL-a.

XVI. Period (19.05.96-23.05.96): Flow rate four times increased. At 19, 20, 22 and 23: NO3- C: e<, w, g and g; NO#-L: vw, w, vw and vw; NO2-L: DL-t<, DL-t<, DL-t and DL-t; NH3-L: SS-a = GW-t, DL-a, DL-a and SS-t; COD-L: SS-a, SS- t, DL-a and DL-a.

XVII. Period (24.05.96-30.05.96): Residence time doubled, on 27 no N2 elimination. At 24, 25, 25, 26, 29 and 30: NO3- C: e<<, e<<, e<<, w and w; NO3- L: vw, vw, b, vw and vw (Consider that at the first three the row were extremely high); NO2-L: GW-t = SS-t, GW-t = SS-t, DL-a, GW-t = SS-t and DL-a; NH3-L: DL-a, DL-a, SS-t, DL-a and DL-a; COD-L: SS-t, SS-t, SS-t, SS-a and SS-a.

XVIII. Period (01.06.96-03.06.96): 1/3 new straw. At 01 and 03: NO3- C: w and w; NO3- L: w and vw<<; NO2-L: missing and DL-t; NH3-L: missing and DL-a; COD-L: missing and DL-a.,

XIX. Period (04.06.96-06.06.96): High Fe chelate, on 05 no N2 elimination. At 04: NO3- C: vw; NO3- L: vw<; NO2-L: DL-t; NH3-L: DL-a; COD-L: missing.

XX. Period (07.06.96-11.06.96): 3/3 new straw, on 08 and 09 no N2 elimination. At 07, 08, 10 and 11: NO3- C: e<, e<, g and g; NO3- L: bb, w, vw and w; NO2-L: DL-t, DL-t<, DL-t< and DL-t<; NH3-L: DL-t<, DL-t<, DL-t and DL-t; COD-L: missing, DL-t<<, DL-t< and DL-t<<.,

XXI. Period (12.06.96-13.06.96): Medium Fe chelate. At 12 and 13: NO3- C: g and m; NO3- L: vw and vw<; NO2-L:DL-t< and DL-t<; NH3-L: DL-a and SS-t; COD-L: DL-t< and DL-t<.

XXII. Period (14.06.96): 1/3 new straw. NO3- C: w; NO3- L: vw; NO2-L: DL-t<; NH3-L: DL-a; COD-L: DL-t<.

XXIII. Period (15.06.96-17.06.96): Smallest Fe chelate, on 15 and 16 no N2 elimination. At 15 and 17: NO3- C: e and m; NO3- L: w and vw; NO2- L: DL-t< and DL-a; NH3-L: DL-a and DL-t; COD-L: DL-t and DL-t.,

XXIV. Period (18.06.96-19.06.96): 1/3 new straw. At 18: NO3- C: g; NO3- L: bb; NO2-L: DL-t; NH3; DL-t; COD-L: DL-a. At 19: NO3- C: e<; NO3- L: bb; NO2-L: DL-t; NH3-L: DL-t<; COD-L: DL-t< (post aeration decreased slightly NO2 and COD, while increased slightly NH3).

XXV. Period (20.06.96): Catalytic amount of acetate introduced. At 20 forenoon: NO3- C: e; NO2-L: b; NO2-L: DL- t; NH3-L: DL-t<; COD-L: DL-t< (post-aeration decreased slightly NO3- L, NO2 and COD, while considerably NH3. At 20 afternoon: NO3- C: e<; NO3- L: bb; NO2-L: GW-t = SS-t; NH3-L: DL-t; COD-L: DL-t< (post-aeration decreased slightly NO2, NH3 and COD).

XXVI. Period (25.06.96-02.07.96): 1/3 new, pre-washed straw, 200 min. residence time (135 mm/h flow rate). At 27: NO3- C: e<; NO3- L: b; NO2-L: DL-t; NH3-L: DL-a; COD-L: DL-t< (post aeration slightly decreased NO2, while considerably COD). At 28: NO3- C: e<; NO3- L: bb; NO2-L: DL-a; NH3-L: DL-t<; COD-L: DL-t< (post aeration decreased slightly NO2 and COD, while considerably NH3). At 30: NO3- C: e<; NO3- L: bb; NO2-L: GW-t = SS-t; NH3-L: SS-t; COD-L: DL-t (post aeration decreased slightly NH3, considerably COD, while increased slightly NO3- L and considerably NO2. At 01: NO3- C: e<; NO3- L: vw (however compair influent conc.); NO2-L: GW-t=SS-t; NH3- L: SS-t; COD-L: DL-t< (post aeration decreased considerably NO3- , NH3 and COD, while increased considerably NO2. At 2: NO3-, NO2 and COD as previous, NH3 changed from DL-t< to SS-t with aeration.

XXVII. Period (03.07.96-06.07.96): 1/3 new straw. At 04: NO3- C: e<; NO3-L: w (nowever consider influx conc.); NO2-L: DL-t; NH3-L: DL-t<; COD-L: DL-t<<

(post aeration decreased considerably NH3 and COD, while increased slightly NO3- L, and considerably NO2. At 06: NO3- C: e; NO2-L: vw; DL-t; NH3-L: DL-a; COD-L: DL-t.

XXVIII. Period (11.07.96-12.07.96): residence time 26 min. (1024 mm/h flow rate). At 12: NO3- C: e; NO3- L: bb; NO2-L: gw-a =ss-a; NH3-L: DL-t<; COD-L: DL-t< (aeration didn't cause considerable changes).

XXIX. Period (18.07.96-31.07.96): residence time 58 min. (466 mm/h flow rate): At 19: NO3- C: g; NO3- L: b; NO2-L: DL-t<<; NH3-L: SS-t; COD-L: DL-t. At 21: NO3- C: e; NO3- L: bb; NO2-L: DL-t<<; NH3-L: DL-t<; COD-L: DL- t<. At 22: NO3- C: g; NO3- L: b; NO2-L: DL-t<<; NH3-L: DL-t; COD-L: DL- a (post aeration made a little worse). At 23: NO3- C: g; NO3- L: b; NO2-L: DL-t<<; NH3-L: DL-a; COD-L: DL-t (post aeration increased little NO2, considerably NH3, while decreased little COD). At 24: NO3- C: g; NO3- L: b; NO2-L: DL-t<<; NH3-L: DL-a; COD-L: DL-t< (post aeration decreased NO2, while increased NH3). At 27: NO3- C: g; NO3- L: b; NO2-L: DL-t<<; NH3-L: DL-a; COD-L: DL-t (post-aeration little decreased NO2 and COD, while little increased NH3). At 31: NO3- C: m; NO3- L: b; NO2-L: DL-t<; NH3-L: <GW-a; COD-L: DL-a (post aeration little increased NO2 and NH3, while decreased COD).

COMMENTS

i.) NH3 was often high in influx (medium; ingredients ?).,

ii.) NH3 may evolved from protein decomposition in straw.,

iii.) NH3 is produced anyhow it would have been eliminated by metabolic assimilation - HOOC-CO-CH2-COOH + NH3 + NADH2 --> HOOC-CH.NH2-COOH + NAD - , however on the expense of NADH2 produced by carbon source dissimilation .

vi.) NH3 and NO2 in effluent would be eliminated by the obligatory water purification post-treatment (thus extra carbon source must not be added).

vii.) Immediately after straw change COD jumps high because of the initial washing out of organic compounds from the new straw (later the value decreases to moderate level, depending on bacterial growth).

viii.) Many times the NO2 and/or NH3 in row are higher than in treated samples (consumed in the column).

ix.) Omitting of N2 elimination generally lead to weaker effect on the next.,

x.) Towards to study the maximum capacity 600 ppm influx NO3- was tried, which was eliminated within 1 h. residence time.

xi.) The system has a relative broad pH resistance, or tolerance, as it remained active both in medium or strong acidic, as well as in medium or strong alkalic conditions.,

xii.) As it is seen post-reactor aeration was introduced into the entire process. It is the first step of the post-reactor treatment to be developed (especially to decrease COD and other unpleasant parameters).

xiii.) introduction into the medium of acetic acid in catalytic amount (10 mgC/L; its C ratio to NO3- nitrogen is 1:3) from 19.06., had a beneficial effect.

xiv.) PO4 depletion of the medium from 24.07 decreased the NO3- elimination and increased the NO2.,

xv.) The changes made in media - acidification of batch I. against fungal contamination (and neutralisation when mixing with bath II. immediately before column) caused decomposition of the iron complex and precipitation of Fe hydroxide leading to Fe depletion and consequently bad results in NO2 from 19.07, however, the system tended to compensate it from 22.07, at least partially.

Newly acquired instruments and equipments:,

- DU-2 Spectrophotometer (Beckman) with flame exciting accessory;,
- DB-G Grating spectrophotometer (Beckman) with recorder;,
- EPIVAL Research microscope (ZEISS) with epi- and trans illumination, and halogen lamp;,
- OH-107 Multifunction Routine Polatograph (Radelkis),
- OK-102/1 Conductivity Meter (Radelkis),
- ProMinent Concept pumps (3 pieces),

Budapest, 08. 16. 1996.

Sincerely yours:

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