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An heterotrophic autotrophic denitrification approach for nitrate removal from drinking water by alfa stems

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ABSTRACT/RESUME

Abstract: *Biological denitrification of drinking water was studiedin up-flow laboratory reactors packed with alfa stems served as the sole carbon source as well as the only physical support for the microorganisms. The highest rates of denitrification were observed in fresh reactors during their first week of operation and the efficiency of the process declined therafter. In the first part, we have analysed the influence ohhydrolyc and volumic load to value the capacity of nitrate prurification in a down flow submerged biofilter then with an experimental design approach, we have analysed the qualitative and quantita-tive aspects of the effects of some factors, concentration of nitrate (80-200 mgl^{-1} and velocity (0.31 mh^{-1} on different responses like the apparentrate of denitrification as well as concentration of nitrite, nitrate and chemical demand on oxygen (DCO)in the reactor outlet.*

I. Introduction

Ecrease of nitrate concentration is often required for dricking water in order to meet standard of 11.29 mg nitrat- N per 1 in water for human consumption. Among various methods available (physical, chemical, physic-chemical and biological) for the removal of nitrate, biological removal (denitrification) is considered to be the most economical and environmental sound and to be feasible on a large scale. Denitrification is the reduction of nitrate to N_2 carried out by aerobic bacteria which, in the absence of dissolved oxygen, can use nitrate nitrogen as a terminal electron acceptor.

Most biological denitrification processes are based on heterotrophic bacteria utilizing organic carbon in the form a simple compound. However, complex carbon sources such as cellulose-rich materials can also be used and we have recently studied the feasibility of using newspaper and cotton as carbon sources for the remediation of nitrate polluted groundwater.

Microorganisms capable of degrading cellulose are widely distributed in nature and usually occur in mixed culture with organisms with organisms which degrade associated polymers. Initial cellulose degradation requires direct physical contact between the enzyme molecules and the surface of cellulose and complete degradation depends on the concerted action of various enzymes which may act in synergism.

Cellulose is a basic component of all plant materials and constitutes the most abundant renewable resource in the word, with an estimated production rate of 4.10^{10} ton per year. It is a linear glucose polymer with neighboring chains and is organized in fibers in close association with lignin and hemicelluloses. Thus, alfa stems is a complex mixtSeveral bacteure of cellulose, hemicelluloses (including xylan), pectins and linins, of which xylans and other xylose polymers constitute about 25%.

The capacity of alfa stems to support water denitrification has been shows by others. Belouanas and al used field and laboratory reactors packed with alfa stems in the treatment groundwater to remove

particulate matter from effluents prior to their application in drip irrigation.
In the development of this water the formation of gases and the special characteristics of the carbon substrate were taken into consideration denitrification system. In oreder to minimize clogging of the reactor due to the entrapment of N_2 bubbles a coarse matrix should be used. On the other hand, a high filling ratio of substrate would be necessary because alfa stems is a bulky non diffusible carbon source which is slow and incompletely degraded. Furthermore, packing of the reactors as well as the removal and disposal of spent substrate should be easily performed. Filling up the reactors with alfa stems only appeared to be the most suitable process. Thus, the aim of this study was to determine if alfa stems can serve as the sole carbon substrate for the denitrification of drinking water as well as the sole physical support for bacterial growth.
In Algeria a study realised By "Agnene National des Resources Hydrique (ANRH) and Blida University, relieved a presence of a higher critical nitrate contamination of water in the zone of Chelif and Metidja, the nitrate concentration was about 200-270 $mg.1^{-1}$ [1].

II. Materials and methods

II.1. Experimental apparatus

The reactors routinely used were PVC columns, 50 cm hight and 08cm diameter, packed with 103g alfa stems with a thin layer of glass wool placed at each end. Slightly larger columns (55 cm hight and 10 cm diameter) were used when aditions of 20g of fresh alfa stems were carried out because there design made it easier to open at the bottom where the new substrate was added ; they were packed with 177 g of alfa stems so that same filling ratio (41 g alfa stems per 1) was used in all experiments. The columns were inoculed with a small amount of alfa stems removed from an active denitrification column and the original inoculums was a mixture of forest ans garden soils. The freshly packed columns were filled with feed solution (tap water amended with 22.6 mg nitrte -N 1-1 and 3mgl-1 phosphate) which was recirculated for 2 days.
After this inoculated period, the reactors were startedvup (day0) in an uplpw mode (Figure 1).
Water velocities v(v=Q/A, where Q is the measured fiow rate and A is the cross section of the column) were calculated in mh-1.
Unless otherwise indicated, the ambient temperature was maintained at 25 1°C. Influent and effluent samples were tested for nitrate, nitrite, amomonia, pH, dissolved organic carbon (DOC)
and bacterial counts.

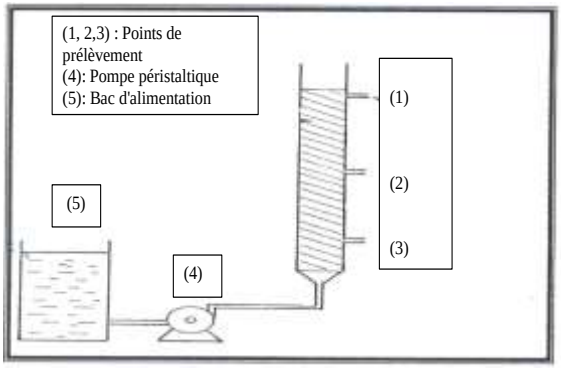

Figure 1. *schematic representation of experimental set*

Chemicals reagents
Potassium nitrate was used to spike tap water.Iron

II.2. Analytical procedures

Nitrate was determined by the method of cataldo and al. (1975) and nitrite and ammonia were assyed according to APHA (1989); DOC was determined by a TOC analyser.

II.3. bacterial counts

Colony forming units were counted by standard plating techniques on R2A agar.
In view of purifying stations possesses a complex bacterial flora able to trait several pollutants, we choused to take the denitrifying bacteria from a sludge of the boumerdes purifying station. In order to favour the development of denitrifying bacteria, the mud is put in a close basin with rich nitrate water and oligoelements (table 1) [2,3]. The device function in discontinue system. A steady of nitrate concentration is made regularly and the basin solution is renew each time chen the nitrate concentration is under the norm, this permits to adapt progressively the bacterial flora and the number of denitrifying bacteria. When this stage attained we pass to the continue system. (figure 2)

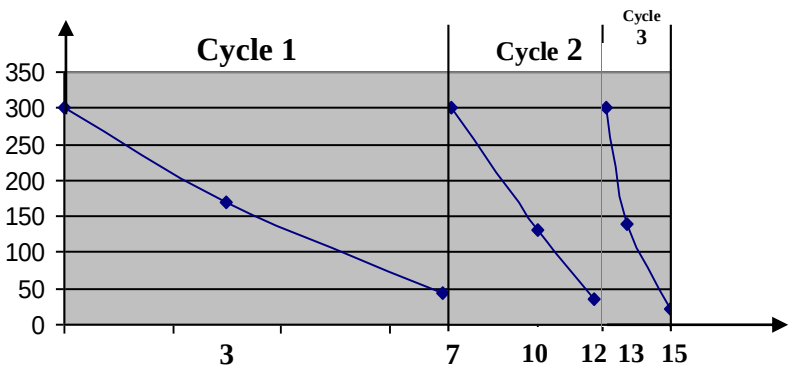

Figure 2. *Denitrification Kinetic at the time of inoculum's preparation [2, 3].*

Table nº1. *concentration of oligo elements*

Oligo elements	Concentration ($g.l^{-1}$)
K_2HPO_4	0,05
$KHPO_4$	0,05
NH_4Cl	0,06
$MgSO_4.7H_2O$	0,16

The ammonium salt was used since it appears that certain denitrifiers use nitrate as a terminal electron acceptor not as a nitrogen source [4, 5, 6].
After the preculture, we propose to cultivate heterotrophe denitrifying bacteria attahed to Alfa stems and coated with polysaccharides. The fist stage of attachement intervenes movement generating appendixes which permits bacteria to approach the attachment surface [6, 7, 8]; these bacteria use the cellulose, lignin and heicelluloses as a carbon source.
The column functions on a closed system. Microorganism's population develops and fixes gradually on the Alfa stems surface.

III. Results and discussions

III.1 Velocity influence on the rate of denitrification at different velocities

III.1-1 influence of height column on the rate of denitrification at different velocities

In order to optimize the velocity in the reactor we have percolate a solution of nitrate which concentration is 100 mg.l-1 along the column in an up flow with different velocities that is to say; 0.3 $m.h^{-1}$; 0.45 $m.h^{-1}$; 0.60 $m.h^{-1}$; 0.80 $m.h^{-1}$; and $1m.h^{-1}$.
E volution of the salvage (residual) value of nitrate according to the column height can be measured thanks to samples points arranged along the column and distances; 50 am; 100 am ; et 150 cm (figure 3,4)
According to the results obtained, we establish (notice) that the rate of denitrification increases when the velocity passage decreases.

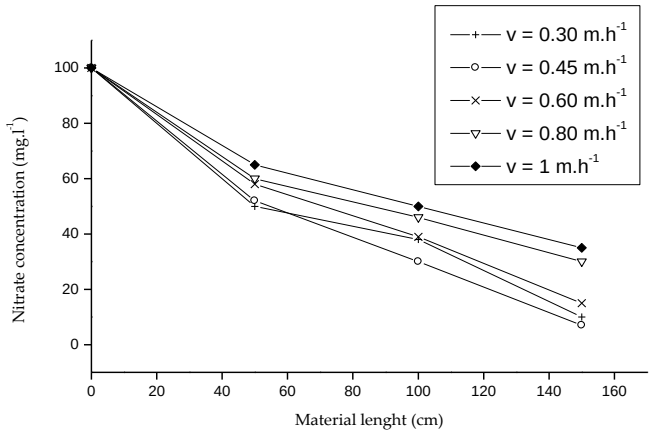

Figure 3. *Evolution of nitrate concentration along the column at different velocities value (pH=8.30, T^o=25ºC, $[NO_3^-]$=100mg.l^{-1})*

III.1-2. Ifluence of height column on the nitrite concentration at different velocities

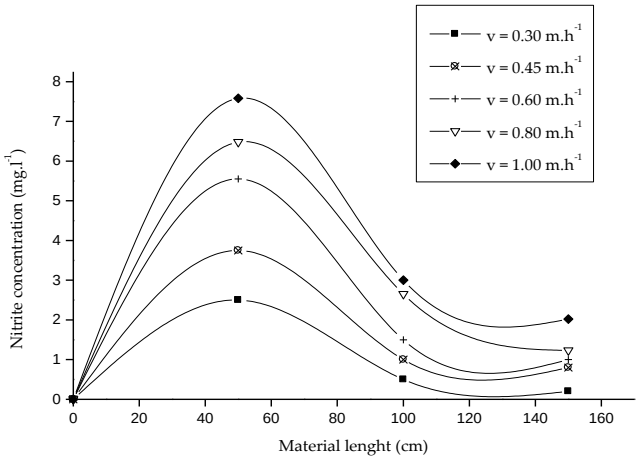

Figure 4. *Evolutionof nitrite concentration along the at different velocities value (pH=8.30, T^o=25ºC, $[NO_3^-]$=100mg.l^{-1})*

III.1-3. Ifluence of initial nitrate concentration on the rate of denitrification:

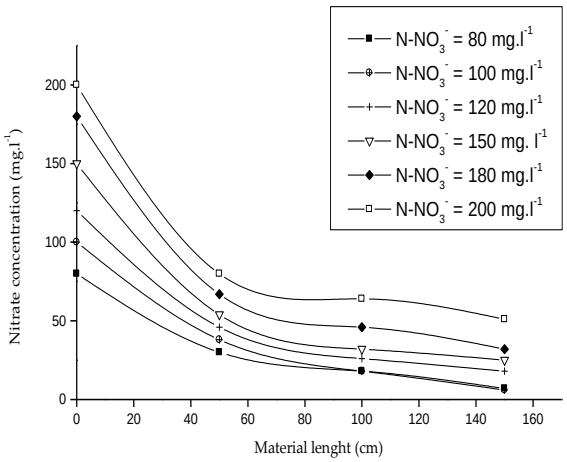

Figure 5. *Evolution of nitrate concentration along the column at different initial nitrate concentration (pH=8.02, Tº=25ºC, v= 0.45m.h^{-1})*

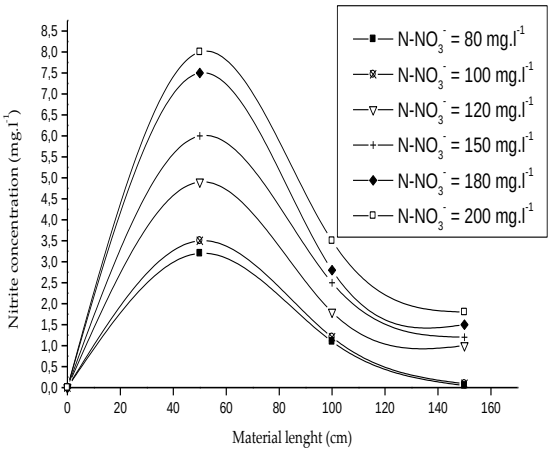

Figure 6. *Evolution of nitrite concentration along the column at different initial nitrate concentration (pH=8.02, Tº=25ºC, v= 0.45m.h^{-1})*

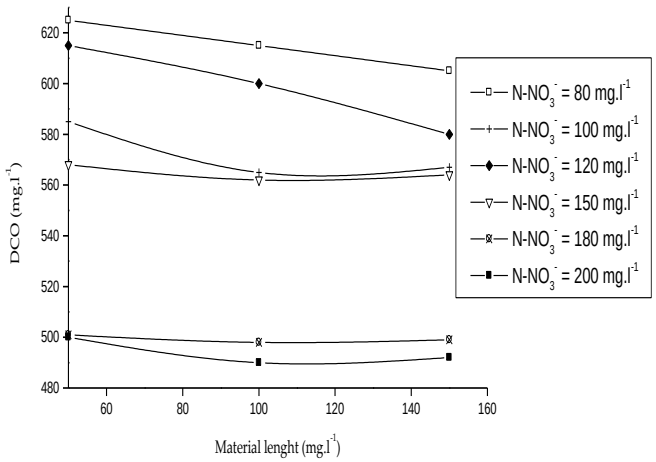

Figure 7. *Evolution DCO along the column at different initial nitrate concentration (pH=8.02, Tº=25ºC, v= 0.45m.h^{-1})*

Nitrates (NO_3-)

The profiles represented on figure 3 shows that the nitrate elimination is realized in an exponential way and there is two functioning phases in the denitrifying reactor;

First phase:

It is situated in the first 60 am of the column; it is rapid and characterized buy the elimination of 70 % of nitrate present in the underworld (sample).

Second phase:

It is characterezied buy a slow elimination of the residual quantity along the columns (60-150 cm).

We can explain this two phases with the presence of denitrifying bacteria in the columns according an exponential profile decreasing from the low to the up of the columns (figure 5,6)

This distribution is due to the up flow of the effluent which causes an important bacteria proportion in the bottom of the column: thers is an accumulation of the bacteria mass near the alimentation [9,10].

Indeed, when the water to treat goes along the column, aero-anerobic bacteria consume the dissolved oxygen in the first way then the one of the nitrate. So the excess of the carbonated substratum permit the rapid elimination of oxygen and an effective elimination of nitrate.

Nitrate can diffuse inside the deep lavers of the biofilm and transformed in the anoxic areas (zones) in spite of the presesnce of oxygen. Enzymes in whole cells or extracts of ordinary, anaerobically respiring denitrifies reduce nitrate to nitrite, nitrite to nitric oxide to nitrous oxide, and nitrous oxide to dinitrogen [11].

Oxygen is the most important regulator of denitrification, rigorous anoxia is not necessary for onset or continuation.
A decrease in the oxygen supply rate to 0.4mmol.1^{-1} or lower permits the on set of denitrification during growth of pseudomonas aeroginosa [12]. Low and al. (1993) found in measurement of denitrification that it was absent until the oxygen concentration was about 1.6 mg. O_2 1^{-1}, found that denitrification was dramatically lowered witrh a slight increase from zero, found that the rapid increase of denitrification is between 1-2 mg O_2 1^{-1}. In our experiences oxygen concentration was 0 mg.1^{-1}.

Nitrite (NO_2):
The nitrite profile along the column shows that these latest appears and there concentration increases to reach a peak, then they decrease to reach the proximate values from zero in the reactor outlet. This is explained buy the fact of the nitrite represent the intermediate stage of the reduction nitrate to the gas nitrogen according to the reaction:

NO3- ⟶ NO2- ⟶ N2

The concentrations of nitrite outlet the column depend on its height, on the hydraulic and volumic load. More the volumic load is high, there is more nitrite outlet the reactor. At lows concentrations of nitrate 80 mg.l^{-1}, 100 mg.l^{-1} we obtain nitrite values under the norm (figure 6).
In order to decrease the concentration of nitrite outlet the reactor, we have to increase the stay's time.

DCO
Evolution of the organic substances in denitrifying water was following with the measure of the DCO in this latest. The figure 7 represents the evolution of DCO according to the column length for different concentrations.
We observed a decreasing profile equivalent to the nitrate one though less pronounced.
There for the DCO reduction is due to the biodegradation pf the substrate (figure 7.
Denitrification is realised by a large species of bacteria, whose optimal conditions of temperature are varied. It's why the temperature range is very wide. We have realised the experiences in temperatures varied between 20 and 25 °C.
Most studies on the influence of temperature on denitrification had done on soils [13]. The temperature is an activate factor until 60 °C and then a deactivate factor after that.
The pH used in experiences was between 8 and 8.50, pH is not limited factor of denitrification [14, 15, 16], but it has an important effect on the gaseous products produced at the time of denitrification, for the values of neutral pH, the final product is the gas nitrogen (N_2).

IV-Conclution
During this study, we studied the feasibility of biological denitrification of synthetic laden water with nitrate, using Alfa stems as consumable support. During the first phase of our study, we favourd the development of the biomass from an activated sludge in order to fix them on the support.
In the second stage we started the treatment and established the profile of different parameters concentrations as nitrate, nitrite, temperature and DCO. This permitted to notice an abatement of nitrate included between 60 and 80% outlet the column.
A regards nitrite, there concentrations increase in the first part of the column and decrease in the second one, but there concentrations outlet the rector is related to the length of the column and the hydraulic charge applied.

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