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Biological Denitrification Heterotrophe of Water With Fixed Biomass Using Alfa Stems as Energy Source

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This work presents the results optimization of the biological process denitrification with fixed biomass using a consumable support (Alfa Stem). In the first part, the influence of hydrolyc and volumic load to value the capacity of nitrate purification in a down flow submerged bio filter was analyzed. Then with an experimental design approach, we have analyzed the qualitative and quantitative aspects of the effects of some factors: concentration of nitrate (80-200 mg L^{-1}) and velocity (0.3-1.0 m h^{-1}) on different responses like the apparent rate of denitrification, as well as concentration of nitrite, nitrate and chemical demand on oxygen in the reactor outlet.

Key Words: Biological treatment, Denitrification, Nitrate elimination, Biological reactor, Denitrifying bacteria.

INTRODUCTION

Several bacteria substitute nitrate for O_2 in a form of anaerobic respiration called denitrification. Nitrite and N_2O are confirmed intermediates: NO may be to and N_2 is the final product. Denitrification is a major mechanism for nitrogen removal from nitrate rich waters, but it requires oxygen poor conditions[1-8].

In aquatic environments, bacteria and other microorganisms attach to solid surfaces producing complex microbial communities, which are referred to as biofilms or periphyton[9]. Zobell[10] discovered that surfaces have a positive influence on bacterial activity and it has later been shown that attached bacteria is more active and often present at higher densities than free living bacteria[9,11,12].

Nitrate is a major pollutant in rivers, reservoirs, ground waters and coastal waters in much of the northern hemisphere. Major sources of NO_3^- in large rivers and ground waters include atmospheric deposition, agricultural run off and sewage effluent[13]. High rates of atmospheric deposition nitrogen result in elevated NO_3^- concentrations even in flowing waters that do not receive agricultural or sewage inputs[14,15].

Increasing pollution of underground and surface water threatens not only the environment but also by extension, the public health. Mineral waste non biodegradable engender the releasing of salts in water (nitrate, phosphate and organic compounds). Nitrogenous pollution, under its different forms (N reduced form, N oxidized form), is one of the key factors of initialization the eutrophication of water accompanied with degradation of its quality and large effects on human health notably: (a) Deterio-

ration of utilitarian value of waters (drinking water problems), (b) Increasing risks of public health (skin disease, viral disease, cancer risks), (c) Biological disequilibrium ecosystem (modification of the flora and fauna, increasing of silting up velocity).

The effect of nitrate on the human health is linked to their transformation to nitrosamines on level with the digestive tube. The nitrites are responsible of risques of methemoglobinemia. Knobelock and Anderson[16] concluded from the work of Hegesh and Shiloah[17] that as little as 12 mm of nitrate-N can significantly increase an infant's methemoglobin level. According to the National Research Concil's (NRC)[18] on nitrate and nitrite on nitrate drinking water, dietary nitrate intake for an adult average 76 mg per day. The United States Environmental Protection Agency reports[19] cited by Avery were contained to evaluating exposures to nitrate in municipal water supplies. The report assumed a maximum nitrate concentration of 10 mg L^{-1}. The European Community also assumed a maximum nitrate concentration[20] of 50 mg L^{-1}.

In Algeria a study realized by Agence Nationale des Ressources Hydriques (ANRH)[21] and Blida University, relieved a presence of a higher critical nitrate contamination of water in the zone of Chelif and Mitidja, the nitrate concentration was about 200-270 mg L^{-1}.

The object of this work consists in treating water polluted by nitrate on a biologic column with optimization of different parameters such as: velocity of the alimentation water and the height of the column. The objective is to study the kinetics of denitrification the fundamental parameters intervening in the bacterial activity, using an organic source of carbon as support (Alfa Stems). We have analyzed of hydraulic and volumic load to value the capacity of nitrate purification, then with an experimental design approach, we have analyzed the qualitative and quantitative aspects of the effects of some factors and measured the concentration of NO_3^-, NO_2^- and COD in the reactor outlet.

EXPERIMENTAL

Treatment trial was made in an experimental device constituted of a column (reactor) full in Alfa stems and an alimentation tray containing nitrate polluted water.

The biological reactor is a glass pipe reaching 150 cm height where only 145 cm are filled with support. Interior diameter of the column is 38 mm and useful volume of the reactor is 1.646×10^{-3} m^3. The column is equipped with three points samples on the height of the column every 50 cm. These stitching allow taking samples in the bed. Efficacy of treatment was estimate analytically with evolution steady of different parameters as: nitrate, nitrite, pH and chemical demand on oxygen.

Alfa stems was used as bacteria support, first treated with sodium hydroxide solution (2 %) for 2 h and then rinsed and check the neutrality of rinsing water. At the time of starting of a biological reactor, the first difficulty is to obtain sludge able to degrade the pollutant. For that reason, inoculation with activate sludge adapted to the substratum and to the operating conditions. In view of the sewage treatment plants generally have an enough complex bacterial flora able to treat several pollutants, we have chosen to take a sample of water from Boumerdes sewage treatment plant.

The column required a preculture. Surveys estimate that 10 % of the recoverable bacterial flora of various soils, sediments and waters are denitrifiers, with *pseudomonas* and *alcaligènes* species[22,23]. Several authors proposed to cultivate *Thiobacillus denitrificans* in columns loaded with sulphur with a specific environment[24]. They fixed 15 days for preculture and during this period water is changed after every 3 d.

In view of purifying stations possesses a complex bacterial flora able to trait several pollutants, we choose 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 (Fig. 1, Table-1)[25,26]. A steady of nitrate concentration is made regularly and the basin solution is renew each time when the nitrate concentration is under the norm, this permits to adapt progressively the bacterial flora and the number of denitrifying bacteria. When this stage is attained we pass to the continue system.

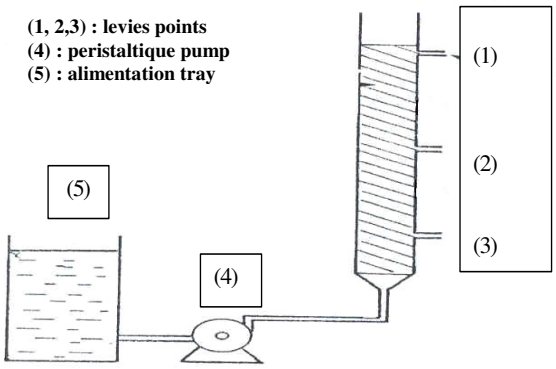

Fig. 1. Representative diagram of denitrification installation

TABLE-1
CONCENTRATION OF OLIGO ELEMENTS

Oligo elements	Concentration (g L^{-1})
K_2HPO_4	0.05
$KHPO_4$	0.05
NH_4Cl	0.06
$MgSO_4 \cdot 7H_2O$	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[27,28].

After the preculture, we propose to cultivate heterotrophe denitrifying bacteria attached to alfa stems and coated with polysaccharides. The first stage of attachment intervenes movement generating appendixes which permits bacteria to approach the attachment surface[29-31]; these bacteria use the cellulose, lignin and hemicelluloses as a carbon source.

The column functions on a closed system. Microorganism's population develops and fixes gradually on the alfa stems surface.

RESULTS AND DISCUSSION

Velocity influence on the rate of denitrification

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 with concentration of 100 mg/L, along the column in an up flow with different velocities *i.e.*, 0.3, 0.45, 0.60, 0.80 and 1 m/h. Evolution of the residual value of nitrate according to the column height can be measured. The samples points arranged along the column and distances 50, 100 and 150 cm.

According to the results obtained (Figs. 2 and 3), it is noted that the rate of denitrification increases when the velocity passage decreases.

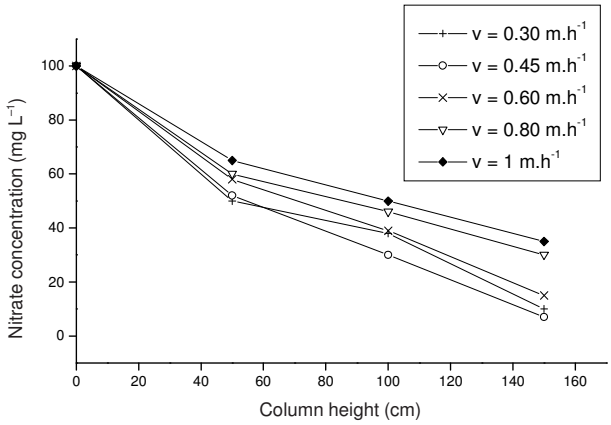

Fig. 2. Evolution of nitrate concentration along the column at different velocities value (pH = 8.30, T = 25 °C, $[NO_3^-]$ = 100 mg L^{-1})

Influence of height column on the nitrite concentration at different velocities:

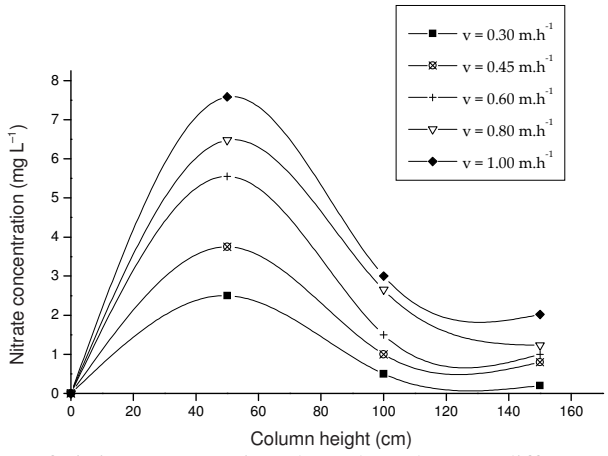

Fig. 3. Evolution of nitrite concentration along the column at different velocities value (pH = 8.30, T = 25 °C, $[NO_3^-]$ = 100 mg L^{-1})

Influence of initial nitrate concentration on the rate of denitrification (Figs. 4 and 4):

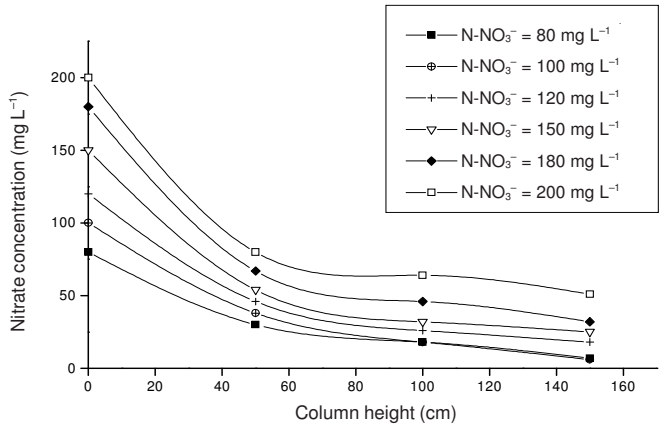

Fig. 4. Evolution of nitrate concentration along the column at different initial nitrate concentration (pH = 8.02, T = 25 °C, v = 0.45 m h^{-1})

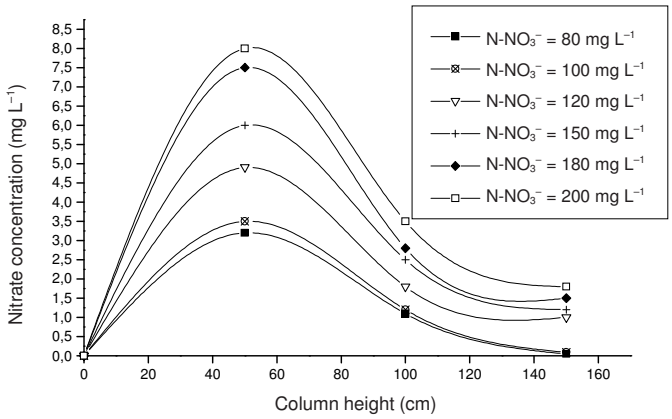

Fig. 5. Evolution of nitrite concentration along the column at different initial nitrate concentration (pH = 8.02, T = 25 °C, v = 0.45 m h^{-1})

Nitrates (NO_3^-): The profiles represented in Fig. 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 cm of the column. It is rapid and characterized by the elimination of 70 % of nitrate present in the sample.

Second phase: It is characterized by a slow elimination of the residual quantity along the column (60-150 cm).

We can explain these two phases with the presence of denitrifying bacteria in the column according an exponential profile decreasing from the lower to the top of the column.

This distribution is due to the upper flow of the effluent which causes an important bacteria proportion in the bottom of the column. There is an accumulation of the bacteria mass near the alimentation[32,33].

Indeed, when the water to treat goes along the column, aero-anaerobic 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 layers of the biofilm and transformed in the anoxic areas (zones) in spite of the presence 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[34].

Oxygen is the most important regulator of denitrification, rigorous anoxia is not necessary for onset or continuation[35]. A decrease in the oxygen supply rate to 0.4 mmol L^{-1} or lower permits the denitrification during growth of *Pseudomonas aeroginosa*[36]. Low *et al.*[37] found in measurement of denitrification that was absent until the oxygen concentration was about 1.6 mg 0_2 L^{-1}, Tiedge[38] found that denitrification was dramatically lowered with a slight increase from zero, Peder[39] found that the rapid increase of denitrification is between 1-2 mg O_2 L^{-1} in present studies oxygen concentration was 0 mg L^{-1}.

Nitrite (NO_2^-): The nitrite profile along the column shows that these 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 by the fact of the nitrite represent the intermediate stage of the reduction nitrate to the gas nitrogen according to the reaction[38]:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2$$

The concentrations of nitrite outlet of the column depend on its height, on the hydraulic and volumic load. More the volumic load, there is more nitrite outlet from the reactor. At lows concentrations of nitrate 80 mg/L, 100 mg/L we obtain nitrite values under the norm. In order to decrease the concentration of nitrite outlet the reactor, we have to increase the stay's time.

Chemical oxygen demand (COD): Evolution of the organic substances in denitrifying water was followed with the measure of the chemical oxygen demand (COD). Fig. 6 represents the evolution of COD according to the column length for different concentrations. We observed a decreasing profile equivalent to the nitrate one though less pronounced. Therefore, the COD reduction is due to the biodegradation of the substrate.

Denitrification is realized by a large species of bacteria, whose optimal conditions of temperature are varied. It's why the temperature range is very wide. We have realized the experiences in temperatures varied between 20 and 25 °C. Most studies on the influence of temperature on denitrification had done on soils[40]. The temperature is an activate factor until 60 °C and then a deactivate factor after that.

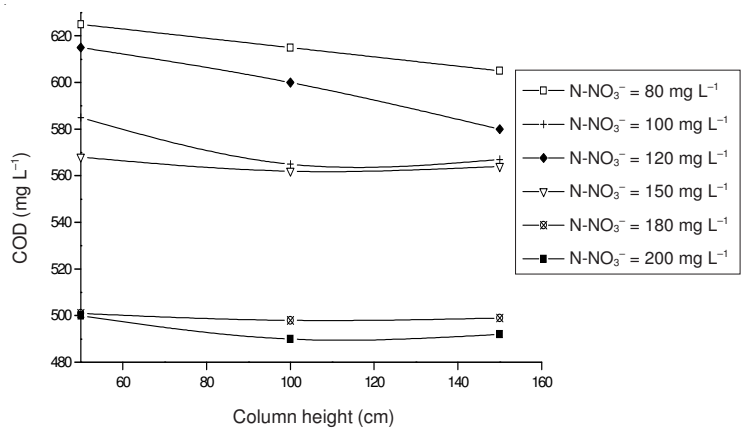

Fig. 6. Evolution COD along the column at different initial nitrate concentration (pH = 8.02, T = 25 °C, v = 0.45 m h^{-1})

The pH used in experiences was between 8 and 8.50, pH is not a limited factor of denitrification[41-43], but it has an important effect on the gaseous products produced at the time of denitrification[43], for the values of neutral pH, the final product is the gas nitrogen[44-46].

Conclusion

In this paper, the feasibility of biological denitrification of synthetic laden water with nitrate, using Alfa stems as consumable support is studied. During the first phase of the study, the development of the biomass is favoured from an activated sludge in order to fix them on the support. In the second stage, the treatment and the profile of different parameters concentrations as nitrate, nitrite, temperature and COD are established. This permitted to notice an abatement of nitrate included between 60 and 80 % outlet the column. In case of nitrite, the concentrations increase in the first part of the column and decrease in the second one. But the concentrations outlet the rector is related to the length of the column and the hydraulic charge applied.

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