

## Alfa stems (*stipatenacissima* L as substrate for water denitrification

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### ABSTRACT/RESUME

*Abstract: Biological denitrification of drinking water was studied in up-flow laboratory reactors packed with alfa stems (*stipatenacissima* L) which 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 thereafter. The addition of fresh alfa stems brought about a temporary improvement of the denitrification performance and a regime of one weekly addition prevented the deterioration of a reactor which was operated for 5 months. The rate of denitrification was affected by the water velocity and decreased at velocities above  $0.054 \text{ m.d}^{-1}$ . Colour and soluble organic carbon associated with fresh alfa stems removed by adsorption on powdered activated carbon.*

### I. Introduction

Surface and subsurface water pollution by nitrate is an important environmental issue, because of both its toxicity and widespread occurrence, thus  $50 \text{ mgNO}_3^- \text{ l}^{-1}$  was set as maximum contaminant level and guide value. Decrease of nitrate concentration is often required for drinking water in order to meet the standard of 11.29 mg nitrate-N per l in water for human consumption [1]. Among various methods available (physical, chemical-physical and biological) for the removal of nitrate, biological removal (denitrification) is considered to be the most economical and environmentally sound to be feasible on a large scale. The removal of nitrate is typically obtained using reverse osmosis, ion exchange and electrodialysis that are considered as expensive processes because of construction and management costs. The main disadvantage of these processes is the generation of nitrate concentrated waste stream. Recently, taking into account the environmental impact concerns, application of biological processes gained an increasing interest. Biological denitrification process via both autotrophic and heterotrophic ways is basically a bacteriologically mediated process where nitrate is

converted into nitrogen gas in the absence of oxygen. Denitrification is the reduction of nitrate to  $\text{N}_2$  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 of a simple compound (e.g. ethanol, methanol, acetate). However, complex carbon sources such as cellulose-rich materials can also be used and we have recently studied the feasibility of using news paper[2]and cotton[3]as carbon sources for the remediation of nitrate-polluted groundwater. Microorganisms capable of degrading cellulose (cellulolytic microorganisms) are widely distributed in nature and usually occur in mixed culture 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 (cellulases) which may act in synergism [4] Cellulose is a basic component of all plant materials and constitutes the most abundant renewable resource in the world, with an estimated production rate of  $4.10^{10}$  ton per year[5]. It is a linear glucose

polymer with hydrogen bonding between hydroxyl groups of neighboring parallel chains and is organized in fibers in close association with lignin and hemicelluloses (cotton is an exception being the purest form of naturally occurring cellulose). Thus, alfa stems is a complex mixture of cellulose, hemicelluloses, pectins and lignins, of which xyans and others xylose polymers constitute about 25 % [6]. Alfa stems resist at the great variation of temperature (-19 °C) in Rogassa region in the Algerian western south, while supporting the very hot summers (+40 °C). Following the observations on the ground and laboratory, showed that the optimal photosynthetic activity of the Alfa takes place at the temperature ranging between 15 and 25 °C. The relatively low temperature lower than 4 °C, slow down the assimilation and delay germination [7] The capacity of alfa stems to support water denitrification has been shown by others [3], used field and laboratory reactors packed with alfa stems mixed with sand or with sand and maërl in the treatment groundwater observed removal of nitrate in alfa stems filters designed to remove particulate matter from effluents prior to their application in drip irrigation.

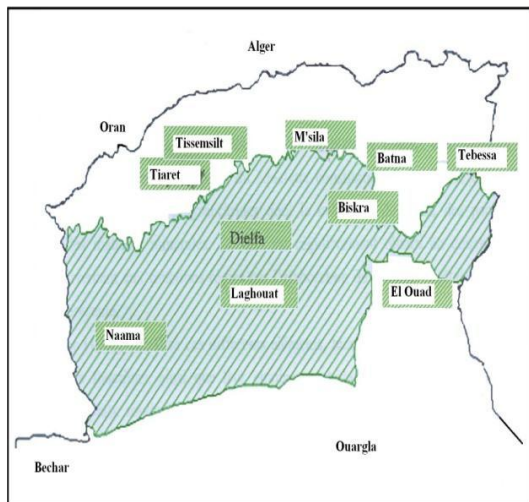


Figure 1. Stepic region presentation

## II. Objective

The main objective of the project was to determine the denitrification rates obtainable under various conditions in deep bed filters supplied with natural gas. The experiments were planned in two stages.

The objective of stage I was to establish a baseline of operation by running the filter containing coarse sand (2-3 mm diameter) with methanol as a carbon source prior to using natural gas (95 % methane, hence will be referred to as methane in the future text) as the entire carbon source.

## III. Materials and methods

### III.1 Experimental apparatus

The reactor routinely used were glass columns, 50 cm high and 8 cm diameter, packed with 103 g alfa stems with a thin layer of glass wool placed at each end. Slightly larger columns (55 cm high and 10 cm diameter) were used when addition of 20 g of fresh alfa stems carried out because their design made it easier to open at the bottom where the new substrate was added, they were packed with 1 g of alfa stems so that the same filling ratio (41g alfa per l) was used in all experiments. The columns were inoculated with a small amount of alfa removed from an active denitrification column and the original inoculum was a mixture of forest and garden soils. The freshly packed columns were filled with feed solution (tap water amended with 22.6 mg nitrate-  $\text{NI}^{-1}$  and 3 mg/l-1 phosphate). After this inoculation period, the reactor were started up (day 0) in an upflow mode (Figure 2).

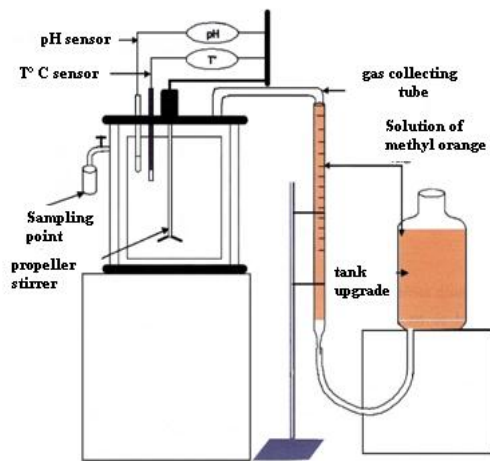


Figure 2. Scheme of semi continuous reactor and gas system collect

Water velocities  $v$  ( $v = Q/A$ , where  $Q$  is the measured flow rate and  $A$  is the cross section of the column) were calculated in  $\text{mh}^{-1}$ .

Unless otherwise indicated, the ambient temperature was maintained at 25 °C. Influent and effluent samples were tested for nitrate, nitrite, ammonia, pH, dissolved organic carbon (DOC) and bacterial counts.

### III.2 Water source

Considering the typical characteristics of groundwater located in the province of Algiers, described by parameters as turbidity (0.4 NTU), nitrate (1.63 mg/l), alkalinity (42 mg  $\text{CaCO}_3 \text{ l}^{-1}$ ), total organic carbon,  $\text{TOC} = 0.3 \text{ mg/l}$ , and UV absorbance at 254 nm,  $\text{UV}_{254} = 0.8 \text{ m}^{-1}$  to attain uniformity throughout the experiments, a synthetic water sample was prepared using tap water spiked with potassium nitrate,  $\text{KNO}_3$  (100

mg/l as  $\text{NO}_3^-$ ) and potassium phosphate,  $\text{K}_2\text{HPO}_4$  (3mg/l as P).

### III.3 Growth area of alfa stems

Alfa stems is a hardy perennial grass of the family of the grasses ones. This is an endemic of the western Mediterranean, which grows on the semi arid grounds of the North Africa and the south Spain[8] estimated the alfa covered surface is in hectares approximately at 4.5 million in Algeria (Figure 1).

In Algeria, the species grows mainly on the high plateaus in mixture with the sparte in an alternation of vegetation studied by the authors [9]. It is the western south steppes of the country that one meets the vast and greatest esparto expanses (1.2 million hectares in the Saida region, Table 1).

*Table 1. Chemical composition of alfa stems*

composition	Proportion(% report/ratio of The absolute dry plant
Cellulose rate	24.00
Hemicelluloses rate	28.00
Lignin rate	19.50
Humidity	10.00
cender	4.50
Extractedwithcoldwater	2.50
Extractedwithbenzene	1.90



*Figure 3. Tuft of alfa stems which decays*



*Figure 4. Compact tuft of alfa stems*

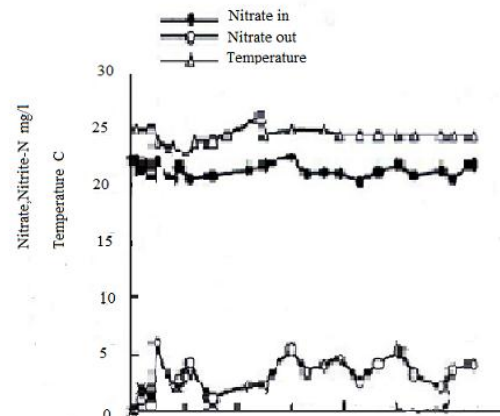
### III.4 Analytical procedures

Nitrate was determined by the method [10] and nitrite and ammonia were assayed according to APHA

## IV. Results and discussion

### IV.1 Nitrate removal and water quality

A freshly inoculated reactor was operated for 2 months during which the water velocity was regulated to allow breakthrough of nitrate up to 6  $\text{mg -NI}^{-1}$  (Figure 2). High rates of nitrogen elimination were observed during the first days of operation when little nitrate and nitrite washed out at a water velocity of 0.086  $\text{mh}^{-1}$  (Figure 5). The soluble fraction of carbon present in the fresh alfa stems allowed rapid microbial growth and the fast colonization of the substrate so that high removal of nitrate was observed at the start-up.



*Figure 5. Concentration of nitrate-N in the influent and concentrations of nitrite*

Breakthrough of nitrite was always gradually lowered to  $0.023 \text{ m}^3 \text{ h}^{-1}$  in order to keep the breakthrough of nitrate within the set limit of  $6 \text{ mg N l}^{-1}$ . This indicated a continuous deterioration of the denitrification performance of the reactor which can be attributed to quantitative and qualitative changes in the substrate. 40 % of the initial weight was lost and 11 g of alfa stems were consumed per g of N eliminated. Due to the physical and chemical heterogeneity of the substrate this ratio should be considered as a very broad estimation only. It can be assumed that all water soluble components and a good proportion of the cellulose and hemicelluloses had been lost by the end of the experiment while lignin and mineral components remained unchanged.

The number of colony forming bacteria in the effluent was in the order of  $10^{-6}$  bacteria  $\text{ml}^{-1}$  and this is within the range found in denitrification with simple carbon sources (Figure 6).

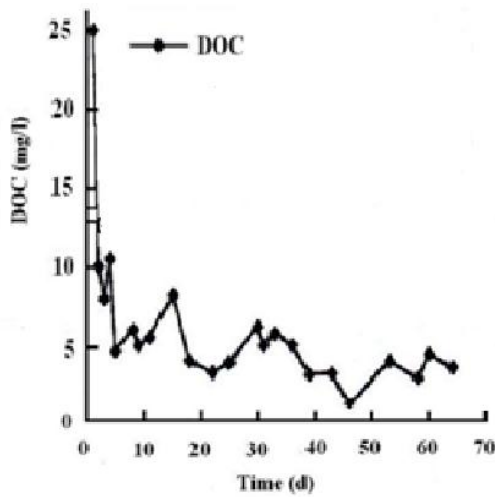


Figure 6. Concentration of nitrite-N in the influent and pH and concentration of nitrate-N

#### IV.2 Effect of water velocity

Breakthrough of nitrate and nitrite started at  $0.054 \text{ m}^3 \text{ l}^{-1}$ , followed by a continuous increase of the former (up to approximately  $12 \text{ mg N l}^{-1}$ ) while the latter stabilized below the concentration of  $5 \text{ mg N l}^{-1}$  (Figure 7).

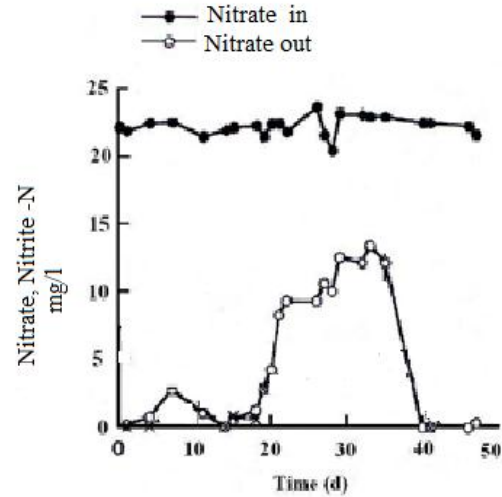


Figure 7. Concentration of nitrate-N in the influent and concentrations of nitrate-N

The highest rate of denitrification ( $0.053 \text{ g N removed l}^{-1} \text{ d}^{-1}$ ) was observed at the water velocity of  $0.054 \text{ m}^3 \text{ d}^{-1}$  and the lowest (approximately  $0.032 \text{ g N l}^{-1} \text{ d}^{-1}$ ) at  $0.092 \text{ m}^3 \text{ h}^{-1}$  and thereafter. The decrease in efficiency was somewhat exaggerated by changes taking place in the substrate. This could be deduced from the breakthrough of nitrite and nitrate when the water velocity was lowered again while complete removal of nitrogen had been achieved earlier at the same water velocity (Figure 8). By day 40 the weight of alfa stems had been reduced by 42 %.

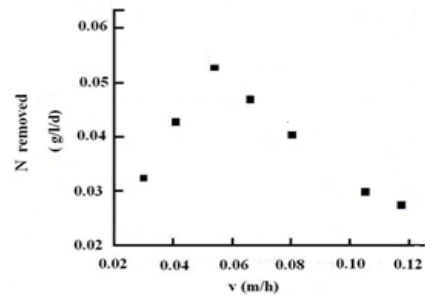


Figure 8. Effect of water velocity on the removal of nitrogen in the reactor

Thus, water velocity plays an important role in the denitrification performance of the system and the reasons for the sharp decrease in efficiency at the higher velocities may include wash-out of bacteria, wash-out of extracellular enzymes and wash-out of solubilized substrate.

## V. Conclusion

In North Africa, the *Stipatenacissima* L, constitutes an essential element of fight against the turning into a desert and an essential factor of the maintenance of balance pastoral; from the economic point of view, it is of industrial interest some: like raw material in water treatment.

Currently the combined action of the many clearing and repeated, the excessive pasture, a prolonged cycle of dryness to which the ignorance of the plant is added partly, make that we attend a progressive regression of the alfa. Very few investments intellectual or financial were consented there, at the moment when the country attaches a great importance to the natural sources.

Safeguarding, the development and the rational exploitation of the *Stipatenacissima* L are major trumps necessary for the development of the Algerian steppe areas.

On the basis of these result, we can affirm that the denitrification performance process can ensure, after a choice of a suitable parameters. In fact, the system is affected by quantitative and qualitative changes of the carbon source. So, the addition of fresh alfa stems temporarily improves the performance.

Water velocity has a marked effect on the denitrification performance of the system.

Alfa stems is a suitable carbon source for water

Water velocity has a marked effect on the denitrification performance of the system.

Denitrification and, at the same time, can serve as the sole support for bacterial growth.

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