FIGHTING AGAINST NITRATE POLLUTION OF THE DAM-RETAINED WATERS THROUGH BIOLOGICAL TREATMENT

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Abstract: Pollution of the water by nitrates was favored by setting husbandry intensive methods of production, which was implemented by an increased use of chemical manures. Most of this pollution is due to the difference in the proportion of nitrates in manures and what is actually uptaken by the plants. In response to this issue, we carried out a research intended for reducing the concentration of nitrates in the water streams supplying the Dam of Beni Haroun, Algeria, in order to avoid the eutrophication fatal effects. This was achieved thanks to a biological treatment, which is one of the cheapest and most efficient for reducing nitrates. In our paper, we used a mixed culture of microorganisms picked up in the El Menia Filtering Station, Constantine, Algeria.

The use of methanol as a carbon source involved a rise in *pH*. In order to prevent the consequences on the degradation performance of the microorganisms, a buffer mixture of acetic acid and sodium acetate was used as a source of carbon. We noticed a growth in bacteria coupled with elimination proportionate to nitrates. During this elimination, the medium *pH* varies slightly, it moves from 7.00 to 7.88. This has improved

denitrification, reducing the treatment time and increasing the denitrification rate. On the other hand, this work allowed to state that the best denitrification rate was obtained for the ratio value C/N that equals 2.5.

Keywords: *biodegradation, denitrification, buffer mixture, nitrate, mixed culture.*

INTRODUCTION

Nitrates are widely used in Algeria as in the rest of the World. They play an essential part as manure [1, 2], for they make up the main nitrogen supply for plants and enhance their growth. Some part of these manures is absorbed by plants; the rest is collected in surface water bodies such as the Dam of Beni Haroun in Algeria or in the underground water. The pollution of waters by nitrates means a double hazard. Nitrates have toxic effects on human health if ingested in too large quantities due to the transformation of the nitrates in the human organism into nitrites and into carcinogenic nitrosamins [3], which can be lethal to infants. This is confirmed by the World Health Organization. Likewise, they take part with phosphates in modifying the biological equilibrium of aquatic systems by causing the phenomenon of eutrophication [4]. The purpose of this paper is to solve this issue by decreasing nitrates levels in the waters.
The technique selected is the biological treatment by the use of a mixed culture of microorganisms taken from El Menia Filtering Plant, Constantine. This technique is one of the most efficient and economic ways to reduce the concentration of nitrates.
The bacterial activity requires the availability of a carbon source [5] such as: ethanol [6, 7], methanol [8, 9], acetic acid [10], acetate [11, 12], fatty acids [13], polyester granules [14, 15], or wheat straws [16]. In our study, we used methanol first, then a buffer mixture of acetic acid and sodium acetate.

MATERIALS AND METHODS

Materials

The microorganisms used during our experiments were taken from the bottom of the secondary decantation pond of the El Menia Filtering Station of Constantine because the denitrifying genes are present in the zones where the concentration of the dissolved oxygen is low. We used a spectrophotometer UV-VISIBLE Techcomp 8500 to measure turbidity of the samples. This allows determination of the bacterial population density. It was also used to measure the evolution of the nitrate concentration. The evolution of *pH* was measured by the means of a HANNA *pH*-meter, instruments HI 8519 N, provided with a joint glass electrode. Concentration of dissolved oxygen was measured with an oxygenometer OXI 92.
With sodium salicylate, nitrates turn to a yellow coloration liable to a spectrophotometric UV-VISIBLE dose at 415 nm [17]. This property has been used for the measurement of the concentration of nitrates in our experiments.

A wave length that equals 600 nm was used to measure the evolution of turbidity along the experiments. This measurement allowed us to determine the evolution of biomass quantity.

Operating Conditions

The sludge taken from the Filtering Station is dissolved by stirring in distilled water.
The recuperated solution after filtering is mixed with a solution containing the nutrient medium [18] (Table 1) and a carbon source. The bacterial growth is followed by measurement of the optic density (at 600 nm) at regular time intervals. The experiments are carried out in a 500 mL reactor containing 200 mL of the nutrient medium, which we inoculated with 5 mL of inoculants already prepared. Anaerobia is achieved by splashing nitrogen until complete deoxygenation of the solution. Measurement of *pH* and concentration of oxygen are achieved with electrodes permanently inserted in the reactor. The reactor is permanently stirred in order to homogenize the reaction medium. It has an outlet meant for sampling without disturbing the reaction medium.

Table 1. *Composition of the culture medium*

Component	Concentration [mg.L^{-1}]
KH_2PO_4	0.2
K_2HPO_4	0.8
KNO_3	250
$(NH_4)_2SO_4$	116
$MgSO_4$	390
Carbon source	500
Mineral solution	1.00

Table 2. *Composition of the mineral solution*

Component	Concentration [mg.L^{-1}]
$FeSO_4 \cdot 7H_2O$	500
H_3BO_3	10
$ZnSO_4 \cdot 7H_2O$	400
$CuSO_4 \cdot 5H_2O$	200
$Na_2MoO_4 \cdot 2H_2O$	1.7
$MnSO_4$	1.6

RESULTS AND DISCUSSIONS

Use of methanol as a carbon source

In order to test the degradation capacities of our microorganism culture, we primarily used methanol as a carbon source for surveying the denitrification kinetics.
The curve representing the evolution of bacterial concentration (Figure 1) shows that the bacterial growth goes through the following four successive stages: latency, exponential growth, slowing down, and finally stagnation.

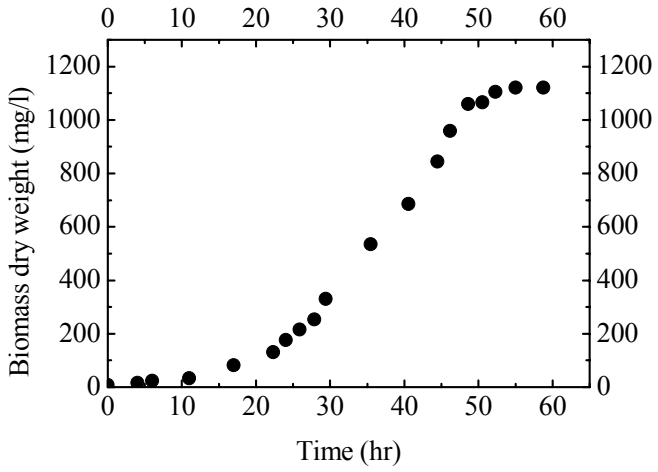

Figure 1. *Evolution of the bacterial growth along denitrification (carbon source: methanol)*

The growth rate and the biomass quantity produced, ΔX, [19] are calculated on the basis of the two following relations:

$$\mu = \frac{\ln X_{\max} - \ln X_0}{t_{\max} - t_0} \tag{1}$$

$$\Delta X = X_f - X_t \tag{2}$$

The calculations showed that:

$$\mu = 0.05\ \mathrm{h}^{-1}$$

$$\Delta X = 1124.2\ \mathrm{mg \cdot L^{-1}}$$

The bacterial growth goes along with a proportional consumption of nitrate ions, as shown in Figure 2.

The bacterial growth stops after the complete consumption of nitrates. This proves denitrification is only a substitute to regular breathing of oxygen. Therefore, nitrates are used as final acceptors of electrons that are driven throughout the respiration cycle [5, 20, 21].

The mean speed of disappearance of the nitrates during the exponential growth stage is:

$$v_{mean} = 3.97\ \mathrm{mg \cdot L^{-1} \cdot h^{-1}}$$

Concentration of nitrates is lower than 50 $\mathrm{mg \cdot L^{-1}}$ (concentration accepted by the Algerian Legislation) obtained out of a 42 h treatment.

Denitrification stops after a 54 h treatment. Concentration of nitrates is then 22 $\mathrm{mg \cdot L^{-1}}$, which gives a denitrification rate of 85.5%.

During the reaction of degradation, we observed an increase of *pH* in our reactor because the biological reaction takes a lot of protons [22]. In the present case, it rises from 7.00 to 8.38 (Figure 3).

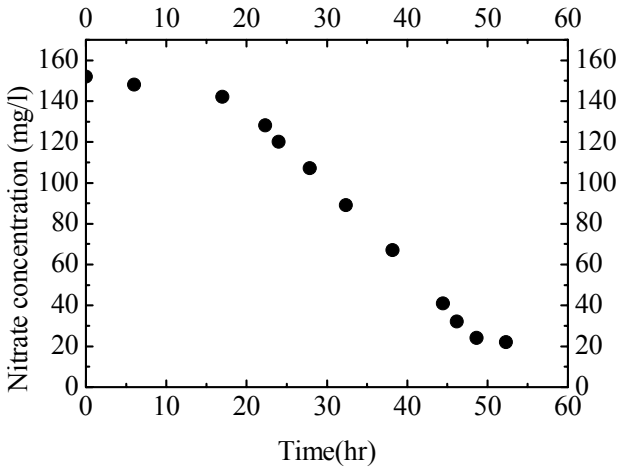

***Fig. 2**: Denitrification kinetics (carbon source: methanol)*

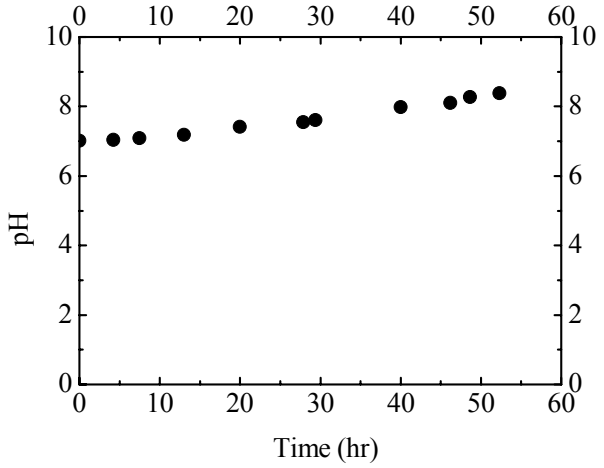

***Figure 3.** Evolution of pH during denitrification (carbon source: methanol)*

Use of a buffer mixture of acetic acid and sodium acetate as a carbon source

The optimal *pH* for a denitrification process usually ranges between 7 and 8 [23, 24]. In order to prevent acidification of the reaction medium we used a buffer mixture of acetic acid and sodium acetate as a carbon source. It caused the increase of the denitrification reaction output. In this case an equal concentration of microorganisms is twice more active in denitrification than with methanol as a carbon source. There is an almost complete consumption of nitrates when the biomass produced equals 537.03 $mg \cdot L^{-1}$ (Figure 4). This result is achieved after 47 h of treatment. This is an evidence that denitrification is an anaerobic process, which uses the oxygen of the nitrates to oxidize the organic substance [25].

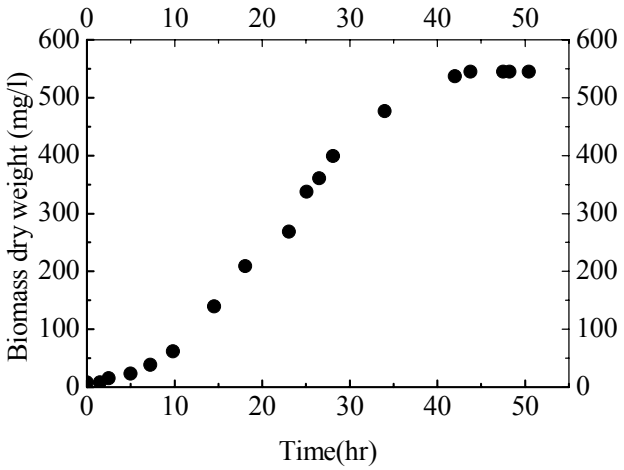

Figure 4. *Evolution of the bacterial growth during denitrification (carbon source: buffer mixture of acetic acid and sodium acetate)*

The mean denitrification speed equals 7.96 $mg \cdot L^{-1} \cdot h^{-1}$ (Figure 5), and *pH* hardly evolves during the treatment (Figure 6).

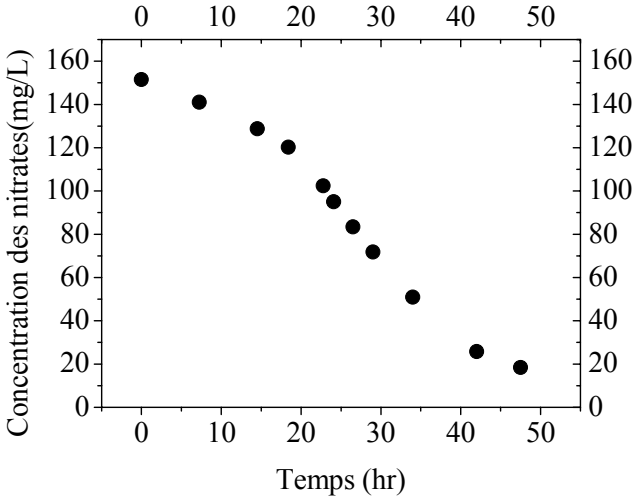

Figure 5. *Evolution of the nitrates during denitrification (carbon source: buffer mixture of acetic acid and sodium acetate)*

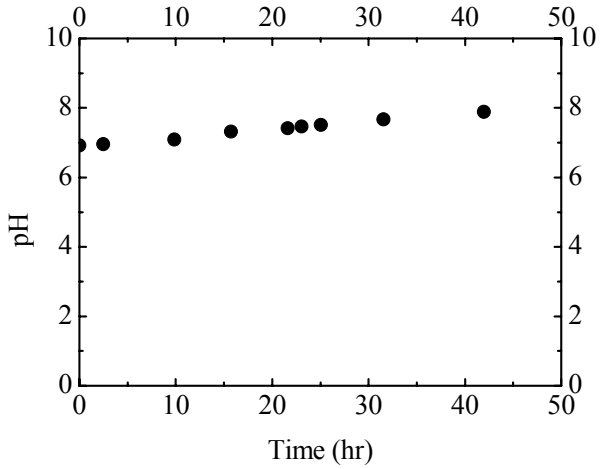

Figure 6. *Variation of pH during denitrification (carbon source: buffer mixture of acetic acid and sodium acetate)*

Effect of the content of nitrate ions

As to optimize the relation of carbon on nitrogen C/N we carried out five cultures in synthetic water inoculated with 10 mL of inoculants prepared earlier, and supplied with a buffer mixture of acetic acid and sodium acetate along with different concentrations of nitrates calculated on the purpose to have different C/N ratios.
In this case, the kinetic evolution observed on Figure 7 shows there is no wide difference in bacterial growth. In addition, we noticed that the biomass quantity produced for the fourth culture C/N = 2.5 is the largest with a growth rate of 0.144 h^{-1}.

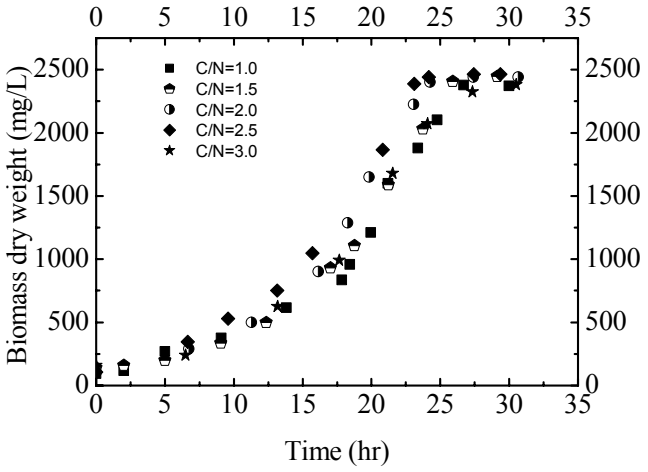

Figure 7. *Evolution of the bacterial growth during denitrification (carbon source: buffer mixture of acetic acid and sodium acetate)*

This bacterial growth is due to consumption of nitrate ions (Figure 8).

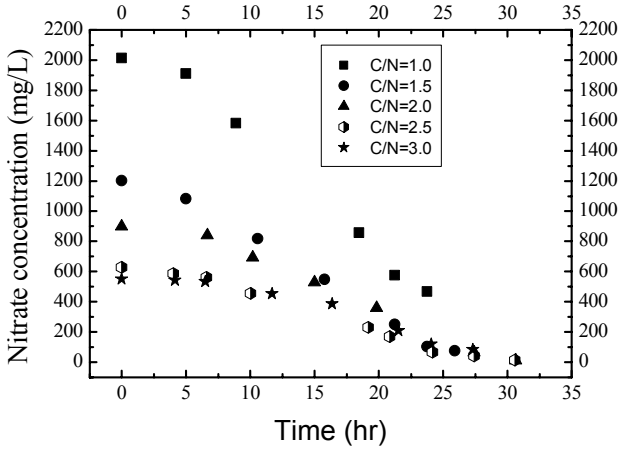

Figure 8. *Denitrification kinetics*
(carbon source: buffer mixture of acetic acid and sodium acetate)

There is a decrease in nitrate concentration until it actually equals zero within 30 hours, especially for the ratio C/N = 2.5. Within these terms, the denitrification rates up to 97.6%. *pH* hardly evolves during the treatment for the five cultures because the media are buffered (Figure 9).

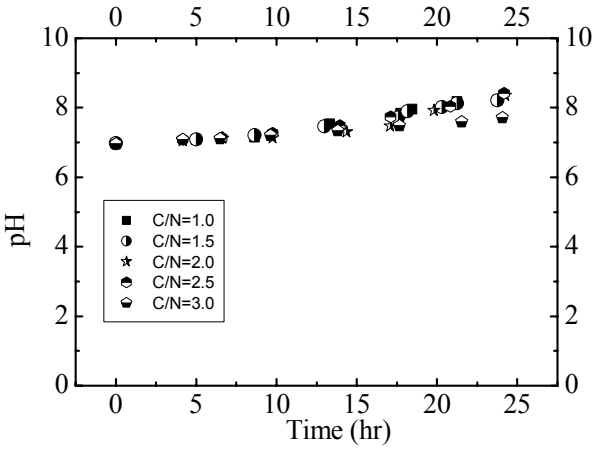

Figure 9. *The evolution of pH during denitrification*
(carbon source: buffer mixture of acetic acid and sodium acetate)

CONCLUSIONS

Pollution by nitrates of waters from Beni Haroun Dam, Algeria, causes the problem of eutrophication. A biological treatment seems to be the most adequate solution to

remediate this problem. During our study, we used a joint culture of microorganisms, which we had taken from the El Menia Filtering Station of Constantine. The use of methanol as a carbon source allowed us to eliminate in a batch reactor 85% of the initial concentration of nitrates. During this treatment, *pH* rose from 7.00 to 8.38. As to prevent the effects of *pH* increase on denitrification output, we used a buffer mixture of acetic acid and sodium acetate instead of methanol as a carbon source. The result was the acceleration of the treatment and an increase in the denitrification rate. An important elimination of nitrates corresponds to ratio carbon on nitrogen (C/N) that equals 2.5, meanwhile *pH* has hardly changed.

REFERENCES

1. Bernet, N., Habouzit, F., Moletta, R.: Use of an industrial effluent as a carbon source for denitrification of a high-strength wastewater, *Applied Microbial. Biotechnology*, **1996**, **46**, 92-97.
2. Zayed, G., Winter, J.: Removal of organic pollutants and nitrate from wastewater from dairy industry by denitrification, *Applied Microbial. Biotechnology*, **1998**, **49**, 469-474.
3. Volokita, M., Abeliovich, A., Inês, M., Soares, M.: Denitriftcation of ground water using cotton as energy source, *Water Science & Technology*, **1996**, **34** (1-2), 379-385.
4. Wang, Q., Feng, C., Zhao, C., Hao, C.: Denitrification of nitrate contaminated groundwater with a fiber-based biofilm reactor, *Bioresource Technology,* **2009**, **100**, 2223-2227.
5. Over, B., Ozgen, S., Yuksel, M.: Biological denitrification of drinking water using *Glycyrrhiza glabra* and *Arunda donax* as the carbon source, *Process Biochemistry,* **2006**, **41** (7), 1539-1544.
6. Dahad, M.F., Kalagiri, J.: Nitrate removal from water using cyclically operated fixed-film biodenitrification reactors, *Water Science & Technology*, **1996**, **34** (1-2), 331-338.
7. Fernandez-Nava, Y., Maranon, E., Soons, J., Castillon, L.: Denitrification of wastewater containing high nitrate and calcium concentration, *Bioresource Technology,* **2008**, **99**, 7976-7981.
8. Foglar, L., Briski, F.: Wastewater denitrification process – the influence of methanol and kinetic analysis, *Process Biochemistry*, **2003**, **39** (1), 95-103.
9. Purtschert, I., Gujer, W.: Population dynamics by methanol addition in denitrifying wastewater treatement plants, *Water Science & Technology*, **1999**, **39**, 43-50.
10. Salem, H., Lebik, N., Cherafa, K. Allia, K.: Valorisation of olive pits using biological denitrification, *Desalination*, **2007**, **204**, 72-78.
11. Almeida, J.S., Reis, M.R.M. Carrondo, M.J.T.: Competition between nitrate and nitrite reduction in denitrification by *Pseudomonas fluorescens*, *Biotechnology. Bioengineering*, **1995**, **46**, 476-484.
12. Huang, H.K., Tseng, S.K.: Nitrate reduction by *Citrobacter diversus* under aerobic environment, *Applied Microbial Biotechnoogyl*, **2001**, **55**, 90-94.

13. Yatong, X.: Volatile fatty acids carbon source for biological denitrification, *J. Environmental Science,* **1996**, **8** (3), 257-269.
14. Mergaert, J., Boley, A., Cnockaert, M.C., Müller, W.R., Wings, J.S.: Identity and potential functions of heterotrophic bacterial isolates from a continuous-upflow fixed-bed reactor for denitrification of drinking water with bacterial polyester as source of carbon and electron donor system, *Applied Microbiololgy,* **2001**, **24** (2), 303-310.
15. Boley, A., Mergaert, J., Müller, C., Lebrenz, H., Cnockaert, M.C., Müller, W.R., et al.: Denitrification and pesticide elimination in drinking water treatment with the biodegradable polymer poly(epsilon-caprolactone) PCL*, Acta Hydrochim. Hydrobiolog*, **2003**, **31** (3), 195-203.
16. Soares, M.I.M., Abeliovich, A.: Wheat straw as substrate for water denitrification, *Water Research,* **1998**, **32** (12), 379-385.
17. Rodier, J. : *L'analyse de l'eau*, 6th édition, **1978**.
18. Patureau, D.: *Etude cinétique et physiologique d'une bactérie dénitrifiant en conditions aérobies. Suivi en réacteur aéré, parfaitement mélangé, en culture pure et en culture mixte associée à une flore nitrifiante*, Doctoral graduation thesis, **1995**.
19. Guiraud, J-P.: *Microbiologie alimentaire*, Ed. Dunod, Paris, **1998**.
20. Nurse, G.R.: Denitrification with methanol: microbiology and biochemistry, *Water Research,* **1980**, **14** (5), 531-537
21. Tiedje, J.M.: Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In *Biology Anaerobic Microorganisms*, (Zenhder A.J.B. – ed.), **1988**, 179-244.
22. Haslay, C.,H.: *Microbiologie des eaux*, Ed. Leclerc, **1993**.
23. Mouchet, P.: Réflexions complémentaires sur l'importance des phénomènes biologiques dans le traitement et la distribution des eaux de consommation, *Technologie des eaux*, **1982**, **424** (4), 7-25.
24. Wang, J.H, Baltzis, B.C., Lewandowski, G.A.: Fundamental denitrification kinetic studies with *Pseudomonas denitrificans*, *Biotechnol. Bioeng,* **1995**, **47**, 26-41.
25. Schwartzbrod, J., Martin, G.: Les microorganismes, agents biogéochimiques, In: *Point sur l'épuration et le traitement des effluents* (Martin G. – Coord.), Ed. tec. & doc. Lavoisier, Paris, **1985**, **Vol. 2-1**, 1-45.