

## Valorisation of olive pits using biological denitrification

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### Abstract

Underground water pollution by nitrates has become alarming in Algeria. A survey carried out revealed that the nitrate concentration is in continual increase. For example, in water samples from the Mitidja plain in 2004, the nitrates concentration (175 mg/l) doubled compared to that found in 2002. Considering their dramatic effects on environment, human health, physicochemical and biological processes of nitrate removal are widely used in domestic treatment and industrial wastewaters. Biological denitrification enables transformation of oxidized nitrogen compounds by a wide spectrum of heterotrophic bacteria into harmless nitrogen gas with accompanying carbon removal. Based on its price and availability, methanol is the most commonly used as an additional carbon source for bacterial denitrification. The study investigated the valorisation of a vegetable residue as a carbon source (olive pits) in water treatment using the denitrification process. In batch tests with the suspended vegetable biomass, the parameters were studied which influence the denitrification process: biomass concentration, initial nitrate concentration, initial pH, temperature and particle size. The results obtained for complete denitrification showed that 500 mg/l of N-NO<sub>3</sub><sup>-</sup>/l were removed within 7 days, the most effective pH values are between 5.95 and 7.91, and an increase of temperature accelerates the process. The effect of the initial concentration influences the kinetics reaction but not the removal. The effect of particle size showed that a fine dimension gives better denitrification because of the importance of the surface area of fine particles.

**Keywords:** Denitrification; Nitrate; Nitrite; Olive pits; Mixed bacterial culture

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## 1. Introduction

The presence and increase of nitrates in underground and surface waters has become worrying, especially as some models predict a regular tendency to the increase if some preventive measures are not taken. In fact, several nitrogenous compounds, including ammonia, nitrite, and nitrate, have been frequently found in drinking water and various types of agricultural, domestic, and industrial wastewater. Nitrates can cause several environmental problems. Nitrates and phosphates can stimulate eutrophication where the pollution is caused in waterways by heavy algal growth, as they are both rate-limiting nutrients for the process. Nitrate-contaminated water supplies have also been linked with outbreaks of infectious disease; excess nitrates in drinking water may cause methemoglobinemia also called “blue baby” disease, in newborn infants. To limit nitrate and nitrite concentrations in drinking water, the international health organisation (OMS) legislation limits the maximum concentration of nitrates to 45 mg/l without nitrites [1].

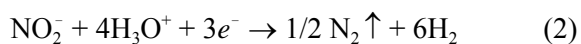
Studies in some regions of Algeria [2] have revealed that contamination often reaches over the maximum level recommended by the OMS. Sidi Bel-Abbes, Birkhadem, Chlef and the Mitidja plains are concrete examples where nitrate concentrations up to 260 mg/l were found in 1990. Nitrate concentrations for different points on the Mitidja plain in 2004 were between 95 and 175 mg/l [2]. Nitrates underground water pollution of the Mitidja is likely due to the use of nitrogen fertiliser quantities distinctly superior to the needs of plants. Indeed, an investigation revealed that the agriculturists of the different localities bring large quantities of nitrogenous fertiliser to the plants because for them the equation is simple: maximum fertilizer is equivalent to a maximum production.

Traditional methods for nitrogen removal are biological processes (denitrification, nitrifica-

tion), chemical processes (breakpoint chlorination, selective ion exchange), and physical operation (ammonia stripping). Other operations or processes are conventional treatment (primary and secondary), biological processes (bacterial assimilation, oxidation ponds), chemical processes (chemical coagulation and adsorption), physical operations (electrodialysis, filtration, reverse osmosis), land application (irrigation, rapid infiltration, overland flow) [3]. Recently the biological processes have proven to be the most competitive and the better adapted to nitrogen treatment because of their ease, efficiency, moderate cost and the possibility of combining carbon and nitrogenous removal pollution.

For the biological processes, autotrophic and heterotrophic denitrification are distinguished. Biological denitrification enables transformation of oxidised nitrogen compounds by a wide spectrum of heterotrophic bacteria into harmless nitrogen gas with accompanying carbon removal. Based on its price and availability, methanol is most commonly used as additional carbon source for bacterial denitrification [4–6]. The nitrates play the role of final acceptor of electrons instead of oxygen, and the implied bacteria (e.g., *Acinetobacter*, *Pseudomonas*, *Moraxella*) have a heterotrophic metabolism, which needs the presence of a carbon directly added or drawn from the wastewater.

The aim was to investigate the applicability of raw olive pits in a biological denitrification process. The aerobic and heterotrophic bacteria reduce the nitric nitrogen in anoxic conditions:



Denitrification is nitrogen removal by recovering the oxygen combined to the nitrates and nitrites in order to replace it in the breathing process of the microorganisms. The electron donor is the carbon of an external organic source.

## 2. Materials

Nowadays, research is oriented toward the valorisation of lignocelluloses materials. Our innovative contribution is the use of olive pits, residue of the food industry, as material because of its abundance and low cost. The olive pit is produced from oil extraction. This biomass is washed with distilled water and dried at 60°C during 24 h and the chemical composition determined. To know the effect of particle size, the biomass was crushed, reduced, and sieved to obtain the following classes: 0.63–1.6 mm and 1.60–2 mm. The main functional groups of olive pits were determined by infrared spectra of the raw biomass. To simulate nitrate pollution, we used synthetic water prepared with a solution of NaNO<sub>3</sub> containing 10 g NO<sub>3</sub>-N/L.

The experiments were performed in 2-L brown closed bottles. The stopper was punctured with a disposable syringe with a needle for sampling. During the denitrification tests, the flasks were agitated on a magnetic stirrer (400 rpm). All experiences were conducted under anoxic conditions (the headspace gas contained O<sub>2</sub> initially, but this was rapidly consumed by bacteria). In the first part, the effect of various nitrate concentrations (100, 200, 300, 400, 500 mg NO<sub>3</sub>-N/L) was examined; in the second run, the pH influence on biological denitrification batch tests was tested at different pH under anoxic conditions. Synthetic water was adjusted to the desired pH by changing the ratio of phosphate salts (K<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O).

During nitrate removal, microbial growth was monitored and the kinetics of microbials was established. To study the kinetics of nitrate removal from the water, the samples were taken from the bottles at a predetermined time and processed immediately. All the experiments were repeated (performed twofold) and data reported represent the average value. Volatile suspended solids, nitrate, and nitrite concentrations measured during duplicate measurements differed by 0.2 to 5 mg/L.

## 3. Analytical methods

Before analysis, the samples were centrifuged at 4000 rpm for 3 min and the supernatant was used for nitrate and nitrite analysis. Nitrate, nitrite detection was monitored spectrophotometrically by absorbance measurements, respectively, at 415 and 435 nm.

Attempts were made to optimize the denitrification process (biomass concentration, initial nitrate concentration, pH, Temperature, particle size) to achieve nitrate removal without nitrite accumulation, and to improve the economical effectiveness of the process.

## 4. Results and discussion

### 4.1. Chemical compounds of the olive pits

The main chemical compounds in percent of dry matter were determined: humidity (Hu), total ash (TA), total nitrogen (T, N, K), total phosphor (TP), total sugars (TS), as well as some mineral elements (Ca, Mg, Mo, Cu, Fe.). The chemical composition of the olive pits is given in Table 1.

The interest of knowing the chemical composition of olive pits lies in its use as suspended particles support of the denitrifying bacteria that must be capable of colonising the surface of the

Table 1  
Chemical composition of olive pits

Parameters, % of dry matter	Value
Hu	10.31
TA	3.51
TP	0.143
TS	9.36
Ca	0.535
Mg	0.011
Mo	0.047
Cu	0.013
Fe	0.045
T,N,K	0.410

support if it has organic compounds, nitrogen, phosphorus, and mineral salts in sufficient quantities. The obtained results confirmed the contribution of olive pits as a nutritive element. In fact, the cellulose, hemicelluloses, and lignine are important:

- cellulose 33–42%
- hemi-celluloses 35–43%
- lignine 15–23%

We note that the percentage of cellulose is important, and therefore the presence of assimilated sugar presence results from the hydrolysis of this.

#### 4.2. Infrared spectral analysis

The infrared spectrum of the raw biomass was established and the pattern revealed a complex and additive impact of its overall chemical texture comprising different functional groups that regulate possible cell interactions such as H-bonding complexation, etc. There were stretching frequencies around  $3400\text{ cm}^{-1}$ ,  $2900\text{ cm}^{-1}$ ,  $1500\text{ cm}^{-1}$ ,  $1700\text{ cm}^{-1}$ ,  $1037\text{ cm}^{-1}$  which indicate the presence of  $\gamma(\text{O-H})$ ,  $\gamma(\text{C-H})$ ,  $\gamma(\text{-NH})$ ,  $\gamma(\text{C=C})$ ,  $\gamma(\text{COO}^-)$ ,  $\gamma(\text{C=O})$ ,  $\gamma(\text{C-C})$ .

#### 4.3. Effect of biomass concentration

To optimise the biomass concentration, the quantity was varied from 0 to 20 g/L while keeping the other parameters constant. Fig. 1 represents nitrate removal as a function of the concentration biomass. A proportional increase of the nitrate removal was noticed until plateau values appeared from 9 g/L, reaching complete denitrification, beyond which the increase of the quantity of the biomass did not affect the rate of denitrification. Considering the fact that the nitrates are acceptors of electrons and the organic matter is a donor of electrons, it is necessary that the contribution in organic matter become sufficient for complete denitrification. If one of these two factors is limiting, denitrification

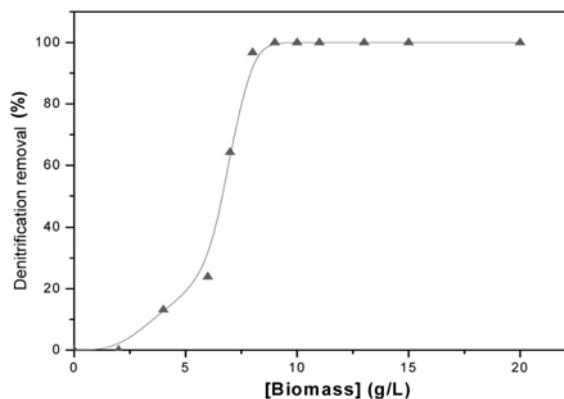


Fig. 1. Effect of biomass concentration on the nitrate removal.  $[\text{N-NO}_3^-]$ : 500 mg/L, pH 7.1.

stops. For an initial concentration of  $[\text{N-NO}_3^-]$  equals 500 mg/L, the optimal concentration of biomass is 9 g/L. For the continuation of our experiments a biomass concentration of 10 g/L was chosen.

#### 4.4. Effect of initial nitrate concentration

Initial nitrate concentrations are presented as a function of time in the range of 0 to 500 mg/L (Fig. 2). As can be seen, the initial nitrate concentration decreases gradually according to time. For the highest initial nitrate concentration, complete denitrification is especially slow. It is deduced that the variation of the initial concentration in the studied range does not affect the nitrate removal but the denitrification rate. The denitrification rate given by the Monod model is [8]:

$$dC_N / dt = k \quad C_N^n C_X^m$$

where  $t$  is time (s),  $C_X$  the biomass concentration (mg/L),  $m$  is a partial order,  $C_N$  is the nitrate concentration (mg/L) and  $n$  is a partial order.

The reduction of nitrate ions was modelled according to the model equation and the model acceptably predicted the nitrate concentration

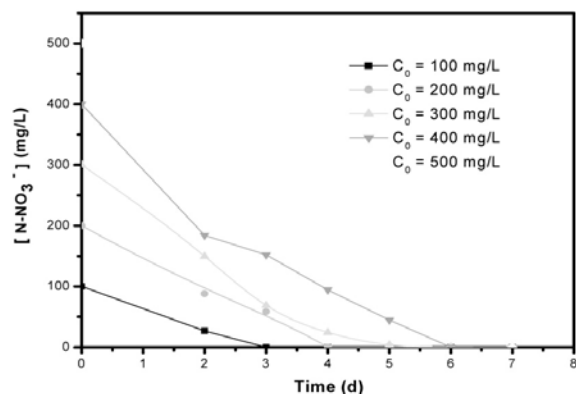


Fig. 2. Effect of the initial nitrate concentration on denitrification. pH 7.01; [biomass]: 10 g/L.

decrease. For the initial nitrate concentration of 300, 400, 500 mg NO<sub>3</sub>-N/L, the predicted nitrate removal based on the Monod model is a curve rather than a straight line. This indicates that the employed model is incomplete for nitrate concentration above 300 mg/L. Denitrification kinetic independent of the nitrate concentration is of a zero order [7].

#### 4.5. Effect of initial pH on denitrification

The pH is an important parameter affecting the denitrification process. Denitrification was tested according to initial pH. This was done by adding quantities of KH<sub>2</sub>PO<sub>4</sub> to vary the acidity and quantities of Na<sub>2</sub>HPO<sub>4</sub> for the basicity. The initial pHs were: 5.7, 5.85, 5.95, 6.81, 7.45, 7.91, and 8.45.

As seen from Fig. 3, denitrification increased with pH; the optimal pH for denitrification is usually within neutral range. In the literature, however, there were other findings: Yang and Bingfallen [9], 7.1; Foglar and Vuković [8], 7.4; and for Lykiardopol et al. [7], the optimum pH was around 7 to 7.5. In our tests a pH between 5.95 and 8.45, nitrate removal range was superior by 60%. Changing the pH in the range of 5.85 to 5.95 caused significant modification as cited in the literature [10]. This phenomenon explains

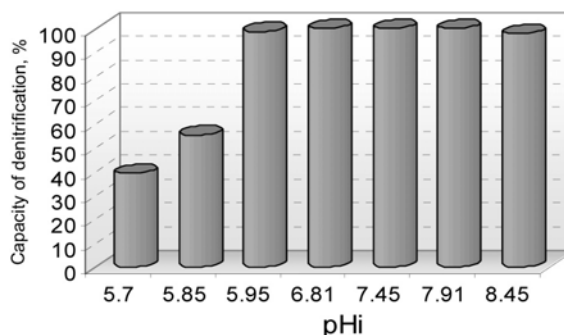


Fig. 3. Nitrate removal for different initial pH. [N-NO<sub>3</sub>]: 250 mg/L; biomass concentration, 10 g/L.

itself by the fact that most enzymes have either a basic group or a weak acid group implied in the catalytic action. In our case, the association of the hydrogen ion (present in an acidic environment) with a weak basis produced a loss of catalytic activity [7], which inhibits the reaction of denitrification.

#### 4.6. Temperature effect

Two similar tests were conducted for two different temperatures: 20°C and 30°C. With the temperature of 20°C complete denitrification was obtained during day 7 and at 30°C it was on day 5. The variation of temperature directly influences the bacteria latency time. The more the temperature is raised, the more the latency time decreases. A minimal latency time means a growth phase reached quickly for a maximum denitrification rate. This was confirmed by the relation giving the maximum growth rate according to  $T$  [7]:

$$\mu_{\max,D,T} = \mu_{\max,D,20^\circ} Y^{(T-20^\circ)}$$

where  $Y$  is the coefficient of temperature.

In the literature, the optimal temperature varies: for Foglar and Vuković [8], the optimal temperature was 35°C. In addition, for Lykiardopol et al. [7] it is around 25–30°C.

#### 4.7. Evolution of nitrites during denitrification

The nitrite concentration is represented in Fig. 4. The nitrite concentration increases during the denitrification and reaches a peak value on the third day, then decreases gradually; on the fifth day denitrification is complete. Monitoring of nitrite concentrations showed that the nitrite concentration points followed the typical pattern of biological denitrification: transient increase in nitrite concentrations (nitrite was produced by nitrate reduction), subsequently followed by nitrite reduction according to reactions (1) and (2) (see above).

The apparition of the nitrites is bound to the disappearance of the nitrates according to reaction (1). The result suggests that nitrite production was insignificant and that denitrification was not inhibited by nitrite ions. Therefore, it was deduced that the formation of the nitrites is only an intermediate step of denitrification and that is worth mentioning because, if there is accumulation of the nitrites — even in a small quantity — the process is not as interesting (if the standard norm of the nitrate is 50 mg/L, for nitrites it is 0.1 mg/L, which explains the danger represented by the nitrites).

#### 4.8. Effect of granulometry

The effect of particle size on the denitrification process was investigated for two sizes: 0.630–1.6 and 1.6–2 (mm). From Fig. 5 it was observed that denitrification is much faster for the smaller size (0.630–1.6 mm). For a fine size dimension, the surface of exchange is more developed, which facilitates the consumption of the organic support by the bacteria; thus, the denitrification process is accelerated. Besides, a fine dimension particle is bound directly to the surface of the developed biofilm on the material support.

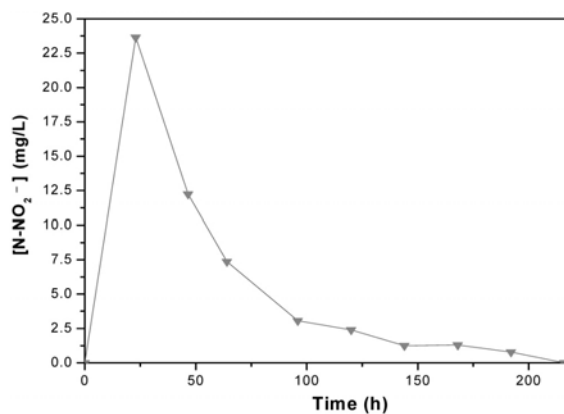


Fig. 4. Nitrite concentration evolution during denitrification. pH 7.45; [N-NO<sub>3</sub><sup>-</sup>]: 500 mg/L; [biomass]: 10 g/L.

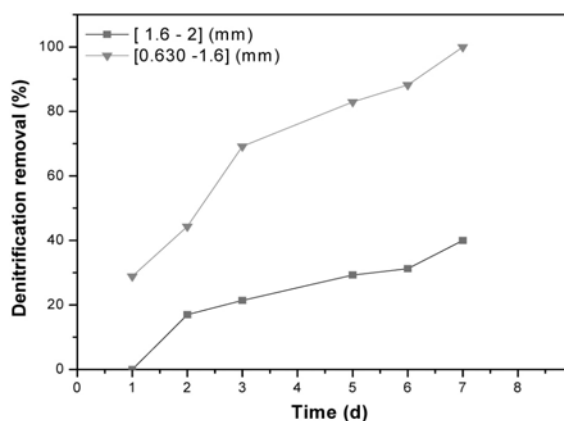


Fig. 5. Effect of particle size on nitrate removal. pH 7.45; [N-NO<sub>3</sub><sup>-</sup>]: 250 mg/L; [biomass]: 10 g/L.

## 5. Conclusions

The results obtained during the experiments for complete denitrification require a pH between 5.95 and 7.91. Elevation of the temperature accelerates the process. The effect of the initial concentration influences the kinetics of the reaction, not nitrate removal. To decrease the length of the process, the effect of particle size was studied, which showed that a fine dimension

particle gives a better denitrification rate. This process of denitrification in batch offers interesting perspectives:

- total nitrate removal without accumulation of intermediate products (nitrites),
- valorisation of the olive pits actually rejected in nature in large quantities,
- high nitrate removal that offers the possibility of use for concentrated nitrate-polluted water.

However, the use of this industrial process again requires more studies to determine the quantity of organic matter that can be dropped by the material support.

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