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Cite this: *RSC Adv.*, 2025, **15**, 37865

Effect of substrate pretreatment on *in situ* heterotrophic denitrification of nitrate-contaminated groundwater

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Received 6th August 2025
Accepted 1st October 2025

DOI: 10.1039/d5ra05750g

rsc.li/rsc-advances

Intensive use of nitrogen fertilizers in Algeria has caused significant nitrate pollution of groundwater, with concentrations reaching 218 mg L^{-1} in the Khemis-el-Khechena region, well above the permissible limit of 50 mg L^{-1}. This study investigates an economical and sustainable biological treatment using date pedicels, an abundant agricultural by-product, as both a carbon source and microbial support for heterotrophic denitrification. Date pedicels were pretreated with 0.5% sodium hydroxide for two hours to enhance biodegradability. Batch experiments showed optimal nitrate removal with 10 g L^{-1} of treated biomass, neutral pH, and a substrate-to-nitrate ratio of 67 g L mg^{-1}. Applied to real groundwater (212 mg per L NO_3^-, pH 7.3), nitrate concentrations decreased to 15.3 mg L^{-1} within seven days, with 4.3 mg per L nitrites detected. A pilot-scale continuous system simulating an *in situ* bioreactor achieved nearly complete nitrate removal from the first day, with minor nitrite accumulation (0.8 mg L^{-1} decreasing to 0.3 mg L^{-1} by day five). Secondary treatment is still required to meet drinking standards, although natural processes such as oxygenation and filtration could further improve water quality.

1. Introduction

Groundwater is considered polluted and unfit for consumption when the concentrations of dissolved elements exceed the maximum permissible limits established by national regulations and international organizations, including the World Health Organization (WHO). Nitrate ions are among the most common groundwater contaminants, and their presence is often regarded as an indicator of pollution.[1] This water pollutant can have several sources, including industrial and agricultural activities, with fertilizers used in agriculture and irrigation, as well as domestic wastewater, being the main contributors today.

Nitrates are highly soluble and are easily transported through rainwater, surface runoff, and infiltration. Consequently, groundwater and even surface water reservoirs often contain elevated nitrate levels, leading to multiple impacts: (i) health-related, as nitrates are a potential cause of methemoglobinemia and a source of nitrosamines,[2] and may contribute to gastric cancer due to nitrate reduction to nitrite in the intestine;[3] (ii) ecological, by promoting eutrophication; and (iii) economic, by increasing the cost of producing drinking water.

Given this concerning situation, the World Health Organization (WHO) has established a maximum nitrate concentration of 50 mg L^{-1} in drinking water.[1] Once contaminated, groundwater is extremely costly to remediate, requiring extraction, nitrate removal treatment, and reinjection or redistribution.

Since then, nitrate removal has been achieved using various physicochemical techniques, including ion exchange,[4] electro-autotrophic denitrification,[5] and reverse osmosis.[6] It should be noted that not all of these processes actually degrade nitrate ions; in many cases, they merely transfer them into a concentrated form. Furthermore, these techniques are non-specific and involve high operational costs.

Biological treatments play a crucial role in nitrate removal processes. Biological denitrification relies on heterotrophic bacteria, primarily from the Pseudomonas genus, which use nitrate as an alternative electron acceptor to oxygen and convert it into nitrogen gas.[7] These heterotrophic bacteria consume organic compounds to obtain energy.

The valorization of lignocellulosic materials in biological water treatment has attracted considerable attention from researchers for two main reasons: environmental protection and economic utilization. One of the main challenges in using lignocellulosic biomass as a carbon source for heterotrophic denitrification is the limited accessibility of cellulose to the enzymes secreted by microorganisms. To enhance this

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accessibility, the lignocellulosic material is pretreated. Depending on their mode of action on the substrate, pretreatments are generally classified into two categories:

• Physical pretreatments, including the use of grinders, irradiation, or thermomechanical methods;[8]

• Chemical pretreatments using alkaline "swelling" agents, such as sodium hydroxide or potassium hydroxide, as well as ammonia and acids.

The pretreatment of plant-based carbonaceous substrates enhances the degradation of cellulose, thereby increasing the efficiency of the heterotrophic biological denitrification process. In this study, our objective is to investigate the effect of soda pretreatment on date pedicels.

Lignocellulosic materials, such as date pedicels, have shown great potential for groundwater remediation and protection. They can be used in *ex situ* biological reactors ('pump-and-treat') or *in situ* within reactive permeable barriers (RPBs), a passive technique for *in situ* groundwater treatment.[9] In this approach, contaminated groundwater naturally flows through a trench filled with the lignocellulosic substrate, driven by the hydraulic gradient, and emerges treated on the other side.

The design of any RPB should begin with laboratory feasibility tests, which aim to select an appropriate substrate and evaluate its performance. Typically, these tests are carried out first in batch kinetic experiments and then in column setups. In the present study, batch experiments were conducted to investigate the influence of various operating parameters on biological denitrification, while a dynamic laboratory-scale pilot system with a fixed bed of soda-pretreated date pedicels was developed to treat nitrate-contaminated groundwater. The main objective is to enhance the accessibility of polysaccharide compounds in date pedicels to the enzymes secreted by denitrifying microflora.

2. Materials and methods

2.1. Influent characteristics

The ground water used is from a shallow domestic well (6 m), located in the region of Khemis-el-Khechna, Algerian, its chemical composition is as follows (Table 1).

2.2. Consumable support material used "date pedicels"

In some countries, date palm by-products, date pedicels are available in significant quantities. The use of the latter in water treatment constitutes a contribution to the efforts made for the recovery of this waste. Date pedicels are part of lignocellulosic materials. We therefore deemed it necessary to provide some information on the composition of the latter. Fig. 1 represents the mass percentage of date pedicels in cellulose, hemicellulose, lignin, total sugars (TS), total nitrogenous matter (TNM) and phosphorus.

The high content of cellulose, hemicellulose, and total sugars (TS) indicates that the hydrolysis of these compounds can release readily assimilable sugars, which in turn promote the growth of denitrifying heterotrophic bacteria. The availability of nutrient resources, including carbon, nitrogen, phosphorus, magnesium, calcium, and iron (Table 2), is a key factor influencing the development of bacterial communities. Furthermore, as reported by Libing Chu and Jianlong Wang,[10] the use of an organic substrate with a diverse composition in

Table 1 Chemical composition of raw water

Parameter	Value (mg L^{-1})
pH	7.30
Ca^{2+}	408.2
Mg^{2+}	178.0
Na^{+}	298.8
K^{+}	—
NO_3^{-}	218.0
NO_2^{-}	0.0
PO_4^{3-}	0.0
SO_4^{2-}	266.5
Cl^{-}	337.9
Fe	0.024
Zn	0.032
Cr	0.000
Ni	0.062
Al	0.580
Cu	0.007
Pb	0.047
Mn	0.000
Cd	—
Pesticides	0.004

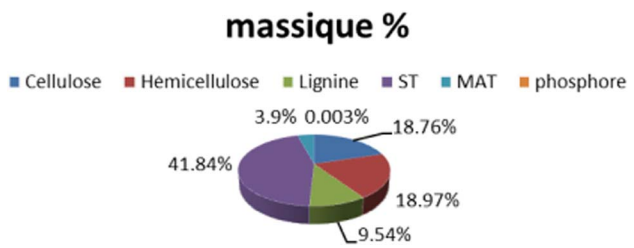

Fig. 1 Results of chemical analysis of date pedicels.

Table 2 Chemical composition of raw date pedicels

Element	Masse (%)
N_2O	0.446
MgO	0.54
Al_2O_3	0.236
SiO_2	1.091
P_2O_5	0.151
SO_3	1.432
K_2O	6.0789
CaO	2.792
TiO_2	0.034
Cr_2O_3	0.109
Fe_2O_3	0.0849
NiO	0.066
ZnO	0.011
SrO	0.016
Cl	2.010
Br	0.004
PAF	85.041
Total	100

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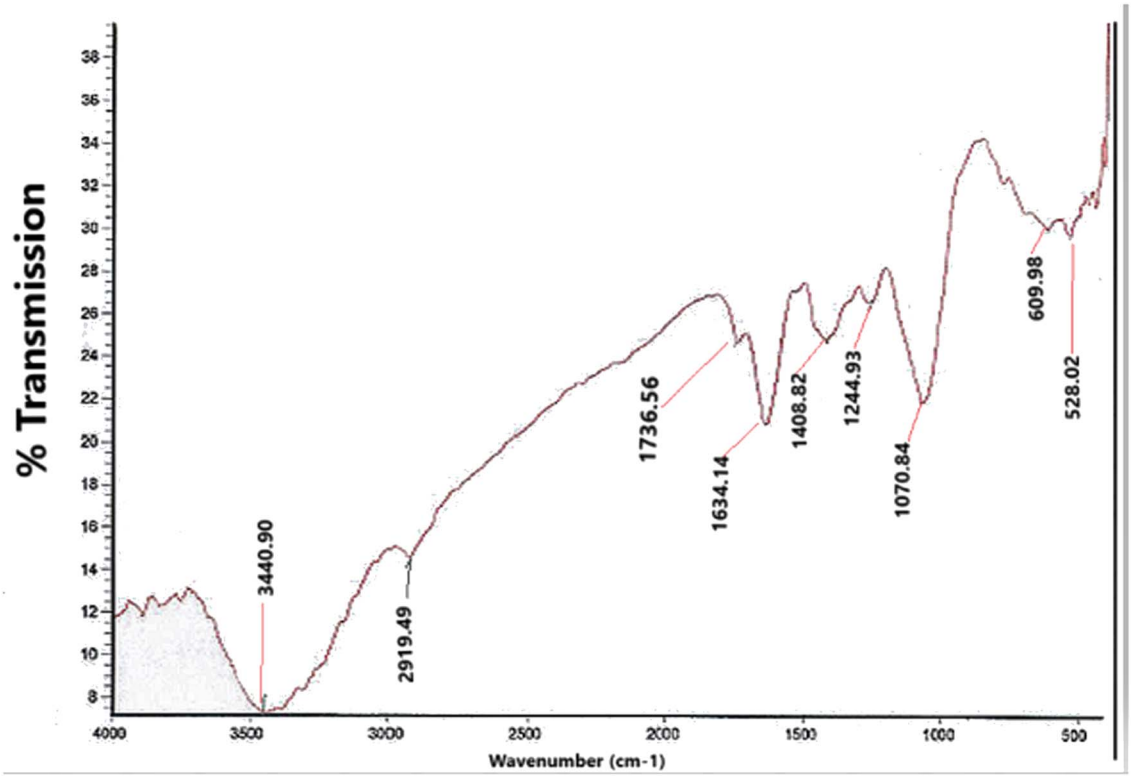

Fig. 2 FTIR spectrum of raw date pedicels.

biological denitrification promotes the growth and stabilization of a bacterial consortium, resulting in higher biological activity compared to that achieved using a carbon source of a single composition.

The raw pedicel substrate was characterized to better understand its chemical and morphological properties. Fourier Transform Infrared Spectroscopy (FTIR) was performed using a Pekim Elmer FTIR2000 spectrometer over the range 400–4000 cm^{-1} (Fig. 2). Major absorption bands were observed at 3446.94 cm^{-1} (O–H, hydroxyl of carboxylic acid, cellulose/lignin), 2919.49 cm^{-1} (C–H aliphatic), 1735.56 cm^{-1} (C=O polyphenolic and aromatic), 1634.14 cm^{-1} (COOH carboxyl), 1244.95 cm^{-1} (C–O–C, O–C–H, C–C–H, aromatic and polysaccharide), 1070.84 cm^{-1} (O–H phenolic and polysaccharide groups) and <1000 cm^{-1} (phosphated and sulfurated groups). These functional groups suggest that raw pedicels can provide a carbon source for denitrifying microorganisms.

2.3. Pretreatment of ligno-cellulose

The highly ordered structure of date pedicel components (cellulose, hemicellulose, and lignin) results in significant crystallinity, which limits enzyme accessibility. Therefore, chemical pretreatment was applied to increase the accessibility of the polysaccharide constituents to enzymatic hydrolysis, thereby enhancing the heterotrophic denitrification process. For the pretreatment, 50 g of date pedicels, cut into small pieces, were introduced into 1 L of an aqueous solution containing a specified concentration of sodium hydroxide (0.1, 0.5, or 1%). The mixture was stirred for 1, 2, or 3 hours. The treated date pedicels were subsequently filtered, washed with tap water until neutrality was reached, dried at 40 °C for 24 hours, and finally ground. The resulting substrate was then used both as a support and as an organic carbon source for the denitrification kinetics experiments.

2.4. Study of the kinetics of biological denitrification

The experiments were conducted in two-liter brown flasks, hermetically sealed with caps fitted with syringes to allow sampling. The flasks were placed on magnetic stirrers to ensure homogeneous mixing.

Batch tests were carried out to evaluate the effect of operating conditions on denitrification kinetics. The working temperature corresponded to the ambient laboratory temperature (26 ± 2 °C). Prior to analysis, all samples were filtered through a 0.45 μm membrane filter. Concentrations of NO_3^-, NO_2^-, PO_4^{3-}, NH_4^+, and organic matter were determined using a UV spectrophotometer (HACH DR 5000, Loveland, CO, USA) following standard methods. Denitrification efficiency was evaluated by calculating the nitrate removal efficiency Y (%) according to eqn (1).

$$Y(\%) = \frac{C_0 - C_t}{C_0} \times 100 \quad (1)$$

C_0 and C_t represent the concentrations of nitrate, nitrite, ammonium, and phosphate at the initial time and at time t, respectively, expressed in mg L^{-1}.

In the present study, we did not perform direct microbiological analyses to confirm the presence of active denitrifiers

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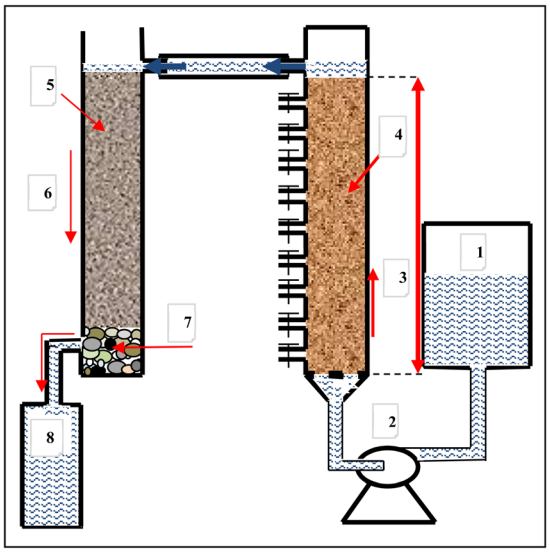

1. Groundwater polluted by nitrates
2. Peristaltic pump ensuring a controlled flow rate.
3. Upward flow through the column.
4. Organic support layer (height: 140 cm).
5. Sand layer for additional filtration.
6. Downward flow towards the outlet section.
7. Gravel layer serving as a drainage medium.
8. Treated groundwater (effluent).

Fig. 3 Experimental setup of the dual-column continuous denitrification system.

(*e.g.*, plate counts, 16S profiling, or qPCR of denitrification genes) due to experimental limitations. However, the observed nitrate removal clearly indicates denitrification activity. We acknowledge that direct confirmation of microbial activity would strengthen the understanding of the process and plan to include these analyses in future work.

To evaluate the influence of soda pretreatment on the denitrification medium, several parameters were systematically investigated. The study focused on the effects of soda concentration, pretreatment time of date pedicels, substrate mass, initial nitrate concentration, and pH on the denitrification kinetics.

2.5. Continuous denitrification system

In situ treatment is the most economical method for removing nitrates from groundwater. One primary approach for *in situ* treatment is the use of an anaerobic biological barrier. This laboratory-scale pilot study implements a heterotrophic biological denitrification technique applied to groundwater with a nitrate concentration of 218 mg L^{-1}.

The process uses date pedicels pretreated with 0.5% sodium hydroxide (NaOH) and ground, serving both as a carbon source and as a support medium for denitrifying bacteria. The experimental setup consists of two columns arranged in series. The first column, measuring 170 cm in height and 3 cm in internal diameter, is filled with 300 g of substrate with a porosity of 0.28. The second column contains sand with a particle size of less than 1 mm and a porosity of 0.5. Sampling ports are positioned along the first column to monitor the variation of nitrate concentration with height (Fig. 3). The system operates with an upward flow through the first column and a downward flow through the sand column. A peristaltic pump, connected to a reservoir containing the water to be treated, supplies the denitrification reactor. The flow velocity was maintained at 0.045 m h^{-1}, which closely approximates the natural groundwater flow rate in the subsurface.

2.6. Preculture

In order to promote bacterial growth and reduce the start-up time of the denitrification reaction, the pretreated and ground support material first undergoes a preculture phase (bacterial seeding). The preculture method consisted of keeping the support in nitrate-polluted groundwater (218 mg L^{-1}) under static conditions for several days, with a daily renewal of the medium. A nitrate removal efficiency of 85% was achieved after 5 days of preculture.

3. Results and discussion

3.1. Batch denitrification tests

The kinetics of denitrification were examined through the evolution of nitrate (NO_3^-), nitrite (NO_2^-), orthophosphates (PO_4^{3-}), ammonium (NH_4^+) and organic matter concentrations.

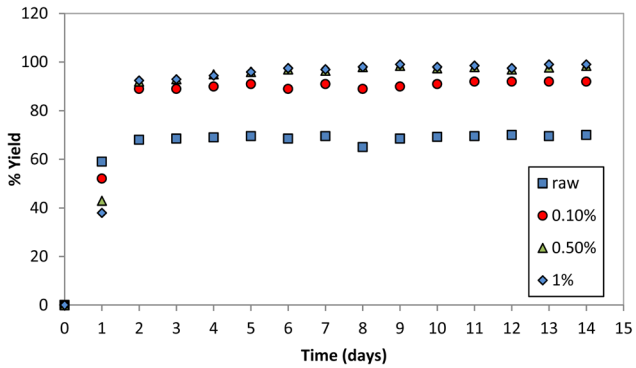

Fig. 4 Influence of soda pretreatment concentration on the kinetics of denitrification ($[NO_3^-]_0 = 150$ mg L^{-1}, $T = 26 \pm 2$ °C, $pH_{initial} = 7.08$, 10 g L^{-1} of the substrate).

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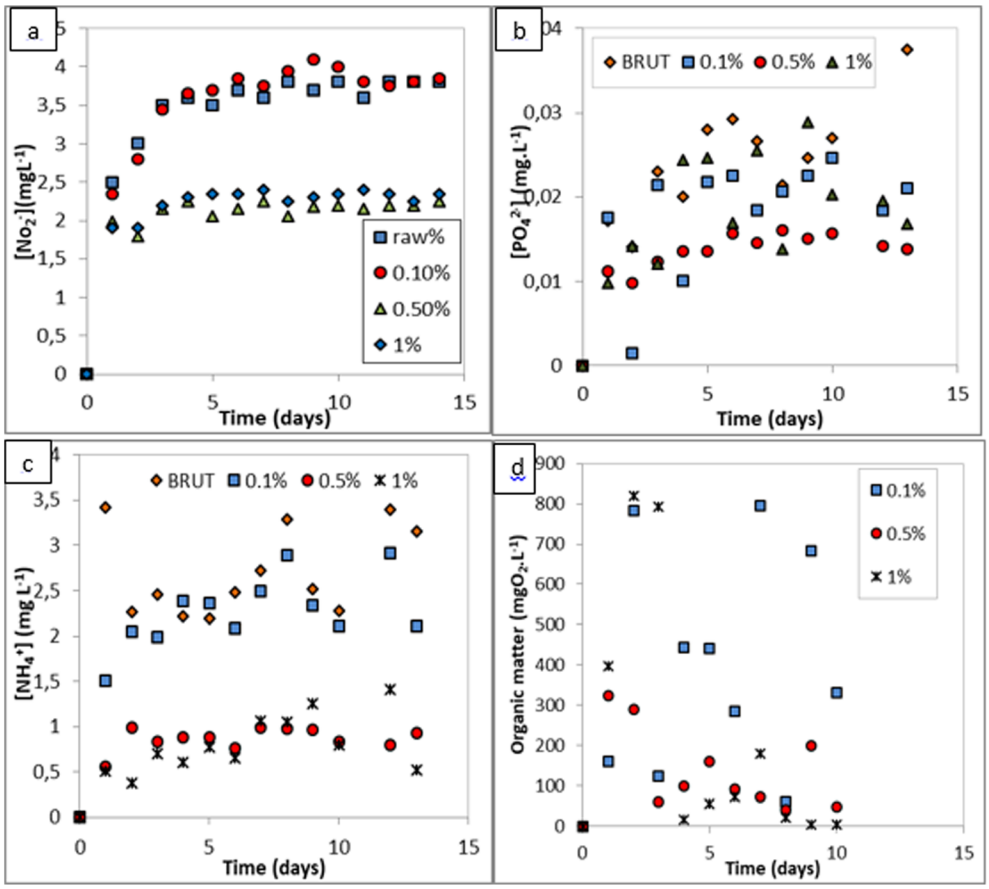

Fig. 5 Evolution of (a) $[NO_2^-]$, (b) $[PO_4^{3-}]$ (c) $[NH_4^+]$ (mg L^{-1}), and (d) organic matter (mg_{O_2} L^{-1}) as a function of time at different soda concentrations [%] ($[NO_3^-]_0 = 150$ mg L^{-1}, $T = 26 \pm 2$ °C, $pH_i = 7.08$, 10 g L^{-1} of substrate).

3.1.1. Study of the influence of the soda concentration. From Fig. 4, it can be observed that the overall trend of the denitrification yield curves is nearly identical, with a slight increase in yield for the supports pretreated with 1% and 0.5% soda. It is clear that bacterial growth is accompanied by a proportional consumption of nitrate ions that are used as terminal electron acceptors transferred along the respiratory chain. Denitrification stops after 48 hours, with an average residual nitrate concentration of 20 mg L^{-1}. This value is below the limit of 50 mg L^{-1} set by Algerian legislation and corresponds to an average denitrification efficiency of 86.66%. Lignin is highly resistant to degradation, and by forming bonds with both cellulose and hemicellulose, it acts as a barrier to solute penetration. Increasing the soda ash concentration from 0.5% to 1% does not significantly affect the denitrification efficiency.

In parallel with nitrate measurements over time, the concentration of nitrites released into the reaction medium was also monitored for the different media. The corresponding results are presented in Fig. 5a.

It should be recalled that nitrite represents an intermediate step in the reduction of nitrate to nitrogen gas (N_2) (eqn (2) and (3)), according to the following reactions:

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \quad (2)$$

$$NO_2^- + 4H^+ + 3e^- \rightarrow 1/2N_2 + 2H_2O \quad (3)$$

The denitrification process can be considered a two-step reaction: the first involves the reduction of nitrate to nitrite, and the second corresponds to the reduction of nitrite to molecular nitrogen. The kinetics of nitrate-to-nitrite transformation (denitration) are faster than those of nitrite-to-nitrogen gas transformation (denitritation).

By examining the evolution curve of nitrite concentration as a function of time (Fig. 5a) for the four reactors, a significant accumulation of nitrites is observed in the reactor operating with the raw support. A nitrite concentration of approximately 19 mg L^{-1} was recorded on the first day, progressively decreasing to 3 mg L^{-1} by the eighth day of treatment.

On the other hand, this phenomenon was not observed in the reactors operating with pedicels pretreated with soda (0.1%, 0.5%, 1%), highlighting the importance of substrate pretreatment. The evolution of orthophosphate, ammonium, and organic matter concentrations in the reaction medium is presented in Fig. 5b–d. The results indicate that microflora activity was intense in all four reactors. The appearance of ammonium in the reaction medium (eqn (4)) suggests that dissimilatory nitrate reduction to ammonium (DNRA) occurred, according to the following reaction.[11]

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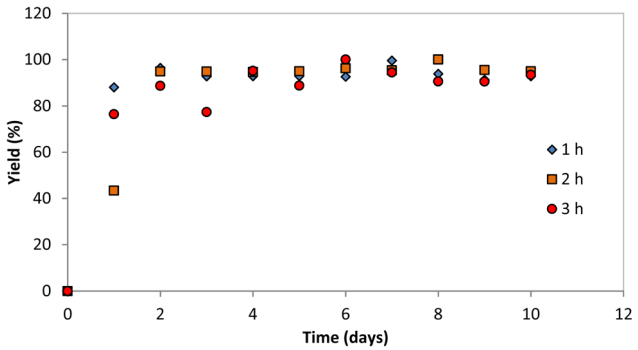

Fig. 6 Evolution of denitrification efficiency as a function of time ($[NO_3^-]_0 = 150$ mg L^{-1}, $T = 26 \pm 2$ °C, $pH_i = 7.08$, 10 g L^{-1} substrate).

$$2CH_2O + NO_3^- + 2H^+ \rightarrow NH_4^+ + 2CO_2 + H_2O \quad (4)$$

Studies have shown that under anaerobic conditions, and when organic matter concentrations are high relative to those of nitrates, dissimilatory nitrate reduction is favored over denitrification.[12,13] The evolution of ammonium and organic matter concentrations observed in this study is consistent with this statement: an increase in organic matter concentration was accompanied by an increase in ammonium, and *vice versa*.[14]

It should also be noted that the ammonium and organic matter concentrations recorded in the reactors operating with raw date pedicels and with pedicels treated with 0.1% soda were higher than those observed in the other two reactors. This can be attributed to the fact that during pretreatment with 0.5% and 1% soda, a substantial fraction of the carbonaceous matter was leached, leading to a reduction for matter available for assimilation by the denitrifying biomass.

3.1.2. Study of the influence of the time of substrate pretreatment. From Fig. 6, it can be observed that increasing the pretreatment time of date pedicels did not have a substantial influence on the average denitrification efficiency. Specifically, the efficiency reached an average of 93.6% for the substrate pretreated with 0.5% soda for 1 hour, 95.7% for that pretreated for 2 hours, and 90.6% for that pretreated for 3 hours.

To determine the optimal pretreatment time of date pedicels with soda, the evolution of nitrite concentration in the reaction medium was monitored (Fig. 7a). According to the results obtained, a strong accumulation of nitrites was observed from the first day of treatment, reaching approximately 36 mg L^{-1} and 22 mg L^{-1} for pretreatment times of one and three hours, respectively. It can be concluded that a pretreatment duration of one hour is insufficient to enable significant assimilation of the organic matter present in the date pedicels, whereas a three-

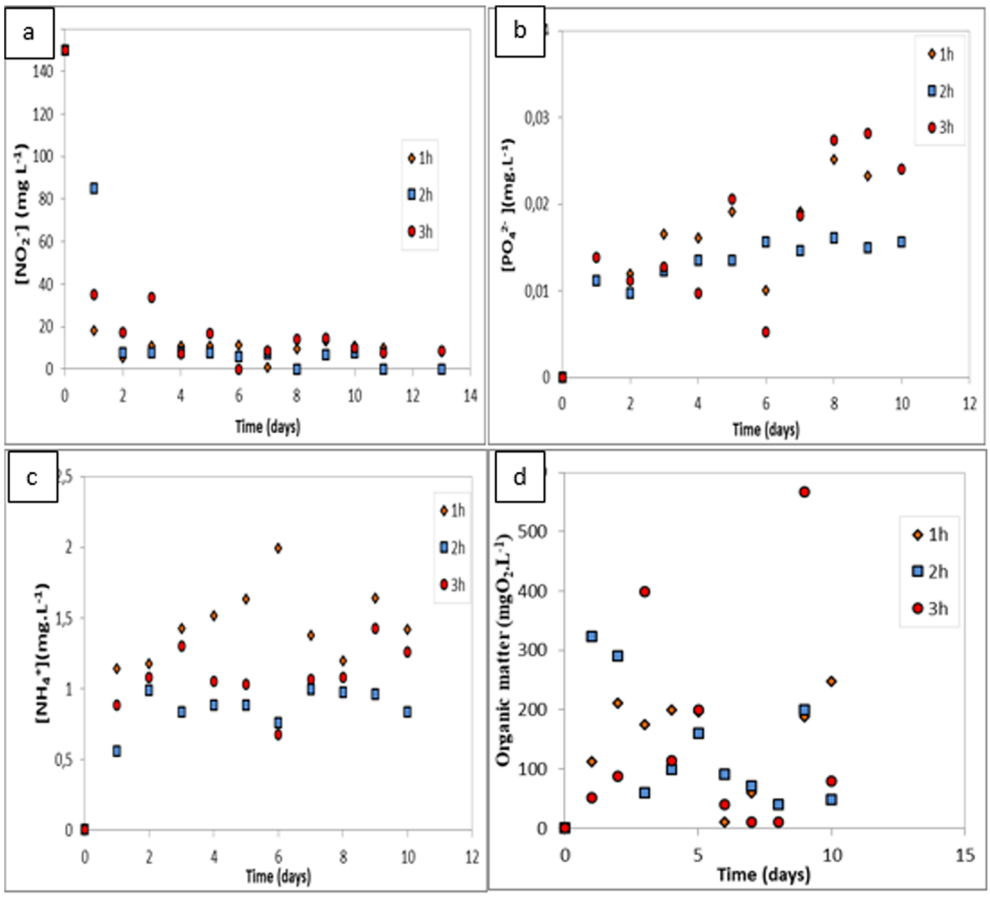

Fig. 7 Evolution over time (days) of (a) $[NO_2^-]$, (b) $[PO_4^{3-}]$, (c) $[NH_4^+]$ (mg L^{-1}), and (d) organic matter $[mg_{O_2}\ L^{-1}]$ for different substrate pretreatment durations (h) ($[NO_3^-]_0 = 150$ mg L^{-1}, 10 g L^{-1} substrate treated with 0.5% soda, $pH_i = 7.08$).

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hour treatment induced the leaching of a substantial fraction of the carbonaceous matter by the washing water (Fig. 7a). Fig. 7b illustrates the evolution of orthophosphate concentration over 10 days for date pedicels pretreated for 1, 2, and 3 hours. The three-hour pretreatment results in the highest and most variable orthophosphate release, particularly after day 6, whereas the two-hour pretreatment yields the most stable and consistently lowest concentrations. Overall, the two-hour pretreatment appears to be the most effective in minimizing phosphorus release during the biodegradation process. Fig. 7c shows that ammonium concentration varies depending on the pretreatment duration of date pedicels.

A one-hour pretreatment leads to the highest ammonium release, whereas a two-hour pretreatment results in more stable and moderate levels. The two-hour pretreatment provides the best balance for controlled biodegradation and efficient denitrification. We can conclude that a one-hour treatment is insufficient to allow significant assimilation of the organic matter present in the date pedicels, whereas a three-hour treatment leads to the loss of a substantial portion of the carbonaceous matter through entrainment in the washing water (Fig. 7d).

3.1.3. Study of the influence of the substrate concentration. To determine the optimal amount of substrate, we investigated the influence of organic support concentration on denitrification kinetics. Several experiments were performed by varying the substrate concentration from 5 to 20 g L^{-1} (Fig. 8).

Nitrate removal is significant for substrate concentrations between 5 and 10 g L^{-1}, but decreases at concentrations above 10 g L^{-1}, as also reported by Wang *et al.*[15] Under anaerobic conditions, with high organic matter relative to nitrate, dissimilatory nitrate reduction is favored over denitrification.[12,13] Overall, nitrate removal increases with substrate concentration up to 5 g L^{-1}, then gradually declines. From Fig. 9, a strong accumulation of nitrite is observe in the reactors containing 5, 8, 15, and 20 g L^{-1} of substrate. Several factors have been shown to contribute this phenomenon, including organic carbon supply, pH, oxygen content, and phosphate concentration.[16] The choice of the initial C/N ratio directly influences the level of nitrite accumulation during denitrification.

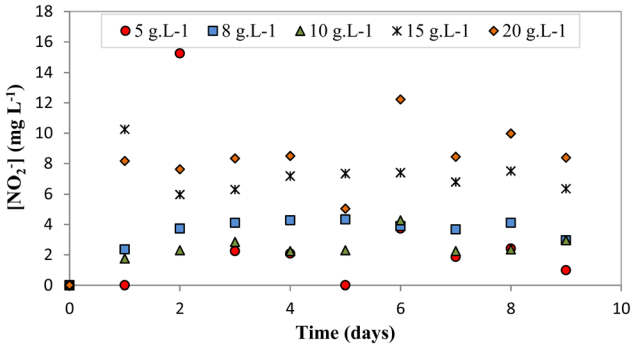

Fig. 9 Evolution of nitrite concentration as a function of time at different substrate concentrations (%) ($[NO_3^-]_0 = 150$ mg L^{-1}, $T = 26 \pm 2$ °C, $pH_i = 7$).

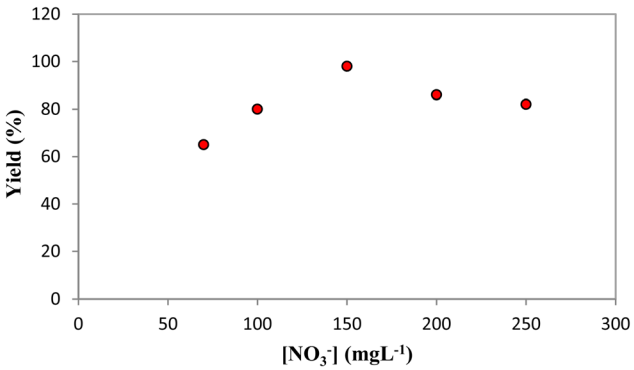

Fig. 10 Average yield as a function of initial nitrate concentration ($pH_i = 7$, $T = 26 \pm 2$ °C, 10 g L^{-1} of substrate).

This is attributed to competition for electrons between nitrate reductases and nitrite reductases.[17] A concentration of 10 g L^{-1} provides more efficient nitrite removal.

3.1.4. Study of the influence of the initial nitrate concentration. The influence of initial nitrate concentration on biological denitrification kinetics was investigated at 70, 100, 150, 200, and 250 mg L^{-1}. The initial nitrate removal rate, determined from the slopes of the denitrification curves (Fig. 10), increases with higher nitrate concentrations. The average denitrification yield rises almost linearly up to 150 mg L^{-1}, then

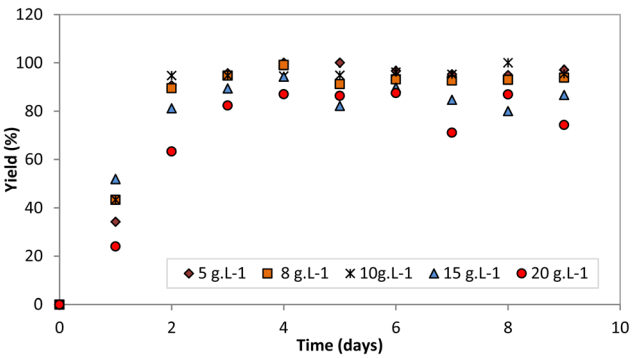

Fig. 8 Evolution over time (days) of denitrification efficiency for soda-treated date pedicels at different substrate concentrations (%) ($[NO_3^-]_0 = 150$ mg L^{-1}, $T = 26 \pm 2$ °C, $pH_i = 7$).

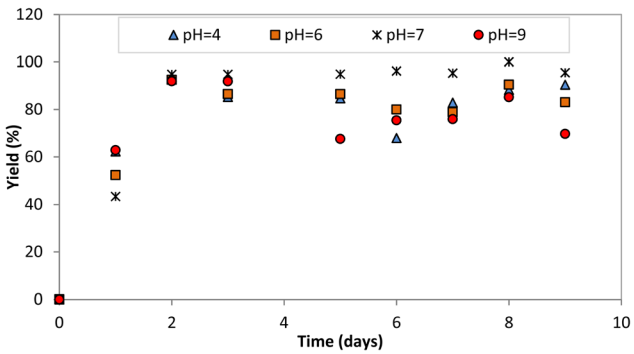

Fig. 11 Evolution of denitrification efficiency as a function of time (pedicels pre-treated with soda) ($[NO_3^-]_0 = 150$ mg L^{-1}, $T = 26 \pm 2$ °C, 10 g L^{-1} of substrate).

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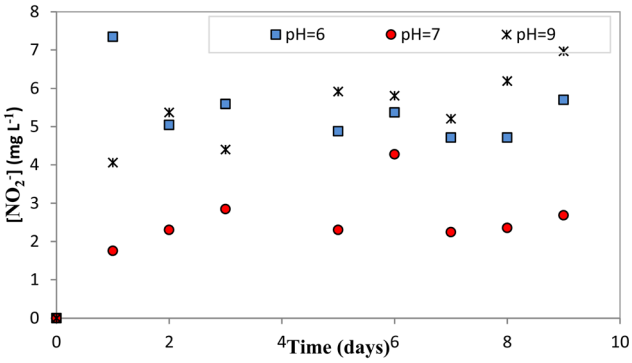

Fig. 12 Evolution of nitrite concentration as a function of time (pedicels pre-treated with soda) ($[NO_3^-]_0 = 150$ mg L^{-1}, $T = 26 \pm 2$ °C, 10 g L^{-1} of substrate).

slightly declines, indicating an optimal substrate-to-nitrate ratio of 10/150.

3.1.5. Study of the influence of initial pH on denitrification efficiency. The effect of the initial pH on biological denitrification was also investigated, and this is adjusted to the desired value by adding hydrochloric acid or soda. The other parameters were kept constant. According to the literature, the optimal pH range for denitrification is between 7 and 8.5.[18] Fig. 11 shows that varying the initial pH of the solution between 4 and 9 does not significantly influence the evolution of the denitrification yield when using the substrate treated by the Fenton process. The average denitrification efficiency ranged between 83 and 86%. Using the substrate treated with soda; the highest denitrification yields were observed at neutral pH.

According to Zhou *et al.*,[18] the pH of the environment plays a major role in nitrite accumulation, as it influences the enzymatic activity of bacteria. Monitoring the evolution of nitrite concentration in the reaction medium at different initial pH values (Fig. 12) clearly shows that acidic conditions favor nitrite accumulation, suggesting that bacterial enzymatic activity is adversely affected.

3.2. Application of the biological denitrification process to well water

Groundwater in the eastern Mitidja region is among the most polluted by nitrates, with average concentrations of around

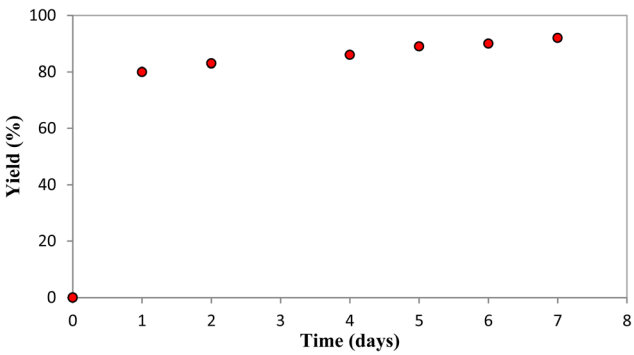

Fig. 13 Evolution of denitrification efficiency as a function of time ($[NO_3^-]_0 = 212$ mg L^{-1}, 10 g L^{-1} substrate, $T = 26 \pm 2$ °C, $pH_i = 7.20$).

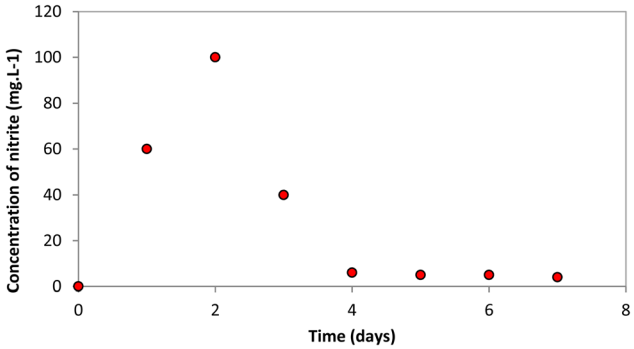

Fig. 14 Evolution of nitrite concentration as a function of time ($[NO_3^-]_0 = 212$ mg L^{-1}, 10 g L^{-1} of substrate, $T = 26 \pm 2$ °C, $pH_i = 7.20$).

120 mg L^{-1} according to the National Water Resources Agency (ANRH) network. A heterotrophic biological denitrification experiment under optimal conditions was carried out on groundwater collected from a private well in Khemis-el-Khechena, a commune located in the eastern Mitidja region. This water is characterized by a pH of 7.20 and a high nitrate content of 212 mg L^{-1}, which greatly exceeds the admissible standard ($[NO_3^-] \leq 50$ mg L^{-1}), making it unfit for consumption. The denitrification efficiency *versus* time curve in Fig. 13 shows that the denitrifying reactor undergoes two distinct phases:

- The first phase is characterized by a marked increase in denitrification yield during the first two days of treatment, reaching 84.70% for the soda-treated substrate. This corresponds to a nitrate concentration below the Algerian drinking water standard of 50 mg L^{-1}.
- In the second phase, the denitrification efficiency stabilizes at approximately 93% for the soda treated substrate.

The monitoring of nitrite concentration over time (Fig. 14) revealed two distinct phases. During the first 48 hours, a rapid accumulation of nitrites was observed, reaching up to 109 mg L^{-1}, as a result of nitrate reduction by bacteria producing the corresponding enzymes. In the subsequent phase, the concentration gradually declined, stabilizing at around 4 mg L^{-1} by the seventh day of reactor operation.

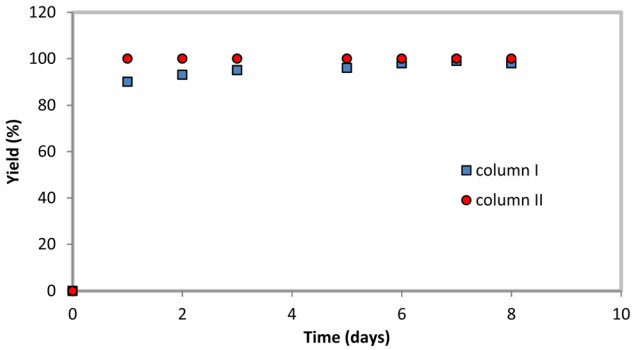

Fig. 15 Evolution of denitrification yield at the exit of the columns, I (soda-pretreated substrate), II (sand) ($[NO_3^-]_0 = 218$ mg L^{-1}, $T = 34 \pm 2$ °C, $pH_i = 7.30$, flow rate = 0.045 m h^{-1}).

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3.3. Continuous denitrification tests

The continuous denitrification of groundwater was studied using a column reactor, with performance monitored through NO_3^-, NO_2^-, PO_4^{3-}, NH_4^+, and organic matter. Two columns arranged in series were fed continuously and monitored daily for one week, after which denitrification performance stabilized. Denitrification yield increased markedly during the first 24 hours, reflecting rapid adaptation of the microflora, and then reached a maximum with nitrate removal nearly complete (Fig. 15). Nitrate removal using soda-pretreated date pedicels was comparable to other plant-based carbon substrates, but exceeded them at initial nitrate concentrations above 200 mg L^{-1}. Therefore, complete nitrate elimination was achieved, underscoring the high efficiency of the denitrification process under the tested conditions. This finding is particularly relevant for drinking water treatment, given that the World Health Organization (WHO) sets the maximum permissible nitrate concentration at 50 mg L^{-1}.[1] Such complete removal ensures compliance with international standards and demonstrates the strong applicability of this method. Nitrite, an intermediate in nitrate reduction to N_2,[19] accumulated up to 3 mg L^{-1} at the outlet of the date pedicel column on the first day, stabilizing at ~1.9 mg L^{-1} by day five, while in the sand column it decreased from 0.8 to 0.3 mg L^{-1} over the same period (Fig. 16a). Orthophosphate concentrations increased to 0.024 mg L^{-1} on day three at the date pedicel column outlet, decreasing to 0.002 mg L^{-1} by day seven, with complete removal observed in the sand column from day six, indicating that phosphorus in date pedicels suffices for microbial energy requirements without additional dosing (Fig. 16b).

Ammonium, formed *via* dissimilative nitrate reduction under strictly anaerobic conditions and governed by C/N ratio (>4 favors reduction, <4 favors denitrification),[20,21] was detected at the outlet of column I but removed within one week in column II, remaining below the drinking water limit of 0.5 mg L^{-1} (Fig. 16c). Conventional ammonium oxidation occurs *via* ammonium monooxygenase, requiring oxygen. Schmid *et al.*[22] observed simultaneous nitrate disappearance, ammonium consumption, and N_2 formation in a fluidized-bed pilot, while Fux *et al.*[23,24] showed nitrite as the preferred electron acceptor according to the reaction:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (5)$$

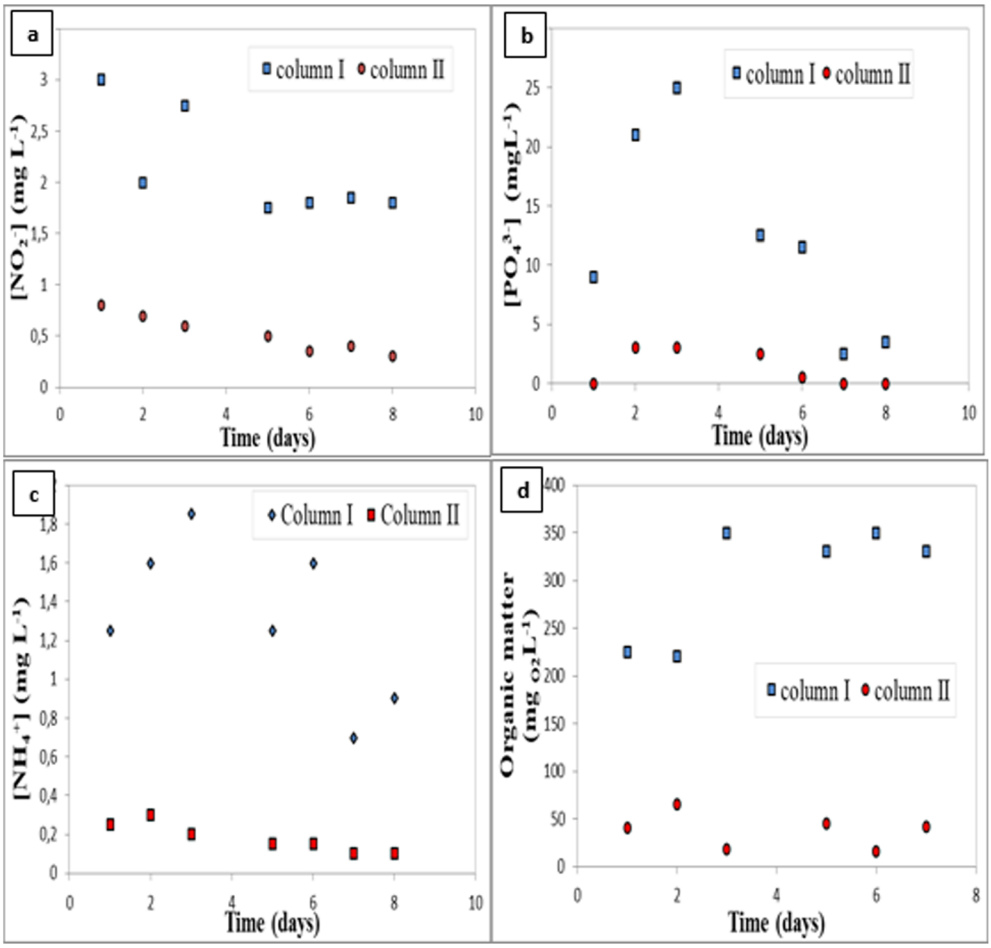

Fig. 16 Evolution over time (days) of (a) $[NO_2^-]$, (b) $[PO_4^{3-}]$, (c) $[NH_4^+]$ (mg L^{-1}), and (d) organic matter [mg_{O_2} L^{-1}] at the exit of the columns, I (soda-pretreated substrate), II (sand) ($[NO_3^-]_0$ = 218 mg L^{-1}, T = 34 ± 2 °C pH_i = 7.30, flow rate = 0.045 m h^{-1}).

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Organic matter initially released from the substrate increased during one week of operation, corresponding to optimal reactor performance; the sand column removed a significant portion, but the post-barrier zone was insufficient to capture all residual organics, limiting secondary denitrification (Fig. 16d).

4. Conclusions

Intensive use of nitrogen fertilizers in Algeria has resulted in nitrate pollution of groundwater. In the Khemis-el-Khechena region (eastern Mitidja), nitrate concentrations reached 218 mg L^{-1}, far exceeding the standard limit of 50 mg L^{-1}. This level of contamination poses significant risks to human health.

The aim of this work is, first, to add value to date pedicels, agricultural by-products with high national availability, and second, to assess the performance of this waste, pretreated with sodium hydroxide, in heterotrophic biological denitrification. Date pedicels contain a high proportion of organic matter, nutrients, and minerals, highlighting their potential as both a support and a substrate for the microflora responsible for biological denitrification.

Our study focused on denitrification in a batch reactor using date pedicels pretreated with sodium hydroxide as both a substrate and a support for the microflora. We found that optimal denitrification occurred when date pedicels were pretreated with 0.5% sodium hydroxide for two hours.

The parametric study allowed us to optimize the operating conditions for the heterotrophic biological denitrification process, including the mass of substrate used, the initial nitrate concentration, and the initial pH. Optimal denitrification was achieved using a treated biomass quantity of 10 g L^{-1}, a neutral initial pH, and a substrate/nitrate ratio of 67 g L mg^{-1}. A batch application of heterotrophic biological denitrification using these substrates, pretreated with sodium hydroxide, was carried out under these optimal conditions for the treatment of groundwater collected from a private well in Khemis-el-Khechena. This water contains no organic matter, has a high nitrate concentration of 212 mg L^{-1} and a pH of 7.30.

On the seventh day of treatment, nitrate and nitrite concentrations were measured at 15.3 and 4.3 mg L^{-1}, respectively. These results prompted a follow-up study using a continuous application on a laboratory-scale pilot, simulating an *in situ* biological reactor composed of substrate pretreated with 0.5% sodium hydroxide for 2 hours. The water flow rate through the reactor was set at 0.045 m h^{-1}, approximating the flow velocity of groundwater in the subsurface.

The feed water was taken from a shallow (6 m) domestic well in the Khemis-el-Khechna region. From the first day of treatment, an almost complete removal of nitrates was observed, accompanied by the appearance of 0.8 mg L^{-1} nitrite, which decreased to 0.3 mg L^{-1} by the fifth day of treatment. Therefore, secondary treatment is required to make this water potable; however, under natural conditions, the aquifer would oxygenate, filter, and purify the water *in situ*. As a perspective, monitoring soluble TOC and COD over time would provide a more precise understanding of carbon availability and its influence on denitrification and phosphate removal. This will be considered in future studies to further optimize the substrate performance.

Treatments other than disinfection of the extracted water would only be effective if the wells were located very close to the denitrification zone. Implementing an *in situ* pilot plant would require further investigation of several critical factors related to durability and treatment performance.

It is therefore necessary to reassess the extent and frequency of nutrient limitations intended to control bacterial growth. The proposed process should be implemented over the long term (approximately one year) to evaluate the suitability of the operating conditions for the purification system's lifespan. This stage is also crucial for assessing production costs.

Author contributions

Salima Dadou: conceptualization, supervision, experimental design, writing – original draft. Amina Djadi: laboratory experimentation, data collection, methodology. Hynda Yazid: data analysis, interpretation, writing – review & editing. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated during the current study are available from the corresponding author upon reasonable request.

References

1 N. Adimalla, P. Li and H. Qian, Evaluation of groundwater contamination for fluoride and nitrate in a semi-arid region of Nirmal Province, South India: special emphasis on human health risk assessment (HHRA), *Hum. Ecol. Risk Assess.: Int. J.*, 2018, **25**(5), 1–18, DOI: 10.1080/10807039.2018.1460579.

2 S. Garcia-Segura, M. Lanzarini-Lopes, K. Hristovski and P. Westerhoff, Electrocatalytic reduction of nitrate: fundamentals to full-scale water treatment applications, *Appl. Catal., B*, 2018, **236**, 546–568.

3 A. Mohseni-Bandpi, D. J. Elliott and M. A. Zazouli, Biological nitrate removal processes from drinking water supply – a review, *J. Environ. Health Sci. Eng.*, 2013, **35**, 1–11.

4 S. Samatya, N. Kabay, U. Yuksel, M. Arda and M. Yuksel, Removal of nitrate from aqueous solution by nitrate-selective ion exchange resins, *React. Funct. Polym.*, 2006, **66**, 1206–1214.

5 S. Yao, L. Liu, S. Zhang and X. Tang, Nitrate removal from groundwater by heterotrophic and electro-autotrophic denitrification, *Water*, 2022, **14**(11), 1759, DOI: 10.3390/w14111759.

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6 R. Epstein, O. Nir, O. Lahav and M. Green, Selective nitrate removal from groundwater using a hybrid nanofiltration–reverse osmosis filtration scheme, *Chem. Eng. J.*, 2015, **279**, 372–378.

7 N. Mothapo, H. Chen, A. Marc, M. A. Cubeta, J. M. Grossman, F. Fred and S. Wei, Phylogenetic, taxonomic and functional diversity of fungal denitrifiers and associated N2O production efficacy, *Soil Biol. Biochem.*, 2015, **83**, 160–175, DOI: 10.1016/j.soilbio.2015.02.001.

8 K. Kuroda, K. Shimomura, T. Ishijima, K. Takada, K. Ninomiya and K. Takahashi, Effective dissolution of biomass in ionic liquids by irradiation of non-thermal atmospheric pressure plasma, *Aust. J. Chem.*, 2017, **70**(6), 731–734.

9 F. Obiri-Nyarko, S. J. Grajales-Mesa and G. Malina, An overview of permeable reactive barriers for in situ sustainable groundwater remediation, *Chemosphere*, 2014, **111**, 243–259.

10 L. Chu and J. Wang, Denitrification of groundwater using PHBV blends in packed bed reactors and the microbial diversity, *Chemosphere*, 2016, **155**, 463–470, DOI: 10.1016/j.chemosphere.2016.04.090.

11 O. Gibert, S. Pomierny, I. Rowe and R. M. Kalin, Selection of organic substrates as potential reactive materials for use in a denitrification permeable reactive barrier (PRB), *Bioresour. Technol.*, 2008, **99**, 7587–7596.

12 C. M. Greenan, T. B. Moorman, T. C. Kaspar, T. B. Parkin and D. B. Jaynes, Comparing carbon substrates for denitrification of subsurface drainage water, *J. Environ. Qual.*, 2006, **35**, 824–829.

13 J. Van Rijn, Y. Tal and H. J. Schreier, Denitrification in recirculating systems: theory and applications, *Aquacult. Eng.*, 2006, **34**, 364–376.

14 Z. Q. Shen, Y. X. Zhou, J. Hu and J. L. Wang, Denitrification performance and microbial diversity in a packed-bed bioreactor using biodegradable polymer as carbon source and biofilm support, *J. Hazard. Mater.*, 2013, **250–251**, 431–438.

15 X. M. Wang and J. L. Wang, Nitrate removal from groundwater using solid-phase denitrification process without inoculating with external microorganisms, *Int. J. Environ. Sci. Technol.*, 2013, **10**(5), 955–960.

16 M. A. Gómez, E. Hontoria and J. González-López, Effect of dissolved oxygen concentration on nitrate removal from groundwater using a denitrifying submerged filter, *J. Hazard. Mater.*, 2002, **90**(3), 267–278.

17 S. Ge, Y. Peng, S. Wang, C. Lu and Y. Zhang, Nitrite accumulation under constant temperature in anoxic denitrification process: effects of carbon sources and COD/NO_3-N, *Bioresour. Technol.*, 2012, **114**, 137–143.

18 M. H. Zhou, W. J. Fu, H. Y. Gu and L. C. Lei, Nitrate removal from groundwater by a novel three-dimensional electrode biofilm reactor, *Electrochim. Acta*, 2007, **52**, 6052–6059.

19 S. Damaraju, U. K. Singh, D. Sreekanth and A. Bhandari, Denitrification in biofilm-configured horizontal flow woodchip bioreactor: effect of hydraulic retention time and biomass growth, *Ecohydrol. Hydrobiol.*, 2015, **15**(1), 39–48.

20 S. Yoon, C. Cruz-García, R. Sanford, K. M. Ritalahti and F. E. Löffler, Denitrification versus respiratory ammonification: environmental controls of two competing dissimilatory NO_3^-/NO_2^- reduction pathways in Shewanella loihica strain PV-4, *ISME J.*, 2015, **9**, 1093–1104.

21 K. Bernat, I. Wojnowska-Baryła and A. Dobrzyńska, Denitrification with endogenous carbon source at low C/N and its effect on P(3HB) accumulation, *Bioresour. Technol.*, 2008, **99**(7), 2410–2418.

22 M. C. Schmid, B. Maas, A. Dapena, K. van de Pas-Schoonen, J. van de Vossenberg, B. Kartal, L. van Niftrik, I. Schmidt, I. Cirpus, J. G. Kuenen, M. Wagner, J. S. Sinninghe Damsté, M. Kuypers, N. P. Revsbech, R. Mendez, M. S. Jetten and M. Strous, Biomarkers for in situ detection of anaerobic ammonium-oxidizing (anammox) bacteria, *Appl. Environ. Microbiol.*, 2005, **71**(4), 1677–1684, DOI: 10.1128/AEM.71.4.1677-1684.

23 C. Fux, M. Boehler, P. Huber, I. Brunner and H. Siegrist, Biological treatment of ammonium-rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant, *J. Biotechnol.*, 2002, **99**(3), 295–306, DOI: 10.1016/S0168-1656(02)00220-1.

24 C. Della Rocca, V. Belgiorno and S. Meriç, An heterotrophic–autotrophic denitrification (HAD) approach for nitrate removal from drinking water, *Process Biochem.*, 2006, **41**, 1022–1028.