Assessment of a novel microalgae-cork based technology for removing antibiotics, pesticides and nitrates from groundwater

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HIGHLIGHTS

- Micropollutants were eliminated in large quantities (95%), but not nitrates (20–58%).
- The prototype's efficiency was highly reliant on the HRT.
- After the cork filter, pesticide transformation products were found.
- Microbial species with a high biodegradation of micropollutants were identified.

GRAPHICAL ABSTRACT

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ABSTRACT

Groundwater pollution has increased in recent years due to the intensification of agricultural and livestock activities. This results in a significant reduction in available freshwater resources. Here, we have studied the long term assessment of a green technology (1–4 L/day) based on a photobioreactor (PBR) containing immobilised microalgae–bacteria in polyurethane foam (PF) followed by a cork filter (CF) for removing nitrates, pesticides (atrazine and bromacil), and antibiotics (sulfamethoxazole and sulfacetamide) from groundwater. The prototype was moderately effective for removing nitrates (58%) at an HRT of 8 days, while its efficiency decreased at a HRT of 4 and 2 days (<20% removal). The combined use of PBR-CF enabled antibiotics and pesticides to be attenuated by up to 95% at an HRT of 8 days, but their attenuation decreased with shorter HRT, with pesticides being the compounds most affected (reducing from 97 to 98% at an HRT of 8 days to 23–45% at an HRT of 2 days).

Pesticide transformation products were identified after the CF, supporting biodegradation as the main
attenuation process. A gene-based metataxonomic assessment linked the attenuation of micropollutants to the presence of specific pesticide biodegradation species (e.g., genus Phenyllobacterium, Sphingomonadaceae, and Caulobacteraceae). Therefore, the results highlighted the potential use of microalgae and cork to treat polluted groundwater.

1. Introduction

Groundwater is the largest freshwater reserve in the world. It represents the most important source of drinking water (it supplies about half of the world’s population) and contributes significantly to irrigation, and therefore to food security, in arid and semi-arid regions (Jia et al., 2019). Nevertheless, there is currently a lot of concern about groundwater pollution from nitrates (NO$_3^-$) and organic micro-contaminants (OMCs) like pesticides and veterinary pharmaceuticals (Nguyen et al., 2020). The abundance and frequency of detection of pesticides and antibiotics has increased in recent years due to the intensification of agriculture and organic soil amendment with manure or biosolids (Kurwadkar, 2017). In Europe, 13% of groundwater monitoring stations exceed the 50 mg/L nitrate limit (91/676/EEC) (EUROSTAT, 2018), reaching concentration levels as high as 500 mg/L due to intensive agricultural practices (Otero et al., 2009). Across Europe, the highest nitrate exceedance rates have been recorded in Belgium (30%), Denmark (26%), Spain (22%), and Cyprus (19%). According to a pan-European study, one or more pesticides occur at concentrations higher than 0.1 μg/L in 7% of monitored European groundwater (EUROSTAT, 2018). Although several pesticides have been identified in groundwater, atrazine and one of its metabolites, desethylatrazine, are the most frequently detected above the drinking water directive in Europe. Nevertheless, some triazine pesticides have been found in the shallow groundwater of the United States at a concentration as high as 40 μg/L (Kolpin et al., 1998).

Current drinking water treatment technologies to remove these compounds are based on separation processes, including advanced oxidation processes, reverse osmosis, ion exchange, electrochemical reduction, electrodialysis, and activated carbon adsorption (Archna et al., 2012). However, these treatments are expensive to build and operate, and they generate a concentrated brine, which poses additional treatment and disposal operations, further increasing process costs. In contrast, biological denitrification processes remove nitrate by converting it to a harmless nitrogen gas. In recent years, biological treatments for nitrate removal have been developed based on heterotrophic microorganisms, but they require the supply of labile organic carbon as an electron acceptor and the presence of specific pesticide biodegradation species (e.g., genus Caulobacteraceae) (Rezvani et al., 2019). Nature-based solutions based on microalgae or cork could solve this issue. Microalgae use sunlight to fix CO$_2$ from the atmosphere through photosynthesis (Taziki et al., 2015). Existing studies on the use of microalgae to remove nitrates from groundwater show that they can be a very effective solution, with removal efficiencies of up to 80% with an incubation time of 3 days (Rezvani et al., 2019). Recent studies from our laboratory have demonstrated that the co-immobilisation of microalgae and bacteria in polyurethane foam (PF) enhances the attenuation of nitrates, pesticides, and antibiotics from groundwater (Ferrando and Matamoros, 2020). The use of cork for the attenuation of nitrates and other pollutants from water has already been tested in various studies, but never for removing nitrates from groundwater. For example, Mallek et al. (2018) suggested that cork is a useful adsorbent for the removal of phenolic compounds and especially for the halogenated phenolic compounds PCP and 2,4-DCP, which only require 4.9 and 29 g/L of cork, respectively, to reduce their concentrations from 1 to 0.1 mg/L. In the same laboratory adsorption study, pharmaceuticals such as diclofenac and triclosan were 100% removed by using 5–10 mg of cork. However, the use of cork as a biofilter for removing nitrate and micropollutants from groundwater has never been investigated. As a consequence, combining photo-biodegradation processes in microalgae systems with biodegradation and sorption processes in cork biofilters might be extremely advantageous in terms of enhancing pollutant attenuation. Furthermore, another important gap in knowledge is the microbiological characterization of the microorganisms that drive pollutants’ biodegradation. For example, recent studies have found that Sphingomonadaceae and Caulobacteraceae genera enhance the biodegradation of certain micropollutants and nutrients from water (Oh and Choi, 2019; Xu et al., 2018).

The goal of this study is therefore to explore for the first time the effectiveness of a novel groundwater treatment based on the use of co-immobilised microalgae and bacteria in combination with a cork filter (CF) for the attenuation of nitrates, pesticides (atrazine and bromacil), and antibiotics (sulfamethoxazole and sulfacetamide) at different hydraulic retention times (HRTs). Furthermore, the study will identify and link bacterial, fungal, and microalgal populations with groundwater pollutant attenuation and the formation of micropollutant transformation products (TPs).

2. Material and methods

2.1. Chemicals and reagents

Sulfamethoxazole (SMX) and atrazine (ATZ) were provided by Fluka Analytical™, and sulfacetamide (SCM) and potassium nitrate (KNO$_3$) were purchased from Sigma–Aldrich, whereas bromacil (BMC) was obtained from PolyScience, Niles, USA and potassium dihydrogen phosphate single-phase (KH$_2$PO$_4$) from Merck KGaA. All described reagents were analytical grade (>95%). Carbon dioxide was supplied from an Alphagaz™ CO$_2$N38 brand carbon dioxide (CO$_2$) cylinder with ≤10 mg/L of O$_2$, ≤5 mg/L of CmHm and ≤10 mg/L of H$_2$O.

2.2. Experimental set-up

The prototype of the water-treatment system consisted of two main units, the photobioreactor (PBR), made of methacrylate (90 mm Øext, 84 mm Øint, 1120 mm height), and the cork filter (CF), made also of methacrylate (90 mm Øext, 84 mm Øint, 400 mm height) but covered by aluminium foil to block the light (Fig. 1). Paulmann SimplLED lights 7.5 m/20 W/12 V DC were used to light the PBR in a 12 h light/12 h dark cycle. The filling height of the PBR was set at 1 m and polyurethane foam cubes (10 × 10 mm) were introduced until the upper half of the container was filled. The PBR was inoculated with a microalgae consortium obtained from a 25 L growing container fed with groundwater and inoculated with surface water from agricultural irrigation channels (Prat de Llobregat, Barcelona, Spain) containing microalgae and bacteria. The microalgal consortium was pre-acclimatised to the growth conditions for more than 6 months before the PBR was stocked with it. Natural granulated cork (2 mm in diameter) was used to fill the CF unit, which was preacclimated with groundwater at an HRT of 8 days for six months.

The most important design parameters of the photobioreactor are CO$_2$-dose, light intensity and quality, HRT, temperature, and stirring system (Huang et al., 2017). CO$_2$ was injected at 0.03% of the total water volume of the PBR (5.8 L); the light generated 105 μmol/m$^2$s, measured inside the methacrylate reactor by means of a photodiode-mounted HD2302.0 and a LP471PHOT Probe of Lighting provided by Delta Ohm. The PBR was continuously stirred by means of a magnetic stirrer (825 rpm).
The prototype was fed with real groundwater collected from a well located in the metropolitan area of Barcelona city with a chemical composition of 60 mg/L of NO₃ and approximately 0.5 mg/L of PO₄³⁻. Groundwater was spiked with nitrates at a concentration of 200 mg/L. Furthermore, because phosphorus is a limiting factor for the growth of microalgae, KH₂PO₄ was added to the groundwater to reach a concentration of 5 mg/L of PO₄³⁻, as described by Rezvani et al. (2017). After 51 equilibration days of the prototype operating at an HRT of 8 days, pesticides (ATZ and BMC) and antibiotics (SMX and SCM) were added at a concentration of 100 μg/L for each one, and the study started (time = 0 days).

Three HRTs (8, 4, and 2 days) were tested by changing the peristaltic pump parameters. The prototype was tested in a temperature-controlled room at 23 ± 5 °C. The pH was monitored every week and maintained around 7 throughout the experiment. The water, along with the contaminants added, was changed every 7 days.

2.3. Sampling strategy

Nitrates, pesticides, and antibiotics were monitored both at the inlet and in the different intermediate sections of the prototype (effluent of the PBR and CF respectively). Samples were collected on days 10, 11, 13, 17, 20, 24, and 27 after the feeding reactor was spiked with pollutants (day 0). After that, the prototype was set to a HRT of 4 days and samples were taken at days 31, 33, 35, 38, 40, 42, 47, and 49. Finally, the prototype flowrate was modified to a HRT of 2 days and samples were collected at days 52, 55, and 57.

Microbial characterization of the prototype was performed during a single sampling campaign in the last week of its operation at an HRT of 8 days. Total bacterial, fungal, and microalgal populations were characterised by collecting 3 samples of foam material and 3 of cork material (mixture of aliquots taken at a depth of between –2 and –10 cm from the CF top) for microbiological analysis. The biomass content (microalgae, bacteria, and fungus) in the foam of the PBR was on average 4 ± 1 mg in dry weight per foam cube.

2.4. General water quality parameters and micropollutants analysis

Nitrates and nitrites concentrations in the water were analysed using Hach Lange Nitrate (LCK339 and LCK340) and Nitrite (LCK341) cell tests on a Hach Lange DR1900 Portable Spectrophotometer.

The determination of ATZ, BMC, SMX, and SCM in aqueous samples was carried out as follows. 1 mL of each water sample was filtered through a 13 mm diameter polytetrafluoroethylene (PTFE) filter with a pore size of 0.22 μm. The filtered samples were then injected into a Nexera X2 ultra high-performance liquid chromatograph (UHPLC) equipped with a photodiode array detector (SPD-M30A) (Shimadzu UK, Milton Keynes, UK). The chromatographic separation was achieved on a coresesshell Ascentis® Express RP-Amide column (10 cm × 2.1 mm, 2.7 μm particle size, Supelco, Bellefonte, USA) with a guard column (0.5 cm × 2.1 mm, Supelco, Bellefonte, USA) containing the same packing material. The flow rate and injection volume were 0.35 mL/min and 25 μL, respectively. A binary gradient elution programme consisting of mobile phase A (water with 0.1% formic acid) and mobile phase B (acetonitrile with 0.1% formic acid) was set as follows: isocratic 0–1 min: 10% of B; 1–10 min: 10–100% of B; isocratic 10–15 min: 100% of B. The column oven and autosampler temperatures were set at 25 °C and 15 °C, respectively. To visualise the UPLC-UV results, Nexera Labsolutions software was used, which is from the manufacturer (Shimadzu Corporation, Kyoto, Japan). The linearity of the analytical methodology ranged from 1 to 200 μg/L with a repeatability lower than 10%.

Additionally, samples from the PBR and CF outlets, as well as groundwater samples, were analysed in an HPLC-Orbitrap to screen for possible TPs. Full scan and all ion fragmentation (AIF) acquisition modes were used with a mass range of m/z 50–1000. AIF as the data-independent analysis was performed using Higher-energy Collisional Dissociation (HCD) fragmentation with collision energies of 10 and 60 eV, and the resolution was set at 50,000 at a scan rate of 2 Hz. Further MS specifications as well as chromatographic conditions are presented in supplementary material (SM section). A list of possible TPs was built based on literature research of previously reported TPs for each of the tested compounds. Then, their exact masses and, when available, previously reported fragments in MassBank (https://massbank.eu) were also collected. The compiled list of compounds and masses can be seen in the SM section. The gathered data was then used in TraceFinder 3.3 EFS (Thermo Fisher Scientific, Bremen, Germany) to conduct a tentative identification of TPs in the analysed samples. Molecular ions (M+1) were detected with mass accuracy below 2 ppm and qualifier fragment ions were also provided.

2.5. Microbial community assessment

The quantification of total bacteria, fungi and microalgae from PBR and CF samples was performed by using PowerSoil™ DNeasy Isolation Kit (Qiagen). Total genomic DNAs were obtained from independent triplicate samples of each material. Gene copy numbers of 16S RNA (total bacteria), ITS1 rRNA (total fungi) and the clade I nosZ gene (typical denitrifiers) were quantified by quantitative real time PCR (qPCR). The metatranscriptomic assessment of microbial populations (microalgae, bacterial and fungal populations) in the PBR and CF samples were characterised by paired-end High Throughput Sequencing (HTS) of V4 18S rRNA, V3–V4 16S rRNA and 5.8S-ITS2-28S rRNA amplicons, respectively. Raw data (R1 and R2 demultiplexed FASTQ files) from 16S RNA (bacteria), ITS2 rRNA (fungi) and V4–V4 18S RNA (microalgae and other eukaryotes) were further processed using Cutadapt and DADA2 software. Further details on microbial community assessment and data curation are described in the SM section.

2.6. Data analysis

The experimental results were analysed statistically using the SPSS v. 24.0 software (Chicago, IL, U.S.A.). Since the samples were non-parametric and independent, the Kruskal–Wallis test, and Mann–Whitney tests were used to examine differences between the removal efficiencies of micropollutants in the different HRTs evaluated and in microbial diversity indexes between materials (foam versus cork).
3. Results and discussion

3.1. Attenuation of nitrates and nitrites

Table 1 shows the attenuation of nitrates according to the different assessed HRTs. After the prototype had been operated with the same influent nitrate concentration for 50 days (200 mg/L), the nitrate removal observed with an HRT of 8 days averaged 58%. The effectiveness of each of the studied units was similar: 28% for the PBR and 30% for the CF unit. However, the attenuation of nitrate was reduced drastically to 12 and 5% at HRTs of 4 and 2 days respectively, showing a great HRT dependence.

The attenuation of nitrates by the PBR is partly or largely attributable to microalgal assimilation for growth. Microalgae are capable of transporting and reducing nitrate, to finally incorporate inorganic N in form of organic N as glutamate (Sanz-Laquè et al., 2015). Denitrification of nitrate by bacteria could also be part of the explanation, as denitrification bacteria have been identified in high abundance (see previous section). The results are also in agreement with previous studies carried out at laboratory-scale with microalgae immobilised in foam showing an attenuation of nitrates of 40–50% with an HRT of 8 days (Ferrando and Matamoros, 2020). Existing studies on the use of microalgae to remove nitrates from groundwater show that they can be a very effective solution, with removal efficiencies up to 80% with an incubation time of 3 days (Rezvani et al., 2019). Fierro et al. (2008) observed that Chitosan immobilisation of Scenedesmus sp. cells resulted in a 70% nitrate removal within 12 h, at a rate significantly higher than free-living cells (20% nitrate removal within 36 h of treatment). The effectiveness of our immobilised consortium in foam, including microalgae, bacteria, and fungi was lower, but this difference can be accounted for by the fact that the studies were conducted in continuous operational mode, whereas the other studies were performed in batch.

The attenuation of nitrates in the CF (30% at a HRT of 8 days) can be mainly explained by the presence of denitrifying bacteria in the biofilm. Our results suggest less effective bioremediation than that achieved by Aguilar et al. (2019), who observed that cork media used in constructed wetlands are very effective for reducing nitrates from agricultural run-off water (80–90% attenuation). This difference probably reflects the source of the water: whereas we were operating the plant with groundwater spiked at 200 mg/L of nitrates, Aguilar et al. treated agricultural run-off containing very low concentrations of nitrates but containing other nutrients and organic matter, which will have enhanced biofilm development.

At the end of the treatment (HRT = 8 days), the quality of the water was still slightly greater than the regulatory limit (50 mg/L) for water intended for human consumption (91/676/EEC). Nevertheless, taking into consideration the effectiveness of the assessed technology, it can be a suitable solution for nitrate polluted groundwater with a nitrate content of up to 100 mg/L.

Regarding nitrates (Table 1), their concentration was reduced by 89% at an HRT of 8 days. This reduction was due to the PBR, with only 2% being removed in the CF. Reduction of the HRT to 4 days resulted in a reduction of the nitrate attenuation by 50% in the PBR, but led to the production of nitrite in the CF, so that overall there was no N removal. Further reduction of the HRT to 2 days, resulted in a 37% decrease in the removal of nitrate and a final overall production of nitrate of 1.9 mg/L. Whereas nitrite removal in the PBR can be explained by microalgae assimilation, bacterial denitrification is suggested as the main mechanism in cork. The nitrite occurrence in the cork filter at low HRT (4 and 2 days) may suggest that the bacterial denitrification process could be hampered by nitrite accumulation (Rajta et al., 2020), due to partial denitrifying processes that could be related to a lower production of C-labile compounds from cork under low HRT.

3.2. Attenuation of pesticides and antibiotics

Fig. 2 shows the concentrations of pesticides and antibiotics in each of the sampling sites (reservoir tank, PBR outlet, and CF outlet) over time at an HRT of 8 days. In the case of the PBR, it can be shown that whereas antibiotic depletion over time was constant (around 50%), the attenuation of pesticides decreased (from 50–66% to 21–35%). These findings suggest that pesticides may accumulate in the foam biofilm until they reach biofilm sorption capacity or that their presence may have an adverse effect on microbial communities, reducing their effectiveness for removing pesticides. Nevertheless, taking into account that the selected pesticides are highly polar (log D<sub>ow</sub> < 2 at pH = 7–8), no sorption into the biomass can be hypothesized. In fact, in previous studies (Ferrando and Matamoros, 2020) we observed that pesticide adsorption to the polyurethane foam was negligible and that assimilation into microalgae was very low (Matamoros and Rodriguez, 2016), suggesting that biodegradation is the main attenuation mechanism.

Regarding antibiotics, our results again agree with previous studies, which have indicated that photodegradation is the most relevant attenuation process in microalgal systems (de Godos et al., 2012; Ferrando and Matamoros, 2020). In contrast to the PBR foam, the concentrations of antibiotics and pesticides at the outlet of the CF were very constant over time, showing no time dependence (between 1 and 2 μg/L depending on the OMCs). The attenuation efficiency of selected OMCs by the cork system was greater than 90% (92–99%). This in agreement with previous laboratory sorption studies that indicate that cork has a great capacity for removing phenolic compounds, especially halogenated phenolic compounds such as PCP and 2,4-DCP, which only require 4.9 and 29 g/L of cork, respectively, to reduce their concentrations from 1 to 0.1 mg/L. de Aguiar et al. (2019) also observed that cork has a great bioadsorption capacity for atrazine and other pesticides from water spiked at 10 μg/L, showing average removal efficiencies of between 60% and 70% following 360 min of incubation at pH 7. Malek et al. (2018) demonstrated that pharmaceuticals such as diclofenac and triclosan at a concentration of mg/L were 100% removed by sorption to cork. New insights into the interactions between cork chemical components and pesticides indicate that lignin moieties are the main components involved in the sorption process (Olivella et al., 2015). Hence, although previous sorption studies have suggested that cork could be used for removing OMCs, our results demonstrate, for the first time, that biofilters filled with cork are a practical solution for removing OMCs from groundwater.

The decrease of the HRT to 4 and 2 days resulted in a significant reduction in the attenuation of selected micropollutants by the prototype (Table 2). Nevertheless, whereas the reduction in attenuation by
the PBR was from 47% to 20% and finally to 13% (on average) as the HRT was reduced from 8 days to 2 days, whereas no or a slight reduction in the attenuation of antibiotics (SMX and SM) was observed. Nevertheless, no atrazine attenuation was observed in free microalgae reactors operated at HRTs of 8, 4 or 2 days (Matamoros and Rodríguez, 2020); for example, the attenuation of atrazine and bromacil decreased from 51% to 75%–31% and 57%, respectively, when the HRT was reduced from 8 days to 2 days, whereas no or a slight reduction in the attenuation of antibiotics (SMX and SM) was observed.

Table 2
Percentage of attenuation of pesticides (BMC and ATZ) and antibiotics (SCM and SMX) in each of the prototype units (PBR and CF). In parenthesis: the attenuation of microcontaminants considering only the CF.

| Compound Name | Section | HRT = 8d (%) | HRT = 4d (%) | HRT = 2d (%) |
|---------------|---------|--------------|--------------|--------------|
| BMC           | PBR     | 34 ± 13 (a)  | 7 ± 2 (b)    | 1 ± 1 (c)    |
|               | CF      | 64 ± 12 (97 ± | 73 ± 5 (80 ±6 | 34 ± 1 (35 ± |
|               |         | 12) (a)      | (a)          | (a)          |
|               | Total   | 98 ± 9 (9)   | 80 ± 5 (b)   | 45 ± 1 (c)   |
| ATZ           | PBR     | 50 ± 11 (a)  | 16 ± 6 (b)   | 6 ± 2 (c)    |
|               | CF      | 48 ± 11 (95 ±| 50 ± 15 (66 ±| 17 ± 4 (18 ±|
|               |         | 8) (a)       | (a)          | (a)          |
|               | Total   | 97 ± 8 (a)   | 66 ± 4 (b)   | 23 ± 3 (c)   |
| SCM           | PBR     | 49 ± 9 (a)   | 26 ± 2 (b)   | 14 ± 1 (c)   |
|               | CF      | 50 ± 8 (96 ±2| 71 ± 2 (97 ±1| 83 ± 2 (96 ±|
|               |         | (a)          | (a)          | (a)          |
| SMX           | PBR     | 53 ± 7 (a)   | 29 ± 3 (b)   | 32 ± 2 (b)   |
|               | CF      | 46 ± 7 (98 ±3| 61 ± 2 (89 ±4| 32 ± 4 (48 ±|
|               |         | (a)          | (b)          | (a)          |
|               | Total   | 99 ± 5 (a)   | 90 ± 4 (b)   | 64 ± 4 (c)   |

Different letters reflect statistical differences between HRTs (p-value<0.05).

the sorption process taking place in the CF. Previous studies carried out with co-immobilised microalgae in foam at a laboratory scale showed a similar reduction in the attenuation of micropollutants (Ferrando and Matamoros, 2020); for example, the attenuation of atrazine and bromacil decreased from 51% to 75%–31% and 57%, respectively, when the HRT was reduced from 8 days to 2 days, whereas no or a slight reduction in the attenuation of antibiotics (SMX and SM) was observed.

3.3. Identification of TPs

Water samples from the system operating at a HRT of 4 days were collected for the identification of TPs. No TPs were detected following the PBR treatment. In contrast, CF treatment resulted in peaks that matched the m/z of two atrazine TPs (2-hydroxyatrazine and atrazine desethyl) and one bromacil TP (5-bromo-3-sec-butyl-6-hydroxy-methyluracil). All identified peaks had a mass accuracy of below 0.6 ppm. The formation of TPs in the CF was expected because this is where the increase in hydraulic loading rate from 0.3 m/d to 3 m/d only reduced the attenuation of atrazine by half (from 61% to 31%) and for fenitrothion and diazinon from 90 to 89% and 87%–84%, respectively.

In the present study, the overall attenuation of antibiotics and pesticides was greater than 97% at a HRT of 8 days, and it decreased slightly at HRTs of 4 and 2 days (Table 2), highlighting the high effectiveness of the combined use of microalgae and cork biofilter for removing polar pesticides and antibiotics from groundwater.

Fig. 2. Evolution of antibiotics and pesticides concentration over time at an HRT of 8 days. Groundwater (GW), photobioreactor (PBR), and cork filter (CF). Sulfacetamide (SCM), sulfamethoxazole (SMX), bromacil (BMZ), and atrazine (ATZ).
often identified in soil or biofilters (Lin et al., 2008) (Ulrich et al., 2017). Another TP is 5-bromo-3-sec-butyl-6-hydroxymethyluracil, which has been registered as a bromacil metabolite in aerobic soil conditions (Lewis et al., 2016) and is also reported as a major bromacil metabolite in plant roots (Jordan and Clerx, 1981). These results confirm that biodegradation of pesticides took place in the CF.

### 3.5. Microbial community assessment

In comparison with other available nitrate-removal technologies such as ion exchange, electrochemical reduction, electrodiagnosis, and activated carbon adsorption (Archena et al., 2012), the nitrate attenuation achieved by the microalgae–cork prototype was only moderate. Nevertheless, the technology developed here has the advantage that there is no need for external energy (membrane-based solutions) or organic matter addition (biological denitrification treatments). Furthermore, future optimization of the prototype, such as the use of other microalgae immobilisation materials (de-Bashan and Bashan, 2010) or the replacement of the cork by wood chips or wheat straw, may increase its effectiveness for removing nitrates (Sailing et al., 2007; Schipper et al., 2010). Finally, the prototype was very effective for removing OMCs, achieving values comparable to those found in membrane groundwater technologies (Plakas and Karabelas, 2012) and greater than those found in rapid sand filtration systems (Hedgegaard and Albrechtsen, 2014).

### 3.5.1. Microalgae population

The floating polyurethane foam cubes were rapidly colonised by visible microalgae biomass. Microscopical examination (Fig. S1) showed that most of the microalgae present were colonial and unicellular green algae, many of which could not be confidently identified at species level, and sometimes even at a genus level. The V4-18S rRNA metabarcoding data gave a much clearer picture of community composition. Both microalgal diversity and richness were quite similar in the floating foam material in the PBR and in the CF (Mann–Whitney, P > 0.05) (Table 3). Microalgal diversity was lower than the bacterial diversity in both materials (microalgae, bacteria, and fungus) is provided in the SM section. In the PBR-cork system.

### 3.5.2. Bacterial diversity

Both bacterial diversity and richness were significantly higher (Mann–Whitney, p < 0.05) in the CF (H: 5.58 ± 0.08, and Chao1: 610.9 ± 3.5) than in the PBR foam (H: 3.86 ± 0.11 and Chao1: 237.61 ± 19.1) (Table 3). Interestingly, bacterial biomass (measured as 16S RNA copies) was abundant and comparable in both materials (t-Test, p = 0.309), accounting for 4–6 × 10^8 16S RNA copies g^-1 (Fig. S2). The bacterial population in CF was statistically more diverse in terms of alpha diversity and in the number of taxonomic groups (at the family level), but not significantly at ASV level (beta diversity, ANOSIM, R = 1, p = 0.1). Fig. 4 shows that the main assigned genera that were significantly abundant in the PBR foam based on LDA (LefSe analysis, Kruskal-Wallis, p < 0.05, LDA score above 4.5) (Fig. S4; Table SS) were Rhodanobacter and Rhodopsuedomonas: the first one is a Gamma-proteobacteria and comprised 10.6% of the reads, whereas the second one is an Alphaproteobacteria and comprised 5.6% of the reads. It is noteworthy that these genera have been previously described as denitrifying bacteria (Dunstan et al., 1982; Gao et al., 2021; Lee et al., 2002; Liu et al., 2020). Furthermore, their abundance could be related to the high nosZ gene population detected by qPCR in the foam material (Fig. S2). Therefore, we suggest that denitrifying activity could take place in the inner parts of the foam biofilms where oxygen would be less available.

In the PBR foam, the dominating taxa was Alphaproteobacteria class (Bradyrhizobiaceae, Sphingomonadaceae, Phyllobacteriaceae, Caulobacteriaceae, and Rhizobiaceae genus) (Fig. S5). For example, recent studies have observed that Sphingomonadaceae and/or Caulobacteriaceae enhance the biodegradation of certain micropollutants and nutrients from water (Oh and Choi, 2019; Xu et al., 2018). Conversely, the most abundant genus in the CF was Simplicispira (Betaproteobacteria, with 6.2% relative abundance), which has recently been described as containing several denitrifying species (Siddiqi et al., 2020). In another recent study, Simplicispira spp. were highly enriched in sequencing batch reactors exposed to synthetic wastewater containing antibiotics such as trimethoprim/sulfadiazine (Krugova et al., 2019). Simplicispira did also occur in the PBR foam, at relative abundances of 1–3%. A further noteworthy aspect is the common occurrence in the CF of Phenyllobacterium (Alphaproteobacteria), Asproborah (Alphaproteobacteria), Actinomarinica (Actinobacteria), and Steroidobacter (Gammaproteobacteria), all of which could also be related to the degradation of micropollutants. Recent studies, such as Liao et al. (2016) and Espin et al. (2020) have described Phenyllobacterium as a ciprofloxacin- and atrazine-degrading bacterium. The denitrifier Steroidobacter (2% of relative abundance in CF) has been correlated positively with sulphonamide degradation (Zhang et al., 2021) including the family Lamiaceae (3% of relative abundance in CF), which includes the genus Actinomarinica, already mentioned above.

The constant lighting received by the PBR foam and the availability of O_2 in excess, due to photosynthesis, could hamper the denitrifying activities of such high facultative denitrifying populations. In contrast, the absence of light and lower availability of O_2 (in the deepest regions of biofilms) in the interstitial water among cork particles in the CF could lead to bacterial denitrifying activity (especially high in TRH-8 days). Total denitrifying bacterial populations were higher in the PBR foam (t-test, p = 0.03), achieving values of 9 × 10^8 nosZ copies g^-1 compared to

| Sampling point | Shannon | Inv. Simpson | Richness | Richness (Chao 1) |
|---------------|---------|--------------|----------|------------------|
| Foam-PBR      |         |              |          |                  |
| (Bacteria)    | 3.86 ± 0.11 | 18.03 ± 18.03 | 197.97 ± 197.97 | 237.60 ± 237.60 |
| Cork Filter   | 5.58 ± 0.08 | 121.73 ± 121.73 | 579.46 ± 579.46 | 610.9 ± 610.9 |
| (Bacteria)    | 5.58 ± 0.08 | 121.73 ± 121.73 | 579.46 ± 579.46 | 610.9 ± 610.9 |
| Foam-PBR      | 0.74 ± 0.66 | 2.03 ± 2.03 | 3.67 ± 3.67 | 5.00 ± 5.00 |
| (Fungi)       | 3.13 ± 0.90 | 7.36 ± 7.36 | 124.67 ± 124.67 | 124.67 ± 124.67 |
| Cork Filter   | 1.34 ± 0.16 | 1.77 ± 1.77 | 58.12 ± 58.12 | 64.91 ± 64.91 |
| (Microalgae)  | 1.31 ± 0.05 | 1.98 ± 1.98 | 45.64 ± 45.64 | 63.18 ± 63.18 |
| Cork Filter   | 1.34 ± 0.16 | 1.77 ± 1.77 | 58.12 ± 58.12 | 64.91 ± 64.91 |

Different letters, for each index and kingdom and materials, reflects statistical differences between materials.
the values in the CF, even though these were also high (5.7 × 10^7 nosZ copies g⁻¹).

In this work, functional gene copy numbers of the nosZ clade I were used to assess the abundance of denitrifying populations because typical denitrifiers are known to reduce N₂O to nitrogen gas at different rates (Hallin et al., 2018). Also, it is described that clade I nosZ consists almost exclusively of the Alpha-, Beta-, and Gammaproteobacteria classes. Considering qPCR and NGS results, we can conclude that both bio-reactors possessed high denitrification capacity, not only because of the nosZ gene copy numbers, but also because the predominant bacterial classes have denitrification potential. Nevertheless, although both systems have a high denitrification capacity, our results show that nitrate attenuation is low (20–60%). This is in agreement with recent findings stating that the transcription rate of denitrification genes depends strongly on environmental conditions (Chon and Cho, 2015), and therefore, having a high oxygen concentration in both systems would reduce it. Consequently, future studies should explore the denitrification effectiveness of the CF operated under oxygen-limiting conditions (water-saturated columns).

3.5.3. Fungal diversity

Our results indicate a high fungal diversity in the CF, probably due to the high organic content of the cork material and cell debris (Table 3). Fungal populations in the CF (Fig. 5) were dominated by Humicola nigrescens (30% of relative abundance). This belongs to the Sordariomycetes, and some genera in this group are producers of phytase to improve phosphorus bioavailability from organic matter (Bala et al., 2014). Also, unclassified Ascomycota (10%), unclassified Chaetomiaceae (8%, Sordariomycetes), Penicillium sp. (3%) and Coniarium duplumascospora (3%) were abundant. It is noteworthy that Coniarium duplumascospora has been shown to play an important role in degrading woody debris and leaves in submerged freshwater environments (Liu et al., 2012). The fungal community in the filter was diverse and could be linked to the utilisation of cork material together with cell debris from detached PBR biofilms that flow to the filter acting as periphyton (a mixture of algae, cyanobacteria, heterotrophic microbes, and detritus attached to solid substrates that are able to release C-labile exudates in aquatic ecosystems). Microbial interactions in the CF could produce additional degradable organic matter from the cork, contributing to additional denitrification processes (Mendonça et al., 2004). Cork material could initially be metabolised by fungi and bacteria, and degradable organic matter could be further used as an electron source by denitrifying microbiota, which have been detected as highly abundant both in PBR and CF.

Previous studies have identified that the Ascomycota (the predominant phylum in the CF) encompasses species (besides Basidiomycota) that also possess ligninolytic enzymes such as laccase (EC 1.10.3.2) (Osono, 2020). Laccase is a well-known enzyme with a high pollutants biodegradation capacity, especially for recalcitrant ones (García-Delgado et al., 2018; Medaura et al., 2021). For example, Cupul et al. (2014) performed enzymatic assays on Basidiomycota ligninolytic fungi with atrazine and observed an increase in laccase activity and atrazine removal. Furthermore, Espanza-Naranjo et al. (2021) showed that different lignin-degraders isolated from leaf litter (such as Fusarium sp., Ascomycota phylum) were also able to degrade atrazine without expressing laccase activity. These findings revealed the importance of other enzymatic capacities that deserve attention, such as cytochrome P450 monooxygenase or unspecific peroxygenases (UPOs), where the latter were studied in sulfonamide degradation by Basidiomycota UPOs (Łeńska et al., 2021).

Overall, our results suggest that isolated bacterial communities from PBR and CF would have high denitrification capacity, but our results indicate that they were only capable of removing nitrates moderately, with removal efficiencies of around 58% at a HRT of 8 days. This is in agreement with the fact that although denitrification capacity existed, bacteria were not capable of performing it due to the predominant aerobic conditions of both systems. Nevertheless, the presence of bacteria and fungus with a high demonstrated capacity for removing micropollutants is in agreement with the high effectiveness observed by both the PBR-CF systems for removing pesticides and antibiotics (>95% at a HRT of 8 days).

4. Conclusions

The results of the study show that the combined use of microalgae and cork filtration is effective for the treatment of groundwater...
Fig. 4. Relative abundances of taxonomically assigned bacterial reads, at class level (above) and genus level (below), in the photobioreactor (PBR) foam and the cork filter of the NDN bioreactor. Relative abundance was defined by the number of reads (sequences) affiliated with any given taxon, divided by the total number of reads per sample. Phylogenetic groups with relative abundance >1% and >2% were categorized as ‘Others’.

Fig. 5. Relative abundance of taxonomically assigned fungal reads, at species level, in the photobioreactor (PBR) foam and the cork filter of the NDN bioreactor. Relative abundance was defined by the number of reads (sequences) affiliated with any given taxon, divided by the total number of reads per sample. Phylogenetic groups assigned to the Fungi with relative abundances >0.5% were categorized as ‘Others’. ‘Unclassified species’ include non-fungal reads, including microalgae.
contaminated by nitrates, and tested pesticides and antibiotics. The main results and key conclusions can be summarised as follows:

- The PBR-CF prototype removes nitrates (58%) and nitrates (89%) at an HRT of 8 days, but it fails at lower HRT (<20%).
- The combined use of PBR and CF enabled attenuation of antibiotics and pesticides up to 95% at an HRT of 8 days, but this decreased with decreasing HRT, with pesticides being the compounds most affected (changing from 97 to 98% attenuation to 23–45% with a reduction of the HRT from 8 to 2 days). We hypothesise that the release of C-labile molecules from PBR and cork material can promote denitrification and micropollutant degradation.
- The identification of atrazine and bromacil TF in CF outputs indicated that biodegradation was the main attenuation process.
- The most abundant microbiological species were the green alga Tetraselmis, in both the PBR and the CF. Nevertheless, molecular analysis confirmed that both bioreactors were enriched in denitrifying populations able to perform denitrification.
- The CF had more bacterial and fungal diversity than the PBR, indicating a higher potential for pollutant biodegradation. The attenuation of micropollutants was linked to the presence of certain microorganism genera and species.

The results are very promising. However, the low efficiency of the system in terms of nitrate attenuation at low HRT values will require further studies, such as testing other materials for both microalgae immobilisation and improving the biofilter system.

Credit author statement

Lorenzo Rambaldo: Investigation, Data curation, Writing – original draft preparation. Héctor Avila: Investigation, Data curation, Writing – original draft preparation. Monica Escolla: Data curation, Writing-Reviewing and Editing. Miriam Guivernau: Investigation, Data curation, Writing – original draft preparation. Marc Vinas: Writing-Reviewing and Editing. Rosa Trobajo: Writing- Reviewing and Editing. Javier Pérez-Burillo: Writing- Reviewing and Editing. David G. Mann: Writing- Reviewing and Editing. Belén Fernández: Writing- Reviewing and Editing. Carmen Biel: Funding acquisition, Writing- Reviewing and Editing. Luigi Rizzo: Writing- Reviewing and Editing. Josep M. Bayonas: writing- Reviewing and Editing. Carme Biel: Funding acquisition, Writing- Reviewing and Editing. Josep M. Bayonas: writing- Reviewing and Editing. V. Matamoros: Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing 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.

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Appendix A. Supplementary data

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References

Aguilar, L., Gallegos, A., Arias, C.A., Ferreraz, I., Sánchez, O., Rubio, R., Saad, M. Ben, Misogia, B., Caro, P., Sahabouh, S., Pérez, C., Morató, J., 2019. Microbial nitrate removal efficiency in groundwater polluted from agricultural activities with hybrid cork treatment wetlands. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2018.10.436.

Arbela Sharma, S.K., Sobel, R.C., 2012. Nitrate removal from ground water: a review. J. Chem. https://doi.org/10.1016/j.jbcb.2014.07.002.

Bala, A., Sapna Jain, J., Kumari, A., Singh, B., 2014. Production of an extracellular phytase from a thermophilic mould Humicola nigrescens in solid state fermentation and application in dephytinization. Bioresour. Biotechnol. https://doi.org/10.1155/2014/593646.

Chon, K., Cho, J., 2015. Abundance and expression of denitrifying genes (narG, nirS, norB, and nosZ) in sediments of wastewater stabilizing constructed wetlands. Environ. Eng. Res. https://doi.org/10.4314/eer.v2015.09.004.

Cupul, W.C., Abarca, G.H., Vázquez, R.R., Salomones, D., Hernández, R.G., Gutierrez, E.A., 2014. Response of ligninolytic macrofungi to the herbicide atrazine: dose-response bioassays. Rev. Argent. Microbiol. https://doi.org/10.1053/57515470994.

de-Bashan, L.E., Bashan, Y., 2010. Immobilized microalgae for removing pollutants: review of practical aspects. Biosens. Technol. https://doi.org/10.1016/j.bite.2009.09.043.

de Aguiar, T.R., Guimaraes Neto, J.O.A., Şen, U., Pereira, H., 2019. Study of two cork species as natural biosorbents for five selected pesticides in water. Heliyon. https://doi.org/10.1016/j.heliyon.2019.e01886.

de Godos, I., Muñoz, R., Guéynez, B., 2012. Tetracycline removal during wastewater treatment in high-rate algal ponds. J. Hazard Mater. https://doi.org/10.1016/j.jhazmat.2012.05.106.

Dunstan, R.H., Kelley, R.C., Nicholas, D.J.D., 1982. Fixation of dinitrogen derived from denitrification of nitrate in a photosynthetic bacterium, Rhodopseudomonas sphaeroides forma sp. denitrificans. J. Bacteriol. https://doi.org/10.1128/jb.150.1.104-1982.

Eparzar-Naranjo, S.B., da Silva, G.F., Duque-Castaito, D.C., Araújo, W.L., Peres, C.K., Boroski, M., Burugli-Santos, R.C., 2021. Potential for the biodegradation of atrazine using leaf litter fungi from a subtropical protection area. Curr. Microbiol. https://doi.org/10.1007/s00284-020-02288-6.

Epin, Y., Arazualla, G., Alvarez-Oritz, M., Gómez-Alaydi, J.J., 2020. Microbial community and atrazine-degrading genetic potential in deep zones of a hypersaline Lake-Aquifer system. Appl. Sci. https://doi.org/10.3390/app10072111.

EUROSTAT, 2018. Archive: Agri-environmental indicator – pesticide pollution of water [WWW Document]. URL https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Archive:Agri-environmental_indicator_-_pesticide_pollution_of_water.

Fernando, L., Matamoros, V., 2020. Attenuation of nitrates, antibiotics and pesticides from groundwater using immobilised microalgae-based systems. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2019.134740.

Fierro, S., del Pilar Sánchez-Saavedra, M., Copacel, C., 2008. Nitrates and phosphate removal by chitosan immobilized Scenedesmus. Biosens. Technol. https://doi.org/10.1016/j.biotech.2007.02.043.

Gao, Y., Manía, D., Mourav, S.A., Lycus, P., Arotsanz, M., Wolisz, K., Lindström, K., Šanchevich, J.P., Ihkken, L.R., Frostegård, Å., 2021. Competition for electrons favours N2O reduction in denitrifying Bradyrhizobium isolates. Environ. Microbiol. https://doi.org/10.1111/1462-2920.15404.

García-Delgado, C., Eymar, E., Camacho-Artivalío, R., Petruccelli, M., Crognaele, G., Gómez Zapata, E., Alonso, A., 2018. Degradation of tetracyclines and sulfonylamides by stevensite-and biochar-immobilized laccase systems and impact on residual antibiotic activity. J. Chem. Technol. Biotechnol. https://doi.org/10.1002/jctb.5697.

Hallin, S., Philipps, L., Leifler, F.E., Sanford, R.A., Jones, C.M., 2018. Genomics and ecology of novel N2O-reducing microorganisms. Trends Microbiol. https://doi.org/10.1016/j.trendsmicrobio.2017.07.003.

Hedegaard, M.J., Albrechtsen, H.J., 2014. Microbial pesticide removal in rapid sand filters for drinking water treatment - potential and kinetics. Water Res. https://doi.org/10.1016/j.watres.2013.09.024.

Huang, Q., Jiang, F., Wang, L., Yang, C., 2017. Design of photobioreactors for mass cultivation of photosynthetic organisms. Engineering 3, 318–329. https://doi.org/10.1016/j.ETYPE.2017.01.020.

Jia, X., Connor, D.O., Hou, D., Jin, Y., Li, G., Zheng, C., Sik, Y., Tsang, D.C.W., Luo, J., 2019. Science of the Total Environment Groundwater depletion and contamination : science of the total environment. Environ. Res. https://doi.org/10.2175/106143017x15023776270584.
Lee, D.Y., Ramos, A., Macomber, L., Shapleigh, J.P., 2002. Taxis response of various denitrifying bacteria to nitrate and nitrite. Appl. Environ. Microbiol. https://doi.org/10.1128/AEM.68.5.2140-2147.2002.

Lemataku, N., Felis, E., Poraj-Kobielska, M., Gajda-Meissner, Z., Hofrichter, M., 2021. Comparison of sulfonamides decomposition efficiency in ozonation and enzymatic oxidation processes. Arch. Environ. Protect. https://doi.org/10.24425/aep.2021.136443.

Lewis, K.A., Tziliakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. Hum. Ecol. Risk Assess. 22, 1050–1064. https://doi.org/10.1080/10807079.2015.1135242.

Liao, X., Li, B., Zou, R., Dai, Y., Xie, S., Yuan, B., 2016. Biodegradation of antibiotic ciprofloxacin: pathways, influential factors, and bacterial community structure. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-016-6054-1.

Liu, F., Hu, D.-M., Cai, L., 2012. Conlarium duplumascospora gen. et. sp. nov. and Jobellisia guangdongensis sp. nov. from freshwater habitats in China. Mycologia 104, 1178–1186. https://doi.org/10.3852/11.379.

Liu, T., He, X., Jia, G., Xu, J., You, S., 2020. Simultaneous nitrification and denitrification process using novel surface-modified suspended carriers for the treatment of real domestic wastewater. Chemosphere. https://doi.org/10.1016/j.chemosphere.2020.125831.

Mallek, M., Chtourou, M., Portillo, M., Monclús, H., Walha, K., Salah, A. ben, Salvado, V., 2018. Granulated cork as biosorber for the removal of phenol derivatives and emerging contaminants. J. Environ. Manag. https://doi.org/10.1016/j.jenvman.2018.06.069.

Matamoros, V., Franco, J., 2018. Assessing the use of sand, peat soil, and pine bark for the attenuation of polar pesticides from agricultural run-off: a bench-scale column experiment. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-018-2213-x.

Matamoros, V., Rodríguez, Y., 2016. Batch vs continuous-feeding operational mode for detoxification of cork boiling wastewaters. Eng. Life Sci. https://doi.org/10.1002/elsc.200420018.

Olivella, M.A., Bazzicalupi, C., Bianchi, A., Fiol, N., Villaescusa, I., 2015. New insights into the interactions between cork chemical components and pesticides. The contribution of n-n interactions, hydrogen bonding and hydrophobic effect. Chemosphere. https://doi.org/10.1016/j.chemosphere.2014.08.051.

Osono, T., 2020. Functional diversity of ligninolytic fungi associated with leaf litter decomposition. Ecol. Res. https://doi.org/10.1111/1440-1703.12063.

Otero, N., Torrento, C., Soler, A., Mencio, A., Masi-Pla, J., 2009. Monitoring groundwater nitrate attenuation in a regional system coupling hydrogeology with multi-isotopic methods: the case of Plana de Vic (Osona, Spain). Agric. Ecosyst. Environ. https://doi.org/10.1016/j.agee.2009.05.007.

Plakas, K.V., Karabelas, A.J., 2012. Removal of pesticides from water by NF and RO membranes - a review. Desalination. https://doi.org/10.1016/j.desal.2011.08.003.

Rajta, A., Bhatia, R., Setia, H., Pathania, P., 2020. Role of heterotrophic aerobic denitrifying bacteria in nitrate removal from wastewater. J. Appl. Microbiol. https://doi.org/10.1111/jam.14476.

Razvanzi, F., Sarrafzadeh, M.H., Ebrahimi, S., Oh, H.M., 2019. Nitrate removal from drinking water with a focus on biological methods: a review. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-017-9185-0.

Razvanzi, F., Sarrafzadeh, M.H., Seo, S.H., Oh, H.M., 2017. Phosphorus optimization for simultaneous nitrate-contaminated groundwater treatment and algae biomass production using Ettlia sp. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2017.08.053.

Rugnini, L., Ellwood, NTW, Costa, G., Falsetti, A, Congestri, R, Bruno, L, 2019. Scaling-up of wastewater bioremediation by Tetradesmus obliquus, sequential bio-treatments of nutrients and metals. Ecotoxicol. Environ. Saf. 172, 59–64. https://doi.org/10.1016/j.ijecoen.2019.01.059.

Salling, W.J.B., Westerman, P.W., Losordo, T.M., 2007. Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. Aquacult. Eng. https://doi.org/10.1016/j.aquaceng.2007.06.003.

Sanz-Luque, E., Chamizo-Ampuola, A., Llanas, A., Galvan, A., Fernandez, E., 2015. Understanding nitrate assimilation and its regulation in microalgae. Front. Plant Sci. Schipper, L.A., Robertson, W.D., Gold, A.J., Jaynes, D.B., Cameron, S.C., 2010. Denitrifying bioreactors-An approach for reducing nitrate loads to receiving waters. Ecol. Eng. https://doi.org/10.1016/j.ecoeng.2010.04.008.

Siddiqui, M.Z., Sok, W., Choi, G., Kim, S.Y., Wee, J.H., Im, W.T., 2020. Simplicissima hankongyi sp. nov., a novel denitrifying bacterium isolated from sludge. Antonie van Leeuwenhoek. Int. J. Gen. Mol. Microbiol. https://doi.org/10.1007/s10483-019-01341-4.

Taziki, M., Ahmadzadeh, H., Murry, A., Lyon S, M.R., 2015. Nitrate and nitrite removal from wastewater using algae. Curr. Biotechnol. https://doi.org/10.2174/221155010466150828193607.

Ulrich, B.A., Vignola, M., Edgehouse, K., Werner, D., Higgins, C.P., 2017. Organic carbon amendments for enhanced biological attenuation of trace organic contaminants in biochar-amended stormwater biofiltrers. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.7b01164.

Wackett, L., Sadowsky, M., Martinez, B., Shapir, N., 2002. Biodegradation of atrazine and related s-triazine compounds: from enzymes to field studies. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-001-0862-y.

Xu, X.J., Lai, G.L., Chi, C.Q., Zhao, J.Y., Yan, Y.C., Nie, Y., Wu, X.L., 2018. Purification of chlorpyrifos: pathways, influential factors, and bacterial community structure. J. Environ. Qual. https://doi.org/10.2134/jeq2006.0503.

Zhang, G., Zhao, Z., Yin, X.A., Zhu, Y., 2021. Impacts of biochars on bacterial community and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae Chlorella minutissima UTEX2341. Bioresour. Technol. 175, 537–544. https://doi.org/10.1016/j.biortech.2017.08.053.