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Biochar from co-pyrolysis of biological sludge and sawdust in comparison with the conventional filling media of vertical-flow constructed wetlands for the treatment of domestic-textile wastewater

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ABSTRACT

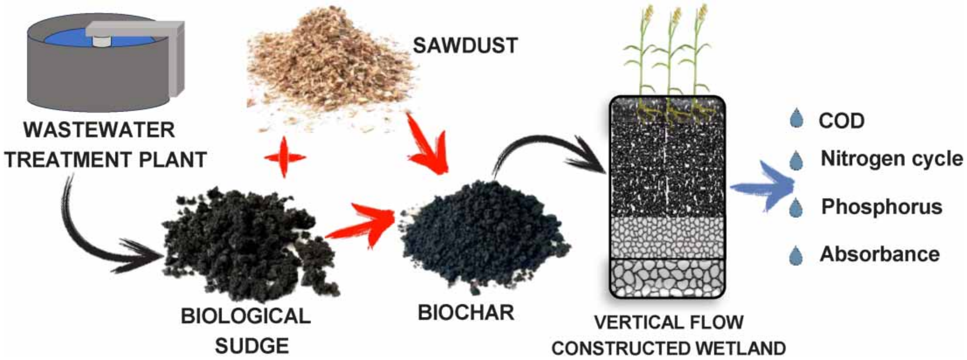
A biochar from co-pyrolysis of a mixture of sawdust and biological sludge (70/30, w/w), providing a high environmental compatibility in terms of water leachable polycyclic aromatic hydrocarbons and inorganic elements, together with a remarkable surface area (389 m^2/g), was integrated into laboratory-scale vertical-flow constructed wetlands (VF-CWs), planted with *Phragmites australis* and unplanted. Biochar-filled VF-CWs have been tested for 8 months for the refining of effluents from the tertiary clariflocculation stage of a wastewater treatment plant operating in a mixed domestic-industrial textile context, in comparison with systems filled with gravel. VF-CW influents and effluents were monitored for chemical oxygen demand (COD), nitrogen and phosphorus cycles, and absorbance values at 254 and 420 nm, the latter as rapid and reliable screening parameters of the removal of organic micropollutants containing aromatic moieties and/or chromophores. Biochar-based systems provided a statistically significant improvement in COD ($\Delta = 22\%$) and ammonia ($\Delta = 35\%$) removal, as well as in the reduction of UV–Vis absorbance values ($\Delta = 32$–34% and $\Delta = 28\%$ for 254 and 420 nm, respectively), compared to gravel-filled microcosms. The higher removal of organic was mainly attributed to the well-known adsorption properties of biochars, while for nitrogen the biological mechanisms seem to play a predominant role.

Key words: agricultural reuse, ammonia, chemical oxygen demand, nitrate, organic micropollutants, urban-textile wastewater

HIGHLIGHTS

- Biochar from sawdust and sludge 70/30 (w/w) was produced and characterized.
- This biochar was used as filling media of vertical-flow constructed wetlands (CWs).
- Planted and unplanted CWs filled with biochar were compared to gravel-based CWs.
- Organic matter, UV–Vis absorption, nitrogen and phosphorus cycles were monitored.
- Biochar-based CWs showed 22–35% higher removal, depending on the parameter studied.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Constructed wetlands (CWs) are well-established biological wastewater treatment systems, particularly suitable in case of domestic wastewater from small communities (Valipour & Ahn 2016). Depending on the direction of the wastewater flow, CW systems have been implemented according to two main design approaches, namely horizontal flow and vertical flow constructed wetlands (VF-CWs), both characterized by a subsurface flow of wastewater through a medium filling the CW bed. Moreover, VF-CWs can be fed according to different approaches, which mainly include (i) the tidal flow consisting in the sequential cycle of a filling phase and a drained phase, (ii) the down-flow with alternate percolation/resting operation scheme, and (iii) the continuous up-flow (Tsihrintzis 2017). CWs are traditionally considered low-cost, low environmental impact, and simple-technology facilities for wastewater treatment, which exploit removal mechanisms active in natural ecosystems (e.g., precipitation, adsorption, aerobic oxidation of organic carbon, nitrification, and denitrification) (Kataki *et al.* 2021). Accordingly, CWs have proved to be very efficient for the removal of conventional water quality macroparameters such as suspended solids, biodegradable organic matter and the various forms of inorganic nitrogen (Vymazal *et al.* 2021). However, recalcitrant organic matter such as organic micropollutants often remain unremoved in this kind of systems (El Barkaoui *et al.* 2023), unless hydraulic retention times of several days are used (Gorito *et al.* 2017), which are unfeasible in the real management of every kind of wastewater treatment plant (WWTP). Furthermore, the biological mechanisms responsible for the removal of poorly biodegradable organic matter appear to be active in CWs only after adequate biomass acclimation, making these systems little or in-efficient in the first weeks of operation. These problems clearly represent a limitation in the use of CWs, especially considering the wide spreading of organic micropollutants in all kinds of wastewater, including domestic ones (Berardi *et al.* 2019; Renai *et al.* 2021).

A possible solution to these issues has been identified in the use of alternative filling materials to those traditionally used in CWs (i.e. sand and gravel), providing a higher surface area and therefore supposed (i) to be able to host higher amounts of microorganisms and (ii) to provide a larger adsorption capacity against organic matter recalcitrant to degradation. Pozzolan (El Ghadraoui *et al.* 2020; Sbahi *et al.* 2022), zeolites (Du *et al.* 2020), and biochar (El Barkaoui *et al.* 2023) have been proposed as unconventional CWs filling media, owing to their high porosity and/or a chemical composition suitable for the removal of various types of contaminants. Among these media, biochar has recently received a great attention as sorbent material for wastewater treatment due to a number of reasons: (i) it is obtained by thermal conversion of waste biomass such as agricultural (Colantoni *et al.* 2016; Kumar *et al.* 2023) and forest management residues (Del Bubba *et al.* 2020; Castiglioni *et al.* 2022), manure (Cao & Harris 2010), and sludge (Gopinath *et al.* 2021); (ii) it contributes to the well establishment of CW plants (Deng *et al.* 2021), as well as of microbial communities (Zhou *et al.* 2020); (iii) it has a highly porous structure, which may promote the adsorption of inorganic ions, such as metals, phosphates, nitrates, and organic matter (Castiglioni *et al.* 2021).

The use of biochar as filling medium for CW implementation has been recently reviewed, highlighting an increasing interest in the scientific community for this topic (Deng *et al.* 2021; El Barkaoui *et al.* 2023). Based on these reviews, biochar integrated in CWs was produced mainly by the thermal conversion of plant waste and its contribution to the treatment performances has been investigated in laboratory and pilot scale systems highlighting an improvement of the removal capacity for conventional macroparameters and some specific micropollutants. However, the use of biochar from sewage sludge as a filling medium for CWs is a poorly described topic in the literature, being that this subject is addressed in only one study (Zheng *et al.* 2022) to our knowledge. In more detail, chemical oxygen demand (COD), nitrogen and phosphorus removal were monitored in laboratory-scale CWs highlighting better performances of the biochar-based system compared to those filled with conventional material.

Based on the aforementioned considerations, this study aimed at investigating the removal efficiency of laboratory scale VF-CWs with biochar produced by co-pyrolysis of a mixture of sawdust and sewage sludge 70/30 (*w/w*), in comparison with systems filled with coarse sand, gravel, and cobblestones, based on the European design of VF-CWs (Tsihrintzis 2017). Both planted (*Phragmites australis* (Cav.) Trin. ex Steud.) and unplanted systems were implemented in order to get information on the influence of plant-biochar interactions on removal performances. These systems were fed with a real effluent wastewater from an activated sludge biological treatment operating in a mixed domestic-industrial textile context and providing treated wastewater for reuse purposes (Fibbi *et al.* 2011). This choice reflects the challenging character of textile wastewater treatment by CWs, mainly related to the need of long hydraulic retention times and/or even the presence of a high adsorptive material as CW substrate, to achieve good removal efficiencies for dyeing agents (Sharma & Malaviya 2022). VF-CW influent and effluent wastewater were monitored for some conventional macroparameters routinely determined in the context of the wastewater. Furthermore, absorbance values at 254 and 420 nm were measured as rapid and reliable screening parameters for investigating the removal efficiency of residual dyeing agents and organic micropollutants absorbing in the UV and visible regions (Ciardelli & Ranieri 2001; Altmann *et al.* 2016), such as organic azo dyes, widely occurring in the industrial textile wastewater.

2. MATERIALS AND METHODS

2.1. Wastewater origin

The wastewater used in this study was the effluent of the clariflocculation stage after the secondary biological treatment of the Baciacavallo WWTP, which receives both domestic and industrial wastewater from the city of Prato and its industrial textile district (see Section 1 of the Supplementary material). In particular, the wastewater treated here was taken before the final ozone-based advanced oxidation stage, which plays a crucial role in the removal of residual colour and trace organic micropollutants before the effluent discharge in the receiving water body. Thus, from this viewpoint, the treatment with VF-CWs simulated the final refining stage of the wastewater. About 1.5 m^3 of wastewater were transported once a week from GIDA to the outdoor laboratory of the Department of Chemistry of the University of Florence (Natural Wastewater Treatment Laboratory, NatLab), which hosts the experimental setup of this study (see Section 2 of the Supplementary material, Figure S1). Since the wastewater supplied by GIDA was quite low in ammonia, it was enriched with reagent grade ammonium chloride (Sigma-Aldrich, St. Louis, MO, USA) to better evaluate the influence of biochar on the nitrification efficiency (57 mg/L, equivalent to about 15 mg NH_4^+-N).

2.2. Design and operation of VF-CW systems

The experimental setup installed at the NatLab consisted of 12 laboratory-scale systems (high-density polyethylene tanks with height = 25 cm and surface area = 0.04 m^2). The outer walls of the tanks have been painted to prevent light from reaching the submerged area, with the exception of a water level control band inside the tank, equipped with a removable lid. Six tanks were filled with coarse sand, gravel, and cobblestones according to the European VF-CWs design (Tsihrintzis 2017) by increasing the grain size from top to bottom and specifically: 3-cm coarse sand Ø = 1–2 mm, 12-cm fine gravel Ø = 7–10 mm, 5-cm medium gravel Ø = 10–14 mm, and 5-cm cobblestones Ø = 30–50 mm (Figure 1(a)). Three of these systems were planted with *P. australis* (G-P), while the remaining three were left unplanted (G-U). Similarly, a further six tanks were filled with biochar, three of which were planted with *P. australis* (BC-P) and the other were unplanted (BC-U). The vertical grain size profile of these systems was the following: 3 cm biochar Ø = 1–2 mm, 6 cm biochar Ø = 2–4 mm, 6 cm biochar Ø = 4–10 mm, 5 cm medium gravel Ø = 10–14 mm, and 5 cm cobblestones Ø = 30–50 mm (Figure 1(b)). The plantation was performed with young plantlets and their rhizomes (two individuals per tank) collected in a natural wetland in the area of 'Parco di Travalle' (Calenzano, 43°52′38.5″N 11°09′21.0″E).

A 15-channel peristaltic pump (Watson-Marlow model 313, Marlow, UK) was used to deliver the wastewater through a 1.5 mm perforated distribution comb installed at the top of each microcosm, at a flow rate of 21 mL/min. Thirteen channels of the multi-channel pump were used for feeding the 12 microcosms and to collect inlet wastewater whenever necessary. Thus, it was ensured that all VF-CWs received the same amount of wastewater and that representative inlet samples of the

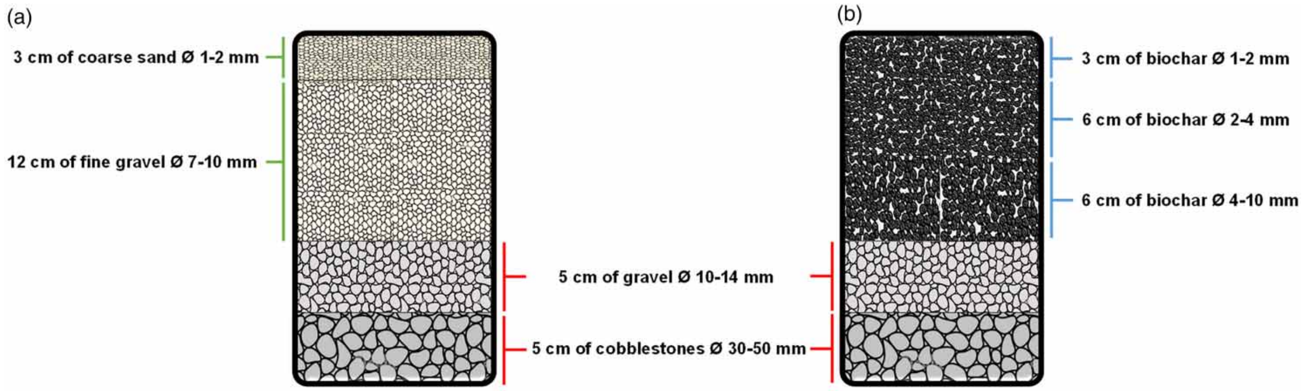


Figure 1 | Schematic representation of the laboratory-scale microcosms filled with sand and gravel (a) and with biochar (b).

wastewater treated in the microcosms were collected. Each system was provided with an outlet tap and a zero-pressure time controlled solenoid valve, which allowed to set the opening interval and the duration, thus permitting the automatic drainage of the microcosms. The microcosms were fed according to the tidal approach by a timer controlling the peristaltic pump with the following 6-h cycle, repeated four times a day: (i) loading at 21 mL/min for 145 min with the solenoid valve closed, which allowed the complete filling of gravel-based CWs up to the limit of the filling medium, while for biochar-based systems the water level was about 2 cm below the upper limit of the substrate; (ii) maintaining saturation for 60 min; (iii) opening of the solenoid valve, rapid draining of the bed, and system left empty for a total time of 155 min. Under these conditions, each microcosm received approximately 12 L of wastewater per day and considering the hydraulic load of a population equivalent (p.e.) equal to 200 L, the design size was about 0.7 m^2/p.e. The duration of the study was about 8 months, from July 2022 to February 2023.

2.3. Biochar production and characterization

The biochar was produced by co-pyrolysis of a 70/30 (*w/w*) mixture of sawdust and biological sludge donated by Romana Maceri Centro Italia S.r.l. (Civitella in Val di Chiana, Italy) and Gestione Impianti di Depurazione Acque S.p.A. (GIDA, Prato, Italy), respectively. The pyrolysis was conducted at 850 °C with a contact time of 60 min in a muffle furnace (Bioclass, Pistoia, Italy) properly modified to allow the thermal conversion of the biomass under N_2-saturated conditions. The biochar was chemically activated by washing with the commercially available BioDea® acid solution donated by Bio-Esperia S.r.l. (Umbertide, Italy). This solution is a by-product of wood waste gasification, and finally washed with deionized water until constant pH. The biochar was finally sieved using ASTM sieves with apertures of 10, 4, 2, and 1 mm (ENCO S.r.l., Venezia, Italy). The biochar was characterized for its environmental compatibility by analysing the parameters reported in the EN 12915-1 European regulation for materials intended for water treatment (European Committee for Standardization 2009), i.e. ash content, and water leachable concentrations of selected elements and polycyclic aromatic hydrocarbons. Further characterizations included elemental analysis, pH of the zero charge point, and study of porosity distribution, i.e. specific surface area (SSA), microporous SSA, and mesoporous SSA. Full details of the analytical protocols adopted for biochar characterization, including those regarding the internal method for PAHs identification and quantification (Supplementary material, Tables S1–S3, Figure S2) (Del Bubba *et al.* 2020) are reported in Section 3 of the Supplementary material, while the results are shown in Supplementary material, Table S4.

2.4. Sample collection and analysis

2.4.1. Sampling

An entire event (approximately 3 L) including one inlet sample and outlet samples of each microcosm was collected once a week for the determination of the following macroparameters: pH, dissolved oxygen (DO), electrical conductivity (EC), total suspended solids (TSS), COD, total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), total phosphorus (TP), orthophosphate (PO_4^{3-}), and absorbance at 254 and 420 nm.

2.4.2. Analysis of physicochemical parameters

Temperature (T), pH, EC, and DO were measured on unfiltered samples in the VF-CW inlet and outlet. In detail, (i) pH was measured using a portable meter, model 3310 (WTW, Weilheim, Germany) equipped with a temperature sensor and coupled with a SenTix® 61 pH-electrode (WTW); (ii) DO was measured with a portable meter HQ40D (HACH Instruments, Loveland, CO, USA) equipped with an LDO10110 sensor coupled with an integrated temperature sensor; (iii) EC was measured using a WTW (Weilheim, Germany) conductivity meter, model COND340i. The values of temperature measured in the inlet and outlet were averaged and used to highlight possible correlations with removal efficiency.

TSS were evaluated according to the method 2540 D of the Standard Methods for the Examination of Water and Wastewater (American Public Health Association & American Water Works Association 1995).

2.4.3. Analysis of chemical parameters

Chemical parameters were determined on wastewater samples after filtration at 0.7 μm using filters (Whatman, Maidstone, England) previously dried in oven at 105 °C for 1 h. All the parameters were determined colorimetrically on the filtrated sample, using a DR/4000 U UV-Vis spectrophotometer (HACH, Loveland, CO, USA) and USEPA approved methods based on HACH reagent kits (for full details see Section 4 of the Supplementary material).

2.5. Statistical analysis

Statistical tests for comparison of mean values of the data obtained were carried out by the Games–Howell non-parametric test using the Minitab® software package version 17.1.0 (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSION

3.1. Characteristics of biochar

The results of the water leaching parameters (Supplementary material, Table S4) highlighted the environmental compatibility of the biochar in terms of release of specific inorganic elements and PAHs into the treated wastewater, according to the EN 12915-1, which should be considered as an important prerequisite for using this material for water treatment. Furthermore, the biochar compiled the aforementioned regulation also for ash content, which represents a parameter that should be low to increase the adsorption performance of the material (Castiglioni *et al.* 2022).

The value of pH_{PZC} was 7.1, i.e. very close to the pH of the real wastewater used in this study (Table 1). Accordingly, the material did not present significant net surface charge in the experimental conditions here adopted, thus providing a poor adsorption capacity by ion exchange mechanism. Interestingly, the biochar showed a quite high SSA compared to data previously reported in literature on biochar obtained by co-pyrolysis of biological sludge and waste vegetal biomass (Cheng *et al.* 2013, 2019; Dai *et al.* 2022a, 2022b). The sum of the *t*-plot microporous (341 m^2/g) and BJH mesoporous (36 m^2/g) areas was very close to the BET total SSA (389 m^2/g), thus indicating a low contribution of macropores to the total surface area. The mixed microporous–mesoporous architecture of the biochar is suitable with the adsorption of organic micropollutants, which commonly have a molecular diameter of few nm. The elemental analysis showed a high percentage of carbon (65.0%) together with a considerable amount of oxygen, indicating the remarkable presence of oxygenated functional groups on the surface of the material and therefore a high adsorption potential through non-covalent polar interactions towards organic micropollutants.

Table 1 | Mean values and standard deviations (in bracket) of pH, electrical conductivity (EC, μS/cm), dissolved oxygen (DO, mg/L), total suspended solids (TSS, mg/L), measured in the inlet and outlet of microcosms filled with gravel (G) or biochar (BC), planted with *Phragmites australis* (P) or left unplanted (U)

Parameter	Inlet	G-P	G-U	BC-P	BC-U	Limit-A	Limit-B
pH	7.6 (0.4)^a	7.5 (0.5)^a	7.6 (0.3)^a	7.7 (0.5)^a	7.7 (0.4)^a	n.a.	n.a.
EC	3038 (828)^a	2991 (846)^a	2955 (774)^a	2940 (804)^a	2920 (833)^a	n.a.	n.a.
DO	1.0 (0.6)	1.3 (0.3)^a	1.2 (0.4)^a	1.6 (0.4)^a	1.5 (0.3)^a	n.a.	n.a.
TSS	8 (9)^a	6 (3)^a	7 (7)^a	5 (4)^a	5 (5)^a	≤80	≤10

Limits A and B refer to the Italian thresholds for treated wastewater discharge in surface water (D. Lgs 152/2006) and treated wastewater reuse in agriculture (D.M. 185/2003), respectively. Within a same parameter, values with different letters are statistically different according to the Games–Howell test for comparison of mean values ($P < 0.05$).

3.2. Physicochemical parameters

Table 1 illustrates the behaviour of the physicochemical parameters here investigated in the inlet and outlet of G-P, G-U, BC-P, and BC-U microcosms. No statistically significant changes were observed for pH, EC, DO, and TSS during the treatment with the four different types of VF-CWs. In contrast, large temperature variations (4–27 °C) were observed in all systems as a function of sampling time, in accordance with the study period covering the summer, spring and winter seasons.

3.3. Chemical parameters

3.3.1. COD removal

COD showed statistically lower concentrations in the effluents of biochar-based microcosms than in the influent (Figure 2), with removal percentages of 35–37% in BC-U and BC-P, respectively. It is worth noting that these removal performances have been obtained with a quite high hydraulic loading (0.7 m^2/p.e.) compared to the dimensioning adopted elsewhere for polishing stages (Ayaz 2008; Martín *et al.* 2013) and should therefore considered as valuable. Conversely, systems filled with gravel did not provide significant reductions of COD, being the mean removal achieved with G-P and G-U only about 13–14%. Thus, the removal in systems using biochar as substrate was about 20% higher than in those filled conventionally, in accordance with the quite high SSA of the material used as VF-CW substrate. The better treatment performance of biochar-based microcosms is a finding already demonstrated in literature for the removal of COD (El Barkaoui *et al.* 2023) and can be explained by the well-known adsorption properties of biochars towards organic compounds (Castiglioni *et al.* 2021). Even though the higher COD removal may also be the result of an increased occurrence of chemoheterotrophic microorganisms in systems filled with biochar (Liang *et al.* 2020) due to its highly porous structure, in this study the COD removal was not correlated with temperature, thus suggesting a poor contribution of biological mechanisms to this process. In addition, it should be considered that the COD determined in the influent wastewater is probably quite recalcitrant, as these waters have already been subjected to biological treatment in the activated sludge WWTP. Hence, the COD removal could be mainly attributed to adsorption mechanisms by biochar rather than biological processes.

3.3.2. Nitrogen removal

As a general consideration, TN influent concentrations were found to be approximately equal to the sum of NH_4^+-N, NO_2^--N, and NO_3^--N, evidencing the absence of a significant contribution of organic nitrogen to this parameter. This finding agrees

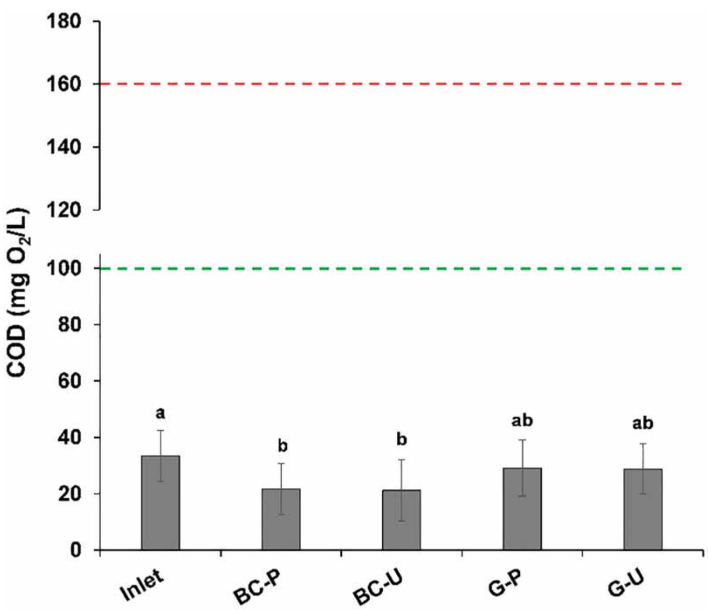


Figure 2 | Mean COD concentrations (mg O_2/L) in the inlet and outlet of microcosms filled with biochar (BC) or gravel (G), planted (P), or unplanted (U). Error bars refer to standard deviation. Bars with different letters are statistically different according to the Games–Howell test for comparison of mean values ($P < 0.05$). Red and green dashed lines refer to the Italian and European thresholds for treated wastewater discharge in surface water (D. Lgs 152/2006) and treated wastewater reuse in agriculture (D.M. 185/2003), respectively.

with the origin of wastewater that was collected after the secondary biological treatment and successive clariflocculation stage of the Baciacavallo WWTP, which provided the degradation of the organic forms of N.

In absence of fortification with ammonium chloride the VF-CWs influent exhibited very low NH_4^+-N concentrations (0.16 ± 0.09 mg/L during the first 2 months of study). Under these experimental conditions, even though the microcosms exhibited appreciable removal percentages of ammonia (i.e. 65–70% and 45–50% for systems filled with biochar and gravel, respectively) influent and effluent concentrations were not statistically different, thus hindering a clear interpretation of the results obtained. Conversely, the use of wastewater fortified with ammonia allowed to highlight interesting differences in the nitrogen cycle between systems integrated with biochar and those filled with gravel. In fact, TN was significantly decreased by biochar-based microcosms, which reduced its concentration below the limit for discharge to surface water (Figure 3(a)). A different scenario arose from gravel-based microcosms, which exhibited effluent concentrations often above this threshold and not statistically different from that found in the inlet. This result was due to the very high decrease in NH_4^+-N observed in BC-P and BC-U effluents, where the removal was about 65% and outlet ammonia concentrations were well below the limits for discharge to surface water (see Figure 3(b)). Conversely, in gravel systems effluent NH_4^+-N concentrations exceeded in few cases this threshold, even though they remained on average below the legal limit. Despite the excellent removal efficiency demonstrated by systems integrated with biochar, the stringent limits provided by the legislation for ammonia nitrogen (i.e. 2 mg NH_4^+-N/L) on the reuse of treated wastewater in agriculture were not met. However, it should be noted that the removal percentages indicated above were achieved through a rather challenging sizing of the microcosms, i.e. approximately 0.7 m^2/p.e. or 2 m^2/p.e., calculated respectively on the basis of the hydraulic or nitrogen load applied to the systems, the latter assuming a population equivalent conversion factor of 12 g N/p.e. per day (Grizzetti & Bouraoui 2006).

The high removal efficiency observed in this study for NH_4^+-N can be explained by the nitrification process. In fact, ammonia oxidizing bacteria (AOBs), such as *Nitrosomonas sp.* and *Nitrosospira sp.*, can survive in environments with DO concentrations as low as 0.3 mg/L (Yue *et al.* 2018), i.e. much lower than those measured in VF-CW effluents, which

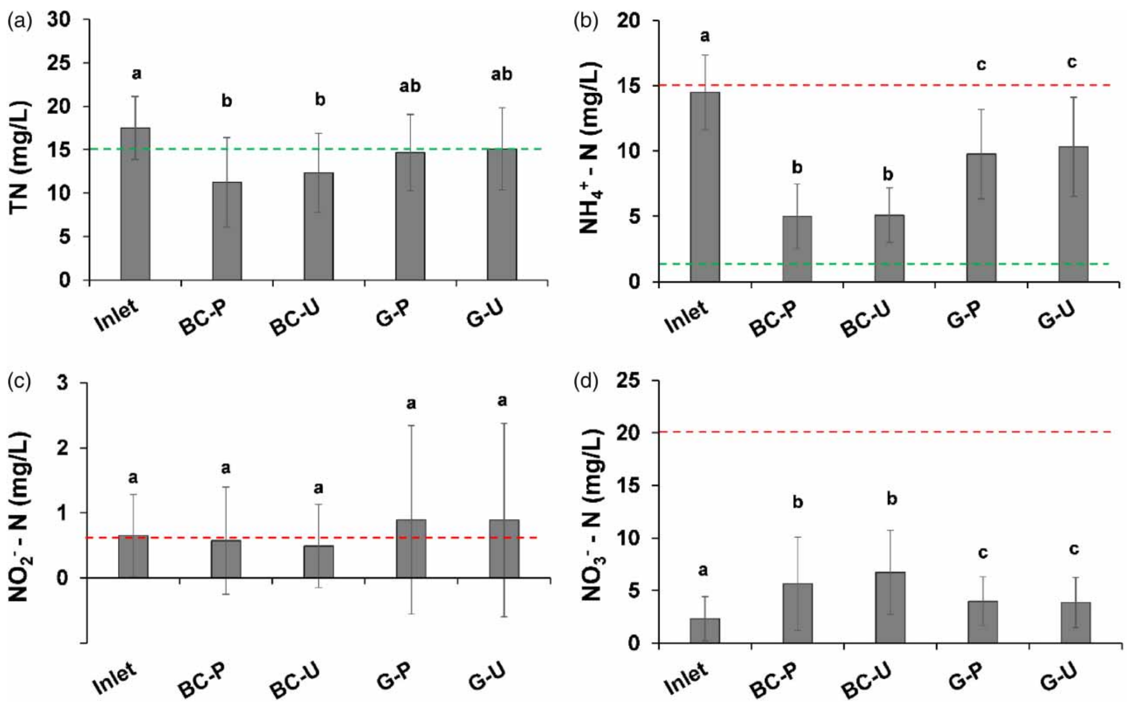


Figure 3 | Mean concentrations (mg/L) of total nitrogen (a), ammonia nitrogen (b), nitrous nitrogen (c), and nitric nitrogen (d) in the inlet and outlet of microcosms filled with biochar (BC) or gravel (G), planted (P) or unplanted (U). Error bars refer to standard deviation. Bars with different letters are statistically different according to the Games–Howell test for comparison of mean values ($P < 0.05$). Red and green dashed lines refer to the Italian and European thresholds for treated wastewater discharge in surface water (D. Lgs 152/2006) and treated wastewater reuse in agriculture (D.M. 185/2003), respectively.

were in the range of 1.2–1.6 mg/L (Table 1). In addition, optimal nitrification conditions have been found to be within the narrow pH range of 7–8 (Tarre & Green 2004), which includes the pH data determined in this study (Table 1). An improvement of ammonia removal without changing the hydraulic loading rate could be perhaps obtained through the installation of an active oxygenation system, so as to increase DO concentrations above 2 mg/L (Tan *et al.* 2013). It should be noted that according to the literature (Tan *et al.* 2013), DO concentrations of about 1–2 mg/L allow to maintain a high nitrification efficiency without compromising the rate of denitrification, thus representing the ideal experimental condition for the removal of TN.

In agreement with existing literature (Liang *et al.* 2020), the higher removal of ammonia exhibited by BC-P and BC-U systems can be explained by an increased nitrification efficiency due to a higher occurrence of AOBs in microcosms integrated with biochar, compared to gravel-based systems. Conversely, based on the biochar characteristics mentioned above (section 3.1), ammonia removal should not be ascribed to cation exchange mechanisms. The temperature measured in inlet and outlet of VF-CWs, which represents an additional key factor in regulating nitrification rate, supported this hypothesis. In fact, in all VF-CWs, ammonia removal increased with increasing temperature, expressed as the mean of the inlet and outlet data, and showed statistically significant linear correlations ($P \leq 0.048$), even though with quite low determination coefficients ($R^2 = 0.22$–0.56). In addition, slightly higher coefficients of determination ($R^2 = 0.28$–0.67) were found using the logarithmic function, due to a greater decrease in purification efficiency for temperatures below 10 °C, as highlighted elsewhere (Del Bubba *et al.* 2000).

It is well known that the removal of ammonia by nitrification leads to nitrate by a two-stage reaction, whose intermediate is nitrite (Del Bubba *et al.* 2000). Nitrite oxidizing bacteria such as *Nitrobacter* are more sensitive to low concentrations of DO than AOBs, due to the higher half-saturation coefficient for DO (i.e. lower affinity) of the former than the latter (Tan *et al.* 2013). Thus, under stoichiometric or even stoichiometric oxygen concentrations can give rise to accumulation of nitrites (Blackburne *et al.* 2008) with serious damage to the organisms living in the receiving water body (Kocour Kroupová *et al.* 2018). It is therefore very important that the nitrification process is as complete as possible. On average, microcosms integrated with biochar did not determine increases in nitrite concentrations during the treatment, unlike what happened for gravel-based systems (Figure 3(c)), highlighting also in this case an important role of biochar in supporting the biochemical processes of contaminants removal. It should also be noted that the mean concentrations of nitrite nitrogen in BC-P and BC-U effluents are compatible with the limit laid down for discharge into surface water, even in the presence of quite high influent ammonia concentrations. Previous considerations on ammonia removal agreed with changes in nitrate concentrations during the treatment. In fact, higher NO_3^--N values were found in BC-P and BC-U compared to G-P and G-U. However, under no circumstances nitrate concentrations exceeded the limits for discharge into surface water, thus highlighting the significant occurrence of the denitrification process. Assuming that reduced nitrogen and oxidized nitrogen are removed largely by nitrification and denitrification (Brix 1997; Del Bubba *et al.* 2000) and knowing the concentrations of the various forms of nitrogen present in the influent and in the effluent, it is possible to calculate the percentage of denitrification (see Equation S2 in Section 5 of the Supplementary material). Supplementary material (Figure S3) illustrates the results obtained for the four investigated microcosms, which highlight a slightly higher denitrification efficiency in planted systems (about 3–8%). This finding confirms the minor role of plants in nitrogen removal. Interestingly, biochar-based microcosms showed a denitrification efficiency about 10% higher than gravel-based systems, in agreement with the previous considerations regarding the higher microbial activity in systems filled with biochar. In this latter regard, it is remarkable that the higher denitrification efficiency observed in biochar-based microcosms allowed to meet the 'Limit-B' for TN (see Table 1), thus making suitable the treated wastewaters for agricultural reuse.

3.3.3. Phosphorus removal

In a similar way to the findings observed for TN, TP was also found very close to the PO_4^{3-} concentrations (Supplementary material, Figure S4), confirming the almost total absence of organic forms of nutrients in the wastewater collected after the Baciacavallo clariflocculation stage. Phosphorus removal was higher in planted microcosms than in unplanted ones (about 30 vs. 10% and 20 vs. 15% in systems filled with biochar and gravel, respectively). Even though these differences were not statistically significant (with the only exception of the removal of PO_4^{3-} in BC-P, see Supplementary material, Figure S4-A), the data suggested that direct plant uptake played an important role for the removal of this parameter. This result agrees with the considerations mentioned above regarding the absence of a net charge on the surface of biochar that exclude a direct role of the material in phosphorus removal.

3.3.4. Absorbance removal

In agreement with literature, absorbance values measured at 254 and 420 nm are parameters correlated to the concentrations of organic micropollutants containing one or more aromatic moieties and/or chromophores, thus representing rapid and reliable screening parameters for investigating their removal efficiency (Ciardelli & Ranieri 2001; Altmann *et al.* 2016). The results obtained here highlighted a relevant role of biochar in the removal of organic micropollutants, as clearly demonstrated by the statistically significant reduction of absorbance values at both wavelengths (see Figure 4). Indeed, regardless of whether the systems were planted or not, the biochar-based microcosms showed much higher mean absorbance reductions than the gravel-filled systems, being about 51–59% and 18–31% at 254 and 420 nm, respectively. The trend of absorbance values measured at the two wavelengths over the entire study period (see Figure 5) provided interesting information suitable to shed light to the mechanism responsible of absorbance reduction. In fact, at 254 nm (Figure 5(a)), in the face of a statistically significant decreasing trend of the absorbance values measured in the inlet, a corresponding decreasing trend was observed in both G-P ($P < 0.001$, $R^2 = 0.43$) and G-U effluents ($P < 0.001$, $R^2 = 0.43$), while the outlets from BC-P ($P = 0.041$, $R^2 = 0.14$) and BC-U ($P = 0.002$, $R^2 = 0.29$) systems showed increasing absorbance values, but remained lower than those determined in the inlet and outlet from gravel-filled microcosms. Overall, these trends suggested a major role of adsorption by biochar for the removal of organic micropollutants responsible of UV-Vis absorbances, in accordance with considerations mentioned above for COD removal. The trends observed at 420 nm (Figure 5(b)), even though more erratic, led to similar conclusions since statistically significant increasing trends were observed for absorbance in the BC-P ($P = 0.021$, $R^2 = 0.18$) and BC-U ($P = 0.002$, $R^2 = 0.31$) effluents.

4. CONCLUSIONS

In this study, a biochar produced from co-pyrolysis of a waste biomass mixture containing a significant percentage of biological sludge was integrated into laboratory-scale vertical-flow CWs as an unconventional filling medium, demonstrating its significant role in improving the removal of organic matter and nitrogen, in comparison with microcosms filled with gravel. The better quality of effluents from biochar-filled microcosms can be the result of a synergistic effect of sorption of

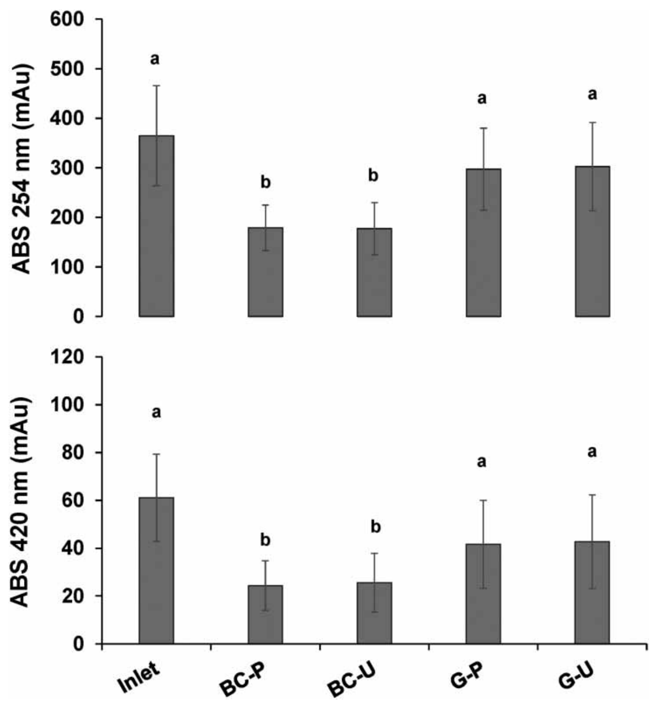


Figure 4 | Mean absorbance values measured at 254 and 420 nm in the inlet and outlet of microcosms filled with biochar (BC) or gravel (G), planted (P), or unplanted (U). Error bars refer to standard deviation. Bars with different letters are statistically different according to the Games–Howell test for comparison of mean values ($P < 0.05$).

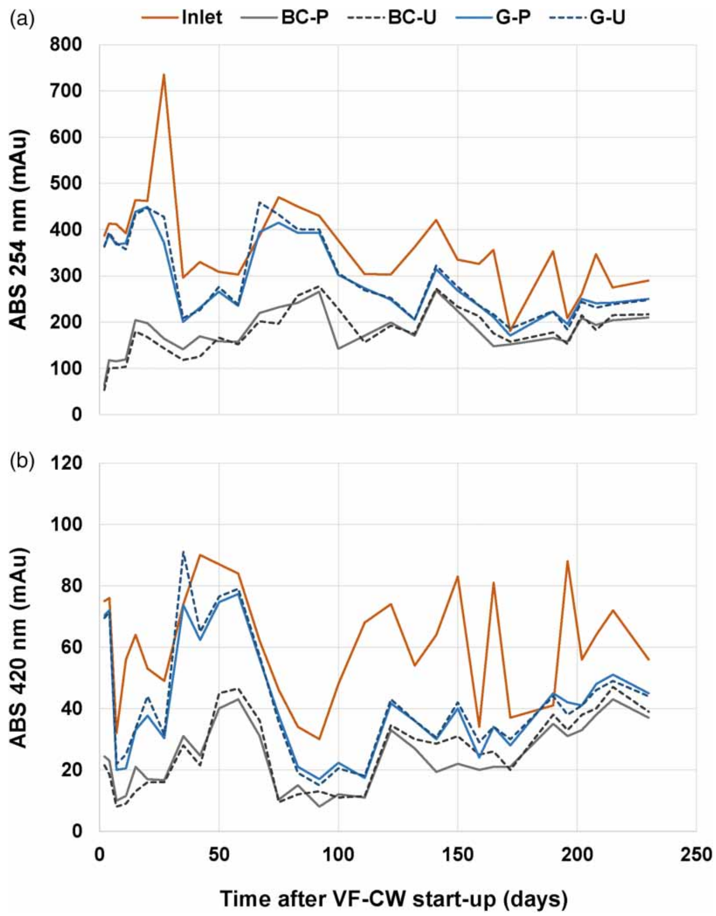


Figure 5 | Trends of absorbance measured at 254 nm (a) and 420 nm (b) in the inlet and outlet of microcosms filled with biochar (BC) or gravel (G), planted (P), or unplanted (U).

organic pollutants by biochar and improved biological processes of nitrogen removal sustained by the highly porous material, while direct assimilation by plants seems to play a minor role. The use of sludge-based biochar as a CWs filling medium for refining treated wastewater in conventional WWTPs represents an approach to wastewater treatment characterized by a high level of circularity. Furthermore, the use of biochar produced from waste (i.e. feedstocks widely available at zero cost) can represent a strategy for the implementation of CWs completely filled with biochar, maximizing the synergistic effects mentioned above, both closely related to the high surface area and to the microporous-mesoporous character of the material. It is remarkable that even using a hydraulic loading rate as high as 300 L/m^2 d, corresponding to a sizing of 0.7 m^2/p.e., and with an influent TN equivalent to 2 m^2/p.e., all forms of nitrogen met the European requirements for wastewater discharge in surface water bodies. Effluent nitrogen concentrations from biochar-based CWs complied also with the TN limit for agricultural reuse, while that of NH_4^+-N was met only in some samples, thus highlighting an insufficient oxygen supply in the microcosms. However, the high and consistent removal of organic micropollutants provided by biochar throughout the study is a strong point in favour of using this type of sorbent material in CWs, since the risk of transfer of organic micropollutants from treated wastewater to edible crops is considered one of the main critical issues of the reuse of such water in agriculture (Renai *et al.* 2020). In this latter regard, further studies are necessary to investigate the behaviour of specific classes of emerging organic micropollutants in biochar-based CWs.

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AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. CWs were implemented by L.V. and I.C. Wastewater to be analysed and the reagents for wastewater analysis were collected by by D.F. Material preparation, data collection, and analysis were performed by M.A., D.P., G.B., L.R., and M.F. The first draft of the manuscript was written by M.D.B., G.B., and D.P. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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