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Research article

Demo-scale up-flow anaerobic sludge blanket reactor coupled with hybrid constructed wetlands for energy-carbon efficient agricultural wastewater reuse in decentralized scenarios

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ABSTRACT

The impact of climate change on water availability and quality has affected agricultural irrigation. The use of treated wastewater can alleviate water in agriculture. Nevertheless, it is imperative to ensure proper treatment of wastewater before reuse, in compliance with current regulations of this practice. In decentralized agricultural scenarios, the lack of adequate treatment facilities poses a challenge in providing treated wastewater for irrigation. Hence, there is a critical need to develop and implement innovative, feasible, and sustainable treatment solutions to secure the use of this alternative water source. This study proposes the integration of intensive treatment solutions and natural treatment systems, specifically, the combination of up-flow anaerobic sludge blanket reactor (UASB), anaerobic membrane bioreactor (AnMBR), constructed wetlands (CWs), and ultraviolet (UV) disinfection. For this purpose, a novel demo-scale plant was designed, constructed and implemented to test wastewater treatment and evaluate the capability of the proposed system to provide an effluent with a quality in compliance with the current European wastewater reuse regulatory framework. In addition, carbon-sequestration and energy analyses were conducted to assess the sustainability of the proposed treatment approach. This research confirmed that UASB rector can be employed for biogas production (2.5 L h^{-1}) and energy recovery from organic matter degradation, but its effluent requires further treatment steps to be reused in agricultural irrigation. The AnMBR effluent complied with class A standards for *E. coli*, boasting a concentration of 0 CFU 100 mL^{-1}, and nearly negligible TSS levels. However, further reduction of BOD_5 (35 mg L^{-1}) is required to reach water quality class A. CWs efficiently produced effluent with BOD_5 below 10 mg L^{-1} and TSS close to 0 mg L^{-1}, making it suitable for water reuse and meeting class A standards. Furthermore, CWs demonstrated significantly higher energy efficiency compared to intensive treatment systems. Nonetheless, the inclusion of a UV disinfection unit after CWs was required to attain water class B standards.

1. Introduction

Planetary resources have experienced great pressures since the boost of anthropic activities following industrialization and technological advance (Graumlich and Steffen, 2018). Notably, water sources have been subject to strong influences from several factors, with pollution and climate change amongst the major causes of fresh water availability reduction (Konapala et al., 2020; Pokhrel et al., 2021). Since agricultural irrigation heavily relies on water availability, these aspects pose irreversible consequences on food production (Qiu et al., 2023). Recently, a wider use of "non-conventional" water resources has been encouraged as a sustainable solution to respond to the increasing water stress

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(Hussain et al., 2019; Mancuso et al., 2020). Wastewater, a continuous by-product, contains essential macro-nutrients such as nitrogen and phosphorous that are essential for growing crops (Mancuso et al., 2022), thereby enabling the adoption of fertigation, the agricultural practice that combines water and nutrient management by delivering water-soluble nutrients directly to crops through irrigation systems (Chojnacka et al., 2020). Consequently, reusing treated wastewater in agricultural irrigation serves a dual purpose through decreasing the need for fresh water and contributing to a reduction in the use of chemical fertilizers (Mainardis et al., 2022; Mancuso et al., 2023; Odone et al., 2024; Ofori et al., 2021). However, prior to be reused in agricultural irrigation, proper treatment of wastewater is essential to prevent adverse effects on human, animal, and environmental health (Mancuso et al., 2021b; Patrolecco et al., 2015). The European Union has recently introduced a new regulation establishing minimum water quality requirements for the safe reuse of treated wastewater in agriculture (Regulation (EU) 2020/741, 2020). Centralized wastewater treatment plants (WWTPs), although being able to treat large water volumes, are not always environmentally and economically beneficial systems, since they might require high levels of energy and resources consumption, as well as skilled human resources (Garrido-Baserba et al., 2022). However, a variety of solutions have captured the attention and interest of the scientific community due to their potential to serve as decentralized treatment options. For example, UASB reactors are typically implemented at the point of wastewater generation, often within or near the facilities producing the wastewater and therefore they can be considered decentralized treatment plants (Capodaglio et al., 2017; Fernández del Castillo et al., 2022). These systems have been widely employed to tackle the challenges faced in efficient treatment of wastewater, since they enable conversion of the wastewater organic matter content into biogas, even at low loading rates and low temperatures, while requiring smaller treatment costs than their centralized counterparts (Mainardis et al., 2020). Nevertheless, UASB reactors produce effluent that frequently fails to meet existing regulations for water discharge or agricultural reuse, necessitating the implementation of additional post-treatment measures (Nair and Ahammed, 2015). Different intensive solutions have been proposed to enhance UASB performance in the last few decades, such as coupling UASB with AnMBR, which can improve UASB effluent water quality (Mehmood et al., 2021; Ozgun et al., 2019; Yoshida et al., 2022). However, the adoption of AnMBR may be cost-prohibitive, primarily due to expenses associated with membrane construction, installation, and maintenance (Saha et al., 2023). CWs serve as another sustainable and decentralized wastewater treatment technology. CWs utilize the combined processes of plant and microbial metabolism, along with selected substrates, for effective wastewater treatment (Mancuso et al., 2021a). With straightforward construction, operation, and maintenance, these solutions emerge as an attractive option for water and nutrient recovery, being extensively studied and applied in recent years (David et al., 2022). These factors permit a flexible distribution of CWs in the territory, closer to the wastewater sources, allowing the saving of resources and space generally dedicated to the collection and re-distribution networks (Melián, 2020). Moreover, their treatment performance remains consistent even through hydraulic loads fluctuation (Nan et al., 2023), and they can be scaled based on treatment needs, providing flexibility and additional cost savings (Rabaey et al., 2020; Zhang et al., 2015). However, CWs may exhibit specific drawbacks, such as a susceptibility to clogging, particularly evident when dealing with untreated raw wastewater (Wang et al., 2021) or insufficient efficiencies when applied as a standalone solution for high-load influents and are therefore usually not suitable for direct reuse (Mosquera-Romero et al., 2022; Vasconcellos et al., 2019). Moreover, achieving effective microbial reduction frequently necessitates disinfection units such as UV radiation, peracetic acid, and others. This need is particularly pronounced when utilizing UASB and CWs, whether alone or in combination (Wendland et al., 2006). Due to the considerations outlined above, there is a burgeoning interest in investigating the integration of intensive treatment systems with natural solutions (Fernández del Castillo et al., 2022).

Therefore, in the present study a novel demo-scale plant, consisting of a UASB reactor, CWs and a UV system has been proposed. The main aim of the research was to evaluate the removal efficiency of each treatment unit, investigating also the capability of the entire treatment chain to produce effluents suitable for agricultural irrigation reuse, according to the new European guidelines. With this purpose, treatment efficiency was evaluated using continuous sample collection throughout the entire investigation period (1.5 years). The proposed treatment chain also aimed at reducing energy consumption of wastewater treatment, recovering energy and nutrients from treated wastewater, and providing a sustainable decentralized treatment solution for wastewater treatment and reuse. Furthermore, an integrative approach was followed, where the carbon sequestration capability of both the CW flora and the UASB was associated with the treatment action of the units, together with the recycling of building waste, used as CW substrate. Finally, considerations on energy requirements by additional processes within the treatment chain were provided.

2. Materials and methods

2.1. Demo-scale plant and experimental tests description

The demo-scale plant (Fig. 1), located at the Imola WWTP (75,000 PE, Northern Italy, 44°21′12.3″N, 11°44′15.1″E), was designed, constructed and implemented to test the impact of the combination of different technologies in diverse possible configurations on wastewater treatment. It had a maximum treatment capacity of 2.5 m^3 day^{-1} and comprised the following treatment units: no. 1 sedimentation tank, no. 1 UASB reactor, no. 2 filters (anaerobic membrane bioreactor and gravity filter), no. 4 CWs (2 horizontal flow (HFCWs) and 2 horizontal flow (VFCWs) CWs) and no. 1 disinfection system (UV lamp unit). The demo-scale plant was equipped with different probes (for pH, total suspended solids, oxidation reduction potential, dissolved oxygen, electric conductivity, temperature recording) and gauges (for water level, flow rate and pressure regulation). Such units were connected to a central control panel that allowed their monitoring and actuation, both remotely and on-site.

During the experimental activity, the combination of different treatment units was aimed at enhancing treatment performances as well as producing effluents suitable for agricultural irrigation reuse. As summarized in Fig. 2, two different configurations were tested: in configuration no. 1, wastewater was treated combining the UASB reactor and the AnMBR, while, in configuration no. 2, the UASB reactor was coupled with either single-stage or hybrid CWs, followed by an UV system.

During the 1.5 years-long experimental activity (including a start-up period of three months), intermediate efficiencies were also monitored by analyzing the effluents from the individual units belonging to the two different combinations. The key monitoring points are presented in Table 1a, along with an explanation of the purpose for which they were considered. The nomenclature of the items shown in Table 1a has been later clarified throughout the text.

The UASB reactor with a volume of 0.9 m^3 (used in all the tested configurations) was composed of: i) a granular sludge bed (lower part), ii) a fluidized zone (middle part) and iii) a biogas separator (upper part). Three sampling taps placed at different heights allowed the sampling of the granular biomass for its monitoring. The produced biogas was measured by a drum-type biogas meter (Ritter, model TG05/5, biogas flow rate range 1–60 L h^{-1}, Germany). A heating element was placed internally, to maintain ambient and/or mesophilic conditions (Jung et al., 2019). The UASB reactor was initially inoculated with anaerobic granular sludge taken from a paper mill wastewater treatment plant located in the Emilia-Romagna region, Italy. Then it was fed with wastewater (characteristics are reported in section 2.2) through four

Fig. 1. Geographical location and aerial view of the demo-scale plant installed within the Imola WWTP, Italy.

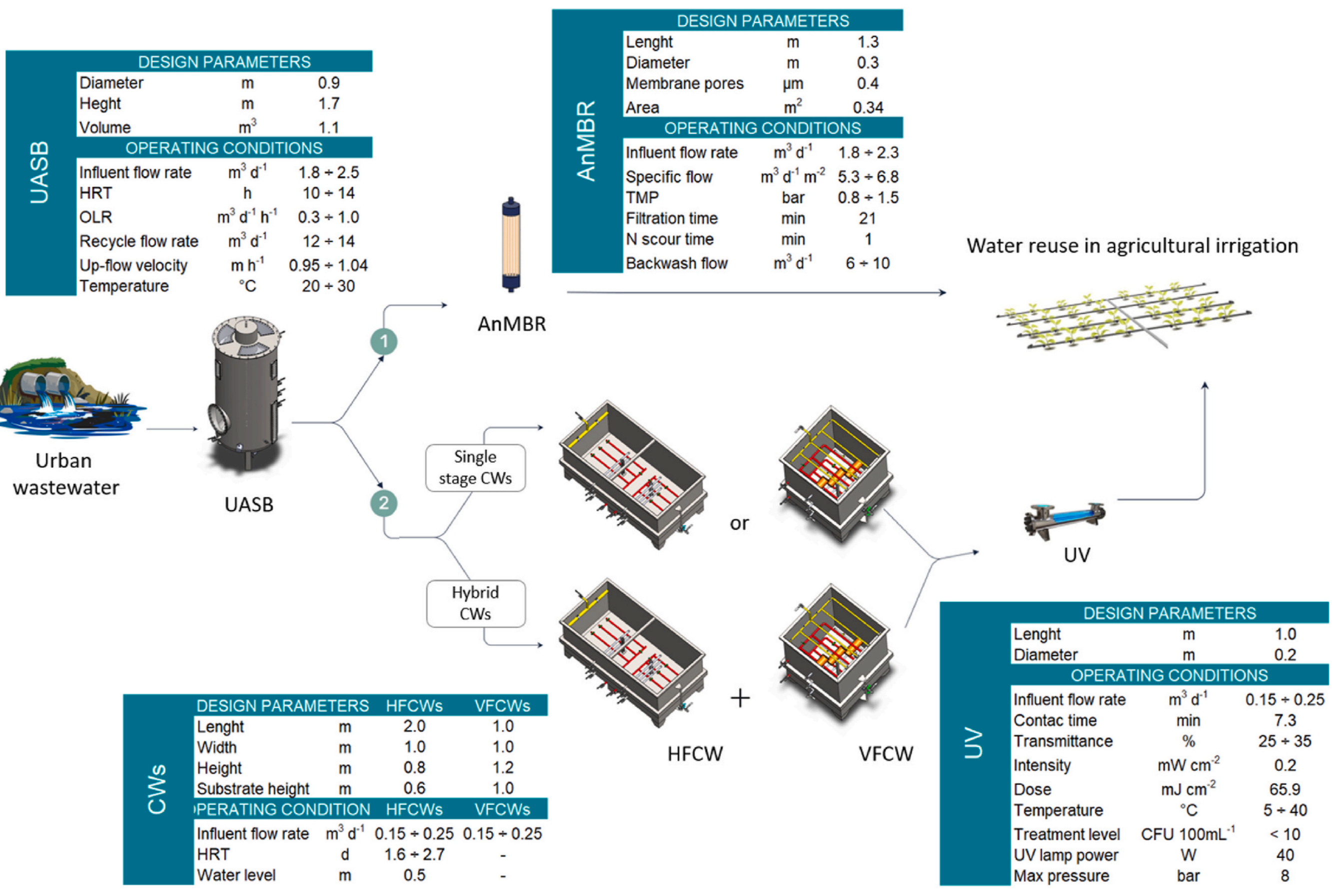

Fig. 2. Tested configurations in the demo-scale plant: configuration no. 1 (UASB + AnMBR) and configuration no. 2 (UASB + CWs + UV).

nozzles placed at its bottom with a flowrate of 1.8–2.5 m^3 d^{-1} and with a recirculation loop (12–14 m^3 d^{-1}) in order to ensure a proper up-flow velocity in the reactor (v_{up} = 1m h^{-1}), selected on the basis of typical values reported in the literature (v_{up} = 0.5–1m h^{-1}) (Mainardis et al., 2020). The temperature within the UASB reactor was regulated by a heating system and it varied between 20 and 30 °C, while the hydraulic retention time (HRT) was maintained between 10 and 14 h, with typical value of HRT for UASB reactors being between 5 and 19 h (Rattier et al., 2022). Moreover, the organic loading rate (OLR) varied between 0.3 and 1 kg COD m^{-3} d^{-1}, depending on characteristics of influent wastewater.

In configuration no. 1, the UASB reactor was followed by the AnMBR unit, which consisted of a tubular ultrafiltration membrane type Memstar UF-0610EDT (0.4 μm) with an area of 0.34 m^2. It was fed with a flowrate of 1.8–2.3 m^3 d^{-1} effluent from the UASB reactor and it operated with a transmembrane pressure (TMP) between 0.8 and 1.5 bar, as those are typical values for TMP reported in the literature (Meng and Li, 2019). The AnMBR filtration cycle was set at 20 min of filtration and followed by 1 min of nitrogen gas sparging to avoid fouling. A backwash flow (6–10 m^3 d^{-1}) with tap water or chemical reagents (NaOCl) was foreseen as a maintenance measure.

In configuration no. 2, the UASB reactor was coupled with four CWs (operated as single-stage or hybrid systems). The two HFCWs and two VFCWs presented identical configuration. They contained crushed stone (10–30 mm) as drainage layer, and recycled construction and demolition waste (HFCWs 5–10 mm; VFCWs 3–5 mm) as main substrate. All the CWs were planted with *Phragmites australis* (reed) with a density of 10 plants per m^2 (Pirrera and Pluchino, 2017). The surface area was 2 m^2 in HFCWs and 1 m^2 in VFCWs, while both systems were fed with

Table 1
List of experiments to assess proper operational conditions.

a) List and aims of plant configurations used during the experimental tests

Plant configuration	Treatment unit	Research objective
a1	UASB reactor	Investigation of UASB used as single unit.
b1	UASB reactor + AnMBR	Investigation of UASB coupled with AnMBR.
c1	UASB reactor + HFCW (NA)	Investigation of UASB coupled with single-stage CWs and influence of CW typology (CWs without aeration).
c2	UASB reactor + VFCW (NA)	
d1	UASB reactor + HFCW (CA)	Investigation of UASB coupled with single-stage CWs and influence of CW typology (CWs continuously aerated).
d2	UASB reactor + VFCW (CA)	
e1	UASB reactor + HFCW (CA)	Investigation of UASB coupled with single-stage CWs and influence of CW aeration mode (CWs continuously, partially and intermittently aerated).
e2	UASB reactor + HFCW (PA)	
e3	UASB reactor + HFCW (IA)	
f1	UASB reactor + VFCW (NA) + HFCW (NA)	Investigation of UASB coupled with hybrid CWs (CWs without aeration).
g1	UASB reactor HFCW (IA) + VFCW (NA)	Investigation of UASB coupled with hybrid CWs (CWs intermittently aerated and without aeration).
h1	UASB reactor + HFCW (NA) + UV	Investigation of UASB coupled with single-stage CWs (CWs without artificial aeration) followed by UV.
i1	UASB reactor + VFCW (NA) + HFCW (NA) + UV	Investigation of UASB coupled with hybrid CWs (CWs without artificial aeration) followed by UV.

b) Explanation of aeration mode in HFCWs and VFCWs

Aeration mode		Description
(NA)	Not aerated CWs	No air was supplied during the experimental tests
(CA)	Continuously aerated CWs	Air was supplied for the whole duration of the experimental tests and through both half of the air distribution network
(PA)	Partially aerated CWs	Air was supplied at the first or at the second half of HFCWs and at the bottom or at the middle of VFCWs, either continuously or intermittently
(IA)	Intermittently aerated CWs	Air was supplied during limited periods of time (15 min per h, air flow rates of 20 and 16 L min^{-1} for HFCWs and VFCWs, respectively), either partially or on the whole air distribution network

wastewater through a horizontal pipe network located above the substrate. Wastewater level in the two HFCWs (saturated systems) was kept 5 cm below the substrate surface for the entire experimentation. With an average working temperature of 24.9 ± 2.2 °C, the single-stage CWs were fed with a flow rate of 7.0 ± 2.4 L h^{-1}; in HFCWs, it corresponded to an HLR of 0.14 ± 0.08 m^3 m^{-2} d^{-1} and to an HRT of 72.7 ± 26.1 h. VFCWs were characterized by an HLR of 0.17 ± 0.04 m^3 m^{-2} d^{-1}. The OLR for the HFCWs and VFCWs resulted to be, respectively, 19.5 ± 7.9 and 22.4 ± 9.3 g COD m^{-2} d^{-1}, while the NLR accounted for 4.3 ± 0.8 and 9.6 ± 1.8 g TN m^{-2} d^{-1}.

Two independent aeration systems were used to apply artificial aeration up to 20 L min^{-1}, calculated considering typical values reported in the literature (Pereyra, 2016), with the air distribution network placed at the bottom of the first and second half in HFCWs, and at the bottom and at 50 cm from the bottom in VFCWs. The air flow rate was manually regulated by means of flow meters (type a/m-95, max air flow rate 20 L min^{-1}, La tecnica-fluidi, Italy). Both types of CWs (HFCWs and VFCWs) were artificially aerated under different operating conditions, with a possibility to vary air flow rates, and to aerate different parts of the systems. Depending on the selected aeration mode, a total of four operating conditions were established. They are summarized in Table 1b.

In configuration 2, to further reduce the concentration of pathogens in the effluent, a disinfection unit, consisting of a cylindrical tube hosting an ultraviolet (UV) lamp (Emiambiente, model UVe® VEGA–10401, Italy), was placed at the end of the treatment chain with the influent flow rate ranging from 0.15 to 0.25 m^3 d^{-1}, resulting in an average contact time of 7.3 min. The intensity was 0.2 mW cm^{-2}, the dose was 65.9 mJ cm^{-2} and the transmittance was between 25 and 35%.

2.2. Analytical methods and wastewater characterization

For wastewater characterization, wastewater samples were collected at the inlet (influent) and at the outlet (effluent) of each treatment unit, and stored at 4 °C before being analyzed. COD, TSS, TN, NH_4^+-N, NO_3^--N, NO_2^--N and TP were selected as the parameters to be monitored throughout the experimental activity. The measurements were performed in accordance with the standard methods of the American Public Health Association (APHA, 2012). Total coliforms, faecal coliforms and *E. coli* determination was performed following the ISO 9308–1:2014 ("ISO 9308–1:2014; Water quality — Enumeration of *Escherichia coli* and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora," n.d.).

The characteristics of the pre-treated Imola WWTP influent (urban and agro-industrial wastewater) are summarized in Table 2.

2.3. Energy and carbon footprint assessment

Since during the experimental activity the demo-scale plant was tested to evaluate the efficiency and sustainability associated with wastewater treatment, the energy consumption of the different treatment units within the tested configurations no. 1 and 2 (Fig. 2) during their operations was evaluated and compared. Moreover, greenhouse gases (GHGs) directly emitted to air were measured through on-site monitoring campaigns. A peristaltic pump and gas bags (5 L) were used to acquire the gaseous samples (three replicates for each point). A hood (130L of volume and 0.25 m^2 of surface), connected with the peristaltic pump, was used to collect and convey the emitted gases into the gas bags. Subsequently, GHGs concentrations (CH_4, CO_2 and N_2O) were determined in laboratory using photoacoustic spectroscopy (Brüel & Kjaer Multi-gas Monitor Type 1302). Results were expressed as

Table 2
Characteristics of the pre-treated wastewater used as influent in the demo-scale plant.

Parameter	Unit	Value
pH	–	7.2 ± 0.1
Total chemical oxygen demand (COD)	mg L^{-1}	245.0 ± 96.0
Total suspend solids (TSS)	mg L^{-1}	73.0 ± 26.0
Total nitrogen (TN)	mg L^{-1}	52.0 ± 7.0
Ammonium nitrogen (NH_4^+-N)	mg L^{-1}	44.0 ± 7.0
Nitric nitrogen (NO_3^--N)	mg L^{-1}	0.5 ± 0.2
Nitrous nitrogen (NO_2^--N)	mg L^{-1}	0.0 ± 0.0
Total phosphorous (TP)	mg L^{-1}	5.3 ± 0.7
Total coliforms (TC)	CFU 100 mL^{-1}	$3.7 \times 10^7 \pm 3.9 \times 10^7$
Faecal coliforms (FC)	CFU 100 mL^{-1}	$2.5 \times 10^7 \pm 3.3 \times 10^7$
E. coli	CFU 100 mL^{-1}	$2.4 \times 10^6 \pm 1.8 \times 10^6$

emitted concentrations (mg L^{-1}) and emitted mass loads (mg d^{-1}) considering the air flowrate. Moreover, different emissions factors (EF) were calculated normalizing the emitted mass load of CH_4, CO_2 and N_2O with the influent mass loads of COD and TN (Marinelli et al., 2021).

3. Results and discussions

3.1. Demo-scale plant investigation

The main objective of all the experiments was to assess the treatment efficiency associated with the implementation of different treatment units belonging to the demo-scale plant. Additionally, the experiments aimed to assess the ability of these units to generate an effluent suitable for agricultural reuse. In this section, the research outcomes of the two tested configurations (configuration no. 1 (UASB + AnMBR) and configuration no. 2 (UASB + CWs + UV)) are reported and discussed. The discussion also focuses on the performance of the single treatment units, since they influenced the performance of the entire treatment chain, and, thus, the effluent characteristics in configurations no.1 and no.2. The complete dataset is reported in Table 3, showing removal efficiency and effluent concentrations.

On one hand, UASB reactor, working at 20 °C and with an average HRT of 12 h, resulted to remove 24.9% of total COD and 28.4% of soluble COD, while producing 2.5 ± 2.8 L of biogas h^{-1}, corresponding to a specific gas production of 18 ± 14 L of biogas per g of influent COD. Similar results were reported by Rattier et al. (2022), which observed a COD removal of 30% at temperature of 23 °C, with an HRT of about 10 h. The authors also noted that a higher HRT (17 h) can enhance COD removal, with levels reaching up 70%. On the other hand, UASB reactor have not led to any TSS, TN or TP reduction in the treated wastewater: TSS concentration was higher in the effluent (82.0 ± 18.6 mg L^{-1}) if compared to the inlet one (73.0 ± 25.6 mg L^{-1}) due to solids washout probably happened due to an up-flow velocity in the reactor higher than 1 m h^{-1}; TN and TP concentrations were the same in the influent and the effluent (52.0 ± 6.9 mg L^{-1} for TN and 5.3 ± 0.7 mg L^{-1} for TP). These results are consistent with the fact that the UASB technology is typically employed for biogas production from organic matter degradation and that its effluent, therefore, requires further treatment steps (Engida et al., 2020; Pandya et al., 2011). Hence, in configuration no. 1 UASB reactor was coupled with the AnMBR to obtain a better quality of the effluent in anaerobic conditions without the necessity of airflow and additional energy consumption. In this study the membrane effluent was averagely characterized by 70 mg COD L^{-1}, 51 mg N L^{-1}, 3.6 mg P L^{-1} and 1 mg TSS L^{-1}, in line with other existing studies coupling UASB and AnMBR (Foglia et al., 2019, 2020).

Testing HFCWs and VFCWs as not aerated and single-stage systems, HFCW (NA) resulted to reduce at the following extent the inlet parameters: COD 89.4%, TSS 82.2%, TN 48.1% and TP 77.4%; while for VFCW (NA) it was 94.3% for COD, 100% for TSS, 32.7% for TN and 96.2% for TP. VFCWs exhibited higher efficiency in the removal of COD, TSS, and TP compared to their HFCW counterpart, but they were generally less effective in removing TN. The unsaturated internal environment probably enhanced the metabolism of plants and bacteria towards the targeted molecules. The lower TN removal might therefore be attributed to increased nitrogen mobilization, such as conversion to nitrates (Abou-Elela et al., 2013; Thalla et al., 2019; Waly et al., 2022).

On the other hand, in the case of continuous aeration, HFCW (CA) allowed the reduction of COD 94.3%, TSS 86.3%, TN 42.3% and TP 86.8%, while VFCW (CA) abated COD 93.5%, TSS 100.0%, TN 30.8% and TP 98.1%. In continuously aerated systems, removal trends were quite the same as those of not aerated systems, indicating that in this case aeration did not affect treatment performance. Regarding the partial aeration application, that could lower energy consumption, the investigation was performed in HFCWs. HFCW (PA) granted the reduction of COD 93.1%, TSS 100.0%, TN 63.5% and TP 81.1%, while HFCW (IA) removed COD 91.8%, TSS 100.0%, TN 87.9% and TP 90.6%. The application of this aeration mode appeared to confer a slight boost to the removal activities. Partial aeration resulted to grant a net enhancement of the performance of the HFCW: a full TSS removal and an increase of about 4% for COD removal, 15% of TN and 4% of TP

Table 3
Removal efficiency and effluent concentration of the demo-scale plant treatment units.

Removal efficiency	%				*Log reduction*		
Plant configuration	COD	TSS	TN	TP	Total coliforms	Faecal coliforms	*E. coli*
a1	24.9	−12.3	0.0	0.0	0.4	0.5	0.4
b1	70.0	99.0	0.0	23.0	6.7	6.9	6.3
c1	89.4	82.2	48.1	77.4	2.0	2.0	2.7
c2	94.3	100.0	32.7	96.2	1.9	2.1	2.1
d1	94.3	86.3	42.3	86.8	3.1	3.2	3.5
d2	93.5	100.0	30.8	98.1	3.6	3.5	4.5
e1	94.3	86.3	42.3	86.8	3.1	3.2	3.5
e2	93.1	100.0	63.5	81.1	–	–	–
e3	91.8	100.0	87.9	90.6	3.2	3.2	4.9
f1	90.6	100.0	78.8	94.3	3.1	3.2	4.9
g1	91.8	100.0	65.4	98.1	–	–	–
h1	89.4	82.2	48.1	77.4	3.3	3.4	3.8
i1	91.8	100.0	65.4	98.1	3.7	4.3	5.3
Effluent concentration	*mg L^{-1}*				*Log CFU* 100 mL^{-1}		
Plant configuration	COD	TSS	TN	TP	Total coliforms	Faecal coliforms	*E. coli*
a1	184.0 ± 54.0	82.0 ± 9.0	52.0 ± 7.0	5.3 ± 0.7	7.2 ± 1.6	6.9 ± 0.9	6.0 ± 1.3
b1	70.0 ± 12.0	1.0 ± 0.0	51.0 ± 11.0	3.6 ± 0.7	0.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
c1	26.0 ± 7.0	13.0 ± 10.0	27.0 ± 3.0	1.2 ± 0.4	5.6 ± 0.7	5.4 ± 0.8	3.7 ± 0.4
c2	14.0 ± 4.0	0.0 ± 0.0	35.0 ± 10.0	0.2 ± 0.1	5.7 ± 1.2	5.3 ± 0.6	4.3 ± 0.5
d1	14.0 ± 2.0	10.0 ± 4.0	30.0 ± 8.0	0.7 ± 0.1	4.5 ± 1.1	4.2 ± 0.9	2.9 ± 0.5
d2	16.0 ± 3.0	0.0 ± 0.0	36.0 ± 8.0	0.1 ± 0.0	3.9 ± 1.2	3.9 ± 0.3	1.9 ± 0.2
e1	14.0 ± 2.0	10.0 ± 4.0	30.0 ± 8.0	0.7 ± 0.1	4.5 ± 0.7	4.2 ± 1.2	2.9 ± 0.8
e2	17.0 ± 11.0	0.0 ± 0.0	19.0 ± 9.0	1.0 ± 0.1	–	–	–
e3	20.0 ± 10.0	0.0 ± 0.0	6.3 ± 3.5	0.5 ± 0.1	4.4 ± 1.3	4.2 ± 0.5	1.4 ± 0.2
f1	23.0 ± 7.0	0.0 ± 0.0	11.0 ± 4.0	0.3 ± 0.2	4.5 ± 0.9	4.2 ± 1.1	1.5 ± 0.2
g1	20.0 ± 3.0	0.0 ± 0.0	18.0 ± 1.0	0.1 ± 0.1	–	–	–
h1	26.0 ± 7.0	13.0 ± 10.0	27.0 ± 3.0	1.2 ± 0.4	4.3 ± 0.4	4.0 ± 1.0	2.6 ± 0.3
i1	20.0 ± 3.0	0.0 ± 0.0	18.0 ± 1.0	0.1 ± 0.1	3.8 ± 3.3	3.1 ± 3.1	1.1 ± 0.5

abatement have been observed.

The aeration of the first half of the CW may have promoted turbulent flows within the systems, allowing a more efficient recirculation and, thus, a stronger interaction between the chemicals and the substrates, plants and microorganisms, resulting in the enhancement of removal efficiency. Intermittent aeration resulted to generally increase the overall performances, in particular towards the nutrients reduction: the intermittence approach may have stimulated particular microbial communities within the unit, potentially broadening the metabolic activity of the biotic factors (Donoso et al., 2019; Fan et al., 2013).

Therefore, although it is known that aeration can enhance CW performances, intermittent application may be the best choice, since it improves an overall performance while requiring smaller compressors functioning times, as compared to the continuous operation, resulting also in energy savings due to the lower amount of required/provided air.

In hybrid CWs, the combination of VFCW (NA) + HFCW (NA) overall reduced COD 90.6%, TSS 100.0%, TN 78.8% and TP 94.3%, while for the opposite combination HFCW (IA) + VFCW (NA), a removal of COD 91.8%, TSS 100.0%, TN 65.4% and TP 98.1% was observed. The combination of the units has slightly enhanced the CWs performance removing some of the measured parameters due to the existence of diverse conditions in these two types of CWs (Dan et al., 2023; Vymazal, 2005).

For the configuration no. 2, where an UV system was implemented at the end of the treatment chain, as expected, the UV unit did not affect COD, TN and TP concentrations (Franco and Azevedo, 2014). However, the ability of CWs to completely eliminate TSS from wastewater allowed unhindered operation of the UV disinfection unit (Friedler et al., 2021).

Microbial parameters, e.g., total coliforms, faecal coliforms and *E. coli*, were monitored in the effluent of each treatment unit. UASB reactor was characterized by very low removal rates. In fact, log reduction was 0.4, 0.5 and 0.4 for total coliforms, faecal coliforms and *E. coli*, respectively. The AD process is usually not highly efficient in removing pathogens due to the absence of oxygen and limited temperature sensitivity, which may allow some pathogens to survive (Amani et al., 2010). Longer HRTs may be required, and additional disinfection steps are often recommended to ensure compliance with pathogen removal standards. While UASB reactor itself reduced the pathogen levels in wastewater to a less extent, AnMBR through the filtration process promoted the complete removal of pathogens by physically preventing them from passing through the membrane, causing them to be absent in treated wastewater (0 Log CFU 100 mL^{-1}) and the removal efficiency to be the highest (log reduction higher than 6).

Single-stage CWs without aeration offered moderate pathogen removal; the involvement of mechanisms such as filtration, plant-uptake, sedimentation and microbial activity allowed a log reduction of about 2.0. The provision of artificial aeration in single-stage CWs enhanced pathogen removal (with log reduction up to 4.5 for *E. coli*, for example). Aerated CWs, employing mechanical aeration, can stimulate microbial activity favoring aerobic bacteria, potentially improving pathogen degradation compared to non-aerated systems (Nan et al., 2020). In particular, intermittently aerated CWs were more performant (with log reduction up to 4.9 for *E. coli*, for example) than continuously aerated CWs, probably due to the fact that the alternating cycles of aeration and non-aeration can enhance oxygen availability during aeration phases, promoting the growth of aerobic microorganisms (Hou et al., 2018). This intermittent exposure to oxygen stimulates microbial activity, facilitating more efficient degradation of pathogens. Additionally, the periods of non-aeration allow anaerobic conditions, which can promote diverse microbial communities that contribute to enhanced pathogens removal (Wu et al., 2016). Hybrid CWs were more efficient in removing pathogens from wastewater, since these systems can provide multiple stages for physical and biological processes, along with optimized treatment conditions (e.g., higher HRTs). The UV disinfection system at the end of the treatment chain enhanced the removal of pathogens (with log reduction up to 5.3 for *E. coli*, for example), ensuring the inactivation of pathogens in residual concentration in wastewater.

3.2. Comparison of effluent concentrations with water reuse standard quality

Effluent concentrations of each treatment unit were compared with the minimum water quality requirements provided by the Regulation (EU) 2020/741 in order to assess their suitability for agricultural reuse. Table 4 provides a summary of the comparison results.

The (EU) 2020/741 identifies as water quality class A with the most stringent limits, encompassing food crops with edible parts in direct contact with reclaimed water and consumed raw, vulnerable to a higher microbiological risk. For water class A, the maximum admissible *E. coli* concentration is 10 CFU 100 mL^{-1}, while in water quality classes B, C, and D, the respective threshold values for *E. coli* concentrations are 100, 1,000, and 10,000 CFU 100 mL^{-1}.

In configuration no. 1 (UASB + AnMBR), the absence of *E. coli* in the AnMBR effluent, observed as 0 CFU 100 mL^{-1}, proved that the treated wastewater met the criteria for water quality class A, indicating its possible safety reuse.

In configuration no. 2 (UASB + single-stage/hybrid CWs + UV), the effluent of UV implemented downstream to the single-stage setups exhibited an *E. coli* concentration of 363 CFU 100 mL^{-1}, demonstrating that the effluent was suitable for water quality class C. On the other hand, the effluent of UV treatment in combination with hybrid CWs resulted in a higher pathogen removal efficiency, ensuring water quality class B with a concentration of 11 CFU 100 mL^{-1}, even though class A standards were not attained, possibly due to a low HRT or an insufficient radiation dose in the UV system.

As only COD data were available, the assessment of BOD_5 to compare with the (EU) 2020/741 limits relied on the assumption of a COD to BOD_5 ratio of 2:1 (Metcalf et al., 2002). Comparing the two configurations no. 1 and 2, both the UASB reactor + AnMBR and the single-stage CWs coupled with UV provided treated effluent of quality B, with BOD_5 concentrations of around 35 and 13 mg L^{-1}, respectively. The CWs, however, demonstrated a higher removal of organic matter compared to the AnMBR. Moreover, in configuration no. 2, the hybrid CWs facilitated an additional decrease in organic matter, leading to an effluent with a concentration of around 10 mg L^{-1}, thus suitable as water quality class A.

CWs are typically adept at removing solids from wastewater due to their natural filtration and sedimentation processes. In fact, single-stage CWs reduced the TSS content to 13 mg L^{-1}, providing an effluent of water quality class B. Moreover, in both the configurations no. 1 and 2, the AnMBR and the hybrid CWs demonstrated the ability to achieve even lower TSS concentrations (0 mg L^{-1}), indicating that treated wastewater was suitable for water quality class A.

Therefore, water quality classes of the two tested configurations were selected considering the lowest class among those defined for the different parameters (*E. coli*, BOD_5, TSS). Consequently, in configuration no. 1, the effluent was classified as water quality class B, while in configuration no. 2, it was classified as water quality class C (single-stage CWs) and B (hybrid CWs).

3.3. Energy and carbon footprint assessment

Fig. 3 shows a comparison of the power absorbed by the units, indicating the kWh m^{-3} consumed daily for wastewater treatment. The power consumed by the UASB reactor was linked to the use of two pumps for feeding and recirculating wastewater, an electric heater to maintain the temperature between 20 and 30 °C, and a biogas meter. In the AnMBR system, power was consumed by the pump feeding the membrane and by other electronic hydraulic components (e.g., solenoid valves and pressure gauges). In not-aerated (NA) CWs, power was consumed by the feeding pump, while in aerated systems (CA, PA or IA),

Table 4
Comparison of treated wastewater quality with the limits imposed by the Regulation (EU) 2020/741.

Regulation (EU) 2020/741				
Parameter	Water quality classes			
	A	B	C	D
E. coli (CFU 100 mL^{-1})	≤ 10	≤ 100	≤ 1,000	≤ 10,000
BOD_5 (mg L^{-1})	≤ 10	≤ 25		
TSS (mg L^{-1})	≤ 10	≤ 35 (Imola WWTP is designed for more than 10,000 PE)		

Observed concentrations in the effluent of the different treatment units			
	E. coli (CFU 100mL^{-1})	BOD_5 * (mg L^{-1})	TSS (mg L^{-1})
a1	986,667	92	82
b1	0	35	1
c1	4,650	13	13
c2	21,173	7	0
d1	850	7	10
d2	80	8	0
e1	850	7	10
e2	-	9	0
e3	28	10	0
f1	32	12	0
g1	-	10	0
h1	363	13	13
i1	11	10	0

* Estimated from COD considering COD:BOD_5 = 2:1.
Note: Red color indicates that treated wastewater was not suitable for agricultural reuse, while the other colors refer to the water quality classes as reported at the table top.

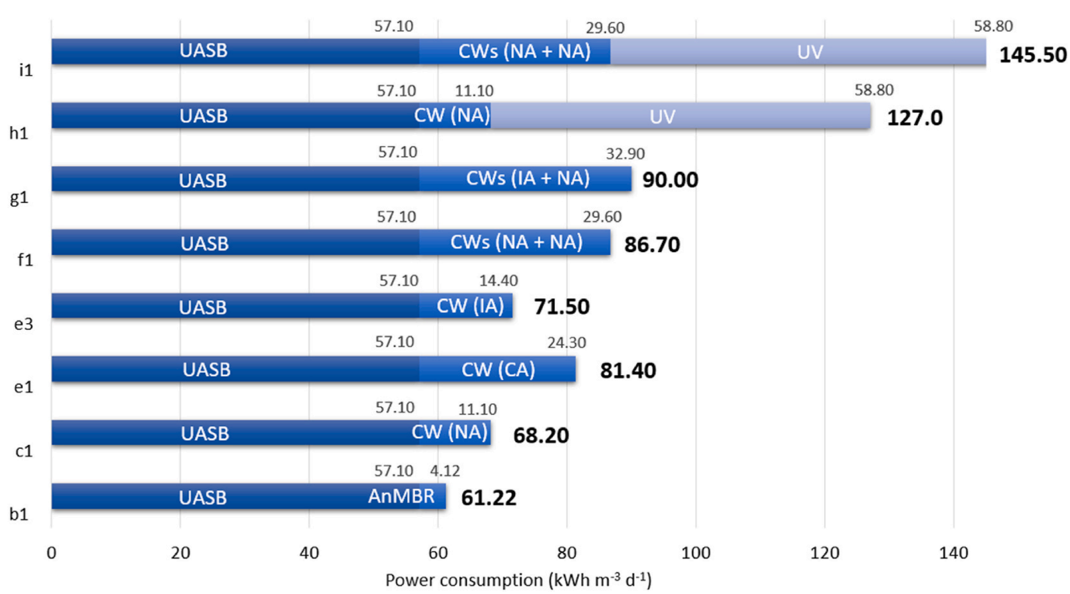

Fig. 3. Absorbed power in the different tested configurations.

power for air compressors in addition to the feeding pump was required. In the UV reactor, power was used by the feeding pump and the UV lamp.

The UASB reactor and the UV reactor were the treatment units that absorbed the highest daily power, (57.10 kWh m^{-3} d^{-1} and 58.80 kWh m^{-3} d^{-1}, respectively. These findings are similar to those reported by Medeirod et al. (Medeiros et al., 2023), indicating a daily energy consumption of 96.38 kWh to treat 1 m^3 of pre-treated influent sewage using UASB reactor followed by disinfection. On the contrary, the AnMBR system had the lowest power requirement (4.12 kWh m^{-3} d^{-1}). However, this value exceeded those reported in other previous studies. For example, Rong et al. (2022), in their AnMBR system, observed an annual net energy demand of 0.10 kWh m^{-3}. The difference in energy consumption may be attributed to several factors such as system design, operational parameters, and environmental conditions. NA CWs absorbed 11.10 kWh m^{-3} d^{-1}. Also for these systems, the observed values of energy consumption were higher than those reported in the literature. For instance, Brunhoferova et al. measured the energy requirement of the CW system as 0.28 kWh m^{-3} d^{-1} (Brunhoferova et al., 2024). Specifically, in this case, the difference was due to the characteristics of the pumps, which notably influenced the absorbed power: 0.28 kWh in the system of Brunhoferova et al. while 2.22 kWh in the current investigation. Nevertheless, in full-scale applications, CWs typically operate with minimal energy requirements (~0 kWh m^{-3} d^{-1}), because these systems can be fed with water using only gravity, rendering the energy consumption by pumps negligible (Fernández del Castillo et al., 2022). In aerated CWs, the absorbed power was influenced by both the pumps used for wastewater supply and the air compressors employed for providing artificial air. Furthermore, in this study, the air flow rate to the CWs was controlled using flowmeters, while the air compressor consistently delivered the same flow rate throughout most of the experimental period, even when it was not entirely required, realizing the surplus of air directly into the atmosphere. For these reasons, in these systems, power requirements increased to 14.40 kWh m^{-3} d^{-1} for IA single-stage CWs and 24.30 kWh m^{-3} d^{-1} for CA single-stage CWs. In PA CWs, power consumption was similar to CA and IA CWs. In hybrid CWs, the total power required was the sum of the single CWs under the specific operating conditions. NA hybrid CWs required 29.60 kWh m^{-3} d^{-1}, while in the case of IA in one of the two systems, power consumption raised up to 32.90 kWh m^{-3} d^{-1}.

In configuration no. 1 (UASB + AnMBR), the power consumption was 61.22 kWh d^{-1} m^{-3}, whereas in configuration no. 2 (UASB + CWs + UV), it was slightly higher, e.g., 127.00 kWh d^{-1} m^{-3} and 145.55 kWh d^{-1} m^{-3} when combining the UASB and UV reactors with not-aerated single-stage and not-aerated hybrid CWs, respectively. The UASB and UV reactors needed the most power, accounting for approximately 40% of the total power required by the entire treatment process. AnMBR and CWs (not aerated systems) required the lowest energy input: these systems accounted for less than 17%.

The higher power required in configuration no. 2 compared to no. 1 was due to the implementation of the UV system, which was necessary to ensure comparable reduction of pathogens. In addition, that configuration also implemented hybrid CW systems therefore also contributing to a higher energy consumption, but, on the other hand, it allowed a higher removal efficiency for the other contaminants (e.g., COD, TSS, TN, and TP as reported in Table 3).

The UASB reactor was monitored to quantify the recovered energy in the form of biogas. The research findings indicated a biogas production of approximately 2.5 L h^{-1} (60.0 L d^{-1}). Converting this biogas volume into power (Lusiana et al., 2021; Perez-Sanz et al., 2019), it amounted to around 0.132 kWh d^{-1} m^{-3}. This amount was lower than the energy required by the demo-scale plant, similar to some of the previous studies (Mainardis et al., 2020; Medeiros et al., 2023). The demo-scale plants operate at a smaller scale, leading to scale effects where inefficiencies related to equipment sizing, typology and operation can contribute to higher energy consumption (Mesquita et al., 2021). Nevertheless, UASB's installation in a WWTP can have numerous benefits, such as reduction of oxygen demand and air supply and lower specific sludge production, resulting in lower sludge processing and disposal costs (Cecconet et al., 2022). However, these findings highlight the need of further studies to assess an overall energy balance in full-scale applications.

With respect to direct GHGs emissions, the results of the monitoring campaign in the CW are reported in Table 5. Plants of CWs play many vital roles in CWs, not only influencing microbial processes and their by-products via the release of oxygen and available carbon from plant roots to the soil, but, also, acting as a significant transport channel for GHG emissions into the atmosphere. In addition, plants fix CO_2 from the atmosphere into sediments via photosynthesis (Yin et al., 2023). CH_4, CO_2 and N_2O concentrations in this study resulted in the order of 0.001–0.002 mg L^{-1}, 0.08–0.16 mg L^{-1} and 0.002–0.005 mg L^{-1}, respectively. Considering the NA CWs, EFs were very low due to the absence of airflow and of stripping phenomena, while in intermittently-aerated CWs, EFs were higher and equal to 9.31E-05 kg CH_4 kg COD^{-1}, to 7.34E-03 kg CO_2 kgCOD^{-1} and to 5.20E-04 kg N_2O kg TN^{-1}. The obtained values of this study are lower than those reported by IPCC 2013 equal to 4.25E-01 kg CH_4 kg COD^{-1} and to 7.90E-03 kg N_2O kg TN^{-1}. This was probably due to the fact that the effect of plants on GHG emissions in CWs is complex, which can either increase or decrease GHG release, depending on the presence, species, richness, growth situation, harvest of plants (Yin et al., 2023).

This research proves that the scalability potential of the proposed treatment configurations in decentralized scenarios is indeed a crucial aspect to consider. In fact, the demo-scale plant, intended primarily for testing purposes, may not prioritize energy efficiency (while ensuring contaminants removal from wastewater) to the same extent as full-scale plants, which undergo extensive engineering optimizations, lowering the overall energy input. Therefore, it is imperative to conduct further investigation to comprehensively evaluate scalability, considering as key indicators treatment efficiency, cost-effectiveness, operational and site-specific conditions.

4. Conclusions

This study examined the combination of intensive and natural systems for wastewater treatment to improve contaminant removal efficiency and reduce costs, as well as their capability to produce effluents suitable for water reuse in agricultural irrigation. UASB, AnMBR and UV disinfection were the intensive technologies, while CWs the natural systems that were investigated. In detail, UASB rector was combined with AnMBR (configuration no. 1) and with CWs and UV (configuration no. 2). The UASB reactor effectively removed organic matter and produced biogas, but had limited impact on suspended solids and nutrients removal. AnMBR enhanced suspended solids removal, with ultra-filtration promoting also pathogens and nutrients removal. Among the tested CWs, VFCWs demonstrated higher removal efficiency compared to HFCWs in most cases, except for nitrogen removal where HFCWs were more effective.

Aeration did not significantly impact treatment performance, with partial aeration slightly boosting removal activities. Partial aeration enhanced treatment performances by removing suspended solids and improving organic matter and nutrient removal. Intermittent aeration generally increased overall performance, especially for nutrient reduction.

Hybrid CWs slightly improved treatment performance exploiting varied conditions in the two different types of CWs (e.g., VFCWs and HFCWs). In both the tested configurations, the effluents exhibited minimal levels of microbial indicators, organic matter, and suspended solids, indicating that treated wastewater was suitable for agricultural reuse. These results can be attributed to the ultra-filtration capabilities of AnMBR, the intricate interplay among soil, vegetation, and microbial consortia in CWs, and the disinfection efficacy of the UV system.

Table 5
GHGs emissions results in the CWs.

Tested units	CH_4			CO_2			N_2O		
Description	mg L^{-1}	mg CH_4 d^{-1}	EF_kg CH_4 kg COD^{-1}	mg L^{-1}	mg CO_2 d^{-1}	EF kg CO_2 kg COD^{-1}	mg L^{-1}	mg N_2O d^{-1}	EF kg N_2O kg TN^{-1}
VFCW (NA) + HFCW (NA)	0.002	0.003	5.38E-08	0.16	0.171	3.94E-06	0.005	0.005	4.54E-07
HFCW (IA)	0.001	6.4	9.31E-05	0.08	446	7.34E-03	0.002	9.2	5.20E-04

As expected, CWs proved to be the most energy-efficient treatment systems compared to the other tested technologies (e.g., UASB, AnMBR, UV), with UASB technology requiring the highest energy consumption. In the context of GHG emissions, plants in CWs played crucial roles by influencing microbial processes and by serving as channels for GHG transport into the atmosphere. They released oxygen and carbon from roots to the soil, while also sequestering CO_2 through photosynthesis. In non-aerated CWs, emission factors remained low due to the absence of airflow and stripping mechanisms, whereas intermittently aerated CWs exhibited higher emission factors. This confirmed the complexity of plant-microbe interactions and their impact on GHG dynamics in CWs.

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CRediT authorship contribution statement

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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