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Performance comparison of microalgae-based and activated sludge with membrane filtration (AS-MBR) for emerging contaminant removal and wastewater reuse

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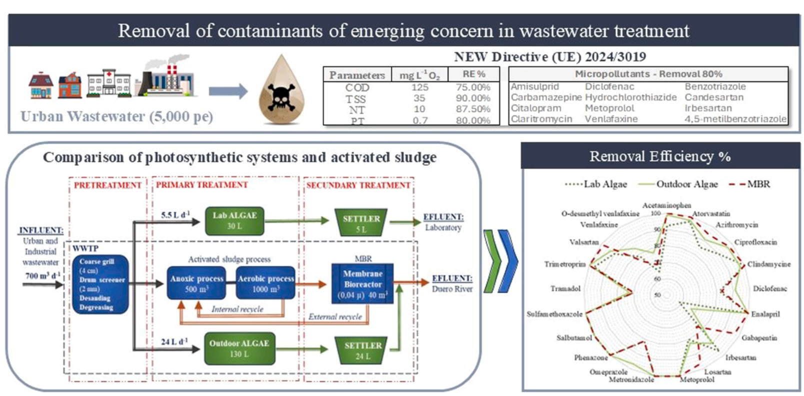
HIGHLIGHTS

- Membrane and microalgae systems reached the pathogen concentration required for water reuse.
- The removal efficiency ranged from more than 50 % to total removal.
- Bioadsorption and photodegradation are the most important elimination pathways in microalgae system.
- Both systems reached the limits stablished in the European regulation for emerging pollutants.

GRAPHICAL ABSTRACT



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ABSTRACT

The removal efficiency of microalgae cultures was compared to that of an activated sludge system with ultrafiltration. Three systems were assessed: an outdoor algae pond, a lab-scale setup simulating open-air conditions, and a full-scale activated sludge facility. High removal rates (>80 %) were observed for hydrophobic, biodegradable or volatile compounds across all systems, despite differences in treatment nature. In microalgae systems, bioadsorption and photodegradation were key pathways, with enhanced removal of UV-sensitive compounds in the outdoor pond. In the activated sludge membrane-assisted system, biosorption and volatilization led to very low pollutant concentrations in the effluent. The membrane bioreactor outperformed conventional activated sludge systems due to higher aeration, sludge age and the elevated solids concentration. The

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three tested systems produce final effluents that met European standards for reclaimed water, in terms of biological contamination, and the limits proposed for emerging contaminant removal.

1. Introduction

There is a growing concern about occurrence and release of new pollutants that can potentially affect human health and the environment [1]. Occurrence of contaminants of emerging concern (CECs) in urban wastewaters and surface waters has been reported in several locations around the world [2–5]. The impacts that CEC may cause to aquatic fauna and flora are of special concern, including diseases and population decline [6]. Additionally, CECs, when discharged into the environment, can re-enter the food chain, causing problems for human health depending on the compound [7,8].

In Europe, the contaminants of emerging concern (CECs) needing observation are published by the European Commission (EU), establishing the priorities and methods for determination. The new Directive (EU) 2024/3019 [9] concerning urban wastewater treatment' discusses micropollutants, focusing on pharmaceuticals, which are of increasing alarm for water quality. The Directive therefore recommends that certain urban wastewater treatment plants (WWTPs) implement additional treatment systems for pollutant removal, emphasizing the need for substantial efforts to overcome the limitations of conventional practices through innovative treatment technologies. The design of conventional WWTPs has focused on the removal of organic matter, nitrogen, and phosphorus from wastewater to prevent river eutrophication. However, most existing facilities lack dedicated processes for the elimination of CECs [10]. Therefore, new treatment approaches for these new compounds, commonly found in urban wastewater, are necessary. The treatments studied for CECs removal mainly consist of various physical and chemical treatments such as adsorption, micro/-ultrafiltration, reverse osmosis, advanced oxidation processes (AOPs), and coagulation, which have shown efficiency in CECs removal but in some cases involved excessive costs of installation and operation [11–15]. Among conventional systems, filtration units, such as membrane bioreactors (MBR) have been proposed for their relatively easy implementation and their compatibility with water reuse systems, since they provide pathogen removal. On the other hand, alternative treatments based on natural systems have been proposed: microalgae culture in wastewater, wetlands and green filters [16, 17]. Among these, microalgae offer additional advantages in terms of sustainability since these systems capture carbon dioxide and produce oxygen through photosynthesis without important energy costs, mitigating greenhouse gases and climate change. Microalgae culture is an environmentally friendly technology due to the use of solar energy and its ability to adapt to different types of wastewaters [18]. Urban wastewater typically contains all the nutrients required for microalgal growth and can facilitate the removal or transformation of CECs into compounds that are less harmful to humans. Furthermore, this treatment can have added value due to the subsequent use of microalgae biomass for transformation into products such as biodiesel, biopolymers and biofertilizers [19]. Besides, algal culture in shallow lagoons mechanically mixed, called High Rate Algae Ponds (HRAP) provides removal of pathogens, providing final effluents of sufficient quality to achieve the requirements established for water reuse (for the purpose of irrigation or different kinds of urban services) [20]. Although, the capacity to reduce the content of organic matter, nitrogen, phosphorus and heavy metals of microalgae-based treatments is well-known, the potential for CECs removal has not been compared with one of the most promising conventional systems: activated sludge with membrane bioreactor, which is known to remove chemical pollutants along with pathogen bacteria [22]. Besides this, most of the previously reported experimentation with algae-based systems is based on lab or pilot-scale outdoor systems, that present very different conditions in terms of illumination and mass transfer, which prevents drawing definitive conclusions [21].

This work studies the elimination of selected pollutants from urban wastewater in non-conventional treatments based on microalgae growth under both experimental conditions: laboratory and outdoor pilot. The performance of pollutant removal was compared with an activated sludge real scale facility provided with a membrane bioreactor. Additionally, the effluents obtained after the different treatments were evaluated also in terms of pathogen removal according to the water reuse regulation in Europe.

2. Materials and methods

2.1. Description of treatments

The study was conducted using two types of systems: microalgae cultures, which included an open (outdoor) system and a laboratory-scale system, and the activated sludge from the wastewater treatment plant (WWTP). The experimental systems were evaluated under temperate climatic conditions, corresponding to the months of September to November at a mid-latitude location. Outdoor system of microalgae was placed inside the Garray wastewater treatment plant (WWTP) located in North Spain (GPS coordinates 41.800136, −2.451801), where the real scale facility of activated sludge serves as sewage treatment. The WWTP collects both urban and industrial wastewater and has a treatment capacity of 5000 p.e. (person equivalents). The wastewater treatments composed of three stages: pre-treatment, activated sludge system with anoxic/aerobic tanks and a membrane bioreactor (MBR) serving as a secondary clarifier. The biological treatment consists on the conventional anoxic/aerobic process with internal recycle addressed to organic matter and nitrogen removal through nitrification/denitrification (Fig. 1). The biomass separation consisted on a membrane bioreactor (Membrane ZeeWeed 5000 Cassette Immersed Hollow-Fiber Ultrafiltration Technology of General Electric Company) [22]. At this point it must be stressed that the effluent samples were taken after the biomass separation module, then the activated sludge system was evaluated as a whole, without intermediate sampling of individual treatment steps. Following the generally adopted nomenclature this system is designed as AS-MBR (Activated Sludge Membrane Bioreactor). A complete description of the system is included in the supplementary material section (SM1).

In the case of microalgae treatments two experimental set-ups were evaluated: and outdoor pond placed in the WWTP and a lab scale pond, where open-air conditions were mimicked. Therefore, the possible differences between outdoor and lab scale experimental were evaluated. The laboratory raceway (Lab Algae) was situated at the laboratory of Chemical Engineering of Department of Valladolid University in Campus of Soria. The system consisted of a raceway with a total volume of 30 L. The surface of the raceway was illuminated with a day-light simulator program depending on two LED modules of visible light spectrum. The LED modules did not include ultraviolet light. Daily and seasonal variations of light intensity and temperature correspond to two conditions: winter and summer, which were coded with an Arduino device (IDE) that modulated the light intensity and temperature by means of a Pulse Width Modulation (PWM). The Lab Algae experimental set-up replicated the environmental conditions that naturally occur in the Outdoor Algae reactor during clear sky days in the selected location. The hydraulic retention time (HRT) chosen was 5 days [21]. Regarding mass transfer characteristics of the Lab Algae system, the empirical values of the reactor were determined for the gas-liquid volumetric oxygen mass transfer coefficient, resulting in a value of $0.39 \pm 0.02\ h^{-1}$. Similar studies conducted on outdoor HRAPs for wastewater treatment have reported values of the same order of magnitude [23]. The system of the laboratory scale plant is described in [18] and supplementary material (SM2). The outdoor microalgae-based system was installed at the municipal WWTP of Garray and consisted of an open raceway reactor (Outdoor Algae) with a working volume of 130 L. This reactor corresponded to the commonly accepted configuration of a High-Rate Algal Pond (HRAP), characterized by a shallow closed-loop channel continuously mixed by a paddle wheel. A complete description of the system can be found in SM2.

2.2. Sampling

Water samples were collected two days per week to determine operating parameters and removal efficiency (RE). The photobioreactors were operated for 120 days, initially filled with wastewater, inoculated and operated at a hydraulic retention time (HRT) of 5 days. The operational conditions were maintained for 120 days to obtain representative steady states. A total of 32 daily values (for each experimental system) were estimated during the 16 weeks of sampling. To obtain representative results, the influent was collected as a 24-hour composite sample. This water was used to fill the influent tank of the scale laboratory raceway, which was changed at each influent sampling (Monday-Wednesday-Friday). Daily composite influent and effluent samples were collected on six different days to analyse the target pharmaceuticals, covering a two-weeks period: 28th and 30th November 2022 and 2nd, 5th, 7th and 9th December 2022 (Monday-Wednesday-Friday for two weeks).

Upon receipt in the laboratory, the samples were immediately stored in the dark at −20°C until transport to the Research Institute of Pesticides and Water (IUPA), University Jaume I, Castellón (Spain), for further determination of CECs. All samples were collected in high-density polyethylene bottles. Measurements of temperature, dissolved oxygen and pH were taken *in situ* in each system.

2.3. Chemicals and reagents

Pharmaceutical reference standards were purchased from Sigma-Aldrich (St Louis, MO, USA), LGC Promochem (London, UK), Toronto Research Chemicals (Ontario, Canada), Across Organics (Geel, Belgium), Bayer Hispania (Barcelona, Spain), Fort Dodge Veterinaria (Gerona, Spain), Vetoquinol Industrial (Madrid, Spain) and Aventis Pharma (Madrid, Spain) and the isotope labelled internal standards (ILIS) were from CDN Isotopes (Quebec, Canada); Toronto Research Chemicals, Cambridge Isotope Laboratories (Andover, MA, USA) Sigma-Aldrich (St Louis, MO, USA) and Cerilliant (Texas, USA). All reference standards presented purity higher than 93 %. Individual standard stock solutions were prepared at concentrations between 50 at 500 mg/L. Intermediate solutions of 10 mg L^{-1} were prepared by dilution with methanol. Mixed working solutions containing all analytes at the μg L^{-1} level were prepared weekly from intermediate solutions by appropriate dilutions with water and were used for preparation of the aqueous standard calibrations and for spiking samples used as quality control. All solutions were stored in amber glass bottles at −20 °C.

HPLC-grade methanol (MeOH), HPLC-grade acetonitrile (ACN), formic acid (HCOOH, content >98 %) and ammonium acetate (NH_4Ac, reagent grade), were purchased from Scharlab (Barcelona, Spain). HPLC-grade water was obtained from distilled water that was passed through a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.4. Analytical methods

2.4.1. Samples characterization

Wastewater treatment efficiency and operational conditions were evaluated using total and volatile suspended solids (TSS and VSS), COD, ammonium nitrogen (N-NH_4^+), phosphorus (P-PO_4^{-3}), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) that were determined according to Standard Methods for the Examination of Water and Wastewater [24] and determined in each sample in Department of Chemical Engineering of Campus of Soria.

Biomass productivity (g VSS $m^{-2}d^{-1}$), in case of microalgae experiments, was determined as volatile suspended solids (VSS) concentration in the bulk liquid, considering the wastewater flow rate and the raceway surface flow rate according to [25] using (1):

$$Biomass\ poductivity = \frac{VSS(Q + Q_E + Q_P)}{A} \quad (1)$$

VSS is the volatile suspended solids concentration of the microalgae cultures (g VSS L^{-1}); Q is the wastewater flow rate (L d^{-1}); Q_E is the evaporation rate (L d^{-1}); Q_P is the precipitation rate (L d^{-1}); and A is the surface area of the raceways (0.16 m^2 for Lab Algae and 0.56 m^2 for Outdoor Algae). Meteorological data were obtained from the net of local weather stations in Soria (https://opendata.aemet.es).

Evaporation rates were determined by the measurement of the decline in depth in batch mode experiments at the tested environmental conditions. The evaporation rate (Q_E) was used in the mass balances and biomass productivity calculations in agreement with de Godos et al. [26].

To evaluate the requirements for water reuse, a method was used for detection and enumeration of Bacteria (*Escherichia coli* and *Enterobacter*

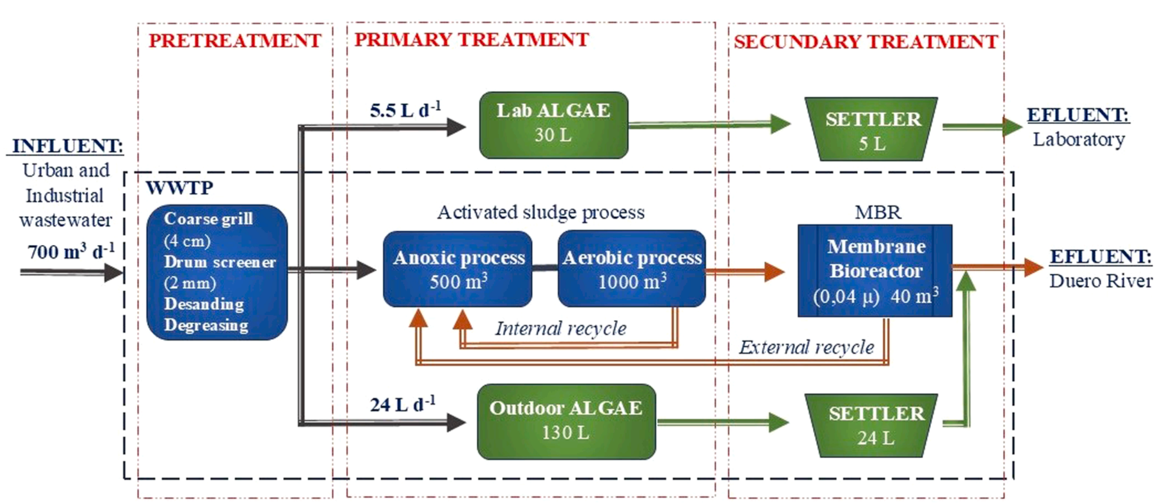

Fig. 1. Scheme diagram of activated sludge and photosynthetic systems.

Faecalis.). The concentration in influent and effluent was determined using the protocol described in the ISO 9308–1:2014) [27] with Chromocult® Coliform Agar (CCA). The method is based on membrane filtration for 100 ml and Petri dishes cultivation, subsequent culture on a chromogenic coliform agar medium, and calculation of the number of target organisms in the sample.

Removal efficiency (RE) was calculated using the average influent concentration as shown in (2):

$$Removal\ efficiency(\%) = 100 \times \frac{(\overline{C}_{IWW} - \overline{C}_{EWW})}{\overline{C}_{IWW}} \quad (2)$$

The physicochemical properties such as molecular weight, octanol-water partition coefficient (K_{ow}), adsorption potential (K_d), Coefficient of biodegradability (K_{BIOD}) and ionization constant (pK_a) of the selected pollutants was obtained from different online sources (see SM4) [28].

2.4.2. *Analysis of the target compounds*

This research focuses on 39 target pharmaceuticals from different families and functions presented. Only 26 of them were found either in influent or effluent wastewater (IWW and EWW, respectively). Table 1 summarizes the compounds (detected and undetected) and classifications. A complete description of the characteristics of each compound is included in the SM section (Table SM2). The study includes a large diversity of substance families and physicochemical properties. The selection of pharmaceutical compounds was based on the following criteria: 1) high consumption, 2) high detection rates in wastewater, 3) potential risks to ecosystems and human health, and 4) the accessibility to the analytical methods. Hence, this research focused on the following groups (G) of pharmaceuticals, which included a large diversity of physicochemical properties: (GI) Analgesics, (GII) NSAIDs, (GIII) Hypolipidemic agents, (GIV) Antibiotics, (V) Antiepileptics/Sedatives/Anxiolytics, (GVI) Antihypertensives, (GVII) Beta-blockers, (GVIII) Antiulcer drugs, (GIX) Antidepressants, and (GXI) Metabolites.

Table 1
Pharmaceutical compounds found in wastewater samples and classification.

Compound (detected)	Family/Function	Compound (undetected)	Family/ Function
Acetaminophen	(GI) Analgesics	Furaltadone	(GIV) Antibiotics
Alprozalam	(GV) Antiepileptics/ Sedative/Anxiolytic	Flumequine	(GIV) Antibiotics
Atorvastatin	(GIII) Hypolipidemic agents	Ioprimide	Diagnostic agent
Azithromycin	(GIV) Antibiotics	Levamisole	(GIV) Antibiotics
Carbamazepine	(GV) Antiepileptics/ Sedative/Anxiolytic	Lorazepam	(GV) Antiepileptics/ Sedative/ Anxiolytic
Ciprofloxacin	(GIV) Antibiotics	Nalidixic acid	(GIV) Antibiotics
Clarithromycin	(GIV) Antibiotics	Norfloxacin	(GIV) Antibiotics
Clindamycin	(GIV) Antibiotics	Oxolinic acid	(GIV) Antibiotics
Diclofenac	(GII) NSAIDs	Pantoprazole	Acid Suppressant
Enalapril	(GVI) Antihypertensives	Primidone	(GV) Antiepileptics/ Sedative/ Anxiolytic
Erythromycin	(GIV) Antibiotics	Roxithromycin	(GIV) Antibiotics
Gabapentin	(GV) Antiepileptics/ Sedative/Anxiolytic	Sulfadiazine	(GIV) Antibiotics
Irbesartan	(GVI) Antihypertensives	Tetracycline	(GIV) Antibiotics
Lincomycin	(GIV) Antibiotics		
Losartan	(GVI) Antihypertensives		
Metronidazole	(GVIII) Antiulcer drugs		
Metoprolol	(GVII) Beta-blockers		
Omeprazole sulphide 4-OH	(GVIII) Antiulcer drugs		
Phenazone	(GII) NSAIDs		
Salbutamol	(GVII) Beta-blockers		
Sulfamethoxazole	(GVIII) Antiulcer drugs		
Tramadol	(GI) Analgesics		
Trimethoprim	(GVIII) Antiulcer drugs		
Valsartan	(GVI) Antihypertensives		
Venlafaxine	(GV) Antiepileptics/ Sedative/Anxiolytic		
O-dimethyl venlafaxine	Metabolite (Velafaxine)		

(GI) Analgesics, (GII) NSAIDs, (GIII) Hypolipidemic agents, (GIV) Antibiotics, (V) Antiepileptics/Sedative/Anxiolytic, (GVI) Antihypertensives, (GVII) Beta-blockers, (GVIII) Antiulcer drugs, (GIX) Antidepressants, and (GXI) Metabolites

The quantitative determination of the 39 target pharmaceuticals was performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with triple quadrupole (Waters Acquity H-Class UPLC, equipped with a binary pump system, interfaced to a triple quadrupole Xevo TQ-STM mass spectrometer, with ESI source (Waters Corp)). The samples were centrifuged and then analysed by direct injection into the LC-MS/MS without any pre-concentration step. The analytical procedure included a 5-fold dilution for influent wastewater (IWW) and 2-fold dilution for effluent wastewater (EWW), and it was as follows: a 200 µL aliquot of IWW was taken (or 500 µL of EWW), and then 750 µL Milli-Q water (or 450 µL Milli-Q water for EWW) and 50 µL of isotope-labelled internal standards (ILIS) mix (at 20 µg/L each) were added (final ILIS concentration in the injected samples, 1 µg L^{-1}). Finally, 50 µL were injected into the LC-MS/MS. Three MS/MS transitions were acquired for each compound, one for quantification and the remaining ones for confirmation of the identity of the compound. Nearly all compounds had their own analyte-ILIS available for an efficient matrix effects correction (36 ILIS out of 39 compounds). The limits of quantification (LOQ) were established by default as the lowest calibration level (5 ng/L), which considering the dilution of the samples was equivalent to 10 ng/L for EWW and 25 ng/L for IWW. The analytical methodology has been supported by the analysis of numerous quality control (QC) samples along several years of application, showing its reliability, robustness and applicability to different water samples around the world ([3,4,29,30]).More information on the analytical methodology applied can be found in SM 3 and the above indicated references.

In this work, a set of QC samples were prepared and analysed together with the sample batch. QCs consisted of real-world wastewater samples spiked with the target pharmaceuticals at two concentration levels 0,1 and 1,0 µg L^{-1}. The results obtained for the wide majority of QCs were satisfactory with recoveries between 60 % and 140 %, and mostly between 70 % and 120 % [31]. Some QCs recoveries at the lower level could not be calculated due to the presence of analytes at high concentrations in the “blank” samples used for QCs preparation.

3. Results and discussion

3.1. *Assessment of conventional wastewater quality parameters*

The operational conditions, removal efficiency of conventional wastewater quality parameters and water for reuse for the Laboratory raceway (Lab Algae), Outdoor raceway (Outdoor Algae) and MBR are shown in Table 2.

The average biomass yield observed in the microalgal systems was comparable to values reported in previous studies involving laboratory and outdoor-scale domestic wastewater treatment systems. Matamoros et al. [16] reported a production of 8 g VSS m^{-2} d^{-1} in cold season for HRAPs operating at 4 HRT, under similar climatic conditions, comparable to Arashiro [25], where the algal biomass productivity from November to January in two HRAP systems, with and without pretreatment were, 15 ± 4 and 10 ± 3 g VSS m^{-2} d^{-1} with 4.5 days of HRT. As expected, COD and TSS removal performances in the AS-MBR were

Table 2
Operational conditions of the Laboratory raceway (Lab Algae), Outdoor raceway (Outdoor Algae) and MBR in WWTP. Removal efficiencies for COD, N-NH_4^+, P-PO_4^{-3}, TSS, *Escherichia coli* and *Enterobacter Faecalis*.

	Parameters	Treatment system		
		Lab Algae	Outdoor Algae	MBR
Operation Condition	Reactor temperature (°C)	14.8 ± 1.7	14.1 ± 2.1	14.2 ± 0.8
	pH	9.6 ± 0.8	9.1 ± 1.1	7.5 ± 0.4
	HRT (days)	5.4 ± 0.8	5.2 ± 1.6	2.7 ± 0.3
	Reactor TSS (mg L^{-1})	714 ± 10	790 ± 32	5204.2 ± 2006.2
	Reactor VSS (mg L^{-1})	276 ± 5	318 ± 9	4036.3 ± 1955.3
	Biomass productivity (gVSS $m^{-2}d^{-1}$)	8.5 ± 0.6	11.2 ± 1.5	
	DO (mg O_2 L^{-1})	9.1 ± 2.9	9.8 ± 2.5	2.5 ± 1.1
Removal Efficiencies (%)	COD	60.5 ± 26.8	68.8 ± 16.1	91.1 ± 4.9
	N-NH_4^+	98.5 ± 1.1	97.6 ± 2.4	72.9 ± 13.9
	P-PO_4^{-3}	82.1 ± 11.5	82.9 ± 8.7	72.2 ± 13.2
	TSS	62.9 ± 16.1	70.1 ± 16.6	97.6 ± 1.5
	E. Coli	99.0 ± 0.1	98.1 ± 0.1	99.9 ± 0.0
	Enterobacter F.	99.9 ± 0.0	99.8 ± 0.0	99.9 ± 0.0

higher than those achieved in microalgae cultures (30–35 % higher on average) due to the strong retention of particles in the membrane, since the size of membrane pores was 0.004 µm SM (SM4. Conventional wastewater quality parameters). Organic matter was effectively removed, with efficiencies above 90 % for COD in the MBR, while the experimental HRAPs did not exceed 70 %. Similar results were reported by Matamoros [21], who achieved COD removal efficiencies between 66 % and 85 % on smaller HRAPs. In the same way, García [32] reported a TSS removal ranging between 60–70 % in a raceway reactor followed by a gravidity settler with a biomass concentration in the culture broth between 200–350 mg TSS L^{-1}. The relatively low TSS and COD removal of microalgae systems is due to fact that approximately 30 % of the algal biomass escapes from the clarifiers, which is consistent with the findings reported by García [32]. Both microalgae systems presented similar nutrient and organic matter removal efficiencies. N-NH_4^+ removal was > 95 % in both cases and much higher than in the MBR process. N-NH_4^+ is the preferred form of nitrogen uptake for most microalgae species [33]. At this point, it is important to notice that photosynthetic systems (algae) presented a considerably higher removal of nitrogen and phosphorus compared to MBR and achieved the discharge limits for urban wastewater and HRAP systems according to the European Directive 91/271/EEC. The MBR demonstrated 72 % removal efficiencies for both phosphorus and nitrogen, while the microalgal reactors achieved superior removal performances, exceeding 82 % for phosphorus and 96 % for nitrogen, respectively. Total nitrogen present in the effluent of the MBR was in the form of nitrate, evidencing a limitation in the denitrification processes which can be attributed to the relative low HRT or to recirculation rate, as reported by Villar-Navarro [34]. The superior performance in nutrient uptake is in accordance to the previous studies devoted to evaluate algae systems [35,36]. The intense nutrient uptake of algae biomass reduces the concentration of soluble nitrogen and phosphorous. Beside this, elevated pH values promote abiotic elimination of NH_3, through volatilization and PO_4^{3-} precipitation, resulting in very low concentrations in the final effluent [26]. Both systems, algae and MBR provided high removal of the pathogen indicator *Escherichia coli* and *Enterobacter Faecalis*. The level of disinfection in all the effluents was compatible with use of water for irrigation of crops in which the edible part is produced above ground and is not in direct contact with water (class B in the reclaimed water classification) of according to the European Regulation (2020/741) [20]. The high disinfection rate with microalgae treatment demonstrates its potential use as a secondary or tertiary treatment combined with conventional wastewater treatment for water reuse.

3.2. Occurrence of CECs in WWTPs

From the 39 target compounds investigated only 26 were found either in influent or effluent wastewater (IWW and EWW, respectively) (Table 1). Only data for the targets that were quantified are shown and discussed in this work. The following compounds were not found in any of the samples analysed: furaltadone, flumequine, ioprimide, levamisole, lorazepam, nalidixic acid, norfloxacin, oxolinic acid, pantoprazole, primidone, roxithromycin, sulfadiazine and tetracycline. The concentrations in the influent of WWTP are shown in Table 3 along with reference values found in previously documented experiments. Concentration data for all samples analysed, including IWW and EWW obtained after different treatment systems, are shown in SM (Table SM3 and Fig. SM6).

The results obtained in this research are in agreement with other studies that have reported the presence of pharmaceuticals in influent and/or effluent wastewater. For most of the compounds the concentrations are in the range of previous studies (Table 3). However, concentrations of acetaminophen, gabapentin and losartan in the samples seem considerably higher, values ranging from 15 to 82 µg L^{-1}, revealing the high consumption of these compounds. It is remarkable that peak values in pharmaceutical concentration in wastewaters are normally reported as a consequence of seasonal consumption or presence of aged population in the area, which could be the case of the town in this study. The average concentrations of compounds identified and quantified in the effluent of the three experimental systems are presented in SM (Table SM2). As it can be seen, 26 out of 39 pharmaceutical compounds were detected in the influent and effluent but only 13 effluent compounds surpassed the limit of quantification (10 ng L^{-1}).

3.3. Removal efficiency of CECs

In general, both microalgal cultures and the AS-MBR system exhibited high CEC removal efficiencies, and comparable trends were observed despite the contrasting biotic and abiotic conditions of the experimental systems. The AS-MBR system presented a considerably more elevated performance compared to the reported values found in conventional activated sludge systems, where biomass is separated in settlers [12]. This may be due to the higher TSS concentration in biological reactors and higher sludge age found in the MBR compared to the mixed liqueur of activated sludge tanks. Therefore, higher removals were achieved for hydrophobic substances that are highly adsorbed in biomass (tramadol, azithromycin, atorvastatin, valsartan, diclofenac, and omeprazole), which are characterized by high K_{ow} values (>3). At this point, it is noteworthy that no physical retention of molecules in the membrane of the MBR system is likely to take place, since the size of the molecules is several orders of magnitude below the pore size. Nevertheless, several authors have reported the retention of hydrophobic compounds as a result of high suspended solid concentration and the biofilm formation in membranes [38, 45].

In case of microalgae treatments, a similar trend to that observed in the AS-MBR was found for most of the pollutants, with very high or total elimination for pharmaceutical compounds such as sulfamethoxazole, salbutamol, phenazone, clindamycin, enalapril and others. A comparative evaluation of algae systems and AS-MBR is showed in fig. 8. In the analgesic group (GI) (Fig. 2), 92 % of acetaminophen was removed in Lab Algae, 99 % in Outdoor Algae, while 100 % was reached in the AS-

Table 3
Compounds and concentrations found in urban wastewater in this work and in prior studies.

COMPOUND	Family/Function	CONCENTRATION (ng L^{-1})					
		INFLUENT *			CR **		
Acetaminophen	Analgesic	82,839.31	±	27,694.73	1570.00	-	56,900.00[f]
Alprozalam	Benzodiazepine		-			-	
Atorvastatin	Hypolipidemic agent	465.40	±	279.36	76	-	220.00[e]
Azithromycin	Antibiotic	3004.00	±	1644.68	260.00[g]	-	22,730.00[b]
Carbamazepine	Antiepileptics/Sedative/Anxiolytic		-		50.00[a]	-	660.00[d]
Ciprofloxacin	Antibiotic	2581.67	±	1560.81	610.00[a]	-	4371.00[b]
Clarithromycin	Antibiotic	<LOQ			59.00[e]	-	360.00[a]
Clindamycin	Antibiotic	253.33	±	123.27	<LOQ[b]	-	101.00[b]
Diclofenac	Analgesic	1057.20	±	724.87	100.00[e]	-	1100.00[a]
Enalapril	Antihypertensive	167.75	±	91.51	<LOQ[a]	-	30.00[a]
Erithromycin	Antibiotic	<LOQ			60.00[a]	-	530.00[f]
Gabapentin	Antiepileptics/Sedative/Anxiolytic	23,800.40	±	10,155.27	210.00[a]	-	4500.00[a]
Irbesartan	Antihypertensive	365.40	±	394.73	510.00[a]	-	870.00[a]
Lincomycin	Antibiotic		-		<LOQ	-	70.00[a]
Losartan	Antihypertensive	1261.80	±	721.23	140.00[a]	-	1552.00[i]
Metronidazole	Antibiotic	443.00	±	184.74	90.00[a]	-	962.00[h]
Metropolol	Beta-blocker	65.33	±	21.78	30.00[a]	-	170.00[d]
Omeprazole sulfide	Antiulcer drug	15,352.40	±	7860.33	500.00[b]	-	15,000.00[c]
Phenazone	NSAID	33.25	±	13.18		-	
Salbutamol	Beta-blocker	51.50	±	30.45	50.00[h]	-	150.00[h]
Sulfamethoxazole	Antibiotic	299.00	±	0.00	40.00[a]	-	5490.00[b]
Tramadol	Analgesic	638.80	±	201.35	800.00[a]	-	2890.00[a]
Trimetroprim	Antibiotic	82.00	±	101.82	10.00[e]	-	230.00[a]
Valsartan	Antihypertensive	7213.80	±	5620.69	1490.00[a]	-	9010.00[a]
Venlafaxine	Antiepileptics/Sedative/Anxiolytic	1250.80	±	498.09	195.70[e]	-	213.00[e]
O-Desmethyl venlafaxine	Metabolite	32,268.20	±	12,607.83		-	

*Concentrations found in this work
**CR (Concentration Reported in prior studies): [a] [37], [b] [5], [c] [38], [d] [39], [e] [40], [f] [41], [g] [42], [h] [43], and [i] [44]
<LOQ:<Limit of Quantification: compound detected in the sample but not quantified (< 25 ng L^{-1}).
-: not detected

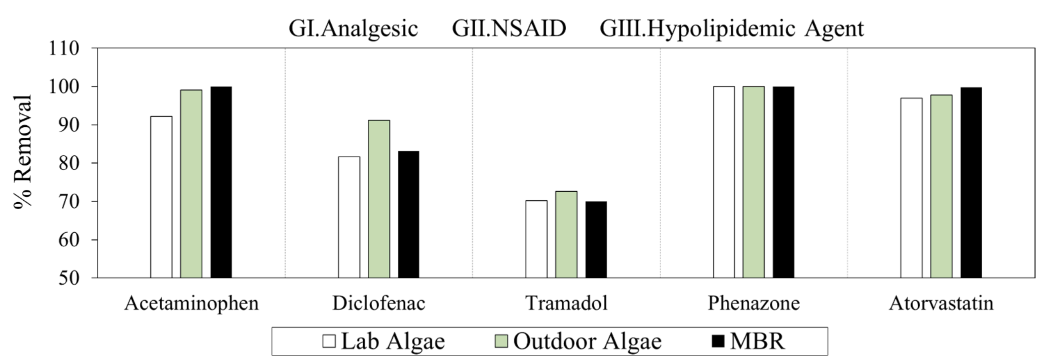

Fig. 2. Efficiency of CECs elimination (%) of different treatment systems of GI. Analgesic, GII. NSAID, and GIII. Hypolipidemic Agent.

MBR. This compound exhibits a high biodegradability, consistent with observations from previous research. [46]. Additionally, prior studies have demonstrated substantial acetaminophen removal in HRAPs [21, 34], and complete elimination in activated sludge systems [47]. Tramadol showed slightly higher removal (3 %) in the outdoor microalgae system (70 % compared to 73 % of lab experiment), likely due to its susceptibility to photodegradation under ultraviolet light. [48]. In case of NSAIDs (GII), diclofenac was extensively removed (82 % in Lab Algae, 91 % in Outdoor Algae and 83 % AS-MBR), while previous studies showed lower eliminations. For instance, Garcia et al. [49] obtained a decrease of 55 % and demonstrated that bio-adsorption/bioaccumulation was the main contributing mechanism since diclofenac was found in the collected algae biomass. Similarly, the study of Matamoros et al. [21] highlighted the influence of HRT on pollutant removal efficiency, with 82 % removal at 4 days HRT and 92 % at 8 days HRT. Contrary, Wilt et al. [50] obtained removals between 40 % and 60 %, attributing its elimination to photo biodegradation due to the lack of diclofenac in the reactors' biomass. In case of activated sludge a different range of removal efficiencies of diclofenac have been documented, with values between 0 % and 81 % in conventional systems and slightly higher in case of AS-MBR with values between 19 % and 98 % [51]. According to several studies, the anoxic–oxic ratios may influence the removal of this compound, which could partly explain the wide variability in reported removal efficiencies. The enhanced reduction observed in this study may be attributed, at least in part, to the relatively high biomass concentration and the hydrophobic nature of diclofenac.

Total removal of the hypolipidemic agent (Fig. 2), atorvastatin, was detected in the activated sludge system, while microalgae also exhibited very high eliminations: 97 % in Lab Algae, 98 % in Outdoor Algae. This compound presents a high lipophilic nature with a Log K_{ow} of 6.36 and higher molecular weight 540 g mol^{-1}. Previous studies have evidenced the adsorption and biodegradation during wastewater treatment. Ottmar [52] demonstrated that atorvastatin is adsorbed during primary treatment and secondary treatment and that both sorption and biodegradation take place during secondary aeration and clarification in conventional activated sludge. Therefore, it is in agreement with the almost total elimination detected in the MBR system.

The removal of antibiotics from group (GIV) is shown in Fig. 3. The removal efficiencies measured across the three systems were comparable and notably high, despite the influent concentrations being lower than those reported in previous studies (Table 2), which could account for the observed high removal rates. In contrast to the other compounds of this class, azithromycin presented a lower elimination with 85 % in Lab Algae, 90 % in Outdoor Algae and 95 % AS-MBR, respectively. This compound is also characterized by a high molecular weight and a high hydrophobic coefficient (Log K_{ow} 4.02). This compound is poorly metabolized, and bioaccumulation may represent a major contributing mechanism. Previous studies have reached the same conclusion, reporting that other organic compounds with simpler molecular structure, higher water solubility and moderate adsorption capacity, are easily degraded by microorganisms [53]. Ciprofloxacin and clindamycin presented high Res, with a more important elimination of the second (85 %-100 % in Lab Algae, 94 %-97 % in Outdoor Algae and 97 %-100 % AS-MBR, respectively). At this point, it must be stressed that these compounds presented very different concentrations in the inlet wastewater, a factor that probably impacts the relative percentage of removal. While ciprofloxacin presented a high influent concentration of 2581.67 ± 1560.81 ng L^{-1}, clindamycin was in the low range of concentration: 253.33 ± 123.27 ng L^{-1}. The differences in the hydrophilic properties of the two compounds, Log K_{ow} of 0.4 (ciprofloxacin) and Log K_{ow} 2.1 (clindamycin), may explain the higher removal of clindamycin. Sulfamethoxazole with a 100 % elimination in the three systems, higher than previously reported experiences in HRAP and AS-MBR systems, was probably due to the low inlet concentration detected in the inlet wastewater (see Table 1). This compound is highly soluble in water with a pK_a 5.5, and it could be found in its anionic form (more stable) due to the pH of the microalgae cultures (9.1–9.6 pH). Moreover, the low sorption potential (Log K_{ow} 0.89) could limit bioadsorption/bioaccumulation due to the electrostatic repulsion of the algal cells. To explain the elimination, Bai and Acharya [54] demonstrated the low efficiency of microalgae to remove sulfamethoxazole by bioadsorption and observed that organic matter associated with microalgae cells could induce indirect photolysis and subsequent molecule breakdown. In relation to trimethoprim, with similar properties to sulfamethoxazole and elevated removals: 97 % in Lab Algae, 100 % in Outdoor Algae and 100 % AS-MBR, the molecule is barely bioadsorbed into biomass according to Bai et al. [54]. However, this compound could be biodegraded and or broken down by indirect photolysis. Regarding the elimination of metronidazole, with a similar low influent concentration (454 ± 11 ng L^{-1}), total removal was found in all the systems. The high hydrophobicity of this antimicrobial (Log K_{ow} −0.01) and its moderate molecular weight 171 g mol^{-1}, suggest low adsorption. Several studies confirmed that metronidazole was not detected in algal biomass after treatment [49]. Therefore, photodegradation seems to be the most likely removal mechanisms as previously demonstrated the study conducted by Tong et al. [55].

Gabapentin is an antiepileptic compound (GV) increasingly common as it can be observed in other studies, with concentration up to 4.5 μg L^{-1} which clearly indicates that municipal wastewater is the main source in the environment [56]. The removal in the microalgae systems (Fig. 4) was 58 % and 66 % in Lab Algae and Outdoor Algae, respectively, with an influent concentration about 23.8 μg L^{-1}. It must be noticed that this value is among the highest found in this study. In contrast, removal in the AS-MBR system was much higher (97 %). In this case, the relatively greater volatility of this compound may explain the improved performance observed. In this regard, aeration in this WWTP is considerably higher than in conventional activated sludge systems, as fine-bubble aeration is applied to the activated sludge, and coarse bubbles are used in the MBR to achieve a homogeneous reactor. This approach enhances the removal of compounds characterized by a relatively high Henry's law constant.

The antihypertensive group (GVI) (Fig. 4), losartan and valsartan had similar behaviour (80 %-90 % in Lab Algae, 82 %-93 % in Outdoor Algae and 96 %-99 % in AS-MBR, respectively). The superior performance of activated sludge could be related to higher biomass concentration that enables the adsorption into bacteria biomass of this hydrophobic substances (K_{ow}>3). In microalgae systems, Outdoor algae presented a higher removal rate probably due to direct photodegradation mediated by UV light, given that these chemicals present photodegradation according to Kaur et al. [57]. Enalapril showed a 100 % RE which can be explained by its lower molecular weight and lower IWW concentration of the antihypertensive group. Irbesartan presented a different pattern than the group (97 % in Lab Algae, 90 % in Outdoor Algae and 76 % AS-MBR). This compound is characterized by a high adsorption potential due to its hydrophobic nature (Log $K_{ow} > 2.7$). Surprisingly, the hydrophobicity did not result in higher eliminations in the MBR.

Beta-blocker removals (GVII.) metoprolol and salbutamol were totally eliminated in the three experiments, which is in agreement with previously reported studies in case of HRAP, where high eliminations are reported, between 70 % and 100 % (Fig. 5). For instance Liu et al. [16] found metoprolol removal of 90 %. In case of metoprolol similar results were reported by Wick et al. [39]. The inlet concentration was in accordance with other studies (35–170 ng L^{-1}) and (50–150 ng L^{-1}), respectively for salbutamol and metoprolol [37, 43]. The elevated removal efficiencies may be attributed to the intrinsic biodegradability of these hydrophilic compounds [50], together with the low influent concentrations of the beta-blockers.

As shown in Fig. 5, enalapril from the antidepressant group was completely eliminated (100 %) in both microalgal and activated sludge systems. It is important to highlight that the influent concentration was considerably higher than values reported in earlier studies (167 ng L^{-1} vs. 0–30 ng L^{-1}) [30]. The hydrophilic characteristics and values of the Henry coefficient 2.63 E^{-11} suggest that this compound could be volatilized in both kinds of systems, although photodegradation can also take place. In contrast, the venlafaxine (antidepressant) showed more limited removals, with higher elimination in Outdoor Algae (84 %) than

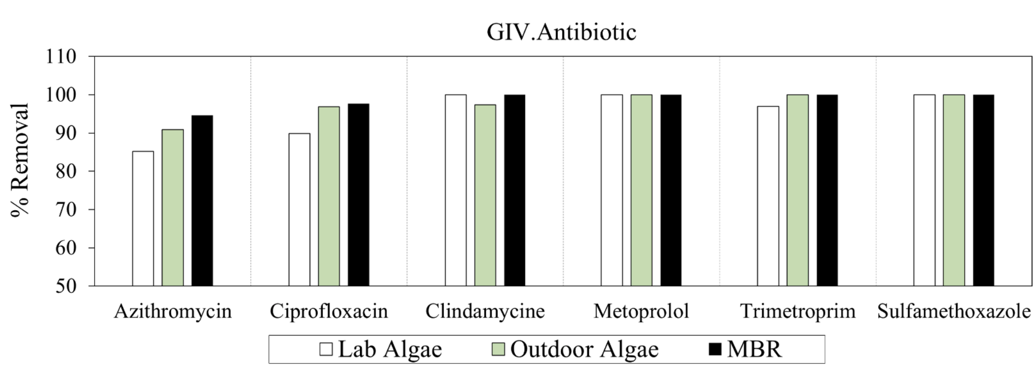

Fig. 3. Efficiency of CECs elimination (%) of different treatment systems of GIV. Antibiotic.

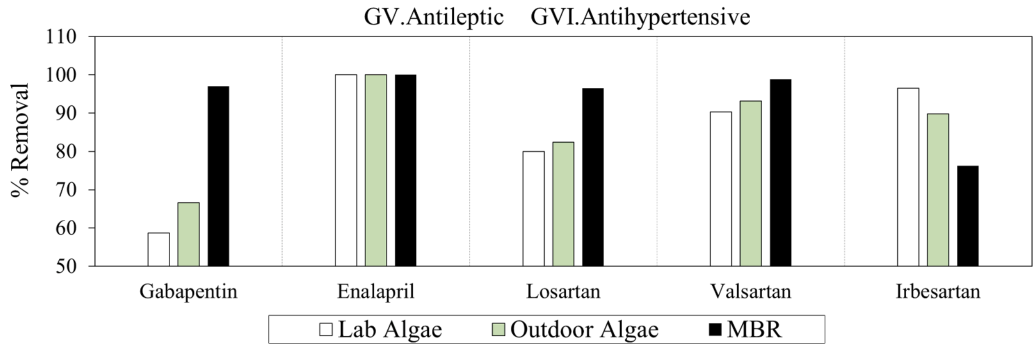

Fig. 4. Efficiency of CECs elimination (%) of different treatment systems of GV. Antiepileptic, and GVI. Antihypertensive.

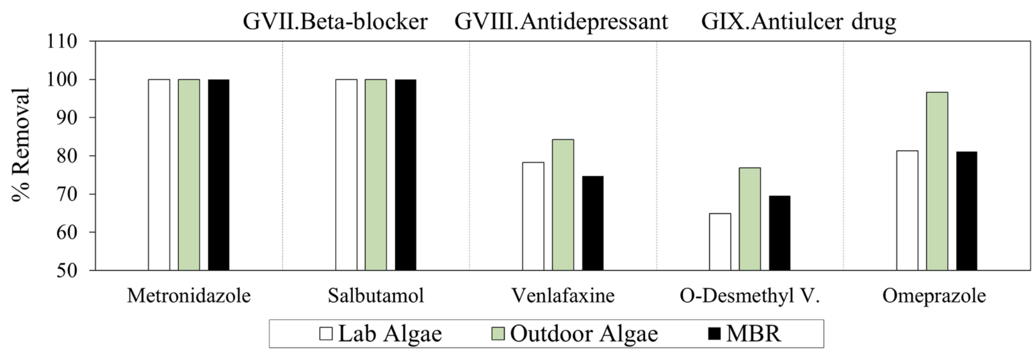

Fig. 5. Efficiency of CECs elimination (%) of different treatment systems of GVII. Beta-blocker, GVIII. Antidepressant, and GIX. Antiulcer drug.

in Lab Algae (78 %) and MBR (74 %). The venlafaxine removal can be attributed to bioadsorption given the hydrophobic nature of the molecule (Log K_d 3.2), although photodegradation has also been suggested in previous studies [48]. The removal differences between venlafaxine and o-Desmethyl venlafaxine can be caused by differences in their hydrophilic characteristics.

Overall, microalgal systems achieved removal efficiencies within the same range as the activated sludge–membrane bioreactor treatment (Fig. 8). This trend was particularly evident for hydrophobic, biodegradable, and volatile compounds, since both treatment configurations favour these removal pathways. The enhanced removal efficiency observed in the AS-MBR systems could be associated with increased volatilization of certain compounds (e.g., gabapentin) and greater biomass adsorption capacity due to the elevated sludge concentration, particularly for azithromycin, valsartan, and losartan. The evaluated conditions corresponded to the temperate season in mid-latitude regions, characterized by average annual temperature and solar irradiance. In the case of the AS-MBR, the removal efficiency of pharmaceutical compounds is expected to remain similar under both colder and warmer conditions. However, in microalgae-based systems, the higher removal efficiency of hydrophobic and photolabile compounds is likely to occur during summer, due to the increased light exposure and greater biomass accumulation in the reactors, in agreement with previous findings. [21].

3.4. Regulation of CEC in urban wastewater treatment

It is worth highlighting the recently approved Directive concerning urban wastewater treatment [9] in which it is established the need of WWTPs meeting the requirements for "quaternary" treatments -those referred to the elimination of micropollutants- in the WWTPs for agglomerations of above 150,000 p.e. and into special areas of above 10, 000 p.e. These requirements imply a mean removal of ≥ 80 % of at least six substances from the following lists: (a) amisulpride, carbamazepine, citalopram, clarithromycin, diclofenac, hydrochlorothiazide, metoprolol, venlafaxine; (b) benzotriazole, candesartan, irbesartan and mixture of 4-methylbenzotriazole and 5-methylbenzotriazole. Moreover, the number of determined substances from category (a) should be twice the number of substances from category (b). In the present work, six of the substances included in groups a) and b) have been monitored in experimental systems and four of them have been detected. Although this regulation will come into force in 2030, the results obtained from the microalgae cultures and the AS-MBR (table 4 and Fig. 6) show that the tested systems reached in most of the cases the target of 80 % of removal, or very close values higher than 74 %. These results evidenced the potential of both technologies, membrane and microalgae culture as complete treatment for wastewater treatment and reuse in the framework of the recent requirements of pollution removal.(Fig. 7)

The RE were mostly higher than 80 % (Fig. 6), suggesting that the systems applied in this work have the potential to be used as additional treatments for the efficient removal of micropollutants in wastewater, according to the requirements of the Directive (EU) 2024/3019.

4. Conclusions

The comparative evaluation of microalgae-based wastewater treatment systems (laboratory-scale and outdoor raceway pond) and an activated sludge–membrane bioreactor (AS-MBR) enabled a comprehensive assessment of nature-based technologies against one of the most advanced urban wastewater treatment processes currently available. The comparison considered the removal performance of CECs, pathogens, organic matter, and nutrients. While the AS-MBR achieved superior removal of organic matter, the microalgal systems demonstrated enhanced nutrient elimination. Both treatment configurations attained high pathogen removal efficiencies, consistent with the second-highest disinfection category defined for water reuse.

Overall, the removal efficiencies for CECs were substantially high across all systems, showing comparable removal trends. The results

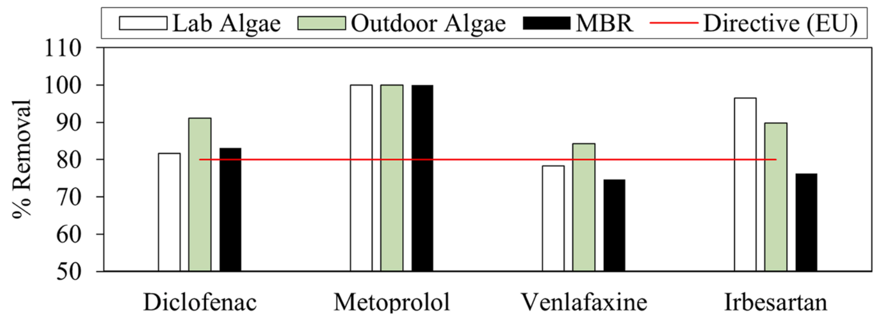

Fig. 6. Removal efficiency of contaminants of emerging concern in Lab Algae, Outdoor Algae and MBR of category a and b of DIRECTIVE (EU) 2024/3019.

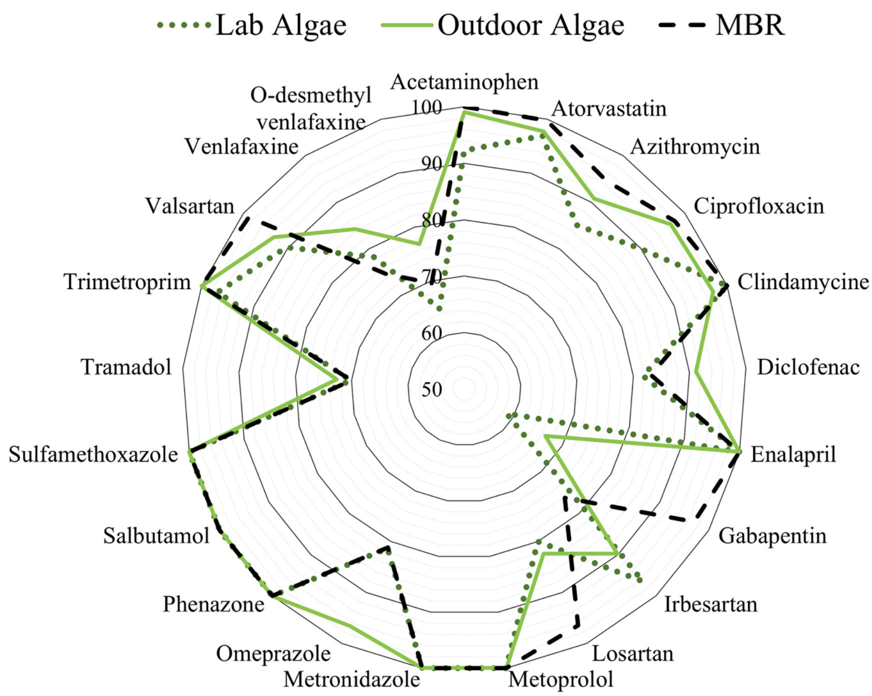

Fig. 7. Removal efficiency (%) of different treatment systems.

indicate that photodegradation and bioadsorption were the dominant removal mechanisms in the microalgae-based systems. Slightly higher removal rates were observed for hydrophobic and volatile compounds in the AS-MBR, likely due to its higher biomass concentration and aeration intensity. In contrast, for photolabile compounds, the outdoor microalgal systems achieved greater removal efficiencies, attributed to direct photodegradation under ultraviolet radiation.

Collectively, these findings demonstrate that microalgae-based wastewater treatment systems represent a competitive and sustainable alternative to membrane-assisted activated sludge processes, as both configurations are capable of meeting the discharge standards established under current European Union regulations.

CRediT authorship contribution statement

Félix Gaspar Gonzalo Ibrahim: Conceptualization, Methodology, Investigation, Writing original draft. **Ana Botero Coy:** Methodology, Investigation, Resources. **Félix Javier Hernández Hernández:** Conceptualization, Investigation, Methodology, Resources, Writing, review & editing, **Raúl Muñoz Torre:** Resources, Writing, review & editing. **Ignacio de Godos Crespo:** Conceptualization, Resources, Supervision, Writing, review & editing.

Environmental implication

Ignacio de Godos, as corresponding author, declares that the study described in the submitted manuscript entitled "Performance comparison of microalgae-based and activated sludge with membrane filtration (AS-MBR) for emerging contaminant removal and wastewater reuse" has been assessed and determined to have no significant environmental implications. It is not expected to cause any adverse impact on the environment. Moreover the study deals with a proposed technology addressed to reduce environmental impact of certain contaminants released to the environment.

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.140553.

Data availability

No data was used for the research described in the article.

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